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Rapid Online Buffer Exchange for Protein Screening

Solutions for high throughput analysis of large biomolecules by native mass spectrometry

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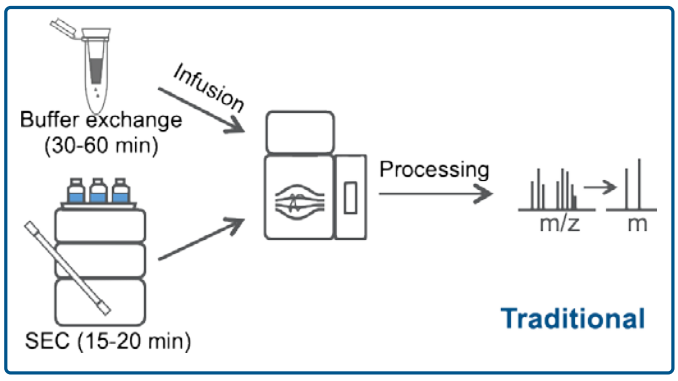
Purpose

- Adopt a workflow for rapid sample screening[1] using novel online buffer exchange (OBE) columns coupled to native mass spectrometry (nMS)
- Determine the identity and purity of proteins and protein complexes for further structural characterization or optimizing upstream/downstream process

Methods

- Proteins were prepared using Thermo Scientific™ VitroEase™ Buffer Screening Kit
- Native OBE- MS analysis was performed using either a Thermo Scientific Vanquish™ Flex UHPLC System coupled to Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap Mass Spectrometer or a Thermo Scientific Orbitrap Eclipse™ Tribrid™ Mass Spectrometer
- Data were analyzed using Thermo Scientific BioPharma Finder™ 4.0 Integrated Software



Primary challenges

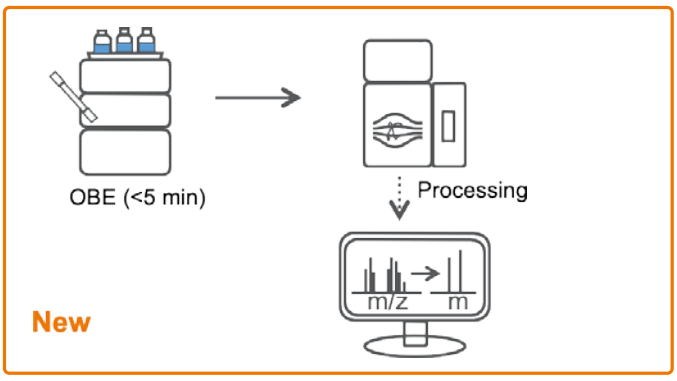
- Non-volatile buffer and salts in protein samples incompatible with MS analysis
- Off-line buffer exchange is time consuming
- Buffer screening with cryo-EM is laborious and expensive

Prior efforts

- OBE using custom made P6 columns[1]
- Online SEC to separate proteins from nonvolatile molecules prior to MS
- Offline buffer exchange to exchange proteins into MS-compatible buffers

Novel approach

- Minimal sample prep with VitroEase™ buffer screening kit
- Fully automated online buffer exchange LC-MS method (< 5 min) using prototype OBE columns



Results

- Obtained protein MW and structural information by OBE-nMS in less than 5 min
- We successfully applied this workflow for fast sample screening strategy for quality control and optimal sample preparation conditions for upstream applications such as cryoEM

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[1]Van Aernum et al *Nat Protoc*. 2020 Mar;15(3):1132-1157

Materials and Methods

	Buffer#	Content (10x)
	1	$C_2H_3NaO_2$ (0.5M), NaCl (1.5M), pH 3.6
	2	$C_2H_3NaO_2$ (0.5M), NaCl (3M), pH 3.6
✓	3	MES (0.5M), NaCl (1.5M), pH 5.5
	4	MES (0.5M), NaCl (3M), pH 5.5
	5	Tris-HCl (0.5M), $Mg(CH_3COO)_2$ (0.1M), NaCl (1.5M), pH 7.2
	6	Tris-HCl (0.5M), $MgCl_2$ (0.1), CH_3CO_2K (1.5M), pH 7.5
✓	7	Tris-HCl (0.5M), $Mg(CH_3COO)_2$ (0.1M), KCl (3M), pH 7.2
	8	HEPES (0.5M), NaCl (1.5M), pH 7.4
	9	HEPES (0.5M), KCl (3M), pH 7.4
	10	HEPES (0.5M), $Mg(CH_3COO)_2$ (0.1M), CH_3CO_2K (1.5M), pH 7.4
✓	11	HEPES (0.5M), $MgCl_2$ (50mM), CaC_2 (50mM), NaCl (1.5M),pH 7.4
✓	12	PBS (1.37M NaCl 270mM KCl, 43mM Na_2HPO), pH 7.4
	13	Bicine buffer (0.5M), NaCl (1.5M), pH 8.5
	14	CAPSO (0.5M), KCl (3M), pH 8.9

Note: The colors in the left column correspond to the colors of the vial caps in the VitroEase kit.

Sample preparation

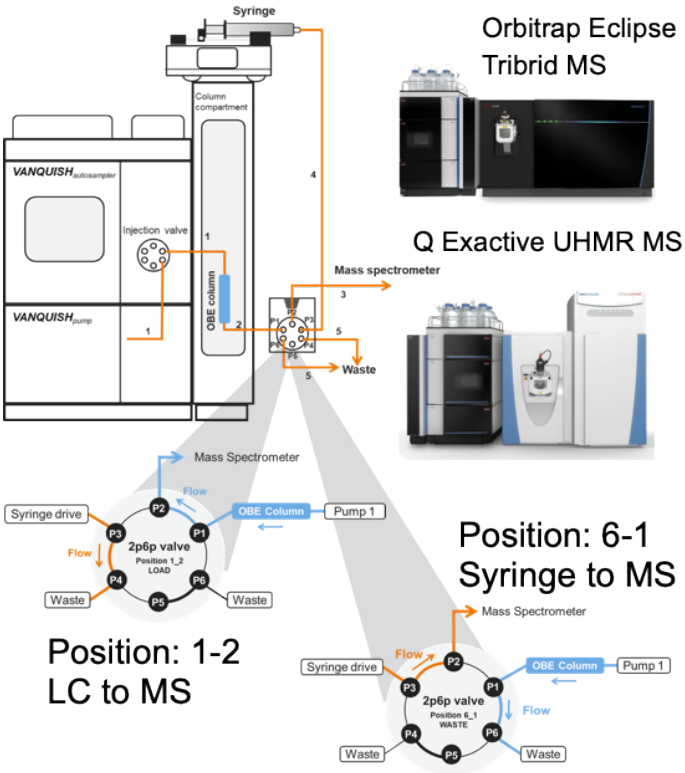
- Buffer screening:
  9 µL protein (1ug/ul) + 1 µL buffer (10×)
- Detergent screening:
  9 µL protein (1ug/ul) + 1 µL buffer (10×) + 1 µL detergent (1 CMC)

VitroEase buffer screening kit (A49856)

	Detergent #	Content (10x)
✓	A	CTAB (0.3%)
✓	B	CHAPS (4.9%)
✓	C	OG (2.7%)
	D	Tween-20 (0.1%)
✓	E	DM (1%)
✓	F	FOM (0.7%)

LC-MS setup

- LC – Vanquish Flex UHPLC system
- MS - Orbitrap Eclipse Tribrid MS Q Exactive UHMR MS



LC method

- Mobile phase: 200 mM AmAc
- Column: OBE 80 Å , 5cm
- Flow rate: 100 µL/min
- Loading: 1 to 2 ug

Divert value

Time	Position	Flow
0	1-2	LC to MS%)
0.85	1-6	LC to waste, Syringe to MS
2.5	1-2	LC to MS
3.0		End of run

MS method

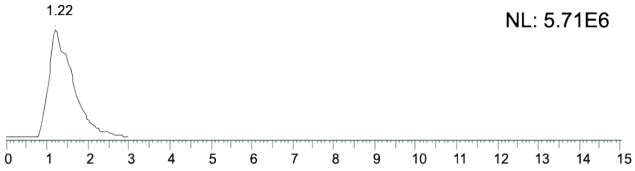
	Eclipse	UHMR
m/z	2000-8000	2000-20000
Source desolvation	Source compenstation 0.1	In-source CID 10 In-source trapping 50
Trap gas	20 mtorr	5

NOTE: Specific MS method is sample-dependent.

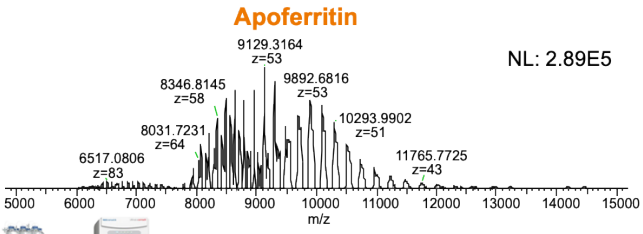
Results: Online Buffer Exchange vs Size Exclusion Chromatography

OBE-3 min

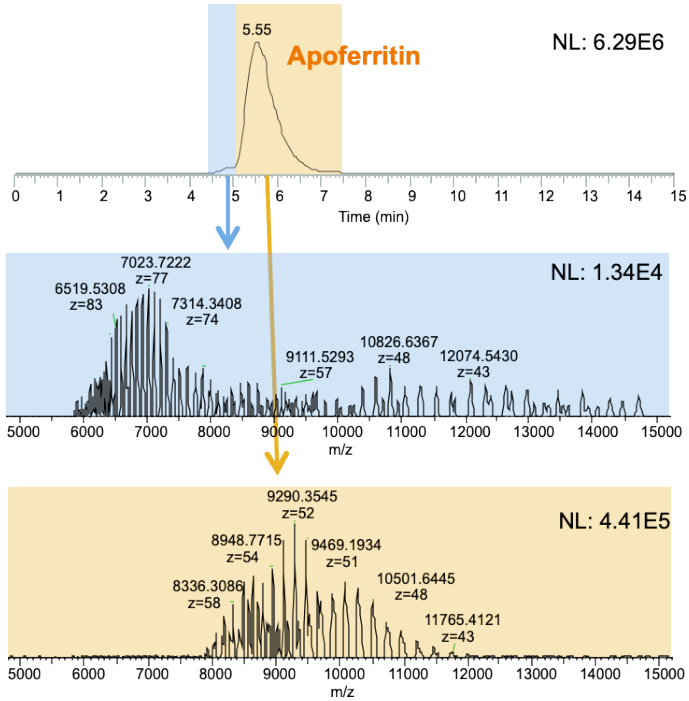
- Fast screening (<5 min) for quality control



- Limited separation provides complete profiles in one spectrum

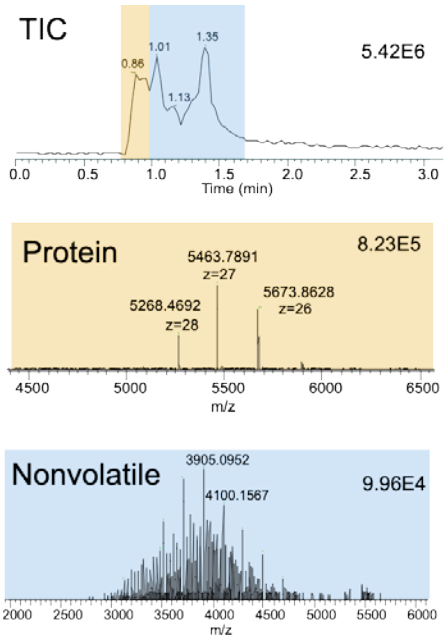


SEC-15 min

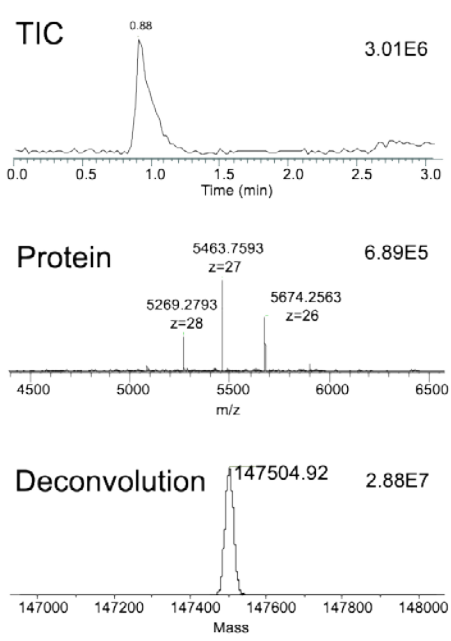


Results: Divert Time Optimization Using Alcohol Dehydrogenases (ADH)

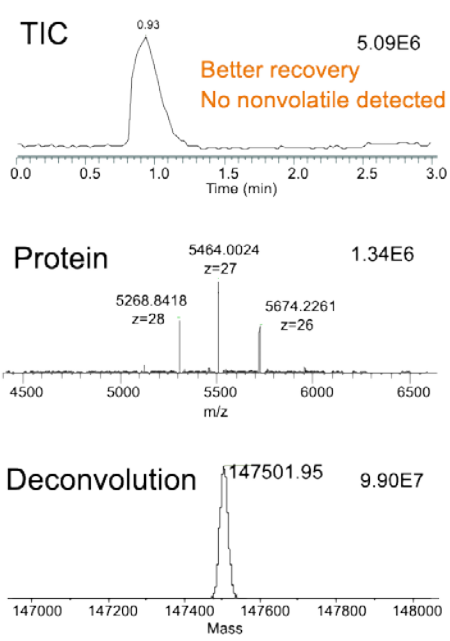
No Divert



Early Divert -0.8 min



Optimized Divert -0.85 min



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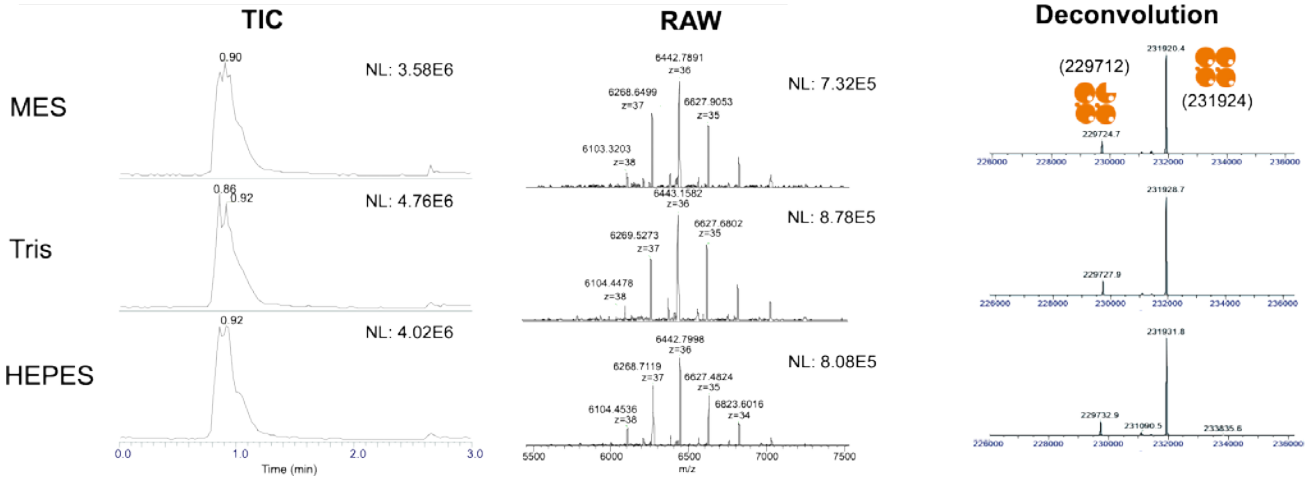
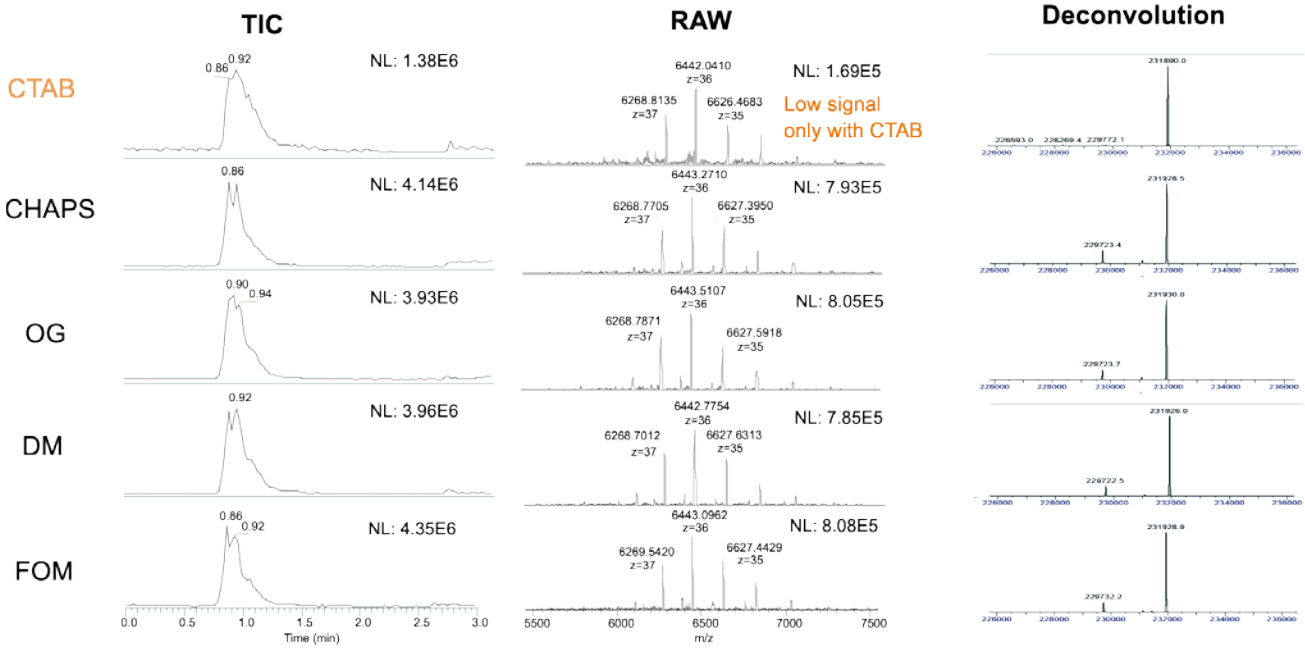
Results: Screening of Pyruvate Kinase Using VitroEase Buffer Screening Kit

	Average MW	Modification
	57942.85	MetOFF, S400A, N-Acetyl
	58018.96	MetOFF, S400A, N-Acetyl, C165 [mercaptoethanol]
	55731.41	MetOFF, S400A, N-Cleavage, N-Acetyl
	55801.40	MetOFF, S400A, N-Cleavage, N-Acetyl, C165 [mercaptoethanol]

Orbitrap Eclipse Tribrid MS



Results: Screening of Pyruvate Kinase using VitroEase Buffer Screening Kit with Detergents



Conclusion

- Developed rapid online buffer exchange coupled to native mass spectrometry workflow using novel OBE column for protein MW and structure screening
- Fully automated method to enable one sample screening < 5 min
- VitroEase buffer screening kit enables efficient cryo-EM sample screening for optimal grid analysis
- Applicable for fast buffer screening of cryo-EM sample as well as optimizing protein process condition

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