BF

Biocenter Finland

ANNUAL REPORT 2014

Biocenter Finland Annual Report 2014

Editors: Olli A. Jänne, Eero Vuorio, Marianna Jokila

Cover: iStockphoto

Photos: Mauri Helenius (pages 20, 46), Eija Hiltunen (pages 52, 62)

Acknowledgements: We are grateful to BF infrastructure network coordinators and technology platform chairs and members for cooperation in compiling this report.

www.biocenter.fi

CONTENTS

FOREWORD

This is the fifth and last foreword that I am writing for the Annual Report of Biocenter Finland (BF), as I retired at the end of 2014. During the four previous years, I have said most of the important things, so this time I will try to be brief. Yet, BF has seen ups and downs during the past five years, and these deserve some comments in retrospect.

Build-up Phase 2010–2012

In 2010–2012, I had the pleasure to be involved in the implementation of the restructuring program that de facto created the BF, as we know it today. I witnessed the excitement of the infrastructure community during the build-up phase, when the 45 M€ of strategic funding brought the infrastructure networks and technology platforms together to plan for joint services and division of responsibilities, provided them with new equipment and salaries for personnel, and subsequently brought national technology services to a new level. The participating biocenters agreed on coordination of technology service development and division of responsibilities at national level. Just like the ESFRI (European Strategy for Research Infrastructures) process in Europe, --

> "--the earmarked funding to BF brought a new kind of momentum to the life science community in Finland. It also contributed to profiling of Finnish universities. The BF concept was duly recognized both in Finland and abroad, and brought fame to Finnish science policy throughout Europe."

During my five years in BF, I was invited to give presentations on the BF concept in the Nordic countries, Greece, Ireland, Italy, Germany, the European Commission, and various ESFRI fora. Also members of our own Scientific Advisory Board and of the evaluation panels for the updated Finnish Research Infrastructure Roadmap efficiently spread the word about the Finnish invention. A survey of BF user community demonstrated that also our users were quite satisfied with the technology services provided.

Subsequent Years 2013–2014

At the end of 2012, the strategic funding from the Ministry of Education and Culture (OKM) ended, although everyone seemed to agree that sustainable funding is the key to the success of research infrastructures (such as EMBL, CERN and ESRF). Fortunately,

the host universities came to rescue the salaries of trained BF technology personnel and the coordination office, but university resources were not sufficient to fund new and updated equipment to a level that maintains state-of-the-art. Therefore, BF was looking optimistically towards the newly established national infrastructure committee (FIRI) of the Academy of Finland as the major source of equipment funding. Upon the very positive statements by the international evaluators, BF was placed on the national infrastructure roadmap. The two first FIRI application rounds have, however, shown that the BF concept is not seen as a suitable model for national funding of life science infrastructure. Although the user community of BF is by far the largest in life sciences, the funding BF received in 2013 and 2014 represented a mere 15–20% of total FIRI infrastructure support to this domain. This disappointing situation also hit host universities, as they received annually only 50 000–100 000 € of FIRI funds for life science equipment. To meet the biggest demands of the user community, all host universities increased their matching funds to allow BF to update at least the most important equipment.

Already in 2014, I predicted that success in obtaining sufficient FIRI funding would be the moment of truth for the future of BF. We failed to reach our goal in 2013 and again in 2014. The fact that Finnish participation in pan-European research infrastructures on the ESFRI roadmap receives more than twice as much FIRI funding as BF, shows that funding of national technology services of BF has not been considered a top priority. The BF concept is not fit for this type of competition. There are probably many explanations for the current dilemma. When priority lists for acquisition of new equipment are drafted as a joint effort of BF infrastructure networks, technology consortia and ultimately the BF board, these lists may not look what the host universities consider their priorities. Indeed, some rectors have confirmed this to be the case. Apparently, the FIRI committee did not like the BF list of prioritized equipment either, since they did cherry-picking and selected individual pieces of equipment from the priority list for funding. Another unresolved problem stems from the national ESFRI nodes competing with BF in the same FIRI calls; they are operational in the same technology areas as BF and often operated by the same individuals. No wonder BF and the ESFRI nodes have been criticized for fishing in the same waters with two nets.

The Future

This all means that life will not be easy for my successor, professor Olli A. Jänne. In addition to the problems of finding funding for equipment, his term coincides with other big changes, such as reorganization of biocenters at University of Helsinki, the planned establishment of the Helsinki Life Science Center, and overall budget cuts due to the difficult financial situation. Maybe the BF concept and the distribution of responsibilities also need to be reviewed. Olli played a central role in the preparation of the strategy document "Restructuring and Development of Biosciences in Finland", which in 2009 resulted in the generous 45 M€ strategic support from OKM to BF. Therefore, I trust he will again be able to convince decisions makers that the current underfunding of infrastructure cannot continue, if the universities want to remain competitive.

> "Life sciences have become increasingly dependent on top-of-the-line equipment, and no one can remain competitive without access to such technologies."

It is striking to note that in Sweden, the Science for Life Laboratory, a concept analogous to that of BF, receives more than ten times the funding BF does, with a promise for more in the upcoming years. I hope the current FIRI policy will be evaluated for its efficacy and success in fulfilling the needs of the national user community.

> "Now that my term has come to an end, I would like to extend my very best thanks to the entire BF community for creating a unique national research infrastructure and for demonstrating its functionality."

I also want to thank all members of the BF SAB for their immensely valuable work. Likewise, I express my thanks to my Board members for their commitment and support, also at difficult times. I am also very grateful to the leadership of BF host universities for their continued support. Another big change coincides with my retirement from BF: Sanna Leinonen, who served as the planning officer of BF for five years also moved to a new position in January 2015 and was replaced by Marianna Jokila. I want to thank Sanna for all the work she did for BF and wish her, Olli and Marianna best of luck in their new challenging positions.

Eero Vuorio,
Director of Biocenter Finland, 2010–2014

UNIVERSITY OF HELSINKI

UNIVERSITY OF EASTERN FINLAND

UNIVERSITY of OULU
OULUN YLIOPISTO

UNIVERSITY OF TAMPERE

Turun yliopisto
University of Turku

Åbo Akademi

Director

Eero Vuorio

Governing Board in 2014 (deputies in parentheses)

Chairman of the Board
John Eriksson, BioCity Turku, Åbo Akademi

Vice-Chairman of the Board
Jyrki Heino, BioCity Turku, University of Turku (Riitta Lahesmaa)

Board members
Olli Kallioniemi, FIMM, University of Helsinki (Janna Saarela)

Pekka Lappalainen, Biocentrum Helsinki, University of Helsinki (Mart Saarma)

Johanna Myllyharju, Biocenter Oulu, University of Oulu (Kalervo Hiltunen)

Tomi Mäkelä, Institute of Biotechnology, University of Helsinki (Pekka Lappalainen)

Tapio Visakorpi, BioMediTech, University of Tampere

Seppo Ylä-Herttuala, Biocenter Kuopio, University of Eastern Finland

Governing Board in 2015

John Eriksson (Chairman of the Board), BioCity, Åbo Akademi;
Johanna Myllyharju (Vice-Chairman of the Board), BCO, University of Oulu (Outi Savolainen);
Jyrki Heino, BioCity, University of Turku (Riitta Lahesmaa);
Olli Kallioniemi, FIMM, University of Helsinki (Janna Saarela);
Pekka Lappalainen, BCH, University of Helsinki (Sampsa Hautaniemi);
Tomi Mäkelä, BI, University of Helsinki;
Tapio Visakorpi, BMT, University of Tampere (Anne Kallioniemi);
Seppo Ylä-Herttuala, BCK, University of Eastern Finland

Director in 2015

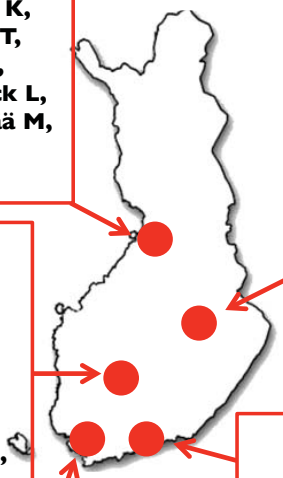
Olli A. Jänne

Biocenter Finland Administration

Planning officer
Sanna Leinonen

BIOCENTER FINLAND FACULTY

In 2014, Biocenter Finland Faculty comprised 274 scientists who belonged to the biocenters in Helsinki, Kuopio, Oulu, Tampere and Turku. Of note, each biocenter has used its own criteria and/or peer review process in assembling the membership (group leaders or principal investigators), and the criteria used varied among the biocenters. Nonetheless, the BF Faculty includes top-tier scientists in each of the scientific fields represented by BF.



Oulu (BCO)

Eskelinen S, Herzig K-H, Hiltunen K, Järvelin M-R, Juffer A, Kietzman T, Kursula P, Lehtiö L, Manninen A, Myllyharju J, Pihlajaniemi T, Ruddock L, Savolainen M, Savolainen O, Sillanpää M, Soininen R, Vainio S,Wei G, Wierenga RK, Winqvist R (20)

Kuopio (BCK)

Airanne K, Auriola S, Carlberg C, Giniatullin R, Goffart S, Gröhn O, Hiltunen M, Hinkkanen A, Jolkkonen J, Kinnunen T, Koistinaho J, Koistinen A, Kosma V-M, Laakso M, Laitinen JT, Levonen A-L, Liimatainen T, Paananen J, Palvimo J, Pihlajamäki J, Pitkänen A, Poso A, Raunio H, Rouvinen J, Tammi M, Tanila H, Tavi P, Töyräs J, Urtti A, Vepsäläinen J, Ylä-Herttuala S (31)

Tampere (BMT)

Aalto-Setälä K, Bova GS, Flodström-Tullberg M, Hultmark D, Hytönen V, Isola J, Jacobs H, Kaguni L, Kallioniemi A, Kulomaa M, Lilja H, Miettinen S, Narkilahti, Nykter M, Pesu M, Rämet M, Schleutker J, Skottman H, Ungureanu D, Visakorpi T (20)

Helsinki (BCH, BI, FIMM)

Aaltonen L, Airavaara M, Aittokallio T, Alitalo K, Anders S, Andressoo J-O, Auvinen P, Bamford D, Butcher S, Castrén E, Di-Poi N, Fagerholm S, Frilander M, Hautaniemi S, Heckman C, Helariutta Y, Hemminki A, Hennah W, Hietakangas V, Holm L, Horvath P, Ikonen E, Iwai H, Jacobs H, Jernvall J, Jokitalo E, Kaila K, Kainov D, Kajander T, Kallioniemi O, Kangasjärvi J, Kaski S, Katajisto P, Kauppi L, Kostiainen M, Kuure S, Kuznetsov S Lappalainen P, Linder M, Lohi H, Lundin J, Löytynoja A, Michon F, Mikkola M, Mustjoki S, Mähönen AP, Mäkelä TP, Nyman T, Ojala P, Otonkoski T, Paavilainen V, Palotie A, Peltomäki P, Permi P, Ripatti S, Saarela J, Saarma M, Saksela K, Salazar CI, Santos H, Schulman A, Shimmi O, Thesleff I, Varjosalo M, Vartiainen M, Verschuren E, Wartiovaara A, Wennerberg K, Widén E, Zhao H (70)

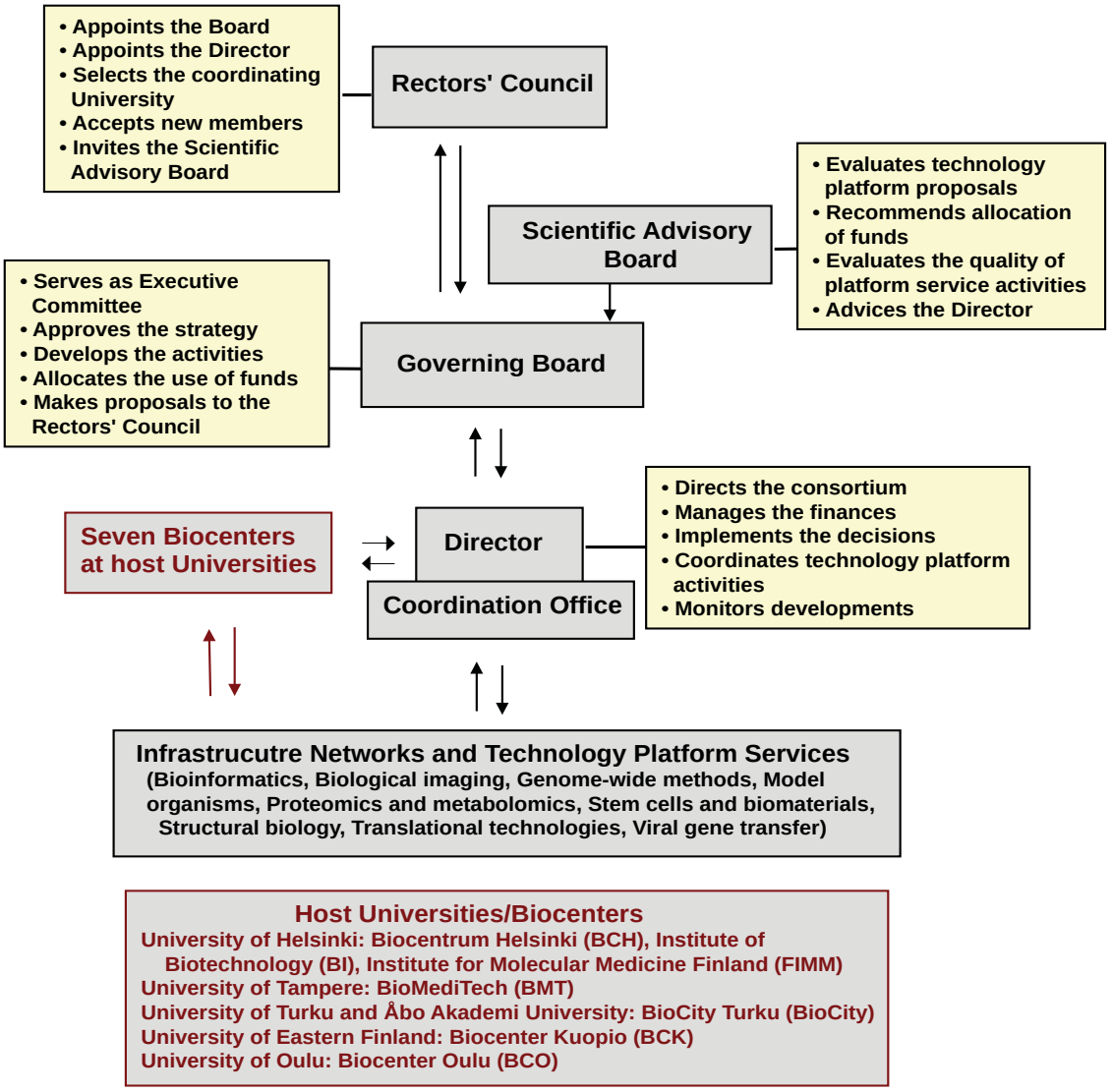
Turku (BioCity)

Abankwa D, Aro E-M, Aro H, Back R-J, Belogurov G, Carpén O,Coffey E, Corander J, Eerola E, Elenius K, Elo L, Eriksson J,Fallarero A, Granfors K, Haapakka K, Haataja L, Hakanen A,Hawkins RD, He Q, Heikkinen T, Heino J, Heino T,Hentunen T, Holmdahl R, Holopainen I, Huhtaniemi I,Huhtinen R, Hukkanen V, Huovinen P, Hupa L, Hytönen J,Häninen P, Hänninen A, Härkönen P, Härmä H,Ilmonen P, Ilonen J, Ivaska J, Ivaska K, Jaakkola P,Jalava J, Jalkanen S, James P, Jartti T, Johnson M, Kallio M, Kangasjärvi S, Kankare J, Kero J, Kiviranta R, Knuuti J,Koskinen P, Kotaja N, Kotilainen P, Kvarnström C, Kähäri V-M, Könönen E, Lahesmaa R, Lahti R, Laitala-Leinonen T, Lamminmäki U, Lapinleimu H, Lassila O, Leder EH, Lehto K, Lehtonen L, Lukkari J, Lund R, Lähdesmäki H, Majamaa K, Mattila P,Meriluoto J, Mertsola J, Meurman O, Mulo P, Mäkelä S, Mäkinen J, Mäntsälä P, Määttä J, Nees M,Niemi J, Nikinmaa M, Nuutila P, Nykter M, Närhi T, Oresic M, Papageorgiou A, Paranko J, Pelliniemi LJ, Peltola M, Peltola V, Peltonen J, Perheentupa A, Petre I, Pettersson K, Poutanen M, Primmer C, Rahman N, Raitakari O, Rantakokko-Jalava K, Rautava P, Roivainen A,Rosenholm J, Ruohola A, Sahlgren C, Salakoski T, Sandler N, Savilahti H, Savontaus E, Scheinin M, Schleutker J,Sillanpää M, Simell O, Sistonen L, Smått J-H, Soukka T, Susi P, Syrjänen S, Säämänen A-M, Söderling E, Tezvergil-Mutluay A, Toivola DM, Toppari J, Törnquist K, Vallittu P, Vehniäinen M, Vuopio J, Vuorela P, Vuorinen T, Väisänen S, Waris M, Westermarck J,Wittfooth S (133)

ORGANIZATION OF BIOCENTER FINLAND IN 2014

Biocenter Finland was established in 2006 by the six Finnish universities housing biocenters, i.e. Universities of Helsinki, Kuopio (now University of Eastern Finland), Oulu, Tampere and Turku, and Åbo Akademi University. Rectors of these universities form the highest decision making body of BF. In practice all decisions concerning the operation of BF are made by the Governing Board comprising directors of the seven biocenters. The Board meets 5–6 times per year.

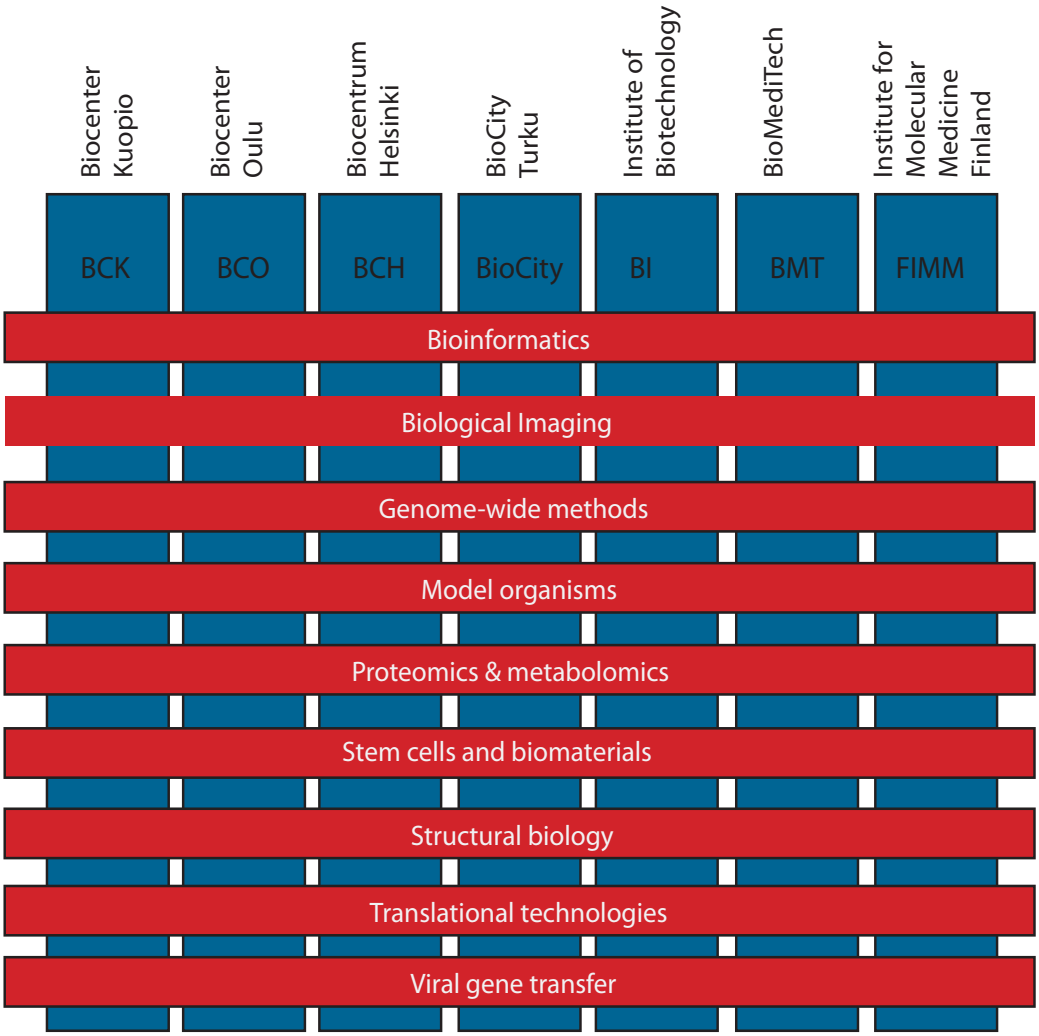
Biocenter Finland Governance Structure



Scientific Advisory Board (SAB) of Biocenter Finland for 2013–2016:

Chair: **Carl-Henrik Heldin**, Ludwig Institute for Cancer Research, Uppsala
Vice-Chair: **Ole Petter Ottersen**, University of Oslo
Members: **Marja Jäättelä**, Institute of Cancer Biology, Copenhagen, **Gunnar von Heijne**, Stockholm University, **Matthias Wilmanns**, EMBL, Hamburg

Biocenter Finland Member Institutes and Infrastructure Networks



Please note that Biocenter Finland, its member institutes and the infrastructure networks will be referred to in the text by acronyms/abbreviations as shown in the diagram above. Additional abbreviations frequently used in the text are: CSC, IT Center for Science Ltd; THL, National Institute for Health and Welfare; VTT, Technical Research Centre of Finland.

In its meeting on January 17, 2011 the Board of Biocenter Finland decided to offer scientists working at the University of Jyväskylä a possibility to participate in the activities of BF infrastructure networks. Subsequently the University of Jyväskylä named members to BF infrastructure networks in those scientific fields where the university is actively engaged in research and technology services.

SCIENTIFIC SUCCESS STORIES BASED ON BIOCENTER FINLAND TECHNOLOGY SER-

Classification of homologues enzymes by bioinformatics analyses

Thiolases are enzymes involved in lipid metabolism. Thiolases remove the acetyl-CoA moiety from 3-ketoacyl-CoAs in the degradative reaction. They can also catalyze the reverse Claisen condensation reaction that is the first step of biosynthetic processes, such as the biosynthesis of sterols and ketone bodies. In human, six distinct thiolases have been identified. Each of these thiolases is different from the other with respect to sequence, oligomeric state, substrate specificity and subcellular localization. Four sequence fingerprints, identifying catalytic loops of thiolases, have been described. Genome searches of two mycobacterial species (*Mycobacterium tuberculosis* and *Mycobacterium smegmatis*) were carried out, using the six human thiolase sequences as queries. Eight and thirteen different thiolase sequences were identified in *M. tuberculosis* and *M. smegmatis*, respectively. In addition, thiolase-like proteins (one encoded in the *Mtb* and two in the *Msm* genome) were found. The aim of the study was to classify these mostly uncharacterized thiolases and thiolase-like proteins. Several other sequences obtained by searches of genome databases of bacteria, mammals and the parasitic protist family of the Trypanosomatidae were included in the analysis. Thiolase-like proteins were also found in the trypanosomatid genomes, but not in those of mammals. To examine the phylogenetic relationships at a high confidence level, additional thiolase sequences were included, such that a total of 130 thiolases and thio-lase-like protein sequences were used for the multiple sequence alignment. The resulting phylogenetic tree identifies 12 classes of sequences, each possessing a characteristic set of sequence fingerprints for the catalytic loops. From this analysis it is now possible to assign the mycobacterial thiolases to corresponding homologues in other kingdoms of life. The results of this bioinformatics analysis also show interesting differences between the distributions of *M. tuberculosis* and *M. smegmatis* thiolases over the 12 different classes.

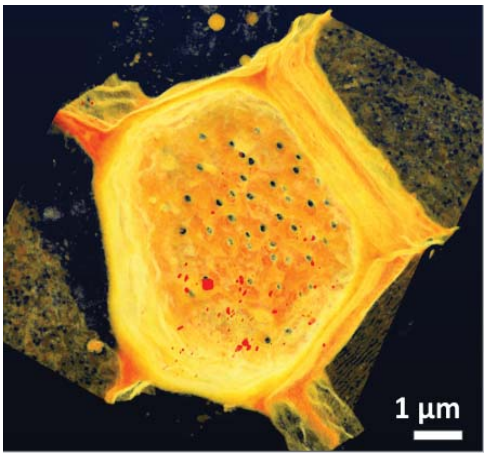
Anbazhagan P, Harijan RK, Kiema TR, Janardan N, Murthy MR, Michels PA, Juffer AH, Wierenga RK. Phylogenetic relationships and classification of thiolases and thiolase-like pro-teins of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*. Tuberculosis (Edinb) 94: 405–412, 2014.

Sieve element morphogenesis in plants

Phloem sieve element cells form a transport network specialized for long-distance allocation of photoassimilates and signaling molecules. This study follows sieve element morphogenesis in *Arabidopsis* and demonstrates that this process occurs through specialized autolysis mechanisms.

Photoassimilates such as sugars are transported through phloem sieve element cells in plants. Adapted for effective transport, sieve elements develop as enucleated living cells. The authors used electron microscope imaging and three-dimensional reconstruction to follow sieve element morphogenesis in *Arabidopsis*. The results show that sieve element differentiation involves enucleation, in which the nuclear contents are released and degraded in the cytoplasm at the same time as other organelles are rearranged and the cytosol is degraded. These cellular reorganizations are orchestrated by the genetically redundant NAC domain-containing transcription factors, NAC45 and NAC86 (NAC45/86). Among the NAC45/86 targets, a family of genes required for enucleation that encode proteins with nuclease domains was identified. Thus, sieve elements differentiate through a specialized autolysis mechanism.

Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, Heo JO, Vatén A, Lindgren O, De Rybel B, Van Isterdael G, Somervuo P, Lichtenberger R, Rocha R, Thitamadee S, Tähtiharju S, Auvinen P, Beeckman T, Jokitalo E, Helariutta Y. Plant development. *Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation. Science 22: 933–937, 2014.



Arabidopsis thaliana sieve plate

Choline is important to sieve development in plants

Multicellular organisms have diverse cellular structures to facilitate cell communication, such as receptors, channels and junction structures. Choline metabolism and transport has been known for a long time as a major factor for cell communication in animals. However, little is known about choline transport in plants. Together, our results indicate that a choline transporter-like protein functions in a choline transport process that is involved in sieve plate and sieve pore formation in plants.

Phloem, a plant tissue responsible for long-distance molecular transport, harbors specific junctions, sieve areas, between the conducting cells. To date, little is known about the molecular framework related to the biogenesis of these sieve areas. This work identified mutations at the CHER1/AtCTL1 locus of *Arabidopsis thaliana*. The mutations cause several phenotypic abnormalities, including reduced pore density and altered pore structure in the sieve areas associated with impaired phloem function. CHER1 encodes a member of a poorly characterized choline transporter-like protein family in plants and animals. The results show that CHER1 facilitates choline transport, localizes to the trans-Golgi network, and during cytokinesis is associated with the phragmoplast. Consistent with its function in the elaboration of the sieve areas, CHER1 has a sustained, polar localization in the forming sieve plates. The results of this work indicate that the regulation of choline levels is crucial for phloem development and conductivity in plants.

Dettmer J, Ursache R, Campilho A, Miyashima S, Belevich I, O'Regan S, Mullendore DL, Yadav SR, Lanz C, Beverina L, Papagni A, Schneeberger K, Weigel D, Stierhof YD, Moritz T, Knoblauch M, Jokitalo E, Helariutta Y. CHOLINE TRANSPORTER-LIKE1 is required for sieve plate development to mediate long-distance cell-to-cell communication. Nat Commun 5: 4276, 2014.

CCBE1, a new target for modulation of lymphangiogenesis and angiogenesis

The link between CCBE1 and VEGF-C in lymphangiogenesis using both *in vitro* and *in vivo* assays was explored in this work. The results show that CCBE1 affects lymphangiogenesis by enhancing the cleavage of VEGF-C by the A disintegrin and metalloprotease with thrombospondin motifs-3 (ADAMTS3) metalloprotease, resulting in the formation of mature, fully active VEGF-C.

The Hennekam lymphangiectasia-lymphedema syndrome is a rare autosomal recessive disease, which is associated with mutations in the *CCBE1* gene. Because of the striking phenotypic similarity of embryos lacking either the *Ccbe1* gene or the lymphangiogenic growth factor *Vegfc* gene, the authors searched for collagen- and calcium-binding epidermal growth factor domains 1 (CCBE1) interactions with the vascular endothelial growth factor-C (VEGF-C) growth factor signaling pathway, which is critical in embryonic and adult lymphangiogenesis. By analyzing VEGF-C produced by CCBE1-transfected cells, it was found that, whereas CCBE1 itself does not process VEGF-C, it promotes proteolytic cleavage of the otherwise poorly active 29/31-kDa form of VEGF-C by the A disintegrin and metalloprotease with thrombospondin motifs-3 protease, resulting in the mature 21/23-kDa form of VEGF-C, which induces increased VEGF-C receptor signaling. Adeno-associated viral vector-mediated transduction of CCBE1 into mouse skeletal muscle enhanced lymphangiogenesis and angiogenesis induced by adeno-associated viral vector-VEGF-C. These results identify A disintegrin and metalloprotease with thrombospondin motifs-3 as a VEGF-C-activating protease and reveal a novel type of regulation of a vascular growth factor by a protein that enhances its proteolytic cleavage and activation. The results suggest that CCBE1 is a potential therapeutic tool for the modulation of lymphangiogenesis and angiogenesis in a variety of diseases that involve the lymphatic system, such as lymphedema or lymphatic metastasis.

Jeltsch M, Jha SK, Tvorogov D, Anisimov A, Leppänen VM, Holopainen T, Kivelä R, Ortega S, Karpanen T, Alitalo K. CCBE1 enhances lymphangiogenesis via A disintegrin and metallo-protease with thrombospondin motifs-3-mediated vascular endothelial growth factor-C activation. Circulation 129:1962–1971, 2014.

A novel method for peptide and oligonucleotide labeling

A novel method allowing very rapid and simple labeling of various peptides and oligonucleotides for various targets is described. This method should be easy to transfer to other sites with only a moderate level of investment.

Seven peptide-based ^{18}F-radiopharmaceuticals for diagnostic applica-tions with positron emission tomography (PET) have thus far entered into clinical trials. Three of these are glycosylated peptides, which may be explained by the beneficial influence of glycosylation on in vivo pharmacokinetics of peptide tracers. Here, a method for labeling peptides with 5-deoxy-5-[^{18}F]fluororibose ([^{18}F]FDR) as a prosthetic group is described. The synthesis of [^{18}F]FDR is effected by a nucleophilic fluorination step by using dried Kryptofix 2.2.2-K2CO3-K^{18}F complex and a subsequent HCl-catalyzed hydrolysis. The conjugation of [^{18}F]FDR to the N-terminus aminooxy (-ONH^2)-functionalized peptides is carried out in anilinium buffer at pH 4.6 and at room temperature (21–23 °C), with the concentration of peptide precursors being 0.3 mM. The procedure takes about 120 min and includes two cartridge isolation steps and two reversed-phase HPLC purification steps. A quaternary methyl amine anion exchange cartridge and a hydrophilic-lipophilic balanced cartridge are used for the isolation of ^{18}F-fluoride and [^{18}F]FDR-conjugated peptides, respectively. The first HPLC purification provides the ^{18}F-fluorinated precursor of [^{18}F]FDR and the second HPLC purification is to separate labeled peptides from their unlabeled precursors. The final product is formulated in phosphate-buffered saline ready for injection, with a radiochemical purity of >98% and a radiochemical yield of 27–37% starting from the end of bom-bardment. The carbohydrate nature of [^{18}F]FDR and the operational convenience of this protocol should facilitate its general use.

Li XG, Helariutta K, Roivainen A, Jalkanen S, Knuuti J, Airaksinen AJ. Using 5-deoxy-5-[^{18}F]fluororibose to glycosylate peptides for positron emission tomography. Nat Protoc 9: 138–145, 2014.

Plasma-membrane protrusions as specialized metastatic engines

Mutations of the tumor suppressor *TP53* are present in many forms of human cancer and are associated with increased tumor cell invasion and metastasis. Several mechanisms have been identified for promoting dissemination of cancer cells with *TP53* mutations, including increased targeting of integrins to the plasma membrane. In tis work, the authors demonstrate a role for the filopodia-inducing motor protein Myosin-X (Myo10) in mutant p53-driven cancer invasion. Analysis of gene expression profiles from two breast cancer data sets revealed that MYO10 was highly expressed in aggressive cancer subtypes. Myo10 was required for breast cancer cell invasion and dissemination in multiple cancer cell lines and murine models of cancer metastasis. Evaluation of a Myo10 mutant without the integrin-binding domain revealed that the ability of Myo10 to transport β_1 integrins to the filopodia tip is required for invasion. Introduction of mutant p53 promoted Myo10 expression in cancer cells and pancreatic ductal adenocarcinoma in mice, whereas suppression of endogenous mutant p53 attenuated Myo10 levels and cell invasion. In clinical breast carcinomas, Myo10 was predominantly expressed at the invasive edges and correlated with the presence of *TP53* mutations and poor prognosis. These results indicate that Myo10 upregulation in mutant p53-driven cancers is necessary for invasion and that plasma-membrane protrusions, such as filopodia, may serve as specialized metastatic engines.

Arjonen A, Kaukonen R, Mattila E, Rouhi P, Högnäs G, Sihto H, Miller BW, Morton JP, Bucher E, Taimen P, Virtakoivu R, Cao Y, Sansom OJ, Joensuu H, Ivaska J. Mutant p53-associated myosin-X upregulation promotes breast cancer invasion and metastasis. J Clin Invest 124: 1069–1082, 2014.

Collagen XVIII in adipocyte differentiation

Collagen XVIII is an evolutionary conserved ubiquitously expressed basement membrane proteoglycan produced in three isoforms via two promoters (*P*). *P2*-null mice that produce only short collagen XVIII developed reduced bulk-adiposity, hepatic steatosis, and hypertriglyceridemia. These abnormalities did not develop in *P1*-null mice that produce medium/long collagen XVIII. White adipose tissue samples from *P2*-null mice contain larger reserves of a cell population enriched in early adipocyte progenitors; however, their embryonic fibroblasts had 50% lower adipocyte differentiation potential. Differentiating 3T3-L1 fibroblasts into mature adipocytes produced striking increases in *P2* gene-products and dramatic falls in P1-transcribed mRNA, whereas Wnt3a-induced dedifferentiation of mature adipocytes produced reciprocal changes in *P1* and *P2* transcript levels. *P2*-derived gene-products containing frizzled-like sequences bound *in vitro* the potent adipogenic inhibitor Wnt10b. The same sequences have previously shown to bind Wnt3a, inhibiting Wnt3a-mediated signaling. *P2*-transcript levels in visceral fat were positively correlated with serum free fatty acid levels, suggesting that collagen α1 (XVIII) expression contributes to regulation of adipose tissue metabolism in visceral obesity. Medium/long collagen XVIII is deposited in the Space of Disse, and interaction between hepatic apolipoprotein E and this proteoglycan is lost in *P2*-null mice.

In sum, these results describe a previously unidentified extracellular matrix-directed mechanism contributing to the control of the multistep adipogenic program that determines the number of precursors committing to adipocyte differentiation, the maintenance of the differentiated state, and the physiological consequences of its impairment on ectopic fat deposition.

Aikio M, Elamaa H, Vicente D, Izzi V, Kaur I, Seppinen L, Speedy HE, Kaminska D, Kuusisto S, Sormunen R, Heljasvaara R, Jones EL, Muilu M, Jauhiainen M, Pihlajamäki J, Savolainen MJ, Shoulders CC, Pihlajaniemi T. Specific collagen XVIII isoforms promote adipose tissue accrual via mechanisms determining adipocyte number and affect fat deposition. Proc Natl Acad Sci USA 111: E3043–E3052, 2014.

Evolutionary dynamics of butterflies and moths

The Glanville fritillary (*Melitaea cinxia*; Nymphalidae) – a butterfly with orange-brown wings that are chequered with black – is a widely recognized model species in metapopulation biology and eco-evolutionary research. It has the putative ancestral karyotype of n=31. The genome comprises 390 Mb of DNA, majority which (70%) was localized to the mapped chromosomes. An integral part of the work was the construction of a high-resolution linkage map, which allowed precise placement of the genes and scaffolds onto the larger chromosomal framework. This, in turn, enabled chromosome level comparative analyses within Lepidoptera. These analyses revealed unique features of the Lepidopteran karyotype evolution, namely the original number of chromosomes, the extremely high level of synteny among different Lepidopteran species and unexpectedly conserved chromosome fusion dynamics among the Lepidoptera.

Comparative analysis confirmed a high level of synteny among the Lepidotera and extended the synteny analysis to cover at least 140 million years. This is a unique observation not seen in other groups of organisms, such as mice and men. A surprising result came from comparative analyses of so-called fusion chromosomes in other Lepidopteran species. In some species, such as in Bombyx and Heliconius, a subset of chromosomes from the original n=31 set has been fused to yield smaller karyotypes. Unexpectedly, the fusions were not random but seemed to prefer those chromosomes that are these smallest in *M. cinxia*. More importantly, the chromosomes appear to maintain their unique identities even after the fusion, keeping a high level of synteny to the original pre-fusion chromosomes and resisting any rearrangements across the fusion boundary.

Ahola V, Lehtonen R, Somervuo P, Salmela L, Koskinen P, Rastas P, Välimäki N, Paulin L, Kvist J, Wahlberg N, Tanskanen J, Hornett EA, Ferguson LC, Luo S, Cao Z, de Jong MA, Duplouy A, Smolander O-P, Vogel H, McCoy RC, Qian K, Chong WS, Zhang Q, Ahmad F, Haukka JK, Joshi A, Salojärvi J, Wheat CW, Grosse-Wilde E, Hughes D, Katainen R, Pitkänen E, Ylinen J, Waterhouse RM, Turunen M, Vähärautio A, Ojanen SP, Schulman AH, Taipale M, Lawson D, Ukkonen E, Mäkinen V, Goldsmith MR, Holm L, Auvinen P, Frilander MJ, Hanski I. The Glanville fritillary genome retains an ancient karyotype and reveals selective chro-mosomal fusions in Lepidoptera. Nat Commun 5: 4737, 2014.

Mouse model for skin blistering

Bullous pemphigoid (BP) is the most common autoimmune subepidermal blis-tering skin disease with a characteristic of itching and blistering. BP typically affects people after 7th decade of their life, and it frequently runs with a chronic course and is associated with significant morbidity and mortality varying between 10.8-41% within the first year after the diagnosis. Systemic corticosteroids are the best-evaluated treatment for BP, but there is an obvious need for development of new therapies, since the long-term use of systemic corticosteroids and current adjuvant therapies are associated with numerous side effects and increased mortality, especially when used for elderly people.

BP patients carry inflammation-triggering autoantibodies against the juxtamembraneous extracellular noncollagenous 16A (NC16A) domain of collagen XVII (ColXVII) involved in in ectodomain shedding. Genetically modified mice lacking the corresponding region (NC14A) of murine collagen XVII appear unaffected until three months of age, when they – similar to BP patients – start to itch, develop crusted erosions, and have circulating IgG and IgA autoantibodies with subepidermal reactivity, indicating autoimmunization against a dermo-epidermal junction component. Therefore, the ΔNC14A mouse strain provides a highly reproducible BP-related mouse model with spontaneous breakage of self-tolerance and development of autoantibodies. Differing from some previous models, the ΔNC14A mice are immunocompetent and mature, which enables long-term monitoring of natural immune responses. In addition, no injection of substances or other stress-causing experimental procedures are required, because symptoms develop spontaneously. Thus, the ΔNC14A mouse strain is an excellent model to study the breakdown of self-tolerance, as well as the early stages and development of autoimmune blistering skin disease. Itching, subepidermal blistering, eosinophilia, eosinophilic infiltrations, and elevated IgE levels closely resemble the key features of BP pa-tients. The ΔNC14A mouse strain is, therefore, a promising candidate for fur-ther studies of bullous pemphigoid pathogenesis and development of novel therapies.

Hurskainen T, Kokkonen N, Sormunen R, Jackow J, Löffek S, Soininen R, Franzke CW, Bruckner-Tuderman L, Tasanen K.. Deletion of the major bullous pemphigoid epitope region of collagen XVII induces blistering, autoimmunization, and itching in mice. J Invest Dermatol 135: 1303–1310, 2015 [Epub ahead of print Oct 13, 2014].

Signal processing in activated T cells

T cell antigen receptor (TCR)-mediated activation of T cells requires the inter-action of dozens of proteins. Here, the authors used quantitative mass spectrometry and activated primary CD4+ T cells from mice in which a tag for affinity purification was knocked into several genes to determine the composition and dynamics of multiprotein complexes that formed around the kinase Zap70 and the adaptors Lat and SLP-76. Most of the 112 high-confidence time-resolved protein interactions we observed were previously unknown. The surface receptor CD6 was able to initiate its own signaling pathway by recruiting SLP-76 and the guanine nucleotide–exchange factor Vav1 regardless of the presence of Lat. These findings provide a more complete model of TCR sig-naling in which CD6 constitutes a signaling hub that contributes to the diversification of TCR signaling.

Collectively, the work demonstrated that functionally interacting protein complexes mediate TCR signalling. In contrast to previous models, the results revealed that CD6 was able to orchestrate TCR-mediated signalling to form T cell-APC conjugates independently of Lat protein. The results provide the basis for future functional studies of the mechanisms that underlie signal processing in activated T cells.

Roncagalli R, Hauri S, Fiore F, Liang Y, Chen Z, Sansoni A, Kanduri K, Joly R, Malzac A, Lähdesmäki H, Lahesmaa R, Yamasaki S, Saito S, Malissen M, Aebersold R, Gstaiger M, Malissen B. Quantitative proteomics analysis of signalosome dynamics in primary T cells identifies the surface receptor CD6 as a Lat adaptor-independent TCR signaling hub. Nat Immunol 15: 384–392, 2014.

Zebrafish model for mycobacterial infection

Tuberculosis is an infectious disease that has spread worldwide. Estimates are that up to one third of the world's population is infected with the causative agent *Mycobacterium tuberculosis*. Most individuals have subclinical infection that has the potential to reactivate. The current Bacillus Calmette-Guérin (BCG) vaccine does not give adequate protection especially against the pulmonary disease. This gap in knowledge is reflected by the inability to develop sufficient diagnostic and therapeutic tools to fight tuberculosis. Tuberculosis research has been hampered by the lack of ideal animal models as *M. tuberculosis* infects naturally only primates. Thus it is currently uncertain what kind of immune response is required to control (or to eradicate) *M. tuberculosis* infection. In the current work, this Mycobacterium marinum-zebrafish model was used to study the favorable T cell responses in mycobacterial infection. Taking advantage of the natural heterogeneity of the zebrafish population, Hammarén and her co-workers found an association between the disease severity and the type of T cell responses. Intriguingly, the expression of Th2 type markers (rather than expected Th1 signature) predicted lower bacterial burdens. This challenges the existing dogma that Th1 cells primarily mediate resistance against mycobacteria. Noteworthy, these results were followed by a notion of other investigators that in human Tuberculosis patients, there is a novel Th2-like subset of cells which inhibits growth of *M. tuberculosis* (Meijgaarden et al, 2015 Plos Pathog DOI: 10.1371/journal.ppat.1004671).

Hammarén MM, Oksanen KE, Nisula HM, Luukinen BV, Pesu M, Rämet M, Parikka M. Ade-quate Th2-type response associates with restricted bacterial growth in latent mycobacterial infection of zebrafish. PloS Pathog 10: e1004190, 2014.

Ovarian endometriosis signatures

New molecular information on potential therapeutic targets and tools for noninvasive diagnosis for endometriosis are important for patient care and treatment. However, surprisingly few efforts have described endometriosis at the protein level. In this work, the authors enumerated the proteins in patient endometrium and ovarian endometrioma by extensive and comprehensive analysis of minute amounts of cryosectioned tissues in a three-tiered mass spectrometric approach. Quantitative comparison of the tissues revealed 214 differentially expressed proteins in ovarian endometrioma and endometrium. These proteins are reported in the article as a resource of SRM (selected reaction monitoring) assays that are unique, standardized, and openly available. Pathway analysis of the proteome measurements revealed a potential role for transforming growth factor β-1 in ovarian endometriosis development. Subsequent mRNA microarray analysis further revealed clear ovarian endometrioma specificity for a subset of these proteins, which was also supported by further *in silico* studies. In this process two important proteins emerged, Calponin-1 and EMILIN-1, that were additionally confirmed in ovarian endometrioma tissues by immunohistochemistry and immunoblotting.

This study provides the most comprehensive molecular description of ovarian endometriosis to date and enumerates the proteins in patient endometrium and ovarian endometrioma by extensive and comprehensive analysis of minute amounts of cryosectioned tissues in a three-tiered mass spectrometric approach.

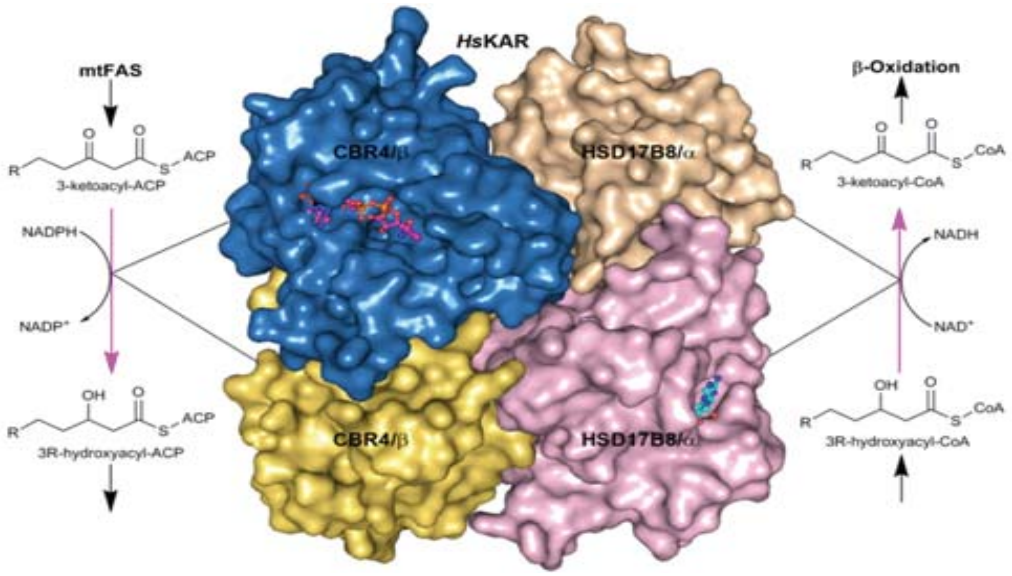
Vehmas AP, Muth-Pawlak D, Huhtinen K, Saloniemi-Heinonen T, Jaakkola K, Laajala TD, Kaprio H, Suvitie PA, Aittokallio T, Siitari H, Perheentupa A, Poutanen M, Corthals GL. Ovari-an endometriosis signatures established through discovery and directed mass spectrometry analysis. J Proteome Res 13: 4983–4994, 2014.

Tetrameric structure of mitochondrial 3-ketoacyl-ACP reductase

The human mitochondrial 3-ketoacyl-ACP reductase (hsKAR) is an α2β2-tetramer. It is involved in the fatty acid synthesis (FAS) pathway, also referred to as the mtFAS, type-2. The α-subunit is also known as 17β-hydroxysteroid dehydrogenase type-8 (HSD17B8) and the β-subunit is also known as car-bonyl reductase type-4 (CBR4). The α and β-chains are homologous, each having about 250 residues. The HsKAR crystals were grown in the presence of 2 mM NAD+. In this crystal form, there are four tetramers per asymmetric unit. The crystal was twinned, having a twin fraction of 0.3. Using the twin re-finement option of Phenix the structure could be properly refined. NAD+ molecules were found to be bound only to the α-subunits. Another dataset was ob-tained from a crystal soaked in fresh mother liquor having 5 mM NAD+ and 5 mM NADP+. This data set was collected at Diamond, beam line IO4. This crystal was not twinned. In this latter crystal form, NAD+ is again observed to be bound to the α-chains, whereas NADP+ is bound to the β-chains. Subse-quently, complementary in vitro and in vivo studies were carried out to provide better understanding of the function of HsKAR.

Surface plasmon resonance studies showed that the affinity of NADPH to the β-subunit is in the micromolar range. The in vivo complementation studies indicated that the β-subunit is also important for binding the ACP moiety of the fatty acyl substrate, thereby establishing that in mitochondrial FAS2, HsKAR catalyzes the NADPH-dependent reduction of 3-ketoacyl-ACP. The α-subunit functions as a scaffold protein. Enzyme kinetic measurements of wild-type and mutated HsKAR showed that the α-chain is a proficient NAD+-dependent dehydrogenase of 3R-hydroxyacyl-CoA. This catalytic activity is present in the peroxisomes, but had not yet been described for any mitochondrial enzyme. The studies suggest that the HsKAR α-subunit could be important by providing auxiliary enzymatic activity for the CoA-dependent degradation of polyunsaturated fatty acids. Via this conversion, 3R-hydroxyacyl-CoA which can arise from the degradation of polyunsaturated fatty acids, can be further degraded by the classical β-oxidation pathway.

Venkatesan R, Sah-Teli SK, Awoniyi LO, Jiang G, Prus P, Kastaniotis AJ, Hiltunen JK, Wierenga RK, Chen Z. Insights into mitochondrial fatty acid synthesis from the structure of heterotetrameric 3-ketoacyl-ACP reductase/3R-hydroxyacyl-CoA dehydrogenase. Nat Commun 5: 4805 , 2014.



The α2β2 tetramer of human mitochondrial 3-ketoacyl-ACP reductase. The ball-and-stick molecules depict the mode of binding of NADP+ (to the β chain, left) and of NAD+ (to the α chain, right). The β-chain is involved in the NADPH dependent fatty-acyl-ACP synthesis pathway, whereas the α-chain is predicted to be important to channel 3-R-hydroacyl-CoA molecules into the β-oxidation degradation pathway of fatty acyl-CoA molecules.

Pluripotent stem cells directly from blood cells

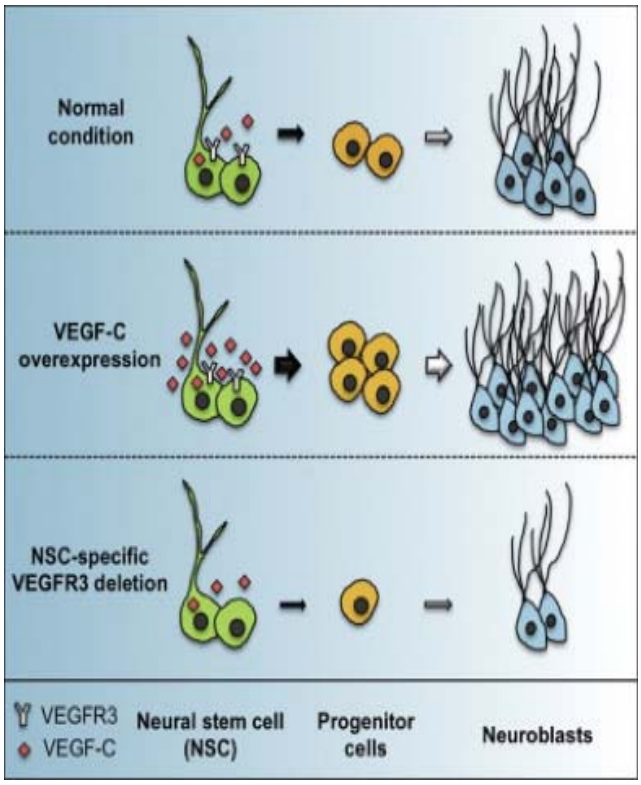
All previously published protocols on blood cell reprogramming have required expansion or activation of certain cell populations with specific cytokines that increases both time and costs of reprogramming. This work shows that by using tetracistronic Sendai virus vectors, freshly isolated or cryopreserved blood mononuclear cells could be reprogrammed directly without cytokine induction.

Generation of validated human induced pluripotent stem cells (iPSCs) for biobanking is essential for exploring the full potential of iPSCs in disease modeling and drug discovery. Peripheral blood mononuclear cells (PBMCs) are attractive targets for reprogramming, because blood is collected by a routine clinical procedure and is a commonly stored material in biobanks. Generation of iPSCs from blood cells has previously been reported using integrative retroviruses, episomal Sendai viruses, and DNA plasmids. However, most of the published protocols require expansion and/or activation of a specific cell population from PBMCs. The authors collected a PBMC cohort from the Finnish population containing more than 2,000 subjects. Here, the authors report efficient generation of iPSCs directly from PBMCs in feeder-free conditions in approximately two weeks. The produced iPSC clones are pluripotent and transgene-free. Together, these properties make this novel method a powerful tool for large-scale reprogramming of PBMCs and for iPSC biobanking.

Trokovic R, Weltner J, Nishimura K, Ohtaka M, Nakanishi M, Salomaa V, Jalanko A, Otonkoski T, Kyttälä A. Advanced feeder-free generation of induced pluripotent stem cells directly from blood cells. Stem Cells Transl Med 3: 1402–1409, 2014.

VEGF-C/VEGFR3 signaling in neural stem cells

Neural stem cells (NSCs) continuously produce new neurons within the adult mammalian hippocampus. The molecular mechanisms of NSC activation remain poorly understood. The authors of this work show that adult hippocampal NSCs express vascular endothelial growth factor receptor (VEGFR) 3 and its ligand VEGF-C, which activates quiescent NSCs to enter the cell cycle and generate progenitor cells. Hippocampal NSC activation and neurogenesis are impaired by conditional deletion of Vegfr3 in NSCs. The role of VEGFR3 signaling is conserved in human neural stem cells, in that NSCs derived from human embryonic stem cells (hESCs), VEGF-C/VEGFR3 mediates intracellular activation of AKT and ERK pathways that control cell fate and proliferation. These findings identify VEGF-C/VEGFR3 signaling as a specific regulator of NSC activation and neurogenesis in mammals. Collectively, these findings are consistent with the possibility that VEGF-C activation of NSCs could improve age-related decline in hippocampal neurogenesis and associated mood defects, such as those seen in Alzheimer's disease.

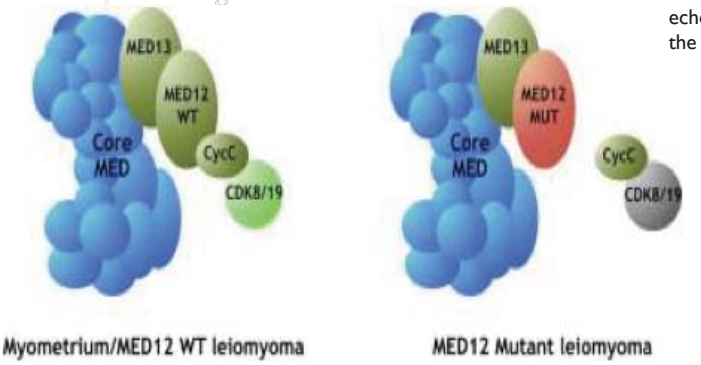


VEGFR3 is required for adult hippocampal neurogenesis, and its signaling converts quiescent neural stem cells into progenitor cells.

Han J, Calvo C-F, Kang TH, Baker KL, Park J-H, Parras C, Levittas M, Birba U, Pibouin-Fragner L, Fragner P, Bilguvar K, Duman RS, Nurmi H, Alitalo K, Eichmann AC, Thomas J-L. Vascular endothelial growth factor receptor 3 controls neural stem cell activation in mice and humans. Cell Rep 10: 1158–1172, 2015

Disrupted transcription complex assembly in leiomyomas

Uterine leiomyomas (fibroids) are monoclonal neoplasms of the myometrium and represent the most common pelvic tumor in reproductive age women. Although benign, they are nonetheless associated with significant morbidity. Somatic mutations in exon 2 of the RNA polymerase II transcriptional Mediator subunit MED12 occur at very high frequency (70%) in uterine leiomyomas. However, the influence of these mutations on Mediator function and the molecular basis for their tumorigenic potential remain unknown. To clarify the impact of these mutations, the authors used affinity-purification mass spectrometry to establish the global protein-protein interaction profiles for both wild-type and mutant MED12. The results indicated that uterine leiomyoma-linked mutations in MED12 led to a highly specific decrease in its association with Cyclin C-CDK8/CDK19 and loss of Mediator-associated CDK activity. Mechanistically, this occurs through disruption of a MED12-Cyclin C binding interface that was also shown to be required for MED12-mediated stimulation of Cyclin C-dependent CDK8 kinase activity. These findings indicate that uterine leiomyoma-linked mutations in MED12 uncouple Cyclin C-CDK8/19 from core Mediator and further identify the MED12/Cyclin C interface as a prospective therapeutic target in CDK8-driven cancers.



Schematic representation of the wild-type and MED12 mutant Mediator complex.

Turunen M, Spaeth JM, Keskitalo S, Park MJ, Kivioja T, Clark AD, Mäkinen N, Gao F, Palin K, Nurkkala H, Vähärautio A, Aavikko M, Kämpjärvi K, Vahteristo P, Kim CA, Aaltonen LA, Varjosalo M, Taipale J, Boyer TG. Uterine leiomyoma-linked MED12 mutations disrupt media-tor-associated CDK activity. Cell Rep 7: 654–660, 2014.

iPSC-derived cardiomyocytes in drug discovery

Familial ventricular tachycardia (CPVT) is caused by mutations in the cardiac ryanodine receptor (RYR2) gene. The gain-of-function mutations of RyR2 cause increased calcium (Ca^{2+}) sensitivity which can lead to spontaneous Ca^{2+} release from sarcoplasmic reticulum, generation of afterdepolarizations, and triggered activity. There is no specific treatment for this disease. A drug for malignant hyperthermia – dantrolene, an inhibitor of sarcoplasmic Ca^{2+} release – functions through RYR1 receptors in skeletal muscle. This drug was used in off-label studies for six CPVT patients, and it abolished the arrhythmias in patients carrying mutations in the N-terminal part of the RYR1 receptor protein but was without any effect in patients with mutations in the C-terminal part of the protein. When dantrolene was applied to iPSC-derived cardiomyocytes from the same individuals, an identical mutation-specific response was observed. Thus, intravenous dantrolene showed antiarrhythmic effects in a subgroup of CPVT patients, and iPSC-derived cardiomyocytes replicated the individual drug responses. In sum, this work demonstrated convincingly that iPSC-derived cells reliably reproduce *in vivo* drug effects and that they could be used to tailor medication in a patient-specific way in the future.

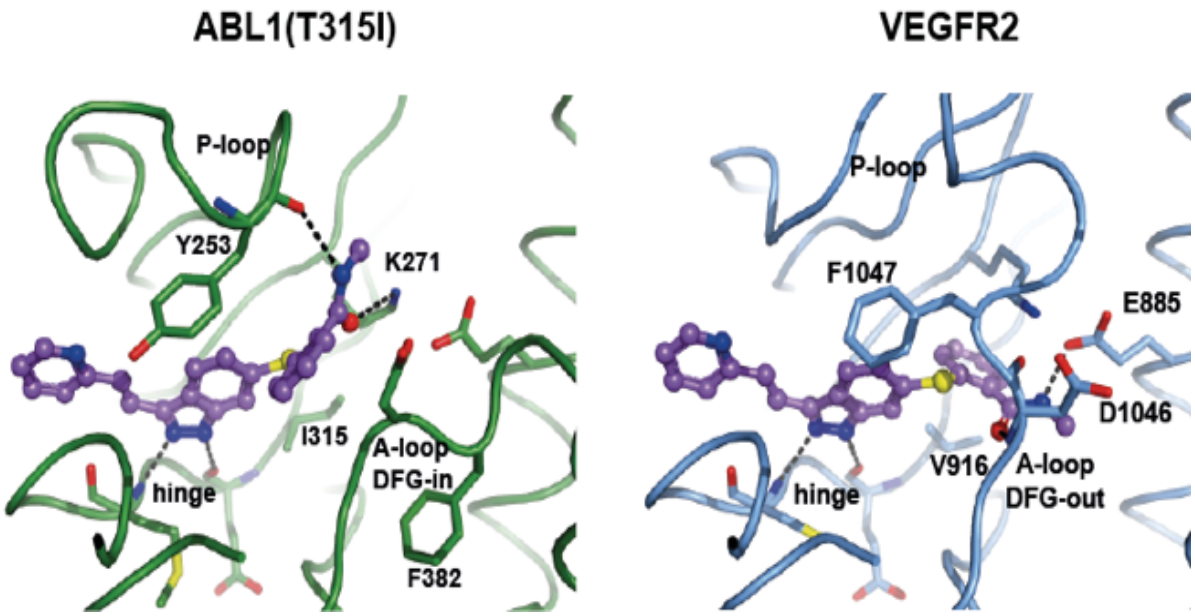
Penttinen K, Swan H, Vanninen S, Paavola J, Lahtinen AM, Kontula K, Aalto-Setälä K. Antiarrhythmic effects of dantrolene in patients with catecholaminergic polymorphic ventricular tachycardia and replication of the responses using iPSC models. PloS One, 2015.

Repositioning of an approved renal cancer drug for treatment of drug-resistant leukemia

The authors of this work discovered that primary patient cells from drug re-sistant cases of BCR-ABL-driven leukemia (Chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) that did no longer respond to the approved BCR-ABL kinase-targeted drugs available, but instead responded to the VEGFR kinase inhibitor axitinib. Following this discovery, they showed with biochemical kinase assays that axitinib selectively inhibited the gatekeeper mutation drug resistant form of BCR-ABL (carrying the T315I kinase domain mutation) at a similar potency as its intended primary target, VEGFR2. Non-mutated BCR-ABL protein on the other hand was not effectively targeted by axitinib. Structural studies showed that the resistance mutation induces a unique kinase domain conformation that conferred a high affinity binding of axitinib. Surprisingly, the binding mode of the inhibitor is strikingly different to ABL(T315I) than to VEGFR2, in terms of the kinase domain conformation, the inhibitor conformation and the inhibitor–kinase molecular interactions, highlighting the unpredictable nature of kinase inhibitor–kinase interactions.

Finally, showing the translational relevance of this finding, a patient with BCR-ABL(T315I)-mutant CML was treated with axitinib with the standard anti-angiogenic dosing used for renal cell carcino-ma, responded to the treatment, and the leukemic cells were significantly suppressed. The discoveries are expected to lead to formal clinical trials of axitinib being used against gatekeeper mutant BCR-ABL-mutant CML, and the molecular-structural discoveries could potentially be used to develop better inhibitors against drug resistant gatekeeper mutants in other clinically targeted kinases such as EGFR, ABL, KIT and PDGFR.

Pemovska T, Johnson E, Kontro M, Repasky GA, Chen J, Wells P, Cronin CN, McTigue M, Kallioniemi O, Porkka K, Murray BW, Wennerberg K. Axitinib effectively inhibits BCRABL1(T315I) with a distinct binding conformation. Nature 519: 102–105, 2015.



Axitinib binds ABL(T315I) in an unexpected manner. Compared to the binding to its primary target VEGFR2, axitinb binds to ABL(T315I) with a different compound and kinase conformations and the molecular interactions are highly divergent between the two complexes.

INFRASTRUCTURE NETWORKS & TECHNOLOGY PLATFORM SERVICES

BIOINFORMATICS

Bioinformatics Infrastructure Network

Coordinator of the network: Sampsa Hautaniemi, BCH
Members: Petri Auvinen, BI; Garry Wong, BCK; André Juffer, BCO; Matti Nykter, BMT; Mark Johnson, BioCity; Imre Västrik, FIMM

www.biocenter.fi

Advances in measurement technologies, such as microarrays, mass spectrometry, deep sequencing and large-scale screening, have made bioinformatics an integral part of biological and biomedical research. These technologies produce huge amounts of data on gene sequences, mutations, protein structures, human diseases and mouse phenotypes into databanks. Technology platforms for imaging both at microscopic and clinical level also provide increasing amounts of data. The task of bioinformatics is to provide tools, such as *in silico* modeling and simulation, to translate multidimensional biological data into knowledge and medical benefits. Thus, the productivity of biomedical sciences and related industries is increasingly dependent on computational methodologies and software. Lack of such software or methodologies is seen as a bottleneck for cutting-edge research exploiting the high-quality Finnish biodata and novel measurement technologies. Therefore, the major objective of the Bioinformatics infrastructure network and the corresponding technology platform is to provide services for both bioscientists and bioinformaticians. Although CSC - IT Center for Science Ltd is not officially part of the BF Bioinformatics infrastructure network, they collaborate actively both at national and European level and CSC is invited to all Bioinformatics network meetings.

Bioinformatics Technology Platform

Chair of the consortium: Sampsa Hautaniemi, BCH, Computational Systems Biology Laboratory
Members: Liisa Holm, BI, Bioinformatics Group; Samuel Kaski, Aalto University, BCH; Garry Wong, BCK, Laboratory of Functional Genomics and Bioinformatics; André Juffer, BCO, Biocomputing and Bioinformatics Core Facility; Matti Nykter, BMT, Bioinformatics Group; Mark Johnson, BioCity, Structural Bioinformatics Laboratory; Imre Västrik, FIMM
External members: Tommi Nyrönen, IT Center for Science, CSC; Harri Lähdesmäki, Aalto University

www.biocenter.fi

http://bioinformatics.biocenter.fi/

Achievements in development and restructuring of technology services during

The bioinformatics network offers a great variety of services in 13 broad categories (Microarray analysis; Genotype data management, hosting and analysis; DNA-sequence archiving and analysis; High-throughput and high-content screening data management; Microscopy image analysis; Protein structure analysis; *In silico* modeling and simulation; Pathogenicity of genetic variants; Immunology and immunodeficiency knowledge bases; Data integration and assorted analysis; Proteomics data analysis; Bioinformatics software development and Anduril; and Server hotel, hosting and scientific IT support). The services in different biocenters have been structured to avoid overlapping activities. All the services have been fully functional and used by the Finnish bioscience community. The consortium has also been in an active dialogue with representatives of other BF technology platforms responsible for production of very large amounts of data that require bioinformatics services for interpretation and storage. Examples of new or improved services are as follows.

- BMT has expanded the services in high-throughput data analysis, including various microarray to high-throughput sequencing studies covering genome, exome, transcriptome, methylome and chromatin level data.
- BioCity together with CSC connected BioCity Turku to CSC IT Center for Science supercomputing via the

Lightpath gigabit link, providing fast, efficient moderate-sized data transfers for use of the IAAS Cloud service, which is sponsored by the ESFRI ELIXIR and BioMedInfra. The Lightpath together with local storage and data buffering capacity was boosted by funding from FIRI and Åbo Akademi University; the product evaluation and bidding process was long but it enabled getting a considerably better product than would have otherwise been possible.

• BI has set up two new web servers. Usage of the servers is free and open to all. BARCOSEL is a web tool for selecting an optimal subset of barcode sequences from a user-defined library. Nucleotide balance is optimized using mixed-integer linear programming. BARCOSEL has been used by BI's DNA-lab. (http://ekhidna2.biocenter.helsinki.fi/barcosel). SANSparallel is a web tool that takes a protein sequence as input and returns homologs from the selected protein database. The original suffix array neighborhood search (SANS) method was improved, re-implemented as a client-server, and parallelized. The method is extremely fast and as sensitive as BLAST above 50 % sequence identity. (http://ekhidna2.biocenter.helsinki.fi/sans)

• FIMM has established new bioinformatics components for pathway enrichment analysis, improved calling of genomic variants, transcriptome normalization, and data visualization. Service outputs have been modified according to the user feedback (collected by interviewing users). In addition, an initial effort has been made towards establishing a clinical microbial metagenomics data analysis service.

• BCO has developed a new software tool for the comparison of any two non-sequential sets of residues. The so-called similarity index is computed from geometrical and physio-chemical considerations. The tool should prove useful in the domain of structural bioinformatics in relation to for instance drug development and design (Garma and Juffer, 2014, submitted).

Because the next generation sequencing (NGS)service – offered by BF Genome-wide Methods network – is the major data producer that is utilized at close to its full capacity, the Bioinformatics network has put a considerable effort into NGS data processing, analysis and interpretation. In fact, the Bioinformatics network members have been working closely and integrating their services with the sequencing service providers in their biocenter. The integration creates for the clients a "one-stop-shop". This may nevertheless manifest itself in decreased visibility of the bioinformatics network, since the users may not be aware of the fact that they are using services of multiple BF networks. In addition to this, the Bioinformatics network has also been supporting data and software services for BF Biological Imaging, Translational Activities, Proteomics and Structural Biology. The Bioinformatics network has presented the services to the biocenters' communities and established a helpdesk. The questions via the helpdesk have been organized in such a fashion that they can be handled by one of the members. Clearly, the helpdesk needs constant advertising, and in the future, the network will merge its helpdesk with that of CSC in order to gain more publicity, efficiency and customers.

The major bottleneck within the network is the availability of qualified personnel to conduct computational analysis. As computing power becomes cheaper and more easily accessible (e.g., the Grid and Cloud computing), the availability of qualified personnel is already a bottleneck, since increased demand for *in silico* modeling and simulation continues. Thus, for a stable and efficient service offering, a commitment to invest in personnel must remain the first priority. Another severe bottleneck of bioinformatics services relates to the staff turnover, stemming from uncertainty about the continuation of funding. Due to the unpredictability of the workload that varies from dataset to dataset, there is difficulty in scheduling projects accurately.

Participation in international, Nordic and European infrastructures

CSC, the Finnish node of the ELIXIR bioinformatics ESFRI infrastructure, is an external member in the BF Bioinformatics network. As such, the CSC's planned

User statistics

Important: BCO provides *in silico* modeling and simulation services where the time for one project may take from weeks to months.

	BCH	BI	BCO	BMT	BioCity	FIMM	Total
Total users	10	6	7	11	52	92	178
a) local	7	6	5	8	39	79	144
b) domestic	3	0	0	3	10	5	21
c) international	0	0	2	0	3	2	7
of which non academic	0	0	0	0	3	6	9
Projects	14	6	7	11	85	92	316
Database & server users / requests	n/a	20 919	n/a	n/a	> 250	5 913	> 27 082
User fees (€)	3 800	0	0	0	44 000	34 500	82 300

service offering in ELIXIR is based on the needs of the BF Bioinformatics network. Members of the Bioinformatics network use the cloud computing pilot service developed and offered by CSC in preparation for ELIXIR. For example, 80% of the processing power of FIMM computer cluster is actually provided by the CSC cloud. Similar arrangements have been implemented at BioCity. In addition, there is currently ongoing work to expand the local cluster using CSC's cloud resources (ePouta service) as they become available (BioCity). Members of the Bioinformatics network use available grid compute resources – both Finnish Grid Infrastructure (FGI) and European Grids Infrastructure (EGI, http://www.egi.eu/) – and train other platforms to use them as well. BCO and CSC host FGI clusters. BCO also relies on other computational resources at the CSC.

BMT works in collaboration with the Cancer Genome Atlas (TCGA). It has provided gene fusion analysis for glioblastoma (http://www.ncbi.nlm.nih.gov/pubmed/24120142), endometrial carcinoma (http://www.ncbi.nlm.nih.gov/pubmed/23636398) and prostate cancer (ongoing) working groups.

Bioinformatics is also in a central role in the EATRIS translational research infrastructure where FIMM leads the Biomarkers services – one of the five service areas of EATRIS. Furthermore, bioinformatics services are needed to analyze the data produced from samples from the BBMRI biobanking infrastructure. Bioinformatics network services are also linked to EC framework program funded projects. For example, the personalized medicine data produced for and used in one of the BioMedBridges projects are processed at FIMM. Bioinformatics services in FIMM are linked to the Tryggve project aiming to establish a Nordic platform for collaboration on sensitive data, funded by NeIC (Nordic e-infrastructure), NordForsk and the ELIXIR nodes in Denmark, Finland, Norway and Sweden. In addition, bioinformatics services are linked to EC framework program funded projects (BioMedBridges) and involved in the EU-LIFE alliance.

Future perspectives

Given the ambitious plans of other BF networks, especially that of Genome-wide Methods, to obtain new equipment that produces massive amounts of sequence data, the need for data storage and analysis capacity is going to increase. In practical terms, it means that there is a need for more disk space, more CPUs, as well as fast connections between the storage and processors (I/O capacity). Especially the latter is crucial and requires a holistic view of the IT system. The network also needs the skilled staff – bioinformaticians and IT experts – to develop, maintain and update the processing pipelines and IT systems.

Utilization of publicly available data sources (ENCODE, TCGA, ICGC, etc.) in bioscience research is clearly an emerging trend. For this, most bioscience groups will require technical analysis support. As a consequence, storage, accessibility and flexible analysis of these vast data collections need to be solved.

Major publications supported by platform services

Ruskamo S, Yadav R, Sharma S, Lehtimaki M, Laulumaa S, Aggarwal S, Simons M, Burck J, Ulrich A, Juffer AH, Kursula I, Kursula P. Atomic-resolution view into structure-function relationships of the human myelin peripheral membrane protein P2. Acta Crystallographica Section D 70: 165-176, 2014.

Yu X, Cojocaru V, Mustafa G, Salo-Ahen OM, Lepesheva GI, Wade RC. Dynamics of CYP51:implications for function and inhibitor design. J Mol Recognit 28: 59-73, 2015.

Kumar H, Lund R, Laiho A, Lundelin K, Ley RE, Isolauri E and Salminen S. Gut microbiota as an epigenetic regulator : pilot study based on whole genome methylation analysis. MBio 5: e02113-14, 2014.

Veskimäe K, Staff S, Tabaro F, Nykter M, Isola J, Mäenpää J. Microarray analysis of differentially expressed genes in ovarian and fallopian tube epithelium from risk-reducing salpingo- oophorectomies. Genes Chromosomes Cancer 54: 276-287, 2015 .

Gundem G, Loo PV, Kremeyer B, Alexandrov LB, Tubio J, Papaemmanuil E, Brewer D, Kallio H, Högnäs G, Annala M, Goody V, Latimer C, O'Meara S, Dawson KJ, Isaacs W, Emmert-Buck MR, Nykter M, Foster C, Neal DE, Cooper C, Eeles R, Visakorpi T, Campbell PJ, McDermott U, Wedge DC, Bova GS. The evolutionary history of lethal metastatic prostate cancer. Nature 520: 353-357, 2015.

Ahola V, Lehtonen R, Somervuo P, Salmela L, Koskinen P, Rastas P, Välimäki N, Paulin L, Kvist J, Wahlberg N, Tanskanen J, Hornett EA, Ferguson LC, Luo S, Cao Z, de Jong MA, Duplouy A, Smolander O-P, Vogel H, McCoy RC, Qian K, Swee Chong W, Zhang Q, Ahmad F, Haukka JK, Joshi A, Salojärvi J, Wheat CW, Grosse-Wilde E, Hughes D, Katainen R, Pitkänen E, Ylinen J, Waterhouse RM, Turunen M, Vähärautio A, Schulman AH, Taipale M, Lawson D, Ukkonen E, Mäkinen V, Goldsmith MR, Holm L, Auvinen P, Frilander MJ, Hanski I. The Glanville fritillary butterfly retains an ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. Nat Commun 5: 4737, 2014.

Wang H, Fewer D, Holm L, Rouhiainen L, Sivonen K. An atlas of nonribosomal peptide and polyketide biosynthetic pathways reveals common occurrence of nonmodular enzymes. Proc Natl Acad Sci USA 111: 9259 - 9264, 2014

Norrmén C, Figlia G, Lebrun-Julien F, Pereira JA, Trötzmüller M, Köfeler HC, Rantanen V, Wessig C, van Deijk AL, Smit AB, Verheijen MH, Rüegg MA, Hall MN, Suter U. mTORC1 controls PNS myelination along the mTORC1-RXR γ-SREBP-lipid biosynthesis axis in Schwann cells. Cell Rep 9: 646-660,2014.

Heinonen S, Saarinen L, Naukkarinen J, Rodríguez A, Frühbeck G, Hakkarainen A, Lundbom J, Lundbom N, Vuolteenaho K, Moilanen E, Arner P, Hautaniemi S, Suomalainen A, Kaprio J, Rissanen A, Pietiläinen KH. Adipocyte morphology and implications for metabolic derangements in acquired obesity. Int J Obes 38:1423-31, 2014.

BIOLOGICAL IMAGING

Biological Imaging Infrastructure Network

Coordinator of the network: John Eriksson, BioCity Turku
Members: Elina Ikonen, BCH; Maria Vartiainen, BI; Olli Gröhn, BCK; Sinikka Eskelinen, BCO; Susanna Narkilahti, BMT; Johan Lundin, FIMM;Varpu Marjomäki, University of Jyväskylä
www.biocenter.fi

Biological imaging ranges from the visualization of ions, molecules, cells and tissues to the non-invasive imaging of full size animals. The importance of imaging has grown tremendously since the development of methods and markers for live cell imaging, such as green fluorescent proteins for confocal microscopy, as well as novel microscopic principles. Different in vivo imaging modalities such as computer tomography (CT), single photon emission computer tomography (SPECT) and magnetic resonance imaging (MRI) has given us tools to visualize structure, metabolism and function in a living organism.

Modern imaging requires sophisticated instrumentation for data acquisition and methods of bioinformatics and data handling for their storage and analysis. The prerequisite for live cell imaging is that the equipment is near to the laboratories and animal centers. Therefore, each biocenter has confocal microscopes, video microscopes, and transmission electron microscopes for imaging of cells and tissues. However, in the Biological Imaging Infrastructure Network of BF, different biocenters have been granted specific spearheaded tasks, which are organized under three technology platforms; those for light microscopy, electron microscopy and in vivo imaging. In light microscopy, Helsinki and Turku focus on new imaging technologies including high-resolution STED, PALM and STORM microscopy as well as high content screening at cellular and molecular level. Turku Bioimaging hosts some of these most recent technologies and has a high-resolution optical imaging core service at the BF level. In electron microscopy, high resolution electron cryo-microscopy, electron tomography and three-dimensional image reconstruction for nanoscale structures are available at the Institute of Biotechnology in the University of Helsinki. *In vivo* imaging platforms include PET instrumentation in Turku, MRI in Kuopio and Helsinki, as well as optical methods in Helsinki and Turku. Since 2011, BF has also supported a small animal molecular imaging (SPECT/CT) platform (RTI unit) in Helsinki.

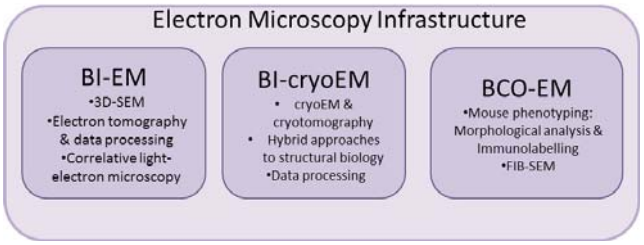
Electron Microscopy Technology Platform

Chair of the consortium: Eija Jokitalo, BI, Electron Microscopy Unit;
Members: Sarah Butcher BCH/BI CryoEM Unit; Raija Sormunen, BCO Tissue Imaging Center

www.biocenter.fi

Achievements in development of technology services

The main common goal of the three EM units forming this consortium has been to restructure and streamline the functioning of the units towards nationally-unique complementary areas. The Helsinki units, BI-EM and BCH-cryoEM, focus on 3D imaging and hybrid methods from molecular models to whole cells and tissues, whereas BCO-EM specializes in the ultrastructural pathology of human and model organisms working closely with the BCO Transgenic mouse core facility. The impact of BF funding has been significant in terms of both renovating the technology platform infrastructure and in retention of highly-trained support staff. During 2014, Biocenter Finland allocations covered six salaries for a total of 72 person months.



Biocenter Finland allocations have resulted in clear improvements in the performance and quality ofthe services, as evidenced by higher scientific impact. The last major instrument investment in this consortium was the installation of a new scanning electron microscope (SEM; Sigma HD VP, Zeiss) – funded by FIRI 2013 and University of Oulu – that was installed at BCO-EM premises at the end of 2014. This will support the continuing development and availability of biomedical SEM research services at University of Oulu, complementing the 24/7 usage of the SEM in Helsinki. Due to instrument development and the introduction of digital imaging on all of the transmission electron microscopes, the quality and turn-around of the work have been significantly improved, with the microscopy time required per specimen greatly reduced. Users now have instant access to the recorded data and so can immediately react to the results from

a specimen, and improve the data saved. Efforts have been made to build long-term data storage solutions that will simultaneously enhance data sharing between the EM units and their users. Overall, the workflow has been streamlined to better fit the current and future needs of the research community.

BF funding has supported an efficient minimal level of experienced microscopy support staff increasing throughput of projects and utilization of advanced EM techniques, which is evident as an upsurge in scientifically demanding projects without increasing the turnover time of the existing services. The consortium members have agreed criteria for pricing including a common price category for all academic work, rather than distinguishing between their own university and others. All members have now implemented the same internet-based booking and invoicing system. This makes statistical analysis of the impact of the investments much easier to follow, as it is fairly automated and has decreased bureaucracy. Standardized laboratory practices such as the reporting and quantification of ultrastructural results have been developed. The network has remained active in providing training, such as personal training for all new users in operation of microscopes and other instruments, supervised specimen preparation and organized lecture and practical courses, e.g., "Recent applications in Biomedical electron microscopy" (Oulu) and "Imaging techniques in biological sciences" (Helsinki).

Participation in international, Nordic and European infrastructures

The broad spectrum of techniques that our consortium covers exceeds the boundaries of the European infrastructure calls.

BI-EM and BCH-CryoEM have joined efforts with the Light Microscopy Unit of the BI to propose a Correlative Light Electron Microscopy (CLEM) platform in the Finnish Euro-BioImaging node application. BCO-EM belongs to the Oulu Bioimaging network (OBI) and is proposing mesoscopic imaging platform in the Finnish Euro-BioImaging node. All sub-nodes are additionally linked together by activities aimed at facilitating image processing, visualization and open-source software production for image analysis.

BI-EM participates in BIIF, BioImage Informatics Finland, which is a network for bioimage analysts, software developers and life scientists who use bioimage informatics as a central toolset and is a partner in EuBIAS (European BioImage Analysis network). EuBIAS is currently preparing a European COST action proposal for getting funding for networking and promoting the organization and development of bioimage informatics in Europe.

BCH-cryoEM is part of the ESFRI Instruct National User Group, and the Instruct-FI National Affiliated Center covering X-ray, NMR, EM (high resolution single particle) and macromolecular complex production and mass spectrometry. BCH-cryoEM is part of the AIROPico FP7 Marie Curie Industry-Academia Partnerships and Pathways with industrial and academic partners that will run from 2014–2018 and includes academic and industrial infrastructure for the development of diagnostics, therapeutics and basic science of picornaviruses.

It is important to note that there is a clear distinction between the two ESFRI calls for development of advanced methods towards international collaboration, and the national BF service platform which is the main priority of this network.

User statistics

	BI-EM	BCH-cryoEM	BCO-EM	Total
Total number of research groups	82	15	35	132
- local academic groups	64	11	21	96
- national academic groups	13	2	8	21
- industrial users	3	0	2	5
- international users	2	2	4	8
Microscope usage (hours)	1847	602	560	3009
Specimens prepared	1240*	354#	1054*	2648

* number of specimens that has been embedded (plastic or cryo), sectioned (room temperature or cryo) and stained (including immunolabelling), excluding duplicates of each step

number of cryo specimens prepared

Future perspectives

BCH-CryoEM aims to upgrade the cryoEM imaging system. There has been a revolution in the detector technology in the past three years with the practical implementation of direct electron detectors, finally replacing film for all applications. The new detectors have two important advantages; the data are collected over a series of frame allowing correction for image movement, and the signal-to-noise ratio is significantly improved. Together, these advantages mean that more data can be collected, and utilized, and the data are of a significantly increased quality after processing than data from CCD or film.This is reflected in a world-wide boom in the number of atomic resolution structures reported in the last three years from cryoEM to over 180 (source: EMDB database). In Helsinki, film has had to be the mainstay for all high resolution EM, but Kodak went bust in 2012 and the film scanners are all obsolete, so the unit is in a desperate situation, living on borrowed time. To make matters worse, the Tecnai F20 microscope has become prone to expensive breakdowns, with over four months of downtime in 2014. The manufacturer has announced that they will no longer perform software updates to it, as it is 15 years old. Hence the whole system needs to be replaced, as the local demand is increasing due to the increasing versatility of the method, especially in combination with other techniques such as light microscopy, X-ray crystallography and small angle X-ray scattering.

This is the only system available to the biological community in Finland, and is one of the few in Europe that is truly open to international users. Access to other systems world-wide is sporadic, on a good will basis, and very costly.

BI-EM is aiming to upgrade its current 3D-EM services with a second, high SEM equipped for serial block face imaging. The current instrument is in constant use, and data series are collected overnight and over weekends without breaks. The current system has already yielded 8 accepted papers, among them Science and Nature Communications papers from the collaboration project with the Helariutta group (Institute of Biotechnology, University of Helsinki). The new system would open up the technology to new type of specimens because of the higher contrast and smaller voxel size gained by the high vacuum system. In addition, efforts in developing image analysis tools will be continued.

InBCO-Oulu, the development of SEM techniques inevitable leads to several kinds of 3-Dmodelling possibilities. This allows us to develop further SEM service for 3-D modelling of large tissue samples.

Major publications supported by platform services

Aikio M, Elamaa H, Vicente D, Izzi V, Kaur I, Seppinen L, Speedy HE, Kaminska D, Kuusisto S, Sormunen R, Heljasvaara R, Jones EL, Muilu M, Jauhiainen M, Pihlajamäki J, Savolainen MJ, Shoulders CC, Pihlajaniemi T. Specific collagen XVIII isoforms promote adipose tissue accrual via mechanisms determinine adipocyte number and affect fat deposition. Proc Natl Acad Sci USA 111: E3043–3052, 2014.

D'Amico G, Korhonen EA, Anisimov A, Zarkada G, Holopainen T, Hägerling R, Kiefer F, Eklund L, Sormunen R, Elamaa H, Brekken RA, Adams RH, Koh GY, Saharinen P, Alitalo K. 2014. Tie1 deletion inhibits tumor growth and improves angiopoietinantagonist therapy. J Clin Invest 124: 824–834, 2014.

Dettmer J, Ursache R, Campilho A, Miyashima S, Belevich I, O'Regan S, Mullendore DL, Yadav SR, Lanz C, Beverina L, Papagni A, Schneeberger K, Weigel D, Stierhof YD, Moritz T, KnoblauchM, Jokitalo E, Helariutta Y. 2014. CHOLINE TRANSPORTER-LIKE1 is required for sieve platedevelopment to mediate long-distance cell-to-cell communication. Nat Commun 5: 4276, 2014.

Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, Heo JO, Vaten A, Lindgren O, DeRybel B, Van Isterdael G, Somervuo P, Lichtenberger R, Rocha R, Thitamadee S, Tähtiharju S, Auvinen P, Beeckman T, Jokitalo E, Helariutta Y. Arabidopsis NAC45/86 direct sieve element morphogenesis culminating in enucleation. Science 345: 933–937, 2014.

Joensuu M, Belevich I, Rämö O, Nevzorov I, Vihinen H, Puhka M, Witkos TM, Lowe M, Vartiainen MK, Jokitalo E. 2014. ER sheet persistence is coupled to myosin 1c-regulated dynamicactin filament arrays. Mol Biol Cell 25: 1111–1126 , 2014.

Magarkar A, Mele N, Abdel-Rahman N, Butcher S, Torkkeli M, Serimaa R, Paananen A, Linder M, Bunker A. 2014. Hydrophobin film structure for HFBI and HFBII and mechanism for acceleratedfilm formation. PloS Comput Biol10.1371/journal.pcbi.1003745, 2014.

Naillat F, Veikkolainen V, Miinalainen I, Sipilä P, Poutanen M, Elenius K, Vainio SJ. ErbB4,a receptor tyrosine kinase, coordinates organization of the seminiferous tubules in the developingtestis. Mol Endocrinol 9: 1534–1546, 2014.

Pandurangan AP, Shakeel S, Butcher SJ, Topf M. Combined approaches to flexible fittingand assessment in virus capsids undergoing conformational change. J Struct Biol 185: 427–439, 2014.

Prunskaite-Hyyryläinen R, Shan J, Railo A, Heinonen KM, Miinalainen I, Yan W, Shen B, Perreault C, Vainio SJ. Wnt4, a pleiotropic signal for controlling cellpolarity, basement membrane integrity, and antimüllerian hormone expressionduring oocyte maturation in the female follicle. FASEB J 28: 1568–1581, 2014.

Vahokoski J, Bhargav SP, Desfosses A, Andreadaki M, Kumpula EP, Martinez SM, Ignatev A, Lepper S, Frischknecht F, Sidén-Kiamos I, Sachse C, Kursula I. Structural differences explaindiverse functions of Plasmodium actins. PLoS Pathog 10:e1004091, 2014.

National Precilinical *In Vivo* Imaging Technology Platform

Chair of the consortium: Olli Gröhn, BCK, Biomedical Imaging Unit and National Bio-NMR Facility

Members: Juhani Knuuti, BioCity, Turku PET Centre; Turgut Tatlisumak, BCH, Biomedicum Imaging Unit

www.biocenter.fi

Achievements in development of technology services

This consortium has created a national multimodal preclinical *in vivo* imaging network, with a clear division of the tasks and core expertise area in each of the contributing Biocenters. During the four years of operation, the platform has completed significant purchase of instruments and established an open access multimodal imaging infrastructure with harmonized user policies and pricing. The investments have made new techniques available for the biomedical research community, and expanded the capacity (both in terms of instrument time and expert service personnel) at each site, so that both the quality and quantity of the services have significantly improved. As a consequence, *in vivo* imaging received one of the best evaluations with either "excellent" or "very good" marks in all categories in the user survey conducted by BF. In addition, imaging was most often mentioned, when the need for the individual infrastructures was queried. Also the feedback was excellent from the proof-of-concept studies of the large-scale pan-European EuroBioImaging ESFRI infrastructure project. The network has reached a fully functional state, and during the year 2014, it has continued to serve the biomedical scientific community with the personnel funded by the participating universities, as recommended by BF. In addition, the network has developed different imaging modalities and multimodal imaging with other funding sources, and the following steps have been taken to improve the availability and promotion of the excellence in different *in vivo* imaging modalities.

PET imaging

Improvements in the capacity of PET tracer production in Turku PET center have been achieved. This is the bottleneck for increasing the capacity required for efficient provision of PET imaging services, as the PET imaging systems were already recently upgraded. The PET tracers need to be produced in a dedicated radiotracer synthesis laboratory that is in close proximity to a cyclotron laboratory. The existing synthesis laboratory has the qualified space for the synthesis, but the number of synthesis devices was limiting for the availability of tracers for preclinical imaging. In 2014, the new tracer production devices that were purchased in 2012 were in routine use, as well as Cu^{64} isotope production. Several novel F^{18} tracers labeling peptides and oligonucleotides were developed and studied.

Preclinical PET imaging is now also available in Kuopio, exploiting the expertise provided by the Turku partner in the implementation of the new imaging modality. Currently, FDG-PET imaging is available for external users, and it is in active use. Furthermore, Kuopio University Hospital has installed a cyclotrone and radiotracer production that will start in 2015–2016, after which a more comprehensive selection of PET tracers will be available also for preclinical imaging.

MR Imaging

Kuopio continues to provide MRI services with three MRI systems operating at 7T and 9.4T exploiting both Agilent DirectDrive and Bruker Biospin consoles, and providing practically all MRI sequences available at the moment for preclinical imaging.

As an indirect consequence of the actions taking place in Kuopio, preclinical MRI has also become available in Oulu. The 4.7T magnet from Kuopio was transferred to Biocenter Oulu, and it became fully functional during 2013 and is now serving as a basic level preclinical MRI instrument.

MRI services have also been provided in Biomedicum Helsinki, with a 4.7T MRI system. The system was closely integrated as a part of the multimodal-imaging platform, in association with optical imaging. However, in 2014 there have been serious technical problems with the MRI equipment, and the service provided by this old instrument (purchased in 2002) has to be discontinued.

Optical Imaging

Advances in optical imaging have taken place in a newly dedicated *in vivo* imaging laboratory in Biomedicum Helsinki. The laboratories were completely renovated in 2013, and they now host the optical imaging systems, including a multichannel intravital two-photon microscope, which allow extended wavelength capabilities to serve the growing use of red-shifted fluorescent proteins in the phenotypic characterization of genetically modified rodent models. In 2014, the system was extensively modified to establish additional organ models for intravital two-photon microscopy. Currently, the system allows acute as well chronic *in vivo* imaging experiments on internal (e.g., brain, kidney, liver, etc.) and external organs (e.g., skin, eye). In addition, experienced core facility personnel were recruited to support the optical *in vivo* imaging activities. Together with previously purchased systems, the *in vivo* imaging instruments now range from

subcellular resolved intravital two-photon imaging to small organisms and whole organs with 3D optical projection tomography, and to whole animal fluorescence and bioluminescence imaging.

The establishment of a National BioCARS center by the Helsinki partner has been accomplished, serving local and national users. Label-free imaging methods, coherent anti-Stokes Raman scattering (CARS), and second and third harmonic generation, will serve as additional spearhead technologies for *in vivo* imaging, offering an important opening also at the international level via the EuroBioImaging ESFRI and EU COST action MicroCoR consortium.

Another aim was to upgrade the optical imaging infrastructure in Turku. Previously, *in vivo* optical imaging was based on bioluminescence and fluorescence imaging. Tomographic optical imaging system was purchased and is now available for the investigators. It allows the co-registration with PET, CT and MRI to yield an anatomical and functional hybrid approach.

User statistics

User statistics and number of research projects (research groups):

	Kuopio	Turku	Helsinki
Total number of projects	32	43	19
Local projects	23	23	19
Other domestic projects	5	6	2
International	4	7	0
Non-academic projects	1	7	0

Participation in international, Nordic and European infrastructures

Parts of the national preclinical *in vivo* imaging network belong to the Euro-BioImaging (EuBI) ESFRI infrastructure that has been included in the Academy of Finland research infrastructure roadmap 2014–2020. The national preclinical *in vivo* imaging network has participated in the preparatory phase of EuBI ESFRI initiative as one of the European sites for 'proof-of-concept' studies in the 'Molecular imaging' work package. The national preclinical *in vivo* imaging network is also linked with other ESFRI initiatives. In EATRIS ESFRI (European Advanced Translational Research Infrastructure in Medicine) Turku PET Centre is one of the two centers providing the imaging tracers. The Helsinki partner is a managing committee member in the EU COST action MicroCoR (Chemical Imaging by Coherent Raman Microscopy) that involves all aspects of coherent Raman microscopy techniques. In collaboration with CSC, FIMM, and the University of Helsinki IT Services, the Helsinki partner has established a centralized platform for data storage, management, visualization, and analysis. CSC has recently expanded its cloud services to offer cluster computing for the biomedical sector, which is to form a part of the Finnish node in the European life science ELIXIR ESFRI infrastructure. This platform will be hosted in the cloud as one of the Ministry of Education and Culture subsidized pilot projects for the ELIXIR infrastructure.

Future perspectives

The importance of preclinical in vivo imaging as a major research tool in translational medicine is increasing. Two general trends can be seen in technological development. First, basic level imaging equipment is becoming more economically feasible making it possible to establish several multimodal preclinical *in vivo* imaging centers in Finland. Second, the state-of-the-art instruments are becoming increasingly expensive and the application of the high-end technologies can only be made in dedicated imaging centers harboring the specific expertise, thus justifying the national centers for the most expensive *in vivo* imaging modalities.

Significant investments have already been made on the instruments. To get the full benefit from these investments, the continuity of the personnel and their salaries has to be guaranteed. Any gap in funding may result in losing these key personnel. The current level of staffing is essential for the sustainability of the network services to the large user community, with increasing needs for sophisticated preclinical *in vivo* imaging technologies.

One of the key issues for the future success is the availability of animal models for human diseases. In each of the three contributing Biocenters, a large variety of rodent models are available. For translational imaging, larger animal models, such as swine, are also needed.

Rapid advances in imaging technologies generate large multidimensional data sets typically in the order of GBs and up to TBs. There is a clear need for centralized data management and standardized visualization and analysis tools. Each of the participating imaging centers is currently developing their own solutions for this purpose, thus there is clearly a need for harmonization and more general solutions to allow remote data management and collaboration within partner units of the BF National preclinical *in vivo* imaging infrastructure network.

PET

The PET imaging component of the consortium is currently of world class. The newest development in this field is a hybrid imaging system that combines PET and MRI. This hybrid system was installed in Turku in 2011. During 2013, the coils and other devices for small animal PET-MRI imaging were purchased, tested and validated, and they are now available for investigators. Despite recent advances in tracer production, the availability and development of new tracers is a continuous challenge and a key issue for the future success. Translational research will also require enough potential to bring novel tracers into GMP level for human applications.

Basic preclinical PET imaging using F^{18}, G^{a68} and Cu^{64} based tracers (such as FDG), should be established also in other participating Biocenters to make multimodal preclinical imaging more available across the country.

MRI

Overall, the situation regarding MRI instrumentation in the consortium is at top-European level with the most advanced instrumentation being located in Kuopio. However, the MRI field is rapidly developing and, for example, new advances in parallel transmit RF technique are becoming available in preclinical MRI scanners in a few years time, requiring upgrades to maintain the availability of the state-of-the-art techniques. MRI microscopy (mice *in vivo*, and *ex vivo* tissue samples) is currently performed on a 9.4T MRI system in Kuopio (magnet purchased 1995, console upgrade 2002). This should be replaced within the next three years with a high field (11.7–14.4 T) MRI scanner.

Basic preclinical MRI should also be developed at different Biocenters to provide multimodality at the local level. In particular, the decommissioned 4.7T MRI system in Helsinki needs a replacement during 2015–2016.

C^{13} Hyperpolarized MRI is probably the greatest breakthrough in the MRI field within the last decade. It allows 10 000 times increased in signal-to-noise ratio and makes possible of dynamic metabolic studies using C^{13}-labeled non-radioactive compounds. FIRI2014 funding was received to purchase a hyperpolarisator in Kuopio.

Optical Imaging

The animal facility has been re-organized at University of Helsinki due to the completion of the new rodent house on the Viikki campus. Both optical multimodal and MRI imaging facilities are located on the Meilahti campus, and convenient animal transfer between the units and campuses has been established. Future perspectives include spearheading the CARS and other label-free technologies and tracking of Raman active probes (e.g., non-fluorescent drug nanoparticles) *in vivo*. The microscope platforms will be further developed for additional modalities, including fluorescence life-time imaging, stimulated Raman spectroscopy, and photo-acoustic microscopy. As stated above, there is a pressing need to purchase replacement for the decommissioned preclinical MRI scanner.

Major publications supported by platform services

Hemminki O, Immonen R, Närväinen J, Kipar A, Paasonen J, Jokivarsi KT, Yli-Ollila H, Soininen P, Partanen K, Joensuu T, Parvianen S, Pesonen SK, Koski A, Vähä-Koskela M, Cerullo V, Pesonen S, Gröhn OH, Hemminki A In vivo magnetic resonance imaging and spectroscopy identifies oncolytic adenovirus responders. Int J Cancer 134: 2878–2890, 2014.

Autio JA, Shatillo A, Giniatullin R, Gröhn OH. Parenchymal spin-lock fMRI signals associated with cortical spreading depression. J Cereb Blood Flow Metab 2014 34: 768–775.

Nissi MJ, Lehto LJ, Corum CA, Idiyatullin D, Ellermann JM, Gröhn OH, Nieminen MT. Measurement of T1 relaxation time of osteochondral specimens using VFA-SWIFT. Magn Reson Med 74: 175–184, 2015.

Jeltsch M, Jha SK, Tvorogov D, Anisimov A, Leppänen VM, Holopainen T, Kivelä R, Ortega S, Karpanen T, Alitalo K. CCBE1 enhances lymphangiogenesis via A disintegrin and metalloprotease with thrombospondin motifs-3-mediated vascular endothelial growth factor-C activation. Circulation 129: 1962–1971, 2014.

den Hollander B, Dudek M, Ojanperä I, Kankuri E, Hyytiä P, Korpi ER. Manganese-enhanced magnetic resonance imaging reveals differential long-term neuroadaptation after methamphetamine and the substituted cathinone 4-methylmethcathinone (mephedrone). Int J Neuropsychopharmacol 18: DOI: http://dx.doi.org/10.1093/ijnp/pyu106, 2015.

Dudek M, Abo-Ramadan U, Hermann D, Brown M, Canals S, Sommer WH, Hyytiä P. Brain activation induced by voluntary alcohol and saccharin drinking in rats assessed with manganese-enhanced magnetic resonance imaging. Addict Biol doi:10.1111/adb.12179, 2014.

Kivelä R, Bry M, Robciuc MR, Räsänen M, Taavitsainen M, Silvola JM, Saraste A, Hulmi JJ, Anisimov A, Mäyränpää MI, Lindeman JH, Eklund L, Hellberg S, Hlushchuk R, Zhuang ZW, Simons M, Djonov V, Knuuti J, Mervaala E, Alitalo K. VEGF-B-induced vascular growth leads to metabolic reprogramming and ischemia resistance in the heart. EMBO Mol Med 2014 6: 307–321, 2014.

Li XG, Helariutta K, Roivainen A, Jalkanen S, Knuuti J, Airaksinen AJ. Using 5-deoxy-5-[18F]fluororibose to glycosylate peptides for positron emission tomography. Nat Protoc ~~2014~~ 9: 138–145.

Light Microscopy Technology Platform

Chair of the consortium: John Eriksson, Turku Bioimaging
Members: Cell Imaging Core (CIC), Eleanor Coffey and Turku Bioimaging (TBI), John Eriksson, BioCity Turku; Biomedicum Imaging Unit (BIU-BCH), Elina Ikonen, BCH; Light microscopy unit (LMU-BI), Maria Vartiainen, BI; Multimodal imaging core (MUIC-BCK), Michael Courtney, BCK; Tissue Imaging Center (TIC-BCO), Lauri Eklund, BCO; BMT imaging facility, Susanna Narkilahti, BMT; also University of Jyväskylä Imaging Facility, Varpu Marjomäki participates in the platform activities.

www.biocenter.fi

Achievements in development and restructuring of technology services

The imaging research infrastructures of Biocenter Finland (BF) have developed to a very prominent position, placing Finland as one of the leading countries among European imaging in terms of available advanced instrumentation. Imaging has nationally a very strong user base, as among all BF infrastructure technologies, imaging is clearly the one in highest demand. The user statistics indicate that the number of light microscopy platform users and user hours are still increasing. This development is partly due to a better open access to advanced technologies, but also a result of light microscopy methods being increasingly applied to different fields of research. Researchers are also gradually becoming aware of their access to cutting-edge technologies, and article reviewers are often demanding the use of such techniques.

From the outset of the BF, there has been a national division into specialized imaging core facilities, with different state-of-the-art technologies being featured. All the units involved in the light microscopy platform are well networked and highly interactive, leading to significant synergistic effects and continuous technology development. To keep the current position, the BF imaging core facilities need funding to be able to provide access to the novel technologies. To this end, the light microscopy consortium anticipates to be the focus area in the BF proposal to the FIRI2015 call.

The long-term goal is to achieve a more seamless interaction with *in vivo* and medical imaging. In this respect, Finland is coordinating a new Network for Bridging Nordic Imaging, financed by Nordforsk. The main aim of this network is to increase interdisciplinary imaging. The first network meeting will take place in August (http://www.bioimaging.fi/nordic-imaging/) and the idea is to, apart from its Nordic perspective, also use this meeting as an instrument to engage the different imaging fields in Finland to better interactions, interdisciplinarity, and collaborations. In this respect, the BF light microscopy can also report on active engagement in the European Bioimage Analysis Network and initiation of the Finnish Network of Bioimage Informatics.

Technology specialization among the Finnish imaging centers

Super-resolution imaging

Super-resolution modalities have been continuously developed with support from BF. Super-resolution centers have been established at the CIC-TBI and BIU-BCH. These units implement STED STORM super-resolution modalities, respectively. A unique initiative in Turku has been to expand the concept of super-resolution microscopy by combining STED with other imaging technologies. In addition to successful combination of STED with atomic force microscopy, also fluorescence lifetime imaging microscopy (FLIM) and fluorescence correlation spectroscopy (FCS) are in progress to be combined with STED. Turku has also developed 3D tomographic STED microscopy (mirror-STED), expanding super-resolution capability from 2D to 3D, and applied it to a biological study. TIRF microscope at CIC-TBI has been in extensive use, and also in special applications such as high-speed live imaging, tile imaging of four labels, and analysis of clinical patient samples. LMU-BI is operating Bayesian localization microscopy and exploring also other techniques that enable super-resolution imaging using standard fluorescent markers and microscopes. A pipeline utilizing the computational resources at CSC aids in this process.

High-content and high-throughput imaging

Both BIU-BCH and LMU-BI have partnered with the BF Genome-wide Methods technology platform to offer complementary high-content imaging services. Close collaboration with FIMM RNAi Technology Center is also important in this context. In addition, LMU-BI and CIC-TBI have developed the Leica SP5 Matrix platform to enhance complementarity between the two units. BIU-BCH, LMU-BI and CIC-TBI work with the CSC to develop solutions for storing large data amounts. Also the BioImageXD software platform (see Image analysis below) is being actively developed to suit better for high-content applications. CIC-TBI acquired a new Cell-IQ high-content automated microscope, which was immediately heavily used in drug development screens. CIC-TBI also initiated projects aimed at significantly improving high-throughput and high-content imaging and image analysis in the years to come.

Label-free technologies

Several units in the network have established various label-free principles, most of which are a continuum between cellular and *in vivo* visualization. BIU-BCH has established a confocal multiphoton CARS (coherent anti-Stokes Raman spectroscopy) platform, as part of a National BioCARS Centre. Additionally, it offers label-free second and third harmonic generation imaging services suited for label-free sample visualization. BIU-BCH is among the first, if not the first, open access CARS microscopy core facilities in Europe. A completely novel label-free technique is photo-acoustic microscopy (PAM), which has been built within TBI in collaboration with Northwestern University, Chicago. PAM system is utilized in cell-biology research, in correlation with optical imaging methods, such as super-resolution STED imaging and *in vivo* microscopy techniques, primarily multiphoton microscopy. First applications with living *Drosophila* larva have been successful. In BMT in Tampere, time lapse imaging systems BiostationCT (Nikon) and Cell-IQ (CMT) have been utilized in studies with multiple cell types and a user training seminar was provided.

Mesoscopic imaging

Light sheet microscopy (for example, selective plane illumination microscopy, SPIM) is an emerging technique that allows exceptionally fast and sensitive imaging of thick living samples. The latest update in mesoscopic imaging is a custom-built light sheet microscope, set-up in collaboration between BCO-TIC and Optoelectronics and Measurement Techniques Laboratory (Department of Electrical Engineering, University of Oulu) in Oulu Bioimaging network. BMT has focused on the imaging of mesoscopic objects (3D cell cultures, biomaterials, Zebrafish and Drosophila) to which the current systems have been optimized with equipment and methods optimizations and user training courses.

Image analysis and processing

Bioimage post-processing in its various forms has continued to grow in importance, as indicated by the coining of a new scientific field, bioimage informatics. Bioimage informatics should be considered at least as important as instrumentation and image acquisition, which is illustrated by the results of a recent European survey. In Finland, a large open source bioimage analysis software package, BioImageXD (http://www.bioimagexd.net/), has been developed as a multidisciplinary collaboration between Turku, Jyväskylä, and others. The first full version of BioImageXD was released in 2012 (Kankaanpää et al., Nature Methods), and since then, the software has been used in approximately 100 scientific publications. In 2014, it was downloaded 300 times every month, and several new features were also developed, such as support for hundreds of new file formats through Bio-Formats (http://loci.wisc.edu/software/bio-formats). In Oulu, new algorithms for segmentation and motion tracking were implemented for time-lapse microscopy (Kaakinen et al. 2014, Journal of Microscopy). A new release of BioImageXD, containing the afore-mentioned features, is planned for 2015. In Turku and Jyväskylä, courses and personal assistance with the software and its usage were available throughout the year.

General bottlenecks

Most life scientists experience bioimage informatics as one of the most necessary but difficult and insufficiently supported areas. There is thus a rapidly growing need for experts and services in quantitative image analysis, which is likely to present a major challenge that needs to be addressed in the near future. There is also need to strengthen rapidly evolving imaging modalities, such as super-resolution light microscopy, to meet current state-of-the-art. Increasing imaging data, storage, transfer, analysis and processing are additional major bottlenecks nationally. Urgent investment needs are not included in this report, since the platform coordinator has been in contact with BF leadership regarding their inclusion in the FIRI2015 application of BF.

User statistics

The user statistics of all facilities from 2014 indicate a clear increase in national and international users and financial turnover, illustrating the increasing importance of light microscopic imaging services in biomedical research, and the synergistic effects obtained through national collaboration and organization.

Participation in international, Nordic and European infrastructures

The BF light microscopy consortium participates actively in the pan-European Euro-BioImaging ESFRI (www.eurobioimaging.eu) infrastructure interim phase and has an excellent possibility to establish European imaging nodes in Finland. In 2014, EuBi Memorandum of Understanding signed countries, including Finland, established EuBi Interim Board that is the decision-making body through the interim phase towards construction. Finland has also an important role in future Euro-BioImaging Hub construction.

A new national network, BioImage Informatics Finland (BIIF), was established for bioimage analysts, software developers and life scientists using bioimage

informatics as a central toolset. BIIF is coordinated by Turku, and it is a partner in the European BioImage Analysis network (EuBIAS) and participates in a European COST action proposal aimed at getting funding for the development of bioimage informatics and networking in Europe. Finland participates also in other European networks, such as the Open Bio Image Alliance, especially through the BioImageXD software project.

Bridging Nordic Imaging network was established in 2014 for efficient collaboration between Nordic countries and to work jointly towards improved options for both biological and medical imaging. Turku is a coordinator of the network, and several LM platform units are actively participating in it. As mentioned above, the first network meeting will take place in August (http://www.bioimaging.fi/nordic-imaging/) with the main aim to engage the different imaging fields in Finland and in the Nordic countries to better interactions, interdisciplinarity, and collaborations.

Future perspectives

While the BF funding has enabled a steady improvement in access to technologies, leading to a continuous increase in user numbers and hours, the aim is to continue this growth not only at the national but also at international level. The main strategy to achieve an increasingly international user base has been the Euro-Bioimaging project. This initiative has been well received and is now prepared in ongoing interaction with the Ministry of Education and Culture and Academy of Finland to establish a Finnish Euro-Bioimaging Node and also a central Hub. These would not only open Finnish infrastructures to international users but also give Finnish researchers unique possibilities to interact and develop joint interests with leading European imaging centers. In terms of technology development, the special interest for the light microscopy platform is to develop different modalities of label-free imaging, ultrafast acquisition and super-resolution reconstruction alongside with image analysis and solving the bottlenecks in image analysis and data storage.

Major publications supported by platform services

D'Amico G, Korhonen EA, Anisimov A, Zarkada G, Holopainen T, Hägerling R, Kiefer F, Eklund L, Sormunen R, Elamaa H, Brekken RA, Adams RH, Koh GY, Saharinen P, Alitalo K. Tie1 deletion inhibits tumor growth and improves angiopoietin antagonist therapy. J Clin Invest 124: 824–834, 2014.

Aspelund A, Tammela T, Antila S, Nurmi H, Leppänen VM, Zarkada G, Stanczuk L, Francois M, Mäkinen T, Saharinen P, Immonen I, Alitalo K. The Schlemm's canal is a VEGF-C/VEGFR-3-responsive lymphatic-like vessel. J Clin Invest 124: 3975–3986, 2014.

Mähönen AP, Tusscher KT, Siligato R, Smetana O, Díaz-Triviño S, Salojärvi J, Wachsman G, Prasad K, Heidstra R, Scheres B. PLETHORA gradient formation mechanism separates auxin responses. Nature 515: 125–129, 2014.

Zhang H, Liu D, Shahbazi MA, Mäkilä E, Herranz-Blanco B, Salonen J, Hirvonen J, Santos HA. Fabrication of a multifunctional nano-in-micro drug delivery platform by microfluidic templated encapsulation of porous silicon in polymer matrix. Adv Mater 26: 4497–4503, 2014.

Rahtu-Korpela L, Karsikas S, Hörkkö S, Blanco Sequeiros R, Lammentausta E, Mäkelä KA, Herzig KH, Walkinshaw G, Kivirikko KI, Myllyharju J, Serpi R, Koivunen P. HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. Diabetes 63: 3324–3333, 2014.

Mohl M, Dombovari A, Tuchina ES, Petrov PO, Bibikova OA, Skovorodkin I, Popov AP, Rautio A-R, Sarkar A, Mikkola J-P, Huuhtanen M, Vainio S, Keiski RL, Prilepsky A, Kukovecz A, Konya Z, Tuchin VV., Kordas K. Titania nan-

User statistics ~~2014~~

	BIU-BCH	LMU-BI	MUIC-BCK**	TIC-BCO	BMT	CIC-TBI	Total
Total number of research groups	71	60		38	19	97	285
- local groups	60	54		28	18	91	251
- other domestic groups	9	6		6*		2	23
- international	2			3	1	3	9
- non-academic				1		1	2
Total instrument hours	10 485	9 171		7 454	3 014	9 942	40 066
Single users	175	188		112	41	269	785
User fees (€)	125 486	100 850		19 965	36 396	155 650	438 347

* Users from Oulu University Hospital (OYKS) marked as other domestic users

** User statistics from MUIC-BCK Kuopio not available for year 2014

ofibers in gypsum composites: an antibacterial and cytotoxicology study. J Mater Chem B 2: 1307–1316, 2014.

Muharram G, Sahgal P, Korpela T, De Franceschi N, Kaukonen R, Clark K, Tulasne D, Carpén O, Ivaska J. Tensin-4-dependent MET stabilization is essential for survival and proliferation in carcinoma cells. Dev Cell 29: 421–436, 2014.

Karikoski M, Marttila-Ichihara F, Elima K, Rantakari P, Hollmen MK, Kelkka T, Gerke H, Huovinen V, Irjala H, Holmdahl R, Salmi M, Jalkanen S. Clever-1/Stabilin-1 controls cancer growth and metastasis. Clin Cancer Res 20: 6452–6564, 2014.

Rajala N, Gerhold JM, Martinsson P, Klymov A, Spelbrink JN. Replication factors transiently associate with mtDNA at the mitochondrial inner membrane to facilitate replication. Nucl Acids Res 42: 952–967, 2014.

Marjomäki V, Lahtinen T, Martikainen M, Koivisto J, Malola S, Salorinne K, Pettersson M, and Häkkinen H. Site-specific targeting of enterovirus capsid by functionalized gold nanoclusters. Proc Natl Acad Sci USA 111: 1277–1281, 2014.

Small Animal Molecular Imaging (SPECT/CT)

Chair persons of the consortium: Raimo K. Tuominen, Division of Pharmacology and Toxicology, University of Helsinki

www.biocenter.fi

Achievements in development of technology services

In 2014, the unit served 12 projects (see user statistics). The total service time of the real-time imaging unit (RTI) was only 22 weeks in 2014. Service was unavailable for 3.5 weeks due to the SPECT/CT camera relocation to the new facilities, and for 3 weeks for maintenance and calibration. Moreover, approx. eight weeks were dedicated to develop each of the two new applications that have already been transferred to actual services. These applications involved heart imaging and parasite labeling as well as imaging of their distribution in rats. In comparison to 2013, the services provided in 2014 increased in time by 15%, in the number of projects by 60%, and in the number of scans by 400%. This growth in services took place despite reduced number of staff at RTI in 2014.

In 2014, the RTI emphasized the applications developed on previous years. Those were: (i) dopaminergic integrity and evaluation in Parkinson's disease models; (ii) evaluation of ocular drug elimination with SPECT/CT; (iii) evaluation of cubosomes and hexosomes as drug delivery nanosystems; (iv) bio-distribution of nanosystems for cancer theranostics; (v) visualization of drug pigment binding in vivo; and (vi) bio-distribution and dynamics of liposomic and silica-based drug delivery nano-systems. During this reporting period, the RTI unit started development of two new applications: imaging of nanosystems for cardiac gene therapy, and imaging of the bio-distribution of Trichinellidae larvae in rats.

The RTI unit service platform has improved. Technology is now efficiently available for pharmacokinetic and pharmacodynamic studies (e.g., assessment of drug delivery systems; bio-distribution studies). In addition, specific methods to measure neuronal integrity – for example, dopaminergic, serotoninergic and inflammatory imaging – for research in neurodegeneration models are getting established, widening the service in different areas of neuropsychopharmacology. The entire unit moved to a new and more functional space, from Biocentre 2 to Biocentre 3 building on the Viikki campus. It now provides a conveniently spaced imaging room together with a separate control room, which leads to much less radiation exposure to the operator and researchers. Moreover, there is now a separate laboratory for radioisotope handling and a separate room for animals.

User statistics

The unit was involved in 12 projects in 2014. The comprised 10 different groups, 9 were domestic, 6 of them being local users, and one user was from abroad. In total, 205 scans were performed. From this work, several articles have been already published where RTI service was essential (see Major publications), and more publications are expected to be out soon.

Participation in international, Nordic and European infrastructures

The laboratory is main partner in the COMPACT project (FP7) 2012–2017, and participant partner in the COST action TD1004.

Future perspectives

Having established a variety of applications in the service portfolio, it is expected that there will an increase on the demand of the service in near future. The unit plans to establish new imaging technical protocols, specifically for neuroscience applications. With regard to drug delivery research, membership in the EU-IMI project COMPACT builds relations to universities and big pharma companies for follow-up research projects. It looks as if the unit is currently under-represented outside the Viikki campus.

The funding from the BF has been essential for the development of the unit. Since the service needs increase, it is important to keep the BF funding at least at the current level which is needed to guarantee proper service. The expectations of RTI to get hardware and software upgrades in 2014 did not occur. Needless to say, these upgrades are mandatory for development

and maintenance of high-end services at RTI. In addition, an in-house availability of animal MRI would be very important in order to achieve higher resolution in imaging of specific tissues. Currently, different areas of the brain and eye are not well defined with the existing instrumentation.

Major publications supported by platform services

Wang CF, Sarparanta MP, Mäkilä EM, Hyvönen ML, Laakkonen PM, Salonen JJ, Hirvonen JT, Airaksinen AJ, Santos HA. Multifunctional porous silicon nanoparticles for cancer theranostics. Biomaterials 48: 108–118, 2015

Subrizi A, Toropainen E, Ramsay E, Airaksinen AJ, Kaarniranta K, Urtti A. Oxidative stress protection by exogenous delivery of rhHsp70 chaperone to the retinal pigment epithelium (RPE), a possible therapeutic strategy against RPE degeneration. Pharm Res 32: 211–221, 2015.

Kallinen A, Todorov B, Kallionpää R, Bäck S, Sarparanta M, Raki M, García- Horsman JA, Bergström KA, Wallén EA, Männistö PT, Airaksinen AJ. Synthesis and biological evaluation of novel 123I-labeled 4-(4-iodophenyl) butanoyl-L-prolyl-(2S)-pyrrolidines for imaging prolyl oligopeptidase in vivo. Eur J Med Chem 79: 436-445, 2014.

Laurén P, Lou YR, Raki M, Urtti A, Bergström K, Yliperttula M. Technetium-99m-labeled nanofibrillar cellulose hydrogel for in vivo drug release. Eur J Pharm Sci 2014 65: 79–88, 2014.

Khabbal J, Kerkelä E, Mitkari B, Raki M, Nystedt J, Mikkonen V, Bergström K, Laitinen S, Korhonen M, Jolkkonen J. Differential clearance of rat and human bone marrow-derived mesenchymal stem cells from the brain after Intra-arterial infusion in rats. Cell Transplant 24: 819–828, 2015.

The imaging research infrastructures of Biocenter Finland have developed to a very prominent position, placing Finland as one of the leading countries among European imaging in terms of available advanced instrumentation.

GENOME-WIDE METHODS

Genome-wide Methods Infrastructure Network

Coordinator of the network: Tomi P. Mäkelä, BI
Members: Outi Monni, BCH; Jorma Palvimo, BCK; Minna Männikkö, Tapio Visakorpi, BMT; Riitta Lahesmaa, BioCity; Janna Saarela, FIMM

www.biocenter.fi

Genome-wide methods including DNA sequencing, RNA analyses, and high-throughput genetic screens have rapidly and profoundly changed basic biological science and biomedicine. Because of the highly specialized and capital-intensive nature of genomics instrumentation and reagent sets these technologies have been developed as core infrastructures providing services to researchers nationally. Genome-wide approaches are a focus area of biocenters in Helsinki and Turku: genetics/genomics and genome-scale biology services are primarily developed in Helsinki and gene expression and regulation services in Turku. Cost-effective access to reagents and libraries enabling knockdowns or overexpression as well as high-throughput facilities is provided national-scale as a collaborative effort of Helsinki biocenters. It is essential to provide tailored services in this area to Finnish scientists also in the coming years to maintain the cutting edge. This development requires both long-term funding to enable recruitment and maintenance of top quality scientists and technicians as well as continuing investments into new technologies. The BF Genome-wide network continues in its role as an expert body to coordinate training efforts, to evaluate the services, to facilitate the use of these services in biocenters throughout Finland, and to integrate these activities internationally. High-content screening services were customized to local research strengths and integrated with imaging and translational technologies.

Development of novel technologies such as RNA interference (RNAi) and the increasing efficiency and speed of DNA sequencing serve as examples of continuous need for new equipment and upgrading of current ones. This requires rapid adaptation of both researchers and the research environment, where highly specialized and capital-intensive instrumentation and reagent sets are optimally developed as core infrastructures providing services to researchers nationally.

Genome-Wide Methods Technology Platform

Chair of the consortium: Tomi P. Mäkelä, BI, Genome Biology Unit (GBU)
Members: Outi Monni, BCH, Biomedicum Functional Genomics Unit (FuGU); Petri Auvinen, BI, DNA Sequencing and Genomics Laboratory (BI-DGEN); Tea Vallenius, BI, Genome-biology Unit (GBU); Riitta Lahesmaa & Riikka Lund, BioCity, Finnish Microarray and Sequencing Centre (FMSC); Janna Saarela, FIMM, FIMM Technology Center; DNA Sequencing and Genotyping Laboratory; High-throughput Screening Facility

www.biocenter.fi

Achievements in development and restructuring of technology services

Genome-scale biology has kept its momentum in 2014, even though no totally new DNA sequencing technologies were released. The existing technologies with their continuously expanding applications – now close to enabling analysis of individual genomes in individual cells – have developed rapidly. The Genome-Wide Methods Technology Service (GMW) platform received recently funding for Fluidigm system, which allows qRT-PCR, dPCR, genotyping and Olink protein interaction analyses in nanoliter volumes. These technologies complement the next-generation sequencing services, since they offer a fast alternative for targeted analysis in small sample volumes. In 2014, GWM launched a new genome-scale instrument in Finland, nCounter Analysis System/NanoString. The technology uses molecular "barcodes" and single-molecule imaging to detect and count up to 800 unique transcripts in a single reaction from a very small starting material without amplification (no cDNA synthesis or PCR). Of note, the GWM NanoString platform represents the first core-based service in Nordic countries. Bioinformatics closely linked to analysis and storage of the omics data has continued developing nearly as fast as the data production in the laboratories. BF is in a key position to provide and develop genome scale biology technologies in Finland. Owing to the very rapid development of DNA sequencing and other genome-wide applications and novel emerging technologies, it is mandatory to have a long-term plan and commitment in securing sufficient funding for personnel and equipment in this field in Finland.

The drastic drop in instrument funding available to BF has caused challenges for staying abreast with the fast-developing area such as genome-scale biology, or even to follow emerging upgrades in technology.

GWM has faced these challenges by a close collaboration and division of tasks within the network. In 2014, GWM organized jointly a symposium of "Genome-scale reagents and services for functional studies", in order to provide training and increase awareness of GWM services among researchers. Division of tasks involves specialized sequence services provided in each GWM node as depicted in the user statistics table. Data analysis pipelines and computational resources crucial for supporting these technology platforms have been established and developed accordingly. The achievements of GWM during the first years of BF funding have demonstrated the strength, power and competitiveness of such a structure and justify for further funding, subject to regular external evaluation. The development of the platforms themselves in all the nodes has been rapid due to the BF funding and additional funding provided by the host organizations. Due to the cutting edge research infrastructure provided by BF, the Finnish scientists have been in an excellent position to apply for European Union or other international funding. The Academy of Finland funding is evaluated by external international panels, who expect access to cutting-edge infrastructure. This has been made available to the entire Finnish research community through GWM. GWM nodes coordinate their activities aiming at optimizing the cost-efficient usage of the funding.

A number of the bottlenecks ought to be solved. In particular, the IT infrastructure needs constant upgrades in terms of capacity, speed and specialized expertise to meet the requirements of high-throughput biology. The interaction between BF networks, especially with the bioinformatics network, has been close and is crucial. Some of the challenges can be solved through a close collaboration with CSC, utilized by all the nodes. Since several applications are ideally performed within the data-producing units, it is essential to reserve funds to develop also such local IT infrastructures. Several sequencers need a near-by rapid storage facility, in which data can be stored prior detailed analysis. The capacity of CSC is not currently sufficient to accommodate the high demand by this network, although recent new developments have made CSC a more suitable producer of computing and storage services. In addition to the constant increase in the capacity and throughput, and need of the genome-wide technologies, automation of the sample preparation processes needs to be increased as well.

Participation in international, Nordic and European infrastructures

The cutting-edge infrastructure developed by BF funding has made Finnish scientists competitive in obtaining not only national but also international funding (e.g., ERC grants). Examples of funding include participation in European research projects (e.g., DIABIMMUNE, NANOMMUNE, PEVNET, SYBILLA, ESTOOLS) and JDRF funded projects or European infrastructures. [EU projects: Systems microscopy, Biomed Bridges, European Innovative Medicines Initiative (IMI), ESFRI networks (EATRIS, BBMRI)]. Nodes are taking part as partners or service providers in several Horizon 2020 applications and NordForsk consortia applications. There are increasing demands for computing power and for storage and archiving in the field of genome-wide methods. GWM has prepared two national level roadmaps of infrastructures in this area (2007 and 2009), and it is in an optimal position to take responsibility as a node for both ESFRI level infrastructures (such as BBMRI) and an upcoming national infrastructures roadmap. In collaboration with CSC, GWM is developing solutions via CSC cloud computing project within the ELIXIR ESFRI program and in several pilot projects using virtual machines solution in complex analyses. These activities are also planned in close contact with the BF Bioinformatics network. Although cloud computing can be used for certain applications, the need for local computing and storage capacity near the data production sites does not cease.

User statistics

Almost 500 research groups used the services provided by GWM nodes in 2014, with user fees exceeding 3 000 000 € as shown in the table. The restructuring and sharing of tasks is evident through comparison of the services in genomics, gene expression, and genome-scale biology. The number of samples analyzed by the nodes increased during the period of 2010–2013, and this has continued in 2014. The amount of work and expertise needed in different services are dissimilar, and therefore, the table only summarizes the total activities.

Future perspectives

The most significant new developments involve single cell analytics, which will bring the existing genome, epigenome and RNA sequencing capabilities to a complete new level. Sequencing technologies are developing rapidly, and novel versions of the second-generation instruments with new properties are launched frequently. The already existing, cost-effective human

genome-scale sequencing is currently lacking in Finland. In 2015, GWM looks forward to developing single cell approaches as a coordinated nation-wide effort, should requisite funds for infrastructure purchase become available. These developments include all phases from the isolation and treatment of single cells to library protocols and library preparation automation, which need to be adjusted to very small sample sizes.

The findings enabled by the current sequencing technologies bring up an urgent need for genome-wide and single cell level follow-up methods, including libraries such as ORF clones, siRNA and shRNA libraries, etc., which have been lagging behind. Moreover, new species and more coverage should be added to the existing genome-scale reagents.

Along with the development and maturation of sequencing technologies, it is expected that genome-wide, deep coverage, allele-specific sequencing applications will become more common, further increasing the demands in sequencing capacity together with data management and processing. GWM services have also been used in several projects involving population-wide sequencing of non-model species genomes. One emerging bottleneck already now seen is arising from both the development of technologies and ever increasing number of samples handled in each node. In many nodes, resources for novel method development or even taking new published methods in production is restricted by the personnel and re-

	BioCity (FMSC)			BI (BIDGEN & GBU)			FIMM			BCH (FuGu)		
Services	Samples	Projects	Groups	Samples	Projects	Groups	Samples	Projects	Groups	Samples	Projects	Groups
Resequencing	16	2	1	83	9	6	639	22	12	344	27	19
De novo				291	112	30				8	1	1
Metagenomics	129	10	2	4534	45	21						
Targeted	6 024	190	78	3	1	1	7 158	190	35	164	11	8
SNP genotyping (QWAS)							2 043	11	11			
Targeted SNP typing	86	1	1				40 007	20	14			
Copy number variation**	82	17	10									
Immunoprecipitates (ChIP-Seq etc)*							460	5	4	32	5	1
RNA sequencing				605	55	21	353	61	19	116	8	6
Gene expression microarrays							18	2	1	348	29	25
Cell microarray screening							171	10	10			
Genome-scale reagents				217	82	33				331	37	24
ORF clooning				62	51	16						
Automated digital slide scanning				4 568	58	10	2 087	9	9			
Pooled & barcode shRNA screens												
High-content analysis (HCA)				1 576	42	19						
Customers												
local		226	78		418	136		455	85		98	57
other domestic		14	12		18	9		46	31		37	15
international		8	4		4	1		4	8		7	3
non-academic groups / units		28	14		15	11		2	2		7	6
TOTAL		276	108		455	157		507	126		149	81
User fees (€), total		667 611			527 238			1 403 210			432 168	

agents. Nevertheless, developing and quickly adapting novel methods, together with further educating the personnel are mandatory, in order to enable provision of cutting edge, state-of-the-art technology services to Finnish scientists.

Major publications supported by platform services

Niemelä EH, Oghabian A, Staals RHJ, Pruijn G, Frilander MJ. Global analysis of the nuclear processing of unspliced U12-type introns by the exosome. Nucl Acids Res 42: 7358–7369, 2014.

Nyyssönen M, Hultman J, Ahonen L, Kukkonen I, Paulin L, Laine P, Itävaara M, Auvinen P. Taxonomically and functionally diverse microbial communities in deep crystalline rocks of the Fennoscandian shield. ISME J 8: 126–138, 2014.

Kopra J, Vilenius C, Grealish S, Härma MA, Varendi K, Lindholm J, Castrén E, Võikar V, Björklund A, Piepponen TP, Saarma M, Andressoo JO. GDNF is not required for catecholaminergic neuron survival in vivo. Nat Neurosci 18: 319–22, 2015.

Joensuu M, Belevich I, Rämö O, Nevzorov I, Vihinen H, Puhka M, Witkos TM, Lowe M, Vartiainen MK, Jokitalo E. ER sheet persistence is coupled to myosin 1c-regulated dynamic actin filament arrays. Mol Biol Cell 25: 1111–1126, 2014.

von Nandelstadh P, Gucciardo E, Lohi J, Li R, Sugiyama N, Carpen O, Lehti K. Actin-associated protein palladin promotes tumor cell invasion by linking extracellular matrix degradation to cell cytoskeleton. Mol Biol Cell 25: 2556–2570, 2014.

Roncagalli R, Hauri S, Fiore F, Liang Y, Chen Z, Sansoni A, Kanduri K, Joly R, Malzac A, Lähdesmäki H, Lahesmaa R, Yamasaki S, Saito T, Malissen M, Aebersold R, Gstaiger M, Malissen B. Quantitative proteomics analysis of signalosome dynamics in primary T cells identifies the surface receptor CD6 as a Lat adaptor-independent TCR signaling hub. Nat Immunol 15: 384–392, 2014.

Morrison VL, James MJ, Grzes K, Cook P, Glass DG, Savinko T, Lek HS, Gawden-Bone C, Watts C, Millington OR, MacDonald AS, Fagerholm SC. Loss of beta2-integrin-mediated cytoskeletal linkage reprogrammes dendritic cells to a mature migratory phenotype. Nat Commun 5: 5359, 2014.

Riihilä P, Nissinen L, Farshchian M, Kivisaari A, Ala-Aho R, Kallajoki M, Grénman R, Meri S, Peltonen S, Peltonen J, Kähäri VM. Complement factor I promotes progression of cutaneous squamous cell carcinoma. J Invest Dermatol 135: 579–588, 2015.

Pihlajamaa P, Sahu B, Lyly L, Aittomäki V, Hautaniemi S, Jänne OA. Tissue-specific pioneer factors associate with androgen receptor cistromes and transcription programs. EMBO J 33: 312–326, 2014.

Gu Y, Bouwman P, Greco D, Saarela J, Yadav B, Jonkers J, and Kuznetsov S. Suppression of BRCA1 sensitizes cells to proteasome inhibitors. Cell Death Dis 5: e1580, 2014.

Haapaniemi EM, Kaustio M, Rajala HL, van Adrichem AJ, Kainulainen L, Glumoff V, Doffinger R, Kuusanmäki H, Heiskanen-Kosma T, Trotta L, Chiang S, Kulmala P, Eldfors S, Katainen R, Siitonen S, Karjalainen-Lindsberg ML, Kovanen PE, Otonkoski T, Porkka K, Heiskanen K, Hänninen A, Bryceson YT, Uusitalo-Seppälä R, Saarela J, Seppänen M, Mustjoki S, Kere J. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. Blood 125: 639–648, 2015.

Tiihonen J, Rautiainen MR, Ollila HM, Repo-Tiihonen E, Virkkunen M, Palotie A, Pietiläinen O, Kristiansson K, Joukamaa M, Lauerma H, Saarela J, Tyni S, Vartiainen H, Paananen J, Goldman D, Paunio T. Genetic background of extreme violent behavior. Mol Psychiatry 20: 786–792, 2015

Kontro M, Kuusanmäki H, Eldfors S, Burmeister T, Andersson EI, Bruserud O, Brümmendorf TH, Edgren H, Gjertsen BT, Itälä-Remes M, Lagström S, Lohi O, Lundán T, Martí JM, Majumder MM, Parsons A, Pemovska T, Rajala H, Vettenranta K, Kallioniemi O, Mustjoki S, Porkka K, Heckman CA. Novel activating STAT5B mutations as putative drivers of T-cell acute lymphoblastic leukemia. Leukemia 28: 1738–1742, 2014.

Nieminen TT, O'Donohue MF, Wu Y, Lohi H, Scherer SW, Paterson AD, Ellonen P, Abdel-Rahman WM, Valo S, Mecklin JP, Järvinen HJ, Gleizes PE, Peltomäki P. Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colorectal carcinoma without DNA mismatch repair deficiency. Gastroenterology 147: 595–598, 2014.

Due to the cutting edge research infrastructure provided by BF, the Finnish scientists have been in an excellent position to apply for European Union or other international funding.

MODEL ORGANISMS

Model Organisms Infrastructure Network

Coordinator of the network: Raija Soininen, BCO
Members: Eero Lehtonen, BCH; Matti Airaksinen, BCH; Heikki Tanila, BCK; Mika Rämet BMT; Matti Poutanen, BioCity; Sergey Kuznetsov, FIMM

www.biocenter.fi

The Model Organisms network comprises two technology platforms, those on mouse and non-mammalian model organisms.

Genetically modified (GM) mice are currently the key model organisms to understand the molecular basis of health and disease in man and to serve as models for human development and diseases, and are expected to have an important role in the development of new therapeutic approaches. Work with GM mice requires high-level expertise, and specific ethical and regulatory issues have to be followed. In Finnish biocenters, GM or "transgenic" mouse core facilities with experienced personnel were established in the 1990's to provide high quality service mainly in the generation of GM mice. Even though large international consortia nowadays systematically produce mutations in genes of the mouse genome, local infrastructure remains essential for providing services and expertise in all aspects of mouse-related issues, especially in customized mutagenesis, rederivation, and archiving of mutant mouse lines, as well as in education. Furthermore, in recent years, services in high-level systematic analysis ("phenotyping") of mutant mice have become more and more in demand. Activities on generation, analysis, and archiving of mutant mice in Finland are organized into the BF FinnMouse technology platform as will be discussed below.

The technology platform on non-mammalian models uses well characterized, simple organisms such as the fruit fly (*Drosophila melanogaster*), the zebrafish (*Danio rerio*) and the nematode *C. elegans* for large-scale genetic analyses of biological regulatory pathways and mechanisms of development. Their use as model organisms is based on the fact that many of the important physiological mechanisms are conserved in evolution, and therefore it is possible to use genetically tractable model organisms also for studies on human genetic diseases.

Emerging technology platform for tissue engineered disease models (TEDM) complements the services of the mouse model, viral gene transfer and drug discovery platforms of BF. TEDM platform is a sequel to LentiGEMM platform developing lentiviral technologies for tissue transductions funded by BF in 2010–2012. In 2013 funding was not available for TEDM platform, but the activities are being supported in 2014.

FinnMouse - National Technology Platform for Generation, Analysis and Archiving of Mouse Models

Chair of the consortium: Raija Soininen, BCO, Transgenic Animals Core Facility
Members: Eero Lehtonen and Petra Sipilä, BCH, Helsinki GM Mouse Unit; Eero Castren, BCH, Neurophenotyping Center (with BCK); Antti Sukura, Finnish Centre for Laboratory Animal Pathology; Heikki Tanila, BCK, Neurophenotyping Center (with BCH); Matti Poutanen, BioCity, Turku Center for Disease Modeling, TCDM

www.biocenter.fi

www.fingmice.org

Achievements in development of technology services

One of the key research tools in understanding mammalian gene function is the laboratory mouse. The scientific community has taken advantage of its fundamental similarity to humans at the genetic level (>95% identity at the exon level), similar physiology and anatomy, its relatively low cost compared to other mammals, and nearly 100 years of genetic study. An extensive toolkit for the manipulation of the mouse genome and the generation of new disease models has been generated.

Since special training of researchers and personnel performing the animal experiments as well as taking care of animals are required, and the units have to be managed according to regulations on the use of experimental animals and GM organisms, core facilities are the only choice. They offer possibilities for both reduction of animal numbers used and refining their life and welfare, following the 3R's principle. Furthermore, in the co-operative, centralized system, the GM animal strains can be used by a large number of researchers.

The FinnMouse technology platform has actively developed GM mouse services, and restructuring resulted in three collaborating core facilities (Helsinki, Oulu, and Turku), providing services in GM mouse technologies to all Finnish scientists. All three units provide elementary services, such as generation and rederivation of GM mice. In addition to the basic services, the units have special service profiles. The Helsinki GM unit specializes in mouse chimera generation by the morula aggregation method, and has started

to offer the generation of GM rat models using new gene modification techniques, along with rat embryo cryopreservation and rederivation, as a nation-wide open access service. The Oulu unit has special expertise in embryonic stem (ES) cells and cryopreservation methods, serving as the Finnish Infrafrontier-EMMA (European Mouse Mutant Archive, www.infrafrontier.eu) node that provides repository services, including cryopreservation and distribution of GM mouse strains to a world-wide user community. In Oulu, generation of GM mice via injections of CRISPR/Cas RNAs has also been started. Turku Center for Disease modeling (TCDM) provides services in generation of gene constructs for GM mouse production, tumor xenografts in immunodeficient mice, and it has collaboration with several pharma and biotech companies.

For phenotypic analyses of mice, Biocenter Oulu (BCO) currently focuses on services in the electron microscopy of mouse tissues (reported separately) and analysis of cardiovascular functions. Also the optical projection tomography (OPT) and basic mouse histopathology services are available. The Finnish Center for Laboratory Animal Pathology (FCLAP), established in 2010 within the Faculty of Veterinary Medicine, University of Helsinki, provides specialist services in laboratory animal pathology, including consultation and diagnostic services, and trains veterinary pathologists. TCDM recently established a cell culture laboratory for generating patient-derived cells, and in 2014, it performed first xenograft studies in immunodeficient mice using these cells. A high-sensitivity steroid profiling in serum and tissues of mice was also established at TCDM. Neurophenotyping Centers (NC) in Helsinki and Kuopio provide services in automated behavioral phenotyping and in specific neurophenotyping tests in both disease models and analyzing roles of specific factors. The Mouse Behavioural Phenotyping Facility (MBPF) in Helsinki was established in 2013 as a core unit, and it has received positive feedback. Thirteen articles with data obtained there were published in 2014.

In 2014, over 40 scientific articles were published in which services of the FinnMouse core facilities were used, emphasizing the importance of the facilities. The web interface www.fingmice.org provides information about services and expertise in generation as well as analyses of GM mice available in Finland. All service facilities are engaged in education of graduate students and postdocs in hands-on laboratory and lecture courses. Visits to partner laboratories and annual personnel meetings speed up the exchange of best practices and new methods, and workshops on specific subjects have been organized for scientists and technical personnel, the most recent one the BCO and Biocenter Kuopio joined Minisymposium on Mouse Models for Diseases and Imaging techniques in October 2014. The FinnMouse network meeting was organized in November 2014.

Participation in international, Nordic and European infrastructures

University of Oulu represents Finland in the ESFRI project INFRAFRONTIER, the European Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes, and is a shareholder in the INFRAFRONTIER GmbH, established in 2013, and is a partner in the EMMA network and in the FP7-Capasities 2013–2016 project Infrafrontier-I3. The FinnMouse platform is, therefore, well positioned to coordinate the national activities with those in Europe.

[Reference: INFRAFRONTIER--providing mutant mouse resources as research tools for the international scientific community. Nucl Acids Res 43: D1171–1175. INFRAFRONTIER Consortium 2015.]

University of Turku is a partner in the European Advanced Translational Research Infrastructure in Medicine (EATRIS). The NordForsk funded network NorIMM, Nordic infrastructure for Mouse Models, www.norimm.org, established to improve communication between GM mouse generation and phenotyping infrastructures in Nordic countries, is coordinated by Oulu University.

Future perspectives

The FinnMouse core facilities are – and continue to be – in a key position to provide knowledge and services in GM mouse models to the Finnish research community, and permanent staff is essential for reliable services and to fulfill the legal regulations. Dedicated experts are also needed to keep in touch with technology development and new innovations, in order to maintain the high quality. Instruments used are in most cases moderately priced but have to be up-to-date. Modern animal facilities are essential and have to be properly equipped and managed with excellent health status; for example, to facilitate transfer of animals between facilities. In most cases, it is not possible to charge the customers a fee that covers all costs, and continued support by host institutes is essential; on one hand, to sustain the quality and reliability of the services, and on the other hand, to maintain the service fees affordable to the researchers.

New methods for genome modification, e.g., the CRISPR/Cas system, will speed up the generation of mutant mice. Collaboration of the units is needed in educating personnel and researchers in these new

User statistics

Research groups / other customers in 2014					
TG/GM unit	Local	National	International	Non-academic	Total
Helsinki	29	3	0	0	32
BCO Oulu	20	7	16	0	43
TCDM Turku	14	6	6	3	29
total GM units	63	16	22	3	104
Phenotyping					
FCLAP Helsinki	13	2	2	1	18
NC Helsinki	8	2	0	0	10
NC UEF	5	1	0	0	6
TCDM Histology	17	5	0	1	23
- Imaging	14	7	9	6	36
- Xenocraft stud.	3	0	0	8	11
- Other	50	11	8	4	73
BCO Histology	19	0	0	0	19
- Imaging (IVIS, OPT, Echo)	12	1	2	0	15
Total Phenotyping	141	29	21	20	211
TOTAL	204	45	43	23	315
GM Services			Univ. Helsinki	Univ. Turku	Univ. Oulu
Pronuclear injection mouse+rat			3+5	2	2
ES cell targeting			2	9	8
Consortia ES cell culture			8	0	36
Cryopreservation of 8-cell embryos			12	4	0
Mouse line rederivation			33	4	6
Blastocyst injection			0	6	31
Recovery of cryopres. embryos			10	10	2
Sperm cryopreservation			3	0	25
Genotyping			0	18	48
Husbandry of mouse colonies			3	25	5
Morula aggregation			8	0	0
DNA construct generation			0	3	0
Cryopres. of IVF-derived 2-cell embryos			0	0	25
Other			2	7	3
Total			89	88	191

Phenotyping services provided	
FCLAP Helsinki	Necropsies (51); Immunohistology specimens (427); Tissue processing (1 197)
NC Helsinki	Behavioral phenotyping (in total 13)
NC Kuopio UEF	Brain microinjections; Video-EEG recording; Brain immunohistology; Behavioral testing (in total 14)
TCDM Turku	Biomarker analyses; Histology and immunohistochemistry; whole animal imaging; Xenografts in mice; Organ-specific expertise for Adipose tissue, Bone, Cardiovascular, Intestine, Reproduction, Thyroid (www.tcdm.fi)
BCO Oulu	Measurements of cardiovascular functions (1 067 animals); In vivo imaging; OPT; Tissue processing for histology (4 389)

techniques and the challenges involved in them.

It is foreseen that rare diseases, where patient material is limited, will be increasingly "modelled" in mice for therapeutic applications. Studies on sophisticated models for diseases, such as cancer and diabetes, will be further advanced.

Technology transfer

TCDM pre-clinical cancer models for prostate cancer (VCaP xenografts in intact, castrated and castrated and arenalecomized immunodeficient mice) are developed and used in collaboration with pharmaceutical industry. Also several proof-of-concept models are tailor-made for companies. The models have been successfully used in screening novel drugs developed by the industry. Altogether, 216 projects were carried out. Furthermore, GM mouse models generated in the Oulu unit have been used in preclinical screenings by Fibrogen Inc. (California, USA)

Major publications supported by platform services

Aikio M, Elamaa H, Vicente D, Izzi V, Kaur I, Seppinen L, Speedy HE, Kaminska D, Kuusisto S, Sormunen R, Heljasvaara R, Jones EL, Muilu M, Jauhiainen M, Pihlajamäki J, Savolainen MJ, Shoulders CC, Pihlajaniemi T..Specific collagen XVIII isoforms promote adipose tissue accrual via mechanisms determining adipocyte number and affect fat deposition. Proc Natl Acad Sci USA 111: E3043–E3052, 2014.

Björkgren I, Gylling H, Turunen H, Huhtaniemi I, Strauss L, Poutanen M, Sipilä P. Imbalanced lipid homeostasis in the conditional Dicer1 knockout mouse epididymis causes instability of the sperm membrane. FASEB J 29: 433–442, 2015.

Hurskainen T, Kokkonen N, Sormunen R, Jackow J, Löffek S, Soininen R, Franzke CW, Bruckner-Tuderman L, Tasanen K. Deletion of the major bullous pemphigoid epitope region of collagen XVII induces blistering, autoimmunization, and itching in mice. J Invest Dermatol 135: 1303–1310, 2015.

Koivisto H, Grimm MO, Rothhaar TL, Berkecz R, Lütjohann D, Giniatullina R, Takalo M, Miettinen PO, Lahtinen H-M, Giniatullin R, Penke B, Janáky T, Broersen LM, Hartmann T, Tanila H. Special lipid-based diets alleviate cognitive deficits in the APPswe/PS1dE9 transgenic mouse model of Alzheimer's disease independent of brain amyloid deposition. J Nutr Biochem 25: 157–69, 2014.

Kopra J, Vilenius C, Grealish S, Harma MA, Varendi K, Lindholm J, Castren E, Voikar V, Bjorklund A, Piepponen TP, Saarma M, Andressoo JO. GDNF is not required for catecholaminergic neuron survival *in vivo*. Nat Neurosci 18: 319–322, 2015.

Li Z, Ma M, Kulesskaya N, Voikar V, Tian L. Microglia are polarized to M1 type in high-anxiety inbred mice in response to lipopolysaccharide challenge. Brain Behav Immun 38: 237–248, 2014.

Lindahl M, Danilova T, Palm E, Lindholm P, Võikar V, Hakonen E, Ustinov J, Andressoo JO, Harvey B, Otonkoski T, Rossi J, Saarma M. MANF is indispensable for the proliferation and survival of pancreatic β cells. Cell Rep 7: 366–375, 2014.

Movérare-Skrtic S, Henning P, Liu X, Nagano K, Saito H, Börjesson AE, Sjögren K, Windahl SH, Farman H, Kindlund B, Engdahl C, Koskela A, Zhang FP, Eriksson EE, Zaman F, Hammarstedt A, Isaksson H, Bally M, Kassem A, Lindholm C, Sandberg O, Aspenberg P, Sävendahl L, Feng JQ, Tuckermann J, Tuukkanen J, Poutanen M, Baron R, Lerner UH, Gori F, Ohlsson C. Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. Nat Med 20: 1279–1788, 2014.

Rahtu-Korpela L, Karsikas S, Hörkkö S, Blanco Sequeiros R, Lammentausta E, Mäkelä KA, Herzig KH, Walkinshaw G, Kivirikko KI, Myllyharju J, Serpi R, Koivunen P. HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. Diabetes 63: 3324–3333, 2014.

Rolova T, Pulli L, Magga J, Dhungana H, Kanninen K, Wojciehowski S, Salminen A, Tanila H, Malm T, Koistinaho J. Complex regulation of acute and chronic neuroinflammatory responses in mouse models deficient for nuclear factor kappa B p50 subunit. Neurobiol Dis 64: 16–29, 2014.

Non-mammalian Models Technology Platform

Chair of the consortium: Mika Rämet, BMT
Members: Pertti Panula, Neuroscience Center Zebrafish Unit Helsinki, Mataleena Parikka, BMT, Tampere Zebrafish Core Facility; Susanna Valanne, BMT, Tampere *Drosophila* Core facility; Tapio Heino, Ville Hietakangas, Osamu Shimmi, Helsinki *Drosophila* facility

www.biocenter.fi

Achievements in development of technology services

With regard to restructuring research related to model organisms, Tampere (BMT) has particularly invested to develop research infrastructure for non-mammalian models (namely, *Drosophila melanogaster* and zebrafish *Danio rerio*) rather than mammalian models during the last five years. Nation-wide, adequate infrastructure and know-how in Helsinki related to both *Drosophila* and zebrafish is important to provide service locally and to enhance quality of Finnish biomedical research at large. Currently, the most important investments in equipment related to routine maintenance of both *Drosophila* and zebrafish have been carried out both in Helsinki and in Tampere. The main challenge related to this consortium is to maintain skilled professionals in the current economical situation of our universities.

Zebrafish

In 2014, both Helsinki and Tampere zebrafish core facilities were operational without major obstacles. This year, the Tampere facility had a part-time coordinator and three technicians. Researchers from several teams from University of Tampere and University of Jyväskylä used the facility. Besides on-going process of creating mutant zebrafish families, the Tampere facility maintains zebrafish lines for scientists, carry out microinjections for production of transgenic zebrafish, and provide assistance in initial phenotypic characterization. Furthermore, the network has organized three times a weeklong in-hands training course for the use of non-mammalian model organisms for research in life sciences. The major recent obstacle for providing

services to the community was the mandatory renovation of the laboratory (from October, 2013 until January, 2014), due to moisture damage and subsequent problems in the air quality. Renovation was completed in January 2014, and thereafter, the zebrafish facility has operated normally.

Helsinki zebrafish facility was fully operational in 2014. Most users have been from University of Helsinki or nearby institutions. The unit maintains fish lines, carries out microinjections and other procedures, including targeted zebrafish mutagenesis using the CRISP-R/Cas9 technology.

Drosophila

In Tampere, a new *Drosophila* Core Facility (http://cofa.uta.fi/fruitfly.html) was built in 2012. This investment was done by the host Institute (Institute of Biomedical Technology, University of Tampere), and the BF-funded Core Facility coordinator, Dr. Susanna Valanne, supervised it. The laboratory includes 12 working stations with stereomicroscopes and carbon dioxide points for anesthetizing flies. Several research teams on the Tampere campus heavily use this unit.

Participation in international, Nordic and European infrastructures

Pertti Panula from University of Helsinki has been a member of the Management Committee of EuFishBioMed (COST Action BM0804), which ended in 2013. He was also a member of the management group of the Nordforsk network BeFiNe 2010–2013. This network included active groups from all four Nordic countries and organized 4 meetings (one in each country) during the grant period, and a number of visits of young scientists between the labs. Pertti Panula also participated in all the Strategic Conferences of Zebrafish Investigators so far.

Future perspectives

On the basis of the support from BF, the network has successfully developed research infrastructure (and know-how) related to use of non-mammalian model organisms in Finland. The most important material investments have been carried out in both Tampere and Helsinki, allowing high-quality services to be provided to the scientific community. Currently, there are many researchers and teams that are using the facilities. The main future challenge is to maintain the skilled personnel to run these operations as the funding situations in general related biomedical research is of concern.

Major publications supported by platform services

Dash SN, Lehtonen E, Wasik AA, Schepis A, Paavola J, Panula P, Nelson WJ, Lehtonen S. Sept7b is essential for pronephric function and development of left-right asymmetry in zebrafish embryogenesis. J Cell Sci 2014 127: 1476–1486, 2014.

Hammarén MM, Oksanen K, Nisula HM, Luukinen B, Pesu M, Rämet M, Parikka M. Adequate Th2 response associates with restricted bacterial growth in latent mycobacterial infection of zebrafish. PLoS Pathog 10: e1004190, 2014.

Hasygar K, Hietakangas V. p53- and ERK7-dependent ribosome surveillance response regulates Drosophila insulin-like peptide secretion. PLoS Genet 10: e1004764, 2014.

Kemppainen KK, Rinne J, Sriram A, Lakanmaa M, Zeb A, Tuomela T, Popplestone A, Singh S, Sanz A, Rustin P, Jacobs HT. Expression of alternative oxidase in *Drosophila* ameliorates diverse phenotypes due to cytochrome oxidase deficiency. Hum Mol Genet 23: 2078–2093, 2014.

Kuuluvainen E, Hakala H, Havula E, Sahal-Estimé, M, Rämet M, Hietakangas V, Mäkelä TP. The cyclin-dependent kinase 8 module expression profiling reveals requirement of Mediator subunits 12 and 13 for transcription of serpent-dependent innate immunity genes in Drosophila. J Biol Chem 289: 16252–16261, 2014.

Saralahti A, Piippo H, Parikka M, Henriques-Normark B, Rämet M, Rounioja S. Adult zebrafish model for pneumococcal pathogenesis. Dev Comp Immunol 42: 345–353, 2014.

Schmid MR, Anderl I, Vesala L, Vanha-aho LM, Deng XJ, Rämet M, Hultmark D. Control of *Drosophila* blood cell activation via Toll signaling in the fat body. PLoS 9: e102568, 2014.

Semenova SA, Chen YC, Zhao X, Rauvala H, Panula P. The tyrosine hydroxylase 2 (TH2) system in zebrafish brain and stress activation of hypothalamic cells. Histochem Cell Biol 142: 619–633, 2014.

Vartiainen S, Chen S, George J, Tuomela T, Luoto KR, O'Dell KM, Jacobs HT. Phenotypic rescue of a *Drosophila* model of mitochondrial ANT1 disease. Dis Model Mech 7: 635–648, 2014.

Zhao X, Kuja-Panula J, Sundvik M, Chen YC, Aho V, Peltola MA, Porkka-Heiskanen T, Panula P, Rauvala H. Amigo adhesion protein regulates development of neural circuits in zebrafish brain. J Biol Chem 289: 19958–19975, 2014.

User statistics

	Groups			Animals Larvae / adult	
	Total	Local	Domestic	Total	Domestic
Zebrafish					
Tampere	6	5	1	14 800 / 8 994	0 / 1 450
Helsinki	12	11	1	45 315 / 739	500 / 0
Drosophila	7	7		~ 500 000	
				~ 1 000 lines	

Tissue Engineered Disease Models (TEDM)

Chair of the consortium: Juha Klefström, BCH, Institute of Biomedicine and Genome Scale Biology Program,

Members: Emmy Verschuren (FIMM) (co-chair); Jukka Westermarck (BioCity) (co-chair); Pipsa Saharinen (BCH); Sergey Kuznetsov (FIMM); Johanna Ivaska (BioCity); Petri Mäkinen (BCK)

www.biocenter.fi

Tissue Engineered Disease Models (TEDM) develops complex *ex vivo* and *in vivo* tissue models compatible with genetic and chemical biology approaches, to benefit biomedical research. The disease focus is in breast and lung cancers and cardiovascular disease. Technology development will focus on systemic, intraductal, and orthotopic somatic cell-based transplantation methods using donor cells from GEMMs (syngrafts) or human diseases, direct virus-mediated engineering of cells in animal tissues and *ex vivo* organoid surrogates. TEDM will restructure this fast developing technology area by bringing leading Finnish groups together to create significant synergy. The focus of TEDM does not overlap with technologies in other BF networks, but complements the existing virus, drug discovery and animal models networks. TEDM will interact with these infrastructures, not by establishing its own core facilities, but by supporting existing core facilities by creating new rechargeable services. This model was already successful in the period 2010–2012, and given the increasing number of Finnish scientists working on complex biological models, TEDM anticipates a solid user base for new TEDM services.

Achievements in development of technology services

Breast, early tumorigenesis. The aim of the breast platform is to develop translationally relevant animal models for the cancer research community in Finland. The new method will be validated during years 2014–2015 to a level that allows for the model to be offered as a core facility-type service by Turku Centre for Disease models starting in 2016. The intraductal injection is a method aiming for to model human ductal carcinoma *in situ* (DCIS) breast cancer. The method mimics DCIS in its normal environment in the mammary gland. This project is fulfills an unmet need. Such a technique is not mastered in many places in the world and is currently totally lacking in Finland even though breast cancer research is a major focus on many campuses within the BF network.

The original method (Behbod et al, 2009) has been improved by leaving out the Y-incision. The reason for this is that drifting of the MCF10.DCIS cell suspension can be seen through the skin by stereomicroscopy. The primary tumors are expected to appear at 4–5 weeks from injection to the inguinal gland, and metastases to emerge at week eight. To master the technique, Johanna Jukkala was trained by Catalina Lodillinsky (Institut Curie, Paris).

Breast, late tumorigenesis. TEDM develops *in vivo* and *ex vivo* models specifically for the purpose of invasion and metastatic centered studies. Methods have been established to manipulate genetically mouse mammary progenitor cells by lentiviral shRNA followed by transplantation of the cells into the cleared fat pad of virgin mice. The transplanted mammary progenitor cells differentiate to epithelial lineages and progenitor populations, generating a functional mammary gland with normal ductal and lobulo-alveolar architecture and enabling studies of tumorigenesis in a transgenic mammary gland surrounded by otherwise normal tissue and its microenvironment.

In addition to the invasion models, TEDM has established a tumor cell transendothelial invasion model that combines tumor cells with cells of the tumor stroma allowing genetic engineering of the cell types and dynamic visualization the cellular interactions. In the model, tumor cell transmigration across an endothelial cell monolayer either with or without a 3-D basement membrane matrix is being monitored using a fully integrated continuous live cell imaging and analysis platform.

Lung, early tumorigenesis. Intranasal delivery of viral particles results in efficient transduction of lung epithelium progenitor cells. This method can generate mouse models for lung disease and development, in their native microenvironment, through Cre-lox activation of germline alleles. A further aim is the cultivation of primary lung tissue usable for *ex vivo* studies, such as bioassays or niche-dependent biology studies.

A protocol for *in vivo* adenoviral Cre (AdCre) delivery using intranasal instillation has been established. High titer viruses produced by the Iowa Gene Transfer Vector Core were found to be most efficient, and are currently routinely used. Further development of technically more challenging intratracheal intubation methodology has been initiated, to improve the accuracy of virus delivery by direct delivery of virus into the trachea. Conditions for short-term culture of organotypic non-small cell lung cancer tissue cultivated as tissue slice cultures were optimized. Detailed expression analyses indicated that proliferation, histotype and DNA damage biomarkers are not maintained after 24 h of expression, suggesting that tissue is short-lived.

Heart, cardiovascular disease. A new platform on

infarcted myocardium has been developed. The model for myocardial infarction, which includes surgical occlusion of the left main descending coronary artery without any major transthoracic surgery, has been set up. Additionally, a lentiviral vector (LV) mediated modification of the infarcted mouse heart was studied and developed. This new model should allow studying of molecular mechanism and therapy of infarcted heart by overexpressing or silencing genes by LV-mediated gene transfer. LVs can transduce infracted heart. However, optimization of the method is still needed to enhance the transduction efficacy, which would enable more extensive and use of the model.

User statistics

Since most of the model systems have been at the developmental stage or recently introduced, they were not offered in 2014 to the BF users at large.

Participation in international, Nordic and European infrastructures

TEDM viral transduction and organotypic lung tissue slicing methods are an integral part of a European Commission-funded technology transfer via the IMI-PREDECT public-private consortium, which includes nine European pharmaceutical companies and twelve academic and SME partner institutions. PREDECT technology assets will be communicated to Finnish research communities interested to pursue somatic *in vivo* and *ex vivo* model technologies for drug discovery purposes. The breast cancer models and methods are also an integral part of a European Commission-funded technology transfer via the IMI-PREDECT.

Future perspectives

The human DCIS model in mice is anticipated to become available for all BF users by 2016. The lung cancer models are expected to be available, via existing core facilities in the local biocenters, by the end of 2015 (virus delivery) or 2016 (organotypic cultures). Models for late tumorigenesis of the breast are foreseen to be available to the Finnish bioscience community in 2015 though existing core facilities in Helsinki. In 2015–2016, the focus will be gradually shifted to genome editing and modulation services, since there is huge demand for these techniques to be implemented in complex biological models.

Major publications supported by Platform services

Lahtela J, Pradhan B, Närhi K, Hemmes A, Särkioja M, Kovanen PE, Brown A, Verschuren EW. The putative tumor suppressor gene EphA3 fails to demonstrate a crucial role in murine lung tumorigenesis or morphogenesis. Dis Model Mech 8:393–401, 2015.

Hakanpaa L, Sipila T, Leppanen VM, Gautam P, Nurmi H, Jacquemet G, Eklund L, Ivaska J, Alitalo K, Saharinen P. Endothelial destabilization by angiopoietin-2 via integrin β1 activation. Nat Commun 6: 5962, 2015.

Turunen MP, Husso T, Musthafa H, Laidinen S, Dragneva G, Laham-Karam N, Honkanen S, Paakinaho A, Laakkonen JP, Gao E, Vihinen-Ranta M, Liimatainen T, Ylä-Herttuala S. Epigenetic upregulation of endogenous VEGF-A reduces myocardial infarct size in mice. PLoS One 9: e89979, 2014.

PROTEOMICS AND METABOLOMICS

Proteomics and Metabolomics Infrastructure Network

Coordinator of the network: Garry Corthals, BioCity Turku

Members: Marc Bauman, BCH; Markku Varjosalo, BI; Antti Poso, BCK; Kalervo Hiltunen, BCO; Vesa Hytönen, BMT; Vidya Velagapudi, FIMM; Janne Ihalainen, University of Jyväskylä

www.ProtMet.net and www.biocenter.fi

The Proteomics and Metabolomics network comprises two technology platforms, one in proteomics and protein characterization, and the other one in metabolomics. Together these platforms represent a large group of skilled researchers offering a diverse range of services, methodologies and applications covering all areas of life science. The network has embarked on an ambitious plan to link independently operating national service laboratories, combining experience and resources to offer a coordinated national technology platforms.

The broad field of proteomics is an essential technology in biosciences that underpins strategically important areas in academia and biotechnology, enabling characterization and temporal and spatial quantitation of proteins at various locations in practically all biological systems. It also affords measurement and discovery of post-translational protein modifications, protein-protein interactions and protein properties, which are amongst the most sought after applications.

Successful proteomics requires both expensive and constantly evolving infrastructures, and a critical mass of expertly trained personnel with skills covering the areas of biochemistry, biomedicine, chemistry and bioinformatics. BF protein and proteomics core facilities provide access to cutting-edge services and knowledge in mass spectrometry based proteomics and protein characterization. The protein-proteome platform is expected to enable the scientific community to take a wide range of societal challenges of a biological and medical nature.

Metabolomics is a rapidly growing field of small molecule analytics, which has applications in different sectors of bio-, health-, and medical sciences. Wide range of metabolites in biofluids and tissues can be currently measured by using metabolomics platforms based on LC-MS, GC-MS or NMR. However, analysis of many important compounds is still challenging, which means that there is a need for major analytical method development in the field of metabolomics in the coming years. The metabolomics analytics within BF network have been welcomed with high interest in national and international scientific forum, which is evidenced by rapidly increasing customer base in each of the facilities.

Protein-Proteome Technology Platform

Chair of the consortium: Garry Corthals, BioCity, Turku Proteomics Facility

Members: Marc Baumann, BCH, Meilahti Clinical Proteomics Core Facility; Markku Varjosalo, BI, Proteomics Unit; Kalervo Hiltunen, BCO, Proteomics and Protein Analysis Core Facility; Vesa Hytönen, BMT, Protein Technologies Facility

www.ProtMet.net and www.biocenter.fi/

Achievements in development of technology services

Overall, Protein Characterization and Proteomics Network (PPN) had a successful year. The number of services provided increased despite reduced funding, and one of the facilities (Oulu) being reorganization. In addition to providing existing services, many of the units were capable of setting up and developing new services and participated in research collaborations. The network has also been active in arranging courses and congresses and participating in teaching.

A new partner from University of Jyväskylä (JYU) joined the network in 2014. Professor Janne Ihalainen's group offers services in fluorescence spectroscopic and vibrational spectroscopic techniques for characterization of proteins and other biomolecules. JYU plans to purchase a time-gated Raman spectrometer, which allows Raman measurements from fluorescent samples and widens thus the possible usage of Raman spectroscopy for biomolecules.

The Turku Proteomics Facility has set up a service for large-scale quantitative phoshoproteomics analysis. At the moment, the facility is investing in analysis of large numbers of small sized clinical tissue samples. Future plans of the facility involve provision of data independent acquisition based quantification. A bottleneck has been in providing high-throughput bioinformatics services due to a lack of dedicated personnel.

The Tampere Protein Technologies continued to offer services both in protein characterization as well as in design and execution of protein production. This combination makes it possible for researchers with limited amount of expertise in molecular biology to approach questions related to protein function and interactions. In 2014, the Tampere facility obtained several novel customers, and the quality of service was

upgraded. Some of the customers are from biotech industry.

The Biocenter Oulu facility focuses on biophysical analyses of proteins and proteomics based on two-dimensional gel electrophoresis (2-DE). Different techniques of mass spectrometry are used as major tools in both areas. In 2014, the Oulu facility and the surrounding research environment moved to the hospital campus and were integrated into the new Faculty of Biochemistry and Molecular Medicine. Owing to this reorganization, operation of the core facility was compromised for a considerable part of the year.

The Institute of Biotechnology proteomics unit provides new cutting-edge analysis services, including characterization of post-translational modifications as well as label and label-free quantitative and systems-wide analyses. The unit has attracted a plethora of new customers, both from academy and industry. In the future, the unit will develop further systems-wide quantitative analyses and plans to start single cell proteomics analyses, which naturally requires the appropriate instrumentation.

The Meilahti Clinical Proteomics Core facility continued to serve with comprehensive clinical proteomic analyses, starting from planning the sample collection at the hospital, sample storage and analysis, and ending with a compact systems medicine and systems proteomic summary of the results. Mass spectrometry imaging, an emerging technology in proteomics, was utilized in a large-scale EU-funded project and several smaller projects. Due budgetary limitations, the unit could not continue glycoproteomic analyses as a paid service.

Training activities

The Oulu facility provided two courses in protein characterization (Biochemical methods II). The Tampere facility has participated in teaching of MSc students, and a course focusing on protein production and mutagenesis was carried out in 2014. The Turku facility arranged a one-week course on proteomics and a couple of two-day workshops, taught on several university courses and organized an international the Nordic Proteomics conference in Turku. Meilahti Clinical Proteomics Core Facility gave three courses on proteomics at the undergraduate and graduate student level.

User statistics

Despite funding reductions, PPN network was capable to serve 174 research groups, which is only slightly less than that of 2013 (185). The volume of the services in terms of service hours was extensive (20639 h in total). Services covered a wide range of expertise, from various types of mass spectrometric analysis to detailed protein characterization services to gel separations and protein production. Overall, PPN network has strong role in protein-focused research in Finland.

Participation in international, Nordic and European infrastructures

PPN consortium members participate in the coordination of ISBE (Infrastructure for Systems Biology – Europe) and INSTRUCT (Integrated Structural Biology Infrastructure for Europe) projects which are on (ESFRI) Roadmap.

In addition, the network was involved in various national Centers of Excellence, FiDiPro projects, as well as national and international funding activities (e.g., ERC, FP7 and COST actions). For example, The Meilahti Clinical Proteomics Core facility has been a partner in two continuing COST actions for using IMS technology in cancer and proteomics for to analyze chemically modified proteins.

User statistics

	BCH Helsinki	BI Helsinki	BCO Oulu	BMT Tampere	CBT Turku	Total
Total number of research groups*	28	46	44	22	34	174
Total number of non-academic groups/units	4	3	4	3	3	17
Local research groups*	15	31	30	6	24	106
Domestic research groups*	7	11	5	18	8	49
International research groups*	2	4	5	4	2	17
Volume of services (instrument time in h)	3 550 h[a, b,c]	3 930 h[a,b]	5 442 h[a, b, c]	2 797 h[a, b, c]	4 920 h	20 639 h
Specifications						
* Research groups (or other customers) who have used the services. ** For proteomics, only MS instrument times recorded; Sample preparation and IT-services, peptide- and protein purification not included. *** For protein production and characterization, experiment design, service coordination and data analysis are not recorded except for non-academic groups.	[a] Mass spectrometry** MALDI-Tof/Tof 330 h Q-Tof 1610 h IonTrap 180 h [b] MS glycoproteoics** Q-Tof 1430 h [c] 2-DE gel-based proteomics 20 gels 50 h	[a] Mass spectrometry** LTQ-Orbitrap Elite 2480 h Q-exactive 1070 h MALDI-Tof/Tof 50 h [b] Edman sequencing 335 h	[a] 256 2D gels: [b] Mass spectrometry (Ultraflex, Synapt): 1500 h [c] Protein characterization ITC 500 h CD: 560 h Biacore: 976 h	[a] Protein production*** Expression 170 h Preparation 163 h [b] Protein characterization *** BLI 187 h DSC 1404 h ITC 72 h Spectrofluorometer 201 h	Mass spectrometry** LTQ-Orbitrap Velos 2094 h Q-exactive 2176h QSTAR Elite 44 h TSQ Vantage 606 h	

Major publications supported by platform services

Turunen M, Spaeth JM, Keskitalo S, Park MJ, Kivioja T, Clark AD, Mäkinen N, Gao F, Palin K, Nurkkala H, Vähärautio A, Aavikko M, Kämpjärvi K, Vahteristo P, Kim CA, Aaltonen LA, Varjosalo M, Taipale J, Boyer TG. Uterine leiomyoma-linked MED12 mutations disrupt mediator-associated CDK activity. Cell Rep 7: 654–660, 2014.

Hyvärinen S, Uchida K, Varjosalo M, Jokela R, Jokiranta TS. Recognition of malondialdehyde-modified proteins by the C terminus of complement factor H is mediated via the polyanion binding site and impaired by mutations found in atypical hemolytic uremic syndrome. J Biol Chem 289: 4295–4306, 2014.

Nassa G, Tarallo R, Giurato G, De Filippo MR, Ravo M, Rizzo F, Stellato C, Ambrosino C, Baumann M, Lietzèn N, Nyman TA, Weisz A. Post-transcriptional regulation of human breast cancer cell proteome by unliganded estrogen receptor β via microRNAs. Mol Cell Proteomics 13: 1076–1090, 2014.

Vehmas AP, Muth-Pawlak D, Huhtinen K, Saloniemi-Heinonen T, Jaakkola K, Laajala TD, Kaprio H, Suvitie PA, Aittokallio T, Siitari H, Perheentupa A, Poutanen M, Corthals GL. Ovarian endometriosis signatures established through discovery and directed mass spectrometry analysis. J Proteome Res 13: 4983–4994, 2014.

Pinon P, Pärssinen J, Vazquez P, Bachmann M, Rahikainen R, Jacquier MC, Azizi L, Määttä JA, Bastmeyer M, Hytönen VP, Wehrle-Haller B. J. Talin-bound NPLY motif recruits integrin-signaling adapters to regulate cell spreading and mechanosensing. J Cell Biol 205: 265–281, 2014.

Vaarala O, Vuorela A, Partinen M, Baumann M, Freitag TL, Meri S, Saavalainen P, Jauhiainen M, Soliymani R, Kirjavainen T, Olsen P, Saarenpää-Heikkilä O, Rouvinen J, Roivainen M, Nohynek H, Jokinen J, Julkunen I, Kilpi T. Antigenic differences between AS03 adjuvanted influenza A (H1N1) pandemic vaccines: implications for pandemrix-associated narcolepsy risk. PLoS One 9: e114361, 2014.

Venkatesan R, Sah-Teli SK, Awoniyi LO, Jiang G, Prus P, Kastaniotis AJ, Hiltunen JK, Wierenga RK, Chen Z. Insights into mitochondrial fatty acid synthesis from the structure of heterotetrameric 3-ketoacyl-ACP reductase/3R-hydroxyacyl-CoA dehydrogenase. Nat Commun 5: 4805, 2014.

Gama JB, Ohlmeier S, Martins TG, Fraga AG, Sampaio-Marques B, Carvalho MA, Proença F, Silva MT, Pedrosa J, Ludovico P. Proteomic analysis of the action of the Mycobacterium ulcerans toxin mycolactone: targeting host cells cytoskeleton and collagen. PLoS Negl Trop Dis 8: e3066, 2014.

Szambowska A, Tessmer I, Kursula P, Usskilat C, Prus P, Pospiech H, Grosse F. DNA binding properties of human Cdc45 suggest a function as molecular wedge for DNA unwinding. Nucl Acids Res 42: 2308–2319, 2014.

Suni V, Imanishi SY, Maiolica A, Aebersold R, Corthals GL. Confident site localization using a simulated phosphopeptide spectral library. J Proteome Res 14: 2348–2359, 2015.

Metabolomics Technology Platform

Chair of the consortium: Seppo Auriola, BCK & Department of Pharmaceutical Chemistry
Members: Tapio Palva, BCH, Metabolomics Unit; Vidya Velagapudi, FIMM, Metabolomics Laboratory

www.biocenter.fi

Achievements in development of technology services

The BF metabolomics units have continued to service in their focus areas: BCK on large-scale non-targeted metabolite profiling, FIMM on targeted quantitative metabolomics, and BCH on plant metabolite analyses. All units have also continued developing their analyses to increase the repertoire of chemicals detected and the type of sample materials used.

One of the focus areas in the BCK unit is metabolomic applications in food and nutrition. For example, a novel biomarker for fish intake was discovered, followed by immediate development of quantitative analysis for this biomarker – now included in the quantitative analytical repertoire. A key development area has been the enhancement of metabolite identification capabilities, for which purpose standard compound collection of 1000 chemicals has been acquired. Additionally, laboratory has set up novel GC-MS system for analysis of Alzheimer's disease biomarkers, as well as developed phospholipid quantitation analyses in serum and CSF samples. Installation of new LC QQQ-MS (funded by University of Eastern Finland, UEF) for targeted analysis of drugs and low level endogenic metabolites allows simultaneous analysis of up to 100 metabolites below nanomolar concentration.

The Viikki unit focuses on plant metabolomics and serves the whole plant research community in Finland. In addition, microbial metabolomics and drug discovery analyses are provided on the Viikki campus. This unit has streamlined its analytical services, including an increase in technical personnel, focused on plant secondary metabolites mainly analyzed with LC-QTOF/MS. In addition, there was increasing demand in metabolic services for additional compounds including volatiles, which required substantial new methods development.

The FIMM unit offers high-throughput targeted quantitative metabolomics analyses and has attracted many research groups locally and internationally. Apart from providing routine services, the unit also does the requisite chemometric and bioinformatics data analyses and participates in research collaborations. The FIMM unit has upgraded several methods, for example, optimized protocols for various types of samples, including different animal and human tissues, extracellular vesicles, bone marrow cells, dried blood spots, *C.elegans*, *drosophila* and yeast, and developed new targeted methods to quantify folate cycle intermediates.

The BF metabolomics units have continued teaching various aspects of metabolite analytics in their units. In UEF, Professor Auriola is responsible for education of mass spectrometry and Dr. Hanhineva is lecturing on various courses within university and other national and international schools on topic "Application of non-targeted metabolite profiling in biosciences". The FIMM unit continued in teaching "Biomedical Applications of Metabolomics" to medical, Ph.D and masters students in different courses offered at University of Helsinki and at other national universities. The BCH unit is responsible for metabolomics and proteomics-part at the Masters level Genomes courses "Genomes" and/or "From Genomes to Gene Functions".

Bottlenecks. All metabolomics units operate with minimal instrumentation. The limited number of instruments causes long waiting periods for the offered services at all units, and whenever there has been downtime due to instrument malfunction, it has caused delays in analyses. Another major bottleneck is within personnel resources, which causes delays in data analyses and bioinformatics. This also hinders development and testing of statistical approaches to validate the best possible practices for data analyses.

User statistics

Metabolomics consortium user base and income in 2014.

	Units			
Users	BCK	FIMM	BCH	Total
Campus	6	7	18	31
National	2	4	2	8
International	2	5	1	8
Total no. of samples	2 684	3 007	1 500	7 191
User fees (€)	146 171	231 284	31 000	408 455

The BCK metabolomics analyses were included in eight publications (2014-2015), in BCH three publications were published out of the performed metabolite analysis, and FIMM unit published 3 articles.

Participation in international, Nordic and European infrastructures

The BF metabolomics consortium has not yet participated in any European infrastructure networks. Instead, all centers have international

collaborative projects where the facility services have been used.

Future perspectives

The BCH unit faces an increased demand for and repertoire of untargeted and targeted quantitative analyses of plant primary and secondary metabolites, necessitating extensive methods development. This situation will be accentuated upon the establishment of the National Plant Phenotyping Infrastructure (NaPPI; part of the current national infrastructure roadmap) in Viikki by the end of 2015. The Viikki unit has currently only one LC-QTOF/MS, perfect for identifying novel, unknown metabolites, but not very suitable for quantifying low abundant metabolites, the main targets in plant phenotyping. In the BCK unit, the major future methodological developments relate to analyses of small sample materials, such as purified exosomes, stem cells, or dried blood drops. For this purpose, nano or micro flow chromatography is needed in combination with an extremely sensitive Q-TOF mass spectrometer. Moreover, to enable analyses of isobaric compounds, the ion mobility separation technique would be useful. The FIMM unit aims at setting up metabolic flux analyses, as customers have already expressed needs to quantify labeled metabolites. There is an urgent and unmet need for a global lipidomics facility for biomedical applications nationally, as such a platform service does not exist in Finland.

Major publications supported by platform services

Hanhineva K, Pedret A, Lankinen M, Schwab U, Kolehmainen M, Paananen J, De Mello V, Sola R, Lehtonen M, Poutanen K, Uusitupa M, Mykkänen H. Non-targeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish and bilberries – a randomized trial (Sysdimet). J Nutr 145: 7–17, 2015.

Bondia-Pons I., Savolainen O, Torronen R, Martinez JA, Poutanen K, Hanhineva K. Metabolic profiling of Goji berry extracts for discrimination of geographical origin by non-targeted liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Food Res Int 63: 132–138, 2014.

Pekkinen J, Rosa N, Savolainen O, Mykkänen H, Poutanen K, Micard V, Hanhineva K. Wheat aleurone processing changes the urinary metabolite profile of mice fed a high-fat diet. Nutr Metab 11: 1, 2014.

Piisilä M, Keceli MA, Brader G, Jakobson L, Jõesaar I, Sipari N, Kollist H, Palva ET, Kariola T. The F-box protein MAX2 contributes to resistance to bacterial phytopathogens in Arabidopsis thaliana. BMC Plant Biol 15: 53, 2015.

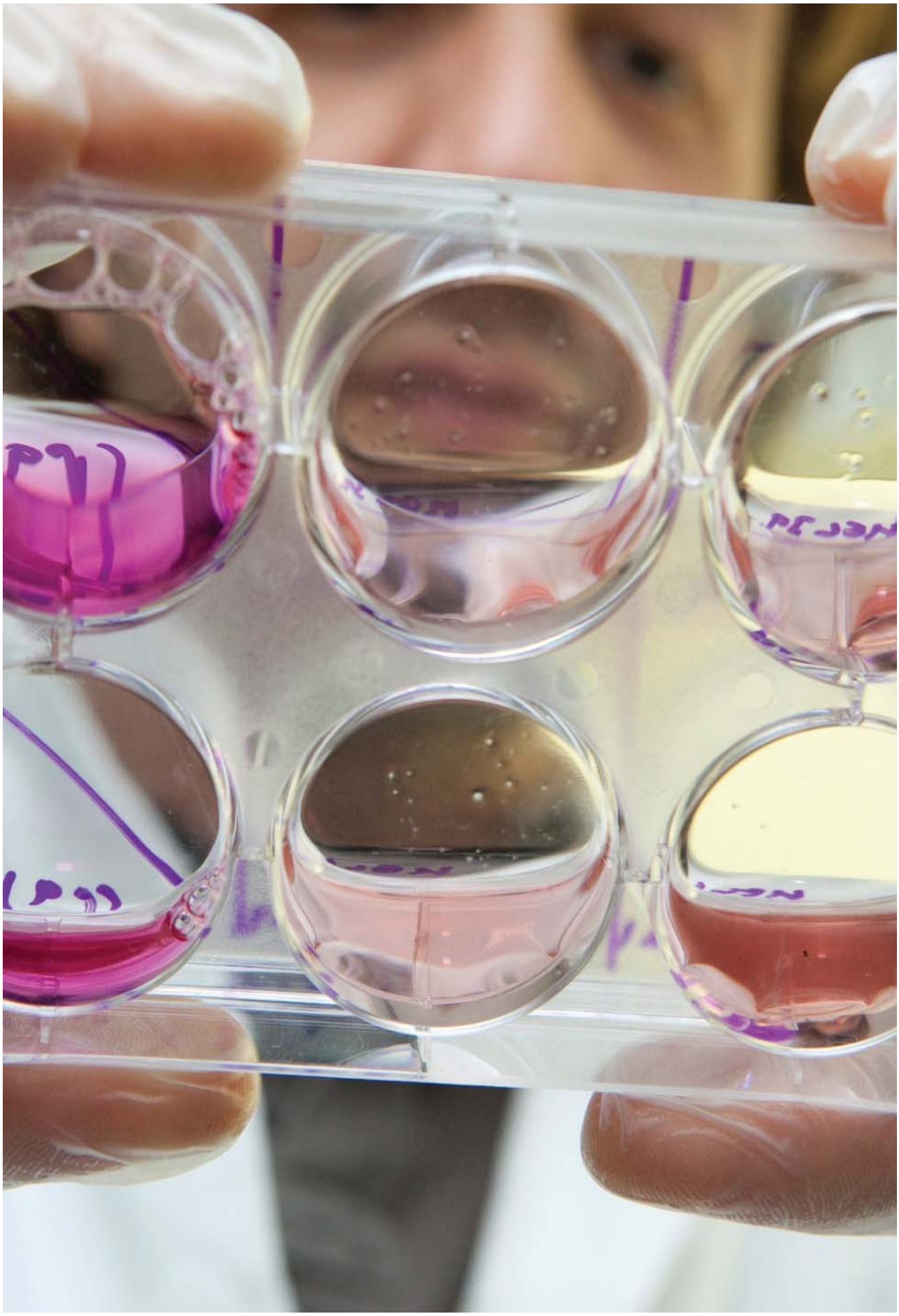
Siipola SM, Kotilainen T, Sipari N, Morales LO, Lindfors AV, Robson TM, Aphalo PJ. Epidermal UV-A absorbance and whole-leaf flavonoid composition in pea respond more to solar blue than solar UV radiation. Plant Cell Environ 38: 941–952, 2015.

Sipari N, Lindfors AV, Strid Å, Aphalo PJ. Are solar UV-B- and UV-A-dependent gene expression and metabolite accumulation in Arabidopsis mediated by the stress response regulator RADICAL CELL DEATH1? Plant Cell Environ 38: 878–891, 2015.

Schrade A, Kyrönlahti A, Akinrinade O, Pihlajoki M, Häkkinen M, Fischer S, Alastalo TP, Velagapudi V, Toppari J, Wilson DB, Heikinheimo M. GATA4 is a key regulator of steroidogenesis and glycolysis in mouse Leydig cells. Endocrinology 156: 1860–1872, 2015.

Roman-Garcia P, Quiros-Gonzalez I, Mottram L, Lieben L, Sharan K, Wangwiwatsin A, Tubio J, Lewis K, Wilkinson D, Santhanam B, Sarper N, Clare S, Vassiliou GS, Velagapudi VR, Dougan G, Yadav VK. Vitamin B12-dependent taurine synthesis regulates growth and bone mass. J Clin Invest 124: 2988–3002, 2014.

Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsström S, Pasila L, Velagapudi V, Carroll CJ, Auwerx J, Suomalainen A. Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol Med 6: 721–731, 2014.



STEM CELLS AND BIOMATERIALS

Stem Cells and Biomaterials Infrastructure Network

Coordinator of the network: Timo Otonkoski, BCH
Members: Katriina Aalto-Setälä, BMT; Ulla Pirvola, BI; Mikko Lammi, BCK; Seppo Vainio, BCO; Olli Lassila, BioCity Turku

www.biocenter.fi

Stem cell research is a rapidly developing area of biomedicine. Recent stem cell technologies have opened up several novel avenues for biomedical research, such as developing disease models, drug development, tissue regeneration and development of functional organoids.

The efforts of the BF network are directed to obtain knowledge and protocols to generate stem cells from different sources. The network aims also to develop adult stem cell-based tissue engineered biomaterial implants and organoids. A special emphasis is put into development of techniques to generate and use the so-called induced pluripotent cells (iPS) from committed permanently differentiated cells. The discovery that somatic cells can be reprogrammed into pluripotency via only a few developmental control genes has opened new horizons for stem cells in, for example, derivation of patient specific cellular disease models for basic and applied research. Propagation of pluripotent cells from patients permits for the first time detailed studies on the molecular biology of human disease mechanisms and the use of such cells for development of novel therapeutics. In the long term, the iPS cells should provide a unique way to develop technologies for obtaining immunologically tolerated cells for cell and tissue transplantation.

The main challenges of the network are: 1) how to channel and validate stem cells to specific cell lineages and functional cell types, 2) how to use these in tissue engineering and regeneration, and 3) how to use these as models for drug screening and organoid development.

Stem Cells and Biomaterials Technology Platform

Chair of the consortium: Timo Otonkoski, BCH, Biomedicum Stem Cell Center (BSCC)
Members: Katriina Aalto-Setälä, BMT; Marjo Yliperttula, Faculty of Pharmacy, University of Helsinki, Viikki Facility; Jari Koistinaho, BCK, Stem Cell Center

www.biocenter.fi

Achievements in development of technology services

Platform partners have continued the development of their stem cell services as described below. Coordination activities between the partners have been limited in 2014. However, plans have now been made to initiate regular activities of The Finnish Stem Cell Network (FSCN), which will pull together all research groups that are actively using stem cell technologies. The BF Stem Cell Platform will be a part of this network, involving those partners who are providing core facility services. The annual meetings of the FSCN will be held in conjunction with the annual meeting of the Finnish Developmental Biology Society.

Funding provided by University of Helsinki enabled the continuation of the patient iPS cell derivation service in Helsinki. In addition, the technologies were actively developed, following the rapid development of the field. Particular areas of emphasis are: (i) chemical reprogramming without genetic manipulation; (ii) genetic integrity during reprogramming; (iii) development of automated methods for the early isolation of reprogrammed cells, and (iv) genome editing of the stem cells. The CRISPR/Cas9 technology has been applied for efficient genome editing in human pluripotent stem cells, allowing the generation of knock-outs or knock-ins (e.g., for the generation of reporter cell lines), correction or generation of single nucleotide mutations, and transcriptional activation of desired genes. Collaboration with the Biomedicum Functional Genomics Unit is expected to enable provision of new core services in this area in 2015. This will initially involve generation of genetic knock-outs in human or mouse cells, including pluripotent stem cells. The Helsinki group has applied for funding from the Academy of Finland together with THL for establishing a Finnish hiPSC biobank to be used to study molecular pathogenesis of obesity.

Maintenance of patient iPS cell derivation service with current methods was possible through financial

support by University of Tampere to BMT. Additionally, hands-on training was provided for two international groups; one from Milan, Italy, and the other one from St. Petersburg, Russia. The main interest of customers has still been in control iPS cell lines. Moreover, an increasing interest of customers was in obtaining differentiated cells and in assays analyzing functionality of the cells (cardiomyocytes). iPS cells were provided to three national and international groups, and differentiated cardiomyocytes to one international and one national group.

Provision of high-quality differentiation of iPSC-derived lines and production of iPSC lines upon request was supported by University of Kuopio. The Kuopio unit was also able provide as service iPSC-derived cells differentiated into functional cardiomyocytes, astrocytes and dopaminergic neurons. BF provided partial support to purchase an automated patch clamp system that is now being set up for characterizing cardiomyocytes and brain cells derived from iPSC lines.

User statistics

The service activities of the platform remained largely at the same level as in the preceding two years. BCH produced 80 iPSC lines for six clients (five academic and one commercial) and provided training in pluripotent stem cell methods to 16 individuals at courses and six individuals through hands-on training. Two of the clients were from abroad. The iPSCs service activities decreased somewhat in BMT, but two additional research groups requested differentiated cardiomyocytes. Hands-on training for iPSC production was provided for two scientists from abroad. Both hands on training were for a month or bit longer. BMT obtained seven new patient samples, and the total number of iPS cells lines produced in 2014 was 32. Differentiated cardiomyocytes were provided to two groups; one in Finland and one in Switzerland. The Kuopio unit obtained 15 new patient samples in 2014 and produced 60 new iPSC lines for three clients (two academic and one commercial), characterized six differentiated iPSC lines for customers, and provided teaching on iPSC methods to 27 individual at courses.

Detailed user statistics are provided in the table below.

Stem cells and biomaterials technology platform user statistics

Stem cell services provided	iPSC lines		Teaching (courses)		Hands-on training		Teratoma		Cell-IQ imaging		Electro-physiology	
	2014	Total 2012-2014	2014	Total 2012-2014	2014	Total 2012-2014	2014	Total 2012-2014	2014	Total 2012-2014	2014	Total 2012-2014
BSCC, University of Helsinki												
Number of customers	6		16		6		2		6		0	0
Academic	5		16		6		2		6		0	0
Non-academic	1		0		0		0		0		0	0
Volume	80[a]	206	16[b]	56	6[b]	20	6	24	1557[d]	8724[d]	0	0
User fees (€)	42 671											
BMT, University of Tampere												
Number of customers	5		0		2		0		0		2	
Academic	4		0		2		0		0		1	
Non-academic	0		0		0		0		0		1	
Volume	32	160	0	15	2	2	0	0	0	0	6	6
User fees (€)	-											
University of Eastern Finland												
Number of customers	3		27		2		1		3	3	2	
Academic	2		18		2		1		0	3	1	
Non-academic	1		9		0		0		0	0	1	
Volume	60	75[a]	27	27[b]	2	5[b]	1	3[c]	120[d]	140[d]	6	6[b]
User fees (€)	25 500											

[a] cell lines, [b] customers, [c] tumors, [d] hours

Participation in international, Nordic and European infrastructures

BCH (Otonkoski) is a partner in one EU (FP7) funded consortium (BETACURE) with a role in the development of iPSC-based models for pancreatic beta-cell hyperfunction and imaging. The Kuopio unit (Koistinaho) is a partner in two EU-funded consortia (ERANET PROTEA; Neuroinflammation Marie Curie ITN program) proving iPSC-derived models for the projects. BMT (Aalto-Setälä) is a partner in two EU-funded consortia (Risky-CAD and Atero-Flux) providing iPSC-based models for atherosclerosis studies.

Future perspectives

While the generation of iPSC lines is becoming a routine technology, it still requires special expertise, experience and facilities. At the same time, know-how and technology development for differentiating cells to true models of human cells and tissues is becoming a bottleneck for taking the full advantage of iPSC methodology. Therefore, the BF Stem Cell Platform services need to focus more on technologies of differentiation and functional analysis of the differentiated iPSCs. BCH focuses mainly on endodermal differentiation to derive functional hepatocytes and pancreatic islet cells. BMT focuses on the differentiation of cardiomyocytes as well as retinal pigment epithelial cells and neurons. The emphasis of stem cell services of BCK is towards neural and muscular cells and diseases.

The iPS cells are ideal cells for biobanking purposes because of their unlimited growth capacity and potential to generate other cell types. Therefore, one area of emphasis needs to be in developing coordinated programs to add banking of iPS cells in current Finnish biobanking initiatives. The BF Stem Cell Platform should play an instrumental role in this.

Due to the challenges in obtaining fully functional and mature cells from pluripotent stem cells, an increasingly important trend in this field is the direct reprogramming (that is, transdifferentiation) of somatic cells into functional cells and their expandable progenitors. Therefore, one area of focus will be the development of direct reprogramming approaches for the generation of endodermal progenitors that could be used as a reliable source for hepatocytes, pancreatic islet cells and intestinal cells. Direct differentiation of mature cells into cardiomyocytes will be a focus in Tampere in collaboration with both national and international collaborators. Other cell types could also be used as future targets. The Kuopio unit (BCK) pursues transdifferentiation of neuronal cells.

New nanofibrillar cellulose based material has been developed for the use in 3D SC culture and the method to release the spheroids from the biomaterial were developed. The material is still available via the Helsinki unit.

Major publications supported by platform services

Polinati P, Ilmarinen T, Trokovic R, Hyötyläinen T, Otonkoski T, Suomalainen A, Skottman H, Tyni TA. Patient specific induced pluripotent stem cells derived RPE cells: understanding the pathogenesis of retinopathy in LCHAD deficiency. Invest Ophthalmol Vis Sci 56: 3371–3382, 2015.

Trokovic R, Weltner J, Nishimura K, Ohtaka M, Nakanishi M, Salomaa V, Jalanko A, Otonkoski T, Kyttälä A. Advanced feeder-free generation of induced pluripotent stem cells directly from blood cells. Stem Cells Trans Med 3: 11402–1409,2014.

Ahola A, Kiviaho AL, Larsson K, Honkanen M, Aalto-Setälä K, Hyttinen J. Video image-based analysis of single human induced pluripotent stem cell derived cardiomyocyte beating dynamics using digital image correlation. Biomed Eng Online 13: 39, 2014.

Spencer CI, Baba S, Nakamura K, Hua EA, Sears MA, Fu CC, Zhang J, Balijepalli S, Tomoda K, Hayashi Y, Lizarraga P, Wojciak J, Scheinman MM, Aalto-Setälä K, Makielski JC, January CT, Healy KE, Kamp TJ, Yamanaka S, Conklin BR. Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia. Stem Cell Reports 3: 269–281, 2014.

Juhola M, Joutsijoki H, Varpa K, Saarikoski J, Rasku J, Iltanen K, Laurikkala J, Hyyrö H, Ávalos-Salguero J, Siirtola H, Penttinen K, Aalto-Setälä K. On computation of calcium cycling anomalies in cardiomyocytes data. Conf Proc IEEE Eng Med Biol Soc 2014:1444–1447, 2014.

Penttinen K, Swan H, Kontula K, Vanninen S, Aalto-Setälä K. Antiarrhythmic effects of dantrolene in patients with catecholaminergic polymorphic ventricular tachycardia and replication of the responses using iPSC models. PloS One 10: e0125366, 2015.

Pomeshchik Y, Puttonen KA, Kidin I, Ruponen M, Lehtonen S, Malm T, Akesson E, Hovatta O, Koistinaho J. Transplanted human iPSC-derived neural progenitor cells do not promote functional recovery of pharmacologically immunosuppressed mice with contusion spinal cord injury. Cell Transplant [Epub Sept 8, 2014] DOI: 10.3727/096368914X684079, 2014.

STRUCTURAL BIOLOGY

Structural Biology Infrastructure Network (BFSB)

Coordinator of the network: Rik Wierenga, BCO
Members: Sarah Butcher, BI; Juha Rouvinen, BCK; Markku Kulomaa, BMT; Tiina Salminen, BioCity; Denis Kainov, FIMM; Jari Ylänne, University of Jyväskylä

www.biocenter.fi

Structural biology and biophysics cover a wide range of topics, from protein production via structure determination to biocomputational analysis. Biocenter Finland Structural Biology network (BFSB) comprises four major disciplines, all focused on experimental determination of macromolecular structures and elucidation of mechanisms by various time-resolved techniques. They are X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, other time-resolved biophysical techniques, including high-resolution mass spectrometry equipment, and electron microscopy. The BFSB activities are continuously aimed at ensuring good facilities for these powerful but expensive technologies. The research activities of the BFSB units are of major importance for the expert teaching and training activities of the next generation of Finnish structural biologists as also highlighted in the annual reports of the respective platforms. In addition it fosters the development of structure based biotech activities.

Many of the BFSB research groups interact with the European structural biology networks, like Biostruct-X and Instruct. Consequently, the BFSB research groups have jointly written an application to become recognized as an Instruct National Affiliate Centre (Instruct-NAC). This application has been approved by the Instruct council. Simultaneously, the FIRI committee of the Academy of Finland has provided the funding for Finland to join Instruct. This will open the much needed funded access to many expert technologies in Europe, as nicely documented on the Instruct-WWW pages. In general, being an Instruct-NAC will help in building the BFSB units further into a coherent and well-funded research community. It will also provide the Finnish life science community with good access to the impressive expertise for structural biology research in the EU, ranging from biocomputational and molecular biology techniques to the large scale facilities for example for EM, NMR and X-ray data collection.

This BFSB network also benefits from central resources, such as CSC – IT Center for Science Ltd. and from the BF Bioinformatics network.

The expert services provided by the BFSB network are organized into two technology platforms, those for X-ray crystallography and for NMR spectroscopy and mass spectrometry. Four of the biocenters have macromolecular X-ray crystallography facilities (BI, BCK, BCO and BioCity), while BI has a significant investment in nuclear magnetic resonance (NMR) spectroscopy, cryo-electron microscopy and novel three-dimensional methods and time-resolved optical spectroscopy (TROS), and BCK in high-resolution mass spectrometry. BFSB partners have achieved an excellent division of labor, and the BF network helps them to communicate efficiently with each other.

In addition to X-ray crystallography and NMR and mass spectrometry technology platforms BF has funded protein service for biophysical and structural characterization since 2012.

NMR Spectroscopy and Mass Spectrometry Technology Platform

Chair of the consortium: Perttu Permi, BI, Finnish Biological NMR Center (FBNMR)
Member: Juha Rouvinen, BCK, High-resolution Mass Spectrometry Facility

www.biocenter.fi

Achievements in development of technology services

NMR spectroscopy. In 2014, several major technological developments have been achieved at the NMR facility. First, the spectrometer console of one 600 MHz NMR spectrometer has been updated to BRUKER Avance III HD, together with a new cryogenic probehead, enhancing the sensitivity and performance. In May 2014, after the change of the head of the NMR facility from Perttu Permi to Hideo Iwai, the 500 MHz spectrometer was decommissioned and transferred to Department of Chemistry, University of Helsinki, in order to host the new 850 MHz spectrometer. At the same time, a new air-compressor was introduced to supply the required air-pressure for the entire NMR core facility. The BF-funded 850 MHz magnet arrived at the end of August and reached the field of 850 MHz (20 Tesla) on September 15. The extremely stable magnetic field of the 850 MHz magnet (<1 Hz/hour drift, compared with typical 8–9Hz/hour) was achieved after a few weeks of the electricity charge, which was followed by one month free-trial service for the users to test various experiments before the first approval. Unfortunately, a cryogenic probehead on 850 MHz spectrometer had to be shipped back and had to wait until 2015, before it became operational again. Due to the

complete changes of the spectrometers from Agilent to Bruker, several user trainings were organized in 2014 by Bruker application scientists. A current limitation for biomolecular NMR re-search in Finland is the lack of a sufficient number of skilled NMR scientists. With the new spectrometers, it is now possible to run various modernized experiments with very high sensitivity (S/N >11000). Moreover, it will be a modern platform for training younger scientists with Biomolecular NMR. The high sensitivity also allows working on proteins with limited concentrations. Due to special design of the new probehead, it has also become possible to record NMR spectra with samples containing high salts (>1 M). N_2-free design of the 850 MHz spectrometer makes it possible to record experiments that last longer than one week. Because of various upgrades, installations and modifications, NMR service functions have been limited in 2014–2015.

Mass spectrometry. Because of the total renovation of the departmental building at the UEF Department of Chemistry, FT-ICR mass spectrometer was temporarily relocated to another room during 2013, but was moved back to the original renovated laboratory in January 2014. The facility has been fully operational since then. The most recent sample introduction systems, the online nano/micro-LC system (Dionex Ultimate 3000) and the ionization robot (Triversa Nanomate; Advion), have been in full use as well. However, the use of a direct surface sampling/ionization technique (desorption electrospray ionization, DESI; Prosolia Inc.) has been postponed because of the silicon compound contamination observed in the mass spectra. Discussions to solve this issue are ongoing with the manufacturer and other users of the same ion source type.

There has been an increase in the number of groups and projects that have used the facility as compared to the last year. Especially encouraging has been a great interest of Finnish industry towards the FT-ICR platform, demonstrated by a number of contract research projects. In the recent TEKES project (ended in 2014), analytical protocols were developed for structural validation of IgG antibodies, especially direct amino acid sequencing, as well as automated ligand screening of proteins using infusion robotics.

Participation in international, Nordic and European infrastructures

As a member of the BF Infrastructure Network on Structural Biology (BFSB), the platform has followed the development of Instruct, the ESFRI infrastructure for structural biology. The platform is part of the National Affiliate Center (NAC) of Instruct (Instruct-FI, https://www.structuralbiology.eu/update/centre/instruct--fi). The Nordisk NMR network was estab-lished with the NMR core facility.

User statistics

	Groups	Metrics
NMR Spectroscopy		
local	13	
domestic	9	
international	2	
non-academic	1	
Total	25	
Mass spectrometry		
local	9	
domestic	14	
international	3	
non-academic	8	
Total	34	3 900 (number of measured spectra)

Future perspectives

The structural studies of biological macromolecules in solution are an essential part of modern structural biology. Both NMR and native mass spectrometry offer such information that cannot be obtained with any other method (e.g., protein dynamics). New developments in this area include the use of solid state NMR to study, for example, membrane proteins in native cells as well as ion mobility mass spectrometry to study very large biomolecular assemblies, such as viruses or DNA. The use of high-resolution mass spectrometry has proven to be very efficient in the analysis of protein materials in respect to heterogeneity and post-translational modifications. The large utilization of these techniques would increase considerably the reproducibility of experiments in life sciences, which has been recognized as a major concern in the field. Structural studies by NMR spectroscopy are typically limited by available samples due to low sensitivity of NMR spectroscopy, requiring relatively high concentrations (sub mM concentration) of target proteins. Ultra-high field (1.2 GHz magnet), new probe design, and dynamic nuclear polarization (DNP) techniques would overcome this limitation by enhancing the sensitivity by a factor of 2–1000. Such enormous improvement in the sensitivity would enable us to investigate a wide range of proteins with sub mM concentration and proteins with the limited availability (e.g., membrane proteins)

Major publications supported by platform services

Kekäläinen T, Venäläinen T, Jänis J. Characterization of birch wood pyrolysis oils by ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry: insights into thermo-chemical conversion. Energy Fuels 28: 4596–4602, 2014.

Safdar, M, Spross, J, Jänis J. Microscale immobilized enzyme reactors in proteomics: Latest de-velopments, J Chromatogr A 1324: 1–10, 2014.

Lehto M, Karilainen T, Rog T, Cramariuc O, Vanhala E, Tornaeus J, Taberman H, Jänis J, Alenius H, Vattulainen I, Laine O. Coexposure with fullerene may strengthen health effects of organic industrial chemicals. PLoS One 8: e114490, 2014.

Niemi MH, Rytkönen-Nissinen M, Jänis J, Virtanen T, Rouvinen J. Structural aspects of dog allergies: the crystal structure of a dog dander allergen Can f 4. Mol Immunol 61: 7–15, 2014.

Taberman H, Andberg M, Parkkinen T, Jänis J, Penttilä M, Hakulinen N, Koivula A, Rouvinen J. The structure and function of a decarboxylating *Agrobacterium tumefaciens* keto-deoxy-D-galactarate dehydratase. Biochemistry 53: 8052–8060, 2014.

Taskinen B, Airenne TT, Jänis J, Rahikainen R, Johnson MS, Kulomaa MS, Hytönen VP. A novel chimeric avidin with increased thermal stability using DNA shuffling. PLoS One 8: e92058, 2014.

Aranko AS, Oeemig JS, Zhou D, Kajander T, Wlodawer A, Iwaï H. Structure-based engineering and comparison of novel split inteins for protein ligation. Mol Biosyst 10: 1023–1034, 2014.

Liu L, Jokela J, Herfindal L, Wahlsten M, Sinkkonen J, Permi P, Fewer DP, Doskeland SO, Sivonen K. 4-Methylproline guided natural product discovery: Co-occurrence of 4-hydroxy- and 4-methylprolines in nostoweipeptins and nostopeptolides. ACS Chem Biol 9: 2646–2655, 2014.

Tossavainen H, Kukkurainen S, Määttä JA, Kähkönen N, Pihlajamaa T, Hytönen VP, Kulomaa MS, Permi P. Chimeric avidin–NMR structure and dynamics of a 56 kDa homotetrameric thermostable protein. PLoS One 9 :e100564, 2014.

Shunmugam S, Jokela J, Wahlsten M, Battchikova N, ur Rehman A, Vass I, Karonen M, Sinkko-nen J, Permi P, Sivonen K, Aro EM, Allahverdiyeva Y. A Secondary metabolite from Nostoc XPORK14A inhibits photosynthesis and growth of Synechocystis PCC 6803. Plant Cell Environ 37: 1371–1381, 2014.

Vestola J, Shishido TK, Jokela J, Fewer DP, Aitio O, Permi P, Wahlsten M, Wang H, Rouhiainen L, Sivonen K. Hassallidins, antifungal glycolipopeptides, are widespread among cyanobacteria and are the end-product of a nonribosomal pathway. Proc Natl Acad Sci USA 111: E1909–E1917, 2014.

Sethi R, Seppälä J, Tossavainen H, Ylilauri M, Ruskamo S, Pentikäinen OT, Pentikäinen U, Permi P, Ylänne J. A Novel structural unit in the N-terminal region of filamins. J Biol Chem 289: 858–8598, 2014.

Tossavainen H, Seppälä J, Sethi R, Pihlajamaa T, Permi P. HN, NH, Ca, Cb, and methyl group as-signments of filamin multidomain fragments IgFLNc4-5 and IgFLNa3-5. Biomol NMR Assign 9: 47–50, 2015.4

Hellman M, Piirainen H, Jaakola VP, Permi P. Bridge over troubled proline: assignment of intrinsi-cally disordered proteins using (HCA)CON(CAN)H and (HCA)N(CA)CO(N)H experiments concomi-tantly with HNCO and i(HCA)CO(CA)NH. J Biomol NMR 58: 49–60, 2014.

X-ray FIX-UP Technology Platform

Chair of the consortium: Rik Wierenga, BCO, Protein Crystallography, Oulu X-ray
Members: Tommi Kajander, BI, Protein Crystallisation Facility, Tassos Papageorgiou, BioCity, Protein Crystallography Core Facility

www.biocenter.fi

Achievements in development of technology services

The FIX-UP Structural Biology (BFSB) technology platform received BF funding for personnel running the facilities in BI Helsinki, BCO Oulu and BCT Turku. The BF investments in structural biology in the previous years have had a major impact on the structural biology research in Finland. The FIX-UP platform members – in Oulu, Helsinki and Turku – have also been able to attract additional funding for extending their infrastructure. In Oulu, additional investments have been made in crystallization devices, such as plate hotels, and equipment for biophysical characterization and enzyme kinetics. Additional investments in Turku have been made in crystallization equipment, such as a nanodispenser; also a plate scanner has been installed. In Helsinki, new equipment for biophysical characterization of membrane proteins has been installed, such as the Blitz instrument for quick measurement of protein-protein interactions, and an assay has been developed for fluorescent labeling and detection of protein complexes in crystals. Helsinki has also, in collaboration with FIMM, tested and demonstrated crystallization of a test protein (lysozyme) with nanoscale acoustic dispensing for automated preparation of crystallization screens with down to 25-nl drop size, potentially enabling the use of only a few microliters of sample per a 96-well plate. BFSB has now been accepted as the Finnish Instruct National Affiliate Center, Instruct-FI, which will foster the further development of the FIX-UP platform. Instruct-FI is also included in Finland's roadmap for research infrastructures 2014–2020.

Services

The goal in service provision is to provide a clear and effective path from a purified protein to a solved crystal structure. To that end:
– FIX-UP provides protein characterization (protein stability screening by fluorescence; protein characterization by chromatography/multiple angle light scattering) crystallization and imaging facilities. The facilities in Helsinki (http://www.biocenter.helsinki.fi/bi/xray/automation/) provide also advice and training on how to proceed with a crystallization project.
– Another important service is the provision of ran-

dom screens for crystallization. These screens are in use in Helsinki, Turku, Jyväskylä and Oulu. Services are also provided to design new screens based on hits within these screens, or FIX-UP can design/produce novel random screens based on demand.
– FIX-UP provides a service enabling data collection and structure determination, including crystal testing, in Oulu and in the regional service center in Turku. Remote data collection sessions at Diamond and the ESRF are now routinely organized in each center. In Turku, a successful in situ data collection (Diamond) was carried out.

Software development

Further development of the web-based imaging software, PiXray with a simple modular architecture to allow visualizing crystallization experiments, has taken place in Helsinki. The images are tagged with screen information from the three platforms to allow design of new crystallization conditions in the Minstrel software. New software to operate communication between Rigaku screen design software and Hamilton screen preparation robot has also been implemented to enable more complex custom screens.

In Oulu, the xtalPiMS data-tracking package, for viewing and annotating the images of crystallization experiments, has been developed further. The xtalPiMS IT-infrastructure allows remote access to its database. A new module to facilitate the shipment of crystals-on-pins to Diamond is now ready. This project is setup in the context of Instruct, in collaboration with staff at the STFC in Daresbury, UK and Diamond in Oxford, UK, and is now continued in collaboration with Diamond.

Training and outreach

Courses at the Masters' level on X-ray crystallography and structure interpretation are offered at each University. Helsinki has also had outreach activities to schools, and in the Spring 2014, a visit of local 4th graders was organized. In Turku, various visits from schools took place.

FIX-UP continues to meet yearly as part of FINNBOX (the Finnish Biological Crystallographers), which has been combined with the national BFSB meetings. In 2014, the meeting was organized by Oulu on April 4. In 2015, it will be organized by Helsinki, on June 9, 2015.

The Oulu X-ray core facility is an integral part of the local Strucbiocat initiative, which is being developed as a multidisciplinary core facility including not only X-ray but also enzymology and biophysical characterization (http://www.oulu.fi/biocenter/strucbiocat). Likewise, the Turku regional data collection center is also part of BioXLabs (http://www.btk.fi/crystallography/cooperation/instruct/bioxlabs, a regional structural biology consortium formed to enhance coordination of activities. In this context, in Turku a demonstration on thermophoresis, which is a new protein characterization tool, was organized that included measurements of user samples.

User statistics

Number of research groups*			
	BI Helsinki	BCO Oulu	Biocity Turku
Campus	10	8	3
Local University	2	-	4
Other Universities	1	-	3
Non-academic	-	-	1
International	1	-	5

*The numbers refer to the number of research groups that have used the core facility infrastructure and expertise. The number of actual users and projects is at least twice as much. The educational activities have not been included in these numbers.

A large number of research projects are supported by the FIX-UP core facilities. Once Finland has officially joined Instruct, it is expected that the number of visiting groups from outside Finland will increase. In Oulu, the BCO core facility has successfully moved from the Linnanmaa campus to the Kontinkangas campus, causing, however, disruption of the services in 2014. The Oulu core facility is much used for research related to approximately 20 projects of the user groups as well as for teaching activities in the context of undergraduate, graduate and postgraduate courses. At least 6 PDB-entries have been submitted in 2014. In Helsinki, crystallization has been performed on at least 30 different protein/macromolecule systems, with a total of 154 crystallization experiments in 2014. At the Turku regional data collection center, the Rigaku micromax has been used on a regular basis for research and educational purposes. Data sets for 12 projects have been collected and 3 structures have been deposited.

Participation in International, Nordic and European infrastructures

FIX-UP is well connected to Instruct. FIX-UP interacts also with other important EU infrastructure networks, such as the Biostruct-X and iNEXT initiatives. There is good access to the ESRF beamlines through the FinnProCC BAG, and through a similar national BAG at Diamond, both coordinated by Kajander, Helsinki. FIX-UP have access to the Biostruct-X synchrotron facilities via a BAG, coordinated by Glumoff, Oulu. Data

collection is also carried out at MAX Lab, DESY and BESSY. Wierenga is a member of the advisory committee of the new PX-beam line of MAX IV. FIX-UP participates in Finnish (FSRUO, Kajander) and European (ESUO, Kajander) synchrotron user organizations on the development of synchrotron radiation for scientific research, as well as we are actively involved in the guidance of the ESRF (Adrian Goldman was Head of Delegation of Nordsync in 2014).

Future perspectives

Each of the three protein crystallography centers of FIX-UP has developed its own specific expertise and research interest. In Helsinki, there is a focus and specialized expertise on membrane protein studies and protein crystallization. In Oulu, the focus is on data collection, including the xtalPiMS development, and quantitative enzymology and biophysical characterization. In Turku, there is emphasis on a combination of protein crystallography and bioinformatics. Each of these centers is committed to reach out to the life science community in Finland. In Helsinki and Oulu, investments in a state-of-the-art plate scanner are very timely, whereas for Turku, the purchase of modern crystallization plate hotels is important.

Major publications supported by platform services

Aranko AS, Oeemig JS, Zhou D, Kajander T, Wlodawer A, Iwaï H. Structure-based engineering and comparison of novel split inteins for protein ligation. Mol Biosyst 10: 1023–1034, 2014.

Bhattacharjee A, Reuter S, Trojnár E, Kolodziejczyk R, Seeberger H, Hyvärinen S, Uzonyi B, Szilágyi Á, Prohászka Z, Goldman A, Józsi M, Jokiranta TS. The major autoantibody epitope on factor H in atypical hemolytic uremic syndrome is structurally different from its homologous site in factor H-related protein 1, supporting a novel model for induction of autoimmunity in this disease. J Biol Chem 290: 9500–9510, 2015.

Haikarainen T, Narwal M, Joensuu P, Lehtiö L. Evaluation and structural basis for the inhibition of tankyrases by PARP inhibitors. ACS Med Chem Lett 5: 18–22, 2014.

Haikarainen T, Frioux C, Zhnag LQ, Li D, Papageorgiou AC. Crystal structure and biochemical characterization of a manganese superoxide dismutase from Chaetomium thermophilum. Biochim Biophys Acta 1844: 422–429, 2014.

Onwukwe GU, Kursula P, Koski MK, Schmitz W, Wierenga RK Human Δ3, Δ2-enoyl-CoA isomerase, type-2: a structural enzymology study on the catalytic role of its ACBP domain and helix-10. FEBS J 282: 746–768, 2015.

Patrikainen P, Niiranen L, Thapa K, Paananen P, Tähtinen P, Mäntsälä P, Niemi J, Metsä-Ketelä M. (2014) Structure-based engineering of angucyclinone 6-ketoreductases. Chem Biol 21: 1381–1391, 2014.

Peterhoff D, Beer B, Rajendra C, Kumpula E-P, Kapetaniou E, Guldan H, Wierenga RK, Sterner R, Babinger P. A comprehensive analysis of the geranylgeranyglyceryl phosphate synthase enzyme family identifies novel members and reveals mechanisms of substrate specificity and quaternary structure organization. Mol Microbiol 92: 885–899, 2014.

Skopelitou K, Muleta AW, Papageorgiou AC, Chronopoulou E, Labrou NE. Catalytic features and crystal structure of a tau class glutathione transferase from Glycine max specifically upregulated in response to soybean mosaic virus infections. Biochim Biophys Acta 1855: 166–177, 2014.

Vahokoski J, Bhargav SP, Desfosses A, Andreadaki M, Kumpula EP, Martinez S, Ignatev A, Lepper S, Frischknech F, Sidén-Kiamos I, Sachse C, Kursula I. Structural differences explain diverse functions of Plasmodium actins. PLoS Pathog 10: e1004091, 2014.

Venkatesan R, Sah-Teli SK, Awoniyi LO, Jiang G, Prus P, Kastaniotis AJ, Hiltunen JK, Wierenga RK, Chen Z. Insights into mitochondrial fatty acid synthesis from the structure of heterotetrameric 3-ketoacyl-ACP reductase/3R-hydroxyacyl-CoA dehydrogenase. Nat Commun 5: 4805, 2014.

Protein Services Technology Platform

Chair of the consortium: Juha Määttä, BMT, Tampere Protein Facility
Member: Olli Ritvos, BCH, Haartman Institute Protein Production Service

www.biocenter.fi

Achievements in development of technology services

Protein production for biophysical and structural characterization in Tampere and Helsinki is a supplement to Structural biology platform services. Protein expression in several organisms offers suitable system for any recombinant protein that is needed with proper scale-up possibility.

Tampere Protein Technologies offers protein expression in *E. coli* and *Spodoptera frugiperda* (baculovirus expression system, BV) cells. In addition, service has special focus on protein interactions. The customers are mainly from universities, and virtually all Finnish universities are represented within the customers. This year, the facility has continued to serve existing users and also new customers. The number of customers who used the baculovirus expression system increased from previous years. Protein technologies made headway in virus like particle expression and purification, which could be one focus of attention in future.

The Helsinki protein production facility offers services for generating recombinant protein expressing mammalian CHO-S and HEK293 cell lines. This facility has its focus on expressing and purifying secretory mammalian proteins in native form or as fusion molecules in milligram to gram quantities. The customer base of the Helsinki facility has increased in 2014 to 16, with local, nation-wide domestic and international clients. Customers need usually both cell line generation and scale-up protein production services, even though there are also several customers whose own cell lines are scaled-up in suspension culture. The facility have been also requested to provide scale-up productions of mouse monoclonal antibodies in chemically defined serum-free media from customers hybridoma cells.

The coordination between the Tampere and Helsinki protein expression platforms offers the choice of three robust protein expression platforms that the clients can use for satisfying their specific protein expression needs. Guidance, counseling and planning aid is provided to help the customers to decide whether to use in parallel several expression methods or to choose the most appropriate recombinant protein expression approaches for their research subject.

Participation in international, Nordic and European infrastructures

So far it has been important to spread awareness of the possibility to use these protein expression services in the BF context on a national scale. The platform has not as yet officially networked with other Scandinavian or European units. The Helsinki facility is currently in discussions with representatives of units providing protein expression services in Sweden (Karolinska institute, Protein Science Facility and the SciLifeLab Drug and Discovery and Development platform infrastructure). Interactions with the Swedish and Finnish National infrastructures within the protein expression area would likely facilitate numerous Finnish–Swedish research collaborations and cooperation to researcher training in this area.

Future perspectives

The Protein Service section of the Structural Biology and Biophysics platform could become a network providing broad technology to screen and express proteins in most efficient way and by that serving the whole Finnish bioscience community. This requires continuous development of processes to enable easily transition from a screening phase to a sufficiently sized and appropriate scale-up protein expression methodology. This work also requires developing, streamlining and perfecting the methodology approaches in protein overexpression and purification.

Major publications supported by platform services

Taskinen B, Zauner D, Lehtonen SI, Koskinen M, Thomson C, Kähkönen N, Kukkurainen S, Määttä JA, Ihalainen TO, Kulomaa MS, Gruber HJ, Hytönen VP. Switchavidin: reversible biotin-avidin-biotin bridges with high affinity and specificity. Bioconjug Chem 25: 2233–2243 2014.

Kukkurainen S, Määttä JA, Saeger J, Valjakka J, Vogel V, Hytönen VP. The talin-integrin interface under mechanical stress. Mol Biosyst 10: 3217–3228, 2014.

Köhler M, Karner A, Leitner M, Hytönen VP, Kulomaa M, Hinterdorfer P, Ebner A. pH-dependent deformations of the energy landscape of avidin-like proteins investigated by single molecule force spectroscopy. Molecules 19: 12531–12546, 2014.

Tossavainen H, Kukkurainen S, Määttä JA, Kähkönen N, Pihlajamaa T, Hytönen VP, Kulomaa MS, Permi P. Chimeric Avidin–NMR structure and dynamics of a 56 kDa homotetrameric thermostable protein. PLoS One 9: e100564, 2014.

Kurppa K, Hytönen VP, Nakari-Setälä T, Kulomaa MS, Linder MB. Molecular engineering of avidin and hydrophobin for functional self-assembling interfaces. Colloids Surf B Biointerfaces 120:102–109, 2014.

Pinon P, Pärssinen J, Vazquez P, Bachmann M, Rahikainen R, Jacquier MC, Azizi L, Määttä JA, Bastmeyer M, Hytönen VP, Wehrle-Haller B. Talin-bound NPLY motif recruits integrin-signaling adapters to regulate cell spreading and mechanosensing. J Cell Biol 205: 265–281, 2014.

Koho T, Koivunen MR, Oikarinen S, Kummola L, Mäkinen S, Mähönen AJ, Sioofy-Khojine A, Marjomäki V, Kazmertsuk A, Junttila I, Kulomaa MS, Hyöty H, Hytönen VP, Laitinen OH. Coxsackievirus B3 VLPs purified by ion exchange chromatography elicit strong immune responses in mice. Antiviral Res 104: 93–101, 2014.

Rangl M, Leitner M, Riihimäki T, Lehtonen S, Hytönen VP, Gruber HJ, Kulomaa M, Hinterdorfer P, Ebner A. Investigating the binding behaviour of two avidin-based testosterone binders using molecular recognition force spectroscopy. J Mol Recognit 27: 92–97, 2014.

Kokkola T, Suuronen T, Molnár F, Määttä JA, Salminen A, Jarho EM, Lahtela-Kakkonen M. AROS has a context-dependent effect on SIRT1. FEBS Lett 588: 1523–1528, 2014.

Taskinen B, Airenne TT, Jänis J, Rahikainen R, Johnson MS, Kulomaa MS, Hytönen VP. A novel chimeric avidin with increased thermal stability using DNA shuffling. PLoS One 9: e92058, 2014.

Wiener Z, Band AM, Kallio P, Högström J, Hyvönen V, Kaijalainen S, Ritvos O, Haglund K, Kruuna O, Robine S, Louvard D, Ben-Neriah Y, Alitalo K. Oncogenic mutations in intestinal adenomas regulate Bim-mediated apoptosis induced by TGFβ. Proc Natl Acad Sci USA 111: E2229–E2236, 2014.

Relizani K, Mouisel E, Giannesini B, Hourdé C, Patel K, Gonzales SM, Jülich K, Vignaud A, Piétri-Rouxel F, Fortin D, Garcia L, Blot S, Ritvos O, Bendahan D, Ferry A, Ventura-Clapier R, Schuelke M, Amthor H. Blockade of ActRIIB signalling triggers muscle fatigability and metabolic myopathy. Mol Ther 22: 1423–1433, 2014.

Myllärniemi M, Tikkanen J, Hulmi JJ, Pasternack AH, Sutinen E, Rönty M, Leppäranta O, Kinnula V, Ma H, Ritvos O, Koli K. Upregulation of activin-B and follistatin in pulmonary fibrosis – a translational study using human biopsies and a specific inhibitor in mouse fibrosis models. BMC Pulm Med 14: 170, 2014.

TRANSLATIONAL TECHNOLOGIES

Translational Technologies Infrastructure Network

Coordinator of the Network: Olli Kallioniemi, FIMM
Members: Kalle Saksela, BCH; Mart Saarma, BI; Asla Pitkänen, BCK; Robert Winqvist, BCO; Jorma Isola, BMT, Noora Kotaja, BioCity; Krister Wennerberg, FIMM; Olli Pentikäinen, University of Jyväskylä

www.biocenter.fi

The network coordinates two technology platforms: (i) Drug Discovery and Chemical Biology (DDCB) for discovery and proof-of-concept validation of therapeutic molecules, and (ii) Tissue Biobanking for biobanking and biomarker research. The DDCB platform focuses on drug discovery and development, and is linked to the European EATRIS and EU-Openscreen infrastructures, coordinated in Finland by FIMM. This platform will further develop several existing strong capabilities in Finland, such as chemoinformatics/structural biology, high-throughput screening, as well as *in vivo* testing. The aim is to facilitate the capabilities for discovering inhibitors to interesting targets, and to carry out proof-of-concept testing *in vivo*. This platform should optimally bridge the gap between academic research and industrial interests to drug discovery.

Finland is well-positioned to play a major role globally in the development of biobanks and biomarker capabilities. Systematic large-scale biobanking activities are ongoing at several sites, such as at the University of Tampere (with Tampere University Hospital) and in Helsinki (Institute for Health and Welfare, THL), University of Helsinki/FIMM and HUS Helsinki University Hospital) and the University of Turku (with Turku University Hospital). The focus of the BF Tissue Biobanking technology platform is on development of virtual microscopy based methods particularly for cancer biobanking. The BF platform is linked through FIMM and THL to the European-level biobanking infrastructure (Biobanking and Biomolecular Resources Research Infrastructure, BBMRI-ERIC). The Finnish BBMRI node comprises not only the large scale Finnish population cohorts, but also numerous investigator-initiated sample collections and clinical data sources and the BF biobanking technology platform. In the future, automation of sample acquisition and fractionation technologies, as well as generation of arrayed tissue and molecular resources will be developed together with demographic and clinical annotation of the samples.

Tissue Biobanking Technology Platform

Chair of the consortium: Johan Lundin, FIMM
Members: Jorma Isola, BMT; Olli Carpen, BioCity

www.biocenter.fi

Achievements in development of technology services

The main goal of the technology platform is to support incorporation of virtual microscopy in medical tissue biobanking projects and biomarker research. The consortium also provides know-how for best histological characterization of biobanked samples and for automated assessment of tissue sample stainings. The platform has been improved further to enable seamless integration of whole-slide images with biobank samples and clinical databases.

The platform has provided the following services.

• Whole-slide cell and tissue sample scanning services. Scanning instruments are available at FIMM, BMT and BioCity Turku. Service is charged for per project or according to a pay-per-slide principle, including the digitization process and data storage. Price per project or slide varies according to volume demands and sample type. Typical price for smaller series of histological slides (<100) is in the range of 25-40 €/slide.

• Access to an online platform for virtual microscopy. The consortium maintains webmicroscopy platforms (fimm.webmicroscope.net, turku.webmicroscope.net, predect.webmicroscope.net, jvsmicroscope.uta.fi). Service for management, storage and access are provided on a project basis and charged for (per working day) according to a cost-recovery principle.

• Access to computational tools, image analysis, clinical informatics. Development and tailoring of image analysis and clinical informatics tools as well as access to the consultation services of a pathologist are charged for (per working day) on a project basis according to a cost-recovery principle. The consortium has implemented analytical tools for image annotation, image analysis (e.g., jvsmicroscope.uta.fi/immunoratio/, jvsmicroscope.uta.fi/immunomembrane/) and for clinical informatics.

The scanning instrument access has been the most important bottleneck at one of the centers (Helsinki) and, since the service at the Meilahti Campus has relied on a single instrument, the service has been sensitive to instrument malfunction or many concurrent requests. Acquisition of a scanner instrument placed within the Biomedicum Imaging Unit at the campus is likely to

improve the situation. The current BF funding allows only 1–2 part time employed persons (one at FIMM and one at BMT) to handle the services, and therefore, longer waiting times may occur.

Activities in this field have increased substantially, and the platform foresees that a high demand for sample digitization and the establishment of new biobanks in Finland will create a need for additional scanning capacity.

User statistics

Total number of research groups who have used the services in 2014: 85+

Breakdown of users (research groups): FIMM research groups 20; domestic 13; international 32; pharmaceutical companies 10, BMT local: 10, domestic 1, international 1.

Metrics:

- FIMM: Total number of scanned slides was approx. 6 000 that constitutes ~ 45 000 000 individual digital images and a data amount of ~165.000 GB.
- BMT: Total number of scanned slides approx. 10 000. The ImmunoRatio software developed at BMT was down-loaded over 4 000 times and ImmunoMembrane down-loaded over 1 000 times. The ImmunoRatio web application is frequently used : over 100 000 images analyzed during 2011–2014.

Participation in international, Nordic and European infrastructures

The Tissue Biobank Technology Platform is used internationally, and it has exceptionally strong links to EU initiatives. For example, a grant of 660 000 € was granted 2013 to develop a platform for the world's largest private-public partnership, the IMI-funded PREDECT project between 9 academic, 3 SME and 7 EU pharmaceutical partners, developing target validation models for breast, prostate and lung cancer, and with a total budget of approx. 20 million €. A webmicroscopy platform has been established for the consortium, where all scanned samples (n>60 000) are made available to the participants (predect.webmicroscope.net). In addition, the webmicroscopy portal is provided as a platform for data sharing within the EU funded projects BioMedBridges (biomedbridges.webmicroscope.net) and Systems Microscopy.

A pilot study has been proposed to the Biomarker Product Group of the European Advanced Translational Research Infrastructure in Medicine (EATRIS) that is one of the ESFRIs. The intention is to assess whether the virtual microscopy methods could be used for biomarker validation and standardization of immunohistochemical staining readouts.

Future perspectives

The global trend towards digital whole-slide microscopy is likely to increase the demand for slide scanning and webmicroscopy services during the next years.

Tasks of the consortium 2015–2016 are as follows.

To maintain and improve the high-performance platform for digital microscopy and associated analytical tools established during 2010–2014, including image servers, software for managing the image data, biomarker analysis functionality, linking of image data to clinical/phenotypic data and return of analysis results to the user.

To improve the sample logistics and scanning services by optimized workflows as well as allocation of person resources to handle samples, scanning and communication with the users. The services will become more clearly defined, and information on digitization time and prices will be made available on the websites of the platform members.

Major publications supported by platform services

Ranki T, Joensuu T, Jaeger E, Karbach J, Wahle C, Kairemo K, Alanko T, Partanen K, Turkki R, Linder N, Lundin J, Ristimäki A, Kankainen M, Hemminki A, Backman C, Dienel K, von Euler M, Haavisto E, Hakonen T, Juhila J, Jaderberg M, Priha P, Vassilev L, Vuolanto A, Pesonen S. Local treatment of a pleural mesothelioma tumor with ONCOS-102 induces a systemic antitumor CD8+ T-cell response, prominent infiltration of CD8+ lymphocytes and Th1 type polarization. OncoImmunology 3: e958937, 2014.

Hemminki O , Parviainen S, Juhila J, Turkki R, Linder N, Lundin J, Kankainen M, Ristimäki A, Koski A, Liikanen I, Oksanen M, Nettelback DM, Kairemo K, Partanen K, Joensuu T, Kanerva A, Hemminki A. Immunological data from cancer patients treated with Ad5/3-E2F-Δ24-GMCSF suggests utility for tumor immunotherapy. Oncotarget 6: 4467–4481, 2015.

Grote A, Abbas M, Linder N, Kreipe HH, Lundin J, Feuerhake F. Exploring the spatial dimension of estrogen and progesterone signaling: detection of nuclear labeling in lobular epithelial cells in normal mammary glands adjacent to breast cancer. Diag Pathol 9 (Suppl 1): S11, 20144

Saeed K, Östling PK, Björkman M, Mirtti T, Alanen K, Vesterinen T, Sankila A, Lundin J, Lundin M, Rannikko A, Nordling S, Mpindi JP, Kohonen P, Iljin K, Kallioniemi O, Rantala JK. Androgen receptor-interacting protein HSPBAP1 facilitates growth of prostate cancer cells in androgen-deficient conditions. Int J Cancer 136: 2535–2545, 2015.

Drug Discovery and Chemical Biology Technology Platform

Chair of the consortium: Krister Wennerberg, FIMM, Chemical Biology Lab

Members: Olli Kallioniemi, FIMM (co-chair); Antti Poso, BCK, Drug Design and Synthesis Laboratory; Adyary Fallarero, BioCity, Drug Discovery of Natural Products Laboratory; Arto Urtti, BCH, Centre for Drug Research (CDR);

Affiliated expert members: Antti Pursula, CSC; Merja Perälä, VTT; Olli Pentikäinen, University of Jyväskylä, Computational Bioscience Laboratory

www.biocenter.fi

http://ddcb.fi/en

Achievements in development of technology services

Drug Discovery and Chemical Biology Technology Platform (DDCB) was formed in 2010 and during the first five years of operation, the services that the platform provides and the user demand for services have been constantly evolving. Initially, the focus was put on traditional drug discovery services such as high-throughput screening, but with time, a key trend has been that services matching future visions biomedical research and biomedicine now are the dominant services of the DDCB platform and they continue to grow.

DDCB purchased diverse library of 3 040 natural compounds and derivatives from TimTec. Overall, the platform now provides more than 5 300 pure natural compounds for screening, a respectable size of natural compound libraries. The host institutes of DDCB supported several instrumentation upgrades, including a microarray scanner and new peristaltic dispensers. DDCB continued to develop the unique joint services of virtual screening from computational/modeling groups with compound cherry-picking and wet lab validation assaying of top hits within the DDCB compound collection with successful discoveries of hits.

DDCB developed phenotypic 3D-cell culture models and analysis tools together with the Light Microscopy Unit and Tiina Sikanen's group (Pharmaceutical Nanotechnology and Chemical Microsystems Unit, Faculty of Pharmacy). A pipeline for reverse-phase protein array antibody-based readouts from high throughput 384-well screening was also developed and implemented using either a pin tool printer or an acoustic dispenser.

A "Clinical DRST" (drug sensitivity and resistance testing) service was established for cancer cell samples, where the highest possible consistency and quality data are required, such as primary patient samples. DDCB made more clearly defined user fee cost models were, including a full cost price, what the user pays for and where the remaining funding comes from.

User statistics

User statistics are presented in a separate table. User statistics highlights:

- Thirty-two scientific articles and at least one patent having utilized DDCB services were published in 2014, while the user/project support continued at a similar high level as the previous year.
- Drug sensitivity and resistance testing services resulted in three new pharmaceutical company-funded research studies.

Participation in international, Nordic and European infrastructures

DDCB has since the start built strong ties to similar research infrastructures in other Nordic and European countries to enable expertise and access to technologies shared between the countries. These collaborations now mean that researchers in Finland can access and get trained to use specialized chemical biology technologies that exist in other countries. An active ongoing effort among the Nordic countries is to build an academic compound collection that could help spark collaborations and discoveries between the Nordic countries. Since 2014, these pan-Nordic initiatives are now supported by a grant from NordForsk (for a Nordic Chemical Biology Consortium)

DDCB is directly linked to three ESFRI roadmap initiatives. First, DDCB coordinates the national participation and plans for construction and operation of EU-OPENSCREEN (www.eu-openscreen.eu), a European research infrastructure focused on the open access development of small molecule "tool compounds" with novel bioactivities. EU-OPENSCREEN operations, scheduled to start in 2016, are expected to be highly complementary to the ongoing operations within DDCB. Second, DDCB also takes actively part in the Small Molecules product platform of the EATRIS translational ESFRI roadmap (www.eatris.eu). CSC and FIMM are involved in the work of BioMedBridges (http://www.biomedbridges.eu) and ELIXIR (www.elixir-europe.org), ESFRI roadmap projects focusing on the coordination and management of biological information

~~DDCB~~ user statistics

	CDR/BCH	UEF/BCK	ÅAU/BioCity Turku	FIMM	Platform total
PI	Arto Urtti	Antti Poso	Pia Vuorela & A. Fallarero	K. Wennerberg & O. Kallioniemi	
Total user research groups	27	14	5	37 (2 jointly with UEF, 2 with CDR)	79
- local	23	7	3	24	53
- national	3	2	1	6	12
- international	1	5	1	5	12
- non-academic				2	2
User fees (€)	1 500 (supply costs)	0 (in silico work only)	0 (all costs supported by the facility)	220 554 (supplies & equipment)	222 054
Key metrics	4 screening projects, 5 virtual screens with exp. validiation, 4 in silico ADME projects	18 virtual screens / molecular modeling projects performed	>3000 new natural compounds acquired	>350 DSRT screens. >400 custom drug plates made. >500 compound aliquots provided	

Future perspectives

Thus far, DDCB has been involved in developing personalized medicine approaches, such as drug sensitivity testing, personalized drug responses, drug repositioning, and studies of individualized drug metabolism and drug transport. Moreover, it has integrated phenotypic bioassay data with molecular profiling data, such as next-generation sequencing, to identify personalized drug response biomarkers. Specialized bioassays such as microbial biofilm assay technologies, label-free screening detection and complex mammalian cell-based assays (co-cultures, 3-dimensional cell culture) in high throughput-capable formats have also been developed by DDCB.

DDCB expects that more "traditional" large-scale high throughput screening projects (e.g., screening more that 100 000 compounds in a project) to an increasing extent will be handled by recently established and emerging European infrastructure efforts such as EU-OPENSCREEN, EATRIS, and to a minor degree the European Lead Factory IMI. However, none of these European infrastructures will provide assay development, optimization and validation services. Finnish researchers to get their projects considered and accepted into these pipelines in European competition, local support will be essential. Therefore, the expertise of the DDCB staff and the access to automation equipment for validation, optimization and proof-of-concept screening (typically in the range of a few thousands to a few tens of thousands compounds screened) is expected to be a key factor in making Finnish research competitive in these European projects.

DDCB provides a number of services that are not covered by other infrastructures and will complement the European initiatives. These services include a unique computational-experimental service platform based on virtual screening followed by picking hits from the extensive DDCB chemical collection and confirmatory follow-up testing. The unique aspect of this service is that there is no need for the user to re-purchase compounds for the experimental validation, which otherwise can cost tens of thousands euros. The proof-of-concept compound distribution is another unique service that is quickly growing and is expected to keep growing. In 2014, DDCB provided over 500 of these aliquots to researchers. Similarly, the service to profile and quality control compounds with mass spectrometry has been critical for many projects and we aim to extend this service to NMR analysis

DDCB expects to continue building on the technological platforms that exist within the DDCB, including an NMR spectrometer, miniaturization and multiplexing using acoustic dispensing, enhanced label-free detection and enhanced high throughput imaging of live and fixed cells. Since the services provided by DDCB are highly specialized and diverse, it is essential to be able to retain and expand on the expert staff that provides the services.

Major publications supported by platform services

Ammad-ud-din M, Georgii E, Gönen M, Laitinen T, Kallioniemi O, Wennerberg K, Poso A, Kaski S, Integrative and personalized QSAR analysis in cancer by kernelized Bayesian matrix factorization. J Chem Inf Model 54: 2347–2359, 2014.

Denisova OV, Söderholm S, Virtanen S, von Schantz C, Bychkov D, Vashchinkina E, Desloovere J, Tynell J, Ikonen N, Theisen LL, Nyman TA, Matikainen S, Kallioniemi O, Julkunen I, Muller CP, Saelens X, Verkhusha VV, Kainov DE. Akt inhibitor MK2206 prevents influenza pH1N1 virus infection in vitro. Antimicrob Agents Chemother 58: 3689–3696, 2014.

Fallarero A, Hanski L, Vuorela P. How to translate a bioassay into a screening assay for natural products: General considerations and implementation of antimicrobial screens. Planta Medica 80: 1182–1199, 2014.

Ghemtio L, Soikkeli A, Yliperttula M, Hirvonen J, Finel M, Xhaard H. SVM classification and CoMSIA modeling of UGT1A6 interacting molecules. J Chem Inf Model 54: 1011–1026, 2014.

Gu Y, Bouwman P, Greco D, Saarela J, Yadav B, Jonkers J, Kuznetsov SG. Suppression of BRCA1 sensitizes cells to proteasome inhibitors. Cell Death Dis 5: e1580, 2014.

Hanski L, Uvell H, Malinovskaja K, Gylfe Å, Laaksonen T, Kolakovic R, Mäkilä E, Salonen J, Hirvonen J, Elofsson M, Sandler N, Vuorela P. Inhibitory activity of the isoflavone biochanin A on intracellular bacteria of genus Chlamydia and initial development of a buccal formulation. PLoS One 9: e115115, 2014.

Haque S, Nawrot DA, Alakurtti S, Ghemtio L, Yli-Kauhaluoma J, Tammela P. Screening and characterisation of antimicrobial properties of semisynthetic betulin derivatives. PLoS One 9: e102696, 2014.

Määttänen A, Fallarero A, Kujala K, Ihalainen P, Vuorela P, Peltonen J. Printed paper-based array platform as substrate for biofilm formation. AMB Express 4: 32, 2014.

Parkkari T, Haavikko R, Laitinen T, Navia-Paldanius D, Rytilahti R, Vaara M, Lehtonen M, Alakurtti S, Yli-Kauhaluoma J, Nevalainen T, Savinainen, Laitinen JT. Discovery of triterpenoids as reversible inhibitors of α/β-hydrolase domain containing 12 (ABHD12). PLoS One 9: e98286, 2014.

Stylianou M, Kulesskiy E, Lopes JP, Granlund M, Wennerberg K, Urban CF. Antifungal application of nonantifungal drugs. Antimicrob Agents Chemother 58:1055–1062, 2014.

VIRAL GENE TRANSFER

Viral Gene Transfer Infrastructure Network

Coordinator of the network: Seppo Ylä-Herttuala, BCK
Members: Akseli Hemminki, BCH; Kari Alitalo BCH; Aki Manninen, BCO; Eric Dufour, BMT; Eleanor Coffey, BioCity; Emmy Verschuren, FIMM; Maija Vihinen-Ranta, University of Jyväskylä

www.biocenter.fi

Gene transfer techniques are an important tool in studies of gene function as well as in the clinical evaluation of new treatments. In research, the most important impact of efficient transient and stable gene transfer methods is the generation of new cell lines or animal models for the basic research of protein functions. Many of these methods are based on utilization of viruses as means to target and deliver genes into appropriate cells. More recently, advances in the RNAi-methodology enable the same delivery method to be used to efficiently silence specific genes in cells.

Successful work with the sophisticated viral methods requires special expertise and strict safety considerations both of which are found in all biocentres in Finland. In particular, the A.I. Virtanen Institute in BCK, specializing in gene transfer methods for drug development, has a long-standing experience with strict regulations and requirements essential for gene therapy based approaches for human patients. Some of their products are already in clinical trials. The AIV Institute is responsible for coordinating the development and production of gene transfer vectors at national level in Finland.

In addition to viral gene transfer and cell therapy platform, BF funded initially "LentiGEMM - Lentiviral platform for creating genetically engineered mouse models" in 2010–2012. The platform changed its name into Tissue Engineered Disease Models (TEDM) in 2013, and the SAB recommended its inclusion in the Model Organism infrastructure network (see pp. 46-47).

Viral Gene Transfer and Cell Therapy Technology Platform (VGTCT)

Chair of the consortium: Seppo Ylä-Herttuala, BCK, National Virus Core Facility, A. I. Virtanen Institute
Members: Kari Alitalo, BCH, AAV Gene Transfer and Cell Therapy Core Facility; Akseli Hemminki, BCH, Oncolytic vector core facility; Juha Klefström, BCH, Functional Genomics Unit (FuGu); Aki Manninen, BCO, Virus Vector Core Facility; Eric Dufour, BMT Virus Vector Facility; Eleanor Coffey, BioCity, Viral Vector Facility.
External member: Maija Vihinen-Ranta, University of Jyväskylä

www.biocenter.fi

Achievements in development of technology services

Viral Gene Transfer And Cell Therapy (VGTCT) network has coordinated virus vector production in different biocenters in such fashion that AAV vectors have mainly been produced in Biomedicum Helsinki (Kari Alitalo); oncolytic vectors in Haartman Institute, Helsinki (Akseli Hemminki); lentiviral vectors in Biomedicum Helsinki (Juha Klefström); siRNA vectors at University of Oulu (Aki Manninen); production of small volumes of adenoviral and lentiviral vectors in BioCity Turku (Eleanor Coffey); small volumes of virus and cell line preparations in BioMediTech in Tampere (Eric Dufour); and large-scale adenoviral, lentiviral, AAV and baculovirus preparations in A.I. Virtanen Institute in Kuopio (Seppo Ylä-Herttuala).

As evidence for the international appreciation and very high scientific level of the VGTCT Network, Finland (that is, Finnish Society of Gene Therapy where VGTCT partners are active members) was selected to organize European Society of Gene and Cell Therapy Annual Congress 2015 that will be held in Helsinki on September 17–20, 2015. This annual meeting is one of the most important scientific gatherings of European and international gene and cell therapy scientists, and it will significantly improve the visibility of gene therapy research conducted in Finland to international researchers and biotech companies.

BF funding has been of key importance for maintenance and development of VGTCT services. Currently, the AAV core facility is able to produce four high-quality AAV preparations with concentration of 10^{12}–10^{13} viral particles per preparation within two weeks. This facility has made a library of commonly used AAV vectors, including GFP, luciferase and other types of control vectors. The advantage of using the

pre-made vectors is significant for the customers to save time. The facility can also provide additional cloning service to modify the available AAV vectors.

Biomedicum Functional Genomics Unit (FuGU) provides lentiviral and retroviral production services. FuGU offers streamlined production of high-titer concentrated lentivirus particles for stem cell and *in vivo* transduction purposes. The vectors for virus production originate either from the customer or from the genome-scale mouse and human TRC1 shRNA libraries licensed for and housed within FuGU. This unit has provided large number of glycerol stocks corresponding to TRC1 shRNA lentiviral backbones as a new service within the Genome-wide Methods Network and tightened its collaboration with Genome Biology Unit on the Viikki campus, University of Helsinki.

The Oulu facility scaled down post-doctoral positions from 1.5 to 0.5 owing to decreased funds. Biocenter Oulu (BCO) funding pays a salary for a M.Sc. level researcher and partial salary for a technician. Such budget reduction affected mainly user training capacity, and the number of virus preparations produced at the BCO Virus Core laboratory has continued to increase. In particular, generation of lentiviral vectors has become widely used at BCO Virus Core. BCO virus core also organized a practical course "Retrovirus-mediated RNA interference" where participants got hands-on introduction to RNAi methodology.

The Turku facility recruited a new technician. CRISPR technology has been developed as a service to local groups. The number of users has increased, which is mainly due to increased usage of lentivectors and retroviruses. Nonetheless, limited funds hinder the development of new viral technologies. The Tampere unit has consolidated its functions; it produced 70% more virus preparations and increases cell line production by 50% in comparison to 2013.

In A.I. Virtanen Institute, bioreactor-based virus vector production was developed further. Wave bag bioreactors have been used for adenoviral large-scale production, whereas solid stage bioreactors have been used for lentivirus and AAV production. This facility differs from the rest of the facilities by being able to produce large-scale vectors for translational studies. The demand of vectors has increased by 30% from the previous year. The Kuopio facility has also given basic training to several domestic and foreign students and also organized a one-week hands-on training course in Spring 2014 together with a Marie Curie AdVance viral vector training program.

Most significant bottlenecks. To meet the demand of downstream purification of large-scale vector production in the Kuopio facility, there is a clear need for a new chromatography and TFF units in the next 12 months, in order to keep up with the increased needs for gene transfer vectors by the customers. A foreseeable future bottleneck requiring attention is the worldwide trend in transition from current unstable RNAi-based approaches to more stable genome editing technologies in the field of reverse genetics. The main bottleneck in the Tampere facility is the absence of support personnel. In Turku facility, a major problem lack of available funds to develop new viral technologies. Similar needs for dedicated staff salaries to maintain high-level services are evident in all VGTCT core facilities.

Participation in international, Nordic and European infrastructures

A.I. Virtanen Institute facility is an integral part of Finnish Academy Center of Excellence and participates in several EU FP7 funded research consortia. BF-related operations are focused on the production of small and large-scale research-grade viral vectors for the use in in vitro and preclinical studies. AAV core and FuGU belong to Finnish Academy Center of Excellence and also participate in several EU FP7 consortia. EU and EFPIA Innovative medicines initiative (BREDECT) involves development of services lowering the bar for Finnish academic–global pharma industrial collaborations in the area of viral gene transfer services. Turku and Tampere core facilities also participate in EU FP7 projects.

Future perspectives

The need for high-quality preclinical and clinical vectors has increased steadily over the last years in the A.I. Virtanen Institute facility, with the prediction that this need will be doubled in EU research community during the next 3–5 years. Therefore, increasing the production and purification capacity of high-quality viral vectors is important for Finnish and European translational medical research and biotechnology applications.

To satisfy better the needs of the customers, acquisition of licenses to extend the range of AAV serotypes is planned. Moreover, future plans will involve constructing modified (shorter) Cas9 vectors (AAV-CRISPR/SaCas9) for *in vivo* gene-targeting studies and developing state-of-the-art genome editing technologies. The Turku facility is developing lentivirus-based CRISPR vectors to allow fast and robust genome targeting. They also foresee expansion of lentiviral production services beyond the local community. Tampere has plans to develop Sendai virus production and technology needed for vaccine research applications.

User statistics

VGTCT provided well-defined services to a large number of customers in 2014. Breakdown of the produced viral lots in VGTCT core facilities is shown in the Table.

	Helsinki			AIV Core	BCO	BMT	BioCity	Total
	AAV	Oncolytic	FuGu	Kuopio	Oulu	Tampere	Turku	
Customers	5	10	46	31	10	8	45**	155
local	2	4	32	10	10	7	44	109
domestic	1	2	12	7			1	23
international	2	2	1	12		1		18
non academic		2	1	2				5
Volume*	52	20	90	89	378	41	147	817
User fees (€)	26 502	NA	27 757	82 010	10 089	NA	14 132	160 419

*number of virus preps produced
**number of scientists, not research groups

Major publications supported by platform services

Husso T, Ylä-Herttuala S, Turunen MP. A new gene therapy approach for cardiovascular disease by non-coding RNAs acting in the nucleus. Mol Ther Nucl Acids 3: e1 DOI: 10.1038/mtna.2014.48, 2014.

Korpisalo P, Hytönen J, Laitinen J, Närväinen J, Rissanen T, Gröhn O, Ylä-Herttuala S. Ultrasound imaging with bolus delivered contrast agent for the detection of angiogenesis and blood flow irregularities. Am J Physiol Heart Circ Physiol 307: H1226–H1232, 2014.

Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, Glasauer A, Dufour E, Mutlu GM, Budigner GS, Chandel NS. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. Elife 3: e02242, 2014.

Eerola K, Rinne P, Penttinen AM, Vähätalo L, Savontaus M, Savontaus E. □-MSH overexpression in the nucleus tractus solitarius decreases fat mass and elevates heart rate. J Endocrinol 222: 123–136, 2014.

Koivisto E, Jurado Acosta A, Moilanen AM, Tokola H, Aro J, Pennanen H, Säkkinen H, Kaikkonen L, Ruskoaho H, Rysä J. Characterization of the regulatory mechanisms of activating transcription factor 3 by hypertrophic stimuli in rat cardiomyocytes. PLoS One 9: e105168, 2014.

Bramante S, Koski A, Kipar A, Diaconu I, Liikanen I, Hemminki O, Vassilev L, Parviainen S, Cerullo V, Pesonen SK, Oksanen M, Heiskanen R, Rouvinen-Lagerström N, Merisalo-Soikkeli M, Hakonen T, Joensuu T, Kanerva A, Pesonen S, Hemminki A. Serotype chimeric oncolytic adenovirus coding for GM-CSF for treatment of sarcoma in rodents and humans. Int J Cancer 135: 720–730, 2014.

Parviainen S, Ahonen MT, Diaconu I, Hirvinen M, Karttunen Å, Vähä-Koskela M, Hemminki A, Cerullo V. CD40-ligand and tdTomato armed vaccinia virus for induction of anti-tumor immune response and tumor imaging. Gene Ther 21: 195–204, 2014.

Aspelund A, Tammela T, Antila S, Nurmi H, Leppänen VM, Zarkada G, Stanczuk L, Francois M, Mäkinen T, Saharinen P, Immonen I, Alitalo K. The Schlemm's canal is a VEGF-C/VEGFR-3-responsive lymphatic-like vessel. J Clin Invest 124: 3975–3986, 2014.

Han J, Calvo CF, Kang TH, Baker KL, Park JH, Parras C, Levittas M, Birba U, Pibouin-Fragner L, Fragner P, Bilguvar K, Duman RS, Nurmi H, Alitalo K, Eichmann AC, Thomas JL. Vascular endothelial growth factor receptor 3 controls neural stem cell activation in mice and humans. Cell Rep 10: 1158–1172, 2015.

Huang Q, Whitington T, Gao P, Lindberg JF, Yuehong Yang Y, Sun J, Väisänen MR, Szulkin R, Annala M, Yan J, Egevad LA, Zhang K, Lin R, Jolma A, Nykter M, Manninen A, Wiklund F, Vaarala MH, Visakorpi T, Xu J, Taipale J, Wei GH. A prostate cancer susceptibility allele at 6q22 increases RFX6 expression by modulating HOXB13 chromatin binding. Nat Genet 46: 126–135, 2014.

MEMBER INSTITUTES

Biocenter Kuopio
University of Eastern Finland
Director: Professor Seppo Ylä-Herttuala
www.uef.fi/bck

Biocenter Oulu
University of Oulu
Director: Professor Johanna Myllyharju
www.biocenter.oulu.fi

Biocentrum Helsinki
University of Helsinki
Director: Professor Pekka Lappalainen
www.helsinki.fi/biocentrum

BioCity Turku
University of Turku and Åbo Akademi University
Director: Professor Jyrki Heino
www.biocity.turku.fi

Institute of Biotechnology
University of Helsinki
Director: Professor Tomi Mäkelä
www.biocenter.helsinki.fi/bi

BioMediTech
(before Institute of Biomedical Technology)
University of Tampere
Director: Hannu Hanhijärvi
www.uta.fi/bmt
www.biomeditech.fi

Institute for Molecular Medicine Finland FIMM
University of Helsinki
Director: Professor Olli Kallioniemi
www.fimm.fi

Biocenter Finland
P.O.Box 56
FI-00014 University of Helsinki
FINLAND

www.biocenter.fi