Baranwal Investigation of the Oligomeric state of NUDT3 AUC Collaboration

Introduction

The analytical centrifuge (AUC) is an instrument with the capability of deriving size, anisotropy, and relative concentration of macromolecules under centrifugal force (1, 2). AUC is a first-principles technique, directly measuring molecular parameters such as sedimentation and diffusion coefficients, and determining partial concentration of solutes in mixtures. A sedimentation velocity experiment was performed to understand any changes in oligomeric state of NUDT3 in various buffer conditions. A high and low concentration was prepared in each buffer condition to observe any mass action.

Materials and Methods

All buffers and NUDT3 aliquots were provided by collaborators at UT Health in San Antonio. Sample analysis was performed at the Canadian Center for Hydrodynamics by spectral analysis and AUC sedimentation velocity. Spectra of the provided buffers were measured using a ThermoFisher Genesys 50 benchtop spectrophotometer, scanning from 200-300 nm in a 1cm quartz cuvette [Figure 1]. A list of the buffers used and the naming system used throughout this report is included in Table 1. A buffer exchange of two aliquots of NUDT3 was performed with buffer 3 in a 3 kDa molecular weight cut-off Millipore Centrifugal Filter Unit. A serial dilution of this buffer and the buffer exchanged protein was scanned in the spectrophotometer to generate an intrinsic extinction spectrum [see Figure 2].

Table 1: Buffer nomenclature

Buffer #	Components	pH value
1	200mM LiSO4 25mM Sodium Acetate	4.5
2	5mM Tris-HCl 50mM KCl	7.5
3	1x PBS	
4	15mM Sodium Acetate 100mM NaCl	4.5
5	25mM MES 100mM NaCl	5.5
6	5mM MES 100mM NaCl	6.5
7	15mM Tris-HCl 100mM NaCl	7.5
8	5mM Tris-HCl 100mM NaCl	8.5

High and low concentrations of NUDT3 were made in each of the 8 buffers at both 69.54 μM and 14.44 μM. All samples were measured using a Beckman-Coulter Optima AUC instrument using an AN50Ti rotor. The experiment was performed at 50,000 rpm at 20 ºC, for 12 hours, measuring in intensity mode with the UV absorbance optics. Cells were assembled with 1.2 cm epon standard 2 channel centerpieces and quartz windows. High and low concentrations were scanned at 295 nm and 239 nm respectively.

Data Analysis

All data analysis was performed using UltraScan-III release 6803 (3). Intensity data was collected and converted to pseudo-absorbance using the UltraScan 4.0 R&D data acquisition module (3, 4). Sedimentation velocity experiments were analyzed initially by two-dimensional spectrum analysis

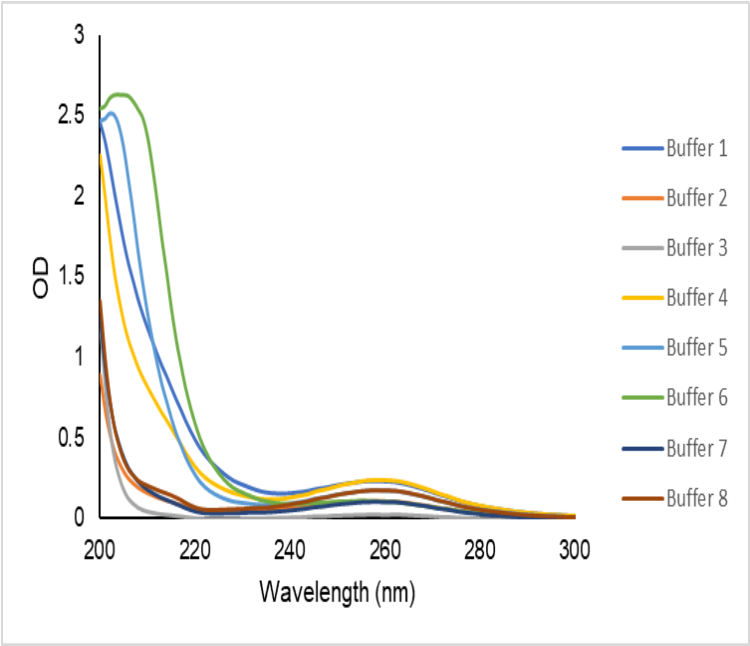

Figure 1: Buffer spectra from 200-300 nm. Potential nucleic acid contamination is seen across all buffers with absorbance peaks at 260 nm. Refer to Table 1 for buffer nomenclature.

(2DSA), removing time- and radially invariant noise, and fitting boundary conditions as explained in (5). Iterative 2DSA models were further refined by 2DSA-Monte Carlo (2DSA-MC) analysis (6). Genetic algorithm analysis was used to remove the false-positive solutes which were caused by noise present in dat., The GA results are further refined by performing the GA-Monte Carlo analysis with 100 iterations and 4 parallel threads as described in (5), presented in [Figure 5].

Results

In the buffer scans, [see Figure 1] potential nucleic acid contamination was seen throughout. A separate peak of a small molecular weight species was also observed in the AUC data [see Figure 4], which could result from the presence of a contaminant. Raw data overlayed with the GA-MC model of the high concentration in buffer 8 is shown in Figure 3. All GA-MC models had

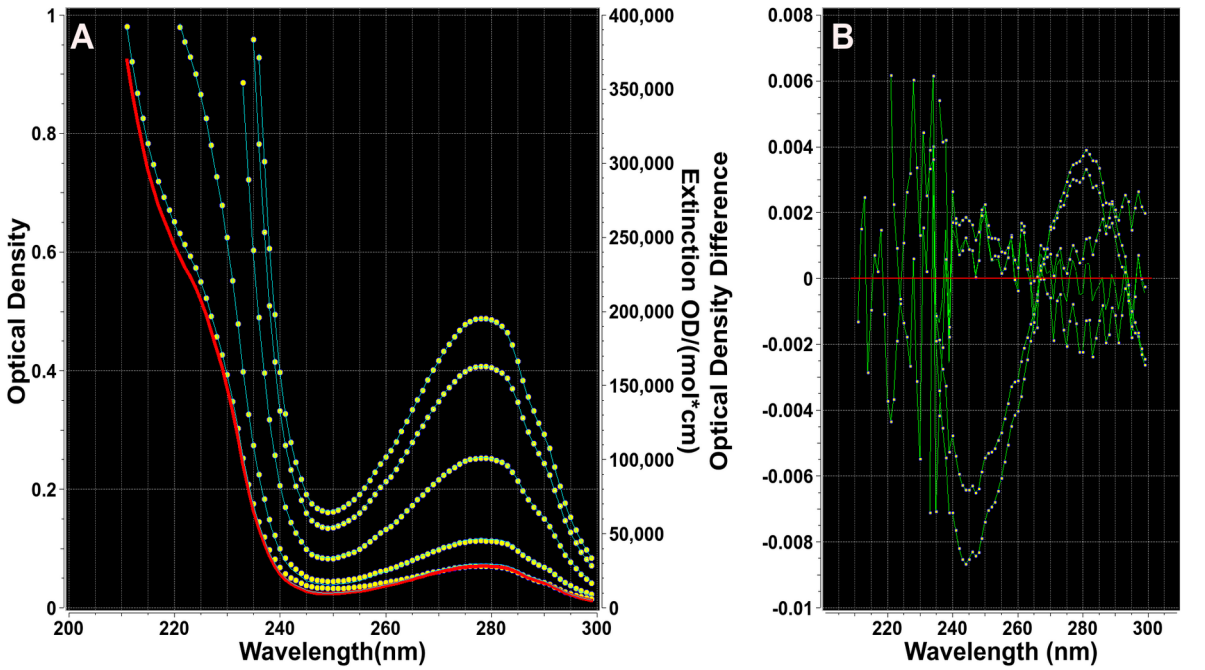

*Figure 2: Absorbance profile of NUDT3 dilution series (A). A global fit (red) of scans was made to generate extinction coefficients for wavelengths between 210-300 nm (secondary y-axis). The fit produced a variance of 8.34 *10^-6 OD with residuals shown in (B).*

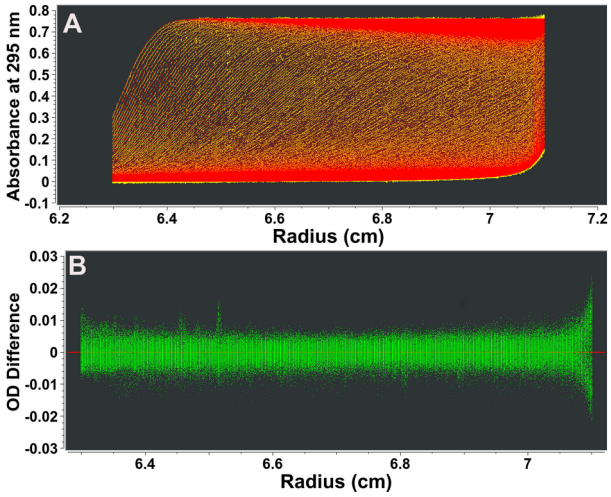

*Figure 3: GA-MC model fit of NUDT3 high concentration in buffer 8 raw data (**A**). The residuals (**B**) plot the OD differences between the raw data and overlayed model, having an RMSD of 3.23 *10^{-3}. This shows that the model is a good representation of the raw data*

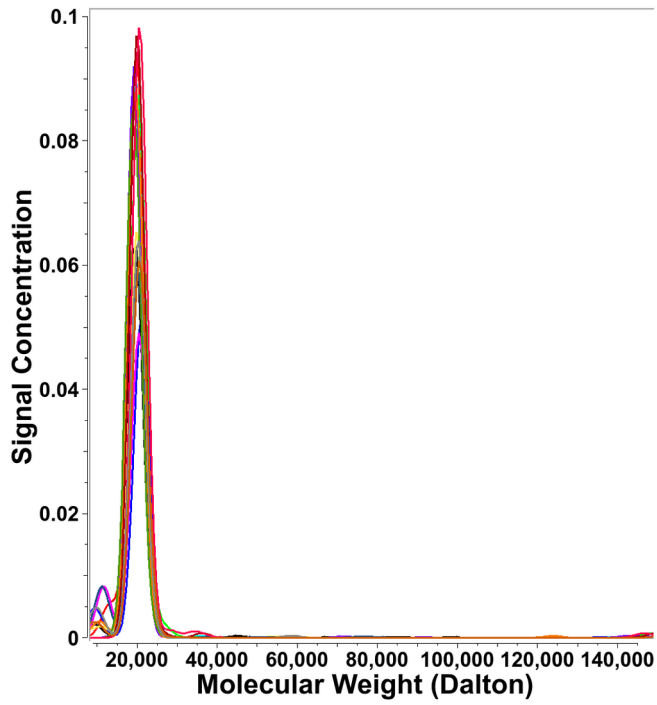

Figure 4: Molecular weight distributions determined with the 2DSA-MC analysis of all concentrations of NUDT3 in all buffers. All species resulted in a single species with a molar mass close to 20 kDa, with a smaller contaminant visible as well. There is no larger species visible at any concentration visible, indicating the absence of dimers or higher order oligomers.

RMSD values below 6.85*10^{-3}, showing a good reflection of each raw data set.

Figure 4 shows a molar mass overlay of all distributions observed across all buffers. This plot highlights the homogeneity of all samples, having only one species at ~20 kDa, and a minor contaminant around 10 kDa, likely resulting from the buffer contamination shown in Figure 1. No larger species are observed, indicating the absence of larger oligomers or aggregates. Figure 5 shows a global pseudo-3D plot of the frictional ratio vs. molar mass derived from the GA-MC analysis across all buffers. Integration of the signal area revealed a single species of 19.799 kDa±0.012 kDa with a frictional ratio of 1.4 and a sedimentation coefficient of 1.9 s.

Discussion

The serial dilutions of NUDT3 in Buffer 3 resulted in an intrinsic extinction profile for NUDT3 that was normalized using the sequence-derived molar extinction coefficient at 280 nm of 27,670 OD/(Mol cm) to derive a molar extinction coefficient profile of NUDT3 (see LIMS uploaded file NUDT3_extinction,csv, spanning wavelengths 211-299 nm). The profile was used to determine exact concentrations of the high and low concentration samples.

All buffer scans exhibited some nucleic acid contamination [Figure 1], but buffer 3, used in the buffer exchange for NUDT3, has the least. This contamination was observed in the AUC experiment, as some lower molecular weight species can be seen in the molecular weight distribution in Figure 4, and Figure 5.

The result from both molecular weight reporting plots [Figure 4 and Figure 5] showed the same molar mass known for the monomeric NUDT3, ~19.8 kDa. This is in excellent agreement with the molecular weight from sequence, projected to be 19.47 kDa. These plots also show that both concentrations of 69.54 µM and 14.44 µM in all buffers are identical, and that there is no evidence of a dimeric form or a higher oligomeric state for NUDT3. These results

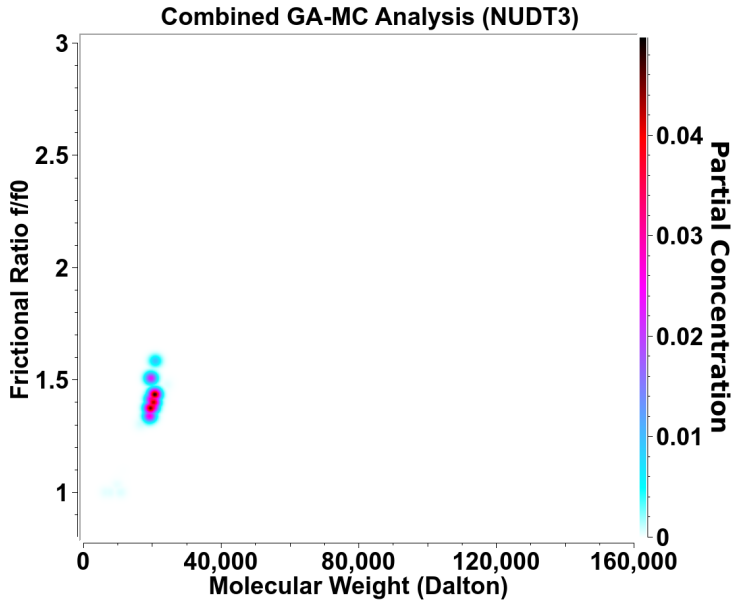

Figure 5: Overlays of the pseudo-3D plots of frictional ratio as a function of molecular weight (Dalton) of all the low and high concentration samples diluted with different buffers generated using GA-MC models. Integration of signal region indicates an anisotropy of ~1.4 and a molecular weight of 19.799 kDa ±0.012 kDa.

unambiguously show that NUDT3 is present only in its monomeric state.

References

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