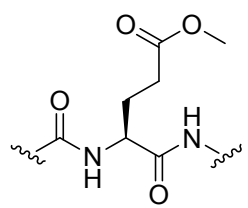
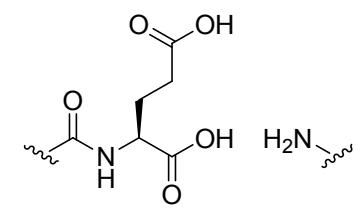


TABLE S3: Enzymatically cleavable linkers

Cleavable linker	Structure	Cleavage products	Cleavage conditions	Advantages	Disadvantages
TEV-cleavable linker ^{1,2}	---ENLYFQG---	---ENLYFQ-COOH $\text{H}_2\text{N-G---}$	TEV protease	High specificity (only cleaves between Gln-Gly of sequence on LHS). => low background	Can get varying cleavage efficiencies, dependent on activity and stability of the protease. ^a
Trypsin-cleavable linker ³⁻⁵	---XXXK/RXXX--- X = any amino acid other than K or R	---XXXK/R-COOH $\text{H}_2\text{N---}$ X = any amino acid other than K or R	Trypsin	Cleave linker and obtain tryptic peptides in a single step.	Protein or peptide cannot be specifically eluted
V8-cleavable linker ⁶			NaOH then V8 protease (aka endoproteinase Glu-C)	Will only cleave after alkaline ester hydrolysis, and therefore orthogonal to protease digestion of target proteins	Not yet been applied to whole proteome analysis

^a activity and stability of the involved protease is applicable to all protease cleavable linkers. Overall, this should not represent a bottleneck.

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