Dendrimer-Based Vesicles

DOI: 10.1002/anie.201101007

Thermosensitive Molecular Assemblies from Poly(amidoamine) Dendron-Based Lipids

Kenji Kono, Etsuo Murakami, Yuki Hiranaka, Eiji Yuba, Chie Kojima, Atsushi Harada, and Kazuo Sakurai*

Molecular assemblies such as vesicles and micelles are attractive as carriers of bioactive molecules. Numerous attempts have been made to assign functions to them and provide the capability to fulfill these functions. In particular, temperature-responsive properties are important as functions of molecular assemblies. Several strategies are available to sensitize them to temperature. For example, temperature-sensitive vesicles have been prepared using a gel-to-liquid crystalline phase transition.[1] Thermosensitive polymers are also used to give temperature-sensitive properties to phospholipid vesicles[2] and polymer micelles.[3]

Recently, we reported a new strategy for temperature sensitization of temperature-insensitive molecules using side groups of thermosensitive polymers.[4] We introduced various alkyl amide groups, which are common with thermosensitive poly(*N*-alkyl acrylamide)s[5] or poly(*N*-vinyl alkyl amide)s,[6] to the periphery of poly(amidoamine) (PAMAM) dendrimers. The resultant PAMAM dendrimers exhibited a lower critical solution temperature (LCST), which indicates that accumulation of *N*-alkyl amide groups in the dendrimer periphery provides the temperature-sensitive property to the originally temperature-insensitive dendrimers. Similarly, incorporation of these groups in chain ends of poly(propylene imine) dendrimers[4a] and hyperbranched poly(glycidol)s[7] provides them with temperature-sensitive properties, which indicates that this approach is generally applicable to providing such properties to temperature-insensitive molecules.

For this study, we sought to expand this strategy to temperature sensitization of molecular assemblies. For this purpose, we used PAMAM dendron-based lipids, which were composed of a PAMAM dendron and two octadecyl chains as the head group and hydrophobic tails, respectively.[8] Figure 1 presents our design of molecular assemblies with temperature-sensitive properties using PAMAM dendron-based lipids having isobutyramide (IBAM) groups, which are

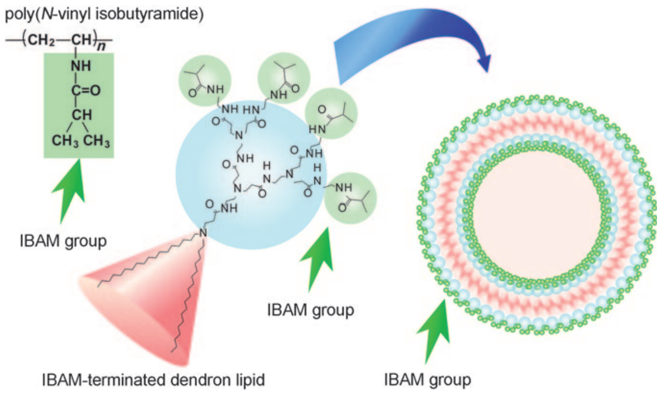
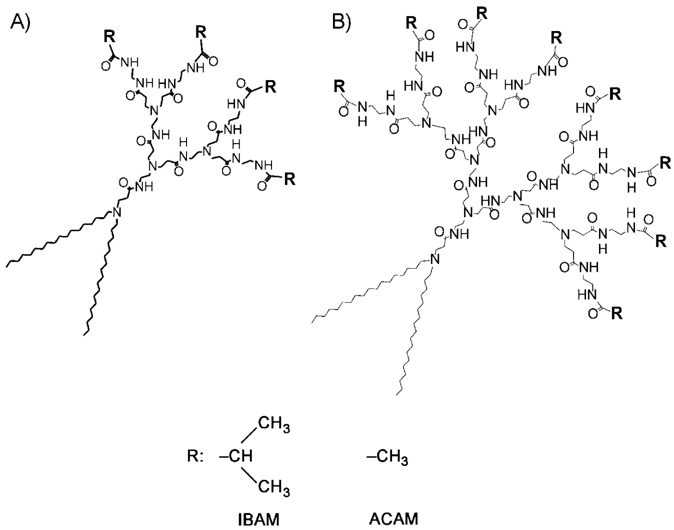


Figure 1. Design of molecular assemblies with temperature-sensitive properties using isobutylamide (IBAM) terminated dendron-based lipids.

common structural units with thermosensitive poly(*N*-vinyl isobutyramide), at every chain terminal of the dendron moiety. When the IBAM-terminated PAMAM dendron lipid molecules were dispersed in an aqueous solution, these molecules spontaneously formed assemblies with IBAM groups accumulating in the surface, which were expected to generate temperature-sensitive properties. We also prepared PAMAM dendron-based lipids with acetamide (ACAM) groups (Scheme 1). Correlation between the terminal groups of the *N*-alkyl amide-terminated dendron lipids and



Scheme 1. Structures of A) PAMAM second generation (G2) and B) third generation (G3) dendron-based lipids with IBAM or ACAM groups.

[*] Prof. Dr. K. Kono, E. Murakami, Y. Hiranaka, Dr. E. Yuba, Dr. A. Harada
Department of Applied Chemistry, Osaka Prefecture University
1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 5998531 (Japan)
Fax: (+81) 72-254-9330
E-mail: kono@chem.osakafu-u.ac.jp
Dr. C. Kojima
Nanoscience and Nanotechnology Research Center
Research Organizations for the Twenty First Century
Osaka Prefecture University (Japan)
Prof. Dr. K. Sakurai
Department of Chemical Processes & Environments
The University of Kitakyushu (Japan)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201101007.

the temperature-sensitive properties of their assemblies in aqueous solution was investigated.

Previously, we achieved incorporation of various alkyl amide groups in every chain terminal of PAMAM dendrimers using the corresponding carboxylic acid chlorides and anhydrides.[4] Based on our previous studies,[4] PAMAM G2 and G3 dendron-based lipids were reacted with isobutyryl chloride, which respectively afforded IBAM-G2-DL and IBAM-G3-DL. ACAM-terminated PAMAM G2-DL (ACAM-G2-DL) was also synthesized by reacting the PAMAM dendron-based lipids with acetic anhydride.

We prepared suspensions of these alkyl amide-terminated dendron lipids by dispersing their dry membranes in 10 mM phosphate solution at pH 3.0, in which tertiary amines of the PAMAM dendron moiety were fully protonated,[9] using a bath-type sonicator. The obtained suspensions were added to a 10 mM phosphate solution of predetermined pH. These dendron-based lipids were shown to form assemblies by dynamic light scattering (DLS): the diameters of IBAM-G2-DL, IBAM-G3-DL, and ACAM-G2-DL assemblies were estimated to be around 60, 200, and 100 nm, respectively, at 20 °C (see Figure S1 in the Supporting Information).

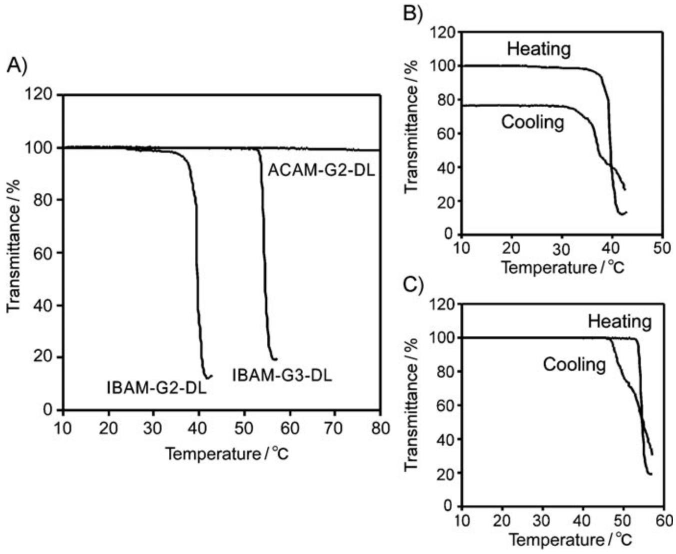


Figure 2. A) Effect of temperature on transmittance ($\lambda = 500$ nm) of IBAM-G2-DL, IBAM-G3-DL, and ACAM-G2-DL suspended in 10 mM phosphate solution of pH 7.0. The temperature was increased at 1 °C min^{-1}. B,C) Temperature-dependent transmittance of IBAM-G2-DL (B) and IBAM-G3-DL (C) suspensions (10 mM phosphate, pH 7.0) upon heating and subsequent cooling. The concentration of the PAMAM dendron-based lipids was 0.2 mg mL^{-1}.

We examined whether assemblies of PAMAM dendron-based lipids with IBAM groups generate thermosensitive properties or not (Figure 2). IBAM-G2-DL suspension was transparent at low temperature and pH 7.0. However, when the temperature was increased, transmittance of the suspension decreased suddenly around 40 °C, which indicated that the lipid suspension became unstable at that temperature. Similarly, a temperature-dependent change in transmittance was observed around 50 °C for the IBAM-G3-DL suspension. This result indicates that these IBAM-terminated lipids form temperature-sensitive assemblies that are stable at low temperatures but which lose colloidal stability above a specific temperature. In contrast, ACAM-G2-DL suspension was stable under the experimental conditions. These results show that alkyl amide moieties of the dendron-based lipids play an important role in generation of temperature-sensitive properties of their suspensions. We have already shown that the IBAM-terminated PAMAM dendrimers of higher than third generation show a LCST and that sufficient accumulation of IBAM groups in the dendrimer surface is crucial for creation of temperature-sensitive properties.[4a] The fact that the suspension of IBAM-terminated PAMAM G2-DL, which has only four IBAM groups, exhibited a cloud point implies that the accumulation of terminal IBAM groups through assembly of IBAM-terminated dendron lipids might be the origin of their temperature-sensitive property.

We also examined the effect of pH on the temperature-sensitive properties of IBAM-terminated dendron lipids (Figure 3). For both IBAM-terminated dendron lipids, the cloud point, which is defined as the temperature at which transmittance decreased to 50 %, increased as the pH of the suspensions increased. Protonation of tertiary amines of the PAMAM dendron moiety enhanced hydration of the lipid head group at lower pH values.[9] Therefore, a higher temperature is necessary to induce dehydration of the surface of the assemblies. The result again demonstrates the importance of the dendron moieties for generation of temperature-sensitive properties of their assemblies.

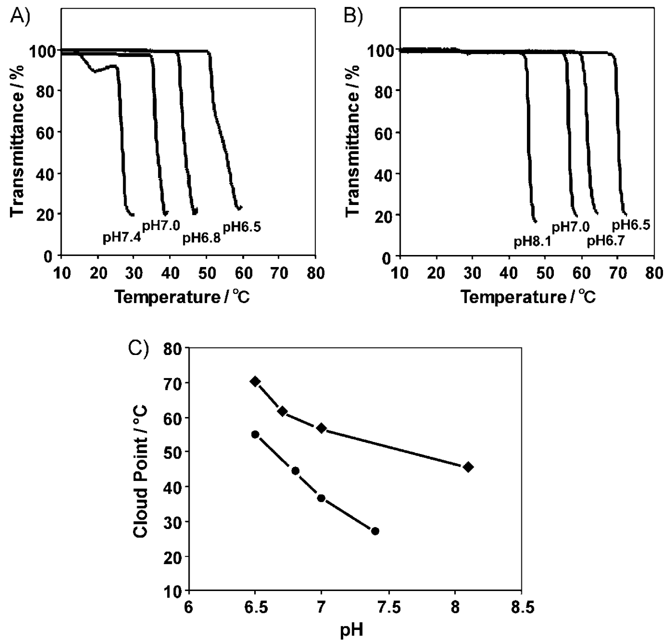


Figure 3. A,B) Effect of temperature on transmittance ($\lambda = 500$ nm) of IBAM-G2-DL (A) and IBAM-G3-DL (B) suspended in 10 mM phosphate solution at various pH values. C) Cloud points of IBAM-G2-DL (●) and IBAM-G3-DL (◆) suspensions as a function of pH. The concentration of IBAM-G2-DL and IBAM-G3-DL was 0.2 mg mL^{-1}.

Next, we examined the morphology of IBAM-G2-DL assemblies formed in aqueous solutions by using transmission electron microscopy (Figure 4A–C). We observed spherical assemblies with a diameter of 50–100 nm at 15 °C. Considering their structural similarity with bilayer-forming amphiphiles, such as dioctadecyl dimethylammonium salts, and the size of their assemblies, it is likely that IBAM-G2-DL forms vesicles at that temperature. However, when the assemblies were incubated for 10 min at 50 °C, which is higher than the cloud point, we observed a fibrous structure about 8–10 nm in thickness (Figure 4C). The IBAM-G3-DL molecules also formed spherical vesicles with diameters of 100–200 nm at 15 °C (Figure 4D). After incubation above the cloud point for 10 min, we again observed spherical vesicles but they were much larger after incubation above the cloud point, which indicated that fusion of vesicles took place during the incubation (Figure 4E). An increase in particle size of IBAM-G3-DL after incubation above the cloud point was also observed using DLS. The average diameter was shown to change from about 250 to about 500 nm (see Figure S2 in the Supporting Information). The morphology of the IBAM-G2-DL assembly was also examined by AFM. We observed spherical particles with diameters around 100 nm at 20 °C (see Figure S3 in the Supporting Information), but their morphology was changed to a fibrous structure at 50 °C (Figure 4F).

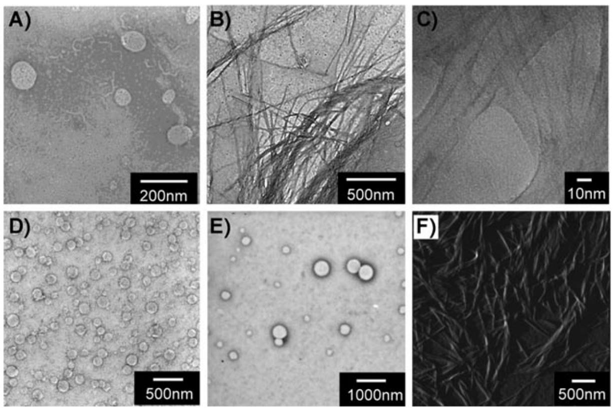


Figure 4. Morphologies of IBAM-terminated dendron-based lipid assemblies. A–E) TEM images of IBAM-DL-G2 (A–C) and IBAM-DL-G3 (D,E) assemblies dispersed in 10 mм phosphate-buffered solution of pH 7.0 at 20 (A,D), 50 (B,C), and 70 °C (E). F) AFM image of IBAM-DL-G2 at 50 °C. The concentration of IBAM-G2-DL and IBAM-G3-DL was 2.0 mg mL^{-1}.

The significant change in the morphology of IBAM-G2-DL assemblies was further analyzed using small-angle X-ray scattering (SAXS; Figure 5). The SAXS profiles show two diffraction peaks at $q = 1.457$ and 2.919 nm^{-1} between room temperature and 34 °C. The relation of these peak positions satisfies the ratio of 1:2, thereby indicating the presence of lamellar stacking with a pitch of 4.3 nm. The broad amplitude overlaying the lamellar peaks can be ascribed to the form factor of one bilayer. Above 36 °C, the scattering resulting from the lamellar form became weaker and new peaks emerged at 47 °C. Since the peak positions satisfy the relation of 1:$\sqrt{3}$:2, the new structure at higher temperatures can be assigned to hexagonally packed cylinders. The SAXS indicates the transition from a vesicle to a rodlike structure.

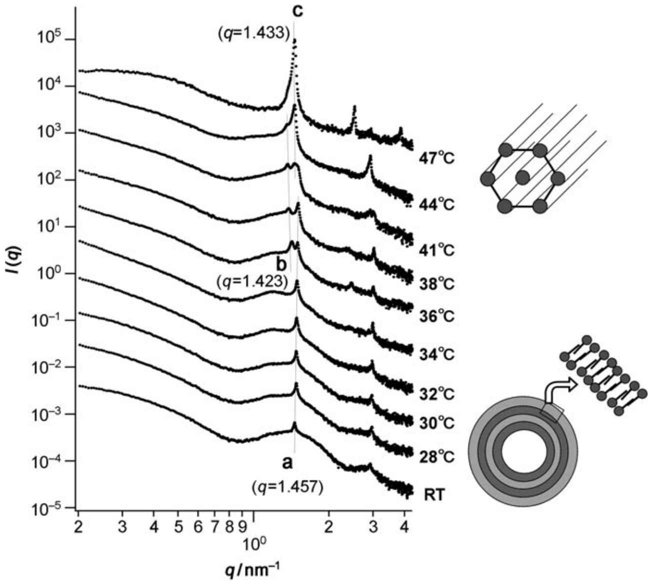


Figure 5. SAXS profiles of IBAM-G2-DL assemblies in 10 mм phosphate (pH 7.0, 0.5 mg mL^{-1}) at varying temperatures. a) The first peak for lamellae appeared at lower temperature, overlapping the vesicle form factor; b) the first peak for another kind of lamellae appeared at an intermediate temperature; c) first peak of hexagonally packed cylinders.

The overall dynamic molecular shape of amphiphiles is known to affect their phase preference.[10] According to this hypothesis, we speculate that the mechanism of the temperature-induced morphological transition for these IBAM-terminated dendron-based lipid assemblies is as depicted in Figure 6. Hydration of the dendron moiety of the IBAM-G2-DL molecule might induce molecular packing suitable to take on a lamellar phase. However, dehydration of the dendron head group might induce shrinkage of the dendron headgroup moiety, which changes its dynamic molecular shape to truncated conic, which is preferable to form an inverted rodlike micelle phase. In fact, an investigation on compression isotherms for an IBAM-G2-DL monolayer formed on the

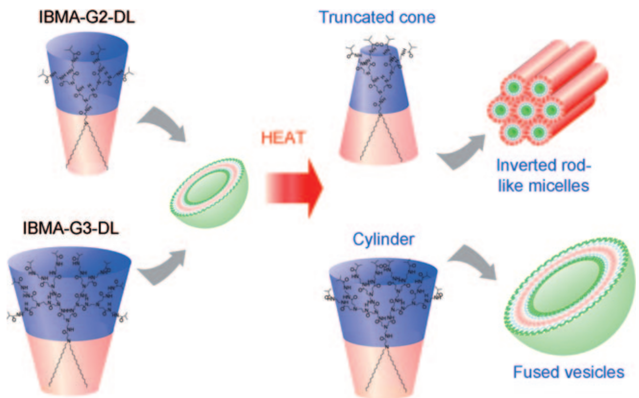


Figure 6. Mechanism for temperature-responsive structural transition of IBAM-G2-DL and IBAM-G3-DL assemblies.

water/air interface showed that a significant decrease in the molecular area from 1.8 to 0.6 $nm^2 molecule^{-1}$ took place at the surface pressure of 40–42 $mN m^{-1}$ above 32 °C, which is close to the cloud point of the IBAM-G2-DL vesicles (see Figure S4 in the Supporting Information). In contrast, IBAM-G3-DL has a head group that is larger than that of IBAM-G2-DL. Therefore, even after dehydration of the dendron head group, IBAM-G3-DL may retain a cylindrical shape that is readily capable of taking on a lamellar phase. However, dehydration of the vesicle surface might increase the hydrophobicity of the surface of the assemblies, thereby promoting aggregation and fusion of the vesicles.

From a practical viewpoint, it is important to control the cloud point of the dendron-based lipid assemblies. For thermosensitive polymers, copolymerization with monomers having appropriate hydrophilicity or hydrophobicity is widely used to control temperature sensitivity.[11] For example, the LCST of copolymers of *N*-isopropylacrylamide and acrylamide increases concomitantly with increasing acrylamide content of the copolymers.[11b] Therefore, we attempted to control the cloud point of the assemblies by using the same strategy. We prepared assemblies consisting of IBAM-G2-DL and ACAM-G2-DL at varying contents. Figure 7 depicts the temperature dependence of transmittance of mixtures of these lipids suspended in phosphate solution. Apparently, their cloud point increased concomitantly with increasing ACAM content from 0 to 0.5. It is likely that the increase in ACAM group content enhanced hydration of the surface of the assemblies, thereby elevating their cloud point. A further increase in ACAM-G2-DL content abolished the temperature-sensitive property of the assemblies because their surface might have become too hydrophilic to undergo a hydrophilic-to-hydrophobic transition.

Finally, we conducted microcalorimetric analysis to examine the temperature-dependent aggregation behavior of alkyl amide-terminated dendron-based lipids. The differential scanning calorimetry measurements for IBAM-G2-DL and IBAM-G3-DL suspended in 10 mM phosphate buffer (pH 7.0) respectively exhibited an endotherm of 8.4 $kJ mol^{-1}$ around 40 °C and of 1.0 $kJ mol^{-1}$ around 60 °C (see Figure S5 in the Supporting Information), which correspond to their cloud points (Figure 2 A). We performed the same measurements for ACAM-G2-DL and ACAM-G3-DL, which showed no endotherm in the same temperature region. This result shows that dehydration of IBAM groups on the vesicle surface might be responsible for the endotherm. A synthetic amphiphile, dimethyl dioctadecylammonium bromide, has structural similarity to the hydrophobic moiety of the dendron-based lipids. Its bilayer membrane reportedly exhibits a large transition enthalpy of 100 $kJ mol^{-1}$ upon a gel-to-liquid crystalline transition.[12] The considerable difference in the transition enthalpies between them is likely to arise from the difference in their transition mechanisms, namely mobility change of the lipid molecules and dehydration of the head group of the lipid molecules.

In conclusion, by using spontaneous association of amphiphiles we have shown that accumulation of IBAM groups, which are common side groups of thermosensitive polymers, affords molecular assemblies with temperature-sensitive properties. These properties were controlled by inclusion of hydrophilic ACAM groups. The dendron lipids can form vesicles that destabilize at a desired temperature. Vesicles of alkyl amide-terminated dendron-based lipids are an entirely new type of temperature-sensitive vesicle that undergoes a temperature-dependent transition through a change in hydration of the vesicle surface. Various types of dendritic compounds are known to form supramolecular assemblies of different shapes and morphologies.[13] Our approach might be applied to such supramolecular assemblies for their temperature sensitization. This methodology could be used as a novel, versatile, and rational strategy for the generation of temperature-sensitive properties, which would contribute to the creation of molecular systems having temperature-responsive functions.

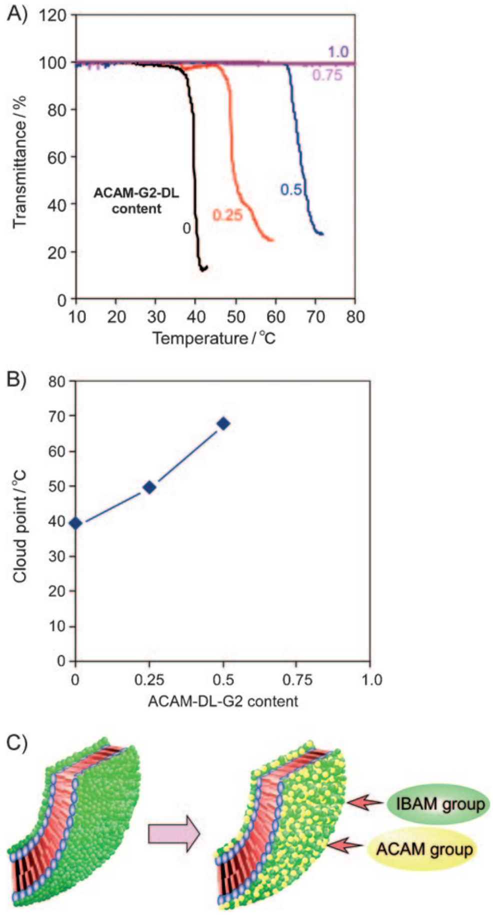


Figure 7. Control of thermosensitive properties of PAMAM G2 dendron-based lipids. A) Effect of temperature on transmittance ($\lambda = 500$ nm) of suspensions of mixtures of IBAM-G2-DL and ACAM-G2-DL with varying compositions in 10 mM phosphate buffer (pH 7.0). B) Cloud point of suspensions of mixtures of IBAM-G2-DL and ACAM-G2-DL as a function of ACAM-G2-DL content. C) Schematic illustration of molecular assembly surface consisting of IBAM and ACAM groups, which affect the thermosensitive property. The total lipid concentration was 1.25×10^{-4} M.

Received: February 9, 2011
Published online: May 20, 2011

Keywords: dendrimers · lipids · micelles · thermosensitivity · vesicles

[1] M. B. Yatvin, J. N. Weinstein, W. H. Dennis, R. Blumenthal, *Science* **1978**, *202*, 1290 – 1293.
[2] K. Kono, *Adv. Drug Delivery Rev.* **2001**, *53*, 307 – 319.
[3] a) J. E. Chung, M. Yokoyama, T. Okano, *J. Controlled Release* **1998**, *53*, 119 – 130; b) J. X. Zhang, L. Y. Qiu, Y. Jin, K. J. Zhu, *J. Biomed. Mater. Res. Part A* **2006**, *76*, 773 – 780.
[4] a) Y. Haba, A. Harada, T. Takagishi, K. Kono, *J. Am. Chem. Soc.* **2004**, *126*, 12760 – 12761; b) Y. Haba, C. Kojima, A. Harada, K. Kono, *Macromolecules* **2006**, *39*, 7451 – 7453; c) Y. Haba, C. Kojima, A. Harada, K. Kono, *Angew. Chem.* **2007**, *119*, 238 – 241; *Angew. Chem. Int. Ed.* **2007**, *46*, 234 – 237.
[5] a) H. Heskins, J. E. Guillet, *J. Macromol. Sci. Part A* **1968**, *2*, 1441 – 1455; b) L. D. Taylor, L. D. Cerankowski, *J. Polym. Sci. Polym. Chem. Ed.* **1975**, *13*, 2551 – 2570; c) S. Ito, *Koubunshi Ronbunsyu* **1989**, *46*, 437 – 443.
[6] a) K. Suwa, K. Morishita, A. Kishida, M. Akashi, *J. Polym. Sci. Part A* **1993**, *31*, 365 – 371; b) M. Akashi, S. Nakano, A. Kishida, *J. Polym. Sci. Part A* **1996**, *34*, 301 – 303.
[7] C. Kojima, K. Yoshimura, A. Harada, Y. Sakanishi, K. Kono, *Bioconjugate Chem.* **2009**, *20*, 1054 – 1057.
[8] a) T. Takahashi, K. Kono, T. Itoh, N. Emi, T. Takagishi, *Bioconjugate Chem.* **2003**, *14*, 764 – 773; b) T. Takahashi, C. Kojima, A. Harada, K. Kono, *Bioconjugate Chem.* **2007**, *18*, 1349 – 1354.
[9] Y. Niu, L. Sun, R. M. Crooks, *Macromolecules* **2003**, *36*, 5725 – 5731.
[10] J. N. Israelachvili, S. Marcelja, R. G. Horn, *Q. Rev. Biophys.* **1980**, *13*, 121 – 200.
[11] a) H. Feil, Y. H. Bae, J. Feijen, S. W. Kim, *Macromolecules* **1993**, *26*, 2496 – 2500; b) H. Hayashi, K. Kono, T. Takagishi, *Bioconjugate Chem.* **1999**, *10*, 412 – 418.
[12] Y. Okahata, R. Ando, T. Kunitake, *Ber. Bunsen-Ges.* **1981**, *85*, 789 – 798.
[13] a) B. M. Rosen, C. J. Wilson, D. A. Wilson, M. Peterca, M. R. Imam, V. Percec, *Chem. Rev.* **2009**, *109*, 6275 – 6540; b) Y. B. Lim, E. Lee, M. Lee, *Angew. Chem.* **2007**, *119*, 9169 – 9172; *Angew. Chem. Int. Ed.* **2007**, *46*, 9011 – 9014; c) K. T. Al-Jamal, T. Sakthivel, A. T. Florence, *Int. J. Pharm.* **2003**, *254*, 33 – 36.

