Using the Empore™ C18 StageTips for High pH Peptide Fractionation in Global Proteomics

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Abstract
This application note demonstrates how the Empore C18 StageTips could be applied to high pH peptide fractionation in global proteomics with significantly increased peptide and protein identification.

Introduction
Extensive off-line peptide fractionation to reduce the complexity of proteome is critical to In-depth global proteome profiling. However, the fractionation of protein samples in the sub-microgram range can be limited owing to sample loss during the fractionation process. Thus, it is essential to develop efficient microscale fractionation strategies. High-pH peptide fractionation is one of the methods among these efforts. The previous methods are either to use Pump-based approach (e.g., using off-line HPLC) which is sophisticated and expensive, or to use beads-based approaches which are unreliable (e.g., need to weight certain amount of beads material and do packing every time). Here, a novel high-pH peptide fractionation method by using Empore C18 StageTips is demonstrated. The results show the method is simple, reliable and robust.

Experiment Setup
HeLa cells were originally obtained from ATCC. Frozen cell pellets were lysed. Lysates were cleared by centrifugation for 2 mins at 4°C; the supernatant was transferred to a new tube and cleared by centrifugation at full speed for 15 mins at 4°C. Empore C18 StageTips (CDS Analytical, Model # 6091) was used for desalting and high-pH fractionation. 10mM ammonium formate, pH 10 and 2% ACN were used as buffer. Analysis of peptide mixtures was performed on an Ultimate 3000 nano LC and Q Exactive mass spectrometer system coupled to a FLEX nano-electrospray ion source (all components were from Thermo Scientific). The raw files acquired by the LCMS were processed using the Proteome Discoverer platform (Thermo Scientific) and the Max Quant software.

Results and Discussion
The results have been showed in Figure 1 and 2. The data shows that high pH fractionation increased the number of protein identification by > 50%, and the sequence coverage of most of the identified proteins increased > 10%. At least five or six fractions should be applied (5%, 10%, 15%/25%, 40%, 80% acetonitrile in 10 mM NH4F, pH=10). Empore C18 StageTips-based high pH fractionation is convenient, easy-to-manipulate and pump-free. Compared with current high pH fractionation methods usually apply off-line HPLC pump, or pack C18 resins into tips, C18 StageTips method is much simple and cost-effective. This method was reported for global proteomics at the first time.

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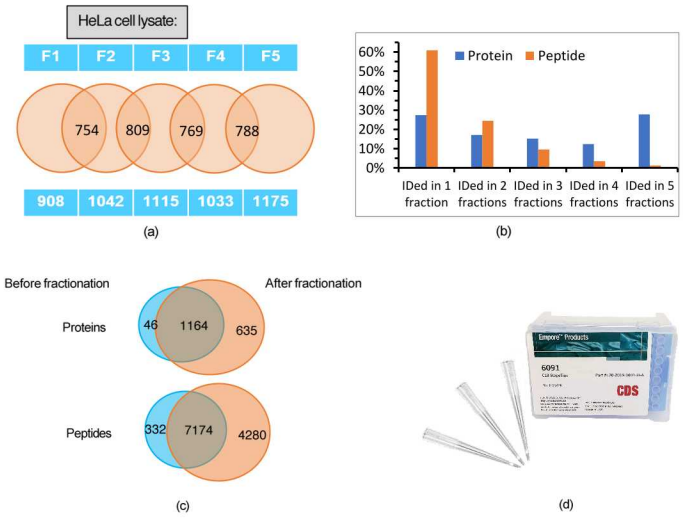

Figure 1: Empore C18 StageTips-based high-pH fractionation significantly increases identification rate on both protein and peptide levels.

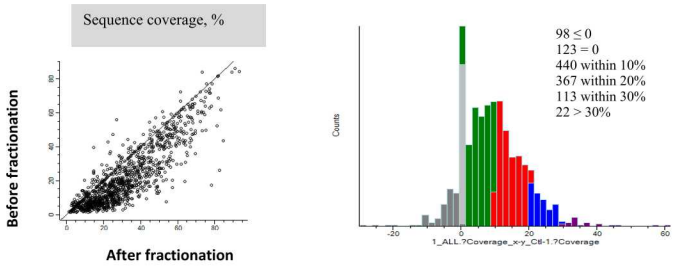

Figure 2: Protein sequence coverage increases after high-pH fractionation