RHODES UNIVERSITY

Basics in steady state and time resolved spectroscopy

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Group Prof. Nyokong

Synthesis of Phthalocyanines and Nanoparticles
Sensors/Electrochemistry
Photodynamic Therapy
Nonlinear Optical Materials

Content

- Steady state absorption spectroscopy

  Absorbance, purity, extinction coefficient ε, aggregation
- Steady state fluorescence spectroscopy

  Fluorescence, Investigation of FRET
- Time correlated single photon counting (TCSPC)

  Lifetime of first excited state, Investigation of FRET
- Laser flash photolysis

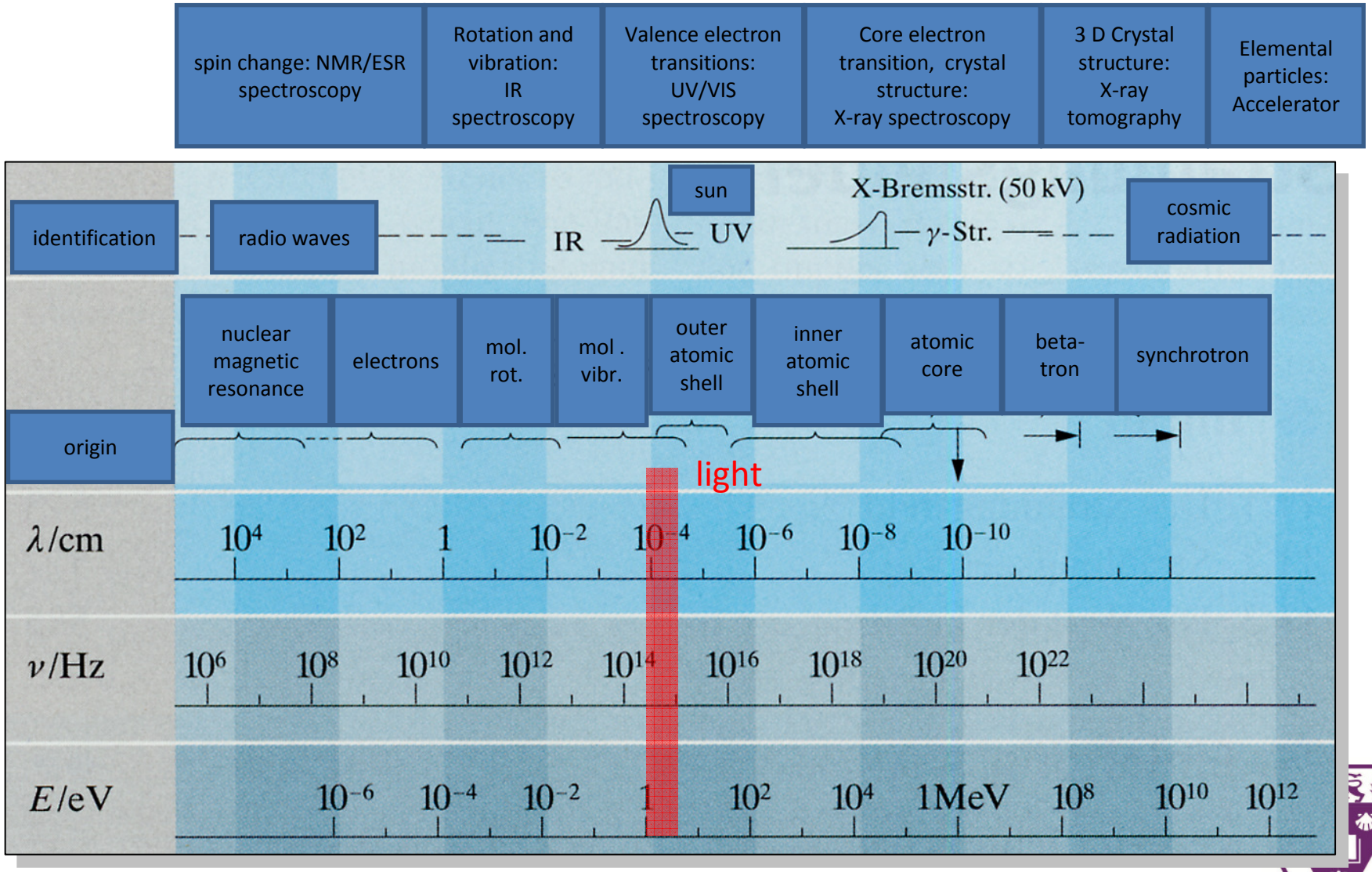
  Population and lifetime of triplet state
- Singlet oxygen luminescence detection

  Singlet oxygen quantum yield
- X-ray diffraction spectroscopy

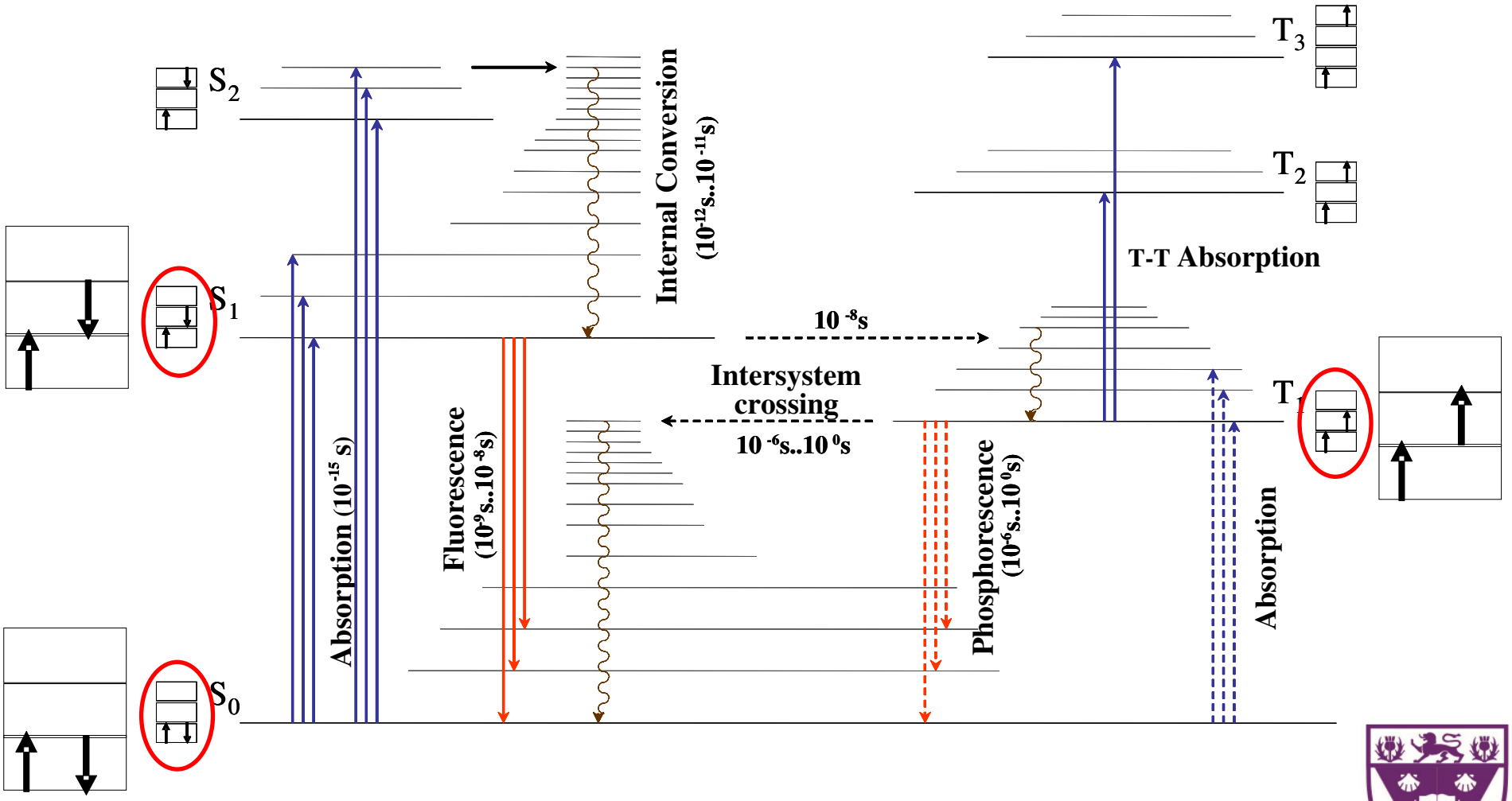
  Structure and size of samples
- X-ray photoelectron spectroscopy

  elemental composition, chemical state and electronic state of the elements in a material

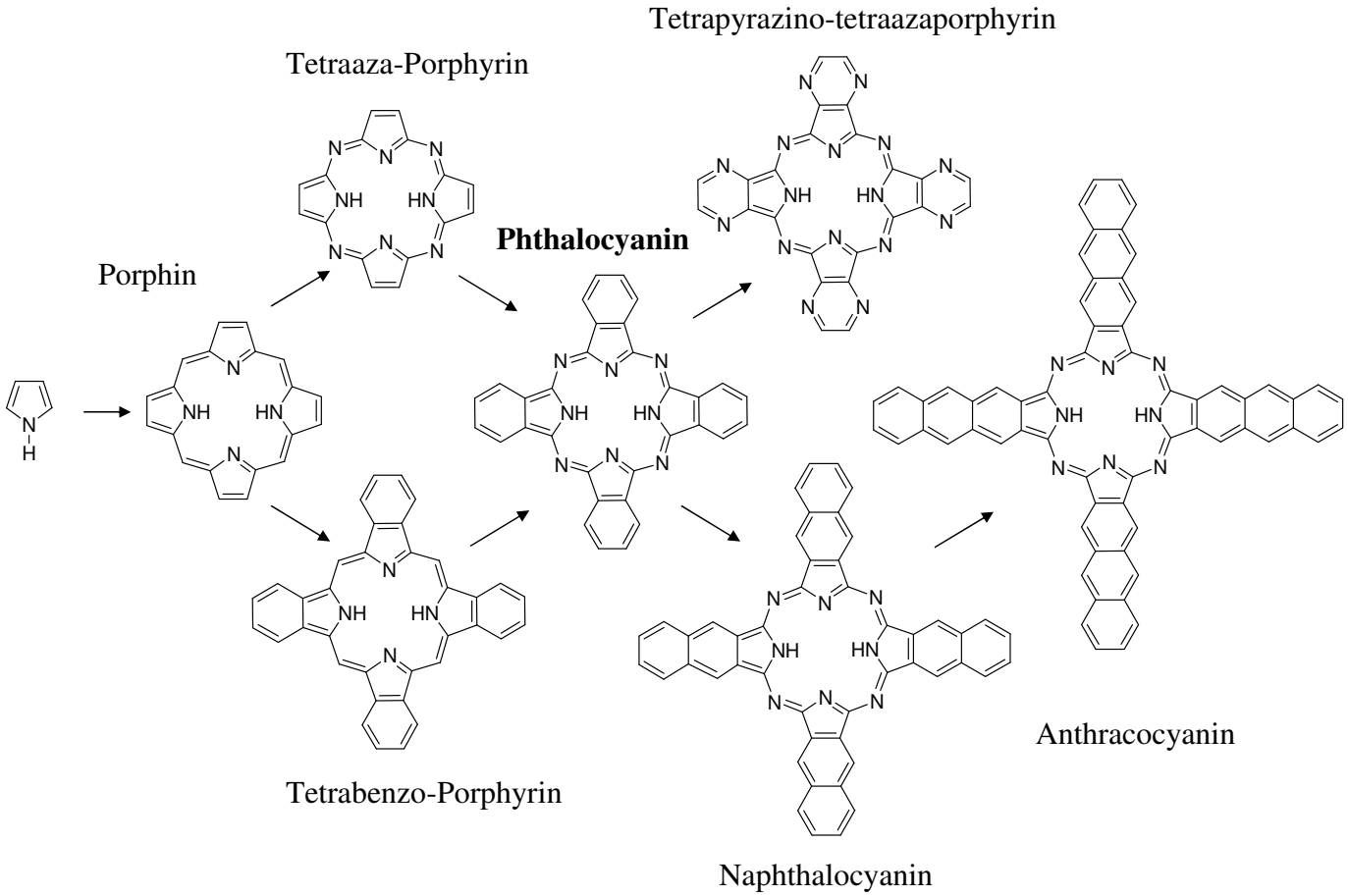
Electromagnetic radiation



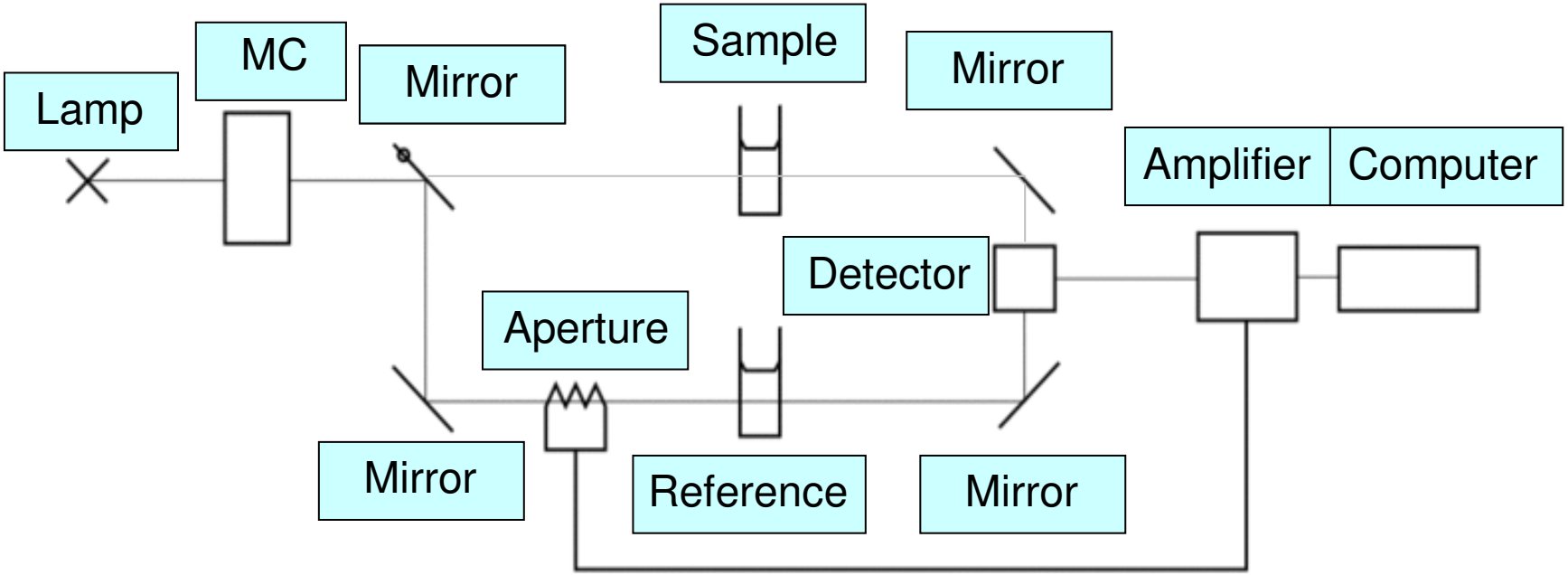
Jabłoński Diagram



Phthalocyanine

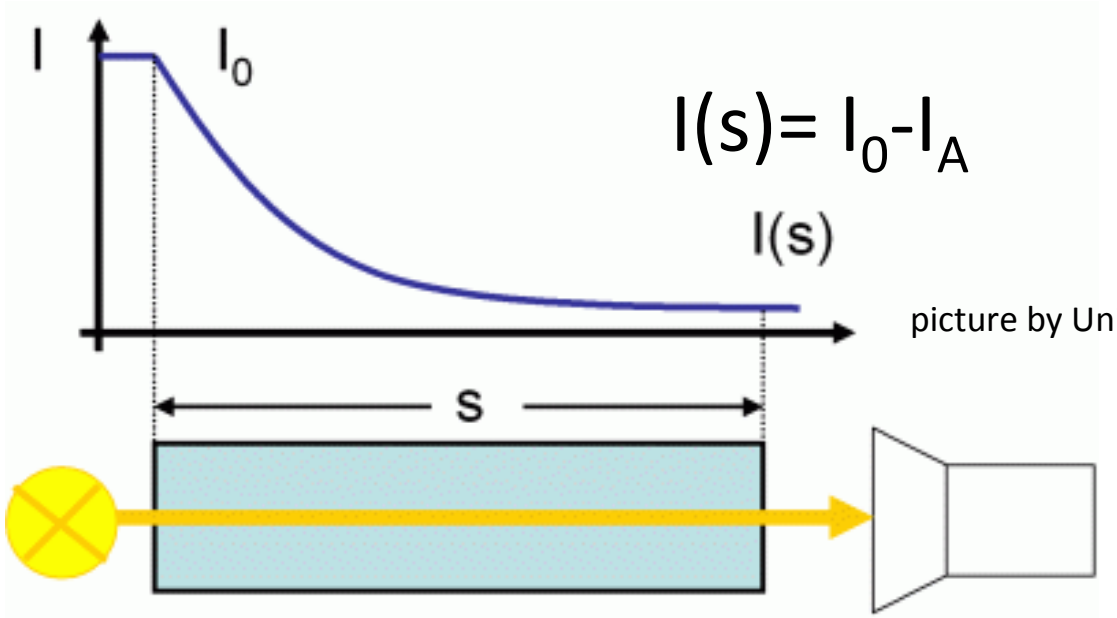


Steady State Absorption



Absorption spectrum shows the fraction of incident light absorbed by the material over a range of frequencies.

Lambert-Beer-Law



$I(s) = I_0 - I_A$

picture by University of Bremen

OD

$I = I_0 \cdot 10^{(-\varepsilon \cdot c \cdot s)}$ or $I = I_0 \cdot e^{(-\sigma \cdot n \cdot s)}$

ε = molar extinction coefficient, $[\varepsilon] = M^{-1} \cdot cm^{-1}$

c = concentration, [c] = mol

σ = absorption cross section of one mol, $[\sigma] = cm^2$

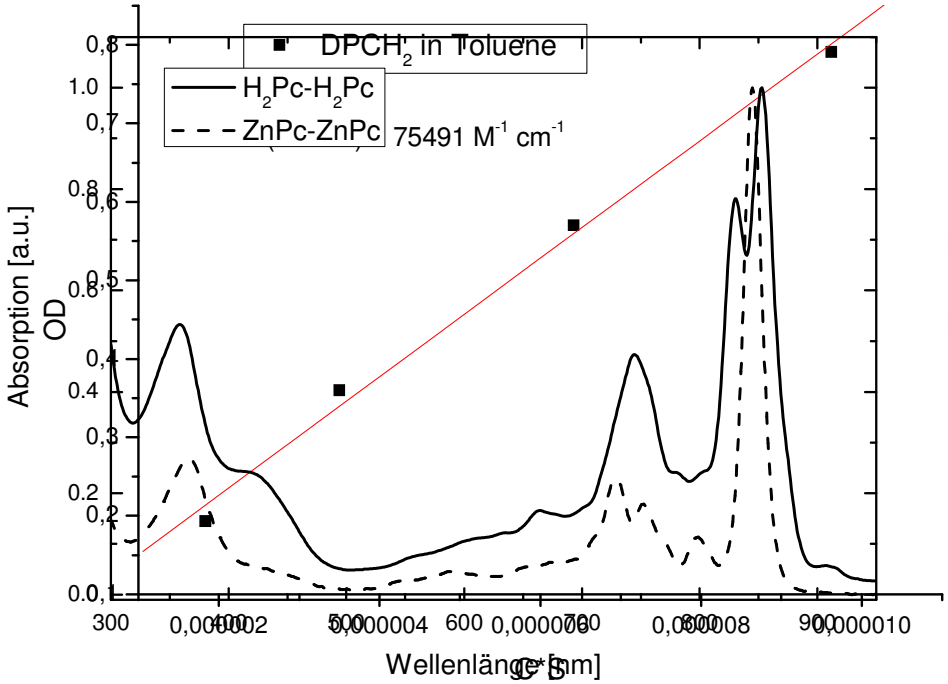
s = pathway, [s] = cm

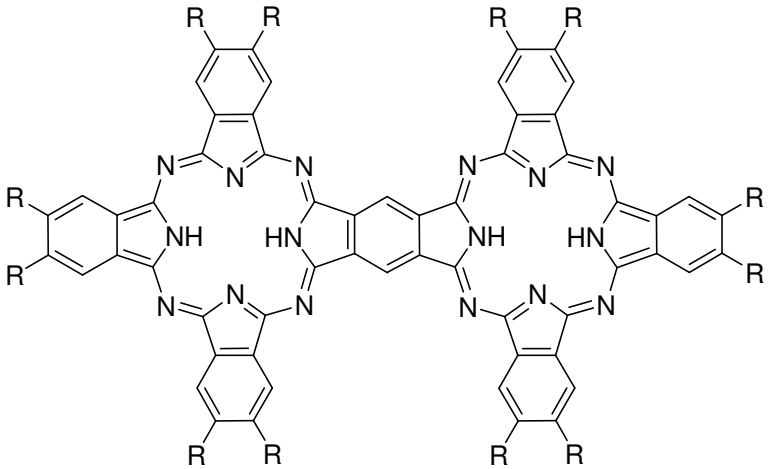
n = number of mol

Determinaton of ε

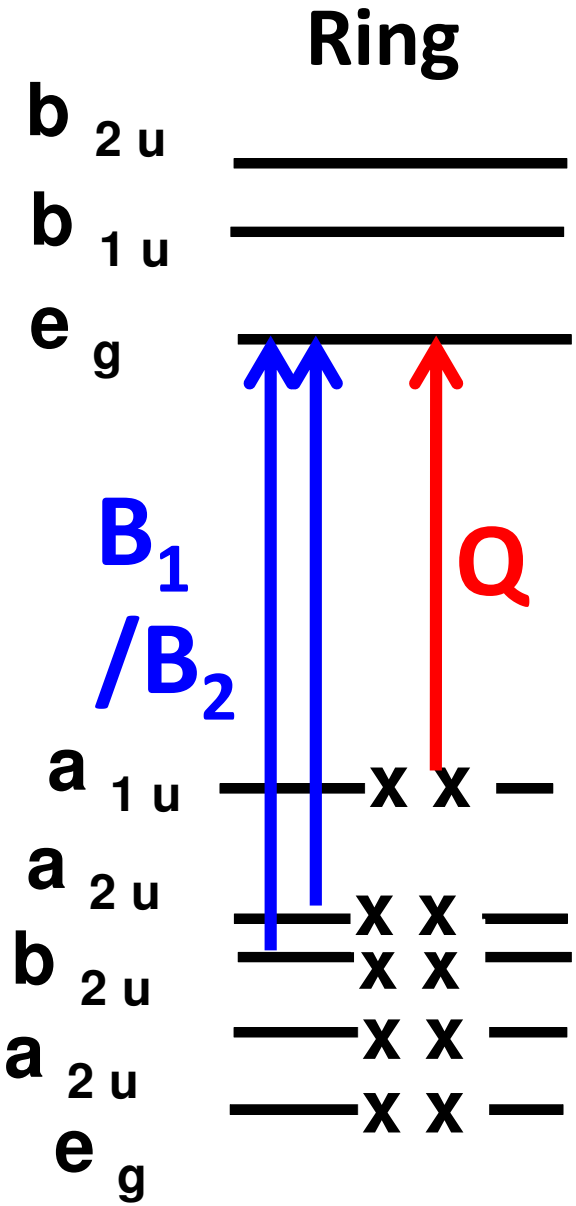
$$I = I_0 \cdot 10^{(-\varepsilon \cdot c \cdot s)}$$

$$OD = -\varepsilon \cdot c \cdot s$$

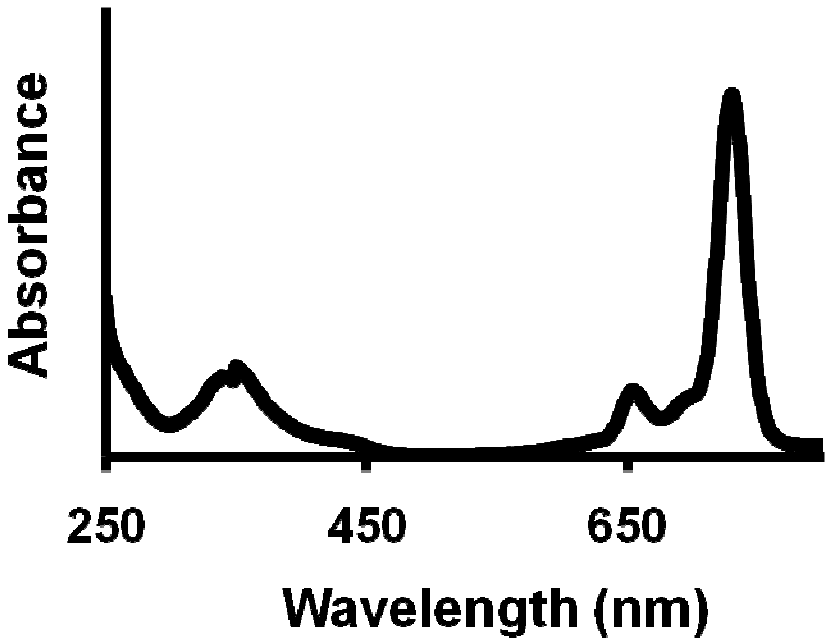




Origin of spectra in Pcs

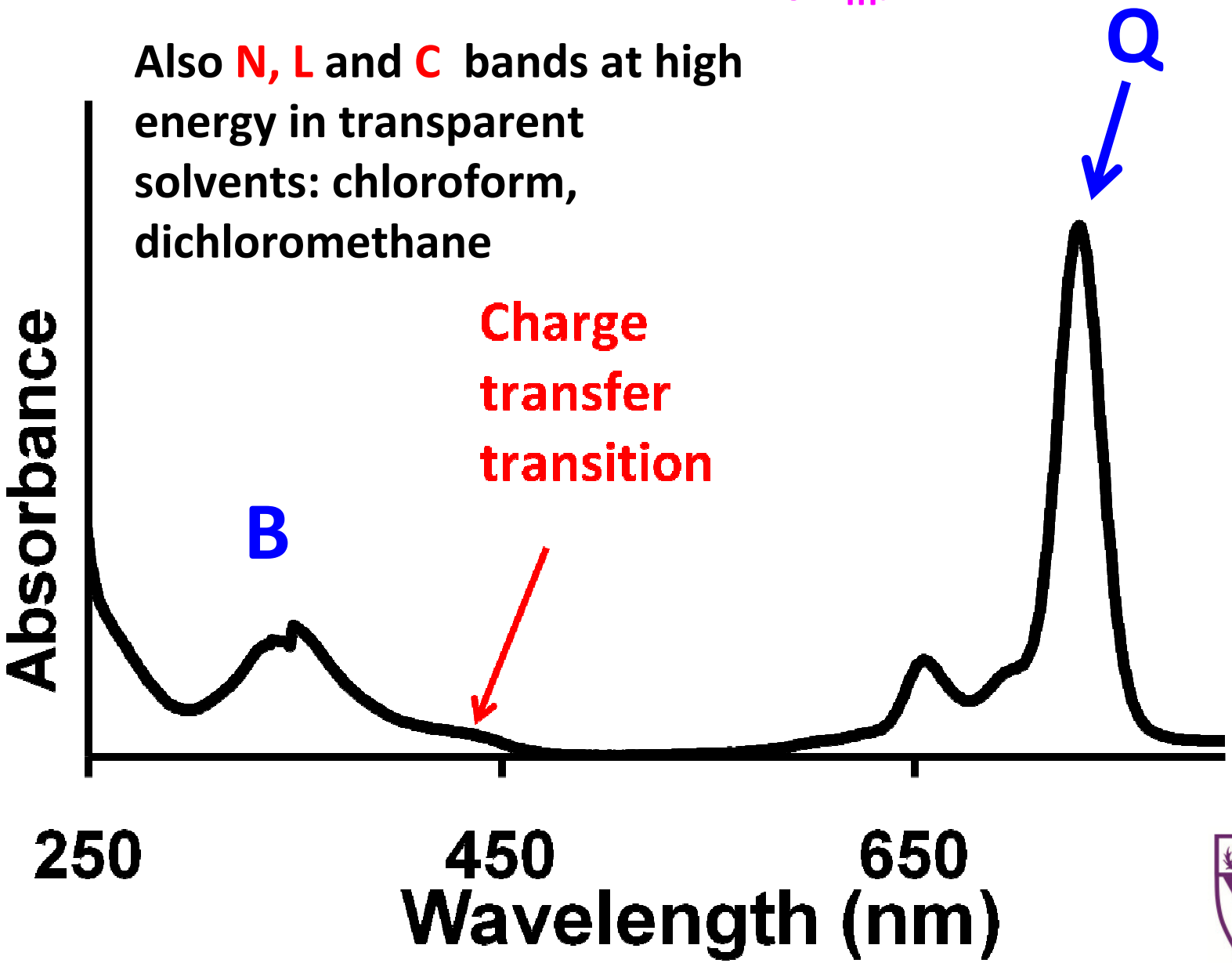


Metallated Pc (D_{4h})



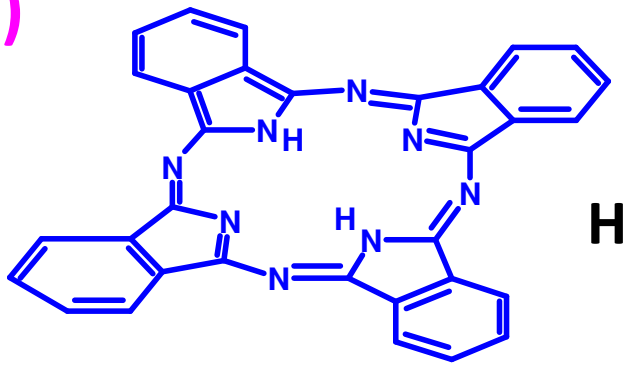
Metallated Pc (D_{4h})

Also N, L and C bands at high energy in transparent solvents: chloroform, dichloromethane

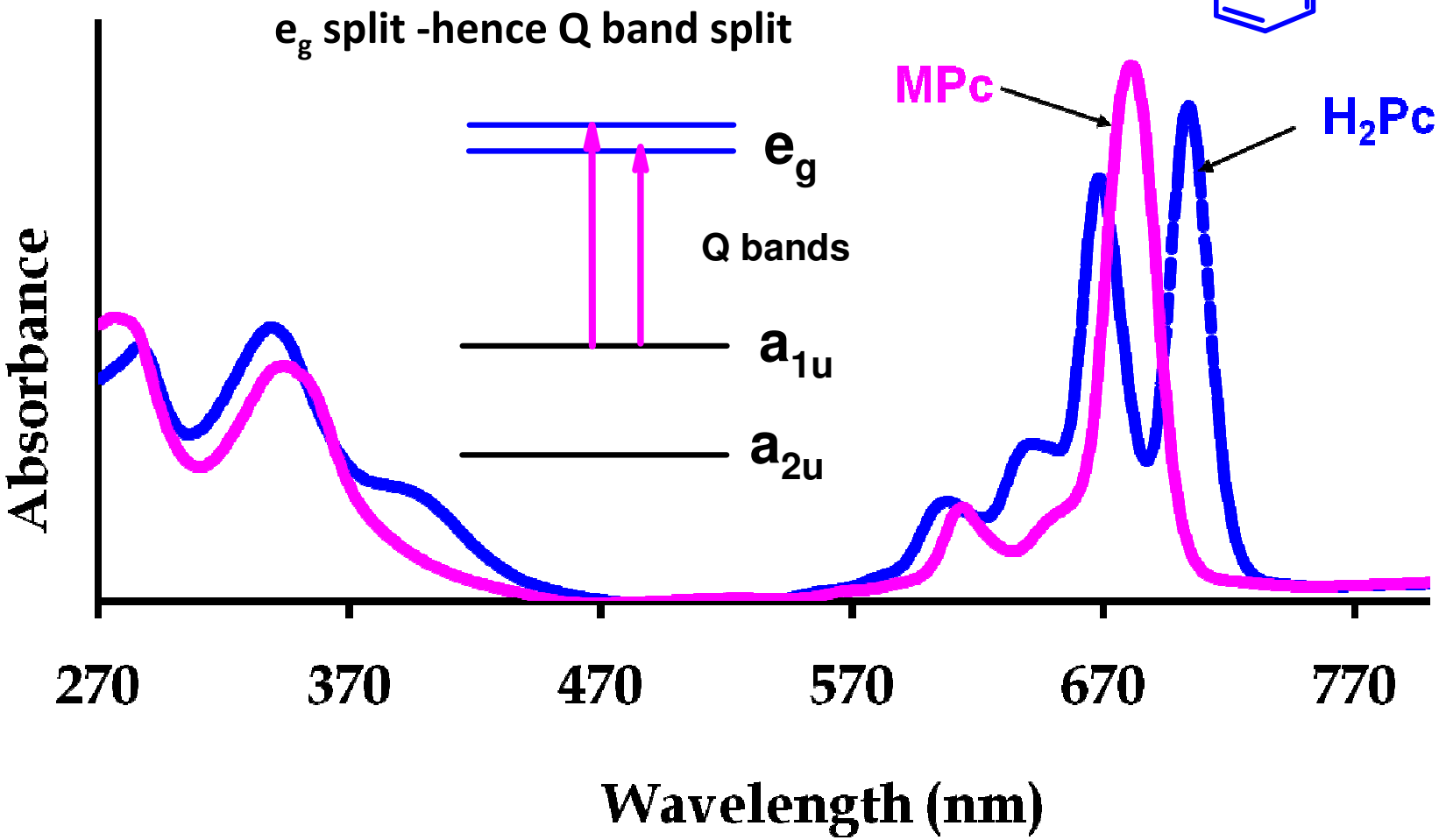


Unmetallated Pc complexes (D_{2h})

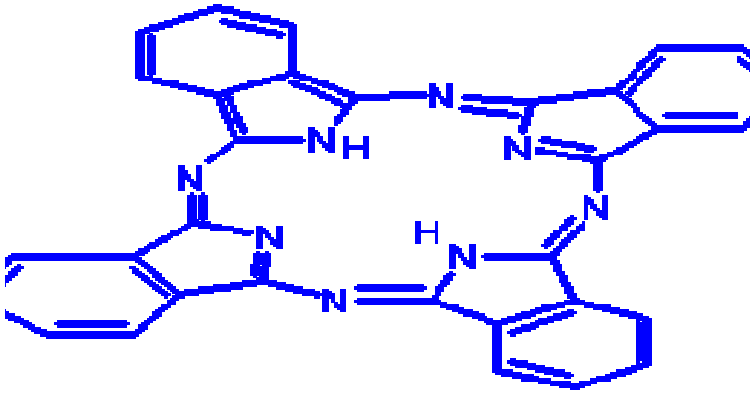
Metallated Pc (D_{4h})

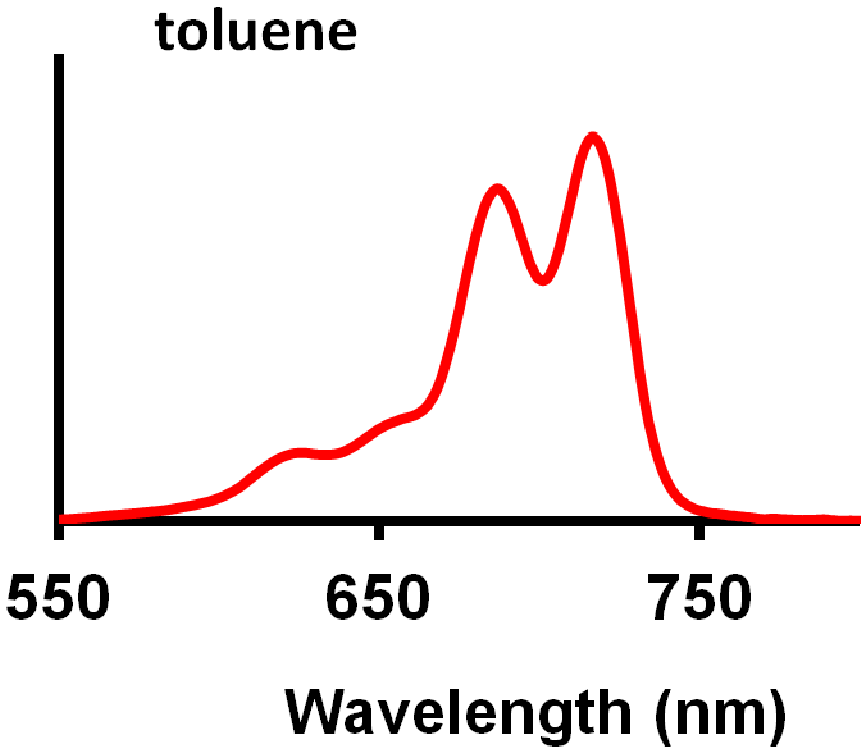


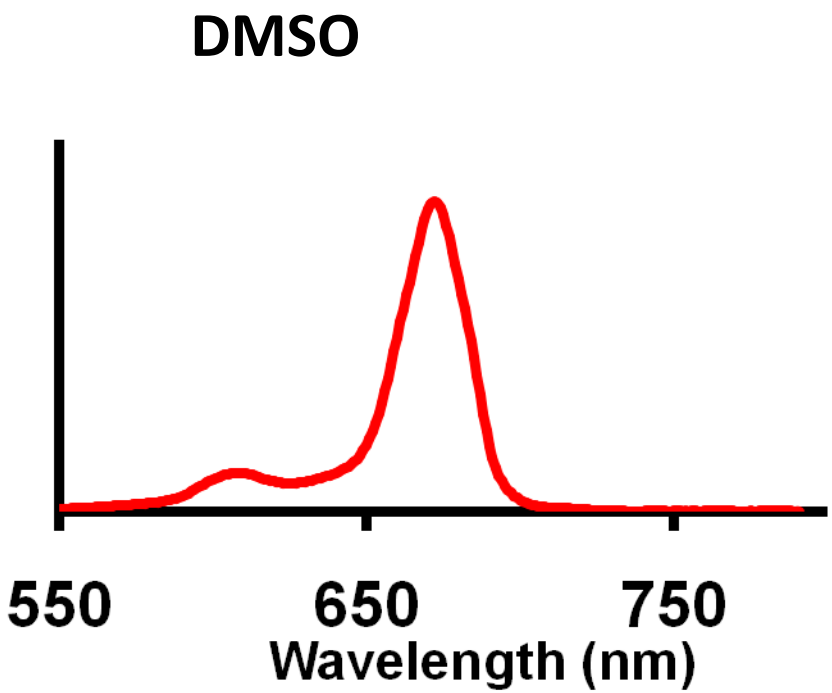
H_2Pc



H_2Pc spectra – Not split in basic solvents (eg DMSO,

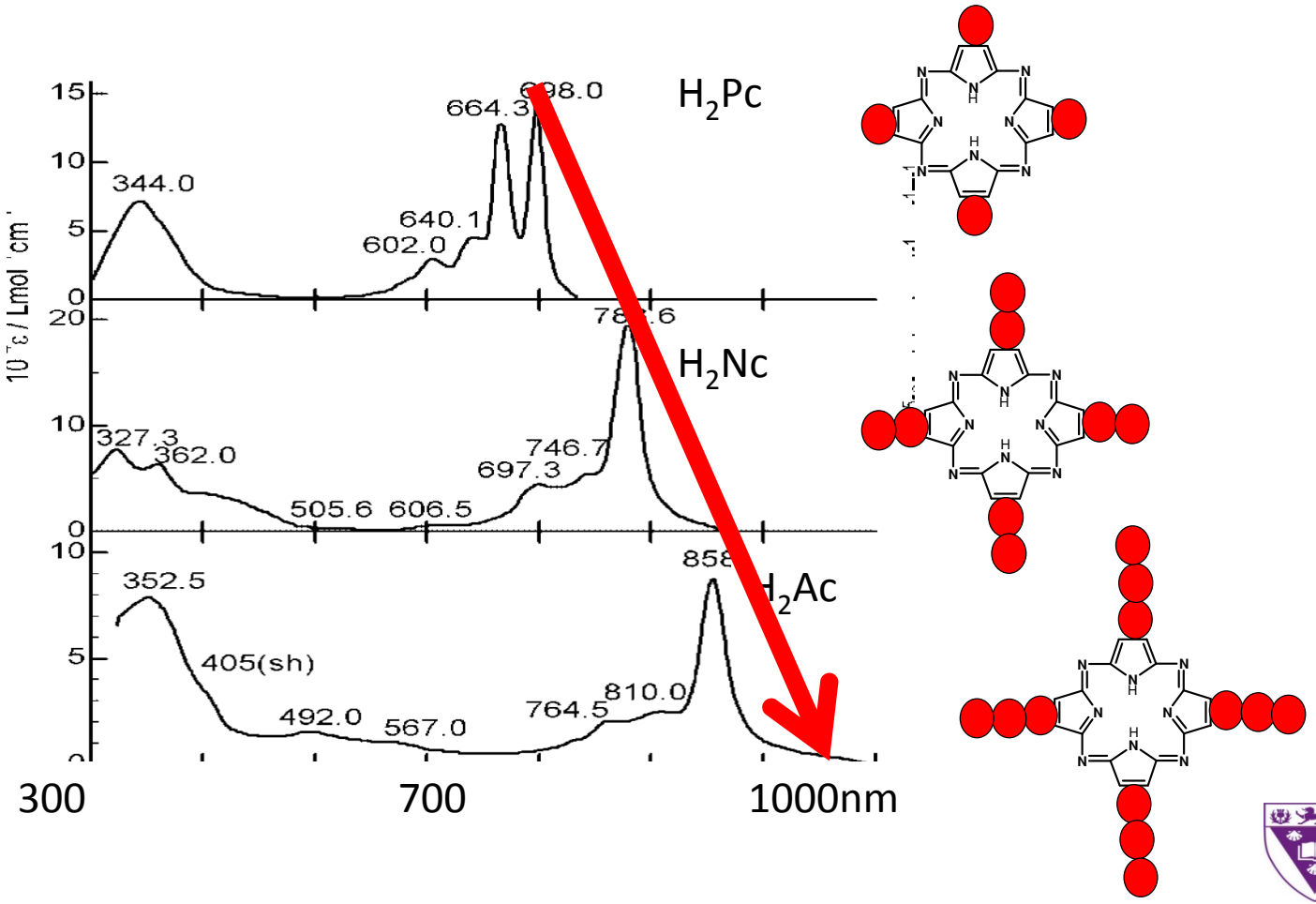




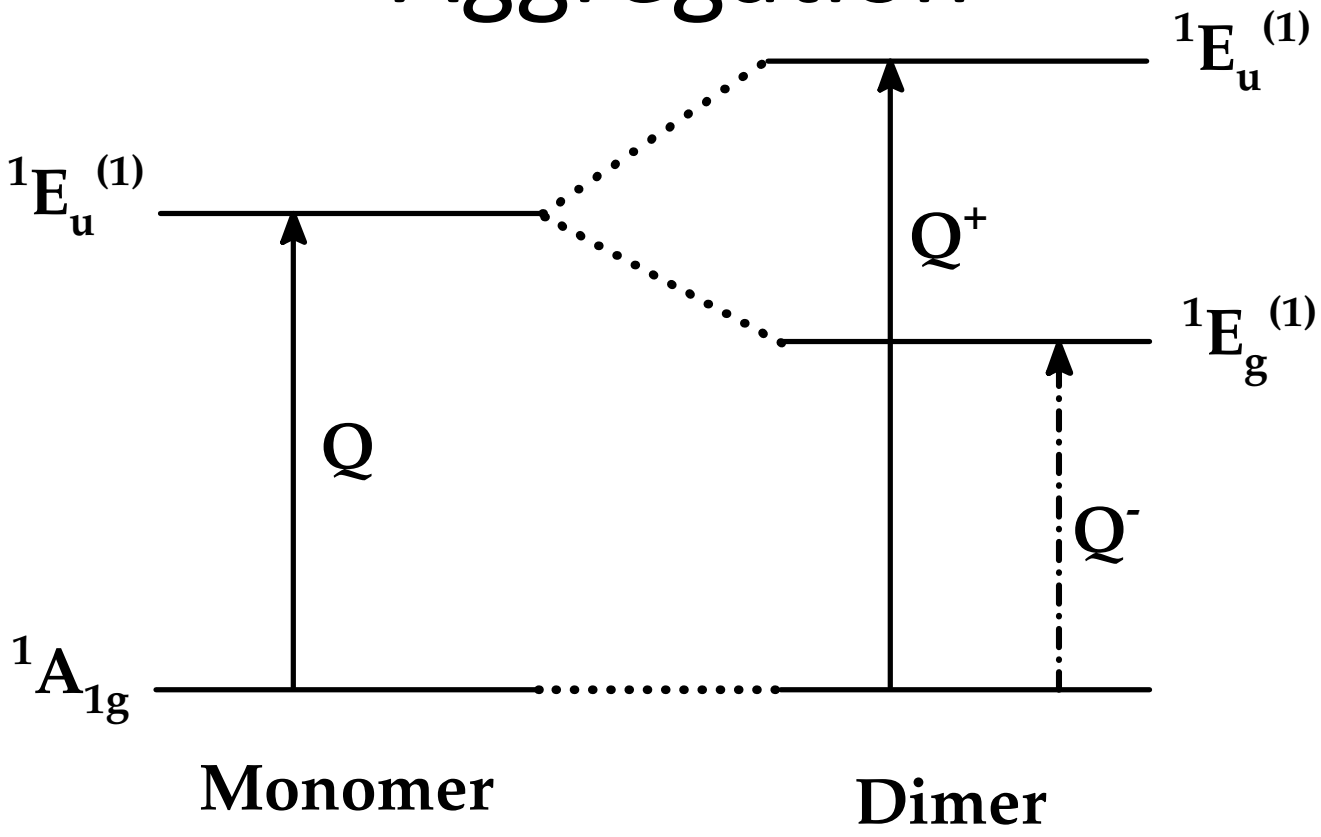


Expansion of the π electron system

The Q band splitting of H_2Pcs becomes smaller at longer wavelength.

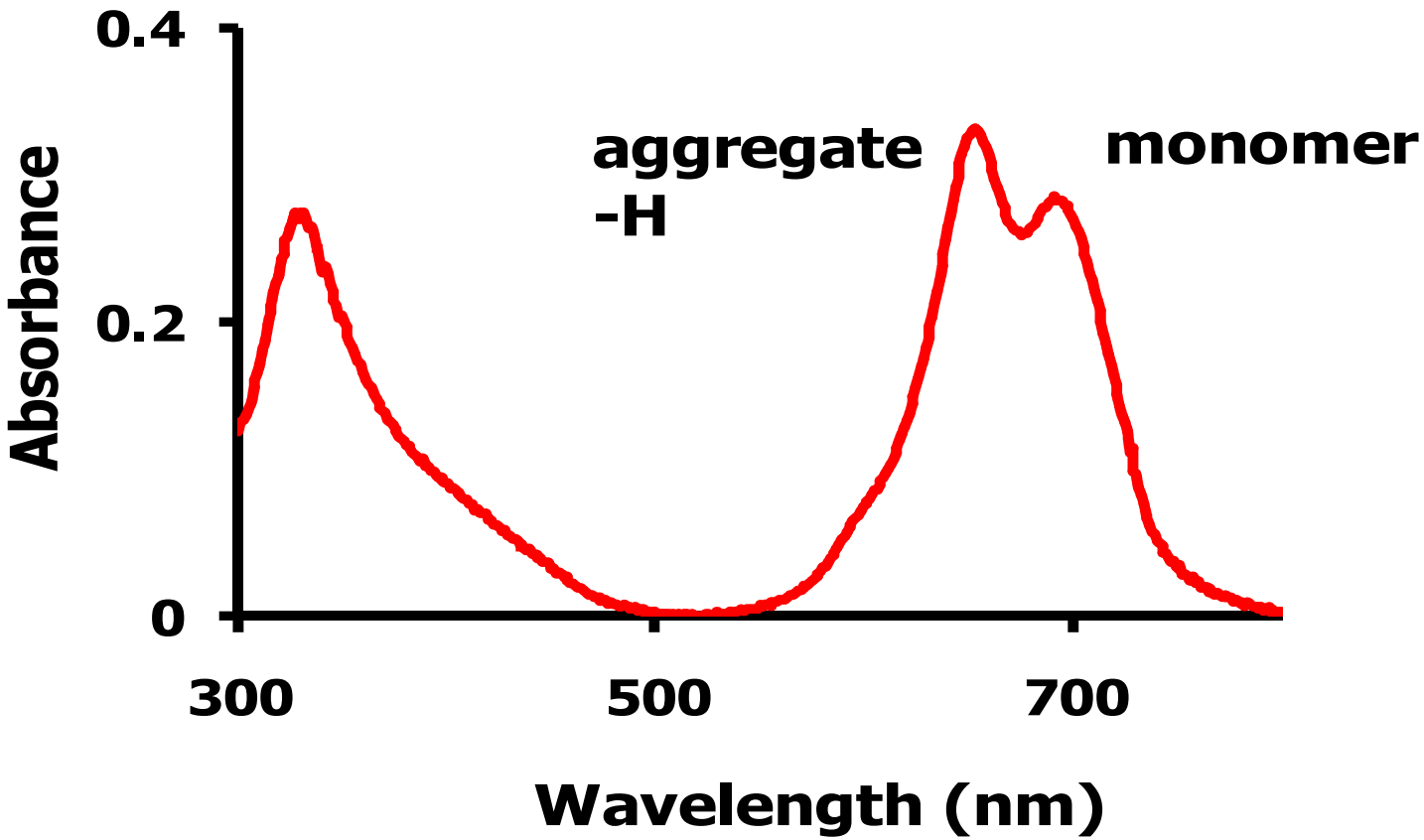


Aggregation

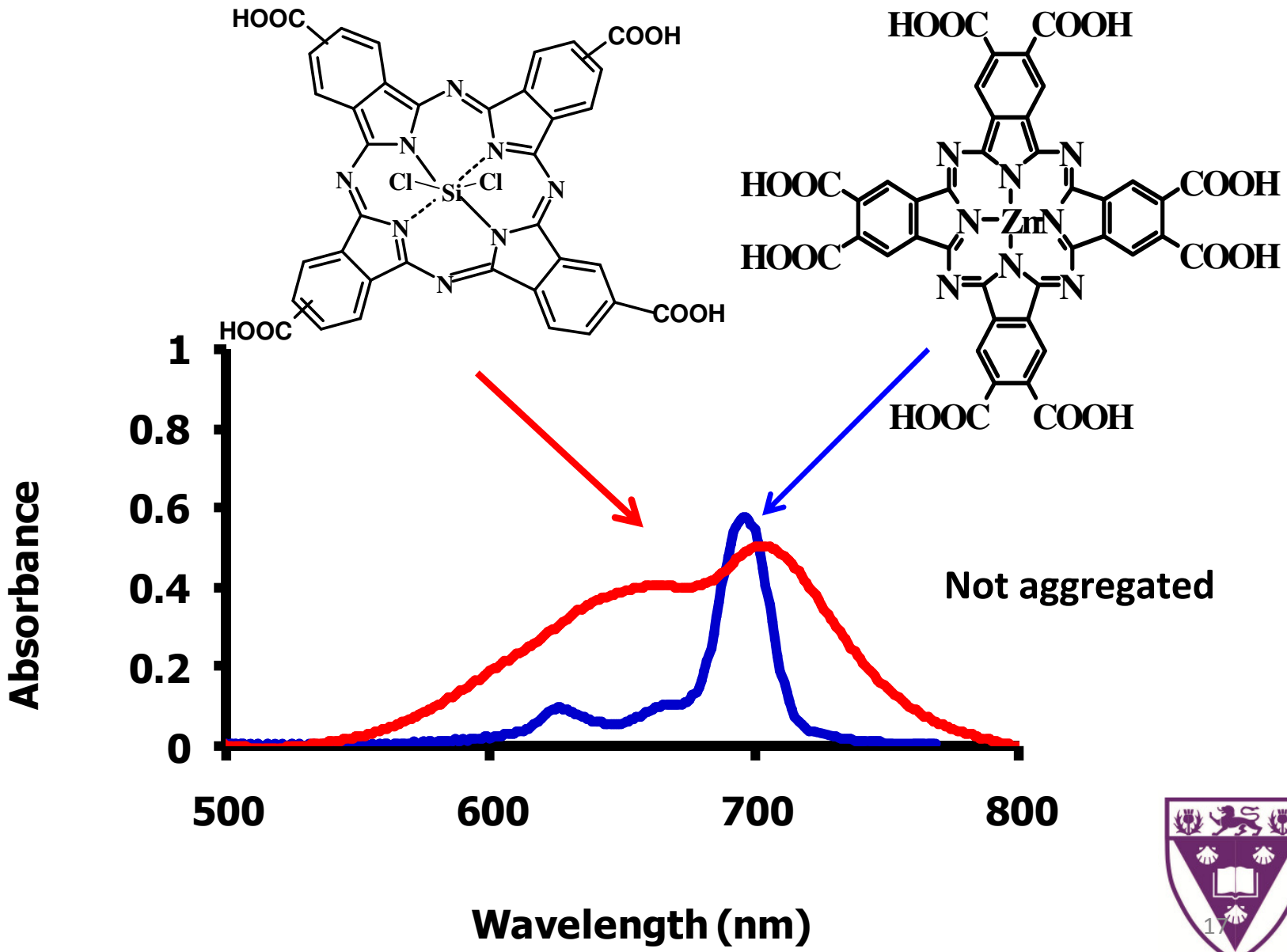


J aggregates – edge to edge: red shifted – NOT COMMON

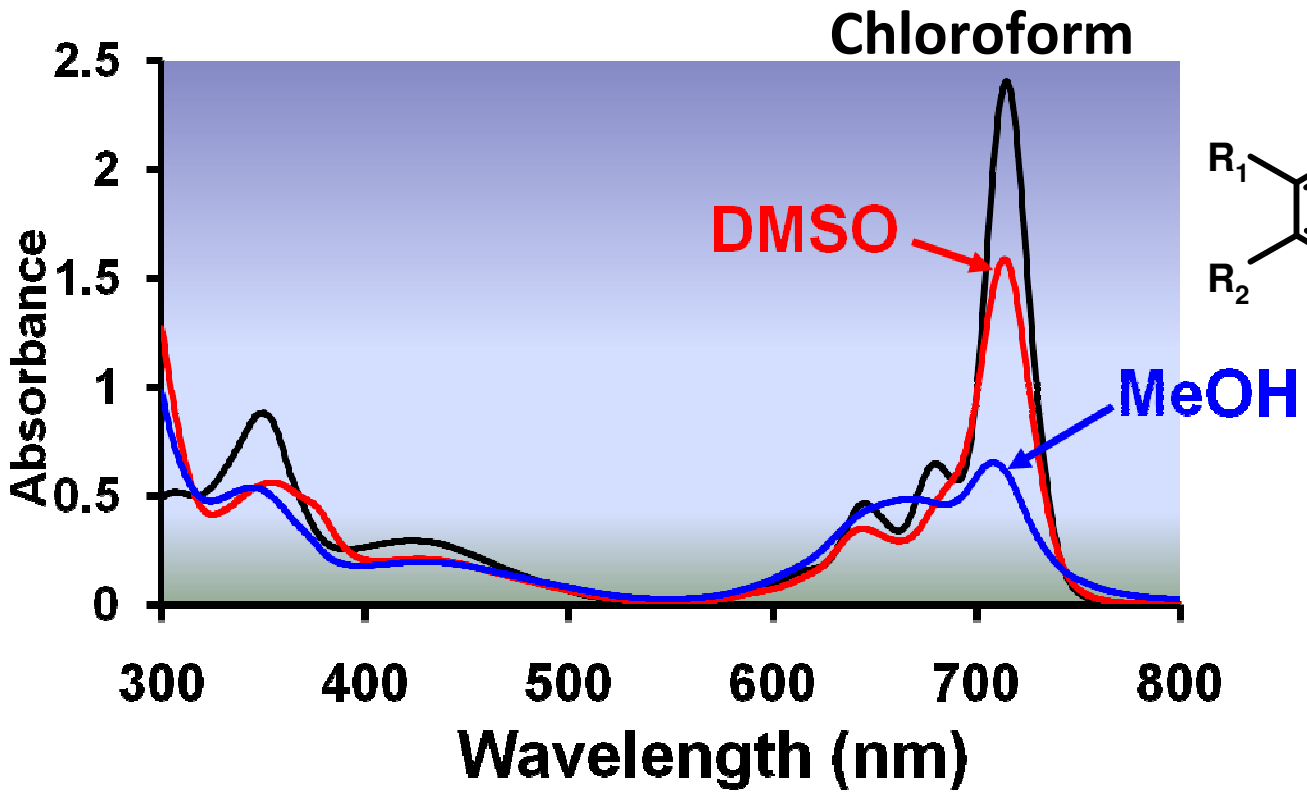
H aggregates – face to face: blue shifted - COMMON

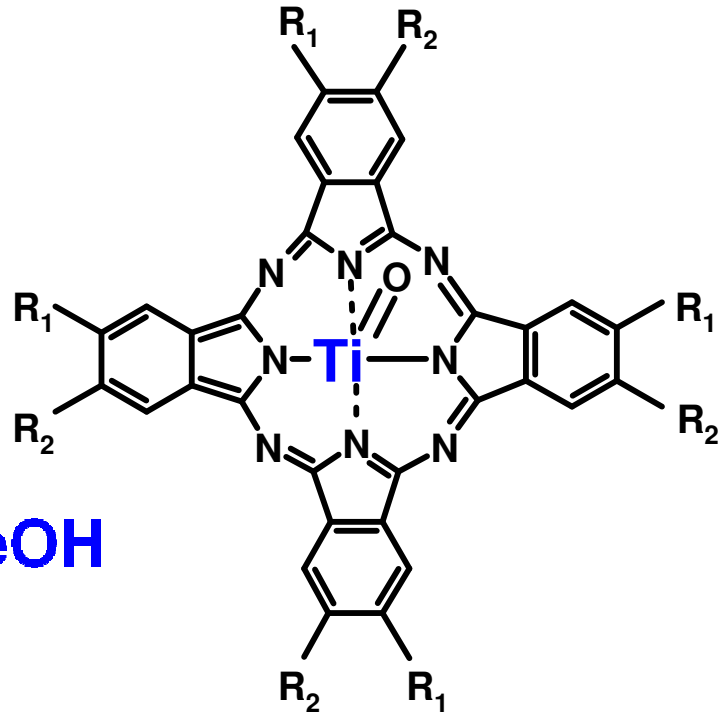

0.4
0.2
0
Absorbance
aggregate
-H
monomer
300
500
700
Wavelength (nm)

Plurality of ligands and aggregation



Effects of solvents on aggregation

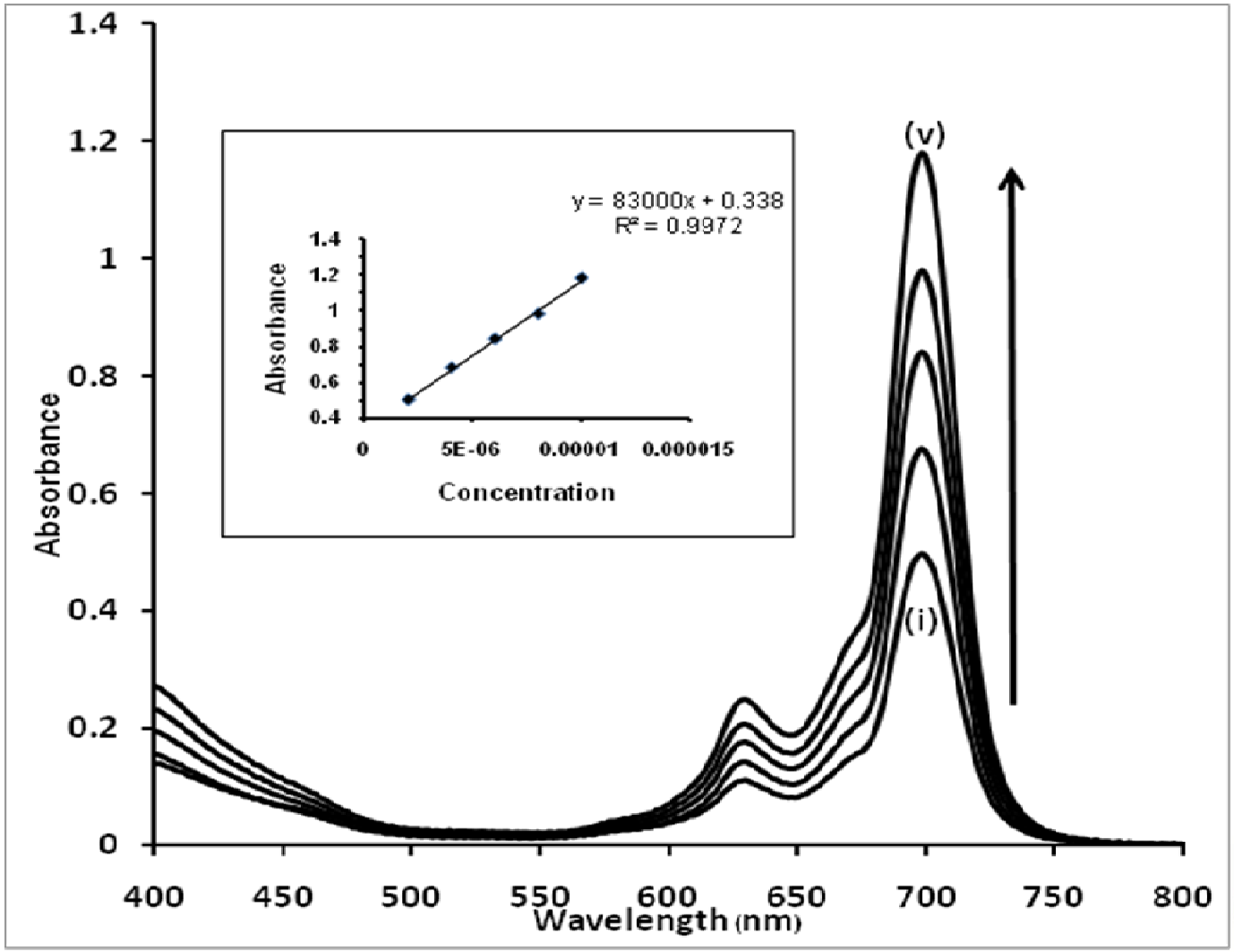




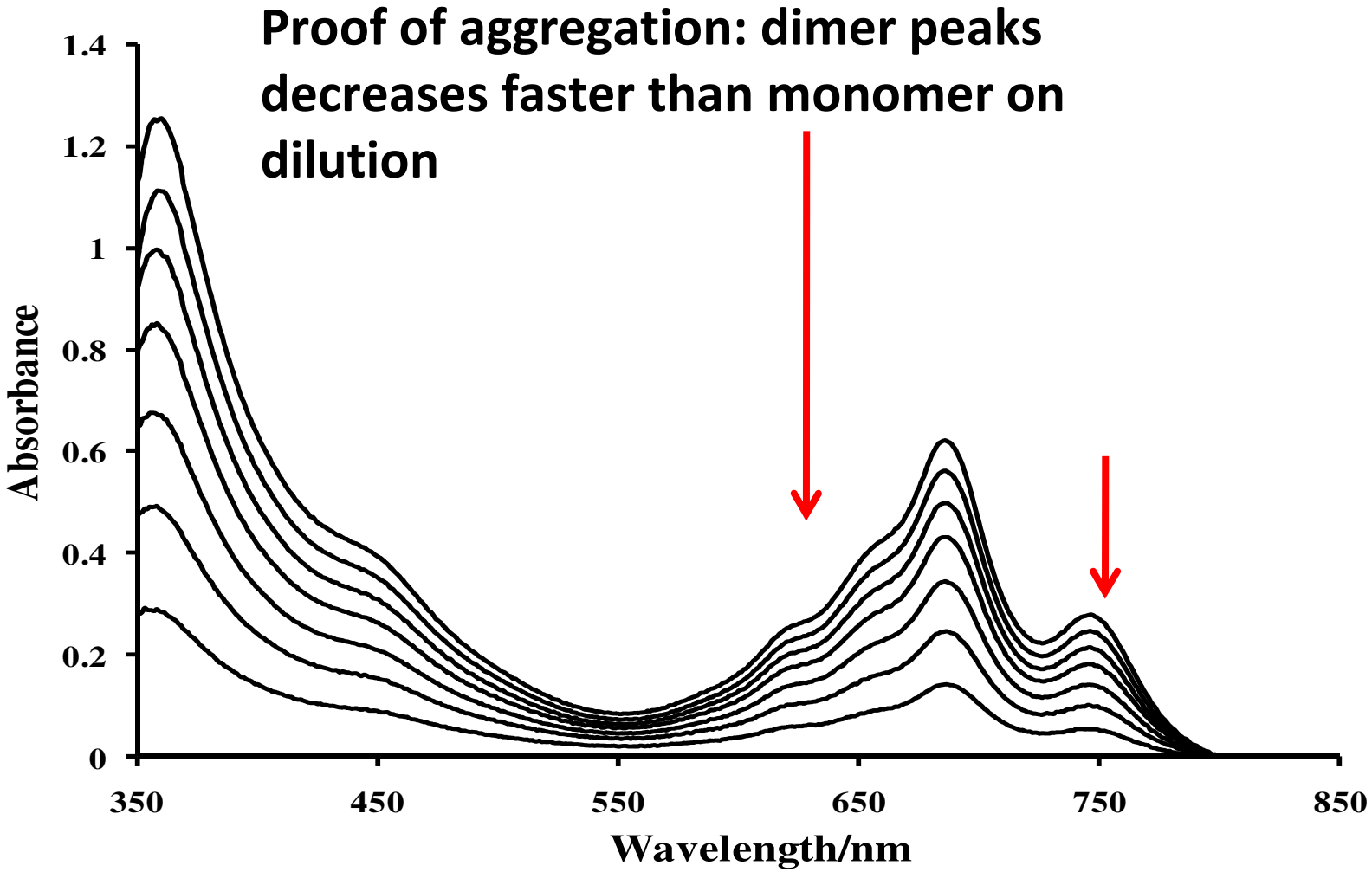
R_2 = $SCH(CH_2O(CH_2CH_2O)_2C_2H_5)_2$

Durmas, Ahsen, Nyokong, Dalton Trans. (2007)1235

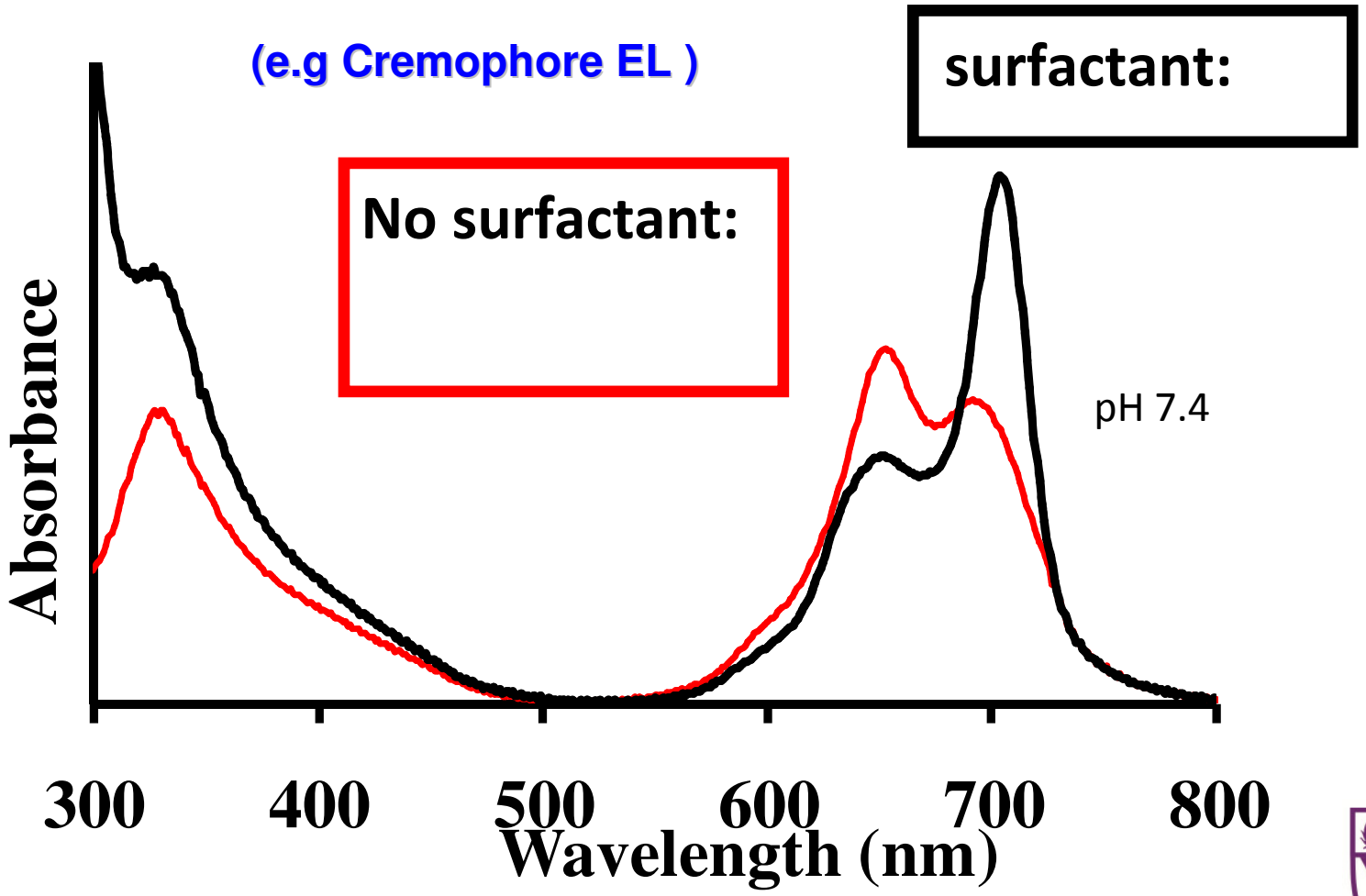
Proof of non-aggregation: Beer's law



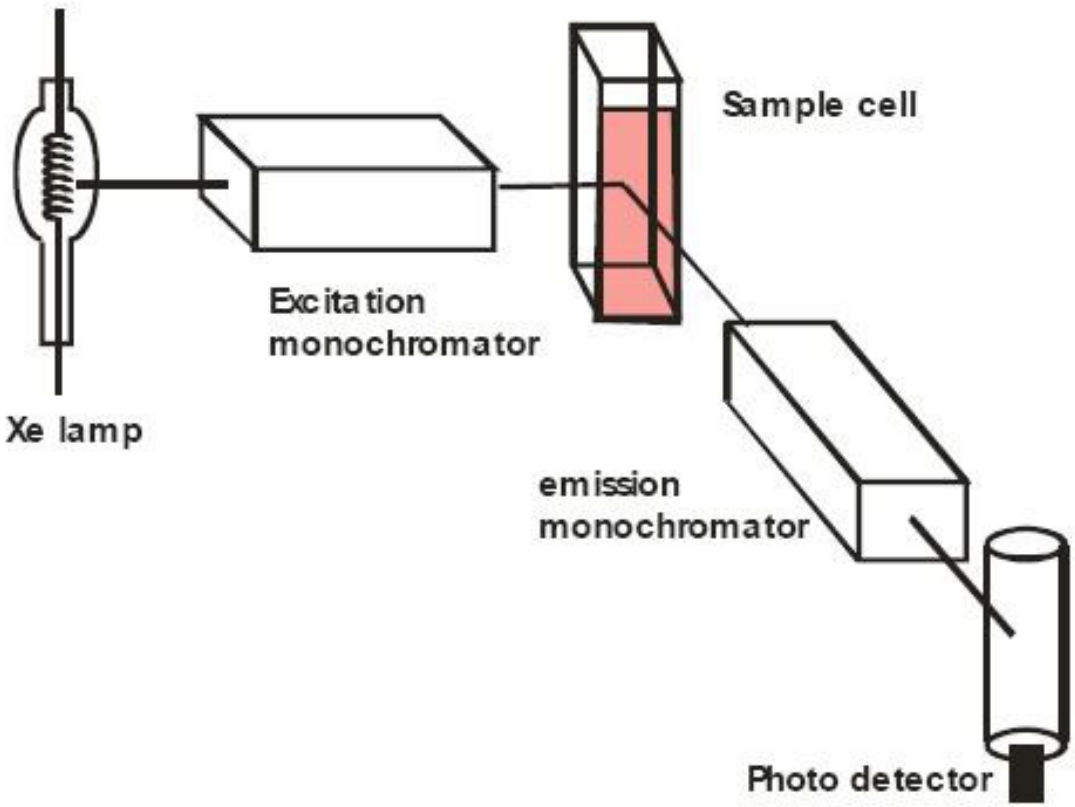
Proof of non-aggregation: Beer's law



Proof of aggregation: Effects of Surfactants



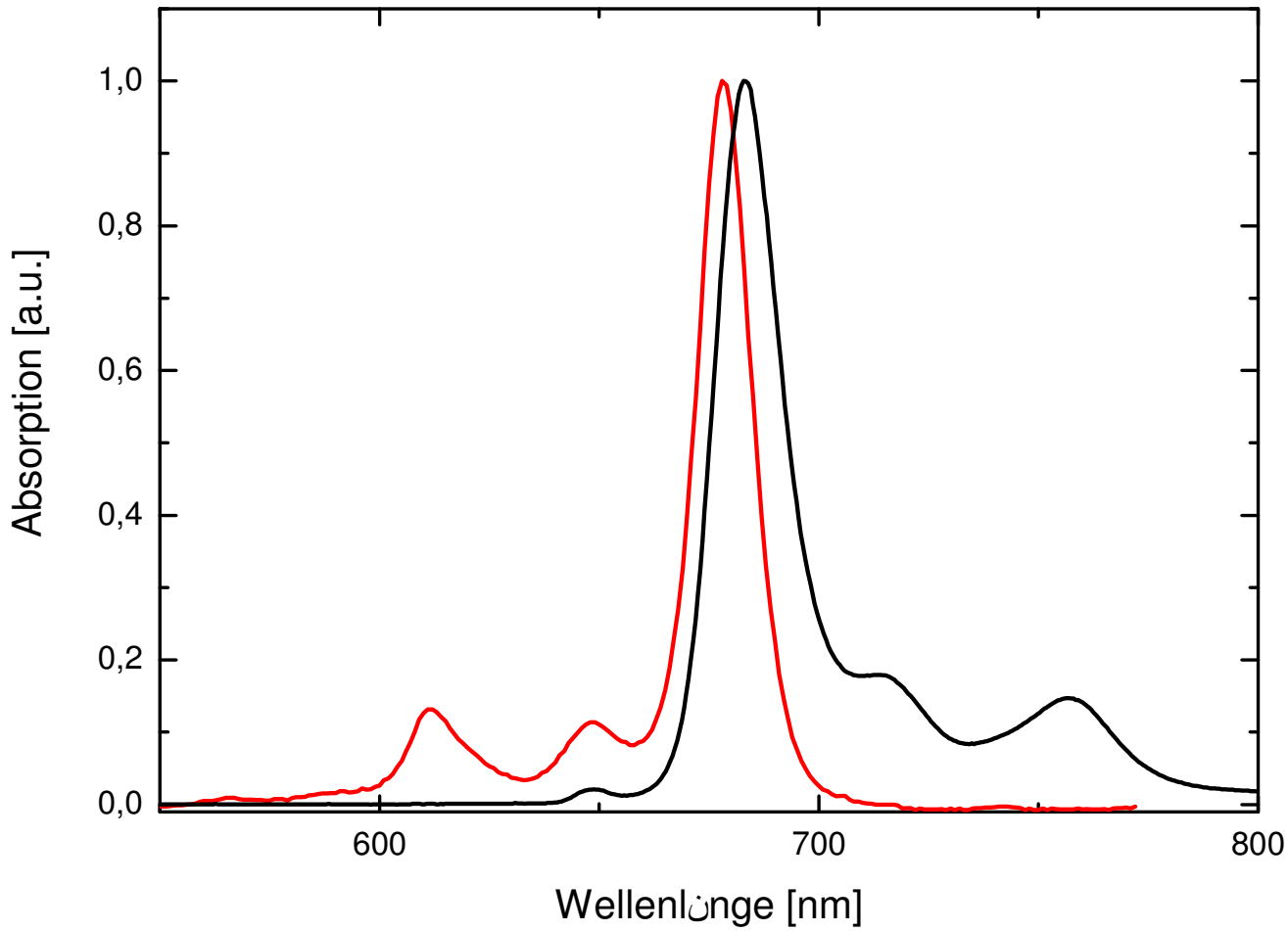
Steady State Fluorescence



Fluorescence spectrum is plot of fluorescence intensity vs. registration wavelength (frequency, energy) at one excitation wavelength.

Picture by Mizower

Steady State Fluorescence

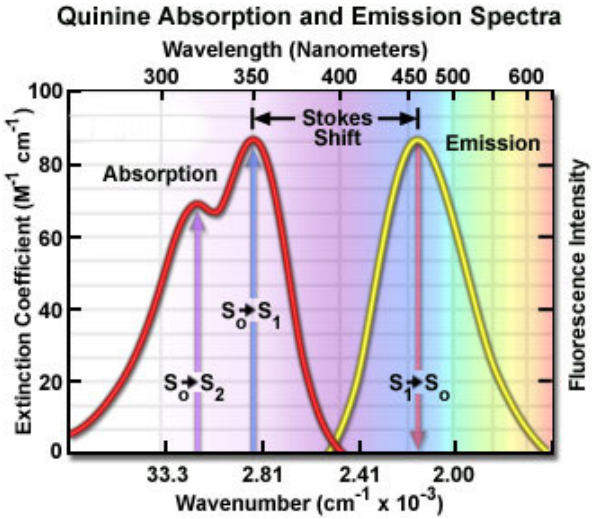


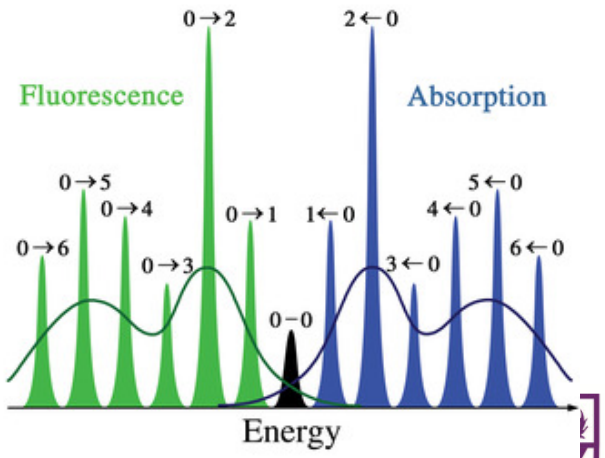
Fluorescence

1. **Stokes Law**: a maximum of fluorescence spectrum is red-shifted compared to a maximum of the corresponding absorption spectrum. (Reasons: Franck-Condon rule)
2. **Mirror Image Rule:** a fluorescence spectrum (plotted in energy scale) strongly resembles the mirror image of the absorption spectrum. (Reason: the vibrational energy level spacing is similar for the ground and excited states)
3. **Universal Relationship (betwenn Abs-Flu):**

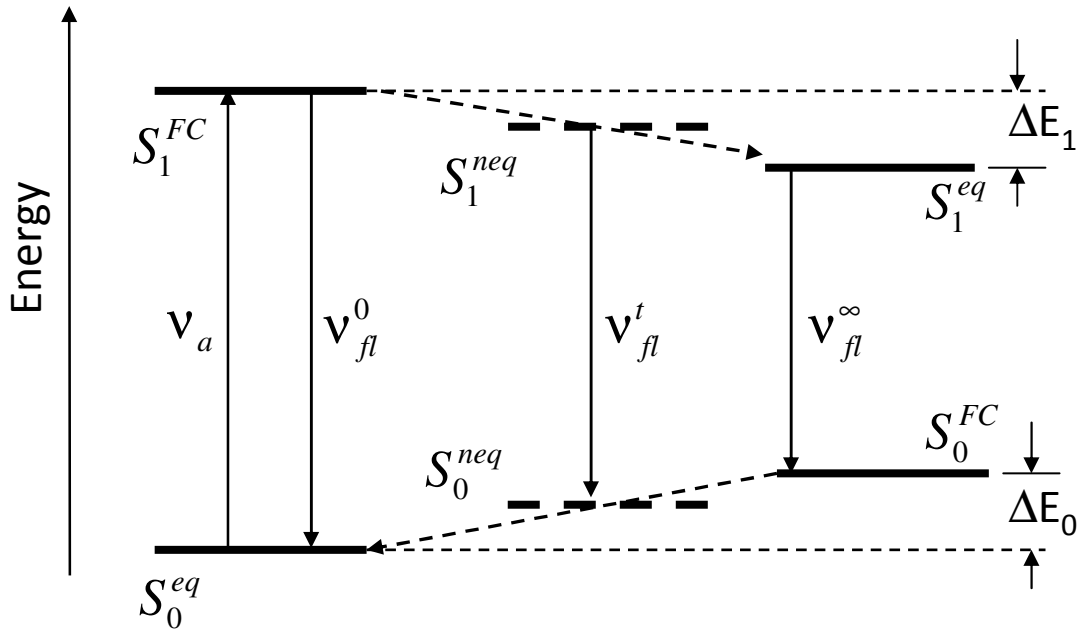
$$W_{fl}(\nu) = C(T)\cdot K(\nu)_{Abs}\cdot \exp(-h\nu/kT)$$

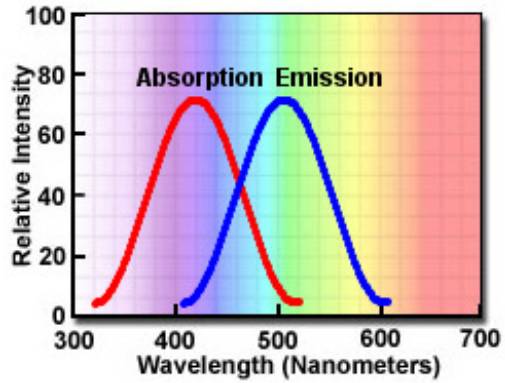
4. **Kasha-Vavilov Rule: the fluorescence spectrum shows very little dependence on the wavelength of the excitation.** (Reasons: the emission occurs exclusively from the lowest singlet excited electronic state)



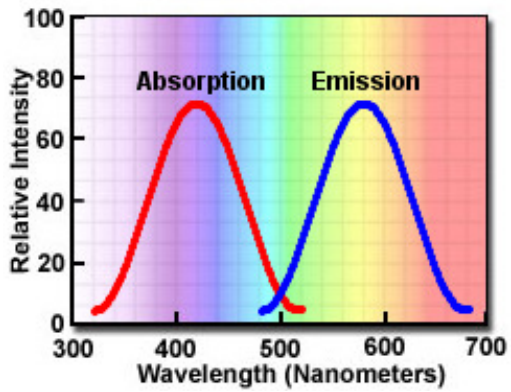


Fluorescence





Low solvent polarity



High solvent polarity

Dependency of the spectral position of a fluorescence band on time

$$\nu_{fl}^t = \nu_{fl}^\infty + \left(\nu_{fl}^0 - \nu_{fl}^\infty\right) \cdot e^{-\frac{t}{\tau_R}}$$

τ_R is solvent relaxation time

Quantum Yield

- yield of product relative to amount of photons absorbed
- sum off all quantum yields in a process is ≤ 1

$$\Phi = \frac{\text{number of product formed molecules}}{\text{number of photons absorbed}}$$

Examples:

Φ_R = Quantum yield of reaction

Φ_{fl} = Fluorescence quantum yield

Φ_{Ph} = Phosphorescence quantum yield

Φ_{ISC} = Intersystem crossing quantum yield

Φ_{IC} = Internal conversion quantum yield

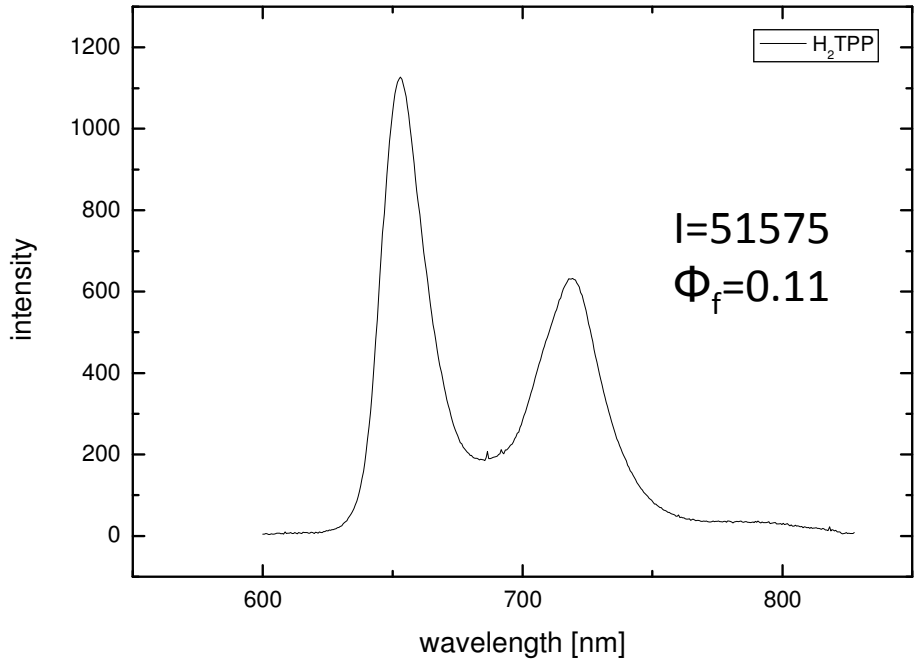
Φ_{Δ} = Singlet oxygen quantum yield

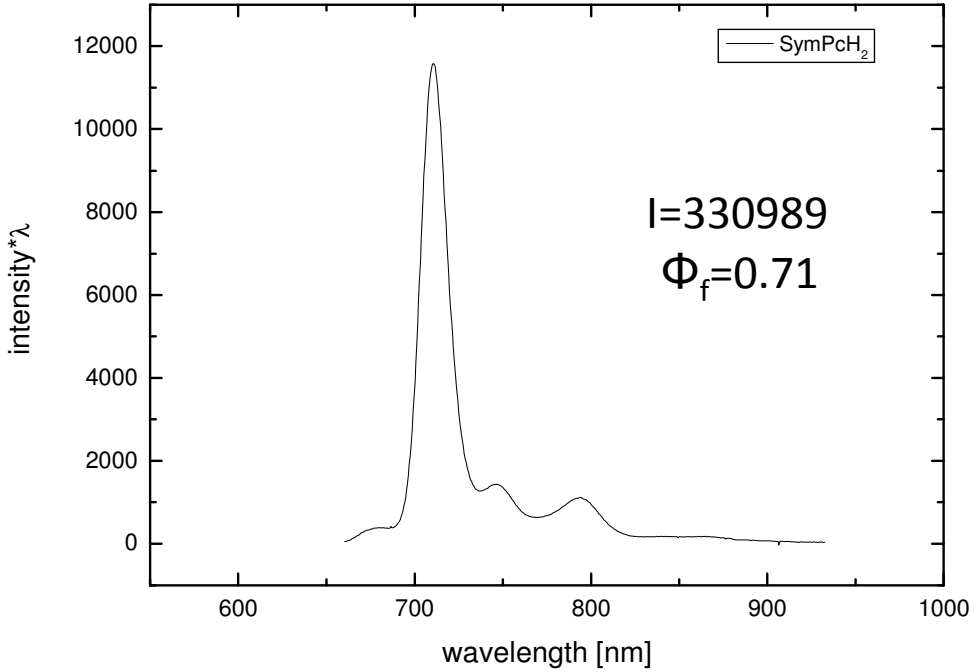
Fluorescence Quantum Yield Φ_f

- absolute measurements: spectrometer with Integrating sphere (Ulbricht Sphere)
- comparative measurements: optical spectrometer

$$\Phi_{fl} = \frac{\text{number of fluorescence photons}}{\text{number of photons absorbed}}$$

Example: comparative measurement





$$\Phi_{sample} = \Phi_{std} \frac{I_{Sample} OD_{std} n_{sample}^2}{I_{std} OD_{sample} n_{std}^2}$$

Polarization of Fluorescence

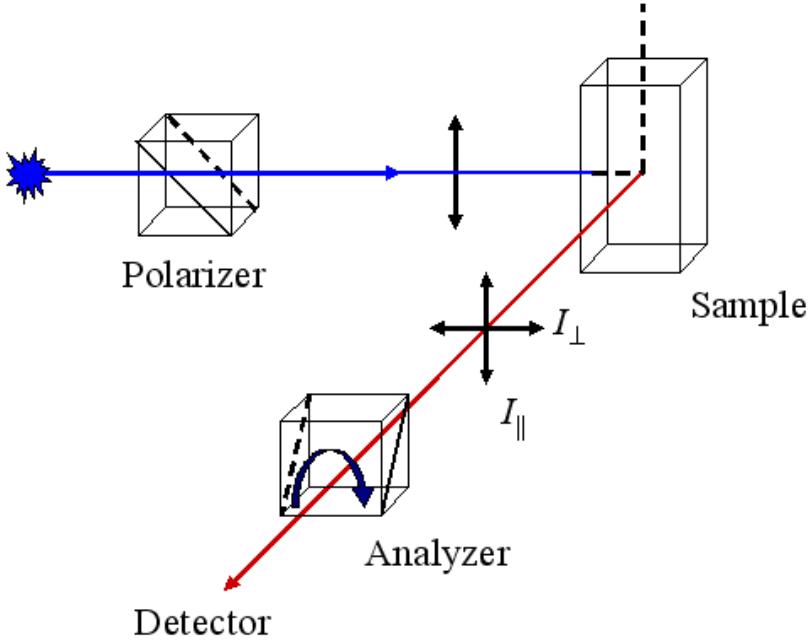
Polarization

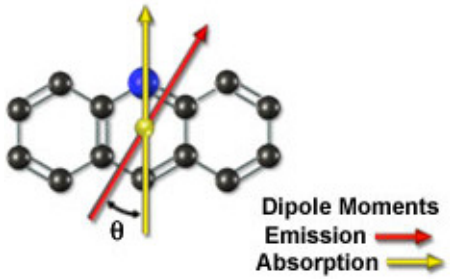
$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Anisotropy

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2 \cdot I_{\perp}}$$

$$r = \frac{2P}{3 - P}; \quad P = \frac{3r}{2 + r}$$

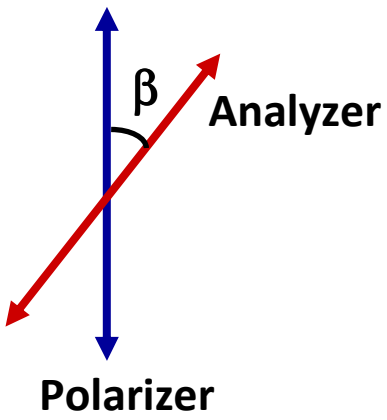




Perrin expression

$$P_0 = \frac{3\cos^2\theta - 1}{3 + \cos^2\theta}; \quad r_0 = \frac{3\cos^2\theta - 1}{5}$$

$$-\frac{1}{3} \le P_0 \le \frac{1}{2}; \quad -0.2 \le r_0 \le 0.4$$



$$r = r_0 \frac{3\cos^2\beta - 1}{5}$$

At β = 54.7° (Magic Angle)

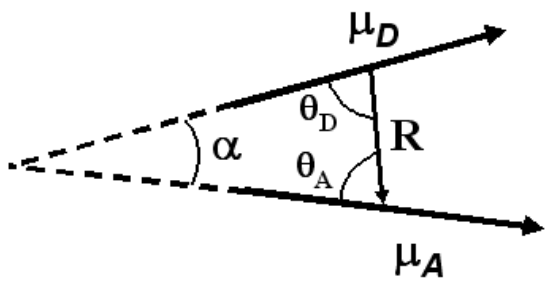
***r* = 0**

Lakowicz, J. *Principles of fluorescence spectroscopy;* Plenum Press: New York, 1999.

Foerster Resonace Energy Tranfer

• **Dipole-dipole** resonance interaction between photoexcited donor molecule (**D**) and acceptor molecule (**A**) (in the most cases A is in the ground state);

Energy of dipole-dipole interaction between donor and acceptor molecules

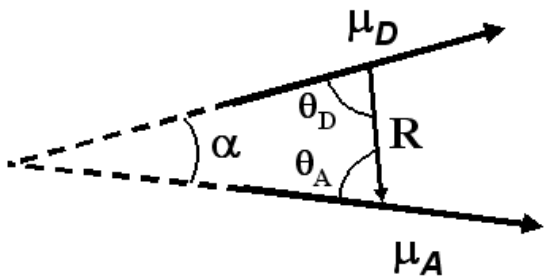


$$M_{dd} = \frac{1}{R^3}\left\{(\mu_D \mu_A) - \frac{3}{R^2}(\mu_D \mathbf{R})(\mu_A \mathbf{R})\right\}$$

Foerster Resonace Energy Tranfer

Rate of dipole-dipole EET

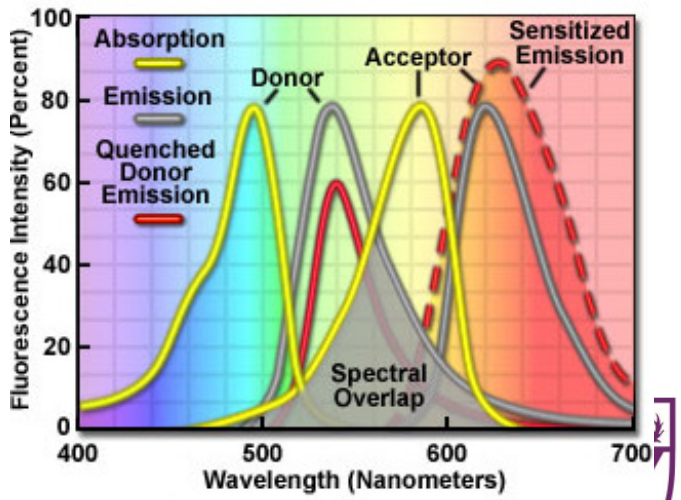
$$k_{DA}^{dd} = \frac{9000 \ln 10 \cdot \chi^2 \Phi_0^D}{128\pi^5 n^4 N_a \tau_0^D R^6} \int I_D^n(\tilde{\nu}) \varepsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4},$$



$$\int I_D^n(\tilde{\nu}) d\tilde{\nu} = 1 \qquad \chi^2 = (\cos\alpha - 3\cos\theta_D \cos\theta_A)^2$$

High probability of EET if:

- Overlap of the fluorescence spectrum of **D** and the absorption spectrum of **A**;
- High extinction coefficient of **A** molecule;
- Short distance between **D** and **A** molecules;
- Right orientation of transition dipole moments **($\chi^2 \neq 0$)**
- High fluorescence quantum yield of **D**.



Foerster Resonace Energy Tranfer

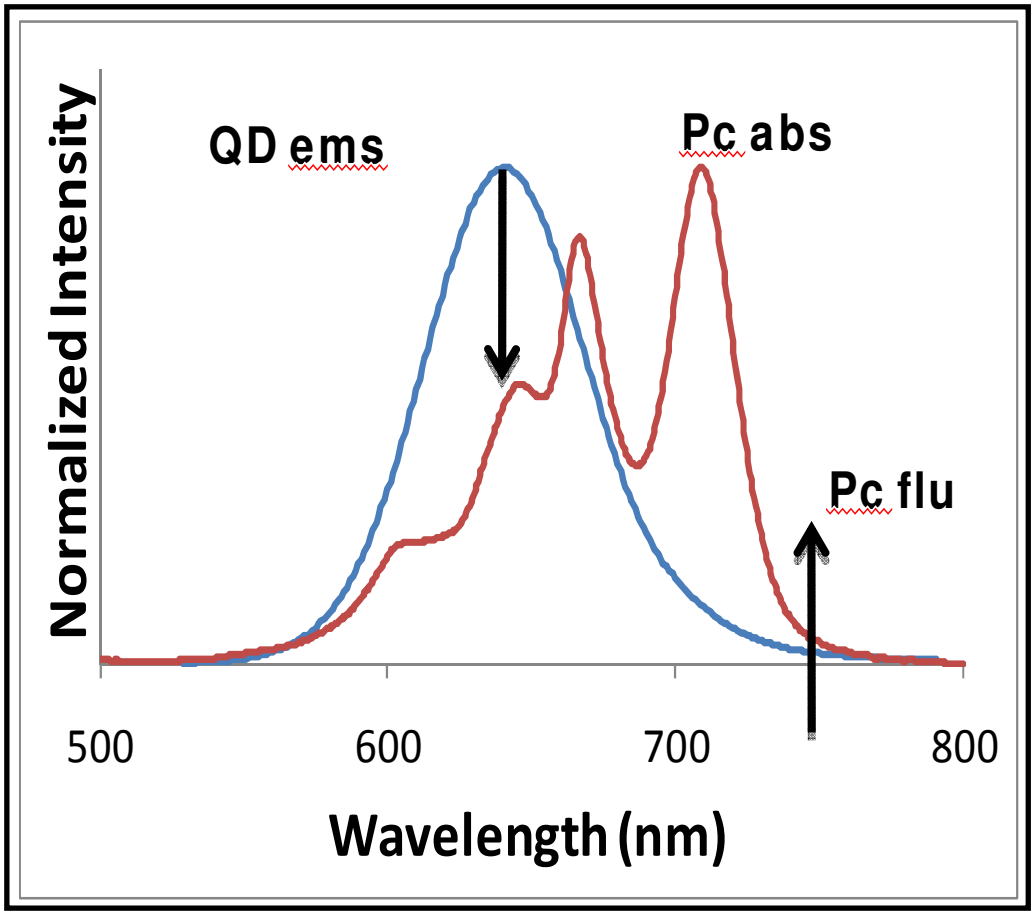
Rate of dipole-dipole EET

$$k_{DA} = \frac{1}{\tau_0^D}\left(\frac{R_0}{R}\right)^6,$$

$$R_0^6 = \frac{9000\ln 10 \cdot \chi^2 \Phi_0^D}{128\pi^5 n^4 N_a}\int I_D^n(\tilde{\nu})\varepsilon_A(\tilde{\nu})\frac{d\tilde{\nu}}{\tilde{\nu}^4},$$

If $R = R_0$, then the fluorescence of the D is quenched by a factor 2:

Factors influencing FRET Efficiency



Good spectral overlap

$$J = \int f_{QD}(\lambda)\varepsilon_{Pc}(\lambda)\lambda^4 \partial\lambda$$

Förster radius

$$R_0^6 = 8.8\times10^{23}\,\kappa^2 n^{-4}\Phi_F^D J$$

FRET efficiency

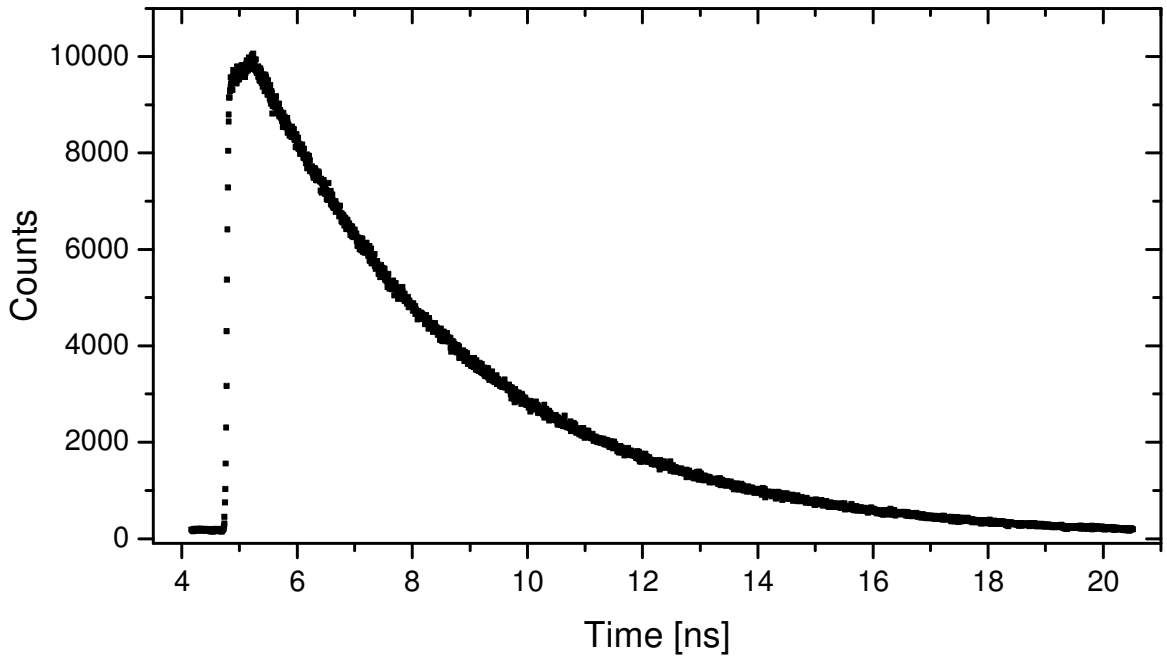
$$Eff_{ss} = 1 - \frac{\Phi_F^{DA}}{\Phi_F^D}$$

$$Eff = \frac{R_0^6}{R_0^6 + r^6}$$

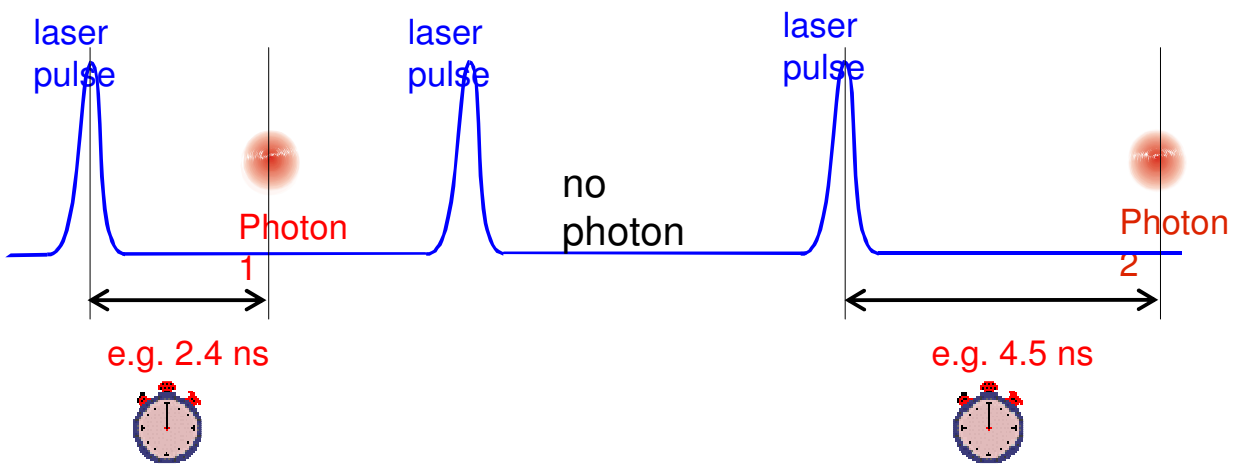
$$Eff_{tr} = 1 - \frac{\tau_F^{DA}}{\tau_F^D} \qquad \tau_i = \Sigma_i \alpha_i \tau_i$$

TCSPC

- Determination of fluorescence lifetime
- Time resolution: 60 ps up to 1 µs
- Measurements with low fluorescence quantum yield possible (<1%)



TCSPC Principle



Photon emission is a stochastic process !

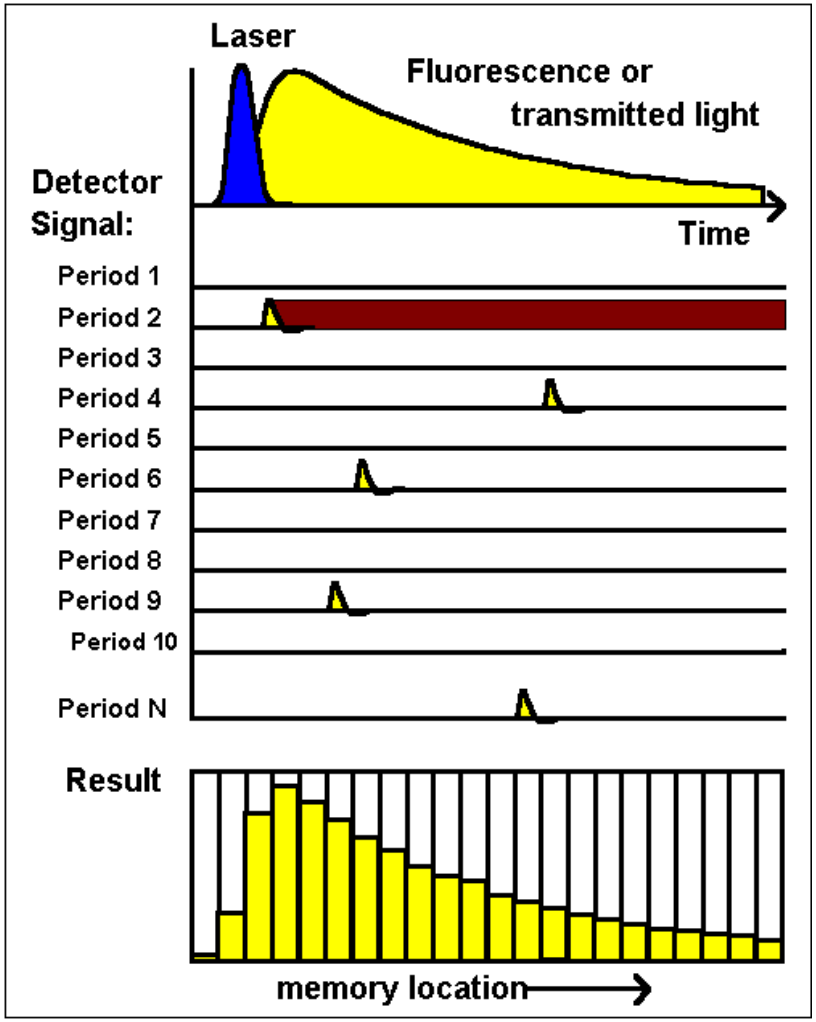
⇒ repeat the dwell time measurement many times,
"count how many photons arrived after what time",
i.e. build a histogram

⇒ time axis is not continuous, but divided into "time bins"

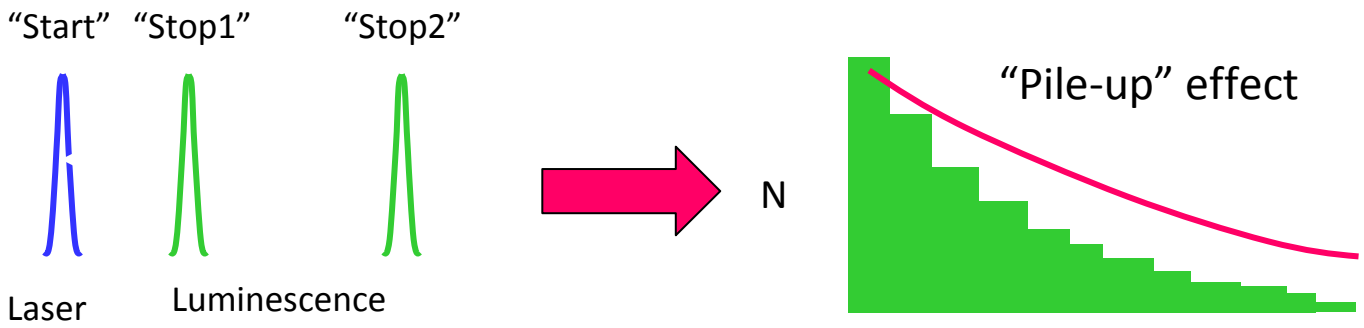
⇒ Note: not more than one photon per laser pulse can be registered !

TCSPC principle

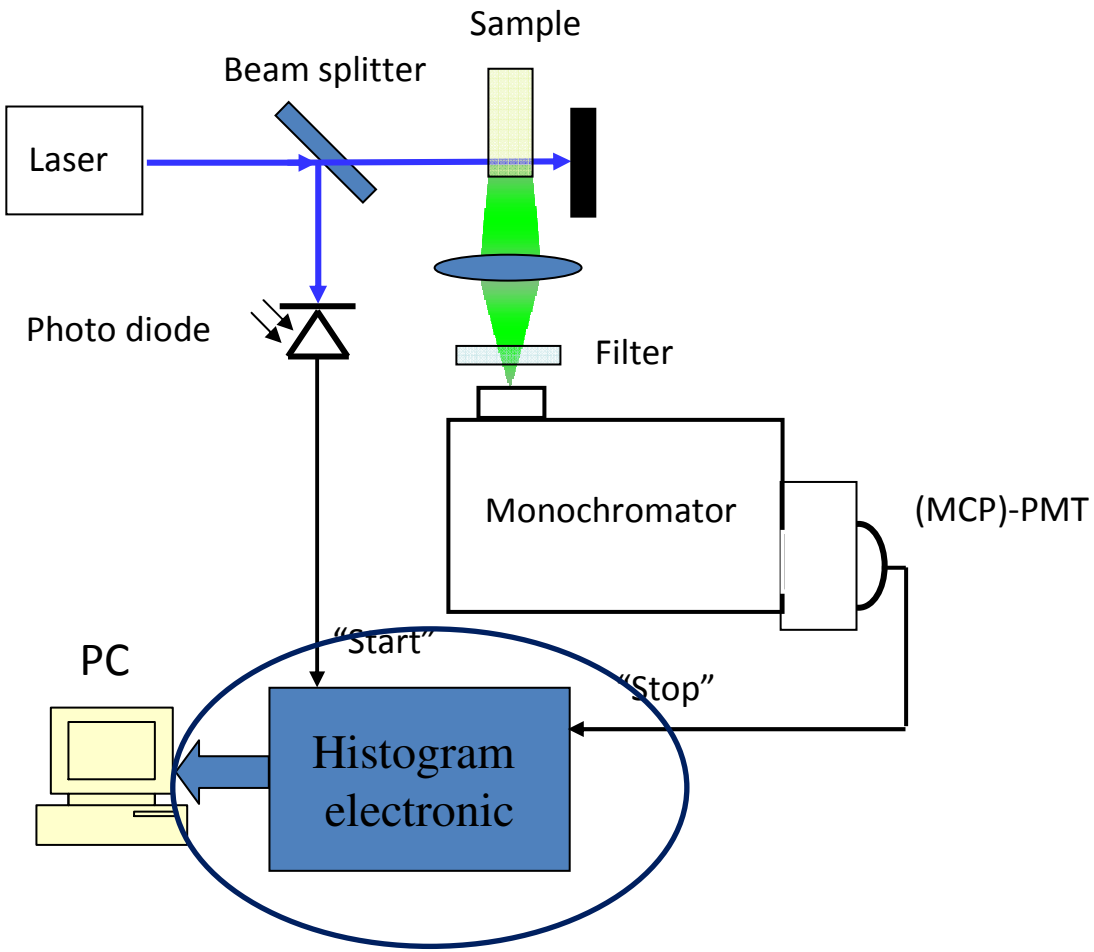
- detection of single photons caused by a *periodic* light signal
- light intensity is so low that probability to dectect one photon in one period is very low
- thus periods with more than one photon are very rare
- the time difference between laser pulse and every detected photon will be measured
- with many pulses (millions) one gets a distribution of time differences which correspond to the fluorescence lifetime
- time resolution up 20ps with a MCP-PMT and 150 ps with a PMT



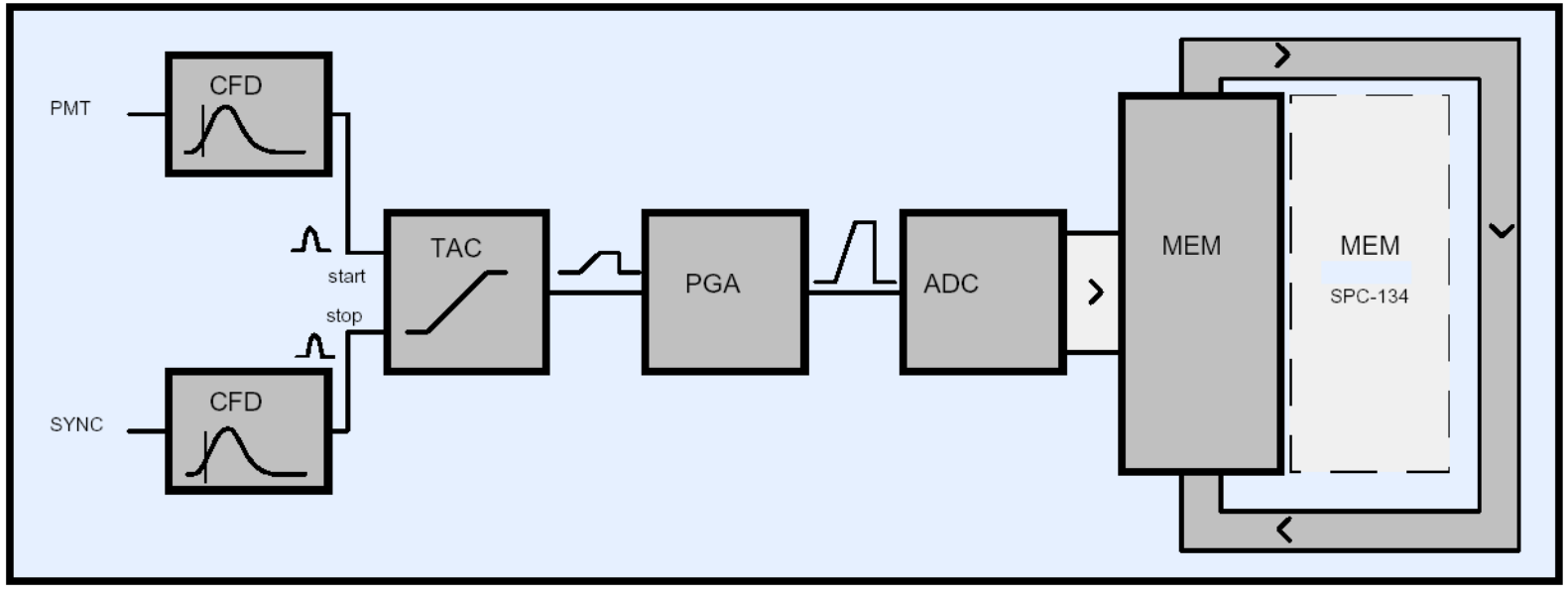
TCSPC principle



TCSPC setup

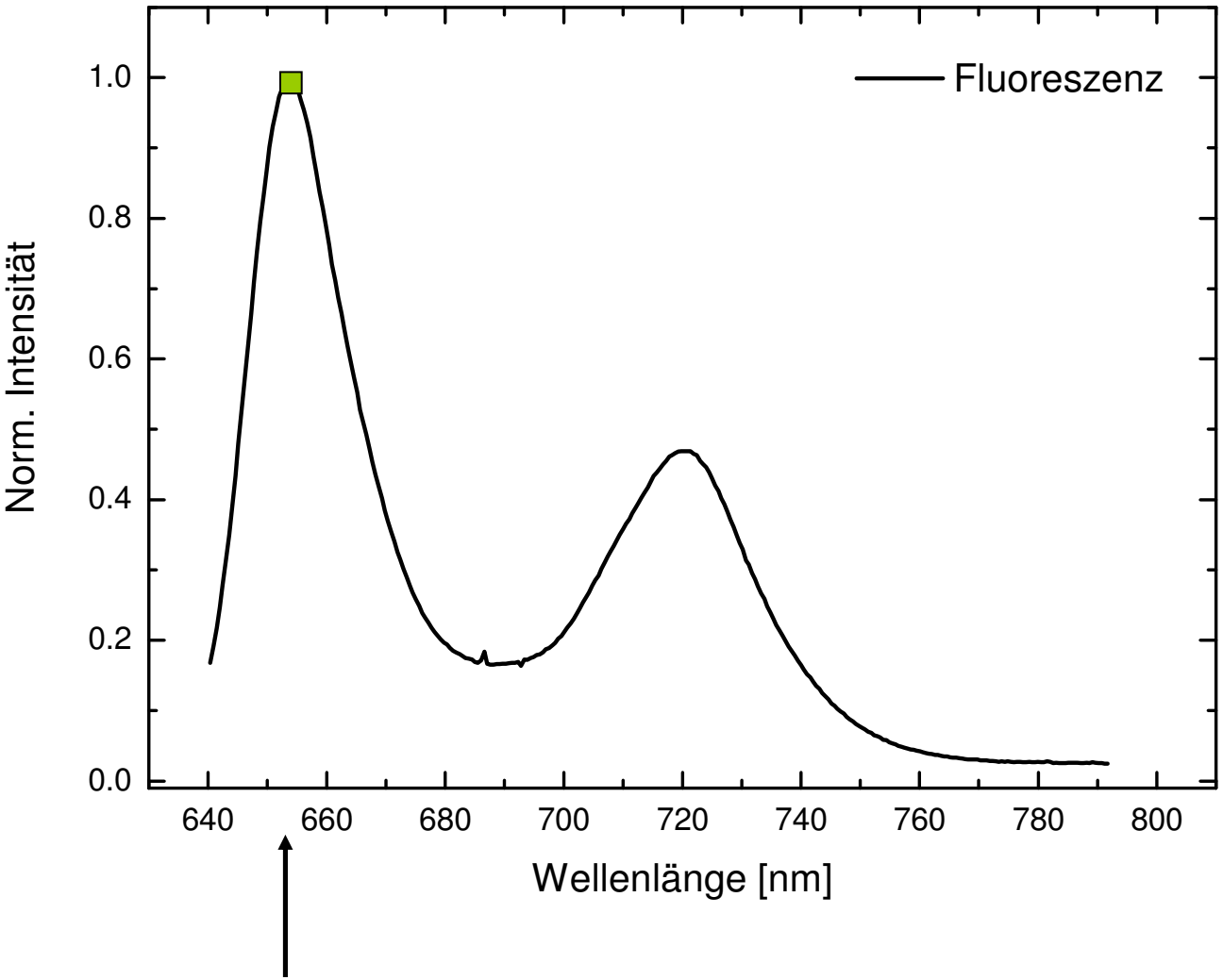

Sample
Beam splitter
Laser
Photo diode
Filter
Monochromator
(MCP)-PMT
PC
"Start"
"Stop"
Histogram electronic

TCSPC schematic diagram

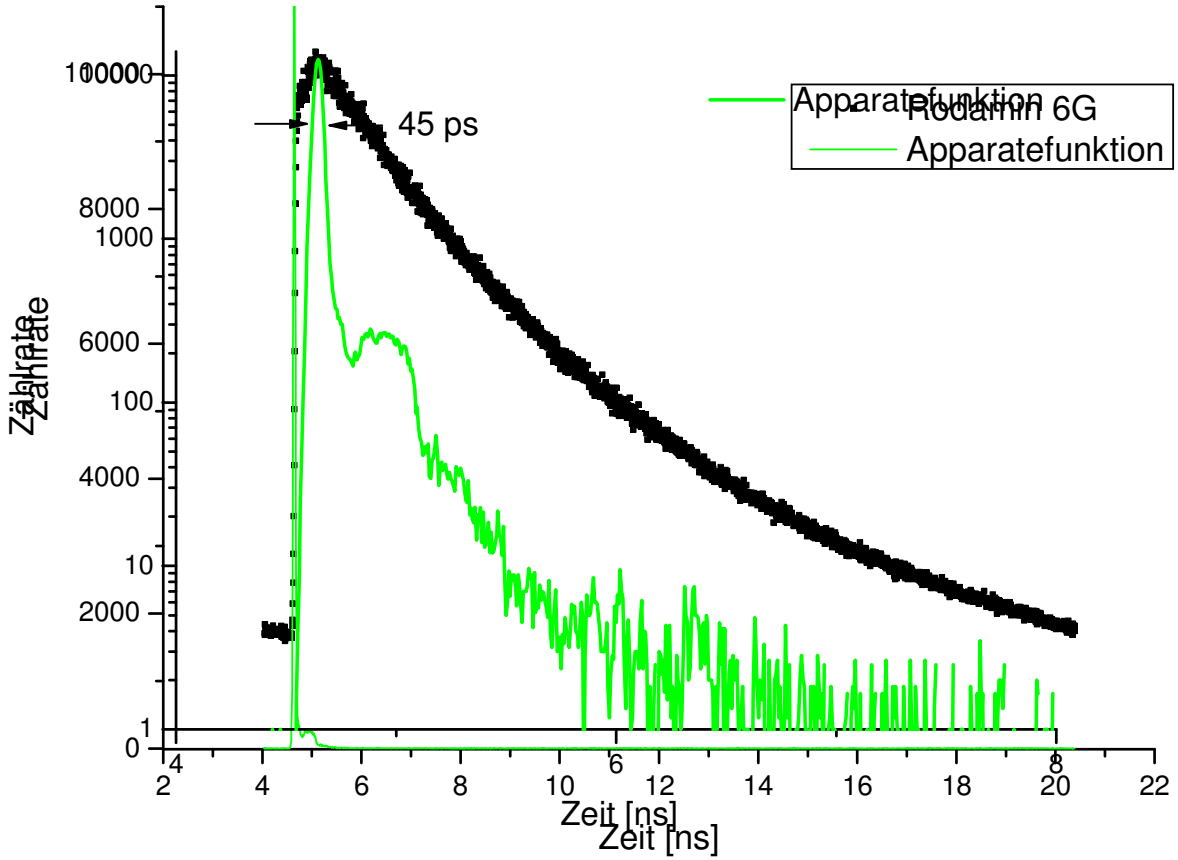


- PMT: pulses from photomultiplier
- SYNC: synchron pulses
- CFD: Constant-Fraction Discriminator
  measures the exact time of detection
- TAC: Time-to-Amplitude Converter
  condensator which loads up in the time between SYNC signal and PMT signal
- PGA: Programmable Gain Amplifier
  amplifies the TAC- output voltage with tunable factor
- ADC: Analog-to-Digital-Converter
  convert voltage to a number between 0 (fastest photons) and e.g. 4096 (latest)
- MEM: Memory
  has in our case 4096 counter, which will be increased by 1 if a photon is detected in the certain time period

Data Analysis



Data Analysis



Data Analysis

Data:

$$d(t_i)$$

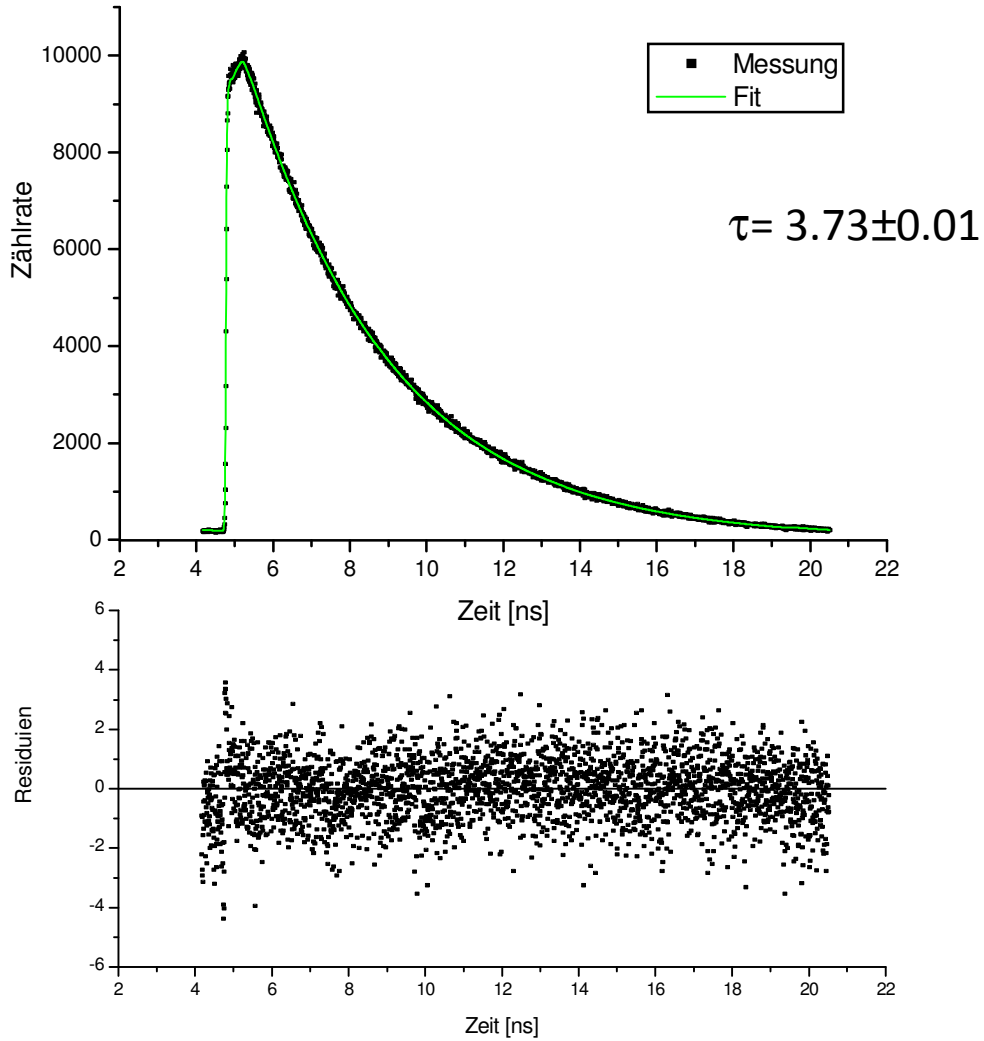
Model function:

$$f(p,t) = \left(\sum_i a_i \exp\left(\frac{t}{\tau_i} \right) \right) \otimes IRF(t - z)$$

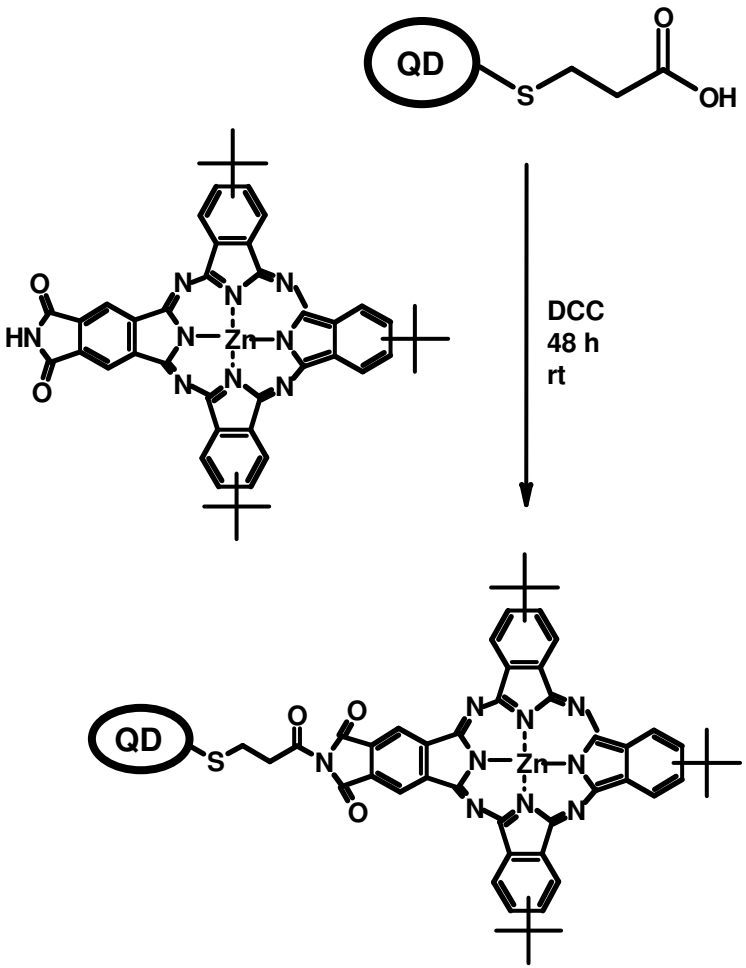
χ^2-value:

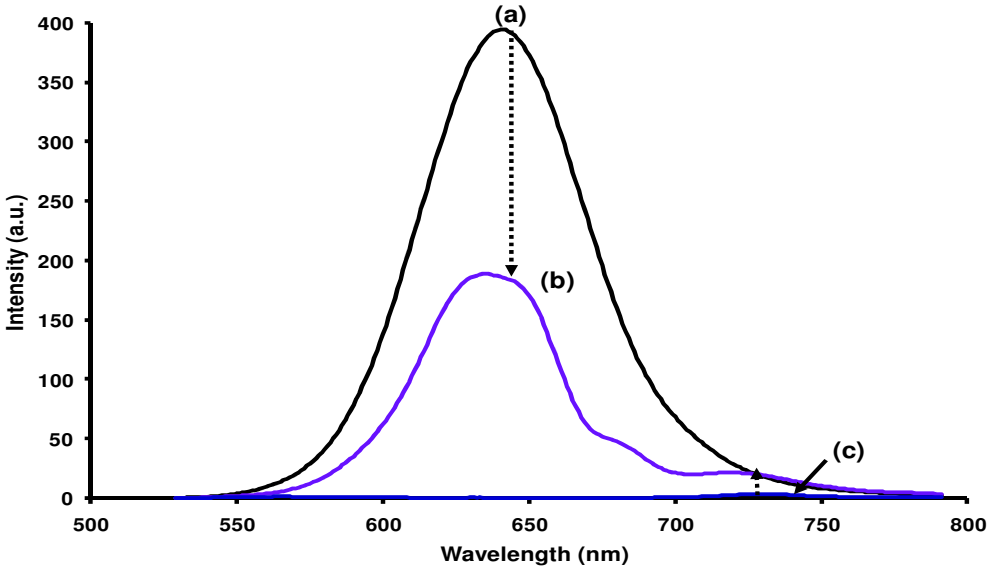
$$\chi^2 = \sum^N \left(\frac{f(p,t_i) - d(t_i)}{\sigma(t_i)} \right)^2$$

Data Analysis



Example





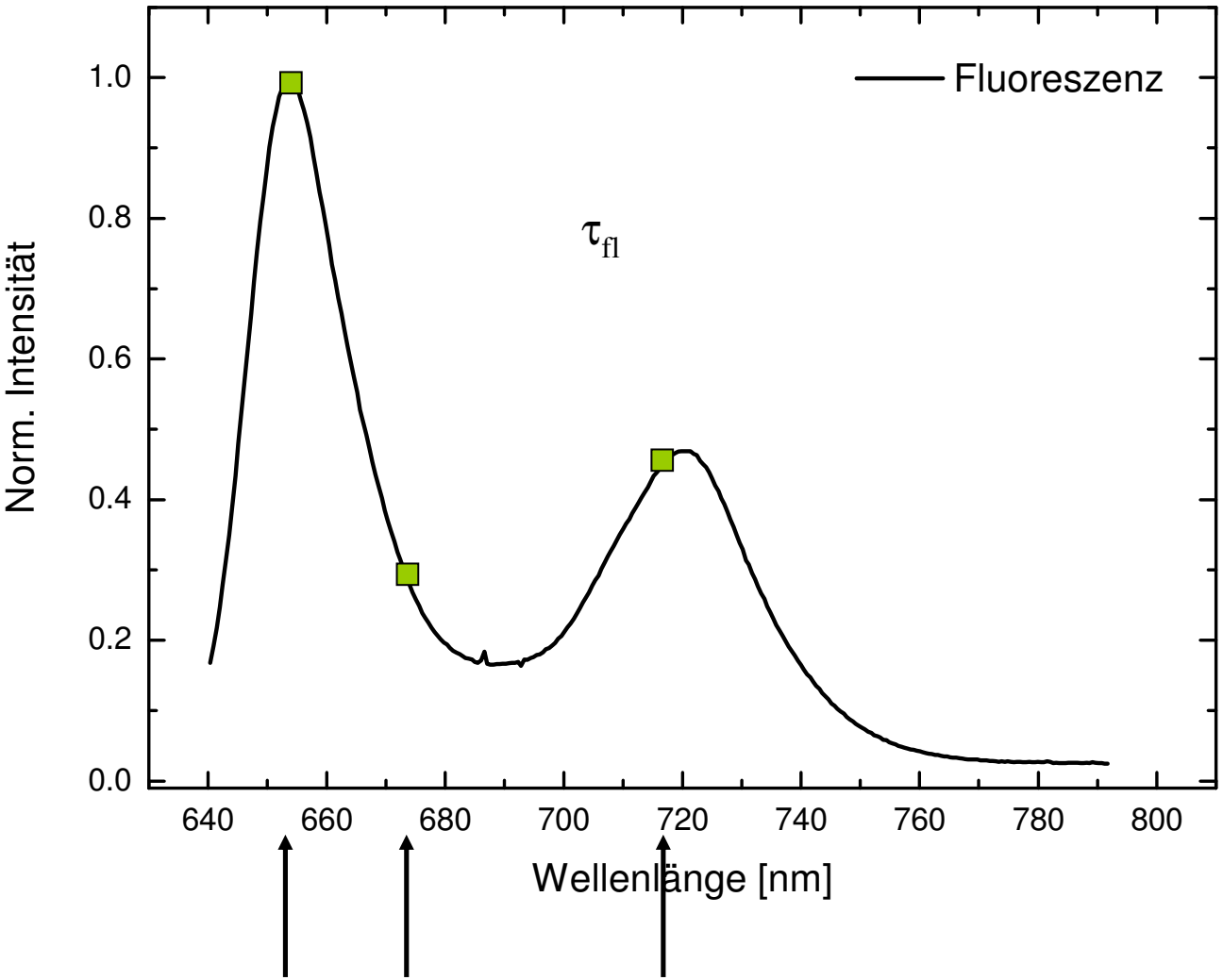
Compound	Relative A_1	τ_{F-1} (ns) (± 0.5)	Relative A_2	τ_{F-2} (ns) (± 0.3)
CdTe MPA QD	0.57 (0.61[a])	26.4 (26.3 [a])	0.43 (0.39 [a])	3.4 (4.3 [a])
QD-ZnttbIPc-linked	0.21	9.6	0.79	1.7

Decay Associated Fluorescence Spectroscopy (DAFS)

- Fluoresence lifetime of a homogenius species is independant from the detection wavelength
- Measurement of time resolved fluroescence at different detection wavelengths
- Data analysis at different detection wavelength is given by the model
- Global fit over all data sets

Result: Fluorescence spectra of differents species with different fluorescence lifetimes

DAFS


Fluoreszenz
τ_{fl}
Norm. Intensität
1.0
0.8
0.6
0.4
0.2
0.0
640
660
680
700
720
740
760
780
800
Wellenlänge [nm]

Data Analysis

Data:

$$D_\omega = \begin{pmatrix} I_1^1 & \mathrm{K} & I_L^1 \\ & I_l^\omega & \\ I_1^M & & I_L^M \end{pmatrix}$$

Matrix of amplitude coefficients :

$$S(\lambda) = \begin{pmatrix} a_1^1 & \mathrm{K} & a_N^1 \\ \mathrm{M} & a_n^\omega & \mathrm{M} \\ a_1^M & \mathrm{K} & a_N^M \end{pmatrix}$$

Matrix of lifetimes:

$$\Pi(\tau_n, \delta t_n) = \begin{pmatrix} \exp(-\frac{t_l}{\tau_1}) \otimes IRF(t, \delta t_1) \\ \mathrm{K} \\ \exp(-\frac{t_l}{\tau_N}) \otimes IRF(t, \delta t_N) \end{pmatrix}$$

N species $1 \langle i \langle N$
M Wavelength $1 \langle w \langle M$
L time channels $1 \langle l \langle L$

Data Analysis

Model matrix:

$$\tilde{D} = S(\lambda) \cdot \Pi(\tau_n, \delta t_n)$$

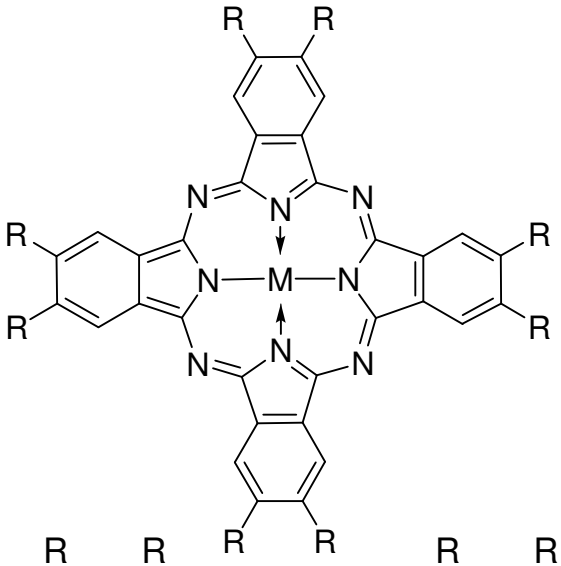
χ^2-Value:

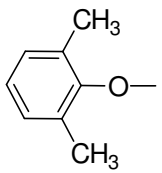
$$\chi^2_{global} = \frac{1}{M} \sum_{\omega} \frac{1}{L} \left\| (D_\omega - \tilde{D}_\omega) \circ \frac{\rho}{\delta_\omega} \right\|^2$$

Example

M= H_2 : H_2Pc

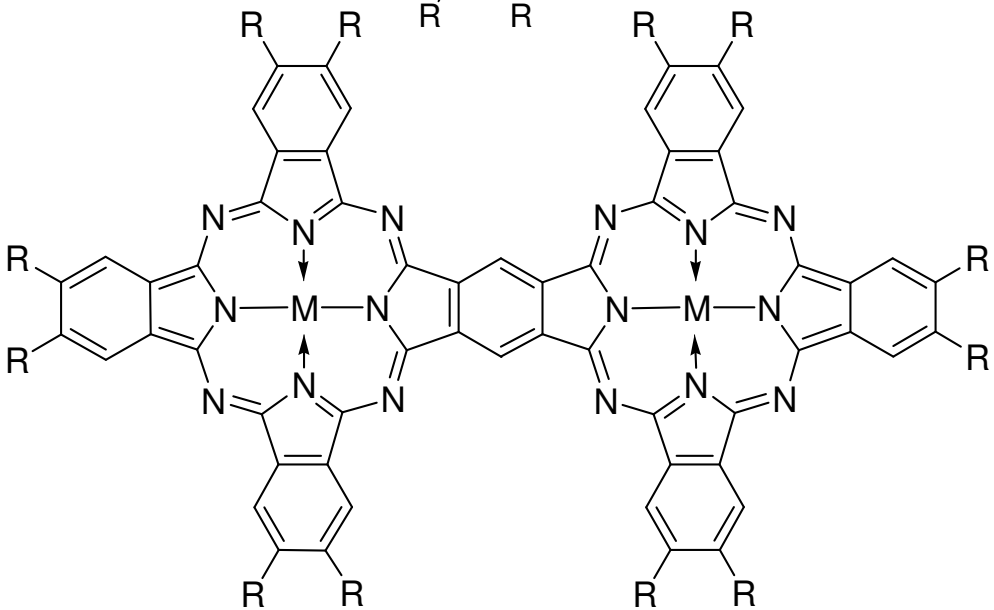
M= Zn : ZnPc



R= 

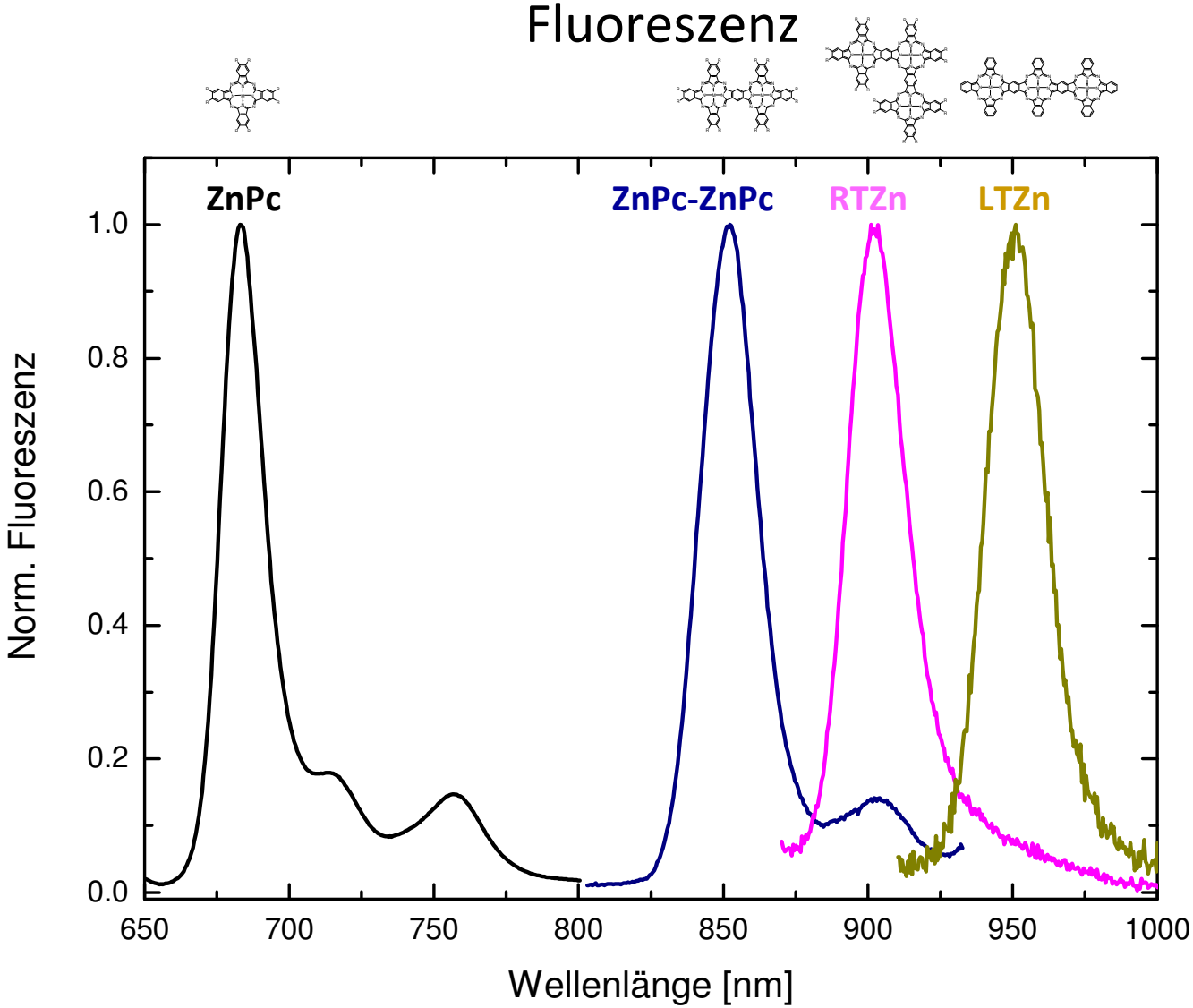
M= H_2 : H_2Pc-H_2Pc

M= Zn : ZnPc-ZnPc



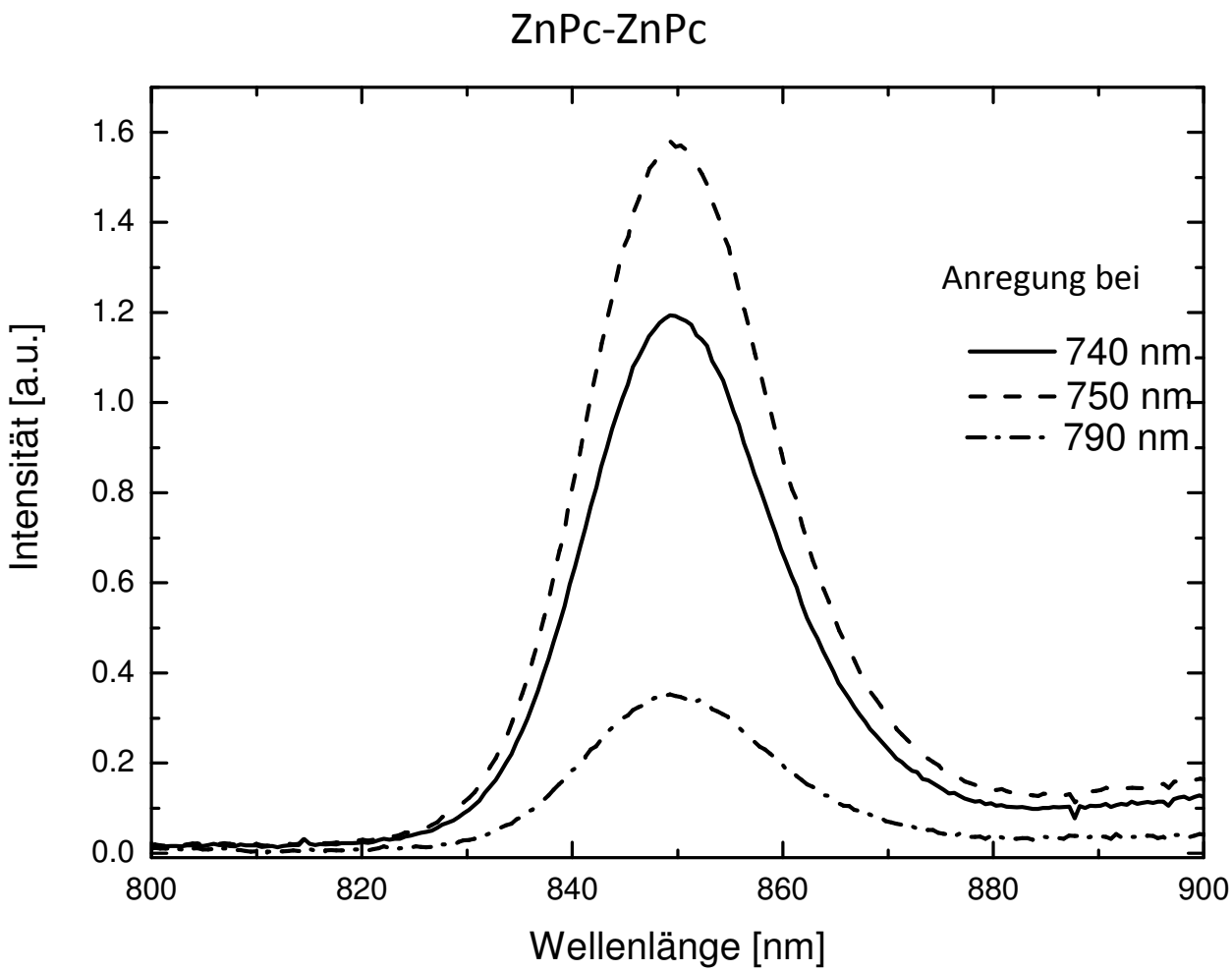
Example

Fluoreszenz

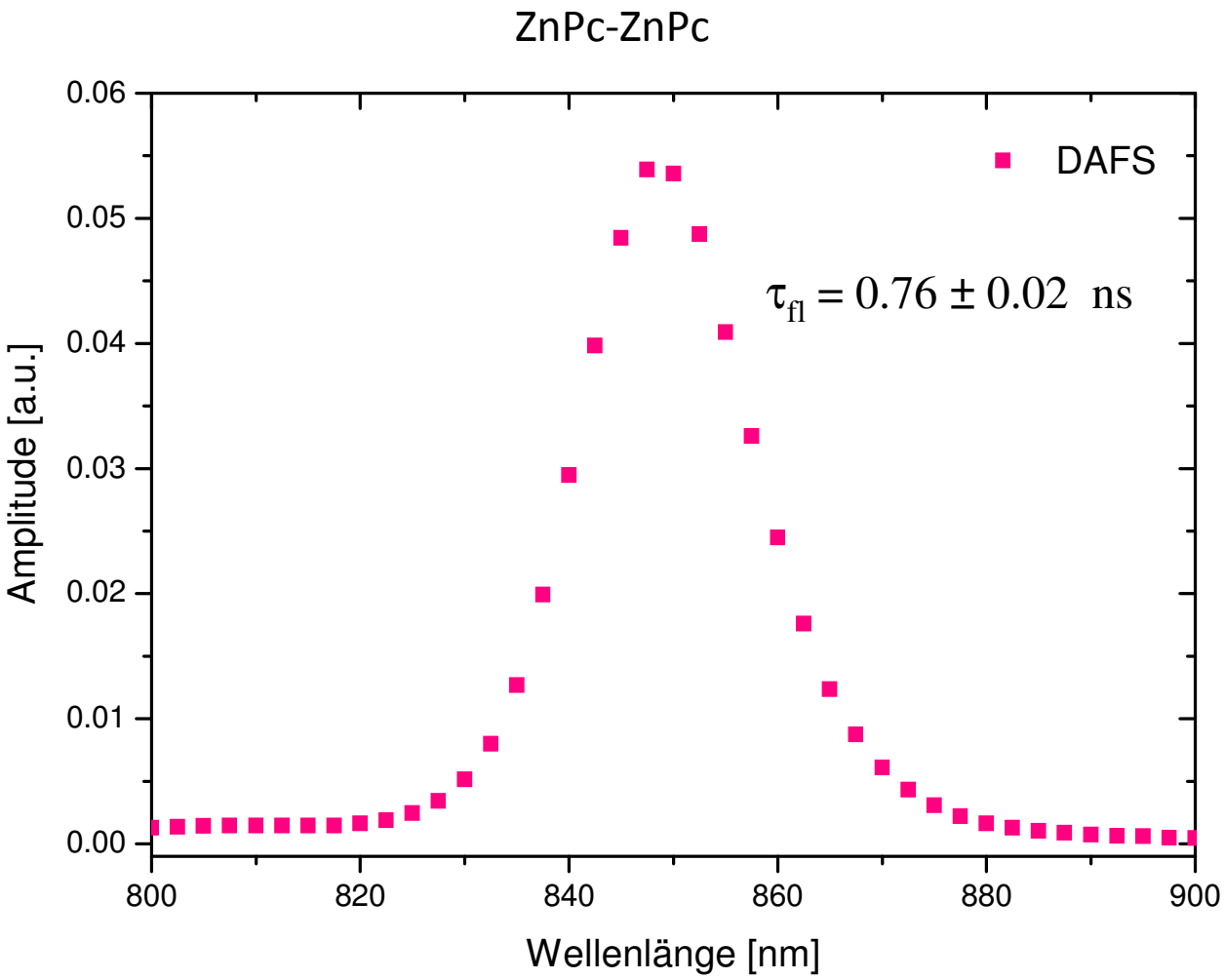


Example

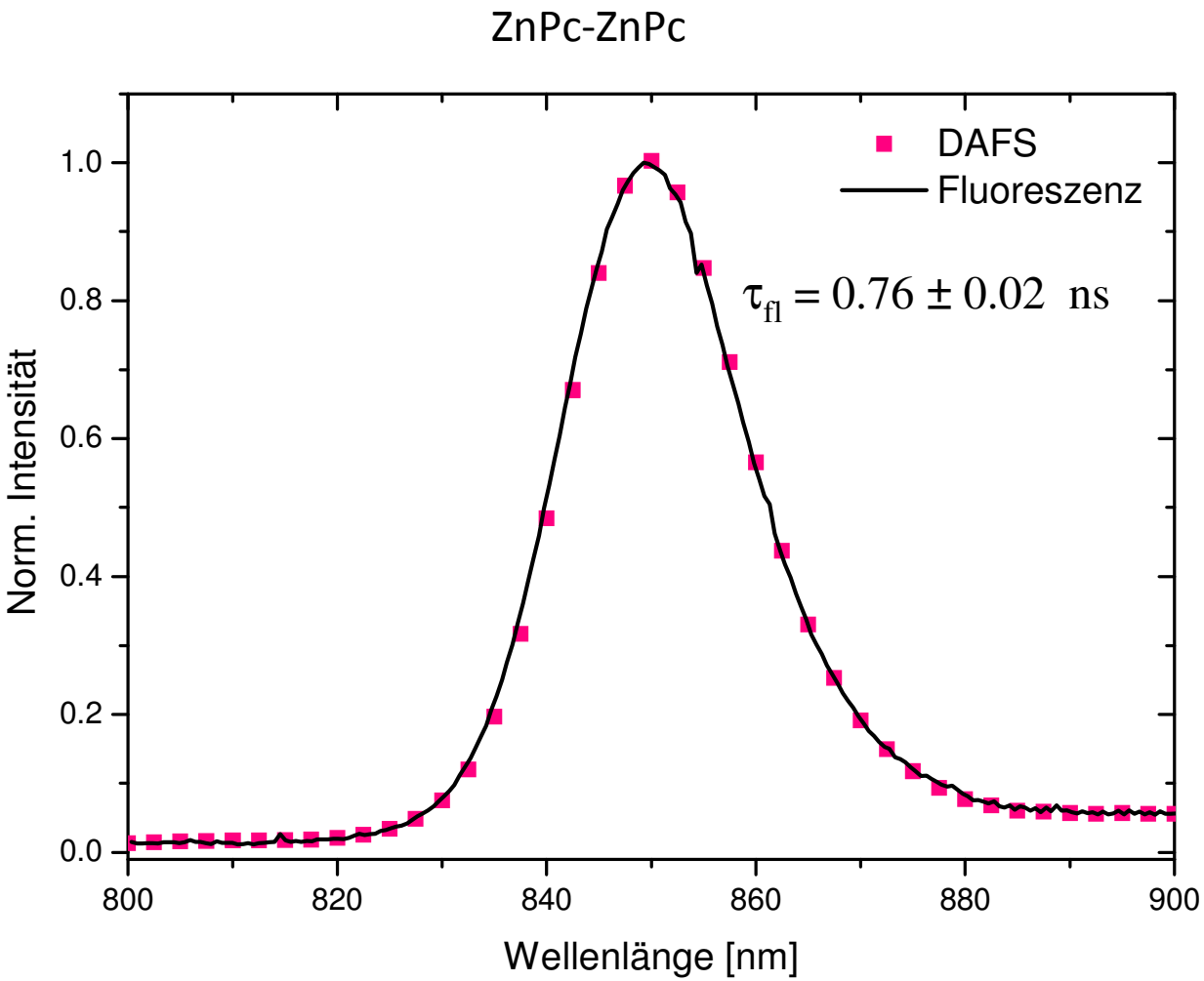
Fluoreszenz



Example


ZnPc-ZnPc
DAFS
$\tau_{fl} = 0.76 \pm 0.02$ ns
Amplitude [a.u.]
0.06
0.05
0.04
0.03
0.02
0.01
0.00
800
820
840
860
880
900
Wellenlänge [nm]

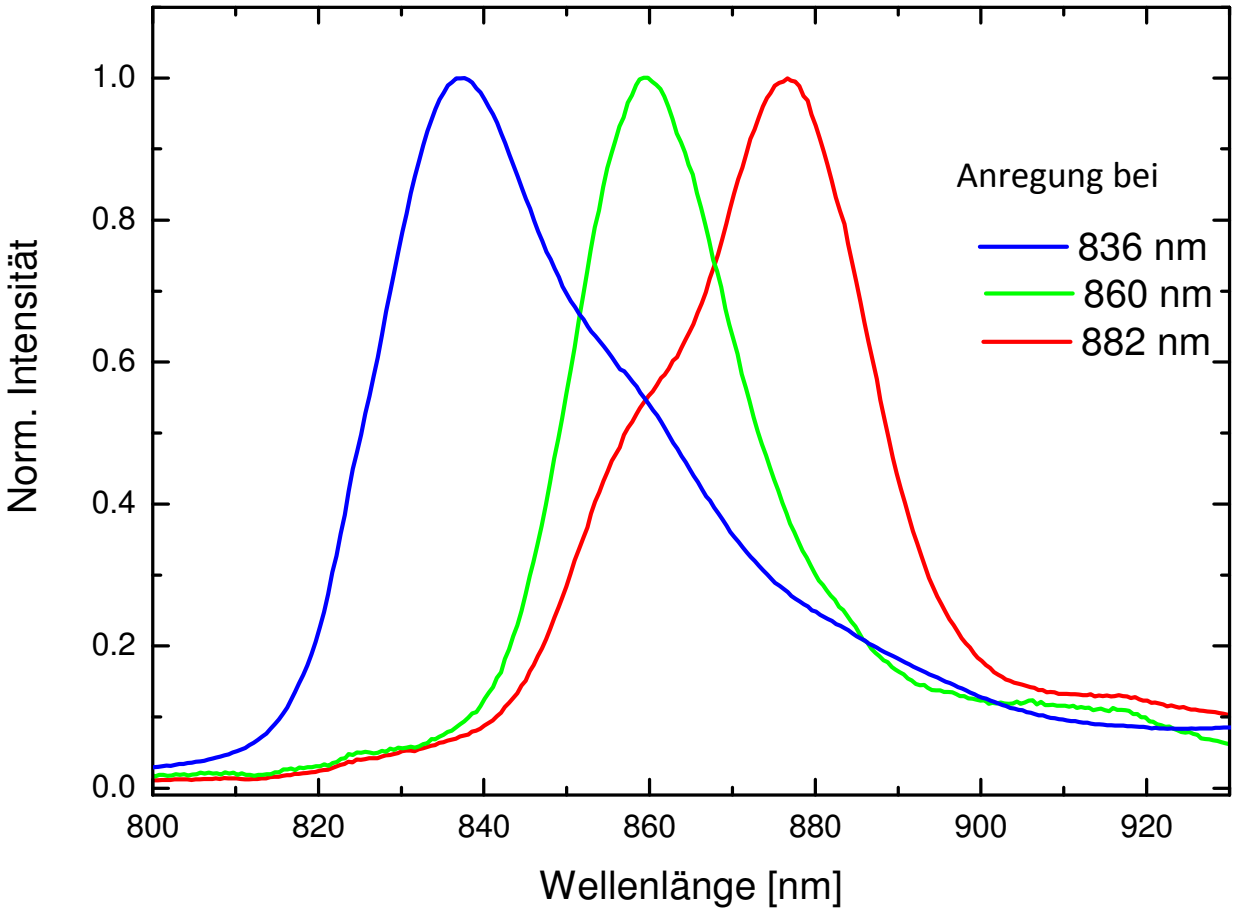
Example


ZnPc-ZnPc
DAFS
Fluoreszenz
$\tau_{fl} = 0.76 \pm 0.02$ ns
Norm. Intensität
1.0
0.8
0.6
0.4
0.2
0.0
800
820
840
860
880
900
Wellenlänge [nm]

Example

Fluoreszenz

H_2Pc-H_2Pc



Example

DAFS