Identification of novel antibiotic targets using covalent inhibitors and residue-specific proteomics

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Background

The antibiotic crisis[1]

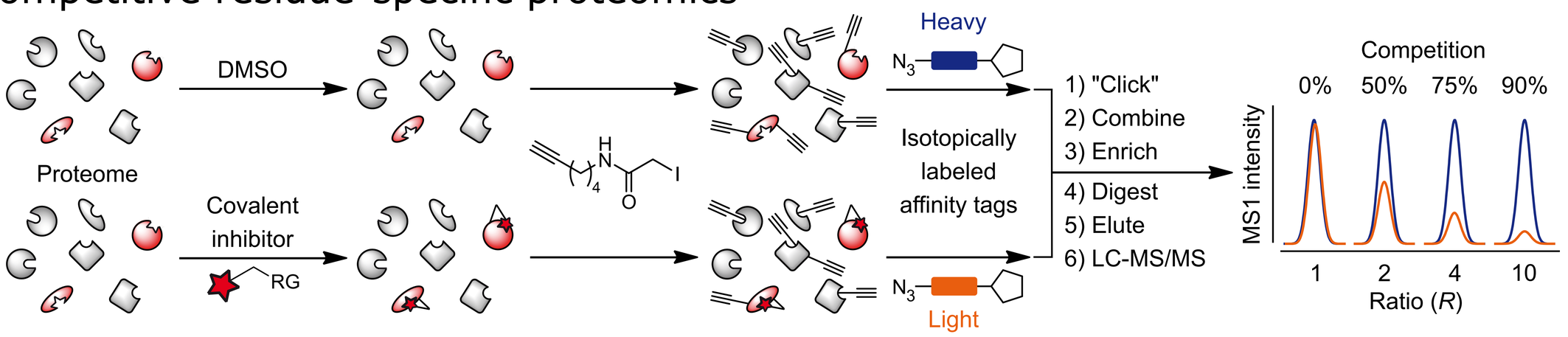
- resistances to all marketed antibiotics
- increasing levels of multi-resistant strains
- declining effectiveness of antibiotic treatment
- antibiotics only target a limited set of pathways
- innovation gap in antibiotic development

Covalent inhibitors[2]

- prevalent as antibiotics (*e.g.* β-lactams)
- increased selectivity and potency
- potentially less prone to resistance development

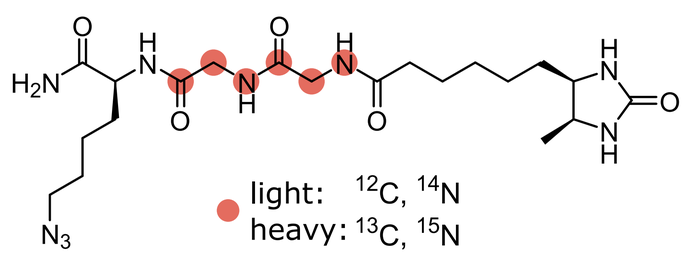
Our approach: identifying new druggable binding sites using covalent inhibitors and proteomic profiling

Competitive residue-specific proteomics[3,4]



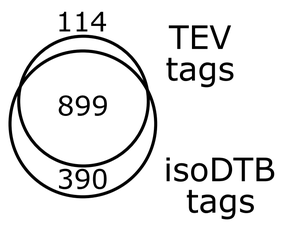
- target identification for unmodified covalent inhibitors
- identification of the exact binding site
- global profiling of ligandable sites with fragments
- quantification of occupancy and affinity

isoDTB tags[4] – smaller, better, faster



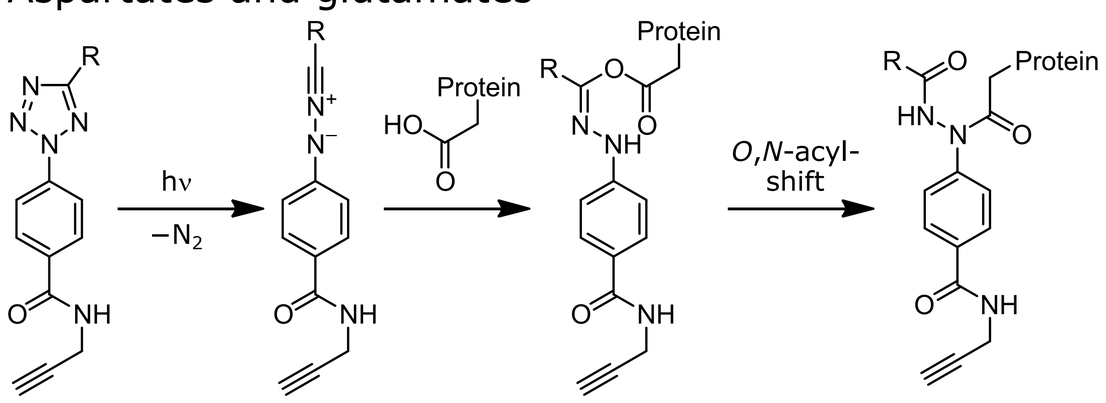
Compared to traditional TEV tags:

- easier synthesis
- shorter workflow
- higher coverage in bacteria
- compatible with non-trypsin digestion

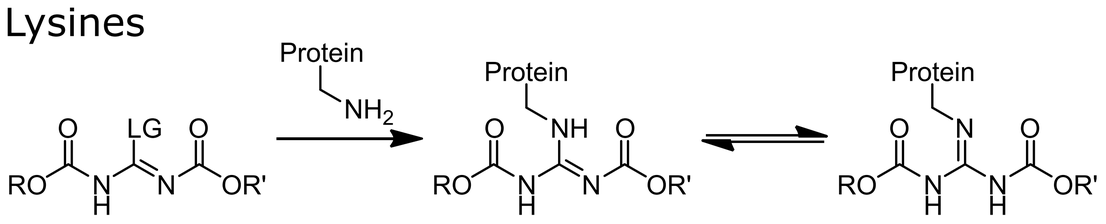


Beyond cysteine

Aspartates and glutamates[5]

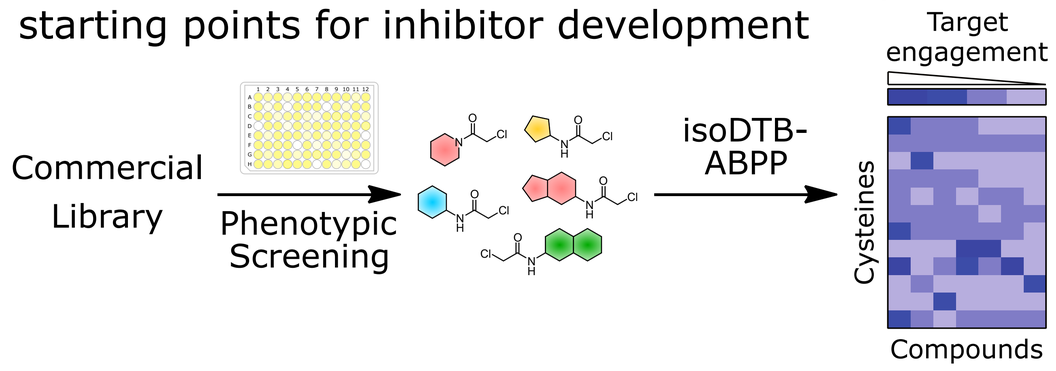


Lysines



Cysteine profiling in bacteria[4]

- cysteine reactivity correlates with functionality
- combination of phenotypic screening and isoDTB-ABPP to identify new antibacterial targets and starting points for inhibitor development



- identification of 268 binding sites on 200 proteins
- **EN106** inhibits HMG-CoA synthase

Conclusion

- isoDTB-ABPP platform allows efficient proteome-wide identification of binding sites of covalent inhibitors
- chemoselective probes for specific nucleophilic amino acids will expand the known druggable proteome and open up new antibiotic strategies

[1] M. Lakemeyer *et al.*, *Angew. Chem. Int. Ed.* **2018**, *57*, 14440-14475.
[2] R. A. Bauer, *Drug Discov. Today* **2015**, *20*, 1061–1073.
[3] K. M. Backus *et al.*, *Nature* **2016**, *534*, 570-574.
[4] P. R. A. Zanon, L. Lewald, S. M. Hacker, *Angew. Chem. Int. Ed.* **2020**, *59*, 2829-2836.
[5] K. Bach, B. L. H. Beerkens, P. R. A. Zanon, S. M. Hacker, *ChemRxiv* **2019**, doi: 10.26434/chemrxiv.11352101.

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