Minireview

# Can we infer peptide recognition specificity mediated by SH3 domains?

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**Abstract Protein interaction domain families that modulate the formation of macromolecular complexes recognize specific sequence or structural motifs. For instance SH3 and WW domains bind to polyproline peptides while SH2 and FHA domains bind to peptides phosphorylated in Tyr and Thr respectively. Within each family, variations in the chemical characteristics of the domain binding pocket modulate a finer peptide recognition specificity and, as a consequence, determine the selection of functional protein partners in vivo. In the proteomic era there is the need for reliable inference methods to help restricting the sequence space of the putative targets to be confirmed experimentally by more laborious experimental approaches. Here we will review the published data about the peptide recognition specificity of the SH3 domain family and we will propose a classification of SH3 domains into eight classes. Finally, we will discuss whether the available information is sufficient to infer the recognition specificity of any uncharacterized SH3 domain. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

*Key words:* Protein module; Target recognition; Interaction network; Peptide repertoire; Protein interaction

## 1. Introduction

The SH3 domain is probably the most widespread protein recognition module in the proteome and more than 1500 different SH3 domains can be identified by search algorithms in protein databases. It is found in proteins that have been implicated in signal transduction, cytoskeleton organization and membrane traffic. All SH3 domains share a highly conserved fold that can be represented as a sandwich formed by two three-stranded β-sheets [1]. One side of the sandwich is rather hydrophobic and constitutes the ligand binding surface (Fig. 1).

The discovery that most SH3 ligands are rich in prolines [2–6] and the analysis of several structures of SH3 domains complexed with their peptide ligands [7–10] led to the formulation of a general SH3-peptide binding model [9,10]. SH3 ligands contain two XP dipeptides, separated by a scaffolding residue (often a proline). The two XP moieties in the core (XP-x-XP) motif occupy two hydrophobic pockets formed by residues that are conserved in most SH3 domains. The third binding pocket is lined by negative residues and can host a positively charged side chain flanking the core motif. SH3 ligands bind to their receptors in a left-handed polyproline type II (PPII) helical conformation in either of two opposite orientations depending on the position of a positive residue in the peptide sequence. Peptides that bind in a type I orientation conform to the consensus RxLPP#P (where # is normally a hydrophobic residue), while peptides that are characterized by Px#PxR (type II) bind in the opposite orientation. The SH3 domain of the protein kinase Abl binds to ligands that have a tyrosine (or a large hydrophobic residue) in place of the positively charged side chain at position P−3 of class I peptides (for residue nomenclature see Fig. 1). This model has served as a framework in the interpretation of SH3 binding experiments and in the identification of SH3 peptide targets on newly discovered proteins.

More recently, however, several exceptions have challenged the generality of this model suggesting that the binding potential of the SH3 domain family might be larger than originally thought. For instance the SH3 domain of amphiphysin 1 was shown to prefer class II peptides containing an arginine instead of an aliphatic residue at position P0 [11,12]. More strikingly, the SH3 domain of Eps8 binds to ligands that contain a PxxDY motif [13] while the second SH3 domain of the immune cell adapter FYB/SLAP was recently shown to form a complex with proteins containing a tyrosine-based RKxxYxxY motif [14]. The assignment of this last domain to the SH3 family, however, still needs structural confirmation since several characteristic SH3 residues are missing from its primary sequence. Finally the minimal sequence required for YAP recognition by the SH3 domain of p53BP2 is VPMRLR [15]. Although these 'atypical' ligands do not contain a classical PxxP signature they still bind to the PPII binding pockets, as demonstrated by mutagenesis experiments. However, it is not clear whether they actually adopt a PPII conformation. One typical example of an SH3-mediated interaction that does not involve a peptide in PPII conformation is the binding of the SH3 domain of p53BP2 to p53 [16]. Furthermore, the SH3 domain of Pex13p, a protein involved in peroxisomal assembly, binds to two very different ligands, one of which, Pex5p, does not contain a polyproline motif and binds in an α-helical conformation to an SH3 region that is different from the PPII peptide binding pocket [17].

Several excellent surveys have recently covered the functional and structural aspects of SH3-mediated interactions [18–20]. Here we will briefly review recent systematic approaches and we will ask whether the vast amount of structural and biochemical information that has been collected makes it possible to infer the recognition specificity of any newly discov-

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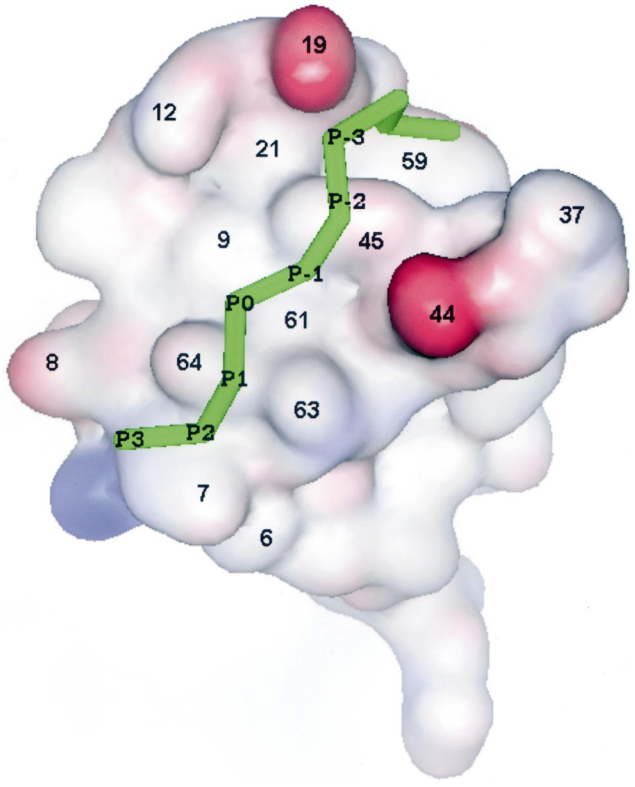


Fig. 1. Surface representation of the Abl SH3 domain bound to the peptide APT**M**P**PP**LP**P** [7]. The surface is colored according to charge with negative residues colored in red and positive ones in blue. Only the α carbon backbone of the ligand peptide is shown. The nomenclature of the residues in the ligand peptide are according to Lim et al. [9] where P0 corresponds to the position of the first Pro in the PxxP motif of class 1 peptides. Residue numbering in the SH3 domain is according to the alignment in Fig. 2.

ered SH3 domain. It is important to stress that, although most SH3 domain partners contain a PxxP motif, the SH3 is an ancient domain that has existed for sufficient time to extensively explore structural and functional diversity. Our analysis will only cover those 'interaction modes' that are mediated by binding to relatively simple short peptides. The significance of this analysis relies on the assumption that most of the natural protein partners of any given SH3 domain contain the preferred proline binding motif, as determined for instance by searching peptide repertoires. Alternatively, other contacts distal to the polyproline core motif may be prominent in determining ligand preference. Examples that support either models have been reported but the relative importance of 'core' and 'distal' interactions in determining partner recognition is not clear [21–24].

Although the scientific literature contains a large number of reports about SH3 domain structures and preferred peptide ligands, this collection may not represent a balanced description of the SH3 domain family recognition specificity. An SH3 domain that happens to bind to a 'classical' RxxPxxP peptide will stand fewer chances of being reported in print than a domain found to bind to an 'odd' peptide motif. In order to provide a general unbiased picture of the SH3 domain family, we have recently characterized the binding potential of the entire SH3 repertoire of the complete genome of the yeast *Saccharomyces cerevisiae* [25].

## 2. The yeast SH3 repertoire

Domain or protein family databases contain more than 1500 SH3 domains. A ψ-blast search of the *S. cerevisiae* proteome reveals a total of 24 proteins containing SH3 domains. Three of these proteins contain multiple SH3 domains. Sla1p has three SH3 domains in tandem while Bem1p and Bzz1p have two.

When all the SH3 domains in the PFAM database are aligned and organized into a phylogenetic tree by the ClustalW program, the yeast domains are represented in most of the branches of the tree indicating that a large fraction of the diversity observed in the SH3 gene family is represented in the smaller yeast SH3 repertoire. SH3 sequences can be aligned relatively easily in the conserved core domain. However, the length of the loops connecting the β-strands differs in size. Most of the SH3 domains have an RT loop of 18 residues and an n-Src loop of four residues. The spread in loop length, however, can be considerable ranging from 15 to 31 residues in the RT loop and from three to 31 residues in the n-Src loop. A similar spread in loop lengths is also represented in the *S. cerevisiae* SH3 family. Thus, the conclusions derived from a detailed study of the binding potential of the yeast SH3 domains are likely to shed light on the rules governing recognition specificity mediated by SH3 domains in general.

## 3. Ligand preference

Twenty-five *S. cerevisiae* SH3 domains were used as baits to select ligands from a nonapeptide library of random amino acid sequence displayed on the capsid of filamentous bacteriophages [26]. Ten to 20 positive clones were sequenced in each panning experiment and the *consensus* sequences reported in Table 1 were deduced from the comparison of the amino acid sequences of the peptides displayed by the selected clones. Four SH3 domains (Cdc25, Hof1, Ydl117W, Yar014C) could not select any ligand from the repertoire. Furthermore, these domains would not bind to the polyproline peptides selected by the other domains. In conclusion, these four SH3 domains, at least when isolated from their protein context, do not bind to any simple linear peptide with micromolar affinity. The vast majority of the yeast SH3 domains selected proline rich peptides. The only exception is the SH3 domain of Fus1p that was found to bind preferentially to peptides conforming to the *consensus* rxxR(ST)(TS)Sl, where x is any amino acid and capital and small letters represent residues that are present in more than 80% and 50% of the selected peptides, respectively. Although this consensus ligand does not contain any essential proline, it still binds to the canonical SH3 domain peptide binding surface as demonstrated by mutation analysis. Most of the *consensus* ligand peptides that we determined could be confidently assigned to class I (+xxPxxP) or class II (PxxPx+) motifs. Myo3p and Myo5p displayed preference for a Tyr, or another aromatic side chain, instead of the positively charged side chain at position P−3. This is reminiscent of the *consensus* ligand of the SH3 domain in the Abl tyrosine kinase. Finally the first SH3 domain of the protein Bem1 would not bind to typical class I or class II motifs but rather selected peptides containing the PpxVxPY *consensus*.

We have constructed structural models of most of the yeast SH3 domains and we have attempted to rationalize ligand

Table 1
Classification of SH3 recognition specificity

| Class | Class *consensus* | SH3 domain | *consensus1* | *Ref* |
|---|---|---|---|---|
| 1R | Rx#PxxP | Rvs167 | Rx#PxpP | [25] |
| | | Nbp2 | PxRPaPxxP | [25] |
| | | Pex13 | RxlPx#P | [25] |
| | | Yhl002 | yRp#PxxP | [25] |
| | | Sla1-3, | hRxpPxpP | [25] |
| | | Yes | RPLPxLP | [30] |
| | | PI3Kp85 | RPLPPLP | [4] |
| | | Src | RPLPx#P | [4,30] |
| | | Hck | RxLPx#P | |
| | | Lyn | RPLPPLP | [4] |
| | | Fyn | RPLPP#P | [4] |
| 2R | PxxPxR | Yfr024 | PpLPxRP | [25] |
| | | Ysc84 | PxLPxR | [25] |
| | | Ygr136 | Px#PxRp | [25] |
| | | Ypr154 | Pp#PxRp | [25] |
| | | PLCg | PPVPPRP | [30] |
| | | CAP | PxPPxRxSSL | [31] |
| | | p53BP2 | RPx#P#R+ | [30] |
| | | Grb2-C | PxxPxR | |
| 1K | +xxPxxP | Sho1 | s+xLPxxP | [25] |
| | | Bzz1-1 | K+xPPpxp | [25] |
| | | Bzz2-2 | ++pPPPp#P | [25] |
| | | Itk/Tsk, | YxKxPPPIP | [32] |
| 2K | PxxPx+ | Crk N | P#LP#K | [30] |
| | | Cortactin | +PP#PxKPxWL | [30] |
| | | Abp1 | +xxPxxPx+PxW# | [25] |
| 1@ | Px@xxPxxP | Abl | PPx@xPPP#P | [4,30] |
| | | Myo3 | Px@pPPxxP | [25] |
| | | Myo5 | Px@pPPxxP | [25] |
| | | Spectrin | @xPPx#P | |
| 2D | PxxDY | Eps8 and rel | PxxDY | [13] |
| X ORS | | Ygr136 | Rx+%xlP | [25] |
| | | Ypr154 | @+RPp%%%P | [25] |
| | | Bbc1 | P+#PxRP | [25] |
| | | | R+xPxpP | |
| | | Boi1 | pPRxPrR# | [25] |
| | | | PxRxPxR | |
| | | Boi2 | pPRnPxR# | [25] |
| | | | PxRNPxR | |
| | | Amph | PxRPxR | [11] |
| | | End | P+RPPxP | [11] |
| | | Fus1 | RxxR(ST)(ST)(ST)L | [25] |
| | | Bem1_1 | PPxVPY | [25] |
| Y | No peptide selected. | Cdc25, Hof1, Ydl117w, Yar014c | | [25] |

[1]For simplicity, for some SH3 domains, only a reduced core *consensus* was reported in this table. Consensus sequences without a reference are unpublished results from our laboratory.

preference. This turned out not to be always straightforward because specificity is largely determined by the residues in the RT and n-Src loops that, because of their variability, cannot be confidently modeled.

Bzz1-1 and Bzz-2 have a preference for an extra positive charge at P−4. Ypr154, Ygr136, Abp1, Boi1, Boi2, Sla1-3 and Bbc1 also like a second positive charge in the ligand peptide. Inspection of their peptide binding surface reveals a higher density of negative residues in the specificity pocket since they all share the characteristic of having a negative residue at positions 19, 20 in the RT loop and/or at position 44 in the n-Src loop. On the other hand, we have not been able to find a simple explanation for the preference for arginine at P+1 in class II peptides bound by Boi1, Boi2 and Bbc1. This is possibly a consequence of the inadequacy of some of the models because of the still insufficient number of structures that describe the interaction between SH3 domains and the cognate peptides.

## 4. Classification of SH3 domains

In Table 1 we have compared the recognition specificity of the recently characterized yeast SH3 domains with that of several other domains, by grouping them into eight classes, according to the similarities of their preferred ligands. Some domains are able to bind to different peptide *consensus* sequences and are therefore assigned to more than one class.

SH3 domains that recognize canonical Rx#PxxP or

Px#PxR motifs are the most numerous and are assigned to classes 1R and 2R, respectively. The distinction into two different classes is somewhat arbitrary since some of these domains bind to both class I and class II peptides. However, most domains show a preference for either peptide orientation and we therefore considered it useful to formally maintain the distinction. Classes 1K and 2K include domains that bind to KxxPxxP or PxxPxK [32], although they sometimes tolerate an Arg at position P−3 of the ligand peptide. The founder of class 1@ is the well studied SH3 domain of Abl which was shown to bind to class I peptides that have an aromatic (or sometimes aliphatic) residue instead of a positively charged one at P-3.

Several other SH3 domains bind to peptides that cannot be confidently assigned to any of the classes that we have defined previously. These domains can be considered members of specificity classes that, at the moment, contain a single element. In the present classification they are grouped into one class, dubbed X or ORS (odd recognition specificity). The SH3 domains of the Eps8 family that bind to the PxxDY *consensus* have been grouped into a separate specificity class since a sizeable number of domains of this family have already been characterized [13,30,31]. This class was named 2D although there is no conclusive evidence that the ligand peptide folds into a PPII conformation and binds in a type II orientation. The last domain class, termed Y, contains those domains for which we have not been able to define any preference for simple linear peptides.

## 5. Searching for classification rules

Fig. 2 shows the alignment of the amino acid sequences of the SH3 domains within the eight classes. Inspection of the alignments reveals some regularity but only few absolute rules. For instance, the AL(YF)D(YF) (positions 5–9 in the alignment), WW (45–46), and PXNY (61–64) motifs, which form the hydrophobic pockets that host the PPII helix, are highly conserved in the domains that bind to typical class I or class II ligands, while the domains that have been assigned to the ORS class deviate to different extents from the canonical pattern. Similarly the (EDT)–(LIV) motif in the RT loop (21–22) is not present in SH3 domains such as the ones of Hof1, Cdc25 and Yar014c that, in our experiments, failed to select simple linear peptides. These and other empirical rules can be implemented in a simple algorithm based on position-specific scoring matrices that permits ranking any SH3 domain according to the probability that it would bind to a polyproline peptide. A similar approach can be used to assign SH3 domains to any of the classes although the limited number of members in some classes renders the statistical approach less significant.

As already pointed out [8,7], the residue at position 21 in the RT loop of an SH3 domain is one of the major determinants of the identity of the residue at position P−3 in the ligand peptide. The vast majority of SH3 domains have either Asp or Glu at position 21. Domains displaying an Asp, without exception, bind to peptides that have a positive residue at P−3. By contrast, those that have a Glu (or Thr, in the case of Abl) bind to peptides that do not contain positively charged residues only if positions 17–19 in the RT loop are not occupied by a second negatively charged side chain. This rule has a high predictive value and is confirmed by experiments carried out with artificial SH3 repertoires obtained by randomization of the ligand binding surface of the Abl SH3 scaffold [27] (Panni et al., submitted). Striking is also the presence of glutamine at positions 12 and 18 in most of the RT loops of the SH3 domains assigned to class 2R. Several other regularities can be revealed by a close inspection of the residues that are conserved in the alignment of the eight specificity classes, although none of these can be used as a strict classification rule. Furthermore, it is sometimes difficult to distinguish whether a residue is conserved in the alignment because it is involved in making specific contacts with a shared ligand, or rather it is only a relic of a common evolutionary history. Particularly instructive is the analysis of the 2D class that includes close relatives of Eps8 that bind to PxxDY peptides. Mongiovì et al. [13] noticed that members of this SH3 class have an Ile at position 64, a position that is normally occupied by a Tyr or a Phe in the vast majority of the members of the SH3 domain family. Since this residue is involved in the formation of one of the hydrophobic pockets, Ile 64 was promptly blamed for the unusual recognition specificity of this SH3 class. However, site-directed mutagenesis has not confirmed the prediction since an Eps8 SH3 in which Ile 64 was changed to Tyr still binds to PxxDY peptides. By panning SH3 repertoires [27] with a peptide that contains the PxxDY motif we have recently shown that the major determinant of PxxDY recognition is the positively charged residue at position 43 in the n-Src loop since all the SH3 domains that are selected from the repertoire share this characteristic. Furthermore, Eps8 SH3 domains that have been mutated in this residue do not bind any longer to PxxDY peptides (Panni and Cesareni, unpublished).

## 6. SH3 domain residues that are not directly involved in peptide binding may affect recognition specificity

The search for rules underlying an elusive recognition code must rely on the assumption that the SH3 domain residues that are involved in ligand recognition can be aligned with confidence and that their 'binding properties' are largely independent of the underlying scaffold. Although this might turn out to be true for the very conserved regions in the hydrophobic pockets, one has to consider that most ligand specificity is determined by the structure of the RT and n-Src loops. These loops are highly variable in length and, as a consequence, model building is less accurate. Furthermore, a couple of recent reports pointed out how SH3 residues that do not make direct contact with the ligand may play a dramatic role in determining the sequence of the preferred partner peptide [15,28]. Although the SH3 domain of p53BP2 binds to class II peptides, as determined by screening of phage displayed repertoires, it also recognizes the VPMRLR peptide in the sequence of its physiological partner YAP [15]. Espanel and Sudol identified two residues that are uncommon at their respective positions in the alignment and could be held responsible for this odd recognition specificity. One is a Trp at position 47 (in the alignment of Fig. 3) while the second is a Leu at position 64. By mutating these residues into Arg and Tyr respectively, they were able to prove that W47R prevents the binding to VPMRLR while enhancing the affinity for typical class II ligands. By contrast, the mutation L64Y has no effect on recognition specificity. Since the side chain of the residue at position 47 is not involved in the formation of

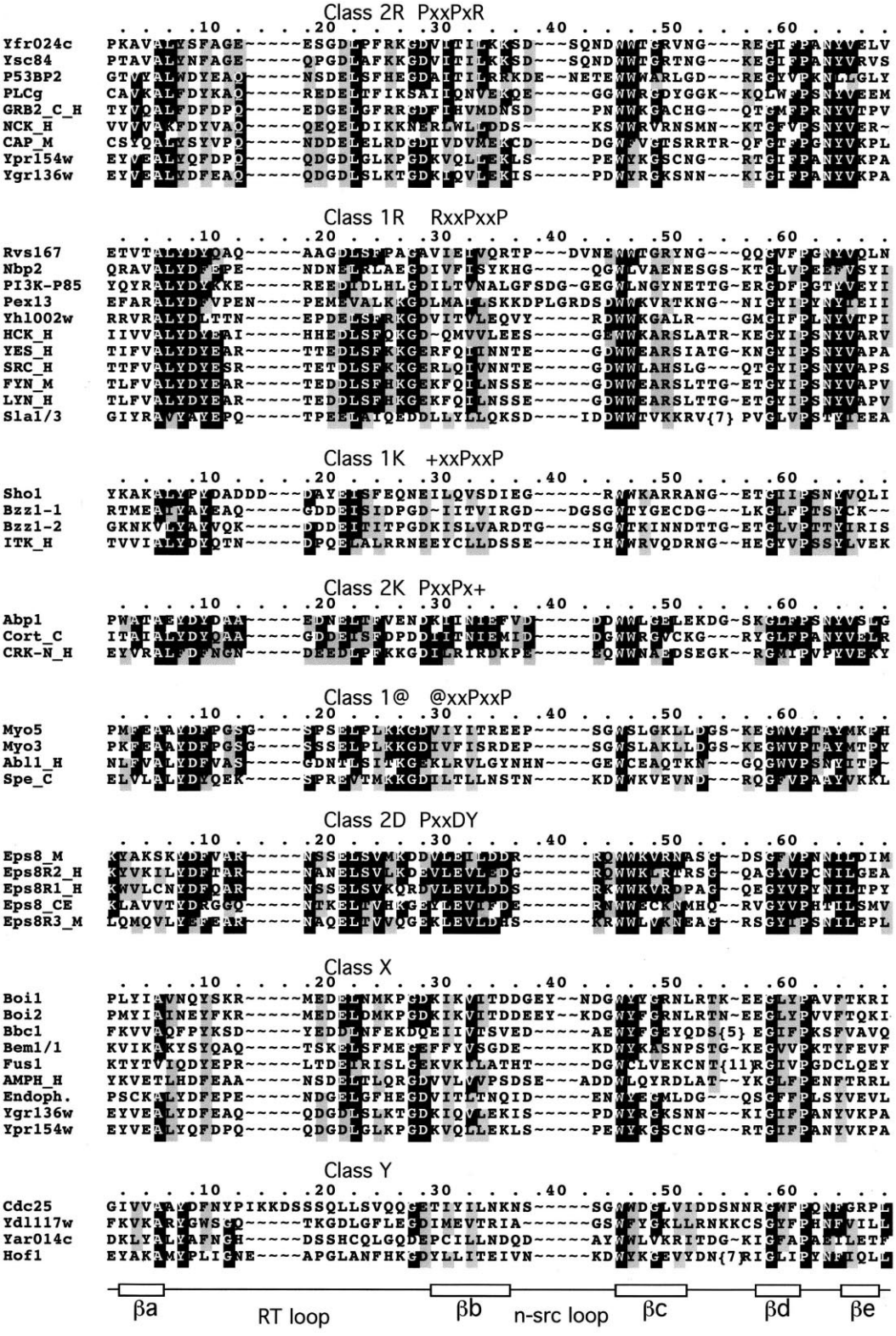


Fig. 2. Multiple alignments of SH3 domains grouped into classes of homogeneous recognition specificity. Residues that are conserved in more than 50% of the sequences have a black background while residues with side chains with similar properties have a gray background.

the ligand binding pocket, this result cannot be rationalized with a simple model and stresses the importance of 'long distance' effects. Similarly the recent determination of the structure of the SH3 domain of the yeast Abp1 protein and site-directed mutagenesis experiments revealed that the Glu at position 6 plays a major role in determining the extended *consensus* of the Abp1 SH3 domain [28]. Also this result is unexpected because the residue at position 6 is not predicted to make contact with the ligand peptide. Notwithstanding these observations, the available data and results from selec-

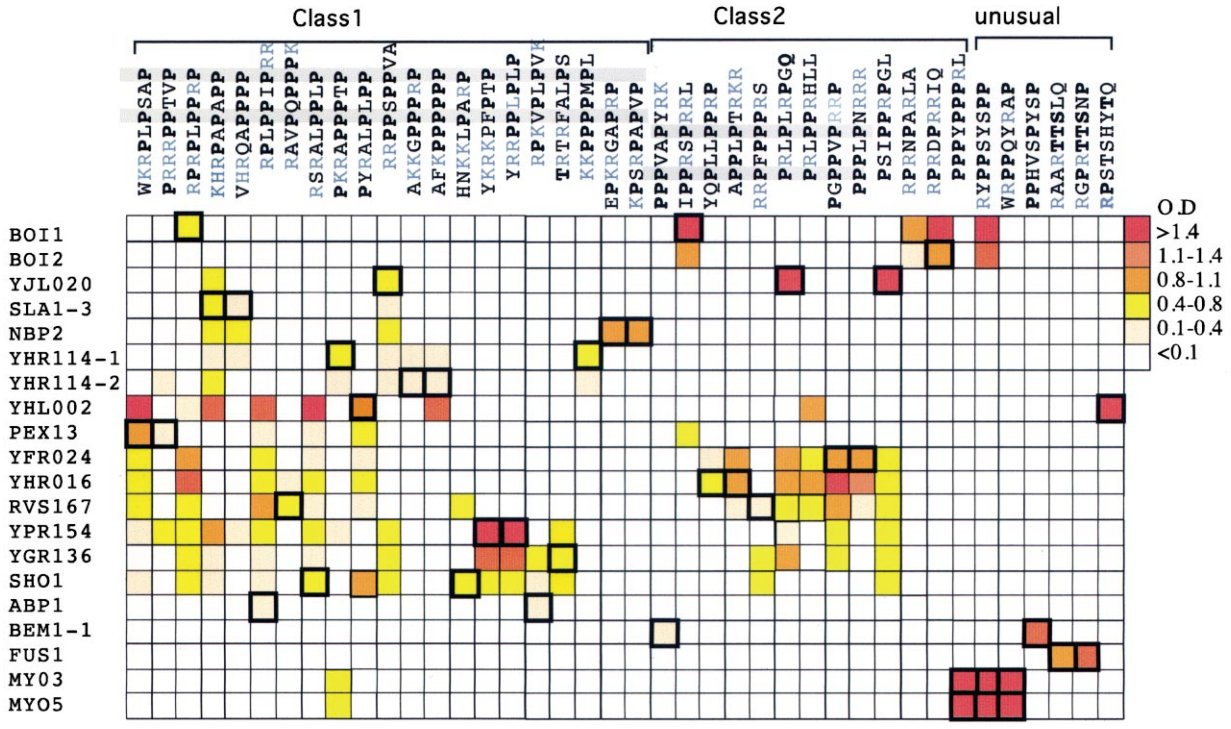


Fig. 3. Cross-reactivity of different SH3 domains. Twenty SH3 domains were tested with 40 different peptides in an ELISA-type experiment. The reactivity of each SH3 domain is reported in a semi-quantitative manner according to the color scale in the lower part of the figure. Colored squares boxed with thick lines indicate the peptides that were selected by the SH3 domain in the corresponding column.

tion of SH3 domain repertoires (Panni et al., submitted) suggest that residues in the peptide binding pockets determine recognition specificity in a way that is largely independent of the underlying scaffold.

## 7. Cross-reactivity

Most of the considerations elaborated above are based on results obtained by screening phage-displayed peptide repertoires. Panning of peptide repertoires makes it possible to determine the preferred ligands of the different bait domains in the specific selection conditions. It is possible, however, that the highly specific *consensus* sequences reported in Table 1 only reflect minor differences in affinities and that SH3 domains may bind, with comparable strength, to peptides displaying different motifs. Thus, we were interested in determining the degree of promiscuity (cross-reactivity) in SH3–ligand interaction. In Fig. 3 we have reported, in a semi-quantitative manner, the results of an ELISA-type experiment in which 20 yeast SH3 domains were tested for their ability to bind to a collection of 40 peptides displayed on filamentous phage capsids. The resulting 'interaction map' in Fig. 3 represents a description of the recognition specificity of the yeast SH3 gene family.

Perhaps reassuringly, most SH3 domains have the highest reactivity when challenged with the partner peptides, that is those that were selected by the panning experiments (boxed). Many of them, however, also reacted with peptides that were originally selected with different domains. As expected, the most specific domains were those displaying a non-conventional *consensus*: Myo3p, Myo5p, Bem1p-1, Fus1p and Abp1p. Also Boi1p, Boi2p, Bbc1p and Sla1p-3 were found to be rather selective although their *consensus* ligands can be easily aligned to class I or class II motifs. The remaining SH3 domains would bind to a larger number of peptides that could be matched to typical +xxPxxP or PxxPx+ *consensus* sequences. Bzz1p-1, Bzz1p-2, Yhl002p and Pex13 would mostly bind to class I peptides while Yfr024, Ysc84, Rvs67, Ypr154, Ygr136 and Sho1 bind to both peptide classes. Collectively these results indicate that SH3 domains have distinct specificities and support the classification that we have outlined (Table 1). However, they also indicate that the class borders are not strict and that some SH3 domains can bind to peptides that are typical of more than one class.

## 8. Conclusions and perspectives

A number of reports over the past few years have indicated that the SH3 scaffold can support a wide variety of different interactions and that it might be difficult to constrain all of them into a single simple interpretative model. However, the recent characterization of the recognition potential of the entire SH3 repertoire of *S. cerevisiae* supports the notion that binding of SH3 domains to simple peptides folded into a PPII helix is likely to govern the formation of a large number of protein complexes [25].

The ability to infer the preferred ligand of any SH3 domain and, as a consequence, to obtain information about likely functional protein partners would represent an important advance. Although some recognition rules begin to emerge, the available data do not support the idea of a simple recognition code whereby the identity of the side chains at a limited number of positions in the SH3 domain binding pocket would determine unequivocally ligand preference.

On the other hand, natural protein partners do not necessarily contain amino acid sequences that exactly match the

sequence of the 'strict' *consensus* ligand, as determined by screening peptide repertoires under highly stringent conditions. An attainable goal would be the ability to infer a broad *consensus*, sufficiently selective to limit the number of candidate partners for further analysis by different more demanding experimental or informatic approaches. At the same time, however, the *consensus* should be sufficiently broad to avoid missing physiological partners that do not contain an exact match to the selective *consensus*.

We have used the published data obtained by panning peptide repertoires with a large number of SH3 domains to define specificity classes and we have shown the emergence of rules that help to tentatively assign any SH3 domain to eight different broad specificity classes. The addition of fresh data to classes that are not sufficiently populated will eventually make it possible to apply, with increased confidence, statistical methods like SPOT [29] or other methods based either on position-specific profiles or on neural network approaches to infer detailed binding specificity within the eight broad classes.

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