so much different from that of DNase I in the complex.
The 350 water positions were assigned on reasonable atomic contact against protein and other water molecules, and about 150 water have small B value than 50. 198 and 139 water molecules have more than one hydrogen bonds, to actin and DNase I, respectively.
Consideration of atomic distance, B value and octahedral coordination, three peaks were assigned as Ca^{2+}ion. One of them is Ca^{2+}binding to ATP of actin and the other two locate in the DNase I region. One of them locates at the same position as Site A in the paper described by Suck.
There are two actin-DNase I contact regions and three actin-actin contact regions. The contact region containing actin 41(G)-44(V) & DNase I 65(T)-67(V) has five solvent bridges between two molecules. Actin-actin contacts are rather weak, the region near to 109(L),112(K),172(H)-176(R) and 370(H)-374(P) forms main contact region and another contact region is between 191(I)-195(R) and 283(K)-286(I) and 374(P). Both regions have two water bridges between adjacent actin molecules.

PS-03.08.11 STUDIES OF MYOSIN LIGHT CHAIN TERTIARY STRUCTURE. By L. Chung*, D.R. Brown#, W. Huang#, B.D. Hambly# & P.M.G. Curmi, *School of Physics, The University of New South Wales, P.O. Box 1, Kensington, N.S.W., 2033, Australia. #Pathology Department, The University of Sydney, N.S.W., 2006, Australia.*

The aim of this project is to produce a model system that facilitates the crystallographic study of the control of myosin force generation by myosin light chains. The regulatory light chain of myosin (LC2) is responsible for the regulation of force generation in smooth- and non-muscle cells. It binds one mole of calcium per mole LC2 , and is activated by phosphorylation. Sequence homology suggests that it is part of the superfamily of calcium binding regulatory proteins that includes calmodulin (CaM) and troponin C (TnC) (Collins,1991 *J. Muscle Res. Cell Motil.* 12: 3-25). Our model system consists of an LC2-synthetic peptide complex that will be suitable for x-ray crystallographic studies. In the long term, we expect to determine the atomic structures of the LC2-peptide complex.
Computer modelling has been used to predict a structural model for the interaction of myosin LC2 with heavy chain (HC). An alpha-helical model of myosin heavy chain was initially built based on a predicted LC2 binding region (Mitchell *et al.*,1989 *J. Mol. Biol.* 208: 199-205). The LC2 portion of the myosin HC sequence was predicted by homology from the binding sequence of myosin light chain kinase given the homology between LC2 and CaM. By means of standard sequence alignment, a sixteen amino acid region of myosin HC for the LC2 binding site was found in the best sequence alignment. This HC segment differs from previous studies. From homology, a model of LC2 was subsequently built by using the x-ray structure of CaM. On the basis of the CaM-peptide complex structure, which has recently been determined (Meador *et al.*,1992 *Science* 257: 1251-5), the model of LC2-HC complex was ultimately made. In this model, the hydrophobic cores of both the N- and C-terminal domains of LC2 naturally interact with the myosin HC hydrophobic residues. HC positive charges fall close to LC2 negative charges. Hence, the predicted binding sequence was supported by modelling the LC2-HC complex.
To verify the results of modelling, biotinylated mutant light chain was mixed with one of a series of 15 residue peptides, that spanned a 50 amino acid region of the myosin heavy chain sequence surrounding the predicted binding site. The peptides differ in sequence by a frame shift of two amino acids. An ELISA-type assay allowed a hierarchy of binding efficiencies to be determined for each of the nineteen 15 residue peptides. From this study, the most likely site for LC2 binding on the myosin heavy chain was determined. We are currently crystallising the LC2-peptide complex.
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03.09 – Receptor and Signal Transducing Proteins

PS-03.09.01 MECHANISM OF THE HYDROLYSIS OF Ras p21.GTP.Mg(H_2O)$_2$.
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The atomic structure of Ras p21 ist stabilized by a great number of intramolecular hydrogen bonds. The structure includes a six stranded beta sheet, five alpha helices and ten loops (H-Ras p21.GppNp.Mg(H_2O)$_{211}$; Symmetry $P3_212$; PDB Dataset 5P21) (E. F. Pai, U. Krengel, G. A. Petsko, R. S. Goody, W. Kabsch, A. Wittinghofer, EMBO J., 1990, 9, 2351-2359). Part of the Ras p21 molecule forms a giant anion hole used as GTP binding site. The triphosphate group of GppNp.Mg(H_2O)$_2$ is held in place by six hydrogen bonds of main chain NH of residues 13 to 18 (loop 1: Gly13-Val14, alpha$_1$ helix: Gly15-Lys16-Ser17-Ala18). Two more hydrogen bonds are formed by Lys16 N, another two by Thr35 (loop 2) and Gly60 (loop 4).
In this crystal structure the conformation of loop 4 is strongly determined by two intermolecular hydrogen bonds between Gln61 (loop 4) to Asp33* and Pro34* (loop 2*) of a neighbor molecule and vice versa because of symmetry. Furthermore the OH group of Tyr32* of this neighbor molecule forms an intermolecular hydrogen bond to O_1 of the gamma phosphate group.
In Ras p21.GDP.Mg.(H_2O)$_{39}$ (Symmetry $P6_522$; PDB Dataset 1Q21) (L. Tong, A. M. de Vos, M. V. Milburn, S.-H. Kim, J. Mol. Biol., 1991, 217, 503-516) loop 2 and loop 4 have different conformations compared to Ras p21.GppNp Mg(H_2O)$_{211}$ and Asp33 as well as Pro34 (loop 2) and Gln61 (loop 4) form different intramolecular hydrogen bonds.
Three water molecules located in the neigborhood of GTP.Mg are necessary for the hydrolysis of GTP.Mg(H_2O)$_2$ to GDP.Mg(H_2O)$_4$ (W175, W189, W171, PDB Dataset 5P21).
The conformational changes of loop 2 and loop 4 caused by the hydrolysis of Ras p21.GTP.Mg(H_2O)$_2$ to Ras p21.GDP.Mg(H_2O)$_4$ have been studied by a time-resolved X-ray crystallographic study of a single crystal (I. Schlichting, S. C. Almo, G. Rapp, K. Wilson, K. Petratos, A. Lentfer, A. Wittinghofer, W. Kabsch, E. F. Pai, G. A. Petsko, R. S. Goody, 1990, Nature, 345, 309-315).
Hydrolysis starts with the movement of the gamma phosphor atom through the plane formed by the three oxygens in the direction of W175 (inversion) (J. Feuerstein, R. S. Goody, M. R. Webb, J. Biol. Chem., 1989, 264, 6188-6190) leading to a transition state where a proton transfer from W175 to O_1 gamma over Gln61 takes place.
A detailed analysis of the observed conformational changes of loop 2 and loop 4 and the conversion of intermolecular hydrogen bonds to intramolecular ones during hydrolysis will be given.
The importance of the highly conserved residues, especially Gly12, for the hydrolysis will also be discussed.
In vivo the rate of hydrolysis is strongly increased by GAP (GTPase activitating protein). We will present a model for possible interaction of GAP by hydrogen bonds with Ras p21 in two different regions of GAP, one "binding domain" and one "docking domain".

