Development of a combined molecular dynamics (MD) and fluorescence-detected resonance energy transfer (FRET) method for structural biology.

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Project Overview: Connecting Simulation and Experiment

- Fluorescence experiments are typically analyzed with a series of assumptions
  - These assumptions are not generally valid, though they are often accepted as so.
  - In particular, we would like to apply fluorescence to membrane proteins and other large complexes where the ‘standard assumptions’ are especially suspect
  - Therefore, we would like to test these assumptions more thoroughly
    - this is what is presented in this poster

- Few fluorescence experiments have been carefully compared with calculation
  - Those few have performed the comparison at intermediate level

- Because the computer simulations can provide information about validity of assumptions mentioned above, we are working to combine computer simulation with fluorescence experiment
  - Results will provide more detail than either alone
  - Comparison can be made at the level of experimental observable requiring the minimum number of approximations.
  - Goal: new technique in structural biology
Background: Previous use of FRET for structural biology

- FRET is fluorescence-detected resonance energy transfer
  - Usually excite a donor (D) molecule with a laser
  - Observe fluorescence emission from an acceptor (A) molecule
  - If D and A are favorably oriented, energy can pass from D to A through a process called Resonance Energy Transfer (RET)
  - Amount of energy transferred from D to A provides information about the relative orientations. If D and A are each attached to a protein, we have learned structural information about the protein.

- Developed by Stryer and others in late 1960s and subsequently used to study:
  - Protein and nucleic acid structure
  - Oligomeric complexes
  - Binding
  - Dynamics
Background:
Assumptions typically used in FRET

• RET is described by Förster Theory
  - Assumes the electronic transition (from ground state to excited state or back) can be described by a dipole -- the Ideal Dipole Approximation
  - Also several other assumptions that are probably fine in our systems.
  - Resulting Förster expression can be greatly simplified (for the purposes of this poster) to
    \[ k_{RET} \propto \frac{\kappa^2}{R^6} \]
    - \( \kappa \) describes the relative orientation of the two dyes
    - \( R \) is the distance between D and A

• Most FRET experimenters also typically assume:
  - The D and A completely sample all orientations faster than energy transfers between them. This means \( \kappa^2 = \frac{2}{3} \)
  - That \( \kappa \) and \( R \) (distance between D and A) are not correlated

These assumptions are not obviously valid in many contexts
Background:
Our system

- Hen egg-white lysozyme (HEWL) is a very generic protein.
  - Small and soluble
  - Well studied (discovered by Alexander Fleming)
  - The first enzyme whose crystal structure was solved
- HEWL happens to bind a fluorescent dye -- eosin
  - Well-defined binding pocket has been studied by several groups
- Genetic modification allows another dye to be covalently attached
  - We have chosen DACM
  - DACM is a good energy donor to eosin for RET

DACM -- donor
Eosin -- acceptor
Lysozyme
Hen egg white lysozyme labeled with fluorescent probes

DACM is green and eosin is magenta
Simulation Details

• We use the AMBER molecular dynamics package
  - Cornell et al. (1994) force field
  - Particle mesh Ewald (PME) for electrostatics
  - Explicit water (buffer region of 20 Å around protein) gives a total of 17706 water molecules.
    - 55,149 atoms total.
    - Explicit counter ions
    - Final simulations are > 10 ns

• Have tested computational results against most MD parameters including box size and presence of counterions using a number of metrics:
  - Convergence of system energy and volume
  - Continuous diffusion of counterions
  - Convergence of simulated DACM and eosin anisotropy
Results:
\( \kappa^2 \) distribution convergence

This plot shows the observed distribution in the \( \kappa^2 \) factor for four different portions of the MD simulation. Overall, the portions are fairly similar, but there are some clear difference among them.
Results:

\( \kappa^2 \) distribution -- comparison to theory

Neither the entire distribution of \( \kappa^2 \) nor any of the sub-distributions look very similar to the theoretical distribution. The theory assumes that both dyes completely sample all possible orientations much faster than RET. Note that our MD has sampled a time interval longer than the DACM fluorescence lifetime. Thus, it is unlikely that the dyes sample all orientations completely in the real system.
**Results:**

**Relative RET rate**

Assuming the MD simulation is reasonable, this plot gives a sense of the error one would make by assuming that $\kappa^2$ is equal to 2/3. The pink line uses $R$ from the simulation and 2/3 for $\kappa^2$. The blue line takes both $R$ and $\kappa^2$ from the simulation. Each point is simply the result of $\frac{\kappa^2}{R^6}$ at each time point.
Results:
Orientation and Distance are Correlated

Plotting the orientation and distance together shows that they are correlated. Again, if the MD simulation is really reasonable, then this assumption (that $\kappa^2$ and $R$ are NOT correlated) is called into question for this particular system. Failure of this assumption is rarely discussed in the literature.
Summary of Lysozyme Results

\[
\langle \kappa^2 \rangle = 0.388 \\
\left\langle \frac{2/3}{R^6} \right\rangle = 0.271 \\
\left\langle \kappa^2 \right\rangle \left\langle \frac{1}{R^6} \right\rangle = 0.157 \\
\left\langle \frac{\kappa^2}{R^6} \right\rangle = 0.119
\]

Our MD results suggest

• The average of \( \kappa^2 \) is not 2/3. This is a weak result because of the poor convergence.

• That \( R \) and \( \kappa^2 \) are highly correlated in this system. This is true regardless of our convergence. This is shown by the difference in the relative RET rate for the two methods of averaging above.

• Normally experiments would be impossible to analyze in this situation. By using our simulations to augment experiment, it will be possible to gain useful data from experiments even when \( \kappa^2 \) is not 2/3 and when \( R \) and \( \kappa^2 \) are correlated.
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