Electronic Supporting Information

Fluorescence resonance energy transfer in recognition - mediated polymer-quantum dot assemblies

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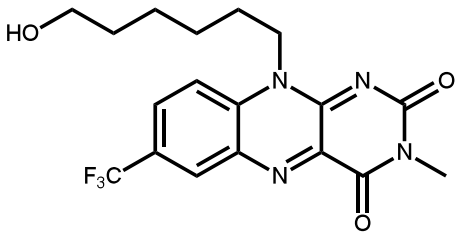
1. Synthesis of poly(MMA-*co*-DAP-*co*-Fl)

Abbreviations:

DAP	2,6-Diamidopyridine
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMSO	Dimethylsulfoxide
EBIB	Ethyl-2-bromoisobutyrate
GPC	Gel Permeation Chromatography
HEMA	2-Hydroxyethyl methacrylate
MMA	Methyl methacrylate
PMDETA	N,N,N',N',N''-pentamethyldiethylenetetraamine
THF	Tetrahydrofuran

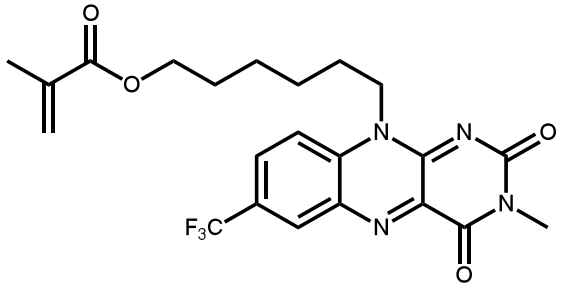
*Synthesis of **1***



7-Trifluoromethane-10-hydroxyhexyl isoalloxane[1] (5.0g, 13.0mmol, 1eq) was dissolved in DMF (10mL) and diluted with acetone (50mL). K_2CO_3 (3.6g, 26.0mmol, 2eq) and iodomethane (4mL, 9g, 64mmol, 5eq) were added and the reaction heated under reflux for 24h. The mixture was cooled to r.t and the solids were removed by filtration. The filtrate was concentrated under reduced pressure and the remaining residue was triturated with diethyl ether (100mL) to precipitate a yellow solid which was collected by filtration. The product was purified by column chromatography (silica) using DCM followed by DCM/acetone as the eluent. Yield = 4.4g (85%). M.P. = 135°C. ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.60 (1H, d, J = 1.35 Hz, ArH), 8.07 (1H, dd, J = 9.0 and 1.3 Hz, ArH), 7.75 (1H, d, J = 9.0 Hz, ArH), 4.70 (2H, t, J = 7.0 Hz, NCH_2-), 3.67 (1H, t, J = 7.0 Hz, O-CH_2), 3.51 (3H, s, CH_3), 1.89 (2H, quin, J = 7.0 Hz, CH_2), 1.66-1.48 (6H, m, CH_2 x3); ^{13}C NMR ($CDCl_3$, 100MHz) δ 159.1 (C=O), 155.7 (C=O), 149.0 (C=N), 138.4 (C=N), 134.9 (C), 134.6 (C), 131.4 (CH), 130.8 (CH), 128.5 (C-CF3, J=34Hz), 122.9 (CF_3, J=273Hz), 116.2 (CH), 62.5 (CH_2),

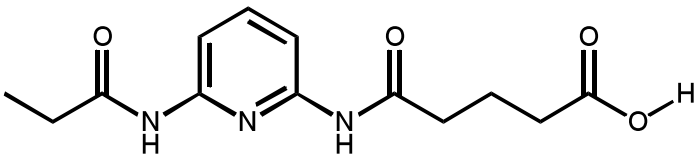
44.9 (CH$_2$), 32.2 (CH$_2$), 28.9 (CH$_3$), 26.8 (CH$_2$), 26.3 (CH$_2$), 25.1(CH$_2$). HRMS FAB(m/z) $C_{18}H_{19}F_3N_4O_3$ [(M+H$^+$)] calculated 397.1483 found 397.1491.

Synthesis of ***2***



Compound 1 (1.0g, 2.52mmol, 1eq), methacrylic anhydride (0.4mL, 3.8mmol, 1.5eq), triethylamine (3.5mL, 25.2mmol, 10eq) and DMAP (30mg, 0.25mmol, 0.1eq) were stirred overnight at r.t in DCM (100mL). The mixture was washed with distilled water (100mL x3), dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by column chromatography using DCM as the eluent. Yield = 760mg (65%). M.P. = 94°C. ^{1}H NMR ($CDCl_3$, 400MHz) δ 8.62 (1H, d, J = 1.5 Hz, ArH), 8.08 (1H, dd, J = 9.0 and 1.5 Hz, ArH), 7.73 (1H, d, J=9.0 Hz, ArH), 6.09 (1H, dt, J=2.5 and 1.0 Hz, CH), 5.56 (1H, dt, J=2.5 and 1.0 Hz, CH), 4.70 (2H, t, J=6.8 Hz, NCH$_2$-), 4.16 (2H, t, J = 6.6 Hz, OCH$_2$), 3.54 (3H, s, CH$_3$), 1.93 (3H, t, J=1.0 Hz, CH$_3$), 1.89 (2H, quin, J=7.8 Hz, CH$_2$), 1.72 (2H, quin, J=7.7 Hz, CH$_2$), 1.64-1.44 (4H, m, CH$_2$ x2);
^{13}C NMR ($CDCl_3$, 100MHz) δ 167.5 (C=O), 159.0 (C=O), 155.5 (C=O), 149.0 (C=N), 138.4 (C=N), 136.4 (C=CH$_2$), 134.9 (C), 134.5 (C), 131.7 (CH), 130.8 (CH), 128.9 (C-CF3, J=35Hz), 125.4 (C=CH$_2$), 122.9 (CF$_3$, J=272Hz), 116.4 (CH), 64.3 (CH$_2$), 45.6 (CH$_2$), 28.9 (CH$_3$), 28.5 (CH$_2$), 27.0 (CH$_2$), 26.4 (CH$_2$), 25.7(CH$_2$), 18.3 (CH$_3$). HRMS FAB(m/z) $C_{22}H_{23}F_3N_4O_4$ [(M+H$^+$)] calculated 465.1745 found 465.1744.

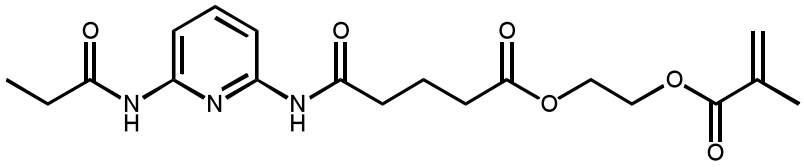
Synthesis of ***3***



N-(6-amino-2-pyridinyl)-propanamide (2.5g, 15.1mmol), glutaric anhydride (3.4g, 30.2mmol, 2eq) and DMAP (183mg, 1.5mmol, 0.1eq) were heated under reflux in dry THF (50mL) for 1h. A cloudy suspension formed during this period. The reaction was cooled to r.t and left for several hours at 4°C. The precipitation was collected by filtration and washed with cold DCM (30mL x2) then dried under high vacuum to provide the product as a white solid. Yield = 3.1g (73%). M.P. = 174°C.
^{1}H NMR (DMSO-d6, 400MHz) δ 12.41 (1H, br s, OH), 10.06(1H, s, NH), 10.03 (1H, s, NH), 7.75 (3H, s, ArH x3), 2.45 (2H, quin, J=7.3Hz, CH$_2$), 2.29 (2H, t, J=7.3Hz, CH$_2$), 1.82 (2H, quin, J=7.3Hz, CH$_2$), 1.10 (3H, t, J=7.3Hz, CH$_3$). ^{13}C NMR (DMSO-

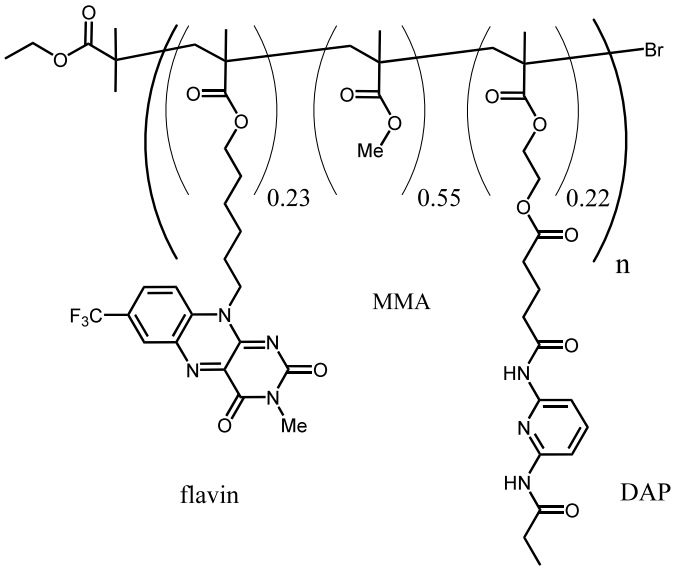
d6, 100MHz) δ 172.8 (C=O), 172.3 (C=O), 167.6 (C=O), 149.6 (HNC=N), 149.3 (HNC=N), 140.8 (CH_2), 109.4 (CH), 109.1 (CH), 36.3 (CH_2), 32.9 (CH_2), 30.8 (CH_2CH_3), 20.8 (CH_2), 9.4 (CH_3). HRMS FAB(m/z) $C_{13}H_{17}N_3O_4$ [$(M+H^+)$] calculated 120.1297 found 120.1293.

Synthesis of ***4***



A suspension of **3** (3.0g, 10.7mmol, 1eq), HEMA (3mL, 24.6mmol, 2eq), EDCI.HCl (3.0g, 15.6mmol, 1.5eq) and DMAP (50mg, 0.4mmol, 0.4eq) were stirred in DCM (100mL) at r.t for 2h. The solution was washed with distilled water (100mL x2), dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by column chromatography (silica) using EtOAc/petroleum ether (7:3) as the eluent to provide the product as a clear colourless oil. Yield = 3.6g (86%).
^{1}H NMR ($CDCl_3$, 400MHz) δ 8.03 (2H, br s, NH x2), 7.89-7.78(2H, m, ArH x2), 7.62 (1H, t, ArH), 6.10 (1H, quin, J=1.5Hz, CH), 5.56 (1H, quin, J=1.5Hz, CH), 4.38-4.29 (4H, m, $COOCH_2$ x2), 2.47-2.35 (6H, m, $COCH_2$ x2 + CH_2CH_3), 2.13 (3H, s, CH_3), 2.02 (2H, quin, J=7.0Hz, $CH_2CH_2CH_2$), 1.19 (3H, t, J=7.5Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100MHz) δ 172.9 (C=O), 172.3 (C=O), 167.6 (C=O), 149.7 (HNC=N), 149.3 (HNC=N), 140.8 (CH_2), 109.4 (CH), 109.1 (CH), 62.7 (OCH_2), 62.2 (OCH_2), 36.3 (CH_2), 32.9 (CH_2), 30.8 (CH_2), 20.8 (CH_2), 18.2 (CH_3), 9.4 (CH_3). HRMS FAB(m/z) $C_{19}H_{25}N_3O_6$ [$(M+H^+)$] calculated 392.1816 found 392.1820.

Synthesis of poly(MMA-*co*-DAP-*co*-Fl) *random copolymer*



Compound **2** (928mg, 2.0mmol, 20eq), compound **4** (728mg, 2.0mmol, 20eq), methyl methacrylate (1.1mL, 10mmol, 100eq), Cu(I)Cl (8.0mg, 0.08mmol, 0.8eq), Cu(II)Br_2

(4.5mg, 0.02mmol, 0.2eq), PMDETA (17.5mg, 0.10mmol, 1eq) were added to a 2 neck r.b flask, dissolved in DMSO (3mL) and degassed by freeze-pump-thaw (3 cycles) using nitrogen gas as a purge. The flask was then placed in an oil-bath thermostatically maintained at 40°C for 5 minutes before adding EBIB (19.5mg, 15uL, 0.10mmol, 1eq) to the reaction mixture using a gas tight syringe under a fast flow of nitrogen. The flask was sealed under nitrogen and stirred at 40°C with periodic removal of aliquots for GPC analysis of polymer development. The reaction was terminated after 2.5h by diluting with DCM (100mL) and washing with distilled water (100mL x3). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to provide the crude polymer as a white solid. The polymer was dissolved in DCM (20mL) and precipitated into diethyl ether (250mL) then left to settle for approx 1h before collecting by filtration to provide the polymer as a yellow solid. Yield = 1.5g (56%). M_n = 10200 g/mol, PDI =1.11. Final MMA:DAP:flavin monomer ratio (determined by ^{1}HNMR peak integration) = 0.55 : 0.22 : 0.23. This was used to calculate approximate values of repeating groups (n=41) of flavin, MMA and DAP.

2. Characterization of poly(MMA-*co*-DAP-*co*-Fl) by GPC and NMR

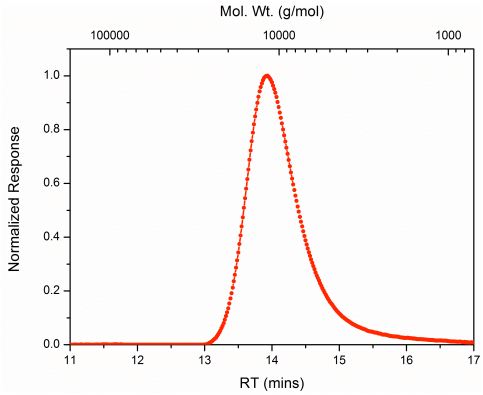


Figure S1 (a). GPC profile of poly(MMA-*co*-DAP-*co*-Fl) using a PL-GPC50 using THF as the eluent at 30°C under a flow rate of 1mL/min calibrated using MMA standards.

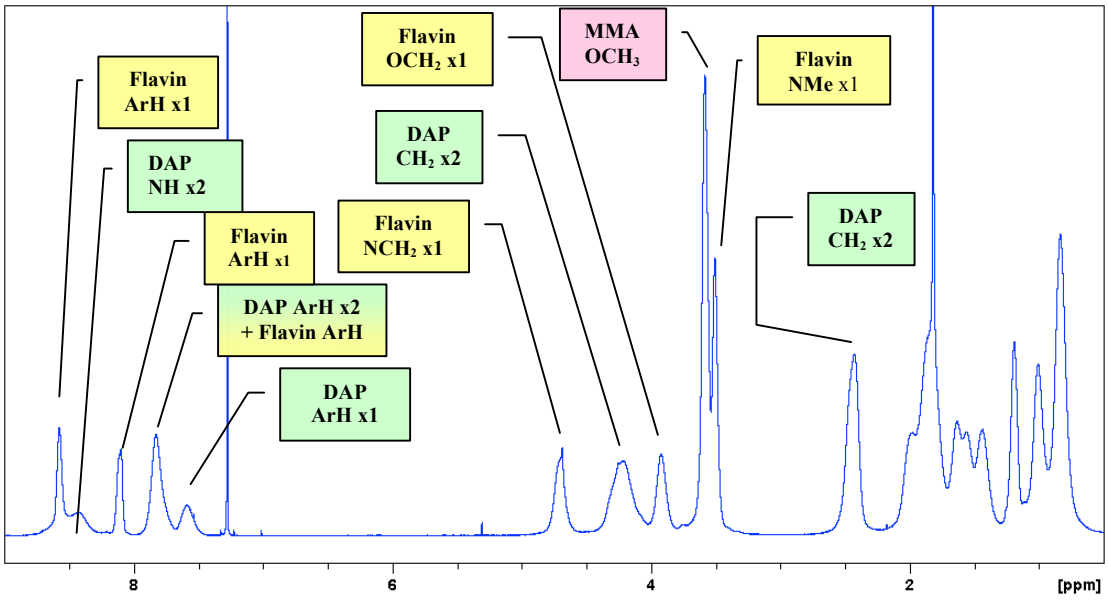


Figure S1 (b). NMR of poly(MMA-*co*-DAP-*co*-Fl).

3. Synthesis of ZnSe quantum dots (QDs)

HDA-capped ZnSe QDs were prepared according to standard hot-injection procedure.[2] In a typical synthesis, 14g HDA was dried, degassed and heated to 315ºC under N_2 flow. 1mL of 1M Et_2Zn in hexane and 1mL of 1M TOPSe stock solution (prepared by dissolving 5mmol of Se in 5mL TOP) were mixed and diluted by another 2mL TOP. The Zn-Se-TOP mixture was rapidly injected into the hot HDA followed by decreasing the reaction temperature to 300 ºC. The reaction was stopped after 30 min growth and the reaction solution yielded light yellow. We carried out extraction and precipitation procedure[3] to purify the ZnSe QDs. Finally, the ZnSe QDs were collected and dispersed in hexane.

4. Ligand exchange on ZnSe QDs

Firstly, thymine functionalized alkanethiol (40mg) and dodecanethiol (60mg) were mixed in 2mL anhydrous DCM. Then 20mg HDA functionalized ZnSe QDs (precipitated by methanol) were dissolved in this ligand solution followed by adding another 3mL DCM. The reaction mixture was stirred under N_2 gas for 2 days at 40ºC. After that, the solvent in the mixture was evaporated. The ZnSe QDs (Thy-QDs) were purified applying hexane-methanol precipitation procedure and redispersed in DCM for further use. For control experiment, N(3)-methylthymine-functionalized ZnSe QDs were prepared following the same procedure.

5. Control experiment (MeThy-QDs and poly(MMA-*co*-DAP-*co*-Fl) mix)

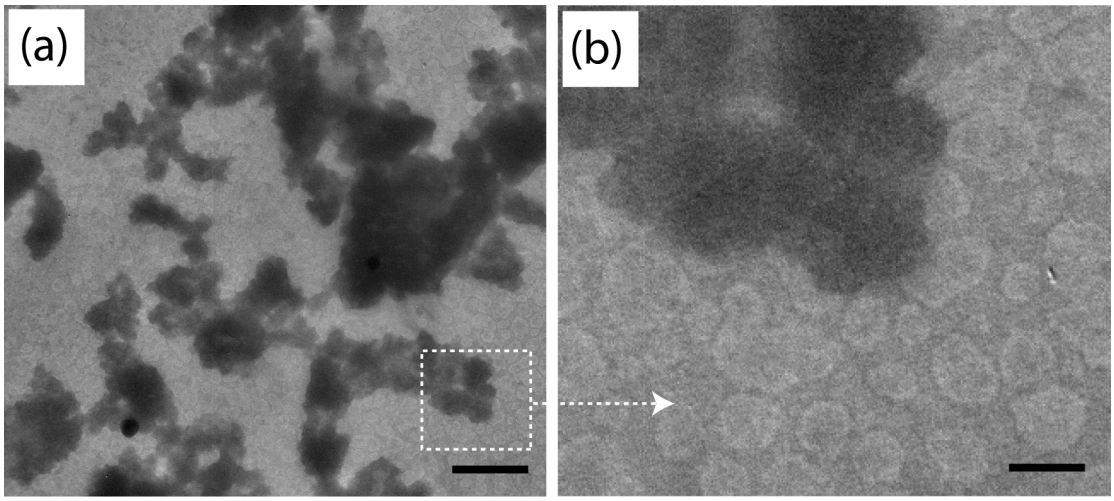


Figure S2. TEM images of MeThy-QDs in presence of poly(MMA-*co*-DAP-*co*-Fl). The scale bar in (a) and (b) are 100 nm and 20 nm, respectively.

6. Characterization of poly(MMA-*co*-DAP-*co*-Fl) by DSC

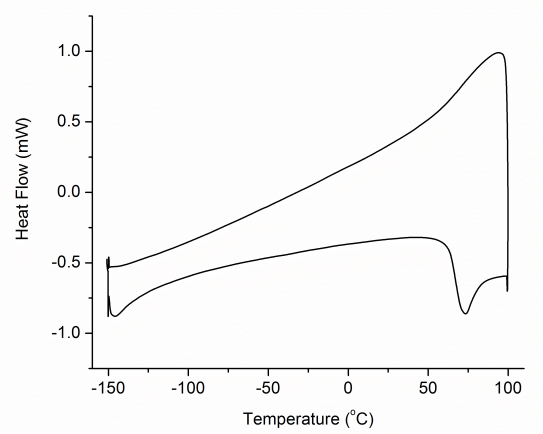


Figure S3. DSC of poly(MMA-*co*-DAP-*co*-Fl).

7. Calculation of FRET efficiency, Forster distance and measured FRET distance between QDs and flavin in Thy-QDs / poly(MMA-*co*-DAP-*co*-Fl) assemblies

FRET efficiency (E) was calculated by [4]

$$E = 1 - \tau_{DA}/\tau_D \qquad [1]$$

Where τ_D and τ_{DA} were the average lifetimes of donor (Thy-QDs) in the absence and presence of the acceptor (poly(MMA-*co*-DAP-*co*-Fl)), respectively.

Forster Distance (R_0) was calculated by [5]

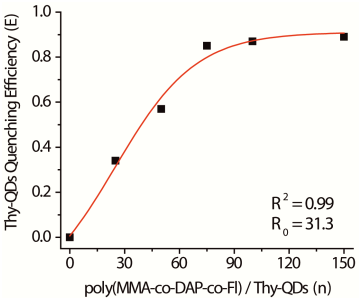
$$R_0 = \left(\frac{[9000 \text{ x } (\ln 10)]k_p^2}{128\,\pi^2 n_D^4 N_A} Q_y I \right)^{1/6} \qquad [2]$$

where Q_Y is the quantum yield of the donor; n_D is the refractive index of the medium (1.4), N_A is Avogadro's number; k_p^2 is the orientation factor (2/3) and I is the integral of the donor–acceptor spectral overlap over all wavelengths.

Measured FRET distance (r) between QDs and flavin was calculated using the single-donor-multiple acceptor FRET model where E can be expressed as [5]

$$E = \frac{nR_0^6}{nR_0^6 + r^6} \qquad [3]$$

where n is the average number of acceptor molecules interacting with one single donor. R_0 is the Forster radius and r is the measured FRET distance.



n	E	r (Å)
25	0.34	60
50	0.57	58
75	0.85	48
100	0.87	49
150	0.89	51

Figure S4. FRET efficiency (E), Forster distance (R_0) and measured FRET distance (r) at different poly(MMA-*co*-DAP-*co*-Fl):Thy-QDs ratios (n).

8. Time-resolved fluorescence measurements

In the absence of flavin, the fluorescence decay of the Thy-QDs has a very non-exponential character. Initial multiexponential decay corresponds to radiative recombination of neutral, single- and doubly-charged excitons.[6] At times longer than ~200 ns, fluorescence decays according to power law with time constant ~-2.5. The nature of the long-time power law asymptotics is still widely debated. Possible mechanism resulting in this phenomenon includes diffusion of the electrons between the dark and bright states,[7] forward and backward transfer of a carrier between the excited state of a quantum dot and a trap state in the surrounding.[8] Further discussion of the QD decay mechanism is beyond the scope of this paper. In this work, we fitted the QD fluorescence decays *F(t)* to mulitexponential function after the convolution with the instrument response function (IRF):

$$F(t) = IRF \otimes \sum_{i=1}^{n} A_i \exp(-t/\tau_i) \qquad [4]$$

where *n* was 3 or 4, depending on the fitting quality. The fitting results are presented in Table S1. Again, we'd like to stress that the individual exponentials have very little physical sense, so for FRET analysis we used the amplitude-weighted average lifetimes of QDs ($\tau_{av} = \sum_{i=1}^{n} \frac{A_i \tau_i}{\sum_{i=1}^{n} A_i}$) and their dependence on the QDs/polymer ratio as a measure of FRET efficiency:

$$E = 1 - \tau_{DA}/\tau_D \qquad [5]$$

Where τ_{DA} and τ_D are the average lifetimes of donor (QDs) in the presence and in the absence of acceptor (flavin).

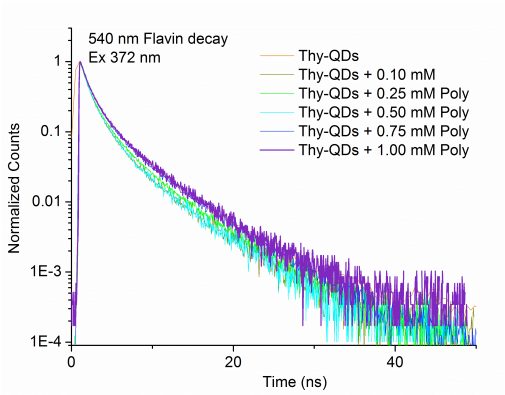


Figure S5. Time resolved fluorescence decay curves of Thy-QDs alone and in presence of poly(MMA-*co*-DAP-*co*-Fl) at excitation 372 nm and emission 540 nm.

Upon increase of the flavin content in the copolymer, the lifetime of the QDs measured at 430 nm decreased (Figure 3d), while the lifetime of the flavin measured at 540 nm practically did not change (Figure S5). This is a classic behaviour of the energy donor-acceptor pair confirming our observation of FRET based on the steady-state emission data from the previous paragraph. The average lifetimes of QDs and flavin are presented in Table S1.

Sample	τ_1, ns[1]	A_1[2]	τ_2, ns	A_2	τ_3, ns	A_3	τ_4, ns	A_4
Thy-QDs	0.083	0.54	0.86	0.29	5.9	0.11	28	0.06
Thy-QDs+ 0.25 mM Poly	0.38	0.80	3	0.16	20	0.04		
Thy-QDs+ 0.50 mM Poly	0.12	0.78	1.22	0.15	6	0.05	25	0.02
Thy-QDs+ 0.75 mM Poly	0.025	0.81	0.41	0.12	2.1	0.05	10.5	0.02
Thy-QDs+ 1.00 mM Poly	0.018	0.75	0.3	0.18	2.4	0.06	13.5	0.01
Thy-QDs+ 1.50 mM Poly	0.062	0.86	1.26	0.13	8.5	0.01		

Table S1. Values of the time constants (τ_i) and normalized (to 1) pre-exponential factors (Ai) of the multi-exponential function fitting the ps-emission transients of Thy-QDs / of poly(MMA-*co*-DAP-*co*-Fl) conjugates in DCM. The excitation wavelength was 372 nm.

[1]The fit quality was inspected using the weighted residuals, and the values of χ^2 which in all cases was < 1.1.

[2]All amplitudes are normalized in a following way: $\sum_{i=1}^{n} A_i = 1$

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[5] I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher and J. M. Mauro, *Nat. Mater.*, 2003, **2**, 630-638.

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