Supplementary Material

Predicting Folding Free Energy and Dynamics Changes Caused by the Mutation Leu(381)Phe

Changes in the folding free energy were predicted *in silico* using 20 NMR models of the large domain 1 (see Supplementary Figure 1A; see also Figure 2A) and then the results were averaged across the 20 models to reach a consensus prediction. Three tools were used: two servers named FoldX and ERIS, and an approach utilizing an in-house server named TINKER. The folding free energy changes were calculated to be very large indicating that the site is very sensitive to the mutation.

Molecular dynamics (MD) simulations were carried out as well. (Detailed results of these simulations are not shown and may be presented upon request). In the majority of the cases, the flexibility as monitored via the root mean square deviation RMSD of the MD snap-shots versus the initial structures, differs drastically between the wild-type and the mutant PSEN1. This observation, again, speaks in favor that the mutation greatly affects the wild-type characteristics.

Predicting Folding Free Energy and Dynamics Changes Caused by the Mutation Arg(280)Ala

Because the NMR structure only covered residues after position 292, new structural models had to be created in order to model the effects of mutation Glu(280)Ala. This was done using the I-Tasser webserver. Of the five plausible models produced, only models 1 and 5 met the criteria to be used for energy calculations when they were superimposed over the known NMR structure using Chimera. The models 1 and 5 had extended structure that aligned well, whereas the

eliminated three models had too compact a structure, which does not match the experimental structure of the large domain 1. Similar to Leu(381)Phe, only the small domain surrounding the mutation site was used in calculations to reduce errors resulting from model uncertainty (see Supplementary Figure B).

Changes in the stability and folding free energy of the protein were completed in the same fashion as for large domain 1, and the results of I-Tasser models 1 and 5 were averaged in order to reach a prediction consensus. It was predicted that the folding free energy changes are very large, but somehow not as large as for Leu(381)Phe. Taken altogether, the mutation Glu(280)Ala is also expected to affect the wild type properties of PSEN1 significantly, but the effect to be less pronounced as compared with Leu(381)Phe.

The MD simulations of the small domain 2 show that the mutant is less flexible than the wild-type. The difference is significant and most probably will affect the function associated with the small domain 2.

Conclusion

Both mutations were predicted to significantly affect the folding free energy and flexibility of PSEN1; however, the changes are predicted to be greater for the Leu(381)Phe mutation than those of the Glu(280)Ala mutation. This, in addition to sequence conservation and known mutation data, leads to the conclusion that the Leu(381)Phe mutation is predicted to be more deleterious than the Glu(280)Ala mutation.