an initially highly concentrated solution of protein contained in a continuously stirred light-scattering cell mounted within the light scattering detector. With this apparatus we obtain a gradient of solute concentration that decreases exponentially with each dilution step, and measure the relative intensity of light scattered at each solute concentration. A concentration gradient may be acquired automatically in less than one hour, and protein is conserved for later study if desired. Initial results obtained from BSA solutions at high concentration (120 g/l) are quantitatively accounted for by an effective hard particle model without significant self-association at concentrations up to this value. The results of analysis of nonideal behavior resulting from nonspecific repulsive interaction between BSA molecules (steric + electrostatic repulsion) agree well with those previously obtained from analysis of the concentration dependence of sedimentation equilibrium and osmotic pressure of BSA solutions under the same experimental conditions.

Enzymes

1093-Pos Mechanism of Enzymatic Activity of β-subunit of the Voltage-Gated Potassium Channels

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Board B69

Oxygen sensitive Kv channels (Kv1.5 and Kv4) bind the auxiliary β-subunits which belong to the aldo-keto reductase (AKR) superfamily. Kvβ1–3 subunits differentially alter the gating properties of the Kv channel and impart or accelerate Kv inactivation when bound to NAD(P)H and abolish inactivation in presence of $NAD(P)^+$. In pulmonary arteries, β-subunits are expressed in a gradient manner and possibly regulate the perfusion ventilation system. We found that Kvβ displays catalytic activity with several aldehydes and ketones. Substituted benzaldehydes and acetophenones were among the substrates with highest catalytic activity (k_{cat}~0.2 min^{-1}) and Michaelis constants in submillimolar range (K_m for p-nitrobenzaldehyde is 162±33 μM; K_m^{NADPH} is 32±11 μM). Structure-activity relationship studies with a series of substituted benzaldehydes and acetophenones revealed importance of substituent in the para-position of benzene ring (nitro-group being best) and acceptance of ketones (p-nitroacetophenone) as substrates. Endogenous aromatic e.g., vitamin K3 as well as aliphatic lipid peroxidation products such as 4-oxo-nonenal, POVPC and eicosanoids such as PGJ_2 were good substrates with K_ms in the micromolar range. Reduction was found to be the preferred direction for Kvβ2 enzyme; as no oxidation of p-nitrobenzylalcohol was detected. Substitution of Tyr90 which is homologous to the catalytic tyrosine in other AKRs, for Phe abolished enzymatic activity of Kvβ2 suggesting that catalytic mechanism of this enzyme is similar to other AKRs. Measurements of the kinetic isotope effects using NADPD showed a $^Dk_{cat}$ for p-nitrobenzaldehyde and nitroacetophenone of 2.0±0.1 suggesting that the hydride transfer step is partially rate-limiting. Limited kinetic isotope effect on k_{cat}/K_m and cofactor binding studies suggest that the release of the cofactor and substrate/product(s) also contribute to rate-limitation. Discovery of high-affinity endogenous substrates (or ligand) suggests that intracellular metabolism maybe kinetically coupled to Kv channel gating by Kvβ subunits.

1094-Pos Crystal Structures Of Human Orotidine-5′-monophosphate Decarboxylase - Implications For Drug Design And Catalysis

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Board B70

The human UMP synthase (UMPS) catalyzes the last two steps of *de novo* pyrimidine nucleotide synthesis and is a malaria and cancer drug target. The C-terminal domain of UMPS harbors the orotidine-5′monophosphate decarboxylase (OMPD) activity, which does not require any cofactors, yet is among the most proficient enzymes known. Based on biochemical and structural data of OMPDs from various micro-organisms several non-covalent mechanisms for decarboxylation through high energy intermediates have been formulated. We describe several crystal structures of the human OMPD in complex with substrate, product, and several novel nucleotide inhibitors. These first structures of a mammalian OMPD outline the requirements potential drugs must meet to maximize ligand efficiency and minimize cross-species activity. Chemical mimic by iodide identified a binding site for the product CO_2, which can be exploited for drug design. Unexpectedly, simple compounds can replace the natural nucleotide and induce a closed conformation of the enzyme, defining a tripartite catalytic site. Structural plasticity of catalytic residues and a covalent OMPD-UMP complex prompt a re-evaluation of the prevailing decarboxylation mechanism in favor of a covalent intermediate. This mechanism can also explain the observed catalytic promiscuity of OMPD.

1095-Pos Are Enzyme Active Sites Built in Multiple Layers?

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Board B71

The importance of "second shell" residues in enzyme active sites is addressed. Much experimental evidence has established the importance of specific residues in the catalytic and recognition capabilities of hundreds of particular enzymes. Nearly all of the residues that have been studied are in direct contact with the reacting substrate in the "first coordination shell" of the substrate molecule in the bound enzyme-substrate complex. Computational evidence, in addition to a very limited set of previous experimental data, suggests that remote residues, particularly those in the "second shell" around the reacting substrate, also contribute to catalytic activity. Residue

conservation and evolutionary trace analyses are reviewed. It is shown that such second shell residues do tend to be well conserved. THEMATICS, an electrostatics-based computational method for the precise prediction of interaction sites in proteins from their 3D structure, often includes some second shell residues in its site predictions. The available experimental data from the literature are reviewed, together with our own data for Nitrile hydratase (NH) from *Pseudomonas putida*, an enzyme for which THEMATICS predicts a double-layer active site and for which mutants of first-shell residues have already been studied. Site-directed mutagenesis (SDM) was performed on the second shell residues and kinetics assays were performed on each of the single mutants and on the wild type enzyme. All of the mutants had K_M values close to that of the wild type. However, three of the second shell mutants exhibited some reduction in k_{cat}. Values for the rate constants, expressed as the ratio k_{cat}(mutant)/ k_{cat}(WT), are: D164N = $7.4 \cdot 10^{-3}$; E168Q = 1.1; E56Q = $4.1 \cdot 10^{-2}$; H71L = $6.3 \cdot 10^{-2}$; Y213F = 0.54. The body of evidence suggests that second shell residues do play a supporting role in enzyme function.

1096-Pos Time-Resolved X-ray Scattering Studies on the Allosteric Transition of E.coli Aspartate Transcarbamolyase

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Board B72

E. coli aspartate transcarbamoylase is a dodecameric allosteric enzyme which undergoes a large scale quaternary structure change up on homotropic and heterotropic allosteric transitions. We have conducted time-resolved x-ray scattering studies of the structural transition in the temperature range 5–25 °C. Advanced synchrotron radiation instrumentation permitted the use of near physiological solution conditions without the use of ethylene glycol which was required in the past but recently found to dramatically influence enzyme activity. We will report the direct observation of the kinetics of the quaternary structural transitions promoted by L-aspartate in the presence of carbamoyl phosphate and the bi-substrate analog N-phosphacetyl-L-aspartate, recorded at 5ms time-resolution. Both the very fast transition from T to R and the reverse transition from R to T after all available substrates have been consumed were observed with high data statistics. It appears that the two-state transition model accounts for the quaternary structural transition even at the earliest stage we have so far recorded. In the presence of an intermediary concentration of the substrates, we have also investigated the effects of allosteric effectors, ATP, CTP and UTP on the kinetics. ATP increases the rate of transition from T to R and shortens the duration in which the enzyme is in the R steady-state. The latter observation is in apparent contradiction to the notion of ATP as an activator. CTP alters both T to R and R to T transitions significantly and the enzyme does not reach the same level of steady state structural state, compared to the comparable condition without CTP. We observed, for the first time, that UTP alone shortens the duration of the steady-state dramatically in contrast to previous studies in which UTP had only synergic effects in the presence of CTP.

1097-Pos Structural Requirements For DcpS (decapping Scavenger) Interactions With 5′ mRna Cap Analogues

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Board B73

Human decapping scavenger protein DcpS takes part in the last stage of mRNA digestion during 3′–5′ degradation pathway. Substrates for scavenger digestion are short (less than 10 nucleotides) capped mRNAs. Scavenger cleaves them on 5‘ end in a specific way, releasing m7GMP mononucleotide. We examined several chemically modified cap analogs to reveal their resistance to cleavage and affinity to both: DcpS protein and its non-active mutant. Substitution of His 277 to Asn led to disabling hydrolytic properties of the enzyme.

Structural analysis has revealed that apo-hDcpS exists as a symmetric homodimer, whereas it forms an asymmetric dimer when bound to its substrate or product. To gain insight into the formation and stability of the hDcpS-cap complex, binding studies using the human scavenger enzyme and mononucleotides or modified dinucleotide cap analogues resistant to enzymatic hydrolysis were conducted using fluorescence titration experiments. Equilibrium association constants and the corresponding Gibbs free energy values were determined for several modified cap analogues selected to investigate particular residues crucial in the enzyme-substrate interaction. Stopped flow experiments were applied to reveal and verify proposed mechanism of association process. Furthermore, conformational changes of protein structure were examined using mass spectrometry methods.

1098-Pos Effect of Radiation on Tgase Activity In Normal and Cancer Cell Lines

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Board B74

Transglutaminases (TGase) are ubiquous enzymes activated only after major disruptions in physiological or homeostatic processes. However, their activity is strongly regulated since overexpression of the enzyme does not change cell survival. In mammalian cells 6 isozymes have been isolated even if the genome there are 8 different isozymes. The most characteristic is Factor XIII, involved in blood clotting activation. TGase-2 has been isolated from keratinocytes in

soluble form or associated to membranes. The enzyme can be activated by physical agents such as ultraviolet or ionizing irradiation or by chemical agents such as the chemotherapeutic drugs. In our study, we have use wild and carcinoma squamous cells irradiated with different doses and radiotheraphy fractioning. TGase-2 has been characterized both in normal and cancer cells before and after irradiation. Before irradiation the activity of the cancer cells is increased a 40% which can be due to structural or metabolic factors. Irradiation of the normal cells with different doses (100, 200 and 600 cGy) and measured at different times after irradiation (1, 6 and 24 hours) changes the TGase activity. The results obtained are compared with those of the cancer cells.

1099-Pos A Crystal Structure of Sucrose Hydrolase from *Xanthomonas Axonophodis* pv. glycines

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Board B75

Amylosucrase from *Neisseria polysaccharea* (NpAS) is a transglucosidase of family 13 glycoside hydrolase which catalyzes the synthesis of amylose-like polymer by hydrolase and glucosyltransferase activity using sucrose as a substrate. Recently, a novel enzyme from *Xanthomonas axonopodis* pv. glycines, with a high sequence similarity of 57% with that of NpAS, was characterized as a member of the newly defined carbohydrate utilization locus which regulates the utilization of plant sucrose in phytopathogenic bacteria. Interestingly, the enzyme named sucrose hydrolase (SUH) exhibits exclusively the hydrolase activity not a glucosyltransferase activity. In order to elucidate structural and functional features of SUH, we carried out crystallographic and site-directed mutagenesis. Four different crystal structures of SUH, including the free enzyme, SUH-sucrose complex, and SUH-glucose complex, reveal structural snapshots along the catalysis. Structural comparisons with NpAS provide that an overall structure of SUH is essentially identical with that of NpAS, and that the active site of SUH in the SUH-sucrose for the Michaelis complex is a pocket-type formed by conformational change, while NpAS has a tunnel-type active site with no indication of the conformational changes by the binding of sucrose. Site-directed mutagenesis was performed such that the active site residues in SUH were replaced with residues crucial for a glucosyltransferase activity in NpAS. Our structural and functional analysis suggests that a pocket-shaped active site and the identity of residues in the vicinity of the active site of SUH are designed to be optimal for the unique hydrolytic activity of sucrose.

1100-Pos The Peroxidase Activity of Human Indoleamine 2,3-Dioxygenase (hIDO)

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Board B76

Indoleamine 2,3-dioxygenase (IDO), a heme-containing enzyme, catalyzes the initial and rate-limiting step of L-Trp via the kynurenine pathway in nonhepatic tissues. Recent Resonance Raman studies showed that the distal and proximal heme environment L-Trp bound human IDO (hIDO) resembles that of peroxidases. In this work, we systematically investigated the peroxidase activity of hIDO in the absence and presence of L-Trp by spectroscopic and kinetic techniques. We found that the peroxidase activity of hIDO is pH dependent. At neutral pH (pH 7.4), hIDO reacts with H2O2 with a rate 30-fold faster than that of myoglobin. Binding L-Trp to hIDO retards the reaction by a factor of 9. Temperature dependent studies showed that the enthalpy of activation is 10.9 kcal/mol for the L-Trp free enzyme, smaller to that of myoglobin (14.2 kcal mol-1), whereas that of the L-Trp bound enzyme is 6.3 kcal/mol, analogous to that of cytochrome C peroxidase (6.3 kcal mol-1). The structural features underlying the peroxidase activity of hIDO were accessed.

1101-Pos Chemosensors Using Phenylenediamines and Aminopyridines

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Board B77

Chemosensors which can detect metal ions such as the Cu^{2+} ion are important because the Cu^{2+} ion is a common pollutant which can cause Wilson's disease. Colorimetric detection of Cu^{2+} is important because Cu^{2+} is the third most abundant heavy metal ion in the human body (after the Fe^{2+} and Zn^{2+} ions). Developing receptors which can facilitate the monitoring and removal of Cu^{2+} from the human body is an active area of research. For example, the copper-zinc superoxide dismutases have been an active research area for the past several years due to their importance to the investigation of ALS disease. Therefore, a series of substituted phenylenediamines and aminopyridines have been efficiently transformed to their carbamate derivatives in high yields under mild conditions for potential applications as chemosensors. Absorbance and fluorescence spectra have been shown to be sensitive to the electron-donating and electron-withdrawing properties of the attached substituents. The copper ion has been shown to cause a bathochromic shift when added to a solution containing a substituted phenylenediamine. Quantum yields have been calculated to be greater for several of the substituted aminopyridines than for the phenylenediamines. However, UV-VIS absorbance measurements recorded as a function of pH and concentration have shown several aminopyridines to be limited to acidic and neutral conditions, due to protonation and deprotonation of both nitrogen atoms, while the phenylenediamines have shown more tolerance to variations in pH. These fluorescent aminopyridines and phenylendiamines show the potential to be valuable tools to screen inhibitors of enzymes such as nucleotide pyrophosphatase, transferases, fatty acid amide hydrolase, and many aminopeptidases by making it possible to use high-throughput assays instead of using radiolabeled substrates which are labor-intensive and costly.

1102-Pos Variolin Receptors for the Selective Binding of Anions

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Board B78

Assay studies previously reported in the literature have shown that variolins, meridianins, and meriolins inhibit protein kinases, including several which are responsible for the production of blood vessels in tumor tissues. These structurally similar classes of indole alkaloids also inhibit the growth of the P388 tumor cell line, and are active against herpes Simplex and the polio virus. Therefore, the properties of these biologically active compounds are an active area of investigation. We have investigated the absorbance and fluorescence properties of several variolin derivatives, and observed the selective binding of halide anions. According to the Job's plot, the binding stoichiometry is one to one. Electron-donating substituents such as the methoxy have shown a fluorescence enhancement, and quantum yields have been calculated to be much higher when the methoxy substitutent is attached. Fluorescence quenching occurs through a photoinduced electron transfer (PET) mechanism. The fluoride anion binds to the receptor via the N-H hydrogens, with the result being an increase of the PET. Proton NMR titration indicates the importance of these N-H bonds in the selective anion binding. Association constants obtained by using a Benesi-Hildebrand plot indicate that the fluoride anion has the greatest association constant, followed by Cl^- and Br^-, in accordance with the basicity of these anions. Consequently, enhanced selectivity for F^- occurs. Therefore, these variolin derivatives show the potential to be useful as fluorescent anion sensors.

1103-Pos Substrate Channeling in the Sulfate Activating Complex: Combined Continuum Modeling and Coarse-grained Brownian Dynamics Studies

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Board B79

This article describes modeling the whole channeling process via numerical solution of the time-dependent Smoluchowski diffusion equation, as well as Brownian dynamics. We have found that the synthesis of APS2- is not a diffusion-controlled process while the phosphorylation of APS2- is diffusion-controlled. Electrostatic plays the essential role in the APS2- diffusion. Furthermore, by combining with coarse-grained Brownian dynamics, substrate channeling process has been studied with reactions in multiple active sites. Our simulations provide a bridge for numerical modeling with Brownian dynamics to simulate the complicated reaction and diffusion biosystems and raise important questions relating with the electrostatically mediated substrate channeling in vitro and in vivo.

1104-Pos Biochemical and Biophysical Studies of the NS3-4A protease/helicase from Hepatitis C Virus

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Board B80

The NS3-4A protein is a bifunctional serine protease/helicase from the hepatitis C virus (HCV) that is responsible for several pro-translational processing of viral proteins. Therefore, NS3-4A has become a prime target for inhibitor design to combat the virus. In my poster, I will present current biochemical and biophysical data on my research pertaining to the progress towards gaining insight into how NS3-4A functions on its substrates.

Molecular Chaperones

1104.01-Pos Single Molecule Fluorescence Studies on the Hsp90

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Board B80.01

The molecular chaperone Heat-Shock-Protein 90 (Hsp90) is one of the most abundant Proteins in unstressed eukaryotic cells. It is involved in the folding and stabilization of several key regulatory Proteins as the tumor suppressor factor p53, stereoid hormone receptor and kinases. The ATPase-activity seems to be of crucial importance for its *in vivo* functions[1]. Recently it has become the target for a new type of anti-cancer drugs that specifically inhibit its ATPase-function[1,2]. The recently published crystal structures[3] of Hsp90 suggest, that the structural changes, which occur during the ATPase-cycle, are in the order of several nanometres and thus accessible with Fluorescence-Resonance-Energy-Transfer (FRET).

Conformational changes of the Hsp90 dimer were observed with single molecule FRET using total internal reflection microscopy. In combination with bulk FRET experiments we could show that - in contrast to previous assumptions - the dimer does only one ATP cycle before it dissociates. To be able to measure several complete ATPase-cycles we stuck the two Monomers together so that the dissociation constant is decreased significantly. The Mutant Hsp90 shows unchanged ATPase and Co-Chaperone-binding (Sba1) characteristics. The single molecule measurements with these modifications reveal further insights into the Hsp90 ATPase cycle.

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