Communicating Across Scales: biophysics and beyond

The Assembly, Dynamics and Organisation of Filaments and Cellular Responses - Organised by Roy Quinlan and Tom McLeish FRS

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One of the most remarkable aspects of biology is the richness of behaviour in the cells and their components. It is this that makes the study of the cytoskeletal filaments and understanding their physical properties essential to appreciating how living systems communicate across scales. The cytoskeleton is a trans-cellular network of filaments that integrates individual cells into their respective tissues and helps coordinate the physiological responses of the organism. The filaments comprise individual proteins just a few tens of nanometers in length and yet these micron/mm/metres long filaments store, dissipate and channel the physical and chemical signals sensed by cells and tissues. The cytoskeleton is both an energy and an information dissipation/storage system for the cell. It is also a major structural asset essential to any tensegrity-based models of the cell and this workshop brought together biophysicists, cell biologists and modellers committed to revealing these biophysical properties.

Detailing the assembly of intermediate filaments *in vitro* has proven a tough scientific problem that has spanned several decades, but of all the cytoskeletal elements, it is this filament system that stands apart from microtubules and microfilaments in their superior biomechanical properties, ability to fission and fuse and exchange subunits and assembly intermediates along the length of the filament.

Workshop Presentations

Although the initial stages of intermediate flament assembly are now reasonably well characterised, the detail of the filament itself and how to regulate the length and width as well as the internal structure are now key experimental goals. Native filaments present significant challenges to our understanding, as exemplified by the hagfish slime intermediate filaments. In these filaments, fusion of individual 10nm filaments to form micron wide filaments is observed, but by completely unknown mechanisms (Fudge).

The potential socio-economic benefit of discovering the biophysical principles are very significant. Lessons from the ancient industries of rope making and weaving evidence the benefits of twisting multiple strands and/or braiding to increase strength. As well as changing the biophysical properties, the limit to the extent of the twist mediated by the ability of individual strands/fibres to slide and yet twisting several strands together can facilitate bending (Prior). So now the importance of understanding whether a filament is a collection of protofilaments or is a single entity becomes a critical question. What is clear is that the intermediate filament and its network of filaments in cells has incredible capacity to withstand stress and strain (Anders).

Individually, filaments can be stretched to the (irreversible) point of undergoing alpha-helix to beta-sheet transition, suggestive of sliding mechanisms accompanied by irreversible secondary structure transitions at the extreme. Cation-assembled filament networks *in vitro* can withstand a 700% increase in strain, providing this is made in a step-wise fashion. This is accompanied by network softening and fluidisation arising from a loss of connectivity between filaments and finally the eventual rupture of the network. The filament network is considered as a mixed Maxwell and Kelvin-Voigt viscoelastic system so the creep and relaxation properties of the intermediate filament network are adequately explained. It is clear that as a network, intermediate filaments and their integration within the trancellular network provides a spectrum of biophysical properties. Two talks from Leube and Goldberg provided examples of these properties.

At the nuclear membrane a supramolecular protein complex (LINC) physically connects the cytoplasmic and nuclear intermediate filament systems and we heard the complexity and functional diversity of the components that control, amongst other things, the spacing between the inner and outer nuclear membranes. Now of course in our reductionist world of science where every cell is a sphere this is a gift not to be ignored. Newtonian principles applied to understanding the regulation of enzymatic activity can now be applied to transcriptional regulation and mechanosensory systems of the cell – an impressive example of scaling indeed from atoms to whole animals – via the cytoskeleton! This included the dynamic behaviour of cytoplasmic filaments that supports both inward flow of polymer, with the necessary localized assembly and disassembly, as well as the positioning and complexity of a more stable filament network according to the functional requirements of the cell. It is this richness of behavior seen for intermediate filament networks and the fact that the transcellular network it forms exhibits such complex behavior in terms of forced oscillations and waves in response to external stimuli (physical and biochemical), their positioning and complexity in terms of bundles, nodes and integration within the cell, properties that likely have significant emergent potential, but as yet undiscovered because the imaging and modeling tools are still in development.

The talk from Bromley and McLeish highlighted this richness in the intermediate filament assembly system. Their approach was synthetic biology based using synthetic polypetides based on coiled coil forming alpha-helices as biomimetics. Here it is clear that aspect of surface geometries and our current abilities to control width, length, subunit registration and uniform, apolar assembly are opportunities for future research. Amyloid fibre assembly (Auer) is related, but a far simpler assembly system compared to intermediate filaments albeit again using synthetic peptides. Modelling of this system to address the questions of nucleation, elongation and thickening of amyloid fibres helps inform amyloid network biomechanics. A step-wise nucleation process that is concentration/volume dependent offers new therapeutic opportunities has come about from simulation analytics. Modeling of the inward flow of keratin filaments (Portet) that accounts for the diffusion, active transport, assembly and disassembly of intermediate filament subunits and filaments is a complex multidimensional problem with potentially 36 different model outcomes. Selection is based on probability and from this a directed active transport model is favoured, where kinesin and dynein enter a tug of war to deliver the movement of intermediate filament particles. Interestingly the biomechanical properties of these particles and filaments affects the outcome of this motor protein tug of war. This is another example of the richness of the behavior in this filament system and why an interdisciplinary team approach is needed.

Future Physics of Life Research Questions

The workshop included several open discussions from which emerged a series of open questions concerning the ability of intermediate filament networks to self-organize, to store and channel biomechanical energy:

- Do the compression/extension properties of individual filaments transfer to bundles and networks?
- How do we explain how cell networks can soft with shear but not so with compression?

Such questions will likely only be answered once we appreciate, understand and then mimic intermediate filament assemblies. The ability to design and fabricate a micron-sized, filament-like network would be a significant step to building and designing a synthetic cell. The advantages of a dynamic network with different assembly states would seem futurist, but filament fission and fusion is an energy independent process and therefore is matter of design, fortitude and vision for the experimentalist as such properties deliver a smart biopolymer that can tune itself to its environment and the changing physical demands.

Here an appreciation and understanding of the 4 dimensional properties of a network (time and space dimensions) is an important experimental goal. How, for instance, the transcellular network of cells contribute to crowding and dragging properties and influence reaction rates / equilibrium constants is currently unexplored, but such a filament network is just a super-scaled catalyst for such reaction. The transcellular mimetic then facilitates reactions by creating nanodomains. How network mesh size influences reaction efficiency can be determined leading to the manufacture of substrate channeling systems using fabricated, synthetic filament networks so that we move artificial crowding agents into controlled and regulated mimics of the cytoplasmic space. In addition to the nanometer/micron scale of these cell-sized bioreactors, we could potentially polymerise the units into millimeter and meter sized bioreactors as the scaling properties are discovered and applied. This is another potential application in addition to potential dynamic, tunable mechanical properties, but illustrates the future for understanding the principles and physics of the transcellular intermediate filament network.