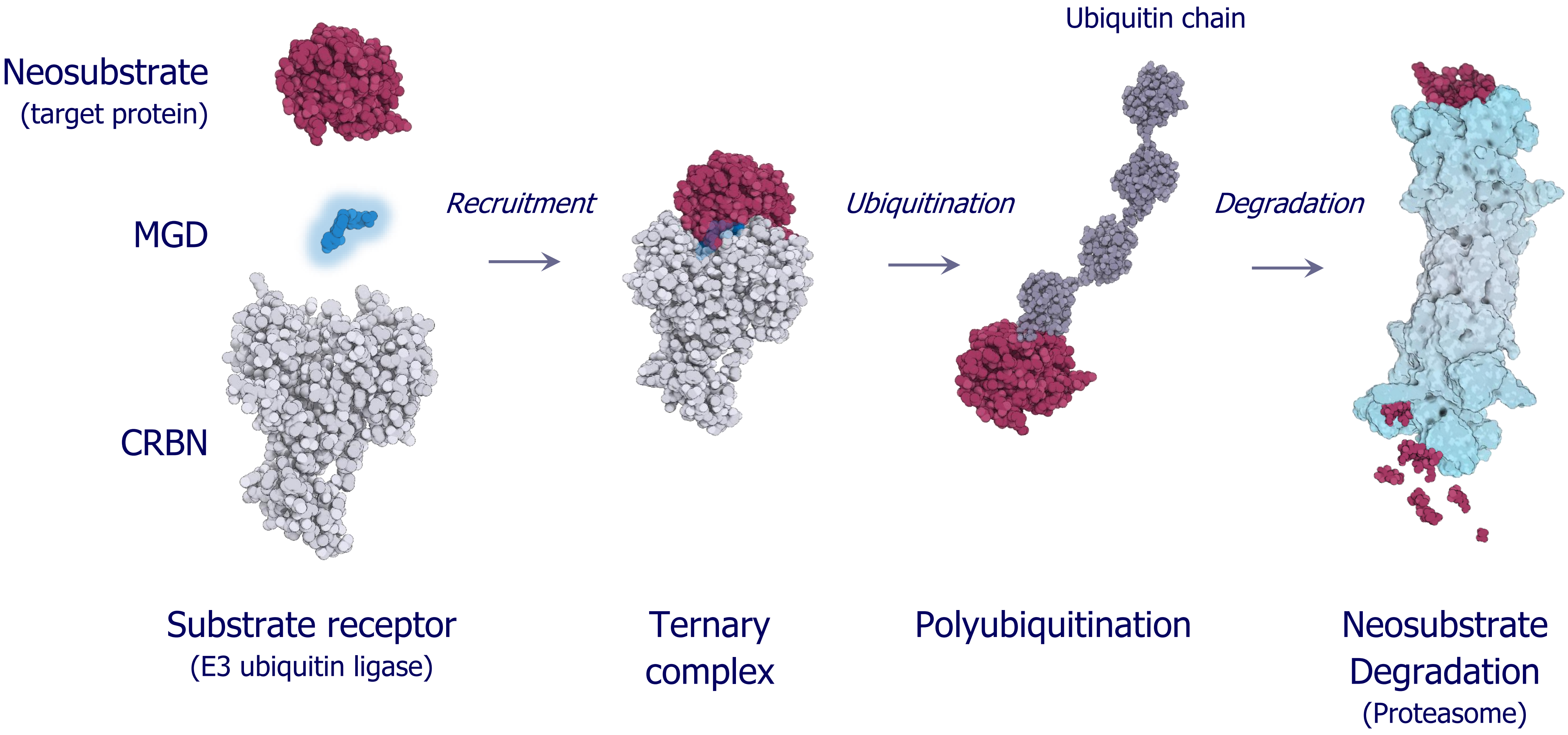
Mining the CRBN Target Space Redefines Rules of Neosubstrate Engagement

Georg Petzold | Keystone Symposia – Proximity-Based Therapeutics | Feb 17th, 2025

Monte Rosa Therapeutics

Molecular Glue Degraders (MGDs) Facilitate Targeted Protein Degradation


Neosubstrate
(target protein)

MGD

CRBN

Recruitment →

Ubiquitination →

Ubiquitin chain

Degradation →

Substrate receptor
(E3 ubiquitin ligase)

Ternary
complex

Polyubiquitination

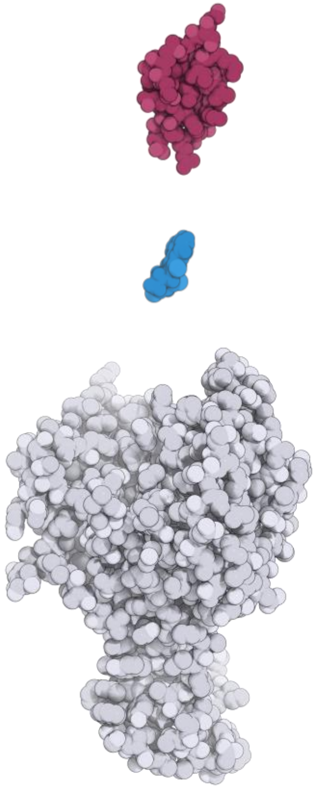
Neosubstrate
Degradation
(Proteasome)

A Rational Approach to Unleash the Full Potential of Molecular Glue Degraders

Neosubstrate
(e.g. IKZF1)

MGD
(e.g. Lenalidomide)

CRBN

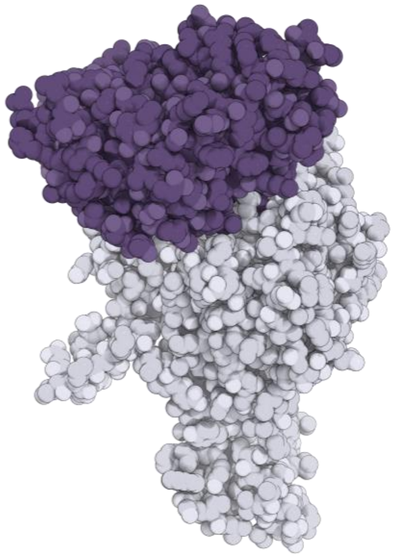


→

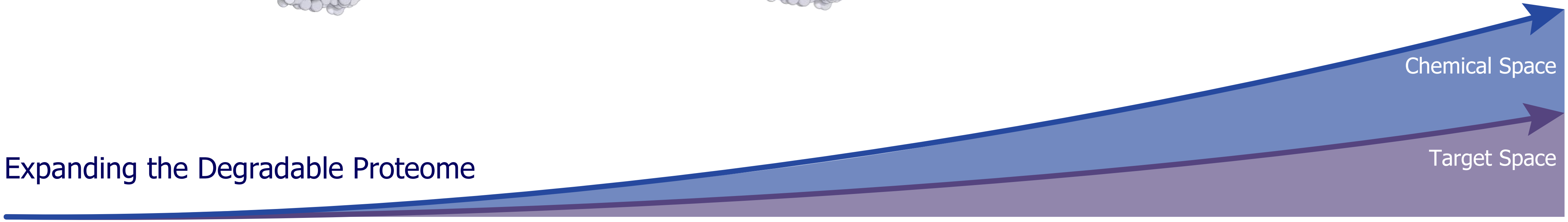
Unraveling the Canonical Degrome

→

Beyond the Canonical Degrome

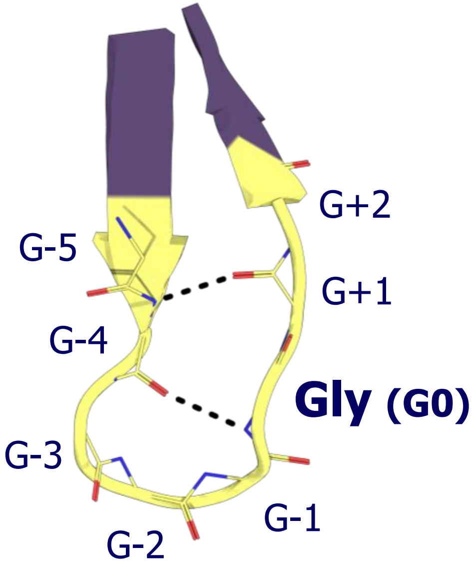


Expanding the Degradable Proteome



Exploring the Canonical Degron Space – Leveraging the G-loop Motif

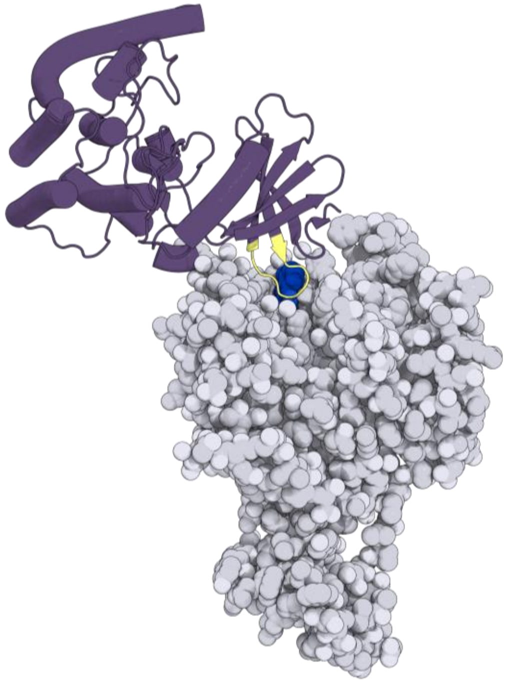
The β-hairpin G-loop



CK1a	INITN**G**EE
GSPT1	VDKKS**G**EK
ZNF692	QCEIC**G**FT
SALL4	VCSVC**G**HR
IKZF2	HCNQC**G**AS

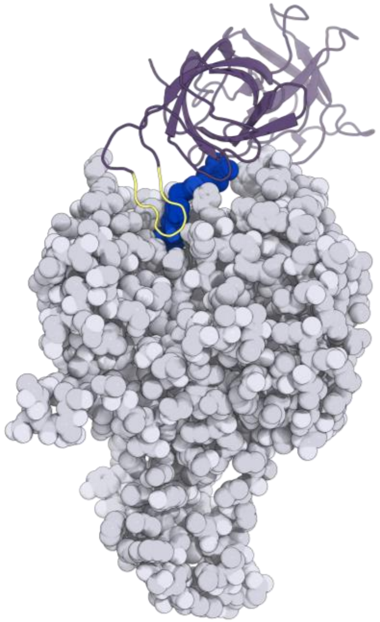
A generic structural motif present in different domain types

CK1α



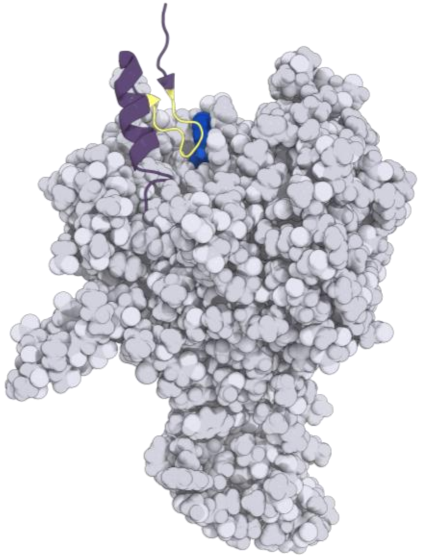
Petzold et al. 2016

GSPT1



Matyskiela et al. 2016

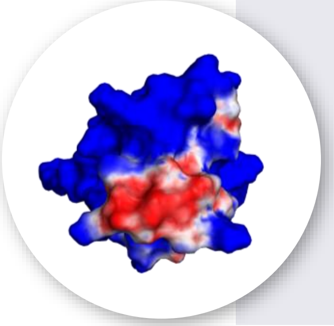
C2H2 ZFs



Sievers/Petzold et al. 2018

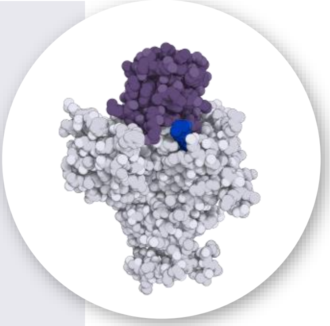
QuEEN™ - Quantitative and Engineered Elimination of Neosubstrates

Breakthroughs enabling rapid discovery of potent, selective, and oral MGDs



AI/ML

In silico discovery using proprietary AI-powered algorithms

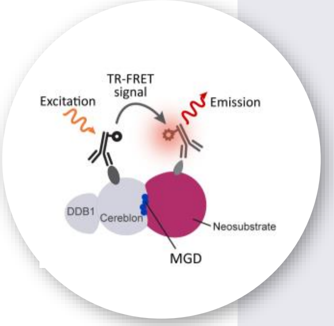


Structure-based Design

Proprietary database of protein structures to enable rapid optimization of MGD chemistry

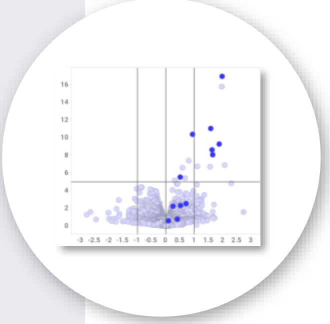
MGD Library

Growing 50K compound library for novel degron and target space exploration



Proximity Screening

Specialized suite of biochemical, cellular and proteomics assays to assess proximity and degradation in high throughput

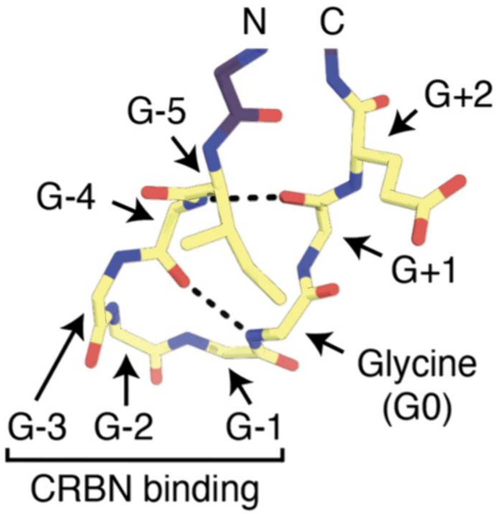


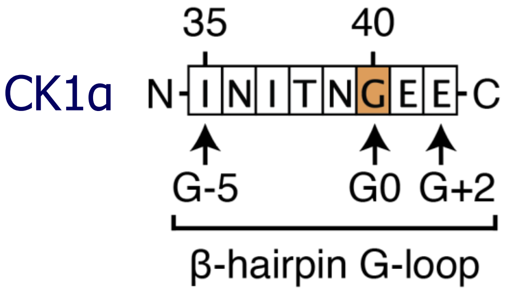
Proteomics

Integrated proteomics engine and database to identify novel targets and explore cellular complex formation and protein degradation

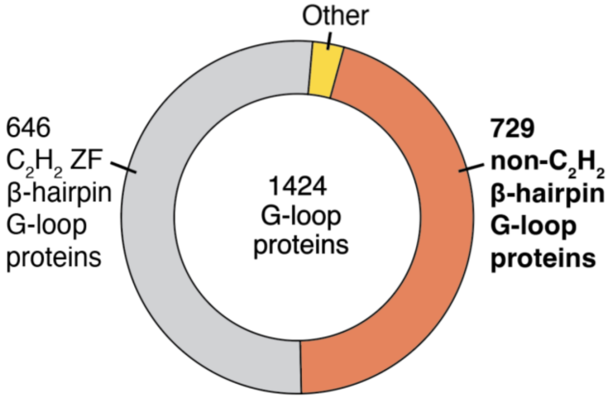
QuEEN™ – Predictions of β-hairpin G-loop Motifs in the Human Proteome

β-hairpin G-loop template

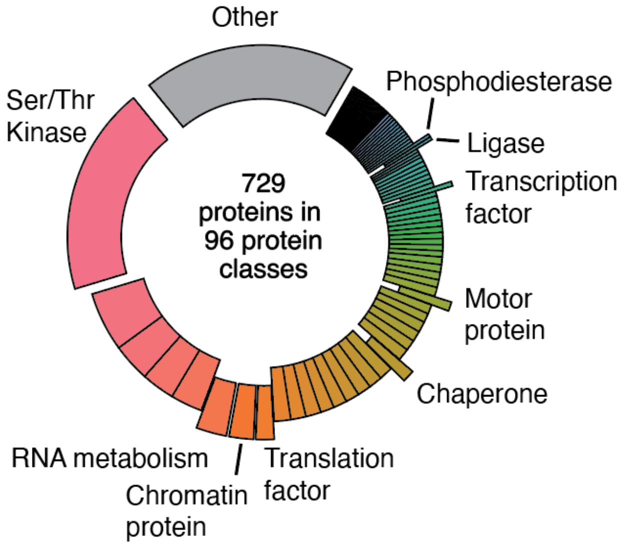




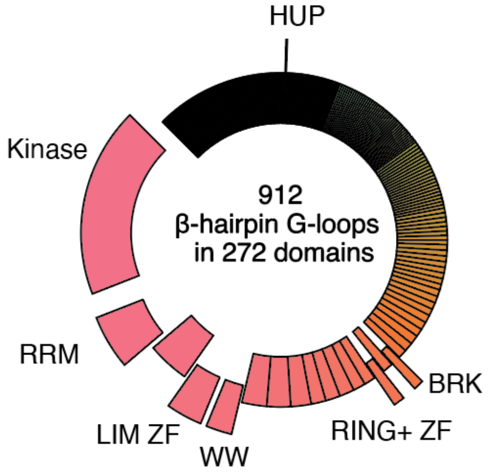
Full length proteins



Protein classes



Domain types



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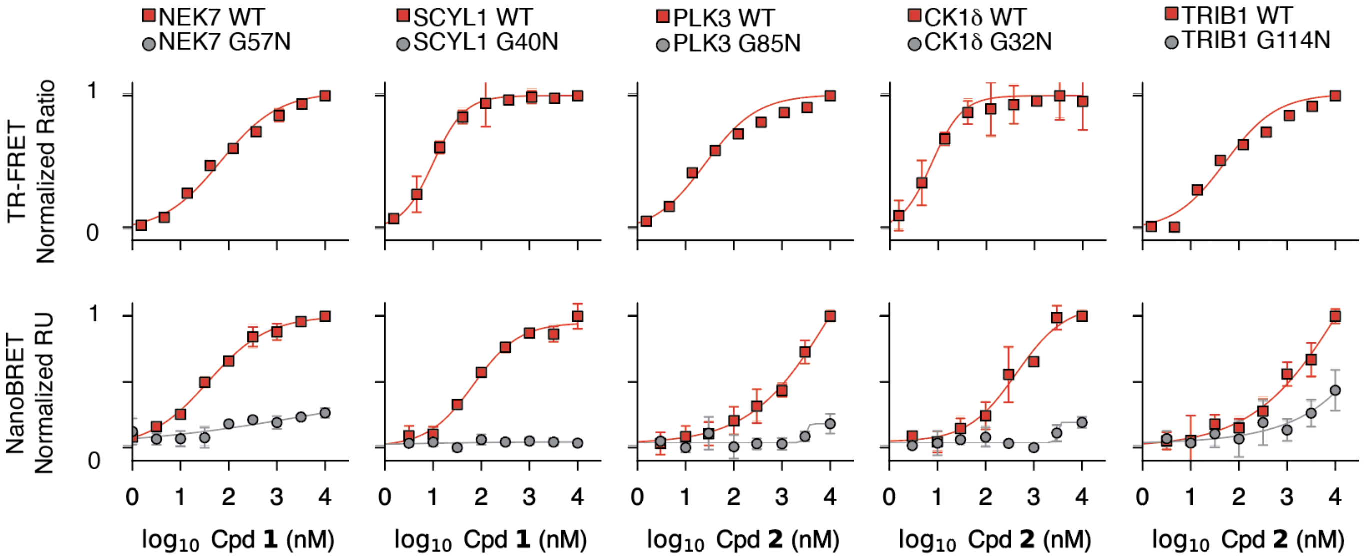
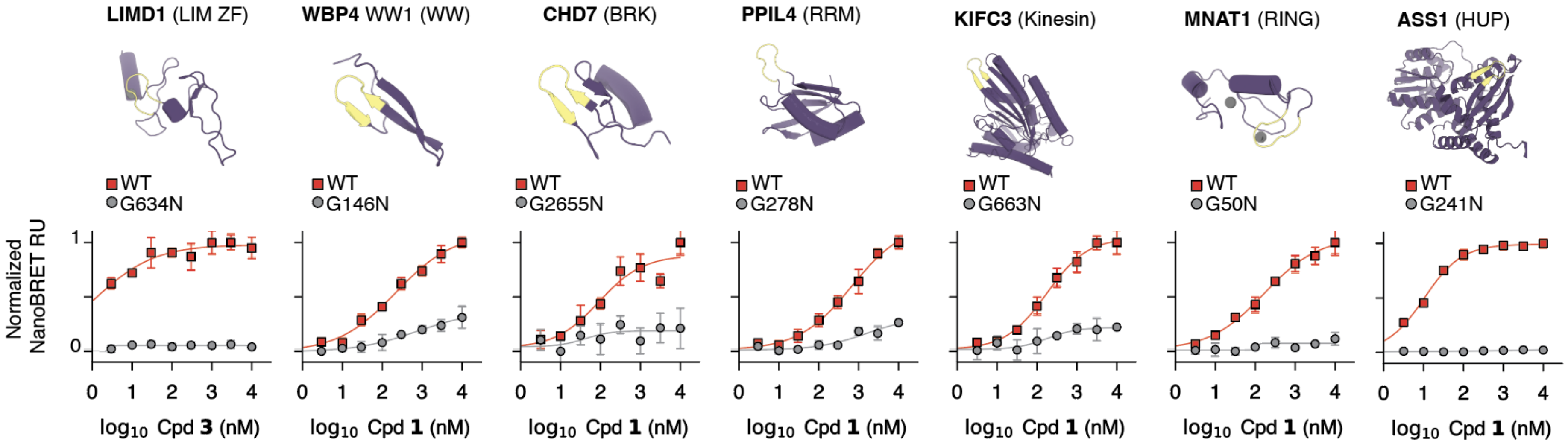
QuEEN™ – Experimental Validation of β-hairpin G-loop Predictions

TR-FRET
(in vitro)

NanoBRET
(in cells)



Predicted kinases



Novel domain types

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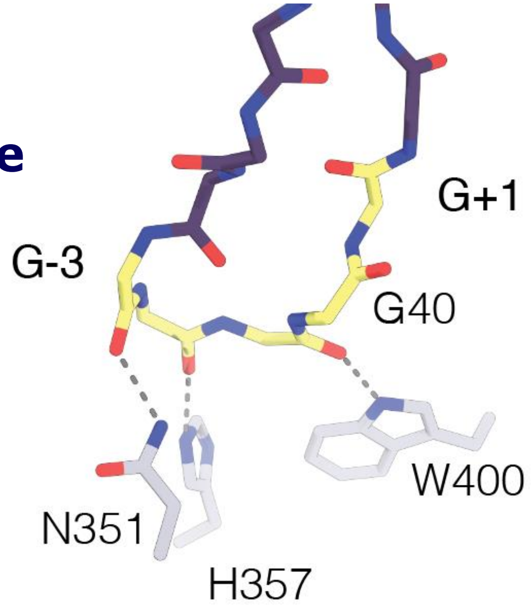
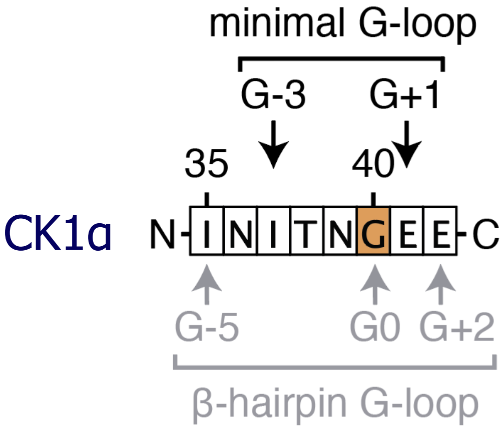
QuEEN™ – Minimal G-loop Motifs Discover New Degrons

Minimal G-loop template

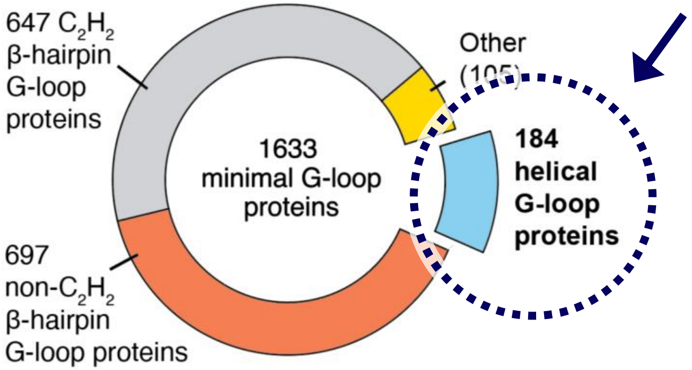


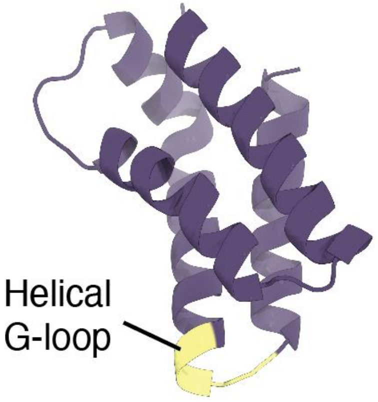
CRBN



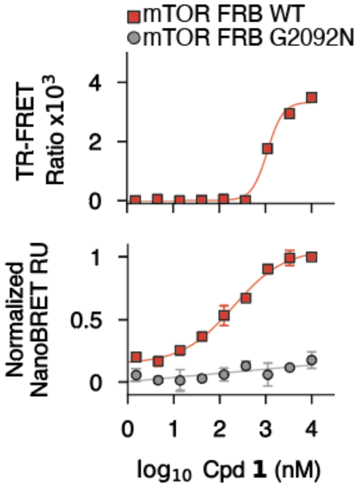
AI/ML

New category:
'Helical G-loops'





mTOR FRB



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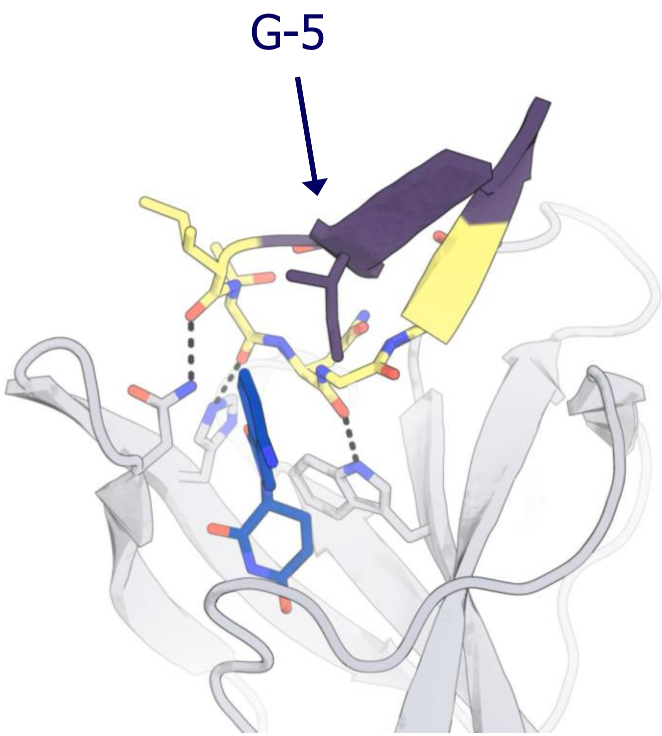
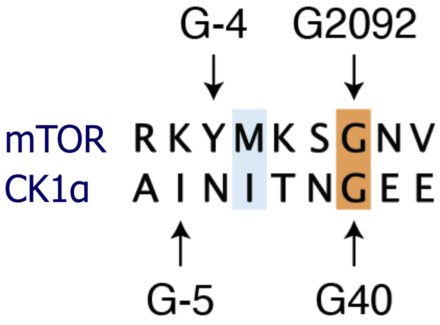
Helical G-loops are Structurally Differentiated from β-hairpin G-loops

β-hairpin G-loop
(1,424 predicted)

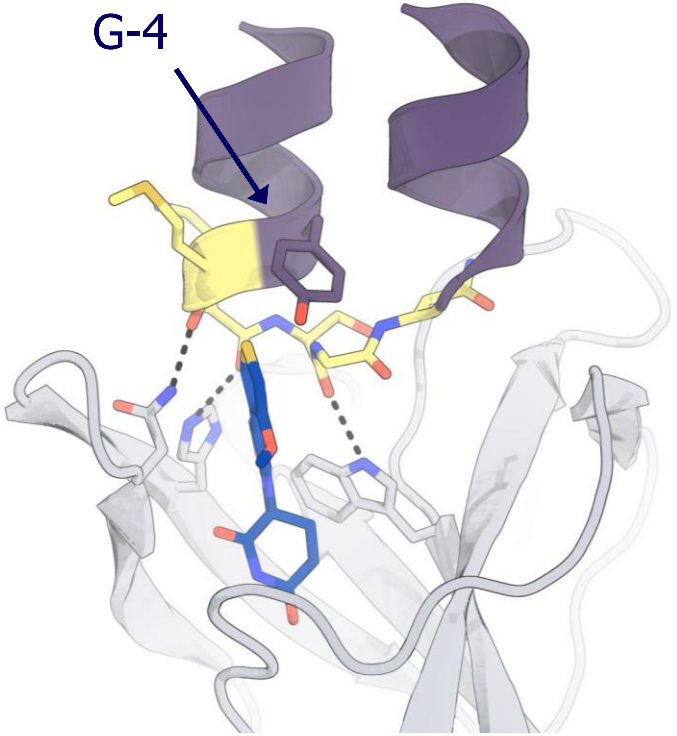
CK1α





Helical G-loop
(184 predicted)

mTOR FRB



G-loop mining creates a meaningful resource: **the G-loop catalogue**

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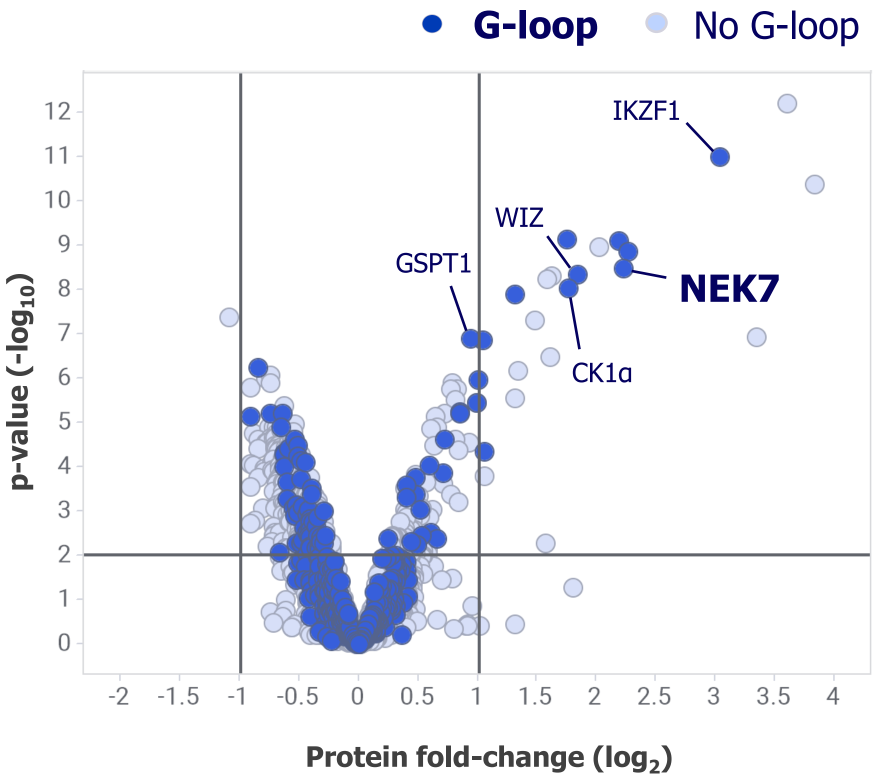
QuEEN™ – Usage of the Computationally Mined G-loop Catalogue

Proximity-ligation
(Cpd **1**)



TurboID-CRBN (U937)

NEK7:

- Immunology target
- Licenses inflammasome assembly
- Kinase-independent scaffolding function

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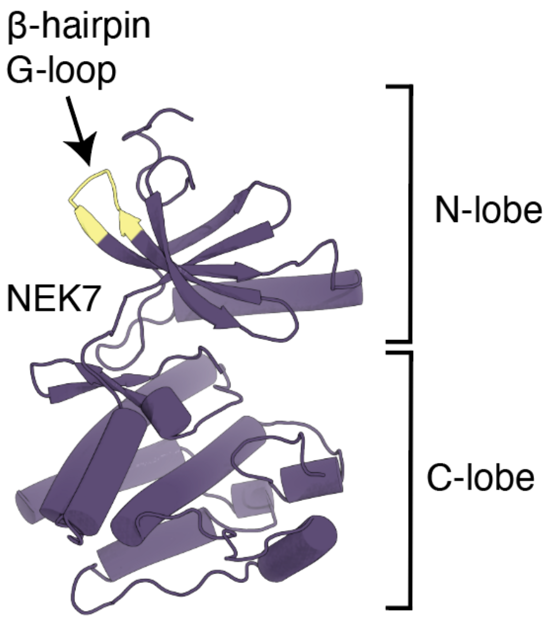
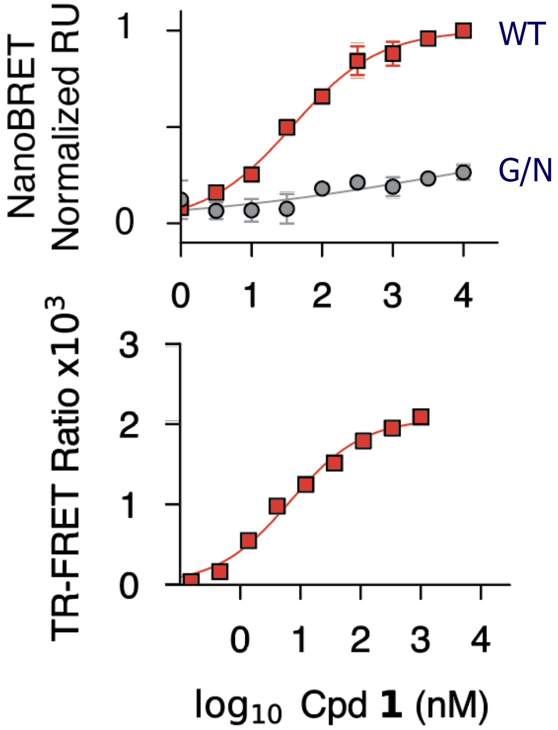
From Promiscuous Recruitment to Potent and Selective NEK7 Degradation



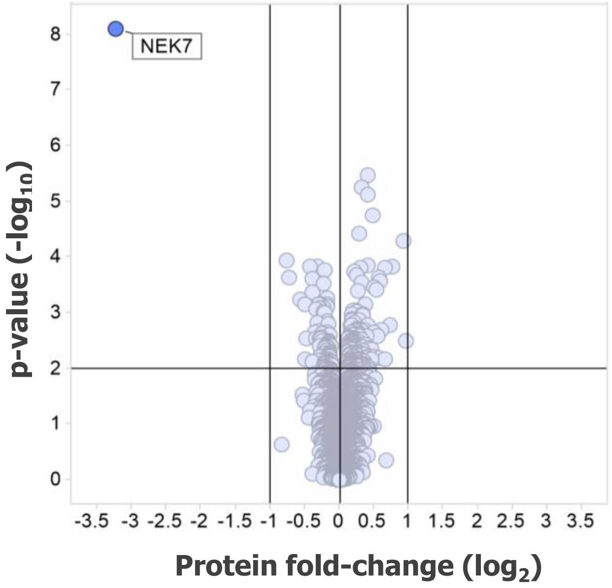
NEK7 recruitment, but no degradation with Cpd **1**



Library Screen

Med Chem

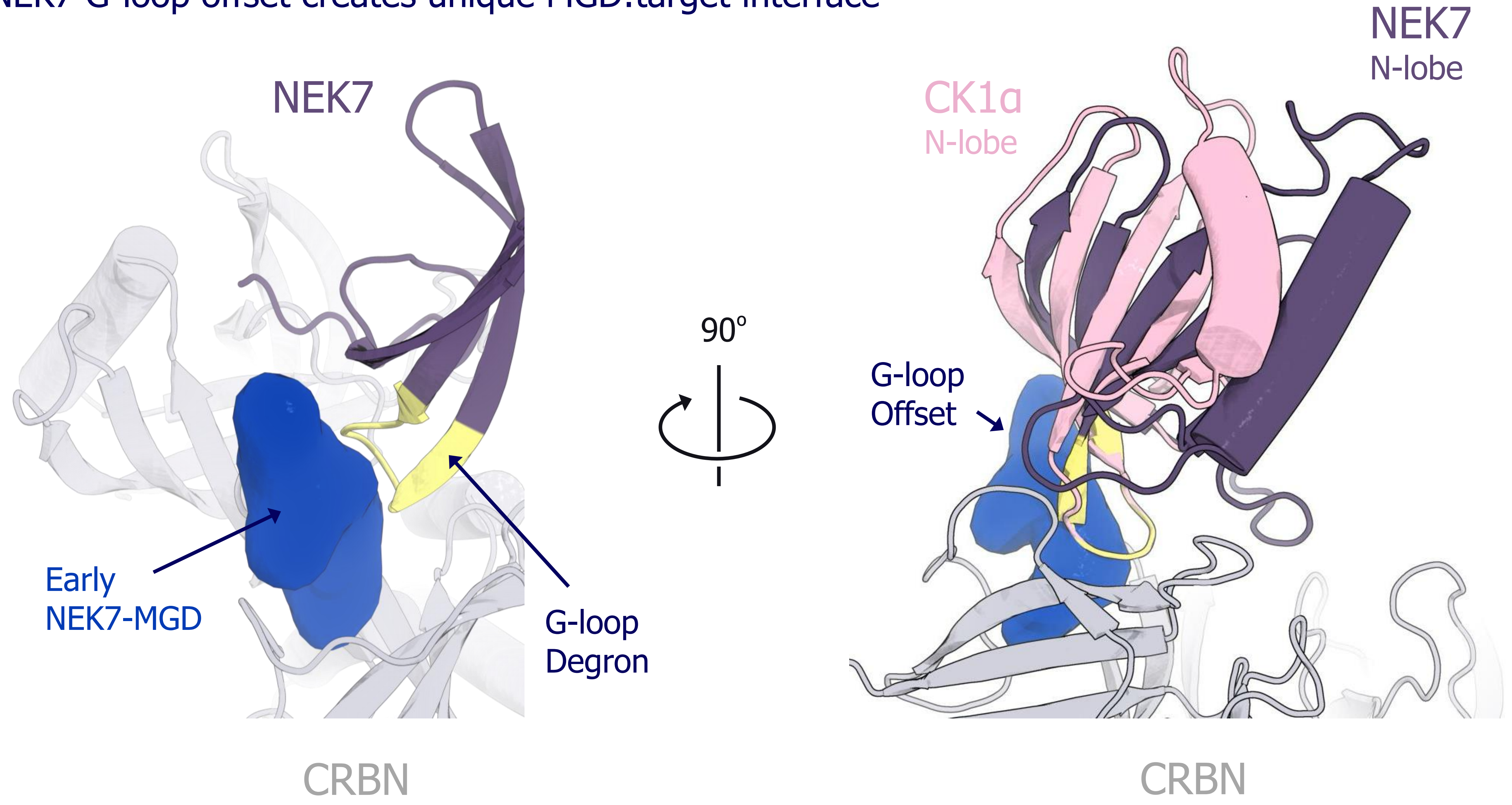
NEK7 degradation with MRT-8102



Global TMT-proteomics

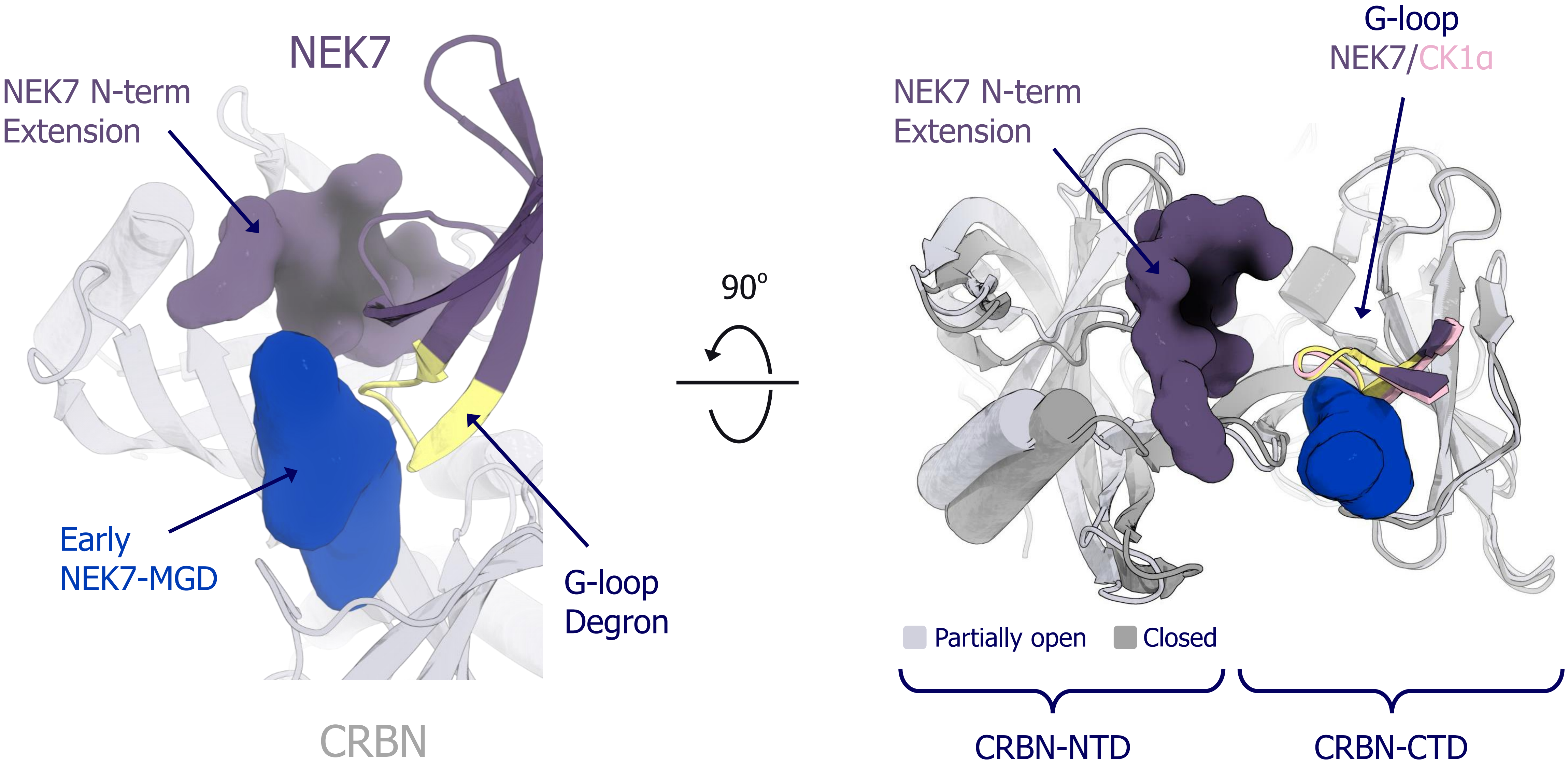
Early Structural Insights into NEK7 Binding Enabled Rational MGD Design

NEK7 G-loop offset creates unique MGD:target interface



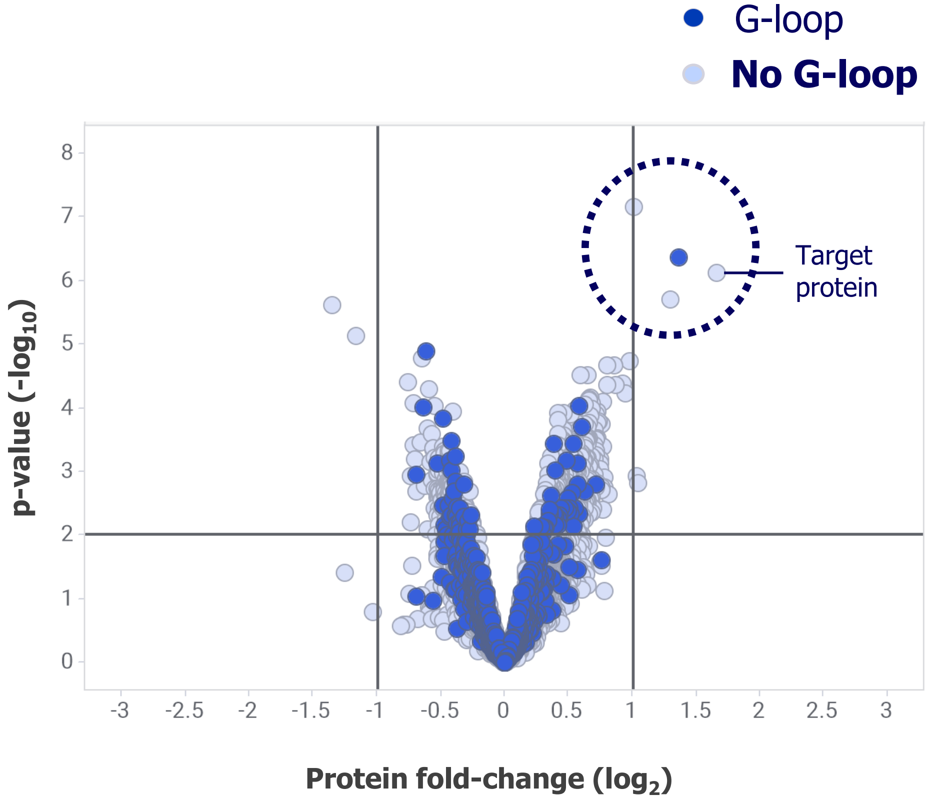
NEK7 Engages CRBN in a Partially Open Conformation

A larger spectrum of CRBN conformations are accessible for TPD



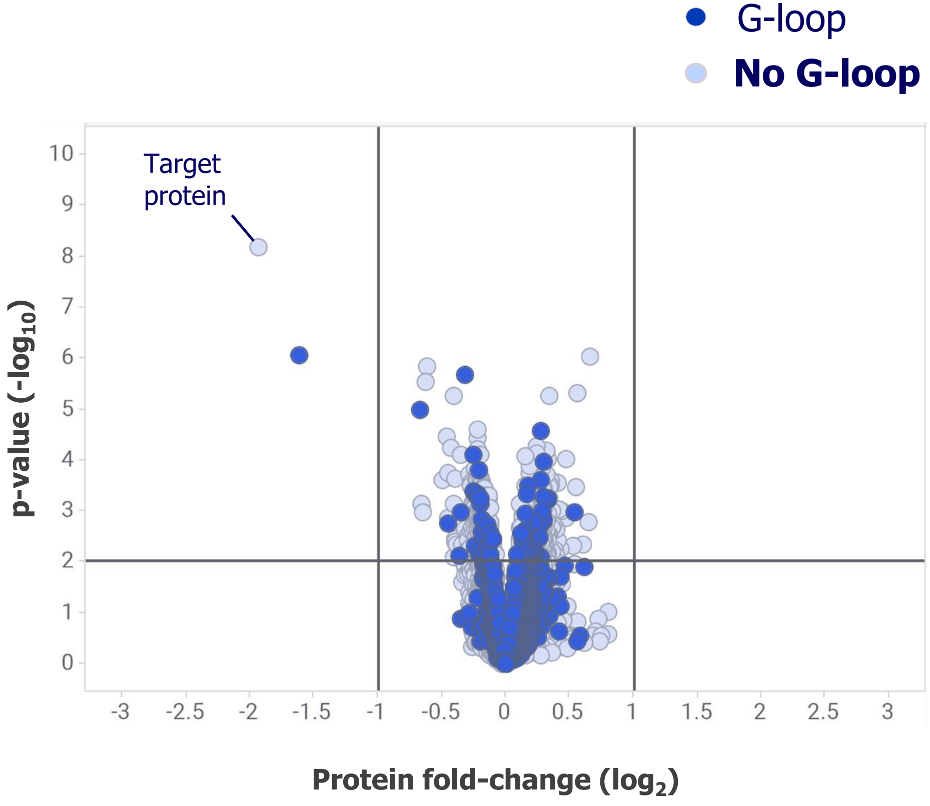
QuEEN™ – G-loop Catalogue Guides Discovery of Non-G-loop Targets

Enrichment of non-G-loop proteins in CRBN proximity-ligation



TurboID-CRBN (CAL51)

Degradation of a non-G-loop target in global proteomics

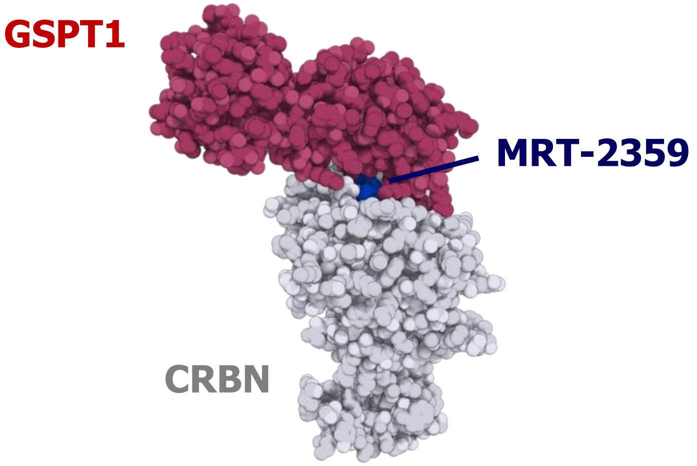


TMT-Proteomics (Jurkat)

QuEEN™ – AI Finds Degrons Using Surfaces

Leverage knowledge of ternary complex structures

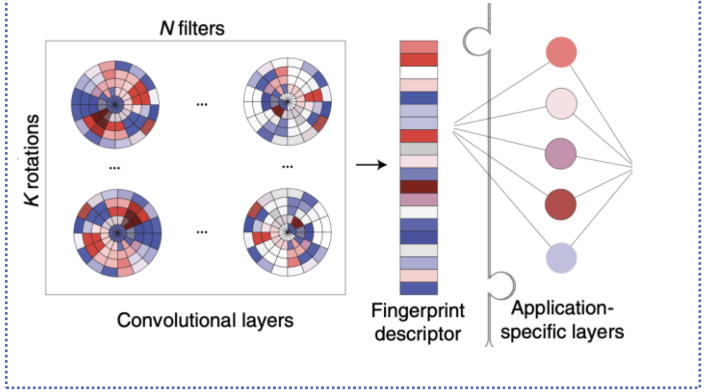
Proprietary ternary complexes
X-ray & cryoEM



Algorithms tailored to degron discovery

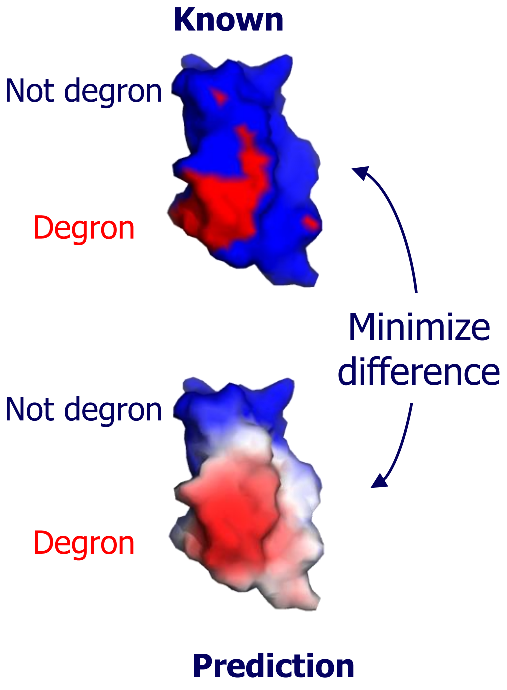
Ultra-fast fingerprint search for surfaces that mimic known degron surfaces

fAIceit-mimicry™



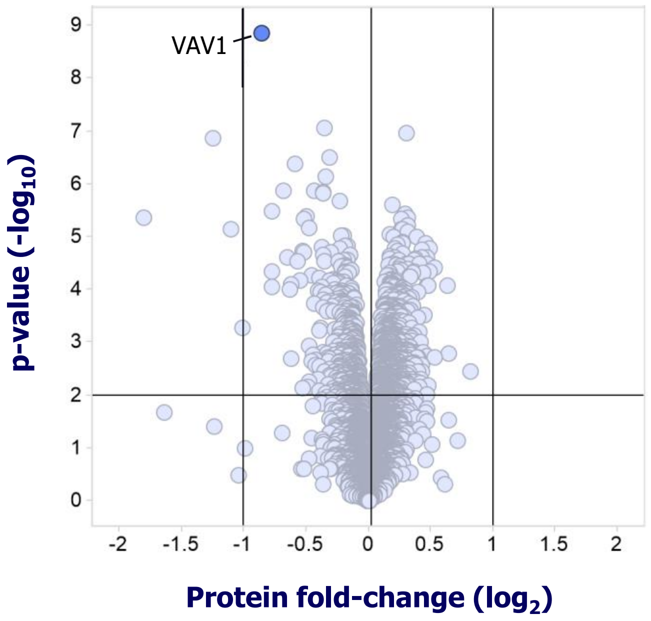
Expand analysis across the proteome

Identify surface patches that match known degron interfaces



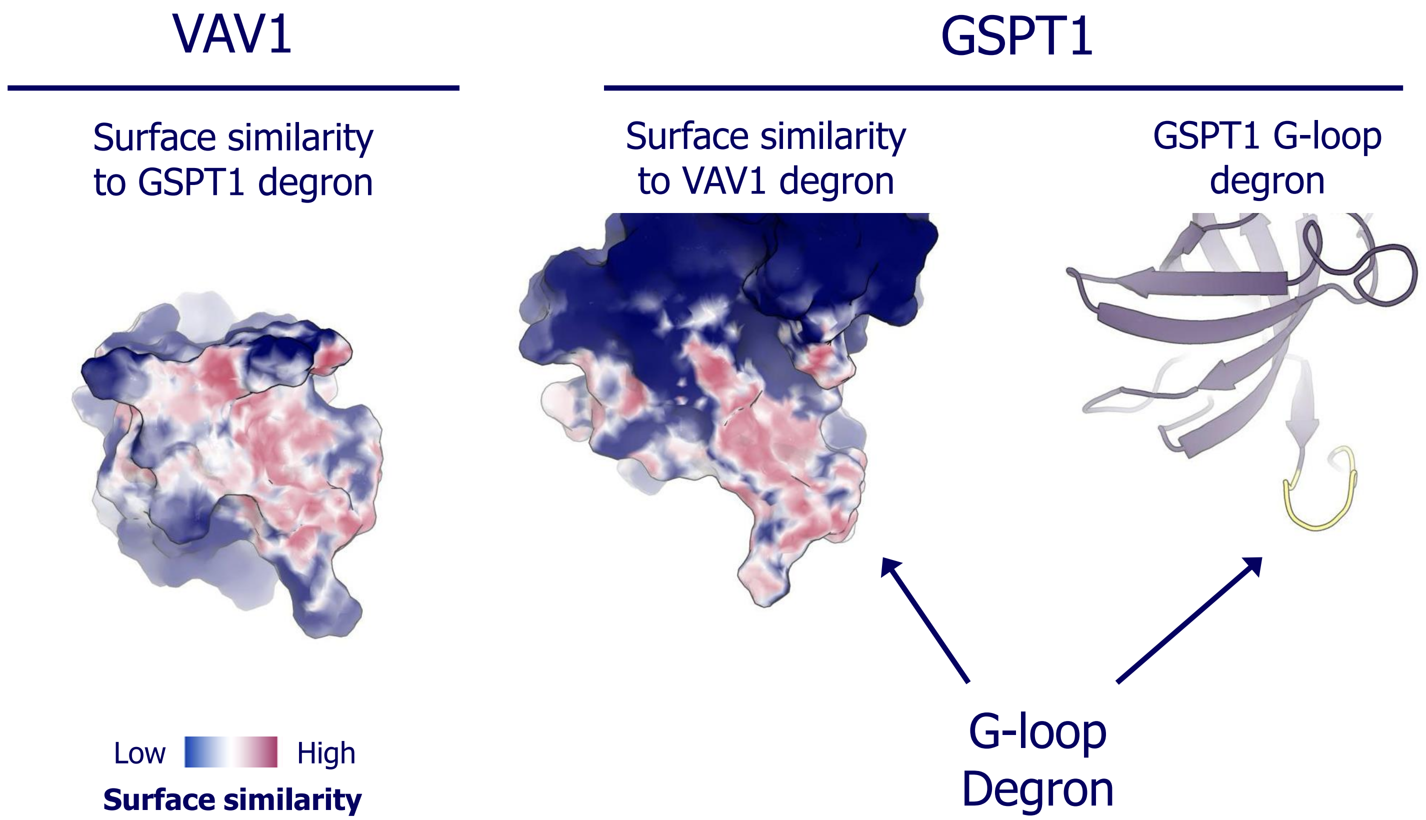
Molecular Surface Mimicry of a Known MGD-induced CRBN Target

Non-G-loop target VAV1 in global proteomics



TMT-Proteomics (MOLM13)

VAV1 shows 'molecular surface mimicry' to the GSPT1 degron



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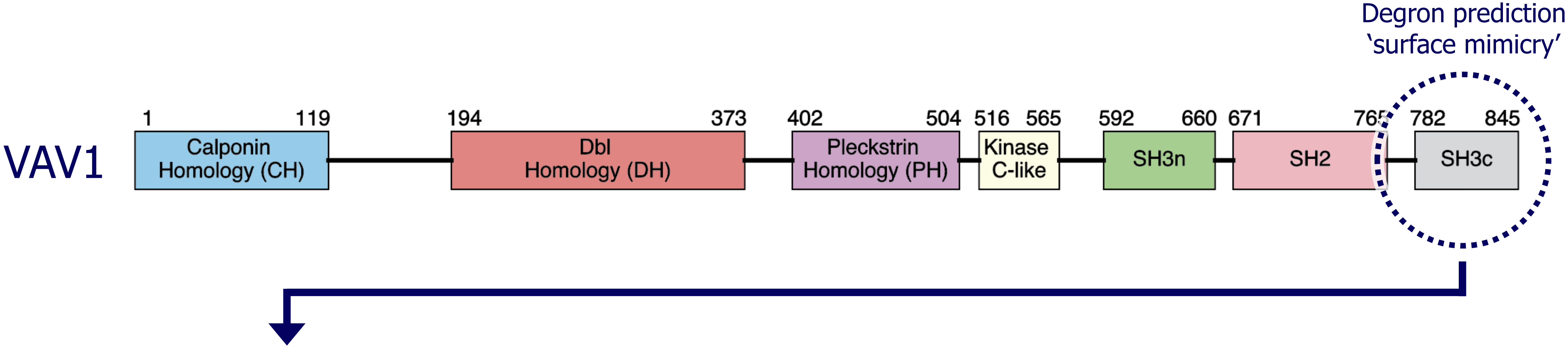
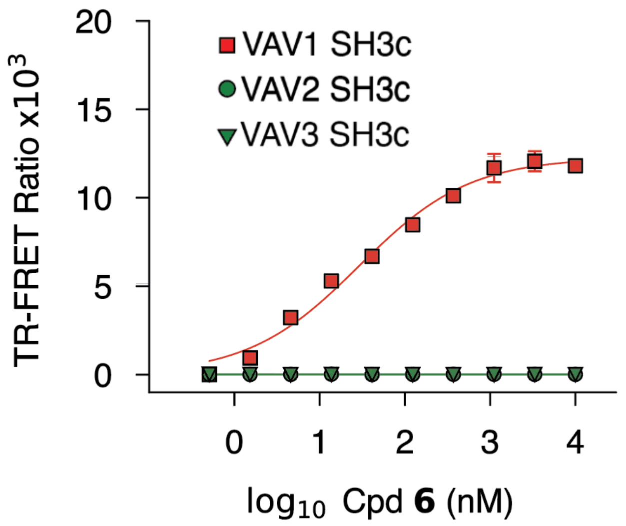
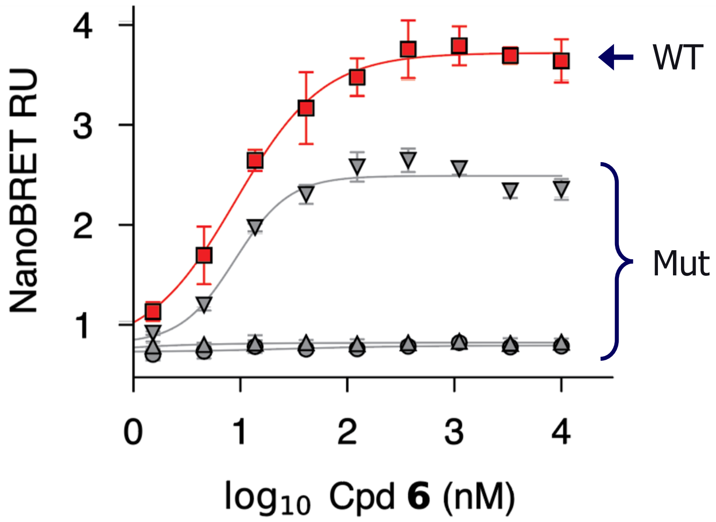
Surface Mimicry Predictions Guide HTS Campaign for MGD Discovery


Degron prediction
‘surface mimicry’

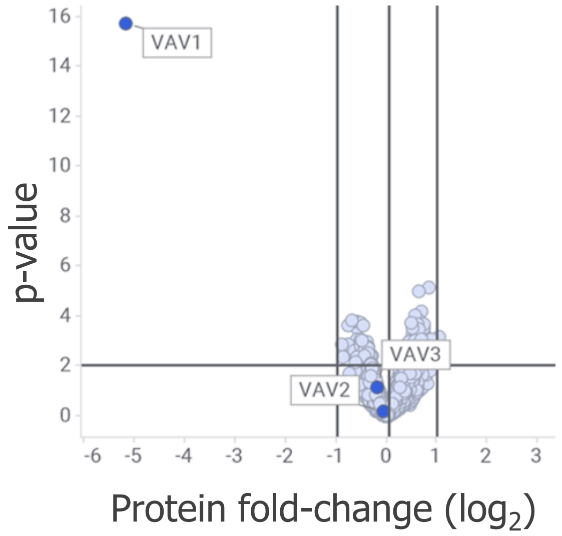
VAV1-SH3c domain
(TR-FRET screen)



Full-length VAV1
(NanoBRET validation)



Proteomics



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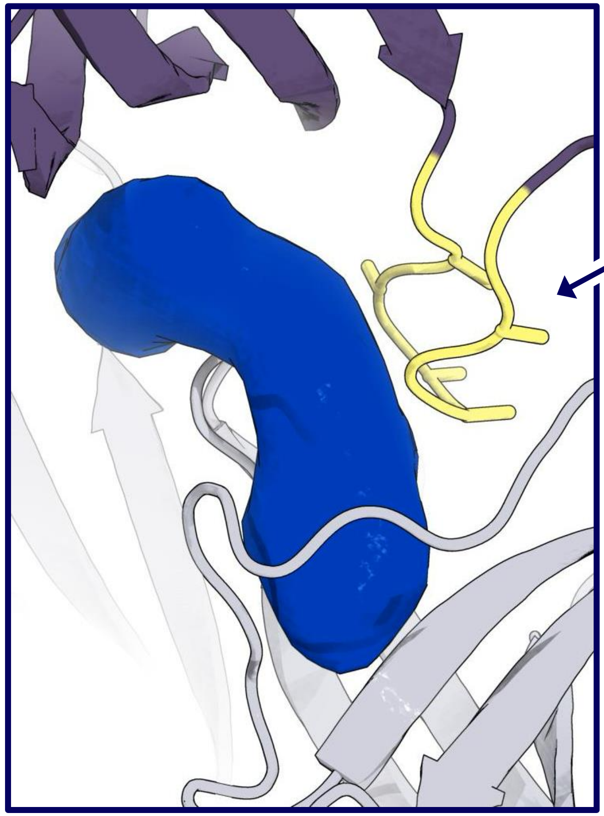
VAV1 Engages CRBN Through an Unconventional Binding Mode

GSPT1

G-loop degron



G-loop interactions

Backbone-driven

CRBN:MGD

VAV1

Novel binding mode

RT-loop interactions

Side chain-driven

CRBN:MGD

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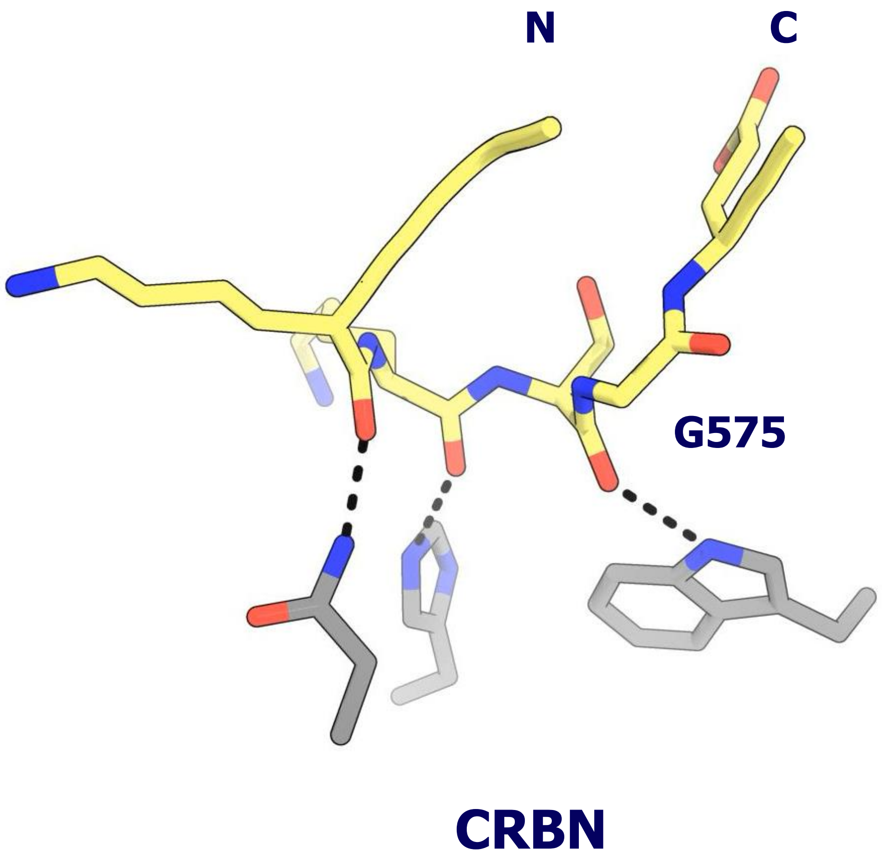
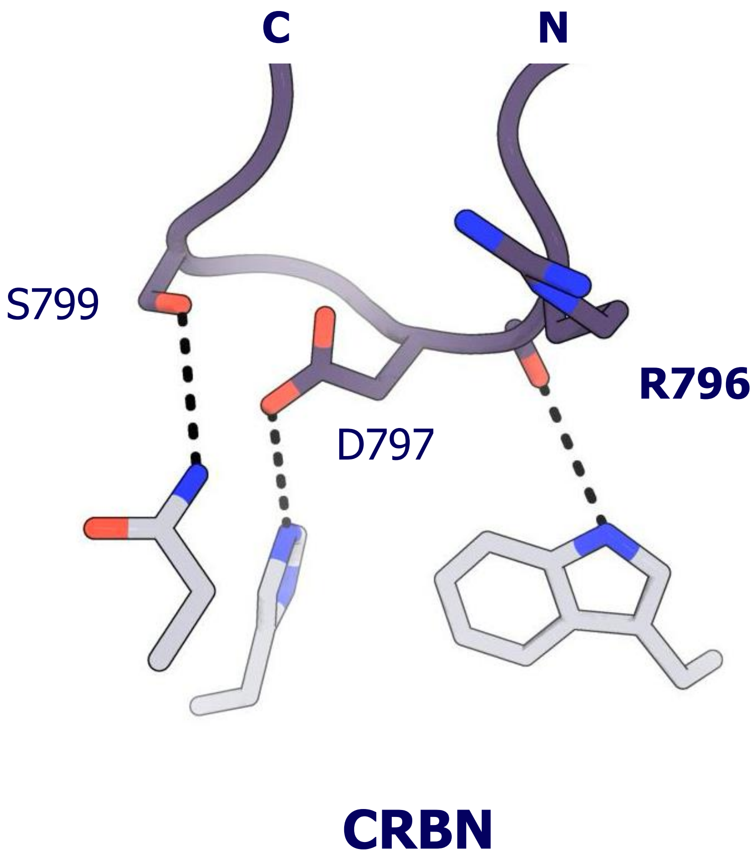
Molecular Mimicry Rationalizes VAV1 Engagement with CRBN

GSPT1
G-loop



VAV1
RT-loop



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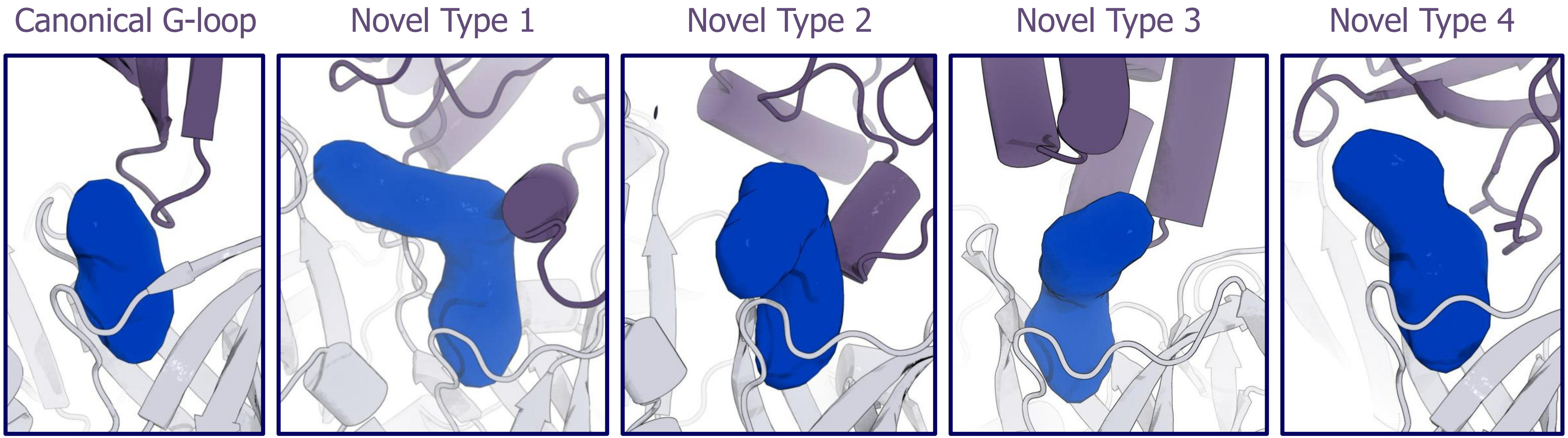
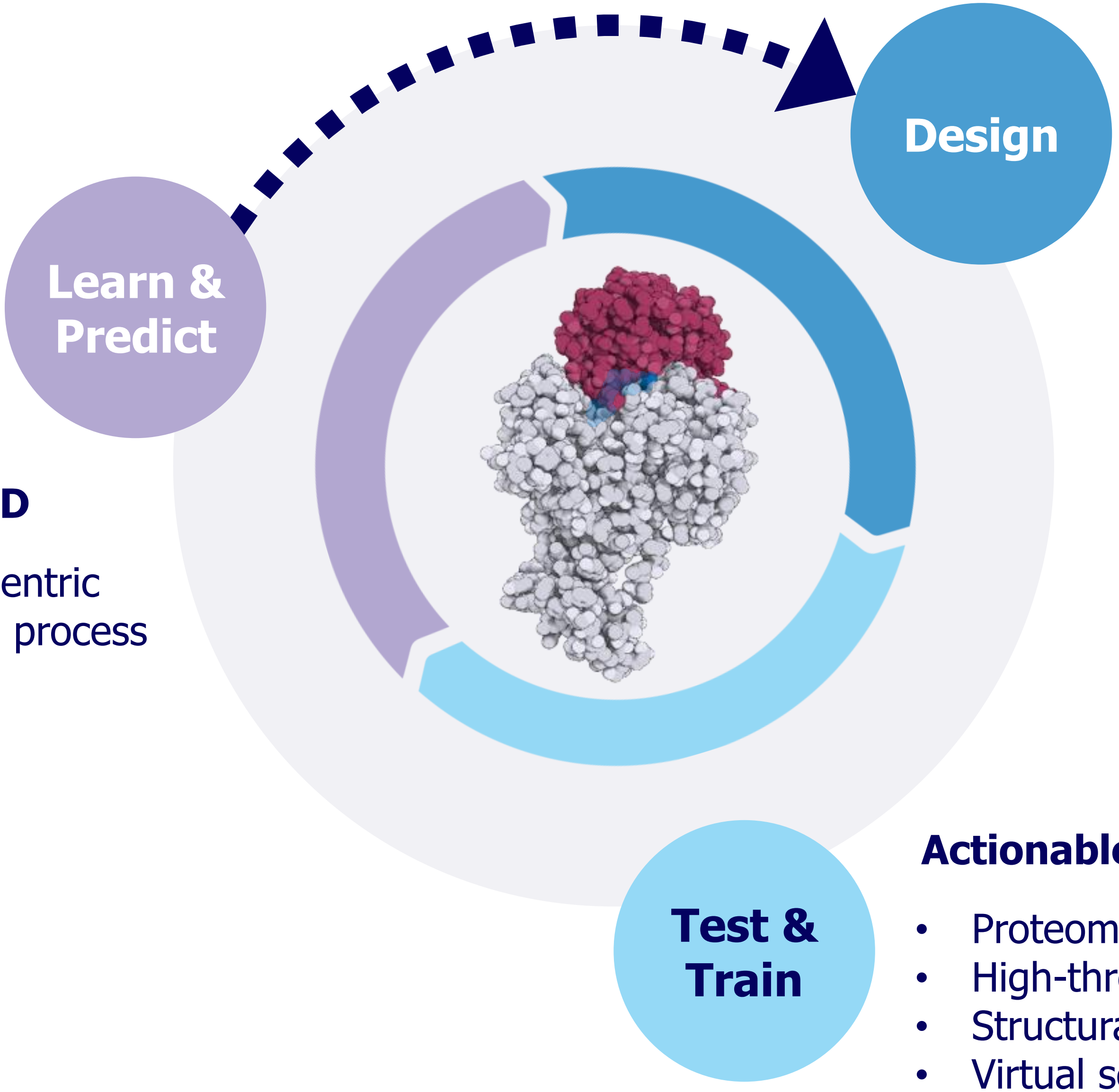
QuEEN™ >18 Novel Binding Modes – Diverse in Structure and MGD



- Target space expansion beyond G-loop degrons
- Overcome species-specific CRBN polymorphisms
- Improved MGD selectivity profiles

QuEEN™ – Iterative Library Design Expands Target Space Opportunities



AI-powered chemistry

Surface-aware MGD generation & optimization

Target ID

Surface-centric discovery process

Actionable data-at-scale

- Proteomics
- High-throughput screens
- Structural biology
- Virtual screens

Thank You to the Global Monte Rosa Team

Boston

Basel

Mining the CRBN Target Space Redefines Rules for Molecular Glue-induced Neosubstrate Recognition." bioRxiv (2024): 2024-10.