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COMMUNICATION

Thermal Responsiveness and Binding Affinity of Cucurbit[7]uril Terminal Poly(*N*-isopropylacrylamide)

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We synthesized cucurbit[7]uril terminal poly(*N*-isopropylacrylamide) (CB[7]-PNIPAM) *via* click reaction of monopropargylated CB[7] and azido terminal PNIPAM. We found that the introduction of CB[7] and charged guest molecules raised the lower critical solution temperature of PNIPAM significantly. And CB[7]-PNIPAM maintained high binding affinity of CB[7].

Cucurbit[n]uril (CB[n], n=5-8, 10, 13-15), a family of synthetic macrocyclic host molecules composed of n methylene bridged glycoluril units, has attracted extensive attention because of its strong binding affinity and good selectivity.[1-8] The outstanding recognition properties of CB[n]s make them widely employed in molecular recognition, self-assembly,[9] molecular switches[10, 11] and pharmaceutical sciences.[12, 13] However, compared with other host molecules like crown ethers, cyclodextrins and calixarenes, the applications of CB[n]s are still limited because of their poor solubility in common solvents and difficult modification. The direct functionalization of CB[n]s was long since thought to be impossible because of their high chemical stability.[14] Kim's group reported the direct functionalization of CB[n]s through oxidation with $K_2S_2O_8$ in water which led to hydroxy derivatives of CB[n]s including perhydroxy CB[n]s (n=5-8),[15] multihydroxy[16] and monohydroxy[17] CB[7]. On this basis, Scherman *et al*. prepared monohydroxy CB[6] through oxidation with $(NH_4)_2S_2O_8$ in the presence of a bisimidazolium guest,[18] and monohydroxy CB[n] (n=5-8) was reported with 5-37% yields using hydrogen peroxide and UV light.[19, 20] Further chemical modification of hydroxy derivatives of CB[n]s was achieved *via* conventional organic transformation routes. In addition, Isaacs's group synthesized monofunctionalized CB[6] and CB[7] derivatives by condensation of glycoluril hexamer with *o*-phthaldehyde and glycoluril bis(cyclic ethers), respectively.[21-23] Among the members of the CB family, CB[7] has drawn much interest because of its ability to form ultra-stable complexes with a variety of guests[7] and its moderate solubility in water (20-30 mM) compared with the poor solubility of CB[6] and CB[8] ($< 10^{-5}$ M)[1]. And the functionalization of CB[7] has made its ultrahigh affinity applied to biomolecule immobilization,[16, 24] protein isolation[25] and supramolecular hydrogels.[26]

Poly(*N*-isopropylacrylamide) (PNIPAM) is an extensively used thermo-sensitive material which exhibits a reversible liquid-solid phase transition with a lower critical solution temperature (LCST) around 32 °C.[27, 28] This phase transition is attributed to the disruption of hydrogen bonding with water and the increasing hydrophobic interactions among isopropyl groups.[29] Stöver's group reported the synthesis of narrow-disperse PNIPAM with high conversion and good molecular weight control at room temperature by atom transfer radical polymerization (ATRP).[30] Aqueous solutions of these PNIPAMs showed a strong decrease of LCST with increasing molecular weight. Our group has reported the synthesis of CB[7] pendent copolymer (poly(4VBOCB[7]-co-NIPAM)) through radical polymerization of NIPAM and 4-vinylbenzyloxy CB[7].[31] The polymer exhibited a LCST at 34 °C, and loading with different guest molecules in the cavity of CB[7] had no influence on its LCST. The copolymer demonstrated a little lower binding affinities (1-2 orders of magnitude lower) to guest molecules compare with CB[7]. And the apparent binding stoichiometry of those guest molecules to the copolymer was lower than those to CB[7]. In this paper, we synthesized CB[7] terminal PNIPAM (CB[7]-PNIPAM) *via* click reaction between monopropargylated CB[7] and azido terminal PNIPAM (N_3-PNIPAM) prepared by ATRP and investigated its thermal responsiveness and binding affinity by turbidimetry and isothermal titration calorimetry (ITC).

The copper-catalysed azide-alkyne cycloaddition (CuAAC) "click" reaction is a convenient method to specifically modify polymers in the side chain or at the terminus because of its excellent rate and selectivity.[32-35] We have reported an "in-water" CuAAC catalyst with good compatibility, stability and high catalytic activity in host-guest system based on CBs.[36]

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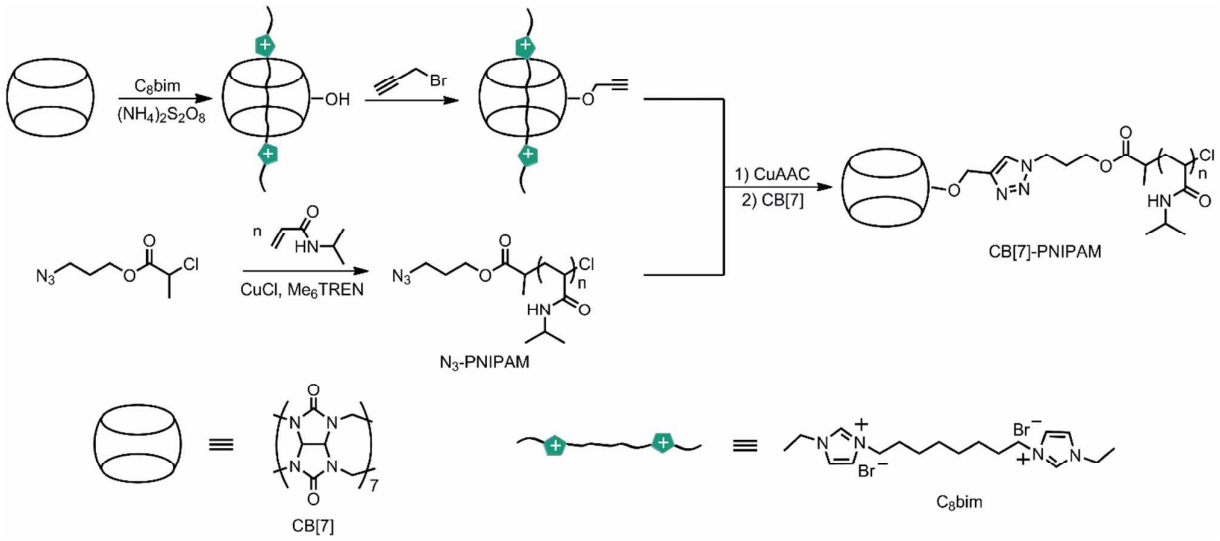

Scheme 1 Synthesis procedure of cucurbit[7]uril terminal poly(*N*-isopropylacrylamide) (CB[7]-PNIPAM).

Hence, monopropargylated CB[7] was designed and synthesized with the support of 3,3'-(octane-1,8-diyl)-bis-(1-ethyl-imidazolium) dibromide (C_8bim) following the synthesis procedures of monopropargylated CB[6][18] and 4-vinylbenzyloxy CB[7]. N_3-PNIPAM was synthesized by ATRP using 3-azidopropyl 2-chloropropanoate/CuCl/Me_6TREN (1:1:1) as the initiating system. C_8bim·CB[7]-PNIPAM was obtained *via* click reaction of monopropargylated CB[7] and N_3-PNIPAM. And C_8bim was removed through dialysis in CB[7] solution according to our previous report.[31] Two CB[7]-PNIPAMs with different polymerization degrees were synthesized following the above steps (Scheme 1), and detailed synthesis procedure could be found in the ESI.

Polymer molecular weights and polydispersity indices (PDIs) of N_3-PNIPAMs were determined by GPC. The M_n values and PDIs of N_3-PNIPAM-1 and N_3-PNIPAM-2 were found to be 16100 g·mol^{-1}, 1.20 and 9200 g·mol^{-1}, 1.15, respectively. Precise molecular weights of CB[7]-PNIPAMs were determined using ^{1}H NMR spectroscopy by comparing the integration of the polymer isopropyl C-H signal at 3.8 ppm with the CB[7] C-H signal at 5.7 ppm. The M_n values of CB[7]-PNIPAM-1 and CB[7]-PNIPAM-2 were found to be 16200 g·mol^{-1} and 7400 g·mol^{-1}. The ^{1}H NMR spectrum of CB[7]-PNIPAM-1 was shown in Fig. 1, more GPC and ^{1}H NMR data plots could be found in the ESI.

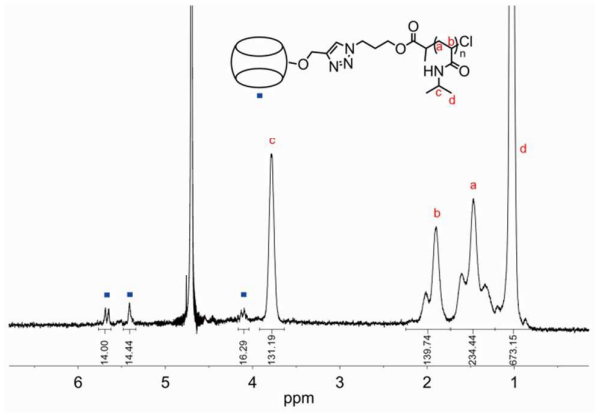

Fig. 1 ^{1}H NMR spectrum of CB[7]-PNIPAM-1 in D_2O. Peaks of CB[7] are marked with blue squares.

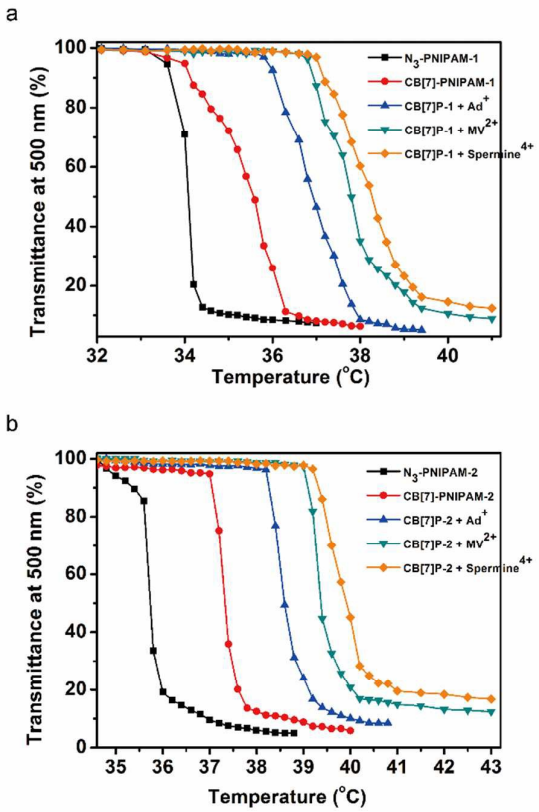

Fig. 2 Transmittance *vs* temperature for 1 wt% solutions of N_3-PNIPAM, CB[7]-PNIPAM and it with different guest molecules (Ad^+: 1-adamantanamine hydrochloride, MV^{2+}: dimethyl viologen dichloride, $Spermine^{4+}$: spermine tetrahydrochloride). a) PNIPAM-1. b) PNIPAM-2.

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Table 1 Binding properties of guest molecules with CB[7] and CB[7]-PNIPAM-1.

Guest molecules	K with CB[7] (M^{-1})	CB[7]-PNIPAM-1		poly(4VBOCB[7]-co-NIPAM)[a]	
		K (M^{-1})	Apparent binding stoichiometry	K (M^{-1})	Apparent binding stoichiometry
L-Phenylalanine	$(1.8\pm0.2)\times10^{6,a}$	$(2.3\pm0.1)\times10^{5,b}$	0.89[b]	$(3.1\pm0.2)\times10^{5}$	0.74
Dimethyl viologen dichloride	$(1.7\pm0.1)\times10^{8,a}$	$(1.4\pm0.2)\times10^{6,b}$	0.87[b]	$(4.8\pm2.9)\times10^{7}$	0.63
Spermine tetrahydrochloride	$(4.8\pm0.6)\times10^{8,a}$	$(6.0\pm1.9)\times10^{7,c}$	0.97[c]	$(3.8\pm0.6)\times10^{7}$	0.54
1,6-Diaminohexane dihydrochloride	$(2.1\pm0.4)\times10^{9,a}$	$(7.8\pm2.1)\times10^{7,c}$	0.97[c]	$(3.8\pm1.1)\times10^{7}$	0.74
Hydroxymethylferrocene	$(3.2\pm0.5)\times10^{9,a}$	$(3.7\pm0.1)\times10^{8,c}$	1.08[c]	$(3.3\pm0.3)\times10^{8}$	0.89
(Ferrocenylmethyl)trimethylammonium iodide	$(4.1\pm1.0)\times10^{12,a}$	$(2.5\pm0.2)\times10^{9,c}$	0.97[c]	$(6.4\pm0.2)\times10^{10}$	0.93
1-Adamantanamine hydrochloride	$(1.7\pm0.8)\times10^{14,a}$	$(8.2\pm1.0)\times10^{10,c}$	0.97[c]	$(3.7\pm1.8)\times10^{12}$	1.03

[a] Cited from references.[31, 37, 38] [b] Determined by ITC experiments at 298 K in pure water. [c] Determined by ITC competing experiments (see Figure S6-S12 in the ESI for full details and data of the ITC experiments).

We wonder whether the introduction of CB[7] and loading with charged guest molecules in the cavity of CB[7] influence the thermal responsiveness of PNIPAM, so the LCSTs of CB[7]-PNIPAMs were investigated using turbidimetry. The plots of transmittances of two polymers and them with different guest molecules at 500 nm *versus* temperatures were shown in Fig. 2. The LCSTs of CB[7]-PNIPAMs were about 1.5 ^{o}C higher than the LCSTs of the corresponding N_3-PNIPAM. Loading with charged guest molecules in the cavity of CB[7] raised the LCSTs of CB[7]-PNIPAMs significantly. And guest molecule with greater charge led to higher LCST. The results confirmed that the introduction of CB[7] and loading with charged guest molecules in the cavity of CB[7] improve the hydrophilicity of PNIPAM. Greater charge meant higher polarity and stronger charge repulsion which hindered the intermolecular interaction, and the two factors led to higher LCST. The LCSTs of PNIPAM-2 were higher than the LCSTs of PNIPAM-1 under all the conditions because of its lower molecular weight. And the influences of CB[7] and guest molecules on the LCSTs of PNIPAM-2 were in accordance with those on the LCSTs of PNIPAM-1. With these results in hand, we could conclude that the LCST of CB[7]-PNIPAM can be adjusted by loading with different guest molecules in the cavity of CB[7] or changing molecular weight of polymer.

The variation of LCST of CB[7]-PNIPAM was totally different from poly(4VBOCB[7]-co-NIPAM). We believed that the disparity stemmed from different polymer molecular weights and PDIs of PNIPAMs polymerized *via* radical polymerization and ATRP. The low molecular weight and narrow distribution of CB[7]-PNIPAM made it susceptible to end group.[30] Furthermore, the ratio of the CB[7] moiety of poly(4VBOCB[7]-co-NIPAM) was only 0.86 mol %. Loading with charged guest molecules in the cavity of CB[7] could hardly change the LCST.

The binding properties of CB[7]-PNIPAM-1 were studied using ITC. In order to contrast CB[7] terminal PNIPAM with CB[7] pendent copolymer we have reported, same guest molecules were chosen. The data for the binding properties of guests to CB[7]-PNIPAM-1 were listed in Table 1, and the data of poly(4VBOCB[7]-co-NIPAM) and CB[7] were also listed for comparison. CB[7]-PNIPAM-1 maintained high binding affinity of CB[7] as did poly(4VBOCB[7]-co-NIPAM). The binding constant of CB[7]-PNIPAM-1 and 1-adamantanamine hydrochloride was up to 10^{10} M^{-1}. Similarly, the binding constants of all the guest molecules with CB[7]-PNIPAM-1 were 1-4 orders of magnitude lower than CB[7], and their binding constant sequences were consistent. Interestingly, CB[7]-PNIPAM-1 demonstrated 1–2 orders of magnitude lower binding affinities to all the guest molecules compare with poly(4VBOCB[7]-co-NIPAM). We believed that the CB[7] moieties of CB[7]-PNIPAM-1 were more likely to be shrouded by polymer chains, because poly(4VBOCB[7]-co-NIPAM) tended to form short blocks due to the great difference on reactivity ratio between styrene based monomer and acrylamide based monomer during polymerization.[39] Different from the significant loss of the apparent binding stoichiometry between the guest molecules and poly(4VBOCB[7]-co-NIPAA), CB[7]-PNIPAM-1 bound to these guest molecules with apparent binding stoichiometry approximate to 1. The steric hindrance and charge repulsion of adjacent CB[7] and CB[7]-guest moieties of CB[7] terminal PNIPAM was far less than CB[7] pendent copolymer. Therefore, the availability of CB[7] moiety of CB[7]-PNIPAM-1 was higher than poly(4VBOCB[7]-co-NIPAM).

In summary, two CB[7] terminal PNIPAMs with different molecular weights were synthesized *via* click reaction of monopropargylated CB[7] and azido terminal PNIPAM polymerized by ATRP. And the thermal responsiveness and

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binding affinity of CB[7]-PNIPAM were investigated and compared with CB[7] pendent copolymer. Different from CB[7] pendent copolymer, the LCST of CB[7]-PNIPAM was about 1.5 ^{o}C higher than N_3-PNIPAM, and loading with guest molecule with greater charge in the cavity of CB[7] led to higher LCST. Similarly, CB[7]-PNIPAM inherited the high binding affinity of CB[7]. Better yet, the CB[7] moiety of CB[7]-PNIPAM was efficiently used. The CB[7] terminal PNIPAM will be useful in the construction of supramolecular graft and block copolymers.

Conflicts of interest

There are no conflicts to declare.

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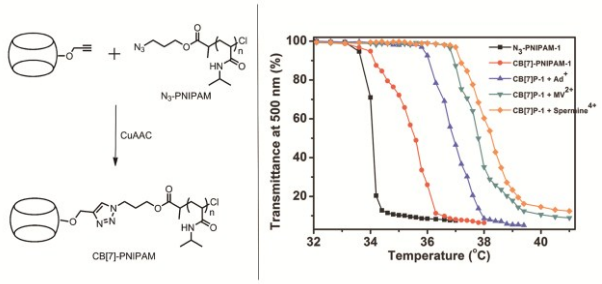

The introduction of CB[7] and guests raised the LCST of PNIPAM significantly, and CB[7]-PNIPAM maintained high binding affinity of CB[7].