SUPPLEMENTARY DATA

AKAP18:PKA-RIIα structure reveals crucial anchor points for recognition of regulatory subunits of PKA

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Experimental

smAKAP-encoding DNA sequences for the insertion into plasmid pCMV6-Entry (Origene, PS100001).

Extended 5' and 3' sequences for restriction enzyme recognition

NheI site XhoI site linker (to stay in frame) AgeI site

smAKAP-WT

CATTGACATTGAGCTAGCatgggctgcatgaaatcaaagcaaactttcccatttcctaccatatatgaaggtgagaag
cagcatgagagtgaagaacccttatgccagaagagagatgtctacctaggatggcttctccagttaatgtcaaagaggaagtga
aggaacctccagggaccaatactgtgatcttggaatatgcacaccgcctgtctcaggatatcttgtgtgatgccttgcagcaatg
ggcatgcaataacatcaagtaccatgacattccatacattgagagtgaggggcctCTCGAGGCACCGGTCATTGA
CATTGA

smAKAP(D72A/Q76A)

CATTGACATTGAGCTAGCatgggctgcatgaaatcaaagcaaactttcccatttcctaccatatatgaaggtgagaag
cagcatgagagtgaagaacccttatgccagaagagagatgtctacctaggatggcttctccagttaatgtcaaagaggaagtga
aggaacctccagggaccaatactgtgatcttggaatatgcacaccgcctgtctcaggatatcttgtgtgctgccttgcaggcatg
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CATTGA

Results
Supplementary figures

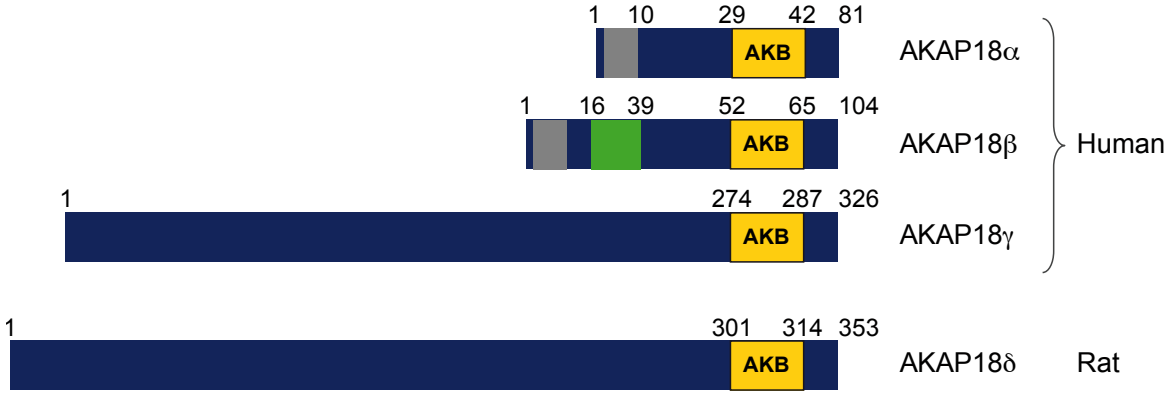


Figure S1. **Alignment of AKAP18 isoforms**

, membrane-targeting domain; , apical membrane-targeting domain; AKB, A-kinase binding domain. Numbers indicate amino acid residues.

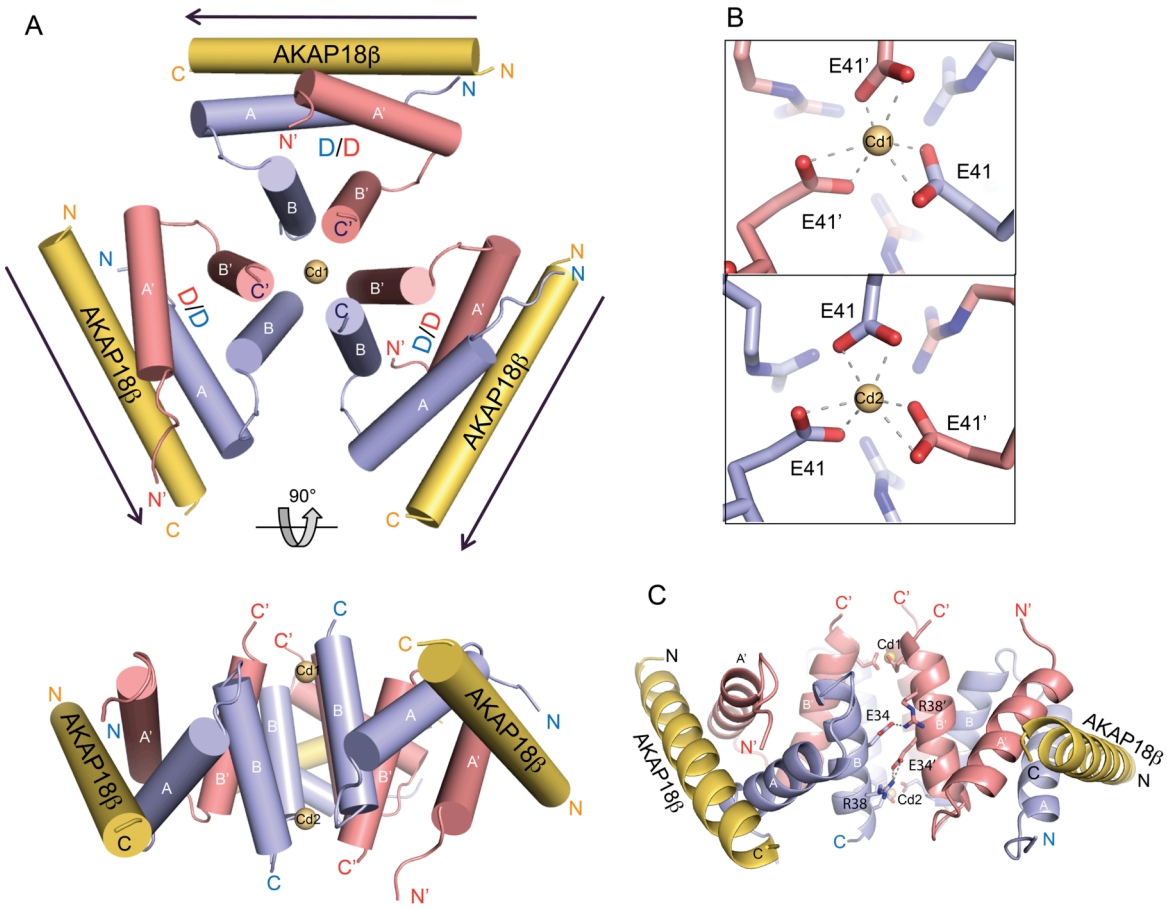


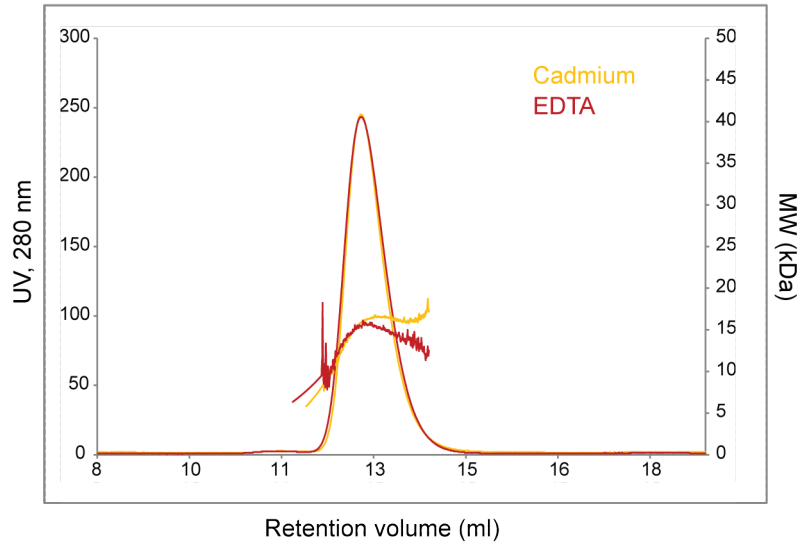
Figure S2. **Arrangement of AKAP18β:RIIα-D/D complex in crystal**

(A) AKAP18β:RIIα-D/D complexes are arranged as two identical trimeric complexes in propeller-like fashion. A pseudo-trimer is displayed at two different view angles with two cadmium ions bound in the centre. The helices A, B and A', B' of the RIIα-D/D dimer are shown as blue and salmon-coloured cylinders, respectively; the AKAP18β amphipathic helix is shown as yellow cylinder. The bound cadmium ion is presented as sphere in wheat colour.

(B) Detailed view of cadmium binding by residue Glu41 of each helix B.

(C) Detailed view into the interface of neighbouring AKAP18β:RIIα-D/D complexes. Arg38 and Glu34 of each helix B of the RIIα D/D domain form salt bridges with Glu34 and Arg38, respectively, of the adjacent D/D domain. Colours in B and C are as in A, and the view is along the blue arrow marked in A (top).

A



B

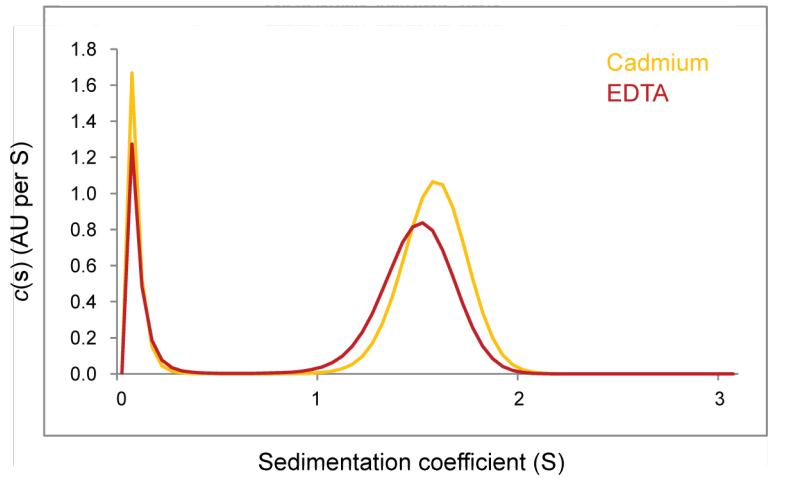


Figure S3. Determination of the molecular weight of the AKAP18β(43-83):RIIα-D/D domain complex.

(A) Static light scattering (SLS) with size exclusion chromatography (SEC) using an analytical Superdex 75 10/300 column. The complex AKAP18β(43-83):RIIα-D/D(1-44) was analyzed either in the presence of 2 mM $CdCl_2$ (yellow graph) or 2 mM EDTA (red graph). The absolute molecular mass determined by SLS (depicted as horizontal lines) was in agreement with the calculated molecular mass of 15 kDa for a monomeric complex independent of the presence of Cd^{2+} ions with 15.7 kDa or 2 mM EDTA with 14.4 kDa.

(B) Analysis of purified complex AKAP18β(43-83)/RIIα-D/D(1-44) in the presence of divalent cations by analytical ultracentrifugation. The buffer contained 20 mM HEPES pH 7.5, 150 mM NaCl, and either, 2 mM $CdCl_2$ (yellow graph) or 2 mM EDTA (red graph). The sample loading concentration was 0.6 mg/ml. Sedimentation coefficients are expressed in Svedberg units, 1 S = 10^{-13} s. In all buffer conditions a single protein species sedimenting at $s_{20,w}$=1.6/1.7 was be detected. This sedimentation behavior is in agreement with the expected sedimentation behavior of a monomeric globular protein species of 15 kDa.

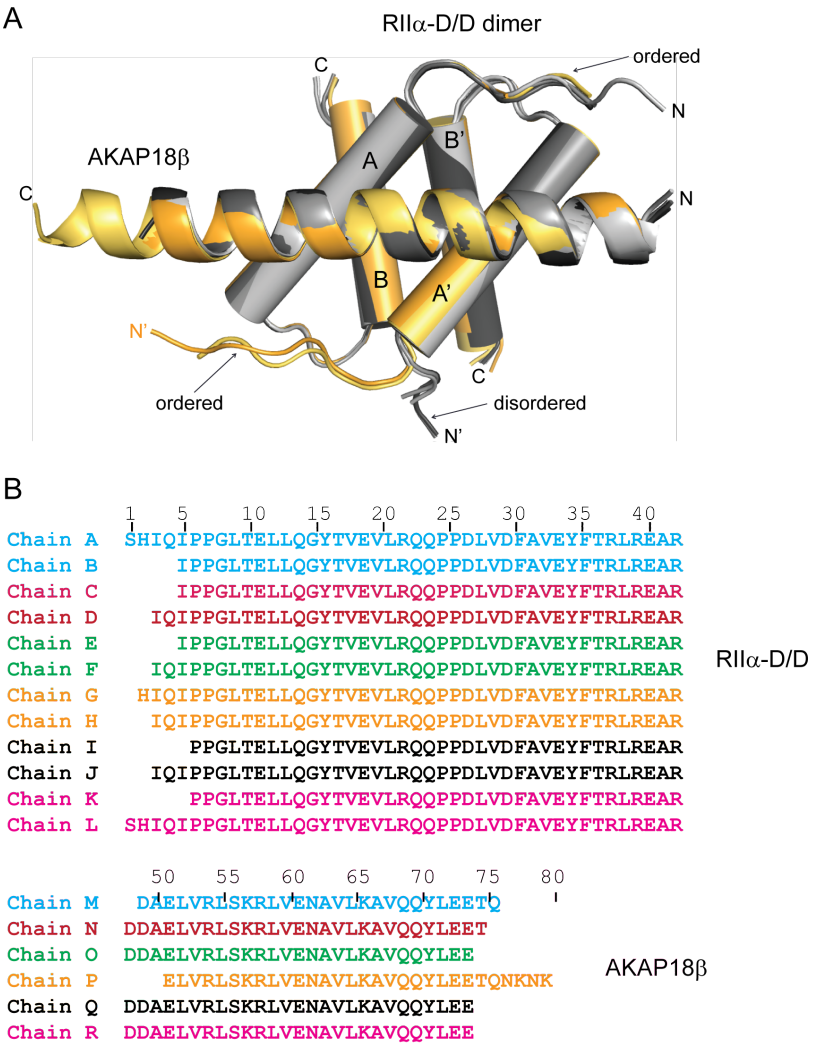


Figure S4. Alignment of the six AKAP18β:RIIα-D/D complexes.

(A) Superimposition of all AKAP18β:RIIα-D/D complexes present in the crystallographic asymmetric unit. The four complexes with a disordered N terminus at the A' helix of the RIIα-D/D dimer are shown in shades of grey, and the two complexes with ordered N termini at the A'-helix are shown in shades of orange (chain A and G). The N termini at the A-helix are ordered in all six complexes.

(B) Presentation of the different chain lengths for all AKAP18β:RIIα-D/D complexes in the crystallographic asymmetric unit. Chains of identical colour for AKAP18β and RIIα-D/D dimer indicate the contribution in forming one complex.