Synthetic Biology: Mapping the Design Principles of Biological Systems and the Rise of Biomimetic Engineering

Dr. George Poste
Chief Scientist, Complex Adaptive Systems Initiative and Del E. Webb Chair in Health Innovation
Arizona State University
george.poste@asu.edu
www.casi.asu.edu

Keynote Presentation at the Six National Academies Synthetic Biology Symposium:
The Royal Society, London • April 13, 2011
Slides available @ http://casi.asu.edu/
Biological Design: “Endless Forms Most Beautiful”: Limitless Diversity From Combinatorial Assemblies of Limited Building Blocks
Technology Acceleration and Convergence in Life Sciences R&D: Mapping the Design Principles of Biological Systems and New Industrial Applications

- A Complex Matrix of Inter-dependencies Across Multiple Disciplines and Industrial Sectors
Systems Biology

- analysis and modeling of entire organisms to define functional inter-relationships between their diverse constituent parts at multiple hierarchical and spatio-temporal scales
- mapping the design “rules” that confer robustness, adaptability and evolvability

Synthetic Biology

- deconstruction of the functions of natural systems to enable rational construction of designed systems to perform novel functions with predictable behaviors
- anticipated technology progression to construct completely synthetic cells/organisms with autonomous replicative, repair and adaptive evolutionary capabilities
Systems and Synthetic Biology:

Understanding the Embedded Information Content and Patterns of Regulated Information Flow and Processing in Dynamic Biological Systems

Digital Biology: “It from Bits”
The Evolution of Technology for the Genetic Modification of Biological Systems

- expression of single genes inserted into single cells
  - rDNA technologies
- expression of multiple linked genes in single cells
  - metabolic process engineering
- multiple independent genes in a complex host
  - genetically modified plants
- semi-synthetic single cells
  - ‘booting up’ synthetic whole microbial genomes in recipient cells
- control of ex vivo growth and differentiation of mammalian/human cell lineages
  - ESCs, iPSCs
- complex histiotypic assemblies on biocompatible/inductive tissue scaffolds
Synthetic Biology: The Next Era in Modification of Biological Systems

Top Down
- modification of existing organisms
- “minimum genomes” and “plug and play” genetics
- transfer of biosynthetic/environmental fitness genes/modules novel pathways/sub-networks

Bottom Up
- directed assembly of higher order structures/organisms via programmed assembly of diverse components

Reprogramming of Cell Fate Decisions
- directed channeling of genomic information pathways in target cells/organisms
- stem cells (embryonic/adult)
- transdifferentiation of cell lineages
- engineered circuits for cis-/trans-control of gene(s)
Application of Engineering Principles to Biological Design and Synthetic Biology

- specification
  - input and output requirements
- standardization
  - unit parts for programmed hierarchical assembly
- abstraction
  - hierarchies of complexity and connectivities
- decoupling
  - design and fabrication
- context and optimality
  - the unique features of biological systems
  - major challenge in the interoperability and exchange of biological components between systems
  - cells adapt expression profiles to optimize cost-benefit solutions
Synthetic Biology and Predictive Biology

- is the analogy with engineering design and defined input:output relationships a vast over-simplification?
- non-linear relationship between genotype (code) and phenotype (expression, regulation, adaptation, selection)
- impact of stochastic events and noise on biological properties/stability of biological systems
- many biological ‘parts’ and pathways exhibit probabilistic behavior depending on their pre-existing ‘state space’
- anthropogenic engineered systems display robustness, but few exhibit adaptive evolvability
- biological systems possess emergent properties expressed only by the entire system and not by any isolated parts
The Development of End-to-End Systems for Applications of Synthetic Biology

**Code**

- Standardized Parts
- Ordered Genome Assembly
- Recipient “Chassis”

**Efficient Insertion**

- Pathway: Network Optimization
- Scale Up and Economic Production
- Oversight: Risk, Regulation and Responsibility
design of a series of microbial chassis with tolerance for insertion of diverse operon networks and construction of novel pathways and network topologies
  – “plug and play”, “cut and paste” genetics
  – “booting up” increasingly complex synthetic genome constructs

minimal or no ‘fitness penalty’ for the engineered phenotype

stability (non-excision) of the introduced construct

‘kill switch’ for safety to prevent contamination of inappropriate environments
Chassis Design
The Challenge of ‘Context’ in Biological Systems

- limited number of microbial species studied in depth to evaluate merits as ‘universal chassis’ candidates
- comparative metastructure analyses of genomes in related species highlight the extent of context-dependent variation in genomic organization/expression
  - *Klebsiella* and *E.coli* exhibit highly conserved ORFs/promoters but large variation in 5' UTRs
Design Parameters for Introduction of Multi-Gene Constructs for Programmed Expression of Complex Synthetic Reactions

- designed operon networks of genes coding for each reaction step
- challenge of balancing upstream and downstream parts of the synthetic metabolic network
  - incorporation of synthetic promoters, regulatory elements, insulators for dynamic regulation of expression based on metabolic flux
  - balance pathway flux via RNA stabilization/destabilization, ribosome binding site affinities
- use of synthetic scaffolds and compartments to optimize spatial interactions of gene products and reduce runoff intermediates
Use of Spatial Controls to Optimize Designed Reaction Pathways for Bioprocess Engineering

From: M. H. Medema et al. (2011) Nature Rev. Microbiol. 9, 131
Use of Temporal Controls to Optimize Reaction Kinetics and Pathway Flux in Engineered Microbial Circuits

From: M. H. Medema et al. (2011) Nature Rev. Microbiol. 9, 131
Synthetic *M. mycoides* Genome Assembled from 1078 Overlapping DNA Cassettes in Three Steps in Yeast and Subsequent Transfer to *M. capricolum*

From: D. G. Gibson et al. (2010) Science 329, 52
“Big Biology”:
The Organizational Scale, Cost and Logistical Complexity
of Design and Construction of Synthetic Microbial Gene Circuits and Genomes

150 person-years
- synthetic pathway for the antimalarial drug precursor, artemisinic acid

400 person-years
- 1.08 mega-base part genome of *Mycoplasma mycoides* JCVI syn 1.0. genome
- D. G. Gibson et al. (2010) Science 329, 52
Protocells and Artificial (Chemical) Cells
Protocells and Artificial (Chemical) Cells

- encapsulation of requisite biomolecular species needed to encode protein expression and regulated metabolic pathways within lipid membrane vesicles and intravesicular membrane-bound compartments
- use of isotonic non-dissipative nutrient environment to sustain metabolic and energy fluxes and sink for draining metabolic waste
- challenge of engineering vesicle fission for reproduction and balanced partition of duplicate genetic and other critical assets
- high barrier for design of adaptation and evolution traits
Microbes: Uniquely Successful Organisms and Adaptive Colonization of Myriad Eco-Niches
Metagenomics: Sampling the Extravagant Functional Diversity of Microorganisms

- estimated 100 billion microbial species
- only 6000 species cultivated and characterized
- massive repertoire of uncharacterized genes/proteins/metabolomes
- metagenomic sampling
  - mass screening of complete genomes of unknown/unculturable organisms
  - high throughput profiling to identify transfer of gene(s) with desired function(s) into ‘universal acceptor’ organisms for industrial production
  - likely need for significant genome editing to overcome the ‘context challenge’ for designed circuits from “extremophiles”
Global Network of Coexisting Microbial Lineages Profiled by 16S RNA Sequences

From: S. Chaffron et al. (2010) 20, 947
Sampling Sites: N. America, Caribbean, Africa and Middle East
Informatics and High Performance Computing: A Fundamental Capability for Future Biosystems Engineering

- analysis of patterns of conservation/diversification in biological components, pathways, networks and functional correlations across evolutionary phylogeny

- progressive comprehension of design rules for automated design of interaction networks to better inform design efficiency and predictability of engineered biological pathways
Informatics and High Performance Computing

- transcending inadequate standards for ontologies data annotation, curation and inter-operability of diverse databases

- computational infrastructure for anticipated rapid expansion to peta-/exa-byte scale datasets and databanks
Informatics and High Performance Computing

- automated design and GUI circuit/network design tools (BIO-CAD)

- modeling and simulation of biological networks of escalating complexity

- development of new mathematical, statistical and computing tools for analysis and modeling of non-linear phenomena
Synthetic Biology: An emerging technology with myriad applications in diverse industrial sectors

<table>
<thead>
<tr>
<th>Healthcare</th>
<th>Public Health</th>
<th>Agriculture</th>
<th>Improved Foods</th>
<th>Novel Materials</th>
<th>Textiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioenergy and Biofuels</td>
<td>Industrial Enzymes</td>
<td>‘Green’ Mfg</td>
<td>Bio-remediation</td>
<td>Clean Water</td>
<td>Devices and Sensors</td>
</tr>
</tbody>
</table>
Bio-inspired Systems Engineering

- complex multi-step syntheses and high performance materials made in completely different ways
- limit depletion of non-renewable resources by traditional industrial manufacturing
- mimic efficiency of natural ecosystems
- limit/eliminate hazard/cost of waste streams
- manufacturing at room temperature in water versus high temperatures and toxic solvents
- highly distributed manufacturing units
Biofuels: The Race to the Pump and Multi-Trillion Markets
The Quest for Sustainable and Stable Alternative Energy Sources


Agricultural Inputs
- Seeds
- Crop protection
- Fertilizer

Biomass Production
- Energy crops
- Sugarcane
- Short rotation forestry

Biomass Trading
- Biomass aggregation
- Logistics
- Trading

Biorefining Inputs
- Enzymes
- Organisms
- Pretreatment chemicals

Biorefining Fuels
- First- and second-generation biofuel production

$15B
Potential revenue by 2020 in USD B

$89B

$30B
$10B

$80B

$65B
$6B

$4B
Biomass power and heat
Downstream chemistry
Biorefining chemicals

Revenue potential: There is significant revenue potential along the entire biomass value chain.
Abundant and Renewable Biomass: An Under-Utilized Substrate for Bio-Based Industrial Feedstocks and Energy

600 million wet tons/year (US)
Synthetic Biology and Bio-Based Manufacturing With Non-Petrochemical Feedstocks

- Speciality Chemicals
  - fine chemicals
  - additives
  - adhesives
  - scalants
  - pulp and paper

- Commodity Chemicals
  - plastics
  - resins
  - paints
  - pigments
  - explosives
  - industrial gases

- Agriculture
  - crop yield, protection and tolerance
  - bioremediation

- (Bio) Pharmaceuticals
  - complex macrocyclics/intermediates
  - vaccines

$1.5 trillion global industry: 70,000 products
Synthetic Biology and Engineering Enhanced Traits in Food, Feed and Fiber Products
Not Immediately Available!
Microbial Genomics and Synthetic Biology: New Technology Platforms for Bioremediation and Improved Efficiency of Wastestream Management
Synthetic Biology and Integration With New Insights Into Microbial Community Dynamics

- Role of Mixed Communities in Bioprocesses and Bioremediation
- Synthetic Bioprocesses and Bioreactor Production Optimization
- Modulation of Biofilm Formation
Site-Specific Profiles of 145 Operational Taxonomic Units (out of total 4962) From Different Full-Scale Methanogenic Granular Sludge Bioreactor for Brewery Wastewater Using Bacterial 16S rRNA Sequencing

Microbial Biofilms

- dominant form of microbial life
- estimated more than 90% of bacteria live in multi-species biofilm communities
- new insights into microbial communication pathways parameters for control of biofilm formation

**Value of Biofilm Promotion and Stabilization**
- bioremediation of water sources and wastestreams
- bioreactor performance optimization with mixed microbial species
- microbial fuel cells
- biosensors and Bio-MEMS

**Value of Biofilm Disruption and Prevention**
- fouling and corrosion
- antibiotic-resistant infections in humans, animals and plants
- design of novel materials with derivatized surfaces/controlled release chemicals to perturb quorum-sensing and other microbial communal signaling pathways
We Are Not Alone: The Human Microbiome – A Barely Understood Factor in Human Health and Disease

- Human body contains 10x more bacterial cells than human cells
- Complex meta-system
  - Host, microbes, viruses, other organisms, metabolites, xenobiotics
  - Is there a core microbiome?
  - How do perturbations affect disease and vice-versa?
  - Modulating the microbiome for improved health/disease Rx
Modification of GI Microbiota for Enhanced Food Conversion Efficiency in Livestock
Engineered Microorganisms as Health Status Sentinels and Therapeutic Delivery Systems

Glass Microbiology Luke Jerram
Decorating Microbes: Engineered Surface Display of Proteins

- optimized performance of bioreactor/bioremediation microbes
- programmable systems for targeted drug delivery
- membrane insertion or C-or N-terminal domain coupling to OMPs, adhesins, virulence factors, autotransporters, ice nucleation proteins
- scaffolds for enzymes/antigens/other proteins that require specific orientation/presentation configuration (cf. solution interactions)
- display of variant molecules generated by directed evolution and substrate profiling of enzymes for biocatalysis
- epitope screening for improved vaccine design
Synthetic Biology and the Exploration of Expanded Functional Chemical and Biological ‘Space’

Incorporation of Non-Natural Components

- nucleotides
- D-amino acids
- tRNA/ribosome engineering

Directed Evolution

- novel genetic sequences and transcription factors
- RNA/protein/peptide variants
- novel tertiary structures/ folds/motifs
Synthetic Biology: Expanding Molecular Structure:Function “Space”

- directed evolution of proteins
  - altered enzyme kinetics, substrate promiscuities
  - novel properties (transport, thermostability; protease resistance, etc.)
  - unique tertiary structures, folds and motifs

- directed evolution of RNAs

- incorporation of non-natural (orthogonal) components
  - modified nucleotides and nucleic acids (XDNAs)
  - D-amino acids, tRNA and ribosome engineering to accept non-natural components
  - non-natural chiralities

- self-sustaining replicants invent complexity and inventiveness
Nano- and Meso-Scale Engineering and Directed Molecular Assembly for Novel Materials, Sensors and Self-Assembling Devices
Directed Molecular Assembly and Construction of Novel Materials

- extensive combinatorial space of novel biotic and biotic:abiotic hybrid assemblies
- nanoscale spatial distribution and “programmable” interactions
  - synthesis and/or self-assembly of novel end-products/higher order assemblies
  - ‘nucleation’ foci for self-assembly and 3-D scaffolds with derivatized surfaces
- sensors with adaptive, reconfigurational and repair capabilities
- molecular motors and miniaturized devices/machines
Directed Molecular Assembly and the Design of Scaffolds for Cell and Tissue Engineering

- derivatized surfaces and 3-D matrices with bound or programmed release of biomediators for induction of specific cell differentiation/reprogramming pathways

Modulated Elastomeric Substrates
J. Fu et. al. (2010) Nature Methods 7, 733

Directed Cell Lineage Pathways and Cell Fate Decision Engineering in ESCs or iPSCs
Cell Therapies and Regenerative Medicine: Replacement, Repair and Regeneration

Reprogramming Rx with ESCs/iPSCs to Generate Specific Cell Lineages as Committed Precursors or End-Stage Differentiated Cells

(Re)Building Complex Histiotypic Structures with Full Homeostatic Controls
Synthetic Biology: Policy Issues
Science and Technology Options Assessment

STOA Conference: Bio-engineering in the 21st Century - 10th November 2010

Making Perfect Life

Realising European potential in synthetic biology: scientific opportunities and good governance

European Academies easac Science Advisory Council

EASAC policy report 12
December 2010
ISBN: 978-3-8647-2868-0
This report can be found at www.easac.eu

Nuffield Council on Bioethics

Emerging biotechnologies

Consultation Paper

April 2011
# Oversight of Synthetic Biology: Risk, Regulation and Responsibility

<table>
<thead>
<tr>
<th>Biosafety: Risk from Legitimate R&amp;D/Industrialization</th>
<th>Biosecurity: Deliberate Use to Cause Harm</th>
<th>Biohackers and Democratization of New Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Biohazard Symbol" /></td>
<td><img src="image2.png" alt="Lock Symbol" /></td>
<td><img src="image3.png" alt="DIY Bio Hack" /></td>
</tr>
<tr>
<td><strong>Screening of Purchases/Supply Transactions</strong></td>
<td><strong>Regulation, Legislation and Codes of Conduct</strong></td>
<td><strong>International Harmonization</strong></td>
</tr>
<tr>
<td><img src="logo1.png" alt="Logos" /></td>
<td><img src="logo2.png" alt="Logos" /></td>
<td><img src="logo3.png" alt="Logos" /></td>
</tr>
</tbody>
</table>

**Logos:**
- Igeneart
- Blue Heron
- IGSC (International Gene Synthesis Consortium)
- IASB (International Association for Synthetic Biology)
- Various international flags
- Other synthetic biology-related logos
Next Era in the Intellectual Transition of the Life Sciences from Phenomenology to the Elucidation of the Design Principles for Complex Biological Systems

Foundation for the Emergence of a New Industrial Ecology with Myriad Applications in Diverse Industrial Sectors and National Security
Synthetic Biology: The Emergence of a New Industrial Ecology

- Cell organisms
  - Prokaryotes
  - Eukaryotes
  - Synthetic cells
- Cell-free systems
- Biomimetic materials
  - Devices
  - Machines
  - Infrastructure

Applications:
- Healthcare
- Agriculture
- Environmental bioremediation
- Energy
- Non-renewable resources
- Novel bioprocesses
- Materials

Production and policy:
- New industries and business models
- Oversight and regulation
- Dual use risk

Design and substrate:
Projected Trajectories for Biosystems Engineering Using Synthetic Biology

- Engineered microorganisms for biomedical applications
- Extremophile design principles
- Novel biosynthetic processes (contained organisms)
- Directed evolution methods
- Bio-inspired materials/sensors
- Reprogrammed human cells/lineages for regenerative Rx
- Environmental engineering (released organisms)
- De novo design of synthetic cells
Mapping the Information Content and Design Rules for Biological Organization and Future Designer ‘Biospace’

- **Design Space**: systems and synthetic biology
- **Coding Space**: metagenomics, phylogenomics
- **Diversity Space**: ecosystems, biogeochemistry, astrobiology/exobiology
- **Unexplored Biospace**: chemical biology, orthogonal components, xenosystems
Reframing the Challenge of “Managing the (Global) Commons”
The Imperative to Understand Complex Adaptive Systems

**Distribution/Control of Resources**
- access
- allocation
- utilization

**Finite/Contracting Resources**
- biodiversity
- non-renewable resources
- unconstrained costs

**Expanding Resources**
- infocosm
- ‘synthetic’ diversity
  - materials
  - genetic

**Drivers**
- economics
- sociology
- politics
- security
- equity
- risk and regulation
- governance and public policy

- sustainability
  - health
  - environment
- designing a new industrial ecology (“lightness”)

- technology acceleration/convergence
- systems and synthetic biology
- de novo design of unique structures and functions
  (exploring biospace)

- designing an industrial ecology (“lightness”)
Progress in Systems and Synthetic Biology Will Depend on New Trans-Disciplinary Knowledge Networks