Development of a yeast internal-subunit eGFP labeling strategy and its application in subunit identification in eukaryotic group II chaperonin TRiC/CCT

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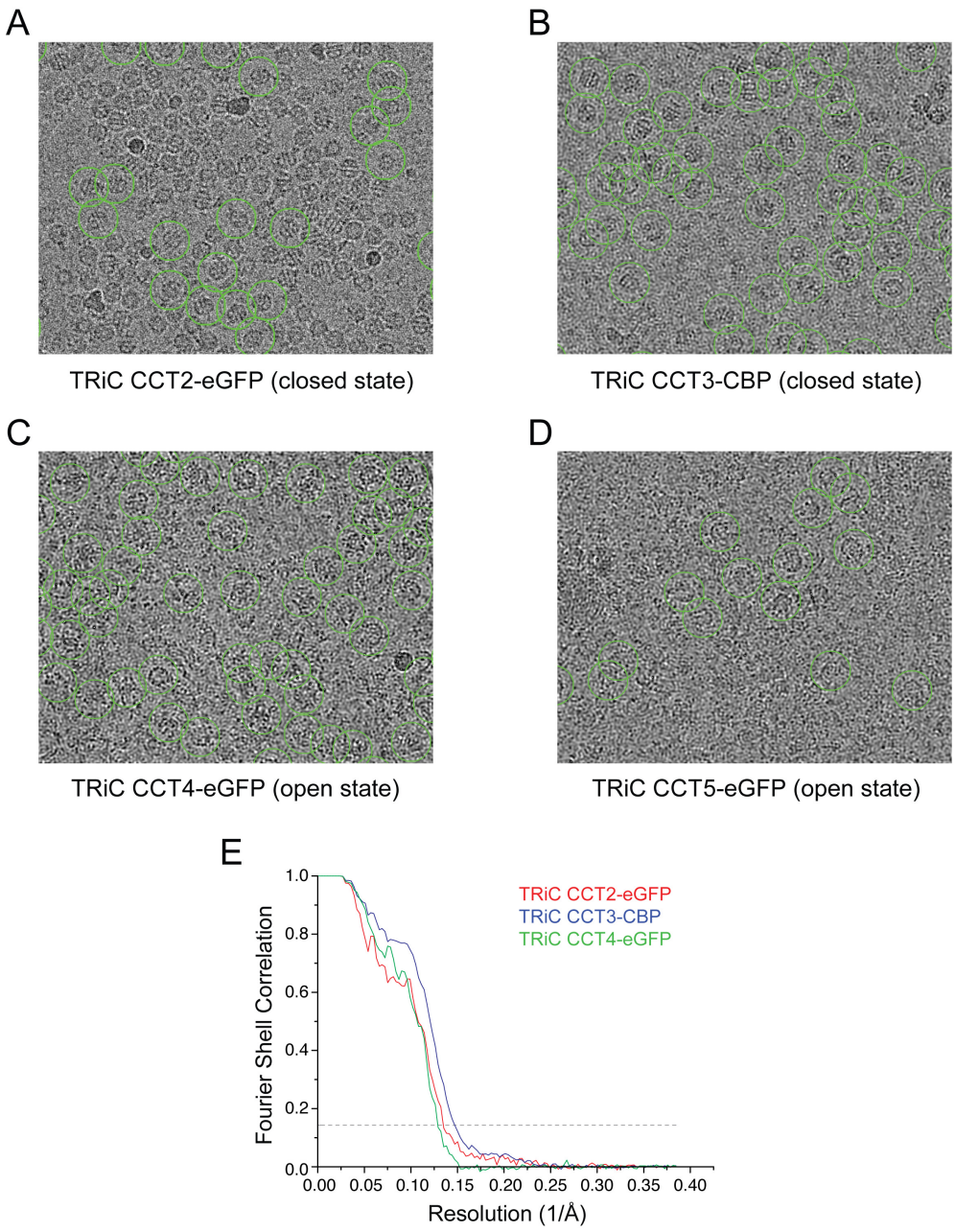

Figure S1 Representative cryo-EM images and resolution estimation. (A, B) TRiC CCT2-eGFP and TRiC CCT3-CBP in the closed state (in the presence of ATP-AlFx). (C, D) TRiC CCT4-eGFP and TRiC CCT5-eGFP in the open NPP state. (E) Resolution estimation of the TRiC CCT2-eGFP (red curve), TRiC CCT3-CBP (blue curve), and TRiC CCT4-eGFP (green curve) maps according to the gold-standard FSC criterion of 0.143.

Table S1 Insertion location of eGFP tag in TRiC subunits, linker information, and cryo-EM 3D reconstructions performed.

	Insertion site	domain	linker	open** state	closed** state
CCT1	K488|P489	E	GSGSG, GSGSG	✔	✔
CCT2	K334|C335	A	GSGSG, GSGSG		✔
CCT3*	P374|K375	A-I	n/a		✔
CCT4	N185|S186	I	GSGSG, GSGSG	✔	✔
CCT5	G263|S264	A	GSGSG, GSGSG	✔	
CCT6	P373|K374	A-I	n/a	✔	✔
CCT7	E150|K151	I-E	GSGSG, GSGSG	✔	
CCT8					

* For CCT3, a CBP tag (containing Strep, CBP and 6xHis) was inserted instead of eGFP tag.

** 3D reconstructions performed in the current study are shown in red, and in previous study in green[28], through which we determined the locations of subunits CCT1, CCT6, and CCT7 in the map.

Table S2 Statistics of cryo-EM data collection, processing, and structure refinement.

	TRiC CCT2-eGFP closed state	TRiC CCT3-CBP closed state	TRiC CCT4-eGFP open state	TRiC CCT5-eGFP open state
Detector	K2 Summit	K2 Summit	K2 Summit	K2 Summit
Defocus range (μm)	-1.5~-3.5	-1.5~-3.5	-1.5~-3.0	-1.5~-3.0
Pixel size (Å)	1.3	1.3	1.3	1.3
Electron dose ($e^-/Å^2$)	25	38	38	38
Particles refined	34,300	42,097	45,451	15,240
Final Resolution (Å)	7.6	6.9	7.9	23.8