

## Defects in the calcium-binding region drastically affect the cadherin-like domains of RET tyrosine kinase

Chunxia Gao, Morten Grøtli, Leif A. Eriksson\*

*Department of Chemistry and Molecular Biology, University of Gothenburg, 405 30  
Göteborg, Sweden*

### SUPPLEMENTARY MATERIAL

**Figure 1.** The binding site for GFL-GFR $\alpha$  ligand interaction.

**Figure 2.** The final structure of RET CLD1-4 for the different systems after 300 ns MD simulations.

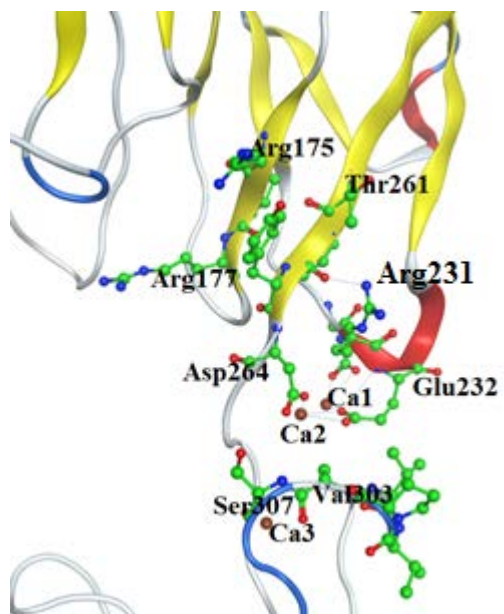
**Figure 3,** Evolution of secondary structure elements for the mutated/modified systems during 300 ns MD simulations.

**Figure 4,** The most populated structures of the calcium-binding site from cluster analysis for all five systems. The mutated residues in R231H, D264K and D300K mutants are colored in pink.

**Table 1.** Distances between the different pharmacophore features.

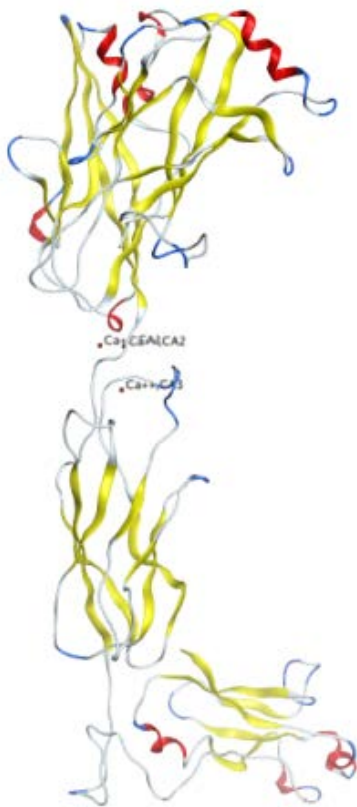
**Table 2.** Angles between the different pharmacophore features

\*email: leif.eriksson@chem.gu.se

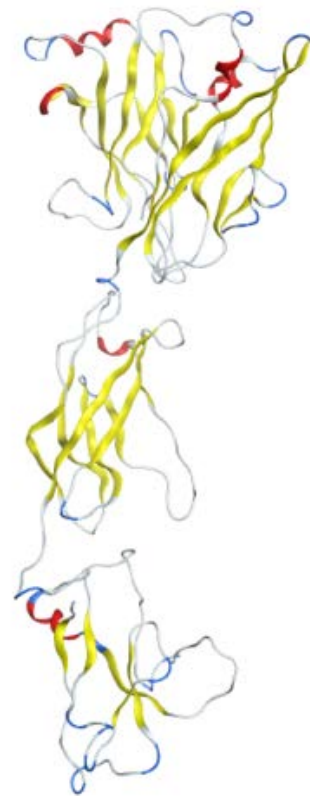


**Supplementary Figure 1.** The binding site for GFL-GFR $\alpha$  ligand interaction.

wildtype



Calcium depleted

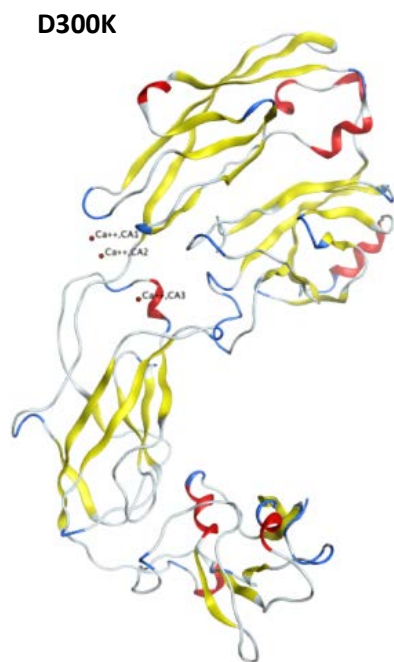


R231H

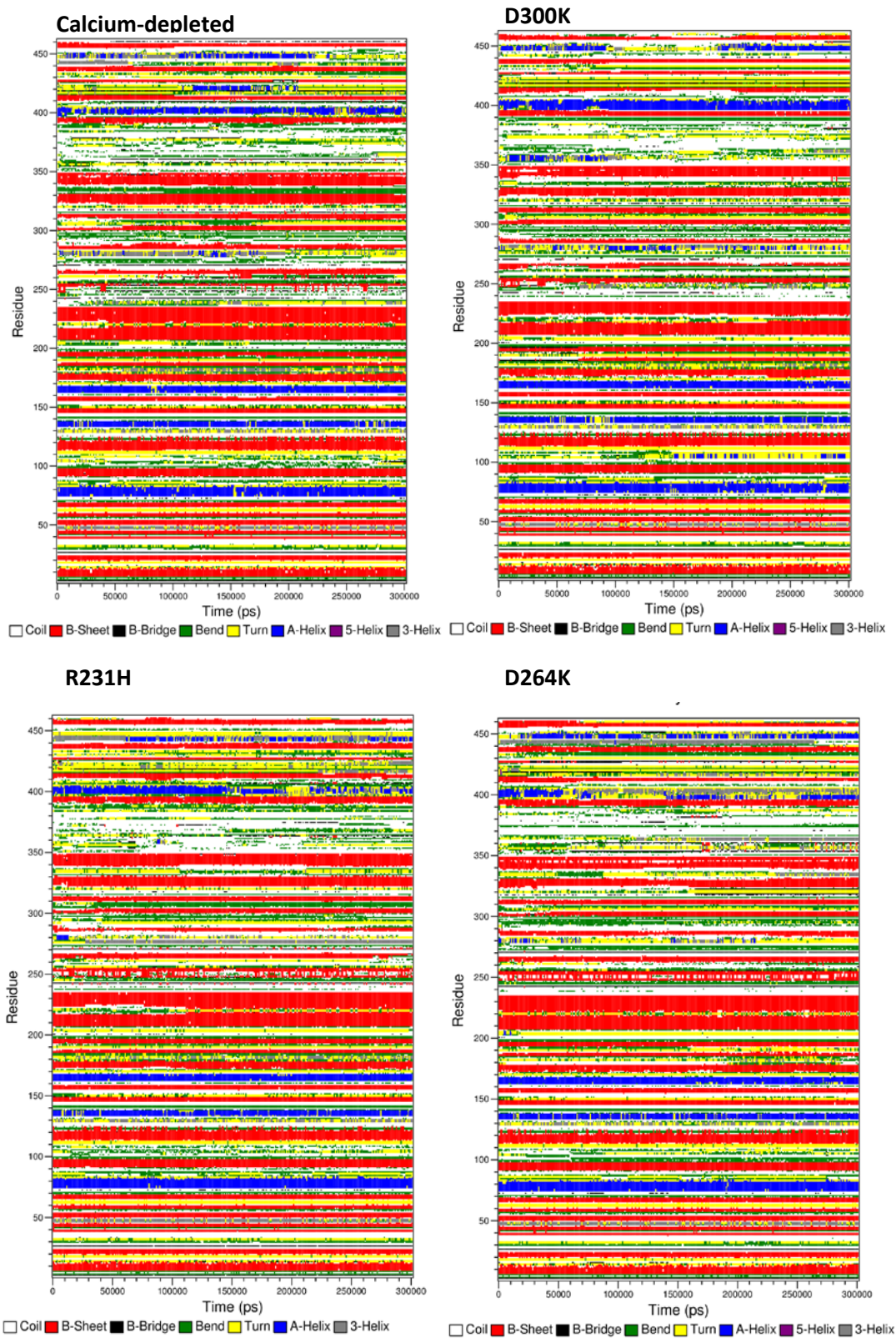


D264K



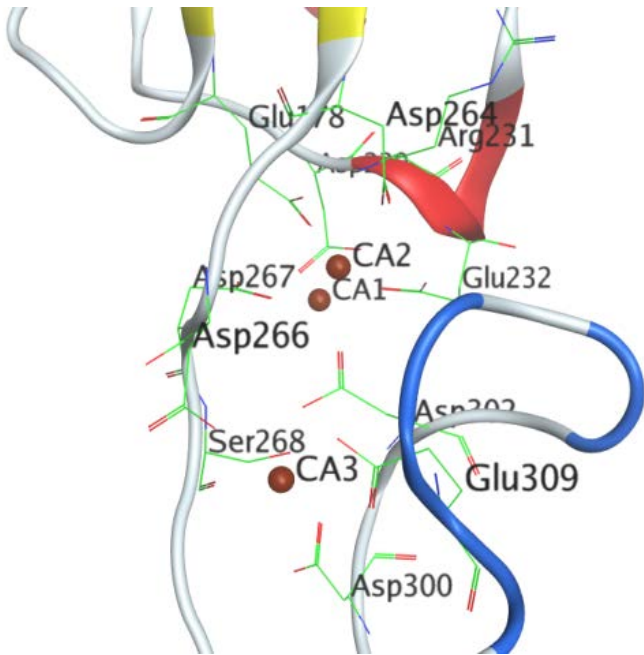


**Supplementary Figure 2.** The final structure of RET CLD1-4 for the different systems after 300 ns MD simulations.

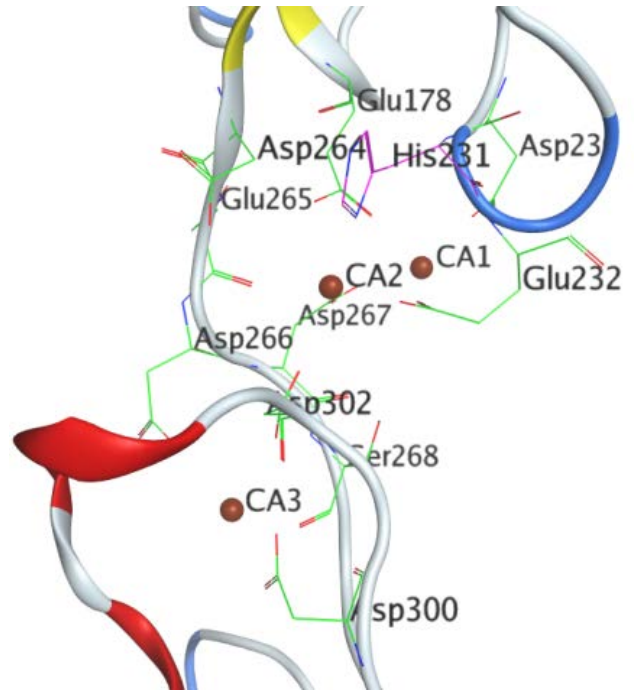


**Supplementary Figure 3**, Evolution of secondary structure elements for the four mutated/modified systems during 300 ns MD simulations.

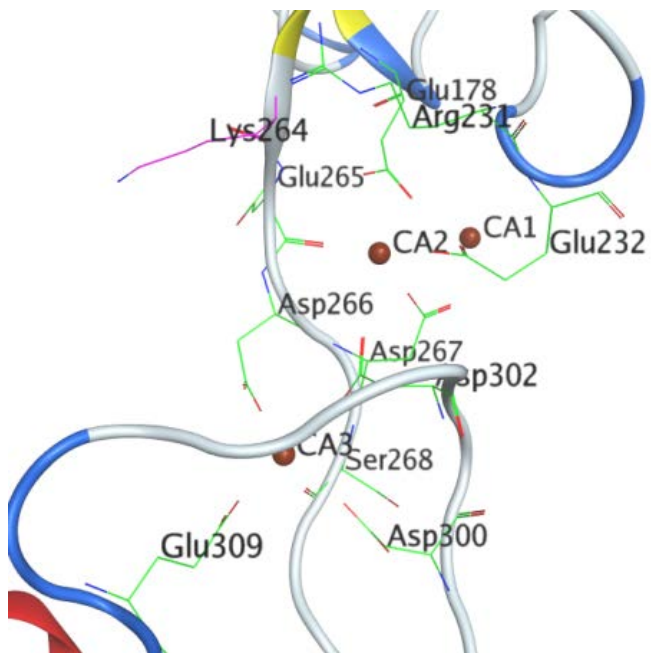
wildtype



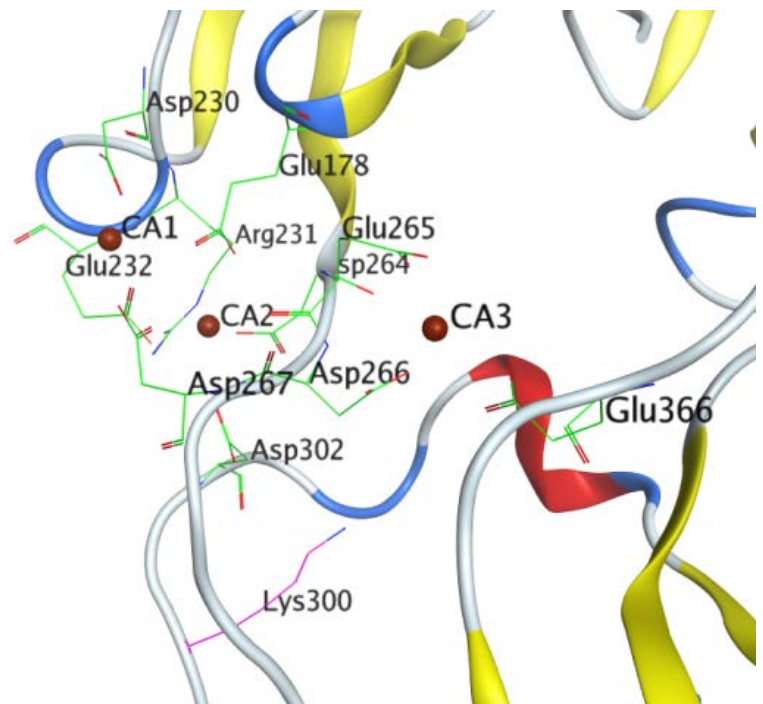
R231H



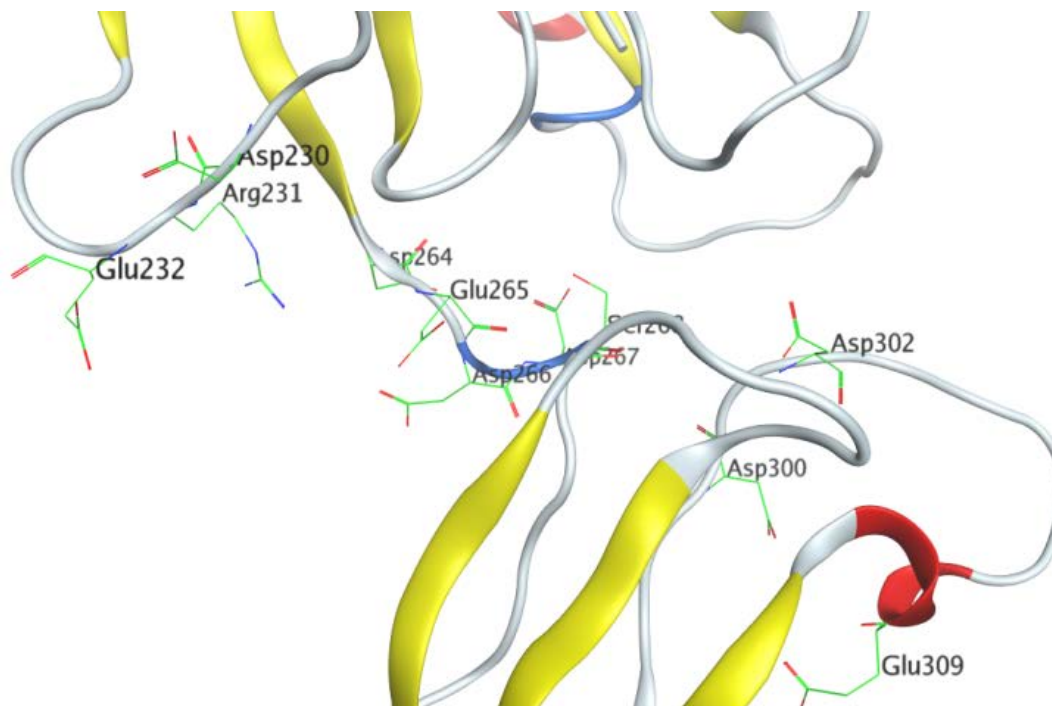
D264K



D300K



### Calcium-depleted



**Supplementary Figure 4**, The most populated structures of the calcium-binding site from cluster analysis for all five systems. The mutated residues in R231H, D264K and D300K mutants are colored in pink.

**Supplementary Table 1.** Distances between the different pharmacophore features.

Site1	Site2	Distance (Å)
D21	D33	4.61
D21	D34	8.149
D21	N39	3.371
D21	N40	14.006
D21	R58	4.956
D21	R60	8.731
D33	D34	4.037
D33	N39	7.847
D33	N40	17.705
D33	R58	4.767
D33	R60	12.502
D34	N39	11.395
D34	N40	19.419
D34	R58	5.673
D34	R60	14.556
N39	N40	12.593
N39	R58	7.316
N39	R60	7.242
N40	R58	14.412
N40	R60	6.268
R58	R60	9.079



**Supplementary Table 2.** Angles between the different pharmacophore features.

Site1	Site2	Site3	Angle (°)
D33	D21	D34	18.2
D33	D21	N39	158.7
D33	D21	N40	138
D33	D21	R58	59.6
D33	D21	R60	136.9
D34	D21	N39	161.4
D34	D21	N40	120.1
D34	D21	R58	43.3
D34	D21	R60	119.1
N39	D21	N40	58.8
N39	D21	R58	121.8
N39	D21	R60	53.3
N40	D21	R58	84.6
N40	D21	R60	17.6
R58	D21	R60	77.8
D21	D33	D34	140.8
D21	D33	N39	9
D21	D33	N40	31.9
D21	D33	R58	63.8
D21	D33	R60	28.5
D34	D33	N39	145.1
D34	D33	N40	109.3
D34	D33	R58	79.8
D34	D33	R60	112.9
N39	D33	N40	38.8
N39	D33	R58	65.7
N39	D33	R60	32.5
N40	D33	R58	40.4
N40	D33	R60	13.5
R58	D33	R60	35.9
D21	D34	D33	20.9
D21	D34	N39	5.4
D21	D34	N40	38.6
D21	D34	R58	36.8
D21	D34	R60	31.6
D33	D34	N39	23.2
D33	D34	N40	59.3
D33	D34	R58	55.8
D33	D34	R60	52.3
N39	D34	N40	38.1
N39	D34	R58	32.9
N39	D34	R60	29.3
N40	D34	R58	23.8
N40	D34	R60	13.5

R58	D34	R60	11.9
D21	N39	D33	12.3
D21	N39	D34	13.2
D21	N39	N40	108
D21	N39	R58	35.2
D21	N39	R60	104.7
D33	N39	D34	11.7
D33	N39	N40	118.2
D33	N39	R58	36.4
D33	N39	R60	111.8
D34	N39	N40	108
D34	N39	R58	24.9
D34	N39	R60	100.3
N40	N39	R58	88.6
N40	N39	R60	19.7
R58	N39	R60	77.2
D21	N40	D33	10
D21	N40	D34	21.3
D21	N40	N39	13.2
D21	N40	R58	20
D21	N40	R60	24.9
D33	N40	D34	11.3
D33	N40	N39	23
D33	N40	R58	12.4
D33	N40	R60	27.7
D34	N40	N39	33.9
D34	N40	R58	9.1
D34	N40	R60	32.8
N39	N40	R58	30.5
N39	N40	R60	22.9
R58	N40	R60	24.4
D21	R58	D33	56.6
D21	R58	D34	99.9
D21	R58	N39	23.1
D21	R58	N40	75.4
D21	R58	R60	70
D33	R58	D34	44.5
D33	R58	N39	77.9
D33	R58	N40	127.2
D33	R58	R60	126.2
D34	R58	N39	122.1
D34	R58	N40	147
D34	R58	R60	160.7
N39	R58	N40	60.9
N39	R58	R60	51.1
N40	R58	R60	16.6

D21	R60	D33	14.6
D21	R60	D34	29.3
D21	R60	N39	21.9
D21	R60	N40	137.5
D21	R60	R58	32.2
D33	R60	D34	14.8
D33	R60	N39	35.6
D33	R60	N40	138.8
D33	R60	R58	17.9
D34	R60	N39	50.4
D34	R60	N40	133.6
D34	R60	R58	7.4
N39	R60	N40	137.4
N39	R60	R58	51.8
N40	R60	R58	139.1