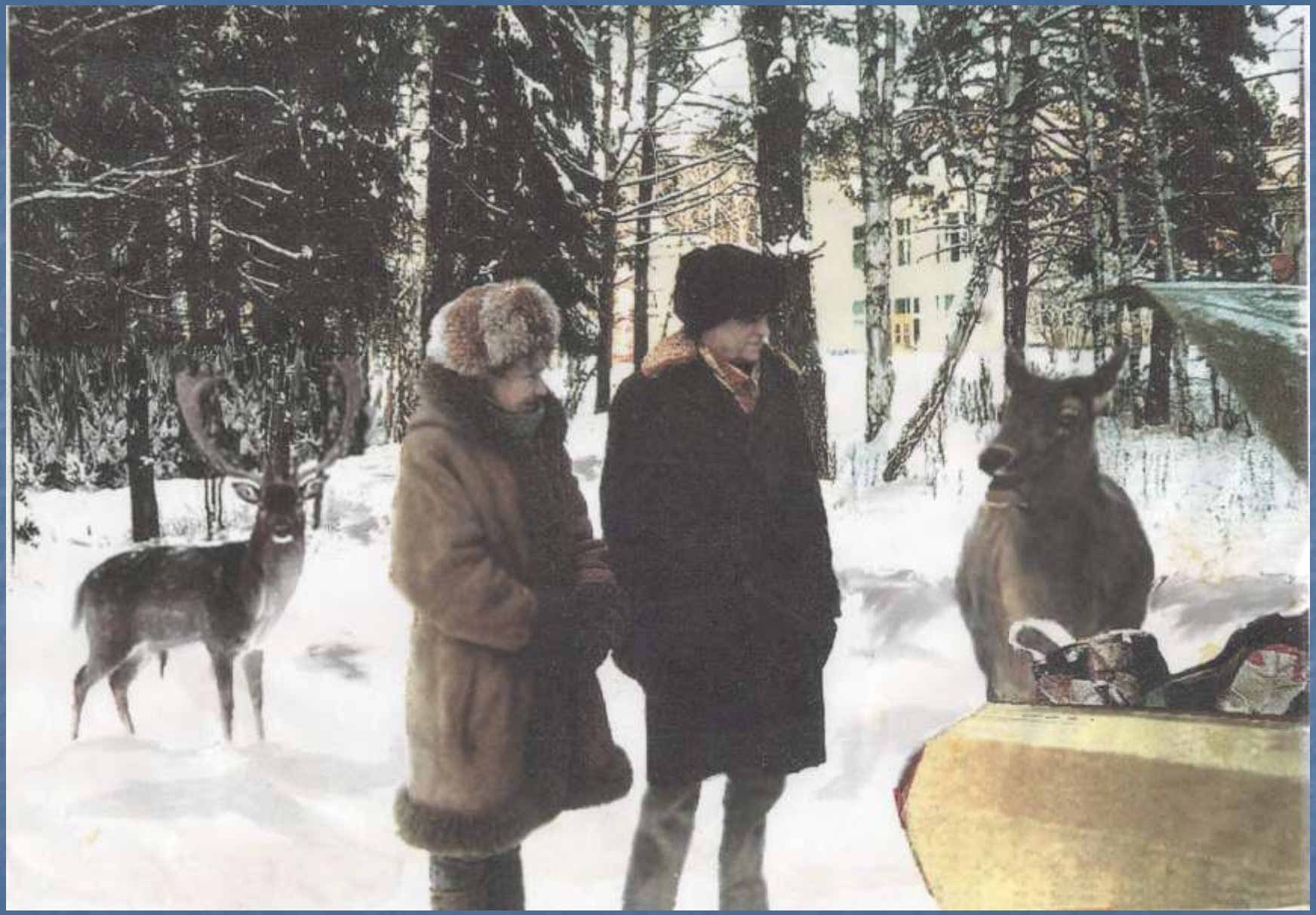
Ben-Gurion University of the Negev, Department of **Chemistry**
Институт Проблем Химической Физики РАН

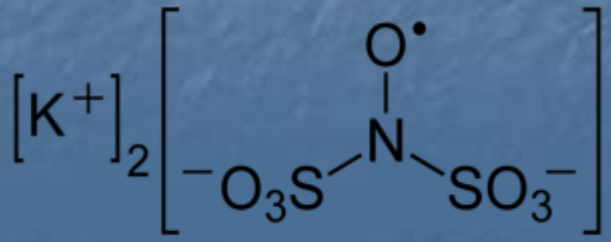
Физические метки в химии и биологии: решающий вклад Семеновской школы химичской физики

Лихтенштейн Г. И.

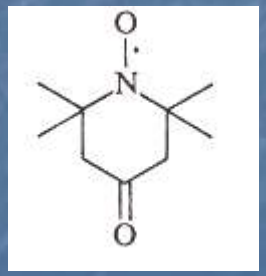


Морис Равель (1875-1937)

Edmond Frémy (1845)



O.L. Lebedev, M. L. Khidekel, and V.A. Razuvaev, 1961



М. Б. Нейман 1961

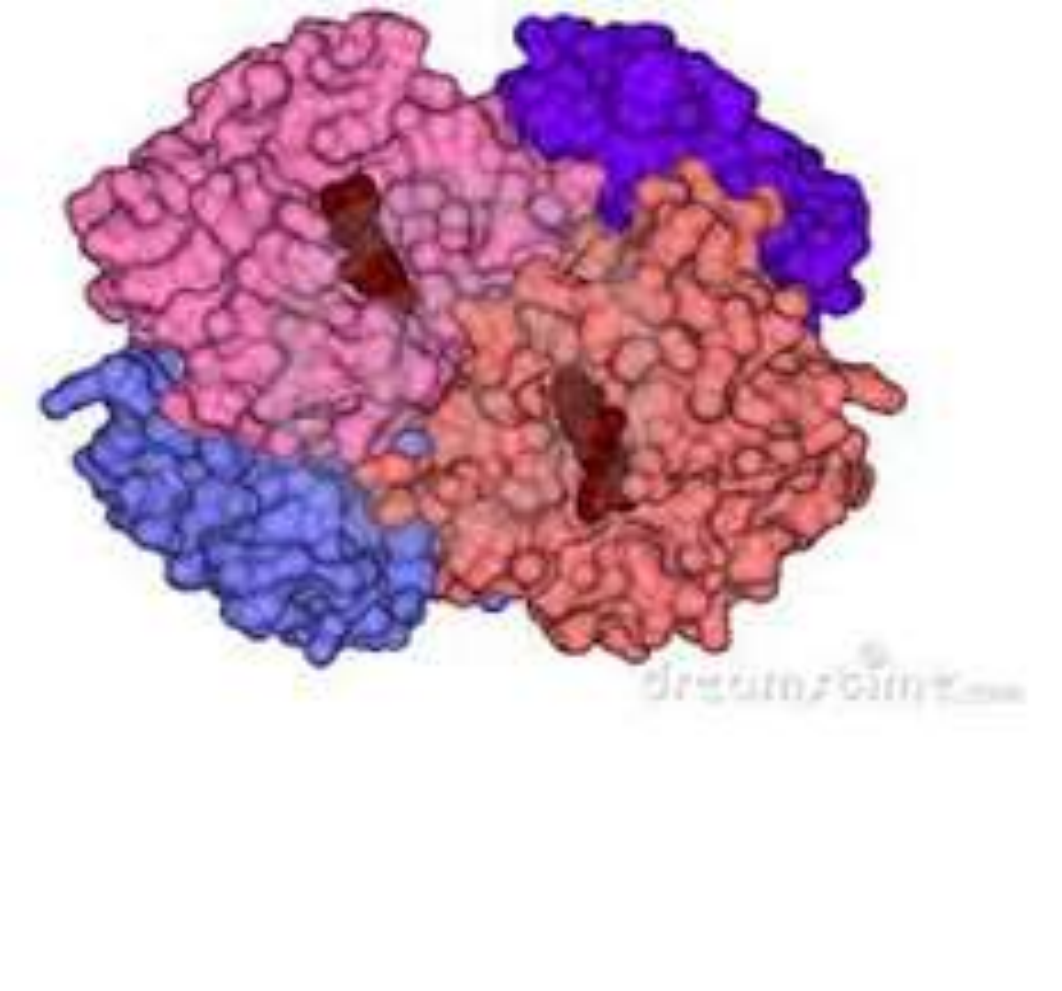
M.B. Neiman, E.G. Rozantzev and Yu. G. Mamedova, 1962

spin labels 47005 references

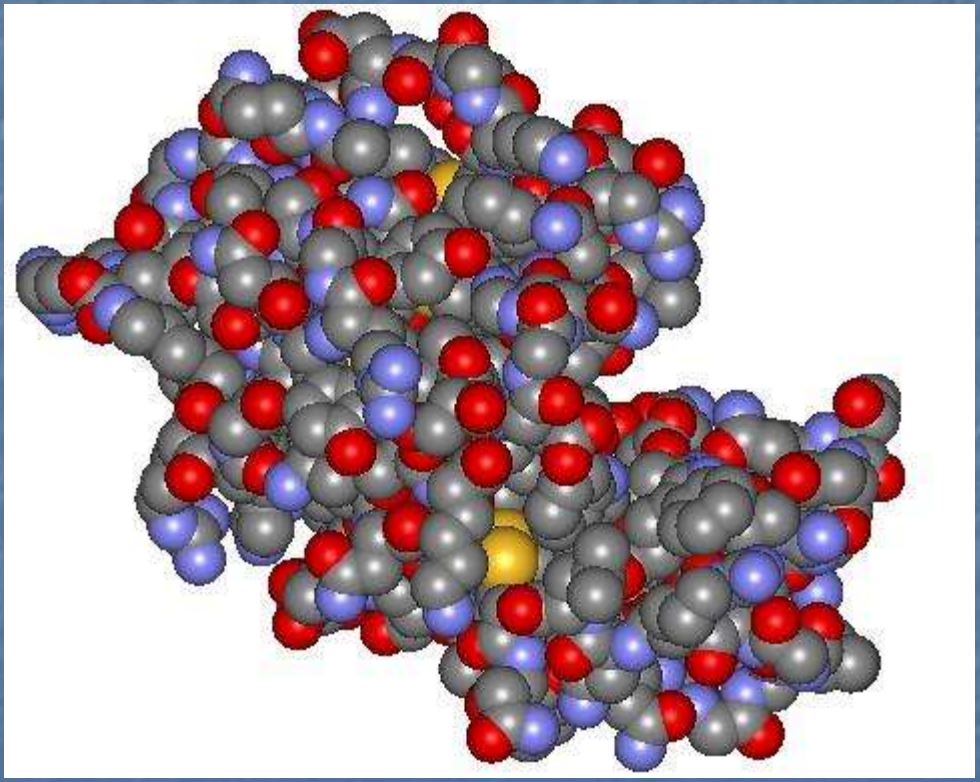
H. M. McConnell 1965

Hemoglobin

S Ohnishi, J C Boeyens and H M McConnell, 1966

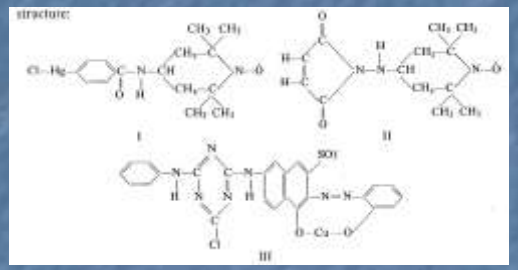


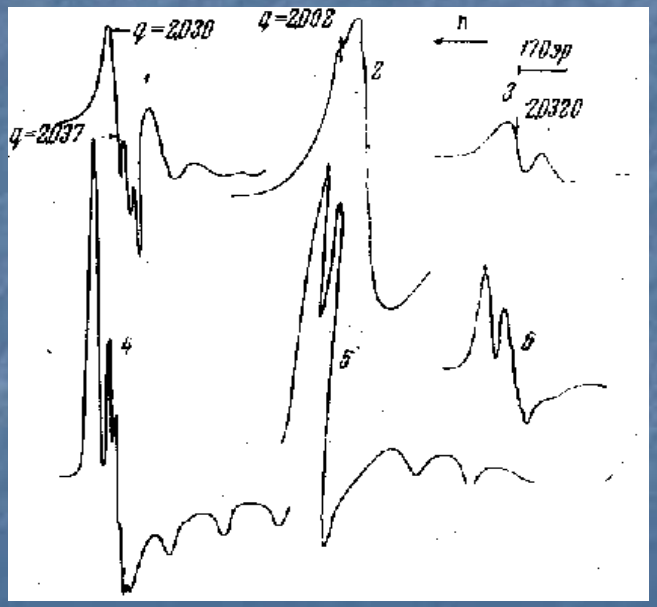
Lysozyme
Likhtenshtein G.I., Akhmedov U.D. 1970



Double Spin Labeling Method (DSLM)

Likhtenshtein G.I. Molekulyarnaya Biologiya 2, 235-240 (1968)

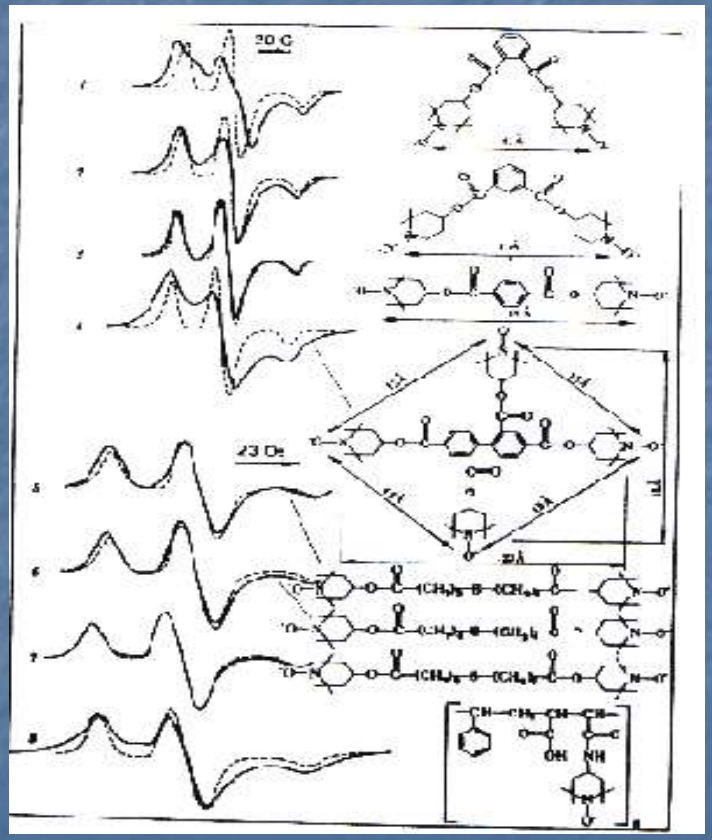




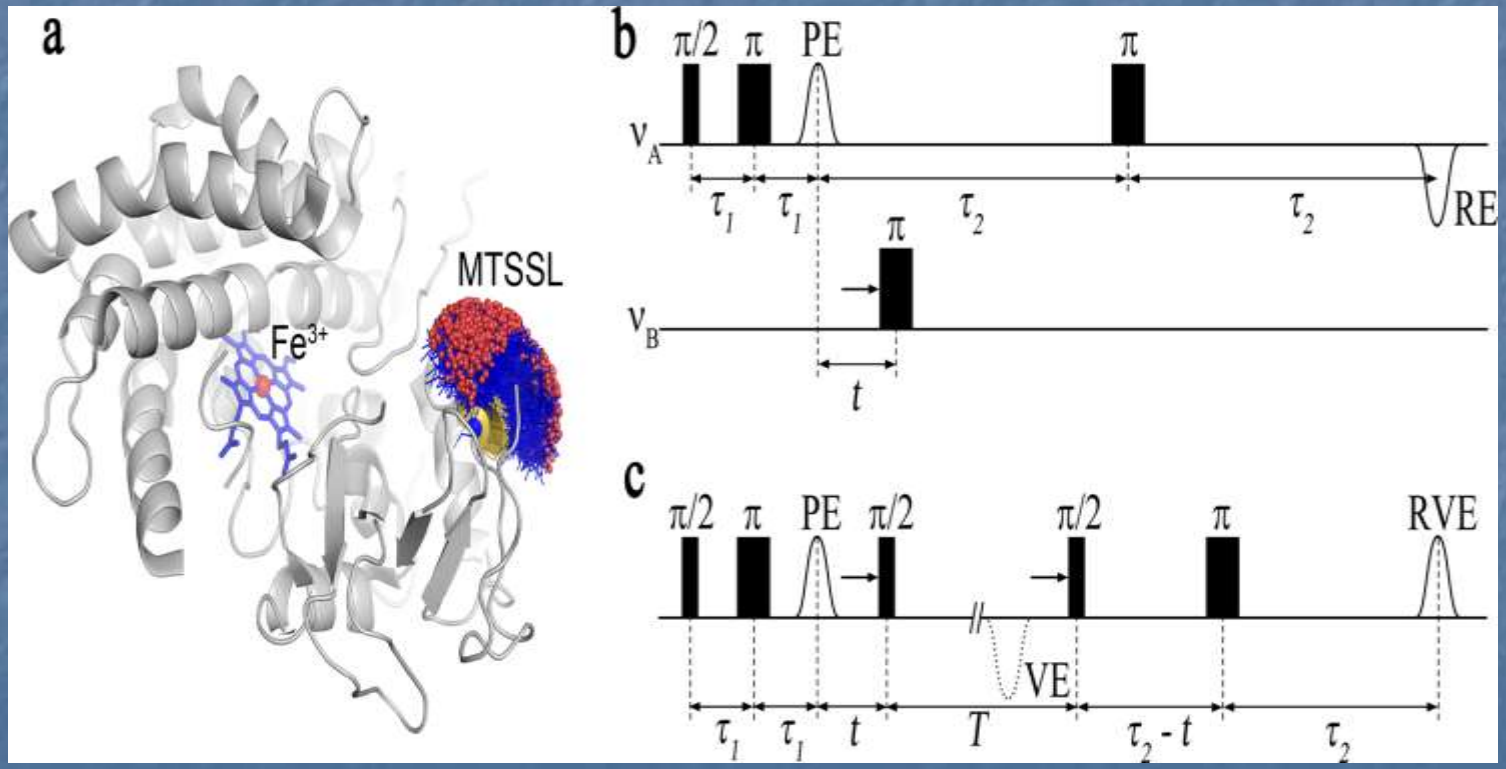
Likhtenshtein, **G. I.** Molek. Biol. **1968**, 2, 234

Kulikov, A. V.; Likhtenshtein, **G. I.**; Rozantsev, E. G.; Suskina, V. I.; Shapiro, A. B. *Biofizika* **1972**, 17, 42 (R < 25 Å)

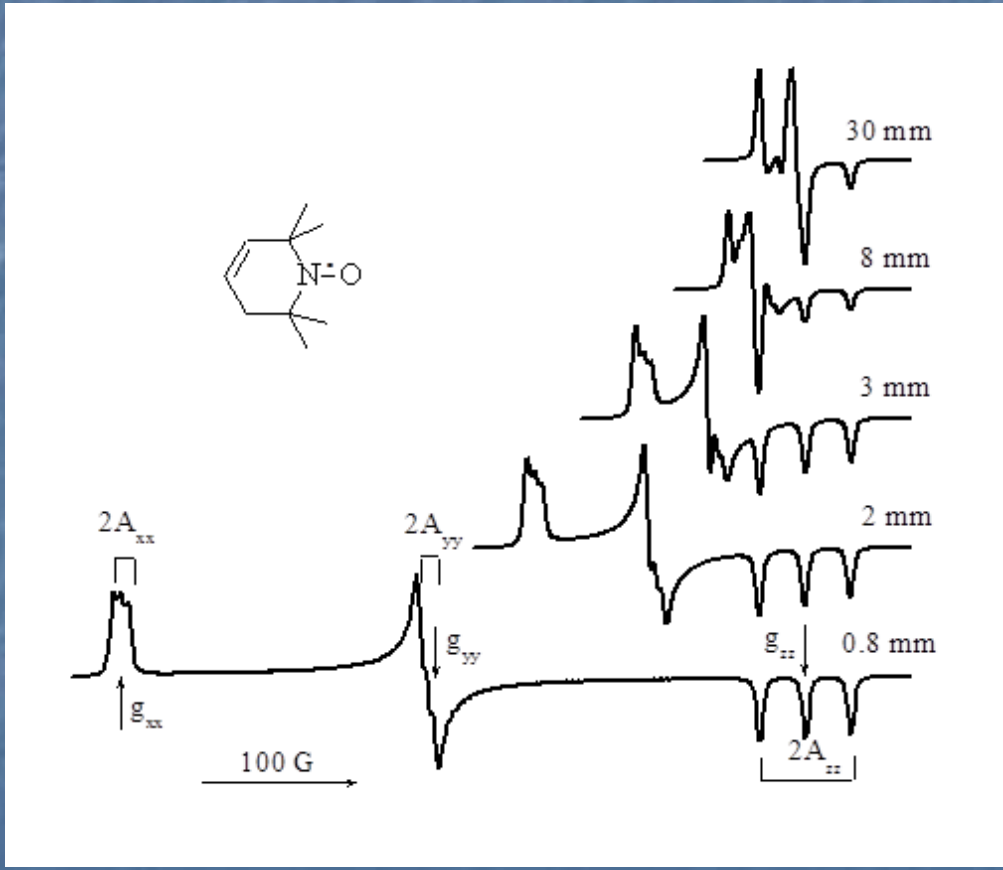
Kulikov AV, Likhtenstein GI. *Biofisika* **1974**, 19, 420 (R < 100 Å)



A. D. Milov A.D., K. M. Salikhov K.M., and Yu. D.Tsvetkov, 1973
Model of MTSSL-labeled cytochrome P450cam
Abdullin, 2016

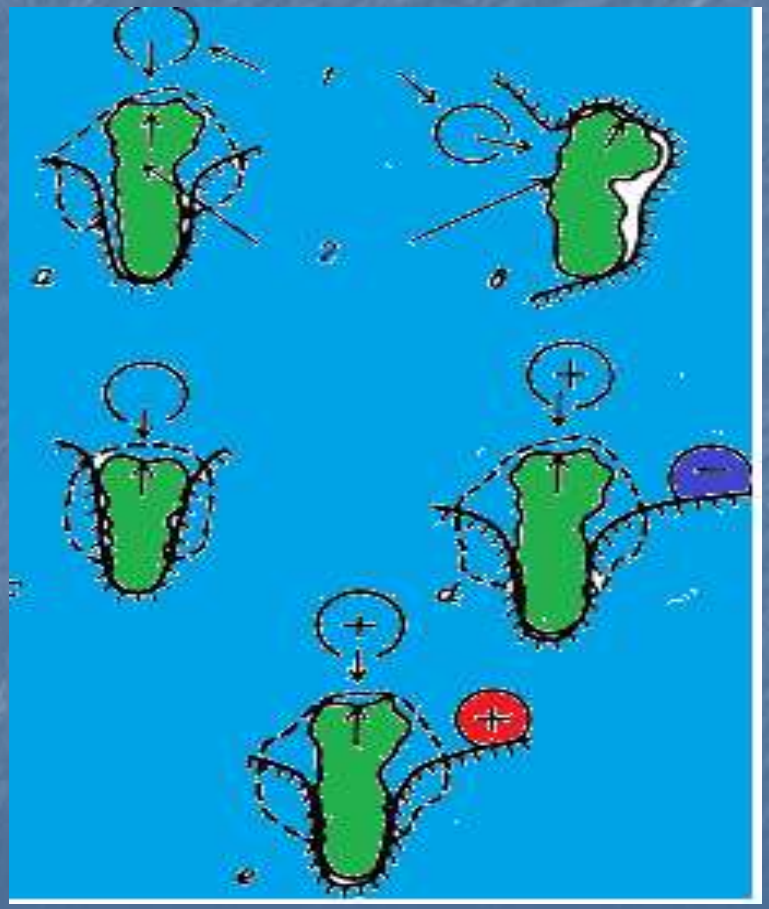


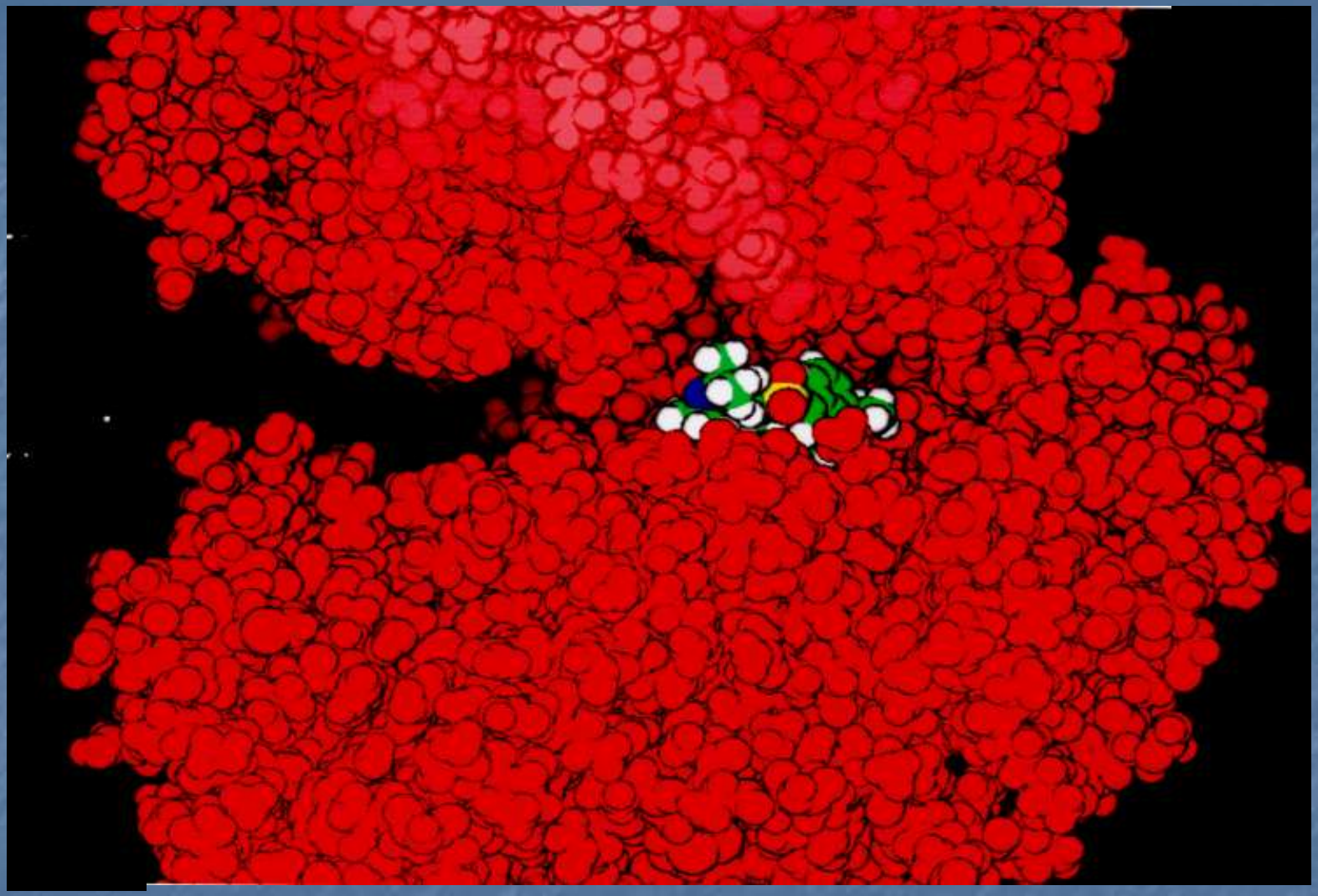
Nitroxide ESR spectra



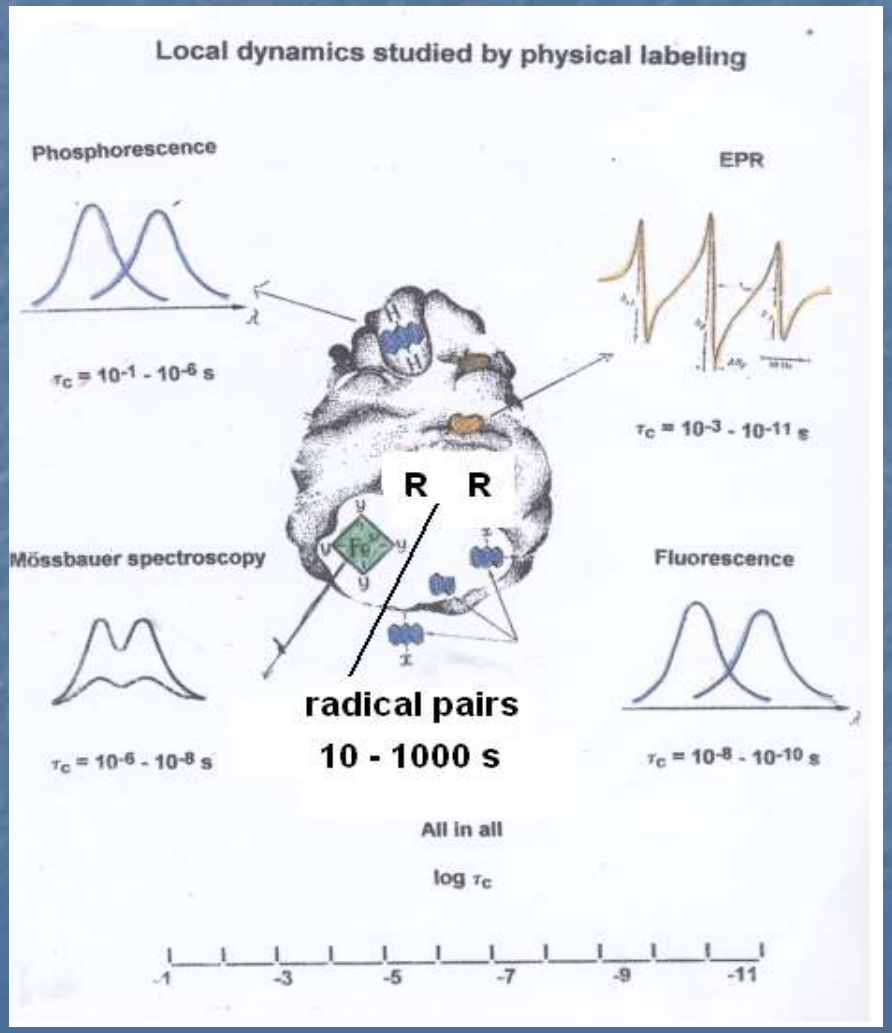
Spin Label – Spin Probe Method (SPM)

Likhtenshtein, G. I.; Grebenshchikov, Yu. B.; Bobodzhanov, P. Kh.; Kokhanov, Yu. V.
Molekulyarnaya Biologiya(Moscow) (1970), 4(5), 682-688

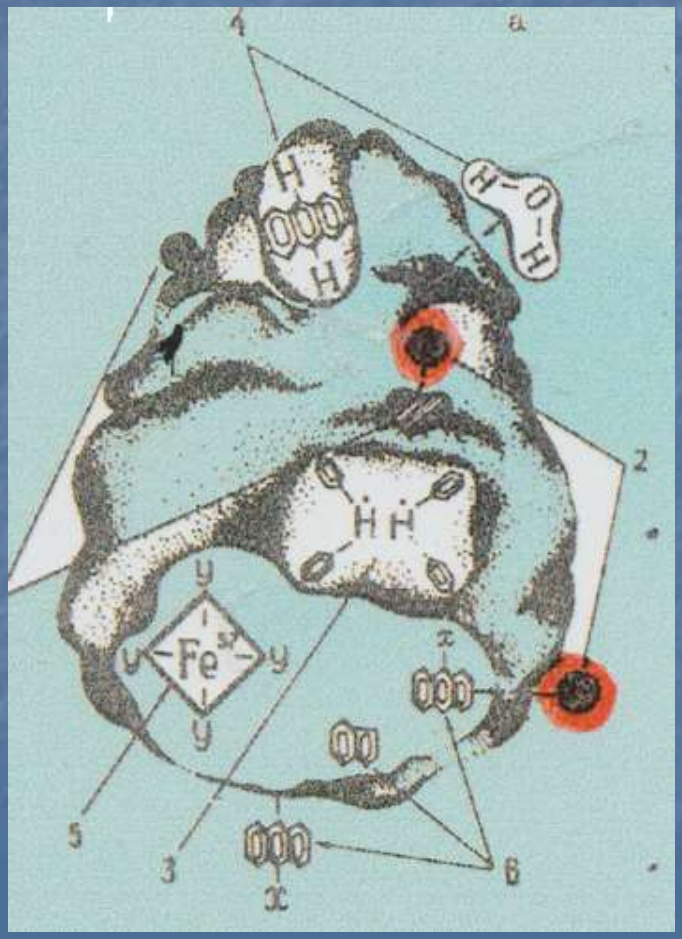


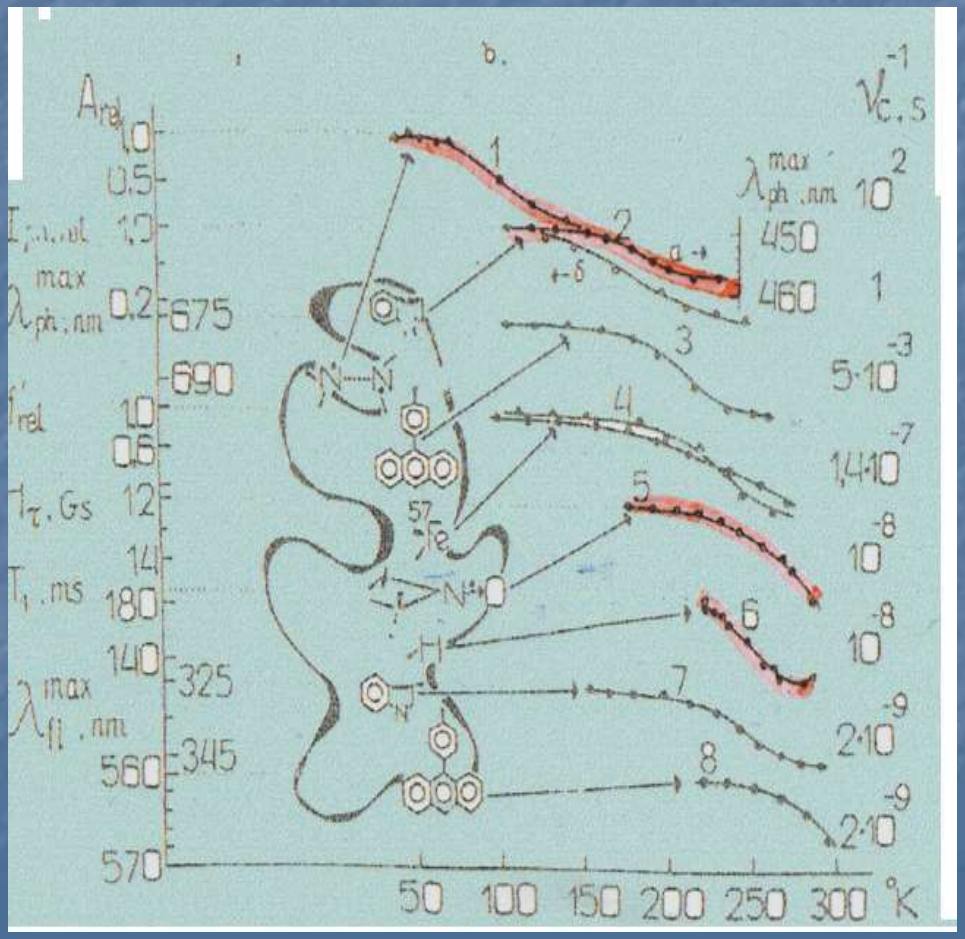


Likhtenshtein, G. I., Grebenshchikov, Y. B., Avilova T V 1972



Protein Dynamics



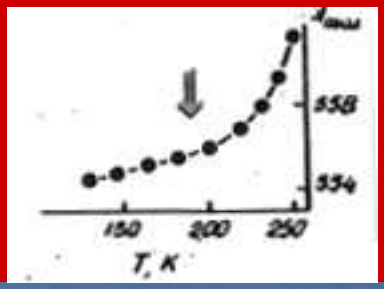


ET between Q-acceptors and Membrane

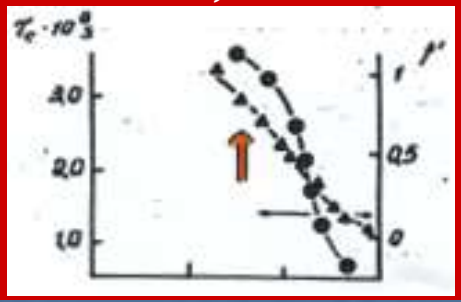
Nanosecond Molecular Dynamics

Mossbauer label **Reaction center** **Fluorescence label**

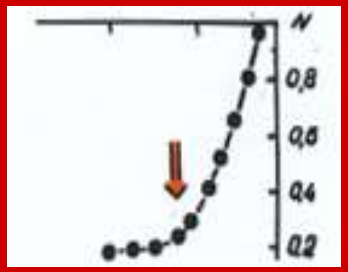
Fluorescence L



SL, ML

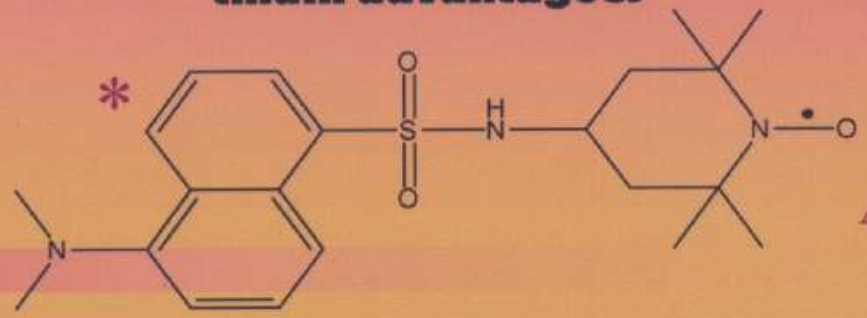


ET



Dual Fluorophore-Nitroxide Molecules

(main advantages)



Donor

Acceptor

Micropolarity
Microviscosity

Micropolarity
Microviscosity

Experiment

Electron Transfer
Chemical Reduction
Photoreduction

Theoretical estimation

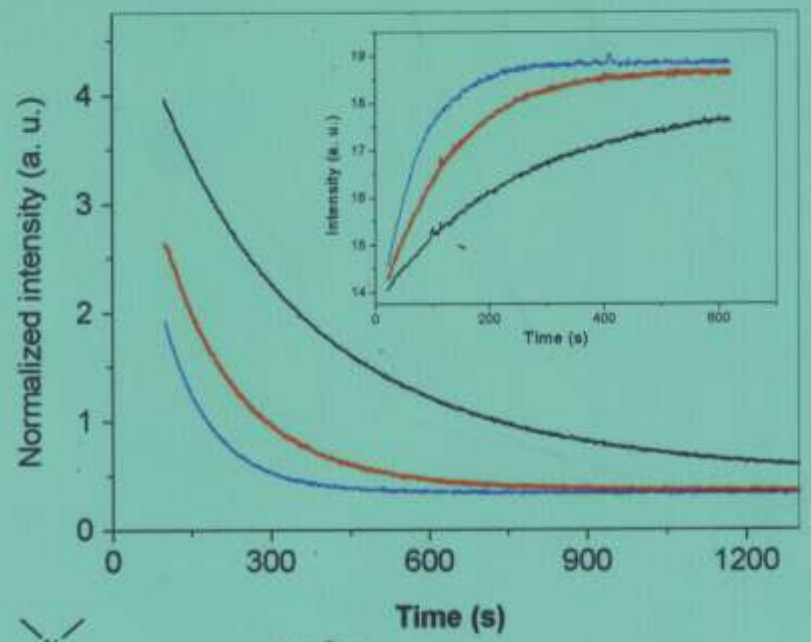
V- resonance integral
ΔG_0 – *Gibbs energy*
λ – *reorganization energy*

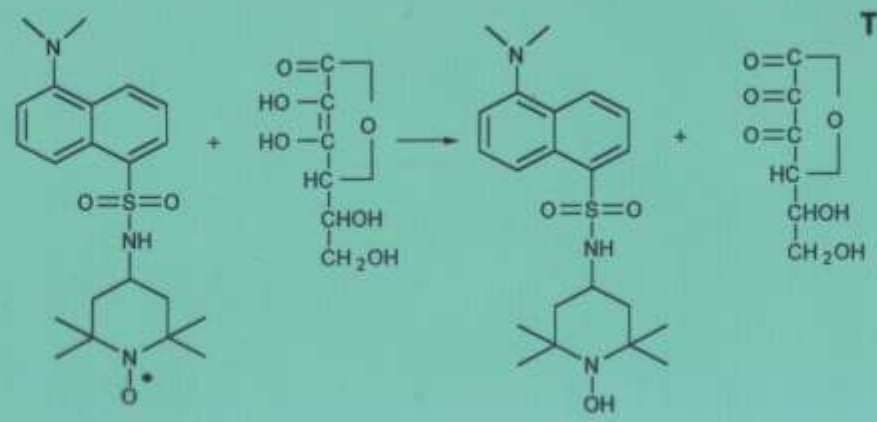
Bystryak I.M., Hideg K., Likhtenshtein G. I. Russian J. Phys. Chem. 60, 1679- (1986)

Determination of the ascorbate content in biological liquids by FN probes

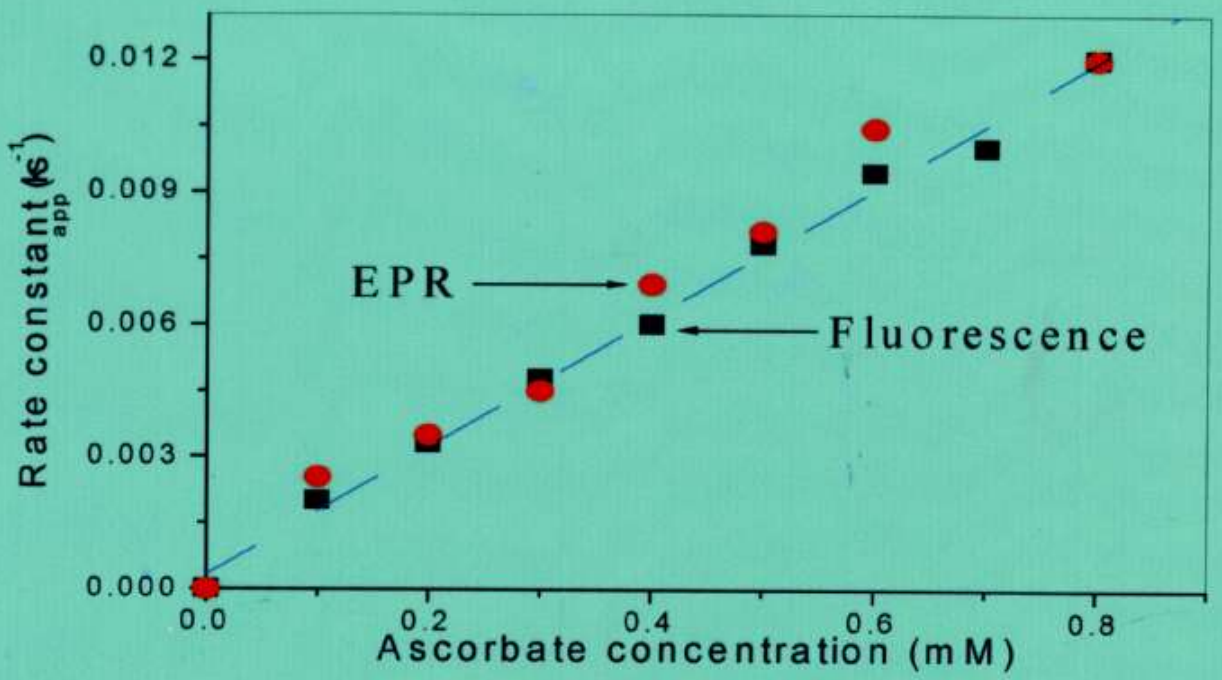
0.1 mM AH_2
0.4 mM AH_2
0.8 mM AH_2

c(F-NO)=0.1 mM)
PBS (pH=7.4)





The plot of k_{app} versus the ascorbate concentration serves a calibration for the determination of ascorbate

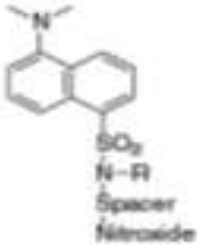


E. Lozinsky et al. J. Biochem. Biophys. Methods 38(1999), 29-42.

Fluorophore (Fl) — Spacer — Nitroxide (˙R)

Pyrene Derivatives (Fl = PYR)

Dansyl Derivatives (Fl = DAN= NR-Dns)



R = H (a)
Me (b)
Et (c)
Ph (d)

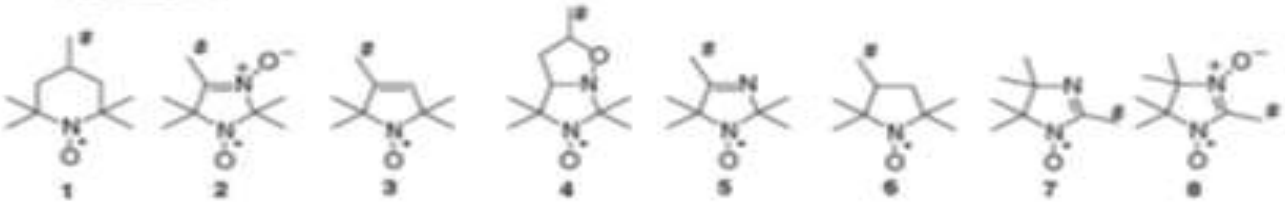
Spacer =

*—CH(CH2Ph)NH— (A)
*—(CH2)2NHCO— (B)
*—(CH2)3NHCO— (C)
*—(CH2)6NHCO— (D)
*—CH2NHCO— (E)
*—CH2CONH— (F)
*—(CH2)2CONH- (G)

*—CONH- (H)
*—NHCO— (I)
*—CH2NHC(=O)C(CH2Ph)=NCO— (J)
*—CH2NHC(=O)CH(CH2Ph)NHCO— (K)

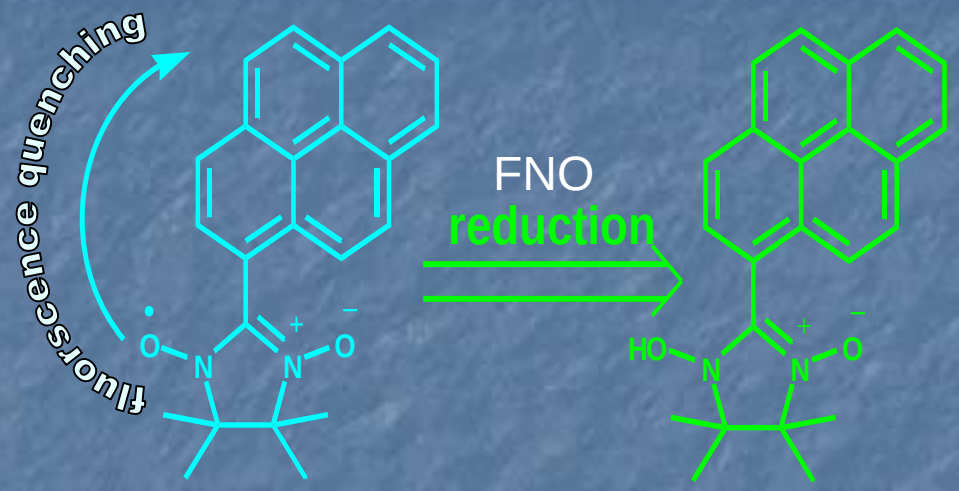
* -Linked to Fl

Nitroxide ˙R =



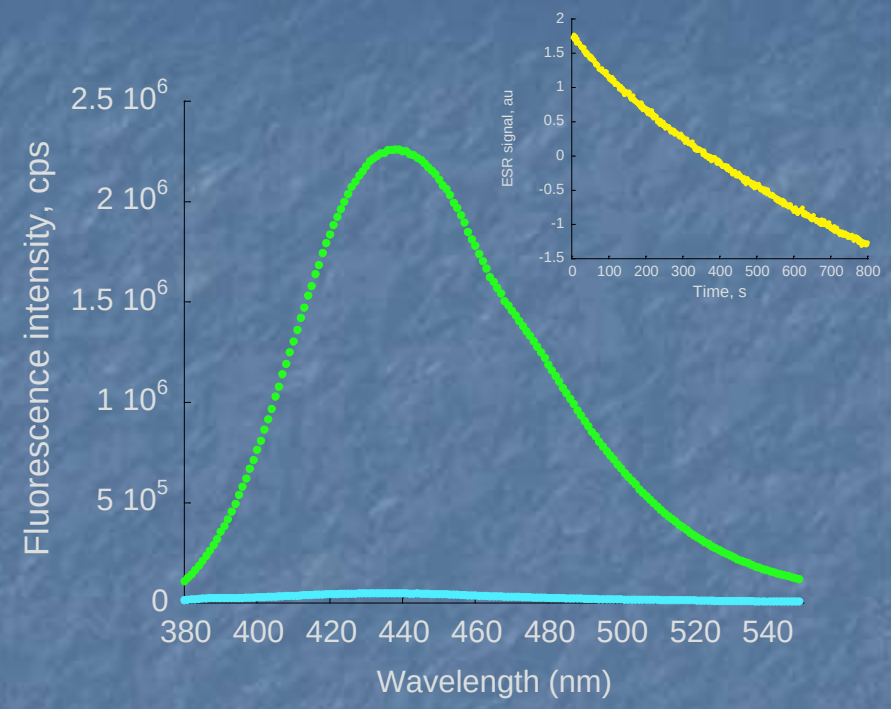
-Linked to spacer

DUAL FLUORESCENT NITROXIDE-NITRONYL PROBE FOR INVESTIGATION OF **SUPEROXIDE** DYNAMICS AND ANTIOXIDANT STATUS OF BIOLOGICAL SYSTEMS

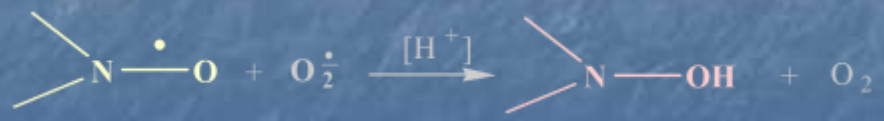


Nitronyl Nitroxide
(fluorescence quenched)

Hydroxylamine
(high fluorescent)

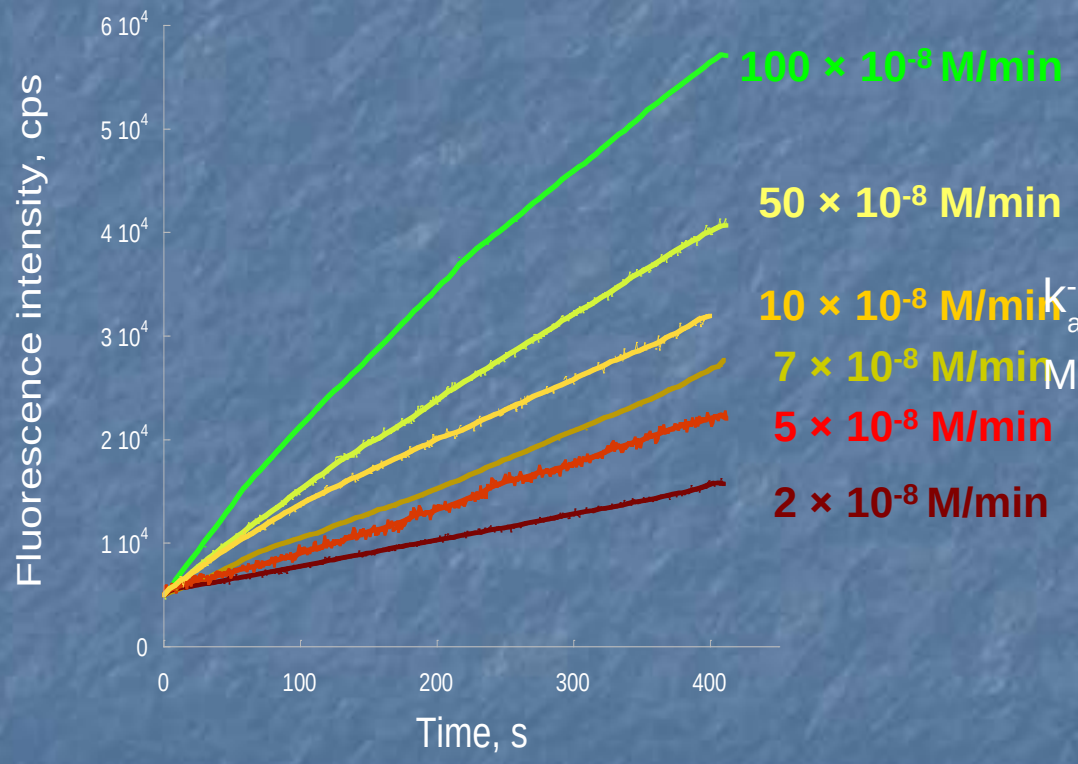


FNO fluorescence before and after reduction by superoxide (10^{-6} M/min)

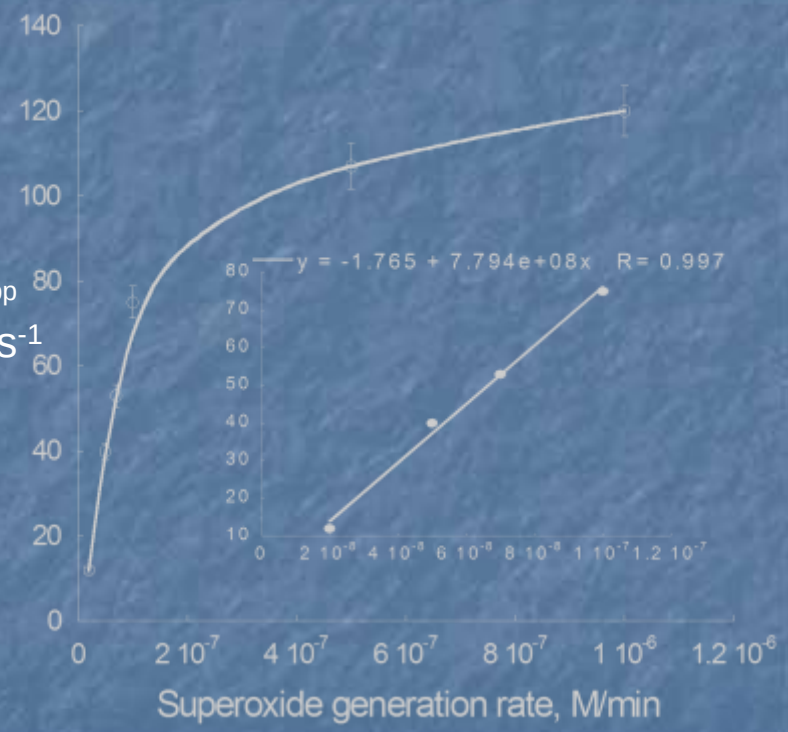


Analysis of superoxide

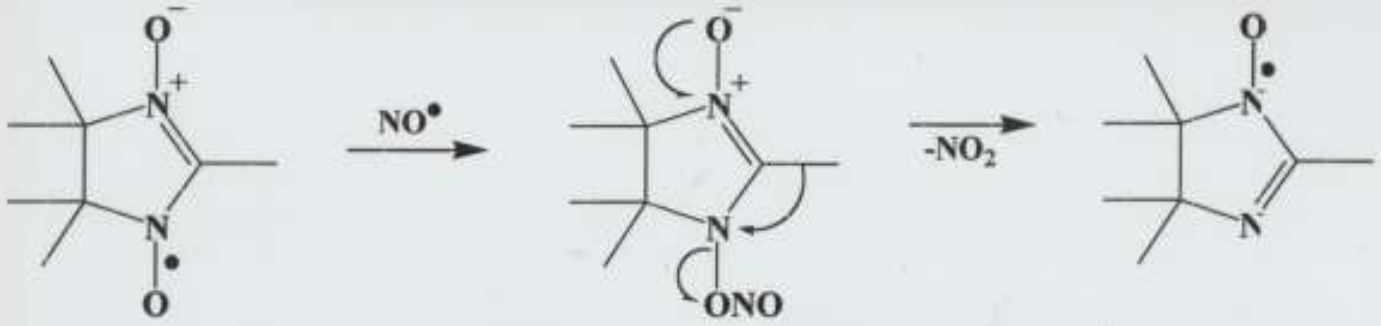
Dose-dependent effect of superoxide on FNO fluorescence kinetics



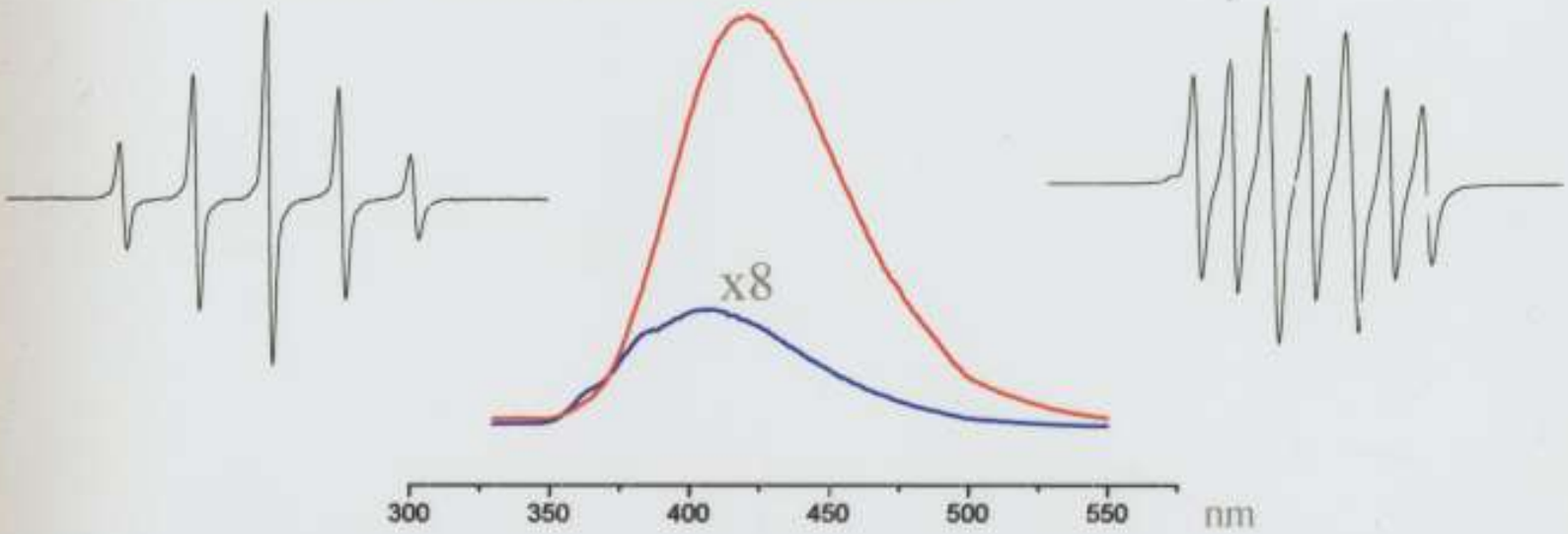
Dependence of apparent rate constant of FNO reduction on the rate of the superoxide production.

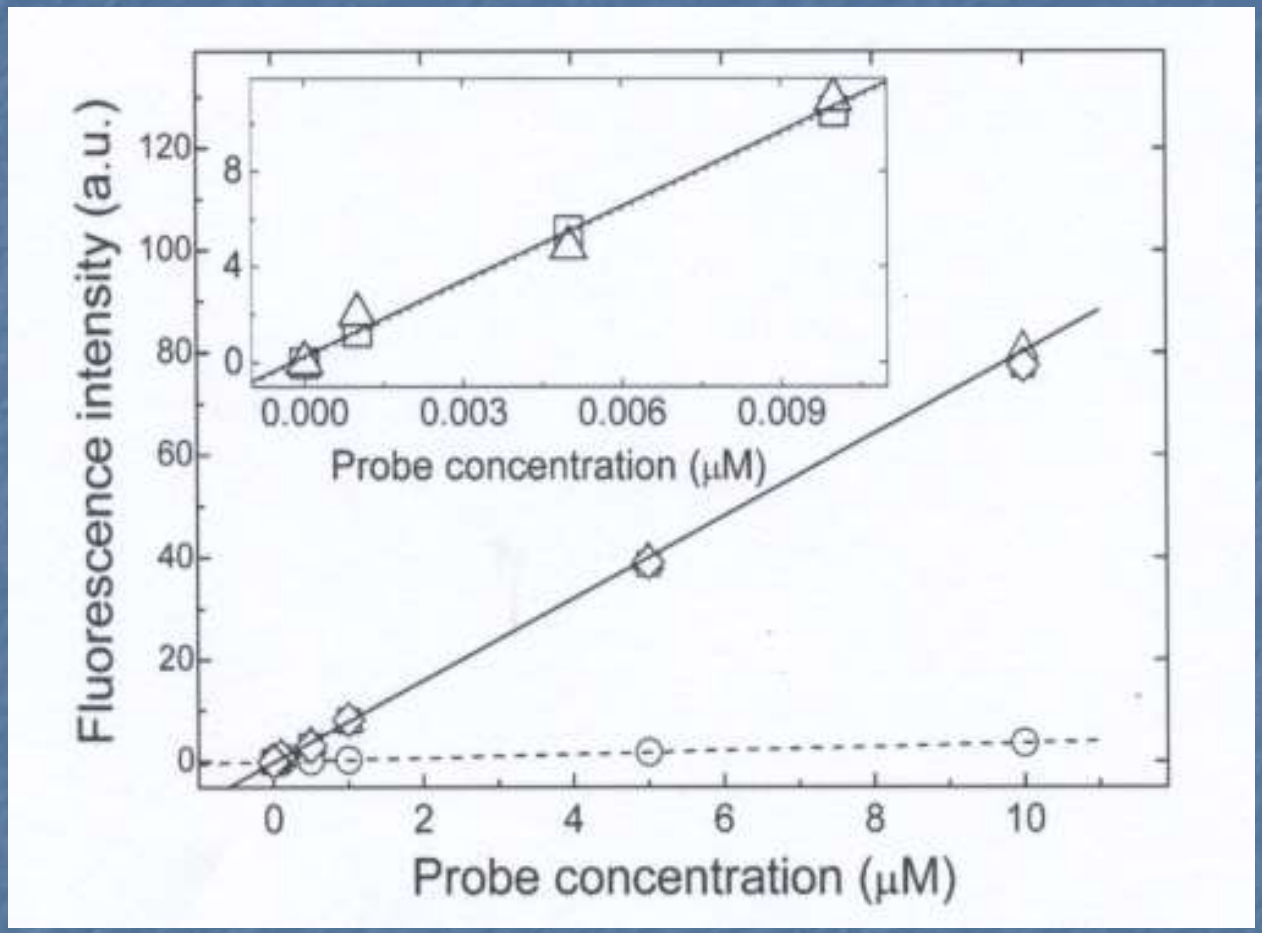


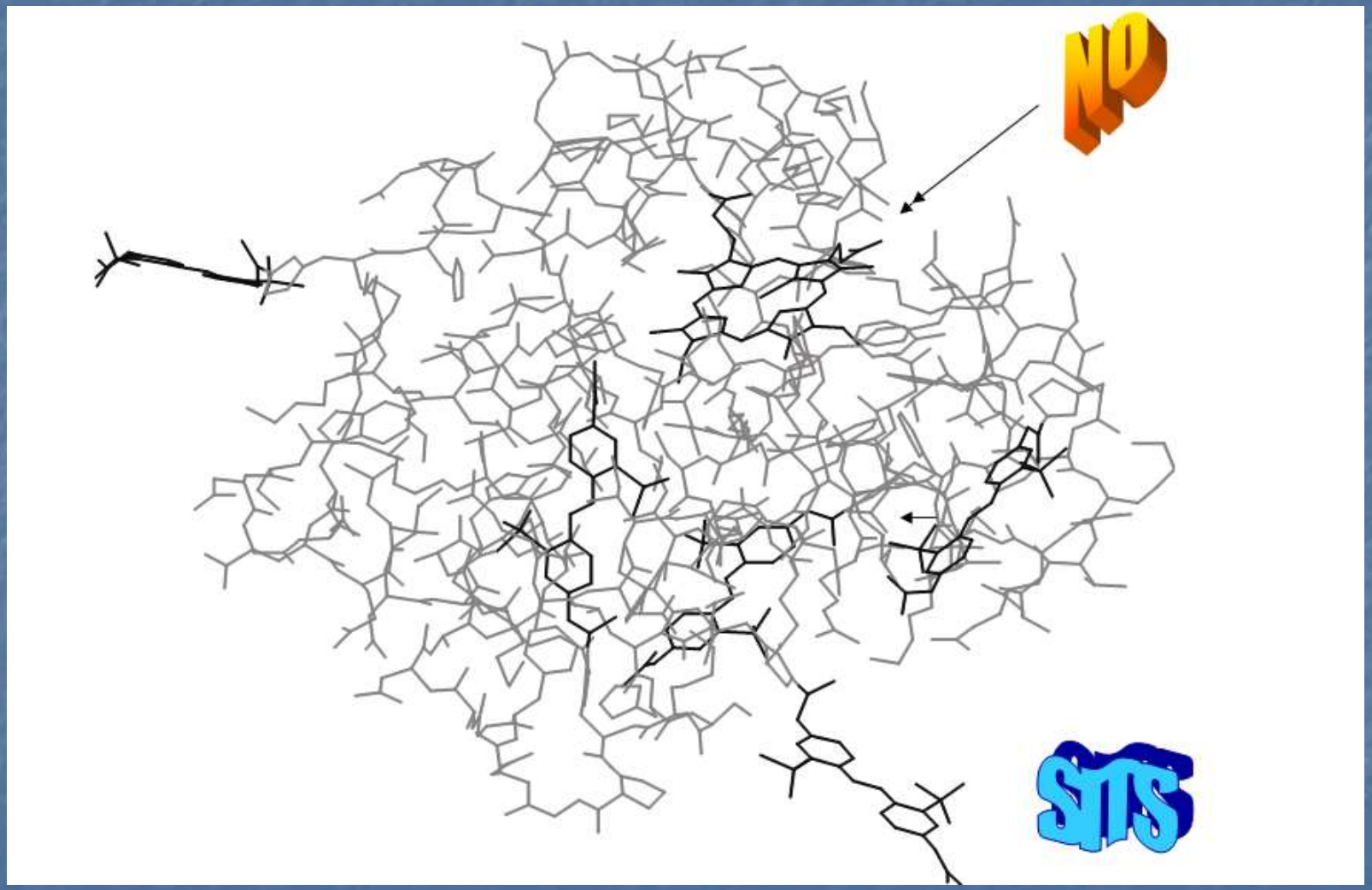
Dual probes as spin traps

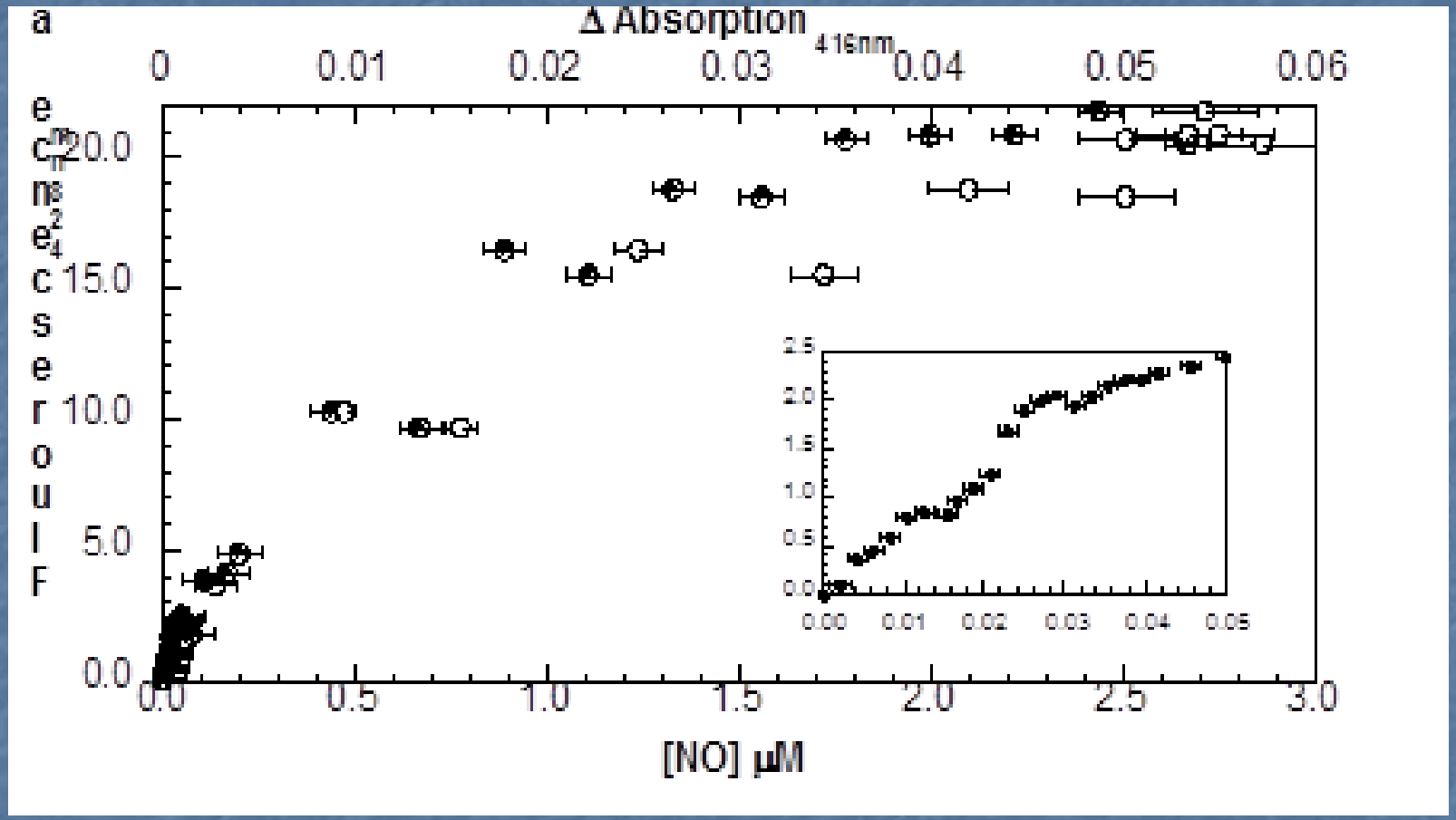


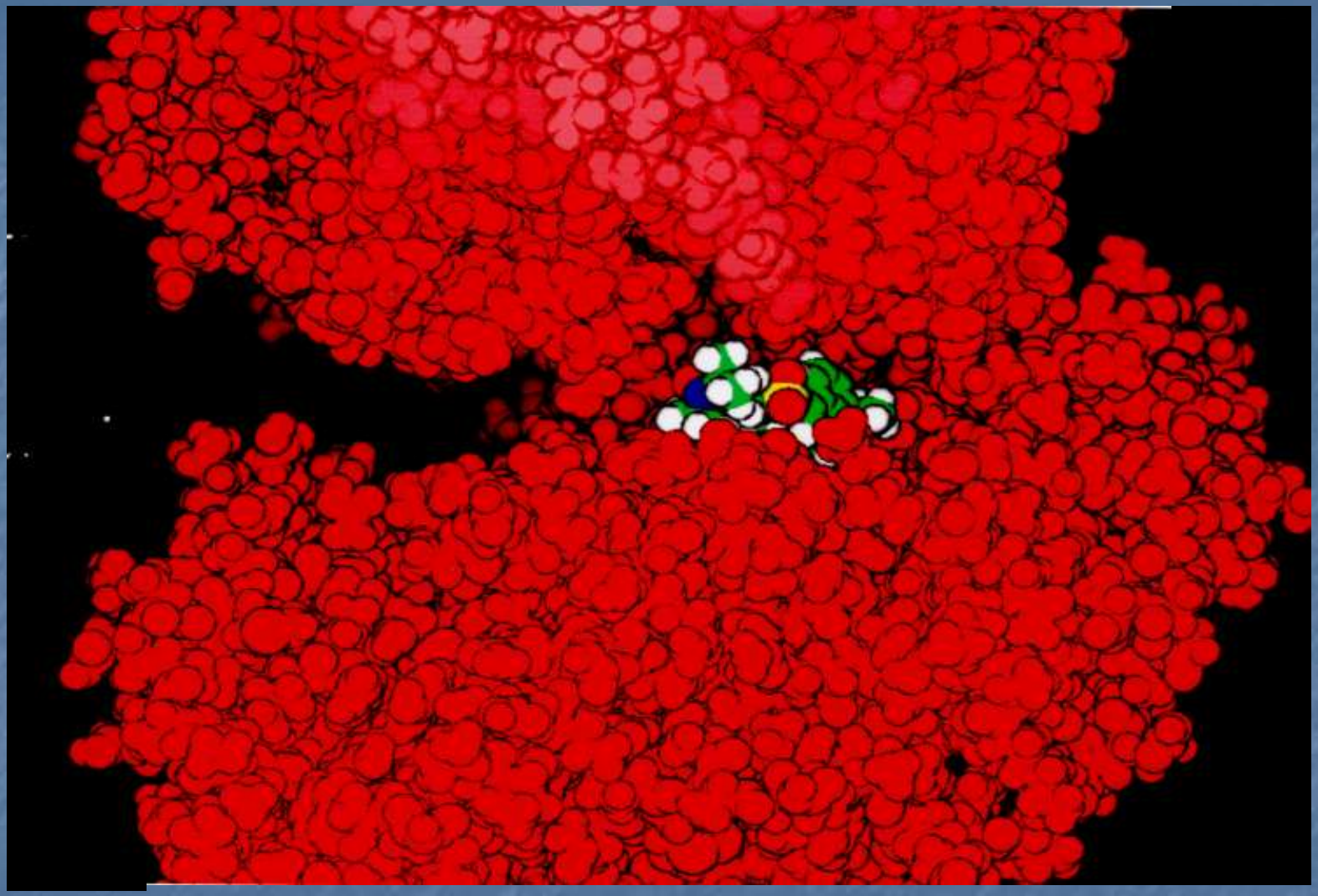
Joseph, J.; Kalyanaraman, B.; Hyde, J.S. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 926-934
Akaike T. et.al., *Biochem.*, **1993**, *32*, 827-832
Balakirev M., Khramtsov V., *Org. Chem.*, **1996**, *61*, 7263-7269



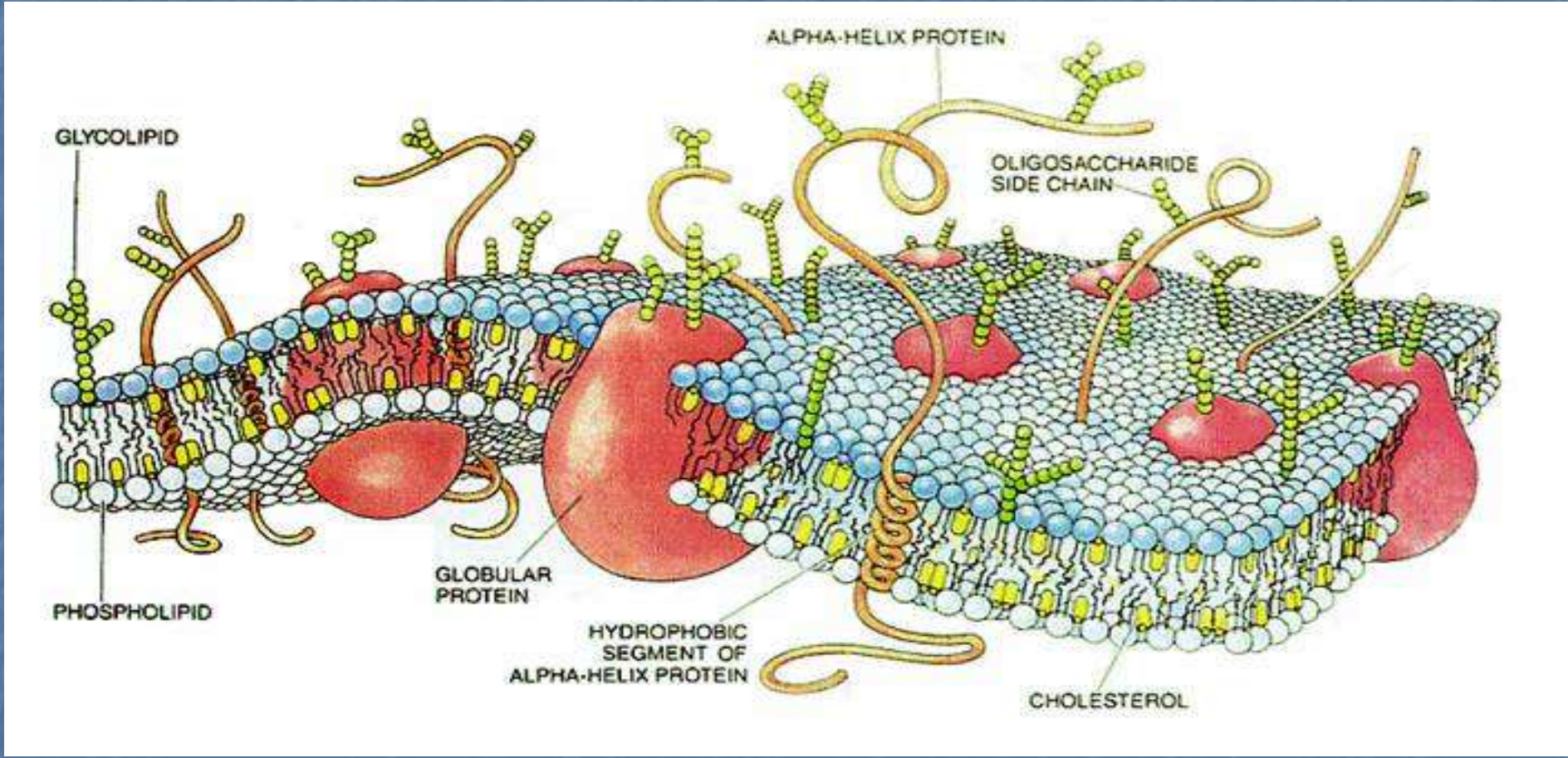

Fluorescence intensity (a.u.)
120
100
80
60
40
20
0
0
2
4
6
8
10
Probe concentration (μM)
8
4
0
0.000
0.003
0.006
0.009
Probe concentration (μM)


NO
SITS


Δ Absorption 416nm
0
0.01
0.02
0.03
0.04
0.05
0.06
20.0
15.0
10.0
5.0
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
[NO] μM
2.5
2.0
1.5
1.0
0.5
0.0
0.00
0.01
0.02
0.03
0.04
0.05

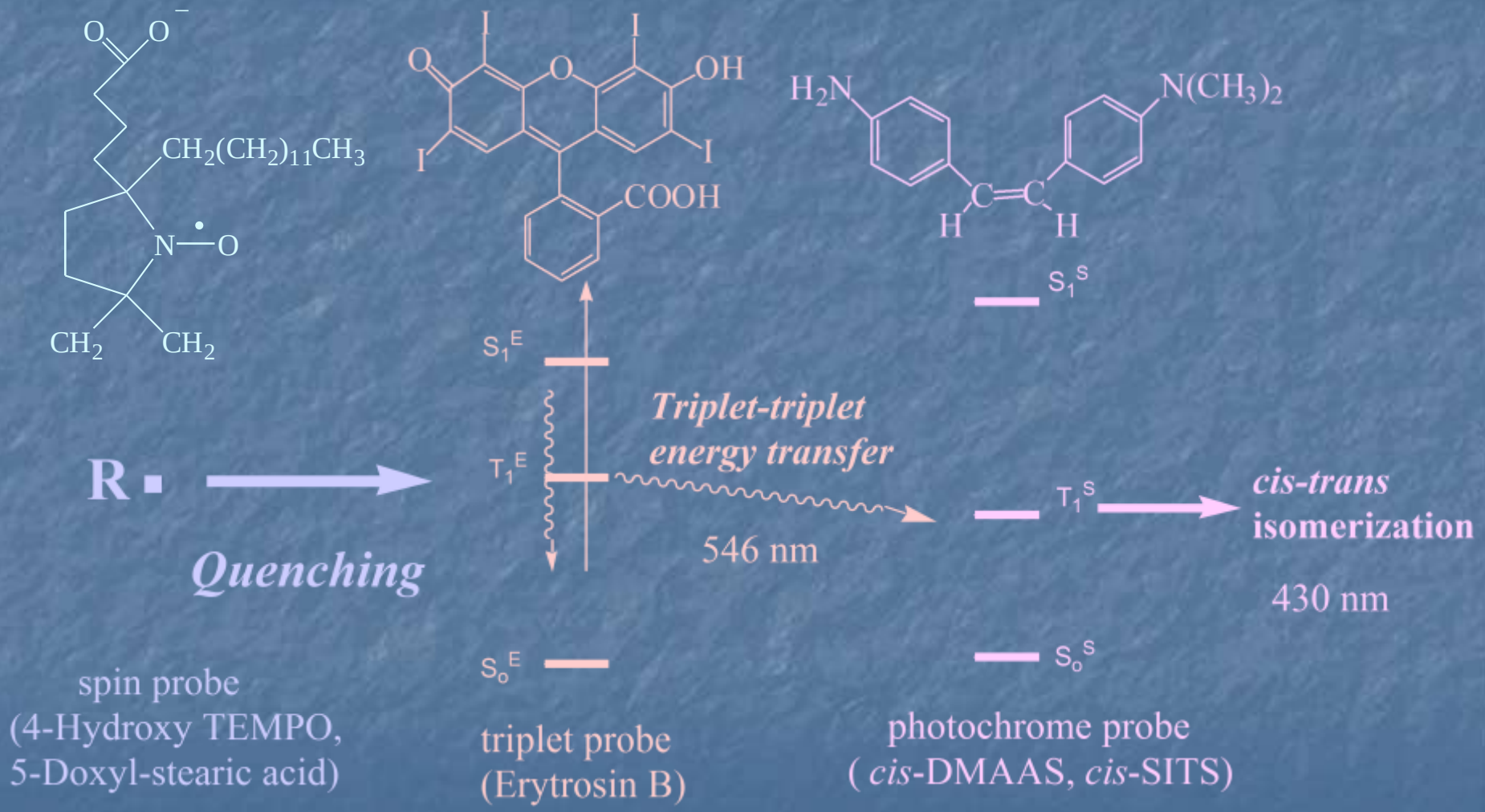


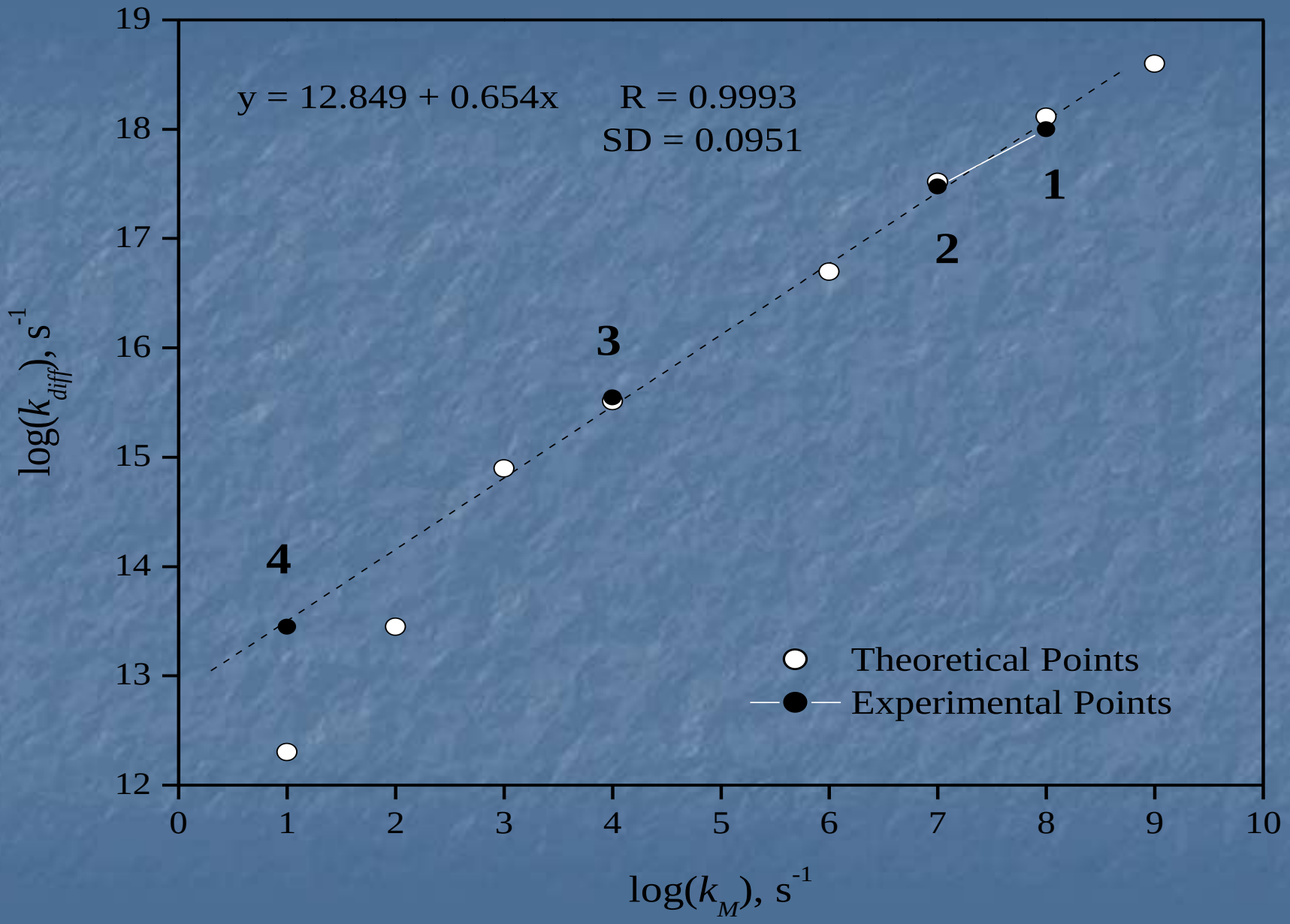
Cell membrane



Quenching of triplet state

CASCADE REACTION in membrane




y = 12.849 + 0.654x
R = 0.9993
SD = 0.0951
1
2
3
4
Theoretical Points
Experimental Points
log(k_diff), s^-1
log(k_M), s^-1
19
18
17
16
15
14
13
12
0
1
2
3
4
5
6
7
8
9
10


Help!!
Spin-label, Sir!
Fe57
O-N
Hg
Hg-C-Hg
Hg
NZYME

Rui Tamura

Albert Beth

Gareth Eaton

Sandra Eaton

Wayne Hubbell

James Hyde

Wolfgang Trimmer

Jack Freed

Igor Grigor'ev

Wolfgang Möbius

Ronald Mason

Sergey Dzuba

Valery Khramtsov

Harold Swartz

Gunnar Jeschke

Yury Tsvetkov

Albert Bobst

Elena Bagryanskaya

Victor Ovcharenko

Yakov Lebedev