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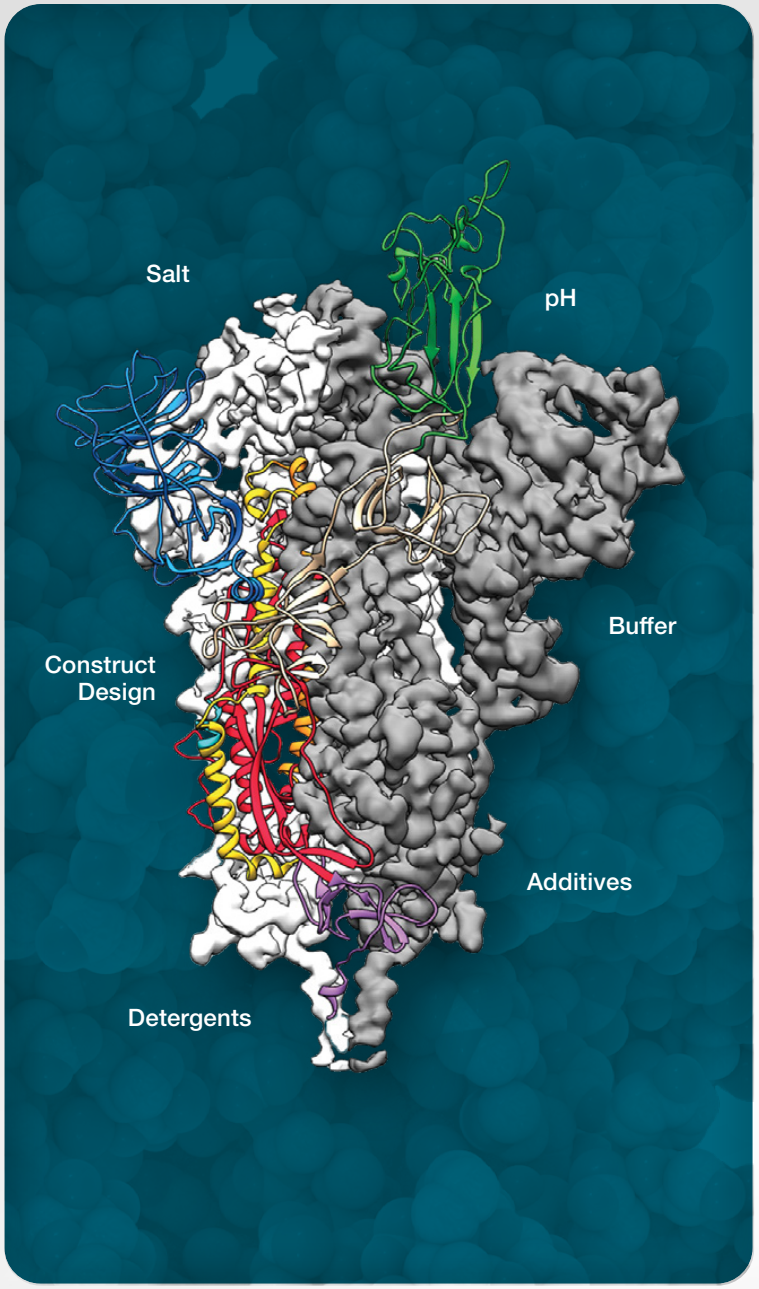
Accelerate structural elucidation

OptiMSe your workflow for faster sample screening



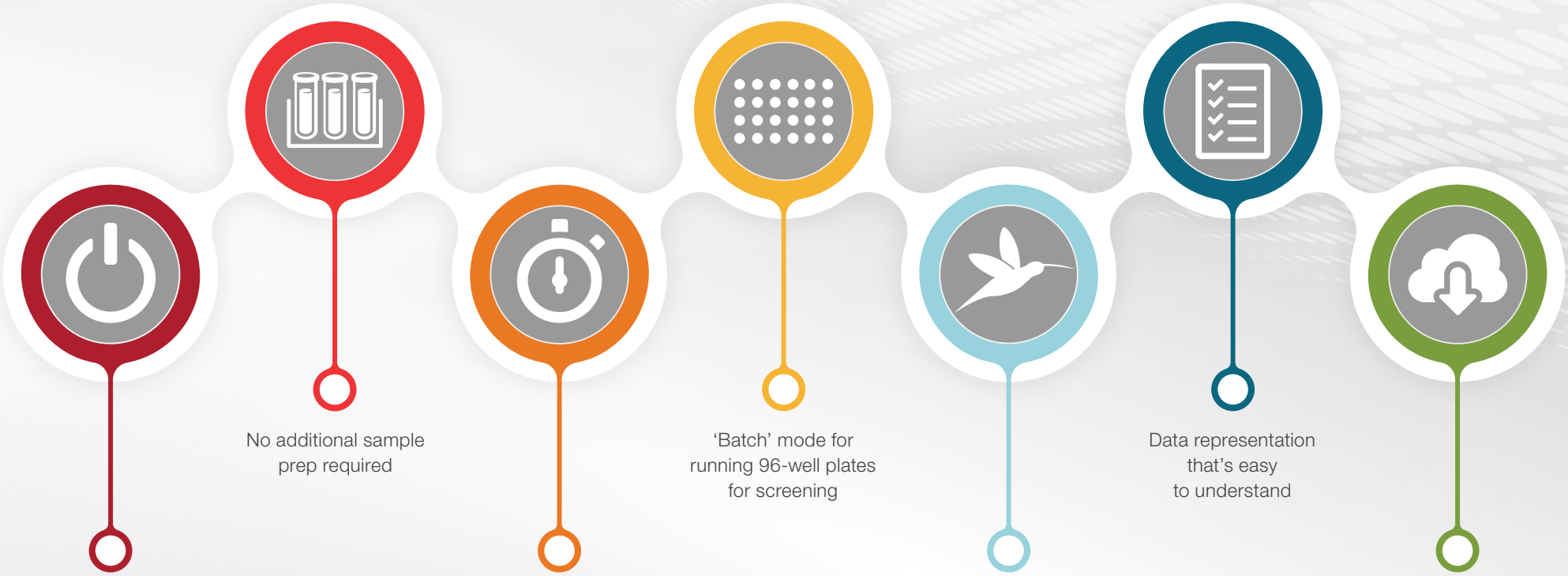
Streamline sample-to-structure with optimized sample preparation

Cryo-EM is a revolutionary tool that is increasingly being used to determine the 3D structure of proteins and their complexes. In the last decade, innovation in hardware and software have allowed cryo-EM to achieve atomic resolution at increasingly higher throughput. As ever more challenging proteins and complexes are being studied by cryo-EM, so increases the need for more robust ways to prepare cryo-EM grids through a process called vitrification, wherein the protein is embedded in a thin layer of vitreous ice.

One aspect that is sometimes overlooked when preparing cryo-EM grids is the correlation between the quality of the sample and the vitrification process. A stable and homogeneous protein sample is much less likely to encounter artifacts during vitrification, like aggregation or dissociation of a protein complex. However, to obtain an ideal sample for cryo-EM, there are many different aspects that require optimization such as ionic strength, use of detergents or additives, and sometimes even construct design.

To find the best conditions, samples are typically vitrified and then screened using a transmission electron microscope. If microscope time is not available, a negative stain screening can be used to embed the sample in a thin layer of heavy metal stain. Unfortunately, this process can introduce artifacts like aggregation and a direct correlation to the results in cryo-EM can be difficult to establish.

The Thermo Scientific™ OptiMSe Workflow is a fast, automated, and intuitive way to eliminate unpromising samples for vitrification and cryo-EM, leveraging the power of native mass spectrometry.



The OptiMSe Workflow

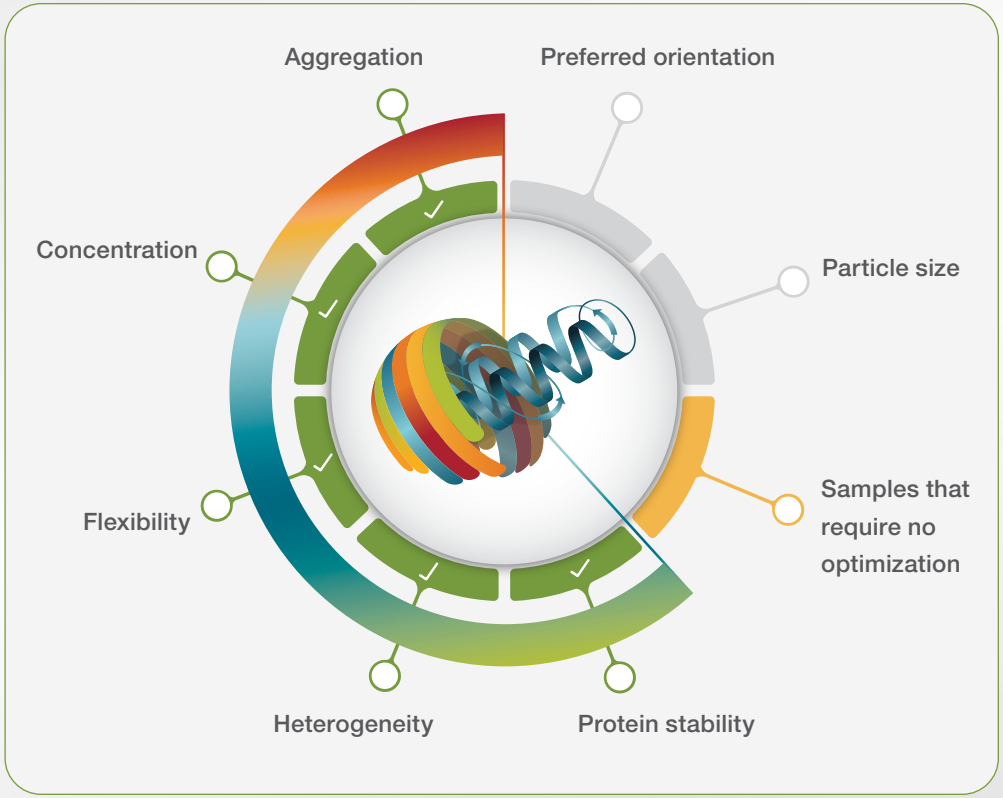
The OptiMSe Workflow
Automated sample screening powered by native MS

Good protein preparation is critical to downstream success for cryo-EM. Optimizing the biochemistry for your protein sample can reduce artifacts during vitrification, such as denaturation, unfolding or reduced structural resolution. The Thermo Scientific OptiMSe Sample Screening Workflow removes guesswork and provides a fully automated sample screening step that can save you time and resources, helping you to select the best quality samples for your microscopic analysis.

Utilize the power of native MS for sample screening

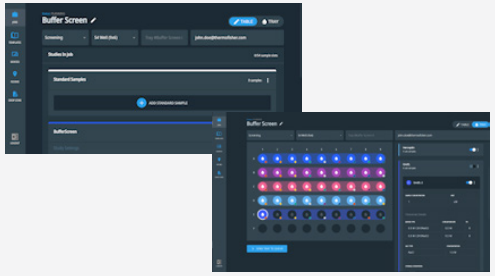
Native mass spectrometry (native MS) is a powerful technique that allows for thorough characterization of proteins and their complexes.[1] Unlike most common structural biology techniques, native MS does not average but rather reveals all proteoforms and conformations at the same time. This requires gentle transfer of the protein from solution to the gas phase through a process called electrospray ionization, which does not perturb the structure of the protein or complex studied, thus revealing the state of the protein in solution.

Cryo-EM sample prep issues



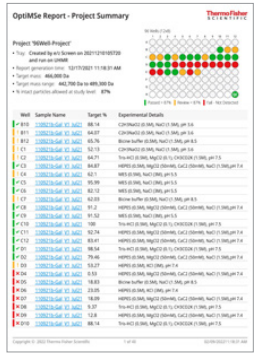
Many of the commonly occurring cryo-EM sample preparation challenges can be identified with native MS

Structural Biologist (Sample Creator)



Thermo Scientific™ OptiMSe SPOT Software

Non-expert report



Mass Spec Operator (Analyst)

Automated data acquisition

OptiMSe 1.0 Software

Instant data processing and scoring

How can native MS help with sample screening?

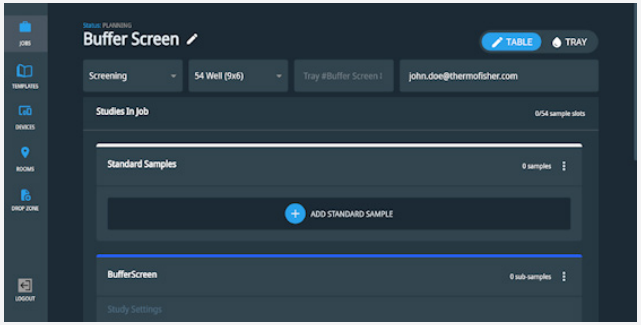
Only a small fraction of samples for cryo-EM will be suitable to yield a structure. Of the seven commonly observed blocking sample preparation issues for cryo-EM success, the OptiMSe Workflow is able to identify five of them and help you move only the most promising samples forward, allowing you to focus on what matters: using your microscopy time to solve structures.

Focus on the results, not the process

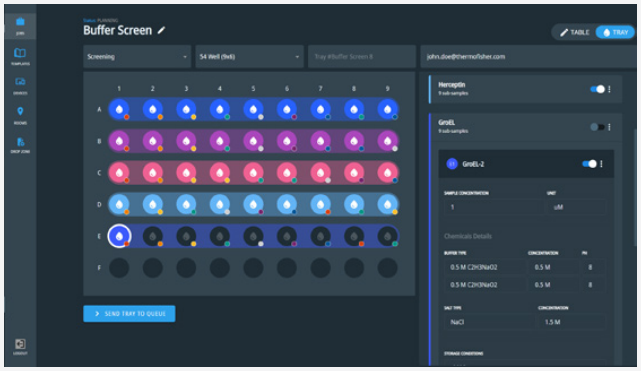
The OptiMSe Workflow was designed to seamlessly integrate in your process. Even if you are not a native MS expert, the OptiMSe Workflow will assist you by preparing LC queues and tuning the instrument while its automated data deconvolution and report generation give you on-the-fly results.

The OptiMSe Workflow
Automated native MS for cryo-EM sample screen

Guided Experiment Design

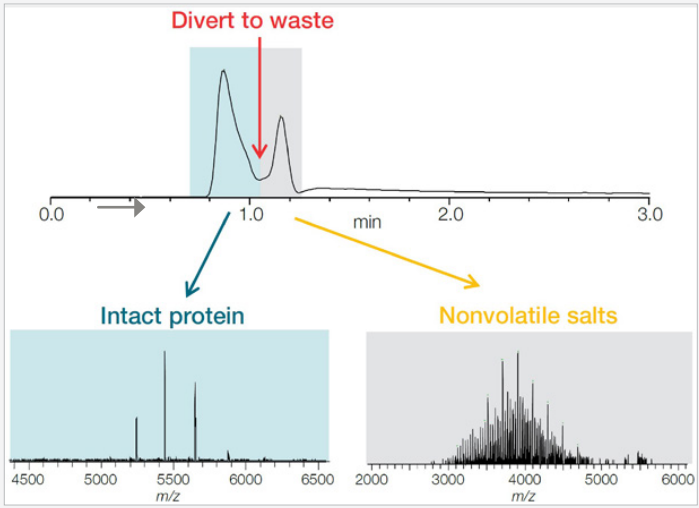


Easily add samples for screening using pre-populated buffer conditions from OptiMSe SPOT Software. The Thermo Scientific™ VitroEase™ Buffer Kit offers numerous buffer combinations and supports custom conditions as well.



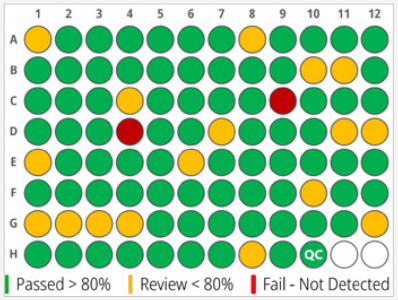
OptiMSe SPOT Software provides electronic screening and visual cues to help you save time and eliminate manual recording.

Online Buffer Exchange ▶ Automated Acquisition



Fast online buffer exchange with NativePAC OBE-1 columns enable 3-5 minute runs.[2,3] When coupled to high resolution accurate mass Thermo Scientific™ Orbitrap™ technology even the most challenging proteins and complexes can be analyzed quickly and easily.

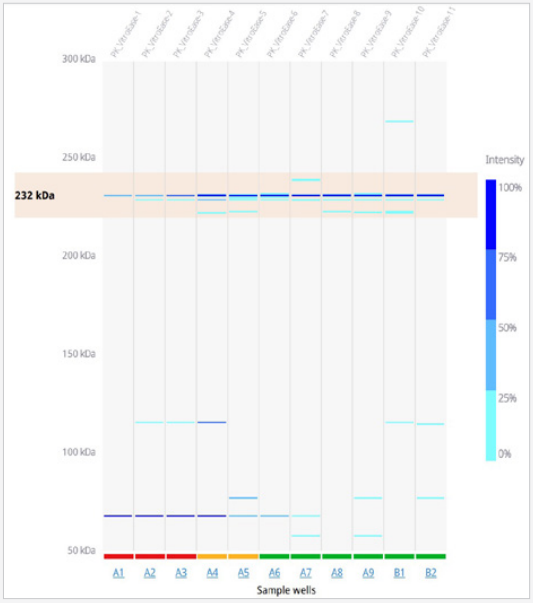
Automated Processing/Reporting



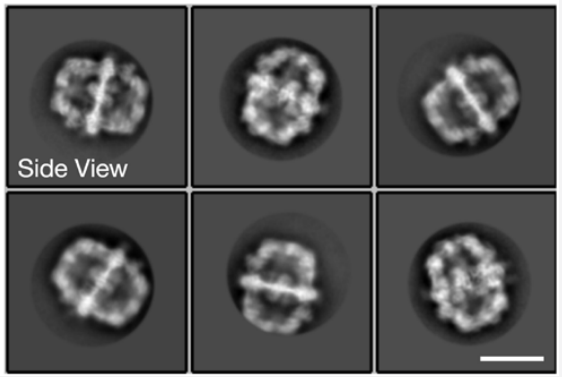
The OptiMSe Workflow determines the best data analysis parameters without user intervention, based on the sample's target mass.

Novel deconvolution algorithms designed specifically for intact protein and protein complex data analysis are deployed automatically and run on acquired data while the subsequent data are collected.

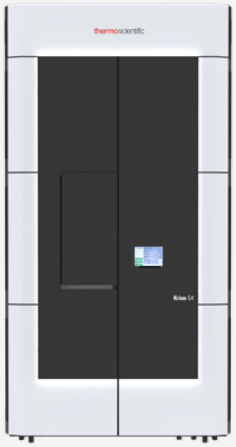
Easily choose the best samples to send for cryo-EM analysis using the intuitive reports generated by the OptiMSe Workflow.



Structural Analysis



Quickly screen out the least promising samples with the OptiMSe Workflow. Reduce your screening time on the microscope so you can focus on the results.



Tune your sample prep for optimal stability

Optimize sample preparation for structural biology with the OptiMSe Workflow

Poor protein sample preparation can sabotage your structure long before the sample reaches the microscope. Shouldn't there be a better way to know what samples are the best candidates for cryo-EM? What if you could screen dozens of different buffers or sample preparation conditions in a single day? With the VitroEase Buffer Optimization Kit and the combined with OptiMSe Sample Screening Workflow you no longer need to guess how to best prepare your sample or rely on "good enough." Don't let time constrains prevent you from finding the optimal sample conditions. Break through the backlog and accelerate your structural elucidation with high-throughput sample screening.

VitroEase Buffer Screening Kit (P/N A49856)

	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

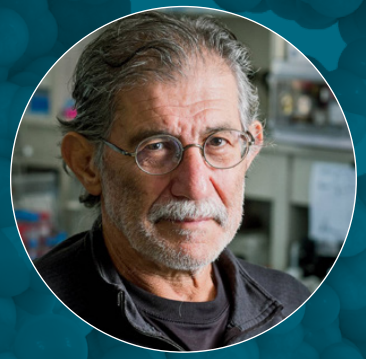
Note: The colors in the left column correspond to the colors of the vial caps in the VitroEase kit.

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)

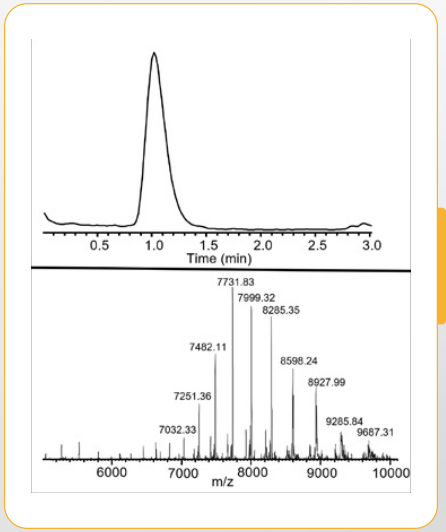
VitroEase buffer screening kit (A49856)

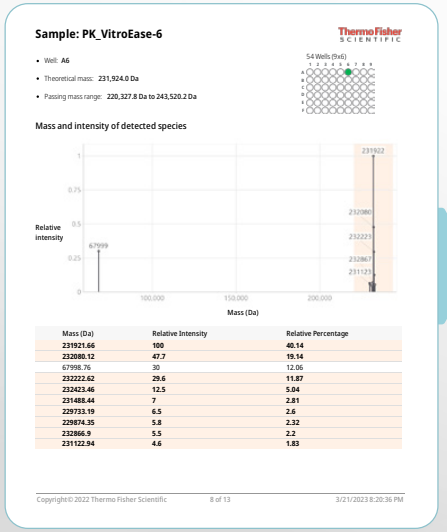
	Detergent #	Content (10x)
✓	A	CTAB (0.3%)
✓	B	CHAPS (4.9%)
✓	C	OG (2.7%)
	D	Tween-20 (0.1%)
✓	E	DM (1%)
✓	F	FOM (0.7%)

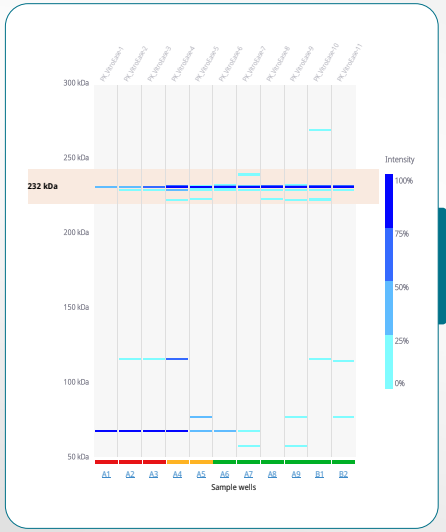


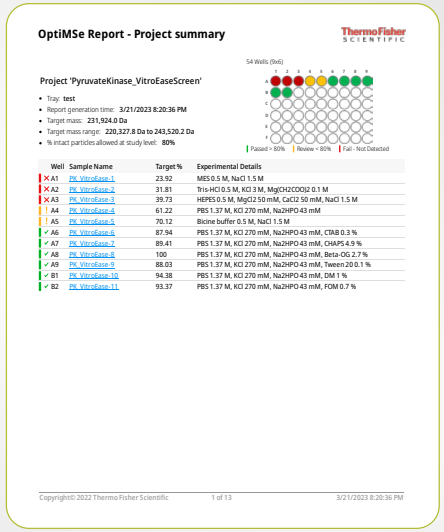
“… I would even say that to do cryo-electron microscopy without native mass spectrometry … is like working with not one hand behind your back, but two hands behind your back…”

Prof. Brian Chait, Camille and Henry Dreyfuss Professor, The Rockefeller University





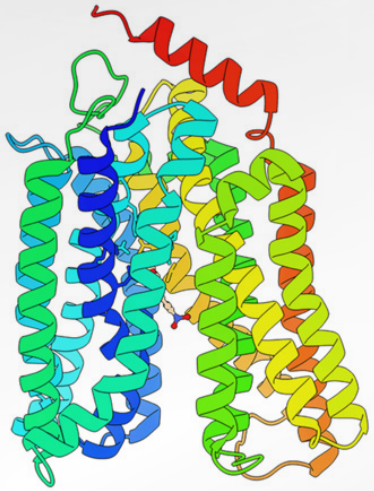




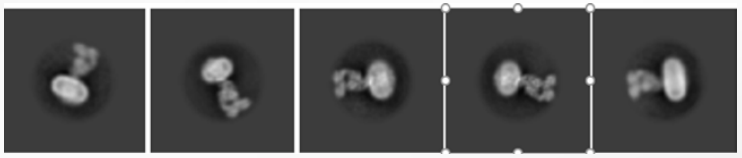
Optimize sample preparation for greater biological insight

The bacterial melibiose transporter, or MelB (54 kDa), catalyzes the transport of small saccharides across membranes. However, the conformational mechanism of saccharide transport remains unclear. In this study researchers were able to utilize nanobody (NB, 14 kDa) binding to stabilize the inward-facing conformation of MelB. This provided an opportunity to better characterize the conformational changes associated with sugar binding and release.

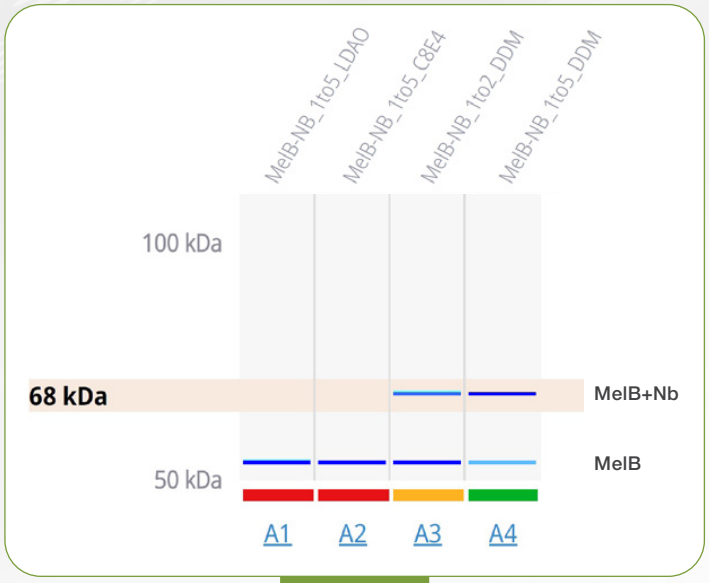
Extensive optimization of the sample preparation was performed. The MelB+NB complex has low binding affinity and is stable only in presence of DDM or UDM detergents and maximum complex formation was detected at NB: MelB ratio of 5:1. The OptiMSe Workflow screening of NB and MelB identified multiple proteoforms and the presence of extra proteins (23.5 kDa). High-resolution structure of MelB-NB725 complex has been obtained with Na^+.[4]

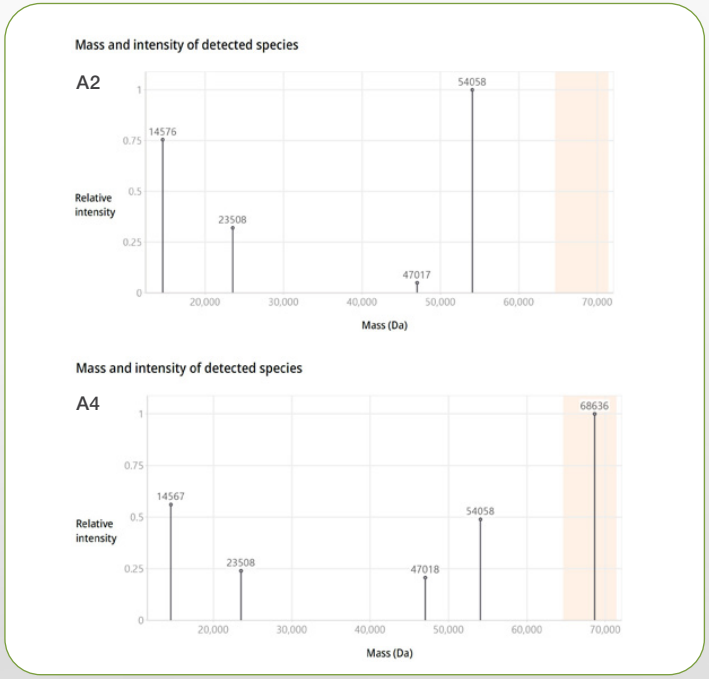


$MelB_{st}$ PDB 7L17



Selected 2D classes of hybrid complex $MelB_{St}$ NB725m, NBFab, and eNB. Grids were prepared with the Thermo Scientific™ Vitrobot™ Mark IV System, and cryo-EM single particles were imaged by Thermo Scientific™ Titan Krios™ Cryo-TEM with a K3 detector at S2C2.

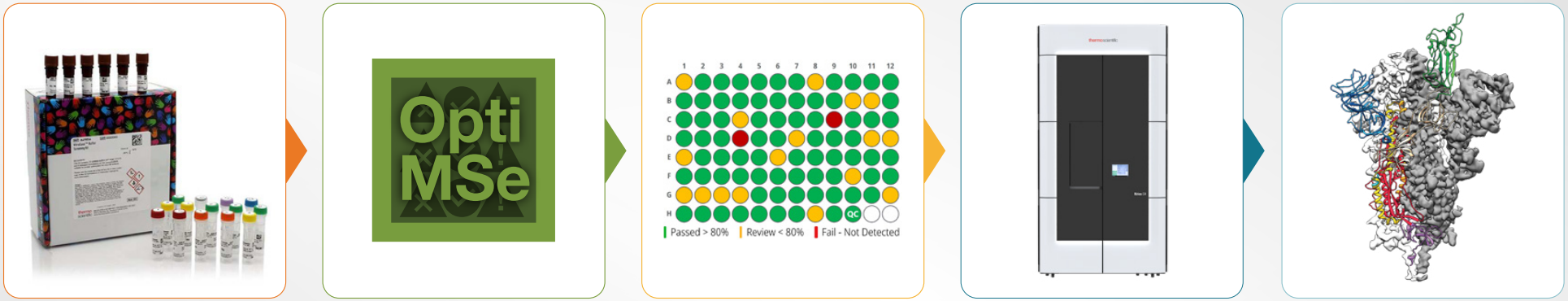




2D images of $MelB_{St}$ NB725m, NBFab, and eNB as well as MelB and Nanobody samples were provided by Prof. Lan Guan, Texas Tech University Health Sciences Center.

> *Mass spectrometry, and specifically native techniques, have great potential to provide valuable information on sample quality and stoichiometry which can feed into the cryo-EM structure determination pipeline. This may make cryo-EM sample preparation and subsequent structure determination more efficient, as well as provide additional insight through this integrative structural biology approach.*
>
> Dr. Stephen Muench, Lecturer in Membrane Proteins, University of Leeds

Accelerate your structure elucidation with the OptiMSe Sample Screening Workflow



- Simplify sample screening and purity determination of proteins and complexes
- Reduce time to result and increase flexibility with automated acquisition and processing
- Enable high-throughput sample screening of hundreds of samples per day
- Confidently select the best sample for downstream analysis from a comprehensive set of sample preparations

1. Olinares PDB, et al. Structure. 2021 Feb 4;29(2):186-195.e6.
2. VanAernum Z.L., et al. Nat Protoc. 2020;15(3):1132-1157
3. Thermo Scientific Technical note #001259. Weijing Liu, et al., (2022) Solutions for high-throughput analysis of large biomolecules by native mass spectrometry
4. Lan G et al. A nanobody-trapped novel conformation of a melibiose transporter MelB by cryo-EM single-particle analysis, COMPA, 2022, NYC

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