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Karyopherins And Condensates

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Abstract

Several aggregation prone RNA-binding proteins, including FUS, EWS, TAF15, hnRNP A1, hnRNP A2 and TDP-43, are mutated in neurodegenerative diseases. The nuclear-cytoplasmic distribution of these proteins is controlled by proteins in the Karyopherin family of nuclear transport factors (Kaps). Recent studies have shown that Kaps not only transport these proteins, but also inhibit their self-association/aggregation, acting as molecular chaperones. This chaperone activity is impaired for disease-causing mutants of the RNA binding proteins. Here we review physical data on the mechanisms of self-association of several disease-associated RNA binding proteins, through liquid-liquid phase separation and amyloid fiber formation. In each case we relate these data to biophysical, biochemical and cell biological data on the inhibition of self-association by Kaps. Our analyses suggest that Kaps may be effective chaperones because they contain large surfaces with diverse physical properties that enable them to engage multiple different regions of their cargo proteins, blocking self-association.

Introduction

Karyopherins transport macromolecular cargos between the nucleus and the cytoplasm through the nuclear pore complex (NPC), controlling the nuclear-cytoplasmic distribution of thousands of molecules [1–6]. Kaps bind cargos and phenylalanine-glycine (FG) motifs in NPC subunits simultaneously to move traffic across the NPC [7,8]. Kaps are divided into importins, which bring cargo into the nucleus, exportins, which take cargo out of the nucleus, and bidirectional transporters, which can move cargo both directions. Many Importins bind nuclear localization signals (NLSs) in their cargos. For example, Karyopherin-β2 (Kapβ2/Transportin-1) binds proline-tyrosine NLSs (PY-NLSs) of many cargos [9,10]. Importin-β (Impβ/Kapβ1) binds some cargos directly, but binds many others

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through its complex with the adaptor protein, Importin-α (Impα/Kapα), which binds the lysine-rich classical-NLS (cNLS) [11–13]. Several importins also bind directly to folded domains of cargos [14–17]. Analogous to importins, some exportins bind linear motifs in their cargos and others bind to folded domains [2,6]. Bidirectional Kaps appear to recognize folded domains [18–20]. The mechanisms of cargo recognition by Kaps have been reviewed extensively elsewhere [6,10].

Many Kaps, including Kapβ2 and Impα/β1, act to control the nuclear-cytoplasmic distribution of RNA binding proteins [11,21–23]. These cargos function in diverse processes, ranging from RNA splicing to DNA repair to transcription. Some of these cargos, including FUS, hnRNP A1 and TDP-43, are mutated in the neurodegenerative diseases amyotropic lateral sclerosis (ALS), frontotemporal lobe dementia (FTD) and multi-system proteinopathy (MSP) [24,25]. The wild type versions of these proteins are predominantly nuclear in healthy cells. In response to stresses, they translocate to the cytoplasm and enter stress granules (SGs), biomolecular condensates that form through self-assembly of stalled translation pre-initiation complexes and associated factors [23,26,27]. Disease-causing variants of the proteins constitutively mislocalize to the cytoplasm, either because of impaired NLSs or physical changes that increase self-association, with concomitant localization in aberrant SGs [27–31]. Kaps co-localize with these aberrant stress granules in patient tissues, animal models and transfected cells [32]. There is therefore substantial interest in understanding the physical mechanisms that drive RNA binding proteins to self-associate, and whether and how Kaps modulate self-association and cellular localization. Here we review recent biophysical, biochemical, cell biological and organismic data that have revealed molecular mechanisms underlying these processes, and their regulation by Kaps, and have suggested potential new strategies for treatment of neurodegenerative diseases.

FET-Kapβ2 binding

The three FET proteins, FUS, EWS and TAF15 (or TAFII68), are ubiquitously expressed in human tissues, mostly localized to the nucleus, bind nucleic acids and function in RNA processing, transcription, splicing, and DNA repair [33]. FUS, TAF15, and to a lesser extent EWS, also localize to SGs in response to stresses [27]. Wildtype and mutant proteins are mislocalized and aggregated in the cytoplasm of neurons in ALS and FTD patients [34–38].

Each FET protein has an N-terminal low complexity (LC) serine-, tyrosine-, glycine- and glutamine-rich region followed by an arginine-glycine-glycine region (RGG1), an RNA recognition motif (RRM) domain, a second RGG region (RGG2), a zinc finger domain (ZnF), a third RGG region (RGG3) and a PY-NLS (Figure 1A). The LC, RGG1, RGG2, RGG3 and PY-NLS are all intrinsically disordered; the 90-residue RRM and the 30-residue ZnF are small globular domains. The LC and RGG regions self-associate, both individually and with each other, to mediate liquid-liquid phase separation (LLPS) and fibrillization [27,39–44]. RRM, ZnF and the RGGs bind RNA and the PY-NLS binds Kapβ2 for transport into the nucleus [45–49]. [9,21,50].

All FET PY-NLSs are similar in sequence (Figure 1B) and structures of the FUS PY-NLS (residues 507–526) bound to HEAT repeats 9–17 of Kapβ2 are likely representative of the

family (Figure 1C) [50,51]. The PY-NLS consists of a structurally conserved N-terminal hydrophobic motif (^{508}PGKM^{511}), a central arginine-rich α-helix and a C-terminal PY motif, all of which make numerous interactions with Kapβ2, affording high affinity (K_D ~ 10–70 nM) [44,50].

ALS mutations are frequently found in the FUS LC and PY-NLS, and slightly less often in the RGGs [23,31,34,52–54]. Mutated sites in the PY-NLS contact Kapβ2 in the cargo complex, explaining why the alterations weaken Kapβ2 binding, leading to aberrant localization and aggregation (see below) [32,42,50]. In EWS and TAF15, ALS mutations are located primarily in the LC and RGGs [55,56], although disease mutations are occasionally found in the PY-NLS of EWS [55].

Self-association of the FET proteins—Upon concentration at low temperature, the FUS LC region forms hydrogels, which contain cross-β fibrils [41]. The solid-state NMR structure of the FUS LC fibrils showed a 57-residue core, which stacks through cross-β interactions (Figure 1D) [57]. Crystal structures of short, 6–8 residue FUS LC peptides show bent coils termed LARKS (low-complexity aromatic-rich kinked segments) stacking into cross-β structures that make non-hydrophobic and water-mediated contacts with neighbors. These structures are distinct from the extremely stable steric zippers of amyloid fibrils, which are canonical β-strands with hydrophobic inter-strand contacts and water-less interfaces [58–61]. These differences were proposed to explain the extreme stability of steric zippers as compared to the sensitivity of LARKS-based hydrogels to changes in temperature and solution conditions. The conformations of the FUS LARKS peptides are different from the same segments in the larger FUS LC core, possibly due to different constructs and experimental conditions (Figure 1D). It remains unknown which aspects of these structures will track to full-length FUS.

Solution NMR and Raman spectroscopy studies of dilute/monomeric and phase-separated FUS LC (residues 1–163) provide yet a different set of information [62,63]. Extensive biophysical studies found no evidence of stable secondary structural elements, and all major residue types seem to participate in transient intra- and intermolecular FUS-FUS contacts [63]. Averaged over time, individual residues appear to contact many residues across the protein to produce a conformationally heterogeneous, dynamically rearranging ensemble of molecules in the condensed phase. Distributed interactions are consistent with observations that all groups of five sequential tyrosine residues tiled across the sequence contribute similarly to phase separation of a FUS LC fusion with a tandem array of SH3 domains [64]. The different constructs, buffer conditions and temperatures used by various labs likely explain the differences in observed FUS LC behavior [57,60–63]. Further research is necessary to determine which of these behaviors are relevant to full-length FUS, where both LC and RGG contribute to self-association.

FUS RGG2 and RGG3 can also phase separate weakly on their own, but greatly promote phase separation of the LC region [42–44,65]. These effects do not require the PY-NLS [44]. Cation-π arginine-tyrosine interactions and the distributed charge in the arginine guanidinium group are important to LLPS [44]. Thus, in full length FUS, LC-RGG, LC-LC

and RGG-RGG interactions contribute to self-association and phase separation. Lessons learned from FUS likely apply to the similarly organized EWS and TAF15.

Kapβ2 inhibits self-association of the FET proteins—Stoichiometric Kapβ2 inhibits LLPS, hydrogel formation or fibrilization of purified FUS [32,42–44]. Kapβ2 also solubilizes phase-separated FUS and preformed hydrogels or solid aggregates that contain fibrils. Kapβ2 has similar effects on EWS and TAF15, all dependent on tight binding to the PY-NLSs [32]. Interference with Kapβ2-PY-NLS interactions by a peptide inhibitor, RanGTP or ALS mutations in the FUS PY-NLS, all diminish the chaperone activity of Kapβ2. FUS without its PY-NLS is solubilized only with >50-fold higher concentrations of Kapβ2 [32,42,44].

Kapβ2 also inhibits FUS condensates in cells [32,42,43]. Co-expression in yeast of human Kapβ2 with FUS eliminated FUS cytoplasmic aggregates (which form since yeast Kapβ2 does not bind FUS) [32]. Kapβ2 overexpression decreased cytoplasmic FUS-positive SGs in HeLa, neural progenitor, HEK293T cells and ALS-patient derived fibroblasts [32]. High affinity binding of Kapβ2 to FUS, rather than nuclear import, is key; a mutant Kap^{TL}, which binds FUS but cannot import it, effectively suppressed FUS-positive SGs in HeLa cells [32], and Kapβ2 can suppress FUS-positive SGs even when nuclear import is blocked with wheat germ agglutinin [42]. Thus, the chaperone function of Kapβ2, rather than the ability to decrease cytoplasmic FUS, is essential to SG disruption.

In patient derived fibroblasts, SG depletion by Kapβ2 coincides with restored expression of FUS mRNA targets that had been depleted by a FUS R521H mutant [32]. In an ALS fly model, decreasing Kapβ2 expression enhanced neurodegeneration caused by FUS, FUS R521H and FUS R518K (bind Kapβ2 slightly weaker) but not FUS P525L (binds Kapβ2 10-fold weaker). Oppositely, increasing Kapβ2 expression in FUS R521H flies increased lifespan [32]. These results suggest therapeutic potential for modulating aggregation of FUS.

How Kapβ2 prevents FUS-FUS interactions—How does Kapβ2 binding to the PY-NLS inhibit FUS-FUS interactions mediated by the LC and RGG regions? It does not act simply as a large object anchored to the FUS C-terminus that blocks interactions through non-specific steric and/or electrostatic effects. Other proteins that bind FUS, such as an antibody raised against the FUS PY-NLS, HDAC1 and PRMT1, do not inhibit FUS self-association [32,42]. When the FUS PY-NLS is replaced with a cNLS or an NES, neither of the respective cognate Kaps, Impα or the 123 kDa CRM1, inhibited LLPS [44]. Dynamic light scattering analysis also showed that Kapβ2 has significant self-adhesion and therefore cannot confer repulsive tendencies to the Kapβ2-FUS complex [44]. Therefore, simply binding of Kapβ2 to FUS is insufficient to inhibit FUS-FUS self-association.

A variety of data rather suggest that Kapβ2, when anchored to the PY-NLS, acts by weakly binding a number of regions distributed across FUS that are involved in self-association. Small angle X-ray scattering (SAXS) analyses showed that FUS compacts upon binding Kapβ2, indicating global changes that extend well beyond the PY-NLS [44]. Consistent with this idea, when Kapβ2 is added to FUS lacking the PY-NLS, NMR analyses showed weak dynamic interactions with the LC, RGG, RRM, and ZnF [44]. Anchoring of FUS PY-NLS to

Kapβ2 should enable these interactions to effectively occur in cis, strengthening them entropically. Together, the FUS-Kapβ2 interactions constrain the FUS chain and interrupt the myriad FUS-FUS interactions (LC-LC, LC-RGG, RGG-RGG) to inhibit and reverse FUS LLPS and fibrillization. Kapβ2 may engage all FUS sites simultaneously, or more likely dynamically samples different collections of contacts that rapidly interconvert. Further physical analyses are necessary to identify the regions of Kapb2 that bind FUS, and understand disruption of FUS self-association in atomic detail.

Kapβ2 chaperone function, methylation state and RNA-binding of FUS—FUS is normally heavily methylated on arginines in healthy cells [66,67], but FUS in neurons of FUS-FTD patients is hypomethylated and accumulates with EWS, TAF15 and Kapβ2 in the cytoplasm [36,67–70]. Arginine methylation weakens LC-RGG and RGG-RGG interactions [42]. Hypomethylated FUS forms solid-like condensates while hypermethylated FUS forms liquid-like condensates; both can be dissolved by Kapβ2 [43].

The FUS RRM and ZnF bind RNAs weakly [45,71] while the RGGs bind with nanomolar affinity [71,72]. Low concentrations of RNA can promote FUS self-association through multivalent interactions that enhance intermolecular networks [47,62]. In contrast, high concentrations of RNA disrupt FET proteins LLPS as they saturate binding sites on individual FUS molecules and prevent intermolecular networks [73]. High RNA concentrations seem important to keep the proteins soluble in the nucleus, while low RNA concentrations in the cytoplasm may cause aberrant assembly in disease. Kapβ2- and RNA-binding to FUS seem mutually exclusive, suggesting that Kapβ2 may inhibit FET self-association in the cytoplasm through blocking interactions with RNA [44]. In the nucleus, RanGTP prevents Kapβ2 from competing with FET-RNA interactions. The regulatory logic of Ran-Kapβ2 interactions and differential concentrations of RNA may act synergistically to maintain FET solubility in the cell.

Kapβ2 and the hnRNP A/B proteins—The Kapβ2 cargos, hnRNP A/B, are splicing factors that bind RNA. They localize mostly to the nucleus in healthy cells, but translocate to SGs upon stress [49,74–76]. Family members hnRNP A1 and hnRNP A2 are mutated in patients with MSP and ALS, where they aggregate into cytoplasmic inclusions, often partially co-localizing with TDP-43 [77]. Both proteins have a similar domain organization: two RRM domains followed by a LC region, which also houses the PY-NLS (Figure 2A). The hnRNP A1/A2 PY-NLSs (residues 273–289 and 301–319, respectively) bind the same site in Kapβ2 that contacts the FUS PY-NLS (Figure 2B, C) [9]. Residues immediately N-terminal to the PY-NLSs (D262V in hnRNP A1, D290V and P298L in hnRNP A2) are mutated in families with MSP, ALS and Paget's disease [77–79]. PY-NLSs of hnRNP A1 and A2 enable both nuclear import and also chaperoning by Kapβ2.

Self-association of the hnRNP A/B proteins—hnRNP A/B proteins can phase separate and form amyloid fibers *in vitro* both alone and in the presence of RNA [41,49,77]. Self-association is driven largely by the LC region, but the RRM domains also likely contribute in the presence of RNA [49,78,80]. Crystal structures and fibril-diffraction guided structural models of hnRNP A1 peptides (^{209}GFGGNDNFG^{217}, ^{234}GGGYGGS^{240} , ^{243}GYNGFG^{248}, ^{246}GFGNDGSNF^{254}, ^{260}YNDFGNY^{266}) showed bent coils/β-strands that

stack in-register into cross-β structures [60,81]. The aspartate residues in some of these peptides stack in-register down the β-sheet, generating a column of negative charges and electrostatic repulsion that could attenuate cross-β fibril stability and afford greater dynamics. One of these aspartates, Asp 262, when mutated to valine or asparagine in disease, could decrease electrostatic repulsion and explain increased fiber formation by the mutant [81].

Solid-state NMR analyses of fibrils of the entire hnRNP A2 LC suggested a 44-residue core within residues 280–341 that forms cross-β structures [82]. The D290V disease variant produces the same cross-β structure but with more stable fibrils, likely through decreased electrostatic repulsion, as in the hnRNP A1 peptide with D262V mutation. Solution NMR analyses of a similar fragment at low concentrations unfavorable to fiber formation showed disordered random coils that sample dynamic and transient intra- and intermolecular interactions [78]. As in FUS, individual residues contact many others, with little apparent specificity or structural preference. Analogous analyses of phase-separated hnRNP A2 LC also showed structurally disordered and dynamic protein [78]. Finally, a very recent solution NMR study analyzed an hnRNP A1 LC fragment (residues 186–320) prevented from aggregation through deletion of a six-residue element (residues 259–264) containing a predicted steric zipper. This fragment does not have persistent secondary structure, but compacts and undergoes LLPS through contacts between aromatic residues distributed across the sequence [83]. The relatively uniform spacing of the aromatics appears to disfavor aggregation to produce a dynamic liquid phase.

Kapβ2 inhibits self-association of hnRNP A/B proteins—Kapβ2 inhibits hnRNPs A1 and A2 from forming hydrogels/fibrils by binding to their PY-NLSs [32]. In cells, Kapβ2 overexpression decreases the number of sodium arsenite-induced hnRNP A1-and hnRNP A2-positive SGs [32]. Expressing Kapβ2 reduced cytoplasmic aggregation of hnRNP A2(D290V), restored it to the nucleus and rescued the fly flight muscles from degeneration caused by the mutation [32]. The mechanism by which Kapβ2 disrupts hnRNP A1/A2 self-association has not been examined. The location of the PY-NLS adjacent to the fiber-forming LC sequences suggests that Kapβ2 may simply sterically block proximal LC-LC interactions. Alternatively, or in addition, Kapβ2 may interact more extensively through weak and distributed contacts, as it does with FUS.

Importinα/β and TDP-43—TDP-43 is mostly localized to the nucleus and functions in mRNA processing, stability, transport and translation [22,84,85]. TDP-43 in neuronal cells under stress also accumulates in SGs [26,86]. Wildtype TDP-43 accumulates into aberrant cytoplasmic aggregates in 90% of ALS patients, ~50% of FTD patients and in Alzheimer's disease patients, regardless of the genetic lesion causing disease [87]. TDP-43 is also mutated in ALS and FTD [87].

TDP-43 contains a globular Dix-like N-terminal domain (NTD) followed by two tandem RRM domains and an LC region (Figure 3A). A bipartite cNLS that binds Impα/β is located in the NTD-RRM1 linker (Figure 3B, C) [22,85]. A putative NES (residues 239–250) was previously reported, but the motif is buried in the RRM2 fold and does not bind CRM1 [85,88,89]. Instead, TDP-43 exits the nucleus via passive diffusion. The 150-residue

disordered LC region has only one tyrosine residue but contains eight FG, FS or FN dipeptide motifs.

Self-association of TDP-43—Several elements of TDP-43 contribute to self-association [49,90]. Its LC region readily phase separates and many fragments within it can form fibrils [90–92]. Like other DIX domains, the NTD oligomerizes in a phosphorylation-sensitive manner, enhancing phase separation of the full-length protein probably by increasing the effective valency of the LC region [93]. RRM1-RRM2 do not self-assemble but may facilitate LLPS in the presence of RNA [90,94,95].

X-ray structures of short LC peptides show either steric zipper or LARKS fibrils, depending on the fragment examined [92]. Cryo-electron microscopy structures of fibrils generated by larger (40–50 residue) LC segments show cores that stack into cross-β arrays (Figure 3D) [96]. Several groups reported solution NMR analyses of the LC region [90,91,97]. Studies of LC segments under conditions favoring either monomer or phase separation both found helical elements flanked by random coils (Figure 3D) [90,91]. Another study of a monomeric 26-residue LC segment found a flexible ensemble with few hydrophobic contacts or β-structure [97]. CD analyses of some LC segments showed initial partial α-helical or coil structure that transformed over time into β-sheet rich aggregates [91,97].

Impα/β inhibits self-association of TDP-43—Impα/β inhibits TDP-43 fibrilization and can disassemble pre-formed fibrils [32]. Tight binding to the cNLS is essential to these effects. The consequences of these activities have not been studied in cells, and no structural or biochemical analyses of the Impα/β-TDP-43 interaction have been reported. Future mechanistic work should examine whether Impα alone can chaperone TDP-43, whether importin binding to the cNLS affects NTD oligomerization and/or RNA-binding to the RRMs, and whether importin makes contacts with other TDP-43 regions outside of the cNLS.

Karyopherins as chaperones—Impα/β and Kap121 can inhibit phase separation of FUS when its PY-NLS is replaced with the cognate ligands of the two importins, the cNLS and IK-NLS, respectively [44]. As described above, Impα/β also blocks TDP-43 fibrillization [32]. It had long been suggested that negatively charged importins could act as chaperones for abundant, positively charged cargos like histones and ribosomal proteins [98]. Structural evidence supporting this suggestion was published recently - Importin-9 wraps around the H2A-H2B histone dimer core to shield it from aggregation [15]. Collectively, these findings suggest that β-Importins may act generally to modulate assembly of aggregation-prone transport cargos. Why are Importins effective chaperones?

β-Importins are large proteins (~100–150 kDa) composed of 20–21 tandem HEAT repeats that form superhelical shapes (Figure 4) [6,99]. Their non-globular architectures produce large surface areas for diverse interactions with many binding partners or cargos. Importins are highly acidic, with long acidic loops and large negatively-charged surfaces on their concave sides, suitable to contact basic elements of RNA-binding proteins such as RGG regions and RRM domains, which often contribute to higher order assembly (Figure 4A). All Kaps also have many hydrophobic patches on their convex spines that bind FG repeats of

nucleoporins. These same patches may be involved in analogous interactions with tyrosine or phenylalanine residues in the LC regions of RNA-binding proteins that mediate self-association. Finally, importins typically bind the NLS sequences of their cargos with nanomolar affinity, enhancing other interactions with the HEAT repeat surfaces that would be weak if they occurred in trans. Together, these properties may enable Importins in general to prevent self-assembly of their RNA-binding cargo proteins.

Many RNA-binding proteins, including FUS, hnRNP A1 nor TDP-43, shuttle between the nucleus and the cytoplasm. All three proteins exit the nucleus via passive diffusion or with bound mRNAs; none are transported by exportins [44,88,89]. When the PY-NLS of FUS was replaced with an NES that binds CRM1, the exportin failed to inhibit LLPS of FUS [44,89]. Thus, it appears that importins but not exportins can chaperone FUS. Differences in physical characteristics between importins and exportins may explain this specificity [44]. Exportins are less negatively charged (isoelectric points 5.0–5.5) and have rather different charge distributions (Figure 4B). While the concave sides of importins have large contiguous negatively charged surfaces, the analogous surfaces of exportins have basic patches. The spatial relationships between NES- and FG-binding sites in exportins are also quite different from those between NLS- and FG-binding sites in importins [100–102]. NLSs bind acidic patches on concave surfaces of importins while NESs bind to a hydrophobic groove on the convex surface of CRM1. These differences may account for the different chaperone activities of importins and exportins.

Future perspectives—Many questions remain about the mechanism of the importins' chaperone function. How general is the mechanism of Kapβ2 chaperoning of Fus, which occurs through distributed, dynamic contacts with many regions of the cargo protein? Is this mode of action used by other importin-RNA-binding protein pairs? How else could importins prevent and reverse protein aggregation? Are importins other than Kapβ2, Impα/β and Importin-9 capable of chaperoning and what proteins do they act on? Do exportins also have chaperone functions, perhaps in the nucleus where they bind protein cargos in the presence of RanGTP? Finally, given the ability of Kapβ2 to abolish cytoplasmic aggregates of FUS, hnRNP A1 and hnRNP A2 in cells and rescue degeneration in fly disease models, could importins be therapeutic targets for neurodegenerative diseases?

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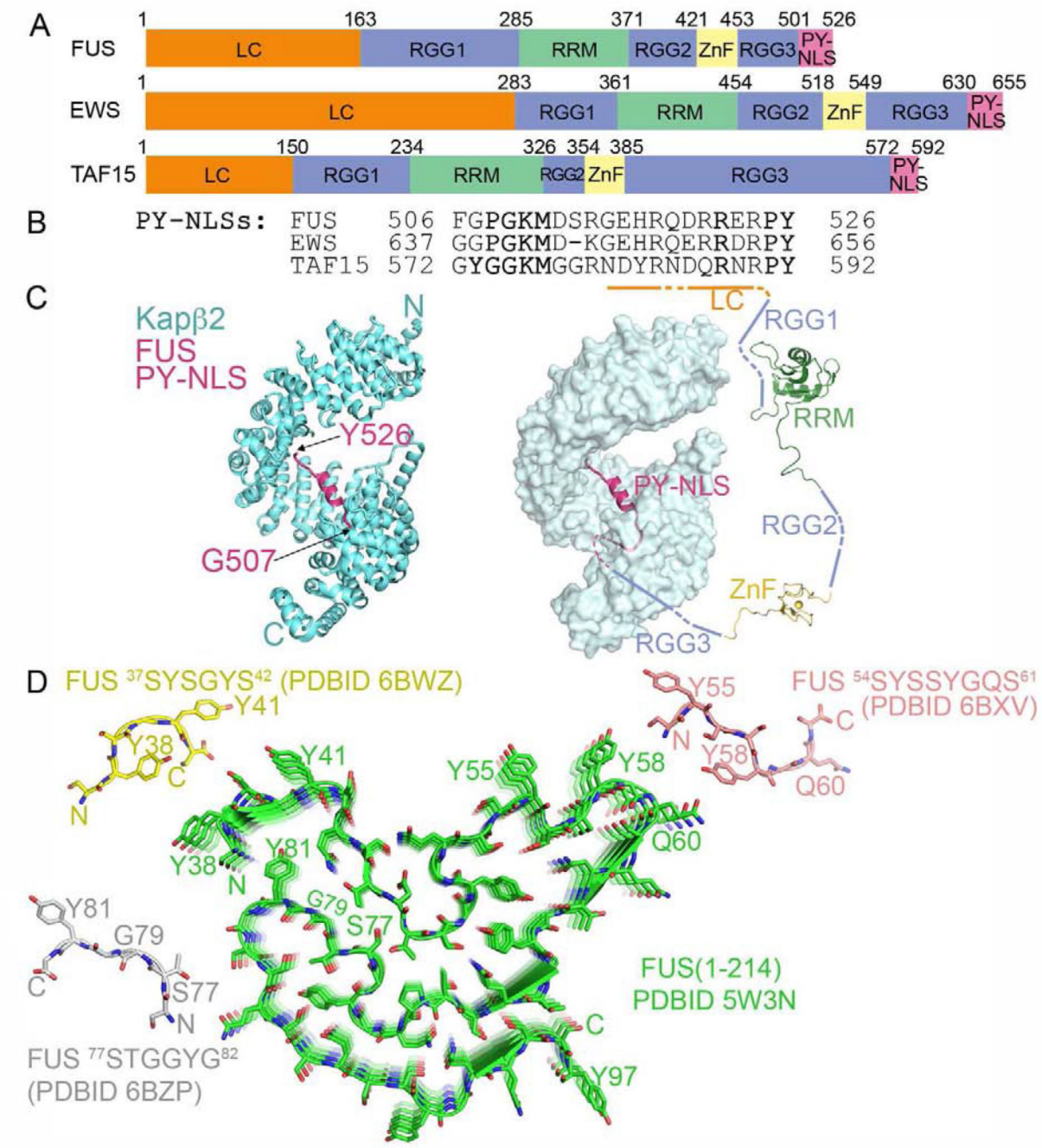

Figure 1. The FET proteins are nuclear import cargos of Kapβ2.

A. Domain organization. **B.** PY-NLS sequences with Kapβ2-binding epitopes underlined. **C.** Structure of Kapβ2 (cyan) bound to FUS PY-NLS (magenta; **4FDD**). Left, cartoon representation of Kapβ2. Right, surface representation with a schematic of FUS domains. RRM (**2LCW**) and ZnF (**6G99**) domains. **D.** Protomers of short FUS LC segments (crystal structures of ^{37}SYSGYS^{42} (yellow), ^{54}SYSSYGQS^{61} (pink) and ^{77}STGGYG^{81} (grey)) and solid-state NMR structure of FUS LC (residues 1–214; green). All four segments form cross-β structures.

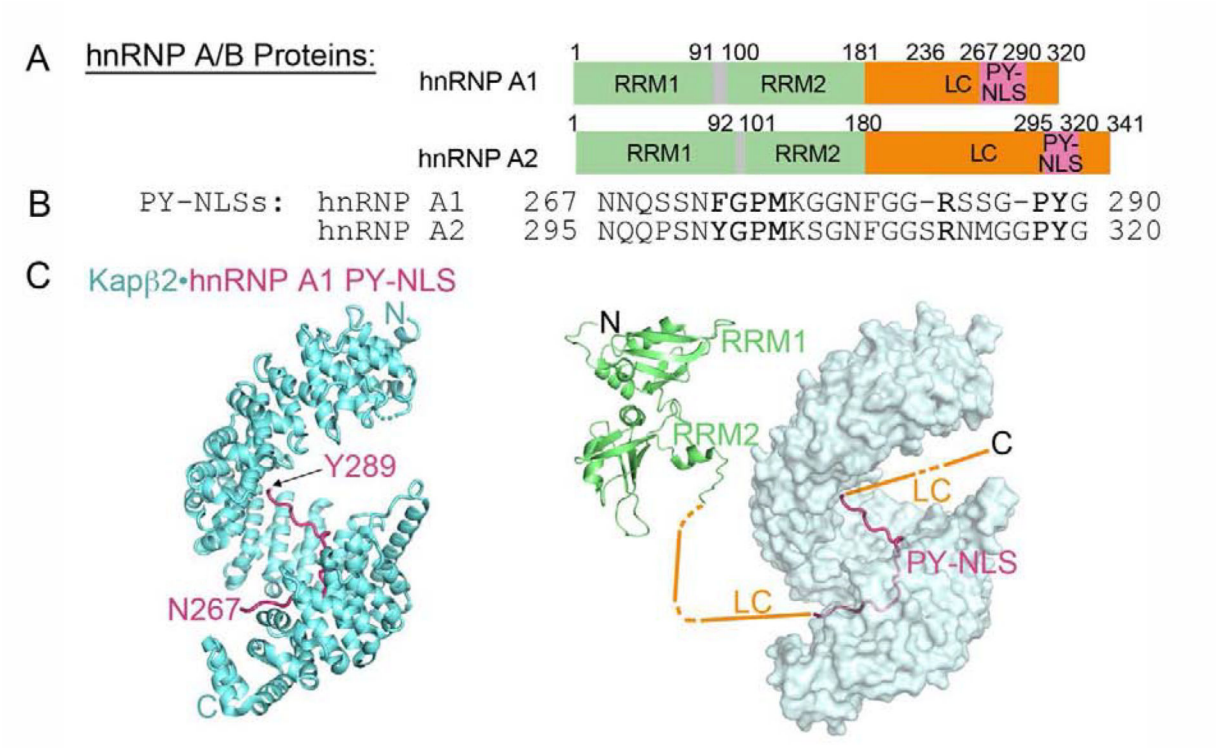

Figure 2. The hnRNP A/B family of proteins are nuclear import cargos of Kapβ2. A, B. Domain organization (**A**) and PY-NLS sequences (**B**) of hnRNP A1 and hnRNP A2. The Kapβ2 binding epitopes of the PY-NLSs are underlined. **C.** Structure of Kapβ2 (cyan) bound to the PY-NLS of hnRNP A1 (magenta); **2H4M**. Kapβ2 is shown with a cartoon representation on the left, and with a surface representation on the right. A schematic of other hnRNP A1 domains are shown in the right panel. Structures of the folded tandem RRM1-RRM2 (**2LYV**) domains are shown.

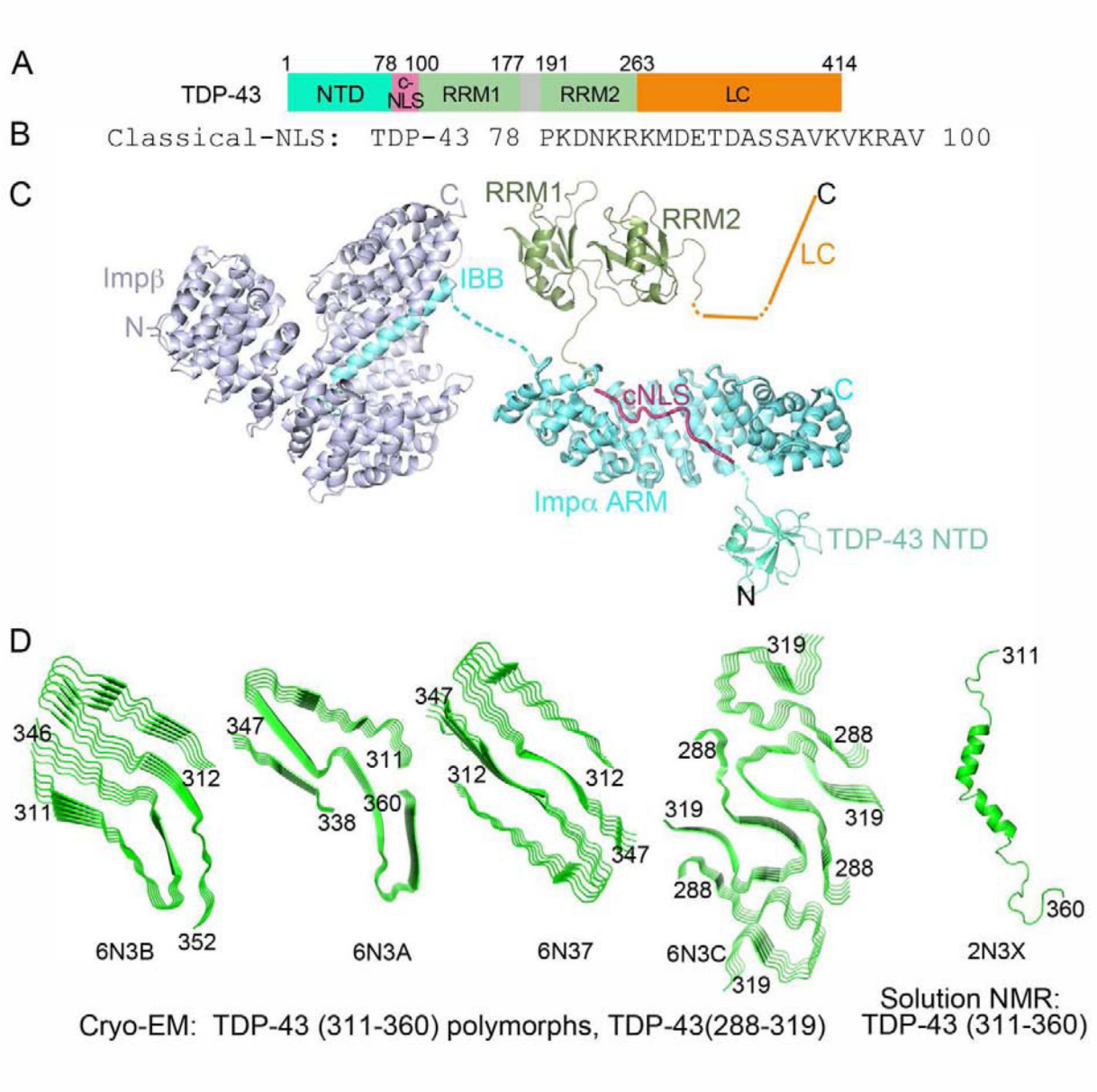

Figure 3. TDP-43 is a nuclear import cargo of the Impα/β heterodimer. A, B.
Domain organization (**A**) and the bipartite cNLS sequence (**B**) of TDP-43 (Impα-binding epitopes underlined). **C.** Structures of Impα (cyan) bound to the bipartite cNLS of Nucleoplasmin (magenta) (**1EJY**) and of Impβ (blue) bound to the IBB of Impα (cyan) (**1QGK)**, along with the schematic of TDP-43 domains: 1) NTD (**5MDI**) connected to the the Nucleoplasmin cNLS (a model for the TDP-43 cNLS), followed by RRM1-RRM2 (**4BS2**) and a schematic of the LC domain. D. Left to right: Cryo-EM structures of TDP-43 segment spanning residues 311–360 (three polymorphic cross-β structures), residues 286–331 which self-associates into one cross-β structure, and the solution NMR structure of monomeric TDP-43 residues 311–360.

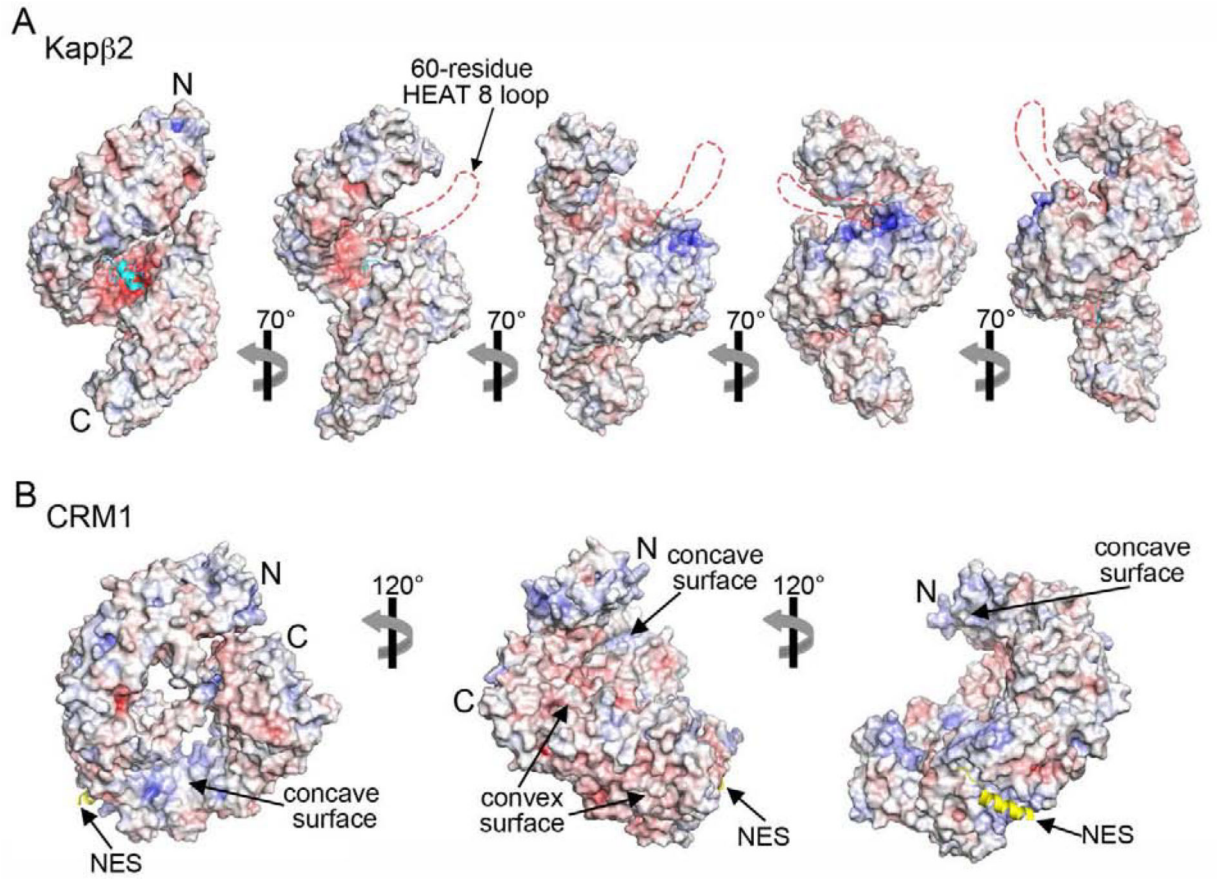

Figure 4. Comparison of charge distributions for an importin versus an exportin.

A. Electrostatic surface potential of Kapβ2 (**4FDD**) in a series of orientations. The bound FUS PY-NLS (cyan) is drawn as cartoon, and the negatively charged disordered 60-residue loop at HEAT repeat 8 is represented with dashed red lines. **B.** Electrostatic surface potential of the exportin CRM1 (3**GB8**) in three orientations. The bound NES of cargo Snurportin-1 is shown in yellow.