



Experiment title: Cambridge MRC Block Allocation Group
Structure of complexes between nucleoporin FxFG repeats and nuclear transport factor 2 (NTF2)

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LS-1525

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14-2

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2

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Preliminary Report:

We collected a 2.8 Å resolution native data for P21212 orthorhombic crystals of a complex between residues 1-442 of human importin-beta and the FF5 FxFG repeat construct of yeast Nsp1p. The lattice constants were $a = 67.25 \text{ \AA}$, $b = 211.79 \text{ \AA}$, $c = 125.82 \text{ \AA}$. The crystals were thin (0.005 mm) plates with the long axis perpendicular to the plane of the plate. The data was distinctly anisotropic and was 98.5% complete to 3.4 Å resolution, 92.4% complete to 3.2 Å resolution and 69.1% complete to 2.8 Å resolution. The data set contained 312925 observations of 144382 unique reflections. Rmerge was 8.9% (18.3% in the highest shell) whereas I/σ was 6.0 (1.7 in the highest shell). However, we have been able to obtain a molecular replacement solution using CNS (R-factor 22.5%, free-R 26.2%, bond rmsd 0.009 Å, angle rmsd 1.5 degrees) that shows the location of the FxFG repeat binding site on importin-beta and have used this information to engineer mutants that reduce the strength of this interaction and which also show reduced levels of nuclear protein import.

A manuscript describing this work has been submitted for publication.