We are currently producing mutants to probe the function of $p13^{suc1}$.

Cyclin A is first observed late in the G1 phase of the cell cycle, and its abundance increases throughout S and G2. It forms complexes with either CDK2 or CDC2. Our structure of a genetically truncated form of cyclin A (residues 171-432) consists of two strikingly similar helical domains, despite low sequence homology. Comparison with the structure of a complex between a fragment of cyclin A and CDK2 [4] showed that in contrast to the major structural changes of CDK2, cyclin remains essentially unaltered when complexed. Thus, cyclin acts as a template, complimentary to the basally active conformation of CDK2 in the complexed structure.

Our poster will describe details of the results mentioned above, as well as the results of on-going work to further characterise these proteins.

[1] Endicott et al., EMBO J. **14**, 1004. [2] Brown et al., Structure **3**, 1235 [3] Bourne et al., PNAS **92**, 10232. [4] Jeffrey et al., Nature **376**, 313.

PS04.10.18 CRYSTALLOGRAPHIC AND BIOCHEMICAL ANALYSIS OF AN INHIBITOR OF APOPTOSIS, BCL-2. Jason W. O'Neill and Kam Zhang, Division of Basic Sciences, Fred Hutchingson Cancer Research Center, Seattle, WA

Programmed cell death (apoptosis) plays an important role in animal development and homeostasis of cell numbers. The primary regulator of human apoptosis is Bcl-2, a novel cellular gene which can function to block most modes of apoptosis. Elevated levels of Bcl-2 protein are strongly correlated with tumorgenesis and is found deregulated in a variety of cancers, including; colorectal (90%), breast (70%), and lymphoma (65%). Bcl-2 may function to inhibit apoptosis through effects on antioxidant protection pathways or by modulating the apoptotic effects of Ca^{2+} release from ER. The regulation of Bcl-2 is thought to occur through phosphorylation and/or heterodimer formation with related promoters of apoptosis, such as Bax. To pursue Bcl-2's function and regulational control: *a)* We have expressed human Bcl-2 in *E. coli* using a pET-His-tag expression vector. Bcl-2 has been successfully purified to high concentrations and is found as a homodimer as measured by dynamic light scattering. Currently we are undergoing crystallization trials. *b)* We also look at the effects of phosphorylation to Bcl-2's function, and how the addition of Bax influences Bcl-2's function using an *in vitro* apoptotic assay. Combining the 3D-structure with the biochemical data will be critical in unraveling Bcl-2's biological mechanism and for its regulation during apoptosis. Furthermore, this knowledge will be extremely useful in structure based design of inhibitors of Bcl-2, which could lead to potent therapeutics for cancers and a broad range of other malignancies.

PS04.10.19 THE STRUCTURE OF PROTEIN TYROSINE PHOSPHATASE-lB COMPLEXED TO A HIGH AFFINITY INHIBITOR. Andrew D. B. Pannifer*, Terrence R. Burke Jr.†, David Barford*, *Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford, OX1 3QU, UK, †Laboratory of Medicinal Chemistry, Bethesda, MD, USA

The 2.4 Å resolution structure of protein tyrosine phosphatase-lB complexed to the high affinity, non-hydrolyzable inhibitor hydroxy-naphthalene difluorophosphonate is presented. The inhibitor is competitive, binding at the active site with a sub-micromolar K_i and acts as a phosphotyrosine analogue. The labile phosphorus-oxygen bond of phosphotyrosine is replaced by a phosphorus-carbon bond in the inhibitor, thereby rendering the phosphate moiety non-hydrolyzable.

The naphthalene group of the inhibitor mimics the phenyl ring of phosphotyrosine, occupying a hydrophobic cleft formed by tyrosine 46 and phenylalanine 182. The phosphate group occupies the established phosphate binding pocket. Specific interactions between the phenolic oxygen of the inhibitor and residues at the active site of the enzyme have also been identified and these are likely to account for the low K_i.

PS04.10.20 CRYSTALLOGRAPHIC ANALYSIS OF A FRAGMENT FROM CHICKEN TENASCIN CONTAINING TWO FIBRONECTIN-TYPE III DOMAINS Klaus Piontek, Daniel Bisig, Peter Weber, Lloyd Vaughan, Kaspar Winterhalter, Laboratory of Biochemistry, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland.

Tenascin-C is an extracellular glycoprotein composed of six identical polypeptide chains which are linked together at their N-terminal ends to form a hexabrachion structure. Each polypeptide comprises a serie of 13.5 tandem repeats homologous to epidermal growth factor followed by 8-13 fibronectin type III (TNfn)-like domains and finally one fibrinogen-like domain at the C-terminus. Alternative splicing of TNfn domains between the domains 5 and 6 leads to the three major isoforms found in chicken. In the smallest isoform (190 kDa) these two domains are adjacent to each other. Tenascin-C isoforms are differently expressed in development and in response to malignancy, leading to the expectation of isoform-specific functions. TNfn5 binds heparin and other iduronic acid-containing glycosaminoglycans, presumably *via* a conserved heparin binding motif. Furthermore, TNfn56 binds to the neural cell adhesion molecule contactin/F11, an interaction which is inhibitable by heparin. To explore these interactions in greater detail we have prepared a fusion protein containing domains 5 and 6 and attempt now the crystal structure determination of this 20 kDa protein.

TNfn56 crystallizes from 27% PEG2000, 100mM Na-acetate, pH 4.5 at RT in a monoclinic ($P2_1$, a=47.7Å, b=54.9Å, c=97.0Å, ß=104.4°, V_m=3.08 $Å^3$/Da, 2 mol/a.u.) and an orthorhombic ($P222_1$, a=45.3Å, b=71.9Å, c=58.0Å, V_m=2.36 $Å^3$/Da, 1mol/a.u.) form. Both crystal forms diffract to about 2.6 Å resolution. The majority of the monoclinic crystals is twinned and the diffraction limit of this form frequently lies only between 3.2 and 4.0 Å. Furthermore, the formation of the orthorhombic form can not be directed. Despite the sparse availability of suitable single crystals we have collected a data set of monoclinic crystals to 2.6 Å resolution utilizing synchrotron radiation (DESY/Germany) and a data set to 2.8 Å resolution on a conventional X-ray source. Presently we attempt the structure determination with the molecular replacement technique and, in parallel, with isomorphous replacement.

PS04.10.21 CURRENT PROGRESS ON CRYSTALLOGRAPHIC COMPLEXES OF TRANSDUCIN. Susan M. Redford, Heidi Hamm‡, Joseph Noel, Structural Biology Lab, The Salk Institute, La Jolla, CA 92037, ‡University of Illinois College of Medicine, Chicago, IL 60680

The opportunity to understand the structure and function of a G protein signal transduction cascade at the atomic level is the underlying goal of this research. The current studies employ peptides that mimic rhodopsin's functionally essential cytoplasmic face in complex with $G_{t\alpha\beta\gamma}$ to probe the structural consequences of receptor/G-protein binding. Recently the heterotrimer structure, $G_{t\alpha\beta\gamma}$ has been reported to 2.0 Å resolution. In these studies, a chimeric Gtα Gtα/iα, which has been *E. Coli* expressed, is substituted for $G_{t\alpha}$. $G_{t\alpha/i\alpha\beta\gamma}$ crystals are then grown in complex with peptides which mimic rhodopsin's cytoplasmic loops 2 and 3.

Signaling cascades provide mechanisms to receive, transmit and amplify cellular messages, and thus are critical pathways for mounting a cellular response to an extracellular stimulus. Heterotrimeric G-proteins have evolved as ubiquitous solutions for signal transduction and amplification throughout many cells, and are involved in processes such as vision, olfaction, hormone signaling, and responses to cytokines. The α subunit has been studied extensively biochemically, as it contains the GTPase function,

and binds rhodopsin, the β and γ subunits, and cGMP-PDE. Diffraction quality crystals of the heterotrimeric complex are grown in microseeded hanging drops containing 10 mg/ml protein, 10% PEG-8000, 50 mM Tris, pH 8.0, 50 mM NaCl, 10% glycerol, and 0.1% β-mercaptoethanol. The space group is C2 with unit cell dimensions of a=133.4, b=91.4, c=83.2, β=120.1°. Progress to date will be reported.

PS04.10.22 REFINED CRYSTAL STRUCTURES OF TNF-ALPHA AND TNF MUTANT R31D. C. Reed*, Z.-Q. Fu*, J. Wu*, Y.-N. Xue, M.-J. Chen and I.T. Weber*. *Department of Pharmacology, Department of Microbiology and Immunology, Jefferson Cancer Center, Thomas Jefferson University, Philadelphia PA 19107, USA.

Crystal structures have been determined of recombinant human tumor necrosis factor-α (TNF-α) and its R31D mutant that preferentially binds to TNF receptor R1 with five times greater affinity than to receptor R2. Crystals of the wild type TNF were of space group $P4_12_12$ and had unit cell dimensions of a=b=94.7 and c=117.4 Å. Refinement of the structure gave an R-factor of 22.3% at 2.5 Å resolution. The crystals of TNF R31D mutant diffracted to 2.3Å resolution, and were of identical space group to the wild type with unit cell dimensions of a=b=95.4 and c=116.2 Å, and the structure was refined to an R-factor of 21.8%. Almost continuous electron density was observed throughout both structures, although the first five residues of the N-termini appear to be disordered. Comparison of the structures of the wild type and mutant TNF showed that the two trimers were similar with an rms deviation of 0.77 Å for main chain atoms, however, the subunits within each trimer were more variable with rms deviations of over 1.05 Å for pairwise comparison of main chain atoms. Model complexes of TNF with receptors R1 and R2 have been used to predict TNF-receptor interactions. The Arg 31 of wild type TNF is predicted to form an ionic interaction with an identical glutamic acid in both receptors R1 and R2. In the TNF R31D mutant, modeling suggested that this interaction is replaced by interaction with a histidine in R1, but there is no equivalent interaction in R2, consistent with the observed greater affinity of the R31D mutant for receptor R1 compared to R2.

PS04.10.23 REFINEMENT OF NATIVE AND MUTANT VEROTOXIN B-SUBUNIT STRUCTURES. Allan M. Sharp, Penelope E. Stein, Amechand Boodhoo, and Randy J. Read. Departments of Biochemistry, and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

The ability of enterohemorrhagic *E. coli* strains to cause hemorrhagic colitis and hemolytic uremic syndrome is a result of the production of a shiga-like toxin or verotoxin. This is an AB_5 type toxin, with a catalytic A-subunit attached to a pentamer of B-subunits that bind the cell surface glycolipid globotriaosylceramide (Gb-3). The solution of the wild-type verotoxin B-subunit pentamer, at 2.2 Å resolution, revealed a predominantly β-sheet structure, built around a central helix-lined pore [1]. Based on the distribution of conserved surface residues, the deep clefts at the interfaces between the subunits were proposed to be the sugar-binding sites. Mutation of a phenylalanine to an alanine residue in the cleft region (Mutant F30A) did eliminate the majority of glycoside binding [2]. Difference Fourier analysis of crystals of F30A at 2.0 Å resolution suggested that there were no major structural differences in the protein away from the mutation site. Further refinement of the native and mutant structures with XPLOR and TNT has reduced their R-factors to 0.195 and has allowed detailed analyses of the features of the structures, their distortion from ideal pentamers by crystal contacts, changes at their putative binding sites, and changes in occupancy at two crystallographic zinc binding sites.

A more recent 2.8 Å crystal structure of the verotoxin B-subunit complexed with Gb3 has shown that there are in fact three different classes of binding sites on the pentamer surface, one of them closely corresponding with the original prediction, and another contacting phenylalanine 30 [3]. The effects of the mutation may aid in elucidating the relative importance of the different sites.

Research supported by the AHMRF, MRC, HHMI.

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PS04.10.24 ATOMIC STRUCTURE OF POTD-THE PRIMARY RECEPTOR OF SPERMIDINE/PUTRESCINE TRANSPORT SYSTEM IN E.COLI. D.G.Vassylyev[1], S. Sugiyama[2], M. Matsushima[3], K. Kashiwagi[4], K. Igarashi[4], K. Morikawa[1], [1]Biomolecular Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka, 565, Japan, [2]Kyowa Hakko Kogyo Co. Ltd., Pharmaceutical Research Laboratories, 1188 Shimotogari, Nagaizumi-cho,Sunto-gun,Shizuoka,411,Japan, [3]Rational Drug Desighn Laboratories, 4-1-1 Misato, Matsukawa, Fukushima 960-12, Japan, [4]Faculty of Pharmaceutical Sciences, Chiba Univ., 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan

The crystal structure of PotD (a periplasmic binding protein which is the primary receptor of polyamine transport system in E.coli) in complex with spermidine has been solved at 2.5Å resolution. The PotD protein (325 amino acids) consists of two domains with a deep cleft (20Å long, 5Å wide, 14Å deep) in the interface between them. This cleft was found to be a binding site of spermidine in the complex. The three positively charged nitrogens of spermidine are recognized by four acidic side chains of PotD in the cleft while five aromatic residues anchor the spermidine methylene backbone by van der Waals interactions. The overall fold of PotD is similar to other periplasmic binding proteins despite the fact of low sequence similarity.

Crystals of the complex belong to the space group $P2_1$(a=145.3Å, b=69.1Å, c=72.5Å, β=107.6, Z=8). The structure was solved by MIR method in combination with solvent flattening and 4-fold N.C.S. averaging and refined at 2.5Å resolution to a final R-factor of 0.199 (R-free = 0.280).

PS04.10.25 CRYSTALLOGRAPHIC ANALYSIS OF THE MAP KINASE P38. Zhulun Wang[1], Jiahuai Han[2] & Elizabeth J. Goldsmith[1]. Department of Biochemistry, UT Southwestern Medical Center at Dallas, Dallas, TX 75235[1]. Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037[2]

The mitogen-activated protein (MAP) kinase cascades are major signaling pathways that transmit extracellular information from the cell surface into the intracellular responses. Various extracellular stimuli, such as, growth factors, heat, UV-irradiation, inflammatory cytokines and hyperosmolarity, activate MAP kineses by dual phosphorylation on threonine(T) and tyrosine(Y). Three distinct MAP kineses signal transduction pathways have been defined so far in mammalian cells based on their differential activation selectivity and substrate specificity. As a new member of the MAP kinase family, p38 has been identified in a stress-activated signal transduction pathway. The three dimensional structure of p38 and comparison of p38 to ERK2 will help to elucidate the mechanisms of specificity determination in this family.

His6-tagged recombinant murine p38(45kDa) protein has been expressed, purlfied and crystallized by vapor diffusion method