**Coil-to- α -Helix Transition at the Nup358-BicD2 Interface
Activates BicD2 for Dynein Recruitment**

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Abstract

Nup358, a protein of the nuclear pore complex, facilitates a nuclear positioning pathway that is essential for many biological processes, including neuromuscular and brain development. Nup358 interacts with the dynein adaptor Bicaudal D2 (BicD2), which in turn recruits the dynein machinery to position the nucleus. However, the molecular mechanisms of the Nup358/BicD2 interaction and the activation of transport remain poorly understood. Here for the first time, we show that a minimal Nup358 domain activates dynein/dynactin/BicD2 for processive motility on microtubules. Using nuclear magnetic resonance (NMR) titration and chemical exchange saturation transfer (CEST), mutagenesis and circular dichroism spectroscopy (CD), a Nup358 α -helix encompassing residues 2162-2184 was identified, which transitioned from a random coil to an α -helical conformation upon BicD2-binding and formed the core of the Nup358-BicD2 interface. Mutations in this region of Nup358 decreased the Nup358/BicD2 interaction, resulting in decreased dynein recruitment and impaired motility. BicD2 thus recognizes Nup358 through a “cargo recognition α -helix”, a structural feature that may stabilize BicD2 in its activated state and promote processive dynein motility.

Impact statement

A cargo-recognition α -helix was identified in Nup358, which is required for activation of a dynein-dependent transport pathway that is essential for brain development.

Introduction

Cytoplasmic dynein is the predominant motor responsible for minus-end-directed traffic on microtubules¹, which facilitates a vast number of transport events that are critical for chromosome segregation, signal transmission at synapses, and brain and muscle development^{2–16}. Integral to the transport machinery are *dynein adaptors*, such as Bicaudal D2 (BicD2), whose N-terminal region (BicD2^{CC1}) recruits and activates dynein-dynactin for processive motility^{17–27}. Also integral to the dynein transport machinery are *cargoes*, which bind to the C-terminal domain (CTD) of BicD2²⁸. Cargoes are required to activate BicD2 for dynein binding, which is a key regulatory step for dynein-dependent transport^{17–27}. In the absence of cargo adaptor/dynein adaptor complexes such as Nup358/BicD2, dynein and dynactin are auto-inhibited and only show diffusive motion on microtubules. Furthermore, in the absence of cargoes, BicD2 assumes a looped, auto-inhibited conformation, in which its N-terminal dynein/dynactin binding site binds to the CTD and remains inaccessible. The CTD is required for auto-inhibition, as a truncated BicD2 without the CTD activates dynein/dynactin for processive motility. Binding of cargo to the CTD releases auto-inhibition, likely resulting in an extended conformation that recruits dynein and dynactin^{17–27}. The crystal structures of the C-terminal cargo recognition domains of three BicD2 homologs have been determined^{27,29,30}; However, to date, there are no detailed

95 structural studies of BicD2/cargo complexes and the structural mechanisms of
96 BicD2-mediated cargo recognition and dynein activation remain poorly
97 understood.

98 The binding partners for human BicD2 include Nup358⁶, Rab6^{GTP 28}, and
99 nesprin 2-G¹⁴. Nup358, also known as RanBP2, is a 358 kDa nuclear pore
100 complex protein with multiple functions³¹. During G2 phase, Nup358 engages in
101 a pathway for positioning of the nucleus relative to the centrosome along
102 microtubules by binding to BicD2, which in turn recruits dynein and dynactin
103 (Figure 1)⁶. This pathway is essential for apical nuclear migration during
104 differentiation of radial glial progenitor cells, which give rise to the majority of
105 neurons and glia cells of the neocortex^{12,13}. A second nuclear positioning
106 pathway is facilitated by BicD2/dynein and nesprin 2-G¹⁴, a component of LINC
107 (Linker of Nucleoskeleton and Cytoskeleton) complexes (LINC)³², which is
108 important for migration of post-mitotic neurons during brain development¹⁴. Apart
109 from its roles in nuclear positioning, BicD2 is also involved in the transport of
110 Golgi-derived and secretory vesicles. In this process, BicD2 and dynein are
111 recruited by the vesicle-associated small GTPase Rab6^{28,33}. Thus, BicD2 plays
112 important roles in faithful chromosome segregation, neurotransmission at
113 synapses as well as brain and muscle development^{6,12–14,16,28}. Mutations in BicD2
114 cause neuromuscular diseases including a subset of spinal muscular atrophy
115 cases^{34–38}, the most common genetic cause of infant death³⁹.

117 Within the Nup358 sequence, there are many intrinsically disordered
118 regions (IDR), which are also commonly found in proteins involved in dynein-
119 dependent transport^{5,40–43}. Although IDRs and IDPs (intrinsically disordered
120 proteins) make up ~30% of eukaryotic proteins and have important physiological
121 functions⁴⁴, they remain the most poorly characterized class of proteins in
122 regards to their structure, dynamics and interactions. IDRs play important roles in
123 dynein biology. For example, an intrinsically disordered region in the dynein light
124 intermediate chain 1 (LIC1) undergoes a coil-to- α -helix transition when it
125 interacts with the N-terminal domain of BicD2, which is an important step in the
126 activation of dynein for processive motility^{40,41,43}. A second, larger interface is
127 formed between the N-terminal coiled coil of BicD2, the dynein tail and dynactin,
128 which promotes activation of dynein for processive motility^{17,18,22,24}. Currently,
129 the mechanism of dynein activation by full length BicD2/cargo complexes is not
130 well understood, since much of the available information pertaining to dynein
131 activation was derived from studies with isolated BicD2 fragments.

132 In addition to BicD2, Nup358 also recruits the opposite polarity motor
133 kinesin-1 via the subunit kinesin-1 light chain 2 (KLC2) which binds to a W-acidic
134 motif with the sequence LEWD in Nup358^{45–47}. While dynein is the predominant
135 motor in G2 phase, kinesin-1 is also actively involved in nuclear positioning in G2
136 phase, modulating overall motility⁶. Such bidirectional transport is also displayed
137 by mitochondria, endosomes, viruses, phagosomes, secretory vesicles, and
138 many vesicles in neuronal axons and growth cones^{2–11,14}. Opposite polarity
139 motors such as BicD2/dynein and kinesin-1 often bind in close spatial proximity

140 to adapter binding proteins such as Nup358, but it is unknown how their overall
141 motility is regulated, likely because their interactions remain poorly characterized
142 by structural and biophysical methods.

143 Here we have determined the structural properties of the interface of a
144 minimal Nup358/BicD2 complex by a combination of NMR spectroscopy,
145 mutagenesis, circular dichroism spectroscopy and small-angle X-ray scattering.
146 These results establish a structural basis for cargo recognition by BicD2 and
147 suggest that Nup358 interacts with BicD2 through a “cargo recognition” α -helix.
148 Direct activation of BicD2 by Nup358 has not been shown before. Here, we use
149 single-molecule binding and processivity assays to show that a minimal
150 dimerized Nup358 construct is sufficient to activate full-length
151 dynein/dynactin/BicD2 complexes for processive motility. Mutations of the cargo-
152 recognition α -helix of Nup358 decreased the Nup358/BicD2 interaction and
153 resulted in decreased dynein recruitment and impaired motility, shedding light on
154 the important role of the cargo-recognition α -helix for the activation of BicD2 and
155 dynein motility. Intriguingly, our results also show that the binding site of BicD2 in
156 Nup358 is spatially close to but does not overlap with the LEWD motif that acts
157 as a kinesin-1 binding site, suggesting that the kinesin and dynein machineries
158 may interact simultaneously via Nup358. Our results thus provide mechanistic
159 insights into the regulation of bi-directional transport by adapter binding proteins.

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Figure 1. A minimal Nup358 domain interacts with BicD2 with micromolar affinity. (a) Nup358 interacts with both BicD2/dynein/dynactin and kinesin-1 (via kinesin-1 light chain 2, KLC2) to mediate bi-directional nuclear positioning in G2 phase of the cell cycle^{6,45,46}. This pathway is essential for a fundamental process in brain development that is required for radial glial progenitor cells to differentiate to the majority of neurons and glia cells of the neocortex¹². (b, c) Schematic representation of the expression constructs BicD2-CTD (b, red) and Nup358-min (c, blue), in the context of the full-length proteins (grey). (c) KLC2 is recruited to Nup358-min via a W-acidic motif with the sequence LEWD⁴⁶. The X-ray structures of the TPR domain of the KLC2 (green, referred to as KLC2 hereafter), fused to a LEWD binding motif (purple)⁴⁸ and the X-ray structure of the BicD2-CTD (red)³⁰ are shown in cartoon representation in c. The α -helical N-terminal domain of Nup358 (dark grey) promotes anchorage to the NPC (d) A representative ITC thermogram of Nup358-min and BicD2-CTD is shown, from which the affinity (dissociation constant K_D) was determined to be $1.7 \pm 0.9 \mu\text{M}$. N: number of sites; ΔS : the change in entropy; ΔH : the change in enthalpy. The experiment was repeated three times. The error of K_D was calculated as the standard deviation from three experiments. In Figure 1 – figure supplement 1, the affinity of Nup358-min-GST (i.e., with the GST-tag intact) and BicD2-CTD was determined by ITC to be $1.6 \pm 1.0 \mu\text{M}$. See also Figure 1 - source data 1 - 4.

Results:

ITC establishes a minimal complex for Nup358/BicD2 interaction

Previously, we have determined an X-ray structure of the C-terminal domain of human BicD2 (BicD2-CTD, residues 715-804), which contains the

185 binding sites for cargoes, including human Nup358 (Figure 1b)³⁰. A complex was
186 reconstituted with BicD2-CTD and a minimal fragment of human Nup358
187 containing residues 2148-2240, which is called Nup358-min^{30,46} (Figure 1c). Here,
188 the affinity of the BicD2-CTD towards Nup358-min was determined by isothermal
189 titration calorimetry (ITC) (Figure 1d, Figure 1 – figure supplement 1). The ITC
190 thermogram fits well to a one-site binding model (with a single equilibrium
191 dissociation constant K_D). The number of sites was determined to be $N = 1.0$,
192 which is consistent with a molar ratio of [Nup358]/[BicD2] of 1. This molar ratio is
193 in agreement with our previously published molar masses obtained from size
194 exclusion chromatography coupled to multi-angle light scattering (SEC-MALS),
195 which showed that Nup358 and BicD2 form a 2:2 complex⁴⁹. The one-site
196 binding model and the 2:2 stoichiometry are in line with Nup358-min binding as a
197 dimer to a single binding site on a BicD2 coiled-coil dimer, although we cannot
198 exclude the possibility that two Nup358 monomers bind to two binding sites on
199 BicD2, where both sites have the same dissociation constant K_D . We also
200 attempted to fit models assuming multiple binding sites and K_{Ds} but those are not
201 supported by the ITC thermogram. The equilibrium dissociation constant K_D was
202 determined to be $1.7 \pm 0.9 \mu\text{M}$, in a similar range as observed for other
203 BicD2/cargo complexes as well as to the previously published affinity of $0.4 \mu\text{M}$,
204 obtained for BicD2-CTD towards a larger fragment of Nup358 (residues 2006-
205 2443, with the GST-tag intact)⁴⁹. An ITC-titration of Nup358-min-GST (i.e., with
206 the GST-tag intact, whereas the GST was cleaved off in the first experiment) with
207 BicD2-CTD yielded a very similar affinity of $1.6 \pm 1.0 \mu\text{M}$ (Figure 1 – figure

supplement 1), demonstrating that the GST-tag does not affect the binding affinity. These ITC results confirm the mapped boundaries of the minimal binding site. The ITC analysis finally revealed that the Nup358-BicD2 interaction is driven by a favorable enthalpy change ($\Delta H = -19.2 \pm 0.7$ kcal/mol), which overcomes the unfavorable entropy change ($\Delta S = -38.2 \pm 5.8$ cal mol⁻¹ K⁻¹).

The dynein-dynactin-BicD2-Nup358^{min} complex moves processively on microtubules

Single-molecule reconstitutions were used to determine if the adaptor-binding protein Nup358^{min} can bind and relieve BicD2 auto-inhibition, which in turn allows dynein-dynactin to be recruited and activated for processive motion. Nup358^{min} (N) and full-length BicD2 (B) were labeled with two different color Quantum dots (Qdots), and tissue-purified dynein-dynactin (DD) was unlabeled (Figure 2a). When dynein, dynactin, BicD2 and Nup358^{min} were all present (DDBN^{min}), 23% dual-color complexes were observed (Figure 2 – figure supplement 1a,c). To increase complex formation, Nup358^{min} was artificially dimerized with a leucine zipper (Nup358^{min-zip}), which increased the number of dual-colored complexes to 35% (Figure 2 – figure supplement 1a,b). The rationale for this strategy was based on our previous observation that dimerization of the *Drosophila* adaptor-binding protein Egalitarian enhanced its affinity for BicD and bypassed the requirement for mRNA cargo for BicD

activation²⁵. All further single molecule reconstitutions thus used the dimerized version of Nup358-min (Nup358^{min-zip}) because of its enhanced affinity for BicD2.

The dynein-dynactin-BicD2-Nup358^{min-zip} (DDBN^{min-zip}) complex exhibited robust processive motion on surface-immobilized MTs, implying that Nup358^{min-zip} relieves BicD2 auto-inhibition to allow dynein activation (Figure 2b, Video 1). As controls, DDB was shown to support few processive events (Figure 2c, Video 2), and DDN^{min-zip} exhibited only diffusive motion (Figure 2d). Nup358^{min-zip} alone does not bind to microtubules (Figure 2 – figure supplement 1d), and thus Nup358^{min-zip} shows diffusive MT binding by virtue of an interaction with dynein-dynactin. The number of processively moving DDBN complexes on MTs is ~5.3 times higher than that of DDB complexes (Figure 2f). The speed and run length of all dual-color DDBN^{min-zip} complexes were analyzed, with the run length obtained from cumulative distribution analysis with a one-phase exponential decay fit, and speed determined from the mean $\pm$ standard deviation (SD). The speed and run-length of DDBN^{min-zip} were quantified as 0.42 ± 0.26 $\mu\text{m/s}$ ($n=123$) and 3.6 ± 0.07 μm ($n=123$), respectively (Figure 2g, h). These motile properties of DDBN^{min-zip} were compared with that of the DDB^{CC1} complex (B^{CC1}, the N-terminal coiled-coil 1 domain of BicD2), a well-established fully active complex^{22,25}. (Figure 2e,f, Video 3). The speed and run length of the DDB^{CC1} complex was 0.53 ± 0.28 $\mu\text{m/s}$ ($n=137$) and 4.8 ± 0.14 μm ($n=137$), respectively, which are significantly faster ($p=0.0022$) and longer ($p<0.0001$) than that of DDBN^{min-zip} (Figure 2g,h). The number of processive events of DDBN^{min-zip} on MTs is approximately half that of the active DDB^{CC1} complex (Figure 2f).

Importantly, the directed motion of $\text{DDBN}^{\text{min-zip}}$ is very different from the auto-inhibited dynein-dynactin complex, which shows only diffusive movement on MTs²⁵. The enhanced number of processive events only in the presence of both BicD2 and Nup358^{min-zip} suggests that Nup358^{min-zip} functionally relieves the auto-inhibition of BicD2 so that dynein-dynactin can bind and move processively on MTs.

Figure 2. Nup358^{min-zip} is capable of forming a dynein-dynactin-BicD2-Nup358^{min-zip} complex ($\text{DDBN}^{\text{min-zip}}$) that is activated for processive motility. (a) Schematic of a $\text{DDBN}^{\text{min-zip}}$ complex bound to a microtubule. BicD2 and Nup358 are shown labeled with two different color Quantum dots (stars). (b) Representative kymograph of the $\text{DDBN}^{\text{min-zip}}$ complex moving processively on microtubules. Nup358^{min-zip} was labeled with a 655 nm Qdot and BicD2 with a 525 nm Qdot. (c) Dynein-dynactin-BicD2 (DDB) complexes bind to microtubules but show very little processive motion. (d) Dynein-dynactin-Nup358^{min-zip} complexes ($\text{DDN}^{\text{min-zip}}$) showed diffusive movement on microtubules. Nup358^{min-zip} was labeled with a 655 nm Qdot. (e) As a control, processive motion of dynein-dynactin in the presence of the N-terminal domain of BicD2 (DDB^{CC1}), with BicD2^{CC1} labeled with a 525 nm Qdot. (f) Bar graph showing the number of processive events of $\text{DDBN}^{\text{min-zip}}$ per min per micrometer MT length. As controls number of processive events are shown for active DDB^{CC1} , DDB and $\text{DDN}^{\text{min-zip}}$ (g, h) Speed and

run length of DDBN^{min-zip} (yellow) were compared with the constitutively active complex DDB^{CC1} (green). Speeds for the two complexes are significantly different ($p < 0.0022$, unpaired t-test with Kolmogorov-Smirnov test), as are the run lengths ($p < 0.0001$, Kolmogorov-Smirnov test). See also Figure 2 – figure supplement 1. For each panel, data were obtained from 3 independent experiments and two protein preparations.

NMR titration mapped the BicD2-binding site to the N-terminal half of Nup358-min

Because our single-molecule processivity assays confirmed that the Nup358-min domain forms a DDBN complex that is activated for processive motility, we characterized the BicD2-binding site on Nup358-min, employing solution NMR, which can provide atomic-resolution information for protein interactions in the native solution state. First, backbone assignment of Nup358-min was carried out using standard triple resonance experiments, 3D HNCO⁵⁰, HNCACO⁵¹, HNCA⁵⁰, HNCACB⁵², and CBCACONH⁵³. 82 of the 89 total backbone amides were assigned in the ¹H-¹⁵N HSQC (Figure 3 – figure supplement 1). Then ¹⁵N-labeled Nup358-min was titrated with unlabeled BicD2-CTD. Figure 3a shows the overlay of the HSQC spectrum of apo Nup358-min with that of the complex, for which ¹⁵N labeled Nup358-min and unlabeled BicD2-CTD were mixed at a 1:1 molar ratio. The HSQC spectrum of the apo Nup358-min is characterized by a lack of dispersion, consistent with an intrinsically disordered protein (IDP) (Figure 1 – figure supplement 1, Figure 3 – figure supplement 1). Analysis of backbone chemical shifts (CA, CB, CO, N and HN) of Nup358-min by TALOS-N⁵⁴ conclusively demonstrates that Nup358-min is a random coil in the apo state (Figure 3 – figure supplement 3). Although the addition of BicD2-CTD resulted in little peak movement in the HSQC, significant peak intensity changes were observed for the N-terminal half of Nup358-min (Figure 3a and 3b). This points to a relatively wide binding region undergoing intermediate to slow exchange on the NMR time scale, due to ligand binding.

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309 **Figure 3. NMR titration mapped the BicD2-binding site to the N-terminal half of**
310 **Nup358-min.** NMR mapping of Nup358 regions involved in BicD2-CTD binding was
311 performed by titration of ^{15}N labeled Nup358-min with BicD2-CTD. (a) The HSQC
312 spectrum of a 1:1 Nup358-min/BicD2-CTD complex (blue) is overlaid on that of apo-
313 Nup358-min (red). The full HSQC assignment is shown in figure 3 – figure supplement 1.
314 Many peaks disappeared in the complex spectrum (selected peaks labeled by residue
315 name and number), indicating that BicD2 binding causes slow to intermediate chemical
316 exchange on the NMR time scale. (b) Plot of the peak intensity change vs the residue
317 number of Nup358. The peak intensities I , as determined by peak heights, of the 1:1
318 BicD2-CTD/Nup358-min complex spectrum were divided by the peak intensities of the
319 apo-Nup358 spectrum (I_0) to obtain I/I_0 . Data points with an I/I_0 of 0.65 or lower are
320 colored red. This plot shows that the N-terminal half of Nup358-min is largely
321 responsible for BicD2 binding. The peak intensities corresponding to the LEWD
322 sequence motif (colored blue) in Nup358, which mediates binding of KLC2, are not
323 affected by BicD2 binding. For the full titration results, please see Figure 3 – figure
324 supplement 2.

325

326 The Nup358-min domain also contains the previously published KLC2
327 binding LEWD motif⁴⁶, but the BicD2 binding site determined here is separated
328 from the LEWD motif by over 30 residues (Figure 3b). Residues 2192 to 2240 of
329 Nup358 show little change in NMR signal between the apo sample and the
330 complex (Figure 3b), indicating that the LEWD motif is not involved in BicD2

binding. Therefore, kinesin-1 and the dynein adaptor BicD2 may bind to separate but spatially close binding sites on the cargo Nup358. The proximity of these motor recognition sites may serve to enable the interaction between dynein and kinesin machineries, for the regulation of bi-directional motility and precise positioning of the nucleus.

CEST suggests a coil-to- α -helix transition at the BicD2-Nup358 interface

Although our results from the HSQC titrations establish the general region of the BicD2 interface in Nup358, the chemical shifts of the bound state were not obtained, due to peak disappearance in the HSQC upon BicD2 addition. To further characterize the BicD2/Nup358 interface with NMR, chemical exchange saturation transfer (CEST) experiments were performed. CEST has recently emerged as a powerful technique in solution NMR for measuring the chemical shifts of NMR invisible states^{55,56}, such as the BicD2-CTD bound state of Nup358-min, whose resonances are absent from the ¹⁵N-HSQC (Figure 3a, b). In CEST, when the invisible state is saturated by a weak and long radiofrequency (RF) pulse (B_1), the saturation of the bound state will be transferred to the free state due to Nup358-min dissociating from the Nup358-min-BicD2 complex, causing a dip in peak intensity when B_1 is on resonance with the bound state chemical shift, generating the minor dip in the CEST curve. If the chemical shifts of bound and unbound state are significantly different, which is for example the

case for interface residues or residues that undergo structural changes upon complex formation, this difference gives rise to a double-dip appearance in the CEST profile (Figure 4c and 4d). The major dip in the profile will be observed at the chemical shift of the free or unbound state (when the B_1 matches the chemical shift of unbound resonance, the “visible state”). The second, smaller dip in the profile peak will be observed at the chemical shift of the bound state (due to saturation transfer from the “invisible state”). In contrast, residues that are not located at the complex interface or do not undergo structural changes upon complex formation will result in resonances without chemical exchange and the CEST profile will only show a single dip (Figure 4a and 4b), corresponding to the chemical shift of the unbound state.

Figure 4. CEST maps chemical shifts of NMR-invisible, BicD2-bound state of Nup358. In the CEST profile curve, E2194 and I2211 have only a single dip in ^{15}N -CEST (a) and $^{13}\text{C}'$ -CEST (b), respectively, due to little chemical shift perturbation upon BicD2-CTD binding. This suggests E2194 is not at the binding interface and does not

undergo conformational transition upon BicD2-CTD binding. In contrast, A2164 shows not only a dip at the chemical shift of the apo HSQC assignment, but also a smaller dip at about 3 ppm to the left in ^{15}N -CEST (c), indicating that A2164 is in the binding region and/or experiences a conformational transition upon complex formation. (d) L2166 has a major peak and then a minor peak about 2.5 ppm to the right. The magnitude and direction of the chemical shift difference suggests a transition from a random coil to an α -helix (see Table 1). (e) Summary of the CEST data obtained across the entire Nup358-min region. Red squares show residues with double dip in CEST profile. Their chemical shift changes from the apo to the bound state indicate that these red residues are part of the α -helical region at the Nup358/BicD2 interface. Blue colored residues show a single dip in CEST profile, suggesting these remain in random coil conformation. White represents residues where data were not obtained, due to low signal to noise ratio or resonance overlap. All the curves with double dips and a sampling of curves with only single dip fits are shown in Figure 4 – figure supplements 1 - 4.

We performed CEST experiments on both the ^{15}N amide and the ^{13}C carbonyl (C') resonances. In ^{15}N CEST NMR experiments, the following residues were identified as interface residues or residues that undergo structural transition because of the double dip appearance in the CEST profile: A2163, A2164, K2165, L2166, I2167, K2178, L2184 (Figure 4 – figure supplement 1). For these residues, CEST indicates a significant chemical shift difference between their bound and unbound states. Using the program RING NMR Dynamics⁵⁷, the CEST data were fit to obtain chemical shift difference between free and bound state ($\Delta\delta$), the exchange rate (k_{ex}) and the fractional minor population (P_b)(See Table 1 – Appendix 1-table 1a, b). The weighted average of the ^{15}N CEST fit parameters resulted in $k_{\text{ex}} = 200 \pm 70 \text{ s}^{-1}$, consistent with slow to intermediate exchange ($k_{\text{ex}} < \Delta\omega$) on an NMR time scale. The weighted average of the minor population (P_b) is 0.04 ± 0.01 , which is consistent with our sample preparation at molar ratio of 1:20 for BicD2-CTD: ^{15}N -Nup358-min. Many peaks in the ^{15}N -CEST suffered from low-signal-to-noise ratio and resonance overlap common in IDPs, preventing the determination of additional chemical shifts of invisible states.

400 To overcome this problem, we further carried out $^{13}\text{C}'$ -CEST experiments based
401 on HNCO, which correlates amides (HN) with the carbonyl (CO) of the
402 **preceding** residue. Here the saturation pulse B_1 is on the carbonyl, and intensity
403 change due to saturation is still reported in an ^{15}N -HSQC-like 2D spectrum. This
404 method provided additional data for residues missed with the ^{15}N CEST due to
405 resonance overlap or low signal-to-noise ratio. For example, the F2183 peak in
406 ^{15}N - ^1H HSQC was overlapped. However, the L2184 peak, a well-resolved signal
407 with high S/N, showed a CEST effect from the saturation of the F2183 carbonyl
408 in HNCO-based $^{13}\text{C}'$ -CEST. With the ^{13}C HNCO-CEST experiment, we were
409 able to observe minor states in the following residues – R2162, A2164, K2165,
410 L2166, R2169, E2171, E2172, L2177, K2181, F2183, and L2184 (Figure 4 –
411 figure supplement 2 and Appendix 1-table 1c). Thus, with CEST, we were able
412 to further map the binding region to residues 2162 through 2184 (Figure 4e).
413 Fitting of HNCO-CEST profiles with RING Dynamics resulted in a global
414 exchange rate of $270 \pm 50 \text{ s}^{-1}$, in reasonable agreement with the value of k_{ex} from
415 ^{15}N CEST. k_{ex} obtained here is much slower in time scale than the rate of coil-to-
416 α -helix transition in model peptides⁵⁸. This suggests that the k_{ex} obtained by
417 CEST include contributions from other events, such as binding and
418 oligomerization, in addition to coil-to- α -helix transition, consistent with similar
419 observations for other binding studies⁵⁶. The weighted average of the minor
420 population (P_b) is 0.06 ± 0.01 , which is again consistent with the ratio in which we
421 mixed the two proteins for the NMR sample. The 18 CEST profiles with double
422 dip appearance all resulted in chemical shift changes in the direction and

magnitude as expected for a coil-to- α -helix transition⁵⁹ (typically the chemical shift for ^{15}N becomes lower in the helical conformation, and higher for ^{13}C , as shown in Table 1). The CEST results suggest that residues 2162 – 2184 of Nup358 undergo coil-to- α -helix transition upon binding to BicD2, revealing that BicD2 recognizes Nup358 through a short “cargo recognition α -helix” which is embedded in an IDP domain.

Table 1: Chemical shift differences from CEST of Nup358/BicD2 and apo-Nup358 ($\Delta\delta_{\text{bound-apo}}$) match closely to $\Delta\delta$ for coil-to- α -helix transition ($\Delta\delta_{\text{helix-coil}}$)*.**

Residue	$\Delta\delta_{\text{bound-apo}}$	$\Delta\delta_{\text{helix-coil}}$
A2163*	-3.6	-2.2
A2164*	-3.6	-2.2
K2165*	-0.8	-1.3
L2166*	-3.8	-1.9
I2167*	-5.7	-1.2
K2178*	-3.6	-1.3
L2184*	-5.5	-1.9
R2162**	1.7	2.3
A2164**	2.8	1.7
K2165**	2.7	2.1
L2166**	2.6	1.6
R2169**	2.9	2.3
E2171**	2.2	2.2
E2172**	3.0	2.2
L2177**	0.9	1.6
K2181**	3.0	2.1
F2183**	3.3	1.5
L2184**	1.0	1.6

*Change in Chemical shifts of amide ^{15}N .

**Change in Chemical shifts of carbonyl ^{13}C .

***Values taken from Wishart, 2011⁵⁹

See also Appendix 1 –table 1

CD spectroscopy confirms formation of an α -helix in Nup358 upon binding to BicD2

To provide further evidence for the coil-to- α -helix transition, circular dichroism (CD) spectroscopy was carried out. The CD wavelength scans of the Nup358-min/BicD2-CTD complex had minima at 208 nm and 222 nm, characteristic for α -helical proteins³⁰ (Figure 5). Such minima were absent in the CD spectra of Nup358-min, as expected for an intrinsically disordered protein. Notably, the minima at 208 and 222 nm were shifted towards more negative values in the complex compared to the sum of the individual spectra of Nup358-min and BicD2-CTD, suggesting that the α -helical content increases upon complex formation.

Figure 5. CD spectroscopy confirms formation of an α -helix in the Nup358/BicD2 complex. CD wavelength scans of BicD2-CTD (red), Nup358-min (blue) and the Nup358-min/BicD2 complex (green) at 4°C are shown. The sum of the individual wavelength scans of Nup358-min and BicD2-CTD is shown in black. The mean residue molar ellipticity $[\Theta]$ versus the wavelength is shown. Experiments were repeated two or three times, representative scans are shown. A characteristic feature of α -helical

structures is a local minimum at 222 nm (dashed line). Based on the values for $[\Theta]$ at 222 nm (shown on the right) and based on our calibration curve (Figure 5 – figure supplement 1), the Nup358/BicD2 complex has a 14 ± 5 % increase in α -helical content compared to the sum of the spectra of BicD2 and Nup358. See Figure 5 – figure supplement 1 and Figure 5 – source data 1.

To quantify the increase of the α -helical content, we determined the difference between the molar ellipticity at 222 nm of the complex and of the sum of the individual proteins, $\Delta[\Theta]$, to be $-4874 \text{ deg cm}^2 \text{ dmol}^{-1}$ (Figure 5), which corresponds to a 14 ± 5 % increase of the α -helical content based on a published thermal unfolding curve of the BicD2-CTD, which was used for calibration³⁰ (Figure. 5, Figure 5 – figure supplement 1 and ref.⁶⁰. We recently determined the experimental error to be $\sim 5\%$ (corresponding to $\Delta[\Theta]$ of $2930 \text{ deg cm}^2 \text{ dmol}^{-1}$)⁶⁰; therefore, we estimate that the α -helical content increases by 14 ± 5 % upon Nup358/BicD2 complex formation, confirming a structural transition from a coil to an α -helix.

The minimal Nup358/BicD2 complex has a rod-like shape that is more compact than the individual proteins

Small-Angle X-ray Scattering (SAXS) experiments were carried out to obtain a low-resolution structure of the Nup358-min/BicD2-CTD complex. The quality of our SAXS data was confirmed by molar mass calculations: for Nup358-min, we determined a molar mass of 12.3 kDa (Appendix 2 – table 1), which matches

487 closely to the calculated mass of a monomer (10.6 kDa). For the Nup358-
 488 min/BicD2-CTD complex, we determined a molar mass of 47.6 kDa (Appendix 2-
 489 table 1), which matches closely to the expected mass of a Nup358-min/BicD2-
 490 CTD complex with a 2:2 stoichiometry (calculated molar mass of 43.0 kDa).
 491 These findings are in line with our previously published SEC-MALS data which
 492 suggest that apo Nup358-min forms monomers, while the Nup358/BicD2
 493 complex has a 2:2 stoichiometry^{46,49}.

496 **Figure. 6. Low-resolution structures determined by SAXS confirm that the**
 497 **complex has a rod-like shape that is more compact than the individual proteins.**
 498 (a) Dimensionless Kratky plots of the SAXS data collected from the minimal
 499 Nup358/BicD2 complex, from Nup358-min and BicD2-CTD (q : scattering vector, R_G :
 500 radius of gyration, $I(q)$: scattering intensity). (b) The pair distance distribution function PI ,
 501 of the Nup358-min/BicD2-CTD complex was derived from the scattering intensity profile
 502 (Figure 6 – figure supplement 1). The molecular weight MW^{61} , the radius of gyration R_G
 503 from the Guinier plot and the largest dimension of the particle D_{max} are shown. (c)
 504 Refined bead model 3D reconstruction of the Nup358-min/BicD2-CTD complex (cyan
 505 mesh). The statistical analysis and supporting SAXS data are summarized in Figure 6,
 506 figure supplements 1-4 and appendix 2. See also Figure 6 – source data 1.

508 A Kratky plot of the SAXS profiles further confirmed that Nup358-min is
509 intrinsically disordered, as the signal increases at high q values instead of
510 approaching zero (Figure 6a). In contrast, the Kratky plots of the BicD2-CTD and
511 the Nup358-min/BicD2-CTD are bell-shaped, suggesting they are folded (Figure
512 6a). Thus, Nup358-min becomes more compact upon complex formation with
513 BicD2-CTD, consistent with the coil-to- α -helix transition observed from NMR and
514 CD spectroscopy.

515
516 The pair distance distribution function $P(r)$ derived from the SAXS profile
517 of the Nup358-min/BicD2-CTD complex has a peak which decays with a linear
518 slope, which is characteristic of elongated, rod-like structures (Figure 6b). Since
519 the width of a rod remains similar throughout its length, the sum of all pair
520 distances will have a linear slope. The maximum particle diameter D_{Max} of the
521 complex is 190 Å, which is identical to the D_{Max} that was determined for the
522 BicD2-CTD sample (Appendix 2 – table 1), suggesting that the overall length of
523 the rod-structure does not change. Bead models of the Nup358-min/BicD2
524 complex were reconstructed from the PI functions and confirm that the complex
525 has a flexible, rod-like structure (Figure 6c). The normalized spatial discrepancy
526 (NSD) is 0.7, suggesting the structural convergence and homogeneity of bead
527 models reconstructed from SAXS profiles (Appendix 2 – table 2).

528

Taken together, these SAXS data suggest that the complex has a 2:2 stoichiometry and is more compact than the apo state, further validating the coil-to- α -helix transition in the Nup358/BicD2 complex.

Nup358 mutagenesis validates α -helix formation at the Nup358/BicD2 interface

Our CEST, CD and SAXS data provided strong evidence that residues 2162-2184 of Nup358 undergo a structural transition from random coil-to- α -helix upon complex formation with BicD2.

To confirm our results, we also carried out alanine mutagenesis for all residues of the Nup358 α -helix and assessed binding of the mutated GST-tagged Nup358-min to BicD2-CTD by pulldown assays (Figure 7 and Figure 7 – figure supplement 1). Eleven mutants displayed diminished binding, which are interspaced throughout the α -helix. An α -helical wheel representation was created, which revealed that these interface residues are clustered on one side of the α -helical wheel (red in Figure 7c). The mutagenesis experiments are consistent with our results from NMR spectroscopy. While interface residues and residues that undergo the transition from coil-to- α -helix will show a chemical shift change, not all residues in the newly formed α -helix in Nup358-min will be at the binding interface, therefore the mutagenesis data provides additional information. Notably, all interface residues that were identified from mutagenesis (red in Figure 7d) also had a double dip in the CEST profile (red in Figure 7d) or were

not assessed (white in Figure 7d). Furthermore, removal of residues 2163-2166 (AAKL) of Nup358-min (Δ AAKL), which had double dips in CEST curves, virtually abolishes the interaction with the BicD2-CTD (Figure 7 – figure supplement 1b). We also removed the α -helix from Nup358-min, and as expected, the resulting Nup358-fragment (residues 2185-2240) shows virtually no interaction with the BicD2-CTD (Figure 7 – figure supplement 1a, last lane). Finally, we successfully assembled a minimal complex of the BicD2-CTD with a Nup358 fragment (residues 2157-2199) that only contained the BicD2 binding site deduced from NMR, which confirmed our mapped binding site (Figure 7 – figure supplement 1c). Furthermore, known BicD2 residues that are important for Nup358 interaction include L746, R747, M749 and R753²⁹, suggesting a potential binding site for the Nup358 α -helix in the center of the BicD2-CTD coiled coil (Figure 7e).

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Figure 7. Mutagenesis of the Nup358 cargo recognition helix. All residues of the Nup358 α-helix were mutated to alanine and binding was assessed by GST-pull-down assays with Purified Nup358-min-GST 27 followed by 27 and BicD2-CTD. The elution fractions were analyzed by SDS-PAGE and the intensities of the gel bands were quantified to obtain the ratio of bound BicD2-CTD/Nup358-min, normalized relative to the wild-type (Figure 7 – figure supplement 1). The binding ratio was averaged from three independent experiments and the error was calculated as the standard deviation. Eleven interface residues were identified, which are colored red and show a reduction of the binding ratio upon mutation by at least three times the standard deviation. In addition, interface residues were required to have a binding ratio that was reduced by 13% or more compared to the wild-type. Alanine and glycine residues were not assessed and are colored white. Residues for which mutations do not affect binding are colored light blue. (a) The sequence of the predicted Nup358 α-helix is shown above a bar graph of the ratios of bound BicD2 to Nup358 from the alanine mutant pull-down assays (WT=1, indicated by the horizontal black line). (b) Representative SDS-PAGEs of elution fractions of the pull-down assays. A representative full dataset is shown in Figure 7 – figure supplement 1. Note that a small gel band at 25 kDa represents GST. (c) Helical wheel representation for (a). (d) Comparison of helical residues in Nup358-BicD2 complex identified by CEST and results from pulldown assay of mutants. Red: CEST positive (double dip in CEST profile) or strong reduction in binding from the pulldown assay with alanine mutation at this residue; blue: CEST negative (single dip in CEST profile) or no effect in mutagenesis; white: data not available. (e) The structure of the Hs

BicD2-CTD³⁰ is shown in red cartoon representation. Four Nup358/BicD2 interface residues that were previously identified by mutagenesis are shown in cyan spheres representation²⁹. See also Figure 7 – figure supplement 1 and Figure 7 – source data 1-3.

Nup358-BicD2 interface is essential for dynein activation

Five mutants that showed a reduced Nup358-min/BicD2-CTD interaction in the pulldown assays were selected for further characterization. Single-molecule binding and processivity assays were used to further explore the impact of Nup358 mutants on dynein recruitment and activation of dynein motility. We assessed the interaction between BicD2 and the following Nup358^{min-zip} mutants: I2167A, M2173A, L2177A, F2180A, and L2184A, in the context of the BN^{min-zip} and the DDBN^{min-zip} complex. The following parameters were quantified for WT and mutant Nup358^{min-zip} constructs: (a) the number of single molecule processive events of DDBN^{min-zip} on MTs, (b) percent complex formation with BicD2 alone or with the DDB complex, and (c) speed and run length of DDBN^{min-zip} on MTs.

The number of processively moving DDBN^{min-zip} complexes on MTs was quantified as the number of dual-color Qdots moving per min per micrometer MT length (Figure 8a). The processive events of DDBN^{min-zip} formed with the Nup358^{min-zip} mutants I2167A, M2173A, F2180A, and L2184A Nup358^{min-zip} were significantly lower than that formed with WT-Nup358^{min-zip} (Figure 8a). DDBN^{min-zip} formed with the Nup358^{min-zip}-L2177A mutant did not result in a lower number

of processively moving complexes, suggesting that there is some plasticity at the Nup358/BicD2 interface.

We next assessed how these Nup358^{min-zip} mutants affected the BicD2-Nup358^{min-zip} (BN) interaction in the absence or presence of dynein-dynactin. BicD2 and WT or mutant Nup358^{min-zip} were labeled with different color Qdots and the number of dual-color BN^{min-zip} and DDBN^{min-zip} complexes non-specifically bound to a coverslip were quantified using TIRF microscopy (Figure 8 – figure supplement 1). Nup358^{min-zip} binds BicD2 alone moderately well, and the percentage of BN was reduced further for the Nup358^{min-zip} mutants (Figure 8b, red). We also quantified how the presence of dynein-dynactin influences the BicD2-Nup358^{min-zip} interaction. Strikingly, in the presence of dynein-dynactin, the formation of DDBN^{min-zip} complexes was about 2-times higher than the BN complexes (Figure 8b, gray). The mutant Nup358^{min-zip} again showed reduced complex formation relative to WT (Figure 8b, gray). The difference between DDBN^{min-zip} and BN^{min-zip} indicates that dynein-dynactin enhances the BicD2-Nup358 interaction.

The motion of DDBN^{min-zip} complexes formed with the five Nup358^{min-zip} mutants was quantified and compared with DDBN^{min-zip} containing WT-Nup358^{min-zip}. DDBN^{min-zip} complexes with Nup358^{min-zip} mutants showed slower speed and shorter run-length compared with WT-DDBN^{min-zip} (Figure 8c, d). There were too few dual-colored complexes formed with the I2167A mutant to

allow speed and run length quantification. Compromised motility in the presence of the five characterized Nup358^{min-zip} mutants suggests formation of a less stable DDBN complex, Even for the L2177A mutant that showed a similar number of processive runs as WT Nup358^{min-zip}. The functional importance of the Nup358 cargo-recognition- α -helix is validated by the impaired motility observed with point mutants of this α -helix.

Figure 8. Nup358 point mutations that diminish the interaction with BicD2-CTD also diminish formation of the DDBN complex. (a) Bar graph of the processive events of DDBN^{min-zip} complexes per min per micrometer MT length. The number of processive events of DDBN^{min-zip} complexes formed with WT-Nup358^{min-zip} is significantly higher ($p < 0.0001$) than those formed with the Nup358^{min-zip} mutants I2167A, M2173A, F2180A, and L2184A but the same as with mutant L2177A (orange, $p = 0.09$). The number of processive events of DDBN^{min-zip} formed with WT-Nup358^{min-zip} was $0.080 \pm 0.0017/\text{min}/\mu\text{m MT}$ ($n = 3$, with n being the number of experiments). In contrast, these values for DDBN^{min-zip} formed with the Nup358^{min-zip} mutants I2167A, M2173A, L2177A, F2180A, and L2184A were $0.020 \pm 0.007/\text{min}/\mu\text{m}$ ($n = 2$), $0.052 \pm 0.006/\text{min}/\mu\text{m}$ ($n = 2$), $0.072 \pm 0.013/\text{min}/\mu\text{m}$ ($n = 3$), $0.044 \pm 0.005/\text{min}/\mu\text{m}$ ($n = 2$), $0.048 \pm 0.0023/\text{min}/\mu\text{m}$ ($n = 2$) respectively. (b) The presence of dynein-dynactin (gray) increases the formation

of BicD2-Nup358^{min-zip} complexes (red). The percent formation of BN^{min-zip} was: 14.41% (n=580) for WT, 3.96% (n=1142) for I2167A, 5.54% (n=1877) for M2173A, 10.49% (n=948) for L2177A, 10.47%(n=412) for F2180A and 6.31% (n=1032) for L2184A Nup358^{min-zip}. N is the total number of Qdots counted. In contrast, these values for DDBN^{min-zip} complexes were 34.92% (n=945), 5.62% (n=986), 19.61% (n=347), 21.87% (n=778), 17.52%(n=890) and 17.13% (n=372), respectively. (c) Comparison of the speeds of DDBN complexes formed with WT-Nup358^{min-zip} and mutant Nup358^{min-zip} constructs. The speed of DDBN formed with Nup358^{min-zip} mutants M2173A (0.23 ±0.13 μm/s, n=58; blue), L2177A (0.32±0.22 μm/s; n=86; orange), F2180A (0.25±0.16 μm/s, n=54; green) and L2184A (0.20±0.13 μm/s, n=41; purple) are significantly slower than that of DDBN-WT (p<0.0001 for mutants M2173A, F2180A, L2184A and p=0.0058 for L2177A), one-way ANOVA followed by Tukey's test). (d). The run length of DDBN^{min-zip} formed with Nup358^{min-zip}-M2173A (1.3±0.07μm, n=58), L2177A (2.3±0.08μm, n=86), F2180A (1.3±0.07μm, n=54) and L2184A (1.5±0.10 μm, n=41) are significantly shorter than that formed with WT-Nup358^{min-zip} (3.6±0.07 μm; n=123 ; data taken from 2h) (with *p-values* p<0.0001 for mutants M2173A, F2180A, L2184A and p=0.0036 for L2177A; one-way ANOVA followed by Tukey's test). See also Figure 8 – figure supplement 1.

Discussion:

Here we elucidate the molecular details of the interface between the dynein activating adaptor BicD2 and the nuclear pore protein Nup358, which links BicD2 to the cell nucleus. First, we reconstituted a minimal Nup358-min/BicD2-CTD complex with μM affinity. Single-molecule processivity assays revealed that a dimerized Nup358-min domain forms a complex with dynein/dynactin/BicD2 (DDBN) that shows robust processive motility on microtubules. Similarly, the small GTPase Rab6a^{GTP} that links BicD2 to membranous cargo promotes processive motility⁶² suggesting that both adaptor-binding proteins act to release the auto-inhibition of BicD2. Dimerization of Nup358-min enhances interaction with BicD2 and with the DDB complex, suggesting that activation is linked to oligomerization, which for Rab6^{GTP} could occur by clustering on the vesicular membrane.

692 NMR titration of BicD2-CTD into ^{15}N labeled Nup358-min mapped the
693 binding region to the N-terminal half of Nup358-min. Furthermore, we obtained
694 chemical shifts for the CA and CB atoms for the apo state of Nup358-min, which
695 confirm that the apo-state is intrinsically disordered. Due to slow chemical
696 exchange on the NMR time scale and fast relaxation in the BicD2-bound state of
697 Nup358-min, this state cannot be directly observed in standard NMR
698 experiments, and thus a powerful solution NMR technique termed CEST, was
699 applied to map the chemical shifts of the “invisible state”. This approach not only
700 identified key residues at the Nup358/BicD2 interface, but also showed that
701 chemical shift changes upon binding are consistent with a coil-to- α -helix
702 transition in Nup358-min. Notably, the coil-to- α -helix conformational change is
703 supported by our results from CD spectroscopy and SAXS, and the BicD2/Nup358
704 interface was validated by mutagenesis. The observed time scale is also in line
705 with other proteins undergoing coil-to- α -helix transitions⁵⁶. However, it should be
706 noted, that in order to obtain higher-resolution structural information, the
707 chemical shifts for the CA and CB atoms of Nup358-min in the bound state
708 remain to be determined in the future. Notably, our functional assays highlight the
709 important role of the interaction between Nup358 and BicD2 in dynein activation.
710 Single-molecule binding assays showed that mutagenesis of the Nup358 cargo-
711 recognition α -helix diminished the interaction of Nup358-min with full-length
712 BicD2 and decreased the formation of dynein/dynactin/BicD2/Nup358 complexes
713 (DDBN). Furthermore, the mutant DDBN complexes that were formed exhibited
714 reduced run length and speed reflecting formation of a less stable complex. Our

data highlight the important role of dynein activating-adaptor/adaptor-binding protein interactions in activating and fine-tuning dynein motility.

Our data establish that BicD2 recognizes its cargo-binding protein Nup358 through an α -helix of ~28 residues. Coil-to- α -helix transitions and folding upon binding, as shown here, have been observed in many studies of IDP/IDR interactions and recognition^{40,56}. We propose that *the dynein activating-adaptor BicD2 recognizes its cargos through a “cargo recognition α -helix”* (Figure 9). It is conceivable that the cargo adaptors Rab6^{GTP} and nesprin-2G are also recognized by similar short α -helices, although this needs to be confirmed by future experiments. Rab6^{GTP} binds to BicD2 with 10-fold higher affinity compared to Rab6^{GDP}^{28,63,64} and associated structural changes are found in the Switch I and Switch II regions of Rab6⁶⁴, which include short α -helices and intrinsically disordered domains and are possibly candidates for the BicD2-binding site. The mapped nesprin-2G binding domain for BicD2 is also predicted to be largely intrinsically disordered⁵. Our data thus suggest a structural basis for cargo recognition by BicD2 and will enable further studies aimed at identifying cell cycle-specific regulatory mechanisms for distinct transport pathways that are facilitated by BicD2^{6,14,28}. For example, our Nup358 mutants that selectively target the interaction with BicD2 can be used to dissect biological roles of the Nup358/BicD2, Rab6/BicD2 and nesprin 2-G/BicD2 pathways in distinct stages of brain development.

A key step in activating dynein motility is overriding the auto-inhibited conformation of BicD2 to allow dynein recruitment. We and others have recently proposed that cargo-binding activates BicD2 for dynein recruitment by inducing a coiled-coil registry shift in BicD2 (i.e. a vertical displacement of the two α -helices against each other by one helical turn)^{27,29,30,60}. In the absence of cargo, BicD2 forms an auto-inhibited state that cannot recruit dynein, with the CTD masking the N-terminal dynein/dynactin-binding site (NTD). When cargo is loaded, BicD2 can directly link dynein with its activator dynactin, which is required to activate dynein for processive motility^{17–27,60}. We recently showed that an F684I mutant of the *Drosophila* (*Dm*) homolog BicD can activate dynein/dynactin for processive motility in the absence of cargo. X-ray structures of the WT and F684I mutant as well as molecular dynamics simulations suggest that this activating mutation causes a coiled-coil registry shift in the *Dm* BicD-CTD^{30,60}. It is tempting to hypothesize that the cargo recognition helix of Nup358 intercalates into the coiled-coil of the BicD2-CTD to stabilize a coiled-coil registry shift in the Nup358/BicD2 complex, resulting in activation of BicD2 for dynein recruitment. This hypothesis remains to be experimentally confirmed.

Nup358-min binds more strongly to the dynein/dynactin/BicD2 complex compared to full-length BicD2 alone (Figure 8b). This observation supports the proposed loop-opening activation mechanism of BicD2^{21,25–27,60} (Figure 9b, c). In the full-length dynein/dynactin/BicD2/Nup358 (DDBN) complex, once Nup358 binding displaces the auto-inhibited loop, dynein and dynactin bind and prevent its reformation. This is not true in the full-length BicD2/Nup358 complex, resulting

in a lower apparent affinity than in the full complex. It is also possible that additional weak interfaces further stabilize the DDBN complex compared to Nup358/BicD2 or the DDB^{CC1} complex. Here we observed a direct interaction of Nup358 with dynein-dynactin that could contribute to enhanced affinity. It has been shown for several cargo adaptors that they interact weakly with the dynein light chains⁶⁵ and the dynein light intermediate chain 1 (LIC1) forms a small interface with the so-called CC1 box of BicD2, which is required for activation of processive motility^{40,41,66}. Cargoes and adaptors often interact through multiple interfaces with motors, and these additional interfaces in many cases enhance overall motility^{2,23,24,40,43,67,68}.

The affinity of the monomeric cargo Nup358-min for BicD2 is enhanced by dimerization by a leucine zipper, similar to what was previously observed for binding of the cargo Egalitarian for *Drosophila* BicD^{25,26}. Thus, it is conceivable that a Nup358-min dimer binds to a BicD2 dimer, which is also in line with our ITC results, which reveal a single K_D . While Nup358-min is a monomer on its own, it oligomerizes to form 2:2 complexes with either the BicD2-CTD or the KLC2, and also forms a ternary complex with both BicD2-CTD and KLC2 with 2:2:2 stoichiometry^{46,49}. We thus propose that cargo adaptor-induced dimerization may potentially be a universal feature of the activation mechanism, since BicD2 and KLC2 both form dimers in active dynein and kinesin-1 motors^{22,24,46,69}. Importantly, NMR titration showed that BicD2 binding has no effect on the NMR signal of the LEWD motif of Nup358 (Figure 3), which can interact directly with KLC2⁴⁶. These data further suggest that the Nup358-min

domain is potentially capable of recruiting both dynein and kinesin-1 machineries for bi-directional positioning of the nucleus, although this remains to be confirmed in the context of full-length proteins and intact motors. Intriguingly, our NMR data show that the BicD2 binding site is only separated by 30 residues from the LEWD motif that acts as KLC2 binding site. The proximity of these binding sites may play a role in coordinating motility for bi-directional transport^{2–4,6,9,10,15,46,70–77}. Another intriguing possibility is that if kinesin binding dimerizes Nup358, this may be the key initial step leading to BicD2 activation and recruitment of dynein-dynactin to form a bidirectional complex (Figure 9). The ternary Nup358/BicD2/KLC2 complex may be a model for other transport modules, in which opposite polarity motors such as dynein and kinesin-1 act together.

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Figure 9. We propose that BicD2 recognizes its cargo through a short “cargo-recognition alpha-helix”, which may also be a structural feature that stabilizes the activated state of BicD2 for the recruitment of dynein and dynactin. (a) Cutaway view of half of an NPC. Each of the eight spokes of the NPC contains four molecules of Nup358 on the cytoplasmic side (*i.e.*, 32 in total), which provide binding sites for dynein (via BicD2) and kinesin-1 (via KLC2). Nup358 makes up most of the mass of the cytoplasmic filaments of the NPC^{78,79} (b) Left panel: Schematic representation of the looped, auto-inhibited conformation of BicD2 which is formed in the absence of cargoes such as Nup358. Apo-Nup358 contains many intrinsically disordered regions (IDR), including the BicD2 binding region. Upon binding of Nup358 to BicD2, a short α -helix is formed at the Nup358/BicD2 interface, which is a structural feature that stabilizes BicD2 in the active state. Binding of Nup358 to BicD2 likely promotes loop opening, which activates BicD2 for dynein/dynactin recruitment²⁵ (c) Schematic representation of the proposed dynein/dynactin/BicD2/Nup358 complex (DDBN) bound to microtubules for processive motility for nuclear positioning. Nup358 has a LEWD motif which recruits kinesin-1 via KLC2. The binding sites for the opposite polarity motor kinesin-1 on Nup358 are indicated by small blue sphere. The location of the LEWD motif which recruits kinesin-1 via KLC2 is indicated. We propose that BicD2 and KLC2 interact simultaneously with Nup358 for precise control of nuclear positioning.

Based on our data, we propose a structural basis for cargo recognition through the dynein adaptor BicD2. Our results establish that BicD2 recognizes its cargo Nup358 through a small “cargo recognition α -helix” which is embedded in an IDR. This region undergoes a structural transition from a random coil to an α -helix upon binding to BicD2. In single molecule TIRF assays, four single point mutations within the cargo recognition helix significantly inhibited the interaction between BicD2 and Nup358^{min-zip}, and impaired run length and speed of the mutant DDBN^{min-zip} complexes on microtubules. Our results may facilitate the identification of regulatory mechanisms for BicD2-dependent transport pathways, which are important for cell cycle control, brain and muscle development and vesicle transport.

Activation of BicD2 is a key regulatory step for transport, as it is required to activate dynein for processive motility. The cargo recognition α -helix may be a structural feature that stabilizes BicD2 in its activated state. We propose that binding of cargo induces a coiled-coil registry shift in BicD2, which promotes loop-opening and activates BicD2 for dynein/dynactin binding. Notably, Nup358 interacts more strongly with the dynein/dynactin/BicD2 complex compared to BicD2 alone, which supports the loop-opening mechanism for activation. We also show that BicD2 and KLC2 bind to spatially close, but non-overlapping binding sites on Nup358, supporting the hypothesis that Nup358 is capable of simultaneously recruiting dynein and kinesin-1 machineries to the nucleus for bi-directional transport.

Methods:

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background (<i>Escherichia coli</i>)	Rosetta 2(DE3)-pLysS	Fisher Scientific	Cat. #: 714033	
strain, strain background (<i>Escherichia coli</i>)	BL21-DE3-CodonPlus-RIL	Fisher scientific	Cat. #: 50-125-350	
recombinant DNA reagent	Human BicD2-CTD pet28a	Genscript Reference 30 https://pubs.acs.org/doi/10.1021/acs.jpcllett.9b01865	Sequence encoding residues 715-804 of human BicD2 cloned into a pet28a vector via the NdeI and XhoI restriction sites.	For the protein sequence expressed from this vector see supplemental methods.
recombinant DNA reagent	Human Nup358-min pGEX-6P-1	Genscript Reference 30 https://pubs.acs.org/doi/10.1021/acs.jpcllett.9b01865	Sequence encoding residues 2148-2240 of human Nup358 cloned into a pGEX-6P-1 vector via the BamHI and XhoI restriction sites.	For the protein sequence expressed from this vector see supplemental methods.

recombinant DNA reagent	Nup358 ^{-min-} pGEX-6P-1	Genscript This paper	Modified Nup358-min pGEX-6P-1 vector that includes a SNAP-tag at its C-terminal domain.	For the protein sequence expressed from this vector see supplemental methods. This vector can be obtained from Dr. Solmaz's lab
recombinant DNA reagent	Nup358 ^{-min- zip} pGEX-6P-1	Genscript This paper	Modified Nup358 ^{-min-} pGEX-6P-1 vector that includes a leucine-zipper that was added at the C-terminus before the snap-tag.	For the protein sequence expressed from this vector see supplemental methods. This vector can be obtained from Dr. Solmaz's lab.
recombinant DNA reagent	Human Nup358 (residues 2157-2199) pGEX-6P-1	Genescript This paper	Sequence encoding residues 2157-2199 of human Nup358 cloned into a pGEX-6P-1 vector via the BamHI and XhoI restriction sites.	Protein overexpression plasmid, which can be obtained from Dr. Solmaz's lab.
peptide, recombinant protein	PreScission protease	Cytiva	Cat. #: 27084301	
peptide, recombinant protein	Thrombin, human plasma	Fisher Scientific	Cat. #: 6051951000U	

chemical compound, drug	^{13}C D-Glucose (U- $^{13}\text{C}_6$)	Cambridge Isotope Laboratories	Cat. #: CLM-1396-PK	
chemical compound, drug	$^{15}\text{NH}_4\text{Cl}$	Cambridge Isotope Laboratories	Cat. #: NLM-467-10	
chemical compound, drug	Complete EDTA-free protease inhibitor cocktail tablets	Roche	Cat. #: 45-5056489001-EA	
software, algorithm	Origin Student 2018	OriginLab		CD spectroscopy
software, algorithm	Origin GE Microcal ITC200	OriginLab		ITC
software, algorithm	BioXTAS RAW software suite (version 2.0.3).	Reference 87		
software, algorithm	ImageJ 1.52v.	Reference 104		
software, algorithm	UCSF Chimera (version 1.14)	Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco. Reference 97		
Chemical compound, drug	Coomassie brilliant blue R-250	VWR	Cat. # VWRV0472-25G	

Chemical compound, drug	Rnase Inhibitor	Promega	N261B	
Chemical compound, drug	Q-dot 525 streptavidin conjugate	Invitrogen	Q10141MP	
Chemical compound, drug	Q-dot 565 streptavidin conjugate	Invitrogen	Q10131MP	
Chemical compound, drug	Q-dot 655 streptavidin conjugate	Invitrogen	Q10121MP	
Chemical compound, drug	SNAP-Biotin	New England BioLabs	S9110S	
Chemical compound, drug	Tubulin protein (X-rhodamine): bovine brain	Cytoskeleton, Inc	TL620M-A	
Chemical compound, drug	paclitaxel	Cytoskeleton, Inc	TXD01	
Recombinant DNA reagent	bicaudal D homolog 2 isoform 2 (<i>Homo sapiens</i>)	this paper	NCBI:NP_056065.1	Protein overexpression plasmid, which can be obtained from Dr. Solmaz's lab.
Biological sample (Bos taurus)	dynein - dynactin	bovine brain		
Biological sample (Bos taurus)	tubulin	bovine brain		
Software, algorithm	Nikon ECLIPSE Ti microscope	Nikon		
Software, algorithm	Nikon NIS Elements	Nikon		
Software, algorithm	Andor EMCCD	Andor Technology		

	Camera	USA		
Software, algorithm	Prism	GraphPad	v7; RRID:SCR_0027 98	
Chemical compound, drug	2- [Methoxy(pol yethyleneoxy) propyl]triMet hoxysilane	J & K Scientific	967192	
Chemical compound, drug	n-Butylamine	Acros Organics	A0344582	
Software, algorithm	Image J FIJI	NIH	1.53c	
Software, algorithm	nmrpipe	NIH, Reference 80		
Software, algorithm	Nmrfam_spar ky	Reference 106		

841

842 **Protein expression and purification**

843 Nup358-min and BicD2-CTD were expressed and purified as previously
844 described^{30,49,60}. For details, see Appendix 3.

845

846 **Pull-down assays**

847 GST-pull down assays of human Nup358-min-GST and human BicD2-
848 CTD were performed as described⁶⁰. For details, see Appendix 3.

849

Isothermal titration calorimetry (ITC)

ITC experiments were performed as previously described⁴⁹. In brief, protein samples were extensively dialyzed against a buffer containing 150 mM NaCl, 30 mM HEPES pH 7.5, 0.5 mM TCEP, and 1 mM MgCl₂. For ITC experiments, BicD2-CTD was placed in the cell of a calorimeter (MicroCal Auto-iTC200, GE Healthcare) and titrated with Nup358-min (either without the GST-tag (Figure 1) or with the GST-tag intact (Figure 1 – figure supplement 1 a) at 25 °C. As controls, titrations of Nup358-min (with or without the GST-tag) into buffer were carried out, which resulted in a flat line (Figure 1 – figure supplement 1 b, c). The corrected ITC curves were analyzed using a nonlinear least-squares minimization method in ORIGIN 7.0 and fitted with the one-site model to determine the number of sites N, the equilibrium binding constant K, the change in entropy ΔS and the change in enthalpy ΔH . The affinity (dissociation constant K_d) was calculated as the inverse of K. Data was analyzed with the Origin software (OriginLab). For the titration of Nup358-min + BicD2-CTD, the protein concentrations were 0.27 mM (Nup358-min) and 0.019 mM (BicD2-CTD), respectively. For the titration of Nup358-min-GST + BicD2-CTD, the protein concentrations were 0.12 mM (Nup358-min-GST) and 0.013 mM (BicD2-CTD), respectively. Protein concentrations were determined by UV absorbance at 280 nm.

Nuclear Magnetic Resonance (NMR)

872 HSQC NMR experiments of ^{15}N -labeled Nup358-min were recorded on a
873 0.2 mM, 440 μl sample with 10% D_2O on a Bruker 800 MHz spectrometer
874 equipped with a cryoprobe at 25 $^\circ\text{C}$. HSQC was taken on a 1:1 mixture of ^{15}N -
875 Nup358min-BicD2-CTD, where the sample was concentrated to keep the
876 concentration of Nup358-Min to 0.2 mM in 20 mM HEPES pH 7.5, 150 mM NaCl,
877 0.5 mM TCEP. Backbone assignments were accomplished using standard triple
878 resonance experiments, and standard NMR processing analysis tools^{80–83}. For
879 details see Appendix 3. ^{15}N CEST NMR was initially performed with a 0.6 mM
880 sample and a 10:1 ratio of BicD2-CTD: ^{15}N -Nup358-min. The temperature was
881 reduced to 20 $^\circ\text{C}$, and the pH was reduced to 6.5 to help improve signal/noise by
882 decreasing the rate of solvent exchange of amide protons. ^{15}N DCEST NMR^{84,85}
883 was performed with the same sample conditions. A second sample with a 20:1
884 ratio of ^{15}N -Nup358-min: BicD2-CTD was utilized to improve the S/N ratio of the
885 weakest peaks. For both experiments, the 800 MHz spectrometer with cryoprobe
886 was used. For CEST, the saturation pulse was 400 ms at both 10 Hz and 20 Hz.
887 The saturation frequency ranged from 118 ppm (the center of the HSQC
888 spectrum) to 126 ppm in steps of 0.25 ppm in CEST. The second attempt was
889 run from 116 to 124 ppm. The DCEST was performed to cover the whole spectral
890 width for amides, with a 10 Hz saturation and a 700 Hz spectral width, and a 20
891 Hz saturation pulse with a 600 Hz spectral width. $^{13}\text{C}'$ HNCO-CEST NMR⁵⁶ was
892 performed with a 0.6 mM sample and a 1:20 ratio of BicD2-CTD:Nup358-Min at
893 20 $^\circ\text{C}$ and pH of 6.5. For this experiment, a 600 MHz spectrometer with
894 cryoprobe was used to minimize the effect of $^{13}\text{C}'$ transverse relaxation. The

saturation pulse was 300 ms at 10 Hz. The saturation frequency was ranged from 170 to 180 ppm in steps of 0.33 ppm. The CEST data were fitted with the program RING NMR Dynamics⁵⁷ and are presented in Appendix 1-table 1.

CD spectroscopy

CD spectroscopy was performed as previously described⁶⁰. For details, see Appendix 3.

SAXS experiments

Nup358-min and BicD2-CTD were purified as described above. The monodispersity of the protein was confirmed by SEC-MALS which is published^{46,49}. Purified Nup358-min and BicD2-CTD were dialyzed against the following buffer: 150 mM NaCl, 20 mM HEPES pH 7.5, 0.5 mM TCEP. The dialysis buffer was used as buffer match for SAXS experiments as well as for dilutions. The following protein concentrations were used: Nup358-min 4 mg/ml, BicD2-CTD 1 mg/ml. To assemble the complex, Nup358-min and BicD2-CTD were mixed in 1:1 molar ratio and incubated for 30 minutes on ice, with a final concentration of 1.3 mg BicD2-CTD and 1.3 mg Nup358-min. The affinity of BicD2-CTD towards Nup358-min is $1.7 \pm 0.9 \mu\text{M}$ (Figure 1), therefore this protein concentration is sufficient for complex formation. To assure monodispersity, SAXS-data were collected for at least 3 protein concentrations for each sample, and we also

collected data of a Nup358-min/BicD2-CTD complex that was further purified by gel filtration, with comparable results (data not shown). Prior to data collection, samples were thawed, filtered (pore size 0.2 μm) and centrifuged (30 min, 21,700 g, 4°C). SAXS data was collected at the beam line 7A1 at the Cornell High Energy Synchrotron Source (CHESS), with a dual Pilatus 100k detector system (Dectris, Baden, Switzerland), at a single detector position, on July 3, 2019 as described previously⁸⁶. Quartz capillary with a path length of 1.6 mm was used as the sample cell (OD=1.5 mm, wall thickness=10 μm). For each dataset, 20 frames were collected at 4°C, with 0.1 s exposure times (wavelength = 9.835 keV, beam dimensions = 250 * 250 μm , beam current = 49.9 mA (positrons), beam flux = 2.4×10^{12} photons/s). Most samples showed no detectable radiation damage, which was monitored by averaging 20 frames.

SAXS data were processed with the BioXTAS RAW software suite (version 2.0.3)⁸⁷. To obtain scattering intensity profiles, 20 data frames were reduced to scattering intensity profiles, placed on an absolute scale, averaged, and the scattering intensity profile of the buffer match was subtracted. The data quality was assessed by Guinier plots, molar mass calculations and dimensionless Kratky plots in BioXTAS RAW⁸⁷⁻⁹¹. Pair distance distribution pl functions were derived from the scattering intensity profiles by the program GNOM⁹² of the ATSAS 3.0.0-1 software suite⁹³ (implemented in RAW⁸⁷). Fifteen bead model 3D reconstructions were performed with the DammiFF program⁹⁴ (implemented in ATSAS/RAW^{87,93}). The resulting models were aligned, grouped into clusters, averaged, and the average model was refined in DammiFF⁹⁴⁻⁹⁶.

Figures of the refined molecular envelopes were created in the program UCSF Chimera (version 1.14)⁹⁷, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from P41-GM103311. PI functions were normalized to the highest signal of each curve.

Protein expression and purification for single-molecule assays

Cytoplasmic dynein and dynactin were purified from 300 gm bovine brain as described in Bingham *et al.*⁹⁸, and tubulin was purified from 200g bovine brain as described in Castoldi and Popov⁹⁹. Purified dynein was stored at -20°C, and dynactin and tubulin were stored at -80°C, in 10 mM imidazole, pH 7.4, 0.2 M NaCl, 1 mM EGTA, 2 mM DTT, 10 µM Mg ATP, 5 µg/mL leupeptin, 50% glycerol. The N-terminal domain of human BicD2 (BicD2^{CC1}) with a biotin tag at its N-terminus was expressed in bacteria as described²⁵. Full-length human wild type BicD2 was expressed in Sf9 cells as described for *Drosophila* BicD²⁵. The Bradford reagent (Bio-Rad, USA) was used to measure the protein concentration. To create a fluorescently labelled version of Nup358-min, the expression vector described above was modified to include a SNAP-tag for fluorescent labelling at its C-terminal domain, which is referred to as Nup358^{min}. Nup358^{min} was expressed and purified with the N-terminal GST-tag intact as described for Nup358-min^{49,100}, using the BL21-DE3-CodonPlus-RIL strain for expression. Nup358^{min} was dimerized using a leucin–zipper (hereafter called Nup358^{min-zip});

the leucine zipper sequence was added at the C-terminus before the snap-tag. The sequences of these two constructs are shown in the Appendix 3. The SNAP tag on Nup358^{min-zip} and Nup358^{min} was biotinylated with SNAP-biotin substrate (New England BioLabs, MA) as described¹⁰⁰.

Single Molecule assay

Dynein, dynactin, BicD2 and Nup358^{min-zip} constructs were diluted into high salt buffer (30 mM HEPES pH 7.4, 300 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 20 mM DTT) and clarified for 20 min at 400,000 x g to remove aggregates. To form the dynein-dynactin-BicD2-Nup358^{min-zip} (DDBN^{min-zip}) complex, BicD2 and Nup358^{min-zip} were mixed with 565nm and 655nm streptavidin Quantum Dots (Qdots) (Invitrogen, CA), respectively, at a 1:1 molar ratio in separate tubes and incubated 15 min on ice. To block excess binding sites on streptavidin Qdots, 5 μ M biotin was added to both tubes. Labeled BicD2 and Nup358^{min-zip} were then mixed with preformed dynein-dynactin complex at a molar ratio of 1:1:2:2 (250 nM dynein, 250 nM dynactin, 500 nM BicD2 and 500 nM Nup358^{min-zip}) and incubated on ice for 30 min in motility buffer (30 mM HEPES pH 7.4, 150 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 20 mM DTT). The dynein-dynactin-Nup358^{min-zip} (DDN^{min-zip}) complex contained Nup358^{min-zip} that was labeled with a 655 nm Qdot. In the dynein-dynactin-BicD2^{CC1} (DDB^{CC1}) complex, BicD2^{CC1} was labeled with a 525 nm streptavidin Qdot. The DDBN^{min-zip}, DDN^{min-zip} and DDB^{CC1} complexes were diluted in motility buffer (30 mM HEPES pH 7.4, 150 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 2 mM MgATP, 20 mM DTT, 8 mg/mL BSA,

0.5 mg/mL kappa-casein, 0.5% pluronic F68, 10 mM paclitaxel and an oxygen scavenger system) to a final concentration of 1.25-2.50 nM dynein for observing motion on microtubules. The oxygen scavenging system consisted of 5.8 mg/ml glucose, 0.045 mg/ml catalase, and 0.067 mg/ml glucose oxidase (Sigma-Aldrich-Aldrich). To analyze the motion of the complexes without any ambiguity, oxygen scavengers were not used in two-color experiments so that the microtubules would photo bleach. Purified tubulin was mixed with rhodamine labeled tubulin at a molar ratio of 10:1 and polymerized as described²⁵. PEGylated glass slides were prepared and coated with 0.3 mg/mL rigor kinesin for microtubule attachment as described²⁵. After rinsing 2-3 times with motility buffer to remove excess rigor kinesin, MTs were added. Excess microtubules were removed by rinsing with motility buffer. Then dynein protein samples were added to the glass surface. Motion of DDBN^{min-zip}, DDN^{min-zip} and DDB^{CC1} were observed using TIRF microscope as described²⁵.

Microscopy and Data Analysis

To detect the motion of Qdot labeled DDBN^{min-zip} and DDB^{CC1} complexes on microtubules, Total Internal Reflection Fluorescence (TIRF) microscopy was used. The TIRF microscope system is operated by the Nikon NIS Elements software and single molecule images were acquired on a Nikon ECLIPSE Ti microscope equipped with through objective type TIRF. The laser lines 488 and 561 nm were used to illuminate 525nm and 655nm Qdots and rhodamine-labeled microtubules. Typically, 300-600 frames were captured at 100 or 200 ms intervals (ten or five frames/s) using two Andor EMCCD cameras (Andor

Technology USA, South Windsor, Connecticut). Individual Qdots were tracked using the ImageJ MtrackJ plugin for run length and speed measurements of single complexes¹⁰¹. Run length is defined as the total travel distance by individual complexes, and speed was calculated by dividing the run length by the total time. To determine the characteristic run length, data were plotted as 1-CDF (1-cumulative probability distribution) with GraphPad Prism Software and fit to a one-phase exponential decay equation $p(x) = Ae(-x/\lambda)$, where $p(x)$ is the relative frequency, x is the travel distance along a microtubule track and A is the amplitude. Speed was reported as mean $\pm$ standard deviation (SD), and run length reported as mean $\pm$ standard error (SE). The number of processive events was calculated by counting the number of moving dual color Qdots per time per μm microtubule. The number of dual color Qdots (DDBN^{min-zip}) were counted on 18-32 microtubules in each case. For measuring the formation of DDBN^{min-zip} and BN^{min-zip} complexes containing WT or mutant Nup358^{min-zip}, the number of dual color and single color (green or red) Qdots were counted on multiple 512 x 512 pixel fields. The DDBN^{min-zip} and BN^{min-zip} complexes were bound to the glass surfaces non-specifically. The percentage of colocalization was calculated from the total number of dual color Qdots divided by all dual and single color Qdots. Statistical significance for two sets of run length data were determined by the Kolmogorov-Smirnov test, a nonparametric distribution. For speed data comparison, an unpaired t-test was performed. For three or more data sets of run length or speed, statistical significance was calculated using one-way ANOVA followed by Tukey's post-hoc test. Statistical differences for binding frequency of

1030 DDBN^{min-zip} containing WT or mutant Nup358^{min-zip} were determined by one way
1031 ANOVA followed by Tukey's post-hoc test.

1032 **References**

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1327

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1346

1347 **Legends for supplementary figures and videos:**

1348 **Figure 1 – figure supplement 1. (a) The ITC thermogram of BicD2-CTD with**
1349 **Nup358-min-GST** (i.e. with the GST-tag intact) is shown, from which the affinity was
1350 determined to be $1.6 \pm 1.0 \mu\text{M}$, demonstrating that the GST fusion has no effect on
1351 binding. (b) The control ITC thermogram for Figure 1 of Nup358-min (syringe) into the
1352 buffer (cell) is shown. Since the titration resulted in a flat line, thermodynamic
1353 parameters were not determined. Note that a brief spike is caused by an air bubble. (c)
1354 The control ITC thermogram of Nup358-min-GST (syringe) into buffer (cell) is shown.
1355 Since the titration resulted in a flat line, thermodynamic parameters were not determined.
1356 (d) Secondary structure prediction of Nup358-min created with the PredictProtein
1357 Server^{102,103}. However, this prediction is not accurate for apo Nup358-min as shown by
1358 subsequent chemical shift analysis. IDP: intrinsically ordered protein domain.

1359 **Figure 2 – figure supplement 1. Dimerization of Nup358^{min} increases the formation**
1360 **of DDBN^{min-zip} complexes.** (a) A leucine-zippered Nup358 (Nup358^{min-zip}) forms DDBN
1361 complex (yellow) at a higher frequency than that of Nup358 monomer (Nup358^{min}) (gray).
1362 The percent formation of DDBN^{min-zip} complex was 35% (n=945), 50% higher than that of
1363 DDBN^{min} complexes (23%, n=514). (b, c) Visualization of complex formation of DDBN
1364 formed with a dimerized Nup358^{min-zip} versus a monomeric Nup358^{min}. Yellow dots
1365 (indicated by white arrows) show the formation of the DDBN complex. Single color green
1366 Qdot indicates BicD2, and red indicates Nup358^{min-zip}. (d) Nup358^{min-zip} alone does not
1367 bind to MTs. Concentration of Nup358^{min-zip} was the same as Figure 2 – figure
1368 supplement 1b,c.

1369 **Video 1:** Dynein-dynactin-BicD2-Nup358^{min-zip} (DDBN^{min-zip}) indicated by dual color
1370 Qdot (yellow) moving on a microtubule track (not seen due to photobleaching) at
1371 2mM MgATP. Nup358^{min-zip} was labeled with a 655nm Qdot (red), BicD2 was
1372 labeled with a 525nm Qdot (green), and dynein-dynactin was unlabeled. Some
1373 single color Qdots are moving long distances, presumably because both Nup358
1374 and BicD2 are present but only one of them was labeled. Movie displayed 12X real
1375 speed. Scale bar 5 μm .

1376 **Video 2:** Fewer dynein-dynactin-BicD2 (DDB) complexes (green) show processive
1377 events compared to DDBN^{min-zip} (see Figure 2f). BicD2 was labeled with a 525nm
1378 Qdot (green), and dynein-dynactin was unlabeled. Movie was displayed 12X real
1379 speed. Scale bar 5 μm .

1380 **Video 3:** Dynein-dynactin-BicD2^{CC1} (DDB^{CC1}) complex (green) moving on a
1381 microtubule track at 2mM MgATP. BicD2 was labeled with a 525nm Qdot (green)
1382 and dynein-dynactin was unlabeled. Movie was displayed 12X real speed. Scale
1383 bar 5 μm .

1384 **Video 4, 5, 6:** These are three replicates of video 1. Dynein-dynactin-BicD2-
1385 Nup358^{min-zip} (DDBN^{min-zip}) indicated by dual color Qdot (yellow) moving on a
1386 microtubule track (not always seen due to photobleaching) at 2mM MgATP. Nup358
1387 ^{min-zip} was labeled with a 655nm Qdot (red), BicD2 was labeled with a 525nm Qdot
1388 (green), and dynein-dynactin was unlabeled. Some single color Qdots are moving
1389 long distances, presumably because both Nup358 and BicD2 are present but only
1390 one of them was labeled. The movie is displaying at 6X real speed. Data were
1391 captured at 200 ms intervals (five frames/s) and the length per pixel was calibrated
1392 as 110 nm.

Video 7, 8, 9: These are three replicates of video 2. Fewer dynein-dynactin-BicD2 (DDB) complexes (green) show processive events compared to DDBN^{min-zip}. BicD2 was labeled with a 525nm Qdot (green), and dynein-dynactin was unlabeled. Movie was displayed 6X real speed. Scale bar 5µm.

Video 10, 11, 12: These are three replicates of video 3. Dynein-dynactin-BicD2^{CC1} (DDB^{CC1}) complex (green) moving on a microtubule track at 2mM MgATP. BicD2 was labeled with a 525nm Qdot (green) and dynein-dynactin was unlabeled. Movie was displayed 12X real speed. Scale bar 5µm.

Video 13, 14, 15: Dynein-dynactin-Nup358^{min-zip} (DDN^{min-zip}) complexes (red) do not show any processive motion on microtubule tracks. Nup358^{min-zip} was labeled with a 655nm Qdot (red), and dynein-dynactin was unlabeled. Image was displayed 6X real speed. Movie was displayed 6X real speed. Scale bar 5µm.

Figure 3 – figure supplement 1: Fully Assigned ¹⁵N-¹H HSQC NMR Spectrum for Nup358-min. Inset shows the assignment of peaks in a crowded region.

Figure 3 – figure supplement 2: Three Points of Nup358:BicD2 Titration. The titration clearly shows a consistent drop in intensity of the peaks in Nup358 that are in the BicD2 binding region. The Nup358 concentration was held constant at 1, and unlabeled BicD2 was added. The intensity ratio is determined dividing the height of the titrated peak by the height of the Apo peak. Error was estimated based on signal/noise for the first two titration points and based on three repeat measurements for the 1:1 complex.

Figure 3 – figure supplement 3: TALOS-N Demonstrates Random Coil for Nup358-min. TALOS-N, a highly accurate program for determining secondary structure based on chemical shifts, was used to determine secondary structure of the apo protein. The majority of the protein heavily favors random coil.

Figure 4 – figure supplement 1: ¹⁵N CEST profiles for Nup358 residues showing exchange between random coil state and an α-helical state. These CEST profiles have a double-dip appearance, showing the bound state, represented by the minor dip as well as the free state, represented by the major dip. This experiment was repeated under slightly different conditions, one with a molar ratio 10:1 Nup358-min to BicD2-CTD and another one with a ratio of 20:1 Nup358-min to BicD2-CTD. The chemical shift range was also shifted by 2 ppm when the 20:1 ratio experimental was run. For example, one can see the presence of both the minor and major dips in L2184 in the “10:1” experiment as well as the “20:1” experiment, although the major dip do not return to baseline. This truncation was due to limited NMR time, necessitated by the stability of the sample. The ¹⁵N CEST curves resulted in the following residues being assigned as part of the α-helical region in bound state: A2163, A2164, K2165, L2166, I2167, K2178, and L2184.

Figure 4 – figure supplement 2: ¹³C' CEST profiles for Nup358 residues showing exchange between random coil state and an α-helical state, showing the minor dip as well as the major dip, representing the chemical shift of the bound state. This experiment was performed using a 20:1 molar ratio of Nup358-min to BicD2-CTD. The following residues, already identified as α-helical residues, using the ¹⁵N CEST experiments, were confirmed with ¹³C' CEST experiments: A2164, K2165 and L2166.

1438 Additionally, the following residues were added to the list of α -helical residues: R2162,
1439 R2169, E2171, E2172, L2177, K2181, F2183, and L2184. To fit the global exchange
1440 rate, only curves with sufficient signal/noise, significant resolution between the major and
1441 minor dips, and curves with sufficient resolution between the minor dip and the end of
1442 the curve were used.

1443 **Figure 4 – figure supplement 3: ^{15}N CEST profiles for Nup358 residues showing**
1444 **NO exchange between the random coil state and an α -helical state.** These ^{15}N
1445 CEST curves show only a single dip at the major chemical shift, indicating that these
1446 sites do not undergo chemical exchange to a bound state. Note that these sites include
1447 residues around the LEWD motif as well as sites all the way to the C-terminus of
1448 Nup358-min.

1449 **Figure 4 – figure supplement 4: ^{13}C CEST profiles for Nup358 residues showing**
1450 **NO exchange between a random coil state and an α -helical state,** showing only a
1451 single dip at the major chemical shift. Note that these sites include residues around the
1452 LEWD motif as well as sites at the C-terminus half of Nup358-min (see Figure 4 in the
1453 main text).

1454 **Figure 5 – figure supplement 1. Calibration curve for the CD signal, based on a**
1455 **published melting curve of BicD2-CTD.** We previously published the thermal unfolding
1456 curve of wild type BicD2-CTD that was recorded by CD spectroscopy at 222 nm (Figure
1457 4E in reference 303030). Here, this curve was re-plotted as mean residue molar
1458 ellipticity $[\Theta]$ versus % protein unfolded, to obtain a calibration curve for the α -helical
1459 content. 0% and 100% protein unfolded represent the values of $[\Theta]_{\min}$ and $[\Theta]_{\max}$ from
1460 the melting curve respectively. At the apparent melting temperature T_M , the protein is
1461 50% unfolded. For purposes of calibration we assume that native, 0% unfolded BicD2-
1462 CTD has an α -helical content of 96% as observed in the crystal structure 303030. We
1463 also used this method recently to assess changes in α -helical content in the *Drosophila*
1464 homolog BicD⁶⁰.

1465 **Figure 6 – figure supplement 1. SAXS plots for Nup358-min/BicD2-CTD complex.**
1466 (a) The scattering intensity profile $I(q)$ is shown as a function of the scattering vector (q).
1467 (b) The Guinier plot (top panel) is shown together with the normalized residual of the
1468 Guinier fit (bottom panel). (c) The pair distance distribution function $p(r)$ is shown (top
1469 panel). The middle panel shows the scattering intensity profile calculated from the $p(r)$
1470 function (red) overlaid with the scattering intensity profile of the actual data (blue). The
1471 bottom panel shows the normalized residual of the fit. (d) The “data” scattering intensity
1472 profile (blue) is overlaid with the “model” scattering intensity profile that was calculated
1473 from the refined bead model 3D reconstruction (red, top panel). The bottom panel shows
1474 the normalized residual of the fit.

1475 **Figure 6 – figure supplement 2. SAXS plots for Nup358-min.** (a) The scattering
1476 intensity profile $I(q)$ is shown as a function of the scattering vector (q). (b) The Guinier
1477 plot (top panel) is shown together with the normalized residual of the Guinier fit (bottom
1478 panel). (c) The pair distance distribution function $p(r)$ is shown (top panel). The middle
1479 panel shows the scattering intensity profile calculated from the $p(r)$ function (red) overlaid
1480 with the scattering intensity profile of the actual data (blue). The bottom panel shows the
1481 normalized residual of the fit.

Figure 6 – figure supplement 3. SAXS plots for BicD2-CTD. (a) The scattering intensity profile $I(q)$ is shown as a function of the scattering vector (q). (b) The Guinier plot (top panel) is shown together with the normalized residual of the Guinier fit (bottom panel). (c) The pair distance distribution function $p(r)$ is shown (top panel). The middle panel shows the scattering intensity profile calculated from the $p(r)$ function (red) overlaid with the scattering intensity profile of the actual data (blue). The bottom panel shows the normalized residual of the fit.

Figure 6 – figure supplement 4. The pair distance distribution ($p(r)$) functions of the Nup358-min/BicD2-CTD complex, Nup358-min and BicD2-CTD were derived from the SAXS-profiles (Figure 6 – figure supplements 1 – 3–) and normalized towards the highest signal.

Figure 7 – figure supplement 1. Interface residues of BicD2 and Nup358 were identified by mutagenesis. SDS-PAGE analyses of elution fractions of the pull-down assays (see Figure 7) to probe the effect of Nup358 mutants on the interaction with BicD2. The molecular weights and band position of standard proteins are indicated on the left. (a) A representative result is shown for all mutants that were quantified. Experiments were repeated three times. Note that a small gel band at 25 kDa represents GST. (b) Pull-down of BicD2-CTD and mutants of Nup358-min. Left panel: the linear sequence motif TEEE which is located outside of the cargo recognition helix was mutated to AAAA, to exclude the possibility that it is engaged in the interaction. Right panel: The sequence motif AAKL was removed to assess the role of the two alanines for the interaction. All four residues showed large structural changes in CEST. (c) SDS-PAGE analysis of the purified Nup358(residues 2157-2199)/BicD2-CTD complex. The individually purified proteins were mixed and the complex was isolated by gel filtration chromatography. (d) SDS-PAGE analyses of purified BicD2-CTD at known protein concentrations. The signals of the protein bands from c were quantified and the intensities of the gel bands were normalized to the 1 mg gel band. The normalized intensities are plotted versus the protein concentration. Note that the curve is linear.

Figure 8 – figure supplement1. Nup358 point mutations that diminish the interaction with BicD2-CTD also diminish formation of the DDBN^{min-zip} complex. (a-e) Visualization of complex formation of DDBN^{min-zip}, formed with WT Nup358^{min-zip} and Nup358^{min-zip} mutants I2167A, M2173A, L2177A and L2184. DDBN^{min-zip} formed with mutant F2180A is not shown because of its similarity to L2184. Yellow dots (some indicated by arrows) indicate the formation of the DDBN^{min-zip} complex, but single color (green or red) Qdots show individual uncomplexed BicD2 and Nup358^{min-zip} molecules, respectively. (f-j). Visualization of complex formation of BN^{min-zip} formed with WT Nup358^{min-zip} and Nup358^{min-zip} mutants I2167A, M2173A, L2177A and L2184.

1520 **SOURCE DATA FILES**

1521 **Figure 1 – source data 1.** ITC thermogram of Nup358-min and BicD2-CTD.

1522 **Figure 1 – source data 2.** ITC thermogram of BicD2-CTD with Nup358-min-GST (i.e.
1523 with the GST-tag intact).

1524 **Figure 1- source data 3.** ITC thermogram of Nup358-min into buffer.

1525 **Figure 1 – source data 4.** ITC thermogram of Nup358-min-GST into buffer.

1526 **Figure 5 – source data 1.** CD spectra.

1527 **Figure 6 – source data 1.** SAXS data.

1528 **Figure 7 – source data 1.** Quantification of the pulldown assay.

1529 **Figure 7 – source data 2.** SDS-PAGE shown in Figure 7.

1530 **Figure 7 – source data 3.** SDS-PAGEs shown in Figure 7 – figure supplement 1 and
1531 SDS-PAGEs that were used for the quantification shown in Figure 7.

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Appendix 1

Appendix 1-table 1

(a) Summary of ^{15}N CEST Curve Fits at 20:1 Molar Ratio of BicD2: ^{15}N -Nup358

Residue	$k_{\text{ex}} (\text{s}^{-1})$	P_b	$\Delta\delta(\text{ppm})$
A2164	704 ± 211	0.07 ± 0.02	-4.3 ± 0.4
K2165	139 ± 162	0.25 ± 0.08	-0.8 ± 0.3
L2166	381 ± 182	0.07 ± 0.03	-3.8 ± 0.3
I2167	975 ± 624	0.02 ± 0.04	-5.7 ± 0.2
K2178	31 ± 35	0.01 ± 0.01	-3.6 ± 0.2
L2184	160 ± 92	0.07 ± 0.03	-5.5 ± 0.2

(b) Summary of ^{15}N CEST Curve Fits at 10:1 Molar Ratio of BicD2: ^{15}N -Nup358

Residue	$k_{\text{ex}} (\text{s}^{-1})$	P_b	$\Delta\delta$ (ppm)
A2163	12 ± 73	0.07 ± 0.05	-3.6 ± 0.2
A2164	228 ± 72	0.09 ± 0.02	-3.6 ± 0.3
K2165	757 ± 273	0.13 ± 0.07	-1.3 ± 0.3
L2166	297 ± 195	0.05 ± 0.02	-3.3 ± 0.6

NOTE: The change in chemical shift in Table 1 was taken from Table S1b for the alanine residues due to a clearer minor peak. For the others, the change in chemical shift in Table 1 was taken from Table S1a. The values used in the weighted averages for k_{ex} and P_b were taken from Table S1a, excluding the K2165 and K2178 with abnormal P_b and significant noise in their CEST curves. Including the K2165 and K2178 values, the weighted average k_{ex} value would be $80 \pm 30 \text{ s}^{-1}$, and the P_b value would be 0.03 ± 0.01 .

Appendix 1-table 2

Summary of $^{13}\text{C}'$ CEST Curve Fits at 20:1 Molar Ratio of [BicD2]:[Nup358]

Residue	$k_{\text{ex}} (\text{s}^{-1})$	P_b	$\Delta\delta$ (ppm)
R2162	313 ± 328	0.05 ± 0.03	1.7 ± 0.3
A2164	474 ± 304	0.07 ± 0.03	2.8 ± 0.2
K2165	208 ± 179	0.05 ± 0.07	2.7 ± 0.1
L2166	391 ± 170	0.04 ± 0.03	2.6 ± 0.2
R2169	140 ± 125	0.06 ± 0.01	2.9 ± 0.2
E2171	359 ± 175	0.07 ± 0.03	2.2 ± 0.2
E2172	8 ± 98	0.22 ± 0.07	3.0 ± 0.1
L2177	540 ± 241	0.03 ± 0.02	0.9 ± 0.3
K2181	316 ± 155	0.06 ± 0.02	3.0 ± 0.1
F2183	464 ± 98	0.06 ± 0.01	3.3 ± 0.3
L2184	238 ± 165	0.08 ± 0.04	1.0 ± 0.2

Appendix 2

Appendix 2-table 1: Summary of SAXS data

Sample	R_G (Å) Guinier plot	R_G (Å) pl function	$I(0)$ pl function/ $I(0)$ Guinier plot	TE / χ^2 pl function	D_{Max} pl function	MW ^{*/**/**} (kDa)
Nup358/BicD2	48.1 ± 0.6	55.5 ± 0.2	0.086/0.086	0.61/1.04	190	47.7/46.0/47.6
Nup358-min	28.4 ± 0.3	30.6 ± 0.4	0.035/0.036	0.69/1.05	120	12.7/12.3/na
BicD2-CTD	42.6 ± 1.0	49.7 ± 0.9	0.066/0.070	0.67/1.10	190	na/na/38.7

R_G : radius of gyration. $I(0)$: scattering intensity at zero angle. TE: total estimate. D_{Max} : maximum particle diameter. MW: molar mass. *MWs were determined from $I(0)$, using glucose isomerase as a standard⁸⁹. **MWs were determined from $I(0)$ without a molar mass standard⁸⁹. *** MWs were determined from the volume of correlation as described by Rambo and Tainer⁶¹. Note that not all methods can be applied to all samples (na). The error of molar masses determined by SAXS is 10%. The calculated MWs are 10.9 kDa for BicD2-CTD and 10.6 kDa for Nup358-min.

Appedix 2-table 2: Statistics of SAXS bead model 3D reconstruction

Sample	Mean normalized spatial discrepancy score (NSD)	χ^2 (refined model) / χ^2 (representative model)	Resolution (Å)	Ambimeter score
Nup358-min/BicD2- CTD	0.70 ±0.05	1.08/1.05	41 ±3	1.23

Appendix 3

Supplementary Methods.

Sequences of expression constructs

Nup358^{min}

MW 56.5 kDa

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKL
TQSMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLK
MFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSS
KYIAWPLQGWQATFGGgDHPPKSDLEVLFGGGLGSDIPLQTPHKLVDTGRAAKLIQRAEEMKS
GLKDFKTFLTNDQTKVTEENKGS GTGAAGASDTTIKPNPENTGPTLEWDNYDLREDALDDSV
SSMSGDKDCEMKRTTLDSP LGKLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLM
QATAWLNAYFHQPEAIEEFVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPA
ATAAVKTALSGNPVPILIPCHR VVQGDLDVGGYEGGLAVKEWLLAHEGHR LGKPGLG

Nup358^{min- zip}

MW 60.3 kDa

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKL
TQSMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLK
MFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSS
KYIAWPLQGWQATFGGgDHPPKSDLEVLFGGGLGSDIPLQTPHKLVDTGRAAKLIQRAEEMKS
GLKDFKTFLTNDQTKVTEENKGS GTGAAGASDTTIKPNPENTGPTLEWDNYDLREDALDDSV
SSMKQLEDKVEELLSKNYHLENEVARLKKLVGERMSGDKDCEMKRTTLDSP LGKLELSGCEQG
LHRIIFLGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNAYFHQPEAIEEFVPALHHPVFQQE
SFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHR VVQGDLDVGG
YEGGLAVKEWLLAHEGHR LGKPGLG

Nup358-min

10.5 kDa

GPLGSDIPLQTPHKLVDTGRAAKLIQRAEEMKSG LKDFKTFLTNDQTKVTEENKGS GTGAAG
ASDTTIKPNPENTGPTLEWDNYDLREDALDDSVSS

BicD2-CTD

10.9 kDa

GSHMYENEKAMVTETMMKLRNELKALKEDAATFSSLRAMFATRCDEYITQLDEMQRQLAAAE
DEKKTLSLLRMAIQQKLALTQRLELLELDHE

Protein expression and purification

Proteins were expressed and purified as previously described^{30,49,60}. Codon-optimized expression constructs and point mutants were created by a commercial cloning and gene synthesis service (Genscript). Sequences are listed in Appendix 3. For purification of isotope-labeled Nup358-min, the previously described expression vector for Nup358-min (which encoded the sequence for residues 2148-2240 of human Nup358 cloned into a pGEX6p1 vector)³⁰ was expressed in the *E. coli* Rosetta 2(DE3)-pLysS strain as described³⁰, with the following modifications: M9 minimal media (3 g KH₂PO₄, 6.8 g Na₂HPO₄, 1 g NaCl, 4 g D-glucose, 1g NH₄Cl, 2 mM MgSO₄, 0.1 mM CaCl₂, 100 µg Ampicillin and 35 µg Chloramphenicol per liter medium) were used for the expression, which was performed at 37 °C. For expression of ¹⁵N labeled Nup358-min, unlabeled NH₄Cl was replaced in the medium by 1g ¹⁵NH₄Cl per liter. For expression of ¹³C and ¹⁵N labeled Nup358-min, 1 g ¹³C D-Glucose (U-13C6) and 1 g ¹⁵NH₄Cl was used per 1 L of M9 media instead of the unlabeled compounds. Isotopes were obtained from Cambridge Isotope Laboratories.

Nup358-min was purified using the previously described protocol⁴⁶, but the cell pellet from a 6 liter bacteria culture was dissolved in 75 ml of the lysis buffer to which 1 ½ tablets of complete EDTA-free protease inhibitor cocktail tablets were added (Roche). In short, Nup358-min was purified by glutathione affinity chromatography and eluted by proteolytic cleavage on the column with PreScission protease. The protein was further purified by size exclusion chromatography as described⁴⁶, using the following buffer: 20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP.

BicD2-CTD was expressed and purified as previously described^{30,46,49}. In short, BicD2-CTD was purified by Ni-NTA affinity chromatography, followed by proteolytic cleavage of the His₆-tag by thrombin. BicD2-CTD was further purified by a second round of Ni-NTA affinity chromatography, followed by size exclusion chromatography in the same buffer as described above. The Nup358 (residues

2157-2199)/BicD2-CTD complex was purified as described for the Nup358-min/BicD2-CTD complex³⁰.

Pull-down assays

GST-pull down assays of human Nup358-min-GST and human BicD2-CTD were performed as described^{59,59}. For the assays, Nup358-min-GST was immobilized on glutathione sepharose beads and incubated with purified BicD2-CTD prior to elution with glutathione. The elution fractions were analyzed by SDS-PAGE (16% acrylamide gels) and stained with Coomassie Blue. ImageJ was used for quantification of gel band bands¹⁰⁴.

Nuclear Magnetic Resonance (NMR)

For backbone assignment, triple resonance experiments (HNCO, HNCA, HNCACO, HNCOCA, HNCACB, CBCCACONH, and HNN¹⁰⁵ were performed on double-labeled ¹⁵N/¹³C Nup358-min at 0.4 mM on a Bruker 800 MHz spectrometer equipped with a cryoprobe. They were performed with non-uniform sampling (NUS) Processing of the data was performed with NMRPipe⁸⁰ and SMILE⁸¹. Further analysis of the data was performed with nmrnam_Sparky¹⁰⁶, including iPine^{82,83}.

CD spectroscopy

Purified proteins at a concentration of 0.3 mg/ml were dialyzed in the following buffer: 150 mM NaCl, 10 mM Tris pH 8 and 0.2 mM TCEP. Data were recorded with a Jasco J-1100 CD Spectrometer, equipped with a thermoelectric control device. A quartz cuvette with a path length of 0.1cm was used. After the buffer baseline subtraction, CD signals were normalized to the protein concentration and converted to mean residue molar ellipticity [Θ]. Thus, the ellipticity Θ (mdeg) was multiplied with the conversion factor 391.51 cm² dmol⁻¹ for all spectra with exception of the Nup358-min spectrum, for which the conversion factor was 362.00 cm² dmol⁻¹. For the Nup358 + BicD2 spectrum, the spectra of Nup358-min and BicD2-CTD were added together prior to conversion to [Θ].

d Secondary structure prediction of Nup358-min

DIPLQTPHKLVDTGRAAKLIQRAEEMKSGLKDFKFTLTNDQTKVTEENKSGTGAAGASDTTIKPNPENTGPTLEWDNYDLREDALDDSVSS

IDP alpha-helix

2162 2184

R2162

A2164

K2165

L2166

R2169

E2171

E2172

L2177

K2181

F2183

L2184

a SAXS-Profile for Nup358/BicD2

b Guinier plot

c P(r) function

d Fit of the refined bead model to the SAXS-profile

a SAXS-Profile for Nup358

b Guinier plot

c P(r) function

a SAXS-Profile for BicD2

b Guinier plot

c P(r) function