Supplementary Information

Chemically Functionalized carbon films for single molecule imaging

Marc C. Llaguno[1], Hui Xu[1], Liang Shi[1], Nian Huang[2,3], Hong Zhang[2,3], Qinghua Liu[2], and Qiu-Xing Jiang[1]

1. Department of Cell Biology, 2. Department of Biochemistry, 3. Department of Biophysics, UT Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, Texas 75390, USA

Supplementary figure legends:

Figure S1 Biochemical purification of His-tagged VCP hexamers

(**a**) Gel-filtration profile in a Superose 6 column. Three fractions from the peak are marked out.

(**b**) The three fractions (1-3) from the gel-filtration were run in a 10% SDS-PAGE gel, and stained with Coomassie Blue. Fraction 3 was used for our experiments.

Figure S2 C1 reference model and resolution determination for 3D reconstruction.

(**a**) After several rounds of angular reconstitution and model refinement by projection matching with C1 symmetry (no symmetry), the map began to show 2-fold symmetry about an axis going through the side open.

(**b**) A 0.5 threshold (red line) corresponds to a spatial frequency of 0.11 $Å^{-1}$ or 21Å resolution.

Figure S3 Handedness determination for the 3D reconstruction of the C3PO on the ssRNA-ChemiC surface

A total of 70 pairs of particle images were selected from the tilt pairs. The two micrographs were aligned in Web, and then rotated in plane such that for both images

the tilt axes (along the holder shaft) are along the Y-axis in the SPIDER coordinate system. The electrons run along the Z-direction in this system. The 3D map (unmirrored) and its mirrored model were first compared with the untilted image in each pair of particles. The Euler angles from the alignment were applied to the 3D maps such that their projections would generate the untilted image. The two maps were then rotated along their Y-axes to the tilted angle that was determined between the tilt pairs (θ =21.29° in this case), or its opposite (-θ). The projections from the rotated maps were then aligned with the tilted image to find out which one matches better. The particles assigned to θ and -θ are surveyed for both the 3D map and its mirror and the statistic numbers are presented.

Figure S4 Analysis of a dataset of negatively-stained C3PO/ssDNA complex in solution.

(**a**) First three eigenimages from the multivariate statistical analysis of the center-aligned images out of the original dataset. The second and third images clearly showed strong C2 symmetry in the molecules.

(**b**) Comparison of the class averages (1), the projections from the reconstruction (2) and the raw data aligned with the same orientation by projection matching (3).
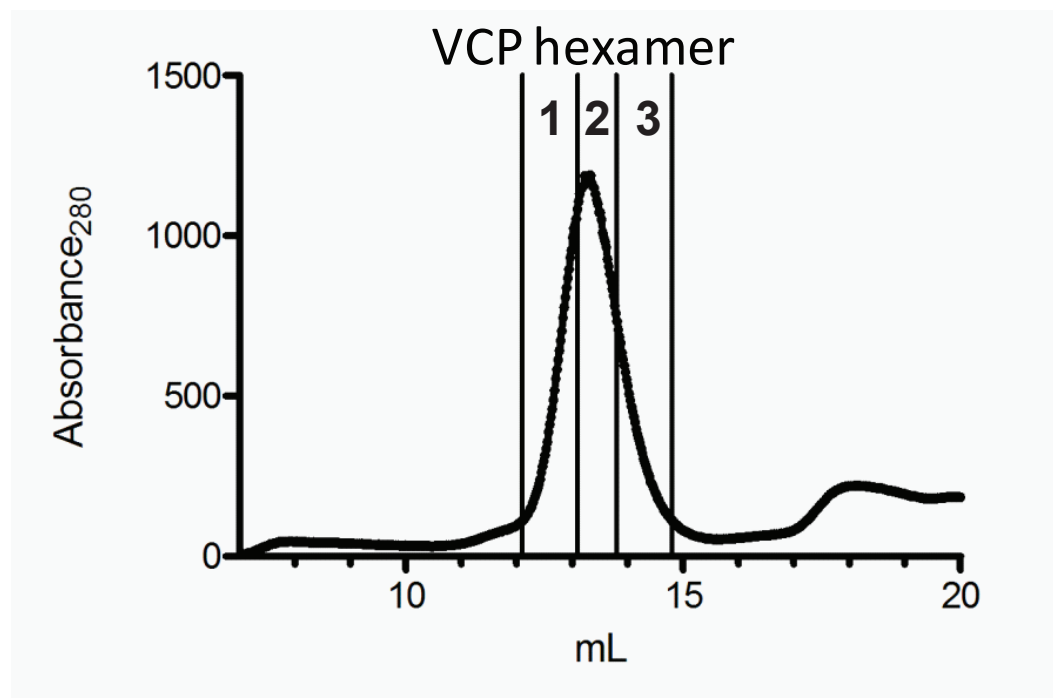
Figure S5 Analysis of the cryoEM dataset of C3PO/ssRNA complex on ChemiC films.

(**a**) First three eigenimages coming from the multivariate statistical analysis of the center-aligned images. The second and third images showed strong C2 symmetry in the molecules.
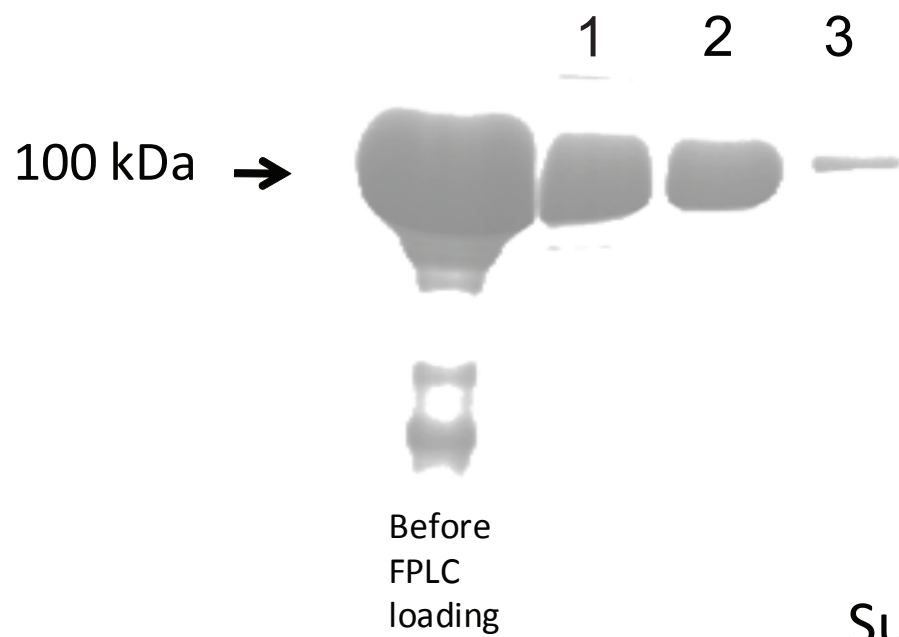
(**b**) Comparison of class averages (1), the projections from the 3D map (2) and raw particle images aligned by projection matching (3).
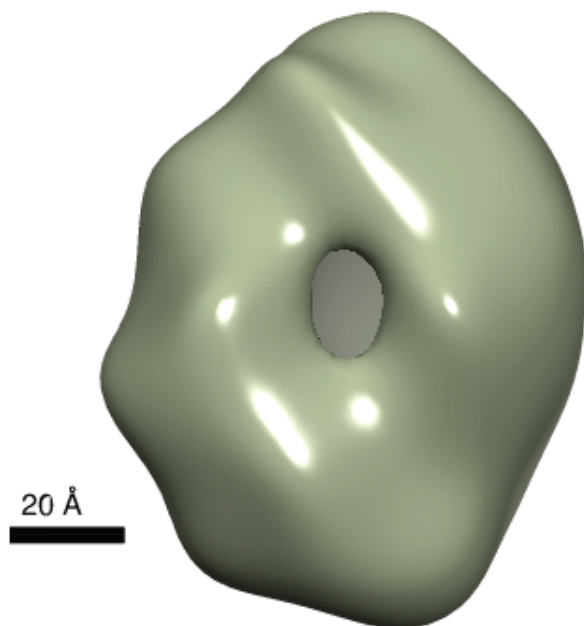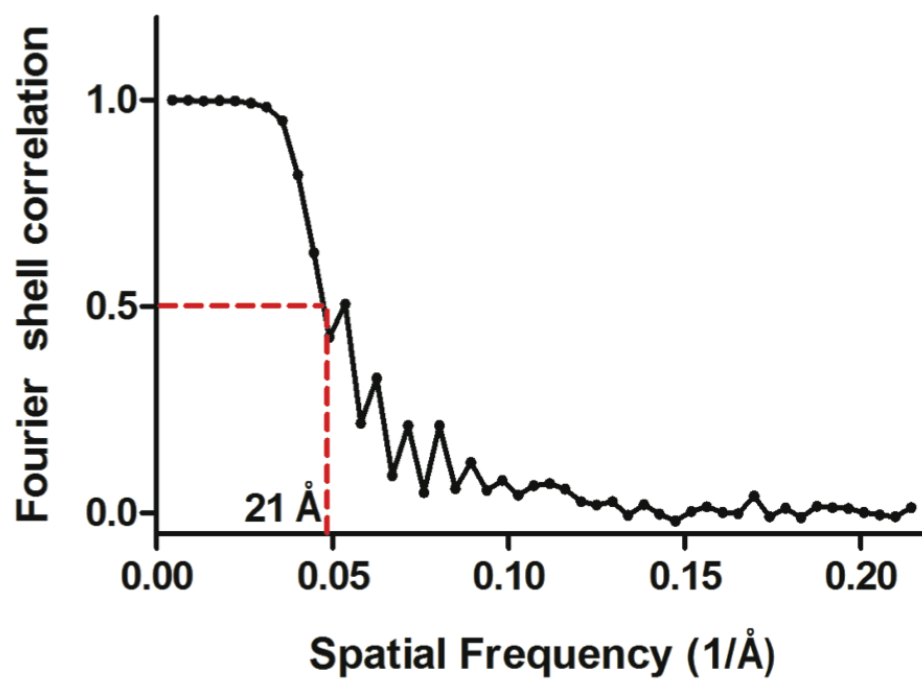
(**c**) Resolution estimation by FSC0.5. The two maps were calculated from two halves of the data and aligned for calculation.
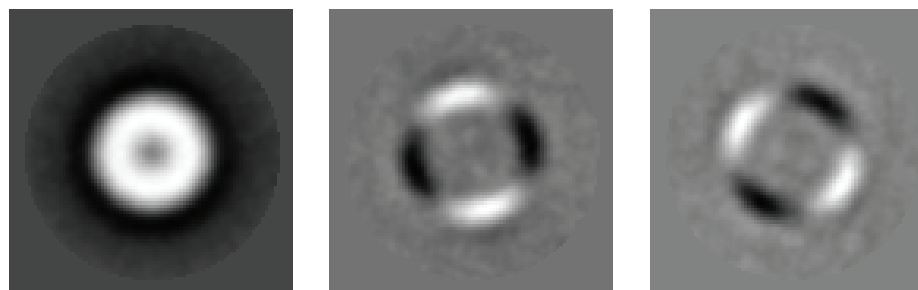
a

VCP hexamer

1 2 3

b

1   2   3

100 kDa →

Before
FPLC
loading

Supplementary Figure S1

a

20 Å

b

Tilt angle(°)	Unmirrored		Mirrored	
	θ	-θ	θ	-θ
21.29	45(64%)	25(36%)	28(40%)	42(60%)

Supplementary Figure S3

a

b

a

b

1 2 3   1 2 3

c

Fourier shell correlation

15 Å

Spatial Frequency (1/Å)

Supplementary Figure S5