

## Thermoresponsive Polymers

Investigations of the Alignment Process of PBPLMG:  $^2\text{H}$  NMR Analysis Reveals a Thermoresponsive  $90^\circ$  Flip of the Polymer

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**Abstract:** The application of anisotropic parameters in NMR-spectroscopy enables the acquisition of spatial and angular information, complementary to those from conventional isotropic NMR-measurements. The use of alignment media is a well-established method for inducing anisotropy. PBPLMG is a recently discovered polyglutamate-based alignment medium, exhibiting thermoresponsive behavior in the lyotropic liquid crystalline (LLC) phase, thus offering potential for deeper understanding of the alignment process. We present one approach for investigating the thermoresponsive behavior by synthesizing specifically deuterated PBPLMG-isotopologues and their subsequent analyses using  $^2\text{H}$  NMR-spectroscopy. It was possible to relate the observed thermoresponsive behavior to a flip of the polymer with respect to the external magnetic field—an effect never observed before in glutamate-based polymeric alignment media. Furthermore, a solvent-induced temperature dependent gelation was verified in THF, which might provide yet another opportunity to manipulate the properties of this alignment medium in the future.

## Introduction

Anisotropy based NMR-techniques applied on structure elucidation of small organic compounds strongly rely on suitable alignment media.<sup>[1]</sup> Organic solvent tolerant homopolypeptide-based alignment media constitute one approach for inducing anisotropy with poly- $\gamma$ -benzyl-L-glutamate

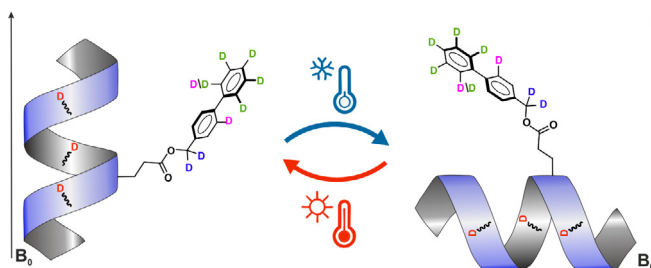
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(PBLG) being the most prominent representative.<sup>[2]</sup> We recently introduced poly- $\gamma$ -*p*-biphenylmethyl-L-glutamate (PBPLMG) as thermoresponsive alignment medium.<sup>[3]</sup> This  $\alpha$ -helical chiral polymer was first synthesized by Feijen et al.,<sup>[4]</sup> who conducted investigations towards  $\alpha$ -helix stability and viscosity behavior. It was concluded that the  $\alpha$ -helix of PBPLMG is less stable compared to PBLG. Additionally, concentration dependent maxima in viscosity were related to the “occurrence of association”. Green et al.<sup>[5]</sup> conducted investigations on the optical properties of PBPLMG, which indicate a thermoresponsive behavior, observable by a sudden change of the specific rotation in various helicogenic solvents as THF (273 K), notably excluding  $\text{CHCl}_3$ . Circular dichroism (CD) spectroscopy in THF and dynamic light scattering (DLS) measurements led to the assumption of supramolecular structures formed by spontaneous association of the polymer aggregates parallel to each other below certain temperatures, comparable to a phase transition.

Preliminary studies in our group<sup>[3]</sup> have revealed thermoresponsive behavior present in  $^2\text{H}$  NMR spectra of PBPLMG-based LLC phases, which is visible as a sudden change in the quadrupolar splitting  $\Delta\nu_{\text{O}}$  of the deuterated solvent ( $\text{CDCl}_3$  and  $\text{THF-}d_6$ ) at the response temperature  $T_{\text{R}}$ . Quadrupolar splittings  $\Delta\nu_{\text{O}}$  obtained from these solvents in PBPLMG-based LLC phases show a change of the size and sign of the splitting by the factor  $-1/2$  at  $T_{\text{R}}$ .

$$\Delta\nu_{\text{O}} = C_{\text{O}} \left\langle \frac{3 \cos^2(\theta_{\text{CD}}) - 1}{2} \right\rangle \quad (1)$$

As can be seen from Equation (1), where  $C_{\text{O}}$  is the quadrupolar coupling constant and  $\theta_{\text{CD}}$  is the angle between the carbon—deuterium-bond vector and the magnetic field  $\vec{B}_0$ , the factor  $-1/2$  indicates an orientation change of  $90^\circ$  with



**Figure 1.** Deuterated derivatives of PBPLMG shall shed light on the question if they undergo a reversible  $90^\circ$  flip with respect to the magnetic field  $\vec{B}_0$  at the response temperature  $T_{\text{R}}$ . One possible orientation of the PBPLMG-isotopologues is shown with directions of C-D bonds matching the signs of the quadrupolar splittings.

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respect to the external magnetic field  $\vec{B}_0$  ( $3 \cos^2(90^\circ) = 0$ ). It would be a very interesting finding, if this change in solvent orientation would also translate to the polymer. This is investigated within this manuscript (Figure 1). One approach to investigate the actual helix or sidechain conformation in homopolymer-based LLC phases dates back to the work of Abe et al.,<sup>[6]</sup> who were able to obtain information on the order parameter  $S$  and the sidechain conformation of the “parent polymer” PBLG in LLC phases via the application of deuterated PBLG-isotopologues and  $^2\text{H}$  NMR-spectroscopy. Extracted quadrupolar splittings  $\Delta\nu_Q$  were translated into angular information of the sidechain using rotational isomeric state (RIS) analysis.<sup>[7]</sup>

Thus PBPMGLG-isotopologues should yield information on the nature of the transition process observed. It is conceivable that this could include a  $90^\circ$  flip of the entire polymer or conformational changes in the sidechains, without affecting the orientation of the helical backbone.

A deuteration at specific positions of PBPMGLG can deliver the essential information needed to understand and ultimately utilize its thermoresponsive behavior.

To determine the influence of backbone and sidechain on the thermoresponsive behavior of PBPMGLG, different positions were labeled for subsequent investigations via  $^2\text{H}$  NMR-measurements. The backbone (red) needs to be labeled to provide a reference of the helix orientation with respect to  $\vec{B}_0$ . The biphenyl function, which is nonexistent in PBLG, might be responsible for the thermoresponsivity, as PBLG itself does not exhibit such behavior. Thus deuteration at the outer phenyl ring appears logical. To investigate the role of the biphenyl rotation on the alignment process and its influence

on the temperature dependence of PBPMGLG, a deuteration of the ortho positions (pink) of the biphenyl group was carried out.<sup>[8]</sup>

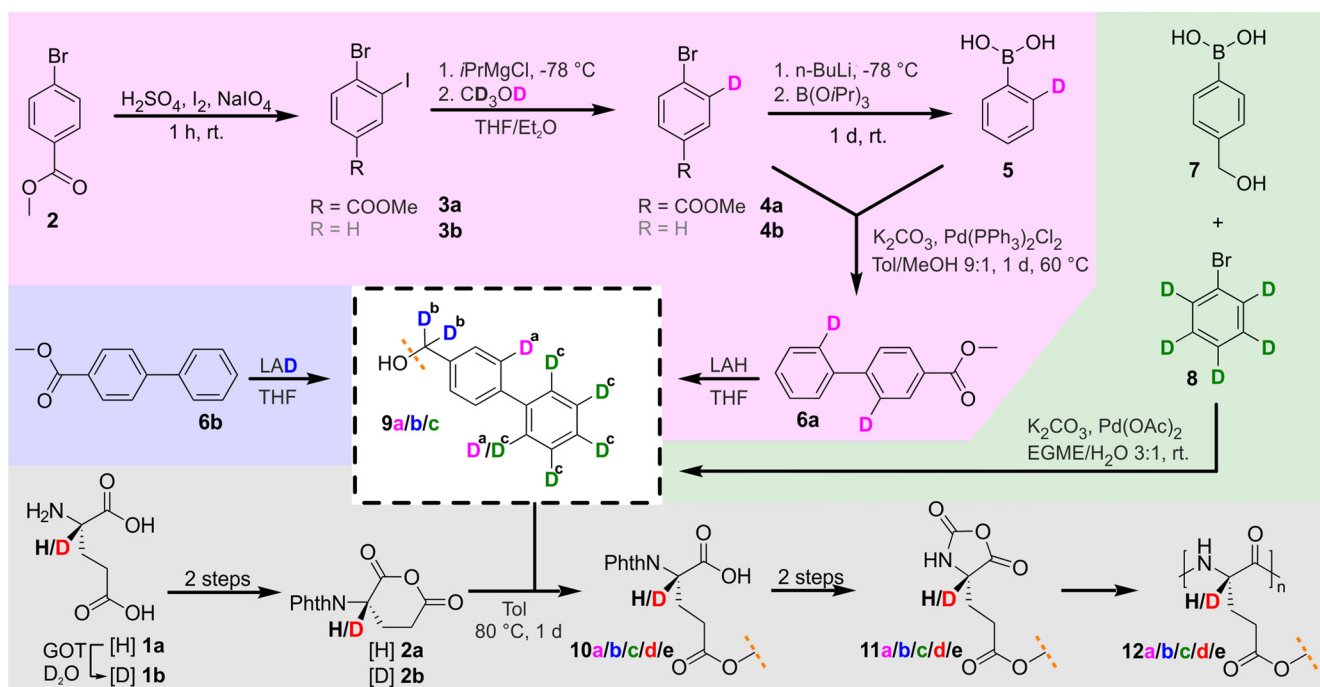
As these positions are absent in PBLG, labeling a function, which is present in both PBLG and PBPMGLG, allows for a comparison of the two LLC phases. Therefore, deuteration of the benzyl group (blue) is necessary.

## Results and Discussion

**Syntheses of PBPMGLG isotopologues:** PBPMGLG is synthesized via a six step protocol according to a literature known procedure<sup>[4]</sup> with minor modifications<sup>[3]</sup> (Scheme 1, gray). As the polymerization is sensitive to even minor impurities, the requirements for the purity of the employed monomer (*N*-carboxyanhydride, NCA **11**) are relatively high.<sup>[9]</sup> The utilization of diffusion-driven crystallization has shown to provide the NCA in acceptable yields and high purity.<sup>[10]</sup>

For the backbone labeled polymer PBPMGLG- $\alpha$ -*d* **12d** (red), interchanging the L-glutamic acid **1a** with L-glutamic-1-*d*-acid **1b** yields the desired product, causing only minimal alterations in the established synthesis protocol. The deuteration of L-glutamic acid at the  $\alpha$ -position was achieved using glutamic oxaloacetic transaminase (GOT) in  $\text{D}_2\text{O}$ , which results in a deuteration degree equal to the excess of  $\text{D}_2\text{O}$  compared to residual  $\text{H}_2\text{O}$  (HDO resp.) in the reaction mixture.<sup>[11]</sup>

Implementing isotopically labeled biphenyl methanol **9a/b/c** into the established synthesis route grants access to the



**Scheme 1.** General syntheses of PBPMGLG-isotopologues via deuterated intermediates. Pink: Synthesis route to PBPMGLG-ortho- $d_2$ ; Blue: Synthesis route to PBPMGLG-methylene- $d_2$ ; Green: Synthesis route to PBPMGLG-phenyl- $d_5$ ; Red: Synthesis route to PBPMGLG- $\alpha$ -*d*; Gray: Established synthesis protocol for PBPMGLG.

other polymers of interest. PBPMLG-methylene- $d_2$  **12b** (blue) was labeled through the use of lithium aluminum deuteride (LAD) in a reduction of the commercially available methyl 4-phenylbenzoate **6b**.

The synthesis of the isotopologue deuterated at the aromatic positions demands a different approach. It is synthesized from already deuterated phenyl precursors through cross coupling reactions. The method of choice for the synthesis of PBPMLG-phenyl- $d_5$  **12c** (green) was the SUZUKI coupling of bromobenzene- $d_5$  **8** with the corresponding boronic acid **7** using a procedure described by Del Zotto et al.<sup>[12]</sup>

The synthesis of PBPMLG-ortho- $d_2$  **12a** (pink) required additional reaction steps due to the necessary regioselective deuteration. Through oxidative monoiodination of **2**, iodine was introduced regioselectively at the corresponding position and subsequently deuterated via halogen-metal-exchange forming an aryl-magnesium-halide, which was treated with deuterated methanol to yield the desired product **4a**. This deuteration was carried out analogously with the commercially available 1-bromo-2-iodobenzene **3b**. The formation of the deuterium labeled phenylboronic acid **5** and subsequent SUZUKI coupling provides the corresponding biphenyl **6a**. After successful reduction with lithium aluminum hydride (LAH), the biphenyl methanol isotopologue **9a** was introduced into the general procedure to form PBPMLG-ortho- $d_2$  **12a**.

**Polymerization of NCAs isotopologues:** The ring opening polymerization of NCAs is a well-established method for synthesizing various polypeptides.<sup>[13]</sup> Triethylamine ( $\text{NEt}_3$ ) is a thoroughly investigated initiator and distinct for its convenient applicability in NCA-polymerization, however, leading to large mean molecular weights  $\bar{M}_n$  and polydispersities  $\bar{D}_M$ .<sup>[14]</sup> Dimethylethanolamine (DMEA) initiated polymerizations follow a different mechanism<sup>[15]</sup> (see SI), thus offering more control over polymer properties such as  $\bar{D}_M$  and  $\bar{M}_n$ .<sup>[16]</sup> As previous studies on PBPMLG were carried out with TEA-initiated polymer; naturally, we first synthesized PBPMLG isotopologues using the same initiator system.

The application of deuterium labeled polymer for the investigation of the thermoresponsive behavior of PBPMLG via  $^2\text{H}$  NMR-spectroscopy turned out to be inherently more difficult than using deuterated solvent molecules as probe. This is due to very fast  $T_2$  relaxation occurring in macromolecules, caused by their longer correlation time  $\tau_c$ . This slower molecular tumbling of macromolecules compared to for example, solvent molecules contributes to a rapidly decreasing FID.<sup>[17]</sup> Thus the smaller the  $\bar{M}_n$ , the less problematic for NMR-measurements. A broadening of lines is already observed in the polymers with deuterated aromatic moieties and is expected to get stronger, the closer the labeled site is located to the rigid polymer backbone. To overcome this challenge, a method to synthesize polymers with shorter chain lengths was utilized. Using DMEA as initiator was successful in that respect.

The LLC phases formed by the shorter and less polydisperse polymers show drastically reduced equilibration times (3 min. (DMEA) vs. 72 h<sup>[3]</sup> (TEA)) in the magnetic field, which is likely due to their extraordinary high fluidity,

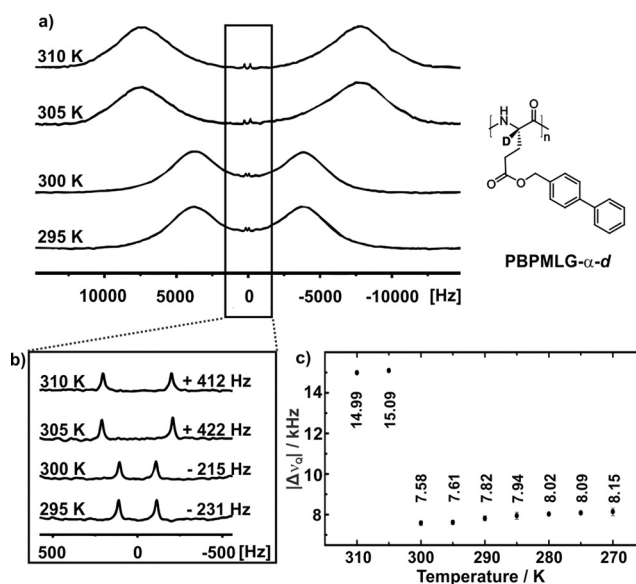
comparable to pure solvent (see SI). Furthermore, the phase stability at lower temperatures and the solubility was found to greatly increase due to the higher fluidity of the LLC samples.

**NMR investigations on LLC phases:** LLC phases of the synthesized PBPMLG-isotopologues were investigated with  $^2\text{H}$  NMR-spectroscopy to relate the temperature dependent change in quadrupolar splitting of the solvent observed previously to a change in the polymer orientation, its helical backbone conformation or the dynamics of the biphenyl mesogene in its sidechain.

**PBPMLG- $\alpha$ - $d$ :** To investigate if the thermoresponsive behavior and the resulting reorientation affects the entire polymer and to get access to a reference for the order parameter with respect to  $\vec{B}_0$ , deuteration in the  $\alpha$ -position of the polymer backbone was carried out.

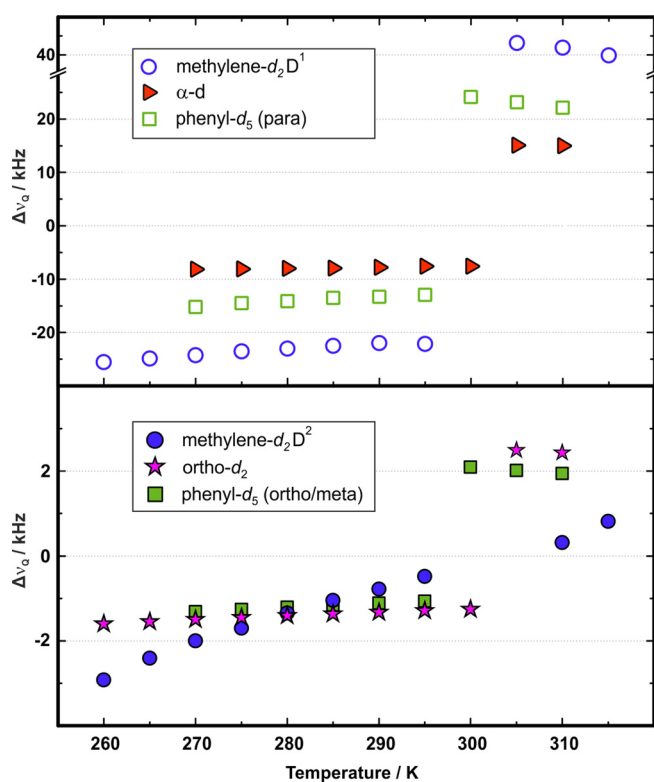
Figure 2 shows temperature dependent  $^2\text{H}$  NMR spectra of PBPMLG- $\alpha$ - $d$  in  $\text{CHCl}_3$ . The thermoresponsive behavior at the corresponding response temperature is clearly visible, proving the effective  $90^\circ$  reorientation of the polymer backbone in the magnetic field  $\vec{B}_0$  between 300 and 305 K. Furthermore, the natural abundance deuterium (NAD)<sup>[18]</sup> signal of the solvent chloroform is also visible (see SI for full spectra). This demonstrates that the solvent of the LLC phase and PBPMLG- $\alpha$ - $d$  display the same response temperature  $T_R$  and the same factor in the change of the quadrupolar splitting  $\Delta\nu_Q$ . It should be noted that the signs of the quadrupolar splittings of PBPMLG- $\alpha$ - $d$  could not be measured due to low sensitivity, while the signs of  $\text{CDCl}_3$  in LLC phases of PBPMLG are well documented and obtained from Q.E.CO-SY.<sup>[3,19]</sup>

**PBPMLG-phenyl- $d_5$ :** A restriction of the biphenyl rotational axis as a function of temperature would give atropisomeric conformations and result in the appearance of addi-



**Figure 2.** a)  $^2\text{H}$  NMR spectra of PBPMLG- $\alpha$ - $d$  in  $\text{CHCl}_3$  (16.6% w/w, DMEA initiated, 92 MHz  $^2\text{H}$  frequency) at various temperatures. b) NAD signal of the solvent chloroform with the corresponding quadrupolar splitting. c) Graphical display of the quadrupolar splitting  $\Delta\nu_Q$  of PBPMLG- $\alpha$ - $d$  at various temperatures.

tional signals below the coalescence temperature  $T_c$  in an anisotropic chiral environment (see SI for description of expectations and a comparison with the parent alcohol). Figure 3 shows the extracted quadrupolar splittings  $\Delta\nu_Q$  from the temperature dependent  $^2\text{H}$  NMR spectra of all polymers.

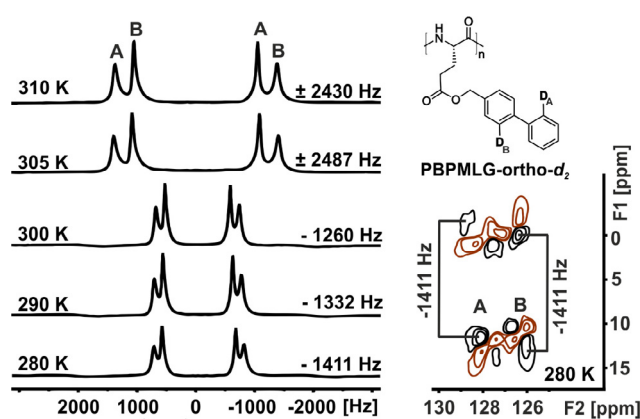


**Figure 3.** Graphical display of the quadrupolar splitting  $\Delta\nu_Q$  of all synthesized PBPMGLG-isotopologues at various temperatures. As only the signs of PBPMGLG-phenyl- $d_5$  (see SI) in both regimes and PBPMGLG-ortho- $d_2$  (below  $T_R$ ) could be measured, the signs of all other polymers are assumed to be negative below  $T_R$ .

For PBPMGLG-phenyl- $d_5$ , ortho and meta deuterons overlap in the  $^2\text{H}$  NMR spectra as observed previously for PBLG.<sup>[20]</sup> Again, the factor of  $-1/2$  is obtained for these isotopologues as well as for the NAD signals of the solvent. A restriction of the biphenyl rotational axis as a function of temperature was ruled out, as no coalescence appears in the spectra, nor are there any observable additional signals below the response temperature (see SI).

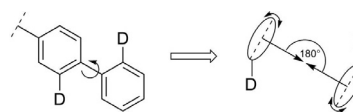
**PBPMGLG-ortho- $d_2$ :** As PBPMGLG-phenyl- $d_5$  was not conclusive concerning the reasons for the reorientation of the polymer and only provides information on the outer phenyl ring, PBPMGLG-ortho- $d_2$  was envisioned to yield information on the relation of both aromatic rings and a possible difference in dynamics. Temperature dependent  $^2\text{H}$  NMR spectra of PBPMGLG-ortho- $d_2$  are displayed in Figure 4.

The deuterons  $D_A$  and  $D_B$  are inherently chemically inequivalent, thus in anisotropic media two doublets are obtained as expected. At first glance, assignment of the signals seems straightforward. Based on the different line broadening the assignment as two doublets with a difference



**Figure 4.** Left:  $^2\text{H}$  NMR spectra of PBPMGLG-ortho- $d_2$  in  $\text{CHCl}_3$  (15.5%  $w/w$ , DMEA initiated, 92 MHz  $^2\text{H}$  frequency) at various temperatures with the corresponding quadrupolar splitting  $\Delta\nu_Q$ . Right: Exemplary Q.E.COSY spectrum (107 MHz  $^2\text{H}$  frequency) at 280 K for the sign determination of the quadrupolar splitting.

in quadrupolar splitting (assignment BAAB) is tempting. As both C-D-bonds point into the same direction (see Scheme 2, following Equation (1) with  $\cos^2(\theta_{CD}) = \cos^2(\theta_{CD} + 180^\circ)$ , the quadrupolar splittings of both deuterons are expected to be identical for identical averaging. The putative different splittings (assignment BAAB) would thus translate into different averaging of the aromatic rings. This would indicate different averaging processes for the two rings.



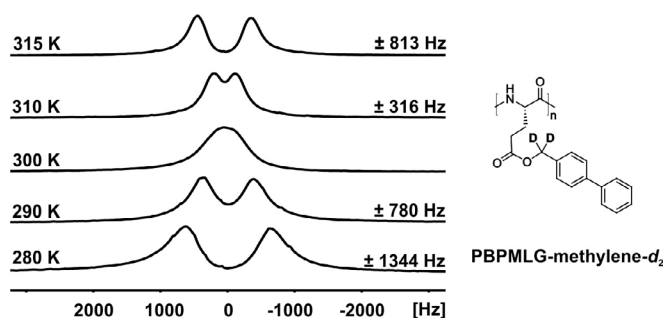
**Scheme 2.** Rotation of the biphenyl-function of PBPMGLG-ortho- $d_2$  and the deuterium-carbon-bond vectors resulting from the rotational averaging process.

When considering the geometry (Scheme 2) and the Q.E.COSY spectrum (Figure 4) the signs of the quadrupolar splittings must be identical and it is thus revealed that two doublets with similar quadrupolar splitting (correct assignment ABAB) are present. This indeed confirms identical averaging.

At the response temperature  $T_R$ , the factor  $-1/2$  reoccurs. Thus the thermoresponsive behavior is unaffected by the rotational axis of the biphenyl-function and confirms free rotation in both aromatic rings (for further considerations on quadrupolar splitting pattern, see SI).

**PBPMGLG-methylene- $d_2$ :** To gain further insights into the dynamics of the sidechain, temperature dependent  $^2\text{H}$  NMR-measurements of PBPMGLG-methylene- $d_2$  were conducted (Figure 5). Here, only one of the diastereotopic deuterons is displayed as the other deuterium signal exhibits a much larger quadrupolar splitting (see SI). Interestingly, the  $^2\text{H}$  NMR spectra do not show a sudden thermoresponsive behavior, but rather temperature dependent quadrupolar splittings over the total investigated temperature range. Due to broad signals and small quadrupolar splittings at the response temperature





**Figure 5.**  $^2\text{H}$  NMR spectra of PBPMGLG-methylene- $d_2$  in  $\text{CHCl}_3$  (16.0%  $w/w$ , DMEA initiated, 107 MHz  $^2\text{H}$  frequency) at various temperatures with the corresponding quadrupolar splitting  $\Delta\nu_Q$ .

range, a change of the quadrupolar splittings with the factor  $-1/2$  cannot be confirmed. This gradually decreasing behavior is identical to that observed for PBLG by Czarniecka and Samulski, who attributed this finding to a temperature dependent intramacromolecular reorientation of the side-chain, causing a significant temperature dependence of the quadrupolar splittings  $\Delta\nu_Q$  of the methylene deuterons.<sup>[21]</sup> The response temperature  $T_R$  can be identified roughly at 300 K from the NAD signal of the solvent thus confirming that the methylene- $d_2$  polymer behaves identically to the undeuterated polymer and the other isotopologues (see SI).

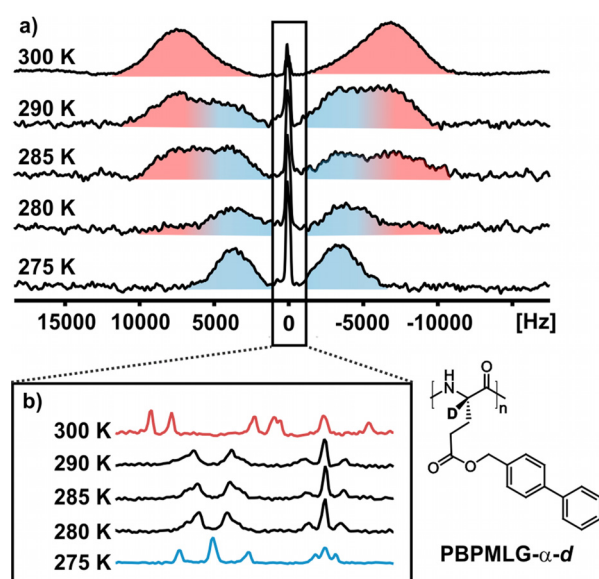
We believe that a change of the global dipole moment of the polypeptide, resulting from a change of the side chain conformation, could be the trigger for the reorientation process.<sup>[22]</sup> The diamagnetic susceptibility of the  $\alpha$ -helix competes with the diamagnetic susceptibility of the phenyl groups in the sidechain.<sup>[23]</sup> Above the response temperature  $T_R$  the diamagnetic susceptibility contribution of the  $\alpha$ -helix could override that of the phenyl groups, resulting in a polymer orientation parallel to  $\vec{B}_0$  as the energetically favored state. As the temperature is decreased, the flexibility of the sidechains is gradually reduced, possibly causing the overall diamagnetic susceptibility to become negative, which results in a perpendicular polymer orientation to  $\vec{B}_0$ . To get more insight into this hypothesis, we measured the induced magnetic moment of an LLC sample of PBPMGLG in  $\text{CHCl}_3$  and indeed observed a sudden change of the magnetic properties of the sample (see SI). This occurs at exactly the same temperature at which the reorientation process is observed in the  $^2\text{H}$  NMR spectra (see SI). Furthermore, thermo- and magneto-dependent polarized optical microscopy studies revealed a drastic change of the texture in the same temperature range, which confirms the observed rearrangement (see SI).

**PBPMGLG-isotopologues in THF:** The thermoresponsive change in the optical properties of PBPMGLG in solution — Green et al. were able to relate to the formation of aggregates — did not occur in  $\text{CHCl}_3$ . Yet, thermoresponsive behavior in LLC phases containing  $\text{CDCl}_3$  as solvent is indeed present. Therefore, we concluded that the reasons for the thermoresponsive behavior in  $\text{CDCl}_3$  are not linked to aggregation. Thus, additional investigations on PBPMGLG in THF, the solvent in which Green et al. observed the change in optical properties, were carried out. As known from preliminary

studies,<sup>[3]</sup> which revealed outstanding enantiodifferentiation properties, the response temperature  $T_R$  for PBPMGLG in THF- $d_8$  is roughly 280 K for TEA-initiated polymers.

**PBPMGLG- $\alpha$ - $d$  in THF:** Figure 6 shows temperature dependent  $^2\text{H}$  NMR-measurements of PBPMGLG- $\alpha$ - $d$  in THF.

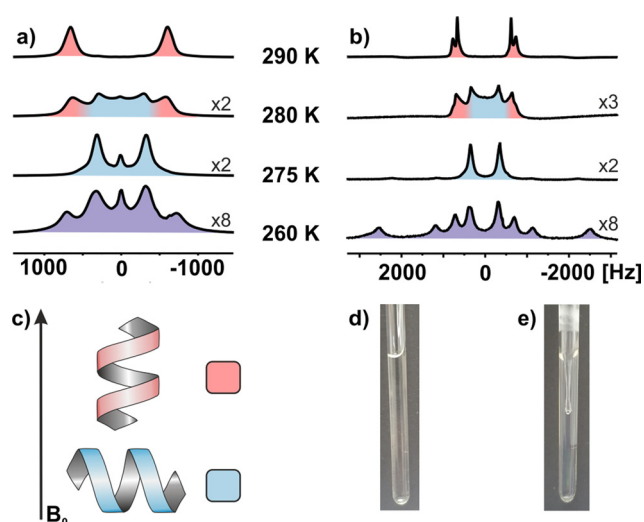
Similar to the  $^2\text{H}$  NMR spectra of PBPMGLG- $\alpha$ - $d$  recorded in chloroform, two doublets of different size for the  $\alpha$ -deuteron are observed at 300 and 275 K respectively. Again, the quadrupolar splittings of both doublets differ by a factor of  $1/2$  (or  $-1/2$ , which could not be proven, because sign determination is not possible). This observation, thus, suggests a  $90^\circ$  flip of the helix with respect to the magnetic field  $\vec{B}_0$  — which was observed in  $\text{CHCl}_3$  — hence, also takes place in THF-based LLC phases. The different states are separated by a transition area, in which both doublets are simultaneously present. Below 275 K the signals become exceedingly broad (for full temperature range see SI).



**Figure 6.** a)  $^2\text{H}$  NMR spectra of PBPMGLG- $\alpha$ - $d$  in THF (25.0%  $w/w$ , DMEA initiated, 92 MHz  $^2\text{H}$  frequency) at various temperatures. b) NAD signal of the solvent THF. Two states are visible; these are separated by a transition area (black): parallel orientation (red) and perpendicular (blue) orientation of the helix with respect to the magnetic field  $\vec{B}_0$ .

**PBPMGLG-ortho- $d_2$  and -phenyl- $d_5$  in THF:** Figure 7 shows temperature dependent  $^2\text{H}$  NMR-measurements of PBPMGLG-phenyl- $d_5$  and PBPMGLG-ortho- $d_2$  in THF. Again, parallel (red) and perpendicular (blue) orientations of the helix with respect to the magnetic field  $\vec{B}_0$  are obtained at 290 and 275 K, respectively, separated by a transition area of approximately 10 K.

Below 260 K additional signals appear in the obtained spectra accompanied by gelation of the previously fully fluid LLC phase. However, using THF- $d_8$  as analyte in PBPMGLG- $d_0$  does not lead to the formation of additional signals but significant line broadening at temperatures of 270 K and lower (see SI).



**Figure 7.** a)  $^2\text{H}$  NMR spectra of PBPMGLG-phenyl- $d_5$  in THF (25.0% w/w, DMEA initiated, 107 MHz  $^2\text{H}$  frequency) and b) PBPMGLG-ortho- $d_2$  (25.0% w/w, DMEA initiated, 92 MHz  $^2\text{H}$  frequency) at various temperatures. c) Helix orientations with respect to the magnetic field  $\vec{B}_0$ ; parallel (red) and perpendicular (blue). d) LLC phase of PBPMGLG in THF at room temperature and e) at approximately 250 K. Gelation is visible due to the formation of the vortex-like air filled area inside the sample.

PBLG is known to form aggregates.<sup>[24]</sup> To further investigate the role of THF, PBLG-based LLC phases in THF with biphenyl methanol- $d_5$  **9c** as analyte were prepared. Temperature dependent  $^2\text{H}$  NMR spectra clearly show a change of the quadrupolar splittings between 280 and 270 K, which is identical to the transition temperature  $T_R$  already observed for PBPMGLG phases investigated (see SI). To the best of our knowledge no such behavior is known for PBLG.

As we can exclude a helix flip of PBLG analogous to PBPMGLG (no sign change of  $\Delta\nu_Q$ ) and due to the absence of this behavior in  $\text{CHCl}_3$ , it can be concluded, that THF is involved in the behavior observed. Moreover, severe line broadening affects the aromatic signals of PBLG in  $^{13}\text{C}$  NMR spectra. This thus seems to be related to the thermoresponsive behavior observed (see SI). The same results are obtained for undeuterated PBPMGLG in THF- $d_8$ .

Polymer flip and gelation of PBPMGLG-based LLC phases in THF take place at the same temperature region (280–260 K), making it difficult to observe and ultimately investigate given effects separately. Sidechain modification as a tool to accomplish a separation of both effects and potentially use these thermoresponsive effects for the five-alignment approach (model free approach, MFA) is under current investigation.

## Conclusion

Successful synthesis of PBPMGLG-isotopologues and further optimization of the polymerization of the NCA-monomers allowed for investigations on the behavior of the corresponding LLC phases via  $^2\text{H}$  NMR-spectroscopy. We have shown that the abrupt reorientation of  $\text{CDCl}_3$  in LLC

phases with PBPMGLG as alignment medium, which results in a sudden change of the quadrupolar splitting  $\Delta\nu_Q$ , is driven by the reorientation of the entire polymer chain. The orientation of the  $\alpha$ -helix from parallel ( $> 300$  K) to perpendicular ( $< 300$  K) with respect to the applied magnetic field occurs without noticeable conformational changes in the backbone. Interestingly, sidechain reorientation is indicated by the temperature dependent quadrupolar splittings in PBPMGLG-methylene- $d_2$ , but is imperceptible in the biphenyl function and the polymer backbone.

The driving force for this thermoresponsive behavior is related to a change in induced magnetic moment of the LC phase as indicated by a sudden change in magnetic properties observed in VSM (vibrating-sample magnetometer) measurements, which most probably results from the subtle balance between the different contributions of the diamagnetic susceptibilities of the aromatic rings vs. the  $\alpha$ -helix. POM experiments further validate our interpretation of the NMR and VSM results. A thermoresponsive  $90^\circ$  reorientation of the entire polymer helix has never before been proven in glutamate-based alignment medium. This knowledge can now be used as design principle and will contribute to future design choices for novel alignment media.

Furthermore, investigations on PBPMGLG in THF have been conducted, showing a temperature dependent gelation of the LLC phase taking place in the same temperature range as the  $90^\circ$  reorientation of the polymer helix with respect to the magnetic field  $\vec{B}_0$  occurs.

Future investigations are focused on separating the effects observed in THF and on implementing the medium into our RDC routine to gain access and further clarify the outstanding enantiodifferentiation properties of PBPMGLG in THF.

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## Conflict of Interest

The authors declare no conflict of interest.

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- [1] a) C. M. Thiele, *Concepts Magn. Reson. Part A* **2007**, *30*, 65–80; b) P. Lesot, C. Aroulanda, P. Berdagué, A. Meddour, D. Merlet, J. Farjon, N. Giraud, O. Lafon, *Prog. Nucl. Magn. Reson. Spectrosc.* **2020**, *116*, 85–154.  
[2] a) P. Doty, A. M. Holtzer, J. H. Bradbury, E. R. Blout, *J. Am. Chem. Soc.* **1954**, *76*, 4493–4494; b) I. Canet, J. Courtieu, A.

- Loewenstein, A. Meddour, J. M. Pechine, *J. Am. Chem. Soc.* **1995**, *117*, 6520–6526.
- [3] S. Jeziorowski, C. M. Thiele, *Chem. Eur. J.* **2018**, *24*, 15631–15637.
- [4] J. Feijen, W. L. Sederel, K. de Groot, A. C. de Visser, A. Bantjes, *Makromol. Chem.* **1974**, *175*, 3193–3206.
- [5] a) M. P. Reidy, M. M. Green, *Macromolecules* **1990**, *23*, 4225–4234; b) S. Yue, G. C. Berry, M. M. Green, *Macromolecules* **1996**, *29*, 6175–6182.
- [6] a) T. Yamazaki, A. Abe, *Polym. J.* **1987**, *19*, 777–780; b) A. Abe, T. Yamazaki, *Macromolecules* **1989**, *22*, 2145–2149; c) T. Yamazaki, A. Abe, H. Ono, H. Toriumi, *Biopolymers* **1989**, *28*, 1959–1967; d) A. Abe, S. Okamoto, N. Kimura, K. Tamura, H. Onigawara, J. Watanabe, *Acta Polym.* **1993**, *44*, 54–56.
- [7] A. Abe, T. Yamazaki, *Macromolecules* **1989**, *22*, 2138–2145.
- [8] a) O. Lafon, P. Lesot, C.-A. Fan, H. B. Kagan, *Chem. Eur. J.* **2007**, *13*, 3772–3786; b) P. Lesot, O. Lafon, H. Kagan, C.-A. Fan, *Chem. Commun.* **2006**, 389–391.
- [9] a) N. Hadjichristidis, H. Iatrou, M. Pitsikalis, G. Sakellariou, *Chem. Rev.* **2009**, *109*, 5528–5578; b) W. D. Fuller, M. S. Verlander, M. Goodman, *Biopolymers* **1976**, *15*, 1869–1871.
- [10] A. Marx, C. Thiele, *Chem. Eur. J.* **2009**, *15*, 254–260.
- [11] a) D. Whelan, G. Long, *Aust. J. Chem.* **1969**, *22*, 1779–1782; b) T. Pathak, N. F. Thomas, M. Akhtar, D. Gani, *Tetrahedron* **1990**, *46*, 1733–1744.
- [12] A. Del Zotto, F. Amoroso, W. Baratta, P. Rigo, *Eur. J. Org. Chem.* **2009**, 110–116.
- [13] a) T. J. Deming in *Peptide Hybrid Polymers* (Eds.: H.-A. Klok, H. Schlaad), Springer Berlin Heidelberg, Berlin, **2006**, pp. 1–18; b) E. Katchalski, M. Sela in *Advances in Protein Chemistry, Vol. 13* (Eds.: C. B. Anfinsen, M. L. Anson, K. Bailey, J. T. Edsall), Academic Press, New York, **1958**, pp. 243–492.
- [14] a) D. G. H. Ballard, C. H. Bamford, *J. Chem. Soc.* **1956**, 381–387; b) C. H. Bamford, H. Block, *J. Chem. Soc.* **1961**, 4989–4991.
- [15] E. Peggion, M. Terbojevich, A. Cosani, C. Colombini, *J. Am. Chem. Soc.* **1966**, *88*, 3630–3632.
- [16] W. Zhao, Y. Gnanou, N. Hadjichristidis, *Polym. Chem.* **2015**, *6*, 6193–6201.
- [17] P. Lesot in *Encyclopedia of Magnetic Resonance (eMagRes), Vol. 2, Issue 3* (Ed.: R. K. Harris), Wiley, Chichester, **2013**, pp. 315–334.
- [18] P. Lesot, *Prog. Nucl. Magn. Reson. Spectrosc.* **2009**, *55*, 128–159.
- [19] P. Tzvetkova, B. Luy, *Magn. Reson. Chem.* **2016**, *54*, 351–357.
- [20] T. Samulski, *J. Phys. Colloq.* **1979**, *40*, 471–474.
- [21] K. Czarniecka, E. T. Samulski, *Mol. Cryst. Liq. Cryst.* **1981**, *63*, 205–214.
- [22] a) A. Saupe, *Angew. Chem. Int. Ed. Engl.* **1968**, *7*, 97–112; *Angew. Chem.* **1968**, *80*, 99–115; b) M. Panar, W. D. Phillips, *J. Am. Chem. Soc.* **1968**, *90*, 3880–3882.
- [23] a) L. Pauling, *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2293–2294; b) L. Pauling, *J. Chem. Phys.* **1936**, *4*, 673–677.
- [24] V. Pokorná, D. Výprachtický, J. Pecka, *Macromol. Biosci.* **2001**, *1*, 185–190.

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