SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Trypsin Digestion.

The protein samples were denatured in a solution of 8.0 M urea in 25 mM ammonium bicarbonate (NH_4HCO_3) at a concentration of 10 μg/μL. To reduce the protein samples, 195 mM DTT in 25 mM NH_4HCO_3 was added to a final DTT concentration of 9.3 mM. The mixtures were then allowed to react for an hour at room temperature. The cysteine residues were then alkylated by adding 195 mM IAA in 25 mM NH_4HCO_3 to a final IAA concentration of 31 mM and allowing the reaction to proceed at room temperature for one hour in the dark. To quench the alkylation reactions, 195 mM DTT in 25 mM NH_4HCO_3 was added for a total DTT concentration of 28 mM. The mixtures were then diluted by adding enough 25mM ammonium bicarbonate so that the final concentration of urea was 0.77 M. To digest the samples, trypsin dissolved in 25 mM NH_4HCO_3 was added to each sample for a protein to trypsin ratio of 50:1. The mixtures were allowed to digest overnight at 37°C. The digestion was quenched by adding enough 10% TFA to reach pH 3. The digested samples were then purified using a C18 solid phase extraction column (200mg/4mL, Ultra-Clean™ SPE C18, Grace, Deerfield, IL). Peptides were eluted from the C18 column in two steps, first in an aqueous solution of 50% ACN and 0.1% formic acid, then in an aqueous solution of 75% ACN and 0.1% formic acid. The fractions were combined before being evaporated to dryness using a vacuum concentrator.

RPLC-MS/MS of Tryptic Digests.

Gradient Separation.

The RPLC-MS run began with a 30 min. loading step at 1 μL/min 100% mobile phase A, followed by a binary gradient at 0.5 μL/min, t = 1 min 0% B, t = 60 min 60% B, t = 61 min 80% B, t= 65 min 80% B at 1 μL/min. This was followed in turn by a 19 min. reequilibration step at 0% B, 1 μL/min. Mobile phase A was composed of 0.1% formic acid in water while mobile phase B was composed of ACN with 0.1% formic acid.

MS/MS Settings.

Dynamic exclusion was enabled with a repeat count of 5, an exclusion duration of 3 min, and a repeat duration of 1 min. The MS and MS/MS data were searched against a small database of 131 proteins, most of which are standard research proteins, using TurboSEQUEST (v.27, revision 13). The search included partially tryptic peptides with 2 or fewer missed cleavages. The mass tolerance settings were 2.00 amu for peptides and 1.00 amu for fragment ions. The Xcorr vs. charge state filter was used at the peptide level. The Xcorr values chosen were 1.50, 2.00, and 2.50 for the +1, +2, and +3 charge states, respectively, as has been previously reported.[1, 2]

HILIC Analysis of Oct 4 peptide.

For hydrophilic interaction chromatography (HILIC) analysis, the peptide TSIENR was run on an in-house 50 μm ID capillary column packed with 3 μm, 200 Å pore size, PolySulfoethyl A stationary phase (LCPackings, Dionex, Sunnyvale, CA). The LC gradients started at 100% mobile phase B (0.2% v/v formic acid in acetonitrile) and ramped to 40% mobile phase B linearly. The same triple quadrupole SRM transitions were monitored as described for the RPLC analysis of the Oct4 peptide.

RESULTS AND DISCUSSION

Modification of Tryptic Digests of Proteins.

	Sequence Coverage		
Protein	**Unmod**	**Hexanal**	**Combined**
cytochrome c	48%	9%	48%
β-lactoglobulin A	46%	64%	70%
BSA	48%	29%	60%

Table 1. Sequence Coverage of Hexylated Tryptic Digests. This table is analogous to Table 2 in the paper, but the results shown here are for hexylated tryptic digests rather than butylated ones. While most modified samples show lower sequence coverage than their corresponding unmodified samples, hexylated β-lactoglobulin A actually shows higher sequence coverage than its unmodified form. As with the butylated digests, the hexylated digests of β-lactoglobulin A and BSA yield an increase in combined sequence coverage. For cytochrome c, however, the combined sequence coverage did not increase from that of the unmodified sample because only one peptide was detected in the hexylated form and this peptide was also found in the unmodified form. This poor result could be due to cytochrome c's small size and its relatively few tryptic peptides, which may not provide a good, representative set to determine the effects of hexylation.

References.

(1) Jiang, X.; Dai, J.; Sheng, Q.; Zheng, L.; Xia, Q.; Wu, J.; Zeng, R. *Mol. Cell Proteomics*. **2005**, *4*, 12-34.

(2) Peterman, S. M.; Dufresne, C. P.; Horning, S. *J. Biomol. Tech.* **2005**, *16*, 112-124.