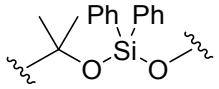
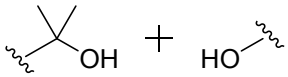
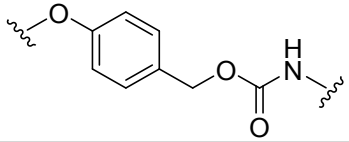
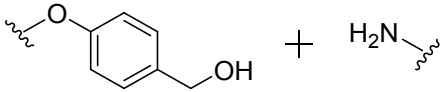
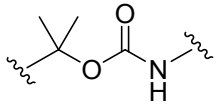
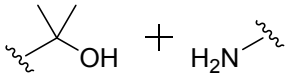
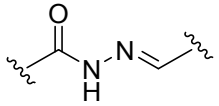
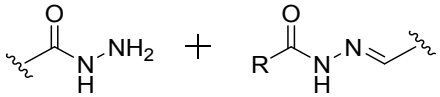
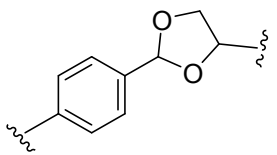
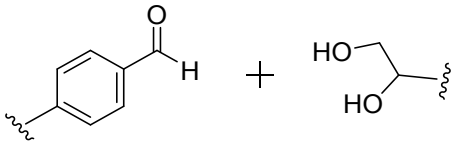
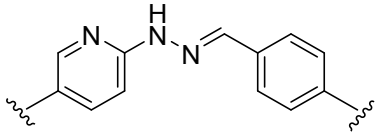
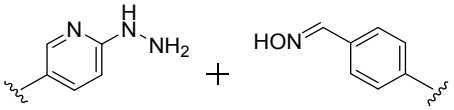
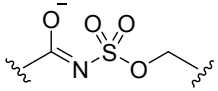
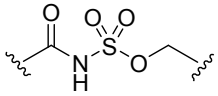
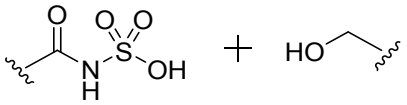
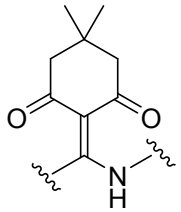
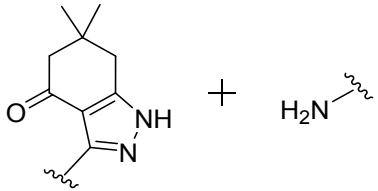
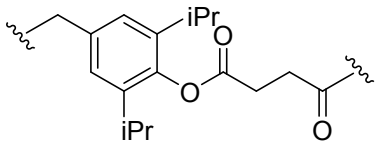
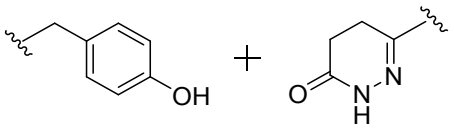
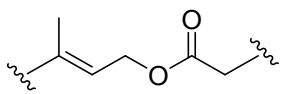
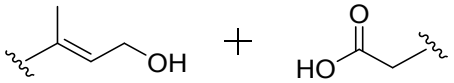
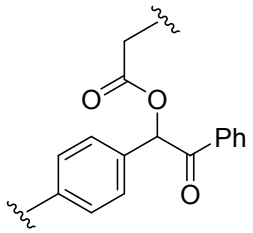
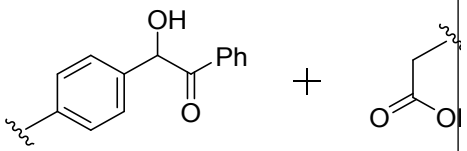
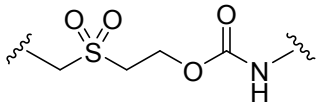
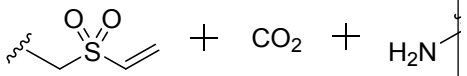
Electronic Supplementary Material (ESI) for Molecular Omics.

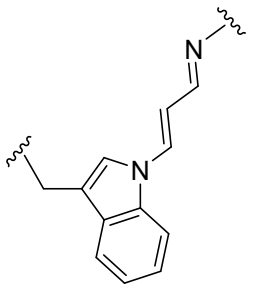
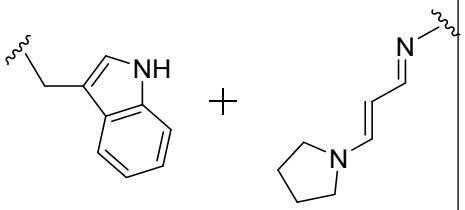
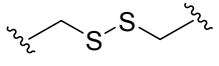
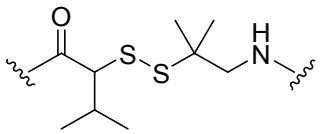
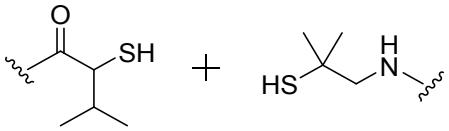
TABLE S1: Chemically cleavable linkers

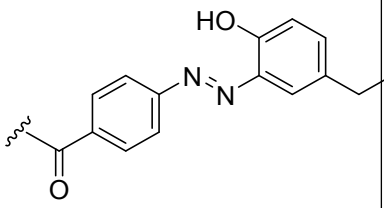
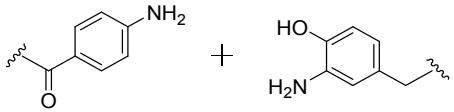
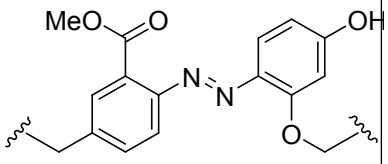
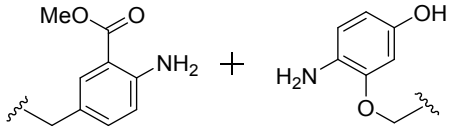
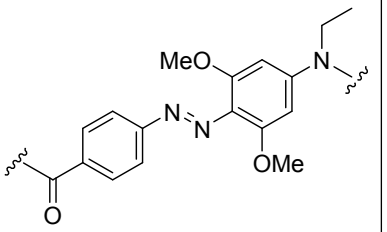
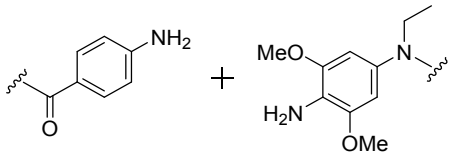
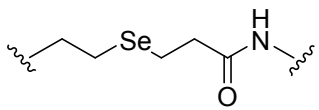
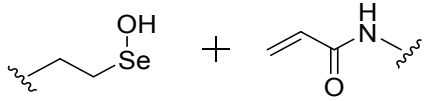
	Cleavable linker	Structure	Cleavage products	Cleavage conditions	Advantages	Disadvantages
Acid-labile	Dialkoxydiphenylsilane (DADPS)[1]			10% HCOOH	mild conditions Stable in aqueous KF solutions of concentrations up to 5 M	
	4-hydroxy-benzyl-alcohol[2]			TFA	Based on well established solid phase chemistry	Strong acid may lead to release of non-specifically bound proteins
	Boc (t-butyl-oxy-carbonyl) group[3,4]			TFA	Based on well established protecting group	Strong acid may lead to release of non-specifically bound proteins
	Acyl hydrazones [5]			Acyl hydrazine pH 4–5	Stable at basic pH (pH 8–10).	Tends to prematurely hydrolyze at neutral pH

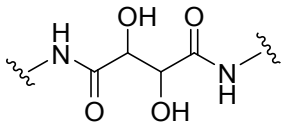
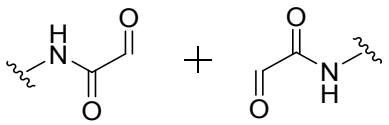
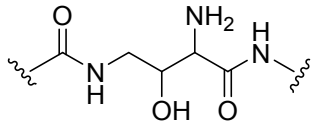
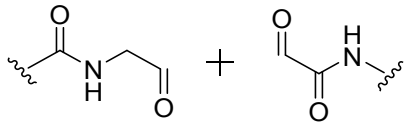
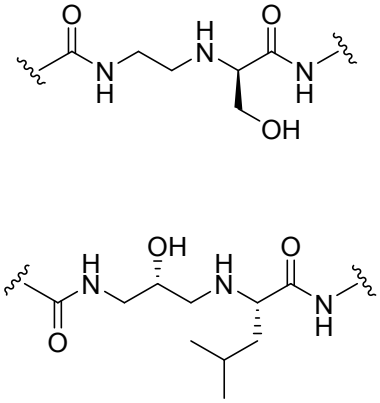
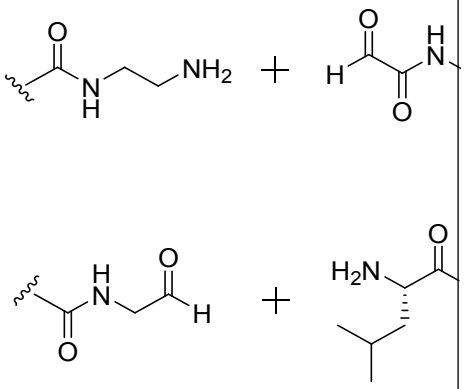
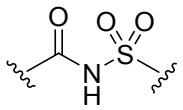
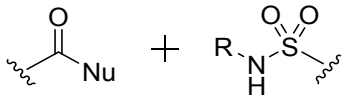
	Cyclic acetals[6]			1M guanidine in 1% TFA at 37 °C for 1 hour	Possibility to reintroduce a label to the released biomolecule	Only tested on lysates spiked with model proteins
	Bisaryl hydrazones [7]			NH_2OH, aniline pH 4–5 Also by ETD (see Table S4)	stable at neutral pH possibility to reintroduce a label to the released biomolecule	high concentrations of aniline (100 mM) are required and full cleavage is not always achieved
	Acyl-sulfamate[8]	 pH 7.6 **Stable**  pH 2.0 **Less stable**		pH 2, 5h @ 37°C	Stable even at 95 °C with 10 mM hydrazine (5 min)	

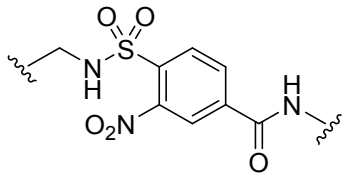
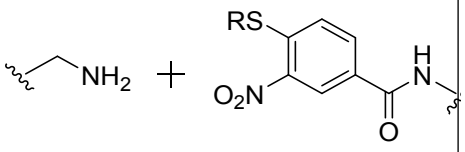
Base-labile	Dde[9]			2% hydrazine	Based on well established protective group chemistry	(DDE) linker has been demonstrated to be unstable as no modified peptides could be detected upon application of a cleavage reagent, due to instability of the linker during on-bead trypsin digestion[10] The conventional way of deprotecting the Dde group in peptide synthesis consists of using 2% hydrazine. This turned out to be not compatible with fluorescent scanning of SDS-PAGE[9]
	Levulinoyl ester[11]			100 mM hydrazine	acid stable and can be removed selectively with respect to other esters	

	Allylic ester[12]			pH 11		
	Benzoin ester[13]			pH 8	Also usable as a photocleavable linker	
	Sulfonyl-based reversible amine[14]			100 mm ammonium bicarbonate, pH 9, overnight at 4 °C	Traceless linker when reacted with amines of proteins.	Long cleavage time

	Indole-based linker[15]			50mM or 100mM pyrrolidine@4°C		
Reductive cleavage	Disulfide[1,9]			DTT TCEP or BME	Commercial building block available	Application in click chemistry is limited (due to reducing conditions during CuAAC); disulfide exchange may occur. Incompatible with reduction/alkylation workflow of MS sample preparation
	Hindered disulfide[16] [17]			10mM TCEP		

	Diazobenzene[18]			25mM $Na_2S_2O_4$ (several elutions)	Short synthesis of building block; linker changes color upon cleavage	Difficult purification + multiple elution steps necessary
	HAZA[19]			1mM $Na_2S_2O_4$ (< 10 s)	Fast cleavage; linker changes color upon cleavage	Multistep synthesis of linker
	Highly labile diazobenzene[20]			0.5mM $Na_2S_2O_4$ (< 5 s)	Very fast cleavage; linker changes color upon cleavage	
Oxidative cleavage	Selenium ether[21–24]			8 mM H_2O_2 for 1 h		

	Vicinal diol[25]			10 mM periodate Neutral pH		
	1,2-Amino alcohols[26]			10 µM periodate		
	Seramox / iso-seramox [27]			1 mM periodate Within 5mins		
Others	Acyl-sulfonamide[28–30]			ICH2CN pH 9, NH4OH **or** pH 5 ICH2COO$^-$ **or** 200mM iodoacetimide pH 8		

	Nitro-sulfonamide[31]			RSH pH 9.2		

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