Physical basis for modulation of folding state in a disordered protein: out of the CPU and into the cell

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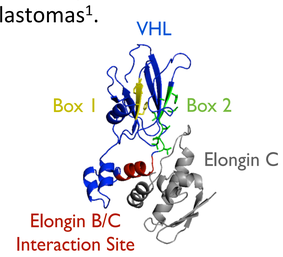
Abstract

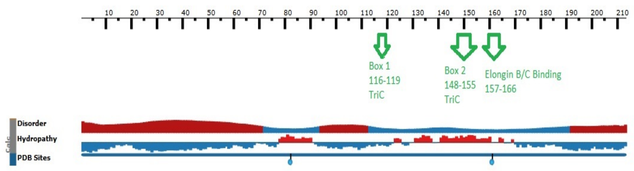
The human von Hippel-Lindau (VHL) tumor suppressor is a model disordered protein that has been studied in conjunction with folding and degradation pathways in the cell. It is thought to interact with different chaperones and requires binding to cofactors like elongin C to become stable in the cell. Here we present 20 pair-swap mutations of the human VHL protein that were transformed into *S. cerevisiae* cells and tagged fluorescently. Of this group, two mutations produced proteins that were stable enough to be detected in large quantities by FACS analysis. One mutation directly affected a known chaperonin binding site (V155/G19), while the other mutation (L201/E173) is not in a known interaction region. A previously-published theoretical model used to predict the degree of exposure of residues in a protein's lowest-energy conformation (its "burial trace") was used to examine the mutant sequences. The L201/E173 burial trace followed the pattern of burying the chaperone interaction sites and exposing the cofactor binding site more than the other mutant and wildtype sequences, similar to the known crystal structure of VHL in complex.

The VHL Protein

The von Hippel-Lindau tumor suppressor protein (VHL) forms part of an ubiquitin ligase complex that targets molecules like HIF-1$^{\alpha}$ for degradation. Mutations in this protein have been linked to a variety of cancers like hemangioblastomas[1].

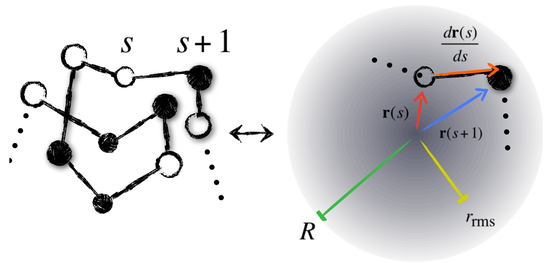


However, the protein interacts with chaperones and achieves stability when bound in complex with its cofactors, elongin B and elongin C[2]. Binding sites are illustrated below, from the VHL RCSB page[3]:



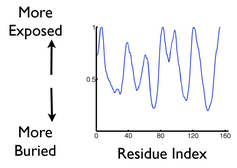
The Model

To study why VHL becomes more stable under this mutation, we will use a previously published model by J. England that uses energy minimization with two components: hydrophobicity of each residue and the energetic cost of stretching neighbor residues.



$$H = \int ds \left[\kappa \left| \frac{dr(s)}{ds} \right|^2 + \psi(s)|r(s)|^2 \right] \quad \text{minimize} \quad r_{rms} = \sqrt{\frac{3R_{max}}{5}}$$

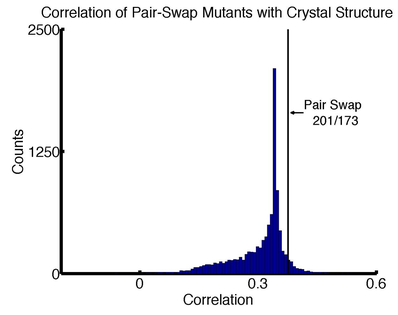
Elasticity between residues — Hydrophobicity

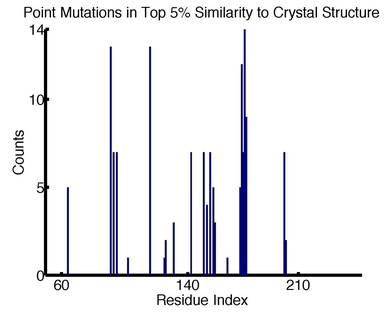


This process gives the "burial trace," which is used to predict which residues are exposed or buried in a protein's lowest-energy conformation on the order of 1 second. This model has previously been used to study allostery in various proteins[4].

Significance of Results

We also calculated each possible pair swap and individual point mutation in [63, 204] and compared them by Pearson correlation to the crystal structure's burial trace.

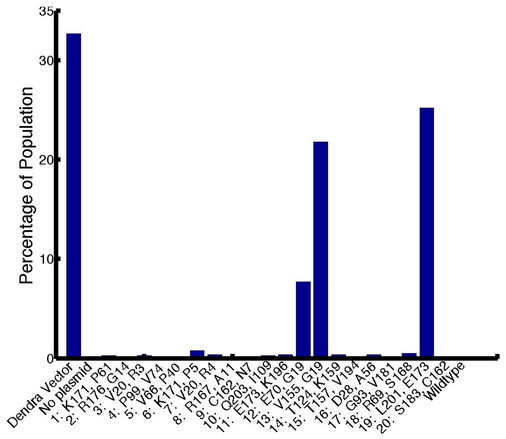




Only 5.88% of all possible pair swap mutations were more similar to the crystal structure for complex-stabilized VHL. Furthermore, the region between 170-180 appears to be a "hot spot" for conformationally changing the protein to match the crystal structure. Another high-scoring residue is 142, which has at least one experimentally-determined stabilizing mutation[5].

Experimentally-Determined Stable Conformations

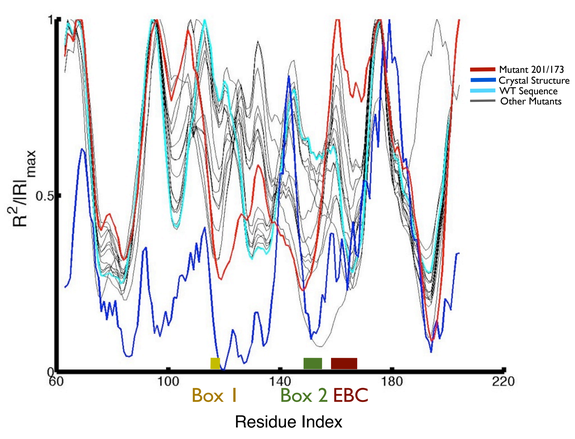
Pure VHL exists in a molten globule form that is unstable in the cytosol. However, some mutations are known to make it stable. Our collaborators created 20 pair-swap mutations in the hopes of finding a stable form in yeast, and analyzed their stability through FACS analysis:



Of these, two (155<->19 and 201<->173) mutations produced VHL protein that was markedly more stable in the cell. The first mutation directly affects Box 2, while the second mutation is outside of any known interaction site.

Exposure Patterns from Model

The burial traces of all 20 pair-swap mutations are shown below, along with the burial trace of the wildtype sequence and that of the crystal structure (1VCB chain C). Since the first ~50-60 residues are highly disordered, the input sequences were truncated to match the crystal structure sequence of residues between 63 and 204.



The 201<->173 mutation has a relatively large correlation with the crystal structure's burial trace, particularly with respect to burying Box 1 and 2 and exposing the elongin B/C interaction site.

Conclusions and References

Based on the computational results, we hypothesize that exposing the elongin B/C interaction site confers stability.

Although VHL is a human protein, a homologue of elongin C exists in yeast and is known to bind to VHL peptides[6].

Current work includes retransforming the mutant VHL into an elongin C-knockout yeast strain to see if this results in decreased stability.

References:

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