Rotation of Membrane Proteins : Determining Correlation Time

- Fluorescence/Phosphorescence
- Saturation Transfer EPR

Hydrophobicity Scales of Amino Acids:

Wimley and White Hydrophobicity Measurements
3 papers:

Anal Biochem.213, 213-217 (1993)
Biochemistry 35, 5109-5124 (1996)
Nature Struc Bio 3, 842-848 (1996)

Fluorescence anisotropy measurements are useful for labeled proteins in solution, but what about proteins imbedded into the bilayer? Recall: <u>Rotational diffusion can only be measured if a significant amount of rotation occurs during the lifetime of the excited state.</u> Membrane imbedded proteins have correlation times as slow as μs to ms.

~ns

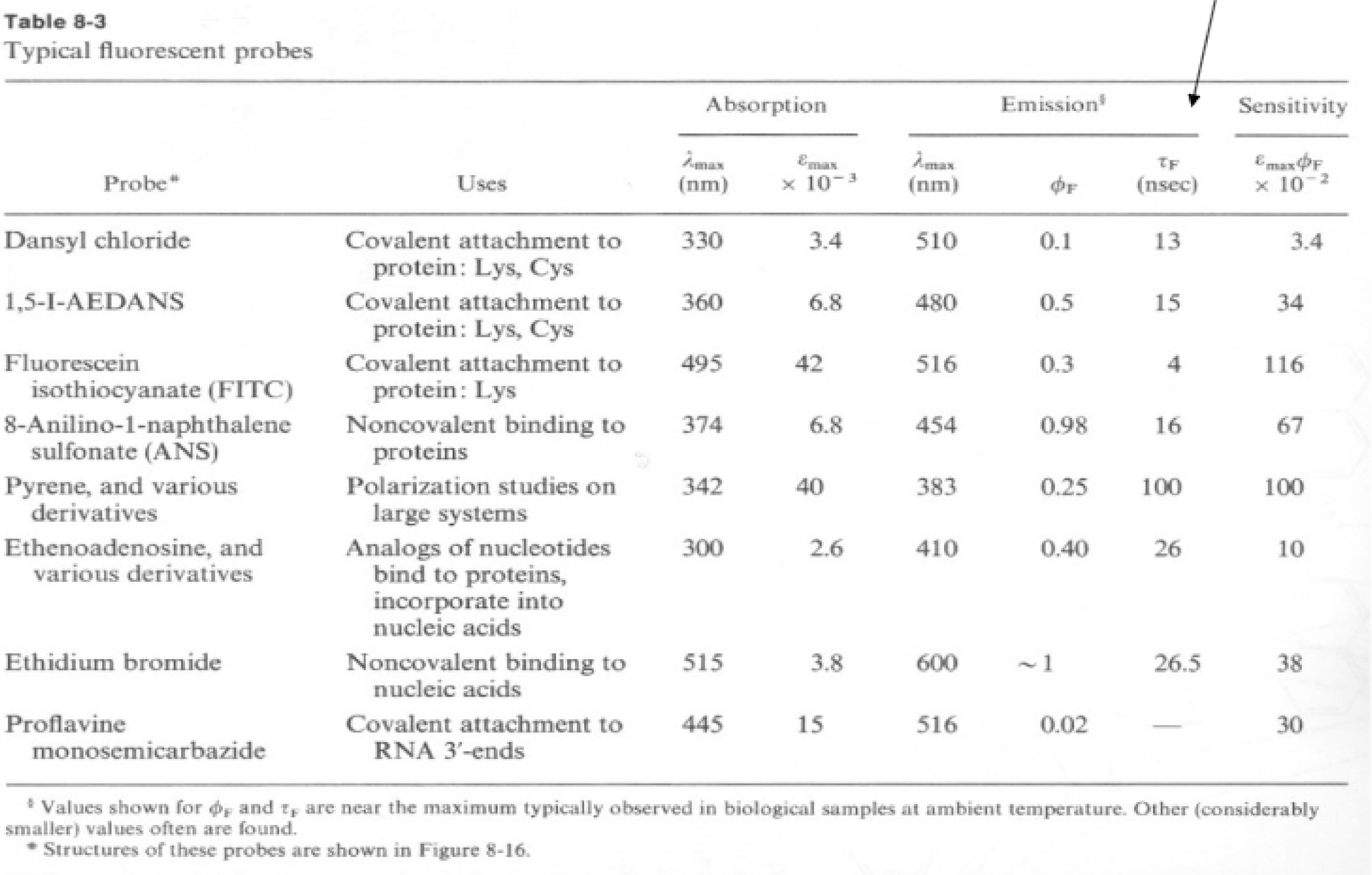

Table 8-3
Typical fluorescent probes

		Absorption		Emission‡			Sensitivity
Probe*	Uses	λ_{max} (nm)	$\varepsilon_{max} \times 10^{-3}$	λ_{max} (nm)	ϕ_F	τ_F (nsec)	$\varepsilon_{max}\phi_F \times 10^{-2}$
Dansyl chloride	Covalent attachment to protein: Lys, Cys	330	3.4	510	0.1	13	3.4
1,5-I-AEDANS	Covalent attachment to protein: Lys, Cys	360	6.8	480	0.5	15	34
Fluorescein isothiocyanate (FITC)	Covalent attachment to protein: Lys	495	42	516	0.3	4	116
8-Anilino-1-naphthalene sulfonate (ANS)	Noncovalent binding to proteins	374	6.8	454	0.98	16	67
Pyrene, and various derivatives	Polarization studies on large systems	342	40	383	0.25	100	100
Ethenoadenosine, and various derivatives	Analogs of nucleotides bind to proteins, incorporate into nucleic acids	300	2.6	410	0.40	26	10
Ethidium bromide	Noncovalent binding to nucleic acids	515	3.8	600	~1	26.5	38
Proflavine monosemicarbazide	Covalent attachment to RNA 3′-ends	445	15	516	0.02	—	30

‡ Values shown for ϕ_F and τ_F are near the maximum typically observed in biological samples at ambient temperature. Other (considerably smaller) values often are found.

* Structures of these probes are shown in Figure 8-16.

Possible solutions to measuring slower correlation times:

1) Utilize the triplet state (optical spectroscopy)
2) Saturation transfer EPR

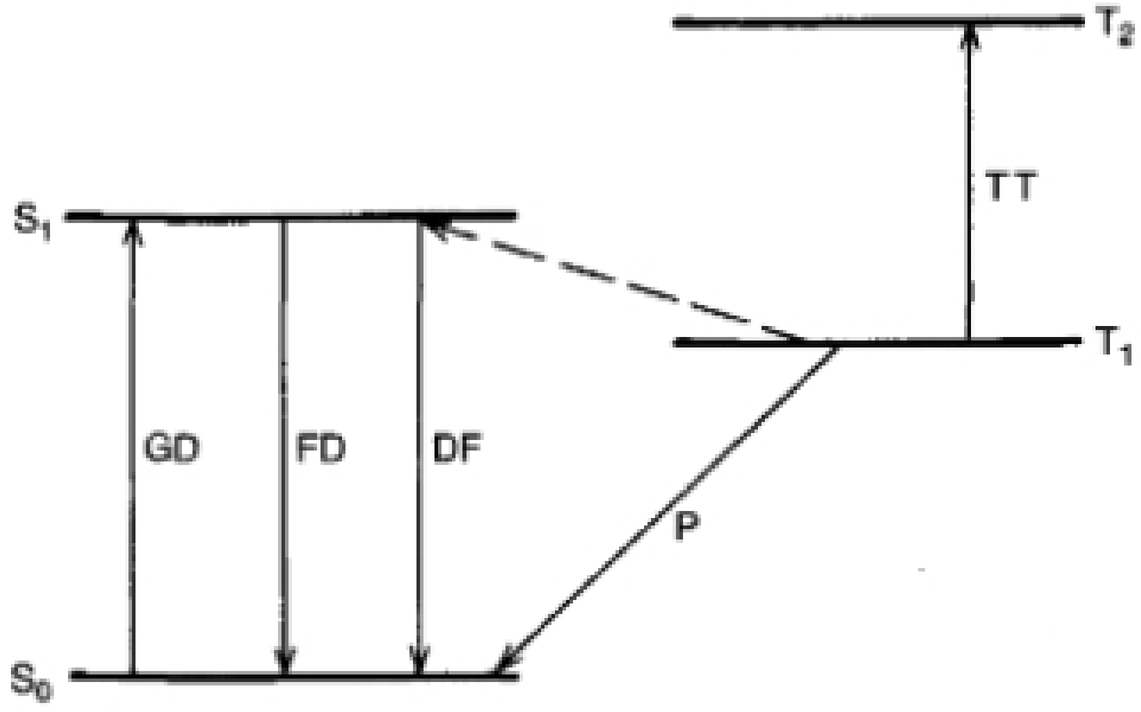

FIGURE 11.2 Electronic energy level diagram illustrating different methods of detecting molecules in the triplet state T_1. Phosphorescence (P) is emission accompanying a radiative transition back to the ground state S_0 via the excited singlet state S_1. Population of T_1 depletes the ground state, resulting in loss of intensity of singlet–singlet absorption (GD = ground state depletion) and prompt fluorescence (FD = fluorescence depletion) under continuous illumination. Finally, molecules in the triplet state undergo triplet–triplet absorption (TT) when illuminated with light of appropriate wavelength. T_1 is populated by intersystem crossing from S_1 after exciting the S_0-S_1 transition. For simplicity, vibrational levels are omitted.

Rotational Diffusion constant for protein in membrane

$$D_R = kT/\left(4\pi a^2 h\eta\right)$$

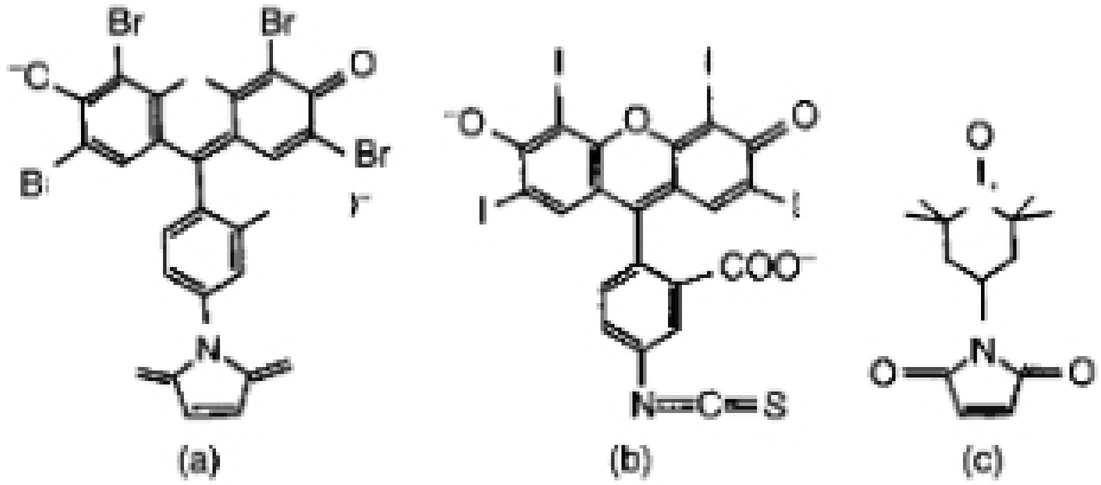

FIGURE 11.3 Commonly used probes for rotational diffusion measurements: (a) eosin-5-maleimide and (b) erythrosine-5-isothiocyanate are employed for optical methods, and the spin label (c) 6-MSL (N-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]maleimide) is employed for ST-EPR.