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## Implications of disease-related mutations in protein-protein interfaces

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### Abstract

Protein-protein interfaces have been attracting great attention due to their critical roles in protein-protein interactions and the fact that human disease-related mutations are generally enriched in them. Recently, substantial research progress has been made in this field, which have significantly promoted the understanding and treatment of various human diseases. For example, many studies have discovered the properties of disease-related mutations. Besides, as more large-scale experimental data become available, various computational approaches have been proposed to advance our understanding of disease mutations from the data. Here, we overview recent advances in characteristics of disease-related mutations at protein-protein interfaces, mutation effects on protein interactions, and investigation of mutations on specific diseases.

### Keywords

Disease-related mutations; Protein-protein interfaces; Binding affinity; Deep learning; Pathogenicity

### Introduction

The protein-protein interface makes crucial contributions to the specificity and strength of protein interactions, which are essential to most biological processes, and determines the mechanism by which proteins fulfill their functions [1]. The loss or alteration of protein functions caused by amino acid mutations can result in diseases and these mutations are hence referred to as ‘disease-related mutations’ [2]. Disease-related mutations have been reported to be enriched at protein-protein interfaces [3, 4, 5, 6], and are more evolutionarily conserved than other surface residues [7, 8, 9]. Therefore, studies of disease-related mutations involved in protein-protein interfaces provide important insights for deciphering

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disease mechanisms and potential treatments. For example, ECLAIR, a unified machine learning framework of Interactome INSIDER [3], computationally predicted protein-protein interfaces of 185,957 binary interactions with previously unresolved interfaces in human and seven other model organisms on a large scale, which extensively makes the downstream studies possible. Cheng *et al.* [10] used the predicted protein-protein interfaces in Interactome INSIDER to demonstrate that network-predicted oncoPPIs, which is a protein interaction with a significant enrichment in interface mutations across individuals, are closely related to patient survival and drug resistance/sensitivity either in human cancer cell lines or in patient-derived xenografts. This finding provides promising prognostic markers and pharmacogenomic biomarkers for potential clinical guidance.

Large-scale studies have reported that disease-related mutations tend to cause large geometrical and physicochemical changes of mutation sites, which in turn affects the stability of protein interactions by changing their binding affinity [6, 7, 11]. In the past few years, many computational methods have been proposed to predict mutation effects on protein interactions, especially their effects on the binding affinity of protein interactions [12]. Although there is often a trade-off between accuracy of experimental methods and efficiency of computational methods, the computational methods have achieved remarkable successes given that experimental methods are generally laborious, costly, and time-consuming. In the following sections, we focus on recent studies on the characteristics of disease-related mutations at protein-protein interfaces, advances in the identification of mutational effects on protein interactions, and investigations of mutations on specific human diseases.

## Mutations at protein-protein interfaces on human diseases

It has been widely demonstrated that disease-related mutations preferentially localize to protein-protein interaction interfaces [3, 4, 5, 13, 14]. Figure 1 shows that a disease-related mutation located at an interface disrupts the interaction and implicates the corresponding disease [9]. Moreover, mutations on the same protein can cause distinct clinical diseases by disrupting its interactions with different partners [3, 4, 5]. David *et al.* [14] examined the frequencies of 2,420 disease-related mutations in three different regions of proteins (protein core, interface, and surface noninterface) and compared the quantified preferences of disease-related mutations at protein interfaces with that of the other two regions. Their results showed that disease-related mutations preferentially occur at interfaces than surface noninterface with an odds ratio (OR) of 1.59. Additionally, a similar observation (OR=1.44) has been reported by another study [2].

Interface residues, which are located over a large surface area, can be distinguished into 'core residues' and 'rim residues' based on their solvent accessibility in the bound state of two interacting proteins [15]. Interface rim residues are partially solvent-accessible and surround the interface core region at which the residues are completely buried as a result of the protein-protein interaction. It has been shown that residues on the interface core region generally make more contributions than those on the rim to protein interactions [2, 13]. Specifically, Navío *et al.* [2] showed this tendency by examining 2,062 residues in the interface core and rim regions. The authors concluded that interface residues that make

an energetic contribution to the protein complex stability or related to human diseases are more likely to locate at the interface core region rather than rim region, and quantified this preference using OR (OR = 2.11). However, disease-related mutations did not show any preference to be located at the interface rim regions than the non-interface surfaces [13]. Moreover, the significance of preference for the neutral mutations, which do not cause any genetic disorders, for interface core vs rim has not been established [2].

The binding affinity change ( $\Delta G$ ) is an important factor to discriminate disease-related mutations from neutral mutations. While random amino acid substitutions including neutral and disease-related mutations tend to decrease the binding free energy [6, 13, 16], disease-related mutations are more likely to cause a decrease in their binding affinity [6, 13]. Jemimah *et al.* [6] performed a statistical analysis to explore the change in binding affinity caused by neutral and disease-related mutations. They found that most of the disease-related mutations decrease the binding affinity and showed that there exists a significant difference in the effect on protein stability between neutral and disease-related mutations.

Mutations may introduce various types of effects, which include reduction in hydrophobic region, overpacking, decrease in electrostatic interactions etc., and cause protein instability and, eventually, loss of protein interactions [14, 17]. For example, an amino acid substitution from a smaller residue to a larger residue could lead to steric clashes. On the other hand, a mutation from a larger amino acid to a smaller amino acid could create a spatial gap. Either of the cases is likely to impair protein stability [17]. Besides, the impact is more significant if the mutation is carried out by energetic hotspots or residues in the protein core [13]. The change of hydrophobicity of residue caused by mutations in the interface region also brings about loss of protein interactions. As interface residues are more hydrophobic than surface noninterface residues [18], their substitution to charged or polar residues could disrupt protein interactions [14]. While destabilization is a more common effect, disease-related mutations could also stabilize the proteins and complexes [19, 20]. This suggests that, although the decrease in binding affinity and protein instability could be the dominant effects of disease-related mutations, they could bring other types of effects, or even opposite effects, to protein structures and protein interactions, which requires more comprehensive analysis to understand their mechanisms.

## Approaches to identifying mutation effects on protein interactions

The change in binding affinity of protein interactions caused by mutations can further affect the stability of protein interactions and the function of proteins involved and, eventually, cause diseases. Effects of mutations on protein-protein binding sites can be assessed by the change in binding free energy, which is one of the most significant factors contributing to pathogenicity [11, 21]. The binding affinity can be quantitatively measured through various experimental methods, including Isothermal Titration Calorimetry (ITC), Förster Resonance Energy Transfer (FRET), Surface Plasmon Resonance (SPR), and Fluorescence Polarization (FP) [11, 22]. These methods provide accurate measures of binding affinity change, however, they are laborious, expensive and time-consuming and, more importantly, not feasible to large-scale datasets in practice. These drawbacks of experimental methods motivated the development of fast and reliable computational approaches to predicting

protein-protein  $G$  in large-scale studies. In the past decades, as shown in Table 1, many computational approaches have been proposed to meet the needs, which can be broadly categorized into classical energy-based methods and machine learning-based methods [12, 23].

Classical energy-based methods typically rely on physical/empirical energies and/or statistical potentials to find the optimal models. Some of these methods, such as FoldX [24, 25], Rosetta [26], CC/PBSA [27], ZEMu [28], Flex ddG [29], etc. use physical energies. The physical energies mainly come from van der Waals, solvation, hydrogen bonds, water bridges, electrostatic, entropy, Lennard Jones interactions. Furthermore, the statistical potentials are also used to predict  $G$ , such methods include BeAtMuSiC [30], contact potentials-based model [31], etc. These statistical potentials describe the correlations between numbers of pairwise inter-residue contacts, pairwise inter-residue distances, amino acid types, backbone torsion angles and solvent accessibilities. The most recently published energy-based methods including BindProfX [32] and SSIPe [33] combined such energies with other information (such as protein interface profiles) to predict  $G$  and have achieved better performance than other energy-based models. However, these methods require the structures of mutated complexes as an input, which in turn limits their applicability drastically.

Machine learning methods capture the relationship between the  $G$  and a set of generally important features extracted from the protein structure, sequence, energy, evolution, etc.. Due to the ever-increasing mutation data availability in public databases such as SKEMPI 2.0 [34] and PROXiMATE [35], a high number of machine learning-based methods have been proposed in recent years. Some of them, such as mCSM-PPI2 [36], iSEE [37], TopNetTree [38], MutaBind2 [39], ELASPIC2 [40], etc., have been developed with structure-based features using extra trees (ETs), random forest (RF), gradient boosting decision tree (GBDT), convolutional neural network (CNN), transformer neural network (TNN), graph neural network (GNN) and so on. Several machine learning-based methods, which give better representations of structures of mutated complexes, typically include the aforementioned energies as features. However, these methods, like the energy-based methods, suffer in terms of their applicability due to their reliance on the input structures, even though the structures of a small portion of mutated complexes can be approximated by modeling 3D structures from sequences using homology modeling. Therefore, some sequence-based methods, such as ProAffiMuSeq [41], MuPIPR [23], SAAMBE-SEQ [42], etc., have been proposed using bidirectional long short-term memory (Bi-LSTM), recurrent convolutional neural network (RCNN), multi-layer perceptron (MLP), GBDT and so on. Over the past few years, deep learning has shown remarkable success and the explosive growth in its application to various fields including bioinformatics [43, 44]. For studying mutational effects on protein interactions, several deep learning models such as TopNetTree, MuPIPR, have been developed and achieves satisfactory performance. Especially, MuPIPR achieves success in an end-to-end manner without the need of hand-crafted features, which indicates the strong representation power and practical advantages of deep learning. Along with further accumulation of data and advances in computational technology, deep learning will lead to enormous opportunities for machine learning-based methods to learn the complicated patterns between various protein features and the  $G$ .

Feature design also plays a crucial role in computational approaches. The knowledge of feature importance would greatly contribute to the understanding of mechanisms of mutation impact on  $G$  and the development of novel computational approaches [12]. Several methods have reported the relative importance of features they used. Specifically, iSEE reveals that the Position Specific Scoring Matrix (PSSM) value of the wildtype amino acid and the difference of PSSM values between mutant and wildtype residues are the most important features. The PSSM captures the evolutionary conservation information of a specific amino acid. In SAAMBE-SEQ, the PSSM-based evolution and conservation scores were also found to be the two most important features. It is worth noting such evolution and conservation information can be obtained through protein sequence without the need for any structural information.

## The pathogenicity of mutations affecting protein interaction on human diseases

Mutations that affect protein interaction interfaces influence the formation of protein complexes. They lead to phenotypic changes and have been demonstrated to play “driver” roles in human cancers as well as other genetic diseases. A vast number of studies have discovered that pathogenic mutations cause different diseases by disrupting protein interactions or changing the binding affinity of specific protein complexes [45, 46, 47, 48, 49, 50, 51]. Kato *et al.* [48] reported that the cancer-derived substitution mutation is likely to occur at highly conserved amino acids of the ubiquitously transcribed tetratricopeptide repeat on the X chromosome (UTX). The authors also observed that several mutations in the tetratricopeptide repeat (TPR) alter the interactions of UTX with core components of mixed-lineage leukemia complexes (MLL3/4 complexes), the interactions with crucial importance in the tumor suppressor function. Yan *et al.* [50] investigated the structural basis of the pathogenicity of Legius syndrome mutations and discovered that these mutations reduce the binding affinity between SPRED1 and NF1. This finding supports the hypothesis proposed in [51] that mutations in SPRED1 could cause Legius syndrome due to an inability to form the NF1-SPRED1 complex. Chen *et al.* [52] showed that missense mutations disrupting interactions of autism spectrum disorder (ASD) proteins are enriched in individuals with ASD and further constructed and analyzed an “ASD disrupted network” by connecting all disrupted pairs of proteins to explore the risk genes of ASD.

The pathogenicity of disease mutations can also be derived from the alteration of binding properties of proteins and their interactome [4, 19, 53, 54, 55], of which little research has been conducted as the perturbations of interactome is much more difficult to analyze than the alterations in single disease proteins. Mehnert *et al.* [53] analyzed cancer mutations in the Dyrk2 protein kinase and found that these mutations significantly change the Dyrk2 interaction network. For more information, readers may refer to Wanker *et al.* [55], which reviewed the studies about the perturbed interactors of the huntingtin (HTT) protein with mutants and their pathobiological roles in the disease.

Moreover, mutations of SARS-Cov-2 proteins that affect specific protein interactions have significant impacts on its infectivity. It is well known that the coronavirus disease 2019

(COVID-19) has stronger infectivity than the SARS coronavirus 2003 [56]. The key factor causing this difference discovered by Wang *et al.* [57] is the mutation of a hydrophobic residue in the SARS-CoV sequence to Lys417 in SARS-CoV-2. It enhances protein interactions between SARS-CoV-2 and the host receptor ACE2 which serves as a major receptor for SARS-CoV-2 in human cells. Besides, several SARS-CoV-2 mutations (e.g., D614G [58, 59], N501Y [60]) were also observed to enhance the binding affinity between SARS-CoV-2 and ACE2. These mutations might either increase the infection rates or be related to a higher case fatality rate from the strains of SARS-CoV-2 found in different countries. Rawat *et al.* [61] performed a mutational analysis for the interactions of three strains of NL63, SARS-CoV and SARS-CoV-2 with ACE2, respectively. They found the mutation of the conserved Gly residue in all three strains (Gly537 in NL63, Gly488 in SARS-CoV and Gly502 in SARS-CoV-2) significantly reduced the binding affinity, which revealed its importance for both stabilizing and interacting with ACE2. Further, the SARS-CoV-2 mutations could also change intraviral protein interactions and might influence the transmission of SARS-CoV-2 and the treatment of COVID-19 [62].

## Conclusion

The significance of protein-protein interfaces has prompted rigorous research and resulted in great insights that enhance our understanding of molecular mechanism of protein interactions. Many studies have demonstrated that disease-related mutations at interfaces may lead to various effects such as destabilizing protein interactions, decreasing binding affinity, etc. Although experimental methods allow us to accurately measure such effects, many computational approaches have been proposed to efficiently study protein functions with large-scale datasets. Especially, deep learning has been given considerable attention due to the accumulation of large data and powerful computational resources, and its strong feature representation ability. The deep exploration of disease-related mutations will enable the novel and rational drug design.

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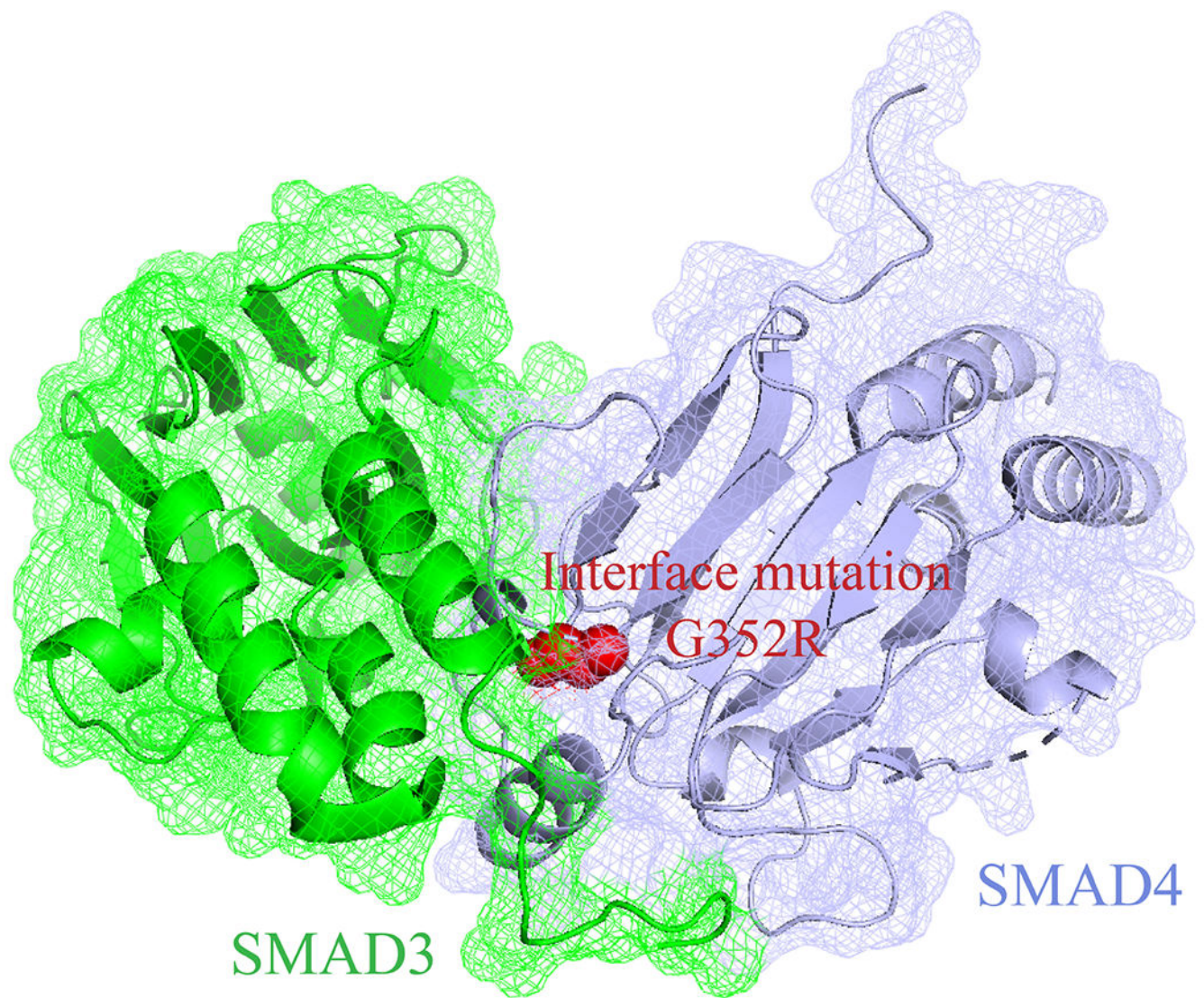


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### Highlights

- Disease-related mutations preferentially localize to protein-protein interfaces.
- Mutations, in general, destabilize protein-protein interactions and protein structures.
- Deep learning shows great advantages and potential in mutation effect prediction.
- Pathogenic mutations may lead to different diseases by disrupting or changing the binding affinity.



**Figure 1. A disease-related interface mutation G352R on SMAD4.**  
This mutation disrupts the SMAD4 interaction with SMAD3, and implicates the TGF $\beta$ /SMAD signaling pathway in the formation of juvenile polyposis.

**Table 1.**Summary of representative computational approaches for  $G$  prediction.

Categories	Names	Webservers/Softwares	Unique features/advantages	References
Energy-based	FoldX	<a href="http://foldxsuite.crg.eu">http://foldxsuite.crg.eu</a>	Uses a rotamer library	[24, 25]
	Rosetta	-	Uses a rotamer library, focusing on alanine mutations	[26]
	CC/PBSA	-	Considers structural flexibility	[27]
	ZEMu	<a href="https://simtk.org/projects/rnatoolbox">https://simtk.org/projects/rnatoolbox</a>	Uses a multiscale method which models flexibility of mutation region	[28]
	Flex ddG	<a href="https://github.com/Kortemme-Lab/flex_ddG_tutorial">https://github.com/Kortemme-Lab/flex_ddG_tutorial</a>	Samples conformational diversity using “backrub” to generate an ensemble of models	[29]
	BeAtMuSiC	<a href="http://babylone.ulb.ac.be/beatmusic">http://babylone.ulb.ac.be/beatmusic</a>	Uses the coarse-grained representation of protein structures	[30]
	Contact potentials-based model	-	Uses atomic and residue contact potentials	[31]
Machine learning-based	BindProfX	<a href="https://zhanglab.dcmf.med.umich.edu/BindProfX">https://zhanglab.dcmf.med.umich.edu/BindProfX</a>	Calculates $G$ as the logarithm of relative probability of mutant residues over wild-type ones	[32]
	SSIPe	<a href="https://zhanglab.ccmb.med.umich.edu/SSIPe">https://zhanglab.ccmb.med.umich.edu/SSIPe</a> <a href="https://github.com/tommyhuangthu/SSIPe">https://github.com/tommyhuangthu/SSIPe</a>	Combines interface profiles derived from structural and sequence homology searches with a physics-based energy	[33]
	mCSM-PPI2	<a href="http://biosig.unimelb.edu.au/mcsm_ppi2">http://biosig.unimelb.edu.au/mcsm_ppi2</a>	Integrates mCSM graph-based signatures, evolutionary information, inter-residue non-covalent interaction networks analysis and energetic terms with ETs	[36]
	iSEE	<a href="https://github.com/haddocking/iSee">https://github.com/haddocking/iSee</a>	Combines structural, evolutionary, and energetic features with RF	[37]
	TopNetTree	<a href="https://codeocean.com/capsule/2202829/tree/v1">https://codeocean.com/capsule/2202829/tree/v1</a>	Integrates topological descriptors with CNN-assisted GBDT	[38]
	MutaBind2	<a href="https://lilab.jysw.suda.edu.cn/research/mutabind2">https://lilab.jysw.suda.edu.cn/research/mutabind2</a>	Combines a set of scoring functions with RF	[39]
	ELASPIC2	<a href="http://elaspic.kimlab.org">http://elaspic.kimlab.org</a> <a href="https://gitlab.com/elaspic/elaspic2">https://gitlab.com/elaspic/elaspic2</a>	Incorporates features generated using pre-trained TNN and GNN, and employs GBDT with a ranking object function	[40]
ProAffiMuSeq	<a href="https://web.iitm.ac.in/bioinfo2/proaffimuseq">https://web.iitm.ac.in/bioinfo2/proaffimuseq</a>	Considers the functional classes	[41]	
MuPIPR	<a href="https://github.com/guangyu-zhou/MuPIPR">https://github.com/guangyu-zhou/MuPIPR</a>	An end-to-end deep learning framework using Bi-LSTM, RCNN and MLP without the need of hand-crafted features	[23]	
SAAMBE-SEQ	<a href="http://compbio.clemson.edu/saambe_websvr/indexSEQ.php#started">http://compbio.clemson.edu/saambe_websvr/indexSEQ.php#started</a>	Employs GBDT on a set of features, and doesn't require the knowledge of interfacial residue	[42]	