Title	**Multi-protein Dynamic Combinatorial Chemistry: A Novel Strategy that Leads to Simultaneous Discovery of Subfamily-selective Inhibitors against Nucleic Acid Demethylases FTO and ALKBH3**
Keywords (up to 5)	Dynamic combinatorial chemistry, differential scanning fluorimetry, self-assembly, thermal tag, demethylase.
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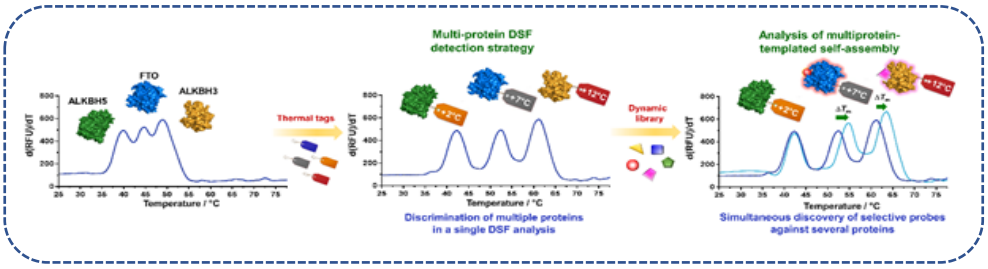
Abstract	Dynamic combinatorial chemistry (DCC) is a powerful supramolecular approach for discovering ligands for biomolecules.[1,2] It is currently of tremendous chemical and biological interest. To date, most, if not all, biologically-templated DCC employ only a single biomolecule in directing the self-assembly process, which severely limits the scope and potential of DCC. Herein, we explored the concept of multi-protein DCC, where two or more target proteins could be used concurrently in the same dynamic system. We envisaged that this will greatly multiply the power and efficiency of DCC. However, such a multi-protein system is extremely challenging to analyse, in part due to the constantly changing nature of dynamic self-assembly. Consequently, to date, there is no report of any methods suitable for studying multiprotein templated DCC. To overcome this challenge, we developed a novel multi-protein detection strategy which combines the discriminatory power of zwitterionic 'thermal tag' with the sensitivity of differential scanning fluorimetry (DSF).[3,4] This strategy enables high throughput analysis of several protein-ligand interactions simultaneously. It is also remarkably sensitive and could differentiate the binding of ligands to different proteins, including structurally-similar subfamily members, which are otherwise challenging to discriminate. However, its greatest appeal lies in its ability to analyse protein-directed self-assembly, where one could assess all thermodynamic parameters of an equilibrating system in a single, rapid experiment. When used in combination with DCC, we were able to achieve simultaneous evolution of ligands against several proteins. This led to the concurrent discovery of subfamily-selective probes against two clinically-important epigenetic enzymes, FTO and ALKBH3. To our knowledge, this study represents not only the first report of multi-protein DSF assay, but also a new strategy for probing dynamic chemical system, which hopefully

	will further our understanding of self-assembly dynamics, and inspire new applications for DCC-based approaches. 
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