CarboMet

Scoping workshop

CCIB, Barcelona, Spain

Wednesday 5th July, 1-6pm

Background briefing paper

1. Polysaccharide based vaccines

The need for continued surveillance of potential new bacterial infections is a widely recognised goal in modern medicine, in particular to protect children, vulnerable adults and the elderly.

The current suite of antibiotics to treat these infections has its limitations and development of antibiotics has stalled in recent years, so we need new vaccination strategies and alternative treatments, to deal with increasing accounts of antibiotic resistance.

Many vaccines work by exposing the body to a harmless equivalent of the bacteria, so that the body can develop antibodies to eliminate those bacteria when exposed to the real thing. These antibodies recognise the harmful bacteria through the presences of an antigen, which can either be a protein or a sugar/polysaccharide (PS) on the surface of the bacteria.

Although not widely used to date PS have provided the basis for a number of successful vaccines, either as isolates from inactivated virus or obtained by chemical synthesis.[1] Examples are successful polysaccharides or conjugate vaccines against *Haemophilus influenzae* type b, *Streptococcus pneumonia*, *Salmonella typhi* and *Neisseria meningitidis.*[2] These vaccines have established a design based on microbial PS as a promising generic strategy, which could be adopted by biopharmaceutical companies.

However, PS vaccine development is still slow due to limitations of current technologies. One bottleneck is the identification of suitable PS structures, in the fast timescale (days) required by the clinic. Whereas we now have fast sequencing methods for DNA and proteins, which have revolutionised modern medicine, carbohydrate sequencing is still very slow and requires highly specialised skills. Here, we propose to address this by developing fast and high-resolution *de novo* carbohydrate sequencing tools that would give us access to high quality structure from easily accessible biological material within days.

The complexity of carbohydrates requires a more complicated sequencing approach than DNA and proteins to detect very subtle differences in three-dimensional structure that are hard to differentiate by some high-throughput sequencing methods. Our strategy proposes to address such issues of use a radically new combination of (bio)chemical and (bio)physical methods including chemical and enzymatic degradation, NMR spectroscopy and mass spectrometry to measure structural fingerprints that will then be used to define high resolution structure using automated bioinformatics tools. In recent years[3], proof-of-principle of key aspects of such *de novo* sequencing methods have been established, and this project aims to combine these technologies take them forward into applications of real clinical challenges.

These are ambitious challenges and will require a large multidisciplinary integrated project recruiting scientists with the biophysical and biochemical expertise to develop the carbohydrate sequencing technology and end-users (clinicians) who provide clinically relevant target polysaccharides and provide feedback on prototype carbohydrate sequencing technologies for use by non-specialists in the clinic.

References:

[1] Hecht et al. *Current Opinion in Chemical Biology* 2009, 13:354–359

[2] Finn *British Medical Bulletin* 2004, 70: 1–14; Vliegenthart *FEBS Letters* 2006, 580, 2945-2950

[3] Both et al. *Nature Chemistry*, 2014, 6, 65-74; Gray et al. *BBA* 2016, 1860, 1688-1709; Gray et al. *Anal.Chem*, 2017, in press; Lombard et al. *Nucleic Acid Res,* 2014, 42: D490-D495; Lundborg et al. *Anal. Chem.* 2011, 83 (5) 1514-1517.

2. Precision diagnosis of disease

All cells, including human cells, have carbohydrates on their surface, in a covering known as the glycocalyx. This coating of glycolipids and glycoproteins controls a variety of fundamental biological processes, for example helping sperm to recognize eggs during fertilization, determining blood groups, or enabling the body to identify diseased cells or infectious agents.

The carbohydrates of the glycocalyx are produced through highly complex biosynthetic pathways controlled by both genes and the environment. The makeup of the glycocalyx is therefore highly sensitive to genetic mutations, changes in gene activation or silencing, or environmental factors such as diet, alcohol consumption or smoking. This makes the glycocalyx a useful target for personalized medicine, including finding new biomarkers for diseases such as cancer, and for patient stratification in clinical trials.

Development of fast and effective diagnostic tools for the detection of pathogens and for monitoring of health and disease will greatly benefit society by both reducing human suffering and cutting healthcare costs. For example; targeted interventions for diseases such as diabetes, or cancer where survival rates strongly correlate with the stage at which the cancer is diagnosed.

As personalised medicine advances there will be a need for diagnostic tools that can rapidly and accurately measure the individual patient's condition in real time through the development of low cost, sensitive, and highly specific diagnostic tools for the detection of glyco-biomarkers in a variety of disease states. This includes companion diagnostics that are used alongside therapeutics firstly to help identify patients with the correct biological characteristics who will respond to treatments, and then to monitor their continued use.

Candidate technology exists in the form of 'glycoarrays' where glycans are displayed on array surfaces to mimic those found on cell surfaces, allowing the study of glycan interactions and identification of biomarkers. However, their development as diagnostic tools is currently hindered by bottlenecks in a number of enabling technologies areas:

Analysis – there is a pressing need for the ability to unambiguously elucidate carbohydrate structure through carbohydrate sequencing capability, needed in both manufacturing (production monitoring, quality control, etc.) and clinical settings. This is a challenge due to the high stereo-chemical diversity of carbohydrates;

Synthesis – efficient scalable methods for production of the required glycans for the arrays, and glyco-standards for benchmarking and quality control purposes are needed. This includes a readily available suite of carbohydrate active enzymes, with a range of activities, on scale, needed for synthesis as well as part of the diagnostic tools and kits themselves;

Bioinformatics - there is a critical need for carbohydrate databases akin to DNA and protein equivalents, that are accessible, contain reliable information in a searchable format, on stable platforms. Long-term management and coordination of the existing provision is urgently required.

Advances in each of these ambitious and highly challenging areas will allow development of the rapid, precision diagnostics needed. This will require a large multidisciplinary integrated project with biophysical and biochemical expertise. There also needs to be strong engagement with clinicians and patient end users to ensure the innovative diagnostic tools and kits developed are readily adopted in clinical settings.

References:

- Flitsch et al. (2015) A roadmap for Glycoscience in Europe – a joint EGSF/IBCarb publication
- Glycobiology: Global Markets for Diagnostics & Therapeutics, September 2016, BCC Research report BIO153A

3. The role of dietary carbohydrates in maintaining a healthy gut microbiome

Promotion of healthy gut microflora is increasingly understood to be important for infant and adult health. Diet is a major influence and the importance of sugars as dietary components, their nutritional value and potential as functional foods is becoming more and more apparent.

The gut microbiota digest and ferment indigestible complex carbohydrates known as prebiotics into short chain fatty acids (SCFAs) and other compounds. These SCFAs are vital energy sources, and have anti-inflammatory and cell regulatory roles. The gut microbiota also have an effect on the immune system and play a role in vitamin production. An imbalance in the gut microbiota, 'dysbiosis' is associated with gut disorders as well as non-communicable diseases such as diabetes, cardiovascular diseases and cancers.

An understanding of the microbial degradation of non-digestible carbohydrates in the gut would allow the design of prebiotics as functional foods that can improve health through the diet. This approach is an alternative to long term medication, especially important for ageing populations, where strategies that can reduce healthcare costs and improve health outcomes are increasingly required.

Prebiotics include plant cell wall polysaccharides (cellulose, arabinoxylan, xyloglucan, mannan and pectin) from fruit, vegetables and whole cereals, and plant storage polysaccharides such as resistant starch. Due to their increased size and complexity, polysaccharides present a major set of challenges in terms of their analysis and characterization. Whereas we now have fast sequencing methods for DNA and proteins, which have revolutionised modern medicine, carbohydrate sequencing is still very slow and requires highly specialised skills. Here, we propose to address this by developing fast and high-resolution *de novo* carbohydrate sequencing tools that would give us access to high quality structure from easily accessible biological material within days.

The identification and characterization of these prebiotic carbohydrates alongside studies into their impact on the gut flora, will lead to structure activity relationship know-how that will in turn allow the development of highly effective prebiotic compounds as functional foods.

For the manufacture of prebiotics, scalable synthesis methods will be required, either through chemical/chemo-enzymatic approaches or, via modification (physical, chemical and/or biological) of natural polysaccharides, and their isolation from complex mixtures. Analytical tools for production monitoring and quality control that can be used by non-specialists will also be needed.

Sequencing of the metagenomes of the gut microbiota remains fragmented and further work is required in order to understand the gut populations. This will also lead to the identification of new carbohydrate active enzymes that due to the diversity and complexity of the sugars available, the gut microbiota rely on for their digestion, that can be employed in the synthesis of prebiotics and other carbohydrates.

This will require a large multidisciplinary integrated project with chemical, biophysical, biochemical and molecular biology expertise to understand and exploit the relationship between carbohydrate utilization, microbial ecology and microbiota metabolism in the gut. There will also need to be strong engagement with consumers to ensure that the development of new functional food products are palatable and consumers are educated on their health benefits. This is an exciting area of opportunity not just in terms of nutrition but also in disease prevention.

References:

- Flitsch et al. (2015) A roadmap for Glycoscience in Europe – a joint EGSF/IBCarb publication
- Thursby & Juge. (2017) Introduction to the human gut microbiota, Biochem. J. 474 1823–1836
- Tailford et al. (2015) Mucin foraging in the human gut microbiome; Front Genet, 6 (81)
- Flint & Juge. (2015) Role of microbes in carbohydrate digestion, Food Science & Technology