P1. Electrophilic Aromatic Selenylation

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Seleninic acids can serve as biomimetic functionality that reacts irreversibly with enzyme active site nucleophiles such as sulfhydryls. We find that seleninic acid adds to electron rich aromatic and heteroaromatic rings to form the aryl selenoether, which in turn can be oxidized to the corresponding selenoxide or selenone. Se-dealkylation leads to the seleninate. These compounds are potential inhibitors of enzymes such as orotate phosphoribosyl transferase and thymidylate synthetase.

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P2. 3D-QSAR Inhibition Model for hASBT using glutamyl-CDCAs

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Inhibition of human apical sodium-dependent bile acid transporter (hASBT) was evaluated using substituted aniline conjugates of glu-CDCAs. Compound inhibitory values, Ki, were measured by testing compounds at various concentrations, using taurocholate as a substrate and stably transfected hASBT-MDCK monolayers. The in silico models of compounds were built using all-atom CHARMM General Force Field. A 3D-QSAR model for hASBT inhibition using conformationally sampled pharmacophore method (CSP-SAR) was built using the 1-D and 2-D probability distributions of various structural descriptors obtained from MD simulations. CSP-SAR models were selected based on multivariable regression and Akaike Information Criterion (AIC) analysis. Models were evaluated by the leave-one-out cross validation method. Interestingly, 2 and 3-amino benzoic acid glu-CDCAs allow intramolecular hydrogen bonding, promoting compound potency; while 4-amino benzoic acid lacked intramolecular hydrogen bond resulting in poor binding affinity. Aniline conjugates of glutamyl-CDCAs were potent inhibitors of hASBT. A highly predictive and robust hASBT-inhibition CSP-SAR model was developed and considers compound hydrophilicity and intramolecular hydrogen bonding on compound activity.
P3. Structure of iodotyrosine deiodinase: Mechanistic implications


University of Maryland - College Park, University of Maryland - College Park, University of Maryland - College Park, University of Wisconsin - Madison, University of Maryland - College Park, University of Maryland - College Park

The need for iodide in biology is almost exclusively limited to its role in thyroid hormones, and the recycling of thyroidal iodide is critical for human health. The flavoprotein iodotyrosine deiodinase (IYD) salvages iodide from byproducts (mono- and diiodotyrosine, MIT and DIT) of thyroid hormone biosynthesis. The original proposal for the deiodination mechanism of IYD included a nucleophilic attack of the iodo group by an active site cysteine. Although this proposal had strong precedence, site-directed mutagenesis proved this wrong. Expression and isolation of IYD was stymied until a truncated version of the wild-type protein was constructed. Further engineering has enabled IYD expression in mammalian, insect, yeast, and bacterial cells. Subsequent crystallographic studies resulted in a structure of IYD at 2.0 Å resolution. The structure verified IYD’s assignment in the flavin reductase/NADH oxidase superfamily and showed that the cysteine residues are not in the active site. Structures of IYD with bound MIT and DIT were also obtained and indicated that the substrates are sequestered within the active site by inducing helical structure in two otherwise disordered regions of the enzyme.

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P4. WRN Helicase Activity is Essential for Its Role in DNA Damage Response

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Targeting the DNA damage response is a strategy for combating cancer, and DNA repair helicases may be a suitable target of chemotherapy to impair the DNA damage response in tumor cells. This hypothesis was explored by screening the National Cancer Institute Diversity Set for inhibitors of Werner syndrome (WRN) helicase, which is mutated in a disorder characterized by genomic instability. Of 500 compounds tested in vitro helicase assay, a compound was identified that potently inhibited WRN helicase activity (IC_{50} <20 mM), but had no effect on helicase activity catalyzed by Bloom’s syndrome, Fanconi Anemia Group J, or RECQ1 helicases. HeLa or U2OS cells treated with a selected WRN helicase inhibitor displayed impaired cell proliferation, elevated
apoptosis and increase in the number of H2Ax foci. These effects were mediated through inhibition of WRN since cells depleted of >90% WRN were resistant to the WRN helicase inhibitor. Isogenic BLM null and corrected cells were equally sensitive to the WRN helicase inhibitor, indicating that the biological effects were not due to inhibition of BLM activity. My results demonstrate for the first time that selective inhibition of WRN helicase activity by a small molecule impairs the growth of cancer cell lines.

To further define its genetic role, yeast rad50 transformed with human WRN was assessed for its resistance to DNA damaging agents. WRN rescued the rad50 sensitivity to the methylmethane sulfonate (MMS), but not to ionizing radiation, suggesting a role of WRN in repair of double strand breaks that occur at blocked replication forks. WRN rescue of rad50 MMS sensitivity was dependent on Exonuclease 1. WRN ATPase/helicase, but not exonuclease activity, was required for rescue of the rad50 MMS sensitivity. Currently, the small molecule WRN helicase inhibitor that I identified is being tested for modulation of WRN rescue of the rad50 DNA repair mutant. Collectively, these studies demonstrate that WRN helicase activity is essential for its role in the DNA damage response to replicational stress.

P5. Estimation of Gluconeogenesis Flux in Fao Rat Hepatoma Cell Line

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We investigated fluxes of gluconeogenesis in a liver cell line using stable isotope tracers and mass spectrometry. We used a detailed metabolic network model and a computational tool for flux analysis (Metran) to elucidate the effects of gluconeogenesis enhancers (cAMP, dexamethasone) and depressors (insulin, oxythiamine) on pathway fluxes. To stimulate glucose production, overnight-starved Fao rat hepatoma cells were cultured in the glucose-free media in the presence gluconeogenic precursors (lactate, pyruvate, glutamine, and glycerol) and isotopic tracers ([U-13C]glycerol, [2H8]glycerol, and 2H2O). Mass isotopomer distributions of glucose produced from the cells was analyzed by gas chromatography mass spectrometry (GC-MS) and the fluxes were estimated using Metran. Gluconeogenesis flux was enhanced by 8-bromo cAMP, dibutyryladenosine cAMP and dexamethansone, while insulin significantly suppressed hepatic gluconeogenesis. The two cAMPs showed differential stimulation of gluconeogenesis pathways from glycerol and lactate as precursors. Through rigorous analysis of mass isotopomer distributions of glucose, we discovered that the hepatoma cells utilize non-oxidative pentose phosphate pathway during gluconeogenesis. These results were independently validated using [U-13C]glycerol and [2H8]glycerol as tracers.
P6. Binding Site Competition of a Single Strand DNA Binding Protein

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Single strand binding proteins, or SSB’s, play a crucial part in DNA replication, recombination, and repair for organisms ranging from bacteria to humans. The gene 32 protein of bacteriophage T4 is well known for its model behavior as a classical SSB. Limited proteolysis of gp32 affords three consecutive domains that are functionally unique—the positively-charged N-domain, the core domain, where ssDNA is known to bind, and the negatively-charged C-domain. The C-terminal domain influences protein-protein interaction and inhibits destabilization of the dsDNA helix. It has been proposed that the C-domain is weakly bound to the core and competes with free ssDNA for binding. This investigation will determine whether the C-domain serves as a DNA mimic by interacting with the core domain binding cleft.

A zero-length chemical cross-linking protocol is currently being employed to bond C-domain, expressed and purified from recombinant E. coli, to core domain; the two-step reaction is performed in vitro, and successful linkage has been seen as a core + C-domain band on SDS-polyacrylamide gels. Addition of ssDNA during cross-linking will create a competitive binding assay. We hope to use this method to determine whether the cross-linked product reflects the native binding position for the C-domain, whether ssDNA interferes with cross-linking, and whether the isolated core + C complex binds to ssDNA. This would demonstrate that binding of ssDNA and C-domain to gp32 core-domain are mutually exclusive.

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P7. Structural and Mechanistic Insights into Fungal Iterative Polyketide Synthase

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Polyketides are a structurally and functionally diverse class of natural products. In fungi, aromatic polyketides are generated by nonreducing, multidomain iterative polyketide synthases (IPKSs). The catalytic domains work in concert to homologate acetate units to enzyme-bound poly-β-keto intermediates, which are regiospecifically cyclized to achieve diverse carbon scaffolds. To facilitate mechanistic investigation, we have adopted a ‘deconstruction’ approach in which mono- to multidomain protein fragments are individually expressed. Reconstitution experiments with selected domains from PksA, the IPKS central to the biosynthesis of the potent environmental carcinogen aflatoxin B1,
revealed the vital role of the product template (PT) domain in cyclization and aromatization of the poly-β-keto intermediates. Furthermore, the thioesterase (TE) domain was identified as a Claisen-type cyclase (CLC) responsible for product release. We report the crystal structures and biochemical analysis of the dissected PT and TE/CLC monodomains from PksA. The 1.8 Å crystal structure of the PT monodomain displays a unique double-hot dog fold. Co-crystal structures with palmitate or a bicyclic substrate mimic illustrate that PT can bind linear and bicyclic polyketides. Analysis of the 1.7 Å crystal structure of the TE/CLC domain affords the first mechanistic insights into this C–C bond-forming class of TEs. Detailed docking and mutagenesis studies of these domains suggest possible mechanisms for substrate binding and catalysis. Comprehensive understanding of IPKS will facilitate rational engineering towards the production of novel polyketides with interesting bioactivities.

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P8. Tuning hydrogel properties of heparinized drug delivery vehicles

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Low molecular weight heparin (LMWH) therapeutics are used as treatments for many diseases including deep vein thrombosis and unstable angina via subcutaneous or intravenous injections. Daily administration of therapeutics could be circumvented by a controlled release vehicle. Here, hydrogels have been synthesized for use as drug delivery vehicles by covalently crosslinking maleimide-functionalized LMWH by Michael addition reactions with thiolated poly(ethylene glycol) (PEG). The extent of heparin modification was characterized via 1H-NMR. The anti-coagulant activity of the modified heparin was determined via factor Xa and IIa coagulation cascade tests, indicating the extent of modification impacts the anti-coagulant activity of heparin. Thiolated PEG was synthesized by esterification of hydroxyl-terminated PEG with various mercapto-acids for facile modulation of ester hydrolysis and thiol nucleophilicity. Such engineering altered gelation kinetics, swelling behavior, heparin release and mechanical strength. The resulting release timescales could be tuned from one day to three months simply by altering the ester’s substituents. The use of these materials in heparin delivery will be discussed.

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P9. NMR Detected pKa Perturbation of A340/341 of the MMLV Packaging Signal

Shawn Barton, Rashmi Singh, Benyam Kinde, Blanton S Tolbert, and Michael Summers
The Moloney Murine Leukemia Virus (MMLV) is a gamma-type retrovirus that is used commonly as a model system to understand the retroviral life cycle. The MMLV genome is composed of two single strands of RNA linked non-covalently via RNA structural elements located within the 5’ non-coding region of the genome. Stem loop C (SLC) of MMLV is a critical RNA element that has been implicated in genome dimerization by forming intermolecular “kissing” complexes with a second stem loop, SLD. In an effort to understand the structural basis of genome dimerization, we investigated structural properties of an SLC fragment that retains features of the intact stem loop: i) (331)GAGC(334) tetraloop and ii) (338)GGAA(341) bulged loop. Both the GAGC tetraloop and the GGAA bulged loop are conserved structural elements across gamma-type retroviral lineages.

Using 1H-13C Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectroscopy, we discovered that the N1 position of adenosine 340 and 341 have atypical pH dependent properties. By monitoring the change in the adenosine C2 carbon chemical shift recorded from HMQC spectra collected at several pH values, we show for the first time that the pKa values of adenosine 340 and 341 is shifted well above the pKa value expected for free adenosine (c.a. 3.8). Additionally, our preliminary NMR data suggests that the perturbed pKas may play a role in modulating local RNA structure at the GGAA bulged loop. These results may imply a hitherto unreported role for a pH induced conformational change regulating the structure and folding of SLC.

P10. JS-K: pathways of anti-cancer action

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JS-K (O2-(2,4-dinitrophenyl)-1-[(4-ethoxycarbonyl)piperazin-1-yl] diazenium-1,2-diolate) is a potent chemotherapeutic agent effective against cancer cells at micromolar concentrations. JS-K can be synthesized in very high yield by the reaction of sodium 1-[4-carboxamido)piperidin-1-yl] diazen-1-ium-1,2-diolate with 1-fluoro-2,4-dinitrobenzene[i]. In cells, JS-K reacts with glutathione (GSH) in the presence of glutathione-S-transferase to form GS-2,4-DNP and nitric oxide (NO)[ii]. Currently, it is not clear if the chemotherapeutic effect of JS-K is due to release of NO from diazeniumdiolate morty. The effect of JS-K and its analogues on different signaling pathways was studied in order to identify the active species and the pathway responsible for the chemotherapeutic effect of JS-K.

P11. Synthesis of inhibitors of the N-acetyl-L-ornithine transcarbamylase arginine in Stenotrophomonas maltophilia

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Stenotrophomonas maltophilia is a gram-negative bacterium responsible for various nosocomial infections, predominantly affecting patients with cystic fibrosis, urinary tract infection or immunosuppression, e.g. after organ transplantation. It is highly resistant to most antibiotics, increasing the time spent in the intensive care unit. Mortality rates of 30% have been reported.

S. maltophilia utilizes a novel enzyme, N-acetyl-L-ornithine transcarbamylase (AOTCase), in arginine biosynthesis, which is the only metabolic pathway for this particular α-amino acid available to the organism. The synthesis of a potential inhibitor of AOTCase, 2-acetylamino-5-(2-phosphono-acetylamino)-pentanoic acid (PALAO), will be discussed.

The optimization of the carbamoylation of L-norvaline, an L-ornithine analogue, will also be presented. L-carbamyl-norvaline is expected to be a competitive inhibitor of AOTCase, based on structural similarity to the known inhibitor acetylnorvaline. In addition, it is not expected to be a substrate for deacetylases that exist in S. maltophilia. It is currently being used to study whether carbamoylated compounds can be taken up by S. maltophilia. Future aims include the synthesis of PALAO-peptides and possibly carbamoyl-NH-PALO-Met-Leu-COOH as well as the testing of their efficacy in entering the bacteria cells and inhibiting growth of S. maltophilia biofilm.

P12. Iron Substituted Neural Zinc Finger Factor-1

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Zinc finger proteins are transcription factors that are ubiquitous in eukaryotes. Neural zinc finger-factor 1 (NZF-1) is a non-classical zinc finger protein that is involved in neuronal development. The unique CCHHC zinc binding domains of NZF-1 are involved in the recognition of the β-RARE promoter element in the β-retinoic acid receptor gene. Zinc naturally binds to this domain, but it can be speculated that iron can bind instead. Iron is very prevalent in the nervous system, as it is important for proper functioning. Misregulation of iron, a highly redox active metal, can have serious consequences. The iron binding affinities for both a single domain (NZF-1-SF) and a double domain (NZF-1-DF) fragment of NZF-1 were examined. Both Fe(II) and Fe(III) were shown to bind to each fragment of NZF-1. Fe(III) bound to both NZF-1-SF and NZF-1-DF with micromolar affinity. Fluorescence anisotropy was used to determine the DNA binding affinity of Fe(III) bound NZF-1-DF. The resulting binding affinity was comparable to that of the zinc bound NZF-1-DF peptide. If Fe(II) bound NZF-1-DF shows similar results, this could suggest possible localized oxidative damage to genes that are regulated by NZF-1.

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P13. Rational design and synthesis of small molecules to mimic α-helices

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α-Helices are important recognition motifs for protein-protein, protein-DNA and protein-RNA interactions. Numerous protein-protein interactions utilize α-helices to control biological events critical in many human diseases, including cancer, HIV, neurological disorders. Mimicry of α-helices using small molecules could allow understanding of mechanism and provide new drug candidates.

We have rationally designed small molecules containing a tetrasubstituted tetrahydronaphthalene core structure, with the correct dimensions to position different substituents that mimic the presentation of the $i$, $i + 3$, and $i + 4$ residue side chains of α-helices. The synthetic scheme proceeds to develop a universal scaffold for α-helix mimic. A key synthetic step is a novel Lewis acid-catalyzed Friedel-Crafts epoxide cycloalkylation. The universal scaffold was rapidly functionalized in four steps to generate small molecule mimics of two turns of an α-helix. These rationally designed small molecules effectively inhibit the α-helix mediated p53•MDM2 (FSDLW motif) interaction, validating the design. This scaffold was readily elaborated to generate mimic of nuclear hormone receptor co-activators (LXXLL motif), the androgen receptor co-activators (FXXLF motif) and other transcriptional domains (FXLL motif). This easily modified scaffold could provide a general tool to study biologically important protein-protein interactions.
P14. Prep of Nitric Oxide-Releasing Polymers for Biomedical Applications

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Polymeric materials are extensively used for the preparation of diverse biomedical appliances. Diazeniumdiolated polymers are currently of great interest for their ability to spontaneously release bioactive nitric oxide (NO) under physiological conditions while rendering physiological effects of NO limited to sites of close physical proximity. Novel strategies for the preparation of N-diazeniumdiolated polyurethanes (PUs) and C-diazeniumdiolated polycrylonitrile (PAN/NO) are reported. Secondary amino nitrogens of alkane diamines inserted within the diol chain extender of PU are diazeniumdiolated and blending this polymer with a biocompatible, relatively nonnucleophilic salt was found to substantially improve diazeniumdiolate stability. In another approach, bromobutylation of the urethane nitrogens, followed by displacing the halides from the bromobutyl side chains with nucleophilic diethylenetriamines, affords PU with pendant amino groups suitable for diazeniumdiolation. These two classes of PUs are found to release NO fluxes as high as 14-19 pmol.cm⁻².s⁻¹ and are considered to be highly thromboresistant materials for the fabrication of biomedical devices such as catheters, components of hemodialysis units, and vascular grafts. Also, the preparation of C-diazeniumdiolated PAN and other polycrylonitrile-based polymers has been achieved by the reaction of NO with the enediamine tautomer of amidines derived by the treatment of these polymers with base. PAN/NO powder releases NO for more than 80 days under physiological conditions and has been found to inhibit the formation of neointimal hyperplasia in rat carotid arteries injured by balloon angioplasty.

P15. Light Dependent phosphorylation of melanopsin

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Melanopsin is a recently discovered photopigment responsible for light dependent regulation of many non-image forming functions including photoentrainment of circadian rhythms, pupillary constriction, suppression of pineal melatonin synthesis, and direct
photic control of alertness. It is found in the mammalian retina expressed in approximately 1-2% of retinal ganglion cells, which are termed intrinsically photosensitive (ipRGCs). We are attempting to understand the manner in which melanopsin activity is regulated following light activation. G-protein coupled receptors (GPCRs) in general are regulated by a class of serine/threonine kinases called G-protein coupled receptor kinases (GRKs). The common deactivation mechanism of GPCRs consists of phosphorylation of the carboxy tail of the activated receptor followed by binding of a small molecule called arrestin. We have demonstrated the light dependent phosphorylation of melanopsin in a heterologus expression system using an in vitro kinase assay and identified candidate GRKs that may interact with melanopsin endogenously using RT-PCR on single isolated ipRGCs. Using crosslinking and co-immunoprecipitation we have also shown a light dependent physical interaction with some of the kinases in vitro. Functional consequences of phosphorylation have been explored using calcium imaging of the light evoked response of melanopsin expressed in HEK293 cells. Further work is being done to confirm in vivo phosphorylation and demonstrate a direct interaction between candidate kinases and the receptor.

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Gene delivery enables mammalian cell manipulation necessary for disease treatment, tissue development, and comprehension of biochemical functions and cellular response. Currently, non-viral gene delivery is accomplished by bolus delivery of cationic polymer-complexed DNA, which is limited by mass transport and deactivation processes. For tissue engineering applications, incorporation of DNA onto biomaterial scaffolds may avoid these limitations and increase cell transfection by maintaining a high concentration of DNA in the cellular microenvironment. Surface immobilization of DNA also allows spatial targeting of cells. Presently, DNA immobilization to a surface is dependent upon molecular interactions between DNA packaging materials and the surface, and requires careful design to support cellular uptake of the DNA. As an alternative, covalent binding of the DNA to a substrate via a labile peptide sequence would allow surface immobilization and greater control over cellular transfection. Our design focuses on chemical functionalization of plasmid DNA to form the vector-surface covalent bond. This is accomplished using a peptide nucleic acid (PNA) and peptide-based surface tethering system. PNA is a DNA analog that sequence-specifically hybridizes with DNA to form a highly stable conjugate but does not interfere with the DNA transcriptional activity. DNA-PNA-peptide (DPP) conjugates may be directly linked to a variety of
biomaterial surfaces. The use of coupling peptides that include cell adhesive and matrix metalloproteinase-1 (MMP-1) degradable sequences enables release and uptake of the complexes in a cell-responsive manner. Formation of the DPP conjugate was demonstrated by agarose gel electrophoresis and fluorimetry. After formation, the conjugates were attached to self-assembled monolayer-functionalized gold model surfaces. Atomic force microscopy (AFM) was used to validate attachment and to observe the structures of the attached conjugates.

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**P17. Role of Rpl4p in Ribosome Biogenesis and Cell Cycle Progression.**

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Eukaryotic ribosome biogenesis is a highly co-coordinated multi-step process that requires coordinated synthesis of several rRNAs and over 50 ribosomal proteins. Until recently, ribosomal proteins were mainly considered only as structural components of the ribosomes. This view has now changed with increasing evidence indicating consistent alterations in r-protein expression levels during tumorogenesis and in certain developmental defects.

Ribosomal protein L4 is a large ribosomal subunit protein that is structurally conserved in all kingdoms of life. Furthermore, there are several reports of alteration in regulation of L4 mRNA during tissue regeneration, interaction of L4 with transcriptional factors, and varied regulation of L4 mRNA in cancer cell lines. This indicates that L4 has key extra ribosomal functions along with its role in ribosomal biogenesis and function. Here we show that in vivo depletion of Rpl4p in S. cerevisiae results in severe loss of 60S ribosomal subunits. Our Northern blot, primer extension, and uracil pulse-labeling experiments suggest this might be due to a block in the processing of the 27SA3 precursor RNA into 5.8S and 25S rRNA as well as a delay in processing of 35S precursor indicating an important role in maturation of the large subunit of the ribosome. More surprisingly, depletion of Rpl4 and select few large subunit ribosomal proteins results in accumulation of multiple budded phenotype. FACS analysis further confirms accumulation of 3N peak representing the multi budded phenotype. Initial microscopic analysis shows that the Rpl4p depleted cells might be getting arrested in late anaphase / early telophase with micro tubules and chromosomal segregation defects. Further experiments are being done to characterize this phenotype and to elucidate the role of Rpl4 in this particular cell cycle defect.

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**P18. Identification and Characterization of Novel p300 HAT Inhibitors**
p300 is a histone acetyltransferase (HAT) with important roles in chromatin remodeling and transcriptional coactivation. Along with its homolog CBP, p300 is involved in many cellular functions, including cell cycle regulation and differentiation, and has been implicated in many diseases, including heart disease, diabetes, HIV, neurodegenerative disorders, and several forms of cancer. Despite its importance, few inhibitors of p300’s HAT activity have been found to date. A recent X-ray crystal structure of p300 HAT in complex with the bisubstrate analog Lys-CoA served as a template for computational docking analysis. We utilized virtual ligand screening (VLS) to identify novel inhibitors of the p300 HAT domain. After an iterative screening process of the VLS hits using spectrophotometric and radioactive assays, a new p300 HAT inhibitor was identified. This compound was found to be a potent competitive inhibitor of p300 vs. acetyl-CoA and noncompetitive vs. peptide substrate, consistent with prediction. Furthermore, this small molecule p300 HAT inhibitor demonstrated high selectivity versus other acetyltransferases tested. Preliminary analysis with this compound supports its ability to block p300/CBP HAT activity in cell culture. Ongoing studies are directed at characterizing the structural basis of inhibition and the range of cellular actions of this compound and related analogs.

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P19. Expression and Purification of GPCR Peptide Fragments

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G-protein coupled receptors (GPCRs) represent the largest family of integral membrane proteins and transduce extracellular signals across the cellular membrane via heterotrimeric G-proteins. Despite the role of GPCRs in various physiological processes and significant interest by the pharmaceutical industry, our knowledge of this class of proteins is limited by the lack of structural and functional data. In order to obtain the structural data via NMR, we have generated a robust system for the expression and purification of GPCR peptide fragments in *E. coli*. The engineered system utilizes an N-terminal ketosteroid isomerase domain, redundant Strep affinity tags, thrombin cleavage
P20. Examining the Importance of Structure of Oximes on Interaction with Bu

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Currently, organophosphorus nerve agent poisoning is treated using an anticonvulsant, which helps alleviate the main symptom of acute exposure, an oxime, which works to reactivate acetylcholinesterase (AChE) after it has bound and been inhibited by nerve agent, and atropine, which blocks acetylcholine receptors to help alleviate the problem of excess signal transfer. Human butyrylcholinesterase (BuChE), an enzyme very similar to AChE, is the lead candidate bioscavenger enzyme for pretreatment against nerve agent poisoning. This enzyme acts by binding and removing nerve agent from circulation before it can reach critical nerve synapses. However, in much the same way that AChE is inactivated by binding with nerve agent, BuChE is a stoichiometric scavenger that is unable to catalyze nerve agent breakdown. Fielded oximes and those currently under development, which are capable of efficiently reactivating nerve agent-inhibited AChE, are not nearly as effective with BuChE; this result is surprising given the structural similarities between the two enzymes. In an attempt to better understand the structural features of the BuChE active site that are important for oxime binding and reactivation after nerve agent inhibition, the thermodynamic parameters of various oxime : BuChE combinations were examined using isothermal titration calorimetry. Inhibition of butyrylthiocholine hydrolysis, reactivation after nerve agent inhibition, and protection against nerve agent inhibition in vitro were tested using variations of the colorimetric Ellman assay. Significant differences in oxime binding (both enthalpic and entropic) were noted, even though the structures of the oximes tested were in some cases very similar.

P21. A Novel Dendrimeric Reagent for Oligomerization of Hemoglobin

Hongyi Cai, Ramachandra S. Hosmane
Discovering a safe and effective blood alternative for emergency transfusion is an unmet scientific goal. Our lab is involved in developing blood substitutes based on hemoglobin (Hb), the natural oxygen-carrying protein within red blood cells (RBC). There are two major problems using Hb outside the RBC, short retention time in circulation and high oxygen affinity. To solve these problems, we first designed and synthesized an intramolecular Hb cross-linking reagent m-BCCEP. However, since the modified Hb is still relatively small, it suffers from potentially facile filtration through the endothelium lining and reaction with vasorelaxant nitric oxide (NO), thus resulting in elevated blood pressure. Our goal is to increase the size of modified Hb via oligomerization employing a dendrimERIC organic reagent. We report herein the synthesis, properties and reactions of a prototypical dendrimeric reagent (1) that has four reactive aldehydic groups at the terminal positions. The reagent has the potential to effect covalent linkage of two, three or four Hb molecules using these aldehydic groups.

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**P22. Synthesis of transition state analogue inhibitors of guanine deaminase**

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There have been reports of abnormally high levels of serum guanase activity in patients with liver diseases, multiple sclerosis, in cancerous kidney and breast cancer tissue cells. These observations suggest that a potent guanase inhibitor is necessary for exploring the biochemical mechanisms of the above metabolic disorders and to understand the specific physiological role played by guanase, and thereby its potential therapeutic use in treating these disorders. Many studies on guanase inhibition have been reported in the literature, but a potent guanase inhibitor with a submicromolar or nanomolar Kᵢ has yet to be discovered. An examination of the intermediate reveals that it contains a quaternary carbon at position-2, the site of hydrolysis, with geminal amino/hydroxy functionalities. So, a series of analogues of the heterocycle containing imidazo[4,5-e][1,4]diazepine ring structure will be investigated as a potential transition state mimic of the deaminase reaction that contains a quaternary carbon attached to two substituents.

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**P23. Ring-Expanded Nucleotides as Potential Anti-Viral/Anti-Cancer Agents**

Mohsan M. Chaudhry, Ramachandra Hosmane
Cancer and viral infection are the predominant factors for the high mortality rates. A number of ring-expanded nucleosides (RENs) and nucleotides (RENTs) have been reported to be highly active against a wide variety of tumor and viral cell lines. RENs/RENTs are a rich source of inhibitors of the enzymes in purine metabolism, and of those utilizing ATP/GTP either as energy cofactors or as nucleic acid building blocks. Most RENs/RENTs synthesized and screened thus far are planar, aromatic compounds. My project explores the effect of non-planar RENs/RENTs on the biological activity. We hypothesize that the non-planar inhibitors will better mimic the transition states of the relevant enzyme catalyzed reactions and therefore, would act as better inhibitors. My project is aimed at synthesizing the appropriate heterocyclic precursors to the ultimate target RENs/RENTs. The synthesis of the target structure starts with the commercially available 4-Nitroimidazole followed by introduction and hydrolysis of the acetal group to form an aldehyde, reduction of the nitro group to an amino group, followed by ring closure. The final reduction of the imino into an amino group will afford the target ring system.

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P24. Chemically ubiquitinated PCNA as a probe for eukaryotic translesion DN

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Modification of cellular proteins by ubiquitin and ubiquitin-like proteins plays an essential role in a number of biological processes including translesion DNA synthesis (TLS), a recently discovered DNA damage tolerance mechanism. The unique enzymatic activity of a class of specialized DNA polymerases enables the synthesis past the damage on DNA template. Equally important is the regulation of eukaryotic TLS by post-translational modification. Our preliminary results suggest that the eukaryotic TLS is strictly regulated, both temporally and spatially, through the monoubiquitination of proliferating cell nuclear antigen (PCNA). The difficulty of obtaining sufficient amount of native ubiquitinlated proteins has hampered the biochemical characterization of the numerous cellular pathways regulated by ubiquitin modification. We have successfully developed a chemical approach of ubiquitinating an essential eukaryotic protein PCNA. With the chemically ubiquitinlated PCNA as probe we were able to address the effects of position and stoichiometry of ubiquitination upon TLS. We have also applied this method for SUMOylating PCNA. Our method can be readily adapted to study other cellular processes involving post-translational modification by ubiquitin and ubiquitin-like modifier.

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P25. Probing the Substrate Specificity of Quiescin-Sulfhydryl Oxidase

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Disulfide bonds stabilize many products of the secretory pathway and play significant roles in a range of cellular processes. Additionally, disulfide bonds are prominent in a lot of the proteins revealed by the Secreted Protein Discovery Initiative as possible new drug targets. The Quiescin-sulfhydryl oxidase (QSOX) enzyme family directly introduces disulfide bonds into protein substrates of higher eukaryotes and aspects of this catalytic reaction are shared among all organisms. It is therefore remarkable that fundamental questions regarding the physiological roles of this enzyme family remain unstudied. To gain a further understanding of the role of QSOX in vivo, we address whether folded or unfolded substrates are the preferred substrates of QSOX. By examining QSOX’s reactivity with folded and unfolded aldolase and pyruvate kinase we demonstrate that QSOX is unable to react with buried thiols and reacts very slowly with isolated surface-accessible thiols in folded substrates. These in vitro results do not support previous suggestions that QSOX functions to oxidize substantially folded reduced protein substrates at late stages of the secretory apparatus.

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P26. Modeling HIV-1 drug resistance and CNS located virus's contribution

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The Acquired Immunodeficiency Syndrome, cause by the Human Immunodeficiency Virus, is responsible for approximately two million deaths each. An estimated 33 million people are infected, with as many as 2.7 million new infections per year. With the advent of Highly Active Anti-Retroviral Therapy, much progress in the management of the disease has been made; however, the emergence of drug resistant strains of the virus still poses a major challenge. Not all anti-retroviral therapeutics are able to cross the blood-brain barrier effectively. This, coupled with the virus’ efficient crossing of the blood brain barrier, prompted studies to investigate the emergence drug-resistant strains of virus derived from the Central Nervous System (CNS). To this end, computer modeling of the HIV-1 infection using a two compartment model consisting of CNS located virus and systemic virus is being carried out. Additionally, in vitro studies are being conducted to investigate how therapeutic agents’ effectiveness correlates to their concentration and how their effects are added. It is expected that a clear correlation between CNS derived HIV-1 and the origin of drug resistant strains of HIV-1 will be seen.
P27. A Study of the Gluconeogenesis Pathway in FAO Hepatoma Cells

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We investigated gluconeogenesis fluxes in rat hepatoma cells to determine the active reaction pathways. Metabolic fluxes were studied using stable isotope tracers and mass spectrometry. We used a computational program for flux analysis (Metran) in order to determine the best network model for the experimental data. To encourage gluconeogenesis, the cells were starved for twenty-four hours and then fed glucose-free media in the presence of gluconeogenic precursors (lactate, pyruvate, glutamine, and glycerol) and isotopic tracers ([U-13C]-glycerol and [2H8]-glycerol). Media samples were collected after fourteen hours and the glucose mass isotopomer distributions were determined by gas chromatography-mass spectrometry (GC-MS). Several network models were analyzed in Metran to estimate the pathway fluxes. The simplest model involved only gluconeogenesis reactions and resulted in a poor fit of the experimental data. Each subsequent model increased in complexity incorporating additional reaction pathways. The most complex model accounted for both the non-oxidative and oxidative pentose phosphate pathway. Metran analysis indicated that the non-oxidative pentose phosphate pathway is active in the hepatoma cells. Both [U-13C]-glycerol and [2H8]-glycerol tracers independently verified these results. Pentose phosphate pathway activity during gluconeogenesis is not observed in primary rat liver cells.

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P28. A Novel Reagent for Synthesizing Hemoglobin Dendrimers

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UMBC, UMBC

The discovery of a suitable alternative to whole blood for emergency transfusions is an unmet scientific goal. Limitations on storage stability of whole blood, as well as the necessity for typing and cross-matching, and the fear of possible transmission of pathogens, reinforce the need to develop a safe and effective blood substitute. Hemoglobin is the oxygen-carrying protein found within red blood cells (RBC), and almost all blood substitutes in clinical trials are based on cell-free hemoglobins that have been chemically modified to enable function outside the RBCs. Without proper
modification, the cell-free hemoglobins will not stay in circulation for more than a few hours and not enough oxygen is delivered to tissues. Intramolecular cross-linking remedies this problem. Even after such modifications, facile filtration through endothelial lining of blood vessels remains a problem. Reaction here with the natural vasorelaxant nitric oxide (NO), results in elevated blood pressure. It is anticipated that increased molecular weight by intermolecular cross-linking via a novel organic linker will prevent NO scavenging and will also increase circulation time. The current status of our work toward this goal will be presented.

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P29. The effect of point mutations of SHP-2 on its structural properties

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Non-receptor protein tyrosine phosphatase SHP-2, contains two tandem SH2 domains at the N-terminus and a PTP domain at the C-terminus. Its catalytic activity can be self-inhibited by the protein-protein interaction of the N-terminal SH2 domain with the PTP domain. Mutations in SHP-2 phosphatase cause hyperactivation of its catalytic activity. These mutations have been identified in various pediatric leukemias and Noonan syndrome. We have conducted explicit-solvent molecular dynamics (MD) simulations to better understand structural and dynamic properties that contribute to the biochemical activity of the wild type SHP-2 protein and its three mutated counterparts (D61G, E76K, and N308D). We also performed Steered molecular dynamics (SMD) simulations to pull the SH2 domain away from the PTP domain. This enabled us to calculate the free energy profile or potential of mean force (PMF) along the distance between the two domain, as well as the free energy of solvation and solvent accessibility and asses the differential properties of all four systems.

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P30. Long Range Interaction between U5 and AUG Region May Regulate the Pack

Sai Sachin Divakaruni, Atheeth Hiremath, Xiao Heng, Michael Summers

University of Maryland Baltimore County

The Human Immunodeficiency Virus (HIV) is a retrovirus that can develop into Acquired Immunodeficiency Syndrome (AIDS), a deadly epidemic that is rapidly spreading among the human population across the globe. Our lab's overall interest is knowing how the virus selectively assembles and packages its unspliced RNA genome against the host cellular RNA pool. Genetic and biological studies suggest that the highly
conserved 5’ untranslated region (5’UTR) serves as a cis-acting packaging signal interacting with the nucleocapsid protein (NC) to mediate genome recognition and encapsidation. The specific structure of the 5’UTR is believed to play a key role during genome packaging. Multiple models have been proposed for residues that overlap with the gag start codon AUG. Recent evidence from our lab indicates that the AUG region may sit in an equilibrium between forming a long range interaction with upstream U5 and itself form a local stemloop (historically called SL4). This equilibrium may alter the entire structure of the 5’UTR and play an important role in genome packaging. We did mutagenesis work in the AUG region, which stabilizes the stemloop structure and therefore inhibits the long range interaction. We are currently in the process of continuing experimentation, including testing this point mutation's effects on long range interaction inhibition, and investigating its interaction with NC protein.

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**P31. Long-Range Interaction in the 5’-UTR of the HIV-1 Genome**

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The replication and infectivity of human immunodeficiency virus type-1 (HIV-1) is dependent on the folding of its RNA genome; however, its structure is not fully understood. Retrovirus genome packaging is mainly regulated by its highly conserved 5’-untranslated region (5’-UTR). This region consists of critical parts such as trans-acting responsive element (TAR), U5 linker, primer-binding site (PBS), and several stem loops (SL1-SL4).

Previous studies through computer-assisted RNA structure prediction, biochemical analyses, and a phylogenetic survey of different virus isolates suggested that SL4 may form a long-range interaction with the U5 linker region. The aim of our project was to determine if SL4 forms such a long-distance interaction with the U5 linker region in the context of the full length 5’-UTR. For that purpose, we generated three mutations in the U5 linker region and synthesized $^{13}$C labeled SL4. The unlabeled 5’-UTR (TAR-SL3, no SL4 region) was titrated into the labeled SL4 and the Nuclear Magnetic Resonance (NMR) Heteronuclear Multiple Quantum Coherence (HMQC) spectra were compared. The absence of a C5-H5 peak in some of the samples, which had previously appeared in the segmentally labeled TAR-SL4, supports that there is a long-range interaction between SL4 and U5 linker region. We are currently investigating the structure of this SL4-U5 linker complex. Our further analysis will demonstrate how mutations in the 5’-UTR will affect HIV-1 genome packaging mechanism.

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P32. Small Molecules to Inhibit Mycobacterium Tuberculosis Dxr

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Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is one of the deadliest infectious diseases, infecting more than 8 million and killing approximately 2 million people each year. About one-third of the world’s population is latently infected with the organism. The emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) has made this disease both difficult and expensive to treat. New TB therapies are needed that will shorten the treatment period and combat both latent and drug-resistant Mtb. A potential target for new drug development is the nonmevalonate pathway of isoprenoid synthesis. 1-Deoxy-D-xylulose-5-phosphate reducto-isomerase (Dxr) is the first committed step in this pathway. Inhibition of Dxr successfully kills other organisms relying on this pathway, such as Plasmodium falciparum, the causative agent of malaria. Fosmidomycin, a natural product, inhibits Dxr but is not effective at killing Mtb. This is likely due to lack of penetration of the Mtb cell wall. The goal of this work is two-fold: to synthesize lipophilic analogs of fosmidomycin in an effort to increase Mtb cell permeability and validate the Dxr pathway as a means of killing Mtb, and to optimize binding of fosmidomycin analogs to the Mtb Dxr active site. The activity of our compounds will be evaluated by measuring Dxr inhibition and inhibition of Mtb cell growth.

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P33. Dynamic culture of human fibroblasts for vocal fold tissue engineering

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The human vocal fold (VF) lamina propria (LP) is a mechanically active tissue. Under normal conditions, it can sustain up to 30% strain at frequencies of 75-1000 Hz. Excessive mechanical stress and deleterious environmental factors can damage vocal fold LP, leading to altered biomechanical properties and biological functions. Although various techniques are available to treat vocal fold disorders, none produce satisfactory
outcomes. We are interested in developing tissue engineering methodologies for the reconstruction of functional vocal fold lamina propria. To this end, we have manufactured and characterized a bioreactor capable of generating vibrational stimuli at human phonation frequencies with varying strain to the cultured cells. In our initial studies, human foreskin fibroblasts were seeded on a collagen I-coated silicone membrane and were subjected to different vibrational stimulation patterns. The cell proliferation, cytoskeleton organization, gene expression and ECM production were systematically analyzed. Our current results show that vibrations at 300 Hz upregulate the gene expression of elastin, MMP13 and TIMP1, whereas vibrations at lower frequencies such as 60 and 140 Hz downregulate the expression of those same genes. Results obtained from this study will help establish the optimum dynamic culture conditions that will trigger the cells into producing vocal fold LP-specific ECM. Our ultimate goal is to engineer a functional VF LP in vitro by 3D dynamic culture of VFF in a biomimetic hydrogel matrix.

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P34. PAED FOLLOWING HPLC-UV FOR RDX AND RDX DEGRADATION PRODUCTS

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Nitramine explosives present significant health and ecological hazards due to their toxic and mutagenic nature. Their persistence to remain in the environment has resulted in substantially contaminating the environment and its surroundings. Their exposure to the environment can be characterized by several pathways including the manufacturing, testing and disposal of ammunitions and explosives.

Trace analysis of explosive contaminants in a variety of matrices has become significantly important for testing bioremediation strategies, biological assays as well as assessing environmental contamination. Royal Demolition Explosive (RDX) is one of few, abundantly used nitramine compounds involved in the production of military explosives. The biotransformation of RDX in the environment results in degradation products that are considered to be dangerous and potentially carcinogenic due to their nitroso components.

A reversed-phase HPLC method is applied for the separation of RDX and RDX degradation products. Photo-assisted electrochemical detection (PAED) following HPLC-UV results in a sensitive, selective method of detection that offers additional selectivity due to a dual detector system setup. PAED is sensitive for the detection of
nitramine explosives which lack rich, UV chromophores resulting in poor signal for absorbance detection.

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**P35. Exploring Non-Mammalian Isoprenoid Biosynthesis: 1-Deoxy-D-Xylulose 5-phosphate**

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Many human pathogens rely upon the methylerthritol phosphate (MEP) pathway for biogenesis of the essential isoprenoid bioprecursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Our long term goal is to understand catalysis of these intriguing biosynthetic enzymes toward the development of new anti-infective agents. 1-Deoxy-D-xylulose 5-phosphate (DXP) synthase is a novel transketolase-like enzyme that catalyzes the first step in the MEP pathway to form DXP from pyruvate and glyceraldehyde 3-phosphate (GAP). Its unique structure distinguishes DXP synthase from its homologs, transketolase and pyruvate decarboxylase, making it a particularly interesting enzyme. We have developed a robust, flexible assay to probe DXP synthase catalysis. We have used this assay to measure DXP synthase kinetic parameters, which are in close agreement with reported values. Moreover, we have initiated substrate specificity studies toward gaining a better understanding of the catalytic potential of this enzyme.

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**P36. Design of Protein Kinase-Inducible Domains as General Sensors**

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Proteins phosphorylation plays central roles in eukaryotic cellular signaling transductions. Misregulation of Kinases and phosphatases is tightly associated with many human diseases, including cancer and heart diseases. Developing new probes is important to understand these complex cellular networks. We are using protein design to develop expressible protein motifs which are switchable from non-fluorescent to fluorescent states upon phosphorylation. Kinase-inducible domain peptides were developed for a series of more than 10 kinases important in cellular signaling, demonstrating the generality of the approach. Kinase-inducible domain peptides have been employed for the detection of kinase activity, phosphatase activity, and kinase inhibitors, in solution and in cell extracts.
We employ an EF hand protein motif, containing a loop and the first turn of the connecting a-helix as our starting point of design. The key design element uses phosphoserine, phosphothreonine or phosphotyrosine as an inducible mimic of a structurally important glutamate residue in the EF hand. Due to the similar electronics and ionic radii of calcium and lanthanides, lanthanides coordinate to the peptides, thereby yielding EF hands with luminescent properties. Fluorescence emission spectra revealed that the nonphosphorylated peptides bound Tb$^{3+}$ poorly, displaying weak terbium luminescence. In contrast, phosphorylated peptides bound Tb$^{3+}$ well and displayed strong luminescence. $^1$H-$^13$C HSQC spectra of phosphorylated pKID-optErk in the absence and presence of La$^{3+}$ indicate that the metal introduces significant changes in chemical shifts, consistent with peptide-metal complex formation.

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**P37. Targeting DNA Ligase IV for degradation by two Adenoviral proteins**

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DNA frequently acquires double stranded breaks, and is subsequently repaired through either non-homologous end joining (NHEJ) or homologous recombination (HR), depending on position in the cell cycle. The final step of NHEJ is ligation of the two DNA ends by a complex comprised of DNA Ligase IV (LigIV), XRCC4, and XLF. Upon infection of a cell by Adenovirus type 5 (Ad5), this last step is inhibited due to the degradation of DNA LigIV. Two Ad5 proteins, E4orf6 and E1B55k, hijack the Cullin5 E3 Ubiquitin ligase complex to target LigIV, as well as p53 and Mre11, for proteasome mediated degradation. It is unknown how E4orf6 and E1B55k specifically target these proteins for degradation; therefore, the focus of this work is to identify the regions of LigIV required for E4orf6/E1B55k-dependent degradation. Through dual infection with Ad5 and transfection with truncated and mutated LigIV, the minimal recognition domain has been narrowed down to the region containing amino acids 600 to 743, which includes one of two BRCT domains in LigIV. Results from co-immunoprecipitations also suggest that XRCC4, a partner protein essential for Ligase IV stability, is not required for targeting. Viruses tend to hijack or mimic host cellular processes, so understanding how Ad5 regulates NHEJ can shed light on the poorly understood area of NHEJ regulation. People with genetic defects in NHEJ are predisposed to cancer. An understanding of the regulation of NHEJ may therefore reveal strategies for reducing cancer incidence.

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**P38. Hybrid Elastin Mimetic Copolymers for Vocal Fold Tissue Engineering**
Biocompatible elastomers with tunable morphological, biological, and mechanical properties are needed for use as scaffolds for the repair and regeneration of soft, mechanically active tissues such as vocal folds. Elastin mimetic hybrid polymers (EMHPs) with alternating molecular architecture have been synthesized for this purpose. The structure of the EMHPs mimics that of natural elastin, containing cross-linking domains of a peptide taken from the amino acid sequence of natural elastin in alternation with flexible, synthetic polymer domains that provide elastic recoil and tunability. The EMHPs were cross-linked to form hydrogels with a compressive modulus of 0.12 MPa when hydrated, and the ability to absorb up to five times their weight in water. Porcine vocal fold fibroblasts (PVFFs) were also shown to grow and proliferate normally in the presence of the cross-linked EMHPs. The effect of replacing varying amounts of the peptide domain with an arginine-glycine-aspartic acid (RGD)-containing peptide in order to promote cell attachment on the EMHPs is currently being investigated, along with the tunability in mechanical properties that can be achieved by varying the molecular weight of the polymer and peptide domains.

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P39. Biochemical effects of DOB, an abasic lesion from DNA

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1,4-Dioxo-2-phosphorylbutane (DOB) is produced in DNA as a result of cleavage of the C4'-C5' carbon-carbon bond following hydrogen atom abstraction from the C5'-position. Using ternary complexes in which DOB is produced from a synthetic precursor, we show that the lesion produces interstrand and intrastrand cross-links. The yields of these products are dependent upon the surrounding nucleotide sequence. Kinetic studies indicate that in the ternary complex, DOB produces cross-links and undergoes β-elimination to give 1,4-dioxobutene. 1,4-Dioxo-but-2-ene alkylates the DNA.

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P40. Evaluation of delayed treatment with novel nerve agent anticonvulsants

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Nerve agents are potential threats for use in terrorist attacks. Administration of standard antidote drugs (atropine, pralidoxime (2-PAM), diazepam) to civilian casualties in a terrorist attack are likely to be substantially delayed by the confusion and possible high number casualties a terrorist attack could generate. This study developed a delayed nerve agent treatment model and evaluated standard treatments plus novel anticonvulsant adjuncts. Rats were surgically prepared with electroencephalographic (EEG) recording electrodes and a microdialysis guide cannulae directed at the basolateral amygdala (BLA) or the piriform cortex (PC). A week later the animals were challenged with the nerve agent soman (180 μg/kg, SC). EEG was monitored for seizure onset; 20 min after seizure onset the animals received standard medical countermeasures: 0.45 mg/kg atropine sulfate, 25 mg/kg 2-PAM and 2.0 mg/kg diazepam either alone or with an adjunctive drug. The animals were monitored for 5 hr after exposure. Microdialysis samples were collected at 15-min intervals at a flow rate of 1.5 μl/min during this time. At 24 hr the animals were anesthetized (75 mg/kg, IP, pentobarbital) and then perfused with saline followed by 10% formalin. The brains were subjected to histological procedures to determine guide cannula placement and evidence of neuropathology. In the absence of an adjunct drug, all animals continued to display EEG seizure activity following standard medical countermeasures, 12 of 19 (63%) were still seizing at 24 hr and all animals displayed profound brain pathology. Adjunctive treatment with the anti-cholinergic procyclidine (5.6 mg/kg) to standard medical countermeasures stopped seizure activity 10.8 (± 0.2) min after administration in 14 of 22 (64%) animals and had additional effects on 24 hr body weight and seizure activity. Animals receiving procyclidine lost 37 g of body weight compared to 44 g in animals not receiving adjunct, and only 6 of 22 (27%) were still seizing at 24 hr. Adjunct treatment with procyclidine appears to have a favorable effect under delayed treatment conditions.

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P41. Small Molecule Binding to Intrinsically Disordered Proteins: Multiple

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We have found that the intrinsically disordered (ID) proteins c-Myc and Id2 contain multiple discrete sites that selectively and independently bind to small molecules. These sites are composed of short contiguous stretches of amino acids and are found in regions predicted to have low disorder probability by the PONDR VSL algorithm. So far, we have identified four such sites in the 85 amino acid bHLHZip domain of the oncprotein c-Myc and three sites on the 41 amino acid HLH domain of the Id2 protein. These proteins are disordered in their monomeric state and only upon dimerization with a partner protein does a stable tertiary structure form. The small molecule inhibitors bind to the ID monomeric proteins, affecting their structure at a local level only, preserving the
overall disorder and preventing dimerization from taking place. Previously we identified three binding sites on Myc for seven selective, structurally diverse small molecule inhibitors; here we identify an additional site on Myc and three binding sites on Id2.

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**P42. Characterization of the metal-dependent deacetylase MshB**

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Mycothiol is the primary reducing agent used by mycobacteria to prevent against oxidative damage. Consequently, enzymes involved in mycothiol biosynthesis are targets for antibiotic development. MshB is a metal-dependent deacetylase that catalyzes the hydrolysis of GlcNAc-Ins to form GlcN-Ins and acetate, a key step in the biosynthesis of mycothiol. We are working towards the biochemical characterization of recombinant MshB from *M. smegmatis* and *M. tuberculosis*. MsMshB has been expressed in *E. coli* and purified using Co-IMAC. Following removal of the His tag, purified MsMshB contains ≥ 1 eq. Co²⁺. Procedures for preparation of apo-MshB and subsequent reconstitution of apo-MshB with divalent metal ions (stoichiometric) have been developed. We are currently expressing MsMshB as a ZZ-tag fusion protein, which will allow for rapid purification of MshB without the introduction of metal ions. Results from these experiments will be used in conjunction with kinetics experiments to identify cofactor preferences of MshB.

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**P43. Pharmacokinetic Parameters and Anti-Nerve Agent Protective Efficacy of**

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Butyrylcholinesterase (BuChE) is the lead candidate bioscavenger enzyme being evaluated for capacity to protect against nerve agent intoxication. To be economically viable, large scale production of this enzyme is required to produce the quantities needed for DOD and civilian personnel. Currently, the most reproducible large-scale source of BuChE is obtained using GMP purification from outdated human blood plasma (Baxter BioSciences). We compared plasma-derived BuChE (pBuChE) to a recombinant version
of the same enzyme (rBuChE) produced by and secreted into the milk of transgenic goats (Pharmathene, Inc.). Both types of BuChE were injected i.m. into guinea pigs to determine \textit{in vivo} retention times. While both BuChE versions reached maximal concentration ($C_{\text{max}}$) in the blood at approximately the same time (24-28 hours), they had dramatically different pharmacokinetic profiles. pBuChE was characterized by a rapid distribution phase (loss of ~ 80 % of the maximal enzyme concentration within 48 hours) and a slow elimination phase (half-life of ~115 hours), while rBuChE had a single phase elimination (half-life of ~72 hours). The median lethal dose (LD$_{50}$) for the nerve agent Russian VX was determined at $t_{\text{max}}$, $t_{1/2}$, and two times $t_{1/2}$ for each BuChE. These data were compared with the pharmacokinetic profiles to determine if a correlation exists between circulatory levels of BuChE and observed efficacy against nerve agent poisoning. For pBuChE, animals were more protected than would have been predicted by observed blood pBuChE levels. The protection afforded by rBuChE correlated well with the detectable blood levels of BuChE.

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P44. Active Site Directed Covalent Modification of Glyoxalase I

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The role of the glyoxalase system in the detoxification of alpha-ketoaldehydes has made it an interesting system for the development of chemotherapeutic agents. It has been shown that inhibitors of glyoxalase I, the first enzyme in the pathway, have the ability to inhibit murine tumor cell growth \textit{in vivo}. Tumor toxicity is attributed to an increase in intracellular concentrations of methylglyoxal, which has the ability to form DNA adducts and prevent DNA synthesis. A new class of compound is presented here, which has the ability to covalently modify the active site of human glyoxalase I (hGlxI). These compounds were predicted to be complete irreversible inhibitors of hGlxI, but the current studies show that irreversible inhibition is limited and the enzyme is able to maintain a fraction of its original catalytic activity. The data presented suggest that it is possible that these compounds covalently modify only one active site of hGlxI and that the other active site maintains a fraction of its catalytic activity. Mass spectrometry experiments show that Cys60 is the site of covalent modification. These compounds are the first reported to have the ability to covalently modify a residue in the active site of hGlxI. Although these molecules are not complete irreversible inhibitors of hGlxI, they could possibly serve as templates for the development of new compounds that may have improved potency.

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P45. Improved Synthesis of V-PROLI/NO and Related Nitric Oxide Prodrugs
The liver-selective nitric oxide (NO) prodrug $O^2$-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO)$^1$ has been extensively studied and was recently shown to reduce arsenic-induced hepatocyte toxicity and apoptosis,$^2$ presumably through the effects of NO. Reduced arsenite hepatotoxicity could mitigate possible complications in cancer chemotherapy and thus use of V-PYRRO/NO may augment the use of arsenic-based chemotherapeutic agents at increased dosage levels. Synthesis of V-PYRRO/NO has been cumbersome with an overall yield of 4% in two steps and has hindered the facile production of a library of its structural analogues. We have developed a method that improved the efficiency of V-PYRRO/NO synthesis in two steps with an overall yield of 40%. This protocol employed Verkade’s Superbase$^3$ (2,8,9-trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane) as a base for the elimination reaction instead of the hydroxide-induced method that was previously used. This dehydrohalogenation method was also applied to the preparation of various V-PYRRO/NO analogues in moderate to good yields (60-81% for the elimination step and 23%-52% overall yields). Recently, we also reported the synthesis of an important V-PYRRO/NO analogue, $O^2$-vinyl 1-[2-(carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (V-PROLI/NO)$^4$. The possible advantage of using V-PROLI/NO over V-PYRRO/NO is that one of its potential byproducts of decomposition, N-nitrosoproline, is a naturally occurring metabolite suggesting that V-PROLI/NO may have a favorable toxicological profile. Using the modified synthetic protocol, we report an improved synthesis of V-PROLI/NO. This protocol will be applied to the preparation of several analogues of V-PROLI/NO that may improve its bioavailability.

Key References:

P46. Hyaluronic Acid-Based Hydrogel Particles (HGP) and Doubly Crosslinked Networks (DXN) with Defined Biological Functions

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We have developed hyaluronic acid (HA)-based hydrogel particles (HGP) and doubly crosslinked networks (DXN) with defined biological functions for use in soft tissue regeneration. HA HGPs were synthesized by in situ crosslinking of HA derivatives carrying complementary functional groups (hydrazide: HAADH and aldehyde: HAALD) within the inverse emulsion droplets. Doubly crosslinked networks were obtained by simple mixing of HGP with HAADH. The visoelasticity of the resulting DXN can be systematically varied by controlling intraparticle and interparticle crosslinking. To render these particles biologically active, Perlecan Domain I (PlnDI) proteoglycan was immobilized to the particles via reductive amination using poly(ethylene glycol) (PEG) as a linker. The conjugated PlnDI can bind to bone morphogenetic protein (BMP-2) specifically. PlnDI conjugated HA HGP showed sustained release of BMP-2 over 15 days. The released BMP-2 was used to stimulate the chondrogenesis of mesenchymal stem cells (MSC). These novel HA-based HGPs and their DXNs are promising materials for soft tissue engineering.

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P47. Assessing Ion Transport Energetics Using Novel Molecular Models

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Molecular modeling is routinely applied today to study a broad range of systems and processes. The underlying potential models, or force fields, introduce the necessary physics of interactions between individual particles within the many-body systems. Electrostatic interactions are treated in a pair-wise Coulomb fashion with interacting sites assigned a fixed charge derived via quantum mechanics. The validity of this approach breaks down, however, for processes where polarization allows for response of the molecular charge distribution to changes in local environment (electric fields). From a physiological perspective, accurate theoretical descriptions of molecular recognition processes such as protein-ligand binding and ion permeation energetics through narrow biological ion channels require an accounting of such effects.
P48. Two Faces of Antiproliferative Factor from IC Patients

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Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS) is a chronic bladder disorder characterized by voiding urge and severe pain caused by thinning and ulceration of the bladder wall resulting in exposure of sensory nerve cells to urinary constituents. To date there is no cure for IC/PBS even though the first written description of a bladder syndrome with symptoms characteristic for IC/PBS was made about 200 years ago. About ten years ago it was shown that urine specimens from approximately 95% of IC/PBS patients contained an antiproliferative factor (APF) that induces many of the same abnormalities in normal human bladder epithelial cells as seen in IC/PBS cells in vitro, including decreased proliferation, increased paracellular permeability, and altered specific gene expression, and which therefore could be a cause of IC/PBS. APF was identified as a sialylated glycononapeptide – Neu5Aca2-3Galb1-3GalNAca-O-TVPAAVVVA. Further studies revealed APF is also able to inhibit proliferation of cancer lines at low nanomolar concentrations. Thus APF is a new and unique lead in anticancer research.

To understand the structural requirements for APF activity we first performed structure-activity relationship studies (SAR) on peptide and carbohydrate segments of APF. To date about 60 derivatives have been synthesized and tested for antiproliferative activity on normal bladder epithelial cells and various cancer cell lines. This presentation will show the recent progress in our work.

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P49. The Mechanism of Action of JS-K, a Nitric Oxide Releasing Agent, in a

Monika Z. Kaczmarek¹, Jami Troxler¹, Joseph Saavedra², Joan Cmarik¹, Larry Keefer³, and Sandra Ruscetti¹
JS-K is a potent anti-cancer prodrug that belongs to the arylated diazeniumdiolate family of compounds, agents that are designed to release nitric oxide (NO) on reaction with glutathione. Previous studies have shown that JS-K is active against human leukemia, multiple myeloma, prostate and liver cancer cell lines as well as xenografts of some of these lines in mice, while having lesser toxicity towards normal cells. In this study, we tested JS-K against murine erythroleukemia (MEL) cell lines from mice infected with the Friend spleen focus-forming virus. These cells grow as tumors when injected subcutaneously into mice and cause meningeal leukemia when injected intraveneously and provide a convenient small animal model to study the effects of anti-cancer agents. Our in vitro studies indicate that JS-K inhibits proliferation of MEL cells with a low IC50 value, and NO appears to be the major component of JS-K’s cytotoxic activity since its metabolites and a derivative unable to release NO failed to block proliferation of the MEL cells. JS-K induces necrosis and an early-stage apoptosis by a caspase-dependent mechanism (caspases 9 and 3 activation). We also observed in JS-K -treated MEL cells a time-dependent increase of FoxO3a, which plays an important role in apoptosis. The increase of FoxO3a levels may also explain the G1-S cell cycle arrest observed in JS-K treated cells since FoxO3a is responsible for activation of the cyclin-dependent kinase inhibitor p21. Studies are in progress to determine if JS-K can block the growth of MEL cells in vivo.

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P50. Screening of Free living diazotroph - Azotobacter from Tondi ecosystem

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Tondi coastal ecosystem contains rich of vegetations like seaweeds and seagrasses; this is due to its shallow nature of the coastal ecosystem. Totally 568 Azotobacter strains were isolated from Tondi coastal water and Tondi coastal sediments during the year 2004 and 2005. Among them 360 strains were from coastal sediments (198 from the rhizosphere sediment and 162 from the non-rhizosphere sediment) and 208 from coastal water. In water samples, the population density of Azotobacter ranged between 3 x 10² and 6.9 x 10³ CFU ml⁻¹ during 2004 and 2005. In sediment samples, the Azotobacter population density was observed to vary from 3 x 10² to 10.7 x 10³ CFU g⁻¹. Screening of the strains was done and it was revealed that, there was a significant good growth at 3 % (89.1 %) of Glucose concentration. Likewise, a remarkable growth was recorded at pH 8.0 (88.9 %) and temperature 28 °C (82.9 %). Based on the colony morphology 6 morphology different
isolates were observed and based on the colour of the colony 4 different colour colonies were observed from the samples. It was also observed that the Tondi coastal sediment harbors more Azotobacter population than Tondi coastal water sample.

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**P51. Microstructure and phase behavior of protein-detergent complexes**

Kelley Kearns, Daniel S. Miller, Abraham M. Lenhoff, Eric W. Kaler

University of Delaware, University of Delaware, University of Delaware, Stony Brook University

The paucity of deposited structures of membrane proteins is due in part to the challenges of making these proteins in sufficiently large quantities, but several additional contributing factors exist as well. The underlying mechanism for the stabilizing role of surfactant in protein-detergent complexes (PDCs) is not well understood, and the interactions leading to crystallization are complicated by the addition of surfactant and precipitants. Several studies suggest that detergent interactions and phase behavior play a major role in crystallization.

Over 80 crystal structures are reported for photosynthetic reaction center in the Protein Data Bank, most of which were obtained using lauryldimethylamine oxide as the solubilizing detergent along with the additive, 1,2,3-heptanetriol. Microbatch phase behavior experiments were employed in order to observe the solution conditions that are achieved during vapor diffusion and to study the effect of each component on the crystallizing solution. In the absence of protein, small amounts of a liquid crystalline (LC) phase are observed at the same solution conditions in which protein crystals are found. Small angle x-ray scattering is used to track changes in microstructure of PDCs and LC phases. Protein-protein interactions are measured via self-interaction chromatography in order to compare different crystallization conditions reported in the literature.

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**P52. Paraoxonase 1 -- Unraveling the Peroxidase Myth**

Akhil Khanal, Brian Bahnson

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Paraoxonase-1 (PON1) is a 40 kDa HDL-associated multi-functional glycoprotein. It has been shown to be anti-atherogenic due to its ability to protect lipoproteins against oxidative modification. Several studies implicate PON1’s peroxidase-like activity as the enzyme’s property that is responsible for reducing phospholipid hydroperoxides and thus
inhibiting oxidation of lipoproteins. In this study, we provide evidence that PON1 is not a peroxidase. Instead, the peroxidase-like activity reported for the enzyme in the literature can be attributed to an "impurity", probably an enzyme, present in human serum and co-purifying with PON1.

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P53. An NMR Protocol for the High-Resolution Structure Determination of Lar

Benyam Z. Kinde, Blanton Tolbert, Yasu Miyazaki and Michael F. Summers

University of Maryland, Baltimore County

One hallmark of all retroviruses is that each virion contains a dimeric genome non-covalently linked at their 5'-end. A smaller fragment (~ 200 nucleotides) encompassing a sub-set of the stem loops has been shown to direct packaging of heterologous RNAs into virion-like particles and is referred to as the CES. We propose that applying a recently developed NMR technique will mitigate the technical challenges associated with NMR structural analysis of large RNAs. A series of NOESY (distance-only) and TROSY based HSQC spectra (global restraints) were recorded for each RNA construct tested. Quantitative analysis of the resulting spectra allowed the extraction of a healthy set of distance-only and two of the global restraints, RDCs and RCSAs. Molecular Dynamics calculations were then performed in three different stages to assess the improvement in the quality of refined structures: a) distance-only b) distance-only + RDC and c) distance-only + RDC + RCSA restraints. Our results demonstrate the improvement gained by incorporating the RDC and RCSA restraints in RNA structure refinement compared to just using distance-only restraints. In particular, we observed a difference in helix orientation for helix one of SLC320-345 when the RDC + RCSA restraints were included.

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P54. Optimization of multilayer surface enhanced Raman scattering (SERS) imaging

Charles K. Klutse, Honggang Li, Brian M. Cullum

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Dynamic intracellular analysis has important applications in areas like biomedical research, defense and security and many others. Although, there are several methods for intracellular analysis, surface enhanced Raman scattering (SERS) is becoming a preferred transduction method for such applications, due to its narrow spectral bandwidth, large SERS enhancement factors and high sensitivity. In our laboratory, SERS-based immuno-
nanosensors are being developed and optimized for real-time, dynamic, and multiplexed analysis of molecular interaction within individual living cells.

These nanosensors are fabricated by drop coating silica nanospheres onto a microscope slide. A film of SERS active metal is deposited on the nanospheres to form metal film over nanospheres (MFON), which are then removed from the slide by mechanical processes. The MFONs are functionalized with antibodies that target specific proteins under investigation. Radiation induced cell perturbation is minimized by the use of a HeNe laser for excitation at 632.8nm. To improve SERS enhancement, different types of metal deposited substrates have been studied with multilayer-MFON (MULTI-FON) substrates demonstrating ideal enhancement.

This talk will evaluate the SERS enhancement of MULTI-FONs with self-assembled monolayers (SAMs) spacers sandwiched between layers of the metal film. Monolayers with different chain lengths and tail groups are used as spacers in order to evaluate the effect of spacer length and chain functionalities on the SERS enhancement. This talk will also discuss functionalization of the SAM MULTI-FON with antibodies to develop immuno-nanosensors. The potential use of these nanosensors for intracellular analysis will be assessed in terms of sensitivity, long-term stability, biocompatibility, reproducibility and signal-to-noise ratio.

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**P55. Disproving a Long-Range Interaction within the HIV-1 RNA Genome**

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The 5´-untranslated region (5´-UTR) of the human immunodeficiency virus type 1 (HIV-1) RNA genome is responsible for many processes that are critical to HIV-1 replication, such as genomic packaging. These elements that are essential to viral replication are dependent on the structure and folding of the RNA genome. A previous study using chemical probing techniques proposed that a long-range interaction may exist between a region of Stemloop 4 (SL4) and both the U5 linker region and a part of the Primer Binding Site (PBS) within the 5´-UTR. Here, we used a novel Nuclear Magnetic Resonance (NMR) spectroscopy approach of examining this long-range interaction within the context of the whole 5´-UTR. To investigate the proposed interaction between the PBS and SL4, we mutated the PBS in a TAR-SL3 fragment so that it lost the ability to base pair with SL4. Both the TAR-SL3 fragment with the mutated PBS and the fragment with the intact PBS were then titrated into labeled SL4. A comparative analysis of the NMR Heteronuclear Multiple Quantum Coherence (HMQC) spectra revealed that the Cytosine H5 peak, which indicates an interaction, was observed in the spectra of both samples. Because the mutation did not affect the spectra, we conclude that there is no interaction between the PBS and SL4. Additionally, our conclusion implies that SL4 most likely interacts with the U5 linker region. Understanding the long-range interaction
in the context of the large RNA will be critical for unveiling the HIV-1 virus genome packaging mechanism.

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P56. From biochemical and structural studies of soluble guanylate cyclase t

Emmanuelle LAFFLY, Jane Macdonald, Elsa Garcin

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The heterodimeric haemoprotein soluble guanylate cyclase (sGC) is the direct sensor and mediator of nitric oxide (NO) signal transduction via the NO-sGC-cGMP pathway. cGMP acts as a second messenger to modulate the activity of kinases, cGMP-gated ion channels and cGMP regulated phosphodiesterases, and leads to vasorelaxation. Aberrant sGC-dependent signalling may be fundamental to the aetiology of a wide variety of cardiovascular pathologies. As a consequence, compounds that activate cGMP production by sGC have a considerable therapeutic potential. Understanding the structural basis for the mechanism of sGC assembly and regulation should facilitate the design of these therapeutic agents. To achieve this goal, I developed a heterologous expression system of full-length bovine sGC (Fl-sGC). Early attempts to produce recombinant bovine sGC in E. coli resulted in misfolded protein accumulation. Indeed, producing soluble protein in Escherichia coli is still a major difficulty in the sGC field. By using a trx-tag (thioredoxin, 109aa) fused to the sGC b subunit, I successfully overexpressed both a and b subunits in a soluble heme-bound native form. Optimization of expression levels by varying bacterial growth conditions including temperature, media, additives and induction, will be followed by purification and characterization of Fl-sGC. So, a crucial step has been achieved, allowing us to pursue structural studies to probe the structure and mechanism of sGC and promote the discovery of stimulators of this physiologically important enzyme.

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P57. Aptamers with Polypurine Tract-Like Sequences as Potent HIV Inhibitors

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Aptamers are synthetic, single stranded nucleic acids with unique three-dimensional structures, allowing the folded sequence to bind specifically to the target protein. Our lab has recently used a unique SELEX (Systematic Evolution of Ligands by EXponential enrichment) technique to demonstrate HIV reverse transcriptase (HIV-RT) can bind with
high affinity to specific primer-template sequences (DeStefano and Nair, 2008). Based on these sequences, which resembled the HIV polypurine tract (PPT), DNA aptamers that demonstrate tight binding and inhibition of HIV-RT \textit{in vitro} were developed. We are currently investigating if aptamers designed in our lab that are potent HIV-1 RT inhibitors \textit{in vitro} can also inhibit viral infection in cell culture. Low micromolar concentrations of aptamers in the absence of a transfection agent inhibited replication in Jurkat cells without significant cellular toxicity. Using fluorescently tagged aptamers, entry of the aptamers into Jurkat cells was observed; this process was enhanced in the presence of HIV-1. It is still unclear if the effect of aptamer inhibition on the replication cycle is due to HIV-RT inhibition or to other factors such as alteration of cellular pathways. Currently the effects of the aptamers on individual steps in HIV replication are being studied. We are looking at cargo proteins as a tool to improve aptamer delivery into cells. The study of these aptamers could help us to understand the interaction between HIV-RT and the different nucleic acid substrates, ultimately allowing for the potential development of small molecule inhibitors that would be useful in the treatment of HIV-1.

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\textit{Larsen, J. D., Kiick, K. L., Sullivan, M. O.}

Department of Chemical Engineering - University of Delaware, Department of Materials Science and Engineering - University of Delaware, Department of Chemical Engineering - University of Delaware

For non-viral gene delivery biomaterials to be efficient, they must meet the contradictory requirements of tightly packaging DNA to prevent degradation by DNAses, yet efficiently releasing DNA once inside the nucleus of a cell. Unfortunately, the majority of non-viral vectors unpack their cargo prior to nuclear entry or do not unpackage it at all, promoting either premature degradation or inefficient transcription. To improve the overall efficiency of non-viral gene delivery, we propose a novel vector that is designed to self-unpackage its DNA upon arrival in the nucleus. To accomplish this goal, we have developed vehicles that mimic the natural histone biochemistry that promotes the unpackaging of chromosomal DNA from the histone octamer. We have incorporated post-translationally modified histone 3, or H3, tail peptides onto a 4-arm PEG backbone. To date, we have explored the effects of the reaction conditions on peptide-PEG conjugate formation, investigated the influence of the N:P packaging ratio on DNA protection, and examined the physical characteristics of the peptide-PEG-DNA complexes. With N:P ratios greater than 10, we form 180 - 200 nm diameter complexes that efficiently protect DNA from serum nucleases. We are currently exploring the ability of these complexes to efficiently transfect cells, with the aim of identifying materials capable of site-specific unpackaging and transcriptional activation of their payloads. Furthermore, we will attempt to further the understanding of the relationship
between the structure of the DNA complexes and the overall efficacy of non-viral gene delivery biomaterials.

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**P59. Metal binding and oxidation studies of classical zinc finger: ZIF-268**

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ZIF-268 is a classical zinc finger protein that utilizes a Cys2His2 ligand set to bind zinc and fold into a characteristic $\beta_2\alpha$ structure. The zinc and DNA binding properties of ZIF-268 are well understood and it is possible to modify the protein sequence to tune DNA recognition. There are several reports that reveal that iron can substitute for zinc in certain zinc finger proteins with varied functional consequences. In order to understand the effect of iron binding on ZIF-268’s function, we have overexpressed a fragment of ZIF-268 that contains three zinc binding domains. Optical titrations of ZIF-268 with ferrous and ferric iron demonstrated that iron at both oxidation states can bind to ZIF-268. DNA binding studies of the ferrous and ferric iron bound ZIF-268 revealed that the iron substituted proteins bind to their target DNA with the same affinity as the zinc bound form. In the presence of H$_2$O$_2$, Fe(II)-ZIF268 generates hydroxyl radicals. Current efforts are focused on understanding the reactivity of these generated hydroxyl radicals.

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**P60. Designed small molecule alpha-helix mimics of the p53-MDM2 interaction**


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p53 is a transcription factor involved in the upregulation of DNA-repair genes in response to cellular insults or DNA damage. p53 interacts with the general transcription machinery or alternatively binds to the repressor oncoprotein MDM2 via its $\alpha_{i\pi\lambda\alpha}$-helical activation domain (AD). MDM2 binding to p53 prevents the transcription of p53-responsive genes including those for cell cycle arrest, DNA-repair, and apoptosis. Developing new methods to understand the interplay between p53 and MDM2 is critical to our understanding of the oncogenic behavior of these proteins. We have developed a p53-based peptide using canonical amino acids featuring a P27S substitution ($K_d = 4.7$ nM) that binds with a 2.3 kcal mol$^{-1}$ higher affinity than the native p53 peptide due to an increase in $\alpha_{i\pi\lambda\alpha}$-helicity of the free peptide. p53-P27S is the highest affinity genetically encodable ligand of MDM2 developed to date. In addition, we have designed small molecules that mimic the presentation of the $i$, $i + 3$, and $i + 4$ residue side chains of
the p53 AD \textbf{(FSDLW)}. These molecules entail a stereospecific display of proteinogenic side chains that mimic the $\alpha_{\pi\lambda\alpha}$-helical interaction between p53 and MDM2. Our compounds bind specifically to MDM2 with affinity comparable to that of p53 relative to a control scaffold. Our highest affinity small molecules inhibit the cell growth of a prostate cancer cell line (LNCaP) but do not affect the cell viability of a prostate cell line that does not express p53 (PC3).

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**P61. MusD RNA transport element contains unusual structural motifs**


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Nuclear export of retroviral or endogenous retrotransposon RNAs is an essential step in the life cycle of these retroelements, requiring direct interaction between protein factors and distinct structural domains within viral and retrotransposon mRNA(s). Studying the structures of these RNA domains and their interactions with cellular or viral factors not only provides insights into the evolution of such elements, but also offers the potential for development of novel therapeutic agents.

We resolve the secondary structure of one such domain that is crucial for nuclear export of endogenous murine LTR retrotransposon, type D (MusD) RNA using a biochemical mapping technique, SHAPE (Selective 2’-Hydroxyl Acylation analyzed by Primer Extension). This approach is based on selective chemical modification of RNA at the 2’-hydroxyl groups of constituent nucleotides, where the degree of reactivity is determined by local flexibility.

Using this technology, a pair of kissing loops and a complex dual pseudoknot within the region of MusD implicated in nuclear export were identified. The existence of these structures was verified by conventional mutational analysis and aiSHAPE (antisense interfered SHAPE), a novel extension of SHAPE in which long range intramolecular interactions are probed with short antisense oligonucleotides. The functional importance of the kissing loops in vivo was confirmed in cell culture experiments involving relevant mutants. Mutations disrupting kissing loop interactions caused absolute inactivation of nuclear export, and compensatory mutations restored this function.

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**P62. Modulating inhibition of bacterial toxins through engineering of homog**

Levenson, E. A., Maheshwari, R., Kiick, K. L.
Functional biological recognition is not only dependent upon exact molecular structure but also upon the spatial presentation and number of ligands recognized. The ability to control how individual structures form larger arrays of defined size, scope, and valency to present to cellular receptors is critical in determining how these systems function and to design strategies to develop therapies for human disease. We used the fidelity of transcription and the template-driven synthesis of the translated protein to make monodisperse biopolymer scaffolds with random-coil conformation and electrostatic charge complementary to the desired receptor. The designed sequence incorporates a pentameric repeat of a solid phase synthesized peptide previously studied. In addition an analogue of methionine, the amino acid homopropargylglycine, was substituted within the translated protein to add terminal alkyne functionality. The alkyne was reacted with an azide-containing galactose through Huisgen 3+2 cycloaddition to form a glycoprotein capable of interacting with the B5 subunit of cholera toxin, a model system. The position of the triazole on the linker, formed by the cycloaddition reaction, is indicated to significantly impact the recognition of the glycopolypeptide by the CT B5. The glycopolypeptide was characterized through size-exclusion chromatography, circular dichroic spectroscopy, and light scattering. Finally, a direct enzyme linked assay was used to find the IC50 of this glycoprotein against the B5 subunit of cholera toxin. These values were compared to other scaffolds of lower valency and alternative structure, and results indicate the potential of these methods for tailoring the interactions of multivalent ligands with protein targets.

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P63. Revive deuterium-labeled reagents for protein quantitation

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Mass Spectrometry (MS)-based quantitative proteomics with stable isotope-labeling reagents is a powerful tool for the systematic understanding of biological processes. Many reagents, including iTRAQ (isobaric Tag for Relative and Absolute Quantitation) and TMT (Tandem Mass Tag), allow us to determine the relative abundance of proteins in an accurate and high-throughput manner. However, because these reagents are made from expensive 13C, 15N, and 18O, their applications are limited by high costs. Although 2H labeled reagent are less expensive to prepare, it is generally believed that they can introduce chromatographic isotope shift in reverse-phase HPLC, thus compromising the accuracy of quantitation. We envisioned that a set of reagents with identical number of 2H should eliminate this adverse effect. To prove this theory, we synthesized six DiART reagents, each of which has the identical structure and molecular
weight, yet contains four 2H atoms in different positions. These reagents were designed for quantitative proteomics by using the same principle behind iTRAQ and TMT technologies.

Our data demonstrated that DiART reagents did not introduce any chromatographic isotope shift and allowed us to accurately determine the relative abundance of these peptides. Because DiART reagents were synthesized easily in 7 steps from low-cost starting materials with an overall yield of 30%, DiART reagents would provide a cost-effective substitute of iTRAQ and TMT. We also demonstrated that DiART reagents are compatible with Mascot, the most widely used software for protein identification with MS.

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**P64. Resilin-like elastomeric polypeptides as cell-responsive biomaterials**

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Resilin, the highly elastomeric protein found in specialized compartments of most arthropods, exhibits low stiffness, high resilience and effective energy storage. Recombinantly-engineered resilin-like polypeptides (RLPs) that possess the favorable attributes of the native resilin would be attractive candidates for the modular design of biomaterials for engineering mechanically active tissues. Facilitated by the recent expression of a repetitive resilin in bacterial hosts, we have designed resilin-like polypeptides which embrace twelve repeats of the putative resilin consensus sequence, as well as cell binding domains, a heparin binding domain and two degradation domains, aiming at maintaining its excellent mechanical properties while incorporating biological function to this matrix. The synthesis of this new polypeptide has been confirmed by SDS-PAGE and amino acid analysis. The crosslinked bio-elastomeric hydrogels display intriguing and tunable mechanical properties and also support the adhesion of NIH 3T3 fibroblasts, which validate the outstanding properties of these materials and indicate their potential in soft tissue replacement.

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**P65. Molecular dynamics simulations of yeast membrane models**

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There has been growing interest in using molecular dynamics (MD) simulations to investigate membrane-protein systems. However, these simulations have heretofore consisted of no more than three types of lipids, greatly simplifying the compositions of actual membranes. CHARMM-GUI, a web-based graphical user interface for performing various CHARMM functions, has recently been used to generate more complex lipid bilayers composed of six different lipids – cholesterol, DOPC, DPPC, POPA, POPE, and POPS – in order to accurately and realistically model yeast membranes. Four bilayers containing varying amounts of chain saturation, cholesterol concentration, and surface tension were created. These yeast membranes were simulated for a total of 170 ns using the atomistic CHARMM27r force field at 303.15 K. MD simulations with a high concentration of unsaturated chains (73%) showed an increase in surface area per lipid and a decrease in the $S_{CD}$ order parameters for DPPC, as opposed to the membrane created with a high concentration of saturated chains (60-63%). Simulations of the more saturated membranes were in a liquid-ordered state and were in agreement with experimental cholesterol-containing membranes. The unsaturated membrane simulation exhibited a larger average tilt angle of cholesterol with respect to the bilayer normal. Moreover, cholesterol in the unsaturated membrane actually oriented parallel to the bilayer surface for periods of less than a nanosecond. This result supports previous observations of parallel cholesterol existing at the center of polyunsaturated fatty acid membranes, and makes us the first to observe parallel cholesterol in a fully atomistic simulation.

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**P66. Macrocyclic peptide-peptoid hybrids designed as PPII helix mimetics**


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Secondary structure provides critical recognition elements for peptide and protein interactions. Recently, left handed polyproline II (PPII) helixes have been attracting significant attention due to their dominant role in cellular signal transduction involving proline-rich motifs (PRMs). In contrast to alpha-helixes, which typically have 3.6 residues per turn and a system of internal $i + 4$ to $i$ hydrogen bonds, PPII helixes are more open and contain three residues per turn with no internal hydrogen bonding. Stabilization of alpha-helixes by ring closing metathesis (RCM) – based hydrocarbon “stapling” has proven to be a promising means of increasing affinity and bio-availability of peptide ligands. In the HIV-1 life cycle, the ubiquitin E2 variant (UEV) domain of the human tumor susceptibility gene 101 (Tsg101) protein is recruited by major structural proteins of HIV-1 to facilitate viral budding. This recruitment involves the direct interaction of the Tsg101 UEV domain with a proline-rich motif in the viral Gag-p6 protein. NMR solution studies of the interaction between the p6-derived 9-mer peptide,
P1E2P3T4A5P6P7E8E9 and Tsg101, have indicated that a key A5P6 di–peptide region binds to Tsg101 in a PPII helix–like conformation. To examine the potential utility of RCM–based hydrocarbon stapling to PPII helix stabilization in the p6–derived 9–mer peptide, we prepared more than 50 macrocyclic peptide–peptoid hybrids. We showed that this approach can stabilize PPII helix conformations to yield more bio-available ligands.

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**P67. Selective delivery of a quinone methide precursor by PNAs**

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Quinone methides (QMs) are electrophilic intermediates that can be generated in vivo to alkylate DNA and function as anti-cancer drugs. DNA conjugate of QM have shown the ability to alkylate target DNA selectively. Analogous peptide nucleic acids (PNAs) conjugates of QM are now being developed in part because PNA can bind to target DNA with higher affinity than equivalent DNA conjugates. PNA derivatives are also advantageous since they are non-ionic and metabolically stable. Direct coupling between an N-hydroxysuccinimide ester of a quinone methide precursor (QMP) with the amino terminus of a PNA was not successful. In contrast, the same QMP was capable of coupling to the PNA after it had been extended at the its N-terminus with 8-amino-3,6-dioxaoctanoic acid. A PNA-QM self-adduct was prepared after initiating QM formation in situ. This self-adduct is stable to purification by HPLC and subsequently capable of selectively alkylating target DNA.

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**P68. Targeting thermosensitive liposomes to B cell lymphoma**


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Targeting liposome encapsulated drugs with single chain antibodies (scFvs) has the potential to increase the efficacy of anti-cancer drugs and to decrease their systemic side effects. The transmembrane protein CD22 rapidly internalizes upon antibody binding and is expressed only in B lymphocytes and the majority of non-Hodgkin’s lymphomas
(NHLs), making it an ideal target for anti-cancer liposomal drugs. Currently, the anti-CD22 monoclonal antibody epratuzumab is in clinical trials for NHLs. We are developing anti-CD22 scFv-conjugated liposomes to deliver anti-cancer agents to B-cell lymphomas. A mutant of the anti-CD22 scFv HA22 (mut-HA22) bearing an engineered cysteine at the C terminus has been developed. Mut-HA22 was conjugated to DPPC and DSPE-PEG(2000)-maleimide liposomes (loaded with calcein and/or containing rhodamine-PE) via the cysteine-maleimide reaction. We used a B-lymphocyte cell line (BJAB) that expresses CD22 receptors to examine binding and uptake of mut-HA22-conjugated liposomes; the T-lymphocyte cell line (SupT1) that does not express CD22 was used as a negative control. Using flow cytometry and fluorescence microscopy, we show that (i) the mut-HA22 recognizes CD22 receptor expressing cells similar to HA22 (ii) mut-HA22-conjugated liposomes specifically bind to the CD22 receptor (iii) mut-HA22-conjugated liposomes bind in a dose dependent manner (iv) mut-HA22-conjugated liposomes have increased association with CD22-positive cells at 37°C, indicating that they are internalized (v) mut-HA22 liposome binding was inhibited in the presence of free mut-HA22. Therefore, our CD22-targeting liposomes are a promising carrier for selective and controlled delivery of anti-cancer drugs.

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**P69. Development of the drude polarizable force field for CHARMM.**

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The Drude polarizable CHARMM force field has been developed over the years focusing in the determination of bonded and non-bonded parameters of the small molecules making the building blocks of the larger biomolecules. The newly developed force field is currently being tested in the simulation of peptides and small proteins in aqueous solution and solid phase. Structural and dynamical properties are compared with available experimental data and results from the additive force field of CHARMM. Extensive discussion of the parameterization strategies of the conformational space is presented.

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**P70. A gain-of-function mutation reveals a novel role of the phosphate tran**

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A detailed understanding of the defense gene networks is critical in advancing our knowledge on the mechanisms of plant defense. However, it remains challenging to identify defense regulatory genes and elucidate their functions. Taking advantage of the unique defense-dependent dwarfism conferred by the Arabidopsis mutant acd6-1, we developed a genetic screen to identify acd6-1 suppressor (sup) mutants, which potentially harbor mutations in novel defense genes. Among the genes identified was SUP3, encoding a phosphate transporter widely conserved in plants. The sup3-1 mutant was found to suppress constitutive defense and small size in acd6-1. In addition, sup3-1 was compromised in basal defense against virulent Pseudomonas syringae strains. We also found that sup3-1-conferred susceptibility could be rescued by exogenous salicylic acid (SA) treatment, suggesting SUP3 acting upstream in SA signaling. Consistent with the role of SUP3 in regulating SA-mediated defense, genetic analysis indicated that sup3-1 acted additively with several known SA regulators, ALD1, EDS5, and SID2, to affect acd6-1-conferred phenotypes. The sup3-1 mutant is disrupted in the fifth exon of the SUP3 gene, leading to the accumulation of a shorter transcript. The sup3-1 mutant is dominant, possibly due to the action of the truncated protein. Transgenic expression of the DNA fragment containing the SUP3-1 region in the wild type recapitulated sup3-1-conferred susceptibility to Pseudomonas infection. In addition, introducing extra copies of the full length SUP3 genomic fragment into the wild type also resulted in enhanced disease susceptibility. These data suggest that SUP3 is a novel negative regulator of basal defense, acting independently of ALD1, EDS5, and SID2 in the SA-mediated defense pathway.

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**P71. Insights into the primary copper binding site of alpha-synuclein**

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α-Synuclein, α-syn, a presynaptic protein implicated in Parkinson’s disease, binds copper(II) ion with submicromolar affinity based on in vitro studies. Tryptophan fluorescence measurements involving mutant proteins (F4W and F4W/H50S) and comparisons to synthetic N-terminal peptides suggest that the primary copper(II) binding site is localized in the first four residues. Moreover, the a-amino terminus is required for Cu(II) binding. New insights on the molecular details of copper coordination environment are gained through circular dichroism analyses. In particular, the presence of a cation-p (Cu/Trp) interaction as well as the effects of methionine oxidation on copper binding will be discussed. Analogous work focused on the ability of α-syn to bind copper(I), the physiologically relevant metal oxidation state, will be addressed as well as the ability of membrane bound α-syn to bind both copper(II) and copper(I).

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P72. NO-Independent activation of sGC

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Nitric oxide (NO) is a small, diffusible molecule produced by the enzyme Nitric Oxide Synthase that acts as a biological messenger and a cytotoxic agent against tumor cells. The primary receptor for NO in the cell is the enzyme soluble guanylate cyclase (sGC). The enzymatic activity of sGC is increased several hundred-fold by the allosteric binding of nitric oxide. sGC is a heterodimeric hemoprotein that catalyzes the conversion of guanosine-5’-triphosphate (GTP) into cyclic guanosine-3’,5’-monophosphate (cGMP) and pyrophosphate. The second messenger, cGMP, then modulates the activity of kinases and ion channels, and ultimately induces smooth muscle relaxation and vasodilation. In cases of endothelial dysfunction, reduced bioavailability of NO or reduced output of the NO-sGC-cGMP pathway can lead to hypertension, cardiovascular diseases and impotence. Current therapies to treat these conditions involve compounds that release NO, but toxicity and tolerance remain an obstacle. An alternate therapeutic strategy is to enhance sGC activity in an NO-independent manner by small molecules known as sGC activators. So far, the molecular details underlying sGC activation by these molecules remain unknown.

Structural studies to identify the binding site(s) of these activators in sGC and to elucidate how they increase cGMP production will provide a platform for structure-based drug design leading to better activators. We will present our latest results combining molecular biology, protein expression and purification, spectroscopy, and x-ray crystallography.

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P73. JS-K inhibits growth of lung adenocarcinoma cells in vitro and in vivo

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JS-K \( (O^2-(2,4-dinitrophenyl)-1\text{-}[\text{4-ethoxycarbonyl}piperazin-1-yl]diazene-1-ium-1,2-diolate} \) is a nitric oxide releasing pro-drug. Its potent antineoplastic activity has been documented in leukemia and multiple myeloma \textit{in vitro} and \textit{in vivo}; however its mechanism(s) of action/molecular target(s) are not completely understood. We investigated the effects of JS-K on growth of human lung cancer cells. We show that JS-K inhibited growth of 20 human adenocarcinoma cell lines, with IC\(_{50}\) concentrations
ranging from 0.3 to 17 μM. JS-K was most effective against cell lines with high levels of endogenous reactive oxygen species (ROS), with IC$_{50}$ values for these cell lines in the range 0.3 - 1 μM. JS-K toxicity correlated significantly with intracellular ROS. Deficiency in antioxidant defense and DNA repair mechanisms appears to sensitize cells to JS-K (levels of peroxiredoxins 1 and 6 and OGG1 correlated significantly with JS-K toxicity).

Two cell lines were chosen for mechanistic studies – H1944 characterized by low level of endogenous ROS and resistance to JS-K, and H1703, with significantly higher levels of ROS and ten-fold greater sensitivity to JS-K. Treatment with JS-K resulted in oxidative/nitrosative stress in the cells with high basal level of ROS, causing significant DNA strand break damage (comet assay). JS-K treatment led to activation of SEK1/MKK4 / SAPK/JNK stress pathway, resulting in apoptotic cell death. Potent antitumor activity of JS-K was also confirmed in lung cancer xenografts. JS-K caused 75% reduction in H1703 tumor growth in vivo. The results indicate that JS-K has potential as a therapeutic agent against human lung cancer.

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**P74. Systematic reconstruction and curation of hybrid metabolic networks**

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It is widely accepted that single metabolic pathways cannot be singularly analyzed and must be combined to form a full metabolic network since proteomic information has shown that genetic modifications pertaining a specific pathway also affect other metabolic processes [Teusink et al]. A genome-scale model presents significant advantages over traditional pathway analysis because it encompasses the entire biochemical network and can predict the effects of genetic modifications in microbial metabolism, such as uptake/secretion rates, exceedingly better than single pathway analysis. However, constructing genome scale models is an extremely laborious task and in every microorganism the genome is not yet fully annotated or has errors which will require some degree of manual correction [Osterman et al]. In our work, we suggest that the development time for network reconstruction can significantly decrease by comparing the metabolisms of closely related organisms. The combined metabolism is described by a hybrid network, obtained from the sum of both individual networks. Our work has shown that hybrid networks have less inconsistencies than traditional single organism networks and, most notably, fewer metabolic gaps. Also, we present several metrics which demonstrate that the expense of increasing network complexity is far outpaced by the improvement in metabolic curation.

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P75. Mutational analysis of a putative base flipping residue R275 in hTDG

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Thymine DNA glycosylase (TDG) is a base excision repair enzyme that excises T from G·T mispairs and removes other lesions, exhibiting specificity for damage at CpG sites. DNA glycosylases use nucleotide flipping to find lesions and cleave the base-sugar bond. Typically, a bulky side chain penetrates the DNA, filling the space vacated by the flipped nucleotide. Our crystal structure of human TDG catalytic domain bound to abasic DNA shows that an arginine (R275) plugs the helical gap and contacts two phosphates flanking the abasic sugar (Maiti A, et al., Proc Natl Acad Sci 105: 8890-8895). Here, we examine the role of R275 in the catalytic mechanism of TDG. Isothermal titration calorimetry (ITC) experiments show that substrate (analog) binding affinity is significantly lower for R275A-hTDG versus hTDG, and lower still for R275L-hTDG. Single turnover kinetics experiments show that $k_{\text{max}}$ is significantly lower for R275A-hTDG versus hTDG, and lower still for R275L-hTDG. Our results are surprising, because two related enzymes, mismatch-specific uracil glycosylase and uracil DNA glycosylase have leucine at the position corresponding to R275 of hTDG. We find that mutation of R275 has no effect on steady-state turnover, $k_{\text{cat}}$, suggesting that R275 cannot account for the exceedingly slow product release observed for hTDG.

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P76. Therapeutic benefit of reactivation of Sarin-inhibited acetylcholinest

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Nerve agents, such as sarin, are potent inhibitors of the enzyme AChE which results in excessive accumulation of acetylcholine (ACh) and overstimulation of central and peripheral cholinergic receptors. Treatment of sarin poisoning consists of an anticholinergic such as atropine to block the effects of excessive ACh and an oxime such as 2-pyridinium aldoxime (2-PAM) to reactivate agent-inhibited AChE. 2-PAM is a quaternary drugs that provide negligible reactivation of CNS AChE. The present study investigated the capacity of the tertiary oxime MINA to 1) reactivate peripheral tissue, blood and brain AChE inhibited by sarin; 2) terminate sarin-induced seizures; 3) prevent neuropathology and the neurobehavioral consequences of sarin exposure; and 4) protect against the lethal effects of sarin. MINA (22-139 mg/kg) administered 15 min after 1LD50 of sarin resulted in a dose dependent reactivation of peripheral tissue, blood and brain AChE after 60 min, whereas 2-PAM (25 mg/kg) reactivated only peripheral tissue.
AChE. MINA (18-56 mg/kg) administered from 0 to 10 min after a 2LD50 sarin challenge resulted in a dose-dependent blockade of electrographic seizures, neuropathology and behavioral debilitation. These sequelae were not blocked by 2-PAM treatment. MINA (35 or 60 mg/kg) administered alone, with atropine (0.5 mg/kg), or with atropine and 2-PAM 1 min after sarin challenge increased the 24-hr LD50 of sarin 3.6- to 15-fold compared to 1- to 3.3-fold for atropine, 2-PAM or atropine plus 2-PAM treatment. Reactivation of brain AChE by the tertiary oxime MINA can mitigate the lethal and adverse neurobehavioral consequences of sarin intoxication.

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**P77. Fluorescence of unmodified oligonucleotides: A tool to probe G-quadrup**

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Fluorescence of unmodified oligonucleotides has not been exploited for guanine-quadruplex (G-quadruplex) characterization. We observe that G-rich sequences have a markedly higher fluorescence than single-stranded or duplex DNA. The fluorescence intensity is, however, weaker than strongly fluorescent dyes like fluorescein. On the basis of their fluorescence spectra, unlabeled oligonucleotides that form G-quadruplexes can be differentiated from similar sequences that do not contain consecutive guanines. Intermolecular quadruplexes formed by the oligonucleotides 5’-T₄GₙT₄-3’ (n = 4 - 10) display a non-linear, but continuous, increase in emission intensity as the G content increases. The sequence 5’-GGGT-3’, which has been proposed to form a monomeric quadruplex and an interlocked quadruplex (Krishnan-Ghosh et al. *J. Am. Chem. Soc.* 2004 126 11009), was compared to the similar sequence 5’-TGGG-3’, the structure of which has not been characterized. Both the maximum emission intensity and the spectral shape differ for these oligonucleotides as a function of sample preparation, indicating that different types of quadruplexes form for both sequences. Addition of fluorophores or radiolabels to oligonucleotides interferes with self-assembly of G-quadruplexes into larger networks/suprastructures. We show that the suprastructure of unmodified G-rich sequences can be probed using fluorescence spectroscopy.

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**P78. Exploring catalytic promiscuity of 1-Deoxy-D-Xylulose 5-Phosphate synt**

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Many human pathogens rely upon the methyerythritol phosphate (MEP) pathway for biogenesis of the essential isoprenoid bioprecursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Our long term goal is to understand catalysis of these intriguing biosynthetic enzymes toward the development of new anti-infective agents. 1-Deoxy-D-xylulose 5-phosphate (DXP) synthase is a novel transketolase-like enzyme that catalyzes the first step in the MEP pathway to form DXP from pyruvate and glyceraldehyde 3-phosphate (GAP). Its unique structure distinguishes DXP synthase from its homologs, transketolase and pyruvate decarboxylase, making it a particularly interesting target. We have anticipated the possibility that DXP synthase may also exhibit distinctive catalytic activities, offering an opportunity to discover new reactions for the generation of diverse molecules that may be useful to study catalysis of downstream enzymes. We have initiated studies to explore the catalytic promiscuity of *E. coli* DXP synthase using a versatile and robust HPLC assay recently developed in our laboratory. Using this assay, we have unveiled new catalytic activities of DXP synthase to reveal a remarkably broad tolerance for alternative acceptor substrates. Our results suggest DXP synthase will be a particularly useful biocatalyst for the production of new molecular entities to study isoprenoid biosynthesis.

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**P.79 Novel site directed affinity reagent for cross-linking human hemoglobin**

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The advantages of a blood substitute continue to grow as disease and viruses proliferate with mutations and the need for blood donations rise, especially in other countries where risks for receiving transfused blood are much greater than in the U.S. Currently, two types of blood substitutes exist; volume expanders and oxygen therapeutics. The project aims to develop an oxygen therapeutic because it uses real hemoglobin to deliver oxygen to deprived tissues. In the project scheme we set forth to create a cross-linking molecule through a series of organic synthetic reactions forming a ten carbon backbone compound affixed with four aldehyde moieties. This allows two or four cell-free hemoglobin molecules to be attached, facilitating efficient oxygen delivery without hemoglobin diffusion into tissues. The first reaction combines 1,10-diododecane with tris(cyanoethyl) phosphate then subject the product to organic reactions forming a tetra-diol followed by a tetra-aldehyde. The aldehyde group provides a connection to the hemoglobin molecule. Preliminary work has attempted the tetra-diol product and the current results suggest that the molecules are possible to achieve. After the success of completing the tetra-diol reaction, the next step requires the development of the tetra-aldehyde and then reaction with amines.

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P80. TSP-1 is a universal inhibitor of soluble guanylate cyclase activation

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Soluble guanylate cyclase (sGC) is the signal transduction enzyme most responsible for mediating the effects of nitric oxide by producing cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). NO-cGMP signaling is vital to proper cardiovascular function. Deficiencies in the production of either NO or cGMP contribute to cardiovascular disorders. Therapies designed to increase in vivo NO levels (such as organic nitrates) have well documented clinical limitations due to drug tolerance. Recently, a variety of NO-independent small molecule activators of sGC have been reported that activate nearly or as well as NO and have promising clinical activities. Previous results from our lab have shown that the secreted matrix protein thrombospondin-1 (TSP-1) binds to CD47 and potently inhibits NO stimulation of sGC. Here we show that TSP-1 signaling via CD47 also inhibits sGC activation by NO-independent sGC activating small molecules. Pretreatment of both porcine vascular smooth muscle cells and washed human platelets with TSP-1 (1ug/ml) significantly inhibited the ability of YC-1 (100uM), BAY 41-2272 (10uM), and meso-porphyrin IX (10uM) to elevate cGMP levels. TSP-1 pretreatment also inhibited the ability of BAY 41-2272 (10uM) to delay thrombin induced aggregation of washed human platelets. This work demonstrates that sGC activation by NO independent sGC activators is greatly diminished in the presence of TSP-1/CD47 signaling. This data coupled with the reported increases in TSP-1 with age, diabetes, ischemia reperfusion, and atherosclerosis implies that the therapeutic potential of NO-independent sGC activating drugs could be compromised in disease states with elevated TSP-1/CD47 signaling.

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P81. Synthesis and characterization of magnetic resonance molecular imaging

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We are interested in developing new magnetic resonance molecular imaging probes for the non-invasive, in vivo evaluation of cancer. Our probes are based on macrocyclic
gadolinium complexes whose signal generation is triggered by interaction with a disease biomarker. This type of pro-agent has been termed “activatable”. We are specifically interested in agents whose signal is responsive to the presence of the cancer biomarker MAP Kinase, which is an important enzyme during rapid cell division in cancer. To accomplish these goals we are currently synthesizing a library of peptide conjugates of macrocyclic gadolinium complexes. Concurrently, we are developing screening assays and characterization methods to identify and fully characterize the library “hits”. We will report on the synthesis and characterization of our magnetic resonance imaging probes, as well as, preliminary results of our screenings.

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P82. Synthesis of masked monophosphate ring expanded nucleosides (RENs)

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The use of Ring Expanded Nucleosides (RENs) has a proven history of successful in vitro treatment of a host of tumors as well as viruses such as HIV and HCV. Literature has shown that the activity of nuceloside analogues is dependent on their ability to undergo in vivo phosphorylation by substrate specific cellular kinases and that most molecules fail during this process. It has also been shown that the very first phosphorylation is the crucial step in converting nucleosides to their tri-phosphate derivatives, and the subsequent phosphorylations are relatively facile. Adding the phosphate through chemical synthesis generates significant ionic character which makes cell membrane penetration impossible, therefore we intend to synthesize masked monophosphate (phosphoramidate) derivatives of our RENs to overcome this deficiency. With this pro-tide technology we hope to enhance the therapeutic potential of our currently active RENs and to explore the possibility of activating those that are currently inactive.

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P83. De novo design of strand swapped beta-hairpin hydrogels

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De novo designed peptides, capable of undergoing a thermally triggered beta-strand swapped self-assembly event leading to hydrogel formation were prepared. Inspired by naturally occurring domain swapping proteins, we designed a Strand Swapping Peptide 1 (SSP1) that incorporates an exchangeable beta-strand domain composed of eight residues
appended to a non-exchangeable beta-hairpin domain. SSP1 adopts a beta-hairpin structure that displays an exchangeable beta-strand region as verified by CD, FTIR and WAXS. The exchangeable strand domain participates in swapping with the exchangeable domain of another peptide affording a strand swapped dimer. These dimers further assemble into fibrils that define the hydrogel. A second peptide (SSP2) containing an exchangeable strand composed of only four residues was also studied. Microscopy and scattering data show that the length of the exchangeable domain directly influences the fibril nanostructure and can be used as a design element to construct either twisted or non-twisted fibril morphologies. Fibril dimensions, as measured by TEM, AFM and SANS indicate a fibril diameter of 6.4 nm, height of 6.0 nm and a of pitch 50.4 nm for the twisted SSP1 fibrils. The non-twisted SSP2 fibrils are 6.2 nm in diameter and 2.5 nm in height. Oscillatory rheology, used to measure bulk hydrogel rigidity, showed that the gel composed of the non-twisted fibrils is more mechanically rigid (517 Pa at 6 rad/s) than the gel composed of twisted fibrils (367 Pa at 6 rad/s). This work demonstrates that strand swapping can be used to fabricate biomaterials with tunable fibril nanostructure and bulk rheological properties.

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**P84. Re-engineering split G-quadruplexes for nucleic acid sensing**

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Biomedical devices or assays that can be used by physicians to detect disease markers at point-of-care or for large-scale screening programs will vastly improve our ability to predict or diagnose diseases at earlier stages, leading to enhancement of the quality of human life and also an increase in life expectancy. Recently the detection of DNA by probes that can reconstitute into active peroxidase-mimicking G-quadruplex DNAzymes, has gained increasing popularity because these approaches afford simple detection of nucleic acids via colorimetric means without the need for expensive proteins such as avidin-horseradish peroxidase conjugates. In this poster presentation, we will reveal salient architectural features of reconstituted G-quadruplex DNAzymes that enhance the catalytic efficiency of these important DNA catalysts. We will also disclose new reaction conditions that protect the DNA peroxidases from oxidative inactivation during catalysis. These discoveries have facilitated a more sensitive detection of DNAs using split G-quadruplex probes.

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**P85. Structural analogues of JS-K, an anti-cancer lead compound**
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$O^2$-(2,4-Dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate (JS-K), a member of the diazeniumdiolate class of nitric oxide (NO) prodrugs, is currently in pre-clinical studies as an anti-cancer drug candidate. JS-K was designed to be activated by glutathione (GSH) to release NO; this reaction was found to be catalyzed by glutathione S-transferase (GST), which is frequently over-expressed in cancer cells. In structure activity relation (SAR) studies of structurally similar JS-K analogues, considerable variation in the anti-proliferative activity was observed, prompting us to expand the library of such analogues and compare their activity with that of JS-K. A number of piperazine and homopiperazine analogues of JS-K were prepared and evaluated. A majority of these compounds dissociated in the presence of glutathione to release nearly quantitative amounts of NO as determined by chemiluminescence. Standard cell viability assays were conducted and several of the JS-K analogues had a nearly identical activity profile ($IC_{50} < 1 \text{ mM}$) to that of JS-K in inhibiting the proliferation of human leukemia HL-60 and U937 cells. Intracellular NO levels formed upon treating HL-60 cells with these compounds were determined using a standard 4-amino-5-methylamino-2',7'-difluorescein diacetate (DAF-FM diacetate) assay; the levels of NO released intracellularly correlated well with the anti-proliferative effects suggesting an important role for cell permeability to release NO in the inhibition of cancer cell proliferation by this class of prodrugs. Finally, this and previous SAR studies provide us insight into a common structural motif for diazeniumdiolate prodrugs that have an anti-proliferative activity that is comparable to that of the lead compound, JS-K.

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P86. Heparin-containing hydrogels for modulating cell responses

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The ability to engineer biologically active materials has advanced dramatically in the last few years. Glycosaminoglycan hydrogels designed for the delivery of therapeutic proteins, such as growth factors, is one of the most important areas of recent development. In this study, in situ crosslinkable and degradable heparin-containing hydrogels were designed for the binding and controlled release of growth factors, and tested as an
ECM mimetic materials for modulating endothelial cell responses. The gelation times and elastic moduli of these gels could be tuned by alternating the chemical and physical properties of the materials.

Cell studies indicate that the proliferation and migration of human umbilical vein endothelial cells (HUVECs) could be modulated by variations in the mechanical properties of hydrogels. Vascular endothelial growth factor (VEGF), which is released from hydrogel, significantly stimulates the spreading and migration of HUVECs on the hydrogel. HUVECs spread and migrate faster on hydrogels with higher moduli (G' = 2,200 Pa) than gels with lower moduli (G' = 500 Pa). This system may therefore provide an ECM mimic matrix for manipulating the local environment and response of endothelial cells (EC) in the engineering of EC-derived structures.

P87. Human Paraoxonase Double Mutants Hydrolyze the Organophosphorus Compounds


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Variants of human paraoxonase 1 (PON1) are being developed as catalytic bioscavengers for the chemical warfare agents soman, sarin, tabun, and VX. It is preferable that the new PON1 variants have broad spectrum OP hydrolase activities to hydrolyze both G-class and V-class agents. PON1 H115W has shown improvements over wild-type PON1 in its capacity to hydrolyze OPs. We improved upon these activities by either adding an additional tryptophan (F347W) for improved substrate binding or reducing bulky groups around the lip of the active site gorge (Y71A). When compared to H115W alone, we found that H115W/Y71A marginally diminished VX catalytic efficiency but improved paraoxon catalytic efficiency by 6-fold. H115W/F347W showed slightly improved VX catalytic efficiency but dramatically less capacity to hydrolyze paraoxon. A cholinesterase protection assay was used to investigate soman hydrolysis. After testing our double mutants against soman, we observed reduced K_Ms for binding and improved k_cat s that were 400 times higher than wild-type PON1. H115W could not hydrolyze soman. We also examined the capacity of these variants to hydrolyze the two stereoisomers of tabun by gas chromatography/mass spectrometry. H115W, H115W/Y71A, and H115W/F347W could catalyze the hydrolysis of tabun. The H115W/F347W mutant showed a preference for hydrolysis of the more toxic P(-) stereoisomer. This is a novel development since all PON1 variants published to date prefer the less toxic P(+) isomers. These data demonstrate the improvements made over H115W alone in developing new activities for the hydrolysis of various OP compounds in efforts to design a better catalytic bioscavenger.
P88. Modeling of interaction between sAnk1 and obscurin in striated muscle

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Binding of obscurin (obsc) to the muscle-specific small ankyrin1 (sAnk1) is important for organizing the sarcoplasmic reticulum in striated muscle. Experimental work investigating this interaction has been performed on obsc_6322-6339 and sAnk1_57-122. We used molecular modeling methods to obtain additional information on this interaction. Initial interaction between obsc_6322-6339 and sAnk1_57-122 was modelled using 10,000 Brownian dynamics simulations followed by the minimization of the complex, with solvation treated using the Generalized-Born method. Obsc is predicted to interact with R67,R68,R69,K73,K100,K101,R104,K105,R108 in sAnk1, in agreement with experimental results. 30ns MD simulations were then performed on six selected obsc-sAnk1 complexes. Results show that the alpha helical conformation of obsc is maintained in the complex, where it is stabilized by the interaction with the positively charged binding groove in sAnk1. Results from the simulations also predicted that K6337 on obsc interacts with D111 on sAnk1, a prediction that we recently confirmed by experiments. The modeling observed the dynamic and heterogeneous properties in sAnk1_57-122-obscurin_6322-6339 interaction.

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P89. CHARACTERIZATION OF THE MYC/PKA PATHWAY IN PROSTATE CARCINOGENESIS

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Gene amplification and overexpression of MYC have been reported in prostate cancer (PCa). Efforts to define the mechanism of MYC-induced carcinogenesis identified Protein Kinase A (PKA) catalytic subunit beta as a direct downstream transcriptional target of MYC. Elevated PKA C beta activity and increased levels of MYC have been reported to be concomitant with the proliferation of prostate epithelial cells. Reversal of MYC-induced tumor phenotype in fibroblasts by inhibition of PKA suggested PKA to be a potential downstream modulator in MYC-induced tumors. To understand the role of MYC in prostate carcinogenesis and the subsequent progression to the androgen independent form, we proposed to dissect the MYC/PKA pathway in prostate cancer cells. Using the Reverse In-Gel Kinase Assay (RIKA) we profiled bona fide substrates of PKA
in the proteome of prostate cancer cells. Initial studies identified MYC binding protein-1 (MBP-1), a transcriptional repressor of MYC and a potential tumor suppressor as a PKA substrate. We will investigate the consequence of PKA phosphorylation on MBP-1 function and its influence on MYC biology in the prostate. Furthermore, pharmacological inhibition of PKA in LNCaP cells led to a decrease in the steady state levels of MYC. A reverse effect was observed on activation of PKA. These results together with supporting data from in vitro kinase assay and mass spectrometric analysis suggest post-translational modification of MYC by PKA. Our data suggests phosphorylation of MYC by PKA to be important in its stability in LNCaP cells. Thus, understanding the molecular pathway downstream of PKA would help us define functional role of MYC in prostate carcinogenesis and would also clarify the role of PKA in prostate cancer.

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P90. Pan-Antagonist for Antiandrogen Resistant Prostate Cancer

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Prostate cancer remains one of the most commonly diagnosed cancers in men. The effectiveness of anti-androgens in the treatment of prostate cancer is well known. However, most patients eventually became resistant to antiandrogen treatment within several years and prostate cancer had progressed into antiandrogen resistant prostate cancer (ARPC). I have designed synthesized and evaluated pan-antagonists of androgen receptors associated with ARPC.

Androgen Receptor (AR) T877A and W741L mutations have been found in patients who experience resistance to antiandrogens, Flutamide and Bicalutamide, respectively. In 2005 Bohl et al published the first X-ray structure of AR W741L bound to bicalutamide in the agonist form. Structure-based computer model was been built and pan-antagonists are designed specifically to be universal antagonists that target not only wild-type AR, but also flutamide and bicalutamide resistant mutants of AR. Based on the design, I synthesized a series of expanded arylsulfone based pan-antagonists with or without long chain extensions. SAR studies were carried out on these ligands. Several ligands with long chain extension bind competitively to mutant ARs as well as wild type AR and also show pure antagonist activities in cell based assays. In particular, PAN52 can evade resistant cancer cell formation in in vitro clonogenic assay which mimic the progression of prostate cancer.

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P91. Inhibition of Pancreatic Phospholipase A2 Activity by Bile Salt

Ying Pan
P92. Tuning protein structure and function via novel electronic and stereo

Anil K. Pandey, Devan Naduthambi, Krista M. Thomas, Neal J. Zondlo

University of Delaware

Protein function is dependent upon protein conformation. Designing peptides having single or multiple switchable motifs for local or broader conformational modification may help understand the basis of protein folding, protein function, and macromolecular complex formation. These switchable entities may behave in contrasting manners under different cellular environments. We have designed and studied peptides that can undergo cis/trans isomerization utilizing aromatic-electronic effects.

A series of proline rich peptides, Ac-GPPXPPGY-NH2 (X = Y, F, W, 4-amino-phe, and electron-rich and electron-poor aromatic amino acids) was synthesized, and analyzed using circular dichroism (CD) and nuclear magnetic resonance spectroscopy (NMR). Multiple motifs were combined to generate a broadly tunable sequence. The electron rich aromatic ring of 4AF may stabilize the electron deficient H’s of the proline ring by CH-π interactions leading to a compact cis conformation, while the corresponding protonated form of 4AF lacks this ability, hence favors more PPII type trans conformation. In addition, dramatic changes in peptide or protein structure may be achieved through utilization of stereoelectronic effects to control protein main chain conformation. We have developed novel chemistry, termed proline editing, which provides us a strong handle to modify and functionalize proline substituted at 4-position by post-translational chemical modifications, which may be utilized to tune protein structure, stability, and function.

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P93. Structural Analysis of the FIV Matrix Protein

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University of Maryland: Baltimore County

The Feline Immunodeficiency Virus (FIV) is a retrovirus and infects domestic cats. Localization of the virus to the host plasma membrane is vital for retroviral replication. Viral assembly and budding at the plasma membrane are mediated by the Gag polyprotein. The Gag polyprotein is eventually processed into five different proteins, one of which is the matrix (MA) protein. The MA protein of the Gag polyprotein plays a significant role in virus assembly due to the co-translational addition of a myristoyl group.
The myristoyl group is used to anchor the MA domain of Gag to the membrane where virus assembly occurs. Our experimentation investigates how the myristoyl group targets FIV-MA to the plasma membrane and its role in viral assembly. Molecular cloning, protein expression, purification, and Nuclear Magnetic Resonance (NMR) are used in the synthesis and structural and functional analysis of the myristoylated and unmyristoylated FIV-MA protein. At this point, we have successfully synthesized pure unmyristoylated MA protein for NMR analysis, which has been confirmed by mass spectrophotometry analysis. Preliminary NMR data of the unmyristoylated MA protein has confirmed protein functionality. In addition, we are in the process of optimizing growth conditions for the myristoylation of the MA protein. The analysis of the data provided by this research will be used to determine the structure of FIV-MA, study virus assembly, and could provide insight into the mechanisms of FIV and other related viruses.

This work is supported by the HHMI Undergraduate Biological Sciences Education Scholars Program Grant #52003756, the NIH (AI) Grant #30917, and HHMI at the University of Maryland, Baltimore County.

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P94. Structural Changes and Sugar Binding in Lactose Permease of E. coli

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Lactose permease (LacY) of E. coli, an important member of Major Facilitator Superfamily (MFS) of membrane proteins, facilitates transport of various sugar molecules across the plasma membrane. Though the structure of LacY open to cytoplasm has been determined, the structure open to periplasm, which is crucial in understanding the detailed mechanism of transport, is unrevealed. Molecular Dynamics simulations with an explicit lipid bilayer were carried out with two different conformations of LacY obtained from previous implicit lipid bilayer simulations. The LacY structure open to periplasm is believed to have the Glu269 residue protonated. This was the only protonation state for which periplasmic opening was observed in implicit bilayer simulations. The same protonation state is used for the explicit bilayer simulations. Simulations were carried out in NPAT ensemble with a suggested experimental area of 65.2 Å² per lipid as well as area of 60 Å² per lipid. Pore radius analysis confirms opening of periplasmic end beyond the opening observed in the implicit bilayer simulations. The helix-helix distances of LacY were compared with the experimental results obtained from DEER experiments (Smirnova et al., PNAS, 2007). Ours is the first simulation study resulting in periplasmic structural changes that agree favorably with the suggested periplasmic open state from experiments. To quantify the affinity of sugars to LacY, free energies of binding for different sugar molecules are calculated using alchemical free energy.
perturbation (FEP) method. The binding affinity calculations are being used in determining the residues that are important in anomeric sugar binding.

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**P95. Protein-Protein Interactions of Rob and SoxS**

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Escherichia coli Rob, SoxS and MarA are monomeric members of the AraC/XylS family of transcription activators. SoxS and MarA are synthesized de novo in response to superoxide stress, salicylate, and antibiotics. Rob is unique, being constitutively expressed at 5,000-10,000 molecules/cell, but maintained in an inactive form consisting of 3-4 intracellular clusters. Dipyridyl and bile salts induce and disperse Rob into a monomeric form that can activate Rob-dependent genes. Once synthesized these transcription activators can activate a set of 40-50 genes that mediate the cells’ defense response.

Rob’s activity is controlled via a sequestration-dispersal mechanism where Rob is maintained in aggregates until induction occurs. Pull-outs were carried out to isolate Rob protein complexes and mass spectrometry was then used to identify potential protein partners of Rob.

Most transcription activators regulate transcription by the classical recruitment method, where the activator binds DNA at a promoter and then recruits RNA polymerase (RNAP). However, genetic and in vitro studies suggest that Rob, SoxS, and MarA activate via pre-recruitment, whereby binary complexes (BC) are formed with RNAP (e.g., Rob-RNAP) that then scans the chromosome for activator-dependent promoter site. SoxS and Rob were captured by the tagged β'-subunit along with the RNAP subunits (i.e. β, β’, α, σ), providing in vivo evidence that some activators form BC with RNAP that then effectively scans the chromosome for activator-dependent sites. Our future goals are to further elucidate the process of BC formation as well as the novel sequestration-dispersal mechanism for regulating the activity of an activator.

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**P95. Chemical Warfare - Lessons Learned**

**Morphological Expressions of Sulfur Mustard Skin Pathology**

John P. Petrali
ABSTRACT

Sulfur mustard (SM), a synthetic vesicating agent used effectively as a major chemical warfare agent in World War 1, continues to be a modern day threat agent of interest to some factions. However, despite its long history of military use and extensive research study, exact mechanisms of its pathogenicity remain investigatively elusive. Consequently, no specific pretreatment or antidotal therapy exists for victims of exposure. Whole body exposure results in ocular, respiratory and cutaneous pathology. Of these, cutaneous pathology has been fairly well characterized in controlled animal model studies, and its assessment is contributing to the basis of understanding of SM injury in exposed tissues not readily accessible for study. This presentation will summarize known histopathological, ultrastructural and immunohistopathological presentations of SM-induced skin pathology in controlled animal studies and in cultured skin equivalents. Emphasis is placed on early pathologies that might be potential sites for therapeutic intervention and for development of possible agent-specific diagnostic strategies.

The views, opinions, and or findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentations. In conducting research using animals, the investigator adhered to the Guide for the Care and Use of Laboratory Animals, National Research Council (National Academy Press, 1996).

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P96. Withdrawn

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P97. Investigations of carbapenem antibiotic biosynthesis.

Ryan M. Phelan, Micah J. Bodner, Michael F. Freeman, Mary L. Raber, Rongfeng Li, Samantha O. Arnett, Kristos A. Moshos, Benjamin J. DiPardo and Craig A. Townsned.
Carbapenem antibiotics are a subclass of the β-lactam family that present both resistance to β-lactamase inactivation, and improved antimicrobial activity compared to earlier generation penicillin and cephalosporin drugs. All clinically used carbapenem antibiotics, such as Imipenem and Meropenem, are derived from the natural product thienamycin and are produced by total synthesis. Penicillins, in contrast, are produced by semi-synthetic means which both lowers cost and increases availability of the antibiotic. Recent investigations of thienamycin biosynthesis are revealing the early and late synthetic steps. Synthetic standards and potential biosynthetic intermediates for this study have been prepared by adaptation of recently developed synthetic methods for the creation of cis-substituted azetidinones. We demonstrate the versatility and robustness of this methodology by developing a new and efficient route for the total synthesis of both N-acetyl thienamycin and epithienamycin A. The methods to produce both cis and trans-β-lactams were used to construct a library of proposed thienamycin biosynthetic intermediates varied in both stereochemistry and oxidation state. Using these newly acquired potential intermediates, the functions of enzymes in the thienamycin biosynthetic path have been identified. Investigations among these have shown the thienamycin N-acetyl cysteamine moiety originates from coenzyme A through modifications by four enzymes, ThnR, ThnH, ThnT and ThnF. Ongoing efforts aim to utilize enzymes with known function, as well as to engineer expanded enzymatic functions, to create a semi-synthetic route to these valuable carbapenem antibiotics.

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P98. Synthesis of Potential Inhibitors of Thymidylate Synthase

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University of Maryland, Baltimore County

Cancer is a group of diseases that causes about 13 percent of all deaths in the world, estimated at 7.6 million in 2007 alone. My research aims to create a set of drugs to inhibit an enzyme in the body fundamental to the out-of-control growth that cancer cells exhibit. This enzyme, Thymidylate Synthase (TS), catalyzes the conversion of Uracil Monophosphate to Thymidine Monophosphate using N5,N10-methylenetetrahydrofolate (THF) as a methyl donor. Cancerous cells need Thymidine to replicate, and therefore proliferate quickly in a TS rich environment. The compounds we propose are potential competitive inhibitor analogs of THF. Already, the necessary intermediates to the final six different THF analogs have been synthesized by condensation of commercially available carboxaldehydes and 1-ethoxy-3-methyl malonate to give UMR-150 (a-f) in 51 percent yield. Subsequent dehydration and decarboxylation of UMR-150 (a-f) with sodium ethoxide in ethanol formed the mono-ester product NP-001 in 63 percent yield. Both compounds have been verified through the use of 1H and 13C nuclear magnetic
resonance spectroscopy. A variety of approaches to ring closure of NP-001 have been unsuccessful, but we suspect that benzyl protection could resolve the issue. Once the final analogues are synthesized, we plan to carry out enzyme assays to determine their inhibitory properties.

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**P99. Engineering an Artificial Salivary Gland**

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Patients with cancer of the upper respiratory tract who receive radiotherapy suffer from xerostomia, a condition that would benefit from development of a functional implantable artificial salivary gland. Salivary gland tissue from surgical patients was assessed by histology and immunohistochemistry to establish the phenotype of normal salivary gland cells including the native basement membranes. Ductal and acinar cells were identified in tissue and cultured cells from dispersed tissue. High levels of laminin and perlecan/HSPG2 were noted in basement membranes and perlecan also was secreted and organized by cultured acinar populations, which formed lobular structures that mimicked intact glands when cultured on Matrigel™ or a bioactive peptide derived from domain IV of perlecan (PlnDIV). On either matrix, large acini-like lobular structures grew and formed connections between the lobes. α-Amylase secretion was confirmed by staining and activity assay. Biomarkers including tight junction protein E-cadherin and water channel protein, aquaporin 5 (AQP5) found in tissue, were expressed in cultured acinar cells. Cells cultured on Matrigel™ or PlnDIV peptide organized stress fibers and activated focal adhesion kinase (FAK). Efforts to devise a three-dimensional culture system with a hyaluronic acid (HA) hydrogel scaffold consisting of PlnDIV peptide are underway. Preliminary studies show self-assembly of acini-like structures with lumen on HA hydrogels containing PlnDIV peptide. The culture system consisting of PlnDIV peptide reported here will aid the development of an artificial salivary gland which will foster formation of functional salivary units capable of secreting salivary fluid and which can be implanted into patients to relieve xerostomia.

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P100. Histone-Mimetic Gold Nanoparticles as Gene Delivery Scaffolds

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The field of gene therapy has garnered significant interest over the past two decades as a method for revolutionizing the treatment of various diseases such as Alzheimer’s, Parkinson’s, and many types of cancer. In recent years, non-viral methods of delivery have received particular attention due to safety concerns and production limitations associated with viral vectors. However, inefficient DNA release is a common cause of ineffective non-viral DNA delivery. A novel solution to this constraint is the development of biomimetic scaffolds capable of regulating DNA accessibility. The presented study involves the design of histone-mimetic gold nanoparticles (HMGNs) as gene therapy packaging materials. Colloidal gold serves as a scaffold for the incorporation of histone H3 tail peptides trimethylated at lysine 4 (H3K4Me3). H3K4Me3 has a high density of positively charged residues that provide a DNA condensation template and impart protection from nuclease degradation. H3K4Me3 is known to be highly enhanced at the transcription start site for essentially all active genes. In addition, recognition of this trimethylated K4-containing peptide sequence by nucleosome remodeling factors has been implicated in mechanisms for chromatin activation. The purpose of the presented work is to assemble and characterize these HMGNs as well as to investigate the influence of HMGN functionalization on DNA binding, protection, and release. To this end, functionalized gold particles have been prepared. Their size and morphology have been characterized by methods such as TEM and SANS. In addition, the self-assembly of H3K4Me3 with plasmid DNA has also been investigated by dynamic light scattering, zeta-potential, nuclease assays, and cell transfection studies. These selected studies are aimed at validating the HMGN approach to gene delivery and will provide the framework for further development of our system.

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P101. Kinetic and Structural Characterizations of NAD+ Synthetase

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Glutamine-dependent NAD⁺ synthetase catalyzes the formation of NAD⁺ from nicotinic acid adenine dinucleotide, ATP/Mg²⁺ and glutamine. This enzyme belongs to the glutamine amidotransferase superfamily whose members utilize molecular tunnels to shuttle ammonia from one active site to another. We report the first structural characterization of a glutamine-dependent NAD⁺ synthetase, a remarkable 600,000 Da
enzyme. Structures with different combinations of ligands are described. In addition, kinetic characterizations of wild-type and mutant enzymes provide insight in the mechanism of regulation of active site coupling and ammonia transfer. The structural and kinetic information acquired lays the foundation for the development of inhibitors of the M. tuberculosis NAD⁺ synthetase.

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**P102. Novel Arginine Mimetics via alpha-Guanadino Acids**

Michael J. Scheuermann, Shalini Balakrishnan, Susan Carr Zondlo, Neal J. Zondlo

University of Delaware

Guanidinium groups are widely used in protein interactions, participating in both electrostatic and hydrogen bonds. However, incorporation of arginine residues into peptide ligands can be problematic because of the linear alkyl side chain of an arginine residue, leading to a decrease in specificity of the ligand to its binding site. Arginine mimetics address this issue by using additional functional groups in tandem with the guanidinium group that is critical for binding. Previous work has shown the ability to guanylate α-amino acids, generating a-guanidino acids, and to incorporate them into small molecules. By creating a peptide with the amino acid Diaminopropionic acid (Dap), we are able to couple these a-guanidino acids onto the Dap residue on the peptide to make novel arginine mimetics that build in greater functionality, in the form of chirality and side chains, than the arginine precursor. In order to test these arginine mimetics, the arginine in a Src homology 3 (SH3) domain ligand was replaced by a Dap residue coupled to one of the following guanylated residues: L-Val, D-Val, L-Phe, D-Phe, L-Trp, D-Trp or Gly. These arginine mimetics were stereospecifically incorporated into peptide ligands as determined by NMR, while retaining binding to the domains SH3 domains of Src and Grb. Binding was further tuned by modifying the chirality and side chain incorporated in the ligand.

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**P103. Synthesis of a novel base analogue for enhanced triplex formation**

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The major limitation of triplex forming oligonucleotides, instability of triple stranded molecules resulted from DNA duplexes other than composed from polypurine-polypyrimidine sequences, was approached. The fragment of HPRT Chinese hamster gene with CG interruption was used as the DNA target site and several N⁴-alkylated
cytosine analogues were screened in the developed binding assay. It was found that amidine type hydrogen donor at a distance of four atoms from N^4 position of cytosine was best in pairing CG interruption. The synthesized third strand oligonucleotide showed similar stability in melting temperature and superior selectivity in cell line experiments to its matched target compared to the oligonucleotide that had thymidene at that position.

P104. Cloning, Expression, Purification & Binding Studies of CR units of LRP

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LRP (low density lipoprotein receptor –related protein) is a member of the low density lipo protein family. The members of the family are involved in many biological pathways including Wnt signaling pathway and clearance of amyloid peptide from the blood stream. They also mediate the internalization of various ligands of different shape and size. In LRP, the ligand binding sites are located throughout the different clusters and the binding is calcium dependent. Each ligand binding site is a ~40 residues, cysteine rich continuous repeat unit. The RAP (receptor associated protein) competes with other ligands and binds more efficiently with LRP. This avoids the premature binding of ligands to LRP before it exports to the cell surface. It has been shown that complement type repeat units 5, 6 and 7 (CR567) are important for the binding of RAP.

In order to study the structural and functional mechanism how RAP potentially inhibits the binding of other ligands and how LRP molecule binds with structurally different ligands using CR units, we made the various expression constructs of CR567 unit of LRP with SUMO tag to express in E.coli and Pichia pastoris in soluble form. Proteins were expressed, purified and characterized with HPLC, mass spectrometry and circular dichroism. ITC and other biophysical methods were used to study binding of the purified proteins with the ligand RAP and lactoferrin, and to optimize expression and purification methods.

P105. NMR Spectroscopy of Streptococcus oralis YC1 Polysaccharide Coating

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This research project centers upon discovering the true structure of a polysaccharide on the cellular surface of the Streptococcus oralis YC1 strain. This strain is a mutant from the Streptococcus oralis C104 strain and its polysaccharide coat contains four beta galactoses, one alpha galactose and one ribitol. The YC1 strain was created by exchanging the Streptococcus oralis C104 UDP-galactose glycosyl transferase gene with that of the Streptococcus pneumoniae 10F’s through the use of restriction enzymes. The sugar chain that was created on the surface of these bacteria due to this change is proposed to be an exact replica of the Strep. oralis C104 except for the linkage position between the alpha galactose and ribitol. The C104 polysaccharide has a linkage of (1,1) while the YC1 is hypothesized to be different. To test this hypothesize, an array of two dimensional NMR spectra prove to be extremely useful. By collecting and analyzing different types of spectra such as COSY, TOCSY, NOESY, HSQC and HMBC, we will determine the structure of the YC1 polysaccharide. This will provide us with a confirmed linkage position between the alpha galactose and ribitol. Ultimately, our findings may result in a better understanding of the biochemistry involved between the polysaccharides of microbes and their environment. It could also possibly lead to the creation of new vaccines that use non-pathogenic bacteria to facilitate immunization against harmful pathogens.

P106. Synthesis and antiviral study of Ring Expanded Nucleosides/tides

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A number of ring-expanded nucleoside (REN) analogs containing the title imidazo[4,5-e][1,3]diazepine ring system exhibited potent in vitro inhibitory activity against a variety of viral NTPases/helicases of the family of Flaviviridae, including the Hepatitis C virus (HCV), the West Nile virus (WNV), and the Japanese encephalitis virus (JEV). The anti-HCV NTPase / helicase activity steadily increased with the increasing length of the alkyl chain at position 6 of the ring mentioned above, and became significantly pronounced when the chain length was extended to 18 carbon atoms. Guided by the rationale offered by molecular modeling studies conducted on the analog mentioned above, and as part of the SAR studies, we made modifications at position 6 of the heterocyclic ring Imidazo[4,5-e][1,4]diazepine ring system with a variety of alkyl, aryl and aralkyl substituents and at position-4 with different alkyl groups. The most potent of the above nucleosides as judged from the biochemical screening studies against enzymes, NTPase/helicase and RdRp, as well as by tissue culture results against the whole virus will be converted into 5’-mono, di- and/or tri-phosphate derivatives.

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P107. NMR Detected pKa Perturbation of A340/341 of the MMLV Packaging Signal

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The Moloney Murine Leukemia Virus (MMLV) is a gamma-type retrovirus that is used commonly as a model system to understand the retroviral life cycle. The MMLV genome is composed of two single strands of RNA linked non-covalently via RNA structural elements located within the 5’ non-coding region of the genome. Stem loop C (SLC) of MMLV is a critical RNA element that has been implicated in genome dimerization by forming intermolecular “kissing” complexes with a second stem loop, SLD.

In an effort to understand the structural basis of genome dimerization, we investigated structural properties of an SLC fragment that retains features of the intact stem loop: i) (331)GAGC(334) tetraloop and ii) (338)GGAA(341) bulged loop. Both the GAGC tetraloop and the GGAA bulged loop are conserved structural elements across gamma-type retroviral lineages.

Using 1H-13C Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectroscopy, we discovered that the N1 position of adenosine 340 and 341 have atypical pH dependent properties. By monitoring the change in the adenosine C2 carbon chemical shift recorded from HMQC spectra collected at several pH values, we show for the first time that the pKa values of adenosine 340 and 341 is shifted well above the pKa value expected for free adenosine (c.a. 3.8). Additionally, our preliminary NMR data suggests that the perturbed pKas may play a role in modulating local RNA structure at the GGAA bulged loop. These results may imply a hitherto unreported role for a pH induced conformational change regulating the structure and folding of SLC.

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P108. Brain Structure Specific Responses in Soman-induced Seizures

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Seizure activity resulting from nerve agent (NA) exposure can rapidly progress to severe stages of status epileptics (SE) consistent with masticatory or generalized convulsive SE. These seizures activate a neural network that includes the mediodorsal thalamus (MDT),
perirhinal cortex (PRC), and entorhinal cortex (EntC). Pharmacological control of this seizure activity is critical in reducing neuropathology and improving survival in casualties. This study evaluated drugs from three pharmacologically distinct classes (muscarinic antagonist, scopolamine; benzodiazepine, midazolam; N-methyl-D-aspartate (NMDA) antagonist, MK-801) injected directly into specific brain areas (MDT, PRC, or EntC) for their ability to block seizures induced by the NA soman. Rats, surgically prepared to record brain electroencephalographic (EEG) activity and implanted bilaterally with cannula toward a designated brain area, were pretreated by microinjection (1 μl/canula) with a treatment drug and intraperitoneal HI-6 (125 mg/kg) thirty minutes prior to subcutaneous injection with 180 μg/kg soman, followed by 2.0 mg/kg atropine methyl nitrate intramuscularly. Animals were then returned to a cage where EEG activity was monitored. Anticonvulsant ED50 values were calculated from an up-down dosing procedure over successive animals. Scopolamine and MK-801 provided comparable anticonvulsant effects, except in the MDT where MK-801 had a significantly lower anticonvulsant ED50 value. Midazolam required significantly higher doses than scopolamine and MK-801 in the PRC or EntC to produce an anticonvulsant effect and was ineffective in the MDT. These findings suggest that NMDA and muscarinic receptor mediated neurotransmission are critically involved in the control of NA-induced seizures within the areas tested.

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P109. Characterization of two eukaryotic UDP-galactopyranose mutases

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UDP-galactopyranose mutase (UGM) catalyzes the conversion of UDP-galactopyranose to UDP-galactofuranose, which serves as the precursor for the galactofuranose (Galf) present on the cell surface of several parasites and the cell wall of bacteria and fungi. Galf has been shown to be essential for microbial survival and pathogenesis. Since Galf is not present in humans, its biosynthetic pathway is an attractive target for the development of novel antibiotics, anti-fungal, and anti-parasitic drugs. UGM is a flavoenzyme, which despite catalyzing a reaction with no net redox change requires the reduced form of the flavin for catalysis. We present the successful expression and purification of active UGM from the parasite Trypanosoma cruzi (TcUGM) and the fungus Aspergillus fumigatus (AfUGM). Both enzymes have higher activity in their reduced form. Size exclusion chromatography experiments show that TcUGM is a homodimer while AfUGM is a pentamer. Mutation of a strictly conserved tryptophan residue indicates that this amino acid plays a role in binding the uridine ring of the substrate. We have also found conditions for the formation of AfUGM diffracting crystals. Using limited proteolysis analyses, it was determined that conformational changes accompany substrate binding only in the reduced, active form of the enzyme.

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P110. Effect of Rapamycin on Filamentous Fungal Cell Walls

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Filamentous fungi are widely used in the bioprocess industry to produce a variety of products resulting in billion dollar returns. Often times in industrial bioprocesses, fungi experience nutrient limitation, and we hypothesize that autophagy, a nutrient starvation response, is induced. We further hypothesize that autophagy leads to changes in the mechanical properties of filamentous fungal cell walls, resulting in thinner, weaker and stiffer walls. We test this hypothesis here, using rapamycin (an immunosuppressant drug) to gratuitously induce autophagy in the model fungi Aspergillus nidulans. Atomic force microscopy (AFM) is used to assess the mechanical properties of the cell wall, electron microscopy is used to assess wall thickness and a novel fragmentation assay is used to determine relative tensile strength of the culture. We will report on these studies and how they support our hypotheses.

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P111. Using MS to Search for HLA-A2 Bound Peptides on Pancreatic Tumor Cells

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Pancreatic adenocarcinoma (PDA) is the 4th leading cause of cancer-related deaths in the United States. Immunotherapy, designed to induce immunological responses directed against antigens expressed by tumor cells, is an innovative approach being developed for the treatment of PDA. Our collaborators have developed an allogeneic granulocyte-macrophage colony-stimulating factor-secreting vaccine capable of stimulating anti-tumor immunity in patients. They identified mesothelin as a potential pancreatic tumor antigen because it is up-regulated in most pancreatic cancers but shows limited expression in normal tissues. Two computer algorithms were used to identify two mesothelin-derived peptides that bind the HLA-A2 molecule. More recently peripheral
blood lymphocytes (PBL) derived from a vaccinated HLA-A2+ patient were used to screen an overlapping peptide library spanning the entire amino acid sequence of mesothelin, resulting in the identification of five more mesothelin-derived peptides of interest.

In the current study, we are now interested in determining if these seven mesothelin nonamers are naturally processed and presented by HLA-A2 on the surface of pancreatic tumor cells. To determine if this is the case, the HLA-A2 molecules and their associated peptides from $1 \times 10^9$ pancreatic tumor cells are isolated using immunoaffinity chromatography. The isolated HLA-A2/peptide complexes are then dissociated and the peptides are isolated by using a 5kD filter. The resultant peptide mixture, representing all peptides presented by HLA-A2, are then analyzed using a Thermo LTQ-Orbitrap mass spectrometer. The presence of the mesothelin-derived peptides are confirmed by comparing the MS$^2$ spectrum from the sample to the MS$^2$ spectrum of the synthetic peptides.

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**P112. ITC Approach Towards Understanding Retroviral Genome Packaging**

Patrice Starck, Yasuyuki Miyazaki, Michael Summers

UMBC, UMBC, UMBC

Understanding retroviral genome packaging could prove advantageous to discovering a new class of retroviral inhibitors. The Moloney Murine Leukemia Virus (MoMuLV) is a simple retrovirus, which is commonly used as a model system for other retroviruses such as HIV. Retrovirus assembly requires two copies of their full-length unspliced genomes be packaged into newly budding virions. One necessary step in retrovirus replication involves the interaction between the nucleocapsid domain (NC) of the GAG poly protein and the 5’UTR of the gRNA. The packaging signal, Psi-site (212-563) of MoMuLV is located in the 5’-UTR of the RNA genome. The mechanism that exclusively packages dimeric gRNA into the virion, is not fully elucidated. It has been shown that a minimal region within the Psi-site, known as the Core Encapsidation Signal, Psi-site$^{CES}$ (278-374), is sufficient to direct genome packaging. Upon dimerization of Psi-site$^{CES}$, the YYG sequence, which contains nucleocapsid (NC) recognition elements, is exposed as a linker. Isothermal titration calorimetry was used to determine the stoichiometry of NC for the monomeric and dimeric conformations of the entire Psi-site. ITC yields information about the dissociation constant (Kd), the stoichiometry, and the free energy of reaction (delta G). This study was conclusive in proving that the dimeric form of the Psi-site has more binding sites for interactions with NC than the monomeric form. Our results suggest the mechanism for how NC binds to the Psi-site to escort the viral RNA to the host cell wall for budding.

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P113. Characterization of ribosomal/ribosomal associated proteins deletions

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Saccharomyces cerevisiae genome harbors five families of long terminal repeat (LTR) retrotransposons. Retrotransposons are mobile DNA elements consisting of two open reading frames, TYA and TYB analogous to gag and pol proteins of retroviruses respectively. The pol protein is produced as a fusion to the upstream gag protein and this requires a +1 programmed frameshift event. Ty1 transposition has to be kept to a minimum for survival of the host cell. A screen identified a set of proteins that restricted Ty1 transposition (Ty1 ‘restriction’ genes) and another that helped in Ty1 transposition (Ty1 ‘helper’ genes). The Ty1 helper genes included some genes that encoded the large subunit and small subunit ribosomal proteins. The list also included other genes that are associated with either translation or ribosome biogenesis. Strains harboring deletion of the above said genes were assayed for Ty1 programmed frameshift activity. Two deletion strains showed a significant increase in frameshift activity while nine of them showed decreased activity. These eleven proteins were further characterized for their misreading phenotype, the subunit level and bulk translation. Interestingly one of the ribosomal proteins that show decreased +1 frameshifting (but increased transposition) is ASC1, homolog of mammalian RACK1, a signaling protein in the glucose response pathway. ASC1 interacts with the ribosome and Gpa2 (protein involved in the glucose response pathway) using the same interacting surface. The main goal of my project is to further test the eleven deletion strains for rRNA processing defects, increased sensitivity to antibiotics and budding defects. We will also further extend our analysis on ASC1 protein by doing a random mutagenesis and isolate mutants that affect the glucose response pathway without affecting its ribosome function.

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P114. Synthesis of Novel Ring Expanded (“Fat”) Nucleoside Analogues

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Adenosine deaminase (ADA) is an enzyme involved in purine metabolism. High levels of ADA have been observed in malignant human lymphocytes, thus making inhibitors of ADA as important therapeutic targets. Coformycin and Pentostatin are the two naturally occurring, extremely tight binding, transition state analogue inhibitors of ADA. However, the severe toxicities of coformycin and pentostatin have greatly limited their clinical use.
Therefore, analogues of coformycin and pentostatin that are less toxic, easily reversible and still potent inhibitors are needed. The main aim of this research is to synthesize 5:8 fused heterocyclic nucleosides as analogs of coformycin and pentostatin for potential inhibition of ADA. The anticipated hydrophobic character of the 8-membered ring of the target 5:8-fused compounds is believed to enhance interactions with the hydrophobic amino acid residues such as valine, isoleucine, and tryptophan present in the active site of ADA.

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**P115. Regulation of Protein Kinase CK2 by Post-translational Modifications**

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Protein Kinase CK2 (formerly known as casein kinase II) is a highly conserved, constitutively active protein kinase with over 300 known substrates. It plays a key role in cell proliferation and has been implicated in several disease states. Despite its crucial role in the cell, its regulation is poorly understood. It has been demonstrated that the catalytic subunit of CK2 is post-translationally modified on its C-terminal tail with phosphorylation and O-linked GlcNAc sites. These dynamic modifications have been shown to modulate protein-protein interactions, cellular localization and stability of other proteins on which they are found. Phosphorylation is also a common way for the cell to regulate the activity of certain kinases. The addition of O-GlcNAc to Ser/Thr residues is involved in regulating nuclear and cytoplasmic proteins in a manner analogous to protein phosphorylation. The role of these modifications on the regulation of CK2 is examined using protein semisynthesis techniques to generate homogenous samples of CK2 possessing the site-specific post-translational modifications. Kinase activity, cellular stability and localization, and protein-protein interactions are examined to determine if they are modification-dependent.

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**P116. Interpretation of p-Cyanophenylalanine Fluorescence in Proteins**

Humeyra Taskent, Juah Chung, Scott H. Brewer, Ryan A. Mehl, Daniel P. Raleigh, and Isaac Carrico

State University of New York at Stony Brook, State University of New York at Stony Brook, Franklin and Marshall College, Franklin and Marshall College, State University of New York at Stony Brook, State University of New York at Stony Brook
The amino acid p-cyanophenylalanine (FCN) has very useful fluorescence properties which make it a powerful probe of protein folding. FCN fluorescence is sensitive to environment, having a high quantum yield when the cyano group is H-bonded, but the factors which control FCN fluorescence are not yet completely understood. We used the N-terminal domain of L9 (NTL9) as a model protein to study FCN fluorescence. The group was recombinantly substituted for F5, a core residue. The fluorescence is low in the folded and high in the unfolded state, however IR spectroscopy indicates that the cyano group is exposed in the folded state and solvated. Mutation of Y25 to F demonstrates that Tyr quenches the native state fluorescence which indicates that caution must be used when interpreting FCN fluorescence in term of solvent exposure. The effectiveness of other side chains as quenchers was probed by examining a set of peptides; GGFcnXA where X was A, H, K, N, or Y. Tyr is the most effective quencher.

P117. Oxygen transfer in process scouting devices and bioprocess design.

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In the past few years, an acceleration of the design and development of novel miniature process scouting devices (0.1ml to 100ml, PSDs) has taken place. Despite their wide use in bioprocess development (i.e. process optimization and process validation), PSDs are considered “black boxes” because they are generally not equipped with sensors. In this study, we show that in-line monitoring of dissolved oxygen (DO) in a T-75 flask based PSD can be achieved during cell cultures and that this information can be linked to the health state of the cells. In this case, we were able to monitor specific oxygen uptake rate fluctuations between 6.3 to 3.3 (x10⁻¹⁰ mmol/cells.h) as a hybridoma cell line evolved between thaw and subculturing up to 33 passages (100 days). This is particularly useful because very early pre-culture in commercial settings is often performed in unmonitored T-flasks prior to scaling-up to spinner vessels and/or bench-scale bioreactors.

P118. Identification and virtual screening of binding sites for BCL6 inhibi

Kenno Vanommeslaeghe,* Gustavo F. Da Silv‡, Alexandru F. Ghetu,† Xiao Zhu,* Shijun Zhong,* Leandro Cerchietti,† Marilyn Matthews,* Jose M. Polo,* Andrew Coop,* Gilbert G. Prive,†,§,¶ Ari Melnick† and Alexander D. MacKerell Jr.*
BCL6 is a potent transcriptional repressor with critical roles in several immunological processes. Additionally, BCL6 has been shown to play a central role in the pathogenesis of diffuse large B cell lymphoma, and inhibitors of BCL6-mediated repression show promise as therapeutic agents for B cell lymphomas. The present work is part of a multidisciplinary collaboration aimed at developing novel anti-cancer drugs that target BCL6. 

In silico screening was performed on a library of commercially available compounds. Starting from X-ray structures, we first performed a Molecular Dynamics (MD) simulation that gave us insights into the flexibility and thermal motion of the BCL-6 protein, information used to account for protein flexibility in the docking procedure. The X-ray crystallography also led to the identification of BCL-6’s protein-protein contact faces, on which the “binding response” algorithm was applied to identify “druggable” binding sites. These binding sites were subjected to a multi-step docking procedure, aimed at identifying promising BCL6 inhibitors. In parallel, the same library was screened for compounds that are chemically similar to known BCL-6 inhibitors. In conclusion, a combination of ligand-based and structure-based drug design strategies was used to identify new leads in the search for anti-cancer drugs that target BCL6.

1. K. F. Ahmad et al., Mol. Cell 2003, 12, 1551–1564

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**P119. Determination of sulfathiazole and lincomycin residues in honey using solid-phase extraction and liquid chromatography-electrochemical detection**

Suma R. Vavilala, William. R. LaCourse

University of Maryland, Baltimore county

Antibiotics are mainly used in veterinary and human medicine for therapeutic purposes. In honeybees, these are used for the prevention and treatment of American foulbrood disease. These drugs also commonly used as growth promoters for food-producing animals. If proper withdrawal times are not observed before the slaughtering of medicated animals, food from these animals may be contaminated with residual amounts of antibiotics. The presence of such residues in food is of toxicological and
regulatory concern as some of these may be carcinogenic, cause allergic hypersensitivity reactions, and reduce the therapeutic effectiveness of these drugs on humans. For these reasons, it is crucial to develop effective methods for trace level detection of these antibiotics.

A HPLC-EC method has been developed for the determination of sulfathiazole and lincomycin in honey. Sample pretreatment was done by solid-phase extraction using OASIS HLB cartridges. The method detection limits were determined to be 30 µg/kg and 160 µg/kg for sulfathiazole and lincomycin respectively. Average analyte recoveries for sulfathiazole 79% to 67% in replicated sets of honey fortified with drug concentration of 100 µg/kg, 200 µg/kg and 800 µg/kg. Average analyte recoveries for lincomycin 67% to 70% in replicated sets of honey fortified with drug concentration of 900 µg/kg, 1000 µg/kg and 2000 µg/kg.

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P120. Potent inhibitors of c Myc Max dimerization through bivalent binding to

Arrole Viacava Follis1, Dalia Hammoudeh1, Huabo Wang2, Edward V. Prochownik2, Steven J. Metallo1

1Chemistry Department Georgetown University Washington DC, 2Children's Hospital Pittsburgh PA

We have shown that selective inhibitors of dimer formation between the oncogenic basic-helix-loop-helix-leucine zipper (bHLHZip) transcription factor c-Myc and its bHLHZip partner protein Max act by binding to the intrinsically disordered (ID) c-Myc monomer. Multiple, independent sites for inhibitor binding were found along c-Myc bHLHZip. We exploited the multiplicity of these sites to generate novel compounds capable of multivalent binding to c-Myc. Despite their disordered protein target, these molecules bind purified c-Myc with low nano-molar affinity, which is orders of magnitude tighter than that of c-Myc's obligate heterodimerization partner Max. The inhibitors effectively disrupt c-Myc-Max dimerization and specific DNA binding; they also inhibit growth of c-Myc overexpressing cancer cell lines in vitro.

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P121. Reversible Quinone Methides Alkylation of DNA

Huan Wang and Steve E. Rokita*

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Quinone methide intermediates (QMs) have been implicated in a range of biological processes and can effectively alkylate numerous cellular components, including
DNA. The reversibility of some QM reactions has recently been illustrated with a bis-functionalized QM-acridine conjugate (bisQMP). The presence of a strong nucleophile such as dA preserves the ability of bisQMP to cross-link DNA under aqueous conditions, whereas other deoxynucleotides show no similar capacity. Oligonucleotides too can preserve QM intermediates by forming intrastrand adducts for later transfer to complementary sequences resulting in selective formation of interstrand cross-links. An oligonucleotide composed of only G and T has a strong ability to trap bisQM and facilitate its cross-linking to a complementary sequence. However, this activity is greatly suppressed when G is replaced by either A or C.

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**P122. Platensimycin analog syntheses and the biological evaluation**

Jingxin Wang, Vincent Lee, Herman Sintim

Department of Chemistry and Biochemistry, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park

Platensimycin, a specific inhibitor of bacterial FabF enzyme, has attracted considerable interest from both chemists and biologists because of its complex chemical architecture and potent antibiotic activities against Gram-positive bacteria. We have provided a simple approach towards platensimycin and analogs. Keys steps in our platensimycin synthesis are, a dynamic ring-closing metathesis reaction, nucleophilic addition to a ketone followed by tandem epoxide ring-opening reaction. A simple two step reductive amination was used to synthesize platensimycin analogs. Initial biological testing indicates that the tetracyclic core of platensimycin is important for the drug’s efficacy.

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**P123. A Photoactivatable UK-1 Analog: Probing Mechanism of Topo II Inhibition**

Dawn N. Ward, Dimitra Kontokosta, and Paul J. Smith

Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore MD 21250

DNA topoisomerase II (topo II) is an enzyme responsible for altering the topology of DNA. It plays a critical role in strand passage events required for separation of daughter chromosomes during cell division; as a result, this enzyme is an important target for the development of anti-cancer agents. UK-1 is a natural product that is cytotoxic to a number of cancer cell lines and an inhibitor of topo II. There are three mechanisms by which topo II inhibitors act: (1) stabilization of the DNA-enzyme covalent complex by hindering relegation of nicked, enzyme-bound DNA; (2) prevention of the enzyme...
association with DNA; and (3) prevention of ATP hydrolysis by binding to the ATPase domain. Currently, UK-1’s mechanism of inhibition towards topo II is unknown. This research focuses on understanding the mechanism of inhibition through the use of a photoaffinity probe. Using photoaffinity labeling, structural information regarding points of contact between a drug and the targeted protein can be obtained. Accordingly, a derivative of UK-1 containing an azido group has been synthesized. Photoactivated azides form nitrenes, which are highly reactive intermediates that can undergo insertion into C-H bonds. The newly synthesized azido-UK-1 compound was incubated with topo II, photolyzed, and the protein was digested with trypsin. The resulting peptides were analyzed using LC-MS and the data was correlated using the Collaboration of MS3D (C-MS3D) program. Progress to date will be reported.

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P124. Natural Product Diversification by Chemoselective Reactions

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Glycosylated natural products are a reliable platform from which many clinically useful drugs have been developed. The difficulties associated with carbohydrate chemistry have impeded progress in understanding the role of the sugar in biological activity. The Thorson lab has adapted the chemoselective reaction between a secondary alkoxyamine and a reducing sugar, neoglycosylation, allows for the rapid generation of natural product libraries. This technology has been used to synthesize a library of 33 podophyllotoxin neoglycosides and their antiproliferative activity has been assessed.

The availability of sugar substrates for neoglycosylation is often cited as a limitation and has stimulated a search for alternatives. Morpholinolactols have the requisite features for participation in a chemoselective reaction similar to neoglycosylation. Moreover, morpholines have been recognized as having desirable pharmaceutical properties and have been used to develop new drugs. A method for the facile synthesis of morpholinolactols using -amino alcohols is demonstrated and our hypothesis that the compounds participate in the chemoselective reaction with the secondary alkoxyamines.

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P125. Requirements for H. pylori NikR-DNA interaction.

Abby L. West, Nujeveen Dosanjh, Sarah L.J. Michel
HpNikR is a nickel binding transcription factor found in *Helicobacter pylori*. HpNikR regulates the transcription of a battery of key *H. pylori* genes including those involved in acid adaptation and nickel ion homeostasis. The promoter sequences recognized by HpNikR differ in sequence and the mechanism by which HpNikR recognizes and distinguishes between these sequences is not well understood. Direct and competitive fluorescence anisotropy (FA) assays were developed to discern metal ion requirements and sequence specificity. These studies revealed that a second metal ion, in addition to nickel, was required for DNA binding. This second metal ion was identified as magnesium, manganese, or calcium. Moreover, HpNikR bound to its target DNA with either tight (nanomolar) or weak (micromolar) affinity leading us to propose that HpNikR utilizes a two-tiered mode of DNA recognition.


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**P126. Light Activated Fluorescent Labeling of Proteins**

Bryan J. Wilkins, Xu Yang and T. Ashton Cropp

University of Maryland

Biochemical activity occurs as a cascade of cellular events which happen at specific intervals depending on the stimulus. This spatial and temporal association is extremely difficult to monitor with typical biochemical assays. Once an agent is introduced to a system the control of a defined instance for its use is lost. Recently, we extend a level of spatiotemporal control to the biarsenical pro-fluorescent dye 4,5-bis(1,3,2-dithiasolan-2-yl)fluorescein (FlAsH) that specifically binds to, and is activated by, the small amino acid tag sequence, CCPGCC. This nonfluorescent molecule exchanges 1,2-ethanedithiol (EDT) for the tetracysteine motif generating a distinctly fluorescent protein-bound complex. We take advantage of the inability of FlAsH to efficiently exchange the thiol-arsenic adducts in the presence of a cysteine mutation by introducing the genetically encoded unnatural amino acid *p*-nitrobenzylcysteine, site-specifically to the tetracysteine tag. These protected (caged) amino acids are decaged upon irradiation at 365 nm, generating natural cysteine. We have successfully shown that FlasH labeling of a protein can be controlled by light where the binding of FlasH to the tetracysteine tag only occurs following UV irradiation.

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**P127. The Oriented, Encodable Adsorption of Proteins Onto Gold Surfaces**

Alison M. Williams and Steven M. Metallo
Georgetown University

There has been increased interest in developing new methods to control the orientation of active proteins onto metal surfaces. In current methods, proteins are attached to metal surfaces covalently (through chemisorption) or non-covalently (through physisorption). However, physisorbed proteins are not stably attached and oriented on the surface. Controlling the orientation of proteins on the metal surface is crucial to preserve the activity of the native protein. Chemisorbed proteins are more stably attached, but are typically oriented randomly, and are often immobilized through residues which may decrease their activity. We have developed a direct and stable genetically encodable method for the oriented attachment of proteins to gold nanoparticles and metal surfaces, through the tetracysteine motif (C-C-P-G-C-C). Agarose gel electrophoresis and fluorescence studies have confirmed that ubiquitin (Ub) and enhanced green fluorescent protein (eGFP) mutants containing the tetracysteine motif bind to the surfaces of gold nanoparticles in an oriented manner. Ellipsometry and confocal microscopy have been employed to study the attachment of proteins on bulk gold surfaces, and to explore the application of the motif in bio-sensors.

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P128. Detection of alcohol metabolites in urine with HPLC coupled with condu

Melinda K. Wilson, Wiliam R. LaCourse

University of Maryland Baltimore, University of Maryland Baltimore County

After consumption of alcohol, the bulk of the ethanol dose (95-98%) is eliminated in a two stage oxidation in the liver, first to acetaldehyde then further to acetic acid. A very small fraction of ethanol (<0.1%) undergo phase II conjugation reactions to produce ethyl glucuronides via UDP-glucuronidase, ethyl sulfates via sulfotransferases, and ethyl phosphate via dephosphorylation of ATP. Modern post-mortem and behavioral toxicology has focused on glucuronides, allowing for easy detection due to high abundance of metabolites from that pathway.

Previous work in this laboratory has used pulsed amperometric detection following a reversed phase separation to detect ethyl-glucuronide, a metabolite of alcohol. This work expands on that, by allowing all three metabolites, ethyl glucuronide, ethyl sulfate, and ethyl phosphate to be detected in a single chromatographic run. All three are ionic in biological matrices, including urine, making them ideal candidates for conductivity detection following ion chromatographic separation.

This poster will outline the development of the ion chromatographic separation of the three metabolites and their subsequent detection using conductivity detection. Analytical figures of merit will be given, and the method will be compared against existing
approaches. Sample preparation will be discussed in detail. This project will have long standing effects in the forensic science community by allowing a confirmed detection of alcohol intake.

P129. Functional Characterization of Mutations in the FANCJ Helicase that Cause Fanconi Anemia and Breast Cancer

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Fanconi anemia (FA) is a recessively inherited disease characterized by congenital abnormalities, bone marrow failure, and a predisposition to cancer. Cells from FA patients are hypersensitive to DNA cross-linking agents such as mitomycin C and cisplatin, suggesting that FA is a DNA repair disorder. FANCJ, one of thirteen genes linked to FA, was originally identified by its association with breast cancer and protein interaction with BRCA1. The FANCJ gene encodes a helicase that is proposed to operate in a BRCA/FA pathway of homologous recombinational repair; however, its roles in cellular DNA metabolism are not well understood. In this study, purified FANCJ recombinant proteins encoded by clinically relevant FANCJ mutations were characterized for their catalytic activities. Biochemical analyses demonstrated that these mutations could be categorized into three groups according to their effects: 1) low, but detectable, helicase and ATPase activity (e.g. R707C); 2) normal ATPase using long (> 50 nt) ssDNA effectors, but abolished helicase activity (e.g. Q255H and A349P); and 3) abolished ATPase and helicase activity (e.g. R798X).

We have focused on characterizing the effect of one disease-causing FANCJ missense mutation, A349P, which resides immediately adjacent to a highly conserved cysteine residue of the Iron-Sulfur cluster domain. The A349P mutation abolished FANCJ helicase activity on duplex and quadruplex DNA substrates by disrupting its ability to translocate on DNA. FANCJ-A349P is defective as an ATPase on short DNA tracts (<10 nt), suggesting that its poor ability to favorably interact with short unwound ssDNA regions and hydrolyze ATP is responsible for its failure to translocate processively on DNA and unwind adjacent structured DNA. Genetic studies demonstrate that the FANCJ-A349P allele poorly complements the cross-link sensitivity of a FA-J null cell line as detected by cisplatin resistance or gamma-H2AX foci formation after DNA damage. Collectively, the biochemical and genetic results demonstrate that the ability of FANCJ to processively translocate and unwind DNA in an ATP hydrolysis dependent manner is
required for its DNA repair function. This study provides the first molecular insight to our understanding of the pathological mechanisms responsible for FA and breast cancer.

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**P130. Anticancer activity of ring-expanded heterocyclic bases, nucleosides**

Min Xie, Ramachandra S Hosmane

University of Maryland at Baltimore County

The ring-expanded nucleoside 4,8-diamino-6-imino-6H-1-beta-D-ribofuranosylimidazo[4,5-e][1,3] diazepine exhibited potent broad spectrum anticancer activities in vitro against a wide variety of human tumor cell lines. Based on this prototype structure, and as part of the SAR studies, we made modifications at the 6 position of the heterocyclic system with various aryl, alkyl, and aralkyl substitutions and at the 1-position with aralkyl and ribosyl substitution. Recently, one of the target compounds containing a long C18 alkyl chain at position 6 was found to exhibit potent in vitro anti-cancer activity against prostate, breast, ovarian and lung cancers. Our current focus is on further enhancing its potency and bioavailability through rational structural modifications coupled with SAR studies.

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**P132. Junction probes: Sequence Detection of Nucleic Acids via TeHyP**

Lei Yan, Shizuka Nakayama, Herman Sintim

University of Maryland

Nucleic acids detection is becoming increasingly important in clinical diagnostics, gene therapy, and a variety of life science research fields. Junction probes (JP), a newly developed nucleic acid detection technology via template enhanced hybridization process (TeHyP), allows the amplified sensing of analytes at isothermal conditions. JP platform separates the analyte recognition domain from the fluorogenic processing domain. It can detect picomolar target DNA and single nucleotide polymorphisms (SNPs). For this poster presentation, we will reveal a new JP design that facilitates the detection of low analyte concentrations in minutes; a remarkable improvement over our first generation technology, which required assay times of several hours.
P133. The role of UBZ domain of polymerase η in translesion DNA synthesis

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Modification of cellular proteins by ubiquitin and ubiquitin-like proteins plays an essential role in a number of biological processes including translesion DNA synthesis (TLS), a recently discovered DNA damage tolerance mechanism. To accomplish TLS numerous proteins are required to function in a highly coordinated fashion. DNA polymerase η has been found to be required for synthesis past cyclobutane pyrimidine dimer (CPD) caused by UV irradiation. Successful translesion DNA synthesis by Pol η relies on monoubiquitination of PCNA, which serves as a signal for recruiting Pol η to the DNA damage site. At present the molecular mechanism of this process has not been fully understood. In this study we investigate the role of Pol η ubiquitin-binding zinc finger (UBZ) domain in translesion DNA synthesis. We generated Pol η with mutations in the UBZ domain and compared their function in a polymerase exchange assay. Our results indicate that these mutations do not significantly affect the polymerase switch between the replicative polymerase δ and the TLS polymerase η. Our results mirror the recent in vivo studies showing that mutation of the Pol η UBZ domain does not affect its localization to the DNA repair foci. Therefore we conclude that UBZ’s role in TLS may not be simply enhancing the affinity between ubiquitinated PCNA and Pol η.

P134. Role of L4 and L22 in Ribosome Assembly, Structure and Function

Sephora Zaman, Megan Fitzpatrick, Lasse Lindahl, Janice Zengel

University of Maryland Baltimore County Department of Biological Sciences

L4 and L22, proteins of the large ribosomal subunit, contain globular surface domains and elongated “tentacles” that reach into the core of the large subunit to form part of the lining of the peptide exit tunnel. Mutations in the tentacles of L4 and L22 confer macrolide resistance in a variety of pathogenic and non-pathogenic bacteria. In an effort to learn more about the roles of L4 and L22 in ribosomal assembly and function, we isolated and characterized erythromycin resistant mutants in E. coli. All of the mutants grew slower than the parent and all showed reduced in vivo rates of peptide elongation.
and increased level of precursor 23S rRNA. Large insertions in L4 and L22 also resulted in accumulation of abnormal ribosomal subunits. Drug binding assays revealed that not all of the L4 mutants are unable to bind erythromycin and not all of the L22 mutants bind the drug. In addition, structural studies using chemical modification followed by primer extension assays reveal changes in rRNA structure due to large insertion mutations in L4 and L22. These results highlight the important role of L4 and L22 in ribosome structure, function and assembly.

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P135. Design the Interface between Biomolecules and Semiconductor Substrate

Xiaochun Zhang, Andrew V. Teplyakov

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A well-characterized interface based upon covalent binding between biomolecules and semiconductor surface was designed using the functionalized self-assembled monolayers (SAM) on Si(111) surface and specific shaped-restricted DNA molecules. This type of interface can serve as a prototype for the future devices in biosensing and single molecule spectroscopy. The spectroscopic and microscopic benchmarks were initially tested using fullerene C60 as a model to understand the attachment chemistry of large molecules with amine-terminated SAM on Si(111) surface. X-ray photoelectron spectroscopy (XPS) and Infrared spectroscopic (IR) studies, supported by computational investigation, verified the covalent attachment of C60 to the amine-terminated SAM on Si(111) surface. The atomic force microscopy (AFM) revealed the topography of the C60-modied surface with molecular resolution. The biomolecule/semiconductor interface was tailored with the same amine-terminated SAM on Si(111) surface and thiol-DNA molecules, which is achieved via a sulfo-succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SSMCC) crosslinker molecule. The shape-restricted thiol-DNA is anchored to the surface through the formation of covalent bonds as confirmed by XPS and time-of-flight secondary ion mass spectroscopy (TOF-SIMS). The AFM is used to visualize the well-defined and selective covalent binding of thiol-DNA molecules on SAM-covered Si(111). In addition, AFM and contact angle measurement are employed to study the change of the surface topography and the change of the surface hydrophilicity following each step of the DNA attachment chemistry on silicon.

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P136. Examination of halogen substituent effects on HIV-1 IN inhibitors

Xue Zhi Zhao,1 Kasthuraiah Maddali,2 B. Christie Vu,3 Christophe Marchand,2 Stephen H. Hughes,3 Yves Pommier,2 and Terrence R. Burke, Jr.1
Integrase (IN) is a key enzyme in the life cycle of human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS). Of the approximately 30 drugs that have been approved by the FDA for treatment of HIV-1 infection, Raltegravir represents the most recently approved member (October 2007) and the only integrase inhibitor. A key component of IN inhibitors is an arrangement of heteroatoms that are hypothesized to chelate divalent metal ions associated with catalytically essential IN residues Asp64, Asp116 and Glu152 (“DDE” motif). Additional aromatic functionality, frequently in the form of a benzyl group linked to the chelating portion of the inhibitor, can make significant contributions to overall binding affinity. The empirical observation that halogen substituents on the ring can enhance potency has led to the hypothesis that this aryl ring may potentially bind in one or more hydrophobic pockets of the IN-DNA complex. Using 2,3-dihydro-6,7-dihydroxy-1H-isoindol-1-one and 4,5-dihydroxy-1H-isoindole-1,3(2H)-dione based IN inhibitors as display platforms, we undertook a thorough examination of halogen substituent effects. Data from this study suggest that dihalo – substituted analogues in general have higher potency than monohalo – substituted compounds, but that further addition of halogens is not beneficial.

P137. Inhibitor design for glycogen phosphorylase

Shijun Zhong1, Nikos G. Oikonomakos2, Alexander D. MacKerell, Jr.1

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2. The National Hellenic Research Foundation (NHRF), Institute of Organic & Pharmaceutical Chemistry, 48 Vassileos Constantinou Avenue, 116 35 Athens, Greece

Virtual screenings of 1 million drug-like compounds were applied to design the inhibitors to defunction glycogen phosphorylase which catalyzes the degradation of glycogen by releasing glucose. Such inhibitors can be used to control the amount of glucose and might help therapeutic curing of type 2 diabetes. The screening process contains a series of steps including two rounds of dockings using different scoring functions and different step sizes in sampling, similarity study and clustering for picking up diverse hits, filtering with empirical rules for selecting lead-like hits. The experimental validation was performed and some active inhibitors have been identified.
P138. Inhibitor design for glycogen phosphorylase

Shijun Zhong¹, Nikos G. Oikonomakos², Alexander D. MacKerell, Jr.¹

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Virtual screenings of 1 million drug-like compounds were applied to design the inhibitors to defunction glycogen phosphorylase which catalyzes the degradation of glycogen by releasing glucose. Such inhibitors can be used to control the amount of glucose and might help therapeutic curing of type 2 diabetes. The screening process contains a series of steps including two rounds of dockings using different scoring functions and different step sizes in sampling, similarity study and clustering for picking up diverse hits, filtering with empirical rules for selecting lead-like hits. The experimental validation was performed and some active inhibitors have been identified.

P139. Binding of N-acetylglucosamine oligmer to Hen Egg-White Lysozyme

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Lysozyme is a well-studied enzyme that hydrolyzes the $\beta$-(1,4)-glycosidic linkage of N-acetyl-D-glucosamine (NAG) oligmer. The active site of hen egg-white lysozyme (HEWL) is believed to consist of six subsites, A-F, which is able to accommodate six sugar residues. To probe the free energetics associated with such binding processes, we present studies exploring the use of polarizable force fields in prediction of binding free energies of complexes of lysozyme and NAG oligsaccharides. Current state-of-the-art methods for computing binding free energetics based on atomistic descriptions of protein-ligand complexes rely heavily on some model of the interactions between constituent species. We present the development of a polarizable force field for the carbohydrate functionality with NAG as the paradigmatic model compound. We discuss condensed phase properties of the NAG monomer as well as polymeric NAG species that are relevant as ligands to naturally occurring receptors (HEWL). The hydration energies of NAG oligmers are computed via a free energy perturbation (FEP) approach and then compared to results obtained from continuum solvent model. These studies will present the first application of polarizable force fields to such systems. Proceeding, we will discuss comparisons of binding free energy calculations using polarizable carbohydrate.
force fields in combination with several continuum approaches for electrostatic solvation free energy estimation. Molecular dynamics trajectories are applied to analyze the binding modes for NAG oligomers at the active site of HEWL.

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P140. Small molecule inhibitors against BCL6 promotes DLBCL cell death

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Oncogenic chromosomal translocation and somatic mutations involving the BCL6 gene is commonly observed in B-cell lymphomas especially in diffuse large B-cell lymphoma (DLBCL). The BCL6 gene expresses a transcription factor that represses expression of regulatory proteins in cell differentiation and apoptosis. Here, we targeted BCL6 for rational drug design. A computational three-dimensional (3-D) database search was used to identify drug-like compounds that bind to a subset of the lateral binding groove on BCL6 to which co-repressors are recruited. Based on the structural and chemical environment of the target binding groove, a preliminary search of a library of more than 1 million compounds produced a final list of 199 compounds. Ten out of the 100 assayed compounds exhibited >20% inhibition of BCL6^{BTB} mediated transcription repression at 50 µM concentration. Similarity search of active compounds yielded active compound 57-6 with improved affinity for BCL6. Crystallographic analyses indicate direct interactions of 57-6 with the originally targeted binding groove. This compound disrupts the ability of BCL6 to maintain survival in DLBCL cells and exhibits anti-lymphoma activity in mice without overt toxic effects. Our \textit{in vitro} and \textit{in vivo} result may together contribute towards the development of a novel anti-lymphoma drug candidate.

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P141. Synthesis and Examination of Nucleoside Adducts formed by α-hydroxy-N-nitrosomorpholine

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N-nitrosomorpholine (NMOR) is a potent carcinogen and mutagen that has been shown to cause liver and esophageal tumors in rats and nasal tumors in hamsters. NMOR has been detected in smokeless tobacco, in many food products, in workplaces of tire, rubber, and leather industries, and in human urine. The metabolism of NMOR occurs in the liver
by P-450 enzymes predominantly through α-hydroxylation. The resulting α-hydroxy-\(N\)-nitrosomorpholine readily decomposes in aqueous media to form a highly reactive diazonium ion that can react with solvent water or alkylate DNA producing 2-ethoxyacetaldehyde adducts. To date, there is only one report that has attempted to examine the nucleoside adducts produced from α-hydroxy-\(N\)-nitrosomorpholine (HONMOR) exposure. The primary aim of this research was to complete a full analysis of the purine nucleoside adducts that are generated from (HONMOR). This was accomplished with the use of a stable precursor, α-hydroperoxy-\(N\)-nitrosomorpholine (HOONMOR), and synthesized nucleoside standards. Experiments were conducted that exposed nucleosides and calf thymus DNA to HONMOR. In the reactions of nucleosides the yields of the 2-ethoxyacetaldehyde (EA) adducts are in order of \(N_7\)-Gua > \(O^6\)-Gua > \(N_7\)-Ade > \(N_1\)-Gua > \(N^2\)-Gua > \(N_3\)-Ade. In the reactions of DNA the yields of the EA adducts are in order of \(N_7\)-Gua ≈ \(O^6\)-Gua > \(N_3\)-Ade > \(N_7\)-Ade > \(N^2\)-Gua.

The second aim of this research was to examine the aqueous stability of the EA fragment in various buffers, on nucleosides, and on duplex DNA to determine if EA could decay into a 2-hydroxyethyl (HE) fragment since a previous report isolated \(N_7\)-2-hydroxyethylguanine from liver DNA in rats treated with NMOR. Our results indicate that the EA fragment is stable in acidic media but readily decays in neutral and basic media. Further examination revealed that the EA decomposes into a HE fragment with its rate of decay stimulated by primary and secondary amines under physiological conditions.

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