



**Temperature- and Salt- Responsive Polyoxometalate-  
Poly(N-isopropylacrylamide) Hybrid Macromolecules in  
Aqueous Solution**

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## Temperature- and Salt- Responsive Polyoxometalate-Poly(N-isopropylacrylamide) Hybrid Macromolecules in the Aqueous Solution

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**Polyoxometalates (POMs) as polar head groups were covalently functionalized with Poly (N-isopropylacrylamide) (PNIPAM) tails. The macromolecular hybrid demonstrates the solution behavior of hydrophilic macroions by self-assembling into blackberry structures at room temperature. It behaves like the amphiphilic surfactant by forming vesicular structure when the temperature is above the phase transition of PNIPAM. The reversible self-assembly is also salt sensitive and the salt-induced smaller vesicular formation is a result of counterion-association.**

To sustain life and maintain biological functions, living systems are able to adapt themselves to the changing environments from the molecular to macroscopic level.<sup>1,2</sup> Inspired by the nature, scientists have been designing and fabricating “smart” materials that can respond to physical and chemical external stimuli.<sup>3,4</sup> Recently, stimuli-responsive formation of self-assembly has been developing rapidly from thin films to nanoparticles for various applications in catalysis, sensors, drug delivery capsules, and et al.<sup>5-9</sup> The typical responsive nanoparticles can be realized by utilizing amphiphilic systems, among which molecular shape amphiphiles,<sup>10,11</sup> especially polymer-tethered molecular nanoparticles (MNPs), are appealing for self-assembling into diverse nanostructural materials.<sup>12-14</sup> Different from small molecular surfactants and amphiphilic diblock copolymers, those hybrids consisting rigid MNPs as polar head groups and hydrophobic polymer chains as tails can be treated as “giant surfactants” and demonstrate unique features in solution.<sup>12</sup>

Poly (N-isopropylacrylamide) (PNIPAM) is a thermo-responsive polymer which exhibits lower critical solution temperature (LCST) phase transition from hydrophilic random coiled conformation to hydrophobic collapsed globular conformation at ~32 °C in water.<sup>15-17</sup> Since the LCST of PNIPAM is near the temperature of human body, it has been recognized as a good model to predict the stability and solution

behaviors of biomacromolecules under various environmental conditions.<sup>18-21</sup> Moreover, thermo-responsive polymers play a crucial role in applications such as tissue engineering, drug delivery and materials with switchable hydrophilic and hydrophobic properties.<sup>22-24</sup>

Polyoxometalates (POMs) are a group of metal-oxide clusters of early transition and actinide metals which have well-defined shapes and charges and vast applications in catalysis, medicine, and materials science.<sup>25-28</sup> More importantly, those molecular clusters such as POMs, polyhedral oligomeric silsesquioxane (POSS) and fullerene (C<sub>60</sub>) attracted increasing attentions because they can provide precisely defined and tunable nanosized structural scaffolds.<sup>29</sup> The development of synthetic methods enabled us to tether POM clusters with organic ligands via covalent modifications.<sup>30-34</sup> Various POM hybrids have been reported to demonstrate amphiphilic features by self-assemble into various supramolecular structures in selective solvents while maintaining the unique properties of POM groups.<sup>35-37</sup> Specific hybrids have been designed to respond to external stimuli, such as pH, metal ions and UV radiation.<sup>38-41</sup>

Herein, we report the design, synthesis and self-assembly of a novel type of POM-polymer hybrid, namely, mono-vacant Keggin-type clusters were chemically linked with PNIPAM oligomeric chains. Using a typical protocol of POMs' functionalization, two silane-terminated oligomeric PNIPAM (M<sub>w</sub>~2,000, RSi(OEt)<sub>3</sub>) chains were tethered to each mono-vacant Keggin cluster (K<sub>7</sub>PW<sub>11</sub>O<sub>39</sub>), under acidic condition (Figure 1). By following previously reported method,<sup>42</sup> the reaction is conducted in acetonitrile into which tetrabutylammonium (TBA) bromide was used to solubilize Keggin clusters and consecutively added PNIPAM and hydrochloric acid dropwise. After the reaction, the solvent was evaporated, and the residue was washed with water to remove extra TBA-Br and dried in vacuum. The hybrid (K2P-TBA) structure was characterized by SEC, <sup>31</sup>P and <sup>1</sup>H-NMR, FTIR,

and MALDI-TOF Mass Spectroscopy. In SEC measurements, the retention time of the hybrid obviously decreased and showed a narrower distribution, suggesting the success of POM functionalization, which is confirmed by the change of chemical shift in  $^{31}\text{P}$ -NMR (Figure S1 and S2).

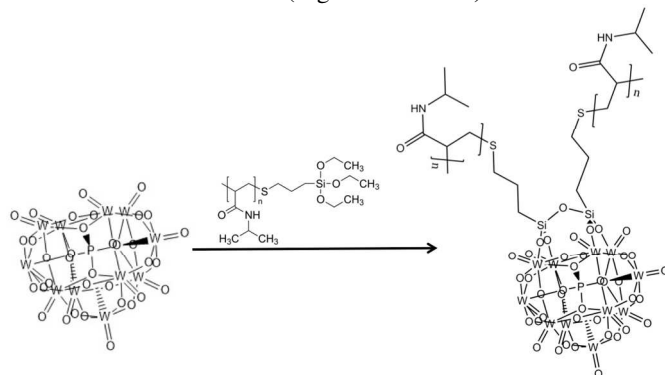


Figure 1. Synthetic scheme employed for synthesizing the temperature-responsive POM-PNIPAM hybrid.

The hybrid with different counterions such as  $\text{K}^+$  ions can be achieved by dialyzing the K2P-TBA solution against salt solution such as KCl. The resulting solution was then dialyzed against deionized water to get rid of the extra ions. Very low scattered intensity for K2P-K solution at room temperature was collected by SLS, indicating that the K2P hybrid existed as discrete molecules (Figure S3). Time-resolved LLS was applied to monitor the aqueous solution of K2P at different temperatures, which could provide information on the thermo-responsiveness of this hybrid. With increasing the temperature to above  $39\text{ }^\circ\text{C}$ , a significant increase of intensity was observed and large assemblies began to form in solution. The scattered intensity as well as the  $R_h$  ( $R_h$  is the hydrodynamic radius measured by dynamic light scattering (DLS)) were keeping going up when further increasing the temperature (Figure S3). The ratio of  $R_h/R_g \approx 1.0$  ( $R_g$  is the radius of gyration of the assemblies measured by static light scattering (SLS)) was obtained for those assemblies, suggesting their hollow, spherical structures. The temperature induced self-assembly should be related to the thermo-response of PNIPAM chains which switched from hydrophilic to hydrophobic state. Consequently, the hybrid owning a charged polar head and two hydrophobic tails behaved similarly with common amphiphiles<sup>43</sup> by forming bilayer vesicles.

POM clusters, as well as other macroions, have been reported for their unique solution behaviors by self-assembling into single-layer, hollow, spherical “blackberry” structures via counterion-mediated attraction.<sup>37, 44, 45</sup> Counterions are important in macroionic solutions and the binding affinity between macroions and counterions varies with the hydration size of counterions.<sup>46</sup> We expect different solution behaviors for the current hybrid systems with different counterions. The K2P-TBA solution was prepared for comparing with K2P-K solution. Although PNIPAM is hydrophilic in water at room temperature and the Keggin polar head is charged, with 3 TBA as counterions, interestingly the fully hydrophilic hybrids can

self-assemble into vesicular structure in aqueous solution. A typical CONTIN analysis of the dynamic light scattering (DLS) results of the K2P-TBA solution ( $0.2\text{ mg/mL}$ ) indicated that the  $R_h$  of the assemblies is  $\sim 40\text{ nm}$  with no angular dependence. The ratio of  $R_h/R_g \approx 1.0$  was obtained for those assemblies, suggesting their hollow, spherical structures, which is also confirmed by TEM measurements (Figures S4). The conductivity and Zeta potential measurements were performed and we obtained the values  $29.4\text{ }\mu\text{S/cm}$  and  $-27.5\text{ mV}$ , respectively, confirming that the vesicles were charged in water and indicating their hydrophilicity. Similar with previous reports, in our study the Keggin heads can be treated as macroions, and TBA counterions associate with Keggin, consequently inducing the attraction between macroions and their self-assembly into blackberry type structures (Scheme 1). This observation suggests that  $\text{TBA}^+$  ions have stronger association with macroions than  $\text{K}^+$  ions in pure water, causing the blackberry formation in the former case but not in the latter case. The effective charges on each blackberry can be estimated based on Equations (1) and (2),<sup>47</sup> which is equivalent to approximately 73, thereby suggesting that most of TBA counterions are closely associated with blackberries.

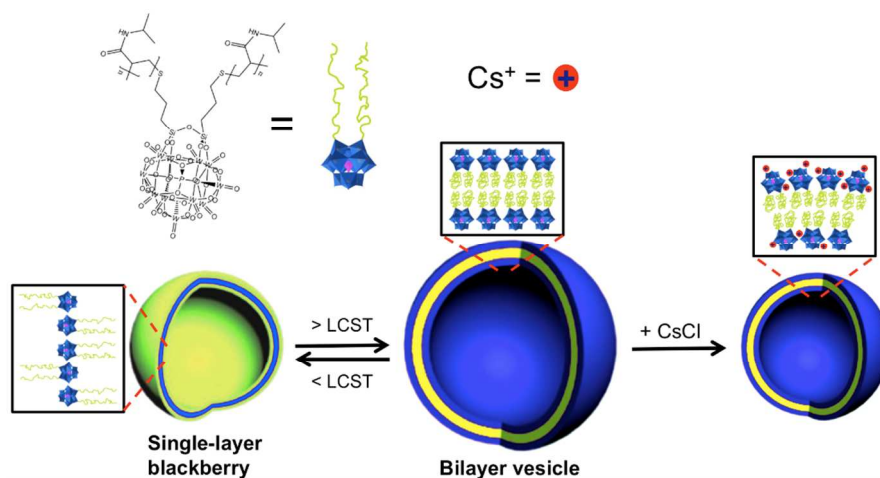
$$Ze = 6\pi\eta R_h \mu (1 + \kappa R_h) / f(\kappa R_h) \quad (1)$$

$$\zeta = 3\eta\mu / [2\epsilon_0\epsilon_r f(\kappa R_h)] \quad (2)$$

in which  $Z$  is the valence of the macromolecules (the effective charge),  $e$  is the elementary charge,  $\eta$  is the sample viscosity,  $R_h$  is the hydrodynamic radius,  $\mu$  is the electrophoretic mobility,  $\kappa$  is the Debye-Hückel parameter, and  $f(\kappa R_h)$  is Henry's function;  $\zeta$  is the zeta potential,  $\epsilon_0$  is the permittivity of free space, and  $\epsilon_r$  is the solvent dielectric constant. Since PNIPAM chains are hydrophilic and do not have special interaction with the Keggin; they should be dangling around the blackberry surface.

With increasing the temperature stepwise from room temperature to  $40\text{ }^\circ\text{C}$ , the scattered intensity of K2P-TBA solution increases gradually but slightly (Figure 2a). The  $R_h$  of assemblies remains almost the same in this temperature range (Figures 2c). From  $40$  to  $41\text{ }^\circ\text{C}$ , a significant increase of intensity was observed and it reached the plateau within 30 minutes. The scattered intensity as well as the  $R_h$  were keeping going up when further increasing the temperature (Figures 2b and 2c). The major changes happened at higher temperatures is due to the change of PNIPAM chains and the transition from blackberry structures to bilayer vesicles was proposed (Scheme 1). Since the phase transition of PNIPAM is a reversible process, the reversibility of the self-assembly of the K2P-TBA hybrid can be expected, which was confirmed by our observation of the scattered intensity returning to the original level of the same temperature (Figure 2a).

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Scheme 1. Graphical representation of the reversible transition from single-layer blackberry structures to bilayer vesicles with changing the solution temperature, and of the salt induced decrease of the vesicular size.

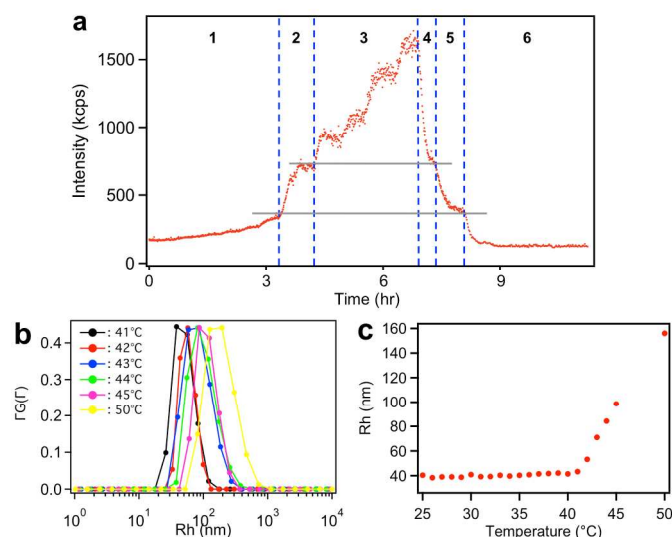


Figure 2. (a) Time-resolved SLS monitoring results of 0.2 mg/mL K2P solution during varying the temperature which is divided into 6 regions (Regions: 1. Starting from room temperature RT, gradually increasing  $T$  to 40 °C; 2.  $T$  is increase to 41 °C; 3.  $T$  is increased to 45 °C stepwise 1 °C by 1 °C; 4.  $T$  is decreased to 41 °C; 5.  $T$  is decreased to 40 °C; 6. Cooling down to RT.). (b) CONTIN analysis of DLS studies on K2P in aqueous solution at various temperatures. (c) Average  $R_h$  of the spherical structures formed by K2P as a function of temperature, measured by DLS.

Extra salts, such as NaCl, RbCl, CsCl and  $\text{CaCl}_2$ , were added into K2P-TBA solutions for studying the ionic effects on the temperature-sensitive self-assembly of K2P. Comparing the sizes of bilayer-vesicles formed in solutions with different extra salts at 45 °C, we found that the sizes of assemblies with extra CsCl and  $\text{CaCl}_2$  are much smaller than that without adding any salt (Table 1). To confirm this, in-situ experiment by adding CsCl stepwise to pre-heated K2P solution was performed. 10  $\mu\text{L}$  CsCl solution (8.3 mM) was added into K2P solution (1 mL,

0.2 mg/mL) after the scattered intensity stabilized at 45 °C, and a significant decrease of intensity from LLS (Figure 3a) was observed. The intensity of the solution after adding extra salt didn't reach the equilibrium immediately, but gradually increased and stabilized at certain level, because the temperature of the solution varied during the salt addition. By adding more CsCl to the solution, the scattered intensity further decreased, as well as the  $R_h$  of vesicles (Figure 3b), demonstrating the salt-responsiveness of the self-assembly of K2P hybrid (Scheme 1).

Table 1.  $R_h$  values of vesicles formed at 45 °C in solutions without and with adding different salts.

	No Salt	Rb <sup>+</sup>	Cs <sup>+</sup>	Ca <sup>2+</sup>
$R_h$ (nm)	100	104	52	61

It's well known that the phase transition of PNIPAM is influenced by the presence of salts, and the effect of alkali metal cations on lowering LCST correlates well to the position of corresponding ions in the classic Hoffmeister series.<sup>48</sup> However, the salt effect on PNIPAM isn't considered as the main reason for the vesicle shrink in our study since the concentration of salt we used is too low to affect the LCST of polymer. Moreover, control experiments of adding the same amount of salt to pure PNIPAM solution indicated no effects on the polymer's phase transition (Table S1). We expect that the salt-responsiveness is relative to the counterion association with Keggin, leading to bigger surface area of the polar head and consequently bigger curvature of vesicles (Scheme 1). The hypothesis can also explain why cations with smaller hydration sizes such as Cs<sup>+</sup> and Ca<sup>2+</sup> can effectively tune the assembly size, but not the ones with larger hydration size such as Rb<sup>+</sup>, as

cations in the former case demonstrate higher affinity to macroions.

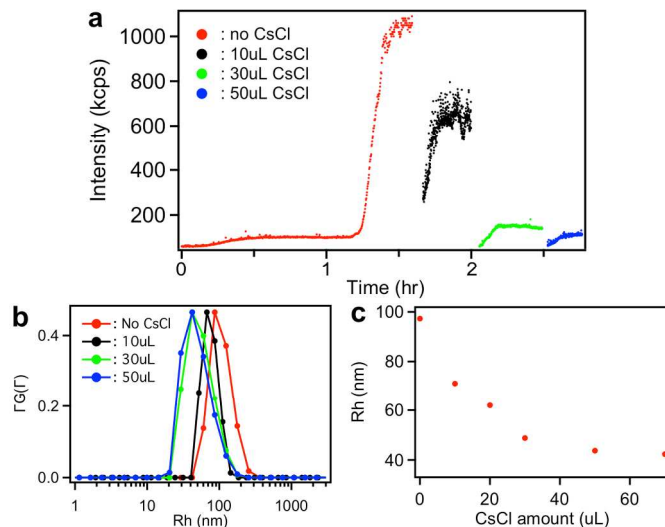


Figure 3. (a) Time-resolved SLS monitoring results of 0.2 mg/mL K2P solution under 45°C during adding CsCl. (b) CONTIN analysis of DLS studies on K2P in aqueous solution with different amount of CsCl. (c) Average  $R_h$  of the spherical structures formed by K2P as a function of CsCl concentration, measured by the DLS.

In summary, a novel type of POM-PNIPAM macromolecular hybrid (K2P), possessing a rigid polar Keggin head group and two temperature-sensitive PNIPAM tails, was designed and synthesized. The obtained hybrid behaves like regular hydrophilic macroions in the aqueous solution at RT by showing the formation of blackberry structures via counterion-mediated attraction. At elevated temperatures, the K2P form amphiphilic bilayer vesicles in virtue of the phase transition of PNIPAM. The vesicle size can be tuned by introducing extra salt into the solution because of the consequent counterion-association with Keggin macroions.

## Notes and references

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