Self-Assembly of Rod−Coil Diblock Oligomers Based on α-Helical Peptides

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ABSTRACT: A detailed study of the supramolecular organization of two series of peptide-based diblock oligomers is reported. The diblock oligomers are comprised of an α-helical oligopeptide rod, which is either based on γ-benzyl-L-glutamate or ϵ-benzyloxycarbonyl-L-lysine, and a short oligo(styrene) coil. The unique feature of these rod−coil molecules is that the conformation of the α-helical peptide rod is sensitive to temperature. As a result, the supramolecular organization of these rod−coil oligomers can be manipulated not only via changes in the block length ratio or chemical composition but also via temperature-induced conformational changes in the rod segments. This makes these molecules attractive building blocks for the development of stimuli-sensitive self-assembled materials.

Introduction

Over the past years, rod−coil type diblock oligomers and diblock copolymers have attracted considerable attention as potentially interesting building blocks for the development of novel self-assembled materials.[1,2] The interest in this class of molecules is driven by (at least) two peculiarities that distinguish them from the common coil−coil type diblock copolymers. One feature that characterizes rod−coil type molecules is that their self-assembly is not only driven by the microphase separation of the constituent blocks, but also by the aggregation of the rod segments. The competition between these two processes can lead to morphologies that are different from those known from coil−coil diblock copolymers. A second feature that distinguishes rod−coil type diblock oligomers and diblock copolymers is their relatively high Flory−Huggins interaction parameter (χ), compared with coil−coil diblock copolymers.[3] The enhanced Flory−Huggins parameter is a result from the difference in stiffness between the rod and the coil segments, and can allow phase separation already at relatively low degrees of polymerization. Whereas "ordinary" coil−coil diblock copolymers typically form phase-separated morphologies with characteristic length scales between 10 and 100 nm,[4] rod−coil diblock oligomers have the potential to self-assemble into phase-separated structures with domain sizes of a few nanometers. Thus, introduction of a rigid segment in a diblock copolymer not only increases the number of possible phase-separated morphologies, but can also allow access to phase-separated structures with length scales not attainable with traditional coil−coil diblock copolymers.

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The possibility to manipulate the structure and properties of self-assembled materials under the influence of appropriate external stimuli is very appealing from a practical point of view. In theory, the temperature-dependence of the Flory−Huggins parameter ($\chi \sim A \times T^{-1} + B$, where A and B represent system specific constants) allows thermally induced order−order transitions in phase-separated diblock copolymers. In practice, however, such order−order transitions are only feasible in the vicinity of the order − disorder transition (ODT, i.e., for diblock copolymers with relatively low molecular weights and/or χ), where the boundaries in the block copolymer phase-diagram have sufficient curvature.[5] Due to the relatively high Flory−Huggins parameter, temperature is unlikely to be a suitable stimulus to induce order−order transitions in rod−coil-based self-assembled materials. This prompted us to search for alternative rod−coil type molecules, whose structure and properties could be manipulated using switching strategies that do not rely on the temperature-sensitivity of χ. Recently, we reported the synthesis and properties of two novel rod−coil diblock oligomers that consisted of an α-helical oligo(γ-benzyl-L-glutamate) rod with a number-average degree of polymerization of 10 or 20 and a short coil-segment containing on average 10 repeat units of styrene.[6] In contrast to the rod segment of most other rod−coil molecules, the conformation of the α-helical peptide segment is sensitive to temperature. Our aim was to exploit this temperature-sensitivity of the peptide's secondary structure to induce order−order transitions in the supramolecular organization of the diblock oligomers. A second feature that distinguishes such peptide-based diblock oligomers are the intermolecular hydrogen-bonding interactions that can occur between neighboring rods. Contrary to the unspecific hydrophobic and $\pi-\pi$ interactions, which provide the major driving forces for the self-assembly of most of the other rod−coil molecules, hydrogen-bonding interactions can allow a directed self-assembly. In our previous report, we were indeed able to demonstrate that the supramolecular organization of peptide-

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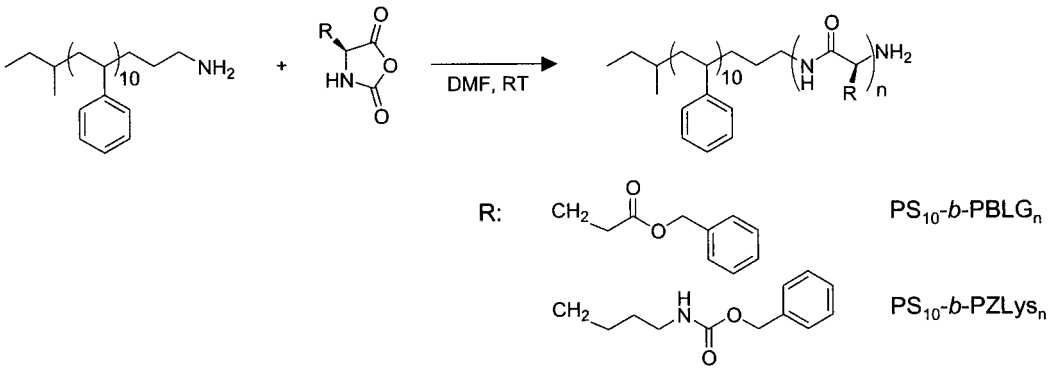
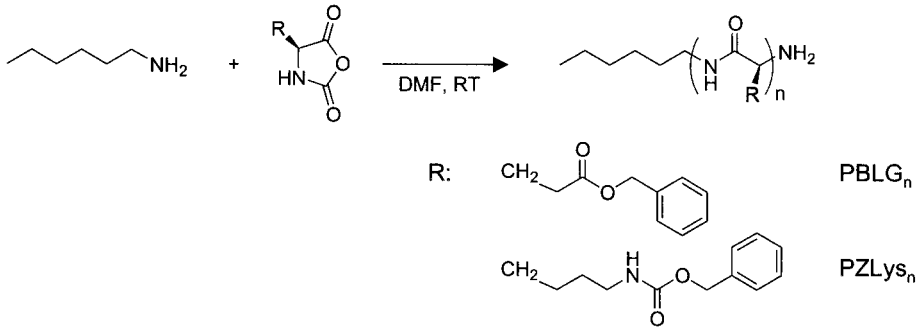
Scheme 1



Scheme 2



based rod−coil diblock oligomers is not only sensitive to variations in the block length ratio but can also be influenced by thermally induced conformational changes in the peptide segment.[6]

In this contribution, we will present the results of a systematic study of the self-assembly of rod−coil type diblock oligomers comprised of an α-helical oligopeptide rod and a short oligo(styrene) coil. Our initial studies were extended to cover a wider range of block length ratios. In addition, to investigate the effect of the type of α-amino acid, a second series of diblock oligomers was prepared whose peptide segment consists of ϵ-benzyloxycarbonyl-L-lysine, instead of γ-benzyl-L-glutamate. Similar to γ-benzyl-L-glutamate, ϵ-benzyloxycarbonyl-L-lysine also has the tendency to form α-helical secondary structures, which leads to a rod−coil type architecture of the diblock oligomers.

Experimental Part

Materials. γ-Benzyl-L-glutamate *N*-carboxyanhydride (Bn-Glu NCA) and ϵ-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (Z-Lys NCA) were prepared according to a literature procedure.[7] The synthesis of the primary amine end-functionalized styrene oligomer and the preparation of the diblock oligomers have already been described in a previous publication.[6]

Physical and Analytical Methods. ^{1}H NMR spectra were recorded at room-temperature on a Bruker Avance 250 spectrometer using the residual proton resonance of the deuterated solvent as the internal standard. GPC analysis was performed at 60 °C with a setup consisting of a Waters 510 pump and a series of three PSS-SDV columns (300 × 8 mm) with pore-sizes of 500, 10^5, and 10^6 Å, respectively. A 0.1 M solution of LiBr in DMF was used as the mobile phase and sample elution was monitored using simultaneous UV and RI detection. Elution times were converted to molecular weights using a calibration curve constructed with narrow-polydispersity poly(styrene) standards. DSC experiments were carried out on a Perkin-Elmer DSC-7 with heating- and cooling-rates of 10 °C/min. A Leitz Laborlux optical microscope equipped with a CCD-camera, a Mettler FP90 central processor, and a Mettler FP82 hot-stage was used to analyze the anisotropic textures. FTIR spectra were recorded on a Nicolet 730 FTIR-spectrometer under nitrogen-atmosphere. Samples were prepared by drop-casting a thin film from a diluted THF-solution onto a piece of silicon wafer. X-ray diffraction experiments were carried out using an 18 KW rotating anode X-ray source (Rigaku-200). A Germanium (111) crystal was used as monochromator to select the Cu Kα radiation. The scattered radiation was collected on a 2D imaging plate system (Mar Research). The reciprocal space fwhm (full-width at half-maximum) was 5×10^{-3} Å^{-1}. The accessible wave-vector (q) range was 0.01 Å^{-1} < q < 1.6 Å^{-1}.

Results and Discussion

Synthesis. The synthesis of the diblock oligomers and the corresponding homooligopeptides is outlined in Scheme 1 and Scheme 2, respectively. The homooligopeptides serve as reference compounds to facilitate the interpretation of the properties of the diblock oligomers. The average length of the homooligopeptides and of the peptide-segment of the diblock oligomers can be controlled via the molar ratio between the monomer (i.e. Bn-Glu NCA or Z-Lys NCA) and the initiator (*n*-hexylamine or the primary amine end-functionalized oligo(styrene)). In this way, homooligopeptides and diblock oligomers were targeted that contained 10−80 α-amino acid repeat units. All the diblock oligomers described in this contribution were prepared using a primary amine terminated styrene oligomer with a number average degree of polymerization of 10 (PS_{10}−NH_2). After precipitation and drying, the materials were characterized by means of ^{1}H NMR and GPC. The results of these experiments are summarized in Table 1 and Table 2 for the Bn-Glu- and Z-Lys-based molecules, respectively.

The results from the ^{1}H NMR experiments indicate that the length of the peptide block linearly depends on the monomer/initiator ratio. This is a typical feature

Table 1. Characterization of the γ-Benzyl-L-glutamate Homooligomers ($PBLG_n$) and the Corresponding $(Styrene)_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ Diblock Oligomers (PS_{10}-*b*-$Pblg_n$)

	yield (%)	M_w[b]	M_n[b]	M_w/M_n[b]	f_{rod}[c]	n_{NMR}[d]	n_{GPC}[e]
$PS_{10}-NH_2$	95	1280	1230	1.04			11
$PBLG_{10}$	70	2990	2820	1.06		8	12
$PBLG_{20}$	71	4800	3970	1.21		16	18
$PBLG_{40}$	73	11100	9800	1.13		34	45
$PBLG_{60}$	67	15200	13850	1.10		54	63
$PBLG_{80}$	70	15300	13880	1.10		69	64
PBLG[f]		154000	75000	2.05			340
PS_{10}-*b*-$PBLG_{10}$	60	3610	3500	1.03	0.63	11	10
PS_{10}-*b*-$PBLG_{20}$	70	6800	5095	1.33	0.79	24	18
PS_{10}-*b*-$PBLG_{40}$	73	7140	5160	1.38	0.85	35	18
PS_{10}-*b*-$PBLG_{60}$	68	10400	8240	1.26	0.88	48	32
PS_{10}-*b*-$PBLG_{80}$	65	14000	11000	1.27	0.91	66	45

[a] Isolated yield, after precipitation and drying. [b] From GPC. GPC experiments were performed in DMF. GPC data of the $(PS)_{10}-NH_2$ refer to a sample that was withdrawn from the oligo(styryl)lithium solution and quenched with MeOH, prior to the addition of the capping agent. This sample was analyzed by means of GPC using THF as the eluent. Mass spectroscopy analysis (FD) of the amine-terminated oligo(styrene) gave M_n = 1030, M_w/M_n = 1.05, and a corresponding to a number-average degree of oligomerization of 9. [c] Calculated using a number average degree of polymerization of 11 for the styrene segment (obtained from GPC) and the peptide block lengths obtained from ^{1}H NMR in combination with the following densities:[8] poly(styrene), d = 1.05 g·cm^{-3}; poly(γ-benzyl-L-glutamate) (α-helix), d = 1.278 g·cm^{-3}. [d] Number average degree of polymerization of the peptide-segment, determined from ^{1}H NMR (DMSO-*d*6). [e] Number average degree of polymerization of the peptide-segment, determined from GPC. [f] Commercial poly(γ-benzyl-L-glutamate) sample.

Table 2. Characterization of the ε-Benzyloxycarbonyl-L-lysine Homooligomers ($PZLys_n$) and the Corresponding $(Styrene)_{10}$-*b*-(ε-benzyloxycarbonyl-L-lysine)$_n$ Diblock Oligomers (PS_{10}-*b*-$PZLys_n$)

	yield[a] (%)	M_w[b]	M_n[b]	M_w/M_n[b]	f_{rod}[c]	n_{NMR}[d]	n_{GPC}[e]
$PS_{10}-NH_2$	95	1280	1230	1.04			11
$PZLys_{20}$	75	6100	5600	1.09		17	21
$PZLys_{40}$	79	10020	6300	1.59		28	23
$PZLys_{60}$	78	14200	10600	1.34		37	40
$PZLys_{80}$	70	17400	13600	1.28		51	52
PS_{10}-*b*-$PZLys_{20}$	76	8700	6300	1.38	0.82	23	20
PS_{10}-*b*-$PZLys_{40}$	76	11600	7650	1.52	0.88	36	25
PS_{10}-*b*-$PZLys_{60}$	35	14300	9200	1.55	0.91	49	31
PS_{10}-*b*-$PZLys_{80}$	52	16300	10300	1.58	0.93	63	35

[a] Isolated yield, after precipitation and drying. [b] From GPC. GPC experiments were performed in DMF. GPC data of the $(PS)_{10}-NH_2$ refer to a sample that was withdrawn from the oligo(styryl)lithium solution and quenched with MeOH, prior to the addition of the capping agent. This sample was analyzed by means of GPC using THF as the eluent. Mass spectroscopy analysis (FD) of the amine-terminated oligo(styrene) gave: M_n = 1030 g/mol, M_w/M_n = 1.05, corresponding to a number average degree of oligomerization of 9. [c] Calculated using a number average degree of polymerization of 11 for the styrene segment (obtained from GPC) and the peptide block lengths obtained from ^{1}H NMR in combination with the following densities:[8] poly(styrene), d = 1.05 g·cm^{-3}; poly(ε-benzyloxycarbonyl-L-lysine) (α-helix), d = 1.208 g·cm^{-3}. [d] Number average degree of polymerization of the peptide segment, determined from ^{1}H NMR (DMSO-*d*6). [e] Number average degree of polymerization of the peptide segment, determined from GPC.

of primary amine initiated polymerizations of α-amino acid *N*-carboxyanhydrides, which are not "living", but do allow quite good control of chain length for peptides containing up to ~100 α-amino acid residues.[9] The deviations of the measured from the targeted chain lengths are due to side reactions (chain termination, chain transfer), which are inherent to these kind of polymerizations.

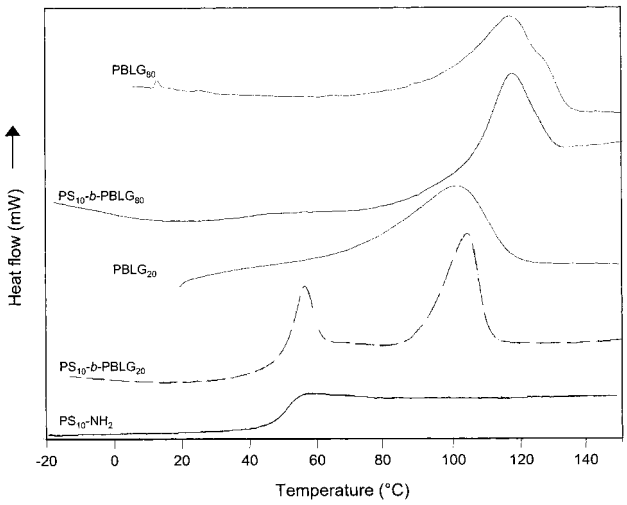


Figure 1. DSC heating traces (first heating cycle, 10 °C/min) for the $(styrene)_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers (PS_{10}-*b*-$PBLG_n$) with n = 20 and 80, the corresponding (γ-benzyl-L-glutamate)$_n$ homooligopeptides ($PBLG_n$), and the primary amine endfunctionalized oligo$(styrene)_{10}$ coil ($PS_{10}-NH_2$).

Thermal Behavior. The thermal behavior of the diblock oligomers was investigated by differential scanning calorimetry (DSC) and optical microscopy. Representative DSC traces of some $(styrene)_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ (PS_{10}-*b*-$PBLG_n$) diblock oligomers as well as the corresponding oligo(γ-benzyl-L-glutamate)$_n$ ($PBLG_n$) reference compounds are shown in Figure 1. The presence of the oligo(styrene) coil in the diblock oligomers is reflected in a glass transition (T_g) between 40 and 50 °C. Due to enthalpy relaxation processes, the T_g of PS_{10}-*b*-$PBLG_{20}$ appears as an endotherm in Figure 1. All of the investigated molecules undergo a first-order transition. For the longer diblock oligomers (n > 20), this transition occurs between 95 and 120 °C, and gradually shifts to higher temperatures with increasing length of the peptide segment. For PS_{10}-*b*-$PBLG_{10}$, this transition occurs at ~60 °C. Except for the T_g, the observed transitions are irreversible and only occur during the first heating run. Optical microscopy studies between crossed polarizers proved that these first-order transitions are not due to isotropization of the material, but represent a solid−solid transition. The (liquid) crystalline textures that were observed remained unchanged until decomposition of the material around 220 °C. The thermal behavior of the PS_{10}-*b*-$PBLG_n$ diblock oligomers and that of the corresponding $PBLG_n$ homooligomers resembles that of the so-called c-form of high molecular weight poly(γ-benzyl-L-glutamate), which is well-known for its ability to form thermotropic and lyotropic liquid crystalline phases.[10] This c-form is a nematic-like paracrystalline phase, which can occur in two modification that only differ in the conformation of the polypeptide chain. DSC experiments have revealed that the c-form of high molecular weight poly(γ-benzyl-L-glutamate) can undergo a first-order transition at ~85 °C, which was assigned to an irreversible change from a 7/2 to an 18/5 helical secondary structure.[11] Since for $n \geq 20$, the $PBLG_n$ oligopeptides as well as the peptide segments of the PS_{10}-*b*-$PBLG_n$ diblock oligomers predominantly adopt an α-helical secondary structure (vide infra), it seems likely that the phase transitions shown

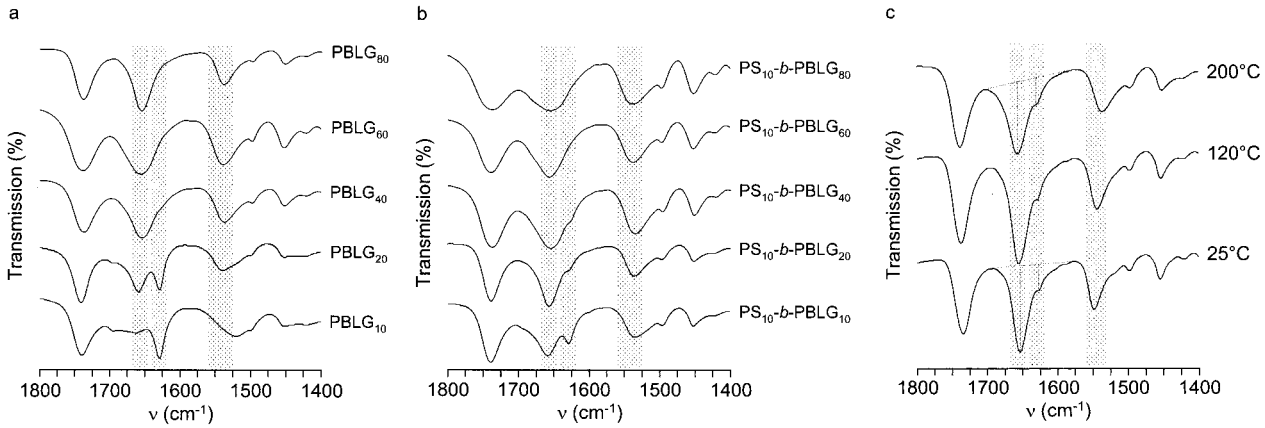


Figure 2. (a) FTIR spectra of the (γ-benzyl-L-glutamate)$_n$ homooligopeptides (PBLG$_n$, with n = 10, 20, 40, 60, 80) at 200 °C. (b) FTIR spectra of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers (PS$_{10}$-*b*-PBLG$_n$, with n = 10, 20, 40, 60, 80) at 200 °C. (c) Temperature-dependent FTIR spectra of a (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_{20}$ diblock oligomer (PS$_{10}$-*b*-PBLG$_{20}$). The amide-bands indicative for the secondary structure of the peptide are marked with shaded bars. The dotted lines in part c serve to indicate the changes in the relative intensities of the two amide I bands.

in Figure 1 represent a similar change in the oligopeptide's secondary structure. At shorter chain lengths (n < 20), the α-helical secondary structure becomes significantly less stable and a large fraction of the peptide segments adopt a β-sheet conformation, which is reflected in the relatively low transition temperature that is observed for PS$_{10}$-*b*-PBLG$_{10}$.

In contrast to the PBLG$_n$ oligopeptides and the PS$_{10}$-*b*-PBLG$_n$ diblock oligomers, no phase transitions were observed by DSC for the corresponding ϵ-benzyloxycarbonyl-L-lysine- (Z-Lys-) based molecules. However, polarized optical microscopy experiments revealed that all of the (ϵ-benzyloxycarbonyl-L-lysine)$_n$ (PZLys$_n$) oligomers as well as the (styrene)$_{10}$-*b*-(ϵ-benzyloxycarbonyl-L-lysine)$_n$ diblock oligomers (PS$_{10}$-*b*-PZLys$_n$) displayed birefringence until their decomposition at ∼220 °C. These observations indicate that some crystalline or liquid-crystalline order must also be present in these materials.

Conformation of the Peptide Segment. Temperature-dependent FTIR studies were performed to obtain information about the conformation of the peptide segment of the diblock oligomers. The question that was addressed in these experiments was to which extent the conformation of the peptide segment was affected by changes in its length, by variations in temperature, by the presence of the oligo(styrene) coil and the chemical structure of the constituent α-amino acid.

The results of the FTIR studies on PBLG$_n$ and PS$_{10}$-*b*-PBLG$_n$ are summarized in Figure 2. The amide I and amide II bands at ∼ 1655 and ∼ 1550 cm^{-1}, respectively, are characteristic for an α-helical secondary structure.[12] For polypeptides possessing a β-sheet conformation, the position of the amide I band is shifted to ∼ 1630 cm^{-1}.[12]

Due to their limited length, the α-helical conformation is only moderately stable for the PBLG$_{10}$ and PBLG$_{20}$ oligomers, and as a result these peptides exclusively (n = 10), or to a large extent (n = 20) adopt a β-sheet conformation at 200 °C. The longer homooligopeptides (n > 20) almost exclusively adopt an α-helical secondary structure, which is insensitive to changes in temperature. Extending the PBLG segment of the diblock oligomers has a similar effect on the conformation of the peptide segment: whereas the FTIR spectrum of PS$_{10}$-*b*-PBLG$_{20}$ and PS$_{10}$-*b*-PBLG$_{40}$ at 200 °C still indicates a small fraction of β-sheet PBLG chains, the peptide segments of the longer homologues (n > 40) exclusively adopt an α-helical secondary structure. Furthermore, comparison of the FTIR spectra of the PBLG$_n$ homooligomers with those of the corresponding PS$_{10}$-*b*-PBLG$_n$ diblock oligomers indicates that the α-helical secondary structure is significantly stabilized upon attachment of the oligo(styrene) coil.

Figure 2c illustrates the effect of temperature on the conformation of the peptide segments. For PS$_{10}$-*b*-PBLG$_{20}$, the relative intensity of the β-sheet amide I band increases in comparison with the α-helical amide I band with increasing temperature. For diblock oligomers with a longer peptide segment (n ≥ 40), effects of temperature are very minor or cannot be observed, and the FTIR spectra only show the amide I band at ∼ 1655 cm^{-1}, indicative of the α-helical conformation.

The effects of temperature and the number of Z-Lys repeat units on the conformation of the peptide segment of PZLys$_n$ and PS$_{10}$-*b*-PZLys$_n$ are illustrated in Figure 3. Also in this case, increasing the number of Z-Lys repeat units from ∼ 20 to ∼ 80 ultimately results in a diblock oligomer whose peptide segment almost exclusively adopts an α-helix conformation (Figure 3b). Comparison of the FTIR spectra shown in Figure 3, parts a and b reveals that, in contrast to the PS$_{10}$-*b*-PBLG$_n$ diblock oligomers, attachment of the PS-coil does not result in a stabilization of the α-helical secondary structure of the Z-Lys segment. Similar to the PS$_{10}$-*b*-PBLG$_{10}$ and PS$_{10}$-*b*-PBLG$_{20}$ diblock oligomers discussed above, temperature also has an influence on the conformation of the peptide segment of the PS$_{10}$-*b*-PZLys$_n$ oligomers. This is shown in Figure 3c for a PS$_{10}$-*b*-PZLys$_{20}$ diblock oligomer, which illustrates the gradual increase of the fraction of peptide segments possessing a β-sheet conformation with increasing temperature. Due to the increased stability of the α-helix with increasing length of the ZLys block, temperature has no influence on the secondary structure of PS$_{10}$-*b*-PZLys$_{80}$. The temperature dependencies of the FTIR spectra of PS$_{10}$-*b*-PZLys$_{40}$ and PS$_{10}$-*b*-PZLys$_{60}$ (not shown here) are intermediate between those of PS$_{10}$-*b*-PZLys$_{20}$ and PS$_{10}$-*b*-PZLys$_{80}$ and also indicate a gradual increase in the fraction of β-sheet peptide chains with increasing temperature.

The data presented in Figures 2 and 3 illustrate the effects of the degree of polymerization (n), temperature, the attachment of the oligo(styrene) block, and the chemical structure of the constituent α-amino acid on

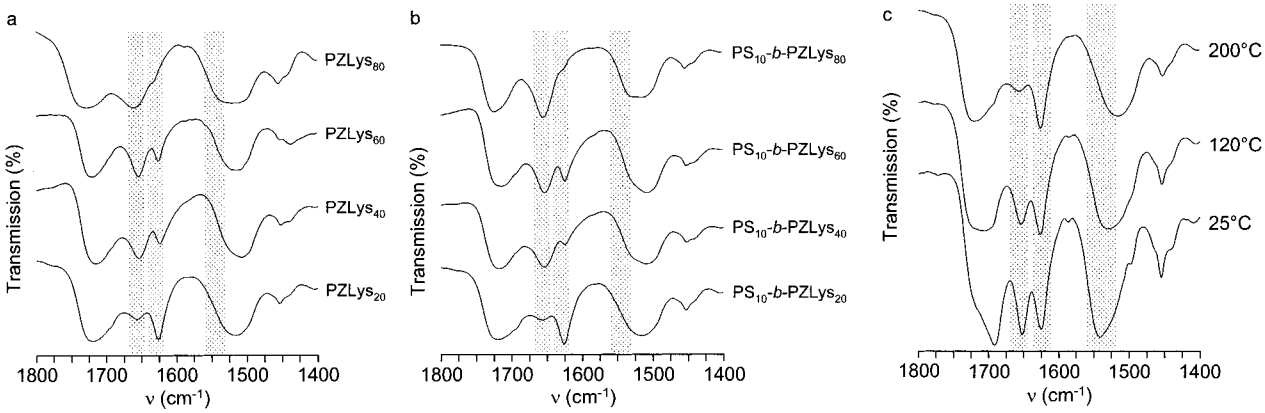


Figure 3. (a) FTIR spectra of the (ϵ-benzyloxycarbonyl-L-lysine)$_n$ homooligopeptides (PZLys$_n$, with n = 20, 40, 60, 80) at 200 °C. (b) FTIR spectra of the (styrene)$_{10}$-*b*-(ϵ-benzyloxycarbonyl-L-lysine)$_n$ diblock oligomers (PS$_{10}$-*b*-PZLys$_n$, with n = 20, 40, 60, 80) at 200 °C. (c) Temperature-dependent FTIR spectra of a (styrene)$_{10}$-*b*-(ϵ- benzyloxycarbonyl-L-lysine)$_{20}$ diblock oligomer (PS$_{10}$-*b*-PZLys$_{20}$). The amide bands indicative for the secondary structure of the peptide are marked with shaded bars.

the secondary structure of the diblock oligomers. Under ambient conditions, the peptide segments of the diblock oligomers largely possess an α-helical secondary structure, indicating the rod−coil architecture of the molecules. For all molecules, increasing the length of the peptide segment results in a stabilization of the α-helical secondary structure. The effect of temperature on the conformation is most pronounced for the diblock oligomers with the shortest peptide segments (n = 10, 20). In these cases a transition from a (predominantly) α-helical to a (predominantly) β-sheet secondary structure can be observed upon increasing the temperature from 25 to 200 °C. Furthermore, the FTIR spectra show that changing the α-amino acid from Bn-Glu to Z-Lys decreases the stability of the α-helical secondary structure. This finding is in agreement with early optical rotation experiments, which also demonstrated that the stability of the α-helix formed by poly(ϵ-benzyloxycarbonyl-L-lysine) is weaker than that of poly(γ-benzyl-L-glutamate).[13] With the exception of PS$_{10}$-*b*-PZLys$_{80}$, the FTIR spectra of the PS$_{10}$-*b*-PZLys$_n$ oligomers show an amide I band at 1630 cm^{-1}, indicative for the presence of β-sheet PZLys chains. This in contrast to the PS$_{10}$-*b*-PBLG$_n$ oligomers, where peptide segments possessing a β-sheet conformation are essentially absent for molecules comprised of 20 or more Bn-Glu repeat units. The lower propensity of Z-Lys toward α-helix formation becomes especially clear upon comparison of Figure 2c and Figure 3c. Whereas the peptide segment of PS$_{10}$-*b*-PBLG$_{20}$ almost exclusively adopts an α-helical conformation, the FTIR spectrum of PS$_{10}$-*b*-PZLys$_{20}$ points toward a β-sheet secondary structure of the Z-Lys block. Another striking difference between the PS$_{10}$-*b*-PBLG$_n$ and PS$_{10}$-*b*-PZLys$_n$ diblock oligomers is the effect of the oligo(styrene) coil on the conformation of the peptide segment. Whereas coupling of a oligo(styrene) segment to the PBLG$_n$ oligomers results in a significant stabilization of the α-helical secondary structure, no such effect is observed for the PS$_{10}$-*b*-PZLys$_n$ oligomers.

Supramolecular Organization. The supramolecular organization of the diblock oligomers was investigated by means of temperature-dependent small-angle X-ray scattering (SAXS) experiments. Below, we will successively discuss the SAXS patterns of the Bn-Glu- and Z-Lys-based diblock oligomers as well as the corresponding homooligopeptides.

The SAXS patterns of the PBLG$_n$ homooligopeptides at 120 and 200 °C are shown in Figure 4. For the longer oligomers (n > 40) a set of Bragg peaks at scattering angles with a ratio of 1:3$^{1/2}$:2 is observed, independent of temperature. Since the FTIR experiments (Figure 2) demonstrated that for n > 40 these peptides exclusively adopt an α-helical secondary structure, both at 120 and at 200 °C, the SAXS patterns indicate a columnar hexagonal arrangement of α-helical oligopeptides. These observations are in agreement with previously published X-ray studies on the so-called C-form of high-molecular weight poly(γ-benzyl-L-glutamate).[11] Above 120 °C, high-molecular weight poly(γ-benzyl-L-glutamate) has been found to form a nematic-like paracrystalline phase in which the 18/5 α-helical polypeptide chains are packed in a near-hexagonal lattice. From the Bragg peak at ∼0.45 Å^{-1}, a lattice parameter of ∼16 Å can be calculated, which corresponds to the intermolecular distance between neighboring poly(γ-benzyl-L-glutamate) chains. The broad reflection at q = ∼ 1.30 Å^{-1} can be assigned to the pitch (∼ 5 Å) of the α-helical polypeptide chains.

Decreasing the length of the peptide chain leads to a destabilization of the α-helical secondary structure. This effect is most pronounced for PBLG$_{10}$, which, according to the FTIR experiments (Figure 2), exclusively possesses a β-sheet conformation at 200 °C. Thus, the Bragg peak at q = ∼ 0.32 Å^{-1} in the SAXS pattern of PBLG$_{10}$ in Figure 4b most likely points toward a lamellar organization of peptide chains with a β-sheet secondary structure. The corresponding d spacing (∼20 Å) represents the width of a lamellae. The reflection at q = ∼1.30 Å^{-1} (∼5 Å) represents the intermolecular distance between adjacent peptide chains within one lamellae.

The FTIR and SAXS data of PBLG$_{40}$ at 200 °C, PBLG$_{20}$ at 120 and 200 °C, and PBLG$_{10}$ at 120 °C represent an intermediate situation. In all of these cases, the FTIR spectra prove that part of the peptides possess an α-helical conformation and part of the oligomers have a β-sheet secondary structure. The corresponding SAXS patterns reflect these data and indicate the coexistence of a columnar hexagonal arrangement of α-helical peptides with a lamellar assembly of β-sheet type peptide chains.

The SAXS patterns of the PS$_{10}$-*b*-PBLG$_n$ diblock oligomers recorded at 120 °C and 200 °C are presented in Figure 5. For the diblock oligomers with the longest PBLG segments (n > 40), one set of Bragg peaks at scattering angles with a ratio of 1:3$^{1/2}$:2 is observed, indicating a columnar hexagonal arrangement of the

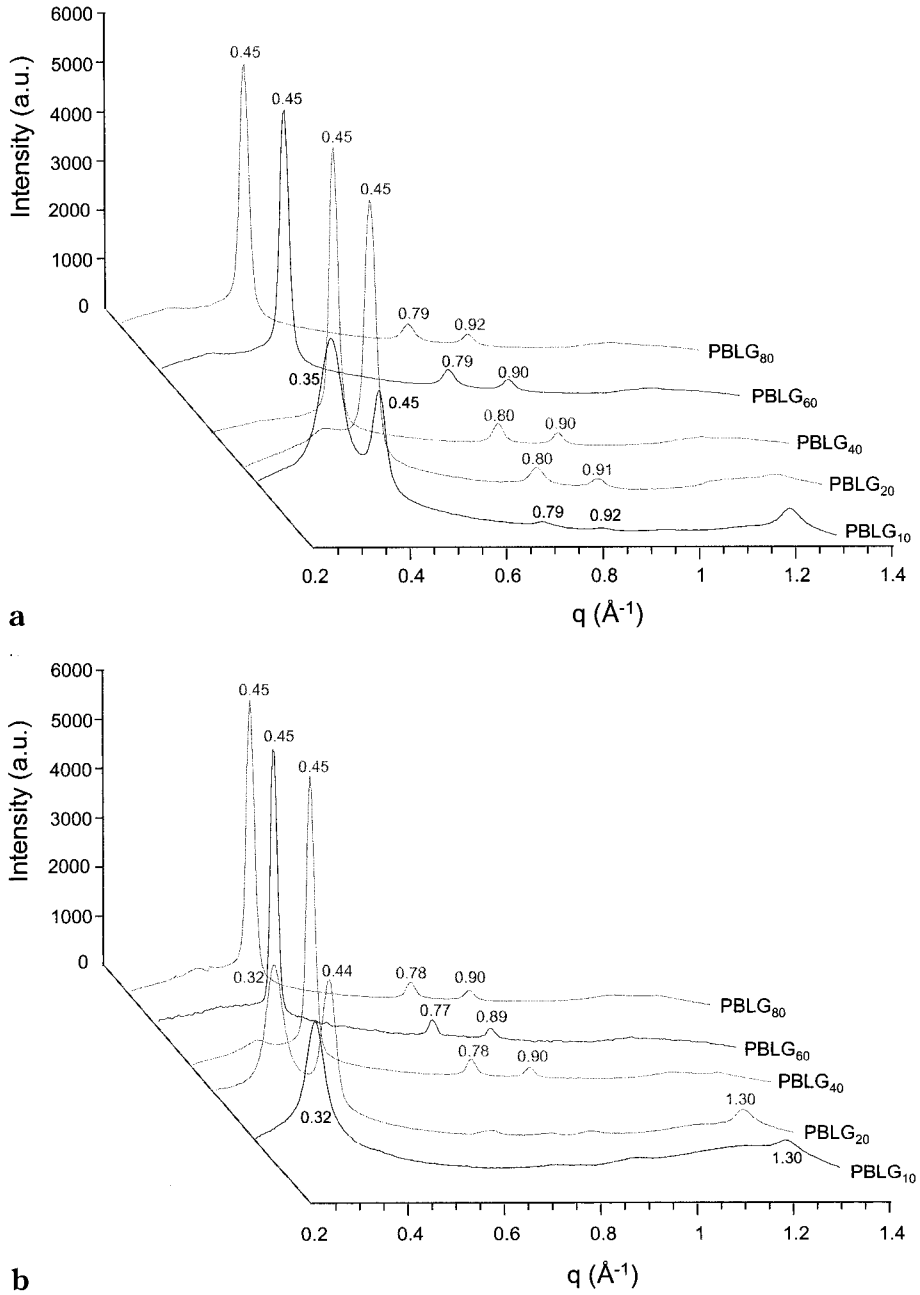


Figure 4. SAXS patterns of the (γ-benzyl-L-glutamate)$_n$ homooligopeptides (PBLG$_n$, with n = 10, 20, 40, 60, 80) recorded at (a) 120 and (b) 200 °C.

molecules at both 120 and 200 °C. The lattice parameter (d = ∼16 Å) which can be derived from these Bragg peaks, as well as the helical pitch (∼5 Å) calculated from the broad reflection around ∼1.30 Å^{-1} are similar to those obtained for the corresponding homooligopeptides (Figure 4). The insensitivity of the supramolecular organization of these diblock oligomers toward temperature is also reflected in the FTIR spectra, which indicate that the peptide segments exclusively adopt an α-helical secondary structure at both 120 and 200 °C.

Upon decreasing the length of the peptide segment, a difference starts to appear between the supramolecular organization of the diblock oligomers and that of the corresponding homooligopeptides. At 120 °C, the SAXS pattern of PS$_{10}$-*b*-PBLG$_{40}$ indicates a columnar hexagonal arrangement, as is also observed for PS$_{10}$-*b*-PBLG$_{60}$ and PS$_{10}$-*b*-PBLG$_{80}$. Increasing the temperature to 200 °C completely changes the SAXS pattern, and only two reflections at q = 0.32 and 1.30 Å^{-1} are observed. In analogy with the SAXS experiments on the PBLG$_n$ homooligopeptides, these reflections are assigned to the width of and the intermolecular distance within lamellae comprised of diblock oligomers whose peptide segments possess a β-sheet conformation. However, whereas the SAXS patterns of the PBLG$_n$ oligomers reflect the FTIR spectra and indicate the coexistence of α-helical and β-sheet secondary structures, the SAXS pattern of PS$_{10}$-*b*-PBLG$_{40}$ at 200 °C only suggests a lamellar ordering of β-sheet type peptide chains, despite the fact that according to the corresponding FTIR spectrum the majority of the peptide chains possess an α-helical conformation. Apparently, already a small fraction of peptide segments with a β-sheet conformation is sufficient to completely prevent a regular arrangement of those diblock oligomers that contain an α-helical peptide block.

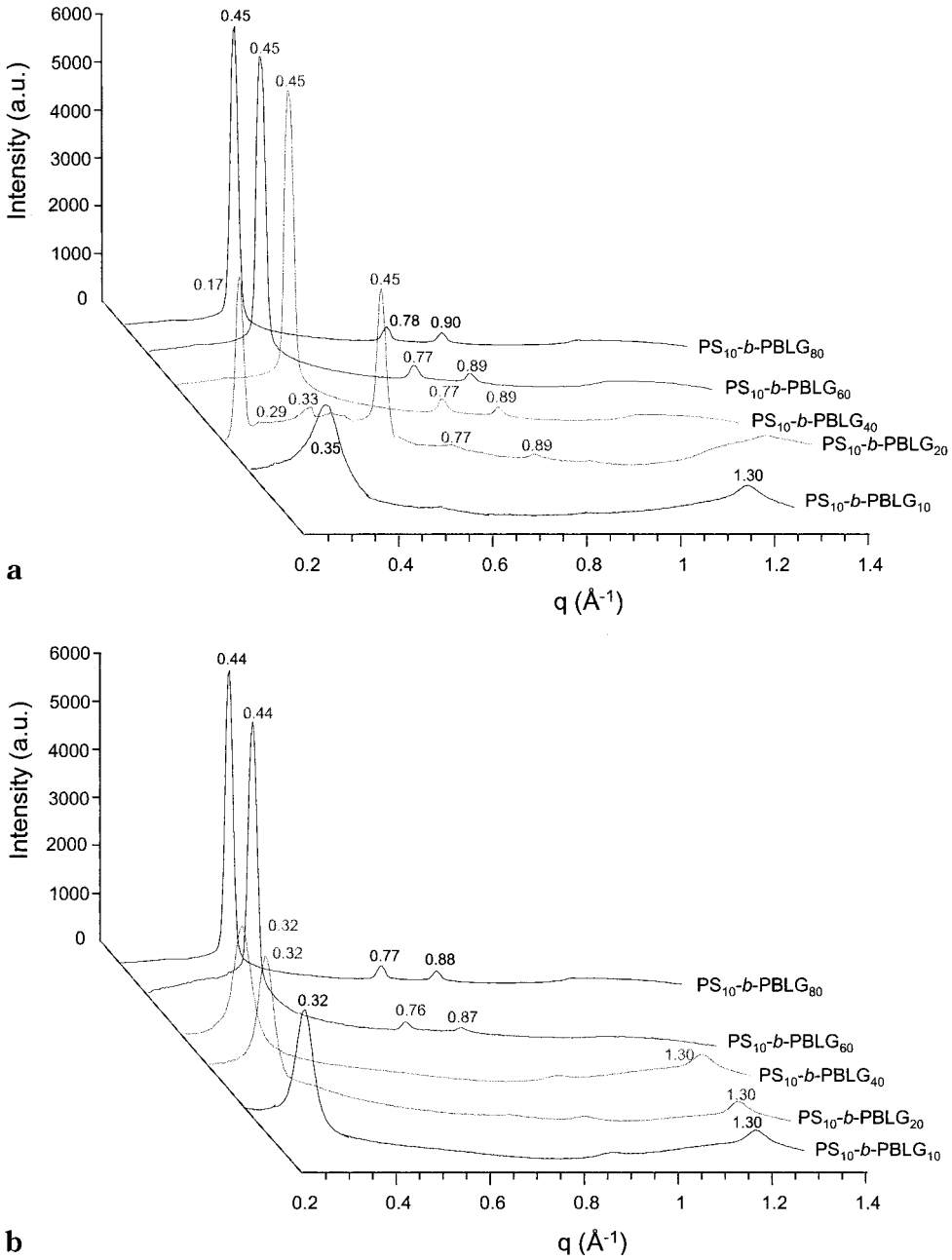


Figure 5. SAXS patterns of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers (PS$_{10}$-*b*-PBLG$_n$, with n = 10, 20, 40, 60, 80) recorded at (a) 120 and (b) 200 °C.

The SAXS pattern of PS$_{10}$-*b*-PBLG$_{20}$ at 120 °C displays two sets of Bragg peaks at scattering angles with a ratio of 1:3$^{1/2}$:2. This diffraction pattern has been previously ascribed to a "*double-hexagonal*" morphology with lattice parameters of 16 and 43 Å, respectively.[6] Upon increasing the temperature to 200 °C, two major signals appear at $q = \sim 0.32$ Å^{-1} and $q = \sim 1.30$ Å^{-1}, suggesting a lamellar assembly of diblock oligomers with β-sheet peptide blocks. Also in this case, only Bragg peaks are observed that point toward such a lamellar organization, even though the FTIR spectrum (Figure 2) indicates that the majority of the peptide segments possess an α-helical conformation.

The FTIR spectrum of the PS$_{10}$-*b*-PBLG$_{10}$ diblock oligomer shows that a considerable fraction of the peptide segments has a β-sheet secondary structure, at both 120 and 200 °C. Accordingly, only Bragg peaks are observed in the SAXS patterns that indicate a lamellar organization of diblock oligomers with peptide segments that possess a β-sheet conformation.

On the basis of the SAXS studies discussed above, "phase diagrams" can be constructed, which describe the supramolecular organization of the peptides and diblock oligomers as a function of peptide chain length (*n*) and temperature (*T*) (Figure 6). The different types of morphologies that are found are schematically illustrated in Figure 7.

The most interesting aspect about the phase diagrams shown in Figure 6 is the fact that the supramolecular organization of certain diblock oligomers (PS$_{10}$-*b*-PBLG$_{20}$ and PS$_{10}$-*b*-PBLG$_{40}$) cannot only be manipulated by varying the degree of polymerization of the peptide segment, but also by changing the temperature. Apart from some exceptions close to the ODT,[5] the latter is not feasible for "ordinary" coil−coil diblock copolymers. These observations nicely illustrate the feasibility

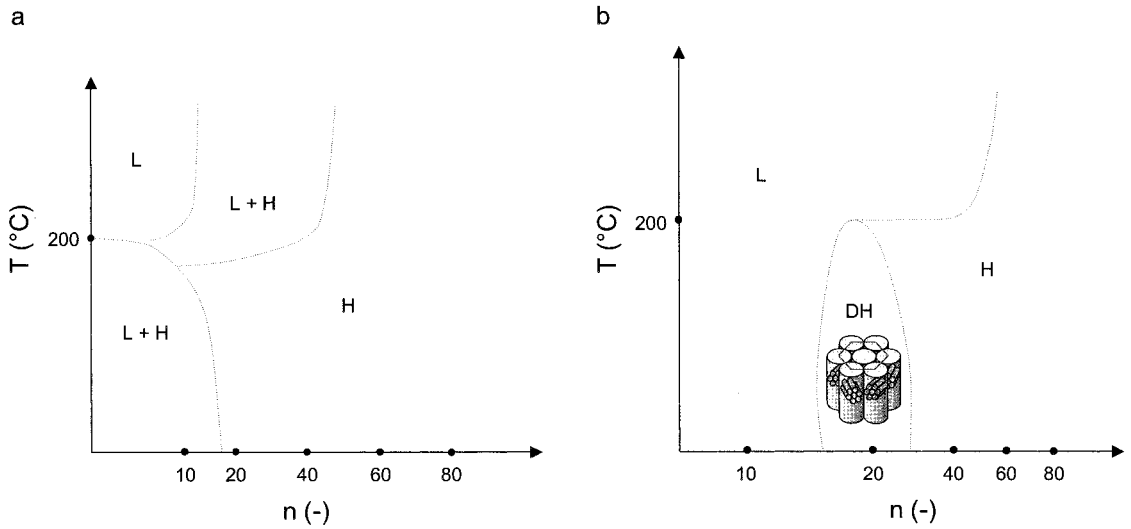


Figure 6. Phase diagrams describing the supramolecular organization of (a) the (γ-benzyl-L-glutamate)$_n$ homooligopeptides (PBLG$_n$) and (b) the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers (PS$_{10}$-*b*-PBLG$_n$) as a function of the number average degree of polymerization of the peptide segment (n) and temperature (*T*). *n* refers to the targeted number average degree of polymerization of the peptide segment. The obtained chain lengths, calculated from ^{1}H NMR, are listed in Table 1. (L = lamellar β-sheet organization; H = columnar hexagonal organization; DH = double hexagonal morphology).

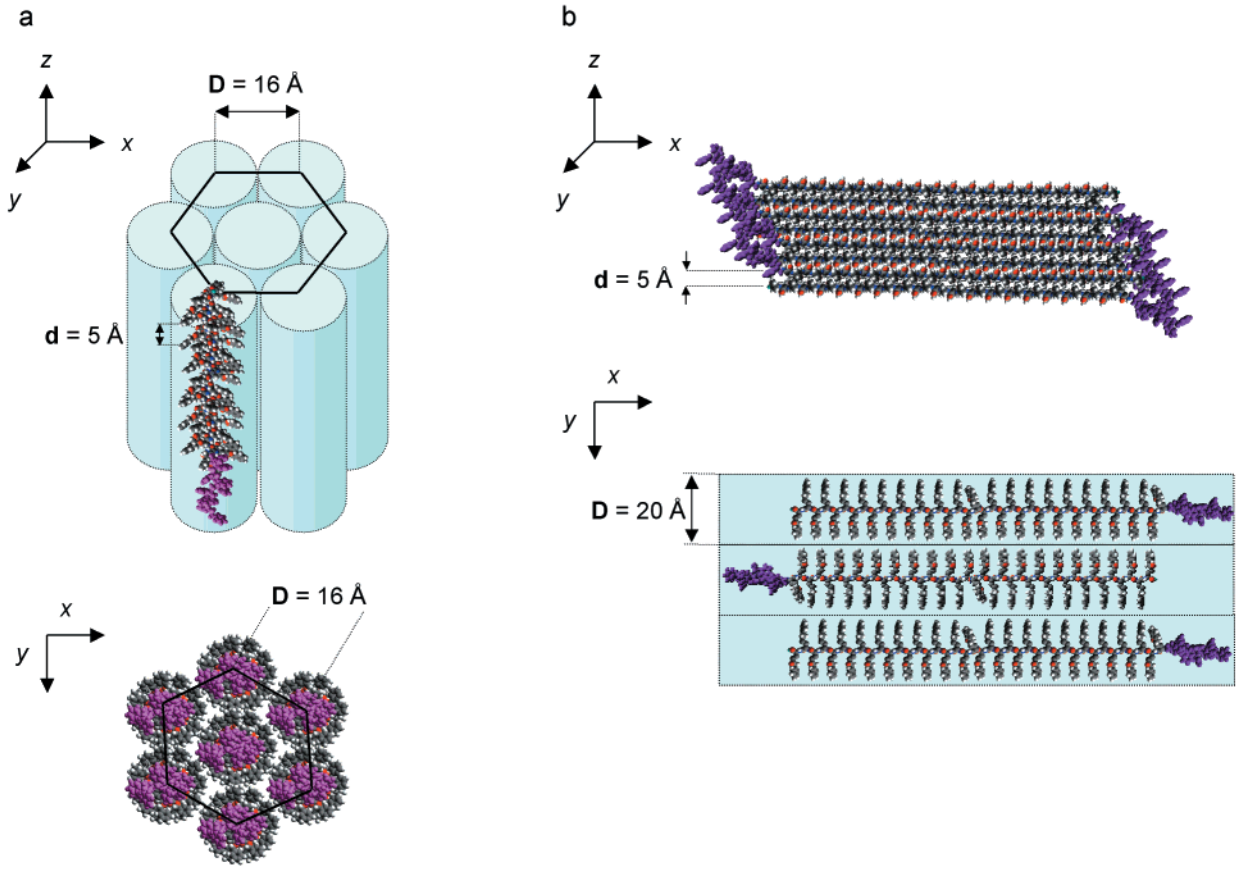


Figure 7. Schematic representation of (a) the columnar hexagonal and (b) the lamellar β-sheet organization of the (styrene)$_{10}$-*b*-(γ-benzyl-L-glutamate)$_n$ diblock oligomers (PS$_{10}$-*b*-PBLG$_n$). In the latter case, the oligomers are stacked on top of each other in the *z* direction with the amide-bonds lying in the *x*−*z* plane.

of temperature-induced conformational changes as an alternative strategy to achieve order−order transitions in the solid-state of diblock oligomers and diblock copolymers.

Upon comparing the phase diagram of the PBLG$_n$ homooligomers with that of the PS$_{10}$-*b*-PBLG$_n$ diblock oligomers, the results of the FTIR studies should be taken into account. For the PBLG$_n$ homooligomers, the SAXS patterns reflect the FTIR spectra (vide supra); i.e., in those cases where the FTIR spectra indicate the presence of a single type of secondary structure, either a purely lamellar β-sheet or columnar hexagonal morphology was found by SAXS. In those cases where the FTIR spectra point toward a mixture of α-helical and β-sheet conformations, the SAXS diagrams indicate the coexistence of the corresponding columnar hexagonal

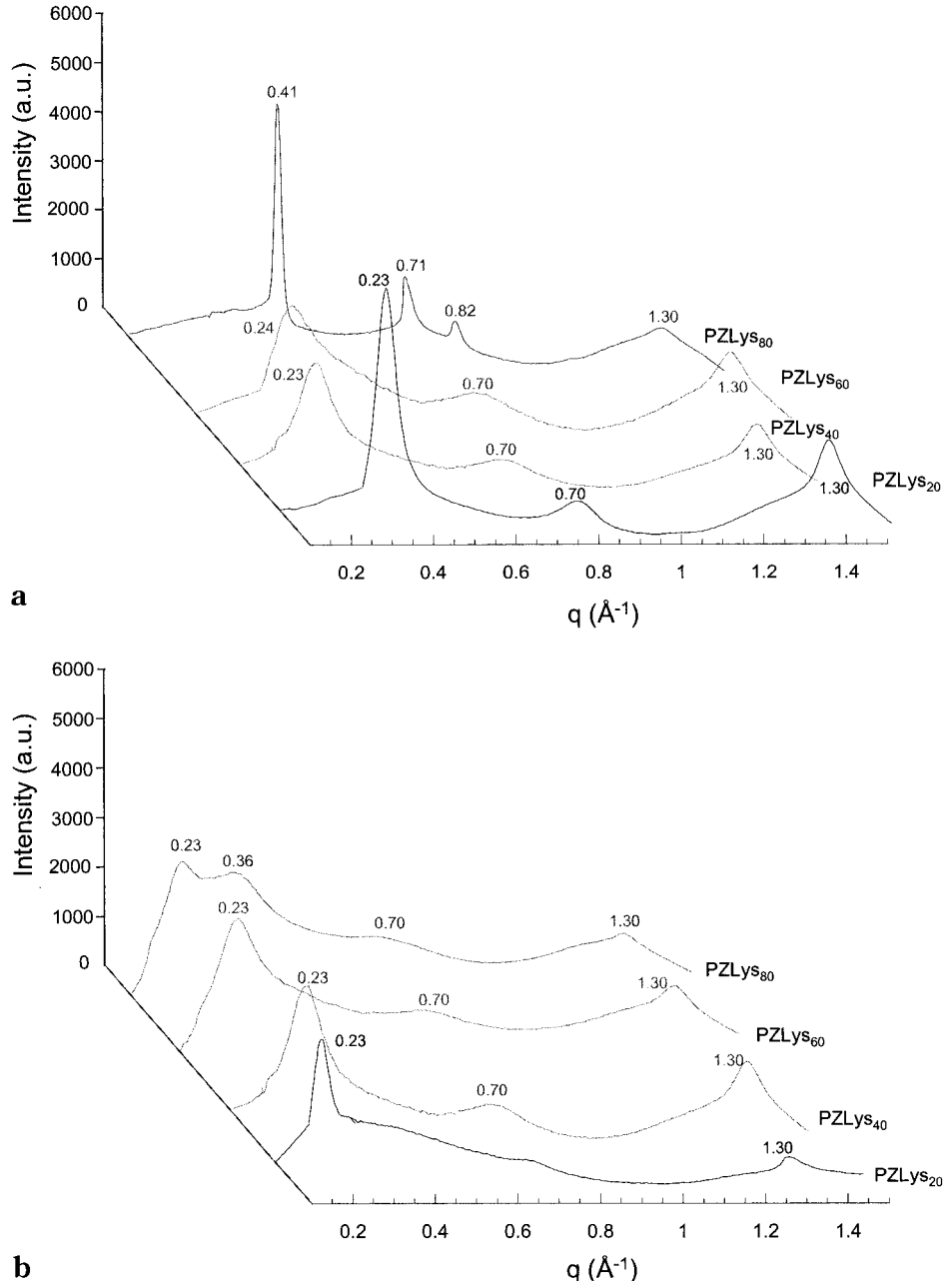


Figure 8. SAXS patterns of the (ϵ-benzyloxycarbonyl-L-lysine)$_n$ homooligopeptides (PZLys$_n$, with n = 20, 40, 60, 80) recorded at (a) 120 and (b) 200 °C.

and lamellar β-sheet morphologies. This situation is different, however, for the PS$_{10}$-*b*-PBLG$_n$ diblock oligomers. For these molecules, the FTIR spectra and SAXS patterns are only in agreement for n = 40, 60, and 80 at 120 °C and for n = 60 and 80 at 200 °C. Under these conditions, the peptide segment of the diblock oligomers exclusively adopts an α-helical secondary structure and the molecules organize in a columnar hexagonal fashion. In all other situations, the FTIR spectra prove the coexistence of peptide segments having α-helical and β-sheet secondary structures. Nevertheless, the corresponding SAXS patterns only indicate the presence of a single type of morphology. Although for n = 10 at 120 °C and n = 10, 20, and 40 at 200 °C the majority of the peptide segments possess an α-helical secondary structure, the SAXS patterns only provide evidence for a lamellar assembly of β-sheet type peptide blocks. Thus, whereas attachment of the oligo(styrene)$_{10}$ block leads to stabilization of the α-helical secondary structure of the PBLG$_n$ segment, it apparently hampers the columnar hexagonal organization of the molecules; even in the presence of a small fraction of diblock oligomers with a β-sheet type peptide segment, a regular organization of molecules having an α-helical peptide block is not possible.

SAXS patterns of the PZLys$_n$ homooligopeptides and the corresponding PS$_{10}$-*b*-PZLys$_n$ diblock oligomers, recorded at 120 and 200 °C, are shown in Figure 8 and Figure 9, respectively.

The FTIR spectra (Figure 3) of both the PZLys$_n$ homooligomers and of the corresponding diblock oligomers indicate that at 200 °C for all of these molecules at least part of the peptide chains possesses a β-sheet secondary structure. In line with the observations on the Bn-Glu-based materials, the corresponding SAXS patterns (Figure 8b and Figure 9b) display two major Bragg peaks at q = ∼0.23 Å^{-1} and q = 1.30 Å^{-1}. The first peak, which corresponds to a d spacing of ∼27 Å,

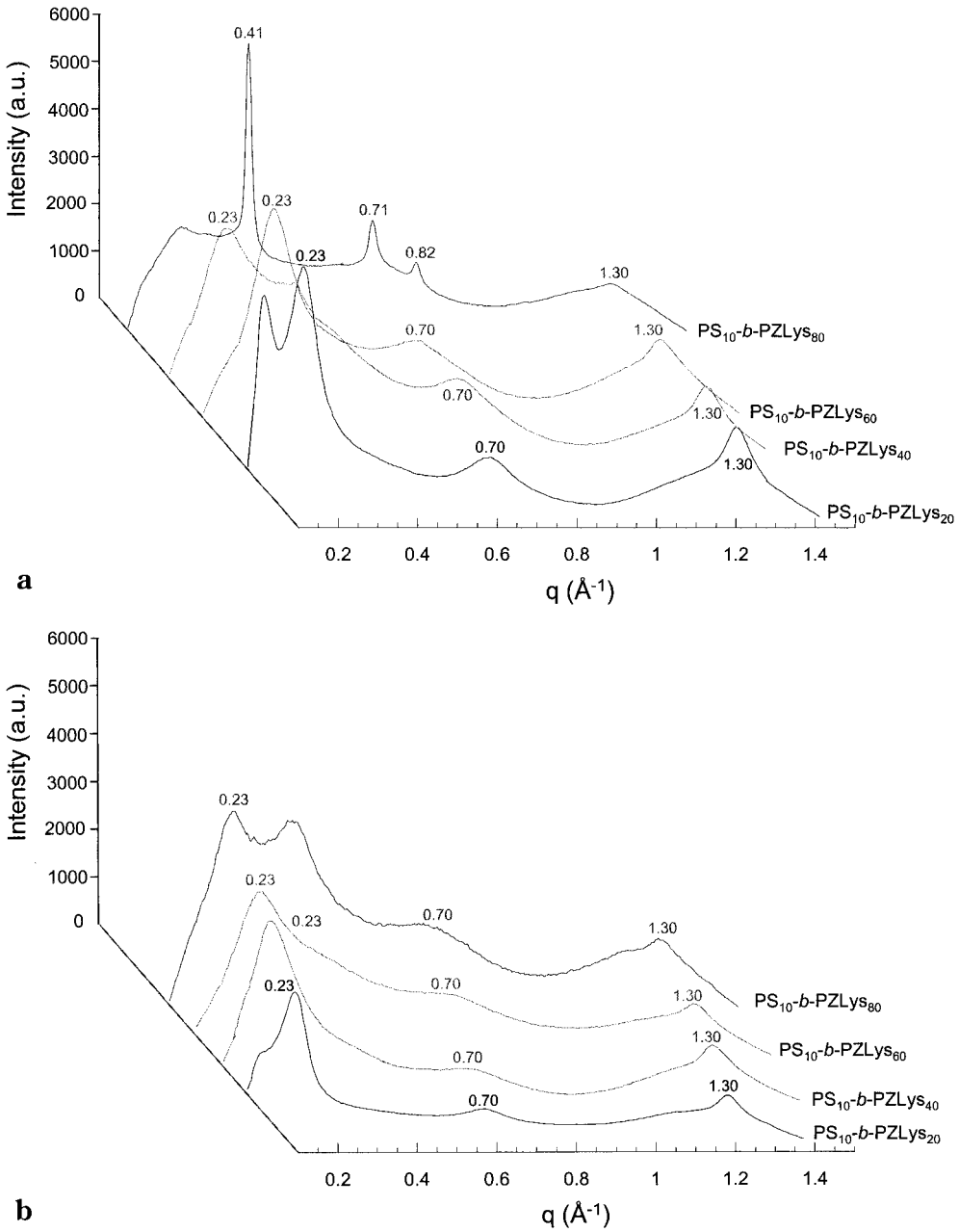


Figure 9. SAXS patterns of the (styrene)$_{10}$-*b*-(ϵ-benzyloxycarbonyl-L-lysine)$_n$ diblock oligomers (PS$_{10}$-*b*-PZLys$_n$, with n = 20, 40, 60, 80) recorded at (a) 120 and (b) 200 °C.

can be attributed to the width of a lamellae formed by stacking of PZLys$_n$ oligomers or PS$_{10}$-*b*-PZLys$_n$ diblock oligomers that are composed of a β-sheet type peptide block. The Bragg peak at q = 1.30 Å^{-1} corresponds to the interchain distance (∼ 5 Å) between adjacent PZLys chains within such a lamellae. The SAXS patterns of the PZLys$_n$ oligomers and the PS$_{10}$-*b*-PZLys$_n$ diblock oligomers, however, show an additional Bragg peak at q = ∼0.70 Å^{-1}, which is not observed for the lamellar Bn-Glu-based molecules. This peak, which corresponds to a spacing of ∼9 Å, roughly correlates with the distance between two neighboring Z-Lys side-chains (see Figure 11). Note that in the β-sheet conformation shown in Figure 11, the α-amino acid side chains are positioned in an alternating fashion on opposite sides with respect to the peptide backbone. The observation of this 9 Å reflection suggests that the α-amino acid side chains of the Z-Lys-based materials possess a higher degree of order than their Bn-Glu containing analogues, for which no such Bragg peak could be observed. We believe that this is due to hydrogen bonding interactions between the carbamate groups of adjacent PZLys chains. Such intermolecular interactions, which decrease the mobility of the side chains, are not possible for the Bn-Glu containing peptides and diblock oligomers.

Only at 120 °C and at chain lengths of ∼80 α-amino acid repeat units, the peptide chains of PZLys$_n$ and PS$_{10}$-*b*-PZLys$_n$ exclusively adopt an α-helical secondary structure. Only in these two cases, the SAXS patterns display a set of three Bragg peaks at scattering angles with a ratio of 1:3$^{1/2}$:2. This indicates the packing of α-helical PZLys chains into a columnar hexagonal lattice with an intermolecular distance of ∼18 Å, The broader signal at q = ∼1.30 Å^{-1} corresponds to an helical pitch of ∼5 Å.

The results of the SAXS studies on the PZLys$_n$ homooligomers and the PS$_{10}$-*b*-PZLys$_n$ diblock oligomers are schematically summarized in the "phase diagrams"

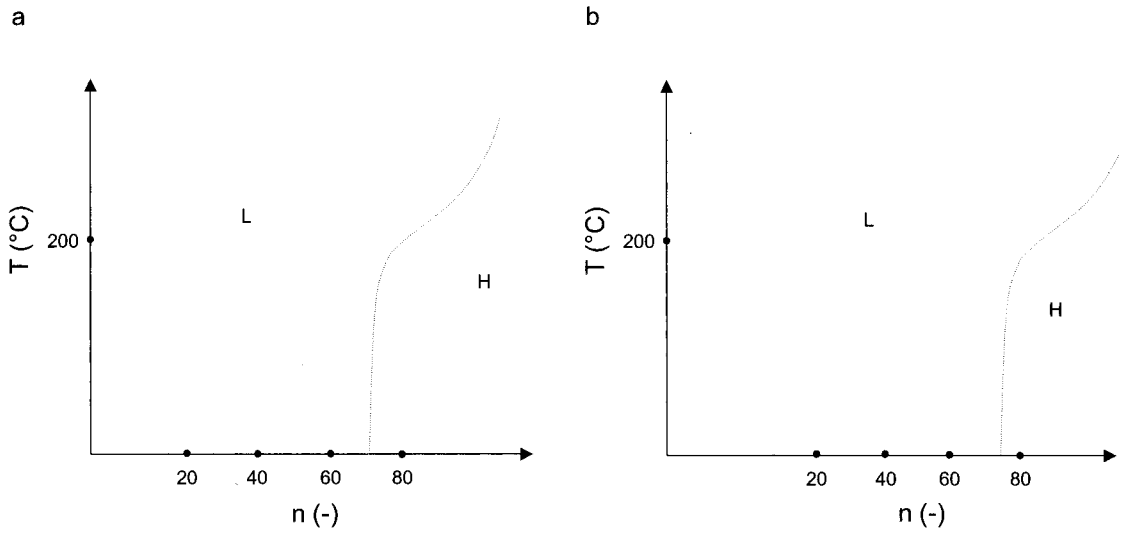


Figure 10. Phase diagrams describing the supramolecular organization of (a) the (ϵ-benzyloxycarbonyl-L-lysine)$_n$ homooligopeptides (PZLys$_n$) and (b) the (styrene)$_{10}$-*b*-(ϵ-benzyloxycarbonyl-L-lysine)$_n$ diblock oligomers (PS$_{10}$-*b*-PZLys$_n$) as a function of the number-average degree of polymerization of the peptide segment (n) and temperature (T). n refers to the targeted number average degree of polymerization of the peptide segment. The obtained chain lengths, calculated from ^{1}H NMR, are listed in Table 2. (L = lamellar β-sheet organization; H = columnar hexagonal organization).

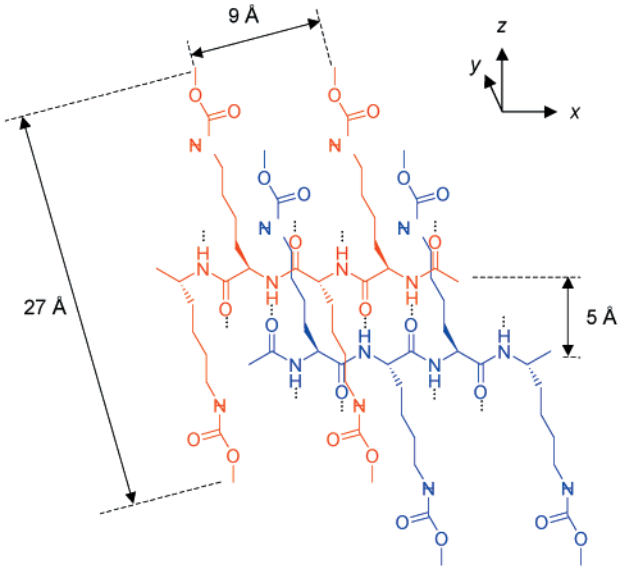


Figure 11. Schematic representation of the lamellar β-sheet organization of the (styrene)$_{10}$-*b*-(ϵ-benzyloxycarbonyl-L-lysine)$_n$ diblock oligomers (PS$_{10}$-*b*-PZLys$_n$). The benzene rings of the benzyloxycarbonyl groups have been omitted for reasons of clarity. The peptide segments are stacked on top of each other in the z direction with the amide bonds lying in the $x-z$ plane. The lamellae are stabilized by hydrogen bonds between adjacent α-amino acid side chains in the $y-z$ plane.

shown in Figure 10. In addition, the β-sheet type lamellar organization of the Z-Lys-based molecules is schematically depicted in Figure 11.

The phase diagrams in Figure 10 again nicely illustrate the proposed concept and show that, given the appropriate length of the peptide segment (PS$_{10}$-*b*-PZLys$_{80}$), it is possible to manipulate the supramolecular organization of the diblock oligomers via variations in temperature.

To discuss the phase diagrams of the PZLys$_n$ homooligomers and the PS$_{10}$-*b*-PZLys$_n$ diblockoligomers and to compare the data with those of the Bn-Glu-based materials, it is useful to consider the results of the FTIR experiments. In a number of cases, the FTIR spectra of the PZLys$_n$ oligomers indicate the coexistence of α-helical and β-sheet secondary structures. The corresponding SAXS patterns, however, only point toward the presence of a lamellar β-sheet type supramolecular structure. This observation is in contrast with the behavior of the PBLG$_n$ oligomers. For these peptides, the SAXS patterns reflect the FTIR spectra, and mixed morphologies were observed in SAXS. Furthermore, the phase diagrams show that increasing the length of the PZLys$_n$ segment induces a transition from a lamellar β-sheet to a columnar hexagonal supramolecular morphology. A similar behavior was also observed for the Bn-Glu-based materials and is due to the increased stability of the α-helical secondary structure with increasing length of the peptide block. However, whereas PBLG$_n$ and PS$_{10}$-*b*-PBLG$_n$ already form columnar hexagonal morphologies at n = 40, approximately 80 α-amino acid residues are needed to allow the formation of this morphology for the Z-Lys containing molecules. The two phenomena discussed above are most likely due to the lower α-helix forming propensity of Z-Lys in comparison with Bn-Glu, which reduces the stability of the α-helical secondary structure.

Conclusions

The styrene-*b*-peptide diblock copolymers discussed in this contribution represent a unique class of stimuli-sensitive materials. Under ambient conditions, the peptide segments largely adopt an α-helical secondary structure, resulting in diblock copolymers with a rod−coil type architecture. Depending on the α-amino acid and the length of the peptide segment, raising the temperature can induce a transition from an α-helical to a β-sheet secondary structure. The supramolecular organization of the diblock oligomers is not only sensitive to variations in the volume fractions of the constituent blocks and their chemical composition (i.e. the type of α-amino acid) as it is the case for most of the "*ordinary*" coil−coil type diblock copolymers, but can also be manipulated by temperature-induced conformational changes. It was found that even a small fraction of peptide segments with a β-sheet conformation is sufficient to induce a lamellar supramolecular morphol-

ogy and suppress the regular organization of those diblock oligomers possessing an α-helical peptide block. Increasing the length of the peptide segment stabilizes the α-helical secondary structure and ultimately allows a regular organization of the rodlike peptide blocks. The block length required to allow such a transition varies from ∼40 repeat units for the PS_{10}-*b*-$PBLG_n$ diblock oligomers to ∼80 α-amino acids for the PS_{10}-*b*-$PZLys_n$ series, reflecting the lower α-helix forming propensity of Z-Lys in comparison with Bn-Glu. Given the appropriate α-amino acid and peptide block length, diblock oligomers were obtained, whose supramolecular organization could be changed from a double hexagonal (PS_{10}-*b*-$PBLG_{20}$) or a columnar hexagonal (PS_{10}-*b*-$PBLG_{40}$, PS_{10}-*b*-$PZLys_{80}$) to a lamellar *β*-sheet morphology by increasing the temperature.

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