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Bind&Bite: Covalently stabilized heterodimeric coiled-coil peptides for the site-selective chemical modification of proteins

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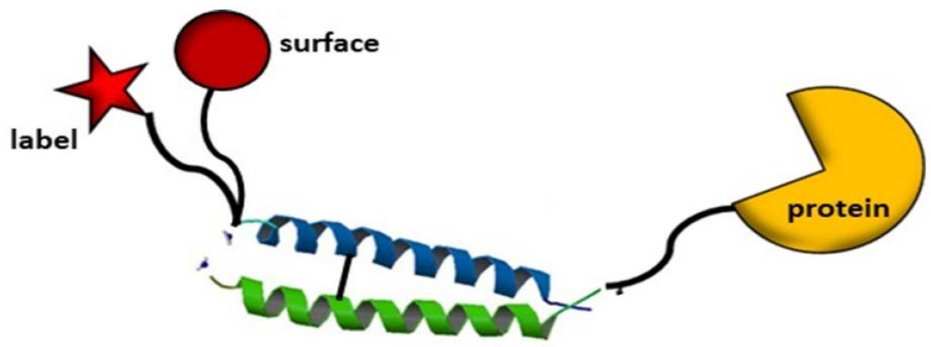
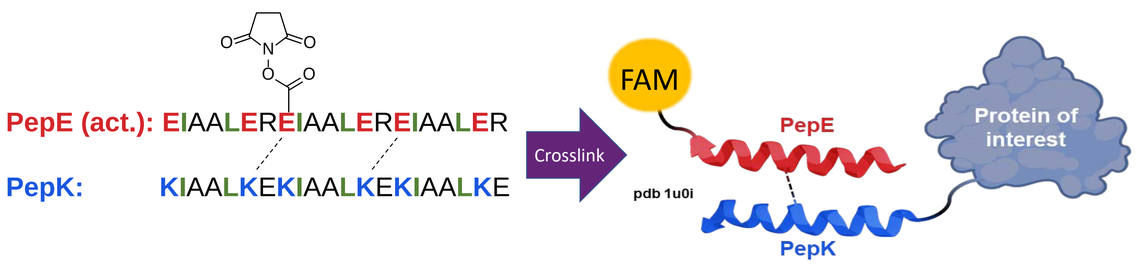
Introduction

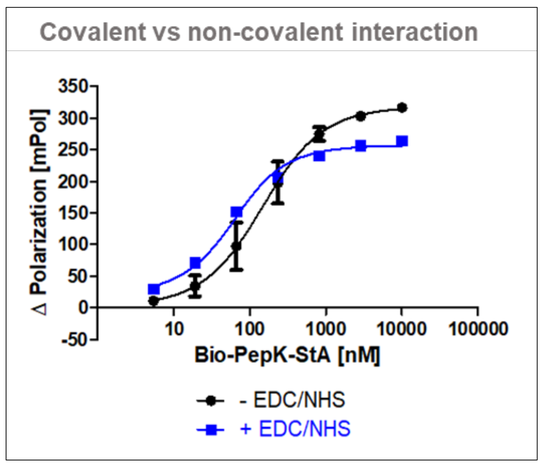
Ensuring site-selectivity in covalent chemical modification of proteins is one of the major challenges in chemical biology, medicinal chemistry and related disciplines [1]. We have modified a pair of heterodimeric coiled-coil peptides [2] to enable enzyme- and cysteine-free, covalent stabilization of the dimer. Fusion of one peptide to the protein of interest, in combination with linking the desired chemical modification to the complementary peptide, facilitates stable, site-selective protein labeling. Covalently crosslinking of the coiled-coil, also allowed for truncation of the peptides by one heptad [3]. This isopeptide/squaramide – based crosslinking strategy, was successfully used to selectively modify the HIV-1 envelope glycoprotein (Env). Furthermore, mutually selective pairs of coiled-coil peptides were identified [3]. Ongoing research explores the Bind&Bite method in a biomedical context, as well its selectivity and versatility for the parallel, concurrent chemical modification of multiple proteins.

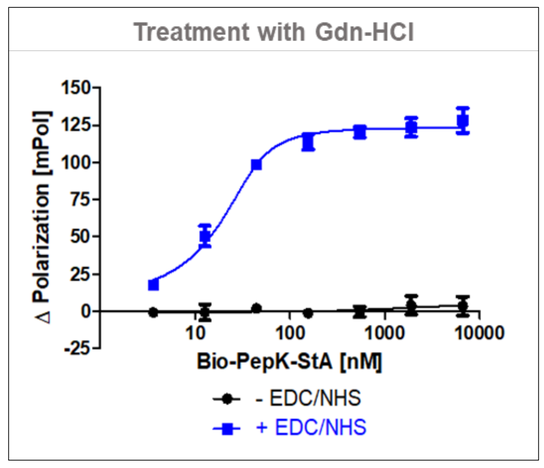


Bind&Bite principle

Activated glutamate residues in PepE can undergo proximity-enhanced isopeptide bond crosslink upon coiled-coil formation:

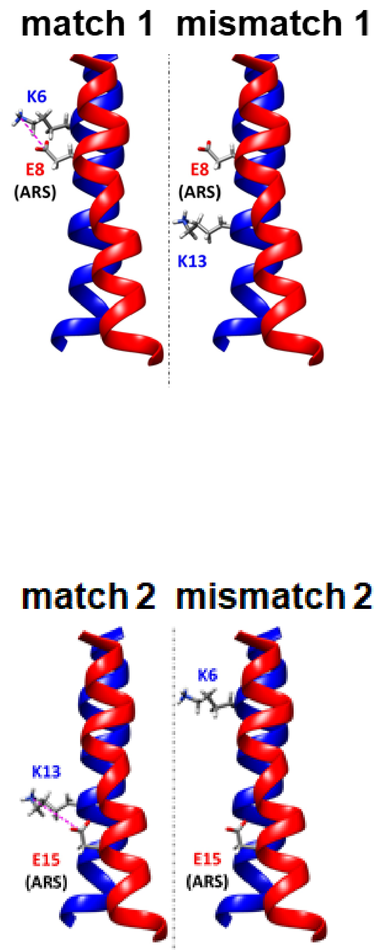


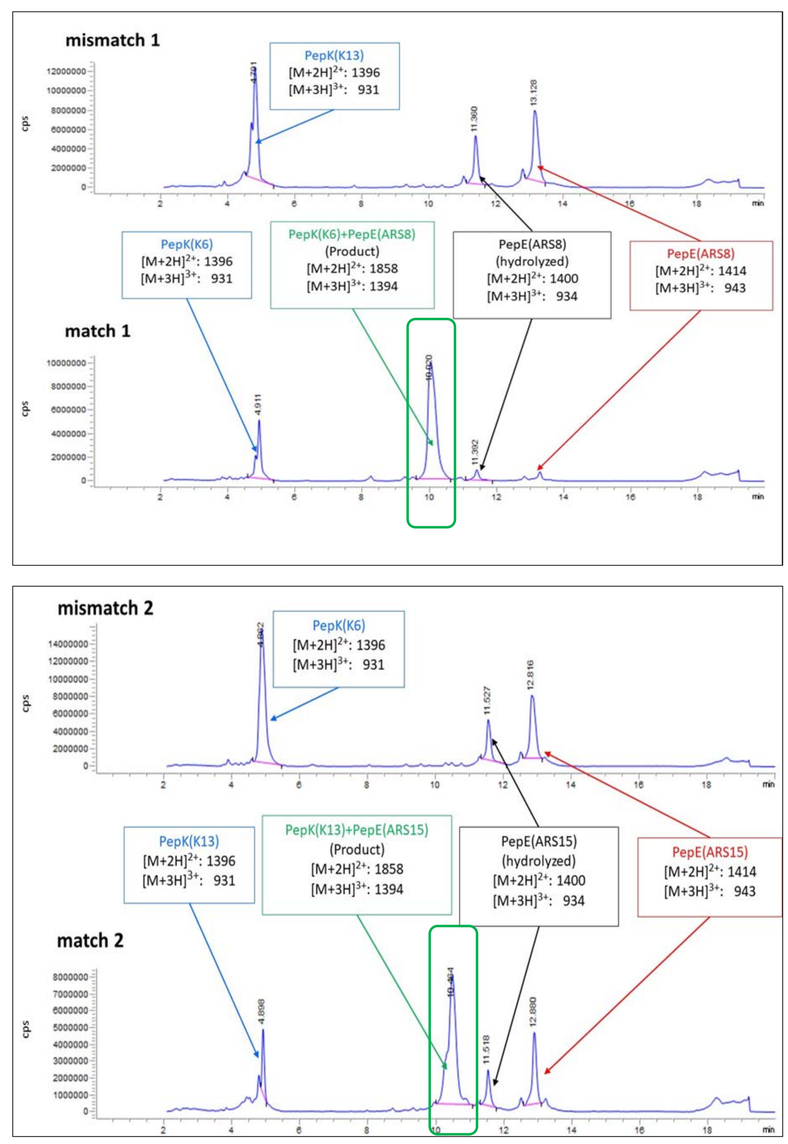




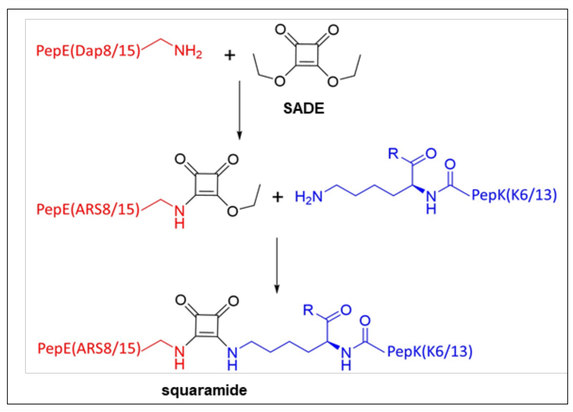
Orthogonal Bind&Bite

Positional scanning of amine-reactive sites (PepE act.) vs. single lysine variants (PepK) yields mutually orthogonal pairs for covalent squaramide crosslinking:



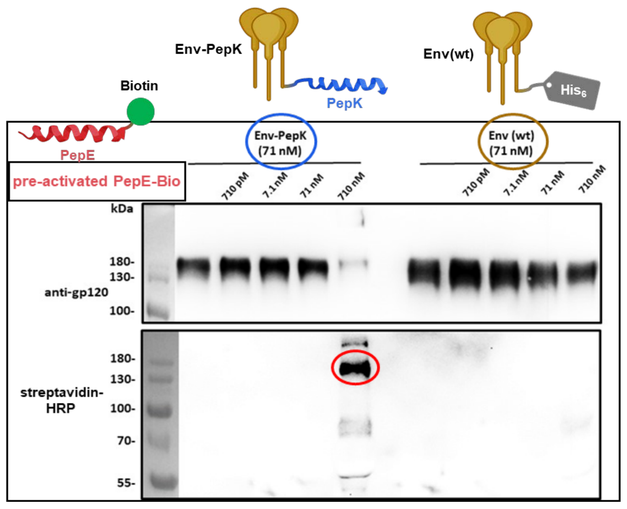


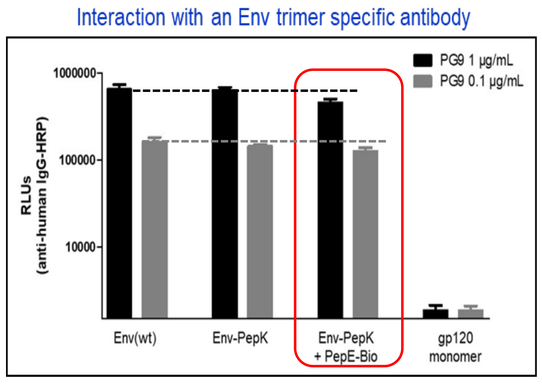
Covalent (squaramide) product formation is only observed for match 1 or match 2, no crossreactivity even after overnight incubation at high concentration (150 µM)
→ Orthogonal pairs



Labeling under challenging conditions

Robust and site-selective biotinylation achieved even in inhomogenous reaction conditions (Labeling of HIV-1 Env in DMEM/10 % FCS):



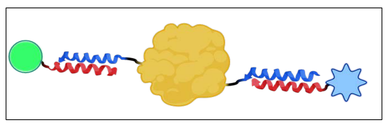


Antibody binding experiments (left):
Labeled protein is functional and retains its trimeric structure yielding responses similar to the his-tagged wildtype protein

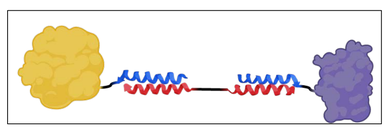
Multiple orthogonal Bind&Bite ligations

Applications of positionally encoded ligations:

Site-selective introduction of multiple tags in one protein:



Controlled heterodimeric protein crosslinking:



References

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