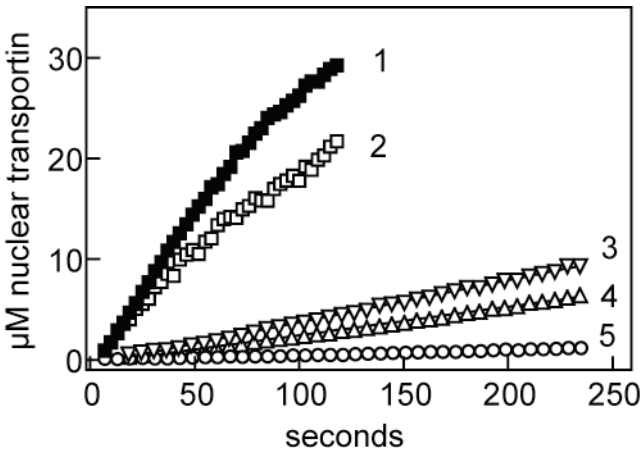


**Supplementary Figure S1: Effect of importin β-derivatives on facilitated nuclear influx of transportin**

2 $\mu$M Alexa488-labelled transportin was added to permeabilised cells and its nuclear accumulation was measured by confocal laser scanning microscopy. Curve 1 depicts the undisturbed influx. Curves 2-5 were obtained after pre-incubating the cells for 5 minutes with either 1 $\mu$M IBB•importin β complex (2), 1 $\mu$M Impβ$^{1\text{-}462}$ (3), 0.2 $\mu$M of the pentameric IBB-nucleoplasmin core fusion complexed to importin β (4), or with 1 $\mu$M Impβ$^{45\text{-}462}$ (5).

Quantitations are shown in the table below. NPC-passage of transportin was inhibited by the Impβ$^{45\text{-}462}$-fragment > 50 times more strongly than by full length importin β (compare curves 2 and 5). Since the experiment was performed in the absence of Ran and GTP, this difference cannot be explained by the failure of Impβ$^{45\text{-}462}$ to bind RanGTP. This is supported by the Impβ$^{1\text{-}462}$-fragment, which shows wild type RanGTP-binding and yet competes transportin influx 6-fold stronger than full length importin β (compare 2 and 3)

We assume that the strong inhibitory potential of Impβ$^{45\text{-}462}$ originates at least in part from its propensity to oligomerise (see Figure 6), which should increase its avidity for NPCs and introduce additional obstacles to the NPC passage of other molecules. These assumptions are consistent with the observation that a multimerised form of importin β (IBB-core$_5$•importin β$_5$) was a 10-fold stronger competitor of transportin import than monomeric importin β (compare 2 and 4).

| Number | Inhibitor (1 $\mu$M) | Nuclear influx rate with inhibitor |
|---|---|---|
| 1 | None | 100% |
| 2 | 1.0 $\mu$M IBB•Importin β | 55% |
| 3 | 1.0 $\mu$M Importin β$^{1\text{-}462}$ | 8% |
| 4 | 0.2 $\mu$M (IBB-core)$_5$•Importin β$_5$ | 6% |
| 5 | 1.0 $\mu$M Importin β$^{45\text{-}462}$ | <1% |