

## NeuCode™ SILAC

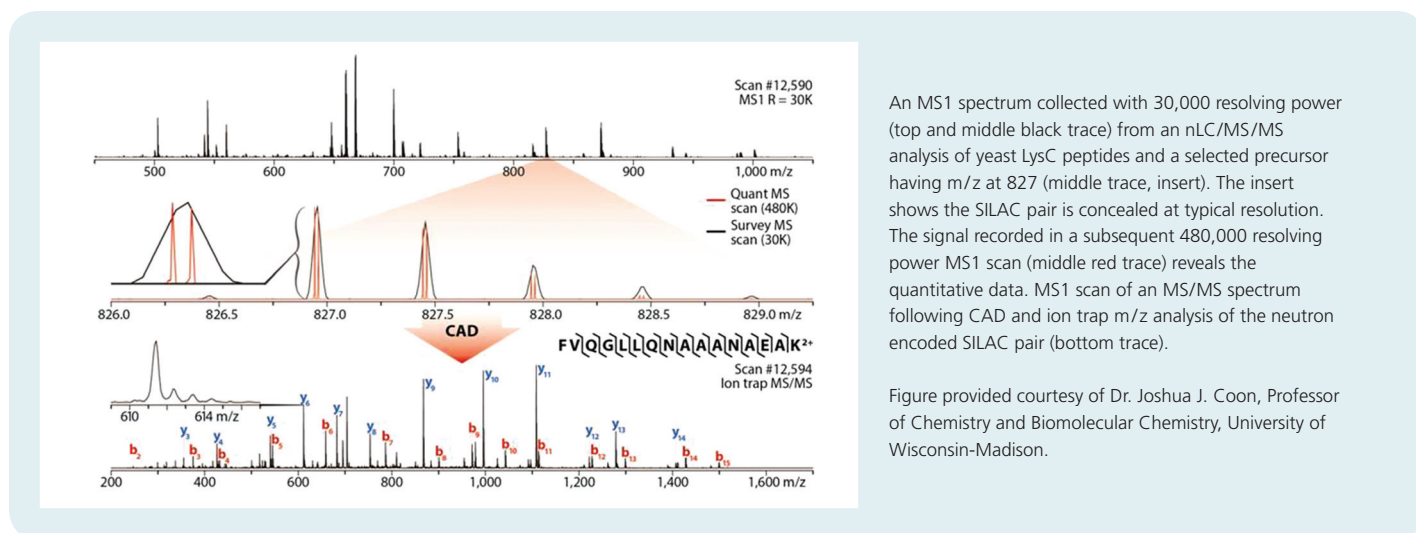
Although SILAC has enjoyed great success as a quantitative tool for MS-based proteomics, it does fall short of the higher levels of multiplexing that are capable using well-known isobaric tagging reagents, such as TMT® and iTRAQ. In fact, SILAC is limited in analyzing two, or at best, three samples simultaneously, thereby making SILAC, although information-rich, an inherently low-throughput technique. Fortunately, metabolic labeling in cell culture has recently been profoundly improved with the advent of NeuCode™ SILAC.<sup>1</sup> NeuCode™ SILAC exploits the subtle mass differences in common stable isotopes, *i.e.* mass defect, which ultimately allows for higher levels of multiplexing than conventional SILAC. NeuCode™ SILAC requires specially chosen isotopologues of lysine and other SILAC-type amino acids so that individual peptide signals become revealed under high mass resolving power (>100,000), thereby providing quantitative

data within a very small mass space (<40 mDa). Under routine resolution settings, however, the quantitative data is concealed and spectral complexity is markedly reduced compared to traditional SILAC. As new NeuCode™ SILAC amino acid isotopologues become available, the multiplexing capabilities of SILAC will continue to increase.

NeuCode™ is a trademark of the Wisconsin Alumni Research Foundation (WARF).

### References

1. Hebert, A.S.; Merrill, A.E.; Bailey, D.J.; Still, A.J.; Westphall, M.S.; Strieter, E.R.; Pagliarini, D.J.; Coon, J.J. **2013**. *Journal Nat Methods*, doi: 10.1038/1nmeth.2378 (epub ahead of print)



An MS1 spectrum collected with 30,000 resolving power (top and middle black trace) from an nLC/MS/MS analysis of yeast LysC peptides and a selected precursor having m/z at 827 (middle trace, insert). The insert shows the SILAC pair is concealed at typical resolution. The signal recorded in a subsequent 480,000 resolving power MS1 scan (middle red trace) reveals the quantitative data. MS1 scan of an MS/MS spectrum following CAD and ion trap m/z analysis of the neutron encoded SILAC pair (bottom trace).

Figure provided courtesy of Dr. Joshua J. Coon, Professor of Chemistry and Biomolecular Chemistry, University of Wisconsin-Madison.