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Structural insights into PROTAC®-induced proximity

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Research Investigator | Platform Biology | Arvinas, Inc.

January 25, 2022 | NESBA Symposium

Safe harbor and forward-looking statements

This presentation contains forward-looking statements within the meaning of The Private Securities Litigation Reform Act of 1995 that involve substantial risks and uncertainties, including statements regarding the receipt of upfront, milestone, and other payments under the Pfizer collaboration, the potential benefits of and the receipt of any related milestones in connection with our arrangements with our collaborative partnerships, statements regarding the potential advantages and therapeutic benefits of bavdegalutamide (ARV-110), ARV-471, ARV-766 and our other discovery programs, the development and regulatory status of our product candidates, such as statements with respect to the potential of our lead product candidates, bavdegalutamide (ARV-110), ARV-471, and ARV-766 and other candidates in our pipeline, and the timing of clinical trials, including the timing to complete enrollment, as well as the presentation and/or publication of data from those trials and plans for registration for our product candidates, and our discovery programs that may lead to our development of additional product candidates, the potential utility of our technology, our plans with respect to submission of investigational new drug/clinical trial authorization applications, the potential commercialization of any of our product candidates and companion diagnostic partnering, and the sufficiency of our cash resources All statements, other than statements of historical facts, contained in this presentation, including statements regarding our strategy, future operations, future financial position, future revenues, projected costs, prospects, plans and objectives of management, are forward-looking statements. The words “anticipate,” “believe,” “estimate,” “expect,” “intend,” “may,” “might,” “plan,” “predict,” “project,” “target,” “potential,” “will,” “would,” “could,” “should,” “continue,” and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words.

We may not actually achieve the plans, intentions or expectations disclosed in our forward-looking statements, and you should not place undue reliance on our forward-looking statements. Actual results or events could differ materially from the plans, intentions, and expectations disclosed in the forward-looking statements we make as a result of various risks and uncertainties, including but not limited to: our and Pfizer, Inc.’s (“Pfizer”) performance of our respective obligations with respect to our collaboration with Pfizer; whether we and Pfizer will be able to successfully conduct and complete clinical development for ARV-471; whether we will be able to successfully conduct and complete development for bavdegalutamide (ARV-110) and our other product candidates, including whether we initiate and complete clinical trials for our product candidates and receive results from our clinical trials on our expected timelines, or at all; whether our cash and cash equivalent resources will be sufficient to fund our foreseeable and unforeseeable operating expenses and capital expenditure requirements; and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, discussed in the “Risk Factors” section of the Company’s Annual Report on Form 10-K for the year ended December 31, 2021 and subsequent other reports on file with the Securities and Exchange Commission. The forward-looking statements contained in this presentation reflect our current views as of the date of this presentation with respect to future events, and we assume no obligation to update any forward-looking statements except as required by applicable law.

This presentation also contains estimates and other statistical data made by independent parties and by us relating to market size and other data about our industry. This data involves a number of assumptions and limitations, and you are cautioned not to give undue weight to such data and estimates. In addition, projections, assumptions and estimates of our future performance and the future performance of the markets in which we operate are necessarily subject to a high degree of uncertainty and risk. This presentation is intended for the investor community only. It is not intended to promote the products referenced herein or otherwise influence healthcare prescribing decisions. Cross-trial comparisons are not based on head-to-head studies and no direct comparisons can be made.

Overview of today's presentation

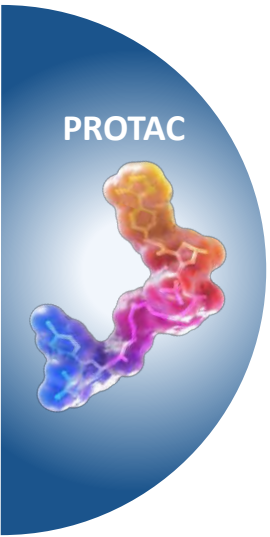
- **Brief background on PROTAC® degraders**

- **CryoEM success stories enabled by our optimized cryoEM workflow**

  - **Structural insights into ARV-471-induced proximity between the estrogen receptor (ER) and the CRBN E3 ligase**

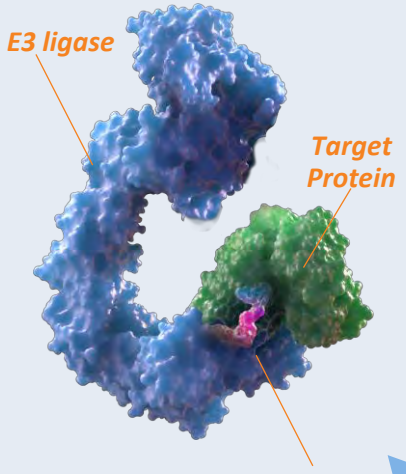
  - **Mechanistic & structural basis of substrate-recruitment by a novel, PROTACable E3 ligase, KLHDC2**

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



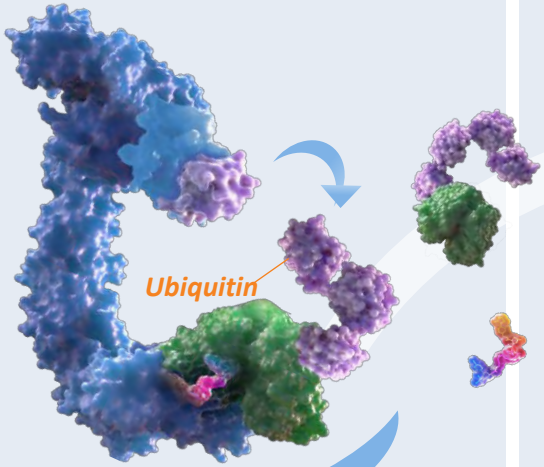
1 Complex Formation

Recognizing disease-causing protein



2 Protein Tagging

Tagging of target protein for degradation



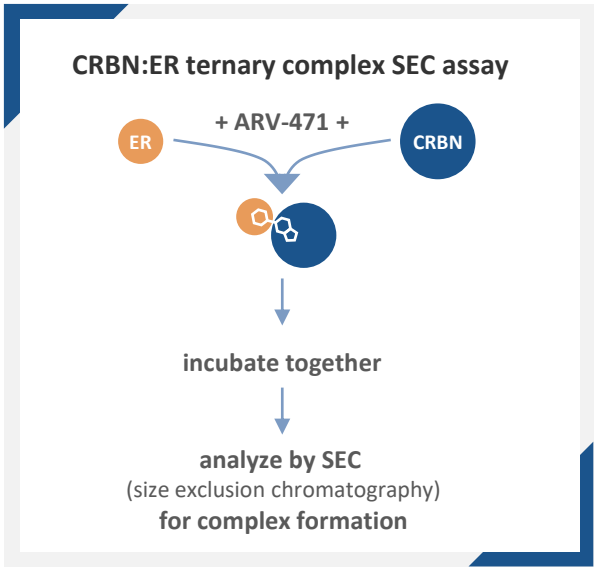
Iterative activity

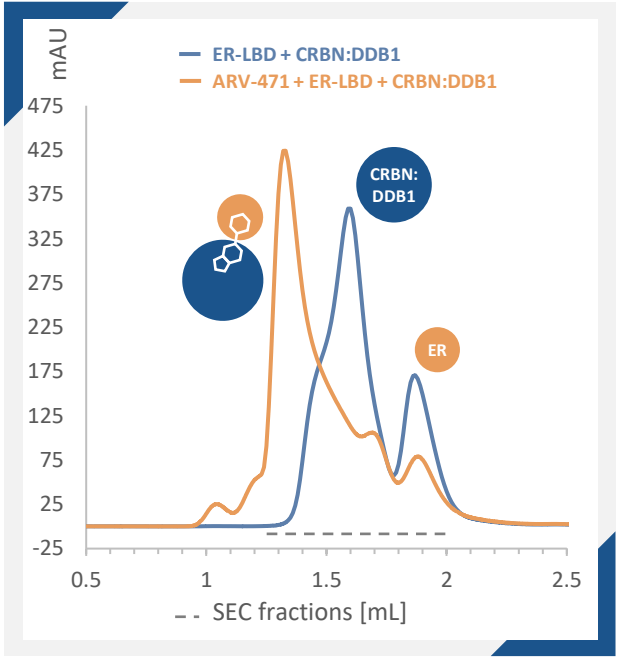
3 Protein Elimination

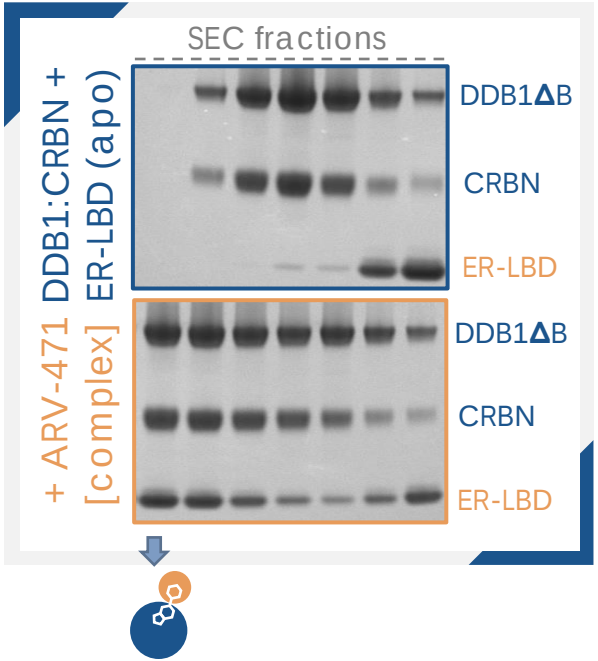
Target protein is degraded by the proteasome

Structural insights into ARV-471-induced proximity between the estrogen receptor (ER) and the CRBN E3 ligase

ARV-471: Induces proximity between CRBN E3 ligase & the estrogen receptor

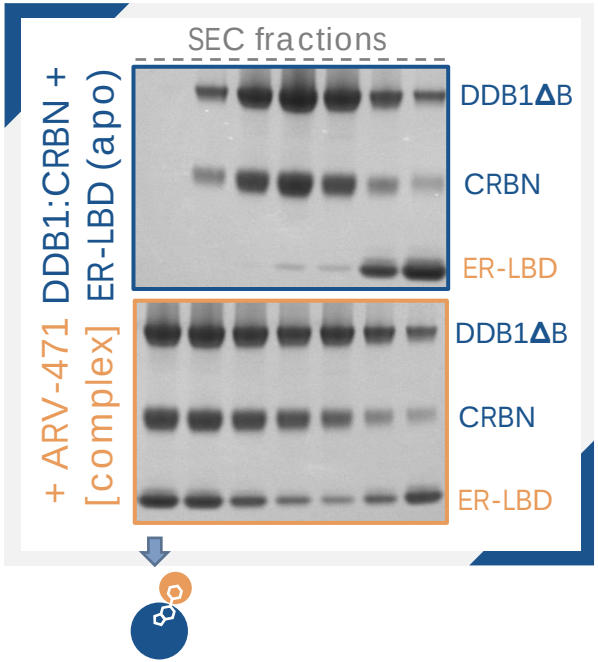




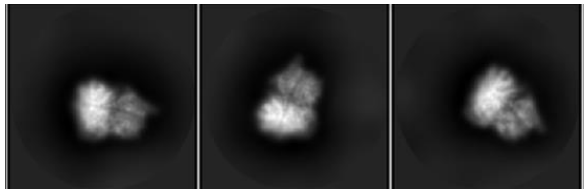


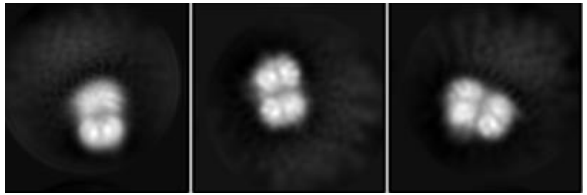
- ER-LBD is pulled into a higher mw complex with CRBN:DDB1 by the presence of ARV-471
- The ER:ARV-471:CRBN ternary complex can be separated by size-exclusion chromatography

Although robust ternary complex formation occurs in solution, this is not the case once frozen.



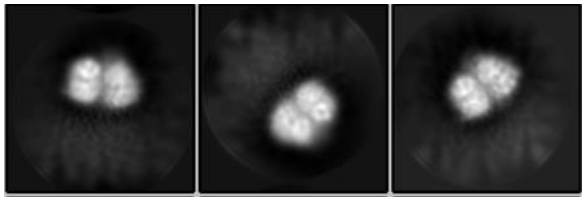
Trip 1





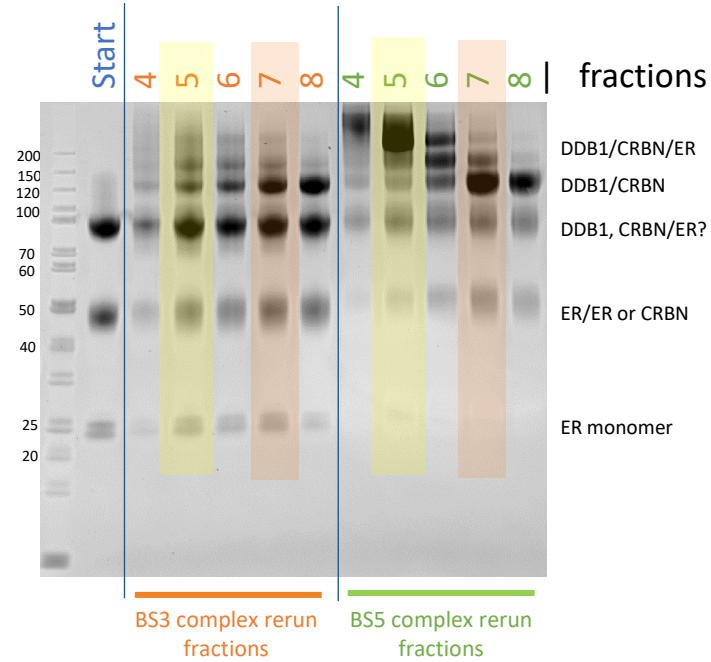
Trip 2

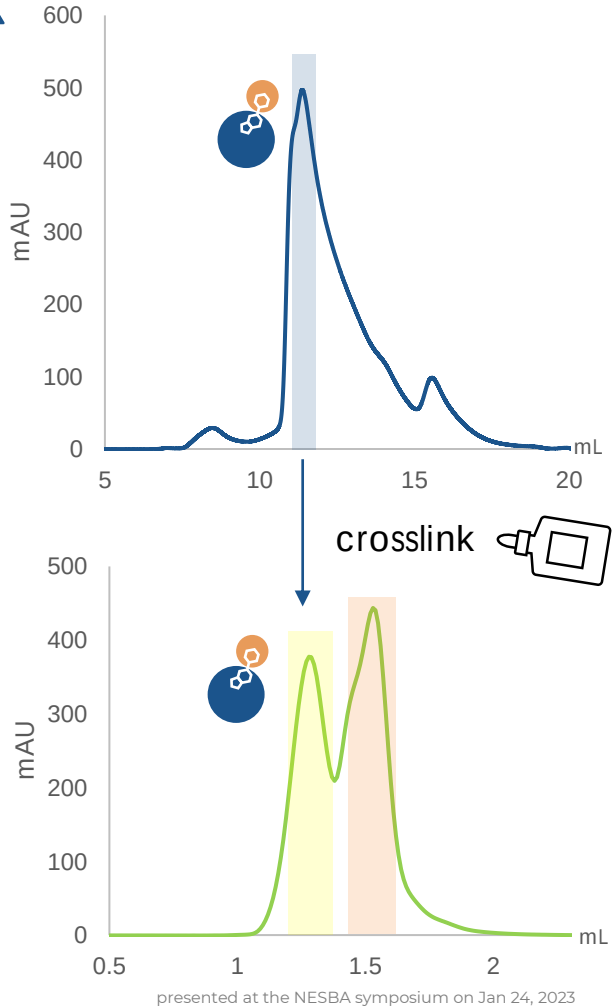
Trip 3…



- 2D classification yields apo-DDB1

Optimization of crosslinking of ternary complex





- ER:ARV-471:CRBN complex can be crosslinked for cryoEM studies

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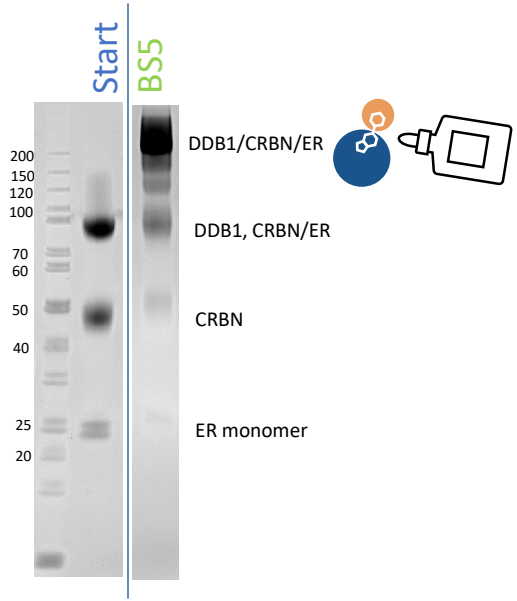
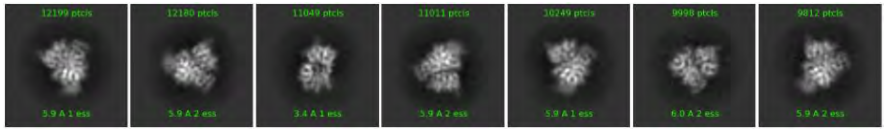
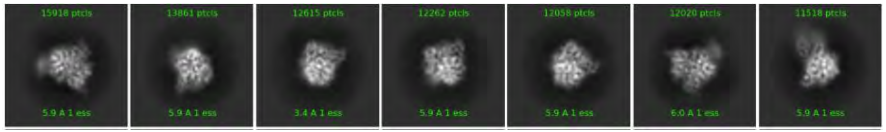
Crosslinked ternary complex also does not show robust ER density.





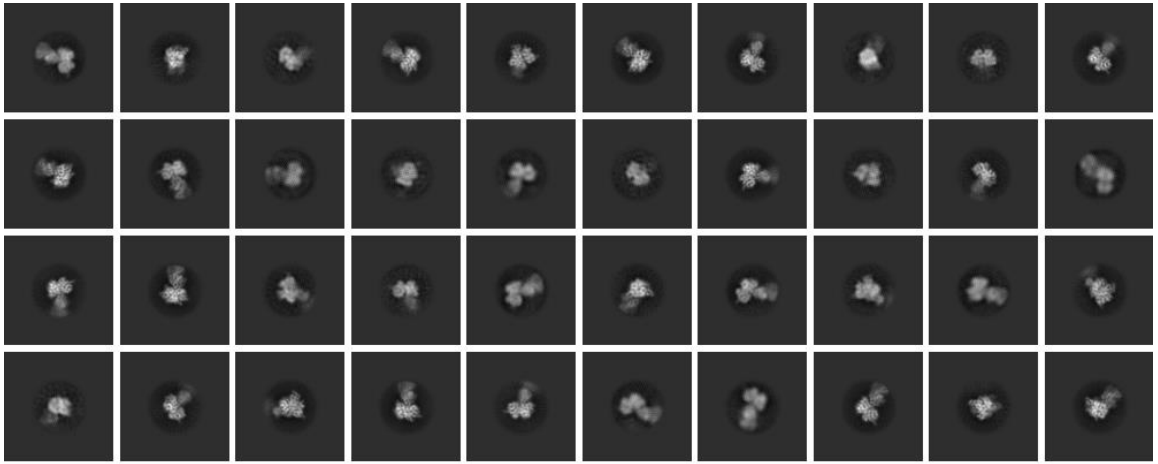
Trip 5

Trip 6…

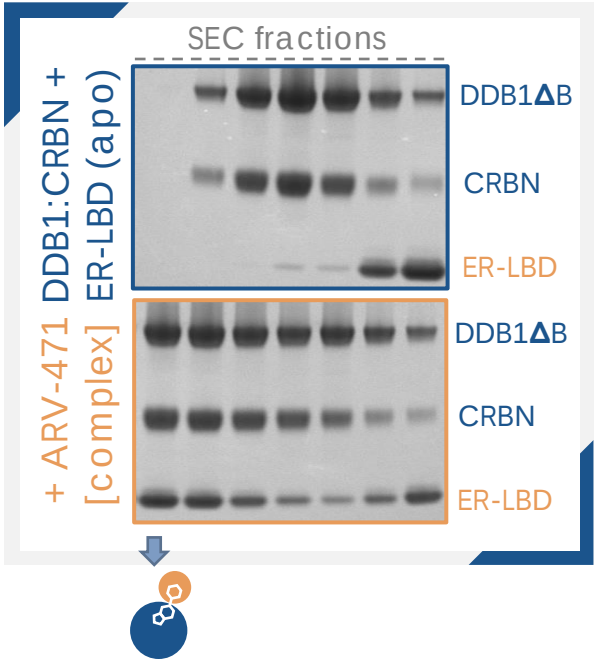


- 2D classification of crosslinked complex yields DDB1/CRBN and little hint of ER

Ultra-fast vitrification yields first evidence of ER in ternary complex by cryoEM



- 2D classification yields ~356k good particles
- Non-uniform refinement
- 3D variability analysis and cluster display



- ER:ARV-471:CRBN complex frozen using Chameleon™ grid prep

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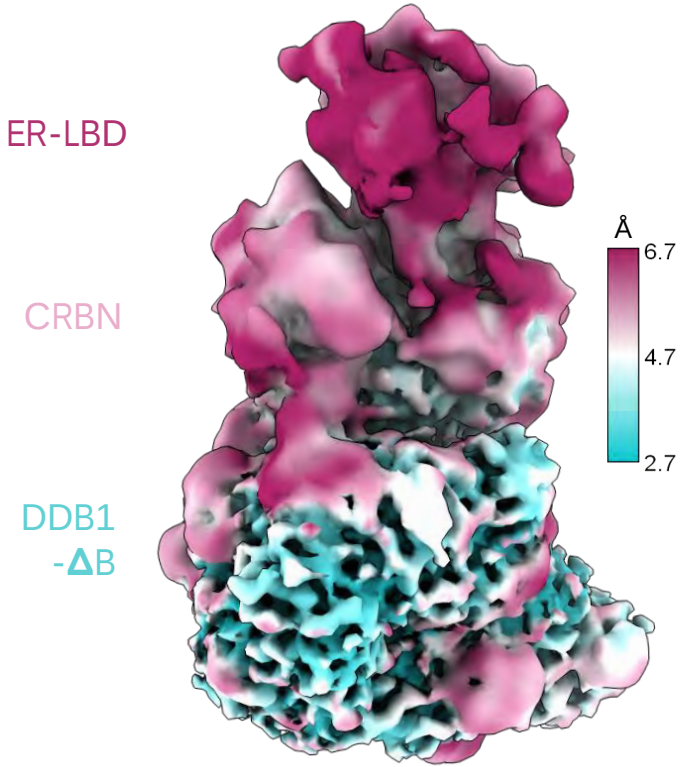
** single particle cryoEM on Krios™ G4 (E-CFEG) / Selectris Falcon 4 / CryoSPARC on AWS / ThermoFisherScientific cryoEM collaboration*

Mechanistic insights into a clinical stage PROTAC

ARV-471: Induces proximity between CRBN E3 ligase & the estrogen receptor, leading to ER degradation



- Highly dynamic ternary complex as imaged by cryoEM
- ER is flexible, and it is not possible to define a single ER binding pose
- **CRBN in "closed"** conformation
- DDB1 resolved to 2.7Å

** Chameleon™ grid prep / single particle cryoEM on Krios™ G4 (E-CFEG) / Selectris Falcon 4 / CryoSPARC on AWS / ThermoFisherScientific cryoEM collaboration*

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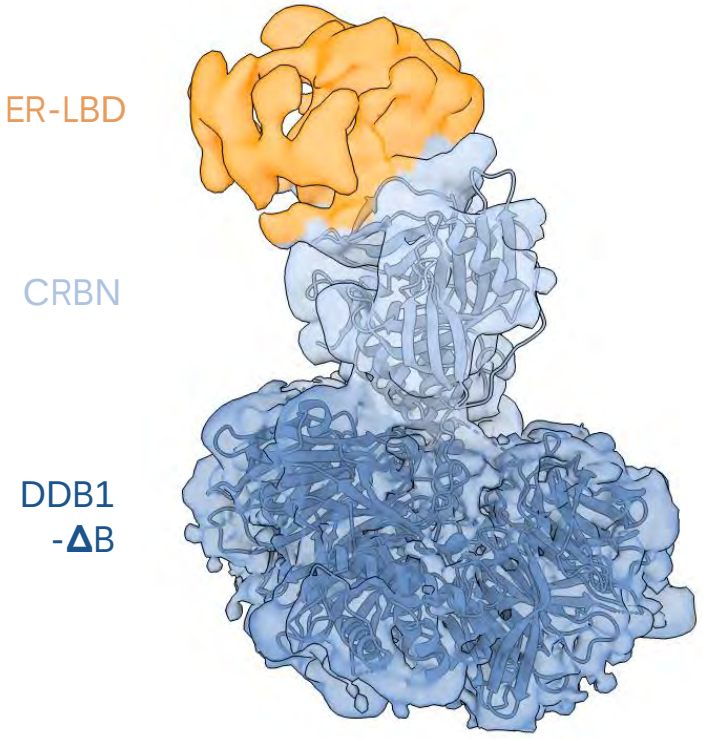

- Highly dynamic ternary complex as imaged by cryoEM
- ER is flexible, and it is not possible to define a single ER binding pose
- CRBN **in "closed"** conformation
- DDB1 resolved to 2.7Å
- ARV-471 not resolved

** ER-LBD + CRBN + DDB1 colored independently, image prepared by ChimeraX / MD fits of ternary complex not shown*

Mechanistic & structural basis of substrate-recruitment by KLHDC2

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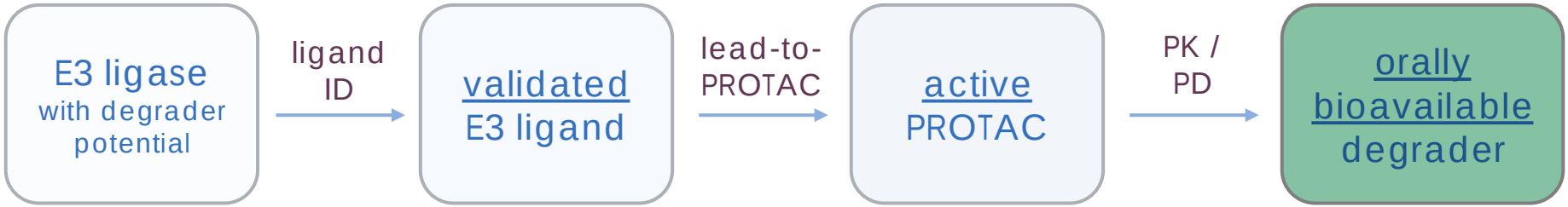
PROTAC discovery – one case study from the Arvinas E3 repertoire

The next frontier is discovering new E3 ligases for TPD – how do we discover them?

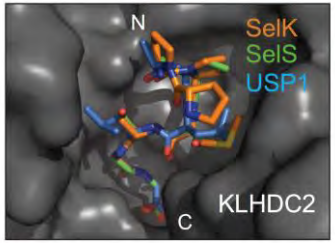


E3 ligand-to-PROTAC discovery → novel $CRL2^{KLHDC2}$ PROTAC degraders

Discovery & characterization of KLHDC2 ligands for PROTAC applications:

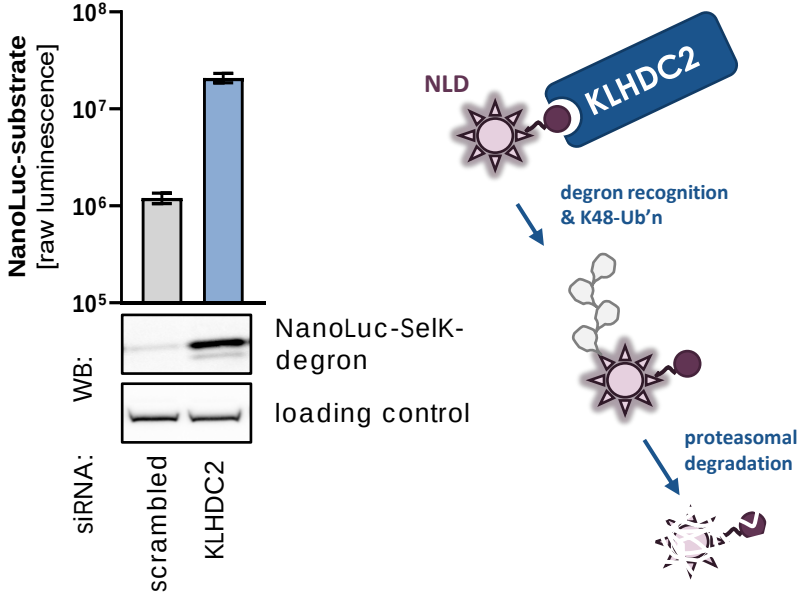
1) Rapid *de novo* ligand design by CADD & ligand evolution
2) Ligand-to-PROTAC conversion & on-mechanism activity validation
3) Mechanistic & structural understanding of E3 assembly

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



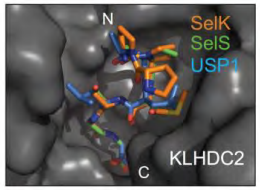
- KLHDC2 is a CRL2-associated substrate receptor
- KLHDC2 has been shown to recognize C-terminal glycine residues as a high affinity degron
- C-term Gly recognition has been structurally elucidated

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

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

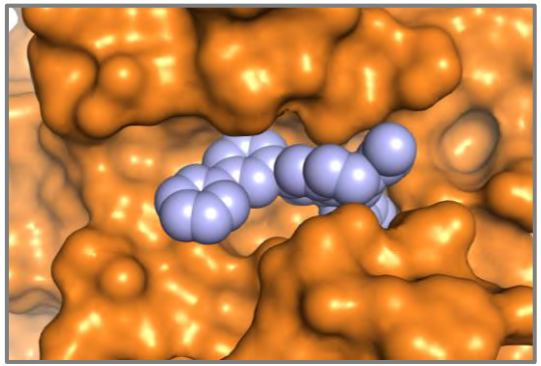
compound Z

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

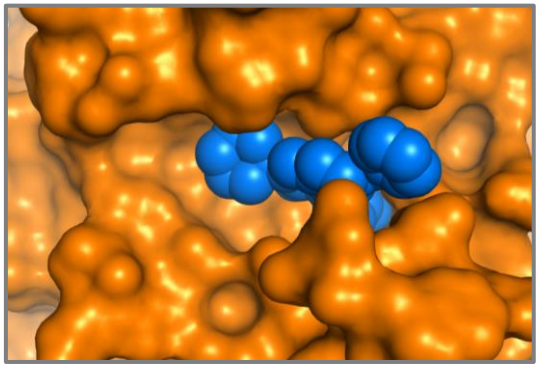
E3-dead

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

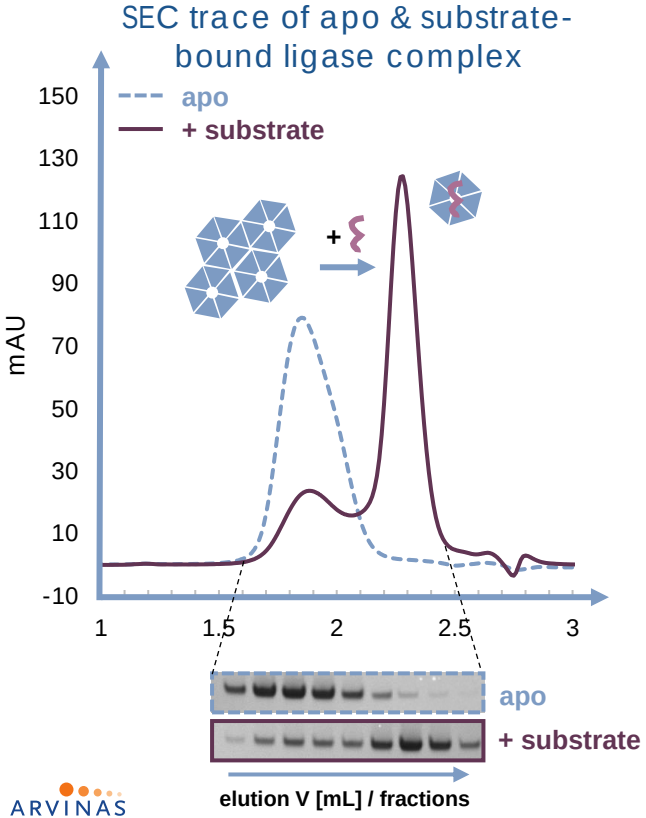


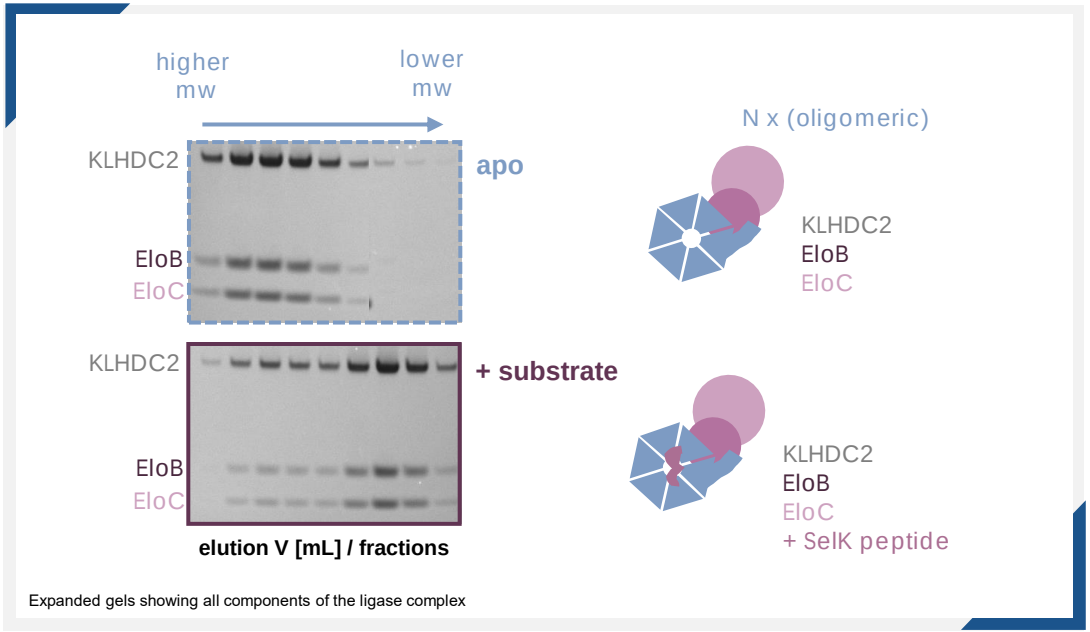
$KLHDC2_{KD}$: compound Y @ 1.8 Å



$KLHDC2_{KD}$: compound W @ 1.6 Å

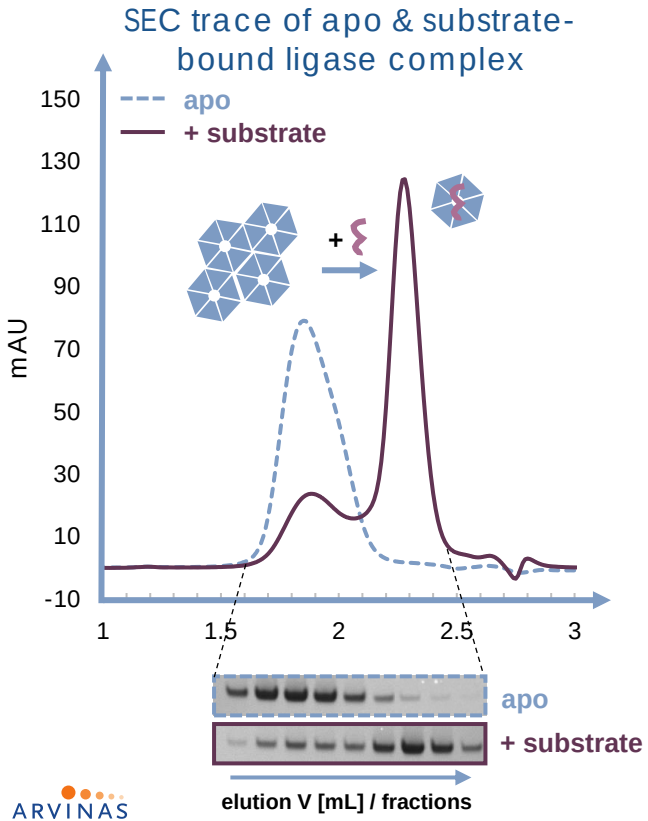
The full-length KLHDC2/EloB/EloC ligase complex is a dynamic oligomer

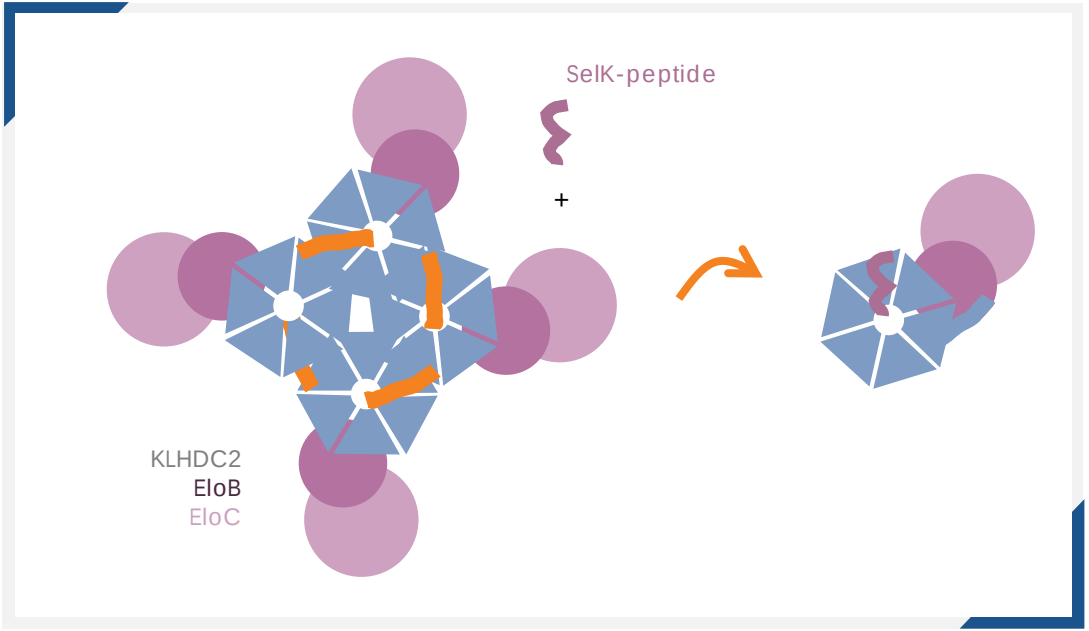




- apo KLHDC2/EloB/EloC ligase complex is oligomeric
- SelK-peptide-bound KBC complex shifts to a smaller size (as by measured by SEC)

The KLHDC2/EloB/EloC complex is self-regulated.

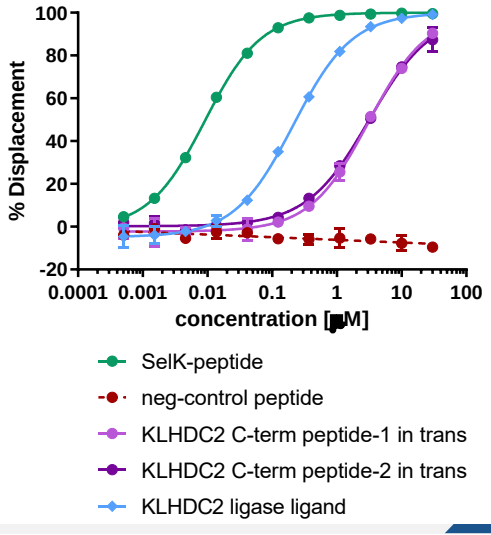




- The C-terminus of KLHDC2 ends in -GlySer
- The substrate (SelK) peptide ends in -GlyGly
- A possible scenario: loosely held together complex via KLHDC2 C-term is outcompeted by a substrate

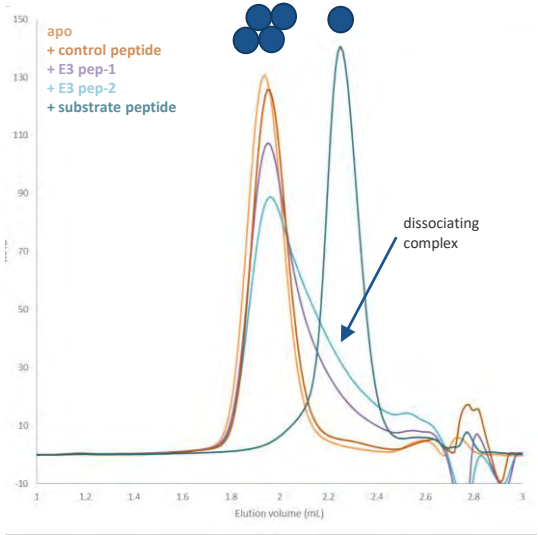
KLHDC2 can bind itself *in trans*

KLHDC2:SelK displacement assay



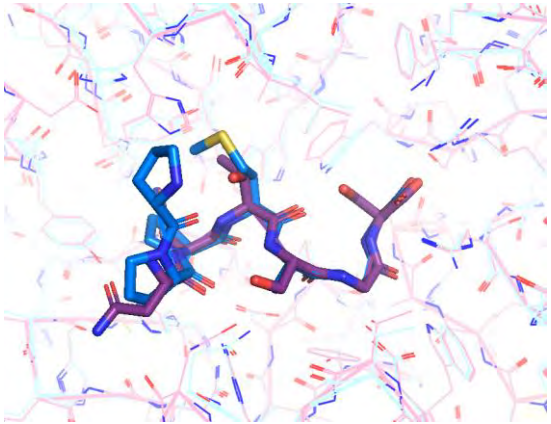
KLHDC2 C-term peptides display low affinity to KLHDC2

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

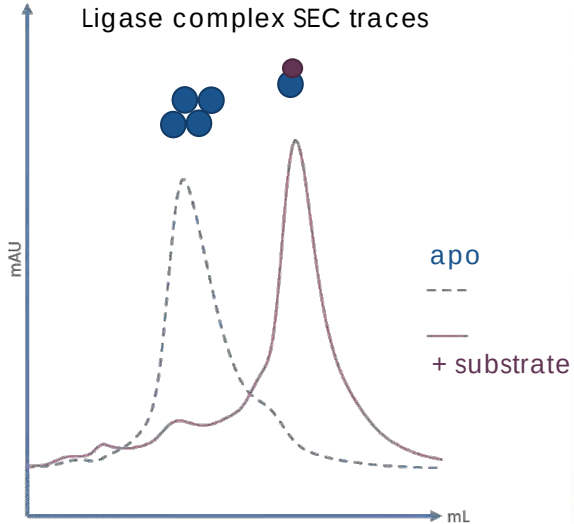
KLHDC2 C-term co-crystallized with $KLHDC2_{KD}$, adopting the conformation of the SelK peptide

Oligomeric KLHDC2 complex organization is dynamic upon substrate binding, which can be recapitulated by small molecule ligand binding

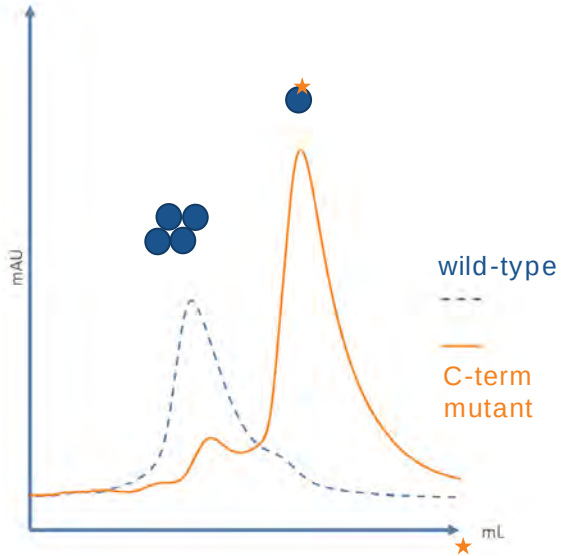
KLHDC2 oligomerization altered by high affinity substrate binding

KLHDC2 oligomerization altered by a C-terminal mutant

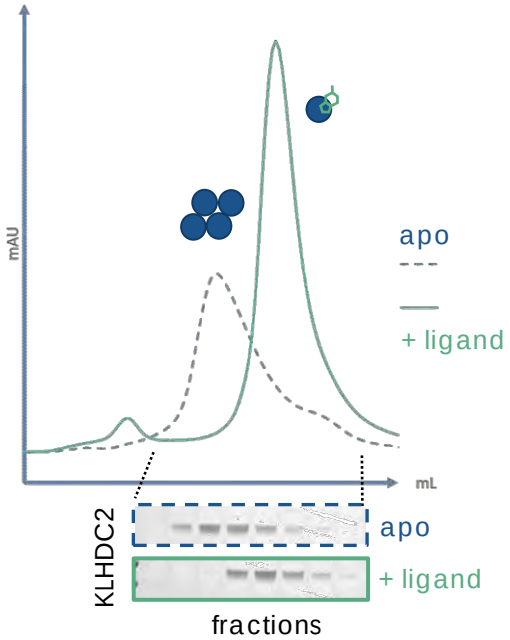
KLHDC2 oligomerization can also be altered by small molecule ligands



KLHDC2/EloB/EloC complex is a dynamic oligomer

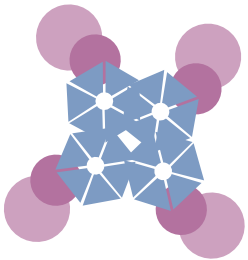


C-terminal KLHDC2 mutant purifies as a monomer

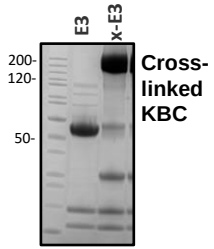


CryoEM structure of the apo KLHDC2/EloB/EloC complex reveals a tetrameric arrangement, consistent with the model

model based on biochemistry



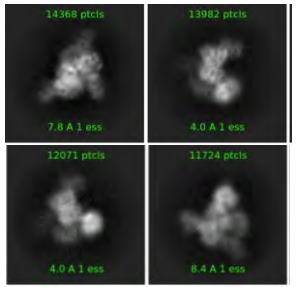
crosslinked apo KBC

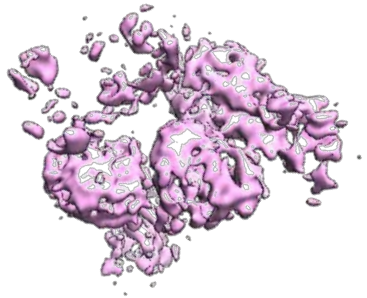


2D images

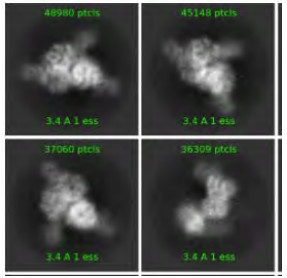
Final map

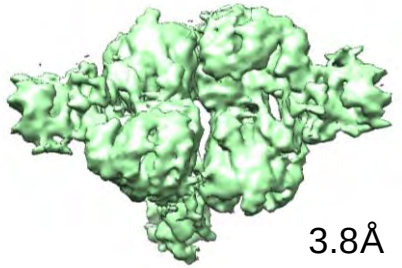
Frozen with Vitrobot™





Frozen with Chameleon™

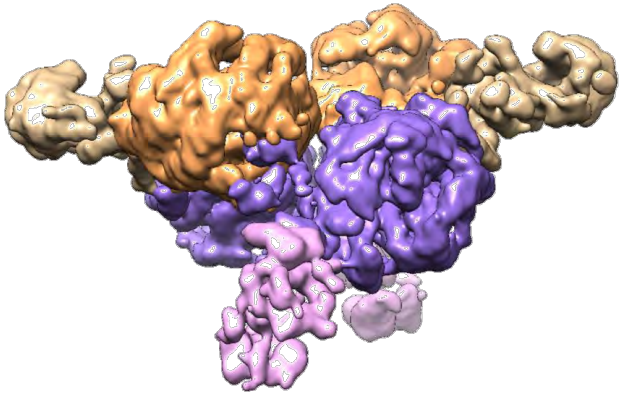




3.8Å

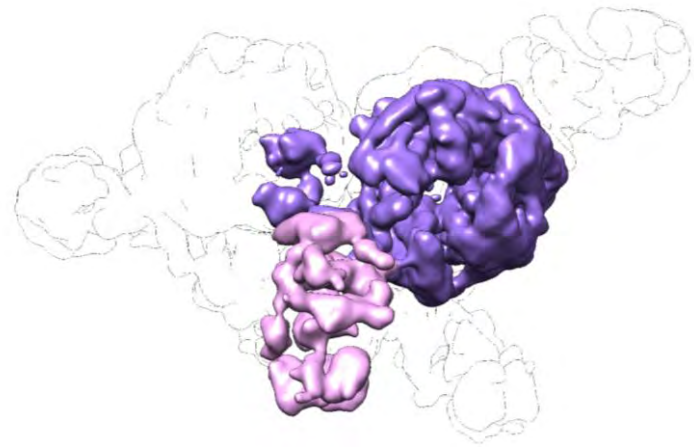
New Slide –new content

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



KLHDC2 EloBC adapter x 2

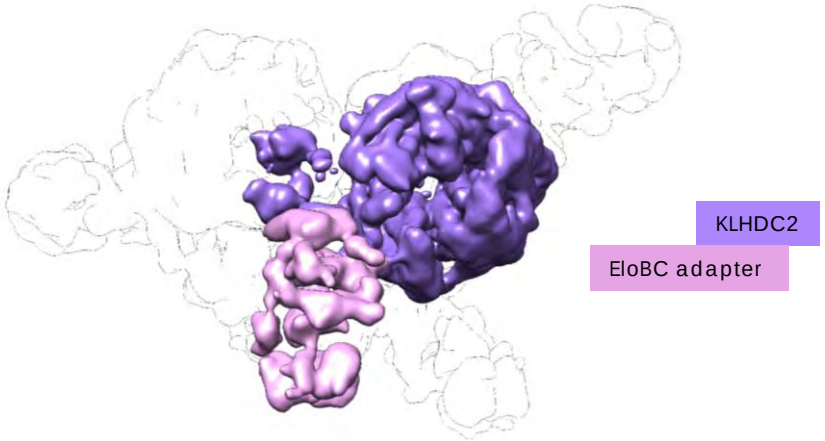
KLHDC2 EloBC adapter x 2



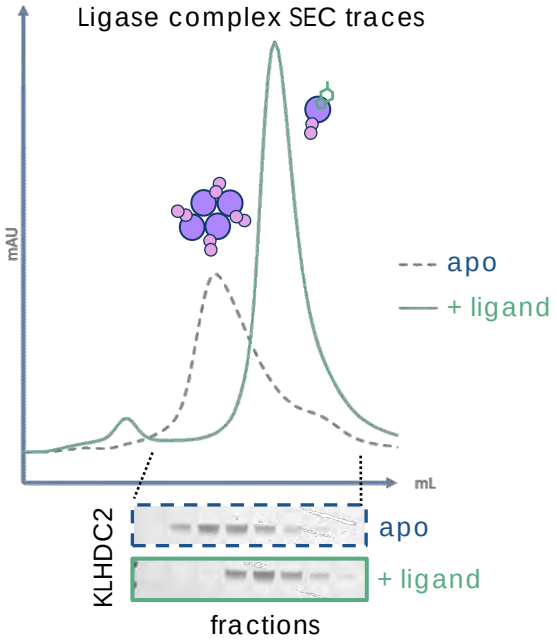
KLHDC2 EloBC adapter

- 4 individual KLHDC2/EloB/EloC complexes have good density & can be visualized in the final complex
- Focusing on one KBC reveals an extended C-terminus of KLHDC2

KLHDC2 targeting small molecules alter oligomeric assembly of KBC



KLHDC2 oligomerization can <u>also</u> be altered by high affinity small molecule ligands

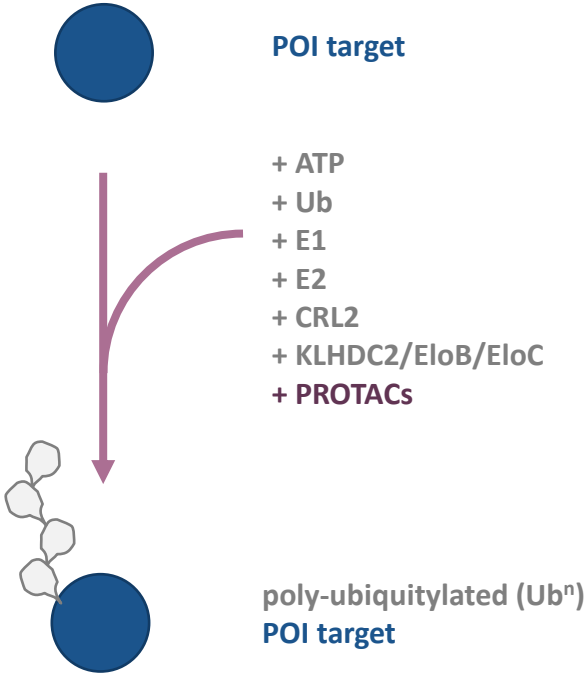


Continuing to look at assembly of:

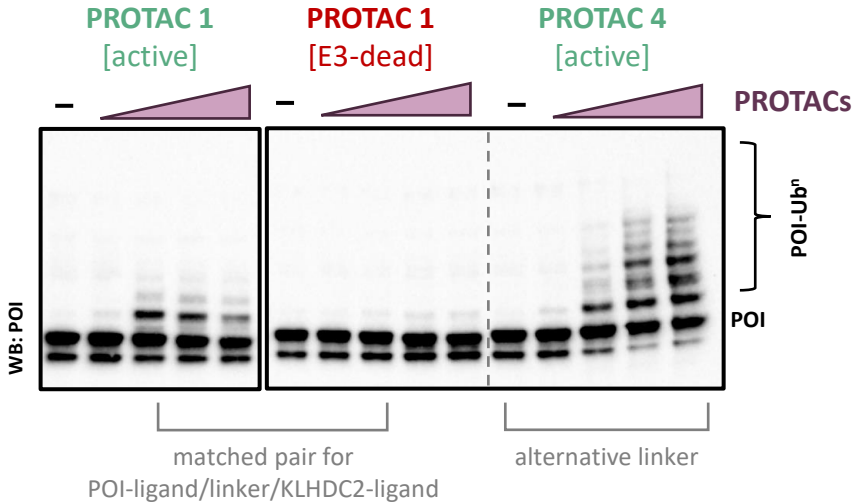
- KBC bound to substrate-peptides
- KBC bound to small molecules
- KBC bound to PROTACs & PROTAC-POI complexes
- KBC bound to full CRL2 complex -/+ substrates/cmpds

→ understanding these offers insight into PROTACs

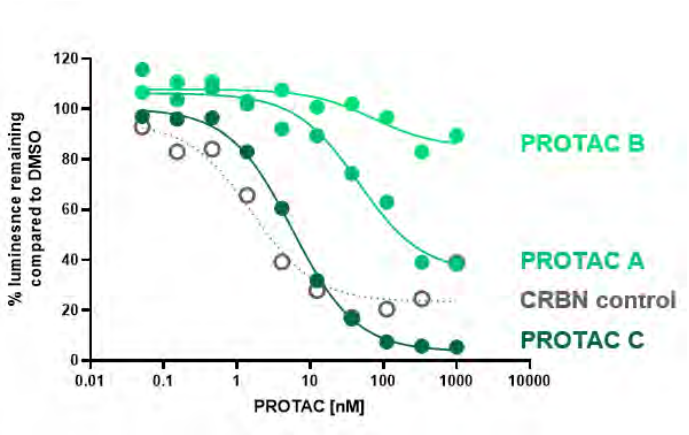
PROTACs based on KLHDC2 ligands ubiquitylate target proteins

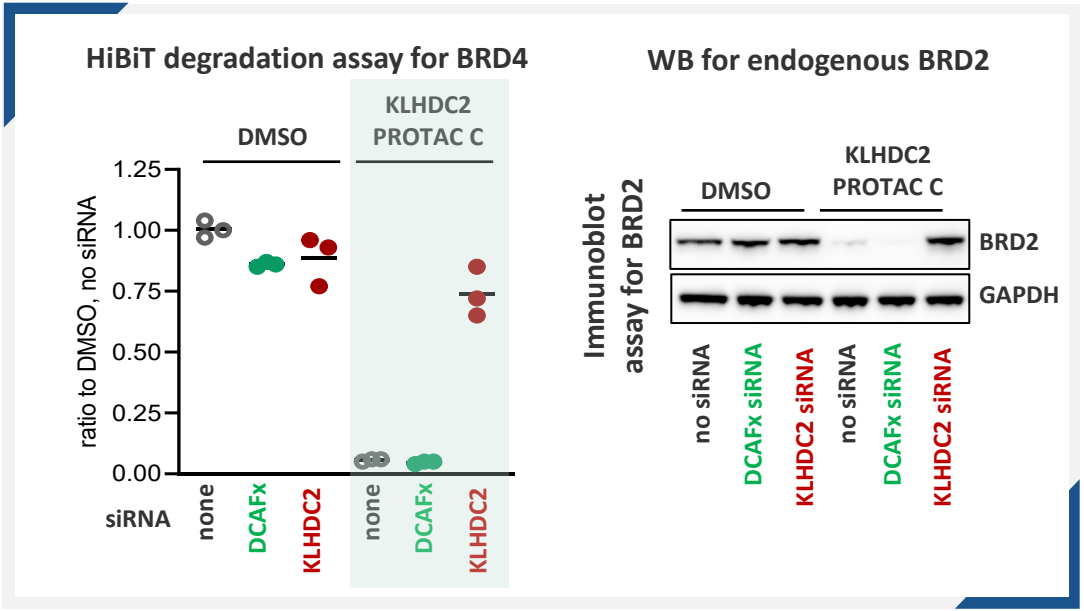


Using purified, full-length KLHDC2/EloB/EloC complex in cell-free, biochemical ubiquitination assays, PROTACs ubiquitylate a target in an KLHDC2-recruitment-dependent manner



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

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

- This 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!