Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Engineering multilayer chemical networks

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1 Materials

Unless otherwise stated, all reagents and catalysts were purchased from commercial sources and used without further purification. 3,4,5-Trimethoxybenzaldehyde, 4-methylbenzenethiol (**2H**), and 1-pentanethiol were purchased from Sigma. Tin(II) trifluoromethanesulfonate, copper(II) trifluoromethanesulfonate, ytterbium(III) trifluoromethanesulfonate and bis(dibutylchlorotin(IV)) oxide were purchased from Aldrich. Dibutyltin(IV) oxide, TFA and TEA were purchased from Fluka. Sulfuric acid 98% was purchased from Anedra. Iron(III) chloride was purchased from Cicarelli. HPLC-grade acetonitrile was purchased from Carlo Erba. Milli-Q H_2O was used after filtration through 47 mm nylon membranes with 0.22 µm pore size (Osmonics Filters Inc.).

2 Instruments and conditions

HPLC

DAD-HPLC measurements were performed on a Hewlett Packard HP 1050 series.

LC conditions used for experiments shown in Tables 1, 2 and sequences I-V

Column 50 mm × 3.0 mm XDB C18 with particle size of 1.8 µm (Agilent).

Conditions: Column temperature: 30 ºC. Injection volume: 0.3 µL. Flow rate: 0.35 mL min^{-1}. Mobile phases: HPLC grade MeCN (channel B) and HPLC grade $MeCN/H_2O/HCO_2H$ (60/40/0.1) (channel C). Gradient elution is given as time (min)/channel B (%): 0/0, 2/1, 4/2, 6/2, 8/2, 9/5, 10/5, 12/8, 13/15, 15/40, 16/40, 17/0, 20/0. Detection at 250 nm.

3 Experimental methodology

3.1 Synthesis of compounds

((3,4,5-Trimethoxyphenyl)methylene)bis(phenylsulfane) (**1A1**) was synthesized according to literature.[1]

1,2-Diphenyldisulfane (**11**) was synthesized according to literature.[2]

S-phenyl thiobenzoate (**B1**): A solution of thiophenol (113 mg, 1.03 mmol) and triethylamine (91 mg, 0.89 mmol) in toluene (3 mL) was brougth to 0°C in a water/ice bath. The mixture was stirred while a solution of benzoyl chloride (120 mg, 0.86 mmol) in toluene (3 mL) was added in a timelapse of 1 min. After 90 min of reaction, the solvent was evaporated under reduced pressure. Flash silica gel column chromatography [Hex:EtOAc 100:0→97:3] yielded 102 mg (55%) of **B1** as a white solid. *R*f = 0.22 [TLC Si gel, Hex:EtOAc 98:2, UV_{254}]. ^{1}H- and ^{13}C-NMR analysis was consistent with structures and in agreement with the literature.[3]

3.2 Preparation of dynamic systems

Stock solutions of compounds **1A1**, **11**, and **B1** in acetonitrile solvent were used for the preparation of dynamic chemical systems.

3.2.1 Libraries shown in Tables 1 and 2 from manuscript

Dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol) and/or disulfide **11** (3.3 mg, 15 µmol) were added to an HPLC vial. Then, acetonitrile was added to reach a volume of 500 µL and the catalyst (15 µmol) was added. Finally, solid 4-methylbenzenethiol (**2H**) (3.7 mg, 30 µmol) was added into the vial and the mixture was stirred (300 rpm) at room temperature. HPLC-DAD analysis was carried out after 24 h of reaction.

When required (specified in the manuscript), before the addition of the thiol **2H**, argon was bubbled into the solution for ca. 2 min, then the thiol was added and mixture was stirred (300 rpm) at room temperature (under argon atmosphere, baloon). HPLC-DAD analysis was carried out at 24 h of reaction.

3.2.2 Dithioacetal exchange (Figures S 1-S 8)

Dithioacetal **1A1** (6 mg, 15 µmol) and one of the Brønsted or Lewis acid catalysts showed in Table S1 were added to an HPLC vial and diluted with acetonitrile (total volume: 500 µL). Solid thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature. Changes in composition were monitored by HPLC-DAD.

Table S1. Amount of acid catalysts added for experiments.

Figure	**Catalyst**	**Concentration**	**Amount added**
Figure S 1	Sulfuric acid	30 mM	1.47 mg, 15 µmol
Figure S 2	Sulfuric acid	3 mM	0.147 mg, 1.5 µmol
Figure S 3	*p*-Toluenesulfonic acid	30 mM	2.85 mg, 15 µmol
Figure S 4	Trifluoroacetic acid	30 mM	1.71 mg, 15 µmol
Figure S 5	Trifluoroacetic acid	300 mM	17.1 mg, 150 µmol
Figure S 6	Tin(II) triflate	30 mM	6.20 mg, 15 µmol
Figure S 7	Tin(II) triflate	3 mM	0.62 mg, 1.5 µmol
Figure S 8	Tin(II) triflate	0.3 mM	0.06 mg, 0.15 µmol

3.2.3 Thioester exchange (Figures S 9-S 13)

An acetonitrile solution (500 µL) of thioester **B1** (3.2 mg, 15 µmol) was added to an HPLC vial. Argon was bubbled into the solution for 2 min and then a catalyst (Table S2) was added. Finally, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature. Changes in composition were monitored by HPLC-DAD.

Table S2. Amount of catalysts added for experiments.

Figure	**Catalyst**	**Concentration**	**Amount added**
Figure S 9	Triethylamine	30 mM	1.52 mg, 15 µmol
Figure S 10	Triethylamine	3 mM	0.15 mg, 1.5 µmol
Figure S 11	Triethylamine	0.3 mM	0.02 mg, 0.15 µmol
Figure S 12	Butylstannonic acid	30 mM	3.13 mg, 15 µmol
Figure S 13	Butylstannonic acid	3 mM	0.31 mg, 1.5 µmol

3.2.4 Oxidation of thiol **2H** (Figure S 15)

Triethylamine (1.5 mg, 15 µmol) or butylstannonic acid (3.1 mg, 15 µmol) was added to an HPLC vial containing an acetonitrile solution (500 µL) of thiol **2H** (3.7 mg, 30 µmol). The reaction mixture was stirred (300 rpm) at room temperature. Changes in composition were monitored by HPLC-DAD.

3.2.5 Disulfide exchange (Figures S 16-S 19)

No Catalyst experiment (Figure S 16): An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol) was poured into an HPLC vial. Then, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature. Changes in composition were monitored by HPLC-DAD.

Catalyzed experiments: An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol) was poured into an HPLC vial. A catalyst (Table S3) was added and then argon was bubbled into the solution for ca. 2 min. Finally, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature. Changes in composition were monitored by HPLC-DAD.

In the experiment with TEA, before the addition of the thiol **2H**, argon was bubbled into the solution for ca. 2 min. After the addition of **2H**, the mixture was stirred (300 rpm) at room temperature (under argon atmosphere, baloon).

Table S3. Amount of catalysts added for experiments.

Figure	Catalyst	Concentration	Amount added
Figure S 16	No catalyst	---	---
Figure S 17	Triethylamine	30 mM	1.52 mg, 15 µmol
Figure S 18	Butylstannonic acid	30 mM	3.13 mg, 15 µmol
Figure S 19	Trifluoroacetic acid	30 mM	1.71 mg, 15 µmol

3.2.6 Simultaneous disulfide and thioester exchanges (Figure 4 from manuscript)

Networked reaction: An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol) and thioester **B1** (3.2 mg, 15 µmol) was poured into an HPLC vial. Triethylamine (1.5 mg, 15 µmol) was added and then argon was bubbled into the solution for ca. 2 min. Finally, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature under argon atmosphere (baloon). Changes in composition were monitored by HPLC-DAD.

Isolated reactions: An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol) or thioester **B1** (3.2 mg, 15 µmol) was poured into an HPLC vial. Triethylamine (1.5 mg, 15 µmol) was added and then argon was bubbled into the solution for ca. 2 min. Finally, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at room temperature under argon atmosphere (baloon). Changes in composition were monitored by HPLC-DAD.

3.2.7 Simultaneous disulfide and dithioacetal exchanges (Figure 5 from manuscript)

Networked reaction: An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol), dithioacetal **1A1** (6 mg, 15 µmol) and $SnCl_2$ (0.14 mg, 0.75 µmol) was poured into an HPLC vial. Argon was bubbled into the solution for ca. 2 min and thiol **2H** (3.7 mg, 30 µmol) was added. The reaction mixture was stirred (300 rpm) at 100°C under argon atmosphere. Changes in composition were monitored by HPLC-DAD after cooling the reaction vial at room temperature.

Isolated reactions: An acetonitrile solution (500 µL) containing disulfide **11** (3.3 mg, 15 µmol) or dithioacetal **1A1** (6 mg, 15 µmol) was poured into an HPLC vial. Tin(II) chloride (0.14 mg, 0,75 µmol) was added and then argon was bubbled into the solution for ca. 2 min. Finally, thiol **2H** (3.7 mg, 30 µmol) was added and the reaction mixture was stirred (300 rpm) at 100°C under argon atmosphere. Changes in composition were monitored by HPLC-DAD after cooling the reaction vial at room temperature.

3.2.8 Sequential, simultaneous and communicating reactions (Figures 6, 8-11 from manuscript)

HPLC-DAD measurements were performed before activation of each reaction of the sequence.

Sequence I

Figure 6A. An acetonitrile solution (500 µL) containing dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol) and disulfide **11** (3.3 mg, 15 µmol) was poured in an HPLC vial. Thiol **2H** (3.7 mg, 30 µmol) was added and inmediately the reaction mixture was injected in the HPLC.

Figure 6B. Solution from the previous step was stirred (300 rpm) at room temperature for 180 min and then it was injected in the HPLC.

Figure 6C. Sulfuric acid (3.7 mg, 15 µmol) was added into the solution and the reaction mixture was stirred (300 rpm) at room temperature for 20 min and injected in the HPLC.

Figure 6D. Argon was bubbled into the solution and triethylamine was added (7.26 mg, 75 µmol). The resulting mixture was stirred (300 rpm) at room temperature for 5 min under argon atmosphere and injected in the HPLC.

Sequence II

Figure 8A. An acetonitrile solution (500 µL) containing dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol) and disulfide **11** (3.3 mg, 15 µmol) was poured in an HPLC vial. Thiol **2H** (3.7 mg, 30 µmol) was added and the mixture was stirred (300 rpm) at room temperature for 150 min and then injected in the HPLC.

Figure 8B. Argon was bubbled into the solution and triethylamine was added (0.15 mg, 1.5 µmol). The resulting mixture was stirred (300 rpm) at room temperature for 20 min (under argon atmosphere) and injected in the HPLC.

Figure 8C. Sulfuric acid (3.7 mg, 15 µmol) was added to the solution and the reaction mixture was stirred (300 rpm) at room temperature for 40 min and injected in the HPLC.

Sequence III

Figure 9A. An acetonitrile solution (500 µL) containing dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol), disulfide **11** (3.3 mg, 15 µmol) and sulfuric acid (3.7 mg, 15 µmol) was poured in an HPLC vial. Thiol **2H** (3.7 mg, 30 µmol) was added and the mixture was stirred (300 rpm) at room temperature for 20 min and injected in the HPLC.

Figure 9B. Argon was bubbled inside the solution before the addition of triethylamine (15 mg, 150 µmol). The resulting mixture was stirred (300 rpm) at room temperature for 10 min and injected in the HPLC.

Sequence IV

Figure 10A. Thiol **2H** (3.7 mg, 30 µmol) was added to an acetonitrile solution (500 µL) containing dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol), disulfide **11** (3.3 mg, 15 µmol), and triethylamine (0.15 mg, 1.5 µmol). The solution was stirred (300 rpm) at room temperature for 20 min and injected in the HPLC.

Figure 10B. Sulfuric acid (3.7 mg, 15 µmol) was added to the solution and the reaction mixture was stirred (300 rpm) at room temperature for 40 min and injected in the HPLC.

Figure 10C. Argon was bubbled into the solution before the addition of triethylamine (2.9 mg, 30 µmol). The resulting mixture was stirred (300 rpm) at room temperature for 20 min and injected in the HPLC.

Sequence V

Figure 11A. An acetonitrile solution (500 µL) containing dithioacetal **1A1** (6 mg, 15 µmol), thioester **B1** (3.2 mg, 15 µmol), disulfide **11** (3.3 mg, 15 µmol), and tin(II) chloride (0.14 mg, 0.75 µmol) was poured in an HPLC vial. Argon was bubbled into the solution and thiol **2H** (3.7 mg, 30 µmol) was added. The reaction mixture was stirred (300 rpm) overnight at 100°C under argon atmosphere and injected in the HPLC.

Figure 11B. Argon was bubbled into the solution for ca. 2 min and then triethylamine (14.5 mg, 150 µmol) was added. The mixture was stirred (300 rpm) at room temperature for 10 min under argon atmosphere and injected in the HPLC.

4 Kinetics based on HPLC chromatograms

4.1 Dithioacetal exchange:

4.1.1 Catalyzed by H_2SO_4 (30 mM)

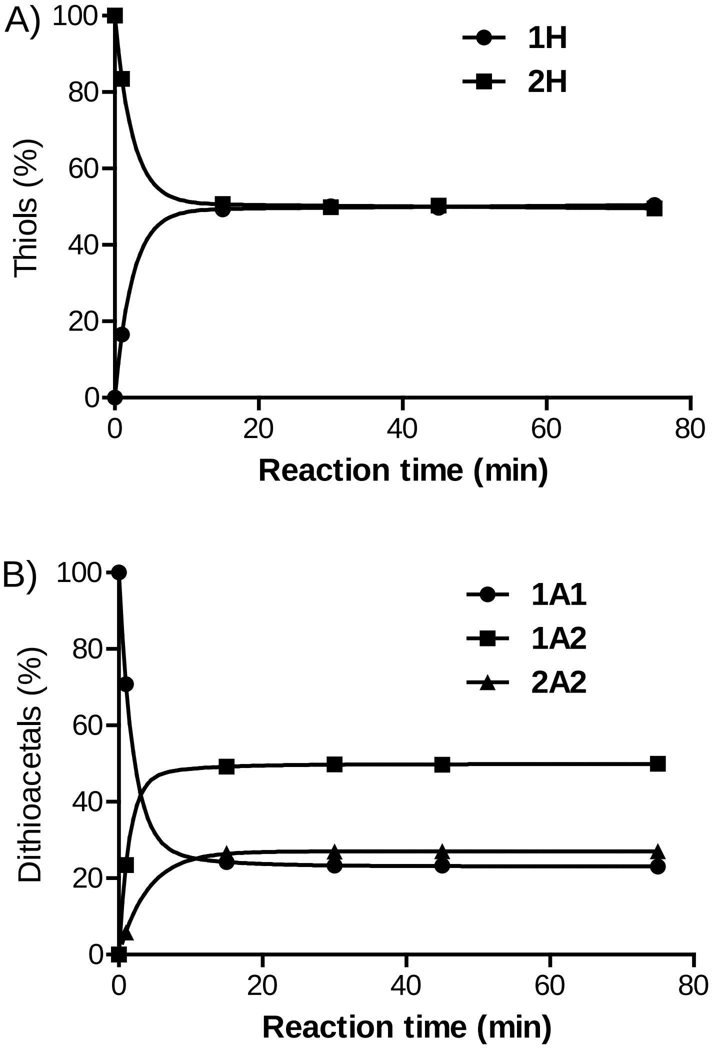


Figure S 1

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by sulfuric acid (15 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.2 Catalyzed by H_2SO_4 (3 mM)

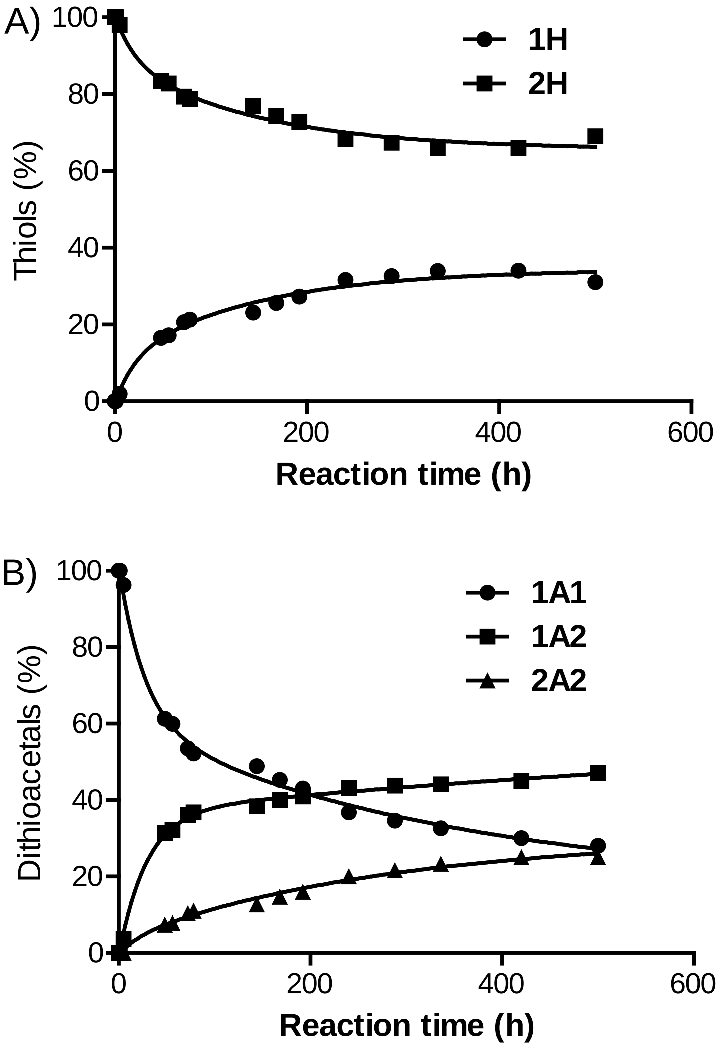
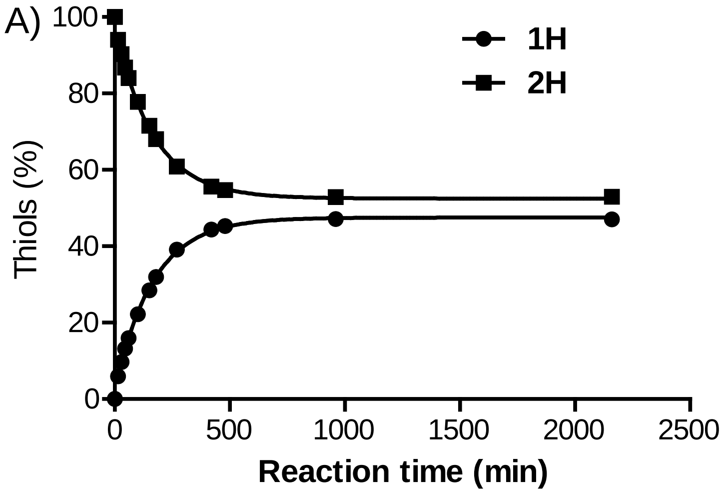


Figure S 2

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by sulfuric acid (1.5 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H + 2H)**; **2H** (%) = **2H*100/(1H + 2H)**; **1A1** (%) = **1A1*100/(1A1 + 1A2 + 2A2)**; **1A2** (%) = **1A2*100/(1A1 + 1A2 + 2A2)**; **2A2** (%) = **2A2*100/(1A1 + 1A2 + 2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.3 Catalyzed by PTSA (30 mM)



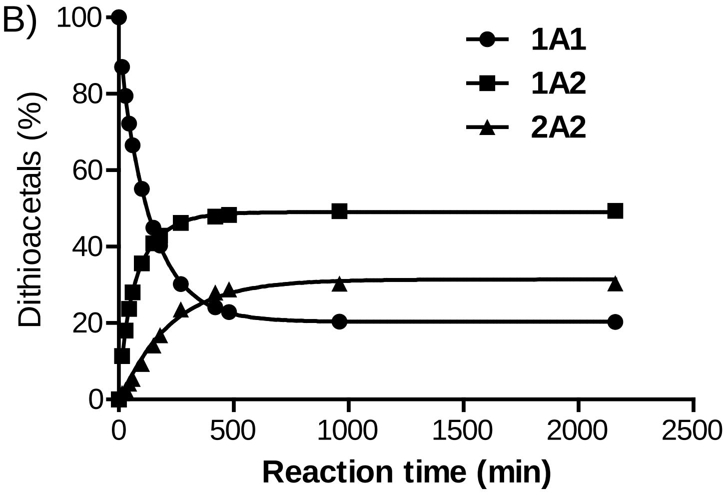


Figure S 3

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by PTSA (15 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.4 In the presence of TFA (30 mM)

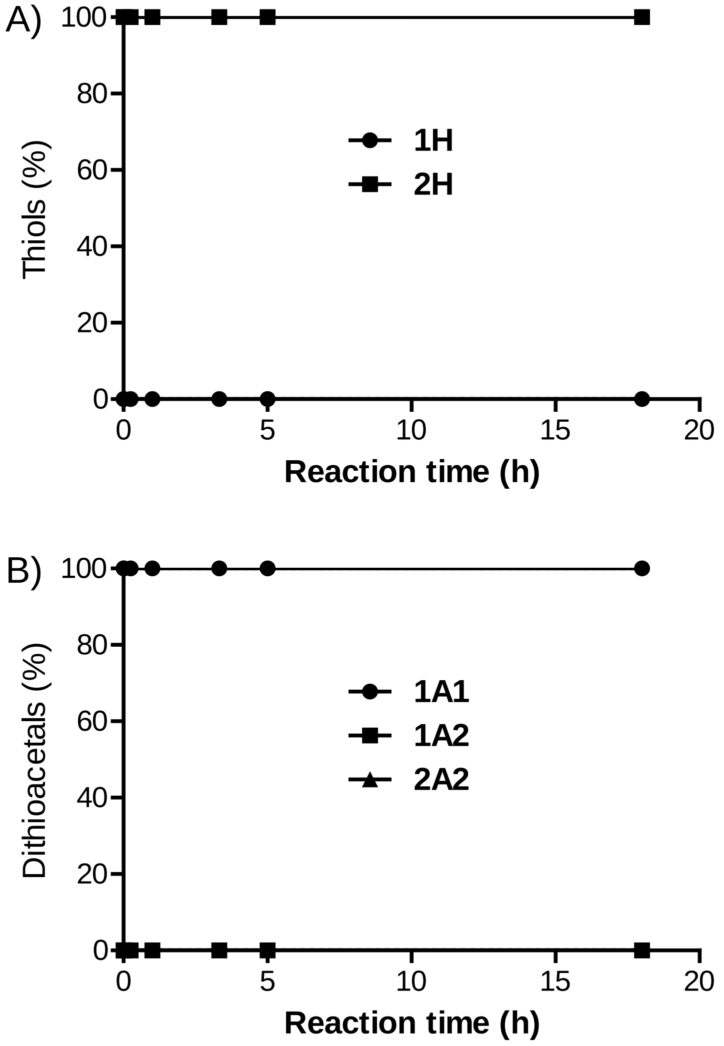


Figure S 4

Relative proportion of A) thiols and B) dithioacetals starting from **1A1** (15 µmol) and **2H** (30 µmol) in the presence of TFA (15 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100**/(**1H** + **2H**); **2H** (%) = **2H*100**/(**1H** + **2H**); **1A1** (%) = **1A1*100**/(**1A1** + **1A2** + **2A2**); **1A2** (%) = **1A2*100**/(**1A1** + **1A2** + **2A2**); **2A2** (%) = **2A2*100**/(**1A1** + **1A2** + **2A2**), as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.5 Catalyzed by TFA (300 mM)

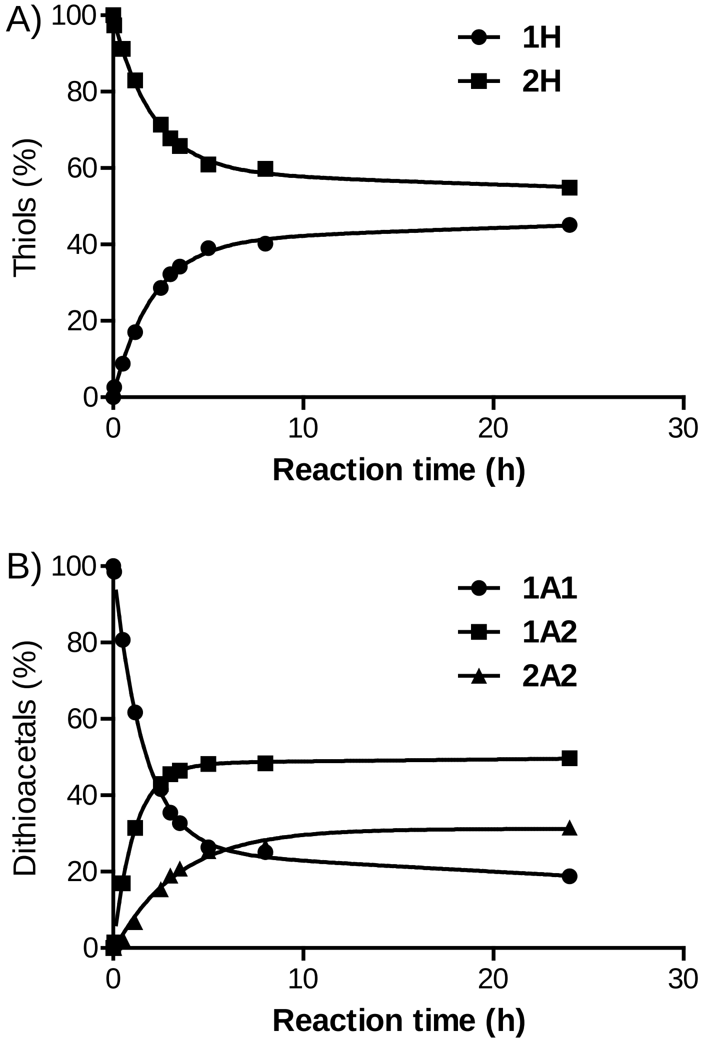


Figure S 5

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by TFA (150 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.6 Catalyzed by Sn(OTf)$_2$ (30 mM)

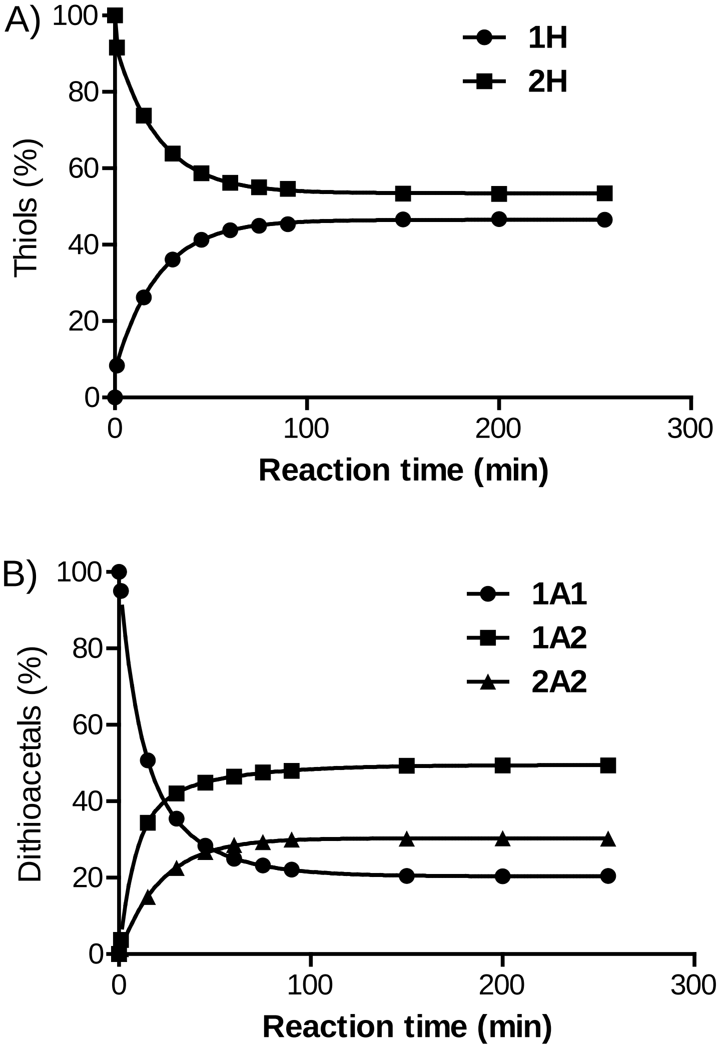
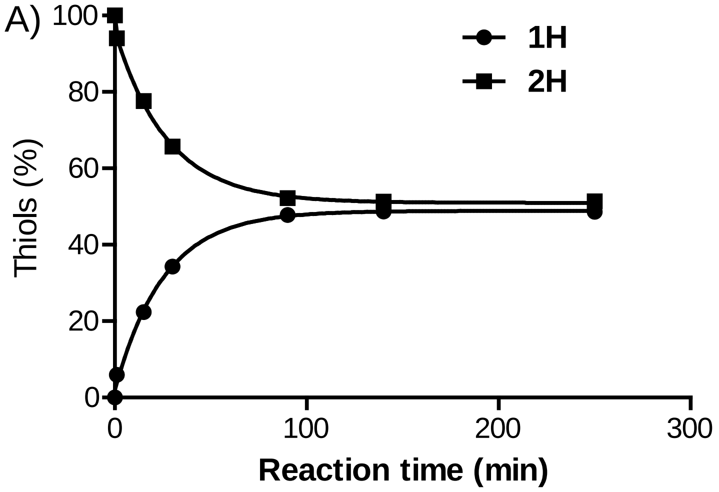


Figure S 6

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 μmol) and **2H** (30 μmol) catalyzed by Sn(OTf)$_2$ (15 μmol). Solvent: acetonitrile (total volume 500 μL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs$_{250}$).

4.1.7 Catalyzed by $Sn(OTf)_2$ (3 mM)



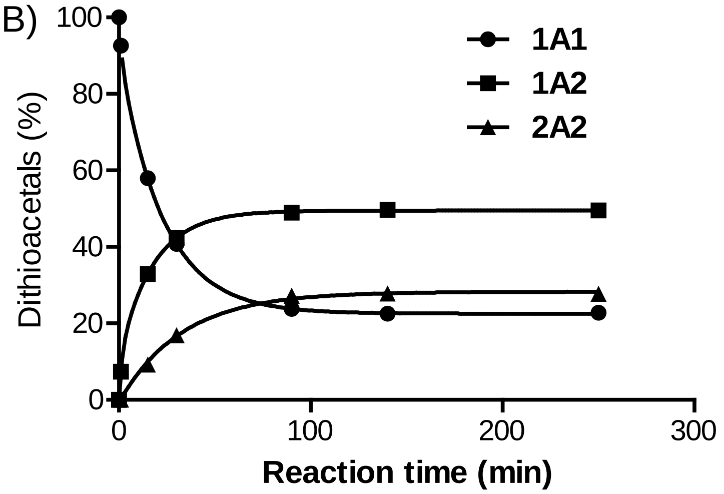


Figure S 7

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by $Sn(OTf)_2$ (1.5 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.1.8 Catalyzed by Sn(OTf)2 (0.3 mM)

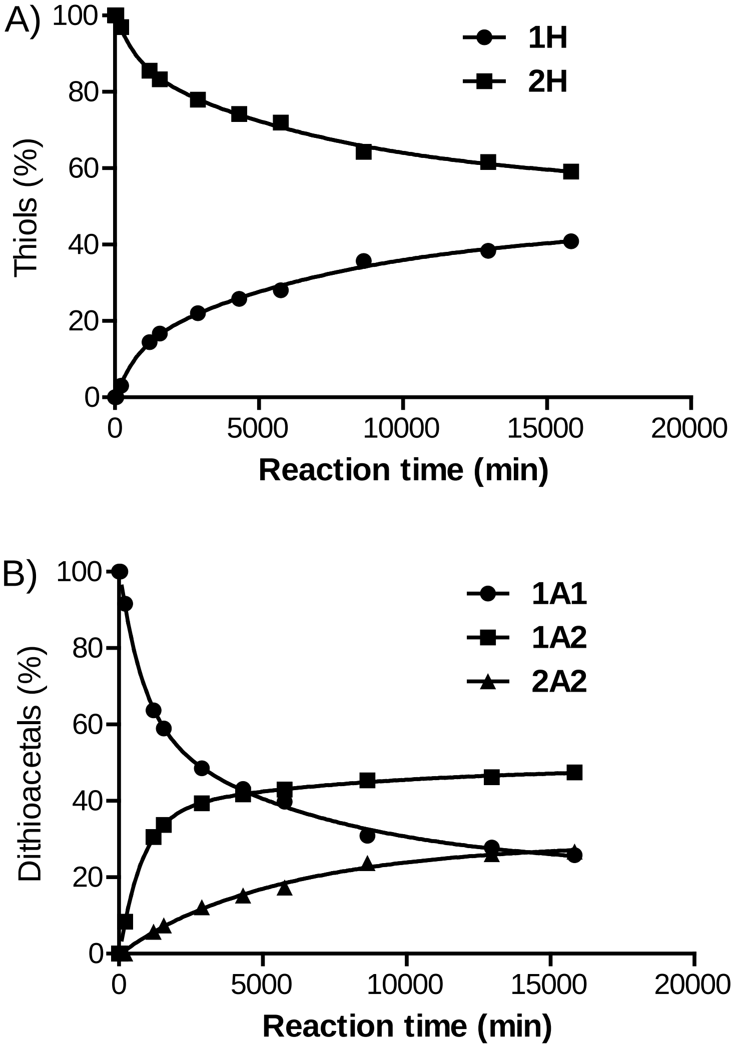


Figure S 8

Relative proportion of A) thiols and B) dithioacetals in the dithioacetal exchange starting from **1A1** (15 µmol) and **2H** (30 µmol) catalyzed by $Sn(OTf)_2$ (0.15 µmol). Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **1A1** (%) = **1A1*100/(1A1** + **1A2** + **2A2)**; **1A2** (%) = **1A2*100/(1A1** + **1A2** + **2A2)**; **2A2** (%) = **2A2*100/(1A1** + **1A2** + **2A2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.2 Thioester exchange:

4.2.1 Catalyzed by TEA (30 mM)

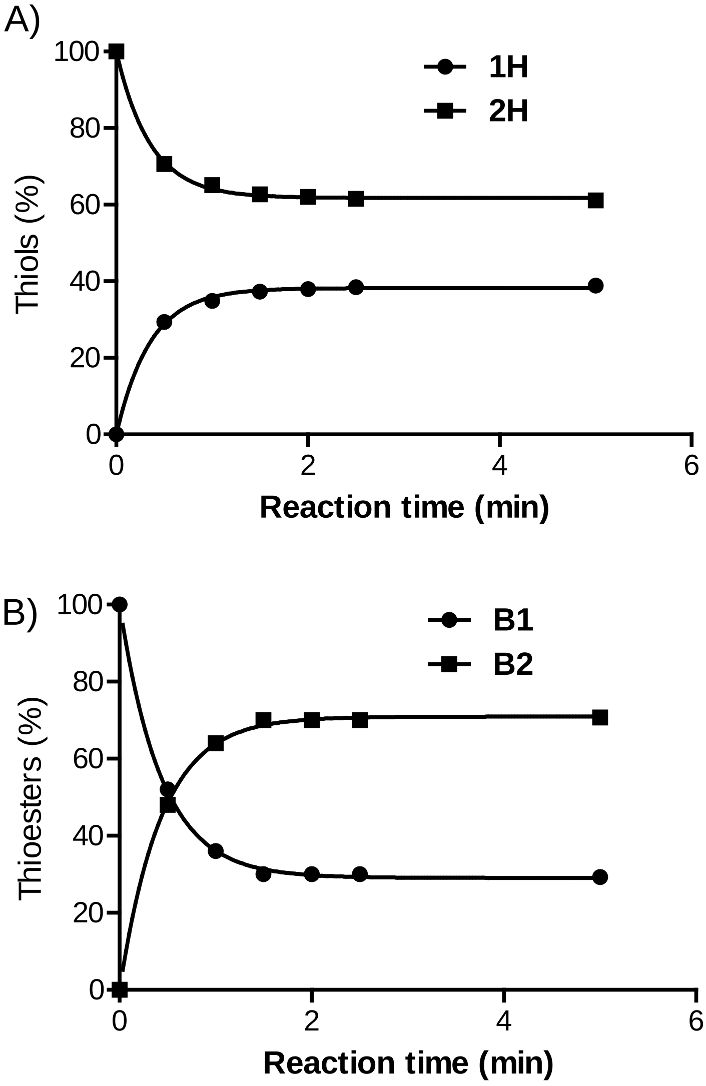


Figure S 9

Relative proportion of A) thiols and B) thioester in the thioester exchange starting from **B1** (15 µmol) and **2H** (30 µmol) catalyzed by TEA (15 µmol). Solvent: acetonitrile (total volume 500 µL). Argon atmosphere. **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.2.2 Catalyzed by TEA (3 mM)

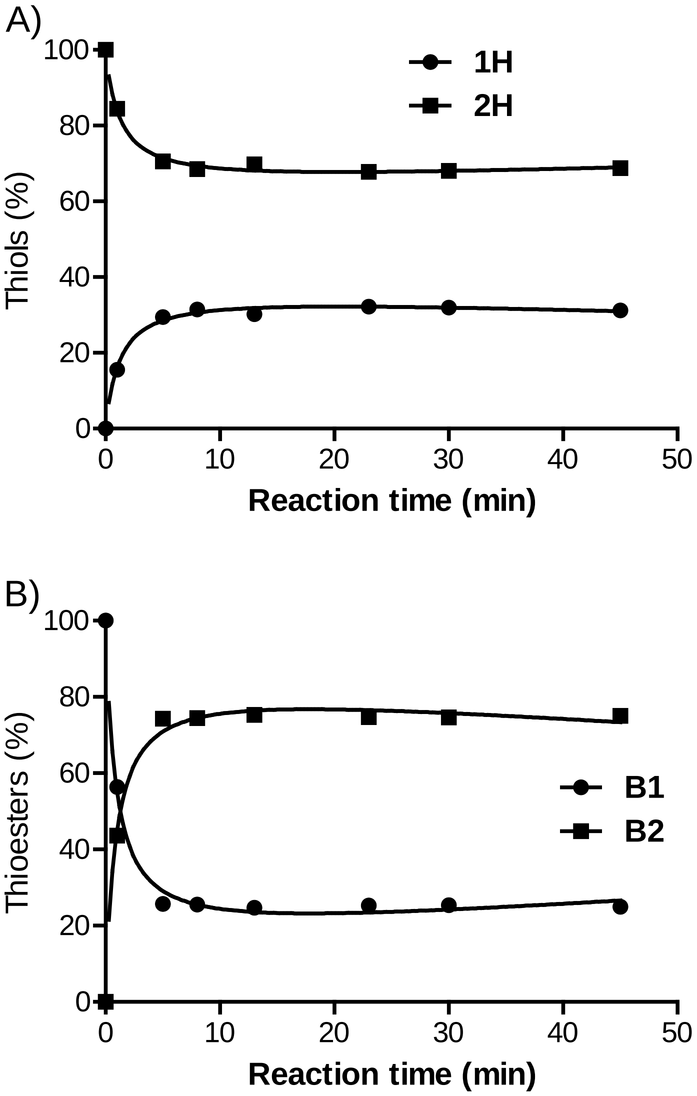


Figure S 10

Relative proportion of A) thiols and B) thioester in the thioester exchange starting from **B1** (15 µmol) and **2H** (30 µmol) catalyzed by TEA (1.5 µmol). Solvent: acetonitrile (total volume 500 µL). Argon atmosphere. **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**, as calculated in base of the HPLC peak area data (Abs$_{250}$).

4.2.3 Catalyzed by TEA (0.3 mM)

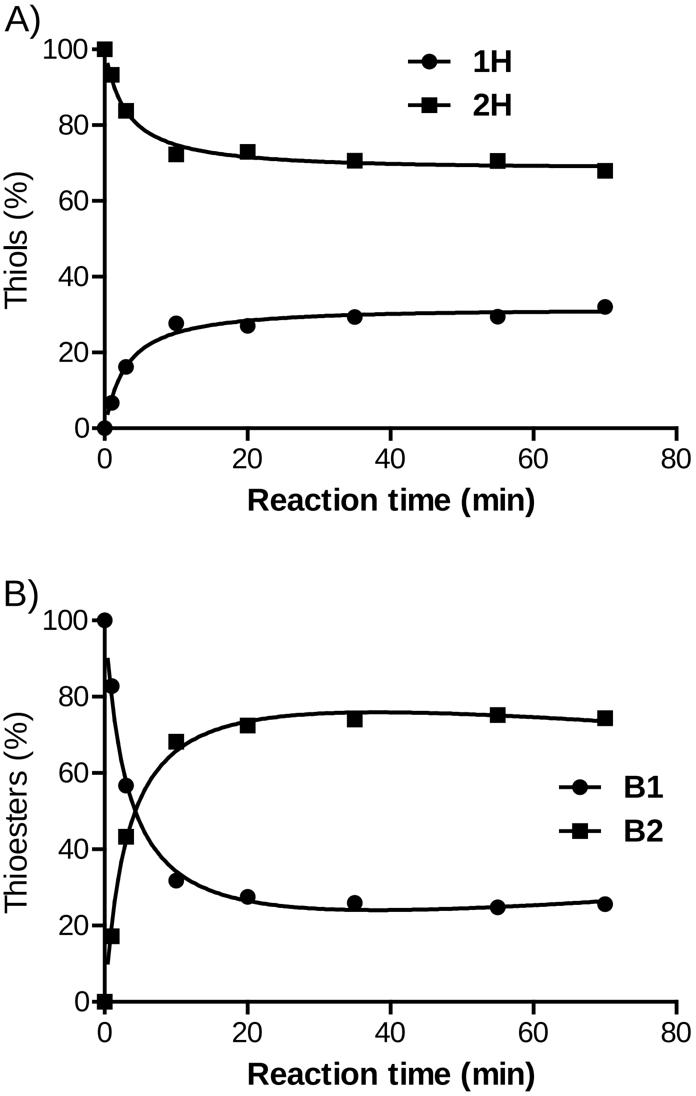


Figure S 11

Relative proportion of A) thiols and B) thioester in the thioester exchange starting from **B1** (15 µmol) and **2H** (30 µmol) catalyzed by TEA (0.15 µmol). Solvent: acetonitrile (total volume 500 µL). Argon atmosphere. **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.2.4 Catalyzed by $BuSnO_2H$ (30 mM)

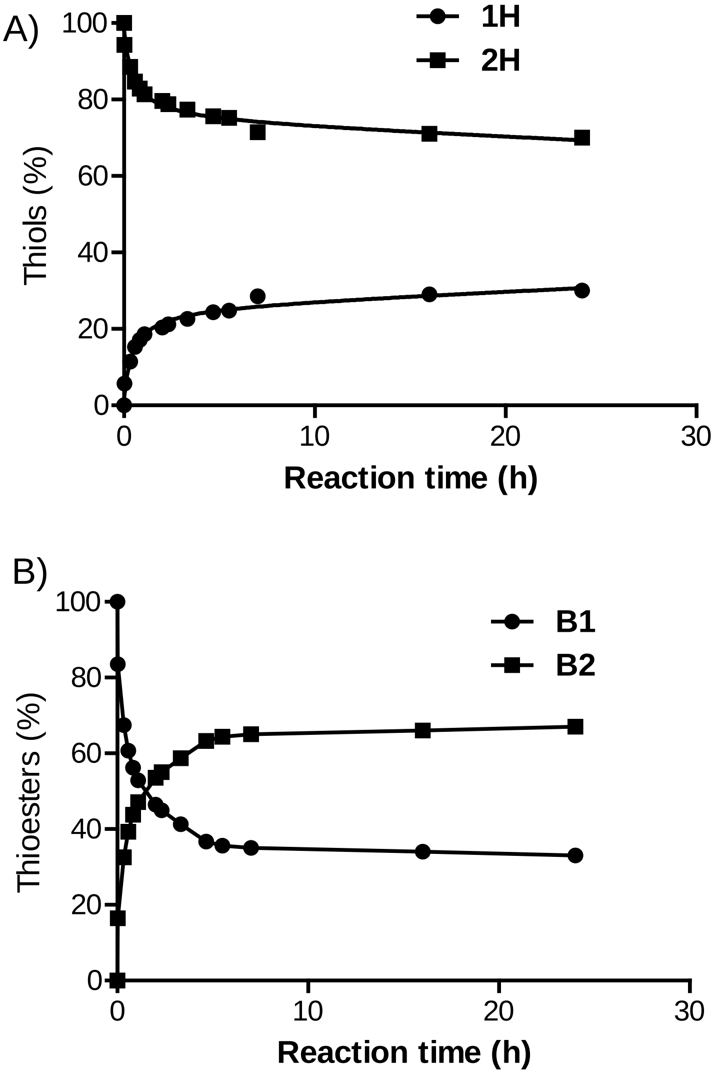


Figure S 12

Relative proportion of A) thiols and B) thioester in the thioester exchange starting from **B1** (15 µmol) and **2H** (30 µmol) catalyzed by $BuSnO_2H$ (15 µmol). Solvent: acetonitrile (total volume 500 µL). Argon atmosphere. **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.2.5 Catalyzed by $BuSnO_2H$ (3 mM)

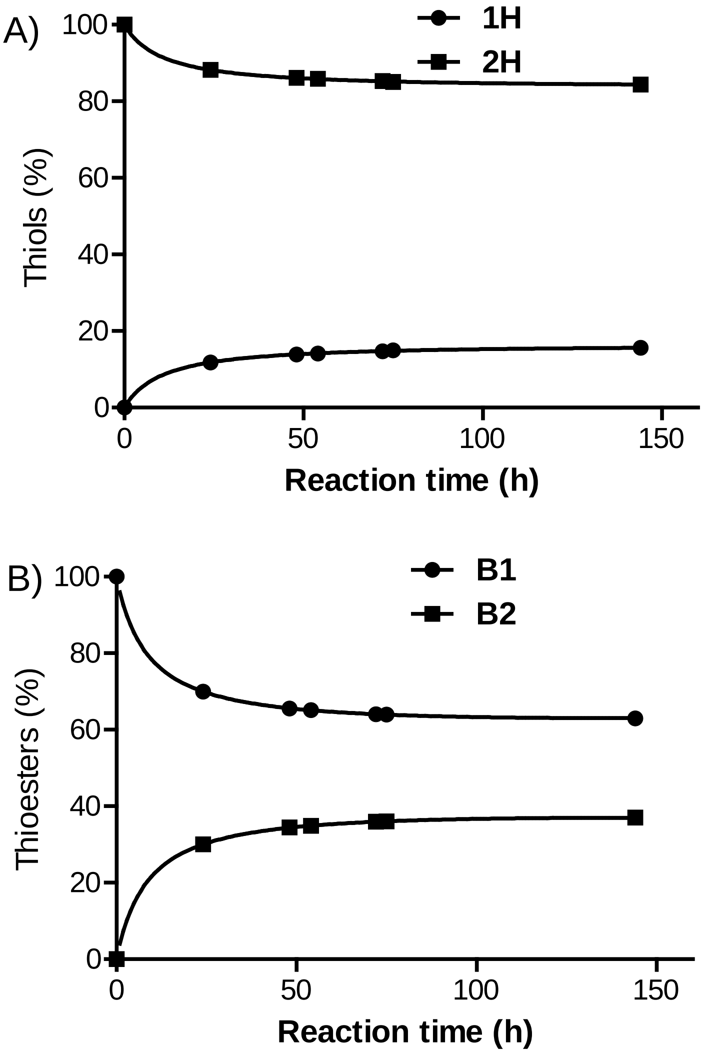


Figure S 13

Relative proportion of A) thiols and B) thioester in the thioester exchange starting from **B1** (15 µmol) and **2H** (30 µmol) catalyzed by $BuSnO_2H$ (1.5 µmol). Solvent: acetonitrile (total volume 500 µL). Argon atmosphere. **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.2.6 Addition of a different thiol (A) or successive additions of BuSnO$_2$H (B and C)

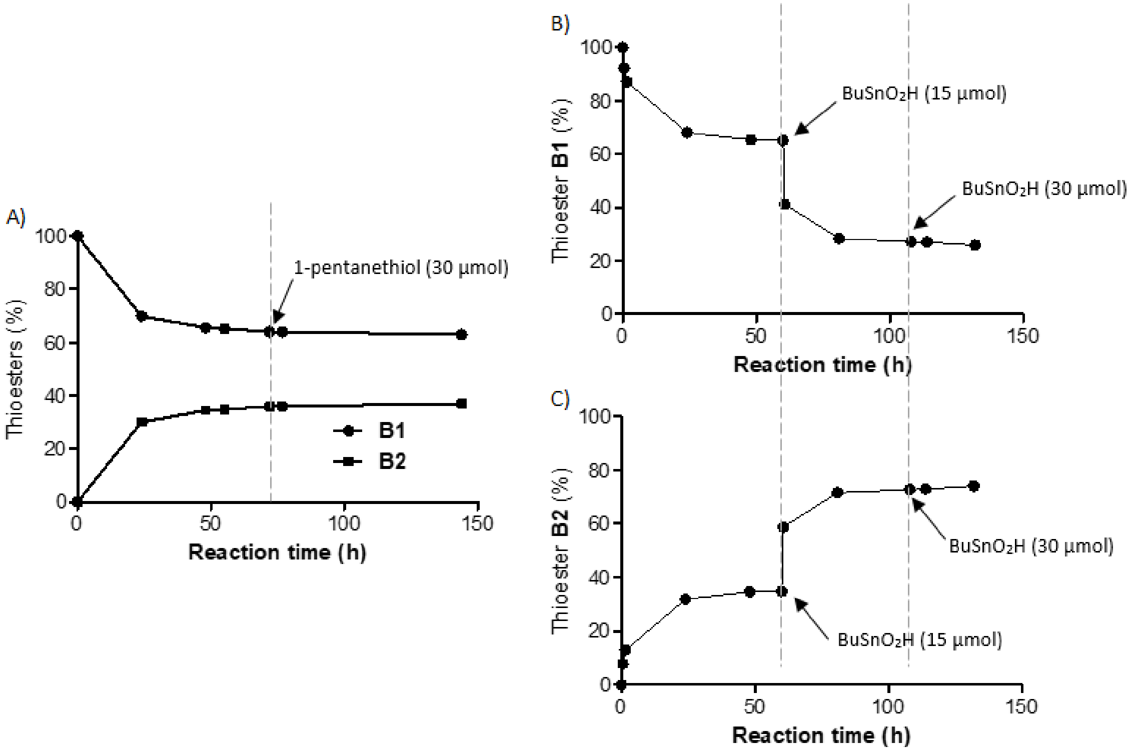


Figure S 14

Kinetics profile of the thioester exchange in two samples of the same reaction starting from thioester **B1** (15 µmol), thiol **2H** (30 µmol) and BuSnO$_2$H (1.5 µmol). Solvent: acetonitrile 500 µL, r.t. A) Sample 1, at 72 hours 1-pentanethiol (30 µmol) was added to the reaction mixture. B and C) Sample 2, successive additions of BuSnO$_2$H were made at two different times: 60 h (13.5 µmol) and 108 h (15 µmol). **B1** (%) = **B1*100**/(**B1** + **B2**); **B2** (%) = **B2*100**/(**B1** + **B2**), as calculated in base of the HPLC peak area data (Abs$_{250}$).

4.3 Oxidation of thiol 2H:

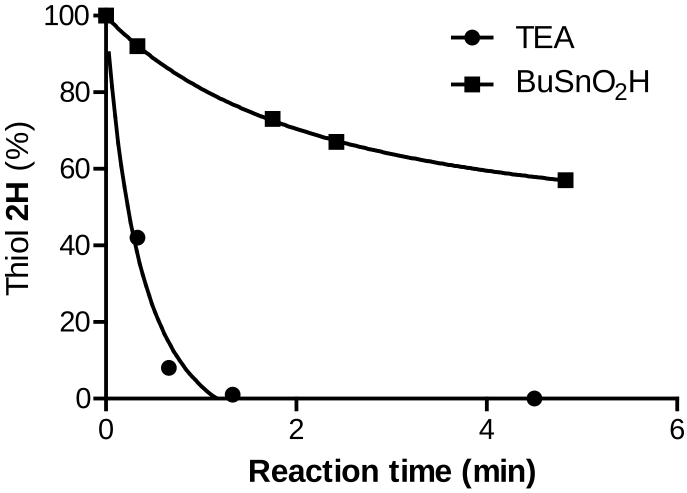


Figure S 15

Effect of butylstannonic acid (30 mM) or triethylamine (30 mM) on the oxidation of thiol **2H** (60 mM). Solvent: acetonitrile (total volume: 500 µL), r.t. Analysis in base of the HPLC peak area data (Abs_{250}).

4.4 Disulfide exchange:

4.4.1 <u>No catalyst</u>

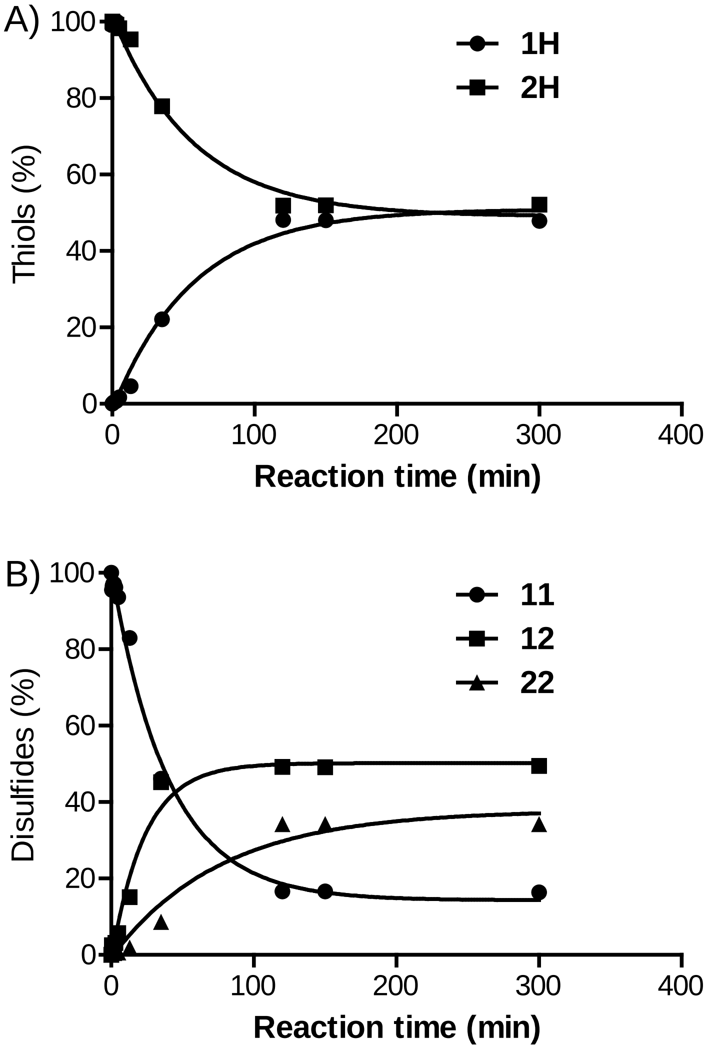


Figure S 16

Relative proportion of A) thiols and B) disulfides in the disulfide exchange in the absence of a catalyst starting from **11** (15 μmol) and **2H** (30 μmol). Solvent: acetonitrile (total volume 500 μL). **1H** (%) = **1H*100/(1H + 2H)**; **2H** (%) = **2H*100/(1H + 2H)**; **11** (%) = **11*100/(11 + 12 + 22)**; **12** (%) = **12*100/(11 + 12 + 22)**; **22** (%) = **22*100/(11 + 12 + 22)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.4.2 Catalyzed by TEA (30 mM)

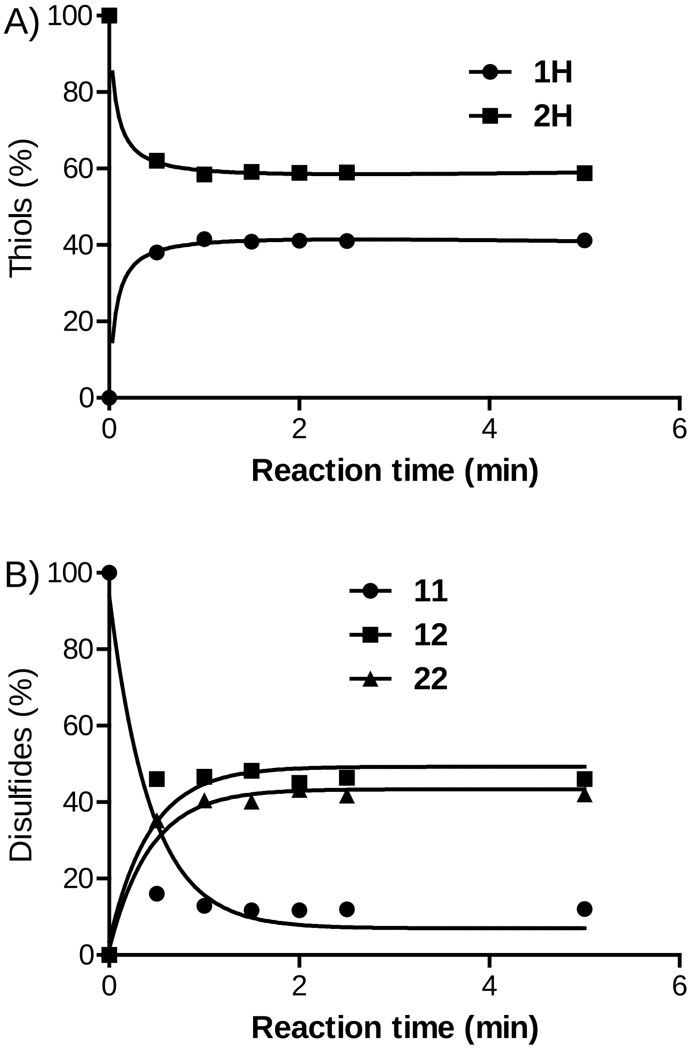


Figure S 17

Relative proportion of A) thiols and B) disulfides in the disulfide exchange starting from **11** (15 µmol) and **2H** (30 µmol) catalyzed by TEA (15 µmol). Argon atmosphere. Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **11** (%) = **11*100/(11** + **12** + **22)**; **12** (%) = **12*100/(11** + **12** + **22)**; **22** (%) = **22*100/(11** + **12** + **22)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.4.3 Catalyzed by $BuSnO_2H$ (30 mM)

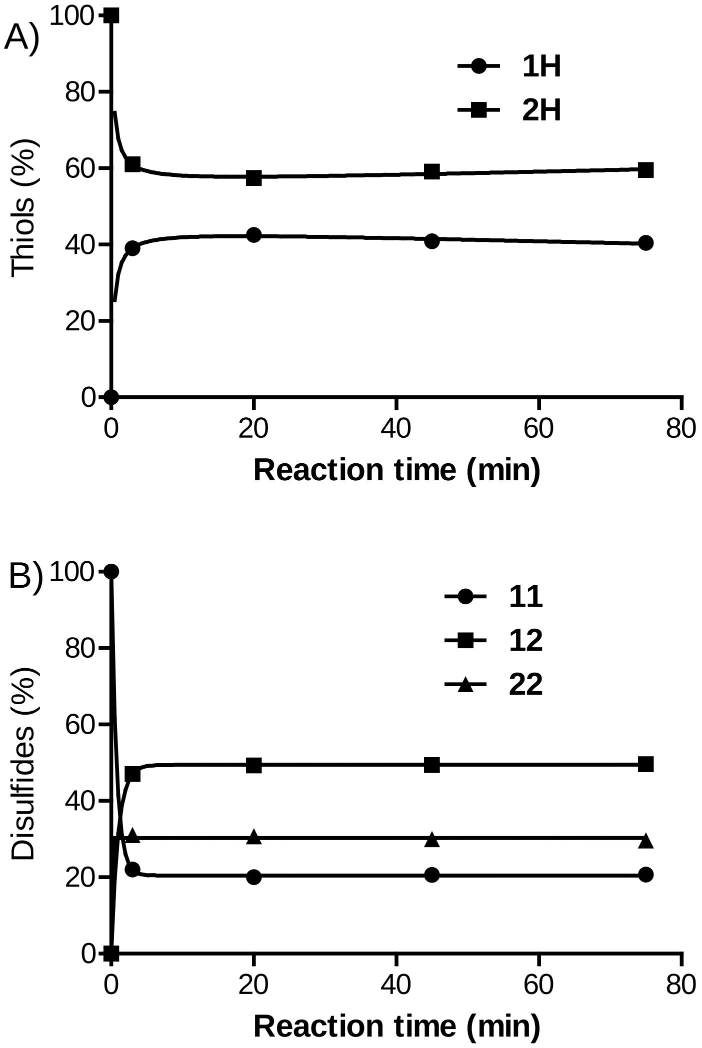


Figure S 18

Relative proportion of A) thiols and B) disulfides in the disulfide exchange starting from **11** (15 µmol) and **2H** (30 µmol) catalyzed by $BuSnO_2H$ (15 µmol). Argon atmosphere. Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H** + **2H)**; **2H** (%) = **2H*100/(1H** + **2H)**; **11** (%) = **11*100/(11** + **12** + **22)**; **12** (%) = **12*100/(11** + **12** + **22)**; **22** (%) = **22*100/(11** + **12** + **22)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.4.4 In the presence of TFA (30 mM)

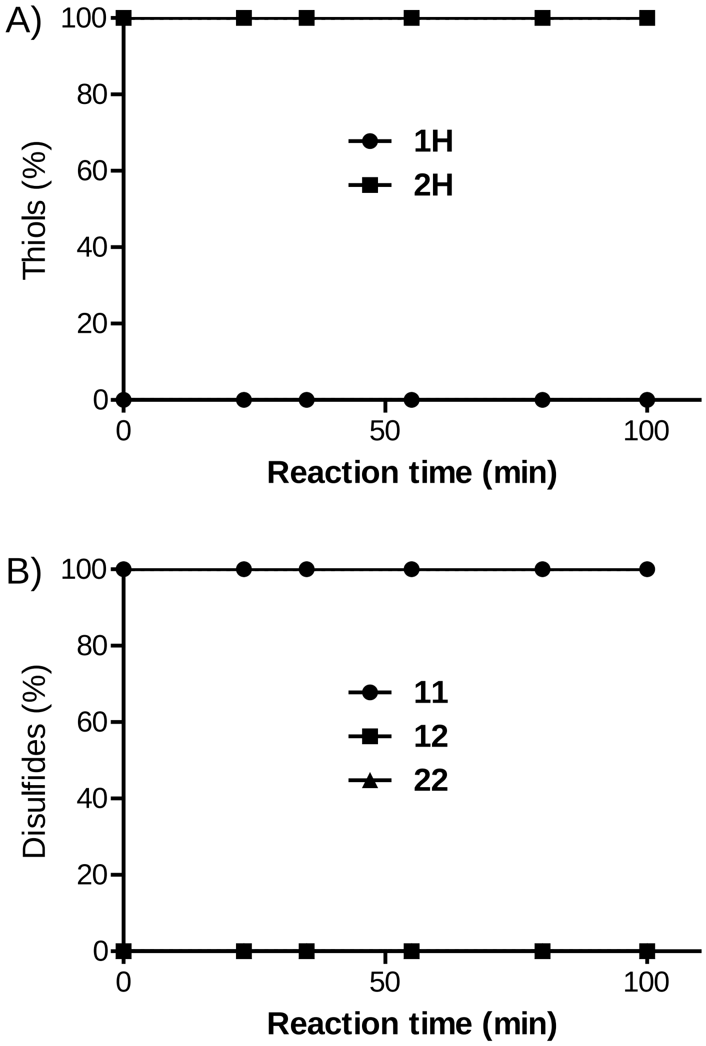


Figure S 19

Relative proportion of A) thiols and B) disulfides starting from **11** (15 µmol) and **2H** (30 µmol) in the presence of TFA (15 µmol). Argon atmosphere. Solvent: acetonitrile (total volume 500 µL). **1H** (%) = **1H*100/(1H + 2H)**; **2H** (%) = **2H*100/(1H + 2H)**; **11** (%) = **11*100/(11 + 12 + 22)**; **12** (%) = **12*100/(11 + 12 + 22)**; **22** (%) = **22*100/(11 + 12 + 22)**, as calculated in base of the HPLC peak area data (Abs$_{250}$).

4.5 Simultaneous disulfide and thioester exchanges:

4.5.1 Catalyzed by TEA (30 mM)

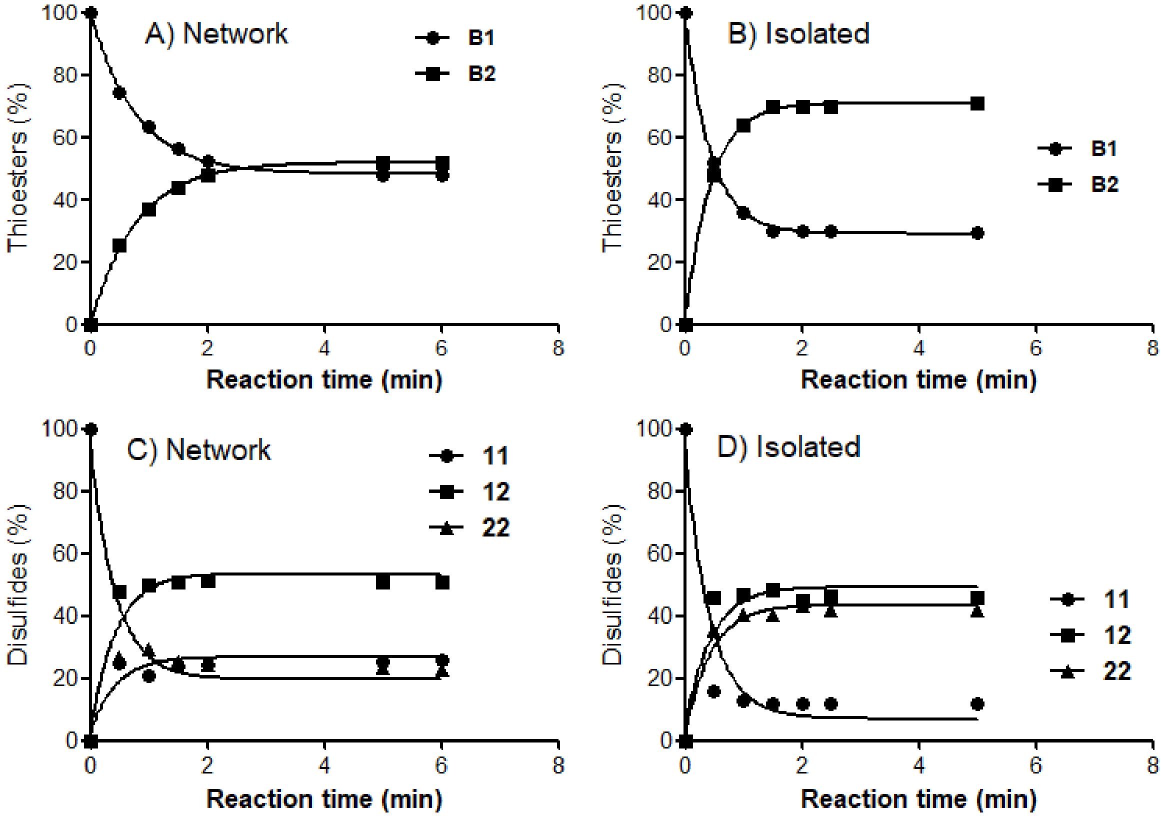


Figure S 20

Network and isolated disulfide and thioester exchanges. A) and C) Starting from **11** (15 µmol), **B1** (15 µmol) and **2H** (30 µmol). B) Starting from **B1** (15 µmol) and **2H** (30 µmol). D) Starting from **11** (15 µmol) and **2H** (30 µmol). All reactions were catalyzed by TEA (15 µmol) under Ar atmosphere. Solvent: acetonitrile (total volume 500 µL). **B1** (%) = **B1*100/(B1** + **B2)**; **B2** (%) = **B2*100/(B1** + **B2)**; **11** (%) = **11*100/(11** + **12** + **22)**; **12** (%) = **12*100/(11** + **12** + **22)**; **22** (%) = **22*100/(11** + **12** + **22)**, as calculated in base of the HPLC peak area data (Abs$_{250}$).

4.5.2 Catalyzed by $BuSnO_2H$ (30 mM)

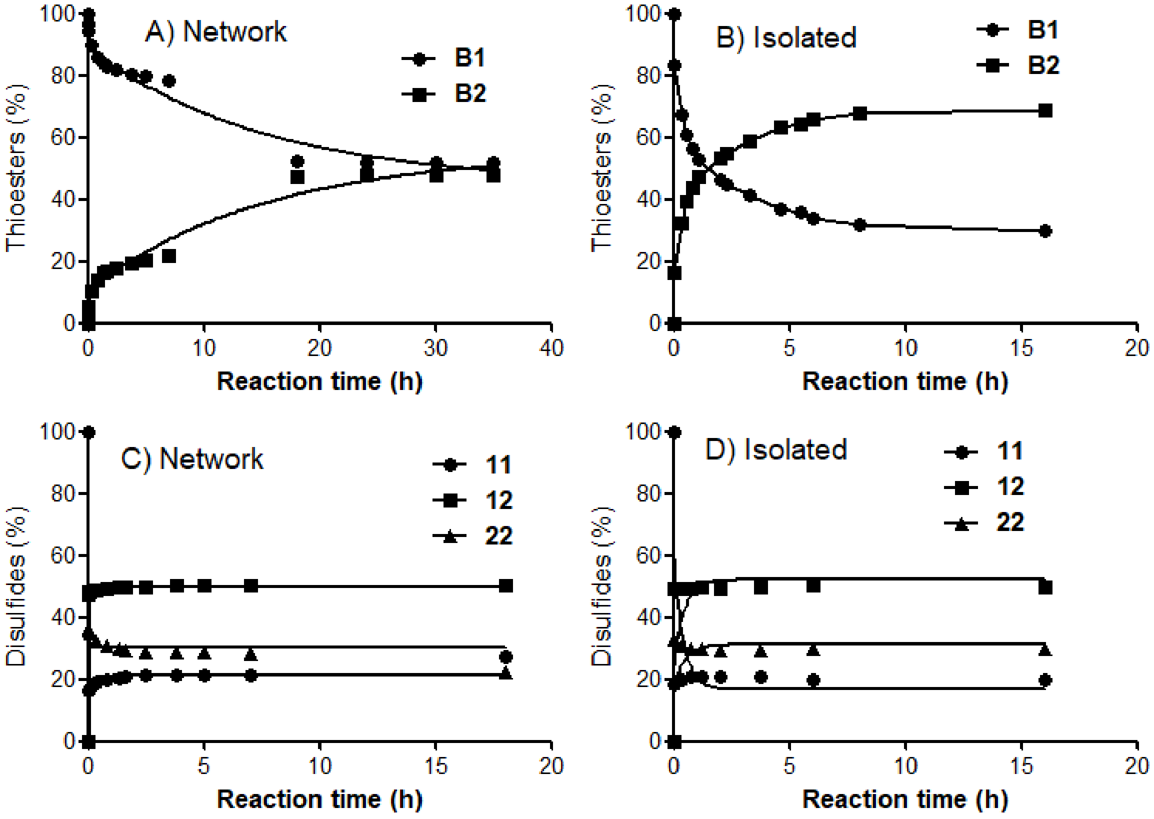


Figure S 21

Networked and isolated disulfide and thioester exchanges. A) and C) Starting from **11** (15 µmol), **B1** (15 µmol) and **2H** (30 µmol). B) Starting from **B1** (15 µmol) and **2H** (30 µmol). D) Starting from **11** (15 µmol) and **2H** (30 µmol). All reactions were catalyzed by $BuSnO_2H$ (15 µmol) under Ar atmosphere. Solvent: acetonitrile (total volume 500 µL). **B1** (%) = **B1*100/(B1 + B2)**; **B2** (%) = **B2*100/(B1 + B2)**; **11** (%) = **11*100/(11 + 12 + 22)**; **12** (%) = **12*100/(11 + 12 + 22)**; **22** (%) = **22*100/(11 + 12 + 22)**, as calculated in base of the HPLC peak area data (Abs_{250}).

4.6 Simultaneous disulfide and dithioacetal exchanges:

4.6.1 Catalyzed by $SnCl_2$ (1.5 mM) at 100°C

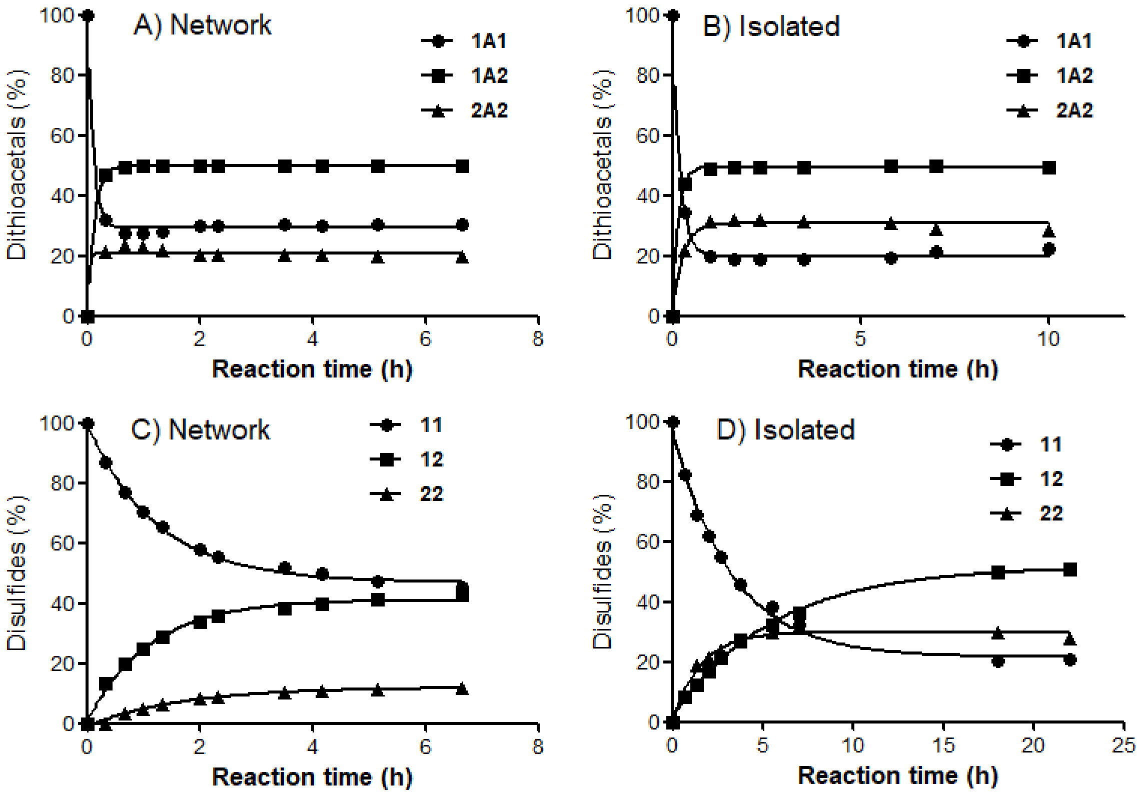


Figure S 22

Networked and isolated disulfide and dithioacetal exchanges. A) and C) Starting from **11** (15 µmol), **1A1** (15 µmol) and **2H** (30 µmol). B) Starting from **1A1** (15 µmol M) and **2H** (30 µmol). D) Starting from **11** (15 µmol) and **2H** (30 µmol). All reactions were catalyzed by $SnCl_2$ (0.75 µmol) at 100°C, under Ar atmosphere. Solvent: acetonitrile (total volume 500 µL).

5 Simulation of dynamic triple-level systems constructed through sequences I-V

Numerical simulations were carried out in order to predict the composition of the equilibrated libraries constructed with sequential reactions. The equilibrium concentrations were calculated with the steady state task of the software COPASI,[4] implementing the mass action (reversible) algorithm in the rate law section of the program.

5.1 *The model*

The implemented model involved the formation of dithioacetals **1A2** and **2A2** from **1A1** and thiol **2H** (eqs. 1 and 2), disulfides **12** and **22** from **11** and thiol **2H** (eqs. 3 and 4) as well as thioester **B2** from **B1** and **2H** (eq. 5).

$$\mathbf{1A1} + \mathbf{2H} \xrightleftharpoons[Kf_{-1A2}]{Kf_{1A2}} \mathbf{1A2} + \mathbf{1H} \tag{1}$$

$$\mathbf{1A1} + 2\,\mathbf{2H} \xrightleftharpoons[Kf_{-2A2}]{Kf_{2A2}} \mathbf{2A2} + 2\,\mathbf{1H} \tag{2}$$

$$\mathbf{11} + \mathbf{2H} \xrightleftharpoons[Kf_{-12}]{Kf_{12}} \mathbf{12} + \mathbf{1H} \tag{3}$$

$$\mathbf{11} + 2\,\mathbf{2H} \xrightleftharpoons[Kf_{-22}]{Kf_{22}} \mathbf{22} + 2\,\mathbf{1H} \tag{4}$$

$$\mathbf{B1} + \mathbf{2H} \xrightleftharpoons[Kf_{-B2}]{Kf_{B2}} \mathbf{B2} + \mathbf{1H} \tag{5}$$

5.2 *The parameter space*

5.2.1 Concentration of starting materials

In the first reaction of one sequence, the concentration of compounds was set to experimental values as follows: [**1A1**] = [**11**] = [**B1**] = 30 mM, [**2H**] = 60 mM, [**1A2**] = [**2A2**] = [**12**] = [**22**] = [**B2**] = [**1H**] = 0 mM. The equilibrium concentrations obtained as result of running the simulation of the first reaction were used as input for set the starting concentration of compounds in the subsequent reaction and son on.

5.2.2 Formation and reversion constants

In order to assume dynamic simulated systems composed by isoenergetic entities, the compositions were biased to obtain statistical distributions of compounds. The value of the constants was set as follows: the formation of the homocompounds **2A2** and **22**, as well as **B2**, were taken as a reference and their formation constants were set to unity, $Kf_{2A2} = Kf_{22} = Kf_{B2} = 1$, while the value of the formation constants of the heterocompounds **1A2** and **12** was double than the value of the formation constants of the homocompounds, $Kf_{1A2} = Kf_{12} = 2$. All the reversion constants $Kf_{-(\mathrm{compound})}$ were set to unity, i. e. $Kf_{-1A2} = Kf_{-12} = Kf_{-2A2} = Kf_{-22} = Kf_{-B2} = 1$.

5.3 Analysis of data

The results were processed grouping each compound in one of four pools: thiols **1H** and **2H**, dithioacetals **1A1**, **1A2** and **2A2**, disulfides **11**, **12** and **22**, and thioesters **B1** and **B2**. The proportion of each compound within each pool is showed below for each sequence in histogram figures.

5.4 *Results*

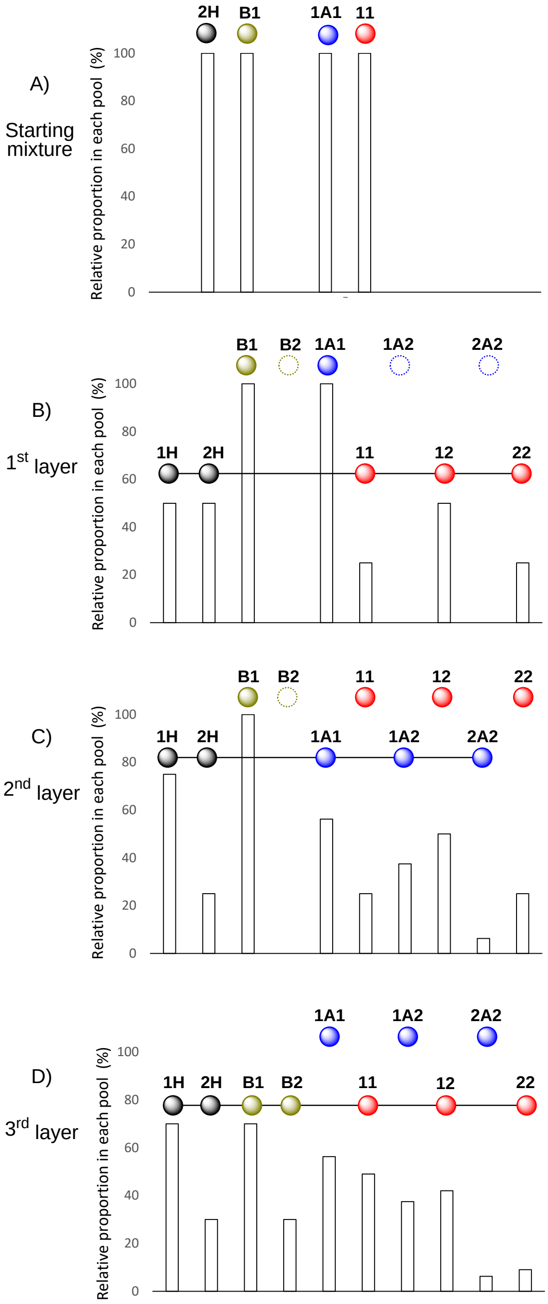


Figure S 23

Simulations showing the expected composition in an ideal system composed by isoenergetic compounds when the exchanges were activated through the sequence I. Histograms shows the relative composition obtained (A) in the starting point before to activating the first reaction, and after activate (B) 1° disulfide exchange, (C) 2° dithioacetal exchange and (D) 3° simultaneous disulfide and thioester exchanges.

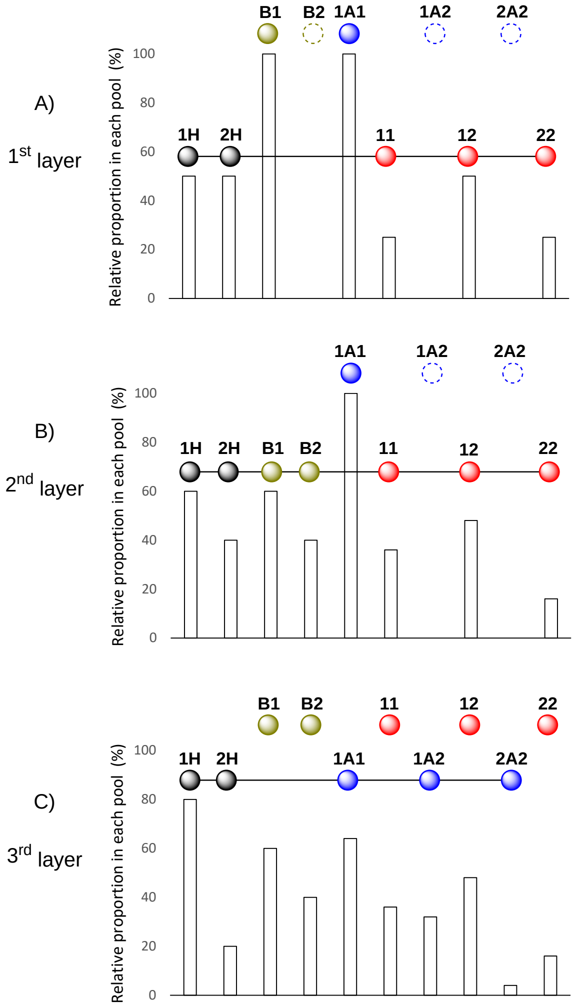


Figure S 24

Simulations showing the expected composition in an ideal system composed by isoenergetic compounds when the exchanges were activated through the sequence II. Histograms shows the relative composition obtained after activate (A) 1° disulfide exchange, (B) 2° simultaneous disulfide and thioester exchanges and (C) 3° dithioacetal exchange.

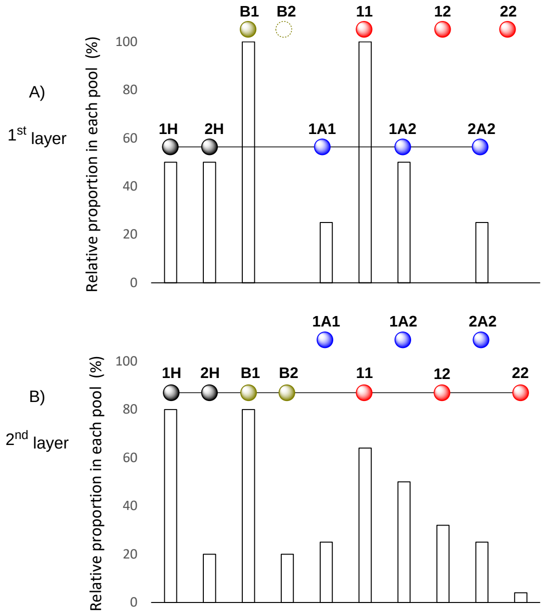


Figure S 25

Simulations showing the expected composition in an ideal system composed by isoenergetic compounds when the exchanges were activated through the sequence III. Histograms shows the relative composition obtained after activate (A) 1° dithioacetal exchange and (B) 2° simultaneous disulfide and thioester exchanges.

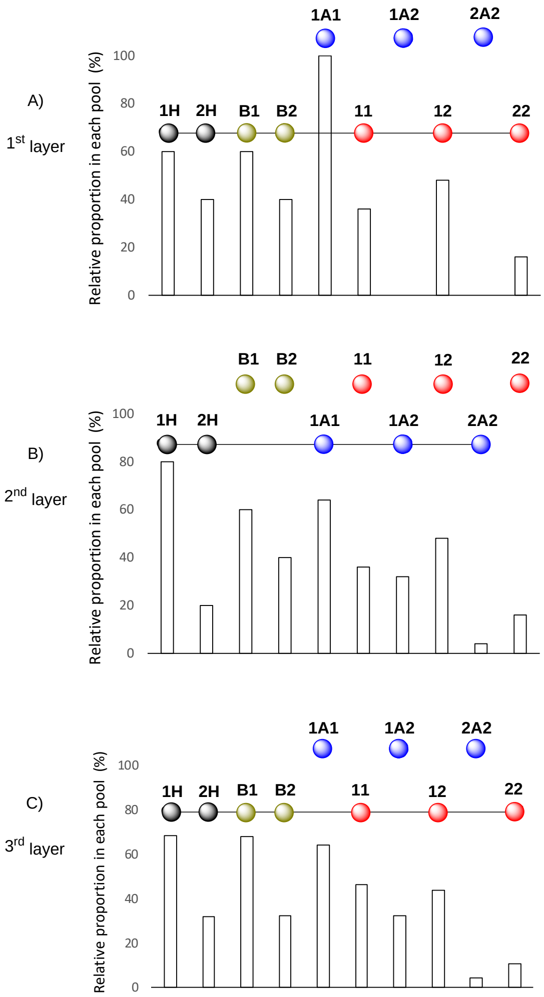


Figure S 26

Simulations showing the expected composition in an ideal system composed by isoenergetic compounds when the exchanges were activated through the sequence IV. Histograms shows the relative composition obtained after activate (A) 1° simultaneous disulfide and thioester exchanges, (B) 2° dithioacetal exchanges and (C) 3° simultaneous disulfide and thioester exchanges.

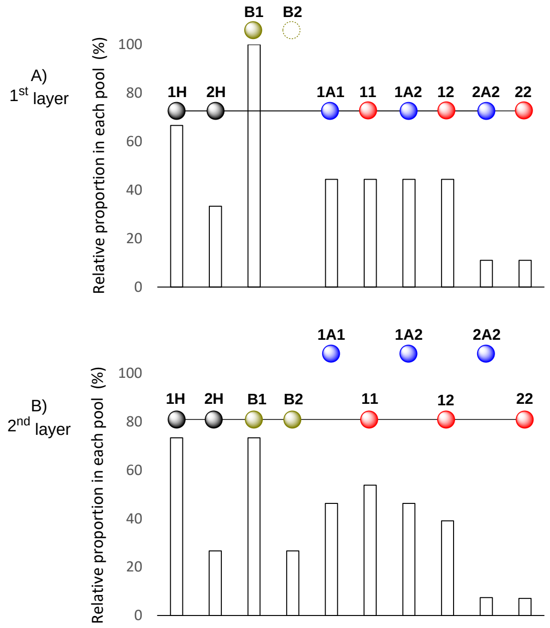


Figure S 27

Simulations showing the expected composition in an ideal system composed by isoenergetic compounds when the exchanges were activated through the sequence V. Histograms shows the relative composition obtained after activate (A) 1° simultaneous disulfide and dithioacetal exchanges and (B) 2° simultaneous disulfide and thioester exchanges.

6 References

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2 A. G. Orrillo, A. La-Venia, A. M. Escalante and R. L. E. Furlan, *Chem. - A Eur. J.*, 2018, **24**, 3141–3146.

3 R. A. Aitken, M. J. Drysdale and B. M. Ryan, *J. Chem. Soc. Perkin Trans. 1*, 1998, 3345–3348.

4 S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes and U. Kummer, *Bioinformatics*, 2006, **22**, 3067–3074.