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Supporting Information

for

Probing the Inner Local Density of Complex Macromolecules by Pyrene Excimer Formation

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A] Fluorescence Measurements

Fluorescence measurements were conducted on polymer solutions having an absorbance of 0.1 at 344 nm, which corresponded to a pyrene concentration of 2.5×10^{-6} M. The solutions were placed in quartz degassing cells and nitrogen gas (Praxair, 4.8-T) was bubbled for 30 minutes through the solutions in THF and toluene or for 45 minutes through the solutions in DMF and DMSO, to remove dissolved oxygen which is a known quencher of pyrene.

The time-resolved fluorescence decays were acquired using the right-angle geometry with a IBH Ltd. time-resolved fluorometer and excited at 344 nm using an IBH 340 NanoLED. The monomer and excimer fluorescence decays were acquired at 375 and 510 nm using a 370 and 495 nm cut-off filter to prevent scattered light from reaching the detector, respectively. For the Py-PC_1A and Py-PNIPAM samples randomly labeled with pyrene and the Py-PNIPAM samples with a number average molecular weight (M_n) of 5.9 and 7.6 kDa, both the monomer and excimer fluorescence decays were acquired up to 20,000 counts at the decay maximum. However, since the Py_2-PNIPAM sample with an M_n of 13.7 kDa formed little excimer, its excimer fluorescence decays were acquired up to a decay maximum of 10,000 counts.

B] Time-Resolved Fluorescence Decay Analysis

The time-resolved fluorescence decays of the monomer and excimer of the polymers randomly labeled with pyrene were analysed according to the fluorescence blob model (FBM) by fitting the decays globally to Equations S1 and S2, respectively.

$$[Py^*] = \left[Py^*_{diff}\right]_{t=0} \exp\left(-\left(A_2 + \frac{1}{\tau_M}\right)t - A_3\left(1-\exp(-A_4 t)\right)\right) +$$
$$\left([Py^*_{k2}]_{t=0} + \left[Py^*_{diff}\right]_{t=0} \exp(A_3) \sum_{i=0}^{\infty} \frac{A_3^{\,i}}{i!} \frac{A_2 + iA_4}{A_2 + iA_4 - k_2}\right) \exp\left(-\left(k_2 + \frac{1}{\tau_M}\right)t\right)$$
$$-\left[Py^*_{diff}\right]_{t=0} \exp(A_3) \sum_{i=0}^{\infty} \frac{A_3^{\,i}}{i!} \frac{A_2 + iA_4}{A_2 + iA_4 - k_2} \exp\left(-\left(A_2 + iA_4 + \frac{1}{\tau_M}\right)t\right)$$
$$+[Py^*_{free}]_{t-0} \exp\left(-\frac{t}{\tau_M}\right) \qquad \text{(S1)}$$

$$[E^*] = k_2 \left([Py^*_{k2}]_{t=0} + [Py^*_{diff}]_{t=0} \exp(-A_3) \sum_{i=0}^{\infty} \frac{A_3^{\,i}}{i!} \frac{A_2 + iA_4}{A_2 + iA_4 - k_2}\right)$$
$$\times \frac{\exp\left(-\frac{t}{\tau_{E0}}\right) - \exp\left(-\left(k_2 + \frac{1}{\tau_M}\right)t\right)}{k_2 + \frac{1}{\tau_M} - \frac{t}{\tau_{E0}}} + [Py^*_{diff}]_{t=0} \exp(-A_3)$$
$$\times \sum_{i=0}^{\infty} \frac{A_3^{\,i}}{i!} \frac{A_2 + iA_4}{A_2 + iA_4 - k_2} \frac{\exp\left(-\left(A_2 + iA_4 + \frac{1}{\tau_M}\right)t\right) - \exp\left(-\frac{t}{\tau_{E0}}\right)}{A_2 + iA_4 + \frac{1}{\tau_M} - \frac{1}{\tau_{E0}}} \qquad \text{(S2)}$$
$$+[E0^*]_{t=0} \times \exp\left(-\frac{t}{\tau_{E0}}\right) + [ED^*]_{t=0} \times \exp\left(-\frac{t}{\tau_D}\right)$$

In Equations S1 and S2, τ_M, τ_{E0}, and τ_D are the lifetimes of the pyrene monomer and of the pyrene excimer formed with two properly and two poorly stacked pyrenyl moieties, respectively. Where τ_M is fixed in the analysis, τ_{E0}, τ_D, and the parameters A_2, A_3, and A_4 are optimized in the decay analysis. A_2, A_3, and A_4 are related to the average number <*n*> of ground-state pyrenes in a *blob*, the rate constant k_{blob} for diffusive encounters between two structural units bearing a pyrenyl label, and the product $k_e \times [blob]$ representing the exchange of ground-state pyrenyl labels between *blobs* according to Equation S3.[1-2]

$$A_2 =< n > \frac{k_{blob} k_e [blob]}{k_{blob} + k_e [blob]} \tag{S3a}$$

$$A_3 =< n > \left(\frac{k_{blob}}{k_{blob} + k_e [blob]} \right)^2 \tag{S3b}$$

$$A_4 = k_{blob} + k_e [blob] \tag{S3c}$$

The end-labeled polymers were analysed according to the model free analysis (MFA), which fits the monomer and excimer fluorescence decays globally according to Equations S4 and S5, respectively. In Equation S4, the parameters τ_i are the decay times and their associated pre-exponential factors (a_i) were normalized to unity ($\Sigma a_i = 1$). The a_i and τ_i values were kept the same in Equations S4 and S5 during the MFA of the fluorescence decays. The index "M" in the molar fractions f_{Mdiff} and f_{Mfree}, which represent the pyrenyl species that encounter each other through diffusion and do not form excimer and emit with their natural lifetime τ_M, respectively, indicates that these pyrenyl species are only detected in the monomer decay.

$$[Py^*] = [Py^*]_{t=0} \left[f_{Mdiff} \times \sum_{i=1}^{n} a_i \exp\left(\frac{-t}{\tau_i} \right) + f_{Mfree} \exp\left(\frac{-t}{\tau_M} \right) \right] \tag{S4}$$

$$[E^*] = [Py^*]_{t=0} \left[-f_{EdiffE0} \times \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_{E0}}} \exp\left(\frac{t}{\tau_i} \right) + \left(f_{EE0} + f_{EdiffE0} \right) \times \sum_{i=1}^{n} a_i \frac{\frac{1}{\tau_i} - \frac{1}{\tau_M}}{\frac{1}{\tau_i} - \frac{1}{\tau_{E0}}} \exp\left(\frac{t}{\tau_{E0}} \right) + f_{ED} \exp\left(\frac{t}{\tau_D} \right) \right] \tag{S5}$$

Equations S5 fits the excimer decay by assuming three possible excimer species. The short-lived ($E0^*$) and long-lived (D^*) excimer species with lifetimes τ_{E0} and τ_D are formed by excitation of well and poorly stacked pre-aggregated pyrenes and are represented by the molar fractions f_{EE0} and

f_{ED}, respectively. The excimer species *E*0* can also be formed by diffusive encounters and are represented by the molar fraction $f_{EdiffE0}$. The index "E" in the molar fractions indicates that these pyrenyl species were only detected in the excimer fluorescence decays.

C] Parameters Retrieved from the Analysis of the TFR Decays

Table S1. Parameters obtained from the fit of the Py(*x*)-C1A monomer decays in toluene and DMF using the FBM program *globmis90bbg* where k_2 is fixed in the analysis.

Solvent	Mol %	k_{blob} (10^{-6} s^{-1})	<*n*>	$k_e[blob]$ (10^{-6} s^{-1})	f_{Mdiff}	f_{k2}	f_{Mfree}	χ^2
Toluene $k_2 = 2.03\times10^{-8}$ s τ_M = 181 ns	1.7	18.5	1.33	9.44	0.65	0.27	0.08	1.13
	2.6	12.9	2.10	7.05	0.72	0.25	0.03	1.24
	3.0	14.3	2.05	6.49	0.71	0.27	0.02	1.14
	6.2	15.0	3.76	10.05	0.48	0.49	0.03	1.29
	6.7	15.5	3.84	6.49	0.42	0.57	0.01	1.22
DMF $k_2 = 1.25\times10^{-8}$ s τ_M = 165 ns	1.7	12.7	0.97	5.07	0.75	0.17	0.09	1.29
	2.6	10.2	1.59	5.30	0.71	0.24	0.04	1.22
	3.0	10.0	1.74	6.03	0.68	0.25	0.07	1.20
	6.2	10.5	3.10	4.26	0.52	0.46	0.02	1.29
	6.7	9.5	3.70	3.84	0.44	0.55	0.01	1.26

Table S2. Parameters obtained from the fit of the Py(*x*)-C1A excimer decays in toluene and DMF using the FBM program *globmis90bbg* where k_2 is fixed in the analysis.

Solvent	Mol %	f_{Ek2}	τ_{E0} (ns)	f_{E0diff}	f_{EE0}	τ_D (ns)	f_{ED}	χ^2
Toluene	1.7	0.28	51	0.68	0.034	150	0.01	1.13
	2.6	0.25	50	0.71	0.000	88	0.04	1.24
	3.0	0.27	51	0.69	0.020	76	0.02	1.14
	6.2	0.47	50	0.46	0.000	74	0.08	1.29
	6.7	0.51	50	0.38	0.086	82	0.02	1.22
DMF	1.7	0.18	58	0.79	0.000	86	0.03	1.29
	2.6	0.25	52	0.73	0.000	109	0.02	1.22
	3.0	0.26	52	0.71	0.000	115	0.02	1.20
	6.2	0.43	50	0.48	0.007	72	0.08	1.29
	6.7	0.51	51	0.41	0.027	73	0.05	1.26

Table S3. Parameters obtained from the fit of the Py(*x*)-PNIPAM monomer decays in THF, DMF, and DMSO using the FBM program *globmis90bbg* where k_2 is fixed in the analysis.

Solvent	Mol %	k_{blob} (10^{-6} s^{-1})	<*n*>	k_e[*blob*] (10^{-6} s^{-1})	f_{Mdiff}	f_{k2}	f_{Mfree}	χ^2
THF	2.7	5.6	1.3	3.07	0.48	0.22	0.30	1.06
$k_2 = 0.91\times10^{-8}$ s	3.6	5.9	1.6	5.15	0.53	0.33	0.14	1.13
τ_M = 184 ns	4.2	7.3	1.4	4.82	0.60	0.33	0.07	1.08
	5.1	5.6	2.2	4.00	0.54	0.43	0.04	1.21
	6.3	4.7	3.0	3.40	0.49	0.48	0.03	1.14
DMF	2.7	10.2	0.84	5.74	0.61	0.17	0.22	1.14
$k_2 = 1.15\times10^{-8}$ s	3.6	10.2	1.15	6.05	0.68	0.23	0.10	1.12
τ_M = 166 ns	4.2	11.0	1.21	6.52	0.72	0.23	0.05	1.13
	5.1	8.0	1.89	5.09	0.65	0.33	0.02	1.06
	6.3	8.8	2.08	6.59	0.61	0.36	0.03	1.07
DMSO	2.7	15.3	0.65	6.63	0.57	0.13	0.29	1.14
$k_2 = 0.97\times10^{-8}$ s	3.6	11.2	0.92	6.33	0.68	0.19	0.13	1.23
τ_M = 136 ns	4.2	12.7	0.93	8.29	0.71	0.18	0.10	1.16
	5.1	7.8	1.52	6.05	0.68	0.27	0.05	1.14
	6.3	8.2	1.72	6.19	0.65	0.31	0.04	1.09

Table S4. Parameters obtained from the fit of the Py(*x*)-PNIPAM excimer decays in THF, DMF, and DMSO using the FBM program *globmis90bbg* where k_2 is fixed in the analysis.

Solvent	Mol %	f_{Ek2}	τ_{E0} (ns)	f_{E0diff}	f_{EE0}	τ_D (ns)	f_{ED}	χ^2
THF	2.7	0.26	50	0.58	0.053	130	0.11	1.06
	3.6	0.32	56	0.53	0.112	150	0.03	1.13
	4.2	0.29	57	0.53	0.152	126	0.03	1.08
	5.1	0.38	57	0.48	0.129	157	0.01	1.21
	6.3	0.42	52	0.44	0.064	95	0.08	1.14
DMF	2.7	0.19	47	0.70	0.013	108	0.09	1.14
	3.6	0.22	49	0.65	0.000	98	0.13	1.12
	4.2	0.21	51	0.65	0.057	92	0.09	1.13
	5.1	0.31	52	0.61	0.029	90	0.05	1.06
	6.3	0.34	51	0.58	0.058	101	0.02	1.07
DMSO	2.7	0.16	48	0.68	0.068	103	0.09	1.14
	3.6	0.19	47	0.67	0.016	94	0.12	1.23
	4.2	0.18	46	0.69	0.000	90	0.13	1.16
	5.1	0.26	49	0.66	0.035	94	0.04	1.14
	6.3	0.29	49	0.62	0.063	98	0.03	1.09

Table S5. Parameters obtained from the fit of the Py_2-PNIPAM monomer decays in THF and DMF using the MFA program *sumegs12bg*.

Solvent	MW (kDa)	DP	τ_1	a_1	τ_2	a_2	f_{Mfree}	χ^2
THF τ_M = 203 ns	5.9	52	46	0.09	149	0.78	0.01	1.12
	7.6	67	51	0.08	161	0.80	0.12	1.09
	13.7	121	47	0.05	180	0.94	0.01	1.01
DMF τ_M = 166 ns	5.9	52	50	0.06	134	0.81	0.14	1.00
	7.6	67	49	0.06	143	0.84	0.10	1.06
	13.7	121	36	0.03	151	0.57	0.40	0.97

Table S6. Parameters obtained from the fit of the Py_2-PNIPAM excimer decays in THF and DMF using the MFA program *sumegs12bg*.

Solvent	MW (kDa)	DP	f_{diff}^{E0}	τ_{E0} (ns)	τ_D (ns)	f_{EE0}	f_{ED}	χ^2
THF τ_M = 203 ns	5.9	52	0.98	70	117	0.000	0.018	1.12
	7.6	67	0.99	73	150	0.012	0.001	1.09
	13.7	121	0.98	81	107	0.017	0.001	1.01
DMF τ_M = 166 ns	5.9	52	0.98	63	153	0.010	0.01	1.00
	7.6	67	0.99	72	123	0.000	0.01	1.06
	13.7	121	0.97	79	156	0.010	0.02	0.97

**Due to the greatly reduced excimer formed in DMF (η = 0.79 mPa.s), the Py_2-PNIPAM samples were not acquired in DMSO (η = 2.0 mPa.s).

C] REFERENCES

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