Supporting information

Time-Resolved Fluorescence Spectroscopy Reveals Fine Structure and Dynamics of Poly(L-lysine) and Polyethylenimine Based DNA Polyplexes

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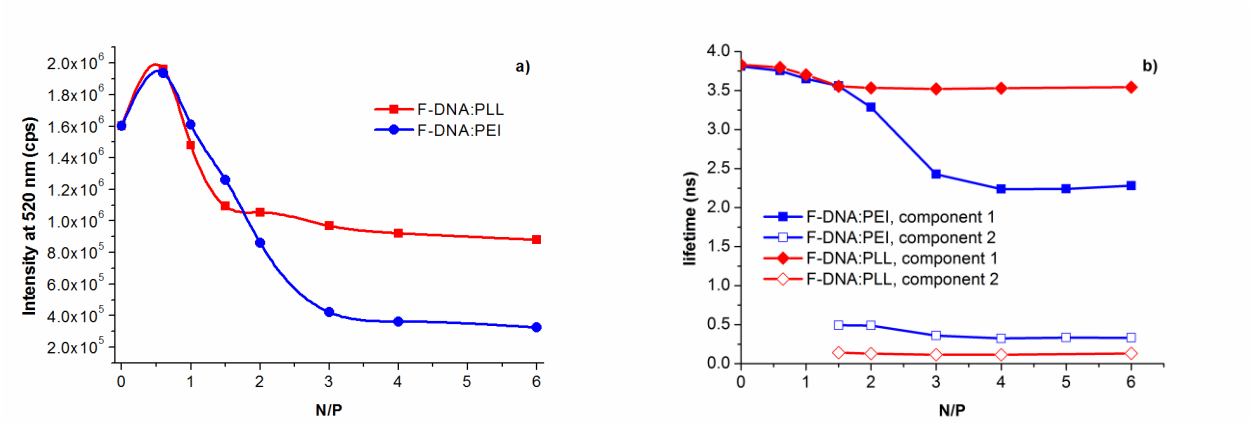

Figure S1. a) Area of the fluorescence spectra for F-DNA:PLL and F-DNA:PEI at different N/P ratios. b) Fluorescence lifetimes for F-DNA, F-DNA:PLL and F-DNA:PEI at different N/P ratios. Excitation wavelength was 483 nm. Lifetimes were calculated using global fitting with equation 1.

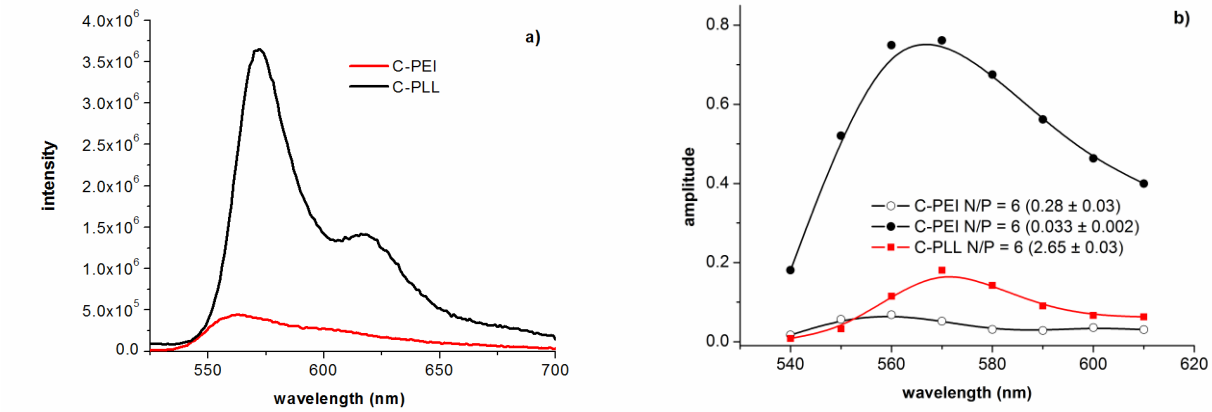

Figure S2. a) Fluorescence spectra and b) DAS of C-PLL and C-PEI at the concentration corresponding to N/P ratio 6. Excitation wavelength was 483 nm. DAS were calculated using global fitting with equation 1 for C-PLL and equation 2 for C-PEI. The lifetimes of each component are denoted in the legend.

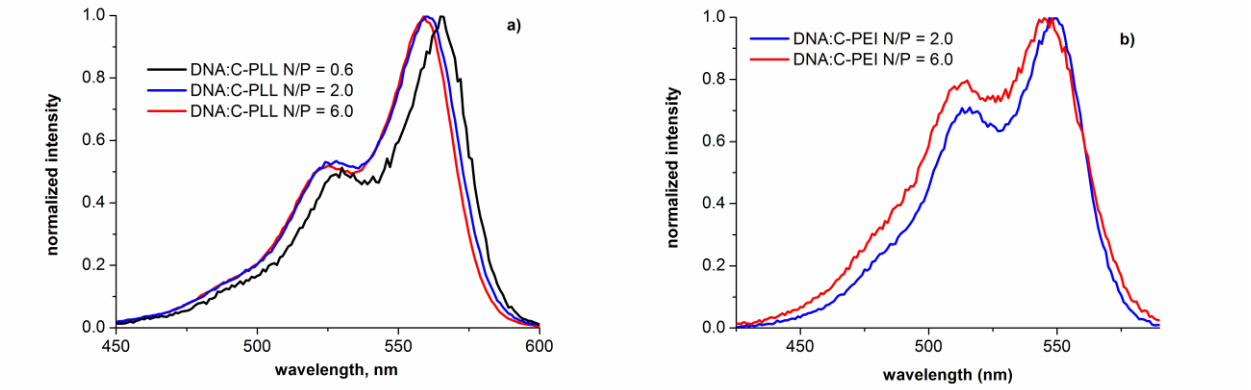

Figure S3. Normalized excitation spectra for a) C-PLL in the presence of unlabeled DNA at N/P = 0.6, 2 and 6 and b) C-PEI in the presence of unlabeled DNA at N/P = 2 and 6. Monitoring wavelength was 610 nm.

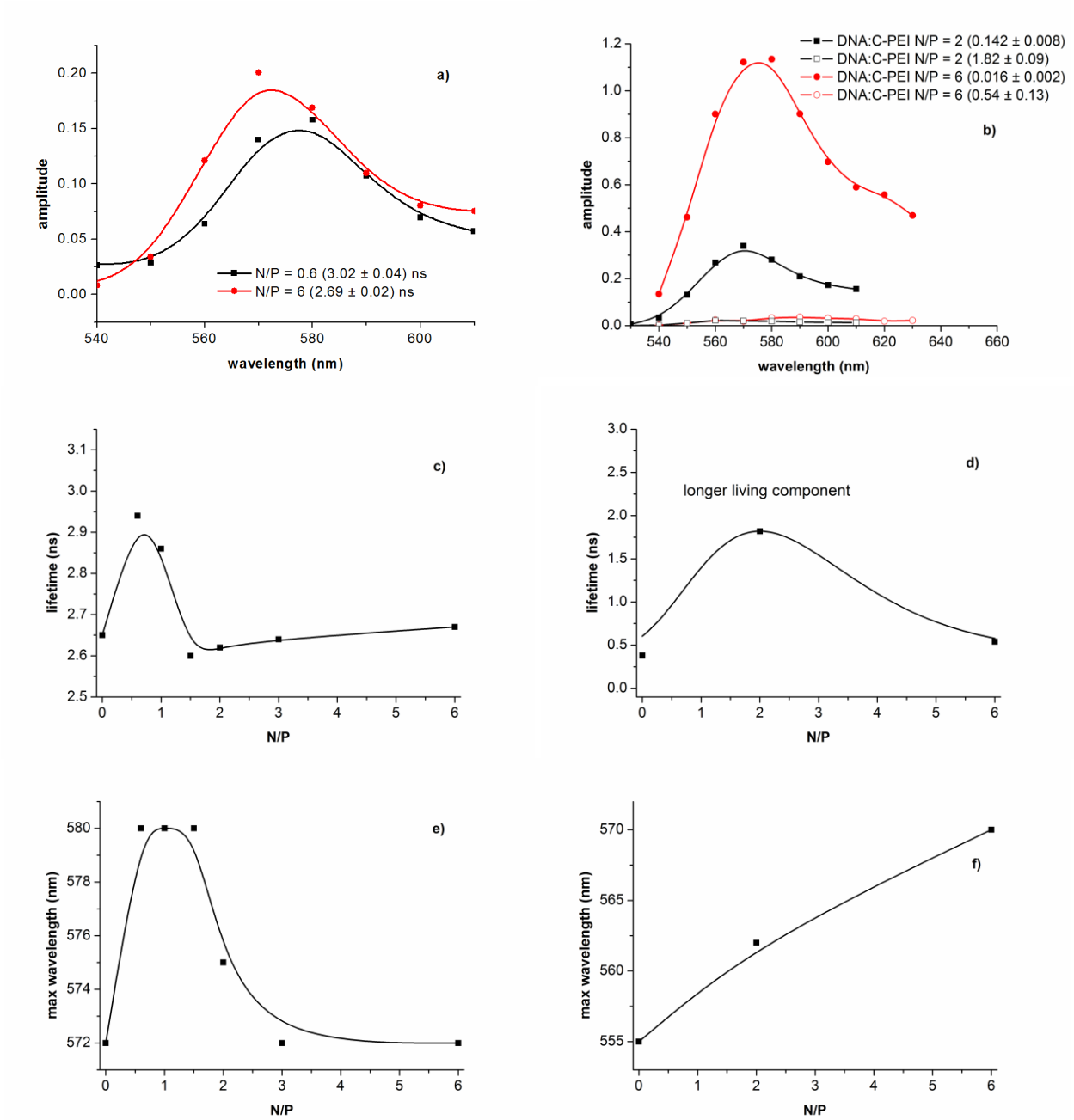

Figure S4. DAS, change in the fluorescence lifetime (0 point corresponds to DNA free system) and the DAS maximum wavelength change for C-PLL (a, c, e) and C-PEI (b, d, f) in the presence of unlabeled DNA. Excitation wavelength was 483 nm. Lifetimes and DAS were calculated using global fitting with equation 1 for C-PLL and equation 2 for C-PEI. The lifetimes of each component in DAS are denoted in the legends.

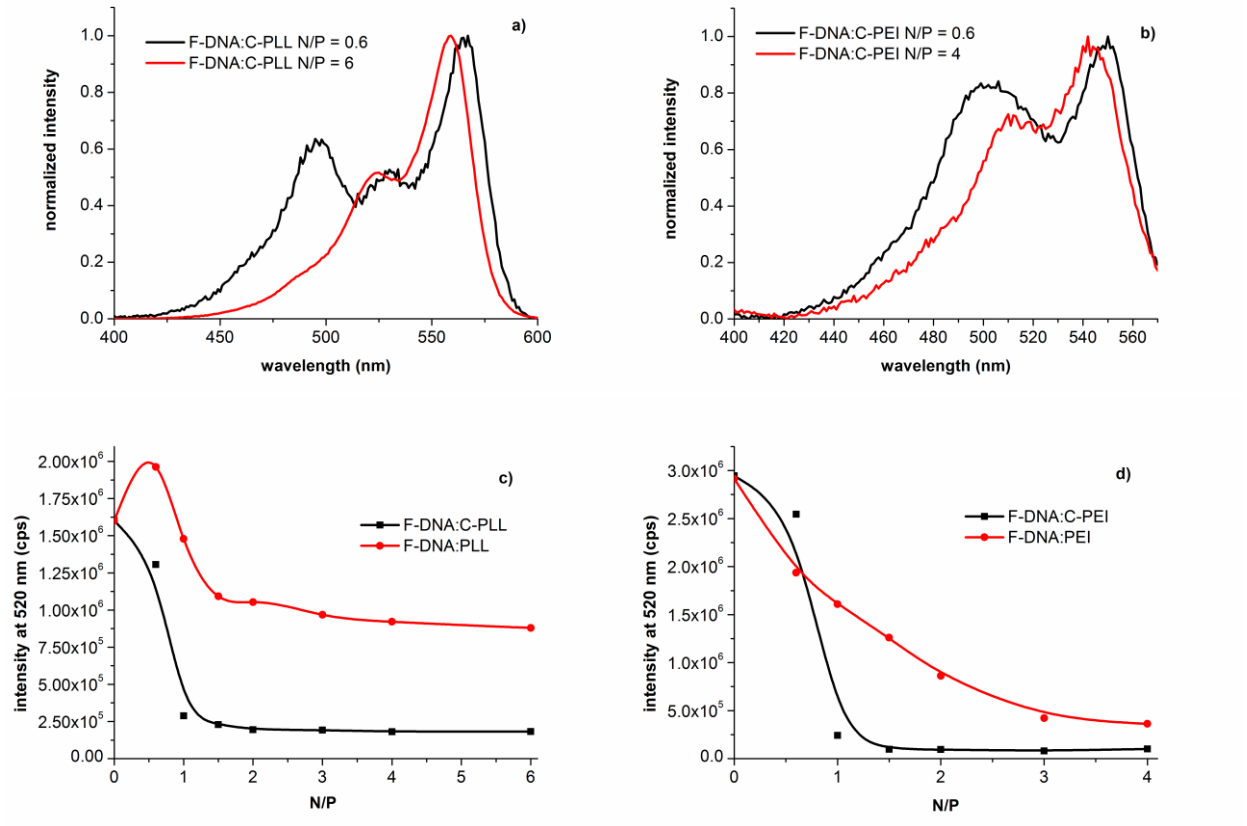

Figure S5. Normalized excitation spectra for a) F-DNA:C-PLL system at N/P 0.6 and 6; b) F-DNA:C-PEI system at N/P 0.6 and 4. Fluorescence intensity as a function of N/P ratio for c) F-DNA:C-PLL and F-DNA:PLL; d) F-DNA:C-PEI and F-DNA:PEI systems at 520 nm, at the maximum wavelength of F-DNA. The excitation spectra were monitored at 610 nm. Excitation wavelength was 483 nm.

Table S1. Lifetimes, β parameters and weighted mean square deviations χ^2 for F-DNA:C-PLL and F-DNA:PLL:C-PLL, F-DNA:C-PEI and F-DNA:PEI:C-PEI at different N/P ratios.

Sample[a]	Fitting model[c]	τ_1 (exp)	τ_2 (st exp)	β	χ^2
F-DNA:C-PEI$_{0,6}$	exp + st exp	3.77 ± 0.03 (55%)	0.10 (45%)[d]	0.391 ± 0.029	1.01
F-DNA:C-PEI$_1$	exp + st exp	3.41 ± 0.19 (1.5%)	0.030 (99%)[d]	0.401 ± 0.005	0.89
F-DNA:C-PEI$_{1,5}$	exp + st exp	3.09 ± 0.28 (1%)	0.043 (99%)[d]	0.426 ± 0.007	1.02
F-DNA:C-PEI$_2$	exp + st exp	3.13 ± 0.23 (1%)	0.022 (99%)[d]	0.439 ± 0.009	0.89
F-DNA:C-PEI$_3$	exp + st exp	3.11 ± 0.22 (1%)	0.028 (99%)[d]	0.499 ± 0.013	0.92
F-DNA:C-PEI$_4$	exp + st exp	3.13 ± 0.04 (1%)	0.035 (99%)[d]	0.544 ± 0.015	0.92
F-DNA:PEI$_2$	exp	3.27 ± 0.05 (100%)	-	-	0.97
F-DNA:PEI$_2$:C-PEI$_3$	exp + st exp	2.88 ± 0.11 (8%)	0.103 (92%)[d]	0.483 ± 0.016	0.82
F-DNA:PEI$_2$:C-PEI$_4$	exp + st exp	3.04 ± 0.14 (3%)	0.055 (97%)[d]	0.505 ± 0.008	0.81
F-DNA:PEI$_2$:C-PEI$_5$	exp + st exp	3.00 ± 0.13 (3%)	0.053 (97%)[d]	0.560 ± 0.017	0.79
F-DNA:PEI$_2$:C-PEI$_6$	exp + st exp	3.00 ± 0.12 (3%)	0.079 (97%)[d]	0.798 ± 0.031	0.81
Sample[b]	Fitting model[c]	τ_1 (exp)	τ_2 (exp)		χ^2

F-DNA:C-PLL$_{0.6}$	exp	3.69 ± 0.05 (89%)	1.60 ± 0.33 (11%)	-	1.11
F-DNA:C-PLL$_1$	exp	2.58 ± 0.06 (47%)	0.46 ± 0.06 (53%)	-	1,39
F-DNA:C-PLL$_{1.5}$	exp	2.60 ± 0.07 (39%)	0.40 ± 0.05 (61%)	-	1,39
F-DNA:C-PLL$_2$	exp	2.68 ± 0.07 (40%)	0.41 ± 0.06 (60%)	-	1,27
F-DNA:C-PLL$_3$	exp	2.72 ± 0.08 (44%)	0.44 ± 0.07 (56%)	-	1,17
F-DNA:C-PLL$_4$	exp	2.71 ± 0.09 (46%)	0.37 ± 0.08 (54%)	-	1,01
F-DNA:C-PLL$_6$	exp	2.76 ± 0.11 (53%)	0.33 ± 0.12 (47%)	-	0,95
F-DNA:PLL$_2$	exp	3.49 ± 0.03 (91%)	0.11 (9%)[d]	-	1.15
F-DNA:PLL$_2$:C-PLL$_3$	exp	2.92 ± 0.04 (68%)	0.46 ± 0.11 (32%)	-	1.45
F-DNA:PLL$_2$:C-PLL$_4$	exp	2.84 ± 0.06 (63%)	0.50 ± 0.12 (37%)	-	1.24
F-DNA:PLL$_2$:C-PLL$_6$	exp	2.73 ± 0.07 (61%)	0.39 ± 0.12 (39%)	-	1.10

[a] for C-PEI containing polyplexes the global fitting of the decay curves at all wavelengths was performed.
[b] for C-PLL containing polyplexes the decay curves monitored at 520 nm were taken for the fitting.
[c] exp: sum of exponents model (eq 1); exp + st exp: mixed model containing exponential and stretched exponential parts (eq 2).
[d] it is not possible to determine the accuracy for the lifetimes ≤ 0.1 ns due to the time resolution of the system.

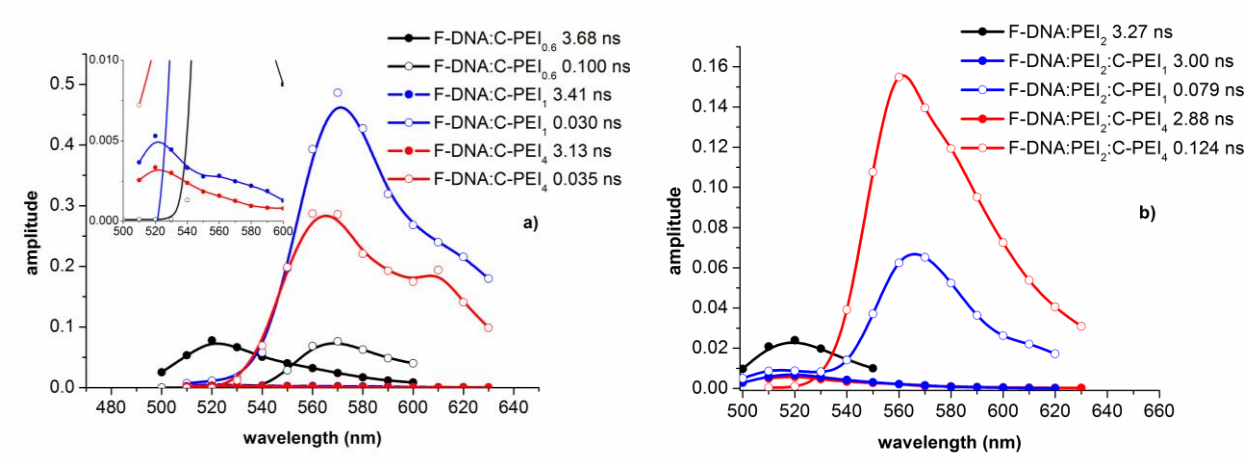

Figure S6. DAS spectra for a) F-DNA:C-PEI and b) F-DNA:PEI2:C-PEI1-4 systems at different N/P ratios, calculated using global fitting with equation 2. Excitation wavelength was 483 nm.