Supporting Information for:

Biomolecular condensation using *de novo* designed globular proteins

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Supplementary Table 1. Amino acid sequences for the proteins used in the study

#	Name	Sequence
1	sc-apCC-4-SP (#1)	M K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE GEPSAQG K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE GPDSV K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE GGTWSGG K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE *
2	sc-apCC-4-#2	M E QLKKIAE QLKKIAQ QLEEIAK QLEEIAK GEPSAQG K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE GPDSV E QLKKIAE QLKKIAQ QLEEIAK QLEEIAK GGTWSGG K QLEEIAK QLEEIAQ QLKKIAE QLKKIAE
3	sc-apCC-4-#3	M E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GEPSAQG E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GPDSV E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GGTWSGG E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK*
4	sc-apCC-4-#4	M E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GEPSAQG K QLKKIAE QLEEIAQ QLKKIAK QLEEIAE GPDSV K QLKKIAE QLEEIAQ QLKKIAK QLEEIAE GGTWSGG E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK*
5	sc-apCC-4-#5	M E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GEPSAQG K QLKKIAK QLKKIAQ QLEEIAE QLEEIAE GPDSV E QLEEIAK QLKKIAQ QLEEIAE QLKKIAK GGTWSGG K QLKKIAK QLKKIAQ QLEEIAE QLEEIAE*
6	sc-apCC-4-LP (#6)	M E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK GEPSAQG E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK GPDSV E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK GGTWSGG E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK *
7	sc-apCC-4-#7	M E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK GEPSAQG K QLKKIAK QLKKIAQ QLEEIAE QLEEIAE GPDSV K QLKKIAK QLKKIAQ QLEEIAE QLEEIAE GGTWSGG E QLEEIAE QLEEIAQ QLKKIAK QLKKIAK*
8	sc-apCC-4-#8	M E QLEEIAE QLEEIAQ QLEEIAE QLEEIAE GEPSAQG K QLKKIAK QLKKIAQ QLKKIAK QLKKIAK GPDSV E QLEEIAE QLEEIAQ QLEEIAE QLEEIAE GGTWSGG K QLKKIAK QLKKIAQ QLKKIAK QLKKIAK*
9	sc-apCC-4-LP-NR-1	M E QLEEIAE QLEEGAQ QAKKAAK QLKKIAK GEPSAQG E QLEEIAE QVEEIAQ QIKKIAK QLKKIAK GPDSV E QLEEIAE QLEEIAQ QVKKIAK QLKKIAK GGTWSGG E QLEEIAE QAEEGAQ QAKKIAK QLKKIAK*
10	sc-apCC-4-LP-NR-2	M E QLEEIAE QLEEGAQ QTKKNAK QLKKIAK GEPSAQG E QLEEIAE QAEEIAQ QSKKIAK QLKKIAK GPDSV E QLEEIAE QLEEIAQ QAKKGAK QLKKIAK GGTWSGG E QLEEIAE QAEEGAQ QAKKIAK QLKKIAK*
11	sc-apCC-4-NR-1	M QLEEIAQ QLEEGAK QAKKAAE QLKKIAE GEPSAQG QLEEIAQ QVEEIAQ QIKKIAW QLKKIAE GPDSV QLEEIAQ QLEEIAK QVKKIAE QLKKIAE GGTSGG QLEEIAQ QAEEGAQ QAKKIAW QLKKIAE GS*
12	sc-apCC-4-NR-2	M QLEEIAQ QLEEGAK QTKKNAE QLKKIAE GEPSAQG QLEEIAQ QAEEIAQ QSKKIAW QLKKIAE GPDSV QLEEIAQ QLEEIAK QAKKGAE QLKKIAE GGTSGG QLEEIAQ QAEEGAQ QAKKIAW QLKKIAE GS*
13	DB-SP-GFP	MKQLEEIAKQLEEIAQQLKKIAEQLKKIAEGEPSAQGKQLEEIAKQLEEIAQQLKKIAE QLKKIAEGPDSVKQLEEIAKQLEEIAQQLKKIAEQLKKIAEGGTWSGGKQLEEIAKQLE EIAQQLKKIAEQLKKIAEGASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFTGVV PILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCF ARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDF

		KEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIG DGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGASPEPQPKP SGDPQSKQTPEPSRSQGMKQLEEIAKQLEEIAQQLKKIAEQLKKIAEGEPSAQGKQLEE IAKQLEEIAQQLKKIAEQLKKIAEGPDSVKQLEEIAKQLEEIAQQLKKIAEQLKKIAEG GTWSGGKQLEEIAKQLEEIAQQLKKIAEQLKKIAE*
14	DB-LP-GFP	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAEQLE EIAQQLKKIAKQLKKIAKGASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFTGVV PILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCF ARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDF KEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIG DGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGASPEPQPKP SGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEE IAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKG GTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGSGGS*
15	sc-apCC-4-LP-[loop3:GFP]	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWMVSKGEELFTGVVP ILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFA RYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGD GPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSGGEQLEEIAE QLEEIAQQLKKIAKQLKKIAK*
16	sc-apCC-4-LP:GS5:GFP	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAEQLE EIAQQLKKIAKQLKKIAKGSGSGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEG DATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQ ERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYIT ADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPN EKRDHMVLLEFVTAAGITLGMDELYK*
17	sc-apCC-4-LP:L:GFP	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAEQLE EIAQQLKKIAKQLKKIAKGSASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFTGV VPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQC FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGID FKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPI GDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
18	DB-LP-GFP-NR-1	MEQLEEIAEQLEEGAQQAKKAAKQLKKIAKGEPSAQGEQLEEIAEQVEEIAQQIKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQVKKIAKQLKKIAKGGTWSGGEQLEEIAEQAE EGAQQAKKIAKQLKKIAKGSASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFTGV VPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQC FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGID FKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPI GDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTSGASPEPQ PKPSGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQ LEEIAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKI AKGGTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAK*
19	DB-LP-GFP-NR-2	MEQLEEIAEQLEEGAQQTKKNAKQLKKIAKGEPSAQGEQLEEIAEQAEEIAQQSKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQAKKGAKQLKKIAKGGTWSGGEQLEEIAEQAE EGAQQAKKIAKQLKKIAKGSASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFTGV VPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQC FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGID FKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPI GDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKTSGASPEPQ PKPSGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQ LEEIAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKI AKGGTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAK*

20	DB-LP-NR-1	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAEQLE EIAQQLKKIAKQLKKIAKGASPEPQPKPSGDPQSKQTPEPSRSQGMQLEEIAQQLEEGA KQAKKAAEQLKKIAEGEPSAQGQLEEIAQQVEEIAQQIKKIAWQLKKIAEGPDSVQLEE IAQQLEEIAKQVKKIAEQLKKIAEGGTSGGQLEEIAQQAEEGAQQAKKIAWQLKKIAEG ASPEPQPKPSGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEP SAQGEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIA KQLKKIAKGGTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAK*
21	DB-LP-NR-2	MEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKKIAK QLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAEQLE EIAQQLKKIAKQLKKIAKGASPEPQPKPSGDPQSKQTPEPSRSQGMQLEEIAQQLEEGA KQTKKNAEQLKKIAEGEPSAQGQLEEIAQQAEEIAQQSKKIAWQLKKIAEGPDSVQLEE IAQQLEEIAKQAKKGAEQLKKIAEGGTSGGQLEEIAQQAEEGAQQAKKIAWQLKKIAEG ASPEPQPKPSGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEP SAQGEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIA KQLKKIAKGGTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAK*
22	KIF5C : DB-LP-GFP	MADPAECSIKVMCRFRPLNEAEILRGDKFIPKFKGEETVVIGQGKPYVFDRVLPPNTTQ EQVYNACAKQIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPQLMGIIPRIAHDIFD HIYSMDENLEFHIKVSYFEIYLDKIRDLLDVSKTNLAVHEDKNRVPYVKGCTERFVSSP EEVMDVIDEGKANRHVAVTNMNEHSSRSHSIFLINIKQENVETEKKLSGKLYLVDLAGS EKVSKTGAEGAVLDEAKNINKSLSALGNVISALAEGTKTHVPYRDSKMTRILQDSLGGN CRTTIVICCSPSVFNEAETKSTLMFGQRAKTIKNTVSVNLELTAEEWKKKYEKEKEKNK ALKSVIQHLEVELNRWRNGEAVPEDEQISAKDQKNLEPCDNTPIIDNITPVTGGSGSGS GGTMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKK IAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAE QLEEIAQQLKKIAKQLKKIAKGASPEPQPKPSGDPQSKQTPEPSRSQGMGVSKGEELFT GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGV QCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKG IDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNT PIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGASPEPQ PKPSGDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQ LEEIAEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKI AKGGTWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGSGGS*
23	KIF5C : GFP	MADPAECSIKVMCRFRPLNEAEILRGDKFIPKFKGEETVVIGQGKPYVFDRVLPPNTTQ EQVYNACAKQIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPQLMGIIPRIAHDIFD HIYSMDENLEFHIKVSYFEIYLDKIRDLLDVSKTNLAVHEDKNRVPYVKGCTERFVSSP EEVMDVIDEGKANRHVAVTNMNEHSSRSHSIFLINIKQENVETEKKLSGKLYLVDLAGS EKVSKTGAEGAVLDEAKNINKSLSALGNVISALAEGTKTHVPYRDSKMTRILQDSLGGN CRTTIVICCSPSVFNEAETKSTLMFGQRAKTIKNTVSVNLELTAEEWKKKYEKEKEKNK ALKSVIQHLEVELNRWRNGEAVPEDEQISAKDQKNLEPCDNTPIIDNITPVTGGSGSGS GGTMGVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLP VPWPTLVTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVK FEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHKVYITADKQKNGIKVNFKTRHNIE DGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITL GMDELYK
24	KIF5C : DB-LP-mCh	MADPAECSIKVMCRFRPLNEAEILRGDKFIPKFKGEETVVIGQGKPYVFDRVLPPNTTQ EQVYNACAKQIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPQLMGIIPRIAHDIFD HIYSMDENLEFHIKVSYFEIYLDKIRDLLDVSKTNLAVHEDKNRVPYVKGCTERFVSSP EEVMDVIDEGKANRHVAVTNMNEHSSRSHSIFLINIKQENVETEKKLSGKLYLVDLAGS EKVSKTGAEGAVLDEAKNINKSLSALGNVISALAEGTKTHVPYRDSKMTRILQDSLGGN CRTTIVICCSPSVFNEAETKSTLMFGQRAKTIKNTVSVNLELTAEEWKKKYEKEKEKNK ALKSVIQHLEVELNRWRNGEAVPEDEQISAKDQKNLEPCDNTPIIDNITPVTGGSGSGS GGTMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEIAEQLEEIAQQLKK IAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGGTWSGGEQLEEIAE QLEEIAQQLKKIAKQLKKIAKGASPEPQPKPSGDPQSKQTPEPSRSQGMVSKGEEDNMA IIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQF MYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKL RGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKA

		KKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKGASPEPQPKPS GDPQSKQTPEPSRSQGMEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGEPSAQGEQLEEI AEQLEEIAQQLKKIAKQLKKIAKGPDSVEQLEEIAEQLEEIAQQLKKIAKQLKKIAKGG TWSGGEQLEEIAEQLEEIAQQLKKIAKQLKKIAK*
25	KIF5C : mCherry	MADPAECSIKVMCRFRPLNEAEILRGDKFIPKFKGEETVVIGQGKPYVFDRVLPPNTTQ EQVYNACAKQIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPQLMGIIPRIAHDIFD HIYSMDENLEFHIKVSYFEIYLDKIRDLLDVSKTNLAVHEDKNRVPYVKGCTERFVSSP EEVMDVIDEGKANRHVAVTNMNEHSSRSHSIFLINIKQENVETEKKLSGKLYLVDLAGS EKVSKTGAEGAVLDEAKNINKSLSALGNVISALAEGTKTHVPYRDSKMTRILQDSLGGN CRTTIVICCSPSVFNEAETKSTLMFGQRAKTIKNTVSVNLELTAEEWKKKYEKEKEKNK ALKSVIQHLEVELNRWRNGEAVPEDEQISAKDQKNLEPCDNTPIIDNITPVTGGSGSGS GGTMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKG GPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQD SSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD GGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYK

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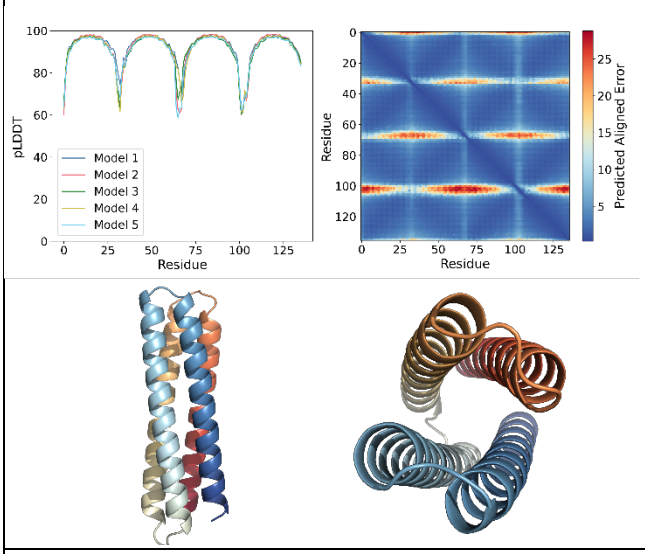
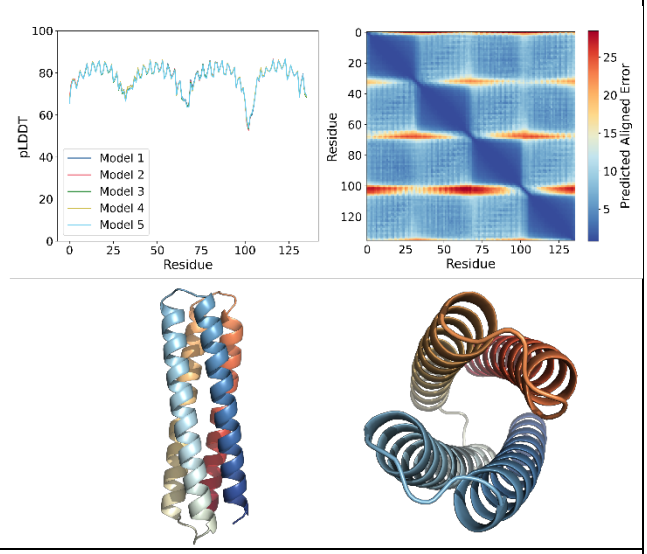
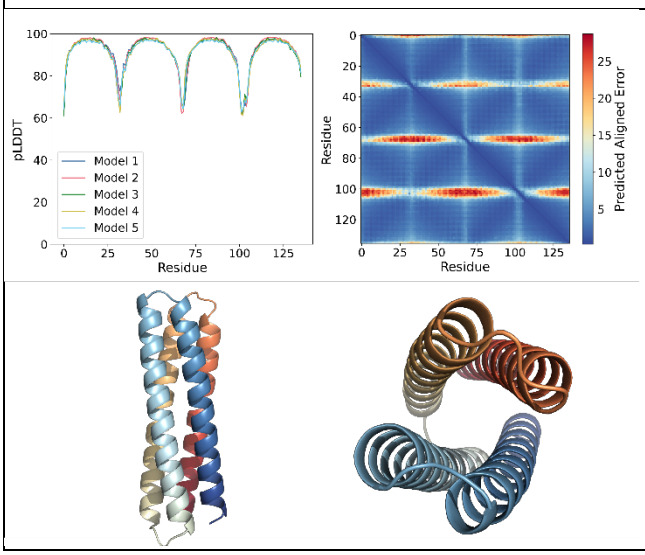
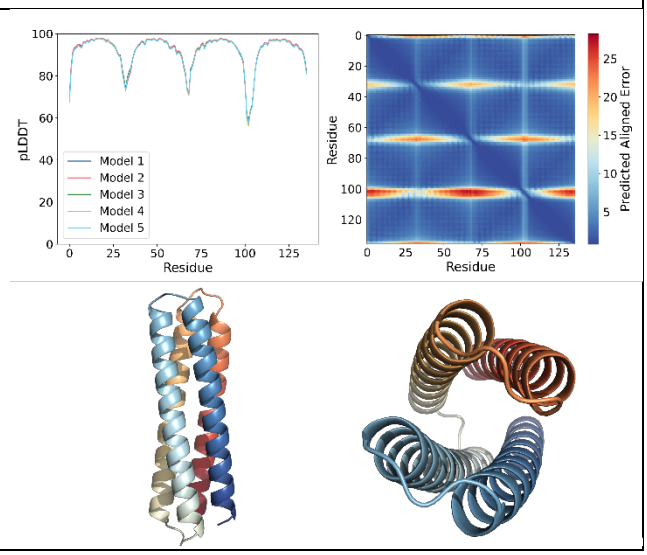
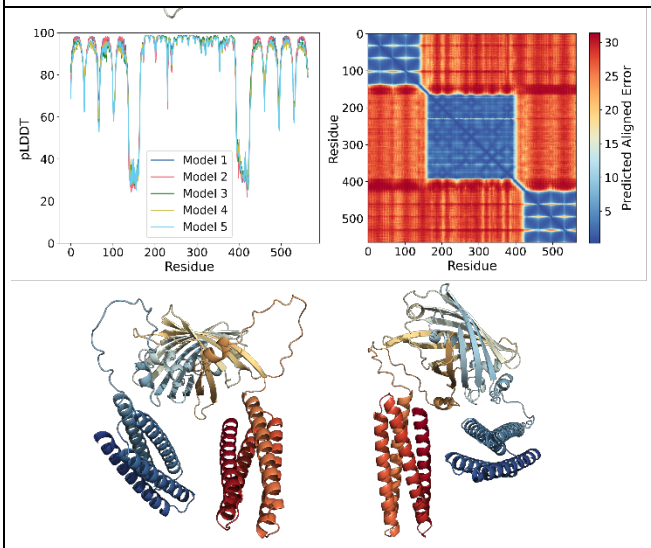
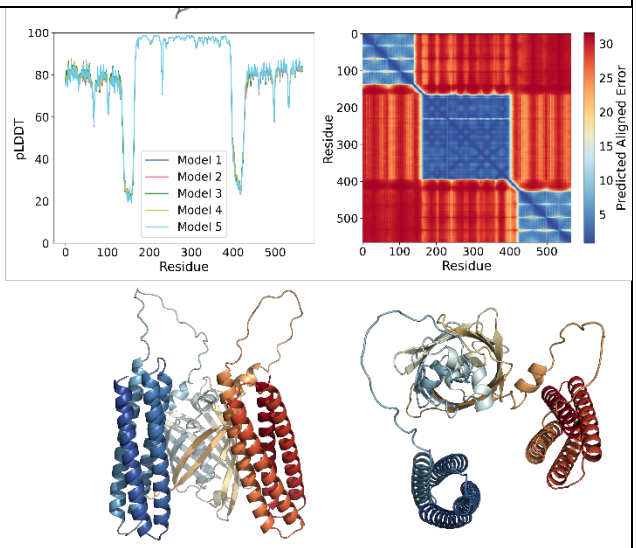
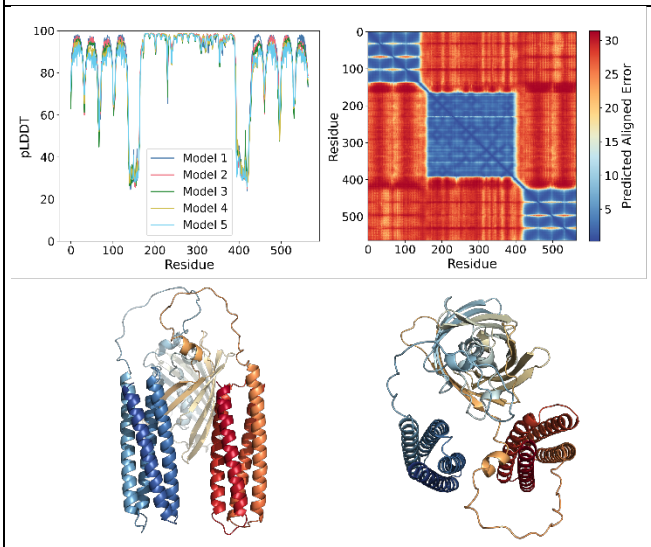
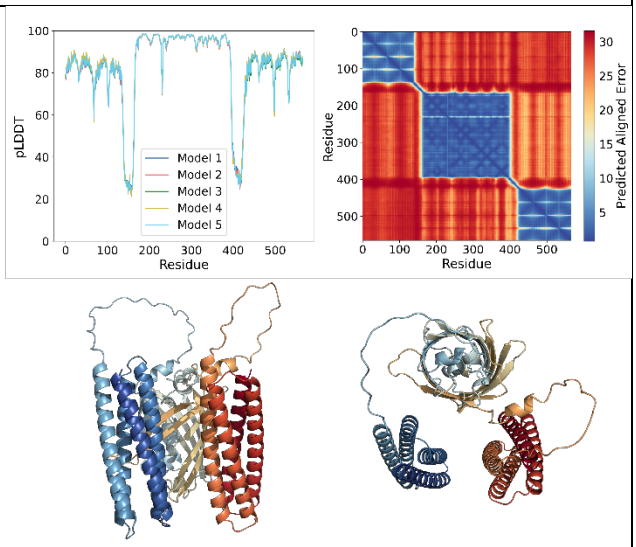
Supplementary Table 2. AlphaFold2 and AlphaFold3 predictions of 3D structures for sc-apCC-4 and DB-GFP variants

AF2	AF3
sc-apCC-4-SP	
	
sc-apCC-4-LP	
	

AF2	AF3
DB-SP-GFP	
	
DB-LP-GFP	
	

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Supplementary Table 3. Literature data for protein dynamics

probe/environment	FRAP recovery half-time (s)	Diff. Coefficient ($\mu m^2/s$)	viscosity (mpa·S)	Source
FUS-GFP/ FUS granules in cytoplasm of HeLa	~ 0.1-1.0		10–100	[1]
$DDX4^{YFP}$ / $DDX4^{YFP}$ BCs	~ 2.5	0.3 ± 0.1 (FRAP)		[2]
$DDX4^{N1}$ + $DDX4^{YFP}$ / *in vitro*	~ 60	0.4 ± 0.1 (FRAP)		[2]
LAF-1 / *in vitro*	161 ± 42 s	0.010 ± 0.003 (FRAP)		[3]
LAF-1 + RNA / *in vitro*	143 ±28	0.011 ± 0.002 (FRAP)		[3]
hnRNPA1-Oregon-green + hnRNPA1 / in vitro	3.72 (fast) and 31.6 (slow)			[4]
hnRNPA1-Oregon-green / stress granules	4.2			[4]
NPM1 / *X. laevis* nucleoli	44 ± 6		740 ± 60	[5]
NPM1 / *in vitro*	16 ± 1			[5]
EGFP / cytoplasm of HeLa	NA	56 ±11 (FCS)	1.93 ± 0.38*	[6]
EGFP / cytoplasm of HeLa	NA	50.6 (FCS)	2.14*	[7]

*** Calculated using the data from literature**

Supplementary Table 4. Summary of fitting the *in vitro* FRAP data for DB-LP-GFP protein.

Conditions: 4 mg/mL protein, Tris 20 mM pH 7.5, NaCl 150 mM, 2% PEG3350, 37 °C.

Model	Parameter	Value	SD	Half-time [s]	R^2	Red. chi^2
Monoeexponential	a	-0.709	0.019	0.6928	0.823	0.002515
	b	1.00	0.05			
	c	0.798	0.003			
Biexponential	a1	-0.64	0.04	0.5311	0.8467	0.002186
	b1	0.58	0.06			
	a2	-0.17	0.04			
	b2	4.09	0.96			
	c	0.815	0.005			
Stretched exponential (Anomalous diffusion)	a	-0.88	0.04	0.4074	0.8387	0.002296
	b	0.73	0.07			
	alpha	0.64	0.04			
	c	0.806	0.003			

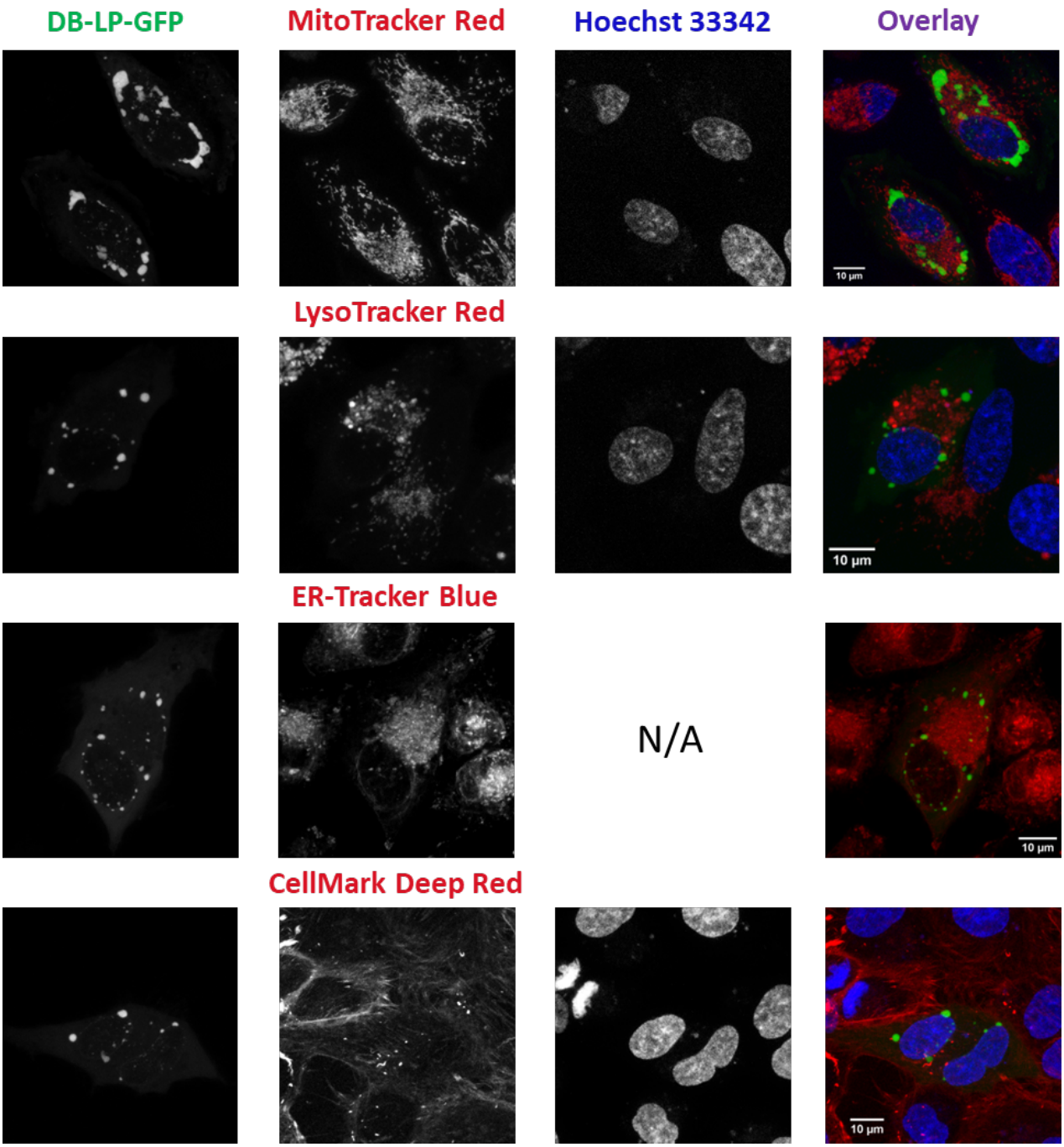
Supplementary Table 5. Summary of fitting the *in vivo* FRAP data for DB-LP-GFP protein expressed in HeLa. The measurements performed 24 hours after transfection at 37 °C.

Model	Parameter	Value	SD	Half-time	R^2	Red. chi^2
Monoexp	a	-0.528	0.014	2.266	0.9077	0.002684
	b	3.3	0.3			
	c	0.690	0.009			
Biexp	a1	-0.45	0.03	1.164	0.9232	0.002262
	b1	4.7	0.6			
	a2	-0.27	0.06			
	b2	0.34	0.12			
	c	0.713	0.012			
Stretched Exp	A	0.89	0.16	1.176	0.9222	0.002276
	k	0.38	0.08			
	beta	0.46	0.10			
	C	-0.100	0.110			

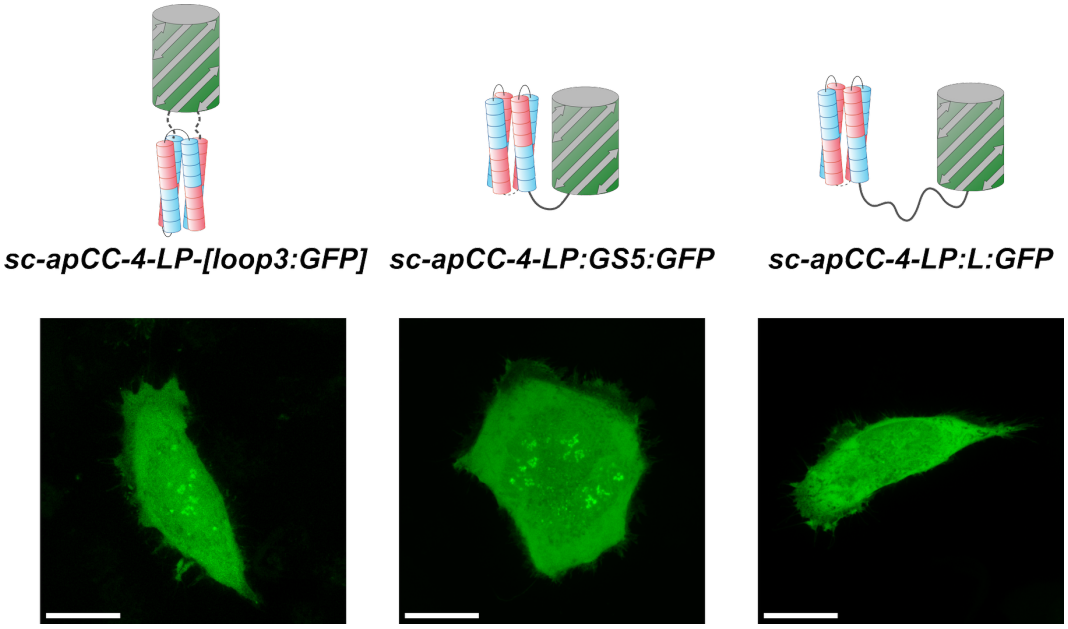
Supplementary Table 6. The results of fitting autocorrelated functions (Fig. 2E) measured for the diffusion of the probe protein using anomalous diffusion model.

Probe / environment	Probe hydrodynamic radius [nm]	Number of molecules (N)	τ_{D1} (slow)	r1(%)	r2(%)	An. factor	Diff. coefficient ($\mu m^2/s$) [fast]	Diff. coefficient ($\mu m^2/s$) [slow]	viscosity (mpaS) [high]
DB-LP-mCherry - / DB-LP-GFP BCs	4.40	6562.51*	22440 ± 1301	52	48	0.67	72.1±2.6	0.36±0.02	143±9
mCherry / DB-LP-GFP BCs	2.1	48.19	236 ± 49			0.57	34.3±7		3.2±0.7
DB-LP-mCherry / cytoplasm	4.40	12.75	342 ± 12			0.74	23.7±0.9		2.18±0.09
mCherry / cytoplasm	2.1	41.69	122 ± 10			0.62	66.5±6		1.63±0.14

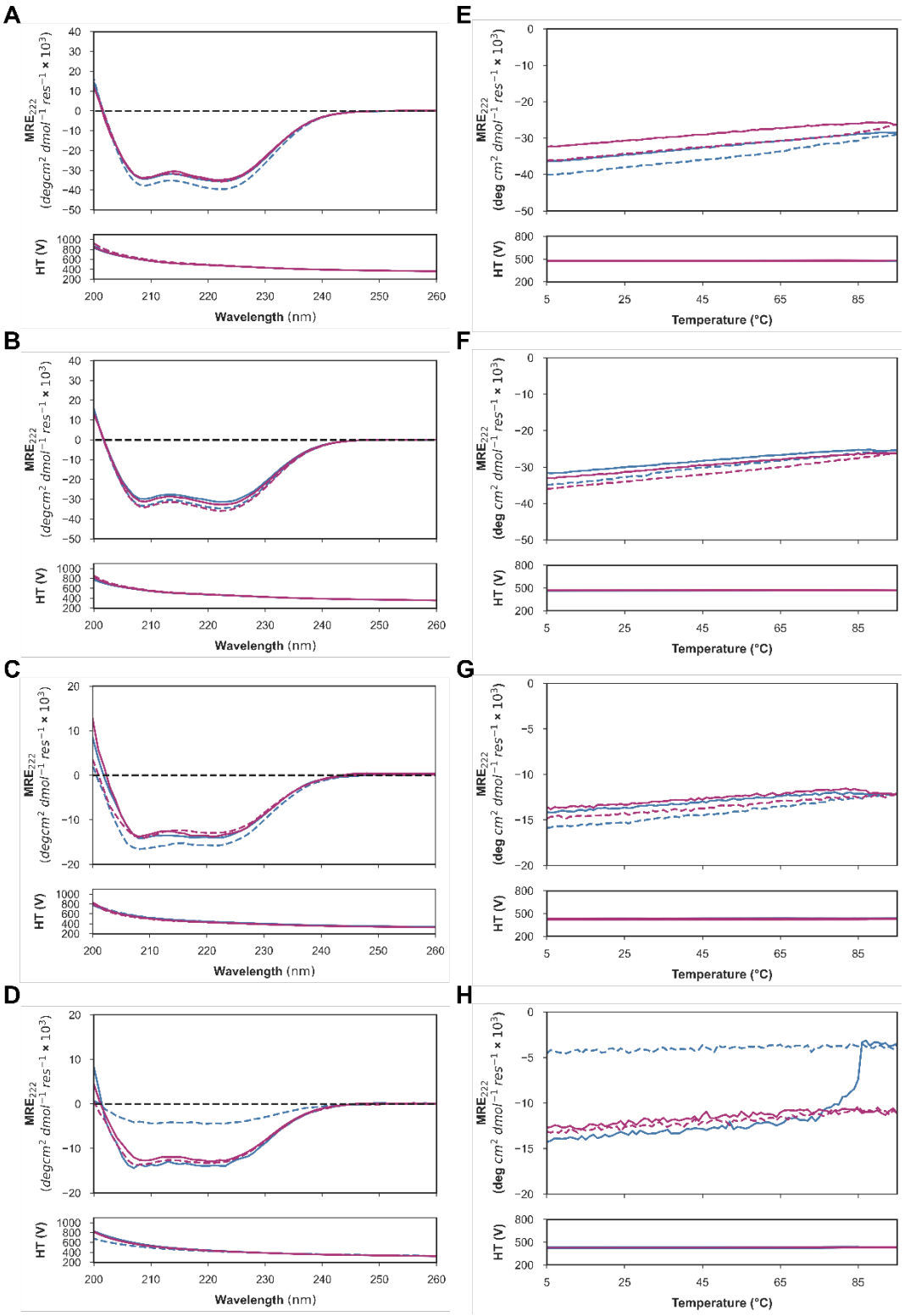
2 Supplementary figures



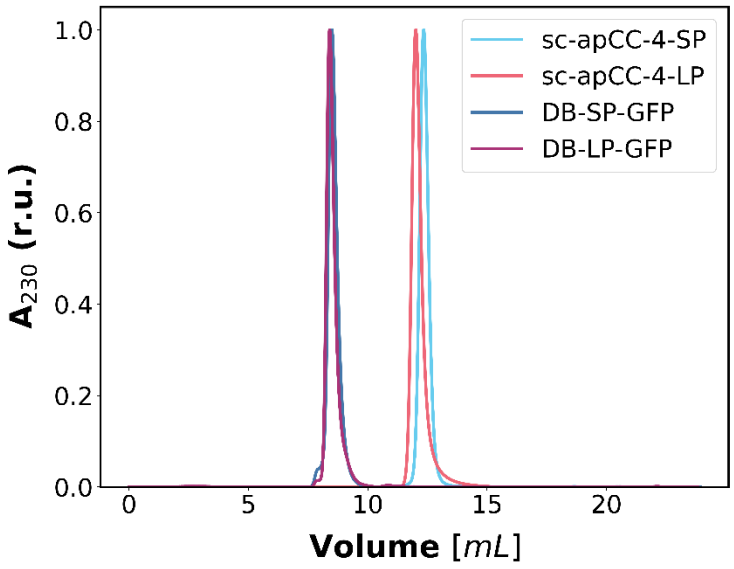
Supplementary Figure 1. Live confocal images of HeLa cells in 24 hours of transfection with the DB-LP protein and stained with organelle-specific fluorescent markers. Columns 1-3 correspond to the green, red, and blue channels respectively. The last column is showing the overlay of all channels in respective colours.



Supplementary Figure 2. Both sticker domains are required for BC formation in live cells. Confocal images of HeLa cells that transiently overexpressed sc-apCC-4-LP fusions to GFP: the loop fusion (1st column) in which GFP was inserted into the 3rd loop of the sc-apCC-4-; the linear fusion (2nd column) in which GFP was fused to the C-terminus of sc-apCC-4-LP with a short 5-residue long flexible GS linker, and another linear fusion with the same 25-residue long linker as in the dumbbell constructs (3rd column) matching the one in DB constructs. Scalebars = 20 μm.

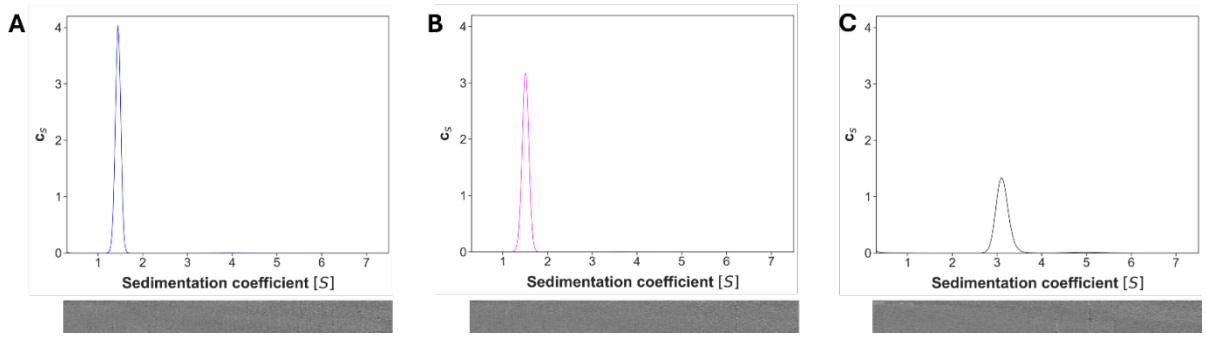


Supplementary Figure 3. CD spectra at 5 °C (A, B, C, D) and thermal response curves at 222 nm (E, F, G, H) of sc-apCC-4-SP (A, E), sc-apCC-4-LP (B, F), DB-SP-GFP (C, G) , and DB-LP-GFP (D, H) at 150 mM (blue line) and 500 mM (dark red line) NaCl before (solid line) and after (dashed line) annealing. Conditions: 50 mM Tris pH 7.5.



65

66 **Supplementary Figure 4.** Analytical size-exclusion chromatography traces for the designer
67 proteins sc-apCC-4-SP, sc-apCC-4-LP, DB-SP-GFP, DB-LP-GFP. Conditions: 50 mM Tris
68 pH 7.5, 500 mM NaCl, 0.8 mL/min, Sephadex 75pg Increase.



69

70 **Supplementary Figure 5.** Sedimentation velocity (SV) AUC traces of the *de novo* proteins
71 designed for this study: A) sc-apCC-4-SP, B) sc-apCC-4-LP, C) DB-LP-GFP. Fits returned
72 weights of 0.98, 1.04, and 1.02× monomer weight, respectively. 80 μM sc-apCC-4-SP and
73 sc-apCC-4-LP, 15 μM DB-LP-GFP. Conditions: 50 mM Tris pH 7.5, 500 mM NaCl, 20 °C.

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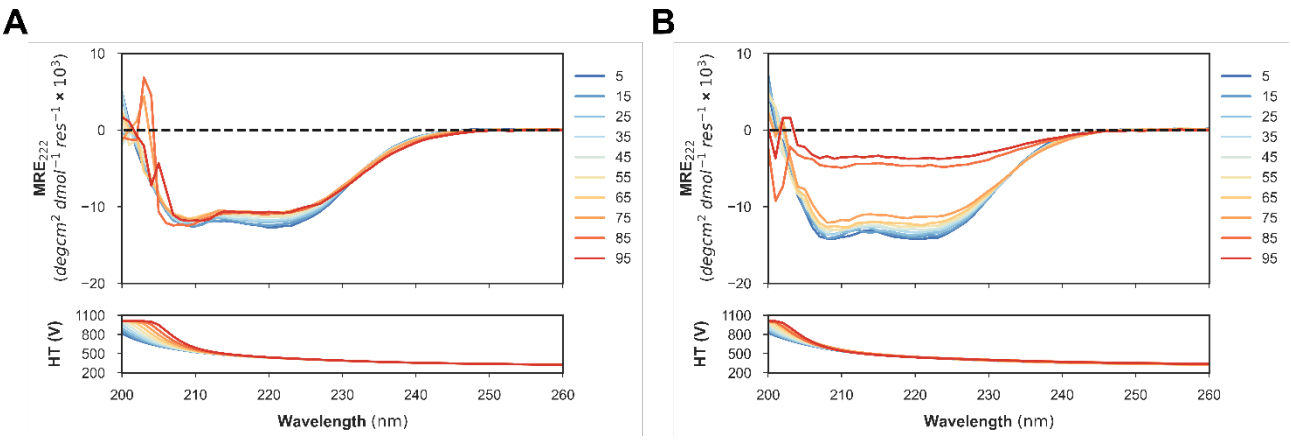
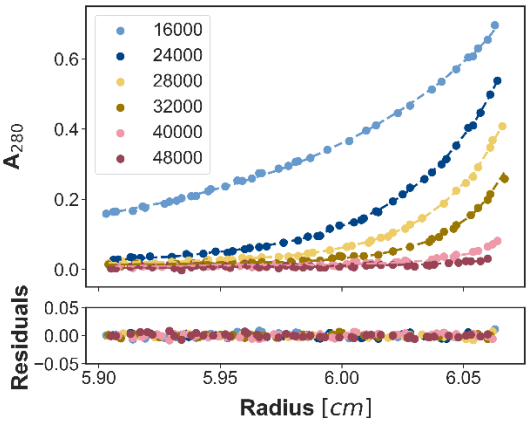
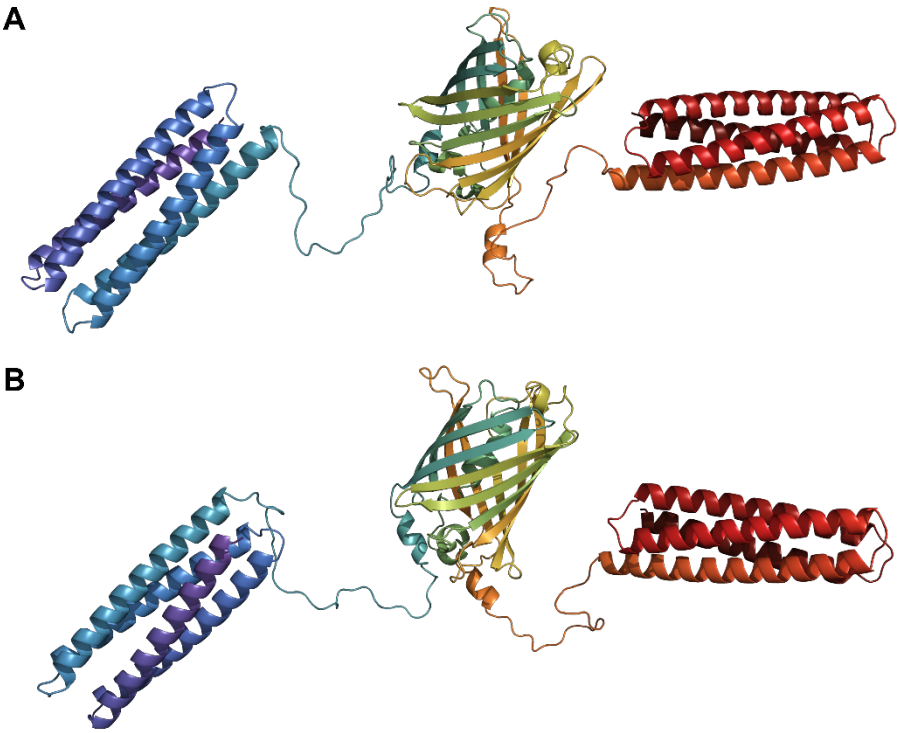


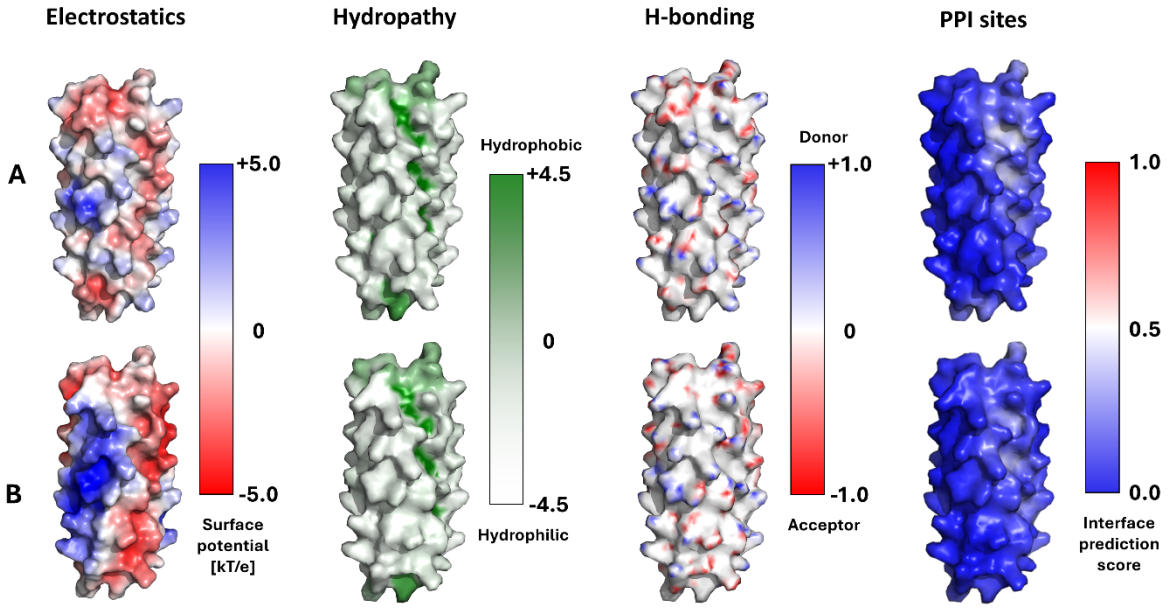
Supplementary Figure 6. CD spectra of DB-LP-GFP protein at 500 mM (A) and 150 mM (B) NaCl at various temperatures upon heating from 5 to 95 °C. Conditions: 50 mM Tris pH 7.5.



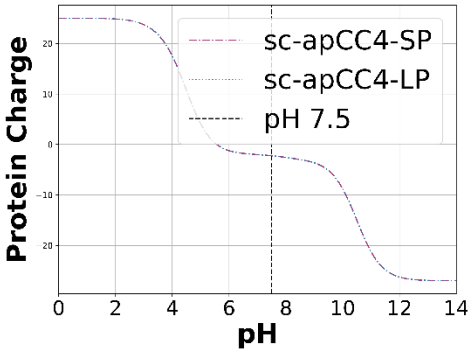
Supplementary Figure 7. Sedimentation equilibrium (SE) AUC data for DB-LP-GFP between 16 and 48 krpm. Fitted single-ideal species model curves are overlaid (dashed lines) and gave a molecular weight of 65.6 kDa which corresponds to 1.03× DB-LP-GFP monomer weight. 15 µM DB-LP-GFP. Conditions: 50 mM Tris pH 7.5, 500 mM NaCl, 20 °C.



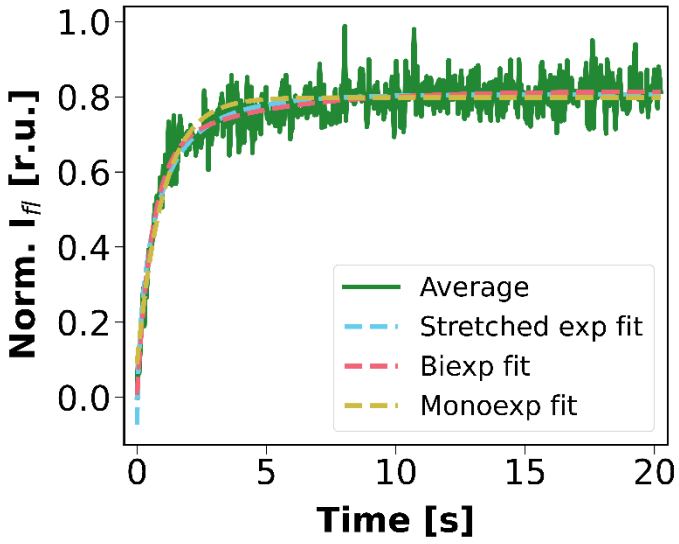
Supplementary Figure 8. The best-fit models for the SAXS data for A) DB-SP-GFP and B) DB-LP-GFP obtained using MultiFoXS (see Methods).



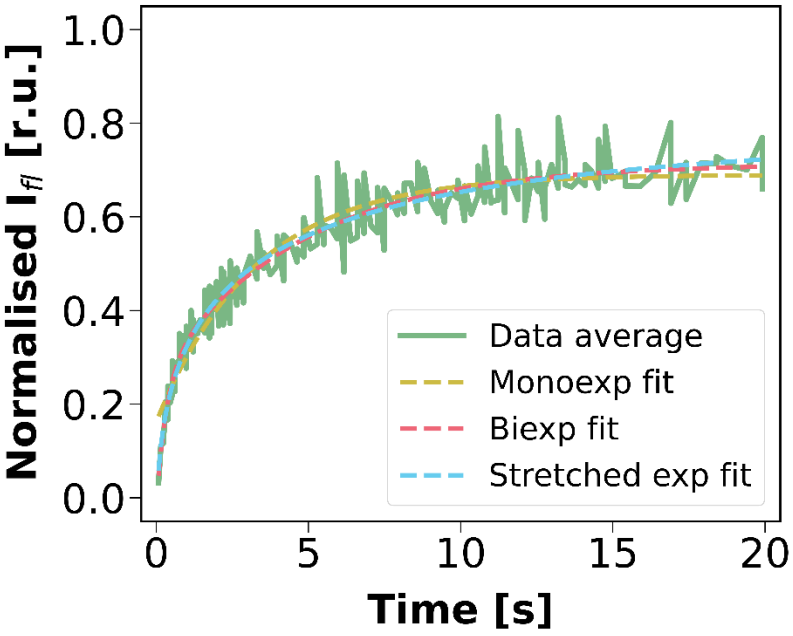
Supplementary Figure 9. APBS[8] and MaSIF[9] predictions for sc-apCC-4-SP and sc-apCC-4-LP.



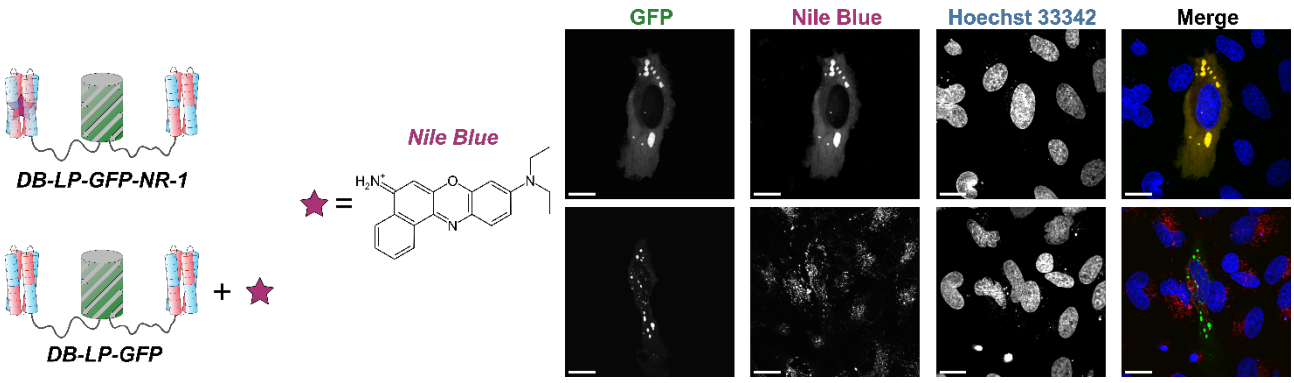
Supplementary Figure 10. PROPKA 3 prediction of the protein net charge at various pH values for sc-apCC-4-SP and sc-apCC-4-LP[10,11].



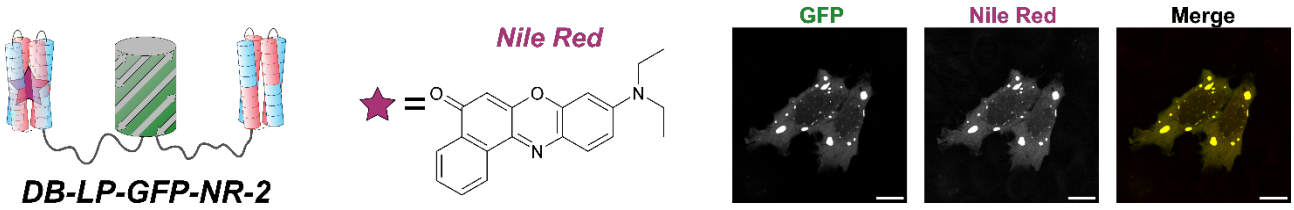
Supplementary Figure 11. Fitting the in vitro FRAP data for DB-LP-GFP protein with mono-, bi- and stretched exponential funcitons. Conditions: 4 mg/mL protein, Tris 20 mM pH 7.5, NaCl 150 mM, 2% PEG3350, 37 °C.



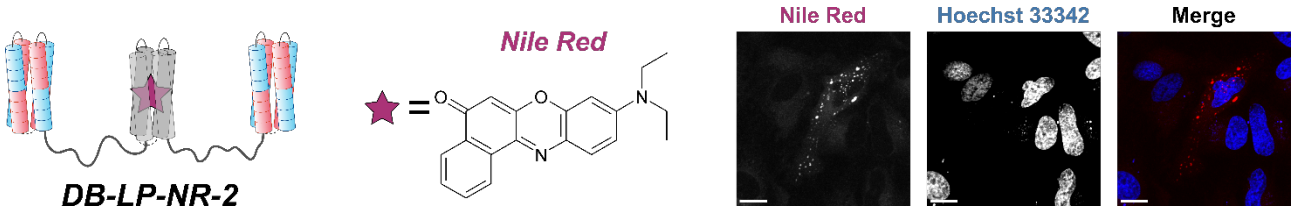
Supplementary Figure 12. Fitting the *in vivo* FRAP data for DB-LP-GFP protein expressed in HeLa. The measurements performed 24 hours after transfection at 37 °C.



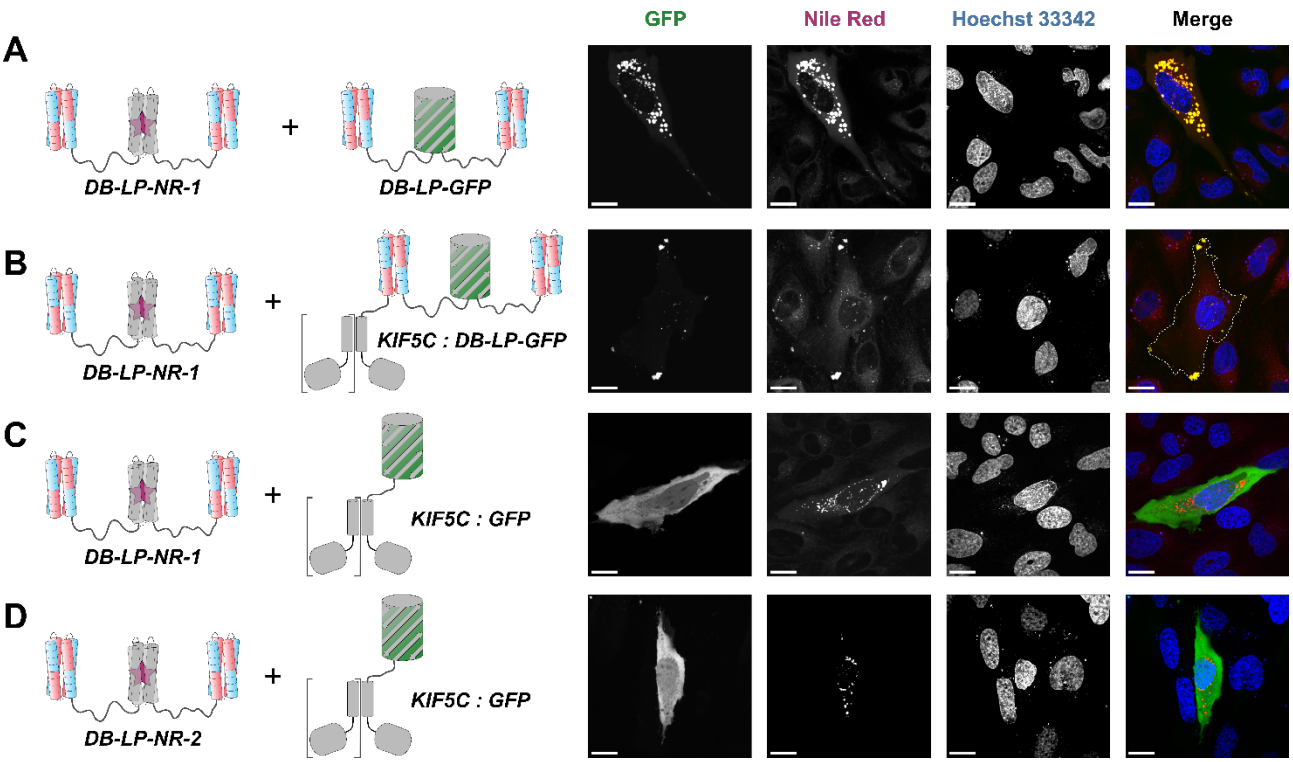
Supplementary Figure 13. Confocal fluorescence images of live HeLa cells expressing DB-LP-GFP-NR-1 (top row) and DB-LP-GFP (bottom row) and stained with Hoechst 33342 and Nile Blue (NB).



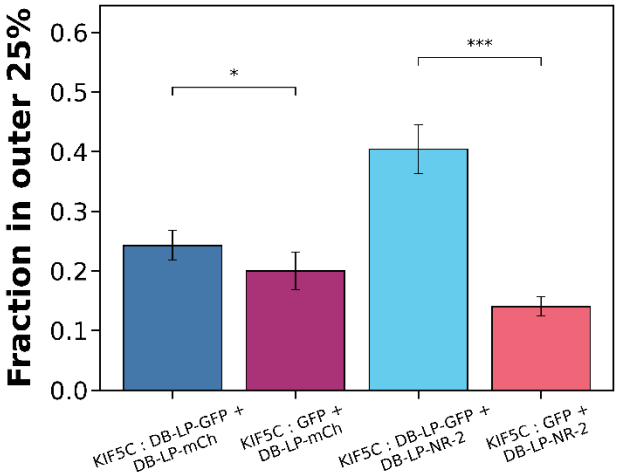
Supplementary Figure 14. Confocal fluorescence images of live HeLa cells expressing DB-LP-GFP-NR-2 stained with Nile Red (NR).



Supplementary Figure 15. Confocal fluorescence images of live HeLa cells expressing DB-LP-NR-2 stained with Nile Red (NR).



Supplementary Figure 16. Confocal fluorescence images of live HeLa cells co-expressing DB-LP-NR-1 with A) DB-LP-GFP, and B) KIF5C:DB-LP-GFP, and C) KIF5C:GFP fusions or D) DB-LP-NR-1 with KIF5C:GFP, and stained with Hoechst 33342 and Nile Red.



Supplementary Figure 17. Quantification of the distribution of the free DB construct (75% by DNA) in the presence of an overexpressed motor either fused to a dummy seed (GFP) or to a full length DB-LP-GFP (25% by DNA). Intensity in the outer 25% of cell was measured in a minimum of 25 cells.

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