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Knowledge-based modeling of peptides at protein interfaces: PiPreD

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Abstract

Motivation: Protein–protein interactions (PPIs) underpin virtually all cellular processes both in health and disease. Modulating the interaction between proteins by means of small (chemical) agents is therefore a promising route for future novel therapeutic interventions. In this context, peptides are gaining momentum as emerging agents for the modulation of PPIs.

Results: We reported a novel computational, structure and knowledge-based approach to model orthosteric peptides to target PPIs: *PiPreD*. PiPreD relies on a precompiled and bespoken library of structural motifs, *iMotifs,* extracted from protein complexes and a fast structural modeling algorithm driven by the location of native chemical groups on the interface of the protein target named anchor residues. PiPreD comprehensive and systematically samples the entire interface deriving peptide conformations best suited for the given region on the protein interface. PiPreD complements the existing technologies and provides new solutions for the disruption of selected interactions.

Availability and implementation: Database and accessory scripts and programs are available upon request to the authors or at http://www.bioinsilico.org/PIPRED.

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1 Introduction

Protein–protein interactions (PPIs) are essential in the shaping of normal and pathological behaviors in cells. A new frontier in drug discovery is, therefore, the development of new therapeutic agents to modulate PPIs (Fletcher and Hamilton, 2006). Although originally considered undruggable, PPIs are arising as promising therapeutic targets as reviewed in Wells and McClendon (2007) and they are attracting considerable attention from biotechnological companies as the '*unmined gold reserve*' in drug discovery (Mullard, 2012). One part of the problem is that proteins usually interact through large and flat surfaces that lack defining physicochemical traits that can be exploited to attain the required affinities. It is becoming clearer that chemistry originating from traditional drug discovery programs is perhaps not the most suited for targeting PPIs (Rubinstein and Niv, 2009), and thus new chemotypes should be sought. It is in this context, and thanks to biotechnology advances that have improved its pharmacokinetic properties (McGregor, 2008), that peptides are gaining momentum as emerging agents for the modulation of PPIs (Gaestel and Kracht, 2009; Marchioni and Zheng, 2009; Rubinstein and Niv, 2009). Besides, peptide-mediated interactions are common in Biology, accounting for up to 40% of the interactions that take place inside the cell (Petsalaki and Russell, 2008). They are also central to number of essential cellular processes such as signal transduction, protein assembly, protein localization and regulation (Neduva and Russell, 2006) and thus are the more natural candidates for the inhibition of PPIs (Mullard, 2012). Moreover, peptides do not preclude traditional chemistry as they can be used as precursors to derive chemical compounds, i.e. peptidomimetics (e.g. p53-MDM2 (Vassilev *et al.*, 2004) and Bak BH3/Bcl-xL (Ernst *et al.*, 2003).)

Computational tools can be used to model the structure of peptides targeting protein interfaces. Such approaches include peptide

growing (Moon and Howe, 1991), linking (Frenkel *et al.*, 1995), property-guided searches (Teixido *et al.*, 2003), optimization (Roberts *et al.*, 2012) and docking (Donsky and Wolfson, 2011; Raveh *et al.*, 2010). While useful, these technologies present a number of limitations: (i) the search is usually restricted to a small region of the interface given the time that will be required to fully explore the entire interface; (ii) the structure of peptides tend to be biased toward linear and extended conformations as its maximized the contacts with the target surface, however, peptides can adopt different conformations including α-helix (Kritzer *et al.*, 2004); (iii) do not explicitly exploit the natural repertoire of peptide sequences, which has been proved to increase the chance to identify bioactive peptides (Watt, 2006); (iv) some methods require the sequence of the peptides, and thus neglecting the discovery of novel sequences; and (v) other methods are based on the optimization of main-chain stretches isolated from its cognate partner(s), and thus cannot be used to explore novel conformations.

Here, we present PiPreD, a novel, structure- and knowledge-based approach to model the conformation of peptides targeting protein interfaces. PiPreD relies on a bespoken library of structural motifs derived from interfaces named iMotifs and it uses native structural elements of the interface being targeted (target-interface), so-called disembodied anchor residues. The search and sampling of peptides covers the entire interface, i.e. a systematic and comprehensive exploration without biases caused by specific location(s) of the target interface or by the conformation of peptides. PiPreD is therefore a good complement to existing methodologies.

2 Methods

2.1 iMotifsDB: a collection of structural motifs derived from protein interfaces

PiPreD is a knowledge-based approach that relies on a library of structural fragments, *iMotifs*, extracted from either protein–protein or protein–peptide complexes. The current library was derived from an initial set of 45 434 complexes downloaded from the protein databank (PDB) (Berman *et al.*, 2003) and interface residues were defined using CSU (Sobolev *et al.*, 1999). Fragments containing at least two nonsequentially continuous interface residues were qualified as iMotifs and used to populate iMotifsDB. Subsequently, iMotifs were clustered to remove structural redundancies following a similar approach as in Lessel and Schomburg (1997) resulting in a final set of 171 306 iMotifs. iMotifsDB is available upon request to the corresponding author both as plain text or MySQL dump formats or downloadable from http://www.bioinsilico.org/PIPRED.

2.2 Structural modeling algorithm

PiPRED encompasses four consecutive steps described below (Fig. 1).

2.2.1 Defining anchor residues and structural diversification or main chain rotamers

Disembodied interface residues or anchor residues are used to drive the modeling of peptides. The anchor residues defined as those mediating the interaction with the protein of interest, also known as contact residues, are selected using CSU (Sobolev *et al.*, 1999). The conformation of main chain atoms is then diversified by deriving inverse rotamers, i.e. a conformational sample of main chain rotamers with respect to a fixed position of the native contacts of the side chain atoms. This step yields alternative conformations maximizing (and subsequently optimizing) the number of modeled peptides.

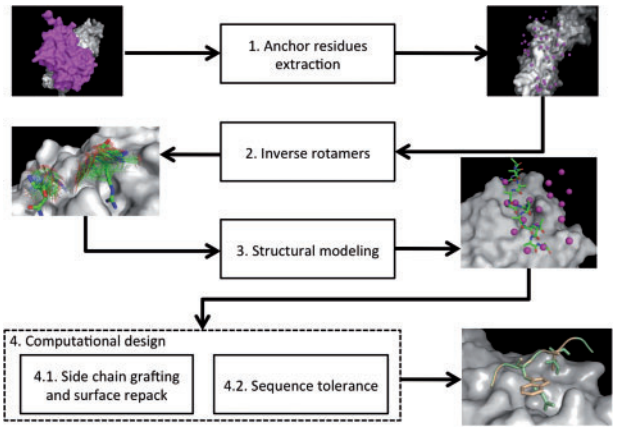

Fig. 1. Overview of the modeling process implemented in PiPreD. The protein structures shown in Figures 1–7 were generated using PyMOL (http://pymol.sourceforge.net)

2.2.2 Structural modeling of peptides using iMotifs

The structural modeling is done using an adaption of our early approach to model loops in protein structures (Fernandez-Fuentes *et al.*, 2006). Drawing analogies between both approaches, anchor residues are the equivalent to stem residues in loop modeling while iMotifs are the equivalent to loop fragments extracted form known protein structures. In the first step of the structural modeling, iMotifs are selected if the difference of the Euclidian distance between start–end Cα-atoms in both anchor residues and iMotifs is smaller than 0.5 Ang (i.e. analogue to distance between stem residues and the first and last residues of the loop fragment in loop modeling). Selected iMotifs are then superposed onto the anchor residues using an iterative structural superposition maximizing the number of structurally aligned residues between anchor and iMotifs. The iteration is performed as follow: (i) it starts by aligning the main chain and Cβ atoms (to preserve orientation of side chains) of anchors and start–end residues; the superposition is discarded if above 1.0 Ang root mean square deviation (RMSD); (ii) the nearest anchor residue is selected if it lies within a Cα–Cα distance of 2.0 Ang to any residue in the iMotif and subsequently added to the set of anchor residues in use. The fitting of iMotif-selected anchor residues is repeated and accepted if the resulting RMSD is smaller than previous RMSD ± 10%; (iii) step 2 is repeated or otherwise stops when there are no more new anchor residues to be included or the resulting RMSD is above the threshold. The native main chain conformation of the anchor residues and the derived inverse rotamers are used to compute the structural superposition. All possible combinations of nonstructurally equivalent pairs of anchor residues are used to direct the modeling.

2.2.3 Computational design of peptides

The last step in PiPreD is implemented within the framework of the Rosetta macromolecular modeling software (Leaver-Fay *et al.*, 2011). Two different protocols have been implemented: the first approach follows the protocol described by Sood and Baker (2006), in which the main chain of peptides is fixed (i.e. main chain conformations are explored at the level of iMotifs) whilst side chains are allowed to rotate and surface residues in the target protein have limited conformational flexibility (i.e. repacking). All 20 possible amino acids are allowed at any position of the peptide with the exception of those defined by the anchor residues where only rotameric changes are permitted. The second approach is based on the work

by Smith and Kortemme (2010), in which structural ensembles are generated using peptide poses by applying backrub motions to derive sequence profiles. The latter is more suited to selected poses for the purpose of generating libraries of sequences for high-throughput screening. The peptides shown in this article as examples were derived using Sood and Baker's (2006) approach.

3.4.2 Scoring of peptides

In the current implementation of PiPreD, the scoring of peptides–interface interaction is ranked using Rosetta score (Leaver-Fay *et al.*, 2011). Rosetta scoring function is a combination of physic-based and statistical-based potentials including several terms that account for a Lennard–Jones potential, a solvation term, orientation-dependent hydrogen bond, backbone and side chain torsion potentials, a short range electrostatic term, and an unfolded reference state (Leaver-Fay *et al.*, 2013). Nonetheless, any structure-based scoring function of choice could be equally used.

3 Results and discussion

3.1 PiPreD comprehensively charts the entire interface during structural modeling

Protein interactions are mediated by large interaction surfaces (Lo Conte *et al.*, 1999). Therefore, targeting a protein interface using peptides presents a combinatorial problem and thus the search is usually restricted to a small region of the interface. However, in PiPreD the selection of anchor residues drive the structural modeling implemented, hence the search and sampling of peptides covers the entire interface, i.e. it is a systematic and comprehensive exploration without any bias both in terms of the size of the specific region of the interface and in the conformation of the interfering peptides (see Fig. 2 and Section 3.2). In the specific case presented in Figure 2: Tweak in complex with the Fab fragment of a neutralizing antibody (Lammens *et al.*, 2013), PiPred delivered around 1.7 M peptides, of which circa 650 000 were helical, resulting from using 25 anchor residues.

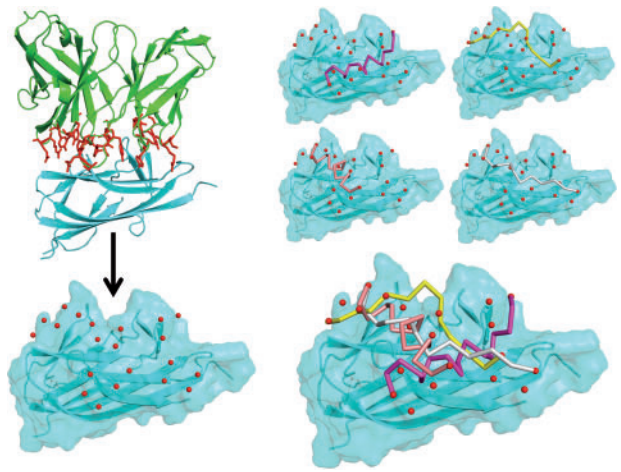

Fig. 2. Overview of the modeling process implemented in PiPreD. On the Top left-corner, a cartoon representation of the Human TWEAK (cyan) in complex with Fab fragment of a neutralizing antibody (green) (Lammens *et al.*, 2013); anchor residues depicted as sticks (red). Lower left corner surface representation of Tweak–Antibody interface (rotated 90°) with anchor residues represented as red sphere. Top right corner, example of four different modeled peptides depicted as cartoons (magenta, yellow, white and gold) and the overlap between them shown in the lower right corner (Color version of this figure is available at *Bioinformatics* online.)

Even considering the large number of potential conformations of the main chain of anchor residues produced by inverse rotamers, i.e. an average of 75 alternative conformations per anchor residues, the comprehensive charting of the interface is qualitatively better (as it covers a large size of the conformational space restricted to the interaction) and faster than a random charting by *ab initio* modeling of main chain conformations. Moreover, there is no need to predefine the size of peptides and/or sequences.

On average, any given selection of anchor residues (two is the minimum) generates on average up to 12 000 candidate iMotifs. This number ranges between a few hundreds to tens of thousands depending mainly on the distance between anchor residues, i.e. the further apart, the less suitable iMotifs are found in the database. As expected, the number of generated peptides correlates with the number of anchor residues, which in turn relates to the size of the interface (Table 1).

3.2 Structural modeling generate peptides not biased toward specific conformations

A clear advantage of the PiPreD is that the modeling process is unbiased toward particular conformations. The modeling relies on a structural library of iMotifs looking for the most favorable conformations given the specific target-interface of a protein. Whether the conformations would be linear/extended or helical will entirely depend on the particular structural environment of the anchor residues and the conformational '*repertoire*' of the iMotif library.

Table 1. Average number of modeled peptides as a function of number of anchor residues

#Anchor residues	Average surface (Ang^2)	# Peptides (in 100 000s)[a]
0–20	1225	11 (6)[b]
20–40	2272	32 (12)
40–60	3902	48 (14)
60–80	5180	50 (13)

[a]Statistics derived from a set of 50 protein complexes of different sizes.
[b]Number of which are helical peptides shown within brackets.

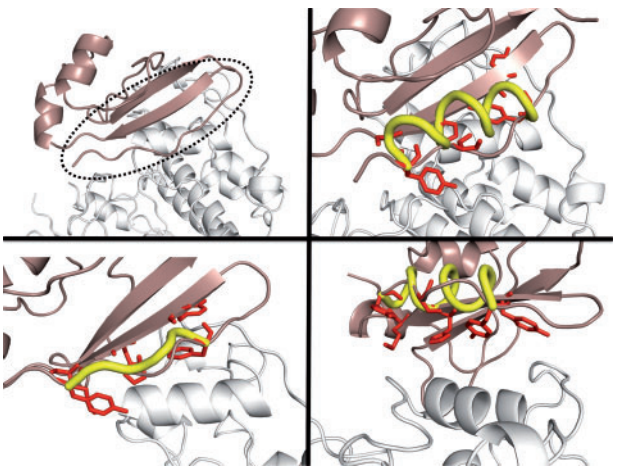

Fig. 3. Example of three orthosteric peptides derived for the CDK2 kinase in complex with the cycle regulatory protein CksHsa. From the left side and anticlock wise, cartoon representation of the protein complex CDK2 (white) CKsHsa (salmon) (Bourne *et al.*, 1996) cartoon representation of three modeled peptide with extended and helical conformation (yellow). Anchor residues depicted in stick representation (red) (Color version of this figure is available at *Bioinformatics* online.)

Figure 3 shows this aspect with three particular examples of modeled peptides. In one of the cases (Fig. 3 lower-left), the peptide has a linear conformation, which closely resembles and recapitulates an existing structural element of the original complex, i.e. an interacting loop located in the interface. In this example, PiPreD is similar to other methods that derive peptides utilizing native regions of the interface [e.g. (London *et al.*, 2010) or (Donsky and Wolfson, 2011)]. However, the other two examples (Fig. 3 top and lower right), the peptides present a helical conformation. This is a new structural arrangement, not observed in the native complex, but that however surrogates an important number of anchor residues, or in other words, an important part of the native interface. In particular, one of the modeled peptides can act as a surrogate of a β-strand and a neighboring loop simultaneously accounting for up to 7 of the anchor residues, i.e. an important percentage of the total number of interactions observed in the interface (Fig. 3).

This is a remarkable example in which, PiPreD yielded 1.5 million peptide designs, where around one third had helical conformation. Besides, this example also shows that PiPreD brings new directions to the field of peptide designs. It is worth noting, that as a lead for drug development, helical peptides are preferred to linear/extended conformations due to higher stability, permeability and resistance to proteases (Walensky *et al.*, 2004).

In summary, not only PiPreD can generate peptides featuring conformations not present in the native complex but also recapitulate existing, native, elements even though they are not taken into consideration during the modeling process, i.e. anchor residues are presented as disembodied elements.

3.3 PiPreD combines native elements of different interfaces into single peptides

Another important feature of PiPreD is that it can integrate and combine multiple interfaces. The only requirement for structural modeling of peptides is the need of the anchor residues driving the modeling. These can be extracted from a single interface or combined using different interfaces, being irrelevant whether they overlap or not. Thus, modeled peptides can integrate anchor residues from independent interfaces and thus reach between different regions with the added value of engineering and designing *de novo* interactions, which were not present in the native complex.

An example illustrating the combination of anchor residues extracted from different interfaces is shown in Figure 4. The biological unit of the TNF-related apoptosis inducing ligand (TRAIL) and Death Receptor 5 (DR5) complex (Cha *et al.*, 2000) is composed of 3 TRAIL and 3 DR5 monomers. Therefore, TRAIL monomers interact both with a TRAIL and DR5 monomers (Fig. 4). To account for this, PiPreD modeling uses the combination of anchor residues from different interfaces. As shown in Figure 4 one for the modeled peptide, a helical peptide, integrates anchor residues both from TRAIL and DR5 monomers while reaching two different interfaces. This has the added value of engineering and designing *de novo* interactions, which were not present in the native complex (Fig. 4), and thus being particularly important when looking at increasing the number of interactions between the peptide and the protein surface.

3.4 Combining anchor residues derived from different cognate partners

There are cases where different partners can bind the same protein by sharing a common interface. In the context of our modeling strategy, it means that anchor residues can be obtained from different cognate partners of the target protein and therefore they can be merged in the modeling step. One of such example is that of RAS, an important protein family involved in the regulation of signaling pathways linked to cell grow and proliferation (Malumbres and Barbacid, 2003), which interact with a range of protein effectors through a common interface called the switch I–II region (Milburn *et al.*, 1990).

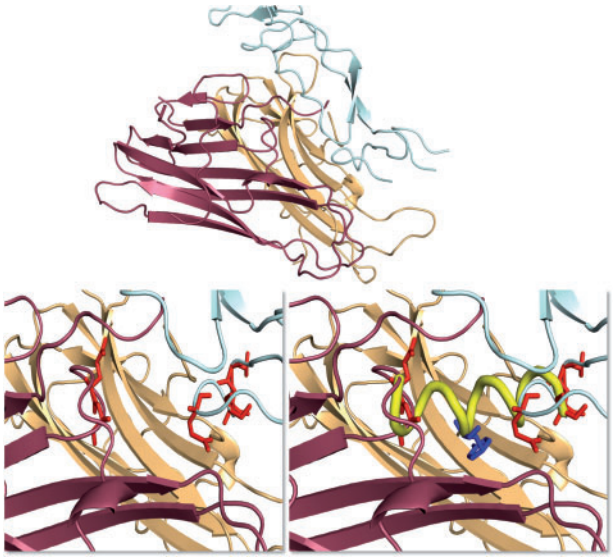

Fig. 4. Example of an orthosteric peptide derived from the two independent interfaces. Cartoon representation of the crystal structure of TNF-related apoptosis inducing ligand (gold and brown) in complex with Death Receptor 5 (cyan) (Cha *et al.*, 2000). Modeled peptide shown in cartoon (yellow) and *de novo* designed side chain and anchor residues depicted in stick representation (blue and red, respectively.) (Color version of this figure is available at *Bioinformatics* online.)

The structures of six RAS-cognate partners were used to extract anchor residues: RAS-p120Gap (PDB code: 1wq1) (Scheffzek *et al.*, 1997), RAS-SOS-1 (PDB code: 1bkd) (Boriack-Sjodin *et al.*, 1998), RAS-RAF (PDB code: 3kud) (Filchtinski *et al.*, 2010), RAS-PLCε (PDB code: 2c5l) (Bunney *et al.*, 2006), RAS-RalGDS (PDB code: 1lfd) (Huang *et al.*, 1998) and RAS-Anti RAS Fv (PDB code: 2uzi) (Tanaka *et al.*, 2007). Interface sizes varies across complexes, being SOS-RAS (Boriack-Sjodin *et al.*, 1998) the complex with the largest interaction interface, which includes 52 residues and cover and area of around 3245 Ang^2; RAS - scFV Antibody (Tanaka *et al.*, 2007) is the complex with the smallest contact surface with 17 interface residues and ~900 Ang^2. The target surface restricted to the intersection of all interfaces covers an area of 1200 Ang^2 and comprises 24 nonstructurally equivalent anchor residues (Fig 5). A few examples of modeled peptides are also shown in Figure 5 and these include anchor residues derived from different partners. The total number of modeled peptides was ~1.5 M, of which over 600 000 were helical peptides.

3.5 Targeting of small interfaces and structural diversity of peptides

Given the nature of the modeling strategy, the number of generated peptides, and thus conformational diversity of peptides, is necessary linked to the number of anchor residues used (Table 1). However, even in the case of small protein interfaces, such as the case of the Myc–Max complex (Nair and Burley, 2003), PiPreD is able to achieve a high conformational heterogeneity among designed peptides (Fig. 6).

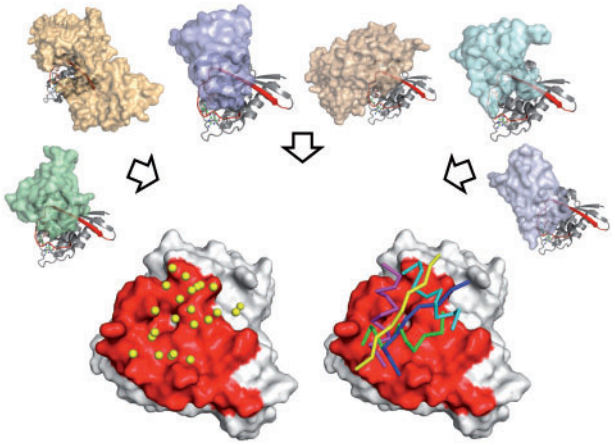

Fig. 5. Example of modeling involving several cognate partners sharing a common interface. Top row from left to right structural rendering of RAS-RAF (Filchtinski *et al.*, 2010), RAS-SOS (Boriack-Sjodin *et al.*, 1998), RAS-PLCε (Bunney *et al.*, 2006), RAS-P120 (Scheffzek *et al.*, 1997), RAS-RAL (Huang *et al.*, 1998) and RAS-scFV (Tanaka *et al.*, 2007). Bottom row, surface representation of RAS (gray) and Switch-I-II depicted in red. Anchor residues and modeled peptides are represented as yellow spheres and ribbon representation, respectively (Color version of this figure is available at *Bioinformatics* online.)

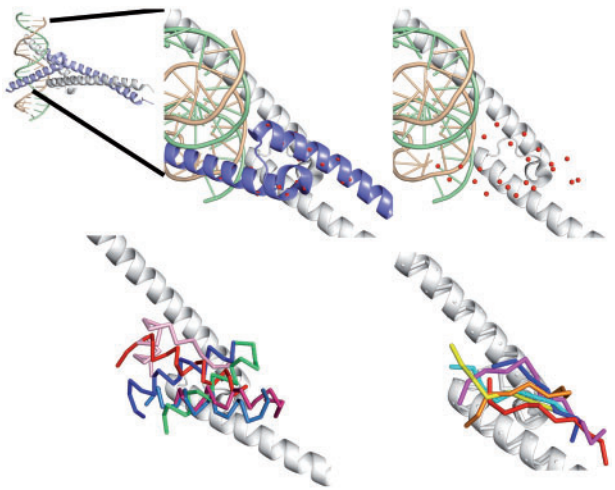

Fig. 6. Example of modeling involving a small interface. Top row from left to right is the cartoon representation of Myc (gray) and Max (blue) complex bound to DNA (green and gold) (Nair and Burley, 2003); anchor residues derived from max shown as red spheres. Bottom row, ribbon representation of Myc (gray) and modeled peptides in helical (left) and linear (right) conformation (Color version of this figure is available at *Bioinformatics* online.)

The interaction between Myc and Max is mediated by an extended helix-zipper and a helix-turn-helix domain. The helix-turn-helix domain comprises 13 anchor residues distributed in a surface of around 690 Ang^2. The modeling of peptides targeting this region generated over 550 000 peptides (over 300 000 of which had helical conformation). As expected around 65% of the helical peptides recapitulate different elements of native cognate partner Max (i.e. helical peptides of different sizes and spanning different regions of native interactions), but also and more importantly, peptides with linear/extended conformation (i.e. conformation not observed in the native complex) and even helical peptides whose conformation and interaction mode was not derived from existing elements of the native complex (Fig. 6).

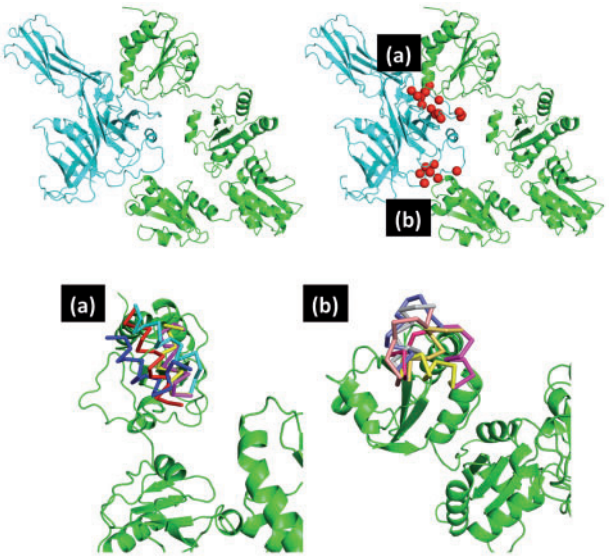

Fig. 7. Example of modeling involving two non-overlapping and distant interfaces. Top row from left to right is the cartoon representation of Tapasin (cyan) and ERP57 (green) complex and the two interfaces labeled 'a' and b' (Dong *et al.*, 2009); anchor residues derived from Tapasin shown as red spheres. Bottom row, ribbon representation of ERP57 (gray) and examples of modeled peptides for site 'a' (left) and site 'b' (right) (Color version of this figure is available at *Bioinformatics* online.)

3.6 Targeting of independent interfaces in the same target protein

The final example presented is the case of large protein complexes where there can be the situation that the same cognate partner interacts with two independent, nonoverlapping and structurally distant protein interfaces. Such example is presented in Figure 7, which illustrates the modeling of peptides to target ERP57 using anchor residues derived from Tapasin (Dong *et al.*, 2009).

As shown, the two interfaces are independent and do not share a common continuous interface of the target protein and account for 60 anchor residues in total (Fig. 7). This example is different to the one presented in Section 3.3 as the interfaces are more distant and do not share a common, continuous, interface of the target protein. The number of modeled peptides, 2.5 M, was slightly lower than expected given the number of anchor residues considered, i.e. potentially one could expect a large number of combinations of pairs of residues but an important proportion of those corresponded to anchor residues from different, distant interfaces (Table 1). Thus, this caused the modeling process to fail finding suitable iMotifs to surrogate these particular instances.

4 Conclusion

In this work, we present a novel, knowledge-based computational method to model and design orthosteric peptides: PiPreD. PiPreD relies on the structural information of the protein complexes and on a bespoken library of structural motifs: iMotifsDB. The residues that mediate the interaction of cognate partner(s) and target protein, i.e. anchor residues, are used to drive a comprehensive and systematic sampling and modelling of peptides derived from iMotifs. PiPreD can be applied to any protein complex for which the three-dimensional structure is known and thus open a new avenue to exploit the repository of protein complexes present in structural databases. Additionally, the technology can be easily adapted to

individual protein structures, i.e. cases where the structure of the complex is not known, since individual aminoacids can be placed on the surface of the target protein structure via single-residue docking [e.g as in (Ben-Shimon and Eisenstein, 2010)]. Finally, this new approach could be used in conjunction with high-throughput peptide synthesis techniques such as peptide microarrays to generate a pool of sequences that are biased toward the targeted surface, i.e. a directed search as opposed to a random peptide screening.

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Conflict of Interest: none declared.

References

Ben-Shimon,A. and Eisenstein,M. (2010) Computational mapping of anchoring spots on protein surfaces. *J. Mol. Biol.*, **402**, 259–277.

Berman,H. *et al.* (2003) Announcing the worldwide Protein Data Bank. *Nat. Struct. Biol.*, **10**, 980.

Boriack-Sjodin,P.A., *et al.* (1998) The structural basis of the activation of Ras by Sos. *Nature*, **394**, 337–343.

Bourne,Y. *et al.* (1996) Crystal structure and mutational analysis of the human CDK2 kinase complex with cell cycle-regulatory protein CksHs1. *Cell*, **84**, 863–874.

Bunney,T.D. *et al.* (2006) Structural and mechanistic insights into Ras association domains of phospholipase C epsilon. *Mol. Cell*, **21**, 495–507.

Cha,S.S. *et al.* (2000) Crystal structure of TRAIL-DR5 complex identifies a critical role of the unique frame insertion in conferring recognition specificity. *J. Biol. Chem.*, **275**, 31171–31177.

Dong,G. *et al.* (2009) Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer. *Immunity*, **30**, 21–32.

Donsky,E. and Wolfson,H.J. (2011) PepCrawler: a fast RRT-based algorithm for high-resolution refinement and binding affinity estimation of peptide inhibitors. *Bioinformatics*, **27**, 2836–2842.

Ernst,J.T. *et al.* (2003) Design and application of an alpha-helix-mimetic scaffold based on an oligoamide-foldamer strategy: antagonism of the Bak BH3/Bcl-xL complex. *Angew. Chem. Int. Ed. Engl.*, **42**, 535–539.

Fernandez-Fuentes,N. *et al.* (2006) A supersecondary structure library and search algorithm for modeling loops in protein structures. *Nucleic Acids Res.*, **34**, 2085–2097.

Filchtinski,D. *et al.* (2010) What makes Ras an efficient molecular switch: a computational, biophysical, and structural study of Ras-GDP interactions with mutants of Raf. *J. Mol. Biol.*, **399**, 422–435.

Fletcher,S. and Hamilton,A.D. (2006) Targeting protein-protein interactions by rational design: mimicry of protein surfaces. *J. R. Soc. Interface.*, **3**, 215–233.

Frenkel,D. *et al.* (1995) PRO_LIGAND: an approach to de novo molecular design. 4. Application to the design of peptides. *J. Comput. Aided Mol. Design*, **9**, 213–225.

Gaestel,M. and Kracht,M. (2009) Peptides as signaling inhibitors for mammalian MAP kinase cascades. *Curr. Pharm. Des.*, **15**, 2471–2480.

Huang,L. *et al.* (1998) Structural basis for the interaction of Ras with RaIGDS. *Nat. Struct. Mol. Biol.*, **5**, 422–426.

Kritzer,J.A. *et al.* (2004) Helical beta-peptide inhibitors of the p53-hDM2 interaction. *J. Am. Chem. Soc.*, **126**, 9468–9469.

Lammens,A. *et al.* (2013) Crystal structure of human TWEAK in complex with the Fab fragment of a neutralizing antibody reveals insights into receptor binding. *PLoS One*, **8**, e62697.

Leaver-Fay,A. *et al.* (2013) Scientific benchmarks for guiding macromolecular energy function improvement. *Methods Enzymol.*, **523**, 109–143.

Leaver-Fay,A. *et al.* (2011) ROSETTA3: an object-oriented software suite for the simulation and design of macromolecules. *Methods Enzymol.*, **487**, 545–574.

Lessel,U. and Schomburg,D. (1997) Creation and characterization of a new, non-redundant fragment data bank. *Protein Eng.*, **10**, 659.

Lo Conte,L. *et al.* (1999) The atomic structure of protein-protein recognition sites. *J. Mol. Biol.*, **285**, 2177.

London,N. *et al.* (2010) Can self-inhibitory peptides be derived from the interfaces of globular protein-protein interactions? Proteins, **78**, 3140–3149.

Malumbres,M. and Barbacid,M. (2003) RAS oncogenes: the first 30 years. *Nat. Rev. Cancer*, **3**, 459–465.

Marchioni,F. and Zheng,Y. (2009) Targeting rho GTPases by peptidic structures. *Curr. Pharm. Des.*, **15**, 2481–2487.

McGregor,D.P. (2008) Discovering and improving novel peptide therapeutics. *Curr. Opin. Pharmacol.*, **8**, 616–619.

Milburn,M.V. *et al.* (1990) Molecular switch for signal transduction: structural differences between active and inactive forms of protooncogenic ras proteins. *Science*, **247**, 939–945.

Moon,J.B. and Howe,W.J. (1991) Computer design of bioactive molecules: a method for receptor-based de novo ligand design. *Proteins*, **11**, 314.

Mullard,A. (2012) Protein-protein interaction inhibitors get into the groove. *Nat. Rev. Drug. Discov.*, **11**, 173–175.

Nair,S.K. and Burley,S.K. (2003) X-Ray Structures of Myc-Max and Mad-Max recognizing DNA: molecular bases of regulation by proto-oncogenic transcription factors. *Cell*, **112**, 193–205.

Neduva,V. and Russell,R.B. (2006) Peptides mediating interaction networks: new leads at last. *Curr. Opin. Biotechnol.*, **17**, 465–471.

Petsalaki,E. and Russell,R.B. (2008) Peptide-mediated interactions in biological systems: new discoveries and applications. *Curr. Opin. Biotechnol.*, **19**, 344–350.

Raveh,B. *et al.* (2010) Sub-angstrom modeling of complexes between flexible peptides and globular proteins. *Proteins*, **78**, 2029–2040.

Roberts,K.E. *et al.* (2012) Computational design of a PDZ domain peptide inhibitor that rescues CFTR activity. *PLoS Comput. Biol.*, **8**, e1002477.

Rubinstein,M. and Niv,M.Y. (2009) Peptidic modulators of protein-protein interactions: progress and challenges in computational design. *Biopolymers*, **91**, 505–513.

Scheffzek,K. *et al.* (1997) The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science*, **277**, 333–339.

Smith,C.A. and Kortemme,T. (2010) Structure-based prediction of the peptide sequence space recognized by natural and synthetic PDZ domains. *J. Mol. Biol.*, **402**, 460–474.

Sobolev,V. *et al.* (1999) Automated analysis of interatomic contacts in proteins. *Bioinformatics*, **15**, 327–332.

Sood,V.D. and Baker,D. (2006) Recapitulation and design of protein binding peptide structures and sequences. *J. Mol. Biol.*, **357**, 917–927.

Tanaka,T. *et al.* (2007) Tumour prevention by a single antibody domain targeting the interaction of signal transduction proteins with RAS. *EMBO J.*, **26**, 3250–3259.

Teixido,M. *et al.* (2003) Development of a genetic algorithm to design and identify peptides that can cross the blood-brain barrier. *QSAR & Combinatorial Sci.*, **22**, 745–753.

Vassilev,L.T. *et al.* (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*, **303**, 844–848.

Walensky,L.D. *et al.* (2004) Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. *Science*, **305**, 1466–1470.

Watt,P.M. (2006) Screening for peptide drugs from the natural repertoire of biodiverse protein folds. *Nat. Biotechnol.*, **24**, 177–183.

Wells,J.A. and McClendon,C.L. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*, 2007, **450**, 1001–1009.