UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION
INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
I.C.T.P., P.O. BOX 586, 34100 TRIESTE, ITALY, CABLE: CENTRATOM TRIESTE

SMR.961 - 23

WORKSHOP ON:
PROTEINS, MEMBRANES and their INTERACTIONS

22 JULY - 2 AUGUST 1996

"Time-resolved spectroscopy - understanding the mechanism of protein function "
PART I

"Diffusional dynamics of unfolded proteins: estimating an upper limit for the rate of protein folding"
PART II

William A. EATON
National Institutes of Health
Public Health Service
Department of Health & Human Services
Building 5, Room 104
20892-0520 Bethesda
U.S.A.

Trieste, July 1996, W. A. Eaton

Lectures on Protein Kinetics and Dynamics

*I. Hemoglobin / Myoglobin - function

II. Dynamics of unfolded protein

III. Observing "fast" protein folding

* Protein folding is not the only interesting area in protein physics and physical chemistry. Techniques are applicable to folding problem

Lecture I: "Time-resolved spectroscopy - understanding the mechanism of protein function"

Question: Can a two-state (MWC, allosteric) model explain hemoglobin kinetics.

Outline of Lecture:

I. The two-state model of Monod, Wyman, and Changeux (MWC)

II. Hemoglobin kinetic data

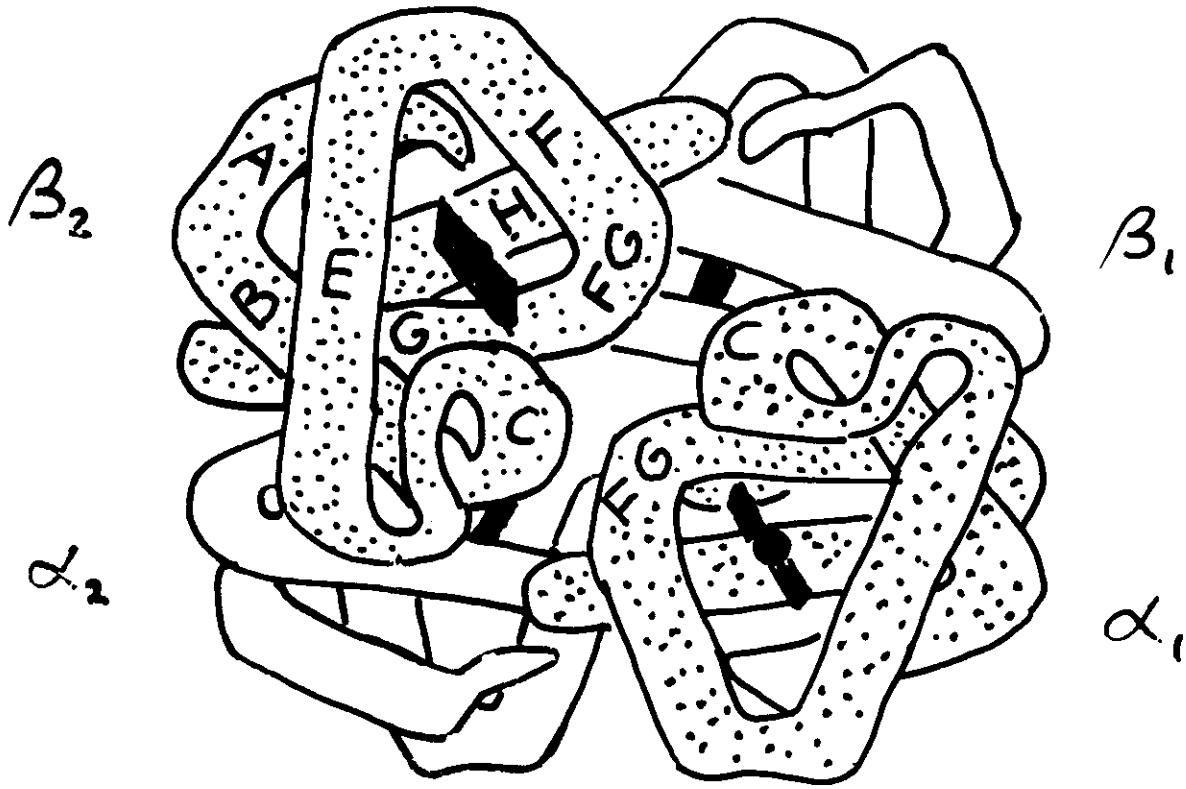
III. Lessons from:
femtosecond/picosecond spectroscopy
molecular dynamics simulations
physics of myoglobin

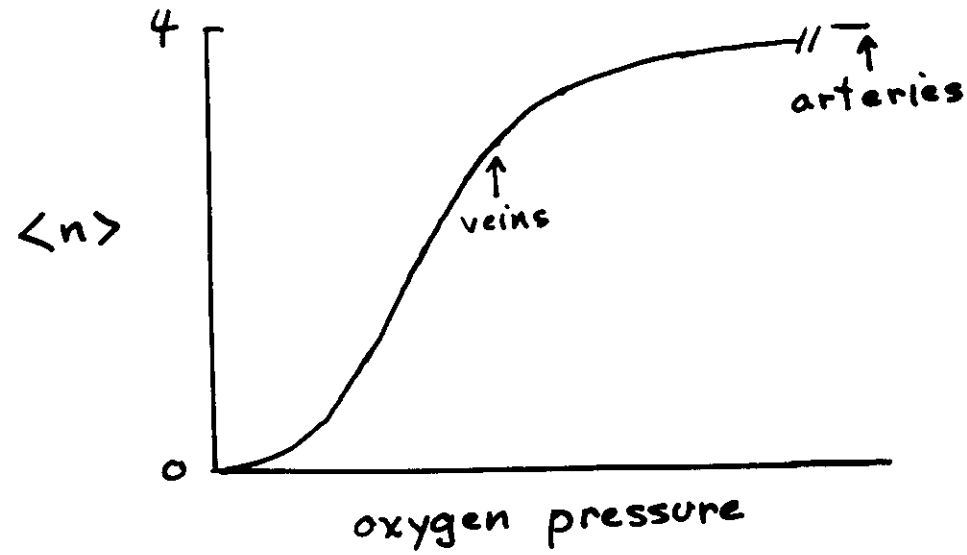
IV. Kinetic model: an "obvious" extension of MWC model.

V. Fitting kinetic data

VI. What next?

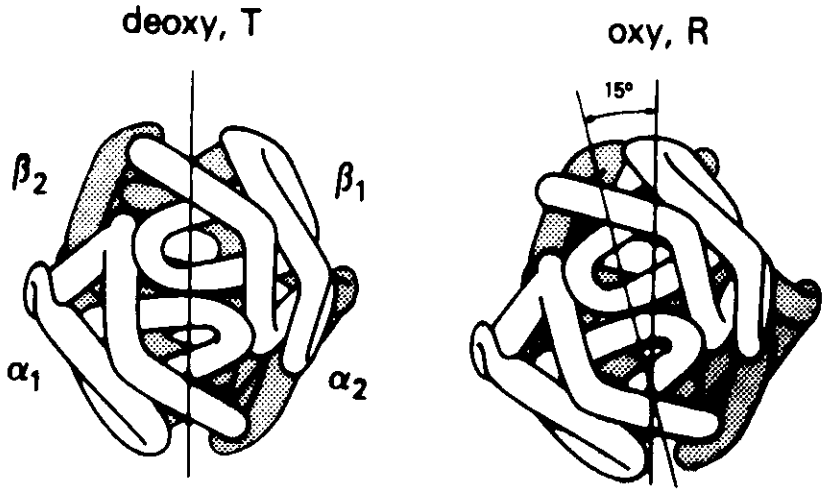
Collaborators: Stephen Hagen
Eric Henry
James Hofrichter
Colleen Jones

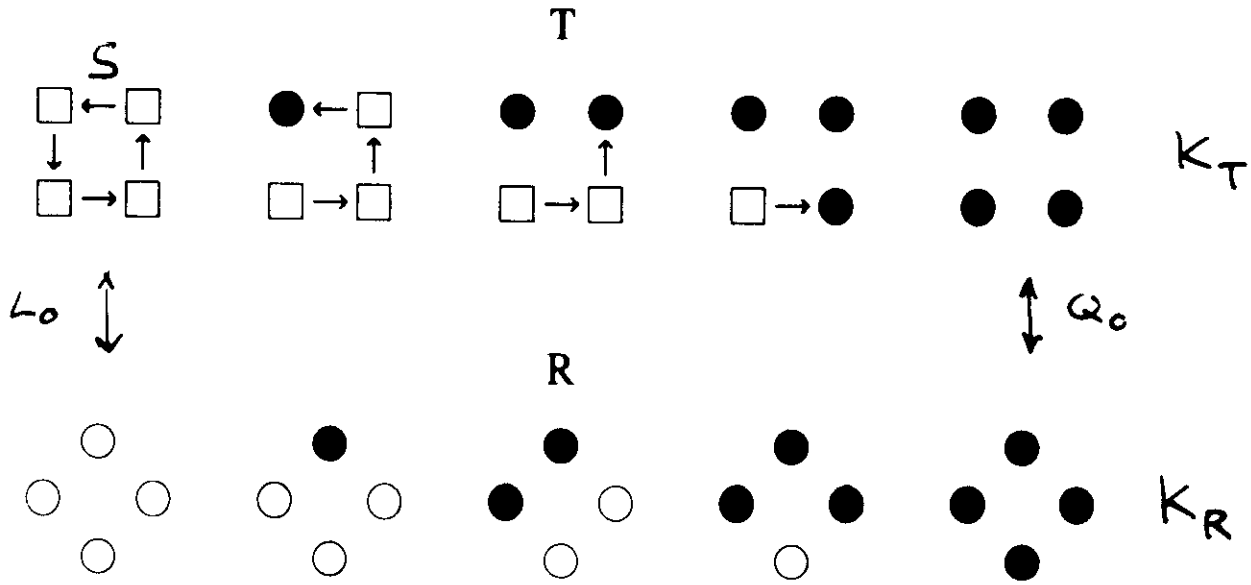

β_2
β_1
α_2
α_1
A
B
E
H
G
C
D
F
G
C
F
G


4
0
<n>
veins
arteries
oxygen pressure

MWC - PSK Model

Monod
Wyman
Changeux
Perutz
Szabo
Karplus





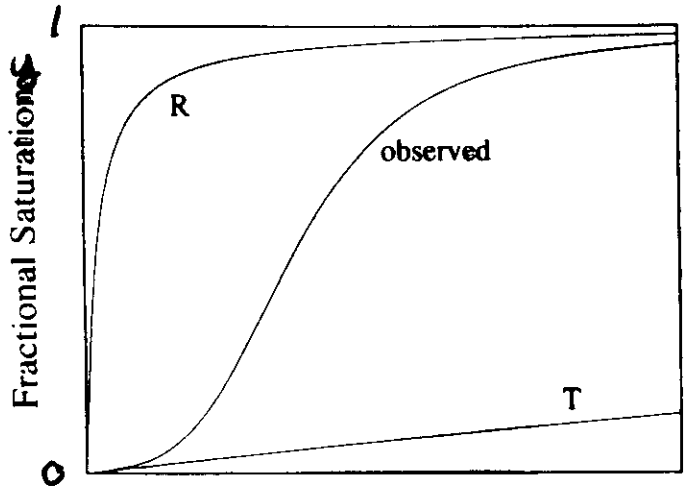
MWC

K_R, K_T, L_0

$L_i = L_0 c^i$

SK

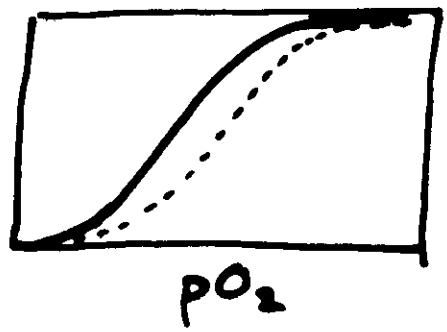
K_R, S, Q_0

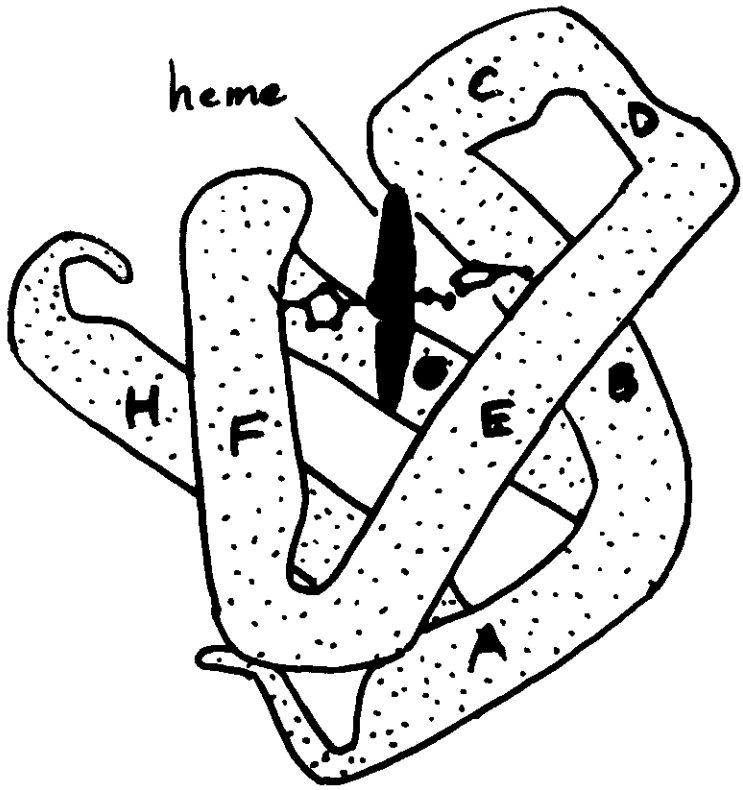


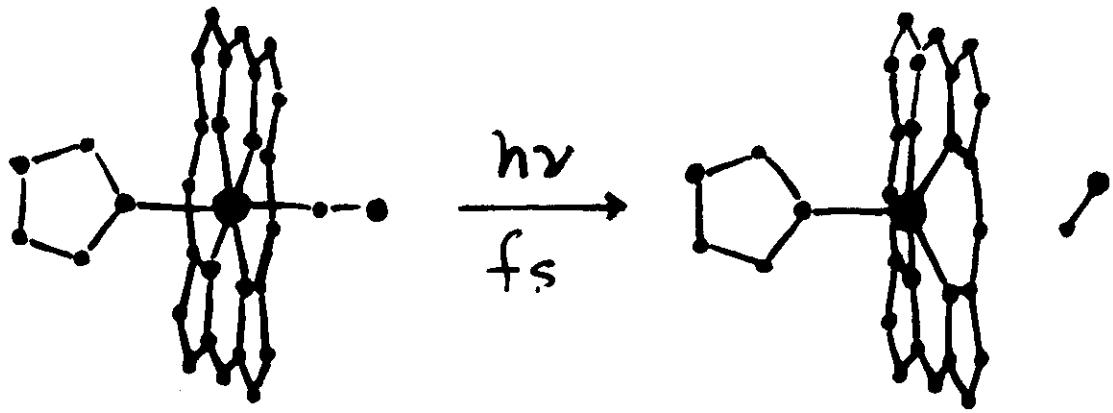
$K_T = K_R / S$

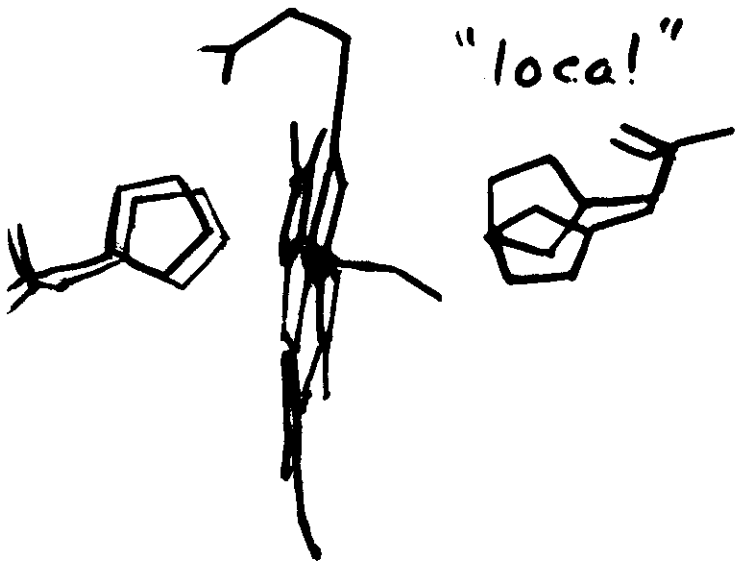
$L_0 = Q_0 S^4$

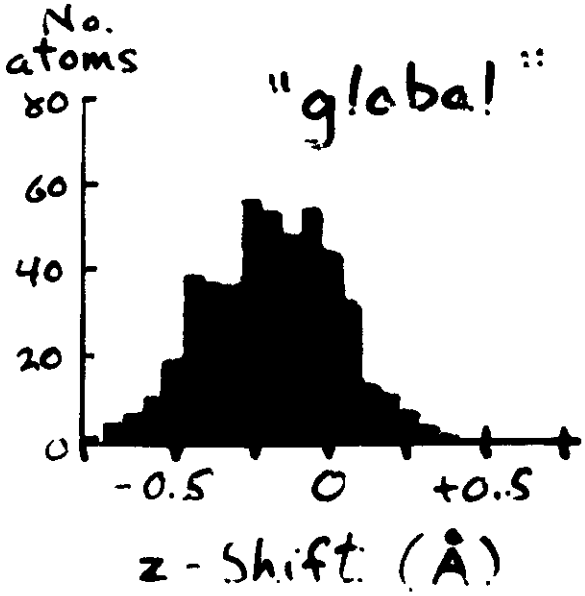
Short History of Hemaglobin Research

1904	Bohr	y 
1920	Roughton	reaction very fast
1925	Adair	tetramer
1935	Pauling	heme-heme interaction
1959	Gibson	$HbCO \xrightarrow{h\nu} Hb^*$ "fast reacting Hb"
1963	Perutz	2 quaternary structures
1965	Monod Wyman Changeux	= 2 affinity states
1970	Perutz	detailed stereochemical mechanism
1972	Szabo Karplus	partition function for Perutz model (modified MWC


heme
C
D
H
F
E
B
A

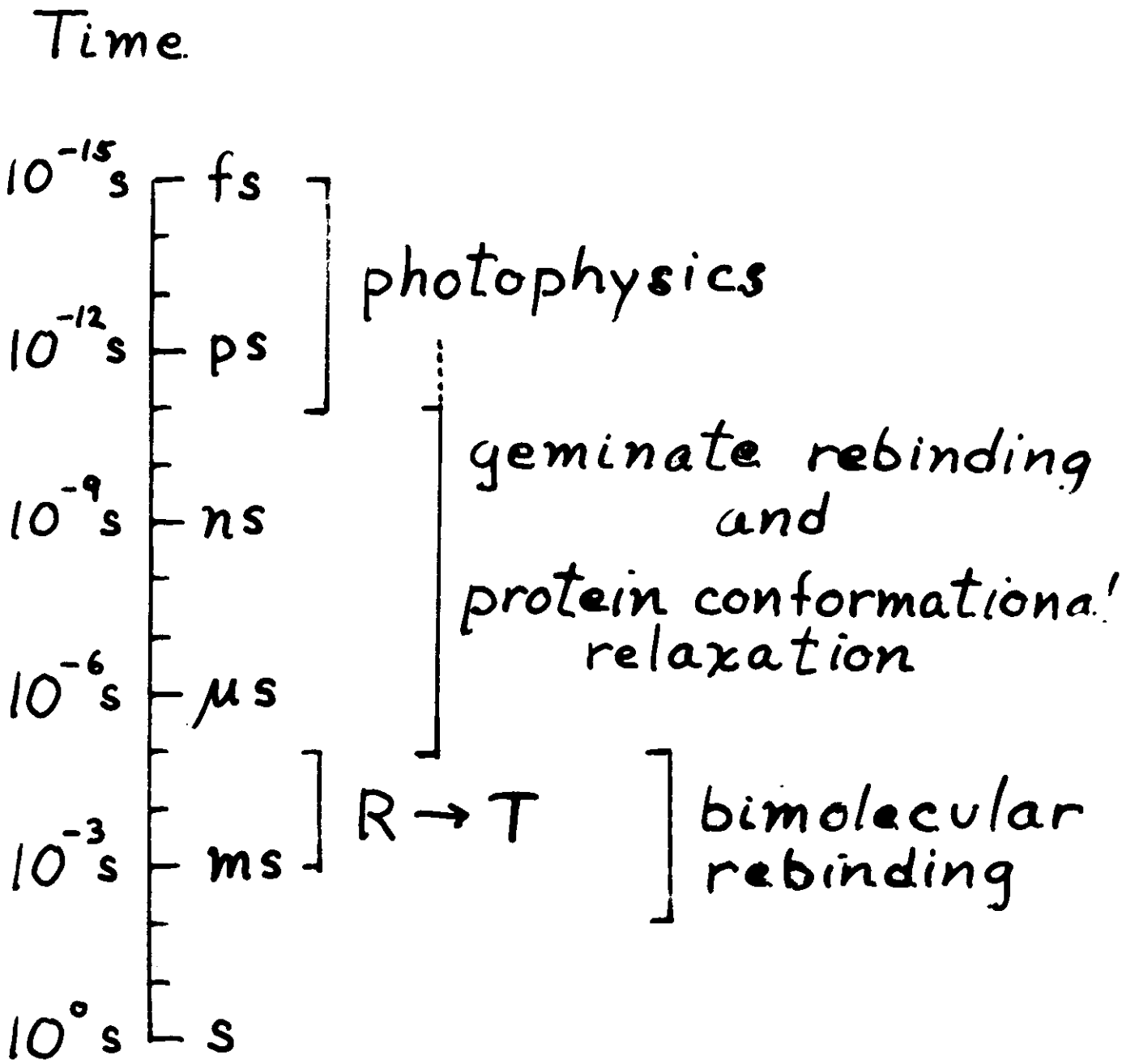

hν
fs

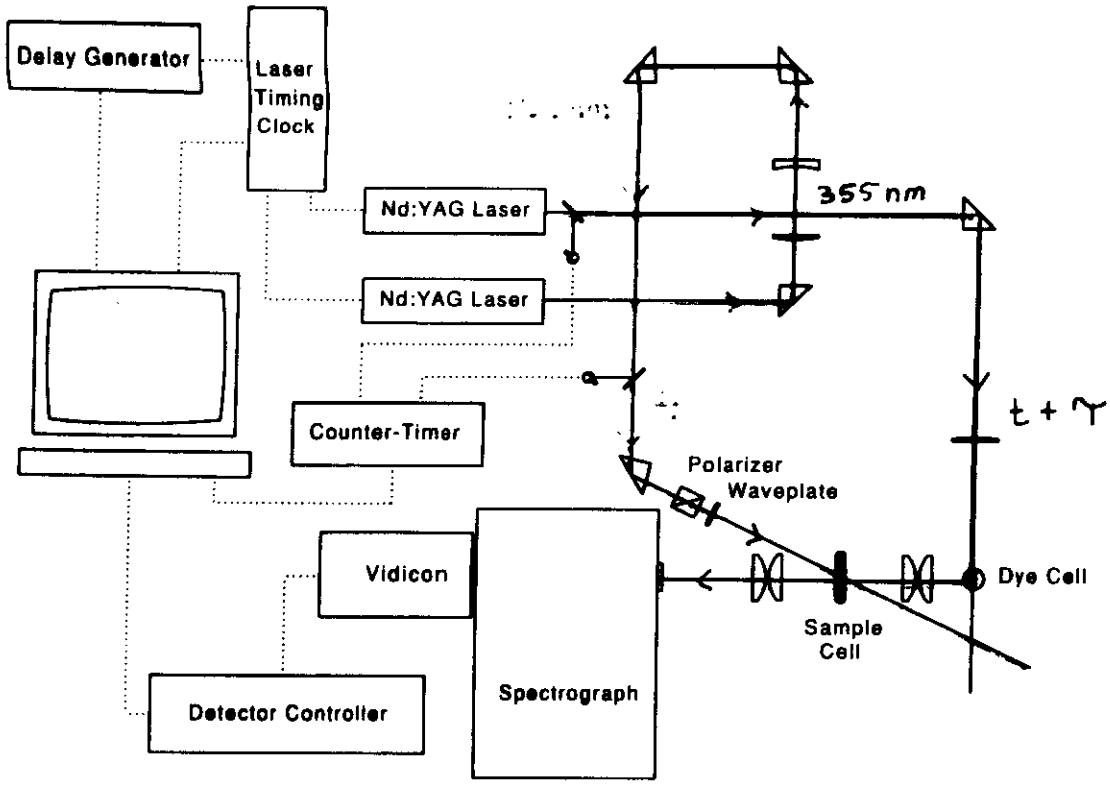

"local"

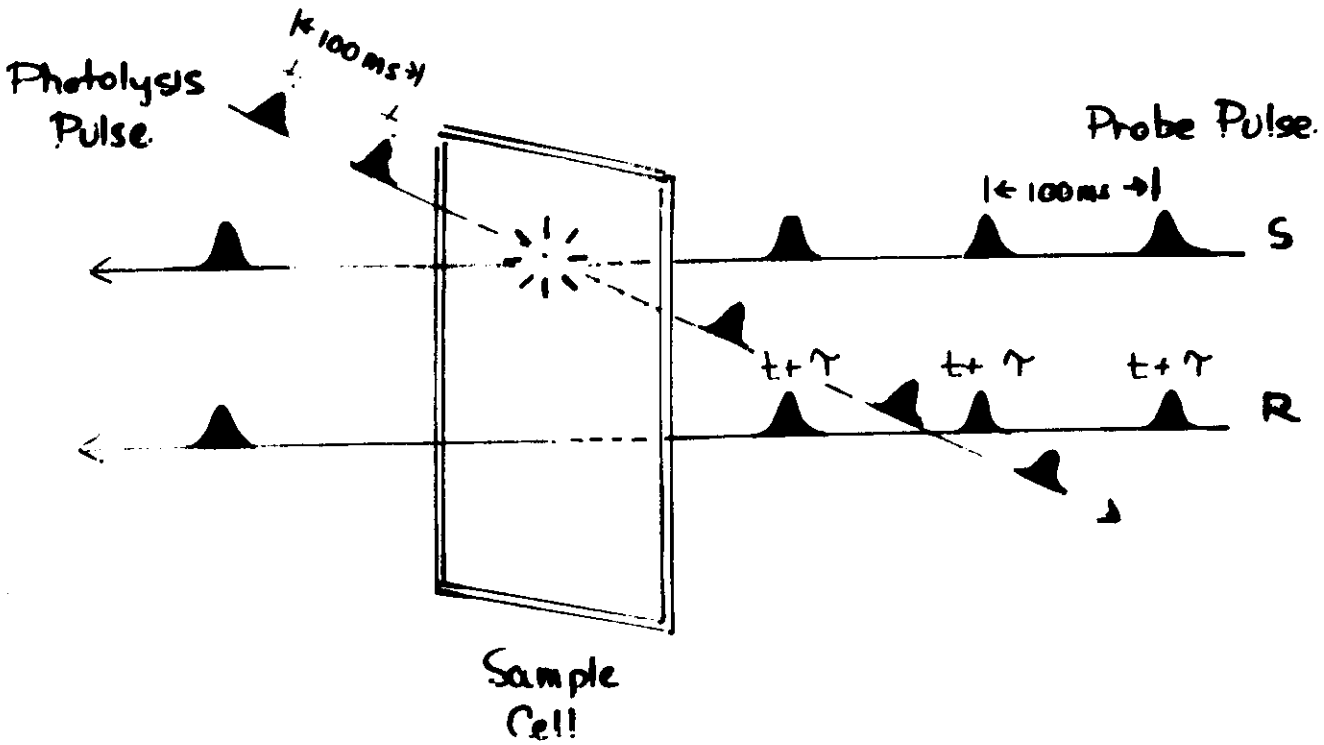

No. atoms
80
60
40
20
0
-0.5
0
+0.5
z - Shift (Å)
"global"

Pulsed Optical Excitation of Hemes and Heme Proteins

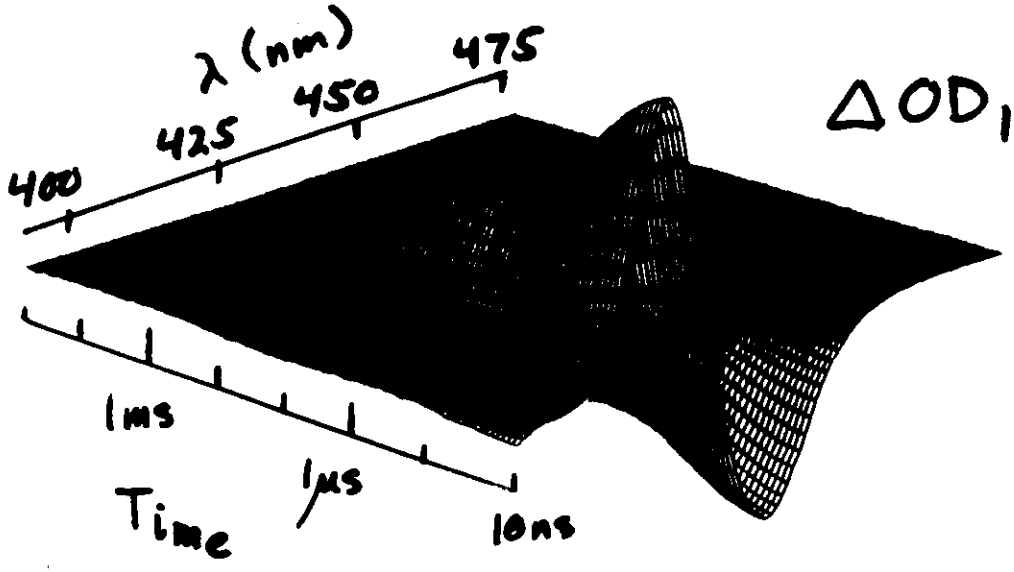
room T



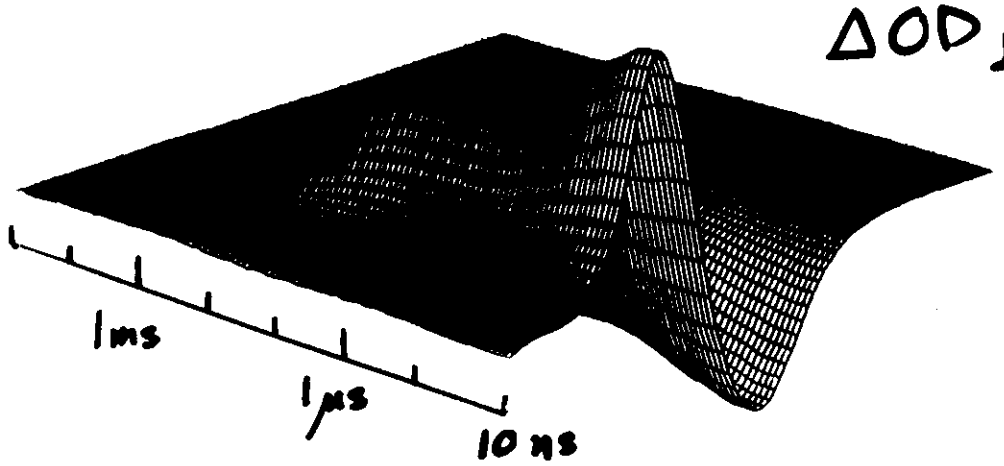

Delay Generator
Laser Timing Clock
Nd:YAG Laser
Nd:YAG Laser
355 nm
Counter-Timer
t + τ
Polarizer
Waveplate
Vidicon
Dye Cell
Sample Cell
Spectrograph
Detector Controller


Photolysis Pulse
|← 100 ms →|
Probe Pulse
|← 100 ms →|
S
t+τ
t+τ
t+τ
R
Sample Cell

Polarized Spectra

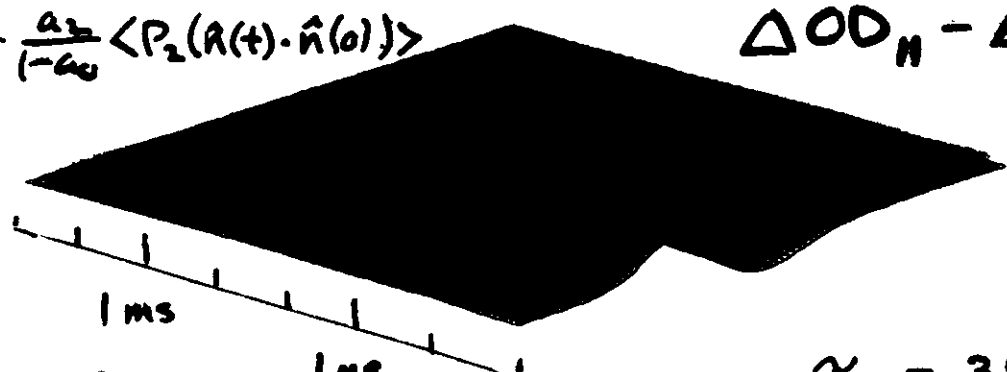


$\Delta OD_{\parallel}$



$\Delta OD_{\perp}$

$$r(x,t) = \frac{1}{10}\frac{a_2}{(-\omega)}\langle P_2(\hat{R}(t)\cdot\hat{n}(0))\rangle$$

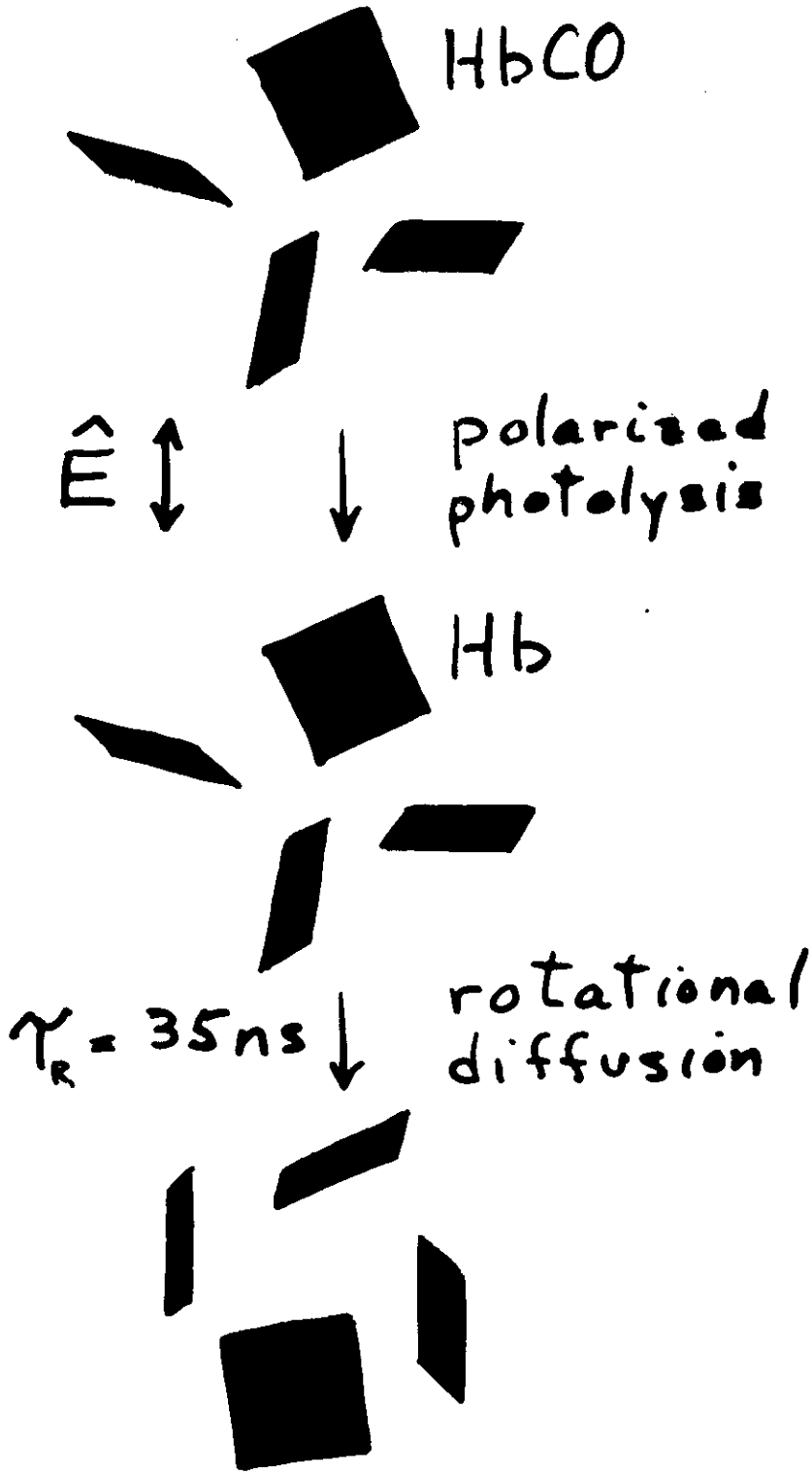


$\Delta OD_{\parallel} - \Delta OD_{\perp}$

$$r(x\to\infty,t) = \frac{\delta^2}{10}\exp(-t/\tau_R)$$

$\tau_R = 35 \pm 1$ ns

Rotational Diffusion Causes Apparent Geminate Rebinding

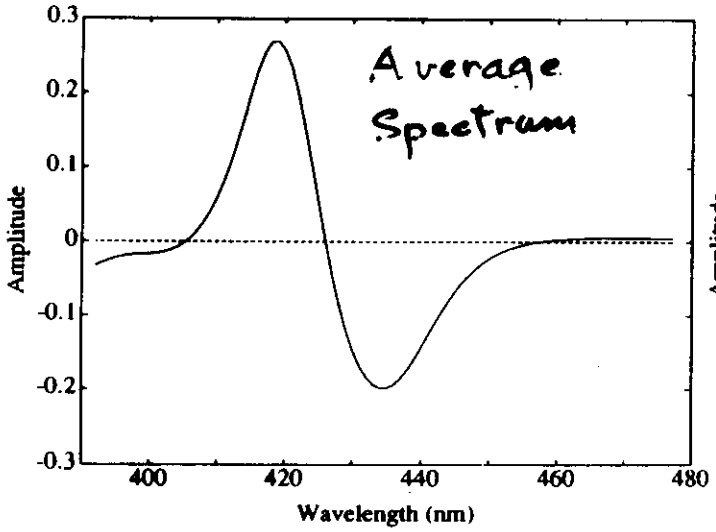


Singular Value Decomposition: $A = U \times S \times V^T$

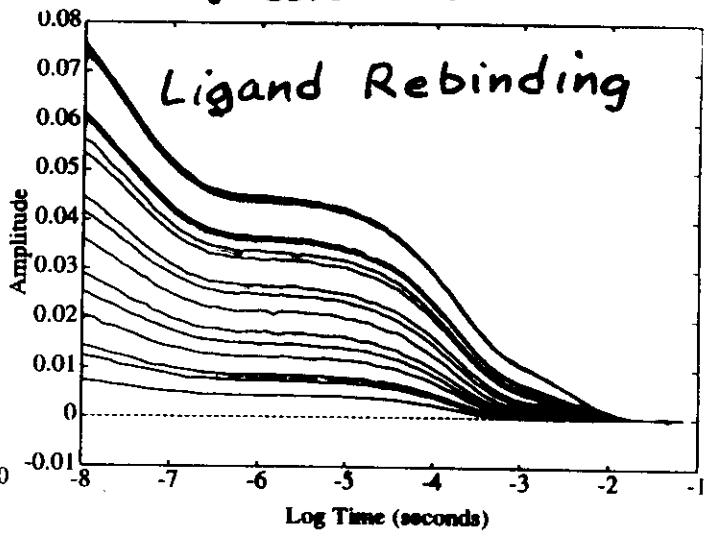
$$\begin{bmatrix} OD(\lambda_1)\,OD(\lambda_1) & \cdots & \\ OD(\lambda_2)\,OD(\lambda_2) & \cdots & \\ \vdots & \vdots & \\ OD(\lambda_n)\,OD(\lambda_n) & \cdots & \end{bmatrix} = \begin{bmatrix} A(\lambda_1)\,A(\lambda_1) & \cdots & \\ A(\lambda_2)\,A(\lambda_2) & \cdots & \\ \vdots & \vdots & \text{NOISE} \\ A(\lambda_n)\,A(\lambda_n) & \cdots & \end{bmatrix} \times S \times \begin{bmatrix} A(t_1)\,A(t_2)\cdots A(t_m) & \\ A(t_1)\,A(t_2)\cdots A(t_m) & \\ \vdots & \\ \text{NOISE} & \\ \vdots & \end{bmatrix}$$

$$A = U \times S \times V^T$$

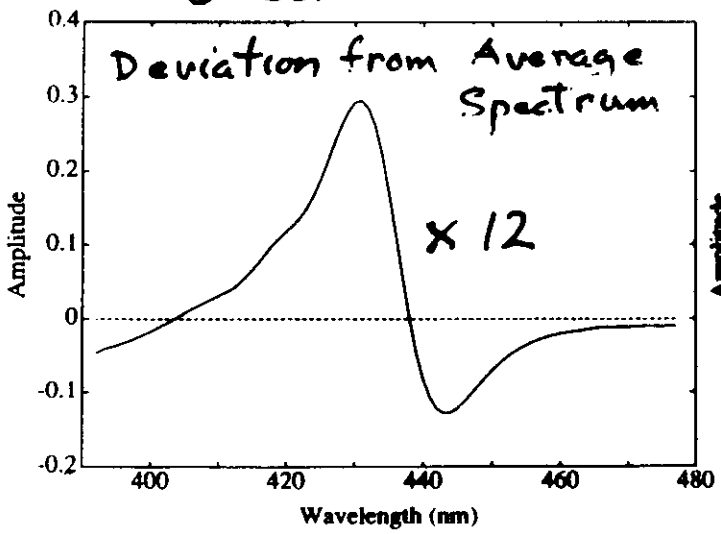
U Column 1



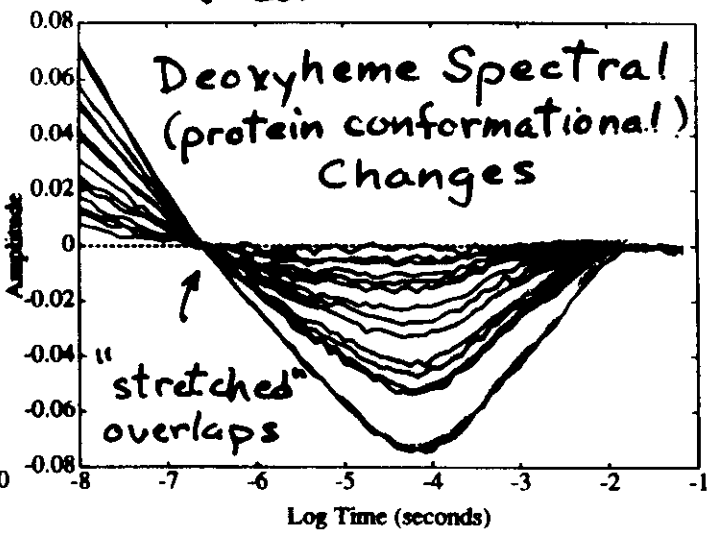
V column 1



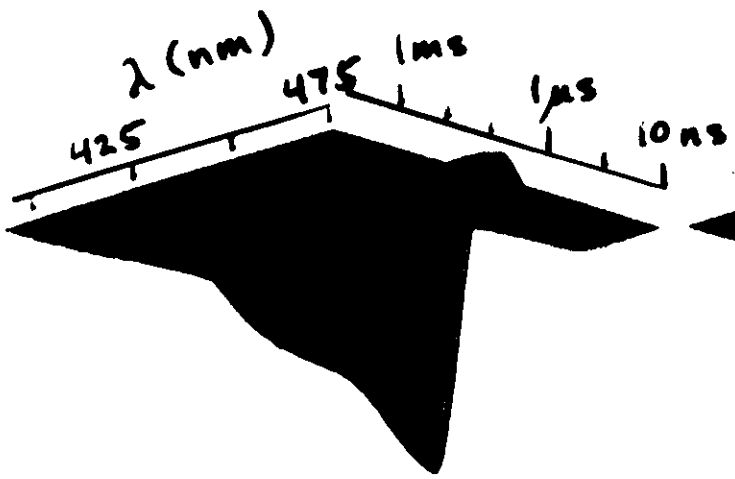
U column 2



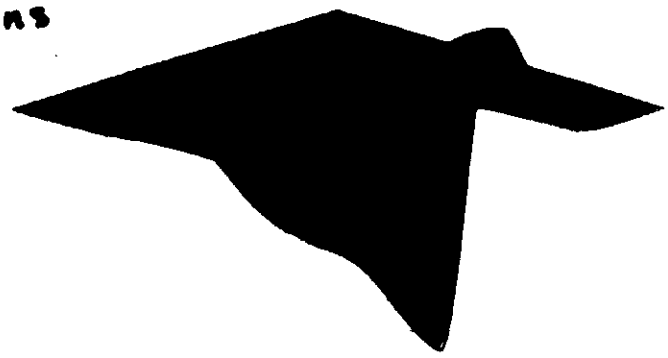
V column 2



Singular Value Decomposition
(isotropic data)



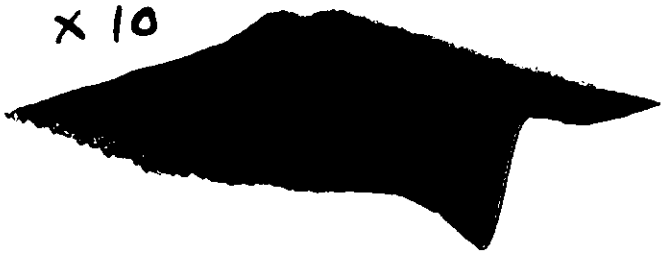
data



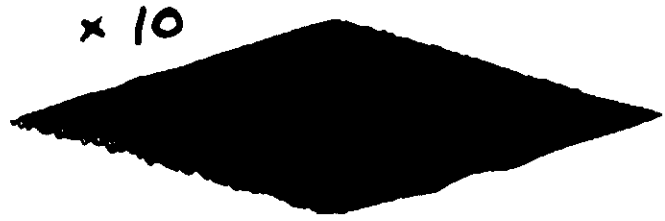
ligand rebinding

best one-component fit

deoxyheme
(protein conformational)
changes

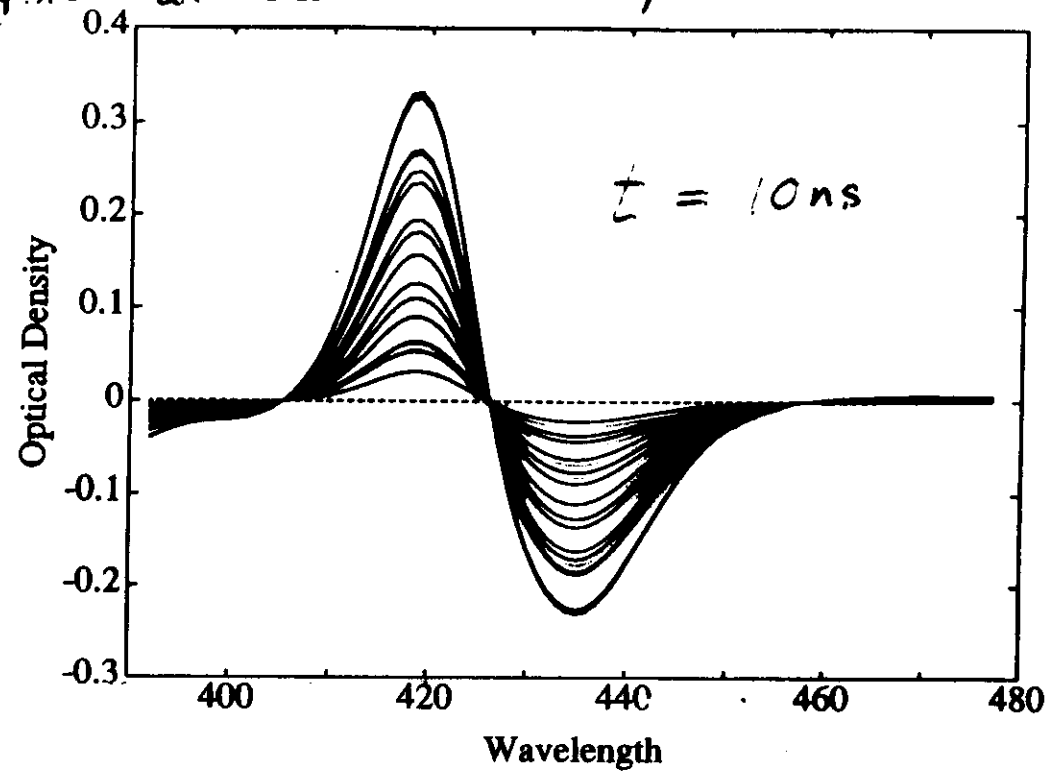


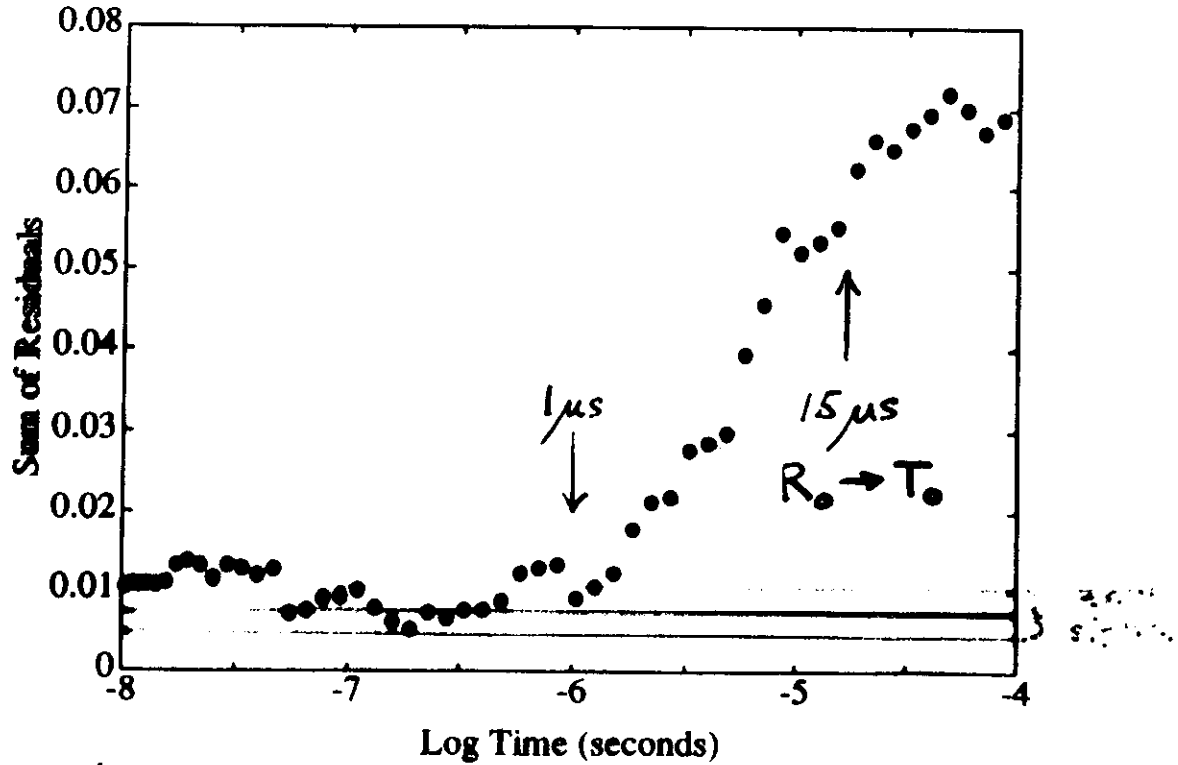
"residual"
second-component



third component

Compare spectra at different degrees of photolysis at each time delay.



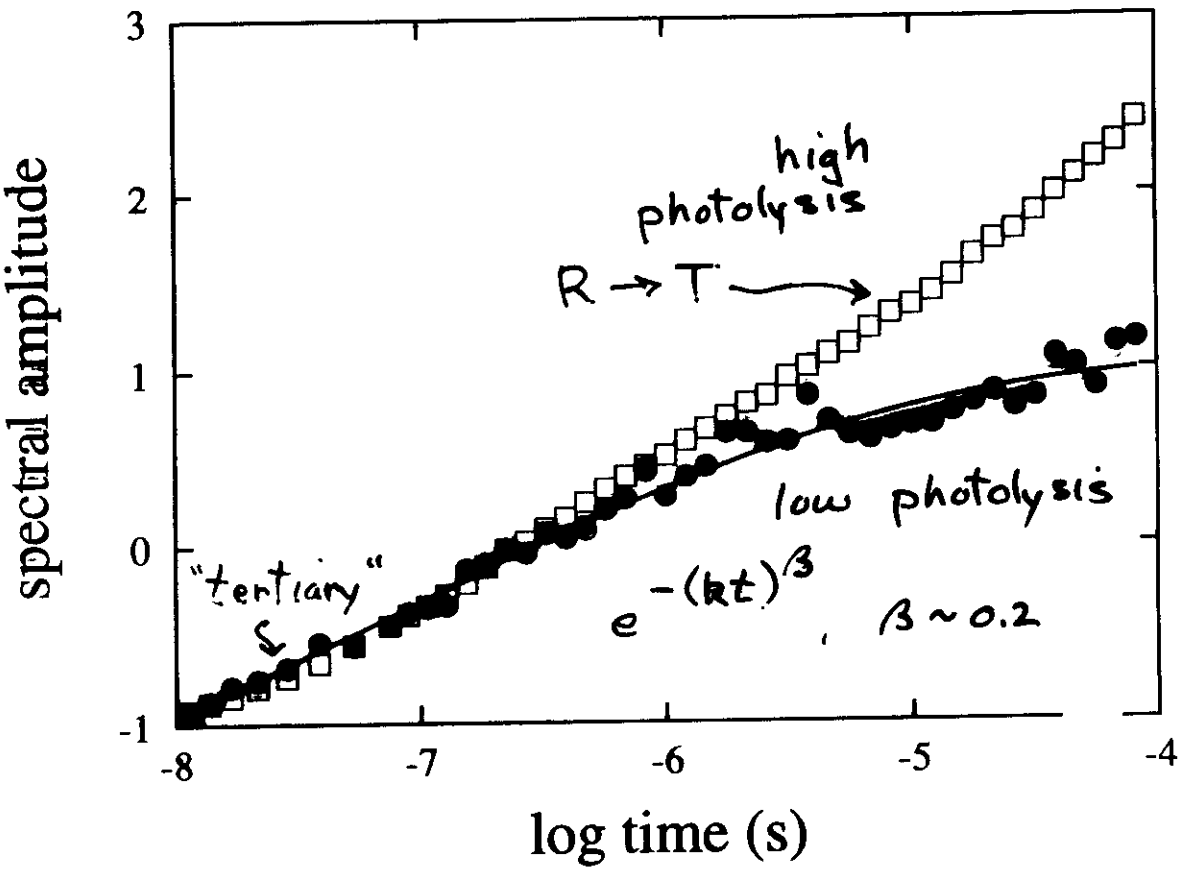


Spectra have same shape (molecule has same structure) at all ligation states until 1μs.

Colleen Jones (NIH)
James Hofrichter (NIH)
Eric Henry (NIH)

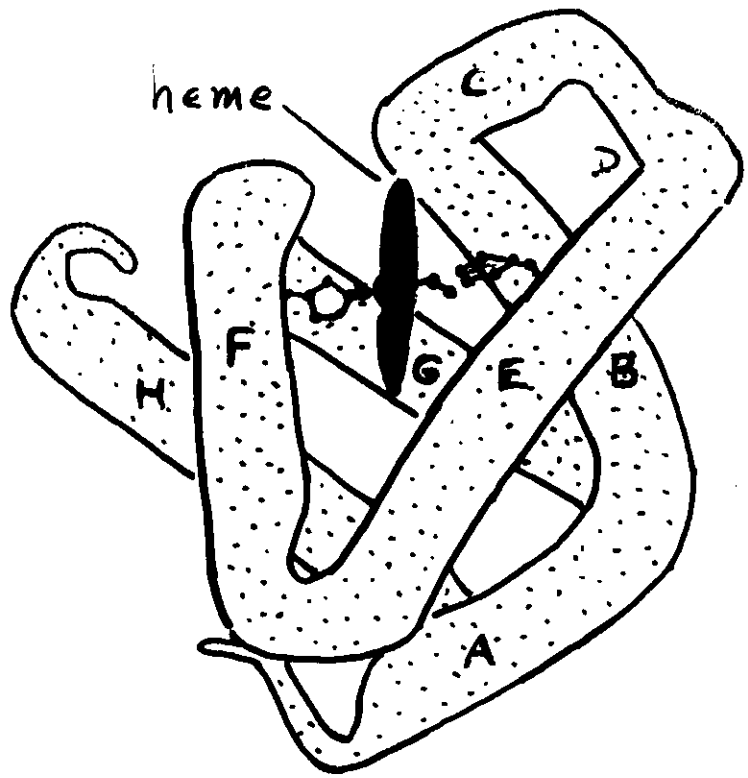
Conformational Relaxation in Hemoglobin

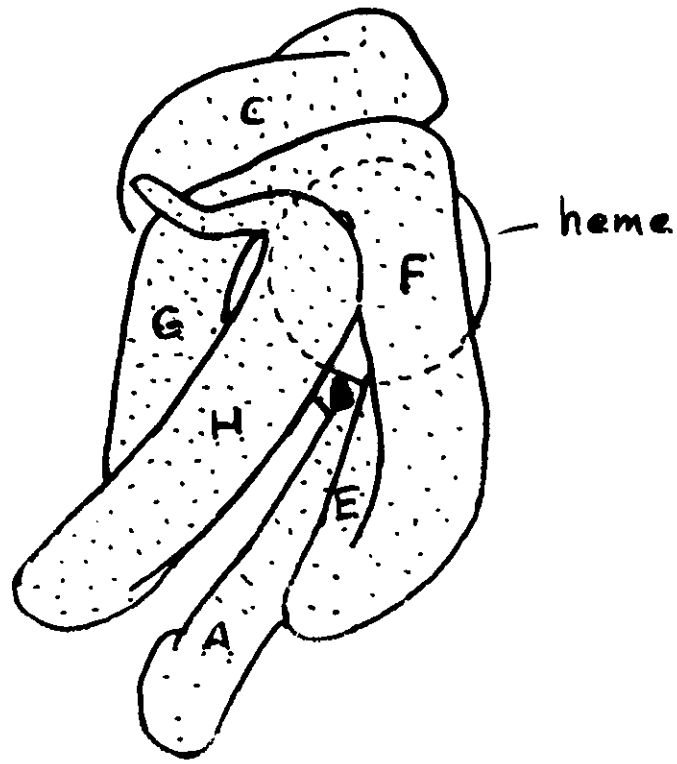
$a_{2u}, a_{1u}(\pi) \rightarrow e_g(\pi^*)$



"Stretched" exponential key to explaining kinetics in terms of a two-state allosteric model."

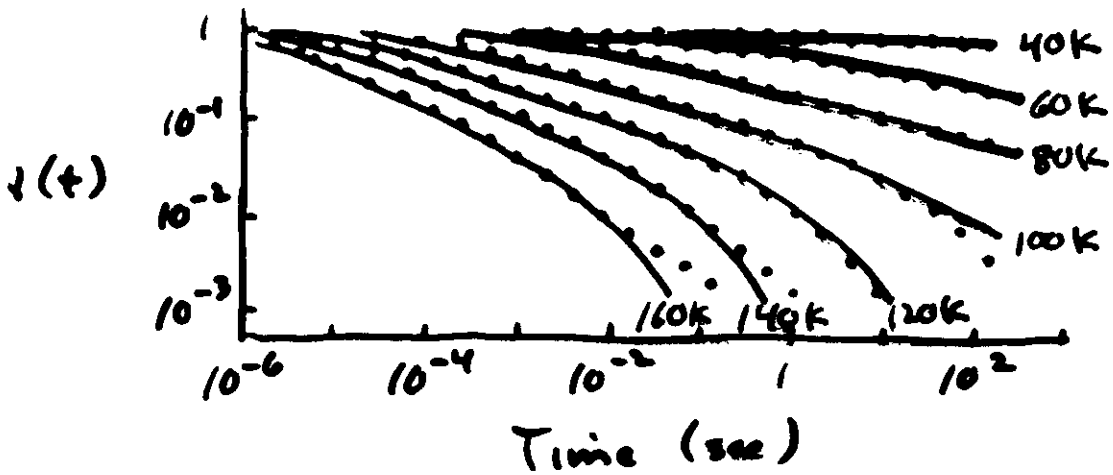
Myoglobin





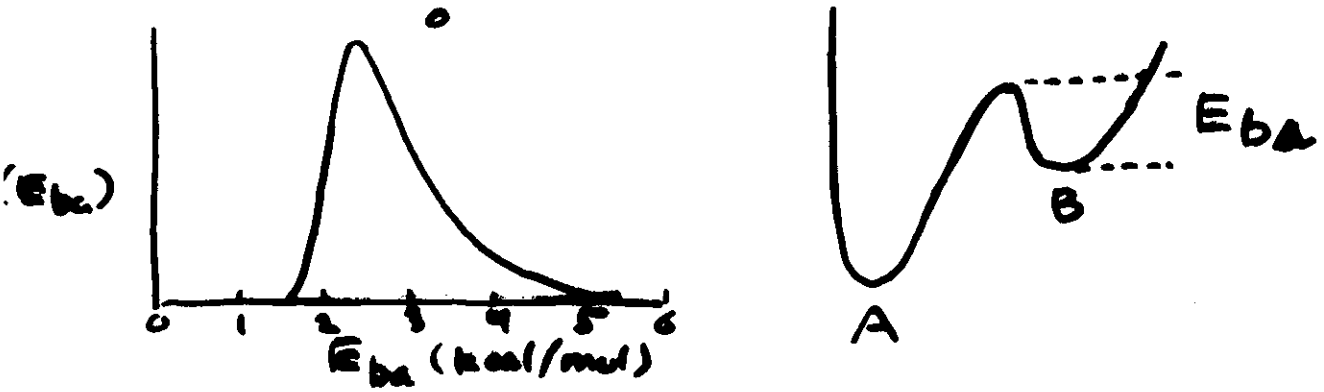
Conformational Substates – 1975:

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.

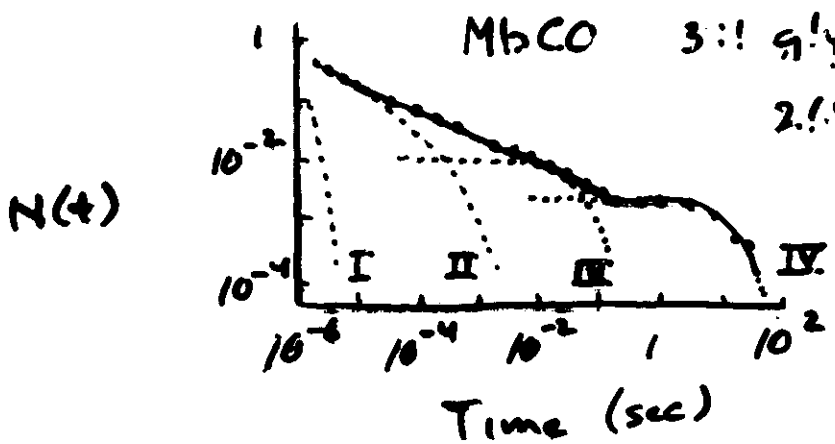
$$N(t) = \int_0^\infty g(E_{ba}) e^{-k_{ba} t} dE_{ba}, \quad k_{ba} = A_{ba} e^{-E_{ba}/RT}$$

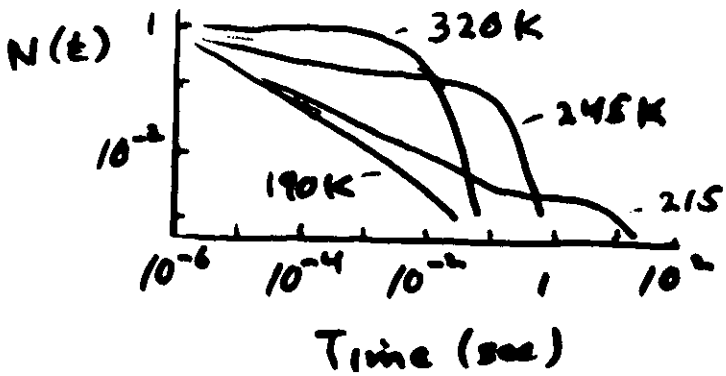


At high temperature, if relaxation among substates is very fast, $k_r \gg k_{ba}^{max}$ – rebinding is exponential

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

Multiple Barriers - 1975



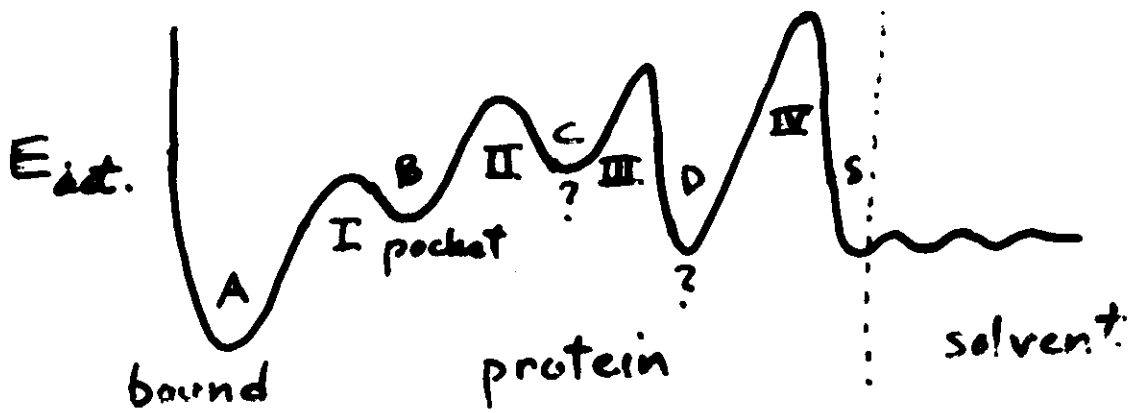


190 K I + II

215 K I + II + III + IV

215 K 245 K II + III + IV

320 K IV



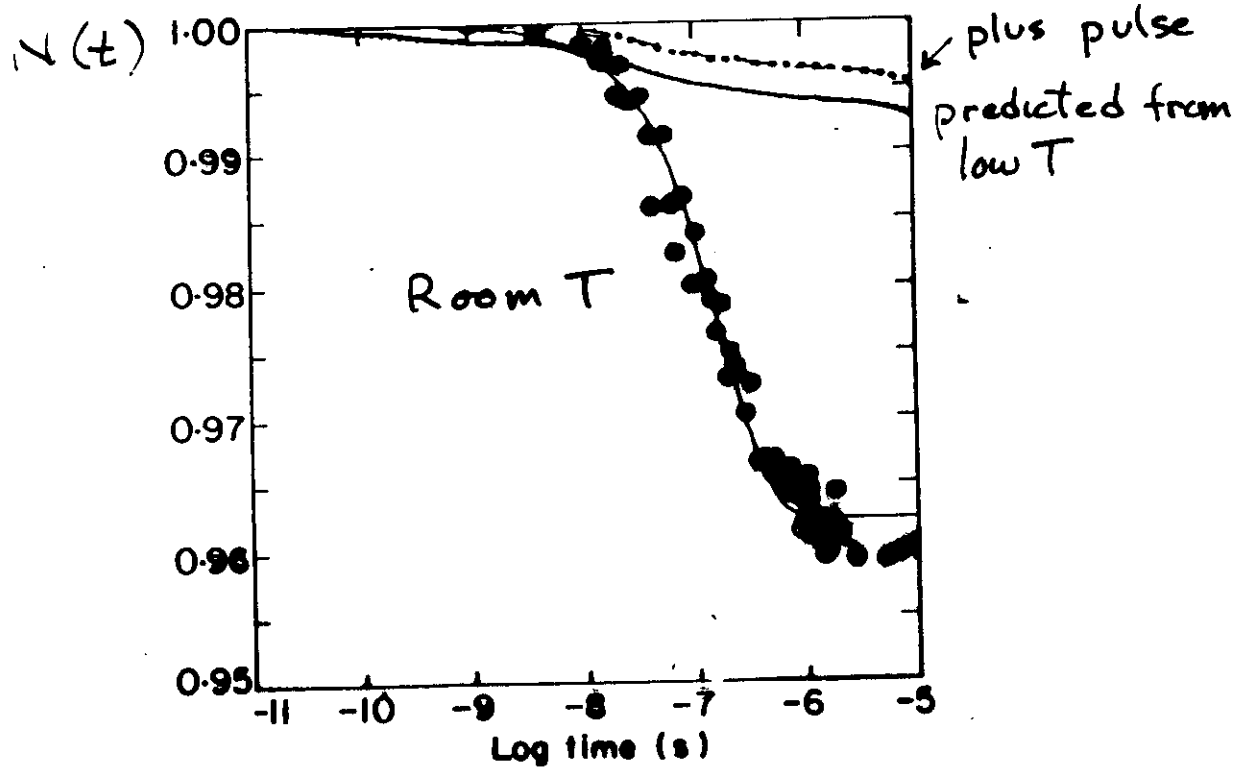
$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \rightleftharpoons S$$

more recently $A \rightleftharpoons B \rightleftharpoons M \rightleftharpoons S$

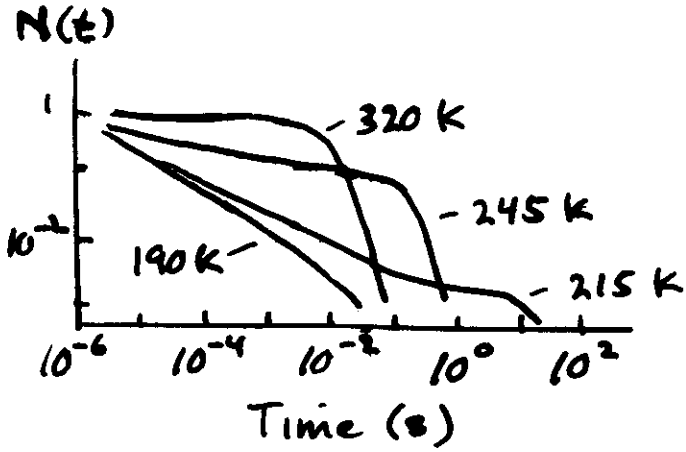
"pocket" "matrix"

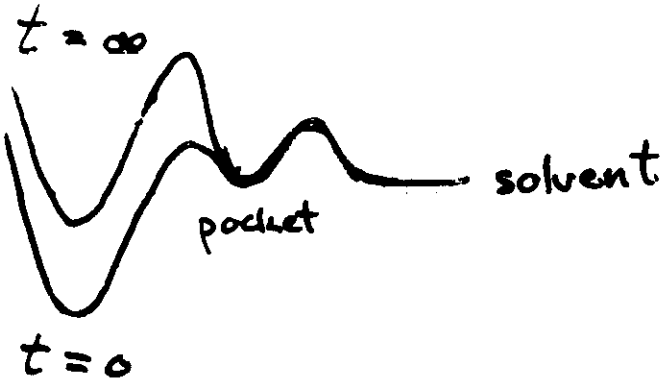
Henry et al., 1983

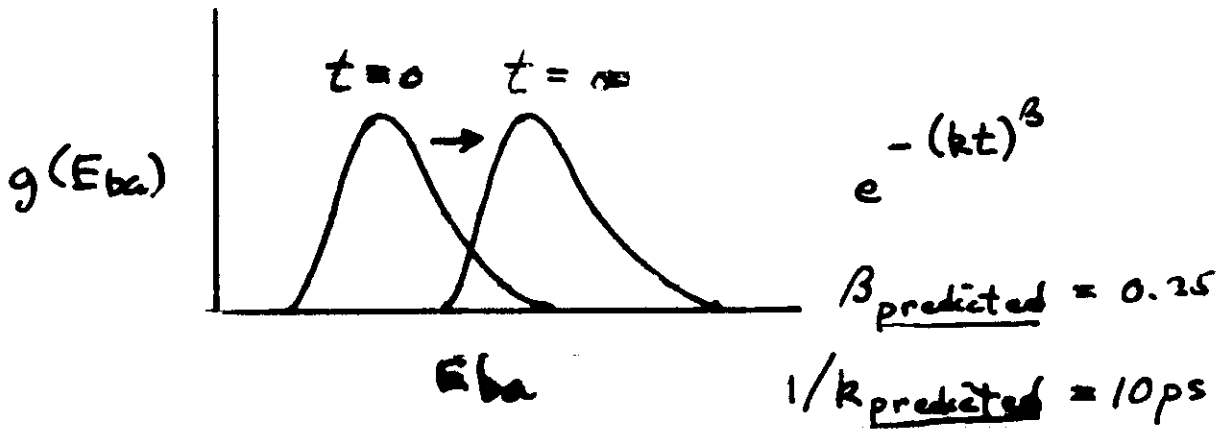
Discovery of geminate rebinding of CO in liquid Mb solution



Forced reevaluation of relation between low T and high T data.







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

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

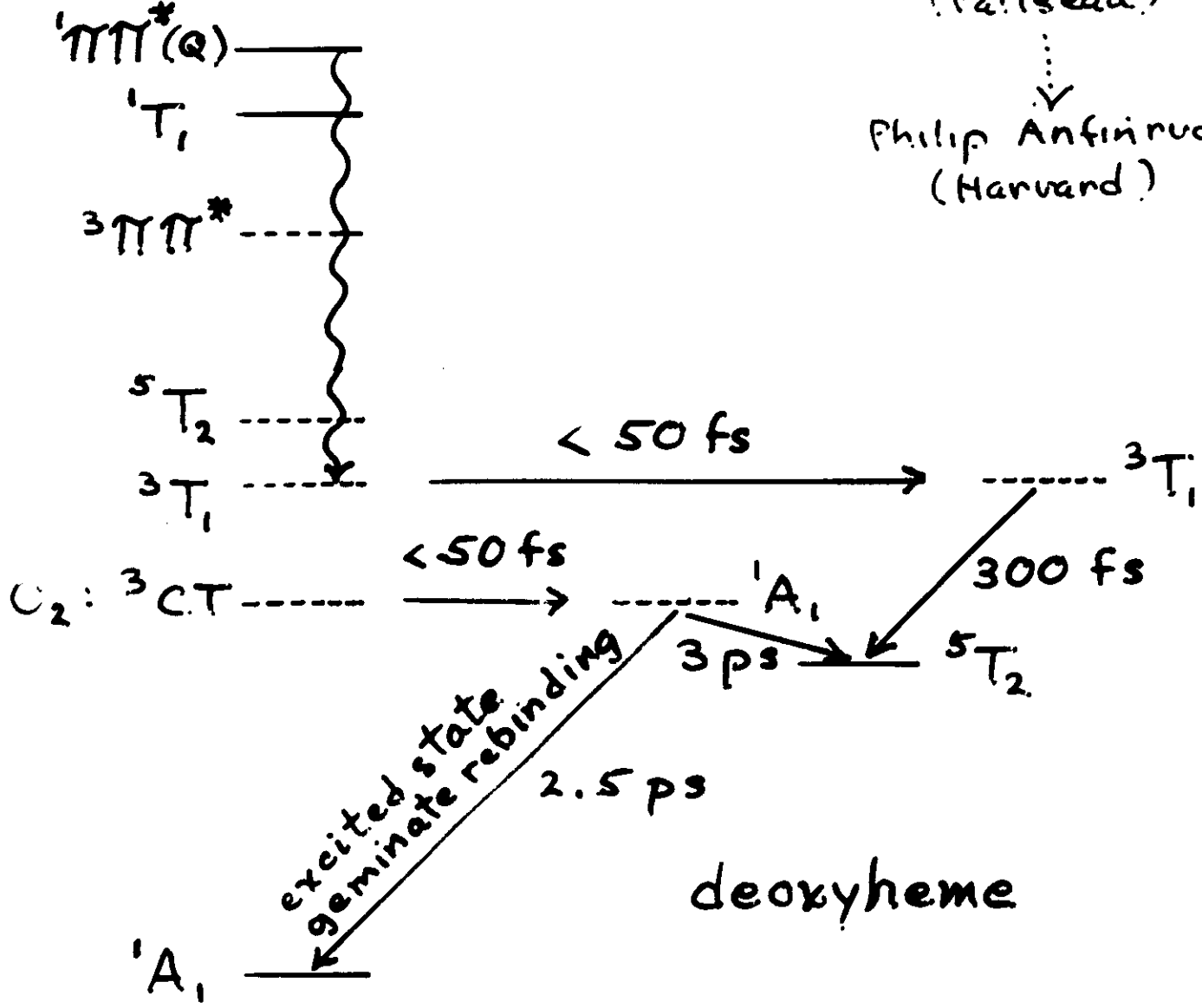
Photophysics

Robin Hochstrasser (U. Penn.)

Jean-Louis Martin (Paliseau)

↓

Philip Anfinrud (Harvard)



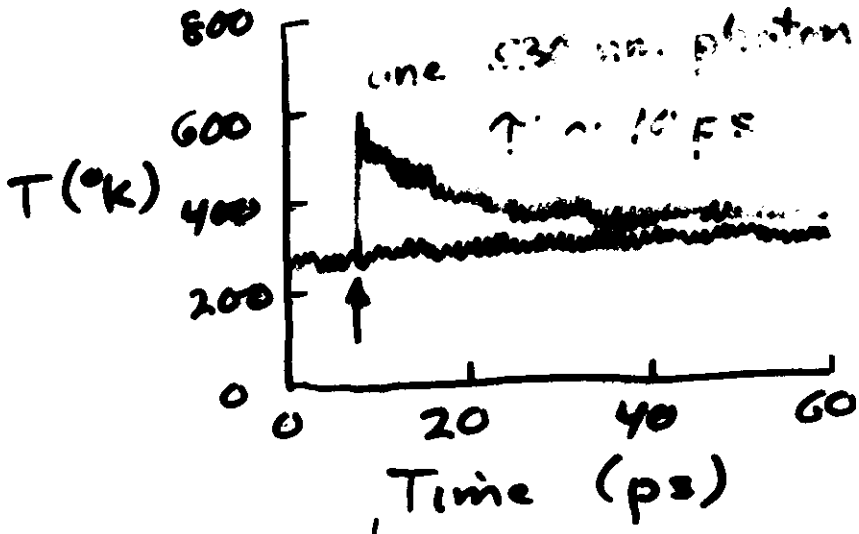
questions:

ccation of excited states ("stone age")

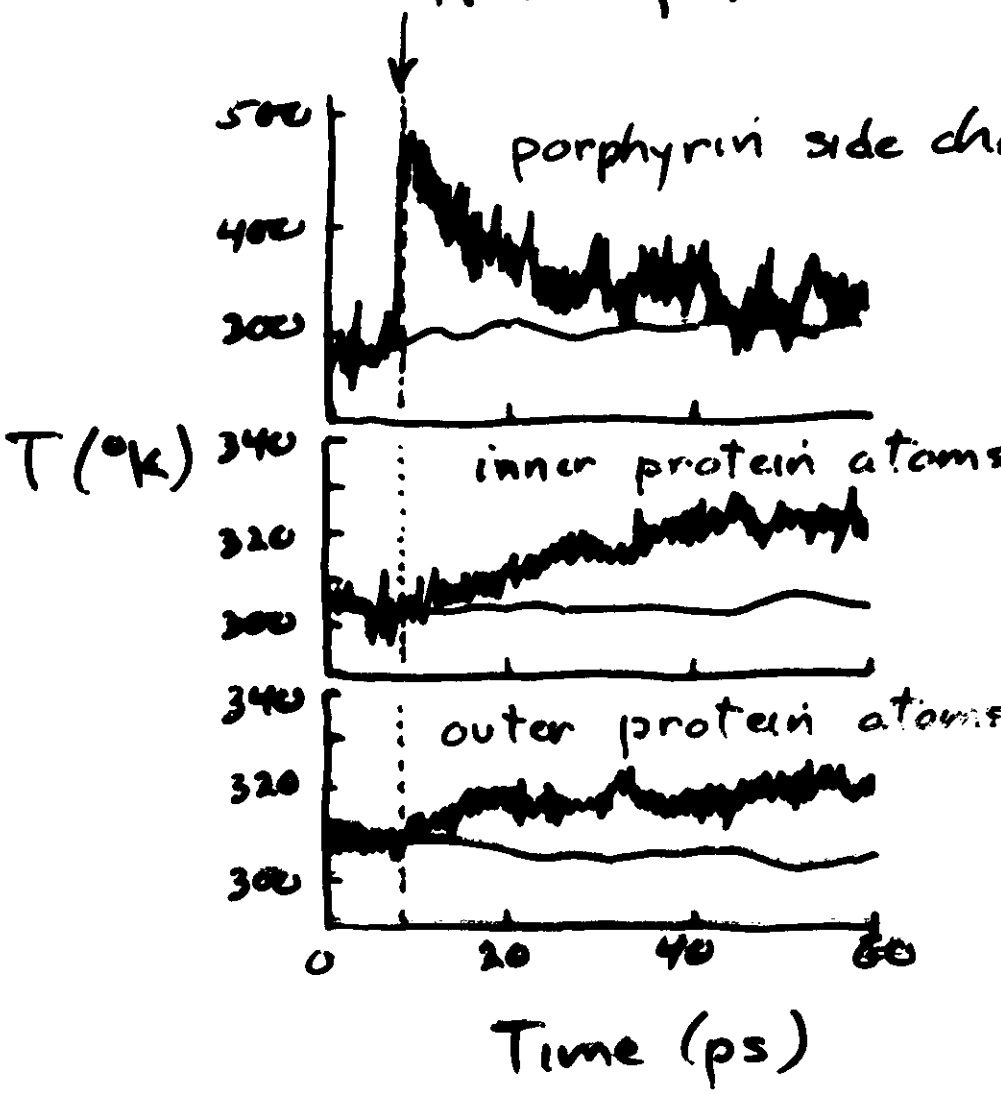
Identification of excited state intermediates

Eric Henry
(NIH)

24 porphin atoms



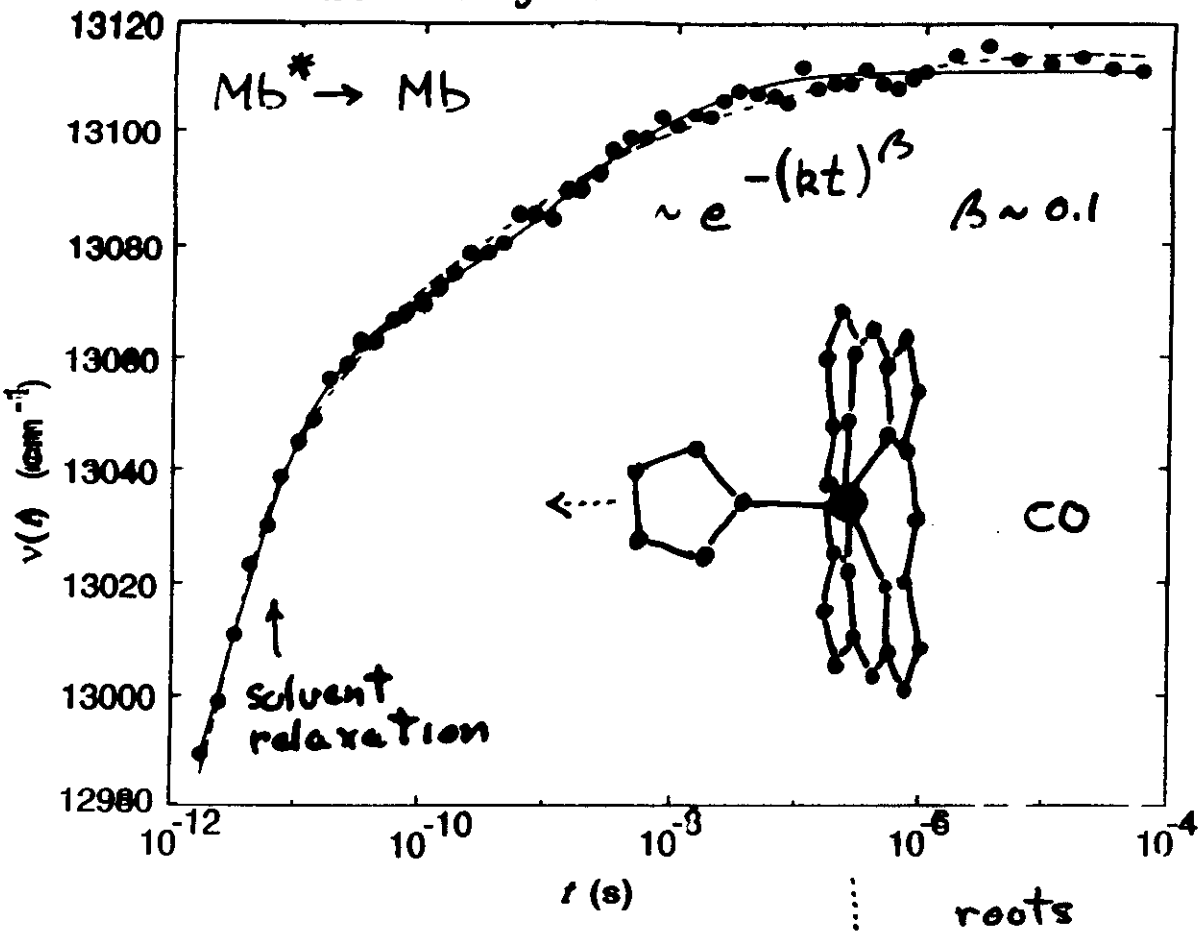
$$\frac{3}{2} N k_B T = \sum_i \frac{1}{2} m_i v_i^2$$



Thermal effects potentially very important at times < 20 ps

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

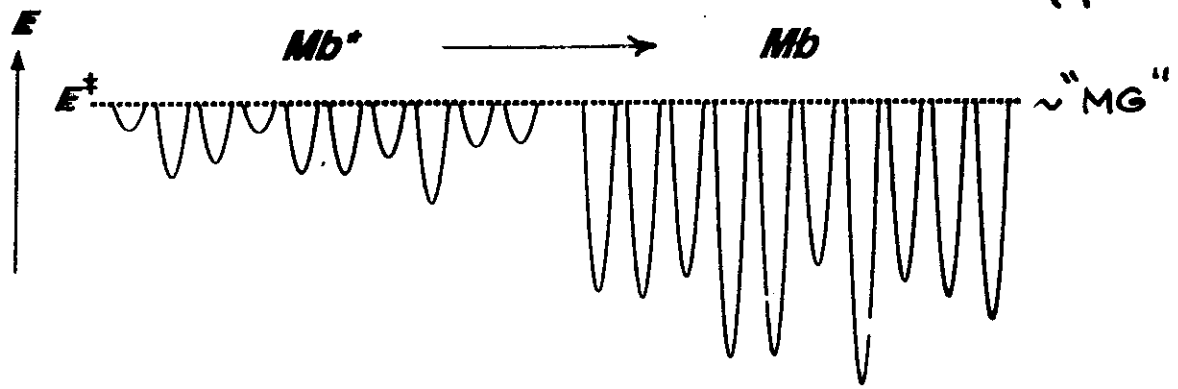
$a_{2u}(\pi) \rightarrow Fe(d_{xz})$ charge transfer



roots

De Dominicis et al. '65
Kopec & Hilhorst '87
Shakhnovich & Gutin '89
Frauenfelder et al. '91
Saven et al. '94

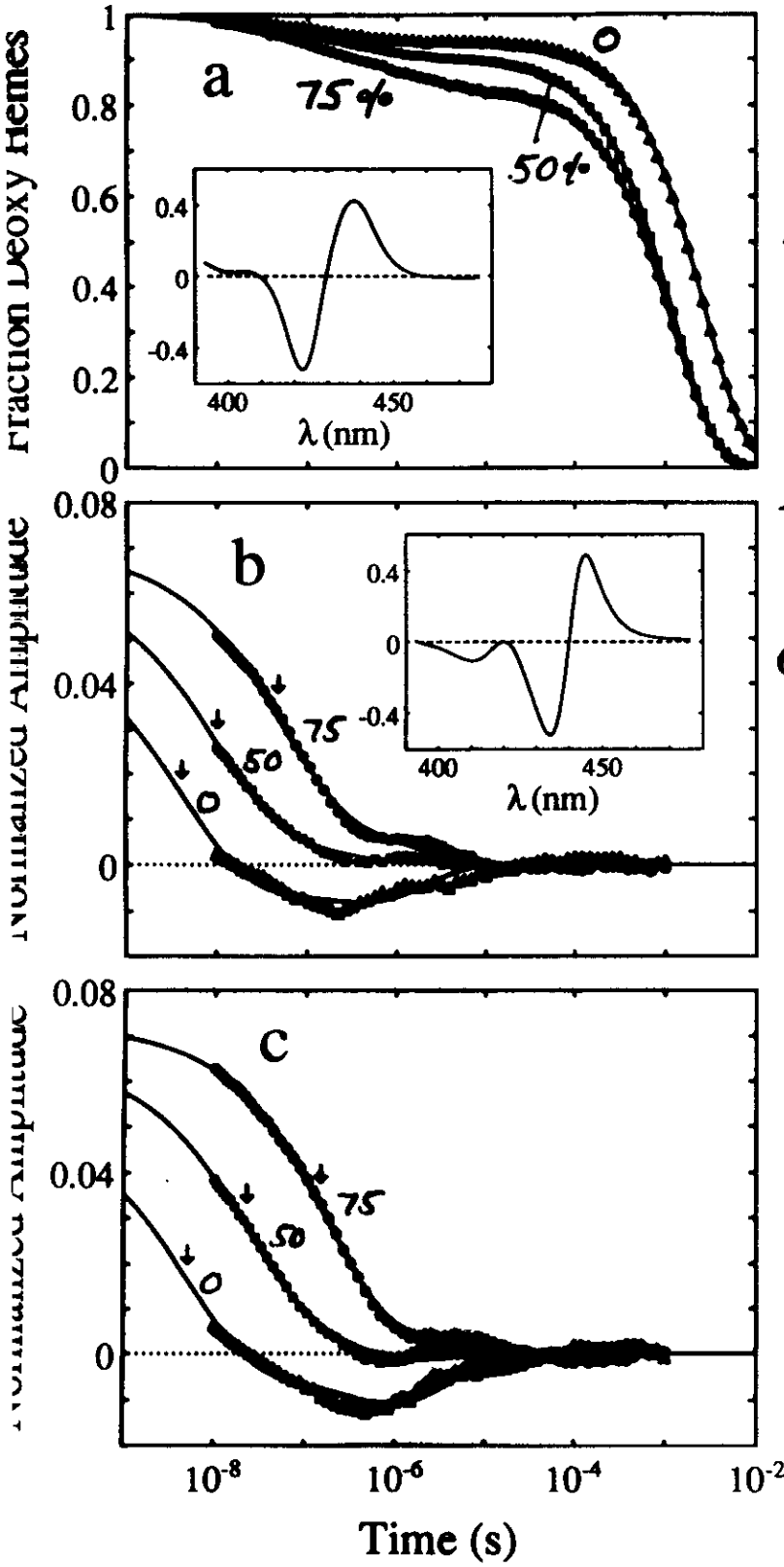
A Model



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

single molecule experiments could reveal connectivity

Myoglobin - CO 0, 50%, 75% glycerol



Ligand Rebinding

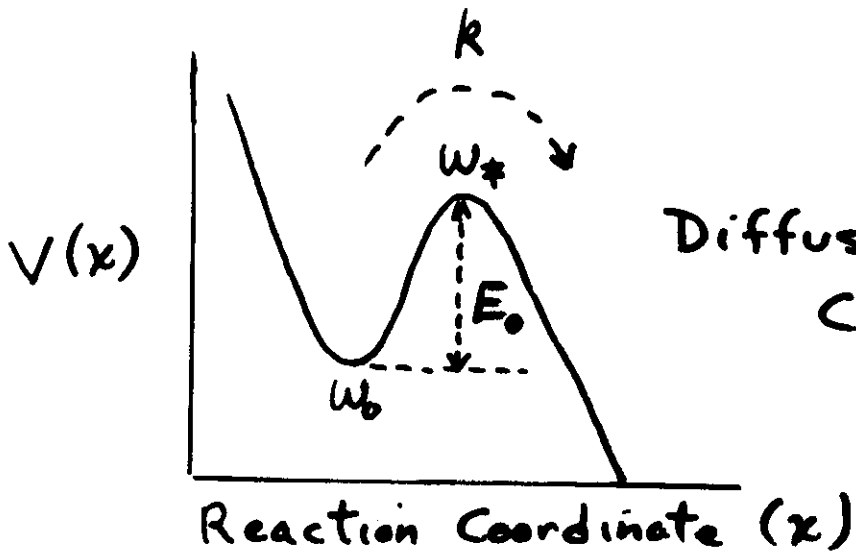
Deoxyheme Spectral Changes = Protein Conformational Changes

20°C

5°C

$A = A_0 \exp(-kt)^{\beta}$

$\beta = 0.6$



Diffusive Barrier Crossing

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, yields Kramers' equation:

$$k = \frac{M\omega_0\omega_*}{2\pi\zeta}\exp(-\frac{E_0}{k_BT})$$

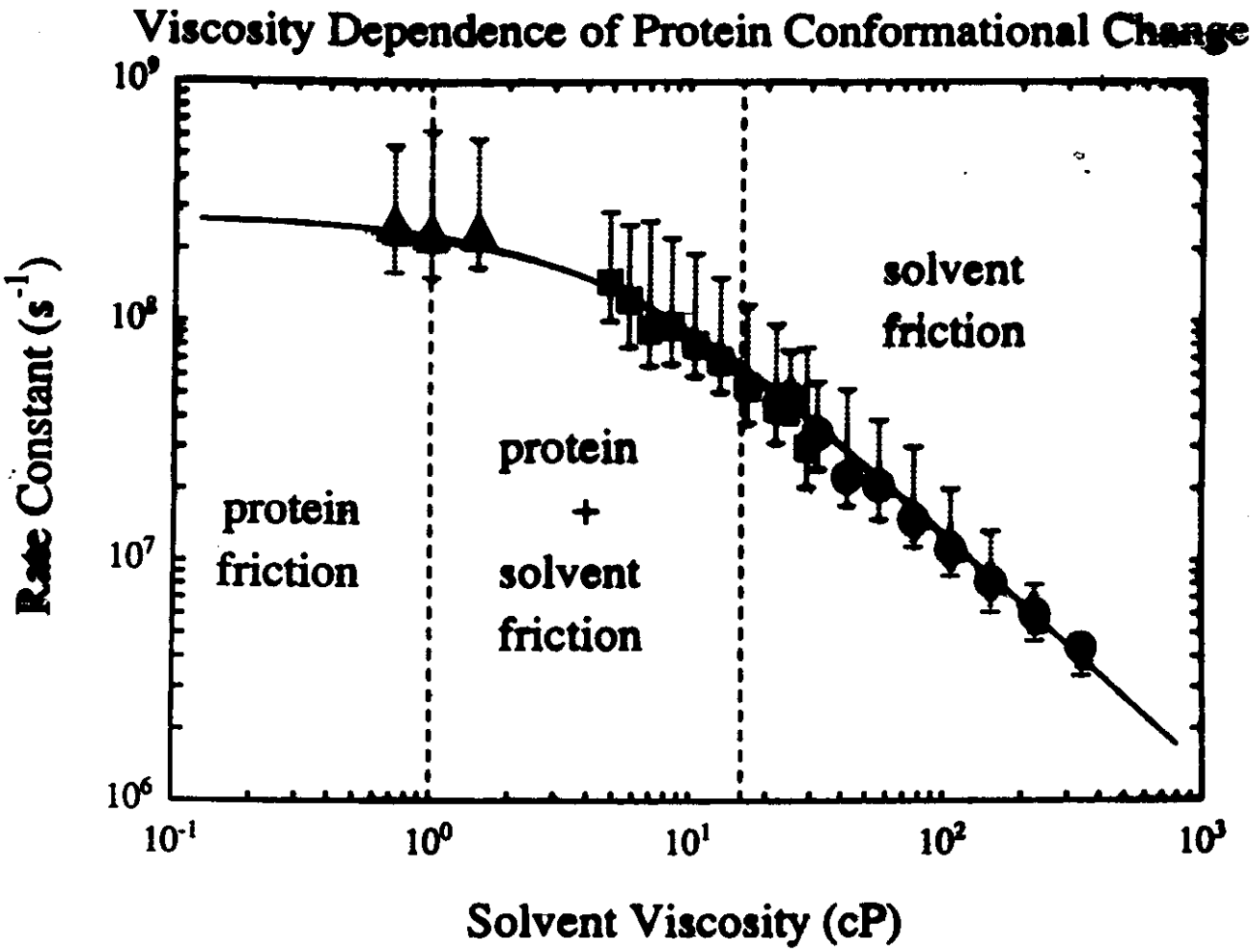
Assuming that protein friction and solvent friction are additive, Kramers' equation is:

$$k = \frac{B}{\alpha\zeta_p + (1-\alpha)\zeta_s}\exp(-\frac{E_0}{k_BT})$$

which, using Stokes' law, becomes:

$$k = \frac{C}{\sigma + \eta_s}\exp(-\frac{E_0}{k_BT})$$

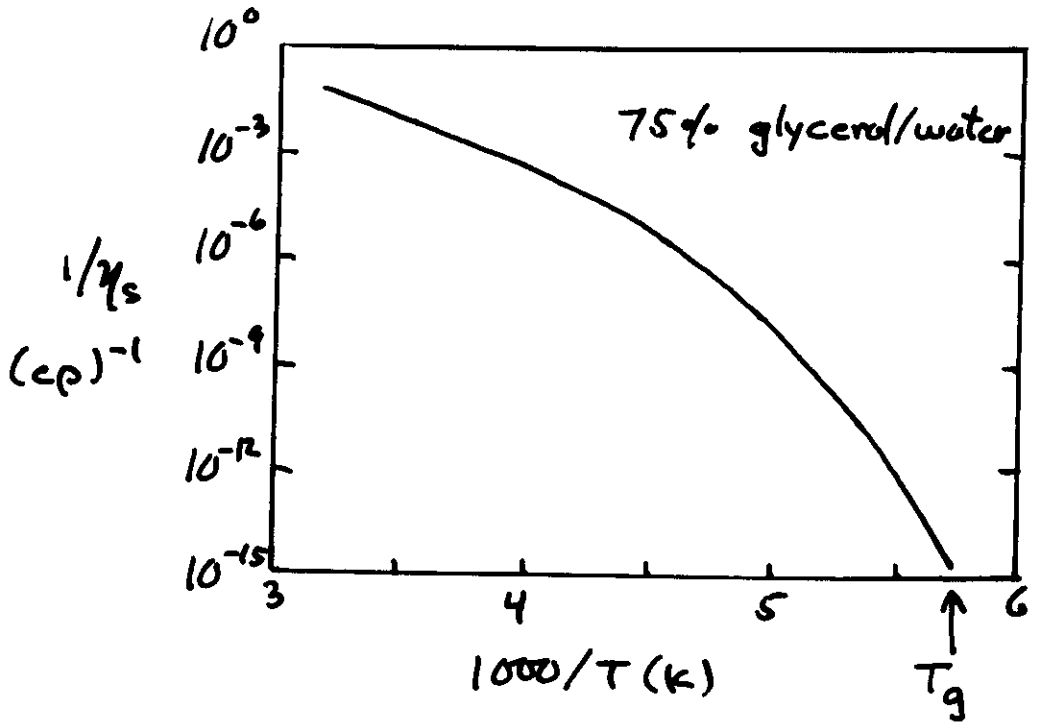
Myoglobin



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

protein "viscosity" ↖ solvent viscosity

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



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

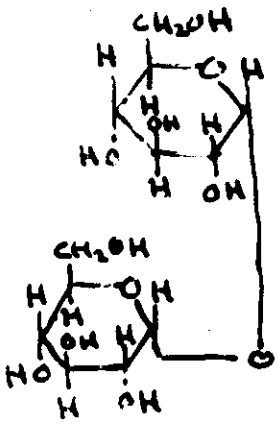
Slowing near T_g due to solvent viscosity
(<u>not glass transition of protein</u>.)

$\eta_s \sim 10^{11}$

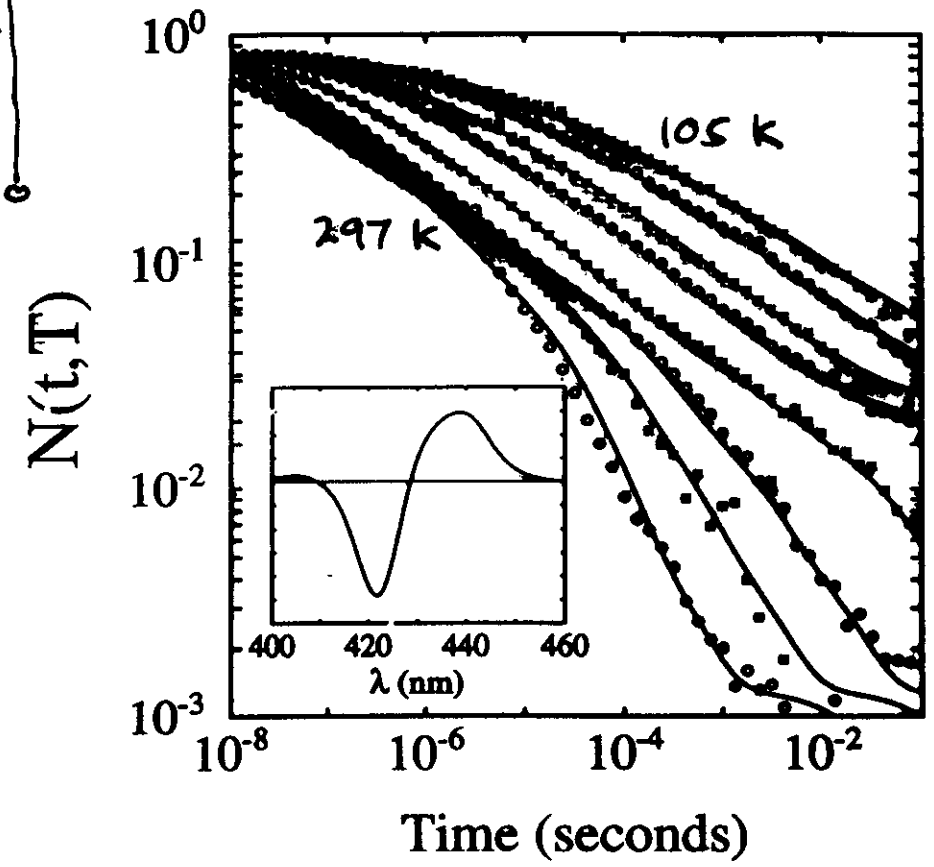
$e^{-E_o/RT} \sim 10^{2}$

Stephen Hagen
(NIH)

Myoglobin in a trehalose glass



/U2/figures/hagen_[redacted]/figure_1



Recall:

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

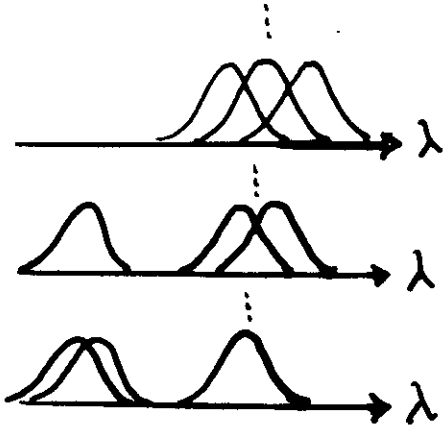
$$= \exp\left[-k_{ba}^{mean} t\right]$$

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

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

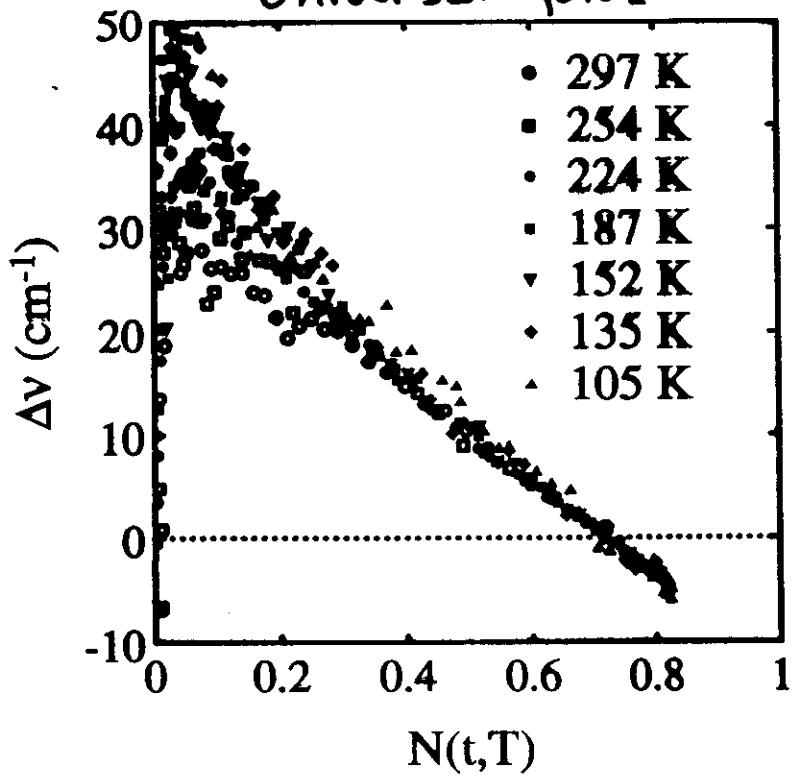
Protein "stuck" in liganded (fast reacting) conformational substates.

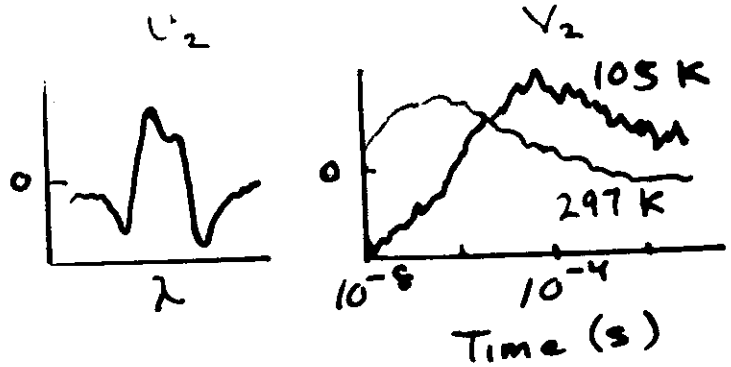
Kinetic hole burning



/U2/figures/hagen_[redacted]/figure_3

universal plot





"global" SVD

Novel results on myoglobin needed to explain more complex hemoglobin

1. geminate rebinding is nonexponential
2. conformational substates
3. conformational relaxation extended in time ($\sim e^{-(kt)^{\beta}}$): explained by "physical kinetics"
4. relaxation slows geminate rebinding,
5. kinetic hole burning from conformational substates

Issues raised:

theoretical: viscosity dependence of conformational relaxation kinetics

experimental: observation of conformational relaxation of single molecule

Linear Free Energy Relation

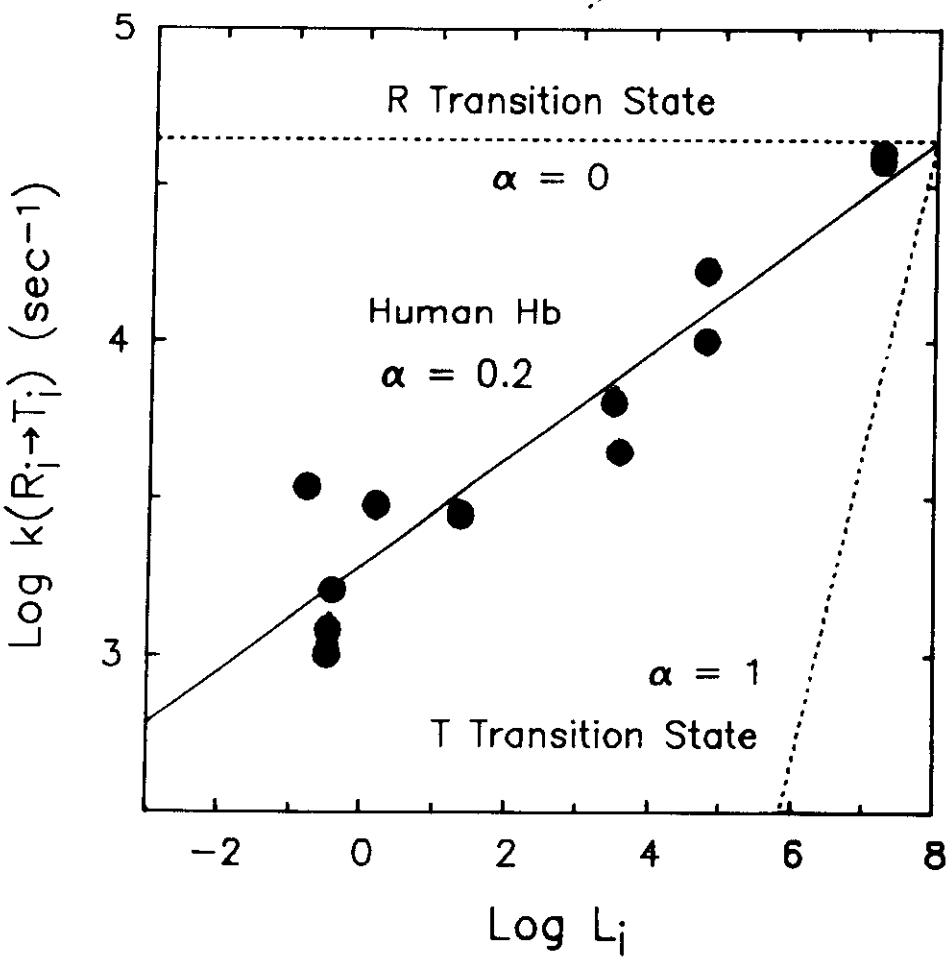
$$\delta \Delta G^{\ddagger} = \alpha \, \delta \Delta G$$

For two-state allosteric model

$$k(R_i \rightarrow T_i) = \gamma (L_0 c^i)^{\alpha}$$

$$k(T_i \rightarrow R_i) = \gamma (L_0 c^i)^{\alpha - 1}$$

$$(d = c^{-\alpha})$$



Transition state appears at 20% of the distance along the reaction path from R to T.

MWC Model with Tertiary Transient

$$\begin{array}{ccccccccc} T_0 & \rightarrow & T_1 & \rightarrow & T_2 & \rightarrow & T_3 & \rightarrow & T_4 \\ \Downarrow L & & \Downarrow Lc & & \Downarrow Lc^2 & & \Downarrow Lc^3 & & \Downarrow Lc^4 \\ R_0 & \rightarrow & R_1 & \rightarrow & R_2 & \rightarrow & R_3 & \rightarrow & R_4 \end{array}$$

$$k(R_i \rightarrow T_i) = \gamma (Lc^i)^{\alpha} \quad (\text{Gibson } d = c^{-\alpha})$$

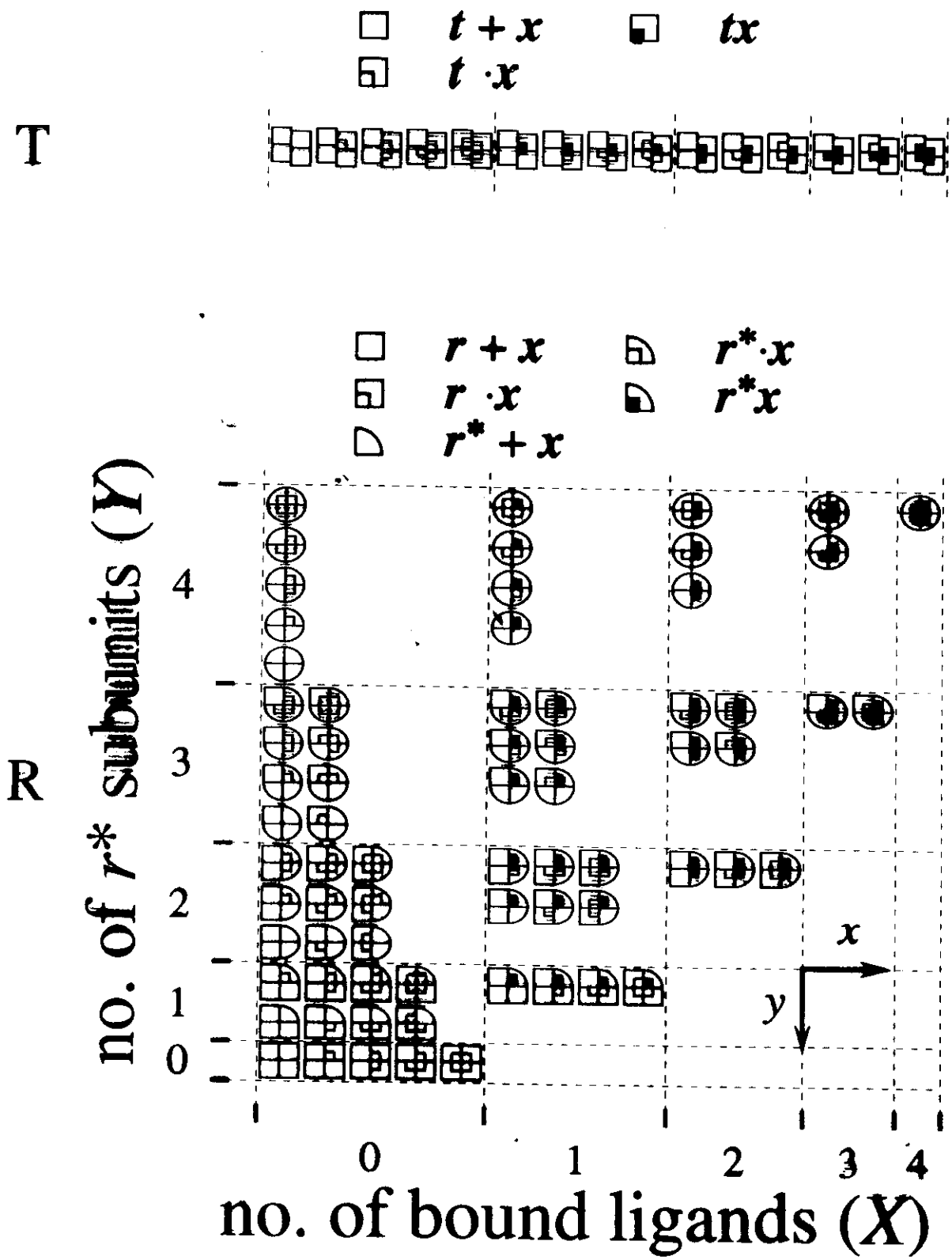
$$\begin{array}{lccc} & k(r^*) & r^* \cdot x & \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} & r^* + x \\ \text{in } R \quad rx & \swarrow & \ell \Downarrow & & \Downarrow \\ & k(r) & r \cdot x & \rightleftharpoons & r + x \end{array} \qquad r^* \rightarrow r : e^{-(kt)^{\beta}}$$

$$\text{in } T \quad tx \xleftarrow[k(t)]{} t \cdot x \rightleftharpoons t + x$$

L, c, γ, α quaternary rates
k, β, ℓ tertiary rates
$k(r^*), k(r), k(t), k_{in}, k_{out}$ ligand binding rates

15 distinguishable species, 3 deoxy spectra
12 model parameters
to describe
37 experimental parameters, 2 deoxy spectra

Parameter space explored by a Monte-Carlo "simulated annealing" (χ^2).



Henry, et al. Figure 3

Integration of rate equations to produce stretched exponential time course (a trick!)

$e^{-(kt)^\beta}$

$$\frac{dr^*}{dt} = -\left[\frac{l}{l+1}\beta k(kt)^{\beta-1}\right]r^* + \left[\frac{1}{l+1}\beta k(kt)^{\beta-1}\right]r + \cdots$$

$$\frac{dr}{dt} = \left[\frac{l}{l+1}\beta k(kt)^{\beta-1}\right]r^* - \left[\frac{1}{l+1}\beta k(kt)^{\beta-1}\right]r + \cdots$$

$$k = k(r^* \to r) + k(r \to r^*)$$

$$l = k(r^* \to r)/k(r \to r^*)$$

$\beta \to 1$

$$\frac{dr^*}{dt} = -k(r^* \to r)\,r^* + k(r \to r^*)\,r + \cdots$$

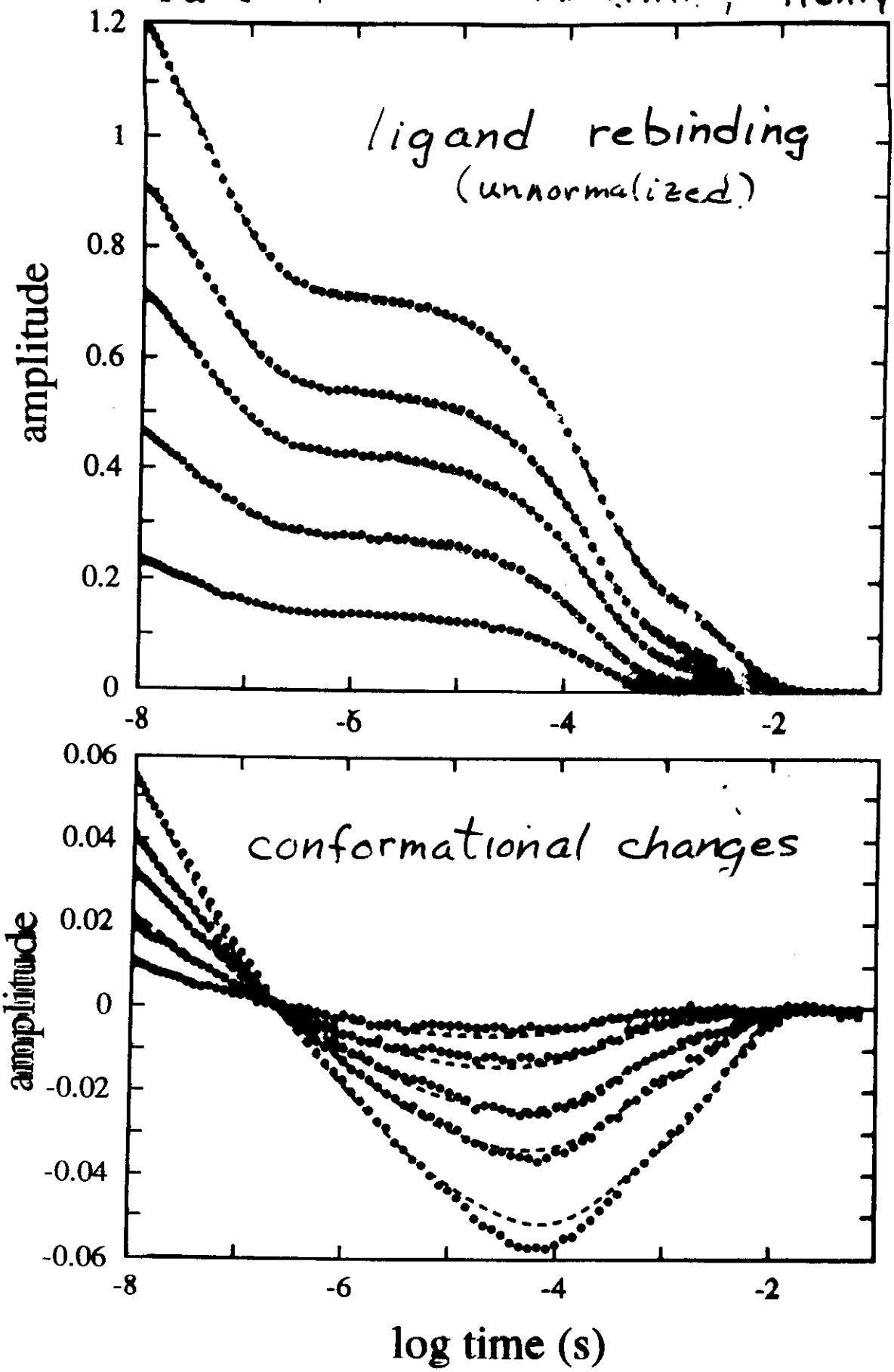
$$\frac{dr}{dt} = +k(r^* \to r)\,r^* - k(r \to r^*)\,r + \cdots$$

---

geminate rebinding:

$$k_{gem}(t) = k_{gem}(r^*)\left[k_{gem}(r^*)/k_{gem}(r)\right]^{x(t)-1}$$

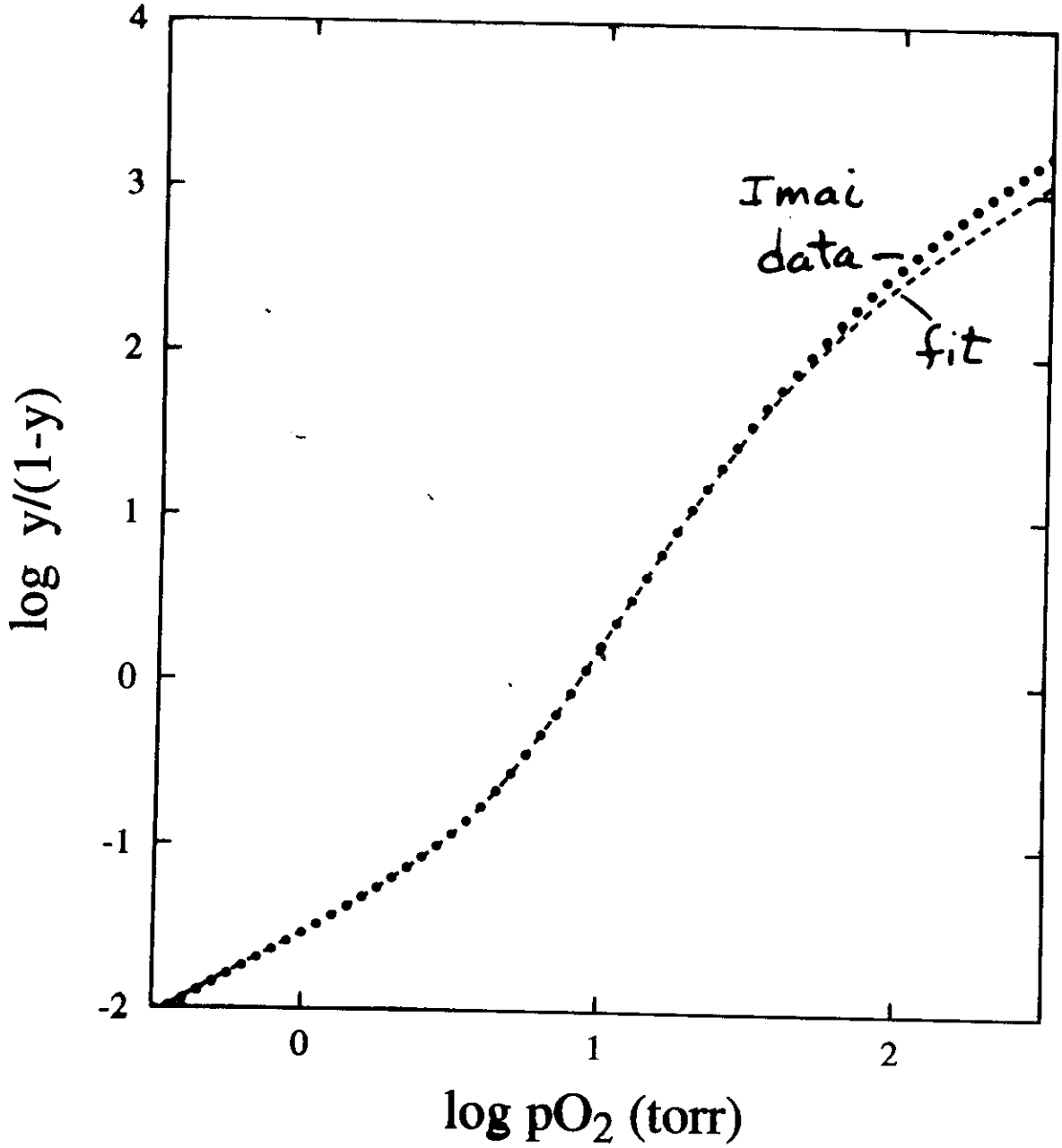
$$x(t) = \exp[-(kt)^\beta]$$


Data of C.M. Jones (NIH), Henry fit
ligand rebinding
(unnormalized)
amplitude
1.2
1
0.8
0.6
0.4
0.2
0
-8
-6
-4
-2
conformational changes
amplitude
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-8
-6
-4
-2
log time (s)

Binding polynomial:

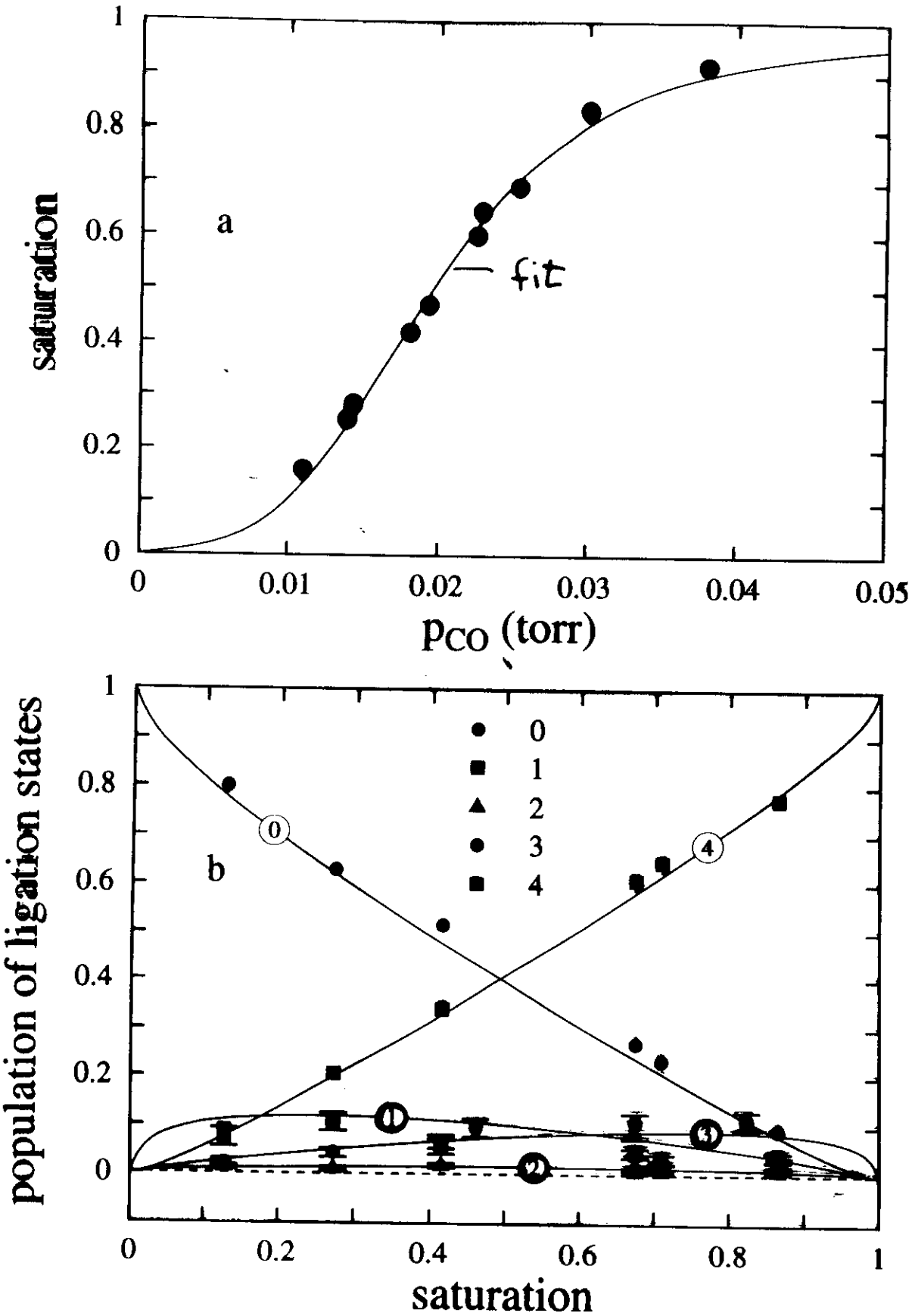
$$Q = \left\{1 + \frac{1}{L} + K_{in}P\left(1 + \frac{1}{L} + K_R\right)\right\}^4 + L\left\{1 + K_{in}P(1 + K_T)\right\}^4$$

Equilibrium oxygen binding curve

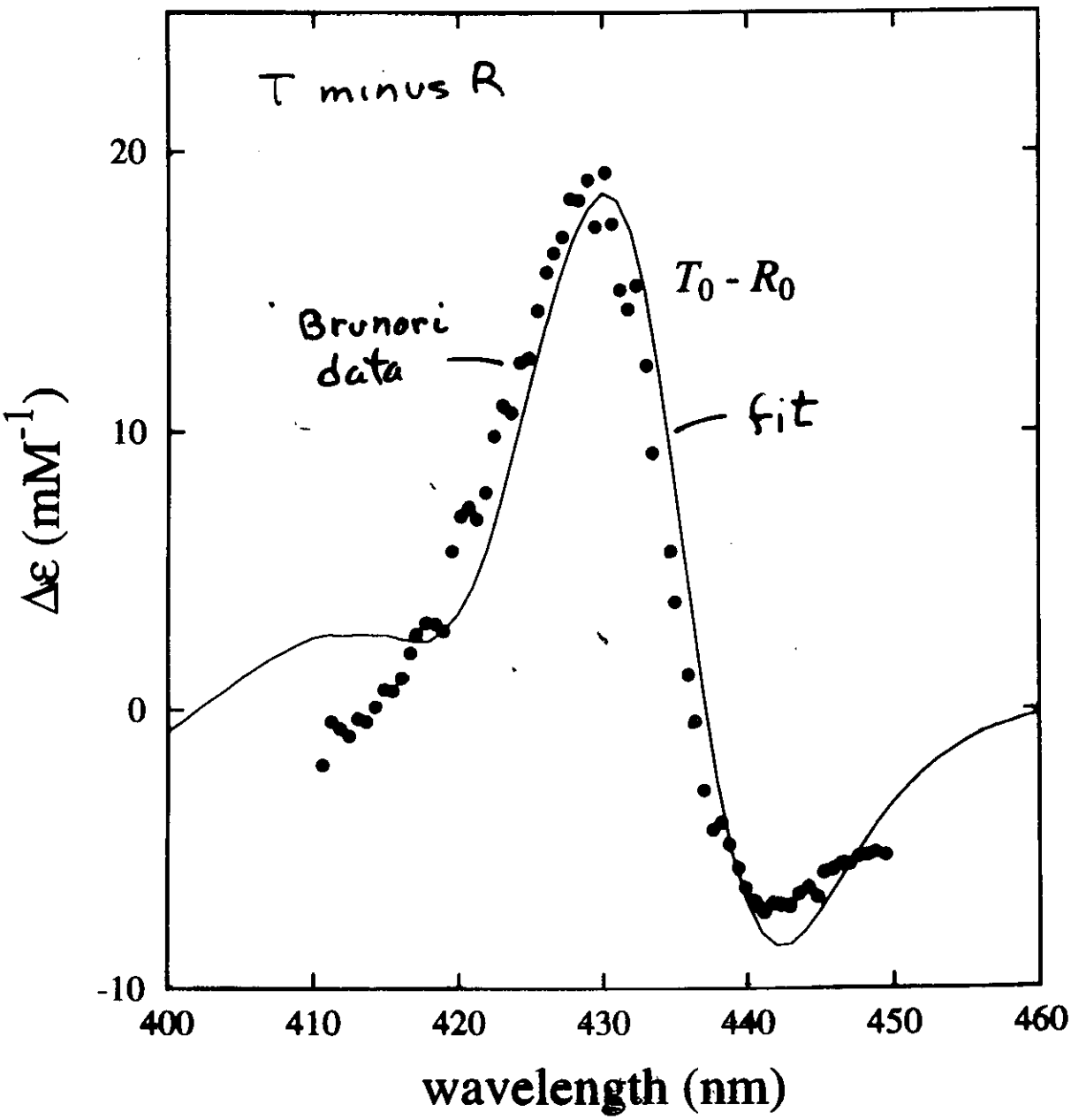


Henry, et al. Figure 8

Equilibrium data of Perrella

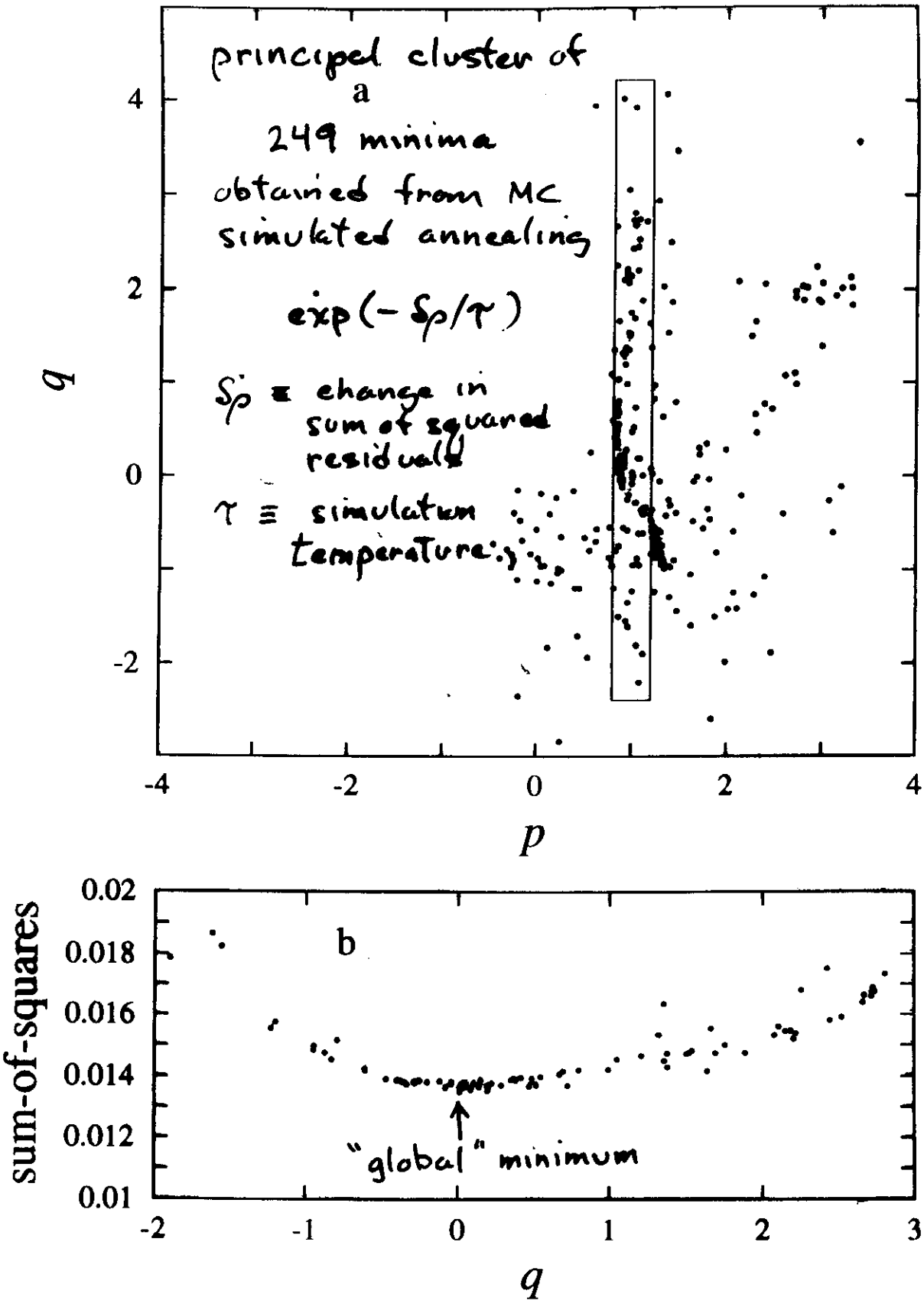


Henry, et al. Figure 9



Henry, et al. Figure 7

Survey of Parameter Space

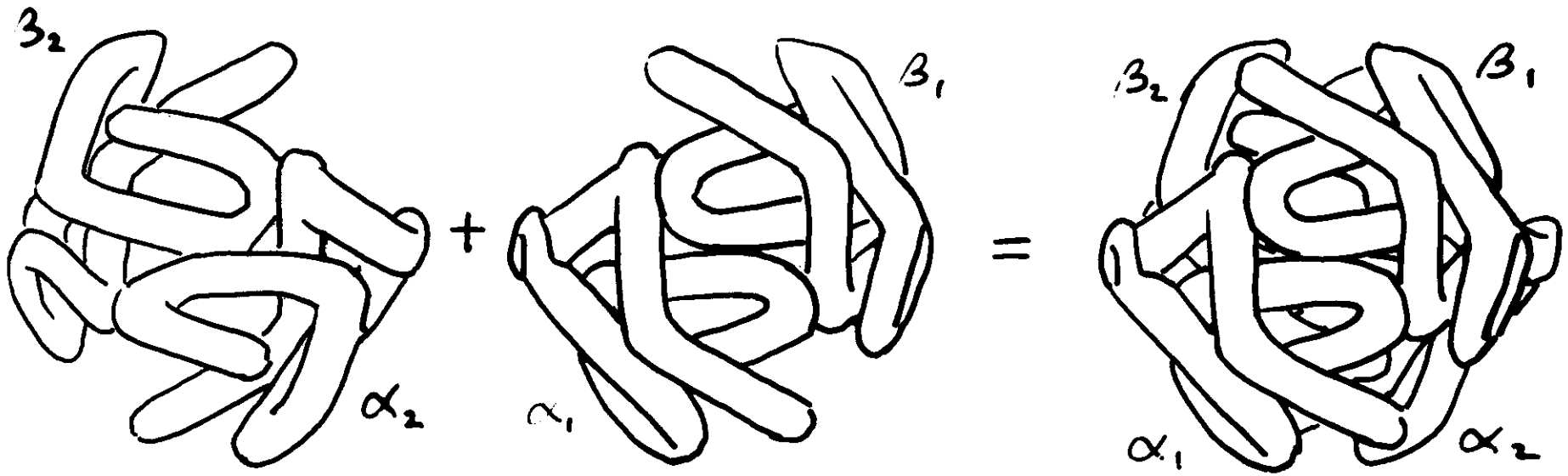


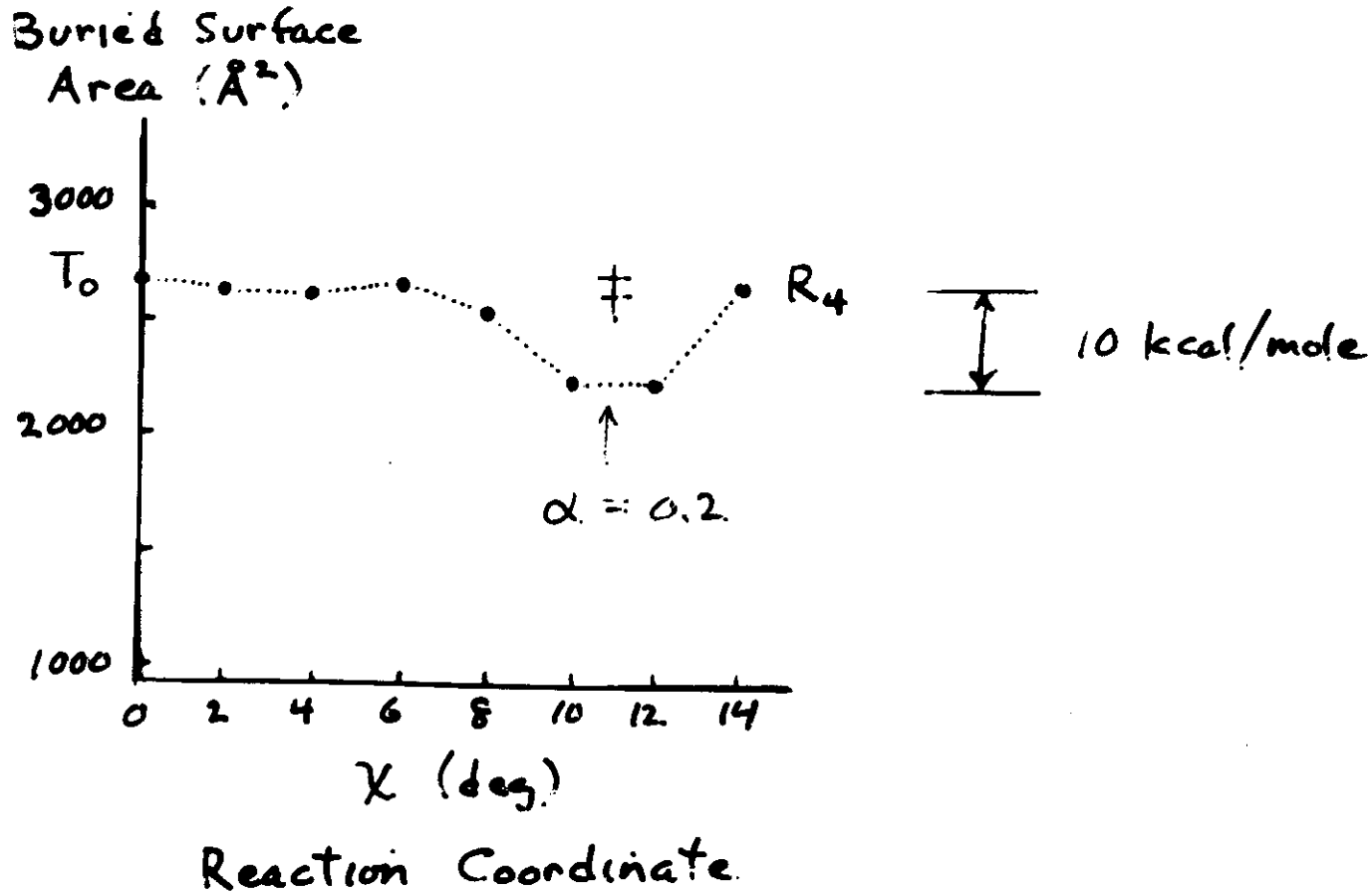
Henry, et al. Figure 6

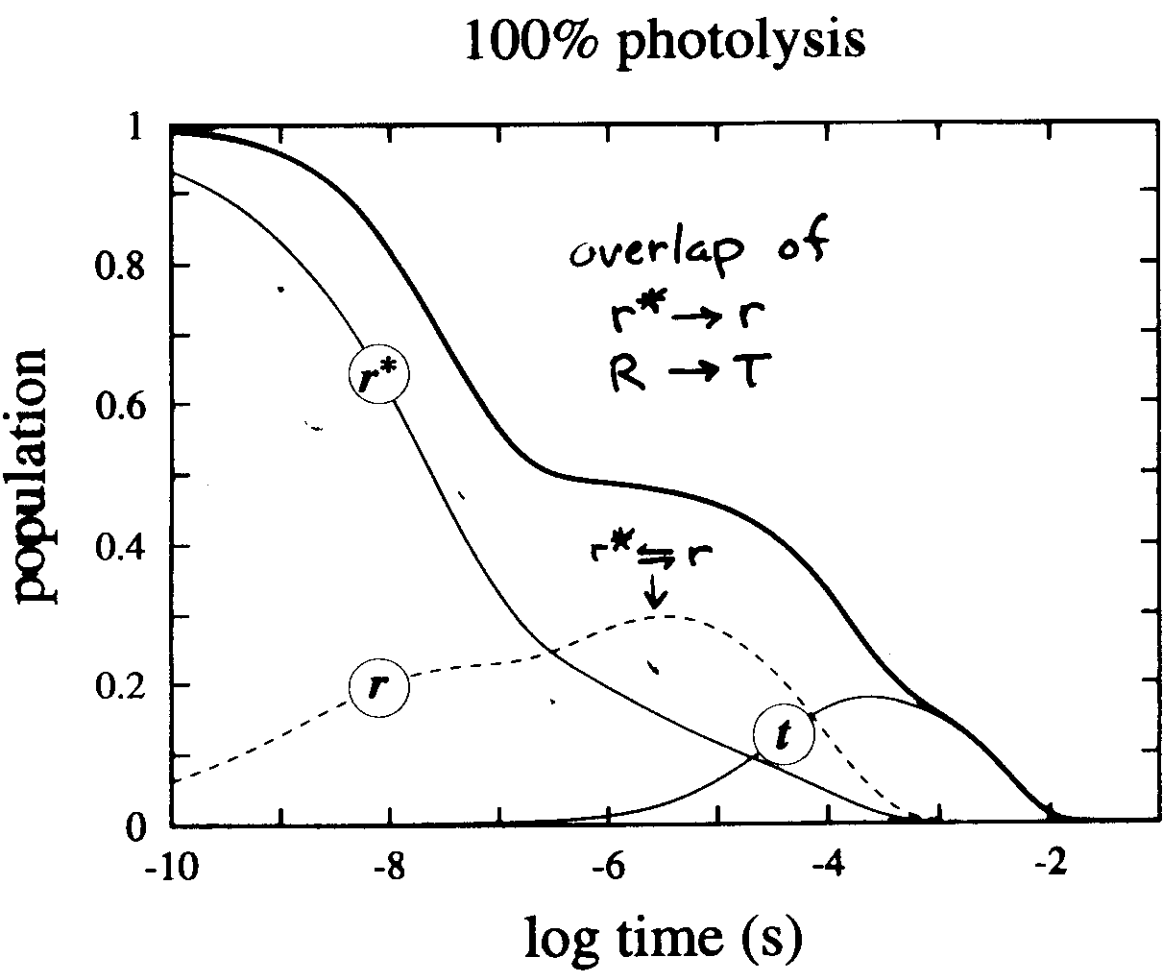
Parameters of Model

	median values	
c	2×10^{-3}	
$L \equiv T_o/R_o$	2×10^{7}	
$\ell \equiv r/r^*$	10	some r^* at equilibrium
$k(r^* \rightarrow r)$	$5 \times 10^{6}\ s^{-1}$	slower than Mb
β	0.3	larger than Mb
$k_{gem}(r^*)$	$1 \times 10^{6}\ s^{-1}$	same as Mb
$k_{gem}(r)$	$2.5 \times 10^{5}\ s^{-1}$	10x faster than Mb
$k_{gem}(t)$	$7.5 \times 10^{3}\ s^{-1}$	additional slowing from $R \rightarrow T$
k_{in}	$1 \times 10^{8}\ M^{-1} s^{-1}$	diffusion-limited
k_{out}	$6 \times 10^{6}\ s^{-1}$	
$R_o \rightarrow T_o$	$6 \times 10^{4}\ s^{-1}$	
α	0.2	test of models e.g. Janin & Wodak, 1985 R. Elber

Janin & Wodak, 1985

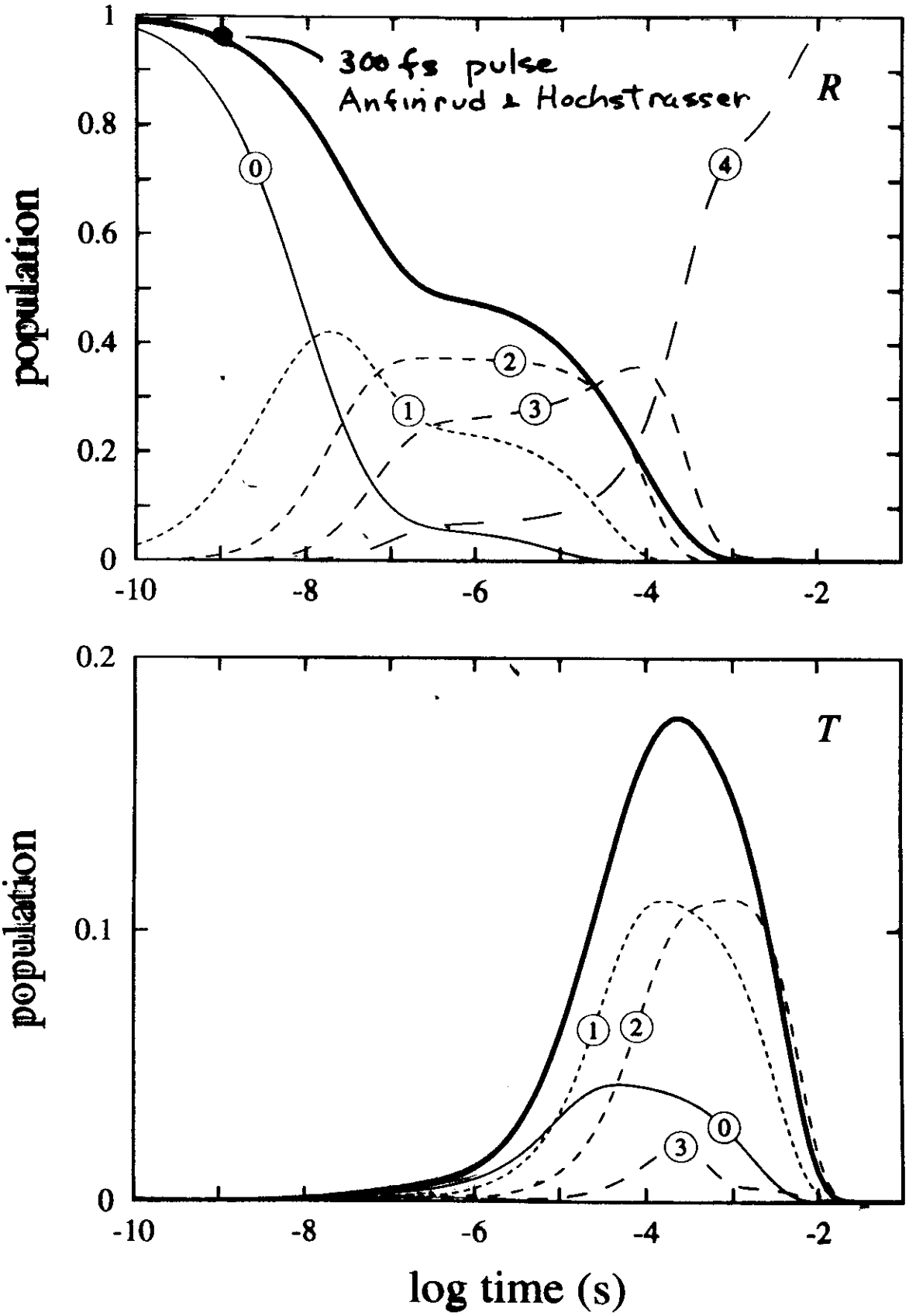



Buried Surface
Area (Å²)
3000
T₀
2000
1000
0
2
4
6
8
10
12
14
χ (deg.)
‡
R₄
α = 0.2
10 kcal/mole
Reaction Coordinate



Henry, et al. Figure 12a

100% photolysis



Henry, et al. Figure 11a

Take Home Lesson

1. Extended MWC model survives most critical test to date.

2. Made possible by:

   medium-tech: nanosecond spectroscopy
   hi-tech: femto/pico-second spectroscopy
   computing power
   new ideas from protein physics

3. Myoglobin remains "hydrogen atom" of biology and hemoglobin the "hydrogen molecule."

what next?

Model the submicrosecond kinetics <u>correctly</u>!

Investigate the condition dependence (i.e PSK model).