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INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
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H4.SMR/916 - 36

SEVENTH COLLEGE ON BIOPHYSICS:

Structure and Function of Biopolymers: Experimental and Theoretical Techniques.

4 - 29 March 1996

Photophysics, Protein Physics and Myglobin -

the Hydrogen Atom of Biology

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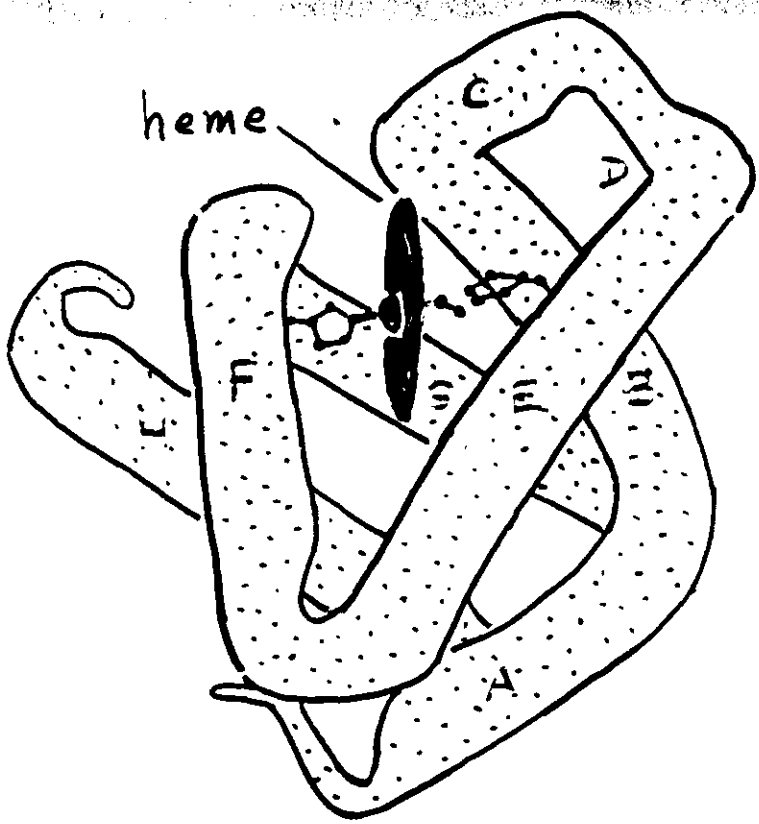
National Institutes of Health
20892-0520 Bethesda
U.S.A.

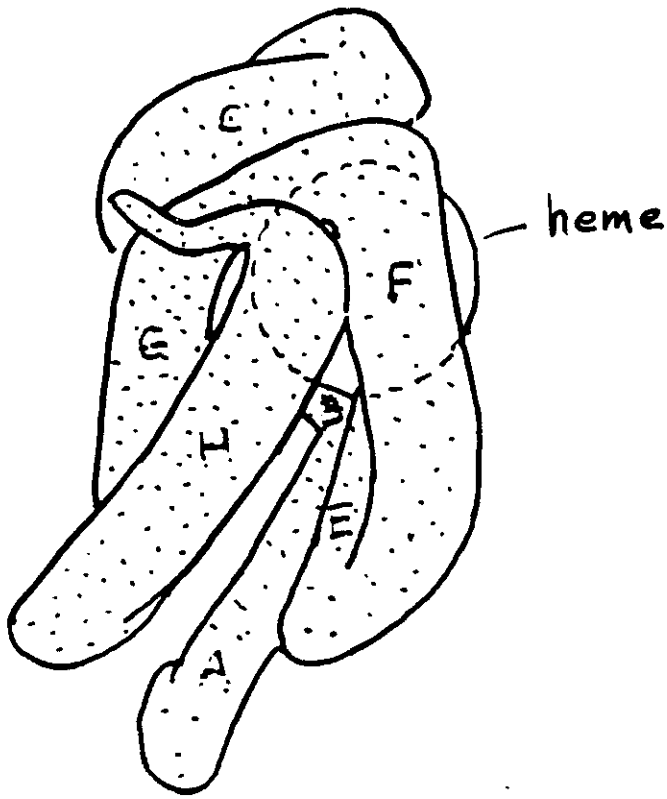
Trieste, March 1996

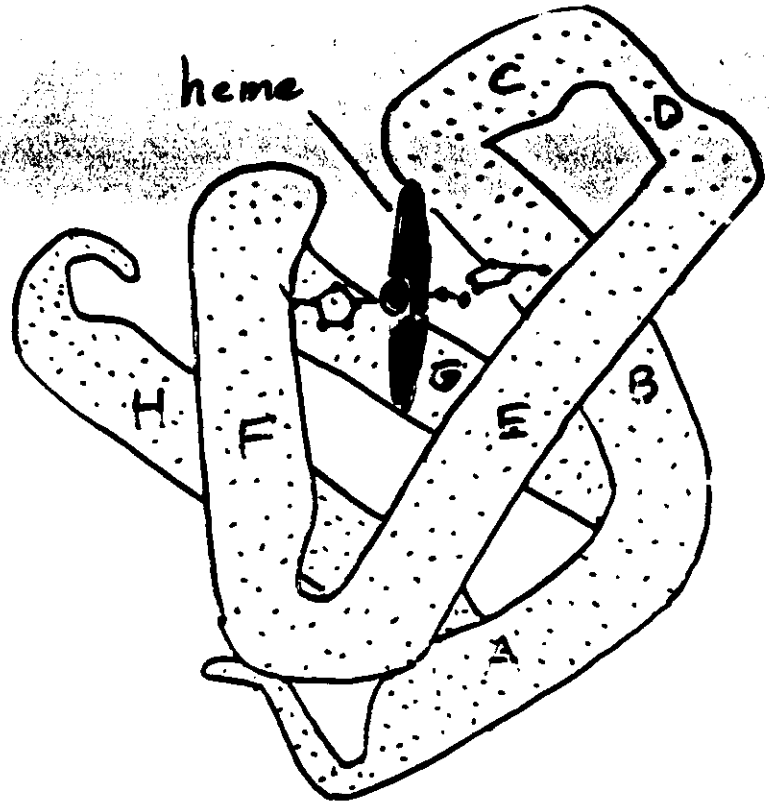
Lecture I

"Photophysics, protein physics,
and Myoglobin -
the hydrogen atom of biology"

Myoglobin






heme
C
D
H
G
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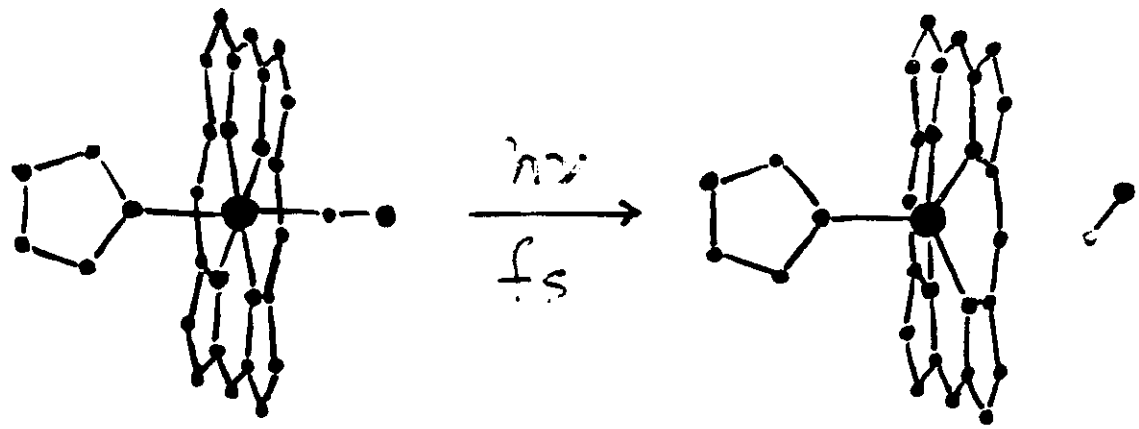
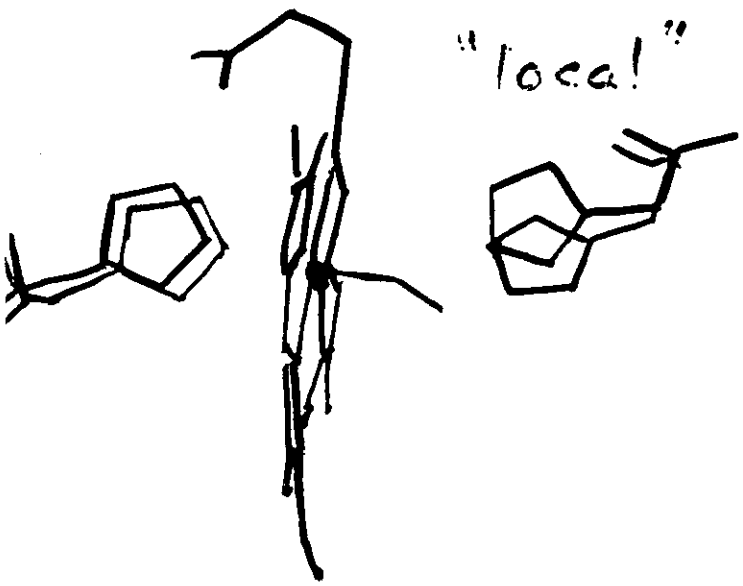
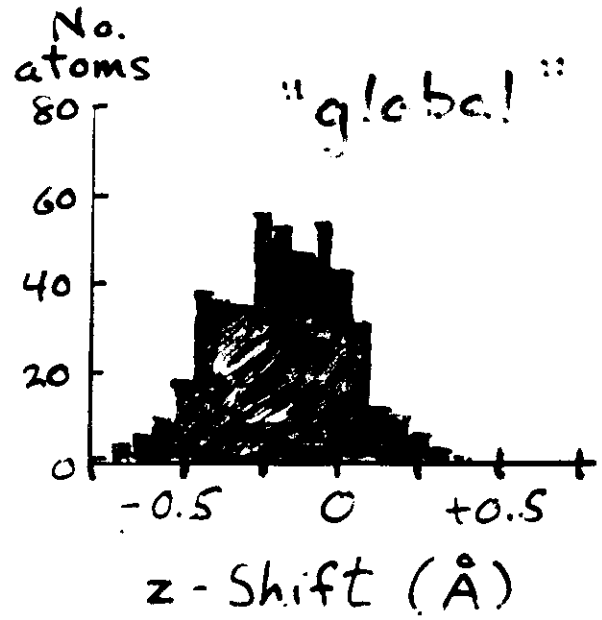
E
A


hν
fs


"local"

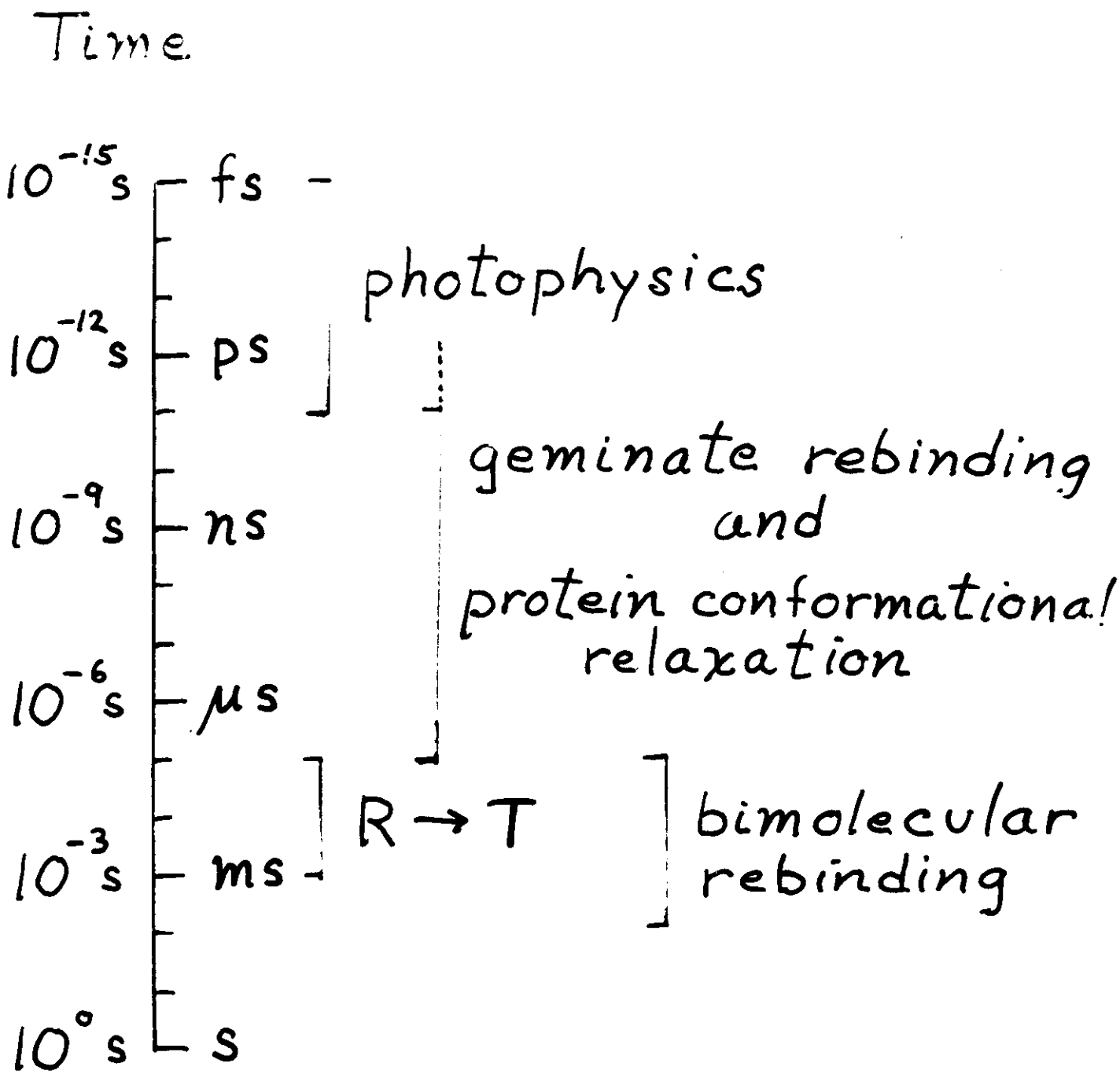

No. atoms
80
60
40
20
0
-0.5
0
+0.5
z - Shift (Å)
"global"

Novel results on myoglobin needed to explain more complex hemoglobin

1. geminate rebinding
2. conformational substates
3. conformational relaxation extended in time ($\sim e^{-(kt)^{\beta}}$) explained by "physical kinetics"
4. relaxation slows geminate rebinding

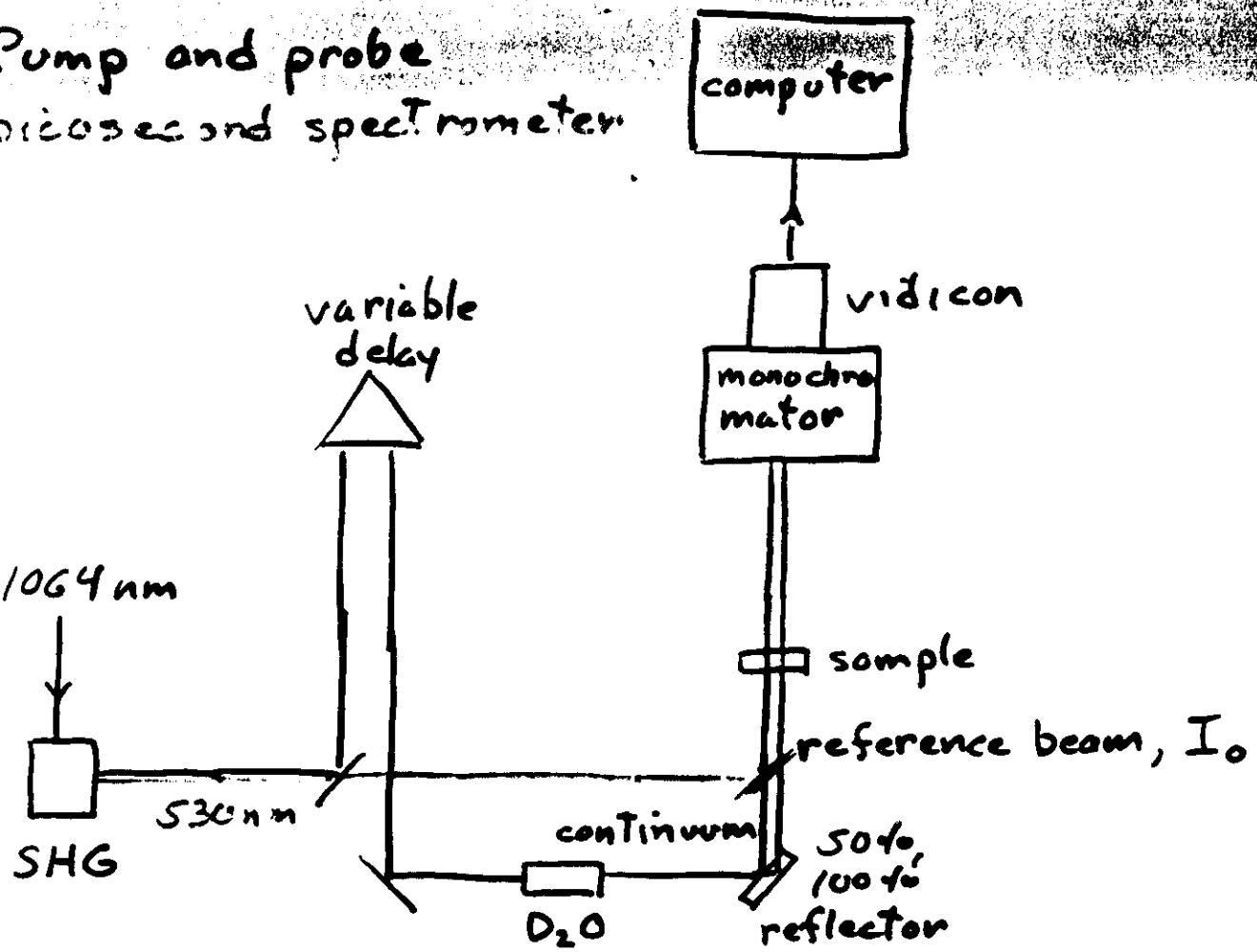
Pulsed Optical Excitation of Hemes and Heme Proteins

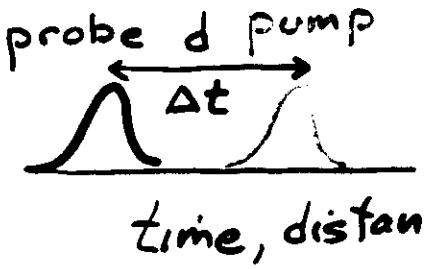
room T



How fast is ligand dissociated?

Pump and probe
picosecond spectrometer



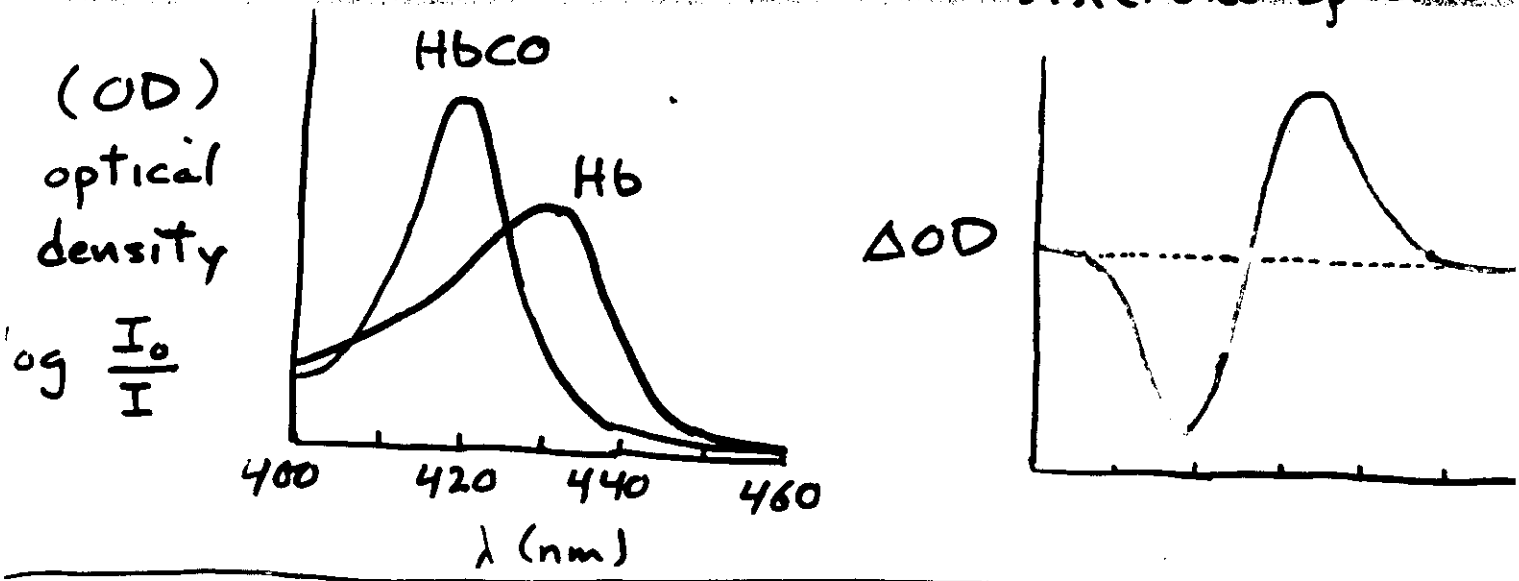


$$\Delta t = \frac{\Delta d}{c}$$

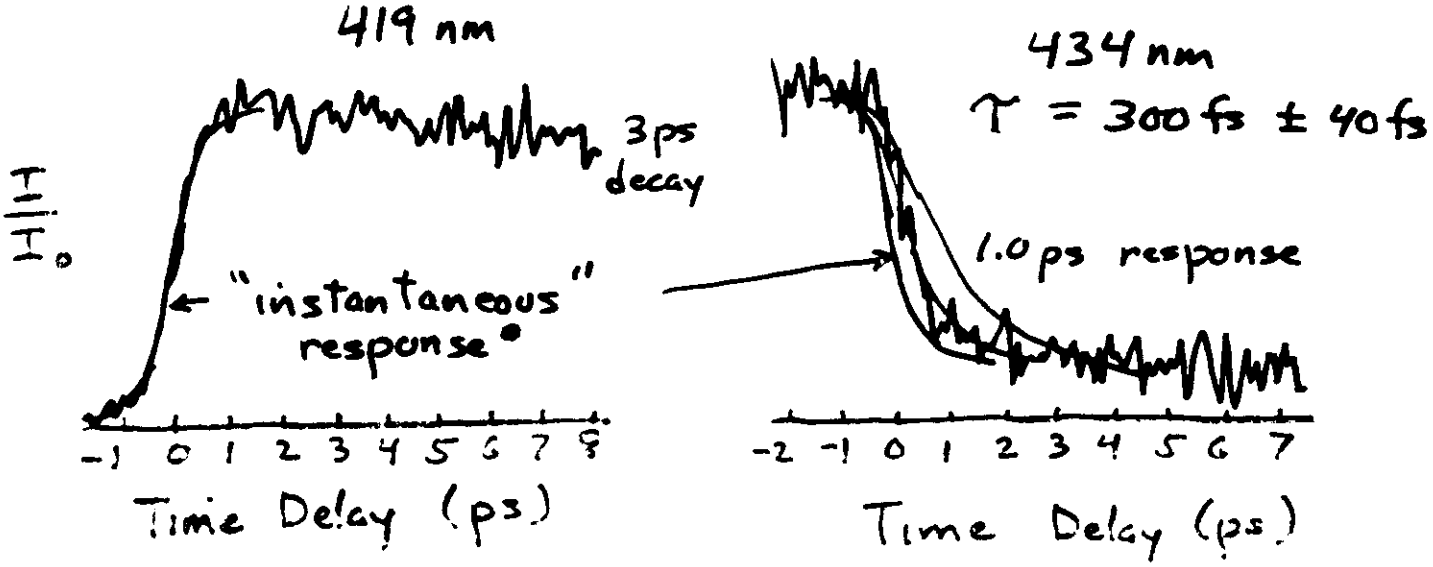
$c = 3 \times 10^{10}$ cm/sec

1 ps = 0.3 mm

B (Soret) bands — Static Difference Spectrum

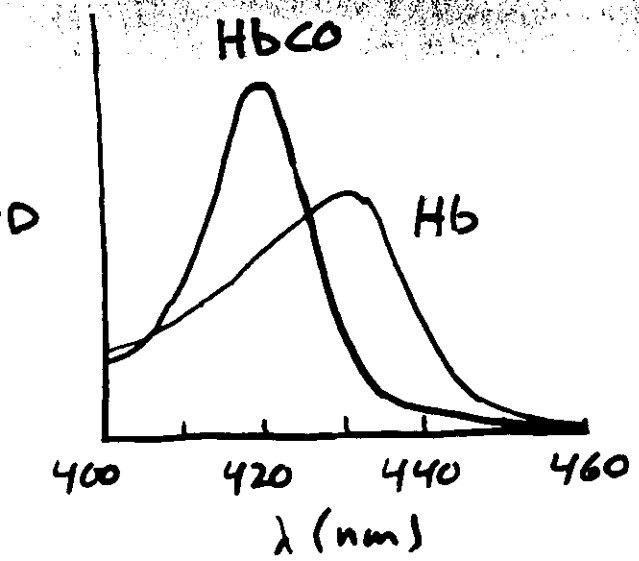


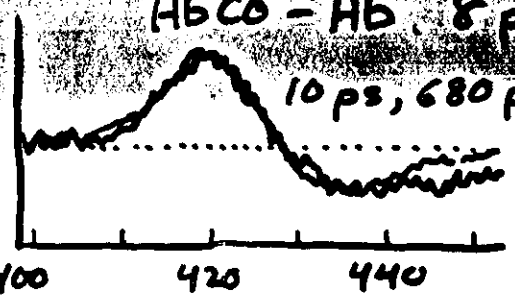
..Martin Excitation with 0.25ps pulse



CO has dissociated and iron displaced within 300 fs.

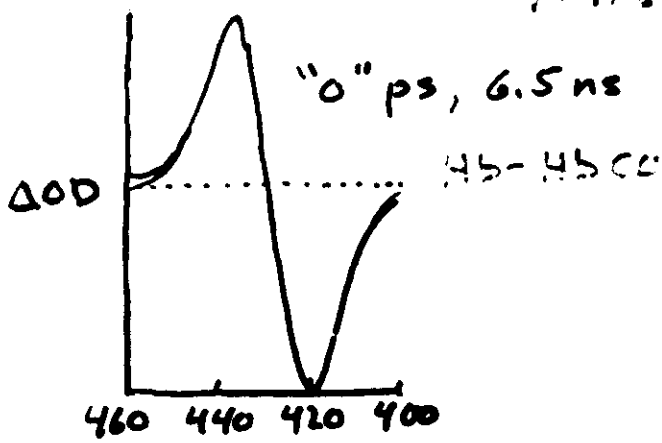
Discovery of geminate recombination





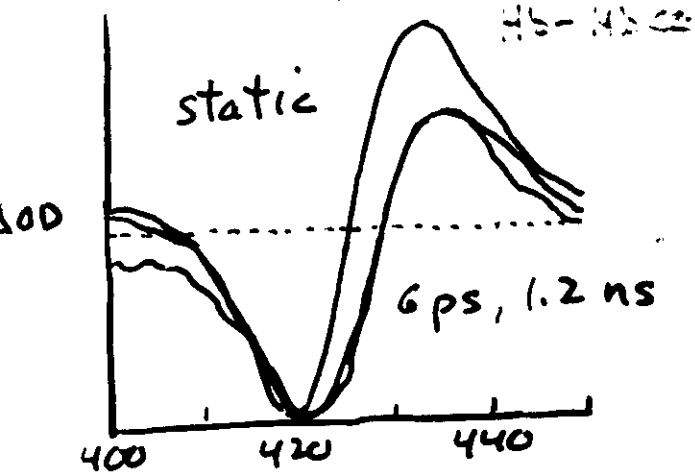
1978 - no geminate rebinding
≤ 6?? ps

1984 - confirmed, 30 ps.
10 Hz

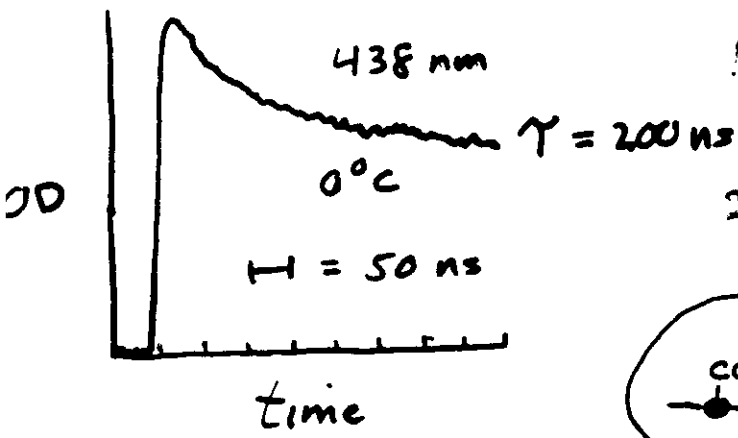


no spectral change

1984 - 8 ps, ? Hz



1979 - 30 ns, scope

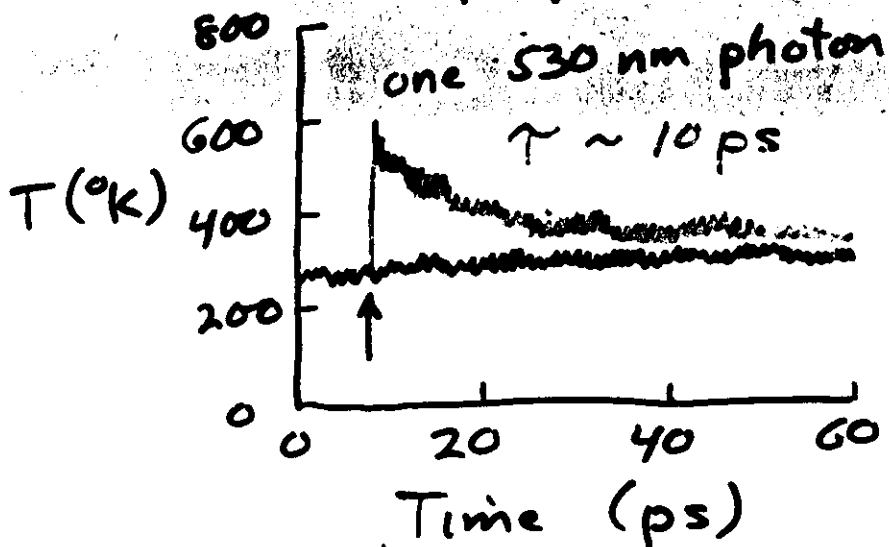


Geminate rebinding
because

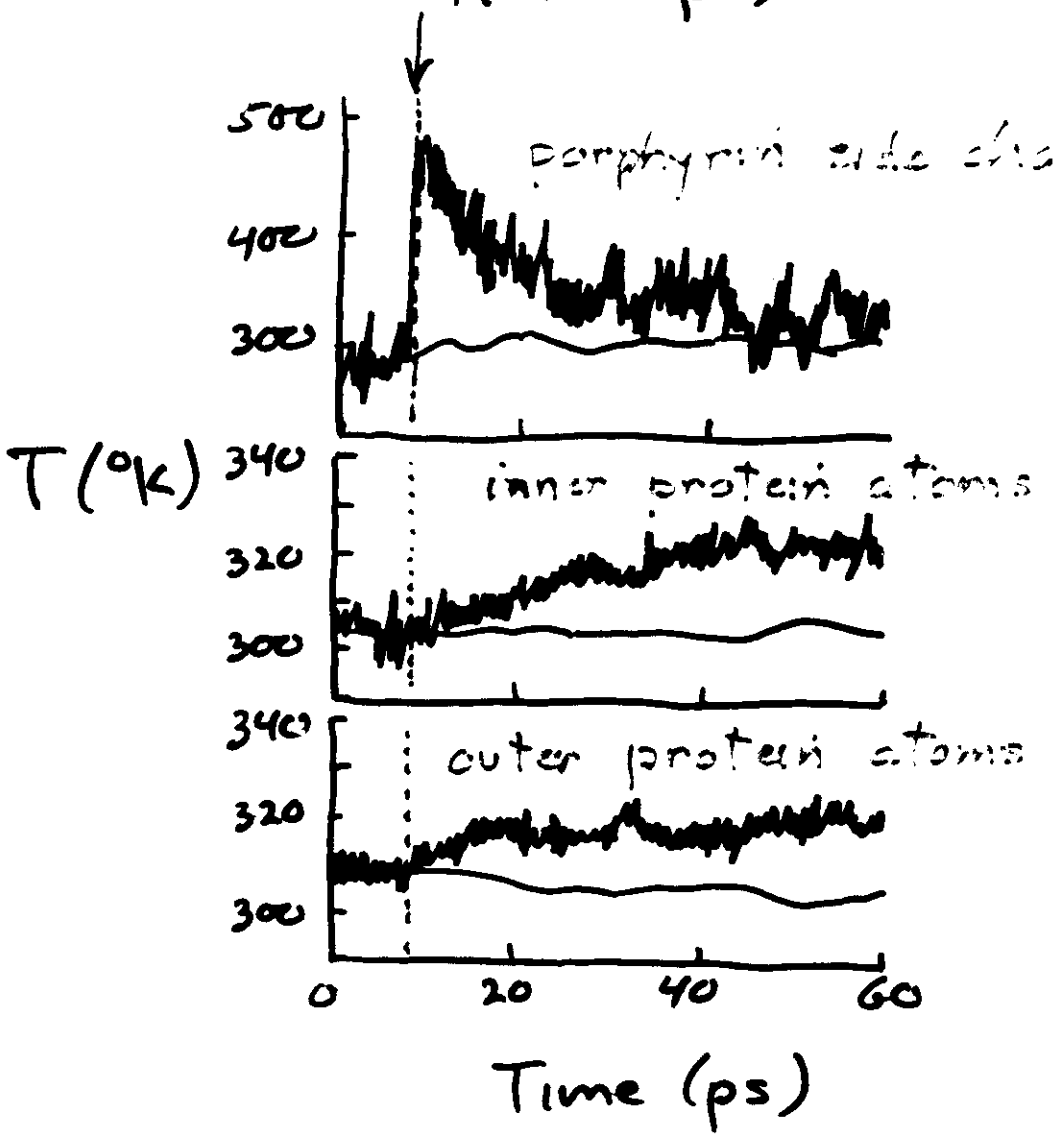
1. $5 \times 10^9\ M^{-1}s^{-1}$ too fast for bimolecular
2. τ pressure independent

Eric Henry (NIH)

24 porphin atoms



$$\frac{3}{2} N k_B T = \sum_i \frac{1}{2} m_i v_i^2$$

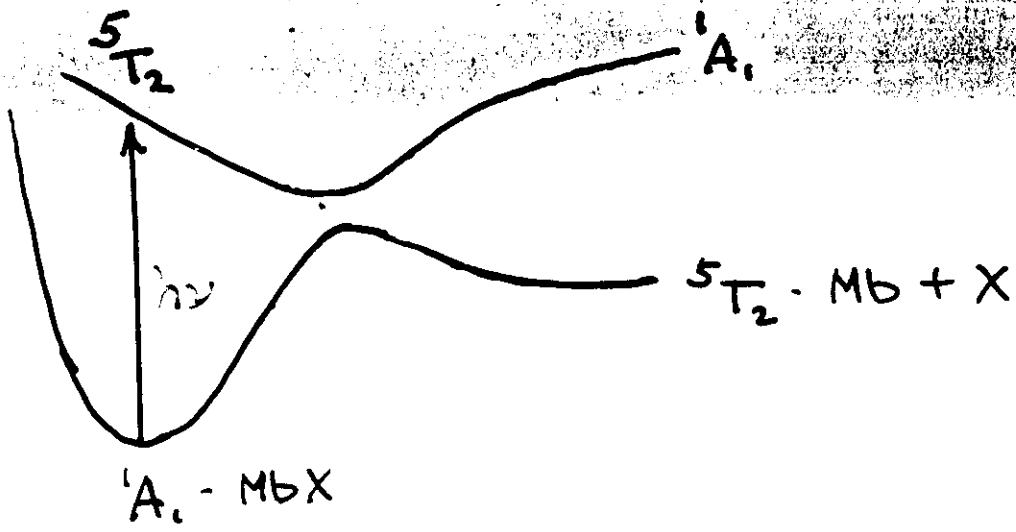


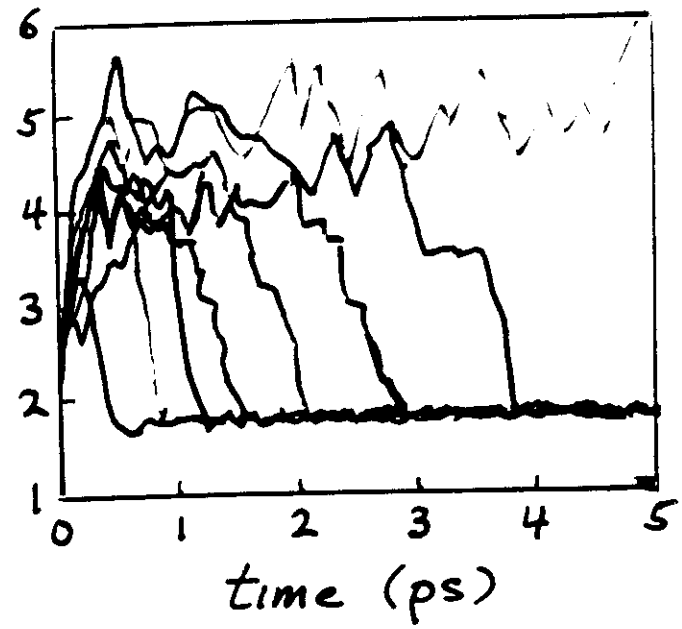
Thermal effects potentially very important at times < 20 ps

QM simulations?

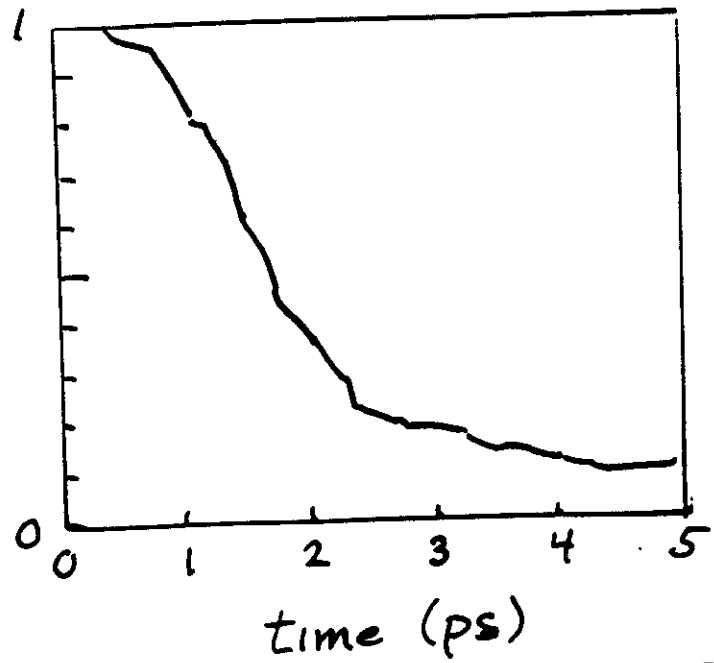
Simulations of Photolysis Experiments

Olivier Schaad
(NIH)





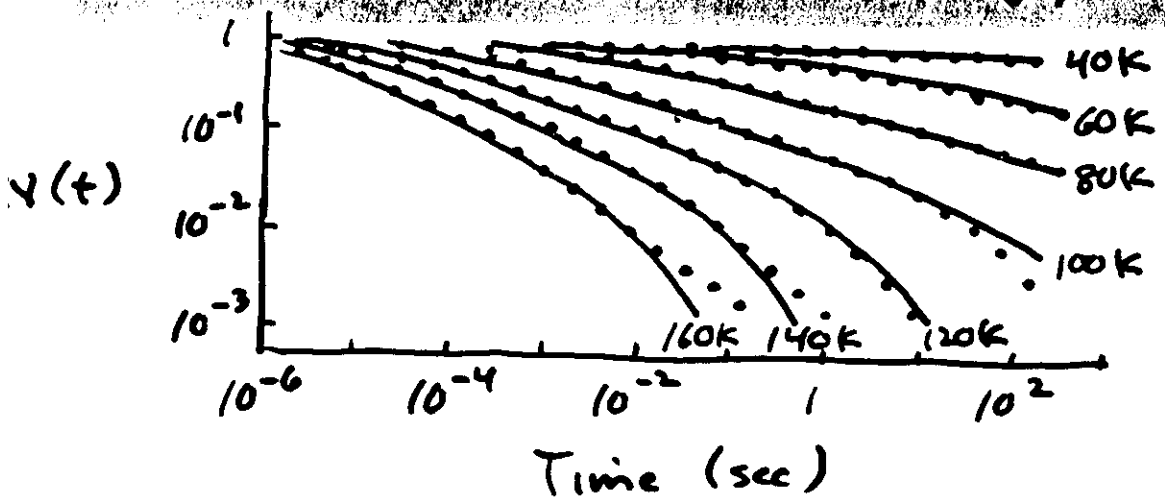
barrierless trajectories



histogram of rebinding times

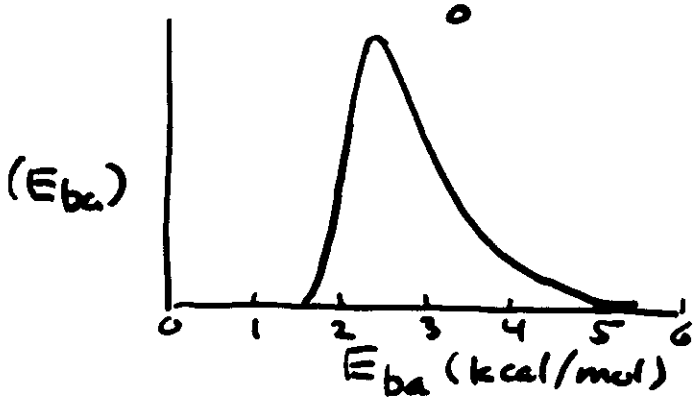
Conformational Substates – 1975.

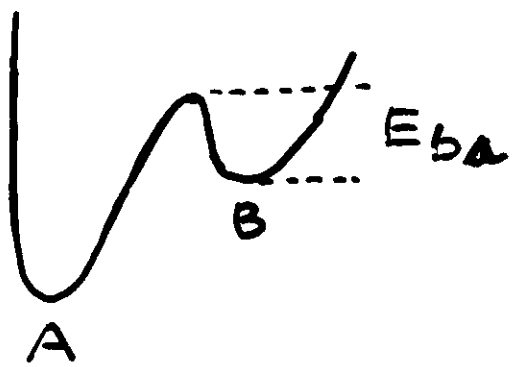
MbCO 3:1 glycerol-water



Basic Idea: multiple conformations each with different activation energy "frozen" in at low temperature, to produce a distribution of rates.

$$N(t) = \int_0^\infty g(E_{ba}) e^{-k_{ba}t} dE_{ba}, \quad k_{ba} = A_{ba} e^{-E_{ba}/RT}$$





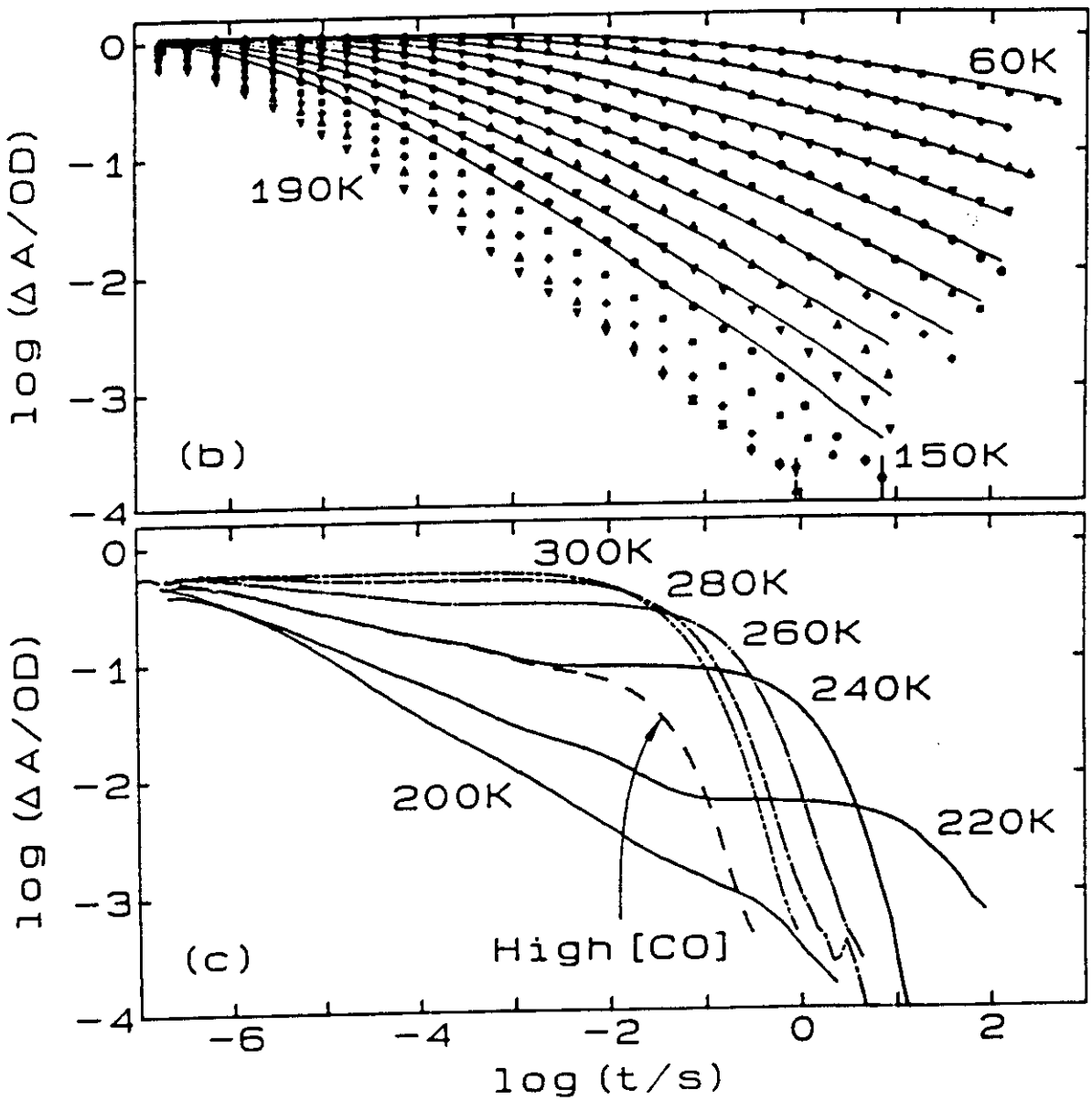
At high temperature, if relaxation among substates is very fast, $k_r \gg k_{ba}^{max}$ – rebinding is exponential

$$N(t) = e^{-\int g(E_{ba}) k_{ba} t \, dE_{ba}} = e^{-k_{ba}^{mean} t}$$

Frauenfelder and coworkers:

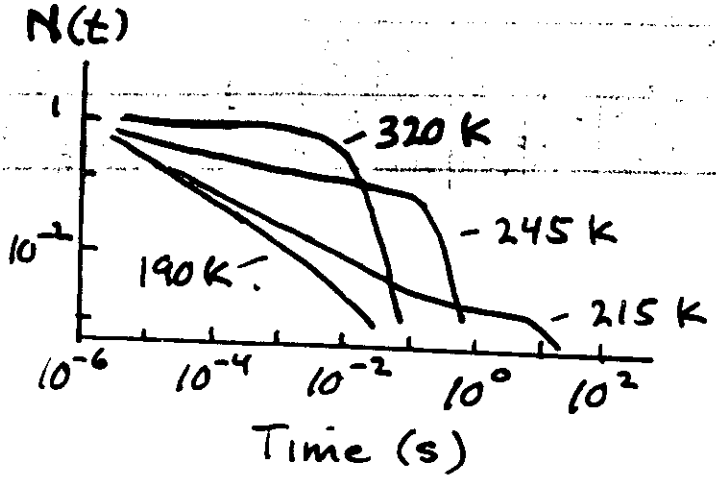
Below T_g : distribution of rates from "frozen" substates.

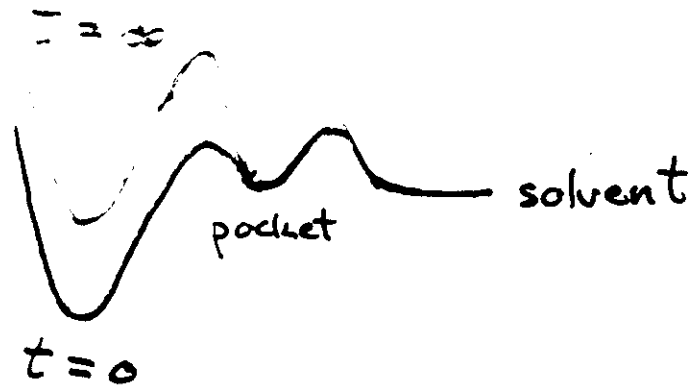
Above T_g : geminate rebinding slows with <u>increasing</u> temperature

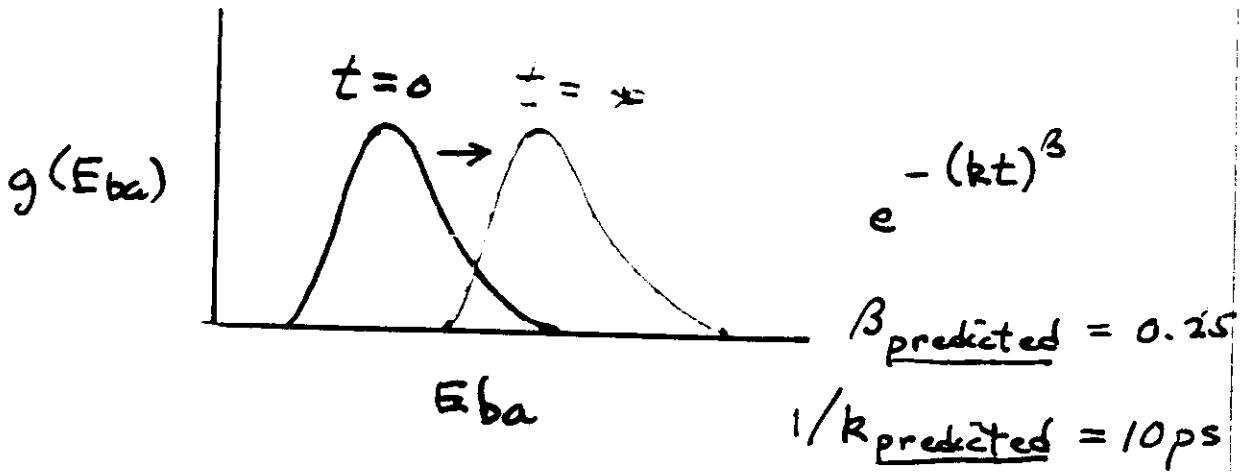


Models: multiple geminate states

$MbCO \rightleftharpoons Mb:CO \rightleftharpoons Mb::CO \cdots \rightleftharpoons Mb + CO$





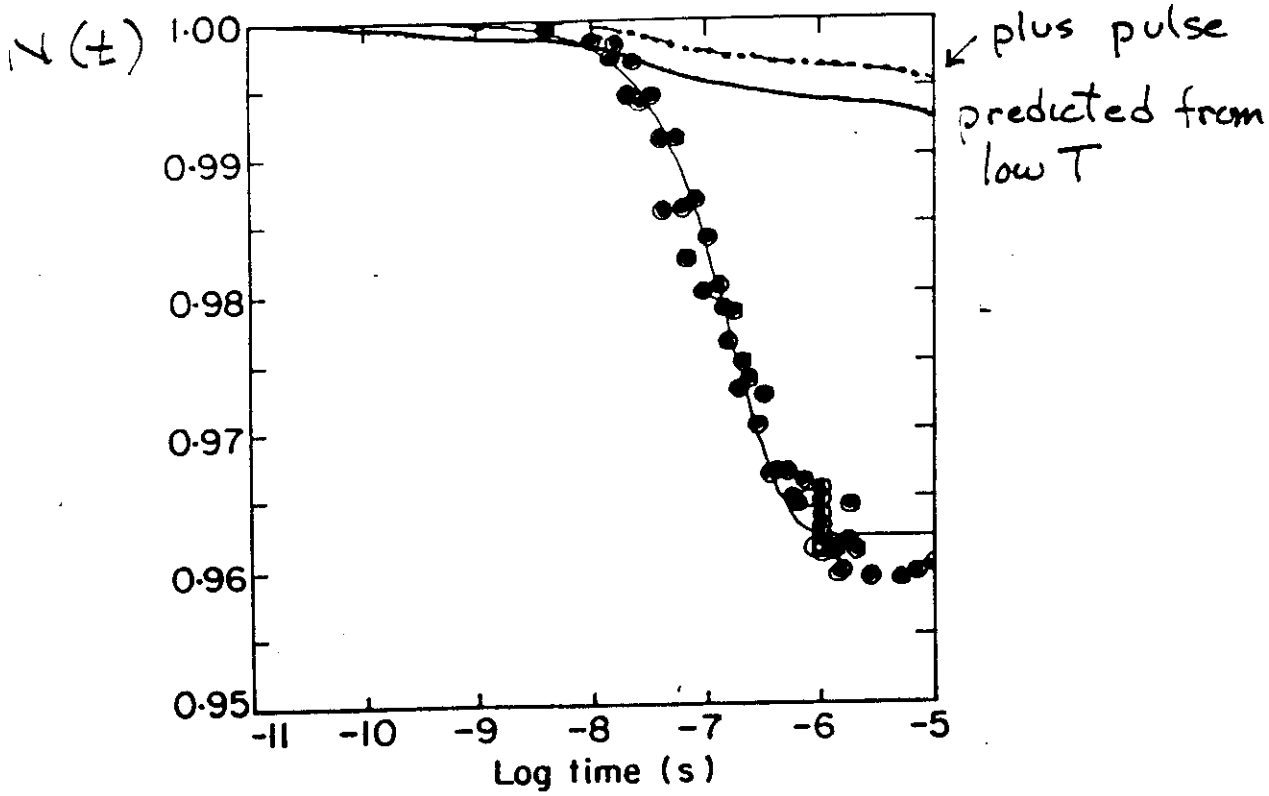


An alternative view (Agmon & Hopfield, 1983)
- protein relaxation shifts barrier height distribution.

Stembach, Frauenfelder et al. 1991 —
predicted kinetics of protein relaxation

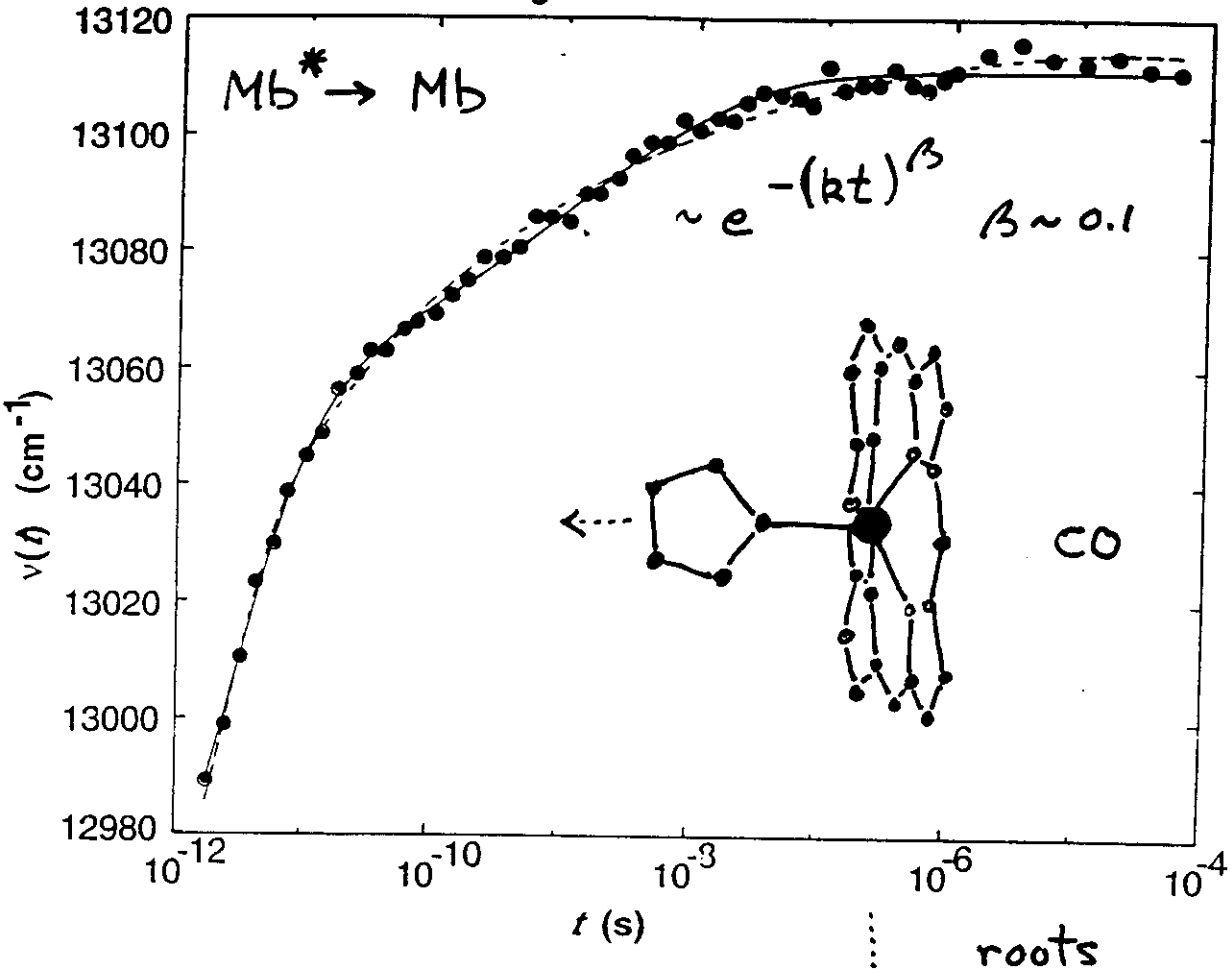
enry et al., 1983

Discovery of geminate rebinding of CO in liquid Mb solution



Stephen Hagen (NIH); Data of Philip Anfinrud (Harvard)

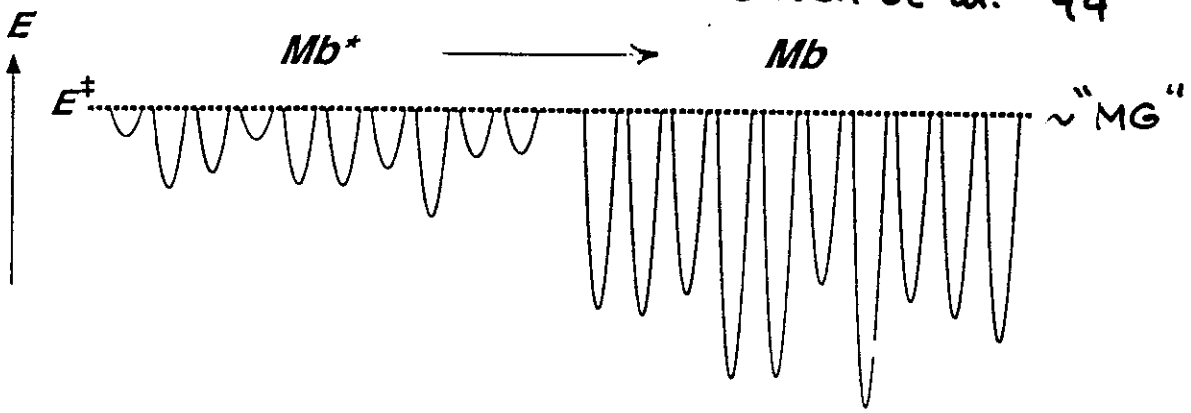
$a_{2u}(\pi) \rightarrow Fe(d_{xz})$ charge transfer



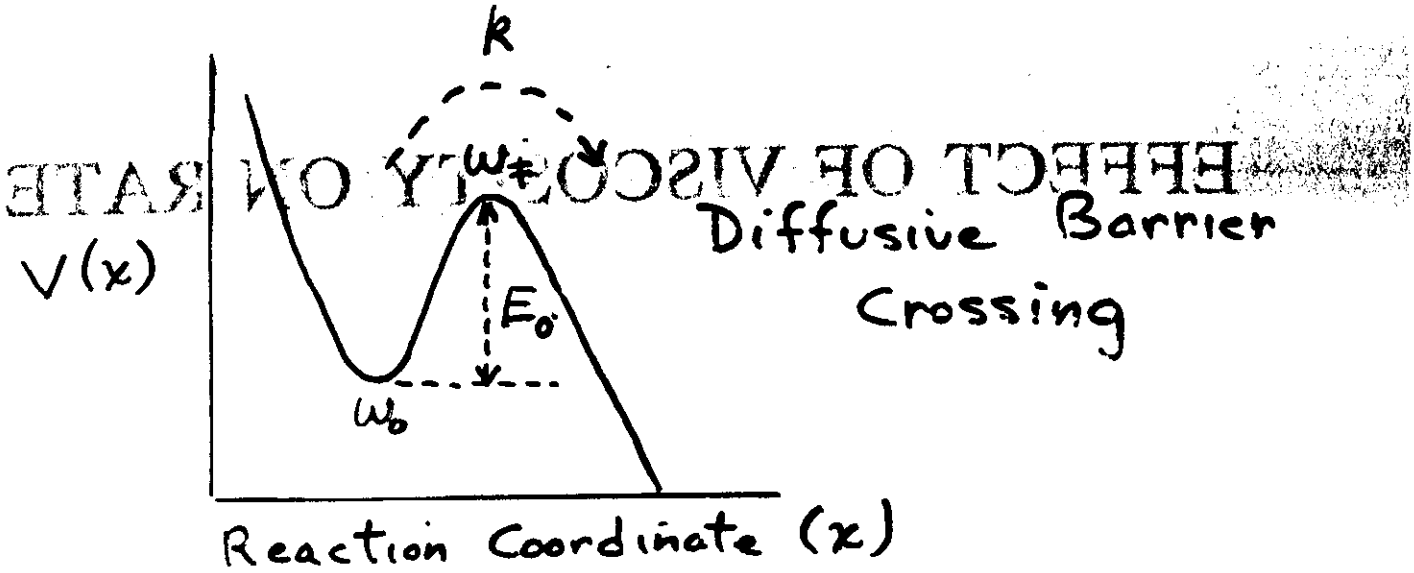
roots

De Dominicis et al. '85
Koper & Hilhorst '87
Shakhnovich & Gutin '89
Frauenfelder et al. '91
Saven et al. '94

A Model



$$k_{ij}(T) = k_0 g(E_j) dE \exp[-(E^{\ddagger} - E_i)/RT]$$



Dynamics described by Langevin equation:

$$M\frac{d^2x}{dt^2} = -\frac{dV}{dx} - \zeta\frac{dx}{dt} + R(t)$$

which, in high friction limit, yieids Kramers' equation:

$$k = \frac{M\omega_o\omega_*}{2\pi\zeta}\exp(-\frac{E_o}{k_BT})$$

Assuming that protein friction and solvent friction are additive, Kramers' equation is:

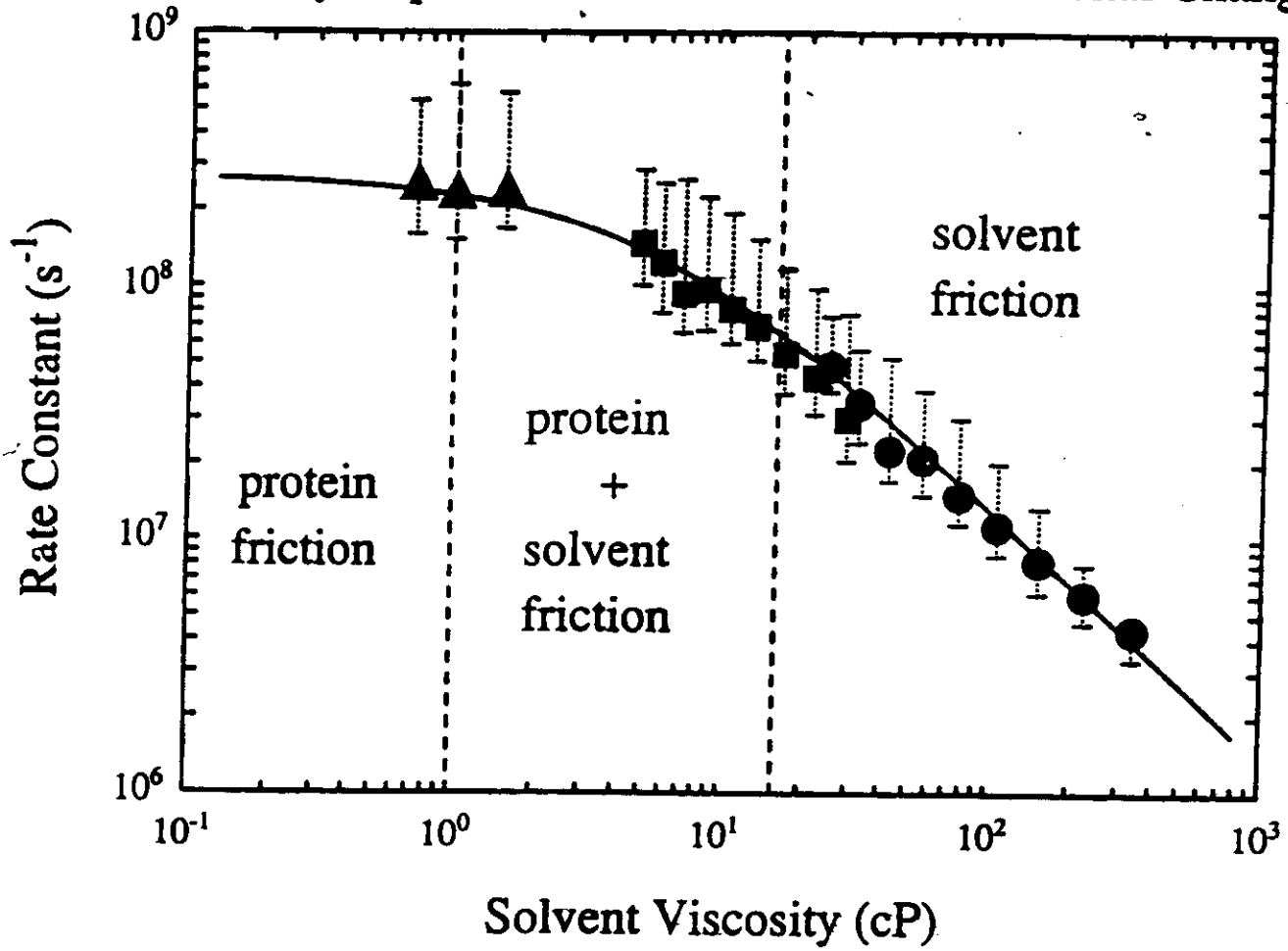
$$k = \frac{B}{\alpha\zeta_p + (1-\alpha)\zeta_s}\exp(-\frac{E_o}{k_BT})$$

which, using Stokes' law, becomes:

$$k = \frac{C}{\sigma + \eta_s}\exp(-\frac{E_o}{k_BT})$$

Myoglobin

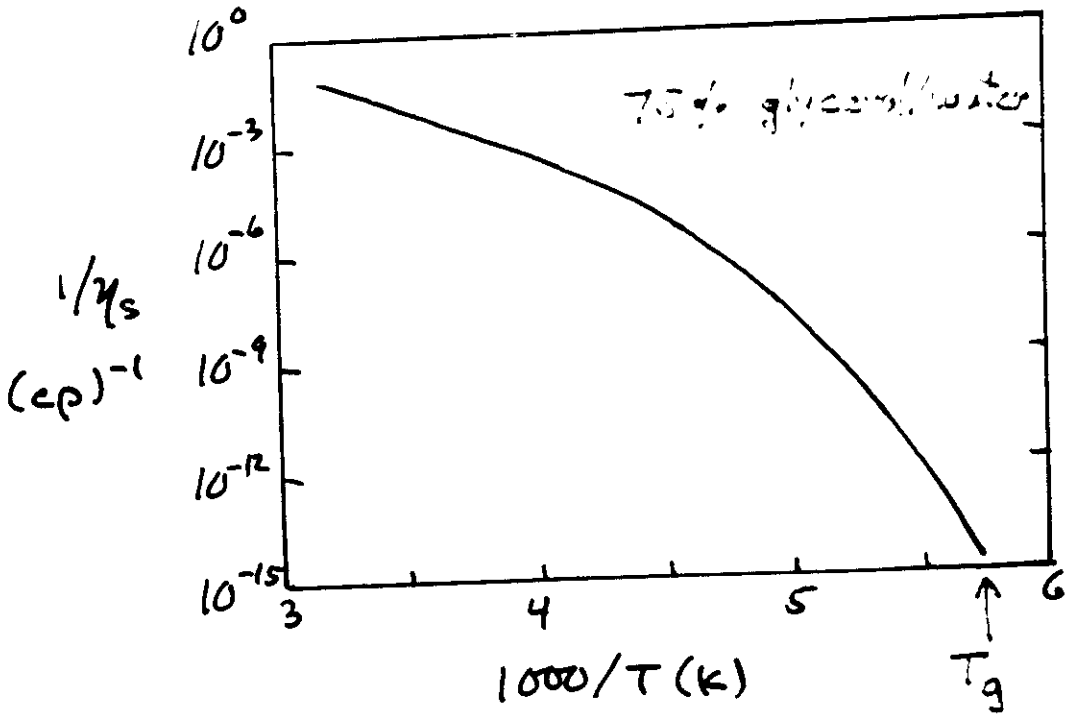
Viscosity Dependence of Protein Conformational Change



$$k = \frac{C}{\sigma + \eta} \exp(-E_a/RT)$$

protein "viscosity" ← solvent viscosity

$$k = \frac{C}{\sigma + \eta_s} e^{-E_o/RT}$$



$\sigma \sim 4$ cp , $E_o \sim 2$ kcal/mole

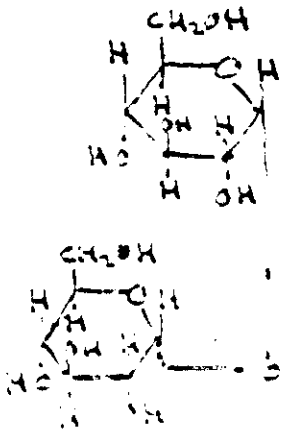
Slowing near T_g due to solvent viscosity
(not glass transition of protein)

$\eta_s \sim 10^{11}$

$e^{-E_o/RT} \sim 10^2$

Stephen Hagen
(NIH)

Myoglobin in a trehalose glass



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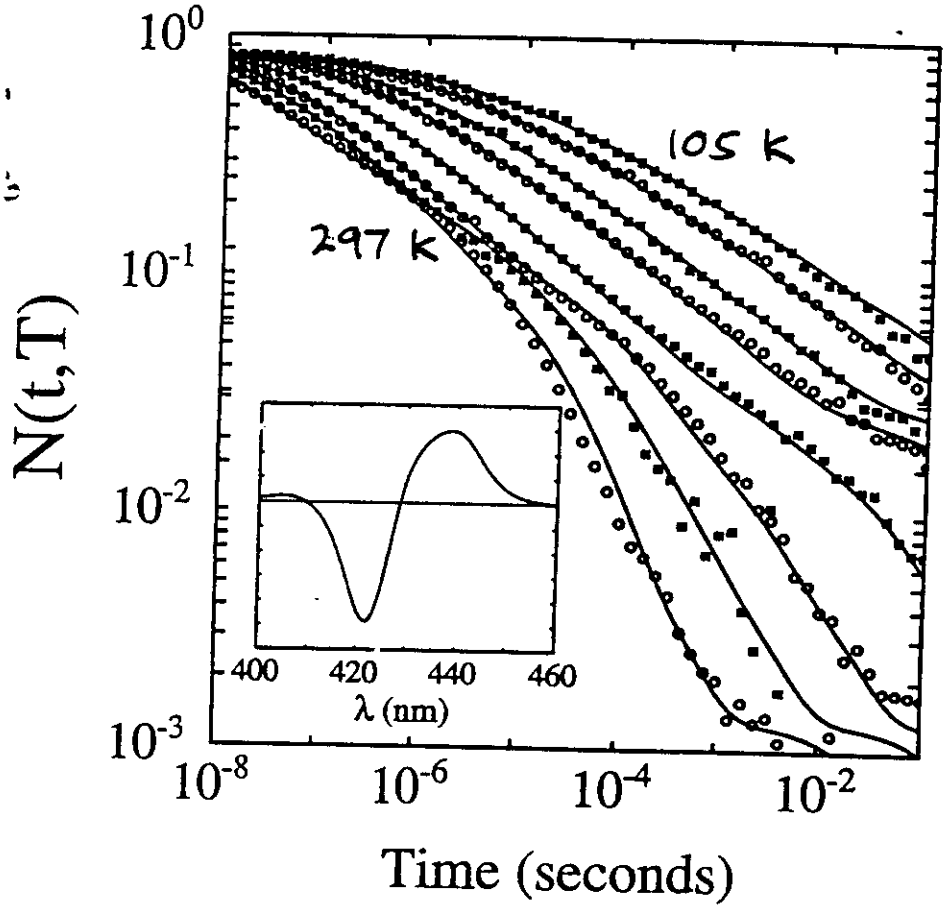

Recall:

$$N(t) = \exp\left[-\int g(E_{ba}) k_{ba} t \, dE_{ba}\right]$$

$$= \exp\left[-k_{ba}^{mean} t\right]$$

$k_{ba}^{mean} = 5 \times 10^7 \ s^{-1}$ unrelaxed protein

$k_{ba}^{water} = 2 \times 10^4 \ s^{-1}$ relaxed protein

Protein "stuck" in "excited" (fast reacting) conformational substates.