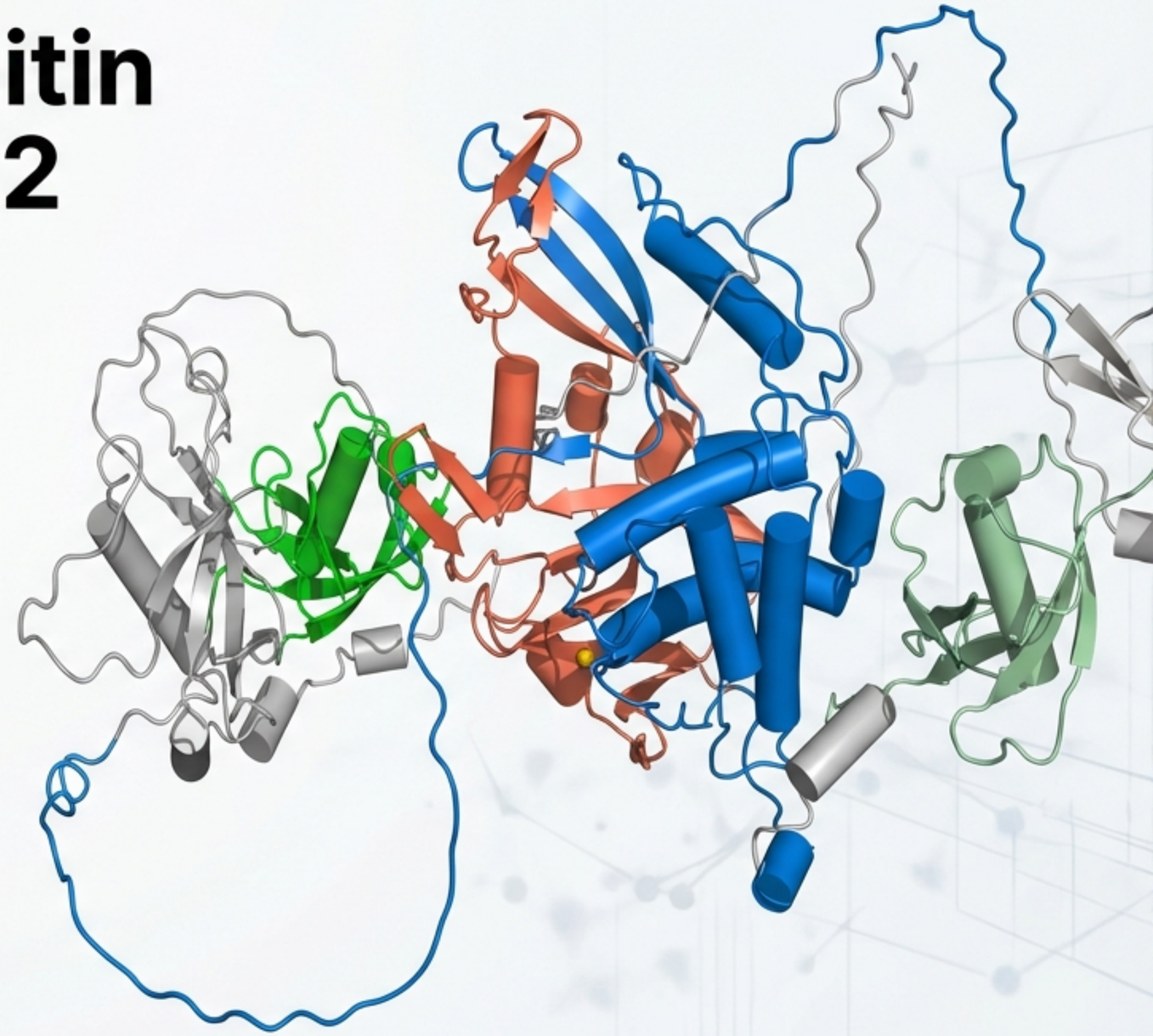
Cracking the Ubiquitin Code: How the UBL2 Domain Directs USP11 Specificity

Uncovering novel regulatory mechanisms and AI-identified therapeutic inhibitors for cancer and neurodegeneration.



Based on research by: Sin-Rong Lee, Han-Hsiun Chen, Ruey-Hwa Chen, and Kuen-Phon Wu.

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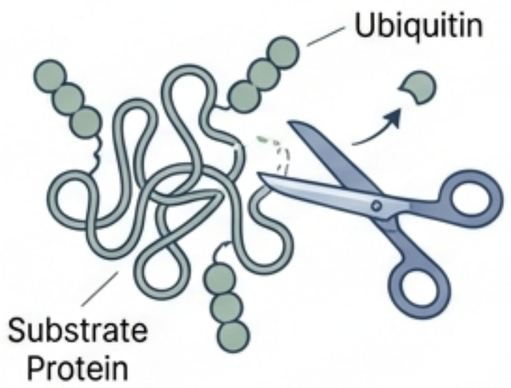
USP11 is a Critical Node in Cellular Health and Disease Progression

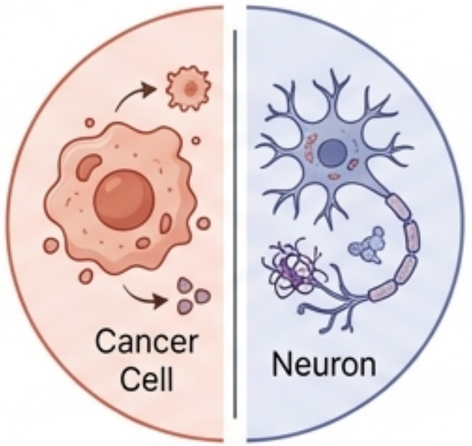
The Molecular Role



The Molecular Role

USP11 is a Deubiquitinase (DUB). It removes ubiquitin tags from substrate proteins, preventing their degradation and altering signaling pathways.

The Disease Link



The Disease Link

Oncology: Promotes tumorigenesis and chemoresistance in prostate, gastric, and ovarian cancers by stabilizing oncogenes (e.g., c-Myc).

Neurodegeneration: Deubiquitinates Tau, leading to toxic accumulation and tauopathy.

The Challenge

The Challenge

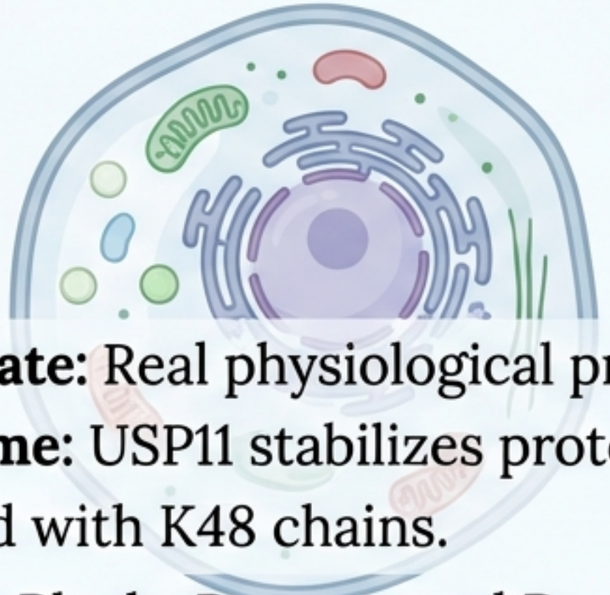
Therapeutic targeting is currently limited by a lack of understanding regarding how USP11 selects its specific targets.

A Decade-Long Discrepancy in USP11 Biology

Previous In Vitro Studies

- **Substrate:** Short Ubiquitin chains (di-Ub)
- **Preference:** K6, K33, K63 (Signaling)
- **Result:** NO activity on K48 chains

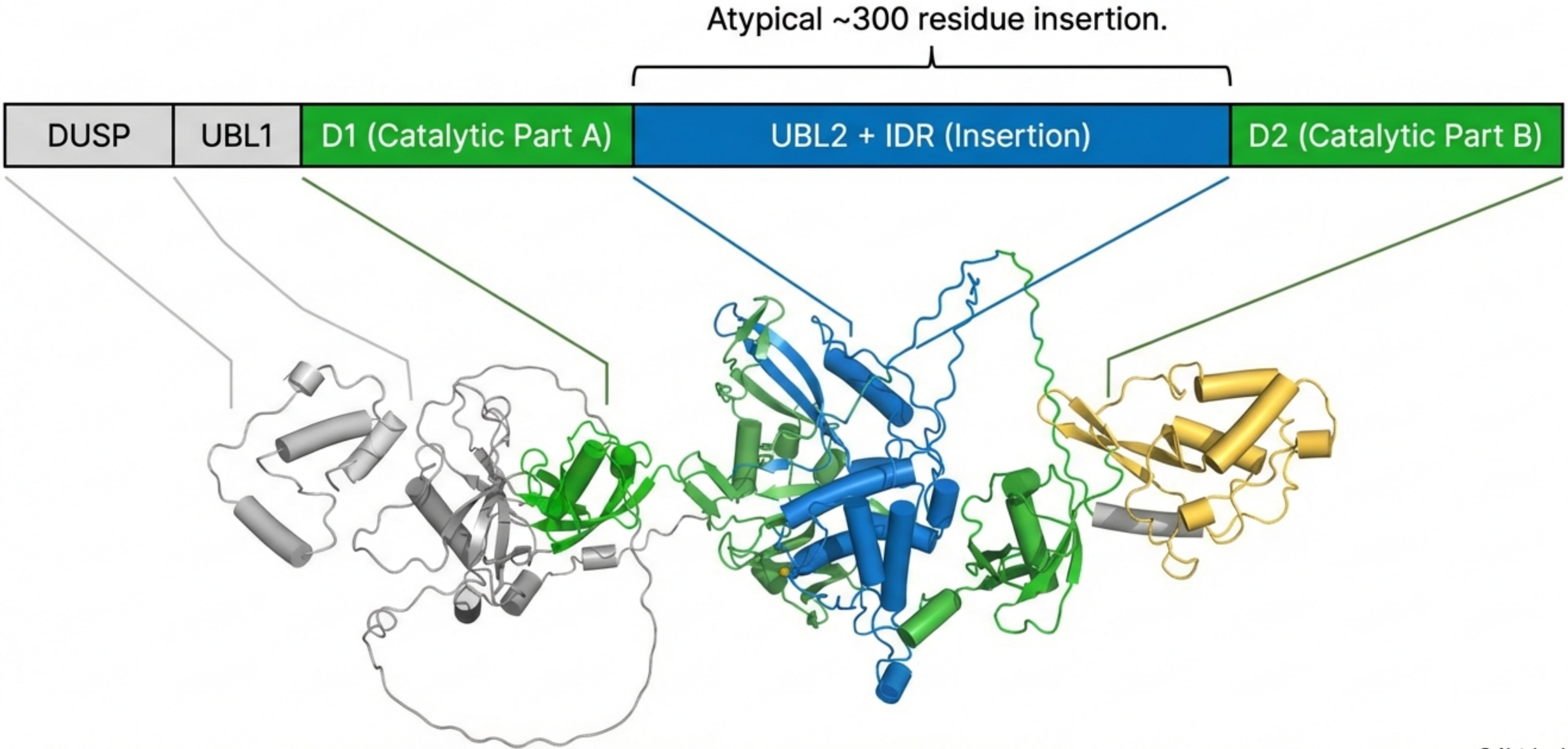
Clinical Reality (In Vivo)



- **Substrate:** Real physiological proteins
- **Outcome:** USP11 stabilizes proteins marked with K48 chains.
- **Result:** Blocks Proteasomal Degradation

The Question: Why is the isolated enzyme ‘blind’ to the very chains it targets in the cell? What structural component is missing?

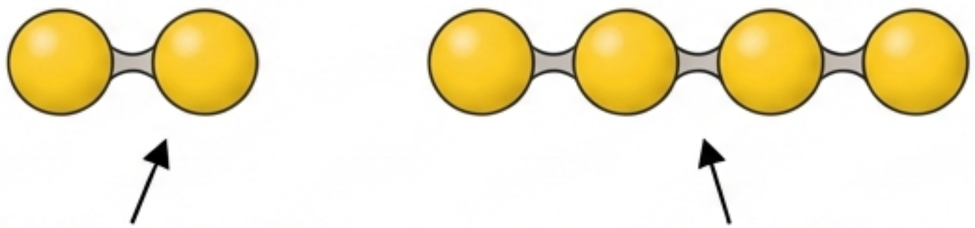
The Suspect: USP11 Possesses a Unique ‘Split’ Catalytic Core.



Length Matters: Using Tetra-Ubiquitin (Ub_4) to Mimic Physiological Conditions

Methodology: The Importance of Chain Length

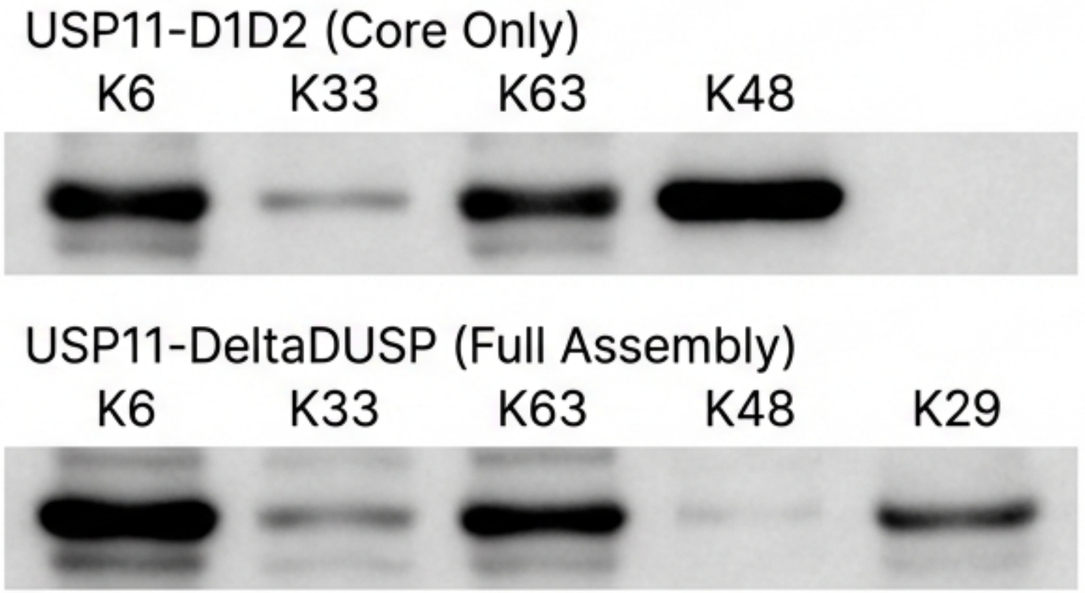
Previous studies used short dimers (Ub_2) and missed the activity. This study utilized tetramers (Ub_4), representing the average chain length in vivo.



Previous Studies: Short Dimers (Ub_2)

This Study: Physiological Tetramers (Ub_4)

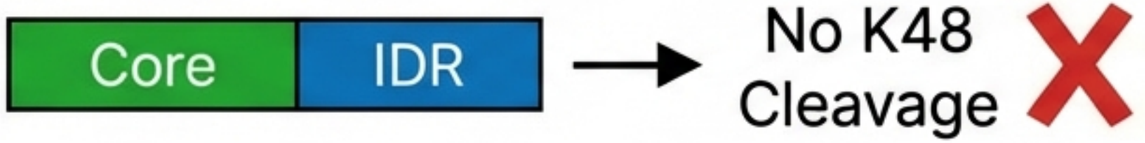
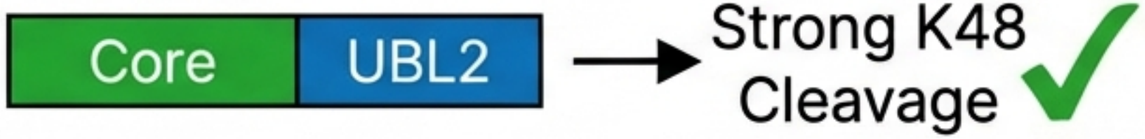
The Evidence: K48 Specificity Revealed



The catalytic core is “blind” to K48 chains on its own. The non-catalytic domains are required to activate K48 specificity.

The UBL2 Domain is the “Key” that Unlocks K48 Specificity.

Process of Elimination

Core + IDR (No UBL2)	
Core + UBL2 (No UBL1)	

Kinetic Analysis

Catalytic efficiency (k_{cat}/K_M) on monomeric substrates is identical between the Core and the Full Assembly.

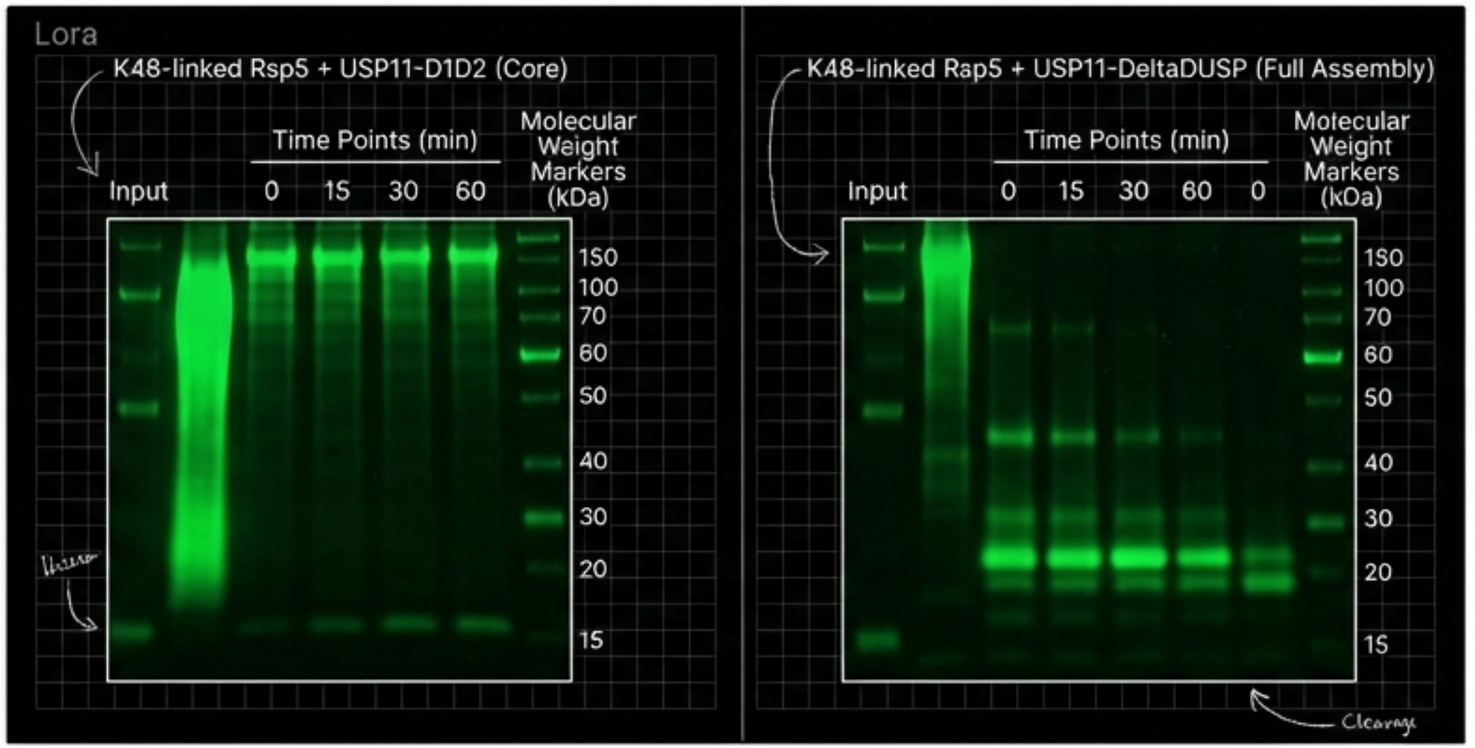
The UBL2 domain acts as a specificity guide, not a catalytic accelerator.

Validation on Complex Substrates: The Rsp5 Experiment.

Testing the mechanism on a real autoubiquitinated enzyme (E3 ligase Rsp5) rather than synthetic chains.

Lab Notebook

Core Only
(D1D2)



With UBL2
(DeltaDUSP)

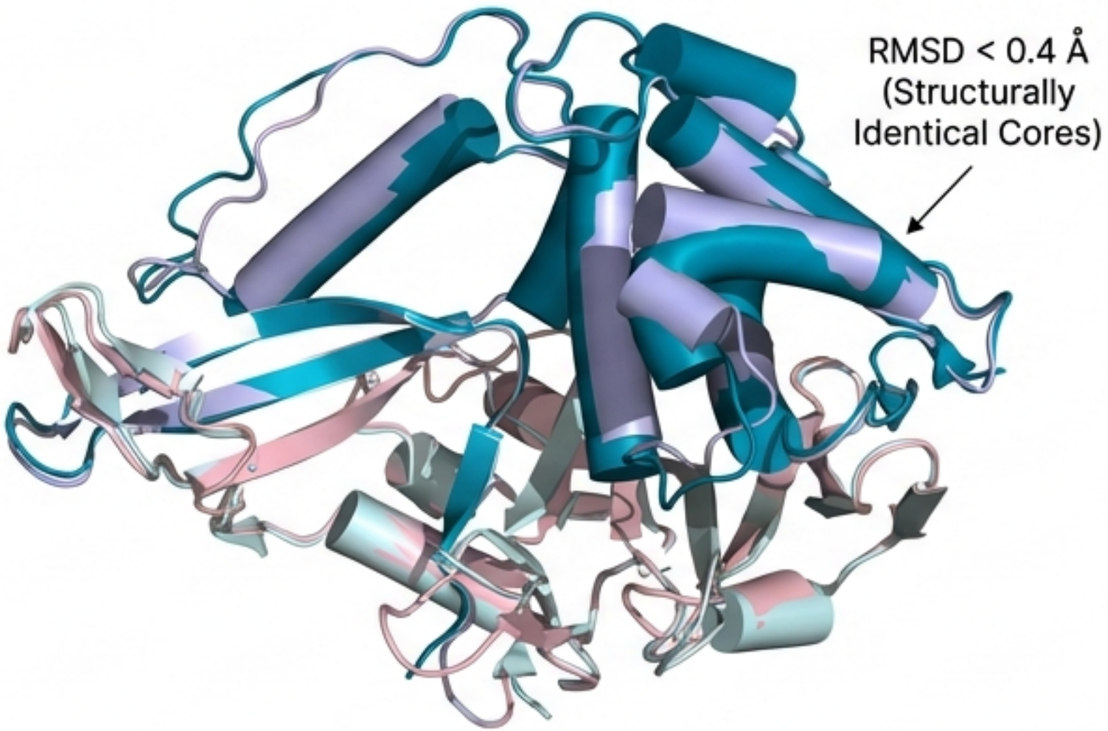
Takeaway Box

USP11 requires the UBL2 domain to strip K48 chains from bulky, real-world protein substrates.

A Mechanism Unique to USP11, Distinct from Paralogs USP4 and USP15.

The Cousins

USP4 and USP15 share the same "Split Core + Insertion" architecture.



The Functional Divergence

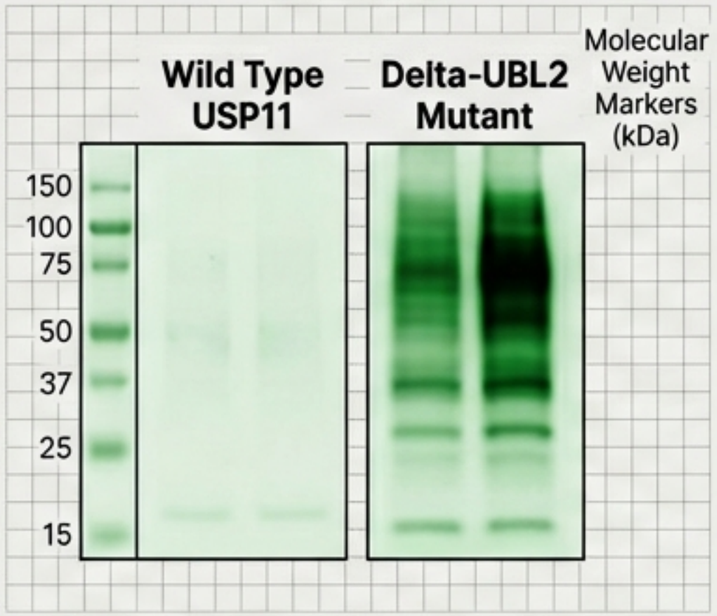
Core Only Activity Comparison	Cleaves K48?
USP4/15 Core Only	✓ YES
USP11 Core Only	✗ NO

Evolutionary Twist: Unlike its relatives, USP11 has evolved to be totally dependent on the UBL2 domain for K48 regulation.

The UBL2 Domain Controls the Fate of Cancer Targets in Cells.

Targets Analyzed: SOX11, PML, c-Myc.

SOX11



Low K48 signal (Substrate is deubiquitinated/Stable).

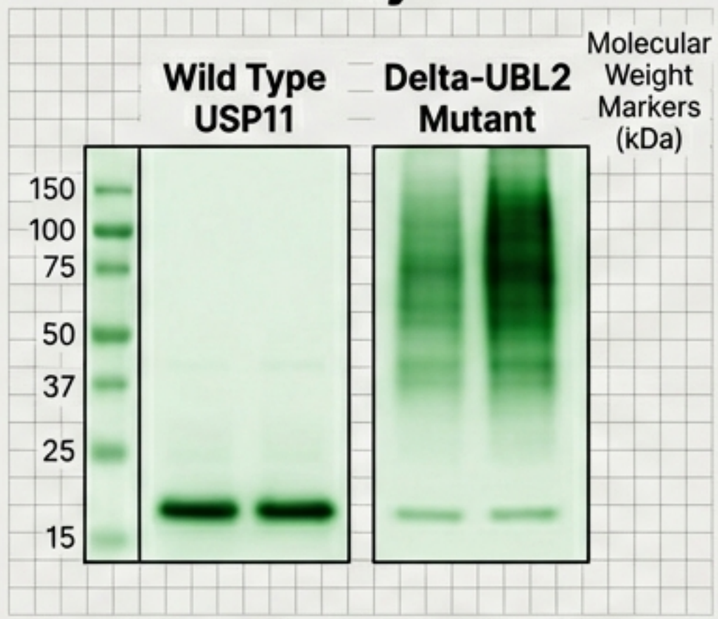
High K48 signal (Substrate remains ubiquitinated/Degraded).

PML

Low K48 signal (Substrate is deubiquitinated/Stable).

High K48 signal (Substrate remains ubiquitinated/Degraded).

c-Myc



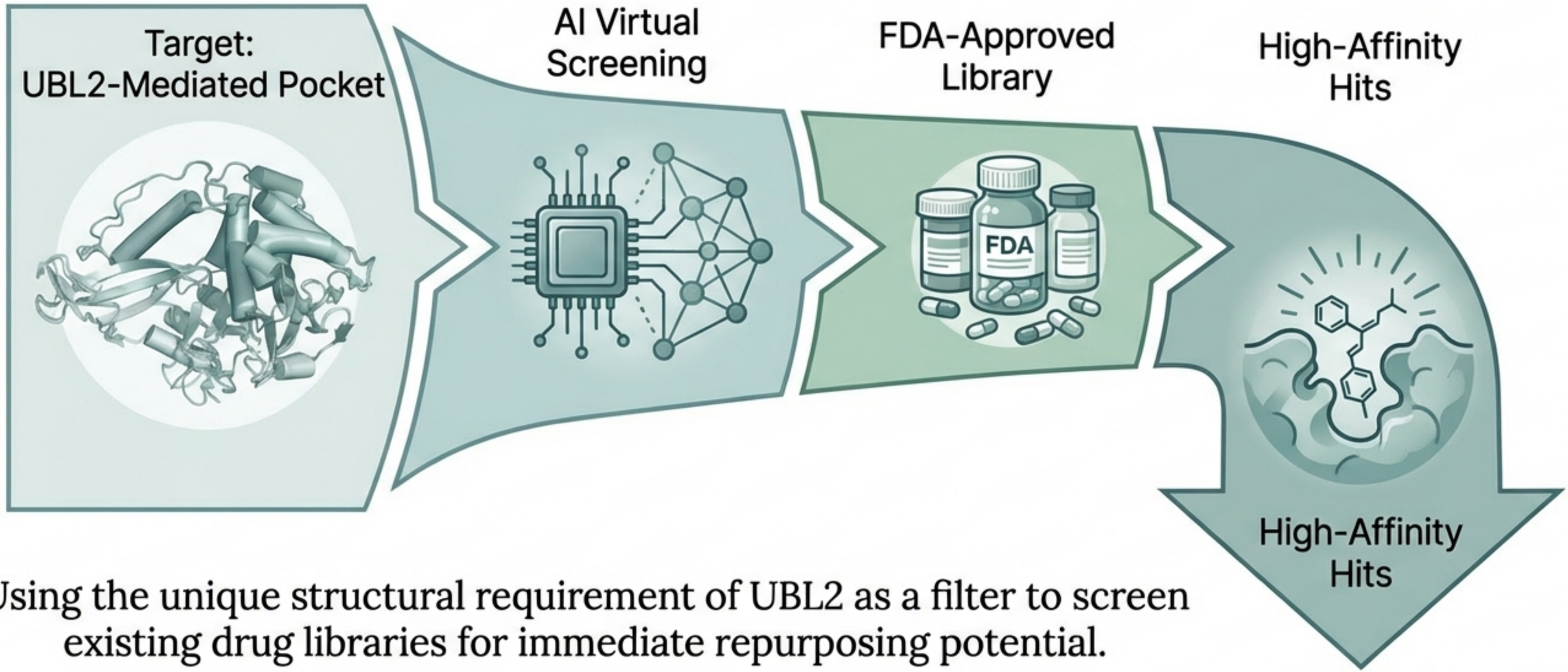
Low K48 signal (Substrate is deubiquitinated/Stable).

High K48 signal (Substrate remains ubiquitinated/Degraded).

Lora

Without the UBL2 domain, USP11 fails to deubiquitinate oncogenes in the cellular environment.

Leveraging Structural Insight for Rational Drug Design

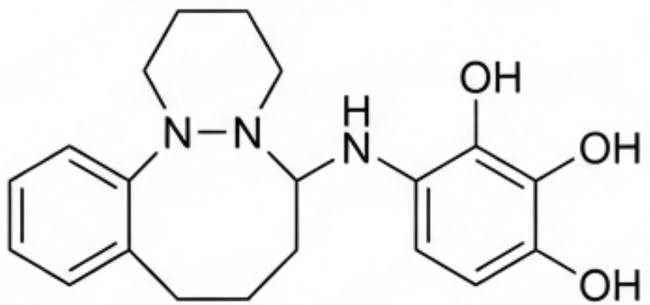


Using the unique structural requirement of UBL2 as a filter to screen existing drug libraries for immediate repurposing potential.

AI Screening Identifies Fenoldopam and Olanzapine as Selective Inhibitors

Fenoldopam

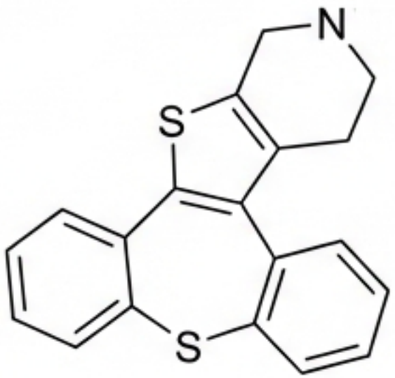
Vasodilator / Dopamine Agonist



Demonstrated significant efficacy in inhibiting USP11 *in vitro*.

Olanzapine

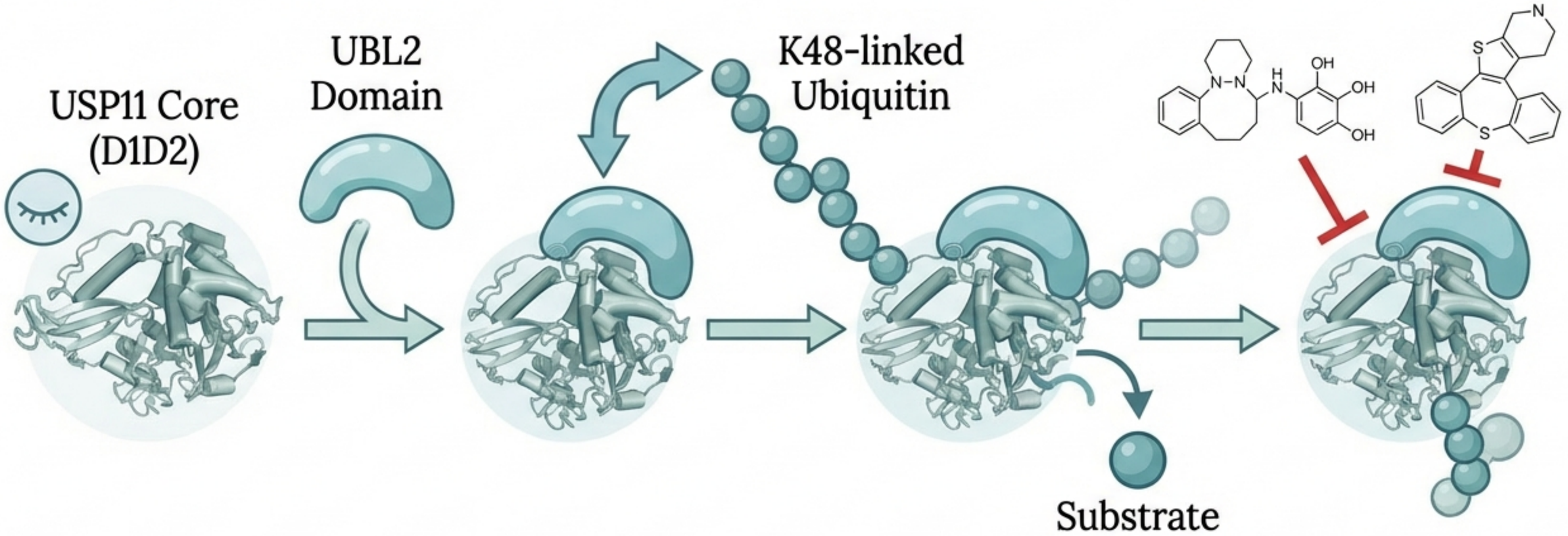
Atypical Antipsychotic



Demonstrated potency in cellular models.

Advantage: Known safety profiles significantly shorten the timeline to clinical trials for cancer and neurodegeneration.

The New Model of USP11 Regulation



Core Alone (Blind to K48)

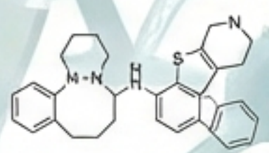
UBL2 Guides Substrate

Substrate Stabilization

Fenoldopam/Olanzapine Block Recognition

A New Avenue for Targeted Cancer & Neurodegeneration Therapy.

- **Solved:** The mystery of USP11 specificity is resolved—the UBL2 domain is the mandatory driver for K48 recognition.
- **Unique:** This mechanism is evolutionarily distinct from paralogs USP4/15, offering a pathway for highly selective drug targeting.
- **Actionable:** AI screening has identified Fenoldopam and Olanzapine as lead compounds ready for optimization and repurposing.



Call to Action: Future Outlook: Developing ‘molecular glue’ degraders and allosteric inhibitors targeting the UBL2 interface.