The Bright, Excited State of Fluorescent Protein Chromophores

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Australia
The GFP Homologues
A Metazoan Superfamily

A. Victoria
GFP

Coral FPs &CPs

FPs for Imaging Applications

Weissleder et al. 2003

Vintersten et al. 2004

Bouvet et al. 2005

Hoffman et al. 2004
The Proteins

- β-can
- Chromophore forms inside
- Soluble
- Oligomerization tendencies

The Chromophores

- Chromophore forms from XXG (often XYG)
- p-hydroxybenzylidene-imidazolinone core (GFP)
- Protein classes map to chromophore structure
**Synthetic Models**

- Spectral properties similar to protein
- Isolated chromophore – good 1\textsuperscript{st} guess

(Yampolsky et al., *Biochemistry*, 2005)

**Color Diversity**

- Protein tunes chromophore
- Similar chromophores in non-homologous proteins

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_{\text{abs}}$</th>
<th>$\lambda_{\text{em}}$</th>
<th>$\Phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DsRed (nat.)</td>
<td>558</td>
<td>583</td>
<td>0.7</td>
</tr>
<tr>
<td>mRFP1 (eng.)</td>
<td>584</td>
<td>607</td>
<td>0.25</td>
</tr>
<tr>
<td>mCherry (eng.)</td>
<td>587</td>
<td>610</td>
<td>0.22</td>
</tr>
<tr>
<td>EqFP611(nat.)</td>
<td>559</td>
<td>611</td>
<td>0.45</td>
</tr>
<tr>
<td>Rtms5H146S</td>
<td>590</td>
<td>636</td>
<td>0.02</td>
</tr>
<tr>
<td>mPlum (eng)</td>
<td>590</td>
<td>649</td>
<td>0.1</td>
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</tbody>
</table>
Why Calculate?

- Chemistry:
  - Understanding photoreactions
- Biotech:
  - Guiding development of new biophotonics tools
- Reef phylogeny:
  - How first principles physics directs evolutionary diversity

Excited State Toolbox

- Ab initio
  - Just the S.E. and a basis set
    - Energies, Geometries, Gradients, Surfaces, Properties
    - MolPro, Columbus, GAMESS, NWChem, Gaussian
- TD-DFT
  - Response of Density to Field
    - Energies, Limited Gradients, Bad Surfaces
    - Turbomole, NWChem, Gaussian
- Semi-Empirical Methods
  - Parametrized models
    - Good for spectroscopy, needs to be reparametrized for anything else
    - MOPAC, GAMESS, Gaussian
Models

*Calculations are expensive – how much do you need?*

- Cut chromophore plus some more
- Hack away
- Pay attention to orbitals ($\pi\pi^*$)
- Pay attention to excitation

The Bright State of FP Models

- HOMO-LUMO $\pi\pi^*$
- $S_1$

*Difference Isodensity*  
*Orbital Structure*
Absorption/Excitation

- Optimize on Ground
- Excitation to bright state
- Maximum
- Vibrational Structure is harder

<table>
<thead>
<tr>
<th>Method</th>
<th>ΔE(eV)</th>
<th>λ(nm)</th>
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<tbody>
<tr>
<td>CASPT2</td>
<td>2.02</td>
<td>563</td>
</tr>
<tr>
<td>EOMCCSD</td>
<td>2.48</td>
<td>500</td>
</tr>
<tr>
<td>TD-DFT</td>
<td>2.41</td>
<td>513</td>
</tr>
</tbody>
</table>

DsRed: 558 nm (2.22 eV)
HBMPDI: 549 nm (2.25 eV)

Consistent with least red of RFPs

HcRed Chromophore States

- **Two conformations**
  - Cis coplanar
  - Trans, noncoplanar
- **Two excitation peaks**
  - 573nm / 2.16 eV
  - 598nm / 2.07 eV

<table>
<thead>
<tr>
<th>Conformer</th>
<th>λ_{abs}(nm)</th>
<th>ΔE(eV)</th>
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<tbody>
<tr>
<td>Cis</td>
<td>525</td>
<td>2.36</td>
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<tr>
<td>Trans, planar</td>
<td>495</td>
<td>2.50</td>
</tr>
<tr>
<td>Trans, nonplanar</td>
<td>515</td>
<td>2.41</td>
</tr>
</tbody>
</table>

**Low pH Rrms5$_{H146S}$**

- Color change $pK_a$ 4.6
- Large shift
- Single isobestic point
- Ambiguous site

<table>
<thead>
<tr>
<th></th>
<th>$\Delta\lambda$(nm)</th>
<th>$\Delta\Delta E$(eV)</th>
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</thead>
<tbody>
<tr>
<td>A$\rightarrow$N</td>
<td>-94</td>
<td>0.53</td>
</tr>
<tr>
<td>A$\rightarrow$Z</td>
<td>+24</td>
<td>-0.11</td>
</tr>
</tbody>
</table>


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**Emitting State FP Models**

- Bond Alternation

CAS(12,11)$^*$PT2: 1.77eV, (700nm)

EOM-CCSD: 2.21eV (561nm)

(DsRed: 595nm (2.08eV))
Unbound and Dark States

- Optimization Constraints
  - Prevent unbound optimization
  - Prevent loss of oscillator strength

Use constraints to mimic matrix environment

Initial \( \pi\pi^* \) Dynamics

- Planar molecules, planar dynamics

Resonance Raman Spectra

Motion away from planarity kills the oscillator strength
**Dynamics with *Ab initio* potentials**

- Ultrafast fluorescence of GFP model

![Graph showing fluorescence decay with time](image)


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**Photoisomerization in FP chromophores**

- FP’s – coplanar
- CP’s – non-coplanar
- No steady-state fluorescence in:
  - Models
  - Denatured Protein

![Graph showing fluorescence spectra](image)

Recap: The Conformations of HcRed Chromophore
Internal Conversion Processes

- IC processes
  - Competition w/ fluorescence
- Need to describe
  - Global surface
  - Multiple surfaces
  - Degeneracies

FP Chromophores Photoisomerize

Twist
Imidazolinone

Twist
Phenoxy
Charge Transfer Isomerization in FP Chromophores

Twist, separate charge
Polarity depends on which bond you twist

Twist Imidazolinone
Electron goes here in ground state

Twist Phenoxy
Electron goes here in excited state

GFP vs. RFP

Acylimines withdraw electrons

Inaccessible Imidazolinone-twist
Stabilized Phenoxy-twist
Phenoxy Isomerization from *Cis* or *Trans* form

- Small/No Predicted Barrier
- Faster internal conversion predicted for *trans* form

Charge Separation at Intersections

- Bond Alternation
- Torsion
Evidence From Synthetic Derivatives

Quantum Yield of Fluorescent Derivatives in water, glycerol and dioxane

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Glycerol</th>
<th>Dioxane</th>
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<tbody>
<tr>
<td>ε=80</td>
<td>n.d.</td>
<td>0.0015</td>
<td>0.003</td>
</tr>
<tr>
<td>ε=40</td>
<td>n.d.</td>
<td>0.023</td>
<td>0.139</td>
</tr>
<tr>
<td>ε=2</td>
<td>0.0001</td>
<td>0.220</td>
<td>0.158</td>
</tr>
</tbody>
</table>

Follenius-wund et al. Biophys. J. 85(3) 1839-1850

Charge Transfer Isomerization

Ethylene in vacuum

Ethylene & point charge
Solvent Dynamics

- Simulated IC in a water cluster

Protein Environment

- Electrostatic Interactions with charged/polar residues
- Which residues are charged?

Toniolo et al. Faraday Disc., 129 149-163
The Protein

Olsen et al. in *Computational Methods in Photobiology* (in press)

![Graph showing energy vs. reaction coordinates](image1)

Hula-Twist

- Proposed by Liu for retinal 1985
- Invoked for GFP by Zimmer 2001
- Dihedrals show coherent oscillations even in vacum – Toniolo 2004

![Hula-Twist molecule](image2)
Different States

• Bright state identified by oscillator strength

\[ f \approx 0.0001 \quad f \approx 1.0 \]

Intersystem Crossing

• El-Sayed’s rules
  – Angular momentum conservation

\[ \pi \pi^* \quad n\pi^* \quad \pi \pi^* \quad \pi \pi^* \]

\[ \pi^2 \quad S_0 \]

\[ \text{S}_0 \text{ Chr.} \quad \text{hv} \quad \text{S}_1 \text{ Chr.} \]

\[ \text{T}_0 \text{ O}_2 \quad \text{T}_1 \text{ Chr.} + \text{T}_0 \text{ O}_2 \]

\[ \text{S}_0 \text{ Chr.} \quad \text{S}_1 \text{ O}_2^* \]
Solvent Effects on the Resonance Raman Spectrum

Altoe et al., J. Am. Chem. Soc., 127(11) 3952-3963

Quantum mechanics predicts 
The Incredible Hulk is Green!
Summary

The Bright State
Spectral properties, transient lifetimes
Cis-Trans photoisomerization
Charge separation/recombination
Solvent & Protein Control

Acknowledgements

CCMS(UQ) Members (particularly)
• Dr. Marlies Hankel
• Dr. Sufan Wang

Dr. Mark Prescott (Monash)
Dr. Sophie Dove (CMS, UQ)

Rowan Gollan (Hypersonics, UQ)
Andrew Denman (Hypersonics,UQ)

Prof. Todd Martínez (UIUC)
Dr. Alessandro Toniolo (Eli Lilly)
Leslie Manohar (US Navy)

Prof. Kazuo Kitaura (AIST)
Dr. Dmitri Fedorov (AIST)
Dr. Toyokazu Ishida (AIST)
FABLS ARC Network RN0460002

UQ Computations Performed at:
• APAC National Facility
• QPSF Partner-share
• CCMS/Hypersonics Cluster Facility

UIUC Computations at:
• The Martínez Lab Group
• NCSA Supercomputing Facility
Tools: Semi-Empirical

- Approx. Shrödinger Eq.
- Good absorption wavelengths
- Bad Surfaces with ready params.
- Cheap

Ab Initio (‘First Principles’)

- Electronic Schrödinger eqn.
- Gaussian LCAO

\[ \hat{H}_{\text{elec}} = \frac{1}{2} \sum_i^N \nabla_i^2 + \sum_{i,j \neq i}^N r_{ij}^{-2} + \sum_A^M \sum_i^N Z_A r_{iA}^{-2} \]

\[ E_{\text{elec}} \Psi(r_1...r_N) = \hat{H}_{\text{elec}} \Psi(r_1...r_N) \]

\[ E_{\text{tot}} = E_{\text{elec}} + \sum_{A>B}^M Z_A Z_B r_{AB}^{-2} \]

\[ \Psi(r_1...r_N) = \sum_l c_l |\psi(r_1)\psi(r_2)...\psi(r_N)| \]

\[ \psi_i(r) = \sum_{\alpha} c_{i\alpha} \phi_\alpha \]

Atom-centred gaussians
Protein Modeling: QM/MM Techniques

• For local chemistry / excitations
• Treat only active electrons via QM

Electrostatics in the Protein

• How to determine the local charges?
  – Continuum electrostatics
  – Empirical trends
    • Problems with unconventional residues
Protein Modeling: Large Scale *Ab Initio* Methods

- Fragment Molecular Orbital
- Divide & Conquer
- ONIOM

Protein Modeling: Other Approaches

- Empirical Valence Bond
- Excited State MM
Fluorescence Phenomena: What Can a Modeler Do?

- $\tau_{fl}$ too long
- Autocorrelation functions are short
- Results for Trp fluorescence in proteins using MD

The Shape of Things To Come
Assessing A Computational Model

- Absorbance
- Vibrations
  - Resonance Raman
- Isomerization Barriers

Synthetic Models

- HB
- DsR
- \( \lambda_{abs} = 477\text{nm (gas)} \), \( \lambda_{abs} = 426\text{nm (H}_2\text{O)} \)
- \( \lambda_{fl} = 490\text{nm (n-propanol)} \)

\[ \lambda_{abs} = 478\text{nm} \]

- KFP
- \( \lambda_{abs} = 520\text{nm (H}_2\text{O)} \), \( \lambda_{abs} = 572\text{nm (DMF)} \)
- \( \lambda_{fl} = 603\text{nm} \)

- FMF

\[ \lambda_{abs} = 565\text{nm (f=0.0008 (H}_2\text{O))} \]
Tools: DFT/TD-DFT

- 1-e: Density Only
- Good wavelengths on the cheap!
- Gradients – Res. Raman
- Bad Surfaces
- Limited Properties

\[ E = E[\rho(r,t)] \]

Chromophore Charge States

**Rtms5 at Low pH**

<table>
<thead>
<tr>
<th></th>
<th>Anion</th>
<th>Neutral</th>
<th>Cation</th>
<th>Zwit.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cis</em>,planar</td>
<td>525</td>
<td>430</td>
<td>477</td>
<td>551</td>
</tr>
<tr>
<td><em>Trans</em>,planar</td>
<td>496</td>
<td>417</td>
<td>456</td>
<td>521</td>
</tr>
<tr>
<td><em>Trans</em>,nonplanar</td>
<td>517</td>
<td>423</td>
<td>496</td>
<td>541</td>
</tr>
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</table>