Ultra Coarse-Graining of Multi-protein Complexes: actin as a case-study

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CMTS Workshop
Challenges in coarse-graining

• ‘Simple’ coarse-graining
  • Isotropic substance
  • Well sampled in simulation
  • Well-described by radially dependent pairwise interactions
  • Spherically symmetrical sites

• Many systems we care about
  • Multiple constituents
  • Orientation dependent interactions
  • Difficult to simulate long enough to fully sample interactions
  • Non-spherical structural subunits
  • Slow exchange dynamics
Actin as a model system

- Introduction to actin system
- Coarse-graining as an analytical method
- Coarse-graining heterogeneity in the actin system
- Identifying and characterizing states
Actin dynamics … why do we care?

Just a track for myosin?

Actin is in more than just muscle

Actin is more than a static track
Actin dynamics … why do we care?

- Present in all eukaryotic cells
- Dynamic networks
- Motive functions
  - Cell motility
  - Cell attachment
  - Cytokinesis
  - Endocytosis
  - Intracellular signaling
- In the nucleus
  - Transcription regulation
  - Chromatin remodeling

http://cellix.imba.oeaw.ac.at/7-actin-can-push/
Actin filaments can drive cell motion by dynamic treadmilling

- Actin filaments are polar
  - Filament ends have different $K_D$ values
  - Nucleotide state changes $K_D$ value
- ATP-bound subunits add to one end
- ATP is hydrolyzed in the filament; $P_i$ released
- ADP-bound subunits dissociate from the other end
Structural Biology of actin

Monomer = 4 subdomains with the nucleotide at the center

- Many high resolution structures for the monomer
  - All with mutations, co-factors, or co-proteins to prevent polymerization
- Nucleotide affects the dynamics of the subunit
  - Proteolysis
  - Fluorescence
- Subunits polymerize
  - Nucleotide affects rates
  - Polymerization accelerates hydrolysis = conformation changes
Structural Biology of actin

Polymerization = subunit flattening.

- Acceleration of hydrolysis suggests conformational change
- No high resolution filament structure
- 2 models
  - refined from cyroEM (Namba) and fiber diffraction (Oda) data
  - Flattening of the subunit

2. Fujii et al., *Nature*, 2010
Actin dynamics is a multiscale problem

Connections between scales – time and size.

Cross-linked network – Munro lab

Single filament

Nucleotide-binding cleft

Monomer
Limitations of MD in the actin system – and CG approaches to overcome them

- Number of atoms and frames involved
  - Thermal motion of individual atoms is not really interesting; collective motions are.
  - More than just an average description – heterogeneity matters

- Timescale – computational power
  - Can’t simulate long enough to see G- to F- transition
  - Can’t observe nucleotide dependent filament dynamics

- Fixed bonding topology
  - Nucleotide cannot hydrolyze within a simulation

Analysis on a coarse-grained level
Identifying and characterizing states
Simulate each state and compare CG variables
Adapt the methodology
  - Coarse graining
  - Stochastic states
Coarse graining for analysis

All-atom data

CG representation

Analysis
How to choose CG sites?

• Essential dynamics coarse graining?
  • Need a consensus model for analysis
  • Non-contiguous domains

• Intuitively – one site per subdomain
  • Non-spherical shape
  • Lose information on internal orientation
  • Noise from less structured regions
Comparing F-actin models using MD data

Analysis using coarse graining: beyond a 4-site model.

Adding more sites offers several advantages

- Reduced noise
- Orientation of subdomains
- Specific contact information

Tailor to the questions
Comparing ED-CG with Intuitive

<table>
<thead>
<tr>
<th>Hand-picked model (CG sites not contiguous)</th>
<th>Memory-optimal contiguous mapping</th>
<th>ED-CG contiguous mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 (N-terminus)</td>
<td>1-10</td>
<td>1-6</td>
</tr>
<tr>
<td>5-33 (SD1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34-39 (SD2)</td>
<td>11-74</td>
<td>7-39</td>
</tr>
<tr>
<td>40-51 (D-loop)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52-69 (SD2)</td>
<td></td>
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</tr>
<tr>
<td>70-147 (part of SD1)</td>
<td>75-102</td>
<td>82-135</td>
</tr>
<tr>
<td>148-179 (part of SD3)</td>
<td>139-185</td>
<td>136-188</td>
</tr>
<tr>
<td>180-219 (part of SD4)</td>
<td>186-218</td>
<td>189-224</td>
</tr>
<tr>
<td>220-235 (TM bind site)</td>
<td>219-231</td>
<td>225-251</td>
</tr>
<tr>
<td>236-251 (Flap)</td>
<td>232-279</td>
<td>252-295</td>
</tr>
<tr>
<td>252-262 (SD4)</td>
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<tr>
<td>263-272 (H-loop)</td>
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<tr>
<td>273-333 (part of SD3)</td>
<td>280-322</td>
<td>296-344</td>
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<tr>
<td>334-349 (Part of SD1)</td>
<td>323-358</td>
<td></td>
</tr>
<tr>
<td>350-375 (C-terminus)</td>
<td>359-365</td>
<td>345-375</td>
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<tr>
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<td>366-375</td>
<td></td>
</tr>
</tbody>
</table>

How does the nucleotide affect the conformation of the subunit?

- All atom simulations of the filament
  - ATP
  - ADP-Pi
  - ADP
- Compare/contrast CG BAT variables
  - Subunit twist - key motion associated with polymerization
How does the nucleotide affect the conformation of the subunit?

- Subunit flattening is important for polymerization
- Nucleotide state affects the stability of this twist
How are these changes in the CG variables related to filament properties?

- Challenges in creating a CG model
  - Complex radial distribution
    - Represents 13 subunits
  - Overlapping minima from essentially harmonic potentials
    - Slow exchange between states
  - Heterogenous subunits

![Graph showing probability distribution for 2-1-3-4 dihedral](graph.png)
Experimental evidence of heterogeneity

• Variable Twist Angles
  Orlova & Egelman, BPJ 78:2180 (2000)

• Six Structural Modes (D-Nase I Binding loop)
  Galkin, Orlova, Schroder, & Egelman

• Heterogeneity in the monomer
  Sagar, A., N. Peddada, et al., BBRC (2013)
Incorporating heterogeneity into a CG model

- Two potential sources of heterogeneity
  - Identity of the sites
  - Interactions between the sites

- How does heterogeneity impact filament properties
  - Persistence length
  - Twist angle
  - Torsional stiffness
Heterogeneity in an elastic network model

Different dynamic domains

\[ \chi^2 = \frac{1}{3N} \sum_{i=1}^{N} \frac{1}{n_t} \sum_{t=1}^{n_t} \left( \sum_{i \in I} \sum_{j \notin I} \left| \Delta r_i^{ED}(t) - \Delta r_j^{ED}(t) \right|^2 \right) \]

Zhang & Voth JCTC 6:2990 (2010)

Essential Dynamics Coarse-Graining

ATP bound

# CG sites=6

Residue index

Ave.

Fan, Saunders, Voth BJ 103:1334 (2012)
Heterogeneity in an elastic network model

Different interaction parameters

Hetero-ENM method of parameterization

\[ U_{ij}(x_{ij}) = \frac{1}{2} k_{ij}(x_{ij} - \bar{x}_{ij})^2 \]

\[ \Delta x_{ij,MD}^2 = (x_{ij} - \bar{x}_{ij})^2 \]

\[ \frac{1}{k_{ij}^{n+1}} = \frac{1}{k_{ij}^n} - 4\alpha (\Delta x_{ii,NMA}^2 - \Delta x_{ij,MD}^2) \]

Fan, Saunders, Voth BJ 103:1334 (2012)
Importance of Heterogeneous Interactions

<table>
<thead>
<tr>
<th>Sets</th>
<th>CG sites</th>
<th>CG interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Set 1</td>
<td>Unique Beads</td>
<td>Unique Interactions</td>
</tr>
<tr>
<td>2) Set 2</td>
<td>Uniform</td>
<td>Unique</td>
</tr>
<tr>
<td>3) Set 3</td>
<td>Uniform</td>
<td>Uniform</td>
</tr>
</tbody>
</table>

Fan, Saunders, Voth BJ 103:1334 (2012)
Limitations of heteroENM approach

- Arbitrary choice of the number of different parameters
  - 13 different sets – one for each subunit in the simulation
- Assumes that each subunit samples only one state
- Difficult to quantitatively compare the parameters for different filaments

Challenge: Identifying and characterizing states
Gaussian Mixture Model

Model each radial distribution function as a mixture of normally distributed states

\[ p_{\text{tot}}(r) = \sum_{i=1}^{N} \pi_i * p_i(r) \]

\[ p_i(r) = \frac{1}{\sigma_i \sqrt{2\pi}} e^{-\frac{(x-x_i)^2}{2\sigma_i^2}} \]

\[ \sum_{i=1}^{N} \pi_i = 1 \]

- Find optimal # of states, N
- Find \( \pi, \sigma, \) and \( \bar{x} \) for each state
- Allows quantitative comparison of complicated distributions
Application to the twist angle

- ATP – flattened major state (-8.6°)
- ADP-P_i – intermediate state (-11.7°) is favored
- ADP – 2 equally populated states: flat (-8.8°); twisted (-15.05°)
Current work: Incorporating states into CG simulation

- General theoretical approach

Challenges:
- Inflation of # of potential states
- Are these states independent?
- How important is heterogeneity in each of these variables?
- Which variables most significantly affect filament properties?
- Moving beyond purely harmonic models

Dama et al JCTC 9:2466 (2013)
Summary

• Selection of CG sites
  • Start with core domains/ regions
  • Isolate highly mobile parts
  • Shape / orientation

• Incorporating heterogeneity
  • Important, esp. with limited sampling
  • Advantages of EDCG and heteroENM

• Identifying states
  • Mixture of Gaussians approach

• Adding states into CG model … still in progress
Acknowledgements

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