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Digging for Buried Amino Acids Unearths New Protein Quality Control Treasure

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Proteins rely on three-dimensional structure for function, yet many proteins are marginally stable and prone to misfolding. In this issue of *Structure*, Brock et al. (2015) present a novel computational modeling method to gain insights into protein stability and misfolding.

To function correctly, most proteins must adopt a proper three-dimensional structure that allows them to operate with high precision and fidelity. However, protein synthesis is not free of errors, and nascent proteins can initially misfold into defective structures. Even when a nascent protein folds properly, post-synthesis exposure to chemical or physical stresses can damage the functional structure and cause misfolding. The stochastic generation of misfolded proteins is a fundamental problem that all cells continuously face.

If left unattended, misfolded proteins can form toxic aggregates that can ultimately lead to cell death. This dire consequence is underscored by the more than 50 human maladies causally linked with aggregation, which include devastating neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's, and ALS (Wang et al., 2008). Normally, the cell is well equipped to manage misfolded proteins through conserved protein quality control (PQC) mechanisms. One of the most important is degradative PQC that typically targets misfolded proteins for destruction by the proteasome (Fredrickson and Gardner, 2012), thereby eliminating structurally defective proteins from the cell and minimizing the potential for aggregation.

Studying what features misfolded proteins present to PQC degradation systems in their abnormal state is a very difficult task. Misfolded proteins are highly recalcitrant to the biochemical and biophysical techniques typically used to probe the structural features of normally folded proteins. Just ask any protein biochemist—the bane of their existence is the aggregation that can occur during the purification of proteins for structural analyses. Yet, aggregation is the "normal" behavior of misfolded proteins that is important to understand for PQC biologists. An additional complicating feature is that when a pool of protein misfolds, different misfolded conformations will likely exist within the pool. This presents a considerable problem for biochemists using structural analyses that rely on a single uniform conformation within a protein pool.

Given the difficulty of working with misfolded proteins by traditional means, it is useful to think outside the typical experimental toolkit used for probing the structure of normal proteins. Computational methods to predict protein structure have a long history; the Ramachandran plot is still used for theoretical models and validations of structure (Ramachandran et al., 1963). Molecular dynamic simulations have yielded powerful insights into what misfolding entails either in intermediate states of folding or when mutations are introduced (Beck et al., 2008; Jonsson et al., 2009). However, molecular dynamic simulations are time-intensive and require considerable computational resources. What would be extremely useful is a simple, rapid, computational method that predicts how residue changes affect the folding and stability of a protein.

In this issue of *Structure*, Brock et al. (2015) developed a novel computational modeling method to explore the structural alterations that could be incurred by residue changes within proteins. The method leverages the strength of their previous computational model that predicts how the burial of hydropathic residues in a protein contributes to the structure (England, 2011). The new method generates a linear trace of buried residues that is computationally derived by considering the hydropathy of a residue in the sequence and the steric constraints due to neighboring residues. One of the key advantages of this new computational method is that it is extremely rapid, taking seconds to perform for short sequences. Thus, many residue changes along the linear length of a sequence can be queried quickly, generating a large ensemble of burial traces that predict effects on protein folding. The patterns that emerge have the power to provide crucial clues for understanding the multiple conformations a protein can adopt.

The authors tested their method with the von Hippel-Lindau protein (VHL), which has been used as a model substrate for PQC because it is prone to misfolding when it does not interact with its partner proteins (Kaganovich et al., 2008; McClellan et al., 2005; Spokoini et al., 2012) (Figure 1). Furthermore, hundreds of mutant versions of VHL have been identified (Nordstrom-O'Brien et al., 2010), meaning that there is an extensive battery of experimental mutations that can be used to validate the computational method. The authors performed a pair-swap mutational analysis in which residues at two different positions are swapped (Figure 1), thus preserving overall residue content but creating positional changes in sequence. Thousands of pair swaps were computationally analyzed to discover those that stabilized the folding of VHL over its native marginally stable state. The authors chose ten candidates to examine in vivo, and their directed choice was based on the assumption that the least structural variability predicted by the modeling would provide the greatest structural stability. To be thorough, the authors also randomly chose ten candidates from the thousands of computationally derived sequences as a control group.

Intriguingly, the most informative mutants that were stable and escaped PQC degradation emerged from the randomly selected set, not the directly selected set. This, of course, could appear as a failure of the method on first blush. The authors noted: "Initially, these results were surprising because… [the]…stabilizing mutations were chosen randomly as part of the control group, while the test group of ten mutants designed to exhibit more stability actually produced less-stable behavior on average" (Brock et al., 2015). This interesting development provides an instructive lesson for both budding and experienced scientists. Highly informed guesses that we use to develop hypotheses can sometimes lead us to choose the wrong fork in the experimental road, but being prepared in mind can favorably lead to a fortunate Pasteurian outcome. Brock and colleagues were open to a successful possibility despite the initial adversity. Once the authors realized that the randomly selected set was still predicted to be more stable in fold than the native state by their novel computational method, the lemons of their directed predictions were made into the refreshing lemonade of discovery using the random set. Although the initial bias in choosing a test data set was flawed, the model actually performed very well with a random sampling. Furthermore, the high frequency of stable mutants in the randomly selected set (4 out of 10) demonstrated the power of the computational method to reveal important fundamental principles of folding.

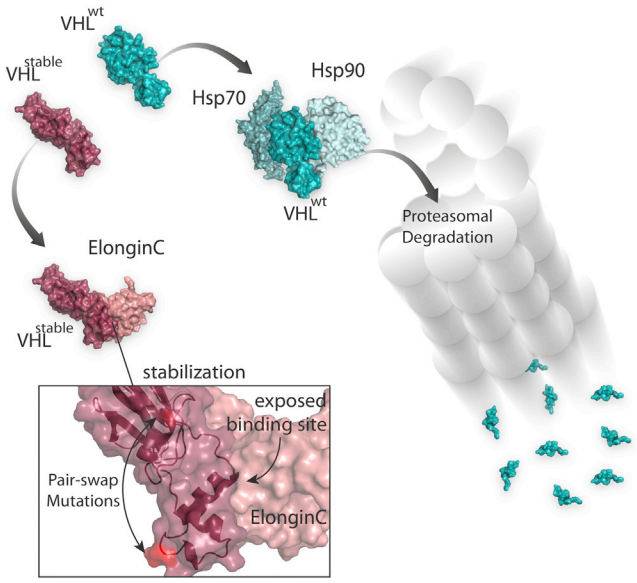

Figure 1. The Folding of VHL

Wild-type VHL (VHL^{wt}) is a marginally stable protein without its binding partners Elongin B and C (McClellan et al., 2005). VHL^{wt} is subsequently targeted for degradation by the proteasome through actions of the chaperones Hsp70 and Hsp90. Using a novel computational modeling method that creates pair-swap mutations, more structurally stable versions of VHL (VHL^{stable}) have been identified that elude proteasome degradation.

It is often tempting to infer that the structural parameters of normal folding will also apply to misfolding. This view can lead to suggestions for applying traditional structural biochemical and biophysical experiments to query protein misfolding, which can be impossible to conduct with aggregation-prone proteins. Computational methods have the power to predict ways to examine misfolded protein behavior. Brock and colleagues are at the forefront of such computational analyses by developing a novel, rapid way to predict protein stability that lends itself to easy in vivo tests.

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