Narrow funnel-like interaction energy distribution is an indicator of specific protein interaction partner.

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Article

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Title

- Full title: Narrow funnel-like interaction energy distribution is an indicator of specific protein interaction partner.
- Short title: Protein interaction prediction using energy analysis

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Abstract

Protein interaction networks underlie countless biological mechanisms. However, most protein interaction predictions are based on interspecies biological evidence (knowledge-based predictions) that are biased to well-known protein interaction or physical evidence that exhibits low accuracy for weak interactions and requires high computational power. In this study, a novel method has been suggested to predict protein interactions using interaction energy distribution. Protein interactions with specific partners can be predicted by investigating narrow funnel-like interaction energy distribution, exhibiting a stable state in a specific orientation. In this study, it was demonstrated that various protein interactions with specific interaction partner, such as protein interaction with kinase and E3 ubiquitin ligases, have narrow funnel-like interaction energy distribution. Moreover, algorithm and deep learning model for prediction of general protein interaction partner and substrate of kinase and E3 ubiquitin ligase were developed. The prediction accuracy of this method is similar to or even better than that of yeast two-hybrid screening, which is the most widely applied interaction screening method. Ultimately, this knowledge-free protein interaction prediction method will broaden our understanding of protein interaction networks.

Teaser

A novel method of determining specific protein interactions was developed by investigating narrow funnel-like interaction energy distribution.

MAIN TEXT

Introduction

40 Most biological activities occur through complex biomolecular interaction networks. After
41 the genome sequencing of several tens of thousands of organisms, understanding biomolecule
42 networks beyond the individual genes is becoming increasingly important. In particular, protein–
43 protein interactions (PPI) are among the most diverse biomolecular networks and the center of
44 system biology. Several experimental techniques, such as yeast two-hybrid screening,
45 coimmunoprecipitation, affinity-chromatography, tandem affinity purification-mass spectroscopy,
46 etc., have been developed to identify PPI (1). However, with the rapid advancement of proteome
47 identification, experimental results cannot keep up with the potential number of protein
48 combinations. Recently, 200 million proteins in various organisms were predicted or identified
49 (2). Meanwhile, the international consortium curated only approximately 1 million PPIs from
50 literatures and datasets (3, 4). Moreover, among the identified PPI, approximately 70% are *Homo*
51 *sapiens*-related (4). Most PPI might still be unidentified. Therefore, *in silico* methods, as well as
52 high throughput experimental approaches, have been developed to broaden our PPI-related
53 knowledge (5).

54 *In silico* PPI prediction methods utilize biological, structural and physical results.
55 Biological evidence includes gene or domain fusion (6, 7), gene neighborhood (6, 8), interolog
56 (9), coexpression (6, 10), coevolution (11), and phylogenetic similarity (6, 12). Although
57 biological result-based PPI predictions have a powerful predictive ability, in addition to being
58 applicable on a large scale, they are highly biased to well-known PPI. Except for PPIs with rare
59 molecular features (gene or domain fusion and gene neighborhood), omics scale interspecies data
60 are required or at least similar protein interactions in other species should be identified to predict
61 PPI using biological results (5, 13). Structural and physical evidence-based PPI prediction utilizes
62 protein–protein interface templates (14), interaction energy (13, 15), and shape complementarity
63 (15, 16). Template-based PPI predictions require known PPIs with similar interface structures (14,
64 17). All these knowledge-based PPI prediction are not enough to discover new PPI, since most
65 PPI might still be unknown. Conversely, PPI predictions with interaction energy and shape
66 complementarity are independent of accumulated large-scale data and known PPIs. However,
67 shape complementarity is different for each PPI type. Obligate PPIs, which are stable only when
68 interacting with each other, have higher shape complementarity, although transient PPIs exhibit
69 low shape complementarity (5, 18). Moreover, PPI prediction using interaction energy require a
70 lot of computational power and is not suitable for large scale prediction. Especially, protein
71 docking programs, which calculate the possible direction of interaction and interaction energy,
72 have a high computational cost (5, 15). Transient PPIs with weak interactions are difficult to

73 predict using interaction energy (15). However, most PPIs are weak, and weak PPIs play a pivotal
74 role in protein interaction networks (19). Therefore, high computational cost and the inability to
75 predict transient PPIs with weak interactions represent the major drawbacks of protein interaction
76 partner prediction using *in silico* protein docking.

77 One of the most difficult PPIs to predict is that of enzyme-specific interaction partners,
78 such as substrates, inhibitors, and regulators, or signaling pathway interactions. Their diversity of
79 complicated signaling pathways (20) makes biological evidence- and structural template-based
80 PPI prediction difficult. Furthermore, due to weak and transient interaction properties, interaction
81 energy and shape complementarity are not useful for the prediction of these PPIs (15, 18).
82 However, understanding PPIs in signaling pathways, such as the interactions of kinases or E3
83 ubiquitin ligase (EUL) with their interaction partners, is one of the most crucial parts of biology.
84 In mammals, kinases and ubiquitination-related enzymes are the most and the fifth most abundant
85 enzymes in signaling pathways, respectively (20). Moreover, in plants, approximately 5% of the
86 protein-coding genes in rice and *Arabidopsis* encode kinases and EULs (21-23). Despite the
87 importance of kinases and EULs, most interaction partner predictions of kinases and EULs are
88 mostly based on biological evidence, which require identified PPI with similar amino acid
89 sequences or domains (24-30). Moreover, because of the lack of identified PPIs, most prediction
90 tools are limited to human kinases or EULs (24, 26-30). Therefore, in most cases, no high-
91 accuracy PPI prediction tools are available for kinases and EULs.

92 In this study, I suggest a new, interaction energy distribution-based PPI prediction
93 strategy. PPIs with a specific interaction partner, such as PPIs of kinase and EULs, exhibit the
94 specificity to maintain sophisticated signaling pathways. For instance, in humans, kinase
95 phosphorylates specific phosphorylation sites ranging from one to hundreds among approximately
96 700,000 potential phosphorylation sites (31). Moreover, most regulators and inhibitors act when
97 they interact with kinases only in a specific orientation (32, 33). Although these PPIs are weak,
98 the specificity of these interactions observed in a specific orientation distinguishes them from
99 non-interacting protein pairs (31, 34). Based on this specificity, it was hypothesized that an
100 interaction energy diagram of PPI with specific interaction partners would display a narrow
101 funnel-like landscape, exhibiting a stable state in a specific orientation (Fig. 1a). In this study,
102 using Rosetta energy function, knowledge (or Boltzmann relation)-based macromolecular energy
103 function (35), the PPI interaction energy distribution was calculated.

104 Although there are considerable differences of interaction energy distributions between
105 interacting and non-interacting protein complexes, setting a classification criterion with high
106 accuracy is difficult. Recent advances in deep learning have made it possible to extract hidden

107 features from complicated data and classify them. (36) Training using interaction energy
108 distribution data of interacting and non-interacting protein complex can be employed to classify
109 the interaction energy distribution of unknown protein pairs into interacting or non-interacting
110 protein interaction energy distribution. This binary classification problem has been successfully
111 resolved in various field using machine learning (37). In this study, deep learning models were
112 developed to predict general protein interaction partners and substrates of kinase and E3 ubiquitin
113 ligase.

114
115
116 **Results**
117

118 **Workflow for the interaction energy distribution of kinases/E3 ubiquitin ligases and**
119 **their interaction partners**

120 To analyze the interaction energy landscape of kinases/EULs and their interaction partners,
121 I retrieved 135 kinase-interaction partners, including regulator, inhibitor, and substrate, and 189
122 EUL-substrate pairs from known databases (3, 38, 39). To construct negative controls, I randomly
123 paired kinases and EULs with different partners with no evidence of the existence of functional
124 interactions in these pairs. Randomly paired partners might later turn out to be real interaction
125 partners, but most likely they would not be interaction partners. Therefore, as a negative control,
126 106 kinase- and 124 EUL-random partner pairs were constructed (Fig. 1B, Supplementary Data 1).

127 Using Phyre2, a server for template-based structure modeling (40), full predicted
128 structures of kinases, EULs, and their partners were obtained (Supplementary Data 2). Because of
129 computational costs, the calculation of every possible protein docking structure is nearly
130 impossible. Therefore, 1,000 possible docking structures per pair were sampled using two docking
131 programs (Table 1, Fig. 1B). RosettaDock uses the Monte–Carlo algorithm with a knowledge-based
132 energy function (35) and includes the structure refinement of the docking structures (41).

133 HDockLite uses the fast Fourier transform (FFT) algorithm with a shape-based scoring function
 134 and does not include structure refinement (42).

135 To analyze the interaction energy landscape (Fig. 1A), I calculated the interaction
 136 energy with Rosetta energy and the following equation:

137

$$138 \text{ Interaction Energy} = \{\text{Rosetta energy of simulated docking structure}\}$$

$$139 - \sum_{\text{Each component in pair}} \{\text{Rosetta energy of single components}\}$$

140

141 As every theoretical protein energy exhibits differences compared with the experimental
 142 data, I ignored energy change because of structure change and considered only the affinity to
 143 minimize error. To indicate interacting positions, I used Interface Root Mean Square deviation
 144 (iRMS, 43). Originally, iRMS was developed to compare simulated docking and native structures
 145 (44). In this study, I slightly changed iRMS. Instead, of the native structure, I set the strongest
 146 interacting structure as a reference structure (Fig. 1C). The redefined iRMS was as follows:

147

$$148 \text{ iRMS} = \sqrt{\frac{1}{N} \sum_{i=1}^N \|x_i - y_i\|^2}$$

149 *N: the number of backbone atom of interface residues*
 150 *x_i : the position of i th backbone atom of interface residues in docking structure*
 151 *y_i : the position of i th backbone atom of interface residues in the strongest interacting structure*
 152

153 As Fig. 1C shows, in the strongest interacting structure, residues closely located within 10 Å
 154 were set as interface residues. In different docking structures, the same residues were in different
 155 positions. iRMS is the root mean square deviation of the backbone atoms of interface residues in
 156 two structures. For every docking structure generated from each enzyme-interaction and random
 157 partner pairs using two docking programs, interaction energy and iRMS were analyzed and plotted
 158 (Supplementary Data 3 and Supplementary Data Figs 1–4: generated using RosettaDock;
 159 Supplementary Data 4 and Supplementary Data Figs 5–8: generated using HDockLite). As
 160 HDockLite does not include structure refinements, the interaction energy calculation indicates that

and false-positive (random pair with narrow funnel-like interaction energy distribution) ratios for each criterion. As per the hypothesis, the narrow funnel distribution of the functionally interacting protein complex was higher than that of random protein pairs in the case of most criteria.

For whole protein pairs, docking structures generated by RosettaDock, HDockLite showed up to 11.9%–20.3% difference of true- and false positives (Supplementary Data Fig. 9). Interaction energy, iRMS, and distribution criteria at the maximum points are described in Supplementary Table 1. As the interaction calculation depends on structure accuracy, I filtered out low-accuracy-predicted structures with less than 70% region with high confidence (>90% confidence, (40) and analyzed the remaining pairs with high-accuracy structure prediction (identified kinase-interaction pair: n = 56 for RosettaDock, n = 58 for HDockLite; random kinase pair: n = 52 for RosettaDock, n = 54 for HDockLite; identified EUL pair: n = 59 for RosettaDock, n = 70 for HDockLite; random EUL pair: n = 62 for RosettaDock and HDockLite). The docking structures, generated by RosettaDock and HDockLite, showed up to 17.9%–26.4% difference of true- and false positives (Supplementary Data Fig. 10). Interaction energy, iRMS, and distribution criteria at the maximum point are described in Supplementary Table 2.

As protein global docking works well for small proteins (48), I again filtered out pairs with large proteins (>700 residues) and analyzed the remaining pairs with high-accuracy structure prediction and relatively small proteins (identified kinase-interaction pair: n = 28 for RosettaDock and HDOCKlite; random kinase pair: n = 26 for RosettaDock and HDOCKlite; identified EUL pair: n = 25 for RosettaDock, n = 36 for HDOCKlite; random EUL pair: n = 28 for RosettaDock and HDOCKlite). For the kinase pairs, the docking structures generated by RosettaDock and HDOCKlite showed up to 44.2% and 48.9% difference of true- and false positives, respectively. For the EUL pairs, the docking structures generated by RosettaDock and HDOCKlite showed up to 35.9% and 27% difference of true- and false positives, respectively (Fig. 3B). Interaction energy, iRMS, and distribution criteria at the maximum points are described in Table 2. In order to investigate the effectiveness of finding specific interaction partners using interaction energy distribution, true- and false-positive rates were compared with those of the yeast two-hybrid screening approach. True- and false-positive rates from genome-scale yeast two-hybrid trials in three species were compared (49). Whole pairs and pairs with high-accuracy structure predictions had similar accuracy to those of the yeast two-hybrid screening (Supplementary Data Figs 9 and 10). Pairs with high-accuracy structure predictions and relatively small proteins showed

significantly better results than those of the yeast two-hybrid screening approach (Fig. 3C). Interaction energy, iRMS, and distribution criteria at the maximum points are described in Table 2.

Interaction energy distribution of other protein complexes

As kinase and EUL PPIs represent only a small part of the whole PPIs, I investigated the interaction energy distribution in other protein complexes. I retrieved 183 curated experimentally determined protein complex structures from the IntAct database (4), then analyzed the PPI between the interacting chains with the interface area in the protein structure using RosettaDock (see Fig. 1B and Supplementary Data 5). The interaction energy and iRMS distribution of the protein complexes are plotted in Supplementary Data Fig. 11. Moreover, I retrieved 110 experimentally determined protein structures, which were unlikely engaged in direct interactions from the Negatome 2.0 database as negative controls (50), then analyzed the PPI using RosettaDock (see Fig. 1B and Supplementary Data 5). The interaction energy and iRMS distribution of the protein complexes are plotted in Supplementary Data Fig. 12.

To confirm whether other protein complexes showed narrow funnel-like interaction energy distribution, I calculated the iRMS averages of simulated docking structures. As per the hypothesis (Fig. 1A), the average iRMS of the interacting protein complexes were significantly smaller than the average iRMS of non-interacting protein pairs (95% confidence using Student's t-test, Fig. 4). Furthermore, I functionally annotated these proteins according to level-2 gene ontology distribution from Generic GO slim (51), then divided the protein pairs according to the related biological processes and calculated the average iRMS for each part (Fig. 4). Almost every division showed smaller average iRMS of the interacting complexes than those of the non-interacting pairs, except for the negative regulation of the biological processes. In particular, protein pairs related to cellular, metabolic, and developmental processes, signaling, response to stimuli, and biological process regulation showed significantly smaller average iRMSs than non-interacting protein pairs (95% confidence using Student's t-test). These processes are well-known to display molecular cascades with specific PPIs. Among those, cellular process-, developmental process-, and response to stimuli-related proteins pairs showed higher differences (99% confidence using Student's t-test). In particular, developmental process-related proteins pairs showed the biggest differences of iRMSs between the interacting complexes and non-interacting pairs (median of average iRMS of the interacting complexes: 22.37 Å, median of average iRMS of non-interacting pairs: 29.6 Å). This result might be related to developmental processes controlled by complex molecular cascades and networks with specific PPIs (52).

Deep learning model to predict protein interaction partners

As I have heretofore demonstrated, there are significant differences in the interaction energy distribution between interacting and non-interacting protein complexes. Thus, I developed deep learning models to predict protein interactions based on complex interaction energy distributions. As described in Fig. 5, the prediction program trained with interaction energy distributions of interacting and non-interacting protein pairs uses deep learning to extract hidden distinguishable features from complex distribution patterns and classify them. Then, based on this interaction energy distribution, the prediction program predicts whether the given protein pair will interact.

To develop the prediction model, protein interaction energy distributions of 183 experimentally determined interacting protein complex from the IntAct database (4) and 110 non-interacting protein pairs from the Negatome 2.0 database (50) were used for training (Supplementary Data 5). Additionally, I tested seven optimizers from the Keras library, including Adam, SGD, RMSprop, Adadelta, Adamax, Nadam, and Ftrl (53), to construct the optimal deep learning model for interaction prediction. Moreover, considering the amount of input data and the findings of previous studies (54, 55), I constructed seven candidate layer structures and tested each. Each of the 49 deep learning models was analyzed with 1–200 epochs (Supplementary Fig. 13). The optimal model for prediction showed an accuracy of 77.6% by utilizing the Adam optimizer containing a 1024-64-2-layer structure and 109 epochs (Table. 3). To assess the predictive performance of this deep learning model, the receiver operating characteristic (ROC) curve from 10-fold cross validation with data shuffling was analyzed. The area under curve (AUC) of this model was 0.834 (Fig. 6), demonstrating a higher predictive performance than that of yeast two-hybrid screening (Fig. 3C).

To apply this deep learning method to predict substrates of EULs and kinases, I retrieved EULs and kinases from various species along with information about their substrates from the Ubibrowser2.0 (25) and PhosphoSitePlus (56) databases. Then, I retrieved predicted structures in the AlphaFold database (57) with less than 700 residues and more than 70% prediction confidence in at least half the region. In total, 63 EULs and their substrates and 214 kinases and their substrates were retrieved (Supplementary Data 7). I then randomly paired enzymes and substrates as a negative control. Among these, pairs with evidence of interactions in the IntAct (4) or STRING databases (6) were filtered out. A total of 63 EUL-random substrate pairs and 203 kinase-random substrate pairs were constructed. Their interaction energy distribution was then analyzed using RosettaDock (41) and HDockLite (42), as shown in Fig. 1. To construct an optimal deep learning model for interaction prediction, 49 candidate models with 7 optimizers in

the Keras library and 7 candidate layer structures were analyzed with 1–100 epochs for EULs and 1–50 epochs for kinases (Supplementary Fig.14–16). The deep learning model trained using the RosettaDock data (Supplementary Figs.14 and 15) performed better than that trained using the HDOCKlite data (Supplementary Fig.16). Therefore, only the deep learning models trained using the RosettaDock data were further analyzed.

For substrate prediction of EULs, the optimal prediction model showed a 77.7% accuracy using an Adam optimizer containing a 1024-2-layer structure and 61 epochs. For substrate prediction of kinases, the optimal model for prediction showed a 61.4% of accuracy with an Adam optimizer containing a 1024-64-2-layer structure and 9 epochs (Table. 3). ROC curves obtained from 10-fold cross validation with data shuffling were then analyzed to test the predictive performance of these deep learning models. The AUCs of these models were 0.711 and 0.601 for EULs and kinases, respectively (Fig. 6). These result demonstrates higher and similar predictive performance than that of yeast two-hybrid screening, respectively (Fig.6 and Fig. 3C).

Discussion

In this study, I demonstrated that the interaction of a protein with a specific interaction partner has a narrow, funnel-like interaction energy distribution. In addition, interaction energy distributions between interacting and non-interacting protein pairs are substantially different. Especially, PPIs related with various signaling pathway showed much narrower interaction energy distribution than those of random pair. Although these PPIs, including PPIs of kinases or EULs, are difficult to predict and validate using experiments and previous prediction methods, the interaction energy distributions had considerably distinguishable traits. I then developed a deep learning model to predict interactions based on the interaction energy distribution. ROC curve analysis showed that general protein interaction prediction and EUL substrate prediction had a good predictive performance, with an AUC of 0.7–0.9. However, kinase substrate prediction only had an acceptable predictive performance, with an AUC of approximately 0.6. For interaction energy distribution analysis, I used whole interactions of kinases, including regulators and substrates. However, when I narrowed those to interactions with only substrates, the weakness of interactions might hamper the prediction. Thus, for further improvement and development of this prediction model, a bigger training data set may be essential. Finally, the deep learning model trained using RosettaDock data performed better than that trained using HDockLite data, indicating that a more accurate interaction energy calculation with structure refinement is necessary to accurately predict protein interactions.

395 using protein structure predicted using deep learning, knowledge-free protein interaction
396 predictions could be achieved.

397 Second, protein size affects the prediction results (Fig. 3, Supplementary Data Figs 9 and
398 10). As global docking works better for small than large proteins (48), interaction energy
399 distribution is affected by protein size. This bias can be improved through high resolution *in silico*
400 protein docking with more docking structures (15). Furthermore, interaction partner prediction
401 with interaction energy distribution can be used in parallel with other interaction partner
402 predictions. Widely used biological result- and template structure-based protein interaction
403 prediction methods are biased by well-studied interaction pairs (5). However, the interaction
404 energy distribution is completely irrelevant for the extent of how well-studied these interactions
405 are. This method is biased by protein pairs with well-predicted structures and of small size (Fig. 3,
406 Supplementary Data Figs 9 and 10). They can be used together to complement each other.

407 To improve this study further, improvement of protein energy function should be
408 considered. There are two kinds of protein energy functions. One is the physics-based energy
409 function, constructed based on classical mechanics and correction terms with perturbations in
410 quantum mechanics, such as the CHARMM force field (70). The other is knowledge-based
411 energy function, using Boltzmann relation to retrieve energy terms from experimentally
412 determined protein structures, such as Rosetta energy (35). These energy functions are developed
413 using a bottom-up approach consisting of each formula and parameter of physical properties like
414 building blocks (35, 70). Terms might also be missing or contain big errors. Therefore, from a
415 holistic point of view, protein energy might be retrieved using the Boltzmann relation from
416 protein structures. To do so, significant amounts of structures are necessary, although the
417 experimentally identified protein structures are limited. However, due to the recent progress in the
418 protein structure prediction-related deep learning field, we can predict distribution of protein
419 structures. In AlphaFold, predicted distance and torsion distribution are generated by deep
420 learning and the potentials are retrieved with predicted distributions using Boltzmann relation
421 without specific physical energy formula (67). Therefore, interaction energy can be generated
422 with a top-down approach using deep learning. Moreover, the Boltzmann distribution is valid in
423 the equilibrium state (71). However, living organisms are dynamic, open, and are in a
424 nonequilibrium state. To describe life phenomena more precisely, nonequilibrium statistical
425 mechanics, such as generalized Boltzmann distribution, would be necessary (72, 73).

426 Another possible interaction energy distribution analysis application is that narrow funnel-
427 like interaction energy distribution can be a specificity indicator. Finding specific interactions is
428 important in drug discovery. Nonspecific interactions can result in side effects (74). Even though

narrow funnel-like interaction energy distribution does not guarantee the exclusion of binding other molecules, it shows that proteins bind to a specific site and can be used as a specific interaction indicator. In particular, several kinases and EULs are considered as targets of multiple drugs, such as anticancer drugs (75, 76). Therefore, this study could be applied in drug discovery as a specific interaction indicator.

Materials and Methods

Protein structure preparation

To analyze kinase interactions, 135 kinase-interaction partner pairs were retrieved from kinase and protein interaction databases (3, 38). Using NumPy's random number generator (77), 106 random, not identified as interacting pairs in literatures and datasets, were generated (Supplementary Data 1). From the E3 ubiquitin ligase-substrate interaction database UbiBrowser (25), 189 E3 ubiquitin ligase-substrate interactions were retrieved. Using NumPy's random number generator (77), 124 random, not identified as interacting pairs in literatures and datasets, were generated (Supplementary Data 1). Using the Phyre2 server (40), protein structures were predicted with the intensive mode option (Supplementary Data 2).

To analyze interacting protein complexes, 183 protein complex structures were retrieved from the protein interactome database IntAct (4). Interacting chains in the protein complexes were analyzed using the interfaceResidue script of PyMOL (78). As negative controls, 110 experimentally determined protein structures, unlikely engaged in direct interactions, were retrieved from the Negatome 2.0 database (51).

Protein docking structure generation using RosettaDock

For RosettaDock, I used the Rosetta 2020.08 bundle (41). Each kinase and E3 ubiquitin ligase protein interaction pairs were merged using PyMOL (78). Kinase or E3 ubiquitin ligase chain IDs were designated as "A" and those of interacting partners as "B" using PyMOL (78). Before kinase and E3 ubiquitin ligase protein pair docking using RosettaDock (41), I optimized their side-chain conformations (prepacking) using the following command:

```
./bin/docking_protocol.static.linuxgccrelease -in:file:s (Merged protein structure file) -docking:partners A_B -dock_pt 3 8 -randomize1 -randomize2 -spin -out:path:all (Output directory)
```

Next, I generated 1,000 docking structures using the following command:

```
./bin/docking_protocol.static.linuxgccrelease -in:file:s (prepacked files) -docking:partners A_B -dock_pt 3 8 -randomize1 -randomize2 -spin -use_ellipsoidal_randomization true -nstruct 1000 -out:path:all (output directory)
```

To analyze interacting proteins complexes and non-interacting protein pairs, I optimized their side-chain conformations (prepacking) using the following command:

```
./bin/docking_protocol.static.linuxgccrelease -in:file:s (protein complex structure file) -docking:partners (chain ID of proteins which participating in interaction)_(chain ID of proteins in
```

opposite side of interaction) -dock_pert 3 8 -randomize1 -randomize2 -spin -out:path:all (output directory)

Then, I generated 1,000 docking structures using the following command:

```
./bin/docking_protocol.static.linuxgccrelease -in:file:s (protein complex structure file) -  
docking:partners (chain ID of proteins which participating in interaction)_ (chain ID of proteins in  
opposite side of interaction) -dock_pert 3 8 -randomize1 -randomize2 -spin -  
use_ellipsoidal_randomization true -nstruct 1000 -out:path:all (output directory)
```

To analyze kinase and E3 ubiquitin ligase interactions using HDockLite (42), I generated 1,000 docking structures using the following command:

```
./hdock (kinase or E3 ubiquitin ligase) (Interaction partner) -out (docking file)  
./createpl (docking file) top1000.pdb -nmax 1000 -complex -models
```

All commands were written in a Linux shell file and executed.

For further analysis, kinase or E3 ubiquitin ligase chain IDs were designated as “A” and those of interacting partners as “B” using PyMOL (78). The graphical representations of the docking structure were exported using PyMOL (78).

Interaction energy and iRMS analysis

To calculate interaction energy, I calculated the Rosetta energy of each protein or complex using the following command:

```
./bin/score_jd2.linuxgccrelease -in:file:s (protein or complex) -out:file:scorefile (score  
file)
```

Then, I calculated the interaction energy using the following equation:

$$\text{Interaction Energy} = \{\text{Rosetta energy of simulated docking structure}\} - \sum_{\text{Each component in pair}} \{\text{Rosetta energy of single components}\}$$

Then, iRMS were calculated using DockQ (43) and the following command:

```
./DockQ.py (Docking structure) (Strongest interacting docking structure) -short >>(iRMS  
file)
```

For interacting protein complexes and non-interacting protein pairs, iRMS were calculated using the following command:

```
./DockQ.py (Docking structure) (Strongest interacting docking structure) -short -  
native_chain1 (chain ID of proteins which participating in interaction) -model_chain1 (chain ID  
of proteins which participating in interaction) -native_chain2 (chain ID of proteins in opposite  
side of interaction) -model_chain2 (chain ID of proteins in opposite side of interaction) -perm1 -  
perm2 >>(iRMS file)
```


All commands were written in a Linux shell file and executed.

Gene ontology analysis

The bioinformatics analysis was performed as described previously (79), with small modifications. Briefly, using OmicsBox v2.0.36, protein sequences were blasted specifically using the NCBI nr database. Functional domains and motifs were distinguished using the EMBL-EBI InterPro database (80). Then, OmicsBox was used to annotate these results. Finally, I summarized gene ontology results according to level-2 gene ontology distribution from Generic GO slim (51) using OmicsBox.

Development of deep learning model and validation

To construct training dataset for general PPI prediction using deep learning, iRMS and interaction energy of 183 protein complex structures, retrieved from IntAct database (4) and 110 non-interacting protein pairs, retrieved from the Negatome 2.0 database (51) were calculated as above. To construct training dataset for prediction of kinase and EUL substrate, 214 kinase-substrate pair and 63 EUL-substrate pair were retrieved from PhosphoSitePlus (56) and UbiBrowser 2.0 (25) database, respectively (Supplementary data 7). Predicted protein structure were retrieved from AlphaFold database (57). Then, 1,000 simulated docking structure were generated using RosettaDock (41) and HDOCKlite (42), as described above. Then, interaction energy and iRMS were calculated as described above.

Deep learning model were constructed using Keras library (53). All data and codes, used for deep learning, are publicly available on GitHub (https://github.com/cjy318/protein_interaction).

References

1. V. S. Rao, K. Srinivas, G. Sujini, G. Kumar, Protein-protein interaction detection: methods and analysis. *International journal of proteomics* **2014**, (2014).
2. A. Bateman, M.-J. Martin, S. Orchard, M. Magrane, R. Agivetova, S. Ahmad, E. Alpi, E. H. Bowler-Barnett, R. Britto, B. Bursteinas, UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Research*, (2021).
3. S. Orchard, S. Kerrien, S. Abbani, B. Aranda, J. Bhate, S. Bidwell, A. Bridge, L. Briganti, F. S. Brinkman, G. Cesareni, Protein interaction data curation: the International Molecular Exchange (IMEx) consortium. *Nature methods* **9**, 345-350 (2012).
4. S. Orchard, M. Ammari, B. Aranda, L. Breuza, L. Briganti, F. Broackes-Carter, N. H. Campbell, G. Chavali, C. Chen, N. Del-Toro, The MIntAct project—IntAct as a common curation platform for 11 molecular interaction databases. *Nucleic acids research* **42**, D358-D363 (2014).
5. O. Keskin, N. Tuncbag, A. Gursoy, Predicting protein–protein interactions from the molecular to the proteome level. *Chemical reviews* **116**, 4884-4909 (2016).
6. D. Szklarczyk, A. L. Gable, K. C. Nastou, D. Lyon, R. Kirsch, S. Pyysalo, N. T. Doncheva, M. Legeay, T. Fang, P. Bork, The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic acids research* **49**, D605-D612 (2021).
7. A. J. Enright, I. Iliopoulos, N. C. Kyripides, C. A. Ouzounis, Protein interaction maps for complete genomes based on gene fusion events. *Nature* **402**, 86-90 (1999).
8. S. Saha, P. Chatterjee, S. Basu, M. Kundu, M. Nasipuri, FunPred-1: Protein function prediction from a protein interaction network using neighborhood analysis. *Cellular and Molecular Biology Letters* **19**, 675-691 (2014).
9. L. R. Matthews, P. Vaglio, J. Reboul, H. Ge, B. P. Davis, J. Garrels, S. Vincent, M. Vidal, Identification of potential interaction networks using sequence-based searches for conserved protein-protein interactions or “interologs”. *Genome research* **11**, 2120-2126 (2001).
10. N. Bhardwaj, H. Lu, Correlation between gene expression profiles and protein–protein interactions within and across genomes. *Bioinformatics* **21**, 2730-2738 (2005).
11. Q. Cong, I. Anishchenko, S. Ovchinnikov, D. Baker, Protein interaction networks revealed by proteome coevolution. *Science* **365**, 185-189 (2019).
12. F. Pazos, D. Juan, J. M. Izarzugaza, E. Leon, A. Valencia, "Prediction of protein interaction based on similarity of phylogenetic trees" in *Functional Proteomics* (Springer, 2008), pp. 523-535.
13. S. Dong, V. Lau, R. Song, M. Ierullo, E. Esteban, Y. Wu, T. Sivieng, H. Nahal, A. Gaudinier, A. Pasha, Proteome-wide, structure-based prediction of protein-protein interactions/new molecular interactions viewer. *Plant physiology* **179**, 1893-1907 (2019).
14. A. Baspinar, E. Cukuroglu, R. Nussinov, O. Keskin, A. Gursoy, PRISM: a web server and repository for prediction of protein–protein interactions and modeling their 3D complexes. *Nucleic acids research* **42**, W285-W289 (2014).
15. M. N. Wass, G. Fuentes, C. Pons, F. Pazos, A. Valencia, Towards the prediction of protein interaction partners using physical docking. *Molecular systems biology* **7**, 469 (2011).
16. B. Dai, C. Bailey-Kellogg, Protein interaction interface region prediction by geometric deep learning. *Bioinformatics*, (2021).
17. Q. C. Zhang, D. Petrey, J. I. Garzón, L. Deng, B. Honig, PrePPI: a structure-informed database of protein–protein interactions. *Nucleic acids research* **41**, D828-D833 (2012).
18. D. Kuroda, J. J. Gray, Shape complementarity and hydrogen bond preferences in protein–protein interfaces: implications for antibody modeling and protein–protein docking. *Bioinformatics* **32**, 2451-2456 (2016).

19. M. Y. Hein, N. C. Hubner, I. Poser, J. Cox, N. Nagaraj, Y. Toyoda, I. A. Gak, I. Weisswange, J. Mansfeld, F. Buchholz, A human interactome in three quantitative dimensions organized by stoichiometries and abundances. *Cell* **163**, 712-723 (2015).
20. S. A. Ochsner, D. Abraham, K. Martin, W. Ding, A. McOwiti, W. Kankanamge, Z. Wang, K. Andreano, R. A. Hamilton, Y. Chen, The Signaling Pathways Project, an integrated ‘omics knowledgebase for mammalian cellular signaling pathways. *Scientific data* **6**, 1-21 (2019).
21. H. Wang, D. Chevalier, C. Larue, S. K. Cho, J. C. Walker, The protein phosphatases and protein kinases of Arabidopsis thaliana. *The Arabidopsis Book/American Society of Plant Biologists* **5**, (2007).
22. C. Dardick, J. Chen, T. Richter, S. Ouyang, P. Ronald, The rice kinase database. A phylogenomic database for the rice kinome. *Plant physiology* **143**, 579-586 (2007).
23. J. Choi, W. Lee, G. An, S.-R. Kim, OsCBE1, a Substrate Receptor of Cullin4-Based E3 Ubiquitin Ligase, Functions as a Regulator of Abiotic Stress Response and Productivity in Rice. *International journal of molecular sciences* **22**, 2487 (2021).
24. K.-Y. Huang, J. T.-Y. Weng, T.-Y. Lee, S.-L. Weng, in *BMC systems biology*. (Springer, 2016), vol. 10, pp. 27-36.
25. X. Wang, Y. Li, M. He, X. Kong, P. Jiang, X. Liu, L. Diao, X. Zhang, H. Li, X. Ling, UbiBrowser 2.0: a comprehensive resource for proteome-wide known and predicted ubiquitin ligase/deubiquitinase–substrate interactions in eukaryotic species. *Nucleic acids research*, (2021).
26. H. Li, M. Wang, X. Xu, Prediction of kinase–substrate relations based on heterogeneous networks. *Journal of bioinformatics and computational biology* **13**, 1542003 (2015).
27. G.-M. Qin, R.-Y. Li, X.-M. Zhao, PhosD: inferring kinase–substrate interactions based on protein domains. *Bioinformatics* **33**, 1197-1204 (2017).
28. H. Wang, J. Qiu, H. Liu, Y. Xu, Y. Jia, Y. Zhao, HKPocket: human kinase pocket database for drug design. *BMC bioinformatics* **20**, 1-11 (2019).
29. J. Gan, J. Qiu, C. Deng, W. Lan, Q. Chen, Y. Hu, Ksimc: Predicting kinase–substrate interactions based on matrix completion. *International journal of molecular sciences* **20**, 302 (2019).
30. B. Xue, B. Jordan, S. Rizvi, K. M. Naegle, KinPred: A unified and sustainable approach for harnessing proteome-level human kinase-substrate predictions. *PLoS computational biology* **17**, e1008681 (2021).
31. J. A. Ubersax, J. E. Ferrell Jr, Mechanisms of specificity in protein phosphorylation. *Nature reviews Molecular cell biology* **8**, 530-541 (2007).
32. P. Jenardhanan, M. Panneerselvam, P. P. Mathur, Targeting kinase interaction networks: a new paradigm in PPI based design of kinase inhibitors. *Current topics in medicinal chemistry* **19**, 467-485 (2019).
33. M. Huse, J. Kuriyan, The conformational plasticity of protein kinases. *Cell* **109**, 275-282 (2002).
34. C. E. Berndsen, C. Wolberger, New insights into ubiquitin E3 ligase mechanism. *Nature structural & molecular biology* **21**, 301-307 (2014).
35. R. F. Alford, A. Leaver-Fay, J. R. Jeliazkov, M. J. O’Meara, F. P. DiMaio, H. Park, M. V. Shapovalov, P. D. Renfrew, V. K. Mulligan, K. Kappel, The Rosetta all-atom energy function for macromolecular modeling and design. *Journal of chemical theory and computation* **13**, 3031-3048 (2017).
36. B. Tang, Z. Pan, K. Yin, A. Khateeb, Recent advances of deep learning in bioinformatics and computational biology. *Frontiers in genetics* **10**, 214 (2019).
37. R. Kumari, S. K. Srivastava, Machine learning: A review on binary classification. *International Journal of Computer Applications* **160**, (2017).

38. K.-Y. Huang, H.-Y. Wu, Y.-J. Chen, C.-T. Lu, M.-G. Su, Y.-C. Hsieh, C.-M. Tsai, K.-I. Lin, H.-D. Huang, T.-Y. Lee, RegPhos 2.0: an updated resource to explore protein kinase–substrate phosphorylation networks in mammals. *Database* **2014**, (2014).
39. Y. Li, P. Xie, L. Lu, J. Wang, L. Diao, Z. Liu, F. Guo, Y. He, Y. Liu, Q. Huang, An integrated bioinformatics platform for investigating the human E3 ubiquitin ligase–substrate interaction network. *Nature communications* **8**, 1-9 (2017).
40. L. A. Kelley, S. Mezulis, C. M. Yates, M. N. Wass, M. J. Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols* **10**, 845-858 (2015).
41. S. Chaudhury, M. Berrondo, B. D. Weitzner, P. Muthu, H. Bergman, J. J. Gray, Benchmarking and analysis of protein docking performance in Rosetta v3. 2. *PloS one* **6**, e22477 (2011).
42. Y. Yan, H. Tao, J. He, S.-Y. Huang, The HDock server for integrated protein–protein docking. *Nature protocols* **15**, 1829-1852 (2020).
43. S. Basu, B. Wallner, DockQ: a quality measure for protein-protein docking models. *PloS one* **11**, e0161879 (2016).
44. M. F. Lensink, R. Méndez, S. J. Wodak, Docking and scoring protein complexes: CAPRI 3rd Edition. *Proteins: Structure, Function, and Bioinformatics* **69**, 704-718 (2007).
45. S. J. Baker, E. P. Reddy, CDK4: a key player in the cell cycle, development, and cancer. *Genes & cancer* **3**, 658-669 (2012).
46. F. Mercurio, H. Zhu, B. W. Murray, A. Shevchenko, B. L. Bennett, J. Wu Li, D. B. Young, M. Barbosa, M. Mann, A. Manning, IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. *Science* **278**, 860-866 (1997).
47. S. Mitchell, J. Vargas, A. Hoffmann, Signaling via the NFκB system. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* **8**, 227-241 (2016).
48. M. D. Daily, D. Masica, A. Sivasubramanian, S. Somarouthu, J. J. Gray, CAPRI rounds 3–5 reveal promising successes and future challenges for RosettaDock. *PROTEINS: Structure, Function, and Bioinformatics* **60**, 181-186 (2005).
49. H. Huang, B. M. Jernak, J. S. Bader, Where have all the interactions gone? Estimating the coverage of two-hybrid protein interaction maps. *PLoS computational biology* **3**, e214 (2007).
50. P. Blohm, G. Frishman, P. Smialowski, F. Goebels, B. Wachinger, A. Ruepp, D. Frishman, Negatome 2.0: a database of noninteracting proteins derived by literature mining, manual annotation and protein structure analysis. *Nucleic acids research* **42**, D396-D400 (2014).
51. G. O. Consortium, The Gene Ontology (GO) database and informatics resource. *Nucleic acids research* **32**, D258-D261 (2004).
52. E. H. Davidson, J. P. Rast, P. Oliveri, A. Ransick, C. Caestani, C.-H. Yuh, T. Minokawa, G. Amore, V. Hinman, C. Arenas-Mena, A genomic regulatory network for development. *science* **295**, 1669-1678 (2002).
53. I. W. Mustika, H. N. Adi, F. Najib, in *2021 4th International Conference on Information and Communications Technology (ICOIACT)*. (IEEE, 2021), pp. 304-308.
54. M. Uzair, N. Jamil, in *2020 IEEE 23rd international multitopic conference (INMIC)*. (IEEE, 2020), pp. 1-6.
55. K. G. Sheela, S. N. Deepa, Review on methods to fix number of hidden neurons in neural networks. *Mathematical problems in engineering* **2013**, (2013).
56. P. V. Hornbeck, J. M. Kornhauser, V. Latham, B. Murray, V. Nandhikonda, A. Nord, E. Skrzypek, T. Wheeler, B. Zhang, F. Gnadt, 15 years of PhosphoSitePlus®: integrating post-translationally modified sites, disease variants and isoforms. *Nucleic Acids Research* **47**, D433-D441 (2019).

57. M. Varadi, S. Anyango, M. Deshpande, S. Nair, C. Natassia, G. Yordanova, D. Yuan, O. Stroe, G. Wood, A. Laydon, AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic acids research* **50**, D439-D444 (2022).
58. I. A. Vakser, Protein-protein docking: From interaction to interactome. *Biophysical journal* **107**, 1785-1793 (2014).
59. J. Janin, K. Henrick, J. Moult, L. T. Eyck, M. J. Sternberg, S. Vajda, I. Vakser, S. J. Wodak, CAPRI: a critical assessment of predicted interactions. *Proteins: Structure, Function, and Bioinformatics* **52**, 2-9 (2003).
60. M. F. Lensink, G. Brysbaert, T. Mauri, N. Nadzirin, S. Velankar, R. A. Chaleil, T. Clarence, P. A. Bates, R. Kong, B. Liu, Prediction of protein assemblies, the next frontier: The CASP14-CAPRI experiment. *Proteins: Structure, Function, and Bioinformatics* **89**, 1800-1823 (2021).
61. A. Kryshtafovych, T. Schwede, M. Topf, K. Fidelis, J. Moult, Critical assessment of methods of protein structure prediction (CASP)—Round XIV. *Proteins: Structure, Function, and Bioinformatics* **89**, 1607-1617 (2021).
62. M. D. Altman, E. A. Nalivaika, M. Prabu-Jeyabalan, C. A. Schiffer, B. Tidor, Computational design and experimental study of tighter binding peptides to an inactivated mutant of HIV-1 protease. *Proteins: Structure, Function, and Bioinformatics* **70**, 678-694 (2008).
63. B. G. Pierce, K. Wiehe, H. Hwang, B.-H. Kim, T. Vreven, Z. Weng, ZDOCK server: interactive docking prediction of protein–protein complexes and symmetric multimers. *Bioinformatics* **30**, 1771-1773 (2014).
64. M. A. Siddiq, G. K. Hochberg, J. W. Thornton, Evolution of protein specificity: insights from ancestral protein reconstruction. *Current opinion in structural biology* **47**, 113-122 (2017).
65. K. A. Boehme, C. Blattner, Regulation of p53 Activity. *Current Chemical Biology* **4**, 1-12 (2010).
66. N. A. Marze, S. S. Roy Burman, W. Sheffler, J. J. Gray, Efficient flexible backbone protein–protein docking for challenging targets. *Bioinformatics* **34**, 3461-3469 (2018).
67. A. W. Senior, R. Evans, J. Jumper, J. Kirkpatrick, L. Sifre, T. Green, C. Qin, A. Židek, A. W. Nelson, A. Bridgland, Improved protein structure prediction using potentials from deep learning. *Nature* **577**, 706-710 (2020).
68. M. Baek, F. DiMaio, I. Anishchenko, J. Dauparas, S. Ovchinnikov, G. R. Lee, J. Wang, Q. Cong, L. N. Kinch, R. D. Schaeffer, C. Millán, H. Park, C. Adams, C. R. Glassman, A. DeGiovanni, J. H. Pereira, A. V. Rodrigues, A. A. v. Dijk, A. C. Ebrecht, D. J. Opperman, T. Sagmeister, C. Buhlheller, T. Pavkov-Keller, M. K. Rathinaswamy, U. Dalwadi, C. K. Yip, J. E. Burke, K. C. Garcia, N. V. Grishin, P. D. Adams, R. J. Read, D. Baker, Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **373**, 871-876 (2021).
69. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Židek, A. Potapenko, Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583-589 (2021).
70. K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *Journal of computational chemistry* **31**, 671-690 (2010).
71. S. A. McDowell, A simple derivation of the Boltzmann distribution. *Journal of chemical education* **76**, 1393 (1999).

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Figures and Tables

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Figure 1. Narrow funnel-like interaction energy distribution hypothesis and workflow for interaction energy distribution analysis.

(A) Schematic diagram of narrow funnel hypothesis. Specific interactions might have narrow funnel-like interaction energy distribution. (B) Schematic diagram of interaction energy distribution analysis workflow. (C) Introduction of redefined iRMS. Residues of the interface area in the strongest interacting structure are colored in red.

Table 1. Docking programs for possible docking structure generation.

	RosettaDOCK	HDOCKlite
Algorithm	Monte-Carlo	Fast-Fourier Transform
Scoring	Knowledge-based Energy Function	Shape-based Scoring Function
Structure Refinement	O	X (rigid body)
Speed	Slow*	Fast**
Reference	(41)	(42)

*For generation of 1,000 docking structures, several hours~several days per interaction pair in personal computer (Intel® Core i7-10700K 3.80GHz with 8 cores and 32GB of RAM)

** For generation of 1,000 docking structures, several minutes~few hours per interaction pair in personal computer (Intel® Core i7-10700K 3.80GHz with 8 cores and 32GB of RAM)

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Figure 2. Structural meaning of narrow funnel-like interaction energy distribution.

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The five strongest interacting structures of CDK4-CCND1 (identified specific interaction pair) and CDK4- $\text{IKK}\alpha$ (random pair). To avoid confusion, CDK4 in each pair is set in the same position and CDK4 in the strongest interacting structure is represented in gray. Each partner is colored in rainbow color in the order of interaction energy.

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Figure 3. Quantitative analysis of narrow funnel-like interaction energy distribution and accuracy of predicting protein interactions by searching narrow funnel-like interaction distribution

(A) Scheme of the strategy to distinguish narrow funnel-like interaction energy distributions. (B) How well narrow funnel interaction energy distribution can distinguish specific interaction partners for each criteria. Among whole interaction pairs, pairs with high-accuracy prediction results ($>70\%$ region with high confidence) and relatively small proteins (≤ 700 residues) were analyzed. Difference ratios between true-positive (identified interacting protein complex with narrow funnel-like interaction energy distribution) and false-positive (random pair with narrow funnel-like interaction energy distribution) for each criteria are shown on heatmap. Due to graphical representation limitations, only the maximum ratio differences are shown for each distribution criteria. (C) Rate comparisons for finding specific interaction partners using the narrow funnel and yeast two-hybrid screening methods. Comparison of genome-scale yeast two-hybrid analysis for three species (49). The criteria for finding narrow funnel-like energy distribution are described in Table 2.

894 **Table 2.** Criteria to distinguish narrow funnel-like interaction energy distribution of interaction
895 pairs with high-accuracy structure prediction results and relatively small proteins.
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	RosettaDock		HDOCKlite	
	Kinase pair	E3 ubiquitin ligase pair	Kinase pair	E3 ubiquitin ligase pair
Interaction energy criteria (kcal/mol)	-3.6	-23.4	26~400	150~151
iRMS criteria (Å)	44.6	39.6~40	44.2	29.2~29.6
Distribution criteria (%)	92.2	0.8	0.2	0.8

Figure 4. Distribution of average iRMS of interacting protein complexes and noninteracting protein pairs.

Distribution of average iRMSs of 1,000 docking structures of interacting protein complexes and noninteracting protein pairs are shown using box-and-whisker plot. Protein pairs divided according to the functional annotation with level-2 gene ontology distribution from Generic GO slim. Each related biological process in level-2 gene ontology from Generic GO slim and the number of protein pairs are notated. Significant differences of average iRMS with 95% confidence using Student's t-test are marked with asterisks and 99% confidence marked with double asterisks.

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Figure 5. Schematic diagram of protein interaction prediction using interaction energy distributions and deep learning.

This diagram describes the protein interaction prediction strategy based on the interaction energy diagram.

Table 3. Highest accuracy of 10-fold validation with data shuffling of candidate models for each protein interaction prediction and optimal deep learning model with the highest accuracy.

		General protein interaction prediction	Kinase substrate prediction	E3 ubiquitin ligase substrate prediction
Accuracy		77.6%	61.4%	77.7%
Optimal model	Optimizer	Adamax	Adadelata	Adamax
	Layer structure	1024-64-2	1024-64-2	1024-2
	Epochs	109	9	61

Figure 6. Predictive performance of deep learning models

Receiver operating characteristic (ROC) curves from 10-fold cross validation when shuffling data in the deep learning model to prediction protein interactions between E3 ubiquitin ligase and kinase substrates. Each area under curve (AUC) value is notated in the chart.

Supplementary Files

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