Strength in **numbers**

Professor Peter Lipke is unravelling the secrets of cell adhesion. In a thought-provoking discussion, here he discusses his research, collaborators and approach to teaching, as well as the steps he is taking to redress the under-representation of minorities among scientists


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As a starting point, what are the overall aims of your research?

Currently we are focused in two areas. First, we want to see if the formation of adhesion surface amyloid patches is a property of the specific sequences in the fungal adhesins or whether the same patch formation might result from sequences that are more like the 'classic' amyloids. Second, we are exploring the consequences of formation of these surface amyloid patches.

Do you believe that amyloid formation may be more widespread than previously thought as a mechanism for cell-to-cell interactions?

Yes, I do. Bioinformatics of cell adhesion protein sequences suggests that the phenomenon of amyloid-dependent adhesion is widespread. We have found, for instance, that anti-amyloids disrupt fungal biofilms, and Jeanine Brady's lab in Florida has a similar result in oral bacterial biofilms.

Who are your main collaborators?

Steve Klotz at the University of Arizona is an infectious disease physician. He has a great sense of medical relevance. His work with Nand Gaur actually inspired us to work with the *Candida* adhesins. Nand was the first to realise that the adhesion proteins were unusual in their physical properties. Yves Dufrêne at Université catholique de Louvain in Belgium has been amazingly productive in his pursuit of the biophysics of these proteins. It seemed like he sent me a new manuscript each week last summer.

Closer to home are several collaborators. My bioinformatics and evolution guru is Weigang

Qiu at Hunter College. Anne Dranginis at St John's University has been instrumental in helping us test the amyloid model with the *Saccharomyces* flocculins, an unrelated set of adhesins. And finally, Jason Rauceo at John Jay College showed us the first evidence of a global cell surface change that turned out to be amyloid formation. He has now been a colleague and collaborator for a decade.

Could you describe your 'student-centred active learning' approach to teaching? How does it differ from traditional lecturing?

I hate listening to lectures, unless they are really delivered well or are directly related to something I really care about. If the lecturer posts the slides, then I wonder why I am sitting there listening to the same material. So I try to make classes a dialogue. I like to challenge students with issues and problems, and I love to have them ask me questions on subjects that they see as related to the subject. I have tricks for making sure every student who shows up in class has to talk. They get points for asking questions and giving answers, whether they are right or wrong. The wrong answers let me know where I and the readings have failed to explain key material.

This approach is easy in small advanced classes, but much harder in large classes. I break large classes into groups of three to five students, and give each group a question or a little problem to solve. I make different students act as group spokesperson for each class, so over the course of three to four classes, everyone is a vocal participant.

What activities do you undertake to help to redress the under-representation of minorities among scientists?

As a parent of children from many different backgrounds, cultures and races, I have experienced too many concrete examples of kids being stereotyped and having decisions made for them based on those stereotypes. Partly because of those experiences, at Hunter College I ran a National Institutes of Health (NIH) undergraduate training programme for under-represented students for 15 years. We placed students in research labs, usually for two to three years; an environment in which most were unfamiliar. Their experiences showed them how good they really were in planning and executing experiments. We also tried hard to persuade them to become research scientists where their skills could maximise benefits to society.

A colleague is running those programmes now at Brooklyn College, so I am concentrating on my own lab and department. My experiences in finding so much talent led me to consciously recruit a highly diverse research group. As I had hoped, lab members have been outstanding in bringing new ideas and approaches to research. The diversity of approaches has reinforced my inclination to try to look at scientific problems in ways that are different.

The ties that **bind**

Research in the **Department of Biology**, **Brooklyn College** aims to dissect the functional roles of amyloid clustering in the process of cell adhesion. This will have important implications in the treatment of infection

AMYLOIDS FORM WHEN large numbers of identical beta strands assemble together, leading to the aggregation of clusters of proteins. This is usually associated with a variety of pathologies, from neurodegenerative disorders such as Alzheimer's and Parkinson's disease, to transmissible disorders including bovine spongiform encephalopathy (BSE) and scrapie.

More recently however, amyloids have been receiving great interest for positive and functional roles. In mammals this includes the storage of hormones, melanin deposition and synaptic remodelling and learning. In addition, they serve crucial functions in microbes and are important in the formation of biofilms, fungal cell coat self-assembly, cell adhesion, the regulation of gene expression and evolutionary variability. Amyloids can also indicate disease states.

CELL ADHESION

Professor Peter Lipke of the Department of Biology, Brooklyn College is a key researcher in this field, having established a significant portion of what is currently known regarding amyloidogenic proteins. He studies the relationship between structure and function in fungal adhesion molecules called adhesins, and is currently establishing the function of amyloid in the regulation of cellular adhesion.

Amyloid-forming sequences lead to the cell surface aggregation of hundreds or even thousands of adhesin molecules into patches of highly ordered molecules. Lipke and his collaborators have termed these amyloid nanodomains, and they exhibit extremely strong and diverse binding with their partner ligands, in contrast to the weak and non-selective binding of individual adhesin molecules. The clustering of such a vast number of adhesins allows the nanodomain to host hundreds to thousands of weak interactions on a small surface area in a process that has been likened to cellular velcro. So far, most fungal adhesins studied by Lipke's group have sequences with the potential to form amyloids.

One of the immediate implications of this process is its ability to answer a longstanding paradox in the study of cell adhesion: why are individual adhesion molecules able to bind a broad range of different ligands very weakly, and yet are responsible for the comparatively strong binding that occurs between two cells, or between a cell and its substrate?

Lipke and colleagues have thus far investigated some of the consequences of nanodomain action and the molecular processes that provide its remarkable binding strength. They have also uncovered that the nanodomain relies on amyloid clustering for its formation and activation. This was demonstrated through specific site mutation of V326N in the conserved amyloid-forming sequence, and application of anti-amyloid perturbants, which both led

AMYLOID-LIKE INTERACTIONS IN YEAST CELL ADHESION

OBJECTIVES

- To understand the formation of adhesion surface amyloid patches
- To explore the consequences of formation of surface amyloid patches
- Ultimately, to translate new understanding of surface amyloids into medically useful treatments

KEY COLLABORATORS

Professor Steve Klotz; **Dr Nand Gaur**, University of Arizona, USA

Professor Yves Dufrêne, Université catholique de Louvain, Belgium

Associate Professor Weigang Qiu, Hunter College, City University of New York, USA

Professor Anne Dranginis, St John's University, City University of New York, USA

Assistant Professor Jason Rauceo, John Jay College, City University of New York, USA

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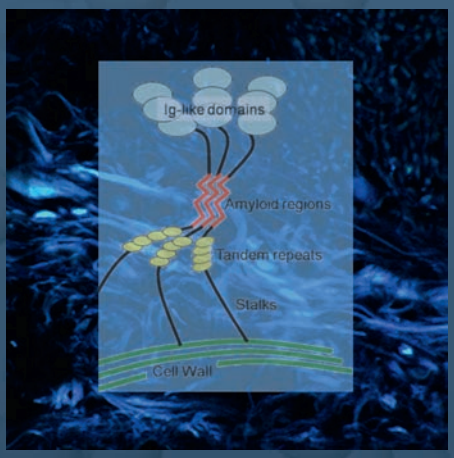
PROFESSOR PETER LIPKE has a BS in Chemistry from the University of Chicago and a PhD in Biochemistry from the University of California, Berkeley. After a postdoc in cell adhesion and developmental biology at the University of Wisconsin, he joined the faculty at Hunter College in 1978. In 2006 he moved to Brooklyn College, where he serves as Chairperson of Biology.

Brooklyn College



A model for amyloid-mediated clustering of a fungal adhesin is superimposed over an image of fluorescent staining of the fungal surface amyloids in tissue from a patient who died of a fungal infection.

to a loss of aggregate formation, alongside a failure for the nanodomain to form and a loss of adhesion activation. In both cases ligand binding still occurred, but lacked the benefits of increased binding strength conferred by the nanodomain structure.

THE MECHANICAL PROPERTIES OF THE NANODOMAIN

In order to understand the mechanical properties of the nanodomain, it is helpful to know some further details about the structure of amyloid clusters. Amyloidogenic proteins aggregate to form a fibre built from beta strands, aligned perpendicular to the fibre and held together by hydrogen bonds acting parallel to the fibre. Each beta strand contains a series of side chains, which are interdigitated with the side chains of adjacent beta strands in a steric formation similar to a zip, and are thusly denoted as having a steric zipper structure. These are held together by van der Waals forces acting between each side chain. Recently it has become possible to conduct experiments in which single molecules can be manipulated and studied. This advance has facilitated the study of the molecular mechanism granted by the steric zipper conformation. By applying forces and stretching modular proteins, it is possible to measure force signatures that reflect the unfolding of a protein's secondary structure.

Lipke's group, in collaboration with Yves Dufrêne's laboratory at Université catholique de Louvain in Belgium, has used this technique to study the amyloid nanodomain zipper formation. They applied single molecule atomic force microscopy to delve into the nanomechanics of amyloids formed by Als adhesin proteins, found in *Candida albicans*. Results revealed that clustering of adhesin greatly increases the strength of ligand binding, and that pulling adhesin away from the cell surface increases the strength of the binding interaction – even when adhesin has been pulled up to 200 nm from the cell, ligands remain bound. Crucially, the latter effect does not occur in the absence of amyloid clustering.

CONSEQUENCES OF FUNCTIONAL AMYLOIDS

Several studies have already been published on surface amyloids, but Lipke believes that they have only scratched the surface, and that novel and important applications lie ahead: "Amyloid surface nanodomains are implicated in biofilm formation, resistance to anti-fungal drugs and innate immune response in infections," he highlights. "It would be exciting to see if our understanding of these surface amyloids result in medically useful treatments."

In order to assess some of the consequences of the adhesion mechanism and demonstrate the formation of functional amyloid *in vivo*, collaborator Professor Steve Klotz and Lipke's group have turned to invasive candidiasis, an often-fatal fungal infection in humans. Last year their groups showed that functional amyloids are present on fungal cell surface proteins in human tissues, and that they play a fundamental role in the progression of infection, promoting strong adhesion, fungal invasion and destruction of the host cell. They have also uncovered that host-expressed immunity receptor Serum Amyloid P (SAP) binds to amyloid on the invading fungi. This finding suggests that the marked suppression of the immune response to invading fungi is due to the presence of amyloid, SAP or a complex of proteins that inhibit the neutrophil response.

IMPLICATIONS

The wider implications of this research are intriguing. Lipke's greater insights into the binding properties of microbes could be applied to devise new treatments for infections, for example by providing a means to disrupt the strong cell-cell interactions of fungi. It is thought that under certain conditions, fungi bind to one another so well using these interactions that they can withstand treatments as diverse as heat killing, inhibition of gene expression or signal transduction. Essentially, disrupting the secondary structure of the adhesion proteins represents a new therapeutic target.

The team has already started testing drugs that disrupt biofilms as a method for reverting fungi back into a dissociated state that is significantly easier to treat. In addition to this, they are investigating the mechanical properties of amyloid nanodomains, providing insight at the nanoscale into the way in which strongly binding interactions can be generated from self-assembling structures. This will likely provide a platform for future technologies to be developed, which take advantage of this elegant self-assembly mechanism.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