Dual-stimuli reduction and acidic pH-responsive bionanogels: intracellular delivery nanocarriers with enhanced release

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I. Aqueous FRP of OEOMA without CMC

A series of FRP of OEOMA in water were carried out under different conditions. As a typical example, the procedure to synthesize FRP-2 (Table S1) under the conditions of OEOMA = 10 mg/mL and VA-44 = 2% of OEOMA at 40 °C is described as follows; OEOMA (0.15 g, 0.32 mmol) was dissolved in water (15 mL) in a 25 mL Schlenk flask. The resulting clear solution was purged with nitrogen for 30 min under magnetic stirring, and then heated to 40 °C in a water bath for 10 min. A nitrogen-prepurged aqueous stock solution of VA-44 (0.3 mL, 10 mg/mL, 9.3 μmol) was added using a syringe to initiate the polymerization. For kinetic studies, samples were withdrawn at different time intervals during the polymerization to determine conversion by ^{1}H-NMR. Polymerization was stopped by cooling down to room temperature.

Kinetic studies of aqueous FRP of OEOMA. A series of FRP of OEOMA in aqueous solution was carried out in the presence of a water-soluble VA-44 azo-type free-radical initiator. VA-44 was selected because of its short half-life at lower temperature ($\tau_{1/2}$ = 10 hr at 44 °C). Important parameters that influence the rate of aqueous FRP were examined; these parameters include the amount of VA-44 and temperature. For kinetic studies, aliquots withdrawn at given time intervals were analyzed for monomer conversion using ^{1}H-NMR in D_2O. Table S1 summarizes the results. First, the amount of VA-44 was varied at 40 °C. The rate of polymerization increased with an increasing amount of VA-44 in the mixture. For example, OEOMA conversion was 63% in the presence of 1 wt% VA-44 after 2 hrs (FRP-1). The conversion increased to 78% with 2 wt% VA-44 (FRP-2), and further to 83% with 3 wt% (FRP-3) under similar conditions. However, molecular weight decreased when the amount of VA-44 increased. Such decrease is attributed to the increase in flux of free radicals generated by thermal decomposition of VA-44 initiators. Next, the polymerization temperature decreased to 30 °C in the presence of 2 wt% VA-44 (FRP-4). The conversion decreased from 78% to 67% by approximately 10%, compared to FRP-2 at 40 °C. For all polymerization reactions, molecular weight distribution of the resulting POEOMA homopolymers was broad as $M_w/M_n > 2.6$, due to polymerization being preceded in uncontrolled manner. These results suggest that the rate of polymerization is enhanced in the presence of larger amounts of initiator at higher temperatures.

Table S1. Characteristics and results for FRP of OEOMA in aqueous solution after 2 hrs.

Recipe	OEOMA (mg/mL)	VA-44 [a] (wt%)	Temp (°C)	Conv [b]	M_n	M_w/M_n
FRP-1	10	1	40	0.63	159,600	2.6
FRP-2	10	2	40	0.78	115,000	3.3
FRP-3	10	4	40	0.83	97,800	3.3
FRP-4	10	2	30	0.67	120,000	3.2

a) Wt ratio based on OEOMA
b) Determined by ^{1}H-NMR.

II. Aqueous FRP of OEOMA in the presence of CMC

For the synthesis of POEOMA-g-CMC, similar procedure for aqueous FRP of OEOMA was conducted in the presence of CMC at 40 °C. For an example of FRP-C1, CMC (0.15 g) and OEOMA (0.15 g, 0.32 mmol) were dissolved in water (15 mL) in 25mL Schlenk flask. The resulting clear solution was purged with nitrogen for 30 min under magnetic stirring and then heated to 40 °C in a water bath for 10 min. A nitrogen-prepurged aqueous stock solution of VA-44 (0.3 mL, 10 mg/mL, 9.3 μmol) was added using a syringe to initiate the polymerization. Polymerization was stopped by cooling down to room temperature.

Preparation of POEOMA-g-CMC. The results of a series of FRP of OEOMA initiated with a water-soluble VA-44 azo-type free-radical initiator in aqueous solution are described in the supporting information. The optimized procedure was applied to the synthesis of POEOMA-grafted CMC (POEOMA-g-CMC). An aqueous FRP of OEOMA in the presence of CMC was conducted at 40 °C under the conditions of OEOMA = 10 mg/mL, VA-44 = 2 wt% on OEOMA, and OEOMA/CMC = 1/1 wt ratio (FRP-C1 in Table 1). Due to the high molecular weight of CMC, the viscosity of its aqueous solution strongly relies on the concentration of CMC. It was found that 1 wt% aqueous CMC solution is suitable for FRP. For more than 10 repeated polymerization reactions, conversion reached 80.4 ± 4.3% on average after 2 hrs. This value is similar to that (conv = 78%) without CMC (FRP-2 in Table S1), suggesting no significant effect of the presence of CMC on the course of aqueous FRP under the conditions. DLS was used to measure the size and size distribution of the resulting POEOMA-g-CMC in water. As seen in Figure 1 (10 mg/mL), DLS trace shows multimodal size distribution with three populations; the major population of small sizes with an average diameter ≈ 23 nm and two minor populations of larger aggregates with average diameters ≈ 86 and 487 nm. These three populations resulted in the number average diameter, D_n = 32.5 ± 11.5 nm and relatively broad size distribution, D_w/D_n = 1.5. Such multimodal distribution with relatively high standard deviation could be attributed to inter-chain or inter-particle coupling reactions, resulting in the formation of undesired aggregates.

The amount of OEOMA (as well as CMC) in the polymerization mixtures decreased from 10 mg/mL to 2 mg/mL and further to 0 mg/mL (CMC only) for aqueous FRP at 40 °C, while other parameters remained constant (OEOMA/CMC = 1/1 w/w and VA-44 = 0.2 mg/mL). Table S2 summarizes the characteristics and conversion data. For all polymerization, conversion reached as high as 80%; however, longer polymerization time was required when smaller amount of OEOMA was added. For example, FRP-C4 with 2 mg/mL OEOMA reached 81% conversion in 8 hrs. DLS results

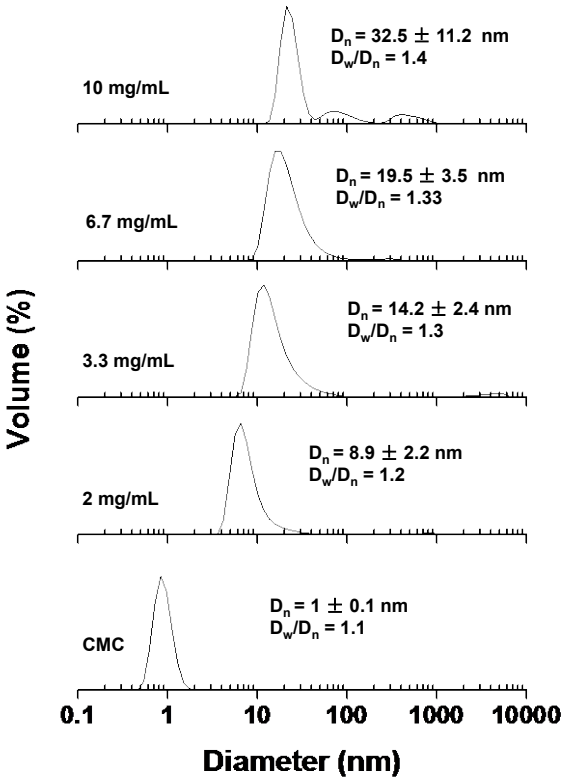
indicate that the sizes of the resulting products prepared in the presence of OEOMA are larger than that (diameter = 1 nm) of CMC only in water, suggesting the occurrence of grafting POEOMA from CMC chains (Figure 1). Interestingly, their diameter decreased with a decreasing amount of OEOMA; 33 nm at 10 mg/mL (FRP-C1) to 20 nm at 6.7 mg/mL (FRP-C2), and further to 9 nm at 2 mg/mL (FRP-C4). Polydispersity also decreased. These results suggest that the probability to inter-chain or inter-particle coupling reaction could decrease with less concentration of OEOMA in aqueous solution.

Table S2. Characteristics and results for aqueous FRP of OEOMA in the presence of CMC at 40 °C.[a)]

Recipe	OEOMA (mg/mL)	Time (hrs)	Conv[b)]
FRP-C1	10	2	0.83
FRP-C2	6.7	4	0.84
FRP-C3	3.3	5	0.86
FRP-C4	2	8	0.81

a) VA-44 = 0.2 mg/mL, OEOMA/CMC = 1/1 wt/wt
b) Determined by ^{1}H-NMR.

Figure S1. DLS diagrams with various concentrations of OEOMA in water from 0 to 10 mg/mL concentrations for aqueous FRP of OEOMA initiated with VA-44 = 0.2 wt% of OEOMA at 40 °C.

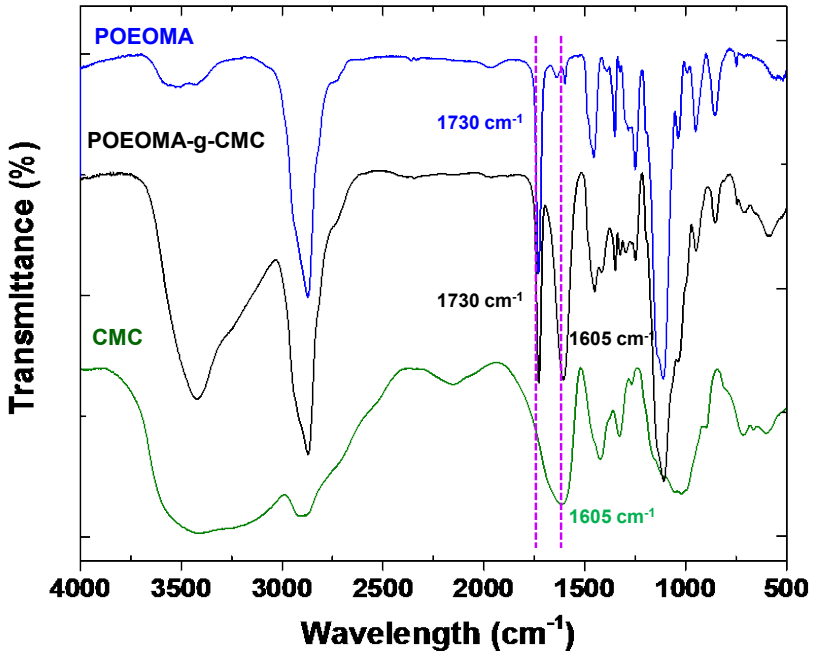


III. Qualitative analysis of POEOMA-g-CMC

Table S3. Solubility test in various solvents at 5 mg/mL concentration.

Solvent	CMC	POEOMA
Water	Dissolved	Dissolved
Acetone	Undissolved	Dissolved
Ethanol: acetic acid (1:1 v/v)	Undissolved	Dissolved

Figure S2. FT-IR spectra of POEOMA-g-CMC, CMC and POEOMA homopolymer.



IV. Loading of Dox

Figure S3. UV absorbance at λ = 497 nm of free DOX in outer water during intensive dialysis.

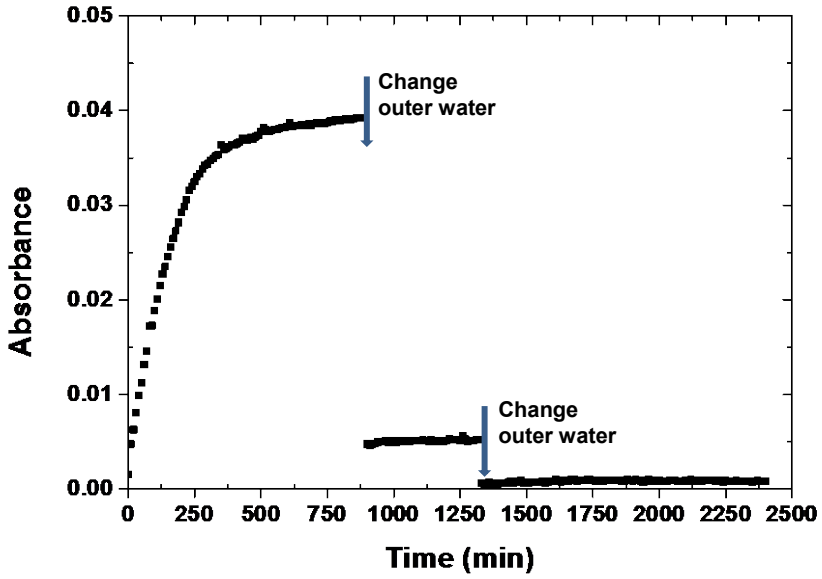
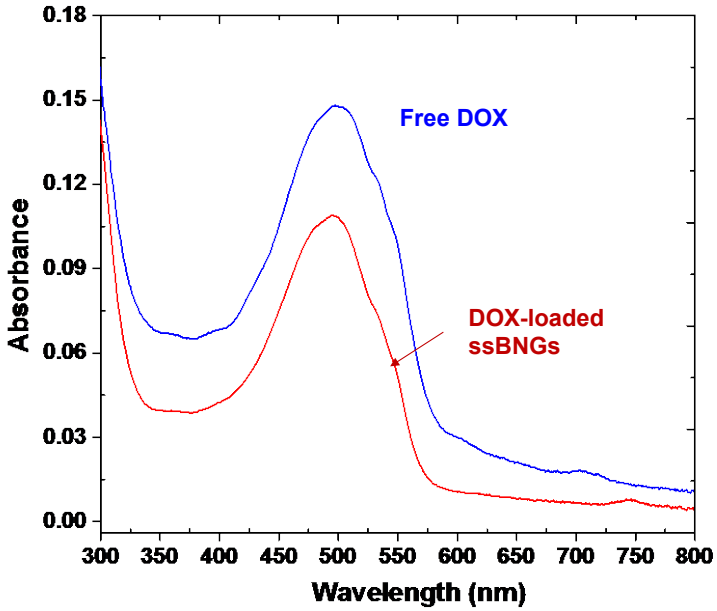


Figure S4. Comparison of UV/Vis spectra of DOX-loaded ssBNGs (0.2 mg/mL) with free DOX (7.7 μg/mL) in water.



V. Bioconjugation for active targeting

Figure S5. UV/Vis spectra of free APTS in aqueous solutions at various pH ranging from 2 to 10.

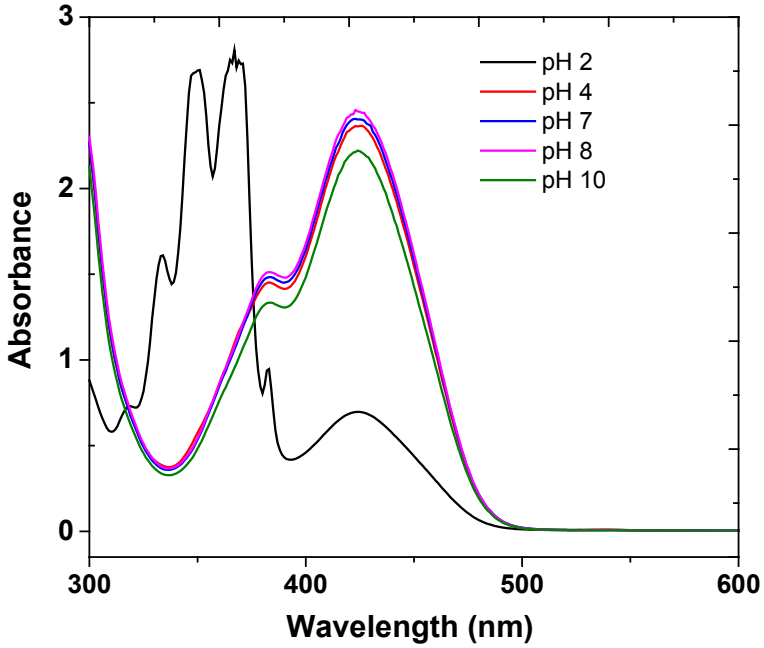


Figure S6. UV/Vis spectra (a) absorbance (b) vs different concentrations of APTS in water (pH = 6.5) to determine the extinction coefficient (ε = 21,700 M^{-1} cm^{-1}).

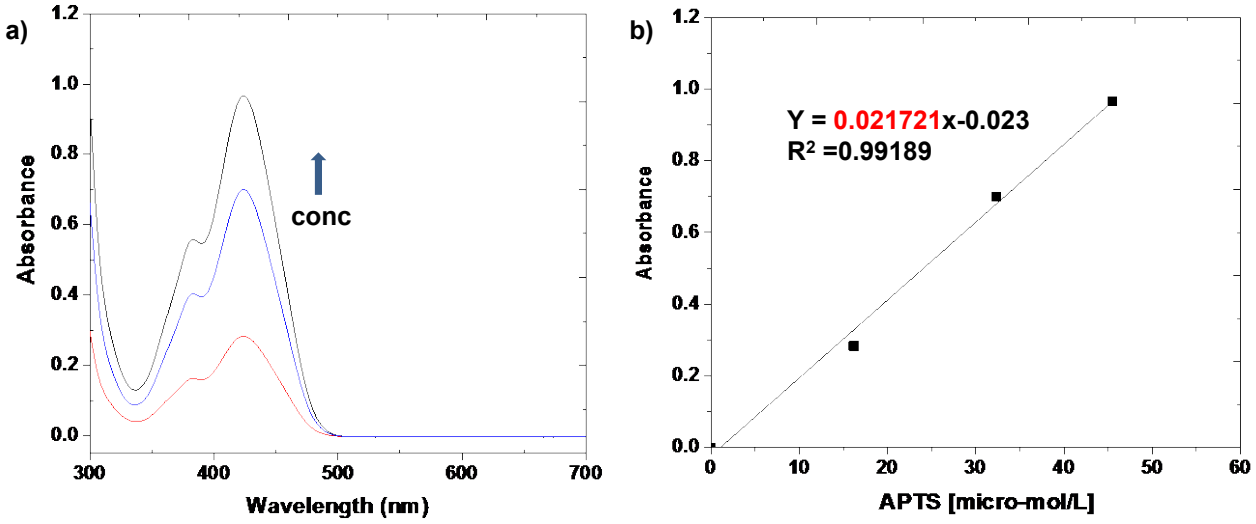


Figure S7. UV/Vis spectra and digital photos (inset) of a mixture consisting of ssBNGs, APTS, and EDC before and after occurrence of EDC coupling reaction. The spectra were recorded upon dilution of the mixtures at 32 μg/mL concentration of ssBNGs.