Sample preparation for cryo EM analysis. cTRiC samples were prepared as described above but starting from 1 μM TRiC in buffer A without glycerol and PEG 8000. Samples were embedded in vitreous ice as follows: 3 μl of TRiC and cTRiC sample respectively were placed onto a washed, glow-discharged 200 mesh R2-1 Quantifoil continuous carbon grid (Quantifoil Micro Tools GmbH, Jena Germany). The grid was blotted and flash frozen in liquid ethane using a Vitrobot (FEI, Hillsboro, Oregon, USA). Grids were stored in liquid nitrogen until imaging.

Image Collection. Ice-embedded images were acquired at an effective magnification of 83,100x on a Gatan 4Kx4K CCD camera (Gatan, Pleasanton CA) using a JEOL2010F electron microscope (JEOL Inc, Tokyo Japan) with a field emission gun operated at 200 kV. The microscope was equipped with the JEOL telemicroscopy software package (FasTEM) (JEOL USA Inc, Peabody MA) and a Gatan Model 626 cryostage (Gatan, Pleasanton CA). In addition, a custom software package developed in-house, JEOL Automated Microscopy Expert System (JAMES), was used to acquire focal pairs of images semi-automatically, at a range of 0.8-2.5 nm underfocus for the first image and a range of 2-5 nm underfocus for the second image.

Generating Reference Free Class Averages. Particle images were selected from the micrographs by hand using the EMAN software program 'boxer'.[1] Using this procedure, 2,875 TRiC particles and 4,522 particles of clipped TRiC were identified for further image processing. Reference free class-averages were generated using the 'refine2d.py' program in EMAN.[2] Therefore 5 iterations of the SVD-based procedure were used to generate 100 reference free class averages from the ice embedded particle images. In the side and top views shown in the figure 1 between 50 and 80 particles from the same orientation were averaged to form the class average.

References:

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2. Chen, D.H., Song, J.L., Chuang, D.T., Chiu, W. & Ludtke, S.J. An Expanded Conformation of Single-Ring GroEL-GroES Complex Encapsulates an 86 kDa Substrate. *Structure* **14**, 1711-22 (2006).