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INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
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H4.SMR/916 - 37

SEVENTH COLLEGE ON BIOPHYSICS:

Structure and Function of Biopolymers: Experimental and Theoretical Techniques.

4 - 29 March 1996

Cooperativity in Hemoglobin - the Hydrogen Molecule of Biology

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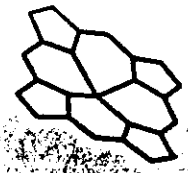
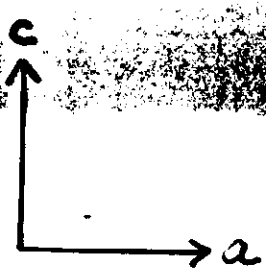
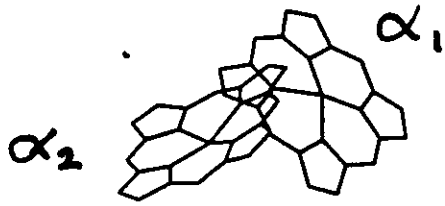
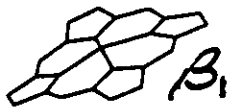
. Mozzarelli (Parma)
. Rivetti (Parma)
. B. Henry (NIH)
. L. Rossi (Parma)

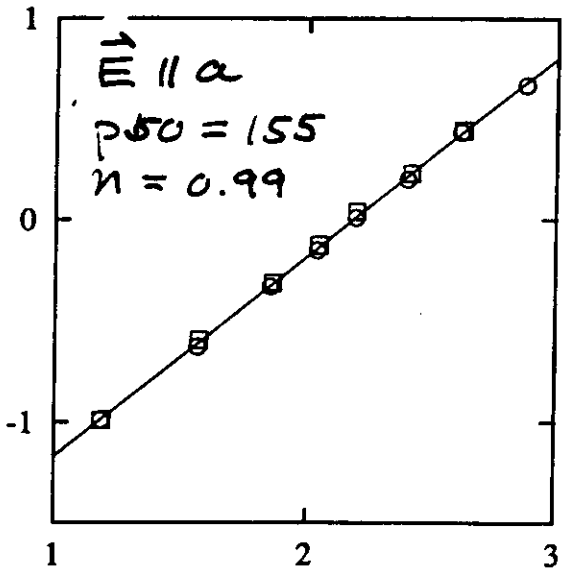


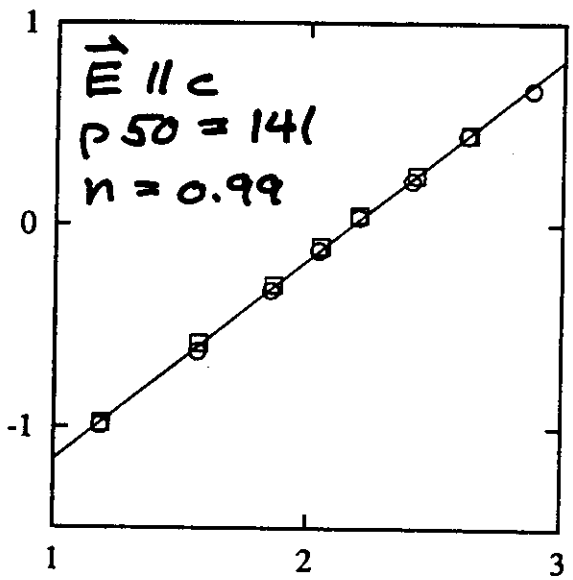


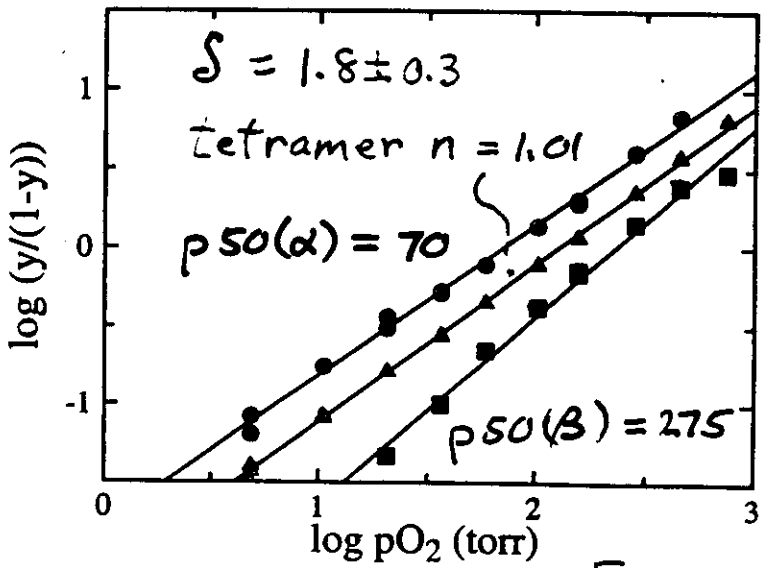
T quaternary





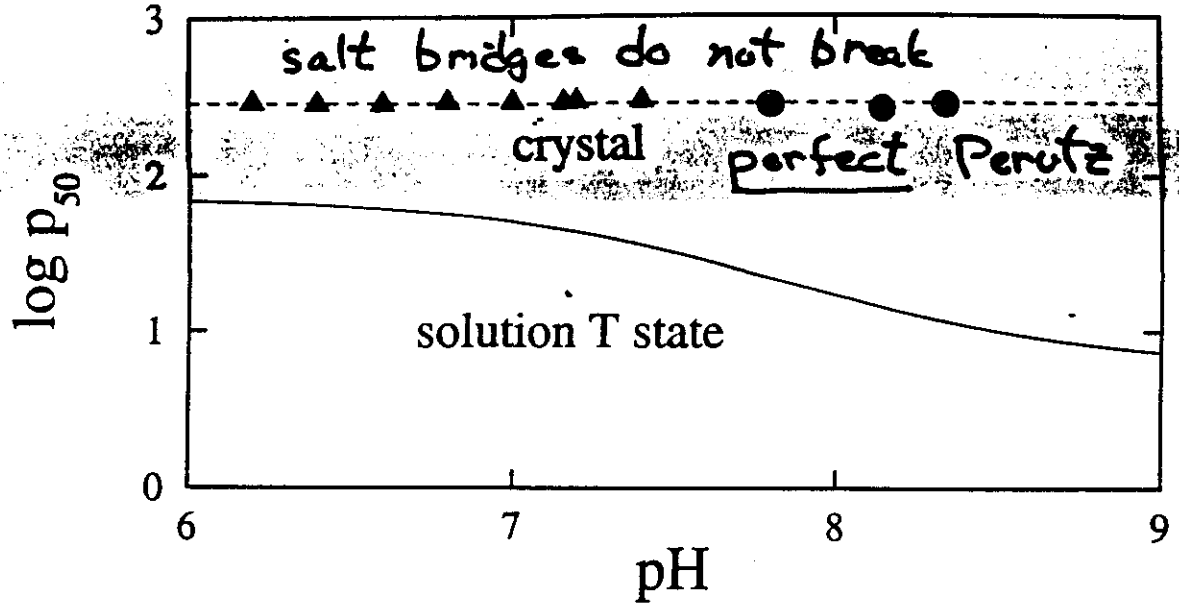


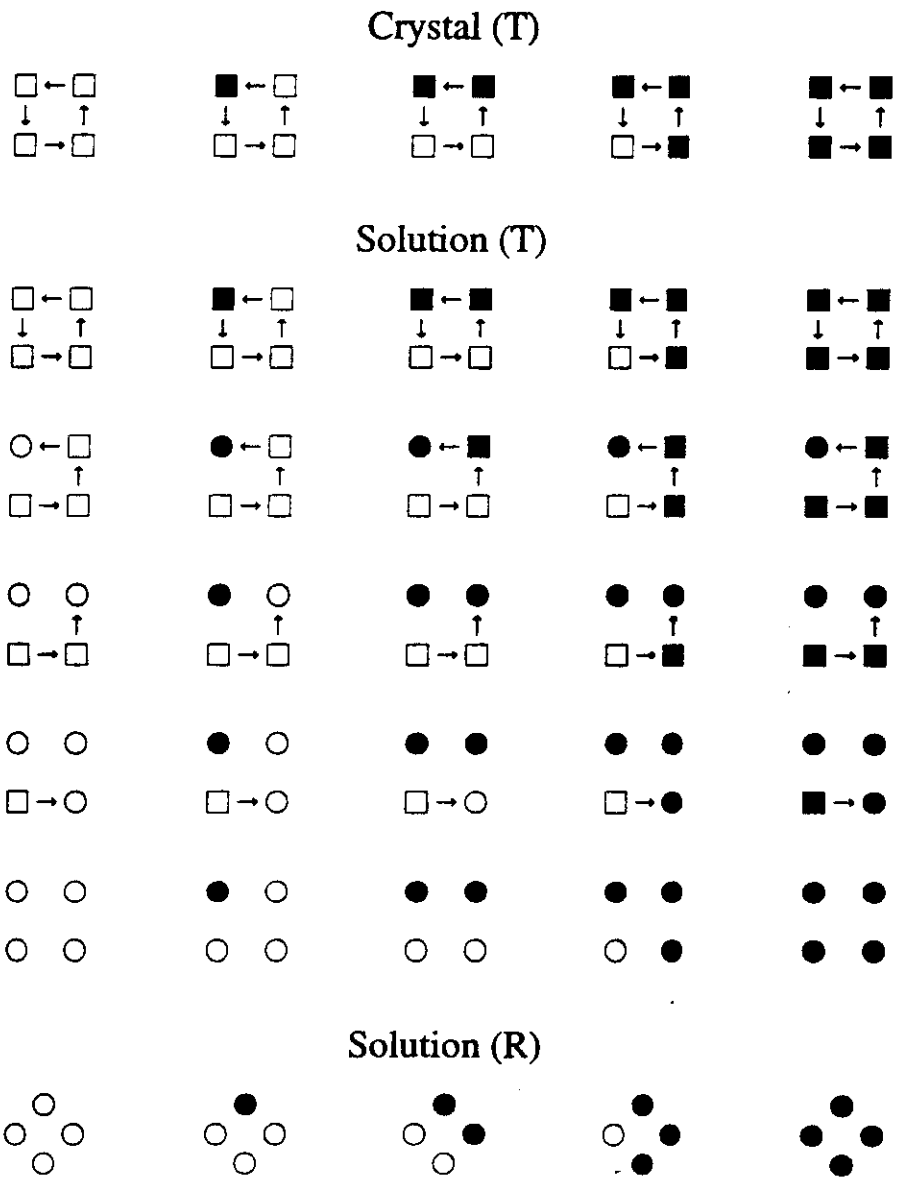


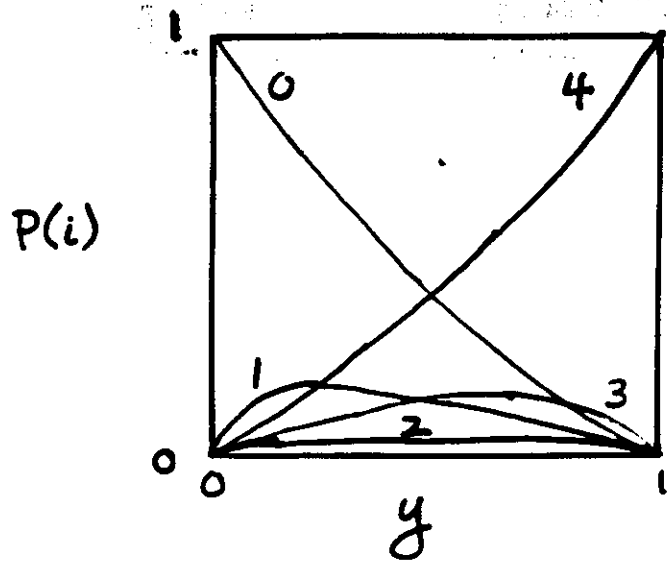


$$\alpha = [1+(K_\alpha+K_\beta)p + SK_\alpha K_\beta p^2]^2 \quad \text{For } n=1,\ q \equiv \frac{K_\alpha}{K_\beta}$$

$$S = (q+1)^2/4q$$


salt bridges do not break
crystal
perfect
Perutz
solution T state
log p_{50}
3
2
1
0
6
7
8
9
pH

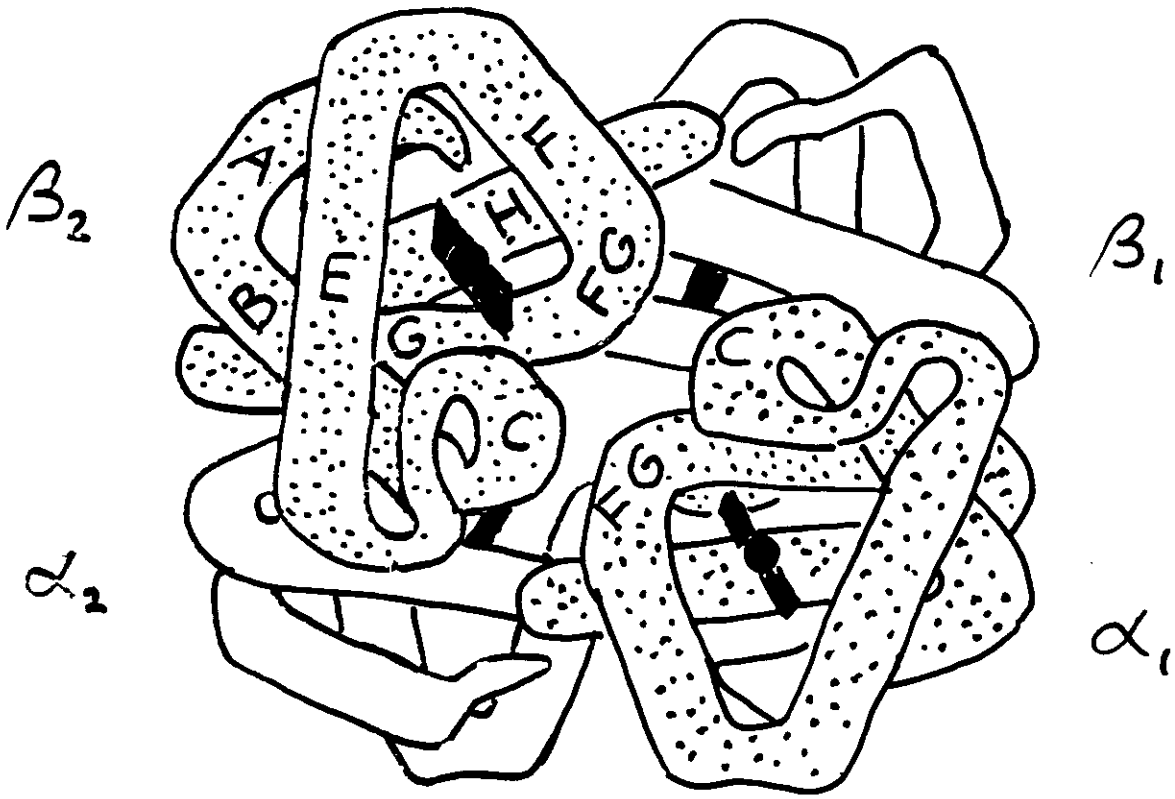

Crystal (T)
Solution (T)
Solution (R)



Intermediates at low population at equilibrium.

Choices:

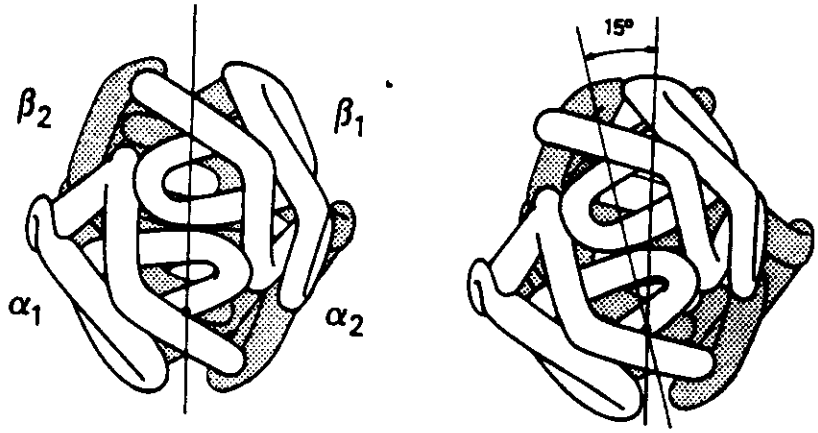
1. Prepare <u>artificial</u> intermediates (can be misleading)
2. Prepare real intermediates by partial photolysis.

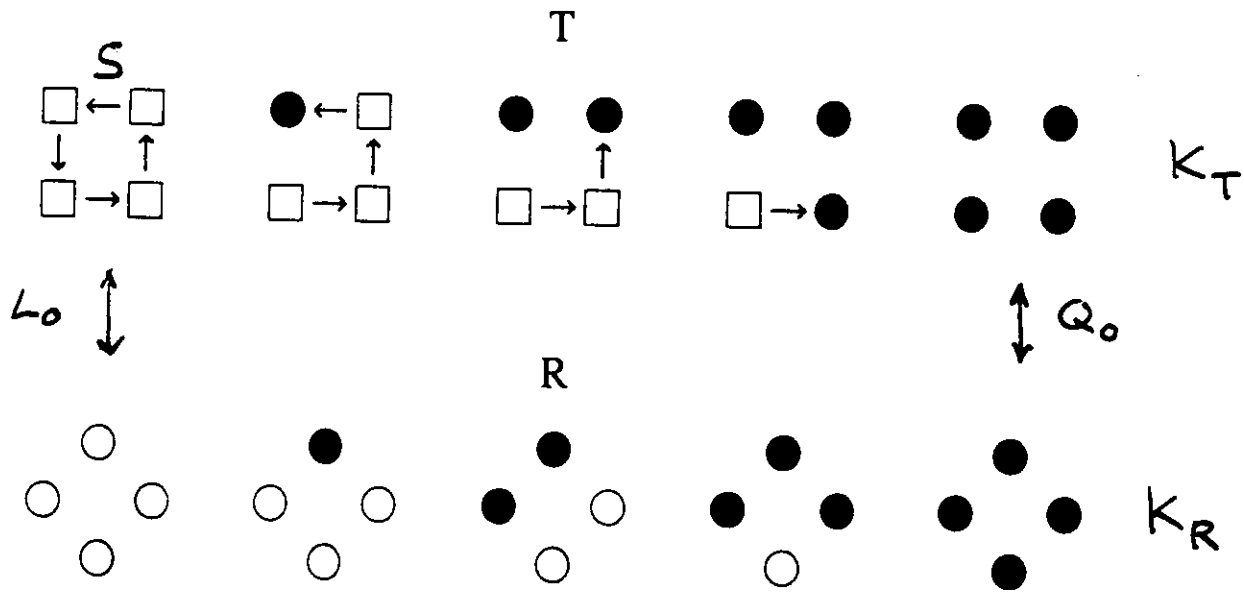

β_2
β_1
α_2
α_1
A
B
E
G
H
L
F
G
C
C
F
G

MWC - PSK Model

Monod
Wyman
Changeux
Perutz
Szabo
Karplus

deoxy, T — oxy, R





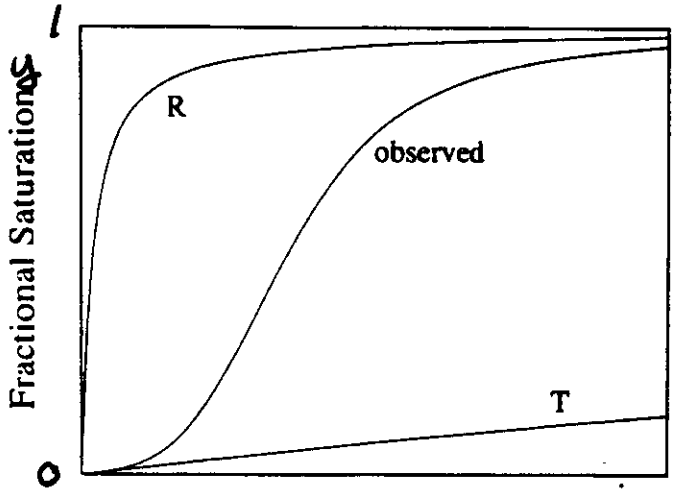
MWC

K_R, K_T, L_0

$L_i = L_0 c^i$

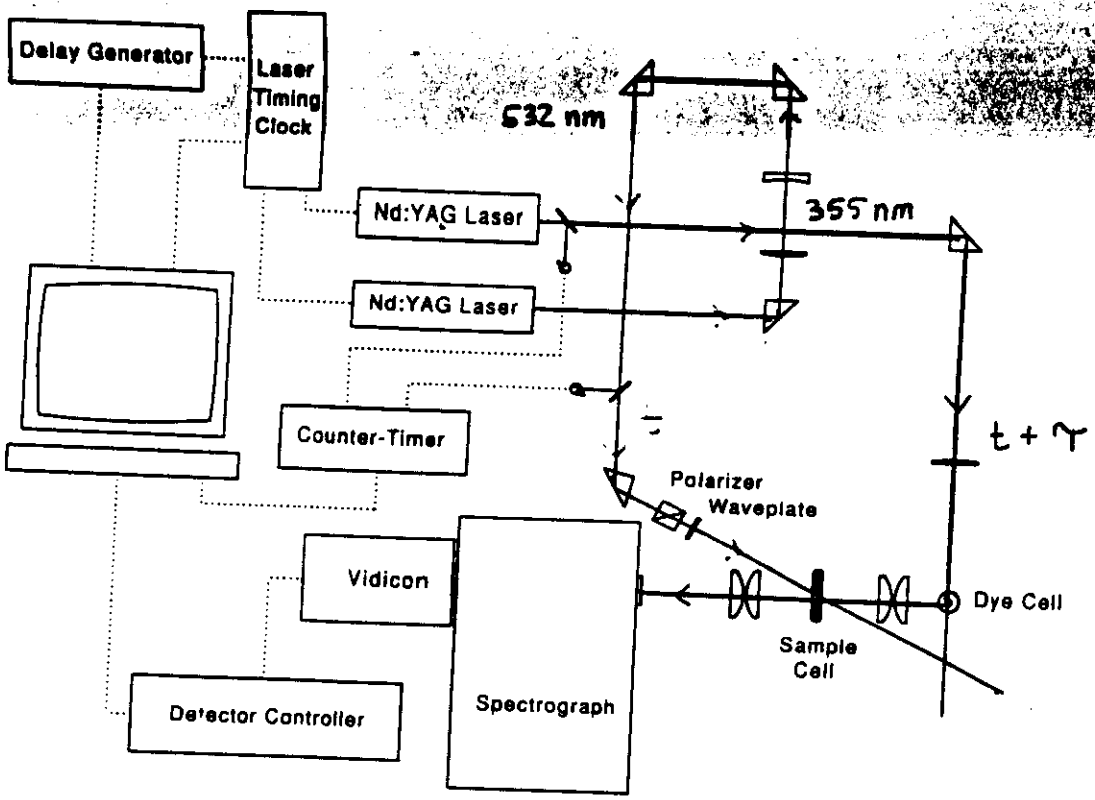
SK

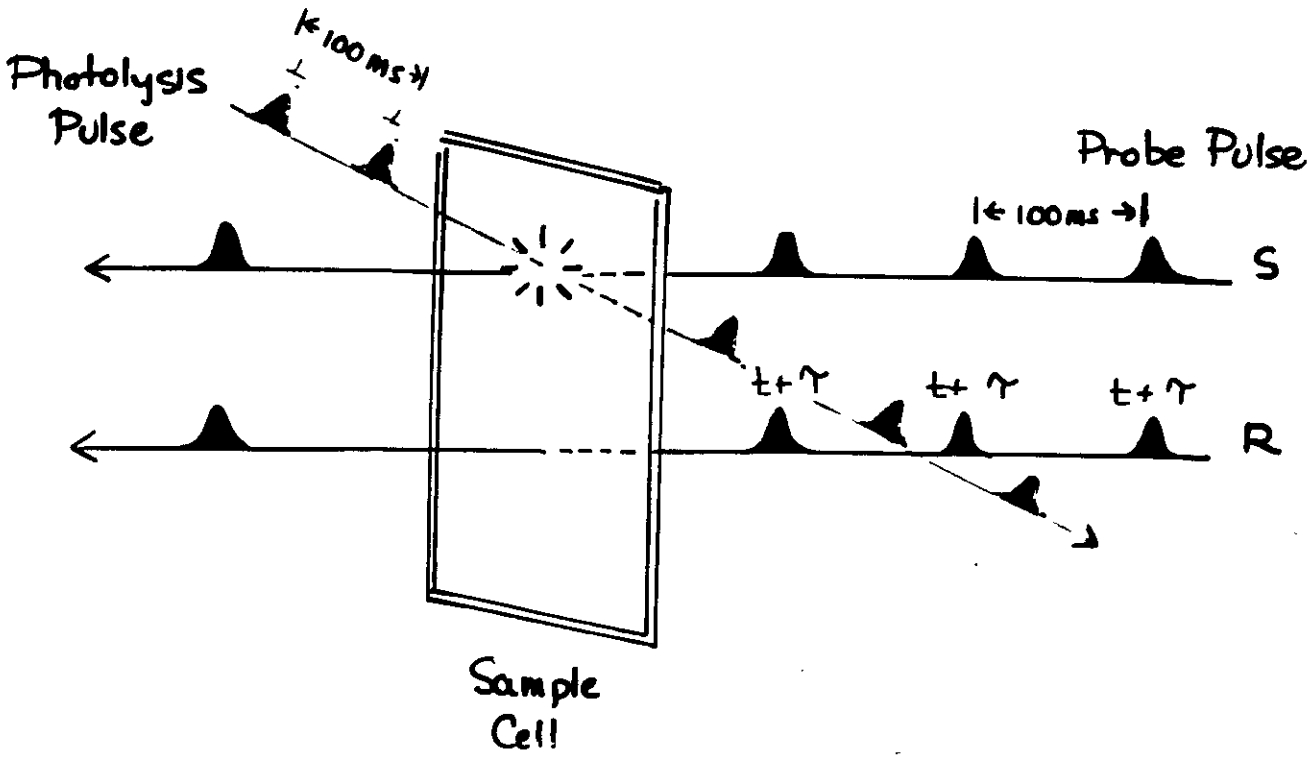
K_R, S, Q_0



$K_T = K_R / S$

$L_0 = Q_0 S^4$

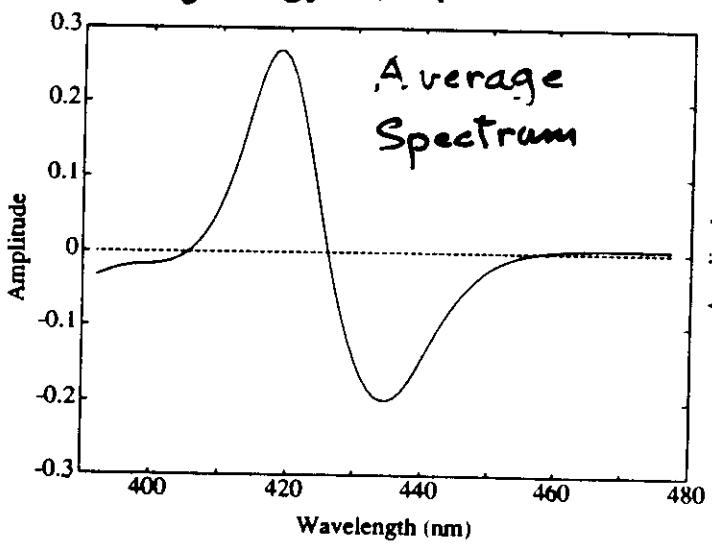

Delay Generator
Laser Timing Clock
532 nm
Nd:YAG Laser
355 nm
Nd:YAG Laser
Counter-Timer
t + τ
Polarizer
Waveplate
Vidicon
Dye Cell
Sample Cell
Detector Controller
Spectrograph


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

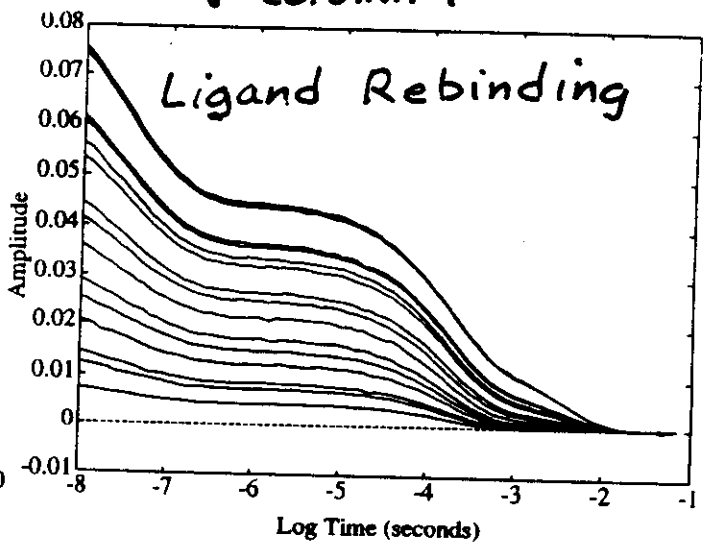
Singular Value Decomposition
(of isotropically-averaged data)

$$A = U S V^T$$

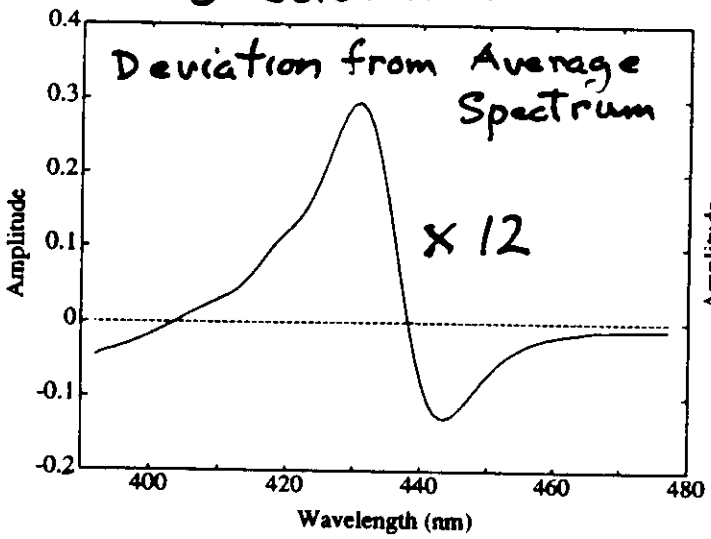
U Column 1



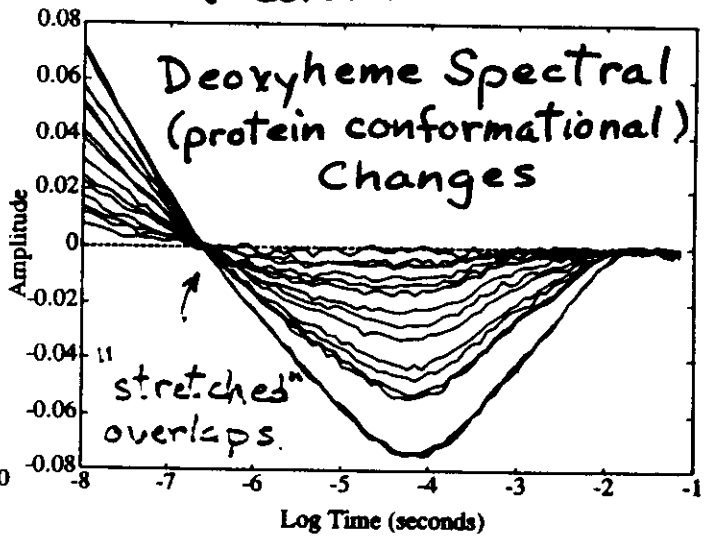
V column 1



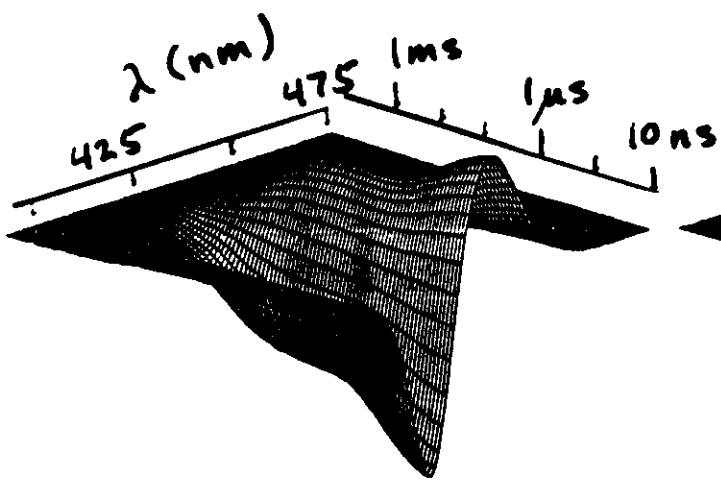
U column 2



V column 2

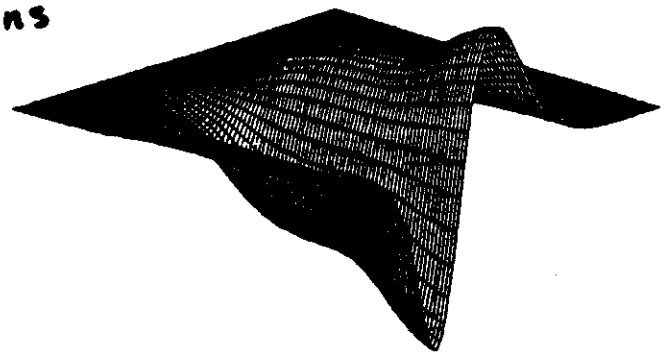


Singular Value Decomposition (isotropic data)



data

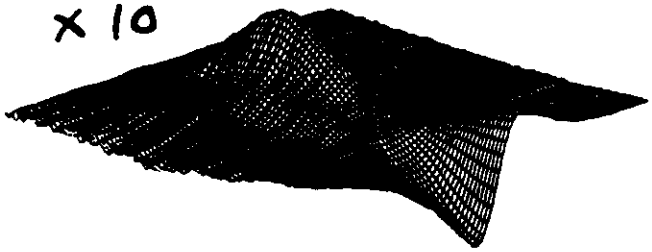
ligand rebinding



best one-component fit

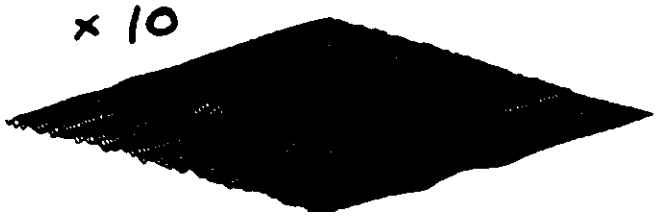
cooxheme (protein conformational) changes

x 10



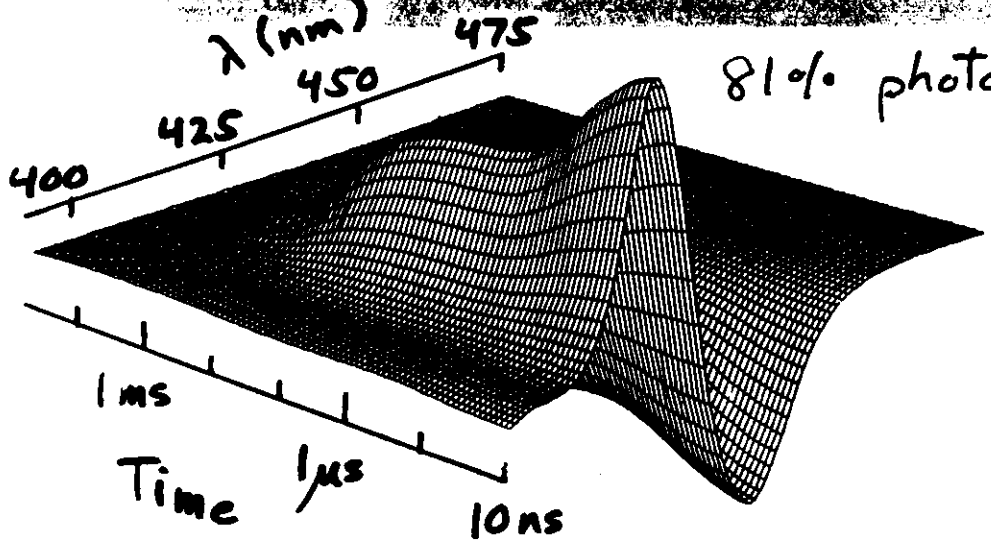
"residual" second-component

x 10

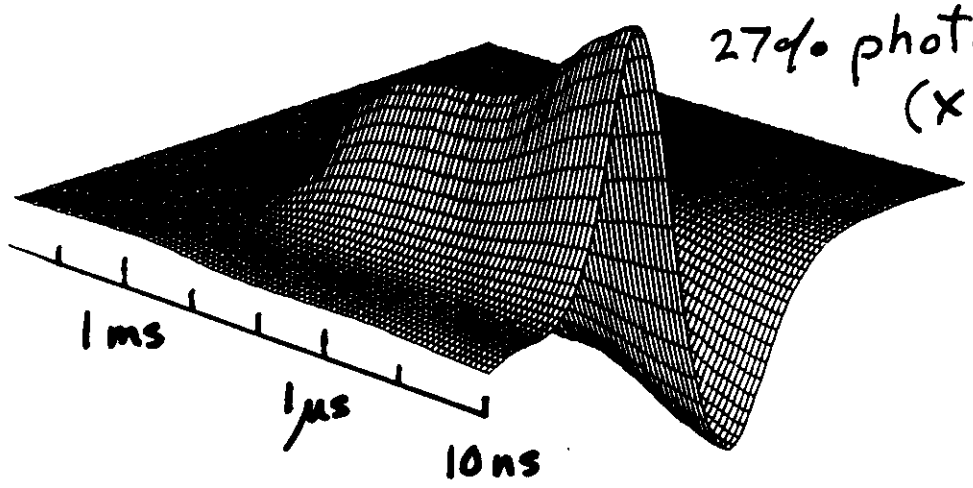


third component

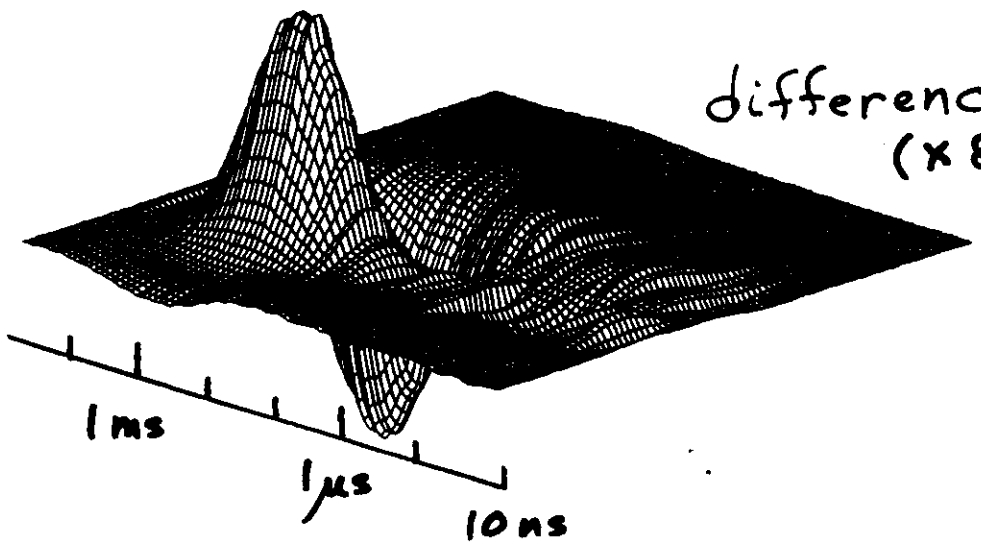
Isotropic Spectra



81% photolyzed



27% photolyzed (x3)

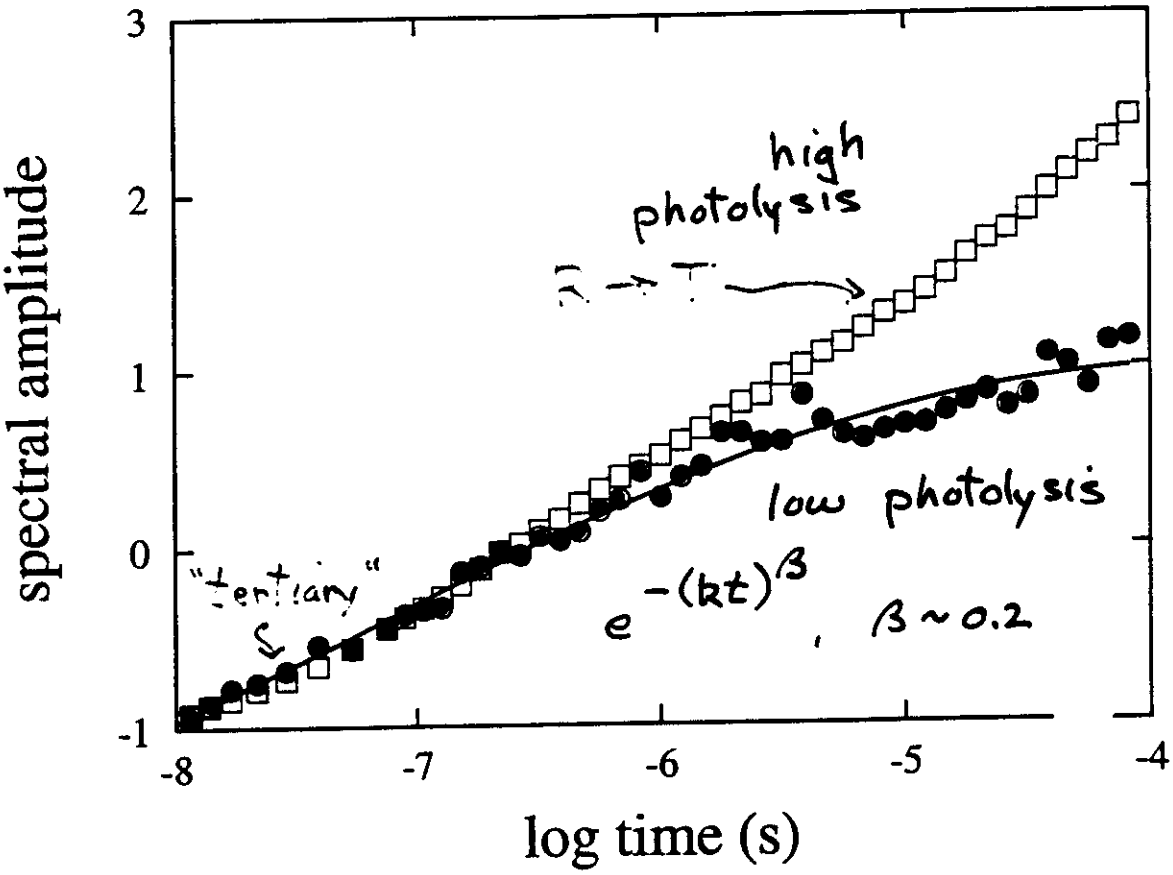


difference (x8)

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."

Linear Free Energy Relation

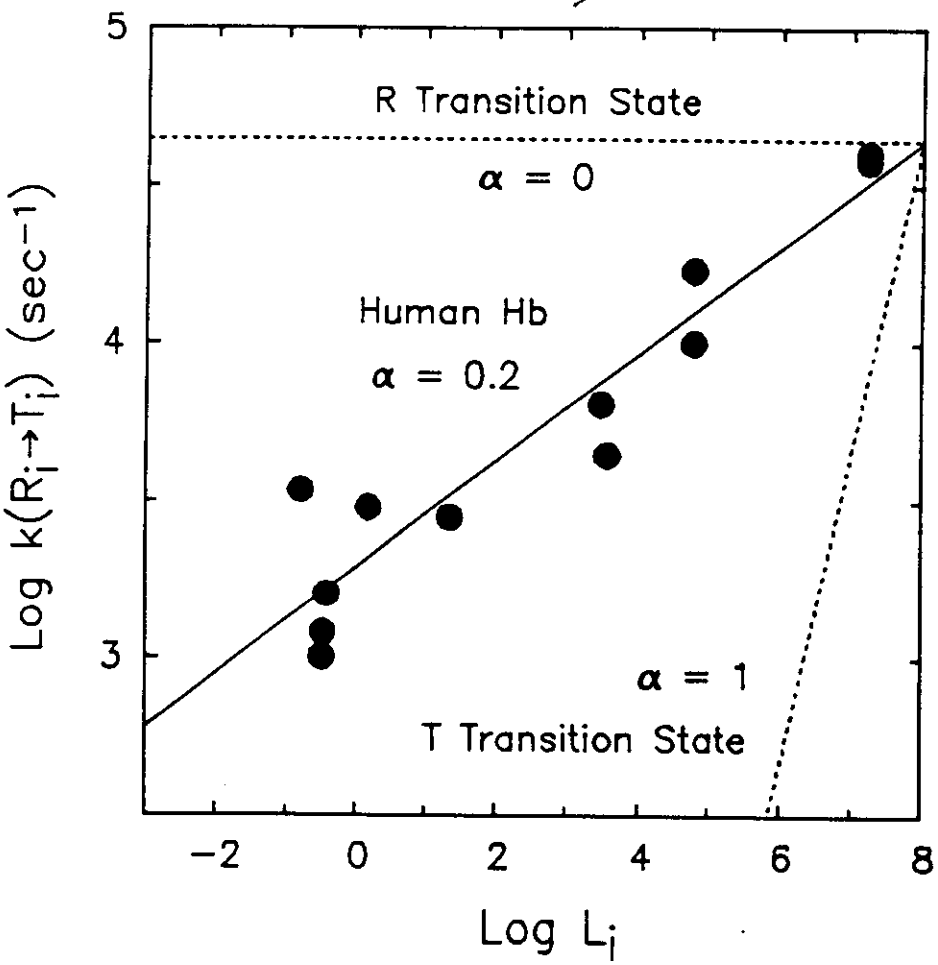
$$\delta \Delta G^{\ddagger} = \alpha \, \delta \Delta G$$

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

$$T_0 \rightarrow T_1 \rightarrow T_2 \rightarrow T_3 \rightarrow T_4$$
$$1 \quad L \quad 1 \quad Lc \quad 1 \quad Lc^2 \quad 1 \quad Lc^3 \quad 1 \quad Lc^4$$
$$R_0 \rightarrow R_1 \rightarrow R_2 \rightarrow R_3 \rightarrow R_4$$

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

in R
$$rx \underset{k(r)}{\overset{k(r^*)}{\rightleftarrows}} r^* \cdot x \underset{k_{out}}{\overset{k_{in}}{\rightleftarrows}} r^* + x \qquad r^* \rightarrow r : e^{-(kt)^{\beta}}$$
$$r \cdot x \rightleftarrows r + x$$

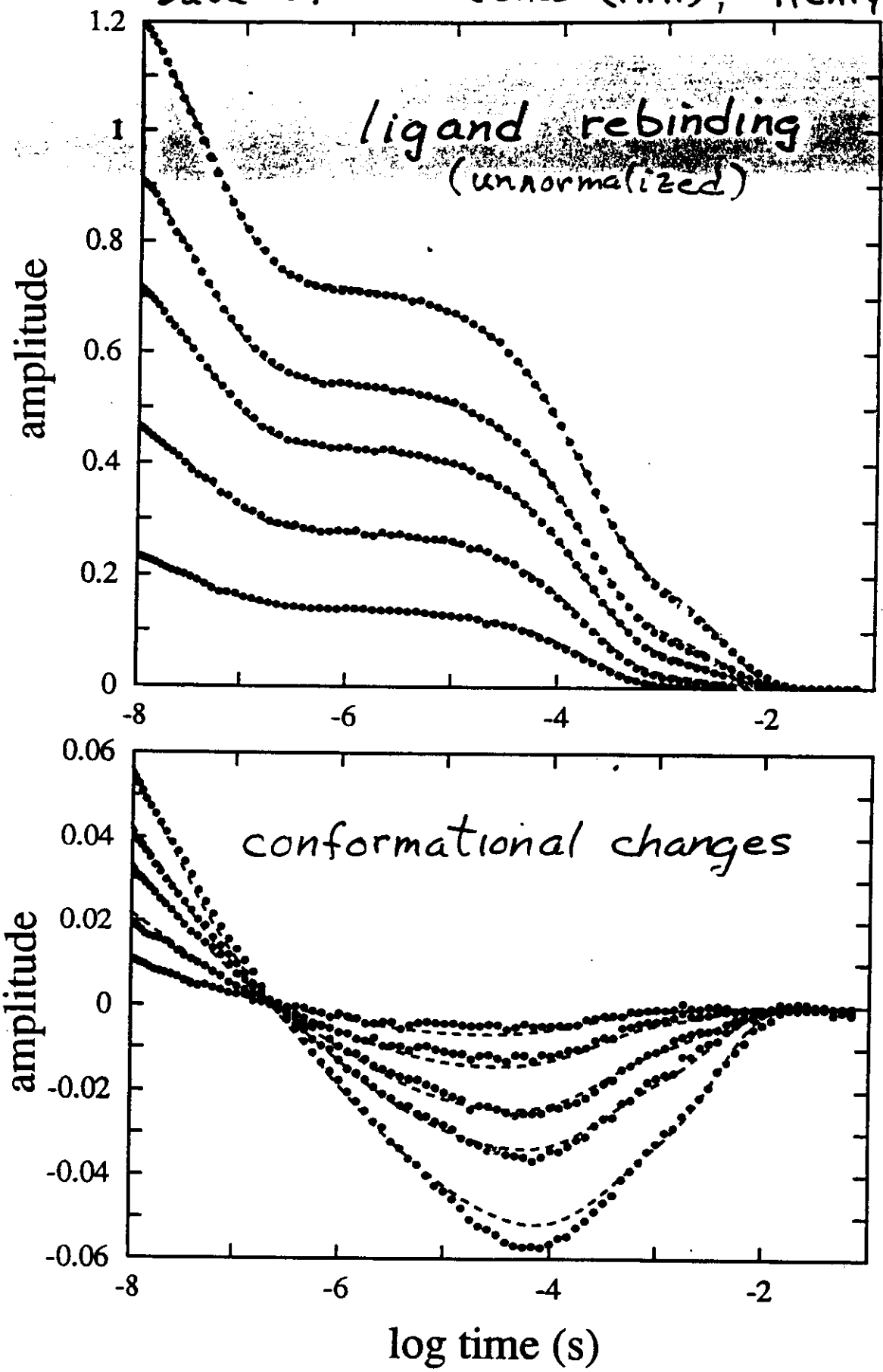
in T
$$tx \underset{k(t)}{\leftarrow} t \cdot x \rightleftarrows t + x$$

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

85 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).

Data of C.M. Jones (NIH), Henry fit



Take Home Lessons

1. MWC-PSK (Hopfield/Shulman/Ogawa/Edelstein) got it right!

2. Hemoglobin remains paradigm for cooperative behavior in multisubunit proteins.

3. Settled by new experiments

   low-tech: single crystal oxygen binding
   medium-tech: nanosecond spectroscopy
   hi-tech: femtosecond spectroscopy

   massive computing power

   new ideas (stretched exponential)