Electronic Supplementary Material (ESI) for Polymer Chemistry

Electronic Supporting Information (ESI)

Biotin-end-Functionalized Highly Fluorescent Water-Soluble Polymers

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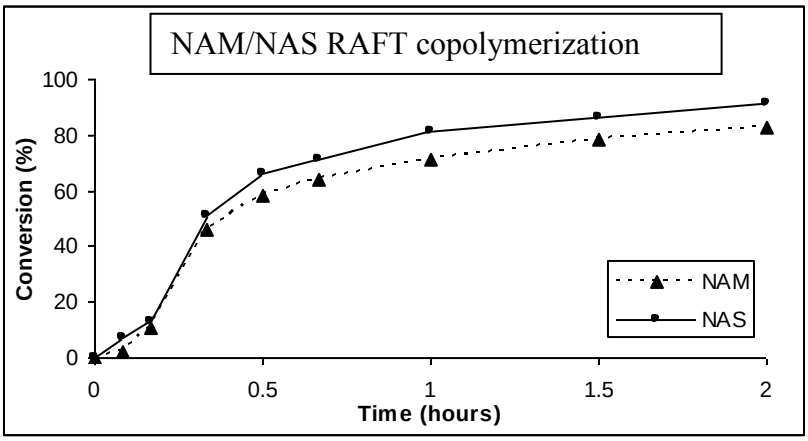


Fig. S1(A) Conversion versus time curve of NAM/NAS RAFT copolymerization (sample P1, 2h).

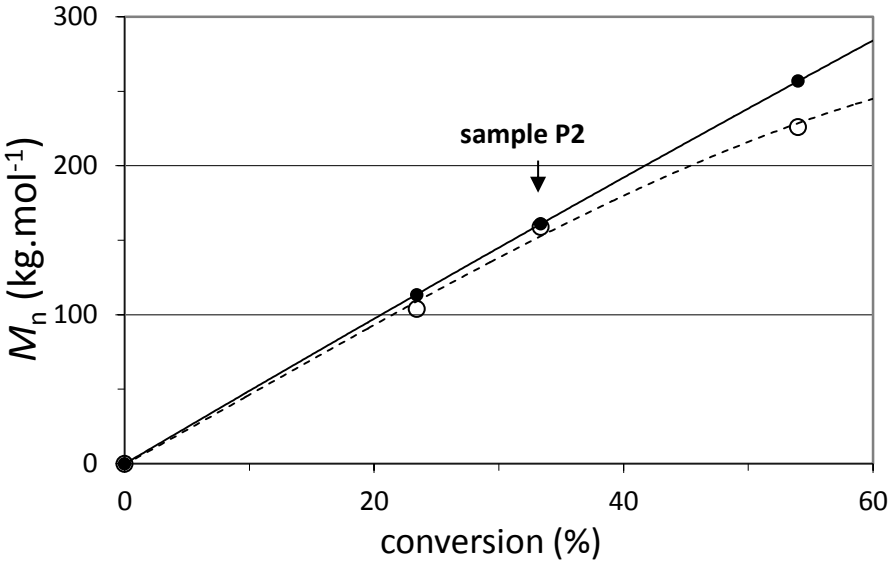


Fig. S1(B) Evolution of molar masses versus conversion for the RAFT copolymerization conducted to produce sample P2: theoretical M_n (●), experimental M_n (○).

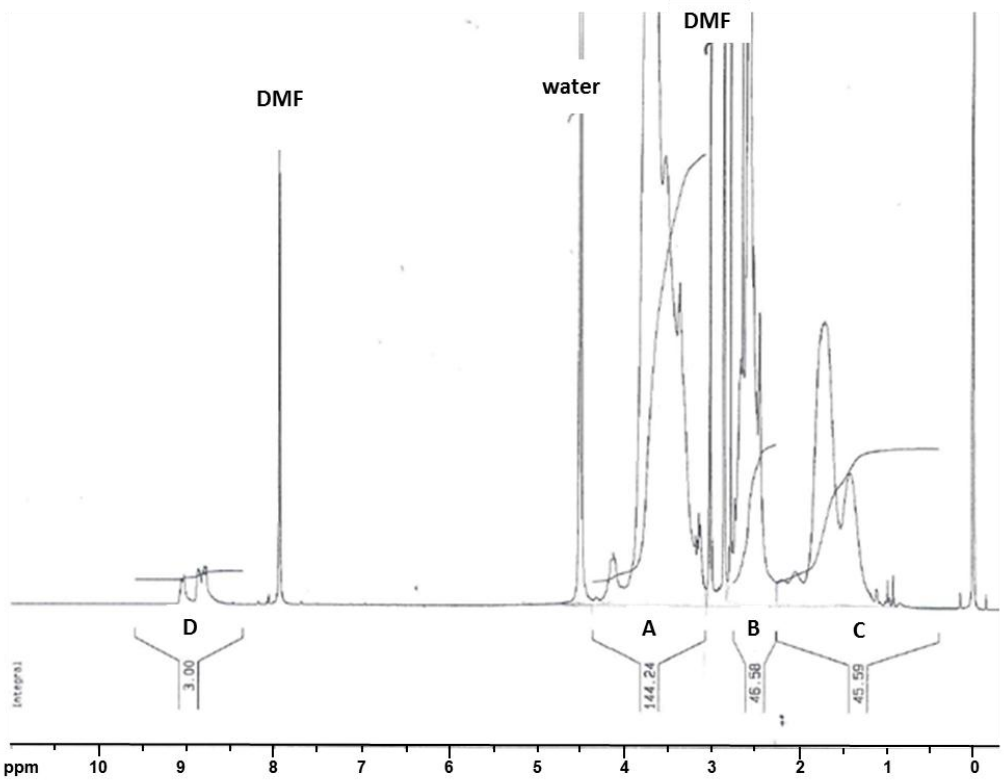


Fig. S2 ^{1}H NMR spectrum (400 MHz, D_2O, TMSP) of labeled poly(NAM-LY) sample C_2.

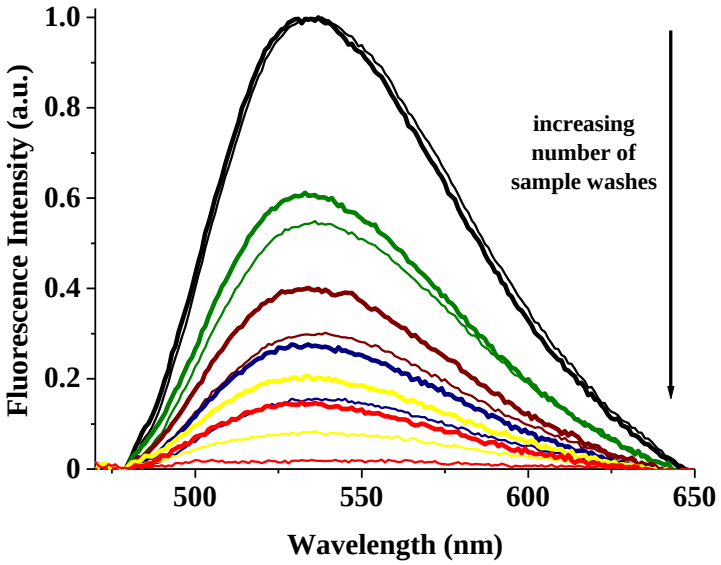


Fig. S3 Fluorescence emission spectra of poly(NAM-LY)-B (heavy lines) and poly(NAM-LY) (solid lines) mixed with PS-streptavidin, using excitation light at 430 nm, before centrifugation/washing (black) and after 1 (green), 2 (wine), 3 (blue), 4 (yellow) and 6 (red) washes.

Table S1 Fluorescence decay curve parameters of the poly(NAM-LY) samples, obtained at 525 nm (excitation at 290 nm).

Poly(NAM-LY) sample (reaction time)	**[LY]** (M)	τ_1 (ns)	A_1	τ_2 (ns)	A_2	$\langle\tau\rangle$ (ns)	χ^2
C1 (96h)	1.00×10^{-6}	5.2	10.7	9.7	11.4	8.2	1.29
C2 (172h)	0.70×10^{-6}	4.3	7.3	8.0	11.9	7.1	1.16
C3 (96h)	0.58×10^{-6}	3.4	3.6	6.9	9.8	6.3	1.05

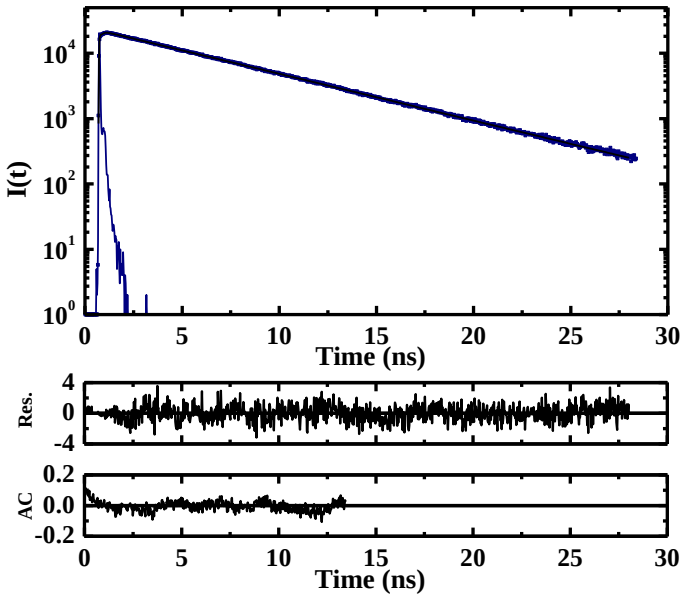


Fig. S4 Fluorescence decay curve for poly(NAM-LY) sample C3 in buffered solutions (50 mM; pH 7.5). Solution was excited at 290 nm and emission was monitored at 525 nm. The solid black line represents the best fit to the data. The lamp profile is also shown in solid blue line. The weighted residuals and autocorrelation of residuals are shown below the decay curve.

Table S2 Decay parameters for LY/AEM phosphate buffered mixed solutions, with constant dye concentration, 10^{-5} M.

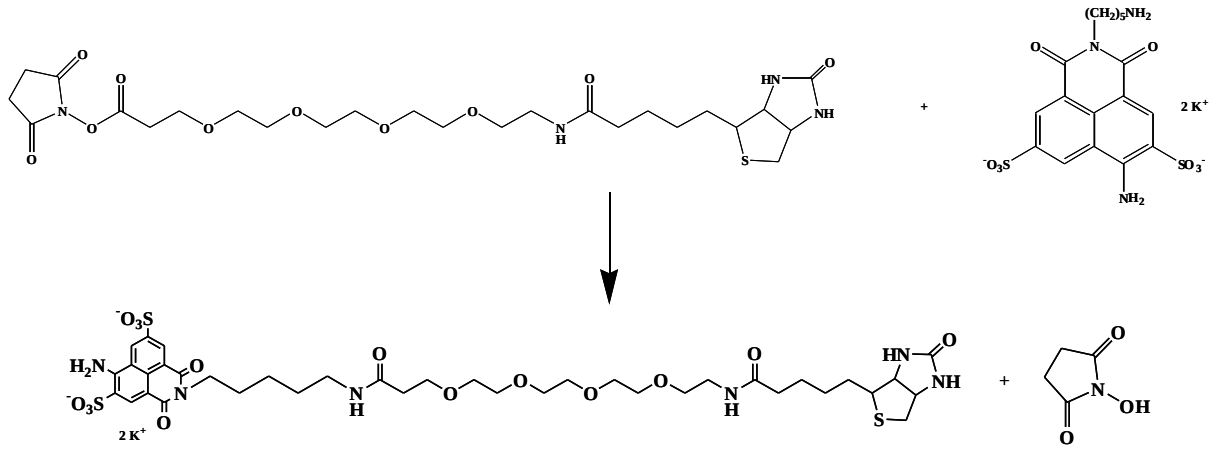
[AEM] (M)	τ_1 (ns)	A_1	τ_2 (ns)	A_2	$\langle\tau\rangle$ (ns)	χ^2
0	6.1	14.1	--	--	6.1	1.11
3.81×10^{-3}	5.8	14.0	--	--	--	1.18
	4.8	3.40	6.1	10.7	5.8	1.10
7.61×10^{-3}	5.3	11.8	--	--	--	1.30
	3.9	1.9	5.5	10.0	5.3	1.15
1.14×10^{-2}	4.8	17.3	--	--	--	1.24
	2.9	1.3	4.9	16.3	4.8	1.07

Table S3 Fluorescence quantum yields, average lifetimes, radiative (k_r) and nonradiative (k_{nr}) rates constant for the poly(NAM-LY) samples and free LY, in phosphate buffer solutions (50 mM; pH 7.5).

Sample	ϕ_F	$\langle\tau\rangle$ (ns)	k_r x 10^8 (s^{-1})	k_{nr} x 10^8 (s^{-1})
Free LY	0.26	6.1	0.4	1.2
C1	0.26	8.2	0.3	0.9
C2	0.22	7.1	0.3	1.1
C3	0.20	6.3	0.3	1.3

Sensitivity to pH assay: comparison of LY-Biotin and Fluorescein-Biotin

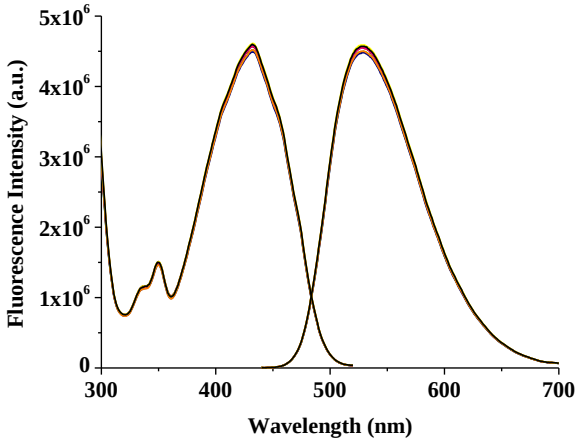
LY-Biotin synthesis. LY-Biotin, LY-B, was synthesized by a stoechiometric reaction between NHS-PEO_4-Biotin (Scheme S1) and Lucifer Yellow cadaverine using the same experimental conditions as for the poly(NAM-*co*-NAS) labeling reaction with LY. NHS-PEO_4-Biotin is a water-soluble analog of biotin vitamin containing a polyethylene oxide (PEO) spacer arm (32 Å). The coupling reaction was performed in dried DMF to avoid NHS ester hydrolysis (competing reaction in aqueous solution). The final product, biotin-PEO_4-Lucifer Yellow cadaverine (LY-B), was dried and re-dissolved in a known volume of DMF.



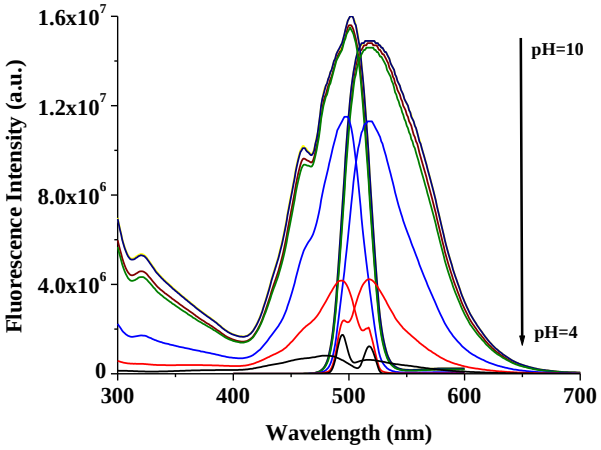
Scheme S1 Reaction of NHS-PEO_4-Biotin with LY cadaverine to prepare LY-Biotin.

Comparison of LY-Biotin and Fluorescein-Biotin. Lucifer Yellow is known to be insensitive to pH changes. In order to confirm this observation in the case of a LY-labeled-biotin (LY-Biotin, ESI Scheme S1), its fluorescence emission was compared to that of a Fluorescein-labeled-biotin (F-Biotin) at different pH values. Fluorescein is also a water soluble dye often used as label in biological studies with absorption and emission wavelength maxima of 494 (absorption) and 518 nm (fluorescence), similar to those of LY. The changes in LY-Biotin fluorescence emission and excitation spectra were compared with those of F-Biotin on the pH range 10 to 4 (Fig. S5A and S5B, respectively).

Knowing the molar absorption coefficient of both dyes, $\varepsilon = (1.16 \pm 0.01) \times 10^4$ $M^{-1}.cm^{-1}$ for LY and $\varepsilon = 75000$ $M^{-1}.cm^{-1}$ for Fluorescein-Biotin[51] (F-B), F-B and LY-B solutions were prepared at approximately the same optical density (0.18 and 0.14, respectively) in potassium phosphate buffer (50 mM) of decreasing pH, from 10 to 4. The final amount of DMF in these solutions was kept below 0.5% to avoid influence of the organic solvent. The F-Biotin emission intensity decreased markedly at pH values below 7, while for LY-Biotin no decrease in intensity or shift in the maximum intensity wavelength were detected.



A



B

Fig. S5 (A) Fluorescence emission (λ_{ex} = 430 nm) and excitation spectra (λ_{em} = 525 nm) of LY-Biotin in potassium phosphate buffer (50 mM), at pH 10 to 4 (LY concentration: 1.34×10^{-6} M) ; **(B)** Fluorescence emission (λ_{ex} = 494 nm) and excitation spectra (λ_{em} = 518 nm) of F-Biotin in potassium phosphate buffer (50 mM), at pH 10 to 4 (F concentration: 1.32×10^{-6} M). Order of decreasing intensity: pH = 10, 8, 7, 6, 5, and 4.