Identification of amino acid residues affecting on the specificity of interaction of protein kinases and small molecular inhibitors

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Functional features of protein molecules frequently depend on the small number of amino acid residues. Such positions can determine the specificity of binding with small molecule ligands. Recognition of functionally significant residues is required for the drug design, the search of drug targets, protein engineering of enzymes, etc.

Protein kinases present the large family of enzymes, which performed the protein phosphorylation thus providing the cell signal transduction, metabolism regulation, control of cell cycle, etc. These proteins are promising drug targets, and their small molecule inhibitors are the perspective drug leads. In spite of the fact that the most inhibitors are bound in the same area of ATP-cleft, they show paradoxical selectivity related to the separate protein kinases. In this reason, these proteins and their inhibitors are attractive objects for the study of mechanisms of protein-ligand specificity.

The methods of 3D modeling are used to locate of ligand-specific positions in both the spatial structure and amino acid sequence. This approach is limited due to incompleteness and biasing of 3D data. The most popular tools are based on the multiple sequence alignment. However, the efficiency of such methods depends on the accuracy of matching of specific residues in an alignment that is not always performed in the case of diverged protein family, such as protein kinases.

In this work, the original method SPrOS [1] was applied for prediction of the ligand-specific residues of protein kinases. The method allows recognizing the positions conserved in the functional subgroups of protein families based on the similarity of position surroundings in sequences, without preliminary alignment. The protein sequences subdivided into the classes were compared with the test protein, providing the estimation of specificity of the individual positions to each class. The studied set included the protein sequences, classified based on the

indices of the protein-inhibitor interaction. The results of prediction were mapped into the protein 3D structures complexed with the inhibitors.

We examined the protein kinases differed in inhibitory spectra, but classes of inhibitory specificity of proteins were intersected each to other. We predicted the positions determining the specificity to different inhibitors in the same proteins.

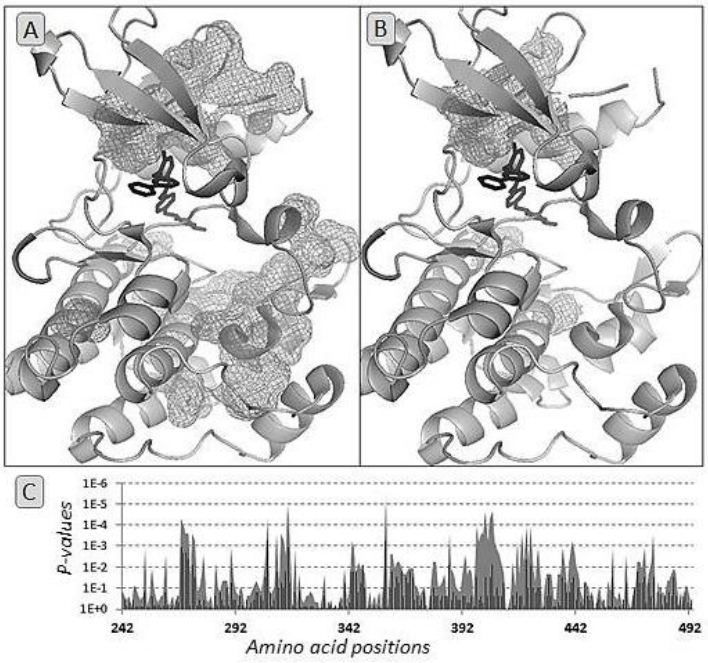

Figure 1. Prediction of amino acid residues determining the specificity of ABL1 kinase to the inhibitor Imatinib Location in 3D structure of residues predicted before (A) and after (B) excluding the close homologies of the test protein. The predicted positions are shown by the mesh. The significance estimates (p-values) of predicted positions (C) are shown by the gray areas (before the excluding procedure) and black bars (after the procedure).

The predicted sets of inhibitor-specific positions were not matched as a whole. But the same fragments of different ligands were shown to be contacted with same predicted positions. The recognized residues were located in the area of ATP-cleft directly contacting with the inhibitor, neighboring regions affecting on the ATP-cleft conformation, and in the distant parts of a protein molecule. By excluding the close homologues of the test protein from the studied set, we predict the less number of significant residues in the distant regions. This procedure allowed the more precise location of amino acid residues associated with ligand

specificity but not with the phylogenic relation.

We demonstrated the efficiency of the suggested approach for detection of the ligand specificity patterns in the protein sequences.

This work was supported by grant RFBR No. 16-04-00491.

1. D.A.Karasev, A.V.Veselovsky, N.Y.Oparina, D.A.Filimonov, B.N. Sobolev (2016) Prediction of amino acid positions specific for functional groups in a protein family based on local sequence similarity, *J. Mol. Recognit.*, **29**: 159-169.