Exploiting disulfide-rich peptides as protein epitope mimics: development of a generalizable conjugation approach for immunogen preparation

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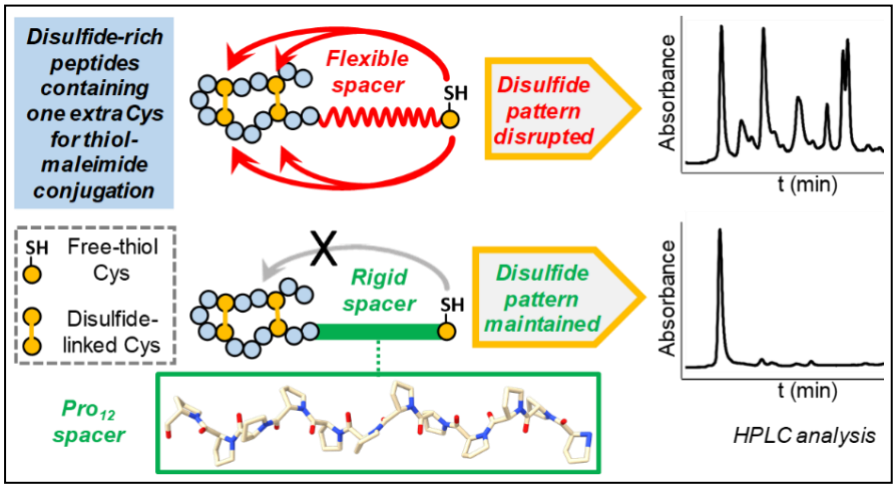
Generation of specific antibodies against peptides by immunization requires their covalent conjugation to protein carriers to override their inherently weak immunogenicity. The vast majority of bioconjugation approaches to achieve peptide-protein constructs rely on thiol-maleimide chemistry[1] and capitalize on a wide array of commercial maleimide-functionalized protein carriers. Disulfide-rich peptides[2] (DRPs) possess a rigid, constrained structure that makes them ideal for designing synthetic mimics of protein regions/domains. For bioconjugation purposes, the introduction of a single spare thiol moiety into a linear peptide antigen is straightforward, while DRPs' disulfide bonds are prone to intramolecular thiophilic attack by the reactive thiolate. This unintended reactivity competes with the desired Michael addition to the maleimide moiety, ultimately disrupting the native disulfide bridging framework. As a result, DRP's tertiary structure will be altered, affording an immunogen that is a poor mimic of the native target. Although a few studies have explored the late-stage introduction of thiol-containing cross-linkers onto DRP antigens for their conjugation onto protein carriers,[3,4] the stability of DRP's disulfide pattern in the presence of an extra thiol has never been examined. To address this, we systematically evaluated the influence of different spacers in "DRP-spacer-thiol" constructs, under thiol-maleimide reaction conditions.[5] Our results highlight how both linker length and flexibility are key to maintain DRP disulfides unaltered, providing a general approach to achieve DRP bioconjugation by thiol-maleimide chemistry. We have applied our approach to a small DRP predicted to closely mimic a surface-accessible epitope of the full LINGO-1 protein, and obtained a very specific antibody response upon immunization: the resulting polyclonal IgG was able to selectively bind the full-length protein in a cellular context, with stringent selectivity across its four homologs.



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