CBMNet
2014–2015

IN FOCUS

CROSSING BIOLOGICAL MEMBRANES NETWORK, A BBSRC NIBB

The University Of Sheffield.

BBSRC bioscience for the future

THE UNIVERSITY of York

WELCOME TO THE FIRST CBMNET IN FOCUS 2014–15

Network Highlights 2014-2015

Projects We've Funded

To date we are pleased to have provided over £175,000 of funding to support 7 innovative Proof-of-concept projects linking academics with industry. We have also funded 5 Business Interaction vouchers worth over £30,000. We have now helped over 30 academics from 20 institutions engage with over 15 industrial biotechnology businesses to secure funding.

Grant Success

The recently announced £3.8M IB-Catalyst award to CBMNet (led by CBMNet co-director Gavin Thomas and the CBMNet Management Board) has brought our first year to a successful conclusion with the promise of some exciting times ahead, exploring new ways to enhance the tolerance of cell-factories to toxic products in 'real-world' fermentations. This award represents exactly what the network was intended to achieve – forging teams of academics and industrialists to work together to create new knowledge and translate this into IBBE 'know-how'.

Another success is data generated from one of our Proof-of-Concept grants going on to develop the basis for a BBSRC LINK grant that was recently awarded. The project, worth over £420,000, entitled 'Bacteria transport and catabolism of human malodour precursors' is between CBMNet co-director Dr Thomas and Unilever.

Congratulations to all our CBMNet members involved in both these grants!

Supporting Our Early Career Researchers

One of the hallmarks of CBMNet is the commitment to promoting IBBE to early career scientists through our Vacation Scholarship Scheme. To date we have supported 7 undergraduates in projects as diverse as 'Enhancing industrial succinate production in *Corynebacterium glutamicum*' and 'Designing a new coat for *E. coli*'. We strongly believe that this scheme is a vital component in raising the profile of IBBE amongst the next generation of bio-scientists. This early exposure to the sector, and in particular the role that membrane function has on IBBE processes, will hopefully translate into attracting new talent to the UK IBBE sector and we will continue to offer these awards in 2016. Data generated from several of these scholarships have already been used to secure PhD funding and further Proof-of-Concept funding.

Looking To The Horizon

In an effort to bring together the best scientists from across the globe we are in the process of co-ordinating a bid for Horizon 2020 Funding, for the challenge 'Optimisation of biocatalysis and downstream processing for the sustainable production of high value-added platform chemicals'. Our application hopes to bring together cutting edge researchers from academia and industry to provide a solution for this increasingly prominent field, which could make some industrial processes significantly cleaner, as well as expand the repertoire of useful chemicals that can be efficiently produced.

For more information on our highlights visit:
cbmnetnibb.net

Director of CBMNet
Professor Jeff Green

How quickly time passes! Just over a year on from the launch and our inaugural members' meeting in September 2014 is perhaps an opportune moment to reflect on the progress of the infant CBMNet and consider the way forward as our BBSRC-NIBB matures.

It has been a busy year. The sterling efforts of the Management Board and our Network Manager Jen ensured that the CBMNet launch meeting was successful bringing together over 100 delegates from academia and industry to Sheffield to engage in productive discussions about the impact of membrane biology in Industrial Biotechnology and Bioenergy (IBBE). This initial meeting was scientifically stimulating, thought provoking and served to cement some extant academic-industry collaborations and initiated several new ones; including at one consortium that created a successful project outside CBMNet remit. Since then we've hosted two focussed meetings centred on 'Efflux pumps' and 'Membrane stress'.

I was struck by the analysis offered by one of our industry members, in which the case was made that the membrane was once the industrial biotechnologists friend, simplifying downstream processing of products that somehow got out into the medium, but now the membrane is increasingly the problem, particularly in getting new substrates into cell factories and getting often toxic products out. It is these pressing issues that CBMNet is here to address by bringing together the academic experts and IBBE practitioners to generate innovative solutions to IBBE problems.

It has been particularly pleasing to take part in those early conversations when new collaborations are being forged and to see the Proof-of-Concept fund or Business Interaction Voucher applications that follow.

So what next? We must continue to build our community and promote the importance of an appreciation of the impact that membrane biology can have on IBBE processes through our project funding streams and our meetings. We are also very much looking forward to our major 2-day symposium *'Membrane transporters in physiology, industrial biotechnology & bioenergy'* at the Microbiology Society's Annual Meeting to be held in Liverpool (21st-22nd March 2016). We have an outstanding programme of international speakers and the final session will be led by industry to promote knowledge transfer to drive new interactions between Microbiology Society scientists at all career stages and industrial stakeholders.

We have lots more exciting workshops, training courses, events and public engagement activities planned for 2016, so watch this space!

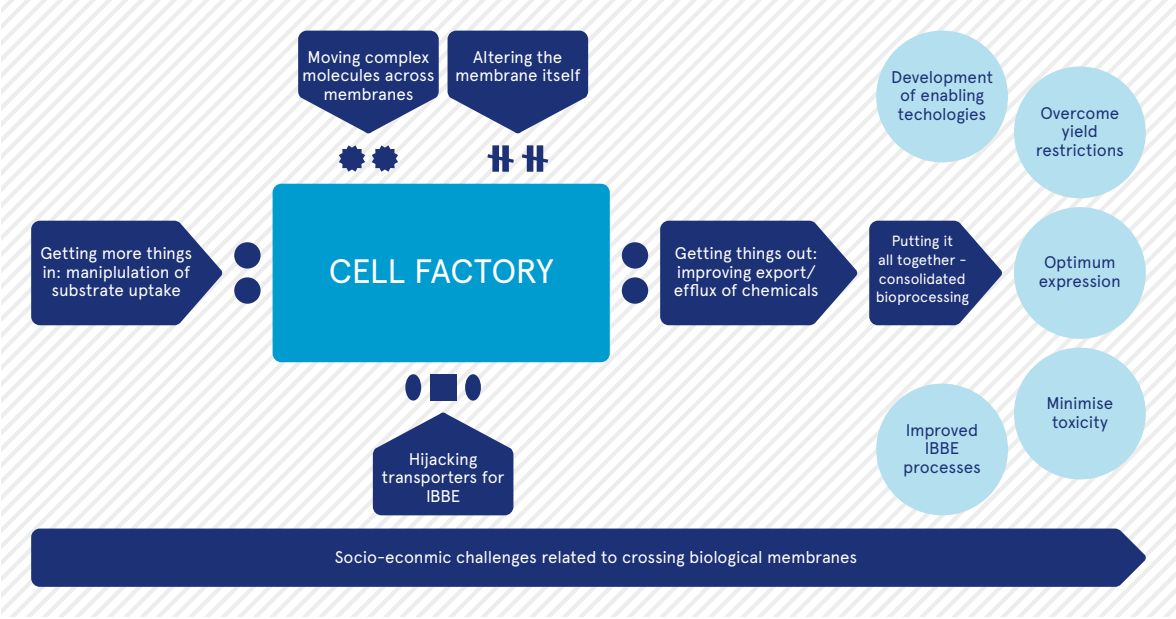
CBMNET 7 THEMES

CBMNet Network Aims

- To foster collaborations between academia and industry dedicated to tackling challenges in Industrial Biotechnology and Bioenergy (IBBE).
- To overcome yield restrictions due to difficulties with transporting substances into and out of cell factories.
- To harness biological resources for producing and processing materials, biopharmaceuticals and energy.

Our Research Remit

We foster collaborations between academia and industry....



CBMNet is focused on enhancing our understanding of how substances are transported into, within, and out of cell factories and translating this knowledge into innovative approaches to enhance IBBE processes. In order to do this we have 7 main research themes.

Getting more things in: manipulation of substrate uptake - The first step in any cell factory-based process must be the transport of reactants across the membrane and into the cell. We aim to use genetic resources and molecular genetics to expand the industry's repertoire of substrate transport options and help optimise the kickstarting of a cell factory-based process.

Getting things out: improving export/ efflux of chemicals - Many industrially useful chemicals are highly toxic. We aim to investigate new applications for natural or reengineered efflux or export systems, exploiting the specialised membrane channels that nature has cultivated to keep the live reactor healthy.

Hijacking transporters for IBBE – We aim to exploit fundamental knowledge of the structure/function relationships of transporters for biotechnology, because transporters have relaxed substrate specificity and thus will recognise and act on modified substrates.

Moving complex molecules across membranes - Secreting and post-translationally modifying complex molecules such as proteins within cell factories can involve passage across several biological membranes, leading to bottlenecks for productivity. By leveraging molecular methods and engineering expertise, we aim to increase the yield of non-native proteins from cells.

Altering the membrane itself - We aim to better understand how cell membranes can be engineered in cell factory systems for heightened resistance to stress and toxicity – improving the lifetime of the cell factory – and efficient transport of reactants and products.

Putting it all together – consolidated bioprocessing - Combining all of the above themes can really leverage our knowledge for improved yield and process efficiency for IBBE.

Socio-economic challenges related to crossing biological membranes – We need to ensure our work has lasting effects on the IBBE sector and beyond by looking at ways to conduct responsible innovation. We aim to study the new markets created by biotechnology opportunities and how these will impact the economy, by considering science and society relations.

CASE STUDIES

> CBMNet introduced us to Dr Graham Stafford and it was clear that his work had potential to significantly impact on the understanding and performance of our industrial platform. Our joint research has provided solutions, but more importantly it has built a longer term relationship between Fujifilm scientists and researchers in Stafford's lab.
>
> – Fujifilm Diosynth Biotechnologies

Proof-of-Concept Funding

Towards an integrated multi-omic assessment of membrane responses during industrially relevant high level protein production and secretion

The Challenge

Escherichia coli is a major workhorse organism used by Industrial Biotechnology as a cell factory for overproduction of diverse commercial protein products. This includes biopharmaceuticals (biologics) and industrial enzymes. However, relatively little is known of physiology during high-level protein production or secretion.

Bacterial "cell factories" can sometimes release these therapeutic proteins prematurely, which could account for the low yield observed in some cases. This research aimed to investigate the phenomenon further, leading to a better understanding of biologic drug production in bacterial cells.

The Research

Dr Graham Stafford is a Senior Lecturer at The University of Sheffield. His research focuses on the microbiology of bacteria, host-pathogen interactions, engineering of bacterial flagella for protein excretion, glycan harvesting enzymes and sugar transport across bacteria membranes. All his work has significant application for industrial Biotechnology.

Dr Stafford, along with colleagues from the ChELSi(Chemical Engineering at the Life Science Interface) institute (Phil Wright) and Fujifilm DiosynthBiotechnologies, applied for CBMNet Proof-of-Concept funding to identify and quantify responses of proteins associated with membrane function that respond to 'secretion stress', with the aim relieving metabolic bottlenecks and improve product yields.

The Result

The inter-disciplinary team achieved the goal of performing a proteomic analysis on real industrial production runs. This has opened up potential for improving Fujifilm's process and discovering new insights into the biology of *E. coli* in industrial fermentations.

The research found alterations in *E.coli* proteome which have identified various metabolic pathways and stress points that targeting to improve protein production and secretion in Fujifilm's strains.

Initial data analysis has shown several sets of proteins that seem to be altered in their expression levels between the control and expression runs.

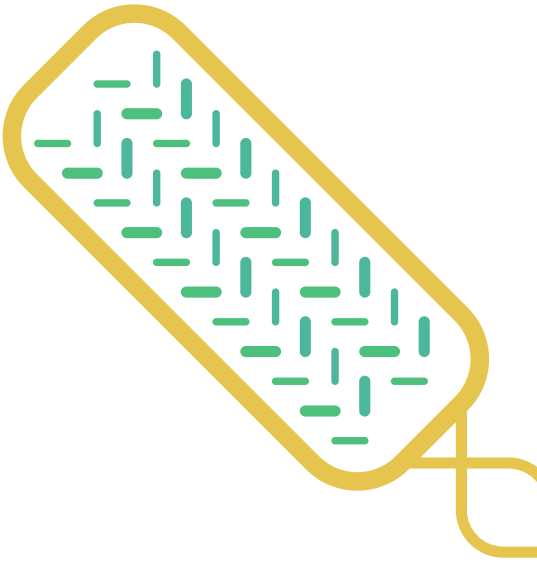
The Future

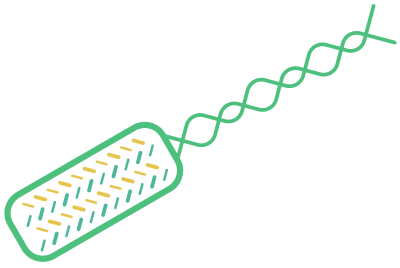
This research has formed the foundation for a wider ranging proposal to identify bottlenecks in recombinant protein secretion in an industrial setting allowing prediction and rational design of *E.coli* membrane function to improve product yields.

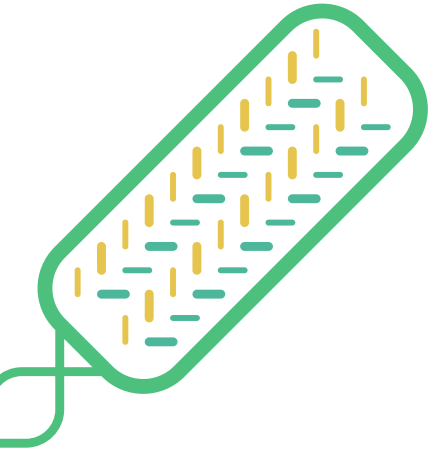
During this project the team discussed other ongoing work on utilization of alternative bacterial secretion systems for production and secretion of protein biologics. The outcome being the signing of new Confidentiality Disclosure Agreement and Material Transfer Agreement for pilot work to be carried out in the lab of Dr Stafford that, if successful, will be the beginning of a new collaborative work with FujiFilmDiosynthBiotechnologies.

This project may also provide the basis for further funding applications (e.g. BBSRC-IPA, IB Catalyst or TSB calls)

This project was funded through the Crossing Biological Membranes Network (CBMNet) by the Biotechnology and Biological Sciences Research Council (BBSRC)







> **The opportunity to examine the cellular behaviour of biologic production strains during a production run has been very exciting, allowing us to learn something about the bacteria but also the processes and considerations taken into account by a top CMO, like Fujifilm Diosynthbiotechnologies.**
>
> – Dr Graham Stafford, The University of Sheffield

Business Interaction Voucher

Understanding the effects of n-butanol on biological membranes

The Challenge

Solventogenic Clostridia are used by Green Biologics to generate n-butanol from a variety of feed-stocks providing sugars for fermentation. However, n-butanol is expensive to purify from the fermentation broth. The cost of in-situsolvent removal is greatly decreased by fermenting at higher concentrations of n-butanol. The transporter (if any) for export of n-butanol from cells is unknown –it is possible that n-butanol can diffuse across membranes. n-butanol is also toxic to Clostridia although the mechanism remains largely uncharacterised. Two possibilities are membrane disruption or deleterious effects on membrane proteins. It is possible that by altering the membrane composition or modifying transport activity we will be able to increase the extracellular n-butanol concentration and reduce production costs.

The Research

Dr Alan Goddard is a Senior Lecturer in the School of Life Sciences at the University of Lincoln. The research in his laboratory focusses mainly on molecules that bind to, and/or cross biological membranes. He uses a variety of model membrane systems and whole cell assays.

Dr Goddard applied for a CBMNet Business Interaction Voucher with Green Biologics Ltd who are based in Oxfordshire. The project aimed to determine the mechanism of toxicity of n-butanol to Clostridia focussing on various aspects of the lipid membrane.

The Result

Dr Goddard's lab obtained results indicating that n-butanol is disruptive to biological membranes, both those synthesised from single lipids and those made from lipids extracted from Clostridia from both high and low n-butanol concentrations. Interestingly, the disruption seems to be most significant at concentrations exceeding the toxic level observed in biofuel generation, suggesting that this may reflect a biologically-relevant toxicity mechanism.

Whilst some of the research was conducted by Dr Goddard, a student on the MSc Biotechnology degree at Lincoln also undertook their dissertation project in this area and gained valuable experience working with model lipid systems and a variety of assays.

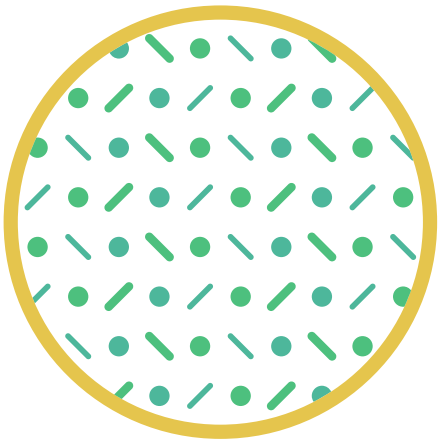
The Future

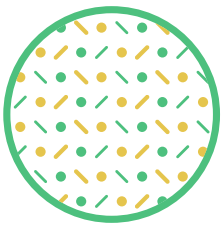
The collaboration and data that has come out of this project will lead to further collaboration between Dr Goddard and Green Biologics Ltd, for example, further CBMNet Proof of Concept funding, Innovate UK funding and, in the longer term, possible Knowledge Transfer Partnership funding.

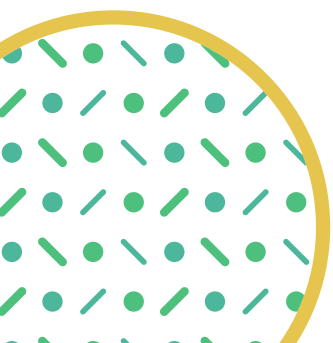
Dr Goddard and members of Green Biologics Limited attended the CBMNet Membrane Stress Meeting in September 2015, and successfully applied for Proof-of-Concept funding to build on the data from this Business Interaction Voucher.

They are now carrying out research to Identify and characterise protective lipid changes under solventogenic stress in Clostridia.

This project was funded through the Crossing Biological Membranes Network (CBMNet) by the Biotechnology and Biological Sciences Research Council (BBSRC)







> With the help of a CBMNet Business Interaction Voucher we have been able to start working with the Goddard lab on artificial and reconstituted plasma-membrane. This system has helped us better understand various aspects of butanol toxicity. I hope we can strengthen the collaboration and this will allow us to improve the renewable process for n-butanol production.
>
> – Dr. Preben Krabben, Green Biologics Ltd

Vacation Scholarship

The function of the aromatic acid transporter VanK

The Challenge

The aromatic acid:H+ symporters (AAHS) are a diverse and widespread transporter family that are responsible for the influx of aromatic acids into bacterial cells.

Aromatic acid transport could be exploited in bioremediation, microbial cell factories and in developing novel orthogonal components for synthetic biology. However, we currently lack the fundamental understanding of protein structure and function that will be required to supvport such applications.

Can we develop in vitro methods to study the transport kinetics and substrate specificity of the AAHS proteins?

The Research

Dr Paul Curnow is a Senior Research Fellow at the University of Bristol. He researches mainly in to membrane proteins, biochemistry and `environmentally friendly' cell-factories.

He applied for a CBMNet Vacation Scholarship which allowed a undergraduate student to be trained in practical techniques common across protein chemistry (microbial culture, IPTG induction, affinity purification, SDS-PAGE, Western blotting, gel filtration) as well as those specific to membrane transport proteins (detergent solubilization, reconstitution, substrate transport assays).

They purified the AAHS transporter VanK after recombinant expression in *E. coli*. The purified VanK was then reconstituted into synthetic proteoliposomes and fluorescence methods were used to assess substrate transport.

The Result

The CBMNet Vacation Scholar showed that VanK could be expressed into *E. coli* membranes, solubilized in a surfactant and purified to homogeneity.

They showed that VanK was an alpha-helical membrane protein that formed a relatively stable homotrimer in surfactant micelles. For functional assays, the Scholar adopted a popular method that used the pH responsive dye pyranine to track the cotransport of H^+.

The student did observe differences in transport data between the full treatment and control samples, although the effect was subtle.

The Future

This was a basic research project that provided excellent training for the CBMNet Vacation Scholar in studying membrane transport proteins *in vitro*.

This included generating publication-quality data that the host lab can build upon in the future.

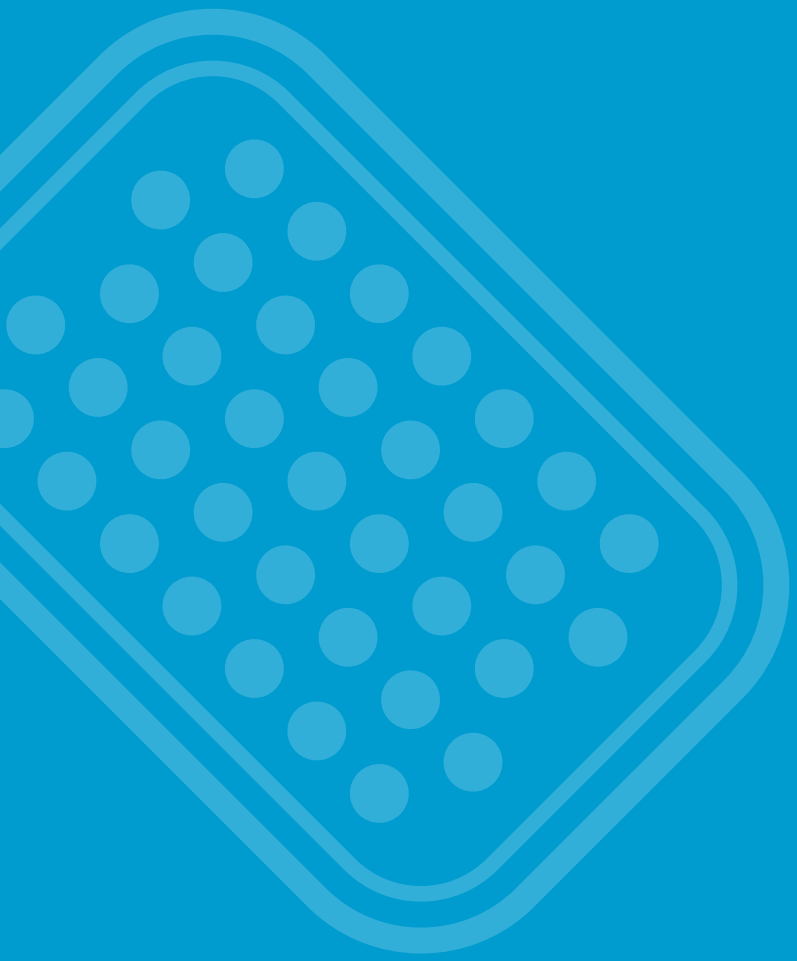
For example, we are now targeting a specific industrial application and will bid for CBMNet Proof-of-Concept funding to enable this.

The student is aiming to undertake a PhD studying membrane transport proteins.

This project was funded through the Crossing Biological Membranes Network (CBMNet) by the Biotechnology and Biological Sciences Research Council (BBSRC)

> **The CBMNet funding was a great way for my lab to explore a new area of research. The success of the project gives us convincing data that we can now use to engage industrial partners within the network.**
>
> **– Dr Paul Curnow, University of Bristol**

SPOTLIGHT ON INDUSTRY



We asked our industrial CBMNet members what they think the grand challenges in crossing biological membranes research are and how we can help provide solutions.

Q&A with industry

DOUG COSSAR – Research Manager Biotechnology, Croda

Douglas.Cossar@croda.com

What Industrial Biotechnology and Bioenergy related project is currently being undertaken by your organisation?

DC Croda is a relative newcomer to IBBE – essentially developing a product pipeline and commissioning a manufacturing capability over the past 6 years. Some of the projects being developed include biosurfactants, specialty natural products, and biopolymers. This work is largely underpinned by significant screening collaborations to identify products of potential interest.

What do you think the challenges related to this project are in the next 1-5 years?

DC Product yield is the most significant challenge. Microbes typically produce only the minimum product required for their needs. In one sense, this is to our advantage since it tends to have evolved highly efficient materials and to foster multifunctionality – both of which are identified key trends in the specialty chemicals industry.

How can other CBMNet members help you and your organisation with your research?

DC The biological membrane presents multiple challenges for a company such as Croda. In some cases, we would prefer that the product is exported to the medium where it can accumulate at much higher levels, so we would be looking for exporters with a particular, or an enhanced, activity. We should also not neglect substrate uptake – both for product-related precursors and nutrient. In a third situation, IB products often present biologically relevant activity – such as anti-microbial, or anti-oxidant. Here, the concerns are about understanding how these products interact with biological membranes.

RICHARD ALLDREAD – Head of Innovation CPI Biologics

Richard.Alldread@uk-cpi.com

What Industrial Biotechnology and Bioenergy related project is currently being undertaken by your organisation?

RA CPI Biologics currently have a number of ongoing projects that are relevant, these include: Manufacturing methods for a nanoparticle drug delivery system; In process formulation of biotherapeutics; Engineering CHO cells for improved process performance; Improved and streamlined biotherapeutic development pathways. There are numerous other projects currently in the planning stages.

What do you think the challenges related to this project are in the next 1-5 years?

RA The biotherapeutic industry will face many varied challenges in the next few years, particularly it will need to adapt to the needs of stratified medicine and adopt more efficient and cost effective ways of selecting, developing and manufacturing therapeutic products. There is likely to be an increase in the diversity of therapeutic products (new protein formats, viral vectors for gene therapy, cell therapy) and these will all challenge our current manufacturing operations.

How can other CBMNet members help you and your organisation with your research?

RA Networks such as CBMNet can help by developing a cross disciplinary approach to solving biological problems and ensuring that biotechnologists have access to and an ongoing dialogue with experts from other fields.

Q&A with industry

GORDON JAMES – Science Leader at Unilever

Gordon.James@unilever.com

What Industrial Biotechnology and Bioenergy related project is currently being undertaken by your organisation?

GJ The most relevant IBBE-related project that I personally am involved in is a collaboration with University of York to identify, clone, express and characterise the bacterial transporters involved in axillary malodour production, focussing currently on malodour precursor uptake systems.

What do you think the challenges related to this project are in the next 1-5 years?

GJ The biggest challenge will be to translate new biological understanding into commercial development opportunities. We have had great success thus far in characterising the transport protein responsible for thiol-precursor uptake in a key axillary bacterium. This is great science, but the ultimate goal for Unilever is to develop a deodorant that measurably reduces underarm odour by inhibiting bacterial uptake of thiol-precursors.

How can other CBMNet members help you and your organisation with your research?

GJ We have an ongoing collaboration with Gavin Thomas, a key CBMNet member of course, and the continuation of this relationship will be key to meeting the above challenge. Additionally, however, we are putting in place collaborations with at least two additional CBMNet members who can add value to the current project in ways that myself & Gavin cannot, for example, by deriving a crystal structure of the thiol-precursor transporter (Simon Newstead, University of Oxford) and designing peptide and peptide-analogue ligands (Beining Chen, University of Sheffield)

IAN HODGSON –Head of Molecular Biology Fujifilm Diosynth Biotechnologies

Ian.hodgson@fujifilm.com

What industrial biotechnology and bioenergy related project is currently being undertaken by your organisation?

IH We have a number of external projects running but one project I am directly involved with is a collaboration with the University of Edinburgh to investigate using *E.coli* turgor pressure regulation to optimise product excretion. *E coli* remains a very versatile expression system used by many of our clients for producing their biotherapeutics proteins. The aim of this project is have product excreted into the growth media while preventing unwanted cytoplasmic leakage. This should provide higher quality material for the start of downstream purification with reduction in host cell proteins and other cell derived species. This collaboration initiated as a CBMNet proof of concept study which then progressed to a collaborative project involving funding from The Industrial Biotechnology Innovation Centre.

What do you think the challenges related to this project are in the next 1-5 years

IH The biggest challenge will be to characterise enough about the impact of turgor pressure on product excretion to be able to know how generally it can be applied and key variables . Additionally at the moment all studies planned at present are laboratory scale so it will be very exciting to see if the data from the project will lead to the need to scale up to full manufacturing fermentation scale at 5000L and above.

How can other CBMNet members help you and your organisation with your research.

IH We have collaborations with other CBMNet members including Louise Horsfall also at University of Edinburgh and with Graham Stafford at The University of Sheffield. We are always looking out for new ideas and approaches which would enable better and more informed development and manufacturing approaches to Biotherapeutics to be achieved. Implementation of these will have potential overall benefits to ourselves and society generally through making new medicines available more quickly and more broadly.

FUNDING & EVENTS

Do you have a great idea for a funded project, but need an industrial or academic partner? We are here to help.

Funding & Events

Proof-of-Concept Funding

These allow consortia to generate the preliminary information required to establish the feasibility of their proposed approaches, with the target of generating competitive bids to IB Catalyst and other relevant funding calls.

Funds available:
Up to £50,000 per project

Next Call: Please see our website for details

Business Interaction Vouchers

These provide £10,000 for academics to work with an industrial partner (who matches the £10,000; can be in-kind). BIVs have proved to be very useful 'confidence builders', leading to longer-term relationships and much larger joint project funding. So, be creative; if you have a piece of work that is in CBMNet remit, then try a BIV!

Funds available:
Up to £10,000 per project

Next Call: Please see our website for details

Early Career Researcher Grants

We provide bursaries to allow ECRs to work with academic and industrial partners and to attend and present at relevant conferences.

Amount available:
Up to £2,500 per grant.

Next Call: Open Call

Open Innovation Meeting Grants

These allow industrial CBMNet members to engage with selected members of CBMNet, in the form of a meeting. For example, the industrial host to hold a confidential meeting to discuss a specific research challenge, to engage with a small, focused group of experts to identify possible solutions. Or, industry host could hold a non-confidential meeting exploring CBMNet relevant scientific theme, with a view to generating project ideas which could form the basis of collaborative funding applications

Amount available:
Up to £2,000 per meeting

Next Call: Open Call

Academic–Industrial Exchanges

To support the development of new teams to transfer knowledge from academia to industry and vice versa. For example, grants can be used for a CBMNet member to visit another member or for CBMNet members to accompany a specialist to a conference.

Amount available:
Up to £1,000 per grant

Next Call: Open Call

Public Engagement Grants

These allow CBMNet members to carry out public engagement events, within the CBMNet remit, on behalf of CBMNet. For example, Café Scientifique, school visits. We are open to new public engagement ideas.

Amount available:
Up to £500 per grant.

Next Call: Open Call

Membrane Transporters In Physiology, Industrial Biotechnology & Bioenergy

March 20–22, 2016
Liverpool

We are looking forward to our major 2-day at the Microbiology Society's Annual Meeting to be held in Liverpool. We have an outstanding programme of international speakers and the final session will be led by industry to promote knowledge transfer to drive new interactions between Microbiology Society scientists at all career stages and industrial stakeholders.

We have lots more exciting workshops, training courses, events and public engagement activities planned for 2016-2019. Please visit our website at **www.cbmnetnibb.net** for more details.

For up to date details about all our funding opportunities, please visit our website at **cbmnetnibb.net**

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So what next? We must continue to build our community and promote the importance of an appreciation of the impact that membrane biology can have on Industrial Biotechnology and Bioenergy processes through our project funding streams and our meetings.

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Professor Jeff Green,
CBMNet Director

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