Electronic Supplementary Material (ESI) for Chemical Communications

Chemical and Light Triggering of Peptide Networks under Partial Thermodynamic Control

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Supplementary Information

1. Experimental section

1.1 Peptides synthesis and characterization. The nucleophilic peptide **N** was synthesized on a Rink-Amide MBHA (4-methylbenzhydrylamine) resin using standard Fmoc-based chemistry, and by coupling of Trt-protected thioglycolic acid as the last amino acid. The templates $\mathbf{T_1}$ and $\mathbf{T_{2C}}$ were also synthesized using the Fmoc method.

The caged template $\mathbf{T_1^{Nv}}$ was synthesized with a specific Lys side chain orthogonally protected by the 4-methyltrityl (Mtt) group. After synthesis of the fully protected peptide, the Mtt group was selectively removed with 1% trifluoroacetic acid (TFA) in dry dichloro methane (DCM), by repeating 10 times for 2 minutes each time. The free ε-amine of the Lys side chain reacted with 6-nitroveratryloxycarbonyl chloride (Nvoc-Cl), *N*-hydroxybenzotriazole (HOBT) as coupling reagent (5 equivalents equivalent each) and *N*,*N*-Diisopropylethylamine base (DIPEA; 10 eq.) in dry DCM containing 1M LiCl, for 3 hours and then again for 12 hours. The caged peptide, equipped with the photocleavable group at the desired position, was obtained after cleavage and global deprotection with the common cleavage mixture (95% TFA).

The electrophile peptides $\mathbf{E_1}$ and $\mathbf{E_2}$ were synthesized on MBHA resin using a modified t-Boc SPPS method, in which 3-triphenylmethylthiopropionic acid (3 eq. relative to resin loading) in dimethylformamide (DMF) was first coupled to the resin, using HBTU as coupling reagent (3 eq.) and DIPEA as base (20 eq.). The Trt group was removed using a TFA:TIS (TIS = Triisopropylsilane) mixture (95:5) twice (5 and 10 min). The synthesis continued using the standard t-Boc-chemistry

procedure, and the crude peptide was obtained after cleavage and global deprotection with a TFMSA/TFA mixture (TFMSA = trifluoromethanesulfonic acid).

Replicating peptides **R_1** and **R_2** were synthesized by ligation. The relevant thioester peptide (1 eq.; 3-5 mM) and the N-terminus nucleophilic peptide (1.2 eq.) were dissolved in 3-(N-morpholino) propanesulfonic acid (MOPS) buffer at pH ~7.5, containing tris(2-carboxyethyl)phosphine hydrochloride (TCEP) as reducing agent. The reaction allowed to proceed at 37 °C for 4-8 hours, until quenched by TFA, and subjected to purification by HPLC.

All the studied peptides were purified by preparative HPLC using a C18 reverse phase column (Dionex 1100) with a step gradient of solvent A (99% water, 1% acetonitrile (ACN), 0.1% TFA) and B (90% ACN, 10% water, 0.07% TFA). The identity and purity of the peptides were analyzed by analytical HPLC (with the same solvent system for elution), MALDI-TOF MS (α-Cyano-4-hydroxycinnamic acid as ionization matrix) and LCMS. Molecular weights (Mw) observed for all peptides were no more than ±2 off the calculated Mw. Only peptides of 95% purity or higher were used for further structure and function analysis.

1.2 Circular dichroism (CD) analysis. Stock solutions (0.7-1.0 mM) were prepared, and then further diluted by MOPS buffer (pH 7.4) to result the desired concentration for analysis (typically 20 μM). The exact concentration of each peptide solution was determined from its UV absorbance at 270 nm, based on the known absorbance of 4-acetamidobenzoic acid (ABA). Measurements were carried out on a Jasco-815 CD spectropolarimeter, at 25 °C, by using a quartz cell with 1.0 mm path length. CD spectra were obtained as the average of three scans and collecting data at 1 nm intervals from 260 to 200 nm. The CD signals resulting from buffer was subtracted from the spectrum of each peptide solution. Data was converted to molar ellipticity (Θ in deg*cm^2*dmol^{-1}) according to the equation: $[\Theta] = \Psi \bullet 100 / (nlc)$, where Ψ is the CD signal in degrees, n is the number of peptide bonds, l is the path length in centimeters, and c is the concentration in decimoles per cm^3. The helical content of each peptide was calculated using CDNN program (provided with the Jasco spectropolarimeter), which was developed to analyze and quantify information content of far UV CD spectra,[1] and proved useful to deduce protein secondary structures.

GnHCl dependent denaturation experiments carried out for **R_1** and **T_1** (20 μM) in 5 mM MOPS buffer pH = 7.4, by increasing the concentration of GnHCl by 0.5M for each scan. The molar fraction of protein at each GnHCl concentration was deduced from the relative intensity at 222 nm, and then

$Gn_{1/2}$ values were calculated from the median in the resulted sigmoidal plot. Unfolding stability difference (ΔΔG in kcal/mol) was calculated using the $Gn_{1/2}$ values as published earlier.[2] GnHCl dependent denaturation experiments were not performed for $\mathbf{R_2}$, since a low signal was observed already in the absence of GnHCl (Figure 1b in the manuscript), and because $\mathbf{R_2}$ degraded quite rapidly when dissolved in neutral pH buffer without excess of thiols.

1.3 Thiol dependant decomposition experiments. Stock solutions of replicators ($\mathbf{R_1}$ or $\mathbf{R_2}$) were prepared by weighing lyphoilized peptides into Eppendorf tubes and dissolving in Millipore water to yield 0.5–1 mM solutions. Experiments were prepared by mixing aqueous solutions containing $\mathbf{R_1}$ or $\mathbf{R_2}$, TCEP (2 mM) as reducing agent, ABA (50 μM) as internal standard, and the small-molecule thiol 2-mercaptoethanesulfonate sodium salt (**S**; 1-5 mM). The mixtures were allowed to equilibrate for 30 minutes under the acidic conditions. After that, the reactions were initiated by the addition of MOPS buffer at pH = 7.4, yielding a total volume of 100 μL. Aliquots (10 μL) were removed at various time points over 24 h, immediately quenched in 5% TFA in water, and stored frozen until subjected to RP-HPLC analysis. The equilibrium constants were calculated from the final concentrations of $\mathbf{E_i}$, **N,** $\mathbf{R_i}$ and **S**, using the following equation:

$$\mathbf{R + S} \overset{\mathbf{K}}{\rightleftharpoons} \mathbf{E + N}$$

1.4 Thioester peptides self-replication experiments. Stock solutions of reactants ($\mathbf{E_i}$ and **N**) and templates ($\mathbf{R_1}$, $\mathbf{R_2}$ or $\mathbf{T_{2C}}$) were prepared by weighing lyphoilized peptides into Eppendorf tubes and dissolving in Millipore water to yield 0.5 – 4 mM solutions. Experiments were prepared by mixing aqueous solutions containing equimolar amounts of $\mathbf{E_i}$ and **N** (100 μM each), TCEP (2 mM), ABA (50 μM), **S** (1 mM), and the desired amounts of the corresponding template peptide. The reaction mixtures allowed to equilibrate for 30 minutes under acidic conditions. After equilibrating, the reactions were initiated by the addition of MOPS buffer at pH 6.8 or 7.4, yielding a total volume of 100-200 μL. Aliquots (10 μL) were removed at various time points, immediately quenched in 5% TFA in water, and stored frozen until subjected to RP-HPLC analysis. The concentrations of the different peptides were calculated from the corresponding HPLC peak areas, and used to show the time dependent reaction profiles. All experiments were repeated at least two times and showed very small variations (≤ 5%) in product formation between consecutive runs. Due to the use of excess of **S** in the experiments, very low concentrations of hydrolysis products of $\mathbf{E_i}$ (≤ 2 mol percent after 8 hours) were observed. Results in Figures 1c and S4 present the amounts of newly formed $\mathbf{R_1}$ or $\mathbf{R_2}$

(ΔR), obtained by simple subtraction of the initially seeded amounts of $\mathbf{R_1}$ or $\mathbf{R_2}$. ΔR usually gets positive values due to formation of the replicators, but in certain cases can become negative, for example when decomposition of initially seeded $\mathbf{R_2}$ is faster than its formation.

1.5 Network experiments. Experiments were prepared by mixing aqueous solutions containing equimolar amounts of $\mathbf{E_1}$, $\mathbf{E_2}$ and **N** (100±10 μM each), TCEP (2 mM), ABA (50 μM), **S** (3 mM for Figures 2a-c; 1 mM for Figures 2e-f), and the indicated amounts of the corresponding template (30-70 μM). The reactions were allowed to equilibrate for 30 minutes, and then initiated by the addition of MOPS buffer at pH = 7.4, yielding a total volume of 100-200 μL. Aliquots (10 μL) were removed at various time points, immediately quenched in 5% TFA in water, and stored frozen. Samples were analyzed by RP-HPLC for monitoring the amounts of $\mathbf{R_1}$ and $\mathbf{R_2}$ as well as unreacted $\mathbf{E_1}$, $\mathbf{E_2}$ and **N** and possible side product. Due to the use of excess of **S** in the experiments, very low concentrations of hydrolysis products of $\mathbf{E_i}$ (≤ 5 mol percent after 24 hours) were observed. Results in Figure 2 in the manuscript present the amounts of newly formed $\mathbf{R_1}$ or $\mathbf{R_2}$ (ΔP) in each of the studied conditions, obtained by simple subtraction of the initially seeded amounts of $\mathbf{R_1}$ or $\mathbf{R_2}$. ΔP usually gets positive values due to formation of the replicators, but in certain cases can become negative, for example when decomposition of initially seeded $\mathbf{R_2}$ is faster than its formation.

1.6 Light-induced network behavior. Experiments were prepared by mixing aqueous solutions of the eloectrophiles $\mathbf{E_1}$ and $\mathbf{E_2}$ (100±10 μM each), nucleophile **N** (150 μM), TCEP (1 mM), ABA (50 μM) and **S** (1 mM), in the presence of $\mathbf{T_1^{Nv}}$ (70 μM). Reactions were initiated by the addition of MOPS buffer at pH = 7.4 yielding a total volume of 200 μL, in the dark. After 45 min, 1 μL (yielding 1 μM) of dithiothreitol (DTT) was added to the solution, and half of the solution was irradiated for 8 min (using 4 PHILIPS-TL 8W bulbs; λ_{max} = 365 nm), while the other half was kept in the dark. Aliquots (10 μL) were removed at various time points, immediately quenched in 5% TFA in water, and stored frozen until subjected to RP-HPLC analysis.

2. Additional Figures

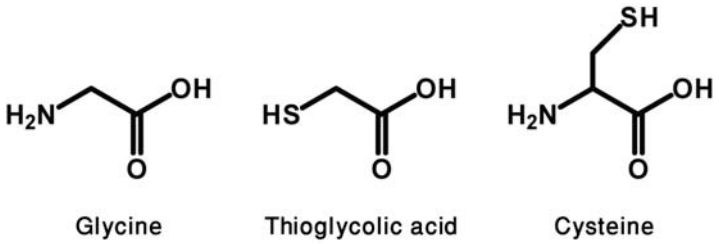

Figure S1. Molecular structures of glycine, thioglycolic acid and cysteine, highlighting that thioglycolic acid may serve as a Gly analog in the peptide backbone.

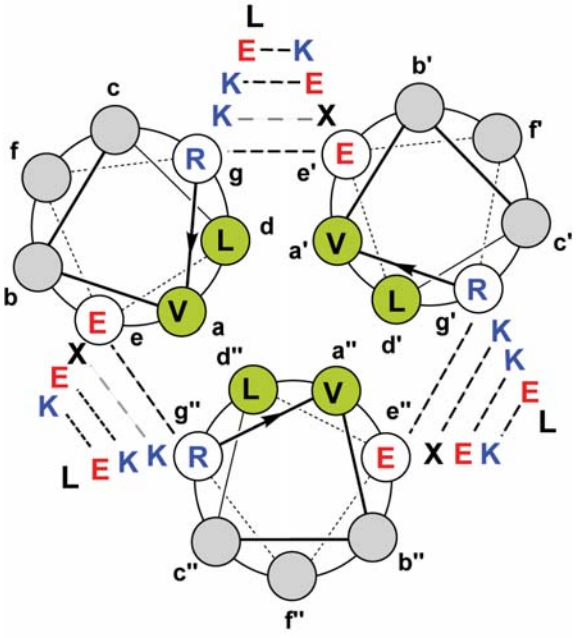

Figure S2. Helical wheel presentation of a trimer coiled coil assembly. Since the hydrophobic core of the studied coiled coils contains Val and Leu residues it tends to form dimeric and trimeric structures, as was evidenced before.[3, 4] The amino acids marked with X, which consist part of the inter-helical recognition interface opposing a Lys residue at position g'8, are either Glu ($\mathbf{R_1}$) or Lys ($\mathbf{R_2}$). Due to charge-charge interactions, $\mathbf{R_1}$ may form stable coiled coils structures while $\mathbf{R_2}$ cannot do that. It has been established before[4] that the ligation rate and replication efficiency can be correlated with the unfolding stability of coiled coils for both dimer template intermediates and the ternary template-product complex (see Figure 1 in the manuscript). In this scheme the thioester bond of $\mathbf{R_1}$ or $\mathbf{R_2}$ is placed between positions *b* and *c*, thus at the solvent exposed regions, and is not expected to affect the folding.

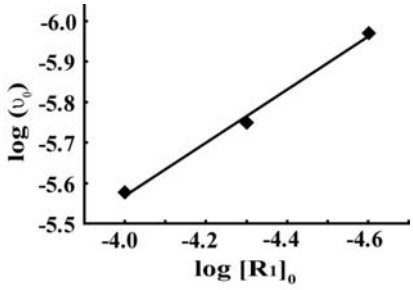

Figure S3. Logarithmic plot of the initial rates extracted from reactions that were initially seeded with different amounts of $\mathbf{R_1}$, as a function of seeded template concentrations. The slope of the linear curve ($p = 0.65$) reflects the reaction catalytic order.[5]

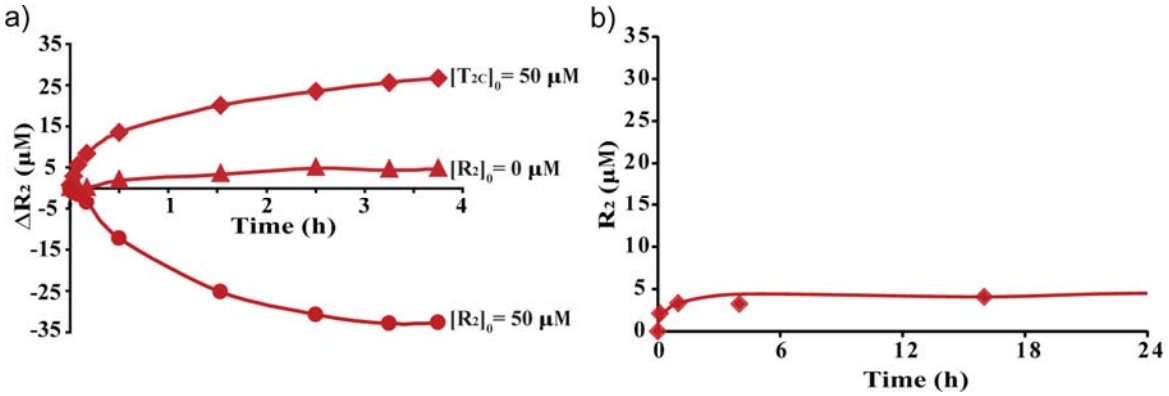

Figure S4. a) Production of $\mathbf{R_2}$ over time in reactions between $\mathbf{E_2}$ and **N** that were initially seeded with 50 μM of $\mathbf{R_2}$ or the template $\mathbf{T_{2C}}$. Production of $\mathbf{R_2}$ over time in the template-free reaction ($[\mathbf{R_2}]_0 = 0$) is shown in panel (a) for rate comparison, and in panel (b) as evidence that no decomposition of $\mathbf{R_2}$ back to starting materials, or due to hydrolysis, was observed.

References

[1] G. Bohm, R. Muhr, R. Jaenicke, *Protein Engineering* **1992**, *5*, 191-195.
[2] O. D. Monera, C. M. Kay, R. S. Hodges, *Protein Sci.* **1994**, *3*, 1984-1991.
[3] P. B. Harbury, T. Zhang, P. S. Kim, T. Alber, *Science* **1993**, *262*, 1401-1407.
[4] G. Ashkenasy, R. Jagasia, M. Yadav, M. R. Ghadiri, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11872-10877.
[5] G. von Kiedrowski, *Bioorg. Chem. Front.* **1993**, *3*, 113-146.