Functional tyrosine residue in the active center of human dipeptidyl peptidase III

The role of Tyr-318 in the catalytic mechanism of metallopeptidase family M49 probed with site-directed mutagenesis

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Human dipeptidyl peptidase III (DPP III) is a member of the metallopeptidase family M49 with an implied role in the pain-modulatory system and endogenous defense against oxidative stress. Apart from active site-zinc ligation, many details of the DPP III (M49 peptidase) mechanism are unknown.

We report heterologous expression of human DPP III and the site-directed mutagenesis results which demonstrate a functional role for Tyr^{318} at this enzyme's active site . The substitution of Tyr^{318} to Phe decreased k_{cat} by two orders of magnitude without altering the binding affinity of substrate, or of a competitive hydroxamate inhibitor designed to interact with S1 and S2 subsites. The results favor the involvement of the conserved tyrosine in transition state stabilization during the catalytic action of M49 peptidases.

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Structural background of the chaperone function of intrinsically disordered proteins

On the chaperone function of ERD14, a LEA protein from *A. thaliana*

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In recent years, more and more intrinsically disordered proteins (IDPs) have been shown to behave as potent molecular chaperones, such as for example fully disordered MAP2 [1], alpha-synuclein [2], some caseins [3] and plant dehydrins [4, 5]. In light of these and other examples, intrinsical disorder has been suggested to account for chaperone activity by an "entropy transfer" mechanism [6]. Although already several examples support this hypothesis, further, systematic experiments are needed.

In the present study we chose to experimentally identify and explore more closely the regions that account for the chaperone activity of ERD14, a plant dehydrin that in our laboratory was shown to be a potent chaperone with an activity comparable to that of HSP90 [5]. According to priorly defined, strongly conserved regions, so called segments [4], and in silico structural predictions, we defined five distinct regions that could be involved in chaperone function. After design and expression of the corresponding deletion mutants in E. coli, their chaperone activities were measured and compared to that of the wild type protein. Additionally to this functional analysis, also an in depths structural study by solution NMR is under preparation. These preliminary results give explicit information on the chaperone function of an intrinsically disordered protein, and may also disclose novel aspects of the physiological action of plant dehydrins in general.

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Fast protein fold estimation based on distance restraints derived from NMR spectra

Identifying protein folds from assigned NMR spectra

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Protein structure determination from nucleic magnetic resonance (NMR) spectra traditionally requires considerable human efforts and might last from several months to years. Even with the advent of automated structure calculation tools and methods utilizing different NMR parameters for quick fold estimation, NMR spectroscopists could benefit from a tool based on distance restraints, the main data source used for high-quality solution structure determination. We have developed a method, called PRIDE-NMR, capable of quick identification of known folds related to distance restraint sets obtained from NOESY-type NMR experiments. We present a thorough evaluation of the PRIDE-NMR approach and discuss its potential uses ranging from aiding *de novo* structure determination to assessing the correspondence between a fold and the restraints used to determine it. The PRIDE-NMR web server is publicly available at http://hydra.icgeb.trieste.it/pride.

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Synthesis and antimicrobial activity of some novel N-[2-p-substitutedbenzyl/phenyl-5-benzoxazolyl)]-2-morpholinoacetamides

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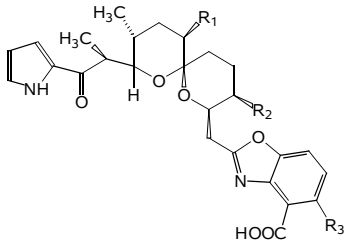
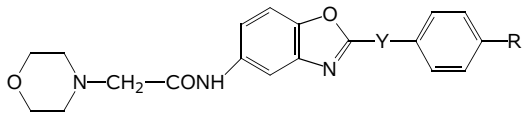
The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of microbial infectious and by drug side effects [1,2]. Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed toward the design of new agents. Benzoxazoles are the structural isosters of natural nucleotides and interact easily with the biopolymers. A benzoxazole derivative; calcimycin (Figure 1) is a carboxylic polyether antibiotic from a strain of *Streptomyces chartreusis* (NRRL 3882). It was found to very active against gram-positive bacteria including some *Bacillus*, *Micrococcus* strains. So that benzoxazoles constitute an important class of heterocyclic compounds with antimicrobial and antibiotic activities[3-5].



R_1=R_2=CH_3, R_3=$NHCH_3$

Figure 1. (Calcimycin)



R=H, CH_3, C_2H_5, $C(CH_3)_3$, F, Cl, Br; Y=-, CH_2

Figure 2.

In the present study, a series of N-[2-p-substitutedbenzyl/phenyl-5-benzoxazolyl)]-2-morpholinoacetamides were synthesized (Figure2). The structures of the new compounds were confirmed by elemental analysis, IR, ^{1}H-NMR and MASS spectral data. The compounds were tested *in vitro* antibacterial and antifungal activity against standard strains and drug-resistant isolates. It was carried out according to broth micro-dilution technique described by CLSI. The minimum inhibitory concentration (MIC) of each compound was determined and compared with the standard drugs.

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Metal ion interaction of a peptide, modelling the metal binding site of human MMP-13

A minimalist model of matrix metalloproteinases

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The matrix metalloproteinases (MMPs), which are zinc-dependent endoproteinases and are able to degrade all kinds of extracellular matrix proteins, have been implicated in a number of normal biological and pathological processes of connective tissue remodeling, such as cardiovascular and neurological diseases, tumor cell invasion and metastasis [1]. In the active site of MMPs the catalytically active Zn^{2+} is bound by three histidines found in the conserved sequence HExxHxxGxxH. In order to develop a minimalist chemical model of these enzymes, we synthesized a pentadecapeptide (Ac-KAHEFGHSLGLDHSK-NH_2) corresponding to the catalytic zinc(II) binding site of human MMP-13, except the first lysine residue, which was inserted to increase the water solubility. Our potentiometric, NMR and kinetic study are aimed to contribute to explore the intrinsic properties of the active site, including the binding of MMP inhibitors. Around pH 7 the peptide binds Zn^{2+} exclusively by its side chain donor groups, with similar stability to other $\{3N_{im}\}$ coordinated Zn^{2+}-peptide complexes [2]. The deprotonation of the zinc-bound water takes place with pK = 7.6. Although, the multi-domain structural organization of MMPs fundamentally determine their activity and selectivity, the naked Zn^{2+}-binding site still possesses hydrolytic activity between pH 7 and 9. Since the multiimidazole environment is characterictic of biological copper, too, for comparative purposes we also studied the copper(II) complexes of the peptide.

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Poster

Structure and Stability of Collagen-like β-Peptide Nanotubes

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Since secondary structure elements are known to play a key role in stabilizing the 3D-fold of proteins for the design of non-natural proteins composed of β-amino acid residues the construction of suitable secondary structural elements is mandatory. Folding analogues of α-helices and β-strands of β-polypeptides were already described.[1] Here we present several collagen-like folds composed exclusively of β-Ala(s). Unlike their natural counterpart, these tubular nanostructures can be composed of more than three polypeptide chains aligned parallel and/or antiparallel. By using *ab initio* and DFT calculations we have optimized a large number of versatile collagen-like antiparallel nanostructures. In these tubular systems, oligopeptide strands are interconnected by $i \rightarrow (i)$ type H-bonds, except for the "closing" set. This latter is called "the H-bond zipper" and is either $(i) \rightarrow i$, $(i+1) \rightarrow i$ or $(i+2) \rightarrow i$ type. Antiparallel, tubular foldamers composed of l number of strands, each of k number of β-amino acid residues (*e.g.* $ap(\beta\text{-}T^{l}_{i})_{k}$, $ap(\beta\text{-}T^{l}_{i+1})_{k}$ or $ap(\beta\text{-}T^{l}_{i+2})_{k}$), are unexpectedly stable supramolecular complexes. Independently of k and l, the local backbone fold of the amino acid residues is usually spiral, abbreviated as "S_P" or "S^*_P". In antiparallel nanotubes the backbone fold can occasionally twist out from S_P or S^*_P type into an alternative local structure. However, the more the local geometry of the strands resembles to S_P or S^*_P, the higher the stability is. Beside the backbone twisting, the overall stability is determined by the type and the geometrical properties of the constituent H-bonds. Interestingly, the higher stability of the tubular systems is enforced not only by maximizing the total number of H-bonds, but also by forming optimum geometrical properties. Finally, we have pointed out, that both the increase of the number of strands and that of their length stabilize the supramolecular complex.

Exploring conformational landscapes of model peptides and their complexes by matrix-isolation techniques

Structural properties of protein building units. A combined experimental and theoretical investigation of natural and non-natural dipeptides

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Using matrix-isolation infrared (MI-IR) and matrix-isolation vibrational circular dichroism (MI-VCD[1]) spectroscopies, conformations of half a dozen small model peptides and their complexes with water have been investigated. The poster summarizes the main results and conclusions of these studies.[2–5] We show that in the case of molecules with multiple low-energy conformers exhibiting strong intermolecular interactions, like the investigated model peptides forming hydrogen bonds, liquid-phase VCD spectra are hard to assign and to compare directly to theoretical predictions, while MI-VCD spectroscopy, combined with matrix-isolation, can be a powerful tool for studying these compounds. In the case of complexes we show that both complexation-induced conformational changes and the so-called chirality transfer phenomenon can be successfully observed by means of MI-IR and MI-VCD techniques helped by electronic structure calculations.

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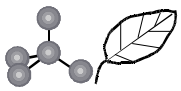

Structural and dynamical characterization of an unstructured myosin fragment (M) upon binding to the dimeric dynein light chain (DLC)

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Class V myosins are involved in short-range intracellular transport along actin filaments. Of the three mammalian myosin-V heavy chain genes, mutations of MYO5A are responsible for the *dilute* phenotype and Griscelli syndrome type1 in mice and humans, respectively. A dynein light chain (DLC) has been identified as a tail domain light chain of myosin-Va (myo5a), which may function as a cargo-binding and/or regulatory subunit of both motor proteins. Various fragments of myo5a tail (M) and DLC were expressed in *E. coli* and human cells, and their DLC-M complexes were analyzed in order to determine the binding sites on each fragment. It was showed already that binding of M occurs on the surface groove on the homodimeric DLC, and two molecules of target M peptide fragments fit in the two binding channels, and there is no communication between the channels.[1]

Our goal in this study is to characterize from the peptide fragment side the structure and dynamics of both the free M fragment and the DLC-M 1:1 complex by means of multinuclear and multidimensional NMR spectroscopy methods. For this purpose ^{15}N labeled, and ^{13}C, ^{15}N labeled, 27 residue long myosin fragments were expressed. Peak assignment is done from 3D measurements, and the relaxation parameters (T_1, T_2 relaxation times and heteronuclear NOE data) determined for each assigned residue show that in free form the M fragment can be considered an IUP. Upon formation of the 1:1 DLC-M complex - monitored by HSQC measurements with successive addition of unlabeled DLC amounts – the M fragment becomes partly structured. Relaxation data reveal that the D14-T23 region is responsible for binding. Furthermore, we discuss the behavior with temperature variation from 280 to 300K for both the free and bound M.

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Poster

Folding kinetics of implicitly solvated alanine oligomers

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In our study, the folding of Ace-$(Ala)_n$-NMe (n = 7, 10, 12, 14, 16, 18, 20) was investigated using an ensemble of molecular dynamics simulations with an implicit solvent model. One hundred independent, 10 ns long simulations started from extended structures with random initial velocities were carried out at 300 K. The average helicities of poly-(Ala) chains were calculated during the simulation period. From these results two processes with different speed (characteristic time) were identified. The characteristic times of the slower processes were used to estimate the chain length dependence of the folding time of poly-(Ala). For the investigated systems, a one-unit elongation of poly-(Ala) chain resulted in 18 % increase of folding time.

The detailed analysis of main chain-main chain H-bonds showed that the folding starts with a very fast process, where the linear extent of the system decreased and unordered structures were formed, which were stabilized by different number of H-bonds [1]. If these H-bonds are not formed between the $(i+n)th$ and ith (n = 2, 3, 4) (local H-bonds), but between any other residues (non-local H-bonds), they will hinder the folding process. The temperature dependence of the folding time and equilibrium helicity was also investigated at 300 K, 350 K, 400 K and 450 K for Ala10, Ala14 and Ala20.

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C- and *N*-glucopyranosyl heterocycles as inhibitors of glycogen phosphorylase

Heterocyclic glucose derivatives as potential antidiabetics

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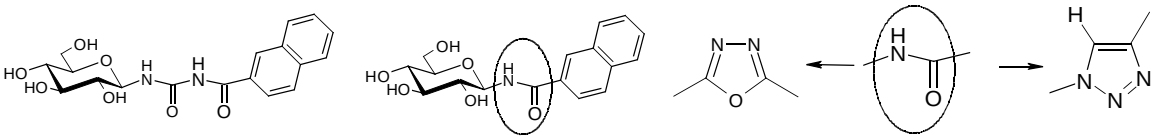
Th*Diabetes mellitus* has become a widespread disease and further exponential growth in its prevalence is predicted. An alternative to the existing methods for the treatment of type two diabetes can be based on the inhibition of glycogen phosphorylase (GP) enzyme. One of the largest groups of inhibitors of GP are the glucose derivatives. It was shown that some *N*-(β-D-glucopyranosyl)amides and -urea derivatives are good inhibitors of GP.



K_i = 0.35 μM (K_i rabbit muscle GP) K_i = 10 μM

In order to overcome some synthetic difficulties and stability problems especially with the acyl-urea derivatives we set out to investigate the non-classical bioisosteric replacement of the NHCO unit with heterocycles like the 1,2,3-triazole and 1,3,4-oxadiazole rings. Three series of 1,2,3-triazole derivatives were prepared from α- and β- as well as 1-deoxy-1-substituted-D-glucopyranosyl azides by using variants of the copper(I)-mediated azide-acetylene cycloaddition („click" chemistry).

For the preparation of 1,3,4-oxadiazoles two routes were studied: a) 5-(β-D-glucopyranosyl)tetrazole was acylated under various conditions, or b) 2,6-anhydro-aldose-acylhydrazones were oxidized to give the target compounds.

Presented will be the synthetic details and preliminary enzyme kinetic as well as crystallographic results characterizing binding of the new compounds to rabbit muscle GP*b*.

The effect of the counter ion on the nature of the Copper(II) complexes containing N,N'-bis(4-dodecyloxy-benzylidene-N-propyl)-piperazine as ligand

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Previous studies [1] have shown that $Cu(L)(ClO_4)_2$, where L stands for N,N'-bis(4-dodecyloxy-benzylidene-N-propyl)-piperazine , has been destroyed by recrystallization and the resulting product was $Cu(L')(ClO_4)_2$ (L'= N,N'-bis(3-aminopropyl-)piperazine [2]. In order to explain this behaviour, the effect of the nature of the anion on the complexes formation is studied.

Here we report the synthesis and spectral properties of the Cu(II) complexes containing N,N'-bis(4-dodecyloxy-benzylidene-N-propyl)-piperazine as ligand and the following anions: perchlorate, chlorate.

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Comparative study regarding the absorption and fluorescence spectra of Zn(II)complexes of tetrapyridylporphyrine and tetra(3-hydroxy-phenil)porphyrine

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Zn(II) porphyrin derivatives are known as useful functional dyes and because they strongly absorb light, they can be used as photosensitizers in photodynamic therapy [1], for the treatment of tumors and malignant tissues.

The present paper was focused on the obtaining and characterization of Zn(II) complexes of 5,10,15,20-Tetrakis(4-pyridyl)-21H,23H-porphine (TPyP) and of 5,10,15,20-Tetrakis(3-hydroxy-phenyl)-21H,23H-porphine (3-OH-TPP), molecules that easily self-assemble into large highly ordered domains.

The Zn(II) complexes were obtained by refluxing for 2-3 hours a large excess of Zn acetate dihydrate with the corresponding porphyrin-base.

Among the investigations, one focus is about the comparative study of the FT-IR, absorption and fluorescence spectra, between porphyrin-bases and their Zn complexes, at various concentrations and in terms of different pH conditions.

In comparison with the porphyrin bases, the Zn complexes, exhibit supplementary emission bands around 600 nm, which are characteristic of the metallated complexes. (Figure 1).

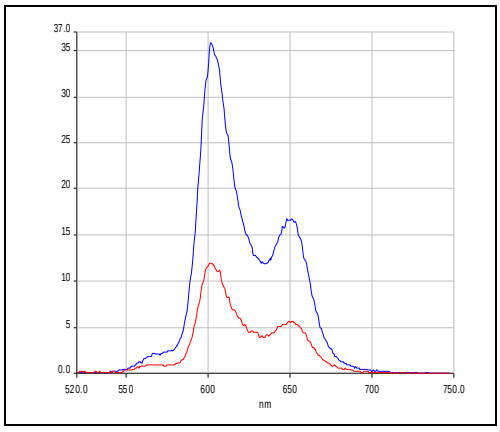

*Fig. 1 Emission spectra of Zn-TPyP in THF at λex= 395 nm, at different concentrations: 1 *10^{-5} M (higher intensity); 1*10^{-6} M (lower intensity)*

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Poster

Synthesis, characterization and antimicrobial activity of some Zn(II) and Ag(I) complexes with chlorhexidine

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Chlorhexidine (CHX), 1,1'-hexamethylene-bis-[5-(*p*-chlorophenyl)-biguanide] is the most frequently used antimicrobial agent of the biguanide class, and is especially used as an antiseptic for the oral cavity and skin [1,2]. At relatively low concentrations, the action of the chlorhexidine is bacteriostatic, whilst at higher concentrations its action is bactericidal.

The mechanism of antimicrobial action of chlorhexidine includes attraction and then the incorporation into the bacterial cell wall, which disrupts the membrane and reduces its permeability [3]. Other demonstrated way of its action is to bind to bacterial DNA, alter its transcription and cause lethal DNA damage.

The synergistic inhibitory effect of some metal ions, such as Zn^{2+} or Ag^{+} with chlorhexidine on various bacteria and fungi was demonstrated by several studies [4].

To this same end our goal was the synthesis, characterization and determination of antimicrobial activity of a number of new complex compounds of Zn(II) and Ag(I) with chlorhexidine.

The complex compounds were prepared from chlorhexidine base, chlorhexidine diacetate and chlorhexidine dichlorhydrate and the following metal salts: zinc(II) chloride, zinc(II) sulfate and silver(I) nitrate.

The complexes have been characterized by elemental and thermogravimetrical analysis, IR, UV-VIS, ^{1}H NMR and ^{13}C NMR spectra.

The chemical form of CHX and the anion of the metal salt have an influence not only on the composition, stereochemistry and stability of the complex, but also on the antimicrobial activity.

Antibacterial and antifungal activities of the complexes have been determined *in vitro*, by the cup-plate agar diffusion method, against various Gram negative and Gram positive bacteria and fungi. The complexes present an activity comparable to that of chlorhexidine and its salts against Gram positive bacteria, but they are more active than the ligands against Gram negative bacteria and fungi tested.

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Poster

RNA hairpin molecule studied by NMR

Structure of the miRNA:mRNA complex

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MicroRNAs (miRNAs) are small RNAs that play important role in developmental timing, differentiation, proliferation and apoptosis. They are produced from endogenous hairpin RNA precursors. One strand of miRNA assembles into RNA-induced silencing complex, which uses the miRNA to identify target messenger RNA (mRNA). The degree of complementarity between miRNA and mRNA is critical for the subsequent cleavage of target mRNA or repression of mRNA translation. Common structural elements of miRNA:mRNA complexes are asymmetric internal loops, mismatch base pairs, bulged A and C residues.

Our study focused on *let-7* miRNA from nematode *C. elegans*. The 21-nucleotide *let-7* regulates the developmental timing from late larval stage to adult by forming two distinct complexes with the 3'-UTR of the *lin-41* mRNA (1). Recently, members of *let-7* family miRNA were isolated in many organisms, also in human, where they can function as Ras oncogene suppressors.

We have prepared 33-nt model complex between *let-7* miRNA and the second binding site on 3'-untranslated region of *lin-41* mRNA. With the use of NMR techniques we have determined the 3D high-resolution structure of this RNA construct (2). NMR restraint computer simulations showed that molecule folds into a stable structure consisting of two stem regions separated by an asymmetric internal loop. Both stems are stabilized by Watson-Crick base pairs. Three uracils in the asymmetric internal loop form a base triple, while the two adenines form AA base pair. The analysis of helical parameters of both stems showed that asymmetric internal loop induces a bend between them, which widens the major groove of the complex. These interesting structural elements make complex between miRNA and mRNA different in comparison to a common A-form RNA.

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Effect of the introduction of a disulphide bridge on protein dynamics and interaction properties of Chicken Liver Bile Acid Binding Protein

Determinants of binding and dynamics properties of bile acid binding proteins by a mutagenesis and NMR approach

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Protein conformational dynamics is an important modulator of binding kinetics, as a varied flexibility in the regions adjacent to the binding site may affect both association and dissociation processes. We have reported [1,2] on the role of chicken liver bile acid binding protein (cL- BABP) in the binding and transport, within cytosol, of insoluble bile acids, important regulators of cholesterol metabolism and of nuclear Farnesoid X receptor activity. Our group has previously reported the NMR structure of apo [1] and holo cl-BABP [3,4], binding bile salts with a 1:2 stoichiometry. NMR 15N relaxation studies revealed a substantial conformational flexibility, on the microsecond to millisecond time scales, mainly localized in the C-terminal face of apo protein, that is quenched in the presence of the bile salts [2]. The effect on backbone dynamics of the introduction of a disulphide bridge on cL-BABP is here discussed on the basis of 15N relaxation data recorded for apo and holo T91C mutant. NMR interaction studies of T91C mutant with different bile acids suggest that the mutation does not alter the binding stoichiometry, although it seems to affect binding kinetics at the superficial site. The present results, combined with data obtained for other cL-BABP mutants and other intracellular lipid binding proteins, contribute to the identification of the molecular determinants of binding and dynamics properties of this protein family.

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New Copper(II) complexes of the polidentate ligand with C_{10} alkyloxy chain. A study concerning the influence of the anion on the nature of the complexes compound

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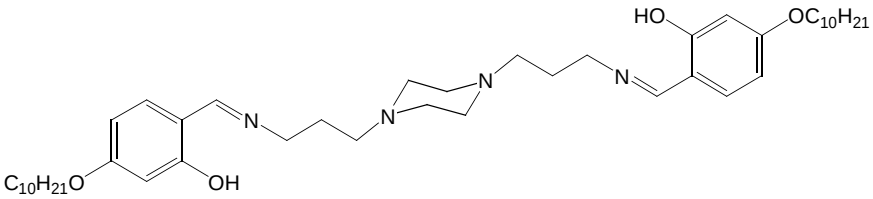

Fig. 1. N,N'-bis(4-decyloxysalicyliden-N-n-propyl)-piperazine

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Syntheses and Monitoring of Manganese Porphyrins. UV-vis, FT-IR and Fluorescence Comparative Characterization.

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Porphyrins can give complexes with metals having oxidation numbers from +1 to +6 [1] with applications as ionophores [2] in anion (salycilate) selective sensor design, for preparing new photocatalytic TiO_2-hybrid systems and in diagnosis and noninvasive therapy of cancer.

The present work is related to the manganese complexes of 5,10,15,20-tetraphenylporphyrin (TPP) and of 5,10,15,20-tetra(3-hydroxy-phenyl)porphyrin (3-OH-TPP), which were prepared by metallation of the porphyrin ligands in either methanol or ethanol at porphyrin:manganese ratios of 1:20. Metallation was accomplished under refluxing conditions for at least 2 hours. The reaction was monitored by UV-visible spectroscopy.

The intense band in the range 460-480 nm in the *hyper* spectra of the above complexes is assigned to the charge transfer arising during the absorption of the light quanta. The stability of manganese porphyrins was studied under strongly acidic and alkaline conditions. The absorbtion spectra of the porphyrin complexes in the organic phase were blue shifted with increasing of pH in the aqueous phase, suggesting the formation of the µ-oxo-dimer [3].

The compounds were also characterized by FT-IR and emission spectra at different pH conditions and a comparison of the effects produced by the nature of the substituents in the porphyrin ligand was done.

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Poster

Synthesis of azadisaccharides as heparanase inhibitors

Targeted selective inhibition of glycosidase enzymes

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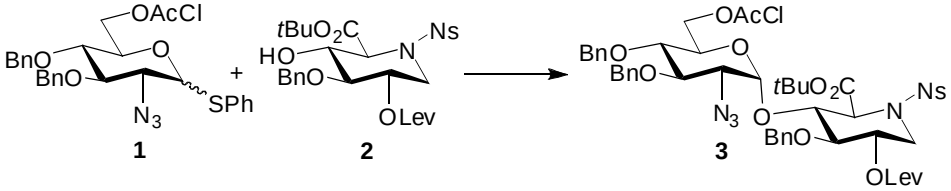
Human heparanase is an endo-β-D-glucuronidase that degrades heparan sulfate (HS) and heparin, these glycosaminoglycans (GAGs) are implicated in a variety of biological processes, such as inflammation, tumor angiogenesis and metastasis.

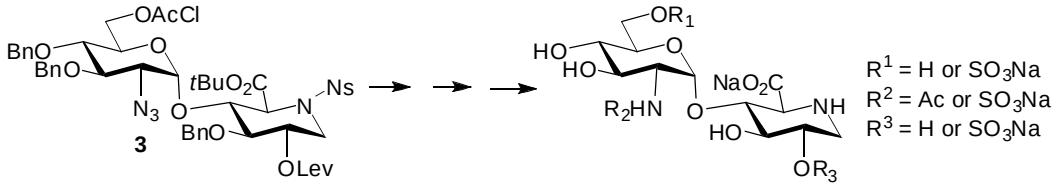
Heparan sulfate proteoglycans (HS-PGs) are covalently linked protein-HS glycosaminoglycan conjugates which are found in the extracellular matrix (ECM) and on the cell surface of most cells and have been demonstrated to be key components of the cell-cell and cell-ECM interactions. HS-GAG binds to and co-localizes with structural proteins, such as fibronectin and collagen in the ECM, providing a framework for the organization of ECM. Therefore cleavage of heparan sulfate by heparanase plays a crucial role in a number of biological processes, including the cell invasion of some malignant solid tumors through basement membranes (1). For this reason, the possibility of heparanase inhibition based antimetastatic cancer therapies is intensely investigated.

Azasugar monosaccharides, nojirimycin and 1-deoxynojirimycin, inhibit several glycosidases. In order to incorporate specificity for heparanase inhibition, we have designed pseudooligosaccharides mimicking structural motifs of heparin and heparan sulfate.

Here we report the synthesis of heparin disaccharides possessing an aza-D-glucuronic acid unit. For the synthesis of the target compounds we extended our orthogonal protection strategy to azasugars and synthesized the central intermediate (**3**), from which different heparin analog azadisaccharides can be derived. Starting from diacetone-D-glucose, a simple and efficient route was developed for the preparation of the partially protected 1,5-dideoxy-1,5-imino-D-glucuronitol derivative (**2**), a versatile glycosyl acceptor for the synthesis of oligosaccharidic heparanase inhibitors. Me_2S_2-Tf_2O-promoted (2) glycosylation of **2** with the thioglycoside donor (**1**) afforded the protected pseudodisaccharide derivative (**3**) stereoselectively.



The protected disaccharide **3** possessed an orthogonal levulinoyl (Lev) group at O-2 of the glucitol and a chloroacetyl (AcCl) group at O-6 of the glucosamine residue. Selective removal of the levulinoyl group followed by sulfation afforded the 2-*O*-sulfated derivative, whereas the removal of the chloroacetyl group resulted in the 6'-*O*-sulfated derivative.



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Poster

Structural and dynamic characterization of intrinsically disordered human securin by NMR

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Securin is the inhibitor of separase, the cysteine protease that initiates chromosome separation at the onset of anaphase in the cell-cycle. A failure in precise synchronization of the action of securin and separase causes unequal chromosome separation in mitosis, which leads to aneuploidy, a frequent genetic aberration in cancer. Thus, detailed structural and functional characterization of securin, in particular of the features relevant for the securin-separase interaction, is not only of theoretical, but also of significant biomedical importance. The challenge in this endeavor, however, is that securin has been previously described to lack a well-defined 3D structure, i.e. to belong to the group of intrinsically disordered proteins (IDPs). Residue-level description of the structural ensemble of an IDP of 202 amino acids in length, containing 24 prolines, has never been accomplished.

Here we report a structural and dynamical characterization of human securin. A combination of ^{1}H detected and ^{13}C detected, *protonless* NMR experiments was used to achieve the successful assignment of its backbone amide resonances, including almost all the prolines that would not have been possible by traditional proton-based NMR alone. Based on this assignment, various NMR observables, such as chemical shift values, ^{15}N relaxation rates (R_1, R_2, ^{1}H-^{15}N NOEs), ^{1}H exchange rates with the solvent (CLEANEX-PM) and ^{1}H-^{15}N residual dipolar couplings could be determined for most residues along the entire length of securin. The analysis of these observables shows that securin is largely disordered, but with several short segments of significant local propensity for order within the C-terminal half of the protein. Most notably, the segment D^{150}-F^{159} has a significant helical tendency, and regions E^{113}-S^{127} and W^{174}-L^{178} also show some deviation from random-coil behavior. Overall, securin contains an N-terminal half, which is fully disordered and a C-terminal half with transient segmental order, with regions probably serving for recognition and inhibition of separase. These findings are interpreted in terms of the enigmatic function and evolution of securin. Our studies therefore pave the way towards delineating the mode of securin function in detail, which will enable to develop specific antagonists to control the action of this proto-oncogene product.

Synthesis of Heparin Tetrasaccharides Based On Orthogonal Protecting Group Strategy

Oligosaccharide Library for the Study of Carbohydrate-Protein Interactions

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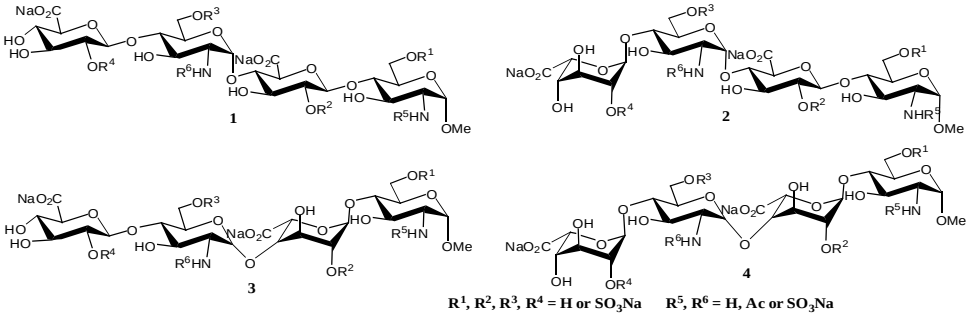
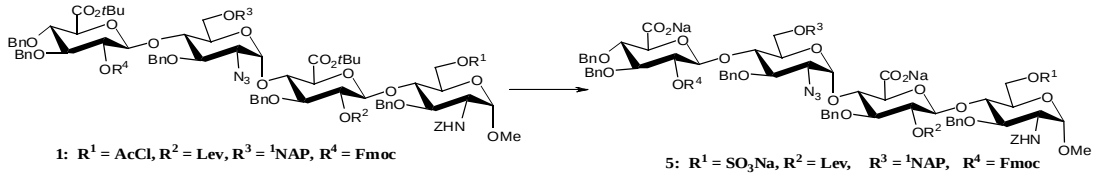
Heparin (H) and heparan sulfate (HS) are sulfated polysaccharides belonging to the glycosaminoglycans. They show a great deal of structural heterogeneity due to variations in their carbohydrate backbone and their highly varied sulfation pattern. Heparin, well-known as a blood anticoagulant, expresses a series of additional biological activities, such as antitumor, antiviral, antiinflammatory and antiasthmatic effects. These activities arise from the interactions of H/HS with a large number of proteins regulating physiological processes. It is generally assumed that different proteins recognize different oligosaccharide units of the highly heterogeneous linear polysaccharide chain [1].

To study heparin–protein interactions homogeneous oligosaccharide fragments are required. We have developed a new synthesis strategy, based on orthogonal protection, for the preparation of heparin oligosaccharides, containing D-glucosamine, D-glucuronic acid and L-iduronic acid units. Here we report the extension of this strategy to the synthesis of heparin tetrasaccharides, by which all the possible heparin tetrasaccharides can be synthesized from only 4 common intermediates, differing in their carbohydrate backbone (**1, 2, 3, 4**).



A new set of orthogonal protecting groups consisting of chloroacetyl (AcCl), levulinoyl (Lev), (1-naphtyl)methyl (^{1}NAP), and (9-fluorenyl)methoxycarbonyl (Fmoc) groups has been introduced. The versatility of this method has been proved by converting the partially protected tetrasaccharide **1**, having six different orthogonal protecting groups at possible sulfation positions, to the sulfated heparin oligosaccharides (**5, 6, 7, 8**).



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β-D-Glucopyranosyl-modified thiosemicarbazones as inhibitors of glycogen phosphorylase

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Glucose derivatives are selective and efficient catalytic inhibitors of glycogen phosphorylase (GP), a target for the design of type 2 diabetes therapeutics. On the other hand, thiosemicarbazones are promising compounds in many diseases, in particular cancer. The present work is aiming to combine these two classes of compounds into one family of organic molecules, and thus a series of β-D-glucopyranosyl-modified thiosemicarbazones have been synthesized (Figure 1). Kinetic experiments showed inhibition (IC_{50} ~90 μM (minimum)) of GP, while crystallographic results for the GP – glucose-thiosemicarbazone complex showed that these derivatives surprisingly bind at the new allosteric site rather than the catalytic site, and stabilise the less active quaternary conformation of the enzyme (Figure 2).

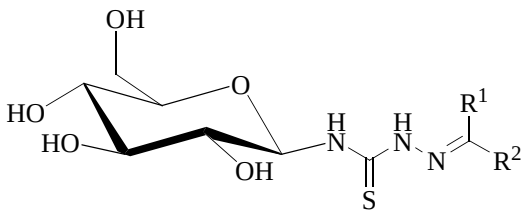

Figure 1. β-D-Glucopyranosyl-modified thiosemicarbazones

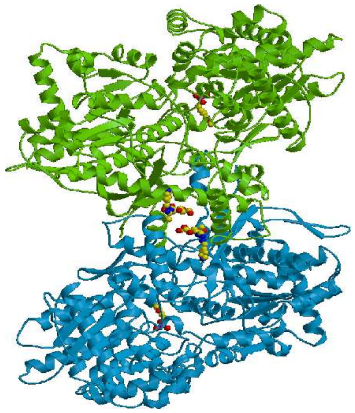

Figure 2. Crystal structure of GP - glucose-thiosemicarbazone complex

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Synthesis and antimicrobial activity of some new triazolo-thiazolyl-thiazolidine-2,4-diones

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1,3-Thiazolidines are the new class of antimicrobial agents with activity against broad spectrum of Gram-positive pathogens including *Staphylococci*, *Streptococci* and *Enterococci*.[1] It has been known that the entrance of arylidene moieties at different positions of the thiazolidine ring enhanced the antimicrobial activity.[2] The thiazole ring is very important in nature. For example, it exists in thiamine, a coenzyme required for the oxidative decarboxylation of α-keto acids. A tetrahydrothiazole also appears in the skeleton of penicillin which is one of the first and still most important of the broad-spectrum antibiotics. [3] Besides, thiazoles and their derivatives are found to possess potent antibacterial [4] and antifungal [5] activities.

On the other hand, compounds containing the 1*H*-1,2,4-triazole ring system are highly active fungicides. Example of such compounds, fluconazole is a antifungal drug bearing the 1,2,4-triazole moiety used in the treatment and prevention of superficial and systemic fungal infections. Also, antibacterial activity data of these structures showed their considerable activity against Gram negative and Gram positive bacteria as well as some strains of fungi. [6]In this study, we have synthesized a new series of 1,2,4-triazolo-thiazolyl- substituted benzyl-2,4-thiazolidinediones as seen in below Formula. Chemical structure of the compounds has been elucidated by their IR, ^{1}H NMR, Mass and elementary analysis data. The synthesized compounds were tested for their antifungal and antibacterial activities in vitro. All the compounds were found active against used microorganisms.

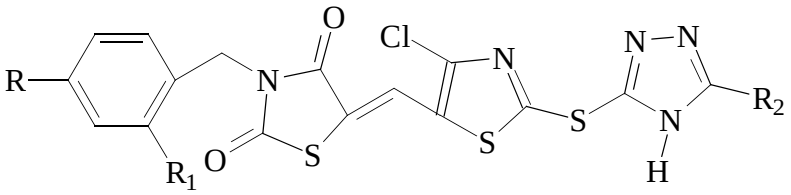

Fig. 1. Formula of fluconazole

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Potential MMPs inhibitor γ-hydroxamate derivatives of folic acid and their binding properties towards zinc(II) and human serum albumin

Binding of hydroxamate-folate MMPs inhibitors to zinc(II) and to albumin

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Folic acid, as a vitamin, is essential to all mammalians and it is required to the biosynthesis of nucleotide bases catalyzed by the dehydrofolate reductase enzyme (DHFR) [1]. Methotrexate (MTX) is a pterine folate analogue, which can inhibit DHFR (antifolate) and is used in cancer therapy. Inhibition of matrix metalloproteinases (MMPs) is also targeted in development of new anticancer agents. Therefore, a series of γ-hydroxamate derivatives of MTX and folic acid as dual target drug candidates was synthesized and tested against different MMPs and DHFR in our last work [2]. A catalytic zinc(II) can be found in the active site of all the MMPs and the inhibition is related to the binding of the inhibitor molecules to zinc centre. The present work is focusing on metal complex formation processes of the γ-hydroxamate derivatives of folic acid with zinc(II). Folic acid and its derivatives are able to bind to serum proteins during their transport, which can modify their actual serum concentration.

Thus, proton dissociation and zinc(II)-complex formation of folic acid and γ-hydroxamate derivatives were studied in a mixture of DMSO-H_2O by pH-potentiometry, UV-Vis, ESI-MS and ^{1}H NMR. Binding of folic acid and γ-derivatives towards the most abundant serum protein, albumin, was also investigated by ultrafiltration, UV-Vis and spectrofluorimetry.

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Generation and evaluation of realistic protein structural ensembles

What do dynamic protein structural ensembles tell us?

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Recent developments in molecular simulations of biomolecules allow the incorporation of multiple experiment-derived restraints yielding a conformational ensemble revealing the internal dynamics of the system as a result [1,2]. The justification of calculating structural ensembles instead of a single ”representative” conformer is that solution NMR measurements ouput the average values for 10^{16}-10^{17} molecules present in the test tube. As more and more types of information can be extracted from present-day NMR experiments, more and more restraints can be incorporated in structure calculations. The two questions arising are how well do calculated ensembles actually correspond to the experimental data available – whether used as restraints or not – and what are the relevance of the visualized “dynamic” features of proteins regarding their biological function. We present example calculations with a modified version of GROMACS allowing the use of backbone order parameter (S^2) restraints on small model proteins such as 35-residue serine protease inhibitors and the Tc5b miniprotein. A complex evaluation procedure for realistic conformational ensembles that can be incorporated into a web server will also be presented.

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A biochemical system for site-specific incorporation of isofroms for all 20 proteinogenic amino acids

A vision of a genetic code for protein NMR

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The outline of a universal cell-free translation system capable of site-specific insertion of any types of labeled amino acids is presented [1]. The system could be an invaluable tool for NMR spectroscopy by making the exclusive and exact labeling of the segments of interest possible. Although the development of such a system requires considerable efforts and can not be expected to be available in the next few years, we argue that recent progress in unnatural amino acid incorporation [2] together with the growing knowledge of the translation apparatus provide clues for overcoming the major difficulties that might arise. The discovery of orthogonal tRNA:aminoacyl-tRNA synthetase pairs [3] and the understanding of the structural basis of tRNA recognition in such systems [4] suggests that the genetic code can be expanded even by 20 additional amino acids. Considering the known rules of codon:anticodon recognition [5], we propose a genetic code coding for the isotope-labeled and unlabeled variants of all 20 amino acids. We also propose a reactor capable of continuous operation enabling separate and specific charging of tRNAs with each labeled and unlabeled amino acid pair. An alternative solution for the site-specific incorporation of any labeled amino acid is also presented. Importantly, incomplete versions of the proposed system could also be useful to study selected functional aspects of a number of proteins, examples of which are also given.

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Synthesis and potent antistaphylococcus activity of some novel 1*H*-benzimidazole-5-carboxamidines

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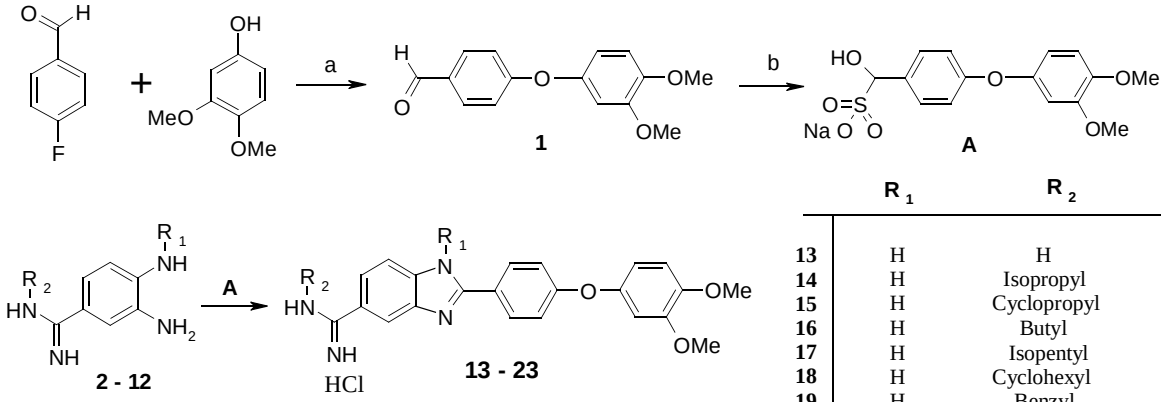
Numerous benzimidazole derivatives containing amidine groups on the benzene ring have been synthesized for their antifungal, insecticidal, herbicidal, antiinflammatory, and potential anthelmintic activities. It is also well known that amides, amidines and combinations of both are present in a variety of antimicrobial, antiparasitic, anthelmintic, antiviral and antitumoral agents. Furthermore, our previous work and that of others showed that benzimidazolecarboxamidines display good antibacterial and antimycotic activity. Taking into consideration these structural features and the expectation of low toxicity without halogen atoms, present, we planned to prepare a series of benzimidazoles carrying 4-(3,4-dimethoxy-phenoxy)-phenyl on the position C-2, with substituted amidines.

For the synthesis of targeted molecules, first unknown compound **1** was prepared by the reaction between the p-fluorobenzaldehyde and 3,4-dimethoxyphenol in good yield, then sodyum metabisulfite adduct of this aldehyde was prepared. Cyclization of compound **A** with several N-(substituted)-1,2-phenylendiamine-carboxamidines gave the final products **13 -23**.

All compounds were tested for in vitro antibacterial activity against, Gram-positive *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) by the macro-broth dilution assay to determine the MIC values of the synthesized compounds. Preliminary results gave promising activity.



	R_1	R_2
13	H	H
14	H	Isopropyl
15	H	Cyclopropyl
16	H	Butyl
17	H	Isopentyl
18	H	Cyclohexyl
19	H	Benzyl
20	H	Dimethylaminoethyl
21	Methyl	Isopropyl
22	Benzyl	Isopropyl
23	Benzyl	Dimethylaminoethyl

Figure 1

Synthesis of 6-methylenesulfonic acid containing analogues of heparin

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Biological significance of the anionic groups containing carbohydrate derivatives is well-known. Heparin is a sulfated glycosaminoglycan and plays important role in the inhibition of blood coagulation.

The pentasaccharide segment (*Figure 1.*) of heparin which has antithrombotic activity was established in the 1980's. Synthesis of simplified analogues of this pentasaccharide which has a longer half-life in human body was developed by Petitou et al[1].

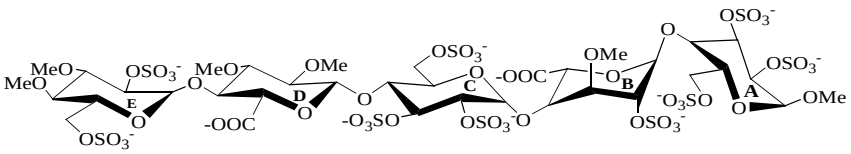
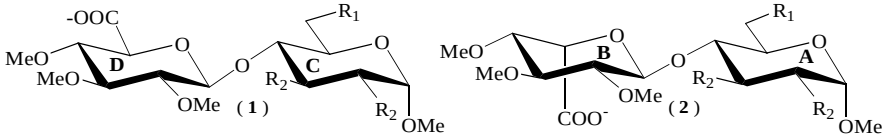


Figure 1.

We are planning the preparation of fragments of simplified heparin pentasaccharide which contains methylenesulfonic acid group instead of sulfate group because of these groups are isosters.

Here, we present the synthesis of sulfated disaccharide fragments (**1a**, **2a**) and the analogous disaccharides with methylenesulfonic acid group at the 6 position (**1b**, **2b**) of the model compounds.



1 a: R_1, R_2: $-OSO_3^-$ **1 b**: R_1: $-CH_2-SO_3^-$; R_2: $-OSO_3^-$
2 a: R_1, R_2: $-OSO_3^-$ **2 b**: R_1: $-CH_2-SO_3^-$; R_2: $-OSO_3^-$

Figure 2.

This work is supported by OTKA (T048798).

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Structure – function relationship of a myosin alpha-helical coiled-coil region

Possible structural basis of different regulation of myosin II isoforms

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The rod region of conventional myosin (myosin II) motors consists of two chains forming a long parallel α-helical coiled coil. The coiled coil is one of the most abundant dimerization (protein-protein interaction) structural motif. Recently, the high resolution structure of the N-terminal region of subfragment 2 (S2N51, a chimera construct containing a C-terminal Leu-zipper) at the head-rod junction of the scallop striated muscle myosin was determined in several crystal forms[1,2]. It was assumed that the stability of this region could be a key determinant for the regulatory ability of myosin II isoforms. The stability of S2 fragments from two regulated and two non-regulated isoforms, respectively, were compared by determing their melting temperatures using circular dichroism spectroscopy and differential scanning calorimetry. The results indicate that the observed melting temperature for these S2N51 chimeras from non-regulated cardiac and vertebrate skeletal myosins are higher than those from the thin filament regulated vertebrate smooth-muscle myosin and especially scallop striated myosin (fig.1). The higher flexibility at the regulated isoforms can be explained by the lack of the stabilizing salt-bridges and the abundance of hydrophilic residues in the hydrophobic core positions of the coiled-coil. The contribution of the salt bridges to the stability was demonstrated through examination of mutant constructs. It seems plain that the isoform-specific instability promotes the off-state conformation of the heads in regulated myosins. Our results suggest that the coiled-coil motif has a dynamic functional role in regulation beyond his well-known role as a structural element.

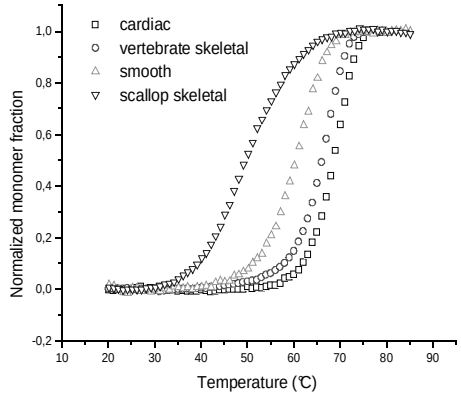

Figure 1
Comparison of the thermal stability of the different isoforms

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Linear scaling semiempirical molecular orbital calculations on the complexation of zinc ions by the Alzheimer's β-amyloid peptide

Theoretical study on zinc-Aβ metallopeptides

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The Alzheimer's β-amyloid peptide, Aβ, found as aggregated species in Alzheimer's disease brain, is linked to the onset of dementia. The toxic properties of Aβ depend on oligomerization and aggregation. In addition, Aβ toxicity is aggrevated by the presence of zinc ions, which affect the secondary structure of the peptide. We report the modeling of possible interactions of zinc ions with Aβ(1-42), one of the most amyloidogenic species known.

The aim of the current study was twofold. First, in order to theoretically handle several Aβ(1-42) monomers together, a quantum chemical method was sought that is capable of handling peptide systems comprised of several hundreds residues. Our tests validated the semiempirical parameterization of zinc termed ZnB, developed by Merz *et al.* originally for treating zinc metalloenzymes, as capable of describing complexation of zinc by Aβ(1-42). Used in conjunction with the linear scaling semiempirical program DivCon, also by Merz *et al.*, fully quantum chemical treatment of Aβ(1-42) oligomers and their zinc complexes is possible, requiring only modest computer resources.

The second part of our project considered several possible structures along feasible pathways of forming proto-aggregate monomers upon complexing zinc with the apo-peptide. DivCon/ZnB calculations probed putative intermediate conformations, which may play role in the eventual pathological mis-folding event. Likely participating ligand residues are suggested, besides the histidines which are the only ones confirmed unequivocally by experiments. Exploring the potential energy landscape at this novel level of theory provides new structural clues for the process.

TK thanks OTKA (Grant No. OTKA/61577) for partial support.

Interaction of the carrier ligands of insulin mimetic metal complexes with human serum albumin

What happens to the antidiabetic metal complexes after absorption?

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In the past few years, numerous antidiabetic complexes of vanadium and zinc were developed and investigated *in vitro* and *in vivo*. The efficiency of these complexes has been already confirmed, but the mechanism of the effect is rather complicated and not completely described. Therefore, it is important to know what happens to the complexes after absorption and how they can interact with the components of the blood. In the case of vanadium ternary complex formation is the most considerable with citrate among the low molecular mass components while in the case of zinc it is with cysteine. Besides, the interaction of vanadium with transferrin,and that of zinc with albumin has predominant role concerning the high molecular mass components of the serum (1,2). Our studies show that it is also necessary to take into account the formation of adducts of human serum albumin and the carrier ligands. In the present work we have determined the stoichiometry and stability of these adducts by ultrafiltration and UV measurements and suggested the most probable binding sites of albumin for the carrier ligands based on spectrofluorimetric and CD methods involving the use of competitive site marker ligands.

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Synthesis and physical properties of the new Ca(II) and Cd(II) model complexes with N, O-chelating ligands in the light of investigations of osteoporosis problems

Model complexes of Ca(II) and Cd(II) with small molecule of ligands — investigation of osteoporosis problems

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Cadmium is a heavy metal, affecting human health through occupational and environmental exposure. Recent studies [1] indicate that this metal may have both direct or indirect effects on bone turnover. An indirectly acted via kidney dysfunction. Directly interfere with calcium effected on an increase loss of bone density, leading to osteoporosis and increased risk of fractures, especially in the elderly an in females. Hypercalcuria, which may prograess to osteomalacia has been taken as a sensitive renal tubular biomarker of low level cadmium expose in the general population. For this reason main goal of ours study is the synthesis of new model Cd(II) and Ca(II) complexes with N, O – donor ligands and the comparison shape of coordination polyhedra for understanding the ability of the Cd^{2+} to mimic the Ca^{2+} ions in biological systems. Our investigations contain chemical and structural characterization of Ca(II) and Cd(II) model complexes with N, O-chelating ligands.

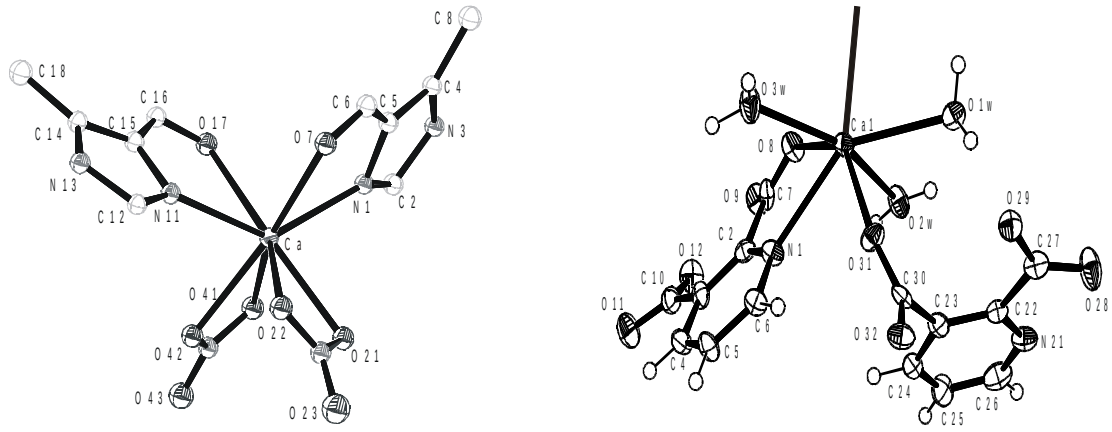

Fig.1.Molecular structure of Ca(II) complexes with 4(5)-methylimidazole-5(4)-carbaldehyde and 2,3-pyridinedicarboxylic acid

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Human dipeptidyl peptidase III acts like a postproline-cleaving enzyme on endomorphins

Degradation of opioid tetrapeptide endomorphin -1 by dipeptidyl peptidase III

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Due to its specific conformation, the amino acid proline imposes many structural restrictions on peptides and proteins, and also restricts the attack by most proteolytic enzymes. Especially sequences with proline in the N- or C-terminal penultimate position were considered to be resistant to non-specialised peptidases.

The dipeptidyl peptidase III (DPP III) is a broad specificity zinc-exopeptidase, with a role indicated in mammalian pain-modulatory system, owing to its high affinity for enkephalins and localization in the superficial laminae of the spinal cord dorsal horn. Our study revealed that this human enzyme hydrolyses opioid peptides belonging to the three new groups, endomorphins, hemorphins and exorphins. The enzymatic hydrolysis products of endomorphin-1 were separated and quantified by capillary electrophoresis and kinetic parameters determined for human DPP III and rat DPP IV. Both peptidases cleaved endomorphin-1 with comparable rate, by liberating the N-terminal Tyr-Pro. This is the first evidence on DPP III acting as a postproline-cleaving exopeptidase.

Synthesis of sulfonic acid containing oligosaccharide mimetics of sialyl Lewis A

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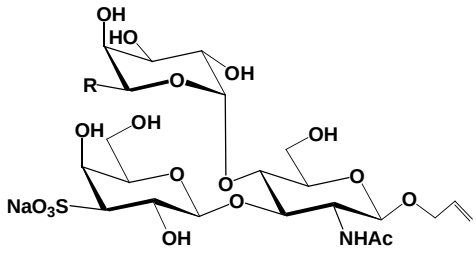
Sialyl Lewis X and Lewis A are regioisomeric tetrasaccharides, which have been identified as tumor-associated antigens, tissue-specifically expressed on carcinoma cells and involved in metastasis. They are ligands of selectins which are carbohydrate recognizing receptors on the surface of endothelial cells responsible for cellular adhesion[1].

To prevent certain pathological processes soluble selectin ligands could be applied as antagonists binding competitively to the selectins and suppressing the adhesion cascade from the beginning. It turned out that sulfate ester group containing Lewis X and Lewis A mimetics are more potent inhibitors than the sialylated Lewis antigens[2].

One of our main goal is to synthesize sulfonic acid mimetics of biologically important anionic carbohydrates. In this point of view we planned the synthesis of sugar sulfonic acid containing sLe^a mimetics having potent anti-inflammatory and anti-metastatic effect.



R = H, CH_3

This work is supported by OTKA (NK48798 and K62802).

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Metal binding of peptides from the His- rich region of histidine-rich glycoprotein

Oligopeptides as probes for the metal binding of histidine-rich glycoprotein

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Histidine-rich glycoprotein (HRG) is a multidomain plasma protein having a central His-rich region (HRR) and it is involved in the control of numerous biological processes, eg. angiogenesis, cell adhesion and migration, fibrinolysis and coagulation. Many of the HRG functions are related to metal ion (mostly Zn^{2+}) binding taking place on the HRR (1).

In order to understand better the role of metal ions in the HRG functions we aimed the characterization of the metal binding ability of HRR by applying two self-synthesized peptide fragments, Ac-HHPHG-NH_2 and its dimer Ac-$(HHPHG)_2$-NH_2, the constituents of the tandem sequence repeats of human HRG. Zn^{2+}- and Cu^{2+}-binding of the two ligands have been studied by equilibrium and spectroscopic (UV-Vis, CD, EPR and NMR) studies. The results show that even the shortest possible model of the histidine-rich region of HRG is able to bind Zn^{2+} and Cu^{2+} *via* only side chain imidazole coordination in the slightly acidic/neutral pH-range whereas the addition of the second -HHPHG- block allows the uptake of a second metal ion coordinated still by the His-imidazole groups, as it is proved by the spectral properties of the species. The decapeptide can bind even a third Cu^{2+} ion nevertheless the observed increase in the CD intensities and the blue shift of the λ_{max} values of the UV-Vis spectra following the formation of the trinuclear species demonstrates the additional coordination of deprotonated amide nitrogen(s). While the decapeptide can keep two Cu^{2+} ions in solution above pH 9.5 with imidazole and amide nitrogen donors in the coordination sphere of Cu^{2+}, Zn^{2+} forms precipitate with both peptides in the basic pH-range. According to our results, the increased number of His units and the possible conformational effects that may operate in the longer peptide did not alter significantly the Zn^{2+}-binding features of the HRR-models.

Acknowledgement: The financial support of the Hungarian Scientific Research Fund (OTKA PF63978, K63606) and the Hungarian Academy of Sciences (János Bolyai Research Grant) is acknowledged.

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Equilibria studies of the reactions between $[Pt(H_2O)_2(dach)]^{2+}$ complex and some sulphur and nitrogen donor biomolecules

Studies of interactions between some sulfur and nitrogen containing biomolecules and metal complexes

Ratomir Jelić[1], Biljana Petrović[1], Predrag Đurđević[1], Živadin D. Bugarčić[1]

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Since some Pt(II)(dach) (dach = 1,2-*trans*-*R*,*R*-diaminocyclohexane) complexes are used as antitumor drugs what is accounted in terms of interactions of the complex with DNA,[1] we decided to study the complex formation equilibria between $[Pt(H_2O)_2(dach)]^{2+}$ complex and some DNA constituents, such as INO, 5'-IMP and 5'-GMP. Also, the well-known anti-tumor activity of platinum complexes is followed with some toxic side effects as a result of interaction with sulphur-donor biomolecules.[2] Then it was very interesting to study the equilibria with L-methionine and glutathione.

The equilibria established in solution of $[Pt(H_2O)_2(dach)]^{2+}$ complex and mentioned biomolecules (L) were studied by glass electrode potentiometric titrations at 298 K. The constant ionic straight of the solutions were adjusted to 0.1 mol dm^{-3} by $NaClO_4$. Aqua complex was prepared by adding of two equivalents of $AgClO_4$ to the solution of $[PtCl_2(dach)]$ complex, heating at 50-60°C and removing the AgCl precipitate. All titrations were done with NaOH standard solution, while the pH interval was between 2 and 11.

As an example, here is presented the distribution diagram of the system $[Pt(H_2O)_2(dach)]^{2+}$ complex with 5'-GMP. ($C_{[Pt(H2O)2(dach)]2+}$ = 2.5 mmol dm^{-3}; $C_{5\text{-GMP}}$ = 5 mmol dm^{-3} ; I = 0.1 mol dm^{-3}). From the Figure 1 can be seen that the most dominant species at the physiological range of pH in Pt-dach-GMP system is (1 1 1) type of complex.

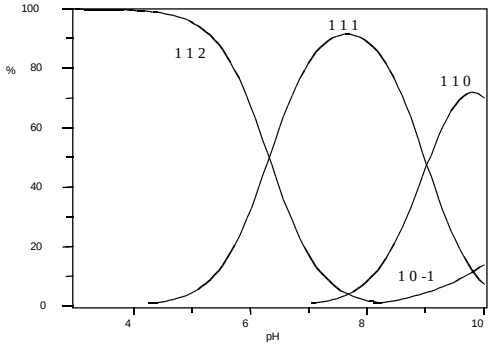

Fig. 1. Species distribution in Pt-dach-GMP system.

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Synthesis and physicochemical properties of novel potential ultrashort acting of novel potential beta-adrenergic blockers

Novel potential ultrashort acting of novel potential beta-adrenergic blockers

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The work is devoted to the synthesis and study of physicochemical properties of some aryloxyaminopropanol derivatives, the compounds of 2-hydroxy-3-[4-(2-metoxyphenyl)-piperazin-1-yl]-propyl-4-[(alkoxycarbonyl)amino]-benzoates, their salts with hydrochloric acid, with one to four carbon atoms in the alkoxy group of carbamoyl group. Their structure was confirmed by elemental analysis, IR, UV and mass spectra. The melting point, solubility, surface activity, dissociation constant and some lipophilicity parameters i.e. – partition coefficient, R_M values from reversed phase thin – layer chromatography and capacity factor obtained from HPLC of studied substances were determined.

Calcium-induced tripartite binding of intrinsically disordered calpastatin to calpain

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The activity of calpain is controlled by both the free intracellular calcium level and its intrinsically disordered endogenous inhibitor, calpastatin, mediated by short conserved segments; subdomain A, B, C. The exact binding mode of calpastatin to the enzyme is yet uncovered. NMR data of the 141 aminoacid long inhibitor, with and without calcium and calpain, have revealed both structural changes and a tripartite binding mode, in which the disordered inhibitor wraps around but contacts the enzyme at three points, enabled by flexible linkers. This unprecedented binding mode permits a unique combination of specificity, speed and binding strength in regulation.

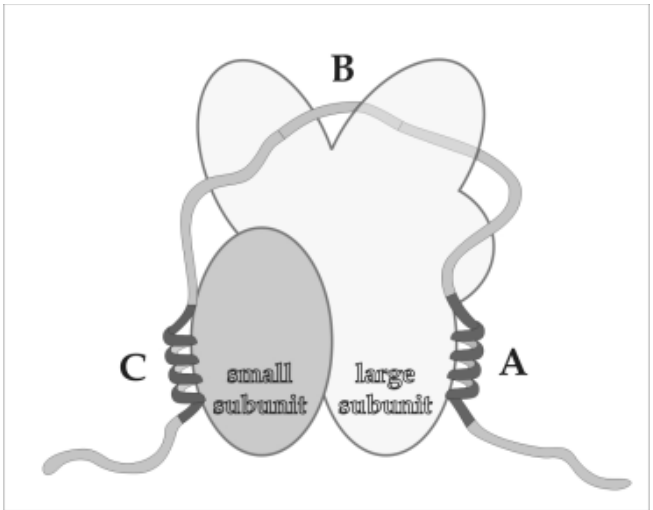

Fig. 1. Schematic representation of calpain:Calpastatin complex depicting the tripartiate binding mode.

Metal ion interaction with the N-terminal part of endostatine, a protein with antitumor activity

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On the Structure and Stability of PNA.DNA and PNA.PNA Duplexes: A Theoretical Study

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Several experimentally determined PNA.DNA and PNA.PNA structures are known in the literature (e.g. Brookhaven Database identification code: 176d, 1pdt, 1hzs, 1pup, 1qry, 1rss, 1pnn, etc.). Their stability related to DNA.DNA duplexes are not well known as well as the rules for the secondary structures. In order to compare the thermodynamic stability, a systematic theoretical study was performed with a wide variety of possible methods: molecular mechanics, molecular dynamics, semiempirical quantum chemical methods (PM6, MOZYME/PM5) in gas phase and with implicit solvation model (GB/SA and COSMO). A comparision of the structural properties of some small duplexes with one or two bases pairs (the initial structures obtained from the experimental results), was performed by the results of *ab initio*, density functional (DFT) methods and dispersion corrected DFT method. On the basis of the calculations, the backbone of PNA has no significiant stabilization effect with the bases, but its geometry can determine the structure of the duplexes.

This work was supported by the Hungarian Research Fund (OTKA K61577). The authors are grateful for the possibility of computations in the High Performane Computer Centre, University of Szeged.

Effect of Metal Ions on the Stability of PNA, PNA.DNA and PNA.PNA Duplexes

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Experimental result were published on the complexes of Zn^{2+} and Cu^{2+} with peptide nucleic acids (PNAs), PNA.DNA and PNA.PNA duplexes. No detailed structural informations were found on these complexes (ML and ML_2 with different charges) in the literature. Molecular mechanics and semiempirical quantum chemical methods (PM6, MOZYME/PM5) in gas phase and with implicit solvation model (COSMO) were applied to suggest structures of the metal complexes with monomers and dipeptide nucleic acid models with different basis (Ac-PNA(A,C,G,T,U)-$NHCH_3$) which structures obtained were refined by methods at higher levels (DFT and dispersion corrected DFT). On the basis of the structure of these small complexes, the position of the metal ions and their effect on the small PNA duplexes were suggested and calculated by semiempirical quantum chemical method.

This work was supported by the Hungarian Research Fund (OTKA K61577). The authors are grateful for the possibility of computations in the High Performane Computer Centre, University of Szeged.

Hydrolysis of RNA by lanthanide complexes

Lanthanide complexes as artificial ribonucleases

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Non-enzymatic hydrolysis of DNA and RNA has attracted much interest, because it is essential for futher developments in biotechnology, molecular biology, therapy and related fields (1-3). A few years ago, the catalytic activity of complexes containing lanthanide ions for hydrolysis of nucleic acids was discovered. In this contribution we wish to report the specific properties of lanthanide Schiff base complexes derived from salicyl aldehyde and biogenic amines (putrescine, cadaverine, spermine and spermine analogues), bound to a DNA oligomer (across succinic acid linker) as the sequence-recognizing moieties which are able to selectively hydrolyse RNA at the target site (Fig. 1.).

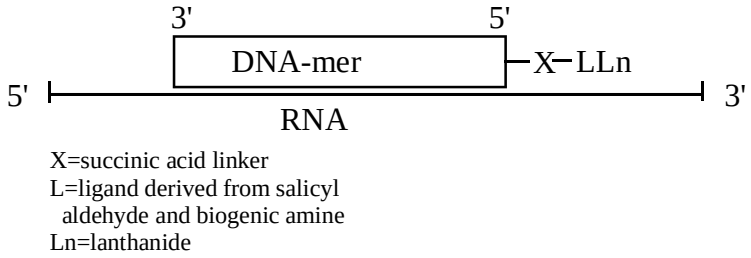

Fig.1.

Hydrolysis of the substrate RNA (^{32}P-labelled at 5'-end) was initiated by the addition of the RNA to the mixture of DNA-merXL and lanthanide(III) salt. When a RNA (^{32}P-labeled at 5'-end) was cut by the lanthanide complexes, fragments of the internucleotide linkages were formed. These fragments were separated by gel electrophoresis. The cleavage of RNA by the lanthanide complexes not covalently bound to a DNA is also investigated.

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Dinucleoside phosphonate-phosphates and nucleoside α-hydroxyphosphonates as potential anti-HIV-1 pronucleotides

Our recent studies on anti-HIV-1 pronucleotides

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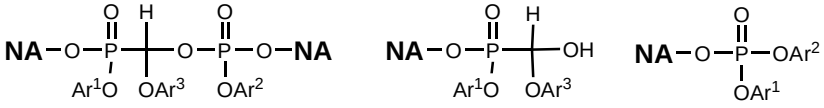
Nucleoside analogues are well established antiviral agents that became in recent years an important part of anti-HIV-1 therapy. In principle, they are prodrugs and have to be converted in a cascade of enzymatic reactions into biologically active species. For nucleoside analogues it involves a stepwise phosphorylation into the respective 5'-triphosphates that are highly active inhibitors of reverse transcriptase, an enzyme that copy viral RNA into cDNA. The most critical step in such an activation process of nucleoside analogues is the first phosphorylation step, i.e. introduction of a phosphate monoester group. If, due to structural modification of an nucleoside analogue, this step fails, the compound remains inactive. Here operates a simple rule: no phosphorylation – no anti-HIV-1 activity. A typical example is 2',3'-dideoxyuridine which, due to it its pour substrate affinity to cellular kinases, cannot be converted into the 5'-monophosphate, and thus is inactive against HIV-1. For similar reason, other nucleoside analogues may lose their anti-HIV-1 potency in thymidine kinase deficient cells (TK^- cells).
To by-pass this crucial and often troublesome in vivo phosphorylation step, efforts were focused on delivery into the infected cells already phosphorylated nucleoside analogues – pronucleotides. These compounds are usually nucleotide derivatives bearing various protecting groups, to ensure proper stability of these compounds and to facilitate their transport through cell membranes. These protecting groups have be removed chemically or/and enzymatically to generate with optimal kinetics, a 5'-phosphorylated nucleoside analogue in the cell. Several type of pronucleotides have been designed with promising pharmacokinetic and pharmacodynamic parameters.
In our laboratories we have been studied as potential anti-HIV-1 pronucleotides, dinucleoside phosphonate-phosphates of type **1**, nucleoside α-hydroxyphosphonates of type **2**, and diaryl nucleoside phosphotriesters of type **3**.



1 **2** **3**

NA = nucleoside analogue e. g. 3'-azido-3'-deoxythymidine (AZT), 2', 3'-dideoxyuridine (ddU), 2',3'-dideoxyadenosine (ddA).

Although both phosphonate-phosphates **1** and α-hydroxyphosphonates **2** posses quite interesting chemical and anti-HIV-1 properties, none of them can act as a pronucleotide. These compounds seem to functioning only as vehicles for delivery of nucleosides into the cell, and this was proved by lack of an anti-HIV-1 activity of ddU derivatives of **1** and **2**. In contrast to this, diaryl nucleoside phosphotriesters **3** appeared to be very promising, non-toxic anti-HIV-1 pronucleotides of controlled stability (pharmacokinetics) and cell membrane permeability (lipophilicity).

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Preparation and study of transition and lanthanide metal complexes of the polyamino-polyphosphonic acids

Biological importance of the transition and lanthanide metal complexes of the polyamino-polyphosphonic acids

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The development of nuclear magnetic resonance (NMR) imaging techniques as a clinical diagnostic modality has prompted the need for new class of pharmaceuticals. These drugs would be administrated to a patient in order to (a) enhance the image contrast between normal and diseased tissue and/or (b) indicate the status of organ function or blood flow. The intensity of the ^{1}H NMR images, largely composed of the NMR signal of the water protons, is dependent on the nuclear relaxation times. Complexes of paramagnetic transition metal and lanthanide ions, which can decrease the relaxation time of nearby nuclei via dipolar interactions, have received most attention as potential contrast agent, and numerous complexes of polyamino-polycarboxylic acid with these metal ions have been studied from this respect in the last decades. The structural similarities between the polyamino-polycarboxylic and polyamino-polyphosphonic (replacement of the carboxylic acid groups by phosphonic acid groups) generally make them more selective towards cations with enhanced coordination capacity.

Some transition (Cu(II), Fe(II), Ni(II), Co(II)) and lanthanide metal complexes of the 1,2-ethanediamine tetramethylphosphonic acid and 1,3-propandiamine tetramethylphosphonic acid were prepared and investigated by IR spectroscopic method and by thermal analysis

Preparation and study of metal complexes of antipyrine derivatives

Biological importance of the metal complexes of the antipyrine derivatives

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Antipyrine and its derivatives exhibit antipyretic and analgesic activity. Their complexes with some metal ions, as Pt(II) and Co(II), have been shown to act as antitumor substances. Also, there have been many reports on some other metal complexes of antipyrine and its derivatives, exhibiting anti-tumor and antipyretic properties. In this respect two of the antipyrine derivatives, the N,N'-tetra-(antipyryl-4-methyl)-1,2-diaminoethane (TAMEN) and the N,N'-bis(antipyryl-4-methyl)piperazine (BAMP) and their metal complexes taken out our interest. Many of their parent and mixed ligand complexes were prepared and studied in both chemically and biologically (e.g. [1-4]).

The complexes were characterized by elemental analysis, UV-, Vis- and IR-spectroscopy. The thermal behavior, and magnetic properties were studied. Also, the biological effect of the complexes was investigated.

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Poster

Effect of pH on tandem protein modules

Complement control module stabilization by protonation

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Complement system is an important enzyme cascade in the blood plasma evolved against invading particles in multi-cellular organisms. According to a recent model it is believed that zymogene activation is accompanied by a large conformational change. In the classical complement pathway, the very first enzyme is C1r, whose two consecutive CCP modules are appointed for conformational change. We have compared 2D NMR spectra at different circumstances of both single CCP modules from C1r. Variation in pH has shown that a polar interaction seems to be important in stabilizing the apical end of CCP2, whereas such an interaction does not significantly contribute to CCP1 stability.

Poster

Merging disulphide bonds for drug design

Interfering with a cancer key protein

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Poster

Deuteration using continuous flow devices

Deuterated biologically relevant molecules for structure determination, in mechanistic studies

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Deuterium-labeled organic compounds and biomolecules have play increasingly important role in structure determination, in mechanistic studies, in the elucidation of biosynthetic pathways and in biochemical studies, where isotopic labeling enables tracking the passage of a sample of substance through biosystems.

Several sophisticated methods are founded on the replacement of hydrogen atoms with its heavier 'isotope' deuterium. These are novel neutron scattering and diffraction methods, state-of-the-art NMR techniques and most importantly mass spectrometry, which has sufficiently high resolution to distinguish among isotopes.

There are several chemical techniques to introduce deuterium into organic molecules including double bond saturation with deuterium gas, as well as deuterium-hydrogen exchange reactions using proper deuterated solvents or reagents (most frequently: “heavy water”) and compounds having loose hydrogen (e.g. next to a carbonyl function) under appropriate conditions.

ThalesNano has developed revolutionary devices (Cube series [1, 2]) to carry out homogeneous and heterogeneous chemical reactions in continuous flow. The first such reactor is H-Cube®, a bench-top standalone hydrogenation reactor, which uniquely combines continuous-flow microchemistry with endogenous on-demand hydrogen generation and a disposable catalyst cartridge system. It allows fast and cost-efficient hydrogenation with superior yield when compared to conventional methods.

In our poster we report various approaches to deuteration using the continuous flow devices together with specific examples.

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Poster

How promiscuous are SGNH-hydrolases?

Activity profile of some bacterial SGNH-hydrolases

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Although lipolytic enzymes are known for decades, the classification of this type of enzymes is a continuous process. Among many defined classes and families, a new one was recently identified: SGNH-hydrolases (1). According to InterPro database (March 2008.), there are 3775 members of this family, with protein sequences originating mainly from genome sequencing projects. However, only 10 SGNH-hydrolases are crystallized and their three-dimensional structures solved (coordinates in PDB database, March 2008.), and very few are biochemically characterized to some extent.

The most thoroughly characterized member of this family is thioesterase I/protease I/lysophospholipase L1 from *Escherichia coli*. Data on substrate specificity of this enzyme show its pronounced substrate promiscuity (2). To examine possible multifunctionality of SGNH-hydrolases, we performed substrate specificity analysis on several members of this family: lipase from *Streptomyces rimosus* (SrLip), esterases from *Pseudomonas aeruginosa* and *Pseudomonas putida* (EstA and EstP, respectively), and acyl-CoA thioesterase from *P. aeruginosa* (TesA). Several types of substrates were tested (34 in total): for esterase, thioesterase, lipase, phospholipase, Tweenase and protease activity.

Among tested enzymes, only SrLip showed significant activity towards a broad spectrum of substrates. TesA was most active towards Tween-detergents and short-chained esters, and EstA and EstP showed activity profiles typical for esterases. After measuring enzyme activity at different substrate concentrations, it was observed that investigated enzymes, characterized as esterases, show classical Michaelis-Menten kinetics, only SrLip showed pronounced interfacial activation.

Our findings show that the lipase from *Streptomyces rimosus* does reveal multifunctionality, i.e. activity towards several substrate types, especially high towards phospholipase substrates and triglycerides.

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Chemical synthesis of tRNA^{Arg3} (s^{2}C32, mnm^{5}U$_{34}$, t^{6}A$_{37}$) *E.coli* anticodon stem-loop sequence (ASL$^{Arg\ 3}$)

Model studies on amplification/restriction of tRNAs decoding capacity: synthesis of native tRNA^{Arg3} (*E.coli*) anticodon stem-loop squence

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tRNA are the most highly modified RNA species in any cell. A number of hipermodified nucleosides is present in the 34 ("wobble"), 37 as well as 32 positions of tRNAs anticodon stem-loop sequences (ASLs), and they strongly influence on various activities of the biopolymer in cell cycles.

A correlated impact of 34, 37 positions on conformation/dynamic of ASLs sequences, and consequently thermodynamics of codon-anticodon interaction has been intensively investigated [1].

Recently, critical role of a status of 32 position on amplification / restriction of tRNAs decoding capacity has been also postulated [2,3].

Arg (R) is coded by six codons, and ASLs^{Arg} are very attractive sequences for multidirectional biochemical / biophysical studies on constraints of tRNAs decoding activity.

2-Thiocytidine (s^{2}C) is the component of tRNA from Procaryota. In this communication we present first in literature successful attempt for incorporation s^{2}C (solid support synthesis; phosphoramidite methodology) into native sequence tRNA$^{Arg\ 3}$ (*E.coli*) (Fig.1a).

Similar approach has been also utilized for synthesis of the analog labeled with fluorescent reporting group at 5'-end (Fig.1b).

a) 5´- A-U-G-G-C-s^{2}C$_{32}$-U-mnm^{5}U$_{34}$-C-U-t^{6}A$_{37}$-A-G-C-C-A-U – 3´

b) 5´-(6-FAM)-A-U-G-G-C-s^{2}C$_{32}$-U-mnm^{5}U$_{34}$-C-U-t^{6}A$_{37}$-A-G-C-C-A-U – 3´

Fig.1. Sequences of ASL$^{Arg\ 3}$ (E.coli) modified with 2-thiocytidine (s^{2}C), 5-methylaminemethyluridin (mnm^{5}U), N-[(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (t^{6}A)

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Poster

High pressure crystal structure of insulin

Pressure as a tool to study insulin folding

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High pressure is a factor that destabilize protein structure and might cause structure fragment displacement or lead to partial protein unfolding [1]. Bovine insulin is a small protein built of two chains of mostly α-helical structure. *In vitro*, under extreme conditions like high pressure or temperature at low pH, it could partially unfold and form amyloid fibers [2,3]. Insulin fibers have β-cross structure that is typical for amyloid fibers associated with molecular diseases. Despite the fact that insulin does not form amyloid fibers *in vivo* it is important to recognize which part of insulin molecule is responsible for fibers formation. Structural changes of insulin induced by high pressure should show which part of molecule is most movable and takes part in insulin amyloid fiber formation.

To investigate structural changes of insulin under high hydrostatic pressure, cubic insulin crystals belonging to the space group $I2_13$ were pressurized in Diamond Anvil Cell in pressure range from 100 to 200 MPa. Diffraction data were collected using Nonius KappaCCD diffractometer with standard Mo sealed tube (55 kV, 30 mA). Crystal structures were solved by molecular replacement.

Structural analysis was based on comparison of insulin structure determined at high and ambient pressure. Difference Distance Matrix (DDM) analysis showed that there are fragments within the insulin that are especially susceptible to displace under high pressure. The structural analysis also showed that under high pressure unit cell volume and B-factors values in crystal structure decrease. It was also demonstrated that the higher the pressure is, the less water molecules can be located in protein structure.

This work was partially founded by grant PB 3170/H03/2007/32 from Ministry of Science and Higher Education (Poland).

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Synthesis and properties of some heteronuclear complexes containing oxalate as ligand in the system: Cu(II)-VO(IV)

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Some heterometallic complexes in the system Cu(II)-VO(IV) have been isolated from aqueous medium at diffrent temperatures.

The solid compounds have been characterised by chemical, thermal and spectral analyses in order to estabilish the nature of the obtained complexes. The electronic spectrum shows the presence of VO(IV) species of C_{4v} symmetry and Cu(II) in octahedral surrounding[1,2]. IR spectrum proves the presence of the coordinated 4,4'-bipyridine and of the oxalate anion V_{4h}.

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Subsite mapping of α-amylase enzymes with molecular modeling

Examination of the substrate binding sites of α-amylase enzymes with molecular modeling

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Carbohydrates are important members of many molecular processes in communication of cells and in the signal transduction. Lots of complex glycan derivatives have antibacterial, antiviral and antitumour activity therefore synthesis of them is very important in the pharmaceutical industry, too.

α-amylases (α-1,4-glucan-4-glucanohydrolases; E.C.3.2.1.1) catalyze the hydrolysis of α-1,4-glycosidic linkages in starch and other related carbohydrates and they play role in degradation of them and also carbohydrate metabolism. They are common enzymes; they can be found in microorganisms, plants, animals and humans. α-amylases are extensively used in clinical chemistry (indicators of pancreatis and salivary disorders) and they play key role in several industries (pharmaceutical; baking industry; beer-making; sewage works also manufacturing process of alcohol, sugar and detergents).

In the last decade several 3D structures of starch-degrading enzymes have been determined by X-ray crystallographic analysis. Together with enzyme kinetic results and bond cleavage frequency (BCF) data we can examine the structure-function relationships of amylases. In recent we develop a computational procedure for interpretation of the functional features of wild type amylases and for prediction of these features of mutant enzymes.

Our work is based on the previously examined α-amylases of which action pattern and subsite binding energies have been determined in the Department of Biochemistry at University of Debrecen. To prepare our computational procedure we used the data of human salivary α-amylases (wild type and W58L, Y151M mutants) and barley α-amylases (AMY1-wild type, Y105A/F/W, T212Y, Y105A/T212Y mutants). The determination of bond cleavage frequencies was carried out with chromophor group linked malto-oligosaccharide substrate series and the products were analyzed with HPLC. The subsite binding energies were determined with the computer program SUMA (SUbsite Mapping of α-Amylases) developed in our department from bond cleavage frequency data.

In this computational model the substrate binding energies of the subsites were calculated using computed interaction energies between enzyme and substrate. For the molecular mechanical calculations the structures built on the basis of the X-ray crystallographic data or homologous modeling were used. To calibrate our model we used the subsite binding energy data determined by SUMA. The values of the correlation coefficients were 0.7-0.9.

To test the computational procedure we determine the experimental action pattern of further α-amylase mutants and compare them to the data predicted using our model. We synthesized the 2-chloro-4-nitrophenyl linked malto-oligosaccharide substrate series (DP 3-11) and the determination of bond cleavage frequency data is under way.

Our procedure can be used to examine and predict the function of different α-amylases (bond cleavage frequencies and subsite binding energies) in a fast computational process instead of the determination of the action pattern in an expensive and time-consuming experimental procedure.

Poster

Four spatial points that define enzyme families

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The entries of the Protein Data Bank were screened for appearances of the Asp-His-Ser and Glu-His-Ser catalytic triads. It was found that the geometric positions *of just four points* relative to the histidine ring well characterize some enzyme families. We constructed 12-dimensional vectors from the Cartesian coordinates of these points and clustered them by the OPTICS algorithm, according to their Euclidean distances. Two, well defined clusters were found, the first one contains 61 structures with an α/ß hydrolase fold, while the second one has 66 structures with trypsin-like and three ones with subtilisin-like folds. All but two of these structures refer to serine proteases. 15 trypsin-like structures are located outside the cluster, for most of these the resolution is relatively low, indicating a lower precision of the structure determination. 29 and 27 structures fall within the α/ß hydrolase and trypsin-like fold clusters, respectively, for which no Structural Classification of Proteins (SCOP) assignment is available. We may suppose that these structures possess the same SCOP fold as all other ones within their cluster. In conclusion, our simple and automatic classification scheme is useful if no EC or SCOP assignments are available.

New high performance liquid chromatographic method for determination of 5-hydroxy-1,4-naphtoquinone (juglone)

Juglone`s natural biosynthesis highlight in fresh leaves from black walnut tree

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Quinones have been the subject of much interest for a number of years due to their various biological activities. Naphtoquinones are deposited in cell plants vacuoles as chromatic pigments.

The aim of our study is to achieve the extraction of juglone (5-hydroxy-1,4-naphtoquinone) from fresh leaves of the black walnut trees (Juglans Nigra). The plant material was extracted by maceration in chloroform at room temperature (1).

In order to determine the juglone content, we developed an HPLC method with UV detection (2).

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Small bioactive molecule interactions in crystalline environment

Inhibitor – enzyme interactions inferred from crystal structures: a comparative study

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The crystal structures of ligand – protein complexes are important sources of information about the interactions between small bioactive molecules and the macromolecules. The knowledge of such interactions helps to understand the mechanism of action of the studied molecules and to elucidate the biochemical processes, which run with their participation. Interesting examples are the enzyme-inhibitor complexes, especially those in which the role of inhibitors is played by drugs or potential drugs, while the enzyme is engaged in some pathological phenomena in living organisms. Anti-inflamatory, anti-HIV and anticancer drugs can be mentioned in this connection. The X-ray structure analysis of the enzyme – inhibitor complex reveals such intermolecular interactions as covalent bonds, co-ordination bonds, ionic bonds, hydrogen bonds, π - π interactions and some other short contacts.

In our studies of the crystalline complexes of urokinase type plasminogen activator (uPA) inhibitors with this enzyme (I-E) and with small picrate anion (I-P) we noticed geometrical similarity between the intermolecular interactions formed by the inhibitors in I-E crystals and in I-P crystals [1,2]. This observation prompted us to check occurrence of such tendency in other systems found in PDB (for inhibitor-enzyme complexes) and CSD (for inhibitors in the environment of small molecules). In this contribution we describe the results of comparison between the intermolecular interactions formed by each of the inhibitors with two different types of molecules (enzyme and small molecules).

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Poster

Monte Carlo analysis of an enzyme kinetic network

Viability tests on eukaryotic cell cycle models

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The cell cycle is the sequence of events by which a growing cell replicates all of its components and divides them into two sister cells. Csikász-Nagy et al. [1] have created a generic cell cycle model that is able to simulate several types of living cells in such a way that for each cell type the differential-algebraic system of equations are identical, but the values of the parameters are different. This common system of differential-algebraic equations contains 14 variables and 86 parameters. In our studies, parameter sets related to budding yeast, fission yeast and mammal cells were investigated. Since measured concentration – time curves are not available for these cells, a parameter set was considered successful, if the corresponding model simulates a proliferating cell. Otherwise, the modelled cell were considered dead. The requirement for a living cell was to produce at least one cell division in the first 500 minutes and in the last 280 minutes, during the 3000 minutes simulation time.

First, the parameters were changed one-at-a-time, while all the other parameters were kept constant at their nominal values. Increasing or decreasing a parameter by four orders of magnitude, the limits were explored where the cell remained still alive. The qualitative results for most parameters were similar for each cell type. This shows that the role of the parameters were basically conserved during the evolution. However, there were 13 parameters with different behaviour for the different types of cells.

To investigate parameter interaction, Monte Carlo analyses of the three cell type models were carried out. For each model, one hundred thousand parameter sets were generated using Latin hypercube sampling assuming log-uniform distribution. In order to explore if a change of a parameter value can be compensated by a systematic change of another parameter to keep the cell alive, correlation of these parameter values was investigated.

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Computational investigation of the interactions of 3- and 5-methyl-6-aminouracils with natural nucleobases

In silico investigation of the interaction of new artificial nucleobases with the natural ones

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In the present study we have theoretically analyzed the hydrogen bonding of two artificial nucleobases (3- and 5-methyl-6-aminouracils, Figure 1a and 1b, respectively) with the natural DNA bases using the DFT method. The molecular orbital analysis of the monomers provides the possibility to distinguish different active parts of the molecules and the interactions energy with natural nucleobases are determined by the extended transition state method [1].

The bonding energy of the two artificial nucleobases with one of the natural DNA bases amounts mostly to values around the Adenine-Thymine pair with a few exceptions which are close to the Gunine-Cytozine pair. For a possible incorporation in the natural DNA, a pair of an artificial base with a natural DNA base must also fit into the Watson-Crick geometry.

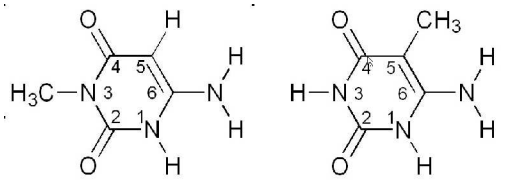

Figure 1: 3- and 5-methyl-6-aminouracils (1a and 1b, respectively)

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Poster

An efficient method for determination of lawsone from *Impatiens balsamina*

Lawsone formation in *Impatiens balsamina*

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Naphthoquinones, compounds of natural origin appears as chromatic pigments in plant cells vacuoles. They have important antimicrobial, antifungical, antiviral and antiparasitic properties. The aim of this work is to achieve the extraction of lawsone (2-hydroxy-1,4-naphtoquinone) from leaves and roots of Impatiens balsamina (1). The plant material was extracted by maceration in methanol and ethyl acetate, under reflux. We developed an HPLC method with UV detection in order to determine the lawsone content (2).

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Reactivity of some biomolecules toward novel monofunctional platinum(II) complex

Reactivity of some DNA constituents and amino acids toward novel metal complexes

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During recent decades the aqueous chemistry of Pt(II) complexes has been extensively studied, owing to the antitumor activity of cisplatin and related compounds.[1] The interactions between Pt(II) complexes and biorelevant molecules are very important from chemical, biological and medical point of view. The platinum interactions with fragments of DNA molecule are responsable for their antitumor activity, but there are many other biomolecules which can also react with platinum complexes. For example, sulfur-containing biomoleculs play an important role in the metabilsm of mentioned complexes, altought binding of cisplatin to intracellular thiols is known to be the reason of its toxicity and other side effects.[2]

The novel monofunctional complex of platinum(II), $[Pt(tpdm)Cl]^+$ (tpdm = terpyridinedimethane) was synthetized and characterized by elementary microanalysis, UV, IR, ^1H NMR spectroscopy. The kinetics of complex formation of $[Pt(tpdm)Cl]^+$ and $[Pt(tpdm)(H_2O)]^{2+}$ complexes with thiourea, L-methionine and 5'-GMP (guanosine-5'-monophosphate) were studied in an aqueous solutions as a function of the nucleophile concentrations and temperature. The aqua complex is more reactive than chloro analoques. The reactivity of ligands follow the order: thiourea > L-methionine > 5'-GMP. The complex, $[Pt(tpdm)Cl]^+$, reacts with L-methionine because it is well known that similar complex $[Pt(terpy)Cl]^+$ does not react with L-methionine. The negative values of entropies of activation for all studied substitution reactions support associative mode of activation.

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Poster

Stability-activity relationships in BS-RNase

Anti-tumor activity and structural properties of BS-RNase

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Bovine seminal ribonucleases (BS-RNase), the only dimeric ribonuclease of the vertebrate RNase superfamily, shows interesting biological properties related to its structural features (1). Besides the catalytic activity, it displays additional biological functions, including antitumor activity *in vitro* and *in vivo*. The protein is made-up by two identical 124-residue subunits held together by two disulfide bridges, and exists as a 2:1 equilibrium mixture of two isoforms with different tertiary structure, with (MxM) and without (M=M) swapping of the subunits N-terminal regions. Upon selective reduction of the interchain disulfide bridges, the M=M form is converted into a monomeric derivative, which retains the ribonuclease activity but is deprived of any additional biological property. The interchange instead of the N-terminal tails prevents the dissociation of the MxM form, which is converted into a so-called non-covalent dimer (NCD). It has been proposed that NCD is produced also *in vivo*, under the reducing conditions of the cytosol, and could represent the bioactive form of the enzyme, since it is able to evade the neutralizing effect of the cytosolic ribonuclease inhibitor (cRI) (2).

To investigate the structural basis of domain swapping in BS-RNase we have designed variants with selected residues replaced with the corresponding ones of bovine pancreatic ribonuclease (RNase A), which shares with BS-RNase 81 % of its amino acid sequence, and can be considered a monomeric counterpart.

A detailed comparison of the structural features of these variants with their biological activity can be helpful to increase the stability of dimeric forms in solution, hence possibly to improve their anti-tumor action. Here, we report the construction of variants, with substitutions at key positions, and a comparison of their cytotoxic activity with the stability of the NCD forms, as evaluated by CD and calorimetric analyses.

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Preparation of uniformly labeled point mutants of Tc5b miniprotein for NMR dynamic studies

Application of the ubiquitin fusion expression system: bacterial expression of uniformly labeled point mutants of Tc5b

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Tc5b is one of the smallest proteins having stable tertiary structure and folding spontaneously while containing only 20 amino acids[1]. The reason for this fast and stable fold was investigated by numerous molecular dynamic simulations but to this date, no NMR dynamic experiments were carried out.

The ubiquitine fusion expression system developed by Kohno *et al.*[2] is a suitable tool to produce polypeptides being too small to be expressed on their own in *E.Coli* cells. By growing the cells on ^{15}N and ^{13}C containing minimal media it is possible to produce uniformly labeled polypeptides for NMR dynamic experiments.

In the work of Hudáky *et al.*[3] on Tc5b and its D9E mutant: Tc6b, it has been suggested that one of the factors stabilizing this protein is the salt bridge between Asp^{9} and Arg^{16}. Our goal was to further investigate the effect of salt bridge on the stability of Tc5b by producing other point mutations of Tc5b in the 9^{th} position: namely D9S and D9N.

In our work we successfully applied the ubiquitine fusion expression system to synthetize Tc5b, and its point mutants: D9E, D9S and D9N via bacterial expression. By applying minimal media we also found an efficient way to synthesize uniformly labeled samples for NMR dynamic experiments.

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Crystal structure of human trypsin 4 complexed with tripeptide aldehyde serine protease inhibitors

A chemical approach to explore the biological function of a Primate-specific serine protease, human trypsin 4

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Human mesotrypsinogen and trypsinogen 4 are encoded by a common gene that is located on chromosome 9 of the Primates only. The two isoforms are formed by alternative splicing and only differ in their propeptide regions, consequently their activation results in proteases of identical structure. Mesotrypsinogen was only found in human pancreas, in contrast, trypsinogen 4 is only expressed in human brain and some epithelial cell lines. The most interesting feature of this serine protease is its resistance to proteineous canonical trypsin inhibitors. Our view is that the inhibitor resistance of this enzyme may be the clue to understand its so far unknown biological functions. The crystal structure of recombinant human trypsin 4 complexed with a small non-peptide inhibitor, benzamidine, provided the first information on the major structural cause of inhibitor resistance (1): The side chain of Arg193 hinders strong binding of proteineous inhibitors (and substrates) to human trypsin 4. This view has been confirmed and refined by a recent report on the crystal structure of human trypsin 4 complexed with bovine pancreatic trypsin inhibitor (2). Along this line we report structures of human trypsin 4 complexed with two strong serine protease inhibitors, Boc-D-Phe-Pro-Arg-H and D-MePhe-Pro-Arg-H (3). Our results show that these tripeptide aldehydes form stable tetrahedral hemiacetal complexes with the catalytic serine, Ser195 and that the hemiacetal oxygen atom points out of the oxyanion hole forming a H-bond with His57. The P3-P1 region, as expected, forms an antiparallel β-sheet with the protease. These interactions, however, do not seem to significantly alter the structure of human trypsin 4 seen in the human trypsin 4 – benzamidine complex (1).

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Poster

Synthesis of C-glycosyl derivatives of hydroquinone, chromanol and tocopherol as enzyme inhibitors or anti-oxidants

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Electrophilic substitution of 1,4-dimethoxybenzene by glycopyranosylium ions, generated from sugar peracetates in the presence of $SnCl_4$ and CF_3CO_2Ag led stereoselectively to β-configured 2-*C*-glycopyranosyl-1,4-dimethoxy-benzenes. Oxidation with ceric ammonium nitrate afforded *C*-glycopyranosyl-1,4-benzoquinones, which were reduced readily to the corresponding hydroquinones.[1] Such novel β-D-glucosyl-hydro/benzoquinones proved to be weak inhibitors of glycogen phosphorylase, due to binding at the active site, as shown by crystallography.[2] This study provided data at the molecular level for designing analogues [3,4] with enhanced affinity.

When extended to dimethyl-1,4-dimethoxy-benzene (*ortho* and *meta*), this route led to the corresponding *C*-glucosyl-dimethyl-hydroquinones which upon reaction with prenyl alcohol or rac. phytol led to glucosyl-chromanols or glucosyl-tocopherols. Deacetylation of the sugar residue allowed the synthesis of a collection of new molecules that have been studied as anti-oxidants.[5,6]

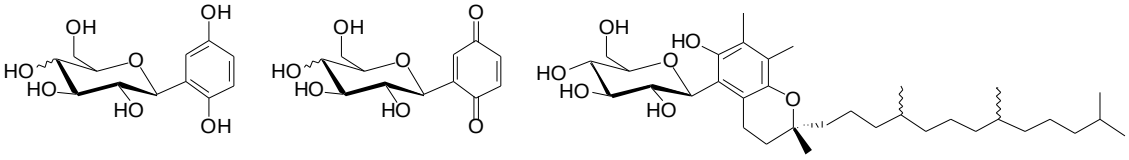

Figure 1

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Poster

Mechanism of DLC binding to multiple protein partners

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Dynein Light Chain (DLC) is a ~10 kDa, homodimeric, conservative protein, which has two mammalian paralogs, DLC1 and DLC2. It was originally discovered as a light chain of the dynein motor protein, however later turned out that a wide variety of proteins (>60) can be regulated by binding to two identical binding grooves of DLC; hence, it is suggested that DLC plays an important role in the protein interaction networks of the eukaryotic cells (it is a "hub" protein). It is involved in diverse biological processes like apoptosis, intracellular transport and cancer development. All DLC binding motifs are localized in a disordered domain of the binding partner and contain a loose consensus sequence. DLC is thought to be regulated by phosphorylation, which shifts the monomer-dimer equilibrium of the molecule strongly to monomers, and therefore eliminates the binding grooves. The thermodynamic and kinetic parameters of DLC interaction with various partner proteins were studied by isothermal titration calorimetry, surface plasmon resonance, and fluorescence polarization techniques. The observed wide range of dissociation constants (from 36 nM to 42 µM) can be partially explained by sequence differences, however, the affinity can also be greatly increased by the dimeric nature of the binding partners. This synergistic phenomenon, called avidity, is most likely caused by a decrease of the dissociation rate constants. Moreover, a dimer partner can apparently bind to the monomeric form of DLC by shifting the monomer-dimer equilibrium back to the dimer state. We also compared affinities of the DLC1 and DLC2 isoforms and found only little differences. The multifaceted binding mechanism between DLC and the partner proteins could be a necessary requirement to fulfill its biological role.

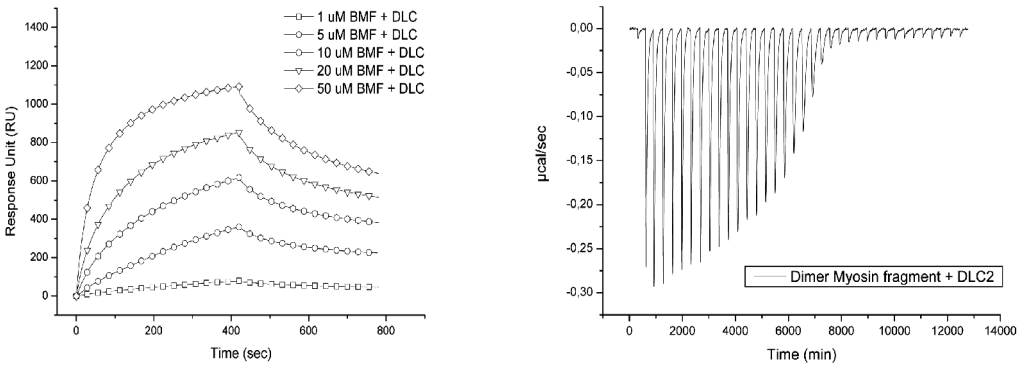

Fig.1. Results of surface plasmon resonance and isothermal titration calorimetry measurements

Computational investigation of a bacterial histidine ammonia-lyase (HAL) model with a completely closed active center

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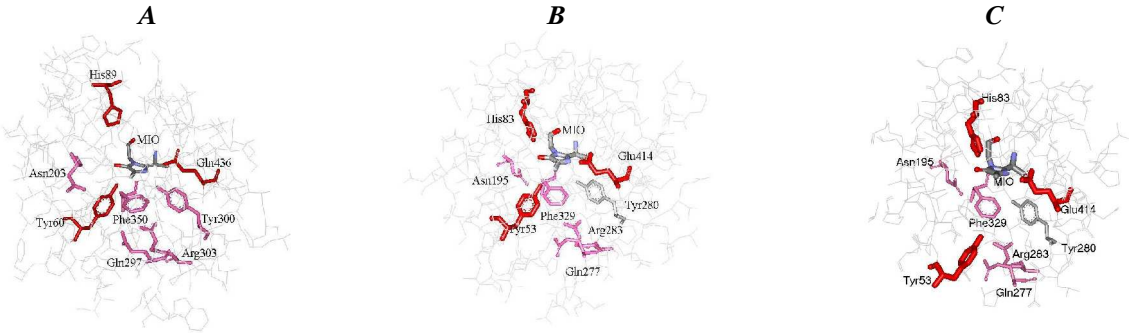
Histidine ammonia-lyase (HAL E.C. 4.3.1.3) is a key enzyme in the degradation of histidine in various bacteria. This degradation begins with deamination of histidine. The deamination requires the essential 4-methylidene-imidazole-5-one (MIO) electrophilic group [1,2,3]. The X-ray structure of HAL from *Pseudomonas putida* (PDB code 1B8F) [1] reveals a partially open, solvent accessible active site which contains the mechanistically relevant Y53 loop region. For a better understanding of the mechanism of ammonia-lyases a closed active center was necessary [4]. Based on the *Rhodobacter sphaeroides* tyrosine ammonia-lyase (*Rs*TAL) structure (PDB code 2O7B, Fig. 1A) [5] we have built *in silico* a partially modified HAL model (Fig. 1B). Our model compared to the HAL X-ray structure (Fig. 1C) affords a better approaching of the Y53 to the substrate and therefore facilitates the β-proton elimination.



*Rs*TAL (2O7B) TAL-*like* HAL model *Pp*HAL (1B8F)

Fig.1. The active sites of the HAL model (B) and HAL (C), TAL (A) crystal structures

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Genetically modified flagellin as a potential tool for display-based target molecule identification

Stable flagellar filaments from flagellin subunits deprived of the variable D3 domain

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The helical filaments of bacterial flagella extend over the surface of the cell membrane and are made of several tens of thousands copies of the flagellin protein (1). Flagellin from *Salmonella typhimurium* consists of 494 amino acid residues. Comparison of the amino acid sequences revealed a high degree of homology in the terminal regions containing about 180 N-terminal and 100 C-terminal residues, whereas the central segments are highly variable. The conserved terminal regions of flagellin subunits are involved in filament formation while the central part of the amino acid sequence forms the D2-D3 domains which project out from the filament core. D3 is the outermost domain which is exposed on the filament surface and involves the most variable segments of flagellin.

The use of recombinant microorganisms with surface exposed protein structures has during the last decade gained a lot of interest since this type of research holds a great promise in different areas such as biotechnology, microbiology and biomedicine (2). In flagellar display technology the dispensable central region of flagellin has been the target position for insertions of foreign peptide/protein sequences to be displayed on flagella. Flagellar display has been used for the display of a random peptide library, for the construction of recombinant vaccines as well as in bacterial adhesion technology. In these studies various segments of flagellin at random positions within the variable region were removed and replaced by the foreign sequence, however, the filaments produced by the fusion constructs usually exhibited significantly decreased stability.

The structure of Salmonella flagellin has been recently solved revealing that the hypervariable D3 domain involves the 190-285 segment (3). In this study we created a deletion mutant flagellin lacking the complete D3 domain. As shown by motility assays on semisolid agar plates, flagellin deficient bacteria exhibited normal swimming ability when complemented with a plasmid carrying the deletion mutant flagellin gene. They produced filaments in the same amount as wild type cells. Purified mutant filaments were resistant against prolonged proteolytic degradation. They possessed thermal stability virtually identical to that of native ones as shown by scanning calorimetric experiments. Our results demonstrate that removal of D3 does not significantly affect filament stability suggesting that replacement of D3 may offer a promising approach for insertion of heterologous segments or domains.

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Receptor-Based 3D-QSAR Study on Matrix Metalloproteinase Inhibition by Sulfonylated Amino Acid Hydroxamates

Combination of 3D-QSAR and Docking in Inhibition Activity Prediction on Matrix Metalloproteinase Enzymes

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The receptor-based 3D-QSAR models of comparative molecular field analysis were derived using bioactive conformations contained by docking compounds to the active sites of MMP-1, MMP-8, MMP-9. Matrix metalloproteinases (MMPs) are members of the zinc- and calcium-dependent endopeptidases known as metzincins. MMP may play a prominent role not only in the stages of tumor progression, characterized by the degradation of the extracelullular matrix, but also in the early phases of cancer development.

Correct binding modes are predicted for set of 39 sulfonylated amino acid hydroxamates of type RSO_2-NX-AA-CONHOH (X=H, benzyl, substituted benzyl; AA=amino acid moiety) using the docking program DOCK. 3D-QSAR models were developed based on these bioactive dock conformations. In combination with developed 3D-QSAR models we could successfully estimate the affinity of MMP inhibitors even in cases where the applied scoring function failed.

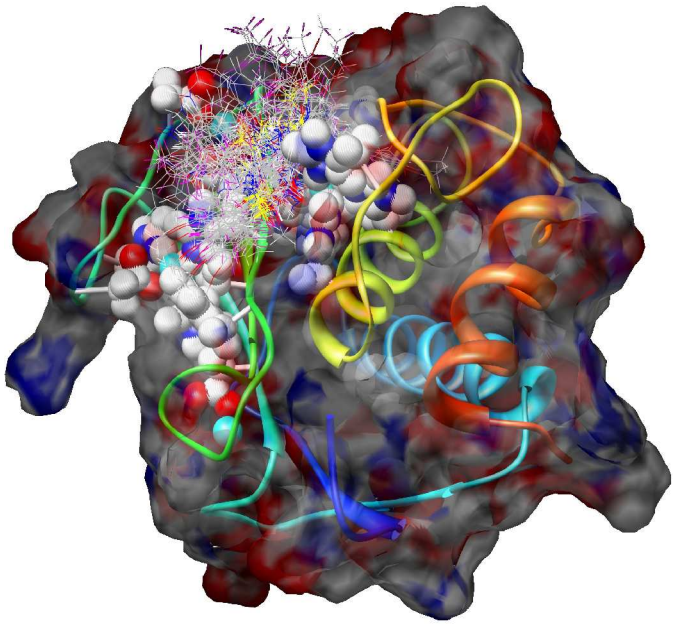

Figure 1 The set of molecules docked to MMP-1

Poster

NMR studies of DNA G-quadruplexes

G-quadruplexes and cation movement

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Nucleic acids show great potential in adopting helical structures. Beside well known right handed double helix, the DNA guanine-rich sequences can fold into four-stranded G-quadruplex structures composed of G-quartets, a planar array of four guanines held together by eight Hoogsteen hydrogen bonds. G-rich sequences can be found in critical segments of eukaryotic and prokaryotic genomes, promoter regions, ribosomal DNAs as well as telomeres in eukaryotes and immunoglobulin heavy chain switch regions of higher vertebrates. The presence of cations seems to be prerequisite for G-quartet formation due to their role in reducing repulsions amongst guanine carbonyl oxygen atoms and additionally enhancing base-stacking interactions.

Our recent studies on the three different G-quadruplex structures have shown that $^{15}NH_4^+$ ion movement from G-quadruplex into bulk solution is mainly influenced by steric restraints imposed by loop residues. In the case of $d(G_4T_4G_4)_2$, a bimolecular G-quadruplex, individual thymine residues of the diagonal loops are positioned around the central ion cavity of the outer G-quartets (1). In such arrangement loop residues do not represent a steric barrier for $^{15}NH_4^+$ ions to enter or leave the interior of the $d(G_4T_4G_4)_2$ G-quadruplex, which results in a relatively fast movement of $^{15}NH_4^+$ ions into bulk solution. Relatively fast $^{15}NH_4^+$ ion movement into bulk solution has been also observed in the case of $d(G_3T_4G_4)_2$ on side of edge-type loop (2,3). On the other hand, steric restraints of diagonal loop of $d(G_3T_4G_4)_2$ G-quadruplex slow down $^{15}NH_4^+$ ion movement for 12 times. In comparison, steric hindrance of T_3 edge-type loops of $d(G_4T_3G_4)_2$ G-quadruplex are responsible for a very slow $^{15}NH_4^+$ ion movement.

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Glucopyranosylidene-spiro-heterocycles as inhibitors of glycogen phosphorylase

Spiro-bicyclic glucose derivatives as potential antidiabetics

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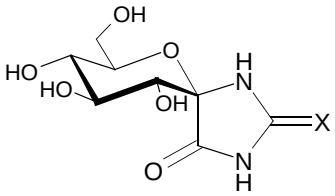
Glycogen phosphorylase (GP) is the rate limiting enzyme of glycogenolysis in the liver, its inhibitors are potential antidiabetics. Spiro-hydantoins **1** and **2** belong to the most effective glucose analogue inhibitors of GP. The rigid spiro-bicyclic structure is highly responsible for the tight binding.



1 X = O, K_i=3.1 μM
2 X = S, K_i = 5.3 μM (rabbit muscle GP)

For the preparation of analogous spiro-oxathiazoles 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose was reacted with hydroximoyl chlorides in the presence of Et_3N. The spiro-cyclization of the obtained hydroximothioates in dry $CHCl_3$ with NBS resulted in a mixture of spiro-epimers (2). In attempts for preparing analogous spiro-oxadiazoles tautomeric ring-opening of the pyranose moiety was observed. Spiro-imidazolones were obtained from 1-azido-2,3,4,6-tetra-*O*-benzoyl-1-deoxy-α-D-glucopyranosyl formamide by reacting the corresponding iminophosphorane intermediate with isocyanates. Spiro-thiazolones were prepared from 1-bromo-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl formamide and thioureas in dry acetone or dry DMF. Acylation and alkylation reactions of the spiro-thiazolones were investigated (3). The cleavage of the protecting groups for enzyme tests was carried out by the Zemplén-method. Preliminary inhibition kinetics results will also be presented.

Generation of nanostructures by map operations

Modeling of hypermolecules

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A map M is a combinatorial representation of a graph embedded on a surface and creates a tessellation on that surface. The map operations (1,2) are topological transformations of a given map. For molecular modeling, the nanostructures are represented by maps - molecular graphs embedded in surfaces of various genuses, thus the surfaces are covered by diverse patterns by the polygons or faces of the graphs. Nanostructures can have the shape of a hollow sphere, ellipsoid, tube or their derivations and combinations, with different coverings. From the nanostructures obtained by map operations (hypermolecules), others structures can be obtained by small covering changes. Then they can be analyzed by tatistical methods.

A computer program, CVNET (3), was made for generating maps or coverings, by map operations. Some algorithms for generalized and chiral operations (4) are presented.

Fig.1. Nanostructures obtained by generalized map operations

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Poster

Backbone mobility of Trp-cage mutants

Skeletal motion of miniproteins

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Tc5b is one of the smallest proteins having only 20 residues with stable tertiary structure and spontaneous folding [1]. The fold of this miniprotein is stabilized by a hydrophobic core and a salt bridge between Asp^{9} and Arg^{16}. Previously, we designed three other Tc5b mutants (D9N, D9S, D9E) [2] to investigate the effect of this salt bridge on the stability of the miniprotein.

In this study, we successfully applied the ubiquitine fusion expression system to synthetize Tc5b, and its mutants via bacterial expression. The reason for this fast and stable fold was investigated by numerous molecular dynamic simulations but to this date, no NMR dynamic experiments were carried out.

We carried out ^{15}N heteronuclear NMR expriments at different temperatures to investigate the backbone dynamics of the four miniproteins. Although these miniproteins are the size of a peptide, our work demonstrated their structural and dynamic properties make them proteins.

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Wrapped or unfolded? On the reliability of predictions of special peptide structural features

Critical evaluation of coiled-coil and disorder predictions

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Coiled-coil and intrinsically disordered regions in proteins are important functional and structural motifs. Coiled-coil is a superhelix formed by two or more α-helices while protein parts marked as disordered lack well-defined three dimensional structure, the atomic coordinates and the torsion angles of such residues can vary significantly. Both structures are abundant in eukaryotic proteomes.

Although prediction from amino acid sequence is a common and very useful method in the characterization of coiled-coil and disordered structures, the algorithms used might confuse these two motifs. In some cases disorder predictors mark coiled-coil regions as disordered and vice versa.

To characterize such discrepancies in detail, we used several widely-used coiled-coil and disorder prediction programs on different databases to determine the rate of correct and false predictions. Our results show misprediction of coiled-coil segments as disordered ones or the reverse can not be neglected in sequence analysis. We show that certain algorithms are more sensitive than others and suggest combinations to maximize the rate of correct predictions. We also discuss the possible functional and structural relevance of predicting protein segments both as coiled-coils and disordered.

Poster

Preparation of neoglycoproteins by reductive amination

Preparation of artificial carbohydrate antigens

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Production of carbohydrate-based antibacterial vaccines is an exciting field in carbohydrate chemistry. Low-molecular-weight oligosaccharide fragments of bacterial cell-surface polysaccharides can mimic the determinants of capsular or O-specific polysaccharides of bacteria. However, oligosaccharides are only haptens, they must be linked to macromolecular carriers, mostly to proteins. Carbohydrate chemistry can produce synthetic conjugates (neoglycoproteins) having well defined structures, without biological contaminations. There are numerous methods for the preparation of neoglycoproteins. We have focused our attention on reductive amination. Aldehydes react reversibily with the amino groups of proteins to form Schiff bases. The Schiff base thus formed may be reduced with $NaCNBH_3$. The aldehyde can be formed at the end of the spacer, or a reducing sugar itself can be the source of the aldehyde group.

First, cellobiose, maltose, lactose and gentiobiose were coupled to BSA, respectively. The spacers were formed from the reducing end of the disaccharides. The degree of incorporation of carbohydrate onto the protein was determined by MALDI-TOF MS. We were able to vary the incorporation level of the sugars (up to 27-38) by varying the molar ratio of the disaccharides to BSA. The results suggested that D-glucose is a suitable candidate as a spacer. For comparison, D-glucose, D-galactose and D-mannose were coupled to BSA, respectively. D-Galactose was the best as a spacer. To avoid unreal conclusions we decided to check this phenomenon by using disaccharides. α-D-Manp-(1→6)-D-Glc, α-D-Manp-(1→6)-D-Gal and α-D-Manp-(1→6)-D-Man were synthesized by standard reactions of oligosaccharide chemistry. The coupling of the synthetic disaccharides to BSA is in progress. Reductive amination which use masked aldehyde as the reactive group has the following advantages: We do not have to modify our oligosaccharides; the reaction conditions are mild; we are able to control the incorporated sugar level.

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Disordered Tails of Homeodomains: Effect on Folding and Binding

Disordered Tails in Transcriptional Regulation

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Disordered N-terminal tails play a crucial role in the function of homeodomain transcription factors by increasing the affinity and specificity of DNA binding. The tail undergoes a disorder-to-order transition upon interactions with DNA. The effect of the tail on the folding mechanisms of Antp and NK-2 homeodomains and their coupling to DNA binding were investigated by coarse grained simulations using the full proteins as well as their truncated forms. In accord with the experimental data, the tail decreases the stability of the free Antp protein due to competing with the intra-molecular contacts in the globular part. This is reflected by larger gyration radius of the core in the presence of tails. In contrast, the tail increases the folding temperature of Antp when DNA is present. The tail anchors the homeodomain to DNA and forms various specific interactions even without native contacts defined. In agreement with ITC results, binding is both enthalpy and entropy driven and both terms are more favorable in case of full proteins. The extended tails bind in the minor groove and a huge amount of water is released from the surface of the DNA upon association and the hydrophobic effect was found to provide a major contribution to the binding free energy. Thus coarse grained simulations gave valuable insights into the mechanism of disordered tails of homeodomains, which perform multiple tasks by fine-tuning specificity and also enhancing binding affinity to DNA.

Poster

Binding modes of metal ions to hyaluronate

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Molecular Modelling Studies on Some Benzazoles as Eukaryotic DNA Topoisomerase II Inhibitors

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Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription, and chromosome condensation, investigation of the inhibitory activities of eukaryotic topoisomerases is widely used in anticancer drug development. Topo II is the target for some of the most active anticancer drugs such as etoposide, teniposide, and doxorubicin used in the treatment of human malignancies [1-3]. In recent years, detailed investigations of bi- and ter-benzimidazole derivatives revealed that these compounds constitute a new class of Topo I and II inhibitors [4]. Work on such compounds indicates that a fused ring system in the structure is critical for the activity.

Many pharmacological studies have resolved receptor active/binding sites using numerous computational 3D-quantitative structure-activity relationship (3D-QSAR) techniques [5]. These methods utilize relevant conformers of ligands to suggest functional groups, the geometry of structural features, and regions of electrostatic and steric interactions essential for activity or fit to the receptor binding/active site.

In previously research paper, inhibition effect of some novel benzazole derivatives on eukaryotic DNA Topo II were investigated [6].

In this study, we applied the CoMSIA (Comparative Molecular Field Analysis) [7] as the 3D-QSAR applications using the Sybyl 7.0 [8] Software in SGI workstation for the lead optimization to the training set of compounds having the eukaryotic DNA Topo II inhibitory activities as log 1/C values. Moreover, the three-dimensional common-features hypotheses are generated by using Catalyst 4.11 [9] for finding the chemical features among a set of some Topo II inhibitor benzazoles.

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