Nonequilibrium dynamics of biofilaments and motor proteins

Wonmuk Hwang Assistant Professor Dept. of Biomedical Engineering, Materials Sci & Eng Program Texas A&M University College Station, TX





Scales & forces in biology

- L < 10⁻⁹m : Atoms & molecules
- 10^{-9} m < L < 10^{-5} m: DNA, proteins, macromolecules
- $10^{-6}m \le L$: Cells, organs, organisms

What (non-bonded) forces exist in the biomolecular world?

- 1. Electrostatic (Coulombic): Stong, but screened
- 2. van der Waals: Can be significant, long-ranged
- 3. Hydrophobic: Long-range, entropic, attractive
- 4. Hydration (solvation) force: Water geometry, repulsive?
- 5. Hydrogen bond: Directional, short-ranged; specificity
- 6. Thermal force: $k_B T = 4.14 \times 10^{-21} \text{ J} = 0.6 \text{ kcal/mol}$

BMEN431/631 Thermodynamics of Biomolecular Systems BMEN 432/632 Molecular & Cellular Biomechanics

- Mechanics at the nanoscale?
- Macromolecular self-assembly?
- Study Cellular 'hardware,' in comparison to cellular 'software'

Localized disruption of protein structure



Cytochrome *c* (PDB: 1YCC)

Aubin-Tam, PNAS 106:4095 (2009)

More global structural disruption when NP is attached to the N- or C-termini: N-C foldon as a `safety-pin' domain? (Maity, PNAS 102:4741 (2005))



How do they fold/form? How do they *function*? How to *control* them?

Amyloid fibrillogenesis: β -sheet self-assembly



Nature 435:773 (2005))

- Amyloid fibrillogenesis: Protein *misaggregation* into fibrils (Selkoe, Nature 426:900 (2003))
- Similar morphology (regardless of the protein sequence)

(Dobson, Nature. 418:729 (2002))

- \bullet Core region of the protein forms $\beta\text{-sheet}$
- Fibril axis perpendicular to β -strands: `Cross- β ' structure
- Functional amyloid / Promising biomaterial (3D cell culture scaffold)

Computational structure prediction



- Analyze solid-state NMR structures
- Predict molecular polymorphism (1 sequence \rightarrow multiple structures); which one is more toxic?
- Develop 'poisoning' peptide that interfere with assembly?

Park, PLoS Comp Biol 5: e1000492 (2009)

Mechanical properties of β -sheet bilayer filament



- Perform Thermal Motion Analysis (TMA), Normal Mode Analysis (NMA), and forced-bending simulations.
- Transverse: Y=5.5~9.7GPa (Persistence length: I_P=1.2~4.8µm)
 Longitudinal: nearly inextensible
- Elasticity governed by backbone hydrogen bond network (elastic core)

How do they form? Oligomer formation





Toroid (11 nm diam.):

Analysis of 1000 dimerization runs involving 5 different peptides reveals a *kinetic trapping* mechanism that affect *structural evolution* of oligomers (structural relaxation time > diffusional encounter time)



Amyloid fibrillogenesis: Multi-staged self-assembly (intermediates: more toxic?)

Elasticity of α -helical filaments

• Normal mode analysis (NMA) of α -helix

Leucine-zipper coiled-coil:



Bending stiffness (κ) drops for longer filaments due to buckling induced by non-specific attractive interactions



Critical buckling length vs persistence length

• Polymer flexibility described in general by persistence length: $I_p = \kappa/k_BT$ k_B: Boltzmann constant. T: temperature



• How to reconcile the effect of nonspecific interactions in the cytoskeleton?

 Regional variations in flexibility of the tropomyosin (coiled-coil): Implications in muscle contraction?

Collagen: long history of study, but still mysterious

- Collagen: Major component of the extracellular matrix- 30% of the protein mass
- Building block of: Skin, bone, vessel wall, ligament, tendon, cornea....
- Each collagen molecule: triple helix composed of 3 α chains (300 nm-long)
- Collagen *turnover* : Relevant to development, aging, wound healing, cancer, cardiovascular disease.....
- •Unstable at body temperature (Leikina, PNAS 99:1314 (2002))
- Mechanical load suppresses collagen degradation (Ruberti, Biochem Biophys Res Comm, 336:483 (2005)); Collagen Mechanoregulation



Tadpole Skin

Alberts, et al, The *Cell (Garland 2002.*)

What molecular properties are responsible for the biological behavior of collagen?

Simulation: Study *microunfolding* of a triple helix near cleavage site



Characterize unwinding: Monitor torsional angles between neighboring triads $\hat{\mathbf{e}}_2$ e₂ $C\alpha$ atoms ê, °e₁ Std. Dev. during ∆t θz **Torsional** angle **Torsional Map (Δt)** 0 2 Triad number

Ravikumar, JoMMS 2:999 (2007)

Region Specific Unwinding Controlled by Water Bridges



Structure of the hydration shell: VERY stable



Collagen assembly, hydration, and turnover

- Collagen imino-poor region (labile domain):
 - Spontaneously unwind at physiological temperatures
 - Local nucleation centers for collagen unfolding?
 - Matrix metalloproteinases (MMPs) utilize unwinding for cleavage
 - \rightarrow Basis for collagen mechanoregulation?
- Water: Role is region-specific!
 - Microscale: Dynamic bridges (lifetime < 3 ps) in the imino-poor domain control unwinding and allow mechanoregulation
 - Mesoscale: Hydration shell as an integral part of the collagen molecule Lubrication layer for collagen assembly
- Hydration map
 - Applicable to studying water-mdeiated interactions between other molecules

· Go higher up:

Prelim. sim, of collagen microfibril 1D period (67 nm)

Physical basis for collagen mechanoreaulation?

MMP-I



Molecular motor toolbox

- New drug target?: cancer, immunosuppression, neurodegeneration, etc.
- Efficient energy converters?
- Design principles of molecular machines?
- How do they generate force??



Vale, Cell 112:467 (2003)

Kinesin as a Mechanochemical Amplifier



Vale & Milligan, Science 288:88 (2000)

8 nm step/ATP (Svoboda, Nature 365:721 (1993))
Force generation mechanism identified by MD simulations: *Dynamic Folding* of a β-sheet domain between the cover strand and the neck linker, the cover-neck bundle. (*Hwang, Structure 16:62 (2008*))

 Static crystal structures are not enough to elucidate dynamic properties.



Calculated force map

• 0.2 nm resolution; 1626 points, 400 ps sampling run on each (total 2.9µs)

500

400

- Temperature independent: enthalpic, not entropic \rightarrow *Power stroke!*
- Less force without the cover strand.



Mutation in the cover strand impairs motility!



Study :

- Dimer motility on microtubule
- Control force generation

The kinesin (karnot) cycle



Hwang, Cell Biochem. Biophys. 54:11 (2009)



Fabricating Kinesin-based delivery/sorting system?

Combine

- Surface patterning (E-beam lithography)
- Self-assembled monolayer (SAM)
- Microtubule assembly
- Electric field

Unpassivated

electrode

Passivated

electrode

to develop controlled in vitro motility system

SAM

formation

exposure

resist

SAM

w/ Winfried Teizer (TAMU Physics)



Noel, ACS Nano 3:1938 (2009), JoVE vol.30 (2009)

silicon

silicon

Electrophoretic adsorption of MTs



Reversible adsorption on disc electrode



MT `antenna'



Alignment of single MT along a ring electrode



5µm



Versatility in patterning (e-beam line width ~18nm)

Noel, ACS Nano 3:1938 (2009), JoVE vol.30 (2009)

TAMU logo

Kaushik: α- helices, Ncd Kinesin The Hwang Blackbury Peach Thin Mint Cheese cake

Wee Wen: Collagen Assembly Wonmuk: Kinesin John: Microtubule Adsorption (PostDoc, Upenn)

Krishna: Collagen

A

Jiyong:Amyloid (Postdoc, UCLA)

(sim)

Jinseon: Electrophoretic Patterning



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Further questions: hwm@tamu.edu