Ultrafast Dynamics of Resonance Energy Transfer in Myoglobin

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General Prospective of RET to Study Protein Dynamics

1948 ~ Theodor Förster’s paper puts RET on the map
Zwischenmolekulare Energiewanderung und Fluoreszenz
“Intermolecular Energy Migration and Fluorescence”
Annalen der Physik (2, 55-75)

1958 ~ Sperm Whale Myoglobin crystal structure solved by
Kendrew et al., Nature (Mar 8) 181 (4610):662-6

1971 ~ Protein Data Bank established at Brookhaven National Laboratory

Today ~ RET has proven to be a powerful tool used to study protein-protein interactions, protein-DNA interactions, protein conformational changes, reaction kinetics, and molecular motors all on diverse time scales
Femtosecond Up-Conversion

\[ I(t) = \sum_{i=1}^{n} I_i e^{-k_i t_i} \]
Resonance Energy Transfer

\[ k_{RET} = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6 \]

\[ R_o^6 = (8.79 \times 10^{23}) \kappa^2 n^{-4} \phi_d J_{da} \]

From Literature:
- \( n \) – Index of refraction
- \( \tau_D \) – Donor lifetime

Can Accurately Calculate:
- \( \phi_d \) – Quantum efficiency of donor probe
- \( J_{da} \) – Integral overlap of donor emission spectrum with absorption spectrum of acceptor
- \( k_{RET} \) – From experimental fitting

Unknowns:
- \( r \) - Distance from donor and acceptor
- \( \kappa^2 \) - Transition dipole orientation factor

\[ \kappa^2 = (\cos \alpha_{DA} - 3 \cos \alpha_D \cos \alpha_A)^2 \]


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Sperm Whale Myoglobin (Mb)
$\kappa^2$ Theory

Trp:

Heme:

Z. Gryczynski et. al., Meth. Enzyme 278 (1997) 538-569

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Solvation Dynamics

Delay Time (ps)

0 10 20 30 40 50

Normalized Fluorescence Intensity (a.u.)

305 nm 310 nm 315 nm 320 nm 330 nm 340 nm 360 nm

W7 Native

Apo Mb


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Apo vs. Holo

No Heme  Heme

Normalized Fluorescence Intensity (a.u.)

Delay Time (ps)

Apo-W14
- 310 nm
- 330 nm
- 340 nm

Holo-W14
- 310 nm
- 330 nm
- 340 nm

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W14 vs. W7

NOTE: Different Time Scales

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### Complete Results

<table>
<thead>
<tr>
<th>Trp</th>
<th>$\phi_d$</th>
<th>$J$ (cm$^3$/M)</th>
<th>$r$ (Å)</th>
<th>$\tau_D$ (ns)</th>
<th>$\tau_{\text{RET}}$ (ps)</th>
<th>$n$</th>
<th>$R_0$ (Å)</th>
<th>$\kappa^2$ Exp.</th>
<th>$\kappa^2$ 1JP6</th>
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</thead>
<tbody>
<tr>
<td>W14</td>
<td>0.17</td>
<td>5.04x10$^{-14}$</td>
<td>15.14</td>
<td>2.6</td>
<td>20</td>
<td>1.33</td>
<td>34.08</td>
<td>0.65</td>
<td>0.75</td>
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<tr>
<td>W7</td>
<td>0.20</td>
<td>5.69x10$^{-14}$</td>
<td>21.40</td>
<td>2.8</td>
<td>111</td>
<td>1.33</td>
<td>36.65</td>
<td>0.76</td>
<td>0.20</td>
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</tbody>
</table>

Z. Gryczynski et. al., Meth. Enzyme 278 (1997) 538-569

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Future Work

- Further mutations will be studied to further probe various regions of Mb
- Conformation dynamics will be studied via photolysis of ligands (CO, NO, O₂)
- Use RET as a tool to study protein dynamics in other heme proteins