Title: Covalent fragments - Not as reactive as you thought.

A key problem in the discovery of small molecule modulators of biological functions is the sheer size of chemical space. One solution to this problem is to focus on screening fragments. Since chemical space grows exponentially as a function of compound size, the sub-space of very small chemical fragments (molecules with up to ~20 heavy atoms) can be much more efficiently covered by a relatively small and diverse library of fragments. Screening fragments however imposes its own challenges, chiefly, the requirement for sensitive assays due to the low affinity of typical fragment hits. Fragments that are able to form a covalent bond with their target protein can overcome this challenge by leveraging the potency of the newly created bond. However, such electrophilic fragments were considered to be non-selective and little to no screening was performed in this space. We have culled a library of 993 electrophilic fragments and have characterized them both by screening a wide panel of cysteine containing proteins, as well as by a new high-throughtput method for evaluation of thiol reactivity. Overall, highly reactive fragments are scarce on the one hand, and can be easily weeded out on the other. We were able to find quality hits for several of the screened proteins and to develop them to more potent and selective probes. Ongoing studies are currently adapting this screening approach to cellular phenotypic screening in which the formation of a covalent bond will further assist in downstream target identification. This study brings to the fore covalent fragment screening as a practical and efficient tool for ligand discovery that we anticipate will be quickly gain traction in chemical biology and drug discovery.