Ultrafast Dynamics of Resonance Energy Transfer in Myoglobin

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General Prospective of RET to Study Protein Dynamics

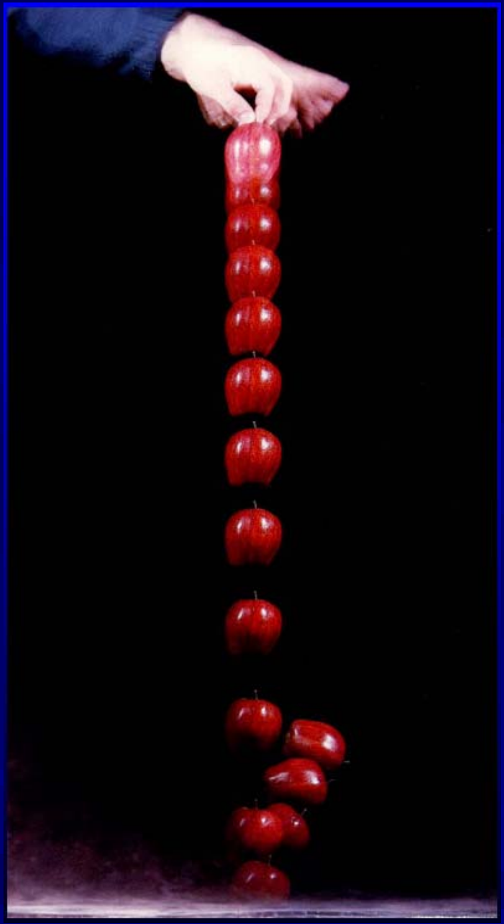
1948 ~ Theodor Förster's paper puts RET on the map
Zwischenmolekulare Energiewanderung und Fluoreszenz
"Intermolecular Energy Migration and Fluorescence"
Annalen der Physik (2, 55-75)

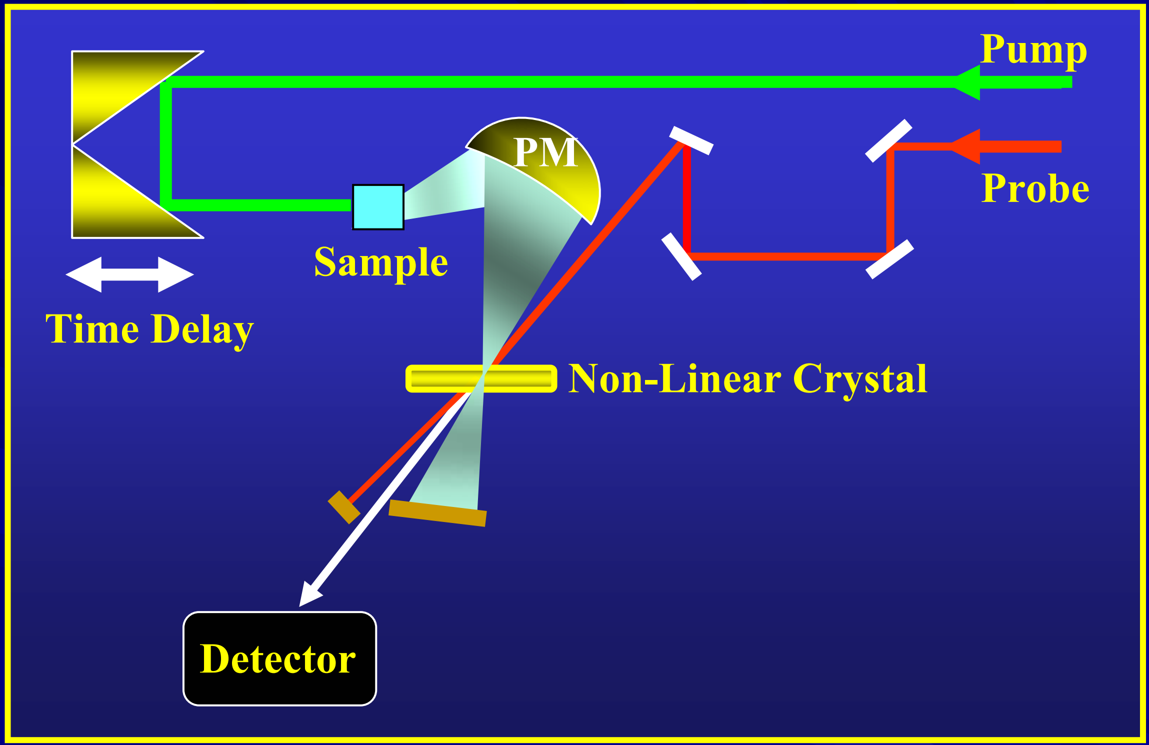
1958 ~ Sperm Whale Myoglobin crystal structure solved by
Kendrew *et al*., *Nature* (Mar 8) **181** (4610):662-6

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

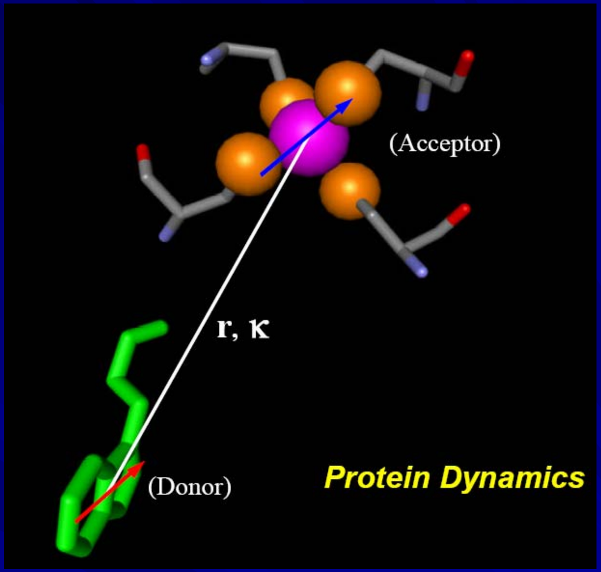




$$I(t) = \sum_{i=1}^{n} I_i e^{-k_i t_i}$$

Resonance Energy Transfer

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



$$R_o^6 = (8.79\times10^{23})\kappa^2 n^{-4}\phi_d J_{da}$$

From Literature:

n – Index of refraction

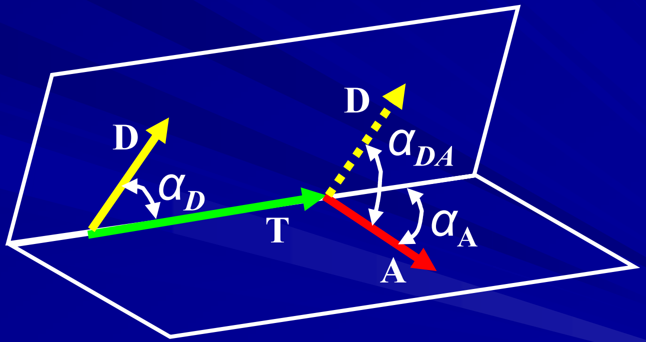
τ_D – Donor lifetime

Can Accurately Calculate:

ϕ_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

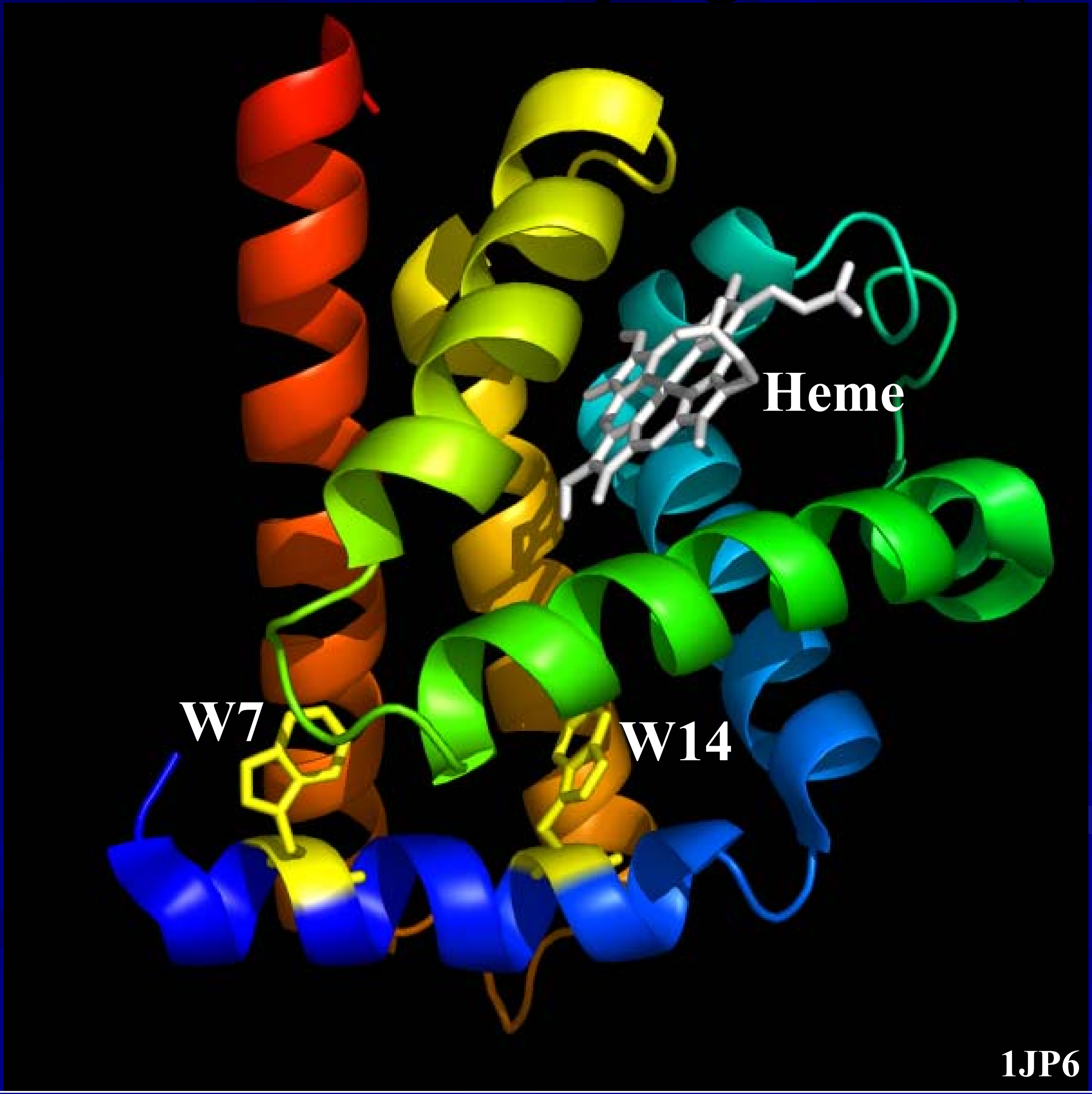
$$\kappa^2 = (\cos\alpha_{DA} - 3\cos\alpha_D\cos\alpha_A)^2$$

κ^2 - Transition dipole orientation factor

Zhong, *PNAS*, January 8, 2002, **99**, 13-18

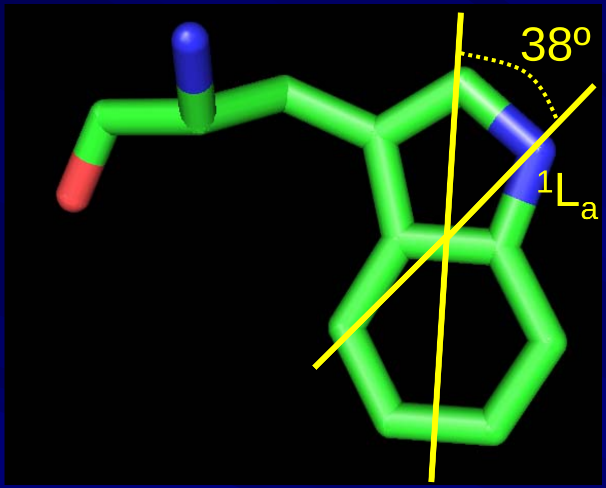
Andrews, DL *et al*, Resonance Energy Transfer, John Wiley and Sons Ltd, Chichester, (1999).

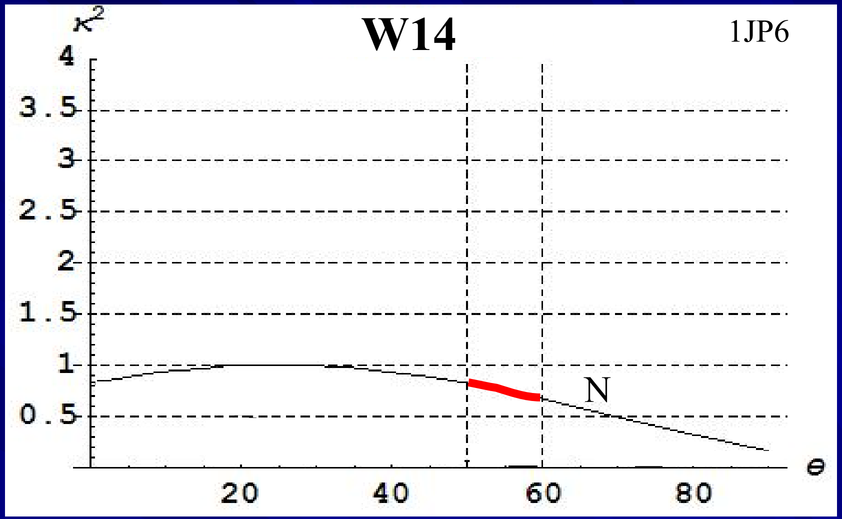
Sperm Whale Myoglobin (Mb)



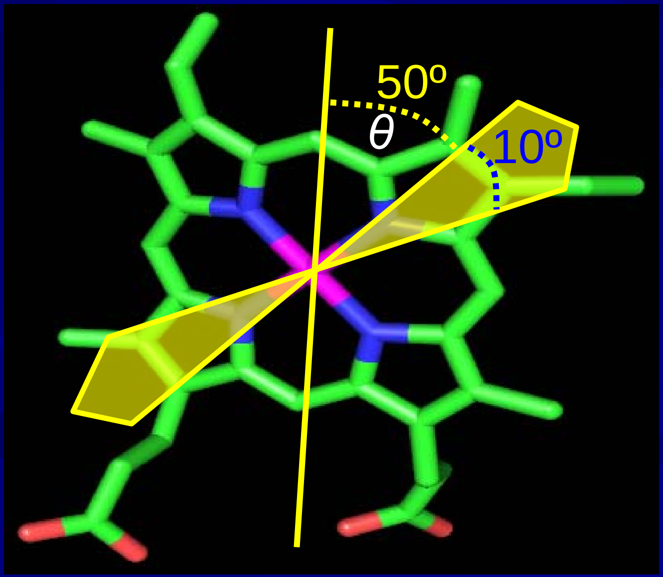
κ^2 Theory

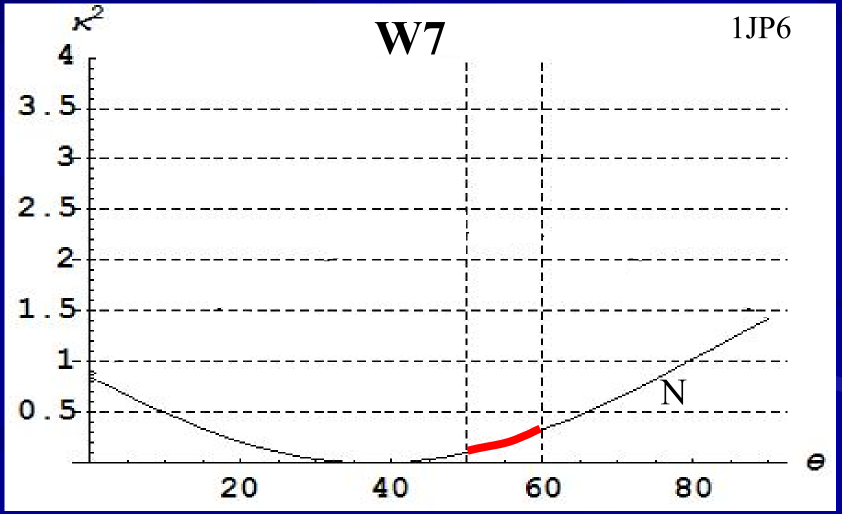
Trp:





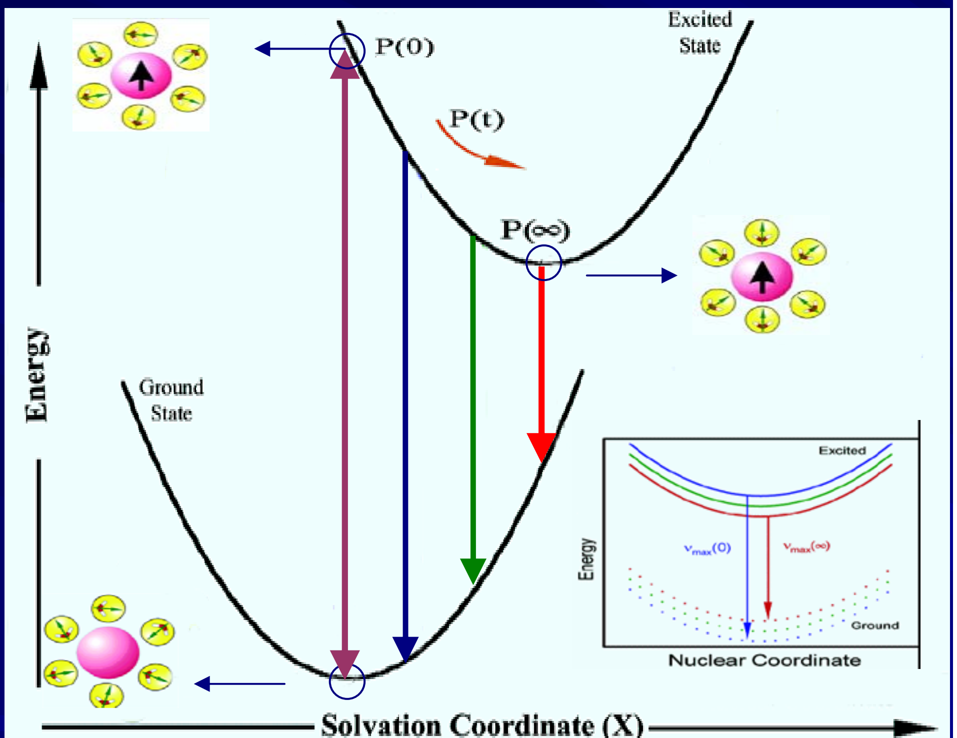
Heme:





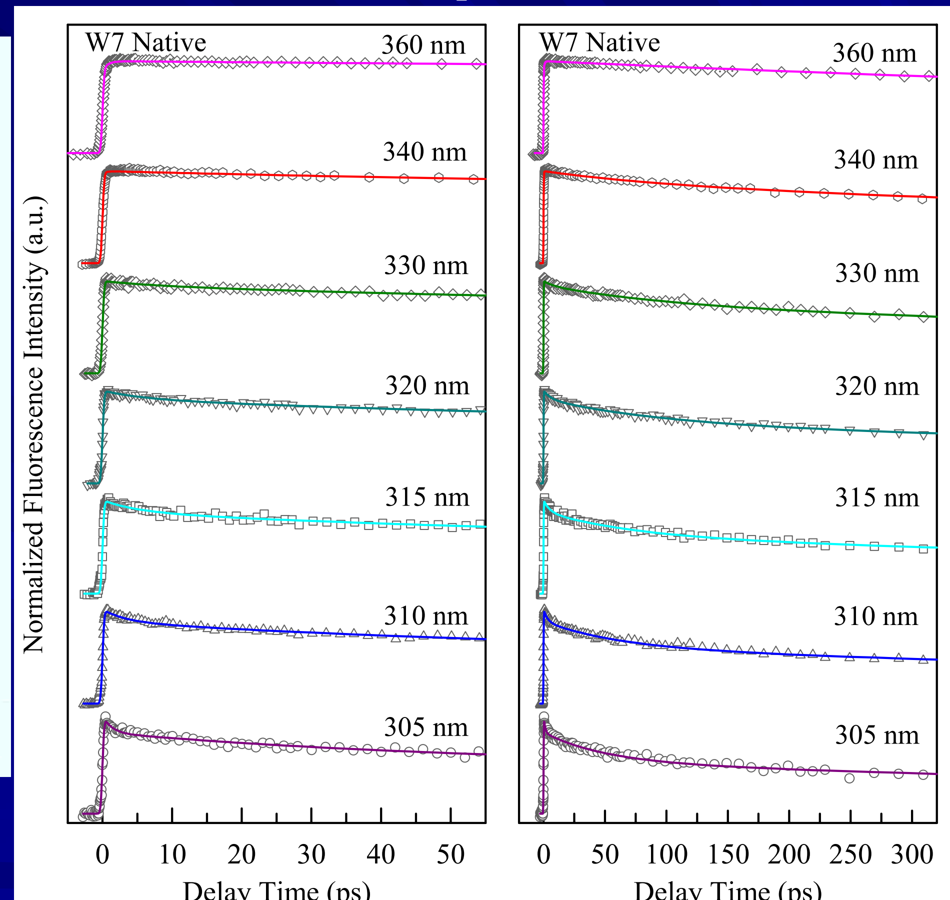
Z. Gryczynski *et. al.*, Meth. Enzyme *278* (1997) 538-569

Solvation Dynamics



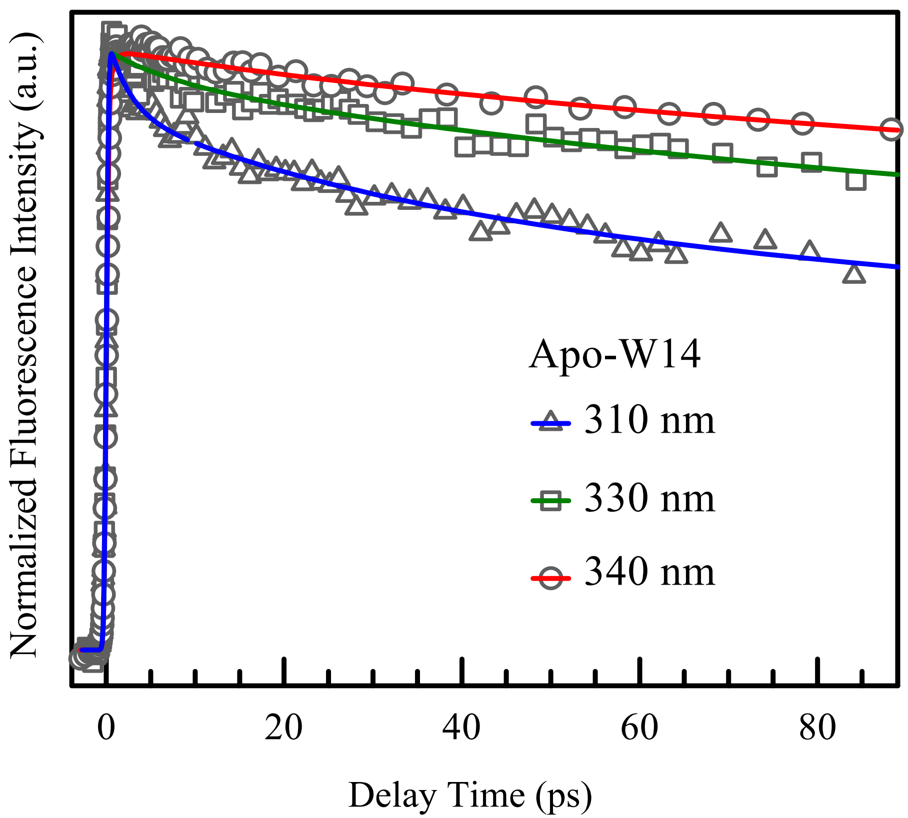
S. K. Pal *et al., J. Phys. Chem. B* **106**, 12376-12395 (2002)

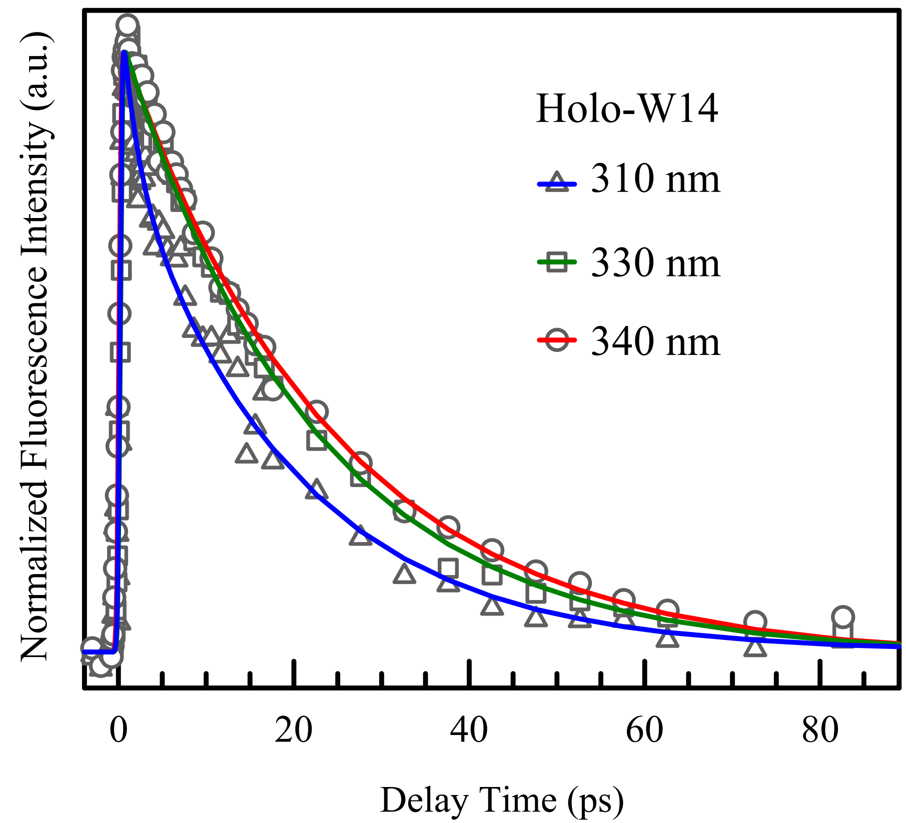
Apo Mb



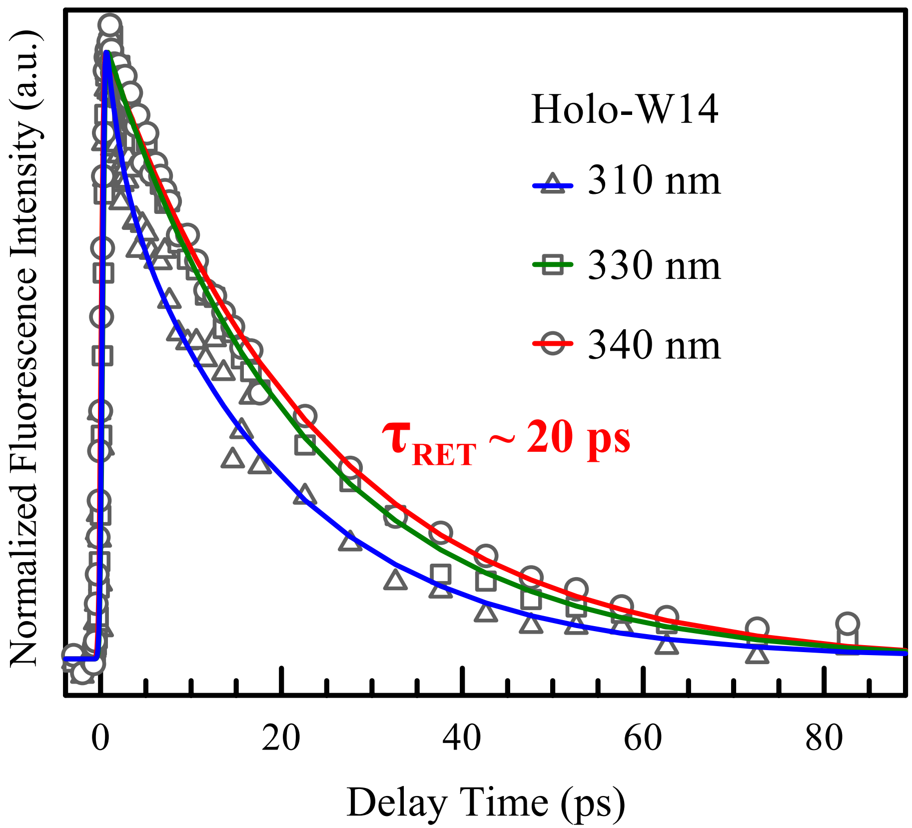
Apo vs. Holo

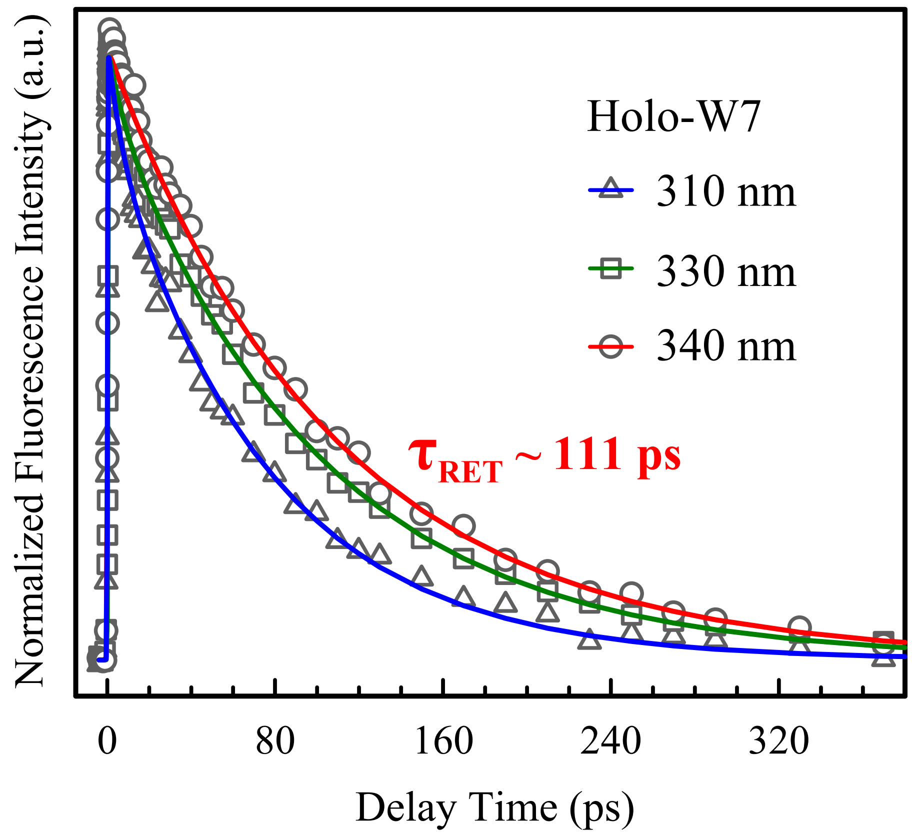
No Heme	Heme





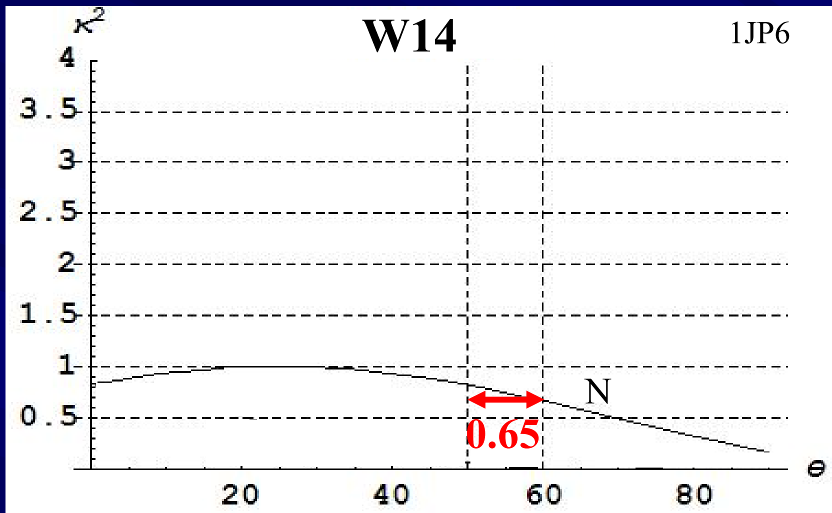
W14 vs. W7

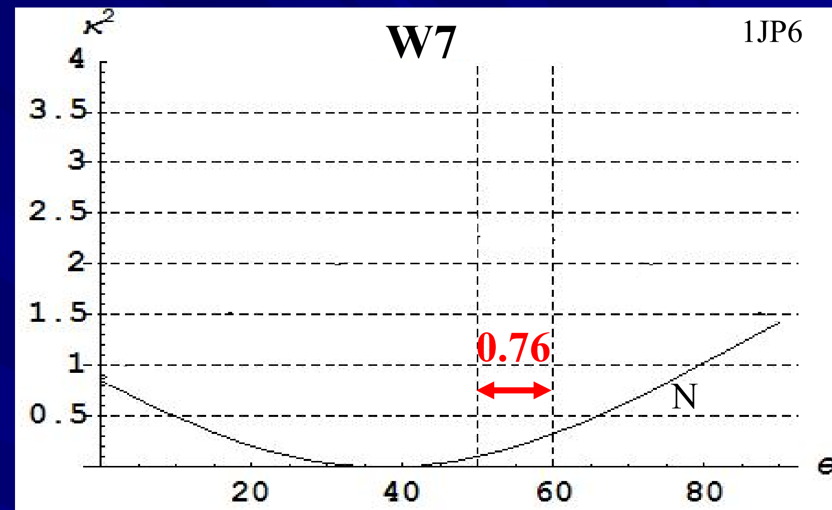




NOTE: Different Time Scales

Complete Results



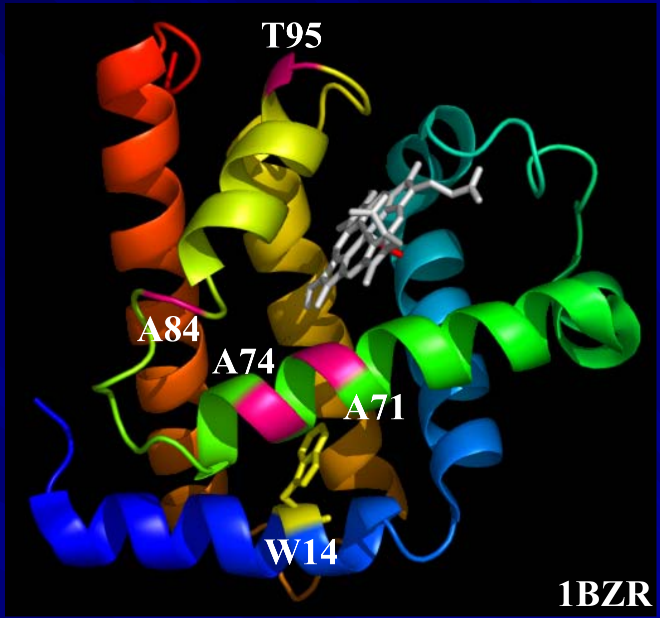


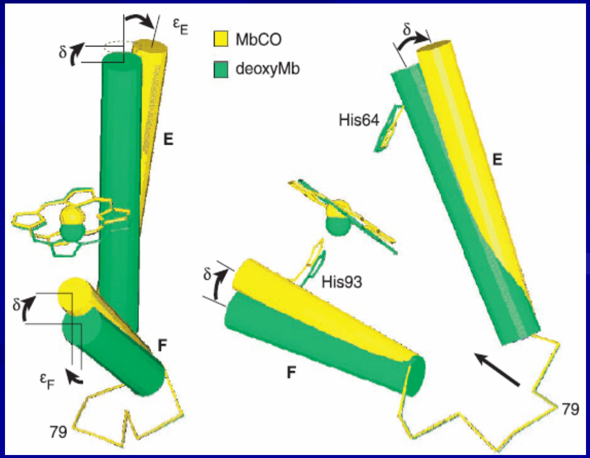
Trp	ϕ_d	J (cm^3/M)	r (Å) 1JP6	τ_D (ns)	τ_{RET} (ps)	n	R_0 (Å)	κ^2 Exp.	κ^2 1JP6
W14	0.17	5.04×10^{-14}	15.14	2.6	20	1.33	34.08	0.65	0.75
W7	0.20	5.69×10^{-14}	21.40	2.8	111	1.33	36.65	0.76	0.20

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

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, O_2)
- Use RET as a tool to study protein dynamics in other heme proteins





S. Kachalova *et al.*, *Science* **284**, 473-476 (1999)