Supporting information for:

Antiparallel Dynamic Covalent Chemistries

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1 Preparation of DCLs for general experiment

Table S1: Volumes (µL) of stock solutions used for preparation of DCLs.

| oxidation level | BC | A (ox.) | A (red.) |
|-----------------|----|---------|---------|
| 0%              | 50 | 0       | 50      |
| 10%             | 50 | 5       | 45      |
| 20%             | 50 | 10      | 40      |
| 30%             | 50 | 15      | 35      |
| 40%             | 50 | 20      | 30      |
| 50%             | 50 | 25      | 25      |
| 60%             | 50 | 30      | 20      |
| 70%             | 50 | 35      | 15      |
| 80%             | 50 | 40      | 10      |
| 90%             | 50 | 45      | 5       |
| 100%            | 50 | 50      | 0       |
2 UPLC/LC-MS analysis procedure

UPLC analyses (for all sets of experiments) were performed on Waters Acquity UPLC H-class systems equipped with a PDA detector. All analyses were performed using a reversed-phase UPLC column (Acquity UPLC BEH Phenyl, 1.7 µm, 2.1 × 150 mm). UV absorbance was monitored at 254 nm. Column temperature was kept at 35 °C. Injection volume was 2 µL.

UPLC-MS was performed using a Waters Acquity UPLC H-class system coupled to a Water Xevo-G2 TOF. The mass spectrometer was operated in negative electrospray ionization mode. Capillary, sampling cone, and extraction cone voltages were kept at 2.4 kV, 30 V, and 3 V, respectively. Source and desolvation temperatures were set at 150 °C and 500 °C, respectively. Nitrogen was used as both cone (5 L/h) and desolvation gas (800 L/h).

Table S2: UPLC method: eluent A: UPLC grade water (0.1 v% formic acid); eluent B: UPLC grade acetonitrile (0.1 v% formic acid).

| time (min) | flow (mL/min) | %A | %B |
|------------|---------------|----|----|
| 0          | 0.3           | 70 | 30 |
| 12         | 0.3           | 50 | 50 |
| 15         | 0.3           | 5  | 95 |
| 16         | 0.4           | 5  | 95 |
| 17         | 0.4           | 70 | 30 |
| 20         | 0.4           | 70 | 30 |
3 Suitability of UPLC-UV for quantitative analysis

Quantification of the system’s behavior was based on UPLC-UV analysis, and thus is strongly dependent on the reliability of such setup. To assure that in our particular system UPLC peak areas are directly proportional to the concentrations of individual library members, we prepared calibration curves for the thiols (A and B), the corresponding disulfides (An and B2), and the starting Michael acceptor (BC). For this purpose, 2.50 mM stock solutions of all mentioned building blocks were prepared in the same manner as for the general experiment (for details see experimental section in the main text). Next, experiments were set up at 5 different building block concentrations: 2.50, 1.25, 0.63, 0.32 and 0.16 mM, by diluting the corresponding stock solutions with borate buffer, and UPLC analysis was performed as aforementioned. Results of those experiments are listed in Table S3.

Table S3: UPLC peak areas (mAU·min) obtained in the calibration experiments.

| concentration [mM] | 2.50 | 1.25 | 0.63 | 0.32 | 0.16 |
|-------------------|------|------|------|------|------|
| A                 | 4310000 | 2112380 | 1030380 | 547300 | 228564 |
| An                | 3666760 | 1941170 | 901472 | 374415 | 153572 |
| BC                | 1235980 | 602258 | 296809 | 154689 | 79170 |
| B                 | 3104820 | 1653640 | 810218 | 383844 | 185857 |
| B2                | 3840300 | 1904720 | 956578 | 474659 | 223182 |

Those values were next plotted against the building block concentration (Figure S1), and a linear relationship was obtained between the concentration and the response of the UV detector for all library members that showed significant peak areas (Table S4).

Table S4: Results of the linear fit of UPLC peak areas as a function of the concentration.

|                        | A             | An            | BC            | B             | B2            |
|------------------------|---------------|---------------|---------------|---------------|---------------|
| Slope [mAU·min·mM⁻¹]   | 1734130       | 1509440       | 494253        | 1249890       | 1541050       |
| ΔSlope [mAU·min·mM⁻¹]  | 11679         | 42735         | 3927          | 30145         | 9953          |
| Intercept [mAU·min]    | -32900        | -53657        | -4655         | 17783         | -11843        |
| ΔIntercept [mAU·min]   | 15058         | 55102         | 5063          | 38869         | 12833         |
| Adj. R-Square          | 0.99982       | 0.99680       | 0.99975       | 0.99768       | 0.99983       |
Figure S1: UPLC peak areas as a function of the building block concentrations plotted for: 

- **a** all the calibration measurements,
- **b-f** separate building blocks.

As can be seen from these results, absorption coefficients of particular species are strongly dependent on the connectivity of the building blocks (thiol vs. disulfide vs. Michael acceptor; for the performed calibrations especially the last (BC) differs significantly from the corresponding thiol/disulfide pair (B/B₂)). These differences, combined with the complexity of
the resulting DCLs (containing altogether over 30 different compounds) prevented us from calculating the absolute concentrations of the library members.

On the other hand, we could still reliably *quantify concentration changes* for individual library members as a function of the oxidation level thanks to the linear relationship between the concentrations and peak areas of the compounds measured. To ensure that this linear relationship holds true for other library members (especially the mixed species), we performed another set of experiments. DCLs with oxidation level set to 0 or 50% were prepared in the same manner as for the general experiment (see the experimental section in the main text). Next, they were diluted two-, three-, or fourfold with borate buffer and analyzed by UPLC. Chromatograms in Figures S2-S5 show that the ratios of all compounds remain unaffected by the dilution, indicating that the response of the UPLC-UV detector is linear for all library members.

Figure S2: UPLC chromatograms of DCL made from A and BC at 0% oxidation level. From top to bottom: diluted four-, three-, twofold, undiluted. Note the different scales.
Figure S3: Expanded section of the UPLC chromatograms of DCL made from A and BC at 0% oxidation level. From top to bottom: diluted four-, three-, twofold, undiluted. Note the different scales.

Figure S4: UPLC chromatograms of DCL made from A and BC at 50% oxidation level. From top to bottom: diluted four-, three-, twofold, undiluted. Note the different scales.
Figure S5: Expanded section of the UPLC chromatograms of DCL made from A and BC at 50% oxidation level. From top to bottom: diluted four-, three-, twofold, undiluted. Note the different scales.
4 General experiment - UPLC analysis

Figure S6: UPLC chromatograms of DCLs made from A and BC at various oxidation levels.
Figure S7: Structures of all possible isomers of observed (by UPLC-MS) library members.
Figure S8: Relative peak area percentages of species observed in DCLs made from A and BC as a function of oxidation level.
5 Mass spectrometric analysis of the library members

Figure S9: Mass spectrum of B (retention time 3.43 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 153.01 [M-H]-; m/z observed: 153.13 [M-H]-.

Figure S10: Mass spectrum of BC (1) (retention time 3.64 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 221.04 [M-H]-; m/z observed: 221.12 [M-H]-.
Figure S11: Mass spectrum of BC (2) (retention time 3.77 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 221.04 [M-H]-; m/z observed: 221.12 [M-H]-.

Figure S12: Mass spectrum of A (retention time 5.02 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 184.98 [M-H]-; m/z observed: 185.09 [M-H]-.
Figure S13: Mass spectrum of **AC** (retention time 5.24 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 253.01 [M-H]⁻; m/z observed: 253.07 [M-H]⁻.

Figure S14: Mass spectrum of **CAC** (1) (retention time 5.44 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).
m/z calculated: 321.04 [M-H]⁻; m/z observed: 321.05 [M-H]⁻.
Figure S15: Mass spectrum of CAC (2) (retention time 5.56 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).
m/z calculated: 321.04 [M-H]⁻; m/z observed: 321.05 [M-H]⁻.

Figure S16: Mass spectrum of BCB (retention time 6.44 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 375.05 [M-H]⁻; m/z observed: 375.02 [M-H]⁻.
Figure S17: Mass spectrum of BCA (retention time 7.81 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized). m/z calculated: 407.02 [M-H]-; m/z observed: 406.96 [M-H]-.

Figure S18: Mass spectrum of BB (retention time 8.17 min) from the LC-MS analysis of a DCL made from A and BC (30% oxidized). m/z calculated: 305.02 [M-H]-; m/z observed: 305.03 [M-H]-.
Figure S19: Mass spectrum of $\text{A}_2\text{C}_2$ (1) (retention time 8.27 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 507.02 [M-H]$^-$; m/z observed: 506.88 [M-H]$^-$. 

Figure S20: Mass spectrum of $\text{A}_2\text{C}_2$ (2) (retention time 8.98 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 507.02 [M-H]$^-$; m/z observed: 506.87 [M-H]$^-$. 
Figure S21: Mass spectrum of $A_2C_2$ (3) (retention time 9.13 min) from the LC-MS analysis of a DCL made from A and BC (0% oxidized).
m/z calculated: 507.02 [M-H]; m/z observed: 506.87 [M-H].

Figure S22: Mass spectrum of BAC (retention time 9.71 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).
m/z calculated: 405.02 [M-H]; m/z observed: 404.95 [M-H].
Figure S23: Mass spectrum of **AB** (retention time 9.65 min) from the LC-MS analysis of a DCL made from **A** and **BC** (30% oxidized). 
m/z calculated: 336.99 [M-H]⁻; m/z observed: 336.98 [M-H]⁻.

Figure S24: Mass spectrum of **A₃C₃**(1) (retention time 10.20 min) from the LC-MS analysis of a DCL made from **A** and **BC** (0% oxidized). 
m/z calculated: 761.03 [M-H]⁻; m/z observed: 760.63 [M-H]⁻.
Figure S25: Mass spectrum of CAAC (1) (retention time 10.84 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized). m/z calculated: 505.02 [M-H]-; m/z observed: 504.86 [M-H]-.

Figure S26: Mass spectrum of CAAC (2) (retention time 10.94 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized). m/z calculated: 505.02 [M-H]-; m/z observed: 504.86 [M-H]-.
Figure S27: Mass spectrum of \textbf{BACB} (retention time 10.80 min) from the LC-MS analysis of a DCL made from \textbf{A} and \textbf{BC} (30\% oxidized).
m/z calculated: 559.03 [M-H]\textsuperscript{−}; m/z observed: 558.81 [M-H]\textsuperscript{−}.

Figure S28: Mass spectrum of \textbf{A}_3\textbf{C}_3 (2) (retention time 11.07 min) from the LC-MS analysis of a DCL made from \textbf{A} and \textbf{BC} (0\% oxidized).
m/z calculated: 761.03 [M-H]\textsuperscript{−}; m/z observed: 760.63 [M-H]\textsuperscript{−}. 

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Figure S29: Mass spectrum of $\text{A}_3\text{C}_2$ (1) (retention time 12.02 min) from the LC-MS analysis of a DCL made from A and BC (30% oxidized).
m/z calculated: 691.00 [M-H]⁻; m/z observed: 690.66 [M-H]⁻.

Figure S30: Mass spectrum of $\text{A}_3\text{C}_2$ (2) (retention time 12.21 min) from the LC-MS analysis of a DCL made from A and BC (30% oxidized).
m/z calculated: 691.00 [M-H]⁻; m/z observed: 690.65 [M-H]⁻.
Figure S31: Mass spectrum of $A_4C_3$ (retention time 12.27 min) from the LC-MS analysis of a DCL made from $A$ and BC (0% oxidized).

m/z calculated: 945.01 [M-H]$^-$; m/z observed: 944.41 [M-H]$^-$.

Figure S32: Mass spectrum of $BAB$ (retention time 12.77 min) from the LC-MS analysis of a DCL made from $A$ and BC (100% oxidized).

m/z calculated: 489.00 [M-H]$^-$; m/z observed: 488.84 [M-H]$^-$.
Figure S33: Mass spectrum of A₃C (retention time 12.63 min) from the LC-MS analysis of a DCL made from A and BC (30% oxidized).
m/z calculated: 620.97 [M-H]⁻; m/z observed: 620.69 [M-H]⁻.

Figure S34: Mass spectrum of BAAC (retention time 13.07 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).
m/z calculated: 589.00 [M-H]⁻; m/z observed: 588.75 [M-H]⁻.
Figure S35: Mass spectrum of **CAAAC** (retention time 13.37 min) from the LC-MS analysis of a DCL made from **A** and **BC** (100% oxidized).
m/z calculated: 689.00 [M-H]-; m/z observed: 688.65 [M-H]-.

Figure S36: Mass spectrum of **BAAB** (retention time 14.07 min) from the LC-MS analysis of a DCL made from **A** and **BC** (100% oxidized).
m/z calculated: 672.98 [M-H]-; m/z observed: 672.65 [M-H]-.
Figure S37: Mass spectrum of BAAAC (retention time 14.35 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).

m/z calculated: 772.98 [M-H]⁻; m/z observed: 772.53 [M-H]⁻.

Figure S38: Mass spectrum of A₄ (retention time 14.81 min) from the LC-MS analysis of a DCL made from A and BC (100% oxidized).

m/z calculated: 734.88 [M-H]⁻; m/z observed: 734.49 [M-H]⁻.
Figure S39: Mass spectrum of $A_3$ (retention time 15.15 min) from the LC-MS analysis of a DCL made from $A$ and $BC$ (100% oxidized). 
m/z calculated: 550.98 [M-H]$^-$; m/z observed: 550.72 [M-H]$^-$. 

Figure S40: Mass spectrum of $A_5$ (retention time 15.67 min) from the LC-MS analysis of a DCL made from $A$ and $BC$ (100% oxidized). 
m/z calculated: 918.85 [M-H]$^-$; m/z observed: 918.31 [M-H]$^-$. 

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6 Preparation of DCLs for redox experiments

Table S5: Volumes ($\mu$L) of stock solutions used for reduction experiments.

|        | A (ox.) + BC | DTT | TCEP | buffer |
|--------|--------------|-----|------|--------|
| 0% (DTT) | 50           | 5   | 0    | 0      |
| 0% (TCEP)| 50           | 0   | 5    | 0      |
| 30% (DTT) | 50           | 3.5 | 0    | 1.5    |
| 50% (DTT) | 50           | 2.5 | 0    | 2.5    |
| 50% (TCEP)| 50           | 0   | 2.5  | 2.5    |
| 70% (DTT) | 50           | 1.5 | 0    | 3.5    |

Table S6: Volumes ($\mu$L) of stock solutions used for oxidation experiments.

|        | A (red.) + BC | NaBO$_3$ | I$_3^-$ | buffer |
|--------|--------------|----------|---------|--------|
| 30% (NaBO$_3$) | 50           | 1.5      | 0       | 3.5    |
| 50% (NaBO$_3$) | 50           | 2.5      | 0       | 2.5    |
| 50% (I$_3^-$) | 50           | 0        | 2.5     | 2.5    |
| 70% (NaBO$_3$) | 50           | 3.5      | 0       | 1.5    |
| 100% (NaBO$_3$) | 50           | 5        | 0       | 0      |
| 100% (I$_3^-$) | 50           | 0        | 5       | 0      |

7 Redox experiments - UPLC analyses

Because of incomplete reduction/oxidation for extreme oxidation levels (0% and 100%), chromatograms of the DCLs at the corresponding oxidation levels obtained in the general experiment were shown as well for comparison.
Figure S41: Comparison of libraries at different intermediate oxidation levels (a 30%; b 50%; c 70%) obtained in the general experiment with libraries obtained in the redox experiments: by addition of reducing agents (TCEP or DTT) to 100% oxidized DCL; addition of oxidizing agents (I$_3^-$ or NaBO$_3$) to a 0% oxidized DCL. For peak assignments, see Figure S6.

Figure S42: Left: Comparison of libraries at extreme oxidation levels (a 0%; b 100%) obtained in the general experiment with libraries obtained in the redox experiments: by addition of reducing agents (TCEP or DTT) to 100% oxidized DCL; addition of oxidizing agents (I$_3^-$ or NaBO$_3$) to a 0% oxidized DCL. Right: comparison with DCLs obtained in the general experiment at oxidation levels close to extremes (a 20%; b 80%). For peak assignments, see Figure S6.
8 Preparation of DCLs for kinetics experiments

DCLs composed of equimolar amounts of A and BC were prepared as described for the previous experiments (see Experimental section in the article), with oxidation levels of the libraries set as 0%, 50% and 100%. UPLC analyses were done for each DCL 10 minutes, 2 hours, 6 hours, 12 hours, 24 hours, and 48 hours after preparation of the DCL. No major changes were observed in compositions of as-prepared DCLs after this time.

9 Kinetics experiments - UPLC analyses

Figure S43: UPLC chromatograms of DCLs made from A and BC. Chromatograms were obtained after (from bottom to top): 10 minutes, 2 hours, 6 hours, 12 hours, 24 hours, 48 hours. Analyses were performed for various oxidation levels: a 0%; b 50%; c 100%. For peak assignments, see Figure S6.
10 Preparation of DCLs for Michael acceptor D experiments

4.0 mM stock solution of D, 4.0 mM stock solution of A and 4.0 mM stock solution of NaBO₃ were prepared. Equal volumes of A and NaBO₃ were mixed to obtain 2.0 mM oxidized A, and left stirring for 3 hours before further use. Simultaneously, A was diluted twice with buffer solution to obtain 2.0 mM unoxidized A.

Libraries were prepared by mixing adequate volumes of D, reduced A, and oxidized A (as listed in Table S7). The volume of each library was 100 µL.

Table S7: Volumes (µL) of stock solutions used for preparation of DCLs for the Michael acceptor D experiments.

| oxidation level | D  | A (ox.) | A (red.) |
|-----------------|----|---------|---------|
| 0%              | 50 | 0       | 50      |
| 10%             | 50 | 5       | 45      |
| 20%             | 50 | 10      | 40      |
| 30%             | 50 | 15      | 35      |
| 40%             | 50 | 20      | 30      |
| 50%             | 50 | 25      | 25      |
| 60%             | 50 | 30      | 20      |
| 70%             | 50 | 35      | 15      |
| 80%             | 50 | 40      | 10      |
| 90%             | 50 | 45      | 5       |
| 100%            | 50 | 50      | 0       |
Figure S44: Building blocks and characteristic representatives of thio-Michael adducts (left), disulfides (right), and intermediate species (middle).
Figure S45: UPLC chromatograms of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark line) with 10% steps.
Figure S46: Expanded section of the UPLC chromatograms (showing D and A) of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark line) with 10% steps.

Figure S47: Expanded section of the UPLC chromatograms (showing AD) of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark line) with 10% steps.
Figure S48: Expanded section of the UPLC chromatograms (showing AA) of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark line) with 10% steps.

Figure S49: Expanded section of the UPLC chromatograms (showing A₄, DAD, AAD, A₃, DAAD, A₅ and DAAD) of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark line) with 10% steps.
Figure S50: Expanded section of the UPLC chromatograms (showing DAAD, A₅ and DAAAD) of DCLs made from A and D at various oxidation levels. Oxidation level goes from 0% (light blue line) to 100% (dark blue line) with 10% steps.
Michael acceptor D experiment - mass spectrometric analysis of the library members

Figure S51: Mass spectrum of A from the LC-MS analysis of a DCL made from A and D (50% oxidized).
m/z calculated: 184.97 [M-H]⁻; m/z observed: 184.77 [M-H]⁻.
Figure S52: Mass spectrum of AD from the LC-MS analysis of a DCL made from A and D (50% oxidized).
m/z calculated: 331.04 [M-H]⁻; m/z observed: 330.84 [M-H]⁻.

Figure S53: Mass spectrum of AA from the LC-MS analysis of a DCL made from A and D (50% oxidized).
m/z calculated: 368.94 [M-H]⁻; m/z observed: 368.92 [M-H]⁻.
Figure S54: Mass spectrum of A<sub>4</sub> from the LC-MS analysis of a DCL made from A and D (50% oxidized). 
m/z calculated: 734.85 [M-H]<sup>-</sup>; m/z observed: 734.83 [M-H]<sup>-</sup>. 
Figure S55: Mass spectrum of (coeluted) DAD and AAM from the LC-MS analysis of a DCL made from A and D (50% oxidized).

DAD m/z calculated: 477.12 [M-H]⁻; m/z observed: 476.93 [M-H]⁻;

AAD m/z calculated: 515.01 [M-H]⁻; m/z observed: 514.95 [M-H]⁻.
Figure S56: Mass spectrum of \( \text{A}_3 \) from the LC-MS analysis of a DCL made from \( \text{A} \) and \( \text{D} \) (50% oxidized).
m/z calculated: 550.89 [M-H]; m/z observed: 550.63 [M-H].

Figure S57: Mass spectrum of \( \text{DAAD} \) from the LC-MS analysis of a DCL made from \( \text{A} \) and \( \text{D} \) (50% oxidized).
m/z calculated: 661.08 [M-H]; m/z observed: 660.99 [M-H].
Figure S58: Mass spectrum of A₅ from the LC-MS analysis of a DCL made from A and D (50% oxidized).
m/z calculated: 918.82 [M-H]; m/z observed: 918.92 [M-H].

Figure S59: Mass spectrum of DAAAD from the LC-MS analysis of a DCL made from A and D (50% oxidized).
m/z calculated: 845.05 [M-H]; m/z observed: 845.07 [M-H].