Abstract 1473

## Modeling Binding of Beta-site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) Inhibitor Aminoquinoline (68 K) for Possible Treatment of Alzheimer's Disease

**Juhi Dalal,** *Nova Southeastern University*

**Shreya Averineni, Pranav Madadi, Emily Schmitt Lavin, Arthur Sikora**

Alzheimer's Disease (AD), affecting approximately 24 million people worldwide, is characterized by the formation of amyloid-β plaques within the brain. Alzheimer's research has been focused on limiting amyloid-β production through developing inhibitors for the enzymes needed within the amyloid cascade. This project focuses on the aminoquinoline class of inhibitors, of which 68 K (PDB: 5i3Y) is the most effective because of its strong Kd and IC50 values. The students of the Honors Protein Modeling class at Nova Southeastern University modeled the interaction between Beta-site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE-1) and 68 K. Using Jmol a model was developed, and 3D printed to show how the inhibitor (68 K) fit into the enzyme's active site. This model highlights important aspects of the interactions between the ligand and the BACE-1 enzyme. 68 K has strong interactions with 32 amino acid residues in BACE1, some of which are intertwined with one another. For example, BACE-1's residues Val69, Pro70, and Tyr71 are known collectively as "the flap". "The flap" is a β-hairpin loop structure that is positioned directly over BACE-1's catalytic dyad, a group of amino acids within the active site of the enzyme. "The flap" is also responsible for regulating access to the enzyme's catalytic dyad (Asp 32 and Asp 228) by a given substrate (or inhibitor). Researchers found the inhibitor 68 K to have interactions with the flap which maximizes the strength of the interaction with BACE-1 residues, thus minimizing the distance between the inhibitor's various functional groups and accommodating their specific polarities. Being able to visualize the protein structure using a 3D model aids in the understanding of how the ligand inhibits this enzyme leading to the progression of AD.

This work was made possible by funding through the National Science Foundation, Division of Undergraduate Education (NSF-DUE) grant number 1725940 for the CREST Project. Nova Southeastern University's Farquhar Honors College and Dept. of Biological Sciences also provided support. Protein model printing was made possible by 3d Molecular Designs.

104026, https://doi.org/10.1016/j.jbc.2023.104026

Abstract 1474

## Regulation of Cullin 2-RING ubiquitin ligases by NEDD8 and CAND1

**Xing Liu,** *Purdue University*

Through recruiting interchangeable substrate receptor modules (SRMs) to the cullin (CUL) core, cullin-RING ligases (CRLs) catalyze the ubiquitination and degradation of diverse proteins that play key roles in a myriad of biological processes in human cells. As the founding member of CRLs, the CUL1-based CRL1s are known to be activated by both CAND1, which exchanges the SRMs associated with the common CUL1 core, and NEDD8 conjugation (neddylation), which modifies CUL1 and alters CRL1 conformation to promote substate ubiquitination. In comparison with CRL1s, CRL2s comprise the CUL2 core that is homologous to CUL1, and SRMs whose composition differ from CRL1s. Because of the structural difference, it is unclear if NEDD8 and CAND1 regulate CRL1 and CRL2 in similar fashions. To uncover mechanisms regulating CRL2s, we studied the CRL2VHL, a ubiquitin ligase well known for targeting HIF1α for ubiquitination and degradation, and for PROteolysis TArgeting Chimeras (PROTACs) induced degradation of disease-causing proteins. We found that neddylation promoted the *in vitro* ubiquitination of the full-length HIF1α and the degron peptide of HIF1α to similar extents, demonstrating that neddylation activates CRL2VHL-dependent ubiquitination regardless of the substrate sizes. In HEK293 human cell-based cycloheximide chase assays, eliminating neddylation failed to stabilize HIF1α due to VHL-independent degradation, but it stabilized the truncated HIF1α containing only the C-terminal Oxygen-Dependent Degradation (C-ODD) domain. This unexpected finding suggests that the cellular activity of CRL2VHL is better reflected by the degradation of C-ODD than that of HIF1α, and we thus analyzed the degradation of C-ODD in HEK293 cells with or without CAND1-knockout (KO). Surprisingly, C-ODD was degraded faster in the KO cells, and similarly, the PROTAC-induced degradation of CRL2VHL neo-substrates also became faster in the KO cells. This inhibitory effect of CAND1 on CRL2-dependent degradation was then explained by our kinetic measurements of CRL2VHL and CAND1 interactions using *in vitro* Förster resonance energy transfer (FRET) assays. We found that CAND1 dramatically increased the dissociation rate of the CRL2VHL complex but hardly accelerated the assembly of stable CRL2VHL, leading to an overall reduction in CRL2VHL assembly. We then hypothesized that this inhibitory effect is important for CRL2 activity in human cells because it allows only proteins that bind CRL2 tightly enough to be ubiquitinated, and thereby, it enhances CRL2 substrate specificity. Using PROTACs that recruit neo-substrates to CRL2VHL with different affinities, we found evidence supporting this hypothesis, showing that eliminating CAND1 from HEK293 cells shortened the half-life of lower affinity substrates but had no effect on the stability of higher affinity substrates. Taken together, we conclude that instead of being an exchange factor that activates other CRLs, CAND1