Supplementary Data

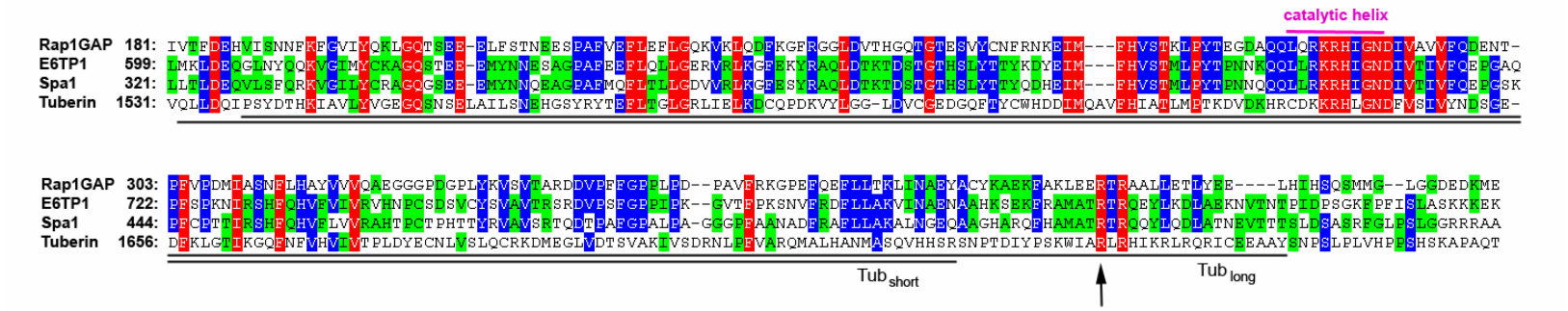
The Rap-RapGAP complex: GTP hydrolysis without catalytic glutamine and arginine residues

Andrea Scrima[1], Christoph Thomas, Delia Deaconescu, Alfred Wittinghofer

Department for Structural Biology, Max-Planck-Institute of Molecular Physiology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

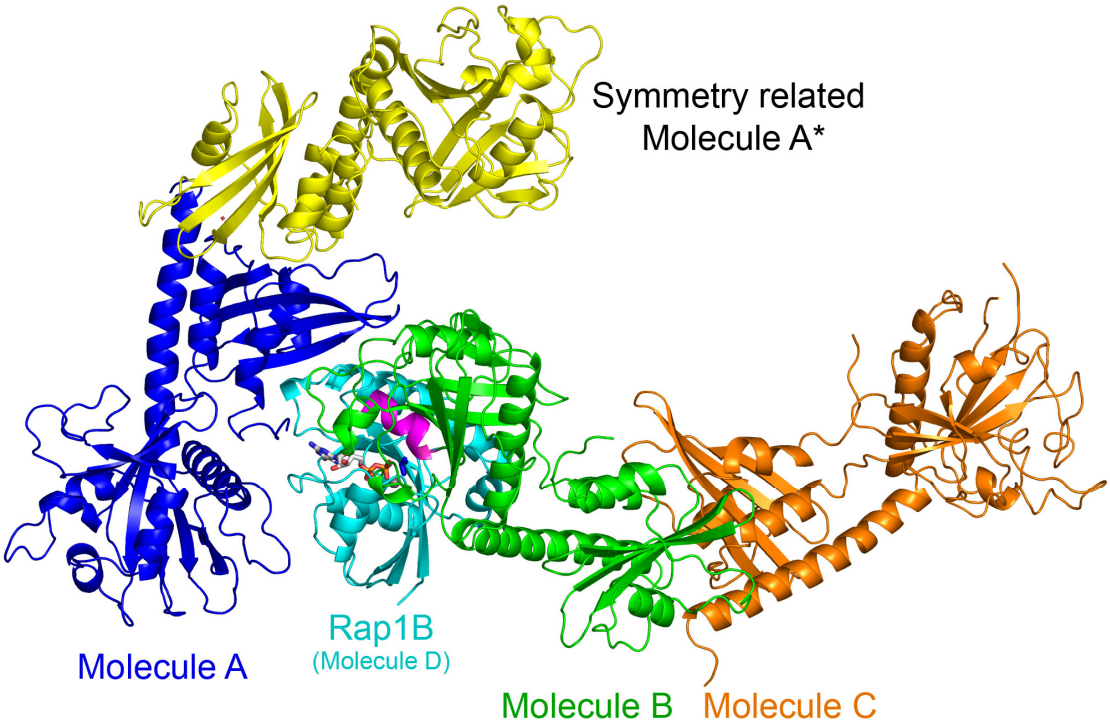
[1]present address: Friedrich Miescher Institute for Biomedical Research , Maulbeerstrasse 66, 4058 Basel, Switzerland

Correspondence:
Alfred Wittinghofer
+49-231-133-2100 (phone)
+49-231-133-2199 (fax)
alfred.wittinghofer@mpi-dortmund.mpg.de



Supplementary Figure S1:

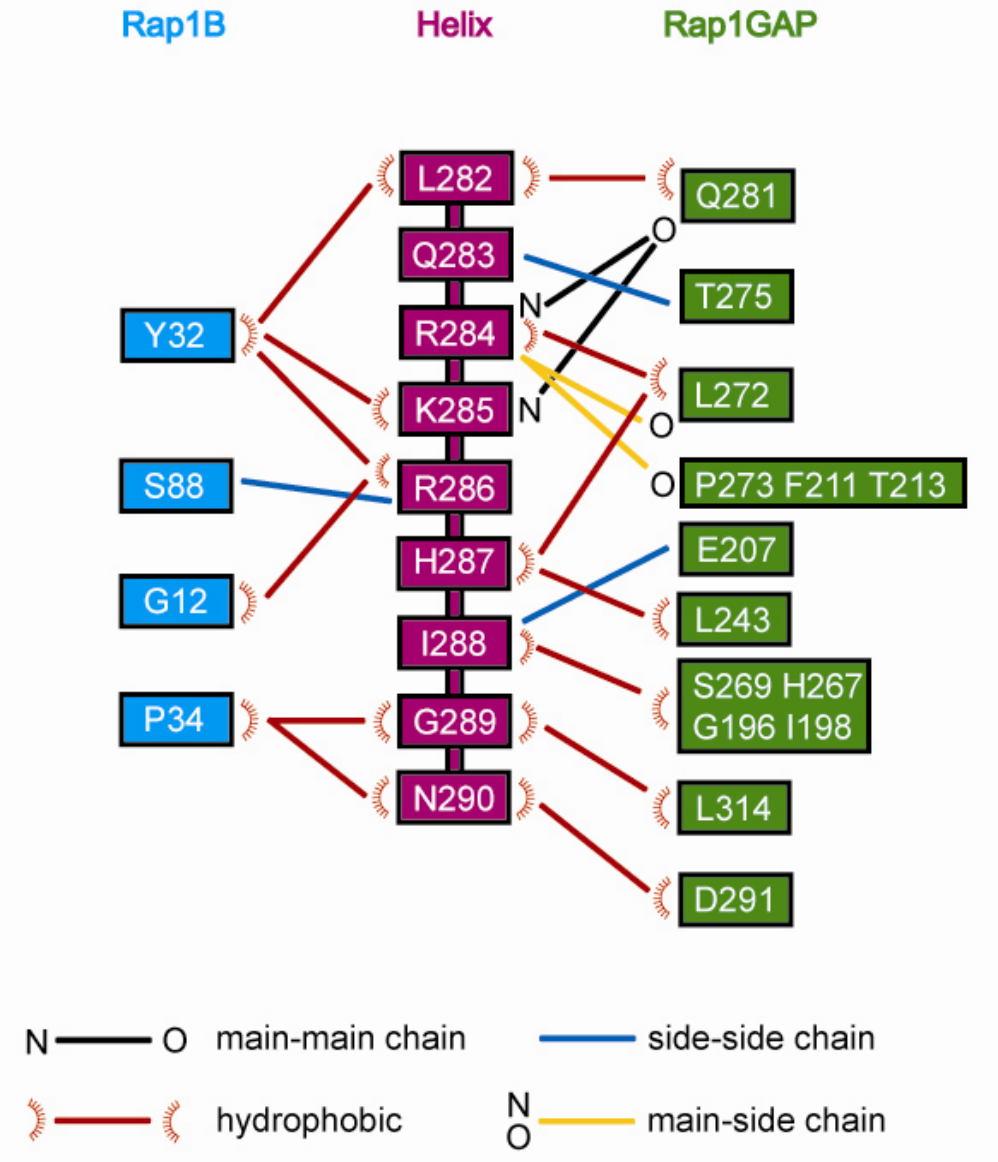
Sequence alignment of the catalytic domain of *Homo sapiens* Rap1GAP (SW P47736), E6TP1 (SW Q9UNU4), Spa1 (SW O60618) and Tuberin (SW P49815). Invariant residues are shown in red, 80 % conserved in blue and 60 % conserved in green. The highly conserved catalytic helix motif is marked in magenta. The Arg 388 (Rap1GAP) / Arg 1743 (Tuberin) is marked with an arrow.



Supplementary Figure S2:

The Rap1B-Rap1GAP complex crystallised in space group P3(1)21. The asymmetric unit is composed of 3 Rap1GAP molecules (A, B, C) and 1 Rap1B molecule (D). Within the asymmetric unit Rap1GAP molecules B and C form a homodimer via the dimerisation domain as already observed before by Daumke *et al.* (2004; PDB-code 1SRQ). Rap1GAP molecule A (blue) forms a homodimer with its symmetry related molecule A* (yellow). Of the three Rap1GAP molecules in the asymmetric unit only molecule B (green) has Rap1B bound, resulting in a 3:1 Rap1GAP:Rap1B stoichiometry. Molecules B and D, representing the Rap1GAP-Rap1B complex, are well defined with average B-factors of 82 and 76 $Å^2$. Rap1GAP molecules A and C are partially highly flexible and ill defined resulting in B-factors of 100 and 120 $Å^2$, respectively. Structural details in the main text are described and analysed based on molecules B and D.

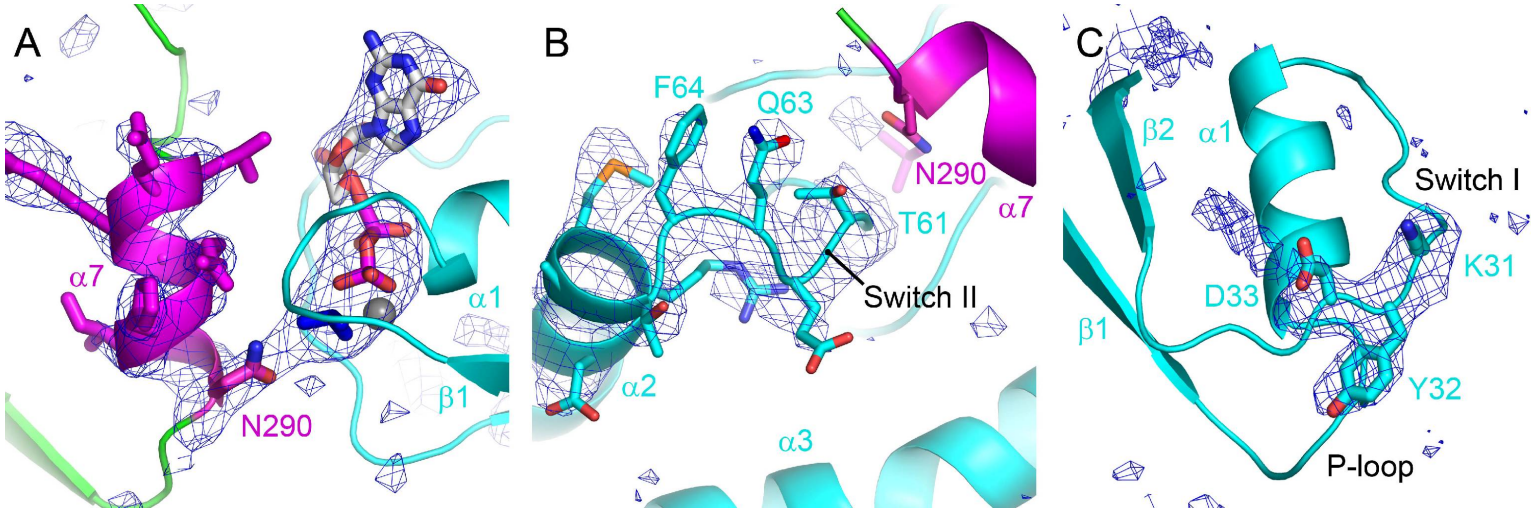
Daumke O, Weyand M, Chakrabarti PP, Vetter IR, Wittinghofer A (2004) The GTPase-activating protein Rap1GAP uses a catalytic asparagine. *Nature.* **429**(6988):197-201.



Supplementary Figure S3:

Schematic representation of the interactions of the catalytic helix (magenta) with the rest of the Rap1GAP catalytic domain (green) and with Rap1B (cyan) Interactions were calculated with Ligplot (Wallace *et al.*; 1995).

Wallace AC, Laskowski RA and Thornton JM (1995) LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Prot. Eng.* **8**:127-134.



Supplementary Figure S4:

Omit maps showing electron density (Fo-Fc contoured at 3σ) for (A) the catalytic helix of Rap1GAP (magenta) and GDP•BeF_3^- (residues 282-290 in Rap1GAP as well as GDP•BeF_3^- omitted); (B) for switch II/α2 of Rap1B (cyan; residues 60-70 omitted) in the alternative conformation (compare Fig. 2b). Panel (C) shows electron density for switch I with a focus on the orientation of Tyr32 (residues 31-33 omitted).