Supplementary Information for

Mass spectrometry reveals protein kinase CK2 high–order oligomerization via the circular trimeric assembly

Wei-Guang Seetoh,* Daniel Shiu-Hin Chan, Dijana Matak-Vinković, Chris Abell*

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom

Corresponding authors

*E-mail: wgs25@cam.ac.uk; ca26@cam.ac.uk

Supplementary Methods

Site-directed mutagenesis

Mutagenesis of *Homo sapiens* CK2$\beta^{1\text{-}193}$ was performed using the Q5 site–directed mutagenesis kit (New England Biolabs) for the E60A/E61A/E63A mutant according to the product protocol. DNA sequencing (DNA Sequencing Facility, University of Cambridge) of mutant clones was performed to ensure correct incorporation of mutation.

Protein expression and purification

Expression vectors encoding sequences for *Homo sapiens* CK2$\alpha^{1–335}$ (cloned into pBAT4), CK2$\beta^{1–193}$ (cloned into pGEX–6P–1) and CK2$\beta^{1–193}$ E60A/E61A/E63A mutant were transformed into *E. coli* BL21(DE3). Fresh transformants were inoculated into LB–ampicillin broth and grown overnight at 37 °C. Subsequent to the inoculation of overnight culture, LB cultures were grown at 37 °C, induced with 0.3 mM IPTG after reaching an optical density of 0.6 (λ = 600 nm) and allowed overnight expression at 30 °C and 18 °C for CK2α and CK2β, respectively. To purify CK2α and CK2β, cell pellets were suspended and sonicated in cold lysis buffer A (50 mM Tris–HCl pH 8.5, 100 mM NaCl, 5 mM β–mercaptoethanol). Debris was removed by centrifugation (20,000 rpm, 30 min, 4 °C). CK2α and CK2β was purified using HiTrap Heparin HP (GE Healthcare) and glutathione sepharose 4B beads (GE Healthcare), respectively. For CK2α, the column was washed with buffer B (50 mM Tris–HCl pH 8.5, 300 mM NaCl, 5 mM β–mercaptoethanol) and the protein eluted over a 300–1000 mM NaCl gradient. CK2α–containing fractions, analyzed by SDS–PAGE, were concentrated and loaded onto a Superdex 75 26/60 (GE Healthcare) column equilibrated with buffer C (50 mM Tris–HCl pH 8.5, 500 mM NaCl, 2 mM β–mercaptoethanol). Fractions containing pure CK2α were combined and concentrated. For CK2β, the beads were washed with cold buffer A and incubated with 3C protease at 4 °C overnight to remove the GST tag. The cleaved protein solution was loaded onto a HiTrap Q column (GE Healthcare) and fractionated over a 0–1000 mM NaCl gradient buffered with 50 mM Tris–HCl pH 8.5 and 2 mM β–mercaptoethanol. Fractions containing pure CK2β were combined, exchanged into buffer C and concentrated.

Binding studies

CK2α and CK2β were mixed to give a final concentration of 30–70 μM and 75 μM, respectively, in 50 mM Tris–HCl pH 8.5 containing 200–750 mM NaCl and incubated at room temperature for 1 hour.

Non–denaturing nanoESI–MS

Spectra were recorded on a Synapt HD mass spectrometer (Waters) modified for studying high masses. Protein samples were exchanged into 0.20–0.75 M ammonium acetate (pH 8.5) solution using Micro Bio–Spin 6 chromatography columns (Bio–Rad) and diluted to a final concentration of 5–10 μM before analysis. 2.5 μL of protein solution was electrosprayed from a borosilicate emitter (Thermo Scientific) for sampling. Typical conditions were capillary voltage 1.8–2.5 kV, cone voltage 60–120 V, collision voltage 10−30 V, with backing pressure 3–4 mbar and source temperature of 20 °C. Spectra were calibrated externally using cesium iodide. Data acquisition and processing were performed using MassLynx 4.1.

Ion mobility spectrometry–mass spectrometry (IMS–MS)

Synapt HD mass spectrometer was used to perform IMS–MS measurements for CK2 complexes. The ion–mobility cell was filled with nitrogen gas at 0.5 mbar pressure and the traveling wave velocity was 250 ms^{-1}. Five wave heights (7.0, 8.0, 9.0, 10.0 and 11.0 V) were used for measurements. The reported collision cross section (CCS) values were an average of the data recorded over all of the wave heights. CCS calibration was performed using exactly identical conditions described above on four proteins (alcohol dehydrogenase, concanavalin A, pyruvate kinase and avidin) and involved validation through comparison with CCS data from literature. The drift times for all charge states were obtained using Micromass MassLynx 4.1 and DriftScope (Waters). Theoretical CCS values from PDB files were calculated using the CCScalc software (Waters), which utilizes a projection approximation (PA) algorithm.

Hydrogen–deuterium exchange mass spectrometry (HDX–MS)

A LEAP autosampler (Leap Technologies) integrated with Acquity UPLC HDX System (Waters) was used to perform HDX–MS. CK2α and CK2β were incubated at a 1:1.2 ratio for 1 hour at room temperature in low (50 mM Tris–HCl pH 8.5, 200

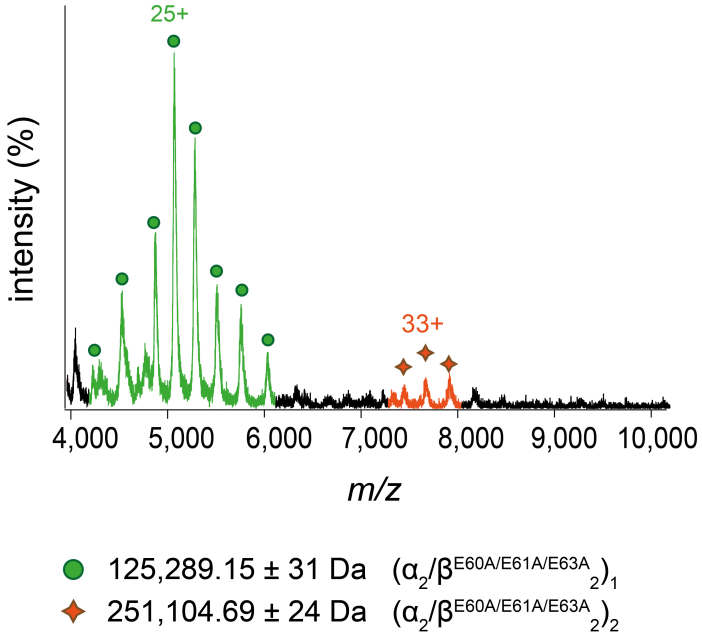
mM NaCl) and high (50 mM Tris–HCl pH 8.5, 750 mM NaCl) salt buffer prior to deuterium labelling. Protein solutions were diluted with equilibration buffer and incubated with D_2O at 18 °C for 0–180 min. Deuterium labelling was stopped by adding an aliquot of the labelled protein solution to an equal volume of quench buffer at 1 °C. Protein samples were digested using an Enzymate BEH Pepsin Column (Waters) and the peptides were trapped on a UPLC Van–Guard Pre–column (Waters) and then separated by gradient elution (8–92% ACN + 0.1% formic acid gradient, 38 μL min^{-1}, 1 °C) from a UPLC C18 BEH column (Waters) followed by electrospray ionization in Synapt G2Si mass spectrometer (Waters). The amount of deuterium in each peptide was determined by measuring the centroid of the isotopic distribution. Data were analyzed using ProteinLynx Global Server v2.5 (PLGS) (Waters) and DynamX v3.0 (Waters). All of the data were derived from at least two independent experiments.

Data availability

Additional data related to this publication can be accessed at the University of Cambridge data repository (https://www.repository.cam.ac.uk/handle/1810/253341).

Supplementary Table 1. Calculated and observed masses for CK2 subunits and complexes generated in varying ammonium acetate concentration

Subunit/complex	Calculated mass (Da)	Observed mass (Da)
α	39,871	39,872 ± 15
β_2	45,891	45,914 ± 36
$\alpha_1\beta_2$	85,761	85,795 ± 34
$\alpha_2\beta_2$	125,632	125,713 ± 59
$(\alpha_1\beta_2)_2$	171,522	171,590 ± 84
$(\alpha_1\beta_2)_2 + \beta_2$	217,413	217,000 ± 45
$(\alpha_2\beta_2)_2$	251,263	251,432 ± 45
$(\alpha_1\beta_2)_3$	257,283	257,799 ± 47
$(\alpha_2\beta_2)_2 + \beta_2$	297,154	297,531 ± 15
$(\alpha_1\beta_2)_3 + \beta_2$	303,174	305,455 ± 36
$(\alpha_2\beta_2)_2 + \alpha_1\beta_2$	337,024	337,785 ± 4
$(\alpha_1\beta_2)_4$	343,044	343,904 ± 27
$(\alpha_2\beta_2)_3$	376,895	378,506 ± 56



Supplementary Figure 1. Native mas spectra of mutant CK2 holoenzyme (10 µM) in 0.40 M ammonium acetate, showing formation of mainly $(\alpha_2/\beta^{E60A/E61A/E63A}{}_2)_1$ and a small proportion of mutant dimeric CK2, $(\alpha_2/\beta^{E60A/E61A/E63A}{}_2)_2$. Charge states are colored and indicated with symbols, each representing a different species. The observed mass (Da) and identity of each species are indicated beside the symbols shown below the spectra. Only the main charge state of each species is indicated in the spectra.