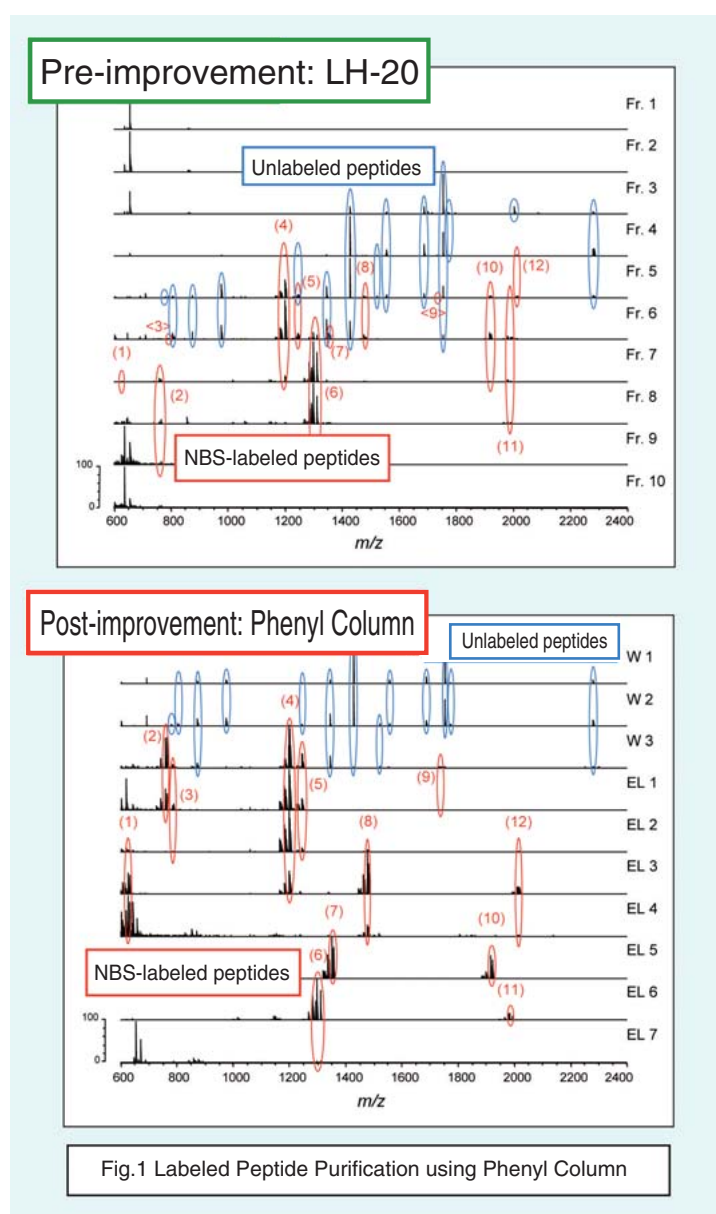


Stable Isotope Labeling Method (NBS*) Protocol Improvement

Previously, we developed a new quantitative proteome analysis method (NBS protocol)¹⁾ based on stable isotope labeling. In the NBS method, a reagent ($^{12}\text{C}_6\text{-NBS}$ or $^{13}\text{C}_6\text{-NBS}$; 2-nitrobenzenesulfonyl chloride) with specific reactivity to tryptophan was utilized to perform labeling and, by introducing a stable isotope, the mass difference between the 2 reagents (=6 Da) was used to quantitate each of the proteins. We modified this NBS protocol, as introduced below, to obtain greatly improved detection sensitivity and also introduce novel methodology for targeted labeled peptide purification and separation²⁾.



There are two main points of improvement.

The first is optimization of the protocol so that either 8M urea or 6M guanidine hydrochloride can be used for the denaturant, thereby keeping sample loss to a minimum. The second improvement is the use of a phenyl column for labeled peptide enrichment, greatly improving labeled peptide purification and separation.

As shown in Fig. 1, it is evident that use of the phenyl column provides efficient separation/enrichment of the NBS-labeled peptides (○) versus the unlabeled peptides (○).

In addition, certain level of separation of labeled peptides is also achieved.

* For details on NBS method, refer to AXIMA Application No. 12 and 16.

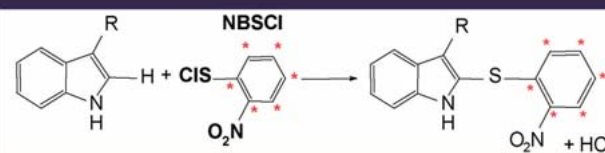
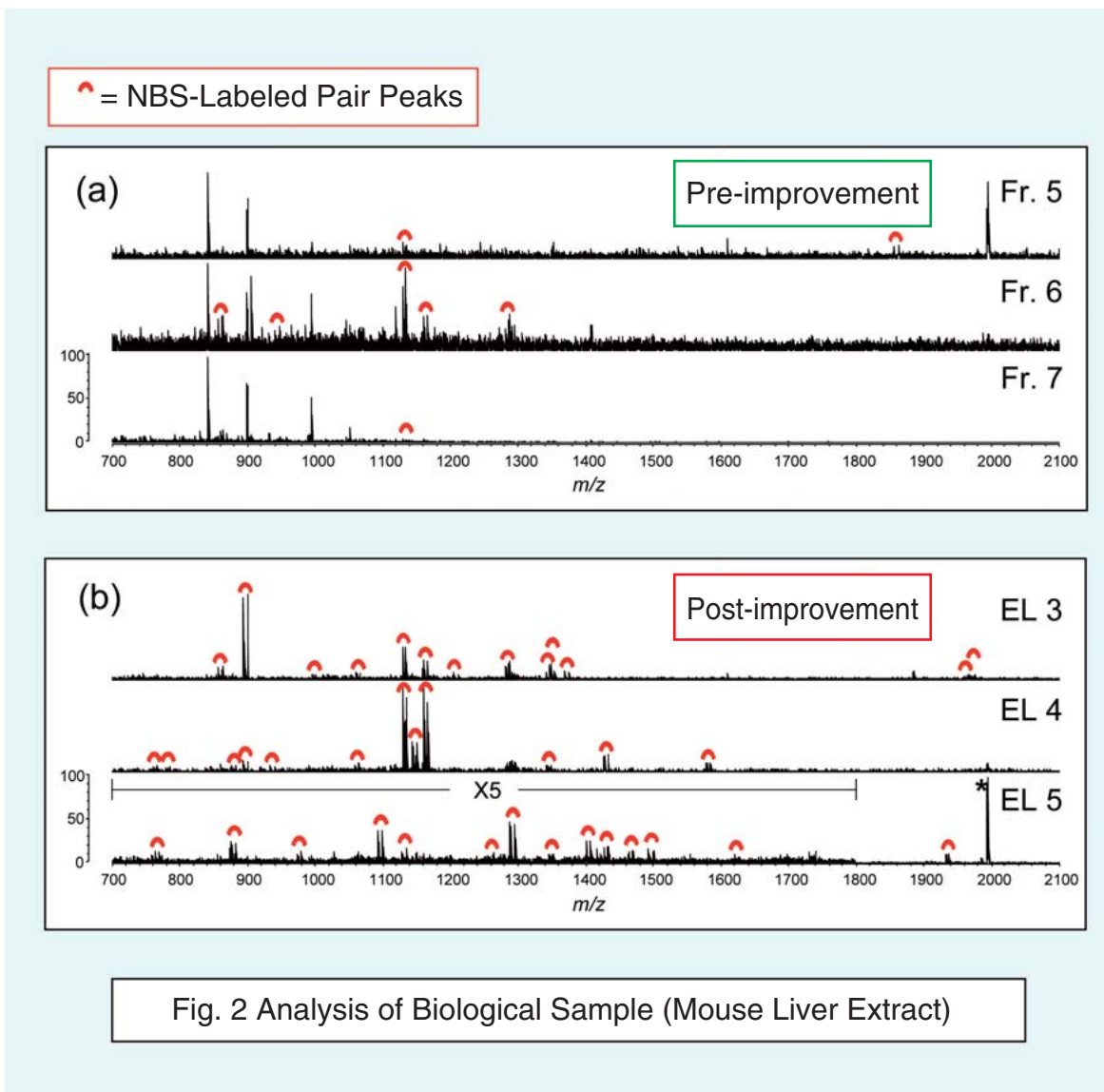


Fig. 2 shows an example of an experiment we conducted using an actual biological sample (protein extract from mouse liver). With the improved protocol (b), sample loss is suppressed and labeled peptide purification and separation are improved, enabling about a five-fold increase in the number of measured labeled peaks (^).

Moreover, a matrix suitable for MALDI-TOF MS measurement of NBS-labeled peptides was discovered³⁾, and using this, detection of labeled peptides at even higher sensitivity has become possible. For details, refer to AXIMA Application No. 23 or Reference 3), below.



References

- 1) H. Kuyama et al., *Rapid Commun. Mass Spectrom.* **17**, 1642-1650 (2003)
- 2) E. Matsuo et al., *Rapid Commun. Mass Spectrom.* **20**, 31-38 (2006)
- 3) E. Matsuo et al., *Proteomics* **6**, 2042-2049 (2006)



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