A Reactive Fragment Screening Platform Utilising Sulfur(VI)–Fluoride Moieties for the Efficient Discovery of Ligands and Tools

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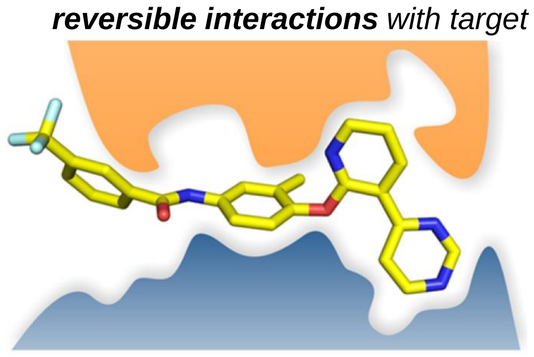
1. What are reactive fragments?

Traditional Screening

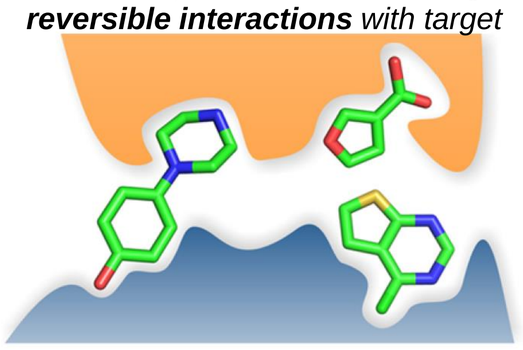
Elaborated structures forming ***reversible interactions*** *with target*



✔ Detectable hits
✘ Inefficient interactions
✘ Challenging to optimise

Fragment Screening[1]

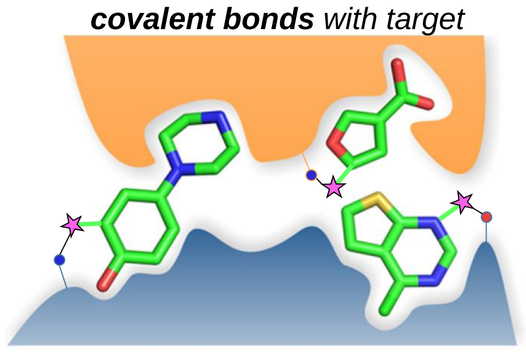
Less complex structures forming ***reversible interactions*** *with target*



✘ Challenging to detect hits
✔ Efficient interactions
✔ Easier to optimise

Reactive Fragment Screening

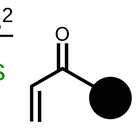
Less complex structures form ***covalent bonds*** *with target*



✔ Detectable hits
✔ Efficient interactions
✔ Easier to optimise

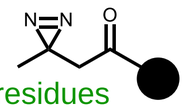
Current established strategies to ***reactive fragment screening****:*

Cysteine-targeting groups[2]



+) High crosslinking yields
–) Limited by the rarity of cysteine in the proteome

Photoaffinity labelling[3]

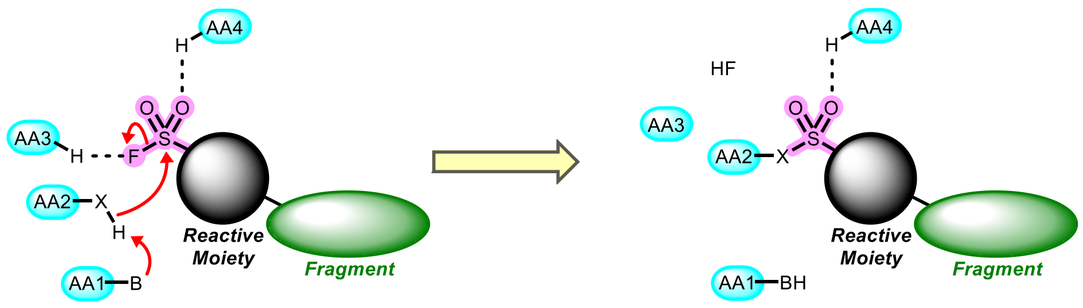


+) Applicable to all AA residues
–) Low crosslinking yields

2. Why are we excited about sulfur(VI)–fluorides?

Aim of my PhD research:

Establish a protocol for generating sulfur(VI)–fluoride (SF) reactive fragment libraries and demonstrate their applicability to proteins of therapeutic interest.

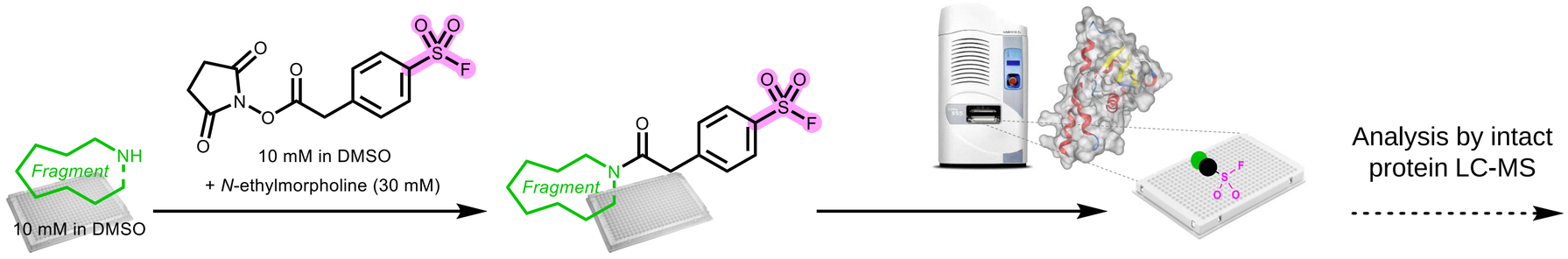


Sulfur(VI)–Fluorides[4]
(target: Lys; Tyr; Ser; Thr; His)

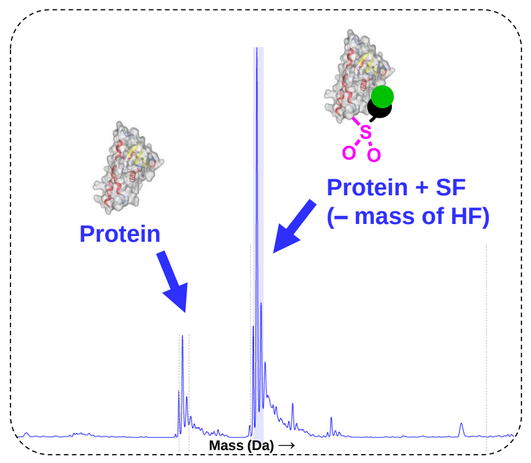
+) High crosslinking yields
+) Applicable to all nucleophilic AA residues

Sulfur(VI)–fluorides offer a nice 'sweet spot' between current established strategies.

3. High-Throughput Chemistry and Direct-To-Biology (HTC-D2B) Approach Established for Reactive Fragment Screening

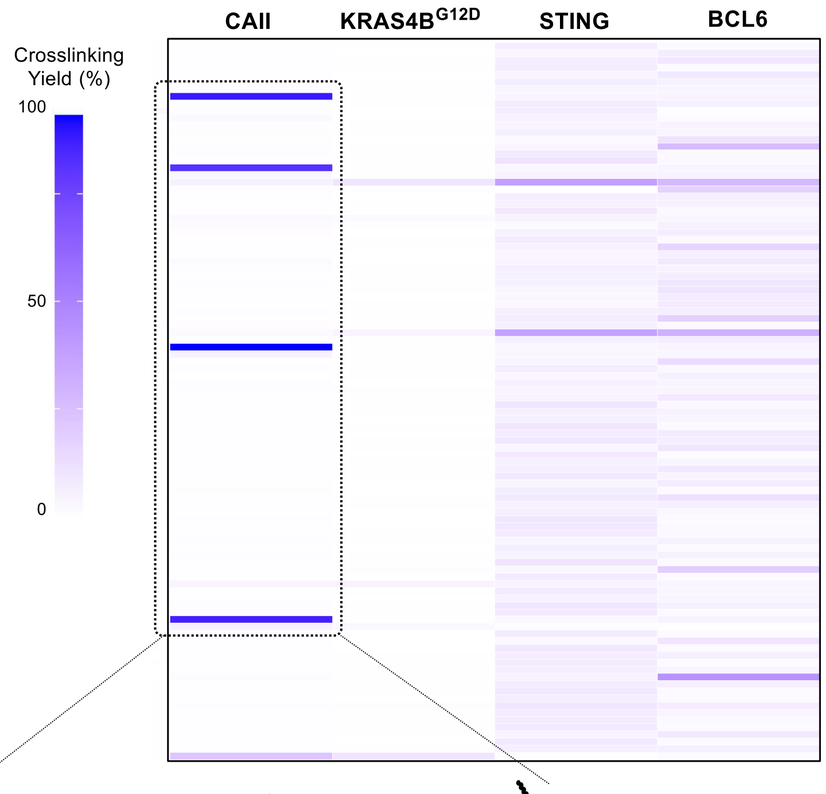


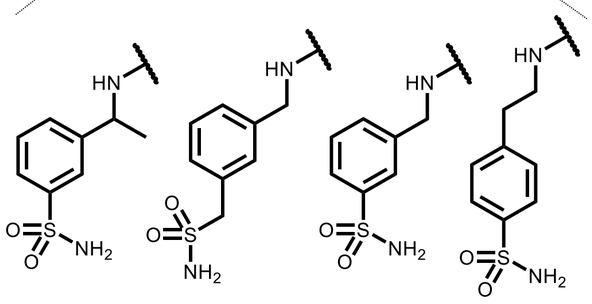
Exemplar mass spectrum of hit SF vs CAII



- HTC: Diverse set of amine-functionalised fragments (10^2–10^3) coupled to reactive moiety in a plate-based format
- No requirement for purification of the reactive fragment library
- D2B: Crude reaction mixtures directly screened against recombinant proteins of therapeutic interest
- Reaction duration: 1 h; Incubation duration: 24 h; analysis duration: 12 h. **SF library generated and screened in <2 days**

4. HTC-D2B Approach vs Four Oncology Targets

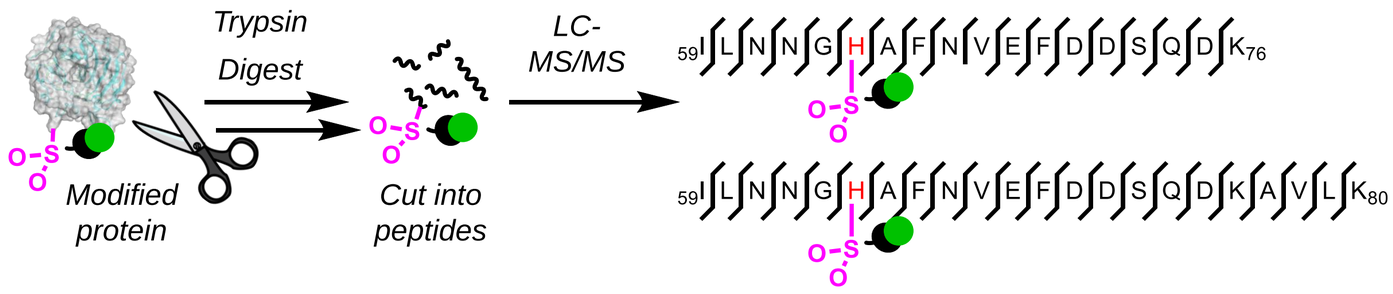




Four high-labelling hits identified against CAII; all contain a **sulfonamide** motif

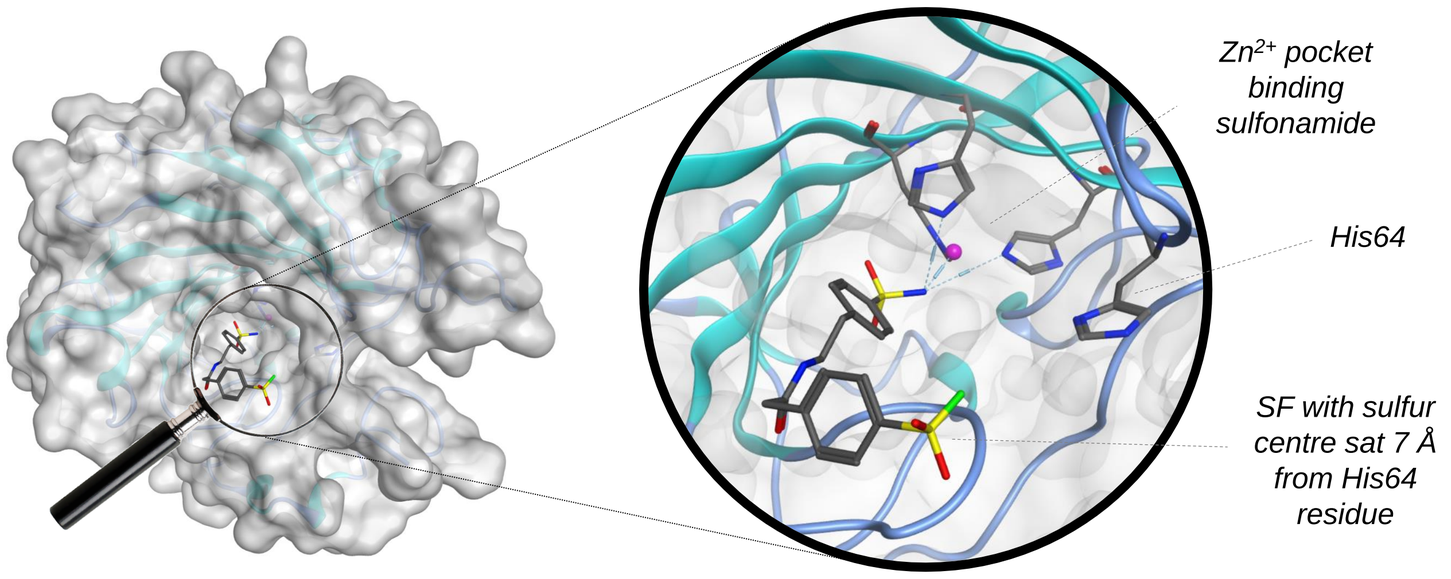
5. His64 identified as major site of modification for SF hits vs CAII

The amino acid residue(s) responsible for crosslinking to these SFs were investigated using **LC-MS/MS**:



His64 identified as major site of crosslinking for hit SFs vs CAII.

Site of modification further rationalised through **crystallography** and virtual **docking** of SF:



6. Conclusions

These results demonstrate the applicability of **reactive fragment screening with sulfur(VI) fluoride moieties** for use in the early stages of drug discovery. It is anticipated that this screening platform will be used in parallel with alternative methods of **ligand/tool discovery** for emerging targets, such as DNA-encoded libraries, and affinity selection mass spectrometry. This will lead to the development of **chemical probes** for the study of diseases and provide starting points for the development of **novel medicines**.[5,6]

References: 1) *Biochem.*, **2012**, *51*, 4990–5003. 2) *J. Am. Chem. Soc.*, **2019**, *141*, 2703–2712. 3) *Angew. Chem. Int. Ed.*, **2020**, *59*, 21096–21106. 4) *Chem. Sci.*, **2015**, *6*, 2650–2659. 5) *J. Am. Chem. Soc.*, **2019**, *141*, 8951–8968. 6) *Chem. Sci.*, **2021**, 12, 12098–12106.

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