PROTEIN-PROTEIN INTERACTIONS AS NEW TARGETS FOR DRUG DESIGN: INTERACTIVE LINKS BETWEEN VIRTUAL AND EXPERIMENTAL APPROACHES

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SUMMARY

Motivation: Protein-protein and protein-ligands interactions play a central role in biochemical reactions, and understanding these processes is an important step in several fields of biomedical science and drug discovery.

Results: Our research is conducted on a number of protein-protein interactions.

We attempt in this report to show interactive links between virtual and experimental approaches in total pipeline “From gene to drug and using modern SPR technology (optical biosensor) for assessing the strengths of protein-protein and protein-ligand interactions.

Availability: Preprint of this paper is available on request from the authors.

INTRODUCTION

Genome sequencing has provided fast growth of our knowledge about proteins present in different live organisms. However this data tell us rather small information about the function of proteins because they often work in complex assemblies of several macromolecules and small ligands. Such complexes play crucial roles in most cellular processes and widely diverse in their activity and size.

Structural and functional description of protein-protein interactions is an important step toward understanding of biological processes. The applied area of such exploration is searching of new targets and creation new generation of highly effective and safety drugs.

Currently there are about 35 000 known structures in PDB, among them about 12 000 structures involving two or more protein chains. Within protein-protein complexes, two different types can be distinguished, homo- and hetero-complexes. It is known from PDB statistics that homo-complexes often exist as dimers, comparatively uncommon – as trimers or high multimeric complexes.

The contact surfaces of the protein complexes have unique structure and properties, so they represent prospective targets for a new generation of drugs (Veselovsky, 2002). Currently many investigations were undertaken to find or design small molecules that block protein-protein (protein-peptide) interactions (Pagliaro et al., 2004) and in particular protein dimerization (oligomerization). We were intrigue to investigate the mechanism of protein-protein interactions and to apply the gained knowledge towards drug design. Our research is conducted on a number of protein-protein interactions. We attempt in this report to show interactive links between virtual and experimental approaches in total pipeline “From gene to
drug” (Fig. 1) (Veselovsky, 2003; Ivanov, 2005) and using modern SPR technology (optical biosensor) for assessing the strengths of protein-protein and protein-ligand interactions.

**Figure 1.** Pipeline “From gene to drug”: integration of virtual and real experiments.

**METHODOLOGY**

**Bioinformatics (in silico) approaches.** Bioinformatics methods and molecular modeling software provide useful tools to help researchers elucidate protein interaction mechanisms by generating 3D models of intermolecular complexes and using scoring functions to select the most likely molecular complex hypothesis and discovering of lead candidates as inhibitors of protein-protein interaction.

**Experimental (in vitro) approaches.** Several proteomics technologies have been developed and adapted to investigate protein-protein interactions. The yeast two-hybrid method allows the mapping of binary or pair-wise interactions, protein chips are suited to detect protein-protein, protein-lipid and protein-ligand interactions. Affinity capturing method based on the chip of optical biosensor (fishing) was coupled to mass spectrometry (MS) protein identification techniques for identification of partners in bimolecular or multimolecular protein complexes. Here, we will highlight the universal character of optical biosensor based on surface plasmon resonance technology (SPR) for solving different experimental tasks in analysis of protein-protein and protein-ligand interactions (McDonnell, 2001).

**IMPLEMENTATION**

**Analysis of oligomerization of L-asparaginase.** This enzyme is widely used in medical practice as therapeutic agents for treatment acute leukemia. However its application is accompanied by several side effects that caused by insufficient enzyme selectivity. The last one is defined by structure of the active site located between subunits of protein tetramer. Hence, the process of asparaginase oligomerization plays a key role in
formation of the active site and defines substrate specificity. In the present work we modeled spatial structure of L-asparaginase from *Erwinia carotovora* (Fig. 2) based on homology with L-asparaginase from *Erwinia chrysanthemi* and the comparative analysis of the interface between subunits was done.

![Figure 2. Experiments with L-asparaginase. (1) – 3D models of monomer and tetramer; (2) – tetramers immobilization on CM5 chip; (3) – sensogram of tetramers dissociation up to monomer.](image)

We also developed experimental approach to study the process of oligomerization of this enzyme using optical biosensor Biacore 3000. Protein was immobilized on a surface of optical chip CM5 and tetramers dissociation up to monomeric condition has been registered. The subsequent restoration of enzyme tetramers was also carried out.

**HIV-1 protease (HIVp) dimerization.** The main function of HIVp is the slicing viral preprotein on mature proteins. The enzyme also aggravates AIDS by damaging the host cell proteins. Many rather effective competitive inhibitors of HIVp are known and some of them are used now in AIDS therapy. Their systematic application as the drugs, however, inevitably promotes the generation of the viral strains that are resistant both to the inhibitor used and to most of its structural analogs. The drug-resistant protease modification is a result of the point mutation i.e. replacement of one amino acid residue in
both identical enzyme subunits. HIVp operates in homodimeric form, each of identical subunits being consisted of 99 amino acid residues. The main interface region in the homodimer represents the antiparallel four-strand β-sheet, which involves the C- and N-terminal peptides of both subunits (Fig. 3).

It is natural to assume that some ligand binding with any subunit can interfere with subunit dimerization. If the binding site coincides or overlaps with the interface region, all the mutations that diminish subunit affinity to a ligand will be also affect negatively inter-subunit interactions. As a result the mutant protease will be form less stable and, consequently, less active dimers. At least two highly specialized and synchronous mutations are necessary to obtain a drug-resistant strain with high inter-subunit affinity. It is obvious that such mutations are highly improbable.

Some years ago we have begun the project on designing inhibitors of HIVp dimerization that are not capable to stimulate the appearance of drug-resistant viral strains. There are few general strategies for the generation of synthetic molecules that directly modulate protein-protein interactions. We have implemented de-novo design using molecular modeling software Sybyl (Tripos Inc.). Constructed structures of lead compounds (peptidomimetic inhibitors of HIVp dimerization) currently are under synthesis. It was necessary to develop biological assay for direct in vitro analysis of interactions of lead compounds with interface site of HIVp monomer. This assay was created based on optical biosensor Biacore 3000. HIVp was immobilized in dimeric form in two channels on optical chip CM5. Than protein dimers in channel 1 were stabilized by chemical cross-linking, while in channel 2 HIVp dimers were dissociated up to monomers. Assay trial experiments were carried out with known test peptide inhibitor (Fig. 4).

It is visible, that inhibitor interacts only with monomeric form of HIVp, which indicate that molecules of inhibitor bind only to subunits interface.

![Figure 3. Analysis of interfaces between two subunits of HIVp.](image)
**Figure 4. In vitro assay for inhibitors of HIVp dimerization.**

**REFERENCES**


