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TABLE S3: Enzymatically cleavable linkers

Cleavable linker	Structure	Cleavage products	Cleavage conditions	Advantages	Disadvantages
TEV-cleavable linker[1,2]	⌇—ENLYFQG—⌇	⌇—ENLYFQ-COOH H_2N-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 H_2N—⌇ 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]	O O H N N H O	O OH O N H OH O H_2N	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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