Abstracts

David BIKARD (Institut Pasteur, Paris, FR)

Studying And Fighting Pathogenic Bacteria with the Help of Crispr

Cas systems have emerged has a powerful biotechnological tool. The Cas9 protein is a RNA-guided nuclease that can be easily reprogrammed to target any sequence of interest. Our work focuses on the development of CRISPR-Cas9 tools to edit bacterial genomes and control gene expression. In particular, we investigate how these tools can be used in high-throughput screens to perform functional screens. Recently we have also shown how CRISPR system can be used as sequence-specific antimicrobial. The Cas9 protein can kill bacteria when directed to cut in their chromosome. Guide RNAs can be programmed to kill Bacteria carrying antibiotic resistance or virulence genes specifically, and the CRISPR system can delivered to bacterial populations using phage capsids.

Jef D. BOEKE (NYU Langone Medical Center)

Building synthetic chromosomes from scratch

Rapid advances in DNA synthesis techniques have made it possible to engineer viruses, biochemical pathways and assemble bacterial genomes. They have also enabled the construction of novel genetic pathways and genomic elements, furthering our understanding of systems-level phenomena. Our current understanding of genomics is solidly within the experimental phase, yet genome engineering is in its infancy. The synthetic yeast genome project, Sc2.0 is well on its way with several of the first synthetic Saccharomyces cerevisiae chromosomes completed. Undergraduate students provide a workforce for synthesis and assembly for some of these chromosomes, though a wide variety of assembly schemes are employed by the various groups building chromosomes. The synthetic genome features several systemic modifications, including TAG/TAA stop-codon replacements, deletion of subtelomeric regions, introns, tRNA genes, transposons and silent mating loci as well as strategically placed loxPsym sites to enable genome scrambling using an inducible evolution system termed SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution). SCRaMbLE can be used as a novel method of mutagenesis, capable of generating complex genotypes and a variety of phenotypes. The fully synthetic yeast genome will open the door to a new type of combinatorial genetics based on variations in gene content and copy number, rather than base changes. We also describe supernumerary designer "neochromosomes" that add new functionalities to cells such as humanized pathways.

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Nico CALLEWAERT (Medical Biotechnology Center, VIB and UGhent, Ghent, Belgium)

Rewiring the synthesis of protein-linked glycosylation in eukaryotic biopharmaceutical expression host

Glycosylation is the enzymatically catalysed modification of biomolecules with carbohydrate structures. For secreted and membrane-integral proteins, it is the most common posttranslational modification. Glycan structures characterize the molecular environment immediately outside of all cell types and hence have critical functions in interactions of any cell with its environment (cell-cell, cell-pathogen, cell-molecule). The field of glycobiotechnology is concerned with understanding and re-engineering of these glycosylation-dominated interactions. In particular, the understanding of the synthetic pathways and functions for eukaryotic N- and O-glycosylation, gained over the past few decades, has enabled the rewiring of these pathways for the benefit of pharmaceutical applications. Based on the conservation of the core pathways between eukaryotes, it has been possible to transfer the efficient synthesis of particular human-specific glycan structures to other eukaryotes such as yeasts and plants. This is enabling the cost-effective production of biopharmaceutical proteins with glycosylation patterns customized to particular therapeutic functionality (e.g. targeting to particular glycan receptors, or customized for particular pharmacokinetic behaviour). I will illustrate our work with regard to the production of human IgG-like glycosylation patterns in yeast[1], and the production of mannose-6-phosphate modified lysosomal enzymes for the treatment of human inherited lysosomal storage diseases[2]. Whereas these earlier synthetic biology endeavours were geared towards efficiently synthesising proteins with complex mammalian glycan structures in other eukaryotes, more recently we have generated mammalian cells and plants in which glycosylation complexity has been reduced to the bare minimum, while still being compatible with eukaryotic cell life and protein productivity. This 'GlycoDelete' technology[3,4] opens up many new structural biology and biopharmaceutical applications that are currently being explored in our laboratory.

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Yvonne CHEN (Dpt of Chemical and Biomolecular Engineering, UCLA)

Engineering Smarter and Stronger T Cells for Cancer Immunotherapy

T cells expressing chimeric antigen receptors (CARs) specific for the B-cell marker CD19 have shown impressive results in the treatment of B-cell malignancies. However, CD19 CAR-T cell therapy remains the only robustly effective T-cell immunotherapy to date. My laboratory is pursuing several strategies to engineer T cells with stronger anti-tumor functions and greater robustness against evasive mechanisms employed by cancer cells. I will discuss the development of multi-input CARs to prevent mutational escape by tumor cells, the design of synthetic receptors to counter immunosuppression in the tumor microenvironment, and the engineering of cytotoxic protein to interrogate intracellular tumor markers. These strategies combine to address critical limitations facing adoptive T-cell therapy, providing potential treatment options for diseases that are otherwise incurable with current technology.

Tom ELLIS (Centre for Synthetic Biology and Innovation (CSynBI) and Department of Bioengineering, Imperial College London)

Engineering Yeast: Synthetic Modularity at the Gene, Circuit, Pathway and Genome Level

Synthetic biology seeks to understand and derive value from biology via its re-design and synthesis using engineering principles. After a decade of work to improve DNA assembly and the control of gene expression, synthetic biology can now tackle cell-scale problems. By applying modular assembly from a kit of parts we can design complex genetic circuits that reprogram how yeast grows or endow yeast cells with new metabolic pathways that produce valuable molecules such as antioxidants and antibiotics. Or is aim to convert yeast into a prototyping factory for new phenotypes, and this will be aided by a modular synthetic version of the *S. cerevisiae* genome that enables evolution of gene content on cue. A part of the global Sc2.0 project to assemble a human-designed yeast genome, our lab is working on assembling synthetic chromosome XI and has already begun exploiting the new possibilities that it offers. After 2 years, we've nearly completed our 665 kb chromosome and have also developed new lab and software tools that will enable the future of genome engineering and yeast synthetic biology.

Luis Angel FERNANDEZ (Department of Microbial Biotechnology, National Center of Biotechnology (CNB-CSIC), Campus Cantoblanco UAM, Madrid)

Engineering synthetic adhesins and injectisomes in Escherichia coli K-12 to target mammalian cells for biomedical applications

One of the aims of synthetic biology is the design of microorganisms with novel capabilities that could be applied for the development of new vaccines, diagnostic sensors, and therapeutic interventions for major diseases such as cancer. This presentation will report the development of two important tools that enable us to precisely program *E. coli* K-12 bacteria to: 1) adhere to specific target cells; and 2) assemble filamentous injectisomes from type III secretion systems (T3SS) that act as "molecular syringes" for translocation of specific proteins into mammalian cells. Firstly, we designed synthetic adhesins based on the display of VHH domains on the bacterial cell surface with an outer membrane b-domain derived from intimin of enterohemorrhagic *E. coli* (EHEC). We generated synthetic adhesins against different antigen targets expressed on the surface of mammaliam cells and have demonstrated the specific adhesion of the engineered *E. coli* bacteria to the target mammalian cells using *in vitro* and *in vivo* models. Using mouse xenotransplants of human tumor cell lines expressing a target antigen, we demostrated that engineered *E. coli* bacteria colonize these tumors more efficiently at lower bacterial doses (Piñero-Lambea et al., 2015). Secondly, in order to express functional injectisomes in a non-pathogenic commensal *E. coli* strain (K-12), we reformatted the operons encoding the structural proteins and chaperones needed for the assembly of filamentous injectisomes from enteropathogenic *E. coli* (EPEC). Our synthetic operon constructs lack secreted effectors and regulatory elements found in EPEC. Five synthetic operons (sLEE1, sLEE2, sLEE3, sLEE4 and escD) were integrated into different sites of the chromosome of *E. coli* K-12 under the control of an inducible promoter (Ptac) using a marker-less strategy. We demonstrated that the resulting strain, named Synthetic Injector *E. coli* (SIEC), assembles functional injectisomes upon induction with IPTG able to translocate proteins into HeLa cells (Ruano-Gallego et al., 2015). Collectively, these results open the possibility to target specific mammalian cells with engineered *E. coli* bacteria and inject heterologous proteins of interest, such as antibody fragments, immunogens, enzymes, transcription factors, or toxins.

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Martin FUSSENEGGER (ETH Zürich, Dpt of Biosystems Science and Engineering, Basel)

Prosthetic Gene Networks for Biomedical Applications

Since Paracelsus' (1493-1541) definition that the dosing makes the drug the basic treatment strategies have lagely remained unchanged. We continue to use a precise prescribed dose of a small-molecule drug, a protein therapeutic or a therapeutic transgene to modulate or complement the activity of a disease-relevant target. However, this treatment concept does neither consider the metabolic dynamics nor the interdependence of the most important pathophysiologies of the 21st century such as obesity, diabetes and cardiovascular disorders. Synthetic biology-inspired prosthetic networks may act as metabolic prostheses that provide the dynamic interventions, the immediate pre-disease action and the multi-target capacity required to meet with the treatment challenges of the future. Prosthetic networks consist of synthetic sensor-effector gene circuits that (i) seamlessly operate in implanted designer cells, (ii) constantly sense, monitor and score metabolic disturbanes in peripheral circulation, (iii) process OFF-level concentrations of pathologic metabolites, and (iv) coordinate an adjusted therapeutic response in an (v) automatic and self-sufficient manner. We will present our latest generation of synthetic mammalian gene circuits and provide a few examples of prosthetic networks operating in animal models of prominent human diseases to highlight the challenges and impact of synthetic biology on future biomedical applications.

Ming HAMMOND (Depts of Chemistry and MCB, UC Berkeley, USA)

Illuminating Bacterial Signaling with RNA-Based Biosensors

My research asks, what can we program RNAs to do *in vivo* beyond base-pairing interactions? Thus, I conceived of starting with riboswitches, natural RNA scaffolds that fold into stable, active structures, and then exploring how to design or evolve new functions. Based on this principle, my research group has made novel RNA-based tools that are robust and context-independent, including biosensors with high fluorescence turn-on that function in all bacteria and suicide exons with high gene activation that function in all plants. In this lecture, I will focus on our development of RNA-based fluorescent biosensors, presenting design principles we have learned, challenges that remain to be tackled, and finally applications to the study of bacterial signaling that have the potential to greatly expand our synthetic biology toolbox

Jim HASELOFF Department of Plant Sciences, University of Cambridge, Cambridge, UK.

Synthetic Biology and engineering multicellular systems.

Synthetic Biology is an emerging field that employs engineering principles for constructing genetic systems. It is providing a conceptual and practical framework for the systematic engineering of gene expression and behaviour in microbes, but also shows great potential for the engineering of multicellular systems. We have used populations of Escherichia coli cells, which exhibit little or no intrinsic coordination of growth, as a model system to study

physical interactions in multicellular systems. This system effectively isolates the effects of cell shape, growth, and division on spatial self-organization. Even these very simple systems show emergent properties, and give rise to striking fractal patterns. Large-scale cellular biophysical models demonstrate that local instabilities are responsible for generating the observed self-organising properties of the system, and confirm the need for multi-scale physico-genetic models of cell growth for understanding and engineering mu lticellul ar systems. We are now exploring a similar approach using a simple plant system, the liverwort Marchantia polymorpha. Marchantia is characterised by morphological simplicity, matched by simple underlying genome structure. Its ease of culture, transformation and analysis make it an ideal system for plant development and synthetic biology. We have developed a battery of computational, imaging and genetic tools to allow clear visualisation of individual cells inside living plant tissues, and are developing a common syntax for plant DNA parts that can be used to reprogram metabolism and development.

Biography: Jim Haseloff is a plant biologist working at the University of Cambridge. His scientific interests are focused on the engineering of plant morphogenesis, using microscopy, molecular genetic, computational and synthetic biology techniques (www.haseloff-lab.org). He and his group have developed new approaches to RNA engineering, quantitative imaging and gene expression in plants, and promote the potential of Synthetic Biology as a tool to engineer new feedstocks for sustainable use. He is a director of the OpenPlant Synthetic Biology Research Centre, a collaborative venture between the University of Cambridge and the John Innes Institute and Sainsbury Laboratory, Norwich (www.openplant.org).

Ichiro HIRAO (Team Leader and Principal Research Scientist, Institute of Bioengineering and Nanotechnology (IBN), Singapore)

Genetic alphabet expansion by an unnatural base pair system toward diagnostic and therapeutic applications using xeno-nucleic acids

Standard nucleic acids comprise four different nucleotide components bearing each of A, G, C or T(U)base as a genetic alphabet. Nucleic acids can be replicated themselves through the complementary base pairings of A–T(U) and G–C, and act as functional molecules, such as catalysts and ligands. However, their functionality is restricted by the limited number of the components, as compared with the 20 standard amino acid components of proteins. If we could expand the genetic alphabet by artificially creating a new unnatural base pair, these extra base components in xeno-nucleic acids might augment their functions.

Recently, several groups reported different types of unnatural base pairs that function as a third base pair in replication and transcription. Among them, we developed a hydrophobic unnatural base pair between 7-(2-thienyl)imidazo[4,5-*b*]pyridine (Ds) and a diol-modified 2-nitro-4-propynylpyrrole (Px) (Kimoto, M. et al., 2009). Chemically synthesized DNA fragments containing the Ds–Px pair are amplified ~10^{28}-fold by PCR corresponding to 100 cycles, and more than 97% of the Ds−Px pairs survived at the initial positions in the amplified DNA (Yamashige, R. et al., 2012).

We applied the Ds–Px pair system to generating Ds-containing DNA aptamers that specifically bind to targets. Then, we found that a few hydrophobic Ds bases efficiently increased the affinity of the DNA aptamers to target proteins, achieving significantly higher affinities than those of the conventional DNA aptamers (Kimoto, M. et al., 2013). Furthermore, the aptamers that we obtained can be stabilized against nucleases by modifying using a mini-hairpin technology that we previously developed (Hirao, I. et al., 1994). Here, I will talk about the unnatural-base DNA aptamer generation for their application to diagnostics and therapeutics.

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Kirsten JUNG

Philipp HOLLIGER (MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus UK)

Synthetic genetics

Synthetic biology seeks to probe fundamental aspects of biological form and function by construction (i.e. resynthesis) rather than deconstruction (analysis). Synthesis thus complements reductionist and analytic studies of life, and allows novel approaches towards fundamental biological questions.

We have been exploiting the synthesis paradigm to explore the chemical etiology of the genetic apparatus shared by all life on earth. Specifically, we ask why information storage and propagation in biological systems is based on just two types of nucleic acids, DNA and RNA. Is the chemistry of life's genetic system based on chance or necessity? Does it reflect a "frozen accident", imposed at the origin of life, or are DNA and RNA functionally superior to simple alternatives.

I'll be presenting recent progress on the development and application of strategies to enable the enzymatic synthesis and reverse transcription and hence replication and evolution of novel synthetic genetic polymers, which we term XNAs. We show that eight different synthetic polymers, based on nucleic acid architectures not found in nature, can also mediate genetic information storage and propagation [1]. Beyond heredity, we demonstrate a capacity for Darwinian evolution by the de novo selection of specific ligands (XNA aptamers) and catalysts (XNAzymes) based on entirely synthetic backbones [1, 2]. Thus, key hallmarks of living systems, including heredity and evolution are not limited to DNA and RNA but can be implemented in synthetic polymers and are likely to be emergent properties of polymers capable of information storage.

I'll also be presenting our progress in the engineering and evolution of RNA polymerase ribozymes towards a general polymerase and self-replication capacity We have discovered RNA polymerase ribozymes that are capable of the templated synthesis (i.e. transcription) of another simple ribozyme [3] or RNA oligomers exceeding their own size (>200 nts) [4], a key milestone on the road to self-replication.

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A synthetic biology approach of translational control

Translation of proteins with a stretch of consecutive prolines leads to ribosome stalling. To overcome this stop, bacteria depend on a specific translation elongation factor P (EF-P), being orthologous and functional identical to eukaryotic/archaeal elongation factor e/aIF-5A (1-3). EF-P binds to the ribosome between the peptidyl-tRNA binding site (P-site) and the tRNA exiting site (E-site) and stimulates peptide bond formation. In their active form both EF-P and e/aIF-5A are post-translationally modified at a positively charged amino acid, which protrudes towards the peptidyl-transferase center. While archaeal and eukaryotic IF-5A depend on hypusination of a conserved lysine, the EF-P modification strategies in bacteria vary. In Escherichia coli and Salmonella enterica a lysine of EF-P is extended by β- lysinylation and subsequently hydroxylated, whereas in Pseudomonas aeruginosa and Shewanella oneidensis an arginine in the equivalent position is rhamnosylated (reviewed in 4). In addition to structural constrains of polyproline stretches, some EF-P dependent proteins require this motif to fine-tune the protein output. Our studies aim to create a synthetic EF-P variant, which is standardized and constitutively active independent of species-specific posttranslational modifications. For this purpose, we replace the conserved lysine with unnatural amino acids, such as pyrrolysine, acetyl-lysine, propionyl-lysine, and butyryl-lysine by using the amber suppression system. All synthetic variants are tested for functionality in vivo using reporter strains. In addition, first results are presented of using the amber suppression system in combination with stalling motifs to generate a translational control tool to fine-tune the protein output.

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Sébastien LEMIRE PhD / Tim K. LU (Synthetic Biology Group, Dpt of Electrical Engineering and Computer Science, Dpt of Biological Engineering, MIT)

Technologies for engineering the microbiome

Understanding the role of the gut microbiome in modulating host health and disease will require technologies for localized and long-term monitoring of microbiome and gut functions in vivo. Furthermore, new strategies are needed for precise modulation of microbiomes to enable new diagnostics and therapeutics, since existing approaches for modulating the microbiome can have significant off-target effects. Synthetic biology can provide new tools for studying and manipulating complex microbial communities.

We have created strategies for engineering commensal gut bacteria, such as Bacteroides thetaiotaomicron, a major and stable member of the human gut microbiome with synthetic gene circuits and we demonstrated that they are still functional in mice stably colonized with the engineered bacterium. This work provides a resource for Bacteroides genetic engineering towards future applications as non-invasive diagnostics and therapeutics in the gut microbiome. Furthermore, we have created technologies for the specific knockdown of bacteria living in mixed microbial communities. For example, we engineered CRISPR-Cas antimicrobials that kill bacteria based on their genetic signatures. In addition, we have built a technology platform for engineering phage host range, which enables the creation of well-defined phage cocktails that can kill specific subpopulations of bacteria within mixed microbial consortia.

We anticipate that these strategies will be useful for the targeted knockdown of bacteria in complex microbiomes to understand the functional role of these bacteria or achieve therapeutic effects.

Pablo I. NIKEL (Centro Nacional de Biotecnología (CNB-CSIC))
Synthetic Morphology Approaches in Pseudomonas putida for Bioremediation of Haloalkanes

Bacterial biofilms are known to outperform planktonic counterparts in several types of whole-cell biocatalysis processes. The transition between planktonic and biofilm lifestyles of the platform strain *Pseudomonas putida* KT2440 (as in many other Gram-negative microorganisms) is ruled by a regulatory network that processes a large number of external and endogenous cues into different levels of the trigger signal cyclic di-GMP (c-di-GMP). This circumstance was exploited for the rational design of a synthetic genetic device that supersedes the processes involved in synthesis or degradation of c-di-GMP in *P. putida* – thus making the bacterium to form biofilms at the user's will. In so doing, the transcription of either *yedQ* (encoding a diguanylate cyclase) or *yhjH* (encoding a c-di-GMP phoshodiesterase) from *Escherichia coli* was artificially placed under the tight control of a cyclohexanone-responsive expression system. The resulting recombinant strain was subsequently endowed with a synthetic dehalogenation operon (spanning two genes from *P. pavonaceae* encoding haloalkane dehalogenases) and tested for 1-chlorobutane biodegradation. Upon addition of cyclohexanone to the culture medium, the thereby engineered *P. putida* cells formed biofilms displaying high levels of dehalogenase activity. These results show that morphologies and physical forms of whole-cell biocatalysts can be genetically programmed while purposely designing their biochemical capacities. Furthermore, the spatial disposition of the bacteria at stake will in fact become an integral part of the design process of geneticallymanufactured catalysts of the future.

Floyd ROMESBERG (Department of Chemistry, The Scripps Research Institute, La Jolla, CA, USA)
A Semi-synthetic Organism with an Expanded Genetic Alphabet

Expansion of the genetic alphabet to include a third base pair not only has immediate utility for a number of applications, such as site-specific oligonucleotide labeling, but also serves as the foundation for an organism with an expanded genetic code. Toward this goal, we have examined a large number of different unnatural nucleotides bearing mainly hydrophobic nucleobase analogs that pair based on packing and hydrophobic interactions rather than H-bonding. Optimization based on extensive structure-activity relationship studies and two screens resulted in the identification of a class of unnatural base pairs that are well recognized by DNA and RNA polymerases. More recently, we have engineered E. coli to import the requisite unnatural triphosphates and shown that DNA containing

the unnatural base pair is efficiently replicated within the cell, resulting in the first semi-synthetic organism that stores increased information in its genome.

Eriko TAKANO (Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, UK)

Harnessing synthetic biology for the production of high-value chemicals

Our ability to readily sequence complete genomes and to manipulate/re-design them on a large scale enables the design and construction of organisms with new functionalities of unprecedented scope ("synthetic biology"). We explore these possibilities in the context of high-value chemical production. Many microorganisms already have the machinery to produce diverse bioactive molecules that can be used in health, agriculture and food (Cimermancic et al., 2014). As a first step towards re-engineering these high-value chemical biosynthesis pathways for enhanced productivity and diversity, we aim to understand the interchangeability of biosynthetic parts (Diez et al., 2015) and to create orthogonal transcription mechanisms (based on signalling molecule circuits (Biarnes-Carrera et al., 2015). In addition, we are expanding our collection of computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters, to enrich our library of parts and building blocks for pathway engineering (Weber et al., 2015). We combine this analysis with high-resolution mass spectrometry analysis, which we also employ for the debugging of the engineered systems (Jankevics et al., 2012). Furthermore, we are using computational modelling (constraint-based descriptions of bacterial metabolism) to identify suitable overproduction hosts and pinpoint biosynthetic bottlenecks to target for further cellular engineering in a synthetic biology strategy (Breitling et al., 2013).

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Danielle TULLMAN-ERCEK (Dpt of Chemical and Biomolecular Engr, UC Berkeley)

Virus Capsids: A Starting Point for Precisely Tunable Nanoscale Containers

Key words: protein engineering, drug delivery, compartmentalization, enzyme kinetics

The encapsulation of biological molecules at the nanoscale has important applications from drug delivery to the scaffolding of engineered metabolic pathways. There are many options for the nano-sized container, but protein-based containers such as viral capsids and bacterial microcompartments are extremely promising for the precise control over production and assembly that these systems offer. Great strides have been made in controlling the encapsulation of enzymes within such systems, yet much is still unknown about how small molecules can move into and out of these protein shells. Moreover, the choice of the viral capsid or microcompartment currently dictates the physical size and shape of the container, as such assemblies are not easily designed de novo. Here we demonstrate that protein engineering can be used to alter MS2 viral capsid permeability and size. We first show that charge interactions enable encapsulation of enzymes such as the reporter alkaline phosphatase [1], which is in turn used to explore the impact of altering the residues that line pores within the capsid shell. We find that manipulating the charge of the pores indeed affects the diffusion-limited reaction kinetics, supporting the hypothesis that such containers can be used to concentrate or sequester intermediates [2]. We next applied directed evolution to develop an MS2-based virus-like particle with a decreased size. Surprisingly, just a single amino acid change is sufficient to alter capsid geometry from a T=3 icosahedron to a T=1 icosahedron, and decrease the diameter of this protein cage from 27 nm to 18 nm. The altered geometry is confirmed by the crystal structure of the mutated VLP. To our knowledge, this is the first time such a remarkable change in geometry and size of a VLP was achieved with a single mutation. Coupled with our study of the pores, these results are quite encouraging: despite the tightly coordinated assembly of protein-based containers, they are highly permissive of changes to the compartment-forming protein and are tunable structures with respect to both size and permeability. This presentation will detail the methods used to engineer the MS2 capsid and discuss the implications of the malleability of these structures.

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