Stable proteins are used in numerous biomedical and biotechnological applications. Unfortunately, naturally occurring proteins cannot usually withstand the harsh industrial environment, since they are mostly evolved to function at mild conditions. Therefore, there is a continuous interest in increasing protein stability to enhance their industrial potential. A number of in silico tools for the prediction of the effect of mutations on protein stability have been developed recently. However, only single-point mutations with a small effect on protein stability are typically predicted with the existing tools and have to be followed by laborious protein expression, purification, and characterization. A much higher degree of stabilization can be achieved by the construction of the multiple-point mutants. Here, we present the FireProt method [1] and the web server [2] for the automated design of multiple-point mutant proteins that combines structural and evolutionary information in its calculation core. FireProt utilizes sixteen bioinformatics tools, including several force field calculations. Highly reliable designs of the thermostable proteins are constructed by two distinct protein engineering strategies, based on the energy and evolution approaches and the multiple-point mutants are checked for the potentially antagonistic effects in the designed protein structure. Furthermore, time demands of the FireProt method are radically decreased by the utilization of the smart knowledge-based filters, protocol optimization, and effective parallelization. The server is complemented with an interactive, easy-to-use interface that allows users to directly analyze and optionally modify designed thermostable proteins. The server is freely available at http://loschmidt.chemi.muni.cz/fireprot.
