Synthesis of ATRP-induced Dextran-\(b\)-Polystyrene Diblock Copolymers and Preliminary Investigation of their Self-Assembly in Water

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Supplementary information

Materials.
Dextran T10 (\(M_n=6600\) g.mol\(^{-1}\); PDI = 1.4 as determined by aqueous SEC using poly(ethyleneoxide) standards) was purchased from Amersham Bioscience. It was dried under dynamic vacuum during one night prior to use. Dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and toluene were purchased from Baker; these solvents were stirred overnight over CaH\(_2\) and distilled prior to use. All other reagents were purchased from Aldrich. Styrene (99%) was stirred overnight over CaH\(_2\) and distilled prior to use. di-tert-butyl dicarbonate (BOC\(_2\)O, 98%), ethylene diamine (99%), 2-bromoisobutyrylbromide (98%), triethylamine (Et\(_3\)N, 99%), sodium cyano-borohydride (NaCNBH\(_3\), 98%), hexamethyldisilasane (NH(Si(Me\(_3\))\(_2\), 98%), copper (I) bromide (CuBr, 98%), N,N,N’,N’’,N’’’-pentamethyldiethylenetriamine (PMDETA, 99%) were used as received.

Syntheses (see Scheme 1).

**Synthesis of 4**
A solution of di-tert-butyl dicarbonate (9.5 g, 0.0435 mol) in 80 mL of THF was slowly added into a stirred solution of diaminoethane (1) (21 g, 0.33 mol) in 80 mL of THF over a period of 2 h at room temperature. After 48 h, the precipitate was filtered off and both THF and excess diaminoethane were removed under vacuum. Water (200 mL) was added to the residue and bis(N,N’-tert-butyloxycarbonyl)-1,2-diaminoethane was removed by filtration. The aqueous solution saturated with sodium chloride and extracted three times with dichloromethane. The organic phase was dried over sodium sulfate and dichloromethane was evaporated under reduced
pressure to give of tert-butyl-N-(2-amino-ethyl) carbonate 2 as a colorless oil (yield = 50%). $^1$H NMR ((d$_6$)DMSO): d¼1.37 ((CH$_3$)$_3$C–), 2.52 (–NH$_2$), 2.73 (–CH$_2$NH$_2$), 2.9 (–CH$_2$NH–), 5.74 (–NH–).

In a second step, 2-bromoisobutyryl bromide (7.776 g, 0.033 mol) was slowly added at 0°C onto a solution of 2 (3.6 g, 0.0225 mol) in THF (40 mL), in the presence of triethylamine (3.5 g, 0.033 mol). The reaction was left for 48 h with continuous stirring at room temperature. Triethylammonium bromide was formed as a white precipitate and filtered off. After removal of the solvent under vacuum, a yellow solid was dissolved in methanol and precipitated into water saturated with Na$_2$CO$_3$, affording 3 (yield = 95%). $^1$H NMR ((d$_6$)DMSO): d¼1.38 ((CH$_3$)$_3$C–), 1.86 ((CH$_3$)$_2$C–), 3.02 (–CH$_2$–NH–COO–), 3.12 (–CH$_2$–NH–CO–), 6.8 (–CH$_2$–NH–COO–), 8.0 (–CH$_2$–NH–CO–).

In a third step, 6.6 g of 3 (0.021 mol) was dissolved in 60 mL of ethyl acetate. HBr in solution in acetic acid (33%) (6 mL) was progressively introduced in the solution, leading to the formation of a white precipitate corresponding to the coupling agent in its ammonium form (4). Yield 90-95%. $^1$H NMR spectrum of 4 is given in ESI 1 below.

**Synthesis of bromoisobutyramide-ended dextran 5**

Dextran T10 (7.9 g, 1.2x10$^{-3}$ mol), N-(2-Amino-ethyl)-2-bromo-2-methyl-propionamide (6.5 g, 0.03 mol), sodium cyano-borohydride (5.5g, 0.09 mol), triethylamine (3 g, 0.03mol) and DMSO/water (9/1, v/v) were introduced in a two-neck round-bottomed flask under N$_2$. The solution was stirred 48 h at 60°C and then 48 h at room temperature. The final product was recovered by precipitation in ethanol. Yield = 95 %. $^1$H NMR spectrum with all assignments is provided in ESI 2.

**Synthesis of Silylated bromoisobutyramide-ended dextran 6**

The silylation reaction of hydroxyl groups of 5 was achieved following the same procedure described in reference 4. Yield 95%. ESI 3 shows the $^1$H NMR spectrum of compound 6, with trimethylsilyl protons around 0 ppm.

**Atom transfer radical polymerisation of styrene for the synthesis of silylated dextran-b-PS diblock copolymer 7**

All copolymers were synthesized as follows. In a flame-dried Shlenk, the silylated dextran ATRP macroinitiator 6 (1g, 6.5*e-5 mol) was dissolved in distilled toluene and stirred during one hour. Styrene (4.545g, 0.043mol), Copper (I) bromide (10.e$^{-3}$ g, 7x10$^{-5}$mol) and PMDETA (10μL, 7x10$^{-5}$ mol) were introduced in the solution. The solution was stirred during 24 h at room temperature. The final product was recovered by precipitation in ethanol.
5 mol) were added under N₂ flow. The solution was degassed three times and put under stirring at the desired temperature (see Table 1). The reaction was stopped by a cooling down the reaction mixture at 0 °C. The copolymer was recovered by precipitation in a large excess of ethanol. A typical ¹H NMR spectrum of copolymer 7 (sample 3 in Table 1) is shown in ESI 4.

**Synthesis of dextran-b-PS diblock copolymer** 8
The deprotection of all copolymers was achieved using aqueous HCl as described in reference 4.

**Characterization**

**Size Exclusion Chromatography (SEC).** Molar masses were determined by SEC with THF as eluent. (1mL/min) at 25 °C, a refractive index detector (Varian RI-4) and a PSS column (8*300 mm, 5µm). SEC columns were calibrated using linear standard polystyrene standards.

**Atomic Force Microscopy (AFM).** Samples for AFM analysis were prepared by evaporation at ambient conditions on substrates starting from solutions in water. Practically, 20 µL of a dilute solution (0.001 wt%) was dropped on a 1×1 cm² freshly cleaved on a mica substrate. Samples were analysed after complete evaporation of the solvent at room temperature. All AFM images were recorded in air with a Dimension microscope (Digital Instruments, Santa Barbara, CA), operated in tapping mode™. The probes were commercially available silicon tips with a spring constant of 40 N/m, a resonance frequency lying in the 270-320 kHz range and a radius of curvature in the 10-15 nm range. In this work, both the topography and the phase signal images were recorded with the highest sampling resolution available, i.e. 512×512 data points.

**¹H Nuclear Magnetic Resonance (¹H NMR).** 400 MHz ¹H NMR spectra were acquired using an Avance DPX 400 spectrometer. For the coupling agent and unsilylated dextran, DMSO-δ6 was used as solvent, while CDCl₃ was chosen for silylated dextran and dextran-b-PS diblock copolymers.

**Dynamic Light Scattering (DLS).** DLS measurements were performed using an ALV laser goniometer, which consists of a 22 mW HeNe linear polarized laser operating at a wavelength of 632.8 nm and an ALV-5000/EPP multiple τ digital correlator with 125 ns initial sampling time. The copolymer solutions were maintained at a constant temperature of 25.0 ± 0.1 °C in all experiments. The accessible scattering angles range from 15 to 150°. However, all the scattering measurements were done from 40° to 120° by a 10° step. The solutions were placed in 10 mm diameter glass cells. The minimum sample volume required for DLS experiments was 1 mL.
Data were collected using ALV Correlator Control software and the counting time was fixed at 300 s for each angle. The relaxation time distributions, $A(t)$, were in the sequence obtained using CONTIN analysis of the autocorrelation function, $C(q,t)$. The relaxation frequency, $\Gamma$ ($\Gamma = \tau^{-1}$) depends generally on the scattering angle, and in the case of a diffusive particle, this frequency is $q^2$-dependent. Consequently, the apparent diffusion coefficient ($D_{app}$) at a given copolymer concentration ($C_p$) is calculated from

$$\frac{\Gamma}{q^2} \xrightarrow{q \to 0} D_{app}$$

where $q$ is the wavevector defined as

$$q = \frac{4\pi n}{\lambda} \sin \left( \frac{\theta}{2} \right)$$

and $\lambda$ is the wavelength of the incident laser beam (632.8 nm), $\theta$ is the scattering angle and $n$ the refractive index of the media. The hydrodynamic radius ($R_H$) is calculated from the Stokes-Einstein relation

$$R_H = \frac{k_B T}{6\pi \eta q^2} = \frac{k_B T}{6\pi \eta D_{app}}$$

where $k_B$ is the Boltzmann constant, $T$ is the temperature of the sample, and $\eta$ is the viscosity of the medium.

The reduced elastic scattering $I(q)/kC$, with $K=4\pi^2 n_0 (dn/dc)^2 (I_0^{90^\circ}/R^{90^\circ})/\lambda_0^4 N_A$, was measured in steps from 40 to 120° in the scattering angle, where $n_0$ is the refractive index of the standard (toluene), $I_0^{90^\circ}$ and $R^{90^\circ}$ are the intensity and the Rayleigh ratio of the standard at $\theta=90^\circ$, respectively, $dn/dc$ is the increment of the refractive index, $C$ is the concentration ($C = 0.083$ mg/mL), and $I(q)$ is the intensity scattered by the sample. Elastic intensity was calculated according to standard procedures using toluene as the standard with known absolute scattering intensity.
ESI 1. $^1$H NMR spectra of the coupling agent in its ammonium form in DMSOd$_6$. 
ESI 2a. $^1$H NMR spectra of the commercial dextran in DMSO $d_6$. 

ESI 2b. $^1$H NMR spectra of the bromoisobutyramide-ended dextran in DMSO $d_6$. 
ESI 3. $^1$H NMR spectra of the silylated dextran-based ATRP macroinitiator in CDCl$_3$. 
ESI 4. $^1$H NMR spectra of the protected copolymer 7 (sample 3 in Table 1) in CDCl$_3$.

ESI 5. Auto-correlation functions obtained for a solution of copolymer 4 in water at different angles at RT. $R_H = 60$nm

ESI 6. Berry diagram obtained for a solution of copolymer 4 in water at RT. $R_g = 60$nm