

Light-fuelled dissipative replication and selection in evolvable biomimetic chemical networks

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Abstract

The concept of chemically evolvable replicators is central to abiogenesis^{1, 2}. Evolvability requires three essential components: energy harvesting mechanisms for non-equilibrium dissipation, effective decomposition pathways and transfer of structural information in the autocatalytic cycles³⁻⁵. We observed a UVA light-fuelled dissipative chemical network displaying sequence-dependent replication and replicator decomposition. The system was constructed with primitive peptidic foldamer components^{6, 7}. The photocatalytic formation-recombination cycle of thiyl radicals was coupled with the molecular recognition steps in the replication cycles. Thiyl radical-mediated chain reaction was responsible for the replicator death mechanism. The competing and kinetically asymmetric replication and decomposition processes led to light intensity-dependent selection far from equilibrium. Here we show that this system can dynamically adapt to the level of energy influx and seeding. The results highlight the feasibility of the complex phenomenon of chemical evolvability with primitive building blocks and simple chemical reactions.

Main

Chemical networks mimicking evolution need to operate dynamically far from equilibrium¹, which is essential for their open-ended adaptive behaviour. The dynamic kinetic stability in such systems results from the balance between the asymmetric formation and destruction processes driven by the dissipation of energy harvested from the environment³⁻⁵. Besides metabolism and compartmentalisation, dissipative replication and selection are crucial components for modelling chemical evolution. Non-equilibrium dynamics occur in dissipative chemical networks^{3, 8, 9}, which rely on an energy-harvesting catalytic cycle covering the entropy production of the system. Engineered dissipative systems^{4, 10} attain off-equilibrium states present in the non-covalent assembly of the building blocks¹¹⁻¹³. The dissipative self-organisation of material can be a source of structural complexity¹⁴⁻¹⁶ linked to the emergence of life.

Chemical¹⁶ or light energy^{17, 18} can promote chemical replication by producing precursors in a protometabolic manner. However, driving replication and replicator decomposition simultaneously with external energy is a current challenge. Our goal was to find a feasible energy-harvesting covalent chemistry that drives both synthetic and breakdown processes asymmetrically depending on the intensity of the energy influx. UV-induced photochemical rearrangement reactions can be carried out with aliphatic disulfides in the organic liquid phase^{19, 20} and an aqueous medium.²¹⁻²³ These works showed that both UVA and UVB induce photolysis, producing thiyl radicals despite the strongly reduced absorbance of the disulfide group in the UVA region^{20, 23}. Thiyl radicals participate in a diffusion-controlled chain reaction and depending on the steric accessibility, the exchange proceeds through radical substitution or recombination. Strikingly, anomalously high reaction rates were obtained for the bulky *iso*-butyl substituent,¹⁹ indicating that the reaction is not always governed by the diffusion-controlled collision of the thiyl radicals. These seminal findings suggest that photochemical disulfide rearrangement facilitates both diffusion-controlled and proximity-controlled mechanisms in a substituent-dependent manner. Since these kinetically asymmetric mechanisms are of different order functions of light intensity (see below), they promise an energy-dependent response, potentially paving the way toward dissipative behaviour.

We set out to test the photochemical disulfide rearrangement with a diverse population of helical foldamers as substituents in the aqueous medium. In order to avoid rapid decomposition of the peptidic chains, the feasibility of the light-induced disulfide rearrangement was investigated at UVA wavelengths. We hypothesised that preferential binding between the foldamer chains could exert side chain-dependent proximity control over the photochemical exchange. In contrast, non-interacting segments should readily enter the diffusion-governed chain reaction. The competition between the two processes was expected to yield a light intensity-dependent composition without damaging the peptidic chains. Seeking potential autocatalytic phenomena, we aimed to investigate the influence of the foldamer-foldamer interactions on the energy-harvesting thiyl chemistry. Designed helical peptides can transfer structural information in autocatalytic processes^{24, 25}, and exponential replication has been achieved for peptidic helices²⁶. Helical foldamers are excellent models because the folding can be readily controlled to generate the biomimetic recognition surface^{7, 27}. Moreover, short peptidomimetic foldamers can self-associate in solution²⁸, which is an advantageous feature in terms of proximity control.

Here, we show that UVA light-fuelled disulfide rearrangement can drive a chemical network to off-equilibrium states in a dissipative manner. We found that cross-/autocatalytic templating plays a dominant role in the exchange processes facilitating replication. A competing breakdown mechanism influenced the replicator concentration and thereby laid the foundation for the adaptive selection phenomenon.

The UVA-induced photochemical disulfide rearrangement is feasible in a foldamer library

We constructed the foldamer library using hexameric β -peptide foldamers designed to fold into compact helical structures (Fig. 1a). The sequences contained two variable positions (Xaa¹ and

Xaa²), where proteinogenic side chains were incorporated. These can successfully probe protein surfaces⁷, and the ordered secondary structure can promote self-association²⁸. We chose 12 different side-chain combinations based on their tendency to self-associate and the ability to bind to hydrophobic patches⁶. We did not pursue other designs to keep the complexity of the sequences at a low level. Cys residues were attached to the C-terminus, which allowed for disulfide linkage between foldamer segments (dimers, MSSM) and glutathione (monomers, MSSG). Before the photochemical experiments, the sequence-dependent association tendency of the foldamer segments was validated in an aqueous medium by relaxing the system to equilibrium with the dynamic covalent thiolate mechanism (Extended Data Fig. 1a). In equilibrium, this setup yielded 78 different dimers and 12 glutathione-protected monomers (Fig. 1a, Supplementary Table 1), and the product distribution was biased toward the hydrophobic dimers indicating sequence-dependent association.

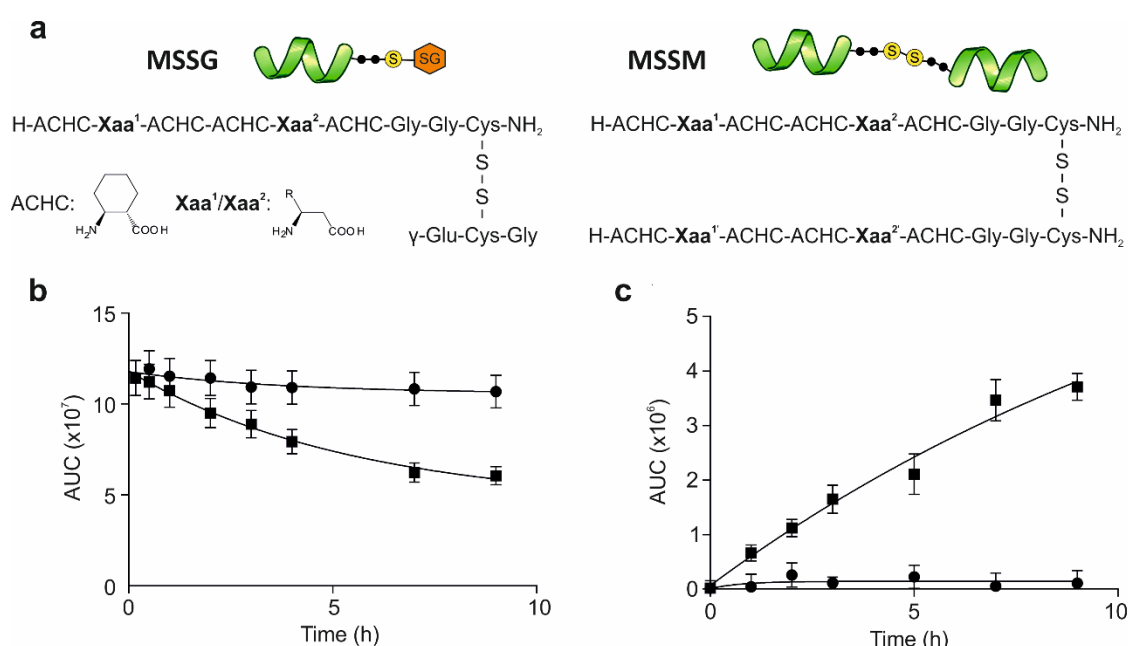


Fig. 1. General sequences of the foldamer disulfide components and their synthesis and break down in the photochemical disulfide-exchange reaction. (a) MSSG: glutathione-protected monomers, MSSM: disulfide-linked dimers. SG indicates the glutathione (γ -L-glutamyl-L-cysteinyl-glycine), and ACHC stands for 1S,2S-2-aminocyclohexanecarboxylic acid, which promotes helical folding. In the highlighted positions Xaa¹ and Xaa², β^3 -amino acids with proteinogenic side chains were incorporated in the following combinations: **IF, KW, LW, QW, RW, RF, SW, TW, VW, WF, WW** and **YF**. One-letter codes correspond to the side chains in the standard α -amino acid notation. (b) Concentration decay of the **RW-RW** dimer as a result of UVA irradiation at 365 nm (square) and without UVA illumination (circle). This reaction was started with a system containing all 10 dimers formed by combining the subset **RW, WF, LW** and **TW**. Dimers concentrations were 10 μ M for each combination. The system also contained 500 μ M oxidised glutathione. (c) Time-dependent concentration increase of the **RW-RW** dimer as a result of UVA irradiation at 356 nm (square) and without UVA (circle). This experiment was started with pure glutathione protected monomers of **RW-SG, WF-SG, LW-SG** and **TW-SG**.

For the photochemical exchange experiments, the presence of free thiol groups was eliminated, and the pH was set to 7.0 to eliminate light-independent thiolate-mediated nucleophilic disulfide exchange. It was essential to avoid the rapid degradation of the peptidic chains; therefore, the disulfide rearrangement reactions were attempted to be driven by UVA irradiation (365 nm) at constant temperature (303 K) according to literature protocols²³. First, we tested the UVA-induced break down of the MSSM dimers (10 μ M each) to MSSG monomers in the presence of oxidised glutathione (500 μ M). In this step, the starting dimers were synthesised by using a limited set of thiol building blocks: **RW-SH**, **WF-SH**, **LW-SH** and **TW-SH**, which allows ten different combinations. The UVA illumination at 5.1 mW cm⁻¹ decreased the concentration of the MSSM components, while MSSG components were produced in a time-dependent manner (Fig. 1b). Second, we started the reaction from the MSSG monomer mixture (**RW-SG**, **WF-SG**, **LW-SG** and **TW-SG**), and the results confirmed the UVA-induced synthesis of the MSSM dimers (Fig. 1c).

Degradation of the peptides was not observed during the experiments. We found no conversion without UV illumination, and nitric oxide blocked the photocatalytic reaction. These observations confirmed the literature findings that the exchange reaction could be induced with UVA light centred at 365 nm, and it proceeds *via* a radical mechanism.

The UVA-dependent reaction contrasts with the absorbance maximum of the disulfide bond at around 280 nm²⁹. However, recent work showed that conformation-dependent stereoelectronic effects around the S-S bond could shift the absorption to the UVA region³⁰. To test this phenomenon, we measured a simple disulfide model, cystamine, which displayed a concentration-dependent absorption tail in the UVA region (Extended Data Fig. 1b). A representative monomer **RW-SG** was also tested, and the absorption tail was observed in the 330 – 500 nm range. In line with the literature, these findings strongly supported the ability of the disulfide group to absorb light energy at around 365 nm and thus, drive the photochemical rearrangement.

Light intensity- and sequence-dependent kinetic asymmetry in the photochemical foldamer-disulfide exchange

Starting from the mixture of pure MSSG sequences (**IF-SG**, **KW-SG**, **LW-SG**, **QW-SG**, **RW-SG**, **RF-SG**, **SW-SG**, **TW-SG**, **VW-SG**, **WF-SG**, **WW-SG** and **YF-SG**) (Fig. 1), we tested the behaviour of the reaction network upon exposure to UVA irradiation. The power density was increased linearly in four steps up to the maximum value available in our setup (5.10 mW cm⁻²). Without irradiation and at 25% light intensity, we could not detect dimer formation. At 50% and above, dimers were observed, and the system attained dynamic kinetic stability at low conversions (without monomer depletion) in 5 h (Fig. 2a). The increasing light intensity caused a non-linear and sequence-dependent growth of the steady-state dimer population (Fig. 2b). We carried out a separate light intensity-dependent experiment, where the UVA intensity of 3.80 mW/cm² was applied first, and after reaching the corresponding stationary state, the intensity was increased to 4.80 mW/cm² (Fig. 2c) for the same sample. The dimer population again displayed a non-linear increase, and a new steady-state was attained adaptively. The light intensity-dependent dynamic kinetic stability strongly supports an open-ended competition

between the break down and synthetic processes, which are of different order functions of the light intensity. Thus, an energy influx-dependent kinetic asymmetry determines the behaviour of the system.

We calculated the light intensity-dependent amplifications for the dimers upon elevating the power density from 50% to 100% (Fig. 2d). For the set of the ten most populated sequences relative to the complete set of dimers, enrichment of aromatic and aliphatic hydrophobic side chains was detected (Fig. 2e). In parallel, depletion of polar and cationic residues was measured at the peripheral positions (1 and 1'). We concluded that the kinetic asymmetry is sequence-dependent as well.

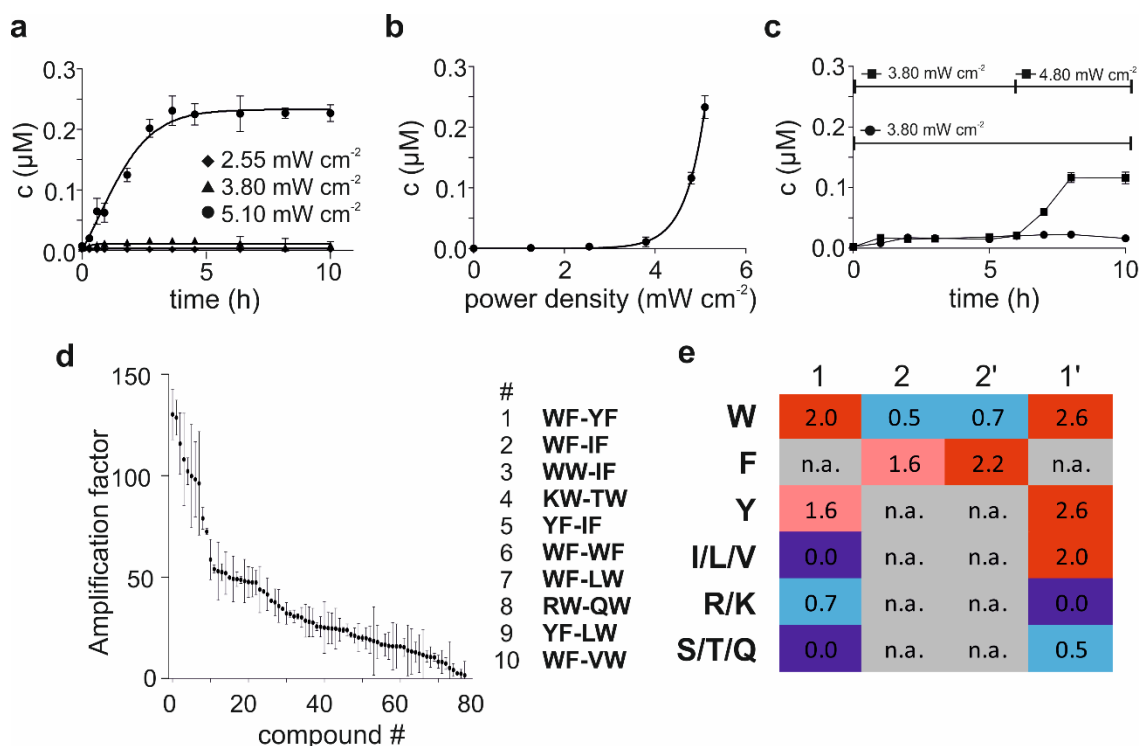


Fig. 2. Light intensity- and substituent-dependent concentrations. (a) Time- and light intensity-dependent concentration of a representative dimer (**WF-YF**) obtained at power densities of 2.55 mW cm⁻² (diamonds), 3.80 mW cm⁻² (triangles), 5.10 mW cm⁻² (circles). (b) Light intensity-dependent steady state concentrations for **WF-YF** (circles). (c) Adaptivity test started with an UVA intensity of 3.80 mW cm⁻² and subsequent increase to 4.80 mW cm⁻² (squares) and the control measurement run with a constant intensity of 3.80 mW cm⁻² (circles). (d) Light intensity-dependent amplifications obtained upon increasing power density from 2.55 mW cm⁻² to 5.10 mW cm⁻² with the ten most amplified sequences indicated (inset). (e) Side-chain enrichments in the peripheral (1, 1') and the central (2, 2') positions for the ten most amplified dimers (n.a. stands for 'not applicable').

The non-linear increase in the steady-state dimer concentration is a striking feature which justified further investigations. We hypothesised that autocatalysis is coupled to the light-driven synthesis routes, which allows the steeply increasing dominance for the synthesis over the break down routes at elevated energy influx. However, the effective light-induced spontaneous seeding and break down reactions are inseparable from the templated auto-/cross-catalysis due to the low complexity of the building blocks. The break down of the seeding

dimer immediately starts upon UVA irradiation, and the consequently diminished rate increasing effect may be difficult to detect given the signal to noise ratio. To circumvent this problem, we synthesised a seeding dimer with a light insensitive thioether coupling unit (**WF-S-YF**, Fig. 3a). We expected that the linker chemistry has only a minor geometric influence on the templating effect, and thus, a sufficient amount of seeding material could induce a significant rate increase for the dimer synthesis. Seeding the mixture with **WF-S-YF** at a concentration of 10 μM led to a marked increase in the synthesis rates (Fig. 3b and 3c), while the seed concentration remained constant. The catalytic effect appeared for the whole MSSM dimer population but was strongly sequence-dependent (Fig. 3d and 3e). The seeding resulted in a 4x increase for the initial rate (measured at 40 min). The six sequences displaying the highest seeding effect included **WF-YF**, which contains the same foldameric building blocks as **WF-S-YF**. Significantly higher concentrations were also found in the steady-state (Fig. 3e) because the autocatalytic synthesis rate determines the dynamic kinetic balance. These results strongly supported the presence of the templated auto-/cross-catalysis, which allows the conclusion that MSSM dimers are produced in a replicator community. Since the auto-/cross-catalysis rates are sequence-dependent, the system exhibits a selection phenomenon; replicators having an effective and large number of templating interactions can dominate the mixture.

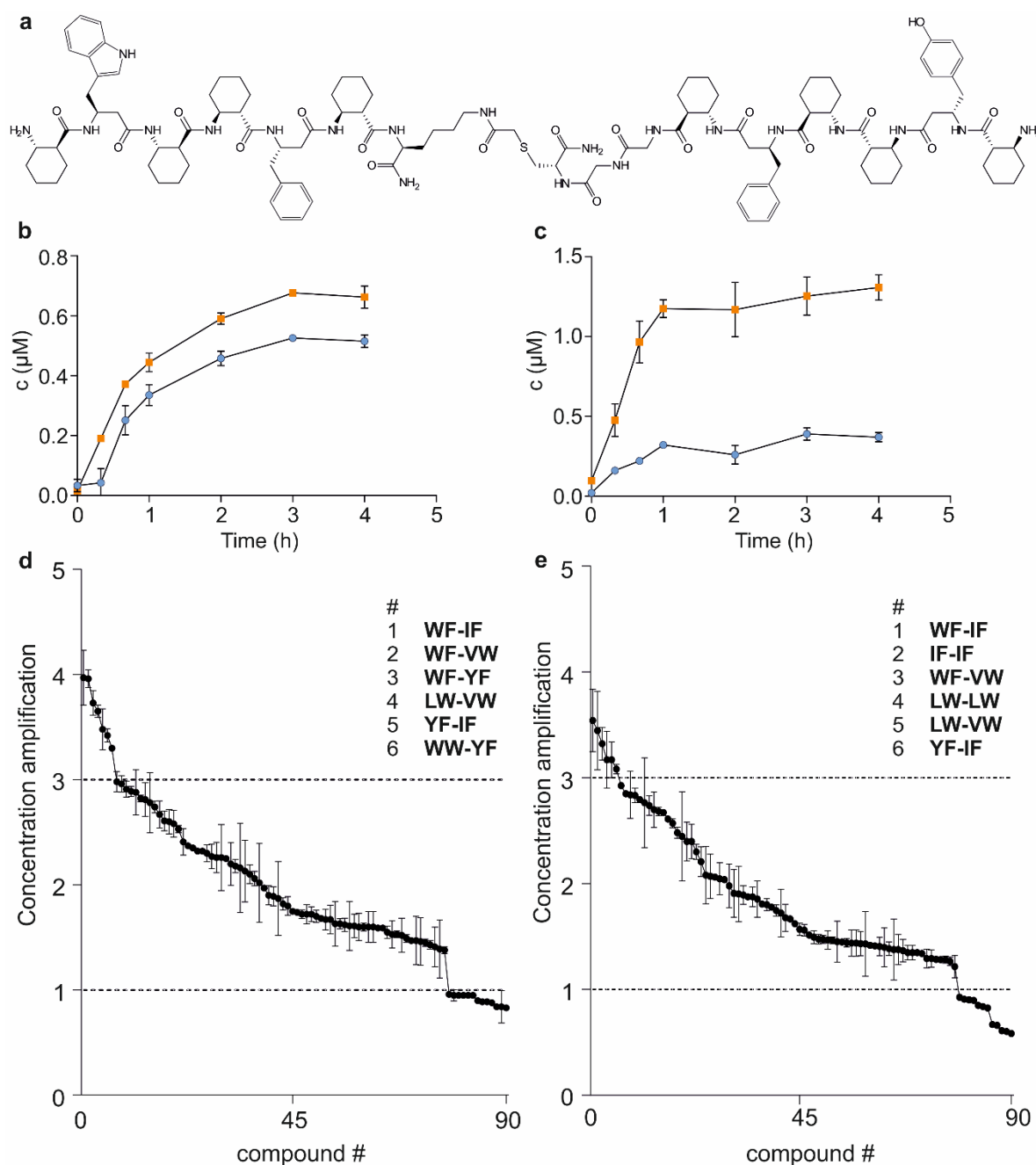


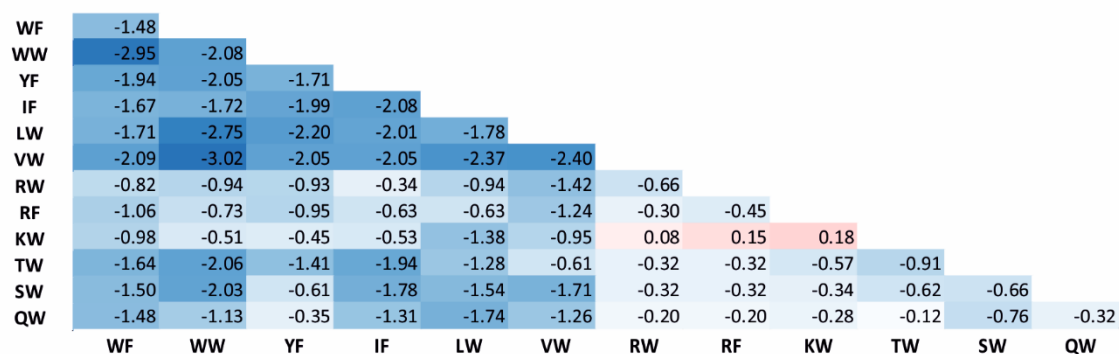
Fig. 3. Catalytic effects of seeding with WF-S-YF. (a) Structure of the seeding dimer (**WF-S-YF**) with the light-insensitive thioether linkage. Time evolution of the concentrations for representative dimers **WF-VW** (b) and **WF-IF** (c) with (orange square) and without (blue circle) the seeding dimer. Concentration amplifications ($c_{\text{seeded}}/c_{\text{control}}$) for the MSSM dimers upon seeding with **WF-S-YF** at 40 min (d) and in the steady-state (e).

Light intensity-dependent replicator concentrations are off-equilibrium in the steady state: dissipative system

We found that the kinetic asymmetry is dependent on the intensity of the energy influx; therefore, the replicator concentrations showed marked changes with the light intensity. This observation is a characteristic of a dissipative system. To gain further evidence for dissipative

replication, we directly compared the dimer concentrations in the equilibrium attained through the reversible thiolate-mediated exchange with those obtained in the kinetically asymmetric photochemical rearrangement. We visualised the significant differences with the logarithm of the concentration ratios ($\log(c_{Dissip}/c_{Equi})$) for each dimer and displayed the values on a heat map (Fig. 4). At 75% light intensity, the steady-state replicator concentrations are well below the equilibrium values (Fig. 4a) due to the strong bias toward the replicator decomposition mechanism. This finding shows that the replicator system is off-equilibrium already at low energy influx, but replicator breakdown is the predominant process. This situation fundamentally changes at 100% light intensity because light energy-driven replication successfully competes with the decomposition mechanism (Fig. 4b). The deviation from the equilibrium concentrations is strongly dependent on the sequence. Hydrophobic sequences are less favoured in the dissipative system, whereas replicators with polar or cationic side chains reach higher concentrations relative to the equilibrium system. Likely, stabilisation of the equilibrium structures and the transition states of replication require different interaction geometry. However, further investigation is necessary to explain this phenomenon. We conclude that both the replicators' overall abundance and sequence-dependent concentration profile deviate from the equilibrium values. This finding, together with the inherent energy-influx dependence, supports the dissipative nature of the system.

a



b

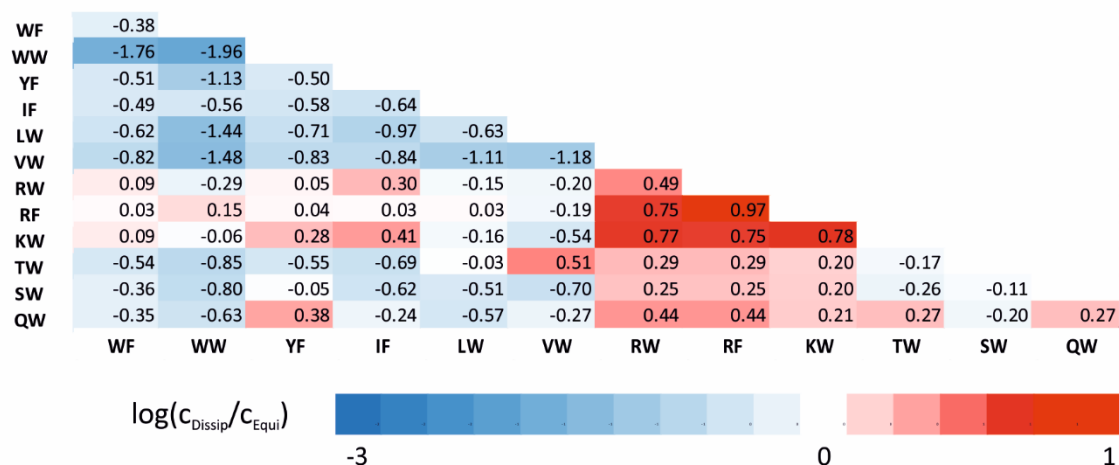


Fig. 4. Light intensity- and sequence-dependent deviation of the replicator concentrations from the equilibrium values. The logarithm of the concentration ratios ($\log(c_{Dissip}/c_{Equi})$) is displayed on a heat map. c_{Dissip} stands for the steady-state concentrations measured in the kinetically asymmetric photochemical exchange at 75% light intensity (**a**) and 100% light

intensity (**b**). c_{Equi} indicates the concentrations obtained in the thiolate-mediated exchange reaction relaxed to equilibrium. The combinations of monomers in the specific dimers are displayed as two-letter codes on the horizontal and vertical axes.

Dynamic model for the photocatalytic dissipative replicator system

The light-induced thiyl radicals participate in a chain reaction as established in the literature^{19, 22} (Fig. 5a, [1]–[4]). In the absence of steric inhibition and preferential binding, the predominant exchange route is diffusion-controlled radical substitution (Fig. 5a, [3] and [4]). Based on the preceding literature observation, the rate equations for dimer synthesis [3a] ($v_{s,ch}$) and breakdown [3b] (v_b) can be computed (eqs. (1) and (2)). The detailed calculations are provided in the Supplementary text.

$$v_{s,ch} = s_{ch}\sqrt{I}[MSSG]^{1.5} \quad (1)$$

$$v_b = b\sqrt{I}[MSSM] \quad (2)$$

The terms s_{ch} and b represent the corresponding rate constants, and I designates light intensity. The central hypothesis of this work is that preferential binding (Fig. 5c, [5]) facilitates the proximity-controlled exchange pathways. In this case, the light-induced homolytic cleavage [6] and the subsequent radical substitution [7] occur within the foldameric complexes. We cannot rule out *a priori* a concerted metathesis with coincident absorption of two photons (Fig. 5c, [8] and [9]). In this case, the cross section of the interaction is not decreased by non-linear two-photon absorption effects because the two disulfides are separately excited. The corresponding rate equations for MSSM formation *via* [7] ($v_{s,p1}$) and [9] ($v_{s,p2}$) can be derived through a binding preequilibrium and the rate-limiting intracomplex steps (eqs. (3) and (4)). See Supplementary text for the detailed computation.

$$v_{s,p1} = s_{p1}I[MSSG]^2 \quad (3)$$

$$v_{s,p2} = s_{p2}I^2[MSSG]^2 \quad (4)$$

The proximity-controlled radical substitution and metathesis rate constants are s_{p1} and s_{p2} , respectively.

The non-linear light intensity-dependence of the steady-state concentration can be modelled with a hyperbolic curve, which can be obtained for this reaction system by incorporating autocatalysis into the model^{31, 32}. Indeed, we confirmed the presence of the templated auto/cross-catalysis (Fig. 5f). This type of templating can exert autocatalysis on dimer formation by both proximity-controlled radical substitution (Fig. 5e, [12]) and concerted disulfide metathesis (Fig. 5c, [14]). The corresponding rate terms ($v_{s,a1}$ and $v_{s,a2}$) can be calculated (eqs. (6) and (7)). Details are provided in the Supplementary text.

$$v_{s,a1} = s_{a1}I[MSSM][MSSG]^2 \quad (6)$$

$$v_{s,a2} = s_{a2}I^2[MSSM][MSSG]^2 \quad (7)$$

The autocatalytic radical substitution and concerted metathesis rate constants are s_{a1} and s_{a2} , respectively.

With eqs. (1)–(7), the steady-state concentration can be expressed (eq. (8)). However, we observed that the steady-state concentrations steeply converge to zero with decreasing light intensity for all sequences (Fig. 2b). This result strongly suggests that the diffusion-controlled radical substitution (Fig. 5a, [3a]) ($v_{s,ch}$) has no detectable contribution to the dimer synthesis ($s_{ch} \approx 0$); that is, the light-independent term is negligible in the numerator of eq. (8). Dimer synthesis, therefore, proceeds predominantly via the proximity-controlled pathways, whereas break down to monomers occurs through diffusion-controlled radical substitution. This observation simplifies eq. (8) to eq. (9).

$$[\text{MSSM}]_{ss} = \frac{(s_{p1}\sqrt{I} + s_{p2}I^{1.5})[\text{MSSG}]^2 + s_{ch}[\text{MSSG}]^{1.5}}{b - (s_{a1}\sqrt{I} + s_{a2}I^{1.5})[\text{MSSG}]^2} \quad (8)$$

$$[\text{MSSM}]_{ss} = \frac{(s_{p1}\sqrt{I} + s_{p2}I^{1.5})[\text{MSSG}]^2}{b - (s_{a1}\sqrt{I} + s_{a2}I^{1.5})[\text{MSSG}]^2} \quad (9)$$

Thus, the light-intensity dependence also appears in the denominator explaining the hyperbolic function observed in the experiments. The hyperbolic approximation is valid only at low conversions ($[\text{MSSG}] \approx [\text{MSSG}]_0$), which holds for our experiments. Beyond the steady-state analysis, the dynamic model incorporating eqs. (2)–(7) was used to numerically simulate and fit the time- and light intensity-dependent data arrays with variable rate constants. Non-linear regressions were carried out simultaneously against all data points measured for the individual dimers. Excellent agreement was found (Fig. 6a, solid curves and Extended Data Fig. 2). The regression against the dynamic model excluding the autocatalytic terms eqs. (6) and (7) failed (Fig. 6a, dashed). Neither the hyperbolic response of the steady state concentration nor the initial conversion rate changes upon the light intensity increase were captured correctly without the autocatalytic terms. This finding strongly supports that the hyperbolic response in the energy influx domain is a characteristic of the templated autocatalysis.

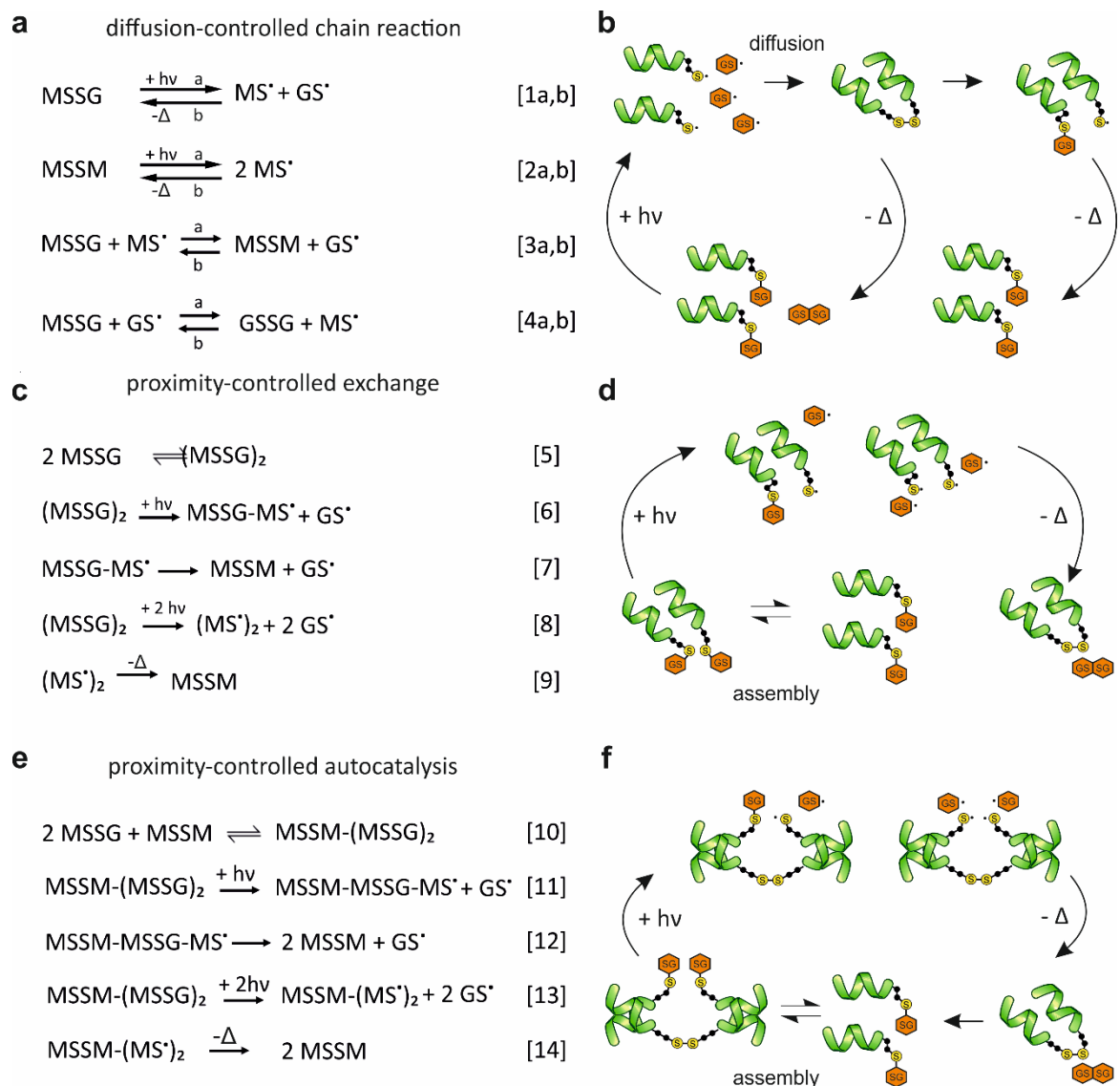


Fig. 5. Steps of the photochemical disulphide-exchange mechanisms. **a)** Diffusion-controlled radical chain-reaction mechanism described in the literature for the photochemical disulfide exchange¹⁹. The separate initiation ([1a] and [2a]), termination ([1b] and [2b]) and chain propagation steps ([3a,b] and [4a,b]) are indicated with the back-and-forth arrows, which do not refer to any preequilibrium or microscopic reversibility. **b)** Schematic representation of the replicator breakdown through diffusion-controlled radical substitution. **c)** The hypothesised proximity-controlled reaction pathway with the foldamer association preequilibrium [5]. The intracomplex steps producing MSSM can be radical substitution ([7]) and concerted metathesis [9]. **d)** Seeding *via* foldamer assembly and subsequent proximity-controlled radical substitution or concerted metathesis. **e)** The proposed proximity-controlled autocatalytic reaction mechanism. **f)** Replication with templated cross-/autocatalysis facilitating the proximity-controlled radical substitution or concerted metathesis.

Time evolution of the reaction rates revealed that the autocatalytic pathway dominates dimer synthesis after 10 min (Fig. 6b). The proximity-controlled, spontaneous (non-autocatalytic) mechanism is effective in seeding but would not produce detectable dimer formation within the observed time frame. Inspection of the resulting rate constants indicated that both the proximity-controlled radical substitution and the concerted metathesis mechanisms are present

in the system. At the level of the individual dimers, however, the mechanism appeared to be dependent on the sequence (Supplementary Table 2). Understanding this substituent-dependent mechanism requires further investigation, which is beyond the scope of the present work.

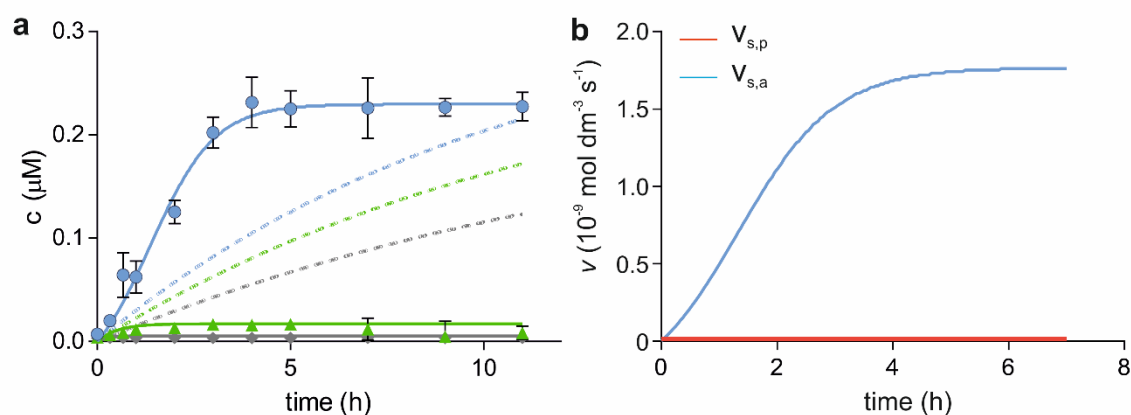


Fig. 6. Effects of templated autocatalysis on dimer formation. a) The best-fitting dynamic models with (solid) and without (dashed curves) autocatalysis. Representative experimental time- and light intensity-dependent data array is shown for the dimer **WF-YF** measured at power densities of 2.55 mW cm^{-2} (grey), 3.80 mW cm^{-2} (green) and 5.10 mW cm^{-2} (blue). **b)** Time evolution of the reaction rates calculated for the proximity-controlled spontaneous synthesis ($v_{s,p}$: red) and the autocatalytic synthesis ($v_{s,a}$: blue) in the best-fitting dynamic model for **WF-YF**.

Discussion

We successfully coupled light-harvesting thiyl radical chemistry with the molecular recognition processes occurring in a foldameric network. Disulfide linkage has a long history, and its nucleophilic exchange mechanism is currently popular in systems chemistry applications^{33, 34}. The photocatalytic rearrangement reaction of disulfides has also been known for decades.¹⁹ However, our work demonstrates the ability of the UV light-induced cleavage-recombination cycle of the disulfide bond to drive kinetically asymmetric processes. The root of asymmetry is the binding between peptidic segments that facilitates a fast intracomplex route for the thiyl-mediated exchange reactions under proximity control (Fig. 5b). In parallel, the diffusion-controlled chain reaction in the background effectively couples disulfides with substituents that rapidly diffuse without preferential binding (Fig. 5d). The weak interactions can exert proximity control because the high-energy radical intermediates rapidly relax to the dimer before entering the chain reaction. This finding suggests that the weak binding between primitive structures facilitates kinetic asymmetry if the dynamic covalent rearrangement is fast enough^{5, 35}. Since the competing mechanisms are affected by the light intensity in different orders, the light not only switched processes on and off, but the energy influx also influenced the extent of kinetic asymmetry. UVA irradiation drives the system to off-equilibrium steady states, ratcheting up the fittest sequences^{3, 36}. Thus, the system meets the criteria of dissipativity.

The modular nature of the foldameric disulfides allowed templating interactions between the foldamer-disulfide dimers and the glutathione-protected monomers. This interaction extends

the proximity-controlled mechanism to an autocatalytic process, thereby facilitating dissipative replication (Fig. 6c). The dissipative replication and the replicator death mechanism asymmetrically compete without physical separation of the pathways^{37, 38}. In this system, the tendency toward assembly into structures of increased complexity is amplified by the dissipative replication. In contrast, rapid diffusion - a property associated with low complexity - promotes decomposition. Thus, the dissipative adaptation^{5, 39} in the present system helps elucidate the chemical mechanisms of spontaneous emergence of complexity by selection. Pioneering experiments with dissipative self-assembling systems established the principles that govern chemical energy⁴⁰⁻⁴² or light-driven^{43, 44} trajectories to off-equilibrium states that generate spatial proximity-based order. Peptide-disulfides in the photochemical exchange reaction can be an additional valuable tool to study the dissipative self-organisation of simple building blocks.

Although the system presented here displays features essential for chemical evolution, prebiotic chemistry is beyond the scope of this study. However, the chemical availability of primitive Cys-containing peptides is supported by prebiotic Cys-catalysed amino acid and peptide synthesis^{45, 46}. These findings make thiol radical-mediated dissipative replication of short, folding peptidic sequences an intriguing mechanism⁴⁷, potentially illuminating energetic aspects of the transition from prebiotic chemical networks to biotic evolution.

Methods

UV-fuelled disulfide exchange reaction. Twelve different MSSG disulfides were dissolved in 20 mM HEPES, 150 mM NaCl, 2 mM CaCl₂ (pH = 7.0), with each disulfide at a final concentration of 10 μ M. The reaction mixture was transferred into quartz cuvettes having a PTFE stopper and kept under an argon atmosphere during the experiment. Solutions were stirred at 150 RPM and kept at a constant temperature of 303 ± 1 K with an air-cooling system (modified Jasco Jetstream 2 Plus Column Thermostat). The temperature was monitored with a laser gun thermometer. Continuous illumination of the samples was carried out with a UVL-28 EL Series UV lamp (Analytic Jena US, Upland, CA). The distance dependence of the power density and the emission spectrum of the lamp were determined prior to the experiments (Extended Data Fig. 3), and the irradiation intensity was controlled by the distance between the lamp and the sample (Extended Data Fig. 4). 200 μ L samples were taken from the reaction mixture at each time point. Placing the sample in the dark froze the reaction; no relaxation to equilibrium occurred. To eliminate any slow non-photocatalytic disulfide exchange reaction in the mixture until the analysis, we added 100 μ L of 10% TFA in water. The product distribution of the samples was analysed using HPLC/ESI-MS measurements.

LC-MS measurements and MS data analysis. LC-MS analysis was performed with a Dionex UltiMate 3000 HPLC system interfaced with an LTQ ion trap mass spectrometer (Thermo Electron Corp., San Jose, CA, USA). Samples were injected onto an AerisTM Widepore XB-C18 (250 \times 4.6 mm, particle size: 3.6 μ m, pore size 100Å) analytical HPLC column using gradient elution 5-80% solution B for 25 min at 0.7 mL min⁻¹ flow rate. Eluent composition was 0.1% formic acid in distilled water (Solution A) and 0.1% formic acid in acetonitrile (Solution B). The MS instrument was operated in the positive-ion mode using the equipped

HESI-II source with the following parameters: capillary temperature: 350°C; spray voltage: 3.0 kV; source heater temperature: 250°C; sheath gas flow: 30 mL min⁻¹; aux gas flow: 10 mL min⁻¹. Mass spectra were acquired in full-scan mode from 200 to 2000 *m/z*. Thermo Xcalibur 2.2 software was used for peak identification and integration. 96% of the foldameric building blocks could be resolved independently by HPLC-MS/MS measurements based on molecular weight, MS fragmentation pattern and retention time depending on the relative hydrophobicity of the side chains. Those components which could not be resolved independently were integrated and averaged. A representative raw file for the library was utilised to create a processing method, where each sample component was associated with a chromatographic peak based on the previously identified mass (*m/z*) and retention time (Supplementary Table 1). Using the ICIS peak detection algorithm, the general detection and integration criteria were: smoothing points: 5; baseline window: 60; area noise factor: 5; peak noise factor: 10. All raw data files were reprocessed with these processing setups together and analysed. Errors in peak identification during the automatic processing were corrected manually.

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Data availability

All data is available in the main text or the supplementary materials. All data and materials used in the analysis are available to any researcher for purposes of reproducing or extending the analysis.

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Dr. Miklós Erdélyi and Prof. Gábor Szabó (University of Szeged) are acknowledged for the calibration of the UV light source used for the measurements.

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Author contributions

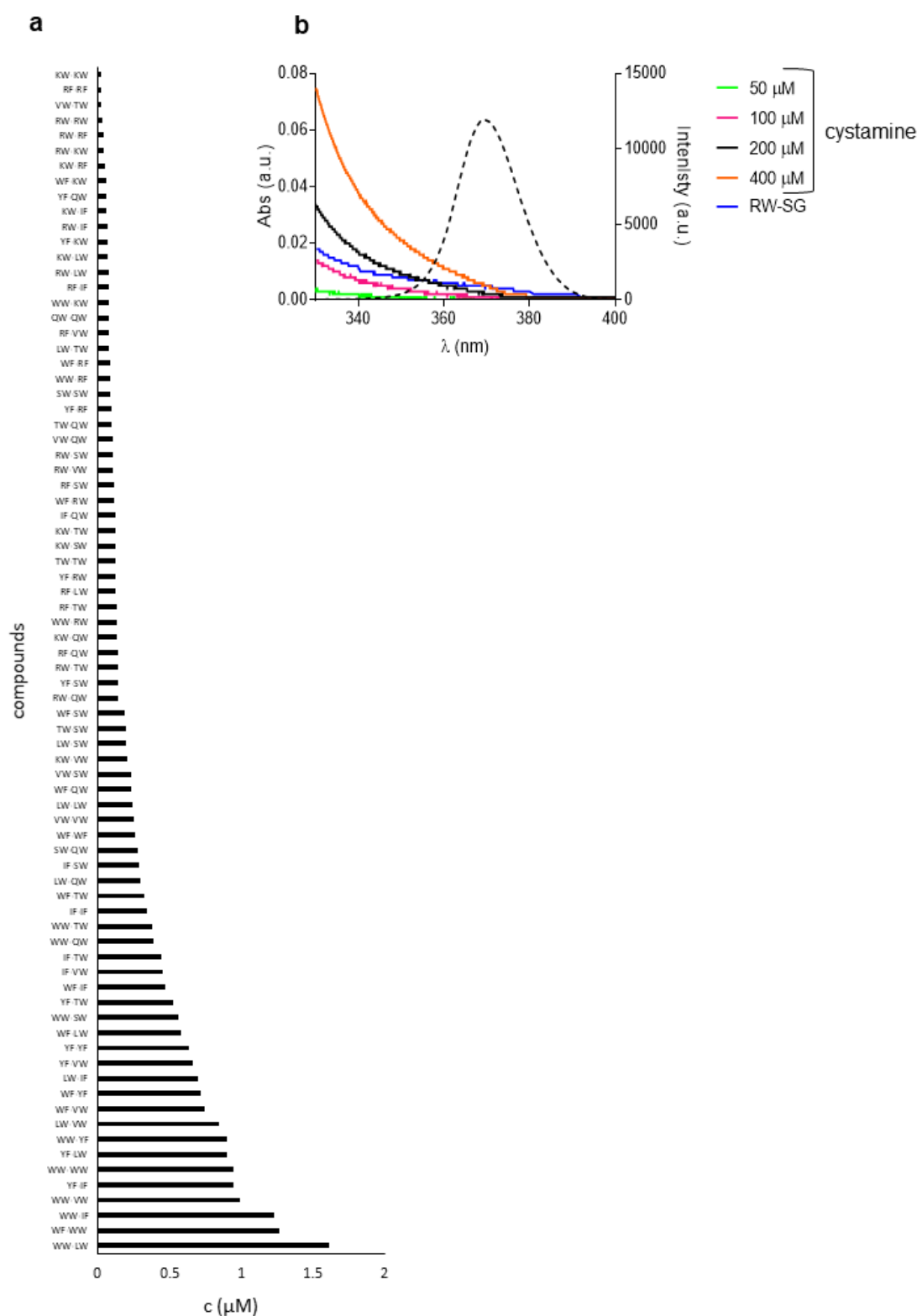
B.M., Á.B. and É.B. synthesised the foldameric building blocks, performed the dissipative chemical network experiments and analysed the data. A.T. and E.W. analysed the foldamer-foldamer interactions, performed inverse seeding and evaluated the data. G.K. and Z.K. carried out foldamer stability tests. G.F. contributed to the formulation of the concept and wrote the manuscript. T.A.M. developed the concept, designed the experiments, evaluated the experimental results and wrote the manuscript.

Competing interests

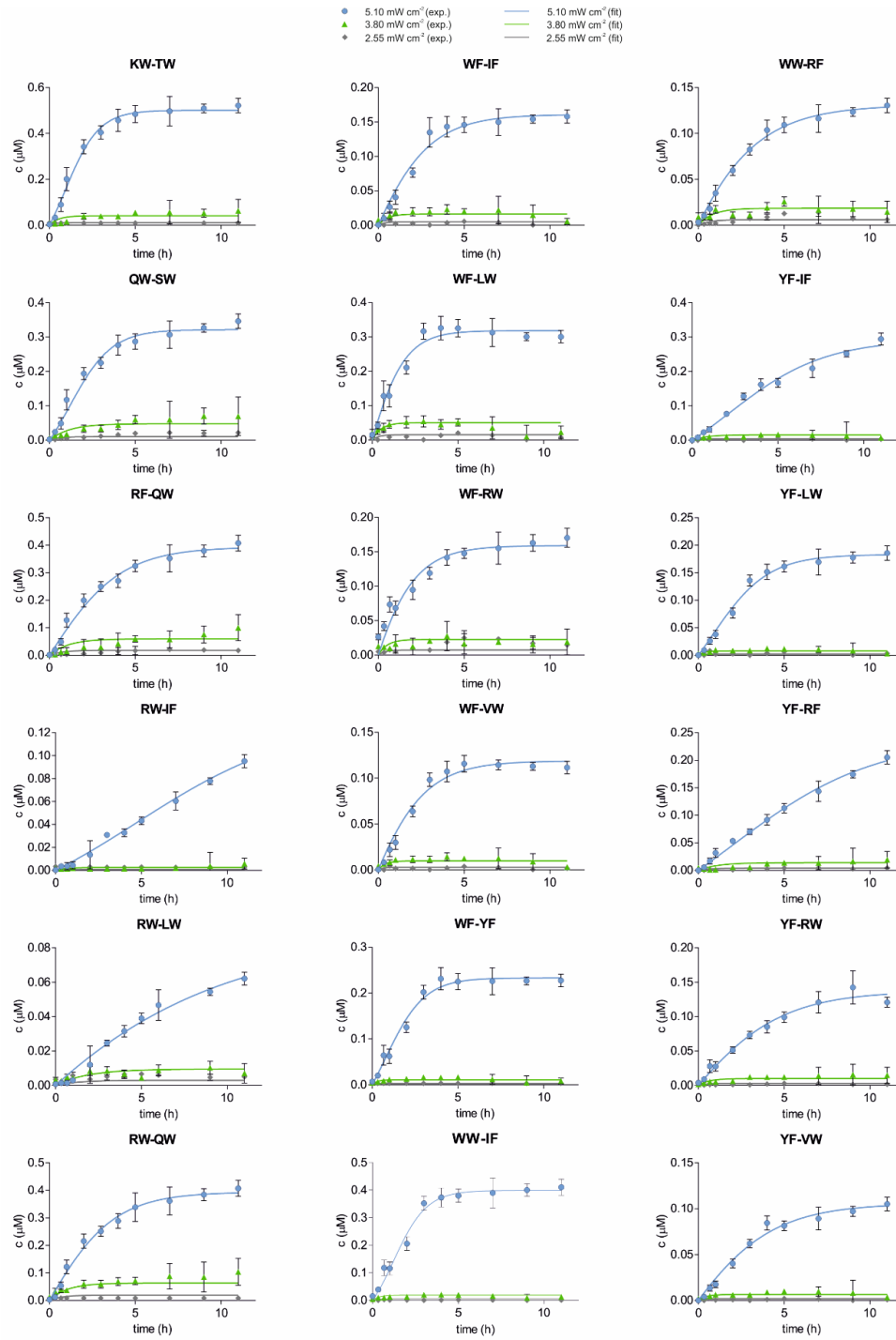
The authors declare no competing interests.

Additional information**Supplementary Information**

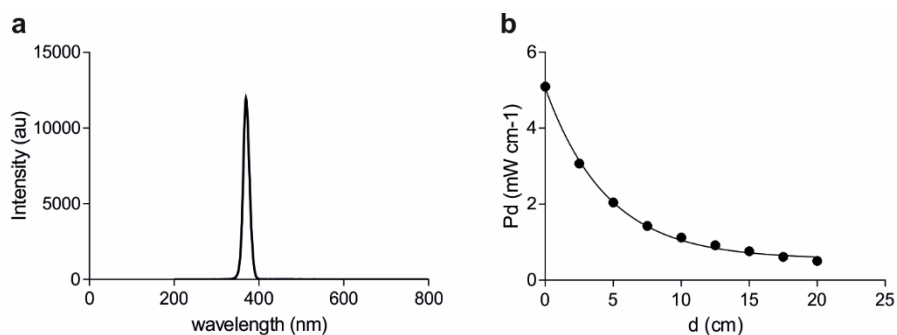
Supplementary Information is available for this paper



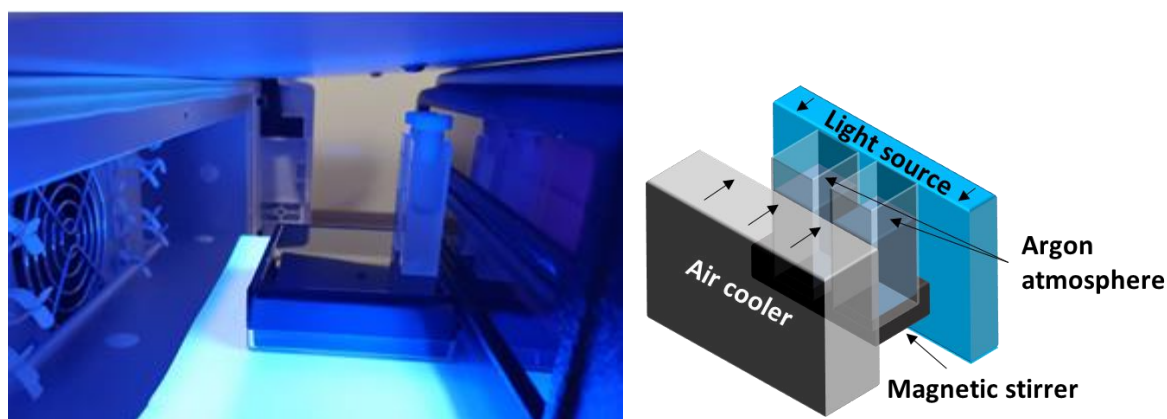
Extended Data Fig. 1. The equilibrium product distribution of foldameric dimers and UV spectra of the disulfide containing samples. a) Equilibrium product distribution was measured in an aqueous solution; for the experimental conditions, see Supplementary Methods. **b)** UV spectra of cystamine solutions in the range of 330-400 nm. UV spectrum of the representative monomer **RW-SG** at the concentration of 120 μ M. The dashed curve shows the emission spectrum of the UV lamp (UVL-28 EL Series UV Lamp) in the range of 330-400 nm.



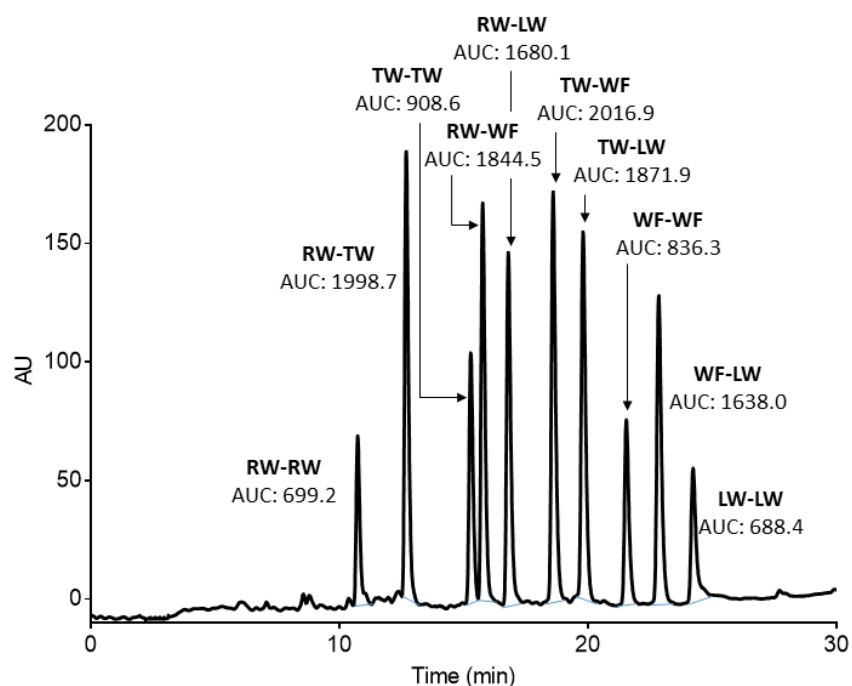
Extended Data Fig. 2. Fitting of the dynamic model to the light intensity and time-dependent data arrays measured for representative replicators. Data at different light intensities are represented as follows: 2.55 mW cm⁻² (gray diamond), 3.80 mW cm⁻² (green triangle) and 5.10 mW cm⁻² (blue circle); fitted curves are depicted with matching color. Non-linear least-squares analysis was used for fitting. Experimental data were obtained from three parallel measurements on three different samples. See Supplementary Table 2 for fitted parameters.



Extended Data Fig. 3. Calibration of the UV light source. (a) The emission spectrum of the UV light source (UVL-28 EL Series UV Lamp), with an emission maximum at 365 ± 5 nm and (b) dependence of the power density (Pd [mW cm⁻²]) on the distance. The power density was measured with an S140C Integrating Sphere Photodiode Power Sensor (Thorlab Inc.). The wavelength range of the Si detector was 350–1100 nm, and the power range was 1 μ W–500 mW (resolution: 1 nW)



Extended Data Fig. 4. Photo and schematic representation of the experimental setup. The reaction mixtures were stirred at 150 RPM in quartz cuvettes having a PTFE stopper and kept under an argon atmosphere during the experiment. Constant temperature (303 ± 1 K) was maintained with an active air-cooling system (column thermostat). The temperature was monitored with a laser-gun thermometer. Continuous irradiation of the samples was carried out by UVL-28 EL Series UV lamp working at 365 nm (Analytic Jena US, Upland, CA), and the power density was varied by changing the distance between the light source and the samples.



Extended Data Fig. 5. Product distribution obtained by slow oxidation in a chaotropic solvent.

Oxidation of the glutathione-protected foldamers **WF-SG**, **LW-SG**, **RW-SG** and **TW-SG** resulted in a statistical product distribution despite their different tendencies to associate. Samples were analysed by HPLC-MS. Conditions of the analytical HPLC-MS measurement: column: Phenomenex Luna-C18 (250 × 4.6 mm); method: 5-80% B during 25 min, flow rate: 0.7 mL min⁻¹, where eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in ACN: water = 8:1.

Supplementary Information for

**Light-fuelled dissipative replication and selection in
evolvable biomimetic chemical networks**

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Supplementary Text

Equation (1): Rate of spontaneous dimer synthesis through diffusion-controlled radical substitution.

In this mechanism, dimers (MSSM) are produced by the reaction of freely diffusing monomer radicals (MS^\bullet) with glutathione-protected monomers (MSSG) as shown in reaction [3a] (Fig. 1b). Here, "MS" corresponds to a single foldameric sequence.



The reaction rate for [3a] ($v_{s,ch}$) satisfies eq. (S1),

$$v_{s,ch} = k_{ch}[MS^\bullet][MSSG] \quad (S1)$$

where k_{ch} is the rate constant for the radical substitution step. In radical chain reactions, $[MS^\bullet]$ can be approximated with a quasi-steady state approach. At low conversions, MS^\bullet is produced preferentially by homolytic cleavage of the starting material MSSG. Therefore, the rate of formation is proportional to the light intensity ($k_I I$) and $[MSSG]$. The chain-termination steps consuming MS^\bullet involve collisions with MS^\bullet and GS^\bullet . Again, the large excess of MSSG at low conversions affords the approximation that reaction [1a] (Fig. 1b) is dominant. Thus, there is a 12× molar excess for $[GS^\bullet]$, that is, $[GS^\bullet] \approx 12[MS^\bullet]$. Consequently, the chain-termination rate can be expressed as a second order function in $[MS^\bullet]$ (eq. (S2)).

$$\frac{d[MS^\bullet]}{dt} = 0 = k_I I [MSSG] - k_t [MS^\bullet]^2 - k_t 12 [MS^\bullet]^2 \quad (S2)$$

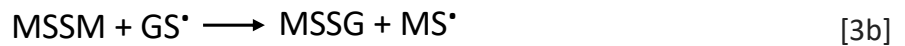
$$[MS^\bullet] = \sqrt{\frac{k_I I}{13k_t}} [MSSG] \quad (S3)$$

Here, k_I is the rate constant of the light-induced homolytic cleavage, and k_t is the rate constant for the chain-termination reaction. Substituting eq. (S3) into eq. (S1) and collecting the concentration- and light intensity-independent terms into the constant s_{ch} , the rate is given by eq. (1).

$$v_{s,ch} = s_{ch} \sqrt{I} [MSSG]^{1.5} \quad (1)$$

Equation (2): Rate of dimer breakdown through diffusion-controlled radical substitution.

The decomposition of MSSM proceeds in this mechanism through the reaction between the freely diffusing glutathione radical (GS^\bullet) and MSSM (reaction [3b]).



The rate equation can be expressed as follows (eq. (S4)).

$$v_b = k_b[\text{GS}\cdot][\text{MSSM}] \quad (\text{S4})$$

For $[\text{GS}\cdot]$, we apply the quasi-steady state approximation again. The source of $[\text{GS}\cdot]$ is the light-induced homolytic cleavage of MSSG and GSSG. The chain-termination steps consuming $\text{GS}\cdot$ involve collisions with $\text{GS}\cdot$ and $\text{MS}\cdot$. Due to the 12 \times excess of $\text{GS}\cdot$ over $\text{MS}\cdot$ in the system studied, the latter reaction can be neglected. Thus, we obtain eq. (S5).

$$\frac{d[\text{GS}\cdot]}{dt} = 0 = k_I I ([\text{MSSG}] + 2[\text{GSSG}]) - k_t [\text{GS}\cdot]^2 \quad (\text{S5})$$

Due to the mass balance for the ‘GS’ moiety, the term $[\text{MSSG}] + 2[\text{GSSG}]$ is constant and equals to the initial concentration of MSSG ($[\text{MSSG}]_0$), which yields eq. (S6).

$$[\text{GS}\cdot] = \sqrt{\frac{k_I I}{k_t} [\text{MSSG}]_0} \quad (\text{S6})$$

Thus, $[\text{GS}\cdot]$ is proportional to the square root of the light intensity, and eq. (S6) can be substituted into (S4). Collecting the constant term into the overall rate constant of b , we obtain eq. (2).

$$v_b = b\sqrt{I}[\text{MSSM}] \quad (2)$$

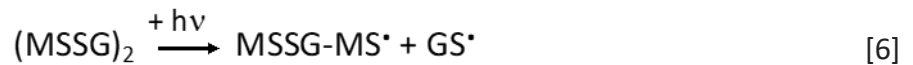
Equations (3) and (4): Rates of dimer synthesis through proximity-controlled mechanisms.

This mechanism begins with the association preequilibrium producing the complex $(\text{MSSG})_2$.



$$[(\text{MSSG})_2] = \frac{1}{K_{D,p}} [\text{MSSG}]^2 \quad (\text{S7})$$

$K_{D,p}$ stands for the dissociation constant. The rate-determining step is the photochemical cleavage of a monomer within the complex (reaction [6]). If the geometry of the complex is advantageous, the resulting high-energy radical can rapidly relax through the intracomplex radical substitution [7].



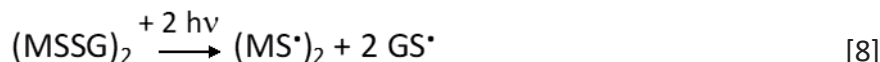
The rate of formation for $\text{MSSG-MS}\cdot$ is proportional to the light intensity ($k_I I$) and $[(\text{MSSG})_2]$. Using eq. (S7), the rate equation can be obtained (eq. (S8)).

$$v_{s,p1} = \frac{k_I I}{K_{D,p}} [\text{MSSG}]^2 \quad (\text{S8})$$

Substituting the constant term with the overall rate constant s_{p1} yields eq. (3).

$$v_{s,p1} = s_{p1}I[\text{MSSG}]^2 \quad (3)$$

Literature results showed that recombination of two thiyl radicals is a possible photochemical exchange mechanism, when radical substitution is sterically hindered¹. Therefore, the intracomplex version of this mechanism cannot be ruled out *a priori*. If the absorption of the two photons is consecutive, the reaction rate remains first order in light-intensity; that is, the functional form of the rate equation is the same as eq. (3). If coincident absorption of two photons produces the diradical intermediate $(\text{MS}\cdot)_2$ and MSSM is formed in a concerted manner (steps [8] and [9]), the probability of the interaction with two photons is proportional to the square of the light intensity.

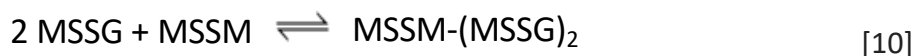


The cross-section of the interaction is not decreased by the non-linear “two-photon absorption” effect, because disulfides in the complex are not coupled quantum mechanically. Moreover, the recombination of the diradical [9] does not involve any activation energy, leading to a very fast reaction. On this ground, we incorporated this mechanism into the model with the rate equation (4).

$$v_{s,p2} = s_{p2}I^2[\text{MSSG}]^2 \quad (4)$$

Equations (5) and (6): Rates of autocatalytic dimer synthesis.

The autocatalytic mechanism starts with a binding preequilibrium between the dimers and the monomers ([10]). The dissociation constant ($K_{D,a}$) determines the concentration of the complex available for the further, rate determining step.



$$[\text{MSSM}-(\text{MSSG})_2] = \frac{1}{K_{D,a}} [\text{MSSM}][\text{MSSG}]^2 \quad (\text{S9})$$

The calculation of the rates for the autocatalytic routes is closely analogous to the non-autocatalytic synthesis pathways described above. For the intracomplex radical-substitution pathway, the rate-determining step is the absorption of a photon, which leads to a linear light-intensity dependence of the reaction rate. Using (S9), eq. (6) is obtained.

$$v_{s,a1} = s_{a1}I[\text{MSSM}][\text{MSSG}]^2 \quad (6)$$

Coincident absorption and the concerted conversion to MSSM is also possible for the autocatalytic complex, which yields a rate equation quadratic in light intensity (eq (7)).

$$v_{s,a2} = s_{a2}I^2[\text{MSSM}][\text{MSSG}]^2 \quad (7)$$

Dynamic model for the foldamer-based photochemical disulphide exchange system

The mathematical framework of chemical evolution^{2, 3} was invoked to analyse the experimentally determined energy-dependent dynamics of the dimer synthesis. The differential equation describing the time- and light intensity-dependent concentration of the dimers ([MSSM]) contained the following rate terms (eq. (S10)): non-autocatalytic (spontaneous) synthesis ($v_{s,p1}$ and $v_{s,p2}$), autocatalytic synthesis ($v_{s,a1}$ and $v_{s,a2}$) and breakdown (v_b).

$$\frac{d[\text{MSSM}]}{dt} = v_{s,p1} + v_{s,p2} + v_{s,a1} + v_{s,a2} - v_b \quad (\text{S10})$$

Fitting the dynamic model to the experimental time- and energy-dependent data arrays

Differential equation (S10) was numerically integrated using the Runge-Kutta (RK4) method to simulate the light intensity-dependent time evolution of a dimer. The synthesis rates are dependent on the actual monomer concentration, which was expressed with [MSSM] using the mass balance (S11).

$$[\text{MSSG}] = [\text{MSSG}]_0 - 2[\text{MSSM}] \quad (\text{S11})$$

The numeric integrations were carried out with the parameters of $[\text{MSSM}]_0 = 0$, $[\text{MSSG}]_0 = 2 \times 10^{-5} \text{ M}$ and $\Delta t = 4.17 \times 10^{-2} \text{ h}$. The light intensities were set to the calibrated values. The rate constants s_{p1} , s_{p2} , s_{a1} , s_{a2} and b were determined by fitting (non-linear regression) the simulated curves against the time- and light intensity-dependent experimental data array.

Supplementary Methods

Synthesis and purification of the foldameric sequences

Foldameric sequences having L-Gly-L-Gly-L-Cys C-terminal segments were synthesised manually by standard solid-phase peptide synthesis with Fmoc/tBu chemistry. Rink Amide AM resin was used as solid support (capacity: 0.71 mmol/g) and HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) as coupling reagent in the presence of DIEA (*N,N*-diisopropylethylamine). Amino acids and coupling reagents were used in excess of 3 equivalents and shaking was applied at room temperature for 3 h. Deprotection was carried out in a DMF (*N,N*-dimethylformamide) solution containing 2% DBU (1,8-diazabicycloundec-7-ene) and 2% piperidine. Cleavage was performed with TFA/H₂O/DTT (DL-dithiothreitol)/TIS (triisopropylsilane) (90:5:2.5:2.5), which was followed by precipitation in ice-cold diethyl ether. The resin was washed with acetic acid and water, filtered, then lyophilised. Peptides were purified by RP-HPLC on a C18 column (Phenomenex Luna C18, 250 x 10.00 mm, particle size: 10 µm, pore size: 100 Å). The HPLC eluents were 0.1% TFA in water (Eluent A), and 0.1% TFA/ 80% ACN (acetonitrile) in water (Eluent B). Different gradient elution was used according to the

hydrophobicity of the peptides. Purity was confirmed by analytical RP-HPLC and ESI-MS measurements.

Synthesis and purification of the glutathione protected monomers

Glutathione-protected monomers were synthesised by oxidative coupling of thiols in the solution phase. Each purified foldamer was dissolved in 20% DMSO (dimethyl sulfoxide) in water separately to 1 mM concentration in the presence of 20× excess of GSH (reduced glutathione) and stirred overnight at room temperature exposed to atmospheric oxygen. The completeness of the oxidation reaction was monitored by HPLC-MS, and the reaction mixture was injected directly onto a semi-preparative HPLC column (Phenomenex Luna C18, 250 × 10.00 mm; particle size: 10 µm; pore size: 100 Å) and purified. Under this reaction condition the amount of the homodimeric foldamer was negligible and could be completely separated from the foldamer-glutathione adduct.

LC-MS analysis of the purified glutathione protected monomers

UHPLC-MS/MS measurements were used to characterize the pure peptides by using an ACQUITY I-Class UPLC™ liquid chromatography system (Waters, Manchester, UK) coupled with a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation was carried out at 25 °C using 0.1% formic acid in water as solvent A and ACN containing 0.1% formic acid as solvent B. The following multistep gradient was used: 5-50% over 20 minutes then 50-80% over 5 minutes and finally 80% solvent B for additional 5 minutes at 0.7 mL min⁻¹ flow rate. Samples were incubated at 5°C until the measurement and 15 µL of the sample was injected into the UHPLC-MS/MS system. The MS instrument was operated in the positive-ion mode using the equipped HESI-II source with the following parameters: capillary temperature: 256°C; spray voltage: 3.5 kV; aux gas heater temperature: 412°C; sheath gas flow: 47.5 mL min⁻¹; aux gas flow: 11 mL min⁻¹; and S-lens RF level, 50.0 (source auto-defaults). Full scan was conducted with a mass range of 150–2000 *m/z* with resolution of 70,000. The ACG (automatic gain control) setting was defined as 3 × 10⁶ charges, and the maximum injection time was set to 100 ms. Data dependent MS/MS was acquired in a mass range of 200-2000 *m/z* with resolution of 17,500. AGC setting was defined as 5 × 10⁵ charges, and the maximum injection time was set to 150 ms.

Preliminary experiments for testing disulphide rearrangement in a foldamer library

Two setups for the starting systems were used for testing. First, four different glutathione protected precursors, **WF-SG**, **RW-SG**, **LW-SG** and **TW-SG** were dissolved in 20 mM HEPES buffer with 150 mM NaCl and 2 mM CaCl₂ (pH=7.0) each one in 10 µM concentration. Second, the 10-membered dimeric library was prepared by slow oxidation of **WF-SH**, **RW-SH**, **LW-SH** and **TW-SH** thiols, which formed the statistical concentration distribution. The components were transferred into the previously defined buffer so that each heterodimer had a concentration of 10 µM. An excess of oxidized glutathione (500 µM) was added to this latter mixture. The reaction mixtures were transferred separately into quartz cuvettes having PTFE stopper and kept under argon atmosphere during the experiment. Solutions were stirred at 150 RPM and kept at constant temperature of 303±1 K *via* air cooling system. Continuous irradiation of the samples was carried

out by UVL-28 EL Series UV Lamp at 365 nm (Analytic Jena US, Upland, CA). Product distribution was monitored with HPLC-MS. To directly test the effect of UV in the disulfide rearrangement, a control experiment was performed from the mixtures and kept under the same condition but without UV-irradiation.

Generating statistical dimer distribution and calibration of the MS-AUC-to-concentration conversion

Statistical product distribution for the dimers were generated in a chaotropic solvent mixture of 20% DMSO:80% water. Glutathione-protected monomers were dissolved (each one at 10 μ M final concentration) and the library was completely reduced with 2 molar equivalents of TCEP (tris(2-carboxyethyl)phosphine). Subsequently, the sample was continuously stirred for 48 h exposed to atmospheric oxygen.

To confirm that oxidation in the chaotropic solvent (DMSO:water) results in statistical product distribution, a subset of monomers was analysed for which self-association tendency is significantly different (Extended Data Fig. 1) (**WF-SG**, **LW-SG**, **RW-SG** and **TW-SG**). The oxidised mixture was analysed using HPLC-UV with the detection wavelength of 210 nm, where the integrated intensity is directly proportional to the peptide concentration. For this system, the oxidation products could be quantitatively analysed in HPLC-UV without problem. As expected, the reaction yielded four homodimers and six heterodimers with statistical concentration distribution (Extended Data Fig. 5).

Next, we performed the same experiment starting from the 12-membered glutathione-protected foldamer disulphide library. Completeness of the oxidation after the TCEP treatment was confirmed, and the final product distribution was quantitatively analysed with HPLC-MS. Due to the large number of components, UV detection could not separate the products. Based on the model experiment, the statistical concentration of each dimer was predicted and then calculated. After the quantitative evaluation of the HPLC-MS chromatograms, the MS-AUC/concentration ratios were calculated for each component (Supplementary Table 4).

Generating equilibrium dimer distribution through thiolate-mediated exchange

The twelve different thiol-functionalized foldameric building blocks (**IF-SH**, **KW-SH**, **LW-SH**, **QW-SH**, **RW-SH**, **RF-SH**, **SW-SH**, **TW-SH**, **VW-SH**, **WF-SH**, **WW-SH** and **YF-SH**) were dissolved at a concentration of 10 μ M in a redox buffer (pH 8.0, 20 mM HEPES, 150 mM NaCl, 1 mM CaCl₂, 3 mM NaN₃, 500 μ M GSH and 125 μ M GSSG). Reaction mixture was shaken at 250 rpm, 37°C for three days in a properly closed vial. Samples (100 μ L) were taken from the mixture every 24 h, quenched with 10% TFA in water (50 μ L) and analysed with HPLC-MS⁴. Product distribution was obtained via quantitative evaluation of the HPLC-MS chromatograms and conversion of AUC-to-concentration was carried out as described above.

Calculation of the light intensity-dependent amplification factor

Light intensity-dependent amplification factors (AF) were calculated for each dimer to determine the sensitivity to the energy influx. The following formula (eq. (S12)) was used:

$$AF_{i(C),int} = \frac{AUC_{i(C),100\%}}{AUC_{i(C),50\%}} \quad (S12)$$

where $AUC_{i(C),100\%}$ is the area under the curve (AUC) of compound i measured at 100% light intensity (5.10 mW cm⁻²) and $AUC_{i(C),50\%}$ is the AUC of the same compound measured at 50% light intensity (2.55 mW cm⁻²). Errors of AF_i (σ_{AF}) were calculated from three parallel measurements with the following general formula (neglecting the correlation between the variables):

$$\sigma_{AFi} = AF_i \sqrt{\left(\frac{\sigma_{AUC1}}{AUC_1}\right)^2 + \left(\frac{\sigma_{AUC2}}{AUC_2}\right)^2} \quad (S13)$$

where $AUC_1 \pm \sigma_{AUC1}$ and $AUC_2 \pm \sigma_{AUC2}$ are the measured variables with uncertainties when AF_i is calculated by AUC_1/AUC_2 .

Calculation of the concentration amplifications for the MSSM dimers upon seeding

Concentration amplifications (CA) were calculated for each dimer to evaluate the catalytic effect of seeding dimer (**WF-S-YF**). The following formula (S14) was used for the calculation:

$$CA_{i,seeding} = \frac{AUC_{i,seeded}}{AUC_{i,control}} \quad (S14)$$

Where $AUC_{i,seeded}$ is the area under the curve (AUC) of compound i measured in the presence of the seeding compound and $AUC_{i,control}$ is the AUC of the same compound measured without the seeding compound.

Calculation of the side chain enrichment

Enrichment (E) of the proteinogenic side chains in the peripheral (1, 1') and the central (2, 2') positions of the ten most amplified dimers was expressed as the frequency of a side chain relative to all side chains in the foldamer system by using the following formula (S15):

$$E_{schi} = \frac{N_{schi,Best}/10}{N_{schi,All}/78} \quad (S15)$$

Where E_{schi} is the enrichment of the side chain i in appropriate position, $N_{schi,Best}$ is the count of the side chain i among the top ten amplified dimers, $N_{schi,All}$ is the count of the side chain i in the complete system.

Supplementary Tables

Supplementary Table 1. HPLC-MS characterisation of dimers (MSSM) and monomers (MSSG) in the system.

Compounds	Calculated molar mass (Da)	Retention time (min)*	Detected ions	
			$[M+2H^+]^{2+}$	$[M+3H^+]^{3+}$
IF-IF	2044.66	17.73	1022.95	682.29
IF-QW	2098.67	15.70	1049.79	700.47
IF-SG	1327.62	14.49	664.84	443.54
IF-SW	2057.62	15.79	1029.89	686.75
IF-TW	2071.65	16.05	1036.42	691.59
IF-VW	2069.67	17.29	1035.41	690.82
KW-IF	2098.71	14.55	1049.79	700.00
KW-KW	2152.76	11.70	1077.40	718.48
KW-LW	2137.75	14.70	1069.44	713.56
KW-QW	2152.72	12.73	1076.93	718.90
KW-RF	2141.74	11.63	1071.41	714.82
KW-SG	1381.69	10.42	691.85	461.56
KW-SW	2111.63	12.73	1056.87	705.04
KW-TW	2125.70	13.09	1063.35	709.34
KW-VW	2123.68	14.26	1063.34	708.73
LW-IF	2084.61	17.84	1042.52	695.41
LW-LW	2123.72	18.00	1062.03	708.28
LW-QW	2137.71	15.84	1069.41	713.77
LW-SG	1366.68	14.76	684.35	456.56
LW-SW	2096.66	15.94	1048.90	700.19
LW-TW	2111.67	16.19	1056.01	704.69
LW-VW	2108.71	17.43	1054.60	703.40
QW-QW	2152.68	13.71	1077.12	718.79
QW-SG	1381.65	11.60	691.83	461.55
RF-IF	2087.69	14.59	1044.38	697.05
RF-LW	2126.73	14.77	1063.94	709.58
RF-QW	2141.70	12.73	1071.37	715.15
RF-RF	2130.72	11.64	1066.67	710.97
RF-SG	1370.68	10.31	686.35	457.89
RF-SW	2100.65	12.80	1050.89	701.43
RF-TW	2114.68	12.77	1057.93	706.26
RF-VW	2112.70	14.28	1056.99	705.03
RW-IF	2126.73	14.77	1064.57	709.87
RW-KW	2180.78	11.80	1091.33	728.04
RW-LW	2165.77	14.91	1083.50	722.76
RW-QW	2180.74	12.90	1090.87	728.20
RW-RF	2169.76	12.22	1085.36	724.15

RW-RW	2208.80	11.97	1104.90	737.47
RW-SG	1409.69	10.56	705.85	470.90
RW-SW	2139.69	12.96	1070.38	714.39
RW-TW	2153.72	13.29	1077.46	718.90
RW-VW	2151.74	14.41	1076.35	718.45
SW-QW	2110.69	13.76	1056.41	704.63
SW-SG	1340.63	11.60	671.32	447.88
SW-SW	2070.58	12.99	1035.86	690.84
TW-QW	2125.66	14.05	1063.25	709.33
TW-SG	1354.64	12.06	678.33	452.55
TW-SW	2083.70	14.13	1042.93	695.92
TW-TW	2098.64	14.35	1049.99	700.56
VW-QW	2122.74	15.35	1063.00	708.79
VW-SG	1352.66	14.05	677.34	451.89
VW-SW	2082.63	15.44	1042.40	695.14
VW-TW	2096.66	15.70	1049.37	699.79
VW-VW	2094.68	16.97	1048.03	698.88
WF-IF	2117.72	17.27	1059.49	706.71
WF-KW	2171.77	14.31	1086.48	724.64
WF-LW	2156.76	17.45	1078.90	719.64
WF-QW	2171.73	15.40	1086.93	724.91
WF-RF	2160.75	14.37	1081.34	721.03
WF-RW	2199.79	14.48	1100.43	734.51
WF-SG	1400.66	14.29	701.34	467.89
WF-SW	2130.68	15.49	1065.95	711.30
WF-TW	2144.71	15.76	1073.20	715.93
WF-VW	2142.73	16.96	1071.97	715.50
WF-WF	2190.78	16.96	1095.93	731.76
WF-WW	2229.81	16.41	1115.44	744.44
WF-YF	2167.74	16.10	1084.52	723.76
WW-IF	2156.75	16.78	1078.99	719.84
WW-KW	2210.80	13.73	1105.93	738.15
WW-LW	2195.79	16.89	1098.46	732.71
WW-QW	2210.76	14.71	1105.98	737.87
WW-RF	2199.78	14.48	1100.43	734.51
WW-RW	2238.82	13.94	1120.36	747.47
WW-SG	1439.67	12.86	720.84	480.89
WW-SW	2169.71	16.08	1086.53	725.38
WW-TW	2183.74	15.09	1092.55	728.13
WW-VW	2181.76	16.39	1091.41	728.06
WW-WW	2268.84	15.81	1135.45	757.47
WW-YF	2206.77	15.53	1103.89	736.72
YF-IF	2094.68	16.41	1047.94	699.11
YF-KW	2148.73	13.54	1075.00	717.42
YF-LW	2133.72	16.57	1067.86	712.64

YF-QW	2148.69	14.50	1074.93	717.02
YF-RF	2137.71	13.54	1069.47	713.51
YF-RW	2176.75	13.71	1088.93	726.82
YF-SG	1377.66	12.98	689.83	460.22
YF-SW	2107.64	14.58	1054.34	703.55
YF-TW	2121.67	14.84	1061.45	707.98
YF-VW	2119.69	16.06	1060.47	707.47
YF-YF	2144.70	15.22	1073.30	715.89

*Analytical HPLC-MS measurement. Column: Aeris Widepore XB-C18 (250 × 4.6 mm). Method: 5–80% B during 25 min, flow rate: 0.7 mL min⁻¹, where eluent A: 0.1% HCOOH in water, eluent B: 0.1% HCOOH in acetonitrile.

Supplementary Table 2. Fitted rate constants for representative dimers.

Compounds	S_{p1} M ⁻¹ (cm ²) W ⁻¹ s ⁻¹	S_{p2} x 10 ² M ⁻¹ (cm ²) ² W ⁻² s ⁻¹	S_{a1} x 10 ⁷ M ⁻² (cm ²) W ⁻¹ s ⁻¹	S_{a2} x 10 ¹⁰ M ⁻² (cm ²) ² W ⁻² s ⁻¹	b x 10 ⁻² (cm ²) ^{0.5} W ^{-0.5} s ⁻¹	RMSD x 10 ⁻⁹ M s ⁻¹
KW-TW	0.00	38.03	0.01	205.16	2.80	19.97
QW-SW	0.00	25.94	0.00	107.88	1.57	15.49
RF-QW	0.00	25.80	0.00	77.01	1.12	24.99
RW-IF	0.00	1.99	0.00	117.49	1.68	3.60
RW-LW	1.27	0.00	0.00	70.29	1.05	4.50
RW-QW	0.00	27.53	18.24	66.25	1.47	18.47
WF-IF	5.43	0.00	247.59	0.00	6.95	10.53
WF-LW	0.00	38.80	100.06	73.31	3.85	22.19
WF-RW	2.73	14.65	0.00	143.86	2.21	17.27
WF-VW	4.39	0.24	304.75	0.00	8.61	6.70
WF-YF	0.00	13.41	270.42	0.00	7.45	11.26
WW-IF	0.00	24.06	0.00	280.08	3.84	20.34
WW-RF	4.38	0.00	155.16	0.57	4.42	15.14
YF-IF	0.00	8.91	0.02	112.28	1.58	11.85
YF-LW	4.01	0.04	270.87	0.00	7.53	7.15
YF-RF	0.00	5.91	0.00	83.24	1.19	6.35
YF-RW	2.83	0.35	177.91	0.08	5.01	6.71
YF-VW	0.00	6.77	0.00	178.50	2.64	5.06

Mean standard deviation of the fitted parameters was estimated with jackknife resampling technique and it was found to be less than 3.4% in each case.

S_{p1} : rate constant of spontaneous synthesis by proximity-controlled radical substitution

S_{p2} : rate constant of spontaneous synthesis by proximity-controlled concerted metathesis

S_{a1} : rate constant of autocatalytic synthesis by proximity-controlled radical substitution

S_{a2} : rate constant of autocatalytic synthesis by proximity-controlled concerted metathesis

b : rate constant of dimers break down via diffusion-controlled radical substitution

Supplementary Table 3. Characterisation of the glutathione-protected monomers.

Compounds	Exact mass (Da)	Detected ions		Retention time (min)*	Retention time (min)**
		$[M+1H^+]^{1+}$	$[M+2H^+]^{2+}$		
IF-SG	1327.62	1328.67	664.84	20.81	14.49
KW-SG	1381.69	1382.69	691.85	16.58	10.42
LW-SG	1366.68	1367.68	684.35	21.79	14.76
QW-SG	1381.65	1382.65	691.83	17.48	11.60
RF-SG	1370.68	1371.69	686.35	16.62	10.31
RW-SG	1409.69	1410.7	705.85	18.03	10.56
SW-SG	1340.63	1341.63	671.32	17.23	11.60
TW-SG	1354.64	1355.65	678.33	19.15	12.06
VW-SG	1352.66	1353.67	677.34	20.11	14.05
WF-SG	1400.66	1401.67	701.34	21.37	14.29
WW-SG	1439.67	1440.68	720.84	17.23	12.86
YF-SG	1377.66	1378.65	689.83	18.94	12.98

*Analytical HPLC-UV measurement. Column: Phenomenex Luna C18 (250 × 4.6 mm). Method: 5–80% B during 25 min, flow rate: 1.2 mL min⁻¹, where eluent A: 0.1% TFA in water, eluent B: 0.1% TFA and 80% ACN in water.

**Analytical HPLC-MS measurement. Column: Aeris Widespore XB-C18 (250 × 4.6 mm). Method: 5–80% B during 25 min, flow rate: 0.7 mL min⁻¹, where eluent A: 0.1% HCOOH in water, eluent B: 0.1% HCOOH in ACN.

Supplementary Table 4. Calibration of the MS Area Under Curve (AUC) to concentration conversion for each component of the system.

compounds	calculated concentration (μM)*	mean AUC	SD	Conversion factor: AUC/μM
IF-IF	0.2083	6738896	167227	32346754
IF-QW	0.4167	14321621	1021416	34371864
IF-SG	5	233796364	12121312	46759273
IF-SW	0.4167	10250563	267887	24601332
IF-TW	0.4167	9781464	99474	23475495
IF-VW	0.4167	14658581	333948	35180567
KW-IF	0.4167	6984610	192959	16763051
KW-KW	0.2083	4688541	196654	22505032
KW-LW	0.4167	10931447	352192	26235452
KW-QW	0.4167	9592254	191099	23021391
KW-RF	0.4167	6041711	280144	14500094
KW-SG	5	93498264	8075947	18699653
KW-SW	0.4167	10307687	34078	24738429
KW-TW	0.4167	11140758	215090	26737798

KW-VW	0.4167	11219383	61459	26926498
LW-IF	0.4167	13317251	59335	31961377
LW-LW	0.2083	5459491	232775	26205601
LW-QW	0.4167	7510246	171240	18024575
LW-SG	5	71564374	1943793	14312875
LW-SW	0.4167	7846422	820359	18831398
LW-TW	0.4167	7877221	1158047	18905315
LW-VW	0.4167	11755384	350708	28212900
QW-QW	0.2083	2505601	113767	12026904
QW-SG	5	154788096	3212397	30957619
RF-IF	0.4167	9685531	98651	23245255
RF-LW	0.4167	9423472	313860	22616315
RF-QW	0.4167	5395324	470128	12948767
RF-RF	0.2083	2600196	66430	12480959
RF-SG	5	76355793	557309	15271159
RF-SW	0.4167	8404764	40877	20171417
RF-TW	0.4167	6458569	88664	15500554
RF-VW	0.4167	9015525	232567	21637242
RW-IF	0.4167	9139403	235650	21934550
RW-KW	0.4167	7268733	135533	17444946
RW-LW	0.4167	9690533	135870	23257261
RW-QW	0.4167	8128642	168034	19508725
RW-RF	0.4167	4839118	14804	11613873
RW-RW	0.2083	2840665	146521	13635212
RW-SG	5	81307536	3537250	16261507
RW-SW	0.4167	9262117	211359	22229063
RW-TW	0.4167	5594796	220034	13427499
RW-VW	0.4167	10268484	436026	24644341
SW-QW	0.4167	7741284	52795	18579067
SW-SG	5	214373675	971923	42874735
SW-SW	0.2083	4460048	9425	21408267
TW-QW	0.4167	6258758	7940	15021007
TW-SG	5	205650252	4735772	41130050
TW-SW	0.4167	7866053	65835	18878512
TW-TW	0.2083	6984610	192959	33526183
VW-QW	0.4167	8395542	149336	20149285
VW-SG	5	218439667	209807	43687933
VW-SW	0.4167	9549648	30622	22919137
VW-TW	0.4167	14321621	1021416	34371864
VW-VW	0.2083	6719439	16227	32253357
WF-IF	0.4167	16802806	261057	40326702
WF-KW	0.4167	12948964	294491	31077488
WF-LW	0.4167	14057330	138666	33737565

WF-QW	0.4167	9308290	140062	22339878
WF-RF	0.4167	9750493	108676	23401163
WF-RW	0.4167	5231109	21871	12554652
WF-SG	5	229629674	1234010	45925935
WF-SW	0.4167	10201405	81613	24483352
WF-TW	0.4167	10261229	335150	24626930
WF-VW	0.4167	12943413	2777026	31064167
WF-WF	0.2083	9182636	141237	44076722
WF-WW	0.4167	7037382	357237	16889704
WF-YF	0.4167	11977596	184803	28746208
WW-IF	0.4167	6697741	203025	16074566
WW-KW	0.4167	7218262	137235	17323816
WW-LW	0.4167	6808518	206582	16340430
WW-QW	0.4167	4956501	97189	11895592
WW-RF	0.4167	5597430	97043	13433822
WW-RW	0.4167	5744039	55944	13785683
WW-SG	5	154424106	809265	30884821
WW-SW	0.4167	7736738	259061	18568158
WW-TW	0.4167	6666086	237986	15998593
WW-VW	0.4167	6666086	237986	15998593
WW-WW	0.2083	2408501	253201	11560822
WW-YF	0.4167	4863880	87381	11673302
YF-IF	0.4167	8352642	1717484	20046325
YF-KW	0.4167	8037313	69720	19289535
YF-LW	0.4167	9072627	56896	21774286
YF-QW	0.4167	6032798	175591	14478703
YF-RF	0.4167	6941704	43753	16660077
YF-RW	0.4167	7672105	108675	18413037
YF-SG	5	177069749	1736459	35413950
YF-SW	0.4167	6309200	101256	15142069
YF-TW	0.4167	6577118	44472	15785071
YF-VW	0.4167	10338711	140452	24812887
YF-YF	0.2083	2466209	13733	11837825

*Calculated from the statistical product distribution

Supplementary Table 5. Concentration amplifications (CA) for the MSSM dimers upon seeding with WF-S-YF at 40 min and in the steady state.

compound #	compound	CA _{i, 40 min}	σ (CA _{i, 40 min})	compound #	compound	CA _{i, steady state}	σ (CA _{i, steady state})
1	WF-IF	3.97	0.26	1	WF-IF	3.54	0.30
2	WF-VW	3.96	0.09	2	IF-IF	3.45	0.37
3	WF-YF	3.73	0.12	3	WF-VW	3.32	0.15
4	LW-VW	3.65	0.06	4	LW-LW	3.17	0.27

5	YF-IF	3.48	0.20
6	WW-YF	3.42	0.06
7	IF-IF	3.30	0.01
8	WF-WF	2.98	0.10
9	IF-VW	2.96	0.08
10	YF-LW	2.91	0.08
11	YF-VW	2.89	0.05
12	LW-LW	2.88	0.22
13	YF-YF	2.82	0.03
14	WF-WW	2.81	0.16
15	LW-IF	2.78	0.29
16	WF-RF	2.74	0.06
17	WW-LW	2.67	0.13
18	WW-VW	2.61	0.09
19	WW-WW	2.60	0.12
20	LW-SW	2.58	0.13
21	WW-IF	2.53	0.04
22	WF-LW	2.41	0.13
23	WW-QW	2.37	0.02
24	WW-TW	2.35	0.01
25	IF-TW	2.32	0.01
26	VW-VW	2.32	0.02
27	YF-SW	2.30	0.08
28	WF-QW	2.27	0.12
29	LW-TW	2.26	0.14
30	VW-SW	2.26	0.31
31	WW-SW	2.25	0.00
32	LW-QW	2.20	0.20
33	WF-TW	2.18	0.06
34	RW-LW	2.16	0.43
35	WW-RW	2.13	0.30
36	IF-SW	2.10	0.09
37	IF-QW	2.06	0.07
38	WF-SW	2.02	0.37
39	VW-QW	1.97	0.02
40	VW-TW	1.90	0.09
41	RF-TW	1.89	0.09
42	RW-RW	1.87	0.35
43	YF-QW	1.82	0.06
44	RF-QW	1.80	0.09
45	SW-QW	1.75	0.02
46	KW-LW	1.74	0.01
47	RW-SW	1.72	0.04

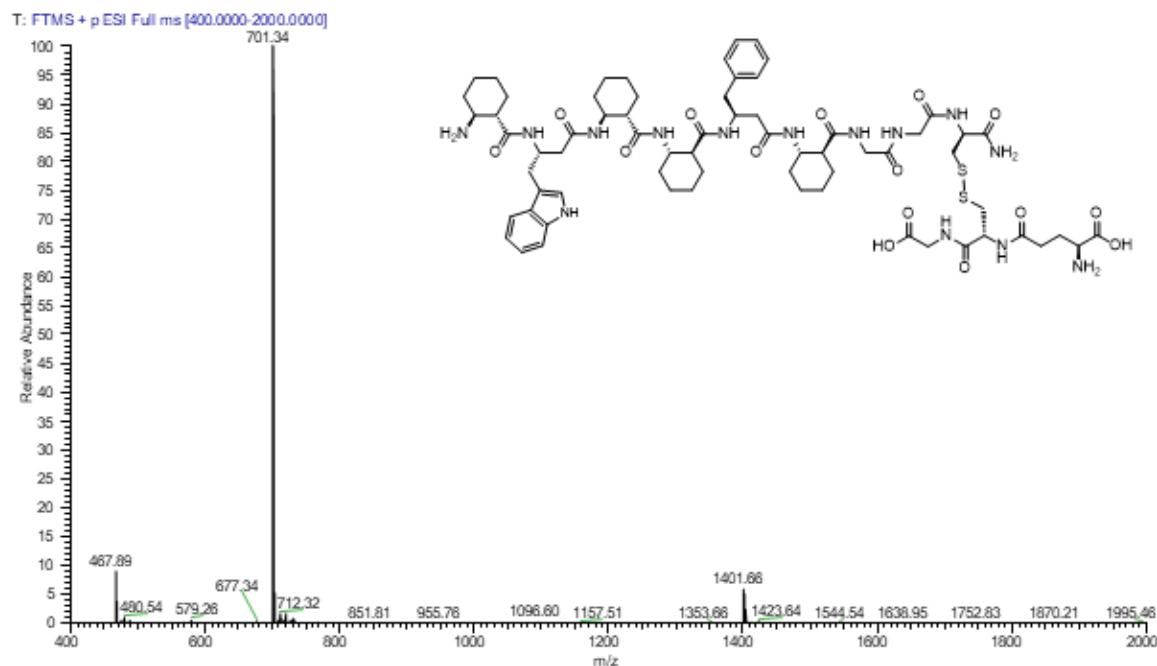
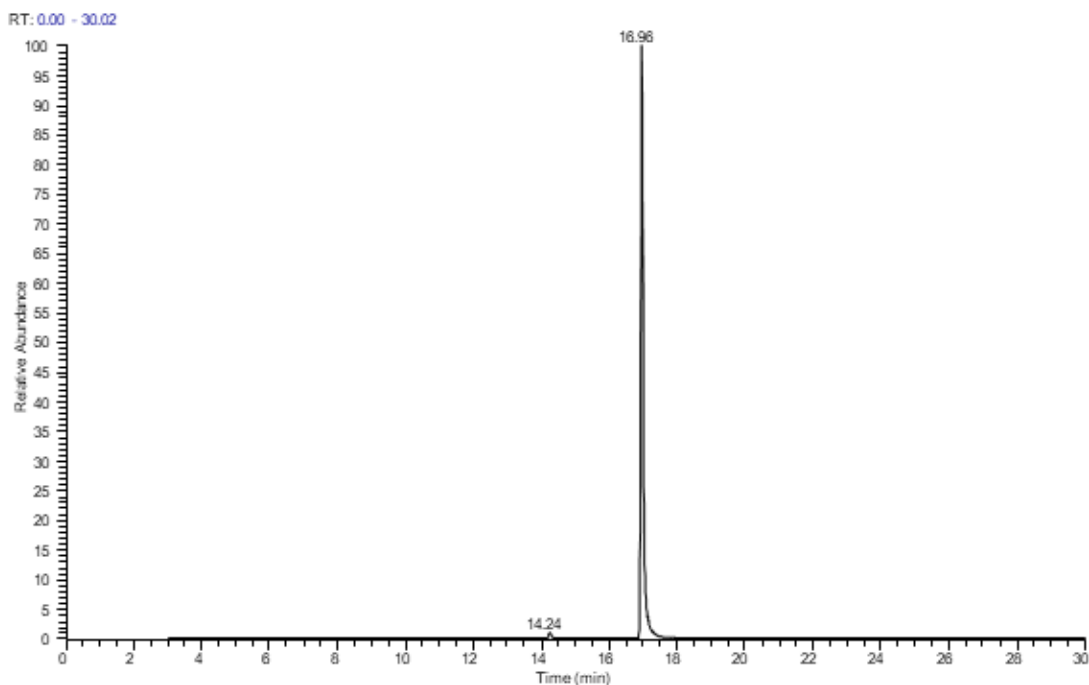
5	LW-VW	3.17	0.17
6	YF-IF	3.08	0.05
7	WF-YF	2.93	0.01
8	WW-YF	2.85	0.00
9	IF-VW	2.84	0.22
10	WF-WW	2.83	0.07
11	YF-LW	2.80	0.01
12	LW-IF	2.77	0.43
13	YF-YF	2.74	0.10
14	YF-VW	2.70	0.17
15	WW-LW	2.68	0.04
16	WW-VW	2.67	0.01
17	WF-WF	2.61	0.01
18	WW-WW	2.57	0.04
19	WW-IF	2.48	0.05
20	LW-TW	2.45	0.42
21	VW-VW	2.40	0.19
22	WF-TW	2.40	0.16
23	WW-TW	2.30	0.07
24	WW-QW	2.21	0.14
25	WW-SW	2.08	0.27
26	WF-LW	2.07	0.21
27	IF-TW	2.06	0.01
28	LW-QW	2.05	0.15
29	WF-QW	2.04	0.00
30	LW-SW	1.98	0.21
31	WF-SW	1.91	0.23
32	WF-RF	1.90	0.26
33	VW-TW	1.89	0.09
34	IF-SW	1.88	0.01
35	IF-QW	1.88	0.09
36	VW-SW	1.86	0.17
37	VW-QW	1.81	0.02
38	YF-QW	1.80	0.06
39	YF-SW	1.78	0.02
40	RW-LW	1.75	0.06
41	SW-SW	1.72	0.23
42	YF-TW	1.68	0.13
43	SW-QW	1.67	0.01
44	WW-RW	1.62	0.01
45	RW-TW	1.57	0.06
46	TW-QW	1.56	0.06
47	YF-RW	1.52	0.01

48	SW-SW	1.72	0.09
49	YF-RW	1.72	0.07
50	YF-RF	1.70	0.06
51	YF-TW	1.68	0.00
52	KW-QW	1.67	0.10
53	QW-QW	1.67	0.14
54	KW-KW	1.63	0.21
55	RW-QW	1.63	0.05
56	KW-IF	1.62	0.06
57	RF-RF	1.61	0.23
58	RF-IF	1.61	0.11
59	WF-RW	1.60	0.13
60	WW-RF	1.60	0.04
61	RF-LW	1.60	0.15
62	RW-IF	1.60	0.15
63	TW-SW	1.59	0.02
64	RF-SW	1.59	0.01
65	RW-TW	1.55	0.06
66	RW-RF	1.53	0.13
67	TW-TW	1.53	0.03
68	KW-RF	1.52	0.16
69	TW-QW	1.49	0.04
70	WW-KW	1.47	0.03
71	RW-KW	1.47	0.23
72	YF-KW	1.46	0.23
73	RF-VW	1.45	0.04
74	WF-KW	1.43	0.04
75	KW-TW	1.41	0.19
76	RW-VW	1.39	0.28
77	KW-VW	1.38	0.03
78	KW-SG	0.96	0.02
79	RF-SG	0.95	0.05
80	SW-SG	0.95	0.01
81	QW-SG	0.95	0.00
82	RW-SG	0.95	0.02
83	TW-SG	0.95	0.02
84	IF-SG	0.90	0.00
85	YF-SG	0.89	0.01
86	VW-SG	0.89	0.02
87	LW-SG	0.88	0.01
88	WF-SG	0.84	0.01
89	KW-SW	0.84	0.16
90	WW-SG	0.83	0.02

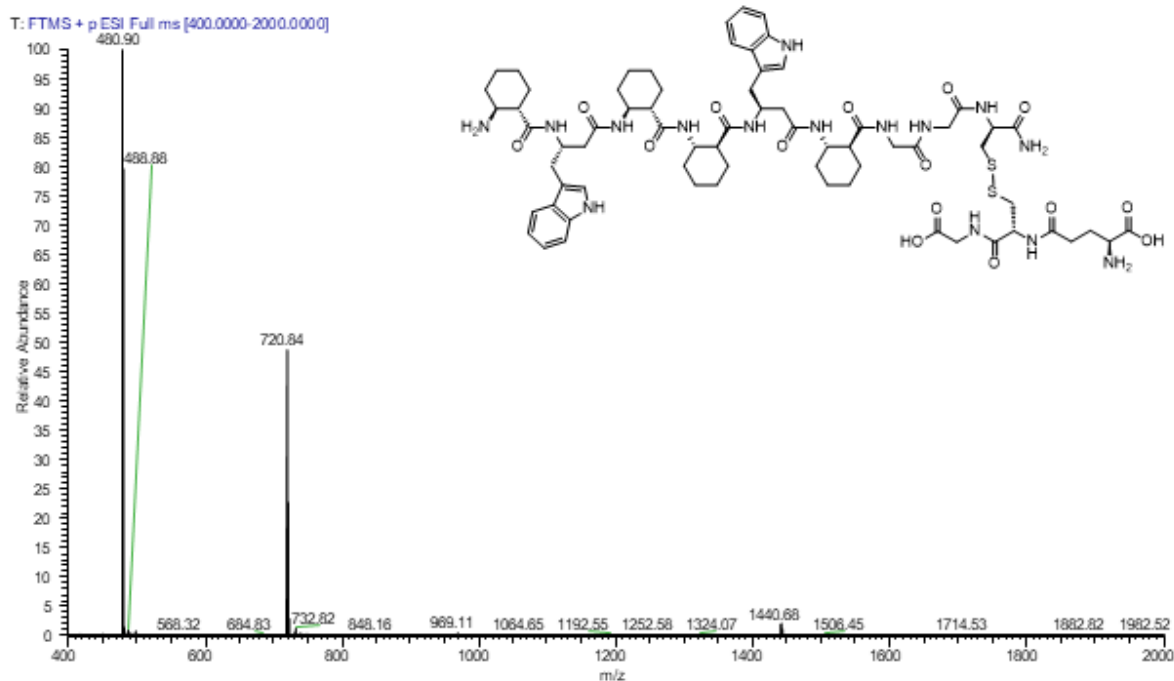
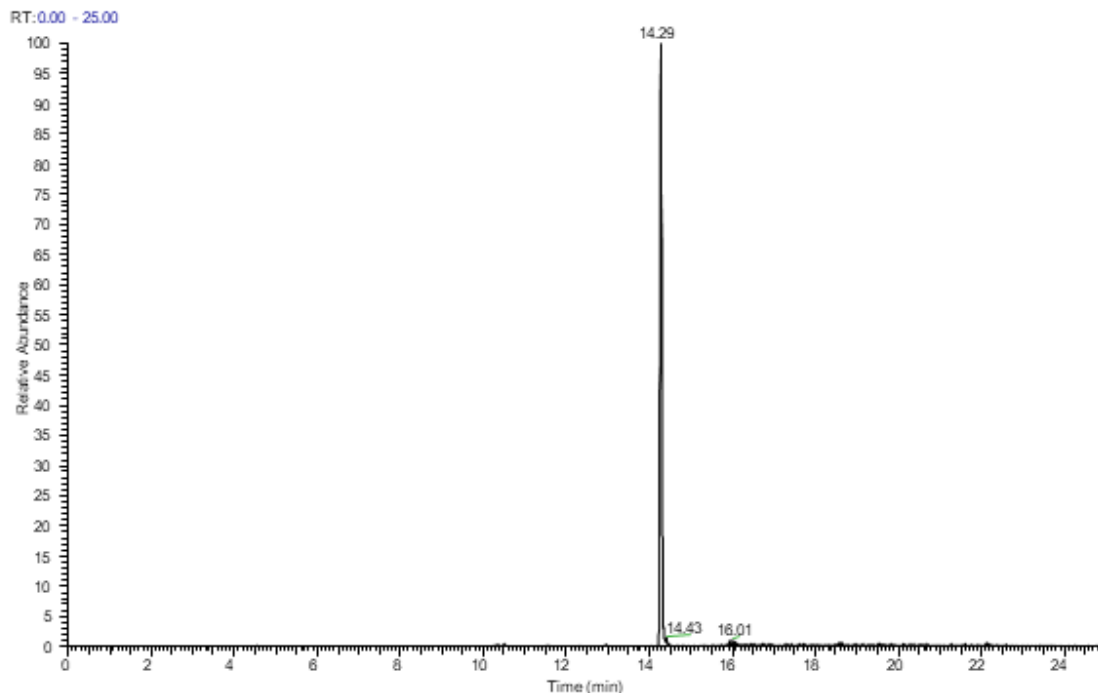
48	RW-QW	1.50	0.06
49	RF-QW	1.48	0.05
50	RF-TW	1.47	0.04
51	TW-SW	1.47	0.09
52	RW-SW	1.47	0.03
53	RW-VW	1.45	0.20
54	KW-VW	1.45	0.01
55	KW-TW	1.44	0.04
56	WF-RW	1.44	0.12
57	WW-RF	1.44	0.12
58	RF-SW	1.43	0.12
59	KW-SW	1.43	0.31
60	KW-QW	1.42	0.02
61	KW-IF	1.41	0.01
62	YF-KW	1.41	0.09
63	RW-RW	1.40	0.18
64	RW-KW	1.39	0.06
65	QW-QW	1.38	0.29
66	TW-TW	1.38	0.14
67	YF-RF	1.37	0.11
68	RF-LW	1.35	0.07
69	RW-IF	1.35	0.07
70	RW-RF	1.35	0.03
71	RF-IF	1.34	0.03
72	KW-LW	1.29	0.09
73	WW-KW	1.29	0.08
74	RF-VW	1.29	0.03
75	KW-RF	1.28	0.03
76	KW-KW	1.28	0.04
77	RF-RF	1.27	0.04
78	WF-KW	1.22	0.11
79	WW-SG	0.93	0.01
80	KW-SG	0.91	0.01
81	RF-SG	0.90	0.03
82	RW-SG	0.90	0.01
83	TW-SG	0.85	0.00
84	SW-SG	0.84	0.00
85	QW-SG	0.83	0.00
86	VW-SG	0.67	0.00
87	WF-SG	0.66	0.01
88	YF-SG	0.61	0.00
89	IF-SG	0.60	0.01
90	LW-SG	0.58	0.01

Peptide characterisation data

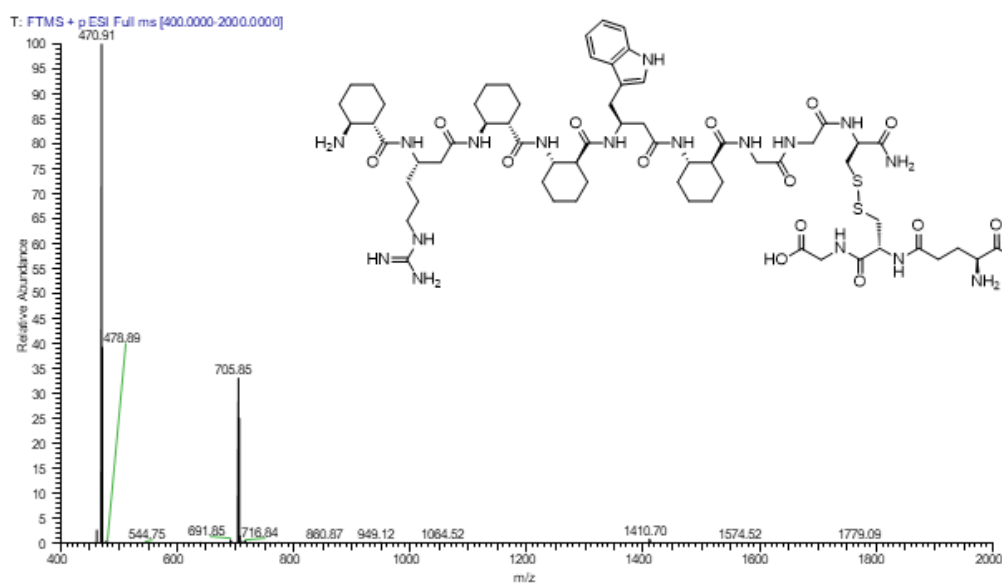
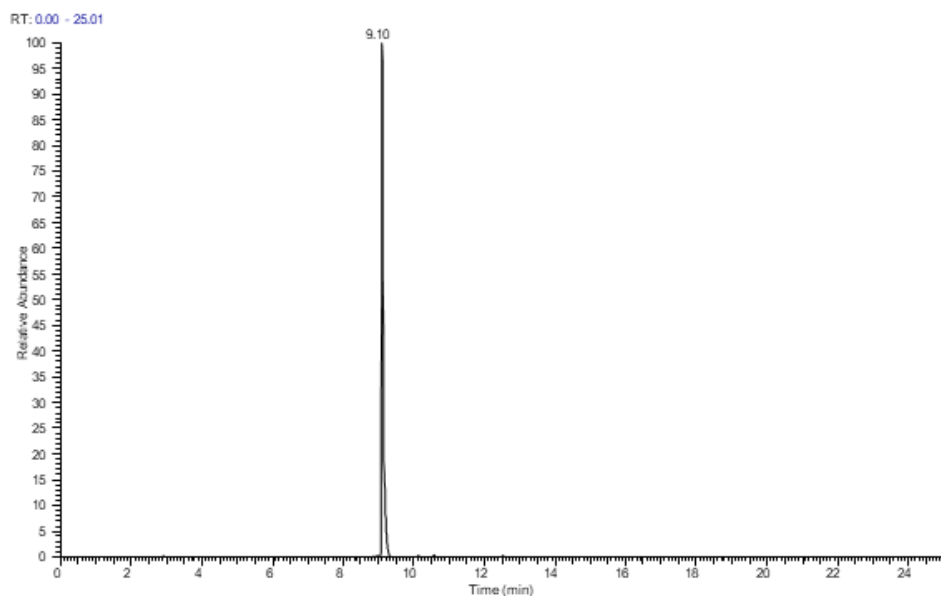
HPLC and MS characterization of the individual monomeric sequences



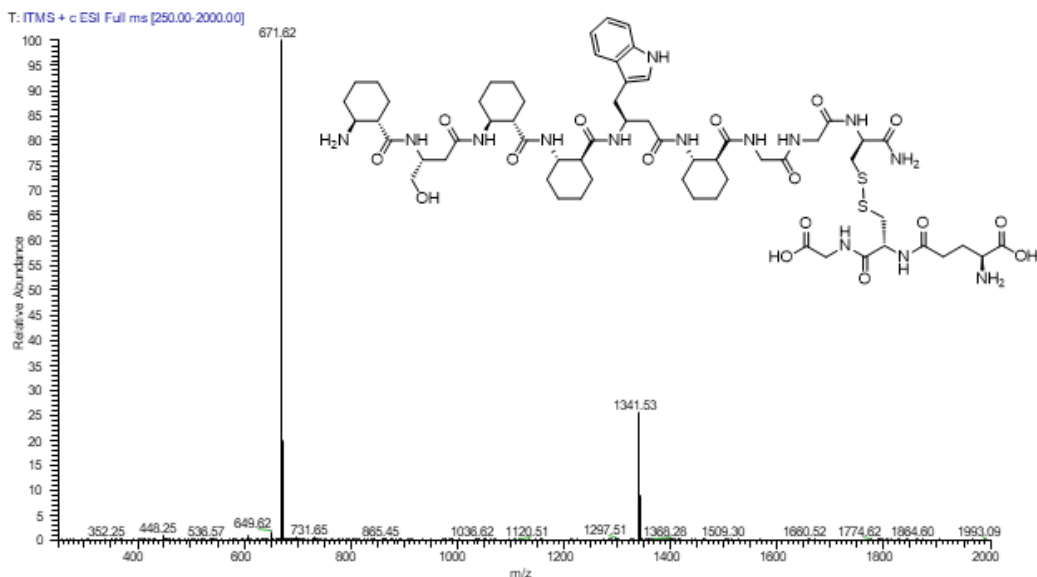
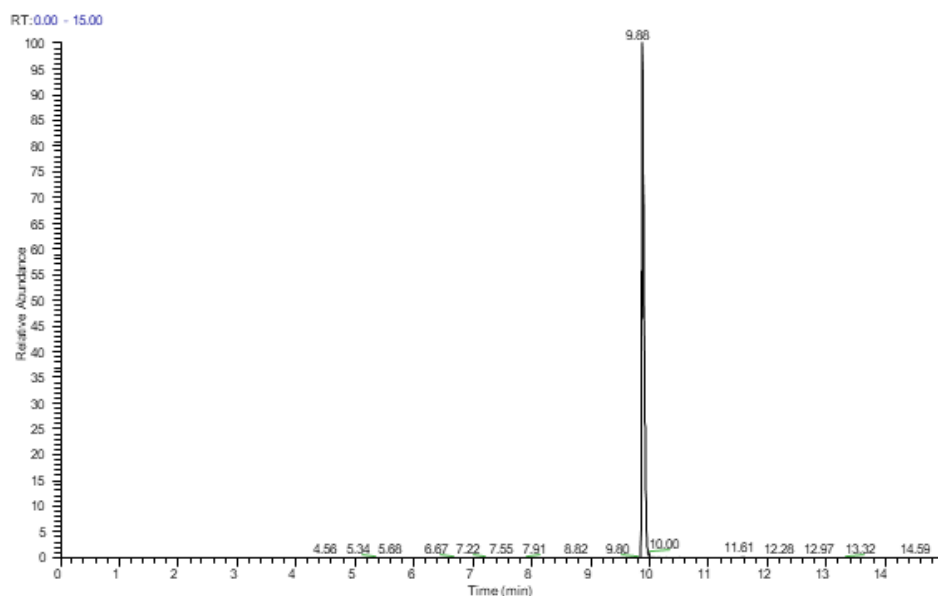
HPLC trace and mass spectrum of the glutathionyl precursor **WF-SG** (exact mass: 1400.66). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 701.3383 (100%), 701.8400 (72.47%), 702.3417 (25.86%), 702.8379 (6.48%); m/z calculated: 1401.67 $[M+H]^+$, 701.24 $[M+2H]^{2+}$; m/z observed: 1401.06 $[M+H]^+$, 701.34 $[M+2H]^{2+}$



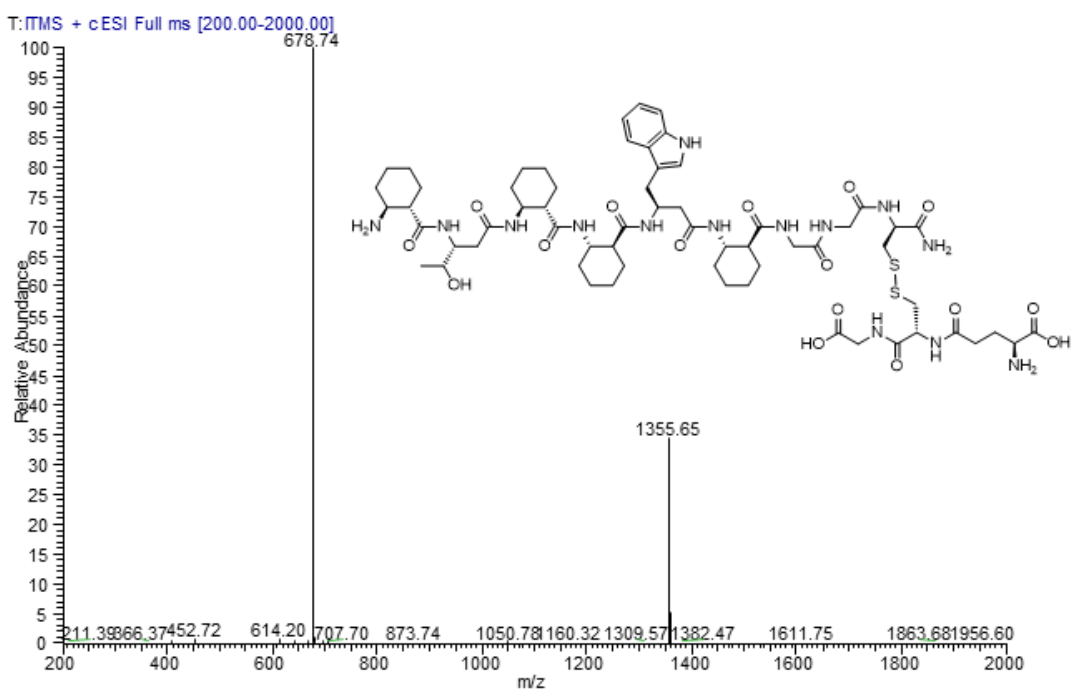
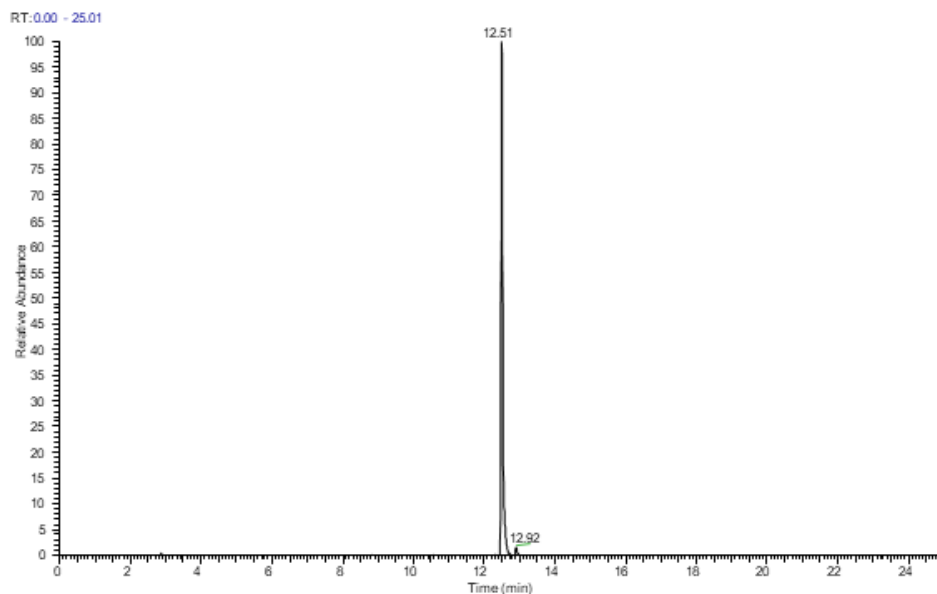
HPLC trace and mass spectrum of the glutathionyl precursor **WW-SG** (exact mass: 1439.67). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 720.8438 (100%), 721.3454 (74.63%), 721.8471 (27.44%), 722.3434 (6.78%); m/z calculated: 1440.51 $[M+H]^+$, 720.76 $[M+2H]^{2+}$, 480.84 $[M+3H]^{3+}$; m/z observed: 1440.68 $[M+H]^+$, 720.64 $[M+2H]^{2+}$, 480.90 $[M+3H]^{3+}$



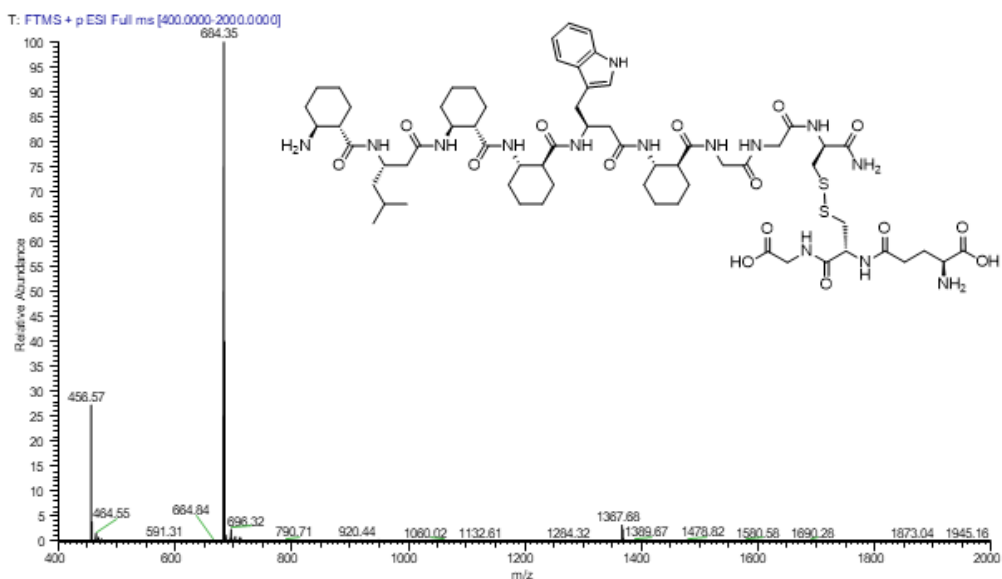
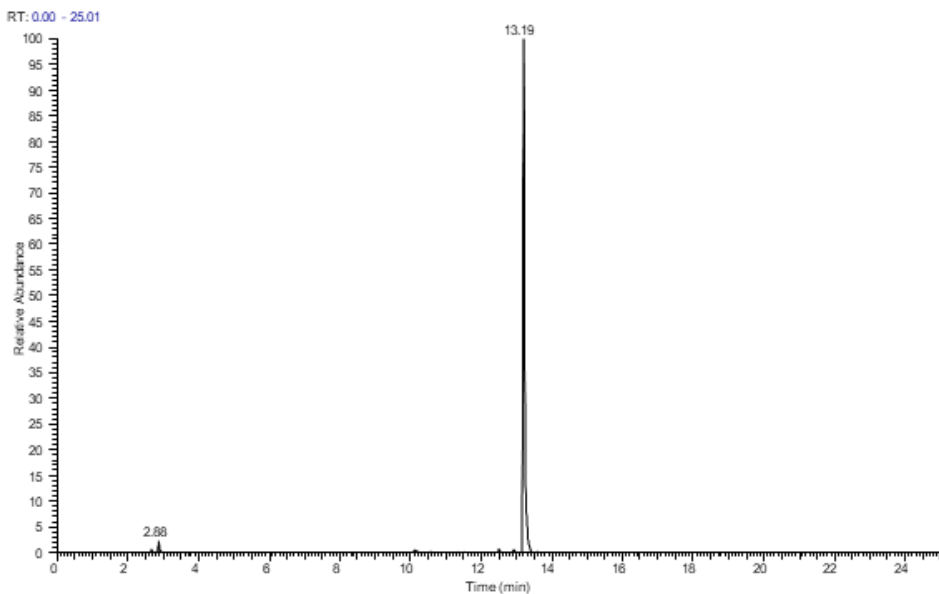
HPLC trace and mass spectrum of the glutathionyl precursor **RW-SG** (exact mass: 1409.69). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 705.3508 (100%), 705.8524 (69.22%), 706.3487 (8.95%), 706.3510 (4.30%); m/z calculated: 1410.49 $[M+H]^+$, 705.75 $[M+2H]^{2+}$, 470.83 $[M+3H]^{3+}$; m/z observed: 1410.70 $[M+H]^+$, 705.85 $[M+2H]^{2+}$, 470.91 $[M+3H]^{3+}$



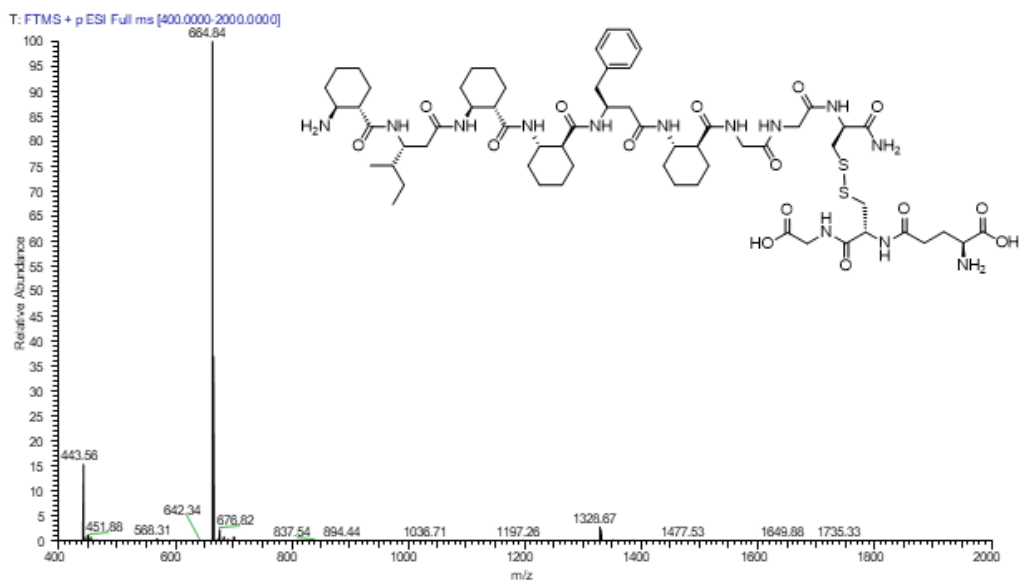
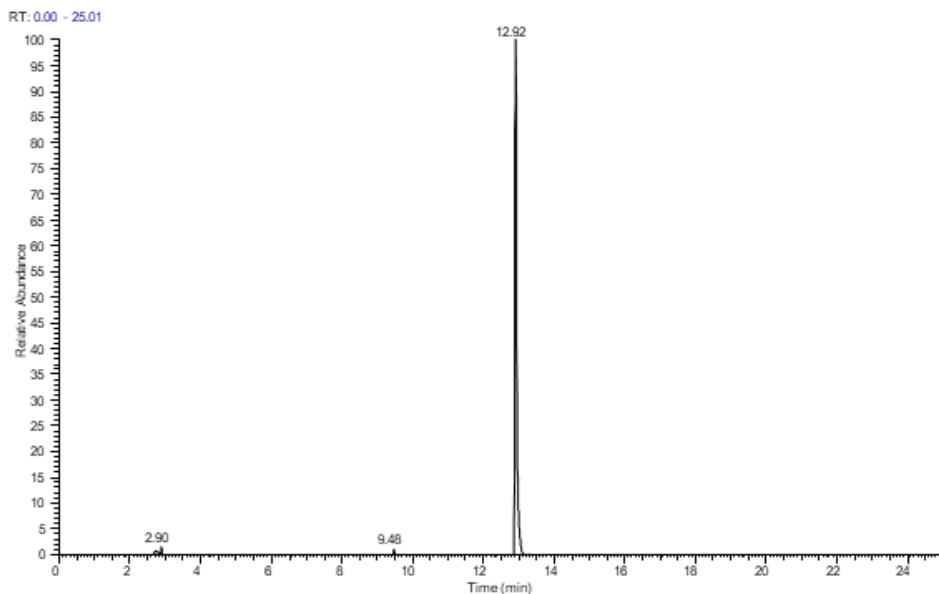
HPLC trace and mass spectrum of the glutathionyl precursor **SW-SG** (exact mass: 1340.63). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 670.8162 (100%), 671.3179 (65.98%), 671.8141 (8.95%), 671.8164 (3.37%); m/z calculated: 1341.38 $[M+H]^+$, 671.19 $[M+2H]^{2+}$; m/z observed: 1341.53 $[M+H]^+$, 671.62 $[M+2H]^{2+}$



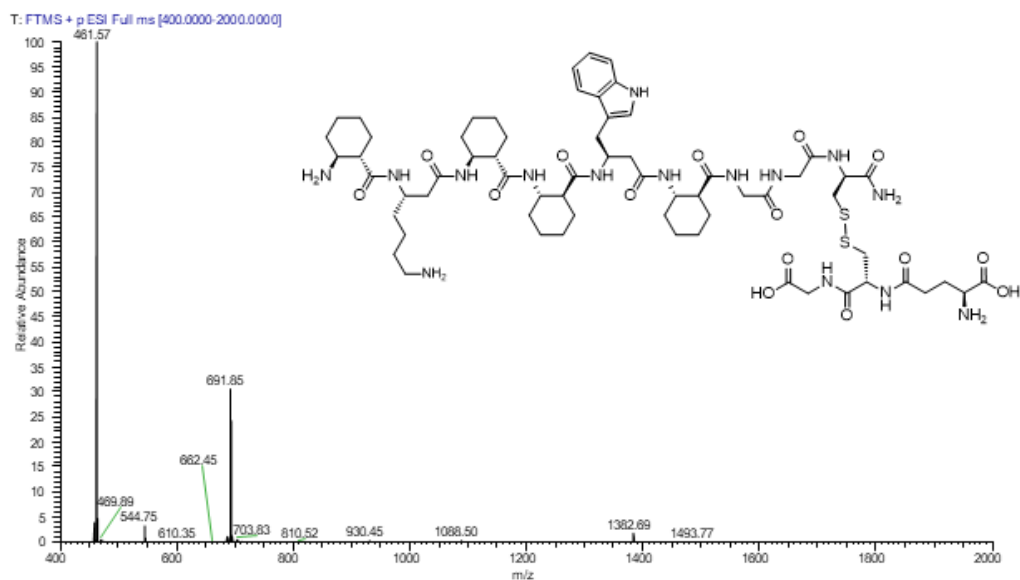
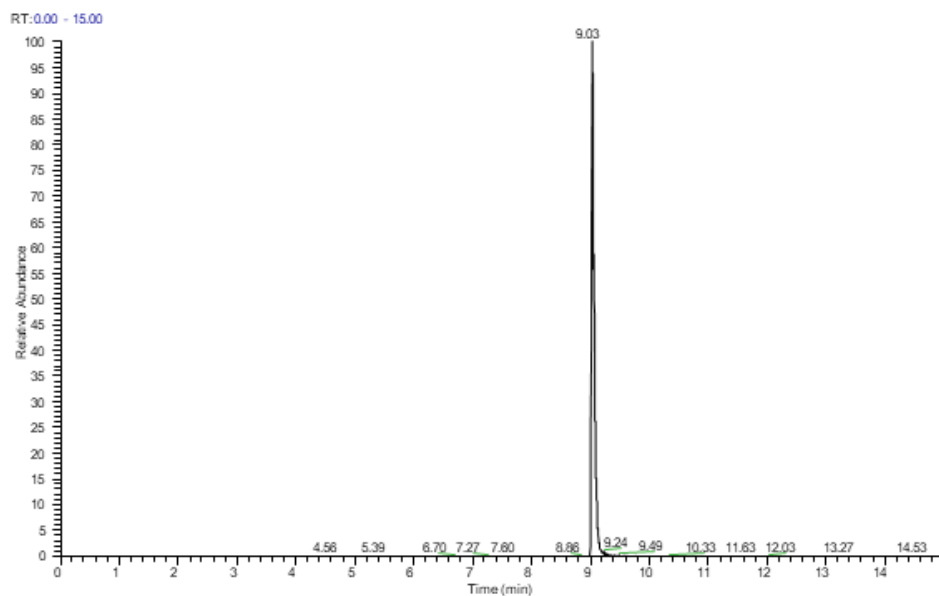
HPLC trace and mass spectrum of the glutathionyl precursor **TW-SG** (exact mass: 1354.64).
 Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 677.8240 (100%), 678.3257 (67.06%), 678.8211 (8.95%), 678.8242 (3.43%); m/z calculated: 1355.41 $[M+H]^+$, 678.21 $[M+2H]^{2+}$; m/z observed: 1355.65 $[M+H]^+$, 678.74 $[M+2H]^{2+}$



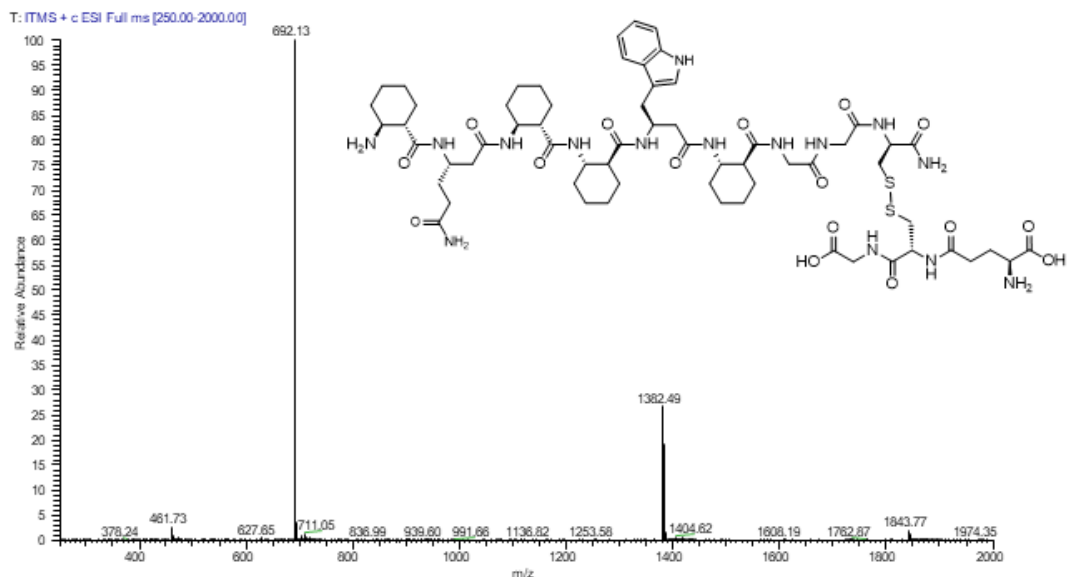
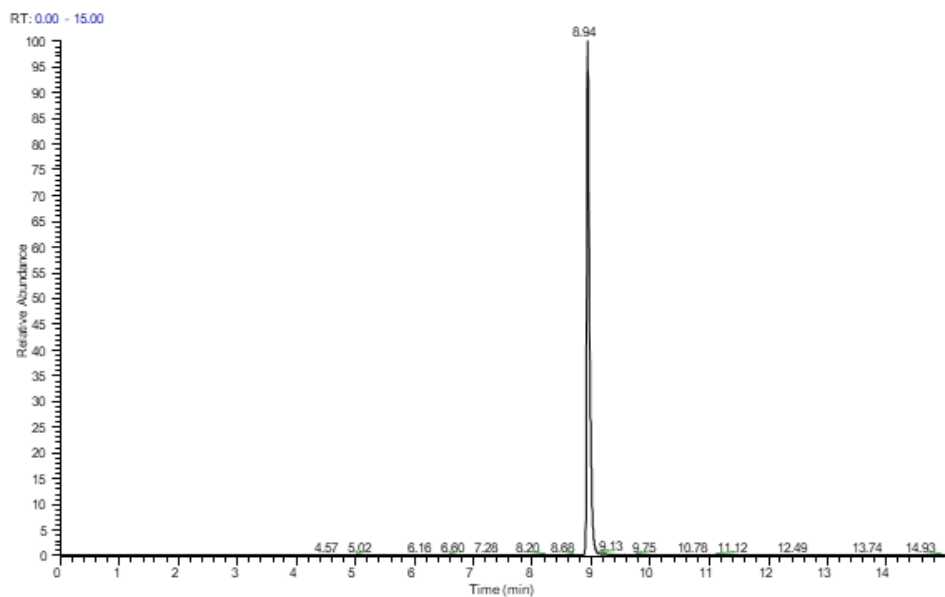
HPLC trace and mass spectrum of the glutathionic precursor **LW-SG** (exact mass: 1366.68). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 683.8422 (100%), 684.3439 (69.22%), 684.8401 (8.95%), 684.8424 (3.54%); m/z calculated: 1367.46 $[M+H]^+$, 684.23 $[M+2H]^{2+}$; m/z observed: 1367.68 $[M+H]^+$, 684.35 $[M+2H]^{2+}$



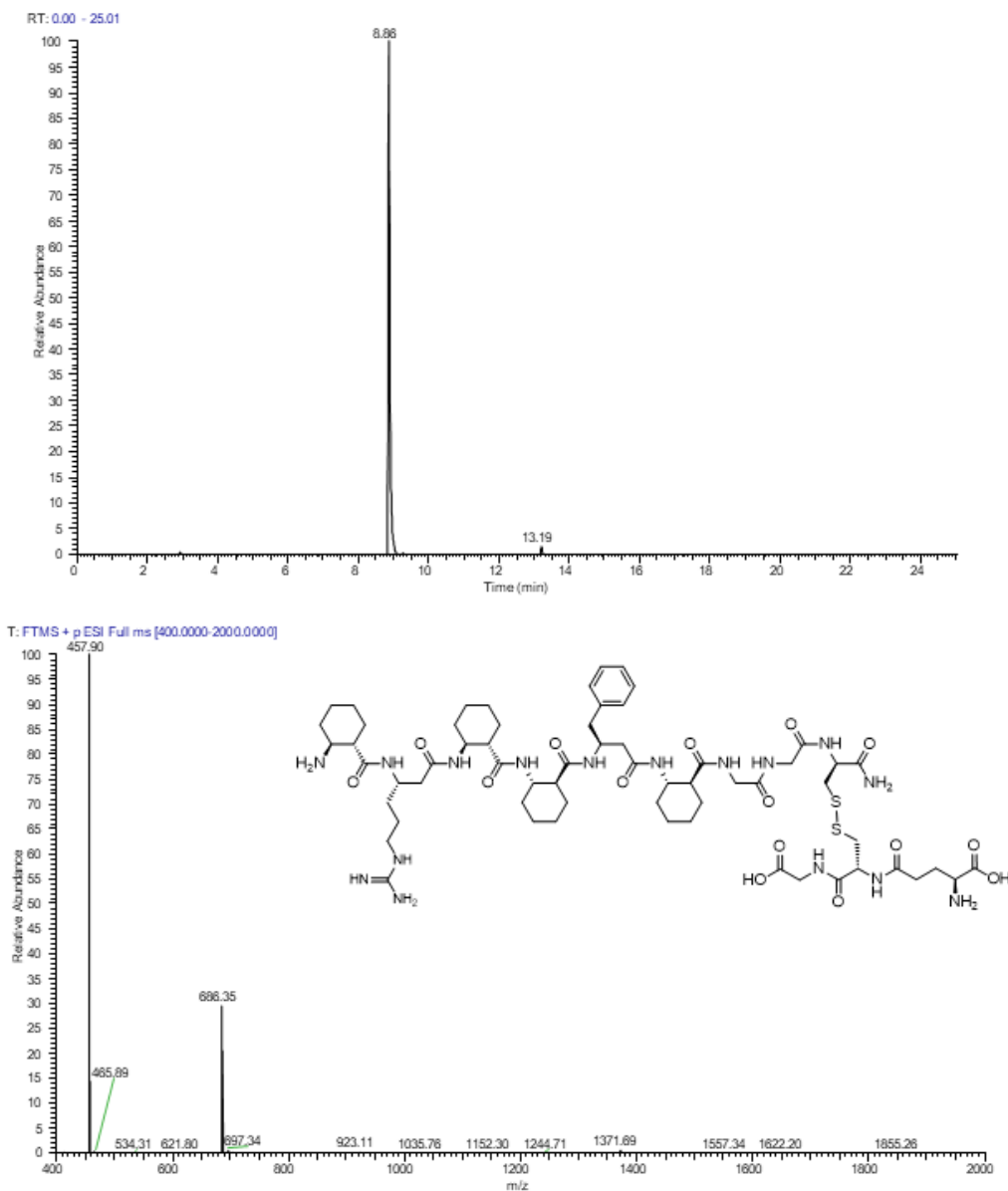
HPLC trace and mass spectrum of the glutathionyl precursor **IF-SG** (exact mass: 1327.67).
 Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 664.3468 (100%), 664.8385 (67.06%), 665.3347 (8.95%), 665.3370 (3.18%); m/z calculated: 1328.42 $[M+H]^+$, 664.71 $[M+2H]^{2+}$; m/z observed: 1328.67 $[M+H]^+$, 664.84 $[M+2H]^{2+}$



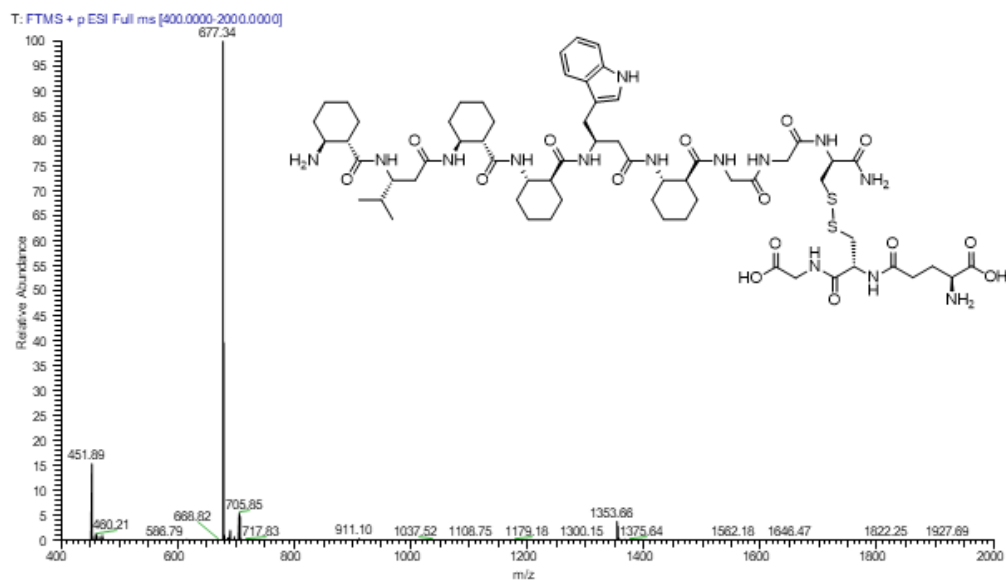
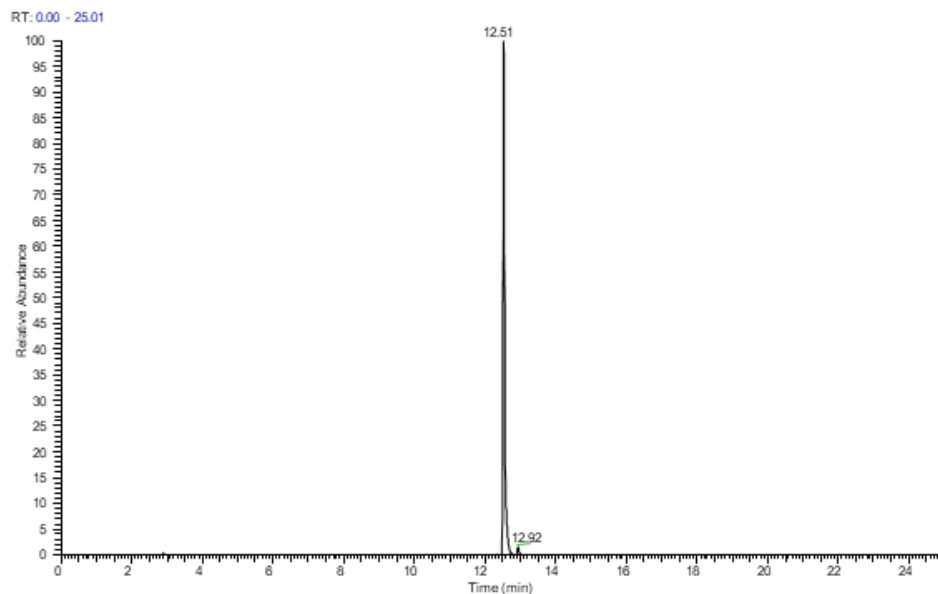
HPLC trace and mass spectrum of the glutathiononic precursor **KW-SG** (exact mass: 1381.69). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 691.3477 (100%), 691.8494 (69.22%), 692.3447 (8.95%), 692.3479 (3.79%); m/z calculated: 1382.47 $[M+H]^+$, 691.74 $[M+2H]^{2+}$, 461.49 $[M+3H]^{3+}$; m/z observed: 1382.69 $[M+H]^+$, 691.85 $[M+2H]^{2+}$, 461.57 $[M+3H]^{3+}$



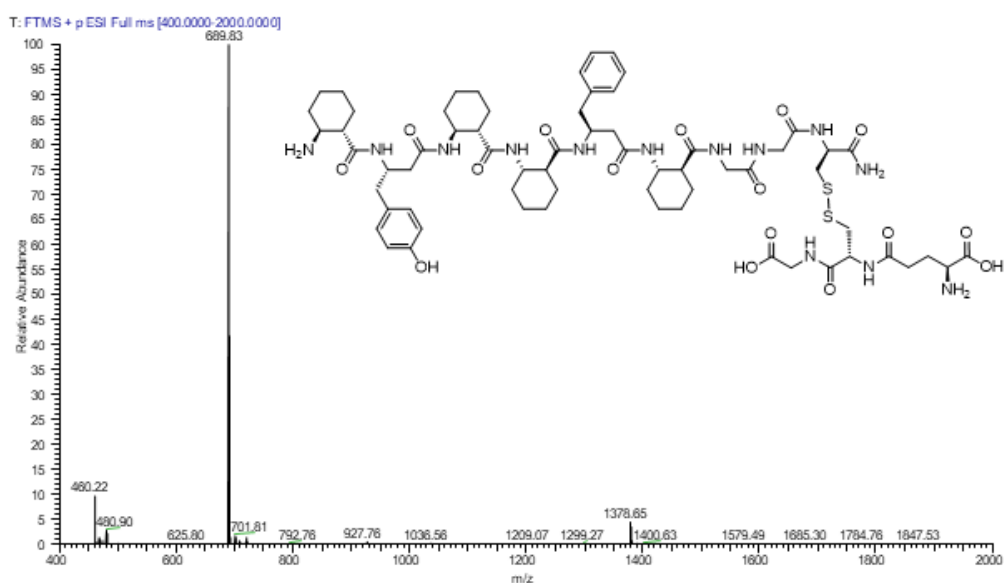
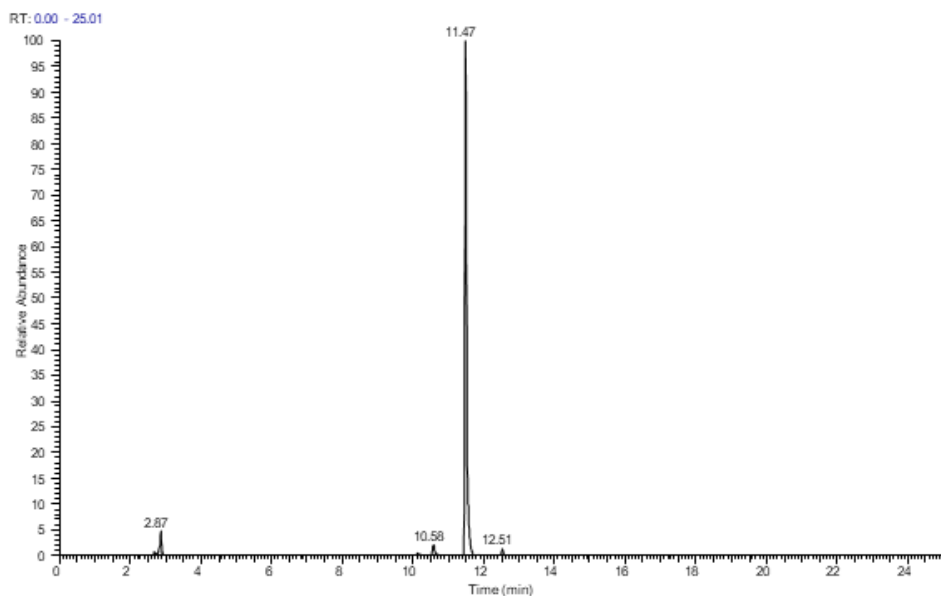
HPLC trace and mass spectrum of the glutathionyl precursor **QW-SG** (exact mass: 1381.65). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 691.3295 (100%), 691.8312 (68.14%), 692.3274 (8.95%), 692.3297 (3.73%); m/z calculated: 1382.43 $[M+H]^+$, 691.72 $[M+2H]^{2+}$; m/z observed: 1382.49 $[M+H]^+$, 692.13 $[M+2H]^{2+}$



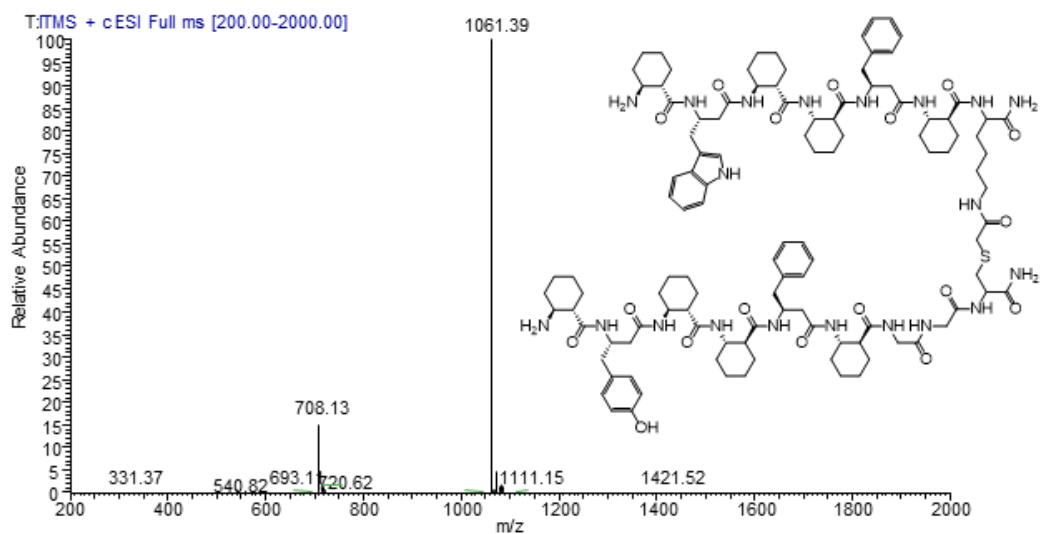
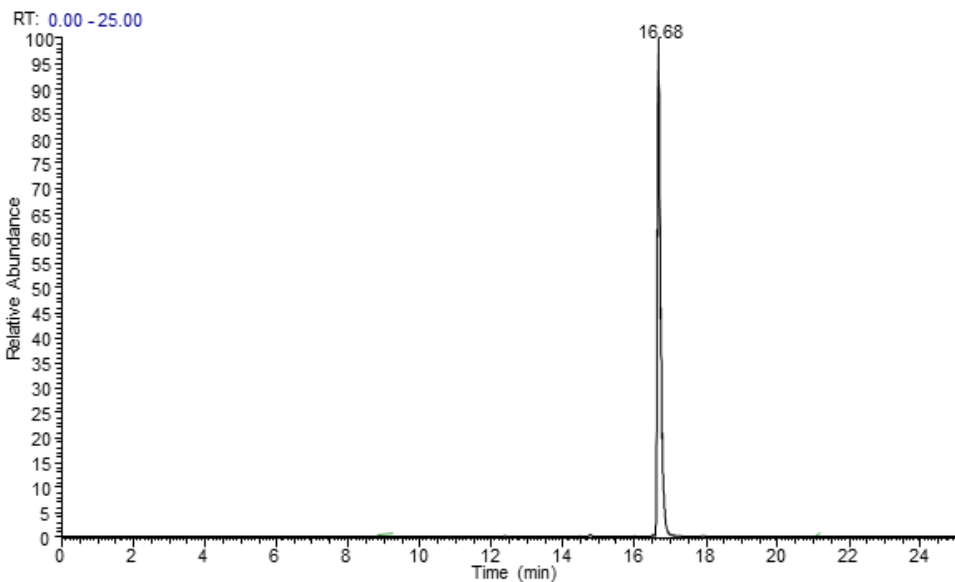
HPLC trace and mass spectrum of the glutathionyl precursor **RF-SG** (exact mass: 1370.68). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 685.8453 (100%), 686.3470 (67.06%), 686.8432 (8.95%), 686.8455 (3.92%); m/z calculated: 1371.45 $[M+H]^+$, 686.23 $[M+2H]^{2+}$, 457.82 $[M+3H]^{3+}$; m/z observed: 1371.69 $[M+H]^+$, 686.35 $[M+2H]^{2+}$, 457.90 $[M+3H]^{3+}$



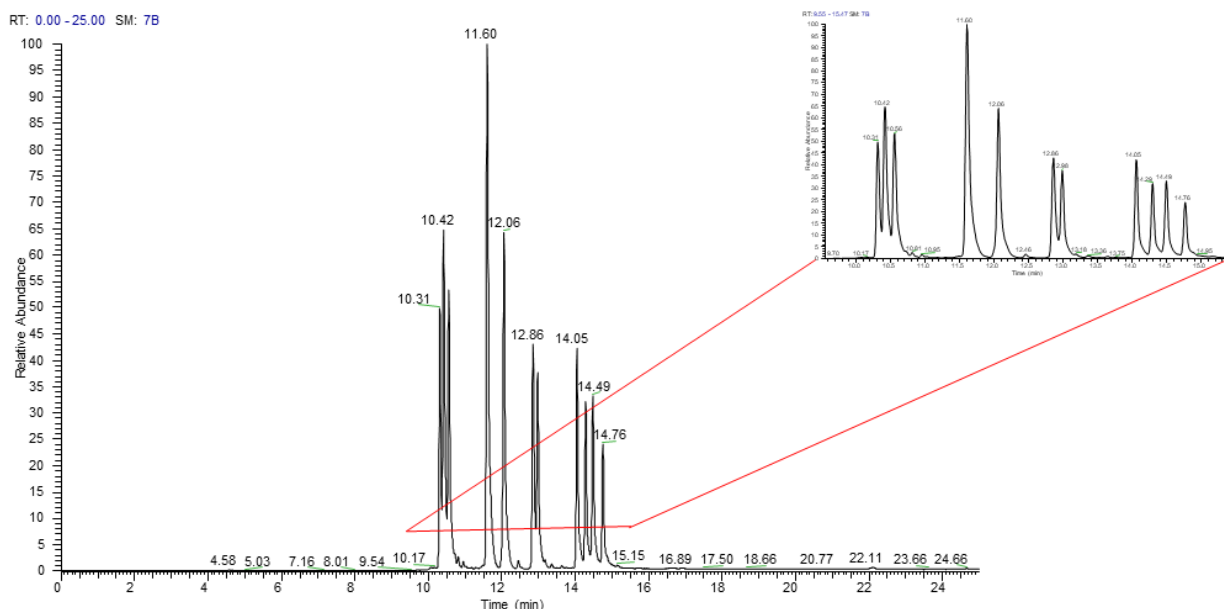
HPLC trace and mass spectrum of the glutathionyl precursor **VW-SG** (exact mass: 1352.66). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 676.8344 (100%), 677.3361 (68.14%), 677.8323 (8.95%), 677.8346 (3.49%); m/z calculated: 1353.43 $[M+H]^+$, 677.22 $[M+2H]^{2+}$; m/z observed: 1353.66 $[M+H]^+$, 677.34 $[M+2H]^{2+}$



HPLC trace and mass spectrum of the glutathionyl precursor **YF-SG** (exact mass: 1377.65). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 689.3264 (100%), 689.8281 (70.30%), 690.3243 (8.95%), 690.3266 (3.34%); m/z calculated: 1378.44 $[M+H]^+$, 689.72 $[M+2H]^{2+}$; m/z observed: 1378.65 $[M+H]^+$, 689.83 $[M+2H]^{2+}$



HPLC trace and mass spectrum of the glutathionic precursor **WF-S-YF** (exact mass: 2119.21). Calculated isotopic profile for $[M+2H]^{2+}$ (species, abundance): 1061.1138 (100%), 1060.6121 (81.82%), 1061.6123 (7.31%); m/z calculated: 2120.22 $[M+H]^+$, 1061.35 $[M+2H]^{2+}$; 707.90 $[M+3H]^{3+}$ m/z observed: 1061.39 $[M+2H]^{2+}$, 708.13 $[M+3H]^{3+}$



Characterisation of the monomer library. Total ion chromatogram of the initial foldamer library containing 12 different glutathione-protected monomers. Conditions of the analytical HPLC-MS measurement: Column: Aeris Widepore XB-C18 (250 × 4.6 mm) Method: 5–80% B during 25 minutes, flow rate: 0.7 mL min⁻¹, where eluent A: 0.1% HCOOH in water, eluent B: 0.1% HCOOH in ACN. For retention time and molar mass of the compounds, see Supplementary Table 3.

References

1. Dinesh Gupta, A.R.K. Reactions of thiyl radicals. XIII. Photochemically induced exchange reactions of liquid alkyl disulfides. *Can. J. Chem.* **58**, 1350-1354 (1980).
2. von Kiedrowski, G. Minimal Replicator Theory I: Parabolic Versus Exponential Growth, in *Bioorganic Chemistry Frontiers*. (eds. H. Dugas & F.P. Schmidtchen) 113-146 (Springer Berlin Heidelberg, Berlin, Heidelberg; 1993).
3. Higgs, P.G. Chemical Evolution and the Evolutionary Definition of Life. *J. Mol. Evol.* **84**, 225-235 (2017).
4. Bartus, É. *et al.* De Novo Modular Development of a Foldameric Protein–Protein Interaction Inhibitor for Separate Hot Spots: A Dynamic Covalent Assembly Approach. *ChemistryOpen* **6**, 236-241 (2017).