Identification of binding pockets in protein structures using a knowledge-based potential derived from local structural similarities Helmer-Citterich, M. BMC Bioinformatics. 2012 No 13, Suppl 4 S17

What has proteomics taught us about Leishmania development? Tsigankov P, Gherardini PF, Helmer-Citterich M, Zilberstein D., Parasitology. 2012. 28:1-12

Leishmania are obligatory intracellular parasitic protozoa that cycle between sand fly mid-gut and phagolysosomes of mammalian macrophages. They have developed genetically programmed changes in gene and protein expression that enable rapid optimization of cell function according to vector and host environments. During the last two decades, host-free systems that mimic intra-lysosomal environments have been devised in which promastigotes differentiate into amastigotes axenically. These cultures have facilitated detailed investigation of the molecular mechanisms underlying Leishmania development inside its host. Axenic promastigotes and amastigotes have been subjected to transcriptome and proteomic analyses. Development had appeared somewhat variable but was revealed by proteomics to be strictly coordinated and regulated. Here we summarize the current understanding of Leishmania promastigote to amastigote differentiation, highlighting the data generated by proteomics.

Accurate multiple sequence alignment of transmembrane proteins with PSI-Coffee. Chang JM, Di Tommaso P, Taly JF and Notredame C. BMC Bioinformatics. 2012. 13(Suppl 4) In press

Methodology optimizing SAGE library tag-to-gene mapping: application to Leishmania. Smandi S, Guerfali FZ, Farhat M, Ben-Aissa K, Laouini D, Guizani-Tabbane L, Dellagi K, Benkahla A.

BACKGROUND: *Leishmaniasis are widespread parasitic-diseases with an urgent need for more active and less toxic drugs and for effective vaccines. Understanding the biology of the parasite especially in the context of host parasite interaction is a crucial step towards such improvements in therapy and control. Several experimental approaches including SAGE (Serial analysis of gene expression) have been developed in order to investigate the parasite transcriptome organisation and plasticity. Usual SAGE tag-to-gene mapping techniques are inadequate because almost all tags are normally located in the 3'-UTR outside the CDS, whereas most information available for Leishmania transcripts is restricted to the CDS predictions. The aim of this work is to optimize a SAGE libraries tag-to-gene mapping technique and to show how this development improves the understanding of Leishmania transcriptome.* ***FINDINGS:*** *The in silico method implemented herein was based on mapping the tags to Leishmania genome using BLAST then mapping the tags to their gene using a data-driven probability distribution. This optimized tag-to-gene mappings improved the knowledge of Leishmania genome structure and transcription. It allowed analyzing the expression of a maximal number of Leishmania genes, the delimitation of the 3' UTR of 478 genes and the identification of biological processes that are differentially modulated during the promastigote to amastigote differentiation.* ***CONCLUSION:*** *The developed method optimizes the assignment of SAGE tags in trypanosomatidae genomes as well as in any genome having polycistronic transcription and small intergenic regions. BMC Research Notes. 2012. 5:74*

Defeating Leishmania resistance to Miltefosine (hexadecylphospho-choline) by peptide-mediated drug smuggling: a proof of mechanism for trypanosomatid chemotherapy Rivas, L./Andreu D. Journal of Controlled Release. In revision 2012

Insights into the uptake mechanism of NrTP, a cell-penetrating peptide preferentially targeting the nucleolus of tumor cells. Radis-Baptista G/Andreu D. Chemical Biology and Drug Design. 2012. Mar 8. doi: 10.1111/j.1747-0285.2012.01377.x. [Epub ahead of print] Blackwell Oxford 2012

AMPA: an automated web server for prediction of protein antimicrobial regions. Torrent M/Andreu D. Bioinformatics. 2012. 28(1):130-131
AMPA is a web application for assessing the antimicrobial domains of proteins, with a focus on the design on new antimicrobial drugs. The application provides fast discovery of antimicrobial patterns in proteins that can be used to develop new peptide-based drugs against pathogens. Results are shown in a user-friendly graphical interface and can be downloaded as raw data for later examination.

T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Di Tommaso P, Moretti S, Xenarios I, Orobitg M, Montanyola A, Chang JM, Taly JF, Notredame C. Nucleic Acids Research. 2011. 39(Web Server issue):W13-7

This article introduces a new interface for T-Coffee, a consistency-based multiple sequence alignment program. This interface provides an easy and intuitive access to the most popular functionality of the package. These include the default T-Coffee mode for protein and nucleic acid sequences, the M-Coffee mode that allows combining the output of any other aligners, and template-based modes of T-Coffee that deliver high accuracy alignments while using structural or homology derived templates. These three available template modes are Expresso for the alignment of protein with a known 3D-Structure, R-Coffee to align RNA sequences with conserved secondary structures and PSI-Coffee to accurately align distantly related sequences using homology extension. The new server benefits from recent improvements of the T-Coffee algorithm and can align up to 150 sequences as long as 10,000 residues and is available from both http://www.tcoffee.org and its main mirror http://tcoffee.crg.cat.

Using the T-Coffee package to build multiple sequence alignments of protein, RNA, DNA sequences and 3D structures. Taly JF, Magis C, Bussotti G, Chang JM, Di Tommaso P, Erb I, Espinosa-Carrasco J, Kemena C, Notredame C. Nature Protocols 2011. (11):1669-82

T-Coffee (Tree-based consistency objective function for alignment evaluation) is a versatile multiple sequence alignment (MSA) method suitable for aligning most types of biological sequences. The main strength of T-Coffee is its ability to combine third party aligners and to integrate structural (or homology) information when building MSAs. The series of protocols presented here show how the package can be used to multiply align proteins, RNA and DNA sequences. The protein section shows how users can select the most suitable T-Coffee mode for their data set. Detailed protocols include T-Coffee, the default mode, M-Coffee, a meta version able to combine several third party aligners into one, PSI (position-specific iterated)-Coffee, the homology extended mode suitable for remote homologs and Expresso, the structure-based multiple aligner. We then also show how the T-RMSD (tree based on root mean square deviation) option can be used to produce a functionally informative structure-based clustering. RNA alignment procedures are described for

using R-Coffee, a mode able to use predicted RNA secondary structures when aligning RNA sequences. DNA alignments are illustrated with Pro-Coffee, a multiple aligner specific of promoter regions. We also present some of the many reformatting utilities bundled with T-Coffee. The package is an open-source freeware available from http://www.tcoffee.org/.

STRIKE: evaluation of protein MSAs using a single 3D structure. Kemena C, Taly JF, Kleinjung J, Notredame C. Bioinformatics 2011; 27(24):3385-91

MOTIVATION: Evaluating alternative multiple protein sequence alignments is an important unsolved problem in Biology. The most accurate way of doing this is to use structural information. Unfortunately, most methods require at least two structures to be embedded in the alignment, a condition rarely met when dealing with standard datasets. RESULT: We developed STRIKE, a method that determines the relative accuracy of two alternative alignments of the same sequences using a single structure. We validated our methodology on three commonly used reference datasets (BAliBASE, Homestrad and Prefab). Given two alignments, STRIKE manages to identify the most accurate one in 70% of the cases on average. This figure increases to 79% when considering very challenging datasets like the RV11 category of BAliBASE. This discrimination capacity is significantly higher than that reported for other metrics such as Contact Accepted mutation or Blosum. We show that this increased performance results both from a refined definition of the contacts and from the use of an improved contact substitution score.

Phosphate binding sites identification in protein structures Helmer-Citterich, M. Nucleic Acids Research. 2011. 39:1231-1242.

Nearly half of known protein structures interact with phosphate-containing ligands, such as nucleotides and other cofactors. Many methods have been developed for the identification of metal ions-binding sites and some for bigger ligands such as carbohydrates, but none is yet available for the prediction of phosphate-binding sites. Here we describe Pfinder, a method that predicts binding sites for phosphate groups, both in the form of ions or as parts of other non-peptide ligands, in proteins of known structure. Pfinder uses the Query3D local structural comparison algorithm to scan a protein structure for the presence of a number of structural motifs identified for their ability to bind the phosphate chemical group. Pfinder has been tested on a data set of 52 proteins for which both the apo and holo forms were available. We obtained at least one correct prediction in 63% of the holo structures and in 62% of the apo. The ability of Pfinder to recognize a phosphate-binding site in unbound protein structures makes it an ideal tool for functional annotation and for complementing docking and drug design methods. The Pfinder program is available at http://pdbfun.uniroma2.it/pfinder.

Phospho3D 2.0: An enhanced database of three-dimensional structures of phosphorylation sites Helmer-Citterich, M. Nucleic Acids Research. 2011. 39 (Database issue):D268-271

Phospho3D is a database of three-dimensional (3D) structures of phosphorylation sites (P-sites) derived from the Phospho.ELM database, which also collects information on the residues surrounding the P-site in space (3D zones). The database also provides the results of a large-scale structural comparison of the 3D zones versus a representative dataset of structures, thus associating to each P-site a number of structurally similar sites. The new version of Phospho3D presents an 11-fold increase in the number of 3D sites and incorporates several additional features, including new structural descriptors, the possibility of selecting non-redundant sets of 3D structures and the availability for download of non-redundant sets of structurally annotated P-sites. Moreover, it features P3Dscan, a new functionality that allows the user to submit a protein structure and scan it against the 3D zones collected in the Phospho3D database. Phospho3D version 2.0 is available at: http://www.phospho3d.org/.

PhosTryp: a phosphorylation sites predictor specific for parasitic protozoa of the family trypanosomatidae Helmer-Citterich, M. BMC Genomics No 12, December 2011 BioMed Central UK2011 614

***BACKGROUND:** Protein phosphorylation modulates protein function in organisms at all levels of complexity. Parasites of the Leishmania genus undergo various developmental transitions in their life cycle triggered by changes in the environment. The molecular mechanisms that these organisms use to process and integrate these external cues are largely unknown. However Leishmania lacks transcription factors, therefore most regulatory processes may occur at a post-translational level and phosphorylation has recently been demonstrated to be an important player in this process. Experimental identification of phosphorylation sites is a time-consuming task. Moreover some sites could be missed due to the highly dynamic nature of this process or to difficulties in phospho-peptide enrichment. **RESULTS:** Here we present PhosTryp, a phosphorylation site predictor specific for trypansomatids. This method uses an SVM-based approach and has been trained with recent Leishmania phosphosproteomics data. PhosTryp achieved a 17% improvement in prediction performance compared with Netphos, a non organism-specific predictor. The analysis of the peptides correctly predicted by our method but missed by Netphos demonstrates that PhosTryp captures Leishmania-specific phosphorylation features. More specifically our results show that Leishmania kinases have sequence specificities which are different from their counterparts in higher eukaryotes. Consequently we were able to propose two possible Leishmania-specific phosphorylation motifs.We further demonstrate that this improvement in performance extends to the related trypanosomatids Trypanosoma brucei and Trypanosoma cruzi. Finally, in order to maximize the usefulness of PhosTryp, we trained a predictor combining all the peptides from L. infantum, T. brucei and T. cruzi. **CONCLUSIONS:** Our work demonstrates that training on organism-specific data results in an improvement that extends to related species. PhosTryp is freely available at http://phostryp.bio.uniroma2.it.*

From sequence to structural analysis in protein phosphorylation motifs Helmer-Citterich. M. Frontiers in Bioscience. 2011. 16:1261-1275

Phosphorylation is the most widely studied post-translational modification occurring in cells. While mass spectrometry-based proteomics experiments are uncovering thousands of novel in vivo phosphorylation sites, the identification of kinase specificity rules still remains a relatively slow and often inefficacious task. In the last twenty years, many efforts have being devoted to the experimental and computational identification of sequence and structural motifs encoding kinase-substrate interaction key residues and the phosphorylated amino acid itself. In this review, we retrace the road to the discovery of phosphorylation sequence motifs, examine the progresses achieved in the detection of three-dimensional motifs and discuss their importance in the understanding of regulation and de-regulation of many cellular processes.

Adaptation of a 2D in-gel kinase assay to trace phosphotransferase activities in the human pathogen Leishmania donovani. Späth GF. J Proteomics. 2011. 74(9):1644-1651

The protozoan parasite Leishmania donovani undergoes various developmental transitions during its infectious cycle that are triggered by environmental signals encountered inside insect and vertebrate hosts. Intracellular differentiation of the pathogenic amastigote stage is induced by pH and temperature shifts that affect protein kinase activities and downstream protein phosphorylation. Identification of parasite proteins with phosphotransferase activity during intracellular infection may reveal new targets for pharmacological intervention. Here we describe an improved protocol to trace this activity in L. donovani extracts at high resolution combining in-gel kinase assay and two-dimensional gel electrophoresis. This 2D procedure allowed us to identify proteins that are associated with amastigote ATP-binding, ATPase, and phosphotransferase activities. The 2D in-gel kinase assay, in combination with recombinant phospho-protein substrates previously identified by phospho-proteomics analyses, provides a novel tool to establish specific protein kinase-substrate relationships thus improving our understanding of Leishmania signal transduction with relevance for future drug development.

Structural framework for the modulation of the activity of the hybrid antibiotic peptide cecropin A-melittin [CA(1-7)M(2-9)] by Nε-lysine trimethylation. Jimenez-Barbero J. Chembiochem. 2011. 12(14) 2177-83

The 3D structures of six linear pentadecapeptides derived from the cecropin A-melittin antimicrobial peptide CA(1-7)M(2-9) [KWKLFKKIGAVLKVL-NH(2)] have been studied. These analogues are modified by ε-NH(2) trimethylation of one or more lysine residues and showed variation in both antimicrobial and cytotoxic activities, depending on the number and position of modified lysines. Since it is expected that these peptides will display a strong conformational ordering when in contact with membranes, we have investigated their structure on the basis of the data extracted from NMR experiments performed in membrane-mimetic environments. We show that inclusion of N(ε)-trimethylated lysine residues induces a certain degree of structural flexibility, while preserving to a variable extent a largely α-helical structure. In addition, peptide orientation with respect to SDS micelles has been explored by detection of the intensity changes of peptide NMR signals upon addition of a paramagnetic probe (Mn(2+) ions).

Refining the eosinophil cationic protein antibacterial pharmacophore by rational structure minimization Boix E/Andreu D. Journal of Medicinal Chemistry. 2011. 54(14):5237-44

Sequence analysis of eosinophil cationic protein (ECP), a ribonuclease of broad antimicrobial activity, allowed identification of residues 1-45 as the antimicrobial domain. We have further dissected ECP(1-45) with a view to defining the minimal requirements for antimicrobial activity. Structure-based downsizing has focused on both α-helices of ECP(1-45) and yielded analogues with substantial potency against Gram-negative and -positive strains. Analogues ECP(8-36) and ECP(6-17)-Ahx-(23-36) (Ahx, 6-aminohexanoic acid) involve 36% and 40% size reduction relative to (1-45), respectively, and display a remarkably ECP-like antimicrobial profile. Both retain segments required for self-aggregation and lipolysaccharide binding, as well as the bacterial agglutination ability of parent ECP. Analogue (6-17)-Ahx-(23-36), in particular, is shown by NMR to preserve the helical traits of the native 8-16 (α1) and 33-36 (α2) regions and can be proposed as the minimal structure capable of reproducing the activity of the entire protein.

Connecting peptide physicochemical and antimicrobial properties by a rational prediction model. Torrent M. PLoS One. 2011. 6(2): e16968

The increasing rate in antibiotic-resistant bacterial strains has become an imperative health issue. Thus, pharmaceutical industries have focussed their efforts to find new potent, non-toxic compounds to treat bacterial infections. Antimicrobial peptides (AMPs) are promising candidates in the fight against antibiotic-resistant pathogens due to their low toxicity, broad range of activity and unspecific mechanism of action. In this context, bioinformatics' strategies can inspire the design of new peptide leads with enhanced activity. Here, we describe an artificial neural network approach, based on the AMP's physicochemical characteristics, that is able not only to identify active peptides but also to assess its antimicrobial potency. The physicochemical properties considered are directly derived from the peptide sequence and comprise a complete set of parameters that accurately describe AMPs. Most interesting, the results obtained dovetail with a model for the AMP's mechanism of action that takes into account new concepts such as peptide aggregation. Moreover, this classification system displays high accuracy and is well correlated with the experimentally reported data. All together, these results suggest that the physicochemical properties of AMPs determine its action. In addition, we conclude that sequence derived parameters are enough to characterize antimicrobial peptides.

Leishmania express a functional Cdc20 homologue. Listovsky, T. Bioch. Biophys. Res. Commun. 2011. 408:71-77

Our knowledge concerning the mechanisms of cell cycle regulation in organisms belonging to the Trypanosometidae family is limited. Leishmania donovani are parasitic protozoa that cause kala azar, a fatal form of visceral leishmaniasis in humans. Here we provide evidence that the L. donovani genome contains a Cdc20 homologue. Cdc20 is a regulator of the Anaphase Promoting Complex/Cyclosome (APC/C) that mediates ubiquitin-dependent proteasomal degradation of key cell cycle regulators in eukaryotes. We show that L. donovani Cdc20 protein (LdCdc20p) can complement a lack of yeast Cdc20 protein in Saccharomyces cerevisiae cells, validating the functionality of LdCdc20p. Furthermore, we demonstrate cyclic expression of LdCdc20p and that it contains an active RXXL destruction motif, a distinctive feature of proteins targeted for proteasomal degradation by APC/C. Finally, in line with the proteasome mediating LdCdc20p degradation, promastigotes exposed to proteasome inhibitor display elevated LdCdc20p levels. Taken together our data indicate that Leishmania regulate their cell cycle by ubiquitin-dependent proteasomal degradation mediated by the APC/C.

Visceral leishmaniasis: elimination with existing interventions. Matlashewski, G. Lancet Infectious Diseases. 2011. 11:322-325

The world's burden of infectious diseases can be substantially reduced by more-effective use of existing interventions. Advances in case detection, diagnosis, and treatment strategies have made it possible to consider the elimination of visceral leishmaniasis in the Indian subcontinent. The priority must now be to effectively implement existing interventions at the community level by actively finding cases in endemic villages and treating them with single-dose liposomal amphotericin B at primary-health-care centres. Once the elimination target of one case per 10,000 population has been reached,

combination therapies involving miltefosine and paromomycin can be introduced to ensure long-term availability of several drugs for visceral leishmaniasis and to protect against resistance.

"Quantitative proteome profiling informs on phenotypic traits that adapt Leishmania donovani for axenic and intracellular proliferation". Pescher P., Blisnick T., Bastin P., and Späth G.F. Cell Microbiol. 2011. 13(7):978-91

Protozoan parasites of the genus Leishmania are important human pathogens that differentiate inside host macrophages into an amastigote life cycle stage. Although this stage causes the pathogenesis of leishmaniasis, only few proteins have been implicated in amastigote intracellular survival. Here we compare morphology, infectivity and protein expression of L. donovani LD1S grown in host free (axenic) culture, or exclusively propagated in infected hamsters, with the aim to reveal parasite traits absent in axenic but selected for in hamster-derived amastigotes through leishmanicidal host activities. Axenic and splenic amastigotes showed a striking difference in virulence and the ability to cause experimental hepato-splenomegaly in infected hamsters. 2D-DIGE analysis revealed statistically significant differences in abundance for 152 spots, with 14 spots showing fivefold or higher abundance in splenic amastigotes. Proteins identified by MS analysis include the anti-oxidant enzyme tryparedoxin peroxidase, and enzymes implicated in protein and amino acid metabolism. Analysis of parasite growth in vitro in minimal medium demonstrated increased survival of hamster-derived compared with axenic parasites under conditions that mimic the nutrient poor, cytotoxic phagolysosome. Thus, our comparative proteomics analysis sheds important new light on the biochemistry of bona fide amastigotes and informs on survival factors relevant for intracellular L. donovani infection.

"Probing the dynamic nature of signalling pathways by IMAC and SELDI-tof MS" Foucher A.L., Späth G.F., and I.K. Pemberton. Archives of Physiology and Biochemistry. 2010. 116(4-5):163-73

One major obstacle to the analysis of signalling pathways is the dynamic nature of signalling response to environmental stimuli. To overcome this limitation we applied immobilized metal affinity chromatography (IMAC) in combination with SELDI-tof MS to investigate the temporal variation of protein phosphorylation. We analysed the phospho-proteome variations in our model organism, Leishmania donovani, in response to changes in pH and temperature, which induce differentiation from promastigotes to amastigotes. Investigation of total cell extracts did not allow promastigote and amastigote life cycle stages to be distinguished. However, using IMAC enriched samples, the pattern and intensity of phospho-proteins analysed distinguished both stages reproducibly. Approximately 61% of the phospho-proteins analysed were significantly different in abundance ($p<0.02$). Of these 61%, 73% showed an increased phosphorylation in promastigotes while 27% showed an increase phosphorylation in amastigotes. The workflow developed is currently being applied to the temporal analysis of environmental stimuli.

Modular architecture of nucleotide binding pockets Helmer-Citterich, M. Nucleic Acids Research. 2010. 38:3809-3816.

Recently, modularity has emerged as a general attribute of complex biological systems. This is probably because modular systems lend themselves readily to optimization via random mutation followed by natural selection. Although they are not traditionally considered to evolve by this process, biological ligands are also modular, being composed of recurring chemical fragments, and moreover they exhibit similarities reminiscent of mutations (e.g. the few atoms differentiating adenine and guanine). Many ligands are also promiscuous in the sense that they bind to many different protein folds. Here, we investigated whether ligand chemical modularity is reflected in an underlying modularity of binding sites across unrelated proteins. We chose nucleotides as paradigmatic ligands, because they can be described as composed of well-defined fragments (nucleobase, ribose and phosphates) and are quite abundant both in nature and in protein structure databases. We found that nucleotide-binding sites do indeed show a modular organization and are composed of fragment-specific protein structural motifs, which parallel the modular structure of their ligands. Through an analysis of the distribution of these motifs in different proteins and in different folds, we discuss the evolutionary implications of these findings and argue that the structural features we observed can arise both as a result of divergence from a common ancestor or convergent evolution.

Superpose3D: a local structural comparison program that allows for user-defined structure representations Helmer-Citterich, M. PLoS One. 2010. 5(8):e11988

Local structural comparison methods can be used to find structural similarities involving functional protein patches such as enzyme active sites and ligand binding sites. The outcome of such analyses is critically dependent on the representation used to describe the structure. Indeed different categories of functional sites may require the comparison program to focus on different characteristics of the protein residues. We have therefore developed superpose3D, a novel structural comparison software that lets users specify, with a powerful and flexible syntax, the structure description most suited to the requirements of their analysis. Input proteins are processed according to the user's directives and the program identifies sets of residues (or groups of atoms) that have a similar 3D position in the two structures. The advantages of using such a general purpose program are demonstrated with several examples. These test cases show that no single representation is appropriate for every analysis, hence the usefulness of having a flexible program that can be tailored to different needs. Moreover we also discuss how to interpret the results of a database screening where a known structural motif is searched against a large ensemble of structures. The software is written in C++ and is released under the open source GPL license. Superpose3D does not require any external library, runs on Linux, Mac OSX, Windows and is available at http://cbm.bio.uniroma2.it/superpose3D.

Cyclosporin A treatment of Leishmania donovani reveals stage-specific functions of cyclophilins in parasite proliferation and viability Späth GF. PLoS Negl Trop Dis. 2010 4(6):e729

Cyclosporin A (CsA) has important anti-microbial activity against parasites of the genus Leishmania, suggesting CsA-binding cyclophilins (CyPs) as potential drug targets. However, no information is available on the genetic diversity of this important protein family, and the mechanisms underlying the cytotoxic effects of CsA on intracellular amastigotes are only poorly understood. Here, we performed a first genome-wide analysis of Leishmania CyPs and investigated the effects of CsA on host-free L. donovani amastigotes in order to elucidate the relevance of these parasite proteins for drug development. ***METHODOLOGY/PRINCIPAL FINDINGS:*** *Multiple sequence alignment and cluster analysis identified 17 Leishmania CyPs with significant sequence differences to human CyPs, but with highly conserved functional residues implicated in PPIase function and CsA binding. CsA treatment of promastigotes resulted in a dose-dependent*

inhibition of cell growth with an IC50 between 15 and 20 microM as demonstrated by proliferation assay and cell cycle analysis. Scanning electron microscopy revealed striking morphological changes in CsA treated promastigotes reminiscent to developing amastigotes, suggesting a role for parasite CyPs in Leishmania differentiation. In contrast to promastigotes, CsA was highly toxic to amastigotes with an IC50 between 5 and 10 microM, revealing for the first time a direct lethal effect of CsA on the pathogenic mammalian stage linked to parasite thermotolerance, independent from host CyPs. Structural modeling, enrichment of CsA-binding proteins from parasite extracts by FPLC, and PPIase activity assays revealed direct interaction of the inhibitor with LmaCyP40, a bifunctional cyclophilin with potential co-chaperone function. ***CONCLUSIONS/SIGNIFICANCE:*** *The evolutionary expansion of the Leishmania CyP protein family and the toxicity of CsA on host-free amastigotes suggest important roles of PPIases in parasite biology and implicate Leishmania CyPs in key processes relevant for parasite proliferation and viability. The requirement of Leishmania CyP functions for intracellular parasite survival and their substantial divergence form host CyPs defines these proteins as prime drug targets.*

"Identification of Leishmania-specific protein phosphorylation sites by LC-ESI-MS/MS and comparative genomics analyses" Hem S., Gherardini P.F., Osorio y Fortéa J., Hourdel V., Morales M.A., Watanabe R., Pescher P., Kuzyk M.A., Smith D., Borchers C.H., Zilberstein D., Helmer-Citterich M., Namane A., and Späth G.F. Proteomics. 2010 10(21):3868-83

Human pathogenic protozoa of the genus Leishmania undergo various developmental transitions during the infectious cycle that are triggered by changes in the host environment. How these parasites sense, transduce, and respond to these signals is only poorly understood. Here we used phosphoproteomic approaches to monitor signaling events in L. donovani axenic amastigotes, which may be important for intracellular parasite survival. LC-ESI-MS/MS analysis of IMAC-enriched phosphoprotein extracts identified 445 putative phosphoproteins in two independent biological experiments. Functional enrichment analysis allowed us to gain insight into parasite pathways that are regulated by protein phosphorylation and revealed significant enrichment in our data set of proteins whose biological functions are associated with protein turn-over, stress response, and signal transduction. LC-ESI-MS/MS analysis of TiO(2)-enriched phosphopeptides confirmed these results and identified 157 unique phosphopeptides covering 181 unique phosphorylation sites in 126 distinct proteins. Investigation of phosphorylation site conservation across related trypanosomatids and higher eukaryotes by multiple sequence alignment and cluster analysis revealed L. donovani-specific phosphoresidues in highly conserved proteins that share significant sequence homology to orthologs of the human host. These unique phosphorylation sites reveal important differences between host and parasite biology and post-translational protein regulation, which may be exploited for the design of novel anti-parasitic interventions.

The Antitumoral Depsipeptide IB-01212 Kills Leishmania through an Apoptosis-like Process Involving Intracellular Targets. Rivas, L./Albericio, F. Molecular Pharmaceutics. 2010. 7(5):1608-1617

IB-01212, an antitumoral cyclodepsipeptide isolated from the mycelium of the marine fungus Clonostachys sp., showed leishmanicidal activity at a low micromolar range of concentrations on promastigote and amastigote forms of the parasite. Despite its cationic and amphipathic character, shared with other membrane active antibiotic peptides, IB-01212 did not cause plasma membrane lesions large enough to allow the entrance of the vital dye SYTOX green (MW = 600), even at concentrations causing full lethality of the parasite. Having ruled out massive disruption of the plasma membrane, we surmised the involvement of intracellular targets. Proof of concept for this assumption was provided by the mitochondrial dysfunction caused by IB-01212, which finally caused the death of the parasite through an apoptotic-like process. The size of the cycle, the preservation of the C2 symmetry, and the nature of the bonds linking the two tetrapeptide halves participate in the modulation of the leishmanicidal activity exerted by this compound. Here we discuss the potential of IB-01212 as a lead for new generations of surrogates to be used in chemotherapy treatments against Leishmania .

Characterization of the leishmanicidal activity of antimicrobial peptides. Rivas, L. Methods in Molecular Biology. 2010. 618:393-420

This chapter describes the basic methodology to assay the activity of antimicrobial peptides (AMPs) on Leishmania, a human protozoan parasite. The protocols included can be methodologically divided into two major blocks. The first one addresses the basic technology for growth of the different stages of Leishmania, assessment of leishmanicidal activity, and monitoring of plasma membrane permeabilization. The second block encompasses the monitoring of bioenergetic parameters of the parasite, visualization of structural damage by transmission electron microscopy, or those methods more closely related to the involvement of intracellular AMP targets, as subcellular localization of the peptide and induction of parasite apoptosis.

Lysine N(epsilon)-trimethylation, a tool for improving the selectivity of antimicrobial peptides. Rivas, L./ Andreu D. Journal of Medicinal Chemistry. 2010. 53(15) 5587-96

The effects of lysine N(epsilon)-trimethylation at selected positions of the antimicrobial cecropin A-melittin hybrid peptide KWKLFKKIGAVLKVL-amide have been studied. All five monotrimethylated, four bis-trimethylated plus the per-trimethylated analogues have been synthesized and tested for antimicrobial activity on Leishmania parasites and on Gram-positive and -negative bacteria, as well as for hemolysis of sheep erythrocytes as a measure of cytotoxicity. The impact of trimethylation on the solution conformation of selected analogues has been evaluated by NMR, which indicates a slight decrease in the alpha-helical content of the modified peptides, particularly in the N-terminal region. Trimethylation also enhances the proteolytic stability of mono- and bis-trimethylated analogues by 2-3-fold. Although it tends to lower antimicrobial activity in absolute terms, trimethylation causes an even higher decrease in hemolytic activity and therefore results in improved selectivity for several analogues. The monotrimethylated analogue at position 6 shows the overall best selectivity against both the Leishmania donovani protozoan and Acinetobacter baumannii, a Gram-negative bacterium of increasing clinical concern.

New benzophenone-derived bisphosphonium salts as leishmanicidal leads targeting mitochondria through inhibition of respiratory complex II. Dardonville C/Rivas L. Journal of Medicinal Chemistry. 2010. 53(4):1788-98

A set of benzophenone-derived bisphosphonium salts was synthesized and assayed for lethal activity on the human protozoan parasite Leishmania. A subset of them, mostly characterized by phosphonium substituents with an intermediate hydrophobicity, inhibited parasite proliferation at low micromolar range of concentrations. The best of this

subset, 4,4'-bis((tri-n-pentylphosphonium) methyl) benzophenone dibromide, showed a very scarce toxicity on mammalian cells. This compound targets complex II of the respiratory chain of the parasite, based on (i) a dramatically swollen mitochondrion in treated parasites, (ii) fast decrease of cytoplasmic ATP, (iii) a decrease of the electrochemical mitochondrial potential, and (iv) inhibition of the oxygen consumption rate using succinate as substrate. Thus, this type of compounds represents a new lead in the development of leishmanicidal drugs.

Sequence inversion and phenylalanine surrogates at the beta-turn enhance the antibiotic activity of gramicidin S. Andreu, D/Cativiela, C. Journal of Medicinal Chemistry. 2010. 53(10):4119-29

A series of gramicidin S (GS) analogues have been synthesized where the Phe (i + 1) and Pro (i + 2) residues of the beta-turn have been swapped while the respective chiralities (D-, L-) at each position are preserved, and Phe is replaced by surrogates with aromatic side chains of diverse size, orientation, and flexibility. Although most analogues preserve the beta-sheet structure, as assessed by NMR, their antibiotic activities turn out to be highly dependent on the bulkiness and spatial arrangement of the aromatic side chain. Significant increases in microbicidal potency against both Gram-positive and Gram-negative pathogens are observed for several analogues, resulting in improved therapeutic profiles. Data indicate that seemingly minor replacements at the GS beta-turn can have significant impact on antibiotic activity, highlighting this region as a hot spot for modulating GS plasticity and activity.

Influence of lysine N(ε)-trimethylation and lipid composition on the membrane activity of the cecropin A-melittin hybrid peptide CA(1-7)M(2-9). Bastos M/Andreu, D. Journal of Physical Chemistry B. 2010. 114(49):16198-208

Although many studies have pointed out the promising role of antimicrobial peptides (AMPs) as therapeutical agents, their translation into clinical research is being slow due to the limitations intrinsic to their peptide nature. A number of structural modifications to overcome this problem have been proposed, leading to enhanced AMP biological lifetimes and therapeutic index. In this work, the interaction between liposomes of different lipidic composition and a set of lysine N(ε)-trimethylated analogs of the cecropin A and melittin hybrid peptide, CA(1-7)M(2-9) [H-KWKLFKKIGAVLKVL-amide], was studied by differential scanning calorimetry (DSC) and fluorescence spectroscopy. The study was carried out using membrane models for mammalian erythrocytes (zwitterionic lipids) and for bacteria (mixture of zwitterionic and negatively charged lipids). The results show that trimethylated peptides interact strongly with negatively charged (bacterial cell model) but not with zwitterionic (erythrocyte model) liposomes. These results are in agreement with the reduction of cytotoxicity and ensuing improvement in therapeutic index vs parental CA(1-7)M(2-9) found in a related study. Moreover, the modified peptides act differently depending on the model membrane used, providing further evidence that the lipid membrane composition has important implications on AMP membrane activity.

“Leishmania major LmaMPK7 protein kinase activity inhibits intracellular growth of the pathogenic amastigote stage” Morales M.A., Pescher P., Späth G.F. Eukaryot Cell. 2010. 9(1):22-30

During the infectious cycle, protozoan parasites of the genus Leishmania undergo several adaptive differentiation steps that are induced by environmental factors and crucial for parasite infectivity. Genetic analyses of signaling proteins underlying Leishmania stage differentiation are often rendered difficult due to lethal null mutant phenotypes. Here we used a transgenic strategy to gain insight into the functions of the mitogen-activated Leishmania major protein kinases LmaMPK7 and LmaMPK10 in parasite virulence. We established L. major and Leishmania donovani lines expressing episomal green fluorescent protein (GFP)-LmaMPK7 and GFP-LmaMPK10 fusion proteins. The transgenic lines were normal in promastigote morphology, growth, and the ability to differentiate into metacyclic and amastigote stages. While parasites expressing GFP-LmaMPK10 showed normal infectivity by mouse footpad analysis and macrophage infection assays, GFP-LmaMPK7 transgenic parasites displayed a strong delay in lesion formation and reduced intracellular parasite growth. Significantly, the effects of GFP-LmaMPK7 on virulence and proliferation were due exclusively to protein kinase activity, as the overexpression of two kinase-dead mutants had no effect on parasite infectivity. GFP-LmaMPK7 transgenic L. donovani cells revealed a reversible, stage-specific growth defect in axenic amastigotes that was independent of cell death but linked to nonsynchronous growth arrest and a significant reduction of de novo protein biosynthesis. Our data suggest that LmaMPK7 protein kinase activity may be implicated in parasite growth control and thus relevant for the development of nonproliferating stages during the infectious cycle.

"Phosphoproteome dynamics reveal heat shock protein complexes specific to the Leishmania donovani infectious stage" Morales M.A., Watanabe R., Dacher M., Chafey P., Osorio y Fortéa J., Scott D.A., Beverley S.M., Ommen G., Clos J., Hem S., Lenormand P., Rousselle J., Namane A., and Späth G.F. PNAS. 2010. 107(18):8381-6

Leishmania is exposed to a sudden increase in environmental temperature during the infectious cycle that triggers stage differentiation and adapts the parasite phenotype to intracellular survival in the mammalian host. The absence of classical promoter-dependent mechanisms of gene regulation and constitutive expression of most of the heat-shock proteins (HSPs) in these human pathogens raise important unresolved questions as to regulation of the heat-shock response and stage-specific functions of Leishmania HSPs. Here we used a gel-based quantitative approach to assess the Leishmania donovani phosphoproteome and revealed that 38% of the proteins showed significant stage-specific differences, with a strong focus of amastigote-specific phosphoproteins on chaperone function. We identified STI1/HOP-containing chaperone complexes that interact with ribosomal client proteins in an amastigote-specific manner. Genetic analysis of STI1/HOP phosphorylation sites in conditional sti1(-/-) null mutant parasites revealed two phosphoserine residues essential for parasite viability. Phosphorylation of the major Leishmania chaperones at the pathogenic stage suggests that these proteins may be promising drug targets via inhibition of their respective protein kinases.

“Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas” Dujardin J.C, González Pacanowska D., Croft S.L., Olesen O.F., and Späth G.F. Trends in Parasitology. 2010. 26(8):395-403

The protozoan diseases leishmaniasis, human African trypanosomiasis and Chagas disease are responsible for substantial global morbidity, mortality and economic adversity in tropical and subtropical regions. In most countries, existing strategies for control and treatment are either failing or under serious threat. Environmental changes, drug resistance and immunosuppression contribute to the emergence and spread of these diseases.

In the absence of safe and efficient vaccines, chemotherapy, together with vector control, remains the most important measures to control trypanosomatid diseases. Here, we review current limitations of anti-trypanosomatid chemotherapy and describe new efforts to safeguard existing treatments and to identify novel drug leads through the three multinational and interdisciplinary European Union Framework Programmes for Research and Technological Development (FP7) funded consortia KALADRUG-R, TRYPOBASE, and LEISHDRUG.

Structural motifs recurring in different folds recognize the same ligand fragments. Helmer-Citterich, M. BMC Bioinformatics. 2009. 10:182

The structural analysis of protein ligand binding sites can provide information relevant for assigning functions to unknown proteins, to guide the drug discovery process and to infer relations among distant protein folds. Previous approaches to the comparative analysis of binding pockets have usually been focused either on the ligand or the protein component. Even though several useful observations have been made with these approaches they both have limitations. In the former case the analysis is restricted to binding pockets interacting with similar ligands, while in the latter it is difficult to systematically check whether the observed structural similarities have a functional significance. ***RESULTS:*** *Here we propose a novel methodology that takes into account the structure of both the binding pocket and the ligand. We first look for local similarities in a set of binding pockets and then check whether the bound ligands, even if completely different, share a common fragment that can account for the presence of the structural motif. Thanks to this method we can identify structural motifs whose functional significance is explained by the presence of shared features in the interacting ligands.* ***CONCLUSION:*** *The application of this method to a large dataset of binding pockets allows the identification of recurring protein motifs that bind specific ligand fragments, even in the context of molecules with a different overall structure. In addition some of these motifs are present in a high number of evolutionarily unrelated proteins.*

Amphibian antimicrobial peptides and Protozoa: lessons from parasites. Rivas, L/Andreu,D. Biochim Biophys Acta. 2009. 1788(8):1570-81

Antimicrobial peptides (AMPs) from amphibians and other eukaryotes recognize pathogenicity patterns mostly related to differences in membrane composition between the host and a variety of bacterial, fungal and protozoan pathogens. Compared to the other two groups, protozoa are fairly neglected targets in antimicrobial chemotherapy, despite their role as causative agents for scourges such as malaria, amoebiasis, Chagas' disease or leishmaniasis. Herein we review the scarce but growing body of knowledge addressing the use of amphibian AMPs on parasitic protozoa, the adaptations of the protozoan to AMP pressure and their impact on AMP efficacy and specificity, and the current and foreseeable strategies for developing AMPs into practical therapeutic alternatives against parasitic disease.

Therapeutic index of gramicidinS is strongly modulated by D-phenylalanine analogues at the beta-turn Andreu D./Cativiela C. Journal of Medicinal Chemistry. 2009. 52(3):664-74

Analogues of the cationic antimicrobial peptide gramicidin S (GS), cyclo(Val-Orn-Leu-D-Phe-Pro)2, with d-Phe residues replaced by different (restricted mobility, mostly) surrogates have been synthesized and used in SAR studies against several pathogenic bacteria. While all D-Phe substitutions are shown by NMR to preserve the overall beta-sheet conformation, they entail subtle structural alterations that lead to significant modifications in biological activity. In particular, the analogue incorporating D-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) shows a modest but significant increase in therapeutic index, mostly due to a sharp decrease in hemolytic effect. The fact that NMR data show a shortened distance between the D-Tic aromatic ring and the Orn delta-amino group may help explain the improved antibiotic profile of this analogue.

“The flagellum-MAP kinase connection in Trypanosomatids: a key sensory role in parasite signaling and development?”. Rotureau B, Morales MA, Bastin P, Späth G.F. Cell Microbiol. 2009. 11(5):710-8

Trypanosomatid parasites are the causative agents of severe human diseases such as sleeping sickness, Chagas disease and leishmaniases. These microorganisms are transmitted via different insect vectors and hence are confronted to changing environments during their infectious cycle in which they activate specific and complex patterns of differentiation. Several studies in Trypanosoma brucei and in different subspecies of Leishmania have shed light on the role of mitogen-activated protein (MAP) kinases in these processes. Surprisingly, several MAP kinases turned out to be involved in the control of flagellum length in the promastigote stage of Leishmania. Recently, a sensory function has been recognized for cilia and flagella in unicellular and multicellular eukaryotes. This review aims to stimulate discussions on the possibility that the Trypanosomatid flagellum could act as a sensory organ through the MAP kinase pathway, with the objective to encourage investigation of this new hypothesis through a series of proposed experimental approaches.