Tailoring Supramolecular Peptide–Poly(ethylene glycol) Hydrogels by Coiled Coil Self-Assembly and Self-Sorting

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Supporting Information

ABSTRACT: Physical hydrogels are extensively used in a wide range of biomedical applications. However, different applications require hydrogels with different mechanical and structural properties. Tailoring these properties demands exquisite control over the supramolecular interactions involved. Here we show that it is possible to control the mechanical properties of hydrogels using de novo designed coiled coil peptides with different affinities for dimerization. Four different nonorthogonal peptides, designed to fold into four different coiled coil heterodimers with dissociation constants spanning from μM to pM, were conjugated to star-shaped 4-arm poly(ethylene glycol) (PEG). The different PEG-coiled coil conjugates self-assemble as a result of peptide heterodimerization. Different combinations of PEG–peptide conjugates assemble into PEG–peptide networks and hydrogels with distinctly different thermal stabilities, supramolecular, and rheological properties, reflecting the peptide dimer affinities. We also demonstrate that it is possible to rationally modulate the self-assembly process by means of thermodynamic self-sorting by sequential additions of nonpegylated peptides. The specific interactions involved in peptide dimerization thus provides means for programmable and reversible self-assembly of hydrogels with precise control over rheological properties, which can significantly facilitate optimization of their overall performance and adaption to different processing requirements and applications.

INTRODUCTION

Hydrogels are highly hydrated polymeric networks that are widely used in various biomedical applications, including 3D cell culture, cell therapy, and bioprinting.1,2 Hydrogels are attractive as cell carriers in injection-based therapies and in bioinks for 3D bioprinting as they can protect cells from damaging shear forces during the injection and the additive manufacturing process. Hydrogels can also offer an extracellular matrix-mimicking environment that supports cell growth and differentiation, which is vital in both tissue engineering applications and 3D cell culture.3–5 Notably, different applications and cell types require hydrogels with significantly different properties. The influence of the mechanical, chemical and structural properties of the hydrogels on cell behavior, stem cell differentiation, tissue integration, and ability to stimulate regeneration cannot be underestimated, and these properties must thus be tightly controlled.5 Hydrogels must also withstand various forms of processing without loss of functionality meaning that they must be structurally robust at the molecular level and display tunable shear thinning and preferably self-healing properties. Although rheological properties of chemically cross-linked hydrogels can be varied using different cross-linking strategies, their synthesis can damage cells, biomolecules and surrounding tissue. By contrast, physical hydrogels can be obtained as a result of supramolecular interactions that typically are not harmful to cells and tissues, making them ideal for biomedical applications. Unfortunately, it is more difficult to rationally tune the rheological properties of physical hydrogels as it requires precise means to control the supramolecular interactions involved in the self-assembly process.

The enormous chemical and structural diversity of peptides make them attractive as supramolecular building blocks for bottom-up fabrication of materials. The increasing knowledge about sequence-to-structure relationships has facilitated design of peptides that adopt well-defined structural motifs, such as coiled coils, helix-loop-helices, and beta-hairpins and have enabled development of self-assembling peptide fibers, tapes, nanoparticles, and hydrogels.7–15 Furthermore, by connecting two or more peptide using short linkers, fairly complex assembled structures can be formed as result of the well-defined folding pattern of each single peptide folding motif.14–18 Peptides have also been conjugated to larger synthetic polymeric backbones, such as poly(ethylene glycol) (PEG), in order to create a wider range of supramolecular hybrid materials and nanostructures,19–21 including peptide–polymer hybrid hydrogels, fibrils, spherulites, and micelles.21–26 PEG is an attractive component in hydrogels and have been extensively investigated for 3D cell culture, and is used in a large number of clinical and pharmaceutical applications.27 By conjugating peptides to PEG, responsive hydrogels can be obtained as a
result of specific peptide-mediated self-assembly. These can in turn be proteolytically degraded, assembled or disassembled, and show other structural rearrangements as a result of changes in temperature, ionic strength or pH.28–31 In addition, peptides can provide highly defined intra- and intermolecular interactions. Highly defined interactions are crucial when developing supramolecular hydrogels with tunable mechanical and structural properties. For example, beta strand-forming motifs are often used for self-assembly of peptide-based hydrogels, although the ability to control the gelation is often rather limited. By contrast, although less prone for gelation, alpha-helical coiled coil forming peptides exhibit much better control of hydrogels, although the ability to control the gelation is often rather limited.32

However, limited progress has been made in exploiting the programmable interactions involved in coiled coil oligomerization and folding for controlling the rheological properties of hydrogels.

We have recently described a set of four 28 amino acid residue peptides that heterodimerize and fold into parallel coiled coils.33 The peptides are nonorthogonal and can form four different heterodimers that display large differences in affinities, which in turn promotes thermodynamic self-sorting. Here, we have conjugated these peptides to 4-armed star-shaped PEGs and investigated their assembly into supramolecular PEG-peptide networks and hydrogels (Figure 1). Four different PEG-peptide conjugates have been synthesized in order to address the hypothesis that mechanical properties of peptide-based hydrogels can be controlled using peptides with different affinities for dimerization. Additionally, we also explore peptide self-sorting using mutually complementary peptides to dynamically tune the self-assembly process of these nanomaterials. The proposed strategy offers means to control the interactions involved in self-assembly of peptide–polymer conjugates and consequently the possibilities to rationally tune dynamical and mechanical properties of the resulting materials.

## EXPERIMENTAL SECTION

### General

Maleimide functionalized 4-armed star-shaped poly(ethylene glycol) (MW = 10 kDa, PDI = 1.04) was purchased from JenKem Technology USA Inc., and is denoted PEG in this paper. PB denotes phosphate buffer (mono- and disodium phosphate, 10 mM, pH 7) unless otherwise stated. PBS denotes phosphate buffered saline (10 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl, pH 7.4).

Peptide concentrations were determined by UV absorbance of tryptophan residues (εTrp = 5690 M⁻¹·cm⁻¹) in a UV-2450 from Shimadzu. PEG-peptide conjugates were always mixed in 1:1 ratios unless otherwise stated.

Peptides. El-, Ev-, Kl-, and Kv peptides with a terminal Cys were ordered and custom-synthesized by GL Biochem Ltd. and used as received, whereas peptides without a terminal Cys were synthesized in house as described previously.33 Briefly, the in-house synthesis was performed using a standard Fmoc protocol with HCTU as the coupling agent. The peptides were purified by reverse phase HPLC and identified by MALDI-ToF spectroscopy with α-cyano-4-hydroxycinnamic acid as the matrix.

PEG–Peptide Conjugation. Cys-containing peptides were dissolved in PBS and reduced in molar excess of dithiothreitol (DTT) for 30 min. DTT was subsequently removed by size exclusion on a PD MidTrap G-10 from GE Healthcare according to manufacturer’s protocol. Reduced peptides were immediately eluted into a solution of PEGs (2:1 ratio of peptide:maleimide). Samples were reacted overnight in PBS, purified by dialysis (MWCO 6–8 kDa, Spectra/Por 4 from Spectrum Laboratories, Inc.) and finally freeze-dried to dry powder. 1H NMR was recorded on a Varian 300 spectrometer and used to verify the conjugation of peptides by the disappearing maleimide (300 MHz, D₂O, δ): 6.86 (s, 2H, C–H), and introduction of new peaks from peptide residues (e.g., Trp (300 MHz, D₂O, δ): 6.86 (s, 2H, C–H), and DCl was added to pK₁₄ and pKᵥ₄, whereas NaOD was added to pE₁₄ and pEᵥ₄, to increase solubility.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded using a Chirascan Spectropolarimeter from Applied Photophysics with a TC 125 temperature controller from Quantum Northwest. CD spectra were assessed from 195 to 250 at 0.5 nm steps. Thermal stability curves were measured by recording complete spectra in steps of 5 °C from 5 to 90 °C at a rate of roughly 5°C per hour. The melting temperature (Tm) was estimated by calculating the derivative of each thermal stability curve. All data were converted to mean residue ellipticity (MRE). All samples were measured at 0.005% (w/v), equivalent to a total peptide concentration of 10 μM, dissolved in PB.

Dynamic Light Scattering (DLS). DLS was measured on a ZetaSizer Nano ZS90 from Malvern Instruments Inc. All individual PEG-peptide conjugates were filtered through 0.2 μm low protein binding filters prior evaluation. Samples were mixed and measured over 4 h for each experiment. Temperature was kept at 20°C and samples were measured at 0.125% (w/v), equivalent to a total peptide concentration of 250 μM in PB.

Turbidity Measurements. The turbidity was measured by recording absorbance spectra with a UV-2450 from Shimadzu. A spectrum was acquired every hour for 12 h, and an aggregation index was calculated by the ratio of A₅₆₀/A₃₉₀.

Light Microscopy. Light micrographs were captured using a Nikon ECLIPSE Ti microscope at 20X using phase contrast enhancement. PEG-peptide conjugates were imaged at 1% (w/v) in PB, equivalent to a total peptide concentration of 2 mM. Samples were incubated overnight before analysis.

Scanning Electron Microscopy. Samples were imaged using a Leo Gemini from Zeiss with an acceleration voltage of 5 kV. The
morphology of the PEG-peptide assemblies was fixed with 1% glutaraldehyde overnight at 4 °C. Samples were sequentially chemically dried using increasing concentrations of ethanol in water (50, 70, 80, 90, 100%) and finally 100% hexamethyldisilazane for 15 min each. Samples were sputter coated with platinum prior to imaging.

**Rheology.** Rheology measurements were carried out on a MCR 102 rheometer from Anton Paar using a 25 mm 1° cone plate at 20 °C. Sample concentration was varied from 1 to 2.5% (w/v). Gel properties were monitored via (1) frequency sweep measurements at fixed strain of 1%, and (2) amplitude sweep measurements at fixed angular frequency of 5 rad/s. Time-dependent gelling was assessed over 30 min each. Samples were sputter coated with platinum prior to imaging.

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**RESULTS AND DISCUSSION**

**Peptides and Peptide Conjugation to PEG.** The peptides EI, EV, KI, and KV (Table 1, Figure 2) are random coils as monomers and designed to heterodimerize and fold into parallel coiled coils at neutral pH. Complementary charged residues at the ε and g position, either glutamic acid (Glu, E) or lysine (Lys, K), favor heterodimerization. In order to vary the affinity for dimerization EI and KI have mostly isoleucine (Ile, I) at position a, whereas EV and KV have valine (Val, V) at this position. The Asn (N) at position a in the third heptad promotes parallel alignment and prevents out-of-register coils. All peptides contain a tryptophan (Trp, W) residue as a chromophore to accurately determine the concentration of peptides in the sample. The peptides are promiscuous and EI, EV, KI, and KV can be combined into four different heterodimers, EV/KV, EI/KV, EV/KI, and EI/KI, respectively. The different combinations show dissociation constants (K_d) spanning from the low micromolar to the picomolar range (Table S1). The large differences in affinities promote thermodynamic self-sorting, which makes it possible to obtain defined stoichiometries of different heterodimers in complex mixtures of peptides and to dynamically exchange peptides in specific and already formed dimers.

To enable peptide conjugation to the maleimide functionalized 4-arm-PEG, an off-heptad cysteine (Cys, C) residue was included at the peptide C-terminal in EI and EV and at the N-terminal in KI and KV. The formation of the thioether bond upon conjugation and complete absence of unreacted maleimides were verified by 1H NMR (Figure S1). The resulting PEG–peptide conjugates were denoted pEV_4, pEI_4, pKV_4, and pKI_4.

**Characterization of Peptide Secondary Structure.** It has previously been shown that coiled coils tend to retain their ability to fold when conjugated to linear poly(ethylene glycol). Pegylation of coiled coil peptides have even been shown to induce folding and to stabilize their secondary structure. Circular dichroism (CD) spectroscopy was used to investigate if conjugation of the peptides to the PEGs affected their ability to dimerize and fold. The peptide secondary structure of all four PEG-peptide conjugates, alone and when combined, was investigated (Figure 3, Table 2, Figure S2). The CD spectra of pEV_4 and pKV_4 showed a distinct minimum at 198 nm, indicating that the peptides are random coil at neutral pH (Figure 3A). By contrast, the CD spectra of pEI_4 and pKI_4 showed presence of α-helical secondary structure, but to varying degrees. pEI_4 showed only minor helicity with a melting temperature (T_m) well below 20 °C (Figure 3B). pKI_4 showed a substantial helicity with distinct minima at 208 and 222 nm. The ratio of the mean residue ellipticity at 222 and 208 nm (MRE_222/MRE_208) was about 0.98 at 20 °C, where a ratio close to 1 or higher is indicative of peptides folded into well-defined coiled coils. The estimated T_m was 43 °C, which is significantly higher than for the corresponding nonpegylated KI (Table S1). The increased helicity of the peptides in pKI_4 compared to the other PEG-peptide conjugates is likely due to the beneficial packing of the Ile residues in the hydrophobic core in combination with the abundance of Lys residues at the dimer interface. The conformational flexibility of the Lys side-chain allows the ε-amine groups to rearrange to maximize their separation enough for peptides to overcome interpeptide charge repulsion. This is less likely to occur for the shorter γ-carboxyls in Glu residues that are abundant in EI. EI is thus more effective in preventing homoassociation compared to KI. In addition, since the nonpegylated peptides only show small to no tendencies of folding and forming homomeric species, it can be concluded that PEG conjugation induces folding of the peptides in pEI_4 and pKI_4. It is, however, not clear from this result alone if the peptides in a PEG-peptide conjugate fold individually or associate intramolecularly within the conjugate, or if the peptide homoassociate intermolecularly with peptides on another PEG-peptide conjugate, thus forming larger networks.

To further study the folding of the peptides in the individual PEG-peptide conjugates, the pH was varied from 4 to 10 and the changes in helicity and T_m of the peptides were examined (Figure 4, Table S2). The corresponding nonpegylated peptides do homoassociate and fold at pH values close to their respective isoelectric point (pI). This is due to protonation/deprotonation of the amino acid residue side chains at ε and g positions, rendering them uncharged and thus reducing the intermolecular charge repulsion. EI and EV (pI ~ 4) homoassociate at acidic pH, whereas KI and KV (pI ~ 10.5) homoassociate at basic pH. As expected, pEI_4 showed a transition from random coil to more α-helical when lowering the pH. However, pKI_4 did not display any large increase in helicity when raising the pH from neutral to basic pH. A minor loss in helicity was seen at acidic pH, but the peptides did not

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**Table 1. Peptide Sequences with Corresponding Heptad Register**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>gabcabcdef</th>
<th>gabcabcdef</th>
<th>gabcabcdef</th>
<th>gabcabcdef</th>
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<td>EI</td>
<td>EIAALEK</td>
<td>EIAALEK</td>
<td>ENAALEW</td>
<td>EIAALEK</td>
</tr>
<tr>
<td>KV</td>
<td>KVSALKE</td>
<td>KVSALKE</td>
<td>KNSALKW</td>
<td>KVSALKE</td>
</tr>
<tr>
<td>EV</td>
<td>EVALEKE</td>
<td>EVALEKE</td>
<td>ENAALEW</td>
<td>EVALEKE</td>
</tr>
<tr>
<td>KI</td>
<td>KIAALEKE</td>
<td>KIAALEKE</td>
<td>KNAALKE</td>
<td>KIAALEKE</td>
</tr>
</tbody>
</table>

"Peptides for conjugation to 4-armed star-shaped PEGs also included an off-heptad Cys residue at the N-terminal in KI and KV and at the C-terminal in EI and EV.

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**Figure 2.** Helical wheel representation of the heterodimeric coiled coils.
completely unfold within the measured pH range. The lack of unfolding of peptides in pKI at acidic pH is probably a result of the conformational flexibility of the Lys ε-amine group as discussed above. Furthermore, a clear difference in maximum helicity could be noted between pEI and pKI close to their respective pI. This difference can be explained by the number of hydrogen bonds that can be formed between the ε and γ amino acid residues in two homoassociated peptides. At acidic pH, each Glu–Glu′ interaction between two homoassociated EI results in formation of two hydrogen bonds. By contrast, two homoassociated KI at basic pH can only form one hydrogen bond for each Lys–Lys′ interaction due to steric hindrance, thus making it less stable compared to two homoassociated EI at acidic pH. Similar behavior as for pEI and pKI regarding folding at different pH has been described in other linear PEG-coiled coil systems with similar peptide sequences.26 Furthermore, a similar trend as for pEI could be seen for pEV with increasing helicity at acidic pH. In contrast, pKV showed no tendencies to fold regardless of pH. This shows that the better hydrophobic core packing provided by Ile compared to Val in pKV, is essential for pKI to be able to homoassociate.

When mixing the different complementary PEG−peptide conjugates, an increased helicity was seen for all combinations (Figure 3C). CD spectra at 20 °C of pEI/pKI and pEI/pKV showed MRE222/MRE208 ratios of 1.00 and 0.99, indicative of well-defined coiled coils. The other two combinations, pEV/pKI and pEV/pKV, showed MRE222/MRE208 ratios that were slightly lower, 0.94 and 0.76, respectively at 20 °C. In the case of pEV/pKV, this is a result of the less favorable packing of the Val residues in the hydrophobic core compared to Ile. For pEV/pKI, on the other hand, the slightly lower ratio indicates that PEG conjugation of KI to a certain extent affects the association with EV in pEV.

Thermal denaturation experiments further confirmed that all complementary PEG−peptide conjugates folded as a result of peptide-mediated intermolecular interactions (Figure 3D). The thermal stabilities of the individual PEG-peptide conjugates were significantly lower as compared to when mixed with a complementary PEG−peptide conjugate. The assembly of pEI/pKI showed the highest thermal stability followed by pEI/pKV, both with Tm > 90 °C. The Tm of pEV/pKI was 73 °C and pEV/pKV was the least stable with a Tm of 51 °C. Except for pEV/pKI, substantially higher Tm values were

### Table 2. MRE222/MRE208 and Melting Temperatures (Tm) of the Individual and Combinations of PEG−Peptide Conjugates at pH 7

<table>
<thead>
<tr>
<th>PEG−peptide</th>
<th>MRE222 at 20 °C (10⁹ deg cm² dmol res⁻¹)</th>
<th>MRE222/ MRE208 at 20 °C</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pEI</td>
<td>-9.1</td>
<td>0.67</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>pEV</td>
<td>-3.8</td>
<td>0.39</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>pKI</td>
<td>-27.4</td>
<td>0.98</td>
<td>42.7 ± 0.2</td>
</tr>
<tr>
<td>pKV</td>
<td>-2.9</td>
<td>0.30</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>pEI/pKI</td>
<td>-34.9</td>
<td>1.00</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>pEV/pKI</td>
<td>-27.8</td>
<td>0.94</td>
<td>73.4 ± 0.2</td>
</tr>
<tr>
<td>pEI/pKV</td>
<td>-33.6</td>
<td>0.99</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>pEV/pKV</td>
<td>-14.3</td>
<td>0.76</td>
<td>51.3 ± 2.8</td>
</tr>
</tbody>
</table>

Figure 3. (A) CD spectra at 20 °C for individual PEG-peptide conjugates and (B) corresponding thermal denaturation curves. (C) CD spectra at 20 °C for combined complementary PEG-peptide conjugates and (D) corresponding thermal denaturation curves. Data points for curves in panel B and D can be found in the Supporting Information.

Figure 4. Mean residue ellipticity at 222 nm at different pH, demonstrating the pH-induced homoassociation of each individual PEG−peptide conjugate at 20 °C.

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obtained for the interacting complementary PEG-peptide conjugates as compared to the corresponding folded non-pegylated coiled coils (Table S1). The higher stability is likely a combined effect of pegylation and positive cooperativity caused by formation of a supramolecular PEG−peptide network. The affinities and thermal stabilities of the nonpegylated coiled coils EI/KV and EV/KI are very similar, but interestingly the thermal stabilities of the corresponding PEG-peptide conjugates differ. Since peptides in pKI4 are already folded, and thus most likely homoassociated to some extent, interactions with the complementary peptides in pEV4 are energetically less favorable as compared to the corresponding interaction between pEI4 and pKV4. The thermal stability of pEV4/pKV4 is, however, higher than for the corresponding nonpegylated coiled coils and pKI4 alone, indicating that the peptides indeed heterodimerize to some extent when combined.

**Self-Assembly of PEG−Peptide Networks.** The ability for the PEG−peptide conjugates to self-assemble into larger PEG−peptide networks was investigated using dynamic light scattering (DLS). DLS measurements were carried out at relatively low concentrations of the PEG−peptide conjugates (0.125% w/v, 250 μM total peptide concentration) to avoid formation of nonergodic hydrogels, which would complicate the analysis. This concentration was, however, still high enough to readily promote and follow the self-assembly process of the PEG−peptide conjugates into larger supramolecular PEG−peptide networks. None of the individual PEG−peptide conjugates homoassociated intermolecularly into large assemblies at neutral pH as indicated by the single relaxation mode at short lag times (Figure 5). When mixing pEV4 and pKV4 the correlation function progressively shifted toward longer lag times with two modes of relaxation (Figure 5D). This suggests a slowing of solution dynamics caused by the formation of larger assemblies with coexisting populations of smaller assemblies. Similar results has previously been reported for assemblies of peptides conjugated to N-(2-hydroxypropyl)methacrylamide.19

The dissociation constant ($K_d$) of the nonpegylated peptide heterodimers are $K_d$(EI/KI) < 0.1 nM, $K_d$(EI/KV) and $K_d$(EV/KI) ≈ 10 nM, and $K_d$(EV/KV) ≈ 1 μM. When all four nonpegylated peptides are combined, the highest and lowest affinity dimers (EI/KI and EV/KV) form at the cost of those

![Figure 5](https://example.com/figure5.png)

Figure 5. (A−C) Schematic representation and (D−K) DLS correlation functions showing the self-assembly and self-sorting of the PEG−peptide conjugates. (D−F) Mixing pEV4 and pKV4 induces self-assembly and formation of a supramolecular PEG network, as seen by increased lag times. Addition of the nonpegylated peptide EI disassembles the PEG−peptide network due to formation and self-sorting into the more thermodynamically favored 4EI/pKV4 over pEV4/pKV4. Addition of nonpegylated KI induces further self-sorting and formation of the thermodynamically favored EI/KI over 4EI/pKV4. This makes pKV4 available to associate with pEV4 resulting in reassembly of the pEV4/pKV4 network. (G−K) Similar as for (D−F) but with other PEG−peptide conjugate combinations. *In (A−C) only one PEG-arm is shown for clarity.
with intermediate affinities (EI/KV and EV/KI) because of the large differences in affinities for heterodimerization.\textsuperscript{33} Interestingly, this ability of the peptides to self-sort was retained when conjugated to PEG. This is indicated by the almost instant disassembly of the pEV\textsubscript{4}/pKV\textsubscript{4} networks upon addition of nonpegylated EI (125 \textmu M), seen as a change from two to one mode of relaxation with lag times comparable to the individual PEG-peptide conjugates (Figure 5E). The nonpegylated EI
thus penetrates the PEG–peptide network and scavenge pKV4 to form the more thermodynamically favored 4EI/pKV4. Subsequent addition of nonpegylated KI (125 μM) leads to formation of EI/KI heterodimers, allowing reassembly of the pEV4/pKV4 network (Figure 5F). The same result was seen when adding nonpegylated KI prior to nonpegylated EI (Figure S3). The disassembly of the initial network by nonpegylated KI was not as rapid as with EI, probably due to the slightly higher stability of KI homomers.

Self-assembly into PEG-peptide networks and self-sorting were seen for both pEV/pKI and pEI/pKV. For pEV/pKI, there was an instant increase in lag times with a progression from one to two modes of relaxation (Figure 5G). Addition of nonpegylated EI instantly disassembled the pEV4/pKI4 network (Figure 5H). pEI/pKV did also show a progressive growth of a PEG–peptide network, which could be disassembled by addition of nonpegylated KI (Figure 5I). However, pEL/pKV4 did only show one mode of relaxation as the network was formed. This was also the case for pEI/pKI4 (Figure 5K). The absence of larger species, as indicated by the single mode of relaxation at relatively short lag times, for these combinations is likely due to assembly of structures that precipitated during the time course of the measurements and thus were too large to be detected by DLS. To verify this, the turbidity changes were monitored over time by measuring the absorbance of the samples. An aggregation index was calculated as the ratio of the monitored over time by measuring the absorbance of the relaxation at relatively short lag times, for these combinations is absence of larger species, as indicated by the single mode of

Formation of Hydrogels. Since pEL/pKI and pEI/pKV4 did assemble into microscopic PEG-peptide networks already at fairly low concentrations (0.125% w/v), it was possible to induce formation of hydrogels by increasing the concentration above 1% (w/v). The pEV4/pKV4 and pEV4/pKI4 did not assemble into hydrogels at these concentrations. Tabletop rheology showed that hydrogels comprised of pEI4/pKI4 were markedly more rigid than hydrogels of pEL4/pKV4 at the same concentration (Figure 7A–C).

Bulk oscillatory rheology was employed to further characterize the viscoelastic properties of the pEI4/pKI4 and pEL4/pKV4 hydrogels. Gel strengths and viscoelastic behavior were assessed by frequency sweep measurements and confirmed the observations from the table-top rheology (Figure 7D,E). Hydrogels formed from pEL4/pKV4 showed storage moduli (G′) of about 200 Pa, whereas G′ for pEI4/pKI4 hydrogels was about a factor five higher, 1000 Pa, at 2.5% (w/v). This G′ interval spans the range of most coiled coil-based hydrogels previously reported 32,35 Furthermore, pEL4/pKI4 showed a typical solid gel-like behavior (G′ > G″), whereas pEL4/pKV4 instead showed a liquid-like behavior (G′ < G″) at low frequencies with a cross point at 0.5 rad/s. Both gels exhibited, as expected, a decrease in gel strength with decreasing PEG-peptide conjugate concentrations. Furthermore, the hydrogels were formed almost instantaneous since more than 50% of the final G′ was reached within 15 s after mixing the two PEG–peptide conjugates (Figure 7F,G). This is more rapid than, for example, the beta-hairpin MAX1 hydrogel at comparable concentrations. 6 One hallmark of supramolecular hydrogels is shear thinning behavior where the viscosity decrease with increasing strain rate due to the disruption of supramolecular cross-links by the applied strain. 39,40 However, they can typically also quickly recover their viscoelastic properties when the strain is discontinued since the supramolecular interactions can reform. This is not seen in chemically cross-linked polymeric networks. 32 As expected, both hydrogels showed a decrease in viscosity as more strain was applied (Figure S5A,B). Furthermore, both hydrogels also showed quick recoveries at concentrations of 2.5 and 2.0% (w/v) (Figure S5C,D), whereas at lower concentrations the hydrogels did not fully recover during the time course of the measurement.

■ CONCLUSIONS

In this work, we have investigated the possibility to modulate the self-assembly process of polymeric networks and hydrogels, using four different heterodimeric coiled coil peptides with different, but defined, affinities for dimerization. The peptides retained their ability to dimerize and fold into coiled coils when conjugated to 4-armed star-shaped PEGs. Depending on the combinations of peptides used, varying assembly kinetics and temperature stabilities were observed. Peptides with high affinities for dimerization resulted in rapid assembly of large supramolecular networks with high thermal stability (Tm > 90 °C), whereas peptides with lower affinities resulted in smaller and less stable assemblies. Hydrogels were obtained at concentrations above 1% (w/v) for two combinations of PEG–peptide conjugates. The self-assembly into PEG–peptide networks were rapid and commenced immediately when complementary PEG–peptide conjugates were mixed, and shear thinning- and self-healing hydrogels were formed within seconds to minutes. The storage modulus of the hydrogels was about 200 Pa when using coiled coils with nanomolar affinities, and about a factor of five higher, 1000 Pa, when using peptides with picomolar affinities. In addition to the possibility to generate hydrogels with different rheological properties, the large differences in affinities for peptide dimerization did enable dynamic modulation of the self-assembly process by means of peptide self-sorting. By sequential additions of nonpegylated peptides with increasing affinities for dimerization, it was possible to disassemble and reassemble the supramolecular PEG–peptide networks. Hence, the possibility to combine multiple complementary coiled coils opens up for numerous strategies to tailor hydrogel properties and to optimize their performance for various biomedical applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioMac.6b00528.

Additional tables, 1H NMR of PEG-peptide conjugates, CD-data and thermal stability curves of individual and
mixed PEG-peptide conjugates at different temperatures and pH, additional DLS-data, turbidity measurements, additional rheology data, purity and identity of non-pegylated EI and KI (PDF)

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Notes
The authors declare no competing financial interest.

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■ REFERENCES

(1) Seliktar, D. Science 2012, 336, 1124−1128.