

Structural bioinformatics

INPS-MD: a web server to predict stability of protein variants from sequence and structure

Castrense Savojardo^{1,†}, Piero Fariselli^{2,†}, Pier Luigi Martelli^{1,*} and Rita Casadio^{1,3}

¹Biocomputing Group, CIG, Interdepartmental Center «Luigi Galvani» for Integrated Studies of Bioinformatics, Biophysics and Biocomplexity, University of Bologna, Italy, ²BCA, University of Padova, Italy and ³Interdepartmental Center «Giorgio Prodi» for Cancer Research, University of Bologna, Italy

*To whom correspondence should be addressed.

†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

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Abstract

Motivation: Protein function depends on its structural stability. The effects of single point variations on protein stability can elucidate the molecular mechanisms of human diseases and help in developing new drugs. Recently, we introduced INPS, a method suited to predict the effect of variations on protein stability from protein sequence and whose performance is competitive with the available state-of-the-art tools.

Results: In this article, we describe INPS-MD (Impact of Non synonymous variations on Protein Stability-Multi-Dimension), a web server for the prediction of protein stability changes upon single point variation from protein sequence and/or structure. Here, we complement INPS with a new predictor (INPS3D) that exploits features derived from protein 3D structure. INPS3D scores with Pearson's correlation to experimental $\Delta\Delta G$ values of 0.58 in cross validation and of 0.72 on a blind test set. The sequence-based INPS scores slightly lower than the structure-based INPS3D and both on the same blind test sets well compare with the state-of-the-art methods.

Availability and Implementation: INPS and INPS3D are available at the same web server: <http://inpsmd.biocomp.unibo.it>.

Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

Contact: gigi@biocomp.unibo.it

1 Introduction

Stability of protein variants may or may not be different for wild type. This information is relevant to understand the relation between protein variants and insurgence of diseases (Ashley, 2015; Lu *et al.*, 2014). Several methods have been developed so far to predict stability change in protein variants, either based on protein sequence or structure features (Folkman *et al.*, 2016; Huang *et al.*, 2007; Laimer *et al.*, 2016; Pires *et al.*, 2014; Teng *et al.*, 2010). Recently we developed INPS (Fariselli *et al.*, 2015), a sequence-based method devised to predict protein stability change ($\Delta\Delta G$) upon single-point variations, well comparing with the state-of-the-art methods. Here we present a web server, INPS-MD that includes the sequence-based INPS, and a new

method INPS3D, exploiting descriptors derived from the protein 3D structure. Benchmark results, performed on two experimentally derived datasets of variations, show that the structure-based INPS3D performs better than the sequence-based INPS when predicting the change in protein stability upon variation. INPS-MD is available at <http://inpsmd.biocomp.unibo.it>.

2 INPS3D

INPS (Fariselli *et al.*, 2015) is based on descriptors extracted from the protein sequence. Briefly, seven features are used to encode a single-point mutation: (i) the substitution score $w \rightarrow m$ derived from

the Blosom62 matrix (Henikoff and Henikoff, 1992); (ii) the Kyte–Doolittle hydrophobicity (Kyte and Doolittle, 1982) scores of native and changed residues (two descriptors); (iii) the mutability index of the native residue (Dayhoff *et al.*, 1978); (iv) the molecular weights of native and changed residues (two descriptors); (v) the difference in the alignment score between the native and variant sequences and a HMM, encoding evolutionary information of the wild type sequence (Fariselli *et al.*, 2015). Sequence descriptors are mapped to $\Delta\Delta G$ values using a Support Vector Regression (Chang *et al.*, 2011) with a radial basis function kernel. In INPS3D, the set of INPS descriptors also includes features derived from protein 3D structure. In particular, the following two additional structure-based descriptors are considered: (i) one descriptor corresponding to the Relative Solvent Accessibility (RSA) of the native residue. The absolute solvent accessibility is computed from the PDB file using the DSSP method (Kabsch and Sander, 1983) and then normalized as previously described in Rost and Sander (1994); (ii) another descriptor encodes for the local energy difference (ED) between native and mutated protein structures. The energy is computed by means of a contact potential previously described in Bastolla *et al.* (2001). Given the set n of neighbors of the changed residue (two residues are considered in contact if the minimal distance between all atoms is $<5 \text{ \AA}$), the contact ED is scored as:

$$\sum_{r \in N} P(r, w) - P(r, m) \quad (1)$$

where w and m are, respectively, the native and the mutated residues, and P is the contact potential defined over pair of residues (Bastolla *et al.*, 2001).

2.1 Web server usage

INPS and INPS3D are included in INPS-MD, available at <http://inpsmd.biocomp.unibo.it>. Here, the user can select the sequence-based INPS or the structure-based INPS3D, when the protein structure is available. In both cases, the user must provide input files and parameters through the input submission form: (i) the query protein in the form of a single FASTA file in the case of INPS, or a valid PDB file in the case of INPS3D. In the latter case, the target PDB chain ID must be specified; (ii) a single file containing the list of point variations relative to the sequence or PDB chain. Upon submission, the server provides the user with a universal job identifier that can be thereafter used to retrieve results. For each variation listed in the input file, the server computes protein descriptors and performs $\Delta\Delta G$ predictions. Upon job completion, results can be visualized online or downloaded in plain-text format.

3 Results and conclusion

INPS and INPS3D are benchmarked on three different sets previously released (Pires *et al.*, 2014): (i) the S2648 dataset, comprising 2648 single-point mutations in 132 proteins derived from ProTherm (Kumar *et al.*, 2006), (ii) a subset of S2648 used as blind test set comprising 351 variations in 60 proteins and (iii) a dataset of 42 variations within the DNA-binding domain of the tumor suppressor protein P53. Table 1 lists results obtained for INPS and INPS3D with a cross validation procedure on the first set and adopting the remaining two as blind test sets. INPS3D outperforms INPS, achieving a Pearson's correlation 7% points higher than INPS and a standard error of 0.1 kcal/mol smaller in $\Delta\Delta G$ prediction. The relative contribution of the two structural features indicates that RSA is more informative than ED. Their combination leads to a further improvement in the performance (Pearson's correlation is 0.58). INPS

Table 1. Performance of INPS and INPS3D

Method	S2648 dataset Corr/SE	Blind test Corr/SE	P53 blind test Corr/SE
INPS	0.52/1.26	0.68/1.26	0.71/1.49
INPS+ED	0.52/1.25	0.68/1.26	0.71/1.45
INPS+RSA	0.54/1.24	0.70/1.18	0.74/1.36
INPS3D	0.58/1.20	0.72/1.15	0.76/1.35

Corr, Pearson's correlation coefficient between predicted and experimental DDG values. SE, standard error in $\Delta\Delta G$ prediction (kcal/mol); ED, energy difference; RSA, relative solvent accessibility.

and INPS3D predictions strongly correlate (Supplementary Figure S1) and well compare with the state-of-the-art predictors when tested on the same datasets (see Supplementary Table S1). INPS3D input includes information derived from multiple sequence alignment and its performance is dependent on the number of aligned sequences. INPS3D is less sensitive than INPS, and even when the number of aligned sequences is <100 , its Pearson's correlation is still 0.5 (Supplementary Figure S2). S2648 dataset contains some redundancy (44 proteins out of the 132 share $>25\%$ identity). In the most stringent per-protein split (Pires *et al.*, 2014), 16 pairs of proteins have sequence similarity between the training and testing sets. However, INPS and INPS3D seem unaffected by this redundancy, since no differences in the cross-validation performances are detected when the sequence similarity is removed. These cross-validation sets, together with the BLAST outputs, are available at the web server page. INPS-MD is a new web server that integrates information from sequence and structure to predict protein stability perturbation upon residue variations and allows the prediction in multi-dimensions.

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