Targeting Protein/Protein Interactions

Protein Kinase CK2 as a Prime Example for a Novel Concept

Eukaryotic protein kinases (EPKs) are predicted to be "the major drug targets of the twenty-first century" [1]. Due to the structural similarity of EPKs a strategy to target their active sites faces serious selectivity problems. A possible solution to overcome this difficulty is to interfere with functionally important interactions of an EPK with regulatory or anchor proteins. The protein kinase CK2 is an ideal object to develop this concept.

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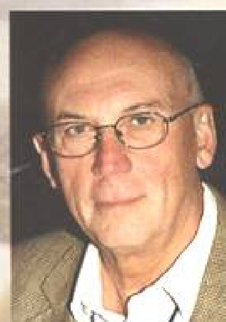

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Protein/Protein Interactions as Targets for Small Molecules

"The molecular sociology of the cell" is the meaningful title of a recent review [2] in which the authors present an overview of macromolecular assemblies and techniques for their investigation. The increasing interest in interactions, rather than in isolated macromolecules documented by this article is further reflected by a current tendency in pharmacology and chemical biology: since many proteins act in combination with protein partners, the protein/protein interactions – more precisely the interfaces between the interaction modules – are regarded as valuable potential targets to be addressed by small molecules [3].

Protein Kinase CK2: A Perfect Test Case

The relevance and the molecular architecture of protein kinase CK2 render this enzyme an attractive study case of such an approach. CK2 is a heterotetrameric serine/threonine kinase with a broad and acidophilic substrate profile [4]. The enzyme is composed of two separate catalytic subunits (CK2α) attached to a dimer of non-catalytic chains (CK2β) (fig. 1A) [5]. CK2α encoding genes are detected in all eukaryotic organisms and essential for their viability [4]. Accordingly, CK2 activity is ubiquitously present in eukaryotic cells, but significantly overexpressed in tumour cells [4]. *Vice versa* CK2α favours the development of lymphoma [4].

Thus, CK2 is biomedically relevant and it is already now subject of industrial drug design efforts [6]. These activities follow the classical strategy to address the canonical ATP-binding site. The principle problem of this approach is the structural similarity of all EPKs in this region so that selectivity of an inhibitor is difficult to achieve. Therefore, in some cases so-called type-II inhibitors have been developed which require the existence of distinctly inactive kinase conformations that can be addressed by the drug [7]. This strategy, however, is excluded for CK2α since due to intramolecular restraints it was never found in an inactive conformation [8].

The CK2α/CK2β interaction may serve to overcome this dilemma: isolated CK2α shows a significant basal activity,

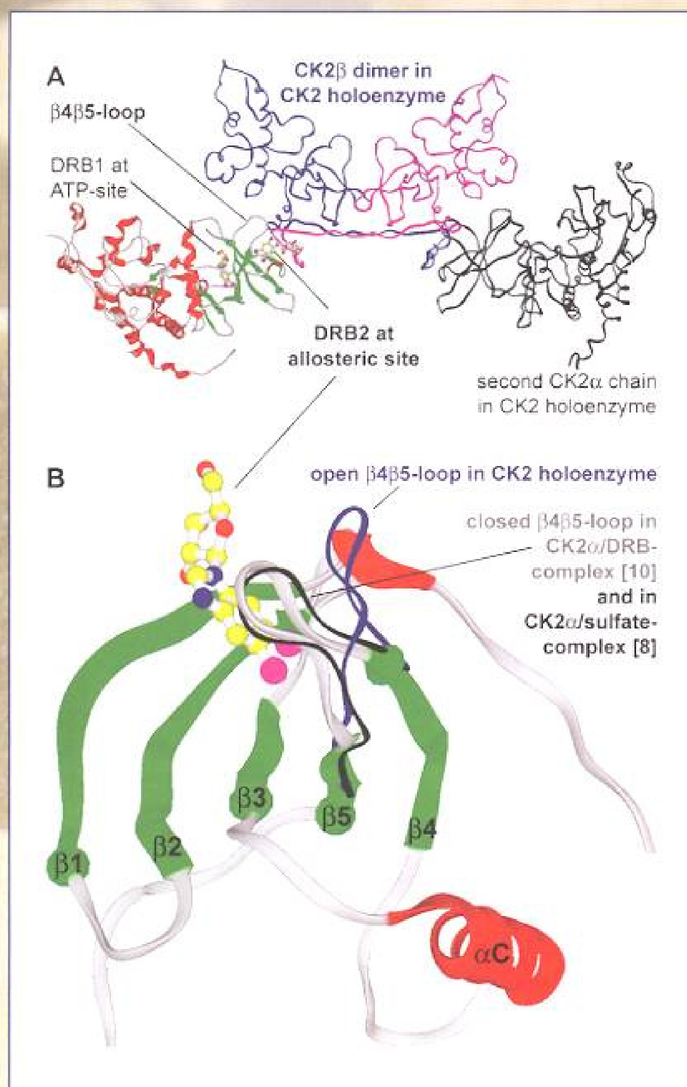

Fig. 1: A) Dual binding of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) to human CK2α [10]. The CK2α/DRB complex structure (left chain with red helices and green strands) was superimposed on one of the CK2α chains of the CK2 holoenzyme [5] to illustrate the location of the DRB2 binding cavity relative to the CK2β dimer (blue and magenta backbone).
B) The two principle conformations of the $\beta_4\beta_5$ loop of CK2α. The main part of the figure was prepared from the human CK2α/DRB structure [10] where the $\beta_4\beta_5$ loop is fixed in a CK2β incompatible conformation similar to all other known structures of isolated human CK2α. In contrast the β4β5 loop is open in the CK2 holoenzyme [5].

but the association with CK2β significantly increases this activity level [4] and changes the specificity and stability of CK2α. Therefore, disturbing this interaction with small molecules may deplete the CK2 activity *in vivo*. This vision is realistic since the CK2α/CK2β interface is relatively small [5].

The CK2β Approach

In a first approach Laudet et al. [9] developed peptidic inhibitors of the CK2α/CK2β interaction. Based on the CK2 holoenzyme structure [5] the authors suggested interaction "hot spots", i.e. residues from the C-terminal tail of CK2β that are particularly important for CK2α binding. Binding studies with linear and cyclised CK2β peptides confirmed that the two aromatic side chains of Tyr188 and Phr190 (fig. 2) are in fact such hot spots. Some of those peptides compete effectively with CK2β for CK2α binding; they disrupt the preformed CK2 holoenyzme and hence significantly change the enzymatic profile. The authors plan to develop these peptides further in the direction of peptidomimetic drugs [9].

The CK2α Approach

The aforementioned approach takes CK2α as a static molecule without conformational changes when CK2β or an antagonist is bound. Yet, several structures of unbound human CK2α have disproven this assumption. In all these structures the $\beta_4\beta_5$-loop as a key element for CK2β binding adopts a "closed" conformation in which CK2β association is sterically prohibited while it is "open" when in the CK2 holoenzyme [5] (fig. 1B). The obvious strategy emerging from this observation is to stabilise the CK2β incompatible conformation of CK2α.

A first step towards this goal was the detection of a small-molecule binding pocket next to the β4β5-loop in its closed conformation. In a co-crystal structure of CK2α with the inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) [10] the latter was observed at two locations: at the canonical ATP-site (DRB1 in fig. 1A) and at an allosteric site formed by the closed β4β5-loop and the outer surface of the N-terminal β-sheet (DRB2 in fig. 1A). Using

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differential scanning calorimetry DRB was shown to have a significant destabilising effect on the CK2 holoenzyme [10].

Crystallography Meets NMR and Calorimetry

Meanwhile further small molecules addressing the DRB2 cavity on the CK2α surface (fig. 1A) could be detected. The saturation transfer decay (STD) NMR technique has proven to be particularly helpful in this context since it allows to rapidly probe the affinity of several compounds simultaneously. In addition to "blind" STD screening trials a full thermodynamic rationalisation of the CK2α/CK2β interaction as provided by the isothermal titration calorimetry method will be essential in the future.

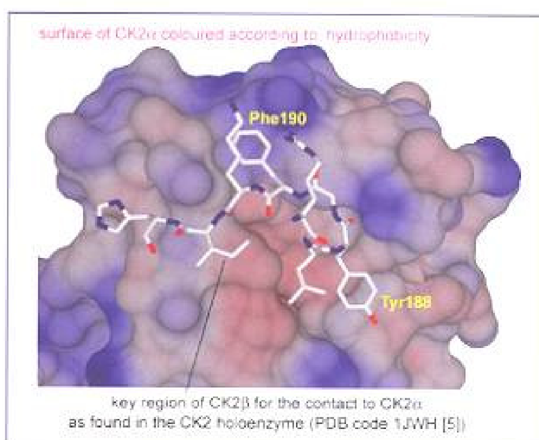

Fig. 2: The CK2α interaction loop in the C-terminal region of CK2β as seen in the CK2 holoenzyme [5]. Based on this structural motif and using Tyr188 and Phe190 as critical constraints Laudet et al. [9] designed cyclic peptides to mimic this region and to antagonise CK2β.

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