Chap. II : Donor-Acceptor pairs for Förster Resonance Energy Transfer (FRET):

1) Introduction:

Demonstrating the wide potentiality of the **RAFT** method to reach specific requirements, *e.g.* predictable molar mass, narrow polydispersity and complex architectures, **RAFT** seemed to be the method of choice for the synthesis of polymeric precursors of new labeled polycations. In this study, the fluorophore grafting on the polycations was selected as method to introduce fluorophores within multilayers. The two alternative processes [1] are i) the alternate deposition of polyelectrolytes and functional molecules and ii) the postdiffusion of the molecules in the multilayers. To the described drawbacks related to these last techniques (molecules need a certain number of charges to be deposited or need a certain time to enter the multilayers), one can argue that there is no specific location of the fluorophores in the stratified films and that a control on the deposited amount of fluorophores is hard to achieve. So that, functional polycations were aimed for use in the Electrostatic Self-Assembly (**ESA**) process. In addition to fulfilling the general macromolecular requirements for the **ESA** process (architecture, charge density), labeled polycations have to be specially tailored to enable the fabrication of multilayered thin films in which the **FRET** phenomenon should be observed and studied.

An appropriate choice of the monomer is required in the strategy for the observation of **FRET** in thin organic films. 4-vinylbenzyl chloride **VBC** was selected as parent monomer for several reasons. It is a styrenic derivative for which a good control was demonstrated when using the **RAFT** process in the free radical polymerization. It can be easily derivatized [2,3] to incorporate cationic or anionic charges as well as fluorophores with different spectral properties, as needed to build a **FRET** system.

The crucial point in the synthetic route to new labeled polycations suited for **ESA** and **FRET** is the choice of the fluorophores. Among the large library of commercial fluorophores, donor-acceptor pairs that fulfil the spectroscopic requirements of **FRET** had to be found. Additionally, the chosen fluorophores must possess a functional group that enable their

grafting onto the polymer chains. Ideally, they have to be small but strong fluorophores. Actually, they should have a high fluorescence quantum yield and be small enough to avoid problems of aggregation that modify the fluorescence behavior negatively in most cases. Furthermore, to build a satisfactory pair of chromophores for **FRET** observation the emission of the donor had to superpose to the excitation of the acceptor fluorophore with a maximum overlap (namely, Förster overlap integral). To avoid any problem of residual excitation of the acceptor chromophore, this last has to present a spectral gap where the excitation of the donor chromophore stands. Last, but not least, they must have a good photostability and a low sensitivity to environmental changes. In reality, a balance has to be found between the size of the molecule and the spectroscopic behavior.

All these requirements for **FRET**, photostability, fluorescence spectroscopy and chemical structures narrow drastically the range of useful dyes. Among this reduced choice, coumarin and perylene diimides derivatives were focused on. Both are well-known class of fluorescent compounds described extensively in literature [4]. Two donor-acceptor pairs sharing a common fluorophore were selected. On the one hand, it is possible to observe separately in each pair the occurrence of **FRET**. On the other hand, when employing the acceptor dye of the first **FRET** system as donor dye in the second one, it is possible to combine the two pairs into a triade (donor- acceptor / donor-acceptor) for the occurrence of a **FRET** cascade. First attempts to observe the **FRET** cascade in self-assembled polymer film will be presented in chapter IV.

2) Förster Resonance Energy Transfer (FRET):

2 a) Introduction:

Fluorescence Resonance Energy Transfer, often known as Förster Resonance Energy Transfer (**FRET**), in reference of the work of Förster [5-7] in the early 50's, is a powerful physical and biophysical technique that permits interactions between fluorophores in close contact to be analysed. The most wide spread application of this phenomenon is its use as "spectroscopic ruler" [8]. Actually, **FRET** is proportional to the inverse six power of the intermolecular distance between the donor and the acceptor molecule. It is also possible to detect changes in the positioning of the fluorophores (relative orientation of the fluorophores within the films, distance between fluorophores) by **FRET** investigations.

Most of the applications of the **FRET** phenomenon are in the field of biochemistry at present. Two strategies are used in order to observe the **FRET** phenomenon linked to biological molecules. The first one uses the intrinsic fluorescence of tryptophan in a protein with a second incorporated fluorophore. The second one is the separate incorporation of complementary fluorophores (extrinsic fluorescence) in different biological entities to observe their interactions [9]. In this way, it is possible to monitor the interaction between protein and small ligands. Such measurements allow to determine ligand-binding constants, to probe ligand-induced structural changes during the binding process, and to map the binding sites of a protein [10]. Bio-membrane fusion may also be studied by monitoring the intrinsic tryptophan fluorescence. To observe *e.g.* the fusion of vesicles (or liposomes), it is often favorable to turn to the extrinsic fluorescence [11]. Numerous examples dealing with interactions of molecules of biological interest can be found in the literature. One can cite *e.g.* the interaction between proteins and nucleic acid [12] (**DNA** and **RNA**) and between proteins [13].

While the observation of the **FRET** phenomenon is a method of choice to investigate interactions between molecules in Life Sciences, this analytical approach is rarely used in Materials Science. Still, it is a very attractive method for investigating materials and objects at the nanometer level.

2 b) Theoretical aspects:

2 b 1) Jablonski diagram for FRET:

The lifetime of a fluorophore in the excited state is about 10^{-9} s. After excitation, competition occurs between fluorescence emission, non-radiative de-excitation and transfer of excitation energy to the surrounding molecules. All these processes are described in the Jablonski diagram (Figure II, 1). **A**: Transfer of energy from a photon to a molecule. For absorption to occur, the energy of the photon must correspond to the energy difference between the ground state of the molecule and one of its excited states. **F** fluorescence is the emission of the excitation energy in form of light (radiative process). The emitting and final states must have the same electronic spin state (they must be singlet - or triplet - states). **P** Phosphorescence is also the emission of the excitation energy via a radiative process. Unlike fluorescence, emitting and final states must have different electronic spin state. **IC** internal

conversion is the relaxation between similar electronic spin states. **ISC** intersystem crossing is the transition between different electronic spin states.

The Förster Resonance Energy transfer is the radiationless transfer of this excitation energy from a donor to an acceptor fluorophore. The main effect of this transfer is that there is suppression or weakening of the emission of light by the donor, while an increase in the emission of light from the acceptor is observed. **FRET** is an interaction that depends strongly on the distance. As a general rule, the energy transfer occurs over a distance of 1-10 nm. But it is not the only factors influencing the efficiency of the transfer. This dependence can be described by equations linking these factors.

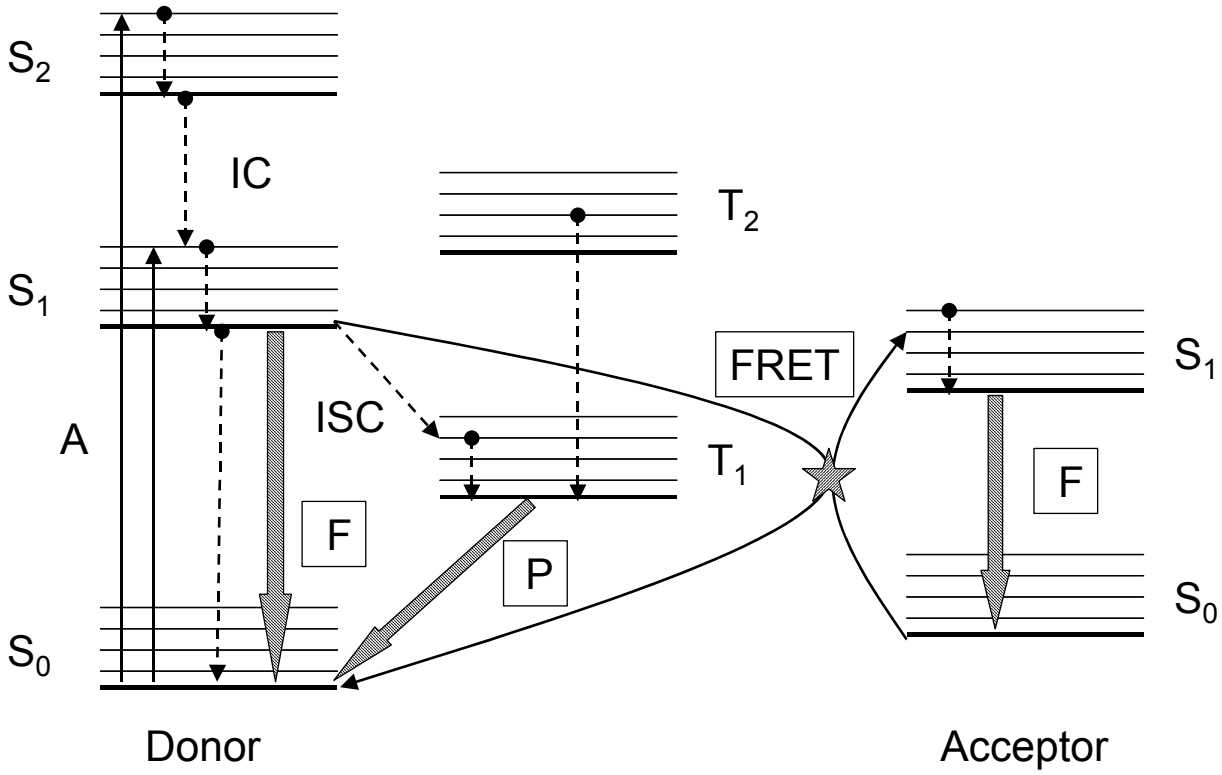


Figure II, 1: Jablonski diagram for FRET phenomenon.

2 b 2) Influencing factors:

The **FRET** phenomenon is based on the dipole-dipole interaction of a suitable donor-acceptor pair. It is possible to describe some key factors influencing this non-radiative process.

The first requirement for a good efficiency of the energy transfer is the spectral overlap between the emission of the donor and the excitation of the acceptor molecule. It is described as the degree of spectral overlap or "Förster overlap integral". It describes the degree of overlap where resonance can occur. In the following equations this factor is noted ***J***. The second factor is the distance. Because the interaction between the donor and the acceptor fluorophore is a dipole-dipole interaction the distance between the centres of the dipoles is critical and proportional to r^{-3}.

$V = -\frac{\mu_1 \mu_2}{4\pi\varepsilon_0} \times \frac{2}{r^3}$ V: potential energy of the dipole-dipole interaction.

However, Förster showed that the rate of energy transfer of the dipole-dipole mechanism is related to the square of this potential energy, and therefore k_{et} is proportional to r^{-6}. The rate constant of transfer k_t is dependent on the distance between the dipoles. This constant can be expressed as:

$$k_t = \frac{1}{\tau_d}\left(R_0 / R\right)^6$$

In this equation, ***R*** is the distance between the dipoles, τ_d is the excited state lifetime of the donor in the absence of the acceptor, and R_0 is the "Förster radius". The "Förster radius" is the distance at which the energy transfer is 50% efficient. The exact value of the Förster radius will depend on the spectral properties of the molecules involved in the process.

Another well-known factor influencing the energy transfer is the relative orientation of the chromophores. The energy transfer occurs through space. The donor-acceptor dipole orientation factor (noted K^2) is the major unknown in **FRET** calculation, but, assuming isotropic distribution of donor and acceptor molecules, a value of 0.66 can be assigned to K^2.

The last factor influencing the quality of the investigation is the large spectral gap that the acceptor must present in the region of the absorption band of the donor. It is possible to gather all the factors to produce the general equation:

$$R_0^6 = 8.785\times10^{-5}\ \frac{K^2\ \Phi_D\ J}{n^4}$$

where N_A is the Avogadro's number, n is the refractive index and Φ_d is the quantum yield of the donor. The efficiency (E) of the energy transfer process may be evaluated with the fluorescence decay time of the donor in the presence (τ_D) and in absence of the acceptor (τ^0_D) or by the quantum yield of the donor in the presence (Φ_D) and in absence of the acceptor (equation 1) [12]:

$$E = 1 - \frac{\tau_D}{\tau_D^0} = 1 - \frac{\Phi_D}{\Phi_D^0} \quad (1)$$

If the donor decay time is not monoexponential, the transfer efficiency can be calculated by the same equation, but the average decay times of the donor in presence ($< \tau_D >$) and in absence of the acceptor ($< \tau^0_D >$) must be used where the amplitude-averaged decay times are defined as [14]:

$$\langle\tau\rangle = \frac{\sum_i \alpha_i \tau_i}{\sum_i \alpha_i} \quad (2)$$

where α is the decay time amplitude

As described above, many factors influence the efficiency of **FRET**. Among all the requirements needed for a good observation of this process, it is most important to pay attention to the Förster overlap integral and to the spectral gap of the acceptor molecule. The choice of a donor-acceptor pair is therefore of main importance.

2 b 3) Case of two-dimensional systems:

In the model developed by Förster [5-7], the spatially mediated non-radiative energy transfer proceeds via dipole-dipole interaction with an R^{-6} distance dependence. The preliminary works for energy transfer experiments done on Langmuir-Blodgett (LB) films [15] allowed to develop the theoretical framework for steady-state energy transfer efficiencies in 2-D systems [16]. In a general way, the dependence of the donor and acceptor distance (d) on the donor fluorescence intensity can be written as [17-19]:

$$\left(\frac{I_d}{I_0}\right) = \frac{1}{1+\left(\frac{d_0}{d}\right)^X}$$

where $\boldsymbol{I_d}$ is the fluorescence intensity for the donor molecules spaced an average distance of $\boldsymbol{d}$ away from the acceptor molecules, $\boldsymbol{I_0}$ is the fluorescence intensity of the donor molecules in the absence of acceptor molecules, and $\boldsymbol{d_0}$ is the critical spacing between donor and acceptor corresponding to a 50% probability of energy transfer.

Both $\boldsymbol{d_0}$ and $\boldsymbol{X}$ are a function of the assumed geometry [17]. $\boldsymbol{X}$ is equal to 6 in the case of a donor molecule which transfer energy to an acceptor molecule in 3-D space. This case was described in the previous paragraph. $\boldsymbol{X}$ is equal to 4 in the case of a plane of donor molecule transferring energy to a plane of acceptor molecules [15].

The very well defined structure of **LB** films was used to verify these theoretical concepts (the inverse fourth power distance dependence for two-dimensional systems). It was reasonable to assume that the fluorophores are distributed within exact two-dimensional sheets. However, electrostatically self-assembled multilayers are supposed to be much more fuzzy with respect to their internal structure (see discussion Chap. IV). This fuzzy structure is caused by the mutual interpenetration of neighbouring layers and is inherent to the method. As a consequence, the distribution of the chromophores within the multilayers is more three-dimensional. So that, the two-dimensional concept was not applied to systems described in this study, but an inverse sixth power distance dependence will be considered in these cases, as this approximation seemed more appropriate.

3) Donor-Acceptor pair:

The choice of the fluorophores for the study was coumarin and perylene derivatives. As it will be described, well-suited pairs of donor-acceptor fluorophores with high Förster overlap integral and sufficient spectral gaps can be found in these classes. In addition to the needed requirements for **FRET** observation, they are relatively stable dyes that can be adapted to the requirements for the planed studies, namely high quantum yield, photostability and low sensitivity to environmental changes.

3 a) Coumarin derivatives:

3 a 1) Description:

Coumarins are members of a class of aromatic lactones which occurs in nature (*e.g.* in lavender, sweet clover grass, apricots, cherries or cinnamon), and which display a wide range of biological activities. This heterocycle is best known for its fragrance (vanilla-like). Coumarins have been synthesized since the 19th century. Their synthesis was carried out first by Perkin [20] and by Pechmann [21]. Nowadays, a large variety of synthetic derivatives is available and well described in the literature [22-25]. Coumarin derivatives have been used to make perfumes and flavors. But they are also used to prepare other chemicals, for instance rodent poison. Some derivatives are used medically because they demonstrated to have blood-thinning, anti-fungicidal and anti tumor activities. They are also used as mesogenic group [26,27] in liquid crystalline technology and as laser dyes [28] because of their excellent photophysical properties (good photostabilities, high quantum yield and the fluorescence spectra moderately sensitive to environment).

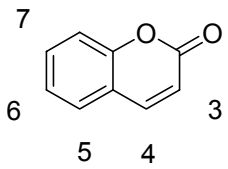


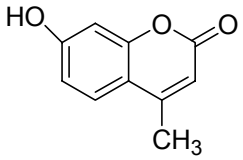
Figure II, 2: general formula of coumarin derivatives.

Coumarin is a small but a strong fluorophore. Coumarins can be synthesized with various substituents. Spectral features can be easily tuned, in particular by the incorporation of substituents in position 4 and 7 (*cf* Figure II, 2). The versatile functionalization of the

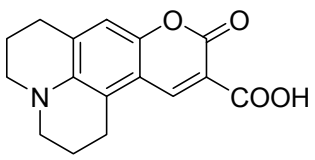
coumarin compounds should enable their fixation on polymer backbones, as will be described in the strategy leading to labeled polycations.

3 a 2) Coumarin-coumarin donor-acceptor pair:

Two coumarin derivatives were selected by virtue of their spectroscopic behavior that makes of them a promising donor-acceptor pair suited for **FRET**. Furthermore, they possess functional groups, *i.e.* hydroxy and carboxylic acid groups, which enable their fixation onto the polymer. They are named dyes **coumarin 4** and **coumarin 343** (Figure II, 3).



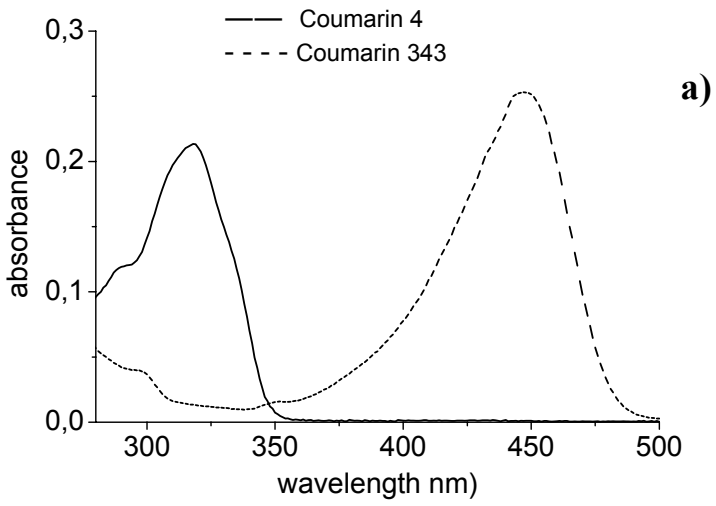
Coumarin 4



Coumarin 343

Figure II, 3: formulas of 7-hydroxy-4-methylcoumarin (coumarin 4) and 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1] benzopyrano [6,7,8-ij] quinolizine-10-carboxylic acid (coumarin 343).

According to their UV-Vis and fluorescence spectra, the **coumarin 4-coumarin 343** donor-acceptor pair is well suited for **FRET** experiments. Looking at the UV-Vis spectra (Figure II, 4a) one can observe that the maximum wavelengths of the two absorption bands are well separated (coum 4: λ_{max}= 318 nm and coum 343: λ_{max}= 449nm). Furthermore, **coumarin 343** has a large spectral gap between 300 and 375 nm. The absorption band of **coumarin 4** is located in the spectral gap of **coumarin 343**.



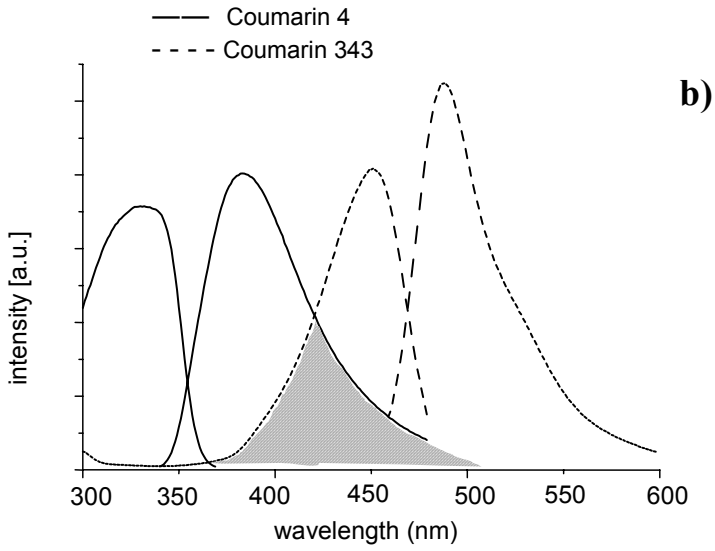


Figure II, 4: a) UV-Vis spectra of **coumarin 4** (**—**) in acetonitrile and **coumarin 343** (---) in ethanol, **b)** fluorescence spectra of **coumarin 4** (—) in acetonitrile (emission with excitation at 325 nm, excitation with emission at 380 nm) and **coumarin 343** (---) in ethanol (emission with excitation at 448 nm, excitation with emission at 490 nm):

The fluorescence spectra of the two fluorophores (Figure II, 4b) show the feasibility of **FRET** experiments. The superposition of the emission spectrum of **coumarin 343** and the excitation spectrum of **coumarin 4** (Figure II, 4b) shows a good overlap known as Förster overlap integral. The excitation maxima are well separated (**coumarin 4**: λ_{ex} = 318 nm in acetonitrile and **coumarin 343**: λ_{ex} = 448 nm in ethanol). The maximum of excitation of **coumarin 4** is located in the spectral gap of the **coumarin 343**.

The spectroscopic behavior of the two fluorophores in solution, make this donor-acceptor pair suited for **FRET**. In order to establish **FRET** as a "cascade phenomenon" involving not only a pair of fluorophores but also three matching fluorophores, it was necessary to complement the pair **coumarin 4** / **coumarin 343** by a fluorescent dye that has a particularly large spectral gap between 300 and 475 nm. Because no appropriate coumarin dye could be identified, another class of fluorophores, namely perylene derivatives, were considered.

3 b) Perylene diimides derivatives:

3 b 1) General considerations:

Perylene diimides derivatives exhibit an attractive spectroscopic behaviour. This widespread commercial dye is a well-known chromophore combining high quantum yield of photoluminescence with excellent photochemical and thermal stability. As for coumarins, a wide range of colours can be obtained by modifying the substituents attached to the perylene chromophore [29].

Low molecular weight perylenes (Figure II, 5) are successfully used as additives in printing inks and paints, in optical switching [30], solar energy conversion [31], liquid crystal displays, light emitting diodes (LED's) [32], laser dyes, polymeric red fluorophores [33]… Certain perylene dyes are also used in Life Sciences as intercalating agent of **DNA** [34] and as fungicide and pesticide. Their spectroscopic behavior makes them well suited for **FRET** observation, notably because they posses a large spectral gap where the excitation of **coumarin 4** and **coumarin 343** are located. Furthermore, the presence of functional groups on the chromophore enables their grafting on polymer backbones via an imide group. The series of 3,4,9,10-perylenetetracarboxylic acid diimides (**PTCAD**) (Figure II, 5) has been the subject of extended research [35-37]. Such dyes are used as ligands that bind and stabilize G-quadruplex of **DNA** structures because they are potential inhibitors of the cancer cell-associated enzyme telomerase. They all show the identical chromophore (basic perylene diimide), but differ in the nature of substituents in the side chains fixed to the imide nitrogens.

3 b 2) Coumarin-perylene donor-acceptor pair:

According to a general procedure [38], **DAPER** was synthesized via reaction of a mixte primary-tertiary amine on the anhydride group, and fully quaternized in order to use it as a model compound (Figure II, 5).

As demonstrated by Figure II, 6, the pair **coumarin 343- DAPER** is well suited for **FRET** observation. According the UV-Vis spectra (Figure II, 6a), the absorption band of **coumarin 343** (λ_{max}= 449 nm in ethanol), as well as the adsorption band of **coumarin 4** (λ_{max}= 318 nm in acetonitrile), are located in a region where **DAPER 01** (λ_{max}^{1}= 430 nm,

λ_{max}^{2}= 460 nm, λ_{max}^{3}= 490 nm and λ_{max}^{4}= 525 nm in trifluoroethanol) has a weak absorbance. Looking closely to the fluorescence spectra (Figure II, 6b), it appears that the excitation band of **coumarin343** (λ_{ex}= 448 nm in acetonitrile) is sufficiently separated from the excitation bands of **DAPER 01** (λ_{ex}^{1}= 430 nm, λ_{ex}^{2}= 461 nm, λ_{ex}^{3}= 490 nm and λ_{ex}^{4}= 526 nm in trifluoroethanol) to allow selective excitation.

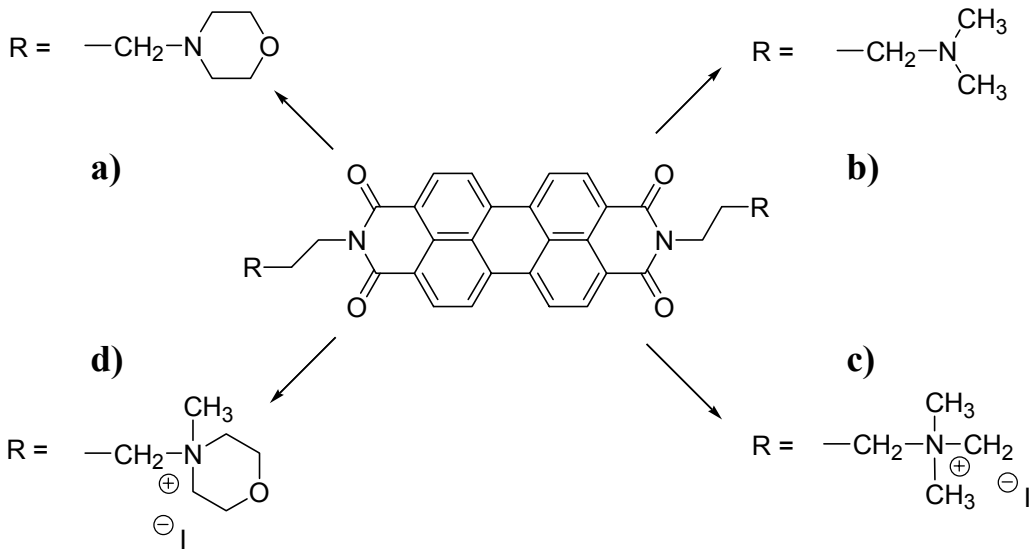
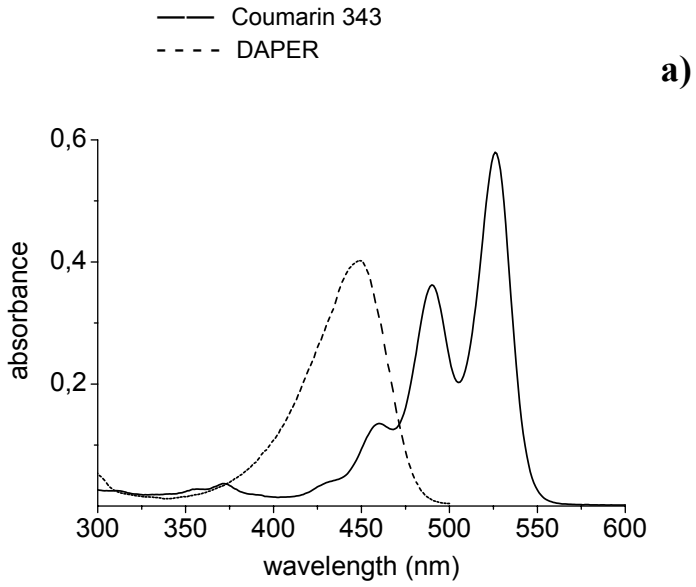


Figure II, 5: formula of 3,4,9,10-perylenetetracarboxylic acid diimide derivatives.
a) **Tel 01**, b) **DAPER**, c) **DAPER 01** and d) **Tel 11**



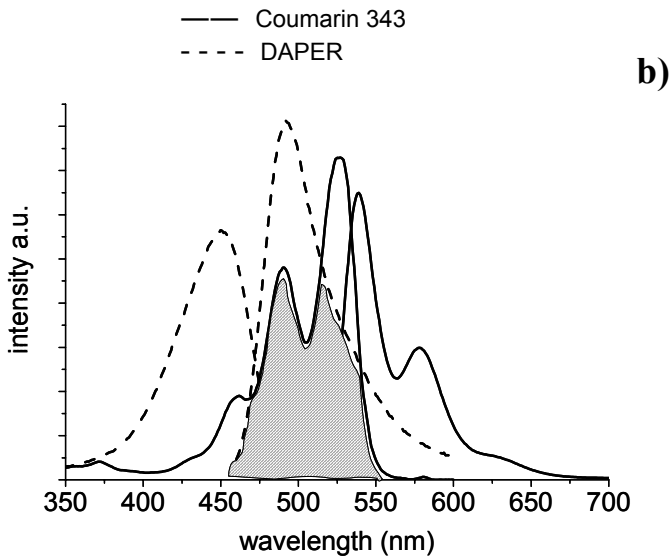


Figure II, 6: a) UV-Vis spectra of **coumarin 343** (---) in ethanol and **DAPER 01** (—) in trifluoroethanol **b)** excitation and emission spectra of **coumarin 343** (----) in ethanol (λ_{ex}= 449 nm and λ_{em}= 490 nm) and **DAPER 01** (—) in trifluoroethanol (λ_{ex}^{1}= 430 nm, λ_{ex}^{2}= 461 nm, λ_{ex}^{3}= 490 nm, λ_{ex}^{4}= 526 nm and λ_{em}^{1}= 540 nm, λ_{em}^{2}= 577 nm, λ_{em}^{3}= 625 nm).

According to these first observations, the **coumarin 343- perylene diimide** donor-acceptor pair is well-suited for **FRET** observations. All the requirements, concerning the spectral gap of the acceptor and the Förster overlap integral, are fulfilled. Furthermore the perylene diimides, though di-functional, can be mono-functionalized in order to become graftable onto a polymer backbone, as will be discussed in the next chapter.

4) <u>Conclusions on FRET</u>:

Two donor-acceptor pairs well-suited for FRET observations were described which can act as donor-acceptor systems. They share a common fluorophore, namely **coumarin 343**. That implies the possibility to use these two pairs to elaborate a transfer "cascade" between three fluorophores from **coumarin 4** to **perylene diimide** with **coumarin 343** as intermediate.

These three complementary fluorophores cannot be deposited by the **ESA** process as such (see Chap. IV). They need to be previously grafted on polycations (or polyanions) to promote their incorporation into multilayered thin films. It is easy to find donor-acceptor pairs suited for **FRET**, but the task is much more complicated when the fluorophores constituting

the pairs have to bear functional groups for grafting. In the subsequent chapter we will see that specific requirements for **ESA** process must be added to the **FRET** requirements. For example, the polycations have to be highly soluble in aqueous media, and the fluorophore content must be kept very low to avoid common problems of self-quenching in thin films. Therefore, the polycations bearing the complementary fluorophores have to be exactly tailored to be able to observe a "cascade of energy transfer" within the multilayered thin films.

5) Experimental part (materials and synthesis of dyes):

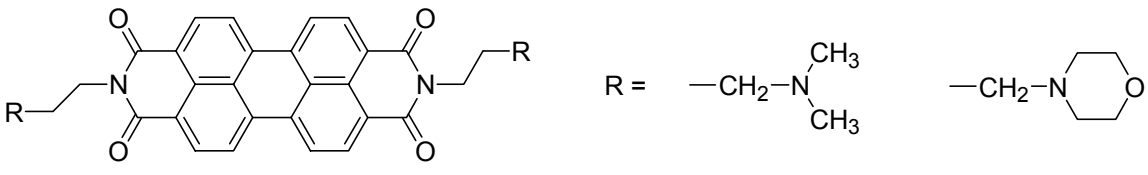
5 a) materials and methods:

3,4,9,10-perylenetretracarboxylic acid dianhydride, methyliodide (99.5%), trifluoroacetic acid-d (99.5 Atom % D) and *N,N'*-dimethylamino-propylamine were purchased from Aldrich and used as received. *N*-(3-aminopropyl)morpholine from Acros Organics was used as received. Chlorobenzene, 1-butanol and methanol were analytical grade solvents from Riedel de Haën.

Fluorescence measurements used a Fluorolog-3 spectrofluorometer Jobin Yvon – Spex system from Instruments S.A., Inc. (USA) (HV= 600, slits: 4 nm, spectra are corrected via correction factors purchased from the factory). UV-Vis measurements used a spectrophotometer CARY 5E (Varian). NMR spectra were recorded at ambient temperature on Brucker 300 MHz. Chemical shifts are given in ppm with respect of the residual resonance of the solvent (11.59 for trifluoroacetic acid).

5 b) Synthesis of dyes:

DAPER and Tel 01:



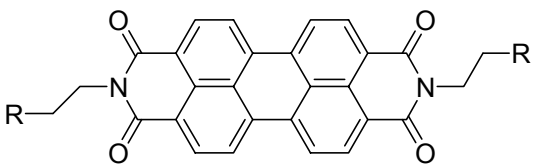
DAPER *TEL 01*

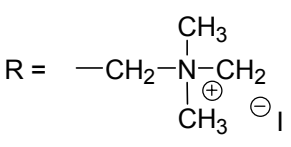
DAPER and **TEL 01** were prepared as described [39] (***caution****: harmful substance for life and environment. Must be handle carefully with gloves*). They were synthesized from 100 mg of 3,4,9,10-perylenetretracarboxylic acid dianhydride ($C_{24}H_8O_6$, M= 392.32, 0.25 mmol) by reaction with 255 mg of *N,N'*-dimethylamino-propylamine for **DAPER** (M= 102.18, 2.5 mmol) and with 360 mg of *N*-(3-aminopropyl)morpholine (M= 144.22, 2.5 mmol) for **Tel 01**, in 20 ml of 1-butanol. The mixture was heated at 90°C for 24h under inert atmosphere. After cooling the reaction medium to room temperature, the product was recovered by filtration. The filter cake was washed with 250 ml of distilled water and 250 ml of methanol. The product was dried overnight under vacuum. The product was obtained as deep purple fibers.

DAPER (M= 560.65 g.mol^{-1}): yield: 89% (124 mg). ^{1}H-NMR in trifluoroacetic acid d_1 (Bruker, 300 MHz, δ in ppm): 8.91 (m, 8H, aromatic), 4.64 (m, 4H, CH_2-N-C=O), 3.58 (m, 4H, $\underline{CH_2}$-N-CH_3), 3.27 (m, 12H, N-CH_3), 2.61 (m, 4H, C-$\underline{CH_2}$-C). ESI mass spectroscopy (positive): M^+ = 561.2. UV-Vis in trifluoroethanol (Varian, Cary 5E): 525 nm (ε = 117 000 l·mol^{-1}·cm), 490 nm (ε = 73 000 l·mol^{-1}·cm), 460 nm (ε = 27 000 l·mol^{-1}·cm), 430 nm (ε = 7 000 l·mol^{-1}·cm).

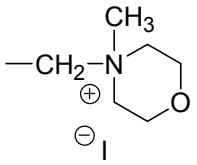
Tel 01 (M= 644,26 g.mol^{-1}): Yield 91% (146 mg). ^{1}H-NMR in trifluoroacetic acid d_1 (Bruker, 300 MHz, δ in ppm): 8.86 (m, 8H, aromatic), 4.70-4.40 (m, 8H, CH_2-O), 4.25 (m, 4H, CH_2-N-C=O), 3.89 (m, 4H, CH_2-N), 3.75-3.40 (m, 8H, heterocycle CH_2-N), 2.59 (m, 4H, C-$\underline{CH_2}$-C). ESI mass spectroscopy (positive): M^+ = 645.2. UV-Vis in trifluoroethanol (Varian, Cary 5E): 525 nm (ε = 110 000 l·mol^{-1}·cm), 490 nm (ε = 74 000 l·mol^{-1}·cm), 460 nm (ε = 26 000 l·mol^{-1}·cm), 430 nm (ε = 6 500 l·mol^{-1}·cm).

Tel 11 and DAPER 01:





DAPER 01



TELL 11

The **Tel 11** and the **DAPER 01** dyes were synthesized according a general procedure previously described [40]. They are synthesized from **TEL 01** and **DAPER** described above by reaction of 100 mg of each with 440 mg of methyliodide (M= 141.94, 3.1 mmol) in chlorobenzene at 140°C for 90 min. The reaction mixture was filtrated hot and the filter cake

was washed with 50 ml of methanol. The crude products were obtained as deep purple fibers from the filter cake.

Tel 11 (M= 928.60 g.mol^{-1}): yield: 61% (88 mg). ^{1}H-NMR in trifluoroacetic acid d_1 (Bruker, 300 MHz, δ in ppm): 8.50-8.00 (m, 4+4 H, aromatic), 4.75-4.30 (m, 12H, CH_2-O and CH_2-N-C=O), 4.70-3.75 (m, 12H, CH_2-N^+ and heterocycle CH_2-N^+), 3.62 (s, 6H, N^+-CH_3), 2.76 (m, 4H, C-$\underline{CH_2}$-C). ESI mass spectroscopy (positive): M $^{2+}$ = 337.3. UV-Vis in trifluoroethanol (Varian, Cary 5E): 525 nm (ε = 66 000 l·mol^{-1}·cm), 490 nm (ε = 41 000 l·mol^{-1}·cm), 460 nm (ε = 15 000 l·mol^{-1}·cm), 430 nm (ε = 2 500 l·mol^{-1}·cm). UV-Vis in water (Varian, Cary 5E): 528 nm (ε = 18 000 l·mol^{-1}·cm), 501 nm (ε = 38 000 l·mol^{-1}·cm) and 470 nm (ε = 18 000 l·mol^{-1}·cm).

DAPER 01 (M= 844.52 g.mol^{-1}): yield: 58% (87 mg). ^{1}H-NMR in trifluoroacetic acid d_1 (Bruker, 300 MHz, δ in ppm): 8.87 (m, 8H, aromatic), 4.52 (m, 4H, CH_2-N-C=O), 3.72 (m, 4H, CH_2-N^+), 3.28 (m, 18H, CH_3-N^+), 2.52 (m, 4H, CH_2-$\underline{CH_2}$-CH_2). ESI mass spectroscopy (positive): M $^{2+}$ = 295.3. UV-Vis in trifluoroethanol (Varian, Cary 5E): 525 nm (ε = 79 500 l·mol^{-1}·cm), 490 nm (ε = 50 000 l·mol^{-1}·cm), 460 nm (ε = 18 500 l·mol^{-1}·cm), 430 nm (ε = 4 500 l·mol^{-1}·cm). UV-Vis in water (Varian, Cary 5E): 528 nm (ε = 9 000 l·mol^{-1}·cm), 501 nm (ε = 20 000 l·mol^{-1}·cm) and 470 nm (ε = 9 500 l·mol^{-1}·cm).

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