SAXS and ITC studies of nucleoplasmin and its complexes with histones and importins

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Nucleoplasmin and its complexes with histones (SAXS and ITC study)

Nucleoplasmin (NP), a histone chaperone, mediates the dynamic exchange of histones in chromatin remodeling processes and assists the nucleosome assembly by modulating the interaction between histones and DNA.

NP is a highly acidic homopentameric protein, each monomer being comprised of a core (120 N-terminal residues) and tail (80 C-terminal residues) domains. The crystal structure of NP core domain is known.

The complexes of NP with two types of nucleosomal histones (H2A-H2B and H5) were studied by SAXS and ITC.

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DAMMIF and BUNCH models of Histones

Nucleoplasmin and its complexes with histones (SAXS and ITC study)

Ab initio MONSA models of Complexes

Isothermal Titration Calorimetry

- ITC is a quantitative technique

- It measures binding affinity ($K_a$), enthalpy changes ($\Delta H$), and binding stoichiometry ($n$) of the interaction between two or more molecules in solution directly.

- From these initial measurements, the full thermodynamics including Gibbs energy changes ($\Delta G$), and entropy changes ($\Delta S$), can be determined using the relationship:

$$\Delta G = -RT \ln K_d = \Delta H - T\Delta S$$

(where $R$ is the gas constant and $T$ is the absolute temperature)
Results of typical ITC experiment
Nucleoplasmin and its complexes with histones (ITC results)

The site-specific cooperative ligand binding model reproduces well both H5 and H2A–H2B titrations, yielding a stoichiometry of five histones per NP pentamer, in good agreement with SAXS data.

Nucleoplasmin and its complexes with histones (SAXS and ITC study)

Small-angle X-ray scattering and ITC reveal that NP pentamer can accommodate five histones, either H2A–H2B dimers or H5, and that NP core and tail domains are involved in the association with histones.

The main difference between the NP/H5 and NP/H2A–H2B models is best expressed in terms of the interfacial area (the number of the dummy atoms belonging to the histone phase contacting those of the nucleoplasmin phase in MONSA models). The ratio of the interface areas (NP core/histone to NP tails/histone) is 0.5 to 0.8 for H5 and 1.8 to 2.3 for H2A–H2B. This means that while H5 interacts largely with the NP tails, H2A–H2B does it with NP core domain.

Nucleoplasmin and its complexes with importins (SAXS and ITC study)

Nuclear import of the pentameric nucleoplasmin (NP1) is mediated by importin α, that recognizes its nuclear localization sequence (NLS), and importin β, that interacts with α and is in charge of the translocation of NP/α/β complex through the nuclear pore.

According to ITC measurements NP pentamer can bind with high affinity 5 importin α/β heterodimers.

The solution structures of α/β heterodimer, NP/α and NP/α/β were reconstructed using SAXS data which reveal a glimpse of a complete nuclear import complex with an oligomeric cargo protein.

Nucleoplasmmin and its complexes with importins (SAXS and ITC study)

\[ \alpha/\beta (1:1) \]

\[ \text{NP}/\alpha (1:5) \]

\[ \text{NP}/\alpha/\beta (1:5:5) \]

DAMMIF and SASREF models

Nucleoplasmin and its complexes with importins (SAXS and ITC study)

The formed multi-domain complex shows an extended shape, and remains stable by virtue of two attachment points: recognition of the NLS by importin α and recognition of the IBB domain by importin β, which allow for conformational flexibility.

This modular and articulated architecture might facilitate the passage of such a big particle through the nuclear pore complex.

EOM analysis

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