Computational modeling of molecular processes in proteins

Alexander Nemukhin

Chemistry Department, Lomonosov Moscow State University, Russia
Institute of Biochemical Physics, Russian Academy of Sciences, Russia
Overview

Methods

Applications

• Transient kinetics in hydrolysis of guanosine triphosphate (GTP)

• Aspartoacylase: Role of protein dimer

• Molecular mechanisms of chromophore formation and decomposition in the green fluorescent protein (GFP)

Concluding remarks

Acknowledgements
Significance of every project can be easily justified

- GTP hydrolysis by human Ras: relevance to cancer
- Aspartoacylase: the key enzyme in brains
- Green fluorescent protein: *in vivo* imaging
Methods

• QM(DFT)/MM for modeling bond breaking and making

• QM(CASSCF and above)/MM for modeling photoexcitation

• QM/MM MD

• Classical MD including Markov State Model and Dynamical Network Analysis

Programs: GAMESS(US)/TINKER
            QChem, NWChem, CP2K, ORCA
            NAMD

Initial source of atomic coordinates: Protein Data Bank (PDB) entries
Application: GTP hydrolysis by Ras-GAP

\[
\text{H}_2\text{O} + \text{GTP} \rightarrow \text{Pi} + \text{GDP}
\]
Application: GTP hydrolysis by Ras-GAP:
GTP hydrolysis catalyzed by Ras-GAP
GTP hydrolysis in Ras-GAP: Initial PDB structure
GTP hydrolysis in Ras-GAP: ES complex restored
GTP hydrolysis in Ras-GAP: Reaction products
The active site: Molecular model of ES
On the use of QM/MM MD
The active site: Molecular model of ES
Molecular mechanism
GTP hydrolysis by Ras-GAP: Gln amide-imide tautomerization

Amide-imide tautomerization

Imide-amide tautomerization
Towards direct simulation of kinetics curves

\[ E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P \]
GTP hydrolysis by Ras-GAP; rates for elementary steps

\[ k_{-1} = 1.1 \times 10^{12} \quad k_{-4} = 7.5 \times 10^6 \quad k_{-5} = 2.1 \]

\[ k_{-2} = 1.3 \times 10^{11} \quad k_{-3} = 8.8 \times 10^5 \]

Slow accumulation and decay of I2

M. Khrenova, B. Grigorenko, A. Kolomeisky, A. Nemukhin
GTP hydrolysis by Ras-GAP: Direct comparison exp vs calc

Calculated \( k_{\text{eff}} = 15 \text{ s}^{-1} \) (exp 19.5 s\(^{-1}\))

Exp: Biochemistry 2003, 42, 3956
Why GTP hydrolysis catalyzed by Ras-GAP is important?

Oncogenic mutations
Human Aspartoacylase (hAsp): Processes in human brain

\[
N\text{-acetyl aspartate}\quad \xrightarrow{\text{hAsp}}\quad \text{Aspartate} + \text{Acetate}
\]
Full Catalytic Cycle of hAsp: Chemistry at the active site

Enzyme active site (QM-subsystem in simulations)

Substrate: NAA
E. Kots, M. Khrenova, S. Lushchekina, S. Varfolomeev, B. Grigorenko, A. Nemukhin,
Substrate deposition and product release

MD calculations with the replica-exchange umbrella sampling technique
hAsp appears as a dimer composed of monomers ASPA, ASPB.
Substrate binding at allosteric sites controls entrance to the active site

Markov State Model for transitions between conformations with open and closed gates to the active site

\[ \text{System} \quad \begin{array}{c|c|c|c} \text{System} & k_o, \text{s}^{-1} & k_c, \text{s}^{-1} & K \\ \hline \text{E} & 6.7 \cdot 10^6 & 6.1 \cdot 10^6 & 1.1 \\ \text{ES}_{\text{act}} & 1.0 \cdot 10^7 & 5.0 \cdot 10^5 & 20 \\ \text{ES}_{\text{inh}} & 6.3 \cdot 10^7 & 1.1 \cdot 10^9 & 0.06 \end{array} \]
Substrate binding at allosteric sites controls entrance to the active site: Dynamic network analysis
Proposed kinetic mechanism: Quantitative description of kinetic data

\[
E \underset{K_1}{\rightleftharpoons} ES \underset{K_1}{\rightleftharpoons} ESS \underset{K_2}{\rightleftharpoons} SESS \underset{K_3}{\rightleftharpoons} SESSS + S + S + S + S
\]

**k_{cat}**

**[S], M**

**V, a.u.**
Three faces of N-acetylaspartate: activator, substrate, and inhibitor of human aspartoacylase

Chromophore formation and decomposition in the Green Fluorescent Protein (GFP)
Chromophore maturation in GFP
Chromophore formation in GFP: Textbooks (R. Tsien et al.)

**Steps of Chromophore Formation**

1. **Step 1:** Formation of the chromophore through cyclization.
2. **Step 2:** Dehydration of the intermediate to form a more stable structure.
3. **Step 3:** Oxidation of the chromophore to complete its formation.

**Key Residues:**
- **Ser65**
- **Tyr66**
- **Gly67**

The cycle illustrates the sequential chemical reactions that convert GFP into its mature chromophore.
Chromophore formation in GFP: Model based on prior work

Chromophore formation in GFP: Starting REAG structure contains tripeptide Ser65-Tyr66-Gly67

Computational protocol: QM(PBE0/6-31G*)/MM(AMBER)
Chromophore formation in GFP: Chain of elementary steps

[Graphical representation of molecular structures and reactions]
Chromophore formation in GFP: Computed QM/MM energy profile for cyclization-dehydration
Chromophore formation in GFP: Modeling oxidation
Chromophore formation in GFP: Computed QM/MM energy profile for oxidation
Chromophore formation in GFP: Comparison to the high-resolution crystal structure (yellow sticks)
Chromophore formation in GFP: Comparison to the results of kinetics studies

<table>
<thead>
<tr>
<th>Reaction Step</th>
<th>Rate constant, s(^{-1})</th>
<th>Experimental (TST) energy barrier, kcal/mol</th>
<th>Calculated energy barrier, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclization-dehydration</td>
<td>0.0038</td>
<td>20.7</td>
<td>17</td>
</tr>
<tr>
<td>Oxidation</td>
<td>0.00015</td>
<td>22.7</td>
<td>21</td>
</tr>
</tbody>
</table>
Chromophore formation in GFP: Summary of scenarios

Modeling photobleaching in GFP

Light-induced reaction with oxygen leads to irreversible photobleaching in FPs
Modeling photobleaching in GFP

Light-induced reaction with oxygen leads to decomposition of the chromophore and irreversible photobleaching in FPs

Chromophore-containing pocket in GFP plus O₂ molecule
First elementary steps: Chemistry

1. 

2. 

3. 

4.
First elementary steps: Molecular models
Excitation to the charge-transfer (CT) state is essential

Excitations in Chro⁻  Excitations in (Chro…O₂⁻)  Occupation numbers  Orbitals

5.61 eV  5.61 eV

4.49 eV  4.49 eV
4.40 eV  4.40 eV  CT  4.40 eV
4.25 eV  4.25 eV

2.59 eV  2.59 eV

0  0
S₀  S₀

Chro⁻(S₁)  Chro⁻(S₀)
2211  2210

[Chro•(D)…O₂⁻(D)]^{S,T}  [Chro⁻(S₀)…O₂(T)]^{T}
2210  21/12
2220  11

Excitations in Chro⁻  Excitations in (Chro…O₂⁻)  Occupation numbers  Orbitals
Evolution of the system after excitation

\[ [\text{Chro}^-(S_1)\ldots\text{O}_2(T)]^T \]

\[ [\text{Chro}\cdot(D)\ldots\text{O}_2\cdot(D)]^{S,T} \]

\[ [\text{Chro}^-(S_0)\ldots\text{O}_2(T)]^T \]

\[ [\text{Chro}^-(S_7)\ldots\text{O}_2(T)]^T \]

1. Geometry of the ground state (S0) of the GFP protein with the oxygen molecule inside the active site.
2. Geometry of the charge transfer triplet state (CT), the point of the triplet-singlet intersystem crossing.
3, 4. Geometries of the chromopore with attached oxygen molecule complex in singlet state.

**Evolution Pathways:**
- Singlet pathway: 40 kcal/mol (peak at 29 kcal/mol)
- Triplet pathway: 41 kcal/mol (peak at 29 kcal/mol)
Evolution of the system after excitation
Computed energy profile
Basic qualitative result – decomposition of the chromophore
MD simulations: exit of benzoquinone from the GFP barrel
Words of Welcome

On behalf of the Local Scientific Committee, it is my pleasure to welcome you to the 11th Triennial Congress of the World Association of Theoretical and Computational Chemists during the week of August 27 to September 1, 2017 in Munich, Germany. With about 1500 registered participants from all over the world and 12 plenary, 215 invited, 136 contributed speakers, as well as over 920 posters, WATOC2017 is the largest WATOC so far.

This shows both the increasing importance of theoretical and computational chemistry across the disciplines, and the central (and easy to reach) location of Munich in the heart of Europe. We have set up an exciting program covering a wide variety of cutting edge research topics ranging from method developments to applications pushing the limits of modern theoretical and computational chemistry, biochemistry, nanotechnology, and materials sciences.

WATOC2017 is held in the city center of Munich with both plenary and parallel sessions in the Gasteig cultural center under one roof. Besides great science, we hope that you will also find some time to explore the city of Munich and its surroundings which offer fascinating possibilities for both cultural and outdoor activities.
Project of 2018  Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains
Designing brighter near-infrared fluorescent proteins: insights from structural and biochemical studies†

Mikhail Baloban,†,‡a Daria M. Shcherbakova,†,‡a Sergei Pletnev,‡b Vladimir Z. Pletnev,‡c J. Clark Lagarias,‡d and Vladislav V. Verkhusha†de

miRFP703 and miRFP709

miRFP670, chromophore I

miRFP670, chromophore II
Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains

Reaction coordinate

Energy, kcal/mol

0
-10
-20
-30

Reag

Cys20
Cys253
W2
W1

I1-A
I2-A
I3-A
I4-A
Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains

Path A

Path B

GAF Cys253

Reag

BV + PAS-GAF

Reag-B

Reag-A

k_{1B} \rightleftharpoons k_{1B}^\text{Reag}

k_1^A \rightleftharpoons k_{1A}^A

k_2^A \rightleftharpoons k_{2A}^A

k_3^A \rightleftharpoons k_{3A}^A

k_4^A \rightleftharpoons k_{4A}^A

Concluding remarks

We are approaching an ambitious goal to simulate kinetic curves for reactions in proteins
Progress in models and methods: GTP-Ras-GAP

Model of 2005

- 1,700 atoms in total
- 43 QM atoms, RHF/6-31G

Ten Years Later: Model of 2015

- 8,000 atoms in total
- 86 QM atoms, DFT(PBE0)/cc-pVDZ
The way forward

Chromophore excitation
(Quantum chemistry)

Modeling photoreceptor proteins
(QM/MM + QM/MM MD)

Chemical reactions in enzyme active sites
(Quantum chemistry)

Chemical reactions in enzymes, allostERIC regulation
(QM/MM + MM + MD + QM/MM MD)

Photoactivated multidomain proteins
(QM/MM + MM + MD + QM/MM MD)

Progress (years, computer power, ... )
Acknowledgements

Dr. Bella Grigorenko
Dr. Maria Khrenova
Dr. Sofya Lushchekina
Dr. Ekaterina Kots

Dr. Igor Polyakov
Prof. Sergey Varfolomeev

Prof. Anna I. Krylov (USC)
Prof. Arieh Warshel (USC)
Acknowledgements

Dr. Bella Grigorenko
Dr. Maria Khrenova
Dr. Sofya Lushchekina
Dr. Ekaterina Kots

Dr. Igor Polyakov
Prof. Sergey Varfolomeev

Prof. Anna I. Krylov (USC)
Prof. Arieh Warshel (USC)

Bella Grigorenko & Arieh Warshel
Moscow 2016