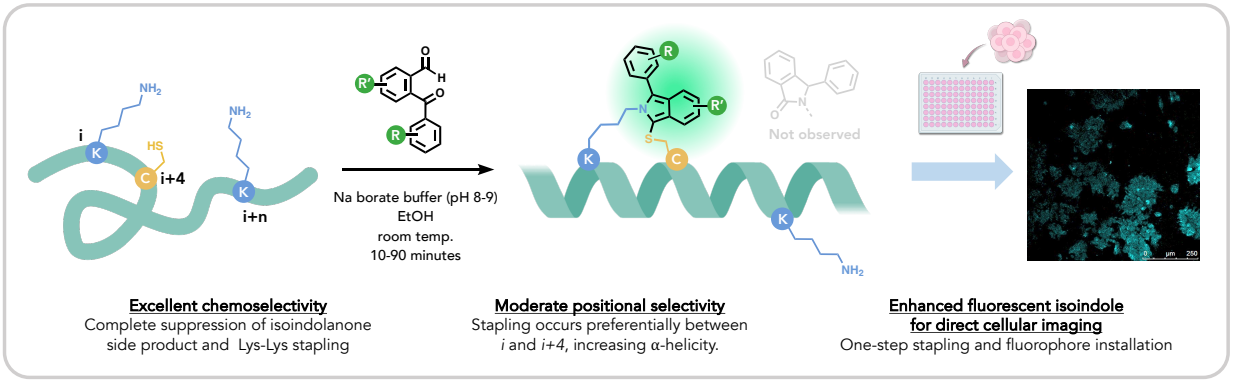
The modulation of peptidic scaffolds through stapling reactions has been established as a powerful tool for recapitulating the bioactivity of native α-helices in targeting protein-protein interactions (PPIs)[1]. However, accessing such helices have largely relied on protecting group manipulations or the use of non-natural building blocks during peptide synthesis. As such, there is a growing need for a stapling strategy involving only natural amino acids in their unprotected states. Herein we report a rapid, mild, and highly chemoselective stapling reaction using a new class of molecular linchpins called 2-ketobenzaldehydes that installs a highly fluorescent thiol-isoindole crosslink. This methodology also exhibited good positional selectivity favoring the helical *i* and *i+4* linkage on fully unprotected peptides in the presence of competing reaction sites, offering exceptional late-stage functionality for easier access of stapled α-helices. In our efforts to further validate this chemistry, we have successfully shown *in vitro cyto*toxicity (IC_{50} = 5.10 μM) equipotent to an all-hydrocarbon stapled peptide candidate[2]. Furthermore, in harnessing the innate fluorescence of thiol-isoindole, this staple can be directly used as a probe for cell imaging in the qualitative assessment of stapled-peptide cell permeability, thus bridging therapeutic potential with analytical probe development.



(1) Li, X.; Chen, S.; Zhang, W. D.; Hu, H. G. Stapled Helical Peptides Bearing Different Anchoring Residues. *Chem Rev* **2020**, *120* (18), 10079-10144. DOI: 10.1021/acs.chemrev.0c00532

(2) Edwards, A. L.; Wachter, F.; Lammert, M.; Huhn, A. J.; Luccarelli, J.; Bird, G. H.; Walensky, L. D. Cellular Uptake and Ultrastructural Localization Underlie the Pro-apoptotic Activity of a Hydrocarbon-stapled BIM BH3 Peptide. *ACS Chem Biol* **2015**, *10* (9), 2149-2157. DOI: 10.1021/acschembio.5b00214.