

A molecular brush with thermoresponsive poly(2-ethyl-2-oxazoline) side chains: A structural investigation

Jia-Jhen Kang,¹ Kaltrina Shehu,^{1,§} Clemens Sachse,² Florian A. Jung,¹ Chia-Hsin Ko,¹

Lester C. Barnsley,^{3,4} Rainer Jordan² and Christine M. Papadakis^{1}*

¹Fachgebiet Physik weicher Materie, Physik-Department, Technische Universität München,
James-Franck-Straße 1, 85748 Garching, Germany

²Professur für Makromolekulare Chemie, Fakultät Chemie und Lebensmittelchemie, Technische
Universität Dresden, Mommsenstraße 4, 01069 Dresden, Germany

³Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum,
Forschungszentrum Jülich GmbH, Lichtenbergstraße 1, 85748 Garching, Germany

⁴Australian Synchrotron, ANSTO, 800 Blackburn Road, Clayton 3168, Australia

Current affiliations:

[§]Heinz-Maier-Leibnitz-Zentrum (MLZ), Technische Universität München, Lichtenbergstr. 1,
85748 Garching, Germany,

*Christine M. Papadakis: papadakis@tum.de, ORCID iD [0000-0002-7098-3458](https://orcid.org/0000-0002-7098-3458)

ABSTRACT

The thermoresponsive behavior of a poly(2-oxazoline)-based molecular brush is investigated in aqueous solution. The molecular brush under study, $\text{PiPOx}_{100}\text{-g-PEtOx}_{17}$, has a poly(2-isopropenyl-2-oxazoline) (PiPOx) backbone grafted with thermoresponsive poly(2-ethyl-2-oxazoline) (PEtOx) side chains. Since the backbone degree of polymerization is only a factor of ~ 6 higher than the ones of the side chains, it features an architecture between a star-like polymer and a comb-like polymer. Its aqueous solution exhibits lower critical solution temperature (LCST) behavior with a cloud point temperature, $T_{\text{cp}} = 40.5\text{ }^{\circ}\text{C}$ at 30 g L^{-1} . The temperature-dependent structural evolution is disclosed using dynamic light scattering (DLS) and small-angle neutron scattering (SANS). An increase of the molecular brush size is found upon heating from room temperature to T_{cp} , which is attributed to the extension of the backbone resulting from the dehydration and collapse of the side chains. Above T_{cp} , the size decreases again, which indicates the collapse of the whole molecular brush. Large aggregates are found to be present in the solution in the temperature range $25 - 50\text{ }^{\circ}\text{C}$. These become more compact, as the temperature is increased across T_{cp} .

KEYWORDS: molecular brushes, thermoresponsive polymers, dynamic light scattering, small-angle neutron scattering

DECLARATIONS

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Introduction

Molecular brushes are densely grafted polymers having a rather rigid conformation due to the steric hindrance between the polymeric side chains on the backbone [1,2]. Their molecular structure can be adjusted by modifying the architectural parameters, e.g., the grafting density and the molecular weights of the backbone/side chains. Due to their versatility in structure along with the possibility to introduce a high number of functional segments, they are attractive candidates for various potential applications, such as carrier systems and photonic crystals [3-7].

The ratio between the backbone and the side chain lengths determines whether the molecular brush is of spherical-like or rod-like shape [8-11]. When the side chains are much longer than the backbone, the molecular brush has an architecture close to the one of a star-like polymer and therefore assumes a spherical shape. On the other hand, when the backbone is significantly longer than the side chains, its architecture is close to a bottlebrush.

It is of interest to study the effect of the conformational changes of stimuli-responsive side chains on the overall brush conformation. For instance, side chains from polymers that exhibit lower critical solution temperature (LCST) behavior in aqueous solution are expected to show strong effects. LCST-type polymers are water-soluble at low temperatures and become more and more hydrophobic, as the temperature is increased. *At the cloud point temperature T_{cp} , the polymers dehydrate, and the chains collapse, which is referred to as the coil-to-globule transition [12,13]. Except for extremely dilute solutions, the collapse of the polymer chains is followed by the formation of aggregates, leading to an increased opacity of the solutions.*

Aqueous solutions of molecular brushes, whose backbones are grafted with LCST-type polymers, also feature LCST behavior. The resulting structural evolution of the molecular brush upon heating depends largely on its architecture [14]. Li et al. investigated the thermoresponsive

behavior of a molecular brush with short backbone and long side chains (degrees of polymerization of the backbone and the side chains of $N_{bb} = 33$ and $N_{sc} = 73$), where the side chains are the LCST-type poly(*N*-isopropylacrylamide) (PNIPAM) [15]. Using small-angle neutron scattering (SANS) in a temperature range from 10 °C to around T_{cp} of PNIPAM (~32 °C), a gradual decrease of the size upon heating was found up to 30 °C, which was attributed to the collapse of the side chains. Above T_{cp} , large aggregates form in the solution. Another study considers molecular brushes which have a backbone that is significantly longer than the PNIPAM side chains ($N_{bb} = 558$, $N_{sc} = 13$) [16]. Dynamic light scattering (DLS) reveals a slight decrease of the hydrodynamic radius from 20 °C to 31 °C, followed by a sharp drop at 32 °C. Under atomic force microscopy, a cylinder-to-globule transition of the brush conformation was observed. This overall collapse was attributed to the enhanced intra-molecular interactions between the side chains upon heating; therefore, as the side chains collapse and attract each other, the backbone coils up, resulting in the globular shape of molecular brush at 32 °C.

Poly(2-alkyl-2-oxazoline)s with short alkyl substituents are another class of thermoresponsive LCST-type polymers, which have attracted attention in biomedical applications because of their biocompatibility and their tunable thermoresponsivity [17-19]. When the substituent is an ethyl group, the polymer, poly(2-ethyl-2-oxazoline) (PEtOx), has a cloud point of $T_{cp} \cong 61 - 70$ °C, depending on the molar mass and the solution concentration [20,21]. Furthermore, short PEtOx chains (with degrees of polymerization below 100) do not exhibit LCST behavior in the range 0 to 100 °C [22,23]. Nevertheless, several studies showed that star-like or comb-like polymers with short PEtOx arms ($N_{sc} < 100$) display LCST behavior [24,25,10,26]. Structural studies were carried out around the cloud points of these molecular brushes with PEtOx side chains using SANS and/or DLS [10,26]. Different dependences of the molecular size on temperature were found: With the

grafting density (fraction of backbone monomers which are attached with a side chain) varying from 70% to 100%, the different N_{bb} to N_{sc} ratios were considered to be the main reason for the size dependence on temperature around T_{cp} . Besides, coexisting individually dissolved brushes and large aggregates were detected from room temperature to T_{cp} in this system.

In the present study, a molecular brush having a poly(2-isopropenyl-2-oxazoline) backbone and PEtOx side chains is investigated in aqueous solution. Its backbone and side chain lengths are $N_{bb} = 100$ and $N_{sc} = 17$; thus, their ratio lies between the ones described above. [At room temperature, the molecular brush is soluble in water.](#) The focus is on the conformational changes of the brushes around T_{cp} and the presence of aggregates. The manuscript is structured as follows: Firstly, the cloud point T_{cp} of the solution is identified by turbidimetry; subsequently, the temperature-dependence of the hydrodynamic radius is investigated by DLS, and finally, the inner structures of the molecular brushes and their aggregates are resolved by SANS.

Materials and Methods

Materials. The molecular brush under study, $PiPOx_{100}$ -g- $PEtOx_{17}$ -NBocPip, is poly(2-oxazoline)-based, featuring a poly(2-isopropenyl-2-oxazoline) (PiPOx) backbone and poly(2-ethyl-2-oxazoline) (PEtOx) side chains, which are end-capped with *tert*-butyloxycarbonyl groups (NBocPip) in order to facilitate the polymer characterization. The chemical structure is shown in Fig. 1a below. For simplicity, it is called $PiPOx_{100}$ -g- $PEtOx_{17}$ in the following. $PiPOx_{100}$ -g- $PEtOx_{17}$ has a high grafting density, i.e. there is a side chain attached on almost every monomer of the polymeric backbone. The number-average molar mass, M_n , is 132 kg mol^{-1} and the polydispersity, \mathcal{D} , is 1.13, as determined by size exclusion chromatography (SEC), see below. The synthesis of $PiPOx_{100}$ -g- $PEtOx_{17}$ was performed according to a modified method of previous

reports [27]. The fully extended length of the PiPOx backbone and the PEtOx side chains of the molecular brushes, are estimated by summing up the monomer length considering the C-C bond length (0.154 nm), the C-N bond length (0.147 nm) and the bond angle (109.5°) [28], to be 25 nm and 6 nm, respectively. Thus, the largest possible length in the longitudinal direction of the brush is the one of the fully stretched backbone plus two times that of the side chains, namely 37 nm.

PiPOx₁₀₀. To 150 mL tetrahydrofurane, 3 mL iPOx (3.18 g, 28.6 mmol, 3 % v/v) were added, and the temperature was equilibrated at -20 °C. Polymerization was initiated by addition of 0.1 mL *n*-butyl lithium in hexane (0.25 mmol, 2.5 M). After 30 min, the reaction was terminated with argon saturated methanol (1.00 ml). Then, the solvent volume was reduced, diluted with chloroform, precipitated from diethyl ether three times and lyophilized from water to obtain white polymer (2.8 g, 88%). ¹H NMR (400 MHz, CDCl₃) δ 4.26 – 4.02 (m, 2H), 3.89 – 3.64 (m, 2H), 2.0 – 1.62 (m, 2H), 1.57 – 0.91 (m, 3H). FTIR: 2942 cm⁻¹ (ν_{as} C-H, m), 1652 cm⁻¹ (ν C=N, vs), 1120 cm⁻¹ (ν_{as} C-O, s), 983-896 cm⁻¹ (oxazoline ring skeleton vibration, s). SEC (RI): *M*_n = 11.1 kg mol⁻¹, Đ = 1.14.

PiPOxOTf₁₀₀. To a stirring solution of 215 mg PiPOx (1.93 mmol, 1 eq.) in 5.8 ml acetonitrile, 314 mg methyl triflate (2.08 mmol, 1.1 eq.) were added at room temperature and stirred for 16 h. The solvent was evaporated, the resulting yellowish solid was dissolved in dry acetonitrile and precipitated twice from dry diethyl ether. After evaporation of remaining solvent, the initiator salt was obtained as yellowish solid (520 mg, 94 %) and processed as soon as possible. Reaction and purification were performed under dry and inert conditions using the *Schlenk*-technique. ¹H-NMR (400 MHz, CD₃CN) δ 5.38 – 4.88 (m, 2H), 4.70 – 4.29 (m, 2H), 3.86 – 3.55 (m, 3H), 2.83 – 2.26 (m, 3H), 1.70 – 1.19 (m, 3H).

PiPO_{x100}-g-PEtO_{x17}-NBocPip. 502 mg PiPO_xOTf (1.90 mmol) was dissolved in 28 mL acetonitrile and the 2.87 mg EtOx (29.01 mmol, 15 eq.) were added. The mixture was stirred at 80°C overnight and cooled to room temperature. After termination with 2.5 mL *N*-Boc-piperazine (250 mg/mL in chloroform, 3.4 mmol, 1.8 eq.) for 24 h at room temperature, an excess of potassium carbonate was added for neutralization. After centrifugation, the polymer was dissolved in methanol/chloroform (3:1) and precipitated in diethyl ether three times. After lyophilization, the polymer was obtained as white solid (2.381 g, 69%). ¹H NMR (400 MHz, MeOD) δ 4.04 – 3.37 (m, 676H), 2.69 – 2.22 (m, 38H), 1.47 (s, 9H), 1.22 – 1.00 (m, 51H). SEC (RI): Đ=1.18, *M_n*=58.7 kg mol⁻¹. SEC (MALLS): Đ=1.14, *M_n* = 132 kg mol⁻¹. The refractive index increment *dn/dc* = 0.076 mol g⁻¹.

Size exclusion chromatography (SEC). SEC measurements were done on a system from Jasco (Groß-Umstadt, Germany) with a PU 2080 HPLC-pump, a JetStream II Plus column oven, equipped with one Gram 3000 8 × 300 mm and one Gram 30 8 × 300 mm column and dimethylacetamide (DMAc) with 5 g L⁻¹ LiBr and 1 vol% H₂O as the mobile phase at 70 °C. The system was calibrated with PMMA standards (PSS, Mainz, Germany). A Dawn DSP laser photometer at λ = 632.8 nm (Wyatt Technology, Dernbach, Germany) and an RI-930 RI detector (Jasco) were used for detection. Samples were dissolved in the mobile phase and filtered through 0.2 μm PTFE syringe filters prior to the measurement. Refractive index increments *dn/dc* were determined using a differential refractometer DR1/b from SLS Systemtechnik (Denzlingen, Germany) in the concentration range of 1 – 5 g L⁻¹ at a temperature of 35 °C.

Turbidimetry. Turbidity measurements were performed on a PiPOx₁₀₀-g-PEtOx₁₇ solution at 30 g L⁻¹ in H₂O, using a Lambda 35 UV/VIS spectrometer equipped with a PTP-1 Peltier System (all from PerkinElmer, Germany) and controlled using the Templab software provided by the instrument supplier. T_{cp} of the molecular brush solution was determined by spectrophotometric detection of the changes in transmission of the polymer solution at $\lambda = 500$ nm, and the transmission values were normalized to that of the sample solution at 25 °C. The heating/cooling rate was 1.0 °C min⁻¹. The given value of T_{cp} was determined as the temperature corresponding to a 10 % decrease in the optical transmission, and the uncertainty for T_{cp} is the temperature difference between two data points, 0.1 °C.

Dynamic Light Scattering (DLS). DLS measurements were conducted on a PiPOx₁₀₀-g-PEtOx₁₇ solution at 30 g L⁻¹ in D₂O. The D₂O solvent was filtered before use to prepare the polymer solution. A LS Spectrometer from LS Instruments (Fribourg, Switzerland) was used, which was equipped with a goniometer and two avalanche photodiode detectors in pseudo cross correlation. The light source was a polarized HeNe laser (Thorlabs, Dachau, Germany) with a maximum power 21 mW and a wavelength $\lambda = 632.8$ nm. The solution was filled in a cylindrical glass cuvette, having an outer diameter 5 mm and wall thickness 0.4 mm. The cuvette was mounted in a decalin bath, connected to a Julabo CF31 *Cryo*-Compact Circulator (JULABO, Seelbach, Germany) for temperature control. Data were collected during a temperature scan from 25 to 41 °C, i.e., up to T_{cp} of the solution, at a scattering angle $2\theta = 90^\circ$. At each temperature, the thermal equilibrium time was set to 10 min, and 20 measurements of a duration of 30 s were carried out. The scan procedure was repeated two times, and after the first time, the sample was left to equilibrate at 25 °C for 2.5 h. The normalized autocorrelation functions $g_2(q, \tau)$ ($q = 4\pi n \times \sin(\theta)/\lambda$ is the momentum

transfer, with n being the refractive index of the solvent, and τ is the delay time) were analyzed using the REPES algorithm implemented in the Gendist software [29,30]. It yields the distribution of hydrodynamic radii $A(R_h)$, which is given in equal-area representation $R_h A(R_h)$ vs. $\log(R_h)$. The R_h values were determined as the center of mass of each peak. *At this, we neglect possible small deviations from diffusive behavior which might be present for anisotropic particles.* The average value of R_h as well as the standard deviation were determined from the 20 measurements at each temperature.

Small-Angle Neutron Scattering (SANS). SANS experiments were conducted on a PiPOx_{100} -g- PEtOx_{17} solution at 30 g L^{-1} in D_2O . Measurements were performed at the KWS-1 instrument of the Jülich Centre for Neutron Science (JCNS) at the Heinz Maier-Leibnitz Zentrum (MLZ), Garching, Germany [31,32]. With the neutron wavelength $\lambda = 5 \text{ \AA}$ ($\Delta\lambda/\lambda = 10 \%$) and sample-to-detector distances (SDD) of 1.5 m, 8.0 m and 20 m, a q -range of $0.02 - 4.5 \text{ nm}^{-1}$ was covered ($q = 4\pi \times \sin(\theta)/\lambda$, where 2θ is the scattering angle). The exposure times at the 3 SDDs were 8 min, 15 min and 30 min, respectively. The sample holder was a quartz glass cuvette (Hellma Analytics) with 2 mm neutron path length, and the cuvette was mounted in a thermostat (Peltier) to control the temperature. Data were collected from $25 \text{ }^\circ\text{C}$ to $50 \text{ }^\circ\text{C}$, and the thermal equilibrium time at each temperature was 3 min. The scattered intensity was recorded by a scintillation detector with an active area of $60 \text{ cm} \times 60 \text{ cm}$ and a spatial resolution of $5.3 \text{ mm} \times 5.3 \text{ mm}$, and the intensity was calibrated to absolute value by plexiglass (poly(methyl methacrylate)). The intensity was corrected for the background from the solvent-filled cuvette, taking the transmissions into account. The such obtained intensity was azimuthally averaged to give plots of scattering intensity versus

momentum transfer q . All operations were carried out using the software QtiKWS provided by JCNS.

Model fitting of SANS data. The SANS data were modeled using the following expression:

$$I(q) = I_{agg}(q) + I_0 P_{mb}(q) S_{HS}(q) + I_{fluct}(q) + I_{bkg} \quad (1)$$

The scattering from large aggregates, $I_{agg}(q)$, was modeled by a [modified Porod term](#), which is given by [33]:

$$I_{Porod}(q) = \frac{I_P}{q^\alpha} \quad (2)$$

I_P is the scaling factor of the [modified Porod term](#). When the aggregates are mass fractals, the Porod exponent α ranges from 5/3 to 3, where $\alpha = 5/3$ indicates loosely connected aggregates and $\alpha = 3$ compact ones. When $3 < \alpha < 4$, the aggregates are compact and α contains information on their surface structure: $\alpha = 3$ is obtained for rough surfaces and $\alpha = 4$ for smooth surfaces [34].

The second term, $I_0 P_{mb}(q) S_{HS}(q)$, is the scattering contribution from the individual molecular brushes. I_0 is a scaling factor. As the form factor $P_{mb}(q)$, we chose the cylinder form factor, $P_{cyl}(q)$, which features the cross-sectional radius R as well as the length L of the cylindrical brushes [35-37]. R and L are distributed according to Gaussian distributions, and their polydispersities are treated as free parameters. The polydispersity is defined as σ/X_{avg} , where σ is the standard deviation and X_{avg} is the central position of the Gaussian distribution. The form factor reads:

$$P_{cyl}(q) = \int_0^{\pi/2} \left[\frac{J_1(qR \sin(a))}{qR \sin(a)} \frac{\sin(\frac{1}{2}qL \cos(a))}{\frac{1}{2}qL \cos(a)} \right]^2 \sin(a) da \quad (3)$$

where J_1 is the first order Bessel function, and a is the angle between the longitudinal axis of the cylinder and the scattering vector. The Percus-Yevick hard-sphere structure factor, $S_{HS}(q)$, was used to describe the spatial correlation between the brushes [38]:

$$S_{HS}(q) = \frac{1}{1+24\eta G(2R_{HS}q)/(2R_{HS}q)} \quad (4)$$

with

$$G(x) = \gamma \frac{\sin x - x \cos x}{x^2} + \delta \frac{2x \sin x + (2-x^2) \cos x - 2}{x^3} + \varepsilon \frac{-x^4 \cos x + 4(3x^2 - 6 \cos x + (x^3 - 6x) \sin x + 6)}{x^5} \quad (5)$$

where the functions γ , δ and ε are defined as:

$$\gamma = \frac{(1+2\eta)^2}{(1-\eta)^4}; \delta = \frac{-6\eta(1+\frac{\eta}{2})^2}{(1-\eta)^4}; \varepsilon = \frac{\gamma\eta}{2} \quad (6)$$

The average distance between the molecular brushes is twice the hard-sphere radius, R_{HS} . The volume fraction occupied by the correlated brushes is the hard-sphere volume fraction, η .

The Ornstein-Zernike structure factor was used to model the concentration fluctuations within the molecular brush at small length scales, $I_{fluct}(q)$ [39]. It reads:

$$I_{fluct}(q) = \frac{I_{oz}}{1+(q\xi)^2} \quad (7)$$

I_{oz} is the scaling factor and ξ the correlation length of the concentration fluctuations.

I_{bkg} is the incoherent background and was treated as a free parameter due to precipitation of the brushes at high temperatures. The scattering length densities (SLD) of the PiPOx backbone, the PEtOx side chains and the NBocPip end groups are calculated from the correspondingly mass densities to be $1.23 \times 10^{-6} \text{ \AA}^{-2}$ [40], $0.88 \times 10^{-6} \text{ \AA}^{-2}$ [41] and $0.74 \times 10^{-6} \text{ \AA}^{-2}$ [42], respectively. The software SasView 4.2.2 was used for model fitting [43], taking the smearing effects into account.

Results and Discussion

To investigate the influence of the thermoresponsive behavior of the PEtOx side chains on the phase behavior and the structure of the $PiPOx_{100}$ - g -PEtOx₁₇ molecular brushes (Figure 1a), turbidimetry, dynamic light scattering (DLS) and small-angle neutron scattering (SANS) measurements were carried out on aqueous solutions of the brushes in dependence on temperature. The polymer concentration was chosen at 30 g L⁻¹, either in H₂O (for turbidimetry) or in D₂O (for DLS and SANS). While turbidimetry indicates the cloud point, T_{cp} , DLS gives the temperature-dependent hydrodynamic radius of the molecular brushes and their aggregates. SANS reveals the shape, size and inner structure of the brushes as well as their correlation.

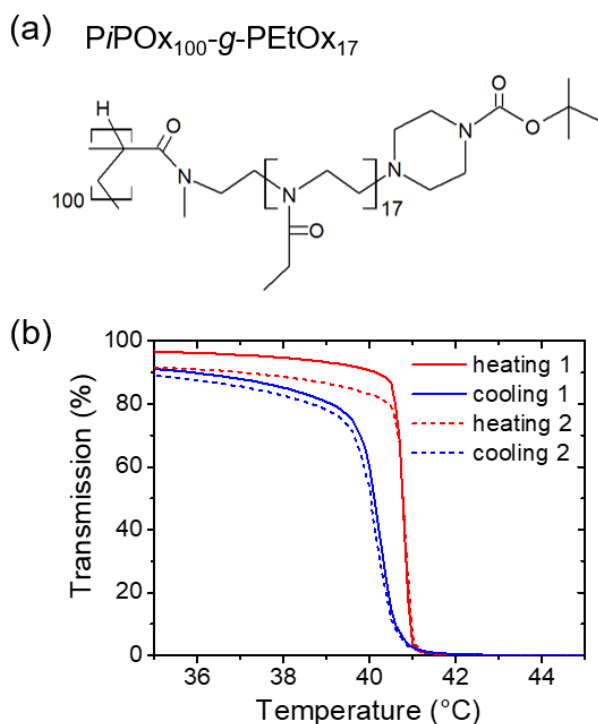


Figure 1. (a) Chemical structure of the molecular brush under study, $PiPOx_{100}$ - g -PEtOx₁₇. (b) Light transmission versus temperature of a 30 g L⁻¹ solution of $PiPOx_{100}$ - g -PEtOx₁₇ in H₂O, measured during two heating and cooling runs, as indicated.

The molecular brush under study consists of a water-soluble PiPOx backbone [44] and PEtOx side chains, which is a LCST-type polymer. Figure 1b shows the light transmission data from temperature-resolved turbidimetry measurements of a 30 g L⁻¹ PiPOx₁₀₀-g-PEtOx₁₇ solution in H₂O. During heating, the transmittance slightly decreases and shows a sharp drop at 41 °C, which is also recovered during the second heating run. The cloud point is determined to be $T_{cp} = 40.5 \pm 0.1$ °C as the temperature where the transmission is 90%. Upon cooling, a hysteresis of ca. 1 °C is observed, which is also reproducible.

Based on previous results [20,21], the short PEtOx side chains ($N_{sc} = 17$) are expected to have a very high cloud point ($T_{cp} > 100$ °C). The T_{cp} of the solution of densely grafted brushes is much lower, which may be due to the close proximity of the side chains [45]. In addition, the fact that the side chains are end-capped with the hydrophobic *tert*-butyloxycarbonyl group [46,47] may also be responsible for the lowered T_{cp} [15].

DLS measurements were carried out from 25 °C to 41 °C, i.e. in the one-phase state, where the solution is optically clear. The heating procedure was repeated twice with an equilibration time of 2.5 h at 25 °C before the second scan. In this temperature range, the normalized intensity autocorrelation curves feature two decays (Figure 2a,c), suggesting two kinds of diffusing particles in different sizes present in the solution. The fast mode has a rather high amplitude and is tentatively assigned to the diffusion of individual molecular brushes. The slow mode is rather weak and is presumably due to the diffusion of large aggregates. The corresponding distributions of hydrodynamic radii R_h (Figure 2b,d) feature two peaks, with the fast one being narrower than the slow one. From the areas under the fast and slow peaks, it is seen that 80 – 90 % of the scattering intensity stem from the fast mode. This implies that the individually dissolved brushes constitute the majority in the solution, and only few aggregates are present. The hydrodynamic radius of the

aggregates is found to range from several micrometers up to several tens of micrometers; however, this length scale is outside the detection range of DLS, thus, the hydrodynamic radius of the aggregates cannot be determined reliably.

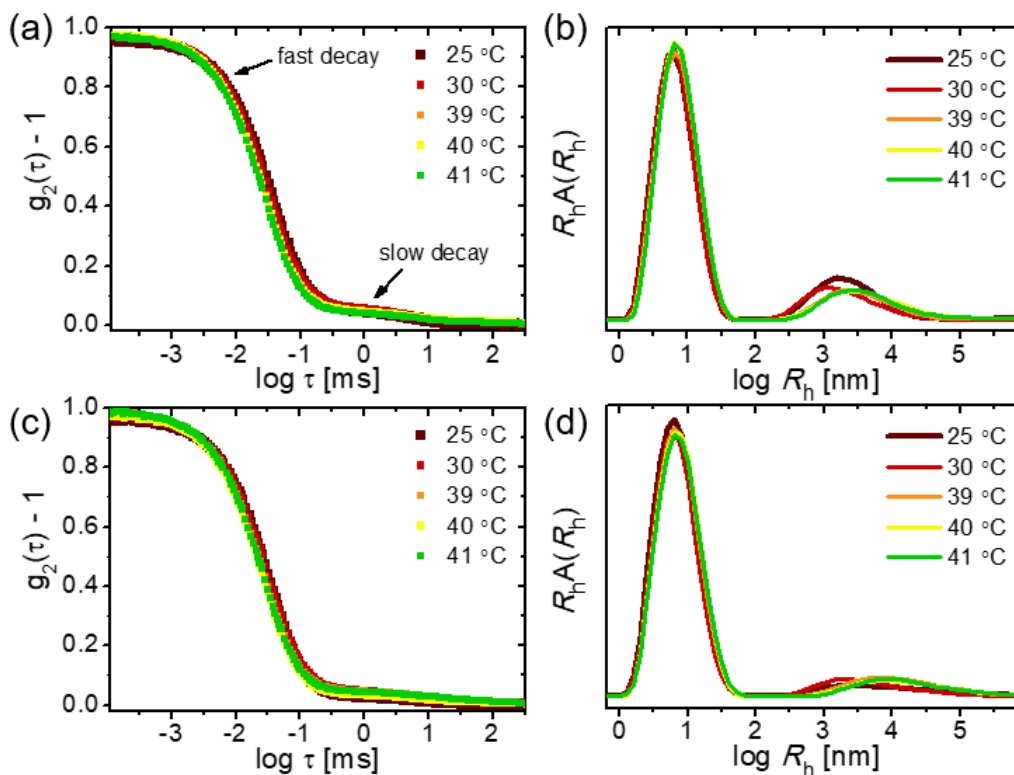


Figure 2. Representative DLS data at selected temperatures from the two heating scans of the 30 g L⁻¹ PiPOx₁₀₀-g-PEtOx₁₇ solution in D₂O. (a) and (c) are the normalized autocorrelation curves from the first and the second scan, respectively. (b) and (d) show the corresponding distribution functions of hydrodynamic radii, R_h , respectively. The temperatures are indicated in the graphs.

The R_h values of the individually dissolved molecular brushes are shown in dependence on temperature in Figure 3, where the results from the two scans are provided. Both scans show a slight increase of R_h from room temperature to T_{cp} , which might indicate a changing brush shape upon heating. As the molecular brushes are supposed to have a rod-like shape, the increase of R_h is most possibly due to an increase of size along the axial direction of the rod.

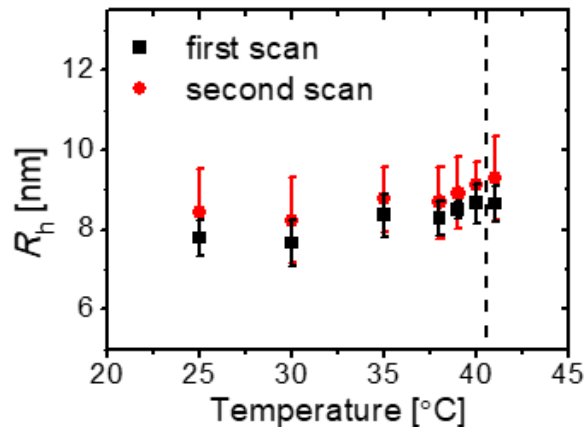


Figure 3. Hydrodynamic radius of the individually dissolved molecular brushes in dependence on temperature from the first (black squares) and the second (red circles) scan. The vertical dashed lines indicate T_{cp} of the solution, determined by turbidimetry.

Comparing R_h in the two scans, it increases from 7.8 ± 0.4 nm (25 °C) to 8.6 ± 0.4 nm (41 °C) in the first scan, and from 8.4 ± 1.1 nm (25 °C) to 9.3 ± 1.0 nm (41 °C) in the second scan (Figure 3). Thus, the thermoresponsive behavior of the individual molecular brushes is reversible within the uncertainties. Nevertheless, this minor inconsistency by ~ 0.5 nm and the higher uncertainties in the second scan might also imply that the solution would need longer than 2.5 h of equilibration at 25 °C to reverse back to the stable equilibrium state at this temperature after having been heated above its cloud point.

To gain information about the structural evolution of the molecular brushes and their aggregates, SANS data were collected in a wide q range and in the temperature range between 25 and 50 °C, i.e. during heating through T_{cp} . Figure 4 shows the scattering data in dependence on temperature. They have several features in common: At the lowest q values ($0.02 - 0.06$ nm⁻¹), the scattering intensity shows a decay, indicating the presence of aggregates in the solution. Comparing data below and above T_{cp} (Figure 4a and 4b), the q -dependence is steeper above T_{cp} . The scattering in

the mid- q region ($0.06 - 1 \text{ nm}^{-1}$) is due to the individually dissolved brushes, and the broad maximum at $q \cong 0.2 \text{ nm}^{-1}$ indicates that they are weakly correlated. Upon heating throughout the entire temperature range, the curves show only weak changes in the mid- q region; only the position of the maximum moves to lower q values up to T_{cp} and to higher q values above, which is an indication of a varying distance between the brushes in the solution. In the high q region ($1 - 5 \text{ nm}^{-1}$), a shallow decay is present, which is assumed to be due to concentration fluctuations within the molecular brushes. This decay remains unchanged with temperature. Above T_{cp} , a steady decrease of the overall scattering intensity is noticeable (Figure 4b), which is due to precipitation. At the end of the SANS measurement ($50 \text{ }^{\circ}\text{C}$), the precipitates are clearly visible in the cuvette. The amount of precipitation is estimated from the loss of the incoherent background. Based on our fitting results, the incoherent background drops from 0.0139 cm^{-1} at $40 \text{ }^{\circ}\text{C}$ to 0.0078 cm^{-1} at $50 \text{ }^{\circ}\text{C}$. Thus, approximately 44% of the polymers in the solution precipitate between 40 and $50 \text{ }^{\circ}\text{C}$. After storage at room temperature for a few weeks, the precipitates had re-dissolved, indicating the reversibility of the transition.

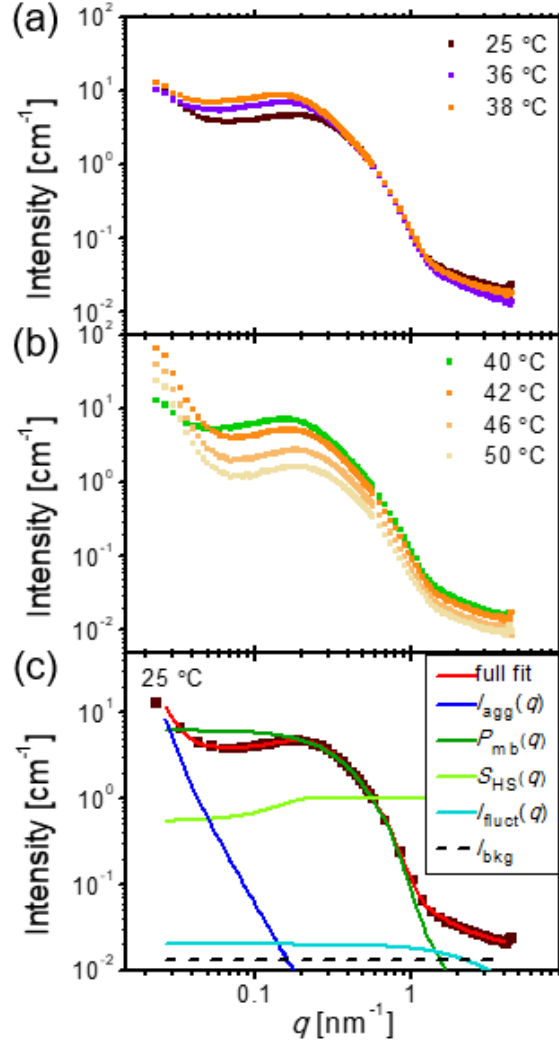


Figure 4. Representative SANS data of the 30 g L⁻¹ solution of PiPOx₁₀₀-g-PEtOx₁₇ in D₂O (a) from 25 °C to 38 °C and (b) from 40 °C to 50 °C. (c) Demonstration of a representative model fit to the SANS data at 25 °C using Eq. 1. For clarity, only every third data point is shown. The different contributions in Eq. 1 are indicated by different colors and line types.

Model fitting of the SANS data enables to extract detailed structural information on the molecular brushes as well as on their aggregates. The Porod law is used to describe the scattering from the aggregates at low q , giving information on their compactness and surface roughness. As for modeling the molecular brushes, judging from the backbone-to-side chain length ratio and the

results from previous studies [10,11,48], the shape of the brushes is most likely to be ellipsoidal or cylindrical. After unsuccessful initial fits of the form factor of ellipsoids, the form factor of cylinders having a polydisperse radius and length gave smooth fits. The flexible cylinder model was tried as well; however, it gave large Kuhn lengths, pointing to a high stiffness of the cylinders, and the model was discarded again. Using the above described model for straight and polydisperse cylinders, the dimensions of the cylinders, i.e., the cross-sectional radius R and the length L are determined. The cylinder form factor is combined with the Percus-Yevick hard-sphere structure factor, and the average distance between the brushes is deduced from the hard-sphere radius R_{HS} , along with the volume fraction occupied by the correlated brushes, η . The scattering due to the concentration fluctuations within the molecular brushes is described by the Ornstein-Zernike structure factor. Figure 4c shows the contributions to the model (Eq. 1) and the overall fit of the SANS data at 25 °C. Fits of the SANS data at the other temperatures as well as the full parameter sets are given in the Electronic Supplementary Material.

The molecular brushes are modeled by the cylinder form factor, and it is found that the cylinder length increases considerably with temperature, as T_{cp} is approached: Between 25 and 38 °C, it grows from ~13 to ~21 nm, which amounts to an increase of 60% of its original value (Figure 5a). In contrast, the cylinder radius stays constant at ~3 nm in this temperature range (Figure 5a). The cylinders thus grow in length when heating from 25 °C to 38 °C, which is in line with the DLS results described above. Upon further heating, the cylinder length decreases from ~21 nm at 38 °C to ~12 nm at 50 °C, while the radius stays unchanged at ~3 nm. Thus, from room temperature to T_{cp} , the brushes extend, whereas further above T_{cp} , the brushes contract. It seems, that they undergo a two-stage conformational change. The reason for the increase of the length of the molecular brushes may be that the PEtOx side chains dehydrate and shrink, resulting in a higher density near

the backbone [49], which leads to a straightening of the backbone of the brushes. The following decrease might indicate the further dehydration of the side chains, resulting in the collapse of the entire molecular brush. It is worth mentioning that this conformational change is not reflected in a change of the cross-sectional radius R , which is probably due to the spatial restrictions in the lateral direction of the brush. A constant cross-sectional radius was also reported in other studies [50]. As for the scaling factor I_0 of the cylinder form factor (Figure 5b), it is unchanged from room temperature to 38 °C, and shows a steady decrease above 38 °C, which is tentatively attributed to the decrease in the number of dissolved molecular brushes in the solution. It could be that, above 38 °C, these brushes become a part of the aggregates, resulting in a decrease of I_0 .

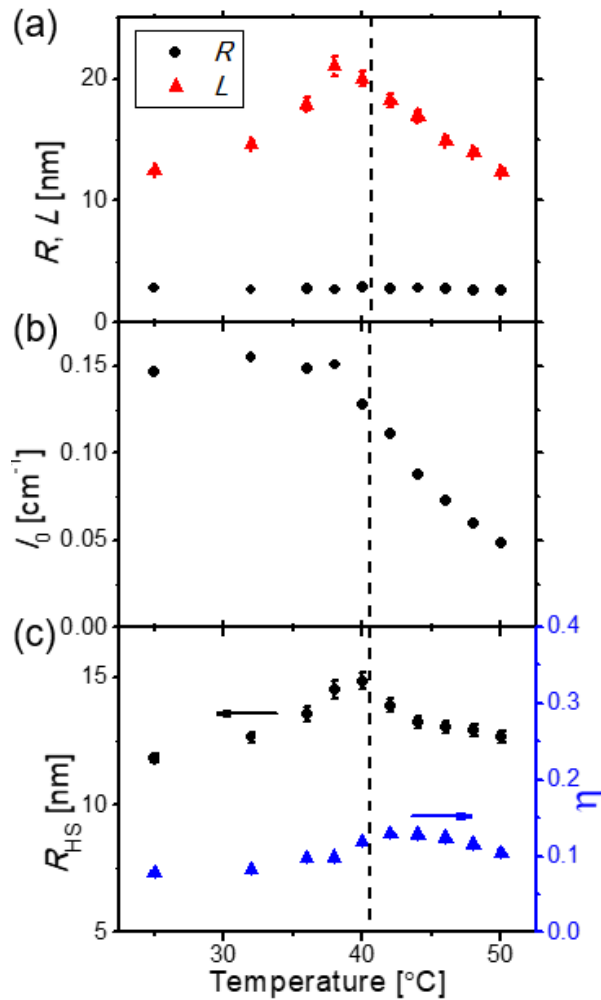


Figure 5. Parameters from model fitting to the SANS data of the 30 g L⁻¹ solution of PiPOx₁₀₀-g-PEtOx₁₇ in D₂O in dependence on temperature. (a) Dimensions from the cylinder form factor: radius R (black circles) and length L (red triangles). (b) Scaling factor of the cylinder form factor I_0 . (c) Parameters from the hard-sphere structure factor: hard-sphere radius R_{HS} (black circles, left scale) and volume fraction η (blue triangles, right scale). The vertical dashed line indicates T_{cp} of the solution.

A rough idea about the PEtOx side chain length may be gained from the cylinder radius, namely ~3 nm at all temperatures. The PiPOx backbone length can be estimated by the cylinder length minus two times that of the side chains, which gives ~15 nm at 38 °C at its most stretched conformation. Comparing the cylinder length and the radius from model fitting (Figure 5a) with the values calculated for the fully stretched backbone and side chains (see the Experimental Section), we conclude that neither the backbone nor the side chains are fully stretched. This holds true even at 38 °C, where the brushes are most extended. This finding is consistent with previous findings [48].

The hard-sphere radius R_{HS} has a value of ~12 nm at 25 °C and gradually increases to ~15 nm upon heating up to 40 °C (Figure 5c). From 40 °C to 50 °C, R_{HS} decreases to ~13 nm. This dependence on temperature corresponds to the one of the cylinder length very well, however, the average distance, $2 R_{HS}$, is always much larger than L ; thus, the brushes are well-separated from each other. Meanwhile, the hard-sphere volume fraction η , i.e. the volume fraction of the correlated brushes, increases from 0.08 at 25 °C to 0.15 at 42 °C, implying a small enhancement of the correlation between the brushes upon heating. Above 42 °C, it steadily decreases to 0.1, which is attributed to the precipitation of the solution. In general, the low η values confirm the weak interaction between the brushes, which is consistent with their large distance.

The scattering features from the hard-sphere structure factor and the form factor, especially the cylinder length, cover a similar q -range (0.06 - 0.6 nm⁻¹) because of the similar length scales of R_{HS} and L . However, the uncertainties of both values are low (Figure 5a, c), and we are thus convinced that they can be determined reliably.

Scattering from large-scale inhomogeneities or aggregates is observed in the entire temperature range. Although the limited q range does not allow for characterization of their size, the Porod exponent α can be evaluated and gives information regarding the compactness of the aggregates. From 25 °C to 36 °C, α decreases slightly from 2.7 to 2.5, and increases abruptly to 3.1 at 38 °C (Figure 6a). It stays at a value of ca. 3.0 and increases above T_{cp} , reaching 3.5 at 50 °C. Thus, below T_{cp} , the aggregates are mass fractals, i.e. they contain voids, while above T_{cp} , they transform into compact aggregates with rough surfaces. Our results are in line with previous studies on other molecular brush systems, which report that aggregates are present; both below and above the cloud point of the solution [50-52,10]. We note that, in our system, not only the temperature-dependent properties of the PEtOx side chains play a role, but also the hydrophobic NBocPip end groups may promote aggregation. The scaling factor of the modified Porod term I_p increases as the temperature approaches 40 °C (Figure 6b), except for the value at 38 °C, however, the uncertainties are large. Considering the fact that, between 25 – 36 °C, there is no additional contribution from the individually dissolved brushes to the aggregates (Figure 5b), the increase of I_p is possibly due to some rearrangement of the aggregates into larger ones with looser structure, thus leading to a slight decrease in their compactness (Figure 6a). Above the cloud point, a drop of I_p is found, which is presumably due to the precipitation.

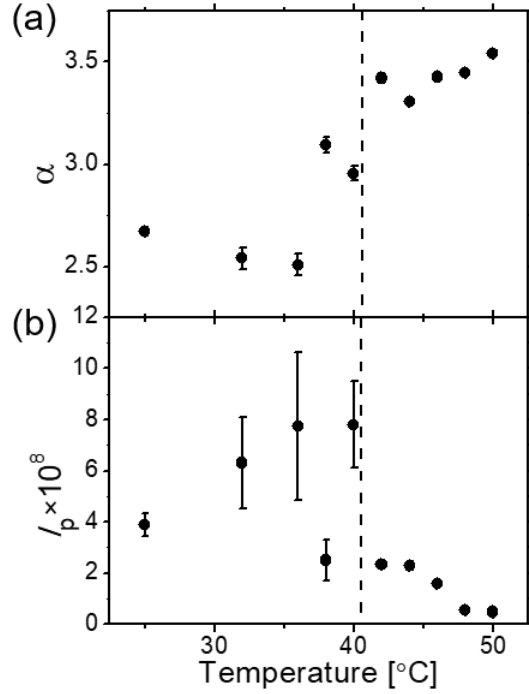


Figure 6. Parameters from the modified Porod term in SANS data of the 30 g L⁻¹ solution of PiPOx₁₀₀-g-PETox₁₇ in D₂O in dependence on temperature. (a) Porod exponent α . (b) Scaling factor I_p . The vertical dashed line indicates T_{cp} of the solution.

The Ornstein-Zernike structure factor describes the scattering from concentration fluctuations in the molecular brushes, most likely from the side chains. The correlation length ξ of these concentration fluctuations is 0.3 nm at 25 °C and increases to 1.6 nm at 40 °C, however, with large uncertainties (Figure 7a). The increasing ξ indicates the dehydration of the side chains as the temperature is increased [39]. Correspondingly, the scaling factor of the Ornstein-Zernike term, I_{oz} , increases sharply, as the temperature approaches the cloud point (Figure 7b), which is consistent with the increase of ξ . Above T_{cp} , the values are scattered, since the cylinder form factor overlaps with the Ornstein-Zernike contribution.

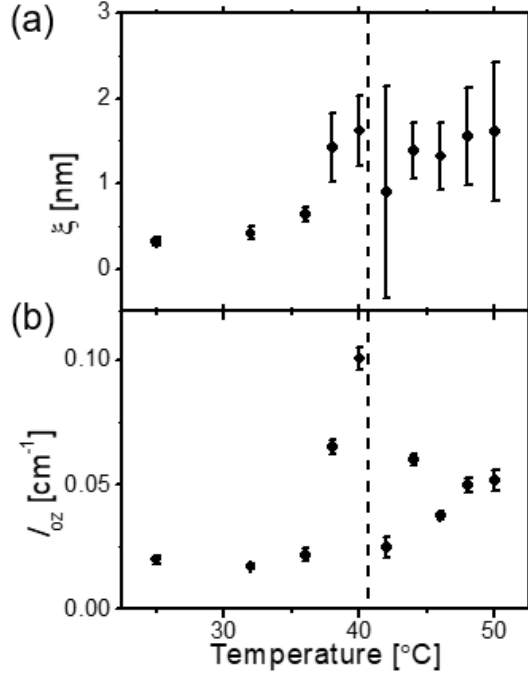


Figure 7. Parameters from the Ornstein-Zernike structure factor in SANS data of the 30 g L⁻¹ solution of PiPO_{x100}-g-PEtO_{x17} in D₂O in dependence on temperature. (a) Correlation length ξ . (b) Scaling factor I_{oz} . The vertical dashed line indicates T_{cp} of the solution.

The proposed temperature-dependent structural changes of the brushes and the aggregates are schematically shown in Figure 8. From room temperature to T_{cp} , the brushes stretch out due to dehydration of the side chains, which is reflected in the increased correlation length of the concentration fluctuations at small length scales. Above T_{cp} , the brushes collapse and shrink. Large aggregates, having sizes in the micrometer length scale, are present in the entire temperature range (from 25 °C to 50 °C, with $T_{cp} = 40.5$ °C). Below T_{cp} , the aggregates are loosely structured, while above T_{cp} , they are compact.

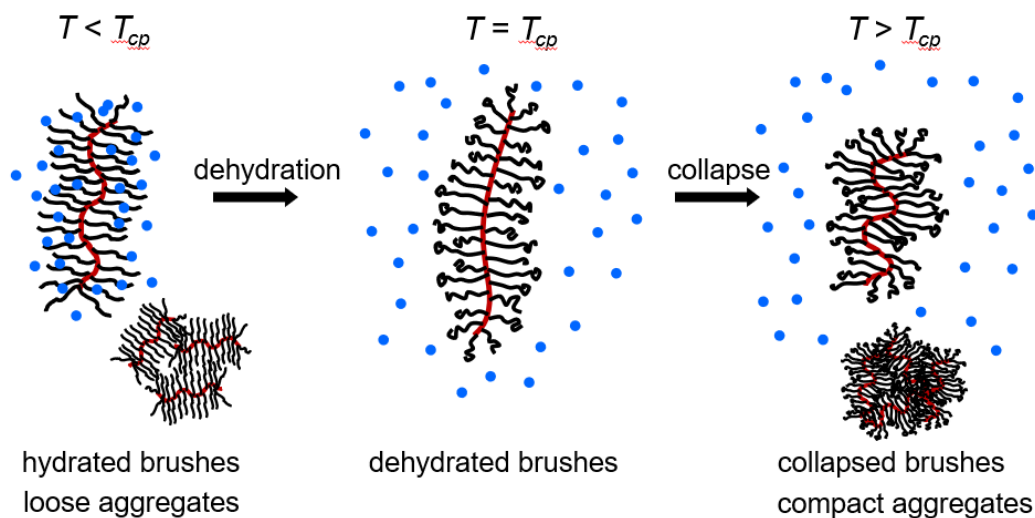


Figure 8. Schematic representation of the structural evolution of PiPOx₁₀₀-g-PEtOx₁₇ in aqueous solution. The arrows indicate the heating process. The blue dots represent the water molecules.

Conclusion

As evidenced by the experimental results from turbidimetry, DLS and SANS, the T_{cp} of the molecular brush with PEtOx side chains is 40.5 °C, thus significantly reduced compared to that of the oligomer PEtOx side chains ($T_{cp} > 100$ °C). The huge suppression is attributed to both, the dense grafting of the side chains to the backbone (virtually one side chain on every PiPOx backbone monomer) and the hydrophobic end groups on the side chains.

In the present study on a molecular brush with a specific ratio between the backbone length and the side chain length, a stretching of the molecular brush is discovered between room temperature and T_{cp} , before the brushes collapse upon further heating above T_{cp} . This two-step conformational change is attributed to the special molecular architecture, which is located between a star-like polymer and a bottlebrush polymer.

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