Captions for Figures and Tables

Figure 1. Different forms of methylation. A) N-methylation (red), O-methylation (blue) and S-methylation (green) could occur on eight amino acid residues. B) The 6 methylation forms occurred on Lys and Arg residues.

Figure 2. Challenges for the analysis of methylproteome. A), The structure alteration caused by methylation is much less significant when compared with other modification forms like phosphorylation, acetylation and glycosylation. B), Methylation introduces mass shifts identical to those of some amino acid substitutions which makes the confident identification difficult.

Figure 3. Strategy for the enrichment of methylpeptides using conventional SCX or High-pH SCX. SCX separation conducted under high-pH condition can alleviate the interference of histidine containing peptides.

Figure 4. Different strategies of metabolic labeling for improving the confidence in methylation identification. A), hM-SILAC. B), iMethyl-SILAC. C), MILS.

Table 1 Recent studies on large-scale analysis of methylproteome (>500 identified sites)

Table 2 The common characteristic ions and neutral losses for methylated peptides

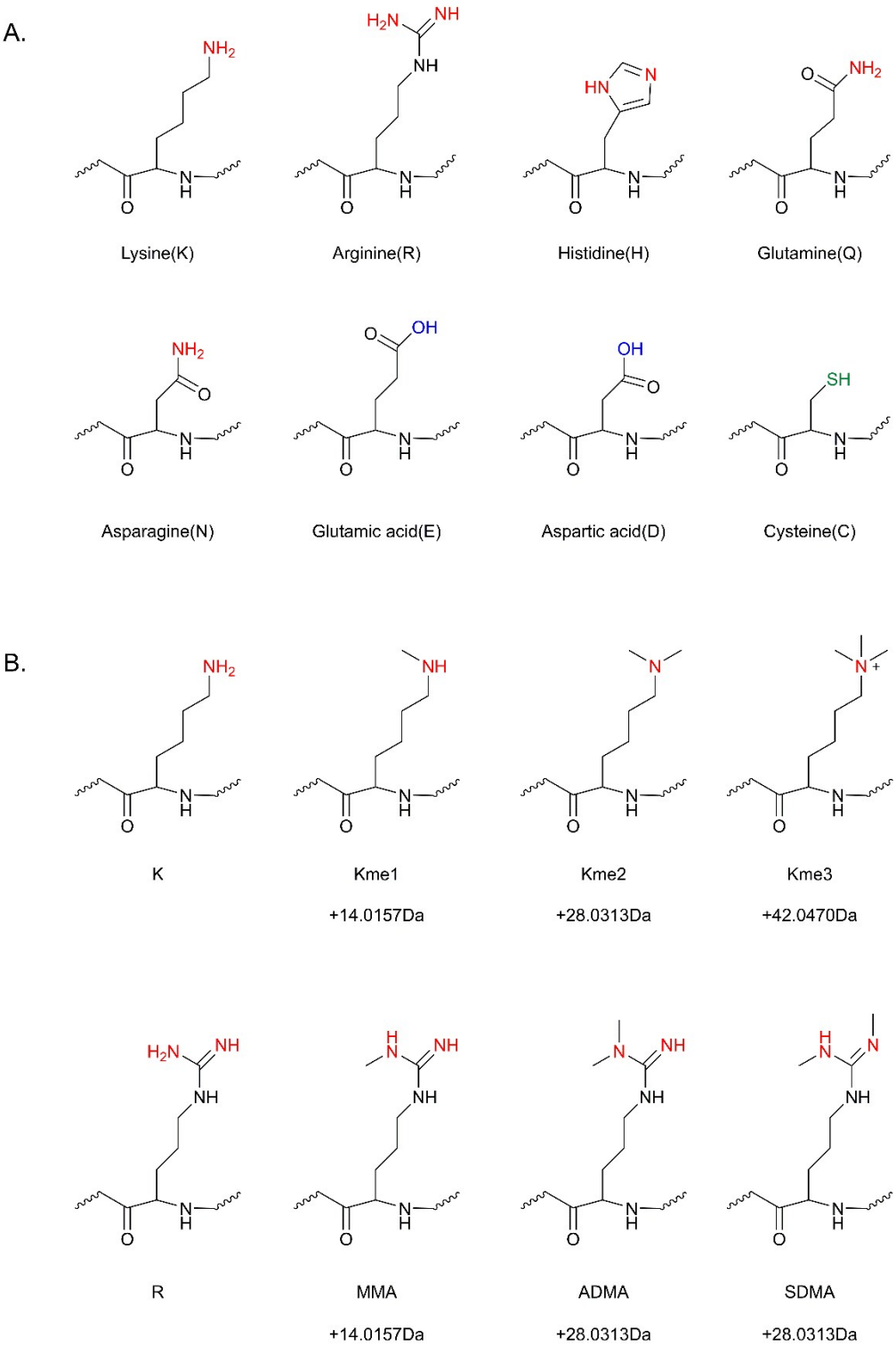
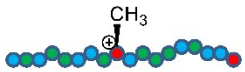
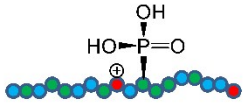
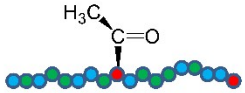
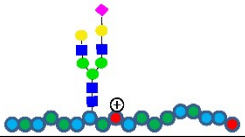
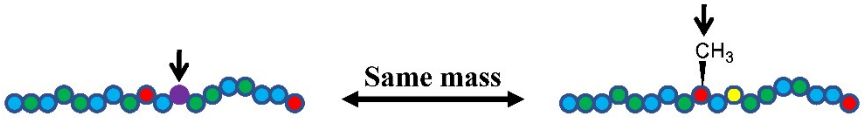


Figure 1. Different forms of methylation. A) N-methylation (red), O-methylation (blue) and S-methylation (green) could occur on eight amino acid residues. B) The 6 methylation forms occurred on Lys and Arg residues.

A.

Modification	Structure	Enrichment methods
Methylation		Lacking efficient enrichment methods
Phosphorylation		IMAC
Acetylation		Immunoaffinity chromatography
Glycosylation		HILIC

B.

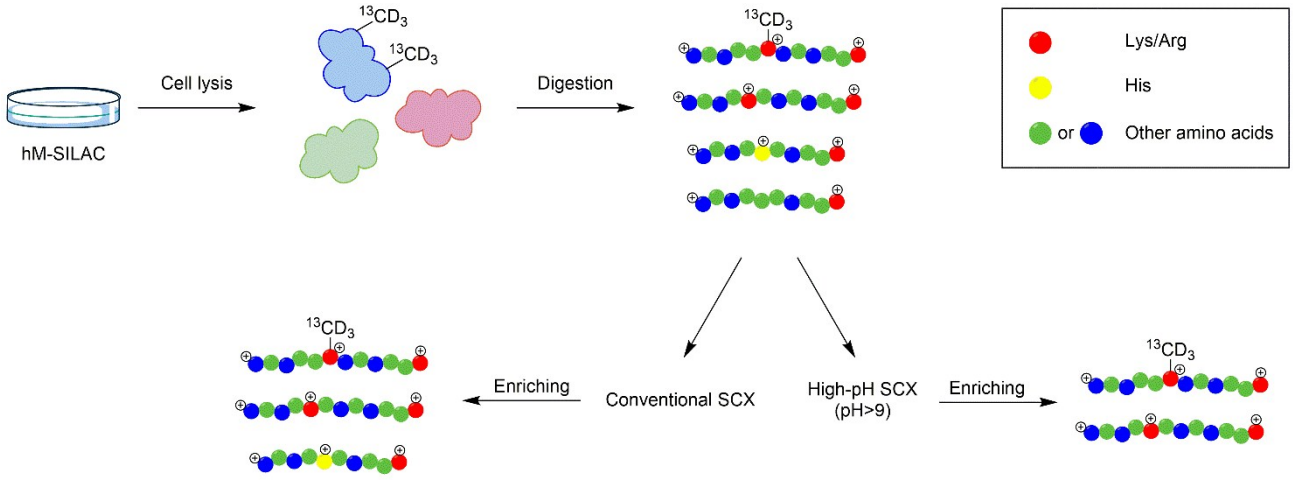


Description	Monoiostopic mass	Composition
Mono-Methylation	14.0157	H(2) C(1)
Asp, Glu mass differential	14.0157	H(2) C(1)
Gly, Ala mass differential	14.0157	H(2) C(1)
Ser, Thr mass differential	14.0157	H(2) C(1)
Val, Leu/Ile mass differential	14.0157	H(2) C(1)
Asn, Gln mass differential	14.0157	H(2) C(1)
Di-Methylation	28.0313	H(4) C(2)
Ala, Val mass differential	28.0313	H(4) C(2)
Cys, Met mass differential	28.0313	H(4) C(2)
Tri-Methylation	42.0470	H(6) C(3)
Gly, Val mass differential	42.0470	H(6) C(3)
Ala, Leu/Ile mass differential	42.0470	H(6) C(3)

K/R Amino acids with mass differential identical to methyl group(s)

Figure 2. Challenges for the analysis of methylproteome. A), The structure alteration caused by methylation is much less significant when compared with other modification forms like phosphorylation, acetylation and glycosylation. B), Methylation introduces

mass shifts identical to the mass differentials between many different amino acids which makes the confident identification difficult.



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41 **Figure 3.** Strategies for the enrichment of methylpeptides using conventional SCX and

42 High-pH SCX. SCX separation conducted under high-pH condition can alleviate the

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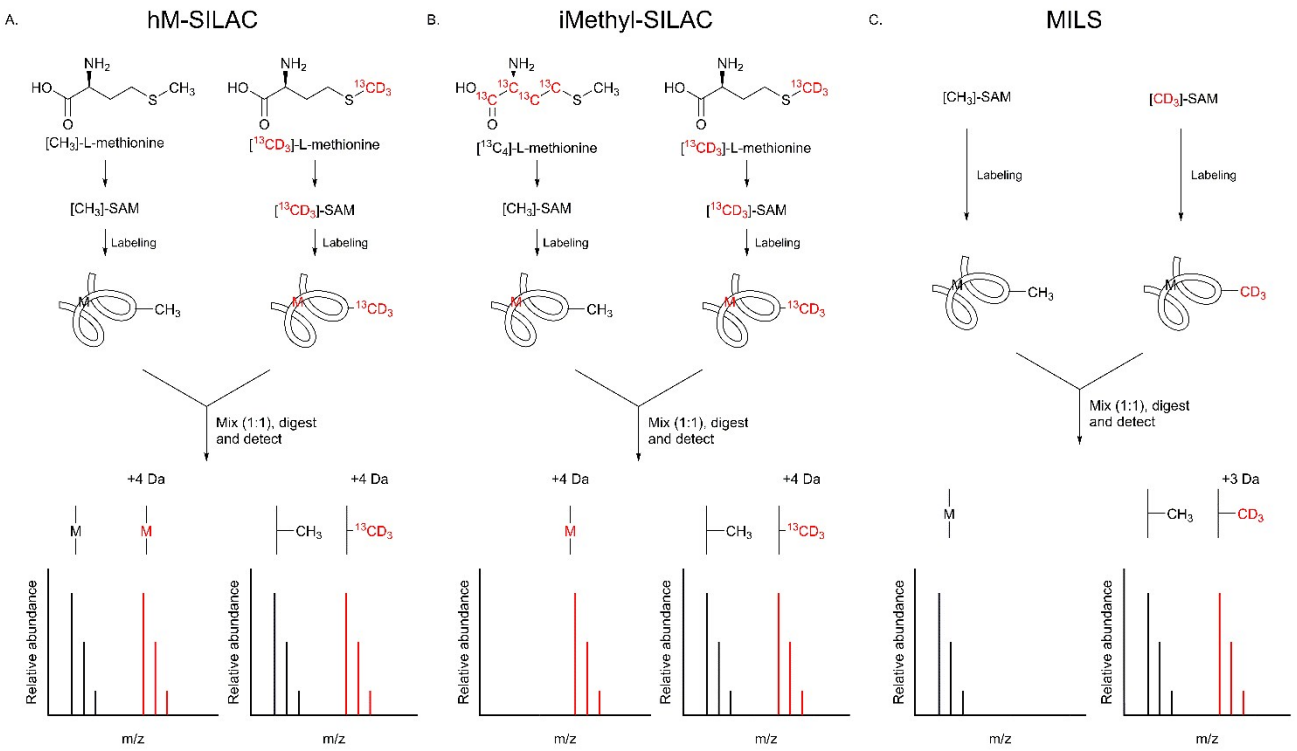

Figure 4. Different strategies of metabolic labeling for improving the confidence in methylation identification. A), hM-SILAC. B), iMethyl-SILAC. C), MILS.

Table 1 Recent studies on large-scale analysis of methylproteome (>500 identified sites)

Organism	Enrichment method	Confidence control	Methylation sites	Ref.(year)
HeLa cells	Immunoaffinity purification at the peptide level coupled with SCX	Global target-decoy FDR estimates	323 Kme1 127 Kme2 102 Kme3	(44) (2013)
T. *brucei*	SCX fractionation	Global target-decoy FDR estimates	649 MMA 683 DMA	(57) (2013)
HCT116 cells	Immunoaffinity purification at the peptide level	Global target-decoy FDR estimates	1473 MMA 497 DMA 132 Kme1 35 Kme2 31 Kme3	(37) (2014)
HEK 293T	Immunoaffinity purification at the peptide level coupled with SCX	Separate target-decoy FDR estimates	1027 MMA	(38) (2014)
T cells	Immunoaffinity purification	iMethyl-SILAC	2400 MMA 465 DMA	(22) (2015)
HEK293	High-pH reversed-phase fractionation and Immunoaffinity purification	Global target-decoy FDR estimates (Separate target-decoy FDR estimates may be used as well)	8030 MMA	(29) (2016)
HeLa cells	SCX prefractionation with immunoaffinity purification	Separate target-decoy FDR estimates	1246 Kme1 59 Kme2 53 Kme3	(45) (2016)
ESCC cell line KYSE-150	SCX prefractionation with immunoaffinity purification	Separate target-decoy FDR estimates	1032 Kme1	(46) (2016)
HEK293	online SCX-RP-MS	Separate target-decoy FDR	77 MMA 163 DMA	(55) (2016)

		estimates	305 Kme1 28 Kme2 66 Kme3	
HepG2 cells	SCX based enrichment	hM-SILAC	218 MMA 587 DMA 35 Kme1 19 Kme2 28 Kme3	(56) (2016)

52 **Table 2** The common characteristic ions and neutral losses for methylated peptides

Types of fragments	Name of ion or neutral loss	mass (Da)	Methylation type	ref. (year)
characteristic ion	Dimethylammonium	46.0657	aDMA	(78) (2003)
characteristic ion	Dimethylcarbodiimidium	71.0609	sDMA or aDMA	(78) (2003)
characteristic ion	Monomethylguanidinium	74.0718	MMA	(82) (2004)
characteristic ion	Immonium ion	98.0970	Kme1 or Kme2	(80) (2004) (81) (2008)
characteristic ion	Immonium ion	112.1126	Kme2	(80) (2004)
neutral loss	Monomethylamine	31.0422	MMA or sDMA	(79) (2004) (30) (2004)
neutral loss	Dimethylamine	45.0578	aDMA	(78) (2003)
neutral loss	Trimethylamine	59.0735	Kme3	(80) (2004)
neutral loss	Dimethylcarbodiimide	70.0531	sDMA	(78) (2003)
neutral loss	Monomethylguanidine	73.0640	MMA	(30) (2004)

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