Electronic Supplementary Material (ESI) for Soft Matter.

Controlling the number of dendrimers in dendrimicelle nanoconjugates from 1 to more than 100

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Experiment section

1. Materials

The diblock copolymer, poly(N-methyl-2-vinyl-pyridinium iodide)-b-poly(ethylene oxide) ($P2MVP_{128}$-*b*-PEO_{477}), was obtained by quaternization of poly(2-vinylpyridine)-*b*-poly(ethylene oxide) ($P2MVP_{128}$-*b*-PEO_{477}) (Polymer Source, M_w/M_n= 1.10, M_n= 34.5 k) following a procedure described elsewhere.[1] The degree of quaternization is about 87% determined by titration with poly(acrylic acid) (PAA, Polymer Source, M_w/M_n= 1.16, M_n= 2.2 k) in 20 mM $NaHCO_3/Na_2CO_3$ buffer at pH 10. The PAMAM dendrimers with sodium carboxylate surface groups were purchased from Sigma Aldrich (G0-G7) or Dendritech Inc. (G8, G9). The original solvent methanol was evaporated carefully under N_2 and the obtained compound was dissolved in nanopure water. The micelles were prepared by mixing solutions of $P2MVP_{128}$-*b*-PEO_{477} and PAMAM dendrimer in 20 mM $NaHCO_3/Na_2CO_3$ buffer at pH 10 ± 0.1.

2. Method

Dynamic and static light scattering

Light scattering at an angle of 90° was performed with an ALV light scattering-apparatus, equipped with a 300 mW cobalt samba DPSS laser operating at a wavelength of 532.0 nm. All measurements were performed at room temperature. Titrations were carried out using a Schott-Geräte computer-controlled titration setup to control sequential addition of titrant and cell stirring. After every dosage, the laser light-scattering intensity (I) and the correlation function were recorded. The hydrodynamic radius and the scattered intensity are studied as a function of the mole fraction of positive charge fraction, $f+$, which is defined as follows:

$$f+ = \frac{n^+}{n^+ + n^-} \tag{1}$$

where n^+ and n^- are the numbers of positive and negative charges.

The light scattering intensity is expressed as the excess Rayleigh ratio R_θ divided by the total polymer concentration. R_θ is obtained as

$$R_\theta = \frac{I_{sample} - I_{solvent}}{I_{toluene}} \times R_{toluene} \times \frac{n^2_{solvent}}{n^2_{toluene}} \quad (2)$$

where I_{sample} is the scattering intensity of the micellar solution and $I_{solvent}$ is the intensity of the solvent. $I_{toluene}$ is the scattering intensity of toluene, $R_{toluene}$ is the known Rayleigh ratio of toluene ($2.1 \cdot 10^{-2}$ m^{-1}) and n is the refractive of solvent (1.333) and toluene (1.497). The total polymer concentration is the sum of the concentrations of dendrimer and diblock copolymer contributing to micelle formation. The CUMULANT method[2] was used to analyze the mean apparent hydrodynamic radius (R_h) as

$$R_h = kTq^2 / 6\pi\eta\Gamma \quad (3)$$

where q is the scattering vector, k is the Boltzman constant, T is the absolute temperature, η is the viscosity of the solvent, and Γ is the measured decay rate (second order cumulant) of the correlation function. The CONTIN method was[3, 4] used to analyze the distribution of particle (C3Ms) radii. The data were analyzed with AfterALV program (AfterALV 1.0d, Dullware), which provides $\Gamma_i W_i$ as default output for each size fraction. Here, the intensity weighted contribution W_i is multiplied by Γ, as suggested by Petr Stepanek for the "equal-area representation".[5] To facilitate comparisons, the absolute $\Gamma_i W_i$ was normalized with the highest value of $\Gamma_i W_i$ for each sample. For angular-dependent light scattering, the intensity was recorded at $30^\circ \leq \theta \leq 140^\circ$ in intervals of 5°.

The Rayleigh ratio can be linked to the concentration and mass of the scattering objects:

$$\frac{K_R C}{R_\theta} = \frac{1}{M} \times \frac{1}{P(qR)} \times \frac{1}{S(q)} \tag{4}$$

where C is the weight concentration of micelles, M is their molecular mass, and R is the radius of the object that contribute to scatter light. $P(qR)$ and $S(q)$ are the form factor and the structure factor, respectively. K_R is an optical constant defined as:

$$K_R = \frac{4\pi^2 n^2}{N_{Av}\lambda_0^4}\left(\frac{dn}{dc}\right)^2 \tag{5}$$

where n is the refractive index of solvent, N_{Av} is Avogadro's number, λ_0 is the wavelength of the incoming beam (532.0 nm), and dn/dc is the refractive index increment of the micelles. dn/dc of the micelles formed with PAMAM dendrimers at different generation is measured by a differential refractive index detector (Shodex RI-71), see the following table,

Generation	2	3	4	5	6	7	8	9
dn/dc*10^4 m^3 kg^{-1}	1.97	1.97	2.02	2.05	1.95	1.94	1.90	1.89

Table S1 Measured dn/dc of Gn-C3Ms formed at different dendrimer generation.

In our experiments, the scattering vector $q = (4\pi n/\lambda_0)sin(\theta/2)$ is approximately 0.023 nm^{-1} ($\theta = 90°$), so that qR is small for the micelles (which have a radius on the order of 25 nm). We therefore assume that $P(qR)$=1. At low concentrations, the structure factor can be approximated as

$$\frac{1}{S(q)} = 1 + 2B_2\frac{C}{M} \tag{6}$$

where B_2 is the second virial coefficient. Substitution into equation 4, we get

$$\frac{K_R C}{R_\theta} = \frac{1}{M} + 2B_2 \frac{C}{M^2} \quad (7)$$

By plotting $K_R C/R_\theta$ versus C, we can obtain the molar mass M from the intercept. In our study, M corresponds to the molar mass of micelles, $M_{micelle}$, from which we can calculate the aggregation number of the micelles, see Figure S1.

Depolarized light scattering

The depolarized light scattering experiments were carried out on the same DLS instrument mentioned above but with a Glan-Laser prism polarizer (Melles Griot, 03 PGL 301/A, extinction ratio < 5 x 10^{-5}) in front of the detector. The detection angle was fixed at 90° and the polarizer was adjusted to transmit only vertically (I_{vv}) or only horizontally (I_{vh}) polarized light, allowing to determine the depolarization ratio

$$\Delta_{vh} = \frac{I_{vh} - I_{vh}^0}{I_{vv} - I_{vv}^0} \quad (8)$$

with I and I^0 denoting the scattering intensity from sample and solvent, respectively.

Small angle X-ray scattering

Small angle X-ray scattering experiments were performed on a Ganesha lab instrument equipped with a GeniX-Cu ultra low divergence source producing X-rays with a wavelength of 1.54 Å and a flux of 1x10^8 ph/s. Scattering patterns were collected on a Pilatus 300K silicon pixel detector (487 x 619 pixels of 172 μm^2) at two sample-to-detector distances corresponding to 730 and at 1530 mm covering a q range of 6.7 x 10^{-2} < q < 4.45 nm^{-1}. The position of the beam center and the q range were calibrated using the diffraction peaks of silver behenate. The liquid samples were contained in 2 mm quartz capillaries sealed and fixed in a stainless steel holder kept at room temperature. The sample concentration was fixed at a dendrimer charge

concentration of 2mM in all cases. The scattering data were corrected for background contributions (such as scattering from the buffer solution), detector response and primary beam intensity fluctuations. The SAXS data were treated and analyzed using the software packages SAXSGUI and SASVIEW. The experimental scattering profiles given in Figure S3 are well described by a polydisperse core-shell sphere model, from which we extract the micellar core size R_{core} and shell thickness H_{shell}.

Determination of the micellar mass and aggregation number

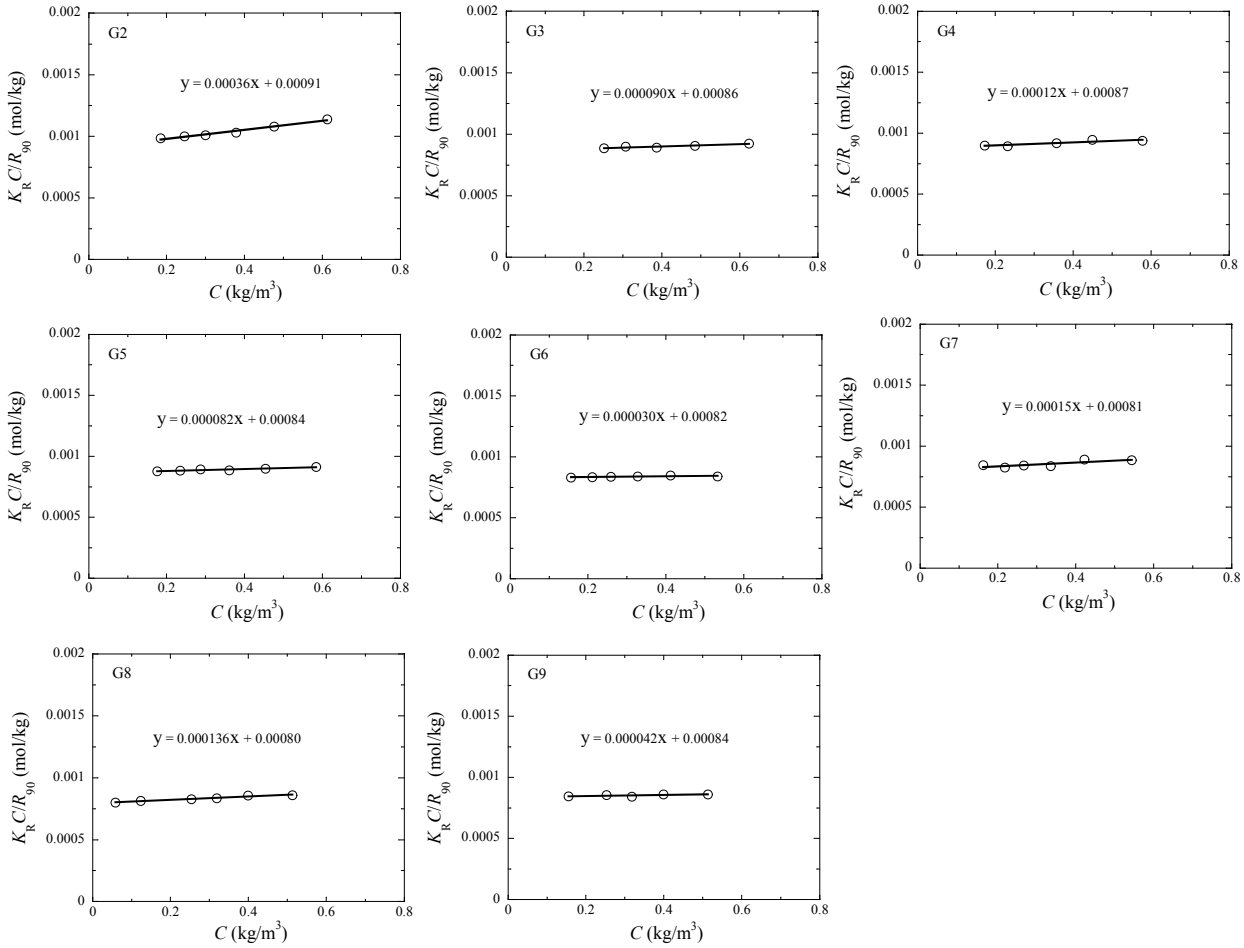

Figure S1 $K_R C/R_{90}$ is plotted as a function of total concentration of P2MVP$_{128}$-*b*-PEO$_{477}$ polymer and dendrimer. ($C = C_{overall} - CMC$, and CMC is obtained from Table S3)

The intercept of the line gives the micellar mass, $M_{micelle}$. Here we take G5 line as an example, to show how to calculate the aggregation number:

$M_{micelle} = 1/0.00084 = 1190.476$ kg/mol = 1190476 g/mol

The charge numbers, Z and molecular weights of dendrimer and polymer are

$Z_{G5} = 128$, $M_{G5} = 26252$, (g/mol)

$Z_{polymer} = 128*0.87 = 111$, $M_{polymer} = 50313$ (g/mol)

By taking into account of the PMC *f*+ shift, we get

$$N_{polymer}*111 / (N_{G5}*128 + N_{polymer}*111) = 0.48 \quad (9)$$

Moreover, the molar masses of dendrimer, polymer and micelle are recorded as follows:

$$N_{G5}*26252 + N_{polymer}*50313 = 1190476 \quad (10)$$

Finally, we combine and solve equation 9 and 10, we find that

N_{G5} = 15.0 and $N_{polymer}$ = 15.8 Then we take 15 and 16 as average dendrimer and polymer numbers within one micelle.

The calculations with other generations follow the same strategy, and micellar mass and aggregation numbers are included in the below table:

Generation	2	3	4	5	6	7	8	9
N_{PAMAM}	108.2	56.1	28.5	15.0	8.0	4.0	2.1	1.0
$N_{polymer}$	15.5	16.1	15.5	15.8	15.8	16.0	16.0	15.0

Table S2 Micellar mass and calculated aggregations numbers of Gn-C3Ms.

Determination of the CMC of Gn-C3Ms

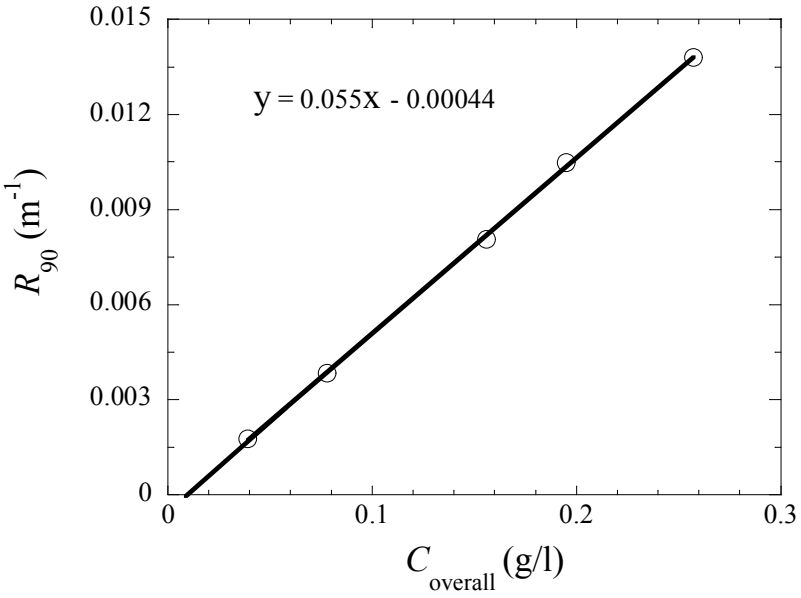

Figure S2 Intensity decay of G2-C3Ms solution upon diluting with $NaHCO_3/Na_2CO_3$ buffer. The open circles are the experimental data and the solid line is the fitting curve.

The intensity is represented as Rayleigh ration R_θ, which is subtracted with the intensity from buffer solution and corrected by the intensity of toluene as a reference, see experimental section, method, equation 2. The CMC is determined by extrapolating the decay line to zero intensity, and calculated from the fitting formula. The CMCs of the micelles formed at other generations are obtained following same strategy, and the numbers are included in the table below :

Generation	2	3	4	5	6	7	8	9
CMC g/l	0.008	0.010	0.010	0.007	0.010	0.010	0.006	0.007

Table S3 CMC of G_n-C3Ms formed at different dendrimer generation.

CONTIN analysis of micellar size and size distribution

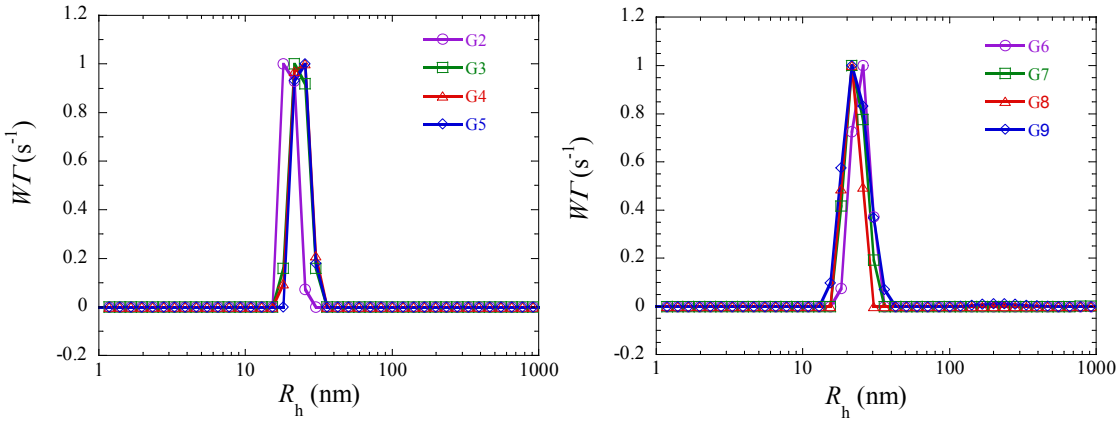

Figure S3 size and size distribution of micelles obtained by CONTIN analysis. For clearity reason, we plot the curve in two plots. In fact, there is no big difference between Gn-C3Ms at different generations.

Determination of the micellar core size and shell thickness

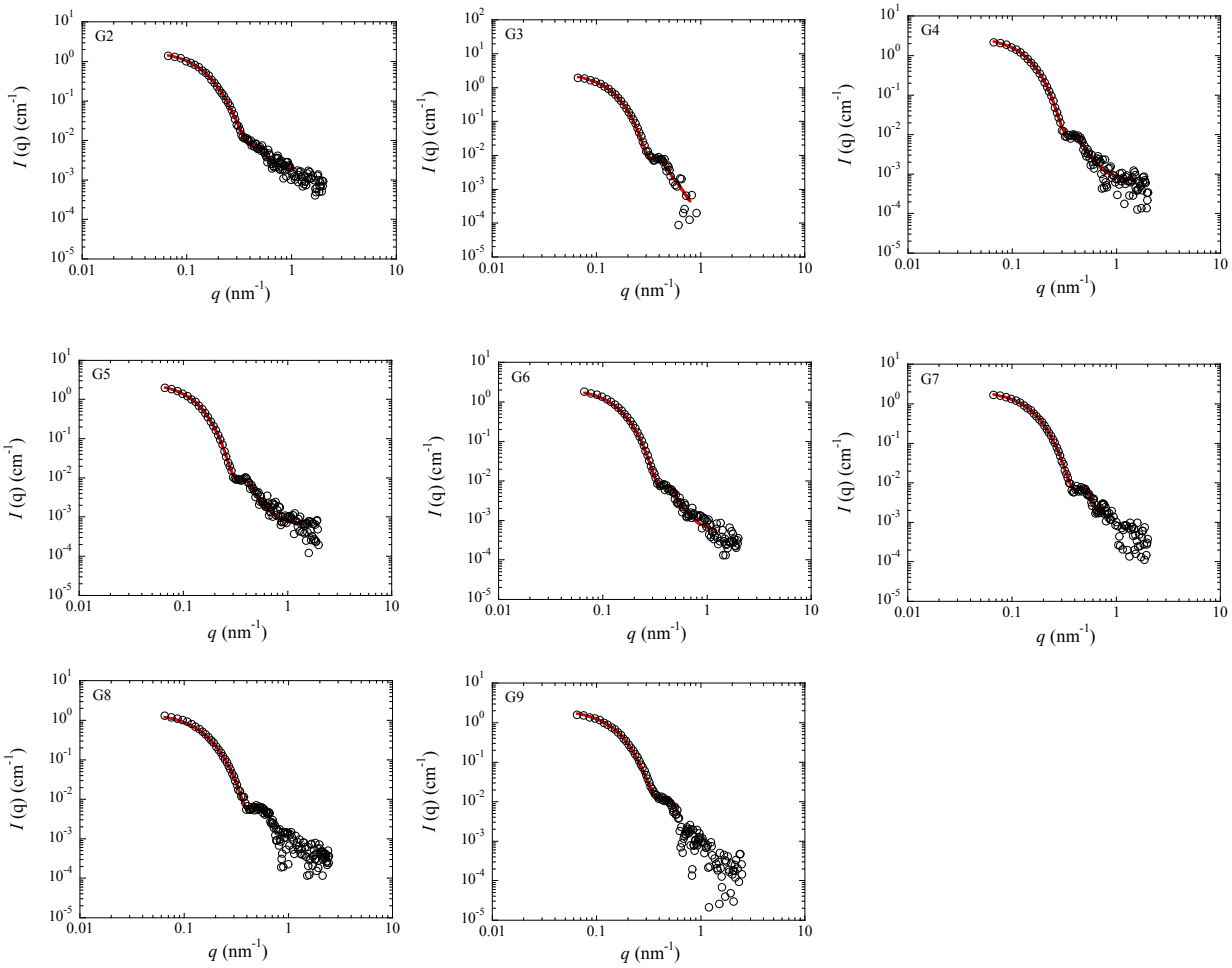

Figure S4 SAXS profiles of Gn-C3Ms at different dendrimer generations. The open circles shows the experimental data and the solid red line corresponds to the fits with a form factor for polydisperse (Gaussian distribution) core-shell spheres. The obtained core radius R_{core} and shell thickness H_{shell} are shown in main text, Table 1. The fitting parameters are adapted from literature:[6]

$\rho_{solvent}$ (10^{10} cm^{-2})	ρ_{shell} (10^{10} cm^{-2})	ρ_{core} (10^{10} cm^{-2})	PD ratio (%) (core and shell)
9.37	9.45	11.11	16

Table S4 Fitting parameters applied in SasView program. ρ: scattering length density; PD: polydispersity.

Reference:

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2. D. E. Koppel, *J. Chem. Phys.*, 1972, **57**, 4814-4820.
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5. P. Stepanek, *In Dynamic Light Scattering: the method and some applications; Brown, D., Ed.; Clarendon Press: Oxford, U. K.*, 1993, **Chapter 4**, 177.
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