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Unusual binding modes of two inhibitors to their target enzymes human leukocyte elastase (HLE) and protein kinase CK2 revealed by protein crystallography

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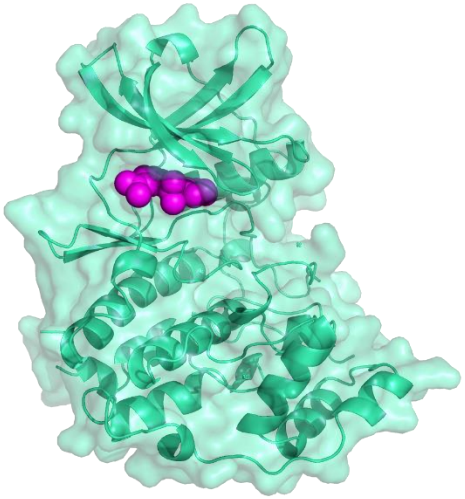
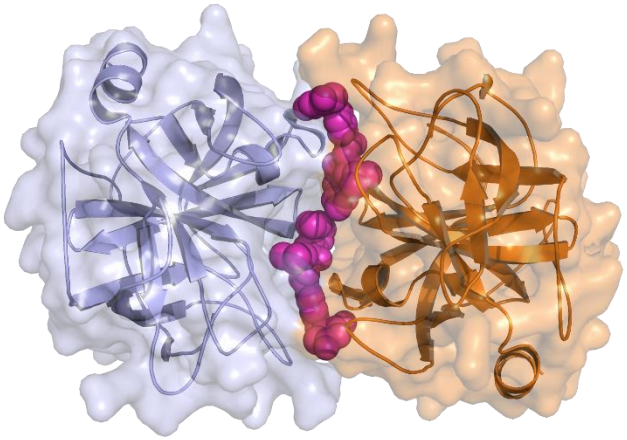
Unusual binding modes of two inhibitors to their target enzymes human leukocyte elastase (HLE) and protein kinase CK2 revealed by protein crystallography



CK2α + inhibitor 4p



HLE + inhibitor CQH

Abstract:

Tumour cells exploit the antiapoptotic activity of **CK2** in order to escape cell death. The indeno[1,2-*b*]indole scaffold is a novel lead structure for the development of CK2 inhibitors addressing the ATP-binding site of the protein kinase subunit CK2α. *In silico* 3D-modelling of the binding modes of a number of indeno[1,2-*b*]indole-type compounds predicted that the hydrophobic side is directed inwards while its hydrophilic part is solvent accessible. In the crystal structure of the CK2α/indeno[1,2-*b*]indole complex we observed a reversed binding mode of the inhibitor. This molecular arrangement requires an inhibitor orientation in which hydrophobic substituents are at the outer surface, which opens the possibility for further modifications.

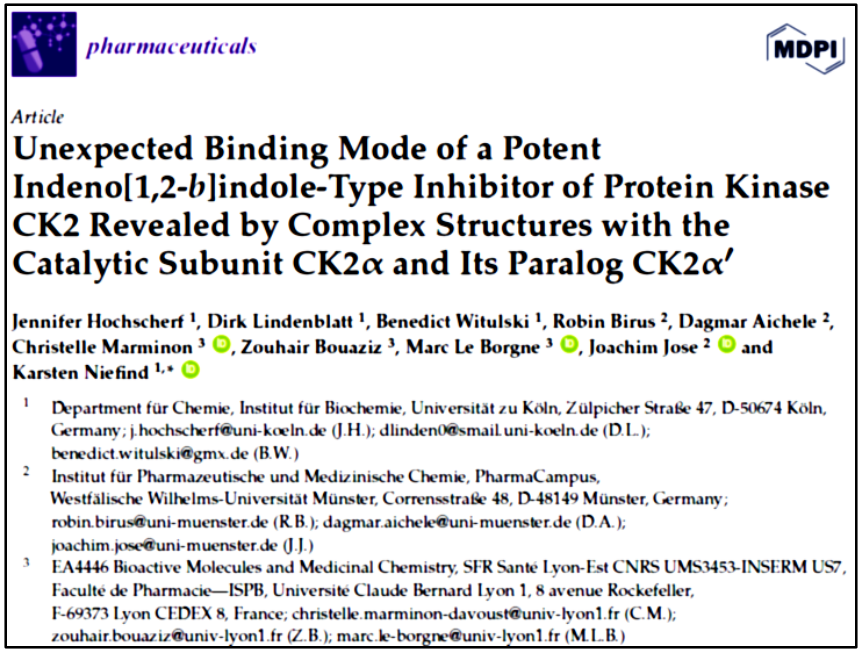
Human leukocyte elastase (**HLE**) is a chymotrypsin-type serine protease produced by neutrophilic granulocytes. The activity of HLE is strictly controlled to avoid proteolytic damage of the connective tissue, which is a particular problem in chronic obstructive pulmonary disease (COPD). Synthetic HLE inhibitors are useful in cases of imbalance of the natural HLE control system and typically block its S1 pocket. We co-crystallized HLE with a 1,3-thiazolidine-2,4-dione derivative inhibitor and observed that the inhibitor is bound to the S2' site. In addition, the inhibitor seems to induce a dimerization of HLE blocking the active site.

Keywords:

protein kinase CK2; eukaryotic protein kinase inhibitors; indeno[1,2-*b*]indole scaffold; human leukocyte elastase; chronic obstructive pulmonary disease COPD; S2’ site

References

The results presented in this keynote lecture were published recently in the following articles:



pharmaceuticals MDPI

Article

Unexpected Binding Mode of a Potent Indeno[1,2-*b*]indole-Type Inhibitor of Protein Kinase CK2 Revealed by Complex Structures with the Catalytic Subunit CK2α and Its Paralog CK2α′

Jennifer Hochscherf [1], Dirk Lindenblatt [1], Benedict Witulski [1], Robin Birus [2], Dagmar Aichele [2], Christelle Marminon [3], Zouhair Bouaziz [3], Marc Le Borgne [3], Joachim Jose [2] and Karsten Niefind [1,*]

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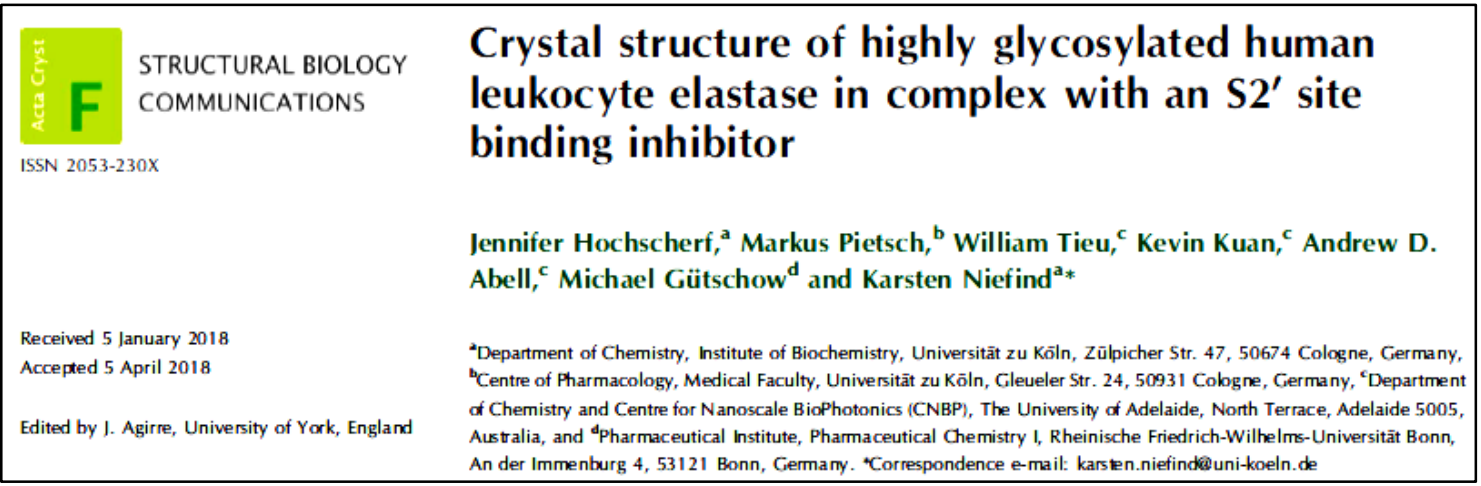
[3] EA4446 Bioactive Molecules and Medicinal Chemistry, SFR Santé Lyon-Est CNRS UMS3453-INSERM US7, Faculté de Pharmacie—ISPB, Université Claude Bernard Lyon 1, 8 avenue Rockefeller, F-69373 Lyon CEDEX 8, France; christelle.marminon-davoust@univ-lyon1.fr (C.M.); zouhair.bouaziz@univ-lyon1.fr (Z.B.); marc.le-borgne@univ-lyon1.fr (M.L.B.)

Hochscherf et al. (2017). Pharmaceuticals, 10; E98



Acta Cryst F STRUCTURAL BIOLOGY COMMUNICATIONS

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Crystal structure of highly glycosylated human leukocyte elastase in complex with an S2′ site binding inhibitor

Jennifer Hochscherf,[a] Markus Pietsch,[b] William Tieu,[c] Kevin Kuan,[c] Andrew D. Abell,[c] Michael Gütschow[d] and Karsten Niefind[a]*

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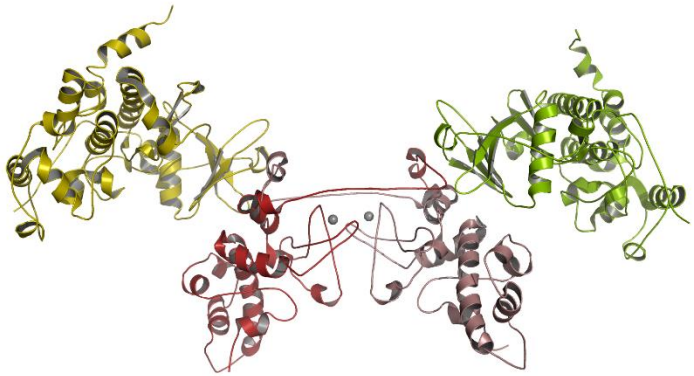
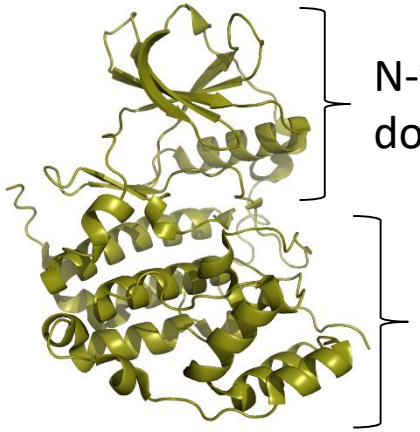
Hochscherf et al. (2018). Acta Crystallogr. F74, 480-489

Protein kinase CK2 – structure and function



CK2$\alpha_2\beta_2$ holoenzyme

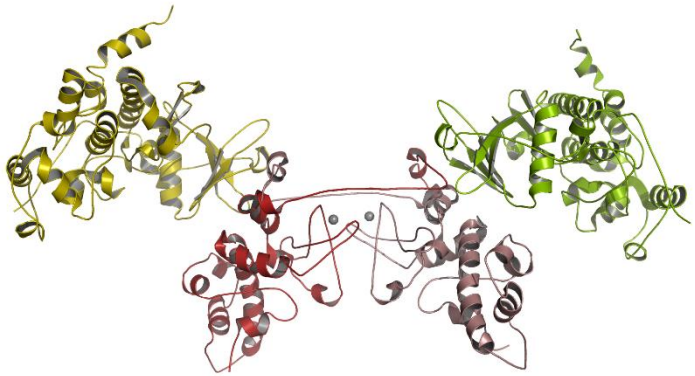
- highly conserved, acidophilic Ser/Thr kinase (CMGC subgroup of eukaryotic protein kinases)
- heterotetrameric:
  - 2 catalytic **CK2α** subunits
  - 2 non-catalytic **CK2β** subunits
- CK2α is constitutively active



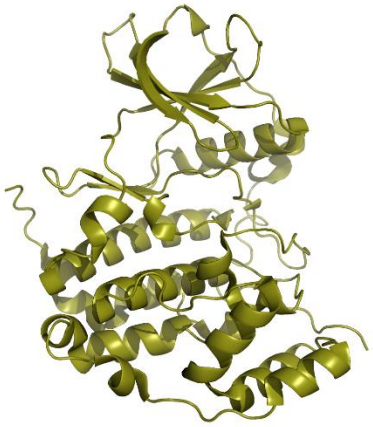
CK2α subunit

- more than 300 substrates *in vitro*
- cell cycle progression
- anti-apoptotic factor
- DNA damage repair

Protein kinase CK2 – human pathologies



CK2 holoenzyme



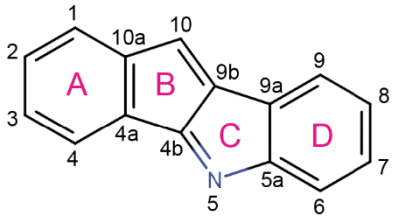
CK2α subunit

- various types of cancer
  - no oncogene
  - elevated activity contributes to cellular environment favorable for neoplesia

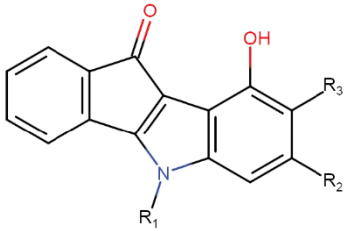
→ **development of CK2-inhibitors**

- neurodevelopmental disorders
  (*de novo* mutations)
- neurodegenerative diseases
- diabetes

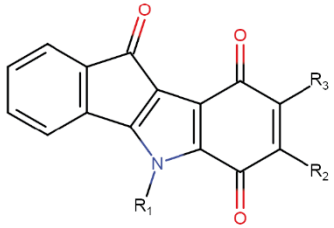
indeno[1,2-*b*]indole compounds – CK2 inhibition



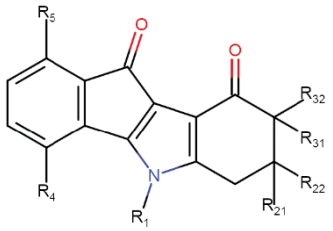
indeno[1,2-*b*]indole scaffold



phenolic scaffold



quinonic scaffold



ketonic scaffold

Bal *et al*. (2004)
***Biochem. Pharmacol.*, 68, 1911-1922**

cytotoxic for leukemia cells
- DNA-intercalator
- DNA-topoisomerase II inhibitor

Weakly affected by drug efflux!

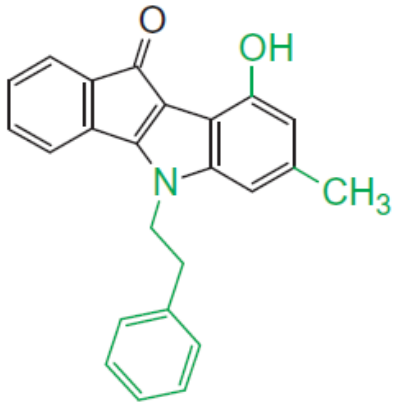
Hundsdörfer *et al*. (2012)
***Bioorg. Med. Chem.*, 20, 2282-2289**

hydrophobic scaffold resembles ATP-competitive CK2 inhibitors
→ collection of compounds with oxogroup at position 10

Tetracyclic ring system offers many functionalization opportunities

inhibitor clusters defined by: Haidar et al. (2017) *Pharmaceuticals*, 10, 8; figures modified from: Hochscherf et al. (2017) *Pharmaceuticals*, 10, 98

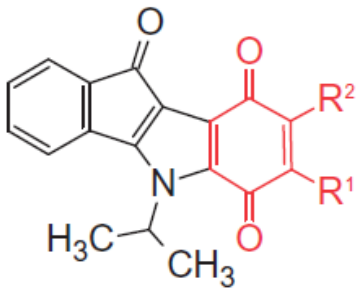
indeno[1,2-*b*]indole compounds – targeted polypharmacology



Gozzi *et al*. (2015)
***J. Med. Chem.*, 58, 265-277**

ABCG2 (also known as: breast cancer resistance protein (BCRP):

- Transporter: efflux of anti-cancer drugs
- multi drug resistance of various types of tumors
- Derivatizations at rings A,C & D



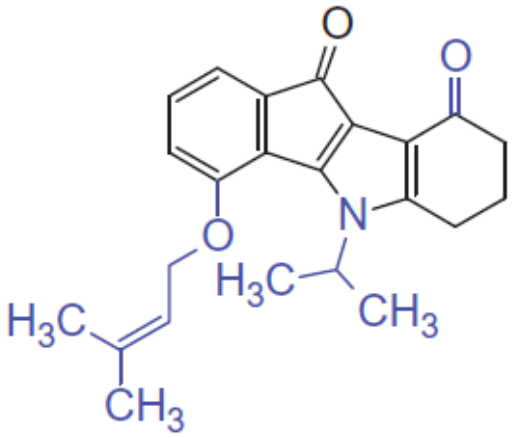
Alchab *et al*. (2016)
***J. Enzyme Inhib. Med. Chem*., 31, 25-32**

CDC25-phosphatases

- Cell cycle key phosphatases
- Cancer-relevant
- Quinonic scaffold

figures modified from: Alchab *et al*. (2016) *J. Enzyme Inhib. Med. Chem*., 31, 25-32

indeno[1,2-*b*]indole derivative inhibitor “4p”



compound „4p“

IC_{50} CK2 = 0.025 µM

IC_{50} ABCG2 = 1.6 µM

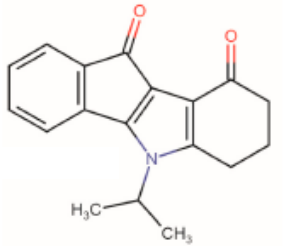
No typical anchor groups of high affinity CK2 inhibitors.

Gozzi *et al*. (2015)
***J. Med. Chem.*, 58, 265-277**

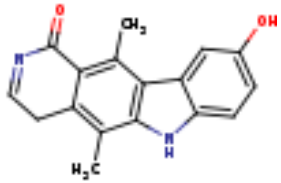
5-Isopropyl-4-(3-methylbut-2-enyloxy)-5,6,7,8-tetrahydroindeno[1,2-*b*]indole-9,10-dione

figure modified from: Alchab *et al*. (2016) *J. Enzyme Inhib. Med. Chem*., 31, 25-32

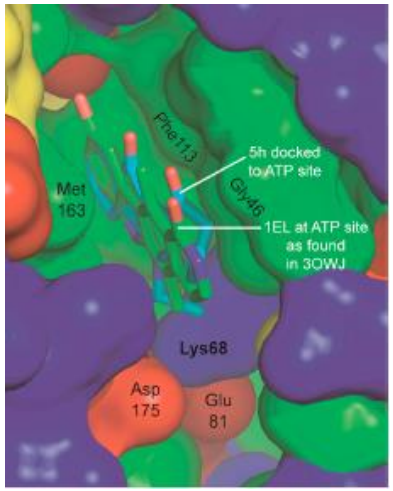
indeno[1,2-*b*]indole-type inhibitors – *in silico* 3D modelling based on a CK2α complex structure with an ellipticine derivative (PDB 3OWJ)



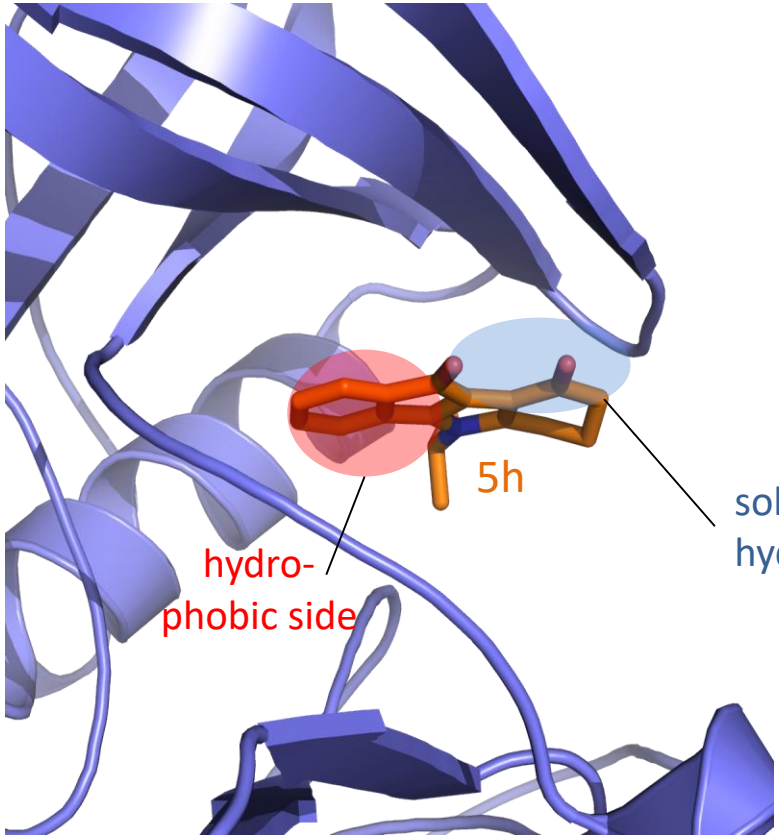
compound 5h



1-oxo-9-hydroxy-ellipticine
(from PDB 3OWJ)

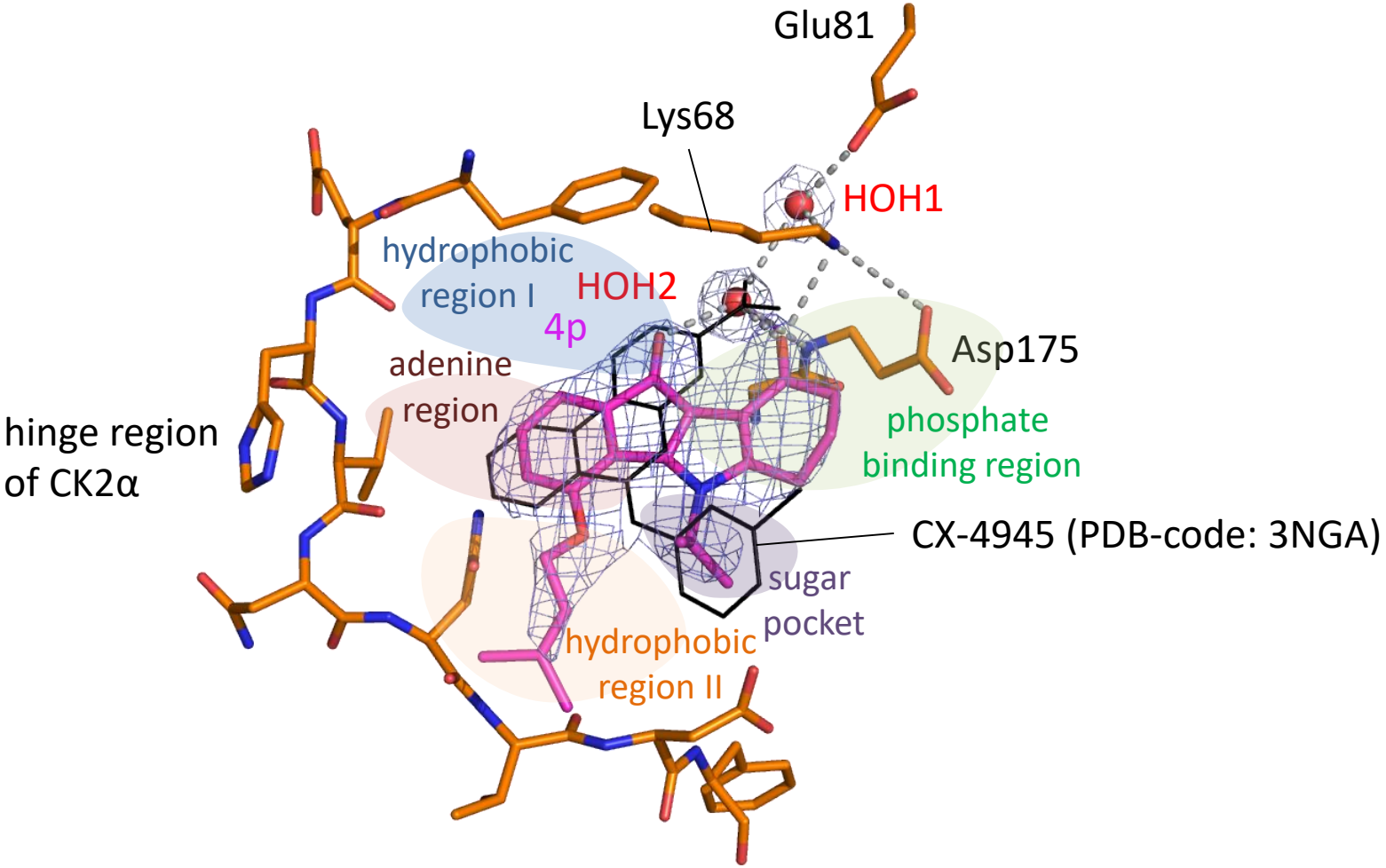


Alchab *et al*. (2015)
Pharmaceuticals, 8, 279-302



orientation of 5h
(inhibitor placed into CK2a active site similar to docking result)

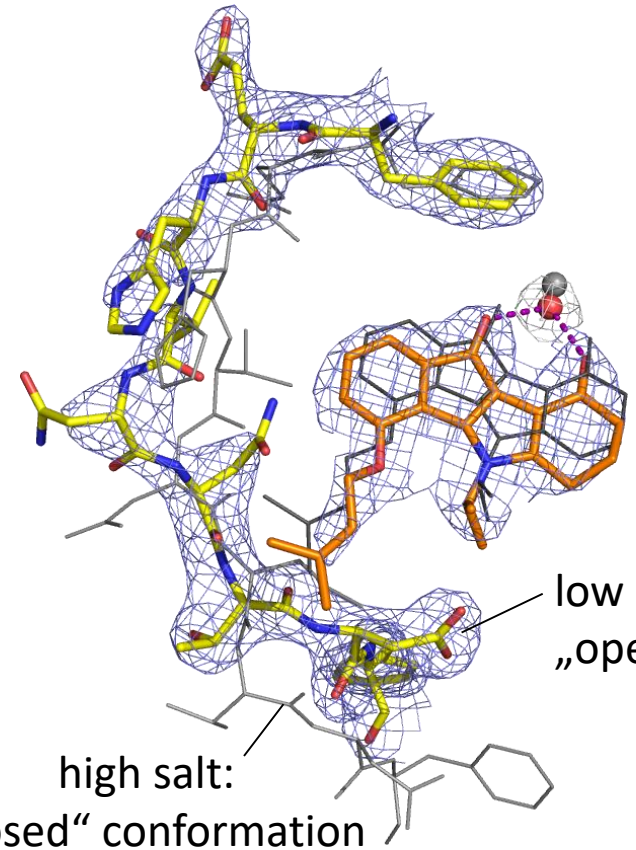
CK2α/4p complex structure with reversed binding mode: "hydrophobic-out/oxygen-in" rather than "hydrophobic-in/oxygen-out"



4p complex structure 5OMY: Hochscherf *et al.* (2017) *Pharmaceuticals*, 10, 98
CX49-45 complex structure 3NGA: Ferguson *et al.* (2011) *FEBS Lett.* 585, 104-110

CK2α/4p and CK2α‘/4p complex structures – similar "hydrophobic-out/oxygen-in" binding mode

I) Comparison of different crystallization conditions



<u>High-salt crystallization condition:</u>
4.2 M NaCl, 0.1 M citric acid, pH 5.5

<u>Low-salt crystallization condition:</u>
0.2 M ammonium sulfate, 0.1 M MES, 25 % (w/v) PEG5000, pH 6.5

4p is not selective with respect to hinge/helix αD conformation

4p complex structures 5OMY & 5ONI: Hochscherf *et al.* (2017) *Pharmaceuticals*, 10, 98

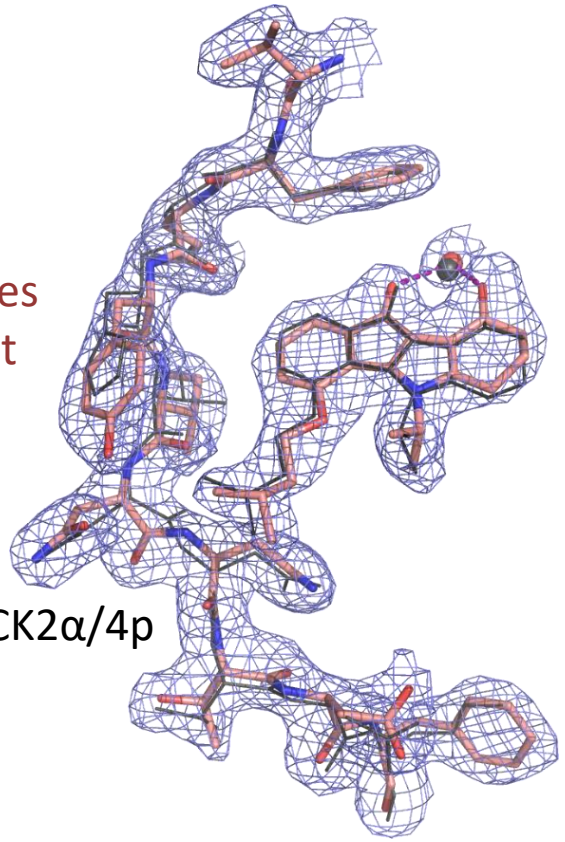
CK2α/4p and CK2α‘/4p complex structures – similar "hydrophobic-out/oxygen-in" binding mode

II) Comparison of different paralogous isoforms

- highly similar sequence and enzymatic characteristics
- differences in C-terminal region
- affinity of CK2α’ and CK2β is lower than the affinity between CK2α and CK2β
- while a knockout of CK2α in mice is embryonically lethal, a knockout of CK2α’ just leads to an impaired spermatogenesis

identical binding mode compared to low salt CK2α/4p complex

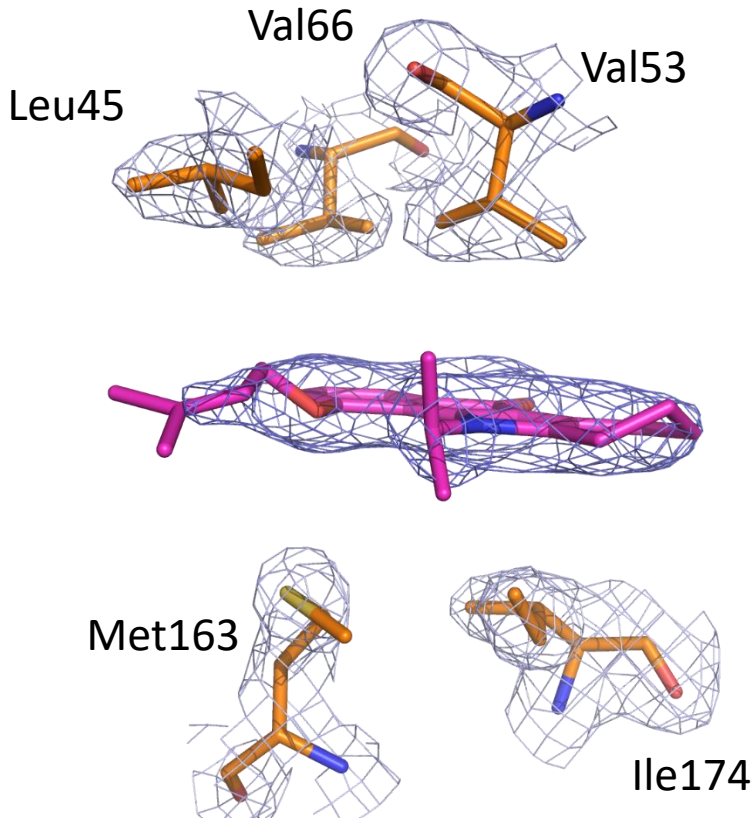
CK2α‘/4p complex (crystallizes in low-salt solution only)



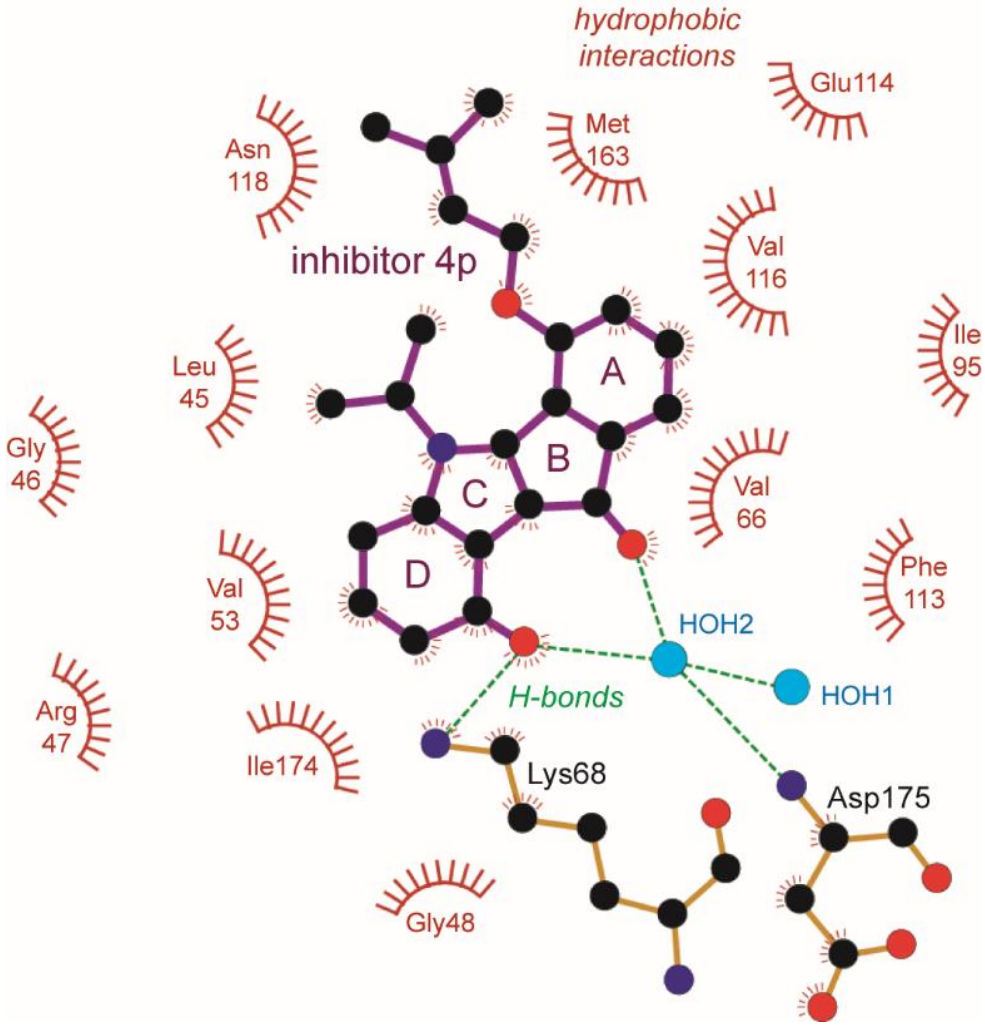
low salt CK2α/4p complex

4p complex structures 5ONI &5OOI: Hochscherf *et al.* (2017) *Pharmaceuticals*, 10, 98

CK2α/4p complex structure – hydrophobic embedding

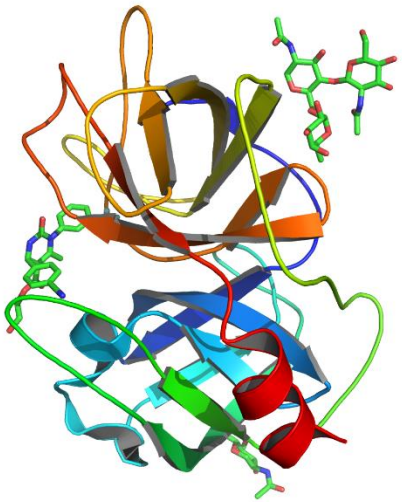


2D-projection of 4p in its CK2α environment

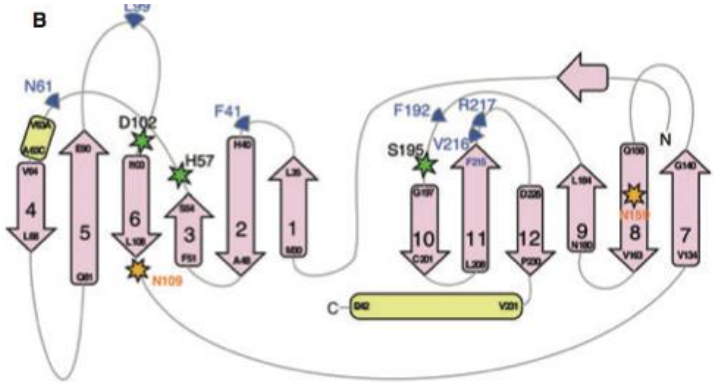


Picture produced with LigPlot+ (Laskowski et al., *J. Chem. Inf. Model.* **2011**, 51, 2778–2786.)

Human leukocyte elastase (HLE) – structure and function



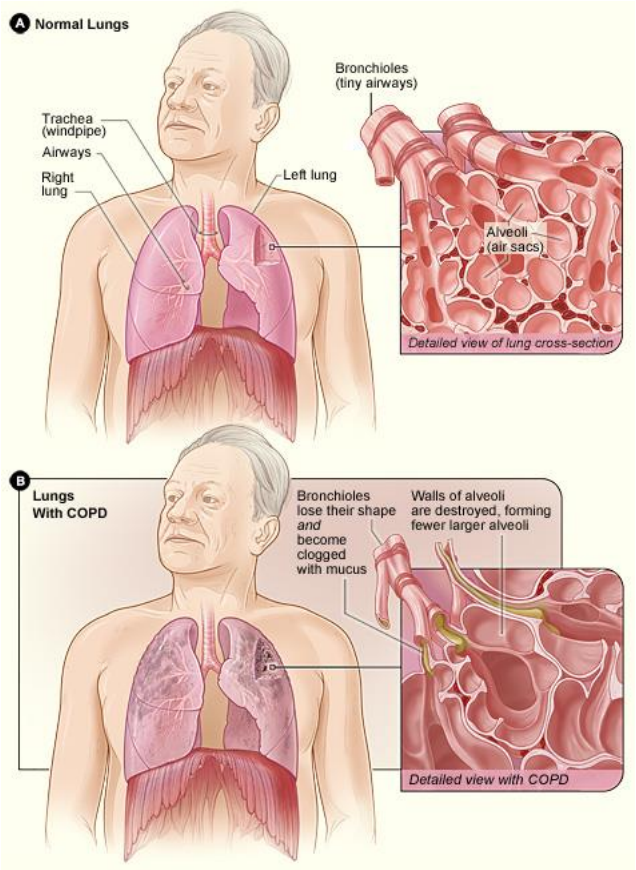
PDB: 3Q76; Hansen *et al.* (2011) *JMB* **409**, 681–691



Hajjar *et al.* (2010) *FEBS J.* **277**, 2238–2254

- chymotrypsin-type serine protease
- two 6-stranded antiparallel β-barrels
- N-glycosylation at 3 Asn side chains
- 4 disulfide bonds
- secreted by neutrophils into the extracellular space during inflammation as part of the innate immune system
- activity strictly regulated to avoid proteolytic damage of the connective tissue
  → inhibited by α_1-antitrypsin (serpin-type protease inhibitor)

Human leukocyte elastase (HLE) – COPD

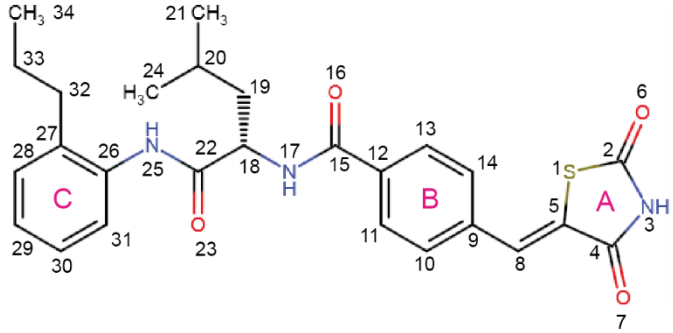


http://www.nhlbi.nih.gov/health/health-topics/topics/copd/

- COPD: Chronic Obstructive Pulmonary Disease
- Risk factors: smoking & other irritants like environmental pollution
- Chronic obstructive bronchitis, emphysema, mucus plugging
- Neutrophils & macrophages secrete a protease cocktail (HLE, proteinase 3, and macrophage-released matrix metalloproteases)
- Protease-anti-protease imbalance

→ **development of HLE-inhibitors**

1,3-thiazolidine-2,4-dione derivative inhibitor “CQH”



compound „CQH“

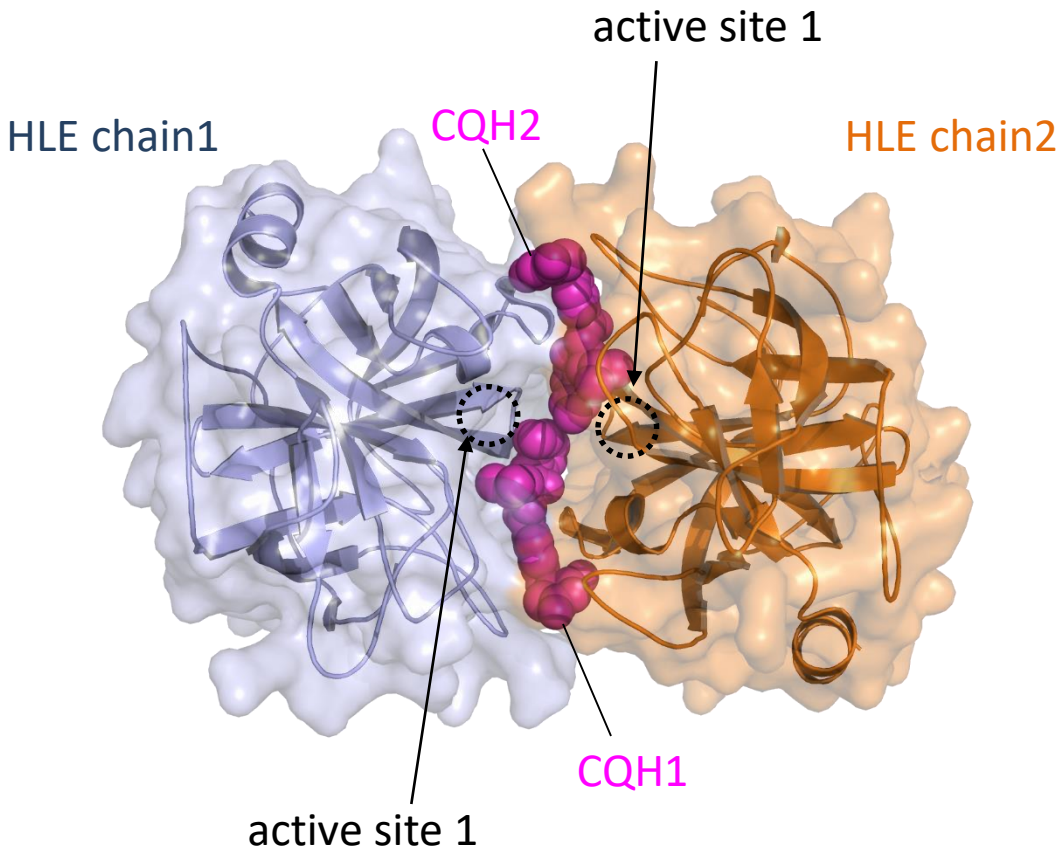
1,3-thiazolidine-2,4-dione derivative

Zvarec *et al.* (2012)
***Bioorg. Med. Chem. Lett.* 22, 2720-272**

$IC_{50,HLE} \approx 0.5\ \mu M$

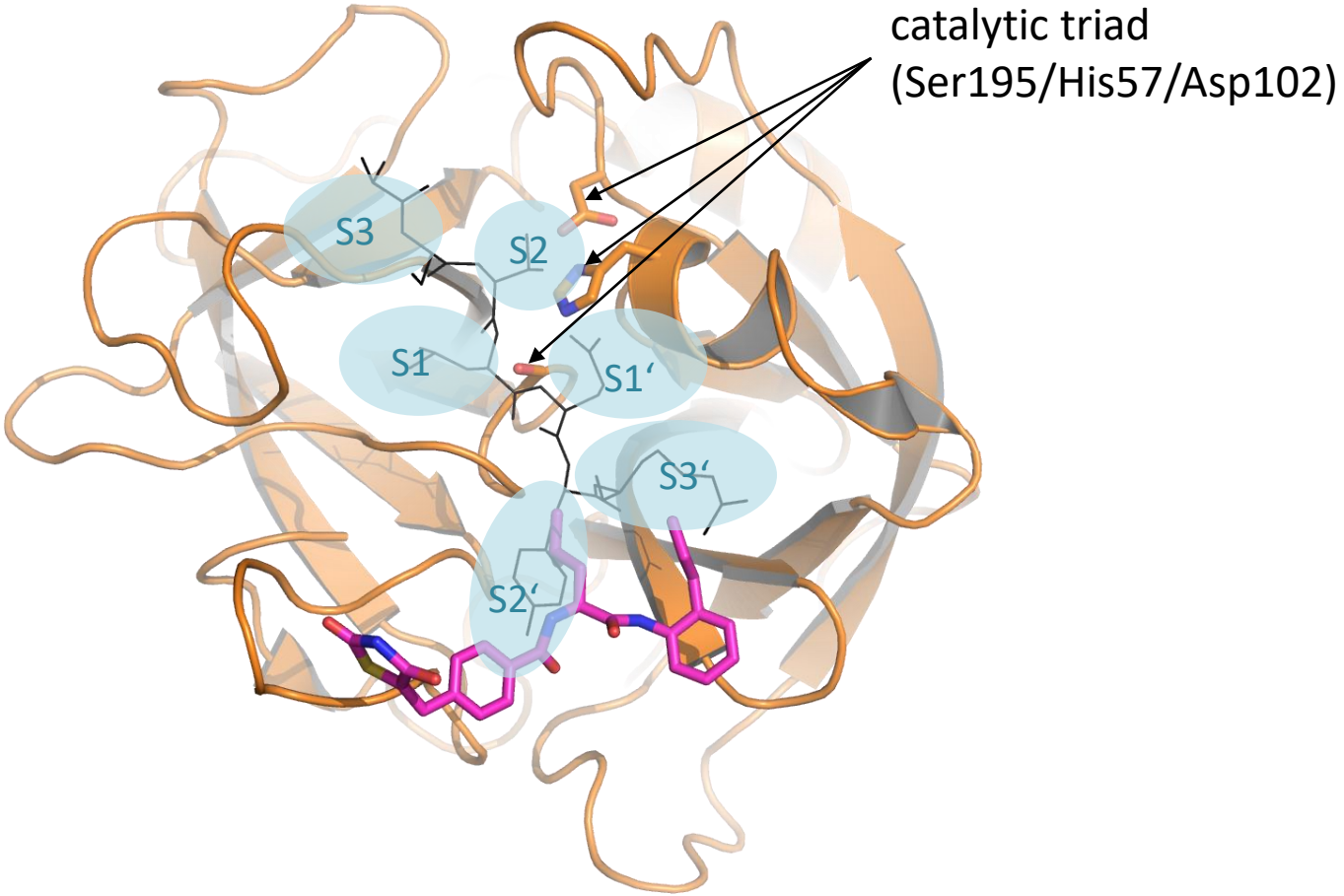
- peptidomimetic
- originally described with antibacterial activity

HLE/CQH complex structure – dimerization blocks access to the active site



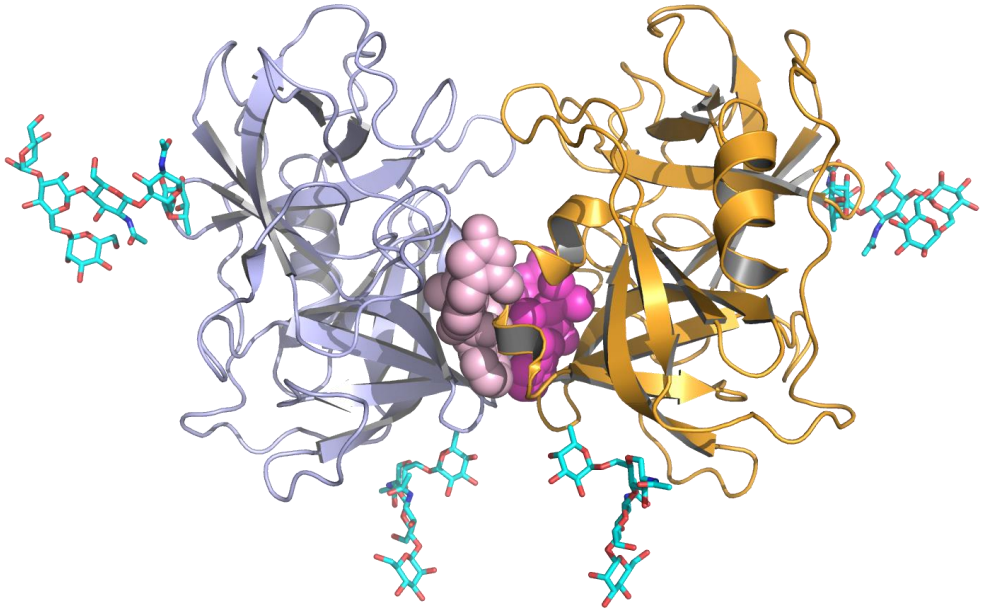
CQH complex structure 6F5M: Hochscherf et al. (2018) *Acta Cryst.* F74, 480-489

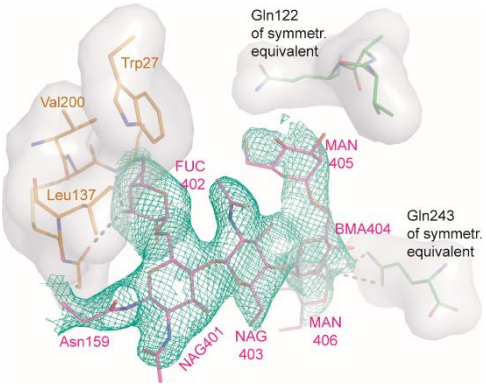
HLE/CQH complex structure – occupation of the S2’ site

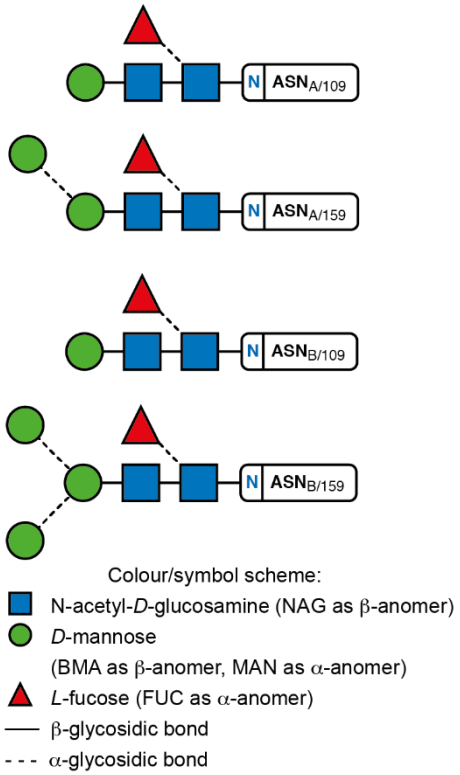


CQH complex structure 6F5M: Hochscherf et al. (2018) *Acta Cryst.* F74, 480-489
1PPF: Bode et al. (1989), EMBO J. 8, 3467-3475

HLE/CQH complex structure – glycosylation







Figures modified from: Hochscherf et al. (2018) *Acta Cryst.* F74, 480-489

Conclusions

CK2α/4p complex:

- reversed binding mode (“hydrophobic-out/oxygen-in“) compared to *in silico* modelling
- different crystallization conditions and usage of paralogues isoform CK2α‘
- no interaction with hinge region
- anchorage through network of hydrogen bonds
- possibility for further modifications to extend inhibitor to the αD-pocket

HLE/CQH complex:

- inhibitor binds to the S2' substrate recognition site
- inhibitor induces the formation of HLE dimers with blocked active sites (so far observed “in crystallo” only and not experimentally confirmed in solution)
- large parts of N-glycan chains are visible

Acknowledgements

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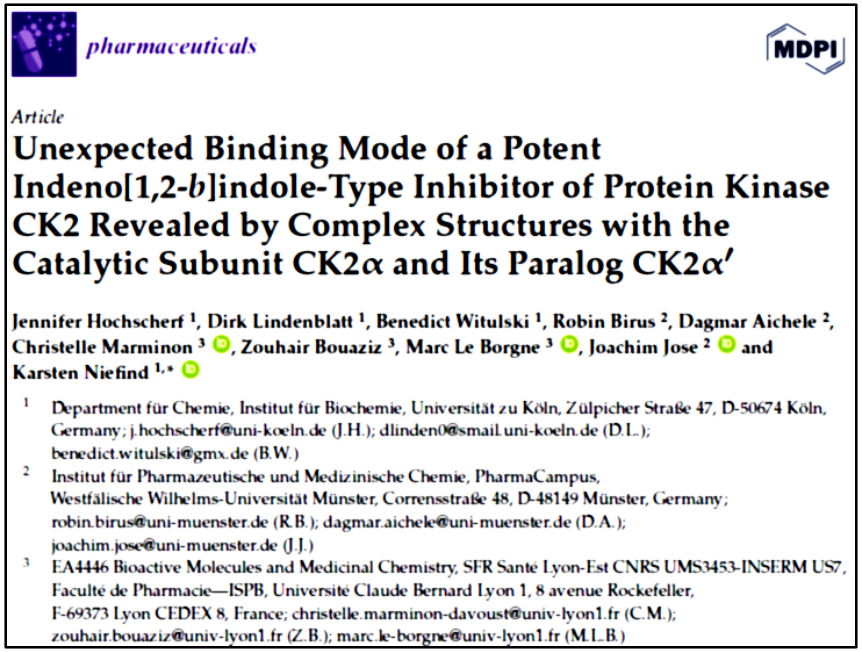

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Article

Unexpected Binding Mode of a Potent Indeno[1,2-*b*]indole-Type Inhibitor of Protein Kinase CK2 Revealed by Complex Structures with the Catalytic Subunit CK2α and Its Paralog CK2α′

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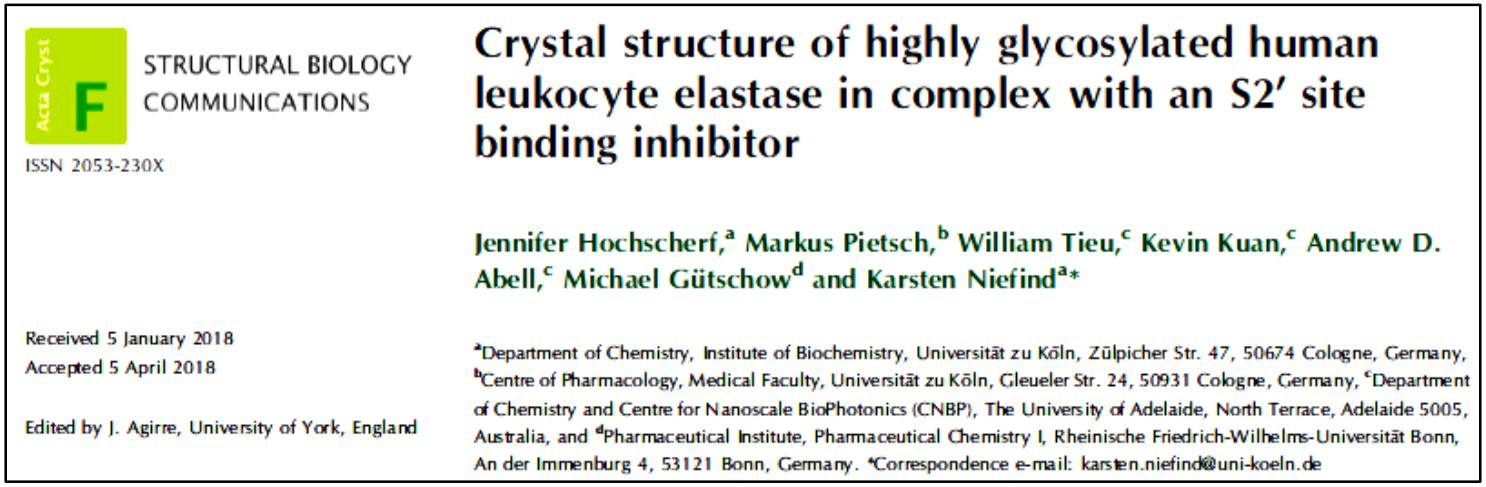

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Crystal structure of highly glycosylated human leukocyte elastase in complex with an S2′ site binding inhibitor

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