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Supplemental Information

Fusion of DARPin to Aldolase Enables Visualization of Small Protein by Cryo-EM

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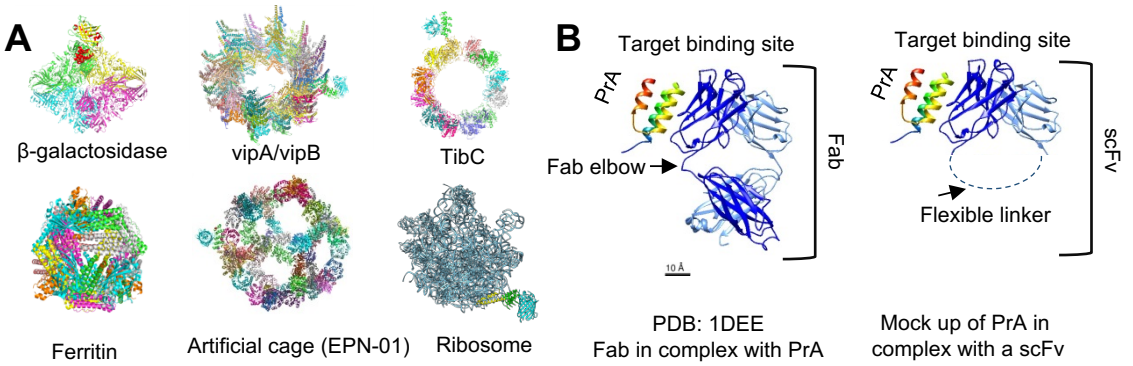
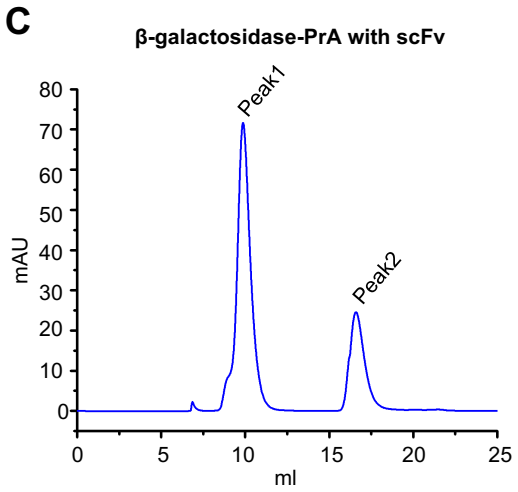
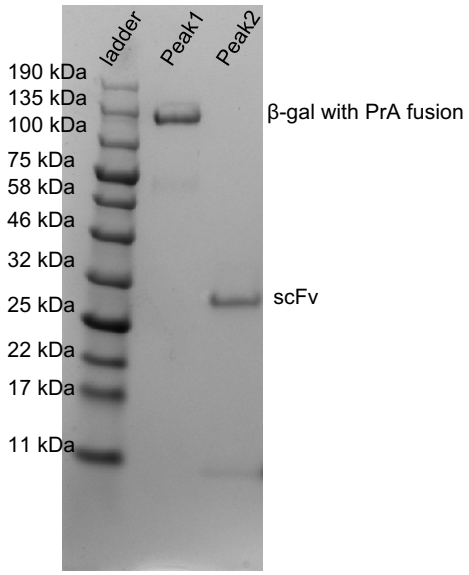
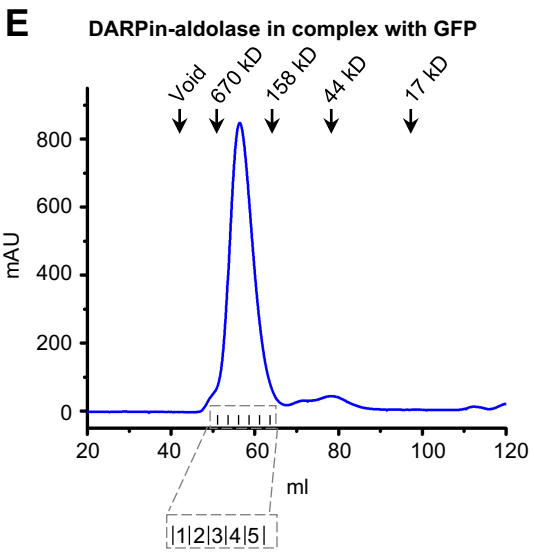
SUPPLEMENTARY FIGURES

Figure S1









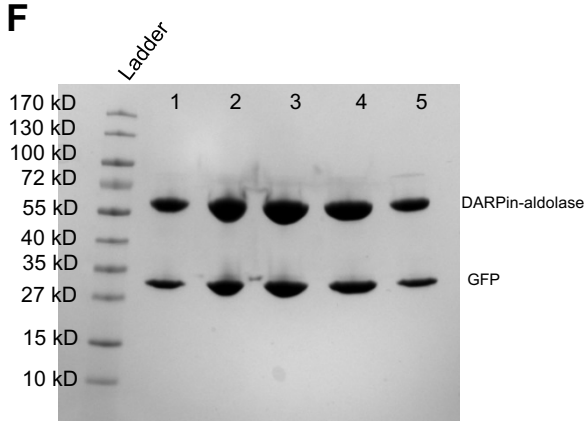


Figure S1. Attempted platform designs and outcomes, related to Figure 1

(A) Models of the six of the platform base proteins tested here. The seventh model (aldolase) tested is shown in Figure 1. The fused DARPin (in green) and target GFP (in cyan) were shown for only one subunit for clarity. (B) Our Protein A/scFv selectable adapter design was inspired by PDB 1DEE (Graille et al., 2000). On the left, PDB 1DEE shows the PrA three helix bundle (represented in rainbow from red to blue as N- to C-termini respectively) in complex with a Fab. On the right, a mock up of a scFv binding PrA (based on PDB 1DEE) is shown. A flexible linker (dashed line) connects the two beta sandwich domains of the scFv. Scale bar, 10 Å. (C) Briefly, the β-gal-PrA fusion protein was mixed with equal copies of scFv and incubated overnight. The mixture was resolved into two peaks (1 and 2) by gel filtration chromatography using a Superdex 200 10/300 GL column (GE Healthcare). (D) SDS-Page gel stained with Coomassie Blue of peaks 1 and 2 from (C) showed that the beta-galactosidase-PrA fusion protein and the scFv appeared in different peaks, suggesting that a stable complex did not form. Bands are labeled with β-gal with PrA or scFv. (E) Gel filtration chromatography of the purified DARPin-aldolase platform in complex with GFP on Superdex 200 10/300 GL column yielded one main peak. The black arrows mark the molecular weight calibration and void volume. Fractions 1 to 5 are labeled. (F) SDS-Page gel stained with Coomassie Blue of fractions 1 to 5 from (E). The bands representing the DARPin aldolase platform subunit and the GFP are labeled.

Figure S2

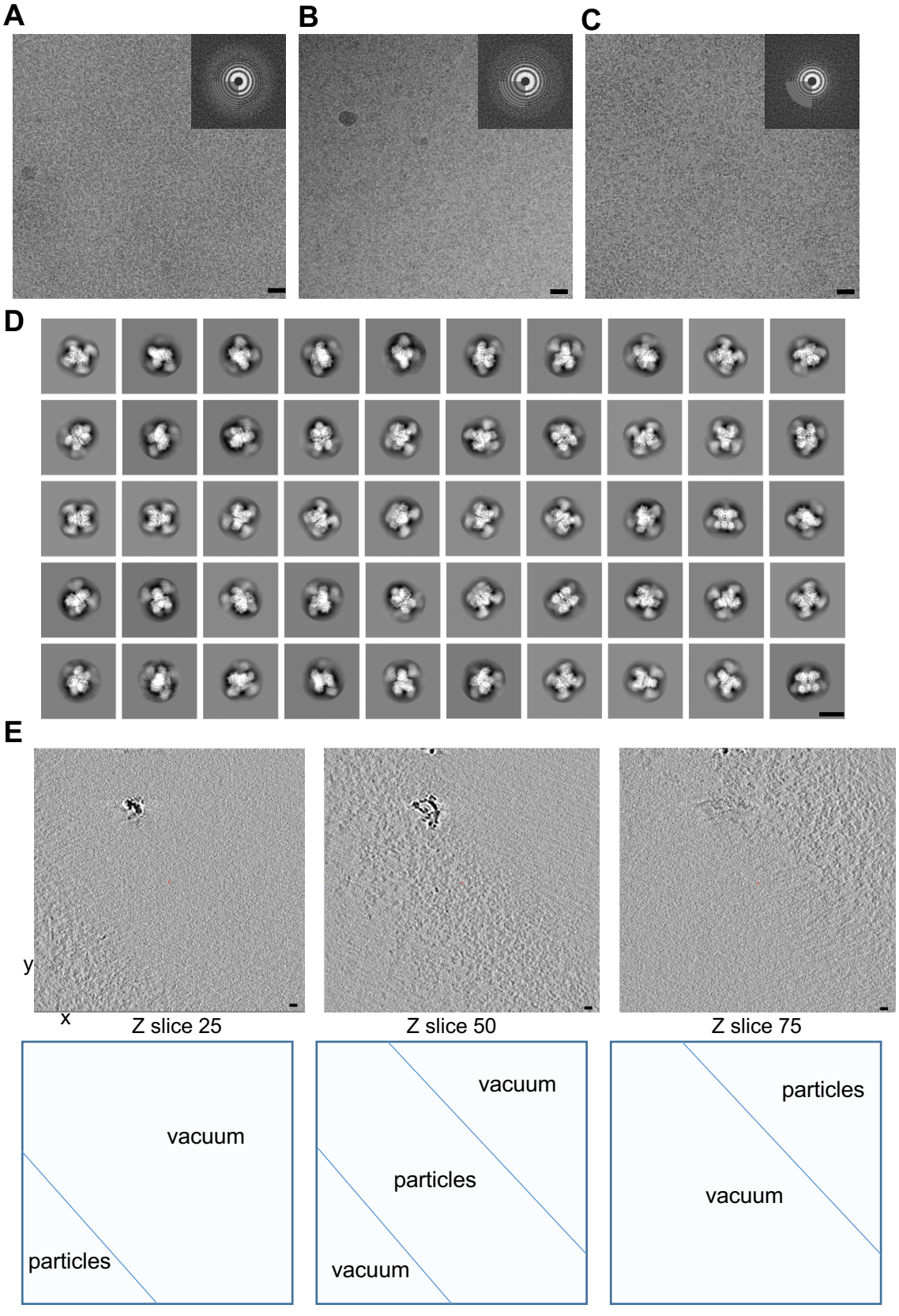

A
B
C
D
E
y
x
Z slice 25
Z slice 50
Z slice 75
vacuum
particles
vacuum
particles
vacuum
particles
vacuum

Figure S2. cryoEM data for the GFP:DARPin-aldolase complex, related to Figure 2

(A)-(C) Motion-corrected, dose weighted micrographs of DARPin-aldolase platform in complex with GFP in vitreous ice (left) with the Fourier transformation (inset). Micrographs were collected at 0˚ tilt in the first session (A), at 0˚ tilt in the second session (B) and at 26˚ tilt in the third session (C). Each micrograph has been low-pass filtered to 10 Å to enhance the contrast. Scale bar, 20 nm. (D) Representative 2D classification results from Relion. The 2D class images are 300 Å by 300 Å. Scale bar, 100 Å. (E) Tomography of the GFP:DARPin-aldolase grids showed a single layer of particles suspended in thin ice. Three Z slices (z=25, z=50, z=75) at a z thickness of 10 slices (to increase contrast) were selected from the tomogram. Axes are X and Y. Scale bars, 10 nm. The tomogram was 100 z slices thick. The ice in the tomogram runs through the ZX plane diagonally with vacuum (empty space) on either side. Below each image is a schematic of which region represents particles in ice and which region represents vacuum above or below the layer of ice.

Figure S3

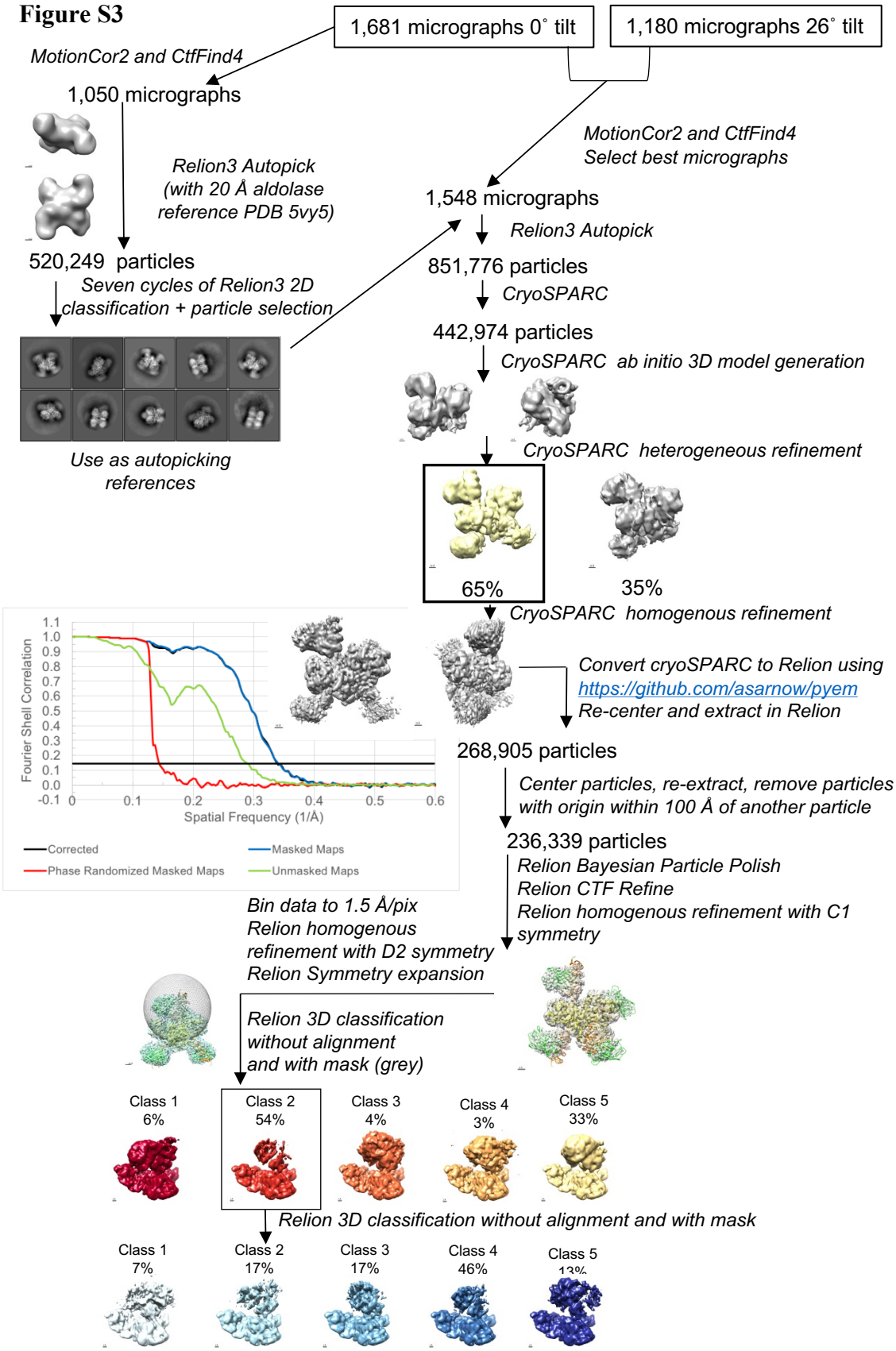

1,681 micrographs 0˚ tilt
1,180 micrographs 26˚ tilt
MotionCor2 and CtfFind4
1,050 micrographs
Relion3 Autopick
(with 20 Å aldolase
reference PDB 5vy5)
520,249 particles
Seven cycles of Relion3 2D
classification + particle selection
Use as autopicking
references
MotionCor2 and CtfFind4
Select best micrographs
1,548 micrographs
Relion3 Autopick
851,776 particles
CryoSPARC
442,974 particles
CryoSPARC ab initio 3D model generation
CryoSPARC heterogeneous refinement
65%
35%
CryoSPARC homogenous refinement
Fourier Shell Correlation
Spatial Frequency (1/Å)
Corrected
Masked Maps
Phase Randomized Masked Maps
Unmasked Maps
Convert cryoSPARC to Relion using
https://github.com/asarnow/pyem
Re-center and extract in Relion
268,905 particles
Center particles, re-extract, remove particles
with origin within 100 Å of another particle
236,339 particles
Relion Bayesian Particle Polish
Relion CTF Refine
Relion homogenous refinement with C1
symmetry
Bin data to 1.5 Å/pix
Relion homogenous
refinement with D2 symmetry
Relion Symmetry expansion
Relion 3D classification
without alignment
and with mask (grey)
Class 1
6%
Class 2
54%
Class 3
4%
Class 4
3%
Class 5
33%
Relion 3D classification without alignment and with mask
Class 1
7%
Class 2
17%
Class 3
17%
Class 4
46%
Class 5

Figure S3. Major steps of the last cycle of cryoEM data processing, related to STAR Methods

Tilted data was collected several months after the initial data collection sessions, so several generations of cryoEM data processing contributed to the final data pipeline in this paper. The major steps in the final cycle of cryoEM data processing are summarized in this flowchart. Scale bars are 10 or 20 Å as indicated per image.