ESRF	**Experiment title:** BAG Barcelona.	**Experiment number**: LS-1805
Beamline: ID14-2	**Date of experiment**: from: 29/11/00 to: 01/12/00	**Date of report**: 31-Aug-2001
Shifts: 6	**Local contact(s)**: Raymond Ravelli	*Received at ESRF:*

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Report:

PhoB is the transcription activator of the two-component signal transduction system which controls phosphate uptake depending on its extracellular levels in many genera of bacteria. In *E.coli,* PhoB contains 229 residues and consists of two functional domains: an N-terminal phosphate receiver domain and a C-terminal DNA-binding and transactivating domain. Under phosphate starvation conditions, PhoR -the transmembrane sensor kinase component- autophosphorylates and transfers its phosphate group to PhoB N-terminal domain, inducing the homodimerization of the molecule. Then PhoB, by its C-terminal domain, binds to its specific DNA target, *pho box*, thus recruiting σ^{70} RNA polymerase subunit to trigger the transcription of the pho regulon genes.

We had previously resolved the crystal structure of the phosphate receiver domain. It presents an α/β *doubly wound* fold where five β-strands form a central parallel β-sheet (β2 β1 β3 β4 β5) flanked by two α-helices on one face of the sheet (α1, α5) and by three on the other face (α2, α3, α4). The active site pocket is located at the C terminus of the β-sheet and contains three acidic residues, Asp53 -the putative phosphorylation site-, Asp10 and Glu9. These residues form an acidic pocked, highly conserved in phosphate receiver domains, and are probably involved in magnesium cation coordination. Another highly conserved residue is Lys105, which is meant to be involved in the activation process that follows phosphorylation. In the crystal structure, two molecules of the receiver form a dimer interacting through helix α1, loopβ5α5 and the N-terminus of α5.

In order to gain further insight into the activation mechanism, we have produced and crystallized the receiver domain of two constitutive-active and double mutants: D53A-Y102C and D10A-D53E. Their structures have been solved by molecular replacement with AMoRe at 1.5 Å and 1.83 Å respectively. The wild-type receiver (code 1B00), with the mutated residues truncated to alanines, was used as search model. Refinement was carried out with CNS, starting with stimulated annealing and then alterning cycles of positional refinement, including temperature factor attribution, and manual building using Turbo-Frodo. Final R-factor of 20.1% and free R-factor of 22.6% for D53A-Y102C mutant; and of 23.3% and 27.5% for D10A-D53E were obtained.

The overall structure of the mutants is the same as that of the wild-type protein. Moreover, both mutants dimerize as the wild type does, therefore confirming the dimerization surfaces previously described. The monomers within a dimer are much more similar in the D53A Y102C mutant than in the D10A D53E or the wild-type. They show no significant differences in helix α3 or the previous β3α3 loop, as happens in the wild-type and the D10A D53E mutant. The three dimers exhibit, however, notable differences in the helix α4, an area of great plasticity, a characteristic related to the protein function.

When comparing the wild-type structure and the mutants, the most important differences are observed in D10A Y102C mutant (global rmsd of the 121 atoms of 1.35Å), which shows major changes involving both the dimerization surface and α4 helix. A new interaction occurs between two highly conserved residues, Trp 54 and Thr 83. These residues link the active site to helix α4, which is part of the signaling surface. In the mutant, the side chain of Trp 54 has rotated 78º, occupying now a position between the protein core and α4, thus displacing this helix by 2.5 Å. This rotation is possible because a Cys -which has an "outer" orientation respect to the Tyr 102 "inner" position in the wild type- has replaced the bulky Tyr 102 side chain. Lys 105 also changes both its side and main chain atoms positions, this way altering the conformation of loop β5α5 and thus the dimerization surface. Therefore, the mutants also confirm that the intramolecular signaling pathway involves the active side residue Lys105, which movement affects the dimerization surface, and residues Thr 83, Trp 54 and Tyr 102 that transfer the movement from the active site to the exposed and mobile helix α4.

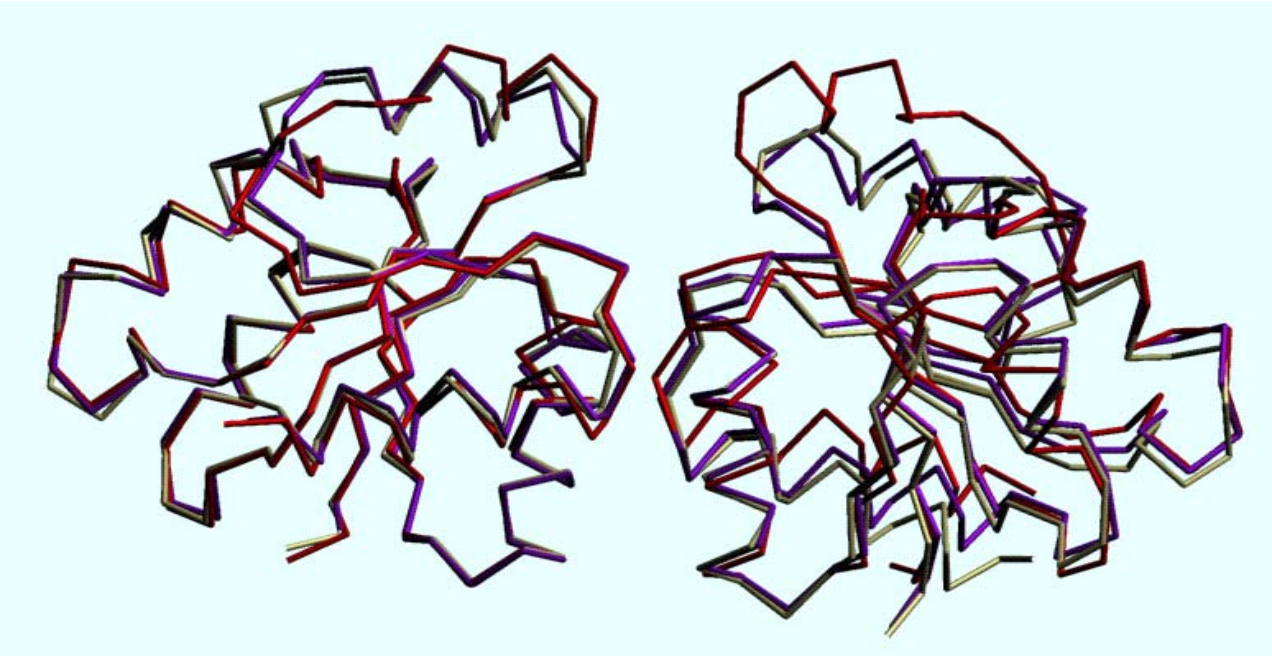

Figure 1. C^{a}-superimpositing of the wild-type (yellow), *D53A Y102 C* the D53A Y102C (red) and D10A D53E (purple) mutants.

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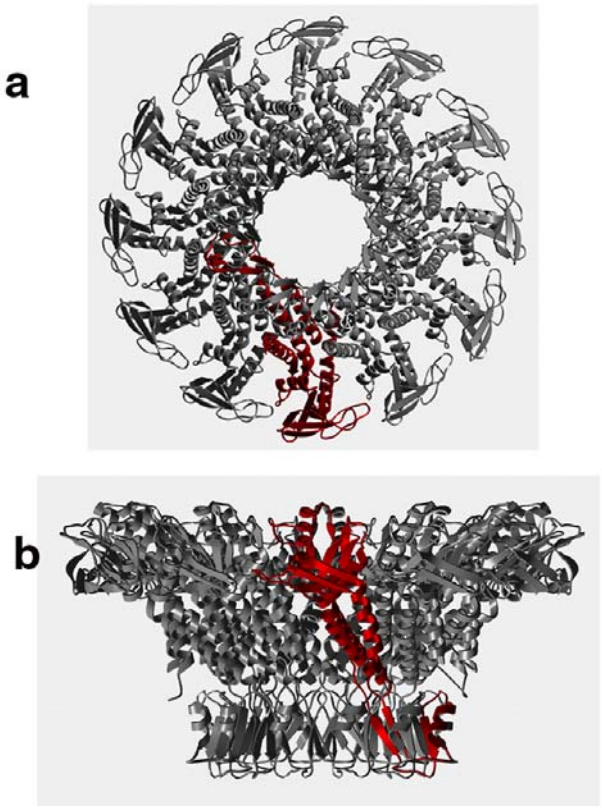
Report:

The three-dimensional crystal structure of the bacteriophage ϕ29 connector has been solved and refined to 2.1 Å resolution. This 422-kDa olimoreic protein connects the head of the phage to its tail and translocates the DNA into the prohead during packaging. Each monomer has an elongated shape and is composed by a central, mainly α-helical domain that includes a three-helix bundle, a distal α/β domain and a proximal 6-stranded SH3-like domain. The protomers assemble into a 12-mer, propeller-like, super-structure with a 35 Å wide central channel. The surface of the channel is mainly electronegative, but it includes two lysine rings, 20 Å apart. On the the external surface of the particle, a hydrophobic belt extends to the concave area below the SH3-like domain, which forms a crown that retainsthe particle in the head. The lipophilic belt contacts the non-matching symmetry vertex of the capsid and forms a bearing for the connector rotation. The structure suggests a translocation mechanism, in which the longitudinal displacement of the DNA along its axis is coupled to connector spinning.

Guasch, A., Parraga, A., Pous, J., Valpuesta, J. M., Carrascosa, J. L., and Coll, M. (1998). Purification, crystallization and preliminary X-ray diffraction studies of the bacteriophage phi29 connector particle. FEBS Lett *430*, 283-7.

Guasch, A., Pous, J., Parraga, A., Valpuesta, J. M., Carrascosa, J. L., and Coll, M. (1998). Crystallographic analysis reveals the 12-fold symmetry of the bacteriophage phi29 connector particle. J Mol Biol *281*, 219-25.

Guasch, A., Pous, J., Ibarra, B., Gomis-Rüth, F.X., Valpuesta, J.M.., Sousa, N. José L. Carrascosa, J.L. and Coll, M. (2001) Detailed architecture of a DNA translocating machine: The high-resolution structure of the bacteriophage ϕ29 connector particle. Submitted.



Ribbon representations of a whole dodecamer of the bacteriophage ϕ29 connector protein. One of the 12 monomers is displayed in red.
Twelve protomers assemble in a propeller-like super-structure that has an external diameter of 146 Å at its proximal part and 77 Å at its distal. The height of the connector is 75 Å. The three helices forming the bundle of the central domain form an angle of about 45 degrees with the 12-fold axis of the particle. This implies that the proximal part of monomer *n*, projects over the distal domain of monomer *n+2*, as seen when looking down the 12-fold axis of the particle. The connector defines a central channel of appropriate size to accommodate a double-stranded DNA molecule. The diameter of the channel is 35 Å at its narrowest part, while the DNA B-form has an average diameter of 23 Å. This leaves about 6 Å between the DNA and the channel wall. The DNA would traverse the channel floating away from the repulsive walls. There is room for a layer of water molecules, between the DNA and the protein.
The structure is shown in its axial (**a**) and lateral (**b**) views.

ESRF	**Experiment title:** The C2 domain of PKCε in complex with acidic phospholipids	**Experiment number**: LS1805
Beamline: ID14-2	**Date of experiment**: from: 29/11/00 to: 1/12/00	**Date of report**: 20-Aug-2001
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Report:

The PKCε isoenzyme, which belongs to the group of novel PKCs, has been implicated in the regulation of different biological processes, including neuronal differentiation, antiviral resistance, hormone secretion and regulation of transporters. In vitro experiments have confirmed the ability of PKCε to bind to negatively-charged phospholipids vesicles in a Ca^{2+} independent manner as all other novel PKCs. However, PKCε differs from other novel PKCs in its oncogenic potential when overexpressed in NIH3T3 fibroblasts. In terms of membrane association, it has been demonstrated that phorbol ester binding promotes a redistribution of the PKCε enzyme from the Golgi to the plasma membrane.

Two different crystal forms were obtained: an orthorhombic form, space group $P2_12_12_1$, for the PKCε-C2 domain in complex with DCPS and a monoclinic form, space group $P2_1$, for the PKCε-C2 domain with DCPA. Diffraction data to resolutions of 1.7 Å and 2.8 Å, for the DCPS and DCPA crystals, respectively, were measured at 100 K. The structure of the isolated domain, solved previously, was used as searching model to solve, by molecular replacement, the DCPS and DCPA crystals.

The central structural feature of the PKCε-C2 domain is an eight-stranded antiparallel β-sandwich with the type II, or P type, topology similar to that found in the C2 domains from phospholipase A2 and from the novel PKCδ .Sheet I of the β-sandwich (composed of strands β3, β2, β5, β6) has a concave surface, whereas the surface of sheet II (consisting of strands β1, β4, β7, β8) has a convex shape. Links that connect strands in the β-sandwich display many variations, in particular at the top end of the β-sandwich where the Ca^{2+} binding regions (CBRs) are located in the C2 domains of classical PKCs.

The different behavior in membrane-binding and activation, between PKCε and classical PKCs, appears to originate in localized structural changes, including the complete rearrangement of the region corresponding to the calcium binding pocket in classical PKCs.

A membrane docking model of the PKCε-C2 domain was inferred taking into account the structural information available for the PKCα-C2 domain docking and the superimposition of the PKCε-C2 domain structure onto PKCα-C2 (Figure 1). Therefore, the proposed model suggest that, despite the absence of Ca^{2+}, a diversity of hydrophobic and electrostatic forces can contribute to the interactions of PKCε-C2 domains with membranes in a way that retains some of the basic features attributed to the C2 domains from classical, Ca^{2+} dependent, PKCs and phospholipases.

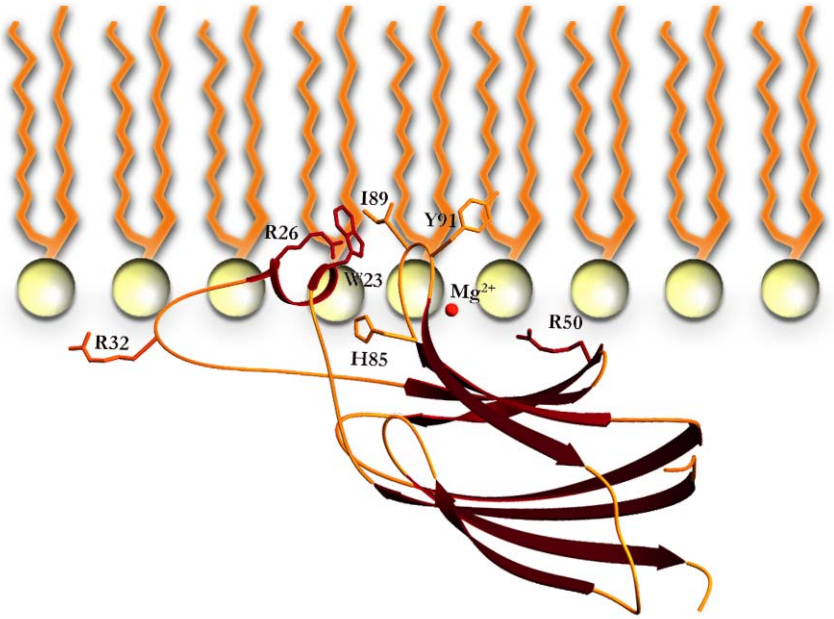

Fig 1. Docking mechanism onto membrane suggested for PKCε-C2 In this model only loop3 appears to penetrate into the lipid bilayer, though loop1 would also be in close contact with the membrane. In the model bulky side chains of Trp23, Ile89 and Tyr91 (explicitly depicted) could reach the inner membrane while conserved basic residues (particularly Arg26, Arg32, Arg50 and probably also His85) would interact with the phospholipids charged heads . The coordination of the Mg^{2+} ion might also facilitate the interaction with the membrane In this model the carboxy end of the C2 domain, to be continued by the pseudosubstrate and the C1 domain in the intact PKC, appears situated apart from the membrane .

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ESRF	**Experiment title:** BAG Barcelona - Human Astrovirus 2 (H-Ast2)	**Experiment number**: LS1805
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Shifts: 6	**Local contact(s)**: Raimond Ravelli	*Received at ESRF:*

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Report:

Astroviruses are a group of non enveloped positive-stranded RNA viruses frequently associated with non bacterial gastroenteritis in humans and animals. Human astroviruses have a world wide distribution, but their real incidence has been probably underestimated because of the lack of sensitive diagnostic assays.

Crystals of Human Astrovirus serotype 2 have a triangular morphology and grow to approximately 100 μm at their largest dimension. These crystals were mounted in capillaries, because the failure of cryo-preservation and tested at the beam line ID14.2.

Crystals diffracted only to 9 A resolution and were stable in the X-ray beam for one exposure. These crystals belong to a monoclinic space group $P2_x$ with a unit cell parameters a=505. A, b=394. A, c=881. A, β=108 º

No data set was collected at the present time.