

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

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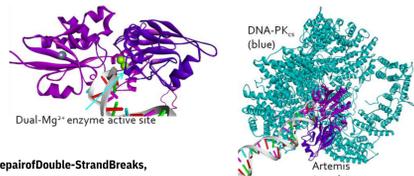
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Introduction

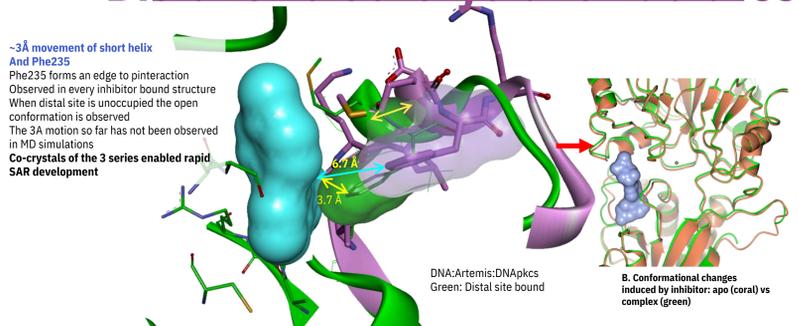
Artemis, a nuclear protein, was first identified as the gene defective in a subset of severe combined immune deficiency (SCID) patients that were hyper sensitive to radiation. Therefore, Artemis is an attractive target for the development of therapeutic to manage various B cell and T cell tumors

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.

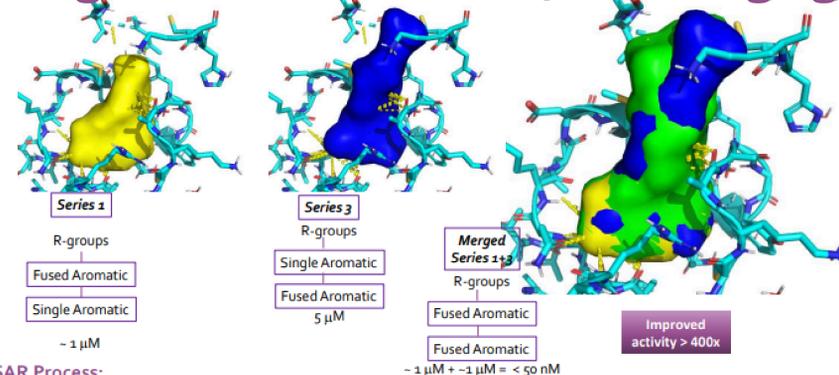


Go Watanabe, Michael R. Lieber, Dynamics of the Artemis and DNA-PKcs Complex in the Repair of Double-Strand Breaks, J Mol Biol, 434, 2022, 167858, https://doi.org/10.1016/j.jmb.2022.167858.

Distal Site Co-Crystal Structures



Fragment Recombination/SAR Merging

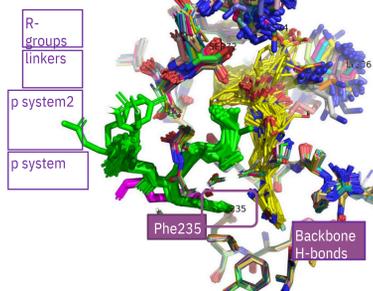


SAR Process:

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.

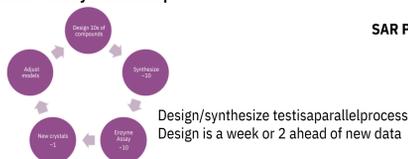
Observations from 89 co-crystal structures

A single ensemble of protein structures represents 4 Series of compounds
A narrow manifold of protein geometries
Camsd ~ 0.4-5 in binding site
Induced fit of Phe235 consistent key pinteraction
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

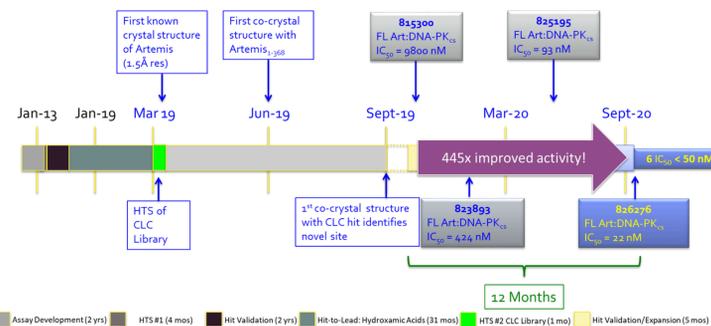


- Efficiency driven by finding a few good chemical series
 - Identifies the structure-activity relationship
- Crystallography identified the correct Protein-Structure-Activity Relationship

SAR Process: Collaborative Structure-Driven SAR Optimization



Acknowledgements



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 timeframe and if successful, proceed with structure enabled compound design of inhibitors for Artemis.

Proof-of-concept questions:

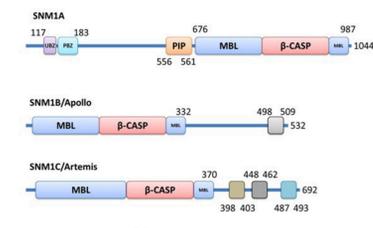
Is Artemis inhibition with a small molecule sufficient to prevent DNA-repair in cells?
Will Metal-binding, active site compounds provide sufficient inhibition?

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



TRF2 binding domain DNA-PKcs binding domain
Autoinhibition DNA ligase IV binding domain

Homology model:

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

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.

Artemis Production

Full length Artemis is 692 amino acids:

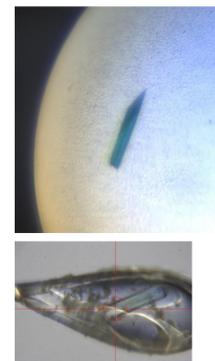
Insect cell expression of 3 different constructs
Residues 1-368 Successful construct
Unsuccessful: Residues 1-470 and 1-506

Purification Scheme

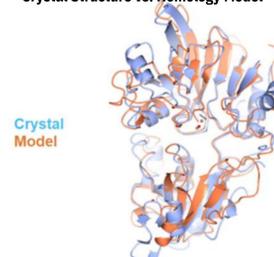
His-affinity column
Dialysis/TEV cleavage
Size exclusion chromatography
Resource S
Final purity was about >95%

Artemis Crystallization Screening

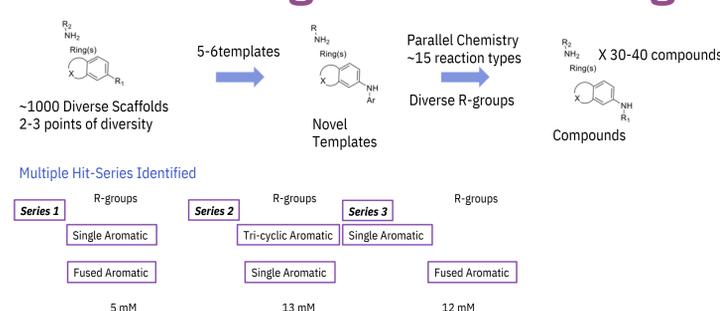
Initial Crystallization in 96-well Screening Format
Protein (only) with different concentrations
Variety of commercially available screens
>7,000 crystallization conditions
Different temperatures (4°C and 23°C)
Digested proteins (limited proteolysis)
Protein with ligands (peptides, DNAs -Cy5 labeled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



Crystal Structure vs. Homology Model



Searching for New Starting Points: Second HTS



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

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

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 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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