Self-Assembly

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Cyclodextrin and Adamantane Host–Guest Interactions of Modified Hyperbranched Poly(ethylene imine) as Mimetics for Biological Membranes

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Supramolecular assembly is fundamental in living systems to ensure transport, barrier formation, templating, and signaling.[1] Self-assembled nanostructures obtained from natural and synthetic compounds enabled to mimic biological membranes or vesicles are of great interest because of their drug, protein, and gene delivery abilities.[2–7] Liposomes[8] for example are artificially prepared vesicles made of lipid bilayers that are also able to mimic biological membranes. They are frequently employed as probe cells or as biomimetic materials in nanomedicine.[9,10] The synthetic design of such vesicles with specific properties such as uniform and targeted size and resistance towards outer stimuli is particularly challenging. Various techniques have been adopted to prepare polymeric,[11] inorganic,[12] and organic–inorganic hybrid[13] vesicles. Recently, we described the self-assembly of cyclodextrin- and adamantyl-modified silica nanoparticles into hollow spheres (vesicles) with uniform size distribution.[14]

Herein, we report the preparation of tubular vesicles of β-cyclodextrin-(CD) and adamantyl-(Ada) modified hyperbranched poly(ethylene imine) (PEI) self-assembled with the corresponding modified fluorescent dye calcein (Cal) in water. This report provides the first description of the preparation, structure, and properties of tubular vesicles from hyperbranched PEI. PEI is well established as backbone in artificial enzymes[15] and used as a vector for in vitro or in vivo cases, for example, for gene delivery.[16] The highly charged cationic polymer acts as a nonviral gene carrier because of fast interactions with negatively charged plasma membranes forming complexes with DNA.[17,18] Furthermore, hyperbranched PEIs offer an increased molecular surface area suitable for chemical functionalization and superior drug loading capacity.[3,19–21]

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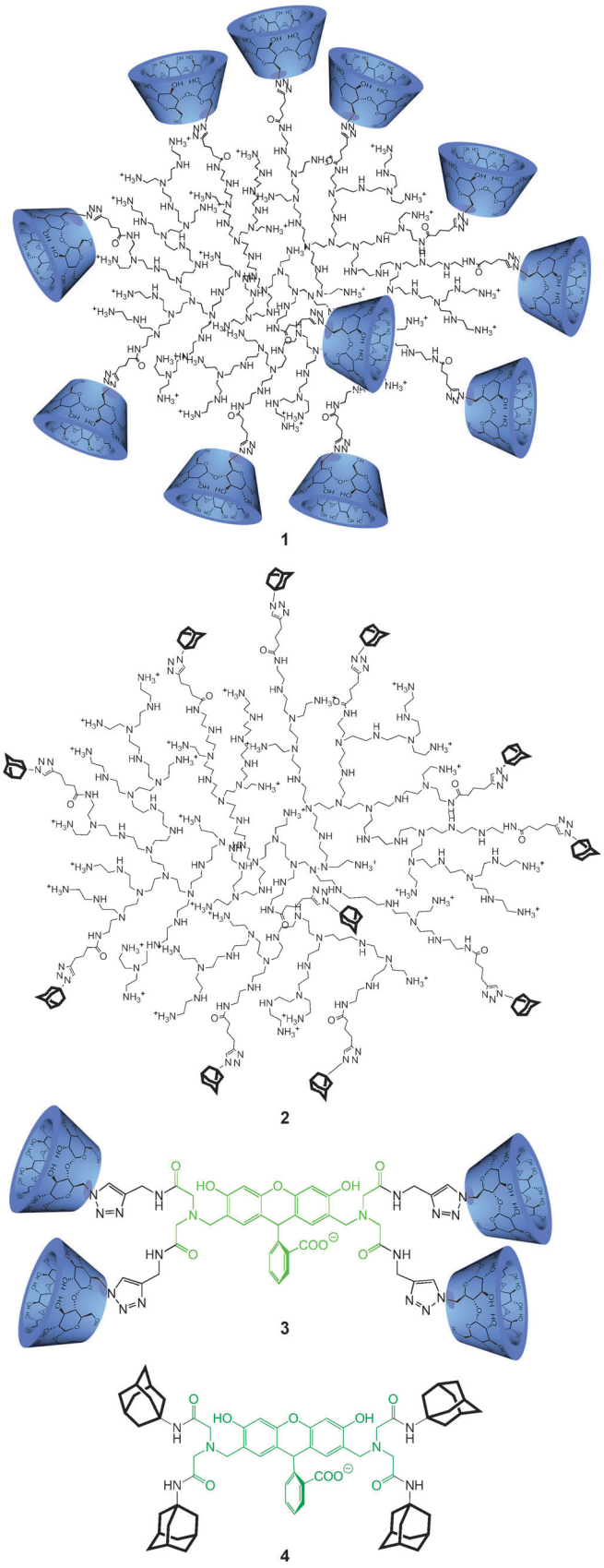
Comparing the novel host–guest assembled systems with the dendrisomes of Percec et al.,[2] they offer the advantage of being much simpler to synthesize. While Percec et al. were utilizing multistep reactions by activation of every single generation to prepare well-defined dendrons, we were able to obtain well-defined vesicles of rather undefined hyperbranched PEI. These polymers can be easily prepared in a one-pot synthesis.

Additionally utilizing the efficient "click reaction" with its advantages, that is, equimolarity, fast timescale, and high yields[22] also facilitates the preparation of these well-defined membranes.

Many factors control the properties of these fascinating hyperbranched materials, including the density of branches and the distribution of chemical moieties located inside the molecular skeleton or at the periphery of the molecule. A cyclodextrin modified hyperbranched PEI **1** was synthesized according to literature.[23] Briefly, the hyperbranched PEI was condensed with 5-hexynoic acid in a polymer-analogous amidation and was subsequently converted with mono-(6-azido-6-desoxy)-β-cyclodextrin by click reaction under microwave-assisted conditions. The analogous guest polymer **2** was prepared accordingly.[24] The cyclodextrin- and adamantyl-functionalized fluorescent dyes **3** and **4** were synthesized by a literature procedure[25] in order to study the supramolecular self-assembly of functionalized PEI with the corresponding dye (Scheme 1).

The hyperbranched PEI in combination with the corresponding fluorescent dye can be dispersed in water through complexation reaction of cyclodextrin with the adamantyl moieties. The dispersions were afterwards stirred for 24 h to ensure equilibration. Complexation of the compounds was studied by 2D ROESY, exemplified for CD-PEI **1** and Ada-Cal **4** in Figure 1. As is apparent from the ROESY plot, strong correlation signals of the inner protons of cyclodextrin C3H and C5H at $\delta = 3.6$ ppm and 3.7 ppm and the adamantyl protons at $\delta = 1.2$ and 1.6 ppm can be observed. These interactions indicate that the CD-PEI/Ad-Cal forms a stable inclusion complex, which is shown in the figure. Furthermore, no correlation signals between the aromatic scaffold and the cyclodextrin cavity are observed. Particle dispersions were characterized for basic morphology by cryogenic transmission electron microscopy (cryo-TEM) and fluorescence microscopy. Figure 2a shows a cryo-TEM image of tubular vesicles derived from system A (CD-PEI **1**/Ada-Cal **4**). Also the cryo-TEM image of the complementary system B (Ada-PEI **2**/CD-Cal **3**; Figure 2b) displays the formation of tubular vesicles.



Scheme 1. Structures of CD-PEI **1**, Ada-PEI **2**, CD-Cal **3,** and Ada-Cal **4**.

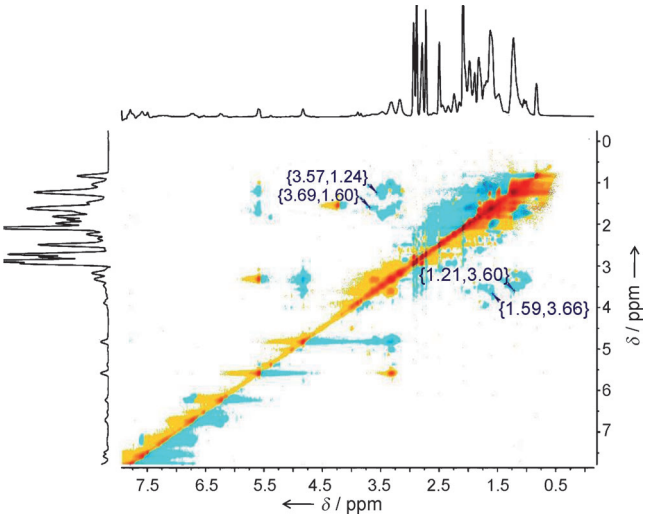


Figure 1. 2D ROESY of Ada-Cal **4** complexed with cyclodextrin-modified PEI **1**.

The estimated structures of the formed tubular vesicles are also schematically shown in Figure 2.

For system A, the more hydrophilic CD-PEI orientates towards the aqueous phase, thus a monolayer of the hydrophobic modified fluorescent dye enclosed by the hyperbranched polymer is formed. In the complementary system B, the hydrophobic Ada-PEI is implemented into the hydrophilic modified calcein resulting in a double layer of fluorescent dye (see 2b) in which the tubular vesicle has a sharp, dark double membrane. However, the vesicles from system B are fewer and not as monodisperse as the ones from system A. Both Figure 2a and b indicate that agglomerated

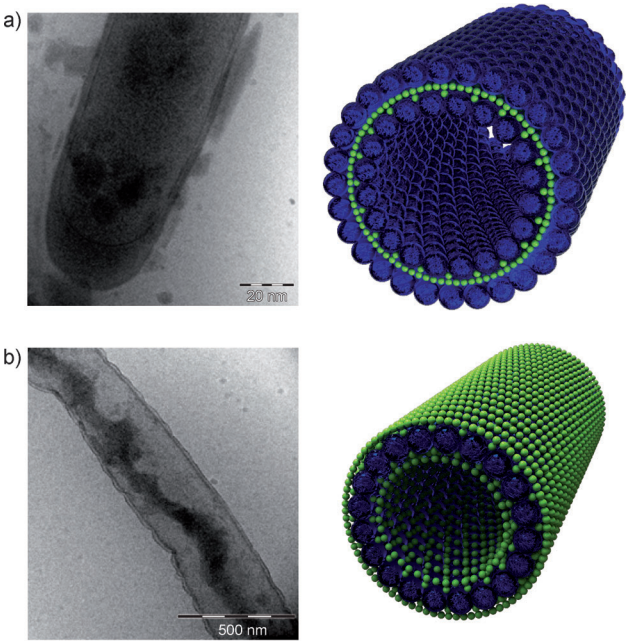


Figure 2. a) Cryo-TEM of system A and B tubular vesicles prepared from CD-PEI **1** (blue)/Ada-Cal **4** (green) and b) Ada-PEI **2** (blue)/CD-Cal **3** (green).

fluorescent dye and PEI are still present in the vesicle since the stoichiometry was not kept equivalent. Thus, hydrophilic compounds dissolved in the aqueous solution still appear in the water-filled vesicle (see dark areas Figure 2a and b). The tubular vesicles exhibit unilamellar as well as multilamellar domain boundaries with a layer thickness of 8 to 16 nm.

The fluorescence image of the supramolecular system of **1** and **4** in Figure 3 also verifies the existence of wormlike strongly fluorescent structures, confirming self-assembly observed by cryo-TEM.

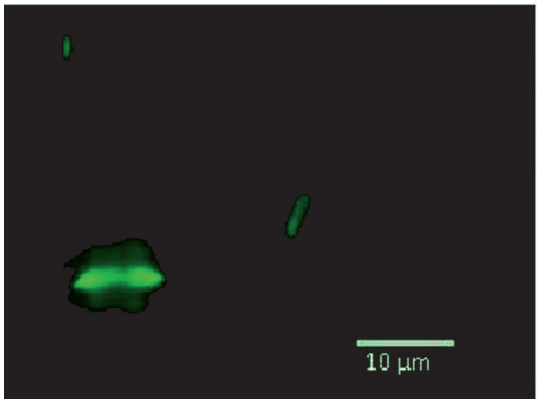


Figure 3. Fluorescence image of CD-PEI **1**/Ada-Cal **4** in water.

To more quantitatively examine the hydrodynamic volume and polydispersity of the systems, we characterized aqueous dispersions by dynamic light scattering. Vesicle solutions with mean hydrodynamic diameters of 88 to 163 nm (Figure S1 to S2 in the Supporting Information) and polydispersities (normalized second cumulants fit) mostly ranging from 0.16 to 0.24 were found, while 0 corresponds to a completely uniform size distribution. In addition, long-term stability of the vesicles was also verified by TEM images taken after several months. Additionally, the particles were stable over a wide range of pH.

Collectively, these results provide powerful evidence that supramolecularly modified hyperbranched PEI produce tubular vesicles with morphologies and properties comparable with advanced liposomes. Self-assembly of these hyperbranched PEI with supramolecular crosslinking fluorescent dyes is qualitatively different from previous work about self-assembled structures of amphiphiles. The self-assembly caused by host–guest interactions is a complex topic that remains the target of detailed experimental and theoretical approaches.

In conclusion, supramolecular functionalized hyperbranched PEIs provide a great potential for engineering of new and exciting materials. Work is in progress to determine: the influence of a) the CD/Ada ratio and b) the molecular weight of PEI units on particle formation.

Addendum after Online Publication (July 18, 2011)

In the course of the publication process of this manuscript, we obtained critical feedback on the EarlyView by Dr. Marc Stuart (Groningen; June 30, 2011) and Prof. Dr. Bart Jan Ravoo (Münster; July 5, 2011). This criticism concerns the displayed cryo-TEM images which bear resemblance with bacteria, particularly *E. coli*. However, at this point we are neither able to prove nor disprove the possible contamination of our sample with bacteria. The displayed structures are not necessarily bacteria since similar structures were also found for synthetic Janus dendrimers.[2] Thus, additional TEM studies of carefully sterilized solutions of our systems will be conducted to clarify this issue. In general, results must be interpreted in the context of the molecular subunits involved and the boundary conditions present. The easy availability of TEM may readily lead to incorrect interpretations of self-organizing structures.[26]

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