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Research Method

DOMAIN INTERACTION PHAGE PANNING (DIPP): A PHAGE DISPLAY BASED METHOD FOR MAPPING AND CHARACTERIZING PROTEIN-PROTEIN INTERACTION INTERFACE

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Abstract: Protein-protein interaction is central to most biological processes in cells and the identification and characterization of such protein interfaces is important for understanding the underlying molecular mechanisms. In this paper we describe a new method, referred to as Domain Interaction Phage Panning (DIPP), to delineate protein-protein interaction interfaces. DIPP takes advantage of displaying potential protein interaction domains or partners without the need for expression and purification of the entire protein. In this method, different overlapping regions of protein are cloned into phagemid vector pCANTAB 5E that display the cloned regions on the surface of the phage. The phage-displayed domains are then panned against the target protein either as a mixture of domains or as an individual domain. The identity of the positive interactor(s) is then established by Enzyme-linked Immunosorbent Assay (ELISA) followed by DNA sequencing of the clones to obtain the protein sequence.

Key words: Phage display; protein-protein interaction; domain interaction phage panning; DIPP; interaction surface

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Introduction

Protein-protein interaction is central to most cellular processes such as enzyme catalysis, signal transduction, immune response etc. (Bahadur and Zacharias, 2008; Reichmann *et al.*, 2007). Protein interfaces comprise of an ensemble of residues (sites) where proteins physically interact via chemical and/or physical forces. Identification and characterization of such protein interfaces is a key to understanding the underlying molecular mechanism of any biological process. The predominant biophysical techniques used in detecting protein-protein interactions and/or further delineating the details of interaction interfaces include X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, cryo-electron microscopy (CryoEM), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and microscale thermophoresis (Bahadur and Zacharias, 2008; Boozer *et al.*, 2006; Jerabek-Willemsen *et al.*, 2014; Pierce *et al.*, 1999; Russell *et al.*, 2004). Additionally, many bioinformatics tools are available to predict potential interaction sites but would require experimental validation (DeLano, 2002; Lise *et al.*, 2009; Neuvirth *et al.*, 2004). In more recent times, the Yeast Two Hybrid (Y2H) method has become a widely used approach to delineate protein-protein interactions in a high throughput manner (Polydorides *et al.*, 2000). However, a major limitation of the classical Y2H method (Fields and Song, 1989) is the requirement for the interacting proteins to be translocated into the nucleus, which therefore makes the technique ineffective in

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studying interactions of membrane and cytosolic proteins (Bruckner *et al*., 2009). To overcome this constraint, alternative Y2H methods such as the SOS and the RAS recruitment systems and the split-ubiquitin system have been developed (Bruckner *et al*., 2009). Despite these advances, there are inherent drawbacks to the Y2H system (Stynen *et al*., 2012) including misfolding and false positives, generally associated with an *in vivo* system.

Another complementary and widely used method is phage display. In this system, originally developed in 1985 by George P. Smith (Smith, 1985), a foreign gene is inserted into the gene(s) encoding the coat protein(s) of a filamentous bacteriophage and the inserted gene then displayed as fusion protein on the virion particle. To date, M13 is the most common filamentous bacteriophage used to display protein (Makowski, 1994; Sidhu, 2001). Typically, random peptides are generally expressed on the surface of M13 bacteriophage as fusions to either the minor coat protein pIII or the major coat protein pVIII (Sidhu, 2001). The pIII gene accepts inserts of short peptides and protein sequences >100 amino acids at the N-terminus (Sidhu *et al*., 2000b) whereas the pVIII gene is only able to tolerate short five to six extra amino acids (Greenwood *et al*., 1991; Makowski, 1993). Random peptide libraries, with a diversity of 10^8 or better, are panned against the target molecule and specific binders are enriched through multiple rounds of binding and phage infection. In general, 3 to 4 rounds of panning are sufficient for selection of positive binders. It has been observed that increasing the number of rounds of panning beyond 4 does not increase the enrichment of specific binders but, in fact, selects for phage with enhanced growth properties (Derda *et al*., 2011; Sidhu *et al*., 2000a). The positive binders are then selected from the enriched phage clones by phage ELISA. In a typical phage based ELISA, the target protein as well as the control protein (such as BSA) is first bound on the well(s) of an ELISA plate and subsequently probed with a phage-peptide library. Phage particles, binding specifically to the target protein, are then detected by HRP conjugated anti M-13 antibody. This facilitates the identification, selection and separation of specific binders from nonspecific binders. Because the phenotype is associated with the genotype, the amino acid sequence of the binding peptide is easily deciphered by DNA sequencing of the phage particle. Thus, combinatorial peptide library screening permits the isolation of peptide ligands that, in many instances, show conservation of motifs in native interacting proteins and facilitate the first step towards identification of natural candidate proteins in vivo (Kay *et al*., 2000; Smothers & Henikoff, 2000). Since its discovery, phage display technique has emerged as a unique and powerful tool for selectively sieving the desired peptides from a diverse pool of peptides in a combinatorial peptide library.

In this paper, we describe a new phage display based method, referred to as ***D***omain ***I***nteraction ***P***hage ***P***anning (DIPP), and demonstrate its versatility for characterizing protein-protein interaction interfaces (Shah *et al*., 2013). DIPP takes advantage of displaying potential protein interaction domains or partners without the need for expression and purification of the entire protein. Therefore, it is especially useful in circumventing problems associated with obtaining pure protein, such as membrane proteins. In the DIPP experiment, one can use the phagemid vector pCANTAB5-E which is an *E. coli* cloning vector containing the filamentous bacteriophage origin of replication (M13 ori) (Figure 1). This replication site, along with Helper phage, provides all the necessary components for the replication of single stranded DNA and packaging of the phagemid DNA into phage particle. Helper phage such as M13KO7 or VCSM13 in the presence of wild type M13 origin preferentially pack single stranded phagemid DNA rather than their own DNA which is secreted into culture media as phage particles. In DIPP, short regions of a given protein are cloned into the phagemid vector between the Sfi1 and Not1 sites in the pIII gene (Figure 1) for display on the phage surface as part of the pIII coat protein. Individual phage displaying a particular domain, or a mixture of phage displaying different domains, are then panned against the target protein. The identity of the binding domain is subsequently established by phage ELISA using an anti-M13 antibody (Figure 2). We illustrate the DIPP technique using two published examples.

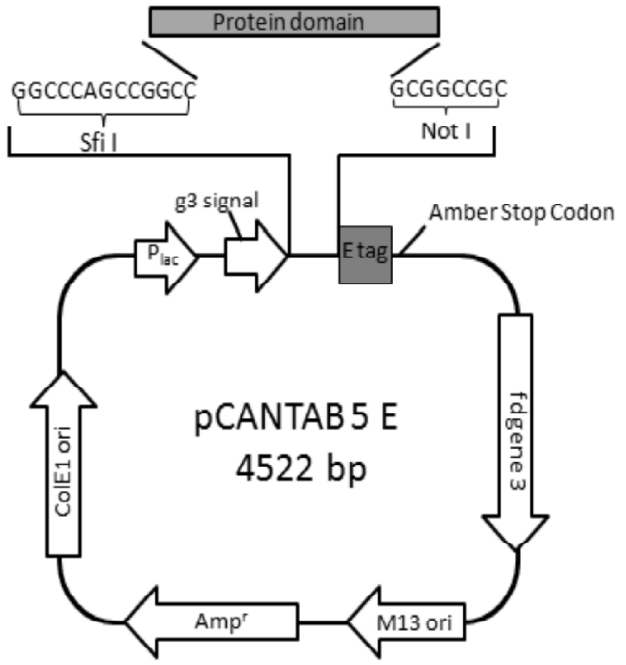

Figure 1: **Schematic representation of pCANTAB5-E phagemid vector for cloning of domain. The plasmid has filamentous phage origin of replication site, ampicillin resistance marker gene, lac inducible promoter followed by signal sequence. There are SfiI and NotI restriction sites available for cloning of various domains followed by the E tag and the amber stop codon.**

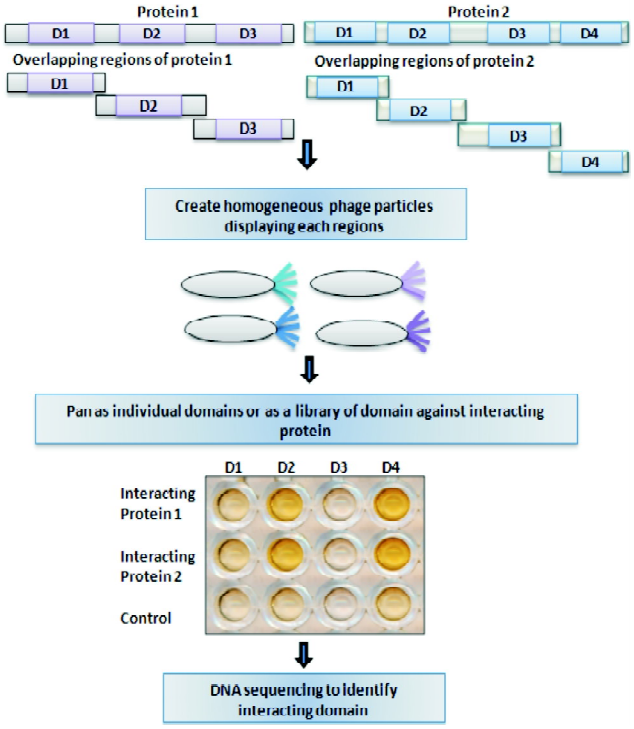

Figure 2: **A schematic flow diagram of the steps involved in Domain Interaction Phage Panning. Individual domains in each protein are indicated at D1, D2 etc.**

Domain interaction phage panning of two RNA binding proteins of Solanum tuberosum

Using this technique, we have determined the interaction interfaces between two RNA binding proteins of *Solanum tuberosum*, i.e., the polypyrimidine tract binding protein (*St*PTB) and Nova-like protein (*St*Nova) (Shah *et al.*, 2013). *St*Nova, which has three K-homology domains (KH domains), was divided into six overlapping regions (D1 to D6) (Figure 3) and each domain was cloned into pCANTAB5E vector. *St*PTB has four RNA recognition motifs (RRM) and was divided into five overlapping domain (P1 to P5) (Figure 3). Using DIPP, we have shown that the linker region between KH2 and KH3 domain (D5) of *St*Nova interacts through the linker region of *St*PTB present between RRM2 and RRM3 (P3) (Figures 4 and 5) (Shah *et al.*, 2013). These interactions were confirmed by protein overlay assays and mutational analysis.

Domain interaction phage panning of Arabidopsis of Binding protein (BiP) and a transcription factor bZIP28

DIPP technique was also successfully employed for identifying the binding site in the interaction between BINDING PROTEIN (BiP), a HSP70 protein and a major chaperone in the ER lumen and bZIP protein, a membrane associated transcription factor (Srivastava *et al.*, 2013). Twelve overlapping peptides from the luminal domain of bZIP28 were used to make phage domain library as shown in Figure 6A. After four rounds of panning against BiP, two peptides (No. 376 and 471) were enriched (Figure 6B). Phage ELISA also confirmed the phage panning results (Figure 6C). Furthermore, these interactions were confirmed by GST pull down experiments (Figure 6D). DIPP showed that BiP binds to the luminal tail of bZIP28, a region with the highest tendency for disorder (Figure 6A) (Srivastava *et al.*, 2013).

Protocol for Domain Interaction Phage Panning (DIPP)

The DIPP method offers a facile tool for high throughput protein-protein interaction studies. However, as with the Y2H method, complementary techniques such as affinity pull-downs and protein overlay assays and

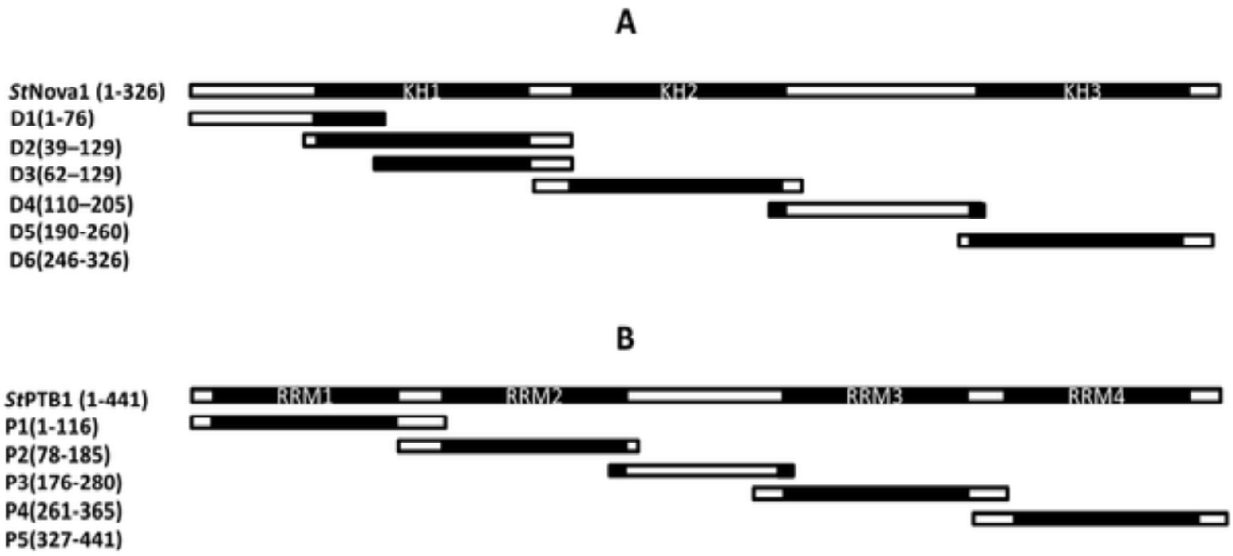

Figure 3: Schematic diagram of StNova1 (A) and StPTB1 proteins (B) showing the arrangement of their respective KH domains and RRMs and their overlapping regions used to construct domain phage library. [Reproduced from Reference Shah *et al.*, 2013 with permission].

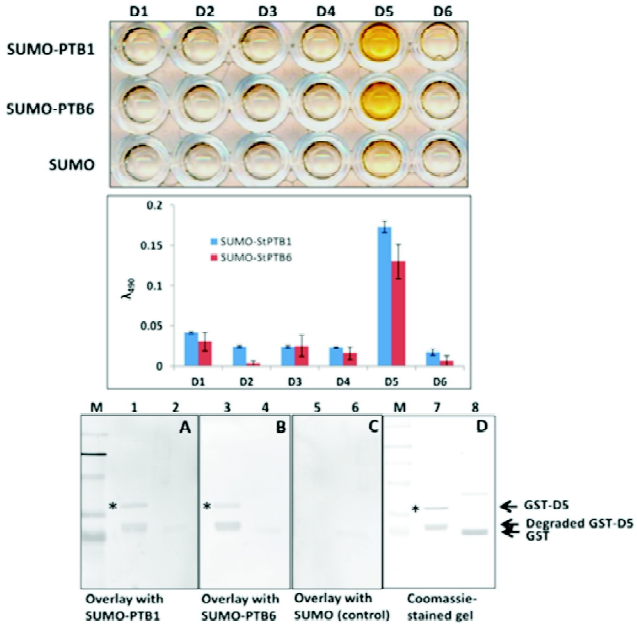

Figure 4: Mapping protein-protein interaction site on *St*Nova1 protein. (*Top panel*) Phage ELISA using *St*Nova1 single domain phage clones (D1 to D6) against SUMO-*St*PTB1, SUMO-*St*PTB6 and SUMO control. (*Middle panel*) Bar diagram showing quantification of phage binding. (*Bottom panel*) A. SUMO-*St*PTB1 protein overlay demonstrating its binding with GST-D5 region (lane 1, indicated with *), GST alone as a control shows no binding (lane 2) B. SUMO-*St*PTB6 protein overlay demonstrating its binding with GST-D5 region (lane 3, indicated with *), GST alone as a control shows no binding (lane 4) C. SUMO protein (as a control) overlay does not bind to either GST-D5 (lane 5) or GST alone (lane 6) D. Coomassie stained SDS PAGE showing protein inputs, GST-D5 (lane 7, indicated with *) and GST (lane 8) used for blotting PVDF membrane for protein overlay experiments [Reproduced from reference Shah *et al.*, 2013 with permission].

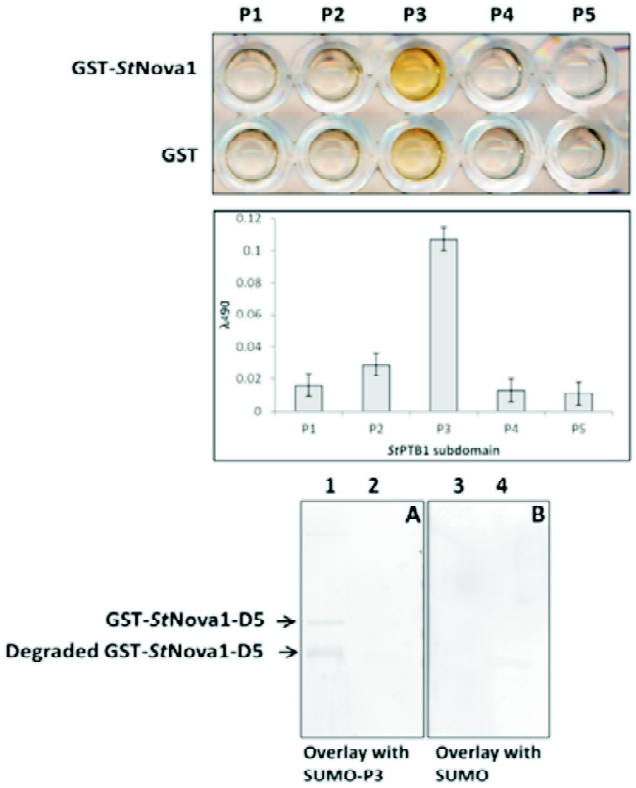

Figure 5: Mapping protein-protein interaction site on *St*PTB1. (*Top*) Phage ELISA using *St*PTB1 single domain phage clones (P1 to P5) against GST-*St*Nova1 protein and GST control (*Middle*) Bar diagram showing quantification of phage binding (*Bottom*) A. SUMO-P3 protein overlay demonstrating binding to GST-D5 region (lane1), GST alone as a control shows no binding (lane2) B. SUMO protein (control) overlay shows no binding to either GST-D5 (lane 3) or GST alone (lane 4). [Reproduced from reference Shah *et al.*, 2013 with permission].

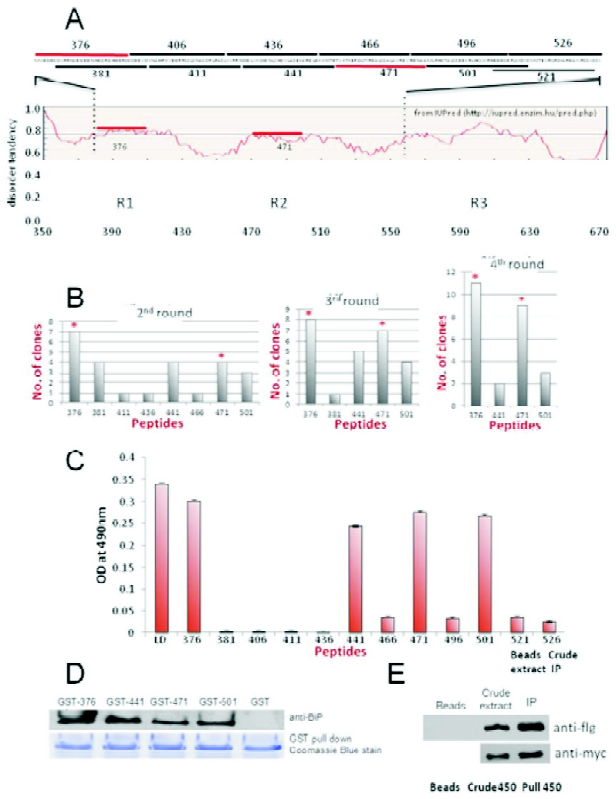

Figure 6: **bZIP28 Peptides Used in Phage Display Library. (A) Overlapping peptides from residues 376 to 555 in the lumenal domain of bZIP28 were displayed in M13 phage. Tendency for intrinsic disorder in the lumenal domain of bZIP28 is plotted against the map of the lumenal domain for bZIP28. The tendency for disorder was determined by IUPred (http:// iupred.enzim.hu/pred.php). (B) Recombinant phages were pooled and panned against immobilized BiP1-His in four rounds of panning. At each round, bound phages were released and the inserts encoding the bZIP28 peptides were sequenced. The frequency in recovering phage expressing the various peptides in progressive rounds of screening is shown. Red asterisks indicate the peptides in phage recovered with the highest frequency in the fourth round of panning. (C) Separate recombinant phage lines were incubated with immobilized BiP1-His, and bound phage were quantified in an ELISA assay. Error bars indicate SE. (D) Overlay immunoblot demonstrating that soluble BiP1-His binds to GST-tagged bZIP28 peptides. The four peptides (441, 471, 376, and 501) enriched in panning were tagged with GST, purified by binding to glutathione beads, eluted, subjected to SDS-PAGE, and transferred to a nitrocellulose filter. The filter was incubated with purified BiP1-His, washed, and incubated with a primary anti-BiP antibody and then a secondary antibody was used to detect BiP binding. The GST-tagged peptides pulled down with glutathione beads and stained with Coomassie blue were used as a loading control. (E) bZIP28 construct containing only the R1 region of lumenal domain (as shown in [A]) binds BiP1-flg in vivo. bZIP28 truncation constructs mycbZIP28D450 (containing region R1) and myc-bZIP28D355 (lacking region R1) were each coexpressed with BiP1-flg in a tobacco leaf transient expression assay. Leaf extracts were subjected to immunoblotting and probed with anti-flg and anti-myc antibodies. [Reproduced from reference Srivastava *et al.*, 2013 with permission]. Note that the figure is copyrighted by the American Society of Plant Biologists].**

mutagenesis experiments must be used to further confirm and verify results.

Materials required

i. Platinum® Pfx DNA Polymerase (Invitrogen Cat. No.1708-013)

ii. 10 mM dNTP mix (solution containing mixture of 2.5mM each of dATP, dTTP, dGTP, dCTP)

iii. SfiI (NEB Cat. No R0123S) and NotI restriction enzymes (NEB Cat No. R0189S).

iv. GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare cat no. 28-9034-70).

v. QIAprep spin miniprep kit (Qiagen cat no. 27106).

vi. T4 DNA ligase (NEB cat no.M0202S).

vii. BSA (Sigma-Aldrich, cat no. 05477).

viii. Carbenicillin (sigma, cat no.C1389), 5 mg / ml solution made in sterile water.

ix. Tetracyclin hydrochloride (Sigma-Aldrich cat no. T3383-25G), 10 mg/ml solution made in sterile water.

x. VCSM13 (Stratagene) or M13KO7 helper phage (NEB, cat. No. N0315S).

xi. 2YT media: 16 g tryptone, 10 g yeast extract and 5 g NaCl into distill water so that the final volume is 1 liter. Media is autoclaved and stored at room temperature.

xii. 2YT agar/carb plate: 2YT, 8 gm agar and 50 mg of carbenicillin per liter.

xiii. PBST: 1X PBS and 0.05 % Tween 20.

xiv. PEG-NaCl solution: 20 % PEG-8000(w/v) (Sigma-Aldrich Cat No. P5413) and 2.5 M NaCl is mixed in distill water and mixture is autoclaved and stored at room temperature.

xv. Anti-M13/HRP conjugate (GE healthcare, cat no. 27942101)

xvi. O-Phenylenediaminedihydrochloride (Sigma-Aldrich, cat no. P1526-10G)

xvii. Hydrogen peroxide solution (30 % w/w) (Sigma-Aldrich Cat. No. H1009-500ml)

The DIPP method consists of the following four key steps:

1. Cloning of individual domains into phagemid vector

2. Construction of domain phage library
3. Phage panning
4. Phage ELISA

STEP 1: Cloning of domains into Phagemid pCANTAB5 E vector

A. Overlapping regions in the protein of interest are first generated by PCR amplification of the cDNA template using forward and reverse primers incorporating SfiI and NotI sites. The amplification is done with Platinum® Pfx DNA Polymerase (Invitrogen) in sterile PCR tubes on ice, as shown below.

Reaction component	*Amount*	*Final concentration*
10X Pfx Amplification Buffer	5	1X
50 mM $MgSO_4$	1 µl	1 mM
10mM dNTP mixture	0.5 µl	2.5 mM each dNTP
Template DNA	Variable	100 ng
Forward Primer with Sfi1 restriction site	Variable	0.3 µM
Reverse primer with Not1 restriction site	Variable	0.3 µM
DNA polymerase	0.5 µl	
Double distilled water	Up to 50 µl	

Note: The amount of 10 X Pfx reaction buffer and $MgSO_4$ may be doubled if amplification is not observed.

B. The PCR reaction is run using the following Program:

Steps	*Temperature*	*Time*	*Number of Cycles*
Initial Denaturation	94	4 min	1x
Denaturation	94	1 min	30x
Annealing	55	30s	
Elongation	72	1	
Final elongation	72	2	1x
Hold	4		

Note: Annealing temperature should be adjusted according to primer melting temperature

C. Sequential digestion of the PCR amplified products and phagemid vector using Not1 and Sfi1 restriction enzymes.

i. The PCR amplified product and phagemid vector are digested using Not1 restriction enzyme at 37 °C for at least 6h for complete digestion of DNA (it can also be kept for overnight digestion for convenience) as described below.

Reaction component	*Amount*
PCR product or phagemid vector	Variable (~10 µg)
10 X NEBuffer 3.1	10 µl
NotI (10 $U\mu l^{-1}$)	1 µl
Double distilled water	Up to 100 µl

ii. The enzyme is heat inactivated by placing the tubes at 65°C for 20 min.

iii. The digested fragments are purified by using PCR DNA and gel band purification system.

iv. The Not1 digested plasmid/PCR product is subsequently treated with SfiI restriction enzyme at 50 °C for 5h. The reaction is set as described in section C(i). For SfiI digestion, NEBuffer 2.1 or CutSmart® buffer is used, as supplied with the enzyme. (It can also be kept for overnight digestion if convenient).

v. The digested plasmid/PCR is run on an agarose gel.

vi. The digested plasmid/PCR products are isolated from the gel using GFX™ PCR DNA and gel band purification kit according to the manufacturer's protocol.

D. The SfiI/NotI digested PCR fragment(s) is ligated into the similarly digested pCANTAB 5E vector as follows:

Two µl of 10X ligase buffer is dispensed into an Eppendorf tube followed by addition of variable amount of pCANTAB 5E plasmid (100 ng) and PCR fragment (in a molar ratio of 1: 3). T4 DNA ligase was added to the reaction and the volume of reaction is then adjusted to 20µl with double distilled water. The reaction mixture is incubated overnight at 16°C [A control reaction is also setup where, instead of the PCR fragment, water is added].

The ligated product (and control) is subsequently transformed into chemically competent *E. coli* cells (such as HB101) for cloning. Each transformation reaction is plated onto LB/

carb plates and incubated overnight at 37 °C. Colonies are then picked, inoculated in 5ml LB/carb media and incubated overnight at 37 °C with shaking at 210 rpm [**Note**: The number of colonies obtained on reaction plates should be 10 times more than colonies obtained on control plate]. Plasmid is isolated from the overnight culture using the Qiagen plasmid prep kit, as described by manufacturer. Positive transformants are confirmed by DNA sequencing.

STEP 2: Construction of domain phage library

A. pCANTAB5E phagemid vectors with insert are transformed into *E. coli* XL1Blue competent cells. Each transformation reaction is plated onto LB/carb plates and incubated overnight at 37 °C.

B. Single colonies from each plate are inoculated into 5ml 2YT/carb/tet media and incubated at 37 °C with shaking at 210 rpm till the OD reaches ~ 0.2–0.3 at 600 λ.

C. XL1 Blue cells are infected with helper phage [VCSM13 (Stratagene) or M13KO7]. Following 1h of incubation, the cell culture (from step A) is transferred to 25 ml of2YT media containing 10 µg/ml of tetracycline and 50 µg/ml carbenicillin and further incubated at 37 °C overnight with shaking at 210 rpm.

D. Next day, cell debris is removed by centrifugation at 8,000 rpm for 10 min. The phage particles are then precipitated from the supernatant using 20% PEG solution containing 2.5 M NaCl so that the final concentration of PEG is 5%. For complete precipitation, solution is kept at room temperature for 20 min with shaking. The precipitated phage solution is centrifuged at 11,000 rpm for 10 min. Supernatant is carefully removed from the precipitate. If needed, tubes with precipitates are centrifuged again at 10,000rpm for 5 min and remaining supernatant is discarded. The precipitate is re-suspended in 1 ml of PBS, and phage concentration determined by measuring absorbance at 268 λ (OD_{268} = 1.0 for a solution containing 5×10^{12} phage per ml). A "domain library" is prepared by mixing equal concentrations of phage particles displaying individual domain. [Note; Phage particles are typically stored in PBS containing 20% glycerol].

STEP 3: Phage panning

A. Protein of interest is immobilized in the wells (typically 5) of a Maxisorp immunoplate (Nunc) by aliquoting 100 µl of protein at a concentration of 10µg/ml in 50 mM $NaHCO_3$, pH 9.6 at room temperature with gentle rotation for 2h.

B. The protein solution is removed and washed once with 1X PBS. Each well is blocked using 300 µl PBS containing 0.2% BSA for 1h followed by 3 washings with PBS containing 0.05 % Tween 20 (PBST).

C. Phage domain library (100 µl) is added to each well and incubated at room temperature for 3h with gentle rotation. The solution is removed and unbound phage removed by washing 5 times with PBST.

D. The bound phage is eluted by incubating with 100 µl of 0.1 M HCl per well for 5 min at room temperature with vigorous shaking. The eluted phage is immediately neutralized by the addition of 1/3rd phage volume of 1 M Tris-HCl buffer pH 8.0.

E. Eluted phage is added toXL1-Blue cells (previously grown to < 0.6 OD) and incubated at 37 °C for 20 min followed by addition of 10 µl of VCSM13 or M13KO7 helper phage (concentration 1x 10^{11}pfu) and further incubated at 37 °C for 30 min. Infected XL1-Blue cells are then transferred into a conical flask containing 50 ml of 2YT media (that includes 10 µg/ml of tetracycline and 100 µg/ml ampicillin) and further incubated at 37 °C overnight with shaking at 210 rpm.

F. Next day, phage preparation is performed as described in Step 2D. This process yields the first round of enriched phage that is used in the second round of panning, etc. The entire sequence is repeated 3-4 rounds, and after 2nd, 3rd and 4th rounds, phage infected XL1-Blue cells are also grown on 2YT carb plates. After each round of panning and infection, there is an enrichment of phage with high affinity for target protein whereas nonspecific phages are washed away. The efficiency of specific binding is monitored by calculating the

"enrichment" ratio which is arrived at by dividing the number of phage that are bound to a well coated with target protein by the number of phage bound to an uncoated or control protein (Sidhu *et al.*, 2000a). If no enrichment is seen the stringency of wash solution can be appropriately increased in subsequent selection cycle(s). Typically, plasmids are prepared from 25 randomly selected colonies after the final round and their DNA sequenced.

STEP 4. Testing binding specificity by Phage-ELISA

A. 100 µl of target protein per well and control BSA (10µg/ml in 50 mM $NaHCO_3$, pH 9.6) are immobilized on the Nunc plate at room temperature with gentle rotation for 2h.

B. The solution is removed from the well and the plate is then washed two times with PBS followed by blocking with PBST containing 0.2% BSA for 1h.

C. This is followed by 3 washings with PBS containing 0.05% Tween 20 (PBST).

D. 100 µl of phage displaying domain (diluted in PBST containing 0.2% BSA) is added to each plate and plate is incubated room temperature with gentle shaking for 2h.

E. The solution is removed; the plate washed three times with PBST and then incubated with 100 µl of anti-M13 HRP conjugated antibody (1:5000 diluted in PBST containing 0.02 % BSA) for 1h at room temperature.

F. The solution is removed and after washing four times with PBST, bound phage in each well is detected by incubating with 50 µl of substrate solution (0.01% hydrogen peroxide + 0.8 mg/ml o-Phenylenediaminedihydrochoride) for ~10 min. Reactions are terminated by the addition of 50 µl of 3M HCl, and absorbance of the developed yellow color is measured at 490 nm (and appropriately adjusted for the control well containing BSA).

Discussion

The two examples presented in this paper establish DIPP as a sensitive method for mapping protein-protein interaction interface. In general, a typical protein–protein interaction is governed by a few amino acids at a protein interaction surface commonly referred to as 'hot spots' (Moreira *et al.*, 2007; Russell *et al.*, 2004). Often, these 'hot spots' consist of a linearly arranged sequence of amino acids although they could also be configured through the three-dimensional structure of the protein. Hence, at the protein-protein interface, only a subset of amino acids contributes predominantly to the total binding energy of the interaction. Our method, alone or in conjunction with other methods such as alanine scanning mutagenesis (Bradshaw *et al.*, 2011; Cunningham and Wells, 1989), can expedite complete mapping of interaction hotspots. In DIPP, once the specific region of interaction has been determined, sub domain libraries can be constructed and the interaction region can be further narrowed down. For example, in the interaction between *St*PTB and *St*NOVA described earlier, once the linker region between KH2 and KH3 (D5 region) of *St*Nova was localized as the binding interface, it was further subdivided into three overlapping subdomains S1, S2 and S3 (Shah *et al.*, 2013). Using DIPP and phage ELISA it was then determined that the S2 subdomain within D5 specifically bound to *St*PTB. Further mutagenesis experiments identified the importance of Gly and Pro residues in the interaction (Shah *et al.*, 2013). In conclusion, having demonstrated DIPP as a simple and elegant method, we are currently exploring the use of biotinylated phage (Smelyanski and Gershoni, 2011) to further optimize the method for high throughput screening of protein-protein interactions.

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TECHNICAL NOTES