

Structural Basis of CK2 Regulation by Autoinhibitory Oligomerization

Graziano Lolli and Roberto Battistutta

Abstract The mechanism of regulation of CK2 differs from those common to other eukaryotic protein kinases and is not entirely established yet. Nowadays, several crystal structures of the tetrameric $\alpha_2\beta_2$ holoenzyme are available, supporting a structural model of an autoinhibitory regulation by oligomerization proposed several years before on the basis of biochemical, biophysical and functional data. Monoclinic crystal forms of the holoenzyme reveal the symmetric architecture of the “free” isolated active tetramers. The dimension and the nature of the α/β interfaces configure the symmetric holoenzyme as a strong complex that does not spontaneously dissociate in solution, in accordance with the low dissociation constant (≈ 4 nM). Hexagonal crystal forms of the CK2 holoenzyme show an asymmetric arrangement of the two catalytic α -subunits around the obligate β_2 regulatory subunits. These asymmetric $\alpha_2\beta_2$ tetramers are organised in trimeric rings and filaments that correspond to the inactive forms of the enzyme, whereby the β -subunit plays an essential role in the formation of inactive polymeric assemblies. The derived structural model of (down)regulation by aggregation contributes to the interpretation of many available biochemical and functional data, although it awaits for a more comprehensive validation at the cellular level. These findings pave the way for the design of novel strategies aimed at the modulation of the CK2 activity.

Keywords Crystal structure • Symmetric CK2 holoenzyme • Asymmetric CK2 holoenzyme • Trimeric CK2 holoenzyme • Oligomerization • Filaments • Activity regulation • Autoinhibition • Crystal contacts • α -Subunit • β -Subunit • $\alpha_2\beta_2$ tetramer • α/β interfaces • CK2 β acidic loop • CK2 α p+1 loop • CK2 β C-terminal tail • Electrostatic interactions • Polyamines • Autophosphorylation

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1 The First Asymmetric $\alpha_2\beta_2$ Holoenzyme Crystal Structure

The first crystallographic structure of the CK2 holoenzyme was determined in 2001 (PDB 1JWH) [1], joining those already known of the isolated α - and β -subunits [2, 3], a great achievement finally solving the puzzling CK2 quaternary arrangement. CK2 tetramer has a butterfly shape with a central β -dimer and the two α -subunits, one on each side and not in contact between them (Fig. 1a). This was also the first structure including the full-length β -subunit that can be divided into a body and a C-terminal tail, the last one not included in the previous structures. CK2 β C-terminal tail has no contact with the body of its own chain but binds both the other CK2 β monomer and one of the α -subunits (Fig. 1a). This tail contributes significantly to the stability of the β -dimer. The β/β interface increases from 543 \AA^2 in the structure of the isolated and truncated β -dimer to 1,766 \AA^2 in the structure of the holoenzyme, in agreement with the obligate nature of the β -dimer. The authors also highlighted that this increase in the interface requires the CK2 β tail in a specific conformation, which is stabilised by the involvement of the same tail in large contacts with the α -subunits and hence depends on the existence of the complete tetramer. The CK2 β tail has then a synergistic character, stabilising both the β/β and the α/β contacts. At the same time, the authors pointed out that this region was not well defined in the electron density and then the very C-terminal amino acids (from Asn206 to Arg215) were left out from the final model, while in the preceding region (from Phe190 to Ser205) the conformations of peptide groups and side chains remain questionable.

Each α -subunit binds both subunits of the β -dimer, the body of one chain and the tail of the other (Fig. 1a); this last interaction is exploring a larger interface (491 \AA^2 vs. 336 \AA^2) and being considered the major determinant of the α/β stability. The structure of the catalytic α -subunit is not significantly affected by the binding of the β -dimer, maintaining its active conformation and confirming previous observations that CK2 β is an environment- and substrate-dependent modulator of CK2 α activity rather than an on/off switch. It was also noted that the tetramer is asymmetric with a relative rotation of 16.4° at the two α/β interfaces for the two halves of the tetramer (Fig. 1a). As a consequence, the two α/β interfaces are different (Fig. 1b) and this was interpreted as an intersubunit flexibility. In the basis of such observation, together with an average size for the α/β interface of 832 \AA^2 , authors proposed that the CK2 tetramer is a transient heterocomplex.

Finally, in the same paper it was pointed out that the inhibitory effect of the CK2 β acidic loop (Asp55–Asp64) observed in previous works [4] cannot be exerted intramolecularly since the distance between this and the closest CK2 α basic cluster (the potential substrate binding site) was >30 \AA .

A few years later, the same authors analysed the crystal packing in the previous structure, showing that the contact between the β -acidic loop and the α -basic cluster was indeed present but between different tetramers [5]. They identified a new CK2 organisation, composed by self-consistent ring-like trimers of tetramers, where the core of the interaction between different tetramers in $(\alpha_2\beta_2)_3$ is formed by the interface between the β -acidic loop and the α p+1 loop (Arg191–Lys198) at the entrance

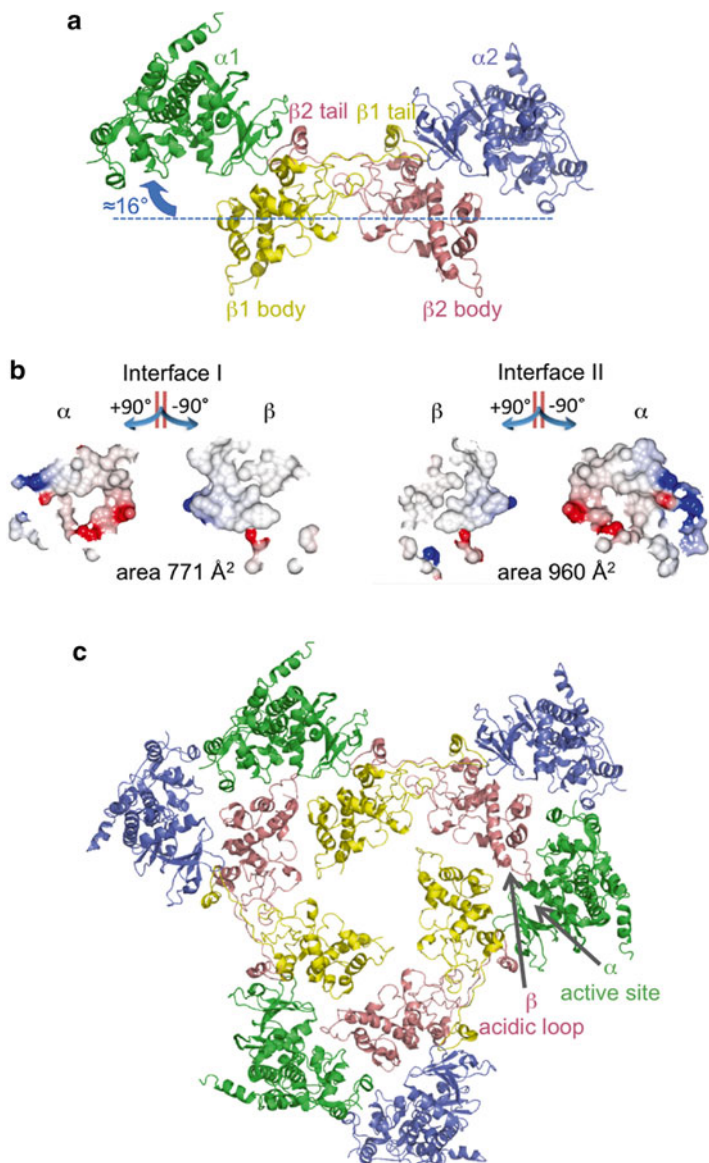


Fig. 1 Structure of the CK2 holoenzyme (PDB, 1JWH) [1]. (a) The CK2 holoenzyme has a butterfly shape with the two catalytic α -subunits not in contact between them. The CK2 β tails have a fundamental role in the holoenzyme both by strengthening the β -dimer and by largely contributing to the α/β interface. The holoenzyme is asymmetric with a relative rotation of 16.4° for the two halves of the tetramer. (b) The asymmetric assembly generates two different α/β interfaces. Adapted with permission from [28]. Copyright (2014) American Chemical Society. (c) The holoenzyme assembly in circular trimers of tetramers has been identified in the crystallographic packing [5]. The core of the interaction is between the β -acidic loop and the α p+1 loop at the entrance of the catalytic site

of the α active site (Fig. 1c). As a consequence, three out of six catalytic subunits of the trimeric ensemble are blocked by the β -acidic loop.

This organisation in trimers of tetramers was able to explain a number of previous observations not easily interpretable solely on the basis of the tetrameric structure. Mutations in the β -acidic loop or in the α p+1 loop generate hyperactive CK2 holoenzyme [4, 6], since trimers of tetramers cannot form anymore. Polyamines like spermine activate CK2 [7–9] by interacting with the CK2 β acidic loop and then disrupting the trimers of tetramers. The CK2 holoenzyme exhibits maximum activity at about 300 mM NaCl or KCl, a property of the holoenzyme and not of the isolated CK2 α [10]. The holoenzyme also needs a minimal NaCl concentration of 250 mM NaCl to bind to phosphocellulose, while it aggregates under low salt conditions [11]. Since the interaction between the β -acidic loop and the α p+1 loop is electrostatic in nature, it is strongly affected by ionic strength. Finally, CK2 holoenzyme has been observed in different ring-like and linear polymeric forms connected via reversible equilibria affected by ionic strength and spermine [12, 13].

2 The Second Asymmetric $\alpha_2\beta_2$ Holoenzyme Crystal Structure

In 2012, we presented a new structure of the holoenzyme (4DGL) crystallised in the same space group and unit cell of the previous 1JWH structure but with higher overall quality [14]. While confirming many features of the 1JWH structure, the 4DGL structure shows significant improvements in important regions such as the α/β interface and the C-terminal tail of the β -subunit. In particular, β -Phe190 leans on the outer surface of the α -subunit N-terminal lobe in 1JWH, while in 4DGL it inserts deeply into a hydrophobic cavity in the α -subunit, known as the secondary or allosteric binding site (Fig. 2a, b). The α/β interaction is then significantly strengthened in accordance with the almost complete abrogation of the binding for the mutant β -Phe190Ala [15]. Also the β C-terminus was built differently with an upstream shift in sequence of two amino acids and extended the α G helix from residues 196–200 in 1JWH to residues 194–207 in 4DGL (Fig. 2b). This again contributes to refine the α/β interface to a better energetic profile (interface increased from 832 \AA^2 in 1JWH to 1,010 \AA^2 in 4DGL) (Fig. 2c). Finally, the β C-terminus was built to the last Arg215 amino acid.

As already noted by Niefind and Issinger [5], the acidic loop of the β -subunit from one tetramer is in contact with the p+1 loop of the α -subunit from another tetramer (Fig. 3a). The interface area is 651 \AA^2 , larger than in 1JWH (538 \AA^2), and greater than normal crystallographic contacts, which have a mean interface of 285 \AA^2 and rarely exceed 500 \AA^2 [16]. This interaction is essential for the organisation of circular trimers of tetramers as previously observed (Fig. 1c), with a positive cooperative effect for their formation and a large interface of 1,953 \AA^2 (651 $\text{\AA}^2 \times 3$).

The newly traced C-terminus of CK2 β is instead responsible for a different inter-tetrameric interaction. This region protrudes from one tetramer and contacts the

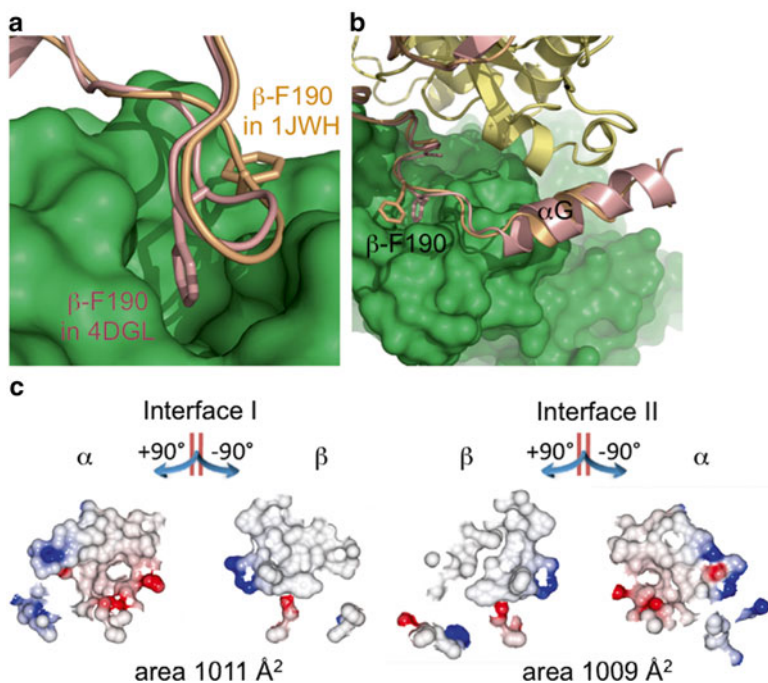


Fig. 2 Details of the α/β interaction in the improved structure of the CK2 holoenzyme (4DGL) [14]. (a) In 4DGL, β -Phe190 (salmon) inserts into a deep hydrophobic pocket on the CK2 α surface (green), differently from the 1JWH structure (orange). Adapted with permission from [14]. Copyright (2012) American Chemical Society. (b) In 4DGL, β C-terminus was built to the last Arg215 and region 194–205 was better modelled with respect to the 1JWH structure; colour code as in (a). Adapted with permission from [14]. Copyright (2012) American Chemical Society. (c) In the improved 4DGL structure, the two α/β interfaces are larger than in 1JWH (Fig. 1b). Adapted with permission from [28]. Copyright (2014) American Chemical Society

C-terminal lobe of an α -subunit of a neighbouring tetramer ending in its ATP pocket (Fig. 3a, b). The interface is large (753 \AA^2) and is complemented by the interaction between the N-terminal lobe and the C-terminal lobe of two α chains from the same two tetramers. The overall interface of $1,254 \text{ \AA}^2$ is then quite extended and again much larger than a normal crystallographic contact. It shows a number of polar contacts, which again makes this interaction susceptible to ionic strength changes. This new interaction is fundamental for a piling organisation of trimers of tetramers (Fig. 3c), and the newly identified polymeric form can represent the filamentous CK2 aggregates described in literature [12, 13]. Moreover, the β C-terminus inserts into the ATP-binding pocket of a catalytic subunit, competing with ATP and stabilising a nonproductive conformation of the α catalytic residues (Fig. 3b). This polymeric form is then expected to be completely inactivated by the combination of the interactions of the α -subunits with the acidic loops and C-termini of β -subunits (Fig. 3a) and by steric hindrance.

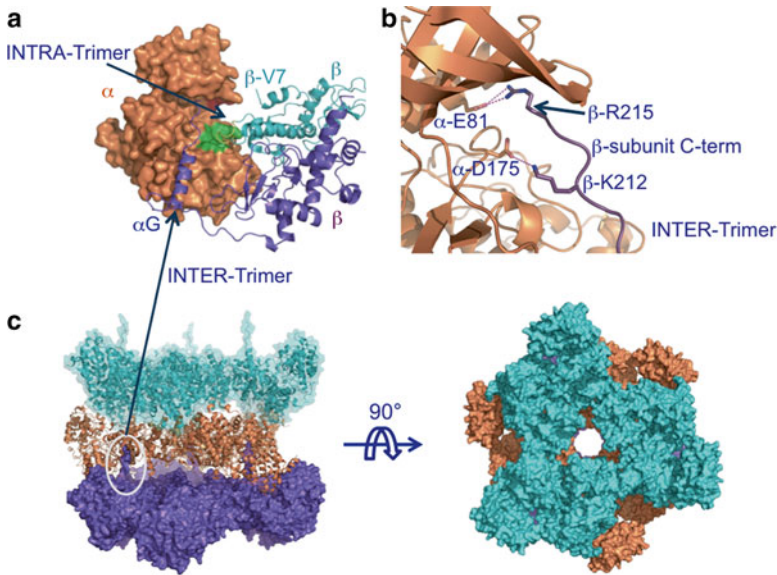


Fig. 3 Oligomeric organisation in the 4DGL structure. Adapted with permission from [14]. Copyright (2012) American Chemical Society. (a) Different inter-tetrameric contacts can be identified. The interaction between the β -acidic loop (β -subunit in cyan with its acidic loop in yellow) and the α p+1 loop (α -subunit in orange with its p+1 loop in green) is responsible for the assembly in circular trimers of tetramers. The interaction between the α G and the C-terminal tail of a different β -subunit (purple) and the α C-terminal lobe and active site (orange) is responsible for a piling organisation of trimers of tetramers. The position of the first amino acid visible at the β N-terminus (Val7) is compatible with the autophosphorylation of the upstream residues Ser2 and Ser3. Phosphorylated residues could then interact with the α -basic cluster (red). (b) Insertion of the β C-terminus into the α active site disrupts the correct orientation of α residues involved in nucleotide binding. (c) In the filamentous organisation, the C-termini of three β -subunits are plugged into the following trimer

This new structure and the hypothesised organisation in trimers of tetramers and subsequently in filaments allow expanding the experimental data previously reported in support of the trimeric organisation by Niefind and Issinger [5]. FRET experiments revealed the existence *in vivo* of two subpopulations of “fast” and “slow-moving” CK2 and showed that different CK2 β dimers can be in close molecular proximity [17]. The downregulating effect proposed for the CK2 β C-terminal tail is in accordance with other *in vivo* observations. CK2 activity is increased in prostate cancer cells by CDK1 phosphorylation of β -Ser209 [18] that engages α -Glu230 in the filamentous organisation, which will then be weakened by the phosphorylation event. Phosphorylation of β -Thr213 by Chk1 is observed in the free β -subunits but not in the tetrameric holoenzyme [19], where the residue is masked in the filamentous oligomers. The downregulatory polymerisation mechanism mediated by the β -subunits is also supported by the observation that transfection of HeLa cells with a degradation-resistant CK2 β strongly inhibits proliferation and counteracts the promotion of proliferation exerted by the isolated CK2 α [20].

It is well known that CK2 autophosphorylates on residues Ser2 and Ser3 of the β -chain [21]. As already observed by Niefind and Issinger [5] and further discussed in [14], the β N-terminus is close to an α catalytic site in the trimeric organisation, so that one tetramer could phosphorylate the neighbouring one (Fig. 3a). Observations in favour of this trans-autophosphorylation mechanism inside the trimeric organisation are the following: autophosphorylation (a) is abolished at high ionic strength [22] and (b) is strongly inhibited by neutralisation of residues in the β -acidic loop [23]; (c) although being in trans, it shows an intramolecular kinetics [24]; and (d) no autophosphorylation occurs in the holoenzyme with the α' paralog, a holoenzyme that does not form oligomers [25]. We also proposed that the β N-terminus, once phosphorylated, could engage the nearby α C basic cluster of the α -subunit from the neighbouring tetramer, further stabilising the ring-like organisation. Autophosphorylation is (e) extensively observed in cells [21], (f) stabilising the enzyme in vivo [26] and (g) having an inhibitory effect, since treatment of CK2 with phosphatases increases its activity [27].

3 The Symmetric $\alpha_2\beta_2$ Holoenzyme Crystal Structures

Very recently, we added to the above picture three additional crystal structures of the $\alpha_2\beta_2$ holoenzyme [28]. In both the 1JWH and 4DGL structures, the $\alpha_2\beta_2$ tetramer is asymmetric. Notably, in the new structures (4MD7, 4MD8 and 4MD9), obtained in monoclinic (rather than hexagonal) space groups, the tetramer is symmetric (Fig. 4a). This reveals that the asymmetry observed in the previous structures is not an intrinsic structural property of the monomeric holoenzyme but rather is generated by the incorporation of tetramers into trimeric rings, whose assembly is not compatible with the symmetric architecture (Fig. 4b). The analysis of the crystal packing of the monoclinic form of the holoenzyme does not seem to support the possibility of stable oligomeric or filamentous forms. Unlike in the hexagonal lattice, where discrete trimeric rings of tetramers are clearly recognisable, in the monoclinic form all contacts between different tetramers have a pure crystallographic nature. Indeed, there is one interaction between the acidic loop of the β -subunit and the basic clusters of the α -subunit of a symmetric tetramer with a significant interface area (around 750 Å²). However, this contact is not responsible for the formation of any closed, self-consistent, oligomeric form of tetramers, nor of a filament of tetramers. Rather, it is responsible for the propagation of two parallel layers of tetramers in two dimensions for the entire crystal lattice (i.e. infinitely), giving raise to a sort of infinite two-dimensional bilayer of tetramers. Hence, it has the characteristic of a crystal lattice contact, making highly improbable, in our opinion, the existence of stable discrete oligomeric or filamentous forms based on this interaction. We then concluded that the new symmetric structures represent the organisation of the “free”, active, monomeric CK2 holoenzyme, the previous 1JWH and 4DGL showing instead the structure of inactive trimers of tetramers. However, it has to be noted that some of the inter-tetrameric interactions typical of the monoclinic crystal packing, observed also for the CK2^{andante} holoenzyme mutant [29],

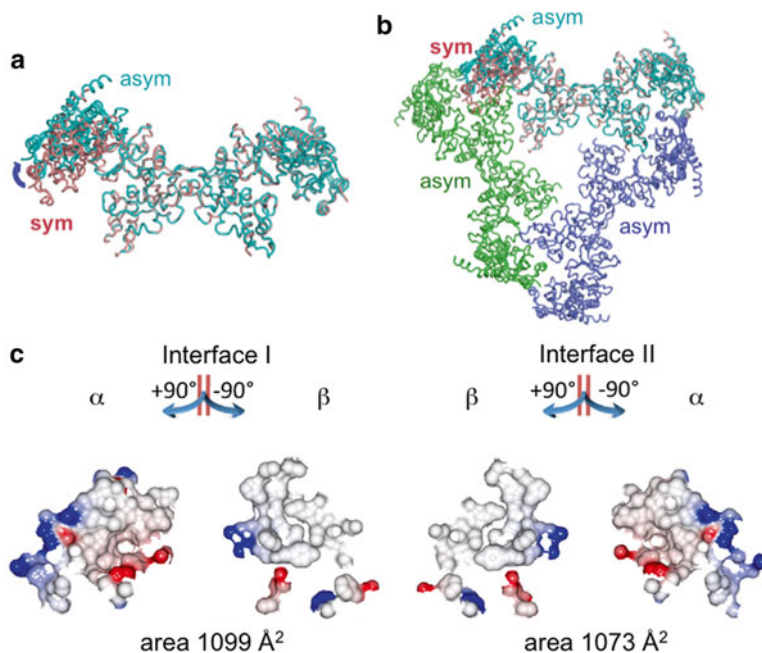


Fig. 4 Structural features of the symmetric tetrameric holoenzyme. Adapted with permission from [28]. Copyright (2014) American Chemical Society. (a) Structure of symmetric tetramer in salmon (4MD7) [28] superposed to the asymmetric 4DGL tetramer (cyan). (b) In 4DGL, the assembly of three tetramers (green, cyan and purple) in trimers of tetramers induces the distortion not present in the symmetric tetramer (salmon). (c) In the symmetric tetramer, the two α/β interfaces are very similar in size and nature

have been postulated to be at the basis of other possible (linear) assemblies with physiological significance [30].

The comparison of symmetric and asymmetric tetramers in terms of their α/β interfaces and then of their stability is of particular relevance since the possibility that the CK2 holoenzyme coexists in equilibrium with its free constituent subunits can represent a regulatory mechanism for its biological function. In 1JWH, the two α/β interfaces are significantly different in size, one being larger (960 Å²) and the other significantly smaller (771 Å²) [1] (Fig. 1b). This suggested that the CK2 holoenzyme might be a transient heterocomplex, spontaneously dissociating in vivo. This seems in contrast with the well-known elevated stability in vitro of the complex ($K_D \approx 4$ nM) [31, 32]. In 4DGL, the better-traced β C-terminal tail generated larger interfaces with similar sizes (1,009 and 1,011 Å²); however, due to the asymmetric assembly, the two interfaces are different in nature with different residues involved [14] (Fig. 2b).

The new symmetric structures have very similar α/β interfaces both in size (1,099 and 1,073 Å²) and chemical nature (Fig. 4c). This was also confirmed by PISA ('protein interfaces, surfaces and assemblies' service at the European Bioinformatics

Table 1 α/β interface parameters in symmetric $\alpha_2\beta_2$ holoenzyme

Parameter	Value	Comment
Mean interface area	1,086 Å ²	740–810 Å ² for weak transient complexes
f_{np} (nonpolar fraction of interface area)	70.5 %	Largely hydrophobic
P -value (PISA solvation energy gain)	0.109	Largely hydrophobic
R_p (residue propensity score)	9.8	Typical of ‘strong’ complexes
f_{bu} (fraction of fully buried atoms)	30 %	High shape complementarity
S_c (shape complementarity score)	0.68	High shape complementarity
I_{gap} (gap volume index)	1.6	High shape complementarity
L_D (atomic density index)	38.7	High shape complementarity

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Institute [33]) that predicted a dissociation pattern in $2\alpha + \beta_2$ from monoclinic structures but in $\alpha\beta_2 + \alpha$ for both 1JWH and 4DGL as a consequence of their asymmetry. The interface size is the most commonly used parameter to infer the stability of a complex; indeed, also its chemical and sterical nature (hydrophobicity and complementarity) needs to be considered [34]. Evaluating a number of parameters (reported in Table 1), we proposed that the CK2 holoenzyme configures as a non-obligate permanent heterocomplex. In obligate complexes, the constitutive protomers are not stable in the isolated form and they simultaneously fold and bind to their partner [35, 36], as in the case of the CK2 β dimer. Obligate complexes have interface areas generally much larger than 1,000 Å² [37]. The CK2 holoenzyme is obviously a non-obligate complex. Permanent protein-protein interactions are strong, with nanomolar or subnanomolar dissociation constants, and irreversible, normally only perturbed by proteolysis [35, 36]. Transient complexes can instead dissociate in their constituent subunits and can be distinguished as weak or strong [35, 36]. In strong transient complexes, a molecular trigger (i.e. phosphorylation or effector molecule) drives the equilibrium through a large conformational change between a high-affinity state (low- or subnanomolar K_D) and a low-affinity state (micromolar K_D). Weak transient complexes have instead micromolar dissociation constants and are characterised by a dynamic equilibrium where interaction is formed and broken continuously; their interfaces are small (mean interface for weak transient complexes was determined in 740 Å² by Nooren and Thornton [37] and in 810 Å² by Dey and co-workers [34]). Given those data, with a $K_D \approx 4$ nM and an interface area of 1,086 Å², CK2 cannot be considered a weak transient complex. It is however impossible to distinguish between permanent and strong transient complexes solely on the basis of the dissociation constant and the interface parameters [34]. Despite that, since no molecular triggers able to alter the affinity of the CK2 protomers have been identified so far and no significant structural rearrangements of the individual subunits are observed during the formation of the CK2 holoenzyme complex, the $\alpha_2\beta_2$ tetramer should be considered a permanent complex, until proven otherwise.

Indeed, isolated α - and β -subunits have been observed in cells [38]. However, as underlined above, crystallographic data, the K_D and the stability of the enzyme in vitro strongly argue against the possibility that the CK2 $\alpha_2\beta_2$ holoenzyme can

spontaneously dissociate in solution. Instead, the existence of “free”, isolated α - and β_2 -subunits can be associated to different factors such as unbalanced expression, differential localization or transport mechanism and to competitive binding with other partners, like the A-Raf, c-Mos and Chk1 kinases, all reported to interact with CK2 β possibly using the same interface explored by CK2 α [39]. As already stated, molecular triggers capable to dissociate the complex have not been identified so far. We also excluded by crystallography that this molecular switch could reside in the phosphorylation of the CK2 α C-terminal tail (phosphorylated by Cdk1 at mitosis [40]).

4 Working Model of CK2 Regulation by Autoinhibitory Oligomerization

The overall regulatory model emerging from structural studies and in accordance with many biochemical, biophysical and activity data is depicted in Fig. 5. When co-localised in time and space, CK2 α and CK2 β dimers spontaneously assemble into an active, symmetric and permanent complex with a $K_D \approx 4$ nM. Enzymatic

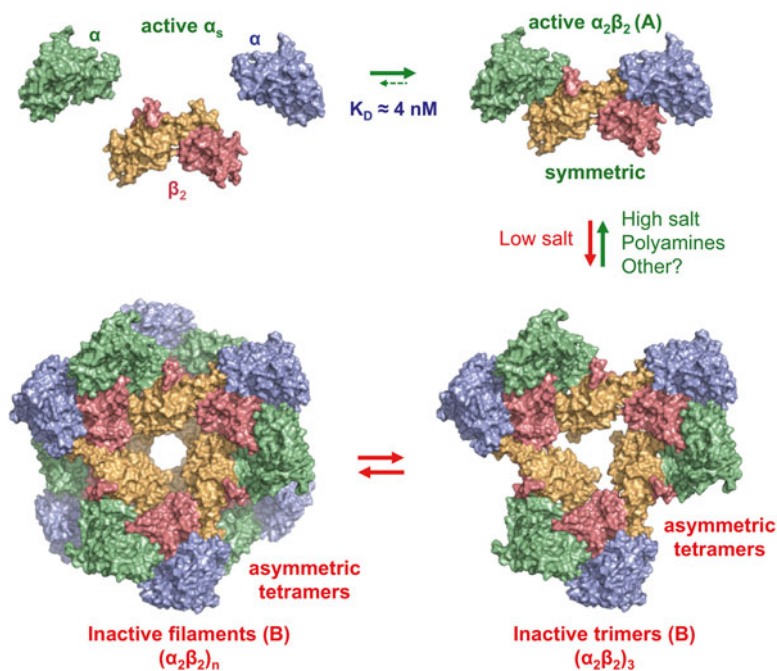


Fig. 5 Proposed model of CK2 regulation. Reprinted with permission from [28]. Copyright (2014) American Chemical Society. CK2 α and CK2 β subunits readily assemble into active and symmetric tetramers. Activity is turned off through organisation in inactive trimers and filaments. Activity is restored upon a depolymerization process induced by variations in the physico-chemical environment, action of substrates or effectors or other events

activity is turned on/off by the equilibrium between the active tetramers and the inactive asymmetric trimers of tetramers that can further polymerise in higher supramolecular assemblies. These represent the latent form of the kinase in accordance with the observation that most of CK2 in crude liver extract is inactive [41]. As for the majority of protein kinases, activity is restored only upon necessity, in this case by a depolymerization process generating active free tetramers. This process can be triggered by different events like β dephosphorylation, physical-chemical variations of the environment (pH, ionic strength, etc.) and/or action of substrates themselves or other effectors (highly charged macromolecules or low-molecular-weight compounds). When the stimuli end, CK2 tetramers are assembled back in trimers and then in fibrous polymers, with the turning off of the catalytic activity. The β -subunit is fundamental in this process, driving both the assembly in trimers of tetramers (via the acidic loop) and in filaments (via its C-terminus), fully deserving the name of “regulatory” subunit.

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References

1. Niefind K, Guerra B, Ermakowa I, Issinger OG (2001) Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme. *EMBO J* 20:5320–5331
2. Niefind K, Guerra B, Pinna LA et al (1998) Crystal structure of the catalytic subunit of protein kinase CK2 from *Zea mays* at 2.1 Å resolution. *EMBO J* 17:2451–2462
3. Chantalat L, Leroy D, Filhol O et al (1999) Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger-mediated dimerization. *EMBO J* 18:2930–2940
4. Boldyreff B, Meggio F, Pinna LA, Issinger OG (1993) Reconstitution of normal and hyperactivated forms of casein kinase-2 by variably mutated beta-subunits. *Biochemistry* 32:12672–12677
5. Niefind K, Issinger OG (2005) Primary and secondary interactions between CK2 α and CK2 β lead to ring-like structures in the crystals of the CK2 holoenzyme. *Mol Cell Biochem* 274:3–14
6. Sarno S, Vaglio P, Marin O et al (1997) Basic residues in the 74–83 and 191–198 segments of protein kinase CK2 catalytic subunit are implicated in negative but not in positive regulation by the beta-subunit. *Eur J Biochem* 248:290–295
7. Hathaway GM, Traugh JA (1984) Kinetics of activation of casein kinase II by polyamines and reversal of 2,3-bisphosphoglycerate inhibition. *J Biol Chem* 259:7011–7015
8. Mamrack MD (1989) Stimulation of enzymatic activity in filament preparations of casein kinase II by polylysine, melittin, and spermine. *Mol Cell Biochem* 85:147–157
9. Leroy D, Heriché JK, Filhol O et al (1997) Binding of polyamines to an autonomous domain of the regulatory subunit of protein kinase CK2 induces a conformational change in the holoenzyme. A proposed role for the kinase stimulation. *J Biol Chem* 272:20820–20827
10. Grankowski N, Boldyreff B, Issinger OG (1991) Isolation and characterization of recombinant human casein kinase II subunits alpha and beta from bacteria. *Eur J Biochem* 198:25–30
11. Hathaway GM, Traugh JA (1979) Cyclic nucleotide-independent protein kinases from rabbit reticulocytes. Purification of casein kinases. *J Biol Chem* 254:762–768
12. Glover CV (1986) A filamentous form of *Drosophila* casein kinase II. *J Biol Chem* 261:14349–14354

13. Valero E, De Bonis S, Filhol O et al (1995) Quaternary structure of casein kinase 2. Characterization of multiple oligomeric states and relation with its catalytic activity. *J Biol Chem* 270:8345–8352
14. Lolli G, Pinna LA, Battistutta R (2012) Structural determinants of protein kinase CK2 regulation by autoinhibitory polymerization. *ACS Chem Biol* 7:1158–1163
15. Laudet B, Barette C, Dulery V et al (2007) Structure-based design of small peptide inhibitors of protein kinase CK2 subunit interaction. *Biochem J* 408:363–373
16. Carugo O, Argos P (1997) Protein-protein crystal-packing contacts. *Protein Sci* 6:2261–2263
17. Theis-Febvre N, Martel V, Laudet B et al (2005) Highlighting protein kinase CK2 movement in living cells. *Mol Cell Biochem* 274:15–22
18. Gotz C, Kartarius S, Schetting S, Montenarh M (2005) Immunologically defined subclasses of the protein kinase CK2 beta-subunit in prostate carcinoma cell lines. *Mol Cell Biochem* 274:181–187
19. Kristensen LP, Larsen MR, Hojrup P et al (2004) Phosphorylation of the regulatory beta-subunit of protein kinase CK2 by checkpoint kinase Chk1: identification of the in vitro CK2beta phosphorylation site. *FEBS Lett* 569:217–223
20. French AC, Luscher B, Litchfield DW (2007) Development of a stabilized form of the regulatory CK2beta subunit that inhibits cell proliferation. *J Biol Chem* 282:29667–29677
21. Litchfield DW, Lozeman FJ, Cicirelli MF et al (1991) Phosphorylation of the beta subunit of casein kinase II in human A431 cells. Identification of the autophosphorylation site and a site phosphorylated by p34cdc2. *J Biol Chem* 266:20380–20389
22. Pagano MA, Sarno S, Poletto G et al (2005) Autophosphorylation at the regulatory beta subunit reflects the supramolecular organization of protein kinase CK2. *Mol Cell Biochem* 274:23–29
23. Boldyreff B, Meggio F, Pinna LA, Issinger OG (1994) Efficient autophosphorylation and phosphorylation of the beta-subunit by casein kinase-2 require the integrity of an acidic cluster 50 residues downstream from the phosphoacceptor site. *J Biol Chem* 269:4827–4831
24. Meggio F, Pinna LA (1984) Subunit structure and autophosphorylation mechanism of casein kinase-TS (type-2) from rat liver cytosol. *Eur J Biochem* 145:593–599
25. Olsen BB, Boldyreff B, Niefind K, Issinger OG (2006) Purification and characterization of the CK2alpha'-based holoenzyme, an isozyme of CK2alpha: a comparative analysis. *Protein Expr Purif* 47:651–661
26. Zhang C, Vilk G, Canton DA, Litchfield DW (2002) Phosphorylation regulates the stability of the regulatory CK2beta subunit. *Oncogene* 21:3754–3764
27. Agostinis P, Goris J, Pinna LA, Merlevede W (1987) Regulation of casein kinase 2 by phosphorylation/dephosphorylation. *Biochem J* 248:785–789
28. Lolli G, Rancio A, Battistutta R (2014) Active form of the protein kinase CK2 $\alpha_2\beta_2$ holoenzyme is a strong complex with symmetric architecture. *ACS Chem Biol* 9:366–371
29. Schnitzler A, Olsen BB, Issinger OG, Niefind K (2014) The protein kinase CK2 (Andante) holoenzyme structure supports proposed models of autoregulation and trans-autophosphorylation. *J Mol Biol* 426:1871–1882
30. Poole A, Poore T, Bandhakavi S et al (2005) A global view of CK2 function and regulation. *Mol Cell Biochem* 274:163–170
31. Raaf J, Guerra B, Neundorff I et al (2013) First structure of protein kinase CK2 catalytic subunit with an effective CK2 β -competitive ligand. *ACS Chem Biol* 8:901–907
32. Raaf J, Bischoff N, Klopffleisch K et al (2010) Interaction between CK2 α and CK2 β , the subunits of protein kinase CK2: thermodynamic contributions of key residues on the CK2 α surface. *Biochemistry* 50:512–522
33. Krissinel E, Henrick K (2007) Inference of macromolecular assemblies from crystalline state. *J Mol Biol* 372:774–797
34. Dey S, Pal A, Chakrabarti P, Janin J (2010) The subunit interfaces of weakly associated homodimeric proteins. *J Mol Biol* 398:146–160
35. Nooren IM, Thornton JM (2003) Diversity of protein-protein interactions. *EMBO J* 22:3486–3492

36. Perkins JR, Diboun I, Dessailly BH et al (2010) Transient protein-protein interactions: structural, functional, and network properties. *Structure* 18:1233–1243
37. Nooren IM, Thornton JM (2003) Structural characterisation and functional significance of transient protein-protein interactions. *J Mol Biol* 325:991–1018
38. Filhol O, Nueda A, Martel V et al (2003) Live-cell fluorescence imaging reveals the dynamics of protein kinase CK2 individual subunits. *Mol Cell Biol* 23:975–987
39. Filhol O, Martiel JL, Cochet C (2004) Protein kinase CK2: a new view of an old molecular complex. *EMBO Rep* 5:351–355
40. St-Denis NA, Derksen DR, Litchfield DW (2009) Evidence for regulation of mitotic progression through temporal phosphorylation and dephosphorylation of CK2alpha. *Mol Cell Biol* 29:2068–2081
41. Baggio B, Pinna LA, Moret V, Siliprandi N (1970) A simple procedure for the purification of rat liver phosphotyrosine kinase. *Biochim Biophys Acta* 212:515–517