

Skin Layer at the Actin Gel-Surface: Quenched Protein Membranes form Flat, Crumpled and Tubular Morphologies

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Filamentous (F) actin together with cross-linking proteins (e.g. α -actinin) form networks, which are a major component of eukaryotic cells involved in key functions including cell shape and mechanical stability. We have reported on the discovery of protein skin-like structures, assembled at the surface of the actin gel (Fig. 1). On the very large micron scale the skin-layers exhibit novel morphologies. Laser scanning confocal microscopy has enabled us to image the 3D structures of the spontaneously formed geometric structures: crumpled and pleated multi-tubular layers (Fig. 1), and remarkable protein tubules (Fig. 2), reminiscent of lipid tubules. Successive magnification reveals the internal structure to be hierarchical consisting of an oriented network of bundles of F-actin (**Hirst & Safinya, *Physical Review Letters*, 93 (1), 018101-1-4, 2004**). On the nanometer scale, synchrotron x-ray diffraction has revealed a distorted square lattice within the Bundle. (**Pelletier et al., *Physical Review Letters*, 91(14) 148102 1-4, 2003**).

Applications: In addition to the spontaneously produced shapes we have developed a process leading to spherical shapes through manipulation. Synthetic versions of these biologically inspired geometric structures may be produced by replacing F-actin with polymeric analogs for a broad range of technical applications, including encapsulation and controlled release, separations, as artificial skin, and templates for nano- and microscale optoelectronic materials.

Fig. 1. 3D reconstruction from laser scanning confocal microscopy data of a micron scale pleated multi-tube protein skin layer formed on the actin-gel surface at the α -actinin/actin molar ratio = 10.

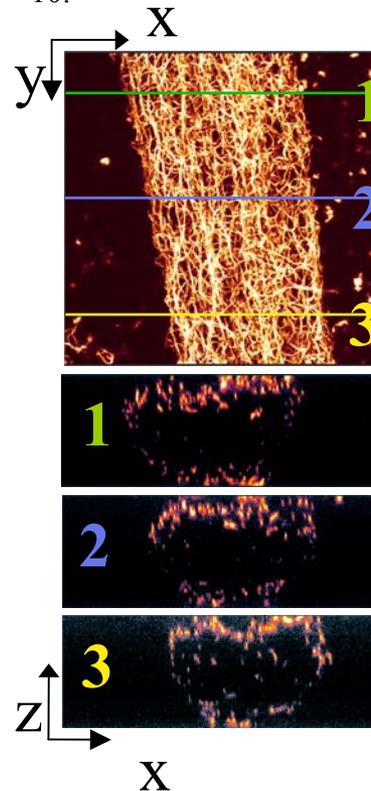
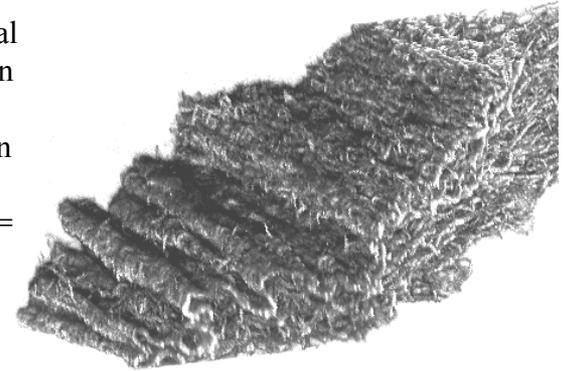


Fig. 2 3D Laser scanning confocal microscopy image of a hierarchically assembled PROTEIN TUBULE. The process involved a skin-layer which separated from the bulk material, forming a large isolated tube at the α -actinin/actin molar ratio = 5. The image shows a small part of a long tube with diameter $\approx 40 \mu\text{m}$. Top x-y view, and transverse (x-z) cross sections through the tube are shown.

With the completion of the Human Genome Project and the emerging proteomics era (dealing with the question of how and for what purpose are nature's building blocks put together) the scientific community is beginning the daunting task of studying the properties of networks of interacting proteins and other biological molecules. Cellular activity results from interactions between proteins or protein molecules with DNA or RNA, leading to the formation of assemblies of biological molecules for distinct functions. For example, in the fundamental process of growth where a cell splits in two, the human genetic material (DNA) has to first undergo a compaction process by more than a factor of 1000 and it does so with the help of "compacting" protein molecules acting in a precise manner (not yet fully understood) on DNA. Cell migration (e.g. which occurs during the process of wound healing or the unfortunate development of a tumor which has metastasized) involves the repeated formation and melting of bundles and networks of filamentous actin proteins within the cell.

Our group's research is inspired by the goal of elucidating nature's fundamental rules of assembly of the building blocks into distinct shapes and sizes (which span lengths from the nanometer to the micrometer scale) and understanding structure-function relations. One may view cells and their inner components as providing the most sophisticated examples of micro-machines. Thus, one expects that the application of the concepts learned from nature will enable us to design and develop miniaturized materials for applications in nanotechnology and biotechnology.

As a model system, our group at the University of California at Santa Barbara has recently focused on studies of the structure of the muscle protein filamentous (F) actin complexed with the cross-linking protein α -actinin. On the nanometer scale (one billionth of a meter), the use of modern methods of x-ray diffraction (provided by the National Facility at the Stanford Synchrotron Radiation Laboratory) has led to the discovery that F-actin bundles contain an unexpected ordered interior comprised of nanometer scale linear pores arranged on a square lattice. On the larger micron scale (1/100th of a human hair thickness), state-of-the-art laser scanning confocal microscopy led to the finding of sheets and tubules of F-actin bundles.

Replacing F-actin with polymeric analogs for improved mechanical strength may produce synthetic versions of these biologically inspired structures. One may envision a broad range of technical applications, including chemical encapsulation and controlled release (using bundles and tubules), separations (where the porous bundles may be used to purify nanometer scale molecules), as artificial skin (using sheets of F-actin bundles in wound healing), and as templates for producing nano- and microscale optoelectronic materials (where inorganic molecules are deposited on the bundles which act as molds).

The work has appeared in two recent issues of the premier physics journal *Physical Review Letters* [volume 91, number 14, 148102, (2003) and volume 93 (1), 018101-1-4 (2004)]. Part of the work was highlighted as the Cover Image of the October 3rd issue in 2003. It is currently featured as the December 2003 Science Highlight on the website of the National Facility Stanford Synchrotron Radiation Laboratory:
(www.ssrsl.slac.stanford.edu/research/highlights_archive/actinin.html or http://www.ssrsl.slac.stanford.edu/research/highlights_archive/actinin.pdf)

Supramolecular Assembly of Biological Molecules

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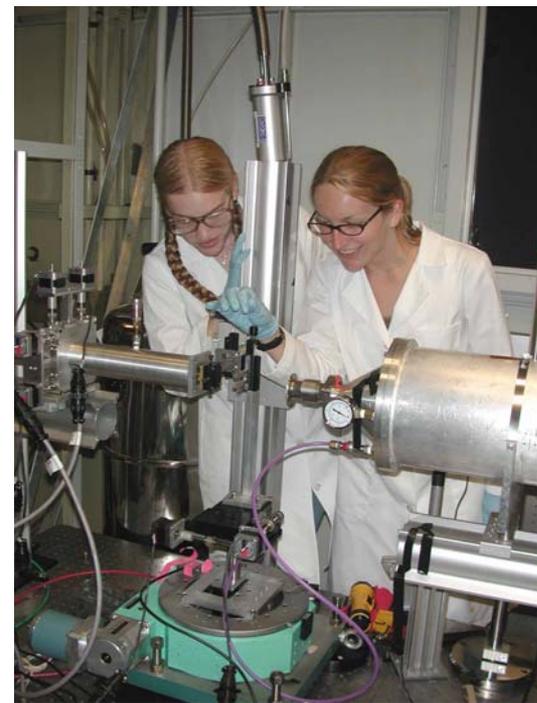
Education: Our educational goals are to train undergraduates, graduate students and postdoctoral researchers, in methods which enable them to discover nature's building blocks and rules for assembling the blocks in distinct shapes and sizes for particular functions. The concepts may lead to the development of advanced materials for applications in electronic, chemical, and biotechnology industries.

[Top: **Heather Evans** (graduate student), and **Uri Raviv** (postdoctoral researcher) imaging samples with a state-of-the-art laser scanning confocal microscope acquired through an NSF major instrumentation grant program.]



Outreach: Having just completed her second year at our local Hancock Community College, **Tracy MacDonough**, is currently a 2004 summer intern in the NSF funded Internships in Nanosystems Science and Engineering Technology (INSET) Program. Her mentor, graduate student Jayna Jones, is training her in modern methods of assembling neurofilaments in order to understand their role in the biophysics of nerve cells. Santa Barbara High School teacher **Claire Carey** (not shown), an AP Biology Teacher, is participating in a new Research Experience for Teachers (RET) outreach program at UCSB. She is learning modern methods in protein purification from graduate students in the Safinya group.

[Bottom: 2004 Summer intern **Tracy MacDonough** (left) and graduate student mentor **Jayna Jones** are preparing a sample for structure studies with the MRSEC funded high resolution small angle x-ray diffraction spectrometer.]



Two invited papers presented at the 2004 American Physical Society March Meeting

(Montreal) related to DMR-0203755

1. The American Physical Society Meeting, "Supramolecular Assembly of Biomolecules", 49, No. 1, 2004 (session B4 5, Montreal, March 22-26) (presented by C. Safinya)

ABSTRACT At present there is a surge in interest in biophysical research in elucidating collective interactions between cellular proteins and associated biomolecules leading to supramolecular structures, with the ultimate goal of relating structure to function. The nerve cell cytoskeleton, provides a rich example of a network of interacting neurofilaments, microtubules, both single and bundles, and filamentous actin, where the structure and structure-function correlations remain poorly understood. We present synchrotron x-ray diffraction, electron microscopy, and optical imaging data, in reconstituted systems of microtubules and neurofilaments from bovine brain and spinal cord, which reveal supramolecular assemblies of bundles, networks and kinetically driven structural transitions from bundles to raft-like assemblies of tubulin rings.

2. The American Physical Society Meeting, "Skin Layer at the Actin-gel Surface: Quenched Protein Membranes With Flat, Crumpled and Tubular Morphologies", 49, No. 1, 2004 (session T8 4, Montreal, March 22-26) (presented by postdoctoral researcher Linda S. Hirst)

ABSTRACT The actin cytoskeleton is a major component of eukaryotic cells involved in key functions including cell shape and mechanical stability. We report on the discovery of a novel hierarchically structure skin-layer formed at the surface of an isotropic gel of filamentous actin bundles at high molar ratios of alpha-actinin, an actin cross-linking protein, to globular actin. Laser scanning confocal microscopy has elucidated the full 3D structure on the micron scale. The protein skin-layer, composed of a directed network of bundles, exhibits flat, crumpled and remarkable, tube-like and pleated multi-tubular morphologies, resulting from stresses due to the underlying gel. These biologically based geometric structures, which may freely detach from the gel, demonstrate potential for the generation of scaffolds with defined shapes for applications in tissue engineering and templating.