Inhibiting CK2α from outside the active site

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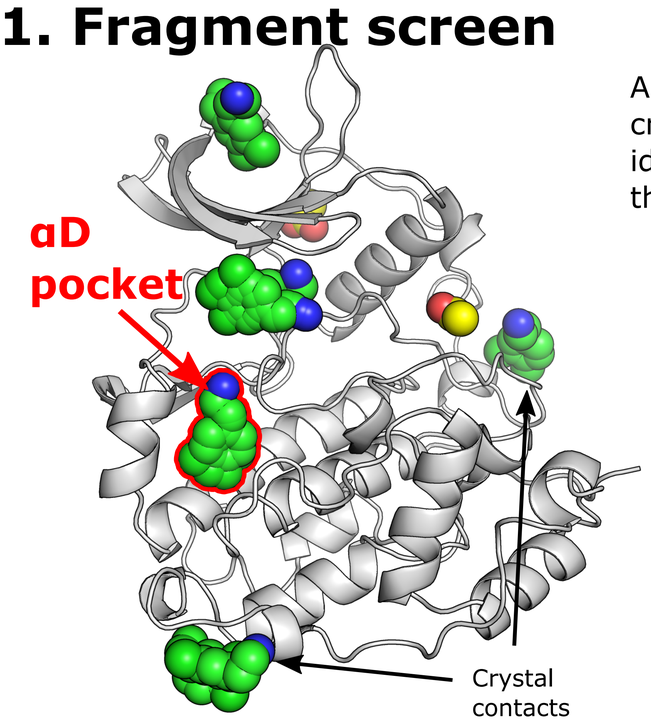
Introduction

CK2 is a highly conserved kinase with pro survival and anti-apoptotic effects on cells. It is often over expressed in cancer cells in which it promotes their proliferation by multiple mechanisms.[1-2] A number of potent CK2α inhibitors, that target only the ATP site, have been shown to inhibit the growth of a range of cancer cell lines and one of these, CX-4945 has progressed to phase II clinical trials.[3] Although described as highly selective CX-4945 inhibits at least 12 other kinases with nanomolar IC_{50}s. Strategies to inhibit CK2α without targeting the ATP binding site offer the promise of enhanced selectivity as well as new mechanisms of action. Here we report the creation and characterisation of a unique inhibitor of CK2α that targets a novel cryptic pocket and was developed using fragment based methods.

1. Fragment screen



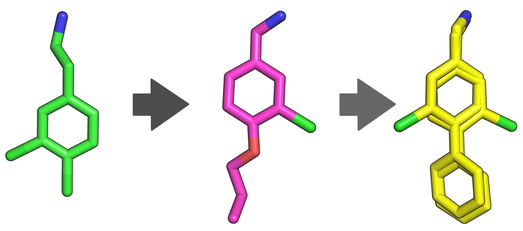
A high concentration X-ray crystallographic screen identified a novel site behind the αD loop.

Scan the QR code to see videos.

1. **Fragment binding**

2. Fragment growth

Aim: Increase selectivity and affinity for the αD pocket



K_d = 630μM → 250μM

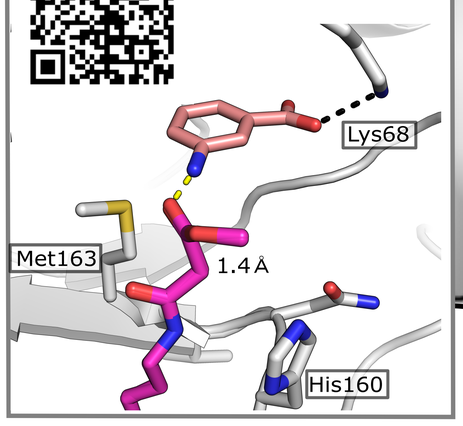
Final fragment has
-significantly increased affinity
-increased selectivity for the αD site

3. Linker design

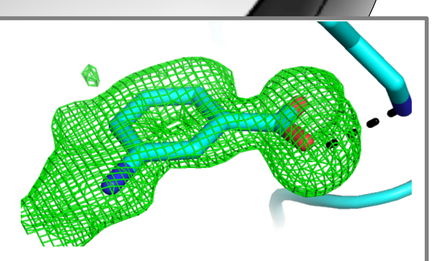
Aim: link the ATP and αD site fragments

A library of linkers was designed and tested.

A channel that connects the 2 sites was discovered, allowing the use of shorter, more efficient linkers.



4. ATP site fragment



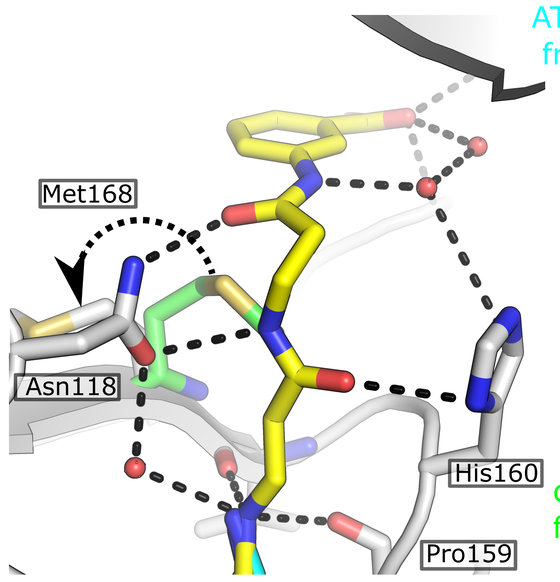
Aim: Identify a fragment in the ATP site to link to the αD pocket.

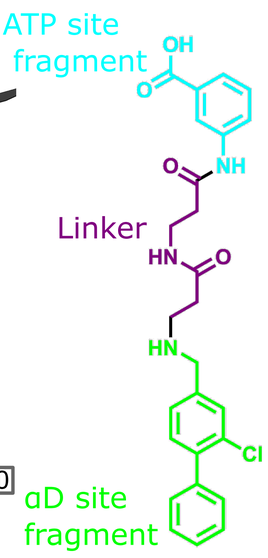
A weakly binding fragment was chosen so that the binding would be dominated by the none conservered αD site therefore giving an inhibitor specifc for CK2α.

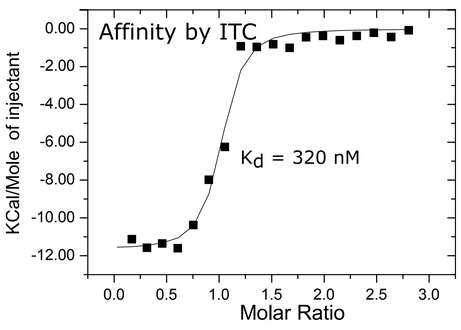
5. Linked compound: CAM4066

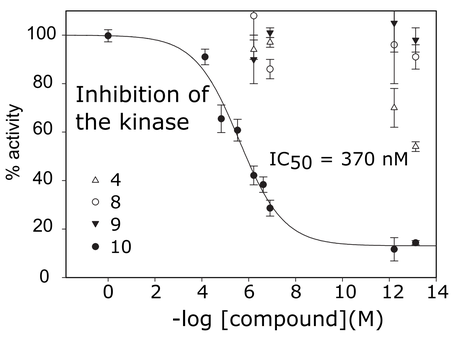
CAM4066 succesfully linked the αD pocket and the ATP site with a greater than 1000-fold improvement on the K_d of the isolated fragment

The linker forms an efficent hydrogen bonding next work with the channel linking to the ATP site



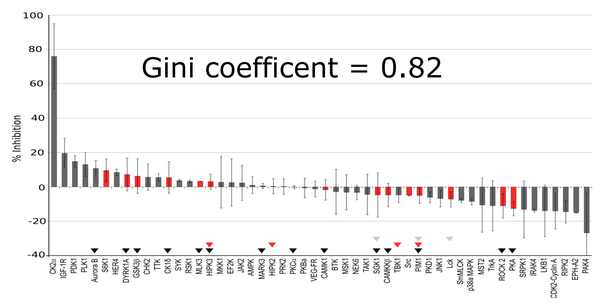




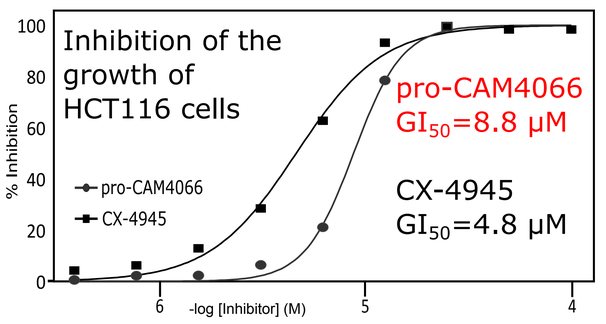


6. CAM4066-Validation

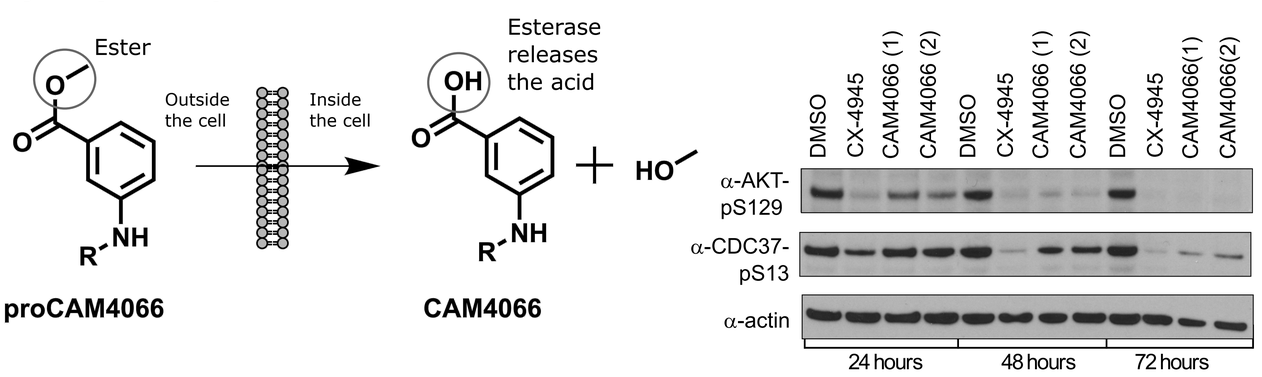
Selectivity screen
CAM4066 was screened against 52 diverse kinases
No significant off target inhibition was detected > Gini coefficient of 0.82
CAM4066 is the most selective CK2α inhibitor to date.



ProCAM4066
An ester prodrug form of CAM4066 was active in cells:
- It inhibited cell growth at silmilar levels to the clinical trials candidate CX-4945.
- It showed good inhibition of specific CK2α phosphorylation targets in HCT116 cells.



Hydroysis of proCAM4066



Acknowledgements

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