Multiscale Approach to Protein Engineering in Bioluminescence Probe Design

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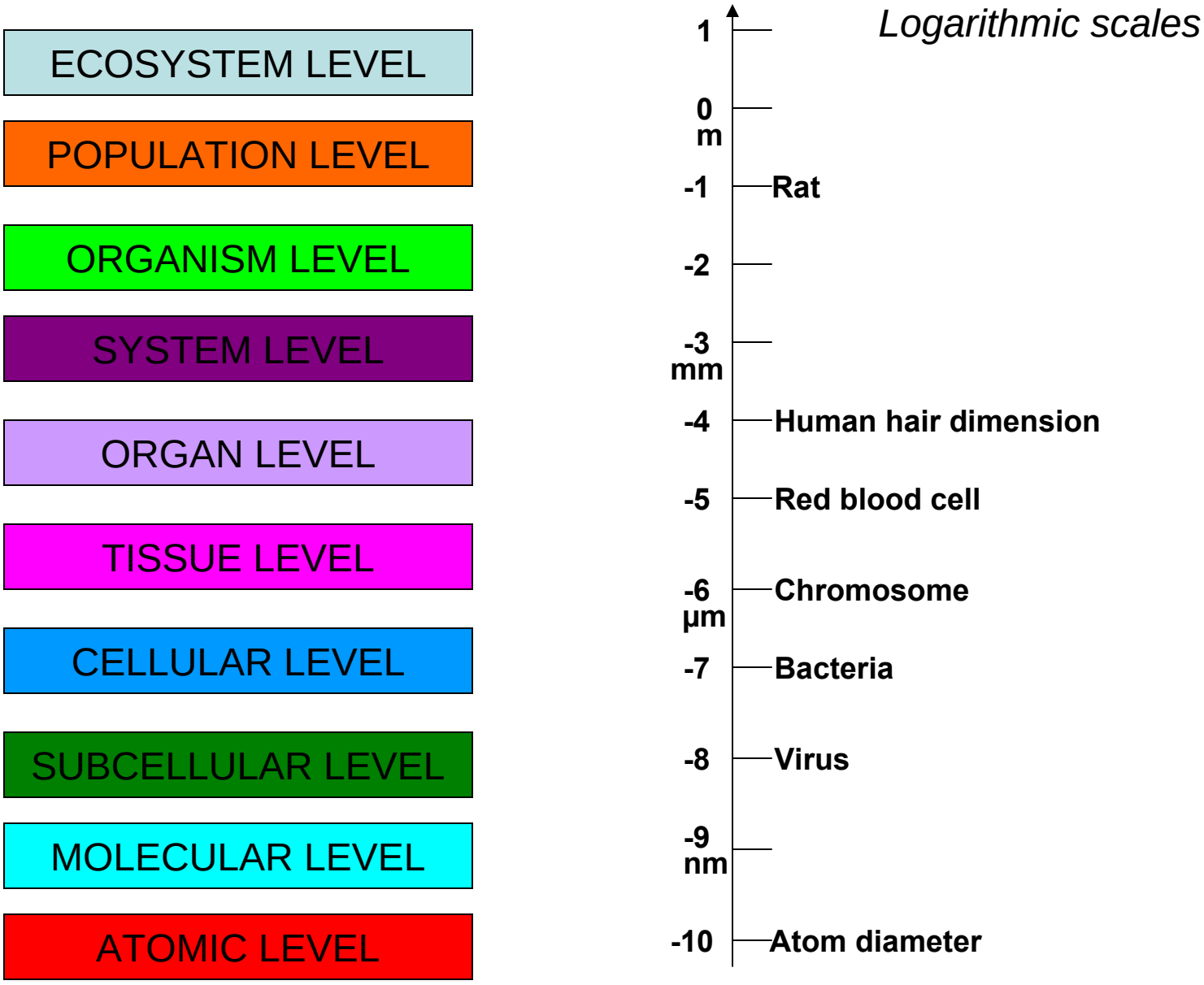
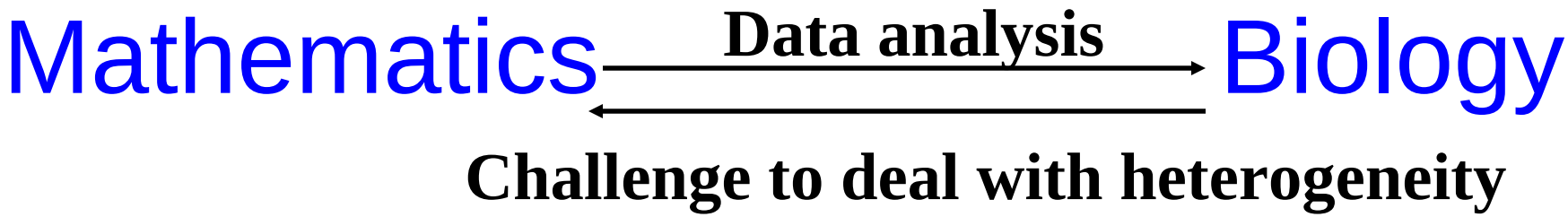
Multiscale in Biology



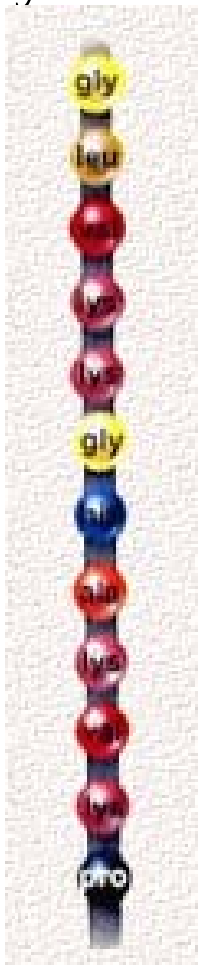

Mathematics
Data analysis
Biology
Challenge to deal with heterogeneity

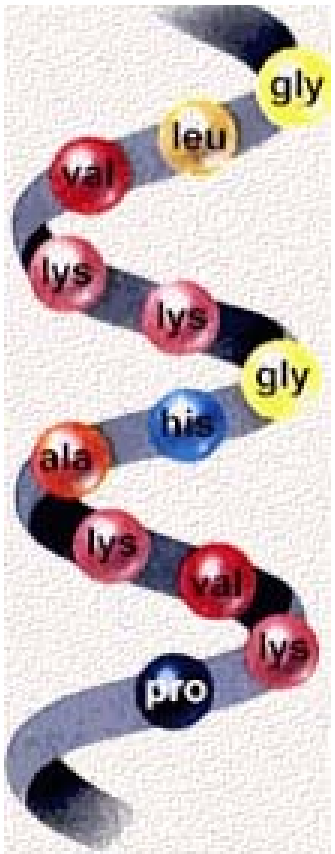
Mathematics Is Biology's Next Microscope, Only Better;

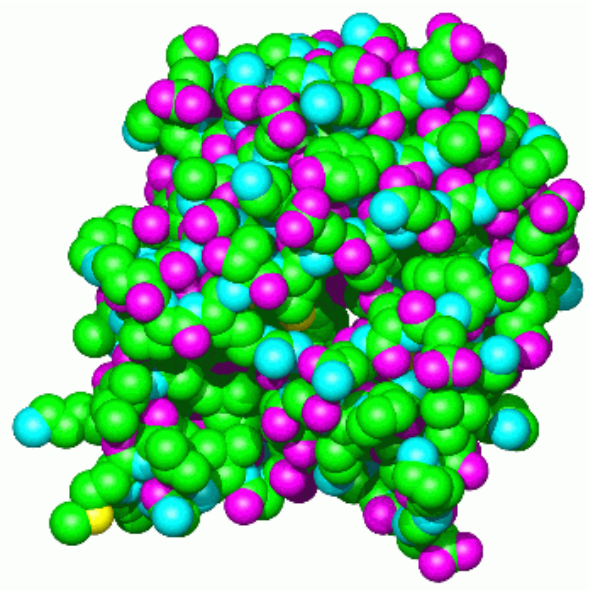
Biology Is Mathematics' Next Physics, Only Better

Protein Structure

- Primary structure
- Secondary structure
- Tertiary structure

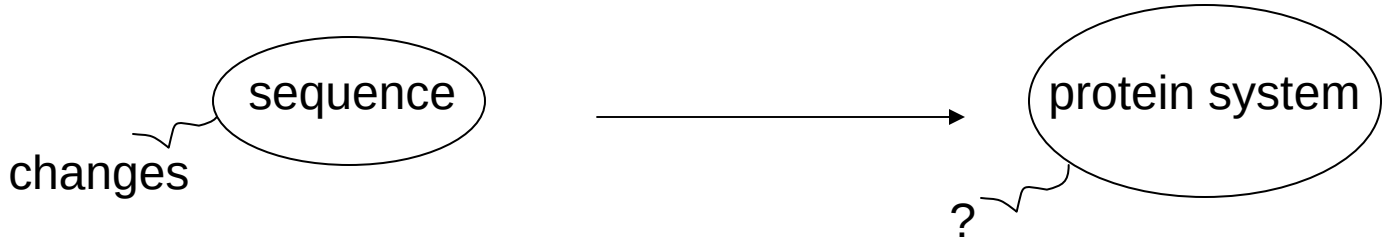






Perturbation experiments in biology:

Mutation



Responses to mutation:

- **no responses or localized responses**
- **cascading effects**

Protein Engineering

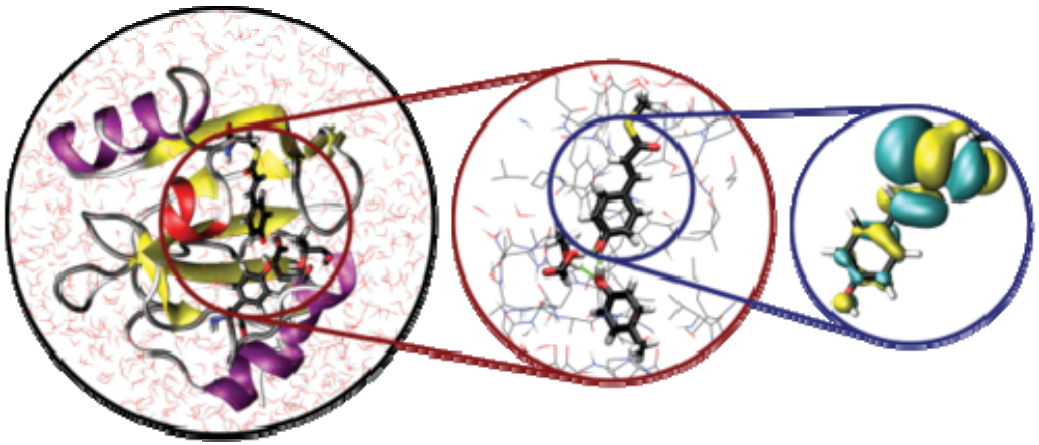
Goal: find the sequence –> desired properties

Idea: alter the sequence –> alter the properties

Methods:

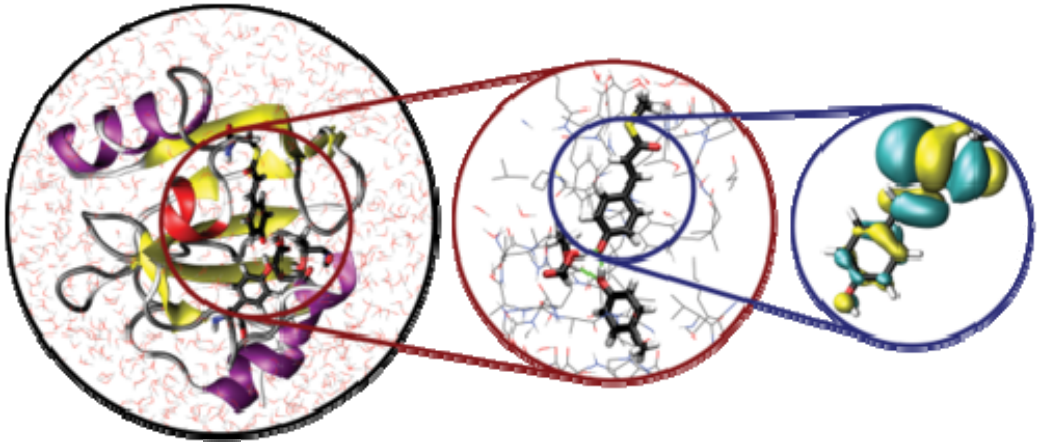
- **rational design**
- **directed evolution**

Multiscale Modeling for Proteins



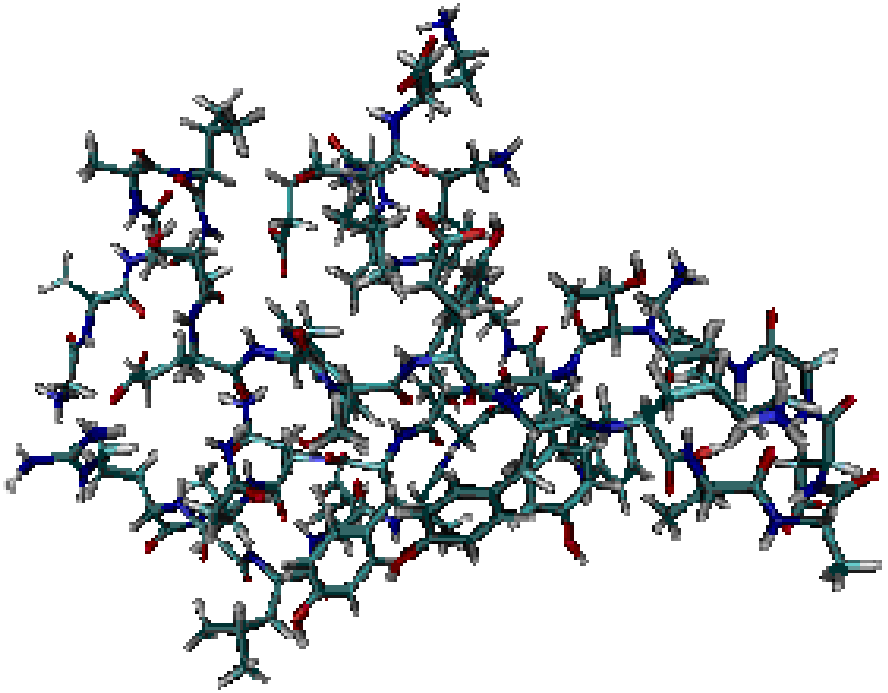
Multiscale Modeling for Proteins

“zoom-in” does NOT work!

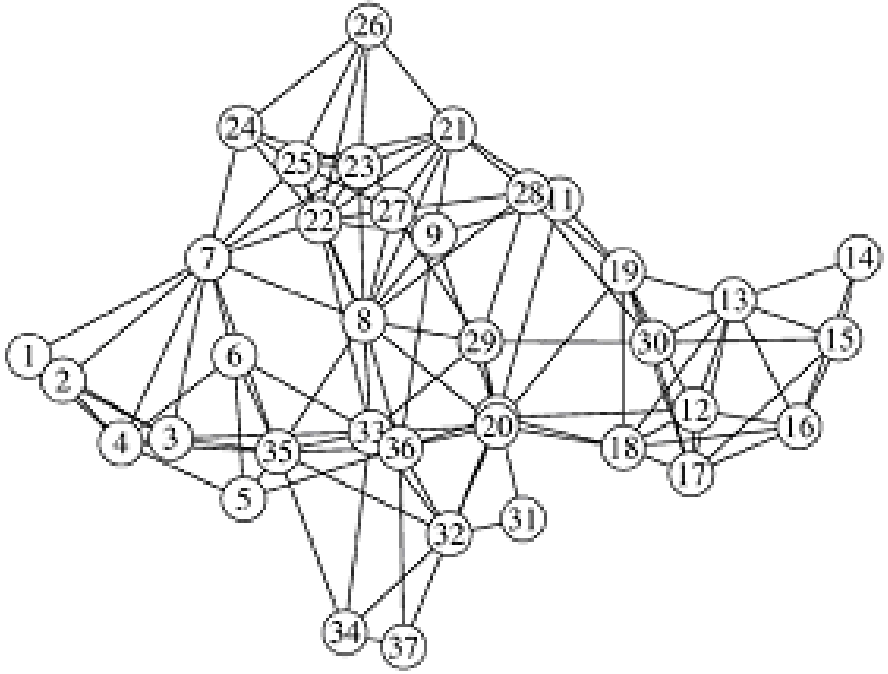


Multiscale Modeling for Proteins

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



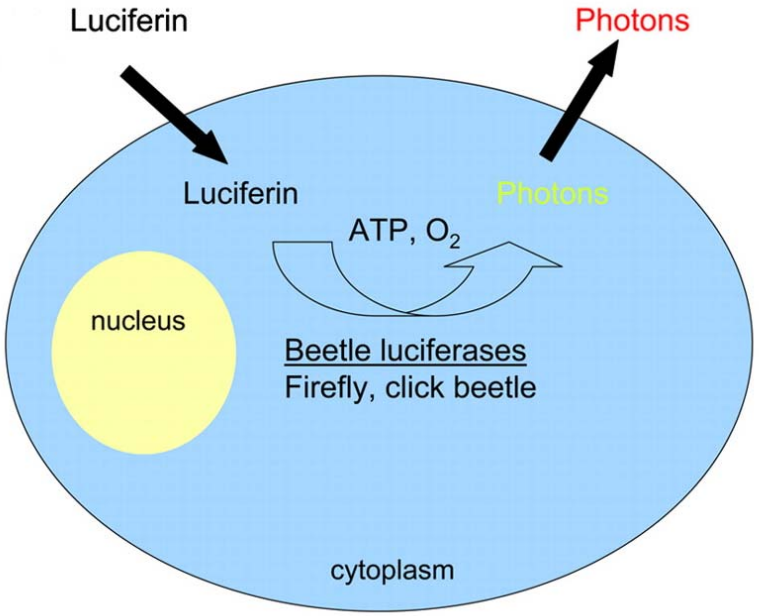
atomistic model
(QM/MM)



network model
(simplified potential)

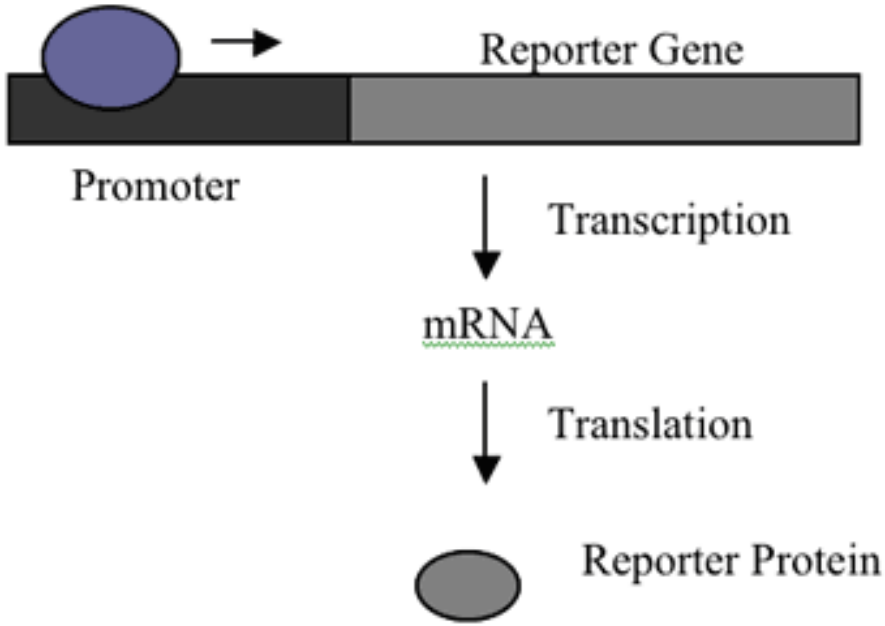
Bioluminescence

- **conversion of chemical energy into light**

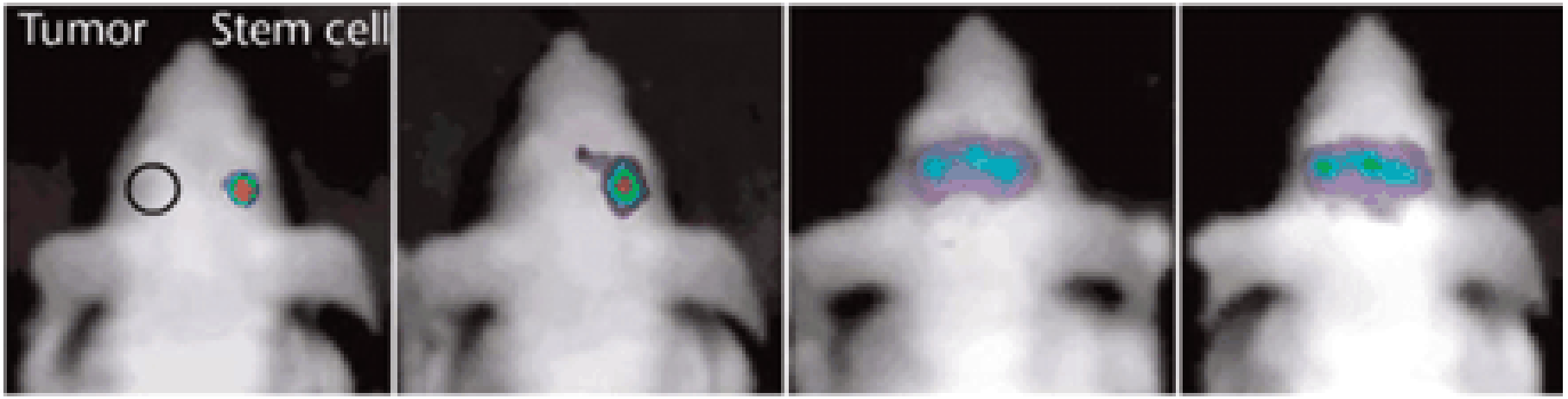


Bioluminescence Reporter Gene Imaging

Insert the reporter gene to the gene of interest to create a gene fusion.



Example of Bioluminescence Imaging



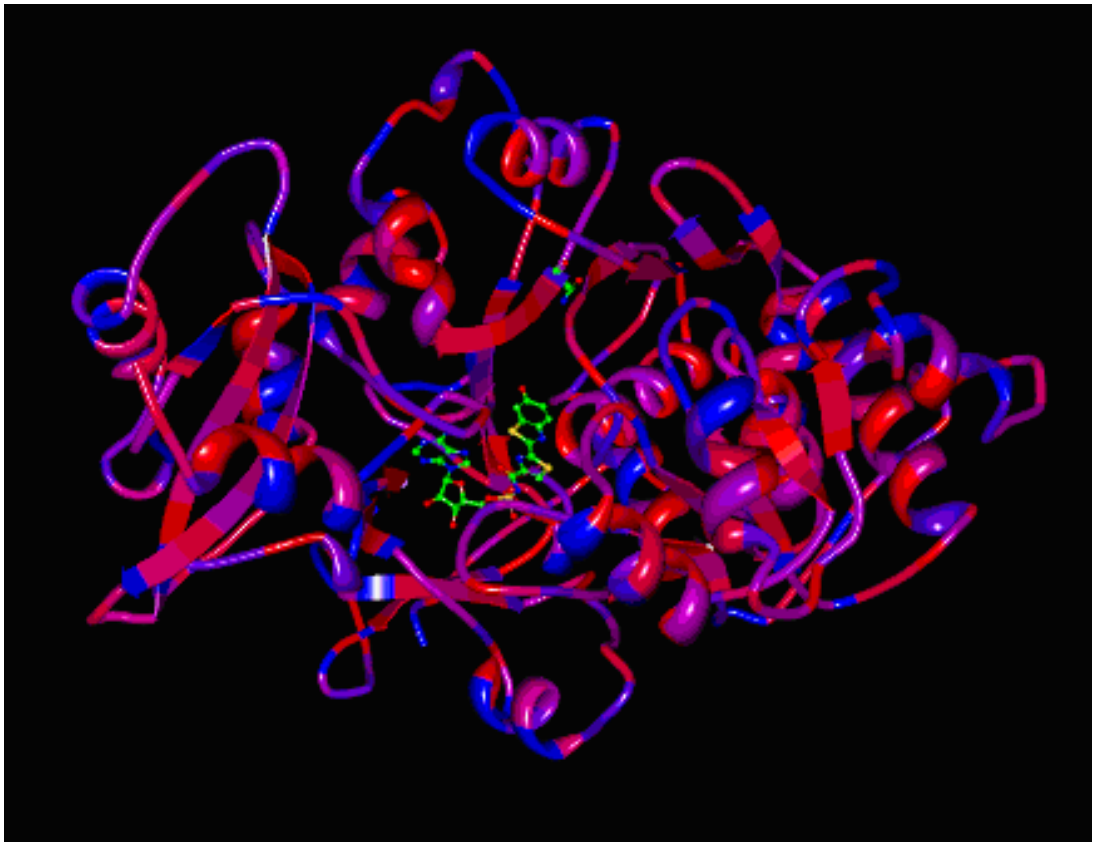
Migration of neural progenitor stem cells (labeled with the luciferin) across the midline towards an implanted brain cancer in a mouse.

A Dzik-Jurasz, *British Journal of Radiology* (2003) **76**, S98-S109

Challenge:

How to achieve Red Emission of Bioluminescence?

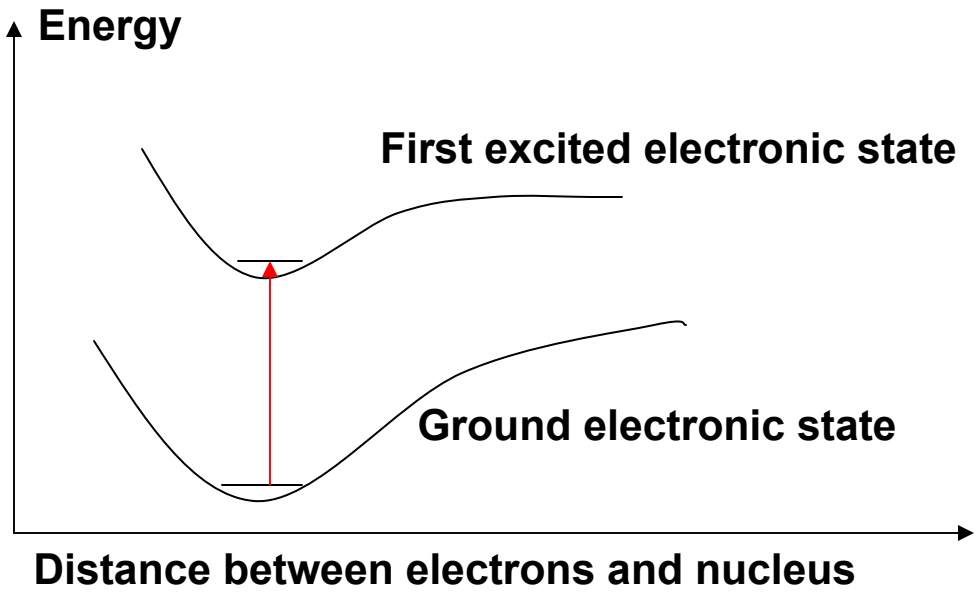
Luciferase•DLSA complex

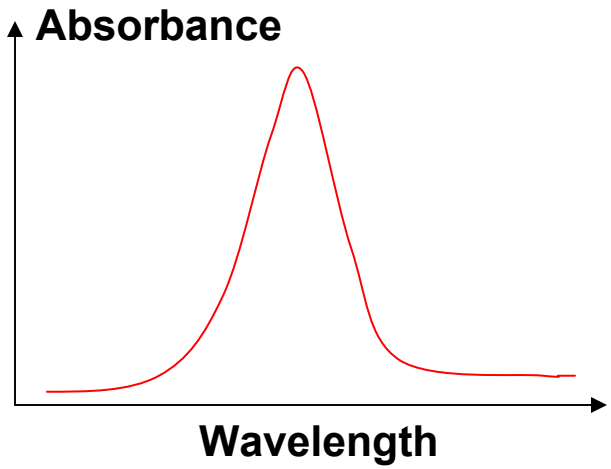


Luciferase (protein): catalyst, 539 residues, 8451 atoms

DLSA: light emitter, 58 atoms

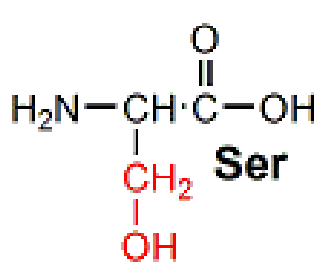
Excitation of DLSA

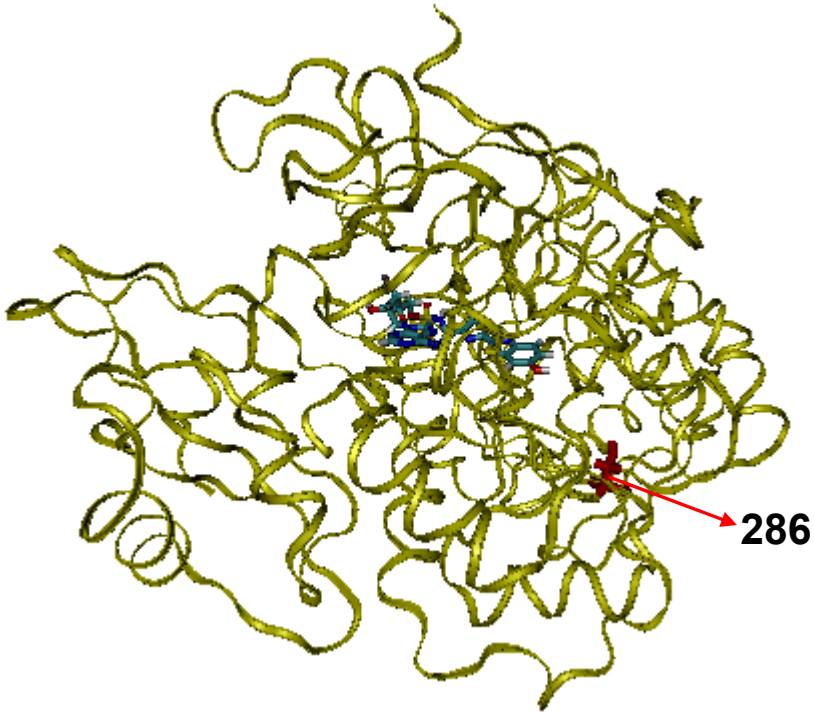


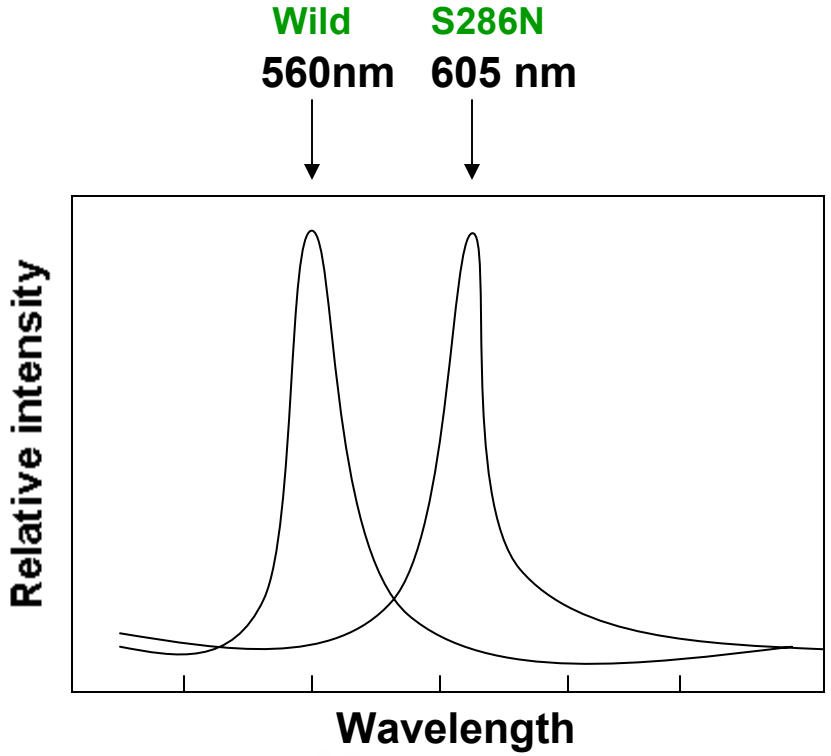


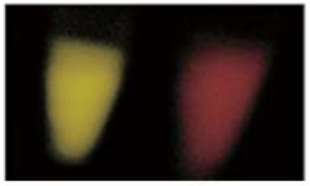
Luciferase: Spectral Shift

Mutation at site 286: Ser ⟶ Asn









Nakatsu et al., ***Nature*** 440, p 372-376, 2006

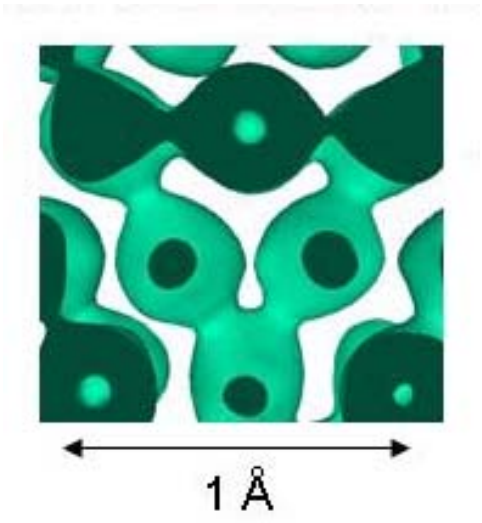
Goal

- **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.**

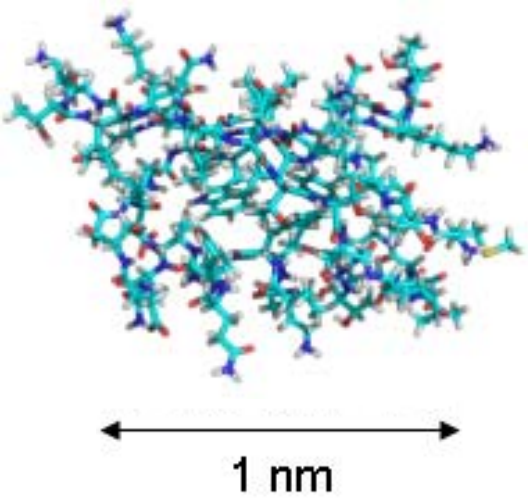
The matter of scale

scale of electrons



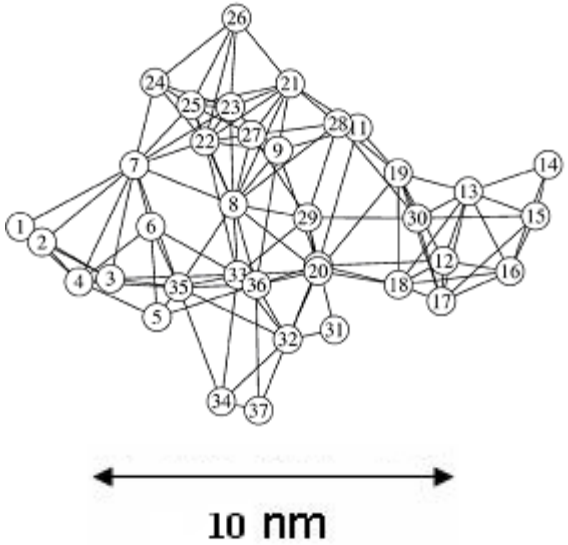
Quantum mechanics
(DLSA)

scale of atoms



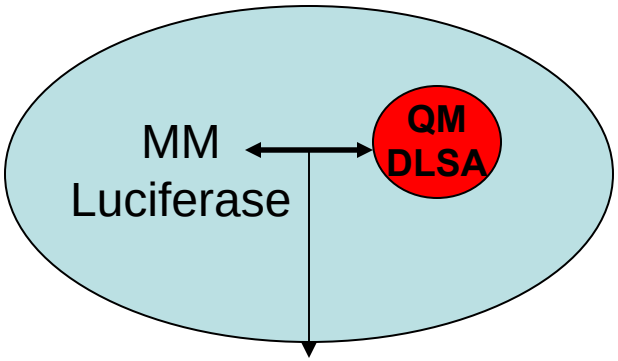
Molecular Dynamics
(protein)

scale of aggregates



Reduced Modeling
(protein/DLSA)

Hybrid Quantum Mechanical/Molecular Mechanical (QM/MM) Approach



QM/MM interaction

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

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

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

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

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

$$\hat{H}_{MM} = \sum_{bonds} k_r (r - r_0)^2 + \sum_{angles} k_\theta (\theta - \theta_0)^2 + \sum_{dihedrals} k_\phi [1 + \cos(n\phi + \phi_0)] + \sum_{atomM}\sum_{M\neq N}\left\{4\varepsilon_{M,N}\left[\left(\frac{\sigma_{MN}}{r_{MN}}\right)^{12} - \left(\frac{\sigma_{MN}}{r_{MN}}\right)^{6}\right] + \frac{q_M q_N}{4\pi\varepsilon_0 r_{MN}}\right\}$$

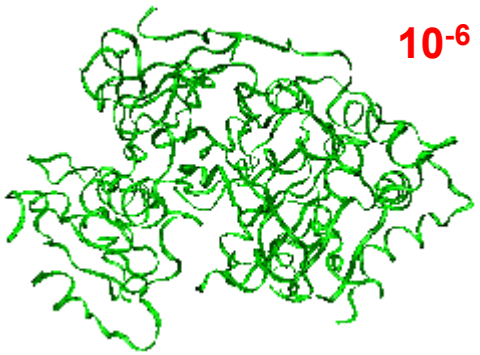
α,β: QM nuclei ***i,j*: QM electron** ***M,N*: MM atoms**

QM/MM Procedure

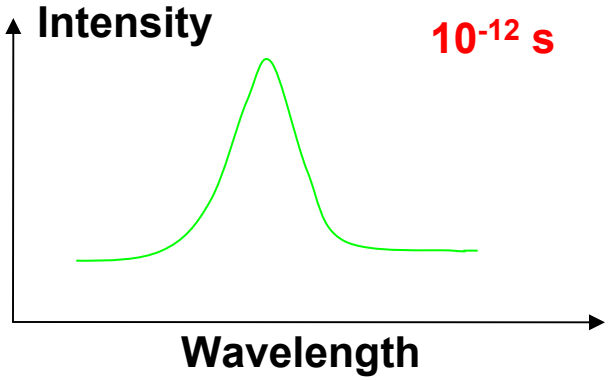
performed by ONIOM program (Gaussian 03)

- **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**

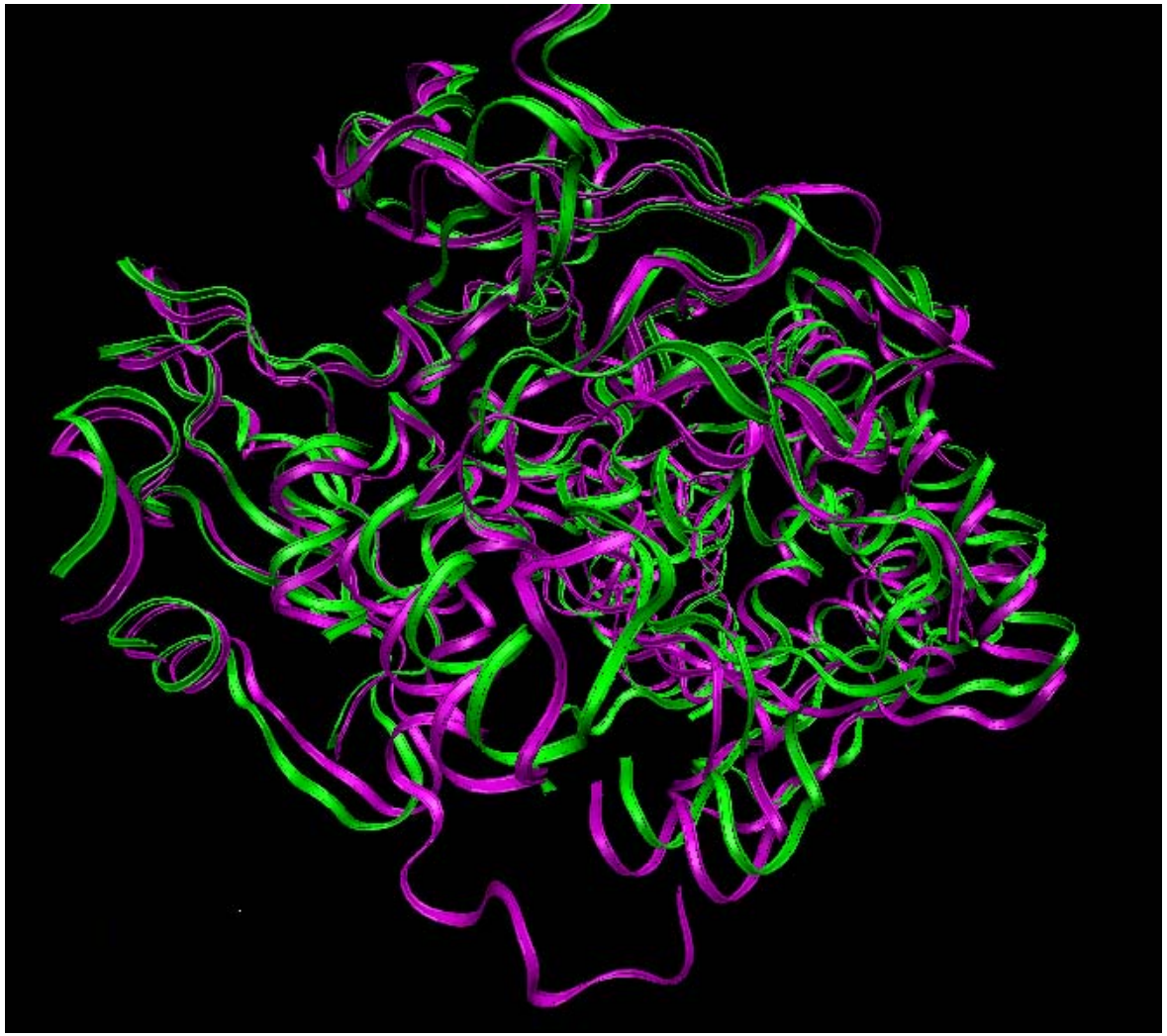
Assumption: time for structural relaxation >> time for electron excitation



$10^{-6} \sim 10^{-3}$ s

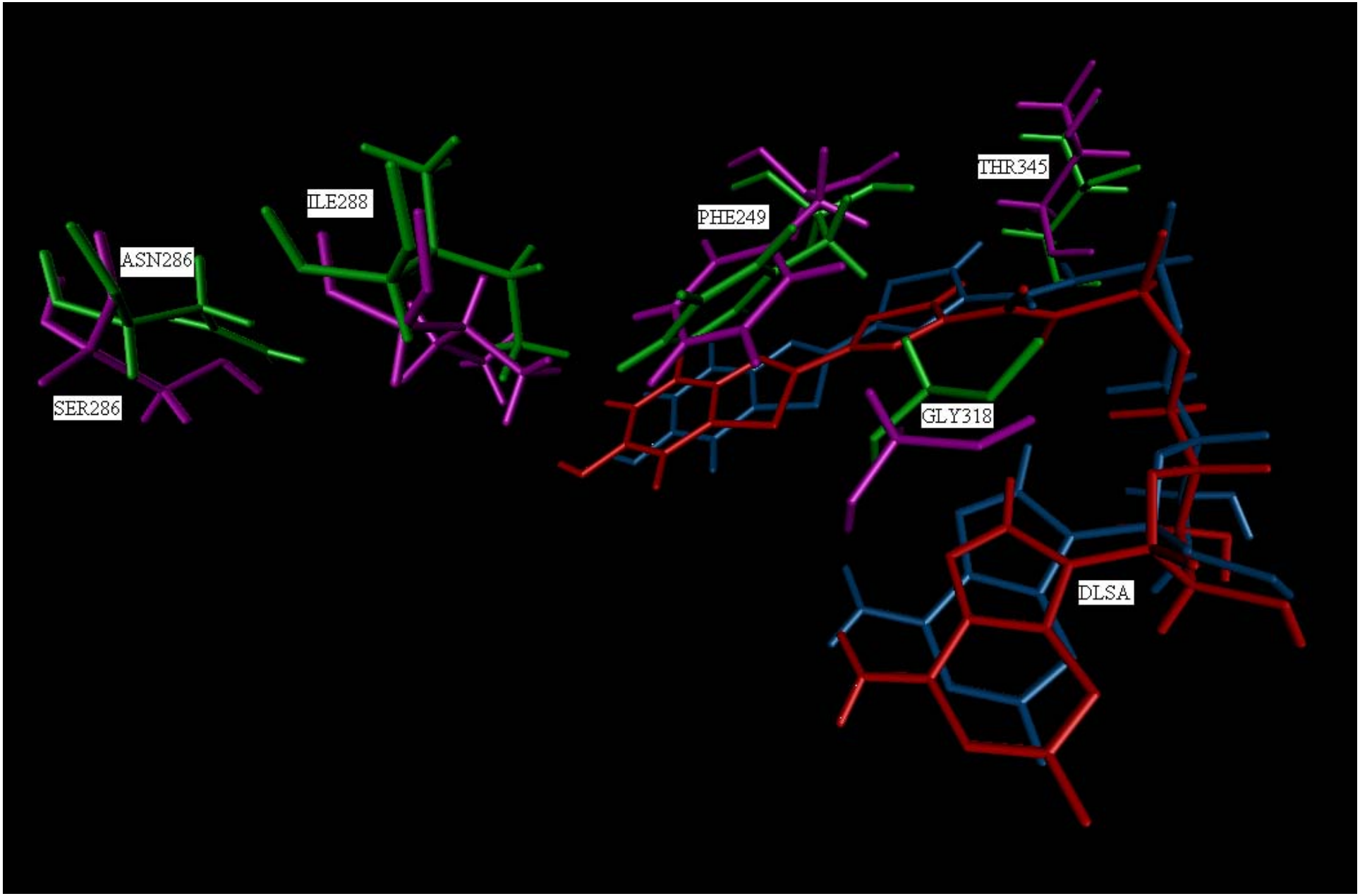


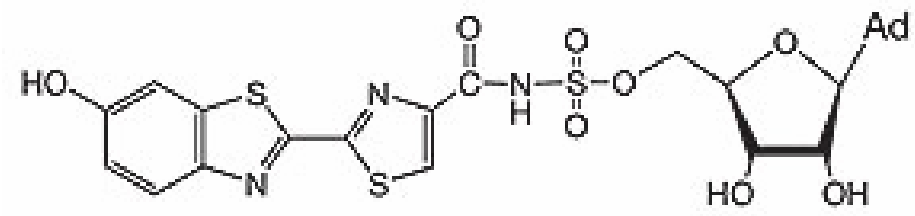
Global Structural Change Caused by Mutation S286N



wild-type

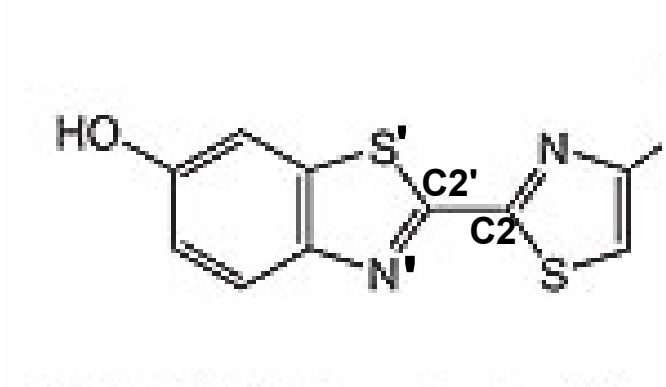
S286N mutant


ILE288
ASN286
SER286
PHE249
THR345
GLY318
DLSA



5′-*O*-[*N*-(dehydroluciferyl)-sulfamoyl]adenosine (DLSA)

	C2'-C2	C2'-N'	C2-S'	C2-N	C2-S	S'-C2'-C2-S	N'-C2'-C2-N
WT	**1.4486Å**	**1.3073Å**	**1.7614Å**	**1.3074Å**	**1.7649Å**	**159.47º**	**163.60º**
S286N	**1.4483Å**	**1.3101Å**	**1.7604Å**	**1.3098Å**	**1.7661Å**	**172.39º**	**174.00º**



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

	Calculated	Experimental Data
Wild-type luciferase	589 nm	560 nm
Mutant (S286N) luciferase	611 nm	605 nm