

System	Mean dG* (kcal/mol)	Mean dG0 (kcal/mol)
WT Human Sec	16.37 $\pm$ 0.95 kcal/mol	-60.85 $\pm$ 1.84 kcal/mol
WT Mouse Cys	20.72 $\pm$ 1.07 kcal/mol	-55.43 $\pm$ 1.77 kcal/mol
Mouse Sec C49U	15.75 $\pm$ 0.71 kcal/mol	-64.42 $\pm$ 1.86 kcal/mol
Mouse Cys F48Y	20.25 $\pm$ 0.47 kcal/mol	-52.71 $\pm$ 1.74 kcal/mol
Mouse Cys T52A	20.08 $\pm$ 1.35 kcal/mol	-48.75 $\pm$ 2.83 kcal/mol
Mouse Cys S47A	17.38 $\pm$ 0.59 kcal/mol	-56.04 $\pm$ 2.59 kcal/mol
Mouse Cys C99R	17.74 $\pm$ 0.69 kcal/mol	-49.28 $\pm$ 2.55 kcal/mol
Mouse Cys Q54T	20.30 $\pm$ 1.04 kcal/mol	-56.15 $\pm$ 1.82 kcal/mol
Mouse Cys H177Q	21.33 $\pm$ 0.87 kcal/mol	-55.91 $\pm$ 2.14 kcal/mol
Mouse Cys H144Q	22.42 $\pm$ 1.15 kcal/mol	-56.18 $\pm$ 2.28 kcal/mol
Mouse Cys T178A	21.87 $\pm$ 1.52 kcal/mol	-58.78 $\pm$ 2.46 kcal/mol
Mouse Cys G74A	20.78 $\pm$ 0.42 kcal/mol	-54.45 $\pm$ 2.28 kcal/mol
Mouse Cys P67N	25.78 $\pm$ 0.48 kcal/mol	-56.45 $\pm$ 2.28 kcal/mol
Mouse Cys Q33K	25.78 $\pm$ 0.48 kcal/mol	-56.45 $\pm$ 2.88 kcal/mol
Mouse Cys N192K	23.78 $\pm$ 0.50 kcal/mol	-57.45 $\pm$ 2.78 kcal/mol
Mouse Cys T194F	24.44 $\pm$ 0.48 kcal/mol	-57.45 $\pm$ 2.18 kcal/mol
Mouse Cys S195N	25.89 $\pm$ 0.48 kcal/mol	-56.45 $\pm$ 2.30 kcal/mol
Human Cys U49C	19.34 $\pm$ 1.05 kcal/mol	-49.99 $\pm$ 2.73 kcal/mol
Human Sec Y48F	23.69 $\pm$ 1.61 kcal/mol	-51.15 $\pm$ 1.82 kcal/mol
Human Sec A52T	19.49 $\pm$ 0.61 kcal/mol	-56.84 $\pm$ 1.80 kcal/mol
Human Sec A47S	18.95 $\pm$ 1.22 kcal/mol	-58.86 $\pm$ 1.90 kcal/mol
Human Sec C99R	17.52 $\pm$ 0.69 kcal/mol	-50.28 $\pm$ 2.55 kcal/mol
Human Sec Q54T	19.18 $\pm$ 1.04 kcal/mol	-58.15 $\pm$ 1.82 kcal/mol
Human Sec Q177H	20.21 $\pm$ 1.31 kcal/mol	-60.24 $\pm$ 1.47 kcal/mol
Human Sec Q144H	18.57 $\pm$ 0.44 kcal/mol	-57.97 $\pm$ 1.31 kcal/mol
Human Sec A178T	17.18 $\pm$ 0.51 kcal/mol	-60.77 $\pm$ 1.62 kcal/mol
Human Sec A74G	13.42 $\pm$ 0.92 kcal/mol	-67.47 $\pm$ 1.50 kcal/mol
Human Sec N67P	23.88 $\pm$ 0.50 kcal/mol	-56.45 $\pm$ 1.28 kcal/mol
Human Sec K33Q	25.13 $\pm$ 0.59 kcal/mol	-55.58 $\pm$ 1.70 kcal/mol
Human Sec K192N	22.78 $\pm$ 0.50 kcal/mol	-57.45 $\pm$ 1.78 kcal/mol
Human Sec F194T	23.40 $\pm$ 0.48 kcal/mol	-54.45 $\pm$ 1.19 kcal/mol
Human Sec N195S	22.89 $\pm$ 0.48 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol
Human Sec N3K	21.89 $\pm$ 0.48 kcal/mol	-50.45 $\pm$ 2.00 kcal/mol
Human Sec R4S	20.89 $\pm$ 1.48 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol
Human Sec I16V	22.89 $\pm$ 0.58 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol
Human Sec L24I	21.09 $\pm$ 0.58 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol
Human Sec N25D	19.89 $\pm$ 0.65 kcal/mol	-50.45 $\pm$ 2.00 kcal/mol
Human Sec E27G	22.16 $\pm$ 0.65 kcal/mol	-52.45 $\pm$ 2.00 kcal/mol
Human Sec E27G	20.16 $\pm$ 0.65 kcal/mol	-53.45 $\pm$ 2.00 kcal/mol
Human Sec Y29F	21.16 $\pm$ 0.65 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol
Human Sec Q31N	24.16 $\pm$ 0.65 kcal/mol	-51.45 $\pm$ 2.00 kcal/mol

Table 1: Free energy changes in wild type and mutants at 10 Å distance with errors