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PNNL-33371

Developing High-Flux Ion Soft Landing with Mass-Selection for Improved Cryo-Electron Microscopy

September 2022

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Abstract

The goal of this project is to redesign and repurpose existing high-flux ion soft-landing (SL) instrumentation developed at PNNL for new structural biology applications by integrating SL, mass-selection/separation of high mass-to-charge species, native mass spectrometry (MS), and cryo-electron microscopy (EM) to generate a workflow enabling structural and functional annotation of proteins and other complex biological samples. Ultimately, this work will result in a new instrument for mass-selection and SL based cryo-EM sample preparation and potentially increase the impact of PNNL and the broader microscopy community through commercial licensing.

Acknowledgments

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1.0 Introduction

Cryogenic electron microscopy (CryoEM) is currently at the forefront of structural biology and structures of both individual proteins and macromolecular complexes may now be solved to near-atomic spatial resolution.[1-2] Despite these advances, one of the largest bottlenecks and challenges limiting the use of the CryoEM technique is sample preparation.[3] Consistently preparing samples that are homogenous in both purity and oligomeric state and have sufficiently high concentration to solve high-resolution structures poses a persistent challenge.[4] Typical methods to overcoming this challenge occur after data collection, on the data processing end, and require large amounts of computational resources. An alternative solution to this problem is to improve the sample preparation pipeline itself, by using advanced mass separation and deposition capabilities to select pure samples by isolating different oligomeric or assembled states of individual proteins or macromolecular complexes. This front-end approach will, in turn, create a high coverage homogenous sample population, thereby reducing requirements for downstream data processing. In this project, we used high-flux ion soft-landing (SL)[5] to select protein ion complexes by their mass-to-charge ratios and cleanup heterogenous samplesolutions for CryoEM characterization.

The overall approach to the project began with redesigning an in-house custom-built SL instrument to adapt it for protein SL.[6] Simultaneously, ion desolvation conditions were optimized for a selection of representative proteins using native electrospray ionization mass spectrometry (ESI-MS). The optimized conditions determined with the analytical mass spectrometer were then used to inform initial settings on the redesigned ion SL system to deposit selected proteins directly onto EM grids. Following deposition, the EM grids were immediately negatively stained and finally imaged on a Titan Krios G3i electron microscope to determine the distribution and coverage of particles.

2.0 Results

2.1 Redesign of the SL System

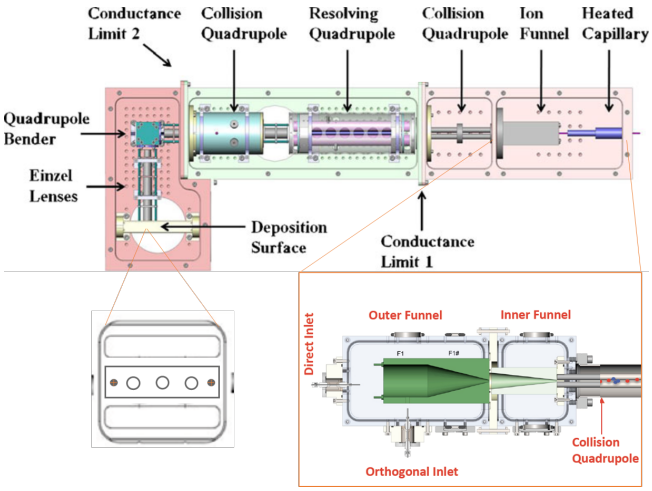

Figure 1. Existing ion SL system at PNNL.

The first deliverable for this project was to redesign the existing custom-built ion SL instrument in the Environmental Molecular Sciences Laboratory (EMSL).[6] Therefore, we set out to reconfigure the existing PNNL SL system (**Fig. 1**) in Dr. Grant Johnson's laboratory in PCSD to adapt it for SL of biological samples including protein complexes. The existing ion SL system was originally used for preparation of well-defined inorganic samples related to DOE missions in chemical catalysis, energy storage, and separations.[7-8] Since the goal of this project was to use ion SL for CryoEM sample preparation, a new sample introduction and transfer arm needed to be designed that would be moved between the ion SL instrument to the CryoEM vitrification system. Additionally, the sample holder needed to be redesigned specifically to accommodate multiple CryoEM grids. A new transfer arm equipped with the newly designed sample holder was designed and built by the EMSL machine shop and successfully installed onto the existing ion SL system (**Fig. 2**). The mass range of the existing ion SL system was also limited and insufficient to select and transmit large *m/z* protein complexes. To extend the mass range of the quadrupole mass filter we purchased a custom-designed low frequency radiofrequency (RF) power supply from GAA Custom Electronics. This novel design incorporated a large induction coil to achieve sufficiently low RF frequencies to transmit heavy protein complexes.[9]

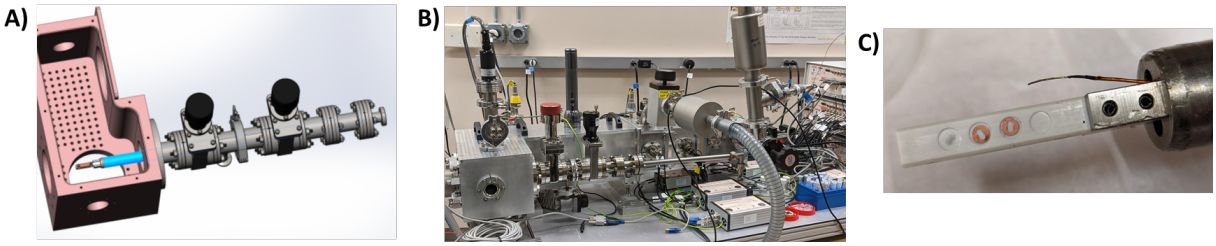

Figure 2. Updates to the ion SL system. (A) Design of the new sample transfer arm and grid holder added to the final deposition chamber (salmon color in Fig. 1 and 2) of the ion SL system. (B) Reconfigured ion SL system with the newly installed grid deposition and transfer arm and (C) the microscopy grid holder designed specifically for holding multiple CryoEM grids.

2.2 Establishing an Integrated Pipeline

Three representative model proteins were initially selected (apoferritin, aldolase, and beta-galactosidase) due to established differences in their monomer molecular weights (20, 45, and 120 kDa, respectively), distinct 3-dimensional (3D) folding (alpha-helix only to alpha-helix and beta-sheet hybrid), and varying numbers of proteins assembling as homomeric oligomers (24-, 4-, and 4-mer, respectively) with different binding strengths. The second deliverable of this project was to demonstrate an integrated workflow that combines mass-selection of protein complexes, native MS, SL, and CryoEM.

Our analytical pipeline starts with determination of the optimum electrospray ionization and desolvation conditions and the *m/z* range to be used for ion SL experiments. This first step was performed using native MS. Specifically, proteins were first buffer exchanged into a 200 mM ammonium acetate solution using Zeba spin desalting columns. Electrospray ionization mass spectra were then collected in the positive ion mode on a Waters Synapt G2s-I mass spectrometer. Representative mass spectra of apoferritin and beta-galactosidase are shown in **Fig. 3**.

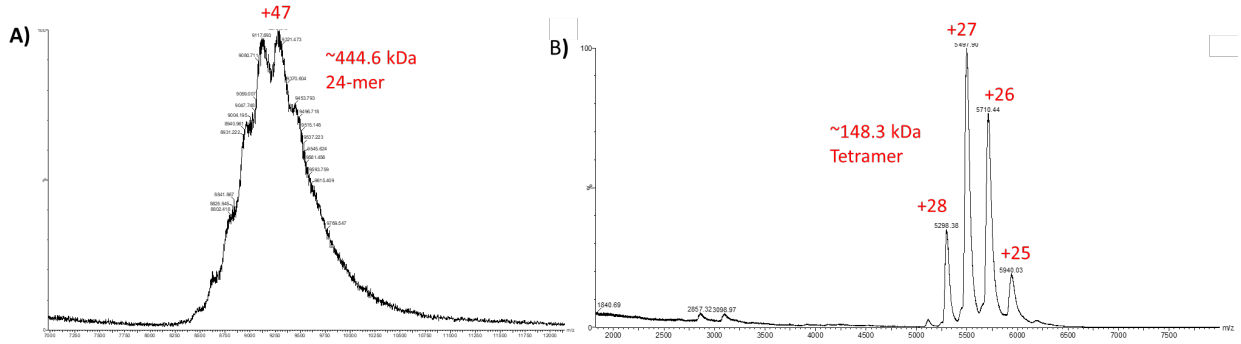

Figure 3. Native electrospray ionization mass spectrum of (A) apoferritin and (B) beta-galactosidase under optimized conditions.

Initial settings for the ion SL instrument were determined using the optimized conditions from native MS screening on the analytical mass spectrometer. Ion SL settings were adjusted to maximize and stabilize the ion current measured at the sample grid holder. At the deposition surface, multiple types of EM grids of varying material and thickness were tested. After each attempt at SL protein complexes, the EM grids were removed from the vacuum environment of the SL instrument through a vacuum load-lock chamber and immediately negatively stained with NanoW to preserve the protein on the grid for subsequent imaging. Grids containing SL protein complexes were then imaged at room temperature using a Titan Krios G3i electron microscope. These efforts led to successful deposition of apoferritin onto an EM grid (**Fig. 4**). Due to artifacts introduced by the negative staining and inconsistent particle coverage across the EM grid we were unable to collect a single particle dataset of the SL apoferritin sample.

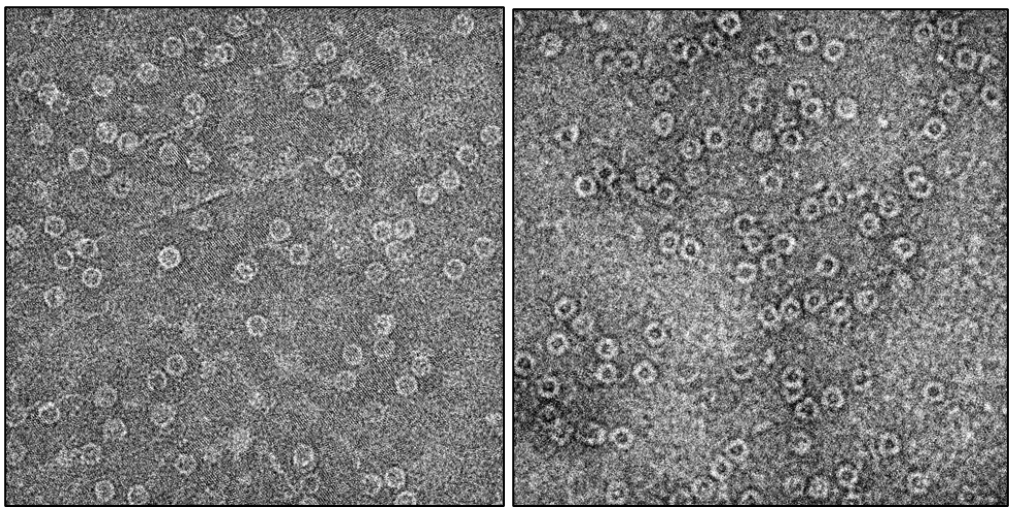

Figure 4. Electron microscopy images of negatively stained SL apoferritin particles on an EM grid.

Other research groups that have attempted ion SL (often referred to in the literature as electrospray-ion beam deposition, ES-IBD) of protein complexes commonly observe partial damage and loss of high-resolution structural features in their deposited samples.[10-12] Recently published literature showed that the addition of a matrix coating to an EM grid may help to alleviate some of this damage to the protein complex.[11,13] Therefore, we attempted to SL proteins onto EM grids coated with different matrices. Our experiments included 5 - 20% glucose, trehalose, glycerol, PEG 2700, and PEG 1000. By the end of this project, we did not observe protein complexes on an EM grid with any of these chemical matrices.

3.0 Conclusions

Over the duration of this project, we successfully redesigned the existing ion SL system at PNNL by incorporating a new sample transfer arm and deposition surface, adapting the system for use with proteins. We also incorporated a new low frequency RF quadrupole power supply that provided the extended mass range necessary for selection and transmission of large protein complexes. Additionally, we established an integrative pipeline using native MS for screening, SL with mass separation, and protein deposition onto an EM grid, followed by imaging of the grid with CryoEM. We were successful in using ion SL to deposit apoferritin onto an EM grid and visualize the negatively stained proteins. Next steps for this research will be to continue fine-tuning ionization and SL parameters, including optimizing matrix conditions on the grids. Upon completion of this optimization, imaging will transition from negative staining and room temperature screening to vitrification and CyoEM data collection to achieve high resolution structures from protein samples prepared by ion SL.

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