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Biochemically validated structural model of the 15-subunit intraflagellar transport complex IFT-B

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Dear Esben,

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by two referees. I had involved a third referee, but as I haven't heard back from this referee, I will move forward with the two reports that we have on hand.

As you can see from the comments below, the referees find the analysis interesting and support publication here. They raise several issues that I would like to ask you to address in a revised version. I think it would be helpful to discuss the comments further and I am available to do so via email or a video call. Let me know what works best for you.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

I thank you for the opportunity to consider your work for publication and I look forward to discussing the revisions further with you.

Yours sincerely,

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

I have attached a PDF with helpful tips on how to prepare the revised version.

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (18th Dec 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. .

Use the link below to submit your revision:

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Referee #1:

This manuscript by Petriman and colleagues synthesizes a large amount of experimental data and alpha fold predictions to produce a plausible model of the IFT-B complex. The paper is a beautiful example of the power of these approaches. It is certain the model has significant shortcomings, but that is expected from this kind of work and does not detract from its importance in any way. The only significant point that I think should be addressed is that the mapping of the ciliopathy mutations onto the structure and the corresponding discussion is incomplete. It is clear from mouse mutations that complete loss of many of the IFT-B proteins yields lethality at about the time of blood flow. In humans, this would be long before the embryos developed to the point of having any of the syndromes described. The map in 9B is a modern version of the classic image of WWII planes that survived bullet strikes to make it back to base. These residues are important as they yield syndromic disease, but they are the sites that can be mutated and still produce a particle that functions well enough to get the embryo through early development. The residues without mutational hits could be unimportant or they could be fundamental to complex formation.

Minor points:
This sentence is a challenge: Given the significant flexibility of the IFT-B complex in solution and the fact that the structural model of the 15-subunit IFT-B complex presented here was assembled by superposing smaller sub-complexes without the context of the IFT train, it is not surprising that a rigid-body manually fit the IFT-B 15mer into the cryo-ET map of anterograde

IFT trains (van den Hoek et al., 2022) resulted in several subunits located outside of the density (data not shown).

Referee #2:

IFT (intraflagella transporter) is a protein complex, which carries cargos from cytoplasm to cilia or from the ciliary tip to cytoplasm. IFT consists of two major complexes, called IFT-A and IFT-B, which make tandem arrays and move between axonemal doublet microtubules and ciliary membrane. Mutation of component proteins of IFT causes defect of cilia growth and thus diseases with various symptoms (ciliopathy). Structure of IFT has been an active topic attracting molecular and cellular biologists interested in cilia and ciliopathy.
IFT-B, which makes an array to be a backbone of anterograde IFT, consists of a number of proteins. Petriman and colleagues modeled the core of this complex, including 15 subunits. Out of the components, several proteins and sub-complexes, such as IFT70/52 and IFt52/46 (Taschner et al. 2014)), IFT25/27 (Bhogaraju et al. (2011)), IFT22/74/81 (Wachter et al. (2019)), IFT80 (Taschner et al (2018)), the CH domain of IFT54 (Weber et al. (2016)), were solved using X-ray crystallography by the same group. They also revealed connection of IFT172/80/57/54/38/20 biochemically (Taschner et al. (2016)). However, an atomic picture of the entire IFT-B complex is still unclear.
In this work, the authors employed alphafold2 program, especially newly developed algorithm of AlphaFold-multimer to predict structures of unsolved components and complex formation. They conducted cross-linking MS experiments as well as pull-down, crystallography and photo-crosslinking to validate predicted complex structures. Theoretical prediction and experimental results were consistent with each other in most cases. When they found contradiction between AF2 and MS results, they carried out biochemical assay to examine which is likely in solution. They succeeded to build an atomic model of IFT-B core particles consisting of 15 proteins. They fit the modeled complex structure to in situ structure of IFT by cryo-electron tomography.
The structure is presented impressively. Together with recent structural works on the IFT-A complex, this work will be a milestone of cilia research. However, there are a few unclearness who the authors reached the final model. This reviewer would ask the authors to clarify these points.

Major points:
Complex of IFT57-CH/IFT172/IFT20/IFT34/IFT80: In their model, IFT57 is folded in extremely extended form (Figs.8&9). The CH domain is far away from the CC domain and located at the end of the whole complex. While the interaction between IFT57-CH and IFT172 was proved by pull-down assay in their past work (Taschner et al. 2016), proof of other connection is not clear (experimentally nor by AF2). There should be an evidence that IFT172 is in proximity to the C-termini of IFT20 and 54. Otherwise it will be weak to conclude with this model, since the CC and CH domains of IFT57 (also IFT54) are connected with disordered structure according to their AF2 analysis. The same should be for IFT80 and the C-terminus of this CC. Could you clarify the logic how these proteins should be complexed in this way?
P.14-15 Second conformation between IFT81/74 helices and IFt52/88/70: To prove/disprove existence of two modes of complex formation (the one in Fig.4 after crosslink and the one in Fig.8), the current AF analysis does not sound convincing enough. The most straightforward way is single particle EM, either cryo or negative stain, and classification to subaverages. Since they formed complexes for MS, they may have done EM. If so, it is worth looking into subaverages to find a sign of heterogeneity. Otherwise this part is rather discussion than conclusion. Besides, the authors could present their AF analysis here more clearly - can they show the 5 AF models (p.15, line 10) in a supplementary figure and explain what are 15 resulting models (line13)?
p.11 Line2-4, Fig.2A "...(Figure 2A). The data show that IFT27/25 and IFT22 have ....a high degree of conformational flexibility ...": The basis of this conclusion is not clear. Which part of Fig2A does "between the N- and C-terminal halves" mean? Is there any factor (such as pLDDT) showing local flexibility?
One general issue is that the modeling was done based on AlphaFold-multimer, which is still in BioRXiv. It will be worth stating the authors thought how trustful this program is for such projects.

Minor points:
Abstract "The large MDa IFT-B complex ....": Probably this sentence should be "The large IFT-B complex".
p.9, line10 "different structural models produced by AF have very similar local structures...": This reviewer could not follow. Please clarify what and what are different and what and what are similar to each other.
p.13, line16 "given that IFT52C/46C is connected to IFT70 via a 4-residue ....with no non-covalent interactions ....": This reviewer could not understand. More explanation needed. Maybe marking the 4-residue covalent linker in Fig.3E helps.
Do the authors have any thought about absence of dimerization of IFT80 in this modeling?
Supplementary materials 1&2: It will be helpful for readers to have an overview of consistency between crosslink and AF, if they show the distance between the pairs of crosslinked residues in the AF-based complex model.
An introductory panel to show which component proteins (and sub-complexes) in this project have been solved in the past and which proteins are known binding to each other.

Hi Esben,

I have received the third report on your study - see below. Most of the points raised are discussion points and I would like to ask you take them into consideration when submitting the revised version.

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Referee #3

This manuscript combines crystallographic and biophysical work from the Lorentzen lab during the past decade with recent AI development in protein structure predictions to produce a complete model of the 15-subunit IFT-B complex. The structure of IFT-B has remained one of the holy grails of structural biology since the complex was discovered in the late 90's and the current manuscript will likely constitute the go-to resource for any researcher interested in IFT-B structure in the future. The work is thorough, methodical and logically organized and there is little to fault in the experimental strategy or quality. The strength of the approach lies in the combination of computational predictions via AlphaFold with biophysical validations, mainly crosslink/mass spec but also protein interaction assays, SAXS and cystallography.
This manuscript does not make for an entertaining light read (at least for non-structural biologists) but it delivers a solid model of the IFT-B complex and the lasting impact of this contribution is without doubt.

As the work is entirely descriptive, the biological significance can at point be difficult to discern for the non-specialist. In my opinion (which can be ignored at will), the paper would benefit from a greater emphasis on the more speculative aspects, chiefly the two conformations of IFT-B and the association with RabL2 and the BBSome.

Main Comments
1- The main point of novelty in the paper is the existence of two possible configurations for IFT-B: one anterograde, one retrograde? The notion of a second IFT-B1 confirmation is interesting but as the interaction surface between IFT74/81 and IFT88 is entirely distinct from that between IFT74/81 and IFT52/46, does one need to invoke a second conformation? Could both interaction take place at the same time in one IFT-B conformer? This question is addressed through a clever modeling strategy that failed to find models where both interactions are satisfied. Nonetheless, given the flexible hinge in IFT46/52 between the domain that associate with IFT70 and those that contact IFT74/81, it would seem that a conformer with both binding modes should be feasible.

2- Foundational work by Xueliang Zhu & Xiumin Yan (EMBO 2020) has placed RABL2 as an IFT-associated inhibitor of BBSome-mediated exit. Together with biochemical work by the Lorentzen lab, their study suggests that RabL2-GTP associates with the IFT-B complex as it enters cilia and that subsequent GTP hydrolysis on RabL2 liberates a site on IFT-B for the BBSome to travel out of cilia. In addition, work by a number of labs (Katoh/ Nakayama and Nachury) has shown that the C-terminus of IFT38 directly contacts the BBSome and that this interaction is critical for BBSome-mediated exit of GPCRs out of cilia. I found it surprising that these interactions were not dicussed. It seemed like a missed opportunity to synthesize multiple lines of results into an interesting cog/lever of the IFT-B machine.

3- One surprising aspect of the paper is the absence of supporting data from negative stain EM of the complete 15-subunit complex reported in EMBO by this lab in 2016? Surely the authors must have tried negative stain of their complex. Even if the data do not allow for precise moleculr docking, it would be interesting to look at the possible hinges that are highlighted in Fig. 8A.

Minor Comments
1- It would seem scholarly fitting to include a mention of the BBSome on p.3: 'IFT-A and -B polymerize into linear assemblies known as IFT trains that move ciliary cargo into and out of cilia'

2- Liew et al. published a back to back paper with Eguether et al. in 2014. Citation needs to be included at the end of this sentence 'IFT27/25 complex (Bhogaraju et al., 2011) involved in BBSome trafficking and hedgehog signalling (Desai et al., 2020; Eguether et al., 2014; Keady et al., 2012)'.

3- The Nachury et al 2010 citation is odd on p.28. 'The occurrence of Bardet-Biedl syndrome (BBS) ciliopathy characterized by obesity, polydactyly, retinal degeneration, and mental retardation is typically caused by mutations or knockouts of genes that translates into proteins of the BBSome complex (Forsyth and Gunay-Aygun, 1993; Nachury et al., 2010).' Do the authors mean to cite the Nachury et al., 2007 paper?

4- 'The BBSome complex functions as an IFT adaptor that removes membrane proteins from cilia (Lechtreck et al., 2013, 2009).' This sentence seems unnecessarily selective in its citations as it only reflects on the Chlamydomonas literature.

5- Regarding the binding interface between IFT25/27 and IFT81/74: does it explain how the interaction can be dynamic? Regulated by GTP binding to IFT27?

6- Comparison to Pigino preprint could use a figure in addition to the paragraph of discussion.

Below is the response to the comments by the reviewers for the manuscript by Petriman et al., with the title 'Biochemically validated structural model of the 15-subunit IFT-B complex'. We thank reviewers for their time and constructive comments.

Referee #1:

This manuscript by Petriman and colleagues synthesizes a large amount of experimental data and alpha fold predictions to produce a plausible model of the IFT-B complex. The paper is a beautiful example of the power of these approaches. It is certain the model has significant shortcomings, but that is expected from this kind of work and does not detract from its importance in any way. The only significant point that I think should be addressed is that the mapping of the ciliopathy mutations onto the structure and the corresponding discussion is incomplete. It is clear from mouse mutations that complete loss of many of the IFT-B proteins yields lethality at about the time of blood flow. In humans, this would be long before the embryos developed to the point of having any of the syndromes described. The map in 9B is a modern version of the classic image of WWII planes that survived bullet strikes to make it back to base. These residues are important as they yield syndromic disease, but they are the sites that can be mutated and still produce a particle that functions well enough to get the embryo through early development. The residues without mutational hits could be unimportant or they could be fundamental to complex formation.

Answer: This is a good point. We have now pointed out on pg. 27 of the revised manuscript that IFT is required for cilium formation and organismal development (and cite the Berberi and Yoder 2011 paper on IFT54 demonstrating this) meaning that ciliopathy variants must be relatively mild. In addition, we made a new Appendix Figure S10 that map benign gnomAD mutations from control healthy patients as a comparison.

We rewrote the first paragraph in the ciliopathy section to read:

IFT is essential for cilium formation and organismic development as highlighted by the Traf3ip1 (encoding the IFT-B protein IFT54) mutant mice that cannot form cilia and thus fail in proper embryonic development (PMID: 21945076). Patients suffering from ciliopathies caused by mutations in IFT-B genes are thus expected to produce viable IFT particles that support at least some degree of cilium formation and function (Braun and Hildebrandt, 2017). To get insights into the structurally distribution of ciliopathy mutations, we obtained all ciliopathy related data from Uniprot, which comprises 327 distinct variants in the IFT-B genes discussed here and mapped these onto the IFT-B complex (Figure 9B). The variants are found together with over 15 prominent ciliopathies such as Bardet-Biedl syndrome, short-rib thoracic dysplasia and asphyxiating thoracic dystrophy. In addition, we extracted presumed benign variants (gnomAD mutations from control healthy patients) for human IFT-B proteins and mapped these onto the Chlamydomonas IFT-B structural model (Appendix Figure S10). We hypothesized that if benign variants are missing in some regions of the IFT-B complex, mutation of those regions may disrupt IFT complex formation and thus ciliogenesis. However, we observed a significant enrichment of disease variants for IFT80 and IFT172 relative to their length (Figure 9B), whereas the benign variants were equally distributed within IFT-B complex (Appendix Figure S10). Although the higher numbers of IFT80/172 disease variants could arise from directed sequencing (and not whole genome/exome data),

this observation does suggest that disease variants tend to interfere with cargo loading, particularly for the most severe ciliopathies. The lack of disease variants in certain regions of the complex could signify either lethality or tolerance, though we had insufficient data to distinguish these possibilities.

Minor points:
This sentence is a challenge: Given the significant flexibility of the IFT-B complex in solution and the fact that the structural model of the 15-subunit IFT-B complex presented here was assembled by superposing smaller sub-complexes without the context of the IFT train, it is not surprising that a rigid-body manually fit the IFT-B 15mer into the cryo-ET map of anterograde IFT trains (van den Hoek et al., 2022) resulted in several subunits located outside of the density (data not shown).

Answer: We have now rewritten this highly convoluted sentence and broken it into two sentences that read: The structural model of the 15-subunit IFT-B complex presented here was assembled by superposing predicted structures of smaller sub-complexes without the context of the IFT train. It is thus not surprising that a rigid-body docking of the IFT-B structure into the cryo-ET map of anterograde IFT trains (van den Hoek et al., 2022) resulted in a relatively poor fit with several subunits located outside the density.

Referee #2:

IFT (intraflagella transporter) is a protein complex, which carries cargos from cytoplasm to cilia or from the ciliary tip to cytoplasm. IFT consists of two major complexes, called IFT-A and IFT-B, which make tandem arrays and move between axonemal doublet microtubules and ciliary membrane. Mutation of component proteins of IFT causes defect of cilia growth and thus diseases with various symptoms (ciliopathy). Structure of IFT has been an active topic attracting molecular and cellular biologists interested in cilia and ciliopathy.
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complex, this work will be a milestone of cilia research. However, there are a few unclearness who the authors reached the final model. This reviewer would ask the authors to clarify these points.

Major points:
1) Complex of IFT57-CH/IFT172/IFT20/IFT34/IFT80: In their model, IFT57 is folded in extremely extended form (Figs.8&9). The CH domain is far away from the CC domain and located at the end of the whole complex. While the interaction between IFT57-CH and IFT172 was proved by pull-down assay in their past work (Taschner et al. 2016), proof of other connection is not clear (experimentally nor by AF2). There should be an evidence that IFT172 is in proximity to the C-termini of IFT20 and 54. Otherwise it will be weak to conclude with this model, since the CC and CH domains of IFT57 (also IFT54) are connected with disordered structure according to their AF2 analysis. The same should be for IFT80 and the C-terminus of this CC. Could you clarify the logic how these proteins should be complexed in this way?

Answer: We agree with the reviewer that the IFT-B2 part of the model containing IFT172 was perhaps not explained well in the original submitted version of the manuscript. It is indeed true that the long flexible linker between CC and CH domains of IFT57 means that the position of IFT172 relative to the IFT-B complex is not fixed by the IFT57 CH-domain. However, the position of IFT172 is fixed through the interaction with IFT80. IFT80 associates with the CH-domain of IFT38 that does not have a long flexible linker and is located close to the IFT54/20 CC domains, which fixes the position of IFT80 along the CCs of IFT54/20. In agreement with previously published experimental evidence for a weak direct interaction between IFT80 and IFT54/20 (Taschner et al., eLife 2018), we do observe a few close contacts between IFT80 and IFT54/20 in our structural model. These contacts may serve to further strengthen the interaction and fix the position of IFT80 relative to IFT54/20.

IFT172 is located near IFT54/20 in our model but the closets contacts are >13Åapart and thus do not support the notion of a direct interaction between IFT172 and IFT54/20. The position of IFT172 within our structural model is thus solely 'fixed' through the direct interactions of the second beta-propeller and the following α-solenoid structure of both IFT172 and IF80. We would like to point out that we provide experimental evidence for this direct interaction in Fig. 6B. The position of IFT172 close to, but not within direct interaction distance of, IFT5420 is thus an indirect effect of the interaction with IFT80 and the interaction of IFT80 with the CH domain of IFT38 and the CC of IFT52/20. We have clarified this on pgs. 18-19 of the revise manuscript.

2) P.14-15 Second conformation between IFT81/74 helices and IFt52/88/70: To prove/disprove existence of two modes of complex formation (the one in Fig.4 after crosslink and the one in Fig.8), the current AF analysis does not sound convincing enough. The most straightforward way is single particle EM, either cryo or negative stain, and classification to subaverages. Since they formed complexes for MS, they may have done EM. If so, it is worth looking into subaverages to find a sign of heterogeneity. Otherwise this part is rather discussion than conclusion. Besides, the authors could present their AF analysis

here more clearly - can they show the 5 AF models (p.15, line 10) in a supplementary figure and explain what are 15 resulting models (line13)?

Answer: We have collected both negative stain and cryo-EM data on several different IFT-B (sub)-complexes. Unfortunately, substantial particle disassembly and heterogeneity on the grids hampered with the collection of high-quality single particle data. To address the reviewers concern regarding the second binding site of IFT81/74 on IFT88/70/52/46 we carried out a pulldown experiment (new Figure 4G), which demonstrated a direct interaction. This new experiment is discussed on pg. 15 of the revised manuscript.

The section on pg. 15 has now been clarified and we have included a figure of the structural model containing both binding sites (and the partial unfolding of CCs) a new Appendix Figure S4C.

3) p.11 Line2-4, Fig.2A "...(Figure 2A). The data show that IFT27/25 and IFT22 have ....a high degree of conformational flexibility ...": The basis of this conclusion is not clear. Which part of Fig2A does "between the N- and C-terminal halves" mean? Is there any factor (such as pLDDT) showing local flexibility?

*Answer: We agree with the reviewer that these concluding sentences on pg. 11 are confusing and not really needed as this section is already concluded with the sentence: '*Thus, we conclude that the main docking site of IFT27/25 is on the C-terminal half of IFT81/74 in agreement with the predicted structural model of the pentameric IFT81/74/27/25/22 complex (Figure 2A).*' We have thus deleted the confusing sentences from the revised manuscript.*

The high degree of flexibility between the N- and C-terminal halves of the IFT81/74 complex is indicated by a) the low pLDDT score hinge regions between N- and C-parts b) the high PAE values between N- and C-terminal halves (Fig. 2D) and the fact that different alphafold runs give very different relative conformation between the two halves (Fig. 2C). This is discussed on pg. 9 in the manuscript.

4) One general issue is that the modeling was done based on AlphaFold-multimer, which is still in BioRXiv. It will be worth stating the authors thought how trustful this program is for such projects.

*Answer: While it is true that the alphafold multimer manuscript (Evans et al., 2021) has still not emerged in a peer reviewed version, several peer reviewed papers have tested and benchmarked the program and the source code is publicly available for anyone to examine. Peer reviewed papers include: Bryant et al., Nature com. 2022 https://www.nature.com/articles/s41467-022-28865-w ; Yin et al., Protein Science 2022 (**https://doi.org/10.1002/pro.4379**).*

Furthermore, most of the IFT-B subcomplex structures shown in our manuscript were initially folded using the 'monomeric' alphafold2 version (Jumper et al., Nature 2021) either with long glycine linkers between subunits or using a large 'gab' between the protein sequences

of different subunits as implemented in: https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2_complexes.ipynb. As the source code for alphafold multimer became available, we re-ran all IFT-B subcomplexes structure predictions using a local installation on our high-performing cluster. These predictions agree well with the previous predictions using older versions of alphafold (the original Alphafold2 release documented in Jumper et al., Nature 2021).

Minor points:
1) Abstract "The large MDa IFT-B complex ....": Probably this sentence should be "The large IFT-B complex".

Answer: corrected.

2) p.9, line10 "different structural models produced by AF have very similar local structures...": This reviewer could not follow. Please clarify what and what are different and what and what are similar to each other.

Answer: We have now re-written these sentences on pg. 10 with clarity in mind. The new section reads: Another hinge region formed between CC VI and CC VII divides the IFT81/74 complex into approximate N- and C-terminal halves (Figure 2A). The hinge region and the fact that no interface is observed between the N- and C-terminal halves of IFT81/74 point to a high degree of conformational flexibility between N- and C-terminal halves of IFT81/74. Indeed, different structural models produced by AF vary greatly in the relative positions of N- and C-terminal halves of IFT81/74 (for three different conformations see Figure 2C).

3) p.13, line16 "given that IFT52C/46C is connected to IFT70 via a 4-residue ....with no non-covalent interactions ....": This reviewer could not understand. More explanation needed. Maybe marking the 4-residue covalent linker in Fig.3E helps.

Answer: Our mistake. The 4-residue linker should not have been part of this sentence. The new sentence only deals with the fact that no interaction interface is observed between IFT70 and IFT52C/46C: However, given that no non-covalent interactions are observed between IFT70 and IFT52C/46C, the position of IFT52C/46C relative to IFT70 is likely quite flexible to accommodate different conformations in solution.

In addition, we have labelled the 4-residue linker in Figure 3E.

4) Do the authors have any thought about absence of dimerization of IFT80 in this modeling?

Answer: As we note on pg. 17 we do not observe an IFT80-homodimer using Alphafold, which is in contrast to our previous crystallographic and solution data (Taschner et al., 2018). The IFT80 dimerization occurs mainly through main-chain and water mediate contacts and Alphafold may thus not be able to extract proper distance restraints from multiple sequence alignments. We note that IFT80 dimerization is also not observed in the Cryo-ET reconstruction of anterograde IFT trains by the Pigino lab. IFT80 homo-dimerization

may be relevant in retrograde IFT trains as now discussed on pg. 24 of the revised manuscript.

5) Supplementary materials 1&2: It will be helpful for readers to have an overview of consistency between crosslink and AF, if they show the distance between the pairs of crosslinked residues in the AF-based complex model.

Answer: We have now compiled an excel sheet (Dataset EV4) that lists all distances between crosslinked residues.

6) An introductory panel to show which component proteins (and sub-complexes) in this project have been solved in the past and which proteins are known binding to each other.

Answer: In panel A of Figure 1 we show an overview of previously determined crystal structures of IFT proteins. In this architectural schematic, proteins 'touching' each other represent previously establishes interactions as does the double arrows in the schematics.

Referee #3

This manuscript combines crystallographic and biophysical work from the Lorentzen lab during the past decade with recent AI development in protein structure predictions to produce a complete model of the 15-subunit IFT-B complex. The structure of IFT-B has remained one of the holy grails of structural biology since the complex was discovered in the late 90's and the current manuscript will likely constitute the go-to resource for any researcher interested in IFT-B structure in the future. The work is thorough, methodical and logically organized and there is little to fault in the experimental strategy or quality. The strength of the approach lies in the combination of computational predictions via AlphaFold with biophysical validations, mainly crosslink/mass spec but also protein interaction assays, SAXS and cystallography.
This manuscript does not make for an entertaining light read (at least for non-structural biologists) but it delivers a solid model of the IFT-B complex and the lasting impact of this contribution is without doubt.

As the work is entirely descriptive, the biological significance can at point be difficult to discern for the non-specialist. In my opinion (which can be ignored at will), the paper would benefit from a greater emphasis on the more speculative aspects, chiefly the two conformations of IFT-B and the association with RabL2 and the BBSome.

Answer: We agree that it is important to keep the right balance of description vs speculation. Our discussion part is properly more speculative than one appreciates at a first glance. The entire discussion of which parts/surfaces of the IFT-B complex structure likely interacts with other complexes such as IFT-A and motors is quite speculative and will require more experimentation to validate. Furthermore, we do speculate that our two different conformations of IFT-B observed correspond to anterograde vs. retrograde IFT trains, another statement that needs validation. We have attempted to glean information regarding BBSome binding on the IFT-B complex by co-folding IFT-B and BBSome subunits

but so far without any luck. We would thus like to refrain from speculation on how the BBSome may associate with IFT trains at this point.

Main Comments

1- The main point of novelty in the paper is the existence of two possible configurations for IFT-B: one anterograde, one retrograde? The notion of a second IFT-B1 confirmation is interesting but as the interaction surface between IFT74/81 and IFT88 is entirely distinct from that between IFT74/81 and IFT52/46, does one need to invoke a second conformation? Could both interaction take place at the same time in one IFT-B conformer? This question is addressed through a clever modeling strategy that failed to find models where both interactions are satisfied. Nonetheless, given the flexible hinge in IFT46/52 between the domain that associate with IFT70 and those that contact IFT74/81, it would seem that a conformer with both binding modes should be feasible.

Answer: We actually believe that the importance of our manuscript is that we now have an almost complete structural model of the IFT-B complex to help explain previous observations and guide future experiments. With respect to the two binding site of IFT81/74 on IFT88/70/52/46, have now examined the second interaction mode in a pull-down that corroborates a direct interaction (new Fig. 4G). Furthermore, we have included an AF model that captures both binding sites but result in significant unfolding of CC regions of IFT81/74 to allow for this (new Appendix Figure S4C).

2- Foundational work by Xueliang Zhu & Xiumin Yan (EMBO 2020) has placed RABL2 as an IFT-associated inhibitor of BBSome-mediated exit. Together with biochemical work by the Lorentzen lab, their study suggests that RabL2-GTP associates with the IFT-B complex as it enters cilia and that subsequent GTP hydrolysis on RabL2 liberates a site on IFT-B for the BBSome to travel out of cilia. In addition, work by a number of labs (Katoh/Nakayama and Nachury) has shown that the C-terminus of IFT38 directly contacts the BBSome and that this interaction is critical for BBSome-mediated exit of GPCRs out of cilia. I found it surprising that these interactions were not dicussed. It seemed like a missed opportunity to synthesize multiple lines of results into an interesting cog/lever of the IFT-B machine.

Answer: RabL2 is indeed a very interesting protein functioning in IFT initiation. We have a lot of data on this component that will be the topic of a different manuscript. We have far been unable to obtain any conclusive data on how BBSomes interact with the IFT complex and would thus like to refrain for speculation at this point.

3- One surprising aspect of the paper is the absence of supporting data from negative stain EM of the complete 15-subunit complex reported in EMBO by this lab in 2016? Surely the authors must have tried negative stain of their complex. Even if the data do not allow for precise moleculr docking, it would be interesting to look at the possible hinges that are highlighted in Fig. 8A.

Answer: We did indeed look at the IFT-B complex by negative-stain EM. The data, however, show very heterogeneous particles that we, unfortunately, we unable to extract any useful information from in terms of class averages or low-resolution envelopes.

Minor Comments

1- It would seem scholarly fitting to include a mention of the BBSome on p.3: 'IFT-A and -B polymerize into linear assemblies known as IFT trains that move ciliary cargo into and out of cilia'

Answer: We have now included a mention of the BBSome and a citation to Nachury et al., Cell 2007 on pg. 3 of the revised manuscript.

2- Liew et al. published a back to back paper with Eguether et al. in 2014. Citation needs to be included at the end of this sentence 'IFT27/25 complex (Bhogaraju et al., 2011) involved in BBSome trafficking and hedgehog signalling (Desai et al., 2020; Eguether et al., 2014; Keady et al., 2012)'.

Answer: Citation inserted.

3- The Nachury et al 2010 citation is odd on p.28. 'The occurrence of Bardet-Biedl syndrome (BBS) ciliopathy characterized by obesity, polydactyly, retinal degeneration, and mental retardation is typically caused by mutations or knockouts of genes that translates into proteins of the BBSome complex (Forsyth and Gunay-Aygun, 1993; Nachury et al., 2010).' Do the authors mean to cite the Nachury et al., 2007 paper?

Answer We have now exchanged the citation as suggested.

4- 'The BBSome complex functions as an IFT adaptor that removes membrane proteins from cilia (Lechtreck et al., 2013, 2009).' This sentence seems unnecessarily selective in its citations as it only reflects on the Chlamydomonas literature.

Answer: We note that the two cited papers using Chlamydomonas were actually the first literature to correctly describe the function of the BBSome in ciliary export while numerous papers using mammalian systems rather suggested a function for the BBSome in ciliary import.

5- Regarding the binding interface between IFT25/27 and IFT81/74: does it explain how the interaction can be dynamic? Regulated by GTP binding to IFT27?

Answer: IFT27/25 interact with IFT81/74 via an extended interface that both utilize 'nucleotide independent' interactions from both IFT25 and IFT27 and IFT81/74 but also likely make interactions via the switch regions of IFT27. The model is thus compatible with a model where the interaction between IFT27/25 and IFT81/74 is at least partly GPT/GDP dependent. However, as Alphafold currently does not include ligands such as GTP in the modelelling, we are not sure of the correct positioning of switch regions and would thus like to refrain from speculation.

6- Comparison to Pigino preprint could use a figure in addition to the paragraph of discussion.

Answer: While we agree that it would be quite useful to have a figure comparing our model to that of the Pigino pre-print it is currently unfortunately not possible as the cryo-ET maps and structural models will only be released once the peer-reviewed version of their manuscript is accepted for publication.

Dear Esben,

Thank you for submitting your revised manuscript. Your study has now been re-reviewed by referee #2. As you can see from the comments below, the referee appreciates the introduced changes.

I am therefore very pleased to accept the MS for publication here. Before sending you the formal acceptance letter there are just a few editorial points to resolve:

- Please add 3-5 keywords

- We need a Disclosure and competing interests statement

- Please submit a "clean" version of the MS file with no marked changes

- Fig 4 is called out before Fig. 3 - this will pose problem for the typesetters as they place the figure close to the first mentioning of the figure in the text. Is there a particular reason for doing so? The same goes with Fig 9 which is called out before Fig 8. We can discuss further. There is a callout for Supplementary Table 1, but no related file.

- Supplementary material 1-3 should be renamed to Dataset EV1-3 with the corresponding legends in a separate sheet (Data EV4 is correct)

- Please insert link in the Data Availability Section.

That should be all - let me know if you have any further questions

Best Karin

Karin Dumstrei, PhD
Senior Editor
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Referee #2:

The authors addressed all the points raised by this reviewer perfectly by an additional experiment and revising the manuscript. This reviewer fully supports publication of the manuscript in the EMBO Journal.

2nd Revision - Editorial Decision **20th Oct 2022**

Dear Esben,

Thank you for submitting your revised manuscript. I have now had a chance to take a look at it and all looks good.

I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a nice study!

best Karin

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