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An artificial virus for nucleic acid delivery

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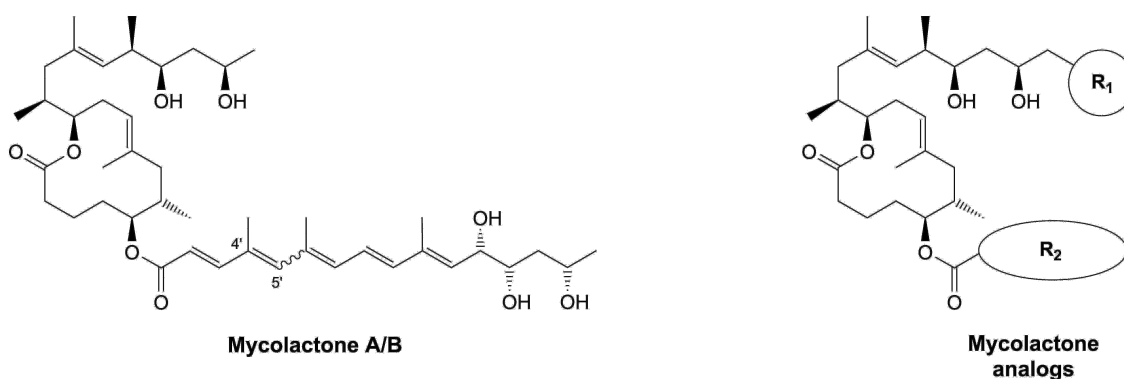
Certain short pieces of DNA/RNA can be used to silence genes and thus halt protein production in living cells. This has important implications both in medicine, as they could be used to treat a wider variety of diseases than conventional drugs, and in biotechnology, as they are important tools to regulating protein expression. Unfortunately, both DNA and RNA suffer from poor cellular uptake and stability in biological media. The development of strategies to overcome this is an important challenge which will ultimately affect science across a variety of disciplines. One promising strategy to enable the use of DNA/RNA for therapeutics is to encapsulate them inside a molecular container, the same strategy employed by viruses. The research presented concerns the development of an artificially designed protein cage to selectively load DNA/RNA molecules within its interior cavity. The ability of the system to load and protect its cargo, enter cells and control protein expression will be described.

Total Synthesis, Target Evaluation and Structure-Activity Studies of Mycolactone and its Analogs

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Mycolactones are a group of macrolides which exhibit cytotoxic, immunosuppressive and analgesic properties. As the exotoxins of the human pathogen *Mycobacterium ulcerans*, mycolactones are central to the pathogenesis of the neglected disease Buruli ulcer, a severe and chronic medical condition characterized by necrotic skin ulcers. Despite extensive research in several academic laboratories, the molecular mechanism of action of mycolactones is still heavily debated and it is not even clear whether the *cis*- $\Delta^{4',5'}$ or the respective *trans*-derivative is the major contributor to bioactivity.



Driven by the desire to understand the action of mycolactones on a molecular level, we prepared a plethora of mycolactone analogs for SAR and target deconvolution studies. By using two distinct biotinylated mycolactone-derived probes in conjunction with real-time PCR, RNA interference and other techniques, we recently identified the mechanistic Target of Rapamycin (mTOR) signaling pathway as the key-driver of mycolactone-promoted apoptosis.[1] By interacting with the intracellular 12 kDa FK506-binding protein (FKBP12), mycolactone A/B inhibits the assembly of the mTORC2 multiprotein complex thereby blocking the phosphorylation of the downstream mediators Akt and FoxO3. The latter triggers the expression of the pro-apoptotic regulator Bim, which finally drives cells into apoptosis. Intriguingly, Bim knockout prevented the typical Buruli ulcer phenotype in *M. ulcerans*-infected mice thus confirming our results *in vivo*.

[1] Raphael Bieri, Nicole Scherr, Marie-Thérèse Ruf, Jean-Pierre Dangy, Philipp Gersbach, Matthias Gehringer,; Karl-Heinz Altmann, Gerd Pluschke, *ACS Chem. Biol.* **2017**, ASAP (March 15, 2017), DOI:10.1021/acscchembio.7b00053.

Development of orally available peptide macrocycles by phage displayX. Kong¹, C. Heinis^{1*}

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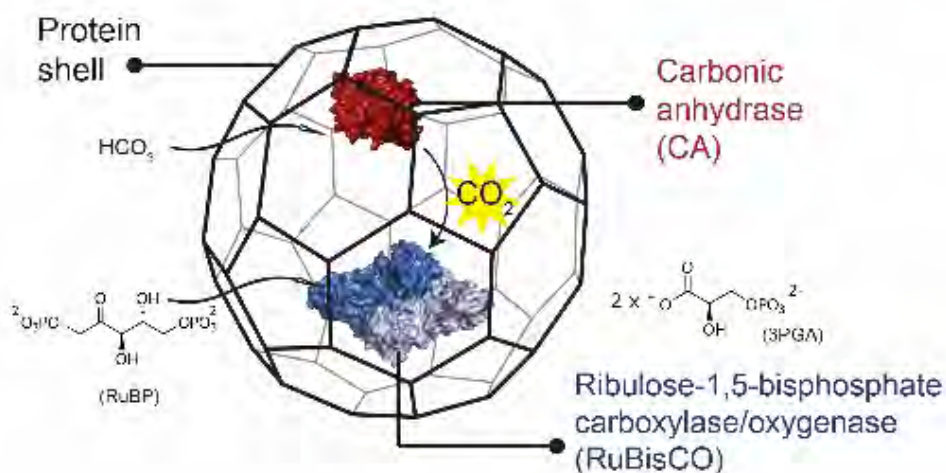
A major challenge in the pharmaceutical industry is the development of orally available peptide-based therapeutics.¹ The oral delivery of protein and peptide drugs is mainly limited by their proteolytic degradation and the poor absorption across the intestinal epithelia.² In this work, we have developed a method for the screening of proteolytic-resistant peptide macrocycles by phage display. In brief, peptides displayed on phage are cyclized in a chemical reaction, exposed to pancreatic proteases, and subjected to affinity selections. Affinity selections against the therapeutic target Factor XIa yielded potent inhibitors with K_i s below 10 nM. Due to the protease pressure during phage display, the peptide macrocycles showed half-lives of > 2 hours in presence of intestinal proteases at physiological concentration (10 mg/mL). Work is ongoing to test the oral availability of the cyclic peptide Factor XIa inhibitor in mice.

[1] K. Fosgerau and T. Hoffmann, *Drug Discovery Today*, **2015**, 20, 122-128.

[2] J. Wang, V. Yadav, A. L. Smart, S. Tajiri, A. W. Basit, *Molecular Pharmaceutics*, **2015**, 12, 966-973.

Bottom-up construction of a primordial organelleS. Mantri¹, D. Hilvert^{1*}¹ETH Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland

Several orthogonal bioreactions take place within membrane bound organelles in eukaryotes and proteinaceous microcompartments in bacteria. These subcellular structures contain sets of enzymes co-involved in metabolic pathways. Towards the goal of creating artificial protein microreactors, we have developed an artificial organelle that emulates the metabolic activity of the carbon fixing organelle of autotrophic bacteria, the carboxysome. Here, we show that the two key carboxysomal enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase (CA), can be efficiently co-encapsulated using our previously reported encapsulation system in a bacterial capsid formed from the protein lumazine synthase (AaLS-13). A statistically significant kinetic effect of co-encapsulated CA on RuBisCO activity was not observed under ambient or oxygen saturated conditions, suggesting that enzyme proximity alone may not be the key determinant in carboxysome function. The capsid shell protected the enzyme from proteolytic damage, however, a factor that could have provided early cyanobacteria with an evolutionary benefit. Our strategy to co-encapsulate different proteins can easily be extended to other sequentially acting enzymes and lays down principles for developing artificial organelles to control bio-synthetic pathways *in vivo*.



Shiksha Mantri, Raphael Frey, Marco Rocca, Donald Hilvert, JACS 2016

Screening Approaches to Understand Cellular Lipid Homeostasis

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Lipids serve multiple functions in cells including: establishing the membrane bilayer, a source of precursor molecules for signalling factors such as hormones, and a general metabolic role in energy storage. In keeping with these critical functions, cells employ intricate and strictly regulated homeostatic mechanics to maintain cellular lipid balance. To interrogate these systems, we have employed several phenotypic screening approaches, using both chemical and siRNA libraries, to identify regulators of lipid transport and biosynthesis in mammalian cell culture models [1-2]. Cellular changes were monitored by automated imaging, and image processing algorithms were used to identify screening hits, with a goal of linking compounds to their respective target proteins and pathways. These screens revealed the Wnt pathway as an unappreciated, and potent, regulator of both cholesterol homeostasis and an activator of lipid droplet accumulation in cells [1].

Lipid droplets are the primary storage organelle for neutral lipids, like triglycerides and sterol esters, in cells. While our screening efforts could conclusively establish the initial part of the canonical Wnt signaling pathway as modulating lipid droplet accumulation, we were unable to identify the signaling mediators that controlled the proximal transcriptional regulators of this organelle suggesting an unknown branch of the pathway was involved. Therefore, we performed *in silico* promoter analyses and RNAseq to identify changes in gene expression linked to the phenotype of lipid droplet accumulation. Together, this examination of transcriptional regulation lead to identification of several candidate factors that may serve as part of the “master” regulatory network controlling lipid droplet biogenesis. We found gene silencing diminished the number of lipid droplets in response to Wnt stimulation, while overexpression of these genes was sufficient to induce lipid droplet accumulation in cells, confirming the role of this transcriptional network in directing lipid droplet biogenesis.

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[2] Moreau, D, Scott, C, and J. Gruenberg, *Chimia*, **2011**, 65(11), 846-848.

Oligonucleotide therapy for treatment of erythropoietic protoporphyriaE. Halloy¹, P. Ćwiek¹, S. Egloff¹, D. Schümperli¹, J. Hall^{1*}¹Institute for Pharmaceutical Sciences, ETH Zürich, Vladimir Prelog Weg 4, Zürich, Switzerland

Erythropoietic protoporphyria (EPP) is a rare genetic disease where patients suffer from extreme skin irritation under natural and artificial blue light [1]. EPP is caused by genetic variations on both alleles of the ferrochelatase (*FECH*) gene [2]: in one, a non-sense or missense mutation prevents synthesis of the FECH enzyme; in the other, an intronic single nucleotide polymorphism (SNP) causes mis-splicing of the pre-mRNA. Low levels of FECH leads to accumulation of its photoreactive substrate protoporphyrin IX (PPIX) in erythroid cells in the blood, in bone marrow and in the liver.

One strategy to treat EPP is to use splice-switching oligonucleotides (SSOs) - oligonucleotides complementary to the FECH pre-mRNA which bind close to the cryptic splice site and “switch” its splicing back to the functional FECH mRNA. The SSOs are composed of 2'-*O*-methoxyethyl (MOE) ribose units [3] linked by a phosphorothioate backbone, the same chemistry employed in recently approved drugs mipomirsen and nusinersen. We have further developed this chemistry with the recent introduction of stereochemically-pure phosphorothioate linkages [4].

A lead SSO sequence was identified by screening a stretch of *FECH* intron 3 in a “minigene” reporter assay. It is presently being investigated in mouse models for its distribution to various tissues, including bone marrow [5]. As delivery to organs other than liver and kidney is a fundamental limitation for oligonucleotide therapeutics, we are also investigating the conjugation of various short peptides to the SSO for enhanced uptake into hematopoietic compartments. The peptide library comprises sequences that are known bind to receptors on erythroid cells, or to locate to the cell nucleus. Conjugates are being tested in erythroid cells and we are quantifying delivery using a technique developed for chemically modified oligonucleotides - chemical-ligation qPCR (CL-qPCR) [6]. Progress on these various aspects of the project will be described.

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