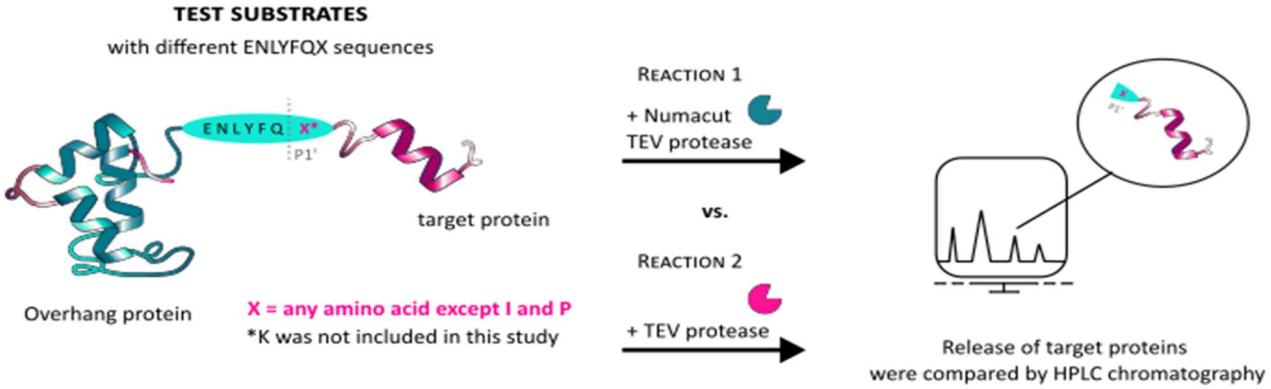
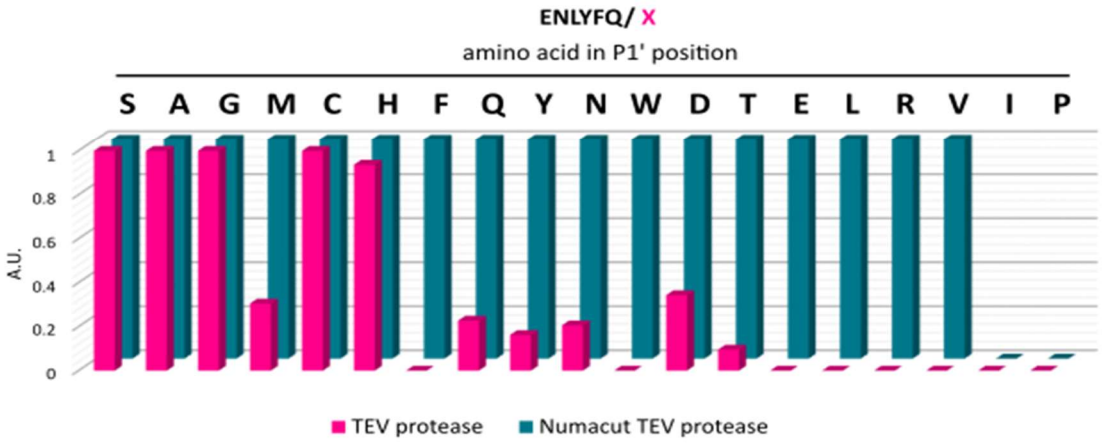
# Numacut™ TEV protease has an increased substrate tolerance compared to TEV protease wildtype

The substrate tolerance of Numacut™ TEV protease was assessed and compared to the TEV protease wildtype. A test substrate consisting of a fusion protein, bearing the classic recognition site of TEV protease (ENLYFQ/S; cleavage between Q & S)) between both the fused proteins was used for the assay development (see below). Afterwards, 19 additional test substrates were generated, containing the classic TEV recognition sequence and instead of S (P1' position), all additional canonical amino acids.

The recognition sites of the TEV protease were inserted between the target protein (located at the C-terminus) and the overhang protein was removed (located at the N-terminus).

Numaferm's standard TEV protease and evolved Numacut™ TEV protease were added to 50 µM of test substrate 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 1h. Cleavage reactions were quenched by addition of 3M guanidium hydrochloride and the release of target protein from overhang protein was quantified by RP-HPLC chromatography. The bar chart below displays the activity of Numaferm's standard TEV protease standardized to the activity of the Numacut™ TEV protease.



The recognition site of the TEV protease (ENLYFQ/S or G) was successfully broadened to ENLYFQ/X (except for I and P) for the Numacut™ TEV protease. It demonstrates that the Numacut™ TEV protease, developed by directed evolution, has a significantly widened amino acid tolerance at the cleavage site compared to the standard TEV protease, 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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