

**A****Comprehensive Prediction Results**

Mutation Site		Structural Features	
Protein:	3J9T	SS element:	Other (turns, coils, etc.)
Chain:	O	Solvent accessibility:	27.57%
Wild type AA:	ILE	Torsion angles:	-115.5°, 20.8°
Residue ID:	178		

**Amino Acid Mutations**

Amino acid	Overall Stability	Torsion*	Predicted $\Delta\Delta G$ (kcal/mol)
GLY	Destabilising	Unfavourable	-0.17
ALA	Destabilising	Unfavourable	-0.09
VAL	Destabilising	Unfavourable	-0.11
LEU	Stabilising	Unfavourable	0.08
MET	Destabilising	Unfavourable	-0.75
PRO	Destabilising	Unfavourable	-0.06
TRP	Stabilising	Favourable	0.28
SER	Stabilising	Unfavourable	0.39
<b>THR</b>	<b>Stabilising</b>	<b>Favourable</b>	<b>0.63</b>
PHE	Stabilising	Favourable	0.53
GLN	Stabilising	Unfavourable	0.37
LYS	Destabilising	Unfavourable	-0.59
TYR	Stabilising	Favourable	0.32
ASN	Stabilising	Favourable	0.35
CYS	Stabilising	Favourable	0.04
GLU	Stabilising	Unfavourable	0.33
ASP	Stabilising	Favourable	0.49
ARG	Stabilising	Unfavourable	0.58
HIS	Stabilising	Favourable	0.92

**B****Comprehensive Prediction Results**

Mutation Site		Structural Features	
Protein:	3J9U	SS element:	Other (turns, coils, etc.)
Chain:	O	Solvent accessibility:	41.62%
Wild type AA:	ILE	Torsion angles:	-120.9°, 148.6°
Residue ID:	178		

**Amino Acid Mutations**

Amino acid	Overall Stability	Torsion*	Predicted $\Delta\Delta G$ (kcal/mol)
GLY	Destabilising	Unfavourable	-1.64
ALA	Destabilising	Unfavourable	-1.13
VAL	Destabilising	Favourable	-1.18
LEU	Destabilising	Favourable	-1.46
MET	Destabilising	Favourable	-1.22
PRO	Destabilising	Unfavourable	-1.35
TRP	Destabilising	Favourable	-1.95
SER	Destabilising	No change	-1.23
<b>THR</b>	<b>Destabilising</b>	<b>Favourable</b>	<b>-1.16</b>
PHE	Destabilising	Favourable	-1.08
GLN	Destabilising	Unfavourable	-0.91
LYS	Destabilising	Unfavourable	-1.64
TYR	Destabilising	Favourable	-1.76
ASN	Destabilising	Unfavourable	-1.79
CYS	Destabilising	Favourable	-2.35
GLU	Destabilising	Unfavourable	-1.79
ASP	Destabilising	Unfavourable	-1.88
ARG	Destabilising	Unfavourable	-1.41
HIS	Destabilising	Unfavourable	-0.96

**C****Comprehensive Prediction Results**

Mutation Site		Structural Features	
Protein:	3J9V	SS element:	Other (turns, coils, etc.)
Chain:	O	Solvent accessibility:	12.97%
Wild type AA:	ILE	Torsion angles:	-112.6°, 132.6°
Residue ID:	178		

**Amino Acid Mutations**

Amino acid	Overall Stability	Torsion*	Predicted $\Delta\Delta G$ (kcal/mol)
GLY	Destabilising	Unfavourable	-3.25
ALA	Destabilising	Unfavourable	-2.23
VAL	Destabilising	Favourable	-3.57
LEU	Destabilising	Favourable	-3.38
MET	Destabilising	Unfavourable	-5.92
PRO	Destabilising	Unfavourable	-4.62
TRP	Destabilising	Favourable	-4.68
SER	Destabilising	Unfavourable	-3.52
<b>THR</b>	<b>Destabilising</b>	<b>Favourable</b>	<b>-3.72</b>
PHE	Destabilising	Favourable	-2.45
GLN	Destabilising	Unfavourable	-7.92
LYS	Stabilising	Unfavourable	0.49
TYR	Destabilising	Favourable	-3.97
ASN	Destabilising	Unfavourable	-2.22
CYS	Destabilising	Favourable	-9.78
GLU	Destabilising	Unfavourable	-2.82
ASP	Destabilising	Unfavourable	-3.61
ARG	Destabilising	Unfavourable	-3.7
HIS	Destabilising	Unfavourable	-3.52

**Supplementary Figure S3. Protein stability prediction (CUPSAT) implicates a destabilizing effect of the mutation in yeast VMA5.**

Thermal protein stability was predicted (<http://cupsat.tu-bs.de/index.jsp>) for the three states of VMA5 reported by Zhao et al. (*Nature* 521:241-245, 2015).<sup>15</sup> CUPSAT predicts stabilization of state 1 (**A**) and destabilization of state 2 (**B**) and state 3 (**C**). These results suggest a fixation of the protein in state 1 preventing the dynamic movement that is most likely necessary for the stability of the active ATPase (Zhao et al. *Nature* 521:241-245, 2015).<sup>15</sup>