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Secret life of importin-β; solenoid flexibility as the key to transport through the nuclear pore

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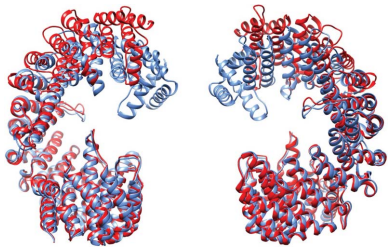
The current issue of *Acta Crystallographica Section D* features 'Impact of the crystallization condition on importin-β conformation' by Tauchert *et al.* (2016), a significant advance in the area of nuclear transport that also has important implications for understanding the limitations of crystallization approaches. Active transport of macromolecules into and out of the eukaryotic cell nucleus occurs through the nuclear envelope (NE)-embedded multiprotein subunit nuclear pore complexes (NPCs). The transport of most cargoes is dependent on solenoid proteins belonging to the Karyopherin β family, of which importin (Imp) β1 is the prototype. Impβ1 is capable of recognizing specific nuclear import cargoes and transporting them across the NPC by interacting with the hydrophobic meshwork constituting the NPC core, formed by phenyl–alanine–glycine (FG) rich nucleoporins (nups). Cargo recognition can occur directly, or indirectly through adaptor proteins such as Impα or snurportin, and in either case complexes are dissociated upon binding of Ran-GTP to Impβ1 on the nucleoplasmic side of the NPC. Since the discovery of Impβ1 (Görlich *et al.*, 1995), structural studies have helped elucidate many aspects of the molecular details of cargo and adaptor binding/release (Christie *et al.*, 2016). Unanswered questions, however, include how Impβs achieve cargo transport across the NPC through interaction with nups (Liu & Stewart, 2005; Bayliss *et al.*, 2000); Tauchert *et al.* provide an important, new slant on this question.

Impβ1 binds to a plethora of different proteins, including cargoes, adaptors, RanGTP and nups. Previous studies have shown Impβ1 to be a solenoid formed by 19 HEAT repeats, each of which comprises two antiparallel helices connected by a turn (Cingolani *et al.*, 1999); HEAT repeats are connected by short linkers and arranged in a superhelix, with very few long distance intraprotein interactions, enabling Impβ1 to undergo extensive changes in tertiary structure (overall protein shape), without alteration to secondary structure (HEAT repeats). Consistent with this idea, the comparison of Impβ crystal structures to date reveals a wide range of conformations varying from very compact, heart-like structures (the 'apo' or nup-bound form) to more relaxed ones (*e.g.* bound to RanGTP).

This observed structural variability has been postulated to be the direct effect of the binding of different partners to Impβ1 causing/inducing changes in Impβ1 folding, but X-ray scattering (SAXS) data shows that Impβ1 alone is more relaxed in solution than in crystal lattices (Fukuhara *et al.*, 2004). Further, molecular dynamics (MD) simulations suggest that the apo form of Impβ1 undergoes remarkable conformational changes in solution, adopting a more extended S-shaped conformation that is quite distinct to that observed in its crystalline form (Zachariae & Grubmüller, 2008; Forwood *et al.*, 2010). Significantly, two drastically different conformations of Impβ1 bound to the snurportin Impβ1 binding domain (IBB) were recently observed in the same crystallographic asymmetric unit (Bhardwaj & Cingolani, 2010); this both highlights Impβ1 flexibility, and importantly underlines the fact that structures obtained in crystals may not reflect the wide range of Impβ1 conformations in solution. Forwood *et al.* (2010) used crystallography/SAXS/MD to show that Impβ1 assumes various different conformations in solution, postulated to result from cumulative incremental structural changes along the entire length of the solenoid, and speculated to be integral to Impβ1's ability to traverse the highly hydrophobic channel of the NPC. Indeed, MD simulations reveal that Impβ1 in water is extremely different from that in 50% 2,2,2-trifluoroethanol, where Impβ1 rapidly becomes more compact (Yoshimura *et al.*, 2014); similar results have been



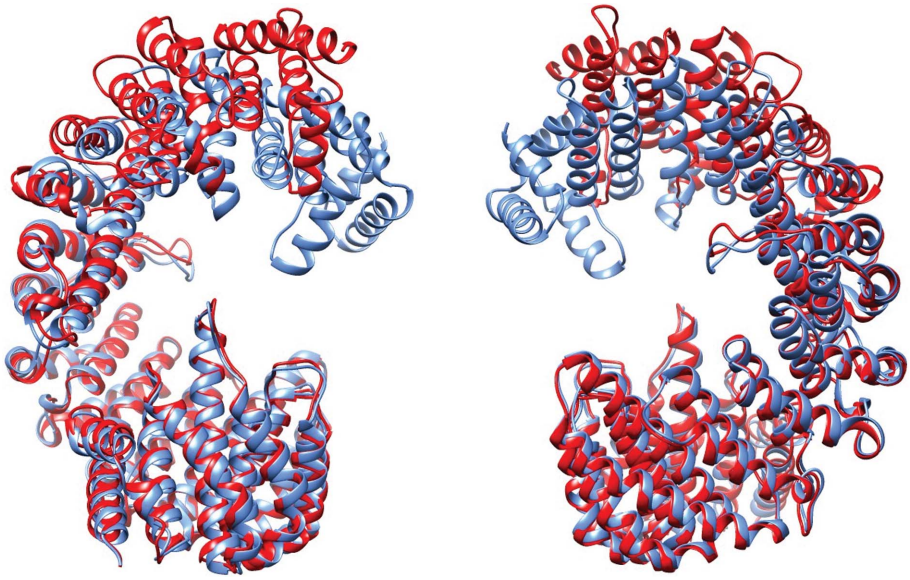


Figure 1
Effect of solvent on *C. Thermophilum* Impβ1 structure. The structures of Impβ1 obtained after PEG precipitation (red) or $(NH_4)_2SO_4$ (blue) precipitation are superimposed. The structure shown on the right is rotated 180°C with respect to that on the left.

obtained with both IBB-complexed and free Impβ1 in water compared to in methanol (Halder *et al.*, 2015). This ability to undergo conformational changes appears to be the key to Impβ1-mediated transport across the NPC, since crosslinking to impair this flexibility impedes nuclear translocation (Yoshimura *et al.*, 2014).

Tauchert *et al.* extend these findings, proffering an interesting alternative point of view regarding the forces determining different conformations of Impβ1 in crystalline form according to the hydrophobicity of the milieu. Tauchert *et al.* solve the structure of Impβ1 from the thermophilic fungus *Chaetomium thermophilum* in two physicochemically different conditions, taking advantage of the serendipitous S107P/V134A mutant which crystallized in the presence of the hydrophilic inorganic salt $(NH_4)_2SO_4$, adopting a much more compact structure than that of its wild-type counterpart crystallized in the presence of PEG (Fig. 1). These findings are confirmed in solution using SAXS, the important overall implication being that solvent hydrophobicity strongly affects Impβ1 conformation, and hence can be of key importance in the dominant conformation crystallized. Further, analyzing previous Impβ1 crystal structures, the authors find a strong correlation between the extent to which Impβ1 takes an extended conformation, and the amount of PEG in the buffer. Importantly, analysis of Impα crystal structure pairs solved in either PEG or $(NH_4)_2SO_4$ indicates that these properties do not apply, since Impα, although structurally related to Impβ1, is less flexible. The polar/apolar regions of PEG would appear to mimic nup FG repeats within the NPC, suggesting that Impβ1 traverses the NPC in an extended conformation, in contrast to what has been proposed previously (Halder *et al.*, 2015; Yoshimura *et al.*, 2014). The only crystal structures obtained so far between Impβ1 and NPC components used short FG-rich nup fragments, and thus do not shed light on the state of Impβ1–nups interaction within the core of the NPC (Liu & Stewart, 2005; Bayliss *et al.*, 2000). Importantly, apart from giving an important new insight into this aspect of nuclear transport, Tauchert *et al.*'s study clearly underlines the need for more extensive Impβ1–nup complex structures, with the proviso that the buffer systems used have to be considered critically (with a grain of salt perhaps?), and ideally should also be analysed in detail using complementary approaches such as SAXS.

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