Supplementary Data

Full Title: Evaluation of Colorimetric Assays for Analyzing Reductively Methylated Proteins: Biases and Mechanistic Insights

Short Title: Colorimetric Analysis of Methylated Proteins

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Supplemental Tables

Table S1 The pathlength of water at various volumes and temperature in a half-area 96-well plate.

Temperature (°C)	Volume (μL)	Average pathlength (cm)	95% Confidence interval (cm)	Number of replicates
25	100	0.568	0.002	26
25	120	0.679	0.003	9
37	120	0.707	0.003	12
25	200	1.102	0.002	10

Table S2 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for BSA in water and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.001	-0.001	-0.001
0.2	0.133	0.123	0.126
0.4	0.255	0.241	0.255
0.6	0.360	0.357	0.373
0.8	0.491	0.484	0.496
1.0	0.617	0.609	0.616
Slope = ε_{280nm} (g^{-1} L cm^{-1})	0.608	0.606	0.615
Intercept (cm^{-1})	0.005	-0.001	0.003
Correlation coefficient (R^2)	0.9996	0.9999	0.9999

Table S3 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for HEWL in water and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.000	0.000	0.000
0.2	0.266	0.264	0.250
0.4	0.530	0.535	0.504
0.6	0.783	0.780	0.743
0.8	1.040	1.035	1.004
1.0	1.306	1.299	1.248
Slope = ε_{280nm} (g^{-1} L cm^{-1})	1.301	1.294	1.249
Intercept (cm^{-1})	0.004	0.006	0.000
Correlation coefficient (R^2)	1.0000	0.9999	1.0000

Table S4 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for ovalbumin in water and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.001	-0.001	0.001
0.2	0.126	0.133	0.112
0.4	0.240	0.247	0.237
0.6	0.367	0.372	0.374
0.8	0.497	0.492	0.506
1.0	0.626	0.624	0.626
Slope = ε_{280nm} (g^{-1} L cm^{-1})	0.624	0.618	0.635
Intercept (cm^{-1})	-0.003	0.002	-0.009
Correlation coefficient (R^2)	0.9998	0.9998	0.9995

Table S5 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for BSA in buffer and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.001	-0.001	0.001
0.2	0.115	0.120	0.113
0.4	0.229	0.233	0.224
0.6	0.344	0.358	0.344
0.8	0.465	0.494	0.467
1.0	0.610	0.636	0.590
Slope = ε_{280nm} (g^{-1} L cm^{-1})	0.602	0.633	0.590
Intercept (cm^{-1})	-0.007	-0.010	-0.005
Correlation coefficient (R^2)	0.9990	0.9991	0.9997

Table S6 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for HEWL in buffer and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.000	0.000	0.000
0.2	0.222	0.231	0.225
0.4	0.454	0.460	0.474
0.6	0.658	0.669	0.678
0.8	0.891	0.917	0.912
1.0	1.092	1.130	1.174
Slope = ε_{280nm} (g^{-1} L cm^{-1})	1.096	1.132	1.162
Intercept (cm^{-1})	0.005	0.002	-0.004
Correlation coefficient (R^2)	0.9998	0.9998	0.9995

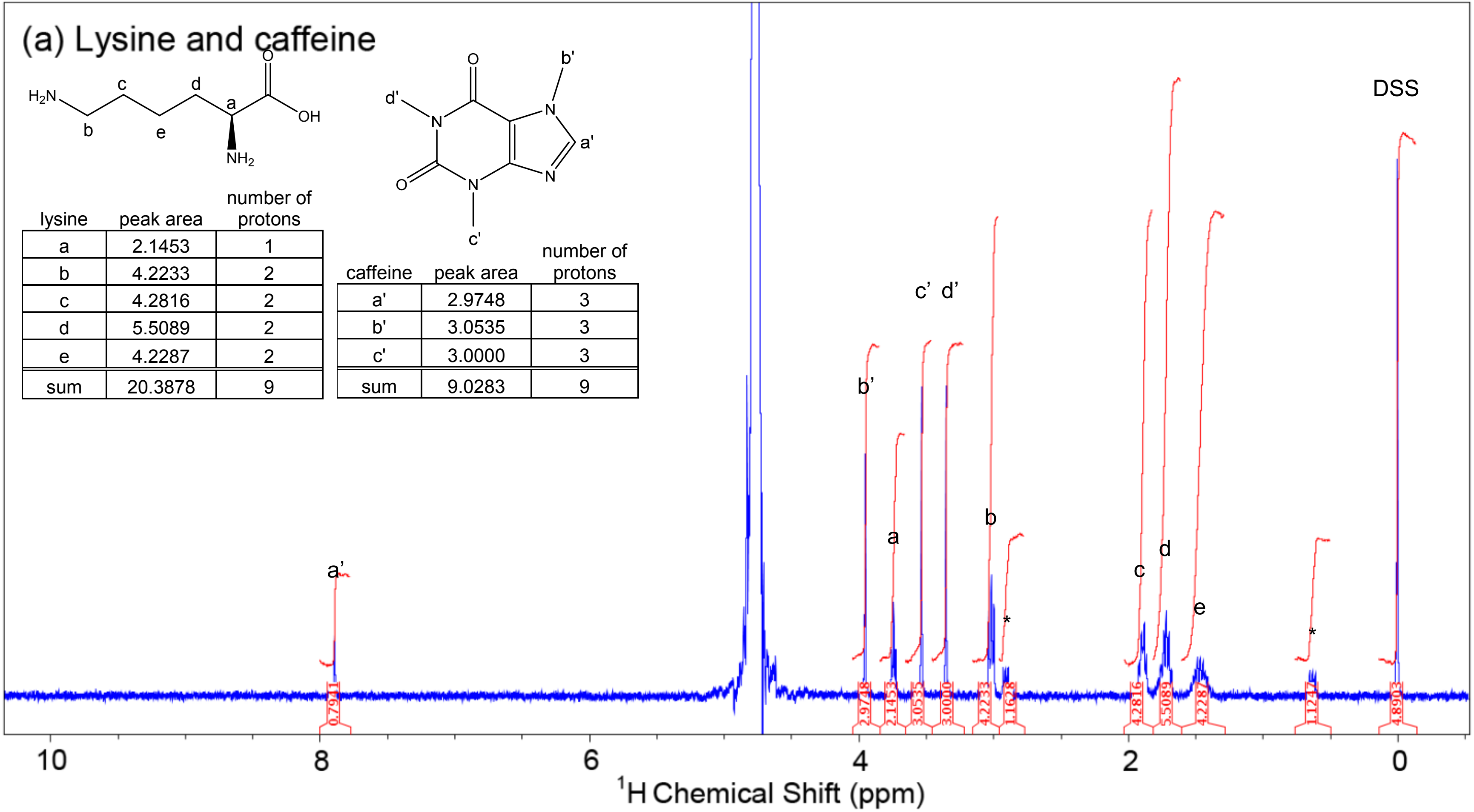
Table S7 Blank and pathlength corrected absorbance data at 280 nm (A_{280nm}/cm) for ovalbumin in buffer and 25 °C with the slope, intercept, and correlation coefficient calculated by fitting the data to a linear equation using the least squares method.

Concentration (g/L) in the well	Sample 1	Sample 2	Sample 3
0	0.000	0.000	0.000
0.2	0.121	0.127	0.121
0.4	0.248	0.250	0.250
0.6	0.375	0.375	0.379
0.8	0.495	0.502	0.507
1.0	0.630	0.650	0.614
Slope = ε_{280nm} (g^{-1} L cm^{-1})	0.628	0.643	0.622
Intercept (cm^{-1})	-0.003	-0.004	0.001
Correlation coefficient (R^2)	0.9999	0.9995	0.9997

Table S8 The center mass, sigma, and correlation coefficient for the Gaussian fits to MALDI mass spectra of BSA, cleaved ovalbumin, and full-length ovalbumin and the apex mass of HEWL.

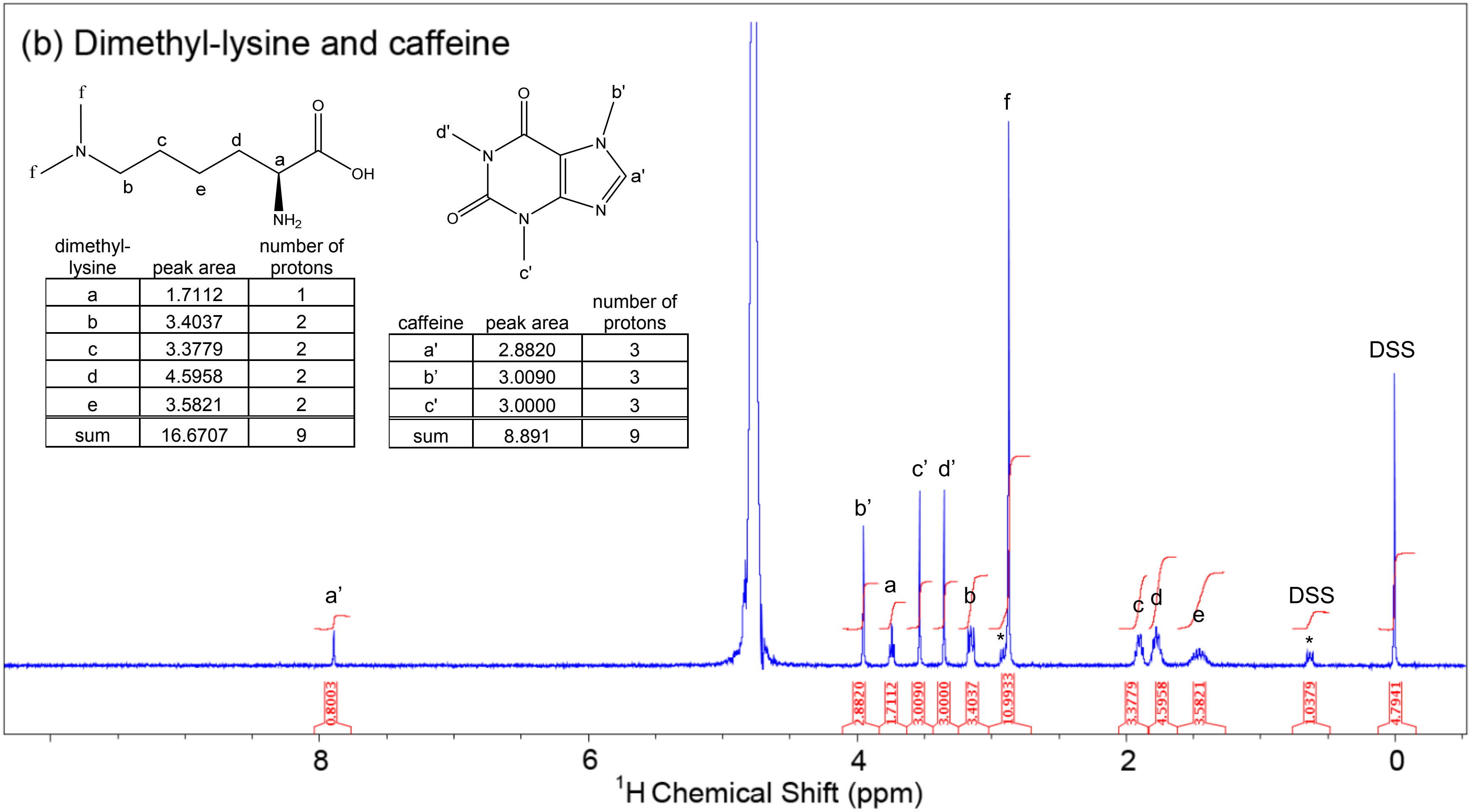
Protein	**Sample**	**Mass (m/z)**	**Sigma (m/z)**	**Correlation coefficient (R^2)**
BSA	(a) Unmodified	66,165	311	0.9433
	(b) Reductively methylated sample 1	67,547	349	0.9870
	(c) Reductively methylated sample 2	67,638	366	0.9765
	(d) Reductively methylated sample 3	67,650	291	0.9689
HEWL	(e) Unmodified	14,298	n/a	n/a
	(f) Reductively methylated sample 1	14,492	n/a	n/a
	(g) Reductively methylated sample 2	14,495	n/a	n/a
	(h) Reductively methylated sample 3	14,497	n/a	n/a
Cleaved ovalbumin	(i) Unmodified	39,899	300	0.9430
	(j) Reductively methylated sample 1	40,398	318	0.9246
	(k) Reductively methylated sample 2	40,403	304	0.7984
	(l) Reductively methylated sample 3	40,420	283	0.8296
Full-length Ovalbumin	(i) Unmodified	44,191	347	0.9042
	(j) Reductively methylated sample 1	44,661	300	0.8326
	(k) Reductively methylated sample 2	44,542	344	0.5071
	(l) Reductively methylated sample 3	44,736	261	0.7069

Supplemental Figure



lysine	peak area	number of protons
a	2.1453	1
b	4.2233	2
c	4.2816	2
d	5.5089	2
e	4.2287	2
sum	20.3878	9

caffeine	peak area	number of protons
a'	2.9748	3
b'	3.0535	3
c'	3.0000	3
sum	9.0283	9



dimethyl-lysine	peak area	number of protons
a	1.7112	1
b	3.4037	2
c	3.3779	2
d	4.5958	2
e	3.5821	2
sum	16.6707	9

caffeine	peak area	number of protons
a'	2.8820	3
b'	3.0090	3
c'	3.0000	3
sum	8.891	9

Fig. S1 ^{1}H NMR spectra of (a) “2 mM” lysine and (b) “2 mM” dimethyl-lysine each with DSS as a chemical shift reference and caffeine (1 mM) as an internal standard to determine the actual concentration of lysine (2.258 mM) and dimethyl-lysine (1.875 mM) using the peak areas (inset tables show the peak areas used to calculate the actual concentration of lysine and dimethyl-lysine).