Enlighten Tutorial: MD analysis

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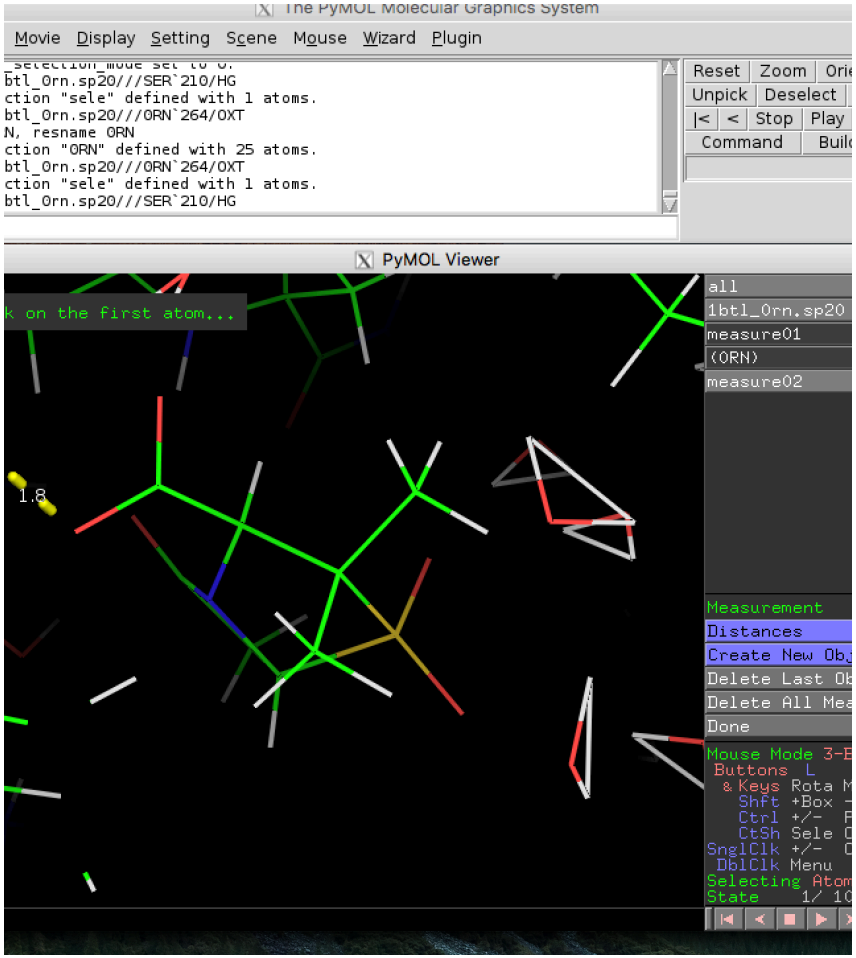
06/06/16

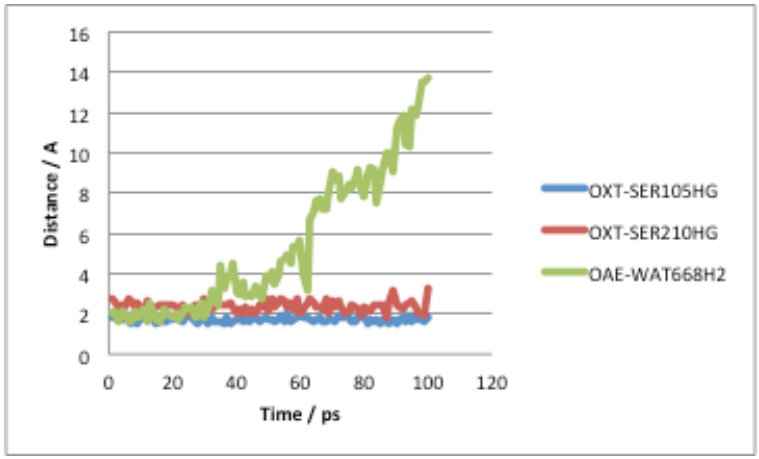
What can I learn from simulations?

- Simulations carried out through *Enlighten* plugin are 'rough and ready'
  - Large part of protein is held fixed
  - Short time scales
- Designed to:
  - Aid identification of interesting interactions
  - Solvation of active site / ligand
  - Effect of point mutations
  - Suggest new experiments / test hypotheses

Identification of interactions

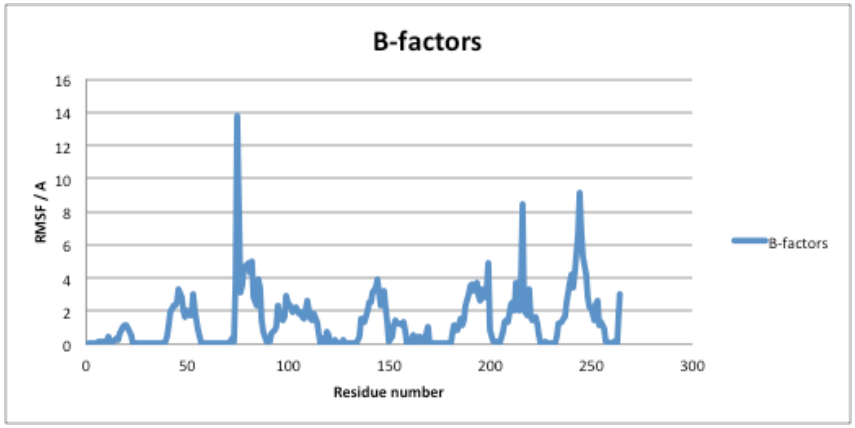


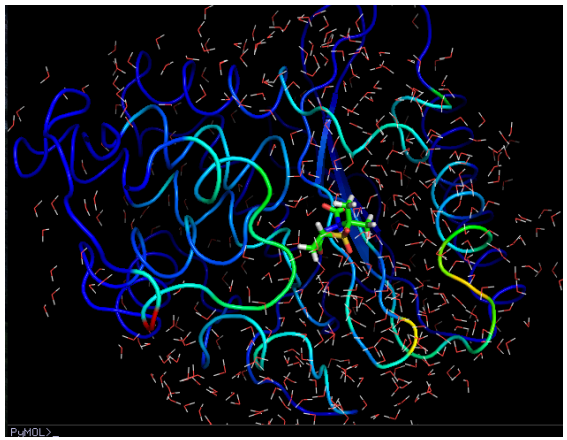
- Watch the simulation several times
- Notice any (un)changing interactions
- Measure distances over the trajectory (cpptraj)



Root Mean Squared Fluctuations

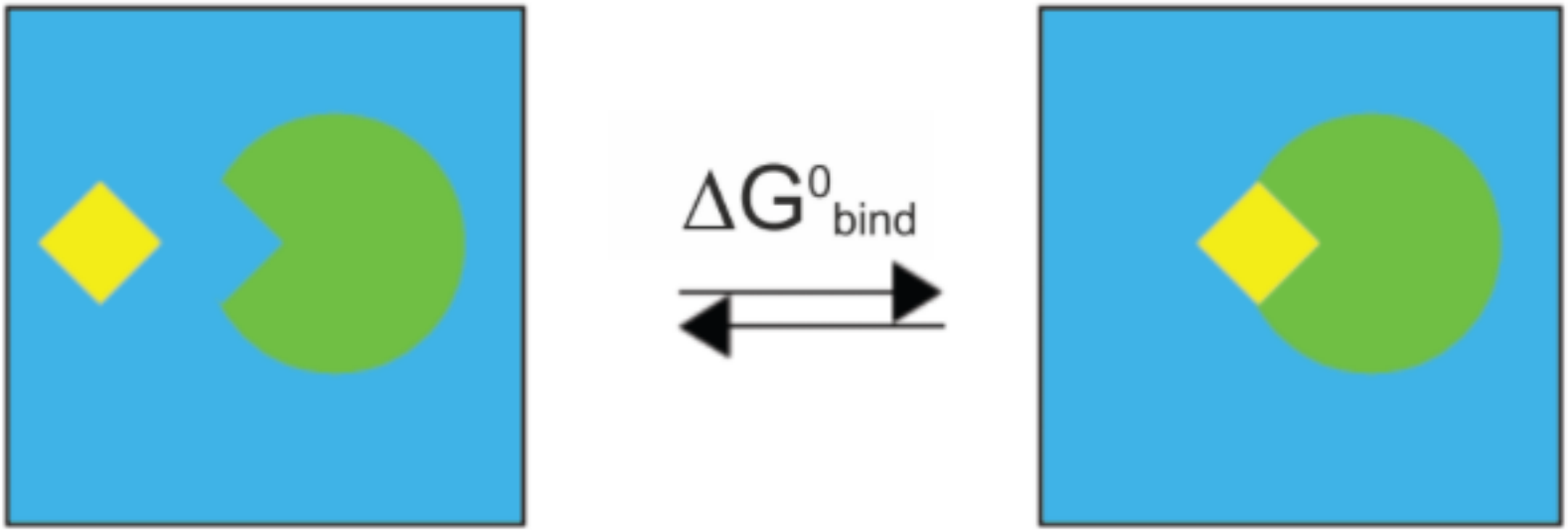
$$\mathrm{RMSF}_i = \left[\frac{1}{T} \sum_{t_j=1}^{T} |\mathbf{r}_i(t_j) - \mathbf{r}_i^{\mathrm{ref}}|^2 \right]^{1/2}$$





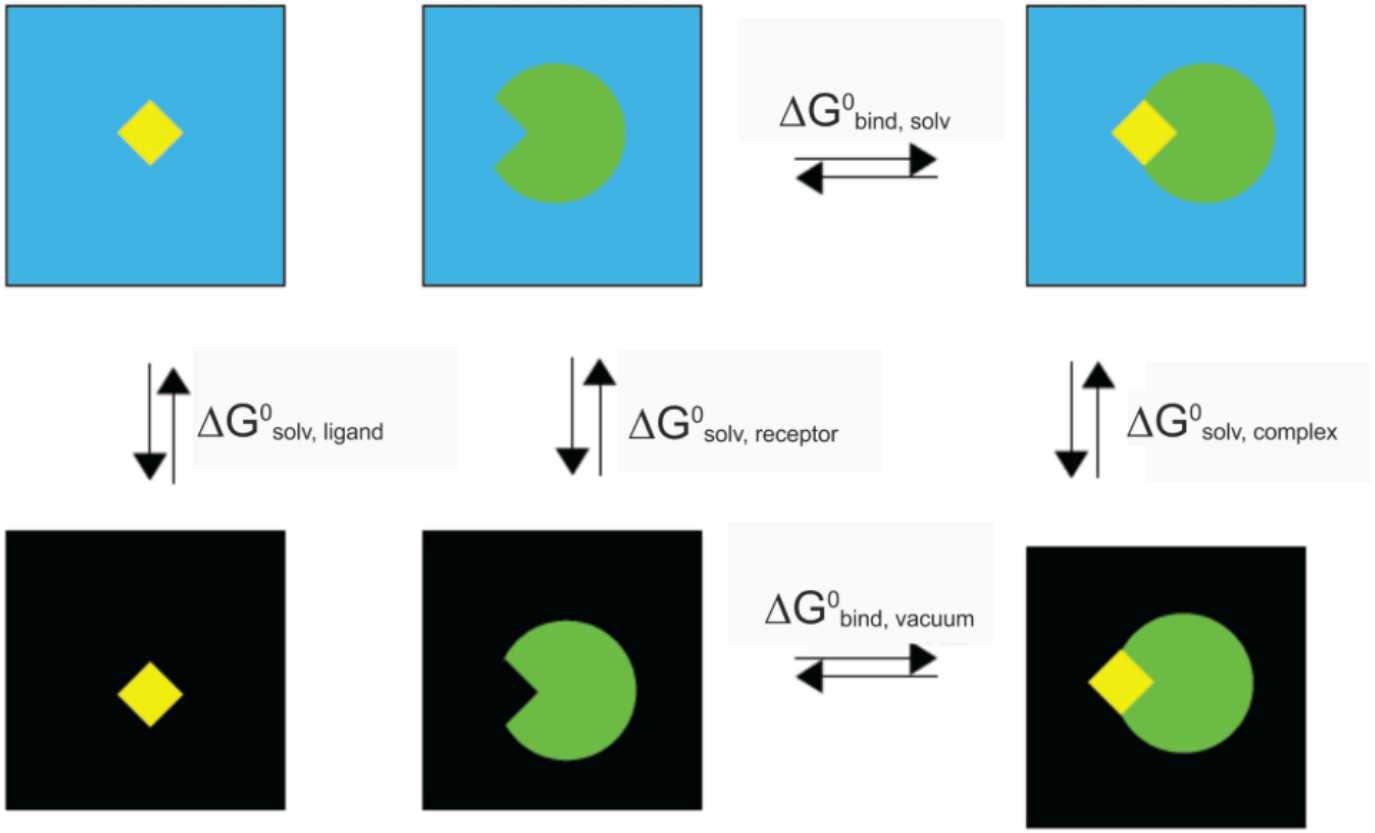
- RMSF is the time averaged deviation of the position of a particle from a reference position
- Reference position is usually the average structure from the simulation
- Measure of protein flexibility →higher values in loop regions compared to helices
- Related to B-factors used in crystallography

Binding energy calculations



Energies calculated using this cycle would be dominated by solvent-solvent interactions → very slow convergence

Binding energy calculations



$$\Delta G^0_{bind,\,solv} = \Delta G^0_{bind,\,vacuum} + \Delta G^0_{solv,\,complex} - (\Delta G^0_{solv,\,ligand} + \Delta G^0_{solv,\,receptor})$$

Binding energy calculations

$$\Delta G^0_{solv} = G^0_{electrostatic,\ \epsilon=80} - G^0_{electrostatic,\ \epsilon=1} + \Delta G^0_{hydrophobic}$$

MM-PBSA/GBSA approach the solvation free energies calculated using either the linearized Poisson Botlzmann or **Generalised Born** methods for 3 states (ligand, receptor & complex) → electrostatic contribution

$$\Delta G^0_{vacuum} = \Delta E^0_{molecular\ mechanics} - T \cdot \Delta S^0_{normal\ mode\ analysis}$$

Entropy term often neglected as the calculations are expensive an can introduce large errors

Binding energy calculations

- MM-GBSA method → fast but approximate
- Important to average over several snapshots
- **Rough guide** to test predictions about mutation before carrying out experiments

WT

Differences (Complex – Receptor – Ligand):

Energy Component	Average	Std. Dev.	Std. Err. of Mean
VDWAALS	-23.9779	2.5531	1.2765
EEL	-31.2224	11.2711	5.6356
EGB	27.6661	8.6280	4.3140
ESURF	-3.3883	0.0397	0.0198
DELTA G gas	-55.2003	11.7523	5.8761
DELTA G solv	24.2778	8.6314	4.3157
DELTA TOTAL	-30.9225	3.4170	1.7085

R244T

Differences (Complex – Receptor – Ligand):

Energy Component	Average	Std. Dev.	Std. Err. of Mean
VDWAALS	-21.7641	2.3832	1.1916
EEL	54.5072	4.7593	2.3797
EGB	-56.3780	1.9694	0.9847
ESURF	-3.1535	0.0861	0.0431
DELTA G gas	32.7431	4.1844	2.0922
DELTA G solv	-59.5315	1.9692	0.9846
DELTA TOTAL	-26.7884	2.2812	1.1406

Binding energy for mutant is smaller (less negative) in agreement with experimental data

Useful Links

- **MM-PBSA/GBSA tutorial:** http://ambermd.org/tutorials/advanced/tutorial3/
- **AMBER have several good tutorials:**

  http://ambermd.org/tutorials/
- AMBER manual for CPPTRAJ instructions:

  http://ambermd.org/doc12/Amber15.pdf
- Practical PyMol for beginners: http://www.pymolwiki.org/index.php/Practical_Pymol_for_Beginners