European Biophysics Journal (2018) 47:789–797
https://doi.org/10.1007/s00249-018-1301-7

ORIGINAL ARTICLE

Multi-wavelength analytical ultracentrifugation of human serum albumin complexed with porphyrin

Courtney N. Johnson[1] · Gary E. Gorbet[1] · Heidi Ramsower[2] · Julio Urquidi[2] · Lorenzo Brancaleon[2] · Borries Demeler[1]

Received: 31 January 2018 / Revised: 26 March 2018 / Accepted: 10 April 2018 / Published online: 19 April 2018

Abstract
The new Beckman Coulter Optima AUC instrument, which features multi-wavelength detection that couples the hydrodynamic separation of colloidal mixtures to spectral deconvolution of interacting and non-interacting solutes present in a mixture, was used to analyze the composition of human serum albumin (HSA) bound to metallo-protoporphyrin. We present new methods implemented in UltraScan that permit Optima AUC-derived multi-wavelength data to be spectrally decomposed in the same fashion as has been made possible for the Cölfen detector earlier. We demonstrate this approach by spectrally separating sedimentation velocity experimental data from mixtures of apo-HSA and HSA complexed to different metallo-protoporphyrins. We further demonstrate how multi-wavelength AUC can accurately recover percentages of metallo-protoporphyrin-bound HSA and apo-HSA from mixtures and how multi-wavelength AUC permits the calculation of molar extinction coefficients for porphyrins bound to HSA. The presented method has broad applicability to other complex systems where mixtures of molecules with different spectral properties need to be characterized.

Keywords Multi-wavelength analytical ultracentrifugation · UltraScan · Spectral analysis · Porphyrin · Human serum albumin

Introduction

Multi-wavelength analytical ultracentrifugation (MWL-AUC) is a recent technique that relies on the ultracentrifuge instrument's ability to collect experimental data at multiple wavelengths during the same run. Collection of such data was originally developed by Cölfen's group in Germany as part of the OpenAUC project (Cölfen et al. 2010). For a review of the history of the Cölfen detector's development and current state, the reader is referred to Pearson et al. (Pearson et al. 2017). Analysis software for this detector was developed and validated by our group (Gorbet et al. 2015) and is available through the open source UltraScan software package (Demeler and Gorbet 2016; Demeler et al. 2017). Sedanal by Stafford and Sherwood (2004) also includes methods for the analysis of MWL-AUC experiments (Walter et al. 2015). Multi-wavelength detection promises to revolutionize the field of analytical ultracentrifugation by adding a second dimension for discrimination between different solutes based on optical properties to the arsenal of solution interaction studies. AUC already excels at the hydrodynamic separation of molecules that are dissimilar in size, shape or density. A second mode of separation can be achieved through optical characterization of individual solutes with unique spectral properties. For the Cölfen designed detector, the accuracy of this capability was first demonstrated by spectrally separating contributions from proteins and DNA (Gorbet et al. 2015). Later, we demonstrated how the same methodology could also be applied to the study of macromolecular interactions by identifying molar stoichiometries of interacting complexes as demonstrated for proteins and RNA (Zhang et al. 2017). These methods share their ability to attribute hydrodynamic

Special Issue: 23rd International AUC Workshop and Symposium.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00249-018-1301-7) contains supplementary material, which is available to authorized users.

✉ Borries Demeler
demeler@biochem.uthscsa.edu

[1] Department of Biochemistry and Structural Biology, UTHSCSA, San Antonio, TX, USA

[2] Department of Physics and Astronomy, UTSA, San Antonio, TX, USA