How to cite: *Angew. Chem. Int. Ed.* **2022,** *61,* e202201791
International Edition: doi.org/10.1002/anie.202201791
German Edition: doi.org/10.1002/ange.202201791

Peptide Hydrogels | Hot Paper

Mediating Oxidation of Thioethers with Iodine—A Mild and Versatile Pathway to Trigger the Formation of Peptide Hydrogels

*Benedikt P. Nowak, Lisa Schlichter, and Bart Jan Ravoo**

Abstract: The development of redox-triggerable peptide hydrogels poses fundamental challenges, since the highly specific peptide architectures required inevitably limit the versatility of such materials. A powerful, yet rarely applied approach to bypass those barriers is the application of a mediating redox reaction to gradually decrease the pH during hydrogel formation. We report a versatile strategy to trigger the formation of peptide hydrogels from readily accessible acid-triggerable gelators by generating protons by oxidation of thioethers with triiodide. Adding thiodiglycol as a readily available thioether auxiliary to the basic precursor solution of a peptide gelator efficiently yielded hydrogels after mixing with triiodide, as studied in detail for Nap-FF and demonstrated for other peptides. Furthermore, incorporation of the thioether moiety in the gelator backbone via the amino acid methionine, as shown for the tailormade Nap-FMDM peptide, reduces the number of required additives.

Introduction

Over the last decade, hydrogels have emerged as one of most promising material classes within the field of soft matter. Traditionally such gels have been built up from covalent polymers, physically or chemically crosslinked to form the essential three-dimensional network that provides the structural integrity of the hydrogel.[1] Nowadays, polymeric hydrogels have been supplemented by low molecular weight analogues. Low molecular weight gelators (LMWGs) form hydrogels upon self-assembly of their molecular building blocks into nanofibrous aggregates which entangle and provide a sample-spanning three-dimensional network.[1,2] While molecular motifs suitable for application as LMWGs are virtually limitless, peptide-based hydrogels have become the most prominent as well as best studied representative of this type of self-assembling materials.[3] This development originates from their ease of preparation via solid-phase peptide synthesis (SPPS), which also allows for straightforward tailoring of the molecular properties since even small changes in the amino acid sequence may result in drastic changes of the assembly properties.[4] Furthermore, vast amounts of natural and modified amino acids are commercially available adding further possibilities of molecular engineering to this type of LMWGs.[3,5,6] Finally, beyond mutation of the gelator motive, manipulation of the chemical environment of the gel is yet another powerful possibility to tune the properties of the soft material.[7]

However, during the preparation of peptide gels special attention has to be paid to the process of gelation. While polymer gelators are usually simply swollen with water to form the corresponding hydrogel, LMWGs are much more sensitive in that regard. To achieve gelation, the LMWG is usually handled at a condition of high solubility and upon application of a trigger transferred into the less soluble, nanofibrous state.[8] The type of trigger used is critical for the material properties of the prepared hydrogel and researchers have extensively studied different pathways to induce the gelation of peptide gelators in order to gain control over the properties of the final material.[6] Here, application of a simple heating-cooling cycle or slow acidification of a basic solution of the LMWG as pioneered by Adams[9] are by far the most commonly used techniques to trigger the hydrogelation of peptide gelators. However, alternative methods such as the addition of calcium salts,[10] enzymatic reactions[11] to cleave solubilizing groups, or redox reactions have been employed successfully. To achieve the later, the redox chemistry of thiols and disulfides is often used since thiol moieties can be incorporated into peptide LMWGs via the amino acid cysteine. Prominent approaches include the reductive linearization of a cyclic pro-gelator,[12] cleavage of a highly solubilizing end group connected to the peptide via a disulfide bond[13] or cross-linking of nanofibers upon thiol oxidation to decrease the critical gelation concentration (CGC).[14] All of these LMWGs require a very specific molecular architecture centered around the optimal positioning of the crucial cysteine residue. The employment of a redox trigger therefore comes at cost of a challenging gelator development and a limited scope of application of such hydrogels.

To overcome this problem researchers have utilized auxiliary reactions that generate protons.[15] Here the gelation is still triggered by a redox input, but as the

[*] B. P. Nowak, L. Schlichter, Prof. Dr. B. J. Ravoo
Center for Soft Nanoscience and Organic Chemistry Institute, Westfälische Wilhelms-Universität Münster
Busso-Peus-Straße 10, 48149 Münster (Germany)
E-mail: b.j.ravoo@uni-muenster.de

decrease of pH is the actual reason for the self-assembly of the gelator and a structurally "simple" and easy to design pH triggerable low molecular weight peptide may be used. This approach has been pioneered by the group of Cameron.[16] In their study Fmoc-VG hydrogel films could be gelated on gold surfaces upon electrochemical oxidation of 1,4-hydroquinone to 1,4-benzoquinone. The oxidation of the auxiliary results in the generation of protons and the subsequent local decrease in pH triggers the gelation process from the alkaline solution. Exploiting the oxidation of 1,4-hydroquinone at the surface of an electrode to generate protons for the gelation of a peptide LMWG, several studies have been published over the last years allowing for various applications of this method.[17] Just recently Adams[18] and co-workers could show that also larger volumes of hydrogel may be realized using this technique. Based on a comparable approach Adams[19] could likewise show that basic solutions of Nap-FF may be gelled upon the addition of dopamine under ambient conditions. In that case, the reaction of dopamine with molecular oxygen to polydopamine as the final oxidation product provides the protons for the acidification of the gelator solution and therefore triggers the formation of the self-assembled gel.

Herein we present a novel, facile and universal approach to redox-triggered peptide hydrogels based on the acidification of a gelator precursor solution caused by the oxidation of thioethers with iodine. In addition, direct incorporation of the thioether moiety into the peptide backbone via methionine residues is possible. To the best of our knowledge a comparably simple yet versatile strategy for redox-triggered gelation is unprecedented.

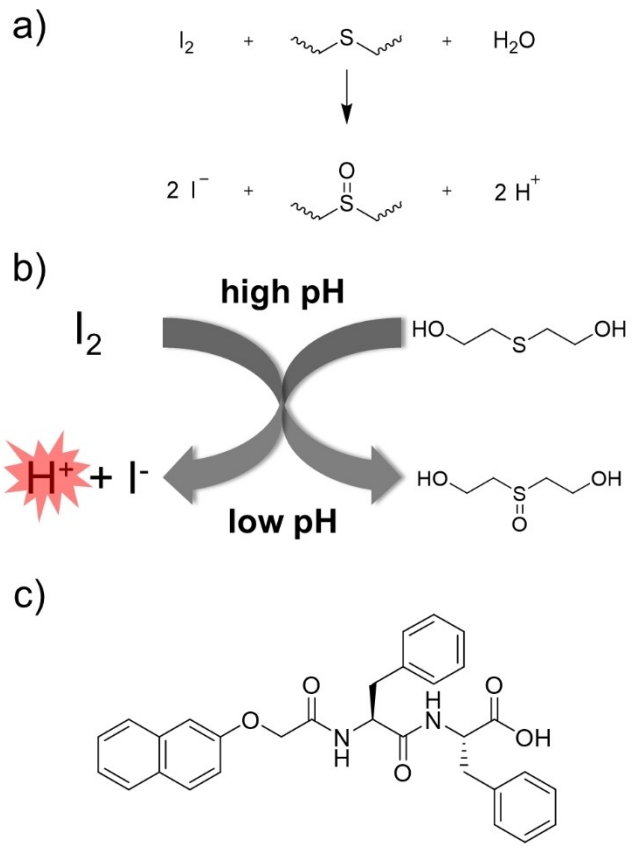

Figure 1. a) General scheme of the reaction of thioethers with iodine. b) Overview of the redox-mediated trigger utilizing the generation of protons during the oxidation of thiodiglycol to the corresponding sulfoxide with iodine. c) Structure of the model gelator Nap-FF.

Results and Discussion

Oxidation of a thioether to the corresponding sulfoxide using molecular iodine as an oxidation agent is a well-known method in organic chemistry and was already presented in 1966 by Higuchi[20] et al. The reaction of a thioether thereby follows the general reaction equation shown in Figure 1a. As evident from the equation, oxidation to the sulfoxide generates two molar equivalents of HI per thioether equivalent acidifying the reaction media with increasing turnover. Hence, a reaction of this type is potentially suitable for decreasing the pH of an alkaline precursor solution of an acid-triggerable gelator in a controlled manner triggering the gelation of the LMWG in the process. In a first step we therefore added an auxiliary thioether component to the basic precursor solution in order to provide sacrificial oxidizable moieties. Thiodiglycol was chosen as a prototypic auxiliary, since the thioether as well as the corresponding sulfoxide are highly water soluble and thiodiglycol is commercially available at low cost making the system applicable for a wide range of users. Nevertheless, we would like to mention that every other water soluble thioether moiety should be in principle suitable as an auxiliary to trigger the gelation widening the scope for this strategy. The working principle of the redox mediated triggering approach is schematically visualized in Figure 1b.

Since molecular iodine exhibits a very low water solubility in accordance with the original publication from Higuchi[20] et al. we used triiodide as a highly soluble iodine source. In all cases a triiodide stock solution of 1 M I_2 and 3 M NaI in MiliQ water was used referred to as a 1 M triiodide solution in the following.

As a well-established standard LMWG, the ubiquitous Nap-FF gelation motive as depicted in Figure 1c was used. Nap-FF is known to form self-supporting hydrogels using different triggering methods including the controlled acidification of an alkaline micellar gelator solution essential for the use within this redox mediated approach.[21,22] Furthermore, Nap-FF is not prone to oxidation with iodine making it perfectly suited as a model compound to demonstrate the functionality of the method. The peptide was synthesized via standard SPPS conditions using naphthoxy acetic acid as the final compound. Further information on the synthesis of Nap-FF is provided in the Supporting Information.

Hydrogels were prepared by dissolving 0.5 wt% Nap-FF ($\hat{=}$10 mM) in alkaline MiliQ (2 µL 12 M NaOH/ml, pH≈12) and adding 1 vol% of thiodiglycol ($\hat{=}$96 mM). Subsequent addition of 1 M triiodide solution lead to the formation of inversion stable hydrogels as depicted in Figure 2a. With just 0.5 vol% of 1 M triiodide solution (corresponding to a 5 mM final solution), self-supporting hydrogels at pH≈6.5

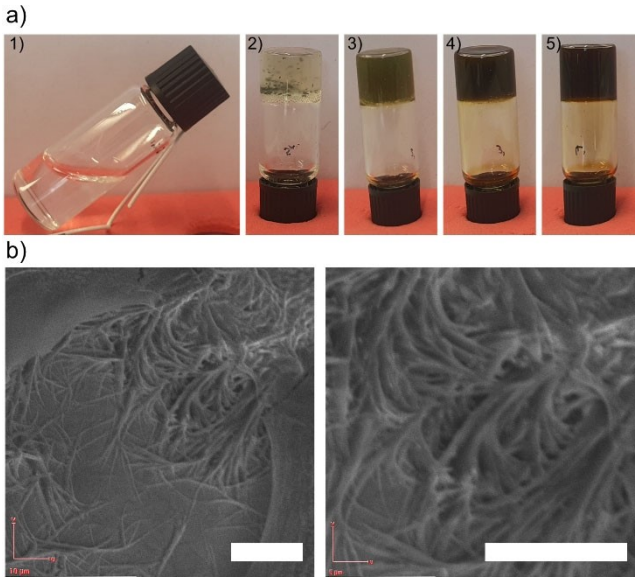

Figure 2. a) Critical triiodide concentration of a 0.5 wt% Nap-FF (≙10 mM) hydrogel with 1 vol% thiodiglycol (≙96 mM) triggered with 1) 0.25 vol% (≙2.5 mM), 2) 0.5 vol% (≙5 mM), 3) 1.5 vol% (≙15 mM), 4) 3 vol% (≙30 mM) and 5) 5 vol% (≙50 mM) 1 M triiodide solution. b) SEM images of a 0.5 wt% Nap-FF (≙10 mM) hydrogel with 1 vol% thiodiglycol (≙96 mM) triggered with 1 vol% (≙10 mM) 1 M triiodide solution. Scale bar = 20 μm.

could be obtained. The decrease in pH as well as the formation of the gel indicate that the redox mediated triggering concept is viable. Furthermore, the rather low concentration of triiodide (ca. 1 molar equivalent) needed underlines the power of this approach. HPLC-MS analysis (Figure S1) of the samples revealed no degradation of the Nap-FF gelator, as expected from the molecular structure. The dark coloration of the gels at higher triiodide concentration is attributed to the remaining triiodide, well-known for its dark brownish colour. The fact that triiodide remains unreacted in the gel may surprise at first glance since an excess of thiodiglycol (96 mM concentration in the precursor solution) is present in all samples. However, as already explained by Higuchi[20] et al. the decreasing pH goes hand in hand with a decrease in reaction rate and the reaction comes to a complete halt when the sample turns too acidic (pH ≈ 4). In addition, we would like to note that even though colourless gels are commonly preferred, the presence of remaining triiodide may be beneficial for certain applications as it should provide the gel with antimicrobial properties.[23] Furthermore, treatment with a scavenging, oxidation sensitive additive or use of alternative oxidants may open up ways towards colourless, triiodide-free gels within future developments based on this approach. To evaluate the oxidation state of the thioether auxiliary, we performed mass spectrometry on a gelator free reference sample (Figure S2). The thiodiglycol sulfoxide is the major oxidation product, but a small amount of the sulfone can also be detected.

Continuing from this successful proof-of-principle, we varied the concentration of thiodiglycol at a fixed triiodide concentration of 2.5 vol% 1 M triiodide solution (corresponding to 25 mM in the gel). The photographs of the related inversion tests are provided in Figure S3. With merely 0.07 vol% (≙7 mM) of thiodiglycol, self-supporting hydrogels can be obtained. The possibility of reducing the amount of the auxiliary to this extent once again stresses the high potential of this approach. Furthermore, the CGC of the hydrogel triggered with 1 vol% thiodiglycol (≙96 mM) and 2.5 vol% 1 M triiodide solution (≙25 mM) could be determined at ≈0.2 wt% as evident from the inversion tests depicted in Figure S4. As shown by the group of Adams,[22] the use of a standard acid trigger such as glucono-δ-lactone likewise yields soft materials with a CGC of ≈0.2 wt% (≙ 4 mM). Thus, even if thiodiglycol (7 mM) and triiodide (5 mM) are required in nearly stoichiometric amounts compared to Nap-FF (10 mM) for redox mediated triggering, the additives do not interfere with the gelation in a sense that significantly weaker hydrogels would be obtained.

To gain further insights into the network architecture of the hydrogels, SEM measurements of the dried samples were performed. The corresponding micrographs are shown in Figure 2b. Here the typical network structure of self-assembled peptide hydrogels is observed. The samples show fibrillar structures which are entangled or merged with neighbouring fibres forming a dense three-dimensional network with distinct pores.

To analyze the mechanical properties of the hydrogels triggered using the redox mediated approach we employed oscillatory rheology. The frequency sweep data of a 0.5 wt% (≙10 mM) hydrogel triggered with 1 vol% thiodiglycol (≙ 96 mM) and 2.5 vol% 1 M triiodide solution (≙25 mM) are provided in Figure 3a. The amplitude sweeps to determine the linear viscoelastic range of the material are reported in Figure S5. As evident from the black data points in Figure 3a the redox-triggered Nap-FF hydrogel shows the characteristics of a viscoelastic solid as expected for such types of gelatinous materials. The storage modulus governs the loss modulus by an order of magnitude and the moduli are frequency independent over the entire range of measured frequencies. This underlines the formation of true and rather stiff hydrogel with a storage modulus of ≈ 20000 Pa. When analysing the reference samples given in blue and red with either component of the trigger missing, the typical rheological behaviour of a worm-like micellar solution is observed.[24] Furthermore, it becomes evident that thiodiglycol as well as triiodide are essential for a successful gelation. This once again stresses the hypothesis that no side reactions occur and the observed gelation is solely caused by the pH decrease in the course of the oxidation of the thioether moiety.

To investigate the possibility of tuning the material properties using the redox trigger, we analyzed the dependence of the final material pH on the concentration of triiodide. We investigated a stirred solution of 0.5 wt% Nap-FF (≙10 mM) with 1 vol% thiodiglycol (≙96 mM) on a pH meter and noted the pH after addition of the corresponding volumes of 1 M triiodide solution. To ensure completion of the reaction especially in the later stages the solution was stirred for 24 h before taking the respective pH value. The

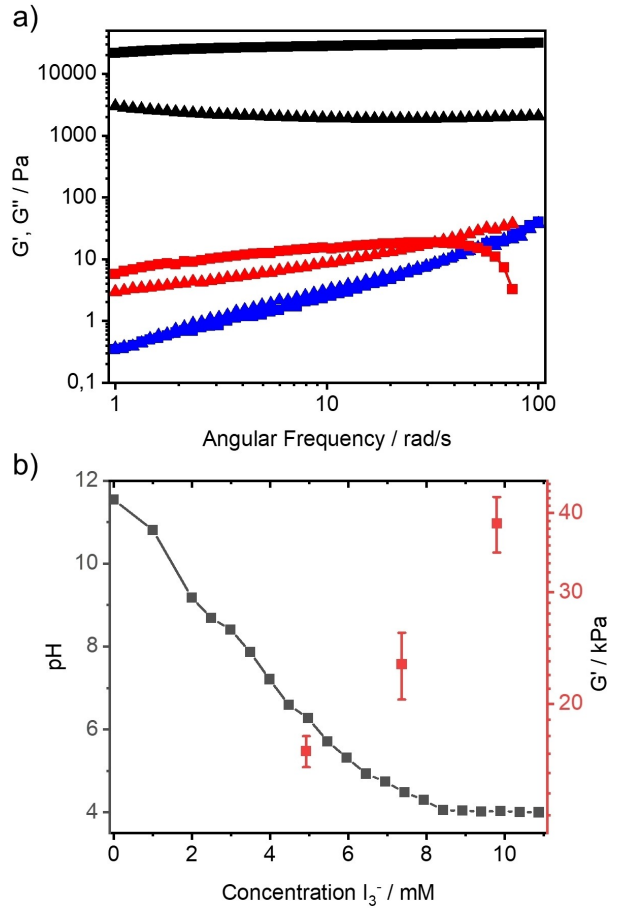

Figure 3. a) Frequency sweep curves measured at 0.5 % strain and 20 °C. Black data points: 0.5 wt% Nap-FF (≙10 mM) hydrogel with 1 vol% thiodiglycol (≙96 mM) triggered with 2.5 vol% 1 M triiodide solution (≙25 mM); red data points: reference sample without thiodiglycol; blue data points: reference sample without triiodide. b) pH dependence of a 0.5 wt% Nap-FF (≙10 mM) sample with 1 vol% thiodiglycol (≙96 mM) on the concentration of added triiodide and mechanical properties of selected, corresponding hydrogels plotted on the second axis. Storage modulus data averaged over the plateau region in frequency sweep measurements with the error bars reflecting the standard deviation.

resulting graphic visualizing the pH in depending on the triiodide concentration can be found in Figure 3b. As expected the pH decreases strongly from pH≈12 with increasing triiodide concentration reaching a plateau at pH ≈4. The fact that a plateau is reached even though a large excess of unreacted thiodiglycol (95.6 mM concentration in the precursor solution) is present supports the hypothesis of a deceleration and halt of the reaction at lower pH. Irrespective of that, the data show that the pH of the material can be precisely controlled by the applied concentration of triiodide. It should be noted that the data from stirred samples are not necessarily identical to the gel state since the difference in the architecture will influence the properties of the gelator causing changes in the pH. However, when comparing the pH of the gels with those predicted from the stirred solution a very high agreement was generally observed indicating those effects may be neglected.

Since the pH is known to be a key factor in terms of the mechanical properties of peptide hydrogels, we measured the rheological properties of distinct samples with different concentrations of triiodide. The corresponding storage moduli extracted from frequency sweep measurements are shown on the second axis of Figure 3b. Again, the expected behaviour is found as an increase in hydrogel stiffness with a decreasing pH caused by elevated concentrations of triiodide can be observed. Therefore, the mechanical properties going hand in hand with the pH of the hydrogel can be precisely manipulated by the addition of different amounts of triiodide facilitating the usability of the gels in different applications.

Next, we studied the gelation kinetics of the redox-triggered Nap-FF hydrogel. For this purpose, a 0.5 wt% Nap-FF (≙10 mM) pre-solution with 1 vol% of thiodiglycol (≙96 mM) was mixed with 2.5 vol% 1 M triiodide solution (≙25 mM) and immediately transferred onto the rheometer set to operate at constant dynamic mechanical conditions. Shear strain and frequency were chosen to show minimal interreference with the gel formation. The obtained time sweep data are provided in Figure 4a). During the first 20 min a strong increase of the moduli is observed, which slowly levels off and terminates after 80 min in quasi-plateau with a storage modulus of ≈10000 Pa. While both moduli show a strong increase a higher slope is found for the storage modulus indicating the rising elastic proportion of the material during the self-assembly of the network structure. These data imply that the main part of network formation is finished in less than 1 h however small changes in the material properties are found to happen even after finishing the measurement after 80 min. To put the obtained kinetics of gelation into perspective, we subsequently analyzed the corresponding change in the pH of the sample. Again, a stirred solution/dispersion was used in order to facilitate measurement of the pH. Figure 4b shows the time dependency of the pH in a system of similar composition. The pH curve nicely reflects the behaviour found in the rheological investigations. Over the first 10 min to 20 min a sharp decrease in pH is found, which gradually levels off after 1 h. After 24 h the pH remains unchanged pointing out the halt of the reaction. The fact that the extremely steep decrease in pH over the first minutes does not transfer entirely onto the rheological data is probably caused by the reduced diffusion speed of the reactive species in the gel state slightly slowing down the acidification and thus the gel formation. Nevertheless, the good agreement of the time-scales of pH decrease and hydrogel stiffness increase once again manifests the acidification as the driving force for the gelation while being caused by the mediating redox reaction.

In order to demonstrate that the trigger with triiodide and thiodiglycol is universally applicable to pH-triggerable hydrogelators not prone to oxidation or interfering with one of the additives, we tested various literature-known peptide gelators available to our laboratory. In each case, 1 vol% of thiodiglycol (≙96 mM) was added to the alkaline precursor solutions and hydrogelation was triggered by the addition of

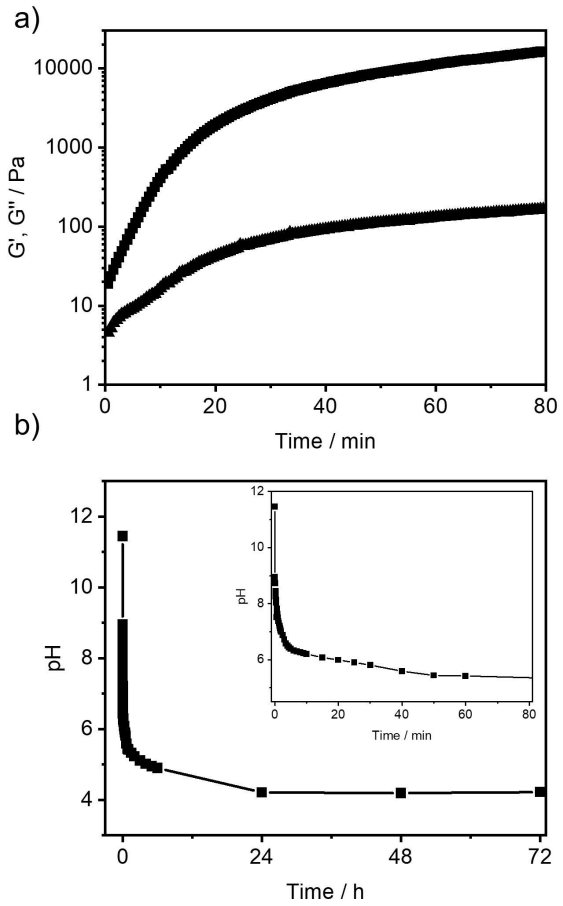

Figure 4. a) Time sweep of a 0.5 wt% Nap-FF (≙10 mM) hydrogel with 1 vol% thiodiglycol (≙96 mM) triggered with 2.5 vol% 1 M triiodide solution (≙25 mM) measured at constant dynamic mechanical conditions of 0.5% shear strain, 5 rad/s frequency and 20 °C. b) Time-dependent analysis of the pH value of a stirred 0.5 wt% Nap-FF (≙10 mM) sample with 1 vol% thiodiglycol (≙96 mM) and 2.5 vol% 1 M triiodide solution (≙25 mM).

2.5 vol% 1 M triiodide solution (≙25 mM). All samples showed a final pH of 4–4.5.

To characterize the successful gel formation frequency sweep tests of the samples were performed after resting overnight. The averaged data over the plateau region of the moduli are provided in Table 1. All samples show dominating elastic properties and loss factors of ≈0.1 indicative for the formation of true hydrogels. It can therefore be concluded that the redox trigger with thiodiglycol and triiodide is well suited for the universal gelation of manifold acid-triggerable gelators.

In order to widen the scope of the redox trigger we reduced the complexity of the system by transferring the oxidizable unit into the gelator. As an abundant platform for the modification of a peptidic gelator with thioether moieties the natural amino acid methionine was chosen. To prove the approach the novel gelator sequence Nap-FMDM was synthesized via standard SPPS. Detailed information on synthesis and characterization are provided in the Supporting Information. Along with the molecular structure of this

Table 1: Rheological data of 0.5 wt% hydrogels (2.0 wt% for Nap-FS) with 1 vol% thiodiglycol (≙96 mM) triggered with 2.5 vol% 1 M triiodide solution (≙25 mM) averaged over the plateau region of frequency sweep measurements at 0.5% strain and 20 °C.

Gelator[a]	G' [kPa]	G'' [kPa]	tan(δ)
Nap-GFYE	26,9 ± 4,8	3,3 ± 0,2	0,13 ± 0,03
Nap-GFFYS	3,6 ± 0,4	0,3 ± 0,0	0,08 ± 0,02
Nap-FGDS	43,3 ± 5,5	3,3 ± 0,7	0,08 ± 0,03
Nap-FS (2 wt%)	1,1 ± 0,2	0,2 ± 0,04	0,14 ± 0,01
Nap-GFYE	12,6 ± 0,9	0,8 ± 0,2	0,07 ± 0,02

[a] Gelator references: Nap-GFYE,[25] Nap-GFFYS,[26] Nap-FGDS (amino acid sequence taken from ref. [27]), Nap-FS[28] and Nap-GFYE.[29]

tailor made LMWG the working principle of the redox mediated trigger when incorporating the oxidation site into the gelator sequence is visualized in Figure 5a. Starting from an alkaline precursor solution of Nap-FMDM, addition of iodine as an oxidation agent (again in form of 1 M triiodide solution) the formation of the corresponding methionine-sulfoxide will generate protons subsequently triggering the hydrogelation of the LMWG. We like to note that this reduction in complexity imposes challenges in gelator development as mentioned in the introduction since methionine residues have to be incorporated into the LMWG structure. Furthermore, it is essential that the corresponding sulfoxide, which presents the actual gelating species, is still capable of self-assembly. As pointed out by Besenius[30] and

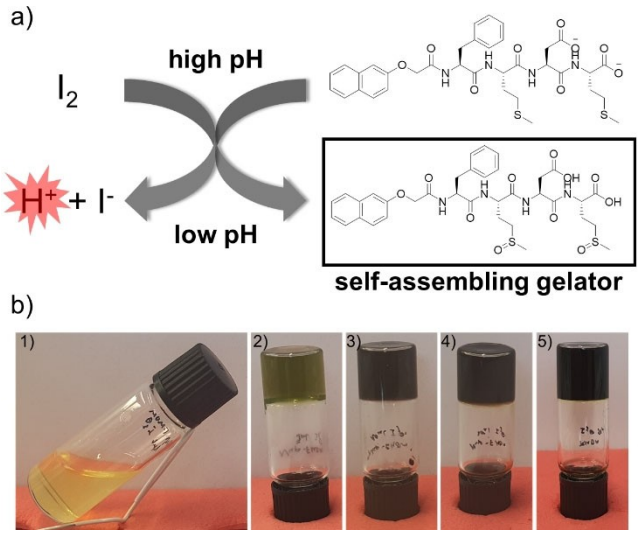

Figure 5. a) Overview of the redox-triggered formation of hydrogels utilizing the generation of protons in the process of the oxidation of the methionine residues on the tailormade peptide gelator Nap-FMDM to the corresponding sulfoxide. b) Critical triiodide concentration of a 0.5 wt% Nap-FMDM (≙7 mM) hydrogel triggered with 1) 0.1 vol% (≙1 mM), 2) 0.5 vol% (≙5 mM), 3) 1 vol% (≙10 mM), 4) 2 vol% (≙20 mM) and 5) 5 vol% (≙50 mM) 1 M triiodide solution.

co-workers, formation of methionine sulfoxide may actually hinder the aggregation of the molecular building blocks due to the increased hydrophilicity.

Figure 5b shows the inversions tests of 0.5 wt % Nap-FMDM (≙7 mM) hydrogels triggered upon the addition of different volumes of 1 M triiodide and after resting overnight. We would like to note that we are aware of the fact that rather than Nap-FMDM itself its oxidation products are largely responsible for the gelation, so that the term "Nap-FMDM gel" is strictly speaking incorrect. However, as evident from the photographs, self-supporting hydrogels are forming already upon the addition of just 0.5 vol % 1 M triiodide solution corresponding to a critical triiodide concentration of 5 mM. The fact that inversion stable gels are obtained proves that the redox-triggered approach is also effective with the oxidizable moieties incorporated into the gelator. To further analyze the Nap-FMDM gel, mass spectrometry was performed on the dried hydrogel triggered with 5 vol % of triiodide (≙50 mM). The corresponding spectra with annotated signals can be found in Figure S6. To our surprise, it was found that even though an excess of triiodide is present, in addition to the di-oxidized peptide (disulfoxide or monosulfone) prominent peaks of the non-oxidized and mono-oxidized peptide (monosulfoxide) can be detected. We attribute this to the aforementioned termination of the redox reaction at low pH, which may be supported by a shielding of the thioether moieties inside the self-assembled nanofibers of Nap-FMDM. To determine the ratio of the different species, HPLC-MS was performed and the corresponding chromatograms can be found in Figure S7. Based on the total ion chromatogram, assuming a comparable ionizability for all species, 13 % of Nap-FMDM remain unoxidized, while 29 % are mono-oxidized corresponding to the monosulfoxide. The largest signal at 54 % can be attributed to a di-oxidized species (either disulfoxide or monosulfone), while a second smaller peak at 4 % with the same mass to charge ratio corresponding to a likewise di-oxidized species is found at shorter retention times (t = 27.8 min). In accordance with the literature[31] and our previous analysis on the auxiliary thiodiglycol, we assume that the disulfoxide is the major oxidation product and the monosulfone is formed to a negligible extent. No signals corresponding to a sulfone-sulfoxide (triple-oxidized) or disulfone (quadruple-oxidized) peptide could be detected.

To further elucidate the self-assembly of the hydrogel, we performed circular dichroism (CD) and SEM measurements on the material (Figure 6). In the CD spectra a strong negative signal at $\lambda \approx 230$ nm along with an intense positive peak at $\lambda \approx 210$ nm is found (see Figure 6a blue data points). This signal pattern is in accordance with a spectrum expected for β-turn like secondary structure even though further analysis would be necessary to resolve the assembly structure of the gelator.[32] However, comparison with an alkaline sample (see red data points in Figure 6a) before addition of triiodide once again underlines the mechanism of oxidation induced pH decrease causing the self-assembly since at high pH the sample just shows neglectable signal intensities. SEM images of the dried gel show homogeneous network structures build up from entangled fiber bundles.

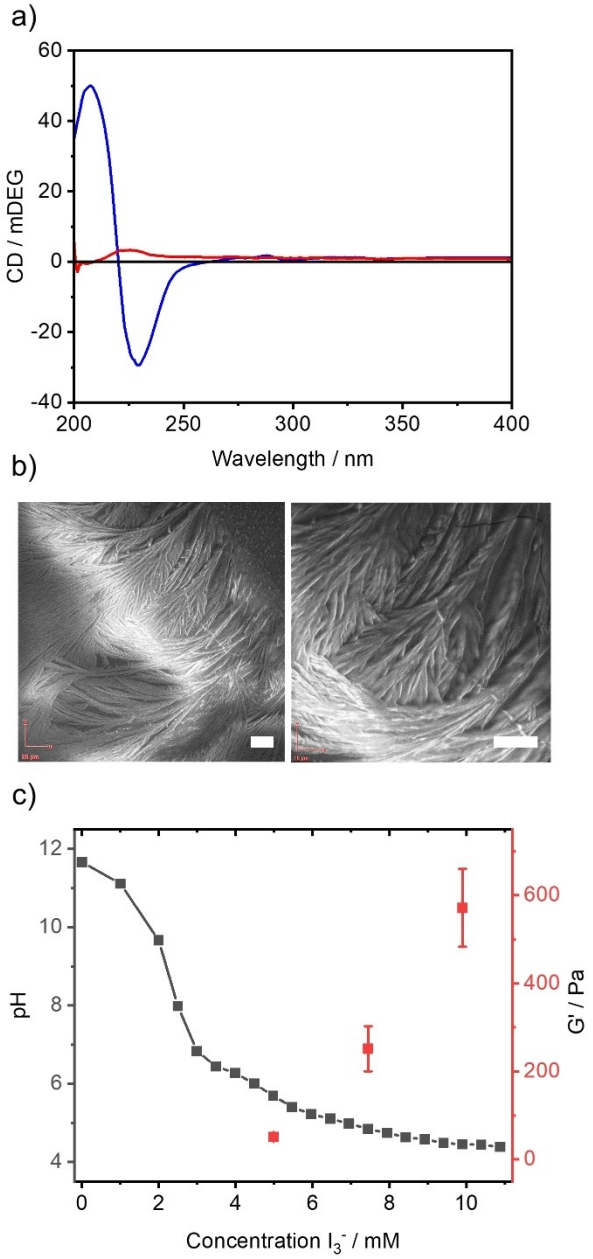

Figure 6. a) CD spectrum of Nap-FMDM; blue data points: 0.01 wt % Nap-FMDM (corresponding to: 0.14 mM) after oxidation with 0.05 vol % 1 M triiodide solution (corresponding to: 0.5 mM) over night (pH ≈ 6); red data points: alkaline reference sample of 0.01 wt % Nap-FMDM without triiodide addition (pH ≈ 12). b) SEM images of a 0.5 wt % Nap FMDM (≙7 mM) hydrogel triggered with 1 vol % 1 M triiodide solution (≙10 mM). Scale bar = 20 μm. c) pH Dependence of a 0.5 wt % Nap-FMDM (≙7 mM) on the concentration of added triiodide and mechanical properties of selected, corresponding hydrogels plotted on the second axis. Storage modulus data averaged over the plateau region in frequency sweep measurements with the error bars reflecting the standard deviation.

Inversion tests at a constant amount of 2.5 vol % triiodide (≙25 mM) added revealed a CGC of ≈0.2 wt % (≙ 3 mM) for the novel Nap-FMDM hydrogelator (Figure S8). To confirm the gelatinous characteristics of the material we

analyzed the mechanical properties of the hydrogel using oscillatory rheology.

The amplitude sweep curves can be found in Figure S9 while Figure S10 shows the frequency sweep data measured at 0.5 % shear strain. Here, as expected the rheological properties for a true hydrogel are observed showing a storage modulus of $\approx$9000 Pa which dominates the loss modulus by an order of magnitude and is frequency independent over the entire measured frequency range. In accordance to the additive based approach the gelation kinetics were likewise analyzed using time sweep measurements at constant dynamic mechanical conditions shown in Figure S11. Similar behaviour to the thiodiglycol/Nap-FF system is found in case of Nap-FMDM with a strong increase of the moduli over the first 10 min followed by a gradual decrease in slope terminating in a quasi-plateau showing just a slight incline after 50 min. Furthermore, the difference between the storage and the loss modulus expands over time indicating the formation of dominatingly elastic, solid-like material. Again, these data correlate nicely to the pH time course measurement of a stirred sample of the same composition (see Figure S12). When triiodide is added the pH rapidly drops from pH$\approx$12 to pH$\approx$6.5 within the first 10 min, levelling of over the following hour and showing a barely noticeable decrease after 60 min. As mentioned earlier, the good agreement of rheology and pH measurement are evidence for the oxidation driven acidification being the reason for the formation of a self-assembled hydrogel. Furthermore, the data obtained for Nap-FMDM and Nap-FF/thiodiglycol are rather similar, pointing out the successful complexity reduction by incorporation of the thioether moiety inside the gelator backbone without compromising the mechanical properties of the gel.

Finally, the possibility of tuning the hydrogel properties by varying the concentration of triiodide was assessed by the means of pH measurements of a stirred gelator solution/dispersion and frequency sweep rheology of the corresponding gels. The previously introduced double y-axis plot for a 0.5 wt % Nap-FMDM ($\hat{=}$7 mM) sample is shown in Figure 6c. As for the material the expected decrease of the pH is found with increasing the triiodide concentration which corresponds to more rigid hydrogels being formed. Furthermore, the gradual decrease of the pH within the region of interest below pH $\approx$ 7 indicated the possibility of finely tuning the acidity as well as the linked mechanical properties of the associated gels to the needs of the respective application. Again, it is to be mentioned that slight differences in pH between the gelatinous state and the stirred sample may arise due to the different assembly architectures. However, pH values measured with indicator strips in the gel state indicate a negligible variance between gel and stirred sample.

Conclusion

An innovative method to trigger the hydrogelation of peptide-based LMWG by translating a redox trigger into a controlled acidification has been presented. The redox reaction is based on the oxidation of a thioether moiety to the corresponding sulfoxide with iodine and generates protons in the process. When thiodiglycol is used as an auxiliary thioether, manifold acid-triggerable gelators can be gelated upon addition of triiodide, which acts as a water-soluble iodine source. As elucidated using Nap-FF as a model gelator, hydrogels are obtained with a high efficiency and the properties of the hydrogel can be fine-tuned by variation of the triiodide concentration. Furthermore, incorporation of the thioether moiety into the LMWGs backbone via the amino acid methionine provides a facile and accessible pathway to reduce the complexity of the system. In that case, the sole addition of triiodide is sufficient to trigger the gelation of the tailormade peptide gelator Nap-FMDM, while maintaining the highly adjustable characteristics of the resulting hydrogel. With this study we aim to provide a readily available and modular toolbox for researchers targeting redox-triggerable peptide hydrogels by circumventing the need for challenging LMWG development and utilizing a mediating reaction instead. We hope that this study aids the development of novel, functional soft materials and widens the application scope of peptide hydrogels.

Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (Ra 1732/14). Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Low Molecular Weight Gelators · Peptide Hydrogels · Redox Triggers · Self-Assembly · Soft Matter

[1] M. de Loos, B. L. Feringa, J. H. van Esch, *Eur. J. Org. Chem.* **2005**, 3615.
[2] a) X. Du, J. Zhou, J. Shi, B. Xu, *Chem. Rev.* **2015**, *115*, 13165; b) L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201; c) C.-W. Chu, C. A. Schalley, *Org. Mater.* **2021**, *03*, 25; d) B. O. Okesola, V. M. P. Vieira, D. J. Cornwell, N. K. Whitelaw, D. K. Smith, *Soft Matter* **2015**, *11*, 4768.
[3] J. Li, R. Xing, S. Bai, X. Yan, *Soft Matter* **2019**, *15*, 1704.
[4] a) S. Mondal, S. Das, A. K. Nandi, *Soft Matter* **2020**, *16*, 1404; b) G. Cheng, V. Castelletto, C. M. Moulton, G. E. Newby, I. W. Hamley, *Langmuir* **2010**, *26*, 4990.
[5] a) A. Dasgupta, J. H. Mondal, D. Das, *RSC Adv.* **2013**, *3*, 9117; b) N. Falcone, H.-B. Kraatz, *Chem. Eur. J.* **2018**, *24*, 14316; c) G. Fichman, E. Gazit, *Acta Biomater.* **2014**, *10*, 1671; d) L.

Li, L. Xie, R. Zheng, R. Sun, *Front. Chem.* **2021**, *9*, 739791; e) D. M. Ryan, B. L. Nilsson, *Polym. Chem.* **2012**, *3*, 18; f) Y. Zhang, M. Barboiu, *Chem. Rev.* **2016**, *116*, 809.
[6] E. R. Draper, D. J. Adams, *Langmuir* **2019**, *35*, 6506.
[7] a) T. N. Tikhonova, N. N. Rovnyagina, Z. A. Arnon, B. P. Yakimov, Y. M. Efremov, D. Cohen-Gerassi, M. Halperin-Sternfeld, N. V. Kosheleva, V. P. Drachev, A. A. Svistunov, P. S. Timashev, L. Adler-Abramovich, E. A. Shirshin, *Angew. Chem. Int. Ed.* **2021**, *60*, 25339; *Angew. Chem.* **2021**, *133*, 25543; b) S. Panja, B. Dietrich, D. J. Adams, *Angew. Chem. Int. Ed.* **2022**, *61*, e202115021; *Angew. Chem.* **2022**, *134*, e202115021; c) S. Panja, B. Dietrich, O. Shebanova, A. J. Smith, D. J. Adams, *Angew. Chem. Int. Ed.* **2021**, *60*, 9973; *Angew. Chem.* **2021**, *133*, 10061; d) A. Wu, Y. Guo, X. Li, H. Xue, J. Fei, J. Li, *Angew. Chem. Int. Ed.* **2021**, *60*, 2099; *Angew. Chem.* **2021**, *133*, 2127.
[8] J. Raeburn, A. Zamith Cardoso, D. J. Adams, *Chem. Soc. Rev.* **2013**, *42*, 5143.
[9] D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen, P. Sanderson, *Soft Matter* **2009**, *5*, 1856.
[10] L. Chen, G. Pont, K. Morris, G. Lotze, A. Squires, L. C. Serpell, D. J. Adams, *Chem. Commun.* **2011**, *47*, 12071.
[11] a) J. Gao, H. Wang, L. Wang, J. Wang, D. Kong, Z. Yang, *J. Am. Chem. Soc.* **2009**, *131*, 11286; b) H. Wang, C. Yang, M. Tan, L. Wang, D. Kong, Z. Yang, *Soft Matter* **2011**, *7*, 3897; c) J. Zhou, X. Du, J. Wang, N. Yamagata, B. Xu, *Front. Chem.* **2017**, *11*, 509.
[12] C. J. Bowerman, B. L. Nilsson, *J. Am. Chem. Soc.* **2010**, *132*, 9526.
[13] a) Y. Shi, J. Wang, H. Wang, Y. Hu, X. Chen, Z. Yang, *PLoS One* **2014**, *9*, e106968; b) C. Yang, D. Li, Z. Liu, G. Hong, J. Zhang, D. Kong, Z. Yang, *J. Phys. Chem. B* **2012**, *116*, 633.
[14] C. Cao, M. Cao, H. Fan, D. Xia, H. Xu, J. R. Lu, *Chin. Sci. Bull.* **2012**, *57*, 4296.
[15] S. Grigoriou, E. K. Johnson, L. Chen, D. J. Adams, T. D. James, P. J. Cameron, *Soft Matter* **2012**, *8*, 6788.
[16] E. K. Johnson, D. J. Adams, P. J. Cameron, *J. Am. Chem. Soc.* **2010**, *132*, 5130.
[17] a) J. Raeburn, B. Alston, J. Kroeger, T. O. McDonald, J. R. Howse, P. J. Cameron, D. J. Adams, *Mater. Horiz.* **2014**, *1*, 241; b) E. K. Johnson, L. Chen, P. S. Kubiak, S. F. McDonald, D. J. Adams, P. J. Cameron, *Chem. Commun.* **2013**, *49*, 8698; c) Y. Liu, Y. Cheng, H.-C. Wu, E. Kim, R. V. Ulijn, G. W. Rubloff, W. E. Bentley, G. F. Payne, *Langmuir* **2011**, *27*, 7380; d) Y. Liu, E. Kim, R. V. Ulijn, W. E. Bentley, G. F. Payne, *Adv. Funct. Mater.* **2011**, *21*, 1575.
[18] C. Patterson, B. Dietrich, C. Wilson, A. R. Mount, D. J. Adams, *Soft Matter* **2022**, *18*, 1064.
[19] E. R. Cross, S. M. Coulter, A. M. Fuentes-Caparrós, K. McAulay, R. Schweins, G. Laverty, D. J. Adams, *Chem. Commun.* **2020**, *56*, 8135.
[20] T. Higuchi, K.-H. Gensch, *J. Am. Chem. Soc.* **1966**, *88*, 5486.
[21] a) A. M. Fuentes-Caparrós, Z. Canales-Galarza, M. Barrow, B. Dietrich, J. Läuger, M. Nemeth, E. R. Draper, D. J. Adams, *Biomacromolecules* **2021**, *22*, 1625; b) F. Aviño, A. B. Matheson, D. J. Adams, P. S. Clegg, *Org. Biomol. Chem.* **2017**, *15*, 6342; c) M. Wallace, A. Z. Cardoso, W. J. Frith, J. A. Iggo, D. J. Adams, *Chem. Eur. J.* **2014**, *20*, 16484; d) K. McAulay, H. Wang, A. M. Fuentes-Caparrós, L. Thomson, N. Khunti, N. Cowieson, H. Cui, A. Seddon, D. J. Adams, *Langmuir* **2020**, *36*, 8626; e) L. Thomson, R. Schweins, E. R. Draper, D. J. Adams, *Macromol. Rapid Commun.* **2020**, *41*, 2000093; f) E. R. Draper, B. Dietrich, K. McAulay, C. Brasnett, H. Abdizadeh, I. Patmanidis, S. J. Marrink, H. Su, H. Cui, R. Schweins, A. Seddon, D. J. Adams, *Matter* **2020**, *2*, 764; g) K. McAulay, B. Dietrich, H. Su, M. T. Scott, S. Rogers, Y. K. Al-Hilaly, H. Cui, L. C. Serpell, A. M. Seddon, E. R. Draper, D. J. Adams, *Chem. Sci.* **2019**, *10*, 7801; h) A. M. Fuentes-Caparrós, B. Dietrich, L. Thomson, C. Chauveau, D. J. Adams, *Soft Matter* **2019**, *15*, 6340.
[22] C. Colquhoun, E. R. Draper, R. Schweins, M. Marcello, D. Vadukul, L. C. Serpell, D. J. Adams, *Soft Matter* **2017**, *13*, 1914.
[23] L. R. Fina, N. Hassouna, G. L. Horacek, J. P. Lambert, J. L. Lambert, *Appl. Environ. Microbiol.* **1982**, *44*, 1370.
[24] E. R. Draper, H. Su, C. Brasnett, R. J. Poole, S. Rogers, H. Cui, A. Seddon, D. J. Adams, *Angew. Chem. Int. Ed.* **2017**, *56*, 10467; *Angew. Chem.* **2017**, *129*, 10603.
[25] B. P. Nowak, M. Niehues, B. J. Ravoo, *Soft Matter* **2021**, *17*, 2857.
[26] B. P. Nowak, B. J. Ravoo, *Faraday Discuss.* **2019**, *219*, 220.
[27] B. P. Nowak, B. J. Ravoo, *Soft Matter* **2020**, *16*, 7299.
[28] Z. Yang, G. Liang, M. Ma, Y. Gao, B. Xu, *J. Mater. Chem.* **2007**, *17*, 850.
[29] Z. Luo, Q. Wu, C. Yang, H. Wang, T. He, Y. Wang, Z. Wang, H. Chen, X. Li, C. Gong, Z. Yang, *Adv. Mater.* **2017**, *29*, 1601776.
[30] a) R. Otter, C. M. Berac, S. Seiffert, P. Besenius, *Eur. Polym. J.* **2019**, *110*, 90; b) D. Spitzer, L. L. Rodrigues, D. Straßburger, M. Mezger, P. Besenius, *Angew. Chem. Int. Ed.* **2017**, *56*, 15461; *Angew. Chem.* **2017**, *129*, 15664.
[31] L. Xu, G. Csekő, A. K. Horváth, *J. Phys. Chem. A* **2020**, *124*, 6029.
[32] S. M. Kelly, T. J. Jess, N. C. Price, *Biochim. Biophys. Acta Proteins Proteomics* **2005**, *1751*, 119.

Manuscript received: February 1, 2022
Accepted manuscript online: March 11, 2022
Version of record online: March 29, 2022