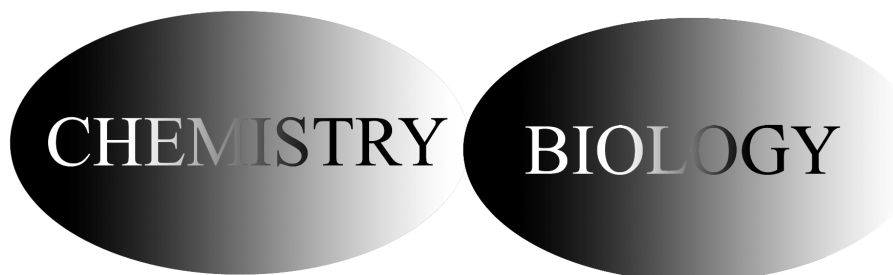


Chmielewski Group Literature Abstracts



Highlight of the Month

Hot-spot mimicry of a cytokine receptor by a small molecule

James A. Wells *PNAS* | October 17, 2006 | vol. 103 | no. 42 | 15422-15427

Protein–protein complexes remain enticing, but extremely challenging, targets for small-molecule drug discovery. In a rare example described earlier, a high-affinity small molecule, SP4206 ($K_d \approx 70$ nM), was found to block binding of the IL-2 α receptor (IL-2R α) to IL-2 ($K_d \approx 10$ nM). Although the protein and small molecule do bind the same hot spot, they trap very different conformations of IL-2 because of its flexible nature. Our studies suggest that precise structural mimics of receptors are not required for high-affinity binding of small molecules, and they show that there are multiple solutions to tight binding at shared and adaptive hot spots.

October 2006

Contributing Editors:

Song-Gil Lee (*PNAS*)

Yannick Fillon (*Angewandte Chemie*)

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Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*JBC*)

Dawn Ernenwein (*ACS Chem Biol/Chem & Bio/Chem Biol & Drug Design*)

Dave Przybyla (*Org Lett*)

Nature

A linguistic model for the rational design of antimicrobial peptides

Nature **443**, 867-869 (19 October 2006)

Christopher Loose, Kyle Jensen^{1,2,3,5}, Isidore Rigoutsos^{1,4} and Gregory Stephanopoulos

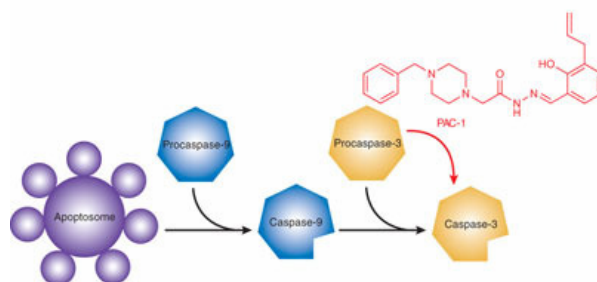
Antimicrobial peptides (AmPs) are small proteins that are used by the innate immune system to combat bacterial infection in multicellular eukaryotes¹. There is mounting evidence that these peptides are less susceptible to bacterial resistance than traditional antibiotics and could form the basis for a new class of therapeutic agents². Here we report the rational design of new AmPs that show limited homology to naturally occurring proteins but have strong bacteriostatic activity against several species of bacteria, including *Staphylococcus aureus* and *Bacillus anthracis*. These peptides were designed using a linguistic model of natural AmPs: we treated the amino-acid sequences of natural AmPs as a formal language and built a set of regular grammars to describe this language. We used this set of grammars to create new, unnatural AmP sequences. Our peptides conform to the formal syntax of natural antimicrobial peptides but populate a previously unexplored region of protein sequence space.

Nature Chemical Biology

Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy

Nature Chemical Biology **2**, 543-550 (2006)

Hoagland, Jung-Taek Kwon⁴, Soon-Kyung Hwang⁴, Hua Jin, Mona I Churchwell, Myung-Haing Cho, Daniel R Doerge, William G Helferich and Paul J Hergenrother



Mutation and aberrant expression of apoptotic proteins are hallmarks of cancer. These changes prevent proapoptotic signals from being transmitted to executioner caspases, thereby averting apoptotic death and allowing cellular proliferation. Caspase-3 is the key executioner caspase, and it exists as an inactive zymogen that is activated by upstream signals. Notably, concentrations of procaspase-3 in certain cancerous cells are significantly higher than those in noncancerous controls. Here we report the

identification of a small molecule (PAC-1) that directly activates procaspase-3 to caspase-3 in vitro and induces apoptosis in cancerous cells isolated from primary colon tumors in a manner directly proportional to the concentration of procaspase-3 inside these cells. We found that PAC-1 retarded the growth of tumors in three different mouse models of cancer, including two models in which PAC-1 was administered orally. PAC-1 is the first small molecule known to directly activate procaspase-3 to caspase-3, a transformation that allows induction of apoptosis even in cells that have defective apoptotic machinery. The direct activation of executioner caspases is an anticancer strategy that may prove beneficial in treating the many cancers in which procaspase-3 concentrations are elevated.

Nature Methods

State-based discovery: a multidimensional screen for small-molecule modulators of EGF signaling

Nature Methods - 3, 825 - 831 (2006)

Mark Sevecka & Gavin MacBeath

As an alternative to conventional, target-oriented drug discovery, we report a strategy that identifies compounds on the basis of the state that they induce in a signaling network. Immortalized human cells are grown in microtiter plates and treated with compounds from a small-molecule library. The target network is then activated and lysates derived from each sample are arrayed onto glass-supported nitrocellulose pads. By probing these microarrays with antibodies that report on the abundance or phosphorylation state of selected proteins, a global picture of the target network is obtained. As proof of concept, we screened 84 kinase and phosphatase inhibitors for their ability to induce different states in the ErbB signaling network. We observed functional connections between proteins that match our understanding of ErbB signaling, indicating that state-based screens can be used to define the topology of signaling networks. Additionally, compounds sort according to the multidimensional phenotypes they induce, suggesting that state-based screens may inform efforts to identify the targets of biologically active small molecules.

ACS Chemical Biology

Conjugating Berberine to a Multidrug Resistance Pump Inhibitor Creates an Effective Antimicrobial

ACS Chem. Biol. 1 (9), 594–600

Anthony R. Ball[†], Gabriele Casadei[†], Siritron Samosorn[‡], John B. Bremner^{‡,*}, Frederick M. Ausubel[§], Terence I. Moy[§], and Kim Lewis

In bacteria, multidrug-resistance pumps (MDRs) confer resistance to chemically unrelated amphipathic toxins. A major challenge in developing efficacious antibiotics is identifying antimicrobial compounds that are not rapidly pumped out of bacterial cells.

The plant antimicrobial berberine, the active component of the medicinal plants echinacea and golden seal, is a cation that is readily extruded by bacterial MDRs, thereby rendering it relatively ineffective as a therapeutic agent. However, inhibition of MDR efflux causes a substantial increase in berberine antimicrobial activity, suggesting that berberine and potentially many other compounds could be more efficacious if an effective MDR pump inhibitor could be identified. Here we show that covalently linking berberine to INF55, an inhibitor of Major Facilitator MDRs, results in a highly effective antimicrobial that readily accumulates in bacteria. The hybrid molecule showed good efficacy in a *Caenorhabditis elegans* model of enterococcal infection, curing worms of the pathogen.

Chemistry and Biology

Sequence Requirements and an Optimization Strategy for Short Antimicrobial Peptides

Chemistry & Biology 13, 1101–1107, October 2006

Kai Hilpert¹, Melissa R. Elliott¹, Rudolf Volkmer-Engert², Peter Henklein³, Oreola Donini⁴, Qun Zhou⁵, Dirk F.H. Winkler⁵ and Robert E.W. Hancock

Short antimicrobial host-defense peptides represent a possible alternative as lead structures to fight antibiotic resistant bacterial infections. Bac2A is a 12-mer linear variant of the naturally occurring bovine host defense peptide, bactericin, and demonstrates moderate, broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria as well as against the yeast *Candida albicans*. With the assistance of a method involving peptide synthesis on a cellulose support, the primary sequence requirements for antimicrobial activity against the human pathogen *Pseudomonas aeruginosa* of 277 Bac2A variants were investigated by using a luciferase-based assay. Sequence scrambling of Bac2A led to activities ranging from superior or equivalent to Bac2A to inactive, indicating that good activity was not solely dependent on the composition of amino acids or the overall charge or hydrophobicity, but rather required particular linear sequence patterns. A QSAR computational analysis was applied to analyze the data resulting in a model that supported this sequence pattern hypothesis. The activity of selected peptides was confirmed by conventional minimal inhibitory concentration (MIC) analyses with a panel of human pathogen bacteria and fungi. Circular-dichroism (CD) spectroscopy with selected peptides in liposomes and membrane depolarization assays were consistent with a relationship between structure and activity. An additional optimization process was performed involving systematic amino acid substitutions of one of the optimal scrambled peptide variants, resulting in superior active peptide variants. This process provides a cost and time effective enrichment of new candidates for drug development, increasing the chances of finding pharmacologically relevant peptides.

Engineering Dehydro Amino Acids and Thioethers into Peptides Using Lactacin 481 Synthetase

Chemistry & Biology 13, 1109–1117, October 2006

Champak Chatterjee^{1, 3}, Gregory C. Patton^{1, 3}, Lisa Cooper², Moushumi Paul¹ and Wilfred A. van der Donk

Lantibiotics are peptide antimicrobials containing the thioether-bridged amino acids lanthionine (Lan) and methyllanthionine (MeLan) and often the dehydrated residues dehydroalanine (Dha) and dehydrobutyrine (Dhb). While biologically advantageous, the incorporation of these residues into peptides is synthetically daunting, and their production in vivo is limited to peptides containing proteinogenic amino acids. The lactacin 481 synthetase LctM offers versatile control over the installation of dehydro amino acids and thioether rings into peptides. In vitro processing of semisynthetic substrates unrelated to the pre-lactacin 481 peptide demonstrated the broad substrate tolerance of LctM. Furthermore, a chemoenzymatic strategy was employed to generate novel thioether linkages by cyclization of peptidic substrates containing the nonproteinogenic cysteine analogs homocysteine and β -homocysteine. These findings are promising with respect to the utility of LctM toward preparation of conformationally constrained peptide therapeutics.

Chemical Biology and Drug Design

Fusion of Fungicidal Peptide dhvar4 to Enterococcal Peptide Pheromone Increases Its Bactericidal Activity against Enterococcus faecalis

Chem Biol Drug Des 2006; 68: 220–224

Xiaofeng Lu^{1,*}, Lin Wan¹, Hao Yang¹, Jie Zhang¹, Shengfu Li¹, Mei Kang², Youping Li¹ and Jingqi Cheng

Bacterial peptide pheromone has a high affinity to its membrane receptor. Fusion of these peptides to pore-forming antimicrobial peptide might enhance its bactericidal activity against pheromone-sensing bacteria. We constructed two chimeric peptides by fusing the pore-forming fungicidal peptide dhvar4 to the C-terminus of enterococcal peptide pheromones cCF10 and cOB1 individually. Comparison on the bactericidal activities against pheromone-sensing bacteria *Enterococcus faecalis* demonstrates that the chimeric peptides cCF10-dhvar4 and cOB1-dhvar4 are more potent than the parent peptide dhvar4. The LD50s of both chimeric peptides (1.0 μ m) are 10 times lower than that of dhvar4 (10.8 μ m). Free peptide pheromone could inhibit *E. faecalis* killing mediated by both chimeric peptides. As same as that of the parent peptide, both chimeric peptides kill bacteria by disrupting its cell membrane. These results indicate that fused enterococcal peptide pheromone increases the bactericidal activity of fungicidal peptide against *E. faecalis* by improving its ability to reach the cell membrane.

Molecular Descriptors of N-Arylhydroxamic Acids: A Tool in Drug Design

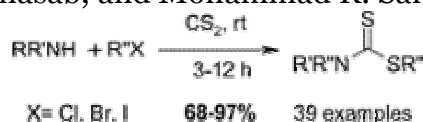
The partition coefficient in the 1-octanol/water system, log P(O/W), is a measure of lipophilicity. It is used as a predictor of solute–membrane partitioning. The objective of this study was to measure the log P(O/W) of five hydroxamic acids. Other molecular descriptors of these solutes, namely molar volume, molar refraction, parachor, polarizability (π^*), hydrogen-bond donor acidity ($\epsilon\alpha$) and hydrogen-bond acceptor basicity ($\epsilon\beta$), have also been discussed. These properties represent the combined effects of a number of intermolecular forces between a solute and its environment.

Organic Letters

Straightforward and Highly Efficient Catalyst-Free One-Pot Synthesis of Dithiocarbamates under Solvent-Free Conditions

Org. Lett., 8 (23), 5275–5277, 2006.

Najmedin Azizi, Fezzeh Aryanasab, and Mohammad R. Saidi



A highly efficient and simple synthesis of dithiocarbamates is possible based on the one-pot reaction of amines, CS₂, and alkyl halides without using a catalyst under solvent-free conditions. The mild reaction conditions, high yields, and broad scope of the reaction illustrate the good synthetic utility of this method. The reaction is a highly atom-economic process for production of S-alkyl thiocarbamates and successfully can be used in high quantities in the pharmaceutical or agrochemical industries.

Switchable Dual Binding Mode Molecular Shuttle

Org. Lett., 8 (23), 5377–5379, 2006.

David A. Leigh* and Andrew R. Thomson

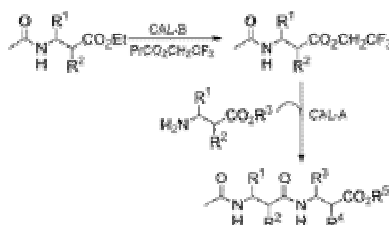


Protonation controls the location of a dual binding mode macrocycle in a [2]rotaxane. In the neutral form, amide–amide hydrogen bonds hold the macrocycle over a dipeptide residue; when the thread is protonated, polyether–ammonium cation interactions dominate and the macrocycle changes position.

Lipases in -Dipeptide Synthesis in Organic Solvents

Org. Lett., 8 (24), 5593–5596, 2006.

Xiang-Guo Li and Liisa T. Kanerva



A number of -dipeptides were prepared by two-step lipase-catalyzed reactions where N-acetylated -amino esters were first activated as 2,2,2-trifluoroethyl esters with *Candida antarctica* lipase B (CAL-B). The activated esters were then used to acylate a -amino ester in the presence of *Candida antarctica* lipase A (CAL-A) in dry Et₂O or i-Pr₂O.

Science

An Essential Role for LEDGF/p75 in HIV Integration

Science Vol 314, Issue 5798, 461-464, 20 October 2006

Manuel Llano, Dyana T. Saenz, Anne Meehan, Phonphimon Wongthida, Mary Peretz, William H. Walker, Wulin Teo, Eric M. Poeschla

Chromosomal integration enables human immunodeficiency virus (HIV) to establish a permanent reservoir that can be therapeutically suppressed but not eradicated. Participation of cellular proteins in this obligate replication step is poorly understood. We used intensified RNA interference and dominant-negative protein approaches to show that the cellular transcriptional coactivator lens epithelium–derived growth factor (LEDGF)/p75 (p75) is an essential HIV integration cofactor. The mechanism requires both linkages of a molecular tether that p75 forms between integrase and chromatin. Fractionally minute levels of endogenous p75 are sufficient to enable integration, showing that cellular factors that engage HIV after entry may elude identification in less intensive knockdowns. Perturbing the p75-integrase interaction may have therapeutic potential.

PNAS

Templated biomineralization on self-assembled protein fibers

PNAS | October 3, 2006 | vol. 103 | no. 40 | 14672-14677

Biological mineralization of tissues in living organisms relies on proteins that preferentially nucleate minerals and control their growth. This process is often referred to as "templating," but this term has become generic, denoting various proposed mineral–organic interactions including both chemical and structural affinities. Here, we present an approach using self-assembled networks of elastin and fibronectin fibers, similar to the extracellular matrix. When induced onto negatively charged sulfonated polystyrene surfaces, these proteins form fiber networks of ≈10-μm spacing, leaving open regions of disorganized protein between them. We introduce an atomic force microscopy-based technique to measure the elastic modulus of both structured and

disorganized protein before and during calcium carbonate mineralization. Mineral-induced thickening and stiffening of the protein fibers during early stages of mineralization is clearly demonstrated, well before discrete mineral crystals are large enough to image by atomic force microscopy. Calcium carbonate stiffens the protein fibers selectively without affecting the regions between them, emphasizing interactions between the mineral and the organized protein fibers. Late-stage observations by optical microscopy and secondary ion mass spectroscopy reveal that Ca is concentrated along the protein fibers and that crystals form preferentially on the fiber crossings. We demonstrate that organized versus unstructured proteins can be assembled mere nanometers apart and probed in identical environments, where mineralization is proved to require the structural organization imposed by fibrillogenesis of the extracellular matrix.

Genome-wide functional analysis of human cell-cycle regulators

Peter G. Schultz PNAS | October 3, 2006 | vol. 103 | no. 40 | 14819-14824

Human cells have evolved complex signaling networks to coordinate the cell cycle. A detailed understanding of the global regulation of this fundamental process requires comprehensive identification of the genes and pathways involved in the various stages of cell-cycle progression. To this end, we report a genome-wide analysis of the human cell cycle, cell size, and proliferation by targeting >95% of the protein-coding genes in the human genome using small interfering RNAs (siRNAs). Analysis of >2 million images, acquired by quantitative fluorescence microscopy, showed that depletion of 1,152 genes strongly affected cell-cycle progression. These genes clustered into eight distinct phenotypic categories based on phase of arrest, nuclear area, and nuclear morphology. Phase-specific networks were built by interrogating knowledge-based and physical interaction databases with identified genes. Genome-wide analysis of cell-cycle regulators revealed a number of kinase, phosphatase, and proteolytic proteins and also suggests that processes thought to regulate G₁-S phase progression like receptor-mediated signaling, nutrient status, and translation also play important roles in the regulation of G₂/M phase transition. Moreover, 15 genes that are integral to TNF/NF- κ B signaling were found to regulate G₂/M, a previously unanticipated role for this pathway. These analyses provide systems-level insight into both known and novel genes as well as pathways that regulate cell-cycle progression, a number of which may provide new therapeutic approaches for the treatment of cancer.

A nucleobase lesion remodels the interaction of its normal neighbor in a DNA glycosylase complex

Gregory L. Verdine PNAS | October 10, 2006 | vol. 103 | no. 41 | 15020-15025

How DNA glycosylases search through millions of base pairs and discriminate between rare sites of damage and otherwise undamaged bases is poorly understood. Even less understood are the details of the structural states arising from DNA glycosylases interacting with undamaged DNA. Recognizing the mutagenic lesion 7,8-dihydro-8-

oxoguanine (8-oxoguanine, oxoG) represents an especially formidable challenge, because this oxidized nucleobase differs by only two atoms from its normal counterpart, guanine (G), and buried in the structure of naked B-form DNA, oxoG and G are practically indistinguishable from each other. We have used disulfide cross-linking technology to capture a human oxoG repair protein, 8-oxoguanine DNA glycosylase I (hOGG1) sampling an undamaged G:C base pair located adjacent to an oxoG:C base pair in DNA. The x-ray structure of the trapped complex reveals that the presence of the 8-oxoG drastically changes the local conformation of the extruded G. The extruded but intrahelical state of the G in this structure offers a view of an early intermediate in the base-extrusion pathway.

Hot-spot mimicry of a cytokine receptor by a small molecule

James A. Wells *PNAS* | **October 17, 2006** | *vol. 103* | *no. 42* | **15422-15427**

Protein–protein complexes remain enticing, but extremely challenging, targets for small-molecule drug discovery. In a rare example described earlier, a high-affinity small molecule, SP4206 (Kd \approx 70 nM), was found to block binding of the IL-2 α receptor (IL-2R α) to IL-2 (Kd \approx 10 nM). Recently, the structure of the IL-2/IL-2R α complex was solved [Rickert, M., Wang, X., Boulanger, M. J., Goriatcheva, N., Garcia, K. C. (2005) *Science* 308:1477–1480]. Using structural and functional analysis, we compare how SP4206 mimics the 83-fold larger IL-2R α in binding IL-2. The binding free energy per contact atom (ligand efficiency) for SP4206 is about twice that of the receptor because of a smaller, but overlapping, contact epitope that insinuates into grooves and cavities not accessed by the receptor. Despite its independent design, the small molecule has a similar, but more localized, charge distribution compared with IL-2R α . Mutational studies show that SP4206 targets virtually the same critical "hot-spot" residues on IL-2 that drive binding of IL-2R α . Moreover, a mutation that enhances binding to the IL-2R α near these hot spots also enhances binding to SP4206. Although the protein and small molecule do bind the same hot spot, they trap very different conformations of IL-2 because of its flexible nature. Our studies suggest that precise structural mimics of receptors are not required for high-affinity binding of small molecules, and they show that there are multiple solutions to tight binding at shared and adaptive hot spots.

A seven-helix coiled coil

PNAS | **October 17, 2006** | *vol. 103* | *no. 42* | **15457-15462**

Coiled-coil proteins contain a characteristic seven-residue sequence repeat whose positions are designated a to g. The interacting surface between α -helices in a classical coiled coil is formed by interspersing nonpolar side chains at the a and d positions with hydrophilic residues at the flanking e and g positions. To explore how the chemical nature of these core amino acids dictates the overall coiled-coil architecture, we replaced all eight e and g residues in the GCN4 leucine zipper with nonpolar alanine side chains. Surprisingly, the alanine-containing mutant forms a stable α -helical heptamer in aqueous solution. The 1.25-Å resolution crystal structure of the heptamer reveals a parallel seven-stranded coiled coil enclosing a large tubular channel with an unusual

heptad register shift between adjacent staggered helices. The overall geometry comprises two interleaved hydrophobic helical screws of interacting cross-sectional a and d layers that have not been seen before. Moreover, asparagines at the a positions play an essential role in heptamer formation by participating in a set of buried interhelix hydrogen bonds. These results demonstrate that heptad repeats containing four hydrophobic positions can direct assembly of complex, higher-order coiled-coil structures with rich diversity for close packing of α -helices.

A missense mutation in *Caenorhabditis elegans* prohibitin 2 confers an atypical multidrug resistance

PNAS | *October 17, 2006* | *vol. 103* | *no. 42* | *15523-15528*

Hemiasterlin is a potent antimetabolic peptide that interferes with microtubule dynamics at picomolar concentrations in cell culture. The molecule largely eludes P-glycoprotein-mediated drug efflux, and an analog is currently being evaluated in clinical trials as cancer chemotherapy. From a nonclonal genetic screen in *Caenorhabditis elegans* we isolated eight independent mutants resistant to a synthetic hemiasterlin analog. In one recessive mutant, *phb-2(ad2154)*, a point mutation in prohibitin 2 (E130K) protects worms from drug-induced injury. Data indicate that direct binding of hemiasterlin to prohibitin 2 is unlikely. In fact, *C. elegans phb-2(ad2154)* was also found to be resistant to numerous other drugs that bind tubulin and to camptothecin, yet this mutant was sensitive to nocodazole and phalloidin. Thus, prohibitin 2 is implicated in a previously uncharacterized pathway of multidrug resistance.

An 85-aa segment of the GB virus type C NS5A phosphoprotein inhibits HIV-1 replication in CD4+ Jurkat T cells

PNAS | *October 17, 2006* | *vol. 103* | *no. 42* | *15570-15575*

GB virus type C (GBV-C) is an apparently nonpathogenic virus that replicates in T and B lymphocytes and is a common cause of persistent human infection. Among HIV-1-infected individuals, persistent coinfection with GBV-C is associated with prolonged survival, and infection of blood mononuclear cells or CD4+ T cells with GBV-C and HIV in vitro results in significantly reduced HIV-1 replication. To date, the viral protein(s) that lead to HIV inhibition have not been identified. The GBV-C nonstructural phosphoprotein (NS5A) is predicted to have pleiotropic effects on cells, including interactions with the IFN-induced dsRNA-activated protein kinase (PKR). We studied GBV-C NS5A to determine whether it is involved in inhibition of HIV replication. GBV-C NS5A protein from an isolate that was cleared by IFN therapy did not inhibit PKR, whereas NS5A from an isolate that was not cleared by IFN inhibited PKR function in a yeast genetic system. Both of these GBV-C NS5A proteins were expressed in a CD4+ T cell line (Jurkat), and both induced a potent, dose-dependent inhibition of HIV-1 replication, thus the effect was independent of PKR inhibition. NS5A induced the release of the chemokine SDF-1 and decreased surface expression of the HIV coreceptor CXCR4, potentially explaining the HIV inhibition. Deletion mapping of the NS5A

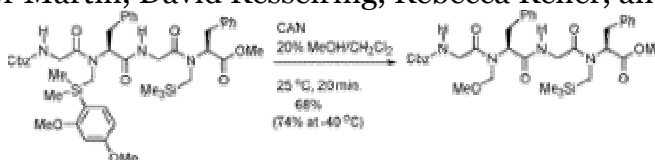
protein found that an 85-aa region between amino acids 152 and 237 inhibits HIV-1 replication. Thus, GBV-C NS5A protein alters the cellular milieu necessary for HIV-1 replication and may provide a previously undescribed therapeutic approach for anti-HIV therapy.

Journal of the American Chemical Society

Building Functionalized Peptidomimetics: Use of Electroauxiliaries for Introducing N-Acyliminium Ions into Peptides

J. Am. Chem. Soc., **128** (42), 13761-13771, 2006.

Haizhou Sun, Connor Martin, David Kesselring, Rebecca Keller, and Kevin D. Moeller*

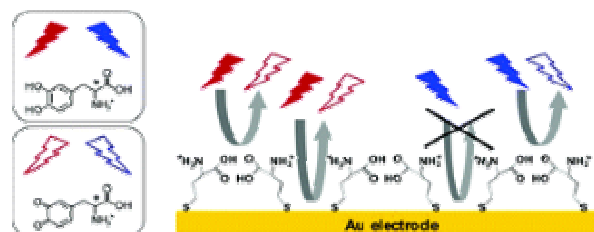


A series of silyl-substituted amino acids have been synthesized, inserted into peptides, and then employed as precursors for oxidatively generating reactive N-acyliminium ions. Both electrochemical and chemical oxidation procedures have been employed. N-Acyliminium ion generation in a solid-phase substrate as well as application to a small library of functionalized dipeptides has been demonstrated. Limitations in terms of how electron-rich the silyl groups can be as well as the compatibility of multiple silyl groups within a longer peptide are defined.

Enantioselectivity of Redox Reaction of DOPA at the Gold Electrode Modified with a Self-Assembled Monolayer of Homocysteine

J. Am. Chem. Soc., **128** (41), 13322-13323, 2006

Takuya Nakanishi,*† Mariko Matsunaga,‡ Makoto Nagasaka,‡ Toru Asahi,†‡ and Tetsuya Osaka



The enantioselectivity of the self-assembled monolayer (SAM) of homocysteine formed on the (111)-oriented gold surface was investigated. We analyzed the redox behavior of 3,4-dihydroxyphenylalanine (DOPA), which is an electrochemically active chiral molecule, by means of cyclic voltammetry at a gold electrode modified with one enantiomeric form of homocysteine. It was demonstrated that the homocysteine SAM of one enantiomeric form blocked the redox reaction of only one enantiomer of DOPA, with cross inversion for the other enantiomer, in acidic solution.

The Open Structure of a Multi-Drug-Resistant HIV-1 Protease is Stabilized by Crystal Packing Contacts

J. Am. Chem. Soc., 128 (41), 13360 -13361, 2006.

Melinda Layten,[†] Viktor Hornak,[‡] and Carlos Simmerling

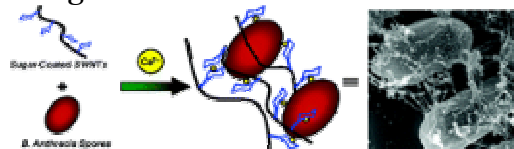


The introduction of HIV-1 protease (HIV-PR) inhibitors has led to a dramatic increase in patient survival; however, these gains are threatened by the emergence of multi-drug-resistant strains. Design of inhibitors that overcome resistance would be greatly facilitated by deeper insight into the mechanistic events associated with binding of substrates and inhibitors, as well as an understanding of the effects of resistance mutations on the structure and dynamic behavior of HIV-PR. We previously reported a series of simulations that provide a model for HIV-PR dynamics, with spontaneous conversions between the bound and unbound crystal forms upon addition or removal of an inhibitor. Importantly, the unbound protease transiently sampled a third fully open state that permits entry to the active site, unlike both crystallographic forms. Recently, a crystal structure of unbound HIV-PR was reported for the MDR 769 isolate (PDB: 1TW7); unlike all previous experimental structures, the binding pocket is open. It is suggested that drug resistance in this strain arises at least in part from the inability of inhibitors to induce closing. We carried out simulations of the MDR 769 HIV-PR mutant and observed that the reported structure is unstable in solution and rapidly adopts the semi-open conformation observed for the unbound wild-type protease in solution. Further analysis suggests that the wide-open structure observed for MDR 769 arises not from sequence variation, but instead is an artifact from crystal packing. Thus, despite being the first experimental structure to reveal flap opening sufficient for substrate access to the active site, this structure may not be directly relevant to studies of inhibitor entry or to the cause of HIV-PR drug resistance.

Unique Aggregation of Anthrax (*Bacillus anthracis*) Spores by Sugar-Coated Single-Walled Carbon Nanotubes

J. Am. Chem. Soc., 128 (41), 13364 -13365, 2006.

Haifang Wang,[†] Lingrong Gu, Yi Lin, Fushen Lu, Mohammed J. Meziani, Pengju G. Luo, Wei Wang, Li Cao, and Ya-Ping Sun



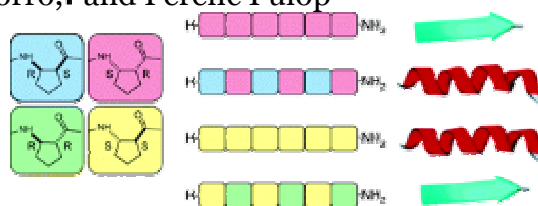
There has been significant interest in the binding of anthrax spores by molecular species, but with only limited success. Proteins and more recently peptides were used.

However, despite the known presence of carbohydrates on the spore surface, carbohydrate-carbohydrate interactions have hardly been explored likely because of the lack of required specific platform for synthetic carbohydrates. We report the successful use of single-walled carbon nanotubes as a truly unique scaffold for displaying multivalent monosaccharide ligands that bind effectively to anthrax spores with divalent cation mediation to cause significant spore aggregation. The work should have far-reaching implications in development of countermeasure technologies

Effects of the Alternating Backbone Configuration on the Secondary Structure and Self-Assembly of α -Peptides

J. Am. Chem. Soc., **128** (41), 13539 -13544, 2006.

Tamas A. Martinek,[†] Istvan M. Mandity,[†] Livia Fülöp,[‡] Gabor K. Tóth,[‡] Elemér Vass,[§] Miklós Hollósi,[§] Eniko Forró,[†] and Ferenc Fülöp

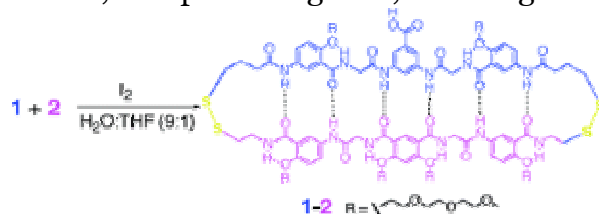


Heterochiral homo-oligomers with alternating backbone configurations were constructed by using the different enantiomers of the cis- and trans-2-aminocyclopentanecarboxylic acid (ACPC) monomers. Molecular modeling and the spectroscopic techniques (NMR, ECD, and VCD) unequivocally proved that the alternating heterochiral cis-ACPC sequences form an H10/12 helix, where extra stabilization can be achieved via the cyclic side chains. The ECD and TEM measurements, together with molecular modeling, revealed that the alternating heterochiral trans-ACPC oligomers tend to attain a polar-strand secondary structure in solution, which can self-assemble into nanostructured fibrils. The observations indicate that coverage of all the possible secondary structures (various helix types and strand-mimicking conformations) can be attained with the help of cyclic α -amino acid diastereomers. A relationship has been established between the backbone chirality pattern and the prevailing secondary structure, which underlines the role of stereochemical control in the α -peptide secondary structure design and may contribute to future biological applications.

Sequence-Specific Association in Aqueous Media by Integrating Hydrogen Bonding and Dynamic Covalent Interactions

J. Am. Chem. Soc., **2006**, **128** (39), 12628 -12629

Minfeng Li, Kazuhiro Yamato, Joseph S. Ferguson, and Bing Gong

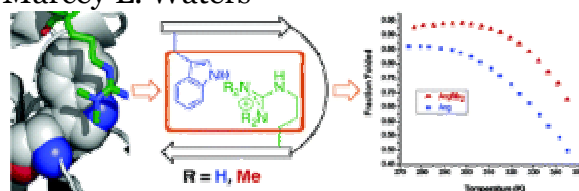


Oligoamide strands that associate in a sequence-specific fashion into hydrogen-bonded duplexes in nonpolar solvents were converted into disulfide cross-linked duplexes in aqueous media. Thus, by incorporating trityl-protected thiol groups, which allows the reversible formation of disulfide bonds, into the oligoamide strands, only duplexes consisting of complementary hydrogen-bonding sequences were formed in aqueous solution as well as in methanol. The sequence-specific cross-linking of oligoamide strands was confirmed by MALDI-TOF, reverse-phase HPLC, and by isolating a cross-linked duplex. This study demonstrates that the sequence-specificity characteristic of multiply hydrogen-bonded systems can be extended into competitive media through the interplay of H-bonding and reversible covalent interactions, based on which a new class of molecular associating and ligating units that are compatible with both polar and nonpolar environments can be conveniently obtained.

Arginine Methylation in a β -Hairpin Peptide: Implications for Arg- π Interactions, ΔC_p , and the Cold Denatured State

J. Am. Chem. Soc., 2006, 128 (39), 12735 -12742

Robert M. Hughes and Marcey L. Waters

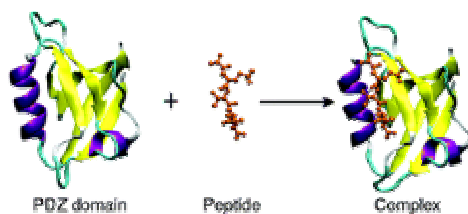


Arginine methylation is a common post-translational modification that plays a role in many cellular processes through mediation of protein-protein interactions. There is still a dearth of structural information as to its role in mediating such interactions, but the available data suggest a possible role of cation- π interactions in the recognition of methylated arginine. Hence, the effect of arginine methylation on its interaction with tryptophan has been investigated within the context of a β -hairpin peptide. Arginine methylation was found to enhance the stacking interaction between the cationic guanidinium functionality of arginine and the indole ring of tryptophan, resulting in structural stabilization of the hairpin. Thermodynamic analysis reveals more favorable entropy of hairpin folding with arginine methylation, a more negative change in heat capacity for folding, and a modest decrease in enthalpic driving force. This is consistent with enhanced stacking and hydrophobic interactions through increased surface area of the guanidinium moiety and greater delocalization of positive charge. In addition, these peptides exhibit significant cold denaturation, which can be accounted for by the inclusion of an expression of temperature-dependent ΔC_p in the thermodynamic analysis.

Thermodynamic Basis for Promiscuity and Selectivity in Protein-Protein Interactions: PDZ Domains, a Case Study

J. Am. Chem. Soc., 2006, 128 (39), 12766 -12777

Nathalie Basdevant,[‡] Harel Weinstein,^{‡§} and Marco Ceruso

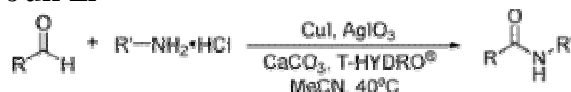


Like other protein-protein interaction domains, PDZ domains are involved in many key cellular processes. These processes often require that specific multiprotein complexes be assembled, a task that PDZ domains accomplish by binding to specific peptide motifs in target proteins. However, a growing number of experimental studies show that PDZ domains (like other protein-protein interaction domains) can engage in a variety of interactions and bind distinct peptide motifs. Such promiscuity in ligand recognition raises intriguing questions about the molecular and thermodynamic mechanisms that can sustain it. To identify possible sources of promiscuity and selectivity underlying PDZ domain interactions, we performed molecular dynamics simulations of 20 to 25 ns on a set of 12 different PDZ domain complexes (for the proteins PSD-95, Syntenin, Erbin, GRIP, NHERF, Inad, Dishevelled, and Shank). The electrostatic, nonpolar, and configurational entropy binding contributions were evaluated using the MM/PBSA method combined with a quasi-harmonic analysis. The results revealed that PDZ domain interactions are characterized by overwhelmingly favorable nonpolar contributions and almost negligible electrostatic components, a mix that may readily sustain promiscuity. In addition, despite the structural similarity in fold and in recognition modes, the entropic and other dynamical aspects of binding were remarkably variable not only across PDZ domains but also for the same PDZ domain bound to distinct ligands. This variability suggests that entropic and dynamical components can play a role in determining selectivity either of PDZ domain interactions with peptide ligands or of PDZ domain complexes with downstream effectors.

Highly Efficient Oxidative Amidation of Aldehydes with Amine Hydrochloride Salts

J. Am. Chem. Soc., 2006, 128 (40), 13064 -13065

Woo-Jin Yoo and Chao-Jun Li



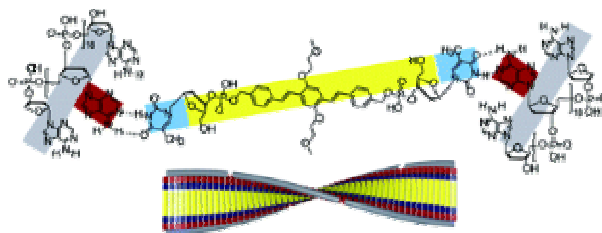
A mild and efficient copper-catalyzed oxidative amidation of aldehydes was developed using amine HCl salts and tert-butyl hydroperoxide as an oxidant.

Molecular-Level Helical Stack of a Nucleotide-Appended Oligo(p-phenylenevinylene) Directed by Supramolecular Self-Assembly with a Complementary Oligonucleotide as a Template

J. Am. Chem. Soc., 2006, 128 (40), 13298 -13304

Rika Iwaura,[†] Freek J. M. Hoebein,[‡] Mitsutoshi Masuda,^{††} Albertus P. H. J. Schenning,[‡] E. W.

Meijer,[‡] and Toshimi Shimizu



The nucleotide- appended oligo(p-phenylenevinylene), {bis[2,5-bis(2-methoxyethoxy)-1,4-phenylene]bis(2,1-ethenediyl-1,4-phenylenemethylene)}bis(2'-deoxy-3'-thymidylic acid) (8), has been synthesized, and self-assembly of the single-component 8 and binary self-assembly of 8 with a complementary single-stranded 20-meric oligodeoxyadenylic acid (9) have been examined in aqueous solutions. Atomic force microscopy (AFM), UV-visible (UV-vis), and circular dichroism (CD) measurements revealed that right-handed helical stacks with 6.4- and 5.1-nm diameters self-assemble from the binary components of 8 and 9 as a template depending on the residual stoichiometry of the two components (thymine (T):adenine (A) = 1:1 and T:A = 2:1, respectively). The concentration of 9 was found to strongly influence the CD spectra of 8 in aqueous solutions. Consequently, we concluded that the one side of the thymine moieties in the stacked assemblies of 8 complexes with a single chain of 9. Complementary T-A base pairs thus formed and induced helical stack of the oligo(p-phenylenevinylene)s in the binary self-assembly. In contrast, self-assembly of the single-component 8 and binary self-assembly of 8 with the noncomplementary 20-meric oligothymidylic acid (10) produced no remarkable formation of fibrous structures like helical stacks.

Angewandte Chemie

Volume 45, Issue 39, Pages 6403-6591 (October 6, 2006)

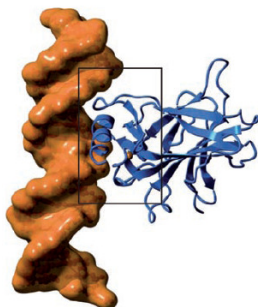
Reviews

Tumor-Suppressor Protein

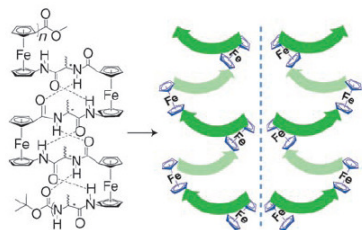
L. Römer, C. Klein, A. Dehner, H. Kessler,*
J. Buchner* ————— 6440–6460

p53—A Natural Cancer Killer: Structural
Insights and Therapeutic Concepts

Front and center: The tumor-suppressor protein p53 is the central element of the control system that many cells use to prevent the development of cancer. An intact p53 network ensures that DNA damage is detected early on. In-depth concepts and the newest advancements in the understanding of the structure and regulation of p53 are discussed. The picture shows the binding of the DNA binding domain of p53 (blue) to the DNA consensus sequence (orange).



Volume 45, Issue 41, Pages 6769-6933 (October 20, 2006)



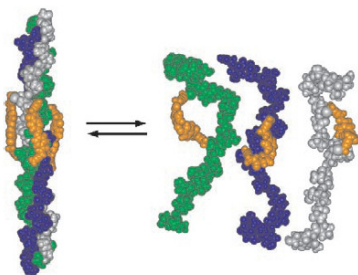
Twists and turns: A series of alanine conjugates of ferrocene amino acid (Fca) were prepared based on the idea that an alternating sequence of a natural α -amino acid and a turn-inducing molecule may result in the formation of a robust model foldamer for β -helical peptides. Right- and left-handed pseudo- β -helical Fca-alanine foldamers were obtained, and their structures were studied in solution and the solid state.

Peptides

S. Chowdhury, G. Schatte,
H.-B. Kraatz* ————— 6882–6884

Rational Design of Bioorganometallic
Foldamers: A Potential Model for Parallel
 β -Helical Peptides

Volume 45, Issue 42, Pages 6935-7097 (October 27, 2006)



At the flick of a switch: Two side chains of a collagen peptide containing (2S,4S)-mercaptoproline at two defined positions are linked with a diiodo azobenzene derivative. With the *trans* isomer of the azobenzene clamp (orange), the peptide folds into the collagen triple helix (green, blue, gray), which unfolds upon irradiation at 330 nm. The light-controlled folding/unfolding processes are fully reversible, making this system well-suited for ultrafast spectroscopic analysis.

Collagen

U. Kusebauch, S. A. Cadamuro,
H.-J. Musiol, M. O. Lenz, J. Wachtveitl,
L. Moroder,* C. Renner — 7015–7018

Photocontrolled Folding and Unfolding of
a Collagen Triple Helix

Journal of Biological Chemistry

Insertion of an Arginine Residue into the Transmembrane Segments Corrects Protein Misfolding

J. Biol. Chem., Vol. 281, Issue 40, 29436-29440

Tip W. Loo, M. Claire Bartlett, and David M. Clarke

Deletion of Phe-508 (Δ F508) in cystic fibrosis transmembrane conductance regulator causes cystic fibrosis because of misfolding of the protein. P-glycoprotein (P-gp) containing the equivalent mutation (Δ Y490) is also misfolded but can be rescued with drug substrates. Whether rescue is due to direct binding of drug substrate to the transmembrane (TM) segments or to indirect effects on cellular protein folding pathways is still controversial. P-gp-drug substrate interactions likely involve hydrogen bonds. If the mechanism of drug rescue involves changes to TM packing then we should be able to identify suppressor mutations in the TM segments that can mimic the drug rescue effects. We predicted that an arginine residue in the TM segments predicted to line the drug-binding pocket of P-gp (I306(TM5) or F343(TM6)) might suppress Δ Y490 P-gp protein misfolding because it has the highest propensity to form hydrogen bonds. We show that R306(TM5) or R343(TM6) increased the relative amount of mature Δ Y490 P-gp by 6-fold. Most other changes to Ile-306 or Phe-343 did not enhance maturation of Δ Y490 P-gp. The I306R mutant also promoted maturation of misprocessed mutants that had mutations in the second nucleotide-binding domain (L1260A), the cytoplasmic loops (G251V, F804A), the linker region (P709A), or in TM segments (G300V, G722A). These results show that arginine residues in the TM

domains can mimic the drug rescue effects and are effective suppressor mutations for processing mutations located throughout the molecule.

Regulation of Steady-state β -Amyloid Levels in the Brain by Neprilysin and Endothelin-converting Enzyme but Not Angiotensin-converting Enzyme

J. Biol. Chem., Vol. 281, Issue 41, 30471-30478

Elizabeth A. Eckman[‡], Stephanie K. Adams[‡], Frederick J. Troendle[‡], Becky A. Stodola[‡], Murad A. Kahn[‡], Abdul H. Fauq[‡], Hong D. Xiao[‡], Kenneth E. Bernstein[‡], and Christopher B. Eckman

The deposition of β -amyloid in the brain is a pathological hallmark of Alzheimer disease (AD). Normally, the accumulation of β -amyloid is prevented in part by the activities of several degradative enzymes, including the endothelin-converting enzymes, neprilysin, insulin-degrading enzyme, and plasmin. Recent reports indicate that another metalloprotease, angiotensin-converting enzyme (ACE), can degrade β -amyloid in vitro and in cellular overexpression experiments. In addition, ACE gene variants are linked to AD risk in several populations. Angiotensin-converting enzyme, neprilysin and endothelin-converting enzyme function as vasopeptidases and are the targets of drugs designed to treat cardiovascular disorders, and ACE inhibitors are commonly prescribed. We investigated the potential physiological role of ACE in regulating endogenous brain β -amyloid levels for two reasons: first, to determine whether β -amyloid degradation might be the mechanism by which ACE is associated with AD, and second, to determine whether ACE inhibitor drugs might block β -amyloid degradation in the brain and potentially increase the risk for AD. We analyzed β -amyloid accumulation in brains from ACE-deficient mice and in mice treated with ACE inhibitors and found that ACE deficiency did not alter steady-state β -amyloid concentration. In contrast, β -amyloid levels are significantly elevated in endothelin-converting enzyme and neprilysin knock-out mice, and inhibitors of these enzymes cause a rapid increase in β -amyloid concentration in the brain. The results of these studies do not support a physiological role for ACE in the degradation of β -amyloid in the brain but confirm roles for endothelin-converting enzyme and neprilysin and indicate that reductions in these enzymes result in additive increases in brain amyloid β -peptide levels.

The Amino Terminus of the Human Multidrug Resistance Transporter ABCC1 Has a U-shaped Folding with a Gating Function

J. Biol. Chem., Vol. 281, Issue 41, 31152-31163

Qun Chen, Youyun Yang, Lang Li, and Jian-Ting Zhang

Multidrug resistance is a serious problem in successful cancer chemotherapy. Studies using model cell lines have demonstrated that overexpression of some members of the ATP-binding cassette (ABC) transporter superfamily, such as ABCC1, causes enhanced efflux and, thus, decreased accumulation of multiple anticancer drugs, which leads to

increased cell survival. Unlike most other ABC transporters, ABCC1 has an additional membrane-spanning domain (MSDo) with a putative extracellular amino terminus of 32 amino acids. However, the function of MSDo and the role of the extracellular amino terminus are largely unknown. In this study, we examined the structural folding and the function of the amino terminus. We found that it has a U-shaped folding with the bottom of the U-structure facing cytoplasm and both ends in extracellular space. We also found that this U-shaped amino terminus probably functions as a gate to regulate the drug transport activity of human ABCC1.

Retrocyclins Kill Bacilli and Germinating Spores of Bacillus anthracis and Inactivate Anthrax Lethal Toxin

J. Biol. Chem., Vol. 281, Issue 43, 32755-32764

Wei Wang[‡], Chandrika Mulakala^{§1}, Sabrina C. Ward[¶], Grace Jung[‡], Hai Luong[‡], Duy Pham[‡], Alan J. Waring[‡], Yiannis Kaznessis^{§1}, Wuyuan Lu^{||}, Kenneth A. Bradley[§], and Robert I. Lehrer

θ -defensins are cyclic octadecapeptides encoded by the modified α -defensin genes of certain nonhuman primates. The recent demonstration that human α -defensins could prevent deleterious effects of anthrax lethal toxin in vitro and in vivo led us to examine the effects of θ -defensins on Bacillus anthracis (Sterne). We tested rhesus θ -defensins 1-3, retrocyclins 1-3, and several analogues of RC-1. Low concentrations of θ -defensins not only killed vegetative cells of B. anthracis (Sterne) and rendered their germinating spores nonviable, they also inactivated the enzymatic activity of anthrax lethal factor and protected murine RAW-264.7 cells from lethal toxin, a mixture of lethal factor and protective antigen. Structure-function studies indicated that the cyclic backbone, intramolecular tri-disulfide ladder, and arginine residues of θ -defensins contributed substantially to these protective effects. Surface plasmon resonance studies showed that retrocyclins bound the lethal factor rapidly and with high affinity. Retrocyclin-mediated inhibition of the enzymatic activity of lethal factor increased substantially if the enzyme and peptide were preincubated before substrate was added. The temporal discrepancy between the rapidity of binding and the slowly progressive extent of lethal factor inhibition suggest that post-binding events, perhaps in situ oligomerization, contribute to the antitoxic properties of retrocyclins. Overall, these findings suggest that θ -defensins provide molecular templates that could be used to create novel agents effective against B. anthracis and its toxins.

Anthrax Edema Factor, Voltage-dependent Binding to the Protective Antigen Ion Channel and Comparison to LF Binding

J. Biol. Chem., Vol. 281, Issue 43, 32335-32343

Tobias Neumeyer[‡], Fiorella Tonello[§], Federica Dal Molin, Bettina Schiffler, and Roland Benz

Anthrax toxin complex consists of three different molecules, the binding component protective antigen (PA, 83 kDa), and the enzymatic components lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa). The 63-kDa N-terminal part of PA, PA63, forms a

heptameric channel that inserts at low pH in endosomal membranes and that is necessary to translocate EF and LF in the cytosol of the target cells. EF is an intracellular active enzyme, which is a calmodulin-dependent adenylate cyclase (89 kDa) that causes a dramatic increase of intracellular cAMP level. Here, the binding of full-length EF on heptameric PA63 channels was studied in experiments with artificial lipid bilayer membranes. Full-length EF blocks the PA63 channels in a dose, temperature, voltage, and ionic strength-dependent way with half-saturation constants in the nanomolar concentration range. EF only blocked the PA63 channels when PA63 and EF were added to the same side of the membrane, the cis side. Decreasing ionic strength and increasing transmembrane voltage at the cis side of the membranes resulted in a strong decrease of the half-saturation constant for EF binding. This result suggests that ion-ion interactions are involved in EF binding to the PA heptamer. Increasing temperature resulted in increasing half-saturation constants for EF binding to the PA63 channels. The binding characteristics of EF to the PA63 channels are compared with those of LF binding. The comparison exhibits similarities but also remarkable differences between the bindings of both toxins to the PA63 channel.