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Mutations at protein-protein interfaces: Small changes over big surfaces have large impacts on human health

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ABSTRACT

Many essential biological processes including cell regulation and signalling are mediated through the assembly of protein complexes. Changes to protein-protein interaction (PPI) interfaces can affect the formation of multiprotein complexes, and consequently lead to disruptions in interconnected networks of PPIs within and between cells, further leading to phenotypic changes as functional interactions are created or disrupted. Mutations altering PPIs have been linked to the development of genetic diseases including cancer and rare Mendelian diseases, and to the development of drug resistance. The importance of these protein mutations has led to the development of many resources for understanding and predicting their effects. We propose that a better understanding of how these mutations affect the structure, function, and formation of multiprotein complexes provides novel opportunities for tackling them, including the development of small-molecule drugs targeted specifically to mutated PPIs.

Contents

1. Protein-protein interactions at the molecular level

Interactions between proteins mediate many biological processes, especially with respect to cell regulatory events requiring high signal-to-noise ratios to transduce information within and between cells (Blaszczyk et al., 2015). Fig. 1 shows an analysis of the range of biological processes in which PPIs are involved in humans. Heavy PPI involvement in critical cellular processes such as metabolism, cell signalling and cell death is indicative of why disruption or stabilisation of PPIs can have significant biological consequences and play roles in the development of diseases such as cancers (Fry and Vassilev, 2005). Residues

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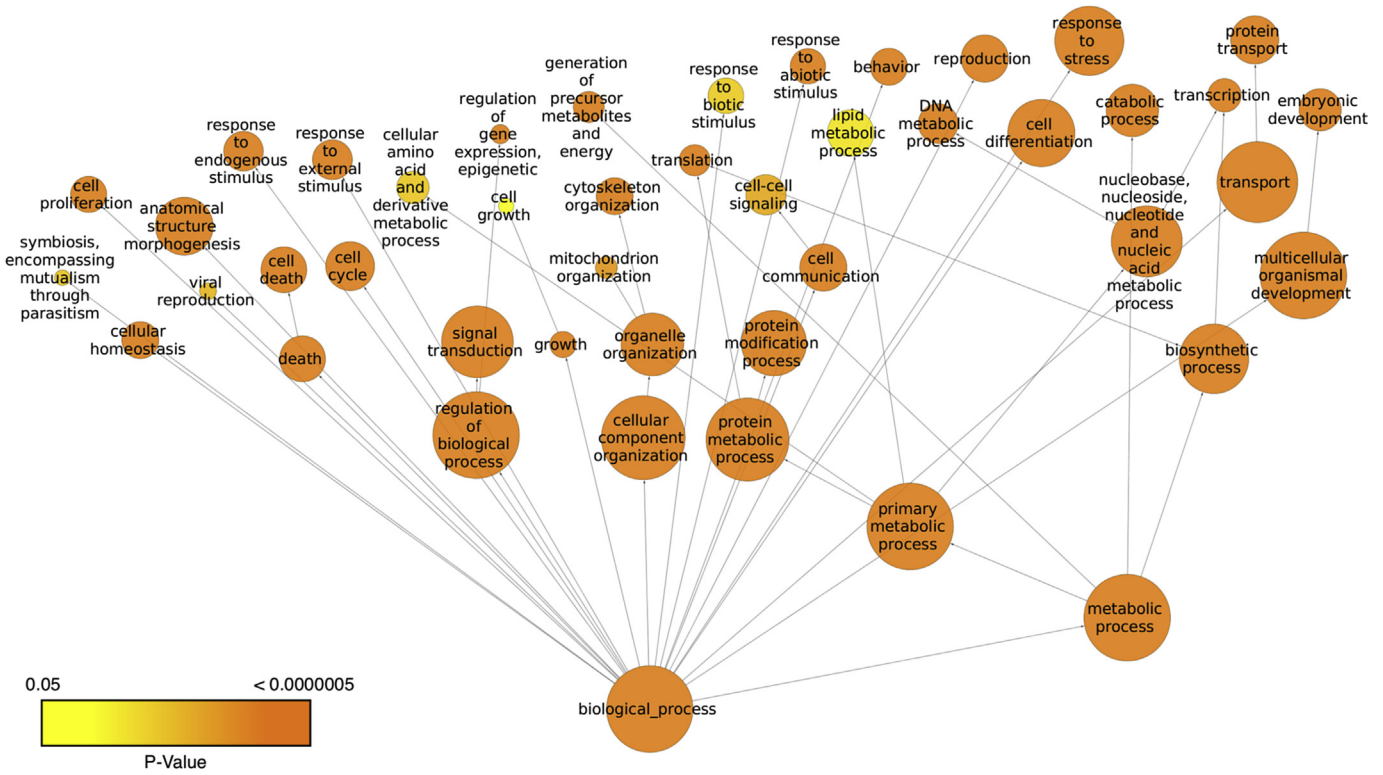

Fig. 1. GOslim term enrichment in the Homo sapiens protein-protein interactome The hierarchical, directional network spanning out from biological process reflects Gene Ontology (GO) (Ashburner et al., 2000; The Gene Ontology Consortium, 2015) biological process terms that were over-represented in a human PPI network constructed from the mentha (Calderone et al., 2013) and HPIDb (Kumar and Nanduri, 2010) databases. Node size was determined by the proportion of genes in the PPI network covered by the GO term. Node colour reflects the adjusted P-value indicating the significance of the over-representation of the term within the PPI network. Generated using the Cytoscape (Shannon et al., 2003) BiNGO plugin (Maere et al., 2005).

involved in protein interactions are under additional evolutionary restraints, and are more highly conserved than surface residues (Chelliah et al., 2004; Innis et al., 2000). It is therefore not surprising that mutations at PPIs are associated with a broad range of diseases. More surprising however is that recent reports show that mutations at PPIs are over-represented amongst disease-causing mutations (David et al., 2012; Engin et al., 2016; Yates and Sternberg, 2013). This raises an interesting idea that mutations affecting PPIs may allow for biological activities to be modulated, causing a disease phenotype, but with a smaller fitness cost compared to the catastrophic effects on protein function caused by many active site or protein-destabilizing mutations. Understanding how mutations modulate protein interactions and thus biological functions raises potential for developing therapeutic interventions targeting interaction mutants.

Protein interactions impart selectivity and sensitivity to biological processes, and may occur either through the co-operative assembly of specific multi-protein assemblies or through the co-operative folding and binding of one binding protomer onto another. The traditional view of protein interaction interfaces (the molecular surfaces through which subunits of multiprotein complexes make contact with one other) as being large, uniformly flat, and chemically featureless, has evolved. Recent studies highlight that interactions involving cooperative folding and binding of small polypeptides make use of distinct concavities (“pockets”) as opposed to the large single volume pockets exploited by small-molecule drugs (Jubb et al., 2015). Furthermore, interactions between larger, globular proteins, while utilising flat binding surfaces overall, make use of small loci of well-defined interaction sites within their large, flat interacting surfaces, even if only via small but well defined pockets fitting a single residue (Jubb et al., 2015). Highly shape and chemically-complementary single residue interactions have been shown to be “anchoring” points in many PPI interfaces (Koes et al., 2012; Koes and Camacho, 2012; Li et al., 2004; Rajamani et al., 2004), and can be important energetic drivers, or “hotspots”, in the assembly of PPI interfaces (Bogan and Thorn, 1998; Clackson and Wells, 1995). The observation that PPI interfaces can have these specific residues or regions that disproportionately drive protein complex assembly has spurred not only in an interest in developing drugs to target these interactions (Winter et al., 2012), but also an appreciation that single mutations can have a significant effects on protein-protein binding affinity. However, it is important to note that assessments of PPI affinity and the impacts of mutations on protein stability and PPI affinity, are complicated by the natural affinity of the interaction and whether the interaction is transient or constitutive/obligate (Liang et al., 2016; Nooren and Thornton, 2003). This can be related to function (Acuner Ozbabacan et al., 2011; Blundell et al., 2000; Perkins et al., 2010), and are important considerations when considering the known and potential impacts of mutations.

2. Mutations altering protein-protein binding affinities: implications for human health

The prevalence of PPIs (Strong and Eisenberg, 2007; Stumpf et al., 2008; Wells and McClendon, 2007) and their importance in a multitude of biological processes (Blundell et al., 2000) make PPIs prime candidates for modulation by disease processes. An especially comprehensive analysis of the structural nature of mutations in cancer has shown that mutations at PPI interface regions play “driver” roles in many cancers, and that specific mutations can herald different patient outcomes (Porta-Pardo et al., 2015). On a

larger, non-structurally-defined scale, genetic analysis of the effect of Mendelian disease polymorphisms on protein stability and interactions has indicated that at least a third of disease-causing polymorphisms affect disease through perturbing protein interactions rather than by destabilising monomeric proteins (Sahni et al., 2015), supporting the analysis of direct effects of mutations on interface affinities to aid in the understanding of human disease. Sahni et al.'s estimates eclipse earlier work using sequence homology mapped to structures to define estimates of 4% of mutations having an effect on protein interactions, across a range of proteins and diseases (Schuster-Bockler and Bateman, 2008). From a biological network viewpoint, lethal mutations have been shown to occur in highly connected nodes of protein interaction networks, and such mutations have disruptive effects on the overall structure of the network without compensatory pathways (Przulj et al., 2004).

In recent years, the structural nature of PPI interface mutations has begun to be understood, building on previous functionally-driven analyses (Yates and Sternberg, 2013). Disease causing mutations at PPI interfaces tend to cause large geometrical and physicochemical changes at interaction sites, affecting interface stability, interface conformational dynamics through stabilisation or disruption of specific conformational states, and affecting direct interactions with partner protomers (Kucukkal et al., 2015). However, not all interface mutations are equal, and the structural location of PPI interface mutations is important with respect to disease. Missense mutations at PPI interfaces exhibit different etiology depending on which region of interfaces they are present in. David and Sternberg (2015) showed that mutations in solvent inaccessible interface "core" regions are more likely to be disease causing compared to mutations in solvent accessible interface peripheral regions, which are as enriched in neutral polymorphisms as non-interacting protein surface residues (David and Sternberg, 2015). Interestingly, there is also a distinction in the mutation profiles of ordered and disordered regions of proteins, wherein disordered regions tend to be tolerant of non-pathogenic variants, but not pathogenic (Mendelian or cancer) variants, suggesting that mutations in more structurally ordered regions in proteins, including PPI interfaces, tend to be more deleterious (Lu et al., 2015). In addition to the published analyses detailed above, new platforms are now available that enable analysis of the structural locations and impacts of mutations from Mendelian disorders and cancer (Lu et al., 2016) (Harry Jubb, Harpreet Saini, Marcel Verdonk and Simon Forbes; manuscript in preparation).

Regardless of the structural environment, mutations of energetic hot-spot residues, which contribute large proportions of the binding free energy in many PPI interfaces, have been shown to be significantly enriched in disease-causing mutations (David and Sternberg, 2015; Jafri et al., 2015; Nemethova et al., 2016). Even for interfaces lacking well-defined hotspots, such as viral capsids, specific point mutations of key residues can still have stark impacts on protein complex assembly (White et al., 2016). For example, mutation of residues at the inter-pentamer interface of foot-and-mouth disease virus have been shown to ablate whole virus assembly (Rincon et al., 2015). Furthermore, just a few mutations in Reston, the only Ebola viruses not pathogenic in humans, disrupt key viral-human protein-protein interactions, which could be restored upon mutation leading to infection of human cells (Pappalardo et al., 2016).

The effects of PPI SNPs has been reviewed from a structural perspective (Yates and Sternberg, 2013), illustrating examples of PPI interface mutations having effects not only on disrupting interaction interfaces directly, but also through the alteration of post-translational modification sites (such as in Parkinsons (Muda et al., 2014)), and intrinsically disordered regions, in addition to effects caused by interface specificity switching. This emphasised the importance of protein structural knowledge in aiding understanding of genotype-to-phenotype relationships by building mechanistic understanding of the effects of polymorphisms (Yates and Sternberg, 2013). *In vitro* and *in vivo* studies including structural analysis have shown that mutations at PPI interfaces can be used to switch interface specificity (Ascher et al., 2014; Kortemme et al., 2004a), illustrating the power of polymorphisms at PPI interfaces with respect to influencing biological processes. This phenomenon is exemplified well by antibody escape mutations such as in HIV1, wherein neutralising antibodies (NAbs) that inhibit viral envelope formation place a selective pressure on HIV such that resistance to Nabs occurs. Antibody escape in HIV1 occurs through specific mutations that directly or indirectly selectively ablate Nab binding while retaining the ability to assemble the viral envelope (Mascola, 2009; Pires and Ascher, 2016; Wei et al., 2003). Such plasticity at PPI interfaces presents therapeutic challenges with respect to tackling drug resistance at what are already very challenging target proteins for pharmaceuticals.

While there are challenges in targeting complex mutations with therapeutics, greater quantities and availability of mutation data are paving the way toward greater understanding of the effects of mutations. Advances in DNA sequencing technologies have led to significant increases in the cost-effectiveness, speed and quality of genome sequence data. This has facilitated the study of genome wide genetic variations in humans thereby helping to understand complex diseases and genetic disorders (Cooper and Shendure, 2011; Welter et al., 2014). The expected growth in the number of characterized disease mutations from next-generation sequencing has driven a need for reliable and high throughput methods of assessing the effects of a mutation within the cellular context, and in particular within the network of interactions made by a protein. It is therefore important to first understand the nature of the interactions made by a protein of interest, and then to assess the likely impact of a disease mutation upon these interactions.

3. Understanding the effects of mutations upon protein-protein interactions: the interactome

Within the context of large networks of interacting proteins ("interactomes"), understanding the molecular mechanisms by which genetic variants affect interaction networks can be very important in establishing the relationship between genotype and phenotype (Pires et al., 2016). This can enable the connection of the molecular mechanisms of diseases to their modes of inheritance (Zhong et al., 2009) and explain how mutations on the same gene might cause different phenotypes by perturbing different interaction interfaces (Wang et al., 2012).

A combination of genome-wide association studies (GWAS) and PPI network-based analysis has been used to understanding of mechanism of complex genetic disorder like schizophrenia and to identify new susceptible genes, gene interactions and molecular pathways (Chang et al., 2015). Network-based approached has been used to identify disease modules (a neighbourhood of interacting disease associated proteins within a PPI network) and its connectivity pattern in complex diseases (Ghiassian et al., 2015). Menche and colleagues have utilised the network-based location of each disease module to determine its biological and clinical similarity to other diseases (Menche et al., 2015).

In order to analyse systematically the full range of effects that a mutation might have, a comprehensive definition and understanding of interaction networks is required, at the levels of where, when and which proteins interact, and the molecular nature of those interactions. While the size of the proteome is relatively well defined, the number of potential interactions and combinations of

interactions can be orders of magnitude higher. The sheer volume and complexity of interatomic data necessitates the need for interactomics databases for data organisation. A number of different generic, specialised and derived interaction resources have been developed to compile distinct information from experimental and computational investigations into PPI's (Table 1). To better understand cellular processes at atomic level, databases such as PICCOLO (Bickerton et al., 2011) and the protein common interface database (ProtCID) (Xu and Dunbrack, 2011) provide comprehensive, atomic level characterizations of PPIs. Structural data are currently a requirement for many methods that can assess the effects of mutations on protein and PPI form and function. To leverage structural knowledge, similar structural annotations of PPIs are accessible in a number of large-scale databases (Table 1).

The organisation and annotation of protein structural information has facilitated the design of various informatics approaches to understand the structural, functional and evolutionary relationships within a network of PPIs (Lee et al., 2009). Combination of structural information and computational tools can serve as a powerful tool to predict new PPIs (Kiel et al., 2008). Experimental characterisation of PPIs can be time-consuming, expensive and technically difficult. Computational approaches allow us more quickly and inexpensively to predict and explore the full interactome. The availability of interactome databases, including databases cataloguing "structural interactomes", has opened up the possibility of large-scale prediction of PPIs (Tsuji et al., 2015; Tyagi et al., 2012; Zhang et al., 2012). The performance of large-scale prediction methods in particular PrePPI (Zhang et al., 2013) has been shown to be overall better than high throughput methods based on a test data set. A range of different methods has been used to predict PPIs (Mosca et al., 2013b; Salwinski and Eisenberg, 2003; Tuncbag et al., 2009), and have been integrated into several databases to increase the coverage and quality of interaction data.

Traditionally, molecular docking methods have been used to predict potential PPI. They use specific combination of spatial sampling and scoring functions to predict most likely binding mode of PPI at structural level. For this purpose, gamut of automatic docking servers has been developed (Comeau et al., 2004; Dominguez et al., 2003; Lyskov and Gray, 2008; Macindoe et al., 2010; Mashiach et al., 2010; Schneidman-Duhovny et al., 2005; Torchala et al., 2013; Tovchigrechko and Vakser, 2006; Viswanath et al., 2014). A specific class of methods have been developed to infer possible interface residues involved in PPI. The inference based methods use the information from structural, surface and residue conservational properties to identify potential interaction interfaces in proteins (Coelho et al., 2016; de Vries and Bonvin, 2006; Glaser et al., 2003; Kufareva et al., 2007; Neuvirth et al., 2004; Porollo and Meller, 2007).

Analysing PPI networks has helped in gaining understanding of the key evolutionary properties and constraints that affect PPIs (Andreani and Guerois, 2014). Based on evolutionary principles, the concept of interologs (equivalent binary interactions in homologous complexes) has been used to infer potential PPIs between species (Walhout et al., 2000). Based on this idea, various computational approaches have taken a network approach to understanding PPIs between many organisms (Brown and Jurisica, 2007; Huang et al., 2004; Persico et al., 2005). Building of putative PPI networks through interolog mapping has been made possible by the development of online tools (Gallone et al., 2011; Garcia-Garcia et al., 2012). However, for species with greater phylogenetic distances, care must be taken while inferring PPIs using interologs (Lewis et al., 2012).

It is worth highlighting that while the amount of experimental interactome information currently available is limited, the amount of biochemical and structural information available to characterize these interactions, and crucially to understand the effects of mutations on these interactions, is rapidly expanding. For example, while the Genome3D initiative identified structural information for the majority of the estimated 20,000 proteins in the human genome (Lewis et al., 2015), less than 10,000 of the greater than 64,000 predicted interactions in the human binary interactome, not even including the vast array of possible pathogen-host protein interactions, have associated structural information (Mosca et al., 2015). The majority of experimental structural information

Table 1
Protein-protein interaction databases.

Experimentally characterised PPI's		
iRefWeb	Consensus/integrated databases	(Turner et al., 2010)
ConsensusPathDB	Consensus/integrated databases	(Kamburov et al., 2013)
BIND	Curated experimental databases	(Bader et al., 2003; Isserlin et al., 2011)
BioGRID	Curated experimental databases	(Chatr-Aryamontri et al., 2015)
DIP	Curated experimental databases	(Salwinski et al., 2004)
HPRD	Curated experimental databases	(Keshava Prasad et al., 2009)
InnateDB	Curated experimental databases	(Breuer et al., 2013)
IntAct	Curated experimental databases	(Kerrien et al., 2012)
MINT	Curated experimental databases	(Chatr-aryamontri et al., 2007)
IID	Tissue Specific Interactions	(Kotlyar et al., 2016)
Pathogen interaction gateway	Pathogen Interactions	(Driscoll et al., 2009)
Virus-host network	Pathogen Interactions	(Navratil et al., 2009)
Computational and experimentally characterised PPI's		
FPCLASS		(Kotlyar et al., 2015)
I2D		(Brown and Jurisica, 2007)
PIP		(McDowall et al., 2009)
STRING		(Szklarczyk et al., 2015)
Structural annotations of PPIs		
GWIDD	Structural annotations of PPIs	(Kundrotas et al., 2010)
IBIS	Structural annotations of PPIs	(Shoemaker et al., 2012)
INstruct	Structural annotations of PPIs	(Meyer et al., 2013)
Interactome3D	Structural annotations of PPIs	(Mosca et al., 2013a)
PICCOLO	Structural annotations of PPIs	(Bickerton et al., 2011)
PrePPI	Structural annotations of PPIs	(Zhang et al., 2013)
PRISM	Structural annotations of PPIs	(Tuncbag et al., 2011)
ProtCID	Structural annotations of PPIs	(Xu and Dunbrack, 2011)
Struct2Net	Structural annotations of PPIs	(Singh et al., 2010)

regarding interactions is between functionally important homo-oligomers, or hetero-oligomers (Ascher et al., 2011; Hermans et al., 2015; Polekhina et al., 2013). While X-ray crystallography and NMR spectroscopy have limitations with respect to the size, dynamics, and molecular environments that can be resolved, for example size limitations on complexes that can be resolved by NMR, and difficulties in crystallising membrane proteins, molecular modelling and cryo-electron microscopy (cryo-EM) are becoming increasingly powerful and valuable tools for shedding light on multiprotein complexes. Thus, the amount of available experimental protein structure data is likely to significantly increase going forward, providing crucial insight into the mechanisms of many other disorders (Pacitto et al., 2015).

4. Understanding the effects of mutations upon protein-protein interactions: structural consequence of mutations

The interfaces through which proteins interact are complex, typically containing many amino-acid residues that collectively must contribute to binding specificity as well as binding affinity, structural integrity of the interface and solubility in the unbound state. While chemical modifications (Chan et al., 2015, 2016; Kaminskas et al., 2013; Landersdorfer et al., 2015) and mutations could disrupt these interactions, the molecular basis behind that was not always clear. It is therefore important to consider the consequences of mutations within a structural context. The predominant source of genetic variations within a protein-coding region comes from single nucleotide polymorphism (SNP). Non-synonymous SNPs (nsSNPs) are the SNPs that change an amino acid sequence of the encoded protein resulting in single amino acid substitution (SAAS). These have been collated in a range of databases. For the purpose of analysis, nsSNP data have been made available through recent projects, including the 1000 Genomes Project (Genomes Project et al., 2010) and the Exome Sequencing Project (Tennessen et al., 2012), which have made available large numbers of nsSNP data from within the general population. Together with databases of disease causing mutations such as HGMD (Human Gene Mutation Database) (Stenson et al., 2009) and OMIM (Online Mendelian Inheritance in Man) (Amberger et al., 2011), genome sequencing projects allow us to begin to interrogate the molecular mechanisms leading to disease.

Studying the effects of such mutations on molecular function is crucial to understanding the link between genetic variation and disease. Experimental methods to study the effects of mutations are often costly, time consuming and challenging, making it infeasible to study the large number of potential amino acid substitutions. Initially, most of the prediction tools considered molecules in isolation, and the effects of mutations upon their stability (Pires et al., 2014a; Topham et al., 1997). However, disease-associated nsSNPs have been shown to occur in hotspots at PPIs (David et al., 2012) and nsSNPs have been shown to affect protein stability, dynamics, and protein interactions with other proteins, nucleic acids and ligands (Kucukkal et al., 2015; Pires et al., 2016). nsSNPs can weaken PPIs by modifying intermolecular contacts, PTMs, altering intrinsic disorder, or introducing novel aberrant interactions (Yates and Sternberg, 2013). The need to be able to evaluate the consequences of the growing number of nsSNPs on the interactome has driven the development of various computational tools to predict the effect of mutation at PPIs.

The first computational tools for analysing mutations at PPIs were used to predict the impact of mutations to alanines for the identification of hotspots (Huo et al., 2002; Kortemme et al., 2004b). These made use of the large number of alanine scanning experimental studies designed to identify key residues mediating these interactions, measuring the changes in binding affinity upon mutation (Fischer et al., 2003; Thorn and Bogan, 2001). While this provided information regarding the importance of a residue at an interface, in order to characterise the broad range of possible mutations on protein-protein binding affinities additional methods were developed to consider mutations to other residues.

One early approach used was the development of free energy perturbation methods based on statistical mechanics, however these techniques are computationally very expensive and often limited to small systems (Gouda et al., 2003; Huo et al., 2002; Kollman et al., 2000). The availability of public mutation databases containing the experimental changes in binding affinities for PPI has facilitated the development of energy function based (Dehouck et al., 2013; Guerois et al., 2002; Kortemme and Baker, 2002) and machine learning based computational tools. These rely upon training and benchmarking the methods against the experimentally measured changes in binding affinities (Moal and Fernandez-Recio, 2012). Machine learning methods can be either supervised (Pires et al., 2014b) or semi-supervised (Zhao et al., 2014). Hybrid techniques that use a combination of molecular mechanics and continuum solvent model have also been developed to calculate relative free energies of structure and complexes (Kollman et al., 2000) and have been used to predict the effect of mutation (Petukh et al., 2015). It has also been used to predict the effect of multiple mutants (Li et al., 2014). Recently we studied the accuracy of these different tools for studying the effects of single-point mutations on protein-protein binding affinity (Ascher et al., 2015).

Analysing nsSNPs within the context of three-dimensional protein structures provides a platform for assessment and to generate hypothesis to explain the impact of SNVs on protein structure and function. To address this, many webservers provide the functionality to map and visualize SNPs on protein structure (Niknafs et al., 2013; Reva et al., 2011; Singh et al., 2008; Yue et al., 2006). Vazquez and colleagues published a web service for the annotation of cancer related single nucleotide variant at PPIs (Vazquez et al., 2015). Various methodologies for annotating the functional impact of mutations specific to cancer with the context of protein structure, function, PPIs and networks has been review elsewhere (Gulati et al., 2013). The structural investigation of nsSNPs within the context of disease network has revealed its link to PPIs (David et al., 2012; Khurana et al., 2013; Wang et al., 2012).

Growing support for the assessment of methods to predict the effects of mutations in PPIs (similar in fashion to CASP for protein structure prediction (Moult et al., 2014) and CAPRI (Janin, 2005) for protein-protein docking) will enhance our understanding and progress the field (Moretti et al., 2013). It is particularly challenging to model PPIs that undergo considerable conformational change upon complex formation (Ascher et al., 2011; Hermans et al., 2015; Tuffery and Derreumaux, 2012).

5. Analysing mutations in genetic diseases

By considering the range of effects that a mutation might have upon a protein's structure and function, we have previously shown that mutations that lead to the development of Alkaptonuria disrupt homogentisate 1,2-dioxygenase activity through three distinct mechanisms: disruption of protomer stability, disruption of hexameric assembly, or disruption of the active site (Nemethova et al., 2016; Pires et al., 2016; Usher et al., 2015). Interestingly, while less than 10% of mutations were linked to changes in the active site, approximately half were associated with changes in oligomer formation.

We recently applied this analysis to mutations that lead to the most common urea cycle metabolic disorder in human, ornithine carbamoyltransferase deficiency (Turner, Blundell and Ascher,

Unpublished data, Fig. 2). Ornithine carbamoyltransferase (OCT) catalyses the conversion of ornithine and carbamoyl phosphate to citrulline during the second step of the urea cycle. OCT is a homotrimer with active sites located at each of the protein-protein interfaces. Nearly 300 mutations have been identified in OCT, with the vast majority leading to either neonatal or late onset OCT deficiency. The mutations are spread throughout the protein and include mutations in the core of each chain, on the exposed protein surfaces, at the protein-protein interfaces, and in the active sites. These mutations were analysed using mCSM-Stability and DUET to evaluate their effects upon protomer stability, mCSM-PPI to assess their effects upon formation of the homotrimer, and mCSM-Lig to predict the effects of the mutations upon substrate binding (Pires et al., 2014b, 2015). Over half of the disease mutations (59%) were linked to changes in protomer stability by mCSM-Stability, and approximately 15% were found to disrupt substrate binding by mCSM-Lig. Interestingly, these were very similar proportions to the disruptive effects observed in AKU.

Only two of the identified neutral polymorphisms were located at the protomer interface (T91I and S96P), however mCSM-PPI did not predict either mutation would disrupt homotrimer formation. This was not surprising considering the extensive interface between the protomers, and that the active sites are located near the interface. By contrast, 10% of the OCT deficiency-causing mutations were predicted by mCSM-PPI to significantly decrease PPI stability and disrupt formation of the active oligomer (Fig. 3). Potentially, the remaining unexplained mutations could alter interaction with TOM-20, and transport into the mitochondria. Characterising the molecular mechanism by which these mutations disrupt OCT structure and activity is an important step to understanding the condition, and developing treatments for it.

6. Using chemical modulators to target mutations at protein interaction interfaces

The importance of PPIs in human disease makes them attractive

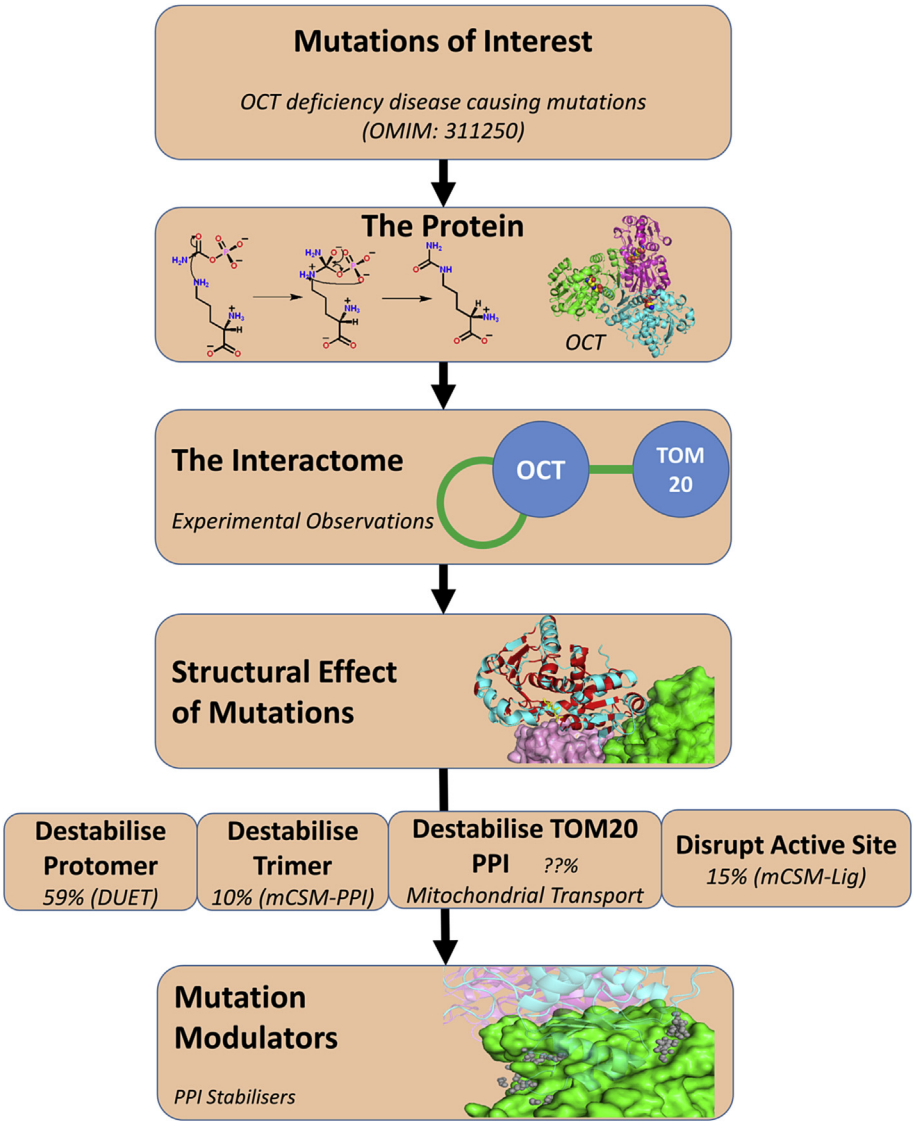

Fig. 2. Workflow for examining the effects of mutations on protein-protein interactions, highlighting OCT deficiency mutations as an example. Characterising mutations requires an understanding of the protein within the context of the interactome (Table 1). This enables the interpretation of computational predictions allowing the molecular mechanism of the mutations to be explored. Many disease associated mutations lead to disruption of key protein-protein interactions. An exciting unexplored possibility is the design of therapeutics targeting interfaces where mutations occur to treat these diseases.

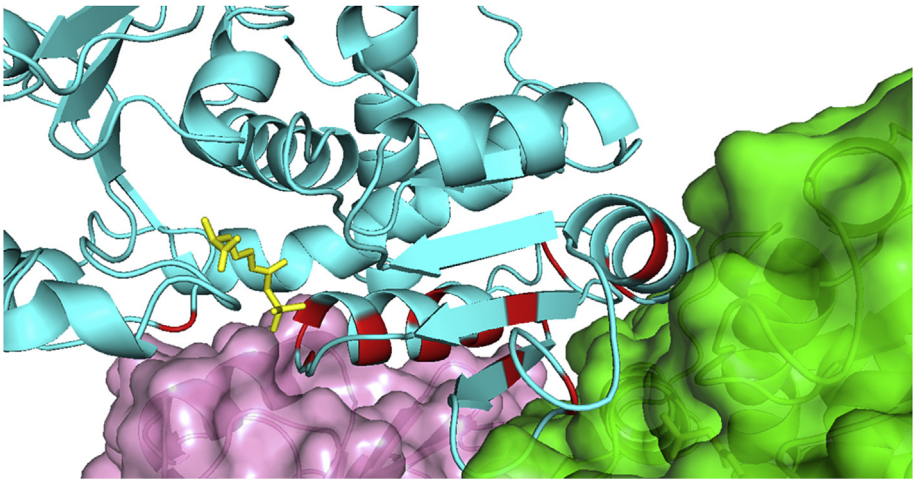

Fig. 3. OCT deficiency mutations (red) that disrupt OCT (PDB ID: 1OTH) activity through destabilization of the homotrimeric structure. The active site is located near the trimer interface, with the cofactor, carbamoyl phosphate, shown in yellow.

targets for the development of novel therapeutics. While PPIs were traditionally viewed as "undruggable", owing to their lack of well-defined, concave binding sites for binding small-molecules, the past decade has seen many successes in modulating the assembly and biological activity of PPIs. PPI modulators, including small-molecules, peptides and larger macromolecules such as antibodies, have been developed as inhibitors or stabilisers of protein complex formation and dynamics (Fischer et al., 2015; Nero et al., 2014; Watt et al., 2014). PPI modulators can act by binding competitively at interface regions (orthosteric binding), at binding sites formed by pre-existing protein complexes ("interfacially") or via interface-distal allosteric sites (Fischer et al., 2015; Thiel et al., 2012). While orthosteric inhibition has been the focus of much early attention and successes in the field (Arkin and Wells, 2004; Sigurdardottir et al., 2015), and has paved the way for further exploration of PPI modulators with respect to driving thinking in computational, fragment-based, and natural product driven drug design (Arkin et al., 2014), more recently the study of PPI stabilizers has also been explored (Ascher et al., 2014; Parker et al., 2011; Parker et al., 2016; Thiel et al., 2012). Many molecules of natural origin, such as rapamycin, brefeldin, forskolin and fusicoccin act by protein complex stabilization (Thiel et al., 2012). Allosteric modulators may specifically affect the dynamics of complex formation, leading to altered oligomerisation patterns (Ascher et al., 2014; Merdanovic et al., 2013). Information about all these different PPI modulators have been accumulated in databases such as TIMBAL (Higueruelo et al., 2013), 2P2Idb (Adamczak et al., 2011) and iPPI-DB (Labbe et al., 2013).

The ubiquity of PPIs across biological processes means that their modulation could have applications across most areas of medicine. However, the relative novelty of the field has meant that so far the most tractable and pressing drug targets have been pursued. Given the preponderance of signalling and PPI mutations in cancer etiology, oncogenic protein surfaces have been the subject of most attention (Nero et al., 2014; Zinzalla and Thurston, 2009), with now a dozen modulators targeting them in clinical trials or already in the clinic. Unsurprisingly, some of the most studied systems in the field, e.g. 14-1-1-3 (Bartel et al., 2014; Milroy et al., 2013), MDM2/P53 (Vassilev et al., 2004) and the BCL-2 family (Walensky et al., 2004), are involved in carcinogenesis, but modulators have already been identified with targets related to infective diseases, neurological disorders and amyloidosis, with some even approved or in clinical trials (Fischer et al., 2015).

The current popularity, interest in, and successes of PPI modulation presents opportunities for using PPI modulators to treat specifically diseases associated with mutations at PPI interfaces, in a variety of diseases. To do so will require an understanding of how mutations in disease can affect and be affected by PPI modulator binding. Fig. 4 presents a forward-looking example of where a

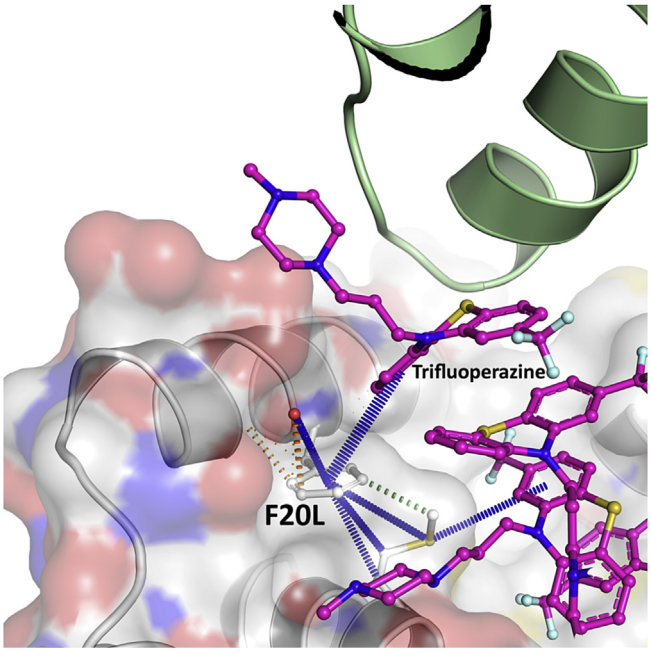

Fig. 4. Human cancer mutation F20L in troponin C in complex with trifluoperazine Troponin C modulates heart muscle contraction in a calcium dependent manner, and several drugs stabilize an open form of the troponin C N-terminal domain that enables heart muscle contraction (Li and Hwang, 2015). F20L missense mutation in human cancers (from the COSMIC cancer mutation database (Forbes et al., 2015)) lies juxtaposed to an interfacially-binding approved drug molecule, trifluoperazine (PDB: 1WRL). F20L lines a large pocket in which three trifluoperazine (magenta) molecules stabilise the formation of a troponin C N-terminal domain homodimer interface (grey and green ribbons). Cursory analysis of inter and intermolecular interactions formed by F20 at the trifluoperazine binding site suggest that mutation to leucine in cancer may affect drug binding by ablating intermolecular aromatic ring interactions (blue), in addition to altering a number of intramolecular interactions (polar shown in orange and hydrophobic in green) in the residues forming the binding pocket.

missense mutation in cancer may influence the binding of an interfacial-binding approved drug. Mining the growing databases of protein structure and of mutations in human disease will enable the identification of such cases, from which we can collectively learn more about the impact of mutations on such modulators, and how we can design chemical modulators to target mutated PPI binding sites. In summary, combining an understanding of the nature and "druggability" of PPIs with the location, nature, and molecular impacts of disease associated mutations, presents powerful therapeutic possibilities.

7. Final thoughts

Understanding and treatment of diseases associated with mutations calls for a detailed understanding of their molecular effects. The prevalence of disease causing mutations that alter PPIs make mutations at PPIs prime targets for therapeutic intervention. Currently, many methods that assess the molecular effects of mutations on PPIs require detailed atomic structures of the proteins involved. Complete maps of PPI networks annotated with biological pathway and protein structural information, will provide a platform from which to understand the molecular nature of PPI networks, in turn guiding predictions of the impacts of mutations on disease biology. Structural understanding and quantification of effects of mutations at PPIs will enable the design of better PPI inhibitors and stabilisers that can target specific disease states, and address cases where further interface mutations lead to drug resistance.

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