# BioProNET

**A Network in Industrial Biotechnology and Bioenergy (NIBB)**

**A BBSRC co-sponsored by the EPSRC**

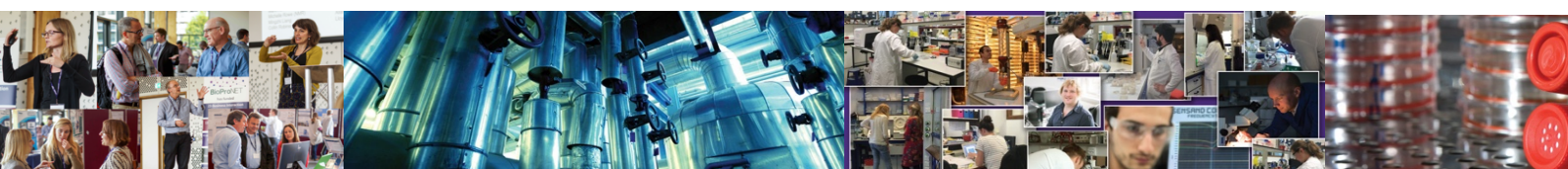
**Summary Report to end of March 2018**

www.biopronetuk.org https://twitter.com/BioProNETUK

## Note on the following report

This report has been prepared by the BioProNET Executive group. The data, figures and material presented is done so using available resources to the Executive group and from information provided to it by the members. We have attempted to obtain evidence to confirm all data and claims made throughout the report and have excluded data where evidence was not available to us. In doing so we have excluded reporting and claiming much wider ranging impacts of the NIBB on the community that we know exist through discussions but for which it is not possible to obtain data to define the exact contribution of the NIBB to a particular output.

BioProNET Executive group, May 2018



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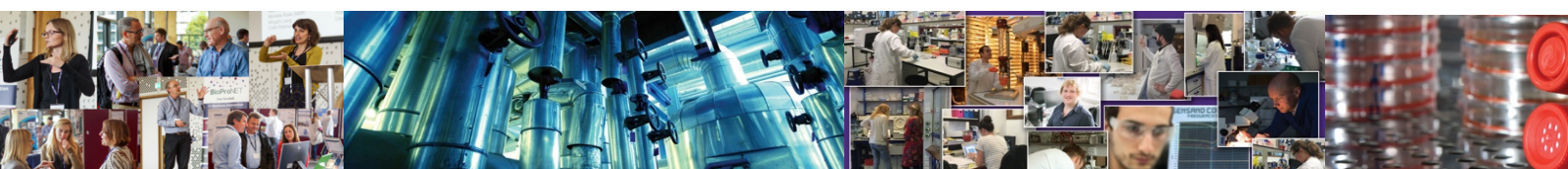


# 1. BioProNET Executive Summary

In 2013 the BBSRC, with support from the EPSRC, announced the commitment of £18M to fund a series of networks to be focused around Industrial Biotechnology and Bioenergy (NIBB). The establishment of these networks was to support, develop and foster collaborations between the UK academic base, industry/end users, social scientists, regulatory authorities, policy makers and NGOs to develop interdisciplinary networks that would (i) identify and then (ii) develop novel approaches to address new and existing research challenges in the networks focus area(s) and (iii) aid in the translation of these ideas into practice. As a result of a successful application by the community to this call, the BioProNET (Bioprocesing Network) NIBB was established as a multidisciplinary network focused upon the area of bioprocessing of biologics/biopharmaceuticals in January 2014.

BioProNET is a unique network that represents a community with major economic and social value for the UK and globally. Biologics encompass a range of molecules of therapeutic (e.g. biopharmaceuticals) and non-therapeutic (e.g. diagnostics, industrial enzymes, drug screening, crystallization / structural studies) use. The UK has been especially innovative in developing processes for commercial scale production (bioprocessing) of therapeutic proteins (biopharmaceuticals) in particular, that have the potential to treat otherwise intractable diseases. The area employs a large work force in the UK, estimated to be over 167,000 people generating a turnover of >£50B. However, the continued development and commercialization of biologics requires further step-changing innovation if the full potential return to the UK is to be realised. Indeed, one of the successes of BioProNET has been the internationalization efforts, which as a result have seen the development of 'copy cat' networks in other countries (see for example The National Institute for Innovation in Manufacturing Biopharmaceuticals, www.niimbl.org where $250M US is to be invested in this area) and underpins the importance of continuing to support such a network in the UK to maintain the international leadership of the UK plc in this theme.

The focus of BioProNET is the biological processes that underpin the development, engineering, manufacturing and monitoring of functionally active biologics to address production of molecules of greater design complexity. The vision and perspectives of multiple scientific disciplines, including life scientists, biochemical engineers, chemists, physicists, mathematicians, computational scientists, and social scientists, have been applied to the research challenges of biological process in the manufacture of biologics, both products of therapeutic use (e.g. biopharmaceuticals) and those of non-therapeutic use (e.g. biosensors, drug development and screening, diagnostics). The specific objectives of BioProNET were to:

- Provide leadership and vision to the UK academic/industrial community in the field of bioprocessing and biologics, ushering in new collaborative models to accelerate innovation and deliver change, ensuring the UK academic research agenda is world leading and the go-to place for collaborative research.
- Facilitate the award of major research funding from UK and international sources in the area of industrial biotechnology and generate outputs of direct benefit to the sector.
- Provide a vehicle for the delivery of Proof of Concept (PoC) studies that would ultimately lead to more competitive, collaborative, cross-disciplinary and integrative research proposals to BBSRC and elsewhere. Through these and other initiatives develop industry partnerships that address the calls of the IB Catalyst fund.
- Create an environment promoting application of new technologies, including synthetic biology,

genomics and systems biology, for the rapid, flexible, predictable (through analytics, robotics, modelling, engineering) and cost-efficient production of high value biologics.

- Inspire and develop the next generation of scientists across the breath of disciplines encompassed by the network.
- Consider the wider impacts of network outputs, promoting public engagement and discussion with the regulatory, environmental, economic and social science communities.

Beneficiaries of BioProNET activity were to include the NIBB academic and industrial bioprocessing and scientific communities and, through improved cost efficiency of manufacture (making UK industry more competitive and attracting overseas investment and overseas businesses to work more with UK industry and academia), the UK economy. Ultimately the commercial supply chain benefits the public, directly via the NHS/patient via cost of goods, and the UK economy via advanced manufacture, IP and investment.

The outputs of the Network were to be;

- Continued development of the UK academic sector to ensure it remains, and grows as, a leading community in bioprocessing and recognised as the "go to" for solving technical bioprocessing challenges.
- Biomanufacturing investment into UK academia and industry, levering further funding and collaboration nationally and internationally, supplying skilled individuals to the workforce.
- The establishment of an internationally recognized and sustainable expanded and integrated cross-disciplinary network of academics, industrialists and other relevant organizations able to address major research challenges in the area of bioprocessing.
- A mechanism for fostering community interactions, enabling rapid responses to research challenges, policy changes, and large research calls.
- The development of large cross-disciplinary grant proposals of scientific excellence from a range of funding sources and calls (UK and internationally) that utilize a wide range of expertise and new technologies including genomics, synthetic and systems biology.
- Generation of new ideas and approaches via sandpit and PoC studies.
- A route for academics to apply their ideas and science to industrially-relevant challenges and consider the societal, environmental, economic and political ramifications of their work.
- High quality research which increases the impact of research previously funded by BBSRC and EPSRC in excellent publications and in valuable IP with pathways to commercialization clearly available through industry partners.
- Useful technologies that can speed development, reduce cost of goods, and ensure the safety and efficacy of new biological products from UK businesses.

As can be seen from the exemplar data and examples described in the remainder of this report, BioProNET has been extremely successful to date in meeting these objectives and deliverables, ensuring that UK Bioprocessing and the interactions between all stakeholders remains the envy of much of the rest of the international community. This has been achieved through a variety of mechanisms (as detailed in the remainder of this report) including, but not limited to;

1. An initial sandpit meeting across disciplines and stakeholders to identify the current and future challenges in the area, providing a roadmap to innovation and TRL developments
2. An ever-growing membership list of diverse stakeholders from all aspects of bioprocessing
3. A large multinational annual scientific meeting of stakeholders from all aspects of the Network, from world-leading academics and industrialists, to early career researchers, supply chain vendors, policy makers and those from different disciplines
4. Tailored early career researcher events to provide training in different aspects of bioprocessing and career development
5. Workshops for smaller groups to aid development of new collaborations and ideas to support grant and project applications
6. Assessing, awarding and monitoring proof-of-concept funding of innovative projects (up to £100K) and Business Innovation awards (BIVs) to provide support for development of larger applications and TRL development
7. Internationalisation events to ensure the UK remains at the forefront of bioprocessing of biologics
8. Development of a BBSRC STARS programme to help train the next generation of leaders in the area
9. Supporting activities in the area through matching expertise to problems/facilitating new interactions, aiding in job creation, facilitating large applications (e.g. EPSRC Portabolomics award to Newcastle) and providing advice and help for members new to the area
10. Holding events with other NIBB where cross-NIBB expertise could aid the community in tackling identified challenges

BioProNET is managed through the executive group (2 Network Managers and two PIs) and a Management Board, details of which are to be found on the website. In order to ensure a transparent management structure with no conflicts of interest, both PIs have ensured they have not been involved in the application for any of the funding available from the network via advertised calls (with the exception of workshops).

The following report provides evidence of outputs from such activities. It is not meant to be comprehensive, but provides documented evidence of BioProNET's continued importance to the community and reinforces the need for such a unique network to remain in place to support the community and the new challenges that will be faced in the coming 5-10 years.

## 2. Membership Summary

From inception, BioProNET has operated open and transparent membership criteria. We have welcomed members from any area that demonstrate an interest in bioprocessing of biologics. Membership can be obtained via application through the website, email contact of the Network Managers, or at any of the meetings we arrange.

Initially (in Jan 2014) the core BioProNET membership was largely derived from Bioprocessing Industrial Club (BRIC) members and totalled just over 200 members. However, since this time the membership has increased steadily with virtually all new members since this time being from outside the BRIC community and including members from the breath of the bioprocessing of biologics spectrum. The growth of the membership since inception is shown in Figure 1.

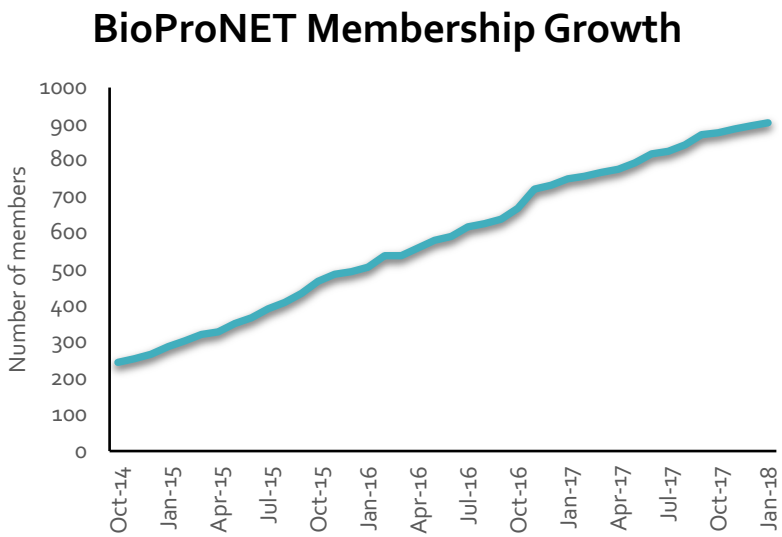
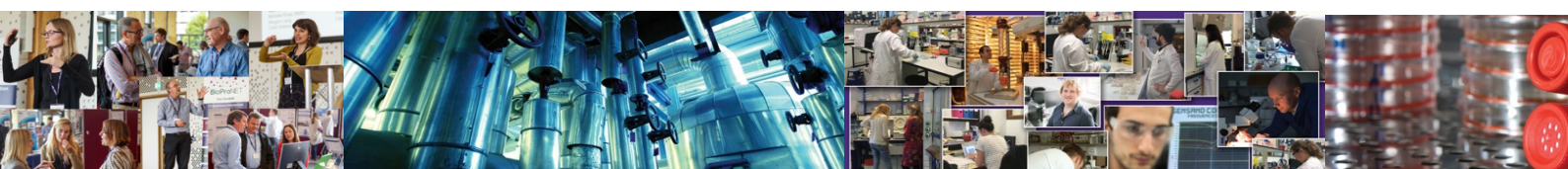



**Figure 1.** BioProNET membership growth from Oct 2014 until the end of 2017.

Some key membership facts include;

- BioProNET now has over 900 active members
- 35% of members are from industry
- 60% are university-based
- 5% are from other institutions including government, RCUK, NGOs, regulatory authorities
- One-third of industry members work at an SME
- 15% of members are international or EU
- Approximately 20% are early career researchers (ECRs)
- Overall, 35% of members say that their work has become more interdisciplinary as a result of BioProNET
- BioProNET membership has resulted in increased motivation of academics to commercialise their research: 65% (13 out of 20 responding to a survey) said they had increased motivation or thinking about starting their own company



We mainly communicate with our membership via

Twitter @BioProNETUK (over 1100 tweets to date and 580 followers)

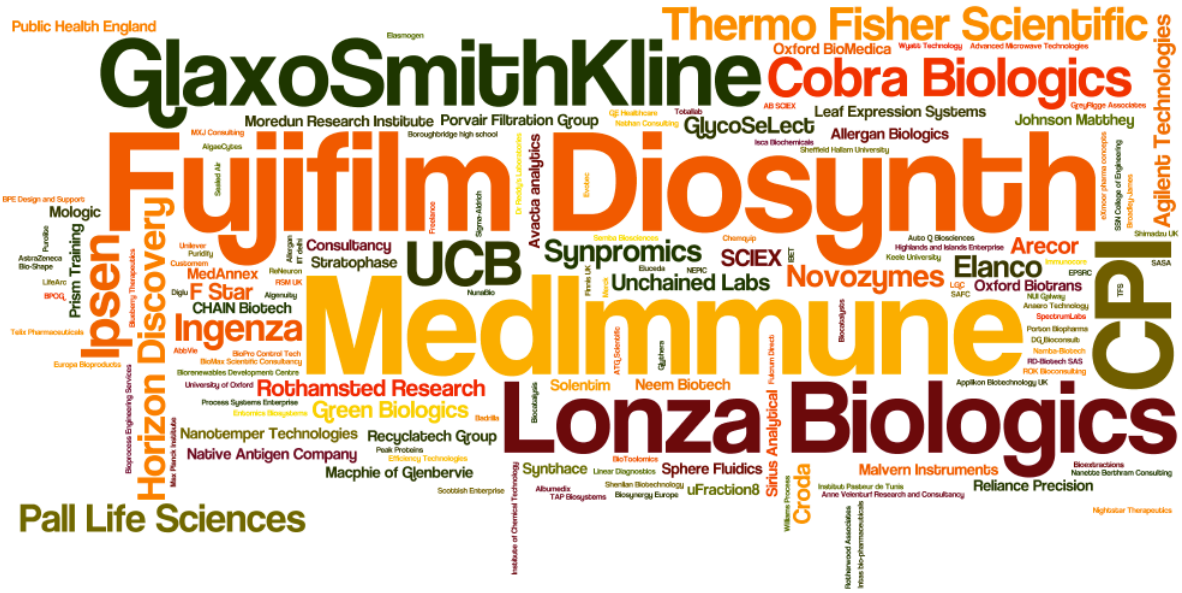
Website http://biopronetuk.org/

Newsletters available here

Email biopronet@biopronetuk.org

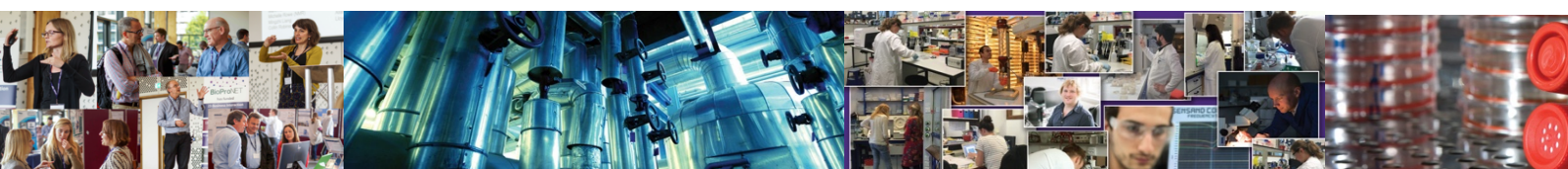
The main website is at **www.biopronetuk.org**

The diversity of companies for which different BioProNET members work is illustrated by the word cloud below.




Wordcloud showing the companies for which individual members of BioProNET work. The larger the size of the text, the more members that are associated with that company.

**Appendix I** lists companies that have BioProNET members.



# 3. Annual BioProNET Scientific Meetings

One of the key mechanisms by which BioProNET has engaged and grown the community is via the annual scientific meeting (see Figure 2 for example). Initially these meetings were co-hosted with the BBSRC/BRIC community but since the completion of BRIC, they have run as successful stand-alone meetings. These annual meetings have now become an established event for the community in the UK and internationally, and have helped foster new collaborations, grant applications, discussions, identification of challenges and opportunities and engagement with other interested stakeholders and aided capacity building for consortia. Members from all aspects of the BioProNET remit have attended and presented. Meetings have had a mixture of invited speakers and speakers (frequently at early career stage, ECR) selected from submitted abstracts. These events have also been an important reporting forum. We have used these large events to report on BioProNET activities and obtain feedback from the community and suggestions for future activities. All project teams funded via BioProNET funding have been requested to present their work at these meetings to the community to act as both a showcase for BioProNET-sponsored events and to provide community transparency to project awards and allow potential users to speak with those involved. The meeting has also provided an important stage for ECRs to present their work, with a number giving oral presentations and around 60 presenting posters at each event.

**Key facts from annual BioProNET scientific Meetings**

- BioProNET have run/hosted 4 Annual science meetings, with a 5$^{th}$ planned for October 2018.
- There have been between 160-200 members attending each meeting
- Approximately one-third of attendees at each meeting have been from industry and about one-quarter have been ECRs
- Feedback shows that these meetings have fostered many new industrial-academic, industrial-industrial and academic-academic interactions
- 93% (26 out of 28) industry respondents who had attended a recent annual meeting said that as a result of BioProNET they had increased motivation to work with academics


**Figure 2**. Attendees interacting during a break at BioProNET's 4$^{th}$ Annual Science Meeting

Evidence of the impact of these meetings is provided in the following quotes from attendees;

*"The annual meeting in particular inspired a lot of ideas in ways to incorporate the latest academic breakthroughs into potential industrial settings."* **Company member**

*"Being aware of what is going on and who is doing what [from the annual meeting] was very useful and yes, did increase my enthusiasm to work more closely with academics"* **Company member**

*"Numerous PhDs and research collaboration ideas initiated as a result of these meetings"* **Company member**

*"Meeting is an excellent route to identifying academics working in relevant fields to our company's research activities"* **Company member**

*"Understanding and interaction with academics particularly is difficult in an industry setting, as focus is always on customer need, rather than research in many situations***" Company member**

"The Annual Science Meeting was an excellent event for increasing awareness of academic groups and identifying collaborations" **Company member**

*"I formed a new interaction with a company (details not given); - based on discussions at this meeting we purchased a nanopore sequencer [PoC awardee for partners with Oxford Nanopre was at the meeting] - received funding as part of the successful Innovative Vaccine Manufacturing Hub bid"* **Academic member**

These meetings have also facilitated company–company interactions. An example of this include

A **company member** stated: *"Through the 2017 annual scientific conference, we engaged with another company to evaluate our novel bioprocess products"*

Such meetings also resulted in many other outputs, many of which cannot be measured. An example of such an output that we have been made aware of is;

BioProNET has also facilitate early career researchers accessing opportunities to develop their own careers via interactions with other members, including senior industrialists and academics. For example, at the 2017 Annual Meeting, an early career researcher, met a senior scientist from a biotech company and as a result of this interaction the researcher was successful in a job application at the company.

**Appendix II** lists the detailed programmes for each of the BioProNET Annual Scientific Meetings.

# 4. Supporting Early Career Researchers (ECRs)

An important focus of the BioProNET NIBB is to help develop the next generation of leaders in the field to ensure the UK remains world-leading in this area. Industry continually emphases that a strong pool of appropriately trained people is essential to maintain their competitive edge and that the quality workforce presents a key reason for locating in the UK. As such, BioProNET has been active in delivering novel workshops and events to support the development of ECRs. BioProNET has designed, organised and hosted 2 dedicated early career events that have provided training for approximately 60 ECRs. This is in addition to design and delivery of a BBSRC-funded STARS programme in bioprocessing.

ECR events have covered/included the following aspects;

- Professional media training (provided by Coconut Communications http://coconutcommunications.tv/)
- CV and interview clinics
- Personal development (e.g. Myers-Briggs)
- Careers talks by bioprocessing experts
- Presentation by the Winner of The Apprentice TV show, Ricky Martin; now head of his own scientific recruitment company https://www.hyperec.com/about-us/meet-the-board
- Peer to peer networking

These events have generated very positive feedback from attendee, including:

*"Excellent meeting, wonderful networking opportunities, best career event I have ever been to, much more opportunities to network than usual"*

*"Really good talks about career choices and progression. Also the CV clinics were very informative and useful"*

*"Refreshing and new rather than dry and abstract as these type of events usually tend to be"*

*"Media training was fantastic and activities were incredibly engaging"*

*"The interviews were very thought provoking, definitely taught me a lot about myself"*

**Figure 4.** Delegates at the BioProNET ECR event in Brighton (2016).

In addition to dedicated workshops, we have supported ECRs to attend other events and held early career sessions at the Annual Scientific meetings so that ECRs can gain experience of presenting their work to key academic and industrial scientists as well as the bioprocessing community

ECR from Sheffield "*BioProNET gave me the chance to identify relevant skills to potentiate my career aspiration. It also gave me an invaluable opportunity to build links and networks across other universities. In general BioProNET provided a good environment to share ideas and advice with other colleagues.*"

BioProNET also maintains a dedicated web page for Early Career Researchers

http://biopronetuk.org/category/early-careers/

**Figure 5**. Early career researcher Eva Pekle receives a prize for her talk at the 2017 Annual Scientific meeting, awarded by Karen Lewis of the BBSRC.

## BioProNET supported BBSRC STARS bioprocessing skills school

http://biopronetuk.org/the-bioprocessing-skills-school/

A recent development for ECRs for BioProNET has been the funding and development of a dedicated residential BBSRC STARS programme. This has been a collaborative project between BioProNET, The Universities of Manchester and Kent, and CPI in Darlington. The first of these ran very successfully in 2017 (Figure 6). This will be repeated in September 2018 and 2019 with the intention of integrating this into subsequent activities. The aims and objectives of the STARS programme are conveyed in the advertising used to attract delegates and is provided below.

**September 2017 BioProNET STARS Information**

Do you want to find out how your career might develop working for industry, the differences between industrial- and academic-driven research and how the industrial environment matches your career ambitions? Here's your opportunity to answer these questions and find out much more about your ability to work in teams and how the innovative, entrepreneurial spirit drives research translation.

At our week-long, intensive residential training programme, designed around the insights and advice of senior industrialists, you will take part in group-based activities and work with real-life industrial case studies. The programme is designed to engage with the process of entrepreneurship, focus on the development of the ability to promote research ideas and their value to audiences and the key importance of the societal impact of industrial biotechnology.

The programme will be held on 11th-15th September 2017, inclusive, at the National Biologics Manufacturing Centre (NBMC, Centre for Process Innovation, CPI, Darlington). Details of the full programme to follow and the sessions will include: drug development, generating the tools for self-awareness, molecular design, manufacture process, formulation, delivery, and presentations from a variety of entrepreneurs in the field.

We are aware that the groups have maintained contact since the September 2017 STARS meeting (one objective of the programme was to build a network that would connect participants with the vision that they would become future sector leaders). One group has developed plans to participate in the BBSRC BiotechYes competition for 2018 and BioProNET has given preliminary approval for support of a face-to-face meeting for this group to formulate their business plan.


**Figure 6.** Delegates and instructors at the first BioProNET STARS Programme, Darlington, September 2017

## Additional ECR Activities

BioProNET has also funded 4 scientific exchanges (at £500 each) that allowed ECRs to visit another lab to learn new techniques.

**Awardees:** Eva Pekle, University of Kent; Luis Martin, Bangor University; Ben Dolman, University of Manchester; Carlos Suarez-Heredi, University College London

**Details can be found on the attached case studies and links below:**

Scientific exchange visit boosts separation technologies collaboration

Exchange visit funding seeds early career researcher collaborations

Our member profiles (Appendix VI) include the profile of early career researcher Charlie Campion, and how membership of BioProNET has facilitated her career development. **Click to download** Charlie (also attached)

*"What's helped me the most [by being a BioProNET member] is having my eyes opened to the variety of careers out there for PhDs – we don't have to limit ourselves to research, whether in academia or industry. Sitting down with people currently in those professions, grilling them about how they got there, what skills they'd be looking for in potential employees, how they manage work life balance etc. has been vital!"*

## Undergraduate Student Projects

BioProNET has also awarded 4 summer studentships to support undergrad students to gain real life bioprocessing lab experience during summer 2018:

- Combining machine learning and metabolic modelling for optimal yeast bioprocessing (Claudio Angione, Teesside University)
- Developing an IP-free plasmid backbone for protein expression (Mark Caddick, University of Liverpool)
- Manufacture of 3D structured designs for the purification of biologics (Simone Dimartino, University of Edinburgh)
- Producing designed protein-DNA conjugates: nanoscale assembly for hybrid biologics construction (Dafydd Jones, Cardiff University)

From a survey of our ECR membership, 74% of respondents said that BioProNET had helped them to gain a gained a better understanding of bioprocessing and the bioprocessing sector.

# 5. Supporting Innovation: BioProNET Funding

Through the core grant, and subsequent funding made available to BioProNET, the network has been able to identify and support high quality projects in the area to generate proof-of-concept data, establish links between industry and academia and to move projects along the TRL pipeline. This funding has been important for engaging and growing the membership, encouraging participation at events, developing collaborations and addressing the aims of the network. The PIs excluded themselves from funding calls in order that a fair and transparent mechanism was followed in allocation. Allocation has been undertaken in line with the details provided in the original application, involving peer review and consideration by the Management Board to rank applications. Through the funding made available BioProNET has funded;

- **26 business interaction vouchers**
- **13 proof of concept grants (most close to £100K)**
- **9 Industrial Biotechnology Seeding Catalyst Awards**

In addition, these awards have **leveraged approx. £870K** of funding (either in-kind or cash) from industrial collaborators. The funding has facilitated the interactions between 17 universities and 29 companies as depicted in Figure 7. Details of awards are given in Appendix 3 (BIVS, PoC) and Appendix 4 (IBSCA funding) which also describes the main outputs from each funded project.

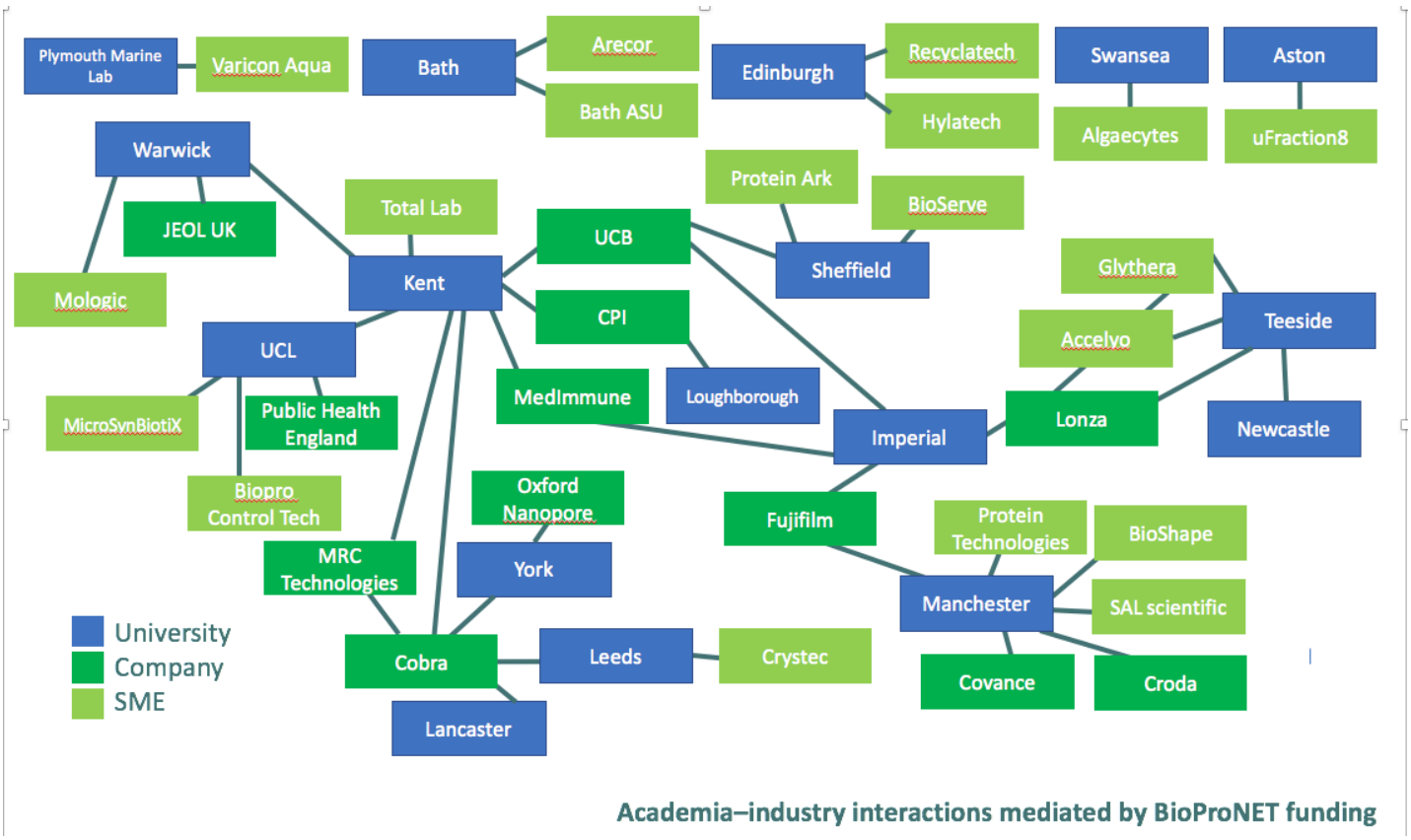



**Figure 7.** Interactions directly fostered by BioProNET funding.

As stated, the funding has been central to facilitating new collaborations and development of approaches to address identified challenges. Quotes from members related to the importance of funding are included below.

Quote from industry partner on a PoC award:

*"Our company  offers the opportunity to develop a process which will support not only the antibody–drug conjugate industry but a partner company's own antibody–drug conjugate product portfolio"*

*"The BioProNET proof of concept scheme is very valuable for young scientists, especially to build their research confidence. Moreover, the quick turnover of the application is without any precedent, and makes doing science much more realistic," Marek Brzozowski, proof of concept awardee.*

Quote from an industrial partner on a BIV project:

*"Academic and industrial collaboration is important to narrow the gap between lab and commercial scale technology. Our project work has certainly produced a promising outcome and as a result, we have much better traction now for securing further private investment."*

# 6. Direct Outputs of BioProNET Activities

BioProNET has stimulated a wide variety of outputs. We provide here examples of different types of output from the network activities. Many outputs have no means of being accurately measured (e.g. collaborations that form we are never told about, jobs created at companies that BioProNET activity has contributed to, international activities as a result of attending a BioProNET event etc). Whilst we are anecdotally aware of many large grants and applications that have been facilitated by BioProNET activities, here we are only reporting examples where we have a direct line of evidence to support outputs.

**Published papers from BioProNET supported research (authors in BOLD received BioProNET funding)**

1. Codon-depedent translational accuracy controls protein quality in *Eschericha coli* but not *Saccharomyces cerevisiae* **Lyne Jossé**, Connor D. D. Sampson, Mick F. Tuite, Kevin Howland, **Tobias von der Haar** doi: https://doi.org/10.1101/200006 (preprint)
2. TatA complexes exhibit a marked change in organisation in response to expression of the TatBC complex Sarah M. Smith, Andrew Yarwood, Roland A. Fleck, **Colin Robinson**, and **Corinne J. Smith** Biochem J. 474, 1495–1508, 2017
3. Protein-Sol: A web tool for predicting protein solubility from sequence Hebditch M, **Alejandro Carballo-Amador M**, Charonis S, Curtis R, **Warwicker J**. Bioinformatics doi: 10.1093/bioinformatics/btx345 May 29 2017
4. A poly-omics machine-learning method to predict metabolite production in CHO cells Zampieri, G., **Coggins, M**., Valle, G. and **Angione, C**. *Proceedings of the The 2nd International Electronic Conference on Metabolomics*, 20–27 November 2017; doi:10.3390/iecm-2-04993
5. Long-term stability and reusability of molecularly imprinted polymers Jozsef Kupai, Mayamin Razali, Sibel Buyuktiryaki, Rustem Kecili and **Gyorgy Szekely.** Polym. Chem., 8, 666-673 (2017) DOI: 10.1039/C6PY01853J
6. Exploring and exploiting the effect of solvent treatment in membrane separations Mayamin Razali, Christos Didaskalou, Jeong F. Kim, Masoud Babaei, Enrico Drioli, Young Moo Lee, and **Gyorgy Szekely.** ACS Appl. Mater. Interfaces, 9, 11279–11289 (2017) DOI: 10.1021/acsami.7b01879
7. Valorisation of agricultural waste with an adsorption/nanofiltration hybrid process: from materials to sustainable process design Christos Didaskalou, Sibel Buyuktiryaki, Rustem Kecili, Claudio P. Fonte and **Gyorgy Szekely.** Green Chem., 19, 3116-3125 (2017) DOI: 10.1039/C7GC00912G
8. A cell culture platform for cryptosporidium that enables long-term cultivation and new tools for the systematic investigation of its biology Christopher N. Miller, **Lyne Josse,** Ian R. Brown, Ben Blakeman, Jane Povey, Lyto Yiangou, Mark Price, Jindrich Cinatl Jr., Wei-Feng Xue, **Martin Michaelis, Anastasios Tsaousis** International Journal for Parasitology https://doi.org/10.1016/j.ijpara.2017.10.001
9. In vitro model for predicting bioavailability of subcutaneously injected monoclonal antibodies. Hanne Kinnunen Bown, Catherine Bonn, Stefan Yohe, Daniela Bumbaca Yadav, Thomas W. Patapoff, Ann Daugherty, **Randall J. Mrsny**. Journal of Controlled Release 273, 13-20 (2018) https://doi.org/10.1016/j.jconrel.2018.01.015

Future publications can be followed here.

http://biopronetuk.org/publications-from-biopronet-funding/

## Further funding obtained

We feel that full definition of the impact of BioProNET activities (funding and meetings etc) on the award of further funding presents a challenge. The consequences of contacts made and ideas sparked by discussions is almost intangible to define and this is especially the case with the industrial sector and the commercial sensitivity around industry-industry and industry-academia contacts that result from the stimuli presented by BioProNET engagements. It is also highly challenging to assign the contribution of BioProNET activity and the activities of others (e.g. the KTN, the National Biomanufacturing Centre/CPI, other complementary NIBB) in building the successes in the bioprocessing sector over the past 4 years. It would probably be fairer to state that BioProNET, the KTN, NBMC and NIBB have supported a general environment of engagement and that as a whole all must take credit for the success.

Given these "health checks" it is illuminating to examine how the sector has prospered and how the overall umbrella of BioProNET has enabled (collaborative) funding that reflects how the sector has been mobilised to look for multi-disciplinary interactions in which industry-academic collaborations have become strong centre-pieces of the research model for bioprocessing.

These facts also have to be read in relation to authenticated additional funding that we are aware has arisen directly as a consequence of BioProNET activity and funding support. £14.82M of additional funding as a result of BioProNET BIV and POC funding has been reported by members and includes 3 PhD studentships, below are some examples.

BBSRC iCASE PhD studentship to Michael Plevin

UCL EPSRC CDT PhDs studentship to -
Karen Polizzi and Dan Bracewell
Mark Wass and Dan Bracewell (both following on from their respective BVs and PoCs)

EPSRC grants - £1.5 M awarded to Paul Dalby

£861 k awarded to Robin Curtis (from PoC_Dec14_Warwicker)

BioProNET member Ian Stansfield attended several BioProNET meetings, which ultimately resulted in him receiving £681 K of funding from the BBSRC and the US NSF for the project 'Synthetic gene circuits to measure and mitigate translational stress during heterologous protein expression'. This is highlighted in the case study that follows on page 20.

| Title | PI | Value |
|---|---|---|
| Bioprocessing Skills School: Embedding the industrial perspective | Alan Dickson, University of Manchester | 99,700 |
| GCRF establishment of biopharmaceutical and animal vaccine production capacity in Thailand and neighbouring South East Asian countries | Colin Robinson, University of Kent | 4,090,772 |
| Polizzi-Dalby DTP student | Karen Polizzi, Imperial College | 100,000 |
| Development of a Novel Membrane Photobioreactor, for cultivation of Haematococcus pluvialis as a Biofilm | Marco Lizzul, Varicon Aqua Solutions | 70,000 |
| Enabling rapid liquid and freeze-dried formulation design for the manufacture and delivery of novel biopharmaceuticals | Robin Curtis, University of Manchester | 861,317 |
| Enabling rapid liquid and freeze-dried formulation design for the manufacture and delivery of novel biopharmaceuticals | Paul Dalby, University College London | 1,519,555 |
| Bioinformatic approaches to identify small molecules do disrupt antibody-host cell protein interactions | Mark Wass, University of Kent | 93,000 |
| Synthetic gene circuits to measure and mitigate translational stress during heterologous protein expression | Ian Stansfield, University of Aberdeen | 681,066 |
| iCASE studentship | Michael Plevin, University of York | 100,000 |
| Validation of a novel preclinical development platform to enable high value therapeutic co-formulations | Nicholas Darton, Arecor | £915,000 |
| Combinatorial genome editing to create enhanced biomanufacturing platforms | Alan Dickson, University of Manchester | 1,730,000 |
| University of Kent and Lonza Biologics | Mark Smales, University of Kent | 204, 568 |
| Synthetic Portabolomics | Natalio Krasnogor, Newcastle University | 4,353850 |
| | | **14.82M** |

**Table 1:** Further funding obtained as a result of BioProNET supported/fostered interactions

**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 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.

## Further grants – for commercialisation

Funding of £70k from Innovate UK was awarded to BIV partner Varicon Aqua Solutions

Innovate UK funding of £70K to the company Varicon Aqua Solutions for the project 'Development of a novel membrane photobioreactor for cultivation of *Haematococcus pluvialis* as a biofilm' (following from BIV_Aug15_Allen).

Innovate UK funding of £915K to Arecor for the project "Validation of a novel preclinical development platform to enable high value therapeutic co-formulations" (Health & Life Sciences Round 2, Application Number: 95404-562765, with Dickson, University of Manchester)

Innovate UK funding of £1.73M to Horizon Discovery for the project "Combinatorial genome editing to create enhanced biomanufacturing platforms (IB Catalyst, with Dickson, University of Manchester [BB/M01701X/1] and CPI)

GCRF funding of £4.1M to a consortium on 'GCRF Establishment of biopharmaceutical and animal vaccine production capacity in Thailand and neighbouring SE Asian countries' (Lead Robinson, University of Kent in collaboration with UCL and Imperial in UK) BB/P02789X/1

University of Kent and Lonza Biologics, £204, 568 awarded by Innovate UK (Mark Smales lead). Application number – 509862.

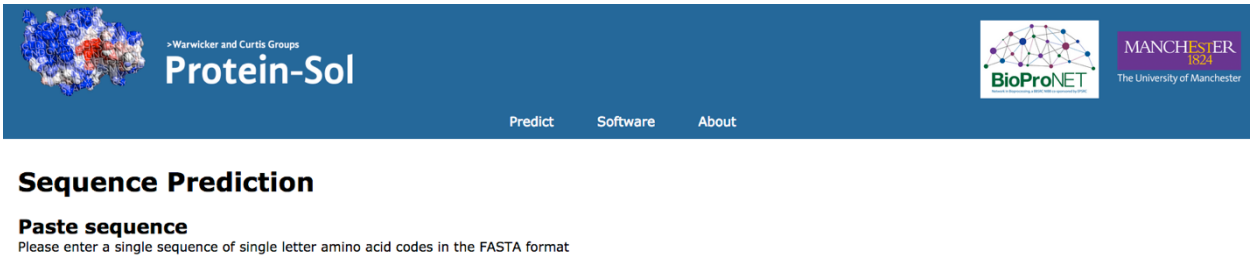
# Direct outputs: Patents, new processes devised and case studies

**We are aware of two patent portfolios developed directly as a consequence of BioProNET support, including** patent application GB1604640.1 by the University of Bath for Methods and the associated apparatus.

The **protein-sol** web tool was developed with BioProNET with proof of concept funding (PoC_Oct15_ Warwicker). This software predicts the likelihood of protein aggregation based on the amino acid sequence of the protein. The software input of amino acid sequence returns the result of a set of solubility prediction calculations, compared to a solubility database. These results are available for download in text form and are interpreted in graphs. To date the software has run 6000 searches from 2000 users.

https://protein-sol.manchester.ac.uk/about

The PDRA employed on the PoC (Dr Alejandro Carbello Amador) has been appointed to a permanent lectureship at Autonomous University of Baja California, and the experience he gained with the POC work was a major factor in his successful employment.




The information above highlights some specific exemplars of outputs from BioProNET funding but the outcomes of the funding are presented in detailed case studies that seek to explain how BioProNET funding has been significant for both a specialist and general audience (Appendix V, http://biopronetuk.org/case-studies-of-biopronet-funding/). Dr Claudio Angione (BIV Jan17 Angione) published a news-style article in Phys.org about the importance of his BioProNET-funded award (available here: https://phys.org/news/2017-12-machine-boost-protein-production-pharmaceuticals.html)

# 7. Indirect Outputs

The impact of BioProNET activities has had indirect value for growth in capability and capacity of the UK IBBE sector by supporting further funding. This generic indirect value can be seen in the quotes from industrial partners on BIV projects, for example

*"The BIV project confirmed the potential production of very valuable by-products from our main process, resulting in an increased interest from our board to extend and accelerate this research path by committing further resources to it".*

*"Academic and Industrial collaboration is important to narrow the gap between lab & commercial scale technology. Our [business interaction voucher] project work with [the academic] has certainly produced a promising outcome and as a result, we have much better traction now for securing further private investment."*

**Other specific examples can be identified** in the manner in which BioProNet supported Prof Natalio Krasnogor (the University of Newcastle) in his successful multi-disciplinary, multi-site grant application entitled 'Synthetic Portabolomics: Leading the Way at the Crossroads of the Digital and the Bio Economies'. This 5-year grant (£4.35M from the EPSRC) started in May 2016 (ET/N031962/1). BioProNET provided the forum for discussions that led to Prof Colin Robinson (University of Kent) bringing together collaborators from UCL and Imperial to develop the successful BBSRC-GCRF grant of £4.8M entitled "Establishment of biopharmaceutical and animal vaccine production capacity in Thailand and neighbouring South East Asian countries" (BB/P02789X/1)

# 8. International Impact

The significance of BioProNET (and the importance of the UK bioprocessing sector has been crystalised by several activities supported by BioProNET. We have invited many international speakers (the majority of whom would rarely, if ever, speak at events in the UK) to speak at our annual scientific meeting. These speakers are individuals who are highly prominent in the world-wide research community. They include Helene Faustrup Kildegaard – Technical University of Denmark; Paula Alves – iBET, Portugal; Leda Castilho – Federal University of Rio de Janeiro, Brazil; Chris Roberts – University of Delaware, USA; Paula Meleady – Dublin City University; Kelvin Lee – University of Delaware, USA; William Barton – Virginia Commonwealth University, USA; Robert Roth – AstraZeneca, Sweden; Mikael Rørdam Anderson Technical University of Denmark; Kathya de la Luz – Centre of Molecular immunology, Cuba; Veronique Chotteau – Institute of Technology Stockholm, Sweden; Hansjörg Hauser – Helmholtz Centre for Infection Research, Germany; Jurgen Hubbuch – Karlsruhe Institute of Technology, Germany; Thomas Scheibel – University of Bayreuth, Germany ; Matthew DeLisa – Cornell University, US; Andreas Schiermeyer – Fraunhofer Institute, Germany ; Martin Jordan – Merck Serono, Switzerland; Mike Betenbaugh - Johns Hopkins, USA; Colin Clarke – Dublin City University; Hitto Kaufman – Sanofi, Germany; Nicole Borth – BOKU, Vienna

In addition, in 2016 (in developing our review of the strategic needs for the future of bioprocessing) we held two internationalisation meetings. In the first we had 30 key industrialists and academics from 9 countries develop a white paper on the challenges, opportunities and international funding schemes for future collaborations across borders. The second meeting was held with a core of 6 international researchers and defined 3 work packages that would represent the work streams to go forward for EU and IMI cross-national funding calls. These activities have led to establishment of strong connections with major international academic-industrial groups including Advanced Mammalian Biomanufacturing Centre (AMBIC, USA, https://www.ambic.org/); European Society for Animal Cell Technology (ESACT; http://www.esact.org), National Institute for Innovation in Manufacturing Biopharmaceutical (NIIMBL, USA; http://www.niimbl.us/index.php), the eCHO Systems international training network (http://www.echo-systems.eu/)

# 9. Public engagement activities

BioProNET has taken part in, and led, several outreach activities. These include

## i. The Big Bang near me

BioProNET are an annual participant at the Big Bang Near Me at Discovery Park in Kent.

## At each event, there are around 800 students aged 11-14

The events aim to inspire students to study STEM (science, technology, engineering and maths) subjects.

Students 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!


**Figure 8.** Getting ready for 'making medicines' at an outreach event at Discovery Park

## ii. Chemistry at Work

We've taken part in two 2-day 'Chemistry at Work' event organised by the Royal Society of Chemistry and Canterbury Christ Church University. At each event, groups of around 15 students learned about plasmid DNA, got hands on-experience of size exclusion chromatography and investigated protein folding with the aid of modelling balloons.

### Through this event, we've reached out to over 300 school children aged 13-15

## iii. Proof of concept awardee Jim Warwicker undertook outreach at a University of Manchester open day. **His group demonstrated his project to A-level students from 8 different schools**. They imitated issues with protein solubility with a bucket of ping-pong balls, of varying degrees of (velcro) stickiness. In addition, they played with tangle toys in order to visualised protein shapes during the protein folding/misfolding process. BioProNET early career researcher Claire Gaffney from University of Manchester ran an event called 'Making Medicines from Cells' as part of British Science Week 2018.

## iv. Artwork

As part of our outreach activities, we commissioned artist Keith Robinson to produce four paintings that explore networks, collaborations and bioprocessing from an artistic perspective. These were unveiled at out Nottingham annual science meeting, have been displayed at BBSRC headquarters and in the University of Kent library gallery.

**Figure 9.** BioProNET artwork installed in the public gallery at the University of Kent library

## v. Prizes and awards

BioProNET co-director Alan Dickson won the Peter Dunnill Award (for outstanding contribution to UK bioprocessing) in 2017 and BioProNET member Barrie Rooney won BBSRC Social Innovator of the Year 2016 (https://www.kent.ac.uk/bio/news.html?view=1332)


**Figure 10.** Alan Dickson receiving the Peter Dunnill award from Prof Nigel Titchener-Hooker at the BIA Annual bioProcessUK conference in Cardiff, November 2017

# 10. Other meetings and events: workshops

We have used small group meetings around specific themes to aid building understanding of specific themes form both an educational and a collaboration-building perspective. In addition to workshops based on specific scientific themes we held a community sandpit meeting within the first two months of the initiation of BioProNET to define the activities and challenges most valid by our community (at the time) and to develop connections (and people) who could be approached for feedback throughout the period of BioProNET development. This activity was repeated mid-2017 (Townhall/strategy meeting) with a key focus group of leaders from academia and industry to take stock of the overall progress attained form BioProNET activities and to develop an clear strategic vision for the challenges that would be faced by the community in the coming 5-10 years.

## Workshop: Overcoming cellular barriers: implications for industrial biotechnology


**Figure 11.** Attendees at the overcoming cellular barriers workshop

This event was organized by BioProNET, CBMnet and BioCatNet

A total of nine new project ideas were identified at the meeting, together with champions to take them forward and the subsequent awarding of 5 business interaction vouchers (from the other co-organising NIBB).

- University of Kent and FujiFilm Diosynth Biotechnologies (2 BIV)
- University of Sheffield and FujiFilm Diosynth Biotechnologies
- University of Leeds and Lucite
- University of Kent and MatTek Corporation

As a result of the event a proof of concept application was submitted to BioProNET

# Workshop: Challenges and opportunities for protein glycosylation

Organized by BioProNET and IBCarb

**We asked attendees what they achieved from the meeting:**

- *Connecting with experts in the field that I would have difficulty identifying*
- *Networking advice and ideas for my project and potential projects, to identify key challenges*
- *Networking and learn about industry perspective*
- *An understanding of the glycoprotein industry and where the current challenges lie, I am new to the field so this was very useful*
- *Better understanding of the academia industry interface & where would be the best point to start collaborative research*

# Details and outcomes from all BioProNET funded workshops:

**1. Cell-free synthesis**

This workshop aimed to garner a better understanding of the current state of the art in cell-free synthesis. It was attended by 28 people and featured a keynote address by Trevor Hallam of Sutro Biopharma (which is available here).

Roundtable discussions focused on the challenges for large-scale manufacturing with cell-free synthesis extracts, the use of different cell types for different applications and ideas for moving forward (a summary of the key roundtable discussion points is available here). As an outcome, it was agreed that the group should consider doing a research presentation focused meeting in conjunction with a BioProNet event that would allow a better understanding of the research landscape in the UK.

"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.

**2. Application of tetrahertz spectroscopy to protein chemistry** – July 2014; was held at the University of Leeds. The aim of this workshop was to foster links between the Terahertz spectroscopy community and protein chemists from both academia and industry.

Forty-nine people attended the workshop from both business and academia. The attendees included staff from Teraview, Fujifilm Diosynth, UCB and MedImmune, and academics from Imperial College London, Queen May University London, University of Sheffield, University College London, University of Bolton, University of Cambridge, University of Leeds, University of Manchester, University of Nottingham and the University of York. Dr Andrew Burnett of the University of Leeds opened the workshop with a talk entitled "Studying protein dynamics and long-range order using terahertz spectroscopy" where he detailed the research carried out at Leeds on protein crystal samples.

**3. Analytics and formulation** – January 2015; was held at University College London and was attended by 17 delegates from academia and industry.

During the course of the day, several key analytical challenges were identified, which were resolved into three topics for taking forward into 'flipchart discussions', namely:

Non-invasive measurement methods
Automated sample prep and analysis from USD systems
Data management and predictability
Each topic was then discussed at a flipchart and people were able to move around between topics (encouraged half way through). The aim was to define potential PoC or IB catalyst type proposals in short form

**4. Downstream processing for biologics manufacture: What are the R&D priorities for the next 25 years?**
The workshop was held on March 18th 2015, and was organised as three parallel discussion groups, with each group tackling a 'big challenge':
**Keeping control** focused on the measurements that need to be made on downstream processes, and on how better, faster measurement might facilitate the transition from batch to continuous processing.
**Bigger target products** sought to discover new ways in which bigger products — such as viruses, conjugates and cells — might be separated from smaller entities.
**Re-inventing chromatography** invited participants to exercise their creativity to invent new, better approaches to what is probably the most widespread, and well-established, separation method in use today.
You can access the workshop report and outcomes by clicking here.

**5. Recombinant protein authenticity: causes and consequences of product heterogeneity**
The BioProNet WORKSHOP ON PROTEIN AUTHENTICITY held on 2nd March 2015 represented a timely event at which to (a) review what we know about decoding and post-translational errors in a range of hosts; (b) assess the impact of reduced protein authenticity on the activity and/or exploitability of high value rPs produced in these expression systems; and (c) develop industry-academic programmes to identify the key parameters that impact on protein authenticity. Such parameters will be key bellwethers of translational stress in the expression platform.
Please click here for a synopsis of the speakers' talks.

**6. Production of pharmaceutical and industrial proteins in microalgae and plants**
This workshop was held in Manchester on **May 1st 2015**. Plants and microalgae are attractive low-cost and scalable production platforms for manufacturing pharmaceutical and industrial proteins. These advantages have driven interest in their use as expression systems for industrial biotechnology. The workshop covered plant and algal expression systems, production systems/bioreactors, downstream processing together with cGMP. An overall aim was to help network the community working on algae, plants and established hosts. The overall objective of this workshop was to identify barriers and potential solutions to facilitate (fast track) the development and use of this emerging field in industrial biotechnology.

**7. Exploiting Genomic Tools in CHO Cells**
This workshop aimed to identify how established/novel genomic tools can be used/developed to improve CHO cell performance by drawing in expertise from other fields or cell systems, bioinformatics and systems biology.
The goals of the meeting were to raise awareness of genomic tools, to identify the genomic tools that need to be developed for CHO, and to formulate specific research ideas that might be pursued together as a community or through smaller collaborations. It was held at the University of Sheffield on April 30th 2015.

**8. Recent breakthroughs and new perspectives of protein aggregation**

Protein aggregation during biopharmaceutical (e.g. monoclonal antibodies) development impacts yield, production costs and must be controlled to ensure the clinical efficacy of the drug product. This workshop discussed common challenges in measuring, controlling and predicting protein aggregation. Additionally, recent advances that will shape the future direction of research were discussed. The outcome of the discussions will be documented in a

commentary put together by the organisers and the event speakers. Some discussion outcomes: From an industrial aspect, aggregation is statistically understood but not mechanically unravelled. Industry have standardised approaches to minimise aggregation or at least can rid of the very bad contenders. Academic collaboration is needed to identify what is the rate limiting factor. It is questioned whether understanding pathways is necessary or finding conditions to limit aggregation would be sufficient. In relation to this, specific topics were: (i) scope of technologies(ii) how to predict aggregation (iii) aggregation at the interface and (iv) novel excipients.

**9. CHO cell platforms: chassis for engineering improved bioprocessing 20/12/2016**

This meeting was held at the Manchester Museum in December 2016. Speakers included Nic Mermod (University of Lausanne, Switzerland); David James (University of Sheffield); Bob White (University of York); Nicole Borth (BOKU, Vienna); Niall Barron (DCU, Dublin); Nico Callewaert (Medical Biotechnology Centre, Gent).

Conclusions from discussions:
Key knowledge requirements:

List of desirable qualities can be made and some individual components known
"Best" CHO as a system unclear; datasets for predictive understanding needed
Recognition and diagnosis – troubleshooting profile and toolbox of criteria
Who could/should do this:
International community activity – multiple industrial/academic centres
Donation of model systems for common ground working – of different product types
Technology is available in different hands – focused interactions over long term
Funding opportunities:
Major and long-term project; combination of industrial and academic funding
Strategic leverage of funding for industry to enable precompetitive research
Existing funding schemes are immediate/short-term – strategic funders influence
Agreed next steps:
Clear interest in specific aspects discussed on the day and good connections made but no group-based approach identified
Industrial influence to develop/maintain continued activity and potential funding calls important

**10. Molecular chaperones: structure, function and applications in bioprocessing**

The meeting attracted 65 registered attendees; participants from 14 different UK Universities (including Oxford, UCL, Manchester, Glasgow, Sussex etc) attended and we were also delighted to have attendees from Holland, Sweden and Italy. We also had representatives from UCB, Oxford Biotrans and the CPI (Centre for Process Innovation) at the meeting although none of these gave formal presentations.

The meeting consisted of a full day of short (20 min) talks but with plenty of time for informal discussion during the lunch and coffee breaks. A total of 13 talks were presented covering a wide range of chaperone-related topics spanning biomedical, fundamental and applied research. Of relevance to the biotechnology/bioprocessing theme were the following talks:

• Reducing the bottleneck of protein translocation: a new generation of E. coli expression hosts (Kirsty Richards)

• mTORC1 signalling, eIF4E/4E-BP1 translation initiation factor stoichiometry and Hsp27 amounts influence recombinant protein productivity from GS-CHOK1 cells (Lyne Jossé)

• Mechanistic insight into co- translational disulphideformation (Philip Robinson)

• Optimisation of the Ero1/PDI interaction for improved protein folding and secretion in yeast (Dave Beal)

**How are you planning on taking these outcomes forward?**

The meeting is part of an annual series and one outcome from this meeting was that, because of the obvious continuing interest in this field of research, it was unanimously agreed that a meeting would be held in 2017. Patricia Van Oosten-Hawle from the University of Leeds agreed to host the next meeting in Leeds in December 2017. In addition, one of the attendees from Holland (Stefan Rudiger) encouraged members of the UK 'chaperone community' to attend the corresponding 'Dutch Chaperone Meeting' that was to be held in Utrecht on 22nd Feb 2017.

Another important outcome of the meeting was that, for the first time in this series of meetings, a focus was put on the application of chaperones in the context of recombinant protein production and we hope that, as a consequence, future meetings will attract more participants from the UK pharmaceutical sector.

## 11. The Versatility of Yeasts

The Kent Fungal Group (University of Kent) organised the 40th annual BYG meeting, entitled "The Versatility of Yeasts", from September 11-13 2017. Thanks to the co-sponsorship received from BioProNET, we were able to attract an unusually large number of participants working on fungal bioprocesses. In total, ten oral presentations discussed work relating to fungal biotechnology from strain engineering for fuel production to the repurposing of standard protein expression species like Pichia pastoris for novel products. Lindsey Male, a PhD student from Aberystwyth University who attended the meeting with a ***BioProNET bursary*** and who presented her work on using new spectroscopy methods for monitoring yeast physiology online during bioprocesses, highlighted the opportunities her presentation had generated for developing her own networks and collaborations.

We have further workshops planned for 2018 on topics that include big data, Genome editing, continuous processing and regenerative medicine.

# 11. Townhall meetings

## BioProNET held its first townhall meeting in June 2014

## This meeting focused on the strategy for the BioProNET network

## Attendees came from

Centre for Process Innovation, University of Nottingham, University of Wolverhampton, University of Kent, University of Leeds, Knowledge Transfer Network Ltd, Loughborough University, Croda Europe Ltd, University College London, Mologic Ltd, University of Manchester, Medimmune, Synthase, Britest Ltd, University of Warwick, GlaxoSmithKline, University of Strathclyde, University of Cambridge, Loughborough University , Linear Diagnostics Ltd, Brunel University, Fujifilm Diosynth Biotechnologies, Avacta, Pall Life Sciences, Actavis Biologics Ltd, Lonza Biologics plc, Newcastle University, University of Birmingham, University of Sheffield, University of Manchester, Imperial College London, Cobra Biologics, University of Aberdeen, UCB, Horizon Discovery, BBSRC, Loughborough University, Glythera, University of Kent, MedImmune, University of Edinburgh, Artos Innovation, University of Sheffield, , Lonza Biologics plc, University College London

## Summary of Outcome(s)/Impact(s)

Topics and groups were identified for workshops; attendees aware of funding opportunities such as PoC and BIV

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

See also the workshop case study of this event.

**Our second Townhall was held three years later in June 2017 at MedImmune in Cambridge**

**This time focusing on the wider BioProcessing strategy in the UK**

**Invitees were key opinion leaders from the following companies -**

Centre for Process Innovation (CPI) , NightStarX, UCB, Independent consultants, University College London, University of Manchester, University of Kent, Loughborough University, Imperial College, BBSRC , Cambridge University, Cobra biologics, Knowledge Transfer Network (KTN), MedImmune, Allergan.

A common theme to emerge was the importance of BioProNET in providing a forum for the engagement of academics and industrialists to identify challenges and develop novel collaborations and approaches towards solving these and keeping the UK bioprocessing sector world-leading. It was acknowledged the competition the UK faces globally in this space, largely as a result of the success of BRIC and BioProNET in this space, and the importance of maintaining this forum and community in the coming years to maintain UK competitiveness.

# 12. Member survey

**To complete parts of this report, we surveyed engaged members from industry and academia (those that attended our most recent annual science meeting) and industry members who had been a partner on a BIV, PoC or IBSCA. In total, about 180 people received a survey. We received 48 replies.**

| **What is the benefit of being a member of BioProNET to me ?** | Academia | Industry |
|---|---|---|
| New interaction with a company | 12 (could include BIV / POC) | 7 |
| Increased motivation to commercialise my research | 11 | NA |
| Or am thinking about creating a company | 2 | NA |
| Received additional funding | 6 | NA |
| Won a prize or award | 2 | 1 |
| My work has become more interdisciplinary | 7 | NA |
| Created new employment | 6 (includes BIV / PoC) | 4 |
| Carried out public engagement activities | 3 | 0 |
| Moved to new employment | 1 | 0 |
| Gained a better understanding of bioprocessing and the bioprocessing sector | 19 | 9 |
| Other | 1 | 3 |
| Increased motivation to work with academics | NA | 28 |
| Our company has funded additional projects | NA | 4 |
| There has been a shift in attitude towards IB | NA | 0 |
| We have devised a new process | NA | 2 |
| We have created a new product | NA | 1 |
| There has been an increase in capacity or capability to use further funding | NA | 4 |
| A patent has been filled or licensed | NA | 1 |
| Total replies | 20 | 28 |

**Appendix I**
**List of companies that have BioProNET members:**

Allergan
Anaero Technology
Anne Velenturf Research and Consultancy
Applikon Biotechnology UK
Arecor
AstraZeneca
ATG Scientific
Auto Q Biosciences
Avacta analytics
Badrilla
Bio-Shape
Biocatalysts
Bioextractions
BioMax Scientific Consultancy
BioPro Control Tech
Bioprocess Engineering Services
Biorenewables Development Centre
BioRN Network
Biosynergy Europe
BioToolomics
Blueberry Therapeutics
BPE Design and Support
BPOG
Britest
Broadley-James
CHAIN Biotech
Chemquip
Cobra Biologics
Centre for Process Innovation
Croda
Crop Intellect
Customem
DG Bioconsult
Dr Reddy's Laboratories
Efficiency Technologies
Elanco
Elasmogen
Eluceda
Entomics Biosystems
Europa Bioproducts
Evotec
eXmoor pharma concepts
F Star
Finnis UK
Fujifilm Diosynth
Fulcrum Direct
GE Healthcare
Gedeon Richter
GlaxoSmithKline
GlycoSeLect
Glythera
Green Biologics
GreyRigge Associates
Horizon Discovery
Howseman Agriculture
Immunocore
Infors-HT
Ingenza
Institut Pasteur de Tunis
Intas bio-pharmaceuticals
Ipsen
Isca Biochemicals
JEOL UK
Johnson Matthey
Leaf Expression Systems
LGC
LifeArc
Linear Diagnostics
Lonza Biologics
Macphie of Glenbervie
Malvern Instruments
MedAnnex
MedImmune
Merck
Mologic
Moredun Research Institute
MXJ Consulting
Namba-Biotech
Nanette Berthram Consulting
Nanotemper Technologies
Nathan Consulting
Native Antigen Company
Neem Biotech
NEPIC
New England Biolabs
Nightstar Therapeutics
Novozymes
NunaBio
Orla Protein Technologies
Oxford BioMedica
Oxford Biotrans
Pall Life Sciences
Peak Proteins
PeptiGelDesign
Plantform Corp
Porton Biopharma
Porvair Filtration Group
Prism Training & Consultancy
Procellia
Process Systems Enterprise
Puridify
Purolite
RD-Biotech SAS
Recyclatech Group
Reliance Precision
ReNeuron
Richter Gedeon
Roche Diagnostics
ROK Bioconsulting
Rothamsted Research
Rotherwood Associates
Varicon Aqua Solutions
RSM UK
SAFC
SAL Scientific
SASA
SCIEX
Scottish Enterprise
Sealed Air
Semba Biosciences
Shenlian Biotechnology
Shimadzu UK
Sigma-Aldrich
Sirius Analytical
Solentim
SpectrumLabs
Sphere Fluidics
Stratophase
Sutro Biopharma
Synpromics
Synthace
TAP Biosystems
Telix Pharmaceuticals
TFS
Thermo Fisher Scientific
Totallab
UCB
uFraction8
Unchained Labs
Unilever
Williams Process
Wyatt Technology

**Appendix II**

**Programmes from previous annual meetings:**

# Cardiff 2014

Day 1
Chair Mark Carver – Fujifilm Diosynth Biotechnologies
**Neil Brewis** – GlaxoSmithKline *Landscape and opportunities in bioprocessing*
**Marcel Ottens** – Delft Institute of Technology, Netherlands *A new paradigm in biopurification process development*
**Shahid Uddin** – MedImmune *Formulation challenges of biologicals*
**Stuart Haslam** – Imperial College, London *Life is sweet - Glycomics and glycoproteomics providing new biological insights*
**Tom Parsons** – University of Oxford *A tag and modification approach to protein modification*
Coffee & Hotel Registration
**Neil Weir** – UCB *Small molecule pharmaceuticals versus biologics - cross fertilisation*

Chair Alan Dickson – University of Manchester
Networks in Industrial Biotechnology and Bioenergy (BBSRC NIBB)
**David Leak** –University of Bath P2P: *From Plants to Products: A network of integrated technologies*
**Nigel Robinson** – Durham University Metals in Biology; *The elements of biotechnology and bioenergy*
**Michelle Stanley** – Scottish Association for Marine Science PHYCONET - *Assessing opportunities and barriers to commercialisation of microalgae for production of high value products*
**Jeff Green** – University of Sheffield CBMNet; *Engineering the cell-environment interface to improve process efficiency*

Posters & Networking Reception
Conference Dinner

Day 2
BioProNET Early Career Researchers Workshop/Breakfast
Amada Weiss - Fujifilm Diosynth Biotechnologies, Malcolm Rhodes - University of Manchester

Chair Ray Field – MedImmune
**Colin Clarke** – Dublin City University *Statistical methods for mining CHO cell omics data: From different ex-pression to integrated multi-level analysis of the biological system*
**Nicole Borth** – Austrian Centre of Applied Biotechnology *The beauty and the challenges of miRNA engineering*
**David James** – University of Sheffield *Design and engineering of CHO cell factories*
**Hitto Kaufman** – Sanofi *Innovation in biologics manufacturing*

10:20 Coffee and Networking
**Workshops on Perspectives Across Expression Platforms, including**
**Nigel Slater** – Cambridge University *Expression meets downstream*
**Phil Wright** – University of Sheffield *Make mine an* E.coli
**Colin Robinson** – University of Kent *Microalgae coming through*
**Tony Hitchcock** – Cobra Biologics *Viruses and vaccines*
**Dan Bracewell** – University College London *Host of worries*
**Peter Levison** – Pall Life Sciences *But what about the purification?*

Chair Mark Smales – University of Kent
**Mike Betenbaugh** – John Hopkins University *Systems biotechnology of mammalian cells*
**Michael Roberts** – Synpromics *Synthetic promoters for custom design gene expression*
**Paula Alves** – IBET, Portugal *Virus-like particles in vaccine development: Insect cell technology as production platforms*
**Dafydd Jones** – University of Cardiff *From backbone to new chemistry, insights into emerging approaches for protein engineering and design*
Close of Meeting, Lunch and Networking

# Manchester 2015

**Day 1**

### Biologics: present and future perspectives

Chair: Nigel Titchener-Hooker, UCL
**Thomas Scheibel** – University of Bayreuth, Germany: *Engineered spider silk proteins: bioinspired polymers for various applications*
**Mark Uden** – GlaxoSmithKline: *Trends in modern bioprocessing – intriguing science meets commercial realities*
**Andy Porter** – University of Aberdeen: *Single domain solomers – simpler and more effective than antibodies?*
Coffee and networking

### Cellular production systems: coping with future demands

Chair: Ray Field, MedImmune
**Matthew DeLisa** – Cornell University, USA**:** *Engineering unnatural biosynthetic pathways for protein modification in bacteria*
**Andreas Schiermeyer** – Fraunhofer Institute, Germany**:** *Plant-based expression systems for the production of recombinant proteins*
**Martin Jordan** – Merck Serono, Switzerland: *Rational media design in microscale-fed batch cultures*
**Ian Hodgson** – Fujifilm Diosynth Biotechnologies: *Yeast expression systems for biologics – recent developments and future trends*
Introduction to BIA MAC – Peter Levison
BioProNET update – Mark Smales & Alan Dickson

### Proof of Concept awardee poster flashes

Karen Polizzi & Rochelle Aw – Imperial College London: *Towards a cell free expression based on pichia pastoris*
Jim Warwicker & Alejandro Carballo – University of Manchester: *Web tools to predict protein solubility and/or aggregation*
Tobias von der Haar – University of Kent: *Gene expression accuracy as a parameter in bioprocessing applications*

Workshops – 4x parallel sessions themed on meeting sessions
**Biologics: present and future perspectives** Chairs Nigel Titchener-Hooker & Brenden Fish
**Cellular production systems: coping with future demands** Chairs Ray Field & Tobias von der Haar
**Bioprocessing of cell products – just like proteins (or not)** Chairs Tony Hitchcock and Owen Thomas
**Emerging technologies for the 2020 bioprocessing agenda** Chairs Paul Dalby, Amanda Weiss & John Liddell
Posters and networking
Conference dinner

**Agenda day 2**

**Bioprocessing of non-recombinant protein based cell products**

Chair: Tony Hitchcock, Cobra Biologics

**Reingard Grabherr** – University of Natural Resources and Life Sciences (BOKU), Austria: *Insect cell culture-based manufacture of bionanoparticles*

**Peter Jones** – Oxford BioMedica: *Scale-up and manufacturing challenges of lentiviral vectors for use in gene and cell-based therapies*

**Sarah Gilbert** – Jenner Institute University of Oxford: *Viral-vectored vaccines – why we need them and how to produce them*

**Farlan Veraitch** – University College London: *Oxygen-controlled bioprocessing of pluripotent stem cells*

**Martin Ebner** – Immunocore: *Immtacs: changing the therapeutic perspective*

Break and networking

**Emerging technologies for the 2020 bioprocessing agenda**

Chair: Paul Dalby, University College London

**Douglas Kell** – University of Manchester: *Speedygenes, genegenie and random forests: three synthetic biology strategies for navigating sequence space intelligently*

**Mike Davies** – Centre for Process Innovation: *Novelty needs characterization: tomorrows analytics*

**Cleo Kontoravdi** – Imperial College London: *Computational tools for implementing 'quality by design*

**Iwan Roberts** – Puridify: *Nanofibres for high productivity purification*

# Nottingham 2016

**Day 1**

Keynote speaker – **William Barton** (Virginia Commonwealth University, USA) *Over-expression of secreted proteins from mammalian cell lines*

**Designing efficient cell-expression systems**

**Chaired by Pete Tessier**

**Robert Roth** (AstraZeneca, Sweden) *Using phenotypic screening to identify regulators of recombinant protein expression*

**Mikael Rørdam Anderson** (Technical University of Denmark) *Networks: the key to understanding and engineering cho protein secretion*

**Neil Bullied** (University of Glasgow) *Optimising the design and production of therapeutic antibodies*

Short presentations from BioProNET Proof of Concept funding awardees:

Talk 1 – **Tim Ganderton** (University of York) *Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins*

Talk 2 – **Stefani Dritsa** (University of Kent) *Analysis of host cell protein impurities using* in silico *approaches*

**Building expression systems into optimised processes**

**Chaired by Robert Roth**

**Kathya de la Luz** (Centre of Molecular immunology, Cuba) *Linking the cell metabolism and recombinant protein expression in mammalian cell lines: cim experiences.*

**Colin Jaques** (Lonza) *Scale-up in the single use age: design matters*

**Veronique Chotteau** (Institute of Technology Stockholm, Sweden) *High cell-density perfusion for biopharmaceutical production – challenges for tomorrow's processes*

## Short presentations from career researchers

**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)
Break – hotel check-in and networking
Poster session (drinks reception, prizes for best posters)
Dinner, with guest speaker Hansjörg Hauser, Helmholtz Centre for Infection Research, Germany

**Day 2**

Keynote speaker – **Pete Tessier** (Rensselaer Polytechnic Institute, USA*) Improved antibody design, evolution and selection methods for minimizing developability issues*

## Molecular characterization of process quality

**Chaired by Jurgen Hubbuch**

**JJ Phillips** (University of Cambridge) *engineering the surface properties of a human monoclonal antibody prevents self-association and rapid clearance in vivo*
**Mike Davies** (F-Star) *overcoming the manufacturing challenges for bi-specific mAbs*
**Lucy Beales** (Mologic) *overcoming development challenges in the development of VLP-based vaccines*

## Upstream meets downstream: an integrated vision

**Chaired by Mikael Rørdam Anderson**

**Dan Bracewell** (University College London) *Nanofibres in bioprocessing: a single-use chromatography format by the use of rapid cycling*
**Jurgen Hubbuch** (Karlsruhe Institute of Technology, Germany)
**Tim Dafforn** (University of Birmingham) *Nanoencapsulation for the production of membrane- and periplasmic-trafficked proteins*

# Warwick 2017


**Figure 13**. Images from the 2017 BioProNET Annual Science meeting held at the Warwick Conference Centre

## DAY 1

### Designing More Efficient Cell-Expression Systems

**Helene Faustrup Kildegaard** – Technical University of Denmark: *Improving cho cell factories with crispr-mediated genome engineering*
**Eva Pekle** – University of Kent: *single cell characterisation of CHO cells*
**Imre Berger** – University of Bristol: *Baculovirus expression: old dog, new tricks*

**David Humphreys –** UCB: *Protein expression demands and demanding protein expressions: protein sciences the biopharma way*
**Stefanie Frank** – University College London: *Engineering spatial segregation within bacterial hosts for bio-therapeutic protein production*
**Karen Coopman** – Loughborough University: *Taking a holistic approach to mesenchymal stem cell culture process design*
**Robyn Emmins** – GlaxoSmithKline: *Embedding the Berkeley lights beacon: a bright future for cell line development*

### Building Expression Systems Into Optimised Process

**Paula Alves** – iBET, Portugal: *Insect cell platforms for production of VLP and difficult to express proteins*
**Eleanor Hanson** – University of Sheffield: *Changes in CHO cell epigenetics throughout cell culture*
**Ray Owens** – University of Oxford: *High throughput cloning and expression of recombinant proteins for structural biology*
**Kathryn Lilley** – University of Cambridge: *Quantitative mass spectrometry to determine the three dimensional relationship of the proteome*
**Tania Selas Castiñeiras** – Cobra Biologics: *Periplasmic recombinant protein production: which signal peptide to use?*

### The Clinic And Beyond

**Leda Castilho** – Federal University of Rio de Janeiro, Brazil: *Production of flavivirus vlps: zika, yellow fever and beyond*
**Jim Faulkner** – Autolus: Process development for autologous cell products
Proof of concept funding awardees (chaired by Mark Smales)
**Martin Michaelis** – University of Kent: *Expanding production time of mammalian cell cultures for biotechnological applications*
**Cleo Kontoravdi –** Imperial College London: *Bioreactor design space identification with product quality constraints*
**Gyorgy Szekely** – University of Manchester*: Molecular imprinting for sustainable downstream processing of biopharmaceuticals*

**Day 2**

### Molecular Characterization Of Process Quality

**Chris Roberts** – University of Delaware, USA: *Mechanistic approaches to stabilization of pharmaceutical proteins*
**Paula Meleady** – Dublin City University: *Quantitative phosphoproteomic analysis of cho cells in response to reduced culture temperature*
**Perdita Barran** – University of Manchester: *Hybrid mass spectrometry approaches to analyse biologics and distinguish biosimilars*
**Chris Sellick** – MedImmune: *Application of custom analytics to support bioprocess development for novel biopharmaceuticals*
**Stephen Perkins** – University College London: *CCP-SAS – its utility for the atomistic modelling of pharmaceutically-important antibody solution structures*

### Upstream Meets Downstream: Rapid Process Development

**Kelvin Lee** – University of Delaware, USA: A *host cell protein that may impact polysorbate degradation*
**Mire Zloh** – University of Hertfordshire: *Effects of excipients on biomolecule structures during dehydration processes at low temperatures*
**Qasim Rafiq** – University College London: *Scale-down approaches to mammalian cell culture process development and primary recovery*
**Rochelle Aw** – Imperial College London: *Increasing ribosome content in pichia pastoris for improved cell free protein synthesis*
**Simone Dimartino** – University of Edinburgh: 3D printed porous media for packed bioreactors and downstream processing
**Peter Levison** – Pall: *Technology advances in continuous bioprocessing from process development into clinical manufacture*

## London 2018 (TBC)

**Day 1**

### *Designing more efficient cell-expression systems*

Welcome, achievements and what next
**Kerstin Otte** – University of Applied Science Biberach, Germany
**Colin Robinson** – University of Kent
**Nate Lewis** – University of California San Diego, USA
**Andrew Peden** (PoC awardee) – University of Sheffield *Developing a tool kit for determining the manufacturability of new therapeutics in CHO cells.*

Lunch

### *Building expression systems into optimised process*

**Gary Finka** – GlaxoSmithKline
**Sophia Hober** – KTH Royal Institute of Technology, Sweden *The Human Secretome Project*
**Tarit Mukhopadhyay** – University College London *Manufacturing the future at less than a $1 a dose and meeting global health needs*
**Natalio Krasnogor** – Newcastle University
**Gary Montague** (PoC awardee) – Teesside University *Improved preservation of biologics by continuous intensified lyophilisation*

Coffee and networking

### *The clinic and beyond*

Caroline Barelle – Elasmogen
Phil Cater – Leaf Expression Systems *Plant produced biologics – process economics*
**Michael Plevin** (PoC awardee) – University of York *Can an archaeal helicase enhance the performance of ananopore DNA sequencer?*
ECR short talk

Drinks reception and poster session
Dinner

**Day 2**

### *Upstream meets downstream* - rapid process development

**Paul Dalby** – University College London *New analytical methods for chromatography OR further downstream to formulation.*
**Yvonne Grenzel** – Max Planck Institute for Dynamics of Complex Technical Systems, Germany *Intensified cell-based viral vaccine processes: from continuous to perfusion and to hybrid systems.*
**Chris van der Walle** – MedImmune *Manufacture and characterisation of nanoparticles encapsulating nucleic acid.*
**Dave Brockwell** (PoC awardee) – *Investigating the effects of hydrodynamic force on the structure and biological integrity of a viral vector gene therapy product.*
ECR short talk
**Pernille Harris** – Technical University of Denmark

Coffee and networking

### *Molecular characterisation of process quality*

**Jonathan Bones** – The National Institute for Bioprocessing Research and Training, Ireland
**Laura Palomares** – National Autonomous University of Mexico
Perdita Barran (PoC awardee) – University of Manchester *Top-down mass spectrometry methods for full characterisation of biopharmaceuticals.*
ECR short talk
**Mike Betenbaugh** – Johns Hopkins University *Glyco-engineering*

Lunch

### Afternoon session – building academia–industry collaborative projects

Chaired by David Humphreys, UCB.

**Appendix III.** Details of BioProNET BIVS and PoC Awards

| Call | Principle Investigator | University | Industry Partner | Title | Public outcomes |
|---|---|---|---|---|---|
| BIV Nov14 | Randy Mrsny | Bath | Arecor | Initial development of novel product concepts with unique pharmacokinetic characteristics | Case study |
| BIV Nov14 | Bob White | York | Cobra | Evaluating enhancement of Secretion for Recombinant Proteins in CHO cells via overexpression of 7SL RNA | |
| BIV May15 | Lorna Ashton | Lancaster | Cobra | Evaluating the use of Raman Spectroscopy to determine topological isoforms of plasmid DNA | Case study<br>Applied for PoC |
| BIV May15 | Anil Day | Manchester | Protein Technologies | Production of therapeutic and industrial proteins in microalgae | Case Study<br>Submitted application to Newton Fund |
| BIV May15 | Corinne Smith | Warwick | JEOL UK | Exploiting advanced electron microscopy to optimise protein and biologic expression platforms | Case study<br>Paper published: Biochemical Journal DOI: 10.1042/BCJ20160952 Title: TatA complexes exhibit a marked change in organisation in response to increased TatBC expression |
| BIV May15 | Yuhong Zhou | UCL | BioPro Control Tech | Development of a crossflow filtration dynamic flux control system to reduce cell harvest time | Case study |
| BIV May15 | Paul Clegg | Edinburgh | Recyclatech | Rapid processing to recover high value microbial by-products | Case study<br>Data has been used to support successful EPSRC application |
| BIV May15 | Tarit Mukhopadhyay | UCL | Public Health England | A pilot study to improve the expression of a *Clostridium difficile* toxin-based fragment in *E.coli* | Applied for BioProNET PoC |
| BIV Aug15 | Lisa Swanton | Manchester | SAL scientific | Enhancing cell growth to allow selection of biopharmaceutical-producer cell lines with favourable properties | Applied for BioProNET PoC<br>Awarded IBSCA<br>Data will be incorporated into SAL's product literature |
| BIV Aug15 | Martin Michaelis | Kent | TotalLab | Monitoring of host cell proteome expression during bioprocessing of CHO cells expressing recombinant proteins | Presented at the BEBPA (Biopharmaceutical Emerging Best Practices Association) conference http://www.bebpa.org/conferences/) on HCPs in San Francisco (May 10-12, 2017) |

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|---|---|---|---|---|---|
| BIV Aug15 | Mike Allen | Plymouth Marine Laboratory | Varicon Aqua Solutions | Design consultation and testing of a membrane photobiorector suitable for advanced biologic production from micro algae | Case study<br>Successful application to Innovate UK |
| BIV Nov15 | Kevin Flynn | Swansea | AlgaeCytes | A bio-process to optimize yields and characterize and test unique exopolysaccharides from two microalgal strains | Case study |
| BIV Feb16 | Michael Plevin | York | Oxford Nanopore | A collaboration to engineer a novel protein nanopore for single molecule DNA sequencing applications | Awarded BioProNET PoC funding<br>Awarded iCASE studentship |
| BIV Feb16 | Tobias von der Haar | Kent | UCB Celltech | Predictive tools for folding-supportive sequence design spaces | Case study |
| BIV Feb16 | Chris Pudney | Bath | Bath ASU | Developing a novel fluorescence-based biopharmaceutical quality control technology | UK Patent Application No. 1604640.1 .<br>Presentation at the 2nd International Antibody Validation Meeting<br>Received EPSRC funding as part of seed-corn funding for a GCRF<br>Secured an EPSRC Impact Acceleration Account (IAA)<br>Produced a short film: https://youtu.be/kHdZBy5xJ2A<br>applied for BioProNET PoC |
| BIV Feb16 | Garry Blakely | Edinburgh | Hyaltech | Industry biotechnology for the production of hyaluronic acid from *Streptococcus equi* | Applied for a PoC from BioProNET (not funded; largely because it was thought that the company might be better placed to fund the study |
| BIV Feb16 | Sourav Ghosh | Loughborough | CPI | Exploring the feasibility of nonlinear acoustic detection technique for online bioprocess monitoring | The work is being written up for journal publication |
| BIV Feb16 | Helen Philippou | Leeds | Crystec | Supercritical fluid processing to improve the stability and delivery of low dose biopharmaceuticals | |
| BIV May16 | James Winterburn | Manchester | Croda | Novel bioprocessing approaches for the separation of product phases | Case study<br>ECR scientific exchange award<br>Shortlisted for BBSRC innovator of the year 2018 |
| BIV May16 | Perdita Barran | Manchester | BioShape | Examining the relationship between charge and zeta potential in proteins using mass spectrometry | |

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|---|---|---|---|---|---|
| BIV Jan17 | Colin Robinson | Kent | UCB Celltech | Fermentation optimisation of biotherapeutic production by *E. coli* 'TatExpress' strains | Ongoing<br>Data has been used in an EU grant application involving both partners |
| BIV Jan17 | Jose Guterriez-Marcos | Warwick | Mologic | Assessing the production of human cysteine knot hormones in plant cell cultures | Ongoing |
| BIV Jan17 | Claudio Angione | Teesside | CPI | A machine learning poly-omics classifier to improve protein production in CHO cells | Ongoing<br>Zampieri, G.; Coggins, M.; Valle, G.; Angione, C. A poly-omics machine-learning method to predict metabolite production in CHO cells . *In Proceedings of the The 2nd International Electronic Conference on Metabolomics*, 20–27 November 2017; Sciforum Electronic Conference Series, Vol. 2, 2017 ; doi:10.3390/iecm-2-04993 |
| BIV Jan17 | Karen Polizzi | Imperial | Lonza | Comparing the productivity of three cell-free extracts based on industrial cell lines | ongoing |
| BIV Jan17 | Saul Purton | UCL | Microsynbiotix | Scale up of vaccine production in a microalgal host for animal trials | ongoing |
| BIVJan18 | Duygu Dikicioglu | Cambridge | Johnson Matthey | Addressing challenges in pre-processing and mining bioprocess development data in biocatalyst manufacturing by machine learning | ongoing |
| | | | | | |
| PoC Oct15 | Tobias von der Haar | Kent | MRC Technologies Cobra | Gene expression accuracy as a parameter in bioprocessing applications | Case study<br>Paper: Codon-dependent translational accuracy controls protein quality in Eschericha coli but not Saccharomyces cerevisiae Lyne Jossé, Connor D. D. Sampson, Mick F. Tuite, Kevin Howland,Tobias von der Haar<br>doi: https://doi.org/10.1101/200006 (preprint) |
| PoC Oct15 | Jim Warwicker | Manchester | – | Web tools to predict protein solubility and/or aggregation | http://www.protein-sol.manchester.ac.uk/<br>paper published<br>Protein-Sol: A web tool for predicting protein solubility from sequence Hebditch M, Alejandro Carballo-Amador M, |

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| | | | | | Charonis S, Curtis R, Warwicker J. Bioinformatics doi: 10.1093/bioinformatics/btx345 May 29 2017<br>Successful proposal to an EPSRC call, led by Paul Dalby at UCL, and including JW and RC at the University of Manchester.<br>Case study |
| PoC Oct15 | Karen Polizzi | Imperial | FujiFilm | Towards a cell-free expression system based on *Pichia pastoris* | Case study<br>Talk at ESACT-UK in January 2016. "From a cell lysate to a protein production system: development of cell-free protein synthesis systems"<br>PhD student from the UCL Centre for Macromolecular Therapies DTP |
| PoC Nov16 | Cleo Kontoravdi | Imperial | MedImmune | Bioreactor design space identification with product quality constraint | Case study |
| PoC Nov16 | Martin Michaelis | Kent | MedImmune | Expanding production time of mammalian cell cultures for biotechnological applications | Paper in International Journal of Parasitology. A cell culture platform for Cryptosporidium that enables long-term cultivation and new tools for the systematic investigation of its biology https://doi.org/10.1016/j.ijpara.2017.10.001 |
| PoC Nov16 | Marek Brzozowski | York | – | Hijacking intracellular storage bodies to create a novel mammalian cell-based expression system for the production of hard-to-express proteins | Case study<br>Talk at 2017 ESACT-UK meeting |
| PoC Nov16 | Gyorgy Szekely | Manchester | FujiFilm | Molecular Imprinting for Sustainable Downstream Processing of Biopharmaceuticals | Long-term stability and reusability of molecularly imprinted polymers Polym. Chem., 8, 666-673 (2017) DOI: 10.1039/C6PY01853J<br>Exploring and exploiting the effect of solvent treatment in membrane separations ACS Appl. Mater. Interfaces, 9, 11279–11289 (2017) DOI: 10.1021/acsami.7b01879<br>Valorisation of agricultural waste with an adsorption/nanofiltration hybrid process: from materials to sustainable |

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|---|---|---|---|---|---|
| | | | | | process design Green Chem., 19, 3116-3125 (2017) DOI: 10.1039/C7GC00912G |
| PoC Nov16 | Mark Wass | Kent | CPI | Analysis of host cell protein impurities using *in silico* approaches | 4 year PhD studentship from the UCL EPSRC Centre for Doctoral Training in Emergent Macromolecular Therapies |
| PoC Nov16 | Andrew Peden | Sheffield | UCB Pharma | Developing a tool kit for determining the manufacturability of new therapeutics in CHO cells | ongoing |
| PoC Nov16 | Michael Plevin | York | Oxford Nanopore | Can archaeal [...] helicases enhance the performance of a nanopore DNA sequencer? | ongoing |
| PoC May17 | Gary Montague | Teesside University | Lonza, Glythera, Accelyo | Improved preservation of biologics by continuous intensified lyophilisation | ongoing |
| PoC May17 | David Brockwell | University of Leeds | Cobra Biologics | Investigating the effects of hydrodynamic force on the structure and biological integrity of a viral vector gene therapy product | ongoing |
| PoC May17 | Perdita Barran | University of Manchester | Covance | Top-down mass spectrometry methods for full characterisation of biopharmaceuticals | ongoing |

### Learning from charge interactions in nature to understand poly-anion/cation complementarity in drug encapsulation

PI: Jim Warwicker, University of Manchester
Partner company: MedImmune (letter of support)

**Project aims**

Nucleic acid-based therapeutics are susceptible to degradation, and so are combined with carrier molecules to improve their formulation. This computer-based project compared charge densities — a key consideration in matching nucleic acid to carrier molecules — in formulations and in naturally occurring complexes of nucleic acids and partner molecules.

**Key findings**

Our major observation was that both positive and negative charge is used by proteins to pack nucleic acid. We infer that negative charge is used to 'shape' nucleic acid conformation, coupled with the counter-ioning effect of positive charge. This finding could impact on the design of nucleic acid delivery systems, since it goes beyond a simple view of charge complementarity.

**Next steps**

A manuscript is in preparation, and data generated from this project will be added to the Protein Sol database.

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### Establishing a prototype process for manufacturing non-therapeutic biologics expressed in plants for the R&D market

PI: Anil Day, University of Manchester
Partner company: Protein Technologies (in-kind funding)

**Project aims**

We have previously developed a low cost, high yield and sustainable leaf-based system for the manufacture of recombinant proteins. This project focused on the steps required to commercialize the technology by streamlining the processing needed to make the final purified and active cytokine protein product from leaf biomass as well as conducting market research and due diligence.

**Key findings**

Our main output was an increased cytokine yield from leaves, identification of a cheaper combination of reagents to enable protein refolding and movement of three key steps (growth of biomass, initial purification from chloroplasts, refolding) from TRL4 to TRL5/6 to produce a prototype process. Our evaluation of the patent landscape concluded we had freedom to operate in this area.

**Next steps**

We have recruited an MSc research student to support the downstream processing steps and a BBSRC Postdoctoral Innovation Fellow to work on the project at the industrial partner.

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### Engineering secretory capacity in *S. cerevisiae* strains to improve recombinant protein production yield

PI: Campbell Gourley, University of Kent
Partner company: Novo Nordisk (letter of support)

**Project aims:**

We have previously identified genetic alterations in the budding yeast *Saccharomyces cerevisiae* that greatly improves its capacity to produce recombinant protein. This project will demonstrate that the identified genetic changes will lead to the increased production of a high value recombinant protein as proof of principle to present to a new industrial collaborator.

**Key findings**

We cloned and expressed human serum albumin in *S. cerevisiae* and are currently analyzing RNA sequencing results to determine the gene expression changes that occur in *S. cerevisiae* cells lacking *STE12*, a mutation that leads to enhanced recombinant protein production.

**Next steps**

We are discussing how best to move our findings into industrial strains in partnership with Novo Nordisk.

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### Enhancing secretory pathway function to allow selection of host cell clones

PI: Lisa Swanton, University of Manchester
Partner company: SAL Scientific (in-kind funding)

**Project aims**

We will set up a new system to measure how quickly cells can secrete recombinant protein, to determine cell growth conditions that maximize the secretion of biopharmaceuticals. We will exploit the 'RUSH' system, whereby a tagged cargo protein accumulates in the ER (by binding to an ER-resident 'hook' protein), which can be released by the addition of biotin to the culture, causing a wave of cargo protein to exit the ER synchronously.

**Key findings**

We generated a set of vectors encoding an ER-localised 'hook' plus several trafficking 'reporters' based on difficult and easy to express variants of erythropoietin EPO. We found that the CHO cell lines had morphology unsuitable for high resolution imaging, so we transiently transfected HeLa cells with each of the constructs developed and examined the localisation of the ER hook and the erythropoietin reporters in the absence of biotin and addition of biotin. The majority of these behaved as predicted – being retained in the ER the absence of biotin and progressing to the Golgi upon addition of biotin. These findings helped move the work between TLR3 and TLR4.

**Next steps**

The academic and industrial partners have secured an iCASE studentship to take the project forward.

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### Pilot production of engineered nucleases with applications in molecular biology and diagnostic devices

PI: Jon Sayers, University Sheffield
Partner company: Bioserv UK (in-kind contribution and outsourcing)

**Project aims**

Nucleases are central to a huge molecular clinical diagnostics industry. Previous attempts to facilitate industry uptake of a 5'-nuclease that is part of the Taq polymerase system have been hampered by our inability to provide sufficient high-quality samples for evaluation. The aim of this project is to scale-up production of our engineered enzyme in partnership with an accredited manufacturer.

**Key findings**

We constructed two inducible, IP-free, expression vectors based on strong bacteriophage promoter sequences suitable for nuclease expression. We evaluated expression of three variant Taq polymerase (TaqPol) proteins in these vectors systems. Protocols were developed for purification of a novel TaqPol-protein, and we passed these on to BioServ UK, together with the specific Taq polymerase overproducers.

**Next steps**

We expect to receive commercially produced prototype enzyme batches soon, which will allow evaluation by end users at TLR 7/8. Mutant TaqPol proteins will be put into crystallization trials for provide pilot data for a for BBSRC grant submission.

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### Optimization of influenza vaccine manufacturing through inhibition of autophagy

PI: Jeremy Rossman, University of Kent
Partner company: Medimmune (in kind funding)

**Project aims**

This vaccine manufacturing process is optimized for spherical viruses, yet influenza viruses can form filamentous virions. This research will disrupt cellular autophagy systems — which are essential for viral filament formation whilst being dispensable for virus replication — in order to produce uniformly spherical morphology that can enhance the efficiency of influenza virus vaccine manufacturing.

**Key findings**

We created a viral mutant that could not interact with the host autophagy system by introducing point mutations in the LC3-interacting region of the M2 protein. Importantly, when this mutant was engineered into a representative live recombinant influenza virus strain, filament morphology was eliminated whilst high-titer growth was retained. While creating a stable cellular system for the inhibition of autophagy, we found that the cells rapidly died during influenza infection; work is underway to see if this will impact vaccine production.

**Next steps**

An MRC research grant application is underway, investigating the intersection between autophagy, apoptosis and influenza virus replication. The data will also form the basis of a manuscript to be submitted to the *Journal of Virology*.

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### Improved microfluidic devices for downstream bioprocess separation of sub-micron targets

PI: Alan Goddard, Aston University
Partner: uFraction8 (in-kind contribution, outsourcing)

**Project aims**

Current separation techniques for the removal of cells or particles from large volumes of liquid can destroy the cells and contaminate the liquid. This project will characterise novel, gentle, low energy devices developed by uFraction8 using fluid flow at very small scales to enable efficient particle separation.

**Key findings**

Liposomes were successfully extruded through devices of 4 different sizes (30, 100, 400 and 1000 nm diameter) without loss of liposome stability during separation. Demonstration that there can be sub-micron separation using these devices has shifted the work from TRL2 to TRL3 and potentially TRL4 if we can enhance the separation and ensure that the particles are completely stable.

**Next steps**

We will build on the data generated here to design chips with varying geometries for enhanced particle separation.

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**Case studies index**

| Order | Funding | Title | Funding ref or PI |
|---|---|---|---|
| 1 | BIV | Scissor technology cuts out a collaboration between Bath and Arecor | BIV_Nov14_Mrsny |
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| 17 | ECR | Scientific exchange visit boosts separation technologies collaboration | Winterburn |
| 18 | ECR | Exchange visit funding seeds early career researcher collaborations | Marriott |
| 19 | Workshop | Sandpit Meeting Builds Collaboration Workshop | None |

Followed by three member profiles

# 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 toinvestigate this. The collaboration brought together expertise in two areas: a new *in vitro* technique — known as Scissor; **S**ub**c**utaneous **I**njection **S**ite **S**imulat**or** — developed by the University of Bath that models events that occur following insulin injection, and Arecor's proprietary technologies for stabilising therapeutic proteins.

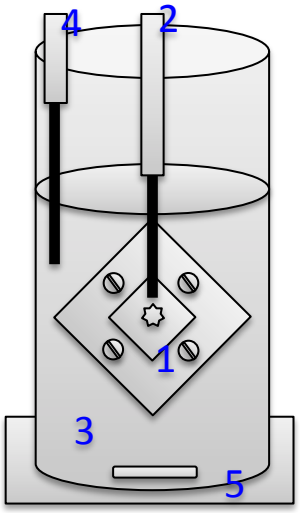
Because this method of stabilising proteins can alter the pharmacokinetic profile, work carried out under the business interaction voucher used the Scissor system to test the pharmacokinetic profile of Arecor's formulations of insulin analogues.

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

# 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 Tavacoli, an investigator on the project from the University of Edinburgh.

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

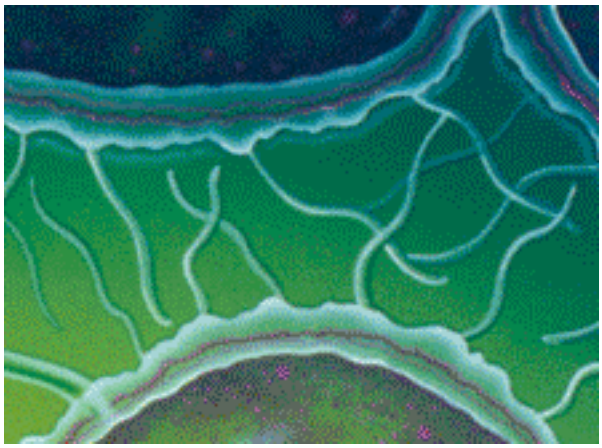
Moreover, the collaborators showed that the capacity of the bacteria to stabilise emulsions and the type of emulsions they could stabilise — oil-in-water or water-in-oil — was probably dependent by the amount of surfactant they hold within their cell walls, which in turn could be controlled by the amount 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 biosufacants 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 Tavacoli. "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."



# 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 Ramen 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 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 Ramen spectroscopy, while at the same time allowing Lorna to access otherwise unavailable plasmid DNA samples.

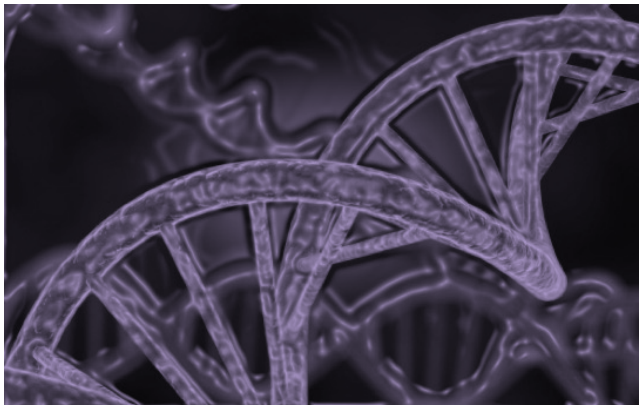
"Raman spectroscopy is sensitive to changes in DNA and RNA structure but is underused in biopharmaceutical analytical R&D"

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".



# 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.

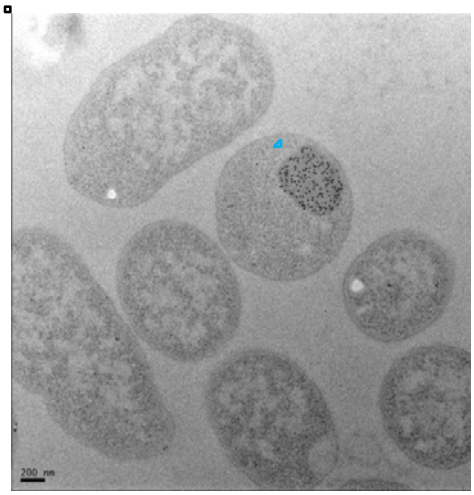
"A better understanding of protein export by the TAT system will facilitate better bioprocessing technologies"

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.




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

# BIV funding lights up collaboration on fluorescent protein expression in microalgae

A business interaction voucher from BioProNET has enabled Anil Day from the University of Manchester to partner with biotech company Protein Technologies. Their project focused on the the expression of a novel fluorescent protein — which could have applications in optical imaging studies in laboratory animals — in microalgae.

The production of recombinant proteins in microalgae could offer a lower-cost alternative to mammalian or bacterial systems. The first step to showing that protein production in microalgae is commercially viable is demonstrating that the desired protein can be expressed in microalgae.

The collaboration provided Protein Technologies, which focuses on protein engineering and the manufacture of recombinant proteins, with access to state of the art expertise to express recombinant proteins in microalgae and plants. "This is an area of considerable interest to Protein Technologies but we were unable to do it in-house due to the expertise and resources required," says Farid Khan of Protein Technologies.

After first generating vectors containing the gene encoding for the infrared protein, the vectors were then transformed into the chloroplasts of the microalgae *Chlamydomonas reinhardtii.* Western blot analysis on total protein from this transgenic strain showed accumulation, albeit limited, of the infrared protein.

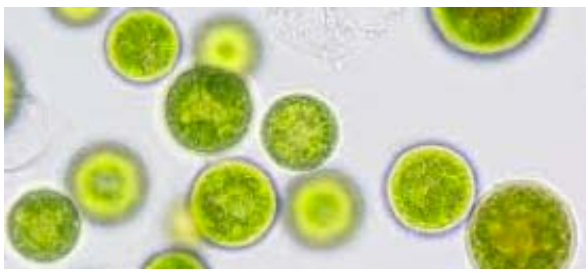
"This first set of transgenic strains of microalgae provides a valuable resource for improving yields by media formulation and changing environmental parameters such as temperature, light intensity and day length," says Anil.

The project also refined methods for detecting the accumulation of the infrared protein in transgenic algae.

Because much higher levels of recombinant protein expression can be obtained in the chloroplasts of plants, genes encoding the infrared protein were then cloned into a vector to allow expression of the protein in tobacco plants. Anil explains that this additional step is ongoing because it takes longer (typically 4-6 months) to isolate stable transgenic plants.

The project has allowed Anil a new insight into his field, "We worked on a commercial protein that had a clear route to market. We would not have considered outside this collaboration," he says.

As a result of this project a proposal led by Protein Technologies including the University of Manchester on the industrial biotechnology applications of microalgae has been submitted to the Newton Fund.



"We worked on a commercial protein with a clear route to market; we would not have considered this outside of this collaboration."

# 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"

# Design consultation and testing of a membrane photobioreactor suitable for advanced biologic production from microalgae

## AIMS

At present, the production of high-value biologics in plankton is uneconomical, primarily due to the demands of mixing and harvesting low biomass concentrations from large volumes. This issue is almost entirely negated by the use of bioreactors that use a biofilm-membrane design, in which the biomass is retained within a high-density, low-volume system. To our knowledge however, there is no commercially available membrane system suitable for the growth of algae as a biofilm.

In this project, Plymouth Marine Laboratory (PML) collaborated with Varicon Aqua Solutions (VAS) to develop a prototype membrane photobioreactor — about 5l volume — for the production of high value biologics from microalgae and assessed biologic production capability using a reference algal system.

## OUTCOMES

We designed and tested a novel solid-state photobioreactor. Growth rates, productivity and algal concentration was measured in several green algal species. Inoculation tests were also carried out to assess the best starting conditions within the bioreactor. We generated a wealth of baseline data for two very different microalgae species and assessed the potential for the system to enter into the competitive market for photobioreactors for the generation of high-value products.

***"The fledgling BioProNET-funded collaboration resulted in a successful application to Innovate UK."*** **Varicon Aqua Solutions**


## MOVING FORWARD

The prototype system will continue to be used at PML for academic research and to provide baseline data to aid VAS with sales of the product. The algal media recipes developed and tested during this work are already generating sales for VAS, and it is envisaged that the PML—VAS relationship will continue to grow. The fledgling BioProNET collaboration resulted in a successful application to Innovate UK to take the technology forward.

# BIV funding grows algae bioprocessing collaboration

BioProNET funding has enabled two teams of scientists with expertise in algal bioprocessing to work together for the first time. Kevin Flynn, Claudio Fuentes-Grünewald and Deya Gonzalez from Swansea University partnered with AlgaeCytes, an SME that is focused on developing and commercializing healthcare ingredients derived from algae.

Each project partner brought a selected strain of algae into the project to compare the bioactivity of certain extracts. Working together using a business interaction voucher funding enabled Swansea and AlgaeCytes to compare functionality of these bioactive properties from their respective algae. "The funding allowed us access to expertise and facilities that were otherwise unavailable to AlgaeCytes as a SME, but growing company," says John Dodd, co-founder of AlgaeCytes.

The focus of the project was algal exopolysaccharides a group of high molecular mass polymers that are secreted by microalgae and typically helps to protect themselves against stress. Exopolysaccharides are known to have antiviral, antioxidant and immunomodulatory activity, and so may have useful medical applications.

The collaborators first established protocols for growing two types of algae under stress conditions in order to maximize exopolysaccharide production. They then used novel downstream bioprocessing techniques to concentrate the exopolysaccharides from large volumes of algal growth media.

Although further studies will be needed to further optimize the production of compounds of interest, the project successfully allowed the collaborators to test the biological activity of exopolysaccharides. "We have encouraging results from preliminary tests of bioactives of algal origin that show their inhibitory activity upon several human cancer cell lines," says Deya. Moreover, the compounds had antioxidant activity on certain cell lines.

"The project has enhanced our research outputs through identification of novel microalgal compounds with potential therapeutic applications," highlights Kevin. The outcomes of this project provide vital proof-of-concept data for grant applications and it is hoped that the data will eventually form part of a patent application as well as supporting publications. "Importantly the project strengthened the relationship between AlgaeCytes and Swansea University for future projects" concludes John.

"The funding allowed us access to expertise and facilities that were otherwise unavailable to AlgaeCytes"

Pictures of the two types of microalgae used in this study growing in biofences at the Centre for Sustainable Aquatic Research at Swansea University. Both types of algae produce exopolysaccharides.

# BioProNET funding drives the use of motor proteins for nanopore DNA sequencing

Business interaction voucher funding from BioProNET has enabled Michael Plevin and James Chong from the University of York to partner with biotech company Oxford Nanopore Technologies. Their project aimed to produce and characterise motor proteins that could be used in a portable hand-held DNA sequencer (see box).

Nanopore-based DNA and RNA sequencing is critically dependent on controlling the movement of the polynucleotide through the pore. The collaborators investigated if a previously untested family of archaeal DNA motor proteins have characteristics that are suitable for use in nanopore-based sequencers.

First, several DNA constructs encoding for different regions of the target protein were made, as well as several mutant variants. Recombinant protein was produced in *E. coli*, with yields exceeding 50mg per litre. Electron microscopy and other studies showed that the purified proteins adopted the expected structure.

The main outcome was the design and optimisation of a 'pipeline' for the production and characterisation of the target proteins. "We incorporated a number of features into this pipeline that would permit the screening of larger numbers of proteins" says Michael. "These included the use of an expression vector that was compatible with ligation-independent cloning, protocols for parallel small-scale expression and solubility tests, structural and oligomeric analyses and newly implemented activity assays."

Indeed, the new activity assays – based on fluorescence rather than previously used radioactivity – showed that the target motor protein catalysed DNA unwinding and strand separation, indicating that the protein is functional. "The BIV funding has enabled work that provided our first evidence that these enzymes can function as motors in a hand-held nanopore-based sequencer," says Andrew Heron from Oxford Nanopore Technologies.

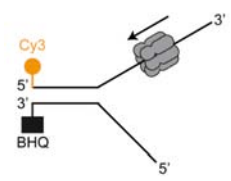
**"The BIV-funded work has provided the first evidence that these enzymes can function as motors in a hand-held nanopore-based sequencer"**

**What is nanopore-based sequencing?**
Nanopore-based DNA and RNA sequencing uses devices that incorporate two protein components: a doughnut-shaped nanopore protein and a motor protein. In the sequencing technology, the recombinant protein nanopore is set in a polymer membrane and an ionic current is passed through the nanopore. The motor protein ratchets single-stranded polynucleotides through the nanopore one nucleobase at a time. The current changes as the bases G, A, T or C pass through the pore and the changes in current can be decoded into a DNA sequence using an algorithm.

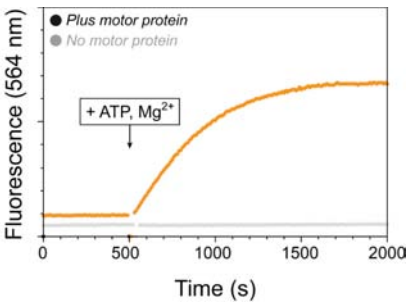
In addition to their use in portable DNA sequencers, the pipeline will be useful for identifying and screening uncharacterised motor proteins to explore their biological structure–function relationship.

The BIV has laid the foundations for an ongoing partnership. "This promising progress motivates us to continue the collaboration to explore DNA motor proteins that improve our DNA sequencing technology, and to continue to support the University of York team," says Andrew. Indeed the partners have been awarded a BBSRC-funded iCASE PhD studentship to investigate the use of hand-held sequencers for characterising DNA motor proteins at single molecule resolution.

They also secured BioProNET for proof of concept funding to develop their target motor proteins — for example in terms of activity, structure and potential to be engineered — for use in portable sequencers.



Fluorescence-based helicase unwinding assay



# Developing a novel fluorescence-based biopharmaceutical quality control technology

***A Business interaction voucher from BioProNET has enabled Christopher Pudney from the University of Bath to partner with Bath ASU to develop a new method for quality control testing of biologics that could be faster and cheaper than current approaches.***

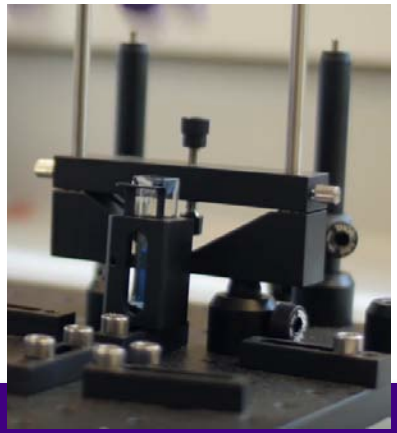
## AIMS

There are a limited number of approaches to perform quality control analysis of biological molecules. We will work with partner company Bath ASU to develop a novel technology — based on quantification of the fluorescence edge shift phenomenon — that gives a library of spectroscopic fingerprints for different biopharmaceuticals and accurately quantifies subtle changes to protein structure. We will test a range of biopharmaceuticals, allowing us to develop our technology to improve speed and accuracy and also to establish the limits of sensitivity.

## KEY RESULTS

We looked at 12 monoclonal antibodies (including humanised and fully human) using our fluorescence edge shift technology. We benchmarked against circular dichroism and dynamic light scattering and found that fluorescence edge shift technology had a similar sensitivity to both traditional approaches, but that we could combine the measurement power of both approaches. So we could measure unfolding (normally assessed by circular dichroism) and aggregation (normally assessed by dynamic light scattering). Our measurements were not destructive, were faster and were at least as accurate as these approaches. So we found our approach could potentially replace these other approaches making quality control faster and less expensive. Most importantly, we have been able to show our approach can actually predict the stability of antibodies and this opens up a whole new range of possibilities for our approach.

***"The BiV let has have a meaningful partnership with an industrial collaborator. The BiV has moved the commercial potential of our technology on immensely and has been key for us going forward."***



## OUTCOMES

1. Patent filing (UK Patent Application No. 1604640.1).
2. Manuscript in review with industrial partner.
3. Presentation at the 2nd International Antibody Validation Meeting
4. EPSRC funding as part of seed-corn funding for a GCRF project
5. EPSRC Impact Acceleration Account award
6. Short film: https://youtube/kHdZBy5xJ2A

**Funded by a BioProNET business interaction voucher**

**Chris Pudney, University of Bath working with Bath ASU**

# Speeding up and slowing down: altering translation speed to enhance protein yield

**The challenge**
One way to increase the yield of recombinant proteins is to increase the speed of translation, through the use of DNA sequences containing codons that are frequently used in the host genome – a process known as codon optimisation. But codon optimisation might reduce activity activity, for example by producing poorly folded protein, especially for proteins that are difficult to express.

**Project aims**
This study aimed to investigate if rhythmically alternating slow and fast protein synthesis might improve that activity of a lead to a novel format antibody, by allowing it to fold correctly.

**The partnership**
Tobias von der Haar, from the University of Kent has developed computer software that models translation and can be used to design DNA sequences that are translated quickly. This was used to design two codon-optimised fast sequences, as well as a rhythmic sequence, in which slowly decoded regions were introduced between defined codon-optimised antibody domains.

The industrial partner, UCB Celltech, synthesised the DNA sequences, cloned them into CHO cells and then analysed expression levels of the antibody.

**Key results**
Expression of DNA constructs that were fully codon optimised reduced expression of the antibody compared a non-codon optimised DNA construct used by UCB. “This is consistent with the current theory that rapid translation, which normally maximises expression levels, can be counterproductive for difficult to express proteins,” explains Tobias.

Expression levels of the antibody encoded by the rhythmic constructs was comparable to that of the UCB construct, supporting the idea that introducing regions of slower translation can help optimise expression levels in multi-domain proteins.

However, the rhythmic construct did not increase expression levels above those obtained by the UCB construct “This indicates that the main limitation in expression levels for this particular antibody is not at the level of translation, but downstream of this – likely molecule assembly in the ER and/ or secretion,” says Tobias.

**Moving forward**
The data from this work has provided a starting point for a more systematic exploration of rhythm-based codon optimisation, including the optimal location of slowly translated sequences within the antibody folded domains.

In addition, analysis is currently underway to ascertain whether the introduction of slowly decoded regions has impacted the quality of the expressed antibody – based on levels of aggregated material and antibody. “If the quality of the expressed antibody has been improved, it provides a great opportunity for us to impact our processes,” says Paul Stephens of UCB.

“This was a unique opportunity to gain exposure to the kind of problems encountered in industry when trying to produce novel molecules.”

# Collaborative development of glycolipid separation technology to reduce costs

**BIV funding from BioProNET has enabled James Winterburn and Ben Dolman from The University of Manchester to work with Croda to scale up a new separation technology for the production of high-value glycolipids. Their novel method increases fermentation productivity and yield, and so has potential to reduce costs.**

**The challenge** – Sophorolipids are glycolipid biosurfactants that are used in environmentally friendly cleaning products and cosmetic/personal care products. Yet current fermentation methods are inefficient as the fermentation must be stopped when product concentrations reach a certain value. The use of sophorolipids could be widened — for use in bioremediation and enhanced oil recovery — if production costs can be reduced.

**Aims** – This project built on the Winterburn Group's previous work showing that separation of sophorolipids from a fermentation broth during production can give higher overall productivities, (patent application 1610932.4**)**. This collaborative project with Croda tested the functionality of the novel separation process at pilot scale.

**The collaboration** – A high productivity sophorolipid fermentation protocol, using *Candida bombicola* as the producer strain and gravity-based separation of the sophorolipid from the fermentation broth, was carried out at the University of Manchester. A gravity-based separator was then designed and built by University of Manchester engineers for pilot scale (30 l) trials at Croda.

Integrated biosurfactant separation

***"The personal development of the research assistant who carried out the work was enhanced through experience of working collaboratively in an industrial environment."***

**Key findings** – Sophorolipid recovery was demonstrated for extended periods, and a total of 550 g sophorolipid was recovered per litre of broth. Both the sophorolipid productivity and the yield were higher than those reported in the literature to date. The separator could recover the phase containing the sophorolipid at over 2 g per hour, a separation rate that makes the system applicable to continuous separation from bioreactors of around 600 times the size, or 200 l working volume, an important achievement given many integrated separation systems cannot be translated from lab scale.

**Outcomes and next steps**

- Paper entitled 'Characterisation and scale up of integrated production and separation of sophorolipids' in preparation
- EPSRC responsive mode grant in preparation 'Advanced glycolipid biosurfactant processing and separation'
- Potential further work with Croda testing the system at larger scales (>100 l) and with other glycolipid biosurfactants.
- Possible BBSRC DTP CASE studentship to grow the collaboration

Find out more here:
https://www.youtube.com/watch?v=jwT22t5lVKQ

Dolman, B.M. *et al.* (2016) *Process Biochemistry*, 54, 162-171
http://dx.doi.org/10.1016/j.procbio.2016.12.021

***"Both the sophorolipid productivity and the yield were higher than those reported in the literature."***

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

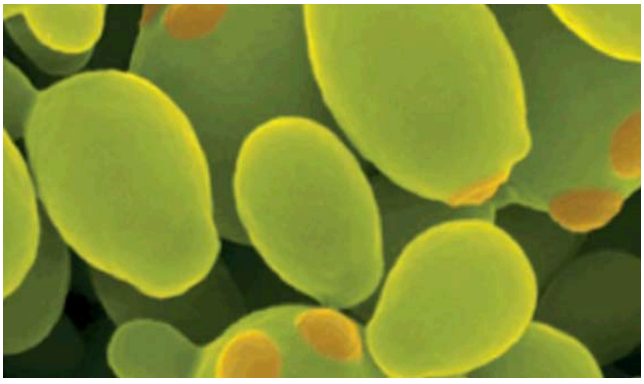
The main phase of the project showed evidence of combined transcription and translation in the extract from the yeast cells. "The most important outcome of the work was that we were able to generate a working cell-free protein synthesis extract from *P. pastoris*", says Karen.

The final titres of GFP and luciferase observed were similar to that observed with a cell extract from another strain of yeast, *Saccharomyces cerevisiæ,* 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.



# 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.

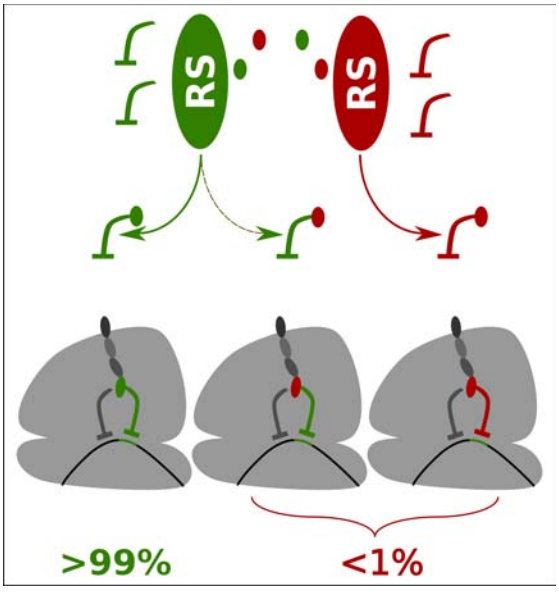
The tools could detect minor variations in the amino acid sequence. "The sequence variations would have escaped detection with standard mass spectrometry approaches, but can be reliably visualised using our novel approach," says Lyne Jossé, who carried out the experimental work.

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 intents 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"



# Hijacking intracellular storage bodies to produce difficult to express proteins

Proof of Concept funding from BioProNET has enabled Marek Brzozowski and Tim Ganderton from the University of York to investigate a novel protein packaging and secretion system, which might be used to make recombinant proteins that are tricky to produce.

Many potentially useful therapeutic or diagnostic proteins are difficult to express in the mammalian cell lines that are, or could be, used for their production. The proteins may be fragile, misfolded, harmful to the host, or require complicated, time consuming purification strategies.

With the aim of creating a novel mammalian cell-based system for the production of difficult to express proteins, the scientists investigated a naturally occurring system of intracellular storage bodies — known as Weibel Palade bodies. In nature these have a crucial role in the storage and controlled secretion of several haemostatic, and very complex, proteins, most notably von Willebrand factor.

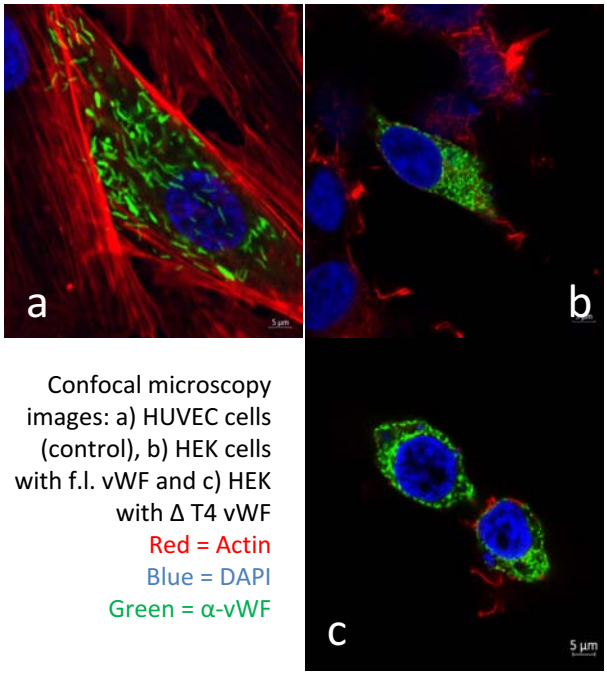
The formation of functional pseudo-Weibel Palade bodies which behave like 'wild type' bodies can be induced in certain non-endothelial cell lines by expression of von Willebrand factor. "The ability of intracellular storage bodies to store, protect, and regulate the release of functionally active proteins, has yet to be exploited as part of a novel production strategy for difficult-to-express proteins," says Tim.

The main part of the work investigated which parts of the von Willebrand factor protein are responsible for promoting accumulation and storage in Weibel-Palade body vesicles "Our aim with is to be able to turn the von Willebrand factor fragment into a Trojan horse for the re-direction and packaging of other proteins into WPB so that it can be hijacked for the production of novel difficult to express proteins," says Tim.

Using green fluorescent protein as a test protein they identified a 'minimal critical region' of von Willebrand factor that was needed to generate functional pseudo-WPB that packaged and secreted the green protein.

As well as demonstrating the feasibility of using Weibel-Palade bodies as part of a protein expression system, the project also generated several novel observations about the fundamental biology of Weibel-Palade bodies. Tim was an invited speaker at the 2017 ESACT-UK meeting, where he presented preliminary results from this project.

Further work studying protein expression levels — to help differentiate stimulated and constitutive protein release — are currently underway. A priority for future studies is to test the system with other proteins. "It will be really interesting to use more relevant proteins — for example, insulin, insulin-like growth factor 1 and erythropoietin to demonstrate effectiveness as a protein expression system," highlights Tim.




Confocal microscopy images: a) HUVEC cells (control), b) HEK cells with f.l. vWF and c) HEK with Δ T4 vWF
Red = Actin
Blue = DAPI
Green = α-vWF

**"The ability of pseudo-Weibel Palade bodies to store and protect functionally active proteins has yet to be exploited as part of a novel protein production strategy."**

# Computational modelling aims to predict cell culture strategies for increasing glycosylation

Glycosylation of therapeutic proteins can affect their efficacy and stability, so predicting culture strategies that optimise glycosylation could boost bioprocessing strategies.

Cleo Kontoravdi and colleagues from Imperial College London used Proof of Concept funding from BioProNET to work with MedImmune to use first-principles modelling and a novel computational method to design optimal cell culture conditions for high glylactosylation.

**What methods did you use?**
We used a previously developed mathematical model that describes the impact of feeding galactose and uridine to cell culture processes on cell growth, antibody productivity and extent of glycosylation. We also applied a new computational method called constrained global sensitivity analysis.

We used these methods to design cell culture experiments that we anticipated would give rise to good-quality protein, with a high mAb titre (470 mg/L and high galactosylation levels (galactosylation index higher than 180).

Our method identified a subset of 346 designs that should, according to the model, result in protein with the desired characteristics. We then tested 12 designs and a control experimentally, and compared the experimental results to the model predictions for each set of conditions.

The addition of sugars (glycosylation) to a therapeutic protein can affect efficacy and stability.

Image: hipnewjersey.com

**What were your main findings?**
That there is considerable mismatch between the experimental data and the values predicted by the model for all three indicators: integral viable cell concentration; antibody titre on day 12 of culture and the galactosylation index.

This was not what we were expecting. We attributed the divergence to negative effect of galactose and uridine feeding on cell growth. Although this is accounted for in the model, the impact was more pronounced than we anticipated. In terms of mAb titre and galactosylation index, only one feeding strategy and, surprisingly, the control (no galactose or uridine addition) met both constraints.

Further analysis showed that there was no clear correlation between the predicted production rate of the antibody and the observed experimental results. Although the feeding strategies resulted in good specific mAb productivity and galactosylation, lower cell growth resulted in a significantly lower final mAb titre.

**What is your take-home message?**
**Although the results are not as encouraging as we would have liked, it is interesting that we can quickly evaluate such a high number of designs computationally and then use a system like the Ambr to test the most promising designs quickly and at relatively low cost.**

**Next steps**
- Use these experimental data to further train our model

- Use these findings to apply for funding from the EPSRC to further develop this approach and collaboration.

- Explore the applicability of the model to other cell lines and glycosylation targets

# Scientific exchange visit boosts separation technologies collaboration

Scientific exchange funding from BioProNET has enabled Ben Dolman, a PhD student from the University of Manchester to visit the lab of Maria Cuellar Soares at the Technical University (TU) of Delft in the Netherlands. The visit was co-funded by the High Value Chemicals from Plants NIBB.

During his PhD, Ben has developed a gravity-based separation system that recovers insoluble sophorolipid biosurfactants from a fermentation broth. This technology aligned with the work of the bioseparation technology group from TU Delft, who are working towards the industrially relevant production of insoluble compounds, in particular terpenoids, and ways to separate them from the production media.

So Ben's exchange visit applied the gravity separation device to the production systems used at TU Delft.

**Scientific results**: The gravity-based separation system could separate insoluble molecules that formed an emulsion with the fermentation broth components, as well as showing that *E.coli* cells continued to grow (in accordance with a previously described model) after they were returned to the bioreactor after the use of the separator.

*"This opens up the application of our system to a whole new range of biomolecules, with the potential to be used as an integrated pre-concentrating step to recover a product-rich emulsion from a bioreactor, and returning the cells and media back to the bioreactor for continued production,"* says Ben.

Ben was also able to enhance the understanding of how fermentation time, cell density, cell growth rate and emulsion formation time can have an effect on emulsion stability and the ease of separation, which complemented the ongoing work on this theme being undertaken at TU Delft.

**New skills:** Ben learned a number of techniques for rigorous analysis of cell behaviour during fermentation.

He also attended the AkzoNobel Imagine Chemistry competition finals during his visit, and benefited greatly from hearing first-hand what AkzoNobel's industrial experts considered the most important steps to be for the separation technology to reach commercialisation.

**New collaborations**: At the Imagine Chemistry Competition, Ben set up interesting collaborations with two of the other finalist teams.

**Exchange of knowledge:** He notes that the placement was also incredibly productive from a research commercialisation perspective, as he had the opportunity to meet employees from Delft Advanced Biorenewables to discuss the industrial application of the separation technology.

*"We were able to share some of the challenges and opportunities that TU Delft and the University of Manchester are both facing bringing our technology to market,"* he says.

Early career researcher Ben Dolman and (inset) the gravity-based separation system that he used at TU Delft

# 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 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.

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

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.

# BioProNET member profile

## Charlie Campion
## University of Manchester

*Charlie is a PhD student at the University of Manchester, working on Industrial Case studentship investigating the effect of oxidation on the immunogenicity of protein therapeutics. She is also passionate about public engagement and is a STEM ambassador.*

### Why did you decide to join BioProNET?

I was recommended BioProNET by colleagues as a good way to keep up-to-date with the current research in my field.

### What BioProNET events have you been to?

I've been to several BioProNET annual meetings over the past 4 years, as well as the early career researcher (ECR) event in Brighton in Sept 2016 and the BioProNET-supported Bioprocessing Skills School in September 2017.

### What have BioProNET events helped you to achieve?

I've had the opportunity to meet people that I wouldn't have meet otherwise, both students and professionals, which is great in itself, but has also helped to improve my confidence when it comes to networking at future events.

What's helped me the most is having my eyes opened to the variety of careers out there for PhDs – we don't have to limit ourselves to research, whether in academia or industry. Sitting down with people currently in those professions, grilling them about how they got there, what skills they'd be looking for in potential employees, how they manage work life balance etc. has been vital!

I've found BioProNET career events really encouraging, as they've often highlighted that we have the skills needed for our future career already, we just need to recognise them and highlight them in job applications.

I've also had opportunities that I wouldn't have got anywhere else; for example practice interviews and CV clinics and being interviewed on camera. I've been able to present data in a more comfortable, relaxed environment.

### What one piece of advice would give to other early career researchers?

It would be to get yourself out there-go to as many training sessions/talks/conferences as you can, make the most of these BioProNET conferences which are a great opportunity to network and learn new things!

### Anything else that you'd like to add?

I feel really supported by BioProNET as an early career researcher, and it's encouraging to know that the issue of the PhD>PostDoc>PI dropout is being taken seriously, and the network is working hard to encourage ECRs to stay in science, without necessarily staying in academic research.

BBSRC EPSRC

# BioProNET member profile

## Michael Plevin
## University of York

*Michael is a Lecturer in molecular biophysics in the Department of Biology at the University of York. He joined BioProNET in November 2015.*

### Why did you decide to join BioProNET?

One of my colleagues in the Chemistry Department at York was awarded Proof of Concept (PoC) funding from BioProNET and told me about the funding opportunities available.

### How has BioProNET facilitated your research?

In February 2016, I was awarded a Business Interaction Voucher (BIV) from BioProNET for a collaborative project working with Oxford Nanopore Technologies (ONT), a biotechnology company that is the world leader in hand-held nanopore-based sequencing devices.

Our collaborative work provided the first evidence that a specific family of archaeal DNA motor protein enzymes can function as the motor protein component of a hand-held nanopore-based DNA sequencer. **Critically, the BIV allowed us to generate the important preliminary data that we needed for subsequent funding applications.**

### What happened next?

The outputs from the BIV enabled us to successfully apply for a BBSRC-funded iCASE PhD studentship. The student will spend the next 4 years working with ONT to develop tools for assaying helicase activity at the single molecule level.

Moreover, we were then awarded PoC funding from BioProNET to investigate if a family of archaeal helicases can enhance the performance of a nanopore sequencer. These awards allowed myself and a colleague, James Chong, to strengthen our collaboration with ONT with projects that will run through the end of 2021.

### What are your future plans for this work?

Over the next year, we'll use our PoC funding to develop a pipeline that will allow us to produce 20-40 new helicase proteins for testing in nanopore sequencing experiments. The outcome of these experiments will determine where the project goes next. We will continue to work with ONT on any interesting findings, including considering joint applications to the BBSRC or other funders for follow-up funding.

### What have you gained from BioProNET science meetings?

I presented findings from the BIV project at BioProNET's2016 Meeting. This was a great opportunity to talk to scientists who were experienced in academic/industry collaborations, and to learn about the type of data that would be needed to apply for the future funding opportunities. And I really appreciated the wide-range of talks at the 4th Annual Science Meeting in 2017; there were many useful things that I could take away.

BBSRC bioscience for the future

EPSRC Pioneering research

# BioProNET member profile

## Andrew Peden
## University of Sheffield

Andrew is a lecturer in the Department of Biomedical Science at the University of Sheffield. He joined BioProNET in July 2016. Dr Peden's BBSRC funded research is focused on elucidating the cellular pathways and machinery required for antibody secretion.

**How did you hear about BioProNET and why did you decide to join?**
I attended the meeting 'Overcoming Cellular Barriers: Implications for Industrial Biotechnology' jointly organised by BioProNET, CBMnet and BioCatNET. At this meeting I had the opportunity to present my research and discuss how it could be applied to aid antibody production in CHO cells. It was immediately clear that BioProNET was a very good forum for meeting members of the bioprocessing community.

**Was it at this meeting where the idea for your proof of concept application was initiated?**
I was involved in leading a breakout session where we discussed the challenges of manufacturing therapeutic antibodies. During this session I has the opportunity to chat with Bernie Sweeney and Paul Stephens, scientists from UCB. From this initial discussion we decided that we should establish a collaboration and apply for proof of concept funding.

**What attracted you the proof of concept scheme?**
Compared to traditional funding methods, the scheme is very flexible and far more agile. It only took a couple of months from the initial submission to find out whether our application had been successful.

**Tell me about your proof of concept project**
The main aim of our project is to develop a toolkit of simple and robust assays that can rapidly monitor and identify problems in protein expression in CHO cells. Using these assays we aim to gain a deeper understanding of the cellular pathways which underpin the manufacturability of secreted proteins.

**What is the role of UCB in your project?**
UCB have been key to this project as they have provide important reagents, cell lines and have kept the project clearly industrially aligned.

**What are the anticipated outcomes of your project?**
At present, it is unclear why certain molecules are more difficult to manufacture in CHO cells than others. We propose that having the ability to monitor levels of pathological cell stress will not only provide a platform for rapidly determining the manufacturability of new therapeutics but also speed up the process of selecting stable clones with the appropriate expression characteristics.

**What have you achieved by attending BioProNET annual science meetings?**
These meetings have been incredibly useful to me as they have given me the opportunity: 1) to gain a better understanding of the challenges facing industry; 2) to meet key academic and industrial scientists from the biomanufacturing community; 3) to discuss ideas and establish new collaborations and 4) to meet with my collaborators more frequently than would otherwise be possible. These meetings have allowed me to fully explore the potential impact of my BBSRC funded research and have helped establish new avenues of research for my group.