Review

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Protein-protein interactions as a target for drugs in proteomics

Protein-protein interactions play a central role in numerous processes in the cell and are one of the main fields of functional proteomics. This review highlights the methods of bioinformatics and functional proteomics of protein-protein interaction investigation. The structures and properties of contact surfaces, forces involved in protein-protein interactions, kinetic and thermodynamic parameters of these reactions were considered. The properties of protein contact surfaces depend on their functions. The contact surfaces of permanent complexes resemble domain contacts or the protein core and it is reasonable to consider such complex formation as a continuation of protein folding. Characteristics of contact surfaces of temporary protein complexes share some similarities with active sites of enzymes. The contact surfaces of the temporary protein complexes have unique structure and properties and they are more conservative in comparison with active site of enzymes. So they represent prospective targets for a new generation of drugs. During the last decade, numerous investigations were undertaken to find or design small molecules that block protein dimerization or protein(peptide)-receptor interaction, or, on the contrary, to induce protein dimerization.

Keywords: Bioinformatics / Dimerizers / Inhibitor of protein dimerization / Protein-protein interaction / Review / Target for drugs PRO 0373

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Abbreviations: BIA, biomolecular interaction; **ID**, inhibitor of dimerization

1 Introduction

Protein-protein interaction is a common mechanism responsible for functioning of numerous physiological processes in the cell; it may be also responsible for the development of pathological processes (for example, Alzheimer's and prion diseases) [1–3]. The protein-protein interaction is regulated by different environmental conditions (temperature, pH, ionic strength *etc.*) and cell mechanisms (by enzymes, covalent modifications, noncovalent ligand binding *etc.*) [4–8].

Depending on the stability and mechanism of protein-protein complex formation, all complexes can be subdivided into temporary (nonobligate, short-living) complexes and permanent stable complexes: proteins are native in oligomeric structures only [9]. They are characterized by different structure and properties of protein interfaces involved in the complex formation. The formation of permanent complexes may be considered as a continuation of protein folding, so their surface properties are similar to the protein core or domain interface [10]. The properties of temporary complex interfaces are unique for each interacting pair of proteins and can be considered as analogues of active site of the enzyme [11].

At present protein-protein contact areas are considered to be new prospective drug targets. The numerous physiological and pathological cell processes depend on pro-

 0173-0835/03/0404–380 $17.50+.50/0

tein-protein interactions, which can be influenced by external compounds. The modern way to design new physiological active compounds consists of three main steps: identification of a prospective target, investigation of its properties and design of a corresponding ligand. So in this article we have considered structure and properties of protein-protein contact surfaces, thermodynamic and kinetic parameters of protein-protein interaction reactions, the methods of computer prediction and functional proteomic validation of contacted pairs of protein, and recent progress in design of compounds capable of modifying the protein-protein interaction.

2 Protein-protein contacts: Structure, composition and forces

The contact surface area of protein-protein interfaces consists of complexes of 6–30% of the monomer surface area and may vary from 550–4900 Å^2. The average value of the contact surface of monomers is about 800 Å^2 [12, 13]. Analysis of these surfaces revealed an increase in the number of arginine, histidine, asparagine, tryptophan, tyrosine and serine [13, 14] and hydrophobic amino acid residues [12, 15, 16].

Analysis of distribution of secondary structures in the interface areas gave the following order: random coil (47%) $>$ α-helix (36%) $>$ β-sheet (17%) [12]. The architecture of permanent complex interfaces is similar to the protein core exhibiting limited sets of protein folding patterns. The distribution of secondary structures in the interface of temporary complexes showed larger variability and resembles exterior protein surfaces with the exception of higher quantity of helix [17].

The main factors responsible for the protein-protein interactions are steric, hydrophobic and electrostatic interactions and hydrogen bonds. Analysis of protein contacts revealed that their interface surfaces are quite complementary to each other [12, 13]. The degree of complementarity depends on the type of protein interaction. Permanent complexes exhibit highest complementarity, whereas temporary complexes are characterized by lower complementarity and antigen-antibody complexes have the lowest complementarity [10, 12, 13]. Usually protein interfaces in protein complexes contain some cavities; their surfaces represent about 10% of total interface surfaces.

The major contribution of the hydrophobic forces to the protein-protein interaction has been demonstrated in numerous studies [17–19]. The average values of contact surface hydrophobicity usually represent a mean of the hydrophobicity of the protein core and its surface [12, 17]. The hydrophobic regions in the contact interfaces are organized as patches. The number of such patches may vary from 1–15; their sizes are within 200–400 Å^2 [20]. Contribution of hydrophobic interaction is higher in permanent complexes than in temporary complexes [12]. However, the temporary complexes of membrane proteins, such as cytochrome P450 2B4, cytochrome b_5, NADPH-specific flavoprotein in contrast to water soluble proteins, are formed by hydrophobic interaction of their membrane parts [15, 16]. In the case of enzyme interaction with peptide inhibitors or substrates the contacted interfaces may have hydrophilic surfaces [12, 21].

Electrostatic forces are the other significant forces involved in protein-protein interactions [21–24]. The charge density varies from 0–12 charged groups *per* interface surface [25]. As a rule, the desolvation cost of the charged groups is lower, since they have favorable interactions with other charges and hydrophilic residues surrounding them [24]. The modern viewpoint suggests the electrostatic complementarity of interacting protein surfaces, instead of charge complementarity [26]. It was proposed that the electrostatic force could promote formation of encounter complexes [27, 28] and defines the lifetime of complexes [4].

The average number of hydrogen bonds is proportional to the area of subunit surfaces: one bond *per* each 100–200 Å^2 [12] or about 10 bonds *per* interface [25, 29]. The major proportion of the hydrogen bonds is formed by side chains of amino acids (about 76% of all hydrogen bonds). The hydrogen bonds in protein interfaces are usually not in the optimal position, so they “are normal or weak in term of energetics” [25]. Some hydrogen bonds are formed between protein contact surfaces and water molecules located near them [18, 25]. Contrary to hydrogen bonds formed between protein surfaces, the protein-water hydrogen bonds are “good” ones [25].

Water molecules are frequently present at the complex interfaces [14, 19, 30]. The number of the water molecules usually varies from 1–50 [14]. They surround the contacting interfaces or are buried in them [14, 31]. In the latter case, they are located in the cavities of the protein interfaces [32, 33]. They form hydrogen bonds with protein groups and other water molecules, resulting in aqueous networks along the protein interfaces [25, 30, 32]. Interface water molecules could stabilize the protein complexes by forming additional hydrogen bonds, by interacting with charges, and by increasing shape and charge complementarity [25, 30, 31].

Usually, complex formation is accompanied by various structural changes. These changes were denoted as “induced-fit” effects [34–37]. Protein-protein interaction

can induce changes in the position of side chains of amino acids, motion of the main chain (especially if it is a loop), or domain [12, 14, 34]. It was shown that the rearrangement in the protein backbone appeared due to low-energy conformational changes, which enable H-bond formation and packing the amino acid residues [38]. The temporary complexes are formed by prefolded proteins, so they have limited conformational freedom for optimization of the subunit structures. It results in formation of cavities, the presence of water molecules at the complex interfaces, nonoptimal hydrogen bond geometry [25, 32, 33] *etc*.

The distribution of forces of protein-protein interaction over contact surfaces is not chaotic. The permanent complexes have hydrophobic surfaces at their interfaces, which insignificantly differ from the protein core [10]. Analysis of morphology of protein-protein interfaces of the temporary complexes showed that they can be subdivided into two types. The first type represents the interfaces with a well defined hydrophobic core surrounded by a ring of polar groups [31]. Water molecules are usually located in these rings [14, 31]. The second type of protein interface has the mixed short hydrophobic patches, polar groups and intersubunit hydrogen bonds [31]. Water molecules are located in the cavities of the protein interfaces [25, 31]. Monomers forming the temporary complexes are existing either in polar solution or in the bound state in the complex. In the latter case, they interact with each other, and their contact areas are shielded from the environment. In this case the hydrophobic surfaces are more optimal. In the polar solution they should be hydrophilic enough to avoid nonspecific aggregation, and whenever possible to shield the hydrophobic area of contact surface from the solvent. This is achieved by arrangement of the charged and polar groups around the hydrophobic area or by decreasing large continuous hydrophobic patches by "dissemination" of polar groups.

3 Thermodynamics and kinetics of protein-protein interactions

The formation of the protein-protein complex may be written as:

$$A + B \underset{k_{off}}{\overset{k_{on}}{\longleftrightarrow}} AB \quad (1)$$

where k_{on} is the second-order rate constant for the association reaction and k_{off} is the first-order rate constant for the dissociation reaction. Their ratio is the equilibrium constant for association (K_a) or for dissociation (K_d) according to the law of mass action that is usually written as:

$$\frac{[A][B]}{[AB]} = K_d = \frac{1}{K_a} = \frac{k_{off}}{k_{on}} \quad (2)$$

although the use of the values of activity of reactants instead of their concentrations is more correct.

The interrelationship between the main thermodynamic parameters characterizing complex formation, such as Gibbs free energy (△G), enthalpy change (△H), entropy change (△S), can be described by the following equations:

$$\triangle G = -RT \ln K_d \quad (3)$$

$$\triangle G = \triangle H - T \triangle S \quad (4)$$

where T is absolute temperature, △G is standard free energy change, R is gas constant. The free energy of protein-protein complex formation is linked to the equilibrium constant or affinity by Eq. (3). So it is possible to estimate the △G value by determining the K_d value. The K_d values for protein-protein complexes are within the range $10^{-4}-10^{-14}$ M, which corresponds to △G values of 6–19 kcal/mol [38].

Changes of Gibbs energy are related to changes of enthalpy (△H) and entropy (△S) (Eq. 4). Change of enthalpy depends on hydrogen bond formation, electrostatic and van der Waals interactions, whereas the change of entropy component depends on changes of freedom state of the system, which consists of three interacting parts such as conformation, solvent and association entropy. Conformational entropy is often subdivided into backbone and side chain contributions [39]. The backbone conformation entropy dominates in protein folding, and often it has very modest contribution to protein-protein interactions when backbone changes are minor [13]. The main contribution of the conformation entropy in the protein-protein interaction is usually the side chain component [39]. The other important components of entropy are the solvent and association entropy. Protein-protein complex formation leads to release of water molecules from the surfaces of the protein interface into the solvent; this results in an increase of solvent entropy [39]. Protein complex formation is accompanied by a reduction of the translational and rotational freedom of partners that results in a change of association entropy [39]. When the net entropy change is positive, the protein-protein interaction is entropy-driven reaction; in the opposite case, the enthalpy is the primary driving force of the interaction. The analysis of 69 complexes showed that in 31 cases the enthalpy is favorable but the entropy of association is unfavorable [13]. There are 18 cases where association is driven by entropy and enthapically opposed. In the remaining 20 cases both enthalpy and entropy favor association [13]. At different temperatures, the leading

driving force of protein-protein interaction in one complex can be different [40]. In most cases, the effects of enthalpy and entropy are opposite. This leads to enthalpy/entropy compensation that results in small changes in $\triangle$G values [39].

When proteins form tight complexes, kinetic measurement of K_d is preferable to equilibrium methods. As showed early the dissociation constant (K_d) represents the ratio of dissociation (k_{off}) and association (k_{on}) rate constants. Typical K_d, k_{on} and k_{off} values vary from 10^{-6} to 10^{-14} M, from 10^4 to 10^8 $M^{-1}s^{-1}$ and from 10^3 to 10^{-7} s^{-1}, respectively. Usually point mutations at interface surfaces reduce the affinity of the complex. As a rule a mutation results in increasing k_{off} values and a minor effect on k_{on} values [38, 41].

The relationship between equilibrium and rate constants depends on the mechanism of protein-protein interaction. The simplest mechanism is a one-step reaction, when proteins form a complex without conformational adaptation to each other (equation of reaction is the same as Eq. 1). In the opposite case, two- or multistep mechanism, when complex formation is accompanied by conformational changes of monomers, the reaction may be written as:

$$A + B \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} AB' \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} AB \tag{5}$$

where AB' is an intermediate complex before conformational changes. Then the K_d value is:

$$K_d = \frac{k_{-1}}{k_1}\frac{k_{-2}}{k_2} = K_1K_2 \tag{6}$$

In this case k_2 must be larger than k_{-2} and this shifts the reaction to the right. When conformational changes are faster (in comparison to intermediate complex dissociation, *i.e.* $k_2 \gg k_{-1}$), Eq. (5) reduces to Eq. (1). Sometimes the minimal reaction scheme for bimolecular complex consists of five steps [42]. So the fit between kinetic and equilibrium data depends on a scheme that better represents the mechanism of protein-protein interaction.

It is tempting to subdivide the process of protein-protein interaction into two possible mechanisms responsible for complex formation and stabilization. The first might underline the protein recognition followed by subsequent complex stabilization due to direct docking of protein monomers. In this case long-distance electrostatic forces determine the oriented factor. However, at this stage the thermodynamic barrier exists and the complex formation constant must be below the diffusion-limited constant (k_D). The second mechanism represents only random collisions of the proteins monomers ($k_{on} \rightarrow k_D$) with subsequent fixation of the complexes formed, which allows overcoming a high thermodynamic barrier. Complex formation is especially favorable when $k_{on} \rightarrow k_D$ and $k_{off} \rightarrow \infty$. Formally this situation is realistic in the case of permanent complexes. In the reality it is much more complex because their formation appears to be a continuation of the folding of three-dimensional structures and cannot be evaluated by such simple thermodynamic considerations.

Numerous data suggest that the recognition and specificity require the directed forces of interaction such as hydrogen bonds and electrostatic forces, whereas the binding energy is probably also determined by hydrophobic forces [2, 19, 33, 43–53]. It is also suggested that the most binding energy is related only to several amino acids from the interface, so called “hot spots” [54–57]. Thus, the same forces are involved in protein-protein interaction, protein folding and ligand-receptor interaction. The dominant factor for permanent complex formation is the hydrophobic force as for protein core folding. Thus such complex formation has some similarities with folding process. On the other hand, the various forces participate in formation of the temporary complexes and those properties of the contact surfaces are more complex.

4 Methods of bioinformatics and functional proteomic investigation of protein-protein interactions

The methods for investigation of protein-protein interaction based on physicochemical approaches, including site directed mutagenesis or chemical modification of amino acid groups participating in such interaction, were discussed in many early reviews [58–61]. Here bioinformatic and functional proteomic methods allowing us to predict and validate protein complexes formation are discussed. There are predictions of interacting proteins based on bioinformatic genome analysis and validation of predicted complexes with the help of the two-hybrid system, combination of optical biosensors and mass spectrometry (“fishing”) *etc*.

4.1 *In silico* prediction of protein-protein interactions

These methods revealing interacting proteins use cross-genome comparisons applied for gene annotation. These methods include well known and new approaches: annotation by sequence similarity, phylogenetic profiling, metabolic pathway mapping, gene neighbor and domain fusion analyses.

Annotation by similarity is based on finding of homologues of the query protein in the annotated protein databases using pair-wise local sequence alignment. Several proteins from the organism under study may share significant similarities with proteins involved in complex formation in other organisms. Most subunits of protein complexes were annotated in such a way. A typical example is prokaryotic transcriptase. The genes encoding chains of transcriptase are conserved in all sequenced bacterial genomes that allow annotation of them as components of transcriptase functional complex in *Mycobacterium tuberculosis* as well [62].

Use of phylogenetic profiling for searching of proteins involved in common metabolic pathway or complex formation (so called "functionally linked proteins") is based on the suggestion that such proteins must be jointly present or jointly absent in different organisms. A phylogenetic profile describes an occurrence of a certain protein in a set of organisms. If two (or more) proteins have identical (or similar) phylogenetic profiles, it may be suggested that these proteins are functionally linked [63].

Another method for detection of functional linkages between proteins is the gene neighbor method. If in several genomes the genes encoding certain proteins neighbor on the chromosome DNA sequence, these proteins tend to be functionally linked [63, 64]. Eisenberg and coauthors [63] reported that this method correctly identifies functional links among eight enzymes involved in the pathway of arginine biosynthesis in *M. tuberculosis*. This method is mainly suitable for prokaryotic genome analysis.

Fused domain analysis suggests that two (or more) separate proteins in one organism are functionally linked (and, most likely, forming the complex) if they correspond to different domains of one protein in an other species [63]. An example of fused domain protein is cytochrome P450BM-3. It includes cytochrome P450 102 and NADPH-cytochrome P450 reductase domains, whereas usually cytochromes P450 and NADPH-cytochrome P450 reductase are separate proteins that interact with each other [65]. Also a group of enzymes involved in peptidoglycan biosynthesis in bacteria is a good example. These genes are often found as clusters in certain bacteria, and two of them are actually fused in *Chlamydia pneumoniae* [64]. Domain fusion phenomenon is quite widespread: more than 6800 probable protein-protein interactions were found in *Escherichia coli* by domain fusion analysis [66]. For this reason domain fusion is frequently mentioned in various investigations for genome annotation [67]. Recently, a similar approach was proposed. It is based on the treatment of protein as a set of conserved domains, where each domain is responsible for a specific interaction with another one [68].

Metabolic pathway mapping can also help in searching for interacting proteins. In many cases it may be suggested that enzymes form temporary complex if they catalyze coupled reactions [69].

The methods mentioned above usually produce some false-positive and false-negative results. Therefore, the joint employment of these methods is recommended to improve the prediction reliability [63, 70]. The main reasons of method-independent inaccuracies of predictions are insufficient experimental data on protein-protein interactions. The incompleteness of genome annotation and errors of gene recognition also may result in wrong predictions of protein-protein interactions. Recently two methods for assessment of accuracy of the protein interaction detection were also proposed [71]. The methods discussed above can aid in the drug discovery. Currently they are mostly used in computer-aided target selection for antimicrobial drug design [64, 72].

Protein binding sites can be mapped onto target protein sequence by similarity with its closest homologues, in which location of such sites is known. Multiple sequence alignment is commonly used for this purpose [73]. The most favorable case is the availability of a 3-D structure of the complex of close homologue with its partner. Then it is possible to carry out molecular graphic analysis of known complex and to design the investigated complex. In this case residues involved into interaction can be revealed more correctly than by sequence analysis only. Finally, it should be noted that the prediction produced by methods of sequence analysis of protein-protein interactions is usually hypothetical and requires experimental validation.

4.2 Yeast two-hybrid system

One of the most commonly used approaches allowing determination of pairs of interacting proteins *in vivo* is the yeast two-hybrid system [74, 75]. For more than a decade this method with numerous variations has been successfully employed for investigating protein-protein, protein-RNA and protein-DNA interactions. It can be used for the analysis of particular complexes and construction of a network of protein interactions. This method is logic continuations studying transcribe activators. Site-specific transcribe activators frequently consist of two separate domains: DNA-binding (BD) and transcribe-activator (AD). In the original scheme of the two-hybrid system, two hypothetically interacting proteins are designed in hybrid proteins bound with BD and AD, respectively. Two hybrid proteins BD-X and AD-Y are jointly expressed in the yeast line carrying a specific DNA site for the transcribe activator. The site is located in the area limiting

5'-end of a gene, whose expression can easily be detected in growing yeast (reporter gene). Interaction of proteins X and Y restored the structure of the transcribe activator, this results in reporter gene transcription and specific phenotype of cells, respectively.

The yeast two-hybrid system is frequently used both for determination of interaction between already known proteins and for screening of genome libraries *i.e.* for revealing partners of interaction of certain proteins. This system can be used for characterizing protein-protein interaction network by extensive screening of each protein expressed in eukaryotic cell [76]. Recently, numerous modifications of this method were proposed. One of them involves the use of gene of the third protein (Z), which is separately expressed and is intended for selection of yeast clones, in which the interaction of this protein with proteins X and Y occurs. The alternative scheme means a participation of protein Z in the prevention of the X-Y complex formation [77]. The next modification is a three-hybrid system. SenGupta and coauthors [78] proposed to use RNA for investigation of RNA-protein interactions. In this method two RNA domains interact with BD and AD hybrid proteins.

A reversible two-hybrid scheme was offered for selection of mutations of medicinal compounds competing for binding sites of the other proteins in the cell. Vidal and coauthors [79] used URA3 gene as the reporter gene. This gene activation is lethal when yeast growth is in a medium containing 5-fluoro-orotic acid. Protein-protein interaction and reporter gene activation leads to cell death. Dissociation or inhibition of complex results in colony formation. This two-hybrid system can be used for high-throughput screening of ligand inhibition or also for dissociating of such complexes.

The classic yeast two-hybrid system has several limitations, since some proteins (for example, membrane proteins) cannot be reconstructed in the nucleus, or additional factors are required to modify expressed proteins to facilitate complex formation. For investigation of membrane protein-protein interaction, alternative nontranscribe two-hybrid systems (cytoplasmic location) are applied. Johnson and Varshavsky [80] have proposed a cytoplasmic two-hybrid system, which can be used for screening of membrane protein interactions. The system consists of a small protein (76 amino acid residues), ubicvintin, whose binding to other proteins in the cell is a signal for proteolysis. Chimeric proteins fused with ubicvintin are quickly destroyed *in vivo* by ubicvintin-specific proteases. The *C*-end of ubicvintin (Cub) is linked with reporter protein and hypothetical proteins participating in the interaction. The *N*-end of ubicvintin (Nub) is linked with another protein. The interaction of two proteins results in ubicvintin complementarity and its proteolysis with release of reporter protein.

Many eukaryotic proteins are exposed by significant post-translated modifications that are important for their main function. In this case the employment of a two-hybrid system is limited by the absence of modifying enzymes in *Saccharomyces cerevisiae*. For example, if protein-"bait" is a component of signalling pathway, the post-translated modifications often form a site of recognition that is necessary for protein interactions in cascade. For proteins of the tyrosine phosphorylation cascade this problem is solved by coexpression of tyrosine kinase in *S. cerevisiae* (variant of three-hybrid system) [81, 82]. The two-hybrid system, associated with membrane, employs proteins from the Ras signalling pathway [83]. The original two-hybrid system is based on activation of transcription by RNA polymerase II (PolII) [84].

Protein-bait and small peptide ($<$ 16 amino acid residues) are also used in the two-hybrid system for revelation of minimal conservative sequence necessary for interaction [85]. Using peptides is ideal for research of genetic network complexes and is already applied for studying pheromone action [86, 87]. At present alternative methods for estimation of interaction of macromolecules *in vitro* are used. The methods allow detection of protein-protein interactions by employment of translation *in vitro* and plotting of products on solid-phase are described. For example, PISA technology (protein *in situ* array), which allows expression of proteins by PCR products and a acellular system of translation. This method is successfully used for studying protein-protein interaction [88]. A method of creation of high-density protein chips with c-DNA expressing libraries in *E. coli* has been developed. These biochips are used for monoclonal antibody selection, their synthetic analogues (aptomers, affibody *etc*.) and also for revealing new partners of protein-protein interactions and in complex experiments for transcriptional and proteomic mapping [89].

An approach for identification of protein-protein interactions in yeast is described by Shevchenko *et al.* [90]; the initial step of this approach is in catching partners on solid-phase with immobilized proteins; protein identification (participating in binding) is carried out by MS/MS. The authors note, that agreement between this method and those of the two-hybrid system does not exceed 14% and this approach is considered as complementary to the existing ones. But the main technology, which will compete with the two-hybrid system in future, is the combination of biomolecular interaction analysis (BIA) with mass spectrometry.

4.3 Combination of BIA and MS analysis for functional proteomics

Combining these two analysis systems reveals the identify of multiprotein complexes, sites of protein interaction and determine rate constants of formation and dissociation of these complexes. These combined methods are based on surface plasmon resonance (SPR-BIA) or resonant mirror (RM-BIA) optical biosensor with different types of MS [91–94]. The more popular optical biosensors (OB) are SPR-biosensor BIAcore (Biosensor, Uppsala, Sweden) and RM-biosensor IAsys+ (Affinity Sensors, Cambridge, UK). Sensor chips of these devices are represented by a glass prism with a gold layer in the case of SPR or with a waveguide in the case of RM. The upper surface of the chip is the bottom of the reaction cell. Immobilized on this surface ligate (low or high molecular weight compound) interacts with its partner ligands added in solution, which induce ligand/ligate complex formation. This complex formation changes the refractive indices, which are registered by resonant laser angle position. Such biosensors allow to definition of ligand concentration in solution at the range up to 10^{-10}–10^{-12} M in real-time regime and to measure kinetic constants of formation and dissociation and to calculate such thermodynamic parameters as K_d and $\triangle$G. By measuring temperature of these parameters, it is possible to calculate other thermodynamic values such as $\triangle$H, $\triangle$S and estimate the contribution of energetic and entropic factors in complex formation and decay. BIA enables registration processes of formation and dissociation of binary and multicomponent complexes in real-time without labels [95]. For example, it was shown that proteins of cytochrome P450-containing mono-oxygenase system from various sources (microsomal fraction, mitochondria or bacteria) are able to form both binary and ternary complexes. The driving forces for complex formation of biomembrane proteins were hydrophobic ones. In the case of water soluble proteins, both types of interaction, hydrophobic and electrostatic interaction, play crucial roles as well as at the decay of formed complexes [16, 22, 96, 97].

Combination of BIA methods with the most efficient MS technique for protein identification has allowed the creation of the "fishing" approach, which is very promising. By using different types of MS the bound proteins and their complexes could be identified very easily in the same manner as proteins after chromatography and electrophoresis [98, 99]. Sonksen and coauthors [100] reported that myoglobin from the mixture of proteins was isolated by immobilizing antimyoglobin antibodies. In our laboratory immobilizing isatin binding proteins were isolated from mitochondrial detergent solubilisate [101]. By immobilizing monoclonal antibodies, HBsAg was discovered in serum of patients with hepatitis B [102, 103]. MS is the most effective method for identification of proteins and these complexes [104]. This method is characterized by very high resolution (10^{-15}–10^{-18} M) and rate. The combination of optical biosensor with MS allows identification of proteins or their complexes formed on the biosensor surface.

There are two ways to transfer the probe from optical biosensor to mass spectrometer. The sensor-chip surface with immobilized protein or a protein complex is analyzed directly in the mass spectrometer. MALDI is usually used for this purpose [91, 94] or the proteins or their complexes removed from the biosensor surface and moved into the mass spectrometer. This can be done on-line by using an intermediate chromatographic column and electrospray ionization (ESI) of the probe [93]. The use of biochips adsorbing spectra of proteins from multicomponent serum of the patients in norm and pathology with subsequent comparative analysis of protein profiles on MS can be helpful for early diagnosis of cancer [105]. Therefore tandem BIA-MS may be considered useful methods for diagnosis of diseases.

5 Protein-protein interaction as drug target: myth or reality?

The formation of permanent protein complexes can be considered as the prolongation of folding of these proteins. The ultimate folding of subunits occur as the part of such complex formation [10, 106, 107]. So this type of complex is less interesting for drug design. The folding procedure itself may be interesting as the drug target [59]. On the other hand, the structure and properties of protein interfaces of the temporary complexes have a dual nature. They share similarity with the protein core and a resemblance to the enzyme active site surfaces. High specificity of the interaction of proteins suggests complementarity of dimer subunits and therefore distribution of their unique properties over protein interfaces. These protein regions can be used as targets for new drug design.

During recent years several results support the validity of such an approach. The cell reaction can be modified by induction or prevention of protein-protein interaction. So investigations for designed compounds were directed in both directions. The compounds that induce protein interaction were called dimerizers [108–110]. Agents preventing this process are inhibitors of dimerization and antagonists of peptide/protein receptors [111–117].

5.1 Dimerizers

Many cell signalling pathways are initiated by protein-protein dimerization. The main idea of dimerizer is induction of interaction between two proteins by small molecules that lead to activation of the cell signalling pathway. Since dimerizers must interact with two separate proteins, they consist of three parts: two anchor groups interacting with the proteins and a long linker between them. Now the most prevalent dimerizer is FK1012 (nontoxic lipid-soluble dimeric form of the drug FK506), but other anchor groups are also used [108, 109, 118–120]. Linkers consist of 5–16 atoms [121, 122]. The concentrations required for induction of dimerization by such compounds vary in a relatively narrow range of 1–10 nM [121, 123]. The effectiveness of dimerizers depends on the anchors affinity [122] and the length of the linkers [121, 122]. At present these systems are used only in the laboratory. Dimerizers can induce cell proliferation [124], transcription [118, 121] and apoptosis [121, 123]. It is proposed to use these systems in gene therapy. Potentially, in clinical practice dimerizers can be used for induction of cell proliferation [124], or for elimination of the transferred gene product and genetically modified cells by dimerizer-inducing apoptosis [121, 123].

The universality of such systems may have important advantages. The same dimerizer can be used for many purposes since its action depends on the constructed target (wild protein with binding domain of dimerizer). For increasing selectivity it is proposed to design the anchor part for the mutant binding domain to exclude the capability of dimerizer binding to normal cell proteins containing wild-type domain [125, 126].

5.2 Inhibitors of dimerization

The most famous example of inhibitors of dimerization (ID) is low weight inhibitors of receptors that have peptides as ligands [2]. Interaction of the protein(peptide) hormones with their receptors is followed by interaction of hormone-receptor complex with other proteins of the signal transduction cascades. Modification of all these interactions can be employed to change cell metabolism. For example, different peptides that terminate all stages of the cascade induced by light adsorption by rhodopsin were identified [117]. There are many examples of successful design of effective nonpeptide ligands for different types of peptide(protein) receptors: vascular endothelial growth factor receptor [127], somatostatin receptor [128], neuropeptide Y receptors [129], thromboxane A2 receptor [130], protease-activated receptors [115, 131, 132].

Numerous investigations were done in attempt to design the IDs for proteins possessing stable domains, which interact with receptors [114, 133–138]. Some proteins act as oligomer complexes, so IDs may prevent formation of the active dimer. Such inhibitors have been discovered for three HIV enzymes (protease, reverse transcriptase, invertase) [117, 139–141], ribonucleotide reductase [142], DNA polymerase of herpes simplex virus [143], human gluthatione reductase [144], phosphatidylinisitol 3-kinase [145] *etc*.

Most discovered IDs are peptides resembling dimer interfaces. The activity of such inhibitors (expressed as IC_{50} or K_i values) vary from low nanomolar [146, 147] to micromolar concentration range [117, 148, 149]. Using site-directed mutagenesis or methods of combinatorial chemistry it is possible to improve inhibitory activity of peptide IDs by optimizing their amino acid composition and sequence [117, 139, 147, 150]. The peptidomimetic molecules or small organic molecules were also recognized as IDs [117, 148, 151, 152].

The most studied protein for ID design is the HIV protease [117, 139, 146, 147, 153, 154]. It was initially found that peptides corresponding to the *N*- and *C*-termini of HIV protease inhibit its activity. The second step consisted in discovery of synthetic peptides with more potent inhibitory activity [117, 154]. These peptides combined by flexible linkers have inhibitory activity up to IC_{50} = 25 nM [117, 153, 154]. Inhibitors with rigid linker ("molecular tongs") were less active (K_i about 0.56–4.5 μM) [139]. The high inhibitor potency was shown for lipopeptides containing peptide, linker and lipid (K_i in the low nanomoles range) [146]. Recently the nonpeptide inhibitor of HIV-1 protease dimerization was designed [155].

In addition to their ability to prevent protein dimerization, some IDs can also cause dimer dissociation. Thus it was found that the imidazole derivative, clotrimazole, induced dissociation of inducible nitric oxide synthase into subunits in the absence of *L*-arginine and tertahydrobiopterin, whereas other derivatives prevented dimerization only [148]. The fungal metabolite, tryprostatin A, induced reversible disruption of the cytoplasmic microtubule assembly of 3Y1 cells [149]. Peptides, inducing dissociation into monomers of HIV-1 protease and integrase were also found [156, 157].

Protein-protein interactions have a great potential as a new class of targets for novel drugs. The inhibitors of dimerization can be applied both for modification of regulator processes and for prevention of formation of the enzyme active form. It is possible to design IDs for numerous cell systems, but, from our point of view, they can be very useful for design of antibiotic, antiviral and

antiparasitic drugs. There are two favorable features of IDs for these classes of drug. Design of antibiotic binding to active site of enzyme of the pathogen organism can be limited by high structural similarity between the human and the pathogen enzymes, whereas the greater structural variability of protein-protein interfaces may supply a target for the effective differentiation between the host and pathogen enzymes [158]. The second favorable feature of IDs concerns the problem of antibiotic resistance of pathogens. One of the preferential mechanisms of resistance is the mutation at the active site of the antibiotic-target enzyme. The mutation of amino acid residue at the active site (but not catalytic residues) can lead to decreasing affinity of the drug with little effect on enzyme activity. Whereas a single mutation in one subunit of protein-protein interfaces often destroy the protein-protein interaction. The conservation of protein complex in this case requires the complementary mutation in both the subunits. The simultaneous coupled double mutation in different subunits is much more unlikely, so it seems that the essential amino acids of protein-protein interface are quite conservative. Correspondingly, it is improbable that pathogens acquire resistance for ID binding to such amino acid residues.

From our viewpoint there are several features of such targets for effective ID design. Low weight compounds will be more effective when the sizes of contacted interfaces are relative small. In this case the binding energy of proteins will be not too high, and the small molecule can effectively compete with subunits. IDs would effectively prevent protein-protein complex formations when they interact with “hot spots” of amino acids of the interface. Recently, a new strategy for increasing the effectiveness of IDs was proposed. At the first step, the ID interacts with its target noncovalently; this brings together the weakly reactive group of the drug and the amino acid side chain of the protein. At the second step, such contacted groups covalently interact with each other [116]. The same strategy was used to design inhibitors of dimerization to HIV protease and Src-homology 2 domain [159, 160].

A new approach for the first step of ID design has recently been proposed. It requires the development of a single chain antibody against one interaction surface of one protein and use of this antibody as a template for design of inhibitors [161]. Recently, several compounds that may act as inhibitors of dimerization and as dimerizers were found. It was shown that some pyrrolidine derivatives are competitive inhibitors of serum amyloid P component (SAP) glycoprotein binding to amyloid fibrils. These low weight compounds are also able to dimerize SAP molecules leading to their rapid elimination in the liver [162].

Thus, compounds directed to the change of protein-protein interactions are the reality. Functional proteomics whose main aim is discovering such interactions may play a crucial role in finding new drug targets in future.

This work was partially supported by Russian Foundation for Basic Research (grants 01-04-48128, 00-15-97926, 01-04-48245), INTAS 01-470, Social Fatherland Medicine Support Foundation (Russia), Janssen Research Foundation. The authors thank Dr. A. E. Medvedev for valuable and helpful discussions.

Received September 16, 2002

6 References

[1] Cohen, F. E., Prusiner, S. B., *Annu. Rev. Biochem.* 1998, *67,* 793–819.

[2] Loregian, A., Mardsen, H. S., Palu, G., *Rev. Med. Virol.* 2002, *12*, 239–262.

[3] Selkoe, D. J., *Trends Cell Biol.* 1998, *8,* 443–457.

[4] Archakov, A. I., Ivanov, Y. D., in: *Biophysics of Electron Transfer and Molecular Bioelectronics*, Pleum Publication, USA 1999, pp. 173–194.

[5] Furukawa, Y., Ishimory, K., Morishima, I., *Biochemistry* 2002, *41*, 9824–9832.

[6] Eyster, K. M., *Biochem. Pharmacol.* 1998, *55*, 1927–1938.

[7] Klemm, J. D., Schreiber, S. L., Crabtree, G. R., *Annu. Rev. Immunol.* 1998, *16,* 569–592.

[8] Markus, M., Benezra, R., *J. Biol. Chem.* 1999, *274*, 1040–1049.

[9] Jones, S., Thornton, J. M., in: Kleanthous, C. (Ed.), *Protein-protein Recognition*, Oxford University Press, New York 2000, pp. 33–59.

[10] Tsai, C.-J., Xu, D., Nissinov, R., *Protein Sci.* 1997, *6*, 1797–1809.

[11] Dmitriev, D. A., Massino, Y. S., Segal, O. L., Smirnova, M. B. *et al*., *J. Immunol. Methods* 2002, *261*, 103–118.

[12] Jones, S., Thornton, J. M., *Proc. Natl. Acad. Sci. USA* 1996, *93,* 13–20.

[13] Stites, W. E., *Chem. Rev.* 1997, *97*, 1233–1250.

[14] Davies, D. R., Cohen, G. H., *Proc. Natl. Acad. Sci. USA* 1996, *93,* 7–12.

[15] Ivanov, Y. D., Kanaeva, I. P., Kuznetsov, V. Y., Lehnerer, M. *et al*., *Arch. Biochem. Biophys.* 1999, *362,* 87–93.

[16] Ivanov, Y., Kanaeva, I., Archakov, A., *Biochem. Biophys. Res. Commun.* 2000, *273*, 750–752.

[17] Tsai, C.-J., Lin, S. L., Wolfson, H. J., Nissinov, R., *Protein Sci.* 1997, *6*, 53–64.

[18] Tsai, C.-J., Lin, S. L., Wolfson, H. J., Nissinov, R., *Crit. Rev. Biochem. Mol. Biol.* 1996, *31,* 127–152.

[19] Wells, J. A., *Proc. Natl. Acad. Sci. USA* 1996, *93*, 1–6.

[20] Lijnzaad, P., Argos, P., *Proteins* 1997, *28*, 333–343.

[21] Stevens, J. M., Armstrong, R. N., Dirr, H. W., *Biochem. J.* 2000, *347*, 193–197.

[22] Ivanov, Y. D., Kanaeva, I. P., Karuzina, I. P., Archakov, A. I. *et al*., *Arch. Biochem. Biophys.* 2001, *391*, 255–264.

[23] Sheinerman, F. B., Norel, R., Honig, B., *Curr. Opin. Struct. Biol.* 2000, *10*, 153–159.

[24] Xu, D., Lin, S. L., Nissinov, R., *J. Mol. Biol.* 1997, *265*, 68–84.

[25] Xu, D., Tsai, C.-J., Nissinov, R., *Protein Eng.* 1997, *10*, 999–1012.

[26] McCoy, A. J., Chandana, E. V., Colman, P. M., *J. Mol. Biol.* 1997, *268*, 570–584.

[27] Camacho, C. J., Weng, Z. P., Vajda, S., Delisi, C., *Biophys. J.* 1999, *76*, 1166–1178.

[28] Vijayakumar, M., Wong, K. Y., Schreiber, G., Fersht, A. R. *et al*., *J. Mol. Biol.* 1998, *278*, 1015–1024.

[29] Lo Conte, L., Chothia, C., Janin, J., *J. Mol. Biol.* 1999, *285,* 2177–2198.

[30] Janin, J., *Structure Fold. Des.* 1999, *7,* 277–279.

[31] Larsen, T. A., Olson, A. J., Goodsell, D. S., *Structure* 1998, *6*, 421–427.

[32] Dall'Acqua, W., Goldman, E. R., Lin, W., Teng, C. *et al*., *Biochemistry* 1998, *37*, 7981–7991.

[33] Vaughan, C. K., Buckle, A. M., Fersht, A. R., *J. Mol. Biol.* 1999, *286*, 1487–1506.

[34] Betts, M. J., Sternberg, M. J. E., *Protein Eng.* 1999, *12*, 271–283.

[35] Kimura, S. R., Brower, R. C., Vajda, S., Camacho, C. J., *Biophys. J.* 2001, *80,* 635–642.

[36] McCammon, J. A., *Curr. Opin. Struct. Biol.* 1998, *8*, 245–249.

[37] Sundberg, E. J., Mariuzza, R. A., *Structure Fold. Des.* 2000, *8*, R137-R142.

[38] Janin, J., in: Kleanthous, C. (Ed.), *Protein-protein Recognition*, Oxford University Press, New York 2000, pp. 1–32.

[39] Brady, G. P., Sharp, K. A., *Curr. Opin. Struct. Biol.* 1997, *7*, 215–221.

[40] Zeden-Lutz, G., Zuber, E., Witz, J., Van Regenmortel, M. H., *Anal. Biochem.* 1997, *246*, 123–132.

[41] Schreiber, G., Frisch, C., Fersht, A. R., *J. Mol. Biol.* 1997, *270*, 111–122.

[42] Mathews, F. S., Mauk, A. G., Moore, G. R., in: Kleanthous, C. (Ed.), *Protein-protein Recognition*, Oxford University Press, New York 2000, pp. 60–101.

[43] Jiang, L., Lai, L., *J. Biol. Chem.* 2002, *15,* 1–48.

[44] Clackson, T., Wells, J. A., *Science* 1995, *267*, 383–386.

[45] Davis, S. J., Davies, E. A., Tucknott, M. G., Jones, E. Y., van der Merwe, P. A., *Proc. Natl. Acad. Sci. USA* 1998, *95,* 5490–5494.

[46] Eubanks, S., Nguyen, T. L., Peyton, D., Breslow, E., *Biochemistry* 2000, *39*, 8085–8094.

[47] Koltzscher, M., Gerke, V., *Biochemistry* 2000, *39*, 9533–9539.

[48] Lin, C. T., Kuo, T. J., Shaw, J. F., Kao, M. C., *J. Agr. Food Chem.* 1999, *47*, 2944–2949.

[49] Martin, C., Hartley, R., Mauguen, Y., *FEBS Lett.* 1999, *452*, 128–132.

[50] Mateu, M. G., Fersht, A. R., *EMBO J.* 1998, *17*, 2748–2758.

[51] Scott, S., Dong, F. M., Kisterswoike, B., Mullerhill, B., *J. Mol. Biol.* 2000, *296*, 673–684.

[52] Thomas, M. C., Ballantine, S. P., Bethell, S. S., Bains, S. *et al*., *Biochemistry* 1998, *37*, 11629–11636.

[53] Zeng, J., Fridman, M., Maruta, H., Treutlein, H. R., Simonson, T., *Protein Sci.* 1999, *8*, 50–64.

[54] Bogan, A. A., Thorn, K. S., *J. Mol. Biol.* 1998, *280*, 1–9.

[55] Hu, Z. J., Ma, B. Y., Wolfson, H., Nussinov, R., *Proteins* 2000, *39*, 331–342.

[56] McInnes, C., Grothe, S., O'Connor-McCourt, M., Sykes, B. D., *Protein Eng.* 2000, *13*, 143–147.

[57] von Kries, J. P., Winbeck, G., Asbrand, C., Schwarz-Romond, T. *et al*., *Nat. Struct. Biol.* 2000, *7*, 800–807.

[58] Lebowitz, J., Lewis, M. S., Schuck, P., *Protein Sci.* 2002, *11*, 2067–2079.

[59] Veselovsky, A. V., Ivanov, Y. D., Ivanov, A. S., Archakov, A. I., *J. Mol. Recognit*. 2002, *15*, 405–422.

[60] Otzen, D. E., Fersht, A. R., *Protein Eng.* 1999, *12,* 41–45.

[61] Spinozzi, F., Gazzillo, D., Giacometti, A., Mariani, P., Carsughi, F., *J. Biophys.* 2002, *82,* 2165–2175.

[62] Cole, S. T., Brosch, R., Parkhill, J., Garnier, T. *et al*., *Nature* 1998, *393*, 537–544.

[63] Eisenberg, D., Marcotte, E. M., Xenarios, I., Yeates, T. O., *Nature* 2000, *405*, 823–826.

[64] Freiberg, C., *Drug Discov. Today* 2001, *6*, S72–S80.

[65] Ruettinger, R. T., Wen, L.-P., Fulco, A. J., *J. Biol. Chem.* 1989, *264,* 10987–10995.

[66] Marcotte, E. M., Pellegrini, M., Ng, H.-L., Rice, D. W. *et al*., *Science* 1999, *285*, 751–753.

[67] Bansal, K., *Bioinformatics* 1999, *15*, 900–908.

[68] Karp, P. D., Krummenacker, M., Paley, S., Wagg, J., *Trends Biotech*. 1999, *17*, 275–281.

[69] Gomez, S. M., Rzetsky, A. *Pacific Symp. Biocomputing* 2002, 413–424.

[70] Butte, A. J., Kohane, I. S., *Pacific Symp. Biocomputing* 2000, *5*, 415–426.

[71] Deane, C. M., Salvinsky, L., Xenarios, I., Eisenberg, D. *Mol. Cell Proteomics* 2002, *1*, 349–356.

[72] Galperin, M. Y., Coonin, E. G., *Curr. Opin. Biotech.* 1999, *10*, 571–578.

[73] Gotoh, O., *J. Biol. Chem.* 1992, *267*, 83–90.

[74] Ito, T., Chiba, T., Ozawa, R., Yoshida, M. *et al*., *Proc. Natl. Acad. Sci. USA* 2001, *98*, 4569–4574.

[75] Auerbach, D., Thaminy, S., Hottiger, M. O., Stagljar, I., *Proteomics* 2002, *2,* 611–623.

[76] Fransen, M., Brees, C., Ghys, K., Amery, L. *et al*., *Mol. Cell Proteomics* 2002, *1,* 243–252.

[77] Drees, B. L., *Curr. Opin. Chem. Biol.* 1999, *3,* 64–70.

[78] Zhang, B., Kraemer, B., SenGupta, S., Fields, S., Wickens, M., *Methods Enzymol.* 1999, *306,* 93–113.

[79] Vidal, M., in: Bartel, P., Fields, S., (Eds.), *The Two-Hybrid System*, Oxford University Press, New York 1997, p. 109.

[80] Johnsson, N., Varshavsky, A., *Proc. Natl. Acad. Sci. USA* 1994, *91,* 10340–10344.

[81] Osborne, M. A., Dalton, S., Kochan, J. P., *Biotechnology (NY)* 1995, *13,* 1474–1478.

[82] Kochan, J. P., Volpers, C., Osborne, M. A., *Methods Enzymol.* 2000, *328,* 111–127.

[83] Aronheim, A., Engelberg, D., Li, N., al-Alawi, N. *et al*., *Cell* 1994, *78,* 949–961.

[84] Marsolier, M. C., Tanaka, S., Livingstone-Zatchej, M., Grunstein, M. *et al*., *Genes Dev.* 1995, *9*, 410–422.

[85] Yang, M., Wu, Z., Fields, S., *Nucleic Acids Res.* 1995, *23*, 1152–1156.

[86] Geyer, C. R., Colman-Lerner, A., Brent, R., *Proc. Natl. Acad. Sci. USA* 1999, *96,* 8567–8572.

[87] Norman, T. C., Smith, D. L., Sorger, P. K., Drees, B. L. *et al*., *Science* 1999, *285,* 591–595.

[88] He, M., Taussig, M. J., *Nucleic Acids Res.* 2001, *29,* e73–3.

[89] Walter G., Bussow, K., Cahill, D., Lueking, A., Lehrach, H., *Curr. Opin. Microbiol*. 2000, *3,* 298–302.

[90] Shevchenko, A., Schaft, D., Roguev, A., Pijnappel, W. W. *et al*., *Mol. Cell Proteomics* 2000, *1,* 204–212.

[91] Ivanov, Y. D., Gara, O. G., Konstantinova, N. I., Moshkovsky, S. A. *et al*., *Vopr. Med. Chem.* 2003, in press.

[92] Catimel, B., Weinstock, N. M., Domagala, T., Nice, E. C., *J. Chromatogr. A* 2000, *869*, 261–273.

[93] Natsume, T., Nakayama, H., Isobe, T., *Trends Biotech.* 2001, *19*, S28-S33.

[94] Krone, J. R., Nelson, R. W., Dogruel, D., Williams, P., Granzow, R., *Anal. Biochem.* 1997, *244*, 124–132.

[95] Berkowitz, O., Wirtz, V., Wolf, A., Kuhlmann, J., Hell, R., *J. Biol. Chem.* 2002, *277*, 30629–30634.

[96] Ivanov, Y. D., Usanov, S. A., Archakov, A. I., *Biochem. Mol. Biol. Int.* 1999, *47*, 327–336.

[97] Ivanov, Y. D., Kanaeva, I. P., Karuzina, I. I., Usanov, S. A. *et al*., *J. Inorg. Biochem*. 2001, *87*, 175–184.

[98] Davis, S., Aldrich, T. H., Jones, P. F., Acheson, A. *et al*., *Cell* 1996, *87*, 1161–1169.

[99] Sakano, S., Serizawa, R., Inada, T., Iwama, A. *et al*., *Oncogene* 1996, *13*, 813–822.

[100] Sonksen, C. P., Nordhoff, E., Jansson, O., Malmqvist, M., Roepstorff, P., *Anal. Chem*. 1998, *70*, 2731–2736.

[101] Ivanov, Y. D., Panova, N. G., Buneeva, O. A., Medvedev, A. E., Archakov, A. E., *Vopr. Med. Chem.* 2002, *48,* 73–83.

[102] Ivanov, Y. D., Gnedenko, O. V., Nikolaeva, L. I., Konev, V. A. *et al*., *Vopr. Med. Chem*. 2001, *47*, 419–425.

[103] Ivanov, Y. D., Gnedenko, O. V., Nikolaeva, L. I., Konev, V. A. *et al*., *J. Microbiol., Immunobiol. Epidemiol.* 2003, in press.

[104] Hochstrasser, D. F., Sanchez, J. C., Appel, R. D., *Proteomics* 2002, *2*, 807–812.

[105] Kathryn, Y., *The Biochemist* 2001, *February*, 19–20.

[106] Srivastava, A. K., Sauer, R. T., *Biochemistry* 2000, *39*, 8308–8314.

[107] Wallace, L. A., Dirr, H. W., *Biochemistry* 1999, *38*, 16686–16694.

[108] Austin, D. J., Crabtree, G. R., Schreiber, S. L., *Chem. Biol.* 1994, *1*, 131–136.

[109] Clemons, P. A., *Curr. Opin. Chem. Biol.* 1999, *3*, 112–115.

[110] Michnick, S. W., *Chem. Biol.* 2000, *7*, R217-R221.

[111] Beeley, N. R. A., *Drug Discov. Today* 2000, *5*, 354–363.

[112] Cochran, A. G., *Chem. Biol.* 2000, *7*, R85–R94.

[113] Cochran, A. G., *Curr. Opin. Chem. Biol.* 2001, *5*, 654–659.

[114] Cody, W. L., Lin, Z. W., Panek, R. L., Rose, D. W., Rubin, J. R., *Curr. Pharm. Design.* 2000, *6*, 59–98.

[115] Freidinger, R. M., *Curr. Opin. Chem. Biol.* 1999, *3*, 395–406.

[116] Way, J. C., *Curr. Opin. Chem. Biol.* 2000, *4*, 40–46.

[117] Zutshi, R., Brickner, M., Chmielewski, J., *Curr. Opin. Chem. Biol.* 1998, *2*, 62–65.

[118] Belshaw, P. J., Ho, S. N., Crabtree, G. R., Schreiber, S. L., *Proc. Natl. Acad. Sci. USA* 1996, *93*, 4604–4607.

[119] Kopytek, S. J., Standaert, R. F., Dyer, J. C. D., Hu, J. C., *Chem. Biol.* 2000, *7*, 313–321.

[120] Smith, K. M., Vanetten, R. A., *J. Biol. Chem.* 2001, *276*, 24372–24379.

[121] Amara, J. F., Clackson, T., Rivera, V. M., Guo, T. *et al*., *Proc. Natl. Acad. Sci. USA* 1997, *94*, 10618–10623.

[122] Keenan, T., Yaeger, D. R., Courage, N. L., Rollins, C. T. *et al*., *Bioorg. Med. Chem.* 1998, *6*, 1309–1335.

[123] MacCorkle, R. A., Freeman, K. W., Spencer, D. M., *Proc. Natl. Acad. Sci. USA* 1998, *95*, 3655–3660.

[124] Blau, A. C., Petrson, K. R., Drachman, J. G., Spencer, D. M., *Proc. Natl. Acad. Sci. USA* 1997, *94*, 3076–3081.

[125] Clackson, T., Yang, W., Rozamus, L. W., Hatada, M. *et al*., *Proc. Natl. Acad. Sci. USA* 1998, *95*, 10437–10442.

[126] Yang, W., Rozamus, L. W., Narula, S., Rollins, C. T. *et al*., *J. Med. Chem.* 2000, *43*, 1135–1142.

[127] Aviezer, D., Cotton, S., David, M., Segev, A. *et al*., *Cancer Res.* 2000, *60*, 2973–2980.

[128] Rohrer, S. P., Birzin, E. T., Mosley, R. T., Berk, S. C. *et al*., *Science* 1998, *282*, 737–740.

[129] Parker, S. L., Parker, M. S., *Can. J. Physiol. Pharmacol.* 2000, *78*, 150–161.

[130] Marusawa, H., Setoi, H., Kuroda, A., Sawada, A. *et al*., *Bioorg. Med. Chem.* 1999, *7*, 2635–2645.

[131] Fujita, T., Nakajima, M., Inoue, Y., Nose, T., Shimohigashi, Y., *Bioorg. Med. Chem. Lett.* 1999, *9*, 1351–1356.

[132] Hoekstra, W. J., Hulshizer, B. L., McComsey, D. F., Andrade-Gordon, P. *et al*., *Bioorg. Med. Chem. Lett.* 1998, *8*, 1649–1654.

[133] Burke, T. R., Yao, Z. J., Gao, Y., Wu, J. X. *et al*., *Bioorg. Med. Chem.* 2001, *9*, 1439–1445.

[134] Davidson, J. P., Martin, S. F., *Tetrahedron Lett.* 2000, *41*, 9459–9464.

[135] Fretz, H., Furet, P., Garciaecheverria, C., Rahuel, J., Schoepfer, J., *Curr. Pharm. Design* 2000, *6*, 1777–1796.

[136] Niimi, T., Orita, M., Okazawaigarashi, M., Sakashita, H. *et al*., *J. Med. Chem.* 2001, *44*, 4737–4740.

[137] Shakespeare, W. C., *Curr. Opin. Chem. Biol.* 2001, *5*, 409–415.

[138] Vu, C. B., *Curr. Med. Chem.* 2000, *7*, 1081–1100.

[139] Bouras, A., Boggetto, N., Benatalah, Z., Derosny, E. *et al*., *J. Med. Chem.* 1999, *42*, 957–962.

[140] Morris, M. C., Robert-Hebmann, V., Chaloin, L., Mery, J. *et al*., *J. Biol. Chem.* 1999, *274*, 24941–24946.

[141] Sourgen, F., Maroun, R. G., Frere, V., Bouziane, M. *et al*., *Eur. J. Biochem.* 1996, *240*, 765–773.

[142] Liuzzi, M., Deziel, R., Moss, N., Beaulieu, P. *et al*., *Nature* 1994, *372*, 695–698.

[143] Digard, P., Williams, K. P., Hensley, P., Brooks, I. S. *et al*., *Proc. Natl. Acad. Sci. USA* 1995, *92*, 1456–1460.

[144] Nordhoff, A., Tziatzios, C., van den Broek, J. A., Schott, M. K. *et al*., *Eur. J. Biochem.* 1997, *245*, 273–282.

[145] Eaton, S. R., Cody, W. L., Doherty, A. M., Holland, D. R. *et al*., *J. Med. Chem.* 1998, *41*, 4329–4342.

[146] Schramm, H. J., Derosny, E., Reboudravaux, M., Buttner, J. *et al*., *Biol. Chem.* 1999, *380*, 593–596.

[147] Shultz, M. D., Chmielewski, J., *Bioorg. Med. Chem. Lett.* 1999, *9*, 2431–2436.

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[148] Sennequier, N., Wolan, D., Stuehr, D. J., *J. Biol. Chem.* 1999, *274*, 930–938.

[149] Usui, T., Kondoh, M., Mayumi, T., Osada, H., *Biochem. J.* 1998, *333*, 543–548.

[150] Pacofsky, G. J., Lackey, K., Alligood, K. J., Berman, J. *et al*., *J. Med. Chem.* 1998, *41,* 1894–1908.

[151] Findeis, M. A., *Biochim. Biophys. Acta* 2000, *1502*, 76–84.

[152] Souroujon, M. C., Mochly-Rosen, D., *Nat. Biotechnol.* 1998, *16*, 919–924.

[153] Shultz, M. D., Chmielewski, J., *Tetrahedron* 1997, *8*, 3881–3886.

[154] Ulysse, L. G., Chmielewski, J., *Bioorg. Med. Chem. Lett.* 1998, *8*, 3281–3152.

[155] Song, M. C., Rajesh, S., Hayashi, Y., Kiso, Y., *Bioorg. Med. Chem. Lett.* 2001, *11*, 2465–2468.

[156] Maroun, R. G., Gayet, S., Benleulmi, M. S., Porumb, H. *et al*., *Biochemistry* 2001, *40*, 13840–13848.

[157] Park, S. H., Raines, R. T., *Nat. Biotechnol.* 2000, *18*, 847–851.

[158] Singh, S. K., Maithal, K., Balaram, H., Balaram, P., *FEBS Lett.* 2001, *501*, 19–23.

[159] Violette, S. M., Shakespeare, W. C., Bartlett, C., Guan, W. *et al*., *Chem. Biol.* 2000, *7*, 225–235.

[160] Zutshi, R., Chmielewski, J., *Bioorg. Med. Chem. Lett.* 2000, *10*, 1901–1903.

[161] Chrunyk, B. A., Rosner, M. H., Cong, Y., Mccoll, A. S. *et al*., *Biochemistry* 2000, *39*, 7092–7099.

[162] Pepys, M. B., Herbert, J., Hutchinson, W. L., Tennent, G. A. *et al*., *Nature* 2002, *417,* 254–259.