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## Direct access to ligand-like sulfonamide libraries via acid-catalyzed sulfinamide crossover

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We introduce sulfinamide crossover as a new dynamic covalent reaction that rapidly generates ligand-like libraries via reversible S–N exchange under mild acid catalysis. The resulting mixtures can be oxidatively locked into medically relevant sulfonamides and directly screened by LC-MS.

Functional groups capable of a crossover (exchange) reaction form the basis of dynamic covalent chemistry (DCC).<sup>1–3</sup> Alkenes, disulfides, imines *etc.* have been extensively explored in the field for the discovery of new receptors,<sup>4–10</sup> materials,<sup>11–20</sup> catalysts,<sup>21–26</sup> and ligands for biomolecules.<sup>27–42</sup> The application of the crossover reaction for the efficient generation of libraries for ligand discovery is of particular interest. Dynamic or pre-equilibrated combinatorial libraries have served as a powerful tool for protein ligand discovery. However, wider adoption of the technology has been hampered by limited diversity of the libraries that can stem from availability of suitable substrates. Also, low ligand-like characteristics, limiting chemistry of the crossover functional groups, and analytical challenges of complex mixtures contributed to the slower progress and broader implementation of DCC in drug discovery. We envisioned that new functional groups capable of crossover reactions that are drug-like, structurally modular, synthetically accessible and compatible with affinity-based screening would overcome the above-mentioned limitations and create a truly general platform for protein ligand optimization and discovery. Here, we report the application of new sulfinamide crossover reaction for the straightforward assembly of ligand-like libraries from simple building blocks, and their use for protein ligand identification by means of affinity selection mass spectrometry (AS-MS).

Recently, we showed that sulfinamides undergo rapid and reversible crossover under mild acid catalysis, forming nearly equimolar mixtures of products while tolerating diverse structural features.<sup>43</sup> Also, mild oxidation of sulfinamides to sulfonamides freezes the dynamic nature of the libraries while providing a medically relevant functional group (Fig. 1a). Importantly, sulfinamide substrates are readily accessible in one-pot reactions from sulfonyl chlorides or thiols and primary and secondary amines.<sup>44–48</sup> An estimated several thousand sulfonyl chlorides or thiols and tens of thousands of primary and secondary amines are commercially available, providing

a rich feedstock for constructing structurally diverse libraries for ligand optimization and discovery. Furthermore, recent advances in sulfinamide synthesis continue to expand their accessibility,<sup>49–58</sup> facilitating the preparation of sulfoximines and sulfonimidamides, which are increasingly prominent scaffolds in medicinal chemistry.<sup>59–63</sup>

To evaluate the robustness of the sulfinamide crossover for library construction, a set of ten sulfinamides **AA–JJ** with drug-like characteristics was prepared for further experimentation (Fig. 1c). The synthesis of the sulfinamides was achieved through either reductive approach starting from sulfonyl chlorides or by oxidative pathway using thiols as a starting material (Fig. 1b). The S- and N-substituents were selected to avoid isobaric compounds in the resulting crossover library, thereby enabling straightforward identification of library members by HPLC-MS. However, such anisobaric design is not strictly required, as both sulfinamides and sulfonamides typically provide diagnostic fragments of S–N bond cleavage even at the MS<sup>1</sup> level. Compounds **DD'** and **EE'** were included because their substituents **D'** and **E** form the known sulfonamide inhibitor **ED'(o)** of a coagulation factor Xa. Also, a structural analog (**I'**) of the N-substituent **D'** with known lower inhibitor potency was included. We subjected sulfinamides **AA'–JJ'** to the crossover reaction and monitored the progress over time. Clearly, the crossover proceeded efficiently and within 4 hours a new library of sulfinamides was detected by HPLC-MS analysis. We also collected 2 h and 24 h timepoints. Comparison of the libraries at given times showed that there was still growth of intensity of a few new sulfinamides going from 2 to 4 hours. On the other hand, the 24 h library showed a slight decline of some signals observed that indicated possible slow decomposition of the library (Fig. S1). These experiments indicated that the 4 h library reached the equilibrium state. Out of 100 theoretically accessible crossover products, we detected 96 compounds by an automated mass search protocol (Fig. 2b). The four undetected crossover products all contain 3,5-difluorophenyl S-substituent (**I**), which we attribute to suppressed ESI ionization due to reduced polarity or altered proton affinity.<sup>64</sup> Nevertheless, the appearance of the corresponding N-substituent **I'** in all other

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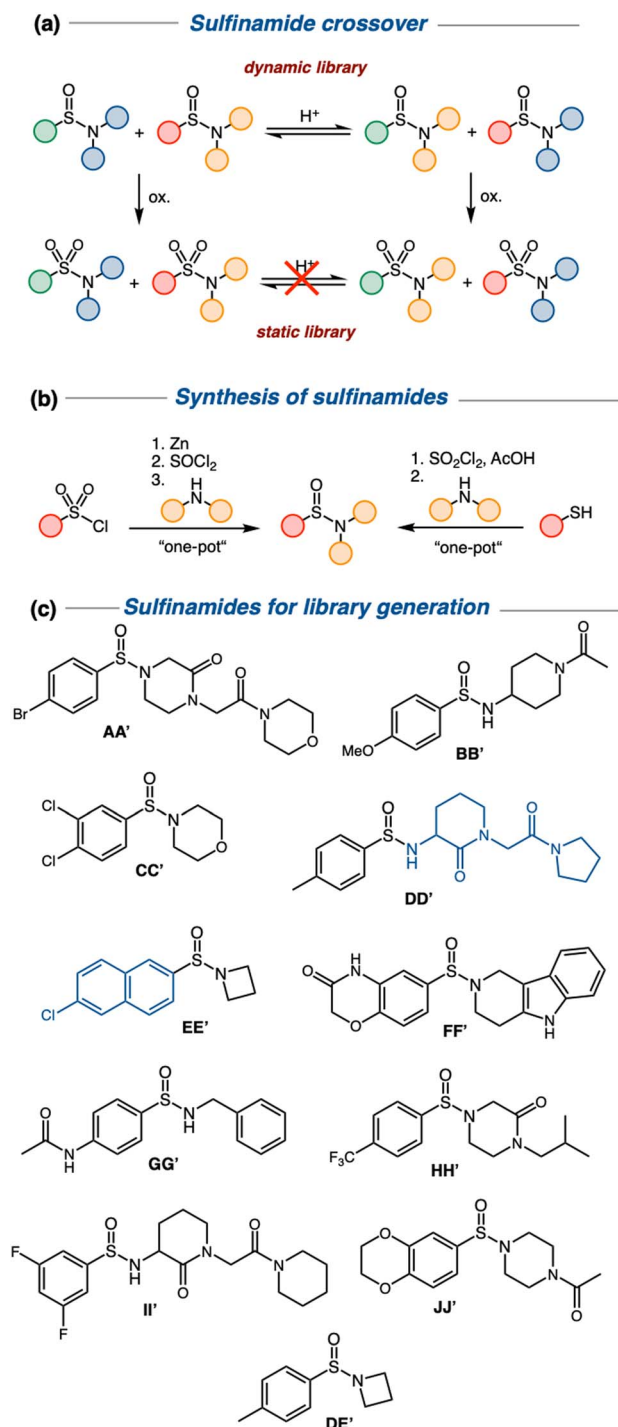


Fig. 1 (a) A scheme showing dynamic nature of sulfenamides under acidic condition and its cessation by oxidation. (b) Methods used for the preparation of sulfenamides. (c) Sulfenamides used for the preparation of libraries by the crossover reaction. Substituents marked in blue form a reported factor Xa sulfonamide inhibitor ED'(o).

crossover products confirms that its precursor underwent the crossover. In general, sulfenamides with electron withdrawing aromatic S-substituents provide signals of lower intensity that is more dependent on the N-substitution. Contrary to that electron donating aromatic S-substituents gave relatively strong signal less dependent on the nature of the N-substituent. The

extracted ion intensities of the compounds in the library span the range of several orders of magnitude, nevertheless even the lower intensity products remained readily detectable.

To further explore the crossover platform, we prepared two additional libraries (B and C) by replacing the starting ligands DD' or EE' with the mixed sulfenamide DE'. In these designs, either the N- or S-substituent present in the known factor Xa inhibitor DE'(o) is omitted and thus enable a further systematic probing of structural elements in the resulting libraries. The crossover reaction provided corresponding libraries with the expected missing analogs, which confirmed both the crossover reaction functionality and an automated mass search protocol robustness (Fig. 2c and d). During the development phase of the crossover reaction, we had synthesized all possible crossover products individually to quantify the molar ratios of the resulting sulfenamides. However, as the library complexity increased, this approach became impractical. Nevertheless, the presence of strong MS signals for the crossover products after the reaction (and their absence beforehand) shows the efficiency of the process and the applicability of the resulting libraries for MS-based affinity selections. To test the utility of the crossover libraries for the protein ligand development, we have oxidized the generated libraries A, B and C with mCPBA to convert sulfenamides to sulfonamides to provide the reported sulfonamide inhibitor ED'(o). HPLC-MS analysis of the oxidized library A showed the presence of 75 out of 100 accessible sulfonamides (Fig. S2–S4). Detailed analysis indicated that particularly ligands with electron rich indole substituent F' were missing in the library. The indole moiety has been described to undergo oxidative modification by mCPBA.<sup>65</sup> Given the excess of the oxidant in the reaction, analogs with the F' substituent were probably oxidatively decomposed. Also, substrates with morpholine substituent C' gave weak signals. As we have observed previously, generally sulfenamides provide stronger signals than corresponding sulfonamides using ESI-MS detection technique. Since sulfenamides with morpholine substituent C' provided detectable but low intensity signals, it is suggested that oxidation pushed these compounds below the detection limit of ESI-MS. Despite these limitations, these experiments indicated that this approach is appropriate for the efficient preparation of sulfenamide libraries with formidable structural diversity.

To demonstrate the utility of the crossover libraries for the protein ligand identification, we selected coagulation factor Xa (FXa) as a model target. Factor Xa is a protease in a coagulation cascade that is a major target for prevention and treatment of blood clots.<sup>66</sup> Compounds DD' and EE' in the original library contain substituent D' and E that form a known sulfonamide inhibitor ED'(o) of factor Xa.<sup>67</sup> We utilized three sulfenamide libraries A(o), B(o) and C(o) for affinity selection mass spectrometry against a coagulation factor Xa (Fig. 3a). The blank experiment contained bovine serum albumin (BSA) instead of factor Xa. The sulfenamide crossover libraries (~100  $\mu$ M total) were incubated with a free FXa (1  $\mu$ M) followed by ultrafiltration step using 10 kDa spin filter columns and ligand elution with methanol. The elution mixture was then analyzed by LC-MS and compared to a blank experiment. The results are summarized in volcano plots in Fig. 3. Screening the library A(o) yielded two



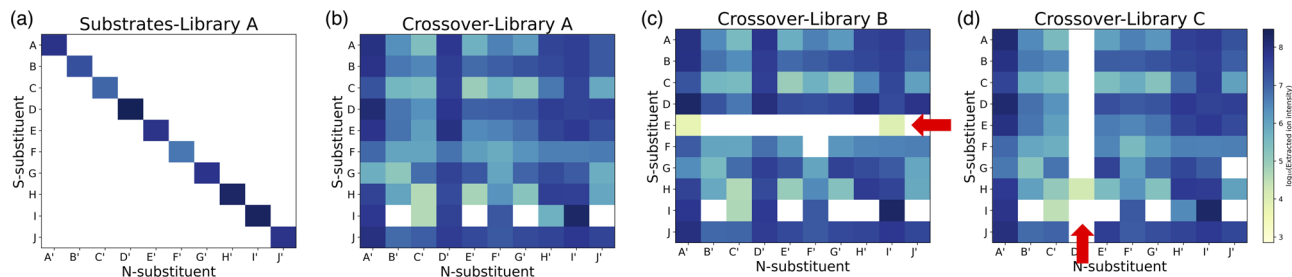


Fig. 2 Heatmap plots showing extracted ion intensities of compounds in (a) substrates for library A, (b) crossover reaction leading to library A, (c) and (d) crossover reactions leading to library B and C. The red arrows indicate the expected missing sulfonamides in the library B and C. Extracted ion intensities are averaged from duplicates.

significant binders: the expected inhibitor **ED'(o)** and the structurally related compound **EI'(o)** containing piperidine ring instead of pyrrolidine moiety. As mentioned above, the compound **EI'(o)** is also a known factor Xa inhibitor, albeit with reduced potency ( $IC_{50}$  32 nM vs. 120 nM).<sup>67</sup> Next, we tested libraries B(o) and C(o) that are missing chloronaphthyl S-substituent E and pyrrolidine N-substituent D' respectively. Screening of library B(o) did not yield statistically significant enrichments. In contrast, library C(o) again provided **EI'(o)** as the dominant enriched binder. Although no individual hits emerged from library B(o), inspection of the enrichment distribution reveals bias toward compounds containing N-

substituent D' or I', mirroring the structural trends observed in library A(o) and C(o) (Fig. 3c). The enriched compound **AD'(o)** has also been reported to form FXa inhibitor, however with  $IC_{50}$  in midmicromolar range.<sup>67</sup> Also, two compounds with N-substituent H' showed bias towards FXa (**BH'(o)** and **AH'(o)**). To validate these observations, we synthesized the significant hits **ED'(o)** and **EI'(o)**, as well as compounds **AD'(o)** and **BH'(o)** biased towards FXa in library B(o) and compound **FJ'(o)** that has not showed any enrichment towards FXa in any library. The inhibition assay against FXa using these compounds corroborated the AS-MS results. **ED'(o)** and **EI'(o)** displayed  $IC_{50}$  values of 82 nM and 300 nM, respectively (Fig. 3e). These values are

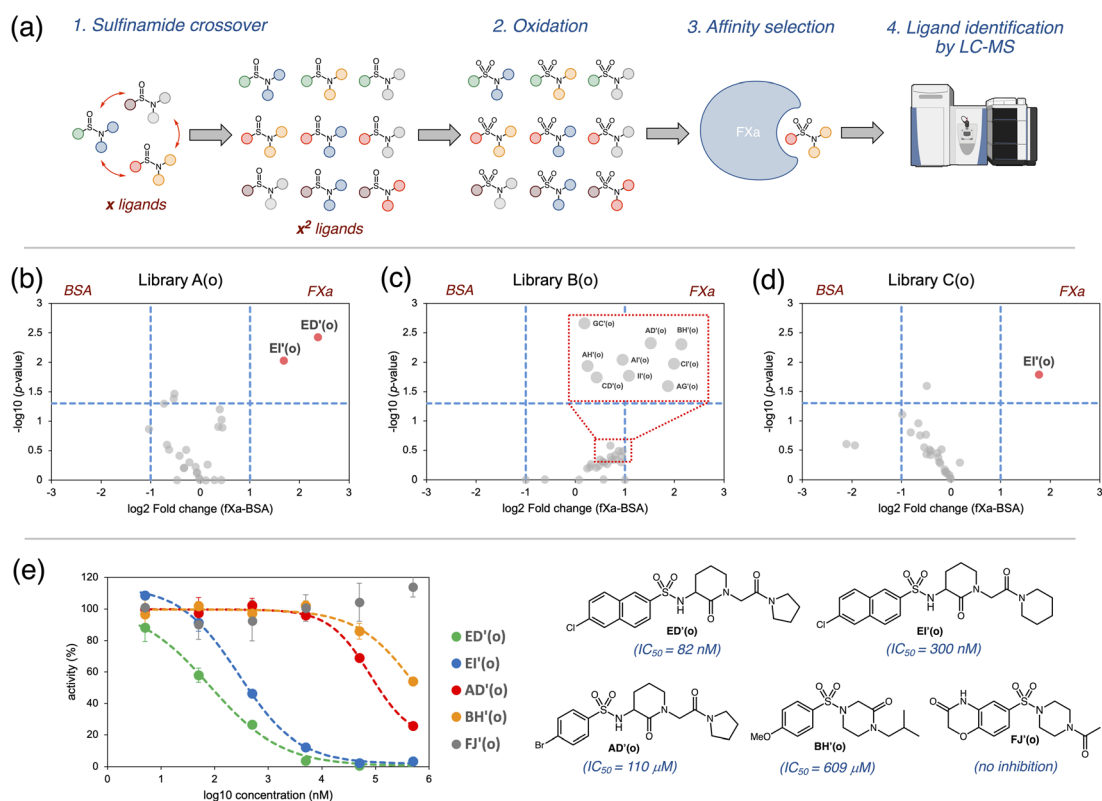


Fig. 3 (a) A schematic representation of the workflow of AS-MS utilizing sulfonamide crossover reaction. (b–d) Volcano plots showing enrichment of ligands to FXa compared to a blank with BSA. Significant hits (red) were defined by false discovery rate (FDR)-adjusted  $p < 0.05$  and  $\log_2$  fold change  $\geq 1$ .  $\sim 100 \mu\text{M}$  ligand library (total) was incubated with  $1 \mu\text{M}$  FXa or BSA. (b) Library A(o). (c) Library B(o). (d) Library C(o). (e) Dose-response curves for selected sulfonamides showing the inhibition of FXa, their structures and  $IC_{50}$  values.



slightly higher than the literature data, which might be reflecting the fact that we worked with racemic compounds whereas the reported values are for optically pure variants. Compounds **AD'(o)** and **BH'(o)** also showed weak inhibition with IC<sub>50</sub> values in high micromolar range. Interestingly, compound **BH'(o)** has not been reported in the literature. Expectedly, compound **FJ'(o)** showed no inhibition of FXa in the tested concentration range. Overall, the screening results demonstrate that the assembled libraries can faithfully reflect ligand–protein interactions in AS-MS enrichment patterns, enabling both confirmation of known pharmacophores and identification of new ligand variants. We note that the objective of this study was not to optimize factor Xa inhibitors, but rather to establish the feasibility of combining sulfinamide crossover chemistry with AS-MS to enable ligand development. Accordingly, the results should be viewed as a proof-of-principle demonstration of the workflow rather than a structure-guided optimization campaign.

Taken together, these results demonstrate that the sulfinamide crossover reaction can be utilized for the straightforward preparation of structurally diverse ligand-like libraries that can be directly screened for protein ligands using AS-MS. These proof-of-principle studies confirm both the chemical robustness of the crossover platform and its functional utility in ligand development, establishing a foundation for broader application of the sulfinamide crossover strategy in discovery chemistry. Importantly, the modular nature of sulfinamides allows their post-synthetic conversion to sulfoximines and sulfonimidamides and thus the sulfinamide crossover potentially provides an efficient gateway to new pharmacologically relevant 3D sulfoximines architectures.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d6ra00651e>.

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