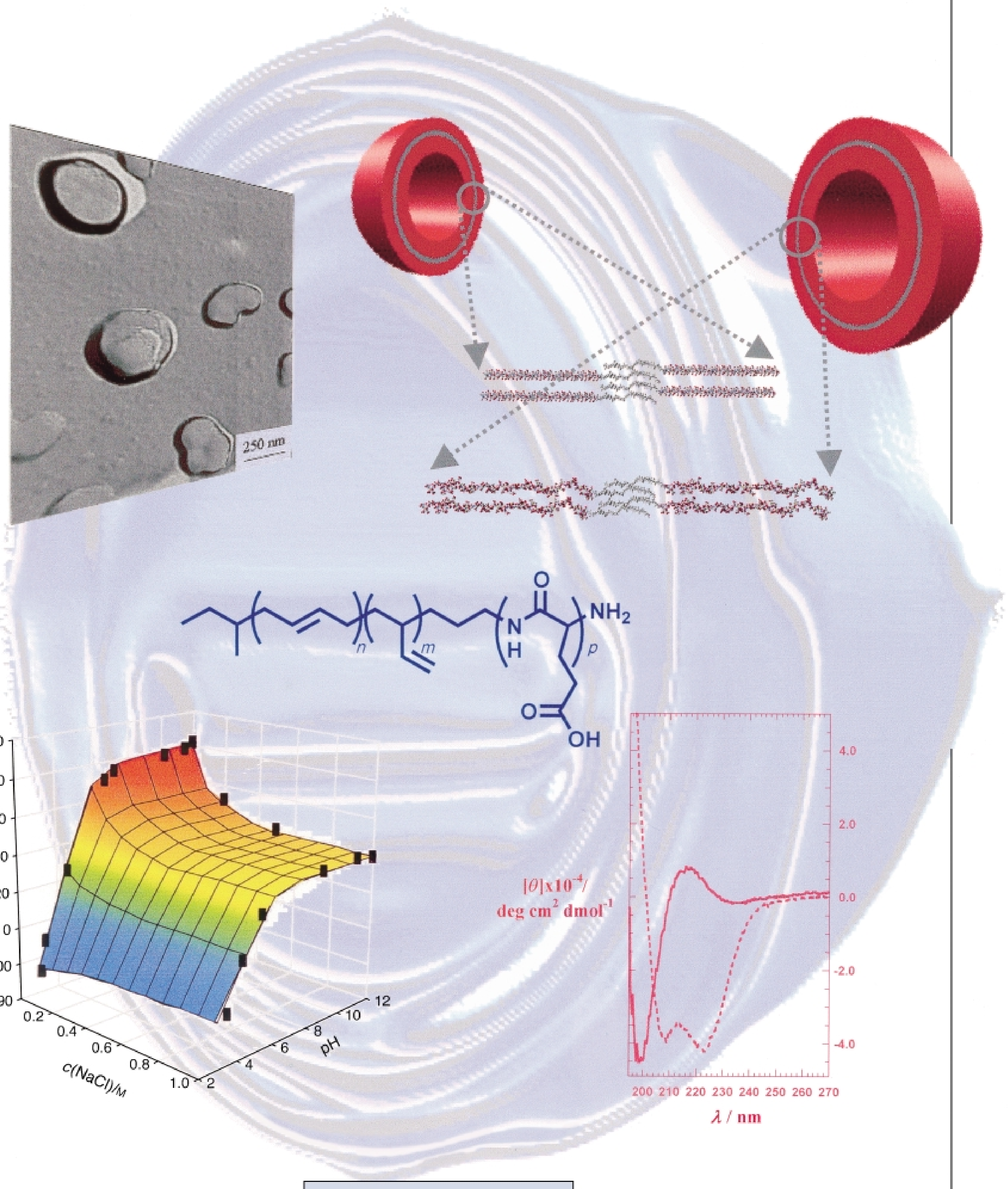
Self-assembly of a peptide-based diblock copolymer has been used to create new shape-persistent stimuli-responsive nanoparticules in water which are like vesicles.



For more information see the following pages.

Water-Soluble Stimuli-Responsive Vesicles from Peptide-Based Diblock Copolymers**

Frédéric Chécot, Sébastien Lecommandoux,*
Yves Gnanou, and Harm-Anton Klok*

Over the past few years increasing attention has been paid to the supramolecular organization of water-soluble block copolymer surfactants and to their potential use in applications such as coatings, drug delivery systems, nanoparticles, and nanoreactors.[1–3] An important issue in making these self-assembled systems useful for specific applications is their capability to respond to external stimuli such as temperature and/or pH.[4] Very recently, Jérôme and co-workers reported the formation in water of three-layer micelles from a polystyrene-*b*-poly(2-vinylpyridine)-*b*-poly(ethylene oxide) (PS-*b*-P2VP-*b*-PEO) triblock copolymer.[5] These authors demonstrated that the pH sensitivity of the P2VP shell can be used to tune the micelle size from a hydrodynamic diameter (D_H) of 75.4 nm at $pH > 5$ to 135.2 nm at $pH < 5$. This effect is based on the electrostatic repulsion between the charged P2VP blocks and is not completely reversible because of the formation of salt with each pH cycle.[5] Another important issue in the design of a self-assembled material is its stability. In fact, most aggregates are only stable within a certain range of concentration, temperature, or pH values. A suitable approach to improve the stability of self-assembled structures is to cross-link one of their blocks.[6]

We report here on the self-organization of a polybutadiene-*b*-poly(L-glutamic acid) (PB-*b*-PGA) diblock copolymer in aqueous solution. Our aim is to exploit the pH sensitivity of the secondary structure of polypeptides to manipulate the size and shape of the supramolecular structures formed by self-assembly of these block copolymers in aqueous medium. In addition to providing the hydrophobic driving forces for the self-assembly, the polybutadiene block may also allow the supramolecular structure to be captured indefinitely by UV-induced cross-linking of its 1,2-vinyl bonds, and thus provide access to novel "shape-persistent stimuli-responsive nanoparticles". Stimuli-responsive nanoparticles have been reported before, and typically rely on changes in the swelling behavior induced by changes in pH value, ionic strength, or solvent quality.[4] The use of polypeptide-based diblock copolymers for the preparation of such structures, however, is unprecedented. In contrast to most other amphiphilic polyelectrolyte (block) copolymers, the hydrophilic peptide block has the ability to fold into a well-defined α-helical secondary structure. More importantly, the swelling behavior of nanoparticles composed of peptide-based block copolymers may be regulated by changes in the pH or ionic strength which induce changes in the conformation of the peptide block; these changes can be precisely controlled by appropriate design of the amino acid sequence.

The diblock copolymer was prepared as shown in Scheme 1. The first step involved the ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (Bn-GluNCA) using an ω-aminopolybutadiene as an initiator followed by a hydrogenation step to remove the benzyl ester protecting groups. The number average degree of polymerization of the polybutadiene block is about 40 and about 100 for the poly(L-glutamic acid) one. The PB_{40}-*b*-PGA_{100} copolymer readily dissolved in water upon addition of one equivalent of NaOH. In a first series of experiments, the critical aggregation concentration (cac) of the block copolymer was determined by using pyrene as a fluorescent probe.[7] Solutions of PB_{40}-*b*-PGA_{100} with concentrations ranging from 10^{-7} to 10^{-5} M were prepared with a constant pyrene concentration of 6.10^{-7} M. Both the excitation and emission spectra were recorded. A red-shift of the pyrene (0,0) band from 335.5 to 339 nm was observed on increasing the copolymer concentration (Figure 1). This change reflects the transfer of pyrene from water into the hydrophobic part of the supramolecular aggregate. A plot of the ratio of the intensity of the signal at 339 nm to that at 335.5 nm ($I_{339}/I_{335.5}$) versus the block copolymer concentration (Figure 1) resulted in a cac value of about 10^{-6} M, a value typical for amphiphilic block copolymers.[3]

The supramolecular organization of the PB_{40}-*b*-PGA_{100} block copolymer in aqueous solutions at pH 11 was investigated by means of dynamic light scattering (DLS) studies. These experiments were carried out with block copolymer concentrations ranging from 0.31 to 2 $g L^{-1}$, both with and without added salt (NaCl), and covered a wide range of angles (from 40 to 140°). The analysis of the autocorrelation functions $C(q,t)$, where q is the scattering vector and t the time, was carried out following the cumulants method (Figure 2).[8] The CONTIN analysis of the same autocorrelation functions showed only one peak,[9] thus confirming the formation of only one population of particles with a very low size polydispersity index (PDI) of 0.04 (± 0.007). A time-dependent study of the hydrodynamic radius of the aggregates at an angle of 90° indicated that a perfect equilibrium state was reached after about ten days of vigorous stirring. The hydrodynamic radius (R_H) of the corresponding equilibrated aggregates was determined by extrapolating $R_H(c)$ to $c \rightarrow 0$ to be approximately 128 nm in the absence of salt and about 114 nm in the presence of 1 M NaCl. This decrease in the particle size reflects the screening of the electrostatic charges upon addition of salt.

In addition to DLS studies, static light scattering experiments (SLS) were performed in water under the same conditions ($0.31 \leq c \leq 2\ g L^{-1}$, $40 \leq \theta \leq 140°$) with added salt (1 M NaCl). A radius of gyration (R_g) of 110 nm was obtained from these studies.[10] The ratio R_g/R_H (where R_H was

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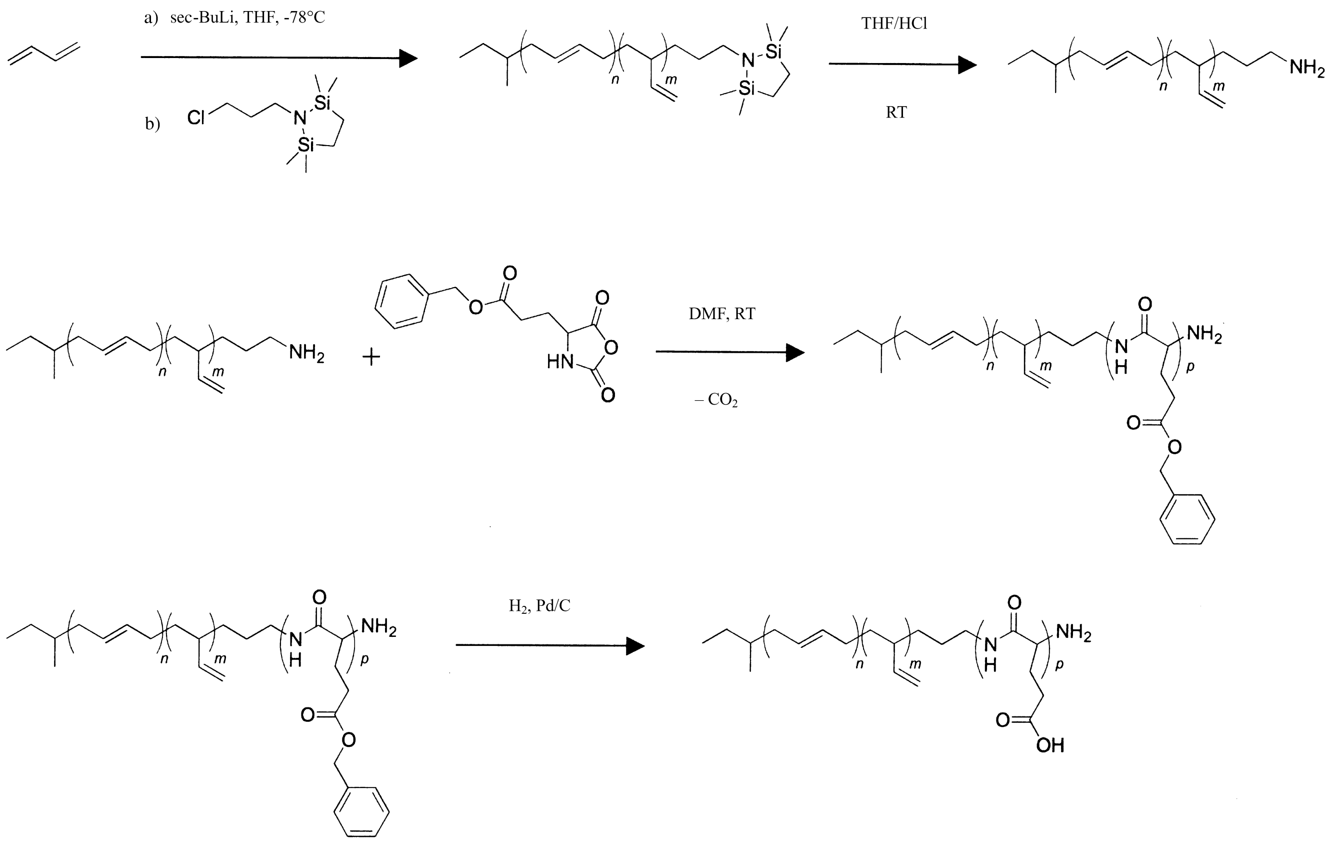

Scheme 1. Reaction scheme for the synthesis of the PB_{40}-*b*-PGA_{100} diblock copolymer by combination of anionic and *N*-carboxyanhydride ring-opening polymerizations.

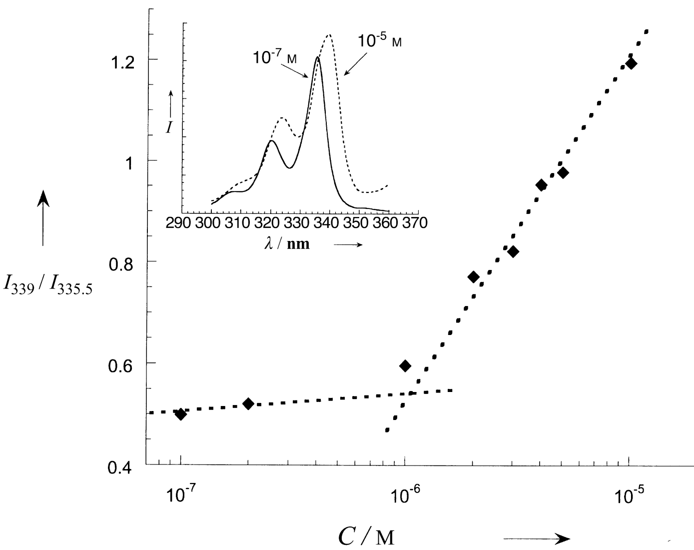

Figure 1. Intensity ratio $I_{339}/I_{335.5}$ obtained from the fluorescence excitation spectra of pyrene plotted versus PB_{40}-*b*-PGA_{100} concentration. Inset: fluorescence excitation spectra of pyrene (6.10^{-7} mol L^{-1}) below (——) and above (- - - -) the cac.

determined in the same conditions (114 nm)) is 0.97/1, which is different from the expected theoretical values for a uniform sphere (0.774/1) or a polymer coil (1.50/1), but is very close to that expected (1.0/1) for a hollow sphere, which suggests there is a vesicle-type morphology.[11] Comparison of the average extended length of a PB_{40}-*b*-PGA_{100} molecule (approximately 25 nm under these conditions) with the measured radii (about 120 nm) also suggests that a vesicle-type morphology (Figure 3) is more likely than a micellar structure. These arguments are supported by freeze-fracture transmission electron microscopy (TEM) experiments (Figure 4). The PB_{40}-*b*-PGA_{100} sample ($c = 1.25$ g L^{-1}) was dissolved in a water/glycerol solvent mixture (7/3) to avoid water crystallization.[12] Aggregates with apparent radii $R_{TEM} \approx 120$ nm were observed by this technique, which are in agreement with the results obtained by DLS and SLS experiments.

Next, the effects of pH changes on the secondary structure of the peptide block of the aggregates were investigated by circular dichroism (CD). The CD spectra recorded in aqueous solution at pH 4.5 and pH 11.5 are shown in Figure 5. The spectrum obtained at pH 4.5, with negative minima at 221 and 208 nm and a positive maximum at 190 nm, is typical of an α-helical secondary structure.[13] PB_{40}-*b*-PGA_{100} gives a doubly inflected CD curve at pH 11.5 with a small positive maximum at 218 nm and a large minimum at 197 nm, which indicates there is a random coil conformation. The observed pH-dependence of the secondary structure of the PGA block of the aggregates is identical to the behavior known for poly(L-glutamic acid) homopolymers.[13]

The effect of pH-induced changes of the polypeptide secondary structure on the size of the aggregates was investigated by DLS studies. Figure 6 shows the R_H value as a function of both the pH value and NaCl concentration. These data illustrate that even at high NaCl concentrations,

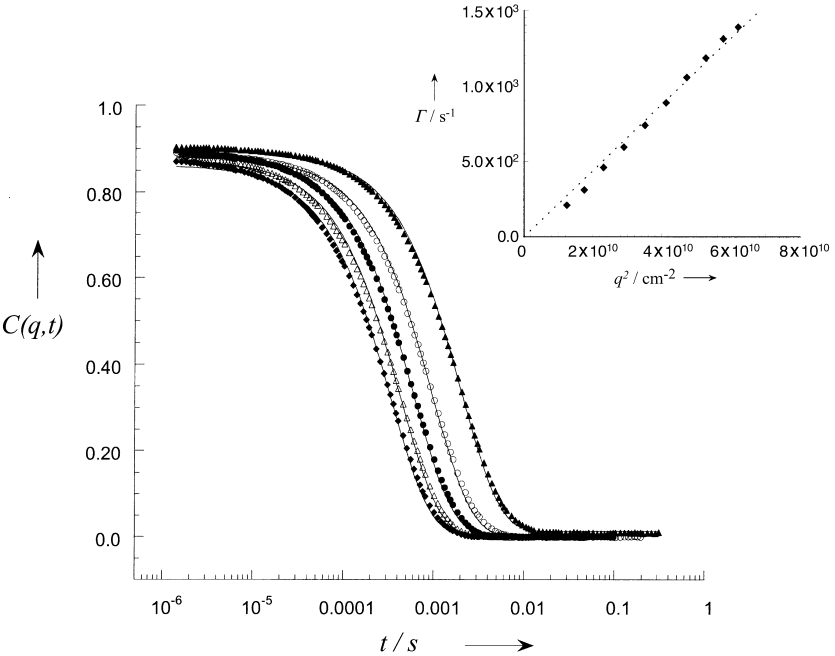

Figure 2. Autocorrelation functions of PB_{40}-*b*-PGA_{100} vesicles in water ($c = 0.625\,g\,L^{-1}$) at different scattering angles: ▲: 50°; ○: 70°; ●: 90°; △: 110°; ◆: 130°. The lines represent data fits with a mono-exponential decay function. Inset: Dependence of the first cumulant frequency (Γ) on the square of the scattering vector (q^2).

when charges on the polypeptide block are completely screened, the size of the aggregates can still be varied by changing the pH value. These pH-induced changes in size (as evidenced from the CD spectra in Figure 5) correlate with a transition from a compact α-helical secondary structure at low pH values to a more extended random-coil conformation in basic media. Such pH-induced size variations are completely reversible and can be repeated numerous times.

In summary, the PB_{40}-*b*-PGA_{100} diblock copolymer forms well-defined vesicular morphologies after direct dissolution into water (basic pH).[14] Dissolution by dialysis and under different pH conditions might certainly lead to other morphologies: this aspect is currently under investigation.[15] The size of the aggregate can be manipulated reversibly by changing the pH value and ionic strength. Even at high NaCl concentrations, where all the charges are effectively screened, pH-induced changes in the polypeptide secondary structure can be used to reversibly vary the aggregate dimensions. Compared with other polyelectrolyte-based block copolymers, this PB_{40}-*b*-PGA_{100} diblock copolymer possesses the unique feature that the polypeptide block is capable of folding into a compact and well-defined secondary structure. It is possible to covalently “capture” the morphology of the system and transform a transient supramolecular self-assembled aggregate into a permanent shape-persistent stimuli-responsive nanoparticle

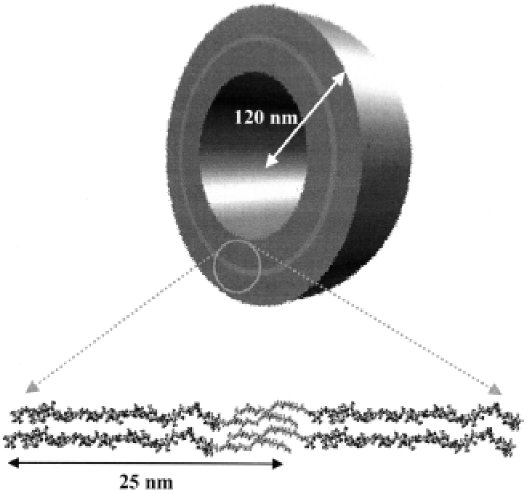

Figure 3. Proposed model for the self-assembly of the PB_{40}-*b*-PGA_{100} into vesicular aggregates.

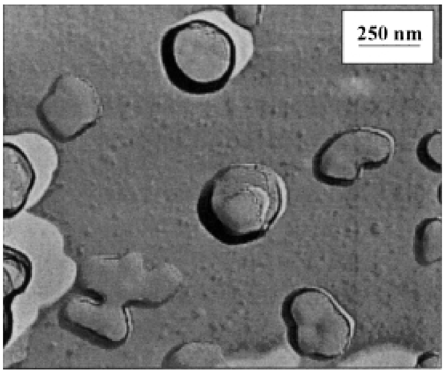

Figure 4. Freeze–fracture TEM image of the PB_{40}-*b*-PGA_{100} aggregates obtained from a water/glycerol (7/3) mixture.

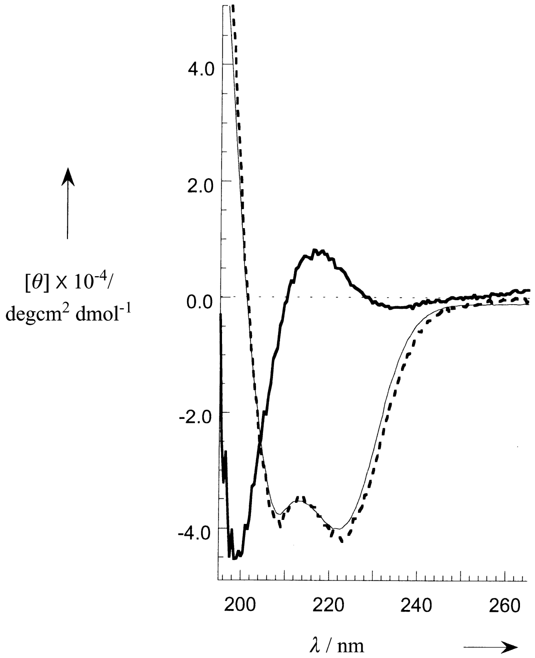

Figure 5. CD spectra of PB_{40}-*b*-PGA_{100} as a function of pH: ——: pH 11.5 (coil conformation), - - - -: pH 4.5 (α-helical conformation). ——: CD spectrum obtained for a poly(L-glutamic acid) homopolymer with a molecular weight of 32 KDa at pH 4.5.

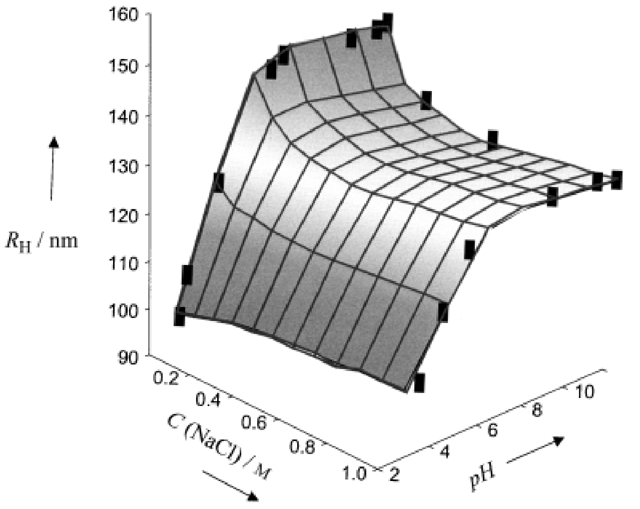

Figure 6. Hydrodynamic radius (R_H) of PB_{40}-*b*-PGA_{100} vesicles measured by DLS studies as a function of ionic strength (NaCl salt concentration) and pH value. ■: experimental data.

by using the 1,2-vinyl bonds present in the polybutadiene. First experiments have shown a moderate shrinkage of the vesicle size after UV curing, which is a typical phenomenon accompanying the cross-linking of polybutadiene molecules. Such nanoparticles might be suitable for a number of applications including the encapsulation and/or the release of hydrophilic as well as hydrophobic active species or their use as sensor nanodevices.

Experimental Section

PB_{40}-*b*-PGA_{100}: Anionic polymerization of butadiene in THF at −78 °C using sec-butyllithium as initiator followed by quenching the oligobutadienyllithium with 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane and acidic aqueous workup gave the ω-aminooligobutadiene. The oligomer functionalized at the primary amine end was then used to initiate the ring-opening oligomerization of Bn-GluNCA in DMF.[16] The length of the γ-benzyl-L-glutamate segment could be controlled through the molar ratio of Bn-GluNCA to ω-aminooligobutadiene initiator to give the required polybutadiene-*b*-poly(γ-benzyl-L-glutamate) block copolymer. After removal of the benzyl ester groups by hydrogenation, the amphiphilic diblock copolymer PB_{40}-*b*-PGA_{100} was obtained. The composition of the block copolymer was analyzed by means of ^1H and ^{13}C NMR spectroscopy and gel permeation chromatography.

SLS and DLS measurements were performed on a ALV5000 goniometer equipped with a ALV5000/E Multiple Tau digital Realtime correlator. The R_H and PDI values of the aggregates were obtained by a cumulant and CONTIN analysis of the experimental correlation functions. TEM pictures were recorded on a JEOL JEM100S microscope working at 80 KV. Samples were prepared by freeze–fracture process on 1.25 gL^{-1} copolymer solutions (7/3 water/glycerol). Fluorescence spectra were recorded on a SAFAS Spectrofluorometer flx spectrometer. CD experiments were carried out on a JOBIN YVON CD6 Spex spectrometer (184–900 nm).

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Dual Biosensing by Magneto-Controlled Bioelectrocatalysis**

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In the last two decades extensive research efforts were directed towards the development of amperometric biosensors based on redox-active enzymes.[1, 2] Ingenious methods to electrically contact redox enzymes with electrode supports by tethering relay units to the protein,[3] the immobilization of

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