DNA-based drug carriers and dynamic proteoids with tunable properties
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Proteoid Dynamers with Tunable Properties

In this chapter, we designed and prepared a range of doubly dynamic proteoid biodynamers based on the polycondensation of different types of amino acid hydrazides with a nonbiological aromatic dialdehyde through formation of two types of reversible C=N bonds (imine and acylhydrazone). We found that the polymerization reaction is driven by the self-organization/folding of the resulting polymers and the side chains of amino acid hydrazides have a strong influence on the rates of polymerization, structures and dynamic properties of the formed biodynamers.

This chapter is adapted from the original publication:
5.1 Introduction

Constitutionally dynamic chemistry (CDC)\(^1\) is characterized by the formation of reversible linkages, either reversible covalent bonds or noncovalent interactions as connections between subunits of a molecular or supramolecular entity, and provides a new way to rationally designed, functional materials. As a consequence of the dynamic character, the resulting constitutionally dynamic materials (CDMs)\(^2\) can present advantages over constitutionally “static” materials, such as self-healing, tunable mechanical and optical properties, bio- or environmental degradability, and stimulus-responsiveness.

Applying CDC to the field of polymer chemistry leads to dynamic polymers, dynamers,\(^{2,3}\) in which monomers are connected by either noncovalent or reversible covalent connections\(^4\) These adaptive materials are formed by polycondensation of ditopic monomers that bear complementary functional groups. Various types of reversible covalent bonds such as thermally activated alkoxyamines,\(^5\) Diels–Alder linkages,\(^6\) imines,\(^7\) boronate esters,\(^8\) and disulfides\(^9\) can be used as the linkage of monomers. Such dynamers are able to undergo changes in response to external stimuli even after polymerization, enabling adaptions in their constitution, length and sequence. As a consequence, their mechanical\(^10\) and optical properties\(^11\) are controllable and may also be adaptable. These modifications can be induced by physical or chemical stimuli, such as light,\(^12\) temperature,\(^13\) pH,\(^14\) electrical field,\(^15\) sound\(^16\) and metal cations.\(^17\)

The incorporation of biologically relevant molecules leads to the generation of biodynamers,\(^18\) in which the functional properties (biocompatibility, recognition, catalysis) of naturally occurring biopolymers and dynamic character of CDMs are combined. Dynamic nucleic acid analogues (DyNAs)\(^18b\) are biodynamers bearing nucleobase residues; they are of great interest for complementary binding to nucleic acid strands, making them good candidate vectors for nonviral gene delivery. Dynamic analogues of polysaccharides\(^18c,\;\!d\) (glycodynamers) have attracted our attention given the involvement of polysaccharides in a wide range of biological or pathological events like immune response, inflammation, cancer, cell adhesion or cell–cell recognition.\(^19\) All these biodynamers have great potential as “smart” biomaterials, which may be utilized in biomedical and bio-engineering fields.

In addition to DyNAs and glycodynamers, we have recently reported dynamic proteoid polymers prepared via the condensation of a water-soluble dialdehyde (nonbiological component) with amino acid hydrazides (biological component), featuring two different types of reversible C=N bonds (imine and acylhydrazone),
thus being doubly dynamic.\textsuperscript{[20]} We found that the reversible polycondensation is driven by the self-organization/folding of the polymeric material formed, reminiscent of the hydrophobic effect, the main driving force for protein folding. Proteoid biodynamers are of particular interest in relation to the issue of how far protein folding has been instrumental in the evolution of the primary sequence towards a well-defined three-dimensional structure.

Based on these considerations, we have performed a comprehensive study on the influence of side chains of amino acid hydrazides on the properties of biodynamers. We report here the design and synthesis of a range of proteoid dynamers featuring a variety of amino acid hydrazides and characterize them in terms of structure, rate of polymerization and dynamic character using a variety of physical techniques.

5.2 Results and discussion

5.2.1 Design and generation of dynamic proteoids

Reversible imine-type bonds have been implemented in biomedical and material sciences to generate self-healing gels, pH-responsive micelles as drug-delivery vehicles and bio-active substances.\textsuperscript{[21]} By linking the monomers with reversible imine bonds, the resulting biodynamers are endowed with a pH-responsive character due to the basicity of the reactive amines. Owing to the different reactivities of the amino and carbonyl groups used, the resulting imine- or acylhydrazone-bearing biodynamers can have different stabilities. The presence of two types of C=N bonds (true imines and acylhydrazones) in one biodynamer affords doubly covalent biodynamers, potentially displaying a third form of dynamic behavior through structure-formation processes (conformational dynamics).\textsuperscript{[22]}

Doubly dynamic proteoids are formed by the polycondensation of a water-soluble dialdehyde and bifunctional amino acid hydrazides in a nucleation-elongation (N-E) manner with component selection.\textsuperscript{[20, 23]} In order to systematically investigate the influence of the amino acid side chain, we chose to use the amphiphilic dialdehyde 1 (Scheme 1), featuring a tricyclic aromatic core and a hexaglyme chain.\textsuperscript{[24]} For reactions performed in an aqueous environment, the hexaglyme moiety endows the biodynamers with water-solubility, as biodynamer formation is driven by hydrophobic interactions involving the tricyclic core and stabilization by the hydrophilic hexaglyme chains, which are solvent-exposed. In addition to π–π-stacking interactions between the dialdehyde core and the side chains of aromatic amino acid hydrazides, the polarity and charge of the amino acid side chain may also play a role. Furthermore, the proteoid biodynamers may be affected by the presence of cations in
the polymerization buffer due to the numerous metal-binding sites and the potential for cation–π interactions. Along these lines, we chose amino acid hydrazides with various types of side chains such as 2–4 with aromatic rings, 5–8 with charged side chains, and 10–11 with different polarity (Scheme 1) as the other component of dynamic proteoids to evaluate various factors that might influence the dynamic character and structure of the resulting doubly dynamic proteoids.

**Scheme 1.** Structures of the dialdehyde 1 and of amino acid hydrazides used.

In our previous study,\(^{[20]}\) we found the mechanism of polymerization to be N-E. The main feature and crucial step of N-E polymerization is nucleation, that is the formation of a critical size of polymer chain (nucleus), after which elongation of the existing polymer becomes more favorable than initiation of a new chain. At pD 5, acylhydrazone formation proceeded readily and went to completion, whereas the amine did not afford the corresponding imine. However, the driving force of polycondensation, which lies in the enhanced stability of dynamic proteoids resulting from folding of the main chain through hydrophobic and π–π-stacking interactions, favored the nucleation and led to the formation of biodynamers. It is still unclear whether the polymer chain necessarily consists of alternating imine and acylhydrazone bonds or of scrambled connections. We also observed that the side chain of the amino acid hydrazide has a direct influence on the structure and physicochemical properties of the resulting biodynamers. This can be ascribed to different degrees of stabilization of the resulting polymer chains depending on the side chain of the building block. Based on our previous experience, we prepared doubly dynamic proteoids by polycondensation of dialdehyde 1 and amino acid hydrazides 2–11 (Scheme 1) in aqueous solution at pD 5 (5 or 10 mM, NaCl, CsCl solution or \(d_3\)-acetate buffer). Under these conditions, both imines and acylhydrazones are efficiently formed to generate the desired dynamic proteoids (Scheme 2). The polycondensation was monitored by \(^1\)H-NMR spectroscopy, and the resulting
dynamic proteoids were characterized by small-angle neutron scattering (SANS), dynamic light scattering (DLS) and cryo-transmission-electron microscopy (cryo-TEM) to provide further information on the shape and size of the biodynamers obtained.

**Scheme 2.** Generation of doubly dynamic proteoids using one equivalent of dialdehyde 1 and one equivalent of amino acid hydrazides. Poly(1-3) was synthesized at a concentration of 5.0 mM in an aqueous solution of 0.1 M NaCl and 0.1 M CsCl at pD 5. Poly(1-5) was synthesized at a concentration of 10.0 mM in aqueous d$_3$-acetate buffer at pD 5. The other dynamic proteoids were synthesized at a concentration of 5.0 mM in aqueous d$_3$-acetate buffer at pD 5. The monomers 1 and 5 and an unfolded heptamer are shown as spacefilling models (hexaglyme groups in monomer 1 omitted for clarity).

5.2.2 Structural characterization of the dynamic proteoids

We investigated solution-state polymer morphologies by SANS, DLS and cryo-TEM, given that mass spectrometry does not provide information on the length of the intact polymers due to the inherent lability of the imine linkages. By using SANS and DLS (see experimental part), we concluded that three different types of nano-structures formed after polymerization, including globular nano-objects ((1-4), poly(1-10), and poly(1-11)), nanorods (poly(1-5) and poly(1-6)) and oligomers (poly(1-7), poly(1-8), and poly(1-9)).
Table 1. Structural parameters obtained from fitting of data presented in Figure 8.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$M_{\text{dimer}}$ (g/mol)</th>
<th>$M_w$ (g/mol)</th>
<th>DP</th>
<th>$R_g$ (Å)$^a$</th>
<th>$R_h$ (Å)$^b$</th>
<th>$R$ (Å)$^c$</th>
<th>ρ ratio</th>
</tr>
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<tr>
<td>poly(1-3) NaCl</td>
<td>678.8</td>
<td>39233</td>
<td>57.8</td>
<td>28.5</td>
<td>40</td>
<td>-</td>
<td>0.7125</td>
</tr>
<tr>
<td>poly(1-3) CsCl</td>
<td>678.8</td>
<td>18912</td>
<td>27.9</td>
<td>16.7</td>
<td>25$^d$</td>
<td>-$^b$</td>
<td>0.67$^d$</td>
</tr>
<tr>
<td>poly(1-4)</td>
<td>652.74</td>
<td>31995</td>
<td>49</td>
<td>36.42</td>
<td>45</td>
<td>30</td>
<td>0.81</td>
</tr>
<tr>
<td>poly(1-5)</td>
<td>643.8</td>
<td>138000</td>
<td>214.4</td>
<td>97</td>
<td>75</td>
<td>60±30</td>
<td>1.29</td>
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<tr>
<td>poly(1-6)</td>
<td>672.8</td>
<td>71738</td>
<td>106.6</td>
<td>57</td>
<td>52</td>
<td>45±15</td>
<td>1.10</td>
</tr>
<tr>
<td>poly(1-7)</td>
<td>630.7</td>
<td>6277</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>poly(1-8)</td>
<td>644.7</td>
<td>3000</td>
<td>4.7</td>
<td>9.32</td>
<td>15$^d$</td>
<td>14</td>
<td>0.62$^d$</td>
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<tr>
<td>poly(1-9)</td>
<td>614.73</td>
<td>4141</td>
<td>6.7</td>
<td>9.31</td>
<td>16$^d$</td>
<td>12</td>
<td>0.58$^d$</td>
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<tr>
<td>poly(1-10)</td>
<td>602.7</td>
<td>21447</td>
<td>35.6</td>
<td>30</td>
<td>36</td>
<td>19</td>
<td>0.83</td>
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<tr>
<td>poly(1-11)</td>
<td>616.7</td>
<td>18489</td>
<td>30</td>
<td>25.2</td>
<td>40$^d$</td>
<td>18</td>
<td>0.63$^d$</td>
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<tr>
<td>poly(1-12)</td>
<td>672.8</td>
<td>71738</td>
<td>106.6</td>
<td>57</td>
<td>52</td>
<td>45±15</td>
<td>1.10</td>
</tr>
</tbody>
</table>

$M_{\text{dimer}}$ = dimer molecular weight, $M_w$ = weight-averaged molecular weight, DP = averaged degree of polymerization, $R_g$ = radius of gyration, $R_h$ = apparent hydrodynamic radius, $\rho = R_g/R_h$ obtained from combining SANS and DLS.$^a$ Obtained from SANS experiments;$^b$ Obtained from DLS measurements; $^c$ Obtained from cryo-TEM experiments. Error bar is 10% for all values; $^d$ The presence of a slow mode due to a few large aggregates prevents any $R_h$, and thus $\rho$-ratio, determination with a very good accuracy. In this case, $R_h$ is obtained by applying the Contin method to our data; $^e$ The amplitude of the slow mode is too large and prevents any analysis of the correlation functions in the short time range and thus determination of the nano-objects $R_h$; $^f$ Cryo-TEM experiments were not performed.
Figure 1. Cryo-TEM images of (a) poly(1-4); (b) poly(1-5); (c) poly(1-6); (d) poly(1-7); (e) poly(1-8); (f) poly(1-9); (g) poly(1-10); (h) poly(1-11). No stain was used and image acquisition was achieved at a 2 μm defocus. Scale bar = 50 nm.
5.2.2.1 Cryo-TEM studies

The cryo-TEM images (Figure 1) give a direct visualization of the structures of polymers and are in good agreement with DLS and SANS results. All the physicochemical and structural features of these polymers are summarized in Table 1. The above results and analysis indicate that three important factors have great influence on the morphology and averaged degree of polymerization (DP) of the biodynamers: (1) Aromatic rings, should enhance the stability of the resulting polymers presumably through π–π-stacking interactions, so poly(1-2), poly(1-3)\(^{[20]}\) and poly(1-4) form globular nano-objects and display medium DPs. (2) Charge to some degree hinders the folding of polymers (poly(1-5), poly(1-6), poly(1-7) and poly(1-8)) by electrostatic force, so that the resulting polymer cannot form globular nano-objects. However, positively charged polymers (poly(1-5) and poly(1-6)) have high DP above 100 and are rod-shaped, whereas negatively charged biodynamers (poly(1-7) and poly(1-8)) feature a DP of less than 10. It appears that interaction between the cations and π cloud of the monomers, and possibly also with the oxygen atoms of the hexaglyme chains, facilitates the formation of the biodynamer while the repulsion between anions and the π cloud (as well as with the hexaglyme chains) may hinder polymerization. (3) Polarity. The stabilities of poly(1-10) and poly(1-11) appear to be enhanced, which may be ascribed to hydrogen bonds (OH-O, OH-N and NH-O) and/or OH–π interactions including the hydroxyl groups in the side chains, therefore, the resulting polymers undergo folding to form globular nano-objects and have medium DP, while poly(1-9) forms oligomers and has a low DP. It indicates that a hydroxyl group in the side chain of an amino acid hydrazide is important for globular nanostructures.

5.2.2.2 Effect of metal cations

The effect of cations in the aqueous solution on formation of the exemplary Tyr-containing biodynamers at 5 mM and pD 5.2 is studied. Interestingly, while the structure of both biodynamers is globular with a ρ-ratio lower than 1 and close to the value predicted for hard spheres (Table 1), the size is much larger for poly(1-3) in NaCl than for poly(1-3) in CsCl. At the same time, whereas the DP is equal to 28 in the presence of 0.1 M CsCl, it reaches a value of 58 in the presence of 0.1 M NaCl. This difference can presumably be ascribed to complexation of Na\(^+\) cations by the hexaglyme chains, which is reflected in a modification in chemical shift between the O-CH\(^2\) signals in D\(_2\)O and in 0.1 M NaCl aqueous solution. In contrast, no such change is observed in the case of 0.1 M CsCl, indicating that complexation does not occur as a result of weaker interactions with the oxygen atoms of the hexaglyme chain.
(Figure 2). Na\(^+\) cations may stabilize poly(1-3) through binding to the oxygen atoms and by cation–π interaction with monomer 1. Taken together, these interactions may play a significant role in the generation of all biodynamers.

**Figure 2.** \(^1\)H-NMR spectra of dialdehyde 1 (signal of hexaglyme part, 5 mM) in D\(_2\)O (blue), aqueous NaCl (0.1 M, red) and CsCl (0.1 M, black; overlapped with blue line) solution.

**5.2.3 Rates of polymerization, dynamic character and selectivity**

**5.2.3.1 Rates of polymerization**

We investigated the influence of the side chains on the rate of polymerization by monitoring the consumption of dialdehyde 1 in polycondensation under acidic conditions (pD 5). Each of the amino acid hydrazides 4–11 was added to an equimolar amount of dialdehyde 1 to generate the corresponding polymer, while (poly(1-2) and poly(1-3) were synthesized as previously described,\(^{[20]}\) and the consumption of dialdehyde 1 was monitored by \(^1\)H-NMR spectroscopy until it was fully consumed and until the spectra no longer changed after 2 d. We found that the factors that affect the structure of the polymers also influence the rates of polymerization.

The generation of poly(1-4) was completed in 2 h (Figure 3). It corresponds to the fastest polymerization and suggests that simple aromatic rings may greatly accelerate the process of polymerization by stabilizing the resulting polymer through π–π-stacking interactions. In the formation of positively charged rod-like poly(1-5) and poly(1-6) (Figure 3), dialdehyde 1 was entirely consumed in 1 d, but the negatively charged poly(1-8) and poly(1-7) that yield only oligomers were hard to generate. Even after weeks, signals from dialdehyde 1 were still visible, which is in agreement with the conclusions from the structural characterization and illustrates that positive charge facilitates polymerization, for instance, through cation-O,N and cation–π interactions. On the contrary, negative charge hinders it owing to electrostatic repulsion with these sites. Meanwhile, polymers poly(1-9), poly(1-11) and poly(1-10) underwent medium or slow polymerization, especially for poly(1-9) and poly(1-10), signals of dialdehyde 1 were still present even after 20 d. It indicates
that polarity does not appear to play an important role for the rate of polymerization. Taken together, our results show that, on the one hand, the aromatic hydrazides undergo the fastest polymerization, while the positively charged hydrazides are also fast but slower than aromatic ones. On the other hand, the negatively charged and other amino acid hydrazides form the resulting polymers at medium or slow rates. All these results also indicate that there is no correlation between the rate of polymerization and size of the resulting biodynamer.

Figure 3. Formation of poly(1-3), poly(1-4), poly(1-5) and poly(1-6). Percentage of unreacted dialdehyde 1 vs time. Poly(1-4) (5 mM), poly(1-5) (10 mM) and poly(1-6) (5 mM) were formed in aqueous $d_3$-acetate buffer at pD 5. And poly(1-3) (5 mM) in 0.1 M NaCl and CsCl aqueous solution at pD 5. For poly(1-3), due to the extreme line broadening observed, it is impossible to integrate the NMR spectra accurately between $t = 300$ min and $t = 930$ min due to the overlapping signals. After polymerization has reached completion ($t = 2$ d), the signal corresponding to dealdehyde 1 was no longer visible and not affected by overlapping signals.

In order to investigate the effect of cations on the rate of polymerization, poly(1-3) was formed in 0.1 M NaCl and CsCl aqueous solution at pD 5 (Figure 3). After 2 d, the polymerization reached equilibrium. When compared with poly(1-4), polymerization of poly(1-5) and poly(1-6) in aqueous $d_3$-acetate buffer, proceeded fast
in the first 300 min, but then slowed down and did not reach completion after 16 h. It suggests that in the first 300 min, the rate may be dominated by interactions between the aromatic rings, after which, the polymerization slows down even if stable and medium-sized polymers are formed and the pD needs to be readjusted to reach completion. Comparing the formation of poly(1-3) in aqueous NaCl and CsCl solution, it is faster with NaCl, presumably complexation of Na\(^+\) by hexaglyme helps to accelerate the reaction, but it slows down in the absence of complexation in CsCl solution.

5.2.3.2 Monomer selection in competitive polymerization

![Figure 4. Competition polymerization of monomer 1 with 2 and 9 at a concentration of 5 mM for each monomer in aqueous d\(_3\)-acetate buffer at pD 5. Parts of \(^1\)H NMR spectrum (400 MHz, 298 K) at different time points: a) 0 h; b) 24 h. After 24 h, the spectra no longer changed. (“▼” signal of monomer 2, “o” signal of dialdehyde 1, “×” signal of monomer 9).](image)

Based on the above analysis, it is clear that the side chains (aromatic ring, charge and polarity) have a strong influence on the rates of polymerization and on the structures of the resulting polymers. We were therefore interested to determine whether these factors may lead to preferential polymerization with monomer component selection, as observed in the condensation of dialdehyde 1 with dihydrazides of different types.\(^{[22]}\) To this end, we performed two three-component
competition experiments. In the first case, dialdehyde 1 was left to react with monomers 2 and 9 at a concentration of 5 mM for each component at pD 5. We chose monomers 2 and 9 based on their different rates of polymerization (fast and slow, respectively) to investigate whether they display similar relative rates in competition experiments. In the second case dialdehyde 1 was reacted with monomers 5 and 8, displaying fast and slow polymerization, respectively, and featuring opposite charge. This system was designed to illustrate whether oppositely charged building blocks might affect the rates in competitive polymerization experiments. We monitored both competition experiments by $^1$H-NMR spectroscopy.

In the first case (Figure 4), dialdehyde 1 was entirely consumed after 1 d, and the system had reached equilibrium. Signals of both monomers 2 and 9 decreased, and monomer 2 was preferentially incorporated into the polymeric chain. Integration showed that 85% of monomer 2 was consumed while only 15% of monomer 9 was incorporated into the biodynamer. This observation is in line with the conclusions from the two-component systems in which building block 2 rapidly afforded true polymers.

**Figure 5.** Competition polymerization of monomer 1 with 5 and 8 at a concentration of 5 mM for each monomer in aqueous $d_3$-acetate buffer at pD 5. Parts of $^1$H-NMR spectrum (400 MHz, 298 K) at different time points: a) 0 h; b) 24 h. After 24 h, the spectra no longer changed. (“▼” signal of monomer 5, “o” signal of dialdehyde 1, “×” signal of monomer 8).

In the second case, we observed that the system reached completion after 1 d
(Figure 5). However, rather than affording poly(1-5) rapidly, a novel three-component polymer was generated preferentially. Based on integration, 40% of positively charged monomer 5 and 60% of negatively charged monomer 8 were incorporated into the resulting polymer. This behavior may be attributed to the fact that the resulting polymer is stabilized through electrostatic interaction between monomers of opposite charge. Thus, electrostatic effects markedly influence competition polymerizations when two oppositely charged monomers are used.

For neutral monomers, the influence of the side chains on the rates of polymerizations is in line with the individual polymerization rates.

Figure 6. Monomer exchange between polymer poly(1-3) and monomer 9 at a concentration of 5 mM for each monomer in aqueous d$_3$-acetate buffer at pH 5. Parts of $^1$H NMR spectrum (400 MHz, 298 K) of solution of dialdehyde 1 and monomer 3 at different time points: a) 0 h; b) 24 h (reached equilibrium), and solution of poly(1-3) and monomer 9 at different time points: c) 0 h; d) 10 d (reached equilibrium). (“▼” signal of monomer 3, “o” signal of dialdehyde 1, “x” signal of monomer 9).

5.2.3.3 Dynamic character

The dynamic character of the biodynamers was demonstrated in a monomer exchange experiment (Figure 6). Adding an equimolar amount of monomer 9 (slow polymerization) to the polymer poly(1-3) (fast polymerization) at pH 5, and monitoring by $^1$H-NMR spectroscopy under acidic conditions showed that the system
reached equilibrium on a certain timescale. Integration showed that only 10% of monomer 9 was incorporated into poly(1–3) at the expense of monomer 3. Besides that, the alternate experiment, adding an equimolar amount of monomer 3 to poly(1–9) gave similar result (Figure 7). Integration showed that 87% of monomer 9 was substituted by monomer 3 from poly(1–3). Therefore, it illustrates that the resulting systems in both cases reached thermodynamic equilibrium. This preferential incorporation of monomer 3 is probably due to the more hydrophobic character and π–π-stacking interactions that stabilize the resulting polymer. The preferential incorporation of 3 over 9 in the biodynamer chain is in line with the polymeric structures and DPs mentioned above, as well as with the relative rates of formation.

![Figure 7](image)

*Figure 7.* Monomer exchange between polymer poly(1-9) and monomer 3 at a concentration of 5 mM for each monomer in aqueous d3-acetate buffer at pD 5. Parts of 1H NMR spectrum (400 MHz, 298 K) of solution of dialdehyde 1 and monomer 9 at different time points: a) 0 h; b) 3 d (reached equilibrium), and solution of poly(1-9) and monomer 3 at different time points: c) 0 h; d) 7 d (reached equilibrium). (“▼” signal of monomer 9, “o” signal of dialdehyde 1, “×” signal of monomer 3).

### 5.3 Conclusions

We have reported herein a range of doubly dynamic proteoid biodynamers based on the polycondensation of different types of amino acid hydrazides with the
nonbiological dialdehyde 1, through formation of two types of reversible C=N bonds (imine and acylhydrazone). We have characterized their structures, rates of polymerization and dynamic character. The polymerization reaction is driven by the self-organization/folding of the resulting polymers. We have demonstrated that the side chains of the amino acid hydrazides have a strong influence on the rates of polymerization, structures and dynamic properties of the resulting biodynamers. The aromatic rings in the side chains can greatly accelerate the polymerization and stabilize the resulting polymers presumably by \( \pi-\pi \) stacking interactions affording globular nano-objects. Positively charged side chains favor the polymerization and yield rod-shaped polymers, while negatively charged groups hinder polymerization leading to the formation of only oligomers. Hydroxyl groups in the side chains can stabilize the resulting polymers and generate globular nano-objects held together presumably by hydrogen bonds; the rates of polymerization, however, are not markedly increased. Finally, the side chain of leucine slows polymerization down, affording oligomers. Interestingly, we found that cations in aqueous solution affect the properties of the polymer and substantially slow down the rate of polymerization. Furthermore, the selective incorporation of the most suitable component from a pool of two monomers into the polymer chain optimizes the properties of the polymer and the results are in line with the relative individual rates of polymerization. In addition, when two oppositely charged monomers are used, electrostatic effects play a major role, favoring the incorporation of both monomers and generating largely neutral polymers. Taken together, the present results provide a basis for the rational design and synthesis of different types of well-ordered structures and adaptive materials offering great potential for utilization in the field of biomaterials science. The resulting responsive materials combine the properties of each monomer, especially the biocompatibility stemming from the adaptability provided by the dynamic character resulting from the reversible covalent bonds. Such dynamic biomaterials might thus find application in both biomedical and bio-engineering fields.

5.4 Experimental section

5.4.1 General experimental details

Starting materials and reagents were purchased from Aldrich or Acros. Yields refer to analytically pure compounds and have not been optimized. All solvents were reagent-grade. Melting points were measured on a Büchi B-540 melting-point apparatus. \(^1\)H- and \(^{13}\)C-NMR spectra were recorded at 400 MHz on a Varian AMX400 spectrometer (400 MHz for \(^1\)H, 101 MHz for \(^{13}\)C) at 25 °C, and \(^1\)H-NMR spectra of
polymerization were referenced to an internal tBuOH standard (1.24 ppm). Chemical shifts (δ) are reported relative to the residual solvent peak. Splitting patterns are indicated as (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, (br) broad. Optical rotations were measured in H$_2$O on a Schmidt & Haensch polarimeter (Polartronic MH8) with a 10 cm cell. High-resolution mass spectra (HRMS) were recorded with a FTMS orbitrap (Thermo Fisher Scientific) mass spectrometer. FT-IR spectra were measured on PerkinElmer FT-IR spectrometer. SANS experiments were carried out on the Pace spectrometer in the Léon Brillouin Laboratory at Saclay (LLB, France). LS measurements were performed on a 3D DLS spectrometer (LS Instruments, Fribourg, Switzerland). Cryo-transmission-electron microscopy (Cyro-TEM) was performed according to standard procedure. 3 µL of suspension was placed on a glow discharged holy carbon coated grid (Quantifiol 3.5/1) blotted and vitrified in a Vitrobot (FEI). Samples were observed in a Gatan 626 cryo-stage in a Philips CM 12 or CM120 operating at 120 keV or in a FEI Tecnai T20 operating at 200 keV. Images were recorded under low-dose conditions on a slow-scan CCD camera.

5.4.2 Synthesis and characterization of the monomers

Monomer 3 was purchased from Aldrich. Monomers 1, 2 and 8 were prepared according to the procedure reported in the literature.[30] Monomers 4, 5, 6, 9, 10 and 11 were prepared based on analogy with procedures reported in the literature for monomer 2. Monomer 7 was prepared based on analogy with procedures reported in the literature for monomer 8. The full characterization of these building blocks is presented below.

**General procedure for amino acid hydrazide synthesis from the corresponding methyl or ethyl ester (4, 5, 6, 9, 10 and 11) (see Scheme 3)**

To a solution of the methyl ester hydrochloride (500 mg, 1 eq.) in ethanol (10 mL), was added hydrazine monohydrate (8 eq.). The reaction mixture was stirred at 25 °C for 20 h. The mixture was concentrated in vacuo, and taken up in dichloromethane/propanol (3:1, 10 mL). The precipitate was filtered off, while the supernatant was concentrated and taken up in water. After lyophilization, the corresponding hydrazide was obtained as a solid.

Synthesis of monomer 7 (see Scheme 3)

To a solution of L-aspartic acid methyl ester hydrochloride (500 mg, 1 eq.) in ethanol (20 mL) was added hydrazine monohydrate (8 eq.). The reaction mixture was left to stir at 25 °C for 20 h. The resulting suspension was centrifuged, and the supernatant discarded. The white pellet was purified by cation-exchange chromatography (DOWEX 50 W x 2, 200–400 mesh size, Na⁺ form) to afford a white solid.

L-Histidine hydrazide (4). [α]D⁰²⁰ = +0.45 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 64–65 °C; ¹H NMR (400 MHz, D₂O, δ): 7.68 (s, 1H), 6.93 (s, 1H), 3.59 (t, J = 7 Hz, 1H), 2.97–2.83 (m, 2H); ¹³C NMR (101 MHz, D₂O, δ): 173.2, 135.9, 132.3, 117.0, 53.2, 30.9; HRMS (ESI) m/z: [M + H]⁺ calculated for C₇H₁₂N₃O, 170.1035; found: 170.1036.

L-Lysine hydrazide (5). [α]D⁰²⁰ = +0.08 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 80–82 °C; ¹H NMR (400 MHz, D₂O, δ): 3.31 (t, J = 7 Hz, 1H), 2.70 (t, J = 7 Hz, 2H), 1.62 (dt, J = 16, 7 Hz 2H), 1.55–1.46 (m, 2H), 1.41–1.24 (m, 2H); ¹³C NMR (101
Proteoid Dynamers with Tunable Properties

MHz, D₂O, δ): 174.7, 52.8, 39.1, 32.9, 26.4, 21.8; HRMS (ESI) m/z: [M + H]⁺ calculated for C₆H₁₇N₄O, 161.1397; found: 161.1397.

L-Arginine hydrazide (6). [α]D²⁰ = +0.18 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 46–48 °C; ¹H NMR (400 MHz, D₂O, δ): 3.45 (t, J = 7 Hz, 1H), 1.76 – 1.53 (m, 4H); ¹³C NMR (101 MHz, D₂O, δ): 175.0, 156.7, 52.8, 40.7, 30.8, 24.2; HRMS (ESI) m/z: [M + H]⁺ calculated for C₆H₁₇N₄O, 161.1397; found: 161.1397.

L-Aspartic acid hydrazide sodium salt (7). [α]D²⁰ = +0.22 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp > 200 °C (decomposition); ¹H NMR (400 MHz, D₂O, δ): 4.07 (t, J = 8 Hz, 1H), 2.69 (m, 2H); ¹³C NMR (101 MHz, D₂O, δ): 176.7, 170.5, 50.1, 38.4; HRMS (ESI) m/z: [M + H]⁺ calculated for C₄H₁₀N₃O₃, 148.0717; found: 148.0716.

L-Valine hydrazide (9). [α]D²⁰ = +0.38 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 186–188 °C; ¹H NMR (400 MHz, D₂O, δ): 3.05 (d, J = 7 Hz, 1H), 1.87 (m, 1H), 0.94 (d, J = 7 Hz, 3H) , 0.90 (d, J = 7 Hz, 3H); ¹³C NMR (101 MHz, D₂O, δ): 175.4, 59.2, 31.8, 18.3, 17.3; HRMS (ESI) m/z: [M + H]⁺ calculated for C₅H₁₄N₃O, 132.1131; found: 132.1131.

L-Serine hydrazide (10). [α]D²⁰ = +0.13 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 50–51 °C; ¹H NMR (400 MHz, D₂O, δ) 3.75 (d, J = 6 Hz, 2H), 3.60 (t, J = 6 Hz, 1H); ¹³C NMR (101 MHz, D₂O, δ): 172.4, 62.8, 54.4; HRMS (ESI) m/z: [M + H]⁺ calculated for C₃H₁₀N₃O₂, 120.0766; found: 120.0768.

L-Threonine hydrazide (11). [α]D²⁰ = +0.08 deg cm³ g⁻¹ (c = 10 mg cm⁻³, H₂O); mp 113–115 °C; ¹H NMR (400 MHz, D₂O, δ): 3.97 (t, J = 6 Hz,1H), 3.28 (d, J = 6 Hz, 1H), 1.20 (d, J = 6 Hz, 3H); ¹³C NMR (101 MHz, D₂O, δ): 173.1, 68.5, 58.9, 18.3; HRMS (ESI) m/z: [M + H]⁺ calculated for C₄H₁₂N₃O₂, 134.0924; found: 134.0924.

5.4.3 Preparation of polymers

Preparation of poly(1-4), poly(1-6), poly(1-7), poly(1-9), poly(1-10) and poly(1-11)

To a solution of monomer 1 (10 mM) in aqueous d₃-acetate buffer (100 mM, 0.4 mL, pH 5), was added a solution of monomer 4, 6, 7, 9, 10 or 11 (10 mM) in aqueous d₃-acetate buffer (100 mM, 0.4 mL, pH 5). The reaction mixture was rapidly mixed, and the pH was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M) and left to stand.
at 25 °C. For LS measurements, samples were passed through a 200 nm syringe filter immediately after mixing.

**Preparation of poly(1-5) and poly(1-8)**

To a solution of monomer 1 (20 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.4 mL, pD 5), was added a solution of monomer 5 or 8 (20 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.4 mL, pD 5). The reaction mixture was rapidly mixed, and the pD was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M) and left to stand at 25 °C. For LS measurements, samples were passed through a 200 nm syringe filter immediately after mixing.

**Preparation of poly(1-3)**

To a solution of monomer 1 (10 mM) in D$_2$O (0.4 mL), was added a solution of monomer 6 (10 mM) or 8 (10 mM) in NaCl or CsCl aqueous solution (200 mM, 0.4 mL). The reaction mixture was rapidly mixed, and the pD was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M) and left to stand at 25 °C.

**Preparation of competitive polymerization experiments (take polymerization of monomer 1 with 2 and 9 for example)**

To a solution of monomer 1 (10 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.4 mL, pD 5), were added solutions of monomer 2 (20 mM) and 9 (20 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.2 mL, pD 5). The reaction mixture was rapidly mixed, and the pD was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M) and left to stand at 25 °C.

**Preparation of monomer exchange system**

To a solution of monomer 1 (10 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.4 mL, pD 5), was added a solution of monomer 3 (20 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.2 mL, pD 5). The reaction mixture was rapidly mixed, and the pD was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M) and left to stand at 25 °C for 1 d to form poly(1-3). A solution of monomer 9 (20 mM) in aqueous $d_3$-acetate buffer (100 mM, 0.2 mL, pD 5) was added, the pD was set to 5 by addition of DCl (1.0 M) or NaOD (1.0 M), and the reaction mixture was left to stand at 25 °C.

**5.4.4 The setup and theory of SANS and DLS**

SANS experiments were carried out on the Pace spectrometer in the Léon Brillouin Laboratory at Saclay (LLB, France). The chosen incident wavelength, $\lambda$, depends on
the set of experiments, as follows. For a given wavelength, the range of the amplitude of the transfer wave vector $q$ was selected by changing the detector distance, $D$. Three sets of sample-to-detector distances and wavelengths were chosen ($D = 1.0$ m, $\lambda = 4.5 \pm 0.5$ Å; $D = 3.0$ m, $\lambda = 6 \pm 0.5$ Å; and $D = 4.7$ m, $\lambda = 9 \pm 0.5$ Å) so that the following $q$-ranges were respectively available: $4.8 \times 10^{-2} \leq q (\text{Å}^{-1}) \leq 4.9 \times 10^{-1}$, $1.1 \times 10^{-2} \leq q (\text{Å}^{-1}) \leq 1.2 \times 10^{-1}$, and $4.6 \times 10^{-3} \leq q (\text{Å}^{-1}) \leq 4.88 \times 10^{-2}$. Measured intensities were calibrated to absolute values (cm$^{-1}$) using normalization by the attenuated direct beam classical method. Standard procedures to correct the data for the transmission, detector efficiency, and backgrounds (solvent, empty cell, electronic, and neutronic background) were carried out. The scattered wave vector, $q$, is defined by equation 1, where $\Theta$ is the scattering angle:

$$ q = \frac{4\pi}{\lambda} \sin \frac{\Theta}{2} \quad (1) $$

The usual equation for absolute neutron scattering combines the intraparticle scattering $S_1(q) = V_{\text{chain}} \phi_{\text{vol}} P(q)$ form factor with the interparticle scattering $S_2(q)$ factor

$$ I(q) (\text{cm}^{-1}) = (\Delta \rho)^2 \left( S_1(q) + S_2(q) \right) = (\Delta \rho)^2 \left( V_{\text{chain}} \phi_{\text{vol}} P(q) + S_2(q) \right) \quad (2) $$

where $(\Delta \rho)^2 = (\rho_{\text{monomer}} - \rho_{\text{solvent}})^2$ is a contrast per unit volume between the polymer and the solvent and was determined from the known chemical composition. $\rho = \frac{\sum_i b_i}{\sum_i m_i} \times 1.66 \times 10^{-24}$ represents the scattering length per unit volume, $b_i$ is the neutron scattering length of the species $i$, $m_i$ the mass of species $i$, and $v$ the specific volume of the monomer (which has been measured on a helium pycnometer and taken to 0.685 cm$^3$ g$^{-1}$ (average value) for all monomers) or the solvent (i.e., 0.9026 cm$^3$ g$^{-1}$ for deuterated water). $P(q)$ is the form factor, $V_{\text{chain}} = N v m \times 1.66 \times 10^{-24}$ is the volume of the $N$ monomers (of mass $m$) in a chain and $\phi_{\text{vol}}$ is the volume fraction of monomer. In the high $q$-range, the scattering is assumed to arise from isolated chains; i.e., $S_2(q) = 0$, and thus $I(q) \propto P(q)$.

In the dynamic light scattering experiments (DLS), the normalized time autocorrelation function, $g^{(2)}(q,t)$, is measured as a function of the scattered wave-vector, $q$, given by $q = (4\pi n/\lambda) \sin(\Theta/2)$, where $n$ is the refractive index of the solvent (1.34 for water at 25 °C), and $\Theta$ is the scattering angle. In our experiments, $\Theta$ was varied between 30° and 130°, which corresponds to scattering wave vectors, $q$, in the range from $7.4 \times 10^{-3}$ to $2.6 \times 10^{-2}$ nm$^{-1}$. The measurements used a 3D DLS spectrometer (LS Instruments, Fribourg, Switzerland) equipped with a 25 mW HeNe laser (JDS uniphase) operating at $\lambda=632.8$ nm, a two channel multiple tau correlator (1088 channels in autocorrelation), a variable-angle detection system, and a
temperature-controlled index matching vat (LS Instruments). The scattering spectrum was measured using two single mode fibre detections and two high sensitivity APD detectors (Perkin Elmer, model SPCM-AQR-13-FC).

In DLS, the experimental signal is the normalized time autocorrelation function of the scattered intensity:\[31\]

\[ g^{(2)}(q,t) = \frac{\langle I(q,0)I(q,t) \rangle}{\langle I(q,0) \rangle^2} \quad (3) \]

The latter can be expressed in terms of the field autocorrelation function or equivalently in terms of the autocorrelation function of the concentration fluctuations, \( g^{(1)}(q,t) \), through:

\[ g^{(2)}(q,t) - 1 = \alpha + \beta \left| g^{(1)}(q,t) \right|^2 \quad (4) \]

Where \( \alpha \) is the baseline (varying between \( 1 \times 10^{-4} \) and \( 2 \times 10^{-4} \) depending on the scattering angle and/or the system) and \( \beta \) the coherence factor, which in our experiments is varying between 0.7 and 0.9 depending on the samples. The normalized dynamical correlation function, \( g^{(1)}(q,t) \), of polymer concentration fluctuations is defined as:

\[ g^{(1)}(q,t) = \frac{\langle \delta c(q,0) \delta c(q,t) \rangle}{\langle \delta c(q,0)^2 \rangle} \quad (5) \]

Where \( \delta c(q,t) \) and \( \delta c(q,0) \) represent fluctuations of the polymer concentration at time \( t \) and zero, respectively.

In our experiments, most of the solutions were characterized by a single relaxation mechanism with a characteristic relaxation time inversely proportional to \( q^2 \). For these solutions, we have also adopted the classical cumulant analysis:\[32\]

\[ \ln g^{(1)}(q,t) = k_0 - k_1 t + \frac{k_2}{2} t^2 + ... \quad (6) \]

Where \( k_1 = 1/\langle \tau_c \rangle \) and \( k_2/k_1^2 \) represents the polydispersity index (PDI). The extrapolation of \( (\tau_c q^2)^{-1} \) to \( q = 0 \), where \( \tau_c \) is the average relaxation time of \( g^{(1)}(q,t) \), yields the mutual diffusion coefficient \( D \). The latter is related to the average apparent hydrodynamic radius, \( R_h \), of the supramolecular assemblies through the Stokes-Einstein relation:

\[ D = \frac{k_B T}{6 \pi \eta_s R_h} \quad (7) \]

Where \( k_B \) is the Boltzmann constant, \( \eta_s \) the solvent viscosity (0.89 cP for water at \( T=25 \, ^{\circ}\text{C} \)), and \( T \) the absolute temperature. From the polydispersity index \( k_2/k_1^2 \) one
can determine the $R$ width distribution. Indeed, the second cumulant $k_2$ provides a quantitative measure for the polydispersity of the diffusion coefficient distribution function, $\sigma_D$, which is defined as:

$$
\sigma_D = \sqrt{\left\langle D^2 \right\rangle - \left\langle D \right\rangle^2} = \frac{k_2}{\left\langle D \right\rangle} \quad (8)
$$

The size ($R_h$) polydispersity can be calculated from the polydispersity of the diffusion coefficients $\sigma_D$.

$$
\sigma_h = \sqrt{\left\langle R_h^2 \right\rangle - \left\langle R_h \right\rangle^2} \quad (9)
$$

For solutions characterized by several relaxation mechanisms (presence of a slow mode) we used the Contin method based on the inverse Laplace transform of $g^{(1)}(q,t)$. If the spectral profile of the scattered light can be described by a multi-Lorentzian curve, then $g^{(1)}(q,t)$ can be written as:

$$
g^{(1)}(q,t) = \int_0^\infty G(\Gamma) \exp(-\Gamma t) d\Gamma \quad (10)
$$

Where $G(\Gamma)$ is the normalized decay constant distribution.

5.4.5 Results and discussions of SANS and DLS

Figure 8 shows variations in scattered neutron intensity as a function of the scattering wave vector $q$ for each of the studied polymers in $D_2O$ in aqueous $d_3$-acetate buffer at pD 5. The solutions were homogeneous, clear, transparent and stable over time. We observed three strikingly different characteristic behavior patterns, which are represented in Figures 8a, 8b, and 8c, respectively, as described hereafter.

5.4.5.1 Nanorod behavior: large objects with high scattered intensity

Figure 8a displays the scattering profile of samples poly(1-5) and poly(1-6). The two spectra are markedly similar, suggesting that morphologically related structures are present in solution. Each scattering profile exhibits a Guinier regime at low $q$ associated with the finite size of the scattering objects, one intermediate regime in which the $q$-dependence of the scattered intensity can be described by a power law with an exponent close to $-1$, a second Guinier regime at the upper $q$ range corresponding to the cross section of the scattered objects, and eventually a dip, which is the initial part of the oscillating term of the shape-dependent form factor of the particle cross section (see Figure 8a dashed lines). The intermediate $q^{-1}$ dependence, which is most apparent for poly(1-5), is characteristic of objects of rigid-rod structure;
whereas the Guinier regimes at high and low $q$ values can be used to extract information regarding the cross section of the rods and the weight-averaged molecular weight ($M_w$)/radius of gyration ($R_g$) of the polymer, respectively. Each of the two spectra fit well to a Guinier model at low $q$ values and to rigid-rod and cross section models at larger $q$, which yield measurements describing both the global ($M_w$ and $R_g$) and local (linear mass density ($\mu$), cross sectional area ($a_c$) and cross sectional radius of gyration ($r_c$)) structural features of the rod-like objects (see Table 2).

Figure 8. SANS profiles collected at 20 °C. Each polymer was prepared by allowing the appropriate monomers to equilibrate and the spectra did not change anymore after 2 d in aqueous $d_3$-acetate buffer at ambient temperature and pD 5 prior to data collection. For clarity, the spectra have been shifted by one log unit along the $y$-axis with respect to each other. The insert shows the plot of $1/I$ vs. $q^2$ and the best linear fit to the data (see Equation (11)). (a) poly(1-6) (5 mM) and poly(1-5) (10 mM) samples; (b) poly(1-4) (5 mM), poly(1-10) (5 mM) and poly(1-11) (5 mM) samples; (c) poly(1-8) (10 mM), poly(1-9) (5 mM) and poly(1-7) (5 mM) samples; (d) poly(1-3) (5 mM) in the presence of NaCl or CsCl at 0.1 M and pD 5.
The data obtained at low-\(q\) values corresponding to relatively large spatial scales can be fitted by the Guinier law shown in equation (11).

\[
\frac{I}{I(q)} = \frac{I}{I(0)} \left( 1 + q^2 \frac{R_g^2}{3} \right)
\]  

(11)

The extrapolation to zero-\(q\) of the scattered intensity, \(I(q^2=0)\), provides a measure of the weight averaged molecular weight (\(M_w\)) of the particle. The insert of Figure 8a shows the plot of \(1/I\) vs. \(q^2\) for both samples. From the best linear fit to the data, \((R_g) = 97 \pm 5\) and \(57\pm 5\) Å for poly(1-5) (10 mM) and poly(1-6) (5 mM), respectively are obtained. The extrapolation of \(I(q)\) to \(q = 0\) is relatively high indicating the presence of large objects, and provides \(M_w = 138 \pm 14\) and \(72 \pm 7\) kDa, which corresponds to a degree of polymerization (DP) of about 214 and 107 for poly(1-5) and poly(1-6), respectively.

**Table 2.** Structural parameters obtained from fitting of data presented in Figures 8a to a low-\(q\) Guinier law, a rigid-rod model, and a high-\(q\) Guinier expression for the form factor of the section.

<table>
<thead>
<tr>
<th>Sample</th>
<th>poly(1-5)</th>
<th>poly(1-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M_{dimer}) (g/mol)</td>
<td>643.8</td>
<td>672.8</td>
</tr>
<tr>
<td>(M_w) (g/mol)</td>
<td>138000</td>
<td>71738</td>
</tr>
<tr>
<td>DP</td>
<td>214.4</td>
<td>106.6</td>
</tr>
<tr>
<td>(R_g) (Å)(^a)</td>
<td>97</td>
<td>57</td>
</tr>
<tr>
<td>(R_h) (Å)(^b)</td>
<td>75</td>
<td>52</td>
</tr>
<tr>
<td>(R) (Å)(^c)</td>
<td>60±30</td>
<td>45±15</td>
</tr>
<tr>
<td>(\rho) ratio</td>
<td>1.29</td>
<td>1.10</td>
</tr>
<tr>
<td>(\times 10^3)</td>
<td>4.41</td>
<td>2.3</td>
</tr>
<tr>
<td>(\Delta \rho^2) (cm(^2))(\times 10^{21})</td>
<td>2.137</td>
<td>2.27</td>
</tr>
<tr>
<td>(\mu) (g/mol/Å)</td>
<td>374</td>
<td>330</td>
</tr>
<tr>
<td>(r_c) (Å)</td>
<td>14.95</td>
<td>12.7</td>
</tr>
<tr>
<td>(a_c) (Å(^2))</td>
<td>493</td>
<td>435</td>
</tr>
<tr>
<td>(I(q^2=0)) (cm(^{-1}))</td>
<td>1.48</td>
<td>0.426</td>
</tr>
</tbody>
</table>

\(M_{dimer}\) = dimer molecular weight, \(\phi\) = monomer volume fraction, \(\Delta \rho^2\) = contrast per unit volume, \(I(q^2=0)\) = scattered intensity extrapolated at \(q=0\), \(M_w\) = weight-averaged molecular weight, DP = averaged degree of polymerization, \(R_g\) = radius of gyration, \(R_h\) = apparent hydrodynamic radius, \(\rho = R_g/R_h\) obtained from combining SANS and DLS, \(\mu\) = linear mass density, \(r_c\) = cross-sectional radius of gyration, \(a_c\) = cross sectional area. \(^a\) Obtained from SANS experiments; \(^b\) Obtained from DLS
measurements; Obtained from cryo-TEM experiments. Error bar is 10% for all values except \( R (\text{Å}) \).

In the intermediate \( q \) regime, the scattering curves can be fitted satisfactorily by a rigid-rod model. Figure 8a shows the fits realized for both polymers by means of the form factor\(^{18d, 24c, 25, 27}\) derived for rigid-rod particles and given by:

\[
P( q ) = \frac{\pi}{qL} \quad (12)
\]

where \( L \) is the contour length of the rod. In the dilute range, the radius of gyration of rodlike particles with large aspect ratio is given by \( R_g^2 = L^2/12 \). For poly(1-5) and poly(1-6) we obtain \( L=336 \) and 197 Å, respectively, in agreement with the model derived for equilibrium polymers that predict a \( C^{1/2} \) dependence of the average length of the self-assembled polymers. Thus, the contour-length difference between the two polymers as determined by SANS could also be due to the concentration of the samples, with polymers derived from 5 mM Lys-containing monomers giving measurably longer chains than those derived from 5 mM Arg-containing monomers. The high \( q \) data can be fitted by a Guinier expression for the form factor of the section:\(^{18d, 24c, 25, 27}\)

\[
V_{\text{chain}}P( q ) = \frac{\pi a_c}{q} \exp\left(-\frac{q^2 r_c^2}{2}\right) \quad (13)
\]

where \( r_c \) is the radius of gyration of the cross section. By fitting the two equations above to the experimental data, the linear mass density (\( \mu \), mass per unit length) of the polymers can be determined, the cross-sectional area, \( (a_c) \) and \( r_c \). From the fits of Figure 8a, we obtain \( \mu = 374 \pm 40 \text{ Da/Å}, \ a_c = 493 \pm 50 \text{ Å}^2 \), and \( r_c = 15 \pm 1.5 \text{ Å} \) and \( \mu = 330 \pm 40 \text{ Da/Å}, \ a_c = 435 \pm 50 \text{ Å}^2 \), and \( r_c = 13 \pm 1.5 \text{ Å} \) for poly(1-5) and poly(1-6), respectively. From the values of \( M_w \) and \( \mu \), the contour length of the polymers is obtained: \( L=M_w/\mu=369 \) and 218 Å for poly(1-5) and poly(1-6), respectively, values in excellent agreement with those of 336 and 197 Å obtained using the rod-like model above and indicating the formation of well-defined rods.

This last point is also confirmed by the so-called \( \rho \)-ratio derived here from combining the particle size characteristics determined from static (SANS) and dynamic light scattering (DLS) measurements.\(^{24a}\) The analysis of the diffusive single relaxation of the scattered electric field correlation function (Figure 9), neglecting Virial effects, allows the determination of the hydrodynamic radius \( R_h \) of the nanorods. The \( \rho \)-ratio is simply defined as \( \rho=R_g/R_h \) and provides also an important indication of the particle
topology. Values of the apparent $R_h$ and $\rho$-ratio (1.29 and 1.10 for poly(1-5) and poly(1-6), respectively) are collected in Table 2.

These experimental values are in very good agreement with the theoretically calculated values for cylinders of length $L$ and diameter $D$ given by the following expression:

$$\rho = \frac{R_h}{R_g} = \frac{1}{\sqrt{3}} \cdot \ln\left(\frac{L}{D} - 0.5\right) (14)$$

Indeed, calculation gives $\rho=1.36$ and 1.13 for poly(1-5) ($L=336$ Å and $D=30$ Å) and poly(1-6) ($L=197$ Å and $D=26$ Å), respectively.

Figure 9. Scattered electric field autocorrelation function, $g^{(1)}(q,t)$, at $\theta=90^\circ$, for solutions of 5 mM poly(1-6) (nanorods), 5 mM poly(1-10) (globular nano-objects), and 10 mM poly(1-8) (oligomers) in acetate buffer at pD 5 and T=20°C. The inset shows the normalized distribution of scattered intensity as a function of the size obtained with the Contin method.

Although cylindrical nanorods formed by poly(1-5) seem a little more compact than those of poly(1-6), the fitting results presented in Table 2 suggest that within the error bars, the local structures of the various rods are rather similar ($\mu$, $a_c$ and $r_c$). Assuming full disk-shaped cross sections with $a_c = \pi R^2$, leads to incompatible results as the obtained geometrical radius of the disk $R=12.2$ Å is smaller than $r_c$. A possible
explanation is the formation of hollow cylinders with a cross-sectional area given by 
\[ a_c = \pi (R^2 - R_i^2) \] and 
\[ r_c = \sqrt{2/\pi} \times \sqrt{(R^2 + R_i^2)} \], where \( R \) and \( R_i \) are the external and internal radii of the section of hollow cylinders, respectively. Using experimental values of \( a_c \) and \( r_c \), we find (\( R=17.4 \, \text{Å}, \ R_i=12.04 \, \text{Å} \)) and (\( R=15.2 \, \text{Å}, \ R_i=9.6 \, \text{Å} \)) for poly(1-5) and poly(1-6), respectively. The thickness of the section given by \( R-R_i \) is equal to \( \sim 5.5 \, \text{Å} \) for both polymers. The formation of cylindrical nanorods presumably can be explained by cisoid conformations of the N–N bonds (acylhydrazones), which allows the winding of the polymer backbone into a regular helical conformation to form the walls of cylinders, leaving the hexaglyme substituents solvent-exposed to stabilize the cylinder. Poly (1-5) is presumably packed in a more compact manner than poly(1-6) owing to the weaker repulsion between cationic sites in the former polymer.

5.4.5.2 Globular nano-objects

Figure 8b displays the neutron scattering patterns obtained for poly(1-4), poly(1-10), and poly(1-11). For these polymers, there is only one Guinier regime associated with the mass and \( R_g \) of the nano-objects. An oscillation associated with the shape-dependent form factor of the particle cross section (indicated by an arrow) is slightly visible for sample poly(1-4) that also exhibits the highest scattering signal. The absence of the \( q^{-1} \)-dependence of \( I(q) \) in the intermediate \( q \)-range indicates that these polymers may not be considered as rigid rods anymore. Values of \( M_w \) and \( R_g \) obtained using a Guinier analysis (see Equation (11)) and neglecting the excluded volume interactions, are collected in Table 3. The difference in \( M_w \) is in line with the difference in rates of polymerization and may be attributed to the difference of hydrophobicity or concentration for poly(1-11) (5 mM).

For poly(1-11), the Guinier plateau is followed by a slight upturn at low-\( q \) associated with the presence of few larger aggregates of \( \sim 100 \, \text{nm} \) (less than 0.01 % in g/cm\(^3\)) as confirmed by DLS measurements. These aggregates display a characteristic slow mode that also prevents any size determination in the short time range with a very good accuracy. The formation of these minority aggregates may result from the presence of impurities. We can thus neglect the presence of this minute aggregated matter and consider the system as a quasi-monodisperse solution (single population) of globular nano-objects. Nevertheless, the electric field correlation function is mainly characterized by a fast diffusive relaxation (see Figure 9) with characteristic time inversely proportioned to \( q^2 \), giving \( R_h \) for poly(1-4), poly(1-10), and poly(1-11) (see Table 3). Indication on the structure and degree of compactness of the nano-objects is provided here by the \( \rho \) ratio. Neglecting the Virial effects, the value of the ratio is lower than 1 for the three samples (comprised between 0.63 and 0.83 that can be
calculated from SANS and DLS) and close to 0.775, a value calculated for homogeneous hard spheres and thus indicates the formation of dense and globular structures, reminiscent of folded protein assemblies. Protein-like copolymers are known to afford spherical objects, if both hydrophilic and hydrophobic monomers are used, as is the case with the globular objects we observed earlier.\textsuperscript{[20, 28]} The distribution width obtained using the cumulant method (see experimental section) is about 1.5 nm, a value characteristic of systems with a low dispersity.

Table 3. Structural parameters obtained from fitting of data presented in Figures 8b, 8c, and 8d to a low-\(q\) Guinier law.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(M_{\text{dlux}}) (g/mol)</th>
<th>(M_w) (g/mol)</th>
<th>DP</th>
<th>(R_g) (Å)\textsuperscript{a)</th>
<th>(R_h) (Å)\textsuperscript{b)</th>
<th>(R) (Å)</th>
<th>(\rho)</th>
<th>(\Phi \times 10^{-3})</th>
<th>(\Delta \rho^2)</th>
<th>(I(q_{=0})) (cm(^{-4})) \times 10^{21})</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(1-3) NaCl</td>
<td>678.8</td>
<td>39233</td>
<td>57.8</td>
<td>28.5</td>
<td>40</td>
<td>0.7125</td>
<td>2.3</td>
<td>1.9</td>
<td>0.195</td>
<td></td>
</tr>
<tr>
<td>poly(1-3) CsCl</td>
<td>678.8</td>
<td>18912</td>
<td>27.9</td>
<td>16.7</td>
<td>25\textsuperscript{e)}</td>
<td>0.67\textsuperscript{e)}</td>
<td>2.3</td>
<td>1.9</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>poly(1-4)</td>
<td>652.74</td>
<td>31995</td>
<td>49</td>
<td>36.42</td>
<td>45</td>
<td>0.81</td>
<td>2.24</td>
<td>1.823</td>
<td>0.1486</td>
<td></td>
</tr>
<tr>
<td>poly(1-7)</td>
<td>630.7</td>
<td>6277</td>
<td>10</td>
<td>9</td>
<td>-\textsuperscript{e)}</td>
<td>-</td>
<td>2.2</td>
<td>1.91</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>poly(1-8)</td>
<td>644.7</td>
<td>3000</td>
<td>4.7</td>
<td>9.32</td>
<td>15\textsuperscript{e)}</td>
<td>14</td>
<td>0.62 \textsuperscript{e)}</td>
<td>4.41</td>
<td>1.883</td>
<td>0.02834</td>
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<tr>
<td>poly(1-9)</td>
<td>614.73</td>
<td>4141</td>
<td>6.7</td>
<td>9.31</td>
<td>16\textsuperscript{e)}</td>
<td>12</td>
<td>0.58\textsuperscript{e)}</td>
<td>2.1</td>
<td>2.123</td>
<td>0.021</td>
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<tr>
<td>poly(1-10)</td>
<td>602.7</td>
<td>21447</td>
<td>35.6</td>
<td>30</td>
<td>36</td>
<td>19</td>
<td>0.83</td>
<td>2.06</td>
<td>1.99</td>
<td>0.1</td>
</tr>
<tr>
<td>poly(1-11)</td>
<td>616.7</td>
<td>18489</td>
<td>30</td>
<td>25.2</td>
<td>40\textsuperscript{e)}</td>
<td>18</td>
<td>0.63\textsuperscript{e)}</td>
<td>1</td>
<td>2.04</td>
<td>0.0429</td>
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</tbody>
</table>

\(\textsuperscript{a)}\) Obtained from SANS experiments; \(\textsuperscript{b)}\) Obtained from DLS measurements; \(\textsuperscript{c)}\) Obtained from cryo-TEM experiments. Error bar is 10\% for all values; \(\textsuperscript{d)}\) The presence of a slow mode due to a few large aggregates prevents any \(R_h\), and thus \(\rho\)-ratio, determination with a very good accuracy. In this case, \(R_h\) is obtained by applying the Contin method to our data; \(\textsuperscript{e)}\) The amplitude of the slow mode is too large and prevents any analysis of the correlation functions in the short time range and thus determination of the nano-objects \(R_h\); \(\textsuperscript{f)}\) Cyro-TEM experiments were not performed.

5.4.5.3 Oligomers

For polymers poly(1-7), poly(1-8), and poly(1-9), there is again only one Guinier regime but with a much lower scattering level (see Figure 8c). Similar SANS analyses were conducted and gave a degree of polymerization ranging between 5 and 10.
depending on the sample and corresponding to oligomers of about 9 Å radius of gyration (see Table 3).

Analysis by DLS of the systems provides information on the composition of the homogeneous solutions at larger scales.\[26, 29\] Despite filtering through 0.2 \( \mu \)m membranes, oligomer solutions contain also minute amounts of aggregates. The scattered field autocorrelation functions are bimodal (see Figure 9). There are clearly two distinct cooperative relaxation processes with characteristic times inversely proportional to \( q^2 \). The fast relaxation mechanism is associated to the diffusion of oligomers with an apparent hydrodynamic radius of \( \sim 15 \) Å (see Table 3), whereas the slower relaxation indicates the presence of large biodynamer aggregates (\( \geq 150 \) nm depending on the samples). As the ratio between the size of aggregates and oligomers is large, the contribution of few large aggregates (less than 0.01 %) to the DLS signal is significant as shown by the height of the amplitude of the slow mode. These aggregates are not visible in the SANS q-ranges. The morphology of the oligomer assemblies may be globular with a \( \rho \) ratio smaller than 1. However, the presence of this slow mode prevents a determination of \( R_h \) with a good accuracy.

Table 4. Percentage of unreacted dialdehyde 1 vs time obtained by monitoring the signals from the aldehyde group by \(^1\)H-NMR spectroscopy.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>poly(1-3) KCl (%)</th>
<th>poly(1-3) NaCl (%)</th>
<th>poly(1-4) (%)</th>
<th>poly(1-5) (%)</th>
<th>poly(1-6) (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>20</td>
<td>96.497</td>
<td>89.43545</td>
<td>84.30847</td>
<td>92.381</td>
<td>91.189</td>
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<tr>
<td>40</td>
<td>90.564</td>
<td>82.73044</td>
<td>74.30847</td>
<td>85.087</td>
<td>83.027</td>
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<tr>
<td>60</td>
<td>84.207</td>
<td>70.51612</td>
<td>58.2303</td>
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<td>80</td>
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<tr>
<td>100</td>
<td>71.309</td>
<td>63.17542</td>
<td>30.77179</td>
<td>68.813</td>
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<tr>
<td>120</td>
<td>66.202</td>
<td>57.45422</td>
<td>17.11547</td>
<td>65.478</td>
<td>60.02</td>
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<tr>
<td>140</td>
<td>63.261</td>
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<td>60.911</td>
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<tr>
<td>160</td>
<td>59.407</td>
<td>48.96322</td>
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<td>28.737</td>
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<tr>
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<td>27.53141</td>
<td>0.16241</td>
<td>31.698</td>
<td>28.179</td>
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<tr>
<td>360</td>
<td>33.312</td>
<td>28.19737</td>
<td>0.16241</td>
<td>30.896</td>
<td>27.438</td>
</tr>
</tbody>
</table>
5.5 Contributions from co-authors

Prof. Dr. J.-M. Lehn and Prof. Dr. A. K. H. Hirsch designed the biodynamer building blocks and Prof. Dr. A. K. H. Hirsch synthesized some of them during her postdoc project. Cyro-TEM measurements were performed by Dr. M. C. A. Stuart. DLS and SANS measurements and analysis were done by Prof. Dr. E. Buhler.

5.6 References


