Accelerating Discovery Success with Structure-Guided Drug Design: A worked example from a challenging DNA-repair target, Artemis

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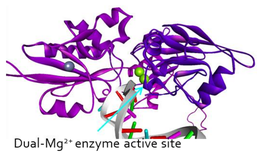
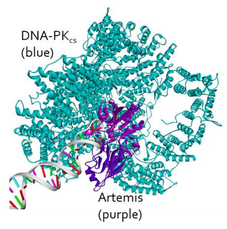
Introduction

Artemis, a nuclear protein, was first identified as the gene defective in a subset of severe combined immune deficiency (SCID) patients that were hyper sensitive to radiation. Therefore, Artemis is an attractive target for the development of therapeutics to manage various B cell and T cell tumors
Artemis-PKcs complex repairs chromosome breaks and in the complex Artemis trims the DNA for joining by ligation. Nonhomologous DNA end joining (NHEJ) major pathway for the repair of double-strand breaks (DSBs) during most of the cell cycle. The DNA-dependent protein kinase catalytic subunit (or DNA-PKcs) tightly regulates Artemis by tethering to DNA-PKcs surface. The complex is activated when it encounters a broken DNA end. The Artemis catalytic domain enters a large cavity in the center of DNA-PKcs.

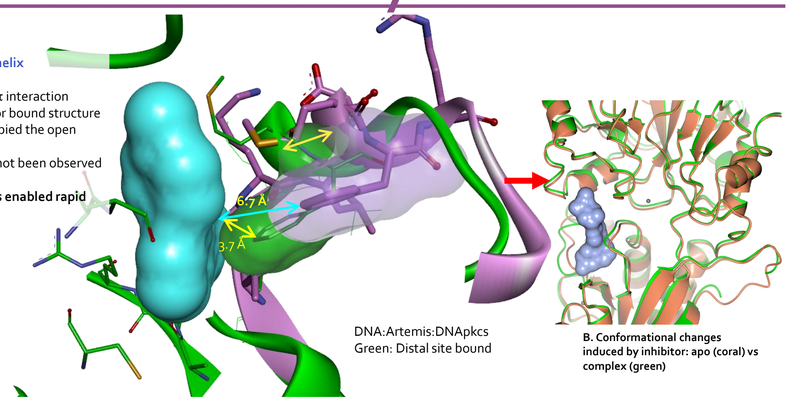




Go Watanabe, Michael R. Lieber, Dynamics of the Artemis and DNA-PKcs Complex in the Repair of Double-Strand Breaks, J Mol Bio,434, 2022, 167858, https://doi.org/10.1016/j.jmb.2022.167858.

Distal Site Co-Crystal Structures

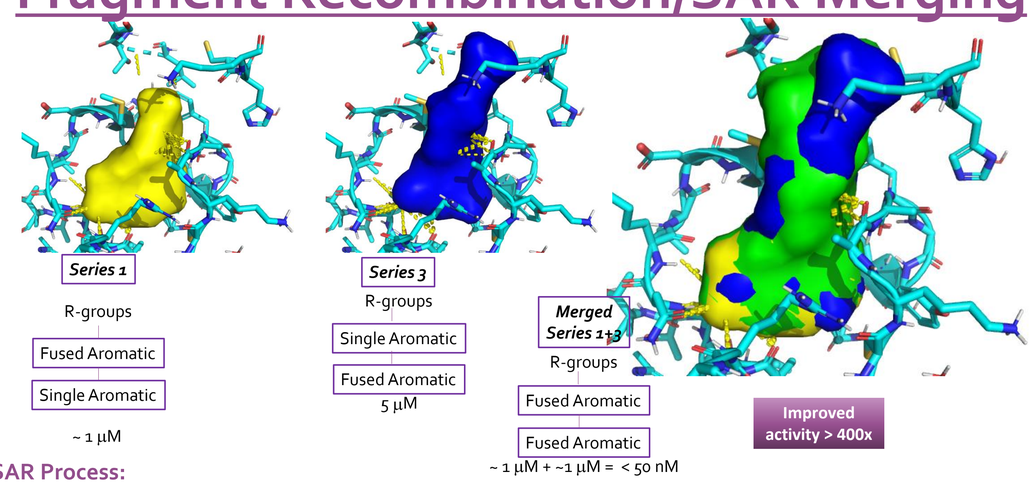
~3Å movement of short helix
And Phe235
Phe235 forms an edge to π interaction
Observed in every inhibitor bound structure
When distal site is unoccupied the open conformation is observed
The 3A motion so far has not been observed in MD simulations
Co-crystals of the 3 series enabled rapid SAR development



DNA:Artemis:DNApkcs
Green: Distal site bound

B. Conformational changes induced by inhibitor: apo (coral) vs complex (green)

Fragment Recombination/SAR Merging



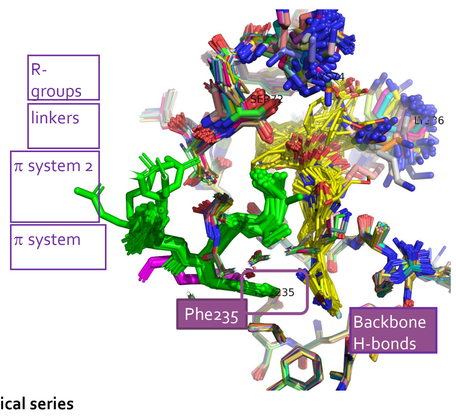
SAR Process:

After arriving at a plateau in activity, ring features from Series 1 and 3 were combined to provide a new series with super-additive activities.

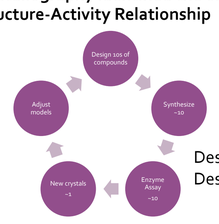
Observations from 89 co-crystal structures

A single ensemble of protein structures represents 4 Series of compounds
A narrow manifold of protein geometries
Cα rmsd ~ 0.4-5 in binding site

Induced fit of Phe235 consistent key π interaction
Low-dielectric portion of binding site-a few key electrostatic interactions
SAR aligns each core in binding site
Diverse cores bind to nearly identical protein conformations
Allows fragment re-combinations



- **Efficiency driven by finding a few good chemical series**
  - Identifies the structure-activity relationship
- **Crystallography identified the correct Protein-Structure-Activity Relationship**



SAR Process: Collaborative Structure-Driven SAR Optimization

Design/synthesize test is a parallel process
Design is a week or 2 ahead of new data

Acknowledgements

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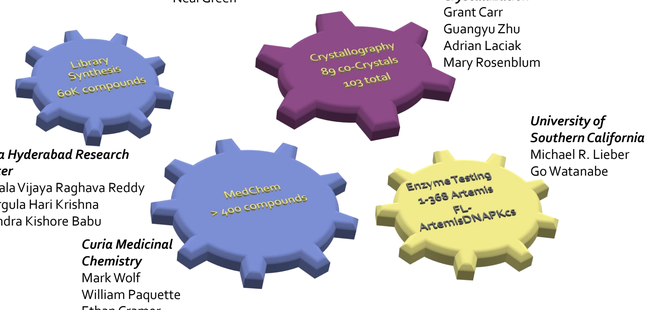
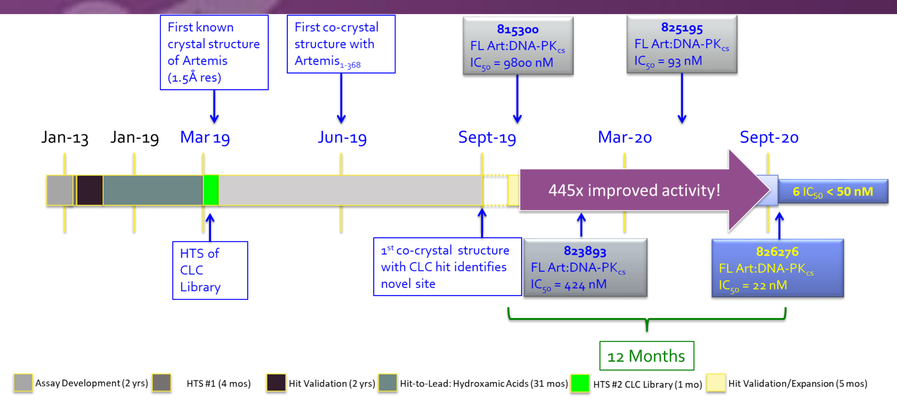




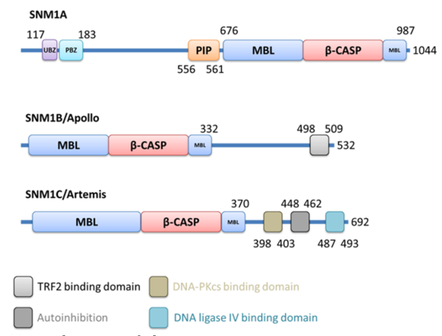
Protein Expression and Structural Biology

Artemis was an unproven target to block DNA-repair in oncology. Structure based drug discovery of inhibitors was the preferred route however after attempts by multiple labs, progress was slow due to difficulties in protein production, lead chemical matter and lack of experimental protein structures. Curia's integrated drug discovery team was asked to try and get a crystal structure within a 6 month time frame and if successful, proceed with structure enabled compound design of inhibitors for Artemis.

Proof-of-concept questions:
Is Artemis inhibition with a small molecule sufficient to prevent DNA-repair in cells?
Will Metal-binding, active site compounds provide sufficient inhibition?
Is Artemis inhibition site "druggable"?
Exploratory research for approximately 6 years lead to low nM inhibitors with single metal binding series.

Goals

Structural enablement
Additional good chemical starting points
Integrated and rapid SAR development
Potent enzyme inhibitors to test in cellular models



Homology model:

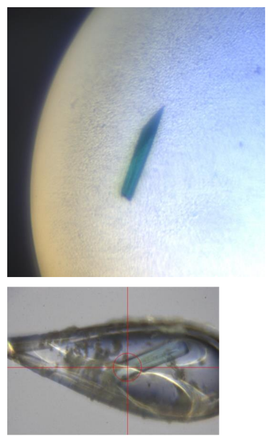
Apollo Nuclease, SNM1A or SNM1B,
32% identity to *Artemis* (SNM1C)

Artemis Production

Full length Artemis is 692 amino acids:
Insect cell expression of 3 different constructs
Residues 1-368 Successful construct
Unsuccessful: Residues 1-470 and 1-506
Purification Scheme
- His-affinity column
- Dialysis/TEV cleavage
- Size exclusion chromatography
- Resource S
- Final purity was about >95%

Artemis Crystallization Screening

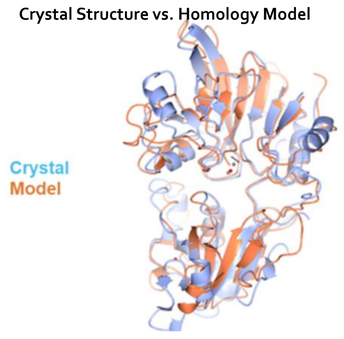
Initial Crystallization in 96-well Screening Format
Protein (only) with different concentrations
Variety of commercially available screens
>7,000 crystallization conditions
Different temperatures (4°c and 23°c)
Digested proteins (limited proteolysis)
Protein with ligands (peptides, DNAs -Cy5 labeled (blue) , inhibitor compounds)



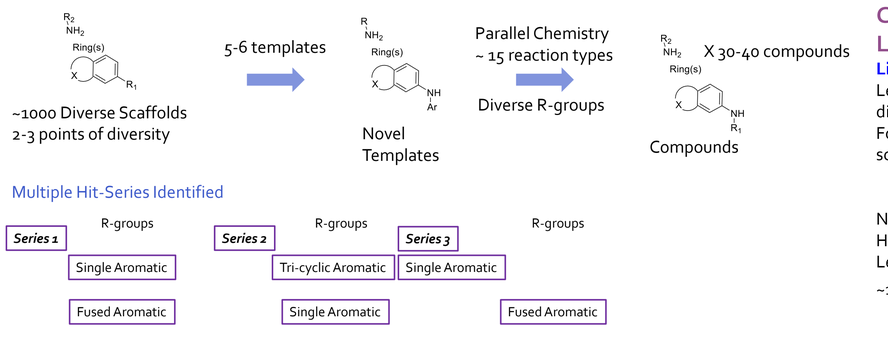
First Artemis-inhibitor Complex Structure: Active Site Inhibitor

Partial density of DNA was observed in structure. DNA binds to Artemis weakly in the structure and prohibited inhibitor diffusion to the active site. Therefore, the DNA molecule was optimized for crystallization.
Artemis inhibitors were soaked into the crystal and electron density of the inhibitor was observed.
The acidic inhibitors bind well to the metal ions at the active site and ~20 complex structures of metal binding inhibitors were obtained. However, there was a lack of consistent interactions with the protein which drove very flexible binding orientations.
Curia obtained the first Co-crystal structures of Artemis.

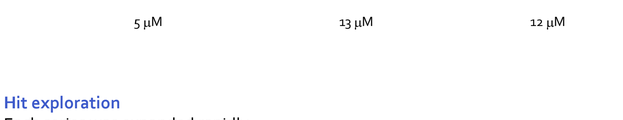
Crystal Structure vs. Homology Model



Searching for New Starting Points: Second HTS



Multiple Hit-Series Identified



Curia Compound Library Consortium (CLC) 60K Library

Library Properties and Composition
Lead-like libraries selected by a 5-member consortium of drug discovery organizations
Focused on producing a structurally diverse set of *lead-like* screening compounds
- No focus on particular biological classes
- No metal binding groups

New compounds not in the literature
High sp3-content, MW < 450; low clogP
Lead-like compound library
~1-2% of Fragment-like compounds

Hit exploration

Each series was expanded rapidly
No obvious metal binding groups
3 Series with Modest SAR: Wide range of IC50's
Without structural information SAR development requires extensive experimentation
10x IC50 improvement over initial hits

Second Co-Crystallization Campaign

Co-crystal structures of each series

The CLC library provided the Hit-series
Crystallography enabled the SAR development by providing the binding site of weak initial hits
Collaborative design and evaluation of compounds with computational support
~2500 design ideas evaluated in these series
- "MedChem" Transformations
- Library enumeration & one-off's
  - ~400 synthesized and tested
- Simple docking and scoring
  - 2-5 crystal structures predicted nearly all geometries

Conclusions

The combination of a novel HTS library and rapid co-crystallization of early hits identified a novel binding site in Artemis distant from the metallo-enzyme active site. This binding site undergoes a small but significant backbone shift that favors non-metal-binding compounds. Co-crystals of new medicinal chemistry analogs allowed a rapid improvement of the inhibition to low nM IC50's. Additionally, this structure-driven SAR identified novel combinations of scaffolds from the original hits. The Curia team of structural biologists, medicinal and computational chemistry was able to improve potency ~500 fold while creating novel chemical matter.

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