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A Protein-Domain Microarray Identifies Novel Protein-Protein Interactions

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SYNOPSIS

Protein domains mediate protein-protein interaction through binding to short peptide motifs in their corresponding ligands. These peptide recognition modules are critical for the assembly of multiprotein complexes. We have arrayed GST fusion proteins, with a focus on protein interaction domains, onto nitrocellulose coated glass slides to generate a protein-domain chip. Arrayed protein-interacting modules included WW, SH3, SH2, 14.3.3, FHA, PDZ, PH and FF domains. Here we demonstrate, using peptides, that the arrayed domains retain their binding integrity. Furthermore, we show that the protein-domain chip can "fish" proteins out of a total cell lysate, these domain-bound proteins can then be detected on the chip with a specific antibody, thus producing an interaction map for a cellular protein of interest. Using this approach we have confirmed the domain binding profile of the signaling molecule, Sam68, and have identified a new binding profile for the core snRNP protein, SmB'. This protein-domain chip not only identifies potential binding partners for proteins, but also promises to recognize qualitative differences in protein ligands (caused by post-translational modification), thus getting at the heart of signal transduction pathways.

Key words: signaling, arginine methylation, Sam68, SmB, proline-rich motifs.

Abbreviations used: PVDF, polyvinylidine difluoride; snRNP, small nuclear ribonucleoprotein; GST, glutathione *S*-transferase; PBST, phosphate-buffered saline with Tween 20; WW, a domain with two conserved tryptophans (W); SH3, src homology 3; SH2, src homology 2; FF, a domain with two conserved phenylalanines (F); PH, pleckstrin homology; PDZ, a domain originally identified in PSD-95, DLG and ZO-1; PTB, phospho-tyrosine binding; FHA, forkhead-associated; KH, ribonucleoprotein K homology; 14.3.3, name reflects their fraction number on DEAE-cellulose chromatography and migration position on starch gel electrophoresis; PGM, proline/glycine/methionine; WBP, WW domain binding protein; Sam68, src associated during mitosis; ELISA, enzyme-linked immunosorbent assay.

INTRODUCTION

As we passage from the genomic to the proteomic era, it is becoming necessary and possible to complement existing techniques of gene expression profiling with chip-size protein microarrays. The development of protein microarrays is in its infancy and as with all emerging technologies there are different approaches being taken to generate such arrays. Initial studies arrayed cDNA expression libraries on PVDF membranes [1]. This approach has been successful in identifying protein binding partners and enzyme substrates [2, 3]. Recent studies have paved the way for the rapid development of high density protein arrays on glass slides by establishing the concentration of protein needed, identifying surfaces for protein immobilization, and demonstrating that fluorophore-tagged proteins can be used effectively to trace and compare protein-protein interactions [4-6]. MacBeath and Schreiber arrayed proteins on aldehyde slides that were probed with fluorophore-tagged proteins to identify protein-protein interactions [5]. A single specific interaction was detected in a field of 10,000 spots. Using a synthetic ligand for FKBP12, this microarray format was also used to demonstrate the feasibility of identifying protein-small molecule interactions. The group led by Pat Brown has also made inroads into the protein microarray field [7], using poly-L-lysine slides. This study focused on the arraying of antibody/antigen pairs. Two different fluorophore-tagged protein solutions were used as probes to assay for relative specificity and abundance of proteins at physiologically relevant concentrations. Recently, nickel-coated slides were used to array GST-HisX6 tagged yeast proteins [4]. In this study 5800 yeast open reading frames were arrayed and new phospholipid and calmodulin-interacting proteins were identified. A different approach was taken by Ziauddin and Sabatini who printed cDNA expression vectors on glass slides and then cultured mammalian cells on the arrayed glass slide [6]. This resulted in small areas of transfection and protein expression where the cDNA was arrayed. This technique has been termed “transfected cell microarrays” and has successfully identified gene products that alter cellular physiology.

The stimulation of cells from outside trigger cascades of signal transduction that result in cellular responses such as growth, differentiation, and movement. These signals are transduced by networks of interacting proteins [8]. As a result of the enormous body of data gathered in recent years regarding protein-protein interactions, it has become clear that a large proportion of protein interactions occur between a domain in one protein and a small motif in its ligand (usually 8-15 amino acids) [9, 10]. These diverse associations are mediated through interactions

of a limited number of modular signaling units or protein domains. Protein interacting domains are classified in the protein family (Pfam) database (http://pfam.wustl.edu/). Protein interactions involving domain are often regulated by post-translational modification (phosphorylation, methylation and acetylation) of the smaller protein motif. The phosphorylation of proteins on serine and threonine residues can regulate 14.3.3, FHA, and WD40 domain binding [11], and the phosphorylation of proteins on tyrosine residues can regulate SH2 and PTB domain binding [12]. In addition, lysine acetylation [13], and lysine and arginine methylation [14-17] have also been implicated in the regulation of protein-protein interactions.

Here we use characterized modular protein domains to generate a chip that can be used to screen for protein interactions. Included in the protein domain array are WW [18, 19], SH3 [20], SH2 [9], 14.3.3 [21], FHA [11, 22], PDZ [23], PH [24] and FF [25] domains. In order to define the parameters of this approach we have focused our attention on the WW- and SH3-domain region of this array. These domains (WW and SH3) bind proline-rich ligands. The signaling molecule Sam68 and the core snRNP protein SmB' are two such proline-rich molecules. Sam68 interacts with SH3 and WW domain-containing proteins [16], and SmB' associates with the WW domains of spliceosome-associated protein FBP21 [26]. Using peptides derived from these proteins and specific antibodies, we have been able to demonstrate distinct and reproducible binding to subsets of protein domains, thus generating domain-binding profiles for cellular proteins.

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EXPERIMENTAL

GST fusion protein purification

GST fusion proteins were overexpressed in *Escherichia coli* DH5α cells (Life Technologies, Rockville, MD) by induction with a final concentration of 0.4 mM isopropyl-β-D-thiogalactopyranoside. Cells were broken by sonication. The resulting lysates were centrifuged and the GST fusion proteins were then batch-purified from extracts by binding to glutathione Sepharose 4B beads (Amersham Pharmacia Biotech, Piscataway, NJ) and washed in phosphate-buffered saline according to the manufacturer's instructions. The purified proteins were eluted from the beads with 30 mM glutathione, 50 mM Tris-HCl pH 7.5, and 120 mM NaCl. The purified proteins were stored in the elution buffer at –70°C in 384 well plates.

Production of protein microarrays

The proteins were arrayed in duplicate using a FLEXYS® robot (Genomic Solutions, Ann Arbor, MI). We used a high density arrayer (HAD) 48 Pin Head (FLX 12021) for arraying. The GST fusion proteins were arrayed from a 384 well plate, which contains 10μl of each protein at a concentration of 1μg/μl. The protein stocks were in elution buffer (no glycerol was added), and each protein was arrayed five times onto the same spot to increase the local concentration of protein. Proteins were spotted onto a glass slide pre-coated with nitrocellulose polymer (FAST™ Slide; Schleicher & Schuell, Keene, NH) to generate a rectangular array (3.1cm x 2cm). The arrayed proteins were air-dried. The protein chip is composed of 20 grids each, in a 5 row by 5 column format, with a distance of 700 microns between spots. Each grid thus contains 12 fusion proteins arrayed in duplicate with GST alone spotted in the middle of the grid. A 384 well master plate containing 10μl of each protein (1μg/μl) was sufficient for arraying 35-40 slides. Thus each spot contains roughly 250ng of fusion protein.

Probing the protein-domain array with a labeled peptides or proteomes

Peptide probes: Peptides were synthesized by the W. M. Keck Biotechnology Resource Center (New Haven, CT). Biotinylated peptides (10μg) were pre-bound to 5μl of Cy3-Streptavidin or Cy5-Streptavidin (Fluorolink™ , Amersham Pharmacia Biotech, Piscataway, NJ) in 500μl PBST. The fluorescent-labeled peptide was then incubated with 20μl of biotin agarose beads (Sigma, St.

Louis, MO) to remove the free Streptavidin label. Arrayed slides were blocked in PBST and 3% powdered milk, followed by the addition of 400µl fluorophore-tagged peptide. Blocking and hybridization were performed in an Atlas Glass Hybridization Chamber (Clontech, Palo Alto, CA). After one hour of incubation at room temperature, the unbound peptide was washed away with PBST, three times for 10 minutes each.

Proteome probes: Human MCF7 cells were grown to 80% confluency. Cells were then scraped into a mild lysis buffer (PBS, pH 7.2; 100mM NaCl; Complete™ protease inhibitor cocktail [Roche, Indianapolis, IN]). Cells were subsequently broken by two 30 second sonicator pulses. The array was first blocked in PBST and 3% powdered milk, and then incubated for one hour with 2 mg/ml of MCF7 cell lysate. The array was washed 3 times for 10 minutes in PBST. Bound protein was then detected by probing for one hour with a primary antibody to the protein of interest, working at a dilution used for Western blot analysis (1:1000). The array was washed 3 times for 10 minutes in PBST. The primary antibody was recognized with an appropriate FITC conjugated secondary antibody. All incubations were performed at room temperature. The anti-peptide Sam68 antibody has been described previously [27]. The anti-SmB' antibody, Ana128, was obtained from ICN Biomedicals, Costa Mesa, CA. When performing endogenous protein profiling, it is important that the primary antibody is raised against a peptide and not against a GST fusion protein, as this would result in cross-reactivity with the arrayed proteins.

Probe detection

Following the washes the slides were centrifuged dry and the fluorescent signal was detected using a GeneTAC™ LSIV scanner (Genomic Solutions, Ann Arbor, MI). A 550nm Long Pass Filter was used for the detection of Cy3 labeled probes and FITC conjugated secondary antibodies. A 675nm Band Pass Filter was used for the detection of Cy5 labeled probes. A positive signal is seen as two dots at varying angles.

RESULTS

Generation of protein-domain microarrays

We have generated and gathered, from fellow researchers, GST expression vectors harboring protein domains as well as proteins of general interest. A list of 212 constructs is shown in **Figure 1**, from which we have generated GST fusion proteins for our master plate. One hundred and forty-five protein domains are represented here and are broken down as follows: 33 WW, 23 SH3, 17 SH2, 23 PH, 23 PDZ, 7 14.3.3, 5 PTB, 4 FHA, 8 FF and 2 KH. An additional 67 GST fusion proteins without canonical protein domains are listed in the "other" section. We have arrayed this set of GST fusion protein in duplicate onto the nitrocellulose film of FAST™ Slides, using a robot. Each spot contained roughly 250 ng of fusion protein. The arrayed proteins were allowed to dry on the nitrocellulose surface and the slide was subsequently stored at 4° C.

Peptide motifs bind specifically to immobilized protein domains

To test the integrity of the arrayed domains, we synthesized biotinylated peptides that are known to bind specifically to certain protein domains. These peptides were conjugated to streptavidin-Cy3 and then used to probe the protein-domain array. The peptides used included the proline-rich P3 motif of Sam68 (which binds SH3 and WW domains [16]) (**Figure 2F**), the PGM motif of the splicing factor SmB' (which binds group III WW domains [26]) (**Figure 2B**), the PPYP motif of WBP1 (which binds group I WW domains [18]) (**Figure 2D**) and the C-terminus of Kv1.4 (which binds a subset of PDZ domains [28]) (**Figure 2C**). Unique binding profiles were detected for each of these peptides in the predicted regions of the array, demonstrating that these arrayed domains are functional and that their binding specificity is intact.

Post-translational modification of a peptide changes the binding profile

Post-translational modifications are key events associated with the initiation or the redirection of signaling pathways. We focused on arginine methylation to assess the ability of the array to distinguish between unmodified and modified peptides. We used the P3 motif of Sam68, which we have shown displays reduced binding to SH3 domain but not WW domains when arginine methylated [16]. The unmethylated peptides were conjugated to streptavidin-Cy3 and methylated peptides were conjugated to streptavidin-Cy5. A mixture of the labeled peptides was then used to probe a single protein-domain array. The unmethylated P3 peptide (**Figure 2F**) bound two WW

domains and six SH3 domains. The methylated P3 peptide (**Figure 2G**) bound the same two WW domains but only three SH3 domains. The SH3 domains that are sensitive to arginine methylation are most obvious when both the Cy3 and Cy5 signals are read simultaneously. Domains that bind the P3 motif regardless of its methylation state are yellow, whereas those domains that are sensitive to methylation are green (**Figure 2H**). A similar approach can be taken to screen for phosphorylation regulated protein-protein interactions.

Detecting the binding profiles of individual proteins from a total cell lysate

Next we attempted to determine the binding profile of endogenous proteins. To do this we established an ELISA-based experiment. First, a total cell lysate from MCF7 cells was used to probe a protein-domain array. The array was then washed with PBST and re-probed with an antibody raised against a protein of interest. Finally, the primary antibody was detected with a FITC-labeled secondary antibody. We chose to look at the binding profiles of two endogenous proteins, Sam68 and SmB'. We have obtained distinct binding profiles for these two proteins using short peptides (**Figures 2B & F**), that represent just a fraction of their entire length (**Figure 3B**). Previous studies had identified the P3 [16, 29] and PGM [26] motifs as the dominant protein interacting regions within Sam68 and SmB'. We thus reasoned that the peptide binding profile should be indicative of how the full-length protein would bind to the domain array, with perhaps a slightly broader binding spectrum for the endogenous protein, as it may harbor additional interacting motifs. Using this ELISA approach we have obtained distinct signals with both αSam68 and αSmB' antibodies (**Figures 3A & C**). Both these molecules are proline-rich and as such bind SH3 and WW domains. The proline-rich sequences of Sam68 and SmB' are different (**Figure 3B**) and they display binding profiles that are distinct, with some overlap. The SH3 and WW domain regions of the probed arrays were enlarged and compared. The pattern of binding observed for the cellular protein is very similar to that seen with the respective peptides (**Figures 3C & D**). Thus, the synthetic peptide binding reflects the binding profile of the endogenous protein.

DISCUSSION

In this report we show that a protein-domain array can be used to detect interactions between peptides and arrayed proteins, as well as between endogenous cellular proteins and the array. Of paramount importance is the fact that this array will not only detect the binding profiles of cellular proteins, but will also identify those post-translational modifications that create or prevent protein-protein interactions. Using this protein-domain array we have identified the binding profiles of two proline-rich proteins – Sam68 and SmB'. Sam68 binds both SH3 and WW domains [16, 29], and thus the observed binding profile was expected (**Figures 3A & C**). The propensity of the core splicing factor, SmB' for WW domain binding has been reported [26], but the degree of SH3 domain binding that SmB' demonstrates is rather unexpected. Splicing factors are localized in nuclear speckle domains *in vivo* [30] and both SmB' and WW domain-containing proteins have been shown to co-localize with SC35 speckles [26]. Recently, two different SH3 domain-containing proteins have also been shown to co-localize with speckles [31, 32]. It is thus possible that SmB' forms an attachment scaffold for WW and SH3 domains-containing spliceosome components.

Traditional methods of domain interaction mapping rely on blot overlay experiments [20, 33-35]. Using this approach, protein interactions are examined by electrophoretically separating fusion proteins containing regions of a protein of interest on denaturing SDS-PAGE gels, transferred to nitrocellulose and incubated with radiolabeled domain-containing fusion protein probes. Such blot overlay experiments have been used to obtain profiles of cellular tyrosine phosphorylation states using SH2 domains [36]. The protein-domain chip can be used for similar mapping and profiling experiments. This chip has the following advantages over the blot overlay approach: 1) a large number of protein domains are screened at a time, 2) conditions are mild (SDS-PAGE is not used) so proteins are more likely to be in their native conformation, 3) the screens are fast (3-4 hours) once the arrays have been generated, 4) different fluorescent labels can be used to evaluate the consequence of post-translational modifications on domain binding, and 5) the ELISA based detection of endogenous proteins is sensitive.

In summary, this protein-domain array could be very effectively used hand-in-hand with a motif-based searching algorithm like "Scansite" [37] (http://scansite.mit.edu/). Short linear sequence motifs predicted to bind domains could be identified within query proteins and experimentally verified using this type of array.

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LEGENDS

Figure 1. **A list of 212 proteins, protein domains and protein motifs that have been purified as GST fusion proteins and arrayed on nitrocellulose coated slides.** The upper panel shows the design of the array. Slides feature a 5x5 gridding pattern. Proteins are arrayed in duplicate and each block harbors 12 different fusion proteins. The middle position (M) contains GST alone as a negative marker and background indicator. The lower panel lists all the arrayed fusion proteins and their position on the slide. The domain type is indicated at the top of each box (WW, SH3, SH3, PH, 14.3.3, PDZ, FHA/PTB/KH and FF). "Other" refers to GST fusion proteins that do not contain domains but are of general interest. Contributors to this array are listed.

Figure 2. **GST fusion proteins, containing protein-interacting domains, retain their binding specificity in a microarrayed format.** Different GST fusion proteins (212) were arrayed in duplicate onto a nitrocellulose slide. (**A**) The array was probed with an αGST primary antibody and detected with a FITC-conjugated 2° antibody. (**B**) The array was probed with a Cy3-labeled SmB' peptide (biotin-PPGMRPPPPGMRRGPPPPGMRPPRP). (**C**) The array was probed with a Cy3-labeled PDZ ligand peptide from Kv1.4 (biotin-SGSGSNAKAVETDV-COOH). (**D**) The array was probed with a Cy3-labeled WBP1 peptide (biotin-SGSGGTPPPPYTVG). (**E**) The key to the arrayed domains. The identity of the interacting domains can be extrapolated from Table 1. (**F**, **G** & **H**) The same protein array was probed with a Cy3-labeled P3 peptide of Sam68 (biotin-GVSVRGRGAAPPPPPVPRGRGVGP) and with a Cy5-labeled P3 arginine methylated peptide of Sam68 (biotin-GVSVR*GR*GAAPPPPPVPR*GR*GVGP) [The asterisks denote asymmetrically dimethylated arginine residues]. (**F**) Detection of Cy3-signal. (**G**) Detection of Cy5-signal. (**H**) The Cy3 and Cy5 signals are superimposed. The yellow signal indicates protein interactions that are insensitive to arginine methylation and the green signals mark protein interactions that are sensitive to arginine methylation (see inset). (**I**) Diagrammatic representation of the signals seen in Figure 2. Single dot indicates a signal of low intensity, double dot indicates a signal of high intensity, single asterisk denotes GST fusion proteins that contain both SH3 and SH2 domains.

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Figure 3. **The protein-domain array detects endogenous protein binding profiles.** (**A-top panel**) The array was probed with an αGST primary antibody and detected with a FITC-conjugated 2° antibody. (**2nd panel**) The array was probed with an antibody to Sam68 and detected with a FITC-conjugated 2° antibody. (**3rd panel**) The array was first incubated with 2 mg/ml of MCF7 cell lysate, and then probed with an antibody to Sam68, followed with a FITC-conjugated 2° antibody. (**4th panel**) The array was first incubated with 2 mg/ml of MCF7 cell lysate, and then probed with an antibody to SmB' (Ana128), followed with a FITC-conjugated 2° antibody. The circled signals represent non-specific interactions detected by Sam68 primary antibody. No non-specific binding was detected with the Ana128 antibody. (**B**) Schematic representation of the Sam68 and SmB' and the position of the biotinylated peptides used as probes. (**C**) An alignment of the WW- and SH3-domain section of arrays probed with a total cell lysate, followed by a specific antibody (αSam68 or Ana128), compared to the binding seen with a nested peptide from these two proteins (**Figures 2B** & **F**). (**D**) Diagrammatic representation of the signals seen in Figure 3C. Single dot indicates a signal of low intensity, double dot indicates a signal of high intensity, single asterisk denotes GST fusion proteins that contain both SH3 and SH2 domains, and double asterisks mark background signals generated by cross-reactivity of the primary antibody.

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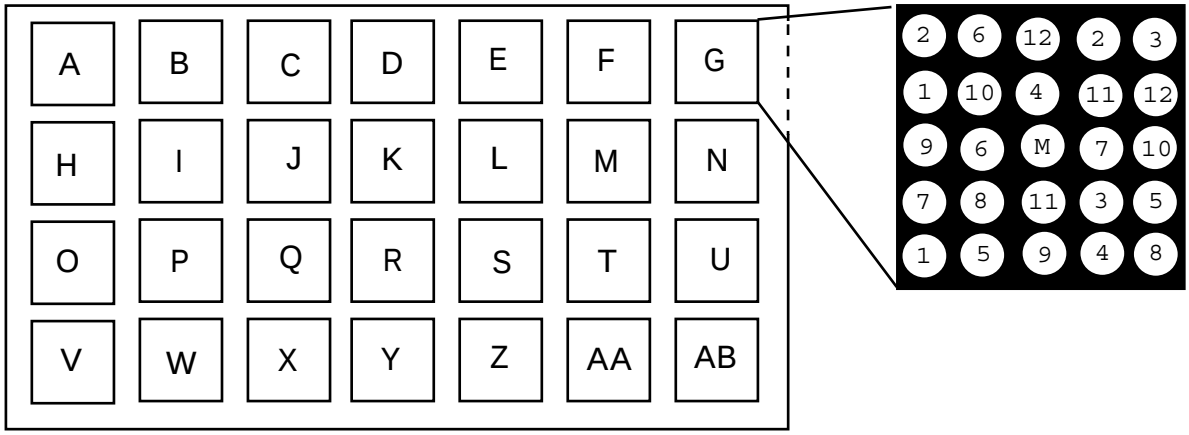


WW	WW	WW	SH3	SH3	SH2
A1 FBP11A (f)	B1 WWOX2(f)	C1 SMN4(q)	D1 Src(f)	E1 p120 ras Gap(o)	F1 Shc (f)
A2 FBP11 (f)	B2 WWOX1(f)	C2	D2 p85(f)	E2 Fyn-3 SH3/SH2(o)	F2 Src (f)
A3 NEDD4(f)	B3 ESS I(q)	C3 HYAP-WW1mutant(q)	D3 Vav c-term(o)	E3 Grb2 c-term(o)	F3 Shc (o)
A4 FBP30AB (f)	B4 Ca150 WWI (a)	C4 HYAP-WW2(q)	D4 Yrk(q)	E4 Nck(o)	F4 G-lck SH3/SH2(o)
A5 FBP30A (f)	B5 Pin 1(f)	C5 Fe65 w.t.(q)	D5 PRMT2(f)	E5 Spectrin(o)	F5 Vav F.L. SH3/SH2(o)
A6 HYPC (f)	B6 YJQ8(q)	C6 HYAP WW1 w.t.(q)	D6 Vav n-term(o)	E6	F6 Grb2(o)
A7 YAP (f)	B7 Ca150 WW2(a)	C7 YAP chicken(q)	D7 Us yes(q)	E7 Abl(o)	F7 p59 fyn SH3/SH2(o)
A8 FBP28(f)	B8 Itck B(f)	C8 Fe65 WW mutant(q)	D8 Yes(f)	E8 SLAP SH3/SH2(o)	F8 Vav SH2-GST(o)
A9 PDGFR Short(f)	B9 RSP II(q)	C9	D9 G-lck SH3/SH2(o)	E9 Ras GAP(o)	F9 PI3K c-term(o)
A10 FBP21(f)	B10 Itck A(f)	C10 DYS WW(q)	D10 Abl(f)	E10 Fyn-2(o)	F10 PI3K(f)
A11 PDGFR Long(f)	B11 RSP I(q)	C11	D11 Vav F.L. SH3/SH2(o)	E11 PLC(o)	F11 Lck(o)
A12 FBP11B(f)	B12 WWOX 1&2(f)	C12 DYS WW mutant(q)	D12 Fyn-1(f)	E12 PLC gamma(o)	F12 Yes(f)

SH2	PH	PH	14.3.3	PDZ	PDZ
G1 PLC SH2/SH2(o)	H1 Dynamin-2(n)	I1 hSos(n)	J1 14.3.3 Zeta(f)	K1 NHERF PDZ2(e)	L1 PDZ CAP1(g)
G2 PI3K n-term(o)	H2 Dynamin-1(n)	I2 Ras GAP(n)	J2 14.3.3 Beta(f)	K2 NHERF PDZ1(e)	L2 PDZ3 CAP1(g)
G3	H3 Akt(n)	I3 PLC (209-572)(b)	J3	K3 Alfa-syn(m)	L3 PDZ8 (z)
G4	H4 Spectrin(n)	I4 PLC (5-180)(b)	J4 14.3.3 Eta(f)	K4 Gamma-syn(m)	L4 PDZ9 (z)
G5	H5 KIAA 0053(n)	I5 PLC (5-295)(b)	J5 14.3.3 Epsilon(e)	K5 Densin PDZ2(i)	L5 PDZ10 (z)
G6	H6 PLC gamma(n)	I6 PLC (570-848)(b)	J6	K6 GST PDZ3(l)	L6 PDZ 11/12 (z)
G7	H7 Pleckstrin(n)	I7 PLC (5-150)(b)	J7	K7 Beta-syn(m)	L7 PDZ 12 (z)
G8F Fyn(o)	H8 PLC delta(n)	I8 PLC (849-1234)(b)	J8 14.3.3 Gamma(e)	K8 AF6 PDZ1(i)	L8 PDZ 13 (z)
G9	H9 DAGK delta(n)	I9	J9	K9 NHERF2(g)	L9 KIT (z)
G10 Abl(o)	H10 DAPP1(n)	I10 Itk(n)	J10 14.3.3 Tau(f)	K10 SAP 97 3x PDZ(f)	L10 KIT delta 976 (z)
G11	H11 Beta ARK-1(n)	I11 PLC (5-208)(b)	J11	K11 NHERF1(g)	L11
G12 SLAP SH3/SH2(o)	H12 Grp-1(n)	I12 PLC Beta3(n)	J12 14.3.3Sigma(e)	K12 SAP 97 F.L.(f)	L12 PDZ CAP2(g)

FHA/PTB/KH	FF	OTHER	OTHER	OTHER	OTHER
M1 RAD 53 FHA2(e)	N1 FBP 11(1)(f)	W1 GST-CTD(w)	X1 HDAC1 (v)	Y1 PRMT1 (f)	Z1 EHR(f)
M2 RAD 53 FHA1(e)	N2 CA 150 (1-3)(f)	W2 Cox2(f)	X2 HDAC2 (v)	Y2 PRMT2 (f)	Z2 EH(f)
M3 PTB2 FE 65 L2(s)	N3 CA 150 (4-6)(f)	W3 MRE11 c-term (f)	X3 HDAC3 (v)	Y3 PRMT3 (f)	Z3 Xiap (124-240)(p)
M4 PTB1 FE 65(s)	N4 FBP 11 (3-4) (f)	W4 TARPP c-term (f)	X4 HDAC4c1 (u)	Y4 CARM (f)	Z4 GAR(f)
M5 PTB DAB2(k)	N5 PRP (1-2)(f)	W5 TARPP F.L. (f)	X5 HDAC4C6 (u)	Y5 PRMT6 (f)	Z5 SMN(f)
M6 QKI KH(o)	N6	W6 FKHRL w.t.(x)	X6 HDAC5R4 (u)	Y6 PABP F.L.(f)	Z6 BARDI 13-638(d)
M7 PTB2 FE 65(s)	N7 p190B(3-4)(f)	W7 FKHRL m(x)	X7 HDAC1 (t)	Y7 PABP hind III(f)	Z7 Histone 3(f)
M8 PTB DAB1(k)	N8 p190B(1-2)(f)	W8 FKHRL N-term(x)	X8 HDAC2 (t)	Y8 PABP c-term (f)	Z8 EVL(f)
M9	N9 p190B(1-4)(f)	W9 hDM2 (x)	X9 HDAC3 (t)	Y9 PABP w.t.(f)	Z9 HPl B F.L.(j)
M10 KI FHA (1-168)(r)	N10	W10 hER alfa(x)	X10 HDAC10 (f)	Y10 PABP R-1(f)	Z10 MENA(f)
M11 SAM 68 KH(o)	N11	W11 Tubby c-term (z)	X11	Y11 PABP R-1.2 (f)	Z11 BARDI 13-777(d)
M12 CHN2 FHA(e)	N12	W12 Tulp3 c-term (z)	X12	Y12	Z12 Profilin(f)

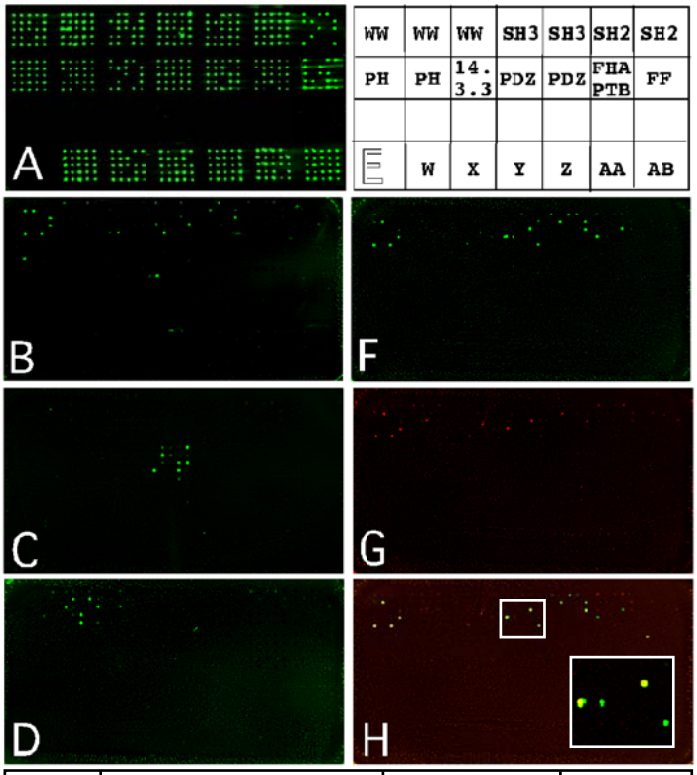
OTHER	OTHER
AA1 p53 F.L.(q)	AB1 WBP 11-5'(f)
AA2 U1A(f)	AB2 SRPK 2-5'(f)
AA3	AB3 Id 28(f)
AA4 DP-1(c)	AB4 Id 10(f)
AA5 p73 beta(h)	AB5 Sap 62 3x motif(f)
AA6 p53 BP-2(q)	AB6 B dystroglican(f)
AA7 E2F4(c)	AB7 3 BPl(f)
AA8 p73 alfa(h)	AB8 11 PGM(f)
AA9 p53 BP-2mt2(q)	AB9 SMB Full(f)
AA10 E2F1(c)	AB10 SmB'(f)
AA11 p53 BP-2mt1(q)	AB11 U1C(f)
AA12 p53 W.T(h)	AB12 WBP 11-3'(f)

VECTOR SOURCE:

a- Gracia Blanco, Mariano.
b- Snyderman, Ralph.
c- Johnson, David.
d- Manley, James.
e- Yaffe, Michael B.
f- Bedford, Mark.
g- Biber, Jürg.
h- Blandino, Giovanni.
i- Borg, Jean-Paul.
j- Kouzarides, Tony.
k- Cooper, Jonathan A.
l- Dobrosotskaya, Irina.
m- Gee, Stephen.
n- Lemmon, Mark A.
o- Richard, Stéphane.
p- Shi, Yigong.
q- Sudol, Marius.
r- Takagi, Masatoshi.
s- Zambrano, Nicola.
t- Chang, Kun-Sang.
u- Jianrong, Lu.
v- Yang, Wen-Ming.
w- Dynan, William.
x- Hu, Mickey.
y- Shapiro, Lawrence.
z- Mancini, Annalisa

FIGURE 1

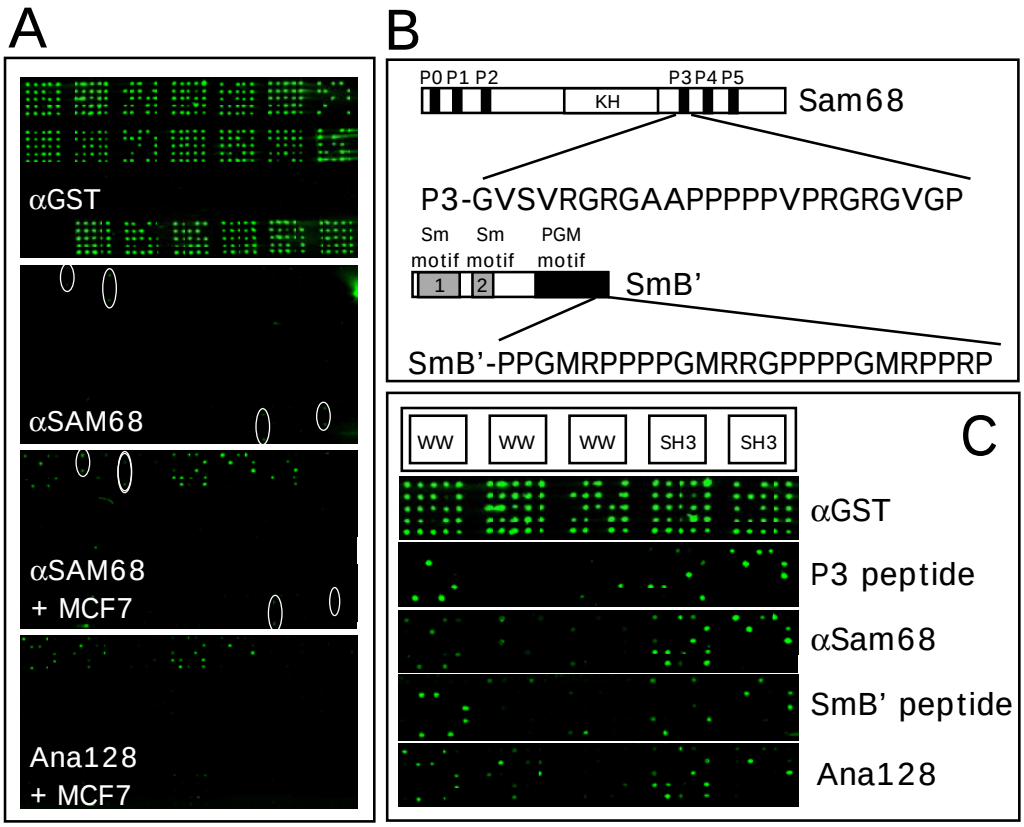
Biochemical Journal Immediate Publication. Published on 23 Jul 2002 as manuscript BJ20020860



Probes \ Domains	Panel	WW													SH3								PDZ					
		FBP 11	FBP 21	FBP 30AB	FBP 30A	Fe 65	SMN4	WWOX 1	RSP I	RSP II	Itck A	Itck B	Nedd4	Yap	US YES	YES	PRMT2	PLC γ	P85	Fyn (1)	Fyn (2)	Fyn (3)*	α-syn	γ-syn	GST PDZ3	β-syn	SAP97 3x	SAP 97 FL
SmB'	B		••	••	••		•	•							••		••	••	••		••							
P3	F			••	••	•									••	••		••	•	•	••	••						
P3 Arg *	G			••	••	•									••						•							
WBP1	D								••	••	••	•	•	•														
Kv1.4	C																						••	•	••	••	••	•

FIGURE 2

Biochemical Journal Immediate Publication. Published on 23 Jul 2002 as manuscript BJ20020860



D

Probes \ Domains	WW													SH3									
	FBP 11	FBP 21	FBP 30AB	FBP 30A	Itck A	WWOX 1-2	WWOX 1	RSP I	RSP II	ESSI	Fe 65	YJQ 8	SMN4	US YES	YES	G-Ick*	PRMT2	PLC γ	P85	Fyn (1)	Fyn (2)	Fyn (3)*
P3 peptide			●●	●●							●			●●	●●			●●	●	●	●●	●●
αSam 68	●		●	●						●		●**	●**	●●	●●	●	●●	●	●	●●	●●	●●
SmB' peptide		●●	●●	●●			●						●	●●			●●	●●	●●		●●	
Ana128	●●	●●	●●	●●	●●	●		●	●					●●	●●	●	●●	●	●	●	●●	●

FIGURE 3