Reverse Micellar Self Assembly and Ligand-induced Protein Motions: Insights from Molecular Dynamics Simulations

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Outline of the talk

• PART – I
  Self-assembly Using All-atom MD: Exploring the Application of Water-in-CO$_2$
  Microemulsions

• PART - II
  Computational Drug Design: Accommodating Receptor Flexibility using MD

• PART – III
  QM/MM and Targeted MD to Answer Relevant Biological Questions
Water-in-Carbon Dioxide Microemulsions
Why Carbon Dioxide?

• CO₂ in the supercritical state is an useful **green solvent**
  - Nontoxic
  - Potentially recyclable
  - Tunable
  - Chemically inert
  - Inexpensive
  - Nonflammable

**Drawback:**
• Proteins, ions, and most catalysts are insoluble in supercritical CO₂ (scCO₂)

**Solution:**
• Water droplets can be formed in scCO₂ by means of suitable surfactants
Surfactants that stabilize W/C microemulsions

- **PFPE surfactant**
  - C • O • F

- **Dichain-F8 surfactant**
  - C • O • P • H • F

- Spectroscopic data show that these surfactants stabilize an aqueous phase in scCO$_2$ by forming reverse micelles (RM)
Structure of a Reverse Micelle

- Surfactant headgroups
- Surfactant tails
- Water
- Solvent
- Counterions
We started MD simulation from a random configuration.

MD Simulation at 25°C and 200 bar
- Water
- CO2
- PFPE
- Counterions

A two-step mechanism:
1. Fast hydration of polar components
2. Slow aggregation of hydrated molecules

=> Calculations involved extensive Force Field Generation
Developing the parameters for the dihedral 
\[ \delta = \text{CF}_2\text{-CH}_2\text{-CH}_2\text{-O} \]

- Start with a model compound

- Computed the ab-initio energy profile for this compound as a function of \( \delta \)

- For each optimized geometry an energy calculation neglecting the contribution from \( \delta \) is performed using SANDER module of AMBER

- Difference in energy between \textit{ab initio} and MM force field was fitted to

\[
V_{\text{dih}} = \frac{1}{2} \sum_n V_n (1 + \cos(n\phi - \gamma))
\]
Torsional parameters

<table>
<thead>
<tr>
<th>Dihedral</th>
<th>$V_n/2$ (kcal/mol)</th>
<th>$\gamma^{(\circ)}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS – CH$_2$ – CH$_2$ – CF$_2$</td>
<td>2.732</td>
<td>0.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>-1.099</td>
<td>30.0</td>
<td>1</td>
</tr>
</tbody>
</table>

**MD Results match well with Experiments**

$$W_0 = 8.4$$

<table>
<thead>
<tr>
<th>Property</th>
<th>MD</th>
<th>Expt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_c$ (Å)</td>
<td>19.2</td>
<td>20</td>
</tr>
<tr>
<td>$A_h$ (Å$^2$)</td>
<td>70.2</td>
<td>76</td>
</tr>
<tr>
<td>$\text{Solv}<em>{\text{Tail}}$ ($N</em>{CO2}/PFPE$)</td>
<td>15</td>
<td>--</td>
</tr>
<tr>
<td>Aq. Core Exposure (%)</td>
<td>12</td>
<td>--</td>
</tr>
</tbody>
</table>
MD Analysis show the existence of water bulk phase

Self-assembled Reverse Micelles in CO₂ entrap protein in native state

$\text{MD at 25°C, 20 MPa}$

$t = 0 \text{ ps}$

$t = 10 \text{ ns}$

$t = 50 \text{ ns}$

$t = 50 \text{ ns}$

W/C microemulsion for Protein Extraction?

S.V. Chaitanya and S. Senapati, 2007 (communicated)
Computational Drug Design: Accommodating Receptor Flexibility
Development of a drug

- Major steps
  - Lead discovery and optimization
  - Preclinical and Clinical testing

- Computational methods can enhance the discovery (lead) process

  => Ligand docking and screening algorithms strive to streamline the process
Protein-Ligand Docking

- Determines how a particular drug lead will bind to the protein target
- Available docking programs (e.g. AutoDock, Dock, FLEXX) include only ligand flexibility
- How to introduce the receptor flexibility?

Relaxed Complex Scheme

- a long MD simulation of the unliganded receptor was conducted to extensively sample the protein’s conformations
- rapid docking of the flexible ligand to the large ensemble of the enzyme’s MD snapshots

=> Example: AChE – TZ2PA6 Docking
Acetylcholinesterase

Physiological Role

• An enzyme present in various tissues, including muscle and red cells

• Terminates cholinergic neurotransmission through hydrolysis of the neurotransmitter acetylcholine

• Among the fastest enzymes known (10,000 molecules/sec)
Acetylcholinesterase binding sites

- AChE has 540 residues

Two distinct binding sites at opposite ends of a 20Å deep gorge: PAS and active center

- **AChE Inhibitors:**
  - Alzheimer drugs e.g. Tacrine
Acetylcholinesterase TZ2PA6 Inhibitors

- Syn is a high affinity AChE inhibitor. Anti is a respectable but weaker inhibitor.
Syn-TZ2PA6 is a better inhibitor than anti

Why is syn a better AChE inhibitor?
Docking Studies of AChE-anti-TZ2PA6

- Flexible anti-TZ2PA6 could not be docked to the X-ray structure of apo-AChE
- When receptor flexibility is incorporated by RCS .....
Docking Studies of AChE-anti-TZ2PA6

- Flexible anti-TZ2PA6 could not be docked to the X-ray structure of apo-AChE
- When receptor flexibility is incorporated by RCS

- Sites of favorable interactions are in good agreement with the crystal structure

- This scheme would be very useful in computer-aided drug design
Anti-TZ2PA6 binds to protein in lock-and-key fashion: Docking + 10ns MD Simulation
Induced Fit in mAChE upon binding syn-TZ2PA6: Docking + 10ns MD Simulation

Docked Complex of syn-TZ2PA6-mAChE: Initial Configuration for MD Simulation
Induced Fit in mAChE
MD Simulation and Docking Results of syn-TZ2PA6+mAChE

Docked Complex of syn-TZ2PA6-mAChE: Initial Configuration for MD Simulation

10 ns Simulation Output along with Superimposed X-ray Structure (yellow)
Simulation Results at a Glance

Solvent-exposed Trp286 in the Complex

- Ligand binding displaces protein residues from the hydrophobic core toward the solvent – A unique AChE conformation

Shape Modification at the Gorge Entrance

• In this unique conformation AChE has an opening at the gorge that is about 2-times larger than usually seen
PART - III

QM/MM and TMD to Answer In-Situ Click Chemistry Question
Polyvalent interactions can collectively be much stronger than corresponding monovalent interactions.

**Click Chemistry:**
- modular
- no side- or by-products
- irreversible

Huisgen 1,3 dipolar cycloaddition:

\[
R_1-N=\begin{array}{c}N\end{array} \oplus + R_2 \rightarrow R_1-N=\begin{array}{c}N\end{array} \oplus + R_1-N=\begin{array}{c}N\end{array} \oplus \text{syn} + R_1-N=\begin{array}{c}N\end{array} \oplus \text{anti}
\]

- Rideout, D. *Science* 233, 561 (1986);
- Sharpless K.B., Taylor, P. *et al.* *Angew Chem Int Ed* 41, 1053 (2002);
- Kolb *et al.* *JACS* 126, 12809 (2004).
Syn and *anti* triazoles derived from TZ2+PA6

- Thermal reaction in the absence of enzyme proceeds very slowly and provides an 1:1 mixture of syn and anti.
- But in the presence of AChE, syn is the sole product and reaction occurs much faster. => WHY?
In Situ Click Chemistry
The Target-Protein as a Reaction Vessel

Why?

**ab initio QM/MM Calculations**

- We aim to look at the mechanism of reaction between azide and acetylene.

- In the process of chemical reactions, only a small number of atoms participate in the bond forming or breaking processes.

- Other atoms serve as a steric and electrostatic environment to influence the reaction active part.

\[
E_{\text{total}} = E_{QM} + E_{MM} + E_{QM/MM}
\]

\[
E_{QM/MM} = E_{ele}^{QM/MM} + E_{vdw}^{QM/MM} + E_{MM-bonded}^{QM/MM}
\]

**QM Method**
\[
E_{QM} + E_{ele}^{QM/MM} = <\Psi | H_{eff} | \Psi >
\]

**MM Method**
\[
E_{MM} + E_{vdw}^{QM/MM} + E_{MM-bonded}^{QM/MM}
\]
QM/MM Setup of *syn*-Complex
QM/MM Setup of anti-Complex
Pseudobond QM/MM Approach

- A one-free-valence atom with an effective core potential is constructed to replace the boundary atom of the MM part and to form a pseudobond with the boundary atom of the QM part.

- The pseudobond is described by QM method.

- It mimics the original bond with same bond length, and have similar effects on the rest of the active part.

- We follow the backward reaction, namely the bond breaking process in TZ2PA6 to obtain TZ2 and PA6.

QM/MM Results

From syn-TZ2PA6-mAChE

From anti-TZ2PA6-mAChE

Phenylphenan-thridinium
Targeted Molecular Dynamics

• Click chemistry and QM/MM results imply that the azide and acetylene have to have a parallel orientation in AChE gorge

• MD simulations of antiparallel TZ2 and PA6 in mAChE could not flip the orientation

• Very large and complex conformational transitions is unlikely to happen in the time scale of ordinary MD simulation

• TMD is a method to induce a conformational change to a known target structure at ordinary temperature by applying a purely geometrical constraint

\[ E = 0.5 \times k_{TMD} \times (\text{RMSD} - \text{TGTRMSD})^2 \]

• WE APPLY TMD ONLY ON PHENYLPHENANTHRIDINIUM MOIETY OF PA6 AND/OR W286
TMD of antiparallel TZ2 and PA6 in mAChE

$k_{TMD} = 2.0 \text{ kcal/(mol Å}^2\text{)}$
TMD of parallel TZ2 and PA6 in mACHe

$k_{TMD} = 4.0 \text{ kcal/(mol } \AA^2\text{)}$
Possible reasons for preferred parallel orientation

Senapati S. et al.  
Conclusions

- The rigid body assumption of the receptor fails to find the correct ligand-receptor binding modes.

- The relaxed complex scheme successfully find correct binding modes by introducing receptor flexibility.

- The enzyme, AChE adopts a low abundance conformation when binds to syn-isomer of TZ2PA6 inhibitor.

- Relaxed complex scheme would be useful in predicting new inhibitors.

- A combined QM/MM and TMD approach could explain why the syn isomer of TZ2PA6 inhibitor is the sole product in AChE environment.

- The architecture of the enzyme’s active site may be responsible for this stereoselective reaction.
Acknowledgements

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