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

H4.SMR/775-19

COLLEGE IN BIOPHYSICS: EXPERIMENTAL AND THEORETICAL ASPECTS OF BIOMOLECULES

26 September - 14 October 1994

Miramare - Trieste, Italy

Protein Dynamics II & III A

William A. Eaton
National Institutes of Health
Bethesda, Maryland - USA

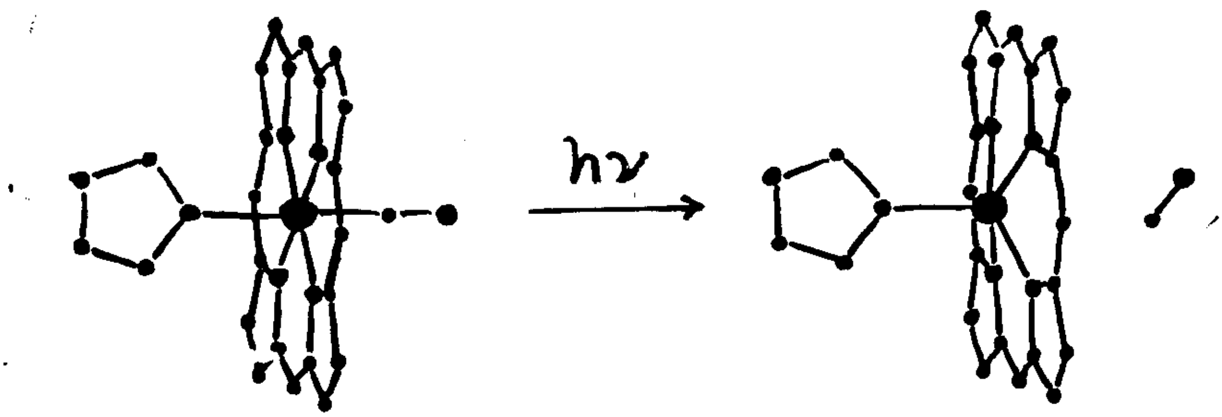
10/11/44

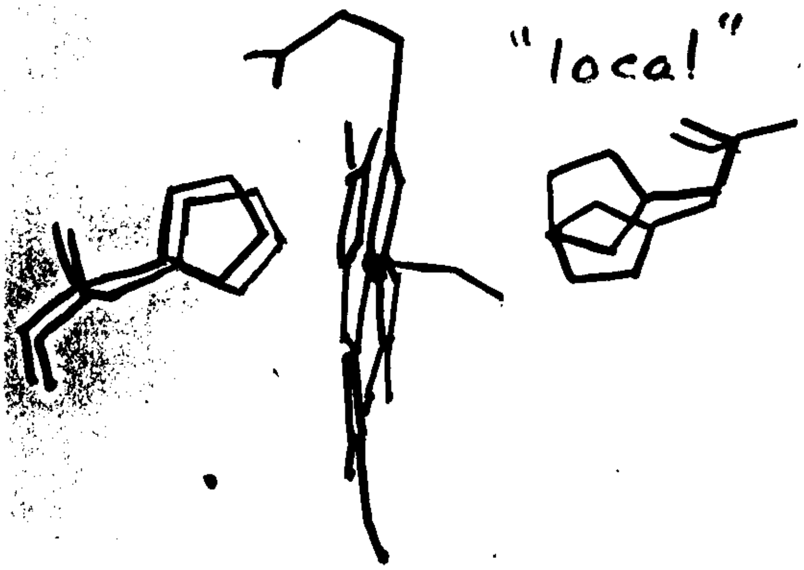
Eaton

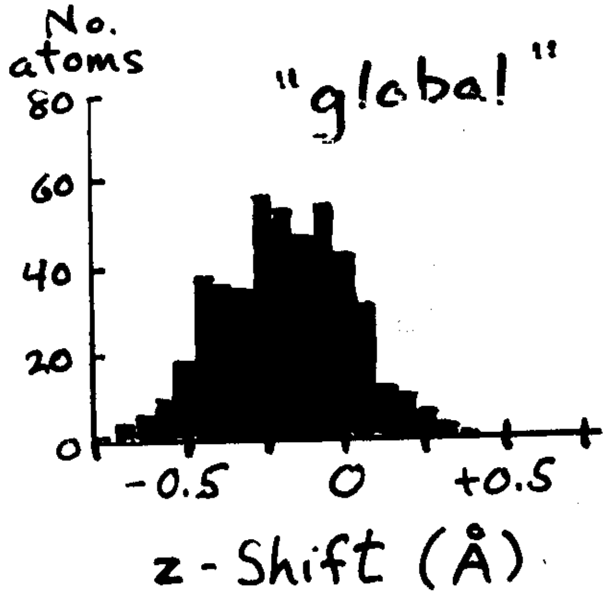
Lecture II

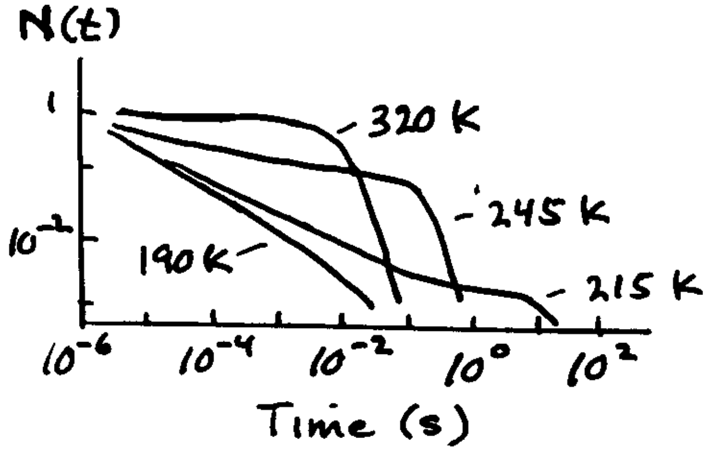
Two "Fairytales"

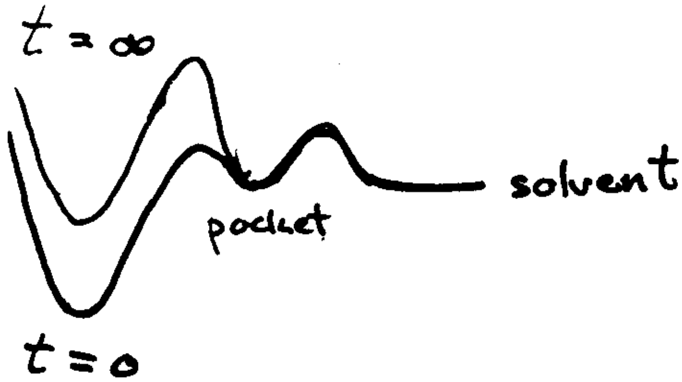

heme
C
D
B
G
E
H
F
A

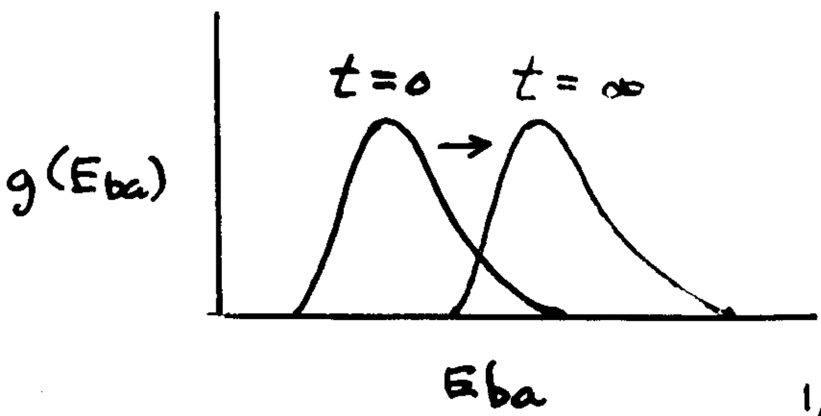

hν


"local"


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







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

$\beta_{\underline{predicted}} = 0.25$

$1/k_{\underline{predicted}} = 10ps$

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

Steinbach, Frauenfelder et al. 1991 —
<u>predicted</u> kinetics of protein relaxation

An finrud

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

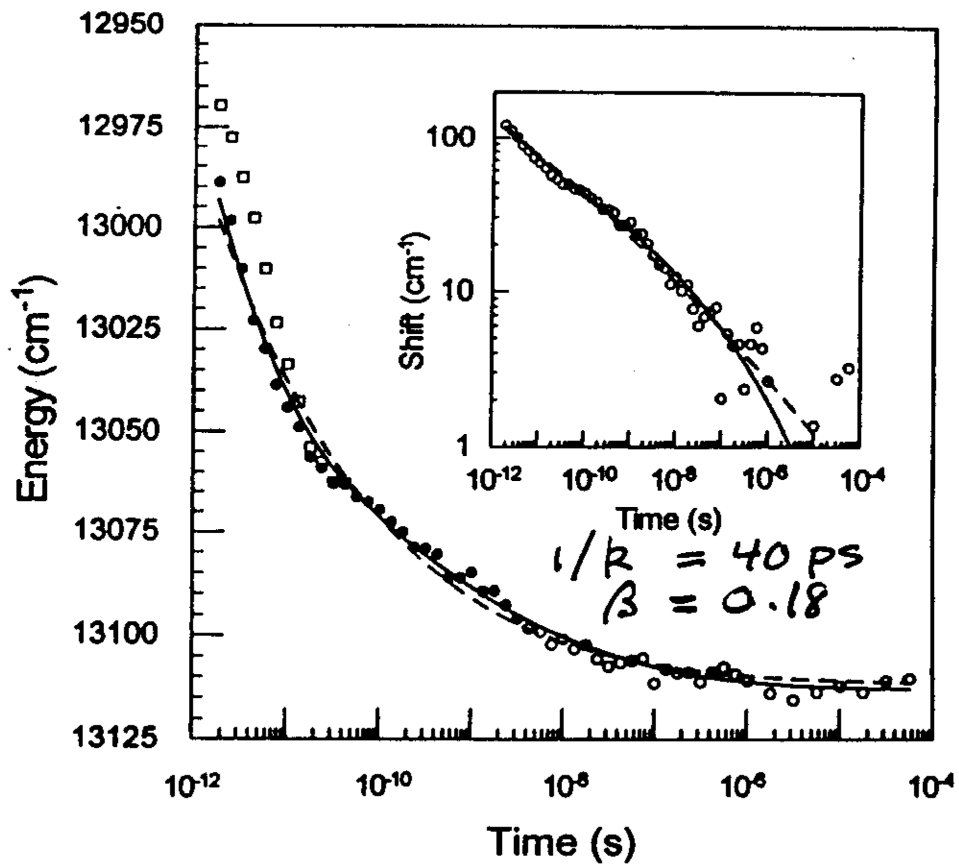
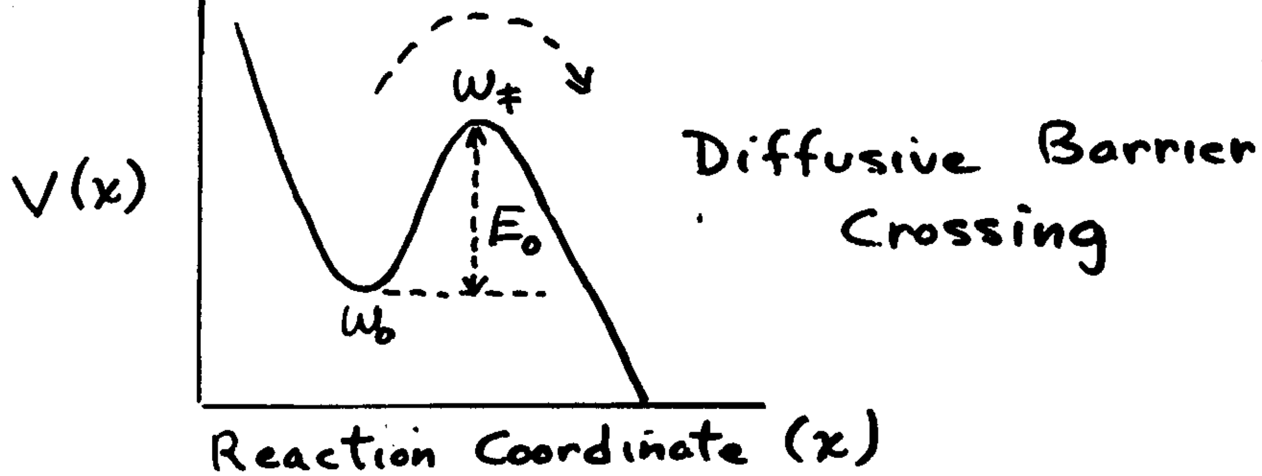


Fig. 2. Time-dependent position of band III with and without compensation for thermal relaxation. The filled and open circles represent data collected with femtosecond and nanosecond pump pulses, respectively. The open squares show data uncorrected for thermal relaxation. Note that the thermal correction is significant only for times shorter than ~20 ps. The data were modeled with a modified stretched exponential function (solid line), and a conventional stretched exponential function (dashed line) over the entire range of times (see parameters in table 1). The inset figure is a log–log plot of the thermally compensated shift of band III relative to equilibrium Mb.



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:

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

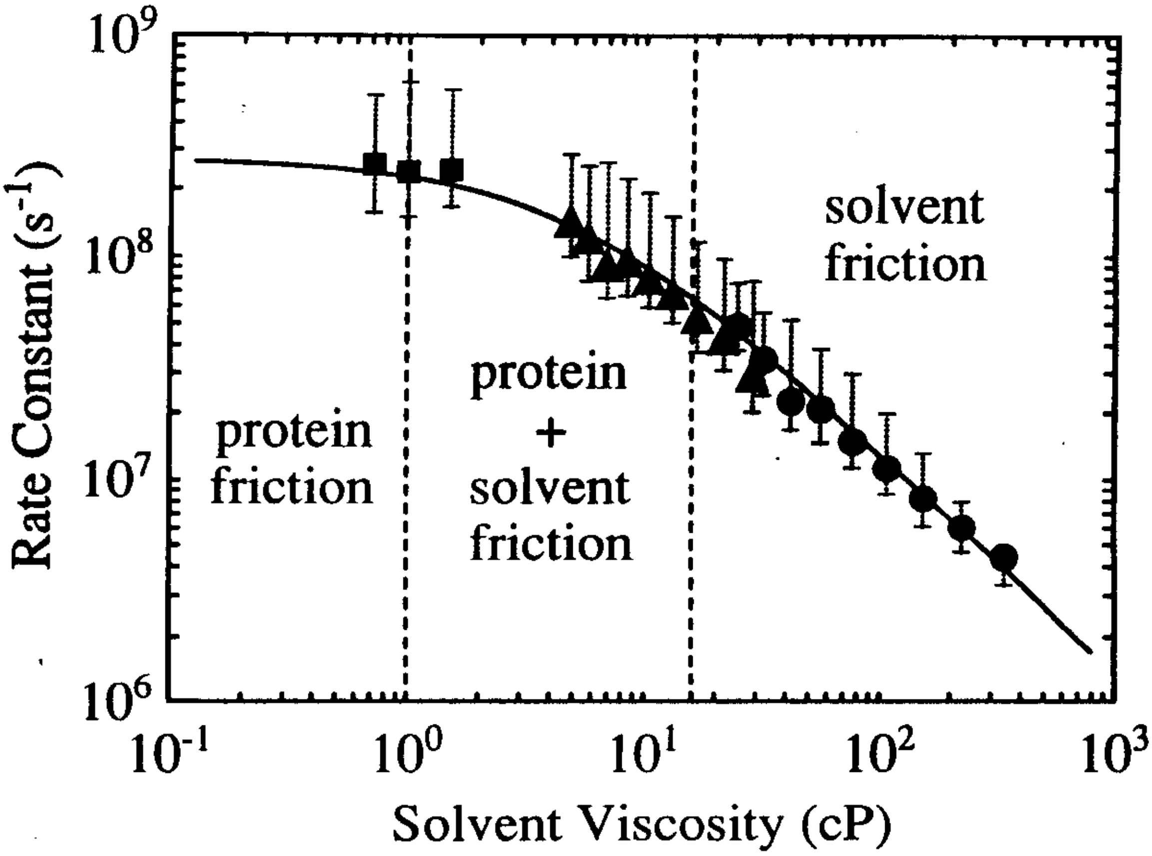
Assuming that protein friction and solvent friction are additive, Kramers' equation is:

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

which, using Stokes' law, becomes:

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

Myoglobin Conformational Relaxation



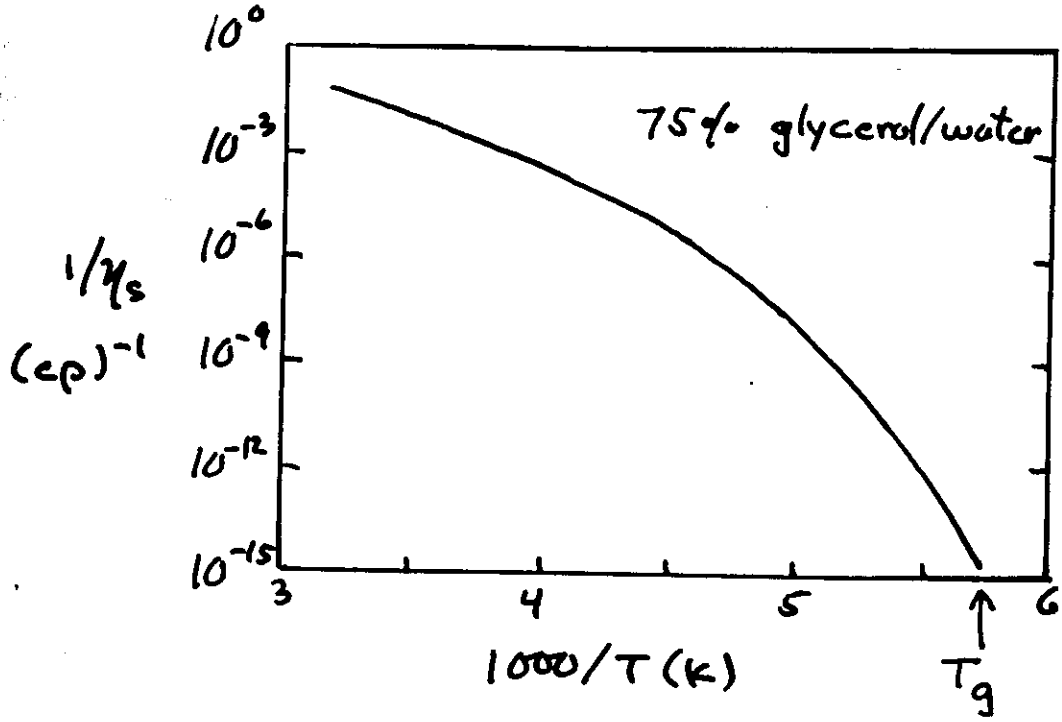
$$k = \frac{C}{\sigma + \eta_s} e^{-E_o/k_B T}$$

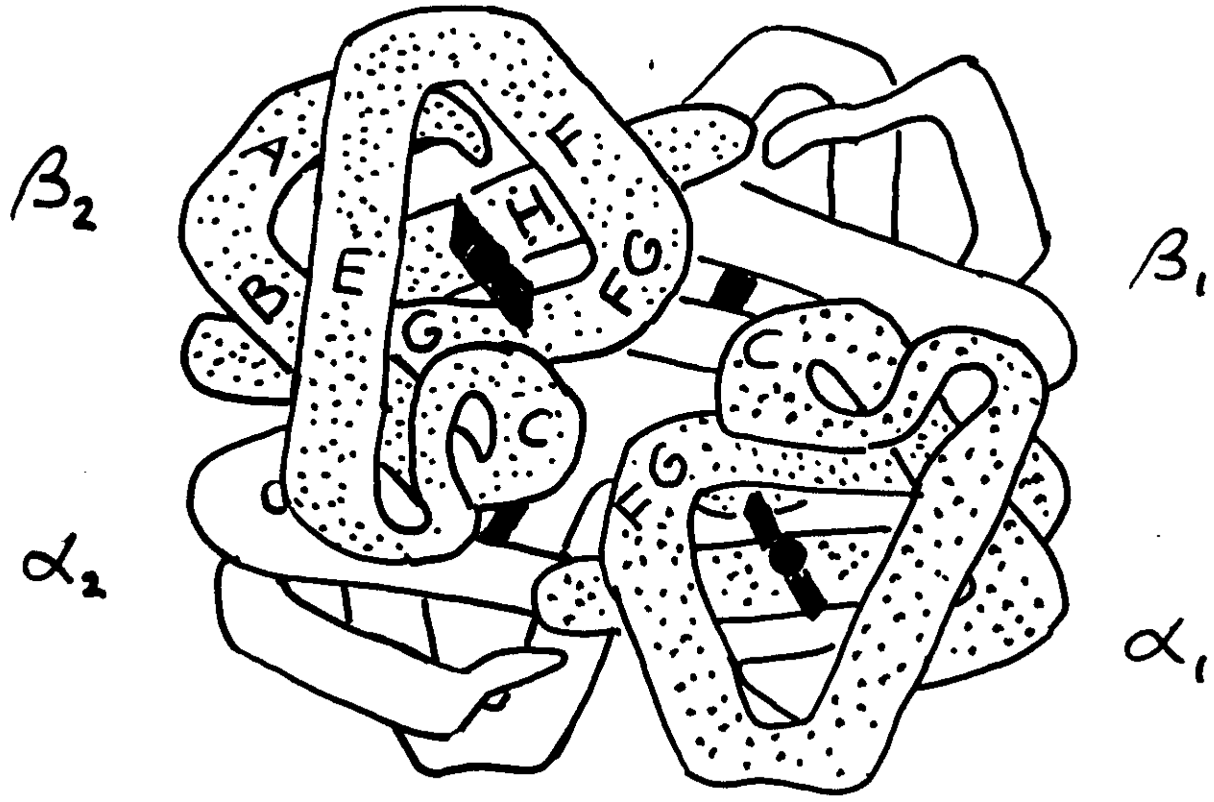
$\sigma = 4$ cP

$E_o = 2.4$ kcal/mole

$C = 7 \times 10^{10}$ cP/sec

$$k = \frac{c}{\cancel{\sigma} + \eta_s}\, e^{-E_0/RT}$$

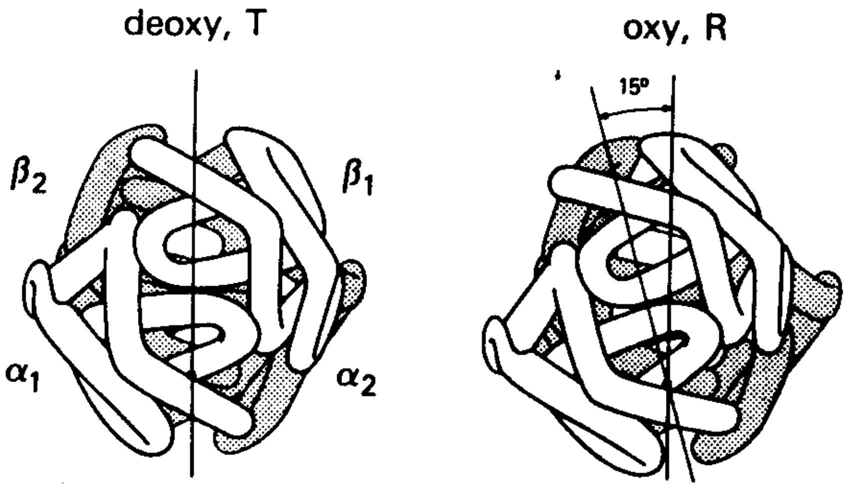


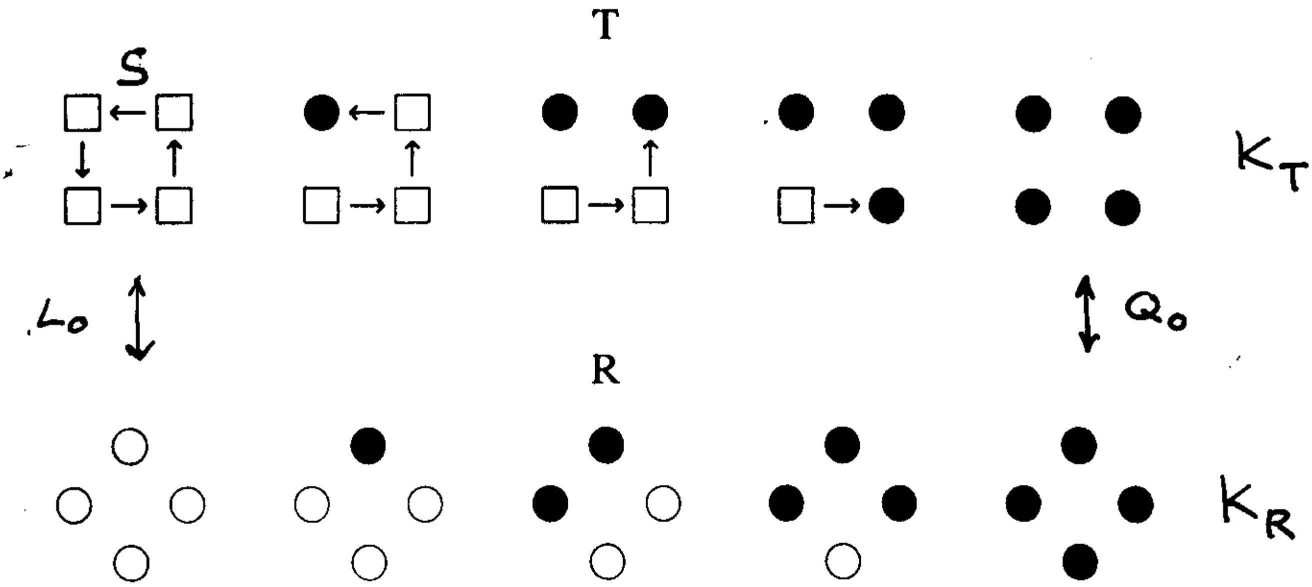


β_2
β_1
α_2
α_1
A
B
E
G
H
F
FG
C
C
FG

MWC - PSK Model

Monod
Wyman
Changeux
Perutz
Szabo
Karplus





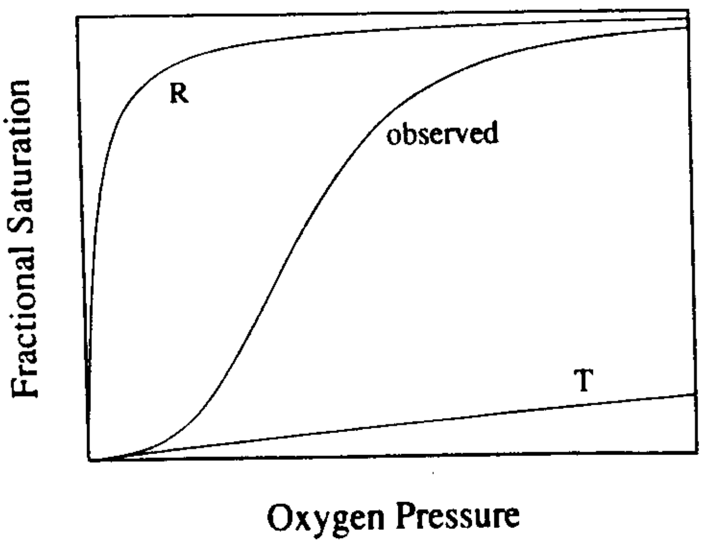
<u>MWC</u>

K_R, K_T, L_o

$L_i = L_o c^i$

<u>SK</u>

K_R, S, Q_o



$K_T = K_R / S$

$L_o = Q_o S^4$

Linear Free Energy Relation

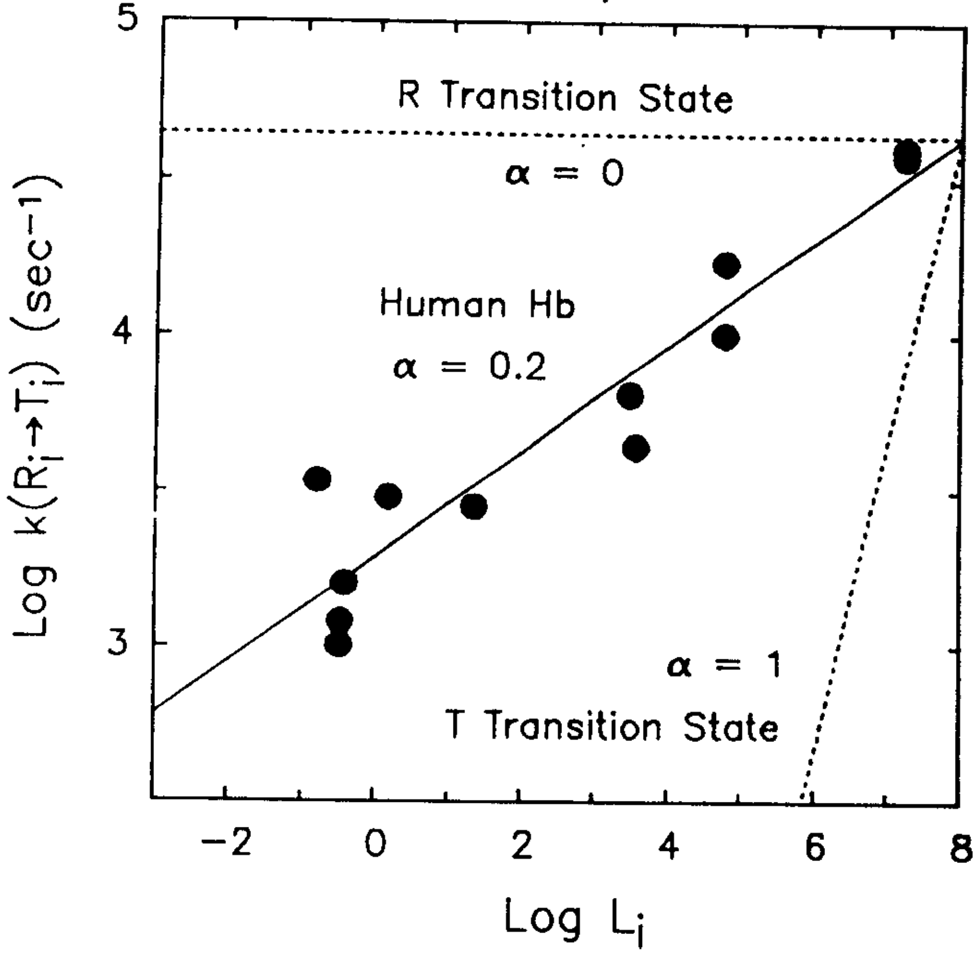
$$\delta \Delta G^{\ddagger} = \alpha\, \delta \Delta G$$

For two-state allosteric model

$$k(R_i \to T_i) = \gamma (L_o c^i)^{\alpha}$$

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

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

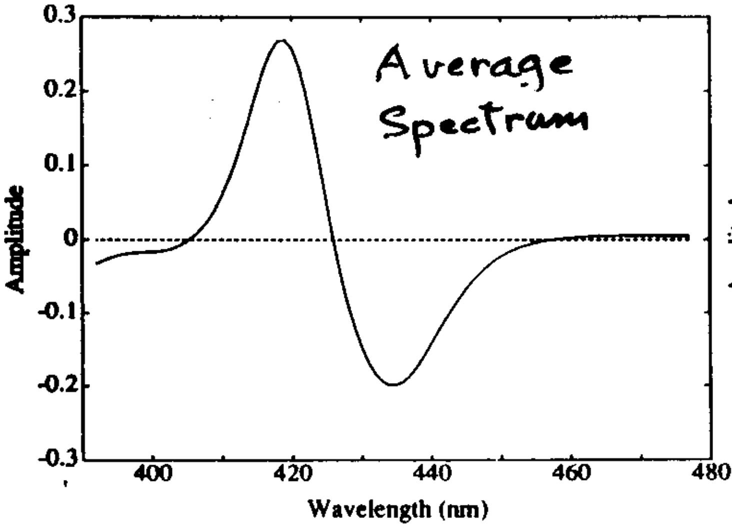


Transition state appears at 20% of the distance along the reaction path from R to T.

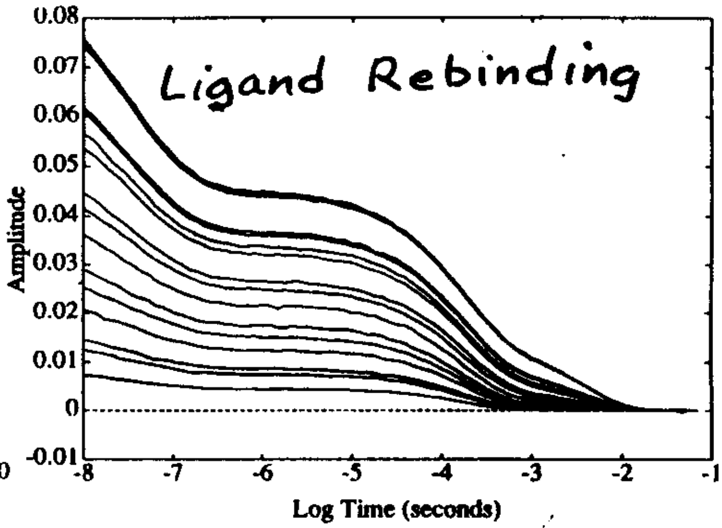
Singular Value Decomposition
(of isotropically-averaged data)

$$A = USV^T$$

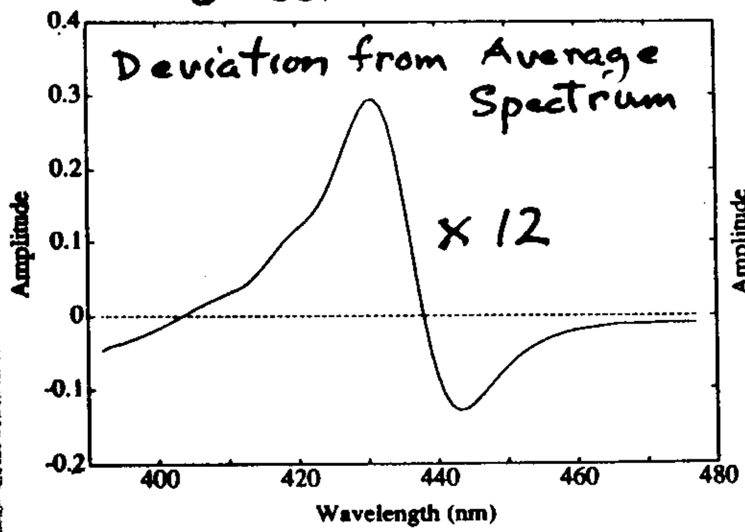
U Column 1



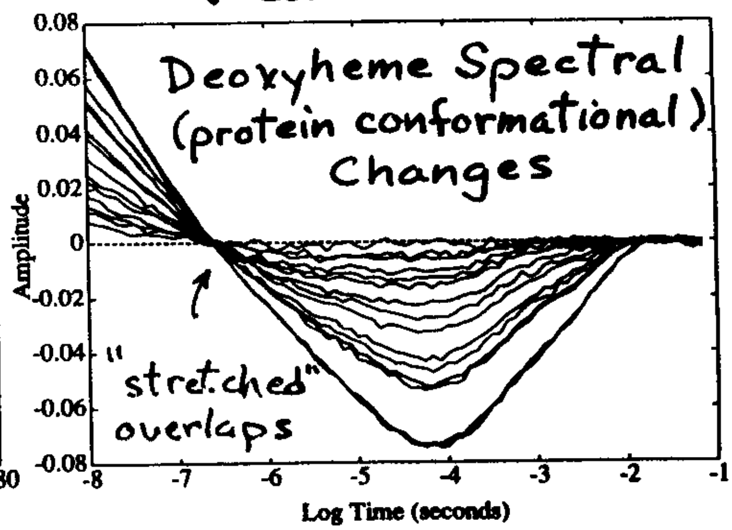
V column 1



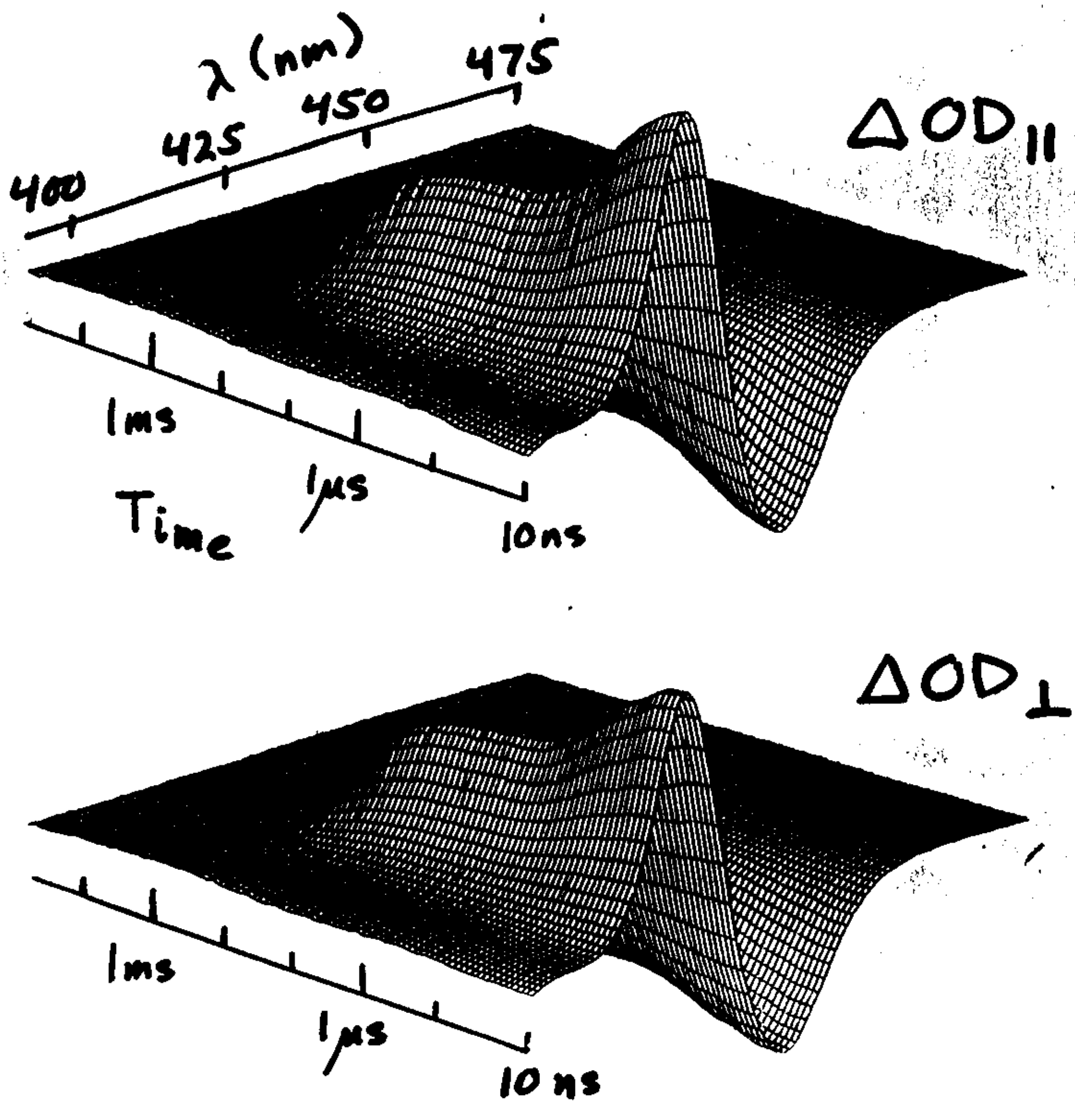
U column 2



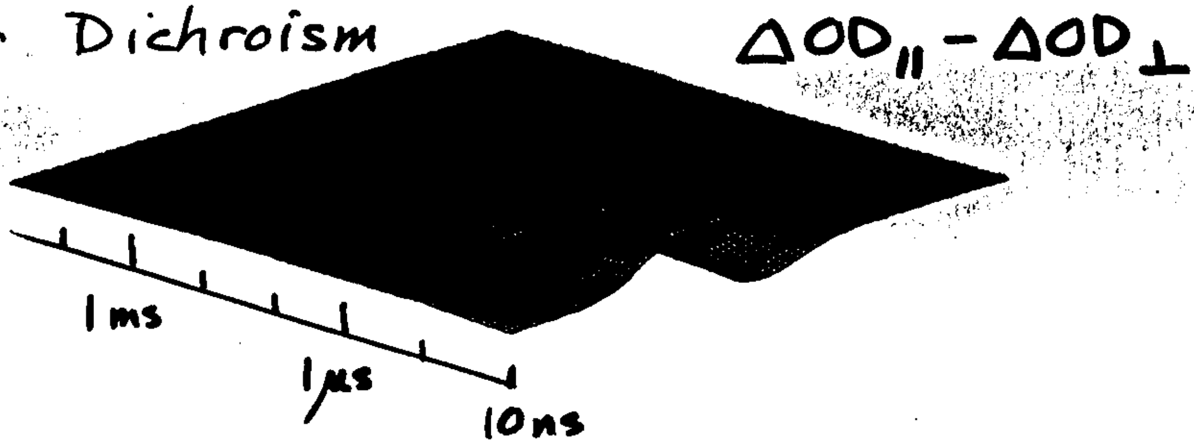
V column 2



Polarized Spectra



Linear Dichroism



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

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

In R

$$\begin{array}{ccccc} & \overset{k(r^*)}{\swarrow} & r^*\cdot x & \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} & r^* + x \\ rx & & \ell \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\ & \underset{k(r)}{\nwarrow} & r\cdot x & \rightleftharpoons & r + x \end{array} \qquad r^* \to r: \; e^{-(kt)^{\beta}}$$

In T

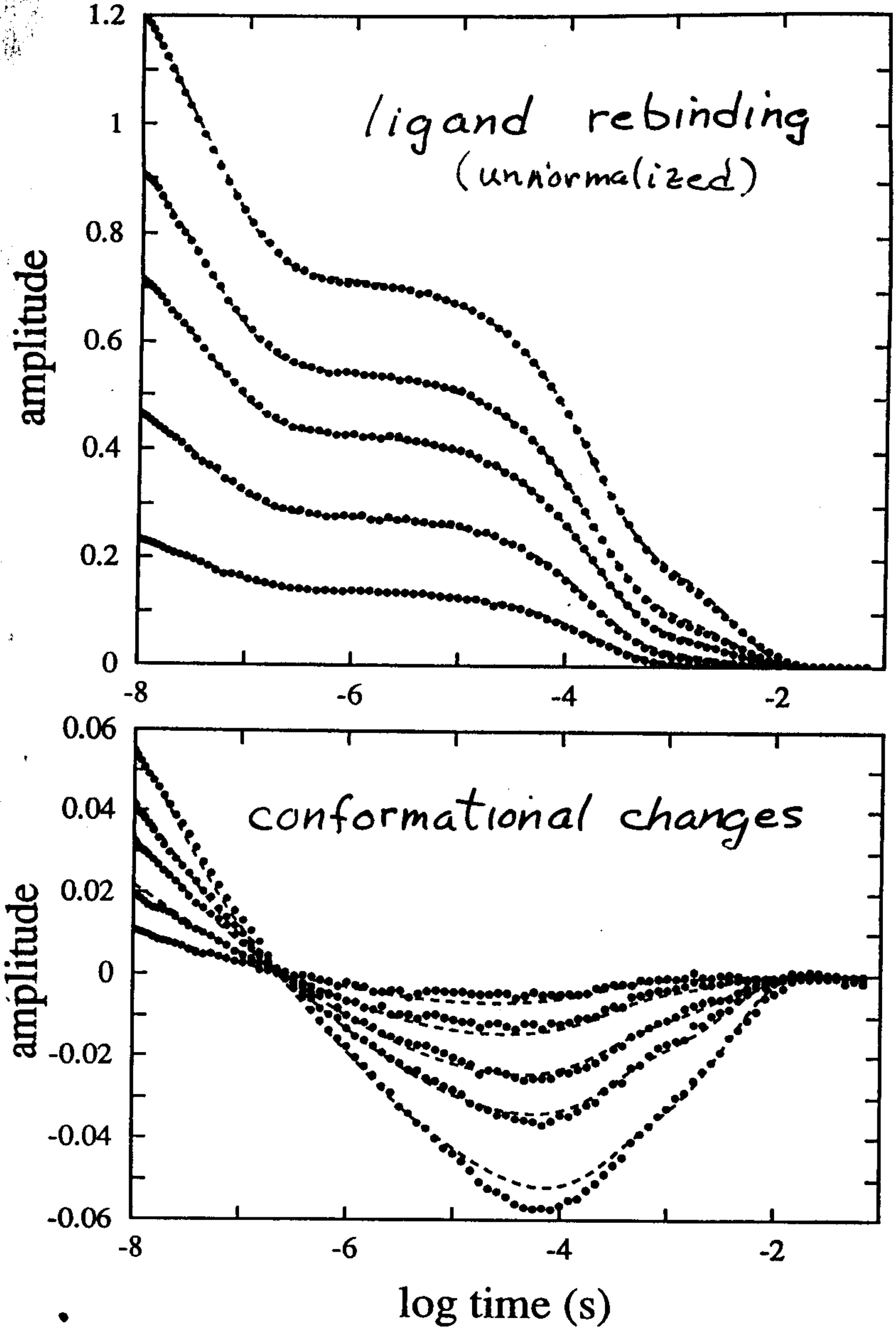
$$tx \underset{k(t)}{\longleftarrow} t\cdot x \rightleftharpoons t + x$$

L, c, γ, α	quaternary rates
k, β, ℓ	tertiary rates
$\left.\begin{array}{l} k(r^*), k(r), k(t) \\ k_{in}, k_{out} \end{array}\right\}$	ligand binding rates

85 distinguishable species, $\underline{\underline{3}}$ spectra

$\underline{\underline{12}}$ model parameters
to describe

$\underline{\underline{37}}$ experimental parameters, $\underline{\underline{2}}$ spectra



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)

PARAMETERS OBTAINED FROM FITS WITH TWO-STATE MWC ALLOSTERIC MODEL WITH TERTIARY TRANSIENT

$L = 2 \times 10^7$ $(\equiv [T_0]/[R_0])$	simultaneously fit to oxygen binding curve of Imai
$c = 0.002$ (CO), 0.003 (O_2) $(\equiv p50(T)/p50(R))$	
$l = 15$ $(\equiv [r]/[r^*])$	r^* is only 6% populated at equilibrium, i.e. "transient"
$k_{gem}(r^*) = 4 \times 10^7\ s^{-1}$	
$k_{gem}(r) = 4 \times 10^5\ s^{-1}$	tertiary relaxation has marked effect on geminate rebinding rate, as in myoglobin
$k_{gem}(t) = 1 \times 10^4\ s^{-1}$	quaternary relaxation also has marked effect on geminate rebinding rate

$k_{in} = 1 \times 10^8\ M^{-1}s^{-1}$	
$k_{out} = 7 \times 10^6\ s^{-1}$	geminate yield determined by ligand exit rate
$k(R_3 \rightarrow T_3) = 500\ s^{-1}$	value constrained to be within a factor of two of the value obtained by Ferrone from modulation experiments at very low light intensities
$\alpha = 0.2$	linear free energy exponent, same as value reported by Eaton et al., 1991
	yields $k(R_0 \rightarrow T_0) = 4 \times 10^4\ s^{-1}$
	$R \rightarrow T$ rate increase by a factor of $15^{0.2} = 1.7$ for every r^* subunit
$k(r^* \rightarrow r) = 3 \times 10^6\ s^{-1}$ $\beta = 0.3$	overlaps quaternary relaxation because of small β

Eaton

Lecture III

Sickle Cell Hemoglobin

Kinetic Equations of Double Nucleation Model

$$\frac{dc_p}{dt} = \underbrace{k_+ c\, c_{i^*}}_{\text{hom.}} + \underbrace{k_+ c\, c_{j^*}}_{\text{het.}}$$

assuming monomer $\leftrightharpoons$ nucleus equilibrium

$$\frac{dc_p}{dt} = k_+ K_{i^*} c^{i^*+1} + k_+ K_{j^*} \phi (c_0 - c) c^{j^*+1}$$

$$-\frac{dc}{dt} = k_+ c_p c - k_- c_p$$

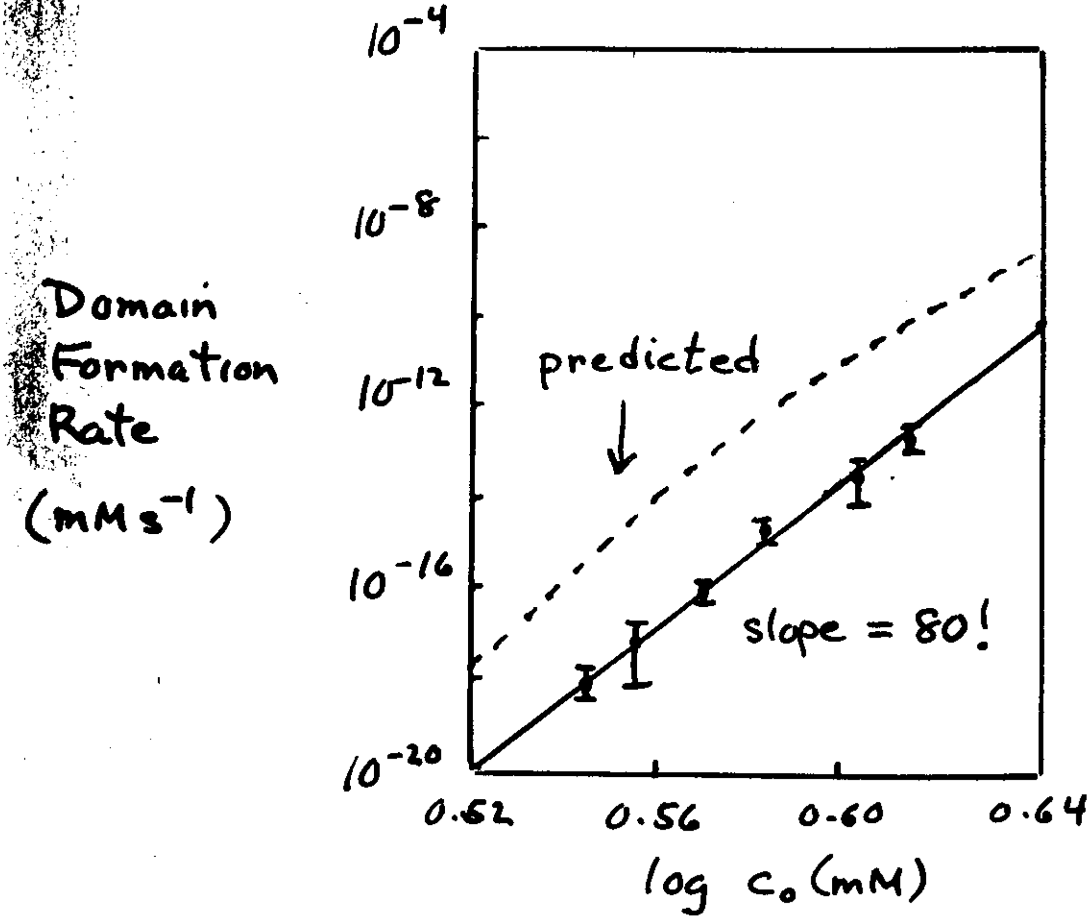
integrating linearized rate equations, $c(t) \sim c_0$

$$c_0 - c(t) = A\left[\cosh(Bt) - 1\right]$$

$$\doteq \begin{cases} \frac{1}{2} A B^2 t^2 & Bt \ll 1 \\ \frac{1}{2} A e^{Bt} & Bt \gg 1 \end{cases}$$

$$A = \frac{K_{i^*} c_0^{i^*+1} \quad \text{hom}}{K_{j^*} \phi c_0^{j^*+1} \quad \text{het}}$$

$$B^2 = k_+^2 (c_0 - c) \phi K_{j^*} c_0^{j^*+1}$$



Domain Formation Rate (mM s⁻¹)
10^-4
10^-8
10^-12
10^-16
10^-20
predicted
slope = 80!
0.52
0.56
0.60
0.64
log c₀ (mM)