# Rapid and Automatable Desalting of Proteins and Protein Complexes by Size Exclusion Chromatography for On-Line Detection by Native Mass Spectrometry

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nMS→SB RESOURCE FOR NATIVE MS GUIDED STRUCTURAL BIOLOGY

## Online Buffer Exchange (OBE) Coupled to Native MS

- Online buffer exchange (OBE) allows for analysis of proteins and protein complexes present in non-volatile buffers in < 3 minutes/sample.

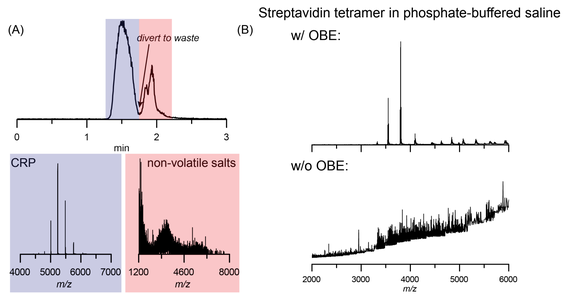


**Figure 1.** (A) total ion chromatogram and mass spectra showing the separation of C-reactive protein (CRP) from non-volatile salts by OBE. (B) Mass spectra of streptavidin stored in a non-volatile buffer with and without the use of OBE.

## OBE Experimental Setup

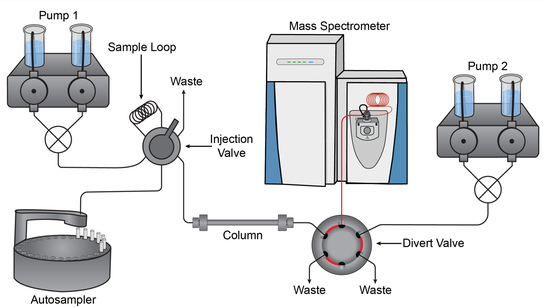


**Figure 2.** An HPLC is used to deliver 100 µL/min of 200 mM ammonium acetate to a size exclusion chromatography (SEC) column (self-packed or commercially available). A divert valve and secondary HPLC pump are used to divert non-volatile salts to waste, preventing them from entering the mass spectrometer (Exactive Plus EMR, Thermo Scientific).

## OBE Removal of Various Non-Volatile Buffers

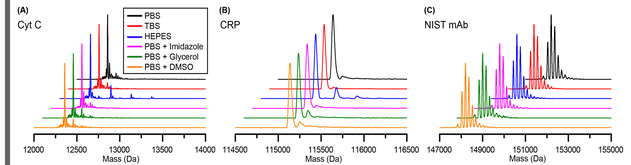


**Figure 3.** Deconvoluted mass spectra of (A) cytochrome c, (B) CRP, and (C) NIST mAb in various buffers (phosphate-buffered saline (PBS), tris-buffered saline (TBS), HEPES, and non-volatile additives (imidazole, glycerol, and DMSO). In all cases, the main peak corresponds to adduct-free protein while minor peaks are due to non-volatile adducts. In the case of NIST mAb, all glycoforms are clearly resolved.

## OBE Performance with Different Columns

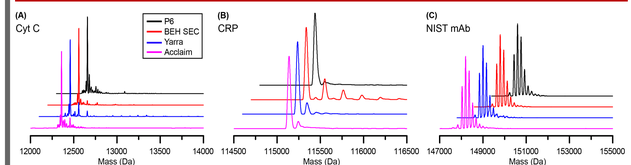


**Figure 4.** Deconvoluted mass spectra of (A) cytochrome c, (B) CRP, and (C) NIST mAb in PBS and desalted using different columns. Self-packed columns (0.75 x 120 mm) with P6 material (Bio-Rad) were compared to Acquity BEH SEC-125 (4.6 x 30 mm, Waters), Yarra SEC-3000 (2.1 x 50 mm, Phenomenex) and Acclaim SEC-300 (4.6 x 30 mm, Thermo Scientific) columns.

## Limit of Detection of OBE Method

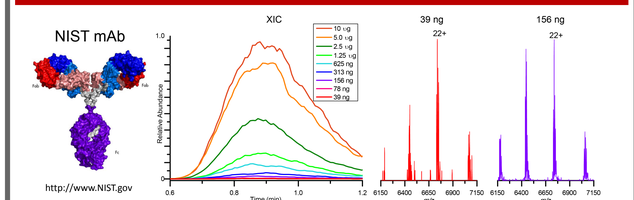


**Figure 5.** Extracted ion chromatograms (XIC) from a series of NIST mAb experiments with different injection amounts. Mass spectra from the 39 ng injection (red) and 156 ng injection (purple) are shown to the right.

## OBE Analysis of an Overexpression Cell Lysate

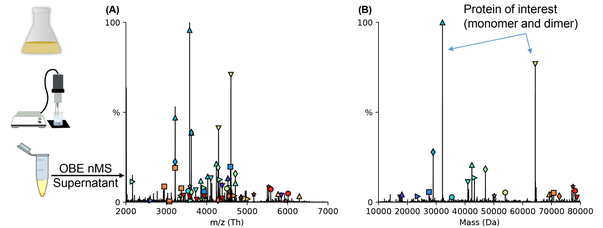


**Figure 6.** Protein overexpression in *E. coli* and subsequent OBE analysis of the crude lysate yields congested spectra (A), but deconvolution reveals monomers and dimers of the protein of interest (B).

## Analysis of Fe-S Cluster Proteins by OBE

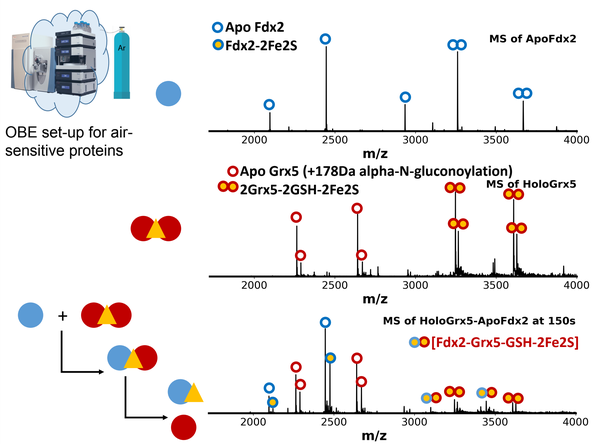


**Figure 7.** Analysis of [2Fe2S] cluster transfer from holo Grx5 homodimer to apo human ferredoxin2 (Fdx2). Spectra of apo Fdx2, holo Grx5 dimer, and transfer reaction products are shown. The products indicate formation of a holo heterodimer [Fdx2-Grx5-GSH-2Fe2S] complex.

## OBE as a Rapid Screening Method of Protein Complexes

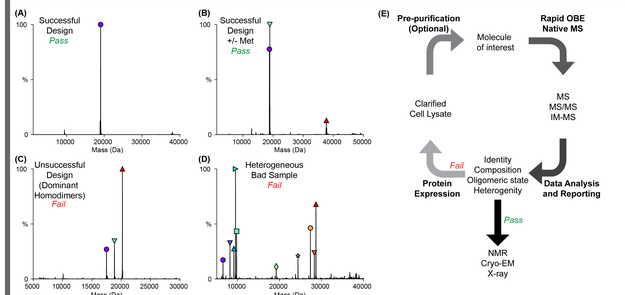


**Figure 8.** (A,B) OBE can be used to rapidly screen protein complexes, for example to differentiate successfully designed heterodimers and (C,D) failed heterodimer designs.[1] (E) OBE can be used to bridge the gap between protein expression and more time-consuming protein characterization techniques.

## Conclusions

- ❖ OBE permits analysis of protein/protein complex samples stored in non-volatile buffers in < 3 min.
- ❖ OBE efficiently removes a range of non-volatile buffers and can be performed using many different types of SEC column types.
- ❖ OBE can be a valuable screening method for bridging the gap between protein expression and more time-consuming structural biology methods.

## References

(1) Chen et al. Programmable Design of Orthogonal Protein Heterodimers. *Nature* **2019**, *565* (7737), 106.

Mass spectra were deconvoluted using UniDec and Intact Mass:

- Marty et al. Bayesian Deconvolution of Mass and Ion Mobility Spectra: From Binary Interactions to Polydisperse Ensembles. *Analytical Chemistry* **2015,** 87 (8), 4370–4376.
- Bern et al. Parsimonious Charge Deconvoluton for Native Mass Spectrometry. *J. Proteome Res.* **2018**, 17 (3), 1216–1226. (https://www.proteinmetrics.com)

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Information Request

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