Understanding multivalent interactions

Professors Christoph Schalley, Beate Koksch and Rainer Haag are dedicating their careers to understanding and harnessing the power of multivalent interactions. Here, they discuss their work and distinct scientific backgrounds in more detail.

Could you provide a brief introduction to multivalency, as well as your goals in this line of research?

CS: Non-covalent bonds are weak. While nature has found ways of combining a large number of weak intermolecular forces to strongly connect biomolecules non-covalently, supramolecular chemistry has not achieved such high binding energies. Consequently, supramolecular chemists have started to use multivalent binding to enhance binding energies and use synergetic effects between binding sites that are tethered together. The Schalley group works on multivalent pseudorotaxanes and rotaxanes to investigate the binding strength increase and cooperativity effects by which one binding event affects the following ones, with the aim of implementing function.

BK: Peptides offer the advantage of a facile de novo design by fine-tuning the primary sequence to obtain morphologies such as peptide nanotubes, amyloid fibrils, peptide nanoparticles and α-helical nanofibres. Peptides are also easier to synthesise and characterise than other polymeric structures, are biocompatible and offer distinct functional groups to which a molecule of interest can be conjugated with arbitrary stoichiometry. Such peptide-based scaffolds allow for multivalent presentation of a variety of ligands. The architectures adopted by peptide nanoassemblies are often retained in the presence of guest molecules, and their potential as biomolecular carriers has applications in regenerative medicine and in the delivery of peptide epitopes and growth factors.

RH: The Haag group works on multivalent inhibitors to prevent infection and inflammation. We design 2D and 3D polymeric scaffolds that mimic the natural situation with respect to ligand type and density as well as scaffold size, in order to effectively compete with the pathogen or the cell.

What are your respective roles in this work and how have your backgrounds shaped your research?

CS: I am a principal investigator (PI) in the Collaborative Research Centre and the spokesperson for all projects involving synthetic multivalent material. As a supramolecular chemist I see molecular recognition phenomena and non-covalent interactions as the basis for the generation of larger programmed structures. Looking into multivalent binding and cooperativity enriches my experience with supramolecular interactions and helps to predict and design functional structures.

RH: I am also a PI and spokesperson. As an organic and macromolecular chemist, I am most interested in controlling function at biological interphases by chemical self-assembly processes and triggering certain mechanisms. My goal is to develop multivalent 2D and 3D polymer platforms for the inhibition of pathogens.

BK: I too work as a PI on the project. As a peptide chemist, I have always been fascinated by self-organisation phenomena, an interest I can actively pursue within this collaboration.

Could you elaborate on your work on multivalent interactions?

BK: Our group develops new peptide-based scaffolds with different properties and architecture for the multivalent presentation of diverse functionalities and ligands. These scaffolds can be designed such that their backbone can respond to environmental changes as pH or metal ion concentration by adapting different conformations. For example, carbohydrate ligands can thus be presented in multivalent fashion in defined distances and with different spacial arrangement.

Why is inhibition of multivalent interactions an important therapeutic target?

BK: Immune system receptors have evolved to distinguish between binding partners based on antigen density, and invading pathogens can be detected according to distinct ligand arrays. Glycans and peptide epitopes are useful diagnostic probes in bioassays and immunogens in vaccines due to their central role in immune response.

RH: Another hypothesis is that we can inhibit the docking of pathogens to cells by mimicking the cell surface functionality on a nanoparticle; we have successfully demonstrated this concept for several virus types. This could eventually lead to new therapeutic agents with higher efficiencies.

How do you see your research developing in the next five years? Do you expect your diagnostic tools to be ready for use in the clinic in the near future?

RH: We hope to raise additional funding to start clinical development of multivalent diagnostic or therapeutic agents. However, the mindset of the pharmaceutical industry – focusing on small molecules and biologicals – is not yet ready for multivalent drugs. Despite this, we are determined that together with several start-up companies we will further advance this great concept!
A new approach to antiviral drugs

MULTIVALENCY, ALSO KNOWN as polyvalency, is a naturally occurring phenomenon which enables strong bonds to be made between molecules. A cornerstone of biochemistry, particularly in protein-carbohydrate interactions, multivalent bonds comprise a large number of weaker, single connections, allowing multiple hooks or ligands on one surface to entangle with loops or receptors on another. In doing so, an interaction results which is significantly more resilient than individual bonds between the equivalent number of monovalent ligands and a multivalent receptor. Consequently, multivalency plays a pivotal role in the self-assembly of matter, recognition processes and signal transduction in a range of biological systems. Unlike covalent bonds, multivalent interactions are also reversible through the sequential separation of individual bonds, making interactions between cells, or with organisms such as viruses, enormously dynamic. However, despite the importance and significant therapeutic potential of multivalency, much is to be discovered about the exact mechanisms that underpin it and how these can be best applied in the treatment of a range of diseases.

In Germany, Collaborative Research Centers (CRCs) are funded by the German Research Foundation (DFG) where cross-disciplinary challenges would benefit from the establishment of temporary research hubs, which may last up to 12 years. CRC 765, based at Freie Universität Berlin, focuses on multivalency. Established in 2008, a team of 20 principle investigators (PIs) intends to elucidate multivalent interactions and implement them in the battle against a variety of aggressive and damaging infections by drawing on the expertise of a range of biologists, chemists and physicists.

VIRUS INHIBITION

One medically significant example of multivalent bonding is virus-cell interaction, whereby a virus establishes multiple connections with a host in order to create strong adhesion, before permeating the cell’s membrane via endocytosis. At present, medicinal approaches to viral inhibition revolve around monovalent drugs that are only capable of targeting viral budding, a latter stage of the viral replication cycle. Additionally, due to the multiple binding sites between the virus and the host cell, monovalent antivirals have limited effectiveness even in high doses.

In response, one team at CRC 765 is developing a range of multivalent antiviral ligands. These efforts, led by PI Professor Rainer Haag, are currently focused on the use of polyglycerol nanoparticles. By incrementally increasing the size of the ligand-functionalised nanoparticles to proportions similar to those of a virus, Haag and his colleagues have been able to successfully measure the effectiveness and differing mechanisms of variously sized multivalent particles’ inhibition of virus-cell binding.

VESICULAR STOMATITIS VIRUS

As part of their ongoing mechanism studies into antivirals, Haag and his fellow researchers have employed a novel binding assay, allowing them to ascertain that surface area-normalised polysulphated gold nanoparticles with a diameter equal to or larger than a virus are particularly effective inhibitors of the vesicular stomatitis virus (VSV). VSV is a potentially economically disastrous disease which causes painful sores around the mouth and feet of cattle, swine and horses and, if untreated, can lead to lameness and refusal to eat.

Having observed that larger nanoparticles can inhibit viral infection by as many as two orders of magnitude more efficiently than their smaller counterparts, Haag’s group deduced that different mechanisms of viral inhibition must be in operation. Following up this work with complementary electron microscopy, the team learned that smaller gold nanoparticles work by adorning the surface of virus particles, whereas the larger gold particles inhibit viral infection by successfully cross-linking and shielding virions. Having reached this breakthrough in understanding, Haag and his colleagues are excited to apply this knowledge in the design of large, biodegradable, multivalently bound nanoparticles aimed at inhibiting a range of viral infections.

MULTIVALENCY MECHANISMS: THE FUNDAMENTALS

The complexity associated with biomedical examples makes understanding multivalency challenging. As such, synthetic systems that can be methodically varied offer an opportunity for comprehending multivalency in greater depth. Consequently, researchers at CRC 765 are first garnering a clearer understanding of the properties and processes underpinning tailormade supramolecular architecture, such as rotaxanes and pseudorotaxanes.

Rotaxanes are mechanically interlocked molecules comprising a bulbously ended axle threaded through a macrocyclic wheel. Owing to the size of the stoppers at either end of the axle, it is not possible for it to become separated from the macrocycle. The non-stopped pseudorotaxane precursors form non-covalently in a chemical equilibrium. By understanding and learning to control the processes by which pseudorotaxanes and rotaxanes form, the researchers at CRC 765 are getting closer to tethering the power of multivalent bonds: “If one prepares multiply threaded pseudorotaxanes, it is possible to systematically vary the number of, and alter the size and rigidity of, the spacers between the
binding sites – parameters which cannot usually be freely chosen in biological complexes,” explains Professor Christoph Schalley, a PI within the CRC. Indeed, multivalency not only enhances binding forces, but also allows molecules to be orientated relative to each other in their complexes. It is thus also a method to control supramolecular geometry, and has proven central to the CRC’s efforts to synthesise controllable molecular machines.

MULTIVALENT PEPTIDIC SCAFFOLD STRUCTURES

A key goal of CRC 765 involves elucidating peptide-based multivalent scaffold structures. Professor Beate Koksch’s group has been developing peptide model systems that enable the systematic evaluation of how mutations in the primary structure (amino acid sequence) influence the stability of the quaternary structures (3D structure and fold). A variety of applications in biomedicine, biotechnology and materials science at the nanoscale are currently being pursued.

In nature, proteins often form multimeric architectures that contain several recognition sites to compensate for weak individual non-covalent interactions. Similarly, oligosaccharide and peptide ligands are often presented multivalently. Thus, molecular recognition is enabled by a synergistic combination of several cooperative binding events. The predictable structural behaviour of self-assembling peptides allows for defined multivalent presentation of a variety of ligands.

In collaboration with other CRC 765 contributors – Professor Peter Seeberger and Dr Bernd Lepenies from the Max-Planck-Institute for Colloids and Surfaces in Potsdam/Golm – Koksch’s group has developed a novel, fibre-forming coiled-coil-based peptide that assembles to display carbohydrate and peptide ligands that are recognised by proteins. The potential of the novel self-assembling peptide to display ligands in bioanalytical assays was illustrated by decoration of the scaffold with a disaccharide glycopote from the surface of the Leishmania parasite. Carbohydrate-specific antibodies that are produced in response to a Leishmania infection are detected more sensitively in human and canine sera due to the multivalent presentation on the self-assembled scaffold. Thus, nanofibres based on coiled-coil peptides are a novel platform for the development of bioassays and diagnostics.

FACING FORWARDS

The research threads touched upon here provide just a few examples of the work conducted at CRC 765. The Center is a hub for multivalency research, and while their findings will no doubt lead to clinical development of multivalent diagnostic and therapeutic agents in years to come, the pharmaceutical industry may take time to catch up with the innovations emerging at the forefront of this exciting field. Nevertheless, the team will continue to attract attention for their work, which could also prove valuable to the advancement of materials science.