Material and Methods

Entry Clone Source: Mammalian Gene Collection (IMAGE consortium clone ID 4102976).

SGC Construct ID: RHOAA-c001

Coding DNA sequence:
ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGGCTGCCATCCGG
AAGAAACTGGTGATTGTTGGTGATGGAGCCTGTGGAAAGACATGCTTGCTCATAGTC
TTCAGCAAGGACCAGTTCCCAGA
GGTGTATGTGCCCACAGTGTTTGAGAACTATGTGGCAGATATCGAGGTGGATGGAAA
GCAGGTAGAATTGGCTTTGTGG
GACACAGCTGGGCAGGAAGATTATGATCGCCTGAGGCCCCTCTCCTACCCAGATACC
GATGTTATACTGATGTGTTTTTCC
ATCGACAGCCCTGATAGTTTAGAAAACATCCCAGAAAAGTGGACCCCAGAAGTCAA
GCATTTCTGTCCCAACGTGCCCATC

ATCCTGGTTGGGAATAAGAAGGATCTTCGGAATGATGAGCACACAAGGCGGGAGCT
AGCCAAGATGAAGCAGGAGCCGG
TGAAACCTGAAGAAGGCAGAGATATGGCAAACAGGATTGGCGCTTTTGGGTACATG
GAGTGTTCAGCAAAGACCAAAGAT
GGAGTGAGAGAGGTTTTTGAAATGGCTACGAGAGCTGCTCTGCAAGCTAGACGTGG
GTGA

Expressed protein sequence:
MHHHHHHSSGVDLGTENLYFQSMAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYV
PTVFENYVADIEVDGKQVELALWDTAGQEDYDRLRPLSYPDTDV
ILMCFSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKDLRNDEHTRRELAKMKQEPV
KPEEGRDMANRIGAFGYMECSAKTKDGVREVFEMATRAALQARRG
(Met1 to Gly184)
The N-terminal residues, **MHHHHHHSSGVDLGTENLYFQS**, derive from the vector.

Vector: pNIC28-Bsa4

Tags and additions: N-terminal, TEV cleavable hexahistidine tag

AKAP13

Entry Clone Source: Collaborator.

SGC Construct ID: AKAP13-c014

Coding DNA sequence:
ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGTCCAAACAGCTGGAAGCAGA
GTCTTGGAGTCGGATAATAGACAGCAAGTTTCTAAAACAGCAAAAGAAAGATGTGG
TCAAACGGCAAGAAGTAATATATGAGTTGAT
GCAGACAGAGTTTCATCATGTCCGCACTCTCAAGATCATGAGTGGTGTGTACAGCCA
GGGGATGATGGCGGATCTGCTTTTTGAGCAG
CAGATGGTAGAAAAGCTGTTCCCCTGTTTGGATGAGCTGATCAGTATCCATAGCCAAT
TCTTCCAGAGGATTCTGGAGCGGAAGAAGG
AGTCTCTGGTGGATAAAAGTGAAAAGAACTTTCTCATCAAGAGGATAGGGGATGTGC
TTGTAAATCAGTTTTCAGGTGAGAATGCAG
AACGTTTAAAGAAGACATATGGCAAGTTTTGTGGGCAACATAACCAGTCTGTAAACT
ACTTCAAAGACCTTTATGCCAAGGATAAGCG
TTTTCAAGCCTTTGTAAAGAAGAAGATGAGCAGTTCAGTTGTTAGAAGGCTTGGAAT
TCCAGAGTGCATATTGCTTGTAACTCAGCGG
ATTACCAAGTACCCAGTTTTATTCCAAAGAATATTGCAGTGTACCAAAGACAATGAAG
TGGAGCAGGAAGATCTAGCACAGTCCTTGA

GCCTGGTGAAGGATGTGATTGGAGCTGTAGACAGCAAAGTGGCAAGTTATGAAAAG
AAAGTGCGTCTCAATGAGATTTATACAAAGA
CAGATAGCAAGTCAATCATGAGGATGAAGAGTGGTCAGATGTTTGCCAAGGAAGATT
TGAAACGGAAGAAGCTTGTACGTGATGGG
AGTGTGTTTCTGAAGAATGCAGCAGGAAGGTTGAAAGAGGTTCAAGCAGTTCTTCT
CACTGACATTTTAGTTTTCCTTCAAGAAAAAGA
CCAGAAGTACATCTTTGCATCATTGGACCAGAAGTCAACAGTGATCTCTTTAAAGAA
GCTGATTGTGAGAGAAGTGGCACATGAGGAG
AAAGGTTTATTCCTGATCAGCATGGGGATGACAGATCCAGAGATGGTAGAAGTCCAT
GCCAGCTCCAAAGAGGAACGAAACAGCTGG
ATTCAGATCATTCAGGACACAATCAACACCCTGAACAGAGATTGA

Expressed protein sequence:
MHHHHHHSSGVDLGTENLYFQSMSKQLEAESWSRIIDSKFLKQQKKDVVKRQEVIYE
LMQTEFHHVRTLKIMSGVYSQGMMADLLFEQQMVEKLFPCLDELIS
IHSQFFQRILERKKESLVDKSEKNFLIKRIGDVLVNQFSGENAERLKKTYGKFCGQHNQS
VNYFKDLYAKDKRFQAFVKKKMSSSVVRRLGIPECILLVTQRITKYPVLFQ
RILQCTKDNEVEQEDLAQSLSLVKDVIGAVDSKVASYEKKVRLNEIYTKTDSKSIMRMK
SGQMFAKEDLKRKKLVRDGSVFLKNAAGRLKEVQAVLLTDILVFLQEKDQKYI
FASLDQKSTVISLKKLIVREVAHEEKGLFLISMGMTDPEMVEVHASSKEERNSWIQIIQD
TINTLNRD
(Lys1973-Asp2342)
The N-terminal residues, **MHHHHHHSSGVDLGTENLYFQS**, derive from the vector.

Vector:pNIC28-Bsa4

Tags and additions: N-terminal, TEV cleavable hexahistidine tag

Expression - RHOA

Expression strain: BL-21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3)).

Transformation: The construct DNA was transformed into homemade chemically competent cells of
the expression strain by a standard heat shock procedure.

Expression: Colonies were used to inoculate 50 mL of LB media containing 50 µg/ml kanamycin and
34 µg/ml chloramphenicol in a 250 ml baffled shaker flask, which was placed in a 37°C shaker overnight. The next
day 3x 10 mL of this starter culture was used to inoculate 3x 1L of TB media containing 40 µg/ml kanamycin in 2L
baffled shaker flasks. When the OD600 was approximately 1.0, the temperature was reduced to 20°C and the cells
were induced by the addition of 0.5 mM IPTG. The expression was continued overnight.

Cell harvest: Cells were harvested by centrifugation and the pellets resuspended in Lysis Buffer and then frozen at -20°C

Expression - AKAP13

Expression strain: BL-21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3)).

Transformation: The construct DNA was transformed into homemade chemically competent cells of
the expression strain by a standard heat shock procedure.

Expression: Colonies were used to inoculate 50 mL of LB media containing 50 µg/ml kanamycin and
34 µg/ml chloramphenicol in a 250 ml baffled shaker flask, which was placed in a 37°C shaker overnight. The next

day 3x 10 mL of this starter culture was used to inoculate 3x 1L of LB media containing 40 µg/ml kanamycin in 2L
baffled shaker flasks. When the OD600 was 0.4-0.5, the temperature was reduced to 20°C and the cells were induced
by the addition of 0.5 mM IPTG. The expression was continued overnight.

Cell harvest: Cells were harvested by centrifugation and the pellets resuspended in Lysis Buffer and then frozen at -20°C

Purification - RhoA
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication on ice. PEI (polyethyleneimine) was
added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.
Lysis Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 0.5 mM TCEP.
Purification:

Column 1: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column.
Column 1 Buffers:

Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5 mM Imidazole, 5% Glycerol, 0.5 mM TCEP.
Wash Buffer 1: As Binding Buffer except 40 mM imidazole and 1M NaCl.
Wash Buffer 2: As Binding Buffer except 60 mM imidazole.
Elution Buffer: As Binding Buffer except 250 mM imidazole.
Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 mL of Binding
Buffer, 50 mL each of Wash Buffer 1 and Wash Buffer 2, and 25 mL of Elute Buffer was passed through to elute the protein.

Purification - AKAP13
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication on ice. PEI (polyethyleneimine)
was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.
Lysis Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, 0.5 mM TCEP.
Purification:

Column 1: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column.
Column 1 Buffers:

Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5 mM Imidazole, 5% Glycerol, 0.5 mM TCEP.
Wash Buffer 1: As Binding Buffer except 40 mM imidazole and 1M NaCl.
Wash Buffer 2: As Binding Buffer except 60 mM imidazole.
Elution Buffer: As Binding Buffer except 250 mM imidazole.
Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 mL of Binding
Buffer, 50 mL each of Wash Buffer 1 and Wash Buffer 2, and 25 mL of Elute Buffer was passed through to elute the protein.

Purification of RhoA:AKAP13 complex
The N-terminal His tag for both proteins was removed using TEV protease.
RHOA and AKAP13 were mixed in a 1:1 molar ratio.
Column 2: S75 16/60 Gel Filtration (GE Healthcare)
Column 2 Buffers:

GF Buffer: 50 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP
Column 2 Procedure: The 1:1 complex was was concentrated to 5 ml volume and injected onto the column.

Concentration:
Fractions from gel filtration were pooled and passed through a gravity column of 5 mL Ni-Sepharose. The flow-through
and an elution fraction with GF Buffer containing 10 mM imidazole were pooled and the complex was concentrated to
5.1 mg/ml (measured by 280 nm absorbance).

Crystallisation:
GDP was added to the concentrated protein sample to a concentration of 1 mM. Crystals grew from a mixture of 50 nL protein and
100 nL of a well solution containing 0.2 M ammonium sulphate,
0.1 M Bis-Tris pH 5.5, 25% (w/v) PEG 3350, using the vapour diffusion method.
Crystals were equilibrated into reservoir solution plus 25% ethylene glycol before freezing in liquid nitrogen

Data Collection: Data was collected at Diamond synchrotron, beamline I02.

Mass spec characterization:
The intact mass of the protein was
confirmed by Electrospray Ionisation/Time-of-Flight Mass Spectrometry (ESI-MS, Agilent Technologies). The purified protein complex had an experimental mass of 37.685 and
30.403 kDa, as expected from primary sequences of CDK12 and CCNK, respectively. Following
CAK treatment the CDK12 mass shifted to 37.768 kDa consistent with a single phosphorylation.
Masses were determined by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase
HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser.
Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with
a gradient of 5-95% methanol in water with 0.1% formic acid.