Multiscale Approach to Protein Engineering in Bioluminescence Probe Design

Yi Mao
Department of Mathematics
Michigan State University

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Multiscale in Biology

Logarithmic scales

-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
1

nm
µm
mm
m

Rat
Human hair dimension
Red blood cell
Chromosome
Bacteria
Virus
Atom diameter
Mathematics $\xrightarrow{\text{Data analysis}}$ Biology

Challenge to deal with heterogeneity
Mathematics Is Biology’s Next Microscope, Only Better;

Biology Is Mathematics’ Next Physics, Only Better
Protein Structure

- Primary structure
- Secondary structure
- Tertiary structure
Perturbation experiments in biology: **Mutation**

Responses to mutation:

- no responses or localized responses
- cascading effects
Protein Engineering

Goal: find the sequence → desired properties

Idea: alter the sequence → alter the properties

Methods: • rational design
          • directed evolution
Multiscale Modeling for Proteins
Multiscale Modeling for Proteins

“zoom-in” does NOT work!
Multiscale Modeling for Proteins

- A whole-system study at all scales
- Multiscale: different resolutions

atomistic model (QM/MM)  network model (simplified potential)
Bioluminescence

- conversion of chemical energy into light
Bioluminescence
Reporter Gene Imaging

Insert the reporter gene to the gene of interest to create a gene fusion.
Migration of neural progenitor stem cells (labeled with the luciferin) across the midline towards an implanted brain cancer in a mouse.

Challenge:

How to achieve Red Emission of Bioluminescence?
Luciferase•DLSA complex

Luciferase (protein): catalyst, 539 residues, 8451 atoms
DLSA: light emitter, 58 atoms
Excitation of DLSA

Energy

Distance between electrons and nucleus

First excited electronic state
Ground electronic state

Absorbance

Wavelength
Luciferase: Spectral Shift

Mutation at site 286: Ser → Asn

Goal

- At the atomic level, explain the physical origin of the spectral shift caused by mutation.

- At the sequence level, design the system (luciferase) with desired optical property.
The matter of scale

- Scale of electrons
  - Quantum mechanics (DLSA)
  - 1 Å

- Scale of atoms
  - Molecular Dynamics (protein)
  - 1 nm

- Scale of aggregates
  - Reduced Modeling (protein/DLSA)
  - 10 nm
Hybrid Quantum Mechanical/Molecular Mechanical (QM/MM) Approach

Hamiltonian: $\hat{H}_{\text{total}} = \hat{H}_{\text{QM}} + \hat{H}_{\text{MM}} + \hat{H}_{\text{QM/MM}}$

$\hat{H}_{\text{QM}} = -\frac{1}{2} \sum_i \nabla_i^2 + \sum_{ij} \frac{1}{r_{ij}} - \sum_{i\alpha} \frac{Z_{\alpha}}{r_{i\alpha}} + \sum_{\alpha\beta} \frac{Z_{\alpha} Z_{\beta}}{r_{\alpha\beta}}$

$\hat{H}_{\text{QM/MM}} = -\sum_{iM} \frac{q_M}{r_{iM}} + \sum_{\alpha M} \frac{Z_{\alpha} q_M}{R_{\alpha M}} + \sum_{\alpha M} \left( \frac{A_{\alpha M}}{R_{\alpha M}^{12}} - \frac{B_{\alpha M}}{R_{\alpha M}^6} \right)$

$\hat{H}_{\text{MM}} = \sum_{\text{bonds}} k_r (r - r_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2$

$\sum_{\text{dihedrals}} k_\phi [1 + \cos(n\phi + \phi_0)] +$

$\sum_{\text{atom}M} \sum_{M \neq N} \left\{ 4\varepsilon_{MN} \left[ \left( \frac{\sigma_{MN}}{r_{MN}} \right)^{12} - \left( \frac{\sigma_{MN}}{r_{MN}} \right)^6 \right] + \frac{q_M q_N}{4\pi\varepsilon_0 r_{MN}} \right\}$

$\alpha,\beta$: QM nuclei  $i,j$: QM electron  $M,N$: MM atoms

QM: $\hat{H}_\psi = E\psi$

MM: $F = -\partial\hat{H}/\partial q$
QM/MM Procedure

performed by ONIOM program (Gaussian 03)

- Start with the crystal structures

- The ground state is optimized at the ONIOM-EE (B3LYP/6-31G*:AMBER) level

- The $S_0 \rightarrow S_1$ excitation energy is computed by TDDFT/B3LYP/6-31G*:AMBER based on the optimized ground state structure

Assumption: time for structural relaxation $>>$ time for electron excitation

\[10^{-6} \sim 10^{-3} \text{ s}\]
Global Structural Change Caused by Mutation S286N
<table>
<thead>
<tr>
<th></th>
<th>C2'-C2</th>
<th>C2'-N'</th>
<th>C2-S'</th>
<th>C2-N</th>
<th>C2-S</th>
<th>S'-C2'-C2-S</th>
<th>N'-C2'-C2-N</th>
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<tbody>
<tr>
<td><strong>WT</strong></td>
<td>1.4486Å</td>
<td>1.3073Å</td>
<td>1.7614Å</td>
<td>1.3074Å</td>
<td>1.7649Å</td>
<td>159.47°</td>
<td>163.60°</td>
</tr>
<tr>
<td><strong>S286N</strong></td>
<td>1.4483Å</td>
<td>1.3101Å</td>
<td>1.7604Å</td>
<td>1.3098Å</td>
<td>1.7661Å</td>
<td>172.39°</td>
<td>174.00°</td>
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</table>
Luciferase Multicolor Bioluminescence Mechanism

- The effect of the protein environment is on the relative angle of two rings of DLSA through van der Waals contacts.

- A more planar two rings of DLSA leads a spectral red-shift.
The Calculated Emission Spectra

<table>
<thead>
<tr>
<th></th>
<th>Calculated</th>
<th>Experimental Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type luciferase</td>
<td>589 nm</td>
<td>560 nm</td>
</tr>
<tr>
<td>Mutant (S286N) luciferase</td>
<td>611 nm</td>
<td>605 nm</td>
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