

## Supplementary materials

### Experimental insight into the structural and functional roles of the ‘black’ and ‘gray’ clusters in recoverin, a calcium binding protein with four EF-hand motifs

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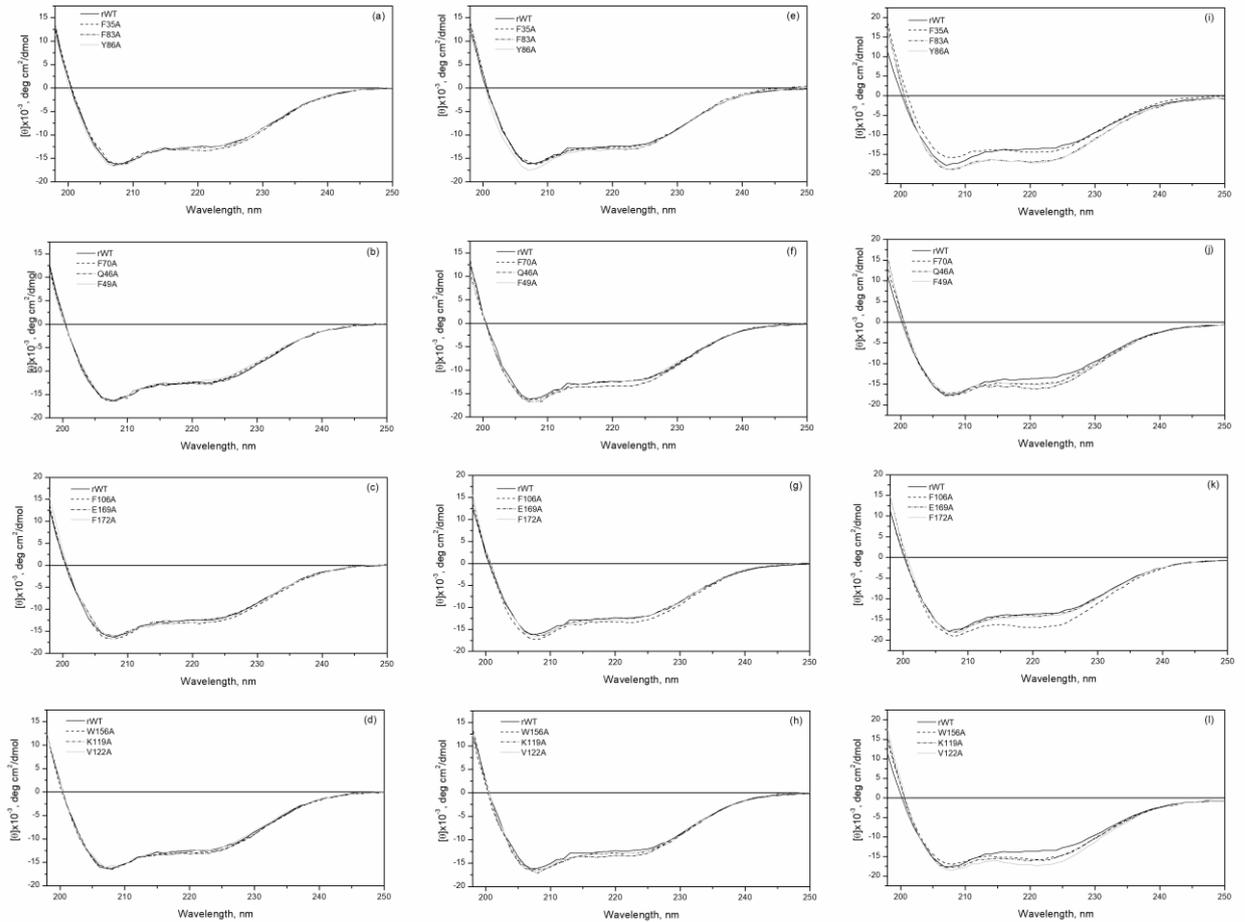
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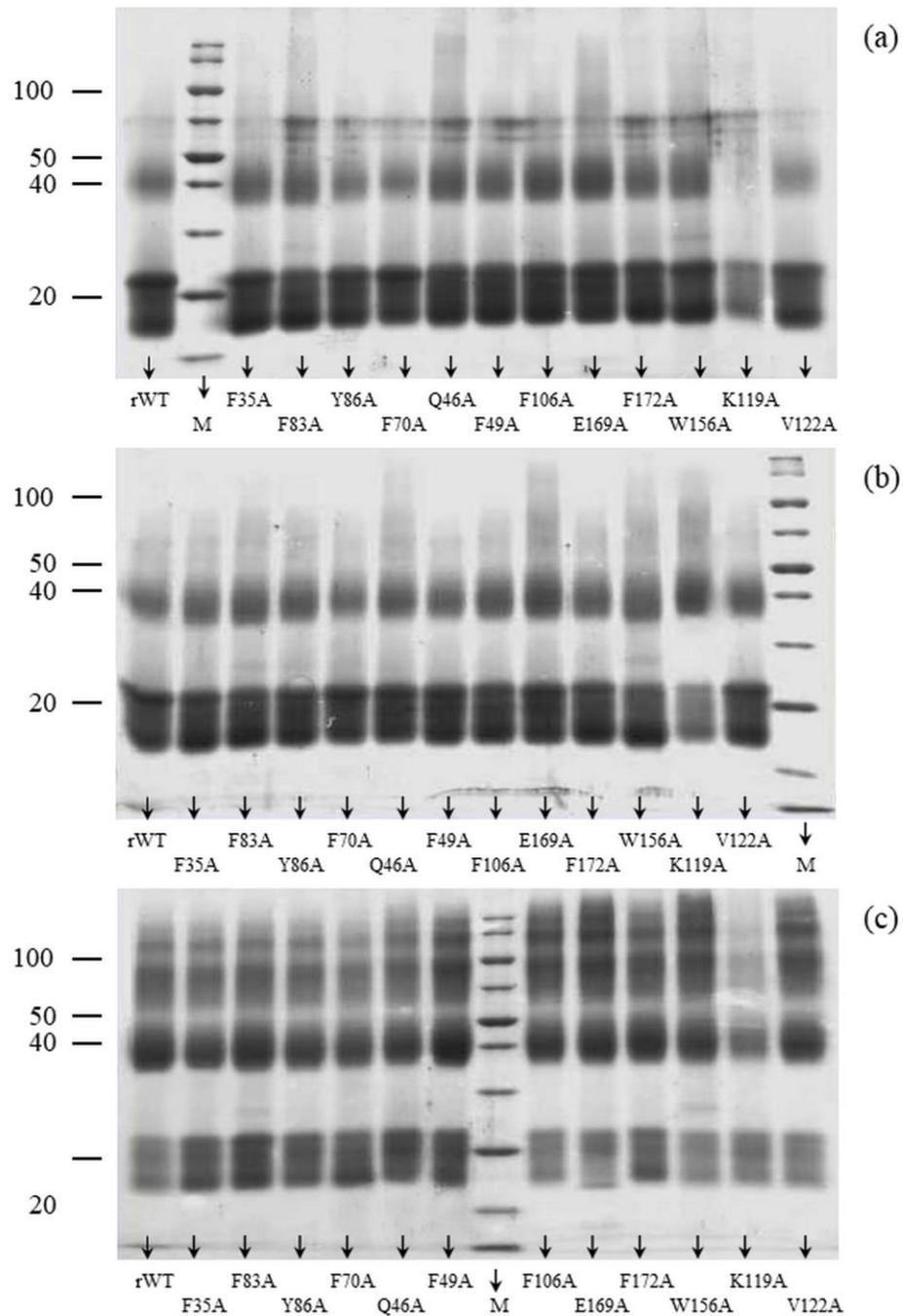
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**Table S1.** The values of  $\epsilon_{280\text{nm}}$  for rWT human recoverin and its cluster mutants (a molar extinction coefficient of 280 nm, calculated using online software ProtParam Expasy (<https://web.expasy.org/protparam/>) and substitutions in the corresponding codons of synthetic oligonucleotides for each mutants of rWT human recoverin.

<i>Protein</i>	<i>Substitution</i>	$\epsilon_{280}, M^{-1}cm^{-1}$	<i>Major molecular mass by the data of mass spectrometry, Da / its % from sample general quantity</i>	
rWT		25,440	23,208 / 89	
N-domain				
Black cluster	F35A	TTC by GCC	25,440	23,138 / 80
	F83A	TTC by GCC	25,440	23,134 / 100
	Y86A	TAC by GCC	23,950	23,116 / 66
Gray cluster	F70A	TTC by GCC	25,440	23,130 / 85
	Q46A	CAG by GCC	25,440	23,152 / 60
	F49A	TTC by GCC	25,440	23,138 / 100
C-domain				
Black cluster	F106A	TTC by GCC	25,440	23,132 / 83
	E169A	GAG by GCC	25,440	23,156 / 100
	F172A	TTC by GCC	25,440	23,131 / 95
Gray cluster	W156A	TGG by GCC	19,940	23,094 / 59
	K119A	AAG by GCC	25,440	23,151 / 55
	V122A	GTG by GCC	25,440	23,179 / 84



**Figure S1.** Far-UV CD spectra of apo (1mM EDTA) (a – ‘black’ cluster of the N-domain, b – ‘gray’ cluster of the N-domain, c – ‘black’ cluster of the C-domain, d – ‘gray’ cluster of the C-domain), Mg<sup>2+</sup>- (1mM EGTa, 1mM MgCl<sub>2</sub>) (e – ‘black’ cluster of the N-domain, f – ‘gray’ cluster of the N-domain, g – ‘black’ cluster of the C-domain, h – ‘gray’ cluster of the C-domain) and Ca<sup>2+</sup>-loaded (1mM CaCl<sub>2</sub>) (i – ‘black’ cluster of the N-domain, j – ‘gray’ cluster of the N-domain, k – ‘black’ cluster of the C-domain, l – ‘gray’ cluster of the C-domain) forms of rWT human recoverin and its cluster mutants at 15 °C. 10 mM Tricine-KOH, 50 mM KCl, 20 μM DTT pH 7.4.



**Figure S2.** Distribution of aggregated forms of recombinant wild type human recoverin and its cluster mutants obtained from experiments on chemical crosslinking by glutaric aldehyde (0.02%) at 20 °C. Protein concentration 1 mg/ml. 20 mM Tricine-KOH pH 7.4, 50 mM KCl, 1 mM DTT; 1 mM EDTA (for apo-proteins), 1mM MgCl<sub>2</sub>, 1 mM EGTA (for Mg<sup>2+</sup>-loaded proteins) or 1 mM CaCl<sub>2</sub> (for Ca<sup>2+</sup>-loaded proteins). The samples were subjected to SDS-PAGE (5% concentrating and 15% resolving gels; 5 µg of protein per lane) and stained with Coomassie Brilliant Blue R-250.