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Structural and physico-chemical effects of disease and non-disease nsSNPs on proteins

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This review emphasizes the effects of naturally occurring mutations on structural features and physico-chemical properties of proteins. The basic protein characteristics considered are stability, dynamics, and the binding of proteins and methods for assessing effects of mutations on these macromolecular characteristics are briefly outlined. It is emphasized that the above entities mostly reflect global characteristics of considered macromolecules, while given mutations may alter the local structural features such as salt bridges and hydrogen bonds without affecting the global ones. Furthermore, it is pointed out that disease-causing mutations frequently involve a drastic change of amino acid physico-chemical properties such as charge, hydrophobicity, and geometry, and are less surface exposed than polymorphic mutations.

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Introduction

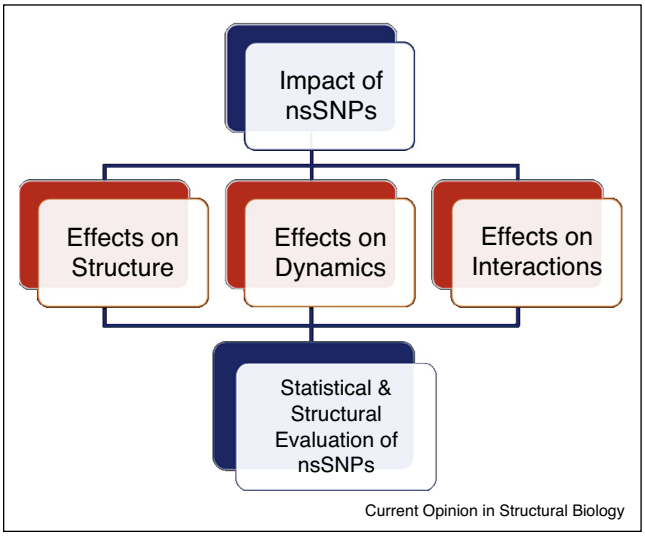
As a natural consequence of the ongoing massive exome sequencing studies, the classification of human genetic variations and its relationship to disease susceptibility and drug response has recently gained remarkable attention and a considerable success has been achieved [1•]. As a result, a variety of tools and databases were developed [2]. In particular, the methods that target understanding the effects of missense mutations on various sequence, structural and functional features have been explored in the hope of deciphering the phenotype–genotype relations [3,4,5•]. Despite the availability of high-quality 3D structures (experimental or homology-modeled), predicting the effect and associated phenotypes of nsSNPs remain a challenging biophysics and bioinformatics problem for various reasons. Perhaps the main reason is that all methods divide the collective effect of mutations into individual contributions from various structural and physico-chemical features. However, many properties used to describe the biological entities are highly interrelated and/or correlated making it difficult to decouple them from each other. With the awareness of this fact, we review how protein structural characteristics and physico-chemical properties have been found to be affected by mutations as summarized in Figure 1.

Impact of mutations on protein structural properties

The effect of nsSNPs on the structural integrity of a protein can be assessed by monitoring or calculating the stability changes upon mutations. The thermodynamic stability of proteins is quantified by the folding free energy (ΔG), and is a result of collective contributions from several structural features including but not limited to H-bonds and salt bridges. The ΔG is typically relatively small, on the order of ~1–15 kcal/mol for a globular protein compared to for example, ~50–200 kcal/mol for a covalent bond. Due to the balance between enthalpic and entropic contributions, the resulting ΔG is relatively small (Figure 2). Mutations change both the energy landscape and the number of accessible conformations in both folded and unfolded states, resulting in the folding free energy change ($\Delta\Delta G$) as shown in Figure 2. It should be mentioned that the change in physico-chemical properties (e.g. polarity, hydrophobicity, solvation energy, etc.) upon mutation affects the free energy of both the folded and unfolded states and depending on balance, the mutation may stabilize or destabilize the protein. However, care should be exercised when classifying the mutations in terms of their degree of stabilization/destabilization based on their $\Delta\Delta G$ upon mutation. A mutation affecting the $\Delta\Delta G$ by several kcal/mol may not cause significant structural changes of a macromolecule having ΔG of ten or more kcal/mol. However, the same magnitude of $\Delta\Delta G$ in a protein with a ΔG of only several kcal/mol may cause large structural changes or unfolding.

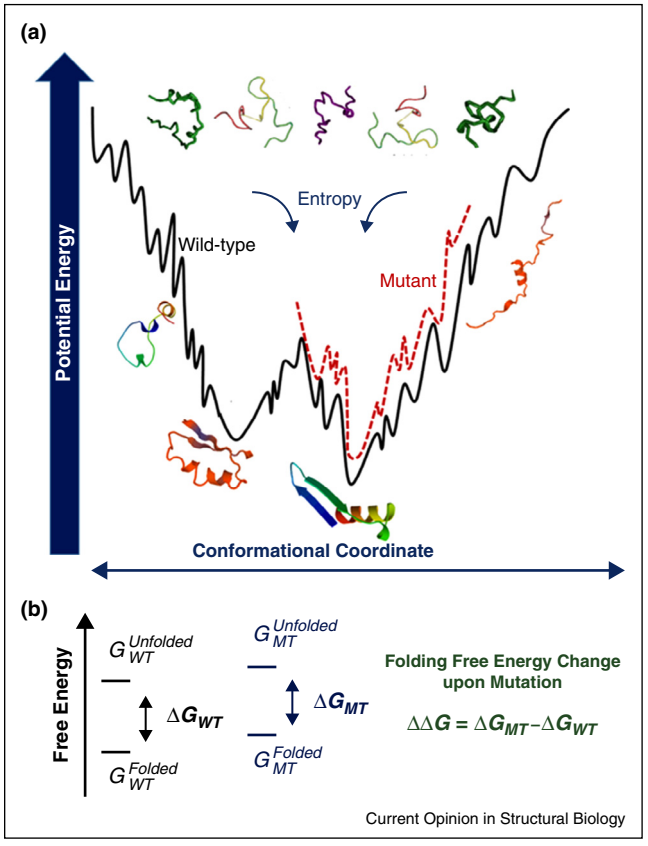
The folding free energy change is measured by various methods monitoring the transition from folded to unfolded (or vice versa) states. The major methods include Circular Dichroism, Differential Scanning Calorimetry, and various spectroscopic measurements [6]. Crucial to mention are the structure determination methods such as NMR and X-ray crystallography. Although they do not deliver the

Figure 1



Block diagram illustrating the impact of SNPs on various protein properties reviewed.

Figure 2



Nonspecific energy landscape for folding of generic wild-type and mutant proteins (a) and folding free energy change due to mutation (b). The graphical interpretation of folding funnel is not straightforward and the main obstacle is the decoupling free energy and conformations, therefore, the present the concept of the folding funnel for an enthalpy-driven process.

$\Delta\Delta G$ directly, they decipher structural changes upon mutations such as changes in H-bonding network and salt bridges. NMR and spectroscopic labeling methods are less demanding compared to crystallography since crystallization is not needed, but still require protein and mutant expression and purification. On the other hand, crystallography requires considerably less concentrated samples compare to NMR. In either case, large scale studies are prohibited.

Furthermore, various computational methods were developed to decipher $\Delta\Delta G$, which can be broadly classified into three categories [3,7•,8]. The first category refers to sequence-based machine learning approaches, however they cannot be used to understand structural consequences. Second category includes the structure-based approaches which include statistical knowledge-based potentials and biophysics-based approaches [3]. Third are the methods that require intensive sampling such as free energy perturbation and thermodynamic integration [9]. Despite the differences, all structure-based methods explicitly modeling folded and unfolded states must address the difficult issue of the nature of unfolded state. It is quite unlikely that the large ensemble of structures representing unfolded state (Figure 2) can be properly modeled and because of that, the unfolded state is either modeled as a completely unfolded chain or a segment of several residues centered at the mutation site [10,11].

In general, the experimental studies are relatively limited due to the cost and time needed for the entire process which often includes protein expression, purification and mutagenesis, followed by thermal or chemical unfolding. Not surprisingly, most of the disease-causing mutations were found to be destabilizing, that is, lowering the folding free energy [12•,13,14]. The degree of destabilization was found to be elevated for mutations that introduce drastic changes such as charged to neutral, relatively rigid to relatively flexible, or aromatic to aliphatic mutation types. For most of the cases, the destabilization was also accompanied by structural changes [12•,13]. Interestingly, some disease-causing mutations can be stabilizing [15••]. This observation highlights the difficulties of predicting the physiological relevance of mutations based on the effect delivered by a single methodology.

In addition, strong salt bridges and H-bonds may contribute to the stability by more than several kcal/mol. However, regardless of the energy contribution, almost always formation of internal bridge or the H-bond is more favorable than not forming it. This is especially valid for buried charged groups, which pay large desolvation penalty that

may not be completely compensated by a favorable salt bridge with another amino acid. In such a case, the salt bridge formation reduces the penalty, but still the presence of salt bridge in the core of the protein may not contribute to the folding energy. Thus, a single mutation that removes or adds a H-bond or a salt bridge may in turn cause local structural change without necessarily affecting the ΔG. Recent experimental studies indicated that disruptions of salt bridges or H-bonds are frequently disease-causing [16]. In some cases, a clinically relevant mutation was found to simply remove the charge of one of the partners (Glu → Gln) while keeping the side chain geometry almost unchanged, but still causing a disease [17]. Overall, in most of these cases, the corresponding mutant protein was still in the native fold, but its functionality was affected by the local changes of geometry within functionally important residues.

Continuing with the recent computational studies, in general, large-scale calculations utilizing structural information indicate the same trend observed in experimental studies: mutations predominantly destabilize proteins. However, mutations can also stabilize the protein [18,19]. Perhaps the reason is that wild type proteins are not necessarily optimized for stability or activity, as indicated by engineering more stable and more active mutants [20]. Thus, while the tendency is that a mutation more likely will destabilize the protein, without detailed analysis one cannot predict the effect on the function without any doubt.

In addition, it was demonstrated computationally that H-bonding and/or salt bridge disruptions are almost always disease-causing if they are in close proximity to the active site [21–23]. In many cases, the mutations were found to decrease the number of intramolecular H-bonds within a protein or the number of H-bonds that the protein makes with DNA or with the solvent [24,25]. Similarly, a deletion [21] or a formation of a new [26] salt bridge were frequently implicated in diseases without being involved in significant changes of the stability of the corresponding protein.

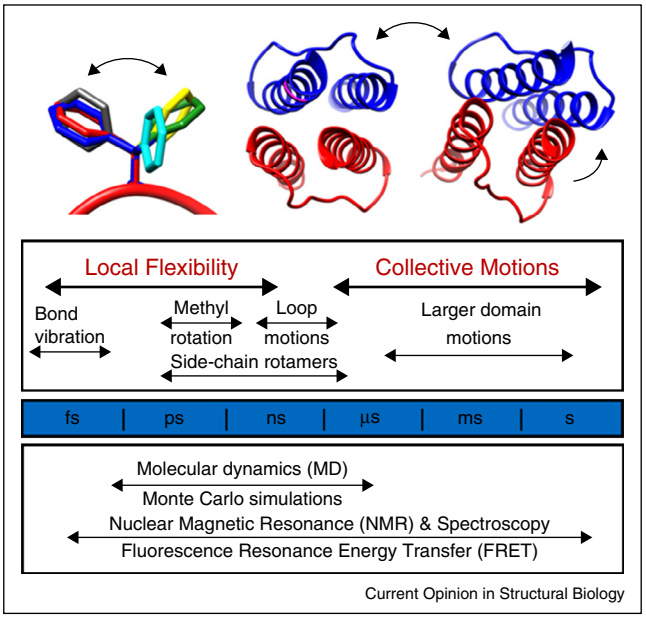
Impact of mutations on protein dynamics

Proteins are not static but rather populate ensembles of conformations. Protein dynamics, both local and global, play an important role in protein function. The local flexibility mainly involves side chain entropy, which depends on the local environment of the residue in question and its side chain length. It often becomes coupled with local interactions such as H-bonding, salt bridges and van der Waals interactions within the protein or with other domains, proteins or nucleic acids. On the other hand, large scale motions involve domain motions. Depending on the nature of the protein motion, the changes between states occur at different time scales. Some conformational changes may involve side chain rotamers occurring fast, while others may be associated with large domain motions taking place at ms time ranges (Figure 3). An important subclass of macromolecular dynamics are allosteric motions [27•,28]. When local interactions are cooperatively coupled in an allosteric pathway, the macroscopic allosteric effects are observed. Considering the effects of mutations on protein dynamics, there is no particular magnitude of the effect that indicates the mutation harmless or disease-causing. For example, if the mutation site is located in an allosteric pathway, depending on the type of the mutation, it may have significant consequences in protein dynamics. In other words, the outcome will depend on how the function is associated with protein dynamics and also the degree of change introduced to that functional protein motion.

Experimentally, the changes of macromolecular dynamics are studied through NMR and other spectroscopic methods including label-based techniques (EPR, IR) and via the change of B-factors in crystallographic structures. The main advantage of NMR and spectroscopic methods over crystallography is that they deliver the time-scale of motions along with atomic resolution structural data. Importantly, the dynamics can be followed in solution in steady-state conditions [29]. On the other hand, FRET is another notable method to study protein flexibility, kinetics and structure particularly for systems that are too large to be studied by NMR [30].

Computationally, the changes of conformational dynamics can be modeled via MD simulations, which provide

Figure 3



The timescale of various protein motions and major methods of study.

atomic resolution exploration of protein dynamics at relatively low cost. However, the MD time scale is still limited to the order of nanoseconds except ambitious simulations [31]. Therefore, large scale explorations with relatively long MD simulations have not been exploited extensively for point mutations including MD combined with advanced sampling. Despite these challenges, there have been a number of MD simulations at various time scales investigating the effects of mutations on dynamical properties. In addition to MD, Monte Carlo (MC) and QM/MM hybrid methods are also used to study protein dynamics.

Continuing with experimental exploration of protein dynamics, recent NMR experiments have indicated that a single mutation can change the occupancy of a given state from several percent in wild-type to more than 50% in the mutant [32,33]. The change of the conformer occupancy was also linked to diseases [32]. This is important because the different conformers in proteins may have different functional roles and mutations may stabilize a particular conformer while destabilizing another [27•]. Several other studies utilizing FRET also revealed changes of stability and dynamics upon mutations with important functional consequences such as affecting the dynamics of hotspots [34•,35].

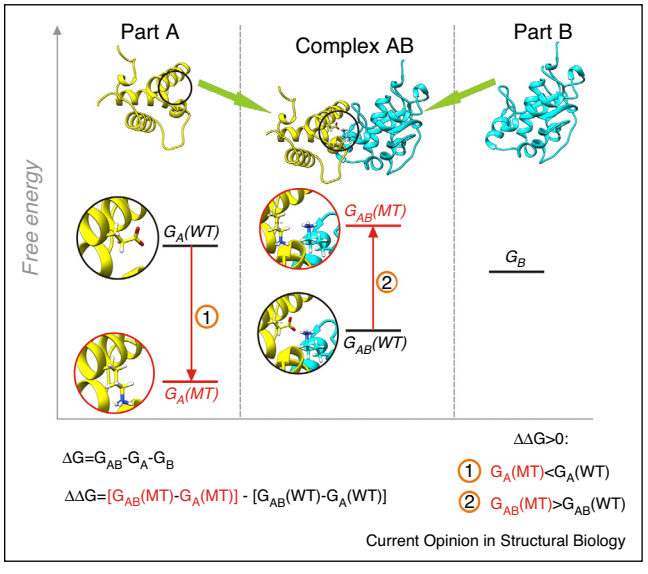
Computationally, several recent molecular dynamics studies of different simulation lengths (ranging from simulation lengths of 1–20 ns, 40–100 ns, up to ~20 μs) have investigated the effects of nsSNPs on protein flexibility as well as time-dependent changes in H-bonding network, and solvent-accessible surface area (SASA) [24,36]. The general strategy is to screen large number of mutations (~10–90) for harmfulness through fast methods/webservers and then the top 2–3 most harmful ones are chosen based on consensus and subsequently studied through MD. After running MD, the protein flexibility can be determined through the principle component and also through comparing the RMSF of individual atoms in wild-type and mutants. Most of the potentially disease-causing mutations studied were found to increase the overall flexibility of the protein at different levels and that was accompanied with a change in H-bonding network and in some cases radius of gyration and SASA [24,36]. In contrast, some disease-causing mutations can also decrease the flexibility of the protein at certain regions [37].

Impact of mutations on protein–protein, protein–ligand and protein–nucleic acid interactions

Practically every macromolecule interacts with partner(s) when performing its biological function. This includes information transfer, immune system operation, inhibition or activation of particular functions, assembly of macromolecular structures into molecular machines among others. On a time scale, the complex can be sustained for the fraction of a second (e.g. proteins involved in electron transfer) to months (e.g. protein–inhibitor interactions) [38••]. Some protein–protein and protein–nucleic acid complexes are termed permanent and once formed, last until the complex is degraded by the cell. Mutations altering such interactions presumably will be more structurally damaging than mutations affecting transient interactions, although both cases may affect the function. Some proteins or regions of proteins are intrinsically disordered before or after the complex formation [39] and these disordered regions may be alternatively spliced [40•] and thus the effects of mutations are difficult to predict.

The affinity of two of more macromolecules to form a complex is measured by the magnitude of the binding free energy as illustrated in Figure 4. A mutation can alter the free energy of both the complex and the unbound monomers and the outcome will depend on the balance of the changes in both states. It is important to distinguish between cases involving hetero-oligomers and homo-oligomers and to be able to make a reasonable prediction about the presence of mutation in the molecules forming homo-oligomers. Besides binding affinity, the binding specificity is also important to consider in regards to protein interactions. Taking into consideration the large number of different macromolecules in living cells, binding specificity plays an important role in recognizing unambiguous binding partners among an overabundance

Figure 4



Free energy change of binding. The free energy change of dimer formation and how it may be affected by a point mutation is illustrated. A hypothetical mutation makes the monomer A more stable (illustrated as change 1), while resulting in less stable complex AB (illustrated as change 2). The corresponding binding free energy change is the balance of these two effects and may make the complex more or less stable.

of other molecules [41,42]. Binding specificity includes multiple factors such as concentrations and compartmentalization of the macromolecules, their shapes, charge and steric complementarity, conformational flexibility, the ability to recognize each other at relatively large distance (mainly through non-specific electrostatic interactions) and to form functioning complex by specific interactions within hot spots [41,43].

Experimentally, mutations were documented to make the binding free energy more favorable by as much as −5.7 kcal/mol or to decrease affinity by as much as +10.0 kcal/mol [44]. This indicates that the effect of nsSNPs may be quite dramatic and can completely abolish macromolecular functionality by altering macromolecular interactions. As it was mentioned in case of folding free energy, the phenotype of the mutation will depend on many factors, perhaps the most important being the ratio of change of the binding free energy and the wild type binding free energy. If this ratio is small, most probably the physiological effect will be small as well.

Typically, the binding free energy changes caused by mutations are measured by monitoring the concentration ratio between bounded and free molecules. Other experimental techniques include isothermal titration calorimetry [45], FRET [46] and surface plasmon resonance [47] among others [48]. It is important to mention that the measurements are done at particular conditions, such as pH and salt concentration, which may not necessarily correspond to the conditions in cell. In addition, the structure determination methods mentioned earlier such as X-ray crystallography can also provide excellent structural perspective on protein–protein, protein–nucleic acid, protein–ATP and similar interactions. Computationally, the methods described earlier for determining folding free energy changes are also utilized to decipher binding free energy changes.

Experimentally, the naturally occurring mutations were found to alter binding affinity in both directions, that is, reducing or enhancing the binding affinity. Recent studies showed that a somatic mutation destabilizes dimer formation [49], while in another case, a disease-causing mutation was demonstrated to stabilize the dimer [50]. Missense mutations associated with cancer [51], Snyder–Robinson syndrome [19], and congenital heart diseases [52] were linked with altered protein–protein and protein–DNA interactions. In general, any deviation in affinity caused by mutation(s), enhanced or reduced, possesses a high risk of developing a disease. Similarly, disease-causing mutations were found to induce structural changes in protein upon binding to other partners such as ATP [37].

Computationally, it was shown that missense mutations associated with cancer alter binding affinity of proteins and make the electrostatic component of the binding energy less favorable [53••]. Similarly, a large-scale study of disease-causing and presumably harmless mutations indicated that disease-causing mutations tend to disrupt the electrostatic component of the binding [54]. In line with experimental observations, it was found that naturally occurring mutations decrease binding affinity of spermine synthase homo-domain causing Snyder–Robinson syndrome [23], while enhancement of the binding between CLIC2 protein and ryanodine receptor causing an X-linked channelopathy [15••,18]. Genomic-scale investigations were also carried out with combined efforts of machine learning and statistical potentials [55]. Molecular dynamics studies, being computationally expensive, were applied to study specific cases only [56], including disruption of salt bridges between protein and substrate [57].

Physico-chemical properties of disease-causing and polymorphic SNPs

Above we outlined the impact of nsSNPs on protein properties broadly categorized as protein stability, dynamics and interactions. Furthermore, a quick comparison between disease-causing and polymorphic mutations listed in HumVar database [58•] along with structures provided in *HumanDisease* and *HumanPoly* disease and polymorphism datasets [59•] reveals that mutations conserving physico-chemical properties (polarity, hydrophobicity, charge and side chain overall geometry) tend to be harmless. Both disease-causing and polymorphic mutation sites are predominantly found to be located in helixes and coil regions, and not so frequently in beta strand regions. The analysis showed that disease-causing mutations tend to cause large changes in the H-bonding and salt bridges at the altered mutation site. Consistent with this observation, the polymorphic mutations induce smaller changes of wild-type H-bond and salt bridges than the disease-causing mutations do. In terms of buriedness, most polymorphic mutations were found to be located at the surface of corresponding proteins, while disease-causing are found to be typically fully or partially buried.

Conclusion

In general, the disease-causing mutations tend to destabilize proteins, weaken the protein binding, and increase the flexibility of proteins, however, it is also documented that harmful mutations may cause enhanced rigidity, increased stability and stronger protein–protein interactions. This indicates that direction of the induced change cannot be used alone to predict the harmfulness of mutations. However, disease-causing mutations tend to cause large changes of H-bonding network and salt bridges as compared with polymorphic ones. In addition, disease-causing mutations typically result in drastic change of physico-chemical properties of mutation site. Consistent with these observations, the disease-causing mutations

were found to cause larger changes in local amino acid environment compared to the polymorphic ones.

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