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Current Opinion in Structural Biology

Constructing structural networks of signaling pathways on the proteome scale

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Proteins function through their interactions, and the availability of protein interaction networks could help in understanding cellular processes. However, the known structural data are limited and the classical network node-and-edge representation, where proteins are nodes and interactions are edges, shows only *which* proteins interact; not *how* they interact. Structural networks provide this information. Protein–protein interface structures can also indicate which binding partners can interact simultaneously and which are competitive, and can help forecasting potentially harmful drug side effects. Here, we use a powerful protein–protein interactions prediction tool which is able to carry out accurate predictions on the proteome scale to construct the structural network of the extracellular signal-regulated kinases (ERK) in the mitogen-activated protein kinase (MAPK) signaling pathway. This knowledge-based method, PRISM, is motif-based, and is combined with flexible refinement and energy scoring. PRISM predicts protein interactions based on structural and evolutionary similarity to known protein interfaces.

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Current Opinion in Structural Biology 2012, **22**:367–377

This review comes from a themed issue on
Sequences and topology
Edited by Christine Orengo and James Whisstock

Available online 9th May 2012

0959-440X/$ – see front matter

http://dx.doi.org/10.1016/j.sbi.2012.04.004

Introduction

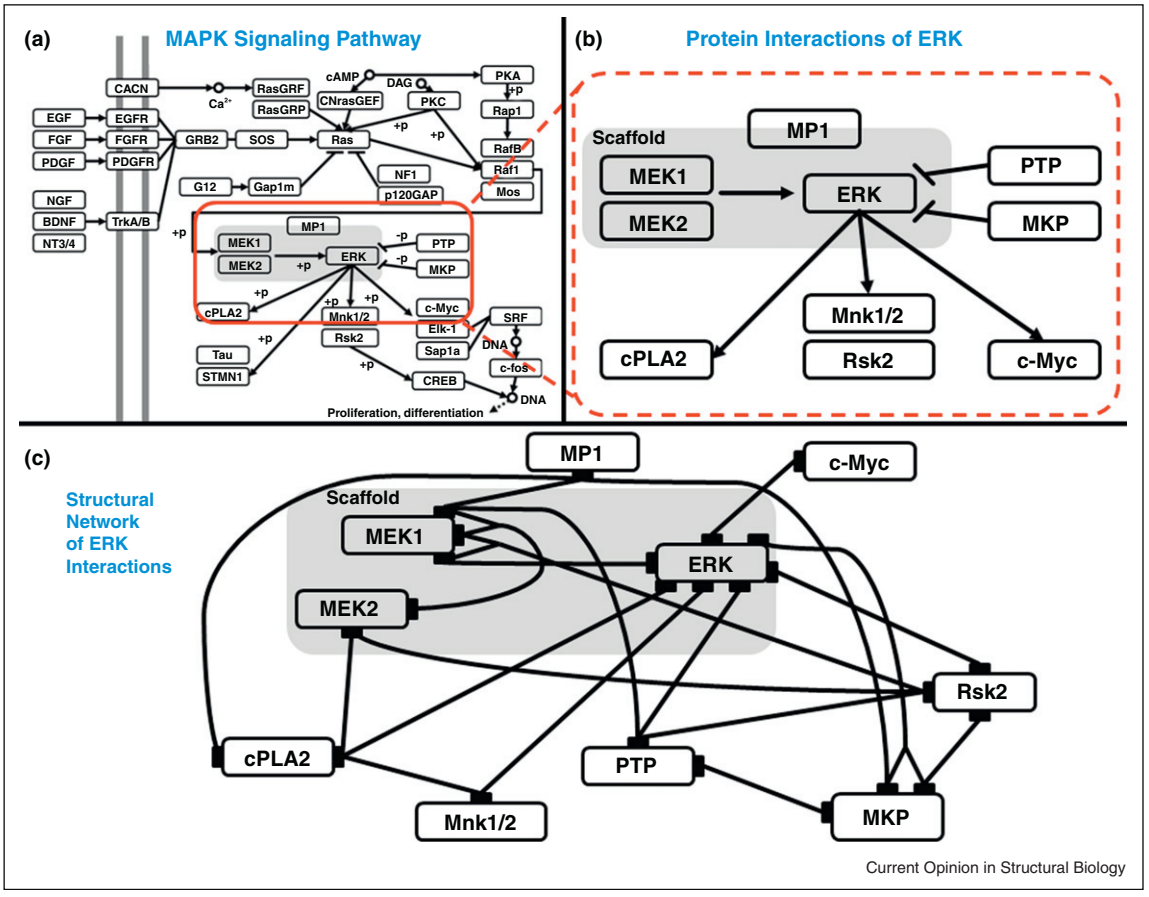
Protein–protein interactions and 'classical' protein interaction networks

Proteins function through interactions, and protein–protein interactions (PPIs) play a crucial role in all biological processes. An interaction with another protein physically perturbs the structure, and the perturbation (or, signal) propagates in the cell [1]. To understand how signals propagate and how regulation takes place, we need to know the interactions of the proteins. Among the first steps toward this goal is the identification of PPIs. Experimentally, identification of pairwise PPIs has been addressed by techniques such as yeast two-hybrid system [2], phage display [3], protein arrays [4], and affinity purification [5], and databases like DIP [6], MINT [7], BIND [8], BioGrid [9], and IntAct [10], have usefully compiled these data. On the basis of these, protein interaction networks (PINs) have been constructed [11,12], on a pathway [13–15] or proteome scale [16–18]. Despite these efforts, 10% the human interactome is known [19]. In PINs, proteins and their interactions are represented as nodes and edges, respectively. Figure 1a presents the mitogen-activated protein kinase (MAPK) signaling pathway. This representation depicts direct and indirect interactions, and signals transmitted from a perturbed source node (a protein) to a sink node (another protein) can be straightforwardly traced through the network edges. Node-and-edge networks help in understanding the communication and functional identification on a large-scale. However, because they do not provide the essential structural details, they are not able to help in figuring out the regulatory mechanisms.

Structural protein–protein interaction networks

A key drawback of high-throughput experiments is in the possible presence of many false positives [20,21]. Methods such as X-ray crystallography [22], nuclear magnetic resonance (NMR) spectroscopy [23], and cryo-electron microscopy (cryo-EM) [24] can provide high resolution structural data. The Protein Data Bank (PDB) [25] provides the structures of proteins and their complexes (over 80 000 structures as of March 2012); however, it is incomplete and does not cover all structures. In addition, it may contain non-biological crystallographic packing interactions [26]. NMR is limited in size [27]. Cryo-EM is informative at relatively low resolution [28]. These limitations can be addressed by complementary experimental and computational techniques. Computational approaches can help to verify experimental observations and predict new ones. Docking and knowledge-based methodologies are the main computational PPI prediction protocols. Docking strategies consider possible structural combinations of query proteins to find the 'best', native bound state. The critical assessment of predicted interactions (CAPRI) [29] demonstrates the performance of docking strategies, and helps in following their progress. ZDock [30], PIPER [31] and GRAMM-X [32] use the Fast Fourier Transform (FFT) approaches in

Figure 1



MAPK signaling pathway and ERK interacting proteins. **(a)** Classical node-and-edge representation, where proteins are nodes and interactions are edges. The network is taken from the KEGG database. Gray lines represent the membrane. Gray box indicates the scaffold for the proteins on it. **(b)** The ERK interacting proteins in the node-and-edge representation. **(c)** The structural network of the ERK interacting proteins. Protein interactions are found using PRISM. Edges which are connected to a node through the same black box imply that the proteins bind through the same binding site, that is, they are competitive. They are connected to different black box on the node, if the binding sites are different. If a binding site overlaps two other binding sites, the edge attaches to the corresponding two black boxes and has a fork shape. Two binding sites are considered as overlapping if the common residues are more than 20% of the smaller binding site. MP1 is discarded from the scaffold shown as a gray box, since the binding sites of MP1 and MEK2 overlap and the complex of ERK, MEK1 and MEK2 can be constructed.

the global search for the binding mode of proteins. RosettaDOCK [33] is generally preferred in the high-resolution refinement step. ATTRACT [34] and FiberDock [35] use normal mode analysis (NMA) to sample conformational backbone variability. Some methods utilize different experimental data to increase their accuracy. MolFit [36] and ATTRACT consider experimentally determined interface residues. ZDock and pyDock [37] block non-interface residues. PROXIMO [38] and MultiFit [39] use radical probe mass spectrometry (RP-MS) and EM data in docking, respectively. HADDOCK [40] utilizes experimental data, mainly NMR, to extract interface information including contacts and relative orientations. PatchDock [41] uses the entire surface; however, if experimental constraints are available they can limit the search. Predicting PPIs by docking is computationally very expensive. Protein size affects the computation time, which makes docking of larger proteins much slower [42]. Moreover, scoring functions in the docking algorithms are still not optimal to predict which proteins interact and how they interact on the interactome scale [43,44]. Consequently, docking approaches are not appropriate for large-scale studies [45,46]. Data derived from known interactions can restrict the solution space in knowledge-based approaches. Homology-based methods can be powerful in predicting interactions (even for unstructured proteins) as shown by the pioneering work of Aloy and Russel [47], who also created a web server (InterPreTS [48]) which scores the predictions based on empirical potentials derived from known interactions. More recently, Kundrotas *et al.* [49] have also predicted the structures of interacting proteins based on sequence

homology. The GWIDD database [50] usefully provides experimental and homology-based models, WSsas [51] functional residues in structural homologs, and IBIS [52,53] considers both structure and sequence conservation. Finally, Multiprospector [54] utilizes multimeric threading, independent of sequence similarity and M-Tasser considers certain protein flexibility [55].

Domain information has also been utilized in protein interaction prediction. Shoemaker *et al.* [56] showed that members of domain families can dock in the same way. Davis *et al.* [57] matched the overall domains and scored predictions by statistical potentials derived from the known interactions. Aloy and coworkers [58] combined the overall structural fold and sequence similarities and developed the 3DID web server [59] which identifies domain-based interactions.

In interface-based approaches, an interaction between query proteins can be achieved fairly reliably if the structure of a similar interface is available, based on the similarity of query protein surfaces to interfaces of known interacting proteins [60]. This concept is similar to homology modeling for structure prediction. Using known data lowers substantially the computational time, making PPI prediction on a large-scale possible. PRotein Interactions by Structural Matching (PRISM) [60,61] is the pioneer interface-based PPI prediction method, which is applicable to structural interactome data. ISearch [62] is based on a similar concept but has a template set of domain–domain interactions. Sinha *et al.* [63] use a template-based docking independent of sequence homology and consider local structural alignments rather than global structural alignments.

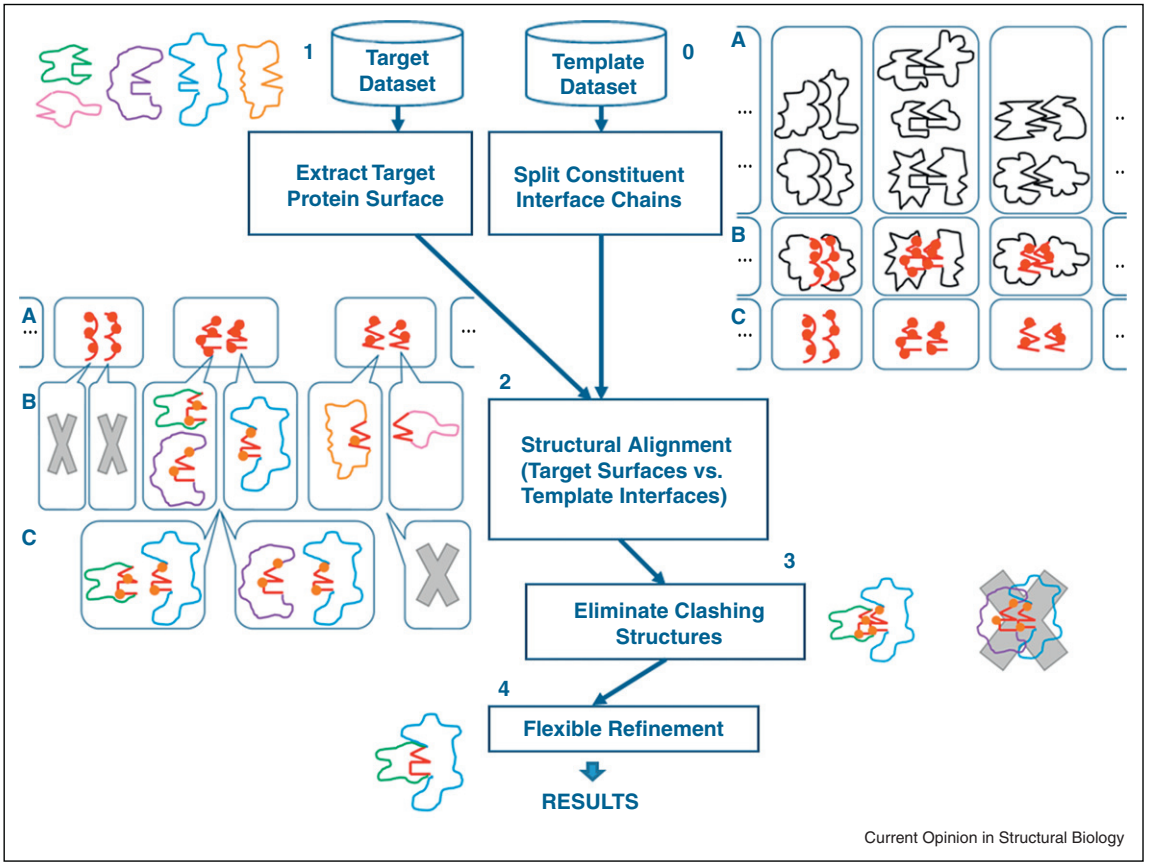
Comparison of the knowledge-based methods is not straightforward because they use different benchmark datasets. Nevertheless, their individual performance shows their success. In the work of Aloy and Russel [47], 59 of 2590 predicted interactions are experimentally verified. Multiprospector [54] predicts 36 homodimers and 15 heterodimers correctly among 40 homodimers and 15 heterodimers. In the work of Davis *et al.* [57], 270 binary and eight multi complexes are experimentally verified among 3387 binary and 1234 multi complexes predicted. ISearch [62] can predict a model for 45 of 59 cases. Sinha *et al.* [63] correctly predict 56% of 372 complexes in their bound forms. PRISM can predict 87 of 88 cases of a docking benchmark dataset [64].

Prediction of PPIs on a large-scale using PRISM, a motif-based PPI modeling method

Proteins have many partners; on average, 5–12 interactions per protein are listed in human PPI databases [65•]. The proteins interact via contacting binding sites. Binding sites are distinct and have limited surfaces [66,67]. Although there are many proteins in nature, the structural variety of their interfaces is limited, which suggests that proteins share binding site structural motifs [68–70]. Protein interfaces can be classified into three groups according to their structures and the global structures of the interacting protein pairs [71]. Type I interfaces are the most common: similar interfaces formed by proteins whose global structures and functions are also similar. The interfaces' architectures are evolutionarily more conserved than the rest of their surfaces [66,72•]. Type II includes similar interfaces formed by proteins whose global structures and functions are different. In Type III, only one side of each interface is similar and the binding sites of the complementary partners are somewhat different. Hub proteins interacting through shared binding sites are good examples of this type [69]. In all cases, interfaces have conserved motifs even if the interacting proteins are structurally and functionally different. This evolutionary feature of interfaces can be used to predict PPIs.

PRISM is an efficient motif-based PPI modeling method [60,61,73••]. Interactions among a group of proteins are predicted based on known PPIs. All known PPIs are assembled into a template set. The interfaces of non-redundant binary interactions in the PDB are extracted and clustered according to the similarity of their structures. Representatives of each group constitute the template set (Figure 2, Step 0). PRISM proposes a potential interaction between two query proteins in a target set based on structural and evolutionary similarity of their surfaces to the complementary sides of a known interface in the template set (Figure 2, Step 2). The structural similarity is determined via geometrical alignment of the structures using MultiProt [74]. If it is known that protein A interacts with protein B (i.e. if there is a representative structure similar to the interface of complex AB in the template set), and the surfaces of proteins A′ and B′ are structurally similar to those of proteins A and B, a potential interaction between A′ and B′ is proposed. The evolutionary similarity is checked by the conservation of hot spots. Hot spots are the residues which are mainly responsible for the affinity and stability of the interaction by contributing significantly to the binding free energy [75]. The strong correlation between hot spots and conserved residues on structurally similar interfaces [76,77•] indicates the importance of hot spots in determining binding sites. Computational hot spots are found via the HotPoint web server [78], and PRISM requires that at least one computational hot spot at each side of the template interface matches structurally with a residue of each target protein when the structures are aligned. Moreover, the matched residues from each interface side should preferably be against each other to guarantee correct matching of the left and right partners. The physical and biological feasibilities of the potential interaction are also queried. If there are many clashes between residues of interacting proteins, the complex is eliminated (Figure 2, Step 3). Flexible refinement is

Figure 2



Flowchart of the PRISM algorithm. PRISM consists of five steps. Step 0: template set organization. Similar interfaces of binary protein complexes are grouped in the same cluster (0A). All interfaces in the same cluster are structurally similar to the representative of the cluster. Red lines represent representative interfaces. Computational hot spots of representative interfaces are found using HotPoint web server, and shown as red dots (0B). Representative interfaces construct the template set (0C). Step 1: surface extraction of target proteins. Multimeric target proteins are split into their monomers, and homologous chains are counted only once. Surface of each non-homologous monomers of target proteins (green, pink, purple, blue and orange proteins) are extracted. Step 2: structural alignment. Target surfaces are aligned onto template structures given in 2A, the same set as in 0C. Green and purple molecules have similar structure to left side of a representative interface. Blue molecule is similar to the right side of that interface. Orange and pink molecules are structurally similar to another representative interface. Structural similarity is not observed for the other template interfaces. Orange dots represent residues matched with a hot spot of the template interface in the structural alignment (2B). Candidate complexes, green-blue and purple-blue proteins, are assigned between two structures similar to each side of a template interface. A complex of orange and pink proteins is not considered, since any residue of the pink protein does not match with a hot spot of the corresponding template interface (2C). Step 3: physical evaluation of candidate complexes. If the residues of the proteins clash, as in the complex of purple and blue proteins, the candidate complex is eliminated. Step 4: flexible refinement. Side chains of interacting proteins are oriented in order to prevent clashes among them. Potential complexes are given as the results, like the complex of green and blue protein.

performed using FiberDock [35] (Figure 2, Step 4). The global energy of the complex is calculated to evaluate the potential complex biologically. PRISM gives the 3D structure of the predicted interactions and can be used to predict PPIs on the proteome scale [73••].

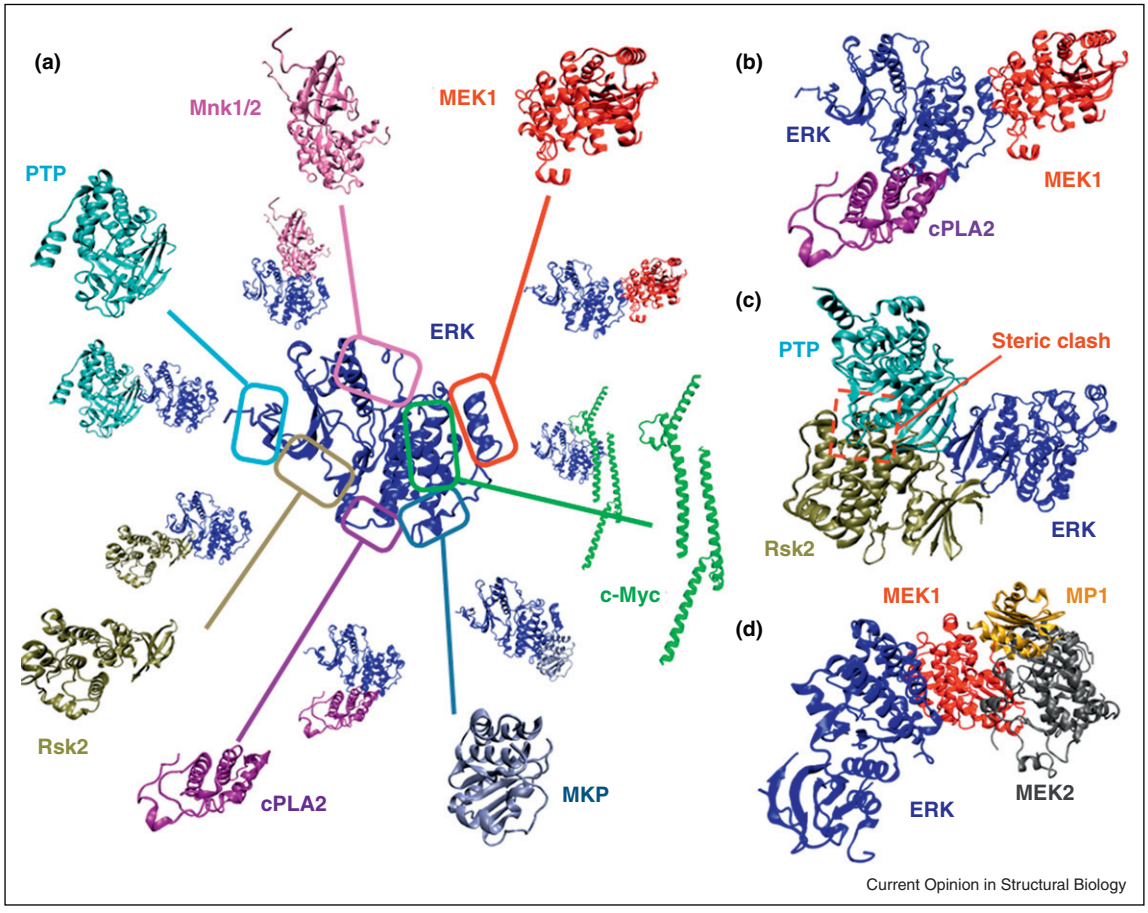
Constructing structural signaling pathways

In the node-and-edge network representation, interaction data are given as binary relation; two proteins are connected by an edge if they interact, and are not connected otherwise. The ERK pathway protein interactions are shown in this representation in Figure 1b, where the interaction data are taken from the KEGG database [79]. However, the binary representation cannot help to elucidate the mechanism in detail, which is essential for figuring out their roles, how the signal flows, and how the function and the regulation are executed in the cell. Structural interaction networks present the nature of the interactions; thus, 'how proteins interact' is characterized in the structural network, in addition to 'which proteins interact' in the classical representation [44,45,80,81••].

Structural networks illustrate the proteins, and the interfaces through which they interact. The structural network of the ERK interacting proteins is represented in Figure 1c, where the interactions are found using PRISM

and the PDB IDs of proteins are taken from the KEGG database (Supplementary Data, Table S1). PRISM predictions with lowest energy values are selected (Supplementary Data, Table S2). In addition to what the classical node-and-edge representation provides, the structural network also shows which partners of a protein are in contact through the same, shared binding sites, and which are in contact through different binding sites of the protein. If two partners bind the same protein surface, it is physically impossible that both partners bind to the protein at the same time. In this case, at any given time, one of these interactions is excluded while the other may exist. However, if two partners interact through different protein binding sites, the protein can bind both partners simultaneously, unless the interacting proteins collide elsewhere. Interface data can be used to elucidate such multi-partner protein interactions. Network studies show that although most of the proteins have one interacting partner or a few partners, a relatively small number of proteins, which are called hubs, have a large number of partners [82]. These hub proteins do not have as many distinct binding sites as the number of their interacting partners [67]. Therefore, some interactions occur through the same binding site, even though the interacting partners can be structurally and functionally different [83,84••]. Identifying overlapping and non-overlapping interfaces [85] helps to determine the interaction behavior in the network, and regulation. The different interfaces also lead to different binding affinities [86••]. Furthermore, it is also physically impossible for two proteins to interact with the third simultaneously, if their residues clash while contacting with the third protein. In such broad, proteome-scale approaches, flexibility and allosteric effects cannot be taken into account.

Figure 3



Protein interactions of the ERK protein. Interactions are predicted using PRISM. Predictions with lowest global energy values are selected. **(a)** Interacting partners and corresponding binding sites of the ERK protein. **(b)** Simultaneous interactions of MEK1 and cPLA2 proteins with ERK. They bind through different sites of the ERK protein. The figure is drawn by aligning ERK–cPLA2 (3i5zA-1lebA) and ERK–MEK1 (1pmeA-3eqcA) interactions onto each other. Only ERK interacting to cPLA2 is shown. **(c)** Clashing in the complex of ERK, PTP and Rsk2 proteins. Although PTP and Rsk2 proteins do not bind through the same site of the ERK protein, their residues clash when they bind to ERK protein at the same time. The figure is drawn by aligning ERK–PTP (2ojgA-1zc0A) and ERK–Rsk2 (2ojjA-2wntA) interactions onto each other. Only ERK interacting to PTP is shown. **(d)** Interactions of ERK, MEK1, MEK2 and MP1 proteins. MP1 and MEK2 interactions are competitive. They are binding through the same site of the MEK1 protein. The figure is drawn by aligning MEK1–MEK2 (3e8nA-1s9iA), MEK1–MP1 (3dv3A-1skoA) and MEK1–ERK (3mblA-1pmeA) interactions onto each other. Only MEK1 interacting to MEK2 is shown.

Figure 3 shows the interactions of the ERK protein. Seven proteins bind at different ERK sites (Figure 3a). Proteins which bind at different sites, like MEK1 and cPLA2, can interact with ERK simultaneously (Figure 3b). However, PTP and Rsk2 cannot bind to ERK at the same time, even though their interactions with ERK are at different sites; this is because of steric clash elsewhere (Figure 3c). Moreover, ERK, MEK2 and MP1 cannot interact with MEK1 simultaneously, because MEK2 and MP1 share a binding site (Figure 3d). In the same manner interface data can be used to construct protein assemblies [81••,87••]. Assemblies are important for cell functions; they increase the effective local concentration of substrates/products, and the efficiency in signaling and cell response. Using pair-wise interactions and steric restrictions, complexes larger than two proteins can be constructed. The complex of MEK1, MEK2, ERK and MP1 given in the KEGG is illustrated in a gray box in Figure 1, and the interactions are shown in Figure 3d. The model structure of the complex is obtained by combining the interactions MEK1–MEK2, MEK1–ERK and MEK1–MP1 which are found by PRISM. The binding sites of MP1 and MEK2 overlap; therefore, it is physically impossible to construct a complex of these four proteins. However, complexes of MEK1–ERK–MEK2 and MEK1–ERK–MP1 can exist.

Introducing mRNA expression data into structural networks further helps in figuring out functional relevance [88]; however, in the absence of structural interaction data, mRNA expression data cannot distinguish between direct and indirect interactions [89].

Structural networks are useful for elucidation of network dynamics; and residue (and atom) level detail can be used to understand their dynamics in the cell [90,91,92••]. At the same time, a main limitation in constructing a knowledge-based structural network derives from the incompleteness of the PDB. About 20 000 PDB structures (as of March 2012) are of *Homo sapiens*. The ones that have a role in signaling processes are just over 3300. Proteins with no PDB structures create 'holes' in the map. However, the number of structures in the PBD is progressively increasing, and as the PDB gets larger the maps will eventually be completed, either with the missing structures, or with highly homologous ones. Until then, these holes can be filled with the help of structural modeling of complexes and the component chains. Single-chain protein structures can be modeled from their sequences using modeling tools, like I-Tasser [93], Swiss-Model [94], Phyre [95], and RaptorX [96].

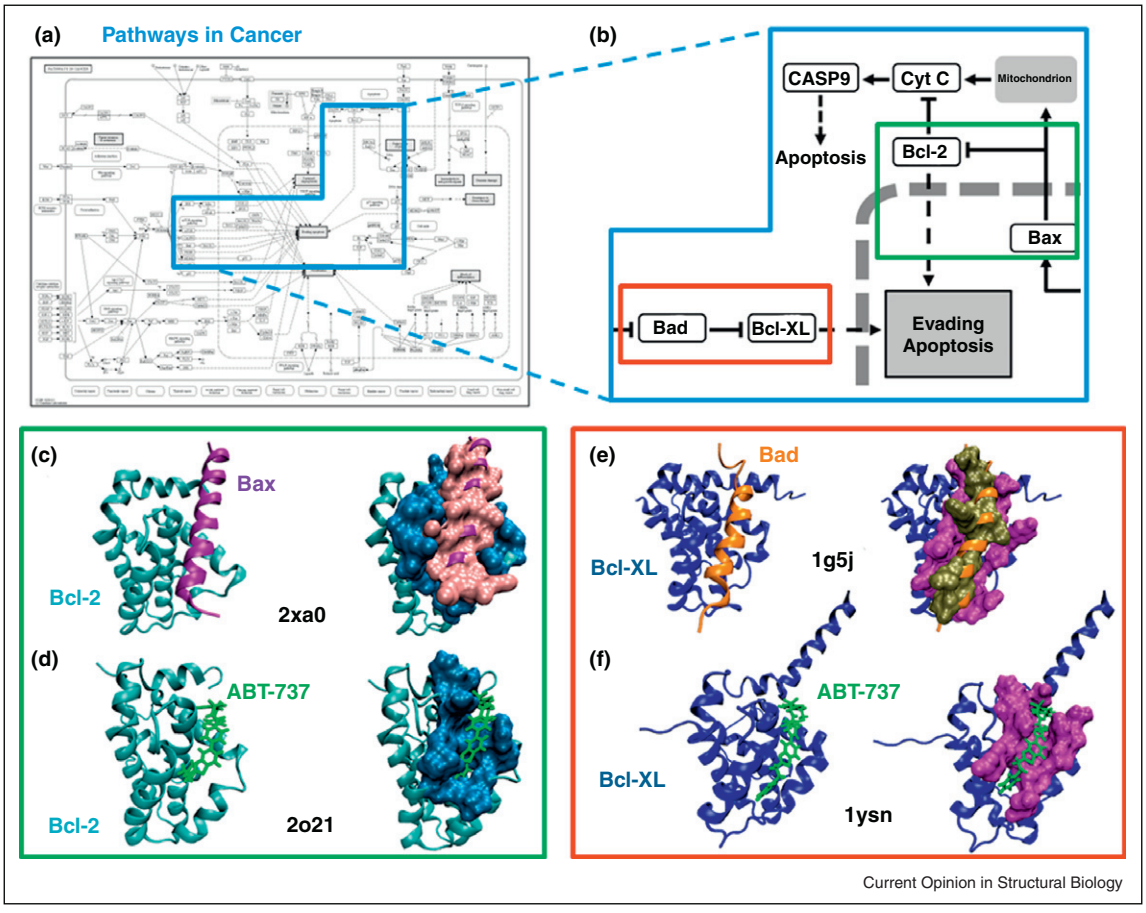
Drug design based on protein interfaces

Identification of protein interactions at the residue-level is also important for drug discovery. Although increasingly drugs target allosteric sites [97,98], most are orthosteric [99••,100]. Peptide inhibitors can mimic the complementary partner of the protein [101,102]. Figure 4c and d shows the interaction of the Bcl-2 protein with the Bax protein and an orthosteric drug-like ligand, ABT-737. Bcl-2 is a member of the B-cell lymphoma 2 (Bcl-2) family of proteins which includes pro-apoptotic and anti-apoptotic proteins. They are central regulators of programmed cell death and their protein interactions have roles in the apoptosis pathway. Pro-apoptotic proteins like Bax and Bad, propagate death signal which activates the family of caspases, cysteine proteases. Anti-apoptotic proteins, like Bcl-2 and Bcl-XL, have protective roles and sequester pro-apoptotic proteins by directly binding to them [103]. They are overexpressed in many cancers, inhibit apoptosis and play a role in tumor initiation, progression and resistance to therapy. Inhibition of these anti-apoptotic proteins targets abnormal cell deaths and offers alternative targets for drug therapies. The interaction of Bcl-2 and Bax proteins is shown in Figure 4c. This interaction is mimicked by the ligand ABT-737 (Figure 4d). It interacts with Bcl-2 through the binding site of Bax. It does not directly initiate apoptosis, but enhances the effect of death signals. It causes tumor regression, increases survival and cures animal models [104].

Drug effects were recently considered on a large scale, pathway-wide or proteome-wide [105,106]. The interacting protein gains or loses a function and the effects propagate in the system. Network analysis can be used to select drug targets and it helps in understanding its global drug effects [107,108]. Perturbing the network by drug combinations can be more effective than targeting individual proteins. Polypharmacology considers cellular robustness and focuses on multi-target drugs [109]. While network analyses increasingly help drug discovery, the absence of network-scale structural data can hamper these efforts. Structural networks, as compared to the classical node-and-edge interaction networks, are more powerful for predictive approaches [81••,110•]: first, because drugs are mostly designed to interact through the binding site of the protein, interface data can dramatically help efficient targeting. The function of the protein can be activated or inhibited by mimicking the interface structure of the complementary protein partner; second, protein–protein and protein–ligand interactions are conserved and often overlap [111•,112], even if the sequences or global structures are not similar. A drug can recognize similar protein interfaces; on average a drug interacts with six protein targets [113]. Interaction with off-targets can be beneficial or harmful to the living organism. Network structural data can help to figure out such potential synergestic or harmful side effects [114•,115].

Figure 4 shows the inhibition of Bcl-2 and Bcl-XL proteins with the same drug-like ligand, ABT-737. This example illustrates that a drug can bind to different proteins with structurally similar interfaces. The overall sequence

Figure 4



ABT-737 inhibition of Bcl-2 and Bcl-XL proteins. **(a)** 'Pathways in cancer' map is taken from the KEGG database. **(b)** Interactions of Bcl-2/Bax and Bcl-XL/Bad on the network. **(c)** Interaction of Bcl-2 and Bax proteins and their contacting residues (PDB ID: 2xa0AC). Cyan structure: Bcl-2 protein, purple structure: Bax protein, blue atoms: interacting residues of Bcl-2 protein, pink atoms: interacting residues of Bax protein. The interacting residue numbers of Bcl-2 are 100, 104, 107, 108, 110, 112, 115, 118, 119, 133, 136, 137, 139, 140, 143, 144, 145, 146, 148, 153, 200, 201, 202, 204 and 205; and the interacting residue numbers of Bax are 57, 58, 59, 60, 62, 63, 64, 66, 67, 68, 69, 70, 71, 73, 74, 75, 77, 78 and 81 (underlined ones are computational hot spots found by HotPoint web server). 64th, 68th, 74th and 78th residues of Bax are found experimentally as hot spots [116]. **(d)** Interaction of Bcl-2 protein and ABT-737 ligand, and their contacting residues (PDB ID: 2o21A). Cyan structure: Bcl-2 protein, green structure: ABT-737 ligand, blue atoms: interacting residues of Bcl-2 protein. The interacting residue numbers of Bcl-2 are 97, 100, 101, 105, 109, 112, 130, 133, 134, 141, 142, 143, 145, 146, 149, 150, 195 and 199. **(e)** Interaction of Bcl-XL and Bad proteins and their contacting residues (PDB ID: 1g5jAB). Blue structure: Bcl-XL protein, orange structure: Bad protein, magenta atoms: interacting residues of Bcl-XL protein, Tan atoms: interacting residues of Bad protein. The interacting residue numbers of Bcl-XL are 97, 100, 101, 105, 108, 112, 115, 116, 126, 129, 130, 133, 134, 142, 143, 145, 146, 150, 198, 199, 203, 204 and 207; and the interacting residue numbers of Bad are 302, 304, 305, 306, 308, 312, 313, 315, 316, 319, 320, 323 and 324 (underlined ones are computational hot spots found by HotPoint webserver). **(f)** Interaction of Bcl-XL protein and ABT-737 ligand; and their contacting residues (PDB ID: 1ysnA). Blue structure: Bcl-XL protein, green structure: ABT-737 ligand, magenta atoms: interacting residues of Bcl-XL protein. The interacting residue numbers of Bcl-XL are 97, 100, 101, 104, 105, 108, 112, 115, 130, 133, 134, 140, 141, 142, 143, 145, 146, 195 and 199.

identity between Bcl-2 and Bcl-XL is only 49%; however, their structures are quite similar. MultiProt [74] gives a root-mean-square-deviation (RMSD) value of 1.41 Å over 131 matched residues when PDB IDs 2xa0A (Bcl-2, 137 residues) and 1g5jA (Bcl-XL, 175 residues) are aligned. The binding sites of these proteins are also structurally similar. When the 62 residues of Bcl-2 interact with Bax in the complex 2xa0, and 70 residues of Bcl-XL interact with Bad in the complex 1g5j are aligned an RMSD value of 1.26 Å for 50 matched residues is obtained.

ABT-737 inhibits the interaction of Bcl-XL and Bad proteins, in addition to inhibiting the interaction of Bcl-2 and Bax proteins. This drug interacts with Bcl-XL through the binding site of Bad, similar to the inhibition of the Bcl-2/Bax interaction [104] (Figure 4e and f). The interacting residues mostly match the computational hot spots and other contacting residues in the Bcl-2/Bax and Bcl-XL/Bad interactions. Overall, these examples show that a drug-like ligand inhibits two different interactions which have structurally similar interfaces.

Thus, considering interface similarity, potential side-effects of drugs can be predicted.

Conclusions

Protein interfaces can provide extremely important and useful data on protein interactions. They give the physical contacts, validate the interaction between them, and illustrate how the proteins interact. A node-and-edge representation of protein interaction networks illustrates which proteins interact. By contrast, a structural representation, which includes detailed interface data of the protein interactions, can help to understand, how function is performed. Moreover, because it is physically impossible for two partners to bind simultaneously at the same binding site structural networks can differentiate between potentially co-occurring and competitive interactions of a protein with its partners. Consequently, structural networks can be used to understand cellular regulation and signal transmission dynamics. Interface data are also crucial for drug discovery. Many drugs are designed to bind at a particular protein interface. However, the drug can interact with other interfaces which are similar to that of the target protein, which can lead to unwanted side effects. Structural networks can help to detect such off-targets.

To construct structural networks, PPIs should be identified on a large scale. Because of experimental limitations, computational methods can be used to complement and extend experiments. Considering computational feasibility, knowledge-based approaches, like PRISM, appear more appropriate to map interactome data, as compared to docking strategies. On the down side, because of technical limitations which exist when carrying out prediction on a large scale, PRISM considers query proteins as rigid bodies; however, in the last flexible refinement step, it uses a docking refinement tool which allows side chains and slight protein backbone re-orientation and optimization. Flexibility and conformational changes upon binding are challenging problems in prediction of protein interactions. Proteins can also change their conformations because of allostery such as that incurred by prior binding events or by post-translational modifications, like phosphorylation and ubiquitination; however, if the structure is present in the template set, PRISM can accurately predict the interactions, and map structural cellular pathways. We expect that with time, knowledge-based approaches will be increasingly used on a large scale. Because structural motifs recur — in single chain proteins and in interfaces — methods which are based on these can be reliable and practical tools.

Acknowledgements

This work has been supported by TUBITAK (The Scientific and Technological Research Council of Turkey), Research Grant Numbers: 109T343 and 109E207, and The Turkish Academy of Sciences (TUBA). Guray Kuzu is supported by a TUBITAK fellowship. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract number HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sbi.2012.04.004.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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