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COMMUNICATION

Light-activated thiomaleimide crosslinking stabilizes collagen mimetic peptide triple helix

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Collagen mimetic peptides (CMPs) are useful self-assembling building blocks for creating tunable biomaterials with structure and function dependent on triple helix stability. We demonstrate rapid, UV-activated stabilization of a CMP triple helix via thiomaleimide self-crosslinking and controlled by light dose, enabling precise *in situ* control and stabilization of peptide assemblies.

As the most abundant proteins in humans, collagens (notably type I) provide structural stability to the ECM through triple helical tropocollagen molecules composed of proline-hydroxyproline-glycine (P-O-G) repeat sequences.[1-6] These molecules assemble into higher-order fibrils that naturally undergo non-enzymatic crosslinking with age and disease.[7, 8] Approaches for mimicking these structures and probing these complex, dynamic crosslinking processes remain a need within the biomaterials community.[9, 10]

Collagen mimetic peptides (CMPs) are synthetic self-assembling molecules that mimic tropocollagen by incorporating $(POG)_n$ repeats to form polyproline type II (PPII) helices that physically assemble into a triple helix.[11, 12] CMPs can be engineered with reactive handles for labeling or incorporation into hydrogels,[13-16] integrin-binding motifs for cell adhesion,[17-19] and charged amino acids for assembly into higher-order fibrils.[17-19] However, maintaining triple helical stability under physiological or environmental stress (e.g., temperature, shear flow) remains a challenge.[20-23] While strategies to enhance stability have been established,[24] such as chemical modifications for intrahelical covalent crosslinking (covalent capture,[13, 25-28] intrahelical “cys-knots”[29]), they often are too slow or lack compatibility for use in situ in many biological applications.

Light-triggered chemistries are widely used to crosslink macromolecules,[30, 31] and a few initiator-free photochemistries have been used to stabilize protein and peptide building blocks in situ under aqueous conditions.[32] For CMPs, UV light has been used to control folding and unfolding of CMP triple helices through modulation of physical interactions within the triple helix via photo-uncaging or photo-isomerization reactions.[33, 34] More broadly, thiomaleimide handles have been used to re-bridge disulfide bonds to stabilize cyclic peptides[35] and antibody fragments[36] using UV light. We hypothesized that thiomaleimides could enable rapid intramolecular covalent crosslinking within CMPs. Such an initiator-free, light-triggered approach would provide spatiotemporal control and operational simplicity for stabilization of the triple helix in minutes. The resulting covalently crosslinked architecture requires no exogenous reagents or purification after crosslinking, offering a streamlined and accessible path for *in situ* stabilization.

In this work, we developed an innovative strategy for fast and tunable stabilization of a CMP triple helix in buffered conditions with application of cytocompatible wavelength and doses of UV light. Specifically, we incorporated thiomaleimide reactive handles on cysteine residues in a CMP designed for the formation of intra-helical crosslinks after exposure to a low dose of long wavelength UV light, resulting in an increase in melting temperature from below to above physiological temperature. In addition, we demonstrate that by increasing the light dose, stability of the triple helix can be tuned until complete consumption of the free thiomaleimide, resulting in further increase in melting temperature. This strategy serves as a template for the design of light-activated CMPs and other assembling peptide and protein structures for their *in situ* stabilization and modification in a range of aqueous applications.

To design a thiomaleimide CMP, we first looked to previously established sequences that had been stabilized with intrahelical crosslinks to inform thiomaleimide placement.[13, 25-27] We employed the same reactive handle placement strategy to design $(POG)_4PC_{mal}GC_{mal}OG(POG)_4$, where C_{mal} is a cysteine modified with a thiomaleimide side chain. Specifically, the

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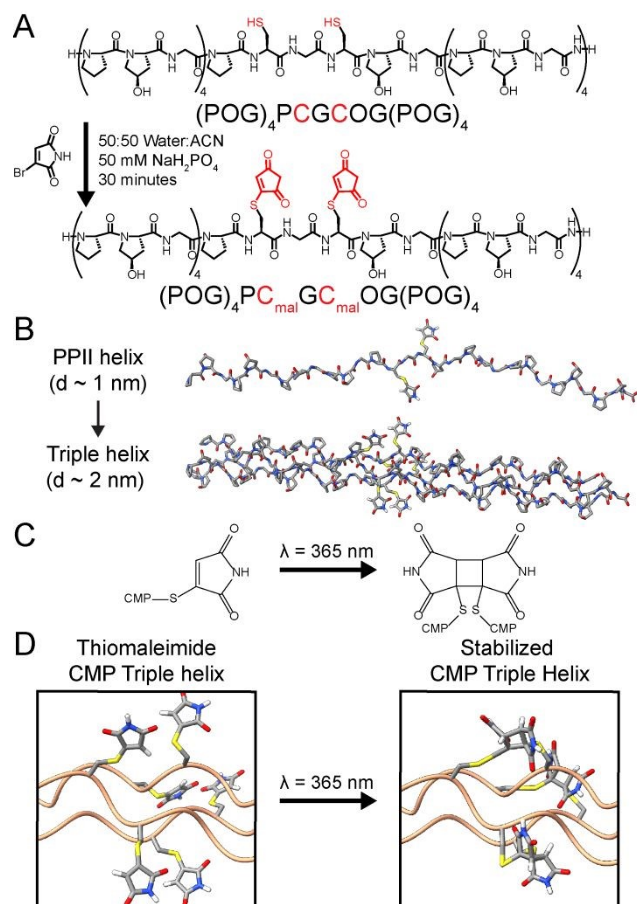
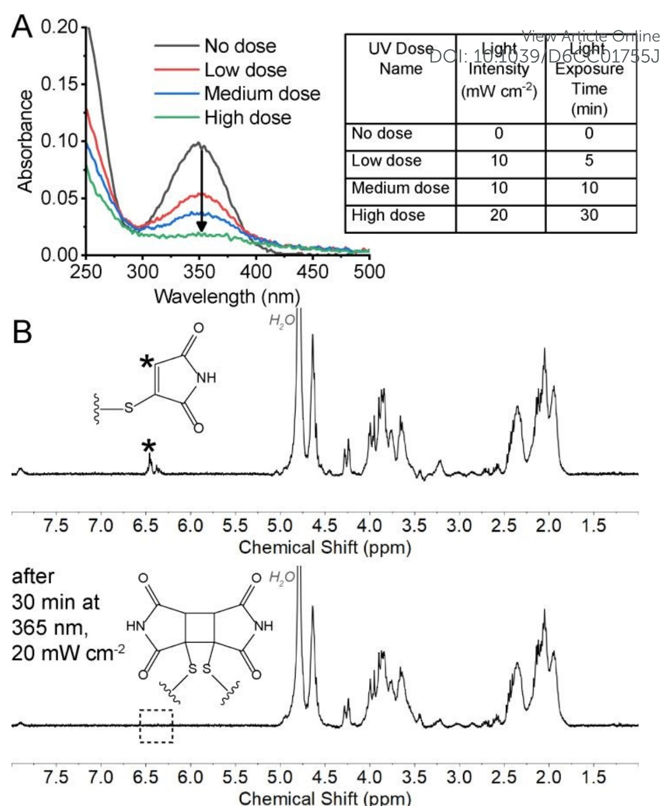


Fig. 1. Thiomaleimide-functionalized CMP triple helix stabilized upon the application of light. A) A CMP integrating cysteines is modified by a bromomaleimide-thiol exchange reaction in buffer to produce a thiomaleimide-functionalized CMP. Note, bond lengths / angles are not to scale and were selected for ease of visual representation. B) Thiomaleimide-functionalized CMPs have a polyproline type II helix secondary structure that allows for self-assembly into a triple helix tertiary structure. C) Chemical structure of the thiomaleimide reactive handles before and after photocrosslinking. D) Visualization of the thiomaleimide-functionalized CMP triple helix being covalently stabilized after UV (365 nm) irradiation.



UV Dose Name	Light Intensity (mW cm^{-2})	Light Exposure Time (min)
No dose	0	0
Low dose	10	5
Medium dose	10	10
High dose	20	30

Fig. 2 Characterization of the thiomaleimide CMP after irradiation by UV light. A) UV-vis spectroscopy of peptide samples with increasing doses of UV light (low = 10 mW cm^{-2} for 5 minutes, medium = 10 mW cm^{-2} for 10 minutes, high = 20 mW cm^{-2} for 30 minutes) showing the disappearance of the characteristic 354 nm thiomaleimide peak. B) 1HNMR spectra showing the disappearance of the thiomaleimide alkene proton signals after UV irradiation

middle two (X-Y-G)$_n$ blocks have cysteines functionalized with thiomaleimides for light-activated covalent stabilization of the CMP triple helix (**Figure 1**). AlphaFold 3[37] and Chimera X[38] were used to generate visual representations of the triple helix and verify that the side changes associated with the cysteine were presented intrahelically. The thiomaleimide reactive handle was chosen because of its ability to self-crosslink upon irradiation (365 nm) by [2+2] cycloaddition in a buffered aqueous environment in minutes.[39] Compared to classical maleimides, thiomaleimides are superior for this application because they undergo self-crosslinking after UV irradiation at a faster rate, have shorter wavelength absorption peaks, and have higher absorption coefficients.[36]

The base peptide sequence (POG)$_4$PCGCOG(POG)$_4$ was synthesized using microwave-assisted solid phase peptide synthesis, with an N-terminal amine and C-terminal amide,[40] and purified by reverse-phase high-performance liquid chromatography. Peptide identity was verified by ultra-performance liquid chromatography mass spectrometry (UPLC-MS) (**Figures S1-2**). Thiomaleimides were then added in solution to the thiolated cysteine side chains on the peptide using previously published methods.[35, 36, 39, 41, 42] Specifically, thiomaleimides were installed on the CMP using a bromomaleimide-thiol exchange reaction in buffered water/acetonitrile after ensuring any disulfide bonds were broken using tris(2-carboxyethyl)phosphine hydrochloride (TCEP). The thiomaleimide-CMP was then purified by dialyzing against deionized water overnight, followed by lyophilization. Peptide identity was again confirmed by UPLC-MS (**Figures S1-2**). Note, we also explored using other light-triggered reactive handles, diazirines and dithiolanes, in similar peptide designs for CMP stabilization; however, these approaches were not pursued further owing to quenching side reactions in solution or ring-opening during peptide cleavage, respectively.

With the thiomaleimide-peptide synthesized, we performed UV-irradiation experiments to form intrahelical covalent crosslinks between thiomaleimide handles and stabilize the triple helix. First, lyophilized peptide was dissolved in a biologically relevant buffer (Dulbecco's phosphate buffered saline) and allowed to self-assemble into triple helices (4 ˚C for 48 hours). Before this assembly step, samples were not heated to avoid hydrolysis of thiomaleimides.[43] After triple helix formation, the peptide was transferred to a glass cuvette (1 mm path length) for irradiation experiments. Three different irradiation conditions were tested using 365 nm light: *i)* low dose (10 mW cm^{-2}, 5 min), *ii)* medium dose (10 mW cm^{-2}, 10

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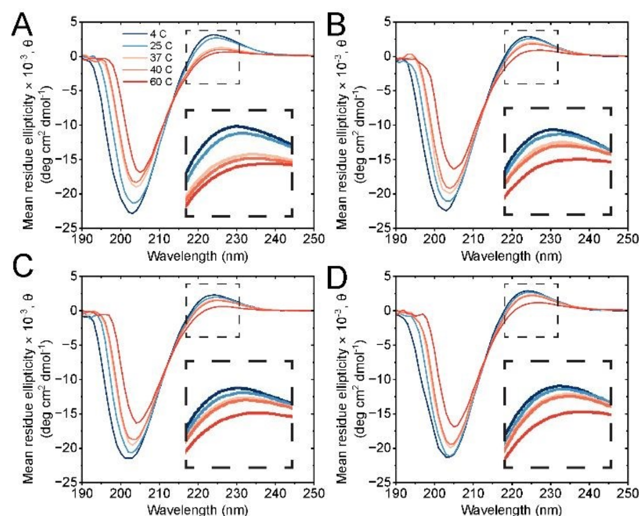

Fig. 3 Representative CD wavelength scans of thiomaleimide-CMP with increasing UV (365 nm) dose, showing the characteristic 225 nm polyproline type II helix peak decreasing in magnitude as temperature is increased (magnified region of interest shown in dashed box). A) No dose, B) low dose (10 mW cm^{-2}, 5 minutes), C) medium dose (10 mW cm^{-2}, 10 minutes), and D) high dose (20 mW cm^{-2}, 30 minutes). All replicates are shown in Figure S4. Note, below 210 nm, high tension voltages were above the highest acceptable value of 600 V; we attribute shifts below this wavelength to noise produced at these high voltages.[50]

min), and *iii)* high dose (20 mW cm^{-2}, 30 min) in comparison to the control (no dose of light). The low dose was chosen as one known to be cytocompatible in several biological contexts;[44-46] the high dose was chosen for complete functional group consumption based on spectroscopic measurements; and the medium dose was chosen as an intermediate between these two that has been reported for use with live cells.[47, 48] To monitor functional group consumption, UV-visible light spectroscopy and ^{1}HNMR spectroscopy were used (**Figure 2**). When comparing the UV-visible light absorbance spectra between the control (no light) and irradiated samples, a disappearance of the characteristic 354 nm thiomaleimide peak can be seen (**Figure 2A**). In addition, ^{1}HNMR spectra showed the disappearance of the C=CH proton peaks (~6.5 ppm) after irradiation (**Figure 2B**).

To confirm the stabilization of the CMP triple helix, circular dichroism (CD) spectroscopy was used, with wavelength (**Figure 3**) and temperature scans (**Figure 4**). As temperature was ramped from 4 to 80 ˚C (1 ˚C min^{-1}), wavelength scan measurements were performed at 4 ˚C, 25 ˚C, 37 ˚C, 40 ˚C, and 60 ˚C. The characteristic collagen-like PPII helix peak (225 nm)[1] was seen in the control (no dose of light) and irradiated samples (**Figure 3**), supporting that photocrosslinking did not disrupt the triple helical structure. This characteristic peak decreased in magnitude as the temperature increased, indicating dissociation or 'melting' of the triple helix. Notably, the low, medium, and high dose conditions show relatively higher magnitudes of the 225 nm peak at 40 ˚C and 60 ˚C compared to the no light condition, indicating a higher triple helical stability after irradiation at these temperatures.

To further probe intrahelical crosslinking, MALDI-TOF measurements were conducted on peptides from the control (no dose of light) and irradiated (high dose of light; 365 nm, 20 mW cm^{-2}, 30 minutes) conditions (**Figure S3**). A peak associated with the molecular weight of a single peptide was observed in the control sample and irradiated sample. Importantly, higher molecular weight peaks were also present in the irradiated sample, corresponding to dimers and triple helices associated with intrahelical covalent crosslinking in addition to higher order multimers. We hypothesize that the multimer peaks are present due to *inter*helical crosslink formation. While the magnitude of the single peptide peak is the highest, the observed relative intensity may be due to higher molecular weight species having lower ionizability and thereby less detection by MALDI. The presence of single peptide peaks in the irradiated sample, which exhibited complete consumption of the thiomaleimide, suggest incomplete formation of intrahelical crosslinks under the conditions probed, as well as the potential for fragmentation during MALDI.[49] Overall, these data confirm peptide connectivity via covalently bonding after irradiation.

To probe how the light-triggered intrahelical covalent crosslinking influenced triple helical stability, temperature scan measurements were made at 225 nm (**Figure 4A**). These plots show the characteristic sigmoidal shape associated with the collagen-like triple helical assembly disassociating or 'melting' for all conditions. Plotting the derivatives of these curves and identifying their minima (**Figure 4B**) reveals melting temperatures (T_m), the temperature at which half of the CMPs in solution are assembled in a triple helix. In this context,

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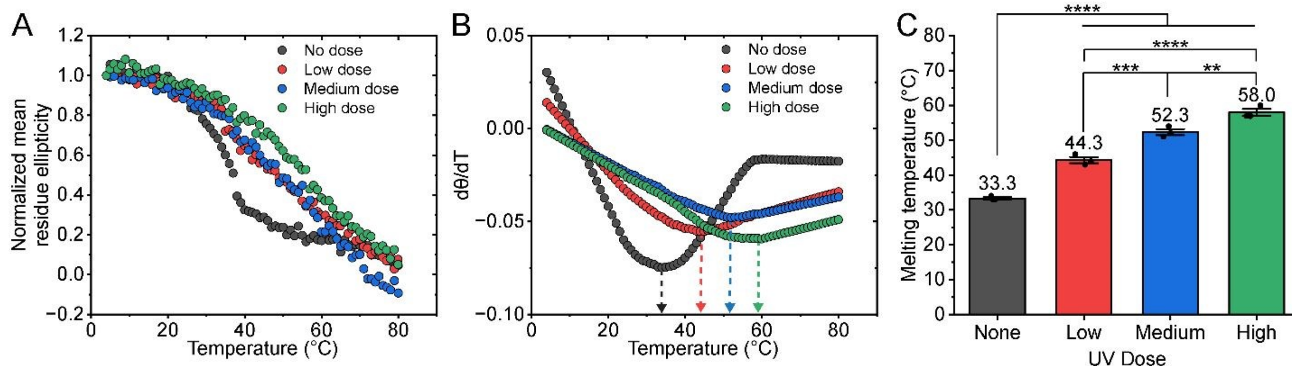

Fig. 4 CD melting temperature analysis of thiomaleimide-CMP with increasing light dose (low (10 mW cm^{-2}, 5 minutes), medium (10 mW cm^{-2}, 10 minutes), high (20 mW cm^{-2}, 30 minutes), and no dose of 365 nm light). A) Representative temperature scan melting curves normalized to their initial mean residue ellipticity values at 4 ˚C. B) Derivatives of representative temperature scan melting curves. Minima of the derivative curves were used to identify the C) melting temperature for each peptide. Means ± standard error for each condition are shown for (n = 3) independent sample measurements. All replicates are shown in Figure S5. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. Statistical significance is shown (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

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melting temperature is a measurement of triple helix stability, with higher melting temperatures indicating higher triple helical stability.[21-23] Indeed, as the dose of light was increased, we observed higher T_ms for the thiomaleimide-CMP. The control CMP (no dose of light) exhibited a T_m of 33.0 ± 0.335 ˚C compared to the low-dose CMP with a T_m of 44.3 ± 0.878 ˚C. As the light dose was increased to medium and high, T_ms of 52.3 ± 0.876 ˚C and 58.0 ± 0.990 ˚C were observed, respectively (**Figure 4C**).

As some degree of interhelical crosslinking was observed for samples irradiated at 0.15 mM (**Figure S3**), samples at 0.015 mM also were irradiated and measured by CD (**Figure S6**). A similar melting temperature of 55.9 ˚C was observed, suggesting that any interhelical crosslinking present has a minimal effect on the cooperative melting associated with the triple helix. Thiomaleimide-CMP refolding behavior was also probed using CD (**Figure S7**). Significant hysteresis was observed for the control thoimaleimide-CMP (no dose of light) after 1 and 2 temperature cycles (4→80→4→80→4˚C), suggesting slow refolding kinetics as expected for physical interactions.[51] Importantly, for the irradiated thoimaleimide-CMP (high dose of light), minimal hysteresis was observed, suggesting a preorganized, covalently stabilized triple helix.[26]

These observations demonstrate that T_m can be tuned with the thiomaleimide crosslinking based on the dose of light. The thiomaleimide-CMP stabilized with a high dose has a T_m comparable to $(POG)_{10}$, which has been reported to have a T_m of 58.8 ˚C in PBS.[52] Since the stabilized thiomaleimide-CMP and $(POG)_{10}$ are both 30 amino acids in length, this result demonstrates that any disruptions to the triple helix introduced by the $PC_{mal}GC_{mal}OG$ block are compensated by the thiomaleimide intrahelical crosslinks formed after UV irradiation. Further, the increase in thermal stability of approximately 25 ˚C after irradiating with the high dose is comparable to previous studies where CMPs of 30 amino acids long are covalently stabilized (e.g., T_m increase of 32.5 ˚C[13] or 44 ˚C[26] with covalent capture of K-E side chains). Overall, our photocrosslinking approach uniquely allows triggered increases in T_m *in situ* under biocompatible conditions using only the thiomaleimide reactive functional handle.

In summary, we have demonstrated an innovative strategy for tunable, light-activated covalent stabilization of a CMP triple helix under biologically relevant conditions. Using a new CMP design $(POG)_4PCGCOG(POG)_4$, we attached thiomaleimides onto cysteine side chains using bromomaleimide-thiol exchange and observed triple helix formation. The triple helical conformation was further stabilized upon irradiation with cytocompatible doses of long wavelength UV light via intrahelical thiomaleimide crosslinks. By increasing the dose of light, the triple helical melting temperature was tuned over a range of approximately two-fold. This initiator-free, operationally simple framework may prove broadly useful for rapid, light-triggered intrahelical crosslinking of a range of assembling peptides for their *in situ* stabilization and modification in biological contexts.

Data availability

The data supporting this article is included in the Supporting Information (SI). References cited in the SI have been listed in the reference list of the main text.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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AlphaFold 3 was used to generate 3D renderings of the molecules shown in Figure 1 and the Table of Contents figure. Further visual and structural edits were made with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from National Institutes of Health R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases.

References

1 M. D. Shoulders and R. T. Raines, *Annual Review of Biochemistry*, 2009, **78**, 929-958.
2 B. Stefanovic, *WIREs RNA*, 2013, **4**, 535-545.
3 M. G. Patino, M. E. Neiders, S. Andreana, B. Noble and R. E. Cohen, *Implant Dentistry*, 2002, **11**.
4 B. An, S.-W. Chang, C. Hoop, J. Baum, M. J. Buehler and D. L. Kaplan, *ACS Biomaterials Science & Engineering*, 2017, **3**, 269-278.
5 M. Gauza-Włodarczyk, L. Kubisz and D. Włodarczyk, *International Journal of Biological Macromolecules*, 2017, **104**, 987-991.
6 V. K. Pálfi and A. Perczel, *Journal of Computational Chemistry*, 2010, **31**, 764-777.
7 J. Bella and D. J. S. Hulmes, *Fibrillar Collagens*, Springer International Publishing, 2017, pp. 457-490.
8 J. G. Snedeker and A. Gautieri, *Muscles Ligaments Tendons J*, 2014, **4**, 303-308.
9 Y. Wu, Y. Xiao, B. Yin and S. H. D. Wong, *International Journal of Molecular Sciences*, 2025, **26**, 9502.
10 M. Rizwan, A. E. G. Baker and M. S. Shoichet, *Advanced Healthcare Materials*, 2021, **10**, 2100234.
11 Y. Xu and M. Kirchner, *Bioengineering*, 2021, **8**, 5.
12 S. A. H. Hulgan and J. D. Hartgerink, *Biomacromolecules*, 2022, **23**, 1475-1489.
13 A. M. Hilderbrand, E. M. Ford, C. Guo, J. D. Sloppy and A. M. Kloxin, *Biomaterials Science*, 2020, **8**, 1256-1269.

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14 C. M. Peterson, M. R. Helterbrand and J. D. Hartgerink, *Biomacromolecules*, 2022, **23**, 2396-2403.
15 C. M. Yamazaki, Y. Kadoya, K. Hozumi, H. Okano-Kosugi, S. Asada, K. Kitagawa, M. Nomizu and T. Koide, *Biomaterials*, 2010, **31**, 1925-1934.
16 R. A. Castro, L. Pradhan, J. Caplan, C. D'Ambrosio and A. M. Kloxin, *ACS Central Science*, 2026
17 I. C. Tanrikulu, A. Forticaux, S. Jin and R. T. Raines, *Nature Chemistry*, 2016, **8**, 1008-1014.
18 L. E. R. O'Leary, J. A. Fallas, E. L. Bakota, M. K. Kang and J. D. Hartgerink, *Nature Chemistry*, 2011, **3**, 821-828.
19 F. W. Kotch and R. T. Raines, *Proceedings of the National Academy of Sciences*, 2006, **103**, 3028-3033.
20 E. M. Ford, A. M. Hilderbrand and A. M. Kloxin, *Journal of Materials Chemistry B*, 2024, **12**, 9600-9621.
21 V. Gauba and J. D. Hartgerink, *Journal of the American Chemical Society*, 2007, **129**, 2683-2690.
22 Y. Xu, in *Methods in Enzymology*, Academic Press, 2009, vol. 466, pp. 211-232.
23 H. P. Bächinger, N. P. Morris and J. M. Davis, *American Journal of Medical Genetics*, 1993, **45**, 152-162.
24 A. J. Kasznel, Y. Zhang, Y. Hai and D. M. Chenoweth, *Journal of the American Chemical Society*, 2017, **139**, 9427-9430.
25 C. C. Cole, B. H. Pogostin, K. A. Cahue, V. H. Vardanyan, T. H. Bui, A. C. Farsheed, J. W. R. Swain, J. T. Makhoul, M. Dubackic, P. Holmqvist, U. Olsson, A. B. Kolomeisky, K. J. McHugh and J. D. Hartgerink, *ChemBioChem*, 2025, **26**.
26 L. T. Yu and J. D. Hartgerink, *Chemical Science*, 2022, **13**, 2789-2796.
27 I. C. Li, S. A. H. Hulgan, D. R. Walker, R. W. Farndale, J. D. Hartgerink and A. A. Jalan, *Organic Letters*, 2019, **21**, 5480-5484.
28 N. B. Hentzen, L. E. J. Smeenk, J. Witek, S. Riniker and H. Wennemers, *Journal of the American Chemical Society*, 2017, **139**, 12815-12820.
29 D. Barth, O. Kyrieleis, S. Frank, C. Renner and L. Moroder, *Chemistry – A European Journal*, 2003, **9**, 3703-3714.
30 C. E. Hoyle, A. B. Lowe and C. N. Bowman, Chemical Society Reviews, 2010, 39, 1355.
31 J. B. Hutchison, P. F. Stark, C. J. Hawker and K. S. Anseth, *Chemistry of Materials*, 2005, **17**, 4789-4797.
32 K. Matsuura and H. Inaba, *Biophys Rev (Melville)*, 2023, **4**, 041303.
33 U. Kusebauch, S. A. Cadamuro, H. J. Musiol, L. Moroder and C. Renner, *Chemistry – A European Journal*, 2007, **13**, 2966-2973.
34 Y. Li, C. A. Foss, D. D. Summerfield, J. J. Doyle, C. M. Torok, H. C. Dietz, M. G. Pomper and S. M. Yu, *Proceedings of the National Academy of Sciences*, 2012, **109**, 14767-14772.
35 D. A. Richards, S. A. Fletcher, M. Nobles, H. Kossen, L. Tedaldi, V. Chudasama, A. Tinker and J. R. Baker, *Organic & Biomolecular Chemistry*, 2016, **14**, 455-459.
36 R. Malde, M. A. Parkes, M. Staniforth, J. M. Woolley, V. G. Stavros, V. Chudasama, H. H. Fielding and J. R. Baker, *Chemical Science*, 2022, **13**, 2909-2918.
37 J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard, J. Bambrick, S. W. Bodenstein, D. A. Evans, C.-C. Hung, M. O'Neill, D. Reiman, K. Tunyasuvunakool, Z. Wu, A. Žemgulytė, E. Arvaniti, C. Beattie, O. Bertolli, A. Bridgland, A. Cherepanov, M. Congreve, A. I. Cowen-Rivers, A. Cowie, M. Figurnov, F. B. Fuchs, H. Gladman, R. Jain, Y. A. Khan, C. M. R. Low, K. Perlin, A. Potapenko, P. Savy, S. Singh, A. Stecula, A. Thillaisundaram, C. Tong, S. Yakneen, E. D. Zhong, M. Zielinski, A. Žídek, V. Bapst, P. Kohli, M. Jaderberg, D. Hassabis and J. M. Jumper, *Nature*, 2024, **630**, 493-500.
38 E. C. Meng, T. D. Goddard, E. F. Pettersen, G. S. Couch, Z. J. Pearson, J. H. Morris and T. E. Ferrin, *Protein Science*, 2023, **32**, e4792.
39 L. M. Tedaldi, A. E. Aliev and J. R. Baker, *Chemical Communications*, 2012, **48**, 4725.
40 E. M. Ford and A. M. Kloxin, *ACS Biomaterials Science & Engineering*, 2021, **7**, 4175-4195.
41 E. Skolia and C. G. Kokotos, *ACS Organic & Inorganic Au*, 2023, **3**, 96-103.
42 M. Aljuaid, Y. Chang, D. M. Haddleton, P. Wilson and H. A. Houck, *Journal of the American Chemical Society*, 2024, **146**, 19177-19182.
43 S. D. Fontaine, R. Reid, L. Robinson, G. W. Ashley and D. V. Santi, *Bioconjugate Chemistry*, 2015, **26**, 145-152.
44 B. D. Fairbanks, M. P. Schwartz, C. N. Bowman and K. S. Anseth, *Biomaterials*, 2009, **30**, 6702-6707.
45 A. Sabnis, M. Rahimi, C. Chapman and K. T. Nguyen, *Journal of Biomedical Materials Research Part A*, 2009, **91A**, 52-59.
46 I. Mironi-Harpaz, D. Y. Wang, S. Venkatraman and D. Seliktar, *Acta Biomaterialia*, 2012, **8**, 1838-1848.
47 B. R. Nelson, B. E. Kirkpatrick, C. E. Miksch, M. D. Davidson, N. P. Skillin, G. K. Hach, A. Khang, S. N. Hummel, B. D. Fairbanks, J. A. Burdick, C. N. Bowman and K. S. Anseth, *Advanced Materials*, 2024, **36**, 2211209.
48 S. J. Bryant, C. R. Nuttelman and K. S. Anseth, *Journal of Biomaterials Science, Polymer Edition*, 2000, **11**, 439-457.
49 A. Wattenberg, A. J. Organ, K. Schneider, R. Tyldesley, R. Bordoli and R. H. Bateman, *J Am Soc Mass Spectrom*, 2002, **13**, 772-783.
50 A. Rodger and D. Marshall, *The Biochemist*, 2021, **43**, 58-64.
51 K. Mizuno, S. P. Boudko, J. Engel and H. P. Bächinger, *Biophys J*, 2010, **98**, 3004-3014.
52 A. V. Persikov, Y. Xu and B. Brodsky, *Protein Sci*, 2004, **13**, 893-902.

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Data availability

The data supporting this article is included in the Supporting Information (SI). References cited in the SI have been listed in the reference list of the main text.

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