Supporting Information

Table S1:

Data collection statistics

$R_{int} = 100\Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|/\Sigma_{hkl}\ \Sigma_i I_i(hkl)$

Data statistics	**CylR2**	**CylR2 T55C**	**CylR2 55R1**
Wavelength	0.8 Å	1.0 Å	0.8 Å
Beamline	SLS-PX2	SLS-PX2	SLS-PX2
Detector	MAR225 CCD	MAR225 CCD	MAR225 CCD
Space group	$P4_1$	$P4_1$	$P4_1$
a	63.412 Å	63.45 Å	63.35 Å
b	63.412 Å	63.45 Å	63.35 Å
c	41.315 Å	41.2 Å	40.97 Å
Resolution	1.21 Å (1.31-1.21 Å)	1.23 Å (1.33-1.23 Å)	1.5 Å (1.60-1.50 Å)
Reflections measured	366600	357078	211815
Unique reflections	48228	48194	25981
Redundancy	7.3 (6.77)	7.41 (6.92)	8.07 (7.38)
Completeness(%)	96.1 (93.6)	96.2 (93.6)	99.0 (98.0)
Mean I/σ (I)	20.94 (5.18)	27.81 (8.86)	31.77 (10.01)
R_{int} (%)	5.2 (31.20)	3.86 (19.99)	3.52 (17.28)

Table S2:

Refinement statistics

Refinement statistics	**CylR2**	**CylR2 T55C**	**CylR2 55R1**
R-factor	13.67%	14.80%	14.15%
Free *R*-factor	16.66%	18.4%	19.67%
Root mean square deviations from ideal geometry			
Bond lengths	0.013 Å	0.013 Å	0.009 Å
Bond angles	2.5°	2.3°	2.0°
No. of protein residues	132	132	131
No. of glycerol molecules	3	2	1
No. of water molecules	167	164	129
No. of MTSL ligands	0	0	2
Ramachandran plot (%)			
Favored	99.22	100	100
Outliers	0.78	0	0

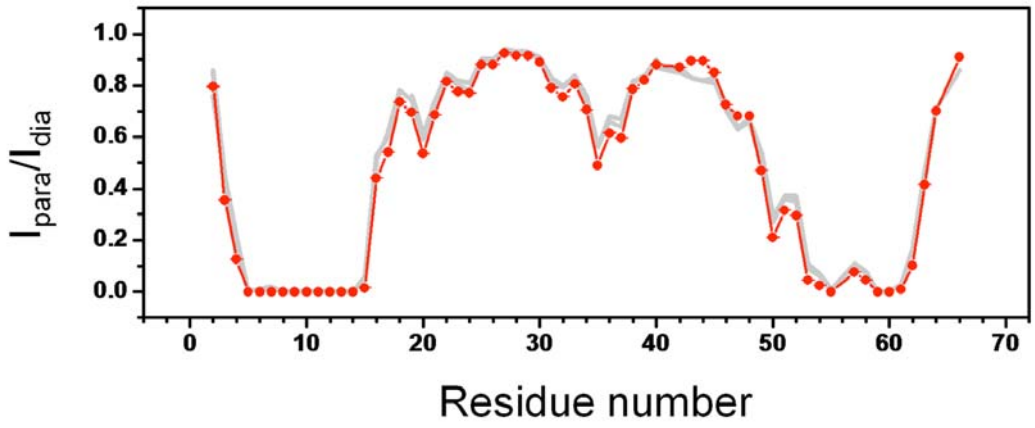

Figure S1. Comparison of paramagnetic relaxation enhancement values, I_{param}/I_{dia}, back-calculated from the dimeric crystal structure of CylR2 55R1 (red) and a single subunit of CylR2 55R1 (grey). Due to the r^{-6} dependence of the PRE and the fact that as the MTSL tag at T55C is not located directly at the dimer interface (Fig. 1a), intensity broadening in CylR2 55R1 is dominated by intramolecular distances.