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► **To cite this version:**

Robert Fagiewicz, Corinne Crucifix, Torben Klos, Célia Deville, Bruno Kieffer, et al.. In vitro characterization of the full-length human dynein-1 cargo adaptor BicD2. *Structure*, 2022, 30 (11), pp.1470-1478.e3. 10.1016/j.str.2022.08.009 . hal-03876788

HAL Id: hal-03876788

<https://hal.science/hal-03876788v1>

Submitted on 20 Apr 2023

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1 ***In vitro* characterization of the full-length human dynein-1** 2 **cargo adaptor BicD2**

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16

17

18 **SUMMARY**

19 Cargo adaptors are crucial in coupling motor proteins with their respective cargos and
20 regulatory proteins. BicD2 is a prominent example within the cargo adaptor family. BicD2
21 is able to recruit the microtubule motor dynein to RNA, viral particles and nuclei. The
22 BicD2-mediated interaction between the nucleus and dynein is implicated in mitosis,
23 interkinetic nuclear migration (INM) in radial glial progenitor cells, and neuron precursor
24 migration during embryonic neocortex development. *In vitro* studies involving full-length
25 cargo adaptors are difficult to perform due to the hydrophobic character, low-expression
26 levels, and intrinsic flexibility of cargo adaptors. Here we report the recombinant
27 production of full-length human BicD2 and confirm its biochemical activity by interaction
28 studies with RanBP2. We also describe pH-dependent conformational changes of BicD2
29 using cryoEM, template-free structure predictions, and biophysical tools. Our results will
30 help define the biochemical parameters for the *in vitro* reconstitution of higher order BicD2
31 protein complexes.

32

33 **INTRODUCTION**

34 Cytoplasmic dynein drives many retrograde microtubule transport events in eukaryotic
35 cells. The main isoform – cytoplasmic dynein-1 (hereafter dynein-1) is involved in cell
36 division, the transport of organelles and vesicles, brain and muscle development, and can
37 also be hijacked by pathogenic viruses to reach cellular locations (Reck-Peterson et al.,
38 2018; Paschal and Vallee, 1987; Dodding and Way, 2011; Wilson and Holzbaur, 2012; Fu

1 and Holzbaur, 2014). It is also a particularly interesting motor in neurons, as it carries
2 essential signals and organelles from distal axon sites to the cell body (Schiavo et al.,
3 2013).

4 Dynein-1 motility and cargo specificity depends on cargo adaptor proteins. Currently there
5 are eleven dynein-1 cargo adaptors known and this number is continuously growing
6 (Olenick and Holzbaur, 2019). Cargo adaptors are characterized by α -helices forming
7 extended coiled-coil domains and they can interact with multiple proteins. Generally, the
8 N-terminal region of the cargo adaptor associates with the dynein motor while the C-
9 terminal domain recruits cargos. Selectivity towards their cargos is achieved by adaptor-
10 specific cargo binding mechanisms (Schroeder et al., 2014; Schroeder and Vale,
11 2016; Gama et al., 2017).

12 The best studied family of cargo adaptors are the BicD proteins. Mammals have two BicD
13 orthologues, BicD1 and BicD2. BicD's form homodimers and based on secondary
14 structure predictions, they fold into the three coiled-coil domains CC1, CC2, and CC3
15 (Figure 1 A). The N-terminal CC1 coiled-coil domain was shown to bind dynactin and
16 dynein via the ARP1 subunit of dynactin and the N-terminus of the dynein heavy chain
17 (Chowdhury et al., 2015; Urnavicius et al., 2015) as well as LIC1 of the dynein motor
18 complex (Schroeder et al., 2014; Lee et al., 2018). These multiple interactions increase
19 the affinity of dynein for dynactin and are required for stable dynein/dynactin/BicD2
20 complex (DDB) formation (Schlager et al., 2014; Splinter et al., 2012; McKenney et al.,
21 2014). It was also shown that the N-terminal region of BicD2 alone is able to induce DDB
22 complex formation with higher efficiency than the full-length protein (Hoogenraad et al.,
23 2003). The CC1 domain of BicD's contains the dynein and dynactin interacting motifs –
24 the CC1 box (Gama et al., 2017; Lee et al., 2018; Celestino et al., 2019) and the spindly
25 motif (Gama et al., 2017; Zhang et al., 2017; Torisawa et al., 2014). The C-terminal
26 domains of BicD's, CC2 and CC3, are usually implicated in cargo recruitment. The BicD1
27 C-terminal CC3 domain can interact with GTP-bound RAB6B (Wanschers et al., 2007;
28 Hoogenraad et al., 2001; Short et al., 2002; Schlager et al., 2010; Matanis et al., 2002)
29 and cytomegalovirus/HHV-5 protein UL32 (Indran et al., 2010). BicD2 is able to recruit
30 RAB6A (Peeters et al., 2013), NEK8 (Holland et al., 2002), DNAI1 (Oates et al., 2013;
31 Peeters et al., 2013), and kinesin KIF5A (Splinter et al., 2010). BicD2 C-terminus is also
32 able to interact with RANBP2 (Splinter et al., 2010) (or NUP358), a component of the
33 nuclear pore complex. The subsequent BicD2 mediated recruitment of dynein-dynactin
34 (Figure 1B) allows the tethering of the centrosome to the nucleus prior to mitosis (Splinter
35 et al., 2010) and drives interkinetic nuclear migration during the development of the
36 human neocortex (Hu et al., 2013; Baffet et al., 2015). It has been shown in vivo and in
37 vitro that BicD2 is recruited to RanBP2 in a CDK1-dependent manner (Baffet et al., 2015)
38 (Figure 1B).

39 Cargo adaptors of the BicD family also interact with themselves. It has been observed by
40 several groups that the C-terminal part is able to make contact with the N-terminal part
41 leading to an autoinhibited state in BicD1 (Terawaki et al., 2015) and BicD (Liu et al.,
42 2013; Wharton and Struhl, 1989) (Figure 1C). There is little known about human BicD2
43 autoinhibition and many features are assumed to be common with the better-studied
44 *Drosophila* BicD ortholog (Sladewski et al., 2018; Stuurman et al., 1999). It has also been

1 suggested that the autoinhibited state can be relieved by cargo binding (Liu et al., 2013;
2 Huynh and Vale, 2017; McClintock et al., 2018; Sladewski et al., 2018). There is evidence
3 of cargo-induced autoinhibition release in *Drosophila* BicD with Egl and mRNA as a cargo
4 (Sladewski et al., 2018). So far no such studies have been done for human full-length
5 BicD1 or BicD2.

6 Understanding the structure, dynamics and reactivity of cargo adaptor proteins is required
7 to advance our understanding of cargo selection, recruitment, and release. Most of the *in*
8 *vitro* reconstitution experiments of the human BicD2 with cargo and/or dynein-dynactin
9 were performed on a truncated protein or the *Drosophila* ortholog. As it is an extensively
10 studied dynein adaptor, there is a need for a protocol for robust production of the full-
11 length human BicD2. The long-standing challenges in characterising cargo adaptors like
12 BicD2 are limited expression levels and solubility which makes them poor targets for
13 structural and functional analyses. Here we describe the recombinant production of
14 human full-length BicD2 and provide novel insights to its *in vitro* behaviour. We validate
15 the dimeric nature and correct folding of our recombinant BicD2. Our results also reveal
16 unexpected pH-dependent conformational changes of BicD2 in the pH range from 6 to 8.
17 We also address the biochemical activity of BicD2 by interaction studies with RanBP2.
18 Our findings lay the ground for further studies on BicD2 reactivity, regulation, and role in
19 motor protein complexes formation.

20

21

22 **RESULTS**

23 **Biochemical characterization of full length BicD2**

24 We succeeded in producing full-length human BicD2 isoform 2 under mild solubilizing
25 conditions (see STAR Methods) and subsequently characterized its biochemical and
26 functional properties. The addition of mild zwitterionic surfactants like CHAPS to the lysis
27 buffer was crucial for obtaining sufficient protein amounts for large scale purifications. Our
28 size-exclusion multi-angle Light scattering (SEC-MALS) analysis indicates a molecular
29 weight of 192 ($\pm 0.7\%$) kDa (Figure S1A), which is slightly larger than the expected value
30 of 186 kDa for a full-length BicD2 dimer. The higher molecular weight is likely caused by
31 the presence of residual CHAPS detergent. BicD2 also elutes at a volume of 1.35 mL on
32 a Superose® 6 Increase 3.2/300 size exclusion column, which is earlier than expected
33 and most likely caused by the increased hydrodynamic radius due to the extended coiled-
34 coil fold of BicD2.

35 We evaluated the effect of buffers on the stability of BicD2 by differential scanning
36 fluorimetry (nanoDSF) to establish an optimal buffer composition. The pH screen revealed
37 that the BicD2 stability varies considerably in the range from pH 6.0 to pH 8.0 (Figure 2A).
38 The melting curves at pH 7.0 and pH 8.0 show clear but distinct inflection points, while
39 the curve at pH 6 indicates partly unfolded protein. Full-length BicD2 carries a net
40 negative charge at all these pH values (Figure S1B). We also performed three SEC runs

1 on full-length BicD2 at pH 6.0, 7.0, and 8.0. The first run after affinity purification at pH
2 7.0 and pH 8.0 was characterized by an elution peak for dimeric BicD2 between 1.3 – 1.4
3 mL and a broad peak of degraded BicD2 around 1.8 mL (Figure S1C). The second SEC
4 run yielded clean BicD2 fractions (Figure 2B). The slightly different SEC elution volumes
5 and the distinct inflection points in the DSC melting curves suggest the possibility of
6 different BicD2 conformations at pH 7.0 and pH 8.0. Moreover, the SEC run at pH 7.0
7 had a significantly higher ratio of full length BicD2 to degraded BicD2 compared to the
8 SEC run at pH 8.0, where degradation proceeds more rapidly (Figure S1C). At pH 6.0
9 BicD2 eluted almost at the end of the column suggesting partial denaturation (Figure 2B).
10 Re-running the BicD2 peak fractions revealed elution peaks at the expected volumes
11 without additional degradation peaks. These results were further confirmed by circular
12 dichroism (CD) experiments (Figure S1D). The spectra for pH 7.0 and pH 8.0 suggest
13 almost pure α -helical content, consistent with the predicted BicD2 fold. The CD spectrum
14 for pH 6.0 does not resemble the α -helical model, but also does not fit into random-coil
15 models. Heating the protein up to 60 °C did not reveal the random coil characteristic
16 peaks (Figure S1E). This suggests that the protein at pH 6.0 adopts a partially unfolded
17 structure.

18 **Influence of pH value on BicD2's conformation and stability**

19 In order to obtain insights into the conformation of BicD2 at pH 7.0 and pH 8.0, we directly
20 visualized BicD2 at these pH values using a cryo-electron microscope. We collected two
21 datasets and applied identical data processing workflows to avoid bias in the
22 interpretation. The representative 2D classes showed that most molecules at pH 8.0 fall
23 into an extended conformation (Figure 2C), whereas the vast majority of BicD2 particles
24 adopt more compact conformations (Figure 2D) at pH 7.0. These results are consistent
25 with the SEC runs where BicD2 elutes at 1.4 mL at pH 7.0 but 1.35 mL at pH 8.0
26 suggesting a shorter hydrodynamic radius at pH 7.0.

27 Next, we wanted to identify amino-acid residues that potentially could be responsible for
28 the pH dependent conformational changes of BicD2. We analysed multiple sequence
29 alignments and focused on conserved histidines as they often have crucial functions in
30 pH-sensitive proteins. An alignment of BicD, BicD1, and BicD2 sequences from all animal
31 kingdoms showed only a poor sequence identity of less than 10%. However, in the C-
32 terminal part of the CC2 domain there is a conserved 'YH' sequence motif and an
33 additionally conserved histidine (H640 in human isoforms) that is present in all vertebrates
34 (Figure 3A). We generated a structure prediction with AlphaFold2 (Jumper et al., 2021)
35 (AF2) for the CC2 and CC3 domains of human BicD2 (Figure 3B) to evaluate where these
36 residues are potentially located within the predicted structure. Our analysis suggests that
37 they are not involved in dimer interactions, but that they are located within the tri-helical
38 interface of H3, H4, and H5. We compared the YH-containing motif of *hsBicD2* (Y538-
39 H539) with its two distant isoforms – *D. melanogaster* BicD and *C. elegans* BicD1. The
40 architecture is very similar for all isoforms. In the case of *hsBicD2*, the additionally
41 conserved histidine residue H640 on helix H5 could interact with residue Y538 on helix

1 H3 (Figure 3C). In order to validate the structure predicted by AF2, we have also
2 compared it to the negative stain class averages reported for *D. melanogaster* BicD
3 (Sladewski et al., 2018) (Figure S2A, B and C).

4 To test if the YH motif and the interhelical tyrosine-histidine pair (Y538-H640) interfere
5 with BicD2's pH dependent behaviour *in-vitro*, we generated the H539A/Y538A and
6 H539A/Y538A/H640A mutants and characterized them by SEC at pH 7.0 and pH 8.0. The
7 fact that the differences in the BicD2 elution peaks do still exist suggests that also the
8 underlying conformational differences are still present. Furthermore, the mutant BicD2
9 proteins are more prone to degradation because the equilibrium between the peaks for
10 full-length BicD2 and its degradation products has shifted towards the degradation
11 products. This effect is more pronounced for the H539A/Y538A/H640A triple mutant
12 (Figure 3D). At pH 7.0 the BicD2 fraction is the most abundant, while at pH8.0 the BicD2
13 fraction significantly decreases, and the protein is prone to degradation. Even though
14 H539/Y538/H640 does not seem to be involved in the pH-induced conformational
15 changes of full-length BicD2, these residues nevertheless are clearly important for the
16 stability of the protein.

17

18 **BicD2's interaction with RanBP2**

19 RanBP2 (or Nup358) is known to recruit BicD2 through a small disordered region between
20 two Ran binding domains (2003-2444) as demonstrated in yeast two-hybrid assays
21 (Splinter et al., 2010). The BicD2 binding domain of RanBP2 (hereafter RanBP2_{BBD})
22 interacts with the C-terminus of BicD2 (715-804) (Gibson et al., 2021) and contains
23 several consensus CDK1 phosphorylation sites, which have been shown to be important
24 for BicD2 binding (Baffet et al., 2015). The individual contribution of these phosphorylation
25 sites to BicD2 binding is unknown. A recent *in-vitro* experiment demonstrated that the
26 RanBP2_{BBD}-BicD2 complex can also be formed in the absence of phosphorylation
27 (Gibson et al., 2021). Since all these experiments used the truncated BicD2 C-terminus,
28 the binding of the full-length BicD2 to RanBP2_{BBD} remains to be characterized. In order
29 to clarify the role of phosphorylation in BicD2- RanBP2_{BBD} complex formation and to
30 confirm the biochemical activity of our recombinant full-length BicD2, we evaluated the
31 interaction with its binding partner RanBP2.

32 We performed double pull-down assays with full length BicD2 and RanBP2_{BBD} as well as
33 with a phosphomimetic variant of RanBP2_{BBD} where the known CDK1 phosphorylation
34 sites had been mutated to aspartates (T2153D, S2246D, S2251D, S2276D, and
35 S2280D). We were able to obtain a BicD2-RanBP2_{BBD} complex in both cases (Figure
36 S3A). However, subsequent SEC runs of the purified complexes indicated that they are
37 not stable and that they dissociate. In order to investigate if RanBP2_{BBD} phosphorylation
38 leads to more stable complex formation, we produced recombinant CDK1 (Veld et al.,
39 2021) and performed an *in vitro* kinase assay on RanBP2_{BBD}. The mass spectrometry
40 analysis revealed eight S/T consensus sites in the BicD2 binding region of RanBP2 of

1 which five overlapped with earlier findings (Baffet et al., 2015) (Figure S3B). We mixed *in*
2 *vitro* phosphorylated or non-phosphorylated RanBP2_{BBD} with full length BicD2 and
3 analysed the samples by SEC. Successful complex formation was only observed for *in*
4 *vitro* phosphorylated RanBP2 (Figure 4A). Next, we investigated if BicD2 can be
5 phosphorylated by CDK1 and if such a modification could potentially strengthen complex
6 formation with RanBP2_{BBD}. The mass spectrometry analysis after BicD2 *in vitro*
7 phosphorylation indicated five phosphorylation sites in regions predicted to be structurally
8 disordered demonstrating that BicD2 can be phosphorylated by CDK1 (Figure S3B). We
9 mixed the *in vitro* phosphorylated BicD2 with non-modified RanBP2_{BBD} and subsequently
10 analysed the sample by SEC. There was no indication of complex formation (Figure 4A).
11 The equivalent experiment with *in vitro* phosphorylated RanBP2_{BBD} did not show any
12 evidence of increased BicD2-RanBP2_{BBD} complex formation compared to non-modified
13 BicD2 – *in vitro* phosphorylated RanBP2 SEC run (Figure 4A). These results suggest that
14 BicD2 phosphorylation does not contribute to the BicD2-RanBP2_{BBD} complex formation.

15 We also analysed the influence of pH on BicD2-RanBP2_{BBD} complex formation. To this
16 end, we mixed full-length BicD2, RanBP2_{BBD} and CDK1 at pH 7.0 and pH 8.0 to induce
17 complex formation and analysed the samples by SEC. We also carried out the
18 corresponding control experiments in the absence of CDK1 (Figure 4B). From the
19 fractions analysed by SDS-PAGE, it is evident that complex formation is enhanced with
20 CDK1-treated samples. Interestingly, the two runs at pH 7.0 (with and without CDK1)
21 showed a broad distribution of RanBP2_{BBD} throughout the elution and only minor increase
22 in the RanBP2 intensity at BicD2 peak fractions in the presence of CDK1. Taken together,
23 these observations suggest that the BicD2-RanBP2_{BBD} complex forms best under slightly
24 alkaline pH which narrows the pool of RanBP2_{BBD} species.

25

26 DISCUSSION

27 The ability of Dynein-1 to bind a wide range of cargoes is mediated by its compatibility
28 with many cargo adaptor proteins, such as BicDs, Hooks, NINs, Spindly, TRAKs, RILP or
29 JIP3 (Olenick and Holzbaur, 2019). Investigating *in vitro* reconstituted complexes
30 between cargo adaptors and dynein/dynactin has led to valuable insights into dynein
31 motor recruitment and activation (Terawaki et al., 2015; Liu et al., 2013; Noell et al., 2019).
32 However most of these studies were done with truncated BicD2 constructs that lack either
33 the dynein or the cargo -binding domains. Many biophysical and structural studies have
34 been hampered by the lack of sufficient amounts of full-length cargo adaptors. Here we
35 report a robust method for the recombinant production of a full-length BicD2 and
36 demonstrate its correct fold and biochemical activity towards its RanBP2 cargo. We
37 believe that other full-length cargo adaptors might benefit from the expression and
38 purification protocols described here and that our results will facilitate *in vitro*
39 reconstitution approaches of motor-cargo complexes.

1 One of the unexpected findings was the strong influence of the pH value on the
2 conformation and stability of full-length BicD2. At pH 6.0, 7.0 and 8.0 BicD2 switches from
3 partially unfolded to compact and to an open conformation. Our cryoEM 2D classification
4 of full-length BicD2 at pH 7.0 indicates that over 50% of the particles fall into compact
5 classes while the majority of particles at pH 8.0 adopt an open conformation. Recent work
6 has provided evidence that the autoinhibited state is characterized by an interaction
7 between the CC2 and CC3 domains (Sladewski et al., 2018). Although we were not able
8 to assign individual coiled-coil domains in our cryoEM 2D class averages due to the
9 intrinsic flexibility of BicD2, there is the possibility that the more compact 2D class
10 averages at pH 7.0 might represent the autoinhibited state and the extended conformation
11 at pH 8.0 the open, activated state. A similar autoinhibited conformation has also been
12 recently described for cargo adaptor Spindly (d'Amico et al., 2022) which suggest that
13 autoinhibition might be a common feature of cargo adaptors.

14 It is not clear if these pH driven conformational changes are relevant only *in vitro* or if they
15 also have a physiological relevance. In principle, the cellular pH value could also trigger
16 pH dependent conformational changes of the BicD2 cargo adaptor. At the cytosolic pH
17 value of ~7.2 most BicD2 molecules would adapt the compact, potentially autoinhibited
18 form and require additional, activating signals to recruit motor proteins and cargo.
19 Conditions that lead to an increase in the intracellular pH, like mitosis and growth factor
20 signalling (Gagliardi and Shain, 2013; Pouyssegur et al., 1985; Chambard and
21 Pouyssegur, 1986), could bypass the need for such signals and activate the BicD2 cargo
22 adaptor on a globular level. Adaptor autoinhibition is not a BicD2-unique phenomenon. A
23 recent study describes a similar behavior for the dynein-1 adaptor Spindly (d'Amico et al.,
24 2022). Cargo adaptors alone are not extensively studied but our work and the one
25 presented by D'Amico et al., (2022) suggests that this behavior might be common for
26 some cargo adaptors and play a role in recruitment of partner proteins.

27 In the case of BicD2 one of the activating signals is believed to be the interaction with its
28 cargos like RanBP2 (Liu et al., 2013; Sladewski et al., 2018; Huynh and Vale, 2017;
29 McClintock et al., 2018). Furthermore, post-translational BicD2 modifications might also
30 influence its reactivity. BicD2 contains several consensus phosphorylation sites (Holland
31 et al., 2002) and an acetylation site in its N-terminal part (Van Damme et al., 2012).

32 We also have identified three conserved residues, H539/Y538/H640 that contribute to the
33 stability of BicD2. Mutating these amino-acid residues to alanines clearly increased the
34 tendency of BicD2 for degradation, which could be caused by auto-cleavage or residual
35 protease amounts in the sample. Our template-free AF2 prediction suggest the possibility
36 of Y538/H640 stabilizing the H3-H4-H5 α -helical bundle via an intramolecular hydrogen
37 bond (Figure S2D). Disrupting it could trigger partial unfolding and subsequent protein
38 degradation. A similar architecture (Figure S2D) was also observed in a pH-sensitive
39 endotoxins and mutating the equivalent histidine-tyrosine pair mutation lead to protein
40 destabilization and degradation (Seale, 2006).

1 We characterized the BicD2 reactivity with its known cargo protein RanBP2. There are
2 conflicting results in the field with respect to the question whether RanBP2
3 phosphorylation is a requirement for the interaction with BicD2 (Baffet et al., 2015; Gibson
4 et al., 2021). Our results now reconcile these reports, because they show that BicD2 can
5 bind both non-phosphorylated as well as phosphorylated RanBP2_{DDB}. In double pull-
6 downs we observed that BicD2 readily interacts with RanBP2 without any protein
7 modification at pH 7.0 and 8.0. These complexes are nevertheless unstable over SEC.
8 This suggests that there is a weak association of these proteins *in vitro* in a wide range
9 of conditions. The CDK1 phosphorylation of RanBP2_{BBD} (but not BicD2) significantly
10 increased the stability of the complex and increase in the pH value improved the complex
11 homogeneity.

12

13 **ACKNOWLEDGEMENT**

14 This research was supported by a LabEx start up grant (ANR-10-LABEX-30-HS) to HS
15 and Boehringer Ingelheim Fonds as well as FRM PhD fellowships to RF. This study was
16 further supported by the grant ANR-10-LABX-0030-INRT, a French State fund managed
17 by the Agence Nationale de la Recherche under the frame program Investissements
18 d'Avenir ANR-10-IDEX-0002-02. The authors acknowledge the support and the use of
19 resources of the French Infrastructure for Integrated Structural Biology (FRISBI) ANR-10-
20 INBS-05 and of Instruct-ERIC. We also thank the IGBMC molecular biology and virus
21 service (Nicole Jung and Thierry Lerouge). This work of the Interdisciplinary Thematic
22 Institute IMCBio, as part of the ITI 2021-2028 program of the University of Strasbourg,
23 CNRS and Inserm, was supported by IdEx Unistra (ANR-10-IDEX-0002), and by SFRI-
24 STRAT'US project (ANR 20-SFRI-0012) and EUR IMCBio (ANR-17-EURE-0023) under
25 the framework of the French Investments for the Future Program.

26

27

28

29 **AUTHOR CONTRIBUTIONS**

30 RF: conceptualization, data curation and analysis, validation, investigation, methodology,
31 funding acquisition and manuscript writing. CC: methodology, investigation and data
32 analysis. TK: methodology, investigation and data analysis. CD & BF: data analysis and
33 discussion. YN, JB and PR: investigation and data analysis. HS: conceptualization, data
34 analysis, supervision, funding acquisition, methodology, manuscript writing and project
35 administration.

36

37 **DECLARATION OF INTEREST**

1 The authors declare no competing interests.

2

3 **STAR METHODS**

4 **RESOURCE AVAILABILITY**

5 ***Lead contact***

6 Further information and requests for resources and reagents should be directed to and will be
7 fulfilled by the lead contact, Helgo Schmidt (schmidth@igbmc.fr)

8

9 ***Materials availability***

10 Plasmids generated in this study are available upon request.

11

12 ***Data and code availability***

13 No cryoEM or x-ray crystal structures were generated in this study. This paper does not report
14 original code. Any additional information required to re-analyze the data reported in this paper is
15 available from the lead contact upon request.

16

17 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

18 *E. coli* BL21(DE3) cell were obtained from New England Biolabs, product number C2527H. The
19 Sf9 insect cells used in this study were obtained from Sigma-Aldrich, product number 71104-M.

20

21 **METHOD DETAILS**

22 ***Cloning and mutagenesis***

23 Human BicD2 isoform 2 and RanBP_{BBD} genes were ordered from Epoch LifeScience and
24 codon-optimized for *Escherichia coli* expression. The genes were subsequently cloned
25 into the pNHD expression vector, a vector used for site-specific pSer incorporation
26 (Rogerson et al., 2015), but both proteins express equally well in a commercial pMMS
27 vector). The BicD2 plasmid was modified to introduce an N-terminal STREP-tag followed
28 by a short 'GSGSG' linker. The RanBP_{BBD} plasmid was fused with a C-terminal double
29 TEV cleavage site, a GSGSG linker, and a 6xHis tag. The H539A/Y538A and
30 H539A/Y538A/H640A mutants were generated by rolling circle PCR. The co-expression
31 plasmid encoding CDK1-cyclinB for insect cell expression was a kind gift from the Andrea
32 Musacchio lab.

33 ***Protein expression and purification***

1 Plasmids for bacterial expression were transformed into *E. coli* BL21 (DE3) chemically
2 competent cells and grown overnight on tetracycline plates. The plate was used for
3 inoculation of the 50 mL LB medium pre-culture which was grown overnight at 37°C for
4 further scale-up. Protein expression was induced at 25°C with 1mM isopropyl-1-thio-β-D-
5 galactopyranoside (IPTG) and incubated for 3h at 37°C. Cells were harvested, washed
6 with 1xPBS, and frozen. BicD2 WT and mutants were re-suspended in lysis buffer
7 containing 1xPBS pH8.0, 10% glycerol, 7 mM CHAPS, 2 mM DTT, 2mM PMSF and
8 cOmplete EDTA-free protease inhibitor cocktail tablets (Roche), and incubated on a roller
9 for 1-2h at 4°C. Cells were lysed by sonication (5x30s with 30s breaks on ice) and
10 centrifuged at 30000 rpm at 4°C for 1 hour. The supernatant was mixed with pre-
11 equilibrated STREP-tactin 4XL beads and incubated on a roller for 30 minutes at 4°C
12 before loading on a gravity flow column. The resin was washed with 10 CVs of the lysis
13 buffer, 10 CVs of the wash buffer (1xPBS pH8.0, 10% glycerol, 7 mM CHAPS), and eluted
14 with elution buffer (1xPBS pH8.0, 10% glycerol, 7 mM CHAPS, 50 mM biotin). Pooled
15 fractions were concentrated to 2 mg/mL and snap-frozen in 100 μL aliquots for further
16 experiments and gel filtration. RanBP2_{BBD} was purified in the same manner as BicD2 with
17 a difference in the buffer used (20 mM Tris-HCl pH8.0 instead of PBS). Both constructs
18 (WT) yielded around 1 mg of protein per litre of culture after the affinity purification step.

19 The CDK1^{CyclinB} plasmid was transformed into *E. coli* DH10MB-MCherry chemically
20 competent cells with a heat shock at 42°C for 30s followed by 6h recovery in LB medium
21 at 37°C with shaking. The cells were then plated on agar plates containing kanamycin (50
22 μg ml⁻¹), gentamicin (7 μg ml⁻¹), tetracycline (10 μg ml⁻¹), Xgal (600 μg ml⁻¹) and IPTG
23 (40 μg ml⁻¹) and positive clones were identified by blue/white selection after 24h
24 incubation at 37°C followed by 24h at RT. The positive clones were grown in LB media
25 containing kanamycin (50 μg ml⁻¹), gentamicin (7 μg ml⁻¹), and tetracycline (10 μg ml⁻¹)
26 overnight. Bacmids were purified using the isopropanol precipitation method. 2ml of Sf9
27 cells at 0.5×10⁶ cells per ml were transfected with 2 μg of fresh bacmid DNA and FuGene
28 HD transfection reagent (Promega) at a ratio of 3:1 transfection reagent to DNA. After
29 72h the 6-well plate was analysed for mCherry fluorescence signal, and the positive
30 conditions (V₀) were directly used for further virus amplifications by adding 2mL of V₀ to
31 50 mL of Sf9 cells at 1x10⁶ cells per mL (V₁) and grown for 72h. The V₁ was then used
32 for infecting another 50 mL of Sf9 at 1x10⁶ cells/mL (V₂). The V₂ virus was further used
33 to infect 500 mL of the Sf9 culture 1x10⁶ cells/mL. After 72h the cells were collected at
34 1000 rpm for 10 min at 4°C. The pellet was flash-frozen in liquid nitrogen and stored at -
35 80°C until purification. The CDK1^{CyclinB} pellet was re-suspended in the lysis buffer
36 containing 50mM Tris-HCl pH8.0, 10mM MgCl₂, 10% glycerol, 0.1% NP40, 0.1 mM EDTA,
37 2mM DTT, 2 mM PMSF, protease inhibitors, and incubated on a roller for 1h at 4°C. The
38 cells were lysed by sonication (2x30s with 30s breaks on ice) and centrifuged at 40000
39 rpm at 4°C for 1 hour. The supernatant was mixed with pre-equilibrated GST-sepharose
40 beads for 30 minutes at RT and loaded onto a gravity flow column. The resin was washed
41 with 10 CV of the lysis buffer, 10 CV of the wash buffer (Tris-HCl pH8.0, 150mM NaCl,
42 10% glycerol, 0.1mM EDTA), and eluted with elution buffer (Tris-HCl pH8.0, 150mM

1 NaCl, 10% glycerol, 0.1mM EDTA, 25mM reduced GST). Pooled fractions were
2 concentrated to 0.5 mg/mL and snap-frozen in 50 μ L aliquots for further experiments.

3

4 **SEC-MALS**

5 Prior to SEC-MALS analysis, the BicD2 dimer was purified on Superose[®] 6 Increase
6 3.2/300 column in SEC buffer (50 mM Tris pH 7.5, 150 mM Potassium acetate, 10 mM
7 Magnesium acetate, 7 mM CHAPS). 90 μ L of BicD2 sample (1mg/mL) was injected into
8 FPLC Ettan Micro LC (formerly GE Healthcare, now Cytiva) coupled with MiniDAWN
9 TREOS MALS detector (Wyatt Technology), Optilab T-rEX (Wyatt Technology) RI
10 detector, and Superdex[®] S200 10/300 GL (formerly GE Healthcare, now Cytiva) column
11 and run at 25 $^{\circ}$ C in SEC buffer (50 mM Tris pH 7.5, 150 mM Potassium acetate, 10 mM
12 Magnesium acetate). The data was processed using the ASTRA software (Wyatt
13 Technology).

14

15 **Circular dichroism**

16 CD experiments were recorded on a Jasco J-815 spectropolarimeter (Easton, MD)
17 equipped with an automatic 6-position Peltier thermostated cell holder. The instrument
18 was calibrated with 10-camphorsulphonic acid. Samples (65 μ L) were prepared in PBS
19 supplemented with CHAPS 7 mM and buffered at various pH (6.0, 7.0 or 8.0). Far-UV CD
20 data were collected in the 182–270 nm range using a 0.1 mm pathlength cell (Quartz-
21 Suprasil, Hellma UK Ltd) at 20.0 $^{\circ}$ C \pm 0.1 $^{\circ}$ C. Spectra were acquired using a continuous
22 scan rate of 50 nm/min and are presented as an average of 10 successive scans. The
23 response time and the bandwidth were 1.0 s and 1 nm, respectively. The spectra were
24 corrected by subtracting the solvent spectrum obtained under identical conditions.

25

26 **CryoEM sample preparation**

27 A 100 μ L BicD2 sample was thawed on ice and equilibrated with 5xPBS at either pH 8.0
28 or pH 7.0. The Superose[®] 6 Increase column was equilibrated for 16h at a specific pH (in
29 PBS buffer only) and the dimer fraction from the SEC taken for grid preparation. Cu/Rh
30 1.2/1.3 300mesh grids were plasma cleaned for 90s at 30% plasma power (80:20
31 argon:oxygen). The protein concentration was adjusted to 1 μ M and applied on the grid
32 and plunge-frozen into liquid ethane in a Vitrobot Mark IV robot (FEI), maintained at 100%
33 humidity and 10 $^{\circ}$ C.

34

35 **CryoEM data collection and image analysis**

36 Datasets for BicD2 at pH 8.0 and pH 7.0 were collected using a Glacios[™] Cryo-TEM
37 operating at 200keV with a K2 Summit direct electron detector (Gatan). Videos were

1 collected in counting mode, with a final calibrated pixel size of 1.078 Å/pixel, 8s exposure,
2 and total dose of $\sim 54 \text{ e}^-/\text{Å}^2$.

3 SerialEM (Mastronarde, 2005) was used for automated data collection and the videos
4 were processed using Relion3.0 (Zivanov et al., 2018). The micrographs were manually
5 filtered giving each dataset ~ 700 micrographs. Particles were picked using the general
6 model in TOPAZ (Bepler et al., 2019) and 2D classified twice. Each dataset yielded
7 around 150k particles used for 2D classification. All data processing steps were done
8 reference-free.

9

10 ***In vitro kinase assays and phosphorylation mapping***

11 BicD2 or RanBP2_{BBD} were mixed with CDK1 at 1:100 molar ratio with 0.1mM MgATP and
12 incubated for 30min at RT. Each sample had a negative control that did not contain
13 MgATP. Each reaction mixture was loaded on SDS-PAGE and stained with pro-Q
14 diamond stain to check for phosphorylated proteins. Phosphorylation sites were analysed
15 with trypsin digest MS using an Orbitrap Elite and Acclaim Pepmap 100 column. The PSM
16 values corresponded to the number of MS2 spectra which made it possible to identify
17 peptides. In the case of BicD2 75% of the sequence were covered (PSM's: 2087) and for
18 RanBP2 85% of the sequence were covered (PSM's: 1195).

19 ***AlphFold2 structure prediction***

20 We used the whole CC2 and CC3 domain of a human BicD2 (residues 265-855) to model
21 a homooligomer. We generated 5 models using the AlphaFold2 (Jumper et al., 2021)
22 advanced notebook from Google Colab. We used MMseqs2 (Steinegger and Söding,
23 2017) (UniRef+Environmental) for MSA generation.

24

25 **QUANTIFICATION AND STATISTICAL ANALYSIS**

26 No quantification or statistical analysis was carried out in this study.

27

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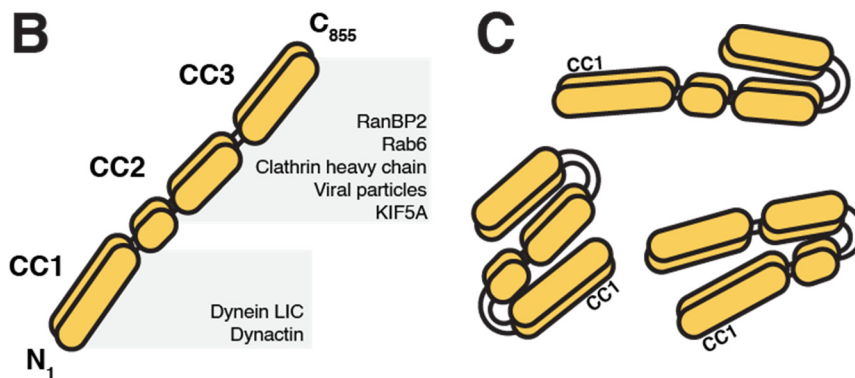
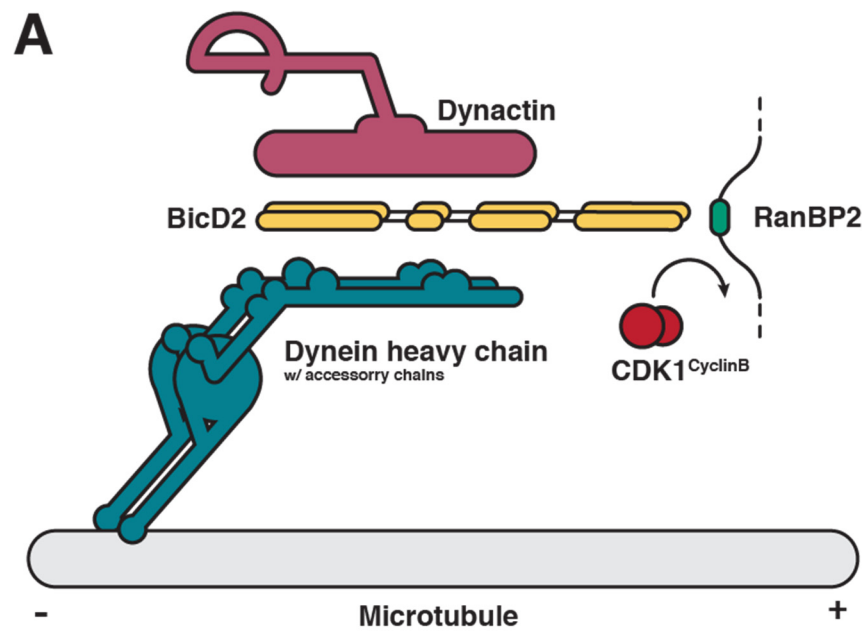


Figure 1. Schematic representation of BicD2 domain organization and function. (A) Domain organization of the BicD2 cargo adaptor dimer. The N-terminal part is responsible for binding dynein and dynactin while the C-terminal part binds the plus-end directed motor kinesin-1 and cargos like RanBP2. (B) BicD2 associates with its RanBP2 nuclear pore cargo and dynein-dynactin. The CDK1^{CyclinB} mediated phosphorylation of RanBP2 triggers the binding of BicD2 to recruit dynein-dynactin to the nucleus during interkinetic nuclear migration in the developing neocortex. (C) Hypothetical BicD2 autoinhibited conformations from the previously reported data.

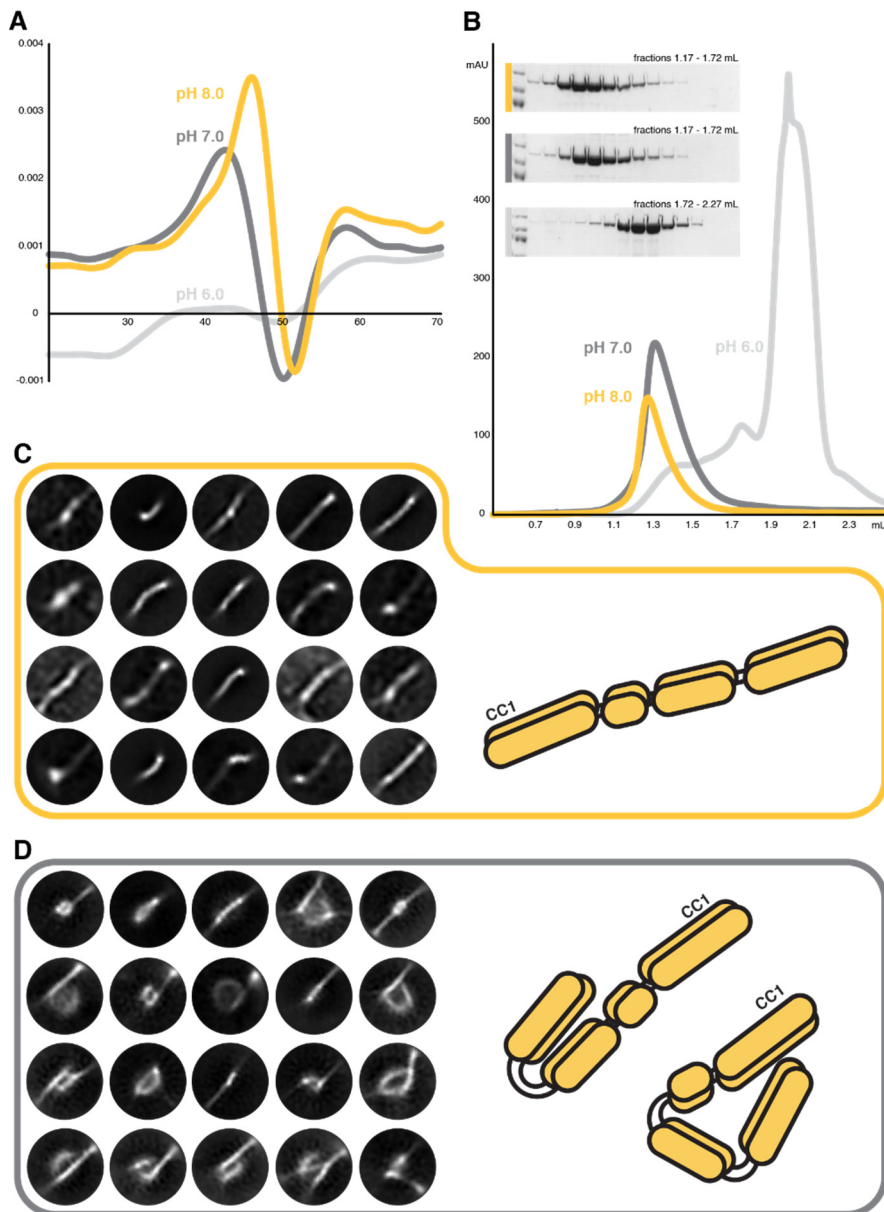


Figure 2. Biochemical and structural characterization of human full-length BicD2 at different pH values. (A) First derivative of the DSF melting curves at three different pH values. The curves at pH 7.0 and pH 8.0 display main inflection point in the range of 47-50°C. The curve at pH 6.0 does not show a distinct inflection point. Each measurement was repeated 3 times. (B) SEC profiles of BicD2 run at three different pH values. Each sample was run twice on a Superose® 6 Increase column to ensure clean peak fractions. (C) and (D) Representative cryoEM 2D classes from ~700 micrographs (per pH condition). The yellow box represents classes from pH 8.0 while the grey box from pH 7.0. In both cases the data was processed with Relion3.1. See also Figures S1 and S4.

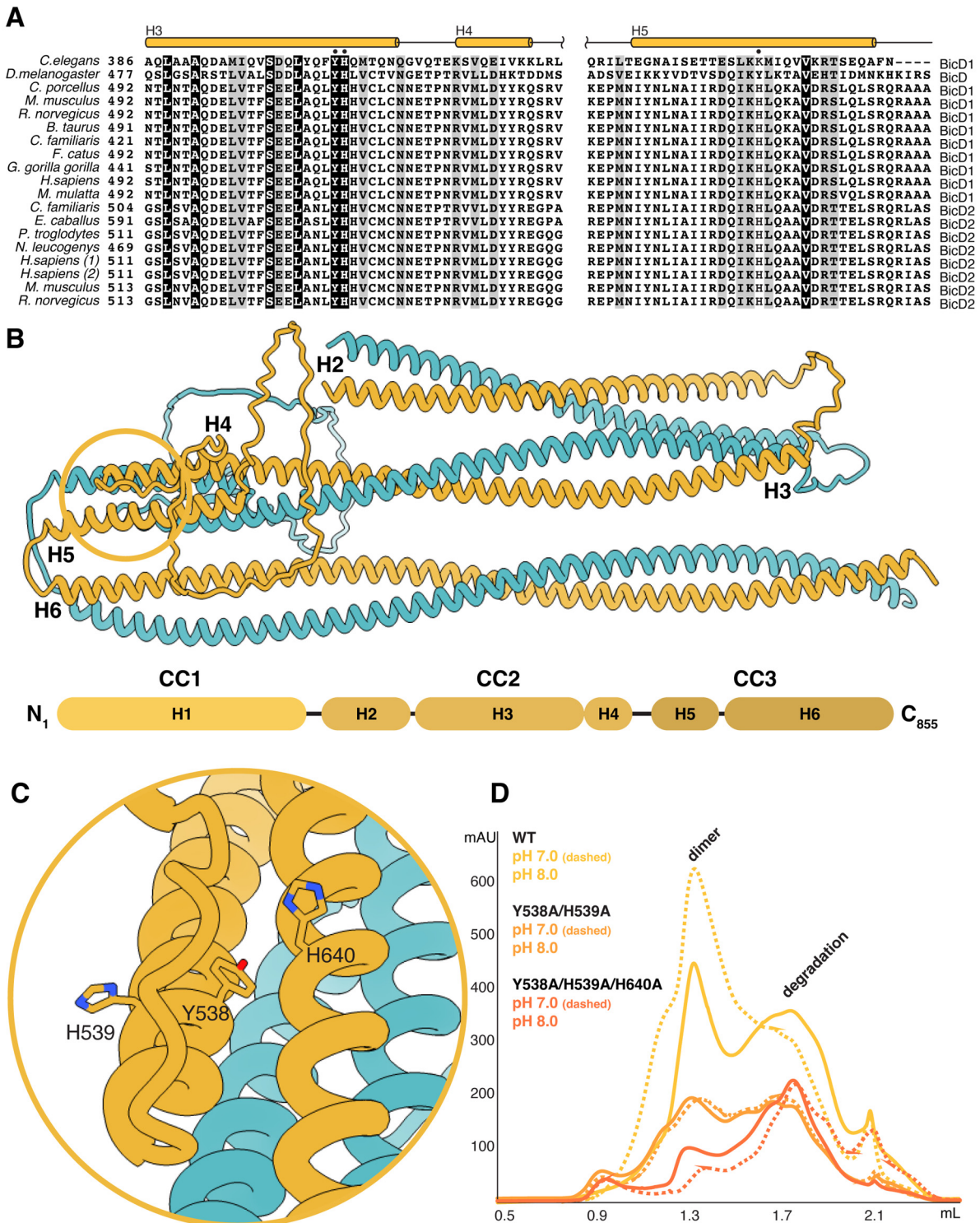


Figure 3. The conserved amino-acid residues Y538, H539 and H640 contribute to BicD2 stability. (A) Sequence alignment of the tri-helical domain (H3, H4, and H5 helix) of *D. melanogaster*, *C. elegans*, and mammalian BicD1 and BicD2 isoforms. The conserved amino-acid residues Y538, H539 and H640 are marked by black dots. (B) Structure of the BicD2 CC2 and CC3 dimer predicted by AlphaFold2. The C-terminal loop of CC3 has been deleted for clarity. (C) Enlarged view of the conserved histidine and tyrosine residues that are crucial for protein stability. (D) Chromatograms of affinity purified BicD2 variants. Yellow - WT, orange - Y538A/H539A double mutant, dark

orange - Y538A/H539A/H640A triple mutant. In all chromatograms the solid line represents pH8.0, and the dashed line pH 7.0. For each construct an identical protein amount was loaded. See also Figure S2.

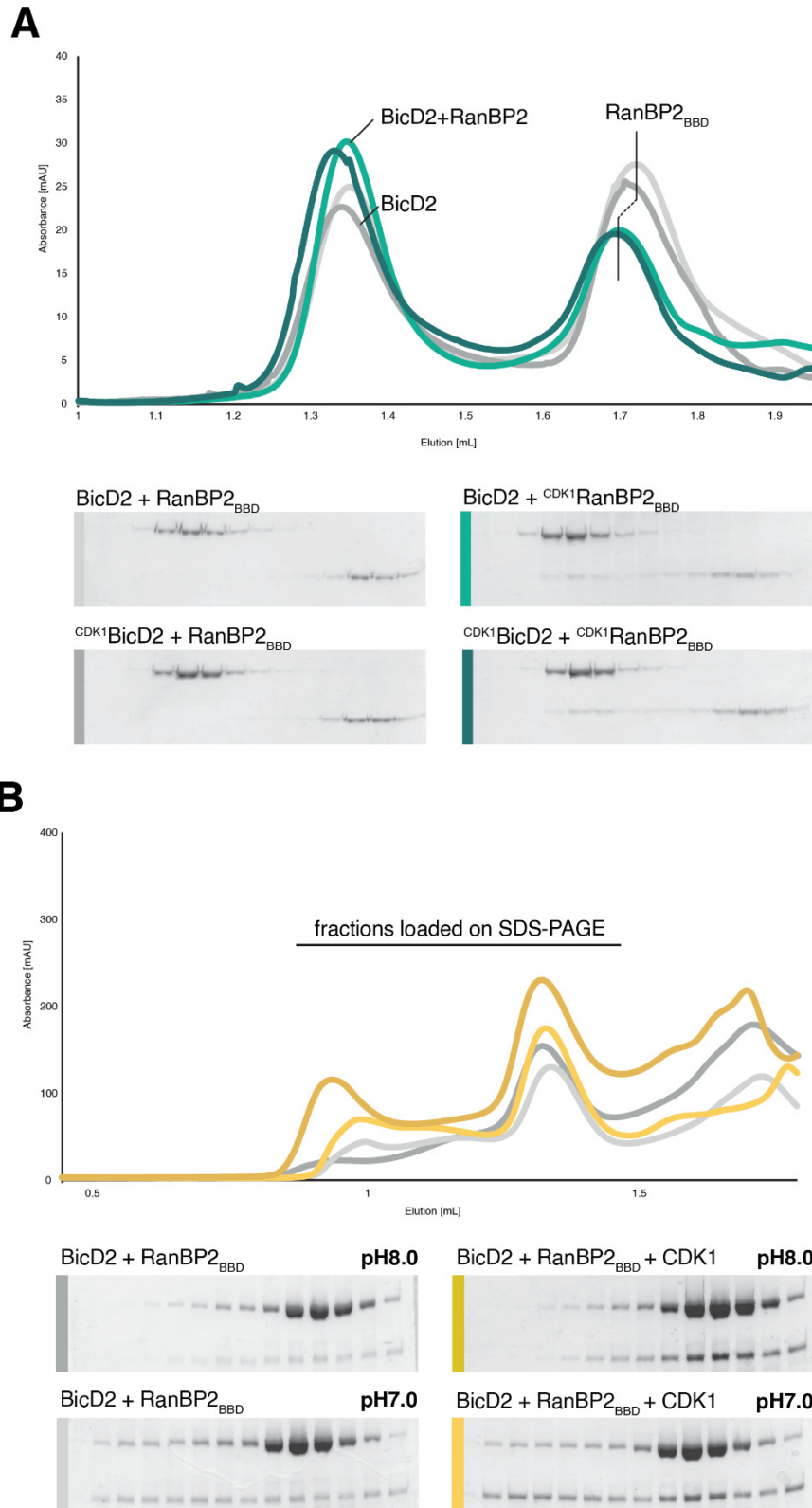


Figure 4. SEC profiles of the BicD2 reaction with RanBP2_{BBD} in the absence and presence of CDK1^{CyclinB}. (A) Purified proteins were mixed at a 1:1 molar ratio and incubated for 30 minutes at RT. In light grey a chromatogram of

unphosphorylated BicD2 and unphosphorylated RanBP2_{BBD}. In dark grey CDK1^{CyclinB} phosphorylated BicD2 with unphosphorylated RanBP2. In light green unphosphorylated BicD2 with CDK1^{CyclinB} phosphorylated RanBP2, and dark green represents both proteins being CDK1^{CyclinB} phosphorylated. All reactions were performed at pH 8.0. (B) Pull-down of affinity purified BicD2, RanBP2_{BBD}, at two different pH values with or without CDK1^{CyclinB}. The dark yellow chromatogram represents the BicD2, RanBP2_{BBD}, and CDK1^{CyclinB} pull-down at pH 8.0, while light yellow shows the same reaction but at pH 7.0. Dark and light grey chromatogram show the same reactions but without activity of CDK1^{CyclinB} complex (at pH 8.0 and pH 7.0 respectively). See also Figures S3 and S4.