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The TPR Motif as a Protein Interaction Module – A Discussion of Structure and Function

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1. Introduction

Many biological functions involve the formation of protein-protein complexes. Indeed, protein–protein interactions are considered to be the center of all functional living cells. Proteins can interact with each other by various structural, chemical and physical means. Some of these interactions can be highly specific, accompanied by high affinity, while some proteins are more flexible and bind diverse proteins as ligands. A common group of proteins that participate in protein-protein interactions and serve as multi-protein complex mediators are the tetra-trico-peptide repeat (TPR) proteins. TPR-containing proteins were found to be involved in many diverse processes in eukaryotic cells, including synaptic vesicle fusion (Young et al. 2003), peroxisomal targeting and import (Brocard et al. 2006; Fransen et al. 2008) and mitochondrial and chloroplast import (Baker et al. 2007; Mirus et al. 2009). In addition, TRPs are required for many bacterial pathways, such as outer membrane assembly (Gatsos et al. 2008), bio-mineralization of iron oxides in magnetotactic bacteria (Zeytuni et al. 2011) and pathogenesis (Edqvist et al. 2006; Tiwari et al. 2009). In addition, mutations in TPR-containing proteins have been linked to a variety of human diseases, such as chronic granulomatous disease and Leber's congenital amaurosis (D'Andrea & Regan 2003).

To date, more than 5,000 TPR-containing proteins were identified in different organisms by bioinformatics tools. Of these, more than 100 structures have been determined and deposited in the Protein Data Bank. These structures demonstrate the tendency of TPR motifs to exist as an independent fold or as a segment of a fold within a protein. The available structures allow the study of a protein-protein interaction platform at atomic resolution. In general, TPR-containing proteins can serve as a study case for protein interactions as they display great binding variety.

In this chapter, we describe the basic TPR sequence and structure, as well as several examples of diverse TPR binding properties. These include:

1. Various amino acid sequences which give rise to the binding of diverse ligands that participate in different biological processes.
2. Variety of structural features and conformations that serve as TPR ligands
3. The role of TPR protein curvature angle in ligand specificity.

4. Protein surface electrostatic potential distribution and the contribution of such distribution to the diverse binding properties of TPRs.
5. Multiple binding pockets which allows TPR-containing proteins to serve as mediators in multi-protein complexes.
6. The broad distribution of homo-oligomerization states in TPR proteins.
7. Ligand computational docking.
8. TPR proteins and biotechnology.

2. TPR sequence and basic structure

The TPR represents a structural motif consisting of 34 amino acids, sharing a degenerate consensus sequence defined by a pattern of small and large hydrophobic amino acids. In this consensus sequence, there are no completely invariant residual positions. The consensus pattern of conserved residues involves positions 4, 7, 8, 11, 20, 24, 27 and 32, in reference to the single motif N-terminal (Fig. 1A). Residues type is highly conserved only at positions 8 (Gly or Ala), 20 (Ala) and 27 (Ala). The rest of the consensus positions displays a preference for small, large or aromatic amino acids rather than for a specific residue. In addition, important structural characteristic conservation can be found at the turns located between two helical segments which contain helix-breaking residues (D'Andrea & Regan 2003). Nowadays, TPR consensus sequences can be identified by most general sequence analysis programs, such as the Simple Modular Architecture Research Tool (SMART) or the PROSITE dictionary of protein sites and motif patterns. At the same time TPRpred is a specially designated tool that uses the profile representation of the known repeats to detect TPR motifs and other patterns of protein repeats.

The canonical unit of the TPR motif adopts a basic helix-turn-helix fold (Fig. 1B). Adjacent TPR units packed in parallel create a series of repeating anti-parallel α-helices that give rise to an overall super-helix structure. This super-helical twist is affected by the type of residue found between adjacent TPR motifs. The unique super-helix fold forms a pair of concave and convex curved surfaces (Fig. 1C). Concave and convex surfaces display some extent of flexibility, as well as amino acid variety, which permits the binding of diverse ligands, usually via the concave surface.

In today's era of complete genome sequencing, one can predict TPR-containing protein dispersion throughout different organisms. Indeed, TPR proteins were found to be common in all forms of life, namely bacteria, archaea and eukaryotes. In nature, TPR motifs can be found in tandem arrays of 3-16 sequential motifs within a given protein (Fig. 1D). Moreover, Kajander et al. (2007) designed and determined the structure of a non-natural recombinant TPR-containing protein incorporating 20 sequential motifs. By using an increasing number of repeat-containing proteins, these authors found a positive correlation between the number of repeats and protein thermo-stability.

3. Ligand binding diversity

TPR-containing proteins bind diverse ligands in different binding pockets. These ligands usually do not share sequence or secondary structure similarities. Despite the lack of a set of defined rules, binding is usually highly specific, with TPR-containing proteins able to identify their ligand within the dense cellular environment. To obtain such diverse binding,
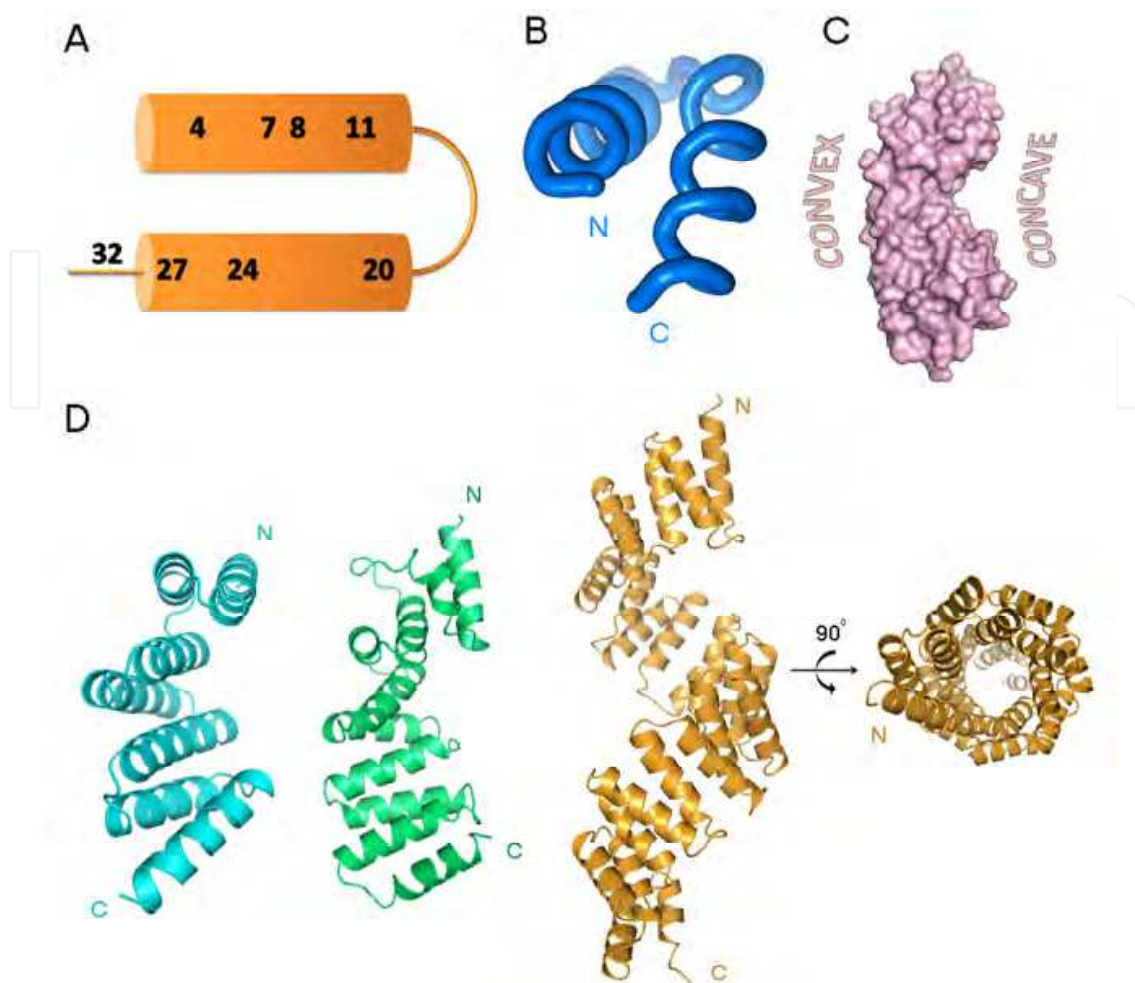
Fig. 1. TPR motif sequence and structure. (A) Schematic representation of the secondary structure arraignment of the 34 amino acids in a TPR motif. Numbers represent the conserved positions of amino acids within the motif. (B) The basic helix-turn-helix fold of a TPR motif canonical unit. (C) Surface representation of a TPR-containing protein displaying concave and convex surfaces. (D) Representative TPR-containing protein structures. From left, the TPR domain of Hop, containing three sequential motifs, MamA, containing five sequential TPR motifs, and the super-helix forming O-linked *N*-acetylglucosamine transferase TPR domain, containing 11 sequential TPR motifs, are shown in side and top view, in blue, green and orange respectively. Images B, C and D were generated by Pymol software (www.pymol.org).

several TPR-distinct folds serve as an interaction platform. This platform can display different surface residues in each binding surface, which later interacts in a specific manner with the ligand. Additionally, residue type influences the electrostatic nature of the binding surface, with, for example, arginine and lysine contributing positive charges, whereas glutamic acid and aspartic acid contribute negative charges. In addition, residues of different hydrophobicity and size can support hydrophobic interactions between the TPR protein and its ligand and therefore enhance protein-ligand specificity. Overall, the available TPR protein structures showing interaction with their ligands show that binding specificity cannot be attributed to a single force and that it is usually achieved by a combination of factors, such as residue type, hydrophobic pockets, charge and electrostatics.

3.1 Ligand sequence diversity

In this section, we describe two relatively simple TPR ligand interactions to demonstrate the multiple interaction types involved in binding and the correlation between TPR binding surfaces to the amino acid sequence of their ligands.

The first released structures of TPR domains bound to their ligand peptides were the two domains of the Hop protein (Scheufler et al. 2000). Hop is an adaptor protein which mediates the association of the molecular chaperones, Hsp70 and Hsp90. The TPR1 domain of Hop specifically recognizes the C-terminal heptapeptide of Hsp70, whereas the TPR2A domain binds the C-terminal pentapeptide of Hsp90 (Figs. 2A&B). Both C-terminal peptides share EEVD sequence ends and were found to bind in an extended conformation. An extended conformation allows the display of a maximal surface to the TPR domain and facilitates specific recognition of short amino acid stretches with sufficient affinity. Examination of solved crystal structures revealed that all electrostatic contacts between TPR domains and both peptides involve the EEVD sequence motif. These interactions include three classes of hydrogen bonding interaction, namely sequence-independent interactions with the peptide backbone, specific interactions with peptide side chains and the carboxylate of the C -terminal residue interaction with the TPR (Figs. 2C&D). The three strong hydrogen bonds formed between the peptide C-terminal carboxylate and the TPR1 and TPR2A domains conserved residues, Lys 8 (Lys 229), Asn 12 (Asn 233) and Asn 43 (Asn 264) respectively, allows the formation of a two carboxylate clamp. This clamp ensures the proper docking of the peptide ends to the TPR domains. Additional peptide residues located at the N-terminal, relative to EEVD motif, are exclusively engaged in hydrophobic and van der Waal's interactions. These contacts were found to be critical for peptide binding with a physiologically relevant high affinity. Other TPR domains which are known to bind Hsp70/Hsp90 proteins also contains identical residues which form the carboxylate clamp, suggesting that these TPR domains bind to the C-terminal carboxylate via a similar network of electrostatic interactions. The Hop and Hsp70/Hsp90 case studies presented here also demonstrate the importance of sequence conservation between TPR domains and their ligands involved in similar cellular functions.

Other example for the importance of TPR-ligand sequence conservation can be found in the peroxin 5 (PEX5) protein from the protozoan parasite, *Trypanasomas brucei*, the agent of human African trypanosomiasis (sleeping sickness). PEX5 is a cytosolic receptor which promotes cargo translocation across the glycosomic membrane, with the glycosome being a peroxisome-like organelle which hosts the metabolic reactions of the parasite. Two domains comprise PEX5, with the C-terminal domain consisting of 7 TPR motifs. The PEX5 C-terminal domain binds either the type 1 (PTS1) or type 2 (PTS2) peroxisomal targeting signal. The more common PTS1 sequence is a C-terminal tripeptide with the SKL amino acid sequence, or variants thereof, such as AKL or SHL. Additional residues upstream to the SKL sequence have also been implicated in binding to PEX5. The crystal structure of the C-terminal domain of PEX5 with five different PST1 fragments revealed that the protein does not fold as a sequential TPR protein with classic super-helical fold, but rather as two distinct sub-domains (Sampathkumar et al. 2008). The N-terminal sub-domain comprises TPR motifs 1-3 while the C-terminal sub-domain comprises TPR motifs 5-7. The fourth TPR motif that serves to interconnect the two sub-domains is only partial ordered and cannot be seen in the electron density resulted from the X-ray determination (Fig. 3A). The disordered nature of the fourth TPR motif implies its flexible tendencies and involvement in ligand-induced conformational

changes that can promote cargo translocation. The PTS1 peptide is bound within the cavity found between the two sub-domains and interacts with residues from both, although the major binding contribution is attributed to the C-terminal sub-domain. The five PEX5 structures bound to five different peptides containing PTS1 sequences indicate that the ligand recognition mechanism involves three critical factors. The first is recognition of the C-terminal
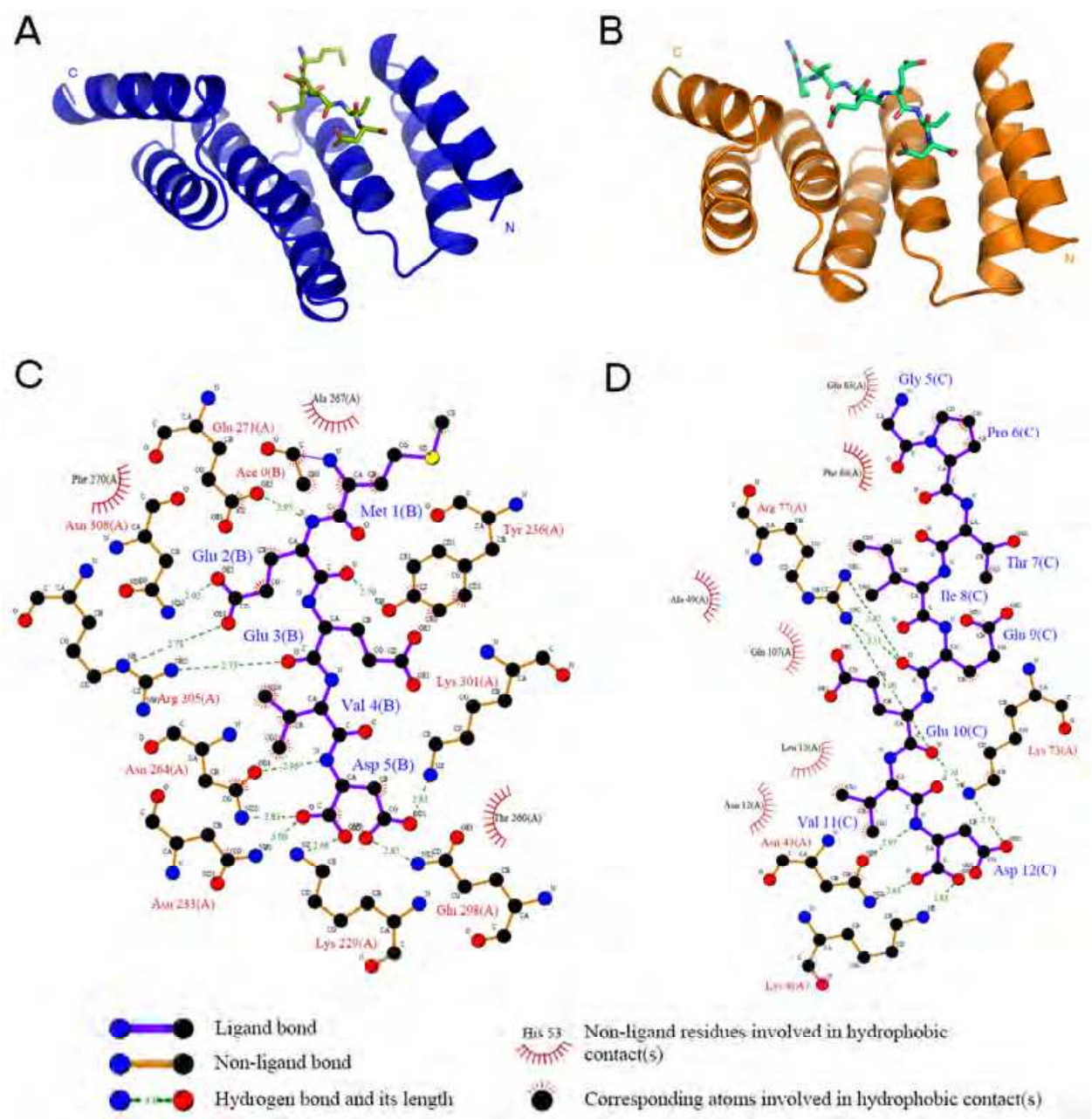

Fig. 2. TPR domains of the Hop protein and their interacting Hsp70/Hsp90 partner peptides. (A) The TPR2A domain of the Hop protein in complex with a Hsp90-derived peptide. (B) The TPR1 domain of the Hop protein in complex with a Hsp70-derived peptide. (C) Schematic representation of the TPR2A-hsp90 peptide interaction. (D) Schematic representation of the TPR1-Hsp70 peptide interaction. Images A and B were generated using Pymol software (www.pymol.org). Images C and D were generated using LigPlot software (Wallace et al. 1995).
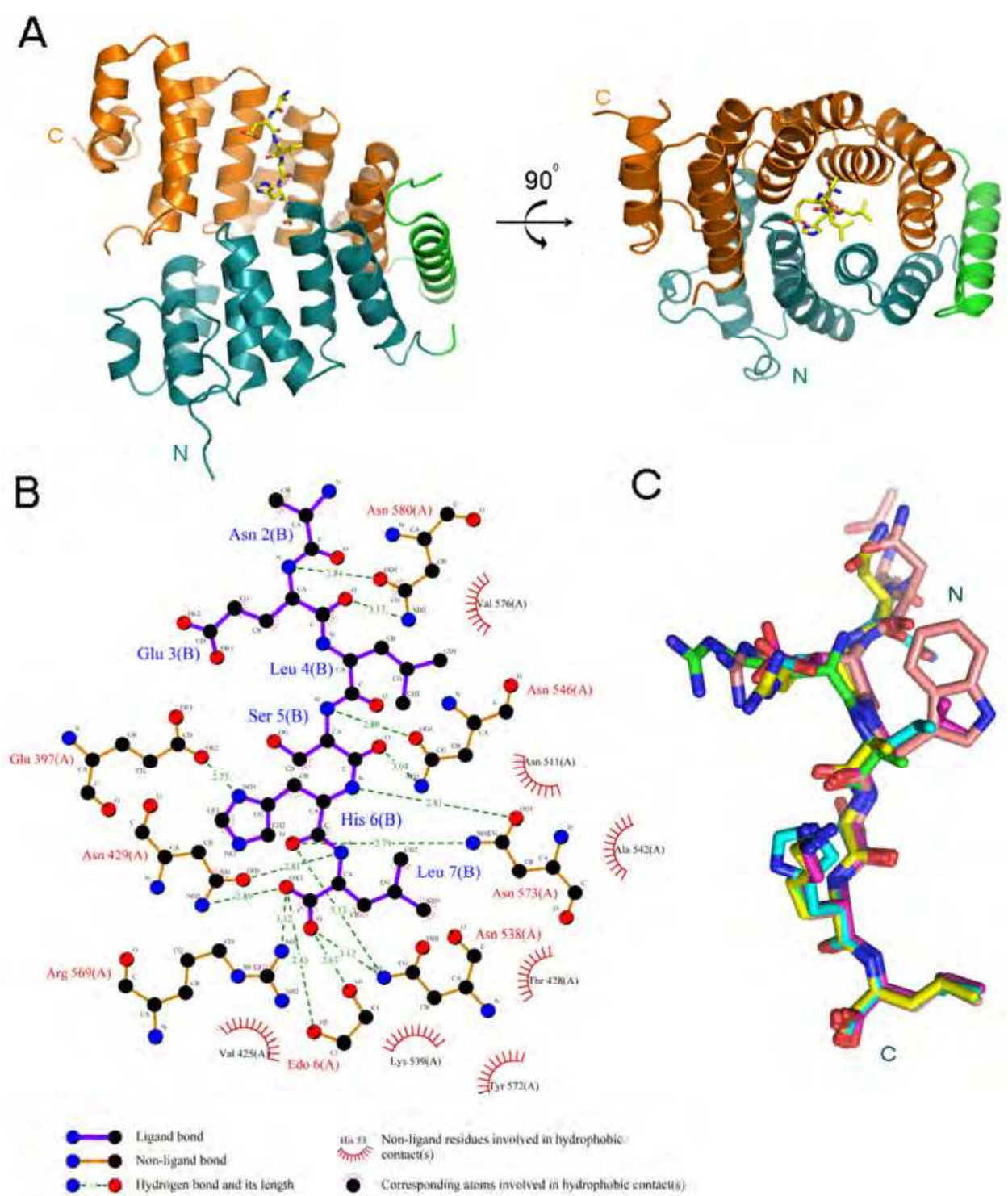
Fig. 3. TPR domains of PEX5 protein and their interacting PST1 partner peptides.
(A) TPR domains of PEX5 in complex with a PST1 peptide (NFNELSHLC). PEX5 is
represented as a cartoon, whereas the N-terminal domain, the fourth TPR motif and
C- terminal domain are colored in blue, green and orange, respectively. The PST1 peptide is
represented as sticks in yellow. (B) Schematic representation of the PEX5-PST1 peptide
interactions. (C) Superposition of five PST1 peptides bound to PEX5, in green, blue, pink,
yellow and light pink. Images A and C were generated using Pymol software
(www.pymol.org). Image B was generated using LigPlot software (Wallace et al. 1995).

PST1 carboxylate in a similar manner to the Hop and Hsp70/Hsp90 complex by two PEX5 Asn residues and a single Arg residue, the second is hydrophobic embedding of the PTS1 C-terminal residue side chain and the third is multiple PTS1 backbone interactions with PEX5 Asn side chains (Fig. 3B). Overall, PTS1 peptides are bound in a similar manner despite substantial differences in amino acid composition. In addition, the spatial positions of the five Asn residues and a single Arg residue of PEX5 involved in backbone binding of PTS1 peptides are similar, emphasizing their significant function in diverse ligand binding (Fig. 3C).

3.2 Ligand secondary structure and length diversity

Secondary structure of a TPR-bound ligand varies between the coiled extended conformation to an α-helix or both. As mentioned previously, an extended conformation maximizes the ligand surface to the TPR domain and facilitates specific recognition of short amino acid stretches with sufficient affinity. Two examples of extended conformation peptide binding are Hop bound to Hsp70/Hsp90 peptides (Fig. 4A) and PEX5 bound to PTS1 peptides. Three TPR-containing proteins bound to their ligands display binding of long peptides that adopt both helical and extended conformations. The first structure of such binding is PscG-PscE in complex with the PscF peptide (Quinaud et al. 2007). PscG-PscE–PscF proteins are members of the bacterial Type III secretion system and play a specific role in formation of the needle that transports virulence effectors into target cell cytoplasm. PscG displays a three TPR motif fold with an additional C-terminal helix, even though its TPR fold could not have been predicted from its sequence. PscG also interacts with PscE through a hydrophobic platform formed by the N-terminal TPR motif of PscG. PscF is composed of two sub-domains, an extended coil (13 amino acids-long) and a C-terminal helix (17 amino acids-long). PscG and PscE fold into a cupped-hand form, whereas the amphipathic C-terminal helix of PscF is bound to the concave surface of PscG. The N-terminal of PscF is bound to the PscG convex surface (Fig. 4B).

The second structure displaying binding of a long peptide which adopts both helical and extended conformations is the TPR-containing protein, APC6, in complex with CDC26 (Wang et al. 2009). APC6 and CDC26 are both members of the multi-subunit anaphase-promoting complex (APC), an essential cell-cycle regulator. The crystallized APC6 TPR domain contains eight full TPR motifs and an additional C-terminal helix. APC6 adopts a solenoid-like structure, wrapping around the entire length of N-terminal region of CDC26 (26 amino acids). The bound CDC26 forms a rod-like structure with the first 12 amino acids adopting an extended conformation and the last 14 amino acids forming a helix (Fig. 4C). Interestingly, as the CDC26 peptide interacts with APC6, an additional non-TPR C-terminal helix with geometry that mimics two helices in a TPR motif appears. This inter-molecular TPR mimic continues the sinuous form of the overall structure and packs against the eight TPR motifs of APC6 to form a four-helix bundle.

A third structure displaying binding of two helices connected by a loop to a TPR-containing protein is Fis1 in complex with Caf4 (Zhang et al. 2007). Both Fis1 and Caf4 participate in the formation of yeast mitochondrial fission complex that controls the shape and physiology of the mitochondria. The Fis1 protein core is composed of two TPR motifs with two additional helices at each motif end and another N-terminal helix arm packed against the hydrophobic groove formed by the protein core. The Caf4 peptide adopts a U-fold with two helices formed at each end connected by a loop. The unique U-fold of Caf4 allows large scale interactions between the peptide and both the Fis1 core at the concave and convex surfaces (Fig. 4D).

Although these are only a few examples of TPR binding modes reflecting secondary structure conformations and peptides lengths, they describe well the diverse nature of ligand types that theTPR platform can bind.
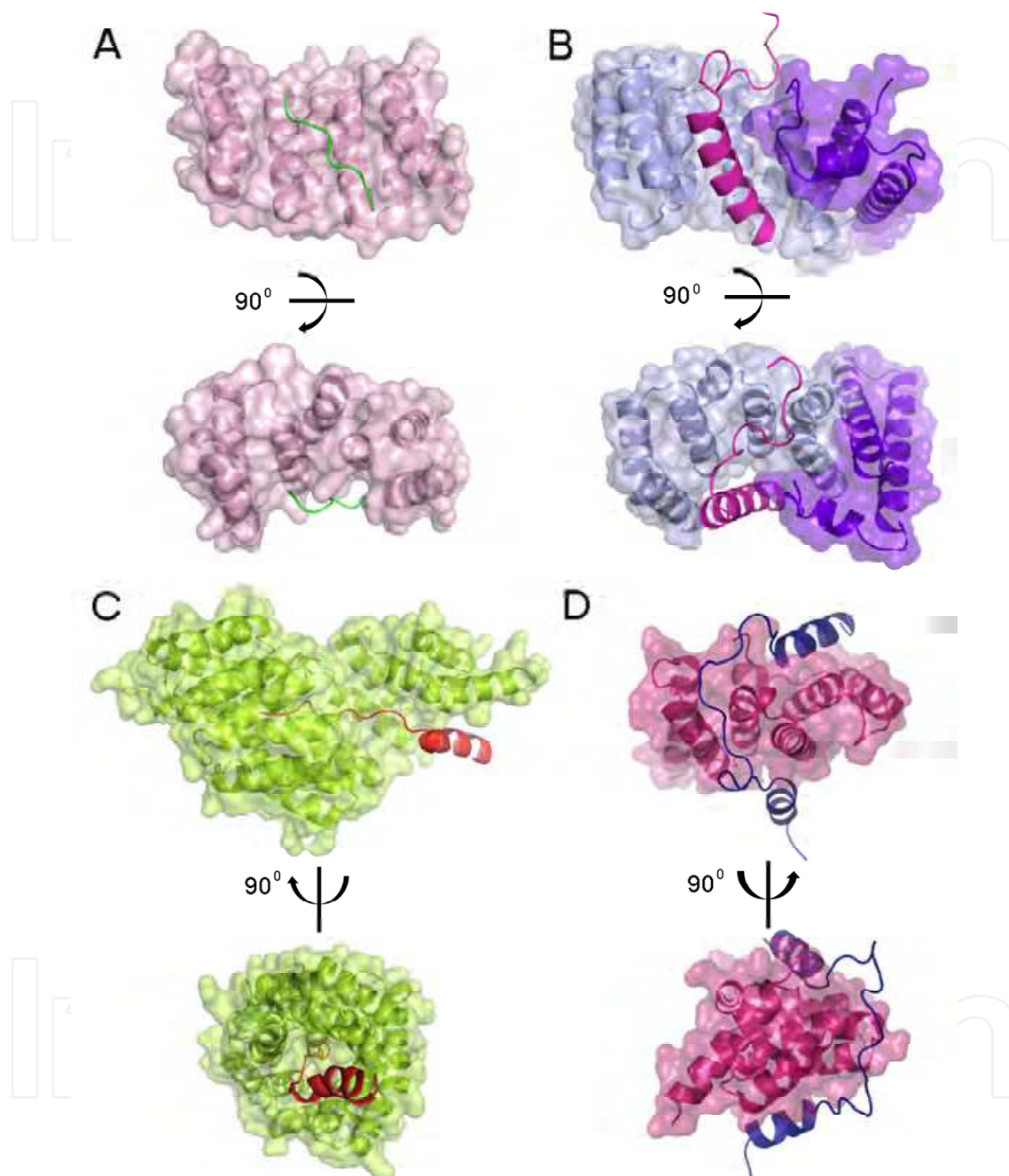

Fig. 4. TPR ligand secondary structure and length diversity. (A) TPR2A domain of Hop is shown in surface representation with secondary structure indicated in light pink, bound to Hsp90, in green. (B) A surface representation of a PscG-PscE dimer shown with secondary structure indicated in light blue and purple, respectively. PscF, in pink, is bound to the PscG-PscE dimer. (C) APC6 is shown in surface representation with secondary structure indicated in light green, bound to CDC26, in red. (D) Caf4 is shown in surface representation with secondary structure indicated in pink, bound to Fis1, in dark blue. Images were generated using Pymol software (www.pymol.org).

3.3 Curvature angle role and diversity

Major diversity can be seen in the overall shape and curvature angle displayed by TPR-containing proteins. There are many factors that determine the overall shape and curvatures angle of a protein. Among these are the number of repeating motifs, their arrangement as sequential motifs or separation into sub-domains, the presence of helix-breaking residues within turns between motifs, as well as residues type, protein function and others. Despite the apparent significance, no extensive study addressing the overall shape and curvature angle displayed by TPR-containing proteins has been reported. Indeed, articles presenting new TPR protein structures hardly refer to this property. The majority of TPR protein structures were determined by X-ray crystallography, a method that favors ordered proteins and can only tolerate a low extant of protein flexibly within the crystal. Since proteins and protein interactions are usually flexible and require the partners to be rigid, the use of X-ray crystallography to study protein interactions can be quite challenging. To overcome this challenge, structural biologists often use a relatively short peptide from the whole binding partner that participates directly in recognition and binding in their experiments. By using a small portion of the binding partner, the overall flexibility is reduced throughout the crystal, a trait that later improves crystallization and crystal quality. Due to method limitations, we usually obtain a close look at the detailed interaction and the forces participating in such interactions but cannot see the complete interaction or the spatial positions of the partners, nor detect remote stabilizing interactions. This incomplete observation of interactions between partners creates substantial difficulty in determining the exact role and importance of the curvature angle. Perhaps in the future, when new structure determination techniques emerge, a better understanding of the role of the curvature angle in TPR protein recognition and binding will be achieved.

4. TPR homo-oligomerization

TPR-containing proteins serve as mediators in the formation of multi-protein complexes. Within these complexes, TPR proteins or domains can be found as homo-oligomers, with oligomerization serving as a crucial factor for realizing proper protein function in the cell. TPR proteins displays a broad range of oligomerization states, such as monomers (Scheufler et al. 2000; Sampathkumar et al. 2008) and dimers (Lunelli et al. 2009; Z. Zhang et al. 2010). It is of note that a complex containing 24-26 TPR protein monomers has been described (Zeytuni et al. 2011). The protein surfaces involved in oligomer formation are diverse, as are the inter-molecule interaction types seen between monomers. In the following section, three interesting protein oligomerization forms will be presented.

The first TPR-containing protein displaying a dimer formation to be considered is invasion plasmid gene C (IpgC), a chaperone that binds two essential virulence factors of the pathogenic bacteria, *Shigella* (Lunelli et al. 2009). IpgC binds the invasion plasmid antigens, IpaB and IpaC which are responsible for epithelial cell invasion, membrane lysis of the phagocytic vacuole, contact hemolysis and macrophage cell death. The IpgC chaperone contains three TPR motifs, with two additional helices at the protein N-terminal and another helix at the protein C-terminal. The protein functional unit that allows efficient substrate binding is a dimer, with dimerization occuring in an asymmetric manner. Such asymmetric dimerization presents an interesting binding mode in which the first helix of one of the monomers binds on the convex surface presented by the other monomer (Fig. 5A). While the
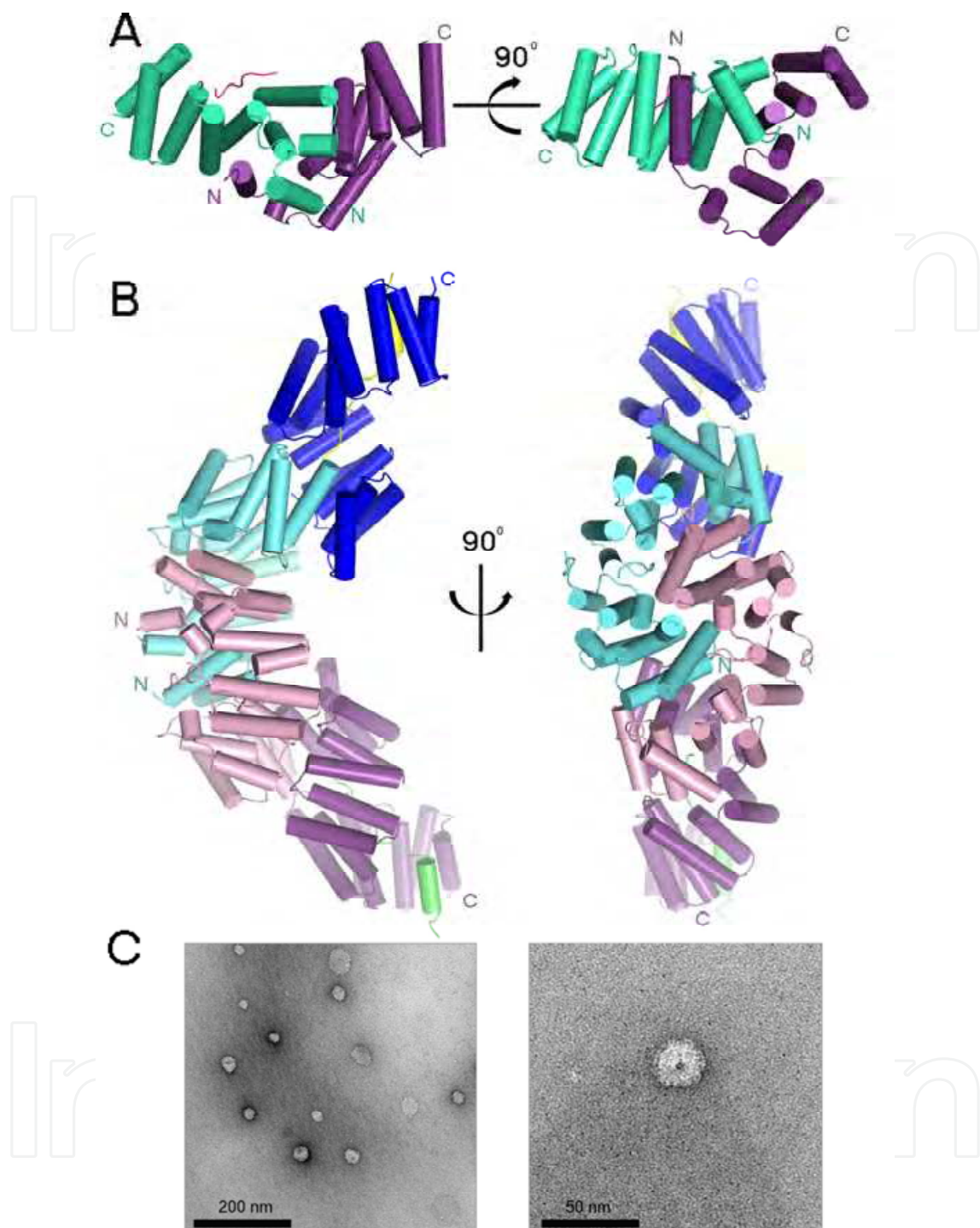
Fig. 5. Homo-oligomerization by -ontaining proteins. (A) Cylinder representation of a IpgC dimer, in green and purple, where one monomer is bound to an IpgB peptide, in pink. (B) The N-terminal domain of Cut9 promotes dimerization. Cylinder representation of the N-terminal domain of each monomer, shown in cyan and light pink. C-terminal domains are in blue and light purple. Hcn1 peptides are colored in green and yellow. (C) Transmission electron microscopy images of negatively stained MamA protein complexes. Images A, B were generated using Pymol software (www.pymol.org). The images shown 4C were taken by Zeytuni N. as described in Zeytuni et al. (2011).

first helix of one of the monomers displays inter-molecule binding, the first helix of the bound monomer appears to be found in a more packed, unbound conformation. The formation of a dimer interface at the TPR concave surface allows the binding of the IpaB peptide at the concave surface in an extended conformation. Moreover, the mode of IpgC dimerization demonstrates the significance of a convex surface as an additional protein interaction platform.

A second TPR-containing protein displaying dimer formation is *Schizosaccharomyces pombe* Cut9, the yeast homolog of human APC6. The Cut9 structure was determined while bound to Hcn1, the yeast homolog of human CDC26 in a similar binding conformation as APC6 in complex with CDC26 (Z. Zhang et al. 2010). Cut9 is composed of 14 TPR motifs forming a contiguous super-helix divided into two functionally and structurally distinct domains, namely an N-terminal domain comprising the first seven TPR motifs and a C-terminal domain comprising the last seven TPR motifs. The Cut9 subunits homo-dimerize through their N-terminal domains to generate a shallow 'V'-shaped molecule (Fig. 5B). The more globular N-terminal dimerization module forms the apex of the 'V'-shape, with narrower C-terminal TPR domains projecting away from the dimer interface. The majority of Cut9 interactions with Hcn1 involve the C-terminal TPR-containing domain. Dimerization of the N-terminal domains forms a tight interface in which the concave surface of each N-terminal domain encircles its dimer counterpart in an inter-lock clasp-like arrangement. In these interactions, the first two TPR motifs of a single monomer interact with residues lining the inner groove formed by the seven TPR motifs of the opposite Cut9 N-terminal domain.

An additional example involving dimerization is the TPR-containing protein, MamA, from *Magnetospirillium* magnetotactic bacteria species. MamA forms large homo-oligomeric complex of 24-26 monomers. This complex is presumed to serve as a wide platform for protein interaction during the iron-oxide bio-mineralization by the magnetotactic bacteria (Zeytuni et al. 2011). MamA contains five TPR motifs with an additional N-terminal putative TPR motif that was found to be responsible for oligomerization and complex formation. Through binding of the first helix of a single monomer to a binding surface displayed on a different monomer, a round-shaped complex of 14-20 nm in diameter with a central pore cavity is formed (Fig. 5C). The structural details of the monomer-monomer interaction remain unclear, since crystallization trials of MamA in complex with peptides of the first and/or second helices proved unsuccessful.

Overall, these three examples of homo-oligomrization by TPR-containing proteins describe additional TPR diversity and further establish the TPR motif as a broad platform for protein interactions.

5. Predicting TPR-ligand interactions

Today, available bioinformatics tools and the well-defined TPR profile allow us to identify TPR-containing proteins with great accuracy through analysis of amino acid sequence. However, these tools still cannot predict TPR-interacting partners and/or the region of interaction. As discussed in section 3.3, the majority of TPR-containing protein structures were determined by X-ray crystallography, a methodology in which the crystallization probability of protein complexes is significantly lower than the probability of crystallization of a single protein. Therefore, the majority of determined TPR proteins were crystallized in a

non-complex form, not bound to their interacting partner, even if that partner was previously identified. In order to predict ligand binding, one should discriminate between two cases, the first involving an unidentified partner protein and the second involving an unknown binding region. In this section, we discuss these two cases and provide several examples of each.

5.1 Interacting protein prediction

A genomic approach for TPR-interacting protein identification was first presented by D'Andrea and Regan (2003). In their study, the authors generated a list of all TPR-containing proteins predicted from the *Saccharomyces cerevisiae* genome. Later, they used the generated list of 22 predicted TPR-containing proteins in protein-protein interaction databases to identify potential binding partners. Their search revealed about 80 potential interacting proteins, some of which are known to participate in multi-protein complex formation. Nevertheless, these authors could not exclude the possibility that these interacting proteins might not all interact directly with TPR domains within the multi-protein complexes.

Another approach uses information derived from the structures of unbound TPR-containing proteins. Certain properties of the binding partner can be deduced from a simple examination of a TPR protein structure, especially from its concave binding pocket, specifically its dimensions, residue composition and electrostatic potential. Although the concave surface serves as the common binding area, the convex surface can also participate in binding and should not be excluded from predictions.

Our first example is derived from the structure of the super-helical TPR domain of O-linked *N*-acetylglucosamine transferase (OGT). The TPR domain of OGT contains 11 motifs with an additional C-terminal helix and forms a homo-dimer through interactions at the convex surface. The inner surface of the elongated super-helix is highly conserved and contains an asparagine ladder. This asparagine ladder bears marked similarity to the array of conserved asparagines in the ARM-repeat importin α- and β-catenin proteins. In both ARM-repeat proteins, the asparagine side chains contribute to binding of the target peptide by forming hydrogen bonds with the peptide backbone. This structural similarity suggests that a similar binding mechanism for the OGT protein. In addition, the extensive surface generated by OGT is likely to represent several overlapping binding pockets which can accommodate multiple substrates (Fig. 6A). Furthermore, partner binding can rely on a mechanism similar to the mode of binding described earlier, in the case of CDC26 bound to APC6 (see Fig. 4C) (Wang et al. 2009).

Another example of TPR partner prediction is derived from the bacterial YrrB protein structure. YrrB is a *Bacillus subtilis* protein containing five TPR motifs with an additional C-terminal helix. The YrrB structure reveals a unique, highly negatively charged deep concave surface containing an aspartic acid array that can accommodate the binding of positively charged residues (Fig 6B). In order to discover new details on the role of the YrrB protein, functional gene cluster localization analysis was performed. Such analysis suggested that YrrB plays a role in mediating complex formation among RNA sulfuration components, RNA processing components and aminoacyl-tRNA synthetases. An opposite charge distribution to the YrrB protein was found in the TPR-containing protein, MamA, a protein that displays a highly positive concave binding surface, implying the electrostatic charge

nature of ligand bindng (Fig. 6C). Although sharing a similar fold, MamA and YrrB demonstrate yet another variation among TPR-containing proteins, namely charge distribution. In general, binding partner identification is not straightforward and can be challenging, as the information obtained from the genetic approach can point to indirect interactions. Indeed, the structural approach can only provide clues for identifying the specific region of the partner involved in binding.
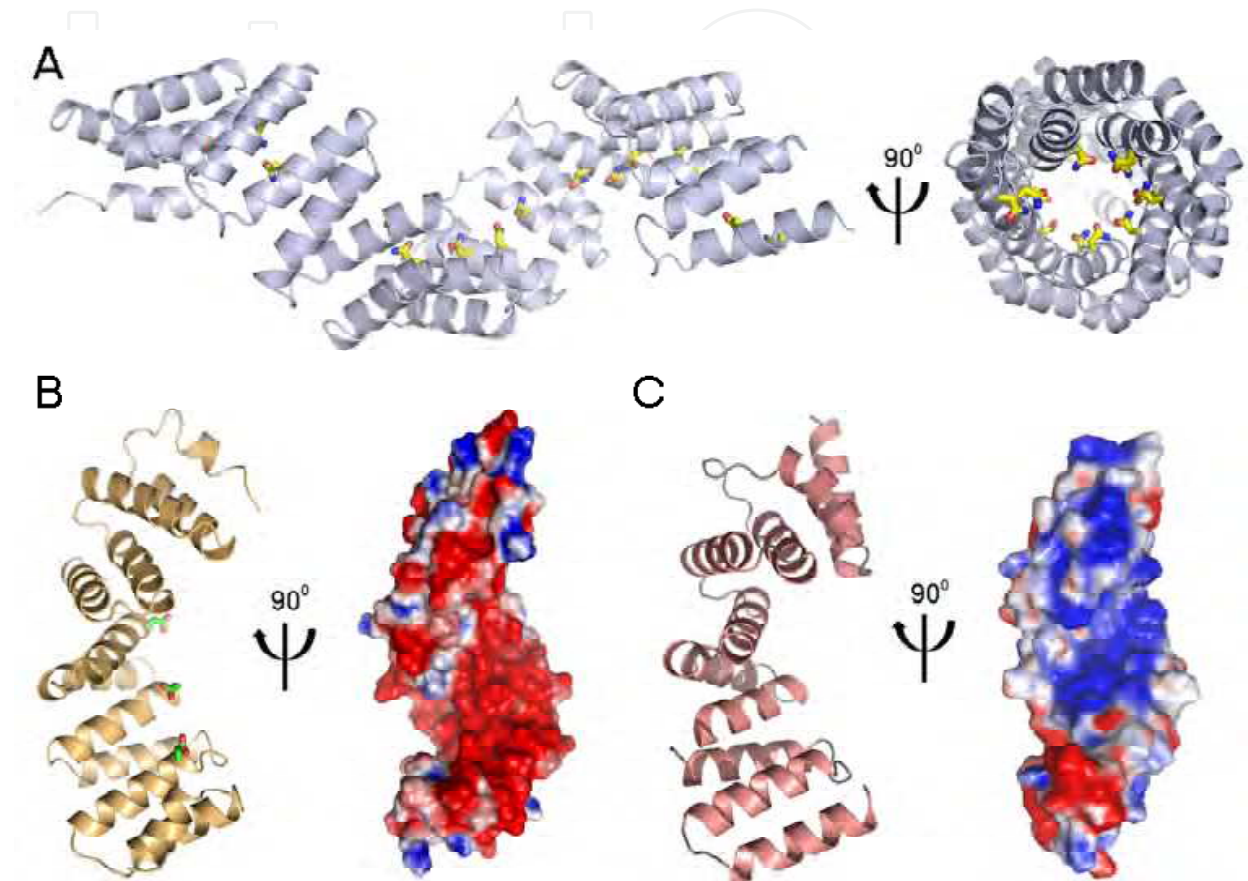

Fig. 6. Structural analysis of concave binding surface. (A) The TPR domain of O-linked *N*-acetylglucosamine transferase is presented as a grey cartoon, while the asparagine ladder within the inner surface of the super-helix is presented as yellow sticks. (B) *left* - YrrB in light orange cartoon. The aspartic acid ladder is presented as green sticks. *right* –Electrostatic surface potential representation, where blue and red represent positive and negative electrostatic potentials, respectively. The YrrB concave surface displays a highly negative potential distribution. (C) *Left* - MamA in light pink cartoon representation. *Right* – Electrostatic surface potential representation, where blue and red represent positive and negative potentials, respectively. The MamA concave surface displays a highly positive potential distribution. Images were generated using Pymol software (www.pymol.org). Surface electrostatic potential calculations were performed using the APBS plug-in (www.poissonboltzmann.org/apbs).

5.2 Interaction region prediction

Interaction region prediction requires at least two components, the TPR protein structure and the identified binding sequence of the partner. Over the past few years, several

attempts to dock ligand peptides onto TPR-containing proteins have been made. These attempts mainly included manual docking of the ligand peptide onto the available TPR protein structure. The resulting models did not, however, consider side chain flexibility and conformational changes due to peptide binding and used limited energy minimization tools (Gatto et al. 2000; Kim et al. 2006). However, with the remarkable development of computational docking servers, more accurate models can be generated for TPR protein–peptide interactions. To date, however, no study has employed these advanced servers to demonstrate TPR-peptide docking, although successful docking have been recorded with other proteins involved in protein-protein interactions, such as PDZ domains and others (London et al. 2010; Raveh et al. 2010; Raveh et al. 2011). Overall, the prediction of interaction region is considered to be more accurate than binding partner prediction, since the former uses algorithms that consider chemical restrains, as well as energy minimization of the final model. In the near future, these tools might aid in overcoming challenges associated with crystallizing proteins with their binding partners and could provide important insight for molecular understanding of binding and recognition.

6. TPR design and biotechnology

The basic TPR fold resulting from its consensus sequence can be considered as a protein scaffold. Redesigning this stable basic scaffold by grafting functional residues involved in binding recognition and specificity enables the introduction of novel binding activities. TPR design includes three major steps. The first stage includes stable consensus scaffold generation by an alignment of natural TPR motifs (D'Andrea et al. 2003). Later, the minimal number of repeating motifs required for thermodynamic stability are determined (Kajander et al. 2007) and finally, functional residues are grafted onto the generated scaffold. An example for a successful designing process was the designed TPR module that binds the C-terminal peptide of Hsp90. This module has been designed by grafting Hsp90-binding residues from natural TPR proteins onto a consensus TPR scaffold to bind Hsp90 with greater affinity and specificity than natural co-chaperones. Introduction of this designed protein into breast cancer cells inhibited Hsp90 activity, presumably by out-competing the interaction of Hsp90 with its natural co-chaperones. Hsp90 inhibition resulted in misfolding and degradation of Hsp90-dependent proteins, such as HER2, and led to cancer cell death (Cortajarena et al. 2008).

TPR binding properties can also be used for specific identification of tagged proteins and can act as a functional substitute for antibodies in a wide range of applications (Jackrel et al. 2009). Conjugation of a peptide ligand sequence to the N or C termini of a desirable protein allows its identification by a TPR-containing protein that can bind the ligand peptide with sufficient affinity. This TPR-containing protein can be conjugated to a reporter protein, such as horseradish peroxidase, biotin or green fluorescent protein and can be later used in one or two-step western blot detection systems, replacing any requirement for antibodies. In addition, conjugation of a TPR-containing protein or domain directly to resin or beads can be used for affinity purification. Immobilizing a TPR-containing protein onto a resin permits the specific binding and enrichment of desirable proteins conjugated to a peptide ligand. Interaction dissociation at the elution step does not require extremely harsh pH conditions as may be needed with the use of antibodies.

7. Concluding remarks

The protein-protein interaction platform generated by TPR motifs can support the binding of diverse ligands. The elegant super-helical fold of TPR-containing proteins presents several binding surfaces that can promote the formation of multi-protein complexes. These binding surfaces can bind chemically distinct peptides in a variety of conformations with sufficient affinity. Therefore, it is not surprising that TPR-containing proteins are widespread across all kingdoms of life, where they participate in diverse cellular processes. From a molecular point of view, the diverse nature of interactions presented by TPR protein structures demonstrates multiple chemical forces involved in ligand binding and can promote the design of novel protein-protein interactions.

Overall, TPR-containing proteins hold great promise for protein engineering, therapeutics and biotechnology, as the basic TPR scaffold can be redesigned to modulate binding specificity and/or affinity towards desirable peptide ligands. As such, TPR-containing proteins can be inhibited by a designed ligand with higher affinity, serve as scaffolds to present proteins in nano-technological applications and more.

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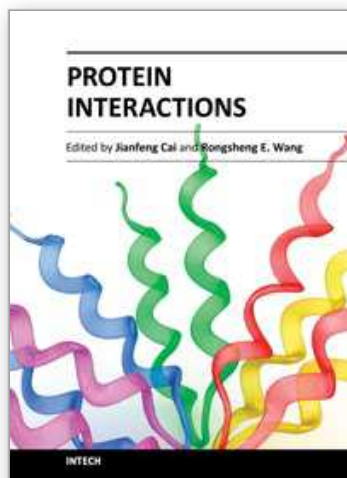
Protein Interactions

Edited by Dr. Jianfeng Cai

Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

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