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Small molecule PROTAC hijacking and structural characterization of an E3 ligase, KLHDC2, for targeted protein degradation

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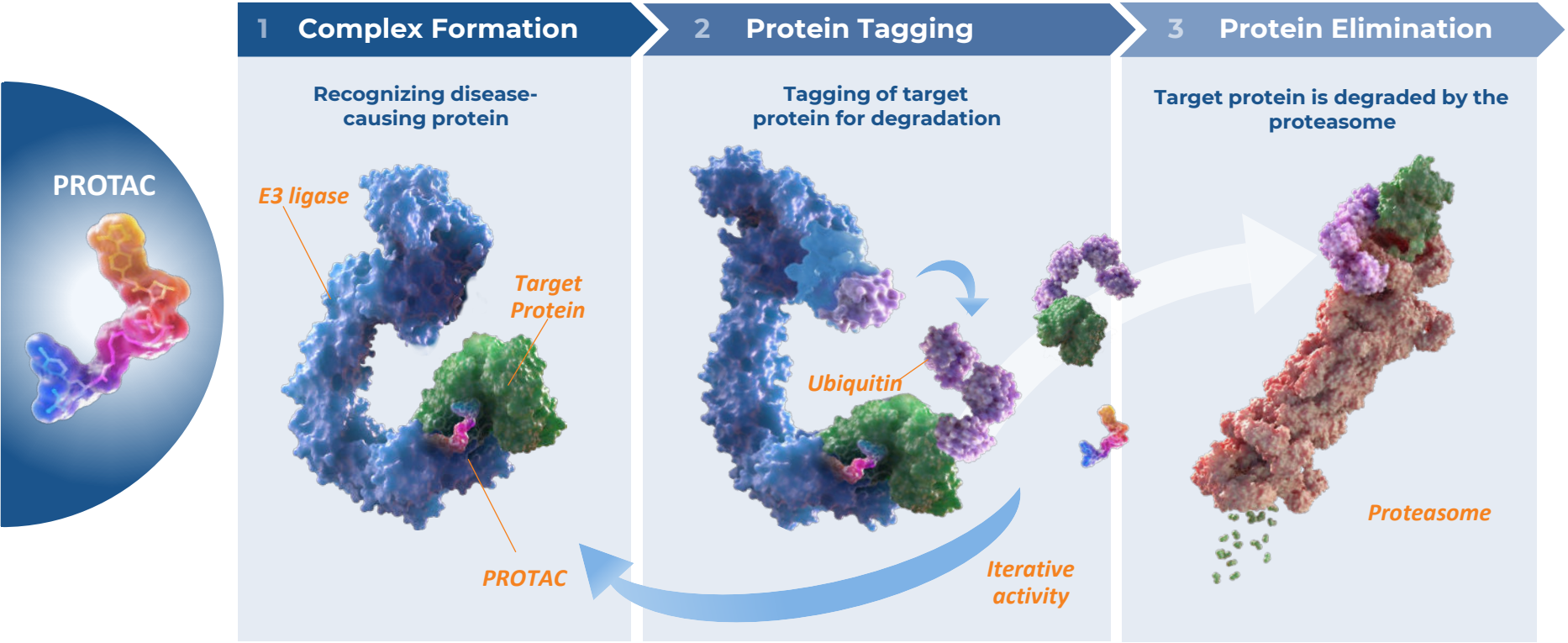
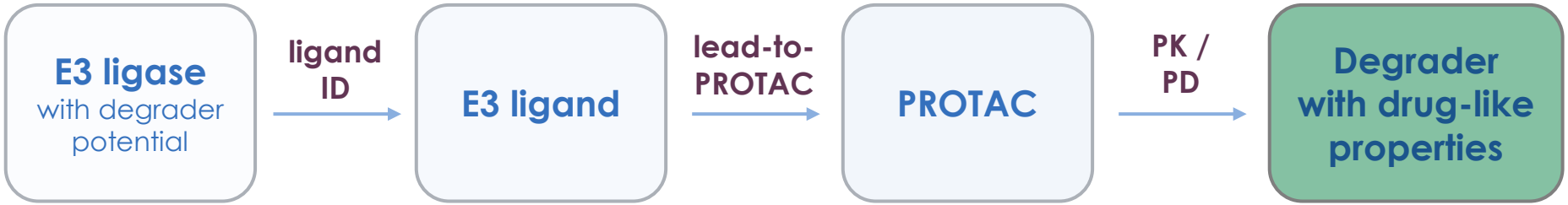
Research Investigator | Platform Biology | Arvinas, Inc.

April 25, 2023 | Ubiquitins, Autophagy & Disease CSHL

PROTAC® protein degraders harness the ubiquitin-proteasome system to induce the degradation of disease-causing proteins



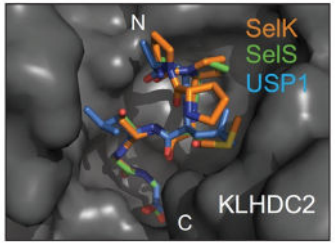
PROTAC® discovery – one case study from the Arvinas E3 repertoire



Discovery & characterization of KLHDC2 ligands for PROTAC applications:

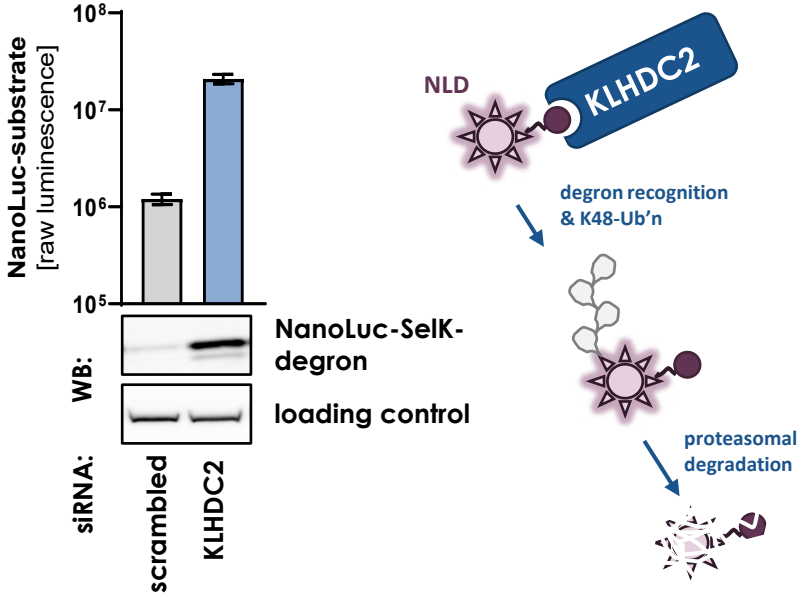
1) Rapid *de novo* ligand design by CADD & ligand evolution
2) Ligand-to-PROTAC conversion & on-mechanism activity validation
3) Mechanistic & structural understanding of E3 assembly

KLHDC2 is an active E3 ligase that can be exploited for PROTAC discovery



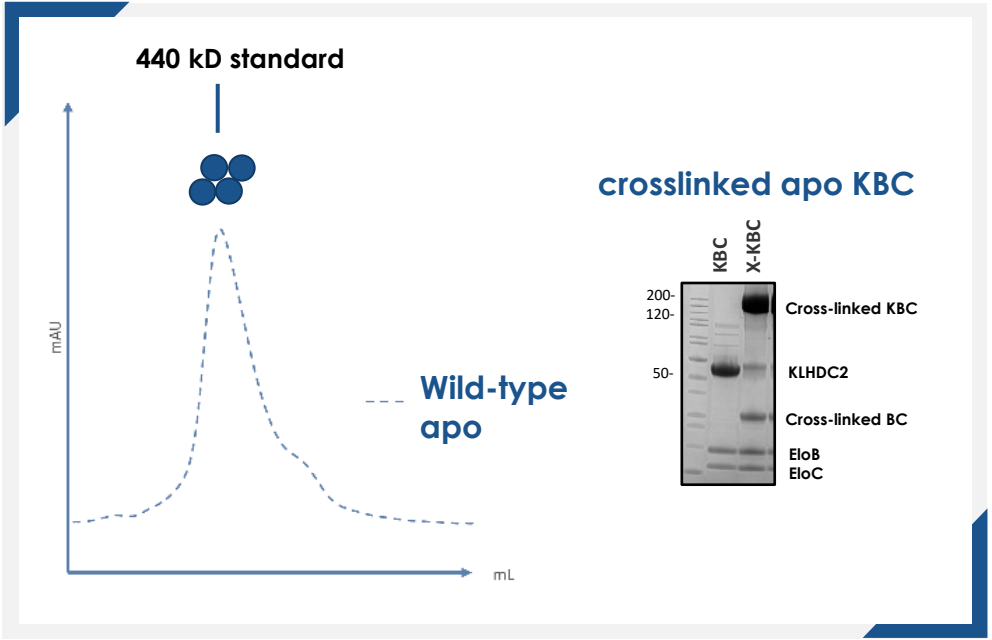
- KLHDC2 is a CRL2-associated substrate receptor
- KLHDC2 has been shown to recognize C-terminal glycine residues as a high affinity degron
- C-term Gly recognition has been structurally elucidated

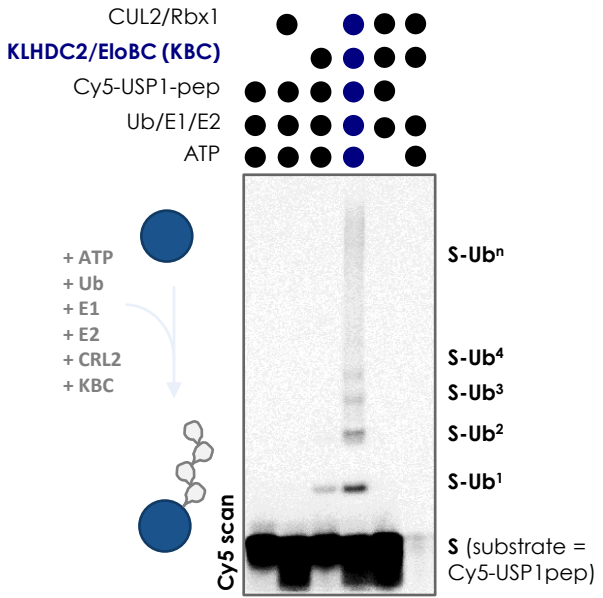
In-house validation of KLHDC2 as a C-terminal degron targeting CRL2 E3 ligase using NanoLuc-degron (NLD) fusions



Rusnac et al (2018) Mol Cell / Lin et al (2018) Mol Cell / Koren et al (2018) Cell

The full-length functional KLHDC2/EloB/EloC complex is unexpectedly large





- **The C-terminus of KLHDC2 ends in -GlySer**
- **The substrate** (SelK) **peptide ends in -GlyGly**
- **Possible scenario: KLHDC2 C-term loosely holds together complex, and is competed by a substrate**

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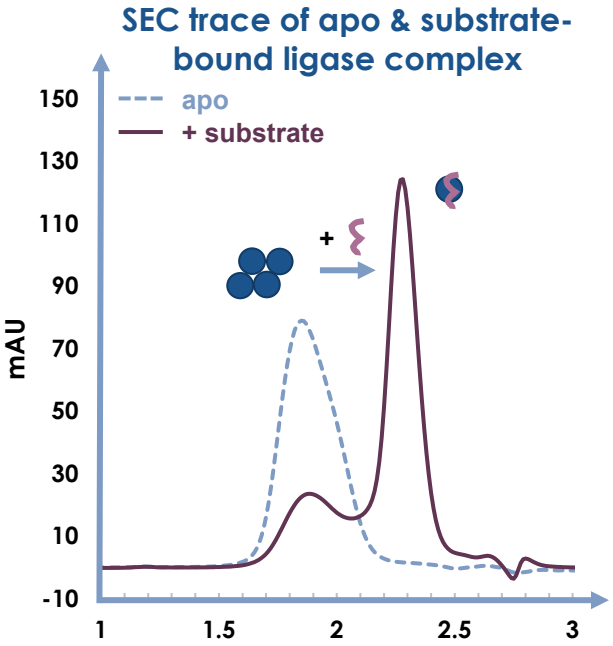
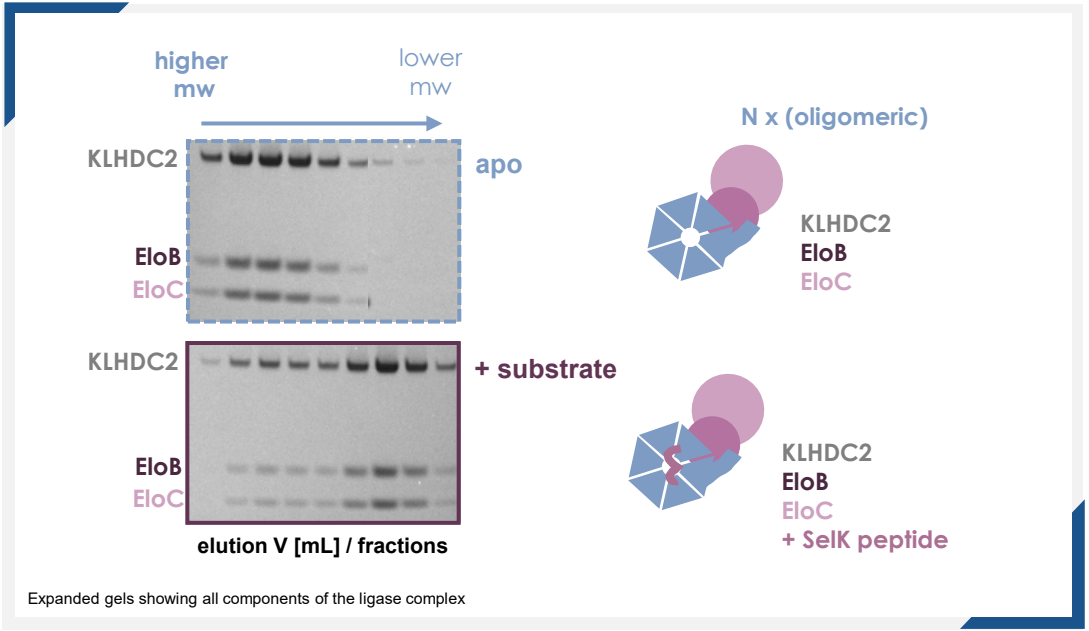
The full-length KLHDC2/EloB/EloC ligase complex is a dynamic oligomer

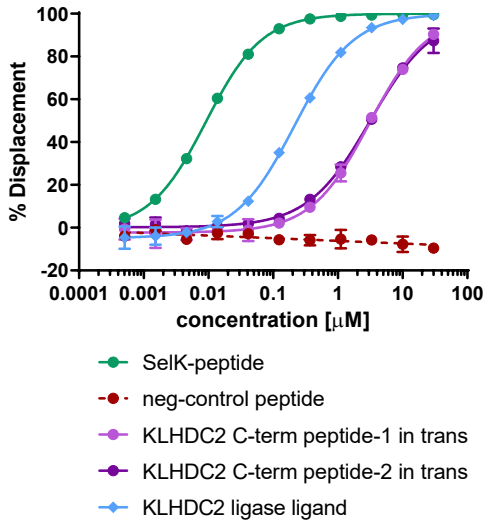




- **apo KLHDC2/EloB/EloC ligase complex is oligomeric**
- **SelK-peptide-bound KBC complex shifts to a smaller size** (as by measured by SEC)

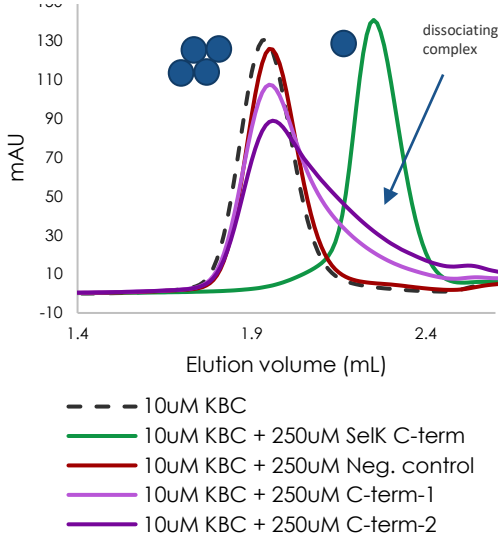
The KLHDC2/EloB/EloC complex can be dissociated by the C-terminus of KLHDC2

KLHDC2:SelK displacement assay



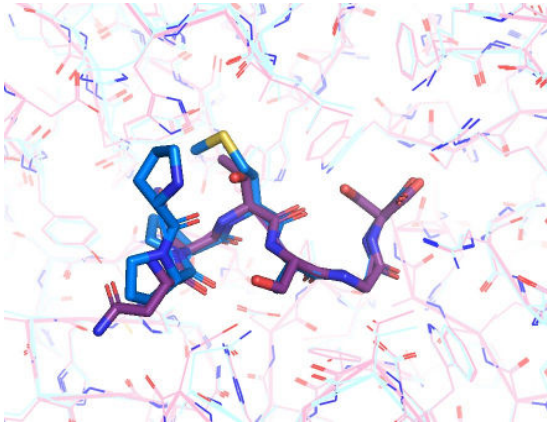
KLHDC2 C-term peptides display low affinity to KLHDC2

SEC traces of KBC + peptide complexes



Low affinity C-term KLHDC2 peptides look to partially dissociate the oligomeric KBC complex

Co-crystal structures of $KLHDC2_{KD}$ + peptides

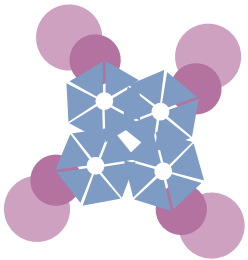


KLHDC2-KD:SelK-Cterm (PPPMAGG) – pdb: 6DO3
KLHDC2-KD:KLHDC2-Cterm (NNTSGS) – Arvinas

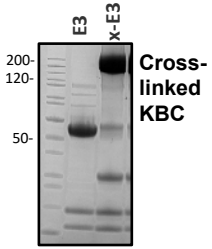
KLHDC2 C-term co-crystallized with $KLHDC2_{KD}$, adopting the conformation of the SelK peptide

CryoEM structure of the apo KLHDC2/EloB/EloC complex reveals a tetrameric arrangement, consistent with the model

model based on biochemistry

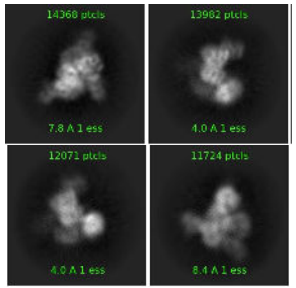


crosslinked apo KBC

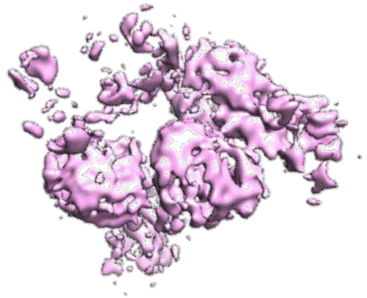


2D images

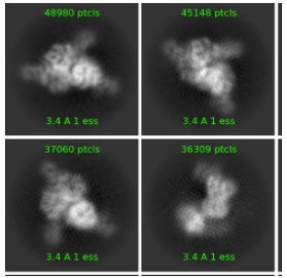
Frozen with Vitrobot®

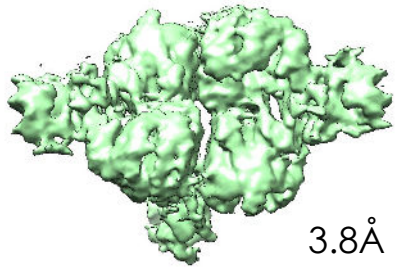


Initial map



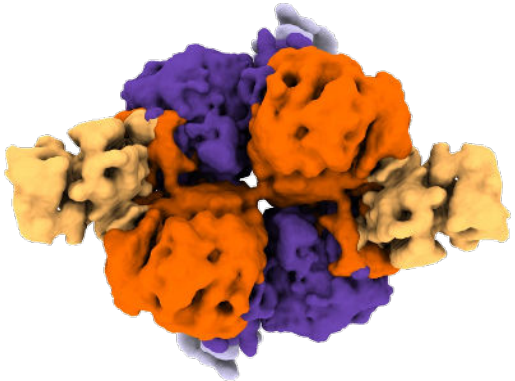
Frozen with chameleon®

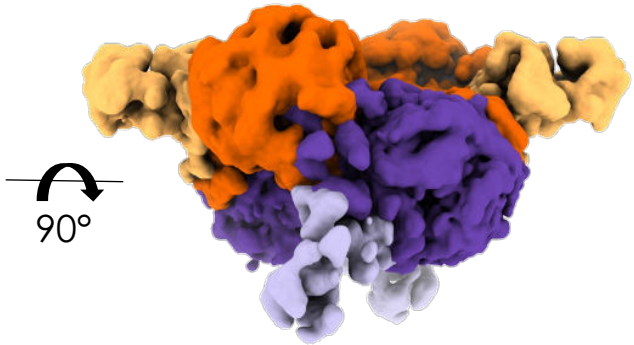


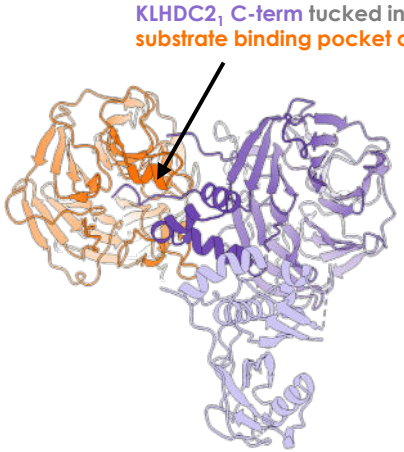


3.8Å

CryoEM structure of the complex supports oligomerization mediated by C-terminus



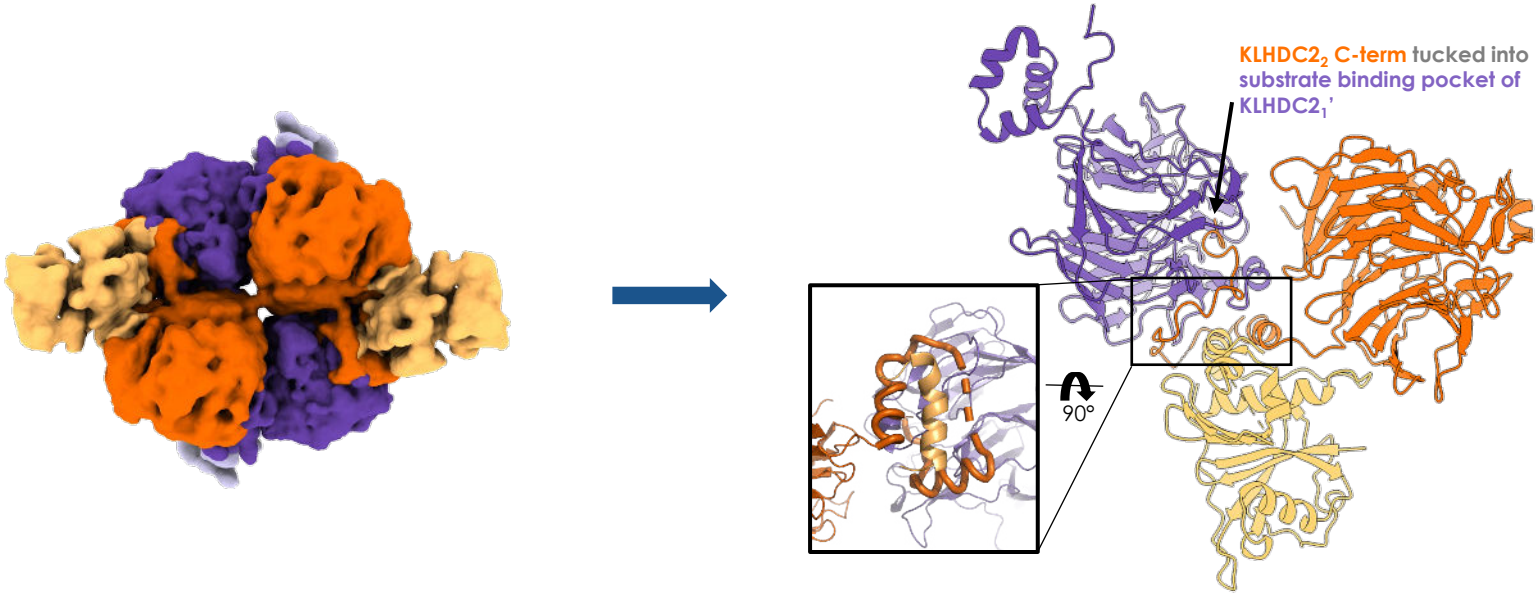




2 x KLHDC2, 2 x EloBC (KBC$_1$)
and
2 x KLHDC2, 2 x EloBC (KBC$_2$)

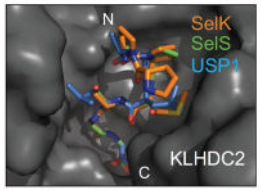
- 4 individual KLHDC2/EloB/EloC complexes can be visualized in the final complex
- Half of KBC are competent to bind Cul2 according to model
- Clear visualization of C-terminus of KLHDC2 in to support daisy-chain arrangement

CryoEM structure demonstrates extensive inter-modular contacts mediated by C-terminus



- Half of KBC components are not competent to bind Cul2 due to dramatic reorganization of BC- and Cullin-box of KLHDC2
- Clear visualization of C-terminus of KLHDC2 to support daisy-chain arrangement

Structure-based, de novo ligand design by CADD & rapid ligand evolution yielded potent and novel KLHDC2 ligands



CADD

de novo ligand design

compound 1

SelK aLISA IC_{50} [nM]: **14000**
SPR K_d [nM]: **20000**
SelK TR-FRET IC_{50} [nM]: ND

compound X

SelK aLISA IC_{50} [nM]: **68**
SPR K_d [nM]: **95**
SelK TR-FRET IC_{50} [nM]: **100**

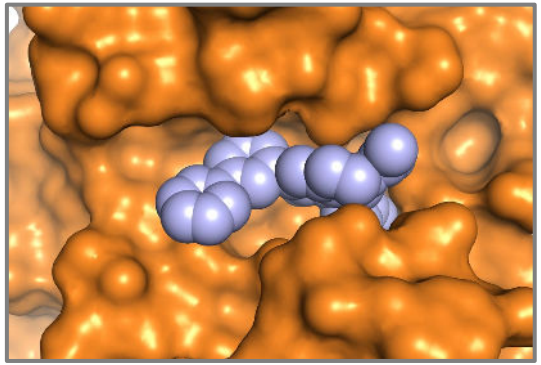
compound Z

SelK aLISA IC_{50} [nM]: **9**
SPR K_d [nM]: **120**
SelK TR-FRET IC_{50} [nM]: **8**

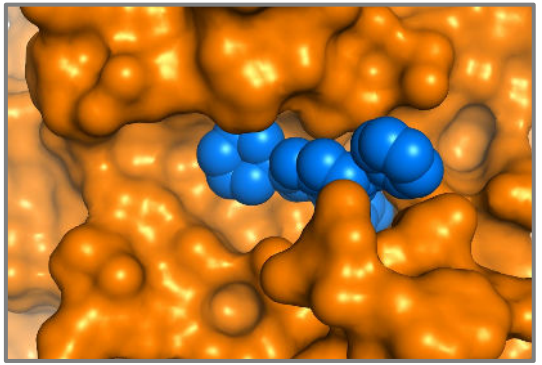
E3-dead

SelK aLISA IC_{50} [nM]: **1000**
SelK TR-FRET IC_{50} [nM]: **810**
SPR K_d [nM]: ND

- **Multiple co-crystal structures solved with our CADD-based KLHDC2 ligands**
- **KLHDC2 ligands extensively occupy and fill the substrate-binding pocket**
- **Crystal structures allow rational design of an E3-dead analogue; and illuminate multiple exit vectors for PROTAC development**

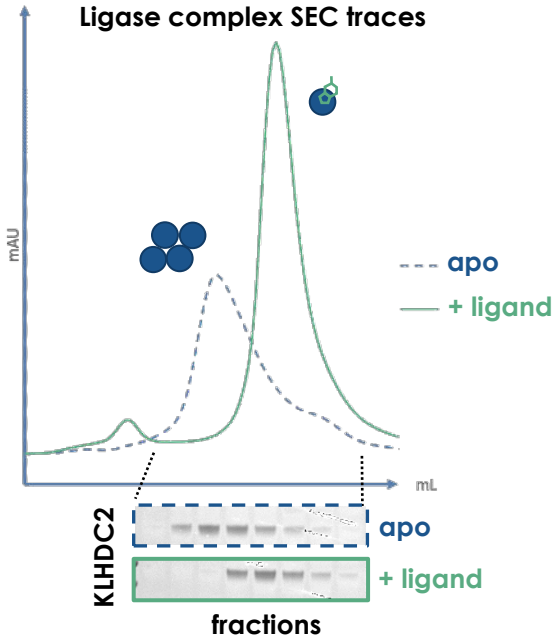


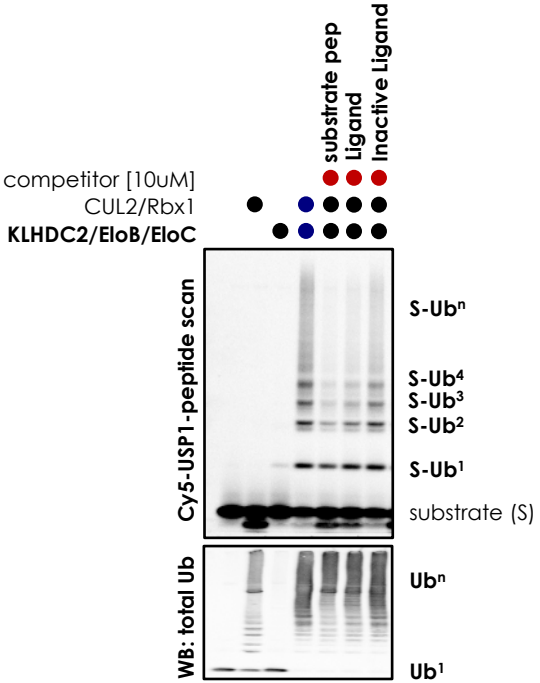
$KLHDC2_{KD}$: compound Y @ 1.8 Å



$KLHDC2_{KD}$: compound W @ 1.6 Å

KLHDC2-targeting small molecules engage full-length KBC





- Small molecule ligands alter oligomeric assembly of KBC
- Small molecule ligands can compete substrate in active KBC complex
- Continuing to look at assembly of KBC bound to substrates, small molecules, PROTACs, and PROTAC-POI complexes

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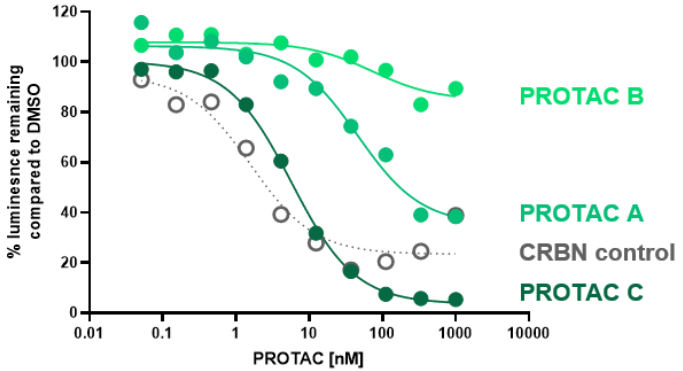
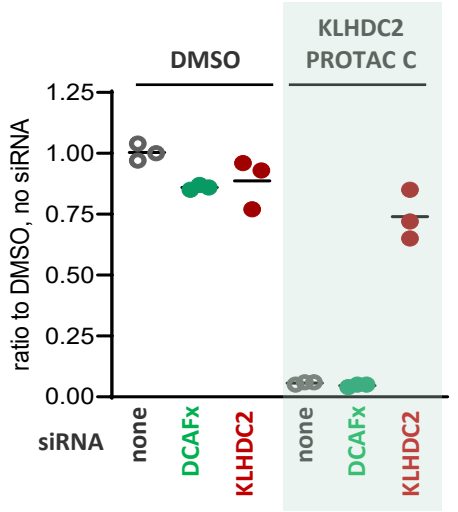
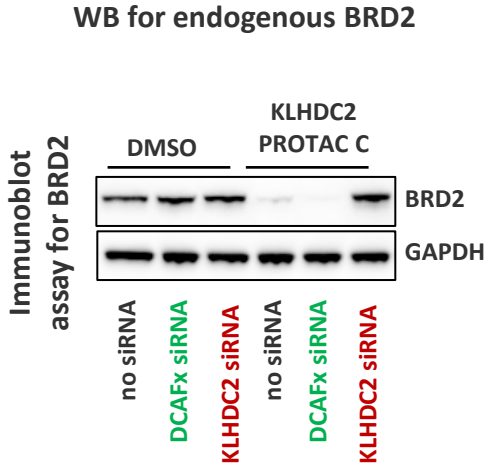
KLHDC2-based PROTAC optimization using JQ1 yields potent pan-BET degraders







- Our novel KLHDC2-based BET-family PROTACs are:
  - ✓ robust → greater than 90% D_{max}
  - ✓ potent → DC_{50} in the low nM range
  - ✓ on-mechanism → sensitive to KLHDC2 siRNA

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PROTAC®-able E3 ligase is now structurally and functionally enabled for TPD

- KLHDC2 E3 ligase can degrade target proteins using our PROTAC technology.
- PROTAC design is enabled by the quaternary structure of this E3 in its full-length, wild-type form.
- Extensive optimization of the protein complex and freezing conditions on the Vitrobot did not permit high-resolution structural determination.
- Freezing on the chameleon® with optimized protein complex allowed high-resolution structural determination.
- We are excited to pursue more high-throughput, streamlined, cryoEM structural determination with the in-house chameleon instrument.

Acknowledgements – the entire Arvinas Team (now 400+!)

Thank you!