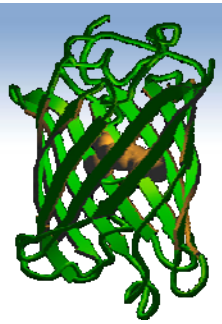


CE und FRET als Tools zur Testung von Inhibitoren der humanen Proteinkinase CK2

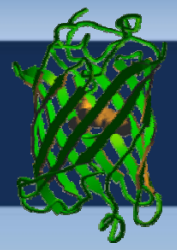
Univ.-Prof. Dr. Joachim Jose

Bioanalytik

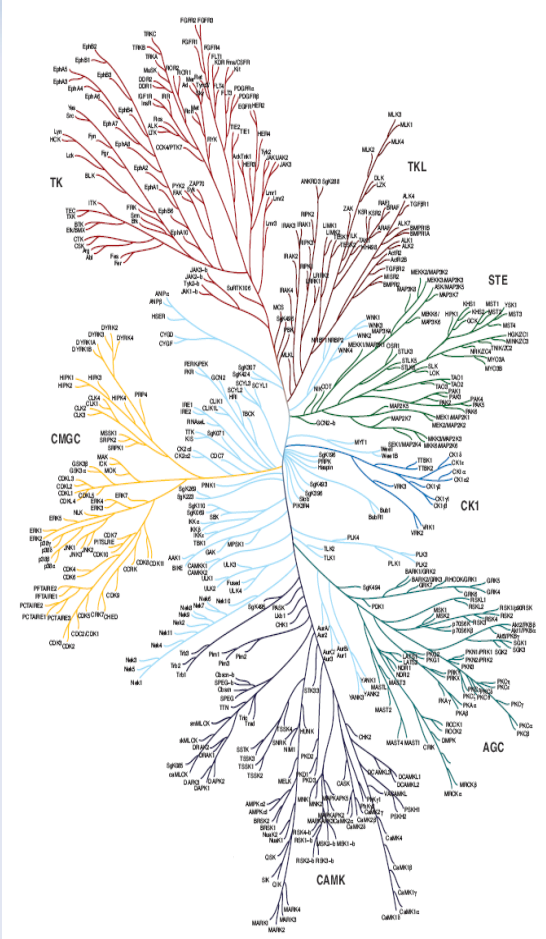
Institut für Pharmazeutische und Medizinische Chemie

HEINRICH HEINE
UNIVERSITÄT
DÜSSELDORF

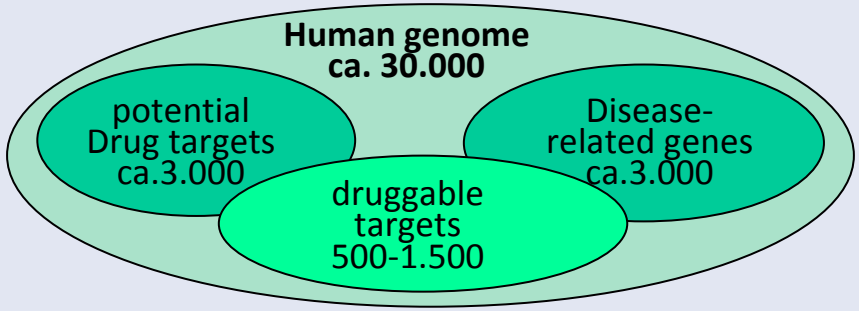
Protein kinases

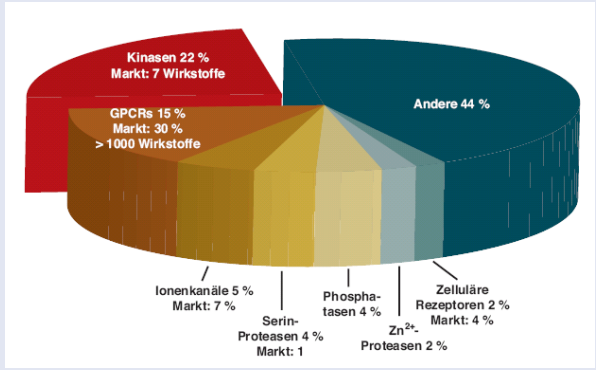


„the largest and best studied superfamily of the human genome“

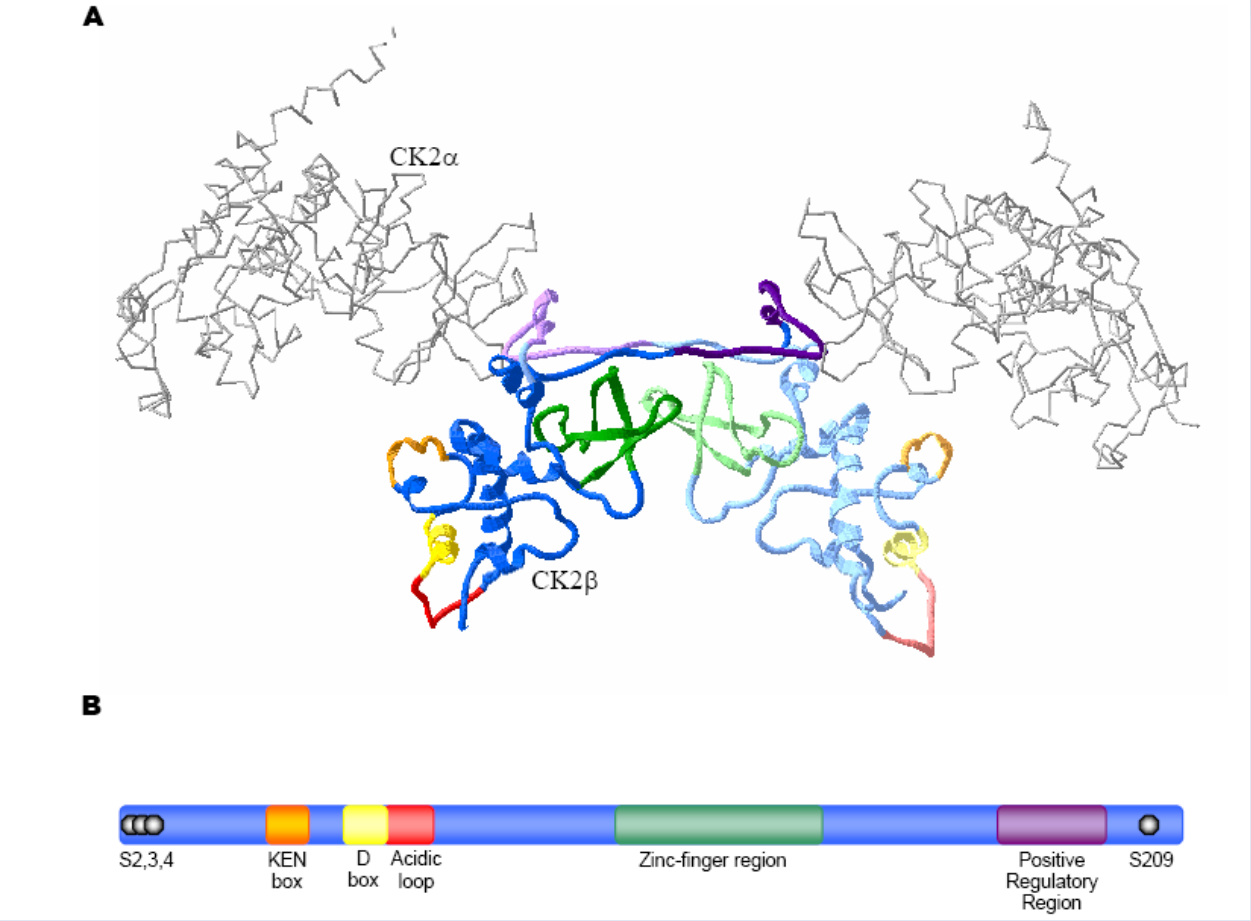


Human „kinome“ consists of 518 kinases



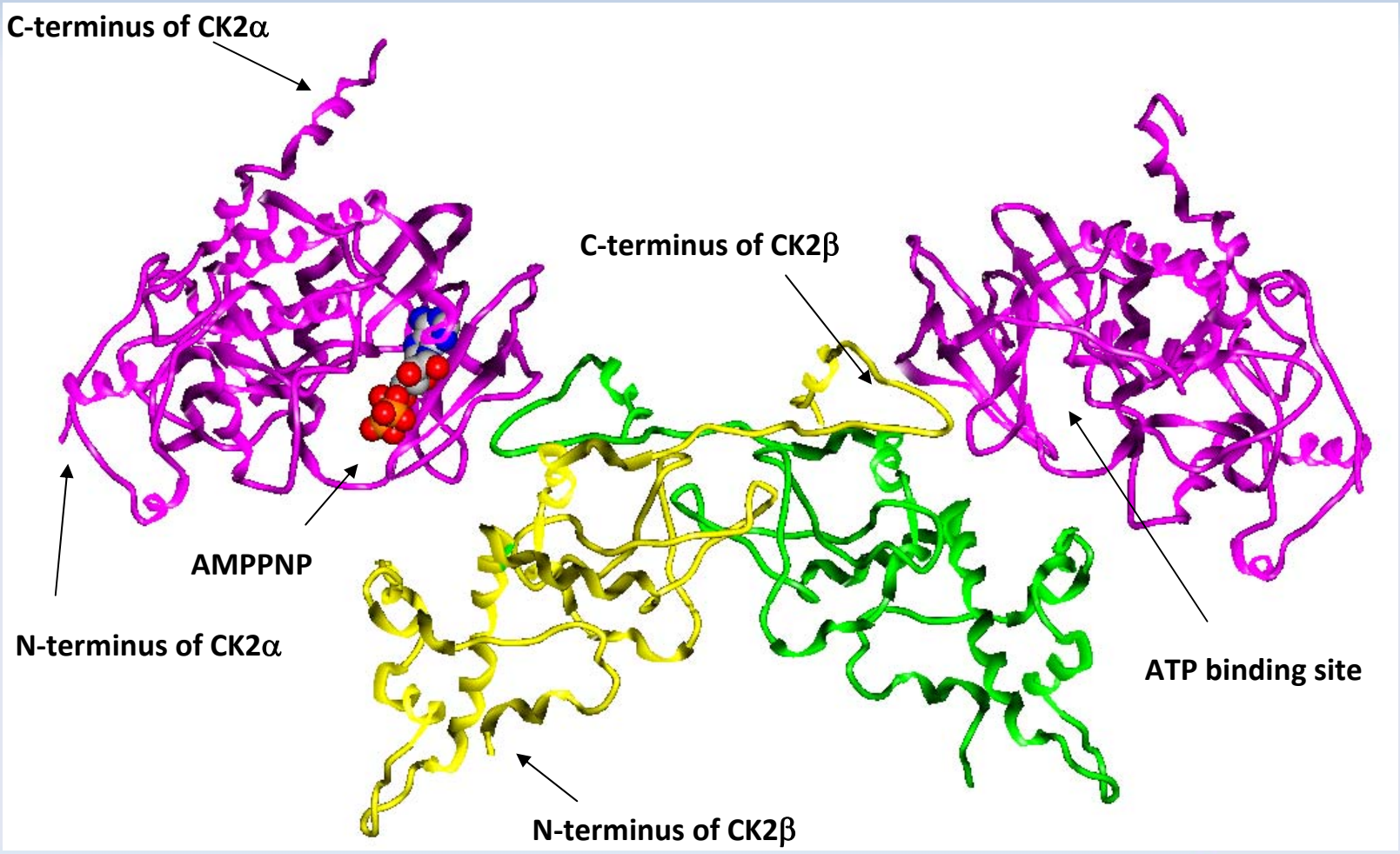


Human protein kinase CK2

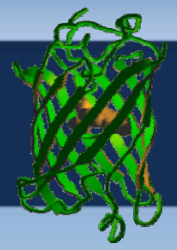


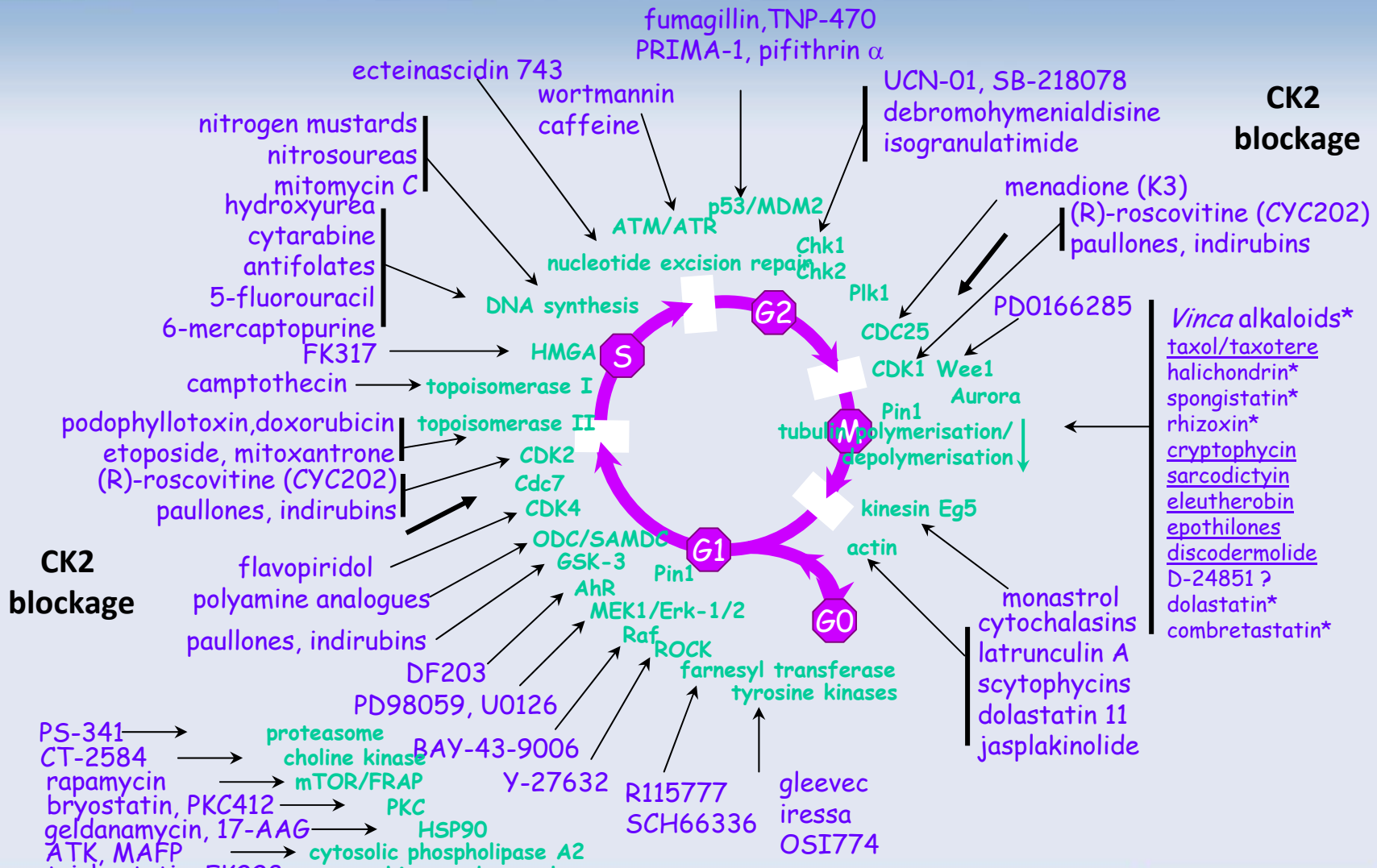
Human protein kinase CK2

Holoenzyme consists of two catalytic and two regulatory subunits

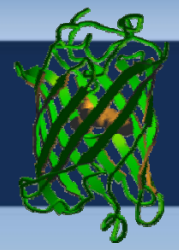


Human protein kinase CK2



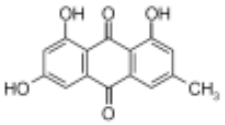


Human protein kinase CK2: a target in neoplastic disease



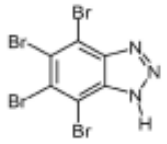
- overexpressed in several cancers, incl. those of:
  prostate (*Yenice et al., 1994, Hessenauer et al., 2003*)
  mammary gland (*Landesman-Bollag et al. 2001*)
  lung (*Daya-Makin et al. 1994*)
  others (*Faust et al., 1996*)
- transformation of lymphocytes into leukemia cells and cells of the mammary gland to malignant cells in an animal model is accompagnied by CK2 induction (*ole-MoiYoi et al. 1993, Seldin and Leder 1995*)
- validation of protein kinase CK2 as oncological target by siRNA silencing (*Seeber et al., 2005)*
- CK2 prevents tumor cells from apoptosis (*Ahmad et al., 2005, Wang et al., 2006*)
- inhibition of CK 2 prevents the progression of glomerulonephritis (*Yamada et al., 2005*)

Known inhibitors of CK2



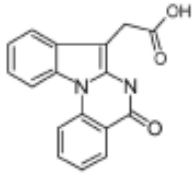
Emodin

IC_{50} value (rat)
0.89 μM



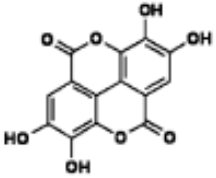
TBB

IC_{50} value (rat)
0.50 μM
1.6 µM (human)



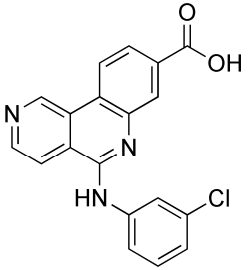
IQA

IC_{50} value (rat)
0.59 μM



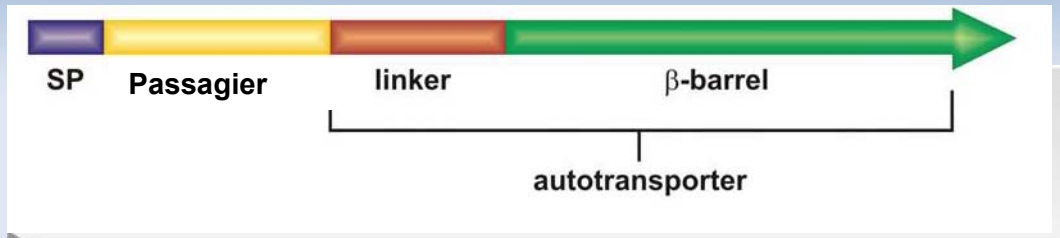
Elagic acid

IC_{50} value (rat)
0.04 μM



CX-4945

Autodisplay



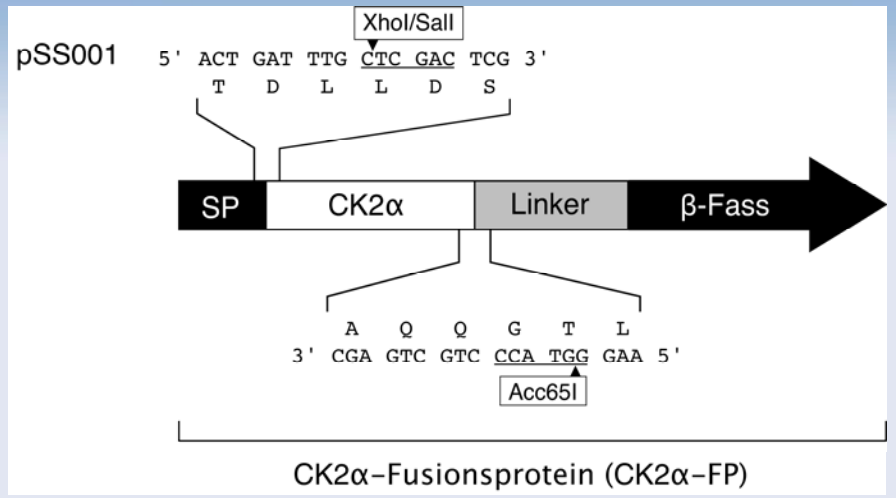
„Cystope tagging“

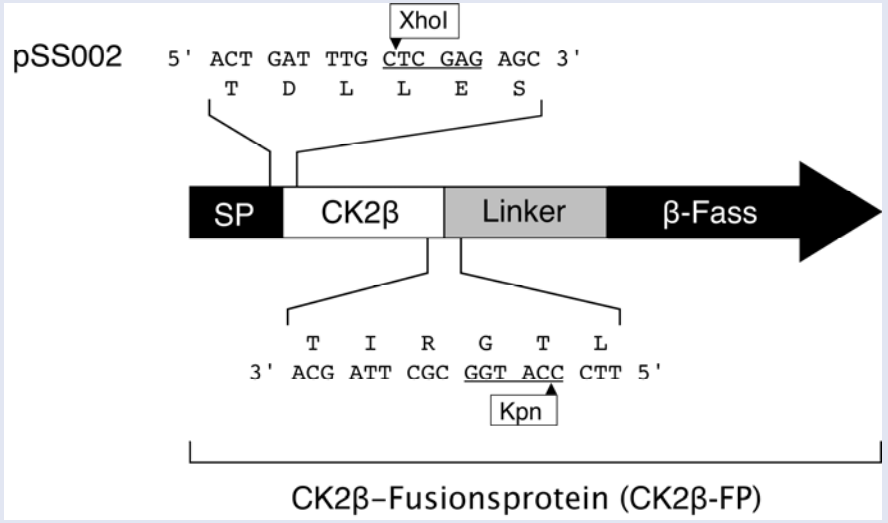
***E.coli* JK 321 (*dsb*A $^{-}$ *ompT* $^{-}$)**
***E.coli* JK 321 (DE3) (*dsb*A $^{-}$ *ompT* $^{-}$)**
***E.coli* UT 5600 (DE3) (*dsb*A $^{+}$ *ompT* $^{-}$)**
***E.coli* UT 2300 (DE3) (*dsb*A $^{+}$ *ompT* $^{+}$)**

C107, 108, 109, 110, 151, 152

Gene (1996), 178:107-110
J Bacteriol (1997), 179:794-804
J Bacteriol (1999),181:7014-7020
ChemBioChem (2003), 4:396-405
Anal Biochem (2004), 331:267-274
Appl Microbiol Biotechnol (2006), 69:607-614
Microbiol Mol Biol R (2007), 71:600-619
Appl Environ Microbiol (2008), 74:4782-4791

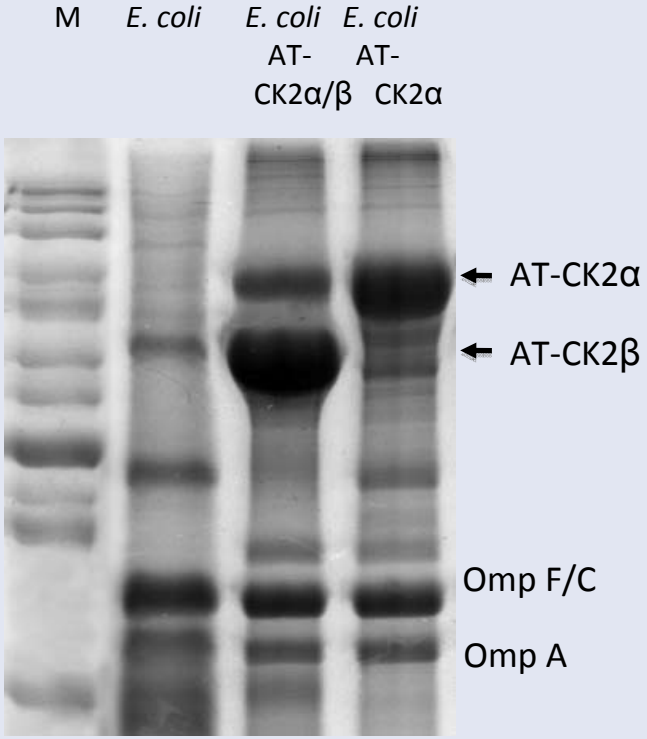
Autodisplay of human CK2

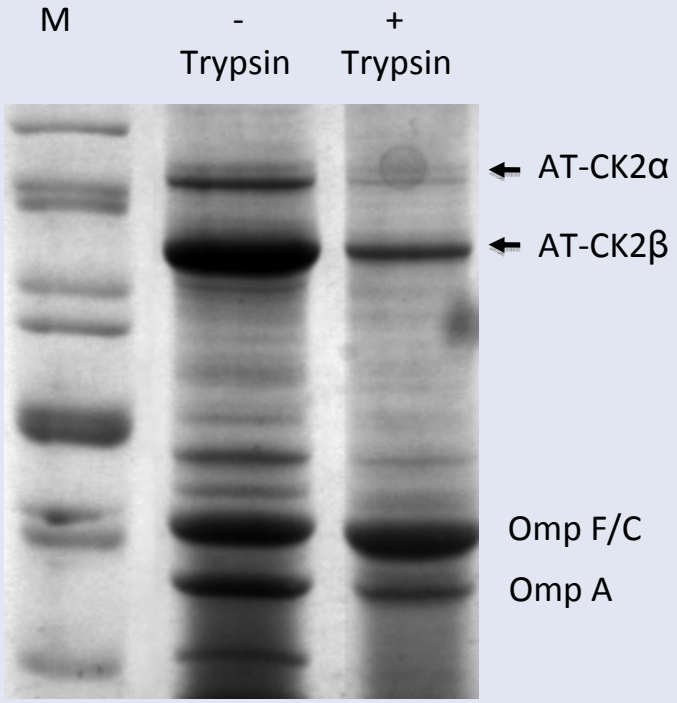




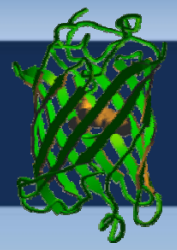
Autodisplay of human CK2

Co-expression of α- und β- subunits

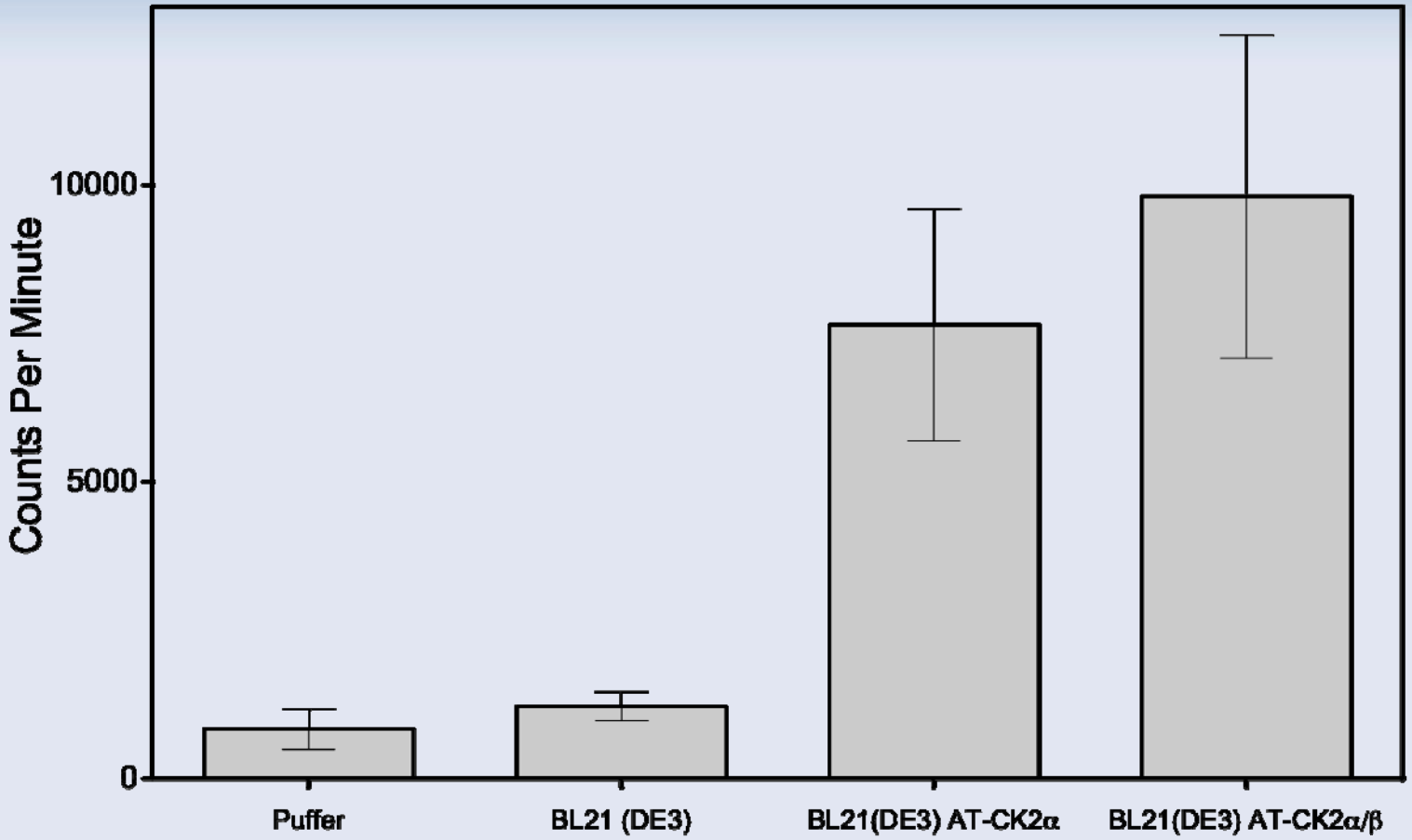




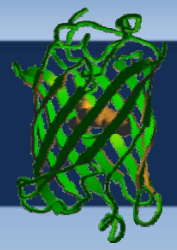
Autodisplay of CK2: enzyme activity



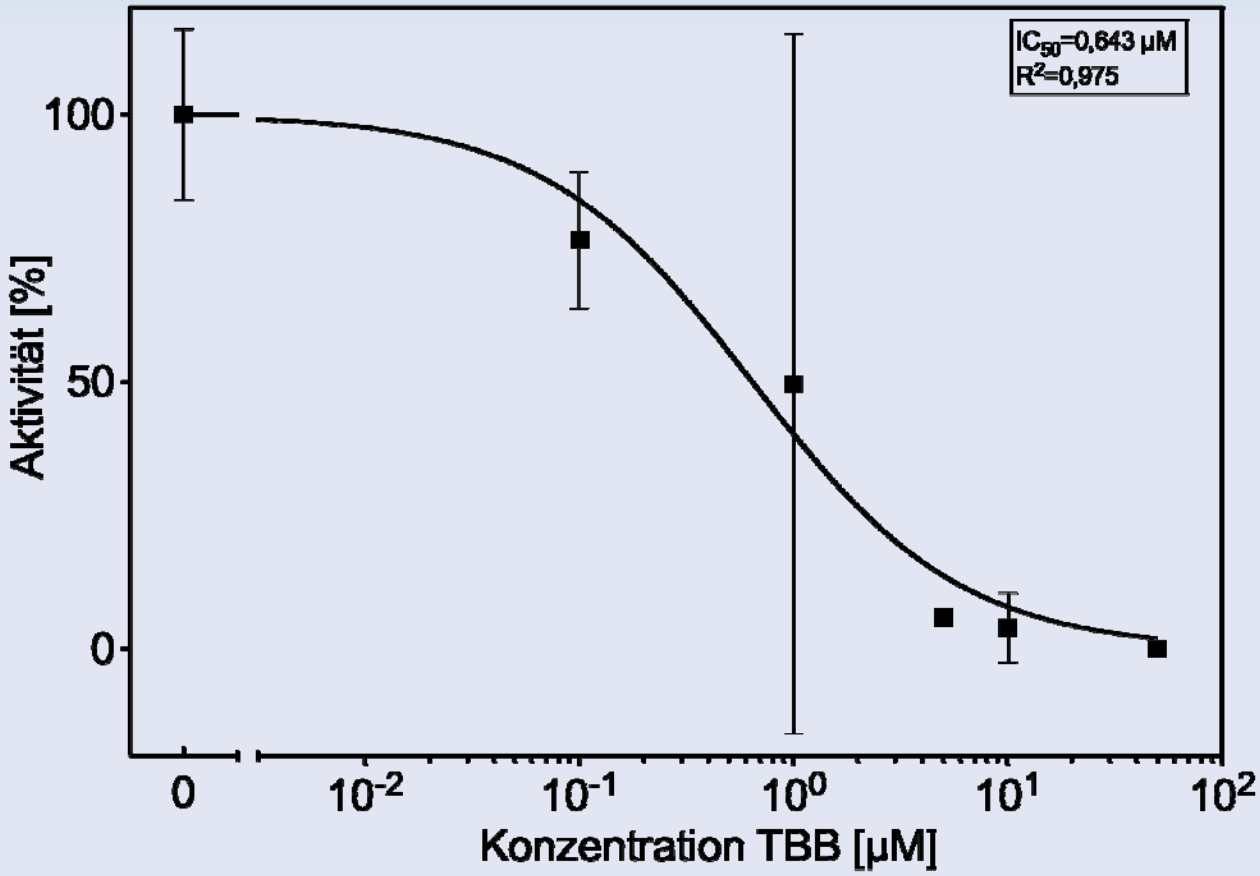
α-subunit separate and α– and β-subunit co-expressed

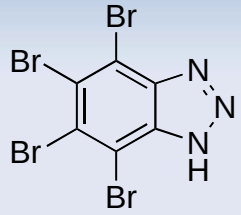


Autodisplay of CK2: IC_{50}-determination



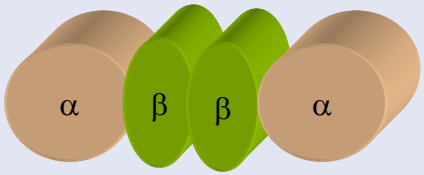
IC_{50} value determination of a known CK2-inhibitor:

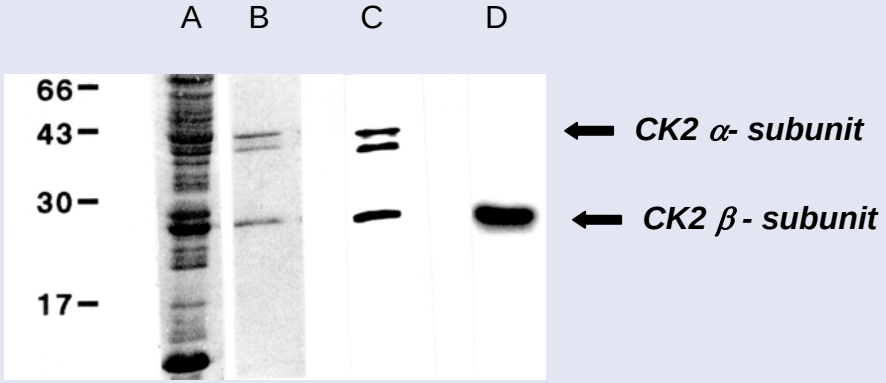




TBB
(4,5,6,7-tetrabromo-benzotriazole)

Recombinant expression and purification of human CK2

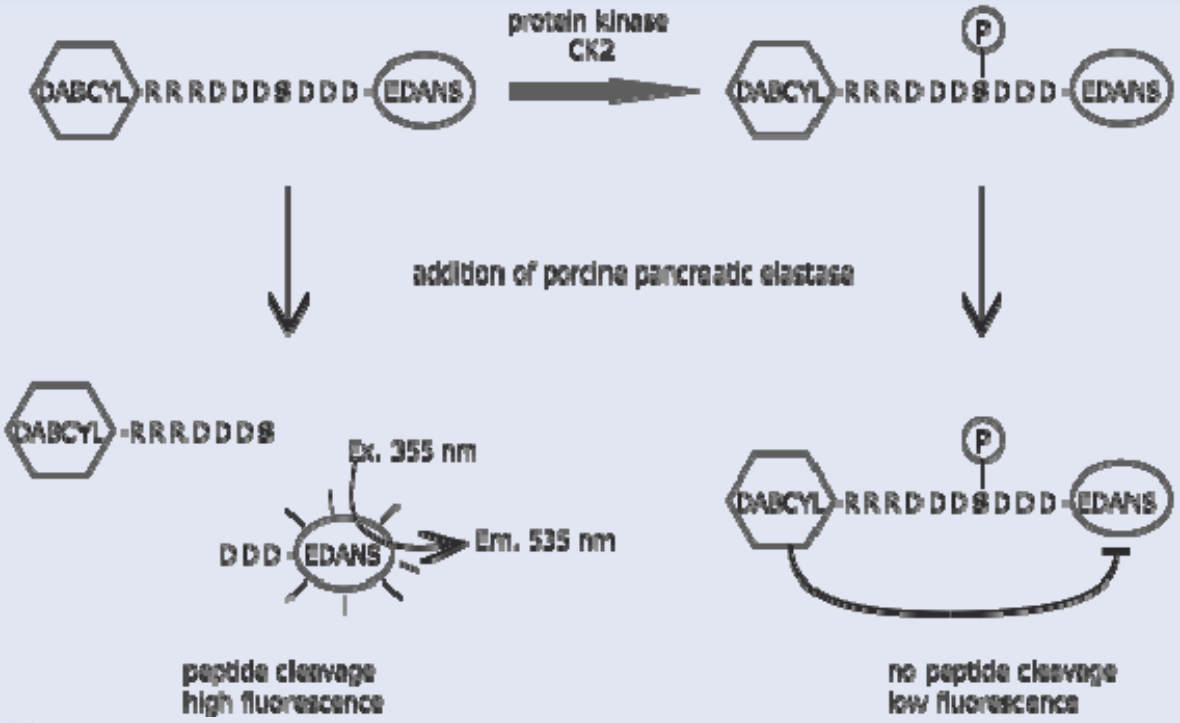




RRRRDDDSDDD **[γ-^{32}]ATP**

Development of a new CK2 assay

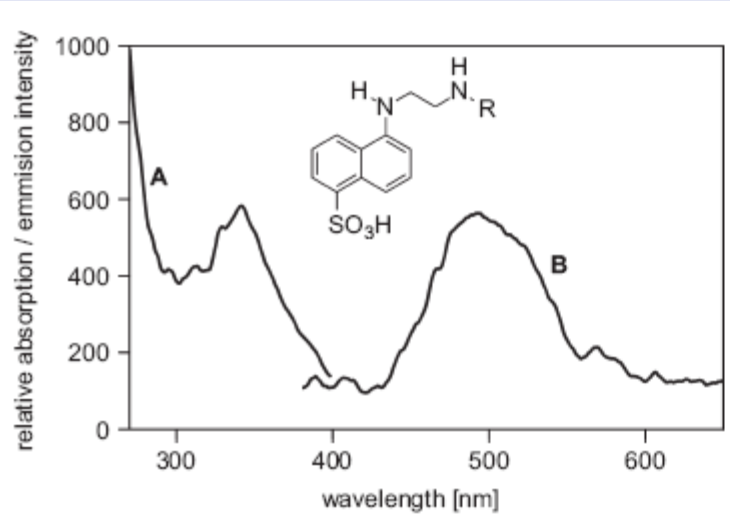
FRET - Fluorescence-Resonance Energy Transfer

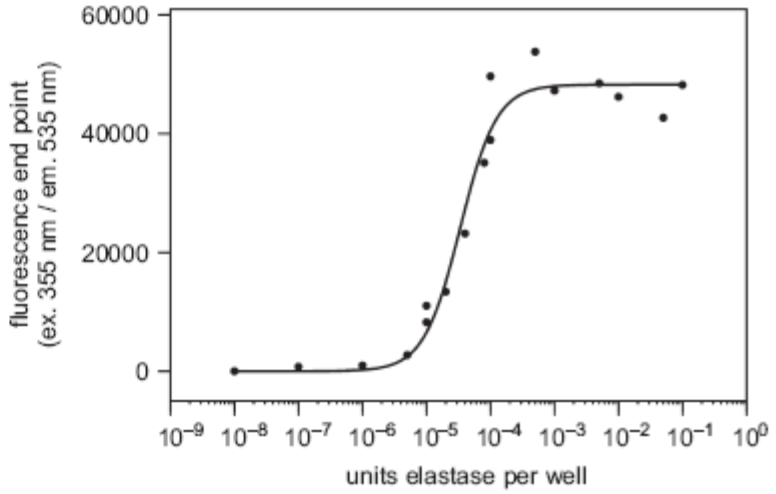


J Enz Inhib Med Chem (2010), 25:234-239

Development of a new CK2 assay

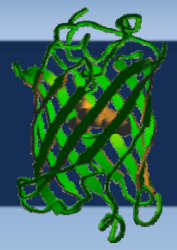
FRET - Fluorescence-Resonance Energy Transfer



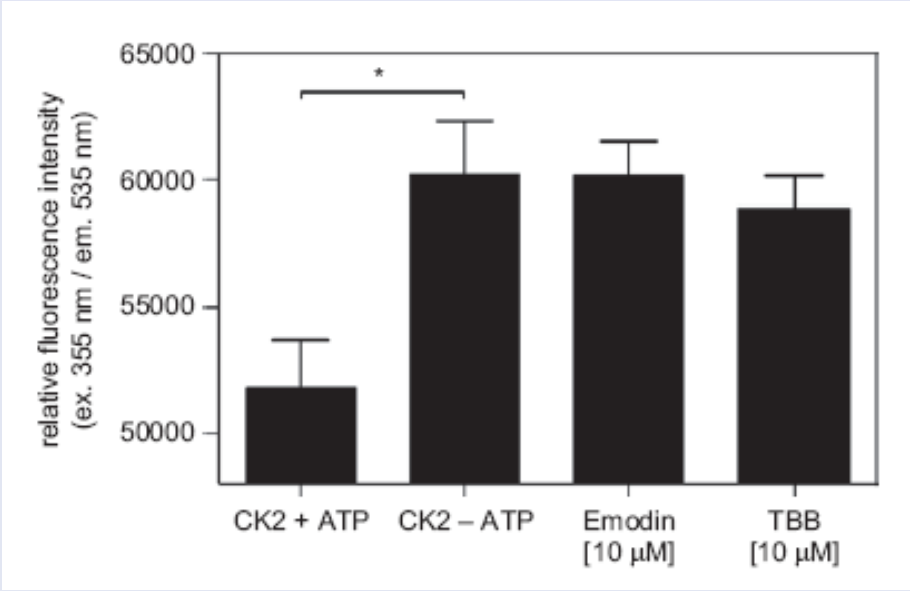


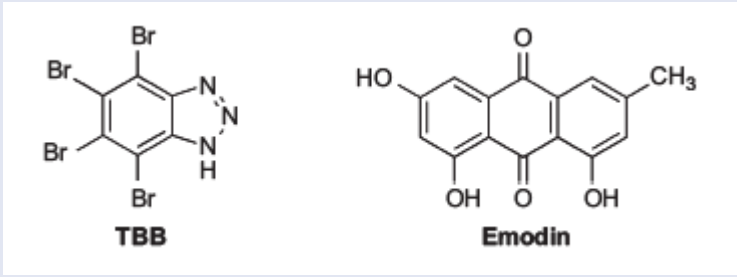
J Enz Inhib Med Chem (2010), 25:234-239

Development of a new CK2 assay



FRET - Fluorescence-Resonance Energy Transfer

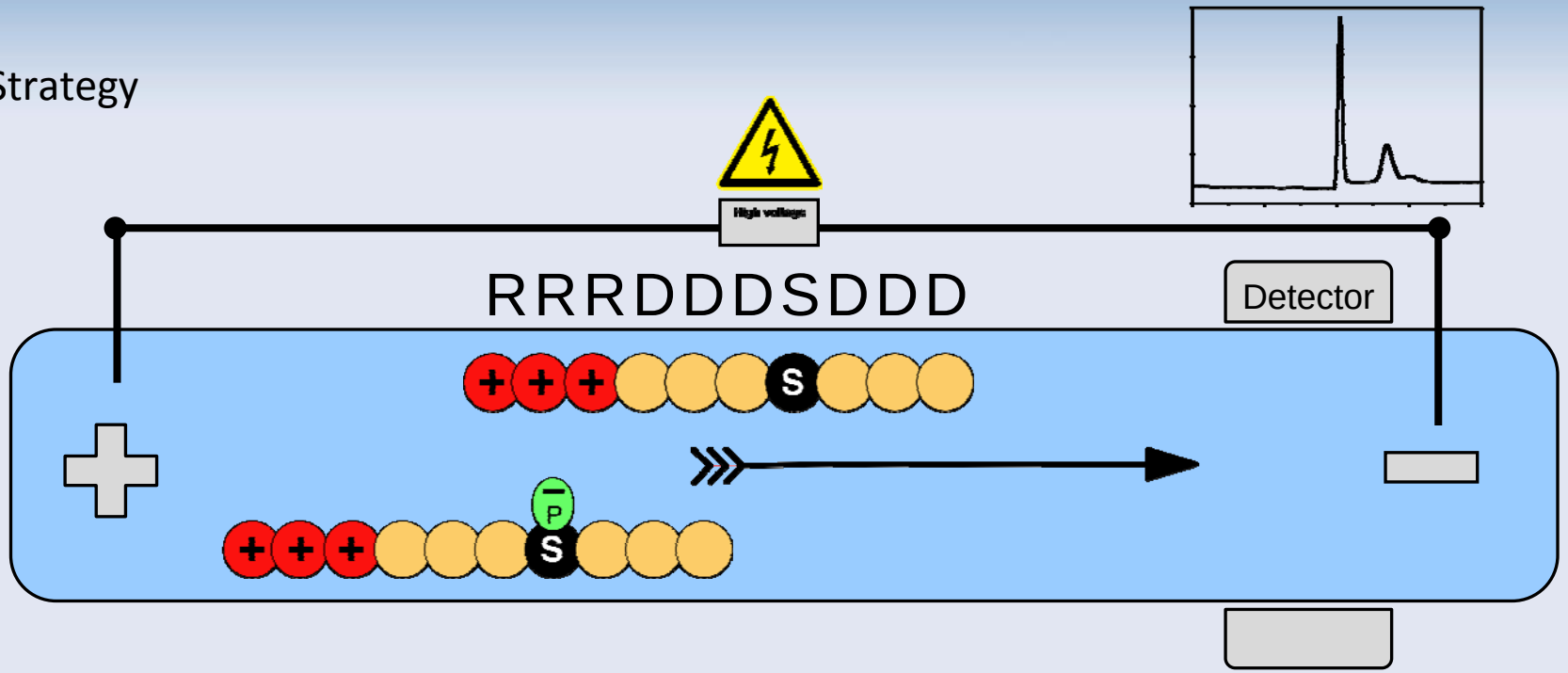




J Enz Inhib Med Chem (2010), 25:234-239

Development of a CE-based CK2 Assay

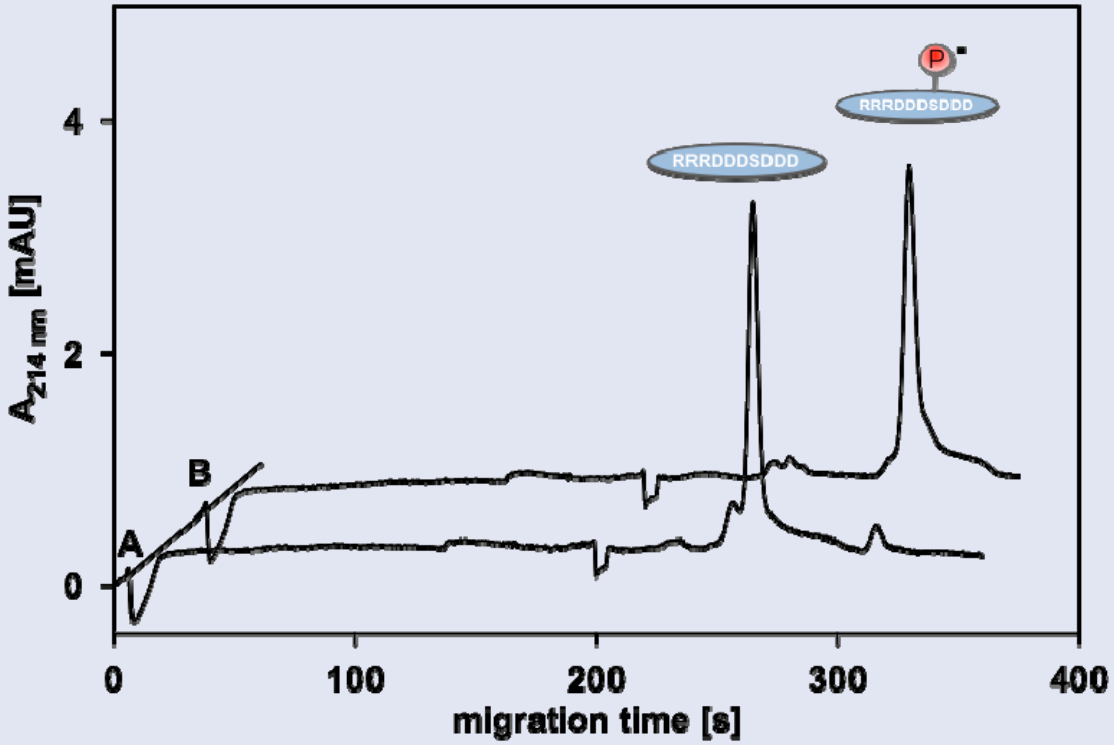
Strategy



phosphorylation → „mobility shift“

Electrophoresis (2010), 31:634-640

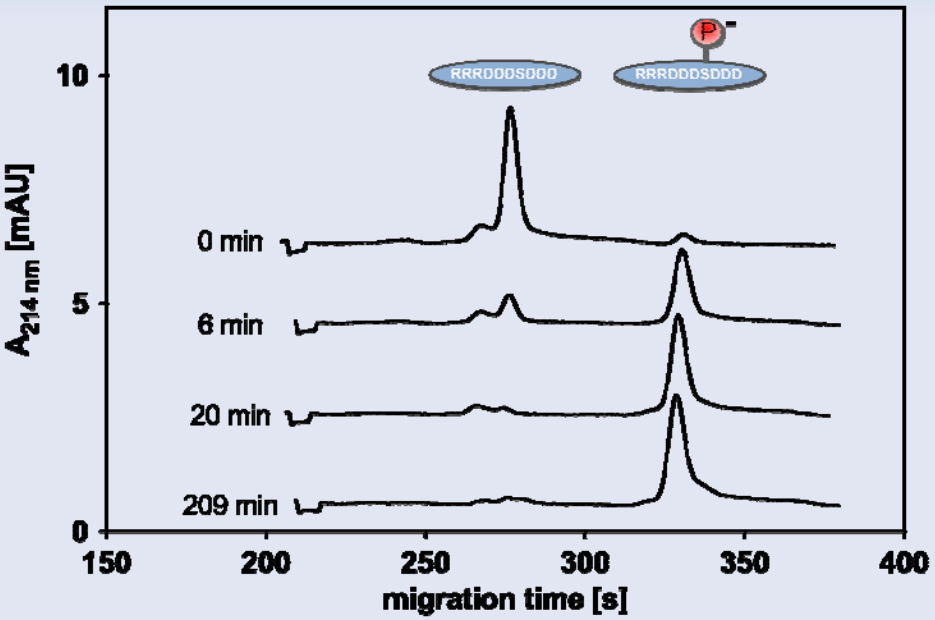
Development of a CE-based CK2 Assay

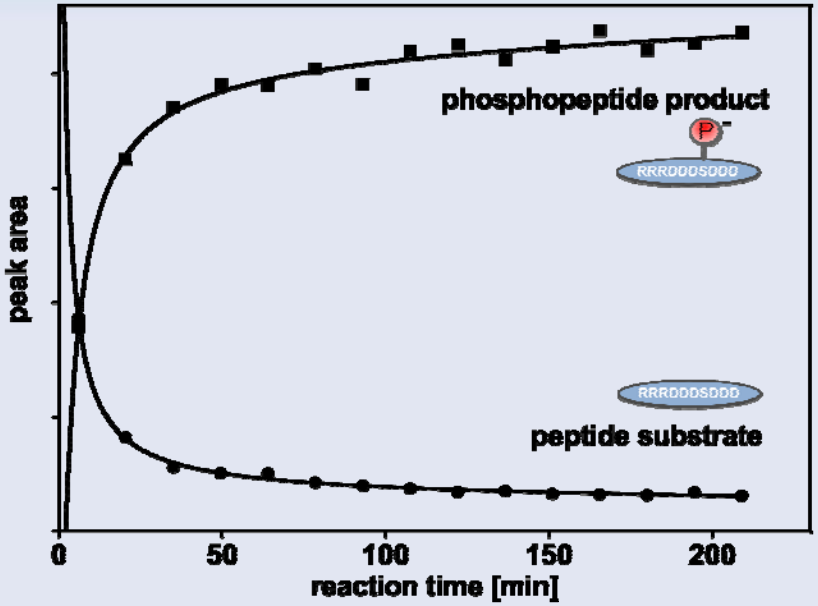


CE-conditions:
30 kV
50 cm length
50 µm I.D.
2 M acetic acid

Electrophoresis (2010), 31:634-640

Kinetic analysis of CK2 reaction

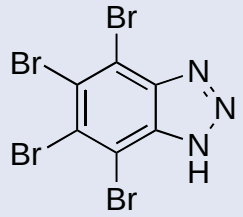




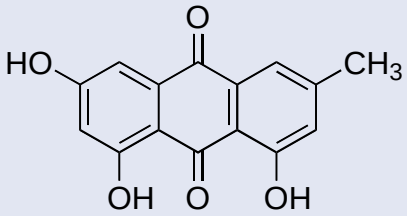
Electrophoresis (2010), 31:634-640

Inhibition test: proof-of-concept

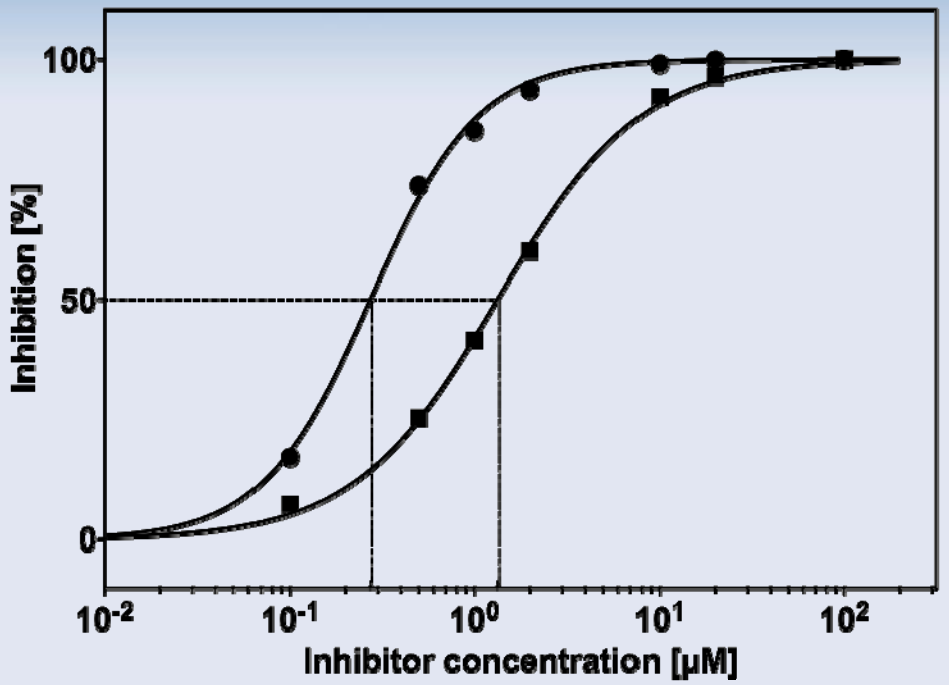
- IC_{50} determination of known CK2-inhibitors:



TBB
(4,5,6,7-tetrabromobenzotriazole)



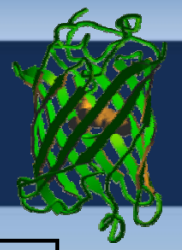
Emodin
(6-methyl-1,3,8-trihydroxyanthraquinone)

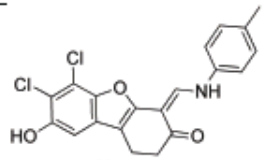
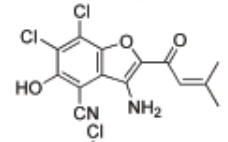
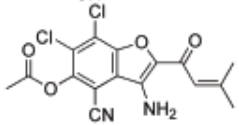
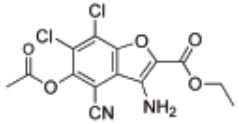
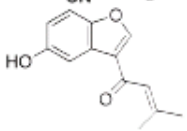
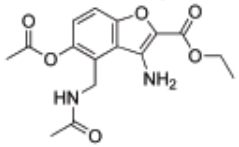


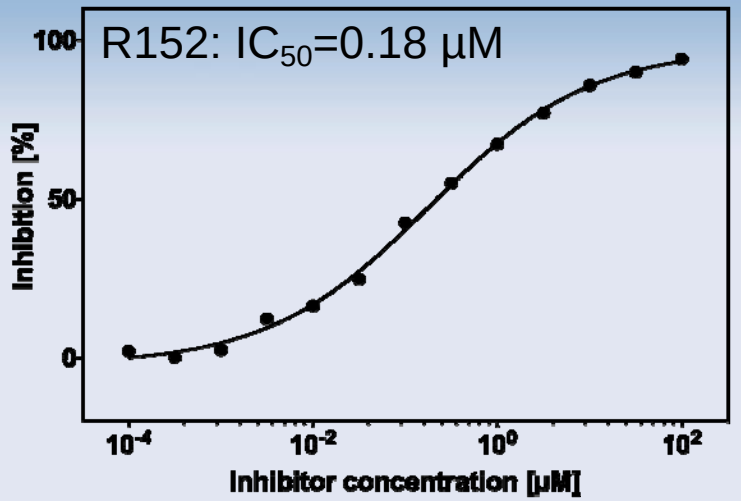
IC_{50} [µM]	TBB (●)	Emodin (■)
CE	0.27	1.33
Radiometric	0.5-1.6	0.89-2

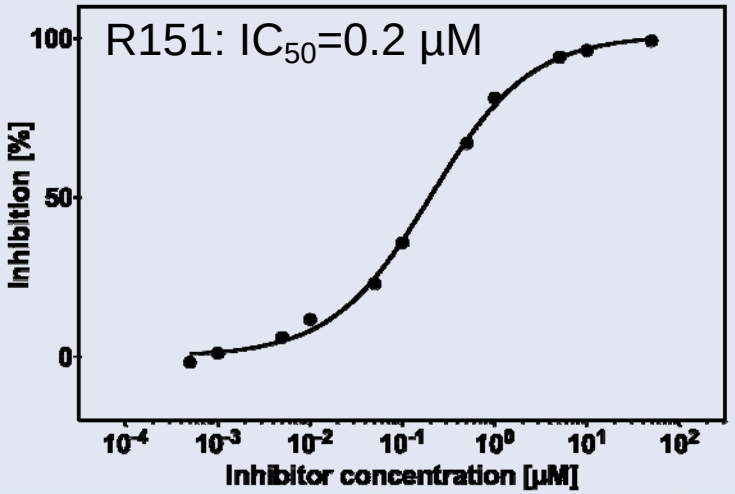
Electrophoresis (2010), 31:634-640

Identification of novel CK2 inhibitors



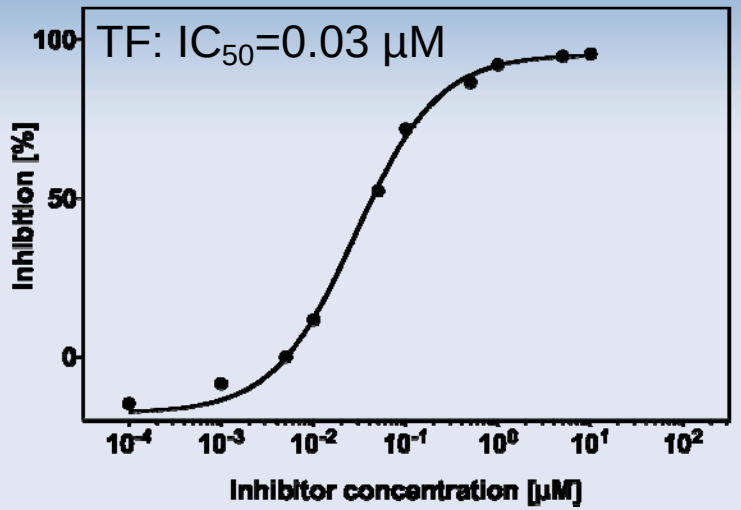
Substanz	Strukturformel	CK2-Aktivität bei 10 µM
TF		< 4 %[1]
Ric151		< 4 %[1]
Ric152		7 %
Ric138		31 %
Ric149		61 %
RicFur		110 %





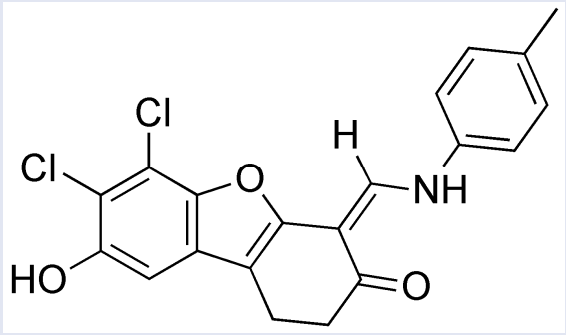
Bioorg Med Chem, submitted

Identification of novel CK2 inhibitors



IC_{50} of TF: on the level of best CK2 inhibitors currently published:

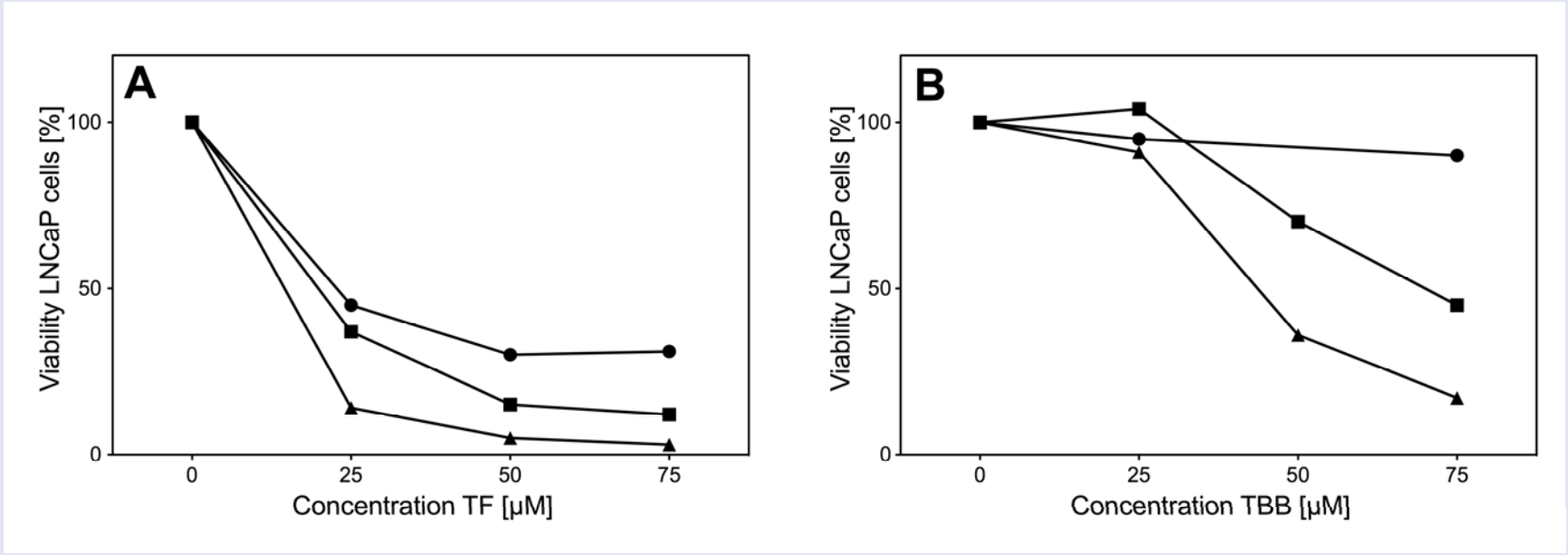
ellagic acid: 0.04 µM
DMAT: 0.14 µM



DE 10 2010 025173.9.

TF based reduction of cell viability

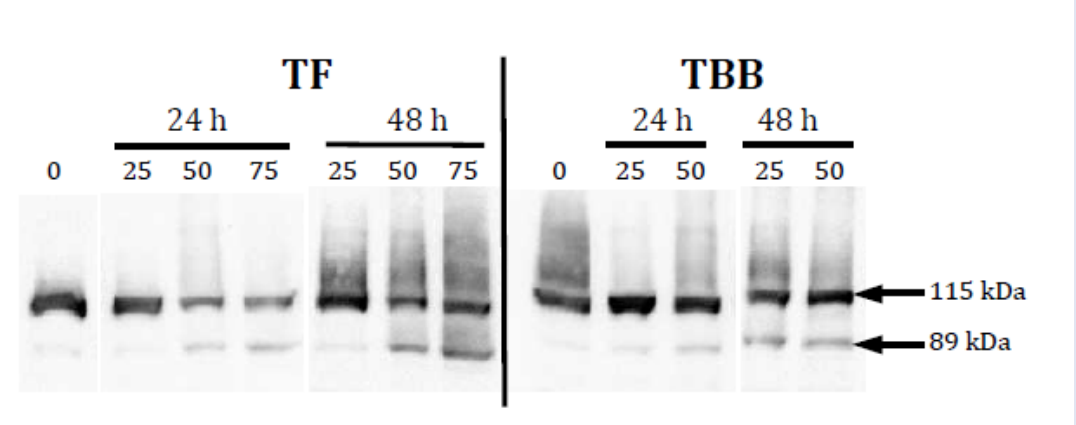
viability of LNCaP cells at different concentrations after
24 h (●), 48 h (■), and 72 h (▲)



DE 10 2010 025173.9.

TF based induction of apoptosis

- PARP (poly-ADP-ribose-polymerase) is cleaved by effector caspase 3
- cleavage results in appearance of a 89 kDa PARP fragment
- indicates point of no return in apoptose induction



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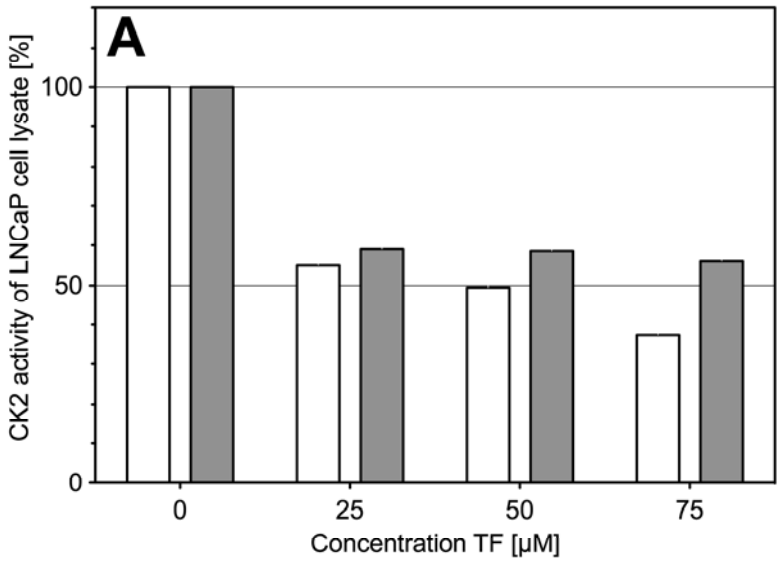
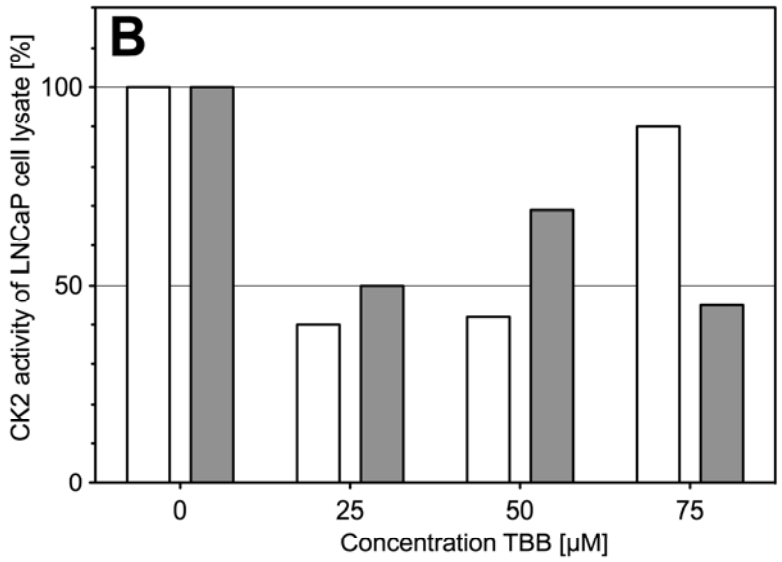
CK2 inhibition in LNCaP cells

- LNCaP cells were incubated with different concentrations of TF or TBB
- radiometric determination of CK2 activity (duplicates)





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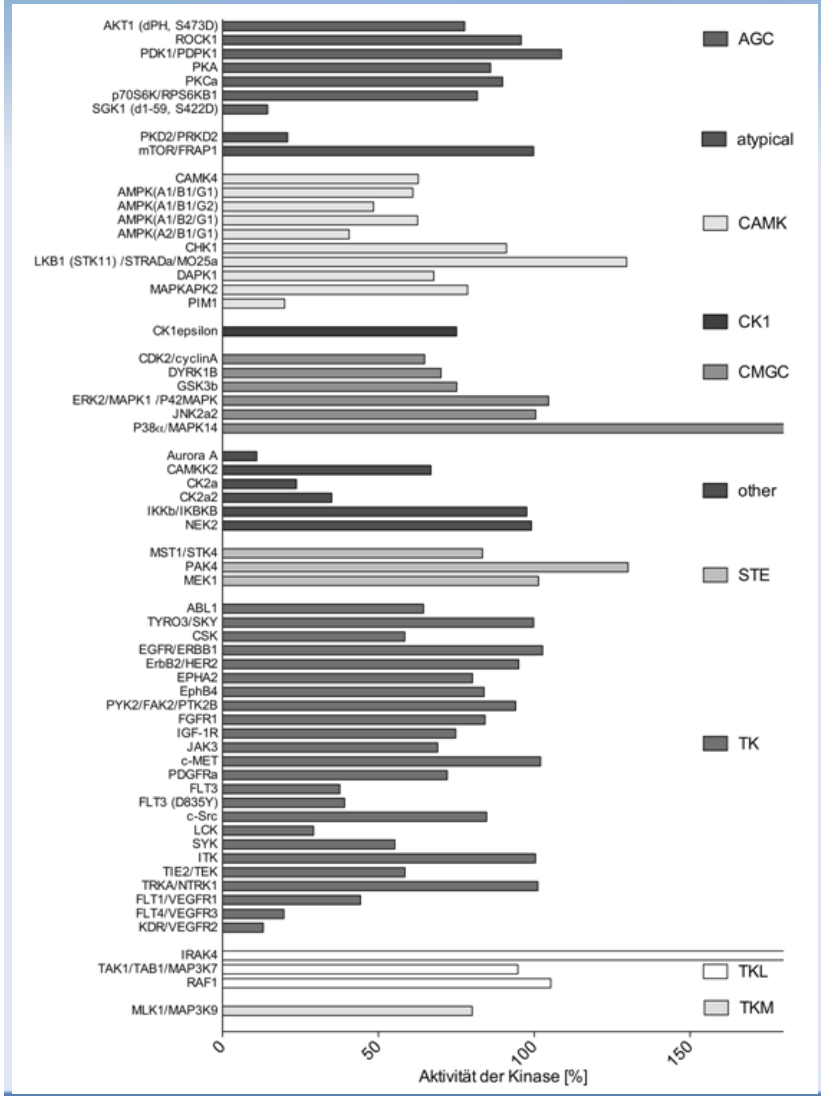
Specificity of kinase inhibition by TF



Protein kinase	activity [10 µM]	UniProt Accession #	function
Aurora A	11,00 %	O14965	cell cycle regulation
KDR/VEGFR2	13,05 %	P35968	angiogenesis
SGK1 (d1-59, S422D)	14,16 %	O00141	stress response cell survival
FLT4/VEGFR3	19,68 %	P35916	cell proliferation
PIM1	19,90 %	P11309	cell proliferation cell survival
PKD2/PRKD2	20,90 %	Q9BZL6	Oxidative stress resistance
LCK	29,20 %	P06239	T-cell differentiation

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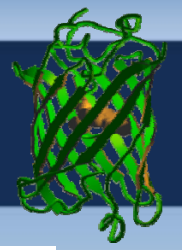
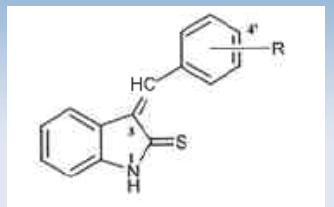
Summary

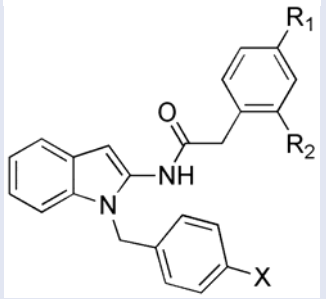


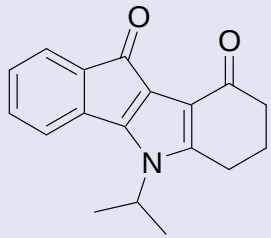
- new access to human protein kinase CK2 for inhibitor testing



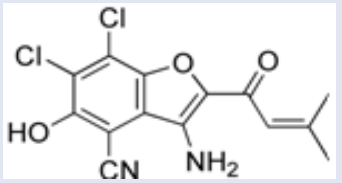
- new inhibitor test assays: FRET-based assay
  CE-based assay

- identification of new potent human CK2 inhibitors:
  indol-2-thione derivatives
  indol-2-acetamide derivatives
  oxazinocarbazoles
  indeno[1,2-b]indoles
  benzofurane derivatives





- TF inhibits CK2 induces apoptosis in prostate cancer cell line



Biol Pharm Bull (2007), 30:715-718
B Cancer 97: 68S-69S
DE 10 2006 047 231 A1, PCT/EP2007/008624

Acknowledgements

Dr. Andreas Gratz, Pharm. Med. Chem., Düsseldorf, Germany

Prof. Dr. Claudia Götz, Medicinal Biochemistry, Homburg, Germany

Prof. Dr. Uwe Kuckländer, Pharm. Med. Chem., Düsseldorf, Germany

Prof. Dr. Süreyya Ölgen, Pharmaceutical Chemistry, Ankara, Turkey

Prof. Dr. Marc LeBorgne, Organic Chemistry, Lyon, France

Acknowledgements