Suitable resolution structures exist of the uncomplexed CaM (Chattopadhyaya, R., et al 1992. *J. Mol. Biol.* **288**: 1177-92, Ban, C., et al. 1993. *PDB*:1osa). However, a high resolution structure of CaM complexed with RS20, a peptide corresponding to the CaM recognition sequence of smooth muscle/non-muscle myosin light chain kinase, is needed. Also, the structure of E84K-CaM:RS20 complex has not yet been determined. We have collected high resolution data, and are determining the structures of both the wild type CaM:RS20 complex (1.73 Å) and the E84K-CaM:RS20 complex (1.90 Å). Once these structures are determined, we believe they will offer insight into the basis for the coupling of CaM's Ca^{2+} binding and peptide binding activities.

(Supported in part by NIH grants GM30861 and T32-GM08320.)

PS04.17.44 DOMAIN INTERACTIONS IN CRYSTALLINS. G. Wright[1], B. Norledge[1], H. Driessen[1], C. Slingsby[1], R. Kroone[2], E. Mayr[3], S. Trinkl[3], A. Basak[1]. [1]Department of Crystallography, Birkbeck College, Malet St. London, UK. [2]Department of Molecular Biology, University of Nijmegen, The Netherlands. [3]Biophysics Institute, University of Regensburg, Germany

Transparency and refraction of eye lenses are dependent on the spatial organisation of the lens α-, β– and γ-crystallin proteins. Random aggregation and phase separation cause sharp discontinuities in the index of refraction leading to light scattering and cataract. The oligomeric β-crystallins and monomeric γ-crystallins form a superfamily of proteins and are an excellent example of how domain swapping can create dimers from monomers as a result of conformational differences in domain linkers. The 21 kDa γ-crystallin family has two branches: the ubiquitous, highly conserved γS, and the more variable branch comprised of at least six members γA - γF in mammals. A predicted model of γS-crystallin shows that it differs from other γ-crystallins mainly in the interface region between domains. In bovine lens γB and γD phase separate at low temperature whereas γE separates at body temperature and consequently is implicated in cold cataract. Our previous crystallographic studies on a single domain of γB show how sequence extensions effect domain interactions.

We will report on crystallographic studies of engineered crystalline. Complete γS-crystallin has resisted crystallization but the isolated N and C-terminal domains have. The C-terminal domain of βB2-crystallin has also been crystallized as well as a mutant γB crystallin with a γE linker. These studies will aid in defining the role of linkers, extensions, and surface hydrophobic patches in determining domain interactions in crystalline.

PS04.17.45 STRUCTURAL AND KINETIC ANALYSIS OF CD4 MUTANTS THAT ARE DEFECTIVE IN HIV BINDING. Hao Wu[1], David G. Myszka[2], Susan W. Tendian[3], Christie G. Brouillette[3], Ray W. Sweet[2], Irwin M. Chaiken[2], Wayne A. Hendrickson[1,2], [1]Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, NY 10032, [2]SmithKline Beecham Pharmaceuticals, King of Prussia, PA, 19406, [3]Southern Research Institute, Birmingham, Alabama, 35205

It is well established by equilibrium binding studies that CD4 interacts with gpl20 in the range of nanomolar affinity. Little is known, however, about the mechanism of this interaction. In this report, we analyzed the native and several mutant forms of the HIV-binding fragment (D1D2) of CD4 using a combination of kinetic, structural and thermodynamic approach. Our real-time binding kinetic data from BIAcore measurements showed that the affinity decrease in HIV-binding defective mutants is mainly due to the decrease in association rate. The predominant alteration of association-rate by neutral mutations may invariantly suggest conformational adaptation in this and many other protein-protein interactions.

PS04.17.46 SHORT HYDROGEN BONDS IN A PROTEIN RECEPTOR-PHOSPHATE COMPLEX: EVIDENCE FROM STRUCTURES REFINED AT 1 Å RESOLUTION. Zhongmin Wang*, Hartmut Luecke†, and Florante A. Quiocho*,‡. *Structural and Computational Biology and Molecular Biophysics Program, ‡Howard Hughes Medical Institute and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030 and †Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, CA 94309

The protein receptor (a phosphate-binding protein or PBP with a mass of 33,000 daltons) serves as an initial and extremely specific component of bacterial active transport. Fully consistent with the stringent specificity of PBP, the initial 1.7 Å structure of the complex shows that the completely dehydrated and sequestered phosphate forms 12 hydrogen bonds (11 with donor groups and 1 with an acceptor group) in addition to one salt link with a guanidinium group. The distance of the hydrogen bond between the donor phosphate O4 oxygen and the acceptor group (an oxygen of carboxylate of Asp 56) is 2.45 Å. This short hydrogen bond has also been observed in the 1.9 Å structure of a fully active mutant PBP in which the donor group Thr141 was replaced by an acceptor Asp residue and in several high resolution structures of other PBP mutants. Moreover, the Asp141 substitution further introduced another short hydrogen bond (2.50 Å) between the phosphate O2 oxygen and an Asp141. In order to cement these findings of short hydrogen bonds, as well as obtain an atomic structure, excellent synchrotron data for the wild-type and the Asp141 mutant PBP were collected to resolutions of 0.98 Å and 1.00 Å, respectively. Using SHELXL-93, full matrix refinement of both structures with hydrogen atoms and anisotropic B-factor for non-hydrogen atoms against the ultra high resolution data confirmed the existence of the short hydrogen bonds. These short hydrogen bonds in the PBP-phosphate complexes could be classified as low barrier hydrogen bonds with energies ranging from 12 to 24 kcal/mole [1,2,3]. Nevertheless, the K_d values of 1 - 10 μM for the complexes do not reflect these high energy hydrogen bonds.

We thank Dr. George M. Sheldrick for providing the program SHELXL and helpful advice.

1. Hibbert, F. and Emsley, J. Adv. Phys. Org. Chem 26, 255 (1990).
2. Cleland, W. W. and Kreevoy, M. M. Science 264, 1887 (1994).
3. Frey, P. A., Whitt, S. A., and Tobin, J. B. Science 264, 1927 (1994).