Dear Editor,

A belt around the waistline of pore membrane (pom) of the nuclear envelope has been detected with electron microscopy. However, its molecular identity, architecture, and function remained unclear. Of the three distinct integral membrane proteins populating the nuclear pore membrane, only pom152 (of yeasts) or gp210 (of multi-cellular organisms) contain a sufficiently large ‘trans’ domain (over 100 kDa) that could form such a belt (Fig. 1a and Supplementary Fig. S1).

Using the Phyre2 program to predict tertiary structures of yeast pom152 and its multi-cellular ortholog, gp210, we first identified ten and fifteen, respectively, immunoglobulin-like (Ig-like) folds which extended over much of these proteins’ large trans domain (Fig. 1a, Supplementary Figs. S2 and S3). We next expressed full-length (FL) pom152 (Ctpom152FL) of the thermophilic yeast Chaetomium thermophilum in insect cells and purified Ctpom152FL with detergent extract. Incubation of purified Ctpom152FL with chymotrypsin revealed a large stable fragment (Fig. 1b), which we identified by mass spectrometry as Ctpom152194-1256 (Fig. 1b). This fragment represents most of the Ctpom152 trans domain, starting ~40 residues downstream of its transmembrane helix (TM) and having lost 14 of its C-terminal residues (Fig. 1a and Supplementary Fig. S1).

The purified Ctpom152194-1256 (detergent-free) was examined by negative-stain EM using uranyl acetate. The electron micrographs showed beaded strings of 37 ± 4 nm, with each bead measuring ~4 nm in length and ~2 nm in width (Fig. 1c) with up to ten beads per string, consistent with our structural predictions. On the grid, the beaded strings assumed various shapes, indicating a large degree of flexibility. To further improve the purity and quality, we expressed a new construct Ctpom152186-1270 in insect cells. Ctpom152186-1270 lacks the upstream transmembrane segment and thus does not require detergent for extraction and purification (Supplementary Information, Fig. 1d). The negative-stain EM of Ctpom152186-1270 also showed a beaded string structure (Fig. 1e) indistinguishable from that obtained for the chymotryptic fragment (Ctpom152194-1256) (Fig. 1c). Size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) of Ctpom152186-1270 (at 6 mg/mL per injection) showed that it behaves as a monomer of 130.3 ± 9.6 kDa (Fig. 1f), close to its theoretical molar mass of 121.7 kDa. Likewise, SEC-MALS measurements carried out at two lower concentrations yielded similar molar masses (129.2 ± 11.2 kDa at 2 mg/mL or 136.5 ± 8.5 kDa at 4 mg/mL).

Our data so far left open the question of whether the trans regions of Ctpom152 could oligomerize into luminal ring. We used purified Ctpom152FL, but removed much of the detergent (Supplementary Fig. S4) before rapidly freezing the sample for cryo-EM. As in the negative-stain images, we saw 44-nm long beaded strings (Fig. 1g, h). Some of these strings displayed sufficient density to unequivocally distinguish ten beads (Fig. 1i). Notably, other upstream elements of FL Ctpom152 were not visible in these cryo-electron micrographs, presumably because of their disordered structures; moreover, we could not detect the clear-cut density differences between the beads (Fig. 1i). We conclude that regions other than the trans domain of Ctpom152 are insufficiently compact to be visible in vitreous ice.
Strikingly, in up to an estimated 10% of images (over 2000 images), full-length Ctpom152 molecules appeared as much longer beaded structures (Fig. 1j), measuring in multiples of 44 nm without consistent punctuation marks between monomers (Fig. 1j). Because the trans segments lacking the N-terminal domain (Ctpom152186-1270 and Ctpom152194-1256) are unable to oligomerize, these data strongly suggested that the longer beaded structures might arise by head-to-tail oligomerization of the trans domains.

Intriguingly, both pre-Ig and Ig10 are amongst the most conserved regions between pom152 homologs.
(Supplementary Fig. S2), indicative of a common mechanism in yeast. To test it, we mapped the interacting sites between recombinant pre-Ig region and purified Ig10 (Fig. 1a, Supplementary Figs. S2 and S5) from both *Chaetomium thermophilum* and *Saccharomyces cerevisiae*. Indeed, both Ctpre-Ig and Scpre-Ig immediately downstream from the TM sufficiently precipitated Ctlg10 and ScIg10, respectively. The remarkable agreement thus strongly suggests that yeast pom152, independent of other poms (pom34 and Ndc1), is capable of assembling into a complete ring.

Taking together, our data here suggest that the large ‘trans’ domain of *Chaetomium thermophilum*, Ctpom152, an integral nuclear pore membrane protein, largely consists of ten closely linked Ig folds. Eight head-to-tail connected trans domains would assemble into a continuous ring with diameter of 100 nm34. Interestingly, our structural predictions of the presence of fifteen Ig folds in gp210 would yield correspondingly larger rings of 150 nm in diameter (Fig. 1k5,6 and Supplementary Fig. S3). Because of the symmetry, the 16 trans domains may form 2 eight-member rings, one situated above, the other below mid-plane, collectively representing the lumenal density around the waist of the pore membrane.

Why should NPC require such a structure? As the only conduit between nucleus and cytoplasm, dilation of the central channel to accommodate oversized particles could destabilize the pore membrane. Intriguingly, the trans domain of pom152 (or gp210) bears striking structural similarity to titin, an abundant protein in sarcomeres that fulfills a number of mechanical functions with the most notable one as a passive visco-elastic spring’. The existence of an elastic ring in lumen thus could provide the pore membrane with the counter force to maintain its integrity6,8,9.

While preparing our manuscript, a paper by Upla et al.9 was published, the results of which were both overlapping and divergent with those of our present paper. Based on 3D reconstruction of negative-stain EM, Upla et al.9 reported that full-length Scpom152 consists of two distinct domains: (1) a large “head” group (comprising of its 375 N-terminal residues) (Fig. S1 and Fig. 4a of Upla et al.9), and (2) a “lumenal” domain consisting of nine beads in a rigid conformation, with the first one much longer than the remaining eight, all distinctly smaller than the head group (Fig. 6a of Upla et al.9). In contrast, our EM structures showed ten beads of about equal size in a highly flexible conformation (Fig. 1g, h, i), and the remaining fragment, terminating around the residue 214 (Fig. 1b and Supplementary Figs. S1 and S2), is insufficiently compact to be detected by cryoEM. Moreover, our biochemical analysis suggested that yeast pom152 can oligomerize in a conserved fashion: our predicted “head” (pre-Ig) of one trans domain directly interacts with the “tail” (Ig10) of another, whereby linking multi-trans domains into a single ring (Fig. 1, Supplementary Figs. S2 and S5). Surprisingly, unlike our model, the Scpre-Ig region, Scpom152214-265 was previously interpreted as part of the “head group” (Upla et al.9), not a constituent of the ring3. Better precision in the ‘head group’ boundary determination, negative stain in the absence of detergent, and higher resolution in 3D reconstruction will be needed in order to resolve the differences between our models.

**Acknowledgements**

We thank Alexandra Coutavas and Jianfeng Sun for technical assistance; Joseph P Fernandez and Henrik Molina (Proteomics Resource Center at The Rockefeller University, funded by the Leona M. and Harry B. Helmsley Charitable Trust for mass spectrometer instrumentation) for mass spectrometry analyses; Devrim Acehan, Mark Ebrahim, and Johanna Soltesz (Electron/ Cryoelectron Microscope Resource Centers at The Rockefeller University) for assistance with negative-stain EM and cryoEM; Xiaochun Li and Thomas Walz for discussion; and Bartlomiej Blus and Elias Coutavas for critical reading and editing of the manuscript. This work was funded by Howard Hughes Medical Institute, Rockefeller University (GB) and Beijing Advanced Innovation Center for Structural Biology at Tsinghua University.

**Author details**

1Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Ave., New York, NY 10065, USA. 2Beijing Advanced Innovation Center for Structural Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China. 3Present address: Calico Life Sciences, 1170 Veterans Blvd, South San Francisco, CA 94080, USA.

**Author contributions**

Q.H., B.Z., H.S., and G.B. designed the study; Q.H., B.Z., K.Y., and H.S. performed the experiments; Q.H., B.Z., H.S., and G.B. analyzed and interpreted the data; Q.H., B.Z., H.S., and G.B. wrote the paper.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Publisher's note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Supplementary Information**

accompanies the paper at (https://doi.org/10.1038/s41421-018-0057-7).

Received: 19 April 2018 Revised: 7 August 2018 Accepted: 8 August 2018 Published online: 18 September 2018

**References**

1. Akey, C. W. & Radermacher, M. Architecture of the xenopus nuclear pore complex revealed by three-dimensional cryo-electron microscopy. *J. Cell Biol.* **122**, 1–19 (1993).
2. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* **10**, 845–858 (2015).
3. Kim, S. J. et al. Integrative structure and functional anatomy of a nuclear pore complex. *Nature* **555**, 475–482, https://doi.org/10.1038/nature26003 (2018).
4. Alber, F. et al. The molecular architecture of the nuclear pore complex. *Nature* **450**, 695–701 (2007).
5. Mahamid, J. et al. Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**, 969–972 (2016).
6. Loschberger, A. et al. Super-resolution imaging visualizes the eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution. *J. Cell Sci.* **125**, 570–575 (2012).

7. Herzog, J. A., Leonard, T. R., Jinha, A. & Herzog, W. Titin (visco-) elasticity in skeletal muscle myofibrils. *Mol. & Cell. Biomech.: MCB* **11**, 1–17 (2014).

8. Solmaz, S. R., Blobel, G. & Melck, I. Ring cycle for dilating and constricting the nuclear pore. *Proc. Natl. Acad. Sci. USA* **110**, 5858–5863 (2013).

9. Upla, P. et al. Molecular Architecture of the Major Membrane Ring Component of the Nuclear Pore Complex. *Structure*, https://doi.org/10.1016/j.str.2017.01.006 (2017).