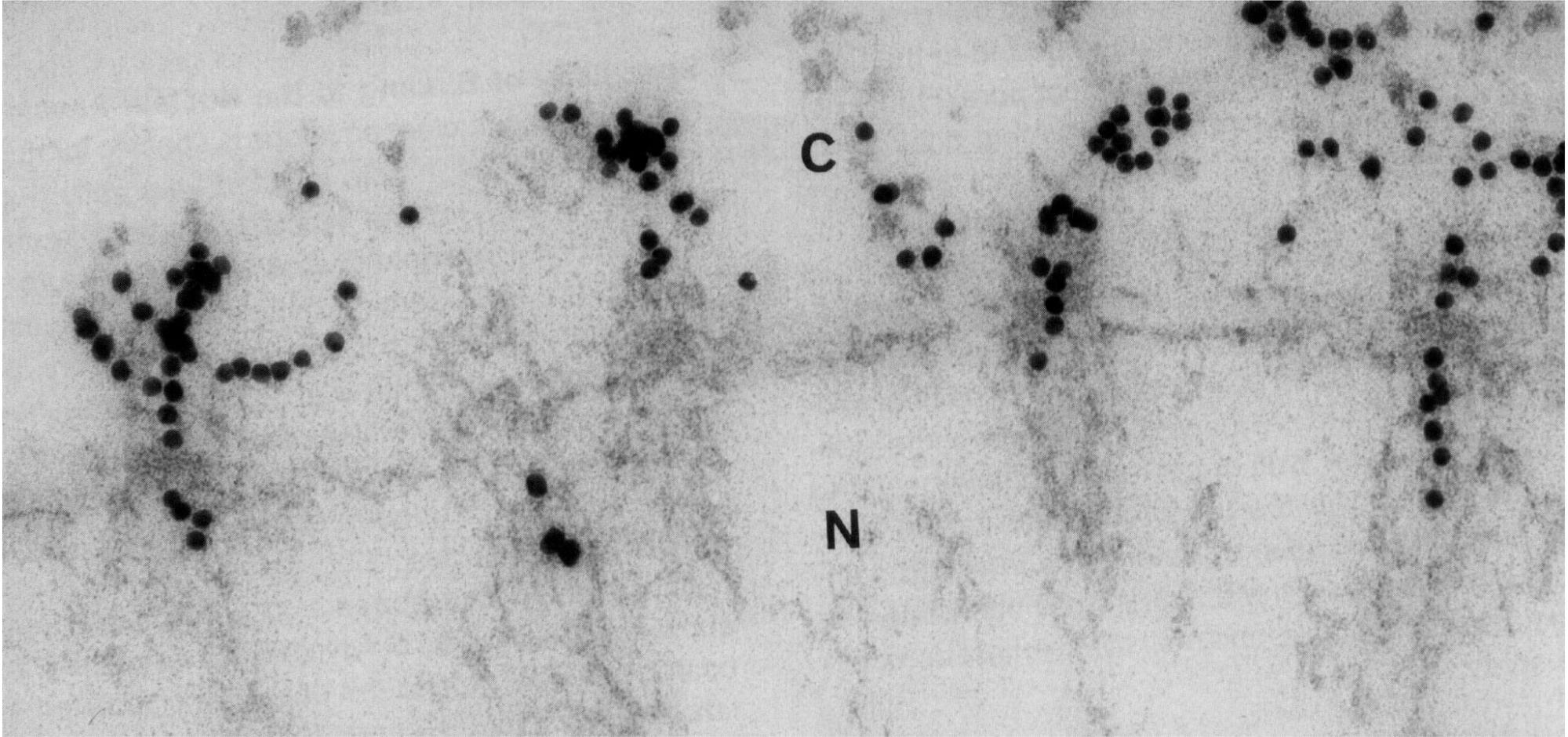


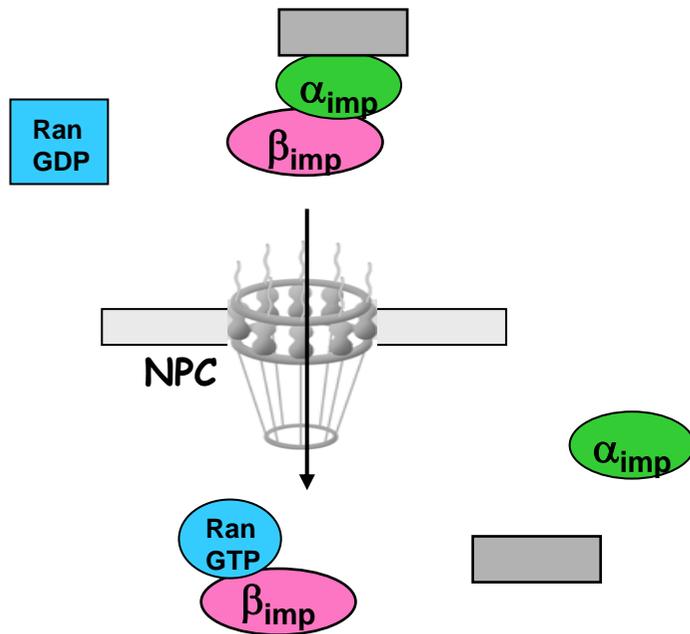
Nuclear targeting by Nuclear Localization Signals (NLS)



Richardson and Laskey (1988)

The nuclear import pathway of proteins containing a classical Nuclear Localization Signal (NLS)

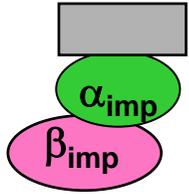
Uptake of NLS-containing protein in the cytosol



Importin β ($Kap\beta$) - transport factor
- binds the NPC

Importin α ($Kap\alpha$) - adaptor
- binds the cargo (NLSs)

Release of NLS-containing protein in the nucleus



Importin α (Kap α) recognizes diverse NLSs

Nuclear Localization Signals:

short sequences (5 to 18 aa)

no strict consensus

positively-charged

SV40 Tag

Histone H2B

c-myc

Nucleoplasmin

KKRK

KKRSK

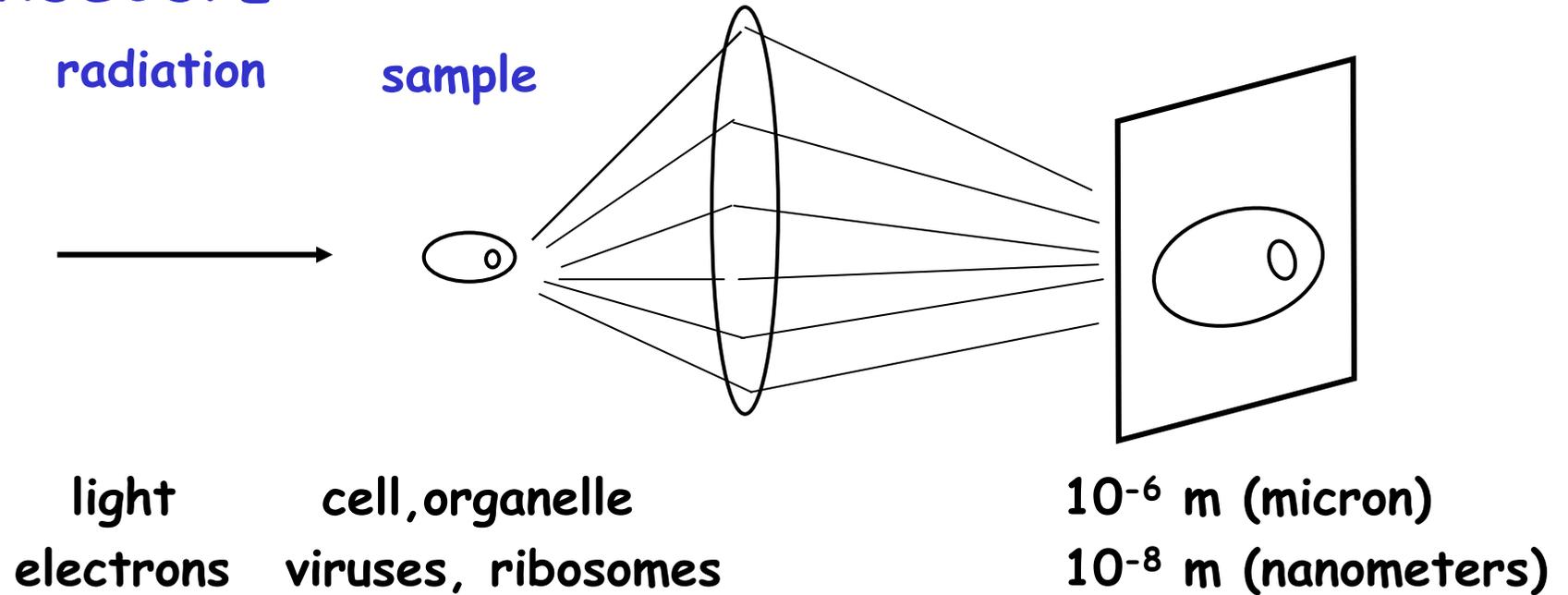
PAAKRVKLD

KRPAAATKKAGQAKKKK

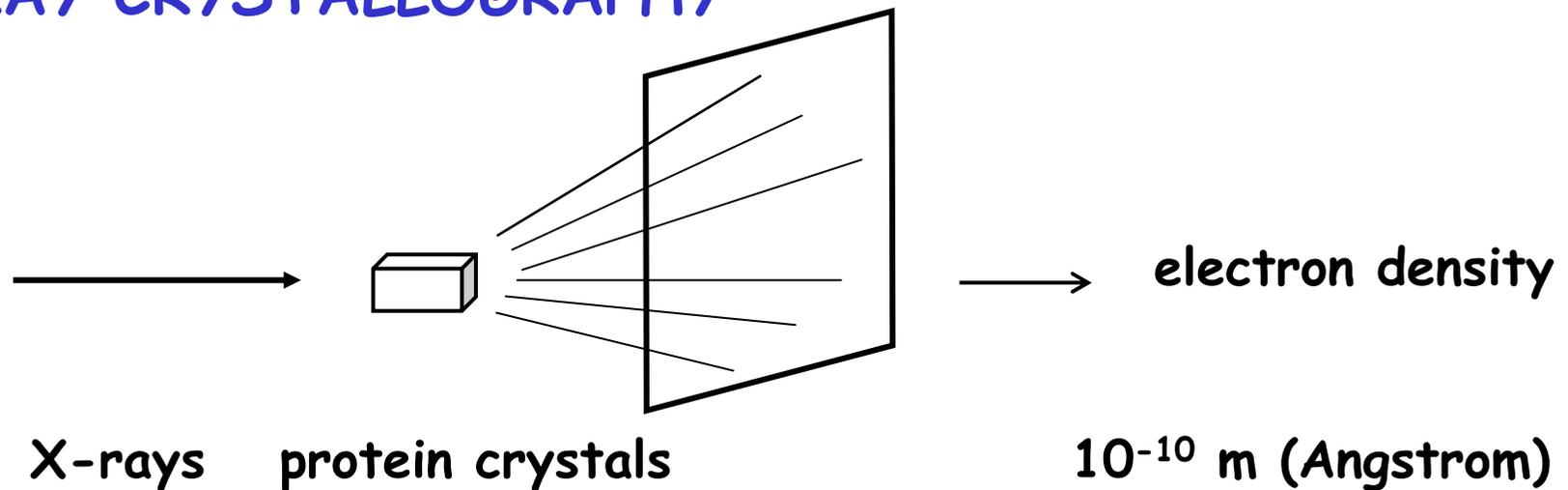
~~KTKRK~~

What determines the recognition at the atomic level?

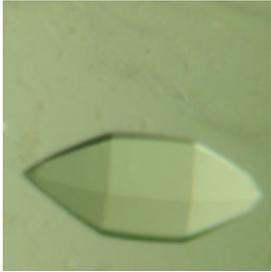
MICROSCOPE



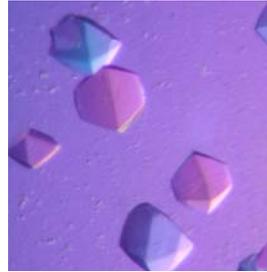
X-RAY CRYSTALLOGRAPHY



Protein crystals contain 10^{12} - 10^{14} molecules



0.4 mm

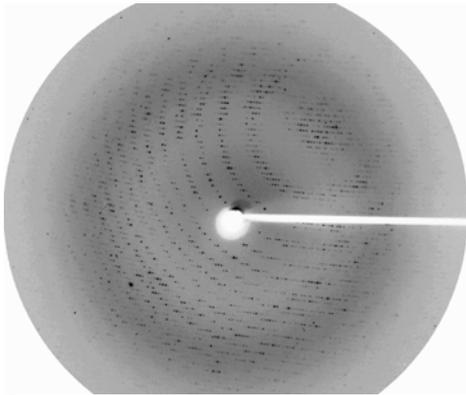


0.15 mm

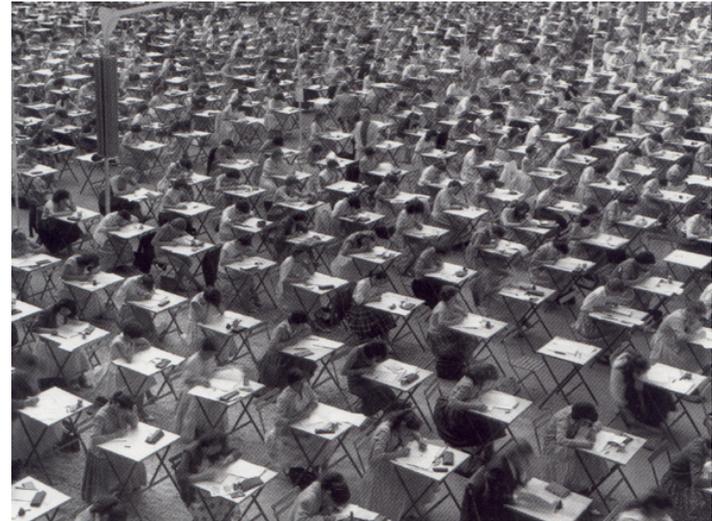


0.05 mm

The molecules have to pack in an orderly fashion for the crystals to diffract to high resolution

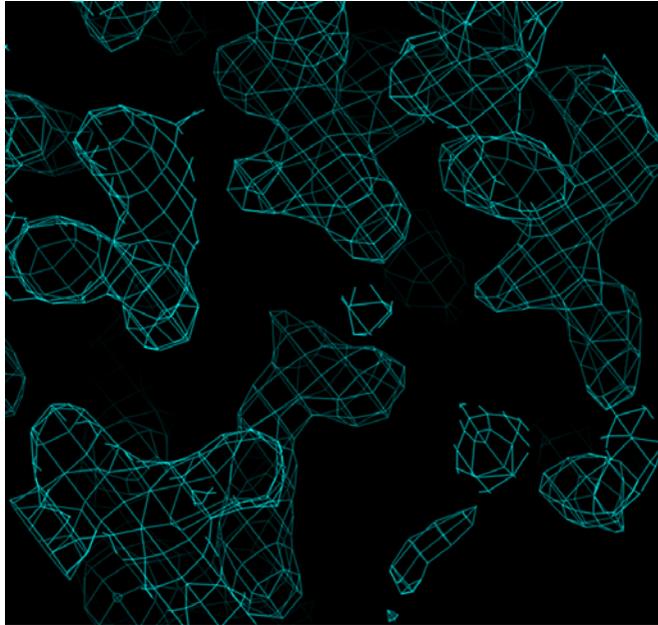


2 Å resolution diffraction pattern
(ESRF synchrotron, Grenoble)

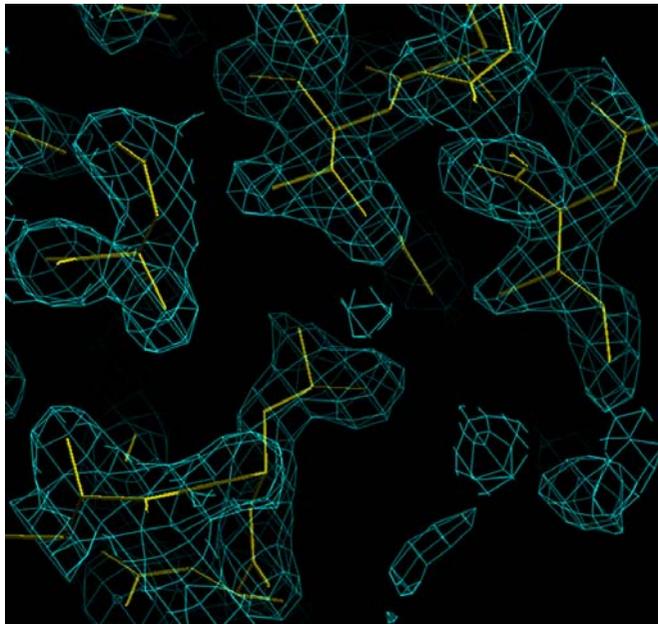


We obtain the structure of the average molecule

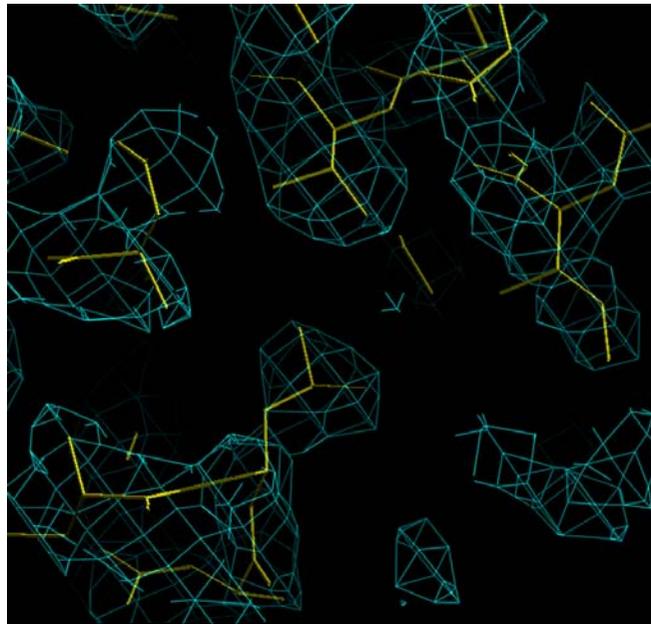
The experimental data: electron density



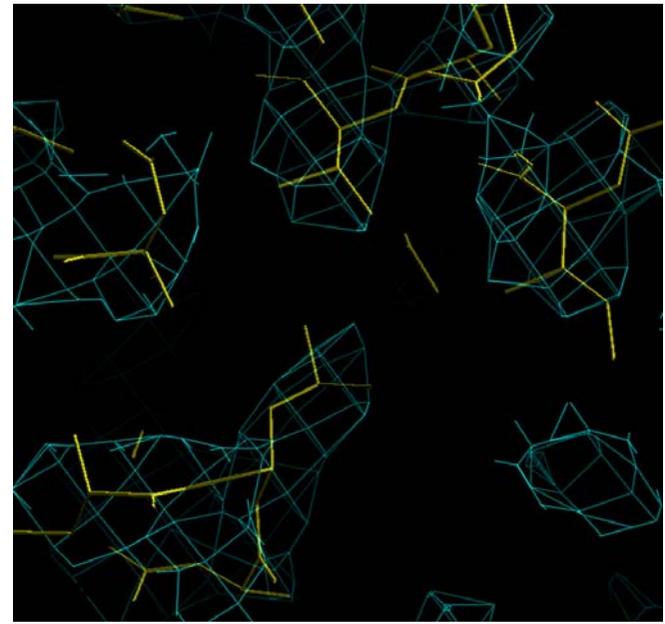
We build and refine a model in the data:



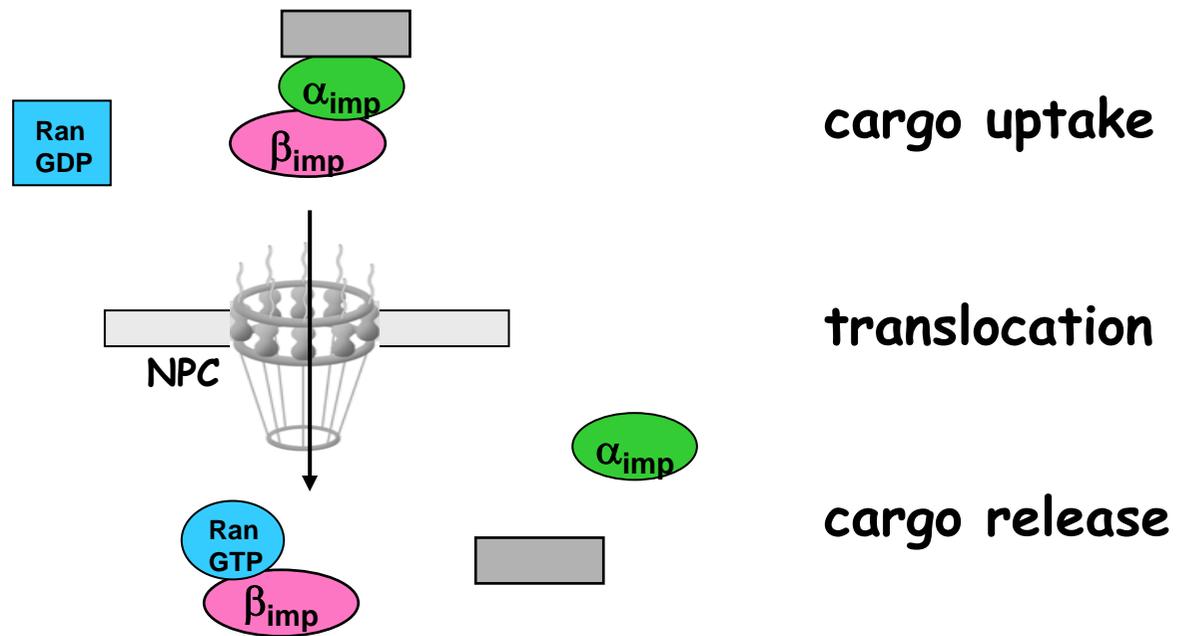
**2Å resolution
accuracy + precision**



**3Å resolution
accuracy**



4Å resolution



Questions we can address by crystallography

How are NLSs recognized with both versatility and specificity?

How does the transport factor dock to the NPC?

How does RanGTP (but not RanGDP) releases the cargo?

Questions we cannot address by crystallography

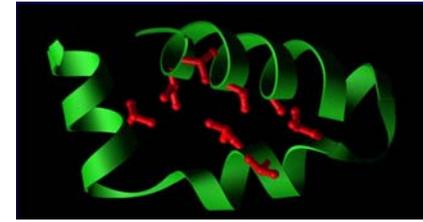
Dynamics of the process

Flexible conformational states

Cargo uptake by importin α (karyopherin α)

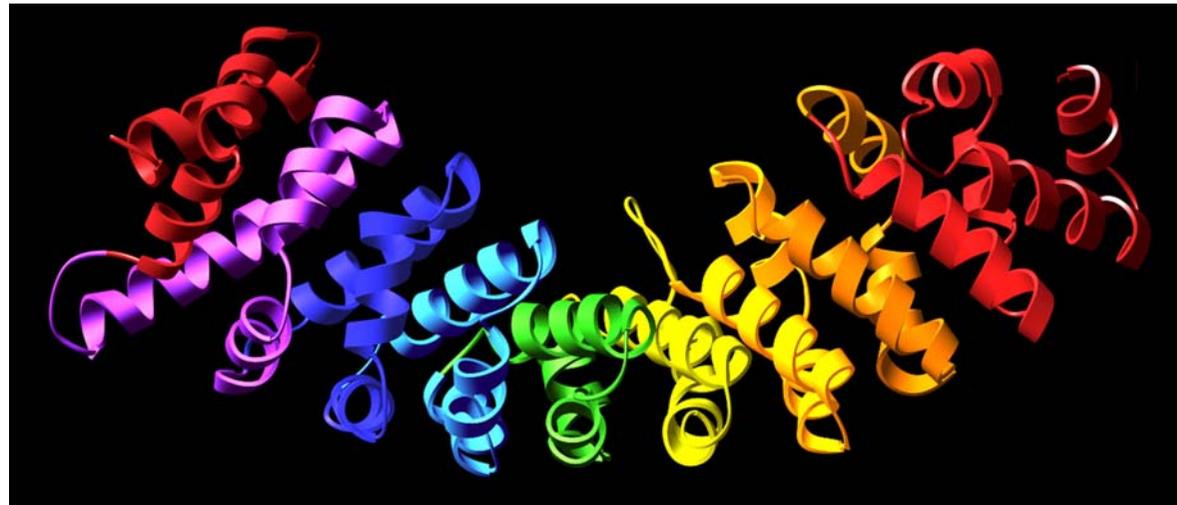


ARM repeat: ~40 residue
3 α -helices



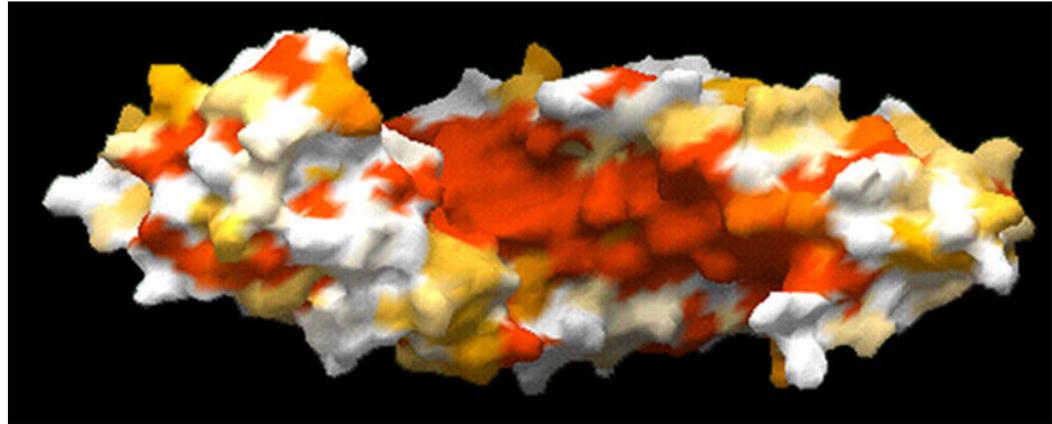
The NLS-binding domain is built by 10 ARM repeats

2.2 Å resolution
 R_{free} 27.5%



The NLS-binding domain of importin α has an elongated shape with a long surface groove

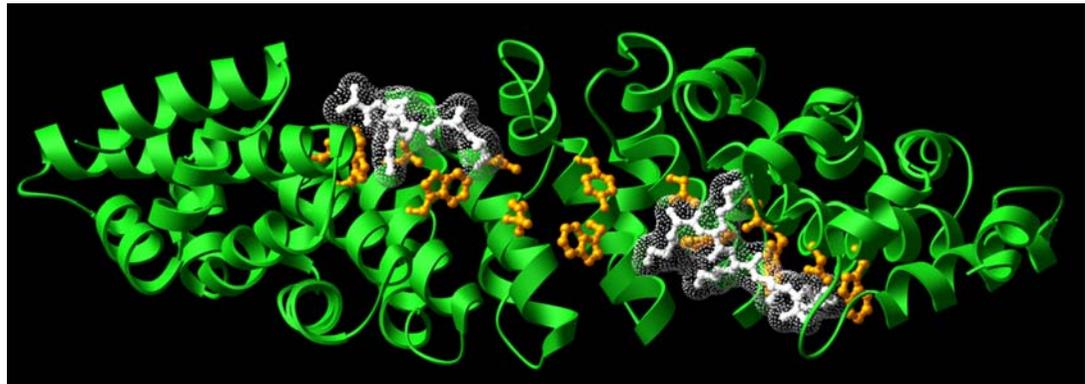
The surface groove is lined by **conserved** residues



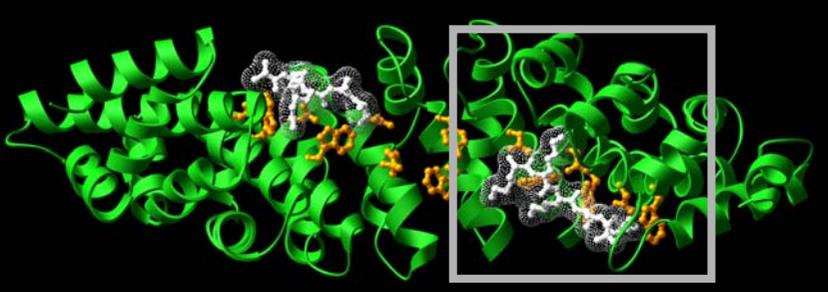
surface coloured by sequence identity



The surface groove is the site of **NLS** binding



SV40 Tag NLS binding



NLS larger binding site

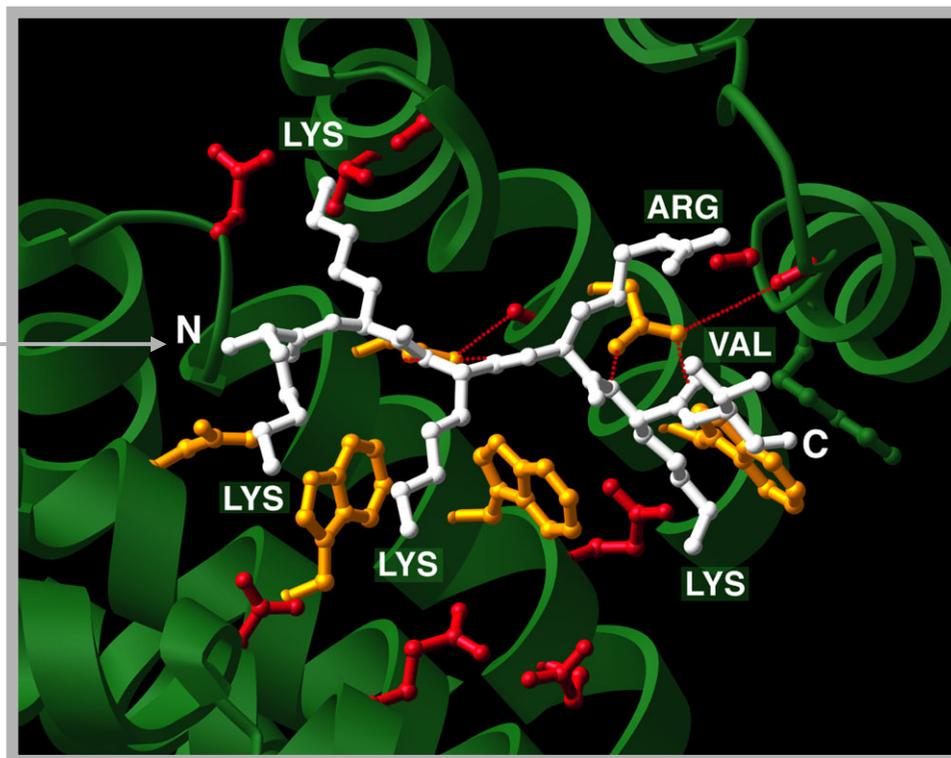
SV40 NLS:

KKKRKV

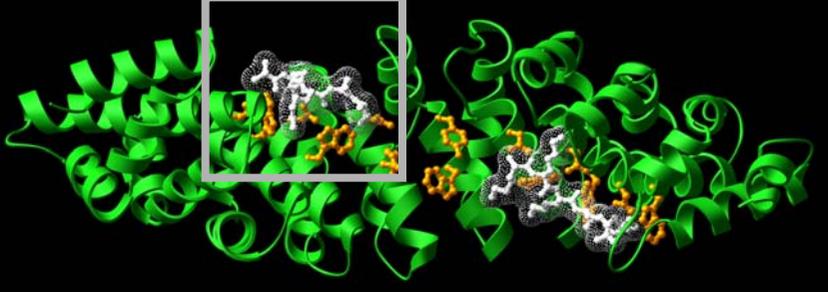
Asn residues

Trp residues

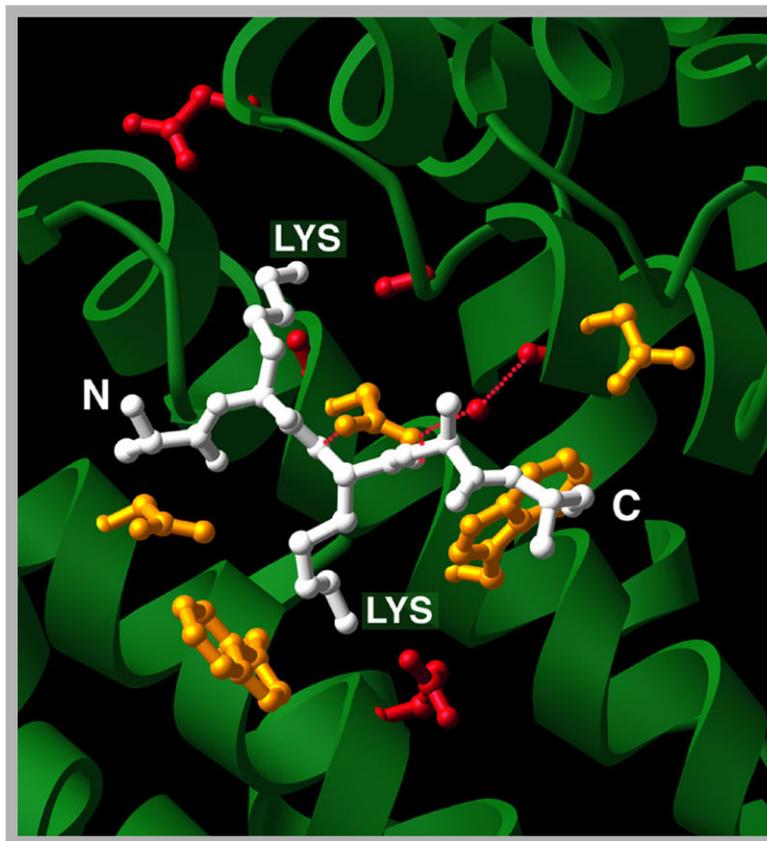
charged/polar residues



5 Lys/Arg are bound



NLS smaller binding site

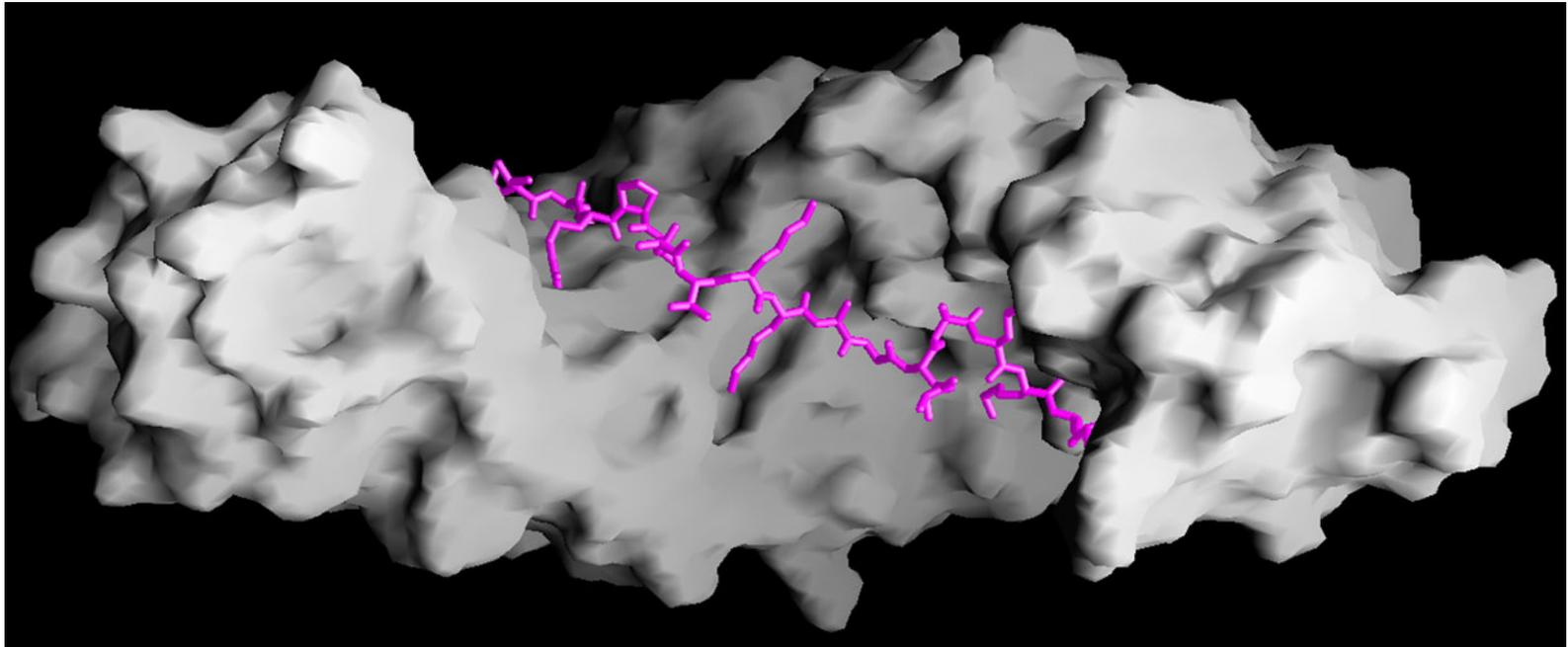


Two Lys are bound

The bipartite nucleoplasmin NLS binds along the whole surface groove

smaller site

larger site



K R P A A T K K A G Q A K K K K

upstream
cluster

linker

downstream
cluster

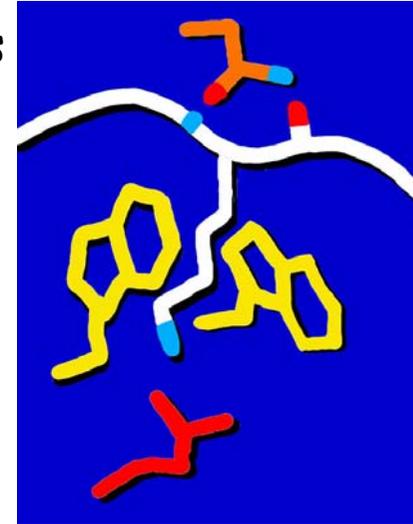
NLS binding

precise chemical recognition

Polar interactions

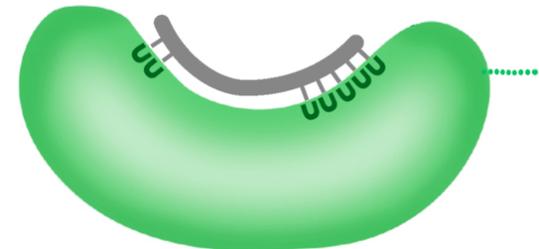
Hydrophobic

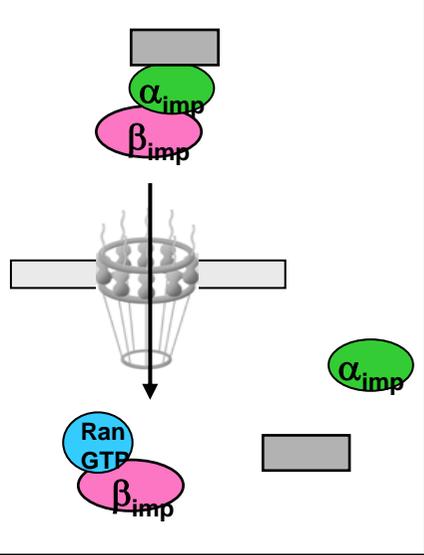
Electrostatic



versatile recognition

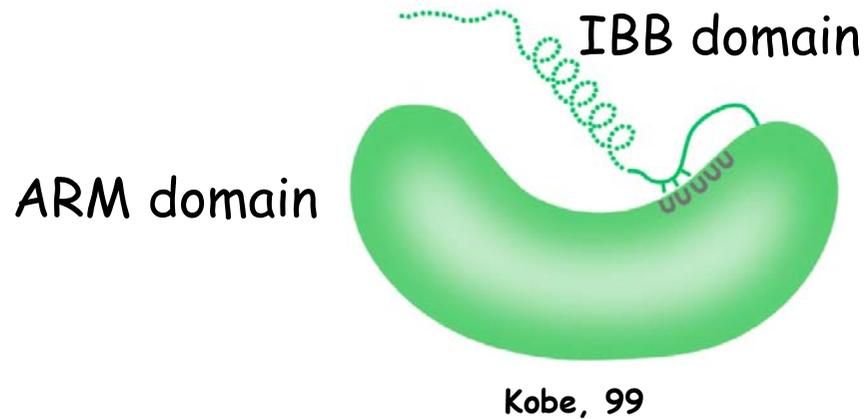
KKRRKV
PAAKRVKLD
KRPAATKKAGQA
KKKK





NLSs bind imp α better when imp β is present

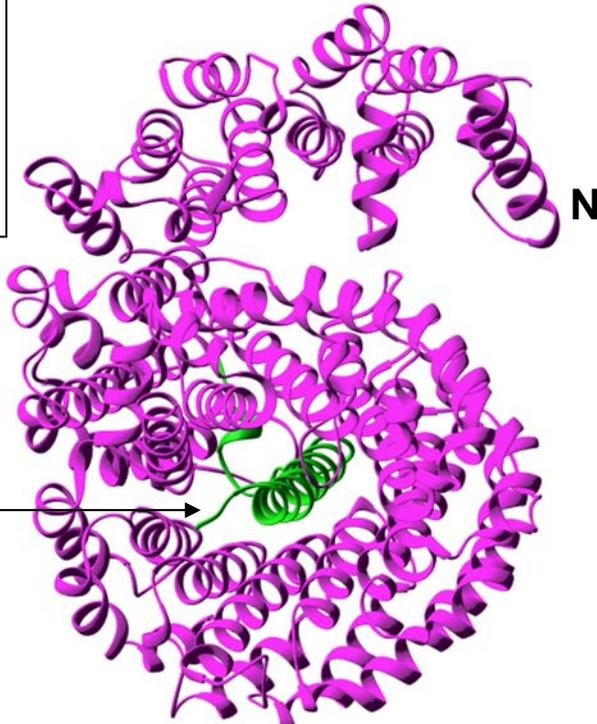
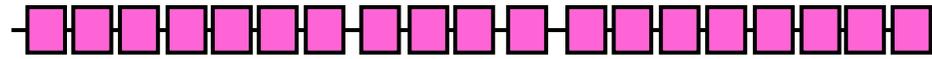
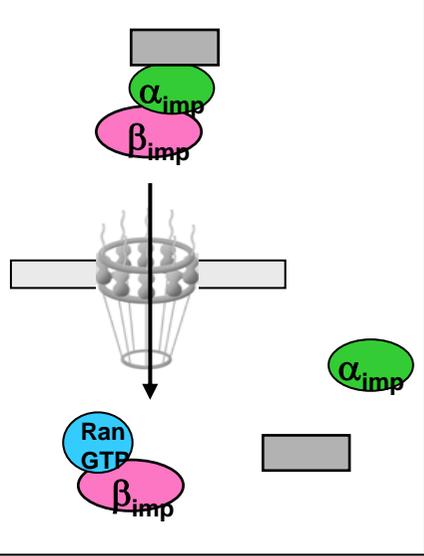
Full-length importin α is autoinhibited



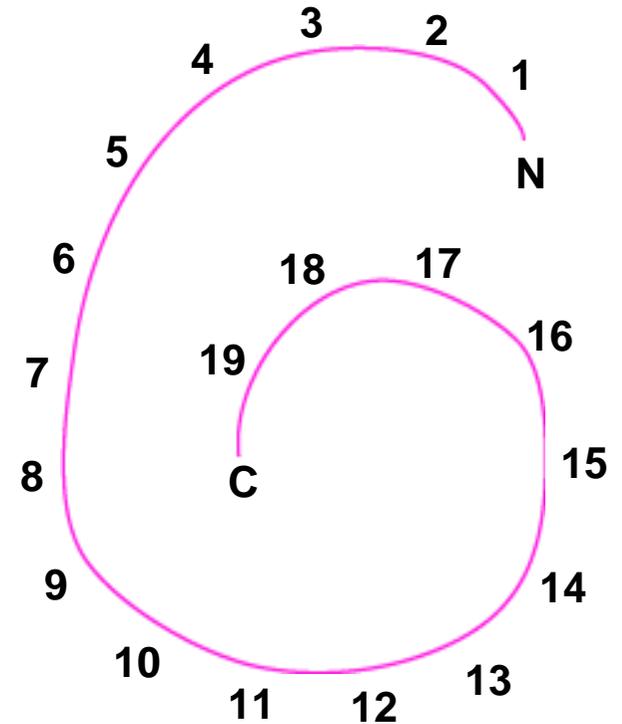
Part of the IBB domain binds to the larger NLS binding site of α

The entire IBB domain binds importin β

Importin β is built by 19 tandem HEAT repeats



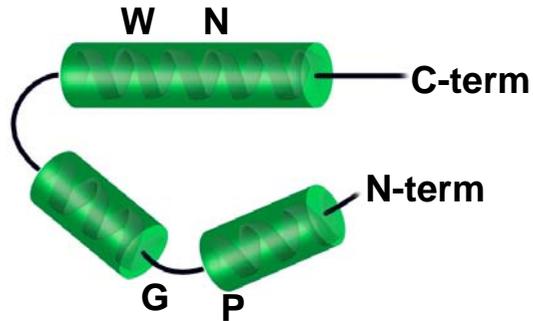
Cingolani *et al.*, '99



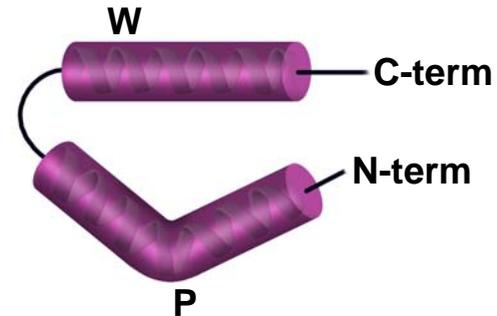
Overall snail-like conformation of importin β , with its C-terminal half wrapped around the IBB cargo

ARM and HEAT repeats are structurally similar

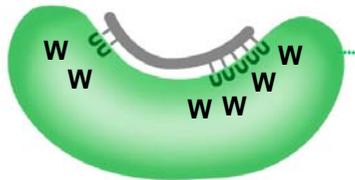
ARM repeat



HEAT repeat



NLS-binding domain of α (10 ARM repeats)

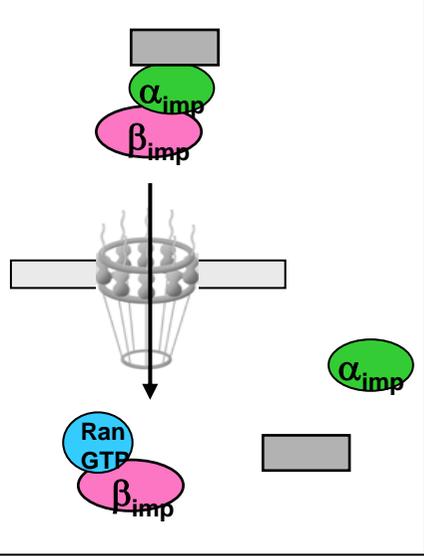


importin β (19 HEAT repeats)

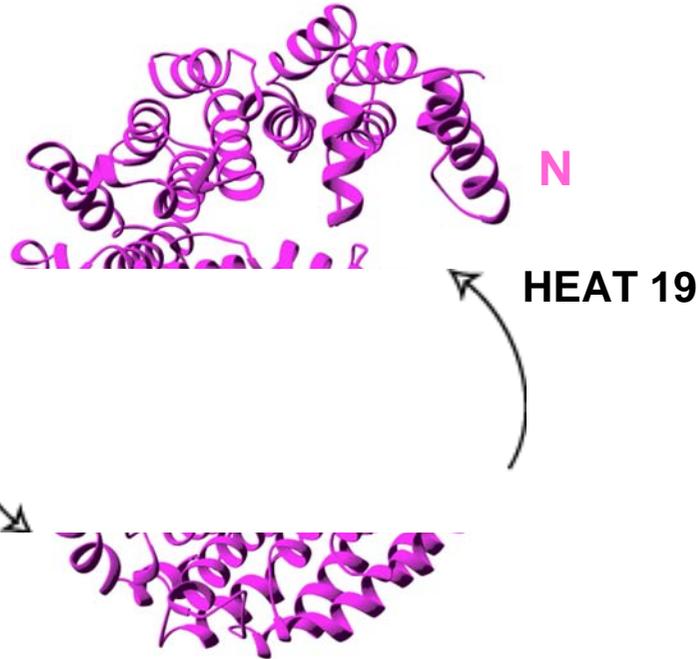


The C-terminal helix of ARM and HEAT repeats line the inner cargo-binding surface of α and β

The import complex docks to the NPC via the binding of importin β to nucleoporins

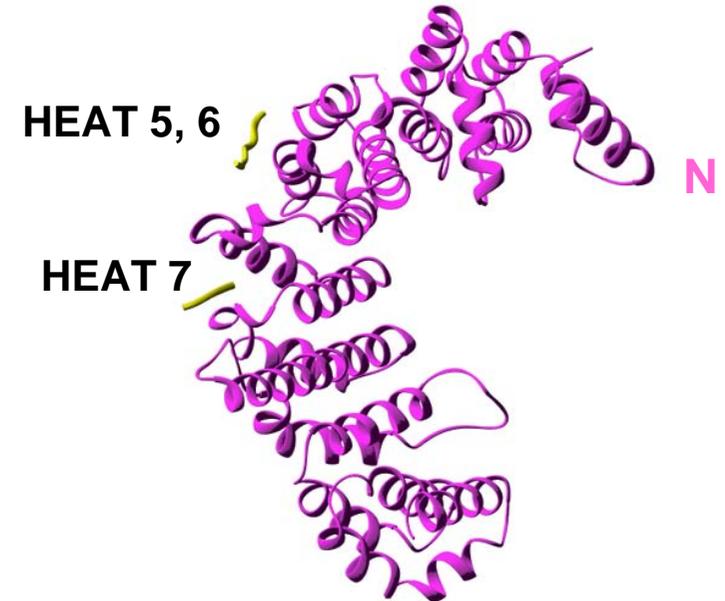


β - IBB domain of α
(Cingolani et al., '99)



The **IBB cargo** binds the C-terminal half of β at its inner concave surface

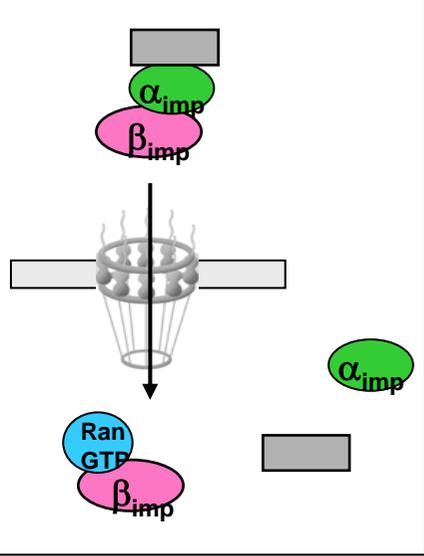
β fragment - FxFG of Nsp1
(Bayliss et al., '00)



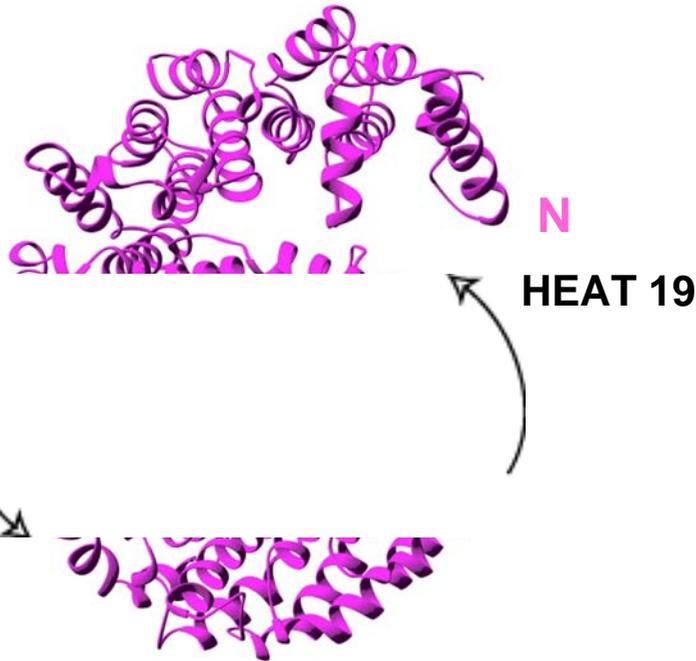
The **FxFG** repeats of a nucleoporin bind β at its outer convex surface

The FxFG nucleoporin and the IBB cargo bind different regions of β

In the nucleus RanGTP binds to importin β and displaces the IBB cargo

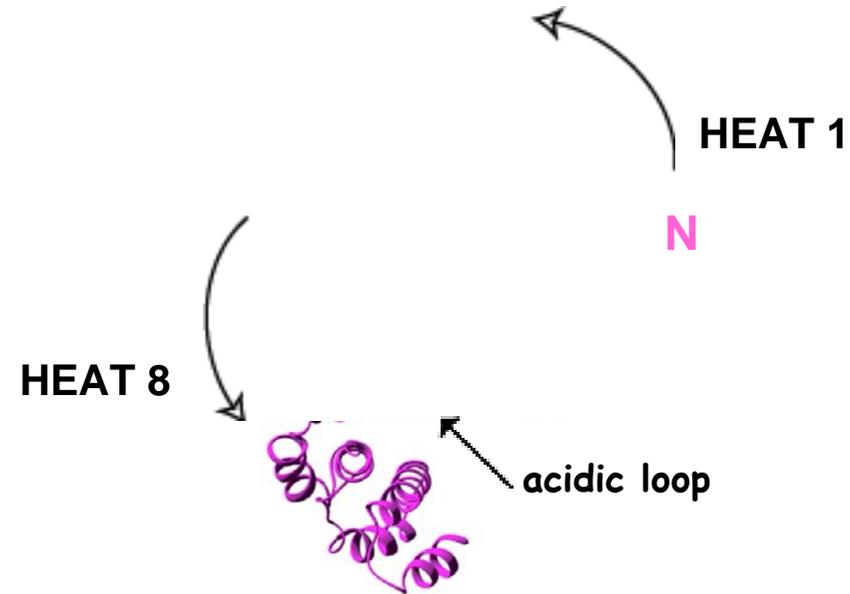


β - IBB domain of α
(Cingolani et al., '99)



The **IBB cargo** binds the inner surface of β between HEAT 7 and 19

β fragment - RanGTP
(Vetter et al., '00)



RanGTP binds the inner surface of β between HEAT 1 and 8

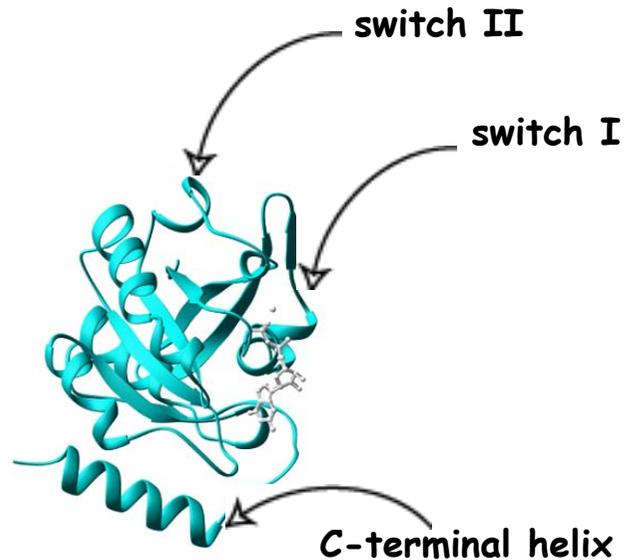
RanGTP and the IBB cargo have overlapping binding sites at the acidic loop of β

In the cytosol RanGDP is unable to bind to importin β

RanGDP

switch I and II move away from nucleotide
C-terminal helix binds to the core domain

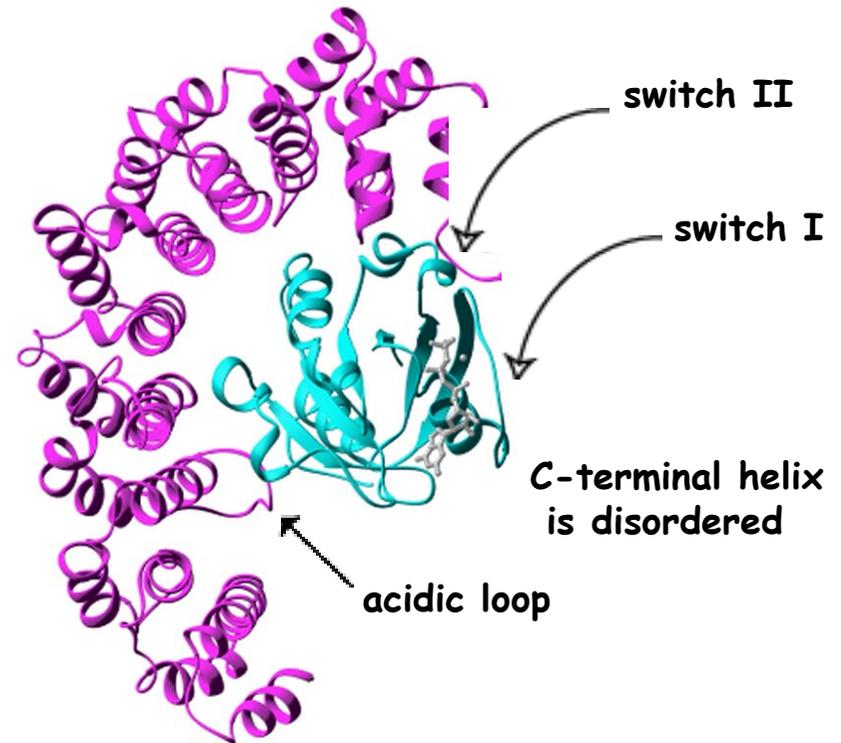
(Scheffzek et al., '95)



RanGTP

switch I and II move close to the GTP γ P
C-terminal helix is displaced from the core domain

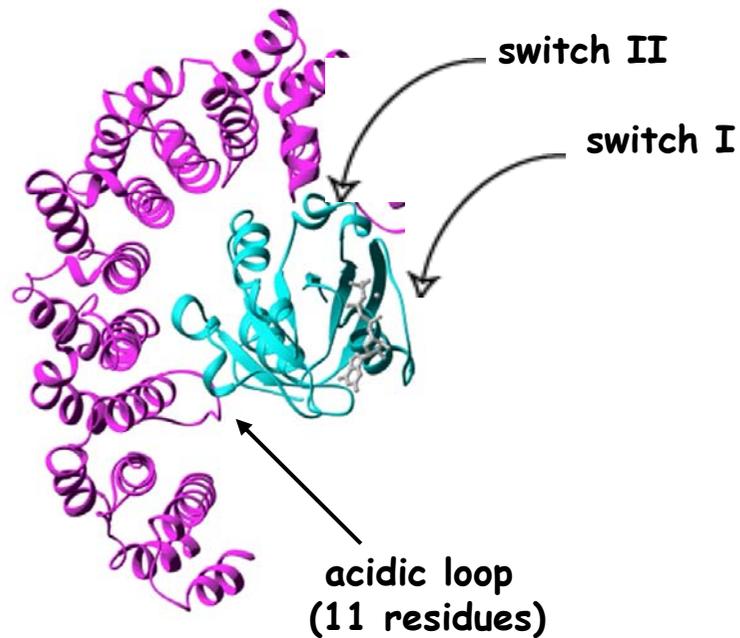
β fragment -
RanGTP
(Vetter et al., '99)



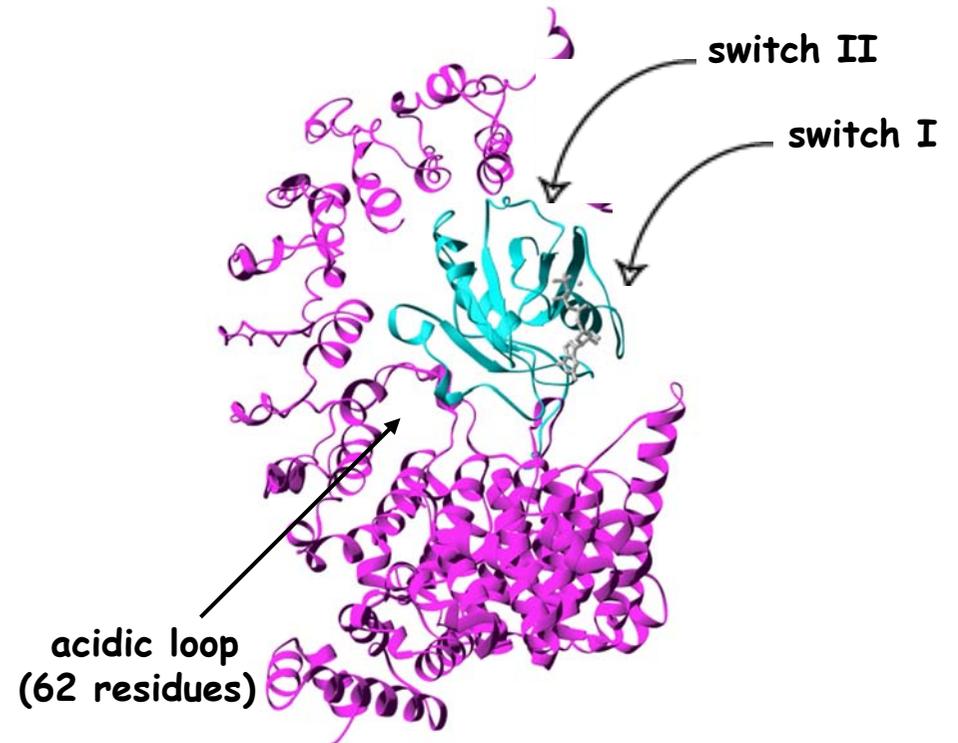
In RanGDP, the conformation of the C-terminal helix is sterically incompatible with β

Similar binding of RanGTP to importin β (Kap β 1) and to transportin (Kap β 2)

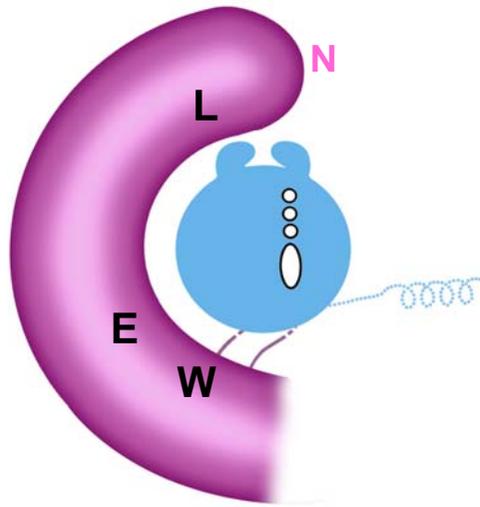
β fragment - RanGTP
(Vetter et al., '99)



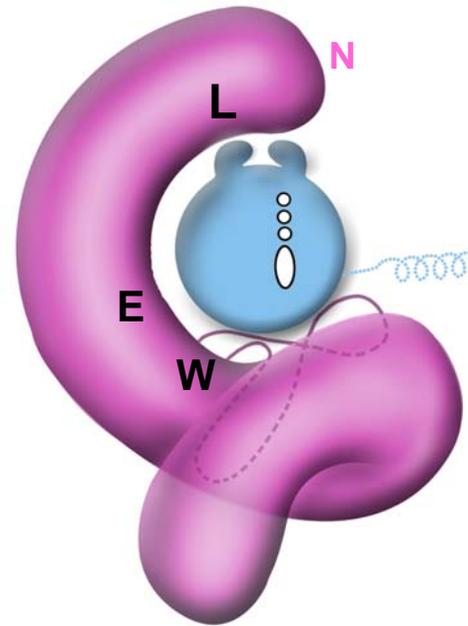
transportin - RanGTP
(Chook and Blobel, '99)



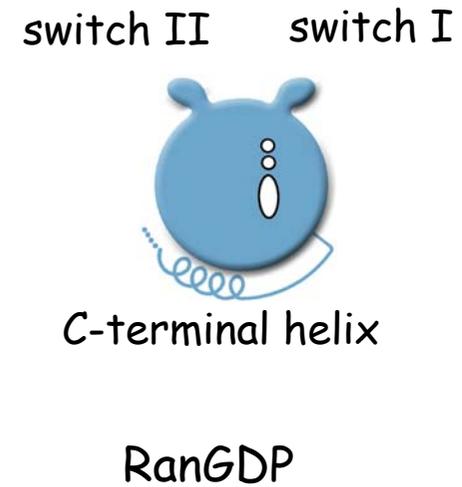
Ran binding to importin β -like transport factors: shape recognition mechanism



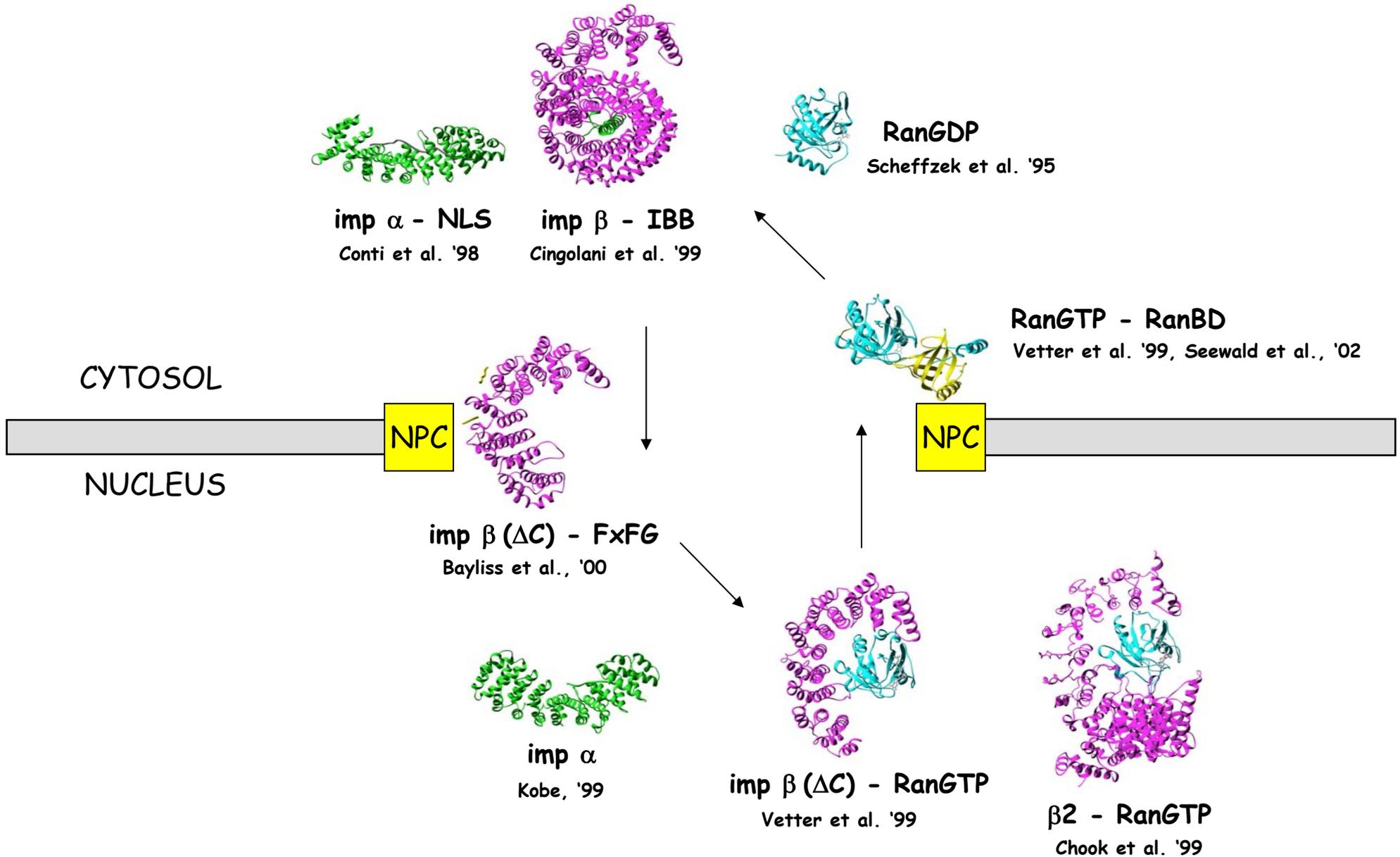
Importin β fragment - RanGTP



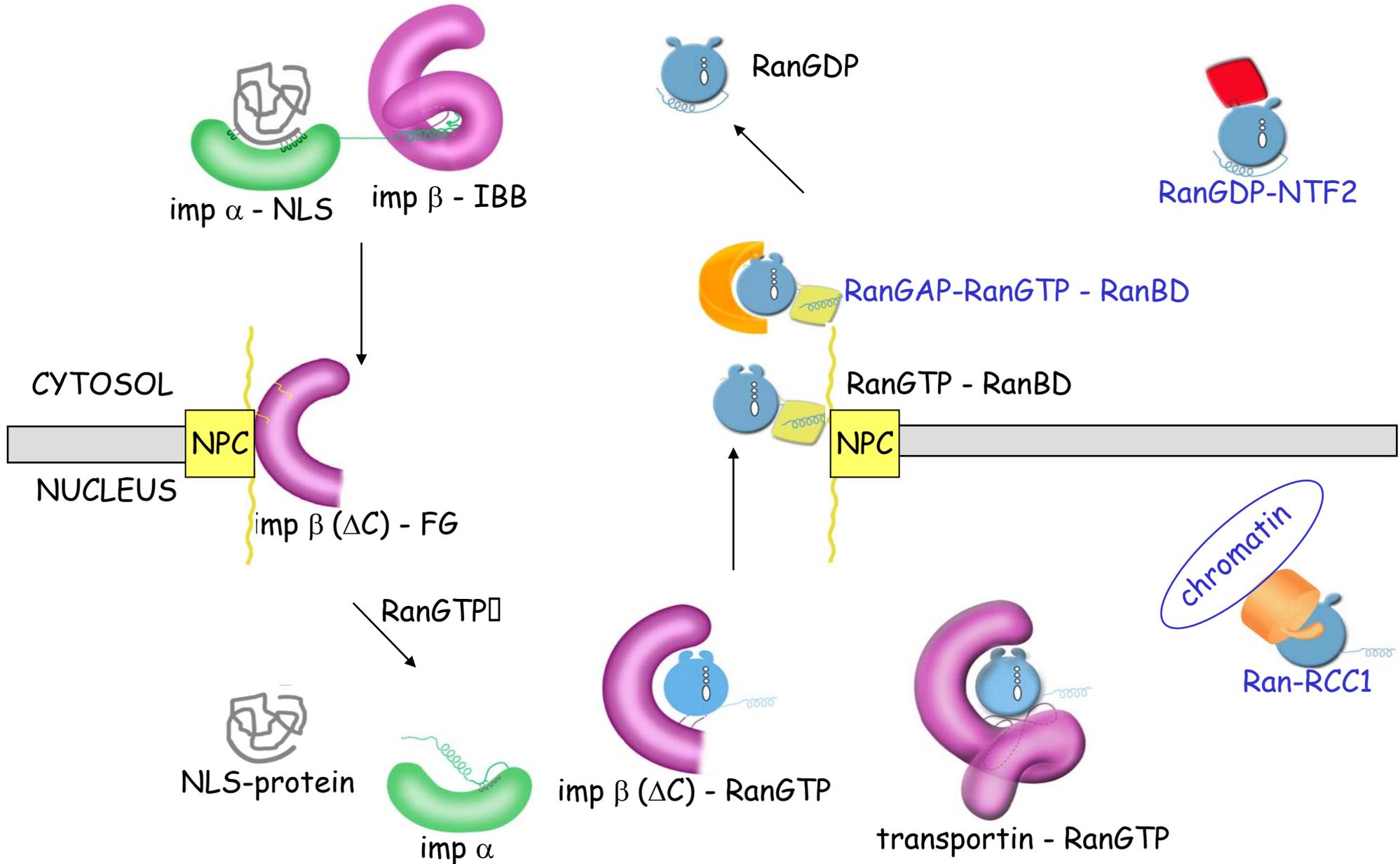
Transportin - RanGTP



Structural studies of NLS-nuclear import: a "dynamic" view



Structural studies of NLS-nuclear import: a "dynamic" view



At least 22 karyopherins in the human genome

IMPORT

Importin β

- IBB domain (Arg-rich) of importin α (adaptor for NLS import) and snurportin (adaptor for U snRNP import)
- BIB domain (Arg/Lys rich) of ribosomal proteins

Transportin

- M9 domain (Gly/Met rich) of hnRNP A1
- TAP N-term domain (Gly/Arg rich)
- BIB domain (Arg/Lys rich) of ribosomal proteins
- mago/Y14, etc.

Importin 13

EXPORT

Xpo-t

- tRNA

CAS

- importin α

Crm1

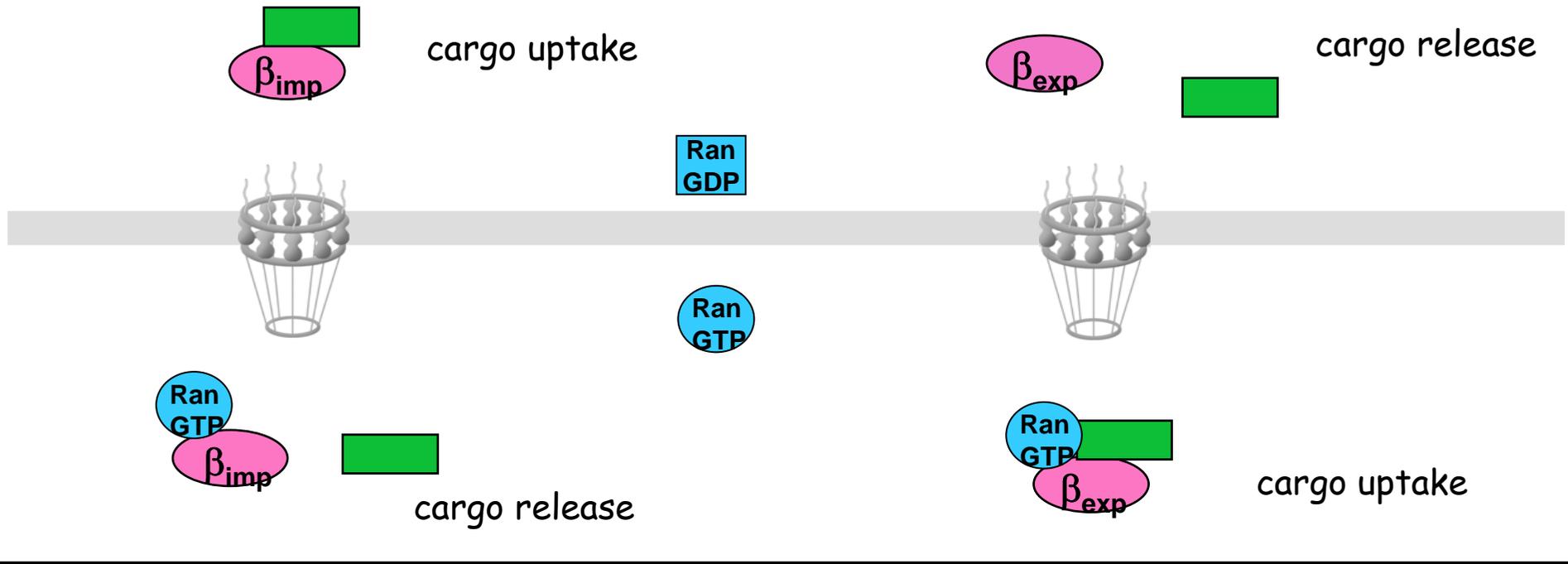
- snurportin
- NES domain (Leu-rich) of proteins with RanBP3

Importin 13

- EIF4A

nuclear import

nuclear export



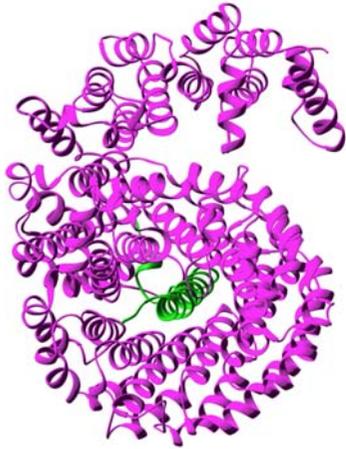
Karyopherins can bind multiple substrates

Substrates can bind multiple karyopherins

Karyopherins can behave as importins and exportins

Different overall conformations of two importins:

Importin β + IBB cargo

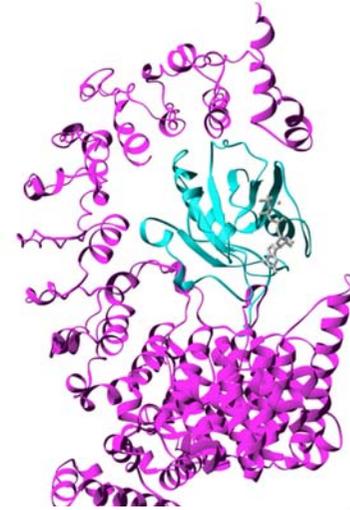


side



front

Transportin + Ran



side

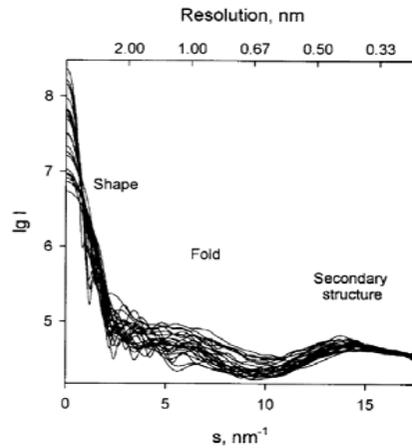


front

Conformational changes as they carry out an import cycle?

Small angle X-ray scattering (SAXS)

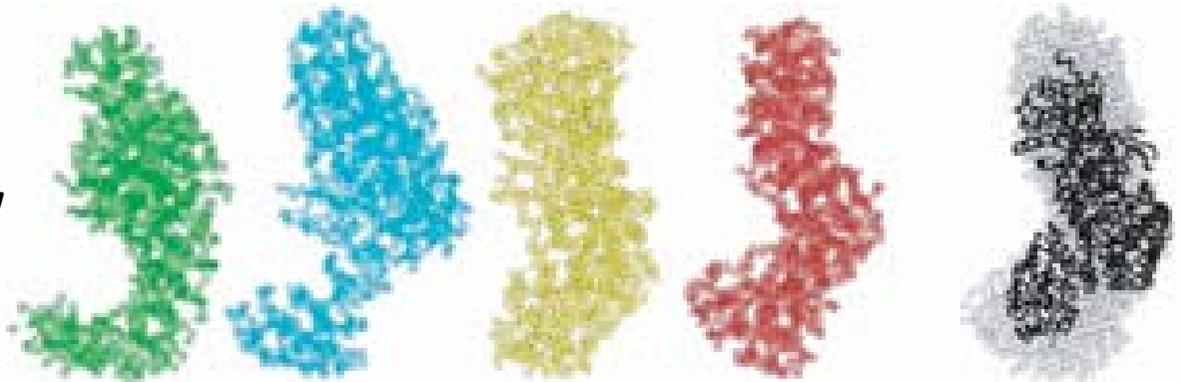
Dmitri Svergun, EMBL-Hamburg



4 independent reconstructions of transportin - RanGTP

average

front view



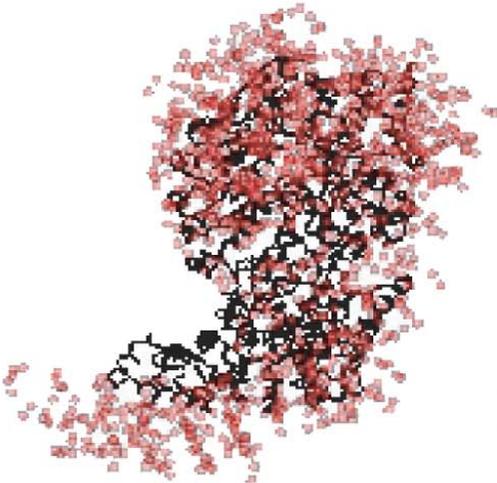
top view



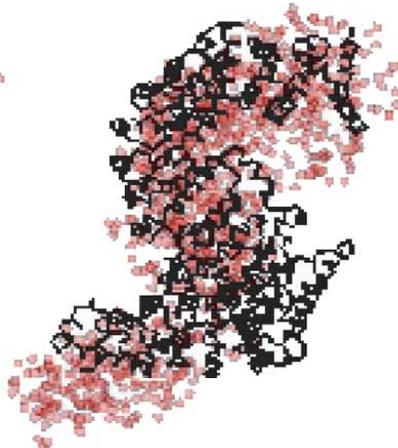
Conformational changes of nuclear import factors



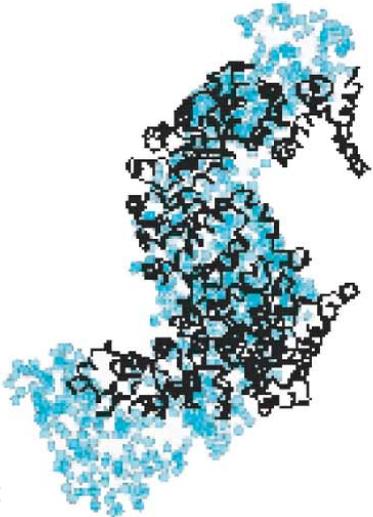
Transportin



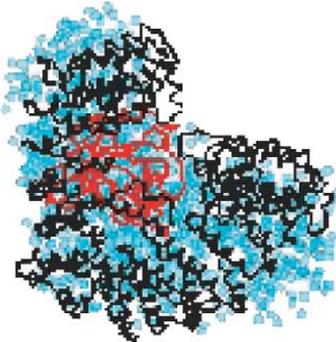
Transportin-RanGTP



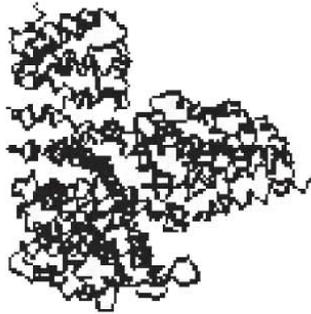
Transportin-M9



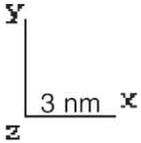
Importin β



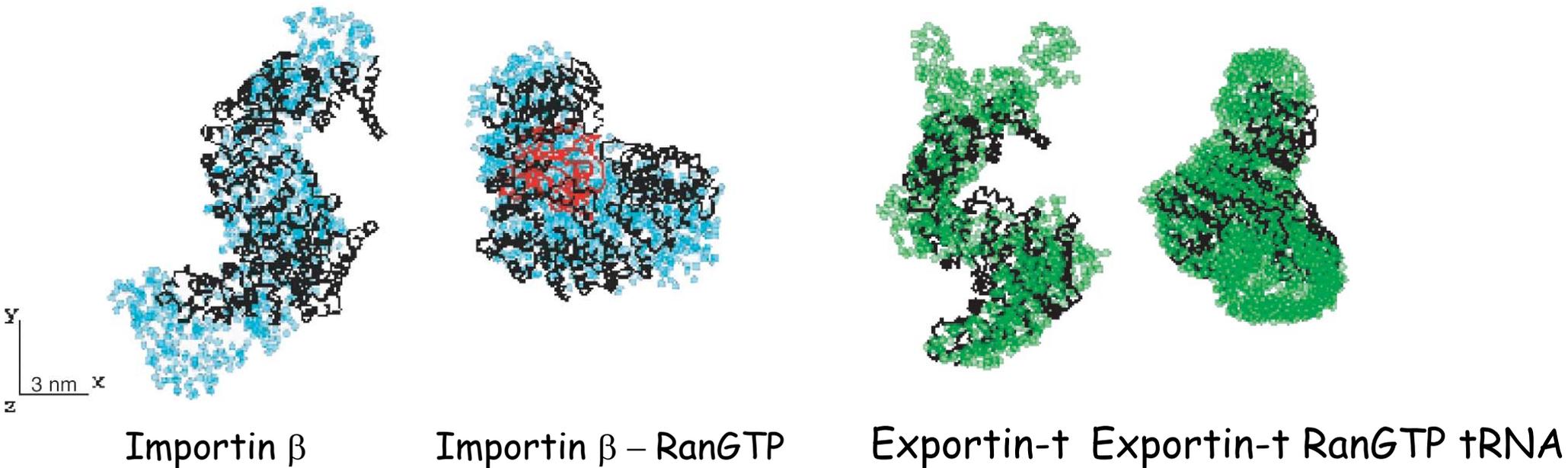
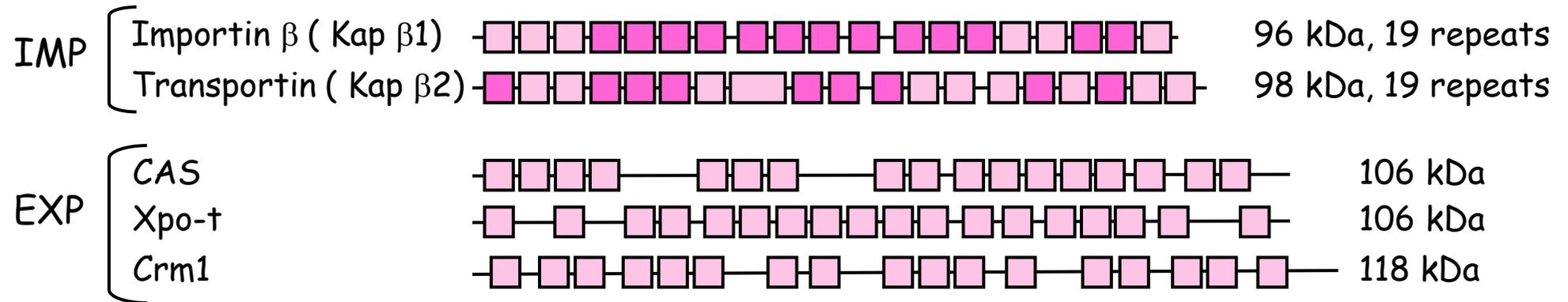
Importin β -RanGTP



Importin β -IBB

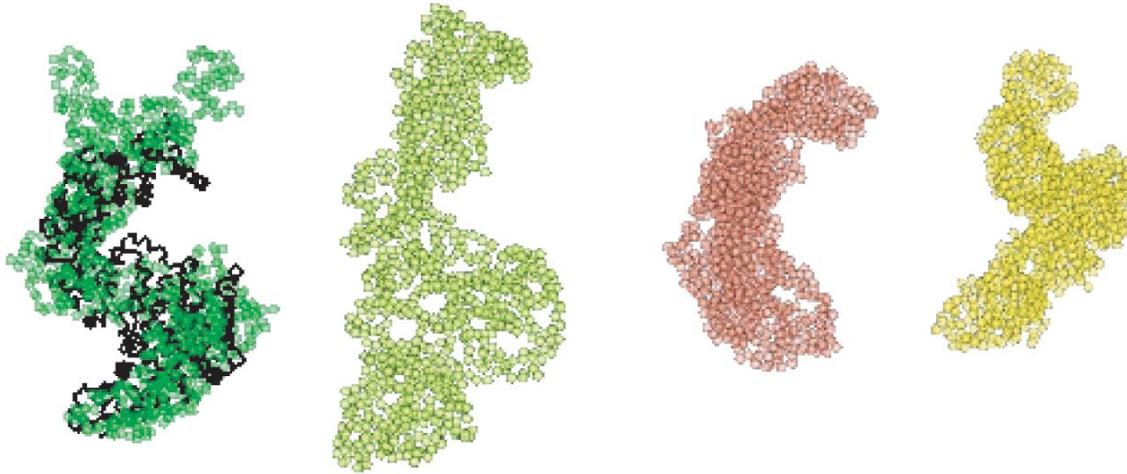


Import-mediators vs Export-mediators

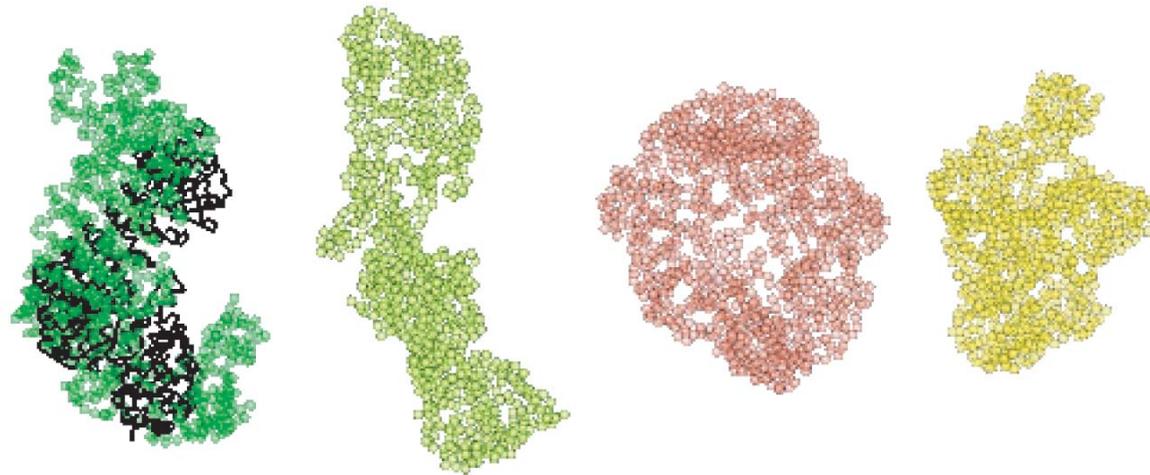


Conformational variability of karyopherins

Side view



Front view



Xpo-t

Los1

Xpo1

Cse1



Elena Conti

SAXS studies: Noemi Fukuhara
Elena Fernandez
Judith Ebert

Dmitri Svergun (EMBL-Hamburg)

NLS studies: John Kuriyan (Rockefeller Univ.)