Supplementary Material:

Supplementary Table 1: PISA interfaces in the crystal structure of Plk-1:DARPin 3H10

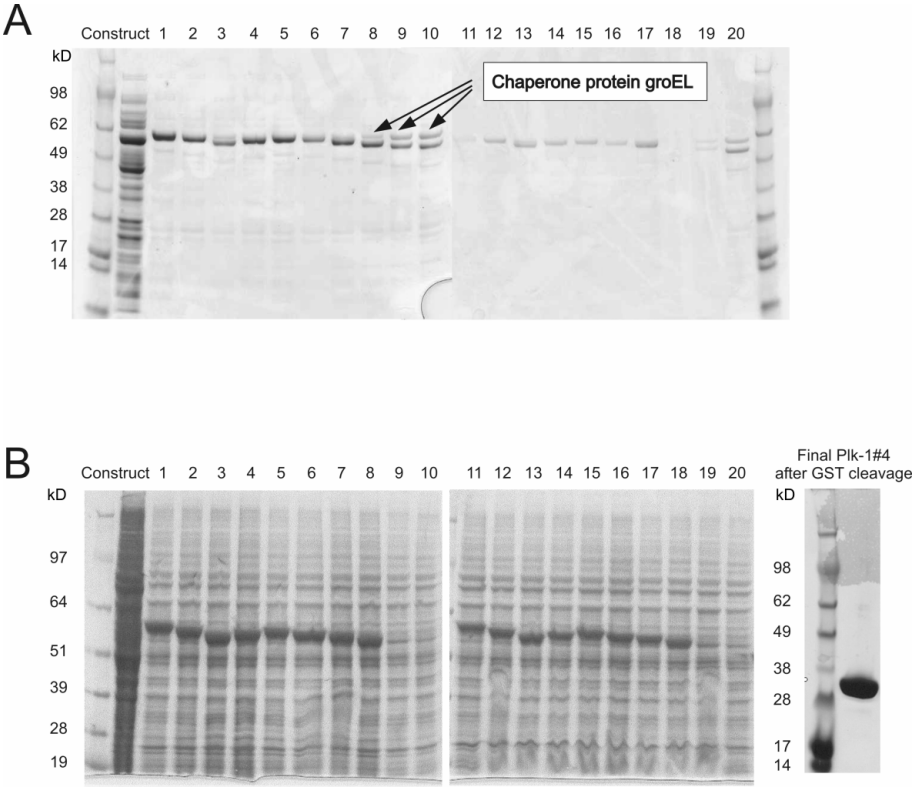
Molecule 1			Molecule 2				Interface	Δ^iG (Δ^tG)	Δ^iG				
Name	N_{at}	N_{res}	Name	Symm. Op.	N_{at}	N_{res}	Area (Å^2)	kcal/mol	P-value	N_{HB}	N_{SB}	CSS	Label
DARPin mol. D	86	25	Plk1 mol. A	x,y,z	89	21	871.4	-4.7 (-14.4)	0.197	14	9	1.00	AD
DARPin mol. C	80	23	Plk1 mol. B	x,y,z	87	21	852.7	-4.0 (-13.0)	0.209	15	5	1.00	BC
Plk1 mol. A	69	21	Plk1 mol. B	x,y,z	66	20	655.6	3.6 (-1.9)	0.786	11	0	0.00	AB
DARPin mol. C	41	13	DARPin mol. D	x,y,z	46	14	469.7	-8.3 (-8.8)	0.071	1	0	0.00	CD
DARPin mol. C	86	23	Plk1 mol. A	-x,y-1/2,-z+1/2	76	21	755.2	-3.2 (-8.6)	0.309	9	3	0.00	AC*
Plk1 mol. B	61	20	DARPin mol. D	-x+1/2,-y,z-1/2	69	22	609.5	-0.8 (-4.4)	0.597	6	2	0.00	BD*
Plk1 mol. A	30	6	Plk1 mol. B	x-1/2,-y+1/2,-z	33	10	278.4	-1.6 (-2.6)	0.408	2	0	0.00	AB*

N_{at} – number of atoms from the specified molecule that take part in the interface; **N_{res}** – number of residues from the specified molecule that take part in the interface; **Symm. Op.** – symmetry operation in the space group $P2_12_12_1$ (including lattice translations) applied to the specified molecule in the asymmetric unit of the crystal structure in order to generate the interface; **Interface Area (Å^2)** – Calculated as one-half of the difference between the total accessible surface areas of the isolated and interfacing structures; **Δ^iG** – calculated solvation free energy gain upon formation of the interface; **Δ^iG P-value** – probabilistic measure of randomness for the calculated solvation free energy gain; **Δ^tG** – estimated total free energy gain upon formation of interface, including contributions from hydrogen bonds and salt bridges across the interface; **N_{HB}** – number of potential hydrogen bonds across the interface. Contribution to free energy is approximately 0.5 kcal/mol per bond; **N_{SB}** – number of potential salt bridges across the interface. Contribution to free energy is approximately 0.3 kcal/mol per bond; **CSS** – Complexation Significance Score, which indicates how significant for assembly formation the interface is. The score is defined as a maximal fraction of the total free energy of binding that belongs to the interface in stable assemblies.

Supplementary Table 2: Interactions in the Plk-1:DARPin 3H10 interface AD [a]

Plk-1 residue	DARPin counterpart	N_{int}	d_{min}, Å	Type of closest interaction
Arg A57	Met D111	7	3.5	weak VdW
Leu A59	Met D111	1	3.7	weak VdW
Arg A134	Met D111	3	3.4	weak HB to carbonyl oxygen
	Thr D112	3	3.8	weak VdW
Arg A135	Tyr D79	2	3.6	weak VdW
	Phe D81	5	3.5	VdW
	Asp D110	5	2.9	SB
	Thr D112	5	3.4	VdW
Arg A136	Val D78	7	2.9	HB to carbonyl oxygen
	Tyr D79	2	3.8	weak VdW
	Met D111	2	3.5	VdW
Glu A140	Thr D46	1	3.9	weak VdW
	Tyr D79	2	3.5	VdW [b]
Leu A141	Tyr D79	2	3.6	weak VdW
Lys A143	Asn D45	5	3.7	weak VdW [c]
	Thr D46	1	3.7	weak VdW
Arg A144	Thr D46	10	2.9	HB
	Leu D48	7	3.5	VdW
	Asp D77	5	2.9	SB
	Tyr D79	9	3.3	VdW
Glu A186	Thr D112	4	3.3	VdW
Arg A313	Asp D122	3	3.7	weak VdW
	Glu D123	9	2.6	SB
Pro A315	Glu D123	5	3.2	VdW
Ile A316	Phe D81	1	3.9	weak VdW
	Met D89	1	3.9	weak VdW
	Glu D123	4	3.1	VdW
Thr A317	Met D89	6	3.5	VdW
	Thr D90	1	3.7	weak VdW
Leu A319	Tyr D79	2	3.9	weak VdW
Thr A320	Val D56	1	4.0	weak VdW
Ile A312	Val D56	1	4.0	weak VdW
	Thr D90	1	4.0	weak VdW

N_{int} – number of intermolecular contacts shorter than 4.0 Å between the two residues; d_{min} – shortest distance; VdW – Van der Waals contact (designated weak if larger than 3.5 Å); SB – salt bridge; HB – hydrogen bond (designated weak if distance between donor and acceptor atoms longer than 3.2 Å). Notes: [a] In addition to the interactions listed here there are at least three more which are mediated via a water molecule, each involving two residues from Plk-1 and one residue from DARPin 3H10: Arg A134, Arg A135 and Tyr D79; Arg A135, Ser A137 and Tyr D79; Lys A143, Lys A146 and Asn D145. [b] Forces side-chain to fold back and form two additional intramolecular HB to Ser A137. [b] The side chain of Lys A143 is ordered, fully extended and masked by this interaction (not unfavorable anymore for crystal contact).



Supplementary Figure 1