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Supplement 1: A Diverse Set of Mutants

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We describe the details of our test set of mutants that we assembled from many different papers.

One group of mutations either destabilized or increased the reactivity of the WT protein. For instance, Bonander et al. (2000) reported destabilization of azurin protein due to the destruction of disulfide bonds (with cystines mutated to other residues). de Antonio et al. (2000) analyzed the effect of mutating tryptophan residues (to phenylalanines) on the functional properties of the ribotoxin α-sarcin, and reported decreased thermostability. Takano et al. (1999) observed destabilization of human lysozyme when foreign terminal residues were introduced, due to the disruption of hydrogen bonding. Ribonucleotide reductase mutations considered by Ormö et al. (1995) had similar destabilizing effects. Chen and Gouaux (1997) achieved increased reactivity in an aqueous solution of bacteriorhodopsin by introducing polar residues in the enzyme surface in Helix D. Carter, Jr et al. (2001) presented a fairly large data set of mutants (76 mutants of five proteins) collected from various previous studies. All of them were destabilizing substitutions made to one or more residues in the hydrophobic core of the proteins in question. We did not include three mutants of T4 lysozyme (PDB code 1L63) from our test set. One of them considered "replacing" residue 71, which is a Valine, with a Valine itself (it is not clear how a SNAPP score was recorded by the authors for this "non-mutant"). Then there were two different sets of data recorded for mutating residue 71 to an Alanine. Even though we identified this change (V71 to A) correctly as a destabilizing one (as indicated by the mutant having a smaller four-body score), it is ambiguous which $\Delta\Delta G$ value to use. Hence we decided to leave out both the V71A mutants. Erwin et al. (1990) engineered salt bridges in subtilisin BPN', resulting in the protein being destabilized. We must note here that a few mutations from this study were not included in our list, as they resulted in mutually disagreeing effects – for instance, these mutants had increased stability and increased enzyme reactivity as well. Lastly, mutations from a study on evolutionally conserved aspartic acid residues in human glutathione S-transferase P1-1 (Kong et al., 1993) were chosen due to the authors reports of decreased thermostability.

Another category of mutants included in our list had stabilizing effects on the WT protein, or decreased its chemical activity. Martin et al. (2001) analyzed mutants that increased the thermostability of a cold shock protein. Pathange et al. (2006) introduced histidine residues on the surface of a protein in order to decrease its enzymatic activity. Hahn et al. (1995) analyzed mutants of glucanase that showed decreased enzymatic activity. In one interesting case, Korkegian et al. (2005) achieved thermostability of cytosine deaminase with the aid of computational techniques. Similarly, Almog et al. (2002) studied thermostabilized mutations of subtilisin BPN'. Siadat et al. (2006) engineered disulfide bonds in Drosophila melanogaster acetylcholinesterase to stabilize it. However, some mutants from this study were not included due to multiple disagreeing effects.

A third group of mutants were selected from papers that reported some increased stability (or reactivity) mutants and some other mutants that showed the opposite effect(s) for the same WT protein. For instance, Ge et al. (2003) studied the activity of a rice lipid transfer protein. We included those mutants that showed increased enzymatic activity. At the same time, mutants whose secondary structure was reported to be destroyed were categorized as destabilizing as well. Certain other mutants were not included in our set due to unspecified "similar activity" to wild type. Suresh et al. (2006) analyzed the effects of C-reactive protein on protecting mice from pneumococcal infection. Two mutations were included from this study: 175A reduced enzymatic activity, while 114A increased the same. Funahashi et al. (2002) reported several mutants of human lysozyme protein. They included mutants with both increased and decreased stability as compared to the WT.

The last group of mutants were selected from studies that used mutagenesis scanning. This is a process in which specific residues (often referred to as *sites*) of the WT protein are mutated with a goal to differentiate functions of particular parts of the protein. Sun et al. (2001) performed active site mutagenesis of methylamine dehydrogenase and reported two groups of mutants – stabilizing and destabilizing. In another case, Huang et al. (1996) performed site-directed mutagenesis of phosphatase 1. Some mutations were categorized as stabilizing due to decreased enzymatic activity and/or increased stability to tested drugs. Others were categorized as destabilizing due to increased enzymatic activity or decreased stability to the tested drugs. Furthermore, some mutations were excluded due to disagreeing effects of the aforementioned cases. Oppermann et al. (1997) conducted site-directed mutagenesis of hydroxysteroid dehydrogenase, and reported the mutations to be either stabilizing or destabilizing based on their enzymatic activity. Mutations that were listed as resulting in "little or no effect" were not considered for our list. Similarly, the mutants considered by Dvir et al. (2003) for human acid-beta-glucosidase using site-directed mutagenesis belonged to two groups: one for decreased enzymatic activity and one for increased enzymatic activity or secondary structure destruction. However, some mutations were required to be excluded based on multiple disagreeing affects or non-reporting of stabilities. Mutants of porphobilinogen deaminase reported by Brownlie et al. (1994), those of lactose permease created by Braun et al. (1997) using site-directed alanine insertion, and those of ribonuclease A reported by Köditz et al. (2004) were all selected into these two groups in a similar manner.

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