# Supplementary Materials: Mechanism of action of non-synonymous single nucleotide variations associated with $\alpha$ -carbonic anhydrase II deficiency

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Residue	Functional role	<b>Reference</b> <sup><i>a</i></sup>					
Trp5	Primary aromatic cluster residue, tertiary CO <sub>2</sub> binding pocket formation, His64	[1–3]					
-	stabilisation when in the "out" conformation						
Tyr7	Primary aromatic cluster residue, active site water network coordination	[1-9]					
Trp16	Primary aromatic cluster residue [						
Phe20	Primary aromatic cluster residue	[1-3]					
Ser29	Enzymatic stability	[10,11]					
Asn62	Active site water network coordination	[4]					
His64	Proton shuttling residue, tertiary CO <sub>2</sub> binding pocket formation	[1,4-8]					
Phe66	Secondary aromatic cluster residue, secondary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Asn67	Active site water network coordination	[12]					
Phe70	Secondary aromatic cluster residue	[1–3]					
Phe93	Secondary aromatic cluster residue	[1–3]					
Gln92	Secondary Zn <sup>2+</sup> ligand	[1,4]					
His94	Zn <sup>2+</sup> coordination	[1,4]					
Phe95	Secondary aromatic cluster residue, secondary CO <sub>2</sub> binding pocket formation	[1,5-8]					
His96	Zn <sup>2+</sup> coordination	[1,4]					
Trp97	Secondary aromatic cluster residue, secondary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Glu106	Orientation of Zn <sup>2+</sup> water ligand molecule for catalysis	[1]					
Glu117	Zn <sup>2+</sup> affinity and catalytic efficiency, secondary Zn <sup>2+</sup> ligand	[13]					
His119	Zn <sup>2+</sup> coordination	[1,4]					
Val121	Primary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Val142	Primary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Phe175	Secondary aromatic cluster residue	[1–3]					
Phe178	Secondary aromatic cluster residue	[1–3]					
Leu197	Primary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Thr198	Deep water molecule stabilisation, catalytic orientation of Zn <sup>2+</sup> water ligand molecule	[4]					
Thr199	Active site water coordination, tertiary CO <sub>2</sub> binding pocket formation	[4]					
Pro200	Tertiary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Trp208	Primary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Phe225	Secondary aromatic cluster residue, secondary CO <sub>2</sub> binding pocket formation	[1,5-8]					
Asn243	Tertiary $CO_2$ binding pocket formation, secondary $Zn^{2+}$ ligand	[1,5-8]					
Arg245*	Enzyme stability	[14]					

Table S1. Identified CA-II residues important for structure, function and stability.

 $^{\ast}$  associated with stability reduction in CA-I

<sup>a</sup> references at end of supplementary data

Table S2. Table of UniProt CA accession numbers for sequences used in motif discovery.

Sequence	UniProt accession
CA-I	P00915
CA-II	P00918
CA-III	P07451
CA-IV	P22748
CA-VA	P35218
CA-VB	Q9Y2D0
CA-VI	P23280
CA-VII	P43166
CA-VIII	P35219
CA-IX	Q16790
CA-X	Q9NS85
CA-XI	O75493
CA-XII	043570
CA-XIII	Q8N1Q1
CA-XIV	Q9ULX7
CA-XV	Q99N23





**Figure S1.** Heat map of all conserved motifs within human  $\alpha$ -CA family and associated UniProt accession. Motif conservation is represented as number of motif sites per total protein sequences. A value of zero shows that motifs are not conserved in any sequence, whereas a value of 1 shows that motif is conserved in all sequences. The prefix 'M' represents the word motif.

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**Table S3.**  $Zn^{2+}$  non-bonded, bonds, angles and dihedral parameters derived within this study.  $K_b$ : bond force constant;  $K_{\theta}$ : angle force constant;  $R_{min}$ : vdW radius;  $\epsilon$ : LJ potential well energy.

# Non-bonded:

Atom	<b>R</b> <sub>min</sub> (Å)	$\epsilon$ (kcal/mol)
M1	1.40	0.02
Y1	1.82	0.17
Y2	1.82	0.17
Y3	1.82	0.17
Y4	1.77	0.15

#### Bonds:

Bond type	K <sub>b</sub> (kcal/mol/Å <sup>2</sup> )	Bond length (Å)
M1-Y4	41.20	2.12
Y1-M1	91.70	1.98
Y2-M1	94.30	1.98
Y3-M1	93.00	1.98

## Angles:

Angle type	$\mathbf{K}_{\theta}$ (kcal/mol/radian <sup>2</sup> )	Equilibrium angle degrees ( $\theta$ )
CC-Y3-M1	56.44	127.30
CR-Y1-M1	38.97	125.70
CR-Y2-M1	53.67	127.46
M1-Y1-CV	39.62	128.05
M1-Y2-CV	54.89	126.22
M1-Y3-CR	54.18	125.48
M1-Y4-HW	44.55	122.59
Y1-M1-Y2	39.89	116.07
Y1-M1-Y3	37.44	115.90
Y1-M1-Y4	31.02	101.10
Y2-M1-Y3	36.29	114.63
Y2-M1-Y4	36.83	105.42
Y3-M1-Y4	30.04	100.62

#### Dihedral:

Definition	Divider	Barrier (kcal/mol)	Phase degrees ( $\theta$ )	Periodicity		
Х -СС-ҮЗ-Х	2	4.80	180.0	2.0		
X -CR-Y1-X	2	10.00	180.0	2.0		
X -CR-Y2-X	2	10.00	180.0	2.0		
X -CV-Y1-X	2	4.80	180.0	2.0		
X -CV-Y2-X	2	4.80	180.0	2.0		
X -Y3-CR-X	2	10.00	180.0	2.0		
CX-CT-CC-Y3	1	0.05	180.0	-4.0		
CX-CT-CC-Y3	1	0.74	0.0	-3.0		
CX-CT-CC-Y3	1	0.20	0.0	-2.0		
CX-CT-CC-Y3	1	0.69	0.0	1.0		

M1: Zn; Y1: His94 NE2 (epsilon nitrogen); Y2: His96 NE2 ((epsilon nitrogen)) Y3: His199 ND1 (delta hydrogen); Y4: O (H<sub>2</sub>O); CC: CG (gamma carbon); CR: CE1 (epislon carbon); CV: CD2 (delta carbon)



Figure S2. RMSD comparison between the WT and variant proteins during MD.

WT	N252D	P236K		P236H	H107Y	,	K180	K18E					WT		1 Marcanet	NICEON		P236R		P236H		H107Y		K18Q			K18E	Variant		Ţ
BCT 02	BCT OF	G B C F A	CO2	Apo 2	R Apo	BCT	Apo CO2	Apo BCT					Apo .	C02	BCT	CO2	BCT	Apo	6 G	Apo -	602	Apo	C02 -	BCT .	CO2	BCT	Ano	I		able
$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	Apo			$< 2.2 \times 10^{-10}$ 0.5124	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	1	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	0.005176	$< 2.2 \times 10^{-16}$	APO 2.2 × 10 <sup>-16</sup>	480		S4. As
$\begin{array}{l} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	BCT	H107Y		$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$^{1} < 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	HIOTY		sociate
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$3.049 \times 10^{-10}$ 0.06527 $3.08 \times 10^{-14}$ $6.491 \times 10^{-07}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-40</sup> 4.226 × 10 <sup>-40</sup>	0.2254 < $2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$8.154 \times 10^{-12}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	CO2			$< 2.2 \times 10^{-10}$ 5.517 × 10 <sup>-07</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	0.005176	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	1	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	000		D and F
$\begin{array}{c} 0.5342 \\ 1.342 \times 10^{-07} \\ < 2.2 \times 10^{-16} \\ 0.04238 \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-30</sup> 0.3209	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$8.154 \times 10^{-12}$ 1	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	Apo			$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$^{1} < 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	A 700		lg distri
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$ \begin{array}{l} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array} $	$< \frac{2.2}{2.2} \times 10^{-16}$ 1	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ 1.765 × 10 <sup>-13</sup>	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	BCT	N252D	ration (Ro)	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	1	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	N252D	deviation (RMSI	
$\begin{array}{c} 1\\ 2.68 \times 10^{-06}\\ < 2.2 \times 10^{-16}\\ 0.1479\end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	0.6927 0.6927	$5.635 \times 10^{-15}$ < 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$3.049 \times 10^{-10}$ 0.5342	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	C02			$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	1	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	ŝ	))	
$ \begin{array}{l} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array} $	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $1.765 \times 10^{-13}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ 0.0001396	$< 2.2 \times 10^{-16}$ 1	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	Apo			$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	1	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	Ano		
$\begin{array}{c} 5.635 \times 10^{-15} \\ 0.0009534 \\ 2.425 \times 10^{-10} \\ 1.059 \times 10^{-10} \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ 3.696 × 10 <sup>-13</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	0.2254 < $2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	BCT	P236H		$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$1 < 0.0 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	P236H		
$\begin{array}{l} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ 1	$< 2.2 \times 10^{-16}$ 0.0001396	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ 2.325 × 10 <sup>-09</sup>	CO2			$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-30}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	3		
$< \begin{array}{c} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	Apo			$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-10</sup>	0.02151	× <u>مدم</u> × ۲۵ 1	< 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-30}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	Ano		
$\begin{array}{c} 0.6927\\ 2.766 \times 10^{-05}\\ < 2.2 \times 10^{-16}\\ 0.3288\end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$222 \times 10^{-10}$	$3.696 \times 10^{-13}$ < 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$4.226 \times 10^{-09}$ 0.3209	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	BCT	P236R		$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	1	0.02151	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	P236R		
$< \begin{array}{c} < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \\ < 2.2 \times 10^{-16} \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$2.607 \times 10^{-10}$ < 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ 4.735 × 10 <sup>-05</sup>	CO2			$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$1 \sim 2.3 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-16</sup>	00		
$2.68 \times 10^{-06}$ $1 < 2.2 \times 10^{-16}$ 0.001236	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ 2.766 × 10 <sup>-10</sup>	0.0009534 < 2.2 × 10 <sup>-16</sup>	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	0.06527 $1.342 \times 10^{-07}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	Apo			$< 2.2 \times 10^{-10}$ 6.227 × 10 <sup>-15</sup>	1	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$1.731 \times 10^{-15}$	< 2.2 × 10 <sup>-16</sup>	A mo		
$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ 1 $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$2.425 \times 10^{-10}$ < $2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$3.08 \times 10^{-14}$ < $2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	BCT	WT		$^{1} < 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	WT		
$\begin{array}{c} 0.1479 \\ 0.001236 \\ < 2.2 \times 10^{-16} \\ 1 \end{array}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	< 2.2 × 10 <sup>-30</sup> 0.3288	$1.059 \times 10^{-10}$ < $2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$6.491 \times 10^{-07}$ 0.04238	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	CO2			$< 2.2 \times 10^{-10}$	$6.227 \times 10^{-15}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$ $< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	0.5124	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-10}$ $< 2.2 \times 10^{-16}$	$5.517 \times 10^{-07}$	$< 2.2 \times 10^{-16}$	< 2.2 × 10-16	001		





**Figure S3.** 3-dimensional (3D) plot of PC1 vs PC2 of the WT and variants proteins as a function of free energy. 2D PCA is projected onto the x-axis and *y*-axis. Free energy is represented in kcal mol<sup>-1</sup>.

Variant	Protein	PC1	PC2	PC3
K18E	Аро	0.5503	0.2500	0.1997
	BĈT	0.4836	0.3165	0.1999
	CO <sub>2</sub>	0.5772	0.2218	0.2010
K18Q	Аро	0.5260	0.2732	0.2009
	BCT	0.4497	0.3598	0.1905
	CO <sub>2</sub>	0.6091	0.2647	0.1261
H107Y	Аро	0.4439	0.3322	0.2239
	BCT	0.5688	0.2764	0.1548
	CO <sub>2</sub>	0.7201	0.1670	0.1129
P236H	Аро	0.5535	0.2628	0.1837
	BCT	0.5195	0.2741	0.2064
	CO <sub>2</sub>	0.4846	0.2977	0.2177
P236R	Аро	0.4693	0.3107	0.2200
	BCT	0.4772	0.3415	0.1813
	CO <sub>2</sub>	0.4218	0.3208	0.2574
N252D	Аро	0.4415	0.3358	0.2227
	BCT	0.4635	0.3607	0.1758
	CO <sub>2</sub>	0.4348	0.3235	0.2417
WT	Apo	0.4673	0.3253	0.2074
	BCT	0.4256	0.3550	0.2194
	CO <sub>2</sub>	0.5399	0.2589	0.2012

**Table S5.** Eigenvalue fraction of each principal component for the active site residues within the wild-type and variant proteins in the absence (apo) and presence (non apo) of  $CO_2$ .



Figure S4. Rg comparison between the WT and variant proteins during MD.



Figure S5. Residue RMSF comparison between the WT and variant proteins.

**Table S6.** Variant residues showing decreases and increases to  $\Delta L$  during MD simulation. Residues from Table reftab:residues are underlined and highlighted in bold. SNV positions are underlined, italicised and highlighted in bold red.

ΔL decrease (Residue accessibility increase)           K18E         His4         His10         Trp16         Ile22         Lys111         Gly155         Leu156         Ala54         Lys158         Val159         Val160           Gly170         Phe230         Asn231         Gly237         Asp161         Leu163         Asp164         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Asp164         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Asp179         Pro180         Asp164         Ser165         Ile166         Asp179         Pro180         Asp164         Ser165         Ile166         Ala173         Asp164         Ser165         Ile166         Ala173         Ser165         Ile166         Ala173         Ser165         Ile166         Ala173         Ser165         Ile164 <th>Variant</th> <th>Аро</th> <th>ВСТ</th> <th>CO<sub>2</sub></th>	Variant	Аро	ВСТ	CO <sub>2</sub>									
K18E         His4         His10         Trp16         Ile22         Lys111         Gly155         Leu156         Ala54         Lys158         Val160           Gly170         Phe230         Asn231         Asn231         Asp161         Leu163         Asp164         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Ser165         Ile166         Asp164         Ser165         Ile166         Ser165         Ile166         Ser165         Ile166         Ser165         Ile166         Ser165         Ile166         Ser165         Ile166         Asp164         Ser165         Ile166		$\Delta L$ decrease (Residue accessibility increase)											
Gly170         Phe230         Asn231         Asp161         Leu163         Asp164           Glu235         Pro236         Glu237         Glu237         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Asp161         Leu163         Asp164         Ser165         Ile166         Asp179         Val49         Ser165         Ile166         Asp179         Pro180         Arg181         Leu163         Asp164         Ser165         Ile166         Ala173         Phe175         Asp164         Ser165         Ile166         Ala173         Phe175         Asp164         Ser165         Ile166         Ala173         Ser165	K18E	His4 His10 Trp16 Ile22	Lys111 Gly155 Leu156	Ala54 Lys158 Val159 Val160									
Glu235         Pro236         Glu237         Ser165 Ile166 Asn177 Phe178           Glu238         His3 Ile22 Ala152         His3 His4         Trp5 Gly6 Gly8         His3 His4 Lys9 Lys24 Lys158           K18Q         His3 Ile22 Ala152         His3 His4         Trp5 Gly6 Gly8         Lys9 His10 Asn11 Lys24           Lys111         Lys111         Asp164 Ser165 Ile166           H107Y         Lys24         Val49 Ser50 Asp52 Gln53         Asp52 Gln53 Gly155 Lys158           Lys111 Leu184         Val159 Val160 Asp161 Val162         Leu163 Ser165 Ile166 Asp179           P236H         Ile22 Asp101 Gly155 Thr199         His3 Gly155 Leu156 Phe178         His3 Ala54 Lys158 Val159           His236 Glu237 Glu238         Asp179 Pro180 Arg181         Leu183 Pro201         Val160 Asp161 Val162 Leu163           P236R         Pro21 Ile22 Phe230 Asn231         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           Glu235         Arg236 Glu237         Glu237         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163           Glu238         Clintcrase (Residue accessibility decrease)         Xasp164 Ser165 Ile166 Phe175           M2         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160		Gly170 Phe230 Asn231		Asp161 Leu163 Asp164									
Glu238         His3 His4 Trp5 Gly6 Gly8         His3 His4 Lys9 Lys24 Lys158           K18Q         His3 Ile22 Ala152         His3 His4 Trp5 Gly6 Gly8         His3 His4 Lys9 Lys24 Lys158           Lys9 His10         Asn11         Lys24         Val159 Val160 Asp161 Leu163           H107Y         Lys24         Val49 Ser50 Asp52 Gln53         Asp52 Gln53 Gly155 Lys158           H107Y         Lys24         Val49 Ser50 Asp52 Gln53         Lys119 Val160 Asp161 Val162           Lys111         Leu163 Ser165 Ile166 Asp179         Pro180         Val159 Val160 Asp161 Val162           P236H         Ile22 Asp101 Gly155 Thr199         His3 Gly155 Leu156 Phe178         His3 Ala54 Lys158 Val159           His236 Glu237 Glu238         Asp179         Pro180 Arg181         Val160 Asp161 Val162 Leu163           Leu183 Pro201         Asp164 Ser165 Ile166 Ala173         Phe175 Asn177 Glu237           P236R         Pro21 Ile22 Phe230 Asn231         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           Glu235         Arg236 Glu237         Val49 Asp52 Gln53 Pro180         Asp161 Val162 Leu163           Glu238         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160           M160 Asp161 Val162 Leu163         Gly155 Leu163 Ile166 Phe175		Glu235 Pro236 Glu237		Ser165 Ile166 Asn177 Phe178									
K18Q         His3 Ile22 Ala152         His3 His4 Trp5 Gly6 Gly8 Lys9 His10 Asn11 Lys24         His3 His4 Lys9 Lys24 Lys158 Val159 Val160 Asp161 Leu163 Asp164 Ser165 Ile166           H107Y         Lys24         Val49 Ser50 Asp52 Gln53 Lys111 Leu184         Asp52 Gln53 Gly155 Lys158 Val159 Val160 Asp161 Val162 Leu163 Ser165 Ile166 Asp179 Pro180           P236H         Ile22 Asp101 Gly155 Thr199 His236 Glu237 Glu238         His3 Gly155 Leu156 Phe178 Asp179 Pro180 Arg181 Leu183 Pro201         His3 Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Ala173 Phe175 Asn177 Glu237           P236R         Pro21 Ile22 Phe230 Asn231 Glu235 Arg236 Glu237 Glu238         Val49 Asp52 Gln53 Pro180 Lys111 Gly155 Leu156 Gln157 Leu183         Asp52 Ile59 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175           N252D         Leu163 Asp164         Lys111 Gly155 Leu156 Gln157 Leu183         Gly155 Leu156 Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly155 Leu156 Leu188           K18Q         Thr35 Lys111 Pro154 Gly155 Leu156 Lys158 Val159 Pro180 Gly182 Leu183 Glu220         Gly25 Glu26 Arg27 Ala54           H107Y         Ser29 Val31 Asp101 Lys111         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54		Glu238											
Lys9         His10         Asn11         Lys24         Val159         Val160         Asp161         Leu163           H107Y         Lys24         Val49         Ser50         Asp52         Gln53         Asp52         Gln53         Gly155         Lys118         Val159         Val160         Asp161         Val162         Leu163         Asp164         Ser165         Ile164         Asp164         Ser165         Ile164         Asp164         Ser165         Ile164         Asp164         Ser165         Ile164         Asp164         Ser165         Ile163         Asp164         Ser165         Ile164         Asp164         Ser165         Ile163         Asp164         S	K18Q	His3 Ile22 Ala152	His3 His4 Trp5 Gly6 Gly8	His3 His4 Lys9 Lys24 Lys158									
Lys111         Asp164 Ser165 Ile166           H107Y         Lys24         Val49 Ser50 Asp52 Gln53         Asp52 Gln53 Gly155 Lys158           Lys111 Leu184         Val159 Val160 Asp161 Val162         Leu163 Ser165 Ile166 Asp179           P236H         Ile22 Asp101 Gly155 Thr199         His3 Gly155 Leu156 Phe178         His3 Ala54 Lys158 Val159           His236 Glu237 Glu238         Asp179 Pro180 Arg181         Val160 Asp161 Val162 Leu163           Leu183 Pro201         Asp164 Ser165 Ile166 Ala173           Phe175 Asn177 Glu237         P236R         Pro21 Ile22 Phe230 Asn231           Glu235 Arg236 Glu237         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           Val49 Asp52 Gln53 Pro180         Asp164 Ser165 Ile166 Ala173           Phe175 Asn177 Glu237         P236R         Pro21 Ile22 Phe230 Asn231         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           Glu235 Arg236 Glu237         Glu235         Arg236 Glu237         Asp164 Ser165 Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160           Asp164 Ser165 Ile166 Pro180 Glu237         Asp164 Ser165 Ile166 Pro180 Glu237         Asp164 Ser165 Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Gly155 Leu163 Leu163           Gly155 Lys111 Lys153 Leu1			Lys9 His10 Asn11 Lys24	Val159 Val160 Asp161 Leu163									
H107Y         Lys24         Val49 Ser50 Asp52 Gln53         Asp52 Gln53 Gly155 Lys158           Lys111 Leu184         Val159 Val160 Asp161 Val162         Leu163 Ser165 Ile166 Asp179           P236H         Ile22 Asp101 Gly155 Thr199         His3 Gly155 Leu156 Phe178         His3 Ala54 Lys158 Val159           His236 Glu237 Glu238         Asp179 Pro180 Arg181         Val160 Asp161 Val162 Leu163           Leu183 Pro201         Asp164 Ser165 Ile166 Ala173           P236R         Pro21 Ile22 Phe230 Asn231         Val49 Asp52 Gln53 Pro180         Asp164 Ser165 Ile166 Ala173           Plator         Glu235 Arg236 Glu237         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159           Val238         Val49 Asp52 Gln53 Pro180         Asp164 Ser165 Ile166 Phe175           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160           Gln157 Leu183         Asp164 Val162 Leu163         Asp164 Ser165 Ile166 Phe175           ΔL increase (Residue accessibility decrease)         Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Pro154 Gly155         Gly12 Pro13 Glu14 Asp19         Ile22 Ala54 Ala152           Leu164 Gly159 Pro180         Gly12 Pro13 Glu14 Asp19         Ile22 Ala54 Ala152           Leu164 Gly20         Leu163 Glu233         Gly25 Glu26 Arg27 Ala54			Lys111	Asp164 Ser165 Ile166									
Lys111 Leu184       Val159 Val160 Asp161 Val162 Leu163 Ser165 Ile166 Asp179 Pro180         P236H       Ile22 Asp101 Gly155 Thr199 His236 Glu237 Glu238       His3 Gly155 Leu156 Phe178 Asp179       His3 Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Ala173 Phe175 Asn177 Glu237         P236R       Pro21 Ile22 Phe230 Asn231 Glu235       Val49 Asp52 Gln53 Pro180 Asp52 Ile59 Lys158 Val159 Val160 Asp161 Val162 Leu163 Ile166 Pro180 Glu237         N252D       Leu163 Asp164       Lys111 Gly155 Gln157 Leu183       Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175         M18E       Trp5 Lys111 Lys153 Leu188       Ala152 Lys153       Gly155 Leu156 Leu156 Leu156 Leu156 Leu188         K18E       Thr35 Lys111 Pro154 Gly155 Leu156 Lys158 Val159 Pro180       Gly12 Pro13 Glu14 Asp19 Leu163 Glu233       Ile22 Ala54 Ala152         H107Y       Ser29 Val31 Asp101 Lys111       Val31 Ala54 Lys158       Gly25 Glu26 Arg27 Ala54	H107Y	Lys24	Val49 Ser50 Asp52 Gln53	Asp52 Gln53 Gly155 Lys158									
P236H         Ile22 Asp101 Gly155 Thr199 His236 Glu237 Glu238         His3 Gly155 Leu156 Phe178 Asp179         His3 Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Ala173 Phe175 Asn177 Glu237           P236R         Pro21 Ile22 Phe230 Asn231 Glu235         Val49 Asp52 Gln53 Pro180 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159 Val160 Asp161 Val162 Leu163 Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111 Gly155 Leu156 Gln157 Leu183         Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175           X18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly155 Leu156 Leu186           K18Q         Thr35 Lys111 Pro154 Gly155 Leu163 Glu230         Gly12 Pro13 Glu14 Asp19 Leu163 Glu233         Ile22 Ala54 Ala152           K18Q         Thr35 Lys109 Pro180 Gly182 Leu183 Glu220         Gly25 Glu26 Arg27 Ala54			Lys111 Leu184	Val159 Val160 Asp161 Val162									
Pro180           P236H         Ile22 Asp101 Gly155 Thr199 His236 Glu237 Glu238         His3 Gly155 Leu156 Phe178 Asp179         His3 Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Ala173 Phe175 Asn177 Glu237           P236R         Pro21 Ile22 Phe230 Asn231 Glu235         Val49 Asp52 Gln53 Pro180 Asp52 Glu237         Asp52 Ile59 Lys158 Val159 Val160 Asp161 Val162 Leu163 Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111 Gly155 Leu156 Gln157 Leu183         Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly125 Thr199 Val31 Ala54 Lys158         Ile22 Ala54 Ala152           K18Q         Thr35 Lys111 Pro154 Gly155 Leu183 Glu220         Gly12 Pro13 Glu14 Asp19 Leu163 Glu233         Ile22 Ala54 Ala152           H180         Thr35 Lys111 Pro154 Gly155         Gly12 Pro13 Glu14 Asp19         Ile22 Ala54 Ala152           H107Y         Ser29 Val31 Asp101 Lys111         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54				Leu163 Ser165 Ile166 Asp179									
P236H       Ile22 Asp101 Gly155 Thr199       His3 Gly155 Leu156 Phe178       His3 Ala54 Lys158 Val159         His236 Glu237 Glu238       Asp179       Pro180       Arg181       Val160 Asp161 Val162 Leu163         Asp179       Pro180       Arg181       Leu163 Asp164 Ser165 Ile166 Ala173       Phe175 Asn177 Glu237         P236R       Pro21 Ile22 Phe230 Asn231       Val49 Asp52 Gln53 Pro180       Asp52 Ile59 Lys158 Val159         Glu235       Arg236       Glu237       Val49 Asp52 Gln53 Pro180       Asp52 Ile59 Lys158 Val159         Val160 Asp161 Val162 Leu163       Ile166 Pro180 Glu237       Val160 Asp161 Val162 Leu163       Ile166 Pro180 Glu237         N252D       Leu163 Asp164       Lys111 Gly155 Leu156       Ala54 Lys158 Val159 Val160       Asp164 Ser165 Ile166 Phe175         M157 Leu183       Glu157 Leu183       Asp164 Ser165 Ile166 Phe175       Asp164 Ser165 Ile166 Phe175         K18E       Trp5 Lys111 Lys153 Leu188       Ala152 Lys153       Gly155 Leu156 Leu188         K18Q       Thr35 Lys111 Pro154 Gly155       Gly12 Pro13 Glu14 Asp19       Ile22 Ala54 Ala152         Leu156 Lys158 Val159 Pro180       Leu163 Glu233       Ile22 Ala54 Ala152         Gly182 Leu183 Glu220       Val31 Ala54 Lys158       Gly25 Glu26 Arg27 Ala54				Pro180									
His236 Glu237 Glu238       Asp179       Pro180       Arg181       Val160 Asp161 Val162 Leu163         Leu183 Pro201       Leu183 Pro201       Asp164 Ser165 Ile166 Ala173         P236R       Pro21 Ile22 Phe230 Asn231       Val49 Asp52 Gln53 Pro180       Asp52 Ile59 Lys158 Val159         Glu235       Arg236       Glu237       Val49 Asp52 Gln53 Pro180       Asp52 Ile59 Lys158 Val159         Val160 Asp161 Val162 Leu163       Ile166 Pro180 Glu237       Val160 Asp161 Val162 Leu163       Ile166 Pro180 Glu237         N252D       Leu163 Asp164       Lys111 Gly155 Leu156       Ala54 Lys158 Val159 Val160       Asp164 Ser165 Ile166 Phe175         Mapped Stress       Cluass       Cluass       Glu237       Glu237         N252D       Leu163 Asp164       Lys111 Gly155 Leu156       Ala54 Lys158 Val159 Val160         Asp164 Ser165 Ile166 Phe175       Glu157 Leu183       Asp164 Ser165 Ile166 Phe175         K18E       Trp5 Lys111 Lys153 Leu188       Ala152 Lys153       Gly155 Leu156 Leu188         K18Q       Thr35 Lys111 Pro154 Gly155       Gly12 Pro13 Glu14 Asp19       Ile22 Ala54 Ala152         Leu163 Glu220       Leu163 Glu233       Ile22 Ala54 Ala152       Ile22 Ala54 Ala152         H107Y       Ser29 Val31 Asp101 Lys111       Val31 Ala54 Lys158       Gly25 Glu26 Arg27 Ala54	P236H	lle22 Asp101 Gly155 Thr199	His3 Gly155 Leu156 Phe178	His3 Ala54 Lys158 Val159									
Leu183 Pro201         Asp164 Ser165 file166 Ala173 Phe175 Asn177 Glu237           P236R         Pro21 Ile22 Phe230 Asn231 Glu235         Val49 Asp52 Gln53 Pro180         Asp52 Ile59 Lys158 Val159 Val160 Asp161 Val162 Leu163 Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111         Gly155         Leu156         Ala54 Lys158 Val159 Val160 Asp161 Val162 Leu163 Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly155 Leu156 Leu156 Leu188           K18Q         Thr35 Lys111 Pro154 Gly155 Leu156 Lys158 Val159 Pro180 Gly182 Leu183 Glu220         Gly12 Pro13 Glu14 Asp19 Leu163 Glu233         Ile22 Ala54 Ala152           H107Y         Ser29 Val31 Asp101 Lys111         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54		His236 Glu237 Glu238	Asp179 Pro180 Arg181	Val160 Asp161 Val162 Leu163									
Pro21         Ile22         Phe230         Asn177         Glu237           P236R         Pro21         Ile22         Phe230         Asn231         Val49         Asp52         Gln53         Pro180         Asp52         Ile59         Lys158         Val159           Glu235         Arg236         Glu237         Val49         Asp52         Gln53         Pro180         Asp52         Ile59         Lys158         Val162         Leu163           Support         Ile166         Pro180         Glu237         Val160         Asp161         Val162         Leu163           N252D         Leu163         Asp164         Lys111         Gly155         Leu163         Asp161         Val162         Leu163           Glu237         Asp164         Lys111         Gly155         Leu163         Asp164         Leu163           Masp164         Ser165         Ile166         Phe175         Asp164         Ser165         Ile166         Phe175           Masp164         Ser165         Ile166         Phe175         Gly155         Leu163         Asp164         Ser165         Ile166         Phe175           K18E         Trp5         Lys111         Lys153         Gly12         Pro13         Glu14			Leu183 Pro201	Asp164 Ser165 Ile166 Ala173									
P236R         Pro21 IIe22 Phe230 Asn231         Val49 Asp52 Gln53 Pro180         Asp52 IIe59 Lys158 Val159           Glu235         Arg236         Glu237         Val49 Asp52 Gln53 Pro180         Asp52 IIe59 Lys158 Val159           N252D         Leu163 Asp164         Lys111         Gly155         Leu156         Ala54 Lys158 Val159 Val160           Asp164         Lys111         Gly155         Leu163         Asp164 Val162         Leu163           M252D         Leu163 Asp164         Lys111         Gly155         Leu163         Asp161         Val162         Leu163           M160         Asp164         Lys111         Gly155         Leu163         Asp164 Val162         Leu163           M180         Thr95         Lys111         Lys153         Gly155         Gly155         Leu188           K180         Thr35         Lys119         Gly155         Gly12         Pro13         Glu14         Asp19         IIe22         Ala54         Ala152           Leu156         Lys158 Val159         Pro180         Leu163         Glu233         Gly122         Ala54         Lys154         Gly25         Gly25         Glu26         Arg27         Ala54           M107Y         Ser29         Val31         Ala54         Lys158         Gl				Phe175 Asn177 Glu237									
Glu235         Arg236         Glu237         Val160 Asp161 Val162 Leu163           Glu238         Ile166 Pro180 Glu237         Ile166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111         Gly155         Leu156         Ala54 Lys158 Val159 Val160           Asp164         Lys111         Gly155         Leu163         Asp161         Val162         Leu163           Main         Asp164         Lys111         Gly155         Leu163         Asp164         Leu163           Main         Asp164         Ser165         Ile166         Phe175         Asp164         Ser165         Ile166         Phe175           Main         Trp5         Lys111         Lys153         Leu163         Asp164         Ser165         Leu166         Phe175           K180         Thr35         Lys111         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           Leu156         Lys158         Val159         Pro13         Glu233         Gly182         Leu183         Glu220         Ile22         Ala54         Ala152           H107Y         Ser29         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54	P236R	Pro21 Ile22 Phe230 Asn231	Val49 Asp52 Gln53 Pro180	Asp52 IIe59 Lys158 Val159									
Clu238         Ine 166 Pro180 Glu237           N252D         Leu163 Asp164         Lys111 Gly155 Leu156         Ala54 Lys158 Val159 Val160           Asp161         Val162         Leu163           AL increase (Residue accessibility decrease)         Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly155 Leu156 Leu188           K18Q         Thr35 Lys111 Pro154 Gly155         Gly12 Pro13 Glu14 Asp19         Ile22 Ala54 Ala152           Leu156 Lys158 Val159 Pro180         Leu163 Glu233         Gly182 Leu183 Glu220         Gly25 Glu26 Arg27 Ala54           H107Y         Ser29 Val31 Asp101 Lys111         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54		Glu235 Arg236 Glu237		Val160 Asp161 Val162 Leu163									
N252D         Leu163 Asp164         Lys111         Gly155         Leu156         Ala54 Lys158 Val159 Val160           Gln157 Leu183         Asp161         Val162         Leu163           ΔL increase (Residue accessibility decrease)         Asp164 Ser165 Ile166 Phe175           K18E         Trp5 Lys111 Lys153 Leu188         Ala152 Lys153         Gly155 Leu156 Leu188           K18Q         Thr35 Lys111 Pro154 Gly155         Gly12 Pro13 Glu14         Asp19         Ile22 Ala54 Ala152           Leu156 Lys158 Val159 Pro180         Leu163 Glu233         Gly182 Leu183 Glu220         Gly25 Glu26 Arg27 Ala54           H107Y         Ser29         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54		Glu238		lle166 Pro180 Glu237									
K18E         Trp5         Lys111         Lys153         Gly157         Gly157         Gly155         Leu183         Asp161         Val162         Leu163           K18Q         Thr35         Lys111         Lys153         Leu188         Ala152         Lys153         Gly155         Leu188           K18Q         Thr35         Lys111         Pro154         Gly155         Gly12         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           Leu156         Lys158         Val159         Pro180         Leu163         Glu233         Gly182         Leu183         Glu220         H107Y         Ser29         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54	N252D	Leu163 Asp164	LysIII Gly155 Leu156	Ala54 Lys158 Val159 Val160									
ΔL increase         (Residue accessibility decrease)           K18E         Trp5         Lys111         Lys153         Leu188         Ala152         Lys153         Gly155         Leu156         Leu188           K18Q         Thr35         Lys111         Pro154         Gly155         Gly12         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           K18Q         Thr35         Lys111         Pro154         Gly155         Gly12         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           Leu156         Lys158         Val159         Pro180         Leu163         Glu233         Gly182         Leu183         Glu220         Gly25         Glu26         Arg27         Ala54           H107Y         Ser29         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54			GIn157 Leu183	Asp161 Vall62 Leul63									
K18E         Trp5         Lys111         Lys153         Leu188         Ala152         Lys153         Gly155         Leu156         Leu188           K18Q         Thr35         Lys111         Pro154         Gly155         Gly12         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           K18Q         Thr35         Lys151         Gly155         Gly12         Pro13         Glu14         Asp19         Ile22         Ala54         Ala152           Leu156         Lys158         Val159         Pro180         Leu163         Glu233         Gly182         Leu183         Glu220         H107Y         Ser29         Val31         Asp101         Lys111         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54			(Desidue essesibility desugar	Asp164 Ser165 lie166 Phe175									
K18E         Irps         Lys111         Lys153         Leu188         Ala152         Lys153         Gly155         Leu186         Leu186           K18Q         Thr35         Lys111         Pro154         Gly12         Pro13         Glu14         Asp19         Ile22         Ala152           Leu156         Lys158         Val159         Pro180         Leu163         Glu233         Gly182         Leu183         Glu220           H107Y         Ser29         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54	V10E		(Residue accessibility decrease										
K18Q         Thr55 Lys111 Pr0154 Gly155         Gly12 Pr013 Glu14 Asp19         He22 Ala54 Ala152           Leu156 Lys158 Val159 Pro180         Leu163 Glu233         Leu163 Glu220         Leu163 Glu233           H107Y         Ser29         Val31 Asp101 Lys111         Val31 Ala54 Lys158         Gly25 Glu26 Arg27 Ala54	KI8E	<b>Ifp5</b> Lys111 Lys155 Leu188	Ala152 Lys155	Gly155 Leu156 Leu188									
Hill         Ser29         Val13         Asp101         Lys111         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54	K18Q	Ihr35 Lys111 Pro154 Gly155	Gly12 Pro13 Glu14 Asp19	liezz Ala54 Ala152									
H107Y         Ser29         Val31         Ala54         Lys158         Gly25         Glu26         Arg27         Ala54		Cl. 182 L 182 Ch. 220	Leu163 Glu233										
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Gly182 Leu183 Glu220	V-121 ALEAL150	$C_{1} = 2E C_{1} = 2C A_{1} = 27 A_{1} = E4$									
Low140 Ala152 Lya152 Val241	H10/Y	Ser29 Vals1 Asp101 Lys111	valst Alas4 Lysts8	Giy25 Giu26 Arg27 Ala54									
Decision         Letter         Lysion         Lysion           B22(11         Lis2         Thr25         Ala152         Lysion         Lysion           B22(11         Lis2         Thr27         Los1(2)         Ana12(4)         Los2(5)	DODALI	Leura Alarsz Lysiss varzar	1122(Thr 271 - 121) = 1(4)	Lys111 GIII248 Lys231									
<b>P230H</b> Hiss Inf55 Ala152 Lys156 Hiss6 Inf57 Leu165 Asp164 Leu156 <u>PRe225</u> Arg226	P230H	HIS3 INF35 AI8152 Lys158	Chu170	Leu156 <u>Priezzs</u> Arg226									
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	D226D	Val109 Val100 His2 Cly6 Tyr7 Thr25 Cly09	Al254 Lou163 Acp164 Chr170	Ala54 Lou156 Chr170									
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1 230K	Sor 99 Lou $100$ Ly $17$ 11055 Gly98	<b>Thr109</b> Cly232 Cly234	Alast Leuiso Giyiro									
$\frac{111177}{11272} \text{ Giv}_{22} \text{ Giv}_{23} \text{ Giv}_{23}$	N252D	$Hic_3$ Thr 25 Clut 63 Pro154	$\frac{111177}{\text{Hig}^2} \text{ Gry252} \text{ Gry254}$	Chy155 Lou156 Arg226									
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1NZ3ZD	Clv155 Lev156 $Clv157$	11155 111155 5e145 Ala152	Giy 155 Leu 156 Aig220									
Lys158 Pro180 Leu183		Lvs158 Pro180 Leu183											

**Table S7.** Variant residues showing decreases and increases to  $\Delta BC$  during MD simulation. Residues from Table S1 are underlined and highlighted in bold. SNV positions are underlined, italicised and highlighted in bold red.

Variant	Аро	BCT	CO <sub>2</sub>						
$\triangle BC$ decrease (reduction in residue communication)									
K18E	Trp5 Gly6 Pro200 Met240	<u>His64</u> His107 Lys113 <u>Glu117</u>	Asn61 Ala77 Leu90 Val162						
	Asp242 <u>Asn243</u>	Ile145 Leu147 Lys153 <u>Thr199</u>	Gly182 Leu183 Val217						
		Ile215 Val222	Phe225						
K18Q	Gly63 <u>His94</u> <u>Glu117</u> <u>His119</u>	Trp5 Gly12 Pro13 Trp16	Trp5 Ala54 Leu60 Asn61						
	Leu147 Pro180 Leu183	Phe66 Asn67 His107 Glu117	<u>His64</u> Phe/0 Gly182 <u>Phe225</u>						
	Asp242 Irp244	Ihr168 Asp242 <u>Asn243</u>	Asp242						
L107V	Val21 Chr62 Hic04 Chr117	Val21 Ala54 Phace Trutto7	$I_{12}$ $A_{12}$ $A$						
H10/ I	$\frac{\text{Vals1 Gly65 } \underline{\text{His94}}}{\text{His110}} = \frac{\text{Glu117}}{\text{Val142}}$	Chr.117 Chr.144 Thr.100	$\frac{11853}{Clus117} = \frac{11804}{Lou147} = \frac{11804}{Thr 100}$						
	$\frac{\mathbf{HISH5}}{\mathbf{Clv}144}$ Let $\mathbf{Val142}$	<u>Glu117</u> Gly144 <u>111199</u>	Val222 Phe225						
P236H	Gly63 Val68 Gln92 Val121	Phe66 Phe70 Gln92 Phe95	Asn61 Ala77 Leu90 Leu147						
1 20011	Val142 Leu147 Val159	<b>Glu117</b> Leu163	Glv182 Leu183 Val222						
	Asp242		Phe225						
P236R	Gly6 His94 Ser105 His119	Tyr51 Ala54 Asn67 Phe95	Ala54 Leu60 Asn61 Glu69						
	Val142 Asp242 Asn243	Glu117 Leu118 Leu147	Gly182 Leu183 <u>Phe225</u>						
	-	<u>Thr199</u>							
N252D	Gly63 His64 His94 His119	<u>His64</u> His107 <u>Glu117</u> Leu147	Leu60 Asn61 Phe70 Ala77						
	Leu156 Pro180 Leu183	Lys153 <u>Thr199</u> Ile215	Gly182 <u>Phe225</u>						
	<u>Thr199 Phe225</u>								
	∆BC increase	(residue communication increa	ise)						
K18E	His4 <u>Trp16</u> <u>His96</u> <u>Thr199</u>	Ser105 Ala116 Leu118 Gly155	Ala54 Ala65 Val68 <u>Phe93</u>						
	Phe230	Leu156 Leu183 Val210 Val217	Phe95 Ala116 Leu118 Val159						
1/100			<u>Thr199</u>						
K18Q	Phe66 H1596 Lys113 Ala116	Gly8 Asn11 <u>H1894</u> Ser105	1yr/ Gly8 Asn11 Ala65						
	11e145 fie215 val217	Chr248	<u><b>PRe66</b></u> valos <u><b>H1596</b></u> valibu						
H107V	Phase Asper Phase Chulle	Thr55 Ala77 Lou90 His94	Ala65 Pha66 Pha93 Pha95						
1110/1	Leu118 Val217	Ser105 Val210	<b>His96</b> Glu106 Ala116 Asp179						
P236H	His64 Phe66 His96 Ala116	Gly63 Phe93 His94 Trp97	Ala54 Ala65 Phe66 Phe93						
1 20011	Gly155 Thr199 Val217	Val134	<b>Phe95</b> Ala116 Leu163						
P236R	Tyr7 Phe66 Phe95 His96	Ala77 His94 Ala116 His119	Ala65 Phe66 His94 Ala116						
	<u>Glu106</u> <u>Thr199</u> Phe230	Val217	Leu163 Phe175 Pro180						
	Arg245								
N252D	Phe66 Asn67 Gln92 Phe95	Ser105 Ala116 Gly155	Tyr51 Ala54 Ala65 Phe66						
	His96 Ile215 Asn243	Leu156 Leu183 Val217	Val68 Ile91 Gln92 Phe93						
		<u>Asn243</u> Trp244	Val142 Val160 Leu163 Ile215						

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