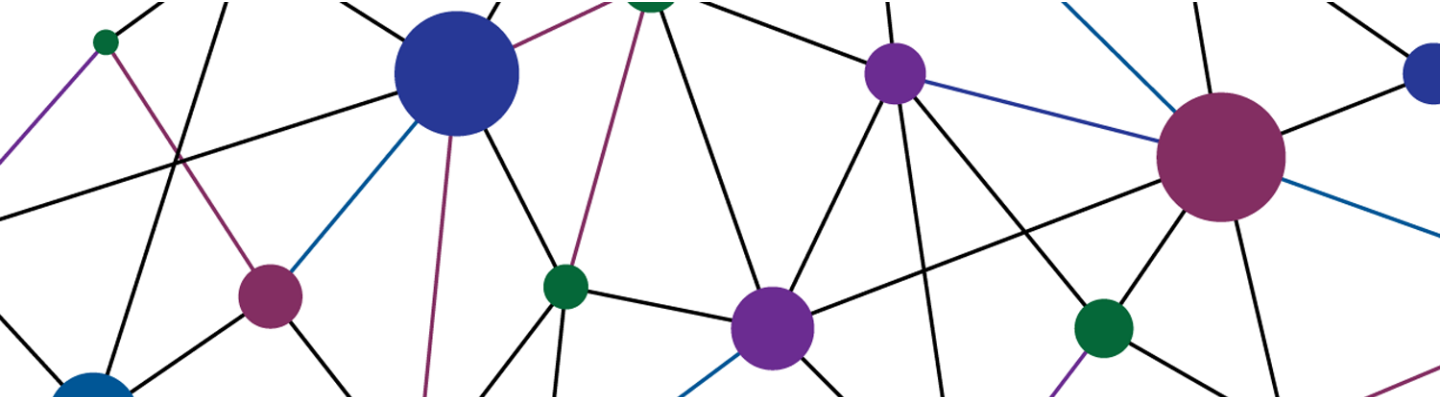




BioProNET

3rd Annual Science Meeting

October 19-20th 2016

**East Midlands Conference Centre
Nottingham, UK**



Welcome to the 3rd BioProNET annual science meeting

Welcome to BioProNET's 3rd annual science meeting, this year held at the East Midland's Conference Centre. We have an exciting line up of speakers, over half of which are international scientists, together with around 165 delegates. We are exceptionally pleased to welcome our two keynote speakers from the USA. Bill Barton is from Virginia Commonwealth University and Pete Tessier is from Rensselaer Polytechnic Institute, New York. These speakers are hosted by some of our early career researchers.

In addition, we have talks from BioProNET funding awardees, short talks from early career scientists and a poster session with over 50 posters that includes a drinks reception and networking session. Over dinner we have guest speaker Hansjörg Hauser as well as the unveiling of the recently commissioned BioProNET artwork by the artist Keith Robinson.

There are several exhibitors present at the conference — Applikon, BioPharma Process Systems, Eppendorf, Europa Bioproducts, Infors HT and Purolite — make sure you visit them for your chance to win a prize! More details on p9.

All of the meeting — presentations, lunch, dinner, posters, exhibitors, refreshments, drinks reception — will be held in the Banqueting Suite & Exhibition Hall in the Conference Centre; the space will be divided into two. Accommodation is in the Orchard Hotel, which is adjacent to the Conference Centre.

In this conference booklet you will find the agenda, speaker biographies, information about the exhibitors, details of the posters and early career and proof of concept talks, as well as some of BioProNET's recent achievements.

BioProNET currently has just over 700 members from academia, industry and other organisations. It is run by an executive group (Mark Smales, Charlotte Harrison, University of Kent; Alan Dickson, Joanne Flannelly, University of Manchester). It is overseen by a management board and executive group; more details can be found on our website www.biopronetuk.org

Enjoy the meeting and mark next year's meeting, Warwick October 10th-11th 2017, in your diary!



@BioProNETUK



www.biopronetuk.org



BioProNET

William Barton



William Barton is an associate professor at Virginia Commonwealth University (USA) in the Department of Biochemistry and Molecular Biology. His research focuses on angiogenesis, receptor tyrosine kinase signaling, protein engineering and method development. Techniques used in his lab include protein crystallography, FRET microscopy, molecular biology and protein chemistry. He obtained his B.S. degree in 1996 from Virginia Commonwealth University, USA, his Ph.D. in 2001 from the Graduate School of Medical Sciences at Cornell University, USA and from 2001–2004 he was a senior research fellow at the Sloan Kettering Institute, New York, USA.

Robert Roth



Robert is an associate principle scientist at AstraZeneca in Mölndal, Sweden. After completing a Ph.D. in Biochemistry at Lund University, Sweden, he started as a postdoctoral research scientist at AstraZeneca in 2003. He first worked on the expression and purification of membrane proteins as part of the initiative to establish this capability within AstraZeneca. Afterwards he had several roles concerning different aspects of protein expression in both prokaryotic and eukaryotic systems. For the last 5 years he has been responsible for the scientific and technical development of protein expression activities, supplying protein reagents to preclinical activities ranging from structural biology to testing of therapeutic proteins in animal disease models.

Mikael Rørdam Andersen



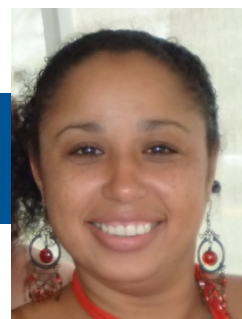
Mikael Rørdam Andersen trained with Professors Jure Piskur (Lund University, Sweden) and Jens Nielsen (Chalmers University of Technology, Sweden), and spent a sabbatical with the United States Department of Energy Joint Genome Institute. He is currently an associate professor and group leader in bioengineering at the Technical University of Denmark. Dr Andersen's research lies at the cross section of computational biology and synthetic biology. He is principle investigator on a project to whole-genome sequence all the 300+ unique species in the industrially and medically relevant *Aspergillus* genus of filamentous fungi. Furthermore, he is the coordinator of a trans-European Marie Skłodowska Curie Innovative Training Networks program on synthetic and systems biology of CHO cells for the production of pharmaceutical proteins.

Neil Bulleid



Neil Bulleid obtained his B.Sc. at the University of Liverpool and Ph.D. at the University of Glasgow in Biochemistry. He currently holds a chair in cell biology at the University of Glasgow and is the Director of the Institute of Molecular, Cell and Systems Biology. He has a Wellcome Trust senior investigator award and project grant funding from the BBSRC. He is an elected Fellow of the Royal Society of Edinburgh and holds a Royal Society/Wolfson merit award and currently is a member of the BBSRC pool of experts. His past achievements include the first indication that specific enzymes and proteins (chaperones) are involved in the folding of proteins into their three-dimensional structure. His research spans many areas of molecular cell biology, such as disulfide bond formation, collagen biosynthesis, MHC Class I assembly, protein folding in the cell, lipid attachment to proteins, oxidative stress and protein degradation.

Kathya de la Luz



Kathya de la Luz completed her Ph.D. at University of Havana, Cuba in collaboration with Simon Gaskell's group at University of Manchester, UK (now at Queen Mary University of London). She is the head of the Protein Analysis Department at the Center of Molecular Immunology in Cuba. She has experience in protein purification and characterization, mammalian cell culture proteomics and metabolic analysis. She has published around 25 papers in reputed journals and serves as an editorial board member.

Colin Jaques



Colin Jaques is a senior principal scientist at Lonza Biologics, leading a team in the Mammalian Process group of the Research and Technology department. Colin has a first degree in biotechnology from Imperial College London and has a Master of Science and a Ph.D. in biochemical engineering from University College London. Whilst in academia Colin's research interests revolved around microbial metabolism, initially studying the sulphur and nitrogen cycles in sewage bacteria and then moving onto industrial production of antibiotics in *Actinomycetes*. Colin has been at Lonza Biologics for 12 years working on the production of therapeutic proteins in mammalian cell culture. He has developed interests in alternative cell line selection systems, platform process development, process robustness, scale-up, ultra-scale-down models and single-use bioreactors.

Veronique Chotteau



Dr Chotteau has over 25 years of experience in mammalian cell culture including 10 years in the biopharmaceutical industry. Her expertise covers process development (perfusion, fed-batch, stem cell bioprocessing, small-, pilot- and commercial-scale, GMP). Between 1996-2008 she worked at Pharmacia Upjohn/Biovitrum, Stockholm (now Swedish Orphan Biovitrum) and had responsibilities including project manager for process development (e.g. recombinant factor VIII ReFacto antibody), business development support for the evaluation of new projects, head of pilot plant and as an expert in animal cell culture development. Since then, her group has focused on cell-based processes for biopharmaceutical production and on stem cell bioprocessing. Her group is involved in several projects of perfusion process at high cell density, metabolic flux analysis and the development of a fed-batch process for biopharmaceutical production.

Hansjörg Hauser



Hansjörg Hauser graduated in biology (Dr. rer. nat.) in 1977 at the University of Konstanz, Germany. He completed his postdoctoral training at the Max-Planck Institute for Molecular Genetics, Berlin, and at the German Cancer Research Center, Heidelberg. In 1981 Hansjörg Hauser became a staff scientist of the Helmholtz Centre for Infection Research, where he is now actively involved in the scientific strategy of the centre. Hansjörg studies events in transcription activation and signal transduction that mediate consequences of infections, as well as translational research concerning gene expression in biotechnology and gene/cell therapies. He is chair of European Society for Animal Cell Technology and the German Collection of Microorganisms and Cell Cultures, as well as a lecturer at the Universities of Oldenburg and the Medical University of Hannover, and guest professor at Lisbon University. He is co-ordinates several EU-funded projects and is a scientific advisor (case to case) for several companies and biotech start-ups.

Peter Tessier



Peter Tessier is the Richard Baruch M.D. Career Development Professor in the Department of Chemical and Biological Engineering and a member of the Center for Biotechnology and Interdisciplinary Studies at Rensselaer Polytechnic Institute in Troy, New York, USA. He received his B.S. in Chemical Engineering from the University of Maine, USA and his Ph.D. in Chemical Engineering from the University of Delaware, USA. Tessier performed his postdoctoral studies at the Whitehead Institute for Biomedical Research at Massachusetts Institute of Technology. Tessier's research focuses on designing, developing and optimizing antibodies. He has received a number of awards in recognition of his pioneering work, most recently including a Humboldt Fellowship for experienced researchers (2014-2015), a Biochemical Engineering Journal young investigator award (2016), and a young investigator award from the American Chemical Society (2015).

Jonathan James Phillips



Jonathan James Phillips is a senior research associate in the Department of Chemical Engineering and Biotechnology, University of Cambridge. His research interests include the structural dynamics of molecular systems, the design, engineering and development of therapeutic proteins, statistical mechanics of protein molecules, structural mass spectrometry, hydrogen/deuterium-exchange, and mathematical and structural modelling and simulation. He previously worked as a post-doctoral research fellow at MedImmune, developing novel methods to understand protein dynamics, and at the University of Sussex and Queen Mary, University of London, where he designed a self-assembling protein scaffold that possessed natural biological functionality. Whilst a research associate at the University of California, Berkeley, USA he developed methodology for the determination of the atomic-level interactions between biological molecules and inorganic surfaces.

Mike Davies



Mike has extensive experience in the development and manufacture of recombinant protein therapeutics. Currently he is vice president, Protein Science at F-star Biotechnology, a clinical stage biopharmaceutical company developing novel bi-specific antibodies (mAb²) for immuno-oncology through the application of its highly efficient modular antibody technology platform. Prior to this he was head of analytical strategy at the National Biologics Manufacturing Centre, a UK-based technology innovation centre that is part of the Centre for Process Innovation and the UK Catapult network. Previously, he worked at Lonza Biologics where he held numerous leadership positions including head of analytical services.

Lucy Beales



Lucy Beales is a senior scientist at Mologic, leading a molecular biology and protein expression team at the York facility. After gaining a Ph.D. in virology at the National Institute for Biological Standards and Control, Lucy spent several years as a post-doctoral scientist at Leeds University and the University of Texas Medical Branch (Galveston, Texas, USA). Lucy then moved to industry where, for the past 10 years, she has led virus-like particle-based vaccine development teams, overseeing the process from concept, through construct design and production to deliver candidate vaccines that are suitable for industrial-scale production.

Dan Bracewell



Daniel Bracewell is Professor of Bioprocess Analysis at University College London in the Department of Biochemical Engineering. He has made major contributions to the fundamental understanding of biopharmaceutical purification operations, generating over \$7 million in research funds including new international research collaborations with India and the USA. He has authored more than 70 peer reviewed journal articles in the area and currently supervises 15 doctoral and postdoctoral research projects; many of these studies are in collaboration with industry. Other outputs from his group include a spin-out company — Puridify — which develops novel separations materials for bioprocessing. Prof. Bracewell received his Ph.D. in biochemical engineering in 1998 from University College London, after undergraduate studies at Imperial College London.

Jürgen Hubbuch



Jürgen Hubbuch is a professor at the Institute of Bio- and Food-Technology in the Department of Biomolecular Separation Engineering at the Karlsruhe Institute of Technology, Germany. His research focuses on all aspects of modern downstream processing: protein purification, formulation as well as analytics in the biopharmaceutical industry. His work ranges from assessing structural parameters of proteins on a molecular level, transport and surface interaction phenomena of proteins, purification and characterization of bio-nanoparticles to industrial process development. He has previously been Head of Department (Separation Engineering) at the Institute for Biotechnology, Jülich Research Centre, as well as a group leader in downstream processing at the Institute for Enzyme Technology, Heinrich-Heine University, Düsseldorf. He obtained his Ph.D. at the Center for Process Biotechnology at the Technical University of Denmark, and his M.Phil. from Heriot-Watt University in Edinburgh.

Tim Dafforn



Tim Dafforn has established himself as an expert in biophysical spectroscopy with a keen interest in synthetic biology. Professor Dafforn has several research interests, including insights into the assemblies that underlie bacterial cell division, a novel method that trivializes the production of membrane proteins enabling advances in bioprocessing, and also the development of a platform bioassay that represents one of the first commercial applications of synthetic biology. He is currently the director of knowledge transfer for the College of Life and Environmental Sciences at the University of Birmingham.

Exhibitors

Visit each exhibitor for your chance to win a prize!

Get each exhibitor to stamp your card (found behind your name badge) then place it in the box.

1. Applikon



Applikon Biotechnology is a world leader in the development and supply of advanced bioreactor systems and is renowned for bringing new technologies to the market.

These technologies offer advantages in high throughput Research & Development applications, as well as pilot plant and production scale processes, where we have system solutions from a few millilitres to 2,000L. At BioProNET we will be exhibiting our Appliflex single use rocking bioreactor and the I line F for smart cell imaging from Ovizio for cell culture applications.

We look forward to meeting you during this conference.

2. BioPharma Group



The Biopharma Group comprises of Biopharma Process Systems (BPS), Biopharma Technologies Ltd (BTL), Biopharma Technology LLC and Biopharma Technologies France (BTF).

Equipment Sales & Service Division: BPS

BPS is a leading supplier of equipment to the pharmaceutical, biotech and process industries in the UK, Ireland and France for freeze drying, solvent removal/evaporation, high pressure homogenisation technologies and industry related equipment.

Our aim is to provide equipment and services that best meet your process requirements and to remain on-hand for assistance thereafter; our in-house service/maintenance department enables us to support you throughout the life of your equipment.

Independent Consultancy Division: BTL

The BTL division provides independent R&D, analysis, process, product and cycle development services, training and analytical instrumentation to the global biopharmaceutical and related industries.

Together with our knowledge of pilot-scale and industrial freeze-dryers we offer a uniquely comprehensive service and training courses covering all aspects of freeze-drying from pre-formulation through to full-scale production and dried product analysis.

Our philosophy is to augment your expertise and work with you to make your project a success.

The BTF division combines elements from equipment sales and access to the expertise of the consultancy division, giving our French-speaking clients a one-stop option.

3. Eppendorf



Eppendorf is a leading life science company. It was founded in Hamburg, Germany in 1945 and has more than 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

Eppendorf - We Know Bioprocessing By exploiting the strong synergies in bioreactor technology and polymer manufacturing, Eppendorf has emerged as a global player and valuable resource to its customers in the bioprocess marketplace. With a comprehensive offering of single-use and traditional products for the growth of mammalian, microbial, insect, plant and algae cells, and working volumes of 60 mL – 2,400 L, the Eppendorf bioprocess portfolio can satisfy the demands of process development through production.

4. Europa Bioproducts



Advancing Glycoscience: Europa Bioproducts is the exclusive European distributor for ProZyme, who manufactures a comprehensive range of reagents used for glycan profiling and characterisation of therapeutic monoclonal antibodies and proteins. ProZyme's flagship product line is the glycobiology portfolio, alongside streptavidin, phycobiliprotein and conjugate offerings. The product range of glycoanalysis products was enhanced in 2003 with the purchase of Glyko. ProZyme has maintained a commitment to invest in, develop, release and support products in the exciting and rapidly-expanding area of glycobiology. At the BioProNET Symposium will present two novel tools for N-Glycan profiling:

Gly-Q Glycan Analysis System Gly-Q is a small, simple, user friendly, low-maintenance capillary electrophoresis system with an easily-replaceable gel cartridge.

Gly-X with InstantPC for LC/MS The Glyko-X InstantPC kit utilizes a novel in-solution protein deglycosylation followed by rapid labelling of released N-glycans with InstantPC dye. After a simple clean up step, the samples are ready for analysis by LC, LC-MS, and other methods. The InstantPC dye delivers unmatched fluorescent brightness and MS signal, which enables a single labelling method to be deployed across different glycan analysis workflows.

Glycan Analytical Services Europa/ProZyme also offers analytical support services to their European customers. We offer screening services for hundreds of samples using UPLC or CE as well as in depth characterisation of N-Glycans using UPLC/MS, exoglycosidases and also domain-specific analysis of antibodies.

5. INFORS HT



INFORS HT have been specialists in shakers, incubator shakers and bioreactors for over 50 years, with a subsidiary present in the UK since 1987. The current headquarters for Infors UK is on a small quiet farm in Reigate. We have local offices in Wigan, Manchester and Edinburgh as well as exclusive distributors in Ireland. All sales personnel have a breadth of laboratory experience and really understand our products, not only from a sales point of view but also how they are best used to optimise performance.

Support starts from the initial sales call to ascertain need, to the sale, and extends to installation and beyond. Our Bioreactor Product Specialist for instance is always available to talk specific applications, help with installation and training and then afterwards, to help with any technical or application modifications. We have extensive service experience and can offer preventative maintenance as well as emergency call-outs or even simple telephone help and advice. Infors UK operates a 'First Visit Fix' policy.

The equipment is versatile, innovative and scalable with quality Swiss engineering at its core. Our comprehensive range of products can meet both everyday needs and specialist applications such as mammalian cell culture, solid state fermentations and algal biofuel solutions. We believe in working closely with our customers to specify, support and improve our products based on input from real experts, i.e. our users.

6. Purolite



Purolite is the only globally-acting company that focuses exclusively on advanced resin technology. The company has more than 35 years of experience in providing resin solutions to their customers, with dedicated R&D and manufacturing facilities in USA, China, UK and Romania.

Purolite Life Sciences, started in 2012, supports R&D and production-scale applications in pharmaceuticals, food production, bioprocessing, fine chemical and other markets.

Brands for Life Sciences include Purolite APIs and adsorbents, Chromalite polymeric resins, Lifetech ECR resins for enzyme immobilization, PuroPhase SPE Reverse Phase products for Solid Phase Extraction, and Praesto agarose-based ion exchange, affinity and plain base resins for MAb processing and recombinant protein purification.

Posters listed by surname of presenting author (bold)

1. Population balance model to understand the dynamics of fed-batch CHO cell culture

S. Alhuthali, S. Fadda, C. H. Goey, C. Kontoravdi

Department of Chemical Engineering, Imperial College London

2. Optimisation of toxin production in *E. coli*

Rachel Atherley

University College London

3. Optimising expression of the anti-HIV antibody VRC01 in *Pichia pastoris*

Rochelle Aw¹, Paul F. McKay², Robin J. Shattock², Karen M Polizzi¹

¹Centre for Synthetic Biology and Innovate, Department of Life Sciences, Imperial College London ²Department of Infectious Diseases, Imperial College London, London

4. Gene maintenance mechanism in chloroplasts for chloroplast biotechnology application

Tengku Nurfarhana Binti Tengku Aziz

University of Manchester

5. Synthetic biology platform development for CHO cell design and engineering

Claire Bryant¹, Joseph Cartwright¹, Claire Harris², Greg Dean², Diane Hatton², David James¹

¹Department of Chemical and Biological Engineering, University of Sheffield, ²Cell Culture and Fermentation Sciences, Biopharmaceutical Development, MedImmune, Granta Park, Cambridge,

6. CamOptimus: Self-contained user-friendly multi-parameter optimisation platform for non-specialist experimental biologists

Ayca Cankorur-Cetinkaya¹, Duygu Dikicioglu¹, Joao M L Dias^{2,3}, Nigel K.H. Slater⁴, Stephen G. Oliver¹

¹Cambridge Systems Biology Centre & Department of Biochemistry, University of Cambridge ²Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge ³Department of Haematology, Cambridge University Hospitals NHS Trust, ⁴Department of Chemical Engineering & Biotechnology, University of Cambridge

7. Microscale refolding of recombinant proteins

Mark Carlile

Faculty of Applied Sciences, University of Sunderland

8. Improving protein yield from mammalian cells by manipulation of stress response pathways

Fiona Chalmers¹, Neil Bulleid¹, Katharine Cain²

¹Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow,

²UCB Pharma, Slough

9. Poster title not available

Alysia Davies

Newcastle University

10. Expression of recombinant proteins in the chloroplasts of microalgae and plants

Anil Day¹, Tariq Ali², Leopoldo Herrera Rodriguez¹, Mohammad El Haj¹, Elena Martin Avila¹, Alejandra Mendez Leyva¹, Elisabeth Mudd¹, Julio Suarez¹, Farid Khan²

¹School of Biological Sciences, University of Manchester ²Protein Technologies, Manchester Science Park

11. Pulling apart alpha synuclein

Ciaran P. A. Doherty^{1,2}, Lydia Young^{1,2}, Oliver Durrant³, Sheena E. Radford^{1,2}, David J. Brockwell^{1,2}

¹Astbury Centre for Structural Molecular Biology, University of Leeds, ²School of Molecular and Cellular Biology, University of Leeds, ³UCB Celltech, Slough

12. Integrated production and separation of sophorolipid biosurfactant

Ben Dolman and James Winterburn

University of Manchester

Posters listed by surname of presenting author (bold)

13. Analysis of host cell protein impurities using *in silico* approaches

Stefani Dritsa¹, Dan Bracewell² and Mark Wass¹

¹School of Biosciences, University of Kent, ²Department of Biochemical Engineering, University College London

14. Exploring protein conformational stability using Raman spectroscopy and 2D-correlation moving windows

Ilokugbe Ettah, Lorna Ashton

Lancaster University

15. B-cell epitope profiling differentiates immunogenic responses to protein aggregates

Tim Eyes¹, Rebecca Dearman¹, Ian Kimber¹, Noel Smith², Jeremy Derrick¹

¹ Faculty of Biology Medicine Health, University of Manchester, ²Lonza, Applied Protein Services, Cambridge

16. The development of high density CHO cell culture manufacturing systems

Isabelle Evie, Paul Young and Alan Dickson

University of Manchester

17. Protein aggregation as a consideration for heterologous protein production in yeast

Sarah A. S. Fareeth, Reem Swidah, Chris M. Grant, Mark P. Ashe.

Faculty of Life Sciences, University of Manchester

18. Bioprocessing biologically synthesised magnetic nanoparticles: production and purification of magnetosomes

Alfred Fernández-Castané^{1,2}, Hong Li¹, Owen RT Thomas¹, Tim W Overton^{1,2}

¹School of Chemical Engineering, University of Birmingham ²Institute for Microbiology & Infection, University of Birmingham

19. The *Bacillus subtilis* TatAdCd system exhibits an extreme level of substrate selectivity

Kelly Frain¹, Colin Robinson¹, Ray Field², Ronald Schoner²

¹School of Biosciences, University of Kent, ²MedImmune

20. Combinatorial genome editing to create enhanced biomanufacturing platforms

University of Manchester

Claire E. Gaffney¹, Samia Akhtar¹, Catherine Page², Bruno Fievet², Suzanne Robb³, Clare Trippe³, Jonathan Welsh³, Julie Anderson³, Rachael Hubery³, Richard Alldread³, Mark Stockdale², Dirk Gewert², Alan J. Dickson¹

¹Manchester Institute of Biotechnology, University of Manchester, ²Horizon Discovery, Cambridge Research Park, ³CPI Darlington

21. Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins

Tim Ganderton, Gill Higgins and Marek Brzozowski

York Structural Biology Laboratory, Department of Chemistry, University of York

22. Translational reprogramming in recombinant Chinese Hamster Ovary cells.

Charlotte Godfrey¹, Emma Hargreaves¹, Gary Pettman², Ray Field², Diane Hatton², Lekan Daramola², Sarah Dunn², Mark Smales¹

¹School of Biosciences, University of Kent, Canterbury, Kent, ²MedImmune, Granta Park, Cambridge

23. Nanofabricating: scale up of ultra-small glycan coated gold nanoparticles

Africa G. Barrientos, Midatech Pharma España and **Juliana Haggerty**

Centre for process Innovation together with project partners Midatech Pharma Group, ProChimia Surfaces, GalChimia, Centre for BioNano Interactions – University College Dublin, Applus+ Laboratories, IFOM - The FIRC Institute of Molecular Oncology, Ecole Polytechnique Fédérale de Lausanne

Posters listed by surname of presenting author (bold)

24. Perfluorocarbons - potential for the successful expansion of human mesenchymal stem/stromal cells in a two phase system

Mariana P. Hanga¹; A. W. Nienow^{1,2,3}, K. Coopman¹; C.J. Hewitt^{1,3}

¹Centre for Biological Engineering, Chemical Engineering Department, Loughborough University, ²School of Chemical Engineering, University of Birmingham, ³School of Life and Health Sciences, Aston University, Birmingham

25. Translational regulation of recombinant protein expression in monoclonal CHO Flp-In cell lines

C. M. Smales and **E. J. Hargreaves**

Industrial Biotechnology Centre, University of Kent

26. Mapping the aggregation behaviour of biopharmaceuticals: a new approach

Sarah Hedberg, J. Heng and D. Williams.

Imperial College London

27. How to get inclusion bodies into a 96 well plate

Fiona Baker¹, Mark Carlile¹, **Charles Heise**², Jonathan Rapley²

¹Department of Pharmacy, Health and Well-Being, University of Sunderland, ²Fujifilm Diosynth Biotechnologies, Billingham

28. Circumvention of an electron bifurcating complex results in increased solvent productivity in *Clostridium acetobutylicum*

Ryan Hope, Klaus Winzer and Nigel Minton

University of Nottingham

29. Identifying opportunities in cell engineering for the production of 'difficult to express' recombinant proteins

Hirra Hussain¹, Alan J Dickson¹, Mark Abbott^{2*}, Robert Roth³, David Fisher²

¹University of Manchester, United Kingdom, ²AstraZeneca, Cambridge, ³AstraZeneca, Mölndal, Sweden *Now at Peak Proteins, Alderley Park, Macclesfield

30. Image correlation spectroscopy analysis: promising screening tools to predict protein aggregation

Maryam Hussain¹, Alain Pluen¹, Robin Curtis¹, Chris van der Walle², Katie Day²

¹University of Manchester ²MedImmune

31. Proofreading of substrate structure by the twin-arginine translocase is highly dependent on substrate conformational flexibility but surprisingly tolerant of surface charge and hydrophobicity

Alexander S Jones¹, Colin Robinson¹, James I Austerberry², Rana Dajani², Jim Warwicker², Jeremy P Derrick², Robin Curtis³

¹School of Biosciences, University of Kent, Canterbury, ²Faculty of Life Sciences, University of Manchester, ³School of Chemical Engineering and Analytical Science, University of Manchester

32. Expanding production time of mammalian cell cultures for biotechnological applications

Lyne Josse, Martin Michaelis, Anastasios Tsaousis, Mark Wass, Emma Hargreaves

School of Biosciences, University of Kent

33. Bio-engineering of *E.coli* flagellar type III secretion system (fTSS) for maximal efficiency of protein secretion

Nitin Kamble, Charlotte Green, Graham Stafford

School of Clinical Dentistry and School of Chemical and Biological Engineering, University of Sheffield.

22. Bioreactor design space identification with product quality constraints

Cher Hui Goey¹, Oleksiy V. Klymenko², **Cleo Kontoravdi**¹

¹Centre for Process Systems Engineering, Department of Chemical Engineering, Imperial College London ²Department of Chemical and Process Engineering, University of Surrey, Guildford

35. Microalgae carbon uptake in microbial consortia

Rahul Vijay Kapoor¹, Gloria Padmaperuma¹, Sara Ortiz de Landazuri¹, Daniel J. Gilmour² and Seetharaman Vaidyanathan¹

¹ChELSI Institute, Advanced Biomanufacturing Centre, Department of Chemical and Biological Engineering, University of Sheffield ²Department of Molecular Biology and Biotechnology, University of Sheffield

Posters listed by surname of presenting author (bold)

36. Development of novel methods for periplasmic release of biotherapeutic products

Julia Kraemer

University of Birmingham

37. Improved downstream operation through formulation innovation

John Liddell, Tibor Nagy¹, James Pullen¹, Nick Darton², Dave Gerring²

¹Fujifilm Diosynth Technologies, ²Arecor

38. Multi-omic modeling of translational efficiency for synthetic gene design

J. Longworth, J. Gonzalez, P. Dobson, J. Noirel, N. Lawrence, M.J. Dickman, D. James

Department of Chemical and Biological Engineering, University of Sheffield

39. Intellectual property of Kyoto University, Japan, in bioprocessing

Takashi Matsuura

Kyoto University European Centre, London Office

40. Controlling terminal sialylation of a monoclonal antibody through culture conditions

Calum McIntosh¹, Karen Polizzi^{2,3}, Alison Mason⁴, Christopher Sellick⁴, Cleo Kontoravdi¹

¹Department of Chemical Engineering, ²Division of Molecular Biosciences, Department of Life Sciences,³Centre for Synthetic Biology and Innovation, Imperial College London, ⁴Department of Cell Sciences, MedImmune

41. The delivery of therapeutic cytokines into the CNS using gold nanocarriers

Conor McQuaid¹, David Male¹, Ignacio Romero¹, Meike Roskamp²

¹Open University, Milton Keynes and ²Midatech, Abingdon

42. Quality control by the *E.coli* Tat protein export system

Daphne Mermans and Colin Robinson

School of Biosciences, University of Kent

43. Molecular imprints for the detection of specific glycoproteins implicated in cancer

Philippa Mitchell, Lewis Hart, Paula Mendes

School of Chemical Engineering, University of Birmingham

44. Enhancing recombinant protein secretion and quality in CHO cell bioprocessing

Ruth Morris¹, Alan Dickson¹, Lisa Swanton¹, Katharine Cain² and Bernie Sweeney²

¹University of Manchester, ²UCB

45. Interrogating recombinant protein expression in CHO cells using inducible systems

Macarena Mosqueira-Dinamarca and Alan Dickson

School of Chemical Engineering and Analytical Sciences, University of Manchester

46. Engineering of the secretory pathway of CHO cell lines

Théo Mozzanino and C. Mark Smales

School of Biosciences, University of Kent

47. Liquid or gel microcarriers as a novel system for expansion of a variety cells

Halina Murasiewicz¹, Andrzej Pacek¹, Alvin Nienow¹, Mariana Hanga², Karen Coopman², Christopher J. Hewitt³

¹Chemical Engineering, University of Birmingham, ²Centre for Biological Engineering, Loughborough University, ³Aston Medical Research Institute, Aston University, Birmingham

48. Protein degradation under inhibition of deglycosylation

Sarah Needs¹, Dominic Alonzi², Martin Bootman¹, Sarah Allman¹

¹Department of Life, Health and Chemical Sciences, The Open University, ²Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford

Posters listed by surname of presenting author (bold)

49. Manipulation and exploitation of microRNAs for enhanced recombinant protein production in Chinese hamster ovary cells

Tulshi Patel¹, Lyne Jossé¹, Mark Smales¹, Robert Young²

¹ School of Biosciences, University of Kent, Canterbury, ²Lonza Biologics, Great Abington, Cambridge

50. Engineering a novel protein nanopore for single molecule DNA sequencing applications

Michael R Hodgkinson¹, Paulina Dubiel¹, Joseph Lloyd², Mark Bruce¹, Andrew Heron¹, James P.J. Chong¹, **Michael J Plevin**¹

¹Department of Biology, University of York, ²Oxford Nanopore Technologies, Oxford Science Park

51. Functional protein surfaces on gold

Timothy Robson

Newcastle University

52. Process improvement of toxin production

Michael Sulu

University College London

53. Rheological and cell population monitoring using an in-line ultrasonic sensor

Joseph Newton¹, **Joanna Vlahopoulou**² and Yuhong Zhou¹

¹Department of Biochemical Engineering, University College London, ²Procellia, North East Technology Park

54. Sequence-dependent protein synthesis quality in two microbial expression hosts

Lyne Josse, Connor Sampson, Kevin Howland, **Tobias von der Haar**

School of Biosciences, University of Kent, Canterbury

55. The protein-sol server for protein solubility prediction

Max Hebditch, Alejandro Carballo, Spyros Charonis, Robin Curtis and **Jim Warwicker**

University of Manchester

56. Society for Chemical Industry

No authors listed

Talks by early career researchers

Manipulation and exploitation of microRNAs for enhanced recombinant protein production in CHO cells

Tulshi Patel¹, Lyne Jossé¹, Robert Young² and C. Mark Smales¹

¹School of Biosciences, University of Kent, Canterbury, ²Lonza Biologics, Great Abington, Cambridge

The key themes for this talk will include CHO microRNAs and how we can use our quality of the bio-therapeutics the cells are producing. The talk will focus on the use of sponge/miR knockdown constructs to enhance mAb productivity and the use of miR over-expression to enhance host CHO cell line growth.

Bioprocessing biologically synthesised magnetic nanoparticles: production and purification of magnetosomes

Alfred Fernández-Castané^{1,2}, Hong Li¹, Owen Thomas¹, Tim Overton^{1,2}

¹School of Chemical Engineering and ²Institute for Microbiology & Infection, University of Birmingham

Biologically synthesized magnetic nanoparticles, namely magnetosomes can be used in a wide range of biotechnological and healthcare applications and represent an attractive alternative to existing commercial magnetic particles. Here, we present a robust platform for the production and purification of magnetosomes from *Magnetospirillum gryphiswaldense* MSR-1. Our work represents a significant advance in manufacturing base magnetosomes, paving the road toward a sustainable and cost-effective bioprocess.

Synthetic biology platform development for CHO cell engineering

Claire Bryant¹, Joseph Cartwright¹, Claire Harris², Greg Dean², Diane Hatton², David James¹

¹Department of Chemical and Biological Engineering, University of Sheffield, ²Cell Culture and Fermentation Sciences, Biopharmaceutical Development, MedImmune, Cambridge

This talk will highlight the development of a high-throughput platform capable of increasing the manufacturability of difficult to express recombinant proteins. It will discuss an investigation into multi-plasmid transfection & stoichiometry.

Mapping the aggregation behaviour of biopharmaceuticals: a new approach

Sarah Hedberg, J. Heng, D. Williams

Imperial College London

We determined the osmotic second virial coefficient, B_{22} , using self-interaction chromatography and we correlated this to the aggregation behaviour observed using regular size-exclusion chromatography and dynamic light scattering. When comparing this data we found very interesting and accurate predictions from our B_{22} values to the aggregation development over time, using only very small amounts of protein.

Combinatorial genome editing to create enhanced mammalian biomanufacturing platforms

Claire E. Gaffney¹, Samia Akhtar¹, Catherine Page², Bruno Fievet², Suzanne Robb³, Clare Trippe³, Jonathan Welsh³, Julie Anderson³, Rachael Hubery³, Richard Alldread³, Mark Stockdale², Dirk Gewert², Alan Dickson¹

¹Manchester Institute of Biotechnology, University of Manchester, ²Horizon Discovery, Cambridge Research Park, ³CPI Darlington

We are using genome editing tools (CRISPR/Cas9 and rAAV) to create a toolbox of engineered CHO cells with enhanced biomanufacturing capabilities, intended to decrease the cost of production for existing biopharmaceuticals, and to broaden their capacity to meet the challenges of novel products. Favourable host phenotypes from single gene edits will be combined to further increase performance, where target parameters include increased biomass, culture length metabolic efficiency, product titre and quality.

Optimising expression of the anti-HIV antibody VRC01 in *P. pastoris*

Rochelle Aw¹, Paul McKay², Robin Shattock², Karen Polizzi¹

¹Centre for Synthetic Biology and Innovate, Department of Life Sciences and ²Department of Infectious Diseases, Imperial College London, London

We have expressed the broadly neutralising anti-HIV antibody VRC01 in *P. pastoris*. We have also shown for the first time that not only is it possible to use the murine IgG1 secretion signal, but that yields are higher than when utilising the alpha-mating factor signal peptide from *Saccharomyces cerevisiae*, which is the most common choice.

Proof of concept talks

Analysis of host cell protein impurities using *in silico* approaches

Stefani Dritsa¹, Dan Bracewell² and Mark Wass¹

¹School of Biosciences, University of Kent, ²Department of Biochemical Engineering, University College London

Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins

Tim Ganderton, Gill Higgins and Marek Brzozowski

York Structural Biology Laboratory, Department of Chemistry, University of York

Save the date 2017!

The next BioProNET annual science meeting will be held at the Scarman Conference Centre, Warwick, October 10–11th 2017



Funding opportunities from BioProNET

Proof of concept

The next call for proof of concept funding is currently open, and closes Friday 4th November. Funding of up to £100,000 is available.

Business interaction vouchers

The next deadline for business interaction vouchers is Friday 13th January.

Funding of up to £10,000 is available, this must be matched by an industry contribution (either cash or in-kind).

Workshop funding

Funding of up to £2,000 is available for collaboration-building workshops; this is an open call.

Scientific exchange

We also have scientific exchange funding for early career researchers of up to £500; this is an open call.

For more information see <http://biopronetuk.org/funding/>

So far, the business interaction vouchers that have awarded by BioProNET have been matched by £165,000 of industry funding, and proof of concept funded-projects have received £167,000 from industry.

See the success stories and case studies towards the back of this booklet to find out more about projects that have been funded by BioProNET



Agenda day 1

11.20	Welcome
11.30	Keynote speaker – William Barton (Virginia Commonwealth University, USA) <i>Over-expression of secreted proteins from mammalian cell lines</i> Hosted by Shraddha Rane and Baojun Wang
12.15	Lunch
	Designing efficient cell-expression systems Chaired by Pete Tessier
13.00	Robert Roth (AstraZeneca, Sweden) <i>Using phenotypic screening to identify regulators of recombinant protein expression</i>
13.25	Mikael Rørdam Anderson (Technical University of Denmark) <i>Networks: The key to understanding and engineering CHO protein secretion</i>
13.50	Neil Bullied (University of Glasgow) <i>Optimising the design and production of therapeutic antibodies</i>
14.15	Short presentations from BioProNET funding awardees: Talk 1 – Tim Ganderton (University of York) <i>Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins</i> Talk 2 – Stefani Dritsa (University of Kent) <i>Analysis of host cell protein impurities using in silico approaches</i>
14.30	Coffee and networking
	Building expression systems into optimised processes Chaired by Robert Roth
15.15	Kathya de la Luz (Centre of Molecular Immunology, Cuba) <i>Linking the cell metabolism and recombinant protein expression in mammalian cell lines</i>
15.40	Colin Jaques (Lonza) <i>Scale-up in the single use age: design matters</i>
16.05	Veronique Chotteau (Institute of Technology Stockholm, Sweden) <i>High cell-density perfusion for biopharmaceutical production – challenges for tomorrow's processes</i>
16.30	Short presentations from early career researchers (7 minutes each) Tulshi Patel (University of Kent) Alfred Fernández-Castané (University of Birmingham) Claire Bryant (University of Sheffield) Sarah Hedberg (Imperial College London) Claire Gaffney (University of Manchester) Rochelle Aw (Imperial College London)
17.20	Break – hotel check-in and networking
18.00	Poster session and drinks reception, prize judging for best posters
19.30	Dinner, with guest speaker Hansjörg Hauser, Helmholtz Centre for Infection Research, Germany and unveiling of BioProNET artwork with artist Keith Robinson

Agenda day 2

- 9.00** Keynote speaker – **Pete Tessier** (Rensselaer Polytechnic Institute, USA) *Improved antibody design, evolution and selection methods for minimizing developability issues*
Hosted by Julia Kraemer and Charlotte Godfrey
- Molecular characterization of process quality**
Chaired by Jurgen Hubbuch
- 9.45** **Jonathan J Phillips** (University of Cambridge) *Engineering the surface properties of a human monoclonal antibody prevents self-association and rapid clearance in vivo*
- 10.10** **Mike Davies** (F-Star) *Overcoming the manufacturing challenges for bi-specific mAbs*
- 10.35** **Lucy Beales** (Mologic) *Overcoming development challenges in the development of VLP-based vaccines*
- 11.00** Coffee and networking
- Upstream meets downstream: an integrated vision**
Chaired by Mikael Rørdam Anderson
- 11.30** **Dan Bracewell** (University College London) *Nanofibres in bioprocessing: a single-use chromatography format by the use of rapid cycling*
- 11.55** **Jurgen Hubbuch** (Karlsruhe Institute of Technology, Germany) *High-throughput downstream process development*
- 12.20** **Tim Dafforn** (University of Birmingham) *Nanoencapsulation for the production of membrane- and periplasmic-trafficked proteins*
- 12.45** Prize giving, lunch and meeting close

BioProNET artwork

Keith Robinson



As part of our outreach activities, BioProNET has commissioned four pieces of artwork by the artist Keith Robinson. These will be unveiled, during the conference dinner.

Keith lives in Surbiton, Kingston upon Thames. His portrait 'Stanley on a Painter's Rag', has recently exhibited in the 2016 B.P. Portrait Exhibition at the National Portrait Gallery in

London: <http://www.npg.org.uk/whatson/bp2016/exhibition/exhibitors-entries/stanley-on-a-painters-rag.php> This was the third time he has been selected for this internationally acclaimed competition.

He has completed many portrait commissions, large and small, and have also exhibited works in numerous exhibitions over the last few years, notably The National Art Open, The Threadneedle Prize, The Lynn-Painter Stainers Prize and The Discerning Eye.

Looking forward, he will be exhibiting 'Younome - A personalized Genome in 25 self portraits' along the King's Mile throughout this year's Canterbury Festival, which runs from 15th October to 5th November.

www.keithrobinsonpainting.com

Don't forget to visit the exhibitors for a chance to win a prize!
Get your card (found with your name badge) stamped by each exhibitor.



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Success stories and case studies

Over the next few pages we've reflected upon some of BioProNET's successes and achievements. There are also several case studies of BioProNET-funded projects.

Early career researchers' event 2016

This year's early career researcher event was held at the Brighton Mercure Seafront in September, and was attended by thirty six BioProNET members.

The programme reflected feedback from delegates at last year's highly successful meeting; the focus was on CVs, cover letters, being interviewed and interviewing, and preparation for job applications, as well as presentation skills.

We were pleased to welcome Martin Popplewell and his team from [Coconut Communications](#) to the event to deliver the media training sessions. Martin has more than 25 years of experience working in journalism, including at the BBC, Sky News and ITN.

In the media training session, delegates worked in small groups (6 people) with a trainer in a practical session where they prepared for an interview, were interviewed and then received a personal review and critic of their performance.

"The media training opportunity to practice what was taught was fantastic"

"The interview clinic was really helpful; so were the talks which helped me think about my career path"



Outreach

BioProNET at Big Bang @ Discovery Park

Earlier this year BioProNET took part in a science fair at Discovery Park in Kent, which aimed to inspire students to study STEM (science, technology, engineering and maths) subjects.

Around 900 school children, aged 11-14 attended the event, and many learned the difficulties in making antibody-based medicines by trying to make replica biologics out of modelling balloons.

The event was covered in a [local newspaper](#) and by [Kent and Medway STEM](#), including some pictures of the students' models. Although the event was called 'Big Bang' we're happy to report that not too many of our balloons burst!

BioProNET at Chemistry at Work

BioProNET, together with the University of Kent School of Biosciences, also took part in a 2-day 'Chemistry at Work' event organised by the Royal Society of Chemistry and Canterbury Christ Church University.

Students learned about plasmid DNA, got hands on-experience of size exclusion chromatography and investigated protein folding with the aid of modelling balloons. Photos and a report of the event can be found here

<http://www.kentandmedwaystem.org.uk/index.php/events/report/792/>



We have funding available if you would like to run your own outreach activity. Contact us at biopronet@biopronetuk.org for more details.

Overcoming cellular barriers: implications for industrial biotechnology

Over 75 BioProNET, BioCatNet and CBMNet members attended this joint NIBB (networks in industrial biotechnology and bioenergy) event, held on July 6th & 7th 2016 in Birmingham.

After an introduction outlining the objectives of the event, attendees completed a 'me profile' describing who they were, their expertise, their dream project and what the next big development in their field of research could be. This was followed by a series of talks from academics and industry scientists in 3 sessions:

Protein trafficking in eukaryotic cells

Protein export from bacterial cell factories

Delivering therapeutic proteins and other compounds

Attendees then spent the rest of the day formulating potential project ideas and developing these new collaborations. This was then followed by a conference dinner where further networking took place, together with some football watching!

The second day focused on 'technology drivers'; after two talks attendees then moved into to groups to further develop project ideas. At the end of day two, 9 project ideas were generated and champions assigned to take these projects forward.



"I thought the selection of talks was excellent. I came across people working in areas I would not normally meet and it was most stimulating."

"This event was great for learning what academic groups are doing and who has the capability and interest in developing collaborative programmes."

New protein solubility predictor funded by PoC award

Proof of concept funding from BioProNET has enabled [Jim Warwicker](#) and colleagues from the University of Manchester to build a webtool that predicts protein solubility. Recombinant biologics often have low solubility, due to their high concentrations, sequence and three-dimensional structure. The accumulation of insoluble protein agglomerates can lead to the formation of aggregates, which can impact biological activity and immunogenicity of a biologic.

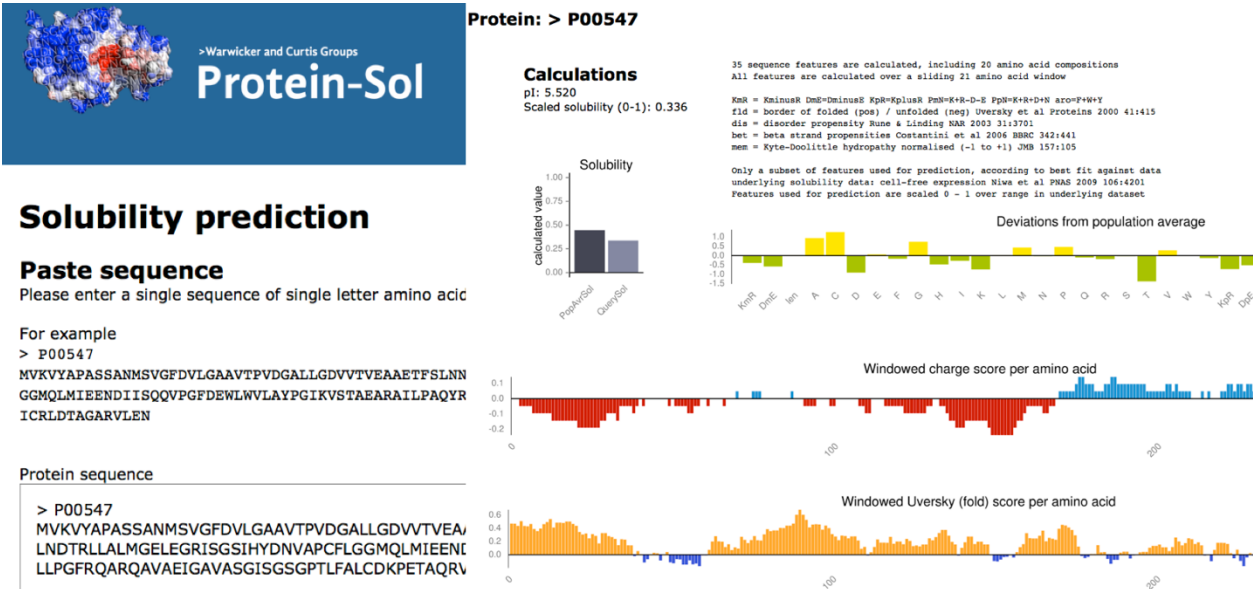
Therefore determining the solubility of a protein and its propensity of a protein to aggregate would be of great use to the biopharmaceutical industry and researchers.

The funding from BioProNET enabled Jim and colleagues to develop existing code into a user-friendly web format. Users (anyone!) can paste a single sequence of amino acids into the tool; the software compares this sequence to a benchmark dataset of proteins with known solubility, and then returns a set of calculations that predict solubility of the protein based on its sequence.

The programme calculates a variety of properties — such as amino acid composition, net predicted charge, predicted pI value, ratio of conservative amino acids, propensity for disorder, propensity for forming beta strands and sheets — that indicate how soluble the entered amino acid sequence is likely to be.

The webtool is available here:
<http://www.protein-sol.manchester.ac.uk/>

The project is already bearing fruit, as it has been used as part of a successful proposal to the EPSRC formulation call. The software is still under development and further improvements, including those based on user-feedback will be added.



BioProNET meetings ignite collaborative project on biologic production

Professor Ian Stansfield from the University of Aberdeen has recently been awarded funding for a collaborative project investigating how to optimize the production of biologics, which was catalyzed by his participation at BioProNET events.

The production of vaccines, antibodies and other proteins in cell lines can induce cellular stress, which can lead to errors in translation — including ribosome frameshift errors. Such mistranslation can compromise the yield and quality of the protein product, and hence the safety and efficacy of biologics. Ian's project will pursue a better understanding of causes of translational error through the design and application of novel reporters of mistranslation.

“Initial discussions on this project were started as a result of the BioProNET sandpit meeting, held in June 2015, when I made initial contact with a scientist from the biotechnology company Fujifilm Diosynth Biotechnologies,” says Ian.

As a result of this networking meeting, Ian co-organized a BioProNet-sponsored workshop on recombinant protein authenticity, together with colleagues Mick Tuite and Tobias von der Haar from the University of Kent. Ian commented “The attendance of scientists from Fujifilm at our BioProNET-sponsored workshop in London consolidated ideas for the project”.

The project includes collaboration partner Professor Phil Farabaugh, a molecular biologist from University of Maryland, USA, and physicist Dr Mamen Romano (University of Aberdeen) who will be mathematically modelling gene expression processes. Ian's group will then use synthetic biology approaches to couple the output from the new mistranslation sensors to recombinant protein expression, in order to autoregulate mistranslation and the quality of the recombinant protein product.

Fujifilm will test these synthetic gene circuits in yeast and *E.coli* to maximise the impact of this research on industrial biotechnology.

More about the project, which is jointly funded by the BBSRC (to Ian Stansfield and Mamen Romano) and the US National Science Foundation (to Phil Farabaugh) [can be found here](#).

PoC study shows protein synthesis errors can cause activity losses in recombinant protein

Proof of concept funding from BioProNET has enabled Tobias von der Haar from the University of Kent and his collaborators to develop a new way of determining the accuracy of protein synthesis. In addition, they were able to use this new technique to show that minor inaccuracies in translation – such as amino acid substitutions – can affect the activity of a recombinant protein.

Cells can be reprogrammed to make many types of recombinant proteins, but this creates additional demand on the cellular protein synthesis machinery that could lead to a decrease in the accuracy of translation and mean that resultant proteins contain more errors compared to endogenous proteins in normal cells. This in turn could lead to changes in the efficacy, bioavailability and immunogenicity of therapeutic and diagnostic proteins.

Working with Cobra Biologics and MRC Technology, Tobias and colleagues sought to establish what effects a loss of translation optimization and decreased protein synthesis accuracy had on the resultant protein. First they developed a new computational tool to generate a database of all possible single-amino acid substitutions in a recombinant protein, as well as LC-MS protocols for analysing mis-incorporated amino acids in a peptide sequence. These tools were then used to analyse recombinant proteins produced in yeast and *E. coli* – two popular bioprocessing hosts.

The tools could detect minor variations in the amino acid sequence. “The sequence variations would have escaped detection with standard mass spectrometry approaches, but can be reliably visualised using our novel approach,” says Lyne Jossé, who carried out the experimental work.

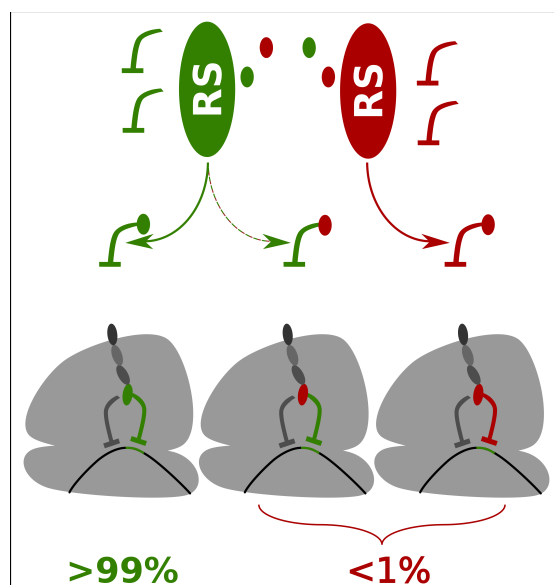
Many of the observed substitutions were shown to be the result of specific biological

mechanisms, such as non-optimal codon usage, that generate specific, predictable translational errors. Interestingly, many other observed errors were universal, occurring in all peptide sequences that were tested from both yeast and *E. coli*. The source of these latter errors is currently not well understood.

A key aspect of this study was the demonstration that errors in protein synthesis can affect the properties of the resultant protein. Surprisingly, a protein translated from a non-codon-optimised DNA sequence had only about 60% of the specific enzymatic activity of the same protein produced from a codon-optimised DNA sequence in *E. coli* (but this was not true for yeast). “To our knowledge, this is the first direct demonstration of DNA sequence-dependent activity differences,” highlights Tobias.

“The collaboration has significantly increased our understanding of the potential issues relating to the production of heterologous proteins in *E. coli*,” says Steve Williams from Cobra Biologics. The study also seeded opportunities for further work – Tobias intends to apply for further funding to investigate the biological mechanisms that cause the observed amino acid substitutions.

“To our knowledge, this is the first direct demonstration of DNA sequence-dependent activity differences”



Warwick and JEOL Strike Gold in Electron Microscopy Collaboration

Escherichia coli is a popular system for the production of recombinant proteins, but little is known about the distribution and shape of structural elements of *E. coli* that drive protein expression and export to the periplasm. To investigate this, Corinne Smith from University of Warwick and colleagues used business interaction voucher funding from BioProNET to collaborate with electron microscope specialist JEOL UK.

The collaboration drew on JEOL's expertise in zero-loss cryo-electron tomography and direct electron detection to investigate the export of human growth hormone by the twin-arginine translocation (TAT) system in *E. coli*. This system is responsible for the export of fully folded proteins — endogenous and recombinant — from the cytoplasm, across the inner membrane and into the periplasm.

“A better understanding of protein export by the TAT system will facilitate better bioprocessing technologies,” says Corinne.

After first using biochemical studies to show that human growth hormone was exported to the periplasm by the TAT machinery, the collaborators then optimised an immunogold labelling procedure to unambiguously identify human growth hormone in *E. coli*.

Electron microscopy data of immunogold-labelled growth hormone showed that a proportion of the protein forms inclusion bodies in the cytoplasm, meaning that it cannot be exported and so would affect the yield of protein. The growth hormone that was available for export at the cytoplasmic membrane was randomly distributed throughout membrane, and did not appear to effect the membrane structure.

“A better understanding of protein export by the TAT system will facilitate better bioprocessing technologies”

Immunolabelling experiments confirmed the formation of inclusion bodies in *E. coli* upon overexpression of recombinant human growth hormone. Scale bar = 200 nm.

Sarah Smith, who undertook the experimental work, gained valuable new skills. “This project gave me training in difficult electron microscopy techniques such as imaging of resin-embedded *E. coli* and electron tomography sections, as well experience of automated image acquisition software, which together which enabled us to gain high resolution data.”

Sarah also showed that a mutant form of growth hormone that cannot be processed for export was randomly distributed in the inner membrane without affecting membrane structure. “In principle this represents a novel way of displaying a protein on the periplasmic face of the *E. coli* inner membrane, which could have applicability in library screening, protein engineering or whole cell biocatalysts”, she notes.

Moving forward, Corinne and Sarah are collaborating with colleagues at University College London to quantify how much human growth hormone can be made by the system, and hope to combine data with results from this study to publish as a paper on a new method of producing proteins in *E. coli*.

“This successful project established a working relationship between JEOL and scientists from the University of Warwick, which will be a catalyst for future electron microscopy-based research projects,” concludes Andrew Yarwood from JEOL.



Dynamic partnership aims to reduce cell harvest time

Cell therapy products and recombinant therapeutic proteins that are produced in cellular systems need to be harvested at the end of the production process. Cell harvesting is often achieved using membrane-based systems, which separate intracellular product and cells from unwanted material in the culture medium or their secreted products from cells.

Business interaction voucher funding from BioProNET has enabled Yuhong Zhou from University College London to work with John Philip Gilchrist of BioPro Control Tech on a project that aimed to reduce the time taken to harvest cells. Reducing cell harvest time could result in a better quality of product and reduced costs. Their project initiated work on a computer-based system that could be used to optimally control the flow of cells and culture medium across a membrane-based separation unit.

“We would not have been able to carry out such a project without the collaborating company,” says Yuhong. “The company developed software and hardware to implement the control method, and we did all the wet laboratory experiments at University College London,” she explains.

Their work centred on a cross-flow filtration membrane system (which has two exit streams) in an ultra-scale down device – so that low volumes (tens of ml) of culture media could be used in the laboratory setting. They aimed to reduce cell harvest time by using the computer-based control system to balance the flux of the culture medium across the membrane against the fouling of the membrane with unwanted

material (which could reduce the efficiency of the membrane).

As a simple preliminary test system, the collaborators used a suspension of Baker’s yeast to generate data on the viscosity of the culture medium at several different cell concentrations, which was then used to develop a mathematical model to control flux. An open-source electronics platform was used as the control system hardware and software was written in house to drive the pressure sensor for online monitoring.

“Our results have provided evidence that the control method has the potential to achieve significant process efficiency”, says Yuhong, noting that further studies will be needed to investigate results in industrially relevant feed systems, such as lysates from *E. coli* or mammalian cell culture broth. Their work also indicates that cost-savings are possible if the control system is integrated into the membrane separation processes.

There are plans to continue the work to further develop the control system and study the application in large scale cross-flow membrane filtration processes. “This work has provided us considerable preliminary data for a new bid for further development of the dynamic control system,” says John Philip. “We would like to collaborate further to develop more sophisticated software for commercial application,” he concludes.

“We would like to collaborate further to develop more sophisticated software for commercial application”



Cobra and Lancaster partnership helps unravel new analytical tool for DNA topology

An increased demand for plasmid DNA in the biopharmaceutical sector — for example, for use in gene therapies — necessitates the use of techniques to analyse the tertiary structure of the DNA, yet current methods are invasive and require a high level of sample preparation.

A business interaction voucher from BioProNET has enabled Lorna Ashton from Lancaster University to work with Cobra Biologics to assess a novel method for determining the topology of plasmid DNA.

The project used Raman spectroscopy; a method for monitoring physiochemical properties of molecules, in which the scattering of light caused by molecular vibrations gives a unique fingerprint of that molecule. It has the advantages of being non-invasive and providing almost real-time information on molecules.

“Raman spectroscopy is sensitive to changes in DNA and RNA structure but is underused in biopharmaceutical analytical R&D”, explains Lorna.

The business interaction voucher enabled Cobra to explore an alternative to current analytical methods by working with Lorna, who has extensive experience of Raman spectroscopy, while at the same time allowing Lorna to access otherwise unavailable plasmid DNA samples.

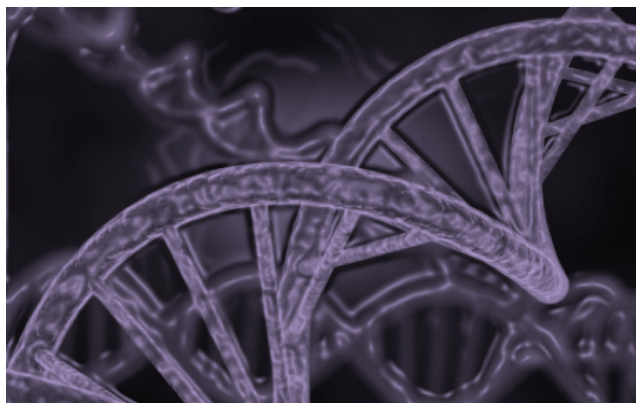
“Raman spectroscopy is sensitive to changes in DNA and RNA structure but is underused in biopharmaceutical analytical R&D”

Cobra provided DNA samples in three topological isoforms — supercoiled, nicked (open circle) and linearised forms — that were verified using two current analytical methods (agarose gel electrophoresis and free-solution capillary electrophoresis) at Cobra.

Then, after method optimization, Lorna determined Raman spectra for each of the isoforms of the plasmid DNA. Next, data processing and statistical analysis were performed to assess any clustering of samples with different topologies.

“The acquired Raman spectra revealed different spectral features arising from the supercoiled, open circle and linearized topologies”, says Lorna. “This indicates that Raman spectroscopy can be used to distinguish the different isoforms.”

However, within the duration of the project it was not possible to assess if Raman spectroscopy could provide quantitative data on the relative amounts of each of the topologies in a sample. Although further work is required to move the project forward, Daniel Smith from Cobra notes that the project has provided “encouraging preliminary data, which that will support continuation of the project in a collaborative manner”.



Collaboration creates a recipe for success in cell-free protein synthesis

Proof of concept funding from BioProNET has allowed Karen Polizzi and Rochelle Aw from Imperial College London to work with Fujifilm Diosynth Biotechnologies on a project that tested if cellular extracts from the yeast *Pichia pastoris* could be used to synthesise proteins.

Protein-based drugs are often synthesised in whole cells. However, the use of cell-free protein synthesis systems — that is, the cell's internal machinery in the absence of the cell wall — has several potential advantages. Compared to whole cell synthesis, this method allows for quicker synthesis, enables the production of proteins that are toxic to living cells and can be scaled to large volumes more easily.

Currently, cell-free protein synthesis extracts from yeast are not commercially available. “This project has proved the concept that *P. pastoris* can be used for cell-free protein expression”, says Ian Hodgson from Fujifilm. “To our knowledge is the first time this has been done.”

As a test system, the scientists investigated the synthesis of green fluorescent protein (GFP) and luciferase. The initial phases of the project determined the best way to lyse yeast cells to release the optimum amount of cellular machinery, and developed a recipe to stabilise RNA transcripts and increase the yield of RNA encoding for the reporter proteins.

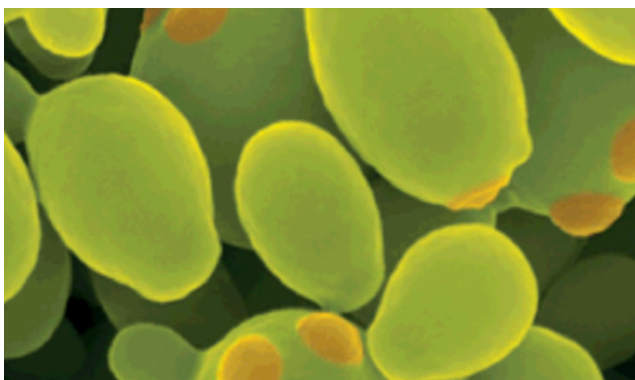
The main phase of the project showed evidence of combined transcription and translation in the extract from the yeast cells. “The most important outcome of the work was that we were able to generate a working cell-free protein synthesis extract from *P. pastoris*”, says Karen.

The final titres of GFP and luciferase observed were similar to that observed with a cell extract from another strain of yeast, *Saccharomyces cerevisiae*, using the same protocol. However, the protein synthesis reaction had a much longer lag phase, and despite initial evidence that the cell-free system was functional, yields of protein were low.

“The project has given us a strong basis to further build upon the results,” highlights Karen. “Optimisation will be key to maximising the productivity of the system.” In addition, the project has benefited the industrial partner. “The project has also allowed Fujifilm to understand some of the factors that would be important in utilising cell-free extracts for commercial use.”

As a next step, Imperial and Fujifilm hope to continue their collaboration by focusing on the production of a more complex, industrially relevant proteins with the *P. pastoris* system.

“The most important outcome of the work was that we were able to generate a working cell-free protein synthesis extract from *P. pastoris*.”



Edinburgh and Recyclatech Join Forces to Recover Microbial By-Products

A business interaction voucher from BioProNET has enabled scientists from the University of Edinburgh to partner with the SME Recyclatech to investigate a new way of recovering useful products from spent media.

Recyclatech uses industrial biotechnology processes that generate large volumes of spent medium, which contains mycolic acid-producing bacteria that contain high value glycolipid. The challenge was to develop a simple, cost-effective way to recover the surfactant-containing bacteria from the large volumes biosurfactants of spent medium.

Together the researchers discovered that the bacteria used by Recyclatech have the capacity to stabilise oil-in-water emulsions. The bacteria can become associated with the oil droplets in the emulsion, and so skimming off the oil droplets from the medium allows the bacteria to be captured and recovered.

“This represents an extremely facile and cost-effective procedure to collect bacteria from a batch reaction,” says Joe Tavecchi, an investigator on the project from the University of Edinburgh.

The biosurfactant can then be extracted from the bacteria using solvents.

Moreover, the collaborators showed that the capacity of the bacteria to stabilise emulsions and the type of emulsions they

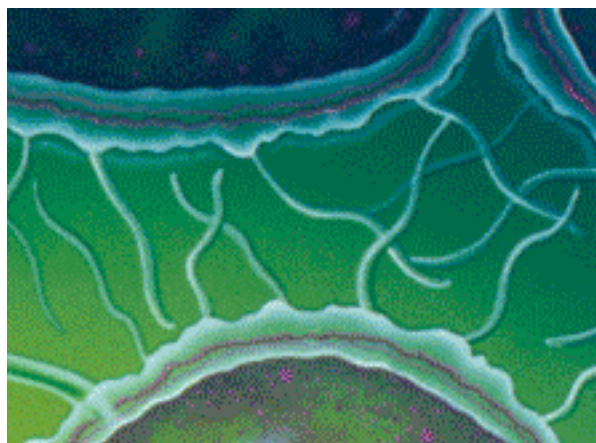
could stabilise — oil-in-water or water-in-oil — was probably dependent by the amount of surfactant they hold within their cell walls, which in turn could be controlled by the amount and type of oil that they were fed.

“Working together with the university of Edinburgh has allowed us to demonstrate biosurfactant production and recovery from our novel bacteria, and has indicated further work to generate different surfactants,” says Nick Christofi, Chief Scientific Officer of Recyclatech.

The extracted biosurfactants can be used in pharmaceuticals, homecare and other products, while intact bacteria have the potential to clean oil from contaminated soils or water.

The outcomes of this work are promising, with initial data being used to support further grant applications and the possibility of scale-up studies. In addition, the collaboration has forged strong links between the partners. “We have been exposed to challenges that industry faces,” highlights Tavecchi. “We intend to channel such a perspective into our future work to increase its impact,” he says.

“We have been exposed to challenges that industry faces; we intend to channel such a perspective into our future work to increase its impact.”



Scissor technology cuts out a collaboration between Bath and Arecor

Insulin is the mainstay of diabetes therapy, with both long-acting and fast-acting formulations on the market. However, a better understanding of what happens to insulin once it has been injected into the body — into the subcutaneous space underneath the skin — will aid the design of new insulin therapies that could lower the incidence of life-threatening hypoglycaemic episodes.

A business interaction voucher from BioProNET enabled Randall Mrsny from the University of Bath to partner with Jan Jezek from Arecor to investigate this. The collaboration brought together expertise in two areas: a new *in vitro* technique — known as Scissor; **Subcutaneous Injection Site Simulator** — developed by the University of Bath that models events that occur following insulin injection, and Arecor's proprietary technologies for stabilising therapeutic proteins.

Because this method of stabilising proteins can alter the pharmacokinetic profile, work carried out under the business interaction voucher used the Scissor system to test the pharmacokinetic profile of Arecor's formulations of insulin analogues.

Results generated using the Scissor system showed clear differences in the behaviour of

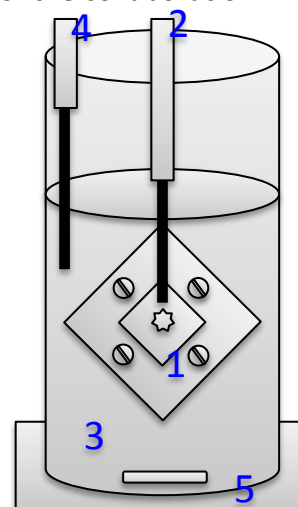
different insulin analogues. For example, differences in the precipitation behaviour of long-acting insulin formulations and fast-acting insulin formulations were observed, with the main differences being in the rate and intensity of the precipitation. These results shed light on the effect of formulation components on the fate of insulin in the subcutaneous space, and consequent differences in their bioavailability. "Further understanding of these effects could lead to the design of fast acting formulations of insulins that have more rapid effects, which is one of the Holy Grails of the diabetes management," says Mrsny.

To disseminate these findings to the wider bioprocessing community, a poster was presented at the BioProNET annual scientific meeting, held in Manchester in October 2015 with almost 180 attendees.

Although the collaborators were unable to optimise the performance of the instrument to follow the release characteristics of long-acting insulin, further studies using an optimised experimental design are being investigated. "The project gave us confidence in the Scissor instrument," says Jezek. "We are already discussing continuation of the collaboration with Professor Mrsny."

"Further understanding of these effects could lead to the design of insulins that have more rapid effects, which is one of the Holy Grails of the diabetes management."

A schematic of the proof-of-concept subcutaneous injection site simulator (Scissor). 1. Simulated subcutaneous injection site; 2. pH probe; 3. Physiological buffer bath; 4. Thermocouple; 5. Stirrer/heater



Exchange visit funding seeds early career researcher collaborations

Luis Martin, a post-doctoral researcher at the BioComposites Centre, Bangor University, has been awarded scientific exchange funding from BioProNET that has enabled him not only to acquire new skills but also to build collaborations.

Luis works on greener ways to obtain purer fractions of glycolipids from fermentation broths. The purification of glycolipids is the main factor that limits the industrial application of new glycolipids. The driving force of his visit was to investigate the possibility of moving from a batch purification process to a continuous one using specialist equipment that was available at the supercritical fluids research group, directed by Professor Ernesto Reverchon at Università degli Studi di Salerno in Italy.

“As a result of the scientific exchange, I was able to understand and master the technique of supercritical counter current fractionation,” say Luis. “Maybe in the future this technique can be imported to our group at Bangor University to complete the versatility of our laboratories.”

Moreover, the exchange strengthened the networking between the two institutions. Luis explains that two Erasmus stays next year have been set up, with two Masters students

“This work will allow the set up of a fruitful collaboration between research groups, sharing valuable experiences within the supercritical fluid world.”

coming to Bangor University, accounting for a total time of one year. “This work will allow the set up of a fruitful collaboration between research groups, sharing valuable experiences within the supercritical fluid world,” he highlights.

But this not all. Once Luis knew that he had secured funding, he attended the inaugural BioProNET early career researcher meeting, where he continued his collaboration drive.

Pravin Badhe, a research assistant at Brunel University met Luis at this event. “The meeting was very helpful for networking; I managed to source access to LC-NMR equipment at Bangor University, which I had been trying to find for nearly 6 months,” he says. Also as a result of the meeting, Kamaljit Moirangthem, a PhD student at the University of Nottingham, was able to set up a collaboration with Luis. “The project is very innovative and has potential to attract future funding,” highlights Moirangthem.

So as a direct result of BioProNET funding and events, the seeds of collaboration for early career researchers are beginning to grow.



Luis Martin from Bangor University and the counter-current column at Università degli Studi di Salerno, Italy

Sandpit Meeting Builds Collaboration Workshops

In June 2014, BioProNET held its inaugural event, a so-called ‘sandpit’ meeting — an event where scientists from different backgrounds come together to discuss challenges and opportunities — that was attended by about 80 delegates, of which about one-third were from industry.

“We felt it that such a meeting was an important way to bring the bioprocessing community together to eek out challenges and key issues,” says Mark Smales, BioProNET director. “We included lots of time for discussions between attendees,” he highlights.

Key to the success of these discussions was the involvement of two professional facilitators, who were able to maximise interactions and dialogue between attendees, and allow discussions to explore new topics.

The discussions identified several themes that attendees thought could be the focus of follow-on workshops that would build collaborations between industrial and academic scientists.

These where:

- Computational bioprocessing
- Continuous processing
- Biologic production in microalgae and plants
- Analytics and formulation
- Synthetic biology tools for bioprocessing
- Protein authenticity and translation
- Cell-free expression systems
- Whole genome tools
- Cells as tools
- Antibody-drug conjugates

Indeed, eight workshops were funded BioProNET; the outcomes of four were presented at BioProNET 2nd Annual Scientific meeting in 2015.

Attendees at the microalgae and plant expression workshop



Production of pharmaceutical and industrial proteins in microalgae and plants

This workshop was organised by Anil Day (University of Manchester), Jags Pandhal (University of Sheffield) and Yuhong Zhou (University College London) and had attendees from seven universities and six companies, and was jointly funded by Phyconet.

The workshop centred on three themes — expression systems; bioreactors, regulation and industry perspective; harvesting and downstream processing — and featured presentations and breakout sessions. Outcomes included a technology assessment, identification of current barriers to progress, the identification of key academic and industry players from both networks, and the establishment of consortia to take projects forward.

Analytics in bioprocessing and formulation

Organised by Paul Dalby (University College London), Gary Montague (Teeside University) and John Liddell (Fujifilm Diosynth Biotechnologies), this workshop had 17 attendees, over half of which were from industry. The group first identified ten key challenges and then grouped these into three themes, which comprised of non-invasive measurements, automated sample preparation and analysis, and data management and predictability.

As well as a professionally written report of the meeting (available [here](#)), other outputs were grant applications to Innovate UK and the EPSRC formulation call.

“We have a new industrial partner that has been very active in our grant application to BioProNET,” says Karen Polizzi “This was largely due to the workshop,” she notes.

Cell-free protein synthesis

This workshop, organised by Karen Polizzi (Imperial College London) and Jose Gutierrez-Marcos (University of Warwick) featured a keynote presentation (available [here](#)) by Trevor Hallam, chief scientific officer of Sutro BioPharma in the USA. This was followed by discussions on the challenges for large scale manufacturing with cell-free extracts and the use of different cell types. “We have a new industrial partner that has been very active in our grant application to BioProNET,” says Karen Polizzi “This was largely due to the workshop,” she notes.

Discussions — such as UK research capabilities and what is best use of technology — on cell free synthesis are continuing and indeed a follow up workshop is being planned for March 2016.

Recombinant protein authenticity

This workshop was organised by Ian Stansfield (University of Aberdeen), Mick Tuite (University of Kent) and Tobias von der Haar (University of Kent). The plenary lecture, entitled ‘improving heterologous protein production through synthetic biology algorithms’ was given by Manuel Santos, University of Aveiro, Portugal. This was followed by talks and discussions focusing on how the detection and mitigation of mistranslation will provide new routes to optimize recombinant protein expression.

“We established that a collaborative research project between academia and industry in the UK needs to be set up to explore the means of detecting errors in recombinant proteins and designing new error-free expression strategies,” says Mick Tuite.

Notes

