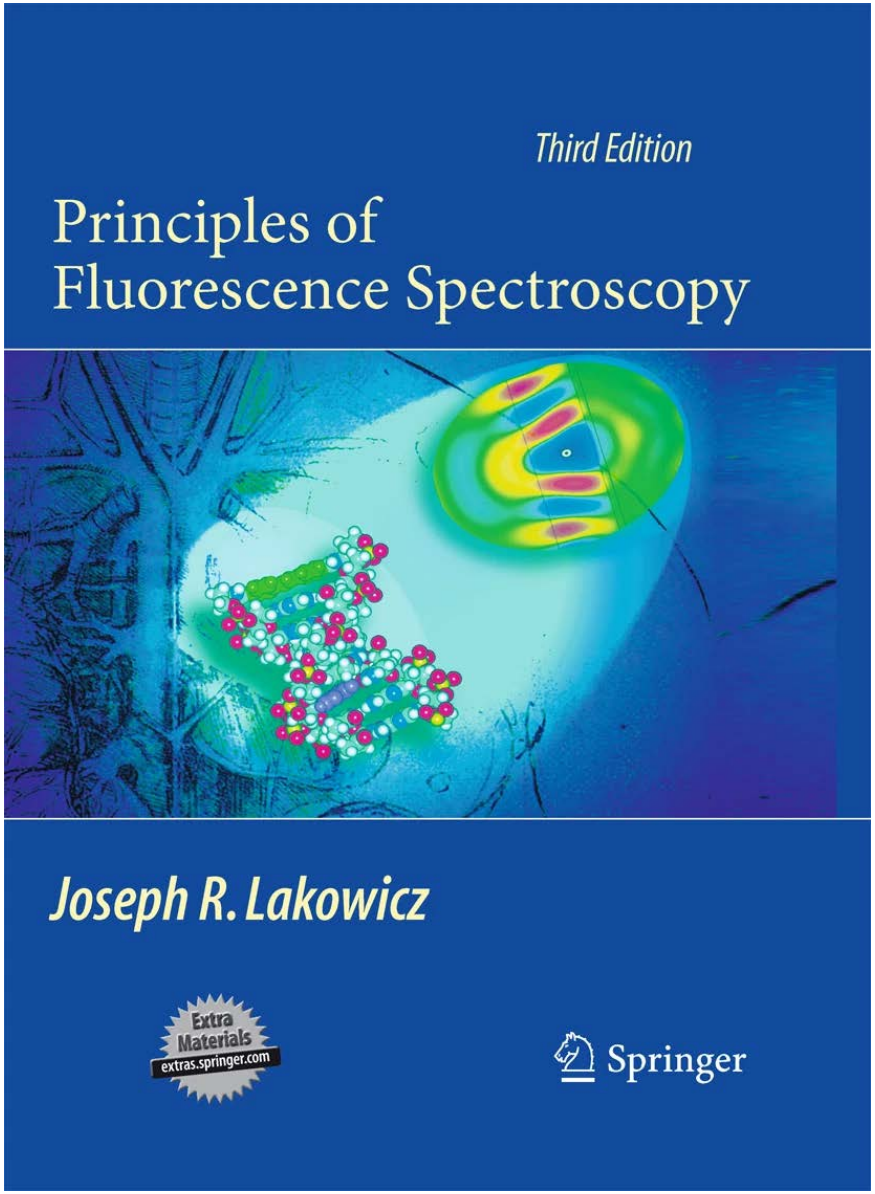
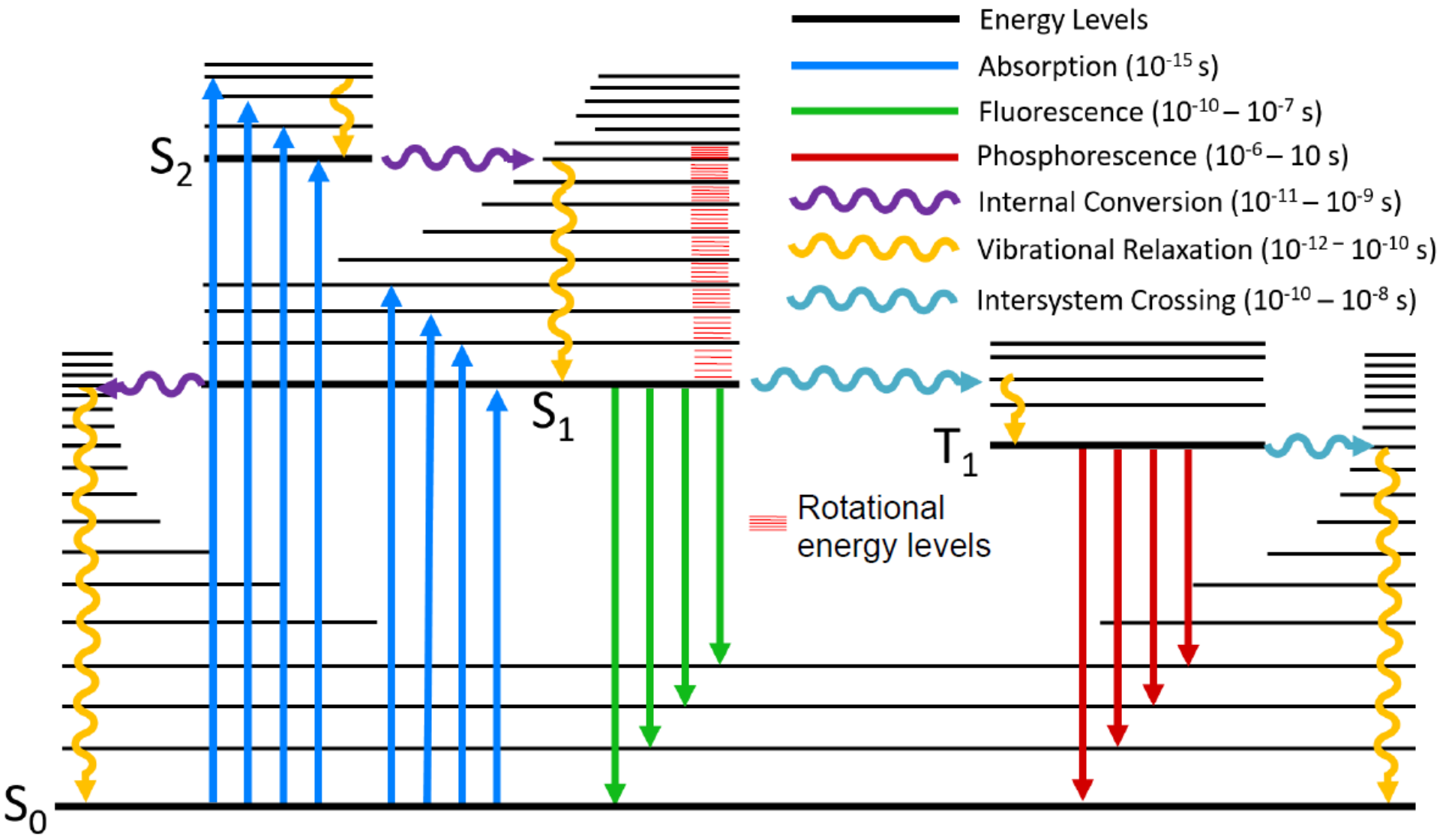
Fluorescence spectroscopy



Jabłonski diagram



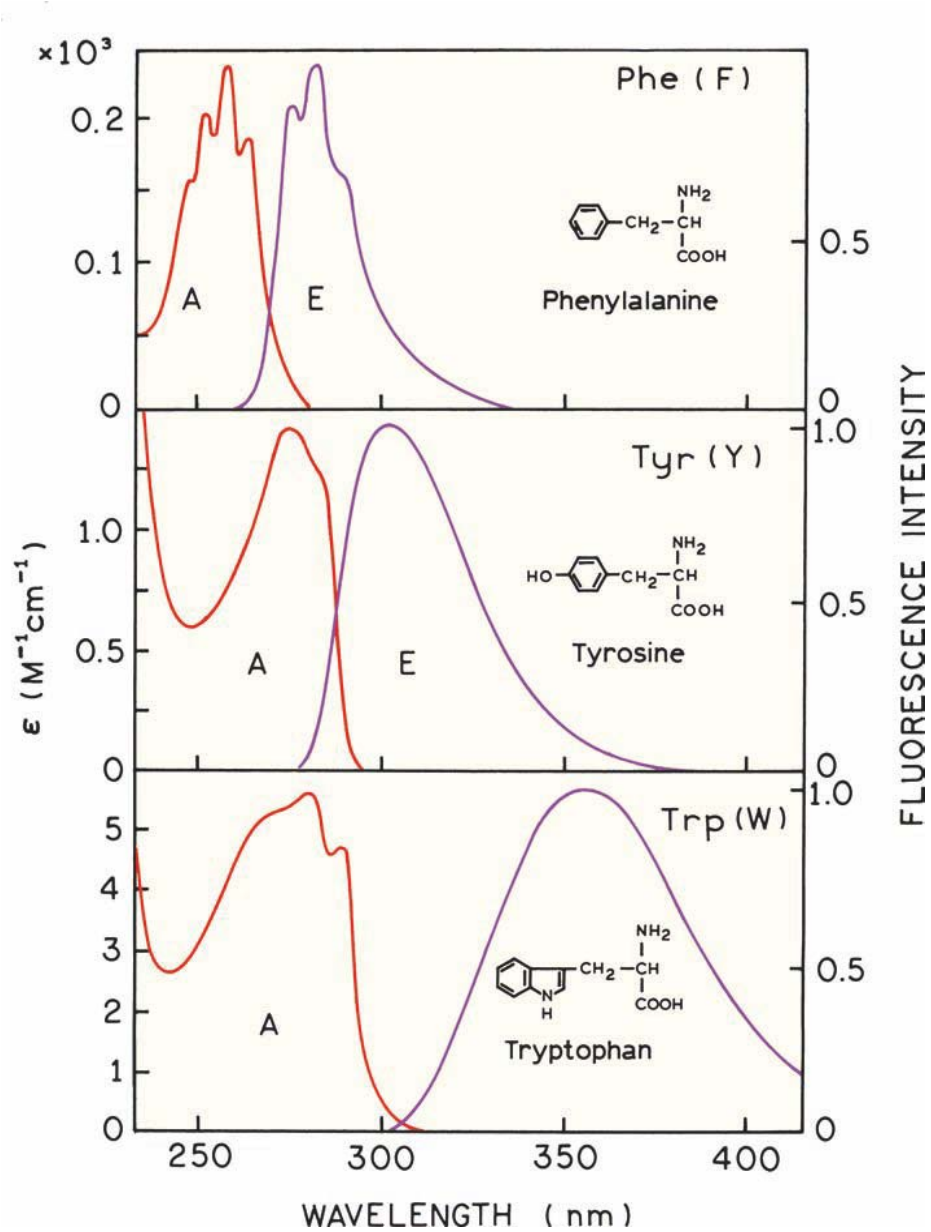
Intrinsic fluorophores in proteins (in H_2O pH 7)

Chromophore	Absorption		Fluorescence			
	λ_{max} [nm]	ε_{max} [M^{-1} cm^{-1}]	λ_{max} [nm]	Quantum yield ϕ	Lifetime τ [ns]	Sensitivity ε_{max} ϕ [M^{-1} cm^{-1}]
Tryptophan	280	5,600	348	0.20	2.6	1,100
Tyrosine	274	1,400	303	0.14	3.6	200
Phenylalanine	257	200	282	0.04	6.4	8

Cantor, C.R. & Schimmel, P.R. Biophysical Chemistry, Part II: Techniques for the study of biological structure and function, 1980, W. H. Freeman and Company.

- Fluorescence in proteins comes from tryptophan and tyrosine, usually dominated by tryptophan
- Tyrosine (and phenylalanine) fluorescence diminished by energy transfer to tryptophan residues in the same protein
- Tryptophan fluorescence can be selectively observed by excitation with λ > 295 nm

Absorption and emission spectra of Phe, Tyr and Trp in H_2O pH 7



Lakowicz, J.R. Principles of Fluorescence Spectroscopy, 3rd edition, 2006, Springer