Filamentous Protein Self-Assembly

Alignment and Structural Studies on Different Length-Scales

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Self-Assembly in Soft Matter – My Research Interests

**Biophysics**

How molecules assemble to form complex functional structures in the cell

- The cytoskeleton – Actin networks/microtubules
- Cell membrane structure and function

**Liquid crystal physics**

Smectic liquid crystals

- What drives the formation of the antiferroelectric/ferrielectric liquid crystal phases?
Techniques
Characterization on different length-scales….

X-ray scattering:

Synchrotron and in-house small angle scattering
(New small angle beam-line in the IMB x-ray lab).

Resonant x-ray scattering
(Synchrotron - Brookhaven National Lab)

Microscopy:

Fluorescence, FRAP

Confocal fluorescence

Electron Microscopy etc…. 
The Actin Cytoskeleton: Research Goals

- To Study and understand the self-assembly of different biological polymers (protein filaments) into structures on different length-scales
- To relate observed structures to form and function in the cell.
- To investigate biologically inspired semi-flexible polymer systems for novel materials.

Technique Development

- Development of micro-fluidic devices for electro/mechanical studies of biopolymers – integrated with microscopy
- New alignment techniques for delicate protein assemblies
Outline

1. Introduction to the cytoskeleton and actin
2. Scattering studies of actin bundles
3. Actin self-assembly on large length-scales
4. Micro-device designs
The Cytoskeleton

• The cell cytoplasm contains a filamentous network of cross-linked proteins –

The Cytoskeleton

• The main components are: actin, microtubules and intermediate filaments

Fluorescently labeled images of the different cellular cytoskeletal components

Kinematic cell research group – U. Frankfurt

The role of F-actin in the Cell

When a cell divides actin forms a contractile ring at the splitting point.

Cells send out long finger-like protrusions (filapodia and lamellapodia) to assist in cell crawling and engulf material.

Actin forms thick fibers called “stress fibers” giving the cell structure – these fibers interact with adhesion proteins to attach the cell to a surface.
Filamentous Actin Assembly

- G-actin (MW 42,000) is the basic unit of actin in the cell.
- G-actin packs together to form long helical filaments called F-actin (filamentous).
- The filaments are ~8nm in diameter and can be up to ~10μm long.

For polymerization to occur the solution must be above a critical actin conc. and Monovalent salt conc.
Large-Scale Actin Structures

Actin can be cross-linked into different structures by different molecules.

The interaction can be electrostatic (counter ion condensation)

or specific (covalently bound proteins)

Salts: MgCl$_2$, SrCl$_2$

Polycations: Spermine (+4), Spermidine (+3), polylysine

Proteins: Actin Cross-linking proteins, α-actinin, filamin, fimbrin etc....

Actin cross-linker α-actinin

Found in stress fibers, filapodia etc..

30 nm

3 nm

Binding head

Alpha helical repeat units

Co-localization of F-actin and α-actinin via double fluorescence
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Small Angle X-ray Scattering (SAXS)

Small Angle x-ray scattering is a powerful structural tool in biophysics

- **Internal molecular packing structures** (i.e. filament spacings in a bundle) - Bragg scattering

- **Molecular-assembly size and shape** (i.e. form factor scattering from rods, spheres etc.)

**On nm lengthscales…..**

**TEM** measures local details,

**SAXS** averages over the whole structure

Different techniques complement each other!!
Looking at Actin complexes with x-ray scattering

Solution Scattering:

Prepare actin samples in solution with other proteins, salts etc…

• Use 2mg/ml actin with varying $\alpha$-actinin/G-actin molar ratios.

• Samples are centrifuged to form a gel-like pellet (~50mg/ml) and inserted into a quartz x-ray capillary.

• Transmission x-ray experiments reveal the packing structure of the actin bundles.
The New SAXS beam-line in the Kasha Laboratory

Small angle transmission x-ray scattering (rotating anode source)

- Area Detector (multiwire)
- Sample in quartz capillary (v. thin glass) – or suitable mount
- Slits/filters
- Focusing optics
- Incident X-ray beam
  1.54Å

For solution scattering the sample is mounted in a transmission geometry.

Glass/ thick samples will attenuate the beam (we need to minimize this).

Scattered x-rays are detected to determine the sample structure.

x-ray pattern on area detector from an unoriented sample
SAXS results for different actin bundles (SSRL – 4-2)

Actin with simple counter-ions

Actin bundles formed at 80mM MgCl₂ only (no linker proteins)

**Int.**

![Graph showing SAXS results with peaks at Q = 0.84 nm⁻¹ and Q = 1.35 nm⁻¹.](image)

- **Q (nm⁻¹):** 0.84 nm⁻¹ and 1.35 nm⁻¹
- **Int.**
- **Hexagonally packed lattice**

a = 2π/0.84 nm⁻¹

b = 2π/1.35 nm⁻¹

a/b ≈ 1/√3

Hexagonally packed lattice
Actin with the cross-linker α-actinin

Actin bundles formed with α-actinin (Meyer and Aebi, J. Cell Biol. 110 2013 (1990))

Square packed lattice!

\[ a/b \approx 1/\sqrt{2} \]
Bundle Structure with cross-linker Conc. (SSRL – 4-2)

The $\alpha$-actinin/actin molar ratio is varied from 1:90 up to 1:2

Spacings are large..

$$\frac{2\pi}{0.19 \text{ nm}^{-1}} = 33 \text{ nm}$$

...about the size of the $\alpha$-actinin molecule

Broad peaks at 0.19 nm$^{-1}$ and 0.28 nm$^{-1}$ indicate a fairly disordered, distorted square packing structure
A solution of F-actin (3000Å long) has slope of ~ -1 (scattering from rods).

A solution of tightly packed bundles has a slope of ~ -2 (scattering from planes).

α-actinin bundles cross-over between -2 and -1 at ~65nm.
Structure of a branching actin/α-actinin bundle

• F-actin filaments packed into a quasi-square lattice arrangement by the cross-linker α-actinin.

• The bundle is loosely packed and fairly disordered.

Pelletier, Podisheva, Hirst, Bouxsein, Li and Safinya
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Actin Gels/Networks

If we look at Actin filaments in solution with different cross-linkers we can observe some interesting structures.

1. Single filaments will form a gel at high concentration.

2. At low concentration filaments appear to form a fluid 3D network with the cross-linker α-actinin.

3. At high α-actinin ratios and low actin concentration the 3D network has gel-like properties.
Actin gels – A little Background

**Single Filament gels**
Actin is a **semi-flexible biopolymer** and at high concentrations will form a gel.

The rheological and mechanical properties of an **entangled actin gel** have been studied in detail (Morse 1998), Isambert and Maggs (1996), Kroy and Frey (1997), Gittes (1998) etc….

**Cross-linked single filament gels**
Appropriate biologically as cross-linkers are always present.

Investigated for various cross-linking proteins, e.g. α-actinin, filamin, scruin, by Pollard, Wirtz, Mackintosh, Weitz, Janmey, Stossel etc..
cross-links may be transient - **Dissociation constant can be varied**

**Bundle gels?**
A new topic producing some fascinating structures which may have importance in cell mechanics and novel materials applications.
Studying Bundle gels (Filament Aggregation)

We can treat the formation of actin networks as a problem in the aggregation of filaments.

Filaments form bundles and simultaneously form a network

How does the structure and affinity of aggregating filaments affect the final structure?

What can we vary?

- **Sticker molecule**
  There are many different cross linking proteins – different dissociation constants/affinities.

- **Filament Length**
  Actin length can be varied with gelsolin – a severing and capping protein.
The Effects of Different Linker Molecules on the Network

Confocal Fluorescence Images

Salt
0.01mg/ml actin 80mM MgCl₂

α-actinin
γ = 1:25 0.01mg/ml actin

α-actinin
γ = 5:1 0.01mg/ml actin

Disordered tangle of filaments
Loosely connected 3D network of bundles
Strongly connected gel-like 3D network of bundles

Cross-linker type and concentration affects the three dimensional network structure
The effects of Filament length on aggregation

Filament length modified by gelsolin – an actin severing and capping protein

• Filament length controls mesh size

• The shortest filaments give a different structure – more like a fractal aggregate

Filament length has a clear effect on the final aggregated structure
Macroscopic Structure Differences – for high and low $\gamma$

Different ratios of cross-linker to F-actin were studied ($\gamma$)

F-actin = 0.01 mg/ml, 100mM KCl

No mixing, Minimize shear

‘Sol-Gel’ transition at $\gamma \sim 1:1$
Structure of the Gel Interior

Confocal Fluorescence Microscopy

We take a stack of 2D images at different focal planes to give 3D information on a sample.

Small section from the ‘Gel’

Isotropic network of bundles
A directed polymer network of actin bundles oriented over large distances forms.

Side branches interact, forming a ladder-like structure.
Interesting Structures form Spontaneously on the Gel Surface

Dense surface layer

Isotropic network

Detached ‘membrane’

Fluctuations quenched
Tubule Formation

• Dense layers wrinkle due to gel shrinkage

• Aligned surface regions lead to tubule formation
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Alignment of self-assembled structures

- Design system to **geometrically confine** filamentous self-assemblies (actin bundles).

- We must take into account the flexibility of the molecule/structure.

Other alignment methods (fibers, shear, magnetic fields)

**BUT** – need large amounts of protein, bundles break up

Bundle = ordered “domain”

Channel walls

Array of channels

We want to align these “domains”
Microchannel Design

• The micro channels are designed to be filled by capillary flow.

• Channels are coated with PEG to resist protein absorption.

• Wells are used to fill the channels with different protein solutions.

• Bundles form in the channels.
Microchannel Microfabrication

**Silicon**

- Standard BOSCH etch process
- High aspect ratio features

Channel structure etched into Silicon wafer

SiO₂ thermally grown on the surface

PEG-Silane absorbed to the surface

**Titanium**

- Chlorine based TIDE etch.
- High aspect ratio.
- Smooth walls robust material
- Thin and strong

Channel structure etched into Titanium foil

TiO₂ Sputtered on the surface

PLL-PEG absorbed to the surface
Filling the Microchannels with actin/α-actinin bundles

• G-Actin is polymerized \textit{in-situ} in the presence of the linker molecule - Like behavior in the cell.

• Bundles grow along the channel, aligned by confinement.

• The wider the channel, the less alignment is observed.
Transmission x-ray scattering from the Micro-device

- We use a **synchrotron x-ray source** – high intensity

- **Small angle x-ray scattering** (SAXS) beam-line

- **High energy x-rays** ~12KeV reduce attenuation from the Si or Ti and minimize sample damage
Microchannel Device X-ray Scattering Results

- The channel system gives highly aligned results with very little material compared to a capillary sample.

- We observe the expected peaks for a quasi-square structure.
Micro-channel X-ray Device Summary

- Actin bundles self-assemble in the micro-channel.
- Observed highly aligned x-ray diffraction patterns.
- Device can be used for very small amounts of protein. ~10nl

By aligning arrays of filamentous structures (i.e. bundles) it will be possible to study the arrangement of different proteins along the filaments.

Microtubules polymerized and aligned in channels.
Future Microdevice Applications:

Microfabricated devices are not just useful for protein alignment!

- **different materials**, Si, Ti, PDMS etc.
  - Surface properties can be tailored and patterned
    - Hydrophobic/hydrophilic,
    - Protein attachment/repulsion (specific or non-specific)

- **Electrodes on 3D structure**.
  - Control flow, induce mixing etc..
  - Incorporate mechanical movement

- **Confine protein self-assemblies**
  - Mechanical measurements
    - I.e. bending rigidity persistence
    - Length, dynamics of biopolymers/bundles
    - (Important for rheology)

The channel system can provide a useful method for studying the effects of molecular **concentration gradients** on proteins.
Other Research Interests
Lipid Self-Assembly

Lipids are amphiphilic and self organize into a variety of structures, i.e. micelles, bilayers, vesicles etc. in solution,

The cell membrane is a lipid bilayer composed of Phospholipids, Sphingolipids and Cholesterol.

Complicated lipid interactions with membrane proteins lead to membrane structure, lipid rafts, ion channels etc..

I am interested in how lipid molecules interact with each other and other molecules.
How does phase behavior lead to complex membrane function?
What is the role of cholesterol in membrane structure?

**Cholesterol** is important in the formation of lipid rafts (domains in lipid mixtures) (modulates phase behavior in lipid mixtures).

**Raft domains are implicated in vital cell functions** (i.e. budding, endocytosis, adhesion, signaling, and protein function)

Lipid bilayers in the retina - stacks of membranes.

Rods and cones respond to light differently but the mechanism is not well understood.

We are looking at the role of membrane composition and cholesterol in retinal cell function.
Phase Structures of Smectic Liquid Crystal Materials

Liquid crystals are ordered fluids, Smectics have a layered structure.

- Antiferroelectric phase (SmC*)
- Ferroelectric phase (SmC*)
- Intermediate (ferrielectric)
- Antiferroelectric phase (SmC*_A)

LCs are birefringent - Different phases have characteristic textures between crossed polarizers.

There is a family of tilted smectic phases – the structures are not well known,

Intermediate (I and II), Alpha phase etc…..
Studying the tilted smectic phases

I want to understand why these phases occur,
to describe their structure in detail ....

and to investigate electrical properties for potential device applications (LCDs, optical modulators etc....)

**x-ray scattering**
(resonant synchrotron scattering)

**Microscopy**, Electrical measurements,

Using free standing films and glass liquid crystal devices.

Free-standing antiferroelectric
Liquid crystal film
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Linker ratio is the key parameter in the ‘transition’ to a bundle gel

F-actin and $\alpha$-actinin combine as rods and stickers

We define two distinct regimes:

**Low $\alpha$-actinin:G-actin ratio ($<<1$)**

- Electrostatic repulsion between negatively charged fibers.
- Attractions between fibers due to long stickers ($\alpha$-actinin).

**High $\alpha$-actinin:G-actin ratio ($>1$)**

- Attractions between fibers due to the stickers dominate.
Rods and Stickers aggregate to form a gel

Diffusion Limited Aggregation (DLA) is dominant actin bundles saturated with stickers (α-actinin) Structures are irreversible.

Cluster-Cluster aggregation cluster growth due to repeated aggregation of two clusters of comparable size.

Difference - The basic unit (a bundle) can show fluctuations between link points and rearrangements
3D FFTs may be used to study scaling

Confocal Microscopy gives 3D data on a network.

We can carry out a 3D Fourier transform.


Good evidence for cluster-cluster aggregation when combined with microscopic observations

Linear fits range from the thickness of a bundle ($\sim 1 \mu m$) to about $35 \mu m$