

Table S1. Refinement statistics. Related to Figure 1.

	NusG-NGN-opsEC	RfaH-NGN-opsEC	RfaH-opsEC
Data Collection and processing			
Microscope	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
Voltage (kV)	300	300	300
Detector	K2 summit	K2 summit	K2 summit
Electron exposure (e ⁻ /Å ²)	69.9	71.0	71.0
Defocus range (μm)	0.8 – 2.5	0.8 – 2.4	0.8 – 2.4
Data Collection mode	Counting	Super-resolution	Super-resolution
Physical pixel size (Å/pixel)	1.07	1.3	1.3
Symmetry imposed	C1	C1	C1
Initial particle images (No.)	514,900	389,200	389,200
Final particle images (No.)	171,900	174,600	107,500
Map resolution (Å) ^a	3.72	3.54	3.70
Refinement			
Map sharpening B factor (Å ²)	- 99.9	- 77.9	- 69.7
RMSD			
Bond lengths (Å)	0.08	0.01	0.01
Bond angles (°)	1.02	1.11	1.04
Ramachandran^b			
Favored (%)	82.2	85.7	82.7
Allowed (%)	17.5	14.0	17.1
Generously Allowed (%)	0.3	0.3	0.2
Disallowed (%)	0	0	0
Molprobity^c			
Clash score	5.18	7.63	6.43
Rotamer outliers (%)	0.29	0.72	0.35
Overall score	1.76	1.92	1.86

^a Gold-standard FSC 0.143 cutoff criteria, as calculated by RELION (Figure S2D) (Scheres, 2012).

^b Procheck (Laskowski et al., 1993).

^c (Chen et al., 2010)

Table S2. Conformational changes of NusG-opsEC and RfaH-opsEC compared to X-EC (6ALF). Related to Figure 1.

Structural module	residues	PyMOL command	Rmsd (Å), # of C α 's		
			vs. EC (6ALH)	vs. NusG-opsEC	vs. RfaH-opsEC
Entire structure	αI : 7-158; 167-234 αII : 4-159; 169-232 β : 3-890; 913-1,341 β' : 16-933; 946-1,126; 1,135-1,373 ω : 2-74	align	0.562 (2,923)	0.641 (2,666)	0.591 (2,732)
		rms_cur ^a	0.701 (3,162)	1.071 (3,159)	0.836 (3,162)
Core module	αI : 7-158; 167-234 αII : 4-159; 169-232 β : 3-30; 140-150; 445-455; 513-713; 786-832; 1,056- 1,318 β' : 343-368; 421-786 ω : 2-74	align	0.488 (1,305)	0.446 (1,274)	0.412 (1,285)
		rms_cur ^a	0.567 (1,369)	0.550 (1,366)	0.508 (1,369)

^a Structures were superimposed over the core module using the PyMOL align command prior to the rms_cur command.