Electronic Supplementary Information

NMR Studies of PEG Chain Dynamics on Mesoporous Silica Nanoparticles for Minimizing Non-Specific Binding

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Additional Experimental Details

Synthesis of PEG_{6-9}-modified Mesoporous Silica Nanoparticle (PMSNs)

The PMSNs were synthesized according to the procedure described previously.[1] In brief, CTAC (2 g, 25 wt%) and TEA (0.8 g) were dissolved in water (20 mL) and stirred magnetically at room temperature for 1 hour. The mixture was then heated to 95 °C and stirred for an additional hour. TEOS (1.5 mL) was added using a syringe pump at a controlled rate of 150 μL/min. After 10 min, $mPEG_{6-9}$-silane (650 μL) was introduced at a rate of 65 μL/min, followed by stirring at the same temperature for another 30 min. After cooling to room temperature, the resulting nanoparticles were collected by centrifugation (45,000 *g*, 45 min) and washed three times with ethanol.

To remove the CTAC template from the mesopores, the nanoparticles were suspended in 50 mL of acidic ethanol (10 v/v% of concentrated HCl in ethanol) and refluxed at 85 °C for 12 hours. This extraction was repeated to ensure the complete removal of CTAC. Finally, the template-free PMSNs were collected by centrifugation (45,000 *g*, 45 min), washed with ethanol and water, and dried using a freeze dryer.

Synthesis of Zwitterion-modified Mesoporous Silica Nanoparticle (PMSNs-Zwi)

Synthesis of zwitterionic sulfobetaine silane (SBS)

Zwitterionic sulfobetaine silane (SBS) was synthesized following our prior published work.[1] The DMASi (2.07 g, 10 mmol), 1,3-propane sultone (1.34 g, 11 mmol), and anhydrous acetone (10 mL) were added to the reaction flask under nitrogen protection. The reaction was stirred vigorously at room temperature for 6 hours. The white precipitate was then centrifuged (4,000 rpm, 10 min) and washed three times with anhydrous acetone. The final product was dried under vacuum and stored under nitrogen.

Yield 75%. ^{1}H NMR (400 MHz, DMSO-$_{6d}$): δ 0.54 (t, 2H), 1.67 (m, 2H), 1.94 (m, 2H), 2.44 (t, 2H), 2.97 (s, 6H), 3.19 (m, 2H), 3.36 (m, 2H), 3.47 (s, 9H). ^{13}C NMR (400 MHz, DMSO-$_{6d}$): δ 5.17, 15.5, 18.8, 47.8, 49.9, 50.2, 62.4, 65.2.

Synthesis of PMSN-Zwi

A sample of PMSNs (50 mg) was redispersed in water (4 mL) under sonication, resulting in a transparent light blue solution, which was purged with nitrogen for at least 15 minutes. SBS (50

mg) was dissolved in water (1 mL) and added dropwise to the PMSN solution. The pH was adjusted to ~9 using 28% aqueous ammonia. The mixture was stirred at 80 °C for 24 hours. The resulting nanoparticles were washed three times with water by centrifugation (45,000 *g*, 45 min), then freeze-dried. The resulting NPs were named as PMSN-Zwi.

Equation for Quantifying PEG Grafting Density from TGA Data

$$\sigma_{TGA} = \frac{\rho\beta N_{AV} d \times 10^{-24}}{6M[1 - \frac{\rho V_p}{3}]} \qquad (Eq.S1)$$

where σ_{TGA} represents the grafting density of $mPEG_{5k}$ chains per nm^2 surface of PMSNs, ρ represents the density of silica (1.85 - 2.20 g/cm^3), β is the weight ratio of $mPEG_{5k}$ in the sample obtained by TGA in units of mg/g, N_{AV} is the Avogadro constant, d (in nm) is the average diameter measured by TEM, M is the molecular weight of the $mPEG_{5k}$ (5000 g/mol) and V_p is the pore volume obtained by nitrogen adsorption/desorption analysis using the BJH method (0.96 cm^3/g). The number 10^{-24} is used for unit conversion. The detailed derivation of this equation is provided in our previous publication.[1]

Derivation of Eq.1 for PEG Grafting Density Quantification

NMR Data Processing and Quantification of Molar Amount of PEG

To estimate the PEG grafting density, we used the integration results of the DCM peak (δ 5.3 ppm, peak 2 in **Figure 1B**) and the PEG repeating unit peak (-CH_2-CH_2O-, δ ~3.6 ppm, peak 1 in **Figure 1B**), using **Eq. (S2)**:

$$\frac{\frac{A_{peak\,2}}{2}}{N_{DCM}} = \frac{\frac{A_{peak\,1}}{4N}}{N_{PEG}} \qquad (Eq.S2)$$

where $A_{peak\,2}$ is the integral area of internal standard peak (2H from DCM), $A_{peak\,1}$ is the integral area of PEG repeating unit (-CH_2-CH_2O-), N is the number of PEG repeating unit (44 for $mPEG_{2k}$ 108 for $mPEG_{5k}$, 220 for $mPEG_{10k}$ used in this study). N_{DCM} and N_{PEG} are the molar amounts of internal standard DCM and $mPEG_{xk}$ (x =2 or 5 or 10) contained in nanoparticles measured by qNMR.

Correction for Pre-Existing $PEG_{6\text{-}9}$ Silane

Since $PEG_{6\text{-}9}$ silane was incorporated into the MSNs prior to the post-grafting of $mPEG_{xk}$ (x = 2 or 5 or 10), $A_{peak\ 1}$, corresponding to the PEG repeating unit ($-CH_2-CH_2O-$), comprises two parts, affecting the integration accordingly. To correct for this, a separate qNMR measurement was performed on $PEG_{6\text{-}9}$ silane-functionalized MSNs (PMSNs) using the same external DCM standard. The integration of *peak 1'* ($A_{peak1'}$) from the $PEG_{6\text{-}9}$ silane repeating unit in PMSNs was measured and subtracted from the integration of *peak 1*:

$$A_{peak\ 2} = A_{peak\ 1} - A_{peak\ 1'} \quad (Eq.\ S3)$$

Combining the results from **Eq. (S2)** with **Eq. (S3)**, the molar amount of long PEG chains (N_{PEG}) in the measured NPs was calculated accordingly.

This methodology enables accurate determination of PEG grafting density while accounting for pre-existing PEG on the nanoparticle surface. It should be noted that this calculation assumes accurate addition and complete dissolution of the DCM internal standard, as well as uniform distribution of PEG on the nanoparticle surface. Care must be taken to prevent evaporation of DCM due to its volatility.

Grafting Density (chains per nm^2) Derivation

Once the molar amount of long PEG chains ($mPEG_{xk}$, x = 2 or 5 or 10) on the nanoparticle surface is determined by qNMR, the grafting density (σ_{NMR}, chains per nm^2) can be determined in conjunction with the total surface area (S_{sph}) and pore surface area (S_{pore}) of the NPs:

$$\sigma_{NMR} = \frac{N_{PEG} N_{AV} \times 10^{-18}}{\frac{m}{\rho V_{sph}}(S_{sph} - S_{pore})} \quad (Eq.\ S4)$$

where V_{sph} is the volume of the single particle by taking the assumption of a sphere, calculated as $V_{sph} = 4/3\pi(d/2)^3$, S_{sph} is the whole surface area of a single nanoparticle, calculated as $S_{sph} = \pi d^2$, S_{pore} represents the void surface of a single nanoparticle, which can be modelled as the base of a cylinder, whose diameter (d_{pore}) is the mesopore size obtained from the BJH data. Consequently, S_{pore} could be calculated by multiplying d_{pore} and the number of pores per nanoparticle (n_{pore}), where n_{pore} is equal to twice the base of each cylinder ($2n_{cylinder}$):

$$S_{pore} = n_{pore}\pi\left(\frac{d_{pore}}{2}\right)^2 = n_{cylinder}\pi\frac{d_{pore_2}}{2} \quad \text{(Eq. S5)}$$

The number of pore cylinders (n_{cylinder}) is calculated by dividing the pore volume of a single nanoparticle ($V_{\text{pore}} \cdot \rho V_{\text{sph}}$) by the cylinder volume (V_{cylinder}), where V_{cylinder} is estimated by using the diameter (d) of nanoparticle as the height:

$$n_{cylinder} = \frac{V_{pore} \times \rho V_{sph}}{V_{cylinder}} = \frac{V_{pore} \times \rho \times \frac{4}{3}\pi(\frac{d}{2})^3}{\pi\left(\frac{d_{pore}}{2}\right)^2 \times d} = \frac{2V_{pore}\rho d^2}{3{d_{pore}}^2} \quad \text{(Eq. S6)}$$

Combining **Eq. (S5)** and **Eq. (S6)** and introducing these terms into **Eq. (S4)** gives **Eq. (1)** in the main text.

Determination of mPEG$_{5k}$ Conformation

The Flory radius (R_F) represents the size of a single PEG chain in solution. The grafting distance (D) is the average distance between grafting sites on the nanoparticle surface. The PEG layer thickness (L) estimates the extended length of the PEG chains in the grafted state. The values for R_F, D, and L of mPEG$_{xk}$ layer were determined using the following equations[2]:

$$R_F = \alpha N^{3/5} \quad (Eq.\ S7)$$

$$D = 2(1/\sigma\pi)^{1/2} \quad (Eq.\ S8)$$

$$L = N(\alpha^{5/3})/D^{2/3} \quad (Eq.\ S9)$$

where α is the monomer length (0.35 nm for PEG)[3], N is the number of PEG repeating unit (44 for mPEG$_{2k}$ 108 for mPEG$_{5k}$, 220 for mPEG$_{10k}$ used in this study), and σ is the measured grafting density of PEG (chains per nm^2).

By comparing these parameters, we can determine the conformational regime of the grafted PEG:

1. If $D > R_F$, the PEG chains are in the "mushroom" regime, individual polymer chains remain separated and do not interact with each other.
2. If $D < R_F$, the PEG chains are in the "brush" regime, where chains extend away from the surface due to interactions between neighboring chains.
3. If $L > 2R_F$, the brush conformation was further defined by Damodaran et al.[4] to be a dense brush.

These parameters provide valuable insights into the structure and behavior of the PEG coating on our nanoparticle system.

Instrumentation

Transmission Electron Microscopy (TEM)

The morphology of different nanoparticles was observed via TEM on a Hitachi HT7700 TEM instrument. Particle sizes were measured using Image-J software and then processed with Origin software to plot size distribution histograms.

Dynamic Light Scattering (DLS)

A Malvern Zetasizer Nano ZS instrument was used for all hydrodynamic diameter measurements. NP samples were dispersed in water at a concentration of ~ 0.1 mg/mL and placed in a ZEN0040 disposable plastic micro cuvette. Each measurement was performed three times at 25 °C with a scattering angle of 173°. Hydrodynamic diameter (d_h), polydispersity (PDI) and intensity distribution plot of each NP sample were obtained using software embedded in the instrument.

Nuclear Magnetic Resonance (NMR)

All NMR spectra were recorded using a 600 MHz Agilent DD2 NMR Spectrometer. All chemical shifts were referenced to the corresponding deuterated solvents peaks.

Thermogravimetric Analysis (TGA)

TGA characterization was conducted on a TA instrument SDT-Q600. Temperature and mass calibrations were done as recommended by the manufacturer. 3-5 mg of dried samples were placed in a ceramic crucible inside an oven continuously purged with nitrogen at a flow rate of 100 mL/min. The temperature of the oven was ramped to 100 ℃, where it was held constant for 30 min to ensure the desorption of adsorbed water. The temperature was then ramped to 800 ℃ at the rate of 10 ℃/min. An empty crucible was run at the same time as a reference. All mass loss values are expressed as wt%.

Ultraviolet-visible Spectrophotometer (UV-vis)

UV-vis measurements were performed on a BioTek Epoch 2 microplate spectrophotometer. All sample dispersions were placed in either BrandTech™ BRAND™ disposable cuvettes with a 1 cm path length or Thermo Scientific Pierce™ 96-well plates.

Supporting Results and Discussion

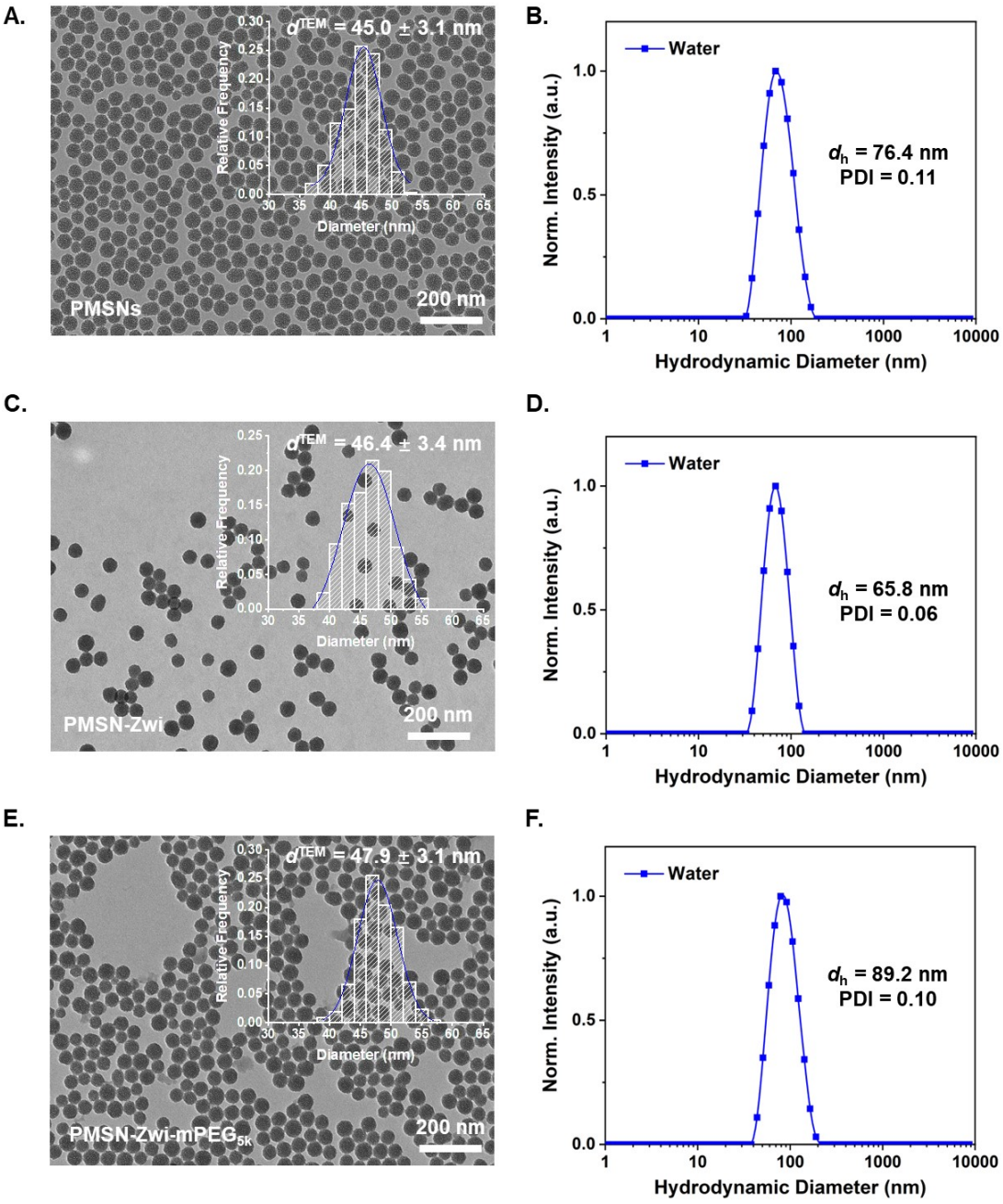

Figure S1. TEM images, size distribution histograms, and DLS results in H_2O of (A-B) redispersed PMSNs NPs, (C-D) redispersed PMSN-Zwi NPs and (E-F) redispersed PMSNs-Zwi-mPEG$_{5k}$ NPs.

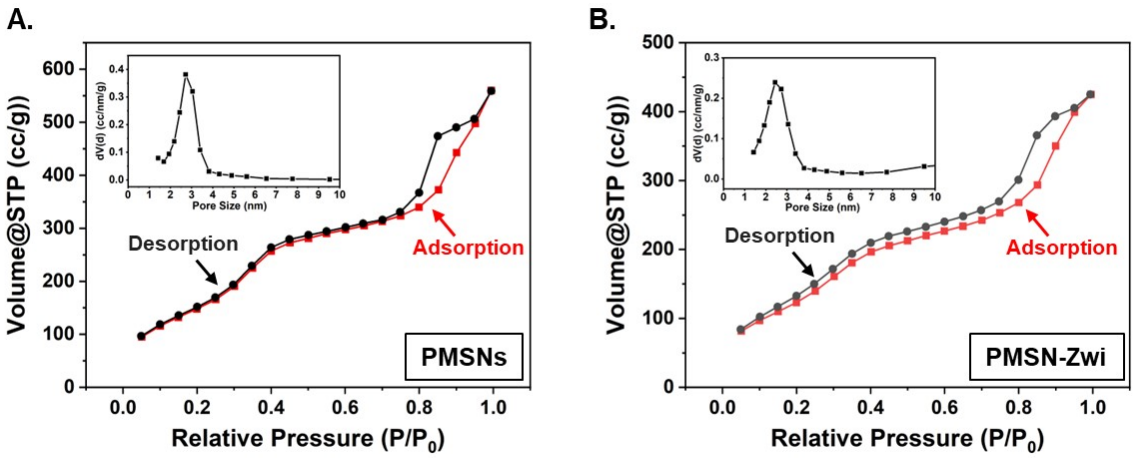

Figure S2. Nitrogen adsorption-desorption isotherms and pore size distribution determined by the Barrett-Joyner-Halenda (BJH) method of (A) PMSNs and (B) PMSNs-Zwi NPs. Reproduced from Ref. [1].

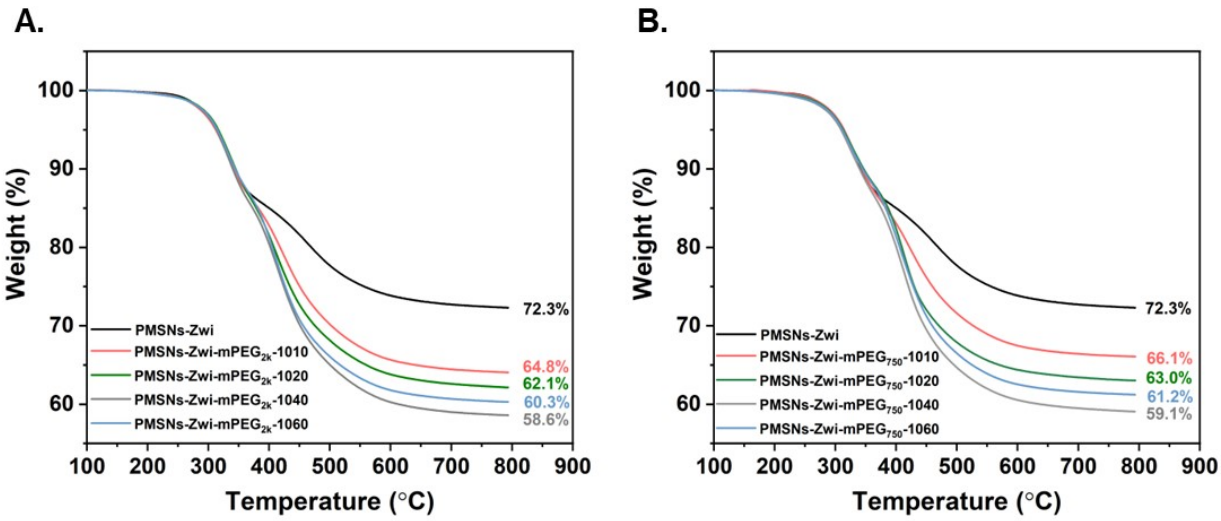

Figure S3. TGA thermal curves of PMSN-Zwi; (A) PMSN-Zwi-mPEG$_{2k}$ nanoparticles with varying mPEG$_{2k}$ silane amounts; and (B) PMSN-Zwi-mPEG$_{750}$ nanoparticles with varying mPEG$_{750}$ silane amounts

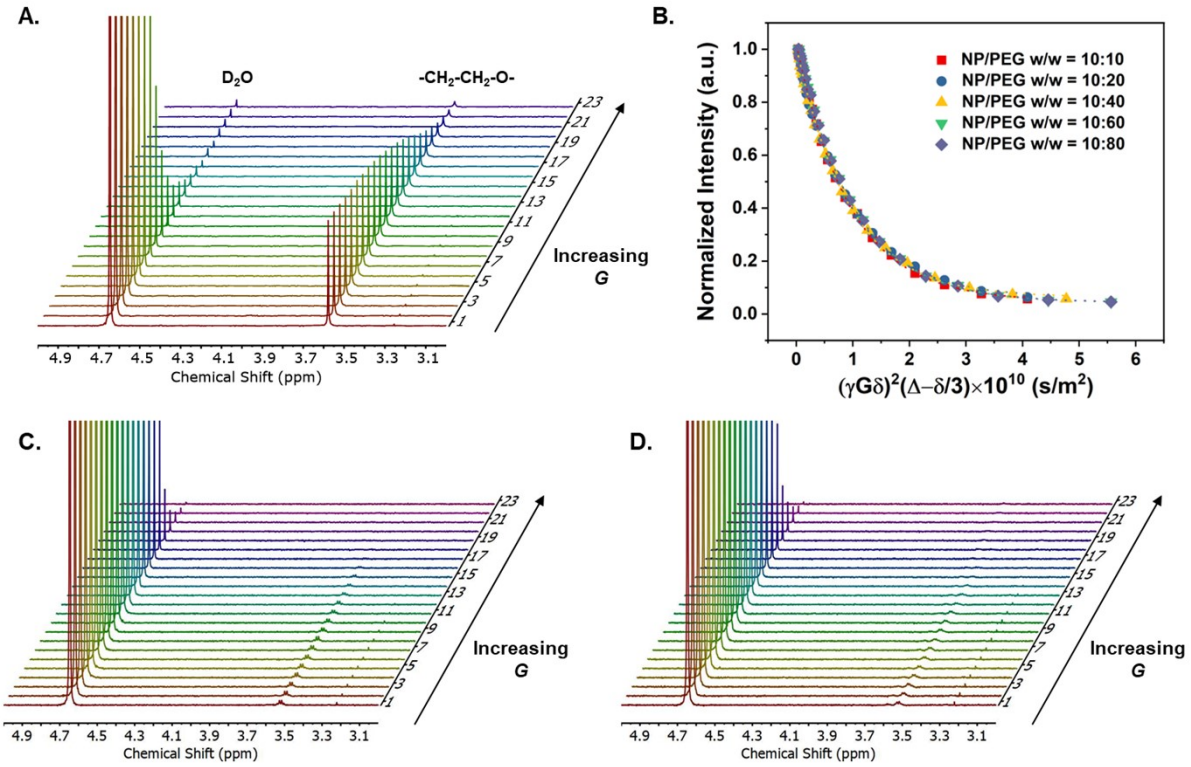

Figure S4. (A) Typical PFGSE ^{1}H NMR spectra of PMSN-Zwi-mPEG$_{5k}$ NPs with NP/PEG w/w ratio of 10:40 as a function of magnetic gradient amplitude (*G*) from low (bottom) to high (top). (B) DOSY fitting of the protons from PEG repeating unit (-CH_2-CH_2-O-, *peak 1*) of the PMSN-Zwi-mPEG$_{5k}$ NP dispersions in D_2O. Dashed curves are the fitted lines with a mono-exponential function. DOSY NMR spectra of PMSN NPs in D_2O at concentrations of (C) 2.5 mg/mL and (D) 10 mg/mL.

We used DOSY NMR to examine the diffusive motion of PMSN-Zwi-mPEG$_{5k}$ NPs with varied grafting densities in D_2O (2.5 mg/mL) at 298 K. The intensities of peaks decrease from bottom to top with increasing magnetic field gradient strength. We chose a range of gradient strengths to allow the intensity of the last spectrum to decay to ~5% of its original value. A total of 23 gradient increments were collected to maintain fitting precision.

PMSN NPs in D_2O at the same concentration as PMSN-Zwi-mPEG$_{5k}$ NPs (2.5 mg/mL) and even higher concentrations (10 mg/mL) were measured by DOSY NMR for reference. As shown in **Figure S4C**, we can see that PEG$_{6\text{-}9}$ signal intensities remained insufficient for diffusion coefficient (*D*s) determination. The possible reasons are: (i) the PEG$_{6\text{-}9}$ chains constitute a much smaller number of protons per chain compared to the long PEG$_{5k}$ chains, and (ii) being covalently bound and very short, the PEG$_{6\text{-}9}$ chains are relatively immobile and likely produce extremely broad NMR signals that fall into the baseline noise. Their signal is severely broadened and very low in intensity, rendering it invisible in the DOSY/T_2 NMR experiments. Meanwhile, the freely diffusing PEG$_{5k}$ (either loosely bound or truly free in solution) dominates the observable signal with a much sharper peak.

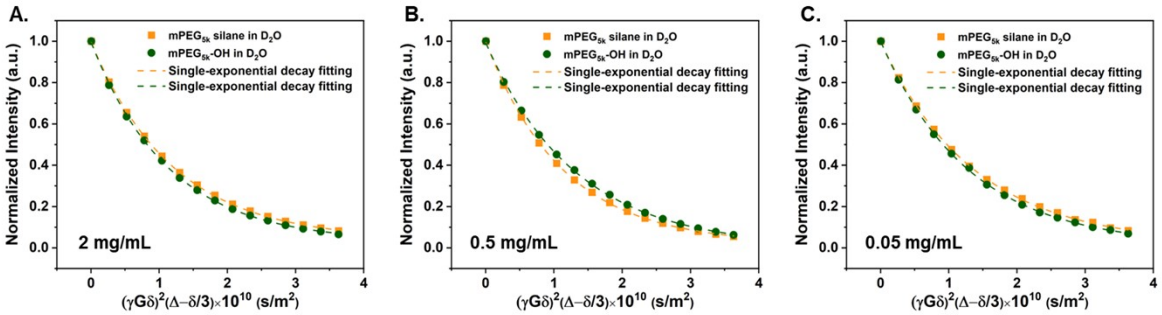

Figure S5. Single-exponential decay curves for protons from the PEG repeating unit (-CH_2-CH_2-O-) of $mPEG_{5k}$ silane and $mPEG_{5k}$-OH in D_2O at varying concentrations: (A) 2 mg/mL, (B) 0.5 mg/mL, and (C) 0.05 mg/mL, with R^2 values ranging from 0.9997 to 0.9999 for all fitted curves.

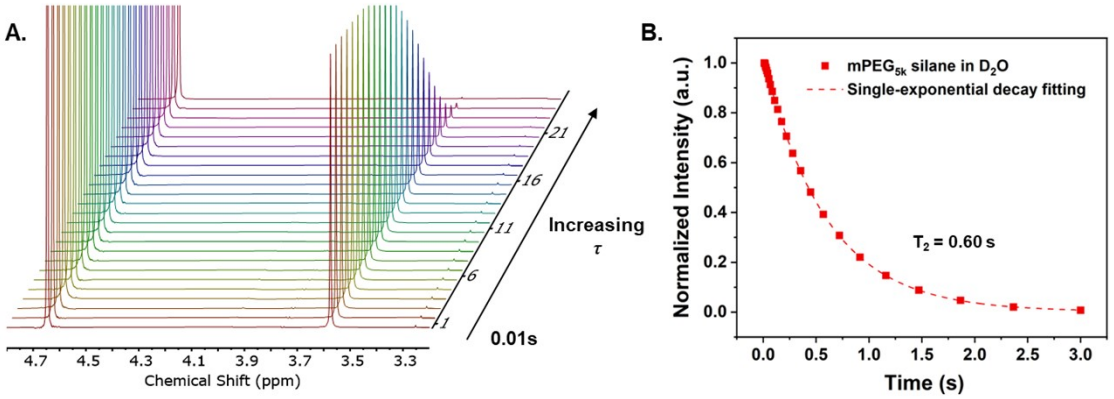

Figure S6. (A) T_2 analysis and (B) fitted curve with a single-exponential decay function ($I = I_2 \exp(-\tau/T_2) + y_0$) for $mPEG_{5k}$ silane in D_2O.

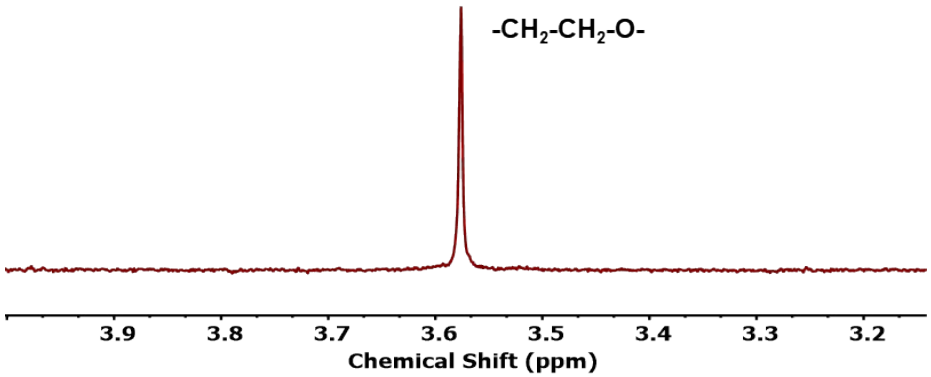

Figure S7. NMR signal of the particle supernatant after an additional H_2O sedimentation-redispersion step following standard purification (ethanol ×2 and H_2O ×1).

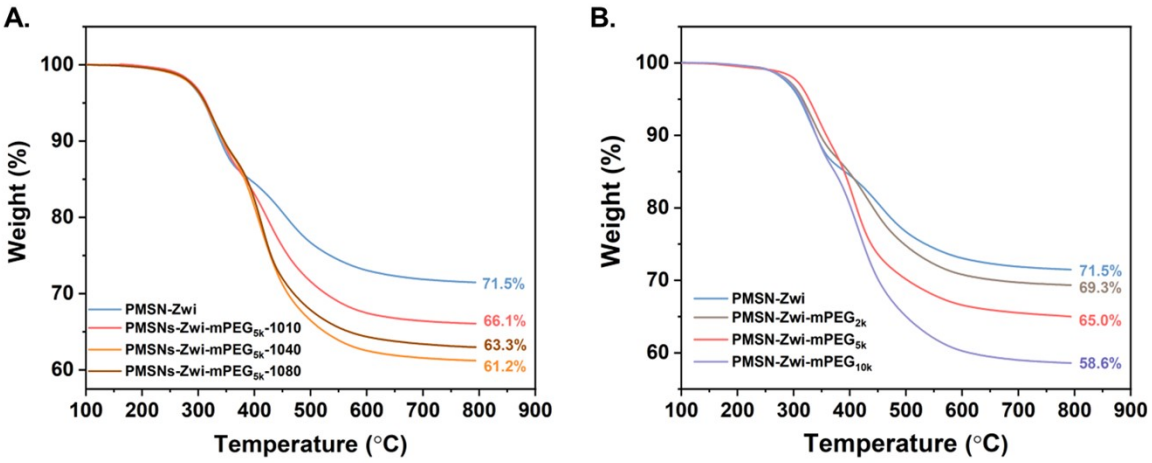

Figure S8. TGA thermal curves of (A) PMSN-Zwi-mPEG$_{5k}$ NPs after two additional water washes following the original purification procedure. (B) PMSN-Zwi-mPEG$_{xk}$ NPs with varying PEG chain lengths (x = 2, 5 or 10) at a consistent grafting density of 0.3 chains/nm².

Supporting Tables

Table S1. Summary of surface areas, pore volumes and pore sizes for PMSNs and PMSNs-Zwi NPs. Reproduced from Ref. [1].

Sample	Surface Area [a] (m^2/g)	Pore volume [b] (cm^3/g)	Pore size [c] (nm)
PMSNs	637	0.96	2.7
PMSNs-Zwi	514	0.66	2.4

[a] Specific surface area was calculated from data in the range $P/P_0 < 0.3$ using the BET method.[5]
[b] Total pore volume was calculated at $P/P_0 = 0.99$ using the BJH method.[6]
[c] Pore diameter was assigned from the maximum of the BJH pore size distribution.

Table S2. Summary of the grafting densities of the $mPEG_{5k}$ layer determined from TGA data in **Figure 1A** and the parameters used to determine their conformations [a].

NP/PEG w/w	Weight percentage [b] (wt%) of $mPEG_{5k}$	Grafting Density [c] (σ_{TGA}, chains/nm^2)	R_F [d] (nm)	D [e] (nm)	L [f] (nm)	PEG Conformation [a]
10:10	6.5/7.2	0.27/0.29	5.8	2.1	11.3	Brush
10:20	8.4/9.1	0.34/0.37	5.8	1.9	12.3	Dense Brush
10:40	12.6/13.3	0.52/0.54	5.8	1.5	13.9	Dense Brush
10:60	11.4/12.1	0.47/0.50	5.8	1.6	13.6	Dense Brush
10:80	10.5/10.9	0.46/0.45	5.8	1.7	13.2	Dense Brush

[a] The prediction of PEG conformation was based on the model of Alexander-de Gennes.[7]
[b] Calculated from mass loss determined by TGA data of two tests.
[c] Calculated using **Eq. (S1)**, where ρ is 1.85 g/cm³,
[d] Flory radius calculated using $R_F = \alpha N^{3/5}$, where α is the monomer length (0.35 nm for PEG)[3], N is the number of PEG repeating unit (108 for $mPEG_{5k}$);
[e] Mean spacing between PEG anchor points calculated using $D = 2(1/\sigma\pi)^{1/2}$, where σ is the average from two tests.
[f] Brush height calculated using $L = N(\alpha^{5/3})/D^{2/3}$.

Table S3. Comparison of the values of the tracer diffusion coefficients (D_s) and hydrodynamic diameters (d_h) calculated from **Figure S5** for $mPEG_{5k}$ silane and $mPEG_{5k}$-OH.

Sample Concentration in D_2O	$mPEG_{5k}$ silane		$mPEG_{5k}$-OH	
	$10^{10} \times D_s$ (m^2/s)	d_h (nm)	$10^{10} \times D_s$ (m^2/s)	d_h (nm)
2 mg/mL	0.83	4.8	0.86	4.7
0.5 mg/mL	0.88	4.6	0.86	4.7
0.05 mg/mL	0.89	4.5	0.90	4.5

Table S4. Fraction of PEG chains removed in the second purification step, calculated from the change in grafting density as determined by TGA and qNMR.

NP/PEG w/w	Grafting density [a] (σ_{TGA}', chains/nm^2)	Free PEG fraction ($_{TGA}$%)	Grafting density [b] (σ_{NMR}', chains/nm^2)	Free PEG fraction ($_{NMR}$%)
10:10	0.22 (0.28)	21%	0.29 (0.39)	25%
10:40	0.42 (0.53)	21%	0.44 (0.57)	23%
10:80	0.34 (0.45)	24%	0.39 (0.51)	23%

[a] Calculated from the TGA using Eq. (S1) after further purification with H_2O. Values in parentheses are the average of the two values determined by TGA before the further purification (see **Table S2**).

[b] Calculated from the qNMR using Eq. (1) after further purification with H_2O. Values in parentheses are the values determined by qNMR before the further purification (see **Table 1**).

Table S5. Summary of grafting densities of the $mPEG_{xk}$ layer (x = 2, 5 or 10) determined by TGA from **Figure S8B** and the parameters used to determine their conformations.

PEG	NP/PEG_{xk} w/w	wt% of $mPEG_{xk}$	σ_{TGA} (chains/nm^2)	R_F [a] (nm)	*D* (nm)	*L* (nm)	PEG Conformation
2k	10:1	3	0.31	3.4	2.0	4.8	Brush
5k	10:10	6.5	0.27	5.8	2.2	11.0	Brush
10k	10:40	13.7	0.28	8.9	2.1	23.1	Dense Brush

[a] $R_F = \alpha N^{3/5}$, where N is the number of PEG repeating unit (44 for $mPEG_{2k}$, 108 for $mPEG_{5k}$, 220 for $mPEG_{10k}$);

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