Synthesis and Application of Sterol-Based Affinity Probes for a Comprehensive Mapping of the Cholesterol Interactome

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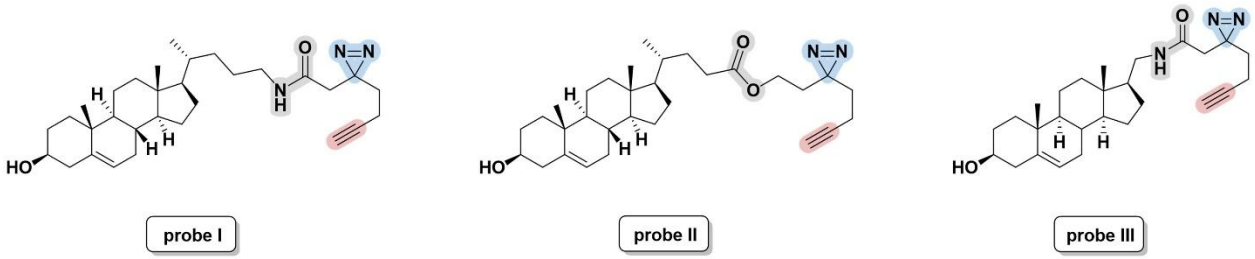
Cholesterol is a structural component of membranes and plays a key role in human health by regulating the fluidity of membranes and affecting signaling pathways.[1] Maintaining cholesterol homeostasis by regulating local cholesterol concentrations, arising from *de novo* biosynthesis or dietary sources is important since a dysregulated cholesterol transport is associated with cardiovascular disease[1] but also Alzheimer's[2] and cancer.[3] In light of the obvious importance of sterol-binding proteins (SBP), Hulce *et al.* introduced a sterol-based affinity probe which showed excellent results.[4] Nevertheless, the chosen design yielded a probe that wasn't fully suitable to bind sterol transport proteins, which our group focuses on. Therefore, we aim to introduce a library of enhanced sterol-based affinity probes which will enable us to bind all cholesterol interacting proteins and identify them by using a mass-spectrometry based chemoproteomic workflow. We synthesized several sterol-based affinity probes that are bifunctional, containing a diazirine (blue), which is transformed into a carbene via UV irradiation for covalent binding to SBPs, and an alkyne (red) for binding to a functional tag via click chemistry (figure 1). These probes are currently being tested for their binding abilities against a panel of recombinant human sterol transport proteins and will soon be investigated in proteomics and imaging experiments.

Figure 1: Examples of bifunctional sterol-based affinity probes from our library.



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