Analytical Centrifuge Facility

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Introduction

The Centre has a Beckman XL-I analytical ultracentrifuge equipped with absorbance and interference optics, two rotors (4-place and 8-place), and velocity and equilibrium cells with a choice of quartz or sapphire windows. We employ a range of data analysis methods, enabling the determination of properties of macromolecules in free solution and the quantification of components in a mixture.

Work carried out in 2001

The instrument was used in a wide range of applications in 2001, including the assessment of aggregation state of highly concentrated small proteins as studied by NMR, the evaluation of virus coat assembly, the effect of buffer composition on the assembly of ribonuclease complexes and determination of their binding affinities, measurement of relative proportions of starting material and complex in mixtures of interacting muscle proteins, study of the assembly mechanism and binding constants of viral packaging RNA, and determination of the association state of an HIV regulatory protein.

Example

One of the results from this last experiment is shown as an example of the kind of analysis that is feasible with this technology.

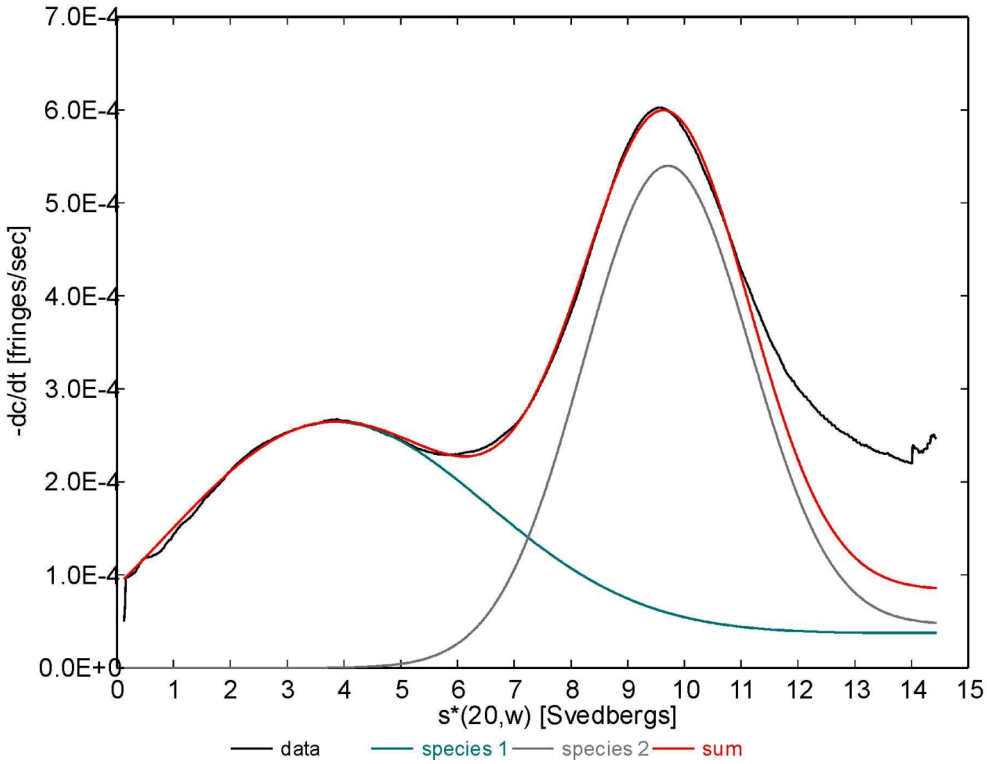

Fig 1. Sedimentation velocity analysis of a 24 kDa HIV regulatory protein centrifuged at 46000 rpm for 4 hours. A group of scans made during the middle of the sedimentation run was analysed by the dc/dt method using the program DCDT+ by J. Philo

Clearly the protein exists in solution in at least two forms, fitted by species 1 and 2. The region of the curve beyond 12S that does not fit to the 2-species model is indication of the presence of aggregate(s). Amounts of the species present are proportional to peak areas, and are calculated by the program as initial loading concentrations, C_0. Knowledge of the starting concentration enables calculation of sample composition in terms of the species described. The diffusion coefficient, D, is related to the width of the Gaussian dc/dt *vs* S peak, and the molar mass of solute, if homogeneous, is proportional to the ratio of S/D. In this example, both peaks are broader than expected for single homogeneous samples, and the conclusion is that there is a dynamic equilibrium between species 1 and 2. Analysis of a higher protein concentration resulted in a larger proportion of the species 2 component and a calculated mass of 88 kDa, a result consistent with the conclusion made. The nature of the distribution could have been investigated further by sedimentation equilibrium, resulting in the most probable degree of association, and an association constant.

Sample conc	**Species**	**Species C_0** (fringes)	**Species proportion of total**	**$S_{20,w}$** (x 10^{-13} s)	**$D_{20,w}$** (x 10^{-7} cm^2 s^{-1})	**MW** (kDa)	**inter-pretation**
2.0 mg/ml (6.6 fringes)	1	2.95	45%	2.97	(45.7)	NA	monomer
	2	1.33	20%	9.44	(12.65)	(66.7)	tetramer
	3	NA	35%	~ 170	NA	NA	aggregate
3.2 mg/ml (10.4 fringes)	1	3.27	31%	3.60	(37.1)	NA	monomer
	2	3.58	34%	9.42	9.58	87.9	tetramer
	3	NA	35%	117	-	NA	aggregate

Table 1. Parameters derived from dc/dt analysis shown in fig1.

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