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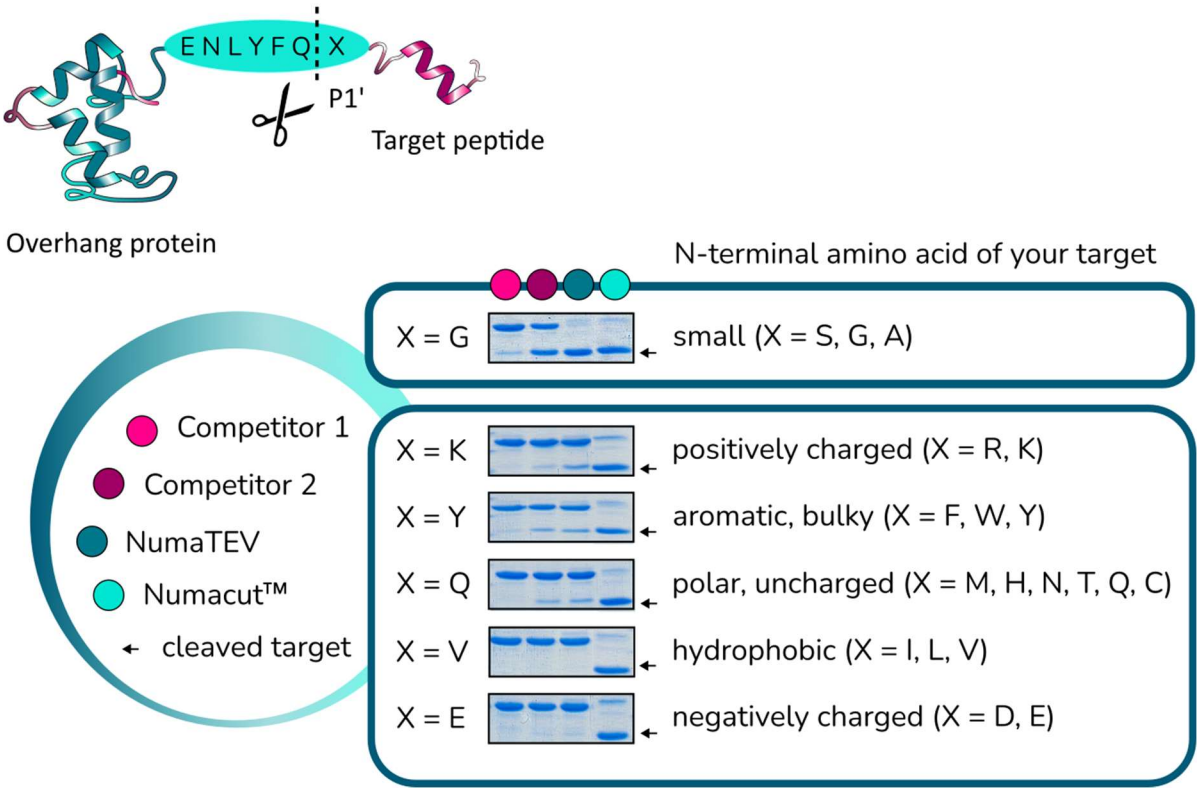
Numacut™ TEV protease exhibits increased substrate tolerance compared to standard TEV protease variants

In established activity assays, the substrate tolerance of the Numacut™ TEV protease (**light blue**) was compared to those of the NumaTEV wild type (**blue**) and standard TEV proteases of competitors (**pink** and **purple**). For this, test substrates, containing the recognition site ENLYFQ/X, were generated where X (also referred to as the P1' position) was substituted for all canonical amino acids. Test substrates incubated with different TEV protease variants are cleaved at the recognition site between Q and X, leading to the release of overhang proteins (N-terminus) from target peptides (C-terminus). For the assay, same units of TEV protease variants were added to 3 µg of test substrates in reaction buffer (50 mM Tris/HCl pH 8.0, 0.5 mM EDTA and 1 mM DTT), respectively, and incubated at 30°C for 3 hours. The cleavage reactions were loaded on 15% acrylamide gels stained with Coomassie blue to assess the cleavage of fusion proteins (upper band) and release of overhang proteins (lower band) for each TEV protease variant. The ability of different TEV protease variants to cleave fusion proteins containing varying ENLYFQ/X motifs is exemplarily shown for X = G, K, Y, Q, V and E.



Quantitative cleavage of fusion protein containing the optimum recognition site (ENLYFQ/G) observed for the Numacut™ and NumaTEV protease indicate superior activity levels compared to standard TEV proteases from competitors. Furthermore, high cleavage efficiencies for hard-to-cleave amino acids in the P1' position (X = K, Y, Q, V and E) were observed for the Numacut™ TEV protease demonstrating that the canonical recognition site (ENLYFQ/S or G) was successfully broadened to ENLYFQ/X (except for P). It shows that the Numacut™ TEV protease, developed by directed evolution, has a significantly widened amino acid tolerance at the cleavage site compared to the NumaTEV and standard TEV protease variants, opening the doors to unique possibilities such as:

- cleavage of overhang protein independently of the N-terminal amino acid.
- Production of native and traceless target proteins without any cleavage scars etc.

No false by-products (due to high sequence specificity)
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