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Characterization of PDZ domain-peptide interaction interface based on energetic patterns

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Abstract

PDZ domain is one of the abundant modular domains that recognize short peptide sequences to mediate protein-protein interactions. In order to decipher the binding specificity of PDZ domain, we analyzed the interactions between 11 mouse PDZ domains and 2387 peptides using a method called MIEC-SVM, which energetically characterizes the domain-peptide interaction using molecular interaction energy components (MIECs) and predicts binding specificity using support vector machine (SVM). Cross-validation and leave-one-domain-out test showed that the MIEC-SVM using all 44 PDZ-peptide residue pairs at the interaction interface outperformed the sequence-based methods in the literature. A further feature (residue pair) selection procedure illustrated that 16 residue pairs were uninformative to the binding specificity, even though they contributed significantly (~50%) to the binding energy. If only using the 28 informative residue pairs, the performance of the MIEC-SVM on predicting the PDZ binding specificity was significantly improved. This analysis suggests that the informative and uninformative residue interactions between the PDZ domain and the peptide may represent those contributing to binding specificity and affinity, respectively. We performed additional structural and energetic analyses to shed light on understanding how the PDZ-peptide recognition is established. The success of the MIEC-SVM method on PDZ domains in this study and SH3 domains in our previous studies illustrates its generality on characterizing protein-peptide interactions and understanding protein recognition from a structural and energetic viewpoint.

Keywords

Protein-peptide interaction; protein binding specificity; molecular interaction energy component (MIEC); support vector machine (SVM); molecular dynamics (MD)

Introduction

Protein-protein interactions play critical roles in biological processes of every living organism and they are often mediated by weak and transient domain-peptide interaction¹. These modular domains are involved in many signal transduction processes². PDZ domain is one of the abundant modular domains^{3–6} and it plays critical roles in the assembly of sub-cellular structures, such as neuronal post synaptic density⁷ and epithelial junction⁸, and the maintenance of cell polarity⁹. The biological function of PDZ domains relies on the specific

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recognition of short peptides from the carboxyl terminus of their substrate proteins. Previous studies have shown that PDZ-binding peptides can be divided into three classes based on the three residues at the carboxyl terminus: class I (X[T/S]XΦCOOH), class II (XΦXΦCOOH), and class III (X[E/D]XΦCOOH) (Φ-hydrophobic residue; X-any residue; T-threonine; S-serine; E-glutamate; D-aspartate)^{10–13}. Peptides recognized by PDZ domains are about 5 amino-acid long¹⁴ while occasionally residues farther away from the carboxyl terminus also contribute to binding specificity¹⁵.

High-throughput technologies such as phage display^{16–19} and protein microarray^{20,21} have been exploited to investigate the binding specificity of PDZ domains and to identify the peptide sequence motif recognized by each domain. For example, phage-display experiments on human and worm PDZ domains by Tonikian et al.¹⁸ revealed 16 specificity classes, conserved between the two species. Stiffler et al.²¹ have purified the majority of mouse PDZ domains and conducted protein microarray experiments to determine their binding specificity against 217 peptides taken from the C-terminal of mouse proteins. A principal component analysis on the peptide sequences recognized by these PDZ domains suggested that PDZ-peptide interactions are continuous in the selectivity space, which challenged the previous view of these interactions falling into discrete classes. A variant of position specific scoring matrix (PSSM) that emphasizes discrimination between binding and non-binding peptides was employed to predict PDZ-peptide interactions. A more sophisticated model was trained on the mouse binding data in a follow-up study by Chen et al.²² to predict PDZ-peptide interactions in *D. melanogaster* and *C. elegans*.

These high-throughput experiments, such as microarray and phage-display, have generated significant amount of data to determine the binding preferences of PDZ domains^{16–21}. However, interpretation of such data is often solely based on amino acid sequence without structural insight to understand the molecular basis of interaction specificity. Although more and more PDZ-peptide complex structures are being solved^{15,23–29}, the number of available complex structures is still a few orders of magnitude less than that of the interacting domain-peptide pairs determined in the high-throughput experiments. Several template complexes shed light on understanding domain-peptide interactions in general but lack of complex structures for each individual domain hinders illustration of how exactly the binding specificity is achieved. Numerous computational methods have also been developed to predict pair-wise PDZ-peptide interactions^{21,22,30,31} or peptide sequence preference of individual PDZ domains³². Most of these methods rely on peptide sequence information such as MDSM^{21,22,31}, or amino acid trigram³⁰, with limited structural information and/or lack of energetic characterization of PDZ-peptide recognition^{22,31}.

We recently developed a computational method based on molecular interaction energy components (MIECs)^{33–35} that aims to decipher and predict binding specificity between protein domains and peptides. In this method, thousands of domain-peptide complexes are modeled based on template structures and then optimized using molecular mechanics. Energetic interaction profile (MIECs) of each domain-peptide complex is obtained from free energy calculation, which includes van der Waals, electrostatic, and desolvation energy components for interacting residues on the binding interface. Combined with either regression method (e.g. partial least squares, PLS) or classification method (e.g. support vector machine, SVM), MIEC-PLS or MIEC-SVM can predict binding affinity of a domain-peptide pair or classify peptides to binder/non-binder category for a given domain. In the previous studies, these methods have been successfully applied to the SH3-peptide recognition^{33,34} and HIV protease–small molecule interactions³⁵.

In this study, we applied MIEC-SVM method to PDZ-peptide interactions. The main purpose of this study is to obtain a generic MIEC-SVM model that can discriminate binder

and non-binder peptides for PDZ domains and quantitatively reveal the molecular basis of the binding specificity from the SVM-MIEC model. To achieve this goal, 2387 PDZ-peptide complexes were modeled. Their MIECs were calculated and used in generating the MIEC-SVM model. The initial cross-validation and leave-one-domain-out (LODO) test showed that the MIEC-SVM model outperformed the previous MDSM-based method^{21,22}. A follow-up feature selection procedure, i.e. selecting the MIECs most predictive of binding specificity, further boosted the performance of our model. This procedure also revealed that the protein-peptide residue interaction pairs at the binding interface fall into informative and uninformative categories. Further analysis on residue pairs showed that the energy distribution between the informative and uninformative pairs was biased, which suggests that these two categories may play different roles in the PDZ-peptide recognition.

Materials and Methods

PDZ domains and binding peptides

In the study of Stiffler et al.²¹, 85 PDZ domains were observed to interact with at least one peptide, among which 12 domains have available structures in the PDB database³⁶. 4 out of 12 domains, PTP-BL(2/5) (1VJ6)²⁶, MAGI-1(2/6) (2I04)²⁵, α 1-syntrophin(1/1) (2PDZ)²⁷, and TIP1(1/1) (3DIW)²⁸, have complex structures with peptides. The rest 8 domains, PAR-6B(1/1) (1NF3)³⁷, Harmonin(2/3) (1V6B), PAR3B(1/3) (1WG6), RGS3(1/1) (1WHD), PDZK11(1/1) (1WI2), ZO-2(1/3) (2CSJ), NHERF-3(1/4) (2D90), and NHERF-3(3/4) (2EDZ), only have domain (PDZ-only) structure without binding peptides (Supplementary Data Table S1). PDZK11(1/1) (PDB code: 1WI2) was left out in the free energy calculations because the molecular dynamics simulation could not reach equilibrium, as manifested by significant RMSD fluctuation (data not shown).

The binding peptides for the above 11 PDZ domains were taken from the study of Stiffler et al.²¹, in which total 217 peptides (Supplementary Data Table S2) were tested in the protein microarray experiments. 86 PDZ-peptide interactions observed between the 217 peptides and the 11 domains were used as the binder (positive) set (Supplementary Data Table S3) to train the MIEC-SVM model. For each binding peptide, 6 non-binding peptides were randomly selected to form the non-binder (negative) set that contained 516 non-interactions. The ratio of 6 between non-binder and binder was chosen to be consistent with that in the test set of Stiffler et al's data²¹.

Modeling PDZ-peptide complex structures

The structures of the 11 PDZ domains were first pre-processed as the following. For X-ray structures, water and ions were removed and only one PDZ chain was kept in the cases that multiple chains were present. For NMR structures, the alpha carbon RMSD was calculated between each structure and the average structure; then the NMR structure with the smallest RMSD to the average structure was selected for the following analysis.

Seven PDZ domains, 1NF3, 1V6B, 1WG6, 1WHD, 2CSJ, 2D90, and 2EDZ, do not have available complex structures in the PDB database. Their complex structures were built by superimposing these PDZ-only structures to other PDZ-peptide complexes and acquired the peptide coordinates from the complex structure (Supplementary Data Table S1). Six PDZ-peptide complex structures^{15,23–25,28,29} (Supplementary Data Table S4) were selected from PDB as the templates because these structures were aligned best to the query PDZ proteins indicated by a minimal C α RMSD (Supplementary Data Table S1) and a maximum number of residues aligned. The structure superimposition was performed by Align Structure module in Discovery Studio³⁸. Because the peptide in the template complex may not be a binder to the query PDZ domain, the peptide was then mutated using SCWRL4³⁹ to the

sequence in the 217 peptides tested in Stiffler et al.'s paper²¹ that had the strongest binding affinity to the query PDZ domain.

The 11 PDZ complexes were optimized by molecular dynamics simulations using AMBER10⁴⁰ and the AMBER03 force field⁴¹. First, *tleap* was used to process the structure and generate the AMBER topology and coordinate files. Missing atoms and hydrogen atoms were added by *tleap*. The structure was solvated in a rectangle box of TIP3P water⁴² extended 12Å away from any solute atom. Counter ions of Na⁺ or Cl⁻ were placed in a grid surrounding the complex structure based on the Coulombic potential to neutralize the system. Second, 5000 steps of energy minimization were performed to relax the system, consisting of 1000 steps of steepest descent followed by 4000 steps of conjugate gradient minimization. Third, after the minimization, molecular dynamics simulation was performed using program *sander*. The first 20ps heated the system from 10K to 300K with a constant volume periodic boundary; the time step was set to 0.5fs. Subsequently, another 20ps simulation was performed to adjust the density of water molecules using a constant pressure periodic boundary; SHAKE⁴³ was applied to constrain bonds involving hydrogen atoms; the time step was set to 1fs. Next, 2ns simulation was performed for system equilibration and data collection; the constant pressure periodic boundary and SHAKE procedure were applied; the time step was 1fs. Throughout the simulation, Particle Mesh Ewald (PME)⁴⁴ was employed to calculate long-range electrostatics interactions and the weak-coupling algorithm was applied to maintain the temperature of the system. The last snapshot of the simulation was relaxed by 5000 steps of energy minimization. The minimized conformation was saved as the complex template for modeling other PDZ-peptide interactions.

To model interactions between the 11 PDZ domains and other peptides, we mutated the peptide in the template complexes using *SCWRL4*³⁹ to other peptides in the positive and negative sets. Restrained by the computational cost, only 5000 steps of energy minimization were performed to optimize each complex. The first 1000 steps used steepest descent method and the rest used conjugate gradient method. TIP3P water⁴² was added to solvate the system.

Energy decomposition analysis

Energy decomposition was performed on the minimized PDZ-peptide complexes using *mm_pbsa* in the AMBER package. For each residue pair between the PDZ domain and the peptide, its interaction energy was decomposed into: van der Waals interaction energy ΔE_{vdw} ; electrostatic interaction energy ΔE_{ele} ; polar contribution to the desolvation energy ΔE_{gb} ; non-polar contribution to the desolvation energy ΔE_{sa} . The dielectric constant of 1 was used to calculate ΔE_{ele} . ΔE_{gb} was calculated using the GB model with parameters developed by Onufriev et al^{45,46}. The interior and the exterior dielectric constants in the GB calculation were set to 1 and 80, respectively. The ΔE_{sa} was estimated according to the solvent accessible surface area (SASA) as $\Delta E_{\text{sa}} = 0.0072 \times \text{SASA}$.

The MIEC profile and the MIEC-SVM model

The molecular interaction energy components (MIECs, Figure 1) were used to capture the energetic characteristics of interactions between peptides and a specific domain family^{33–35}. Here, to construct a generic model for all the PDZ domains, we needed to identify a set of interacting domain-peptide residue pairs at the interface that may determine the binding specificity. Sequence alignment for the 11 PDZ domains (Figure 2) was generated by ClustalW⁴⁷. The result from ClustalW was manually adjusted based on the structure to ensure correct alignment of the PDZ binding region¹⁴ which contains the carboxylate-binding GLGF loop (residue 39–42), $\beta 2$ (residue 43–47), and $\alpha 2$ (residue 105–113)¹⁴.

The interaction residue pairs were defined by a 6Å distance cutoff between any two residues at the domain-peptide interface and total 44 domain-peptide residue pairs were so identified. In this study, we also included residues pairs between adjacent peptide residues in the positions from 0 to -4 and these interactions reflected the conformational preference of the peptide. Therefore, there were 48 interaction residue pairs in total. For each interaction residue pair, ΔE_{vdw} , ΔE_{ele} , ΔE_{gb} , and ΔE_{sa} were calculated using energy decomposition analysis. The number of MIECs for one domain-peptide was $(44+4) \times 4 = 192$.

Support vector machine (SVM) was chosen to classify peptides to binders/non-binders based on the MIECs. In this study, the software package LIB-SVM⁴⁸ was used. We have conducted three tests to assess the performance of the MIEC-SVM models. First, 500 runs of 3-fold cross validation were performed on all the data to select the best-performed MIECs and assess the contributions of individual positions (leave-one-site-out test). Second, 500 runs of 5-fold cross validation were used to compare the prediction accuracy of our model with the Stiffler et al.'s study²¹. Third, 500 runs of leave-one-domain-out cross validation were carried out to assess the generality of the MIEC-SVM model.

The prediction accuracy of our models were evaluated using sensitivity (SEN), specificity (SPC), accuracy for positive (ACC+), accuracy for negative (ACC-), and Matthews correlation coefficient (MCC).

$$SEN = \frac{TP}{TP+FN} \leftrightarrow$$

$$SPC = \frac{TN}{TN+FP} \leftrightarrow$$

$$ACC+ = \frac{TP}{TP+FP} \leftrightarrow$$

$$ACC- = \frac{TN}{TN+FN} \leftrightarrow$$

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FN)(TP+FP)(TN+FP)(TN+FN)}} \leftrightarrow$$

TP, FN, TN, and FP represent the count of true positive, false negative, true negative, and false positive, respectively.

Results and Discussion

Modeling of PDZ-peptide complexes and calculation of MIECs

The structures of the 11 PDZ domains used in this study were obtained from PDB database. Complex structures were built for the 7 PDZ domains whose complex structures were not available. All 11 complex structures were then optimized by molecular dynamics. For each optimized PDZ-peptide structure, virtual mutagenesis was performed to mutate the peptide

in the optimized structure to one of the 217 peptides, which generated 2387 PDZ-peptide complex structures. These complex structures were then optimized by energy minimization.

MIECs between PDZ-peptide residues pairs at the interaction interface, including van der Waals, electrostatic, non-polar and polar part of desolvation, were calculated to capture the energetic characteristics of the binding. To select the MIECs for the best prediction of binding specificity, we tried numerous combinations of MIECs. The performance of each combination was evaluated by 500 runs of 3-fold cross-validations with random partition of binders and non-binders to training and test sets (Table 1, Supplementary Data Table S5). Based on the previous studies^{33,34} and the test for PDZ-peptide interactions (data not shown), RBF kernel was chosen for SVM because it performed better than the other kernel functions in cross-validations.

Cross-validations were used to select the best combination of MIECs, consisted of van der Waals, electrostatics, polar part of desolvation, non-polar part of desolvation, and the peptide internal interactions (model 12 in Table 1). The sensitivity, specificity, and Matthews correlation coefficient (MCC) for this combination were 59.6%, 88.5%, and 0.438, respectively (Table 1). Note that inclusion of peptide internal interactions is only an empirical option that indirectly accounts for conformational preference of the peptide. As shown in Table 1, adding this term only moderately improved the performance of the model (model 6 and 12, MCC increased from 0.431 to 0.438). This is most likely because the peptides binding to PDZ domains are short (5 residue-long) and do not form specific secondary structure. In contrast, for the SH3 domains, including this term in the model improved the performance more significantly (model 6 and 12 in Table 1 of³⁴, MCC increased from 0.374 to 0.523), which may be due to the left-handed polyproline type II helix formed by the peptides binding to the SH3 domain. It is also worth pointing out that the dataset for cross-validation was unbalanced because non-binders was six times of binders. Therefore, different weights were assigned to positive and negative classes in SVM. In the current study, weight $k_+=4$ was used for the binder class and $k_-=1$ was remained for the non-binder class. The optimal k_+ value was selected based on a systematic cross-validation test using k_+ from 1 to 30. (Supplementary Data Figure S1) For the LODO test (see next section), k_+ was selected as 4 by cross-validations on all 11 leave-one-domain-out datasets (Supplementary Data Figure S7), which was consistent with the k_+ value selected from the entire dataset.

Generality of the MIEC-SVM model

To assess the generality of the MIEC-SVM model on predicting the binding specificity of PDZ-peptide interactions, we conducted a leave-one-domain-out (LODO) cross validation. In this test, the interaction data associated with one domain was completely removed from the training set and used as the test set for the model trained on the remaining data. This test mimics the situation in reality to predict the binding specificity of a new PDZ domain that is not included in training. In total, 11 LODO cross validations were performed (Table 2, Supplementary Data Table S5). The average sensitivity, specificity, and MCC were 63.7%, 78.9%, and 0.343, respectively. The average accuracies for the binders and non-binders were 33.7% and 93.2%, respectively. Compared with our previous work of SH3 domains³⁴, the MIEC-SVM model performed slightly worse, which may be due to the much smaller training data available for each PDZ domain (Supplementary Data Table S1). On average, the binders and non-binders for each PDZ domain in this study were 8 and 48, respectively. As a comparison, each SH3 domain in our previous study³⁴ had 27 and 540 binders and non-binders, respectively. A larger training dataset no doubt helps characterize the domain-peptide binding specificity better.

Important residues for PDZ-peptide specific binding

To identify residues important for the binding specificity of PDZ-peptide interactions, we conducted leave-one-residue-out (LORO) tests. If leaving out a residue significantly deteriorated the performance of the MIEC-SVM model, we considered this residue important for binding specificity. In each test, one of the 19 PDZ and 5 peptide residues involved in calculating MIECs was excluded, i.e. all the interactions involving this residue were removed from the MIEC matrix. Then cross-validations were performed on the new MIEC-SVM model to assess the importance of the left-out residue. The difference between the MCC of the LORO model and that of the default using all the 24 residues reflected the contribution of the left-out residue to the prediction accuracy.

Not surprisingly, this LORO test showed that four peptide residues at the C-terminal are important for the binding specificity (their MCC differences >0.02 , Figure 3a and Supplementary Data Table S6). Indeed, these four residues fit to the binding pocket of the PDZ domain and establish the peptide binding specificity (Figure 4a and 4b), which is consistent with the knowledge that residues at the carboxyl terminal of a peptide are important for PDZ binding^{14,18}.

Three residues on the PDZ domain were identified as important for the binding specificity. Their residue numbers in the sequence alignment were 45, 71, and 105 and their MCC differences were 0.020, 0.034, and 0.013, respectively (Figure 3b, Supplementary Data Table S6). The structural roles of these three residues in the PDZ-peptide binding are illustrated in Figure 4c and 4d. In PDZ domains, residue 45 is often one of the three hydrophobic amino acids: Ile, Leu, and Val (Figure 2). It is part of the hydrophobic pocket to stabilize the side chain of peptide residue 0. In addition, the hydrogen bonds between residue 45 and peptide residue -1 are crucial for the formation of the well-known anti-parallel sheet between $\beta 2$ of the PDZ domain and the peptide. Residue 71 is located in $\beta 3$ strand and tends to interact with the side chain of residue -3 of the peptide^{23,24,26,28,29} through either electrostatic^{28,29} or hydrophobic^{23,26} interactions, which is consistent with the LORO test results. Residue 105 is in the $\alpha 2$ of PDZ domain and is often a histidine (7 out of 11, Figure 2). The hydrogen bonds formed between residue 105 and Thr/Ser on class I peptide -2 position was known critical to distinguish class I^{14,24,26,27,29} and class II peptide binding¹⁵, which supports the important role of residue 105 in determining binding specificity.

Feature (residue pair) selection to boost the model performance

LORO test revealed that not all the residue pairs were informative for the discrimination of binders and non-binders (Supplementary Data Table S6). The initial residue pairs were selected based on the distance between the residues of the PDZ domain and the peptide, i.e. any PDZ-peptide residue pair closer than 6Å was included in the MIEC calculation. To systematically remove noisy pairs from the model, we conducted feature selection using the sequential backward selection method⁴⁹. First, SVM model was trained using all residue pairs. The MCCs for the cross validation and leave-one-domain-out tests were recorded. Second, residue pairs were left out from the MIEC calculation one in a time, and cross validation and leave-one-domain-out (LODO) tests were performed using the new MIECs. We removed all the residue pairs whose removal improved the performance of the MIEC-SVM model in both cross-validation and LODO tests, as measured by MCC change, i.e. $MCC_{LORO} - MCC_{ALL}$ larger than 0 in both cross-validation and LODO tests.

As a result, 16 residue pairs were removed and 28 were kept (Supplementary Data Table S7). This feature selection procedure was fast and straightforward, but significantly improved the prediction accuracy. The MCCs for 3-fold cross validation and LODO test

were boosted to 0.487 and 0.426 (Table 3, 4, Supplementary Data Table S5), compared to 0.438 and 0.343 without feature selection, respectively. Especially, significant improvement on ZO-2(1/3) (2CSJ) was achieved by feature selection. Our analysis illustrated the importance of feature selection, which also sheds lights on understanding the PDZ-peptide binding specificity (see below).

We noticed that the PDZ domain of Harmonin(2/3) (1V6B) did not perform as well as other domains. We found that the binding site of uncomplexed Harmonin(2/3) (1V6B) is quite different from the other 10 PDZ domains: the β 2 strand and α 2 helix are much closer to each other in Harmonin(2/3) than in other proteins; GLGF motif region takes a flat conformation in Harmonin(2/3) but not in other PDZs. It is likely that the Harmonin(2/3) (1V6B) PDZ domain may undergo substantial conformational change upon peptide binding, which requires molecular dynamics longer than the one we conducted to model the complex structure.

To further test the generality of the MIEC-SVM model, we modeled two additional mouse PDZ domains that were arbitrarily selected from the mouse proteome and not included in the initial dataset: PSD95-2 and CHP110-2. We predicted their binding specificity using the model trained from the 11 PDZ domains. The MCCs for the two domains are 0.505 and 0.42, respectively (Table 5). Note that the maximal sequence similarity of both PSD95-2 and CHP110-2 are 68.8% to α 1-syntrophin(1/1), but they only share less than 1/3 of binding peptides (4 out of 12 between PSD95-2 and α 1-syntrophin(1/1) and 5 out of 18 for CHP110-2). Therefore, the satisfactory performance of the model further validated the generality of our model.

Comparison with other methods

We compared our method with the study of Stiffler et al.²¹. Because Stiffler et al. used a sequence-based method, they were able to train their model on 16058 interactions, and tested it on 3552 interactions. Essentially, they conducted a cross-validation of roughly 5-fold (the ratio between their training set and the test set was 4.52) because they trained their model on the same PDZ domains used in the test set against different peptides. The sensitivity (48%), specificity (88%), and accuracy for the positive class (38.5% calculated from their reported TP/FP=0.63) obtained from such cross-validation were satisfactory²¹.

For a comparison, we performed 500 runs of 5-fold cross-validations with random partitions of binders and non-binders. The sensitivity, specificity, and accuracy for positive interactions were 63.2%, 88.7%, and 50.0% respectively, which all outperformed the model of Stiffler et al.²¹. This comparison suggests that the MIEC-SVM model is able to capture the energetic patterns of the interaction interface of PDZ-peptides.

Following Stiffler et al.'s work, Chen et al.²² developed a modified sequence-based model combined with the structural information from one selected PDZ domain. We run Chen et al.'s model on the 11 PDZ domains in our dataset. For a fair comparison, the ratio of binder and non-binder was kept as 6. The prediction was repeated 500 times with non-binder randomly sampled each time. Without feature selection, our MIEC-SVM model had comparable MCC (0.438) as Chen et al. model (0.433). With feature selection, our MIEC-SVM model outperformed (MCC=0.487) the Chen et al. model (Table 3). It is worth of noting that the interactions between the 11 PDZ and the 217 peptides were included to train the Chen et al. model²².

Sequence and binding specificity similarity of the PDZ domains in the dataset

It is critical to confirm that the generalization capability of our model was not due to presence of similar PDZ domains in our dataset. We checked the similarity of the 11 PDZ

domains in terms of their interface sequence (residues involved in the interactions) similarity, full sequence similarity (Figure 5 and Supplementary Data Table S8), structural (RMSD of interface) similarity (Table S8), and binding specificity similarity (overlaps of binding peptides, Figure 7).

Interface sequence similarity is shown in Table S8 and Figure 5. Sequence similarity is calculated as the percentage of identical residues in the sequence. Interface sequence contains 16 PDZ residues that form 28 informative pairs with 5 peptide residues. The number of identical residues was obtained from the multiple sequence alignment. The interface sequence similarity is moderate: the average value is 27.7%. Note that the interface contains several conserved residues in most of the PDZ domains (whole GLGF loop, whole β 2, and His at the beginning of the α 2). As a comparison, the 82 PDZ domains in the Chen et al. dataset have an average interface sequence similarity of 38.3% (Figure 5). We also performed t-test for the two distributions of interface sequence similarity. The p-value was 1.34×10^{-7} , which showed the means of the two distributions were significantly different. Therefore, the 11 PDZ domains in our dataset have even lower pair-wise similarity in the binding sites than all the 82 PDZ domains in the Chen et al. dataset. The maximal sequence similarity distribution between our dataset and Chen et al.'s dataset was also compared (Figure 6). The maximal similarity of PDZ domains in our dataset is significantly smaller than those in Chen et al.'s study.

The full sequence and RMSD similarity are shown in Table S8. The average full sequence similarity in our dataset is 23.1%, lower than the interface sequence similarity. The RMSD similarity between PDZs was calculated between interface residues on the minimized PDZ structures after the MD simulation. The average RMSD similarity in our dataset is 1.346 Å.

The specificity similarity between two PDZs was calculated using the number of shared binders. As a comparison, the average specificity similarity in our dataset and Chen et al.'s dataset are 0.618 and 1.332, respectively. We also calculated the percentage of overlapping peptides among the non-redundant binders of any pair of PDZ domains and show the distribution in Figure 7. It is clear that 11 PDZ domains in our study have lower binding specificity similarity than the 82 PDZ domains in Chen et al. study.

Insights into PDZ-peptide interaction from MIEC and residue pair selection

We further investigated the MIECs of informative and uninformative PDZ-peptide residue pairs. The average MIECs of all the 86 PDZ-peptide interacting complexes (Supplementary Data Table S7) were calculated for all the 44 PDZ-peptide residue pairs. Obviously, residue pairs did not contribute equally to the binding energy. We found that 22 pairs with the most favorable interaction energies contributed about 90% of the total binding energy. The 28 informative pairs contributed 53% to the total binding energy compared to 47% contributed by the 16 uninformative ones. For all the peptide positions, residue 0 alone contributed about 45% of the total binding energy, while residue -1, -2, -3, -4 contributed 13%, 19%, 14%, and 9% respectively.

Peptide residue 0 was involved in 13 interaction pairs (Figure 8a). Although it contributes significantly to the total binding energy, most of its residue pairs (7 out of 13) were uninformative (Supplementary Data Table S7). It is owing to many non-specific backbone interactions formed between peptide residue 0 and PDZ residues. Note that 7 uninformative pairs contribute significantly to the total binding energy ($\Delta G_{\text{total}} < -1.0 \text{ kcal/mol}$) and their total contribution consists about 33% of the average total energy, compared to 12% contributed by the 6 informative pairs. Specifically, PDZ residues 40, 41, 42, and 43, known as GLGF motif^{13,14} or carboxylate-binding loop¹⁴, interact strongly with peptide residue 0, manifested by the MIECs of these 4 residue pairs: large polar ($\Delta G_{\text{polar}} < -1.5 \text{ kcal/mol}$) and

non-polar interaction energies ($\Delta G_{\text{non-polar}} < -0.8 \text{ kcal/mol}$, except residue 42). Only residue pair 0–40 was identified as informative pair (Figure 8b). For the uninformative pairs 0–41, 0–42, and 0–43, previous studies showed that hydrogen bonds are formed between their backbone atoms^{15,23–29,50}, which is consistent with their insignificant contribution to the binding specificity. PDZ residue 32 and 113, mostly Lys or Arg for the 11 PDZ domains form strong polar interactions with peptide residue 0 ($\Delta G_{\text{polar}} = -3.1$ and -3.0 kcal/mol , respectively). Previous structural analysis showed that these two residues stabilize carboxyl group of the peptide residue 0 through salt bridges with the backbone atoms^{29,51,52}, which supports the uninformative pairs of 0–32 and 0–113 found by feature selection. We also observed that such polar interactions were not always formed in all the 86 PDZ-binding complexes, which suggests that these interactions may be sensitive to conformational flexibility. PDZ residues 43, 45, 109, and 112 are likely to be hydrophobic, such as Phe, Val, Leu, and Ile, as shown in the multiple sequence alignment (Figure 2). Strong non-polar interactions have been observed for residue pairs 0–43, 0–45, 0–109, and 0–112 in this group ($\Delta G_{\text{non-polar}} = -2.3$, -1.2 , -1.1 , and -1.7 kcal/mol respectively). Previous structural analysis showed that these four residues form a hydrophobic pocket to interact with the hydrophobic side-chain of peptide residue 0^{24,26,27,29,53}, which is normally Leu, Val, or Ile. 45 and 109 formed informative interaction pairs with peptide residue 0 probably because these two residues are located at the edge of the binding pocket (Figure 8b).

Peptide residue –1 interacts with 10 PDZ residues (Supplementary Data Figure S2a). All the interaction pairs were identified as informative except –1–74. The interaction between –1 and 74 is moderate (average $\Delta G_{\text{total}} = -1.5 \text{ kcal/mol}$, Supplementary Data Table S7). Multiple sequence alignment showed that the position 74 contains diverse amino acids, including aliphatic, aromatic, polar, and positive charged amino acids (Figure 2). These two observations may explain why this pair is uninformative. Contradictory to the reports that the side chains of –1 and 74 form hydrophobic contacts^{27,29}, the –1–74 pair showed weak non-polar (average $\Delta G_{\text{non-polar}} = -0.5 \text{ kcal/mol}$, Supplementary Data Table S7) and for a few cases strong polar interactions (average $\Delta G_{\text{non-polar}} = -1.0 \text{ kcal/mol}$, Supplementary Data Table S7). The 9 informative pairs (Supplementary Data Figure S2b) contribute about 11% of the average total binding energy, compared to 2% contributed by the uninformative pair –1–74. Amino acid choice at residue –1 is quite broad and no general pattern has been found^{13,14}. In the MIEC-SVM model, the energetic characterization avoids the arbitrariness of summarizing occurrence of amino acids in a peptide or PDZ position. Regardless of amino acid types, as long as an amino acid forms favorable MIECs, it contributes to the PDZ-peptide binding.

Peptide residue –2 are involved in 10 interaction pairs (Supplementary Data Figure S3a), including four informative ones: –2–45, –2–46, –2–71, and –2–105 (Supplementary Data Figure S3b). Residue 45 shows both strong non-polar and polar energies ($\Delta G_{\text{non-polar}} = -1.5 \text{ kcal/mol}$, $\Delta G_{\text{polar}} = -3.9 \text{ kcal/mol}$) due to the hydrogen bonds formed between the backbone atoms and side chains⁵⁴, which explains its roles in binding specificity determination. Residue 105 shows a strong interaction with peptide residue –2 ($\Delta G_{\text{total}} = -1.7 \text{ kcal/mol}$) and this interaction is indeed important in determining the PDZ-peptide binding specificity¹⁸: residue 105 is critical to form hydrogen bond with residue –2 for recognizing class I peptides^{14,24,26,27,29} and it is also part of the hydrophobic pocket crucial for the binding of class II peptides^{50,54}. In total, the 4 informative pairs contribute about 11% of the total average binding energy, while the 6 uninformative pairs contribute about 8%.

All 6 interaction pairs involving residue –3 were identified as informative (Supplementary Data Figure S4), indicating the important role of residue –3 in determining the binding specificity. These 6 informative pairs contribute about 14% of the total average binding

energy. The strong polar interactions between peptide residue -3 and PDZ residue 71 ($\Delta G_{\text{polar}} = -2.4 \text{ kcal/mol}$) stabilizes the side-chain of residue -3, which is consistent with the previous observations^{18,24,27,29}. Residues 44 and 105 were also reported to affect the amino acid choice in position -3¹⁸.

Among the 5 interaction pairs formed by peptide residue -4, three of them (-4-47, -4-105 and -4-106) were identified as informative and they contribute about 6% of the total binding energy, compared with 3% contributed by the two uninformative pairs (Supplementary Data Figure S5). It is worth pointing out that residue -4 forms strong non-polar interaction with residue 105 ($\Delta G_{\text{non-polar}} = -1.7 \text{ kcal/mol}$), which suggests another important interaction formed by residue 105 besides its specific recognition of residue -2. Our analysis here is resonant to the previous studies on the roles of residue -4 in binding specificity^{24,29}.

Conclusion

In this study, MIEC-SVM was applied to PDZ domain and successfully discriminated binding peptides from non-binding peptides. Compared with sequence-based methods of predicting PDZ binding specificity, MIEC-SVM takes advantage of structural information to better characterize the PDZ-peptide interactions. The MIECs are free energy terms and the matrix is fully filled regardless of what amino acids interact with each other. In contrast, the contact matrix used in other methods has to consider the combinations of amino acids, e.g. Ala at peptide position -1 contacts with Ala/Ile/Leu et al. at PDZ position 1. Due to the availability of limited binding peptides, the contact matrix is often sparse because not each combination of amino acid pairs is observed in the relatively small samples of binders. Therefore, fully filled in MIEC matrix require less data to train the SVM model and thus avoids over-fitting problem, which reduces noise and improves prediction accuracy. Compared with energy-only-based calculations, MIEC-SVM employs the SVM to filter out noisy interactions as well as to better capture the local interacting environment such as the interior dielectric constant variation³³⁻³⁵. The satisfactory performance of MIEC-SVM on various systems (PDZ, SH3 and HIV protease³³⁻³⁵) illustrates its generality on deciphering binding specificity of proteins from a structural and energetic viewpoint.

For a PDZ domain to achieve binding specificity, it needs to distinguish (A) between binding peptides and ones not binding to any PDZ domain; (B) between its own binders and peptides binding to other PDZ domains. The residue pair selection shows that only 28 out of 44 pairs at the interaction interface are informative to binding specificity although the remaining 16 pairs still contribute significantly to binding. Using only the 28 informative pairs, MIEC-SVM achieved better prediction accuracy. Most of the previous identified important residue pairs for PDZ-peptide binding are informative. Given these observations, we hypothesize that the PDZ-peptide residue pair interactions fall into two categories: binding affinity, i.e. the 16 uninformative pairs that discriminate PDZ binding peptides and other peptides; binding specificity, i.e. the 28 informative pairs that discriminate among PDZ binding peptides. This hypothesis is waiting for experimental test, which requires point mutations on both PDZ and peptide residues to decipher the contribution of each residue pair to binding specificity. The feature (residue-pair) selection in the MIEC-SVM model is straightforward but provides great insights into understanding the binding specificity. We expect such analysis would shed light on deciphering protein recognition in other systems as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
The MIEC-SVM method
(1) The complex structures formed between PDZ and different peptides were modeled by homology modeling and MD simulations. (2) Residues close to the interaction interface were selected. PDZ residues: red balls. Peptide residues: blue balls. To consider the conformational preference of peptides, the interactions of adjacent peptide residues were also calculated. (3) Molecular interaction energy components (MIECs) were computed for all binders and non-binders by energy decomposition analysis. The fully-filled MIEC matrix was then used to train a SVM model to classify peptides into binders or non-binders.

Figure 2.

Multiple sequence alignment for the 11 mouse PDZ domains.

The multiple sequence alignment was generated by ClustalW with manual adjustment. The alignment is colored using the ClustalX coloring scheme. The 19 selected residue positions in our MIEC study are colored in purple in the *Selected_residues* row.

Figure 3. Leave-one-residue-out (LORO) test results for (a) peptide residues; (b) PDZ residues. The PDZ residue numbers are taken from the multiple sequence alignment (Figure 2).

Figure 4.

Important residues for the PDZ-peptide interactions. Peptide residues shown in the complex structure of (a) PAR-6B(1/1) and (b) PTP-BL(2/5). PDZ residue numbers are taken from the multiple sequence alignment. PDZ surface is colored by electrostatic potential and peptide is shown in stick. The important PDZ residues shown in the complex structure of (c) PAR-6B(1/1) and (d) PTP-BL(2/5). All figures were generated using UCSF Chimera 1.4.1⁵⁵.

Figure 5.
Interface sequence similarity.

Figure 6.
Maximal sequence identity between PDZ domains in our and Chen et al's datasets.

Figure 7. Binding specificity similarity. The bars show the portion of overlapping peptides and the curves are the accumulated counts.

Figure 8. PDZ-peptide residue pairs involving peptide residue 0 (shown on the PAR-6B(1/1) structure). (a) PDZ residues interacting with peptide residue 0. (b) PDZ residues forming informative residue pairs with peptide residue 0. PDZ residue numbers are taken from the multiple sequence alignment. Residues interacting strongly (<-1.0 kcal/mol) and weakly (≥ -1.0 kcal/mol) with peptide residue 0 are colored in red and yellow, respectively. Figures were generated using UCSF Chimera 1.4.1⁵⁵.

Table 1

Selection of MIEC components in predicting PDZ binding specificity

Model	MIEC component ^a	SEN	SPC	ACC+	ACC-	MCC
PDZ-peptide interactions only						
1	ΔE_{vdw} , ΔE_{ele}	0.537	0.883	0.445	0.920	0.390
2	ΔE_{vdw} , ΔE_{ele} , ΔE_{gb}	0.542	0.891	0.463	0.921	0.406
3	ΔE_{vdw} , ΔE_{ele} , ΔE_{sa}	0.576	0.885	0.464	0.926	0.422
4	ΔE_{vdw} , ΔE_{ele} , ΔE_{polar} , ΔE_{sa}	0.558	0.897	0.482	0.924	0.428
5	ΔE_{vdw} , ΔE_{ele} , ΔE_{desol}	0.548	0.887	0.458	0.922	0.405
6	ΔE_{vdw} , ΔE_{ele} , ΔE_{gb} , ΔE_{sa}	0.575	0.890	0.475	0.926	0.431
PDZ-peptide interactions plus intra-peptide (adjacent residue pair) interactions						
7	ΔE_{vdw} , ΔE_{ele}	0.583	0.876	0.449	0.927	0.415
8	ΔE_{vdw} , ΔE_{ele} , ΔE_{gb}	0.584	0.876	0.450	0.927	0.414
9	ΔE_{vdw} , ΔE_{ele} , ΔE_{sa}	0.602	0.881	0.465	0.930	0.436
10	ΔE_{vdw} , ΔE_{ele} , ΔE_{polar} , ΔE_{sa}	0.555	0.897	0.483	0.924	0.428
11	ΔE_{vdw} , ΔE_{ele} , ΔE_{desol}	0.572	0.884	0.462	0.925	0.419
12	ΔE_{vdw} , ΔE_{ele} , ΔE_{gb} , ΔE_{sa}	0.596	0.885	0.472	0.929	0.438

^a ΔE_{vdw} , ΔE_{ele} , ΔE_{gb} , and ΔE_{sa} are van der Waals, electrostatic, polar part of desolvation, and non-polar part of desolvation energies, respectively. ΔE_{polar} is the sum of ΔE_{ele} and ΔE_{gb} . ΔE_{desol} is the sum of ΔE_{gb} and ΔE_{sa} .

Table 2
The MIEC-SVM performance evaluated by the leave-one-domain-out cross validation

Domain Name (PDB ID)	Binders ^a	SEN ^b	SPC ^c	ACC ^d	ACC ^e	MCC ^f
PAR-6B(1/1) (1NF3)	2	0.995	0.866	0.610	0.999	0.720
NHERF-3(3/4)(2D90)	7	0.939	0.897	0.624	0.989	0.714
MAGI-1(2/6) (2I04)	2	0.880	0.762	0.409	0.979	0.496
NHERF-3(1/4)(2EDZ)	11	0.844	0.782	0.406	0.969	0.483
TIP1(1/1) (3DIW)	6	0.602	0.845	0.418	0.928	0.389
α 1-syntrophin(1/1) (2PDZ)	6	0.730	0.764	0.350	0.945	0.381
PAR3B(1/3) (1WGG)	3	0.947	0.574	0.282	0.986	0.373
PTP-BL(2/5) (1VJ6)	20	0.762	0.544	0.218	0.933	0.215
RGS3 (1/1) (1WHD)	14	0.537	0.537	0.163	0.874	0.052
ZO-2(1/3) (2CSJ)	13	0.043	0.954	0.132	0.857	-0.005
Harmonin(2/3) (1V6B)	2	0.000	0.970	0.000	0.853	-0.038
Average	8	0.637	0.789	0.337	0.932	0.343

^aNumber of binders for each PDZ domain

^bSensitivity

^cSpecificity

^dPrediction accuracy for binders

^ePrediction accuracy for non-binders

^fMatthews correlation coefficient.

Table 3
The MIEC-SVM performance evaluated by cross validation before and after feature selection

	SEN ^a	SPC ^b	ACC+ ^c	ACC- ^d	MCC ^e
No feature selection	0.596	0.885	0.472	0.929	0.438
After feature selection	0.648	0.890	0.504	0.938	0.487
Chen et al's model	0.605	0.948	0.359	0.980	0.433

^a Sensitivity

^b Specificity

^c Prediction accuracy for binders

^d Prediction accuracy for non-binders

^e Matthews correlation coefficient.

Table 4

The MIEC-SVM performance evaluated by leave-one-domain-out (LODO) test after feature selection

Domain Name (PDB ID)	Binders ^a	SEN ^b	SPC ^c	ACC ^d	ACC ^e	MCC ^f
PAR-6B(1/1) (1NF3)	2	1.000	0.849	0.578	1.000	0.697
NHERF-3(3/4)(2D90)	7	1.000	0.949	0.786	1.000	0.862
MAGI-1(2/6) (2I04)	2	0.950	0.695	0.377	0.990	0.484
NHERF-3(1/4)(2EDZ)	11	0.762	0.877	0.522	0.959	0.551
TIP1(1/1) (3DIW)	6	0.790	0.757	0.368	0.956	0.420
α 1-syntrophin(1/1) (2PDZ)	6	0.755	0.816	0.422	0.953	0.461
PAR3B(1/3) (1WGG)	3	0.823	0.772	0.406	0.964	0.466
PTP-BL(2/5) (1VJ6)	20	0.823	0.630	0.273	0.956	0.321
RGS3 (1/1) (1WHD)	14	0.659	0.521	0.188	0.901	0.127
ZO-2(1/3) (2CSJ)	13	0.479	0.887	0.414	0.912	0.342
Harmonin(2/3) (1VdB)	2	0.000	0.962	0.000	0.852	-0.047
Average	8	0.731	0.792	0.394	0.949	0.426

^aNumber of binders for each PDZ domain

^bSensitivity

^cSpecificity

^dPrediction accuracy for binders

^ePrediction accuracy for non-binders

^fMatthews correlation coefficient.

Table 5

Prediction results of two additional mouse PDZ domains.

Domain Name	Binders ^a	SEN ^b	SPC ^c	ACC+ ^d	ACC- ^e	MCC ^f
PSD-95(2/3)	12	0.438	0.967	0.726	0.912	0.505
CHP110(2/3)	18	0.490	0.922	0.516	0.916	0.420

^aNumber of binders for each PDZ domain

^bSensitivity

^cSpecificity

^dPrediction accuracy for binders

^ePrediction accuracy for non-binders

^fMatthews correlation coefficient.