Peptide Self-Assembly

DOI: 10.1002/anie.201106080

Cooperative Hierarchical Self-Assembly of Peptide Dendrimers and Linear Polypeptides into Nanoarchitectures Mimicking Viral Capsids **

Xianghui Xu, Hui Yuan, Jing Chang, Bin He, and Zhongwei Gu**

Supramolecular self-assembly is regarded as ubiquitous and essential phenomenon during the early stages of life.[1] In the past decade, self-assembly inspired from nature has been evolved as an effective and practical strategy for nanoarchitecture fabrication.[2] With respect to the formation of soft matter, advances were achieved in the self-assembly of linear polymers, but the self-assembly of spherical macromolecules such as dendrimers is still at an early stage.[3] The regulation of dispersive dendrimers into ordered nanoarchitectures as potential biomacromolecules remains challenging research work.[4] Peptide dendrimers possess not only the general characteristics of typical dendrimers, but also certain unique properties of globular proteins.[5] Initiating hierarchical self-assembly of globular or linear polypeptides may provide a powerful approach to fabricate supramolecular structures with transfer or delivery functions in medical applications as virosomes in cellular environment.[6]

Most dendrimers lack the driving forces for self-assembly;[3] therefore, chemical or physical interactions were explored as driving forces.[4b–e] Herein, we report a novel approach to regulate the cooperative self-assembly of peptide dendrimers and linear polypeptides into capsid-like nanostructures. In our cooperative self-assembly strategy, the end-functionalized linear polypeptides were utilized to first self-assemble with peptide dendrimers to form amphiphiles driven by electrostatic interactions and/or hydrogen bond formation. The amphiphiles then self-assembled into virosome-like nanoarchitectures with controllable morphologies and biological functions.[7] This supramolecular self-assembly was called cooperative self-assembly owing to the involvement of multiple components and steps.[8] The self-assembled nanoparticles were called peptidesomes.

Poly(L-lysine) dendrimers with polyhedral oligomeric silsesquioxane nanocubic cores were used as model peptide dendrimers.[4a,9] Generation 2 poly(L-lysine) dendrimers with 32 peripheral groups were synthesized. Hydrophobic poly(L-leucine) was used as the second component to regulate the

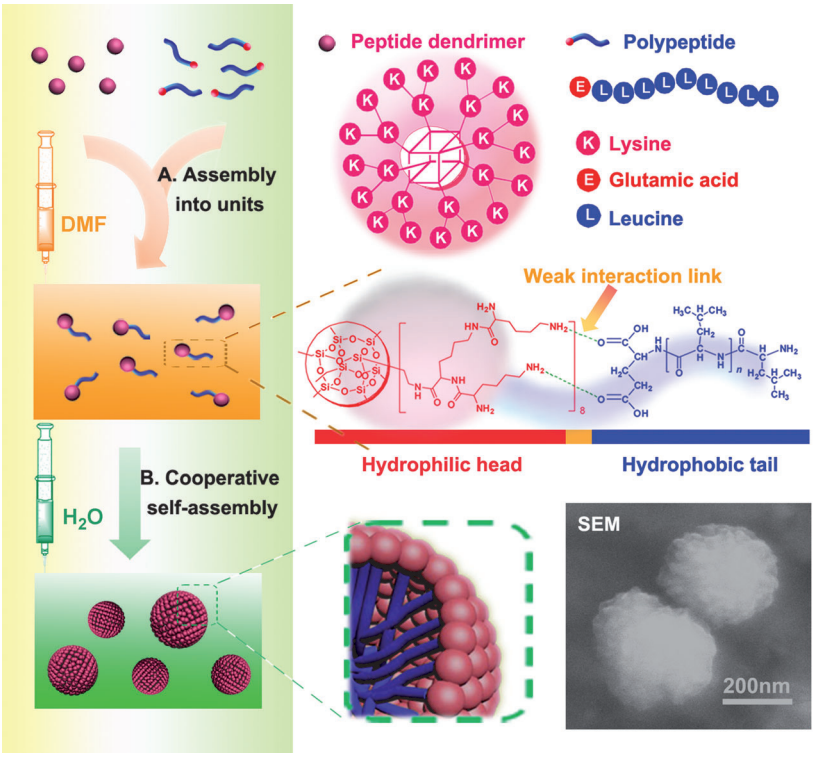

Figure 1. Schematic illustration of the cooperative self-assembly of poly(L-lysine) dendrimers and linear poly(L-leucine) into hierarchical peptidesomes. Step A: peptide dendrimers were linked with linear polypeptides to form amphiphiles through weak interactions in cosolvent; Step B: the amphiphiles self-assembled into hierarchical peptidesomes in aqueous phase. The red ball and blue line represent generation 2 poly(L-lysine) dendrimer and linear poly(L-leucine), respectively. Their chemical structures and synthetic routes are shown in the Supporting Information.

[*] X. Xu, H. Yuan, J. Chang, Prof. B. He, Prof. Z. Gu
National Engineering Research Center for Biomaterials
Sichuan University
Chengdu, Sichuan 610064 (P.R. China)
E-mail: bhe@scu.edu.cn
zwgu@scu.edu.cn

[**] This research work was supported by the National Basic Research Program of China (National 973 program, No. 2011CB606206), National Science Foundation of China (NSFC, No. 51133004, 50830105), International Collaboration Project of Ministry of Science & Technology (MOST, No. 2010DFA51550), Program for New Century Excellent Talents in University, Ministry of Education (MOE, NCET-10-0564), International Cooperation Project of Sichuan Province (No. 2009HH0001). We thank Prof. Lei Jiang for helpful discussions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106080.

cooperative self-assembly of polypeptides. The detailed synthesis and characterizations of the compounds are shown in the Supporting Information.[10] As shown in the schematic route (Figure 1), the first-step self-assembly gave amphiphiles with the poly(L-lysine) dendrimer as hydrophilic head and the linear poly(L-leucine) as hydrophobic tail (Step A). After that, the amphiphiles self-assembled into stable hierarchical peptidesomes in water (Step B), which were capsid-like nanoarchitectures.

The peptidesomes formed by cooperative self-assembly were investigated. The mass ratio of poly(L-lysine) dendrimers/poly(L-leucine) was 10:1, and the total mass concentration was 100 $\mu g\,mL^{-1}$. In the transmission electron microscopy (TEM) image (Figure 2a), the sizes of the nanoparticles were mainly in the range between 250 to 300 nm. The hierarchical peptidesomes were composed of small spherical particles. The size of the small spherical particles was comparable to that of a single dendrimer. Such well-organized hierarchical self-assembly was rarely reported in the self-assembly of soft matters.[11]

The diameters and morphology of the peptidesomes measured by atomic force microscopy (AFM) were consistent with the results of TEM (Figure 2b) and scanning electron microscopy (SEM, Figure 2c). Figure 2c1 and Figure 2c2 show the detailed surface pattern of the self-assemblies; it seemed that the rugged structure resulted from the aggregation of poly(L-lysine) dendrimers. The sizes of the self-assembled nanoparticles determined by different microscopy methods were also in agreement with the results of dynamic light scattering (Figure S13 in the Supporting Information). Interestingly, the appearance and size of these self-assembled nanoparticles are similar to those of viral capsids of, for example, a rotavirus or an adenovirus, the capsids of which are analogously built of peptide units.

A series of experiments was carried out to clarify whether the cooperative self-assembly followed the design strategy. Viscosity measurements were performed to demonstrate the primary self-assembly of the two components in a cosolvent (Figure 3a).[12] According to the basic characteristics of

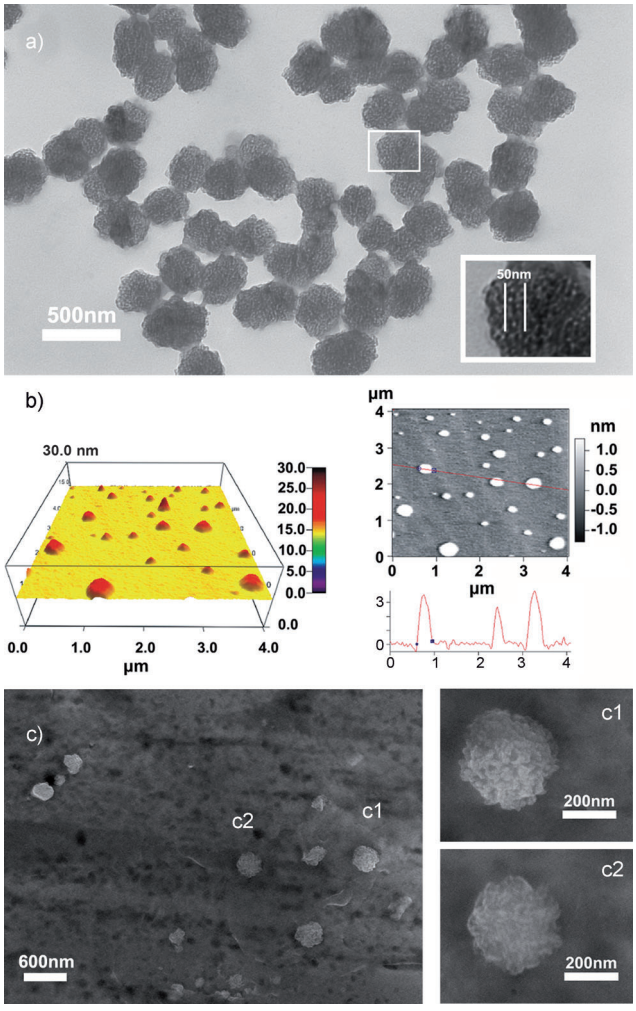

Figure 2. The morphology of peptidesomes assembled from poly(L-lysine) dendrimers and linear poly(L-leucine) (10:1 (w/w), 100 $\mu g\,mL^{-1}$). a) TEM images of the peptidesomes; b) AFM image of the peptidesomes (left: 3D view, right: top view and the size profile along the red line); c) SEM images of the peptidesomes; c1, c2 are the magnified peptidesomes in c).

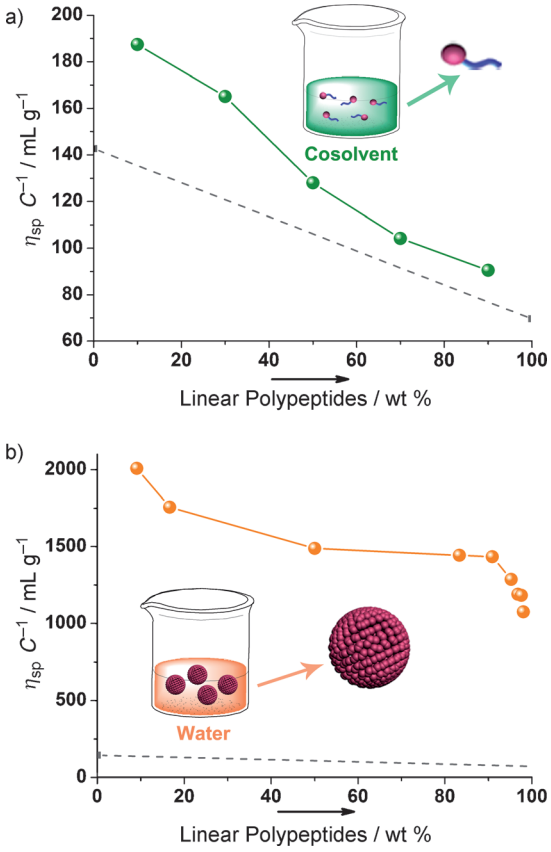

Figure 3. Viscosity measurements of self-assembly in solutions containing peptide dendrimers and polypeptides in different ratios. The portion of linear polypeptide is plotted on the x-axis. a) Viscosity of the first-step self-assembly of peptide dendrimers and linear polypeptides in cosolvent (DMF); b) viscosity of the second-step self-assembly into hierarchical peptidesomes in water. The dotted gray lines are the variation trend of the viscosity of the solutions without interactions. The total concentration of each sample was 100 $\mu g\,mL^{-1}$. η_{sp} = specific viscosity and C = total concentration of the sample in $g\,mL^{-1}$.

Ubbelohde viscosity, if there was no interaction between the two components, a linear decrease of the total viscosity around the dotted gray line in Figure 3a would be expected. The observed values were much higher than the values on the gray line. Therefore, it was concluded that there was interaction between peptide dendrimers and linear polypeptides, thus resulting in an increase of the viscosity. When the peptidesomes were formed in water at the secondary self-assembly stage, further aggregation caused a substantial increase of the viscosity (Figure 3b). The dramatic increase was attributed to the supramolecular interactions among the assembly units.

The turbidity test also supported the above-mentioned conclusion.[13] With the addition of the hydrophilic dendrimers, the turbidity of hydrophobic-peptide solution was reduced significantly. This reduction of the turbidity was caused by the formation of linkages between the linear peptides and the peptide dendrimers through noncovalent interactions, which increased the solubility of the linear polypeptides (Figure S16 in the Supporting Information). Circular dichroism (CD) spectroscopy was used to characterize the secondary structure of the hierarchical peptidesomes. The result showed that the secondary structure of the self-assembled nanoparticles was attributed to the abundant amino acid residues among the peptide dendrimers and linear polypeptides, which facilitated the hierarchical self-assembly into capsid-like nanoarchitectures (Figure S17 in the Supporting Information).

Moreover, the morphology of the self-assembled nanoparticles could be controlled by the total concentration and ratio between the peptide dendrimers and the linear polypeptides. The average size of the nanoparticles increased from 300 to 800 nm when the ratio of peptide dendrimers to linear polypeptides was changed from 10:1 to 1:5 (w/w) while the total concentration was 100 μg mL^{-1}. The morphology of the nanoparticles changed from spherical to fusiform (Figure 4a,b,c). Figure 4d shows the size of the nanoparticles at different total concentrations. At each concentration, the size of the nanoparticles increased with increasing the portion of linear polypeptide. The nanoparticles were bigger at a higher total concentration. All the self-assembled peptidesomes exhibited hierarchical nanoarchitectures.

As a preliminary biomedical application, the hierarchical peptidesomes were used as gene vectors.[14] Cells that express the green fluorescent protein (GFP) after transfection with a complex of peptidesomes and the DNA encoding GFP are presented in Figure 5. Strong green emission of GFP expression and flow-cytometry analysis of the peptidesomes demonstrated that the transfection efficacy in the presence of serum was comparable to that of poly(ethylene imine) (PEI, 25 kDa) in the absence of serum (Figures S20 and S22 in the Supporting Information). The biocompatibility of the peptidesomes and peptidesomes/DNA complex was excellent as shown in Figure 5d and Figure S18 in the Supporting Information.

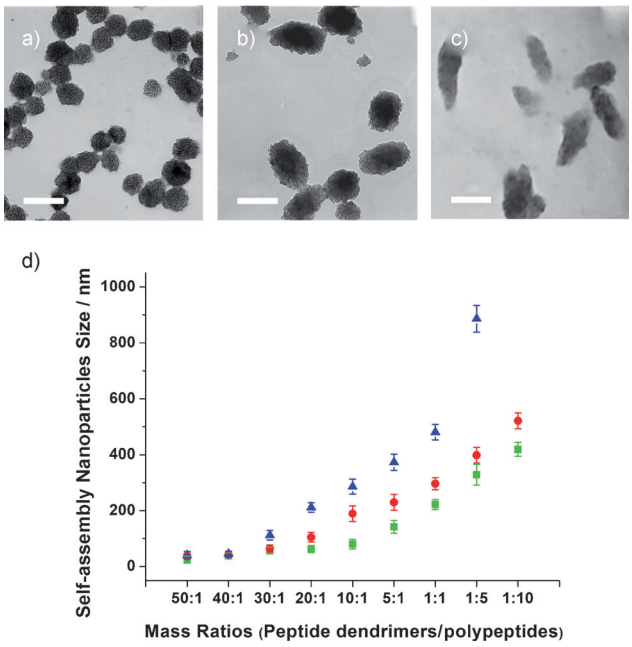

Figure 4. Size and morphology of the self-assembled peptidesomes. a–c) TEM images of the peptidesomes (peptide dendrimers/linear polypeptides ratios (w/w) = 10:1 (a), 1:1 (b), 1:5 (c); the total concentration was 100 μg mL^{-1}); the scale bars correspond to 500 nm. d) Sizes of self-assembled nanoparticles plotted against the ratios of peptide dendrimers/linear polypeptides at total mass concentrations of 10 (green squares), 50 (red circles), and 100 μg mL^{-1} (blue triangles).

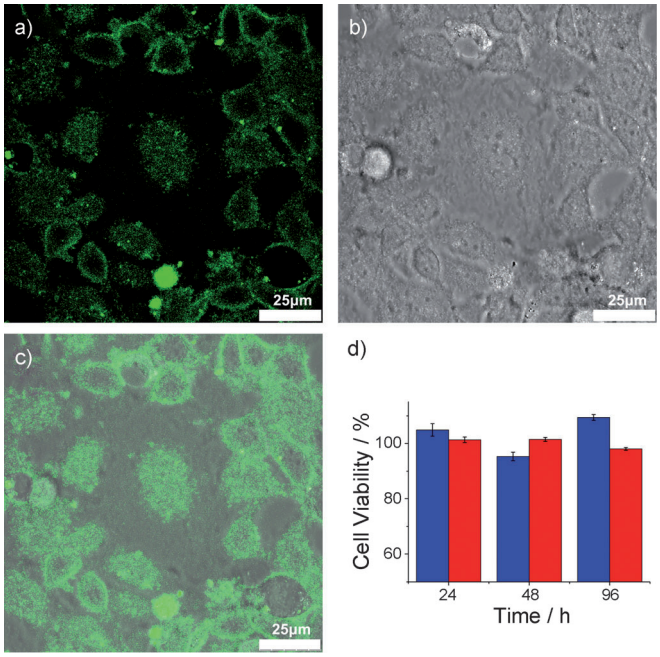

Figure 5. Confocal laser scanning microscopy (CLSM) images of GFP expression in HEK 293 cells after incubation with peptidesomes/DNA complex; a) GFP expression; b) bright field image; c) overlay of images in a) and b); scale bars correspond to 25 μm. d) Cell viability of NIH/3T3 cells in the presence of peptidesomes (blue) and in the presence of the peptidesomes/DNA complex (red) determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay with a total concentration of peptidesomes or of peptidesomes and DNA of 100 μg mL^{-1} (means ± standard deviation (SD), $n = 6$).

In conclusion, we have successfully demonstrated a versatile strategy for cooperative self-assembly of globular poly(L-lysine) dendrimers with linear poly(L-leucine). After a two-step hierarchical self-assembly, the nanoparticles possessed a capsid-like biomimetic nanoarchitecture. The hierarchical peptidesomes exhibited high gene-transfection efficiency as nonviral gene vectors. The strategy of the hierarchical self-assembly could also be applied for fabricating versatile nanoarchitectures. A detailed study of the cooperative self-assembly, such as precise regulation of the morphology and medical applications, is being explored and will be reported in our future publications.

Received: August 27, 2011
Revised: November 15, 2011
Published online: February 8, 2012

Keywords: dendrimers · gene vectors · nanostructures · peptides · self-assembly

[1] a) J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* **2001**, *409*, 387–390; b) G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295*, 2418–2421; c) U. J. Meierhenrich, J. J. Filippi, C. Meinert, P. Vierling, J. P. Dworkin, *Angew. Chem.* **2010**, *122*, 3826–3839; *Angew. Chem. Int. Ed.* **2010**, *49*, 3738–3750.

[2] a) L. Jiang, M. J. Liu, Y. M. Zheng, J. Zhai, *Acc. Chem. Res.* **2010**, *43*, 368–377; b) D. Philp, J. F. Stoddart, *Angew. Chem.* **1996**, *108*, 1242–1286; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154–1196; c) J. S. Moore, M. L. Kraft, *Science* **2008**, *320*, 620–621; d) V. Percec, G. Ungar, M. Peterca, *Science* **2006**, *313*, 55–56; e) R. F. Service, *Science* **2005**, *309*, 95–95.

[3] a) M. Lee, B. K. Cho, W. C. Zin, *Chem. Rev.* **2001**, *101*, 3869–3892; b) D. E. Discher, R. D. Kamien, *Nature* **2004**, *430*, 519–520; c) H. G. Börner, *Prog. Polym. Sci.* **2009**, *34*, 811–851; d) B. M. Rosen, C. J. Wilson, D. A. Wilson, M. Peterca, M. R. Imam, V. Percec, *Chem. Rev.* **2009**, *109*, 6275–6540; e) J. K. Kim, S. Y. Yang, Y. Lee, Y. Kim, *Prog. Polym. Sci.* **2010**, *35*, 1325–1349.

[4] a) J. M. J. Frechet, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4782–4787; b) V. Percec, M. R. Imam, T. K. Bera, V. S. K. Balagurusamy, M. Peterca, P. A. Heiney, *Angew. Chem.* **2005**, *117*, 4817–4823; *Angew. Chem. Int. Ed.* **2005**, *44*, 4739–4745; c) V. Percec, D. A. Wilson, P. Leowanawat, C. J. Wilson, A. D. Hughes, M. S. Kaucher, D. A. Hammer, D. H. Levine, A. J. Kim, F. S. Bates, K. P. Davis, T. P. Lodge, M. L. Klein, R. H. DeVane, E. Aqad, B. M. Rosen, A. O. Argintaru, M. J. Sienkowska, K. Rissanen, S. Nummelin, J. Ropponen, *Science* **2010**, *328*, 1009–1014; d) B. M. Rosen, M. Peterca, C. H. Huang, X. B. Zeng, G. Ungar, V. Percec, *Angew. Chem.* **2010**, *122*, 7156–7159; *Angew. Chem. Int. Ed.* **2010**, *49*, 7002–7005; e) Z. Cheng, L. J. Thorek, A. Tsourkas, *Angew. Chem.* **2010**, *122*, 356–360; *Angew. Chem. Int. Ed.* **2010**, *49*, 346–350.

[5] a) L. Crespo, G. Sanclimens, M. Pons, E. Giralt, M. Royo, F. Albericio, *Chem. Rev.* **2005**, *105*, 1663–1681; b) C. C. Lee, J. A. MacKay, J. M. J. Frechet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517–1526; c) D. A. Tomalia, *Prog. Polym. Sci.* **2005**, *30*, 294–324; d) T. Darbre, J. L. Reymond, *Acc. Chem. Res.* **2006**, *39*, 925–934; e) B. Helms, E. W. Meijer, *Science* **2006**, *313*, 929–930.

[6] a) S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171–1178; b) Y. R. Yoon, Y. B. Lim, E. Lee, M. Lee, *Chem. Commun.* **2008**, 1892–1894; c) Y. B. Lim, K. S. Moon, M. Lee, *Angew. Chem.* **2009**, *121*, 1629–1633; *Angew. Chem. Int. Ed.* **2009**, *48*, 1601–1605; d) X. B. Zhao, F. Pan, H. Xu, M. Yaseen, H. H. Shan, C. A. E. Hauser, S. G. Zhang, J. R. Lu, *Chem. Soc. Rev.* **2010**, *39*, 3480–3498.

[7] a) G. Zuber, E. Dauty, M. Nothisen, P. Belguise, J. P. Behr, *Adv. Drug Delivery Rev.* **2001**, *52*, 245–253; b) E. Mastrobattista, M. A. E. M. van der Aa, W. E. Hennink, D. J. A. Crommelin, *Nat. Rev. Drug Discovery* **2006**, *5*, 115–121; c) Y. B. Lim, E. Lee, M. Lee, *Angew. Chem.* **2007**, *119*, 9169–9172; *Angew. Chem. Int. Ed.* **2007**, *46*, 9011–9014; d) Y. B. Lim, M. Lee, E. Lee, Y. R. Yoon, M. S. Lee, *Angew. Chem.* **2008**, *120*, 4601–4604; *Angew. Chem. Int. Ed.* **2008**, *47*, 4525–4528.

[8] a) D. Y. Chen, M. Jiang, *Acc. Chem. Res.* **2005**, *38*, 494–502; b) D. S. Turygin, B. Konig, M. Subat, O. A. Raitman, V. V. Arslanov, M. A. Kalinina, *Angew. Chem.* **2006**, *118*, 5466–5470; *Angew. Chem. Int. Ed.* **2006**, *45*, 5340–5344; c) R. K. O'Reilly, A. O. Moughton, *J. Am. Chem. Soc.* **2008**, *130*, 8714–8725; d) E. Moulin, N. Giuseppone, F. Niess, M. Maaloum, E. Buhler, I. Nyrkova, *Angew. Chem.* **2010**, *122*, 7128–7132; *Angew. Chem. Int. Ed.* **2010**, *49*, 6974–6978; e) S. N. Che, H. B. Qiu, *Chem. Soc. Rev.* **2011**, *40*, 1259–1268.

[9] a) B. He, H. Yuan, K. Luo, Y. S. Lai, Y. J. Pu, G. Wang, Y. Wu, Z. W. Gu, *Mol. Pharm.* **2010**, *7*, 953–962; b) Z. R. Lu, T. L. Kaneshiro, X. Wang, *Mol. Pharm.* **2007**, *4*, 759–768.

[10] a) J. A. Hanson, C. B. Chang, S. M. Graves, Z. B. Li, T. G. Mason, T. J. Deming, *Nature* **2008**, *455*, 85–88; b) N. Hadjichristidis, H. Iatrou, M. Pitsikalis, G. Sakellariou, *Chem. Rev.* **2009**, *109*, 5528–5578; c) C. Y. Yang, B. B. Song, Y. Ao, A. P. Nowak, R. B. Abelowitz, R. A. Korsak, L. A. Havton, T. J. Deming, M. V. Sofroniew, *Biomaterials* **2009**, *30*, 2881–2898.

[11] a) M. M. Maye, I. I. S. Lim, J. Luo, Z. Rab, D. Rabinovich, T. B. Liu, C. J. Zhong, *J. Am. Chem. Soc.* **2005**, *127*, 1519–1529; b) S. Nayak, L. A. Lyon, *Angew. Chem.* **2005**, *117*, 7862–7886; *Angew. Chem. Int. Ed.* **2005**, *44*, 7686–7708; c) F. Gröhn, *Soft Matter* **2010**, *6*, 4296–4302.

[12] a) H. J. Dou, M. Jiang, H. S. Peng, D. Y. Chen, Y. Hong, *Angew. Chem.* **2003**, *115*, 1554–1557; *Angew. Chem. Int. Ed.* **2003**, *42*, 1516–1519; b) K. Köhler, G. Forster, A. Hauser, B. Dobner, U. F. Heiser, F. Ziethe, W. Richter, F. Steiniger, M. Drechsler, H. Stettin, A. Blume, *Angew. Chem.* **2004**, *116*, 247–249; *Angew. Chem. Int. Ed.* **2004**, *43*, 245–247; c) O. A. Scherman, G. B. W. L. Ligthart, R. P. Sijbesma, E. W. Meijer, *Angew. Chem.* **2006**, *118*, 2126–2130; *Angew. Chem. Int. Ed.* **2006**, *45*, 2072–2076; d) S. C. Zimmerman, T. Park, *J. Am. Chem. Soc.* **2006**, *128*, 11582–11590.

[13] a) I. Willerich, F. Grohn, *Angew. Chem.* **2010**, *122*, 8280–8285; *Angew. Chem. Int. Ed.* **2010**, *49*, 8104–8108; b) J. del Barrio, L. Oriol, C. Sanchez, J. L. Serrano, A. Di Cicco, P. Keller, M. H. Li, *J. Am. Chem. Soc.* **2010**, *132*, 3762–3769; c) G. Askarieh, M. Hedhammar, K. Nordling, A. Saenz, C. Casals, A. Rising, J. Johansson, S. D. Knight, *Nature* **2010**, *465*, 236–238.

[14] a) E. Wagner, Y. Nie, M. Gunther, Z. W. Gu, *Biomaterials* **2011**, *32*, 858–869; b) R. Liu, D. Li, B. He, X. Xu, M. Sheng, Y. Lai, G. Wang, Z. Gu, *J. Controlled Release* **2011**, *152*, 49–56.

