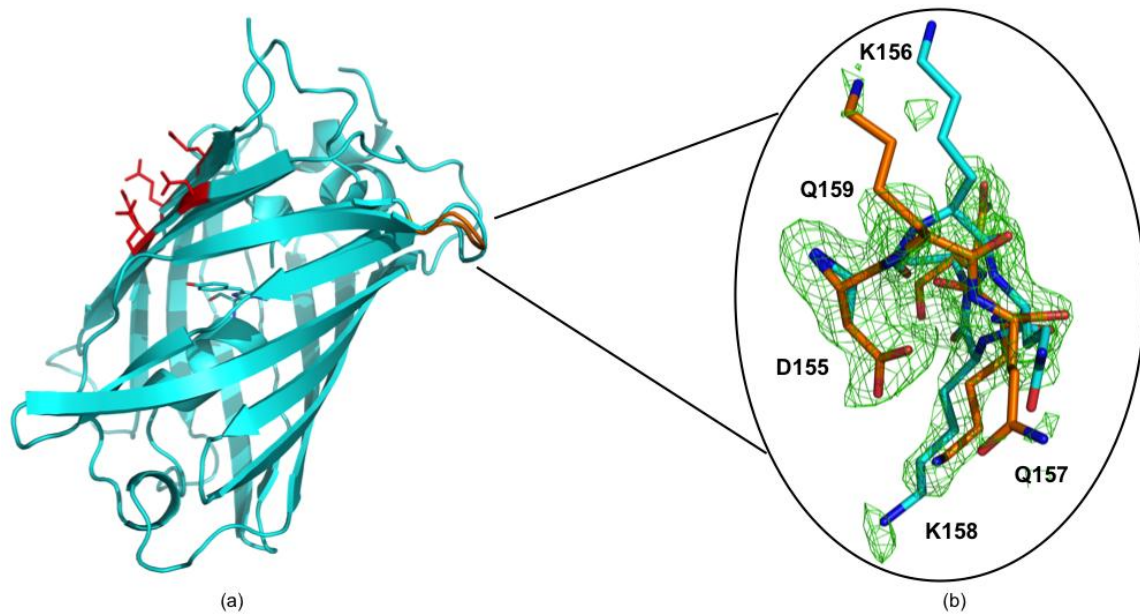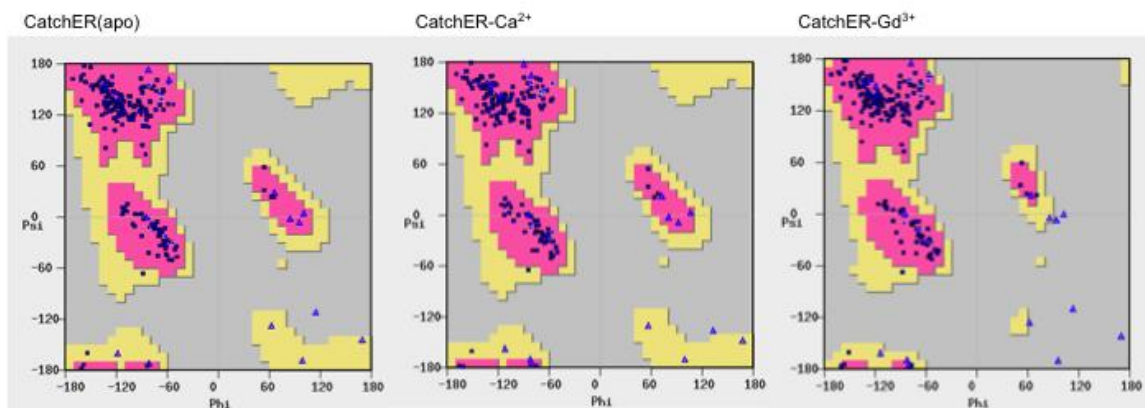**Supplementary Figure S1.** Fluorescence response of CatchER to Ca^{2+} and Gd^{3+} excited at 395 nm (a and c) and fitted normalized intensity (b and d). CatchER experiences a large fluorescence increase when titrated with Ca^{2+} and with Gd^{3+}. The normalized fluorescence was fitted with the 1:1 binding equation to give K_d values of 315.4 ± 40.0 μM for Ca^{2+} and 53.0 ± 4.0 μM for Gd^{3+}.

(a) (b)

Supplementary Figure S2. (a-b) Flexible loop region comprising residues 155-159. CatchER-Gd^{3+} is shown in a cyan cartoon representation with the mutated residues of the designed Ca^{2+} binding site in red sticks and chromophore in cyan bonds. The alternative conformations of residues 155-159 are indicated in cyan and orange. In the circle, F_o-F_c omit map (in green) for the two conformations of residues 155-159 contoured at 2.0σ. In EGFP (PDB code: 4EUL) and GFP structure (PDB code: 2WUR), only the conformation in orange was observed.

Supplementary Figure S3.Structure validation by Ramachandran plots for the three CatchER structures, as performed by PROCHECK CCP4i suite program (Potterton *et al.*, 2003);

Supplementary Table S1. Data Statistics vs Resolution

CatchER(apo)

Resolution Å							
Lower limit	Upper limit	Average I	Average error	Norm. stat	Linear Chi**2	Square R-fac	R-fac
50.00	3.58	5088.0	187.5	37.2	0.971	0.052	0.064
3.58	2.84	2732.9	116.4	21.1	0.968	0.066	0.076
2.84	2.48	1118.7	50.3	13.5	0.974	0.072	0.081
2.48	2.25	754.5	33.6	12.6	1.050	0.076	0.081
2.25	2.09	584.7	32.0	12.6	0.931	0.088	0.091
2.09	1.97	427.2	25.7	12.3	0.960	0.103	0.107
1.97	1.87	277.3	19.7	11.7	0.956	0.123	0.119
1.87	1.79	176.9	14.8	11.3	1.041	0.156	0.147
1.79	1.72	120.3	13.2	11.2	1.015	0.197	0.183
1.72	1.66	95.3	12.4	10.9	0.987	0.228	0.224
All reflections		1171.9	51.8	15.6	0.985	0.068	0.068

CatchER-Ca^{2+}

Resolution Å							
Lower limit	Upper limit	Average I	Average error	Norm. stat	Linear Chi**2	Square R-fac	R-fac
50.00	2.59	15117.6	803.1	245.3	0.901	0.060	0.071
2.59	2.05	4838.3	172.0	34.5	1.098	0.074	0.084
2.05	1.79	2084.5	106.1	23.6	0.955	0.098	0.110
1.79	1.63	811.5	39.3	11.3	1.094	0.128	0.147
1.63	1.51	448.4	23.4	9.4	1.124	0.157	0.175
1.51	1.42	262.5	16.1	8.6	1.122	0.210	0.224
1.42	1.35	163.4	12.6	8.5	1.077	0.281	0.295
1.35	1.29	120.5	11.3	8.8	1.108	0.317	0.331
1.29	1.24	87.4	10.7	9.2	1.063	0.351	0.370
1.24	1.20	64.1	10.5	9.5	1.013	0.392	0.408
All reflections		2389.8	119.8	36.6	1.061	0.081	0.076

CatchER-Gd³⁺

CatchER-Gd^{3+}

Resolution Å							
Lower limit	Upper limit	Average I	Average error	Norm. stat	Linear Chi**2	Square R-fac	R-fac
50.00	3.83	2142.8	130.3	21.5	0.951	0.065	0.071
3.83	3.04	1484.5	109.5	15.9	0.980	0.086	0.088
3.04	2.66	611.6	47.1	8.3	0.951	0.091	0.094
2.66	2.42	379.2	29.5	6.3	0.912	0.093	0.092
2.42	2.24	283.8	22.6	5.9	0.930	0.102	0.101
2.24	2.11	217.9	16.8	5.3	1.048	0.115	0.107
2.11	2.00	171.4	13.7	5.0	0.973	0.118	0.108
2.00	1.92	122.6	10.2	4.7	0.989	0.131	0.114
1.92	1.84	78.8	7.1	4.4	1.004	0.162	0.149
1.84	1.78	52.8	6.1	4.7	1.008	0.185	0.156
All reflections		572.3	40.4	8.4	0.974	0.085	0.079

This table is from SCALEPACK

References

Potterton, E., Briggs, P., Turkenburg, M. & Dodson, E. (2003). *Acta Cryst.* D**59**, 1131-1137.