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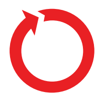
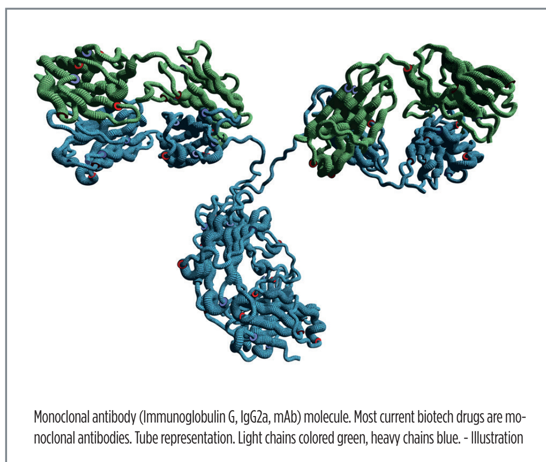


Analysis of Protein-Protein-Interactions
by analytical ultracentrifugation

INTRODUCTION

Protein-Protein-Interactions (PPI) are fundamental to the function of proteins and the life of a cell. PPIs can be divided into homo- or heterocomplexes whether being present as dimers or multimers. Additionally, these interactions are classified as strong and long-lived or weak and transient [1]. Moreover, PPIs can also be a result of an artefact during preparation and storage of proteins. Structurally, globular proteins are able to interact due to preformed surfaces or with an induced binding surface. In case of protein-peptide-interactions, the binding is mediated whether with a discontinuous or a continuous epitope [2]. The knowledge of the strength and type of interaction between two or more proteins is pivotal. The analysis of PPIs and thus understanding the role of proteins and protein complexes is of major interest not only for basic research but also for drug discovery.

Analytical ultracentrifugation (AUC) is an expectional technique for the investigation of PPIs due to the fact the proteins are analyzed in solution with good chances to not influence the protein´s binding behaviour. Strength, stoichiometry, dynamics and reversibility can be monitored by AUC [3]. Both optical systems are suitable for the analysis of absorbing or weakly absorbing proteins and particles at a large concentration range. Both methods, sedimentation equilibrium (SE) and sedimentation velocity (SV), give insights into the nature of PPIs and are complementary to each other [4].

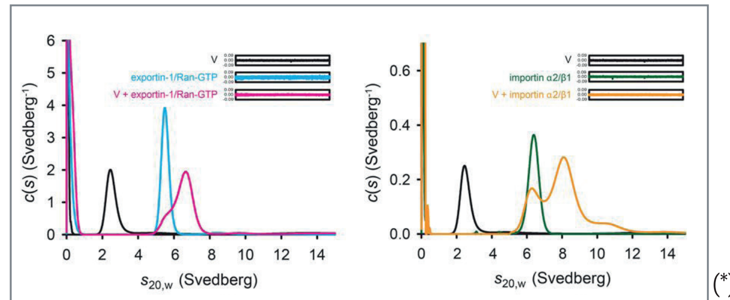


Monoclonal antibody (Immunoglobulin G, IgG2a, mAb) molecule. Most current biotech drugs are monoclonal antibodies. Tube representation. Light chains colored green, heavy chains blue. - Illustration

EXAMPLE 1

Interaction between HeV V protein and cytoplasmic host proteins drives viral pathogenesis

The presented work by Atkinson and colleagues [5] describes the interaction of the V protein of the Hendra virus (HeV) with nuclear import proteins, which is crucial for its viral pathogenesis. The Hendra virus belongs to the single-stranded negative-sense RNA virus family and causes a lethal disease in humans for which there is no vaccine available. HeV replicates entirely in the cytoplasm of the host cell, but some proteins seem to be transported via the nuclear envelope. In order to be translocated, proteins larger than ~40 kDa have to pass the envelope via nuclear pore complexes.



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Atkinson SC et al. (2018) Scientific Reports 8, 358

One nuclear import pathway is controlled by the importin superfamily. Atkinson and colleagues analyzed the nuclear import and export mechanism of the V protein. They demonstrated that import and export of the V protein is mediated by importin α1/β1 and exportin-1/Ran-GTP. With a sedimentation velocity experiment, they proved that the V protein binds to both protein complexes during nuclear import and export. The V protein features a sedimentation coefficient of 2.5 S. Importin α2/β1 shows an s-value of 6.4 S and exportin-1/Ran-GTP an s-value of 5.1 S. By incubating V protein with equimolar concentrations of whether the import or export proteins, it binds directly to them as seen by the increase in the sedimentation coefficients. Small molecule inhibitors can abrogate the interaction of the viral protein to importin α2/β1 thus offer one possible treatment option against HeV infection.

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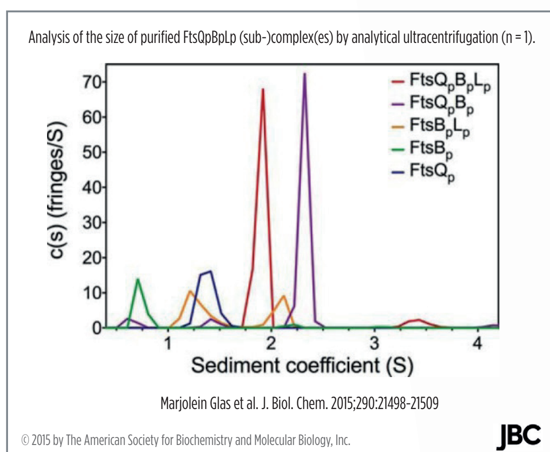
EXAMPLE 2

The trimeric complex FtsQBL controls the formation of the bacterial divisome

The publication by Glas and colleagues [6] gives insight into the complex formation during bacterial cell division, which is a series of well-defined steps including cell constriction, septic wall synthesis and finally cell segregation. The gram-negative divisome of *E. coli* is a macro-molecular complex of up to 10 essential and more or less 15 accessory proteins. A pivotal role in the divisome formation plays the FtsQBL complex where the FtsQ protein seems to be the central player. It is an attractive target for PPI inhibitors to block bacterial cell division. The analysis of the FtsQBL complex is complicated due to its membrane-bound nature. Glas et al. analyzed the complex formation by sedimentation velocity experiments of the periplasmic parts of all three proteins.



Their work revealed that the trimeric complex features a 1:1:1 stoichiometry at 1.9 S corresponding to 51 kDa as well as ternary complex of dimers at 3.4 S corresponding to 123.1 kDa. Whereas FtsQB seems to form a dimer of dimers with a sedimentation coefficient peak at 2.3 S (65 kDa). Knowledge of complex formation of FtsQBL will support the development of inhibitors in order to obstruct the formation of the bacterial divisome.

OPTIMA AUC

- First-principle technique that does not depend on a matrix and does not require standards
- Samples are analyzed in their native state with almost no buffer restrictions
- One experiment reveals information about shape, diameter, mass, stoichiometry, purity, formulation heterogeneity, aggregation, association and conformation of a protein or protein complex
- Optical systems:
  • Rayleigh Interference
  • UV absorbance
- Sample volume:
  max. volume for 2-sector centerpieces: 450 µl
  max. volume for 6-channel equilibrium centerpieces: 120 µl
- Wavelength range: 190 – 800 nm
- Molecular weight range:
  10^2 Da (i.e. Peptides/Oligosaccharides) - 10^8 Da (i.e. Viruses/Organelles)
- Concentration range:
  UV absorption:
  0.005 – 1-2 mg/ml Lutenizing Hormone
  Interference:
  0.025 - 4-5 mg/ml BSA

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