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Click chemistry for advanced drug discovery applications of human protein kinase CK2

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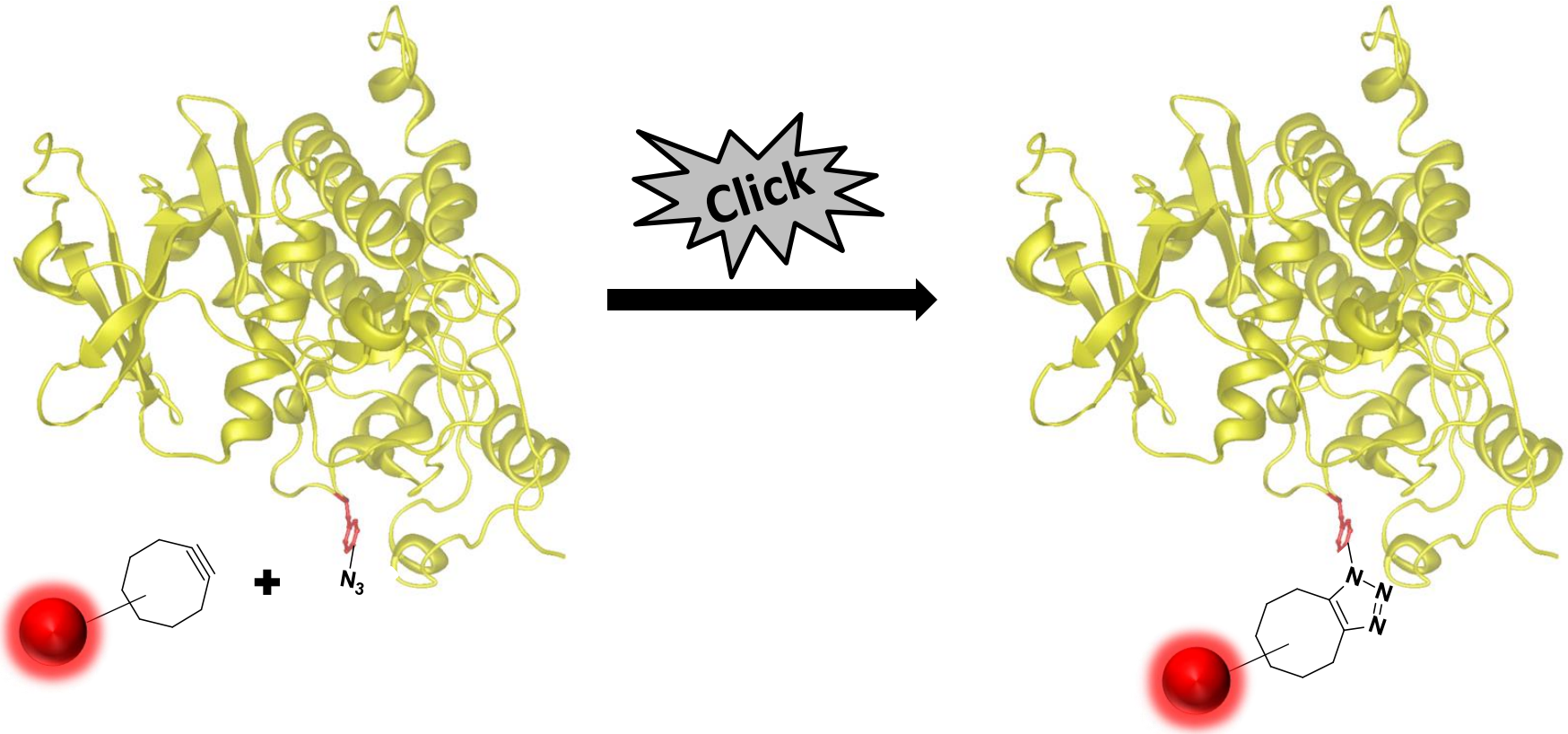
Click chemistry for advanced drug discovery applications of human protein kinase CK2



Abstract:
Human CK2 is a heterotetrameric constitutively active serine / threonine protein kinase and plays an important role in current cancer research [1]. The kinase is composed of two catalytic CK2α subunits and two regulatory CK2β subunits. Most protein-protein interaction (PPI) studies or screening assays are based on fluorescence detection and require the labeling of the target enzyme by a fluorophore. Unfortunately, through labeling by commercial applications the catalytic subunit CK2α loses activity. Furthermore, the labeling ratio of the protein sample differs and is not exactly reproducible.
The solution for this problem was a bioorthogonal click reaction of the protein kinase. By expanding the genetic code, the unnatural amino acid para acidophenylalanine (pAzF) could be incorporated into CK2 [2]. Performing the SPAAC click reaction (Strain-Promoted Alkyne-Azide Cycloaddition) by the use of DBCO 545 (dibenzylcyclooctyne fluor 545) led to a specifically labeled human protein kinase CK2 [3].
This site specific labeling does not impair the phosphorylation activity of the kinase, which was evaluated by capillary electrophoresis. The innovatively labeled kinase in combination with the Autodisplay technology could be a significant advancement for inhibitor screening assays by flow cytometry and for CK2α/CK2β interaction studies [4].

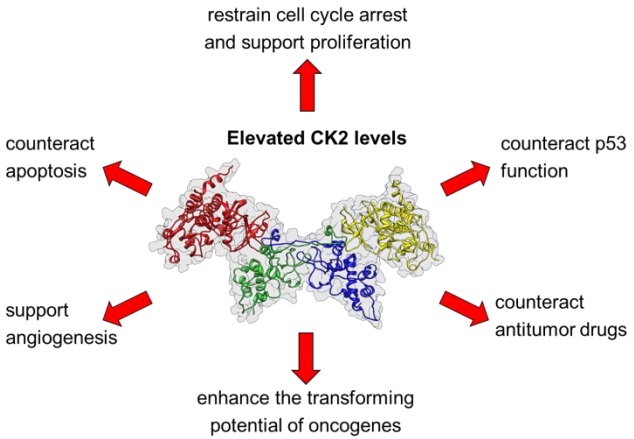
Keywords: CK2; Click chemistry; Unnatural amino acid; Drug discovery; Autodisplay

References:
1. Trembley, J.H. et al.: *Biofactors* **2010**, 36(3): 187-95.
2. Chin, J.W. et al.: *J. Am. Chem. Soc.* **2002**, 124(31): 9026-7.
3. Mbua, N.E. et al.: *Chembiochem*. **2011**, 12(12): 1912-21.
4. Jose, J., Meyer, T.F.: *Microbiol. Mol. Biol. Rev*. **2007**, 71(4): 600-19.

Introduction

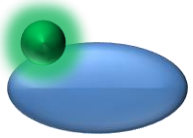
Human protein kinase CK2

- heterotetrameric protein kinase: $\alpha_2\beta_2$
- highly pleiotropic
- constitutively active
- overexpressed in many different tumors
- important role in cell survival and neoplasia



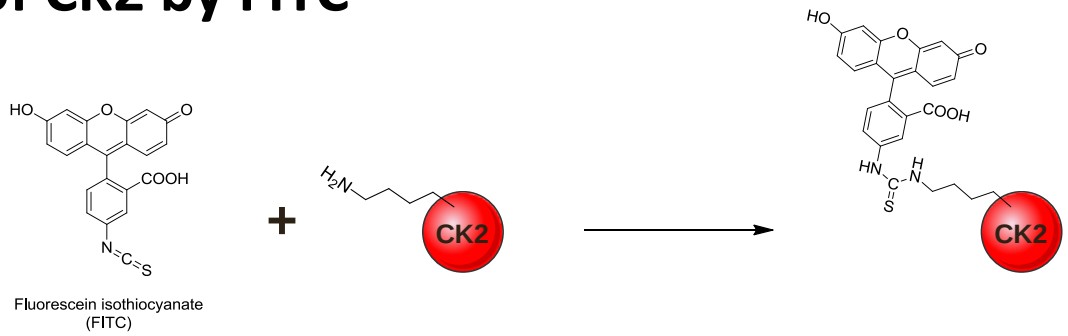
Need for fluorophore labeled proteins

- visualization of proteins
- analysis of protein-protein interaction (PPI)
- enzyme activity assays
- screening assays for new binding partners / inhibitors

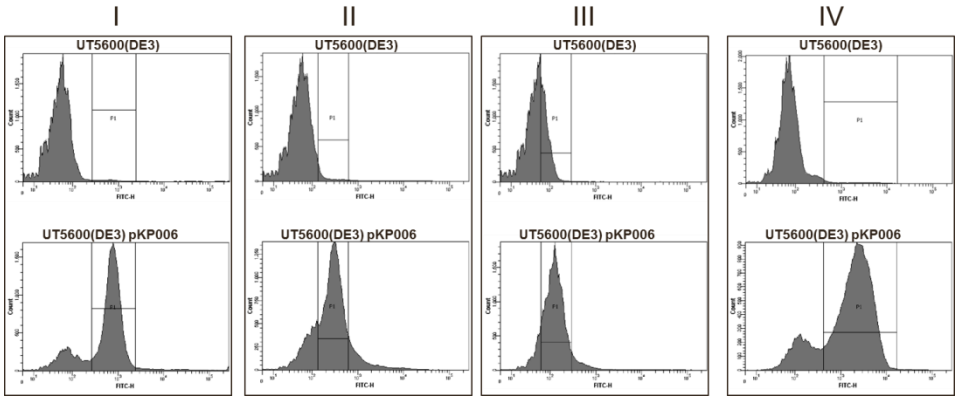


- flow cytometry
- microscopy
- ELISA
- FRET
- fluorescence anisotropy
- …

Labeling of CK2 by FITC

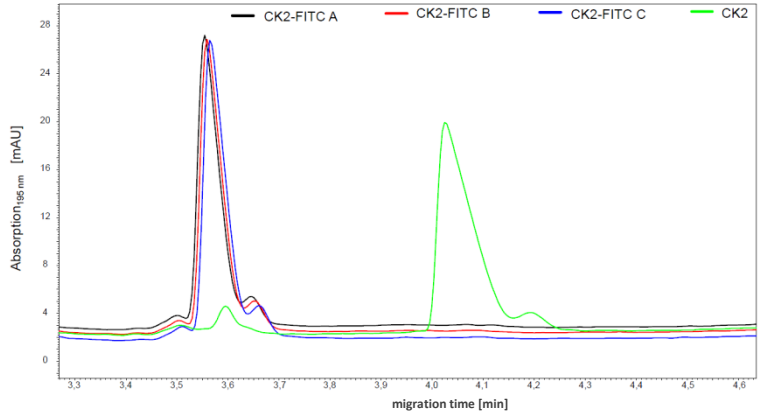


Different CK2-FITC give different results



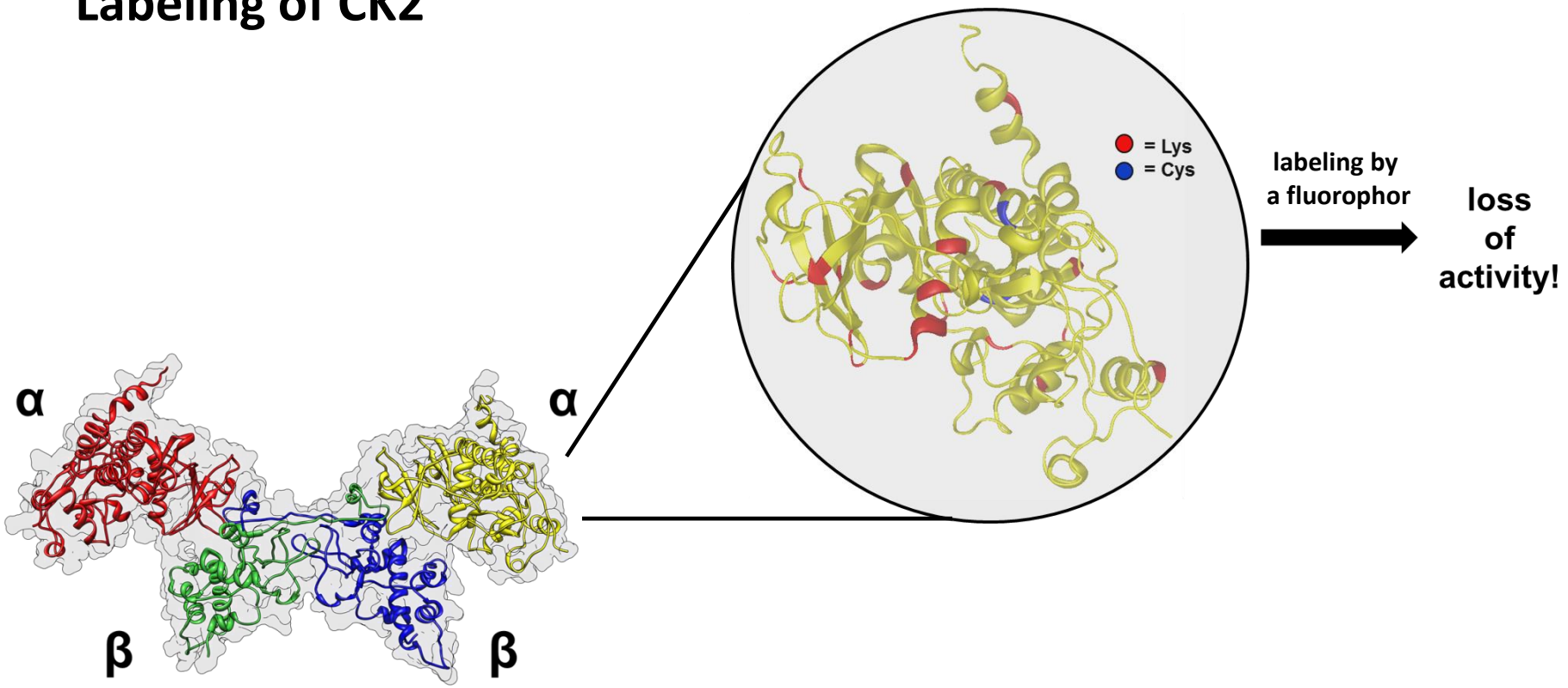
Binding affinities of different CK2-FITC batches (I - IV) were analyzed by flow cytometry. Labeling of CK2 by FITC leads to a different labeling ratio of each batch and is not exactly reproducible. UT5600(DE3) and UT5600(DE3)pKP006, a short αS1-casein fragment, were used as a non-binding and as a binding control, respectively.

No activity for CK2-FITC



Phosphorylation activity of three different batches (A - C) of CK2-FITC were tested by capillary electrophoresis assay (illustrated on slide 12). No activity was detectable after labeling by FITC. Unconjugated CK2 served as a control (green).

Labeling of CK2



Structure of heterotetrameric CK2 holoenzyme (PDB: 1JWH).

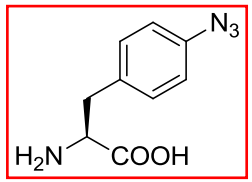
Yellow, red: catalytic α-subunits Green, blue: regulatory β-subunits

Zoom: Visualization of sidechains (Lys + Cys) of CK2α, which would be attacked by commercially available labeling applications.

Modifications of lysine or cysteine residues lead to a heterogenous mixture of labeled CK2 and a wide distribution of CK2-to-fluorophor ratio. No control of the stoichiometry is given. The consequences are distinct affinities, stabilities and phosphorylation activity.

A promising approach: Expanding the genetic code

- Site-specific incorporation of an unnatural amino acid (uaa) into CK2α *in vivo.*

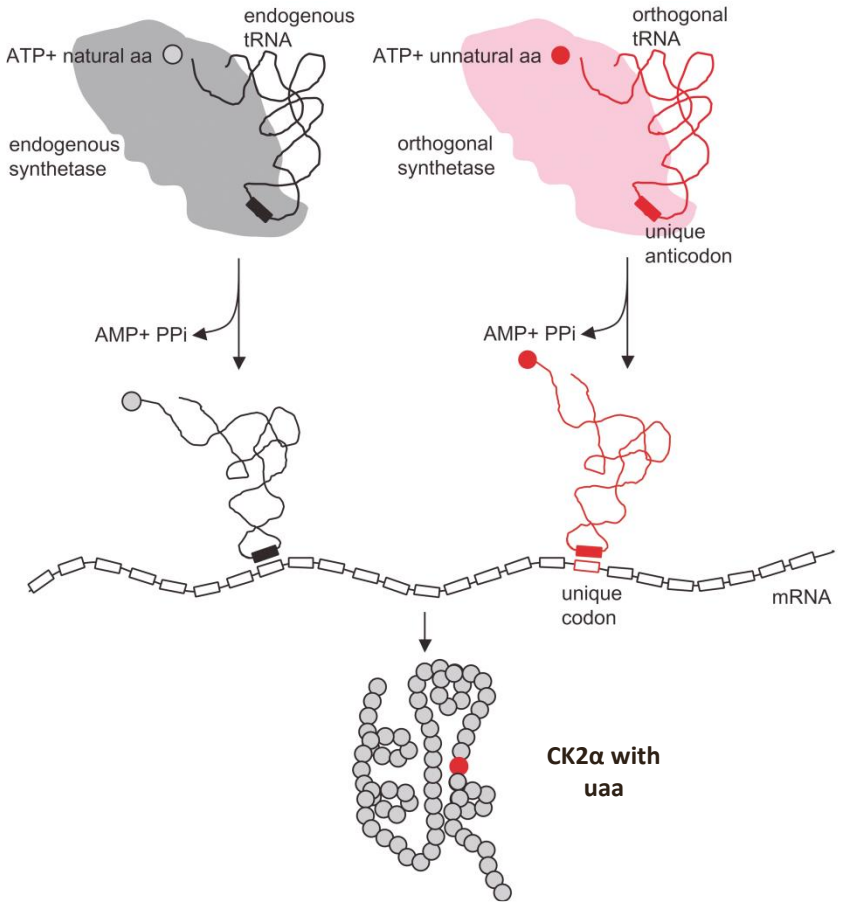


***p*-azido-L-phenylalanine (pAzF)**

- Orthogonal aminoacyl-tRNA synthetase acylates the orthogonal tRNA with uaa

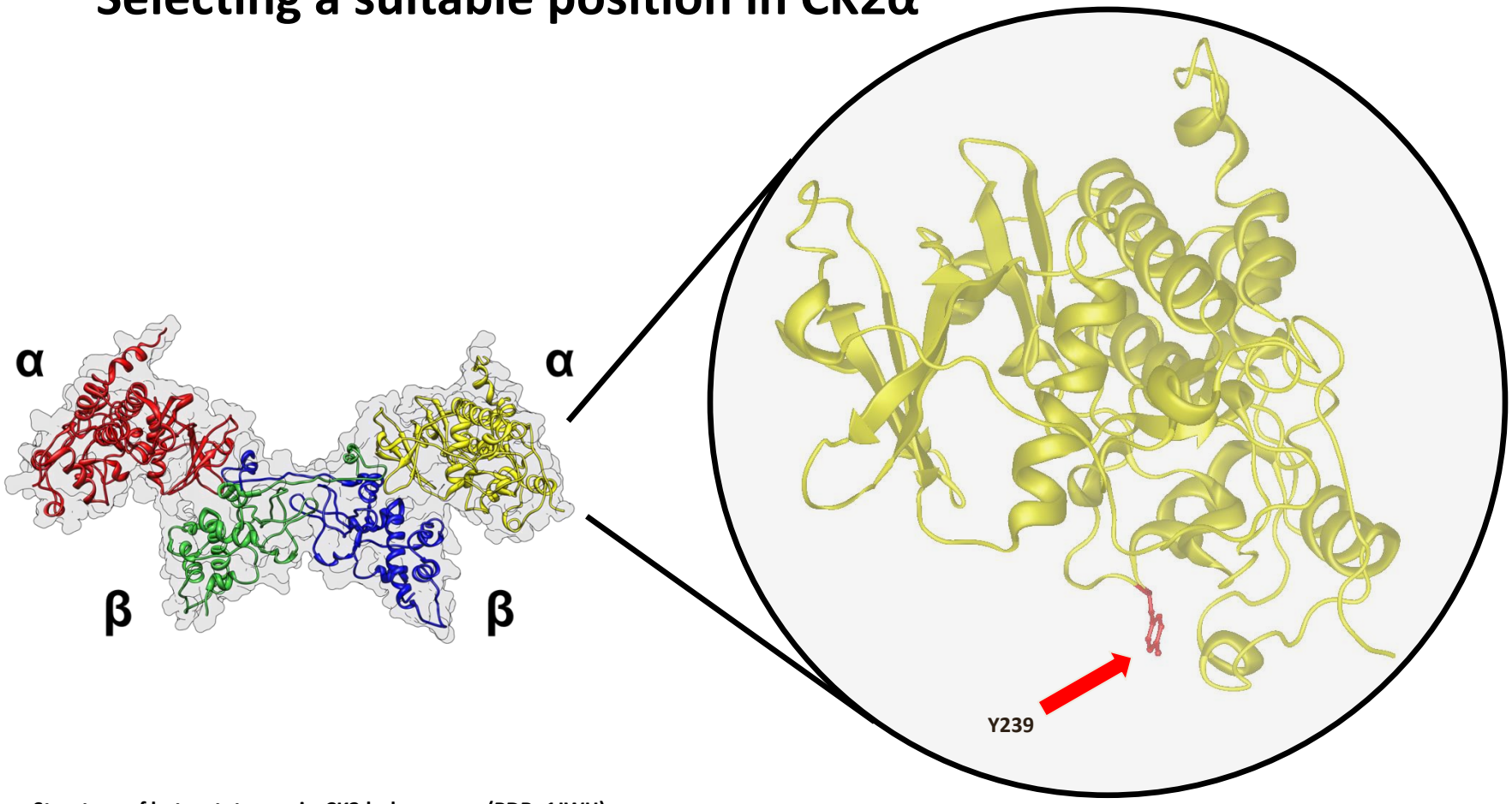
- Acylated orthogonal tRNA inserts the uaa at the position specified by the unique codon

UAG (amber)



Wang, L. and Schultz, P.G.: *Chem. Commun.* **2002**, 7(1): 1-11.

Selecting a suitable position in CK2α



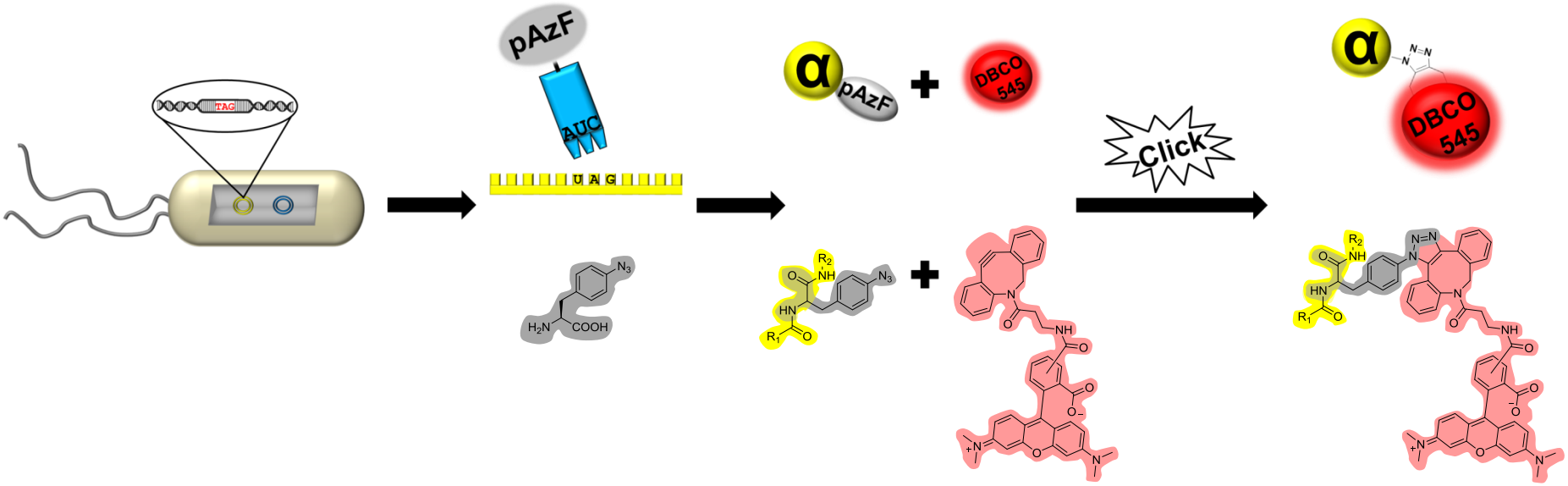
Structure of heterotetrameric CK2 holoenzyme (PDB: 1JWH).

Yellow, red: catalytic α-subunits Green, blue: regulatory β-subunits

Zoom: Illustration of Y239 in CK2α , which was chosen for the specific incorporation of the unnatural amino acid pAzF.

This position represents a sufficient distance to the ATP-binding pocket of CK2α and should not interfere the interaction with the β-subunit.

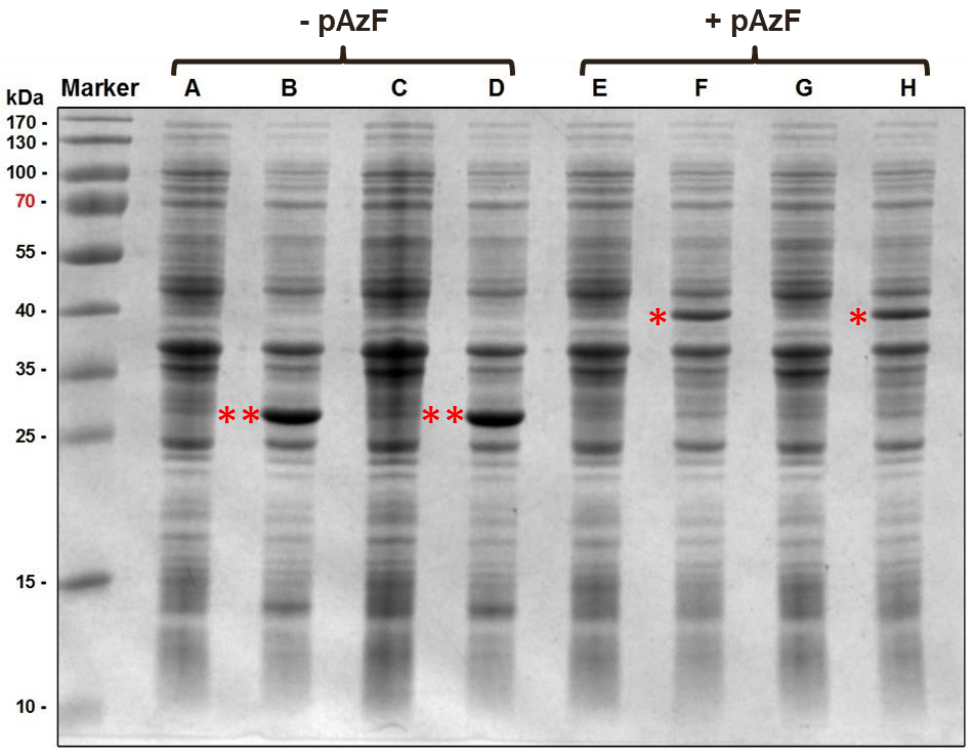
Strategy for labeling CK2α



Incorporation of the unnatural amino acid para-acidophenylalanine (pAzF) into the catalytic CK2α-subunit. In the DNA sequence of CK2α, the position Y239 was chosen and mutated to the amber nonsense DNA codon TAG. The unnatural amino acid pAzF was incorporated at this unique position by suppression of the nonsense codon with an amber suppressor tRNA. By Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) with dibenzylcyclooctyne-fluor 545 (DBCO545) a specifically labeled CK2 was obtained.

Results and discussion

Incorporation of pAzF into CK2α

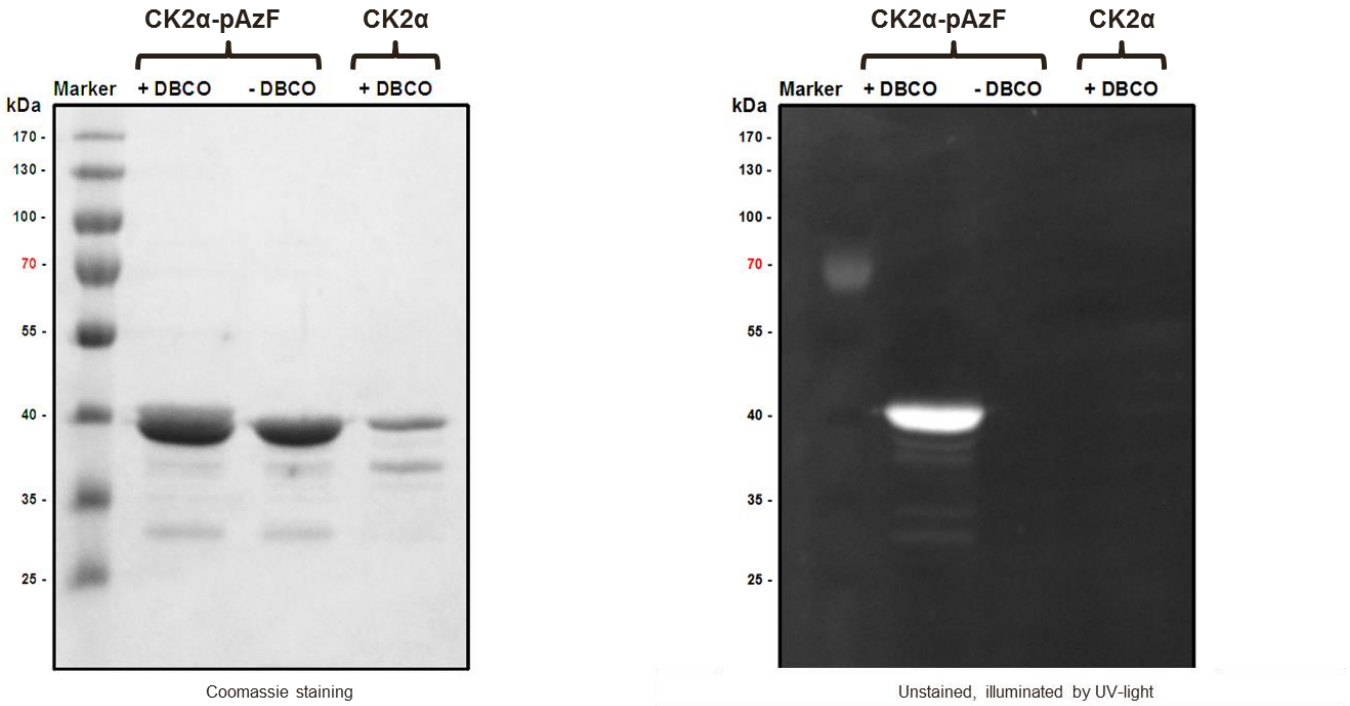


Tube	IPTG	arabinose
A & E	-	-
B & F	+	-
C & G	-	+
D & H	+	+

SDS-PAGE analysis of the incorporation of pAzF into CK2α. All possible combinations of adding pAzF, IPTG and arabinose to *Escherichia coli* BL21(DE3) cells expressing the mutated CK2α (IPTG) and the amber suppressor tRNA/ aminoacyl-tRNA synthetase (arabinose) are given. Theoretically, full-length CK2α (*) should only be expressed in lane H, i.e. when all components are present. The full-length protein can, however, also be produced in tube F because arabinose might not be essential. The truncated CK2α (* *) appears in lane B and D, because of the present nonsense DNA codon TAG and the lack of pAzF.

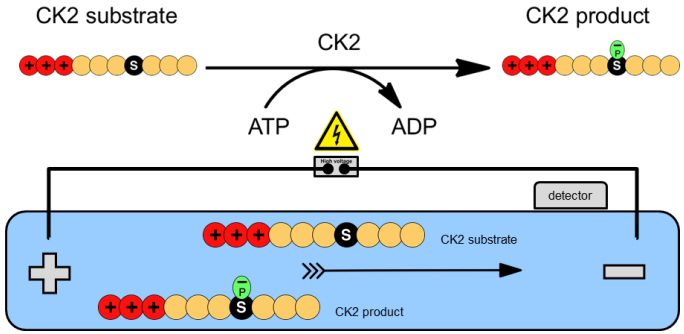
Results and discussion

Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC)

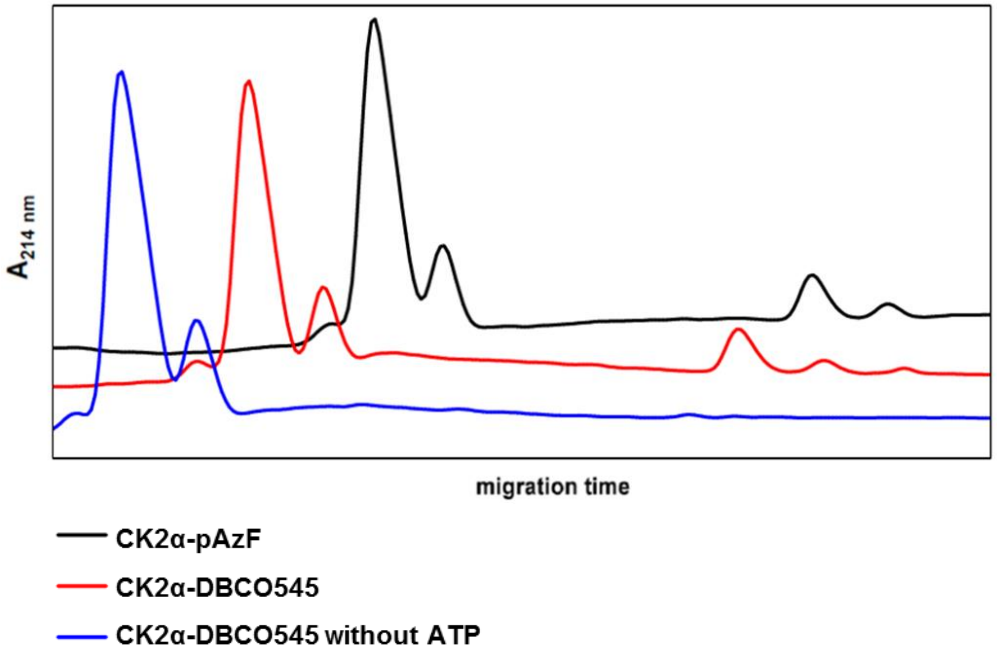


SDS-PAGE analysis of SPAAC. Performing the click reaction by the use of purified CK2α-pAzF and the fluorophor DBCO545 results in a fluorescent protein band. CK2α without the unnatural amino acid served as a control for the specifity.

Activity measurement by capillary electrophoresis



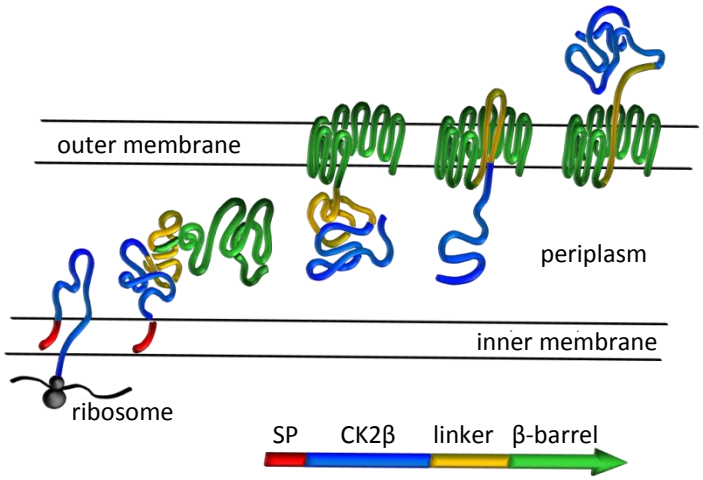
Gratz, A. et al.: *Electrophoresis* **2010**, 31(4): 634-40.



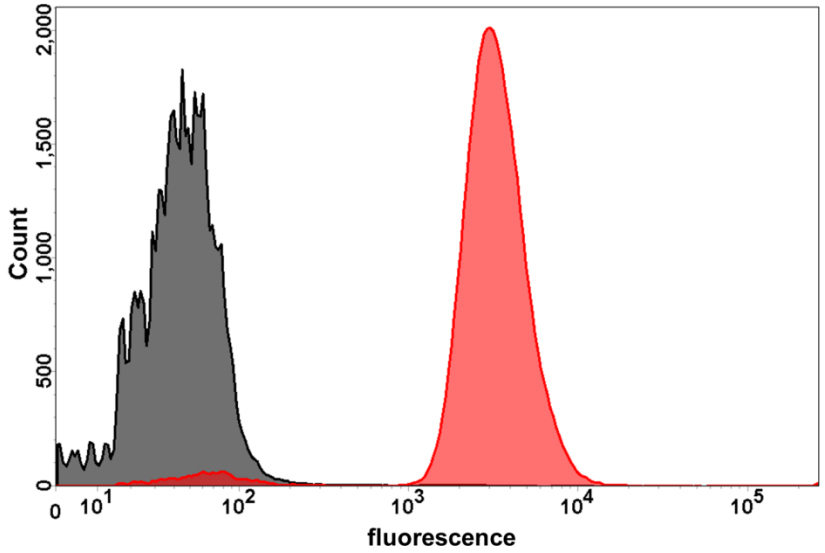
Activity measurement by capillary electrophoresis assay. CK2α-pAzF and CK2α-DBCO545 were tested on phosphorylation activity towards the CK2 substrate peptide RRRDDDSDDD.

Interaction of the specifically labeled CK2α with the surface displayed CK2β-subunit by flow cytometry

Autodisplay of CK2β



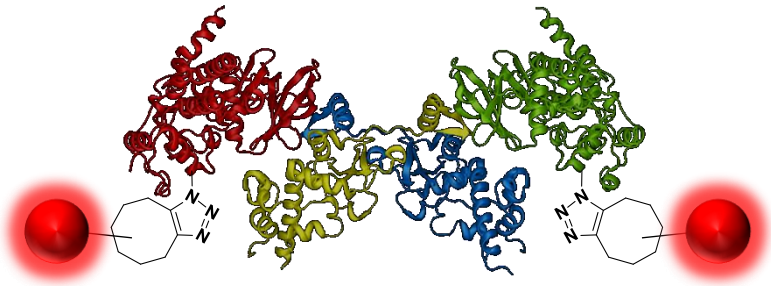
Flow cytometry

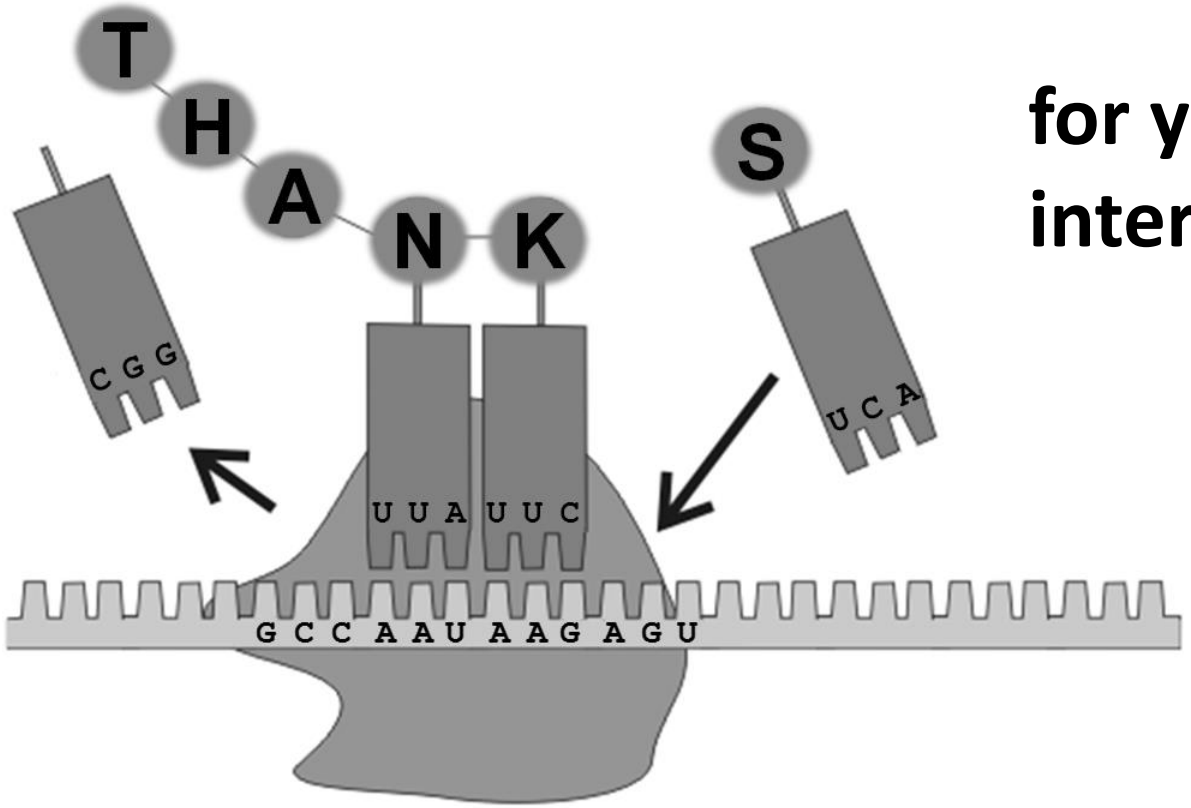


Interaction between purified CK2α-DBCO545 and surface displayed CK2β. Proof of interaction between the purified specifically labeled CK2α-subunit and the CK2β-subunit (red), which was surface displayed on *Escherichia coli* by the Autodisplay technology. As a non-binding control, a surface displayed protein of similar size was used (grey).

Conclusions

It could be shown that the unnatural amino acid pAzF was successfully incorporated into the purified CK2α-subunit. The followed SPAAC click reaction leads to a specifically labeled CK2α. Interaction of the catalytic α-subunit with the CK2β-subunit could be verified by flow cytometry. The phosphorylation activity of this protein kinase was confirmed by the capillary electrophoresis based assay. This provides an excellent starting point for inhibitor screening assays by flow cytometry and for CK2α/CK2β interaction studies.





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