

Supplementary Table 1. Cryo-EM data collection

Data set	D93N-I	D93N-II	D93N-III	D93N-IV
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Camera	K2 Summit	K2 Summit	K2 Summit	K2 Summit
Data collection and processing				
Magnification	22,500	22,500	22,500	22,500
Voltage (kV)	300	300	300	300
Electron Exposure ($e^-/\text{\AA}^2$)	80	80	70	70
Exposure time (sec)	12	16	14	14
Defocus range (μm)	-0.5 to -2.3	-0.5 to -2.5	-1.0 to -2.5	-0.5 to -2.5
Pixel Size ($\text{\AA}/\text{pixel}$)	1.059	1.059	1.059	1.059
Micrographs collected (no.)	3145	3213	489	4248
Particles picks (no.)	986,519	1,397,986*		1,728,005
Refined particles (no.)	139,412	214,315*		282,329

* Data sets II and III were merged and processed together.

Supplementary Table 2. Cryo-EM single-particle analyses and model refinement/validation statistics

Map	Hsp90:Hsp70:Hop:GR	Hsp90A _{NTD-MD} : Hsp70C _{NBD}	Hsp90B _{NTD-MD} : Hsp70S _{NBD}	Hsp90B _{NTD-MD} : Hsp70S _{NBD} :Hop _{TPR2A-TPR2B-DP2}	Hsp70S _{NBD} :Hop _{TPR2A}	Hsp90AB _{CTD} :Hsp70C _{SBD-β} : Hop _{DP2} :GR _{Helix 1}
EMDB ID	EMD-23050	EMD-23053	EMD-23054	EMD-23056	EMD-23055	EMD-23051
PDB ID	7KW7					
Classification type	Global	Focused (signal subtraction)	Focused (signal subtraction)	Focused (signal subtraction)	Focused (signal subtraction)	Focused (signal subtraction)
Resolution (Å) at FSC 0.143	3.57	3.77	3.53	3.85	3.64	3.46
Estimated B-factor (Å ²)	-107.5	-156.4	-91.5	-150.0	-116.4	-112.6
Refined particles (no.)	85619	35024	56945	37845	60433	43626
Coordinates refinement						
Initial models used (PDB code)	Hsp90: 3T0H, 3Q6M, 5FWK Hsp70: 3AY9, 4PO2, 4EZQ Hop: 3UQ3, 2LLW GR: 1M2Z					
Resolution (Å) at FSC 0.5	3.99					
Map sharpening B-factor for modeling (Å ²)	-80					
Model composition						
Non-hydrogen atoms	21854					
Protein residues atoms	21796					
Ligand atoms	58					
Mean B-factor (min – max) [Å ²]						
Protein residues	202.8 (18.8 – 600.0)					
Ligands	118.4 (62.8 – 202.0)					
Model geometry (r.m.s.d.)						
Bond lengths (Å)	0.0209					
Bond angles (°)	1.73					
Ramachandran plot						
Favored (%)	98.71					
Allowed (%)	1.25					
Disallowed (%)	0.04					
Validation						
MolProbity score	0.76					
Clash score	0.82					
Poor rotamers (%)	0.00					

Supplementary Table 3. Homologous residue numbering for residues involved in Interface I & II of Hsp90:Hsp70 in the GR-loading complex

	Hsp90 α	Hsp82	Hsc82	Hsp70	Ssa1
Interface I residues	S53	S39	S39	Q156	Q154
	D57	D43	D43	D160	D158
	R60	R46	R46	V163	T161
	Y61	Y47	Y47	I164	I162
	L64	L50	L50		
	Y216	Y203	Y203		
Interface II residues	E332	E312	E308	R171	R169
	G333	G313	G309	N174	N172
	Q334	Q314	Q310	T177	T175
	E336	E316	E312	D213	E210
	K414	K394	K390	D214	D211
	K418	K398	K394	I216	I213
	K419	K399	K395	F217	F214