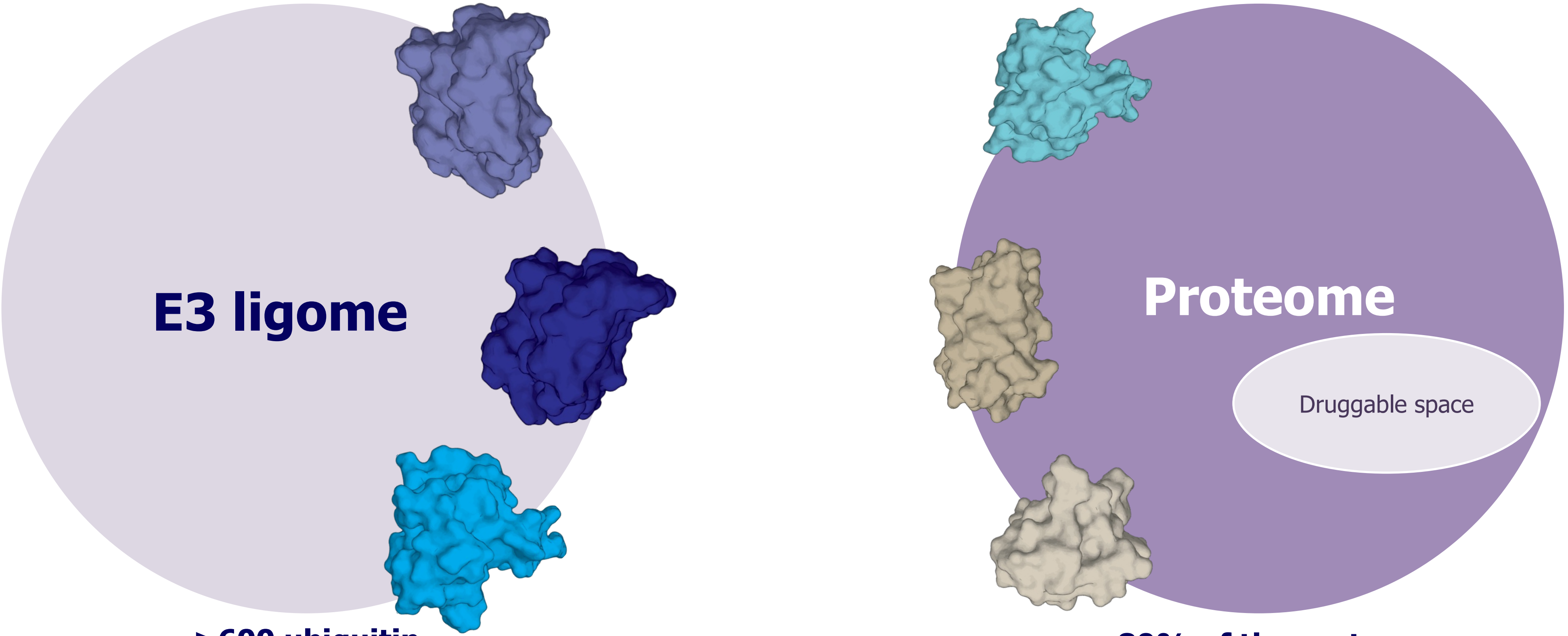
# AI Applications for Molecular Glue Degraders: From Degron Discovery to *in silico* Screening

Pablo Gainza | 5th Annual Targeted Protein Degradation Summit | October 26th, 2022

Monte Rosa Therapeutics

# Molecular Glue Degraders (MGDs) – Drugging The Undruggable

## Pairing E3 ligases to the target space for MGD-induced degradation



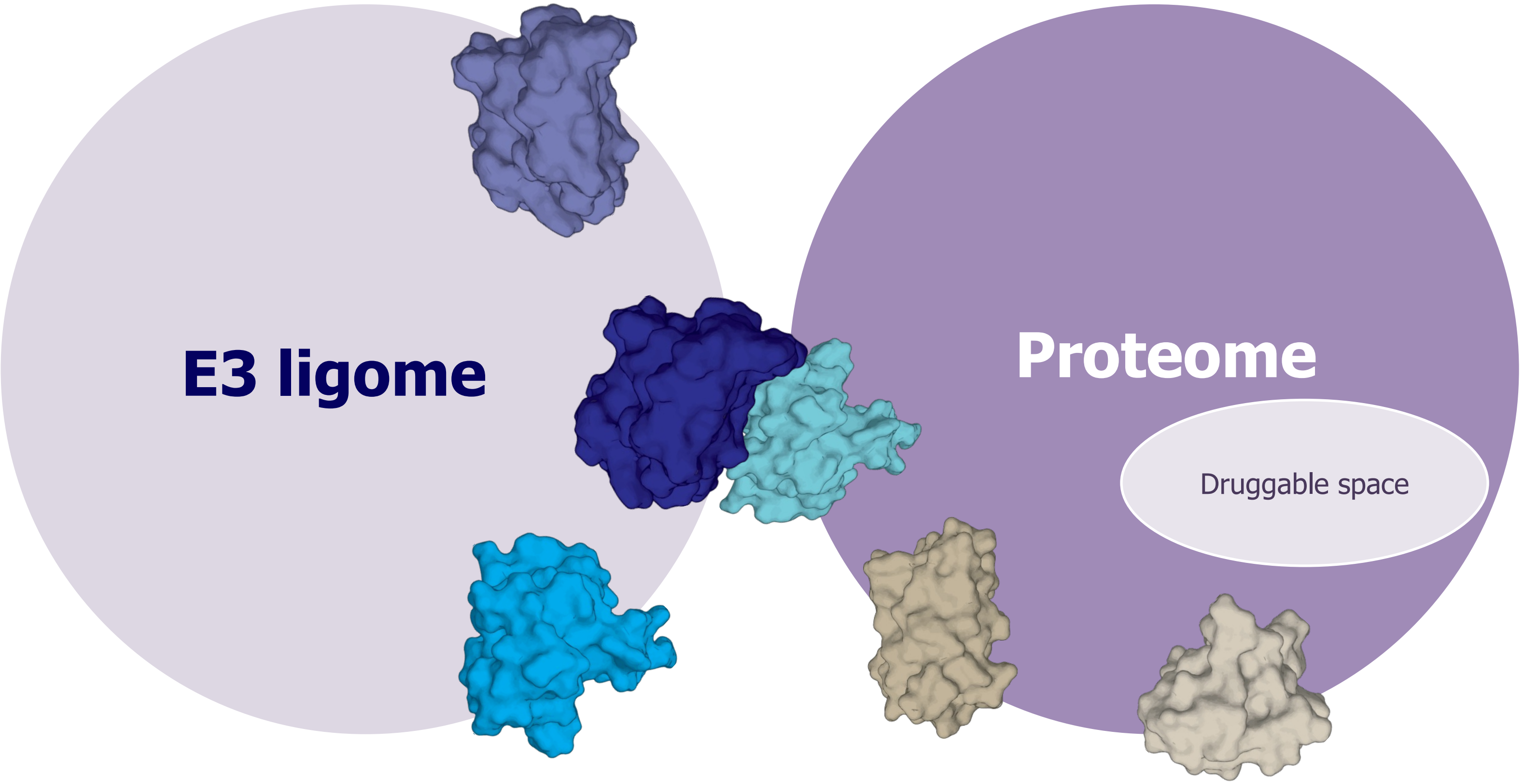

**>600 ubiquitin E3 ligases**

**>80% of the proteome is undruggable**

AI/ML *in silico* predictions (AI/ML)

# Molecular Glue Degraders (MGDs) – Drugging The Undruggable

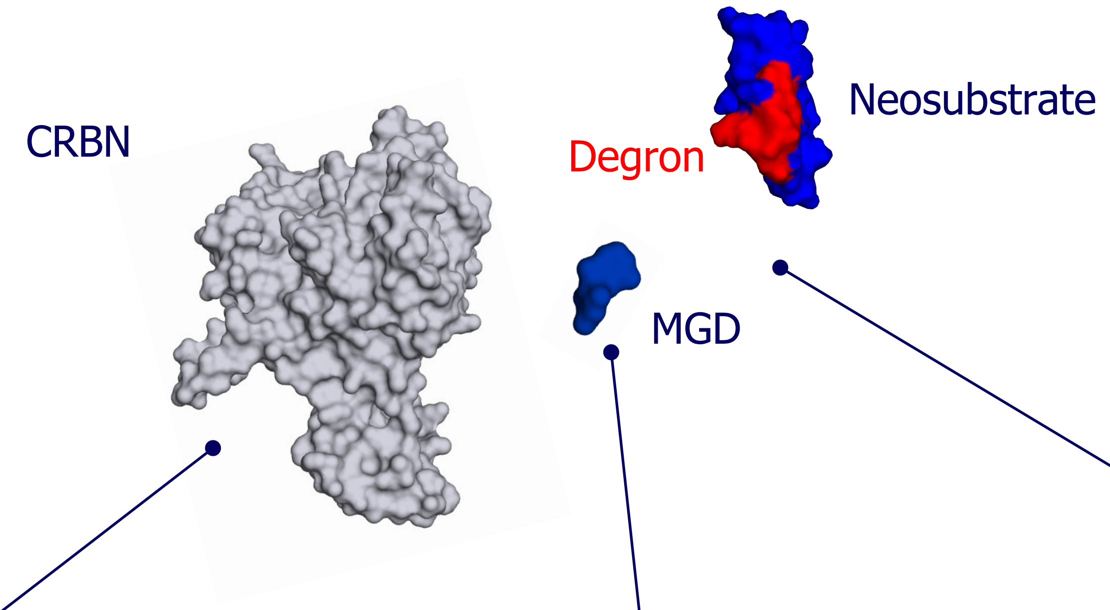
## Pairing E3 ligases to the target space for MGD-induced degradation




AI/ML *in silico* predictions (AI/ML)

# Essential Ingredients For Glue-based Protein Degradation

## Cereblon (CRBN) as a template for future E3 ligase platforms




**Ligandable E3 ligase**
with a reprogrammable surface

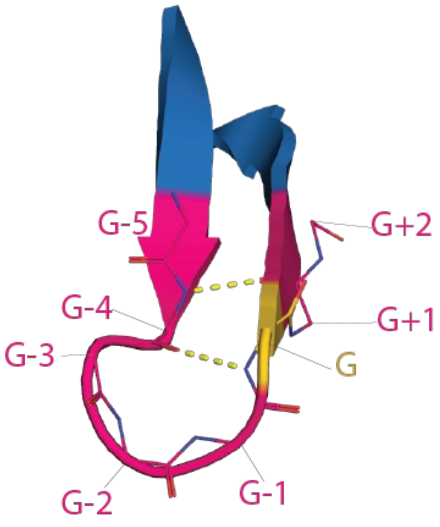
**MGDs** to create a neosurface
specific for the target

**Targets** complementary
to the neosurface

# Known CRBN Neosubstrates Share a Common Structural Motif

## The canonical G-loop: a beta-hairpin with an alpha-turn and a conserved glycine

**G-loop motif**

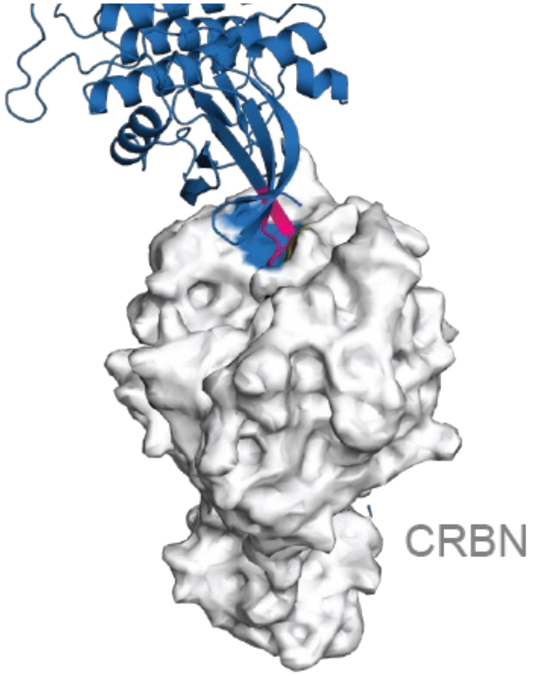



H-bond

| | |
|---|---|
| CK1a | INITNGEE |
| GSPT1 | VDKKSGEK |
| ZNF692 | QCEICGFT |
| SALL4 | VCSVCGHR |
| IKZF2 | HCNQCGAS |

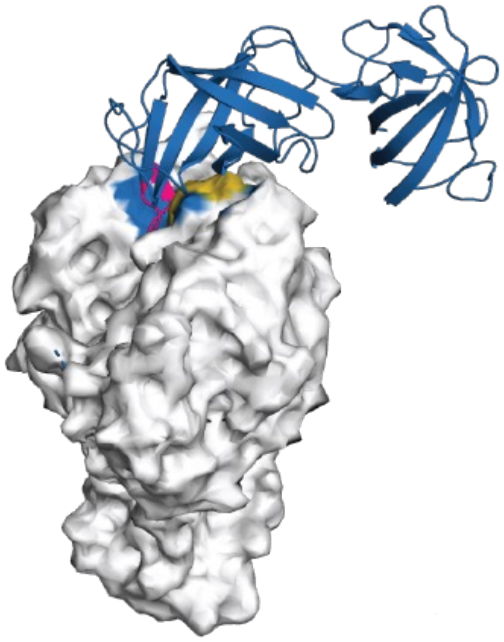
**Structurally characterized neosubstrates in the public domain**

CK1$\alpha$



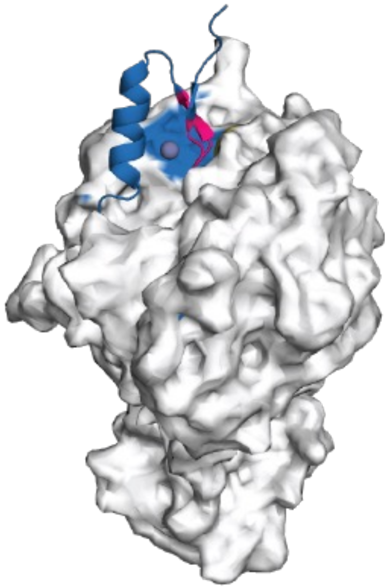

*Petzold et al. 2016*

GSPT1



*Matyskiela et al. 2016*

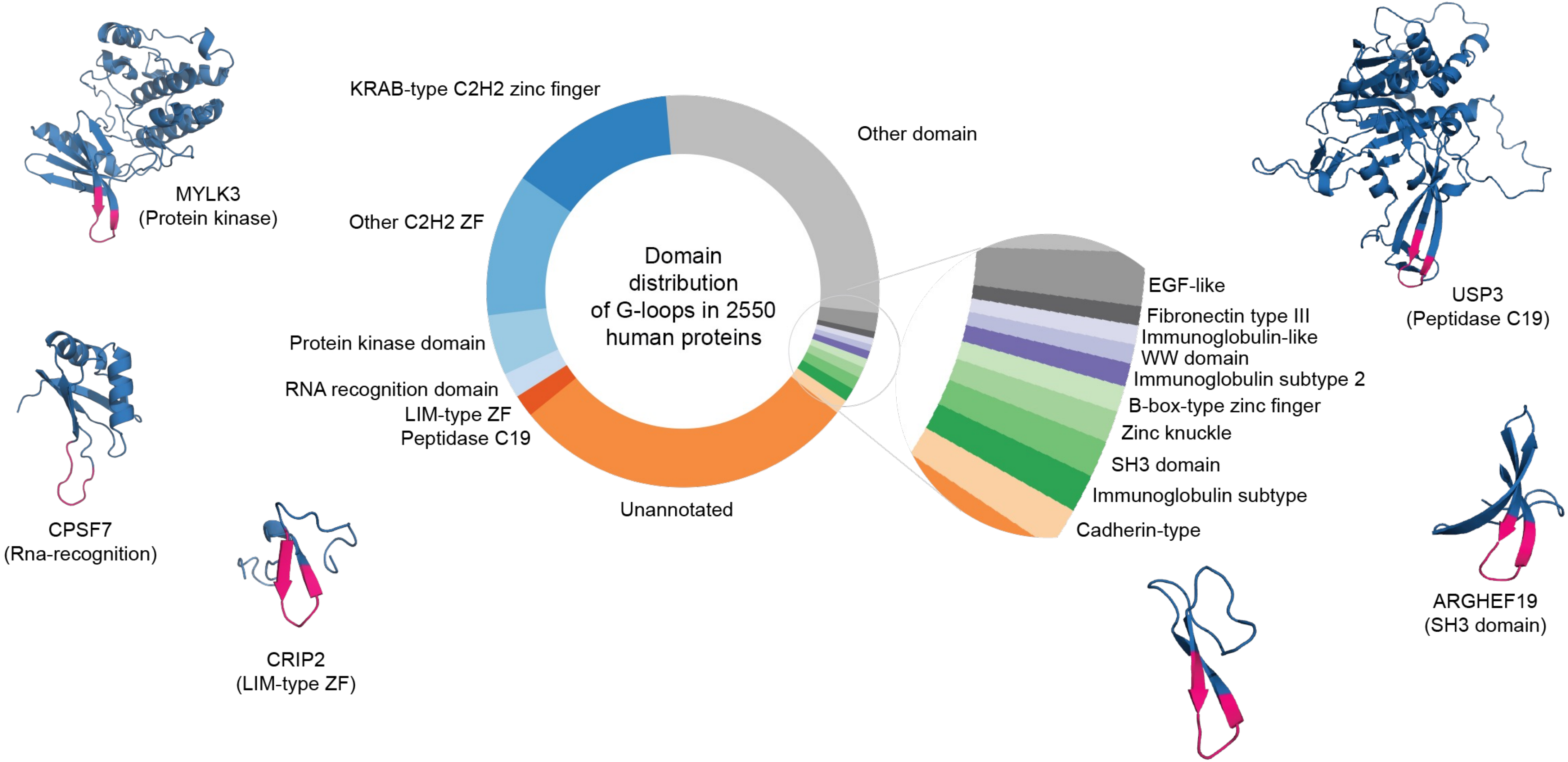
C2H2 ZF



*Sievers/Petzold et al. 2018*

# Canonical G-loops in the Human Proteome

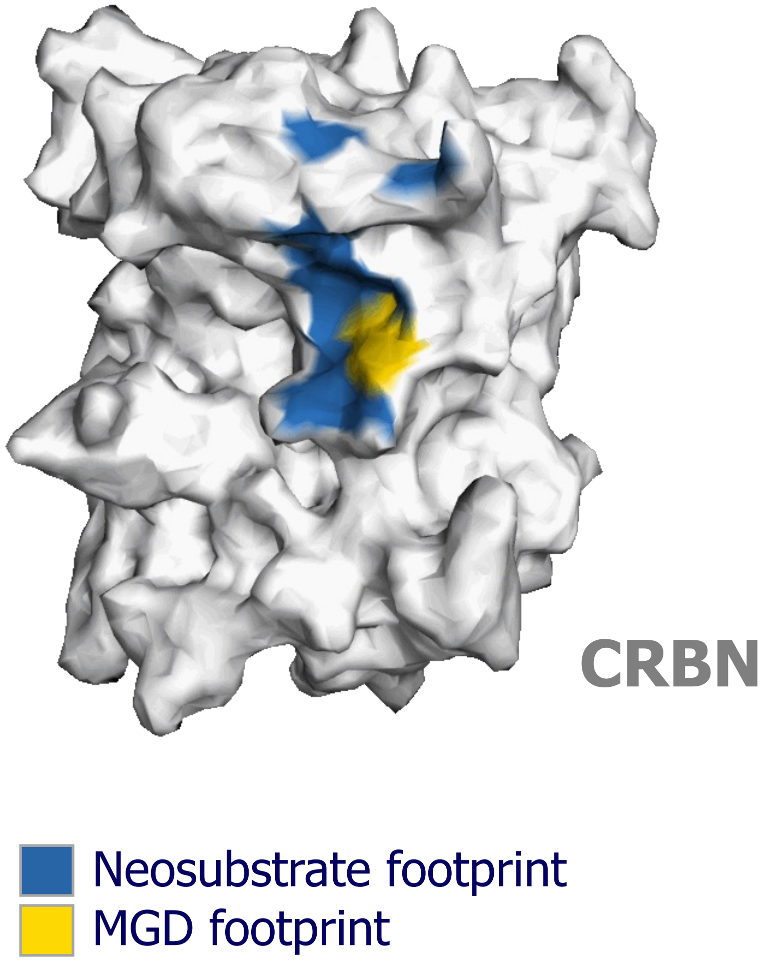
## Over 10% of human proteins contain a G-loop like structure, most in undruggable domains*




*Based on AlphaFold2 structures

# The E3 Ligase Neosurface Drives Neosubstrate Recruitment

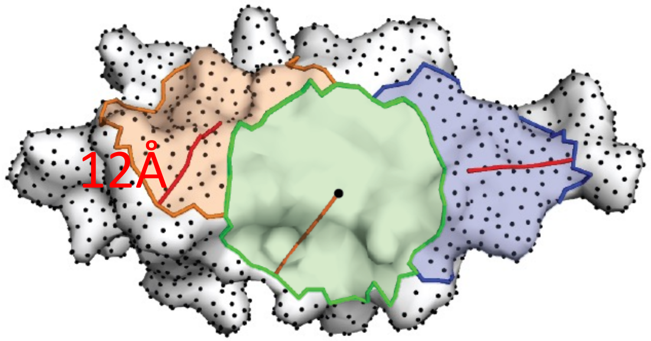
## The molecular surface is the best model to understand and predict neosubstrate interactions




- G-loops present a limited description of the CRBN target space.
- Rationally expanding chemistry creates diverse E3 ligase neosurfaces, enabling recruitment of new canonical and non-canonical targets
- Our geometric deep learning platform **fAIceit ™** effectively leverages surfaces to predict neosubstrates for CRBN and beyond.

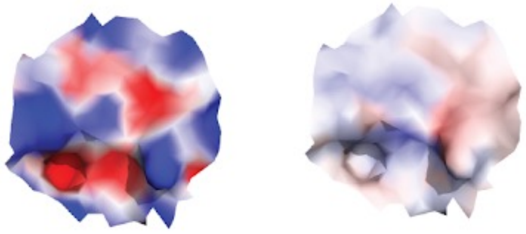
# Molecular Surface Interaction Fingerprints

## Geometric deep learning applied to protein surfaces




Patch

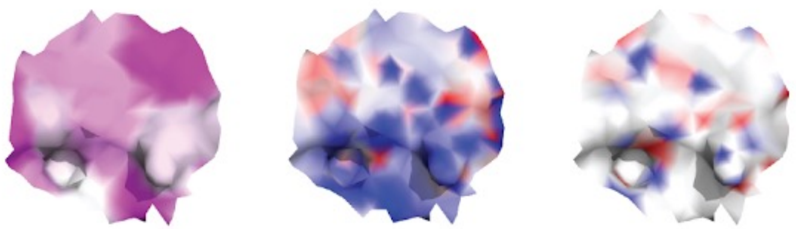
Geometric features



Shape index

Distance-dependent curvature

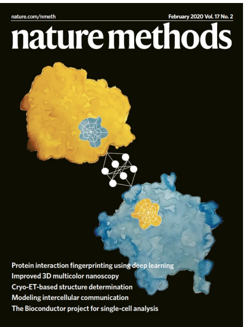
Chemical features



Hydropathy

Continuum electrostatics

Free electrons/ protons



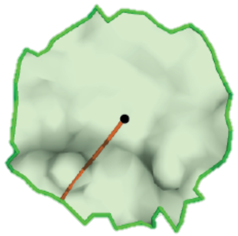

*Gainza et al. Nature Methods 2020*

# Molecular Surface Interaction Fingerprints

## Geometric deep learning applied to protein surfaces

Geometric features

Chemical features



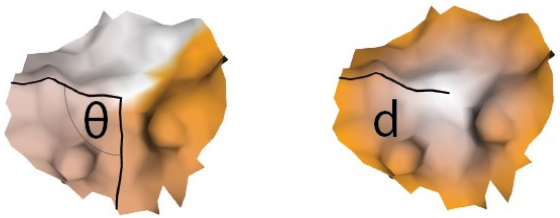
Shape index

Distance-dependent curvature

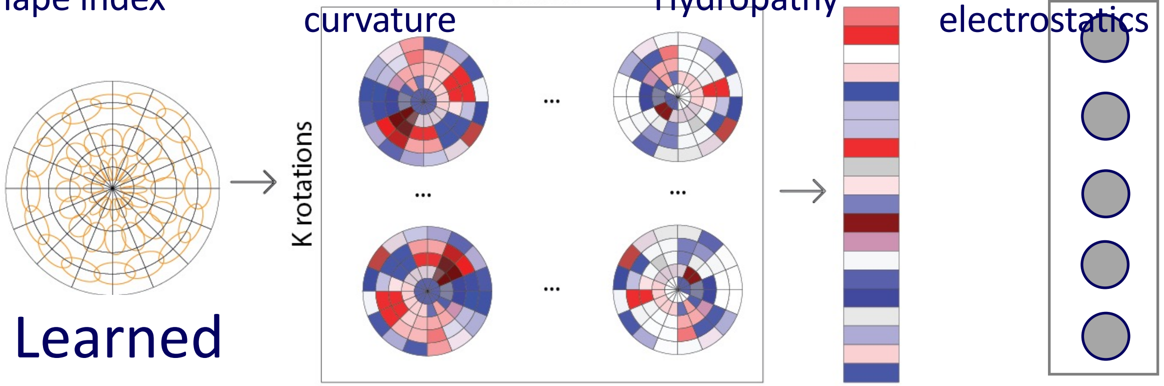
Hydropathy

Continuum electrostatics

Free electrons/ protons

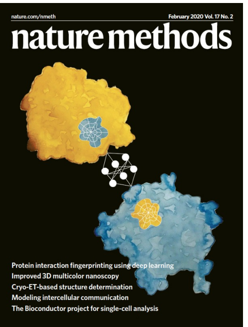



Geodesic polar coordinates




Learned polar grid 'soft pixels'

Task-specific layers

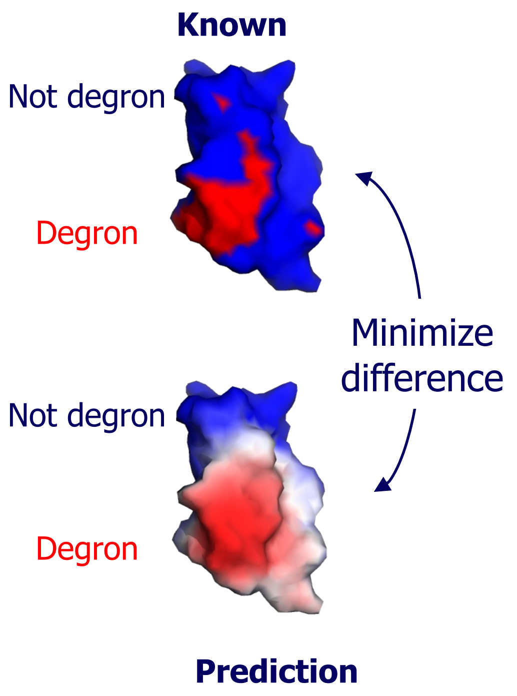


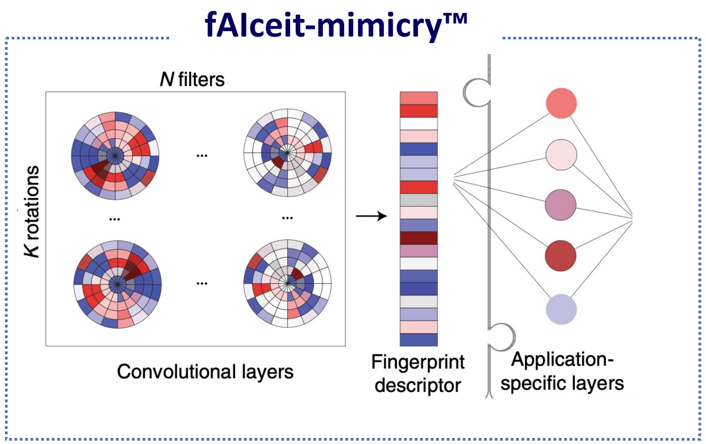

*Gainza et al. Nature Methods 2020*

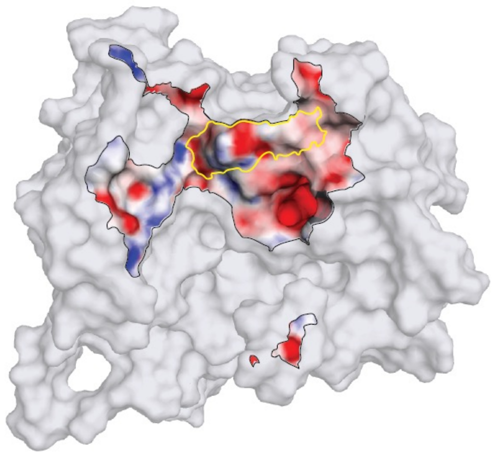
# Monte Rosa AI finds Degrons Using Surfaces

## Fast, proprietary algorithms tailored to molecular glue discovery

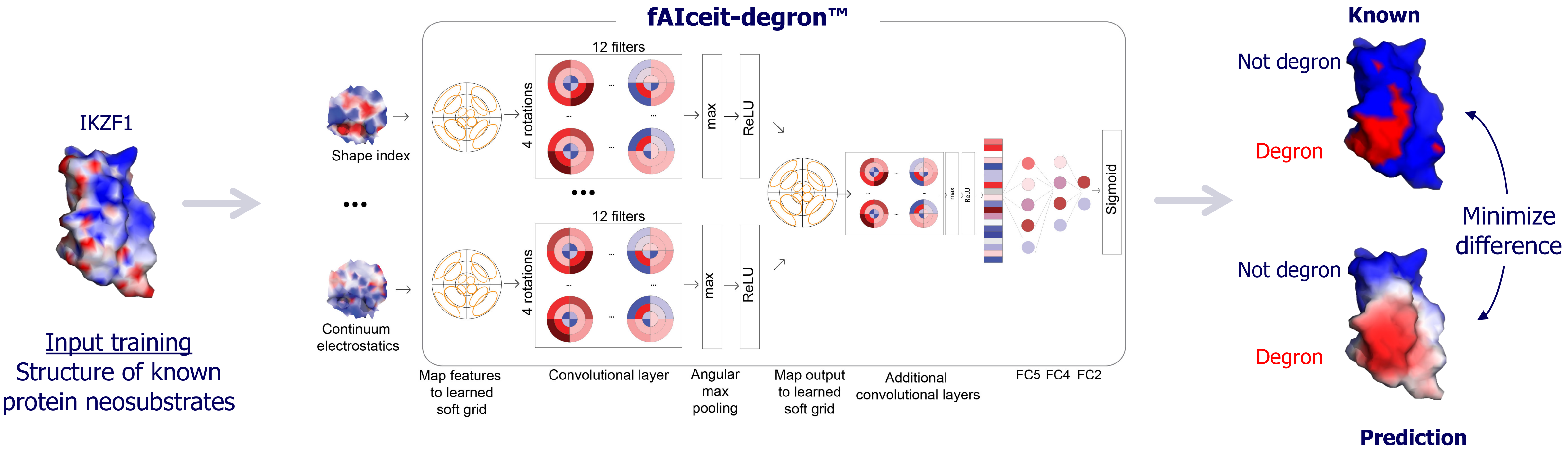
| **fAIceit -degron™** | **fAIceit -mimicry™** | **fAIceit -complementarity™** |
|---|---|---|
| Search for surface patches that match known degron interfaces, labeling degrons on targets | Ultra-fast fingerprint search for similar surfaces, finding surfaces that mimic known degron surfaces | Ultra-fast fingerprint search for complementary surfaces, such as for E3 ligase - neosubstrate matchmaking |



# fAIceit-Degron Optimized to Learn CRBN Degron Features From Known Degron Surfaces

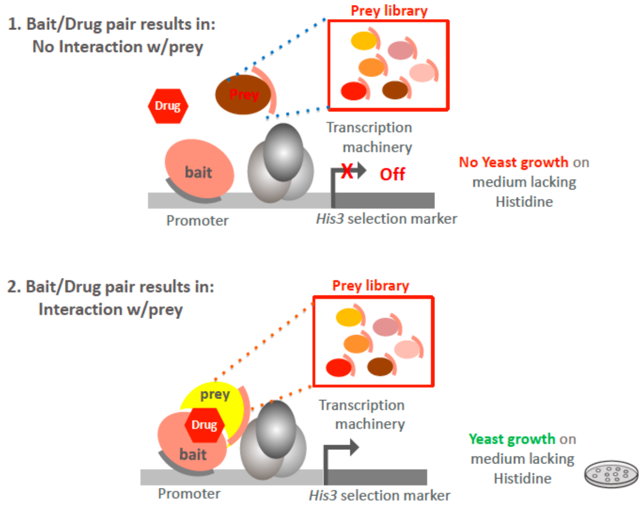



fAIceit-degron classifies protein surfaces for the presence of degrons.
fAIceit-degron creates a feature-rich surface characterization and uses 3 layers of geodesic convolution with deep vertexes to classify input surfaces.

# Validating fAIceit-degron on Novel Degrons (not in Training Data)

**Yeast-3-hybrid proximity assay**

Identifies MGD-induced interactions between CRBN and cDNA library-derived targets. Maps degrons to individual domains



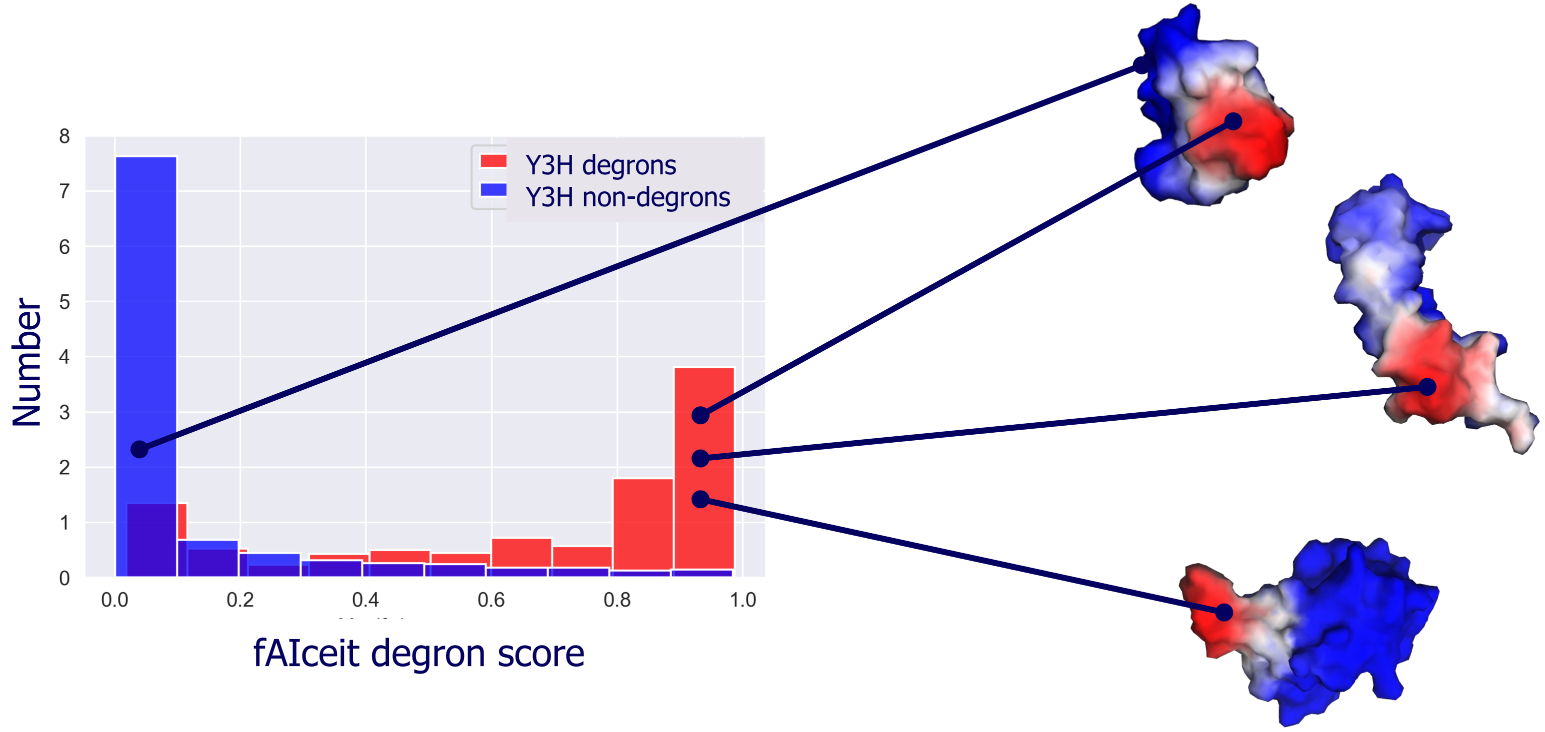

With Hybrigenics

**Benchmarking fAIceit with Y3H**

Y3H experiments identify 8 novel G-loops from 5 distinct domain classes

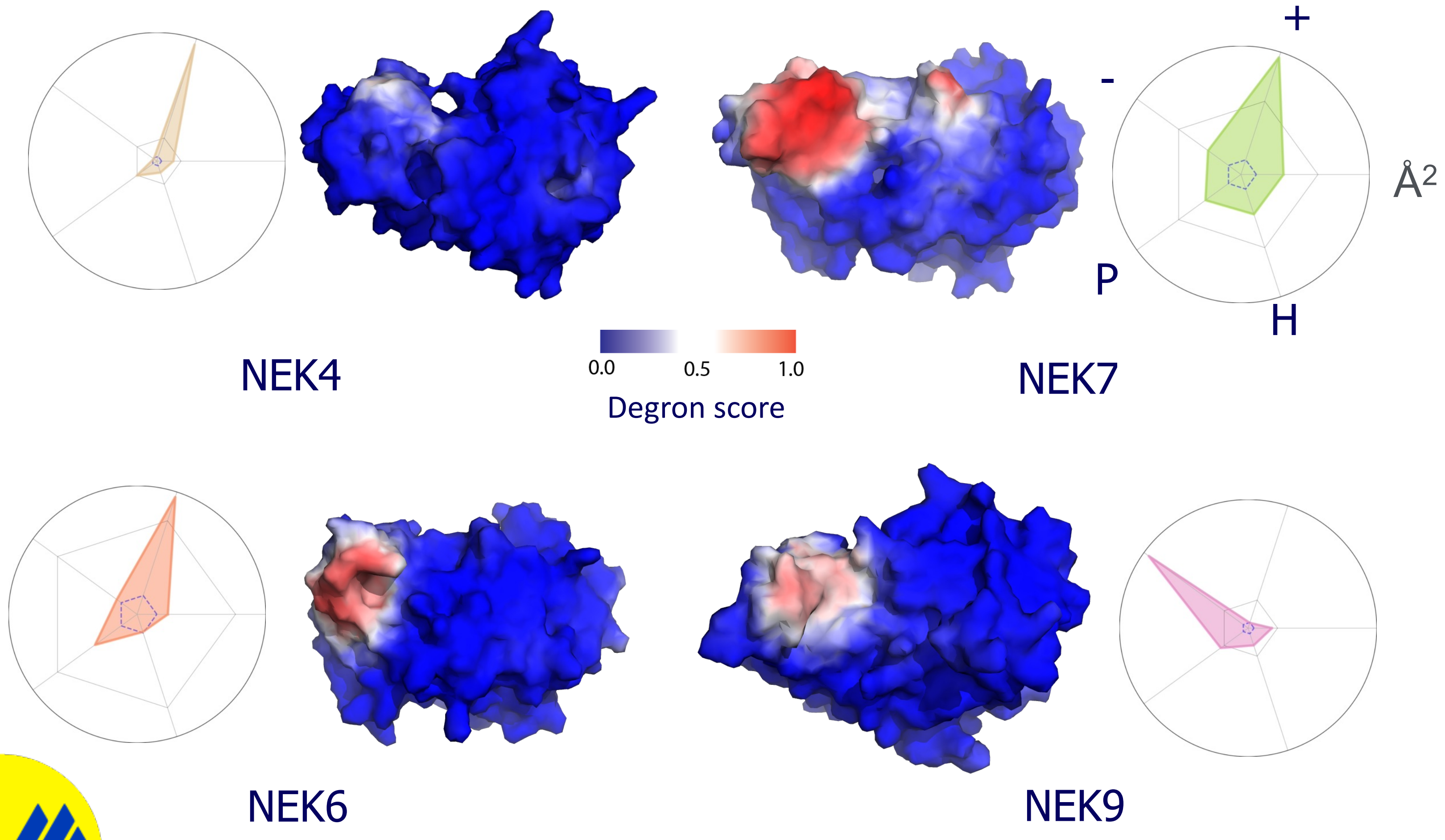
**Y3H and fAIceit results agree**

Y3H results agree with fAIceit degron predictions

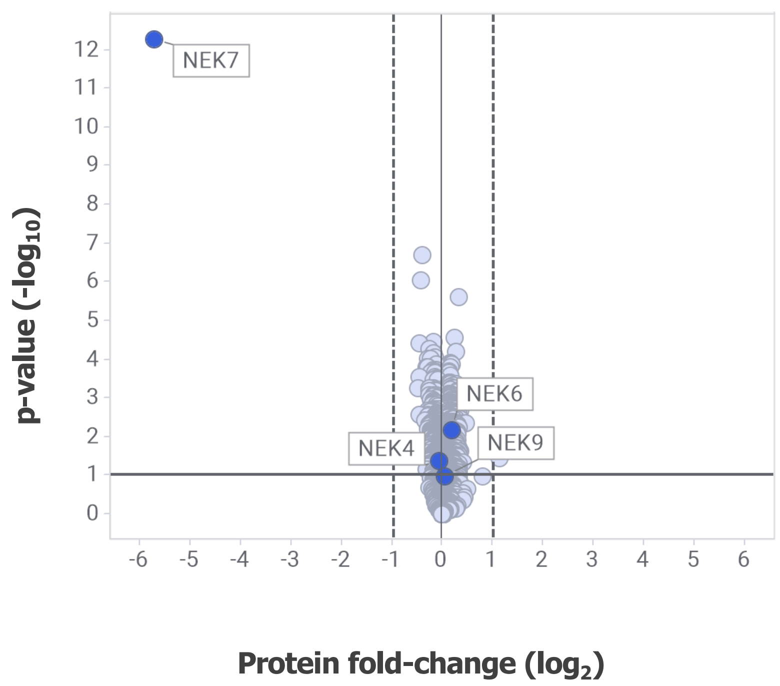


# fAIceit-degron Finds and Characterizes Degron Surfaces

## NEK7 has a unique G-loop surface, enabling selective MGD degradation




**MGD-induced NEK7-selective degradation**

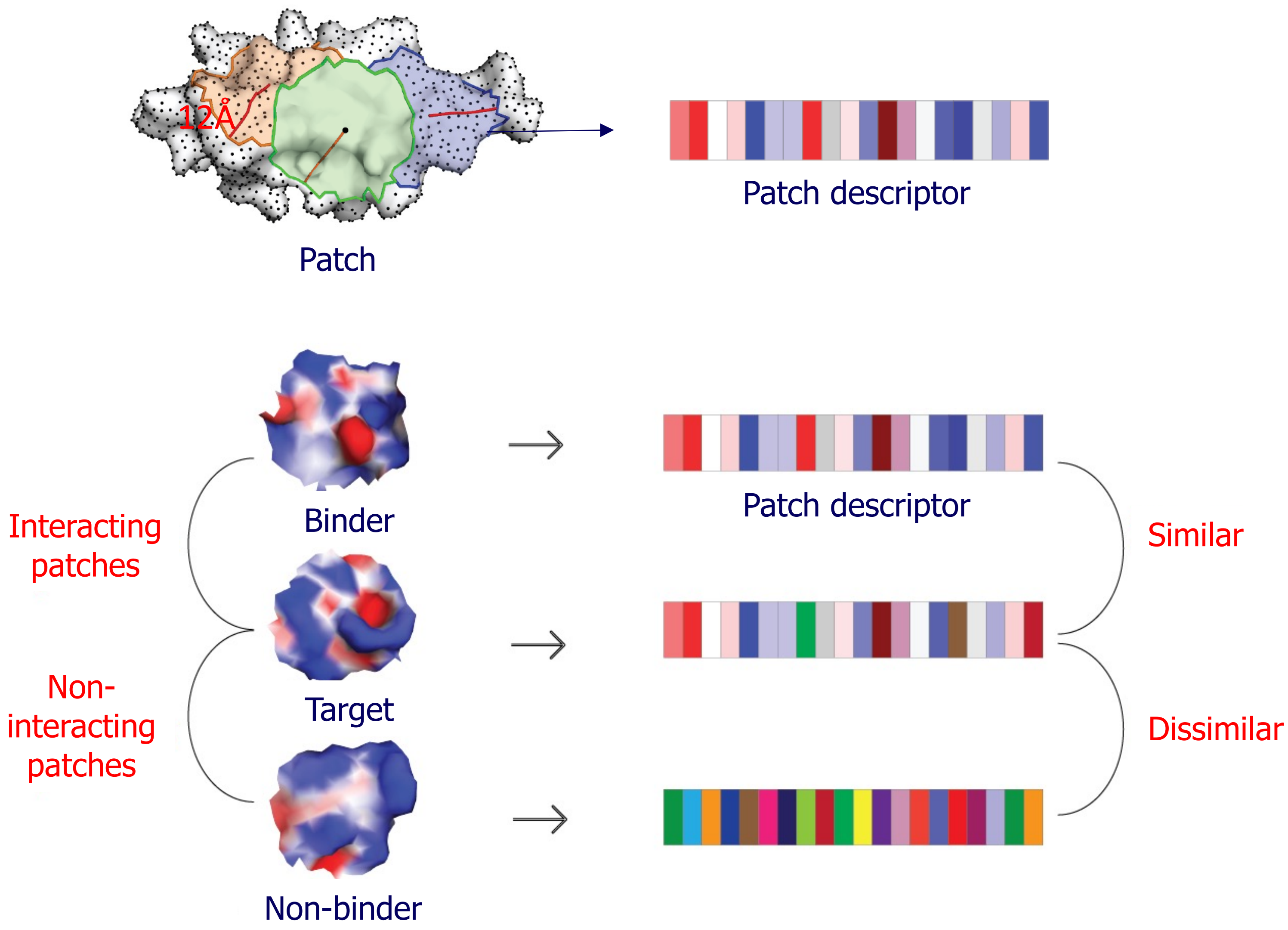



Mass-spectrometry TMT proteomics
U937 24hr post treatment

Å² degron area; P polarity; H hydrophobicity; + positive area; - negative area

# Encoding Protein Surfaces as Fingerprints

## Enables ultra-fast, proteome-wide search for similar & complementary fingerprints



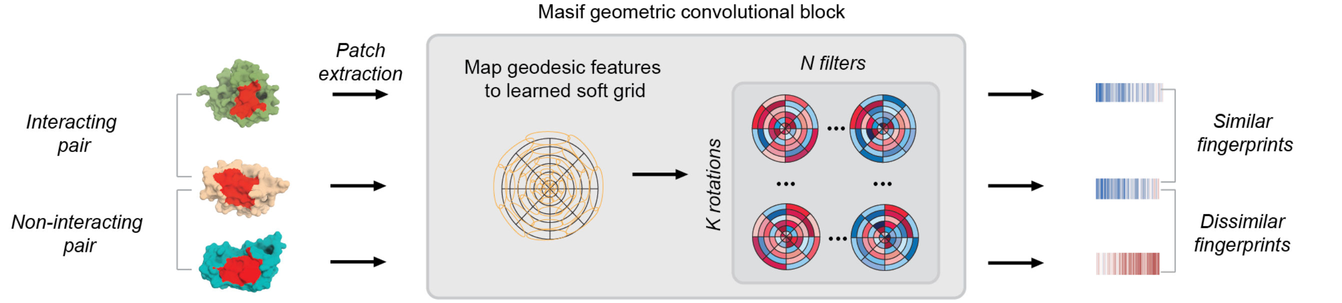

*Gainza et al. Nature Methods 2020*

# Fingerprint Encoding Enables Ultra-fast Search

## Proteome-wide queries of complementary (or similar!) surfaces

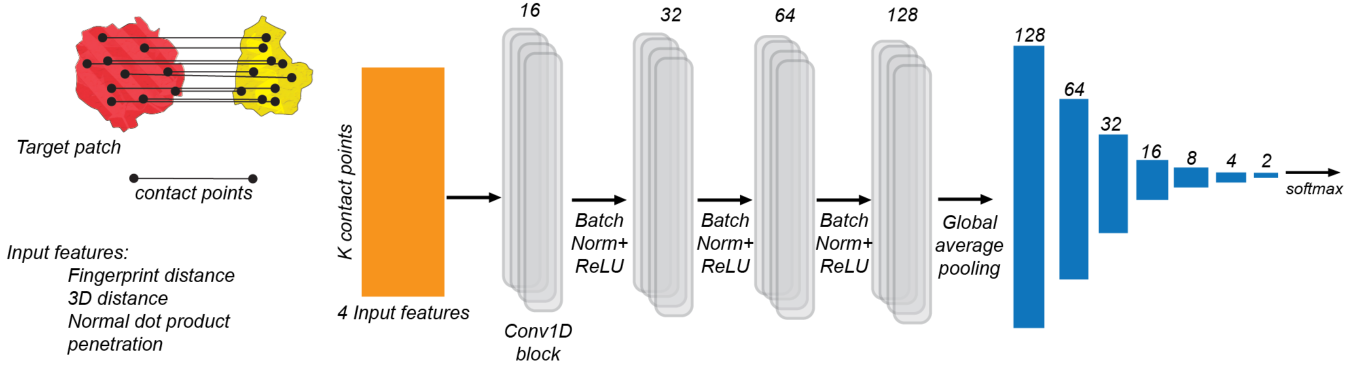
### Searching using surface fingerprints

1. Encode surfaces as fingerprints




2. Align fingerprints using RANSAC

3. Score post-alignment interface using neural network




### Fingerprint matching is blazingly fast

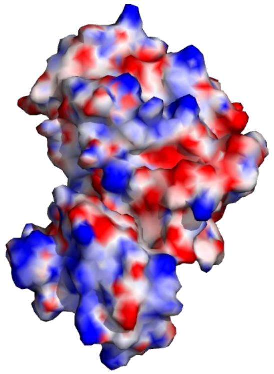
| Method | | MaSIF-search | Zdock + ZRank2 |
|---|---|---|---|
| # solved complexes in top | 100 | 67 | 77 |
| | 10 | 56 | 63 |
| | 1 | 43 | 45 |
| Time (min) | | 39 | 159,902 |

>4,000x (!!) faster than state-of-the-art docking tools

*Gainza et al. Nature Methods 2020*

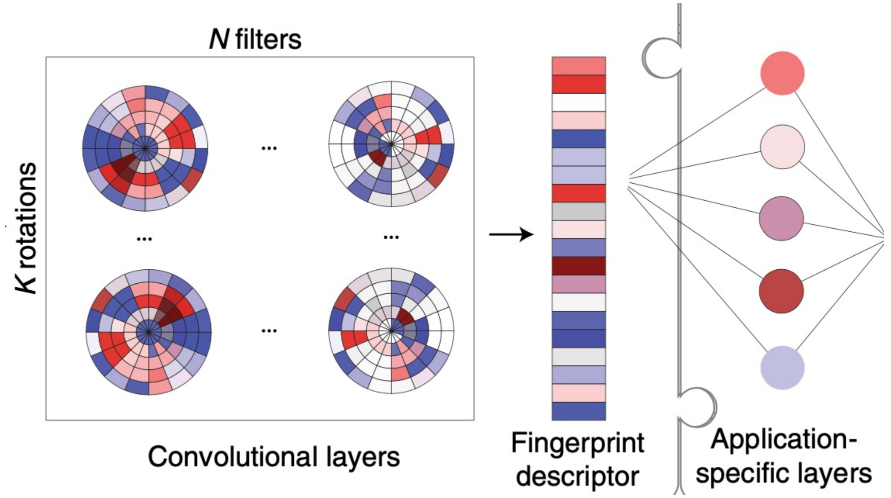
# Proteome-wide Fast Matching of Degron Surface Mimics

## Match surface fingerprints, not G-loops

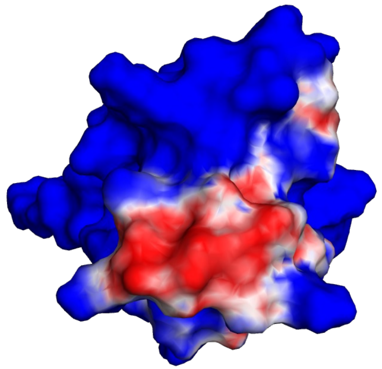


*billions of patches*

**fAIceit-mimicry™**




*scored surfaces*

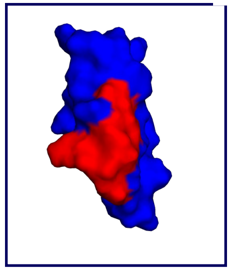


Input
human proteins

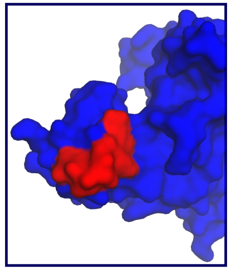
Output
degron mimics

*Match surfaces*

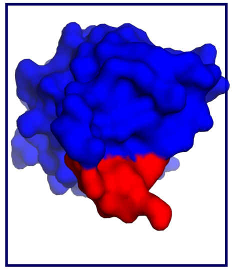
Known **degron** surfaces



IKZF1



CK1a

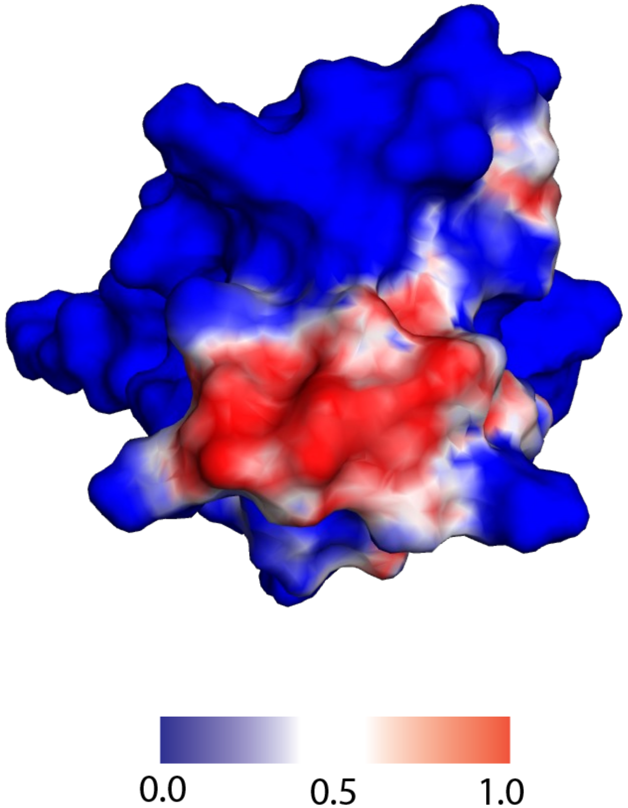


GSPT1

...

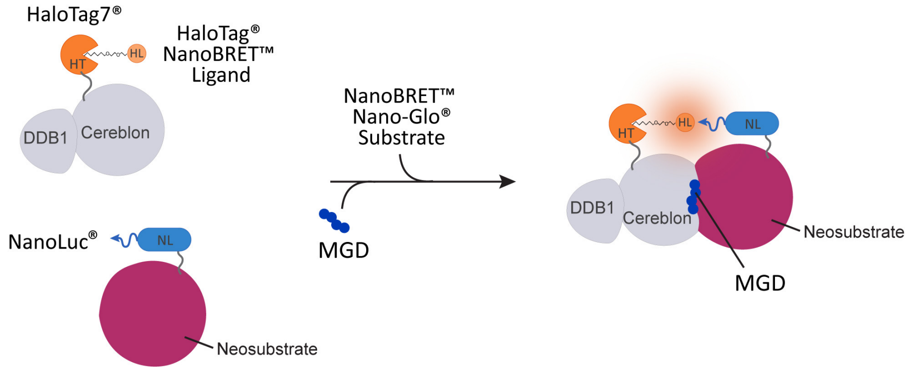
# fAIceit-Mimicry Search Identifies Novel Degrons

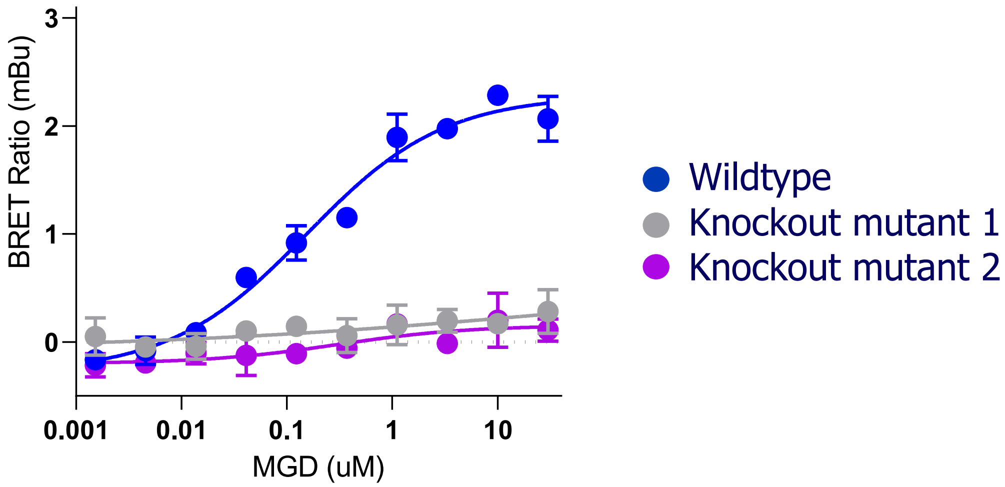
**A predicted non-hairpin, non-canonical degron in an established oncology target**




Surface similarity to C2H2 ZF degron

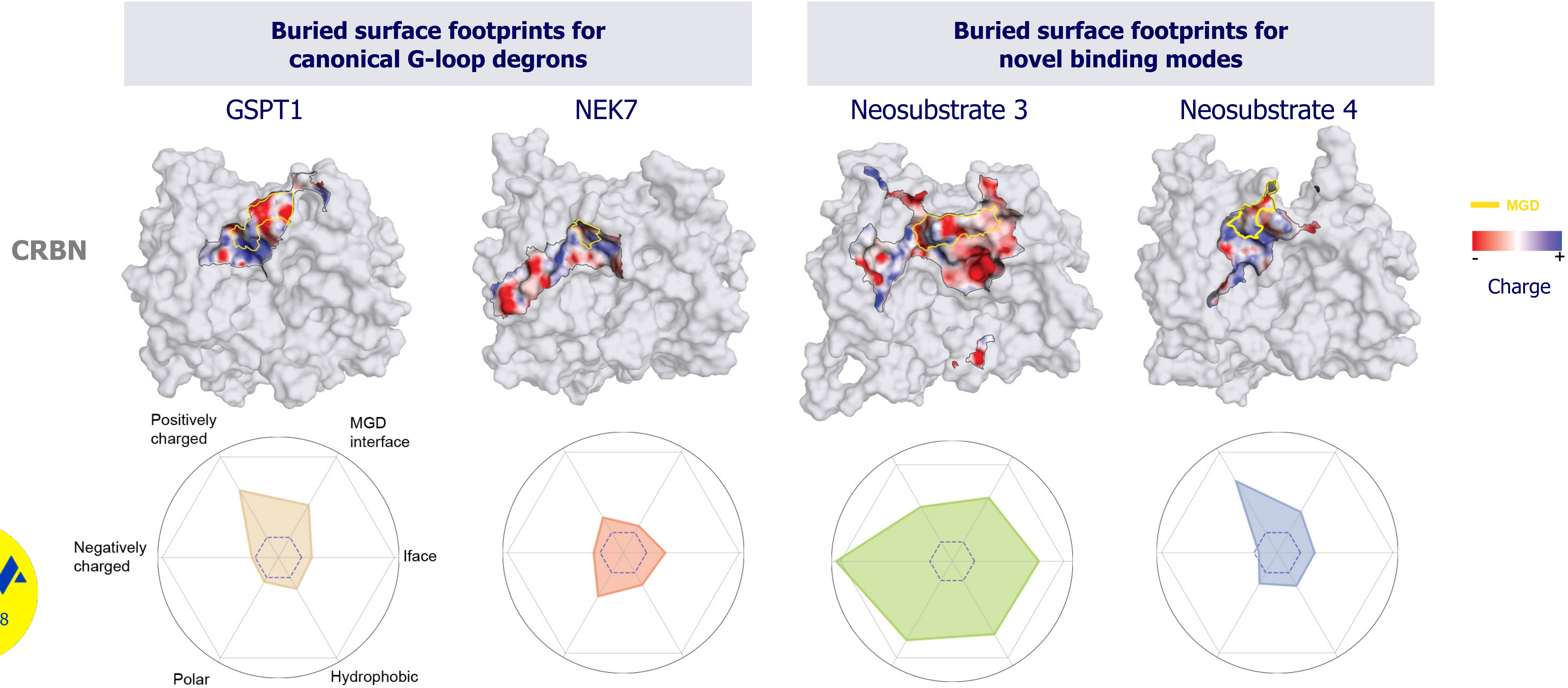
**NanoBRET confirms prediction and binding mode**



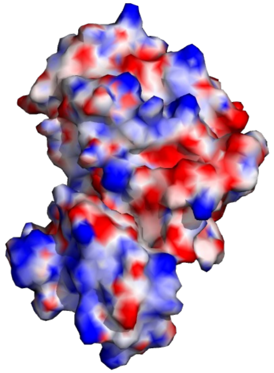
# E3 Ligase Neosurface Footprint Defines the Target-Complementary Surface

## The neosurface can be used to find novel neosubstrates



# fAIceit-Complementarity Finds Proteins Complementary to E3 Ligases

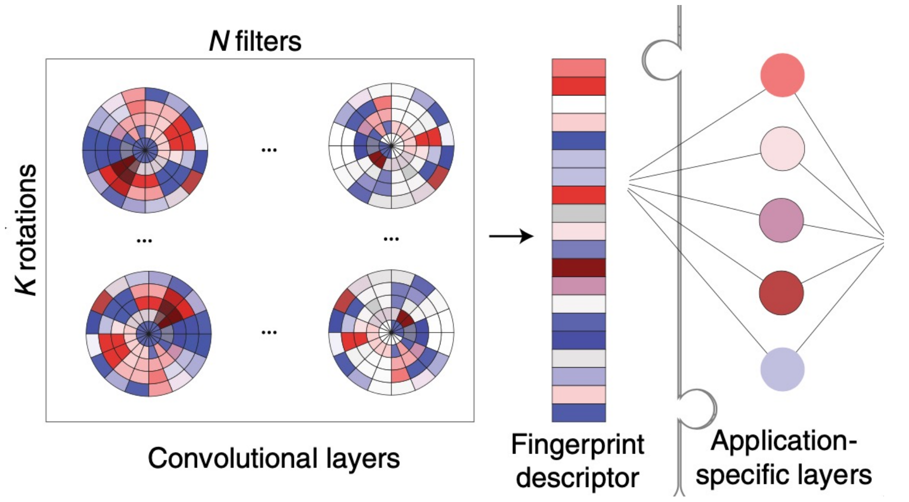
## The E3 Ligase footprint is encoded as a fingerprint for fast E3-target matchmaking



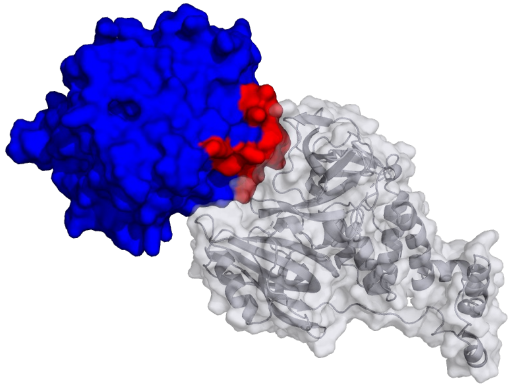
Input
human proteins

*billions of patches*

**fAIceit-complementarity™**



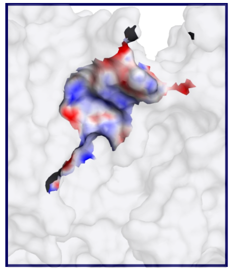

*scored surfaces*



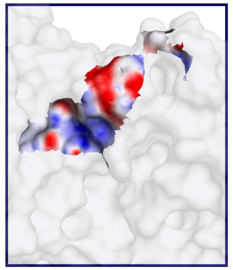
Output
degrons engaged with novel binding modes

*Complement match*

E3 ligase surface footprint

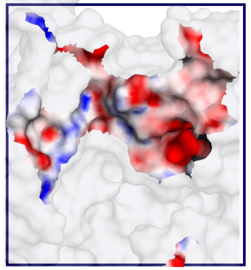


$MGD_1$



$MGD_2$

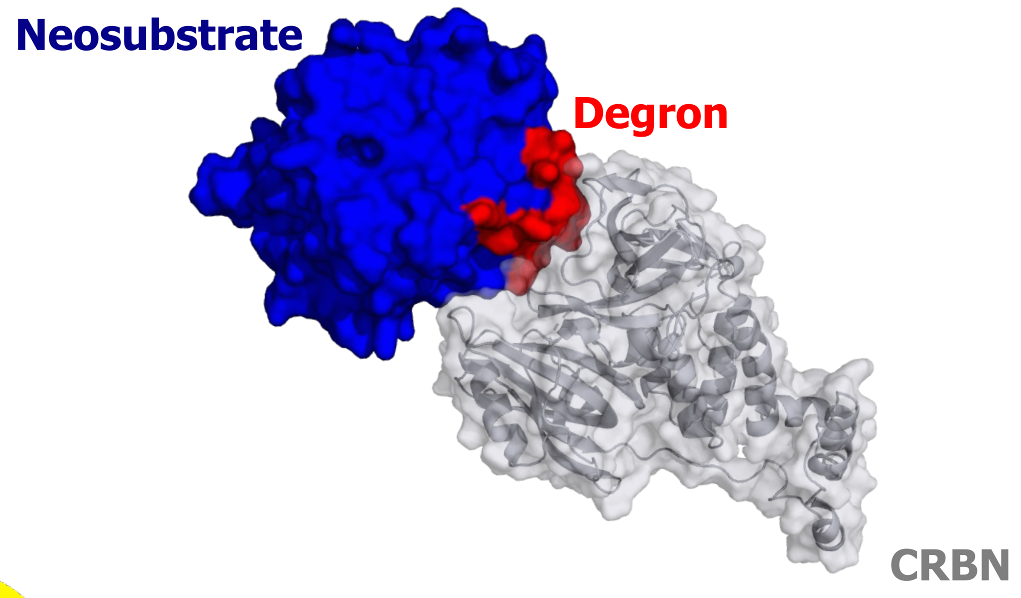
...



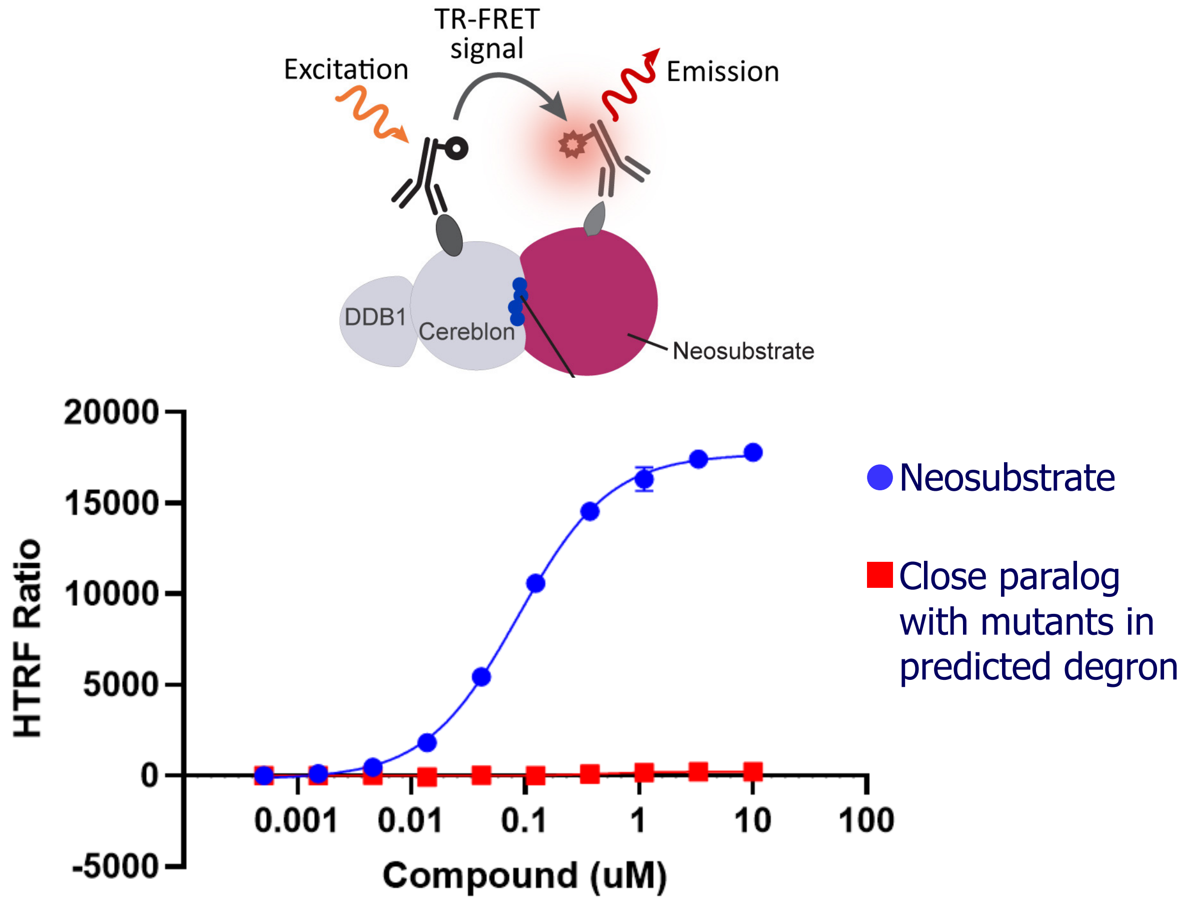
$MGD_N$

# fAIceit-Complementarity Expands Target Space to Non-Canonical Degrons

**Predicted novel non-canonical degron with CRBN surface complementarity**



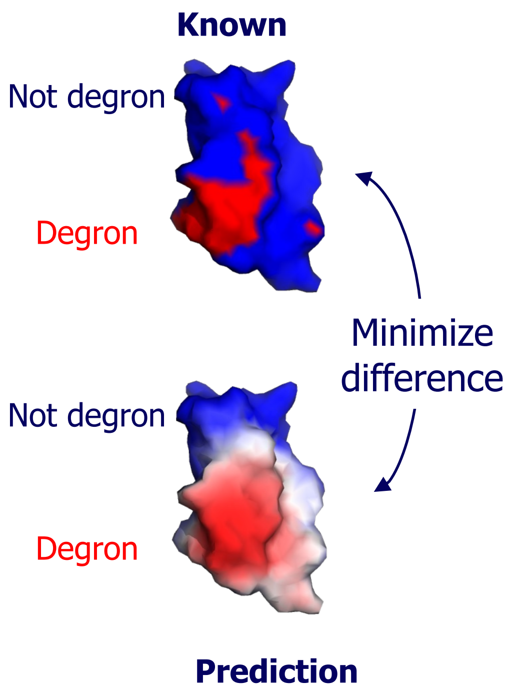

**HTRF confirmation**

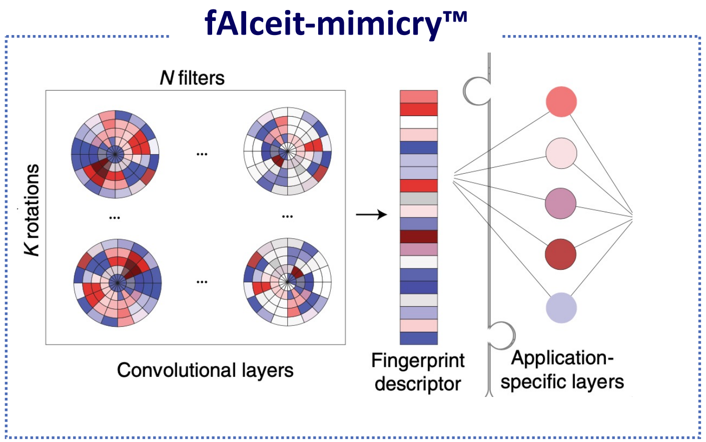


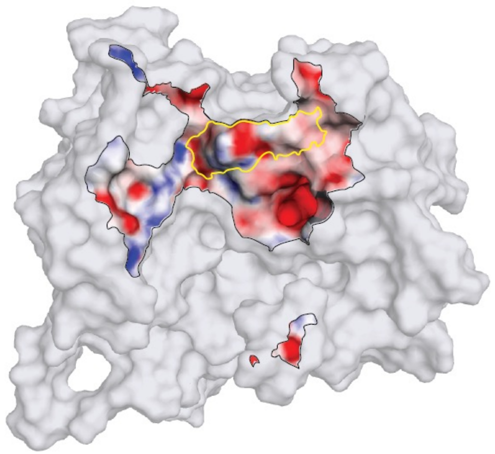
# Monte Rosa AI finds Degrons Using Surfaces

## Fast, proprietary algorithms tailored to molecular glue discovery

| fAIceit-degron™ | fAIceit-mimicry™ | fAIceit-complementarity™ |
| --- | --- | --- |
| Search for surface patches that match known degron interfaces, labeling degrons on targets | Ultra-fast fingerprint search for similar surfaces, finding surfaces that mimic known degron surfaces | Ultra-fast fingerprint search for complementary surfaces, such as for E3 ligase - neosubstrate matchmaking |




Thank you
Monte Rosa Therapeutics