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

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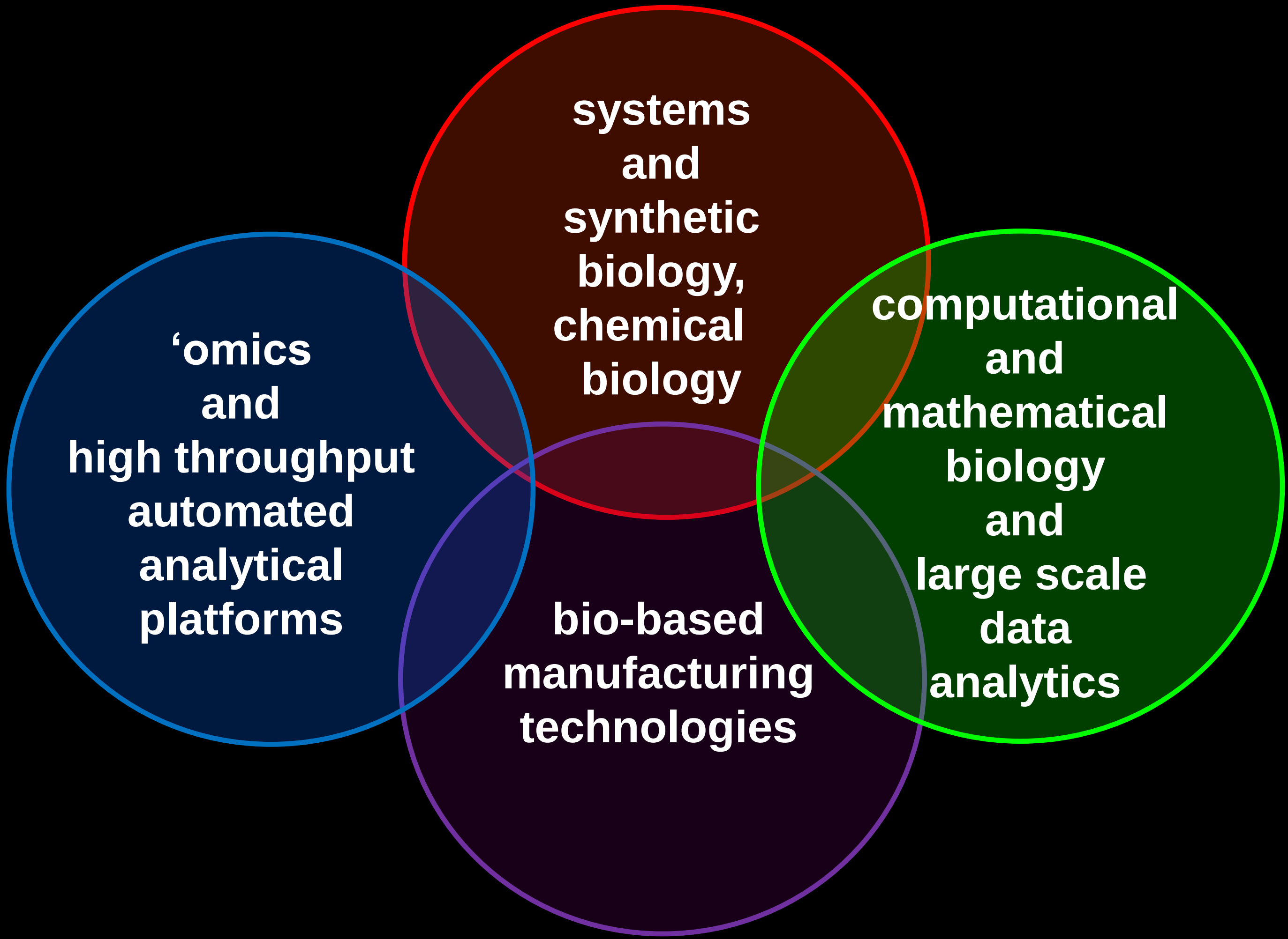
Slides available @ http://casi.asu.edu/

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 and Synthetic Biology: Blurred Boundaries and Shared Conceptual and Technological Horizons

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

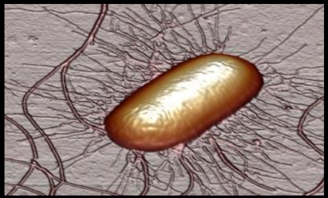
- deconstruction of the functions of natural systems to enable rational construction of designed systems to perform novel functions with predictable behaviors
- anticipated technology progression to construct completely synthetic cells/organisms with autonomous replicative, repair and adaptive evolutionary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

Systems and Synthetic Biology:

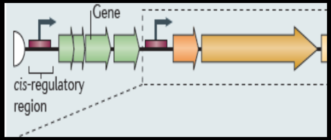
Understanding the Embedded Information Content and Patterns of Regulated Information Flow and Processing in Dynamic Biological Systems

Digital Biology: “It from Bits”

The Evolution of Technology for the Genetic Modification of Biological Systems

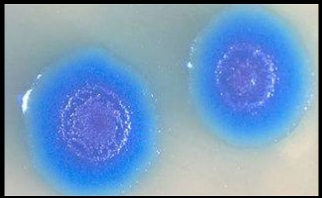


- expression of single genes inserted into single cells
  - rDNA technologies

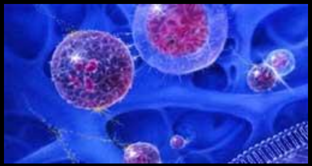


- expression of multiple linked genes in single cells
  - metabolic process engineering

- 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

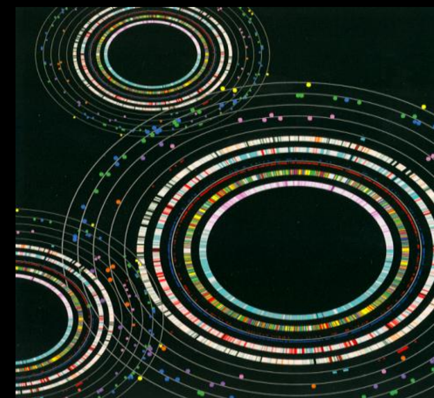
- specification
  - input and output requirements
- standardization
  - unit parts for programmed hierarchical assembly
- abstraction
  - hierarchies of complexity and connectivities
- decoupling
  - design and fabrication
- context and optimality
  - the unique features of biological systems
  - major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

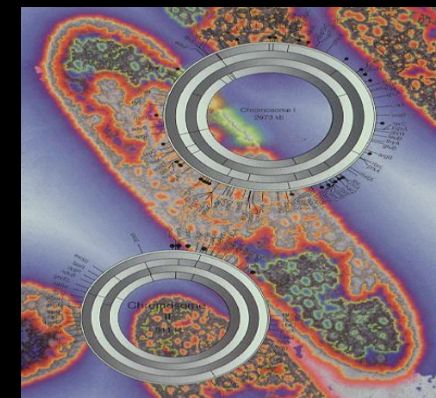
Synthetic Biology and Predictive Biology

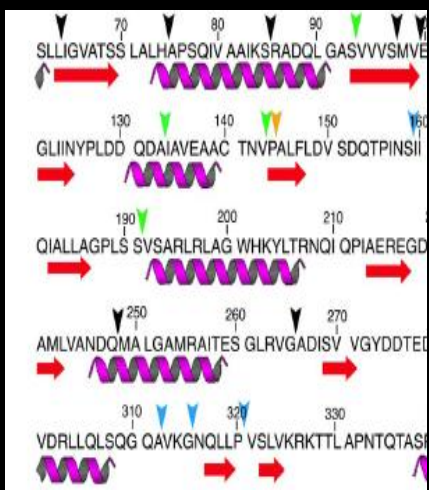
- is the analogy with engineering design and defined input:output relationships a vast over-simplification?
- non-linear relationship between genotype (code) and phenotype (expression, regulation, adaptation, selection)
- impact of stochastic events and noise on biological properties/stability of biological 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

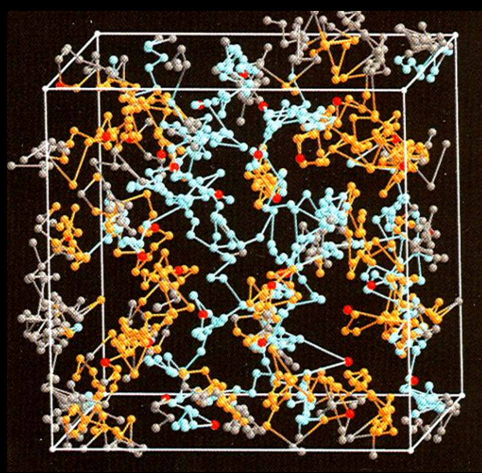
The Development of End-to-End Systems for Applications of Synthetic Biology

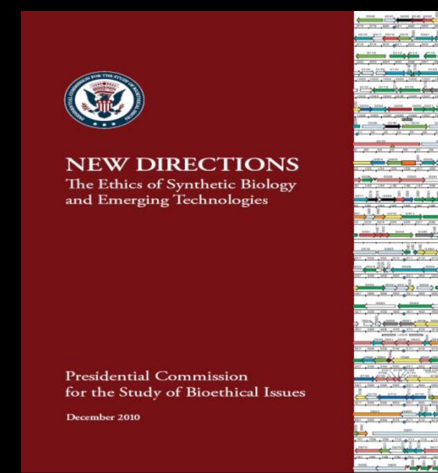
Code	Standardized Parts	Ordered Genome Assembly	Recipient "Chassis"











Efficient Insertion	Pathway: Network Optimization	Scale Up and Economic Production	Oversight: Risk, Regulation and Responsibility

Chassis Specifications for Synthetic Biology

- 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

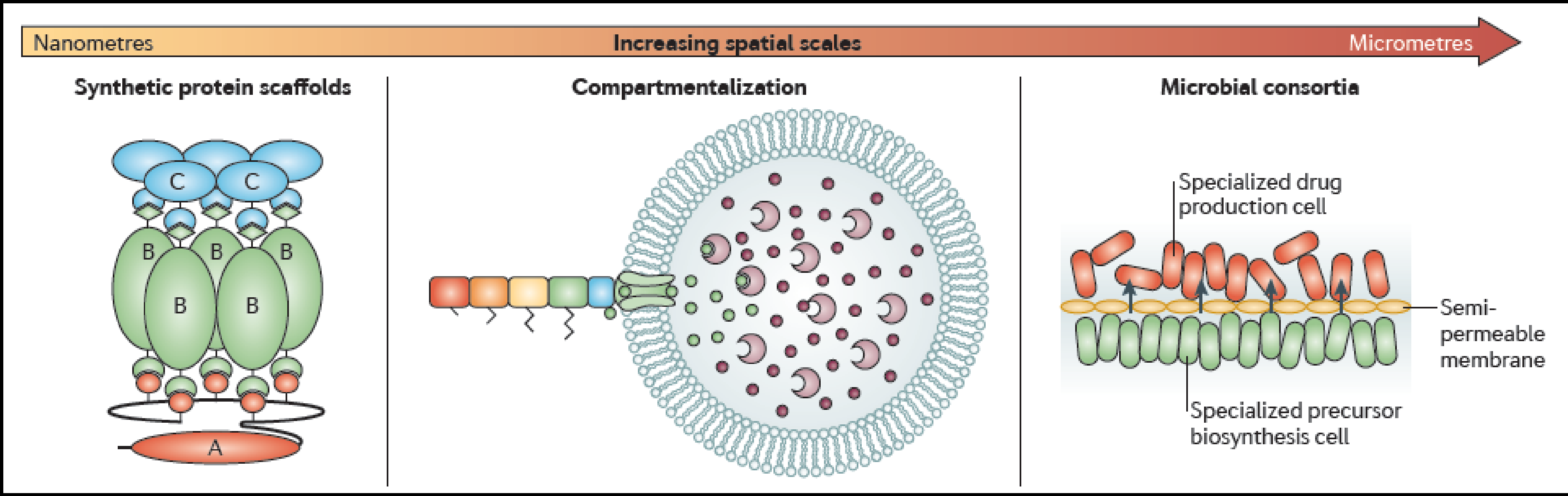
Chassis Design
The Challenge of ‘Context’ in Biological Systems

- 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

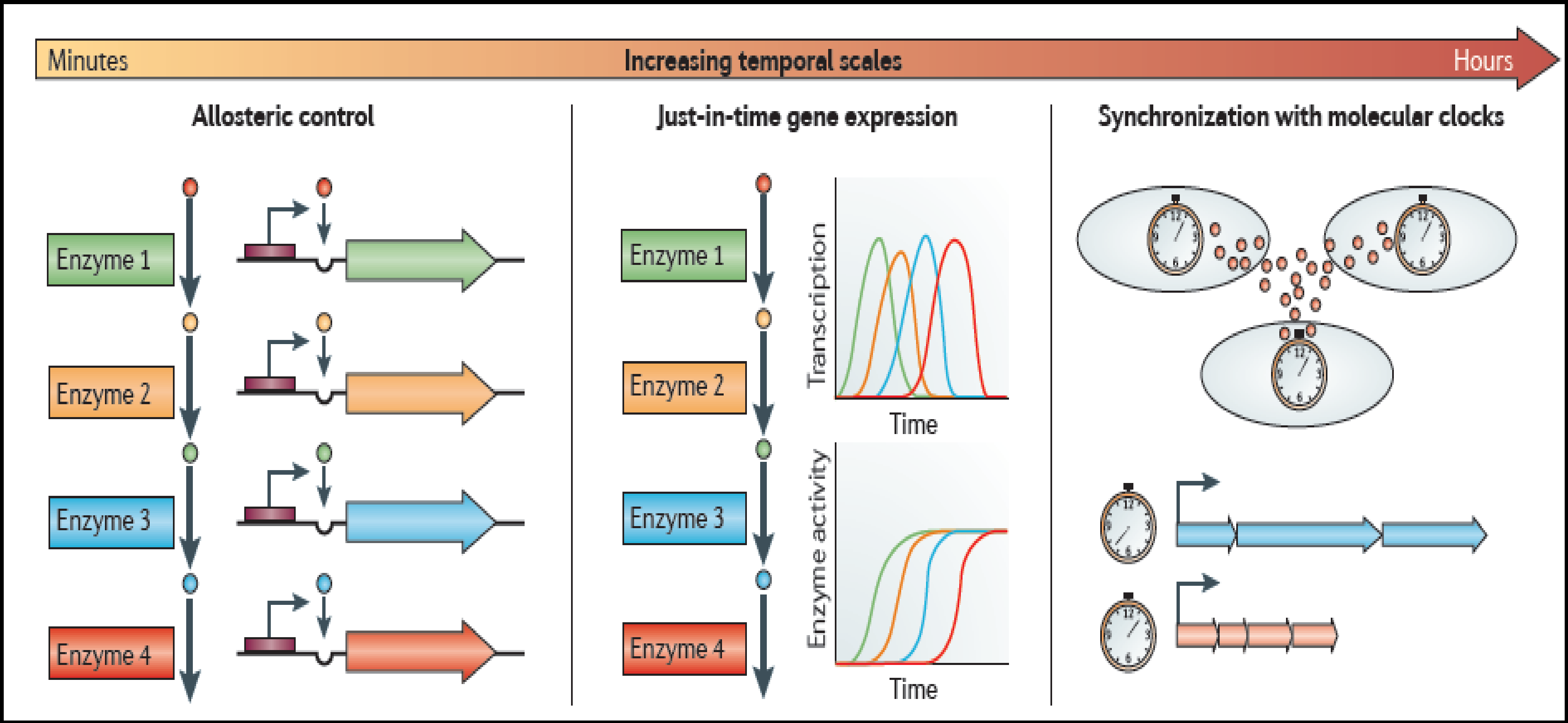
Use of Spatial Controls to Optimize Designed Reaction Pathways for Bioprocess Engineering



From: M. H. Medema et al. (2011) Nature Rev. Microbiol. 9, 131

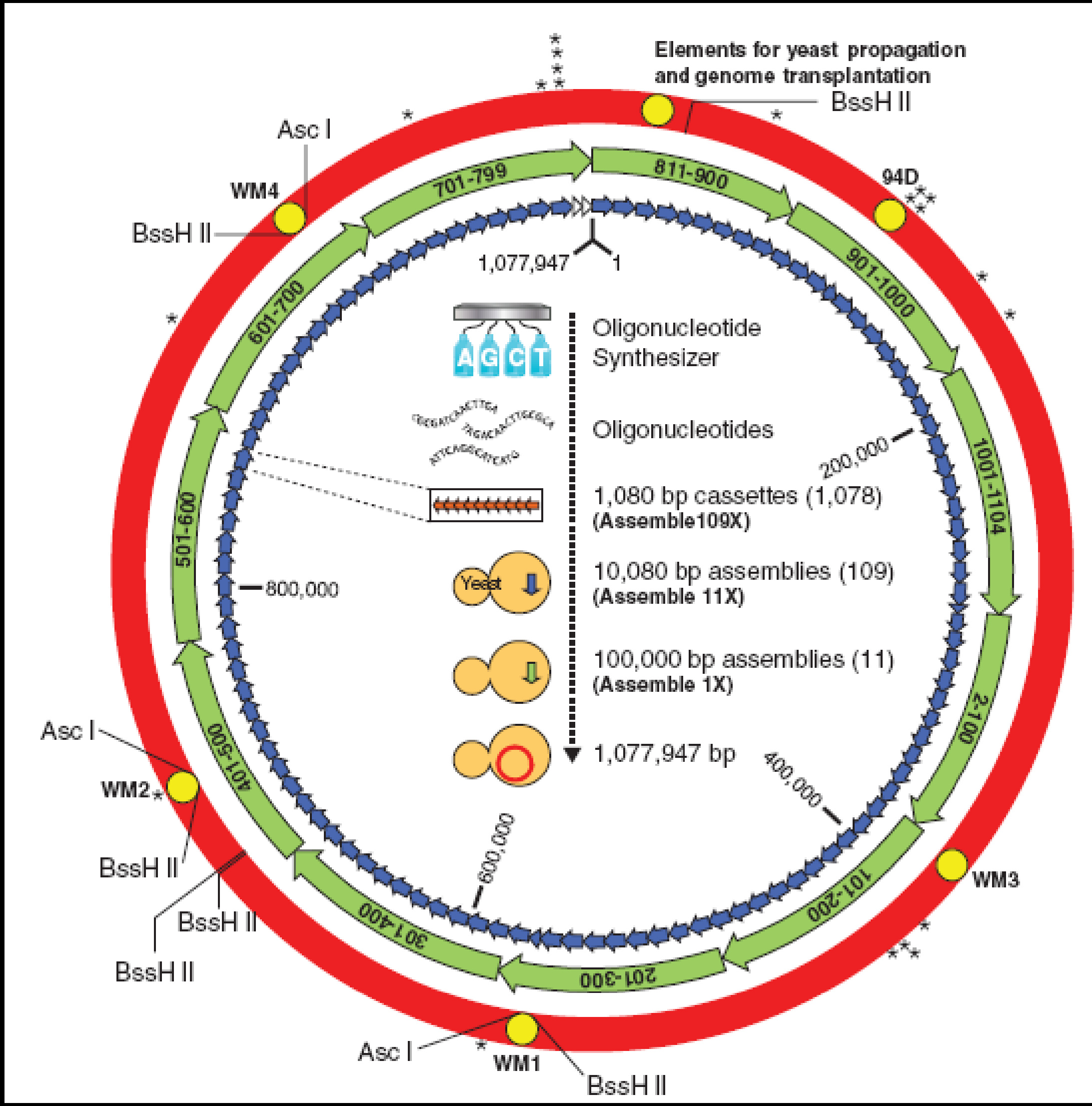
http://www.nature.com/nrmicro/journal/v9/n2/pdf/nrmicro2478.pdf

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

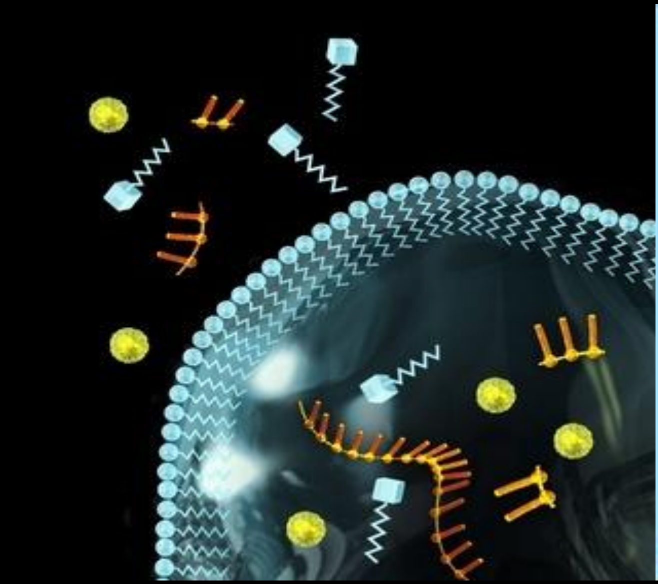
150 person-years

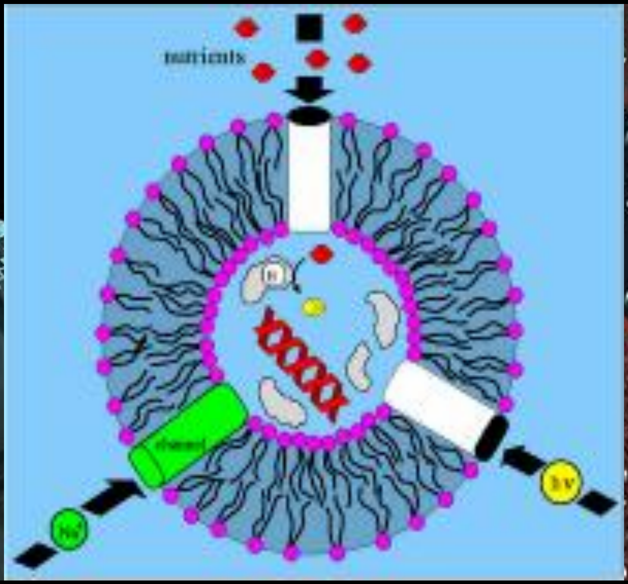
- synthetic pathway for the antimalarial drug precursor, artemisinic acid
- D. K. Ro et al. (2006) Nature 440, 940

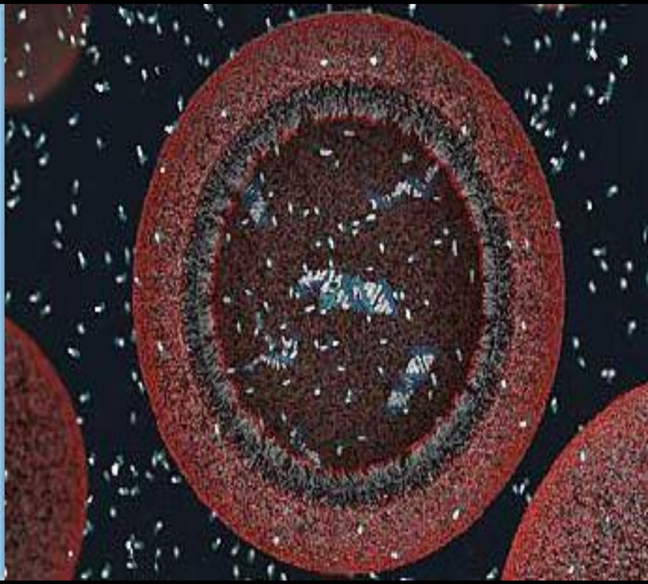
400 person-years

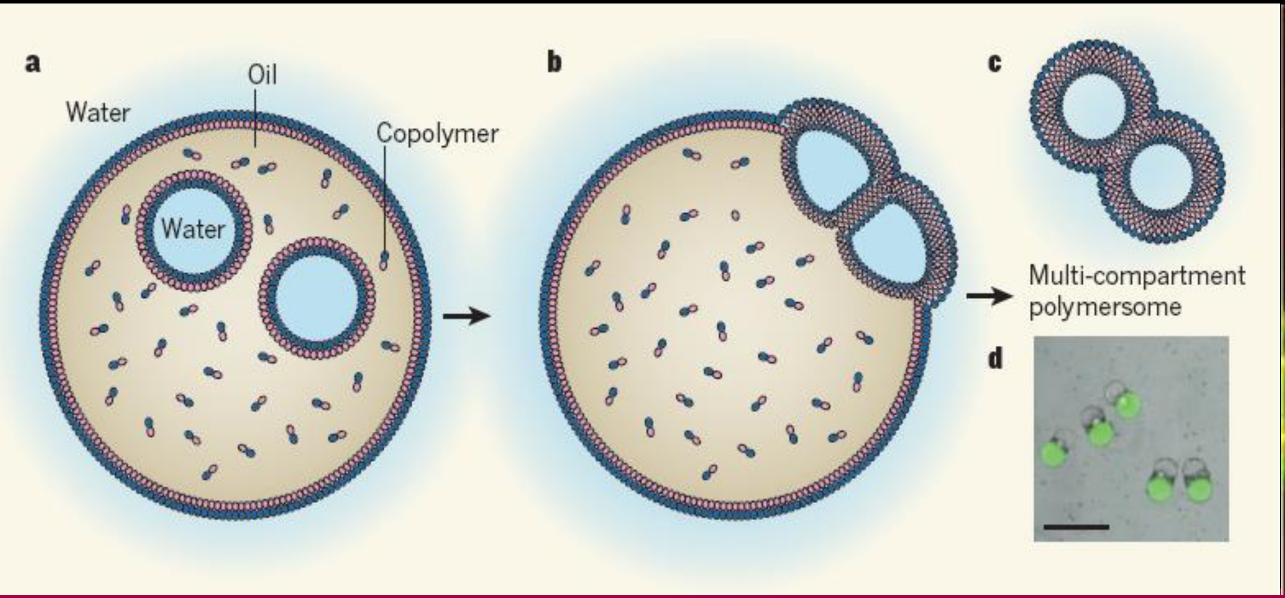
- 1.08 mega-base part genome of *Mycoplasma mycoides* JCVI syn 1.0. genome
- D. G. Gibson et al. (2010) Science 329, 52

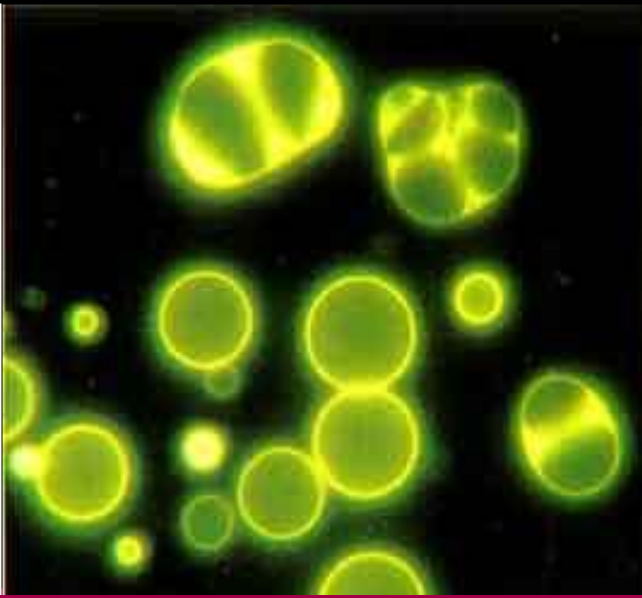
Protocells and Artificial (Chemical) Cells







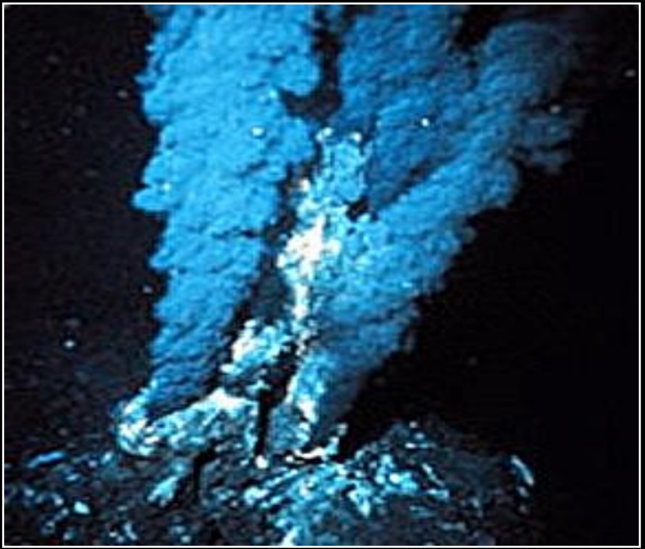


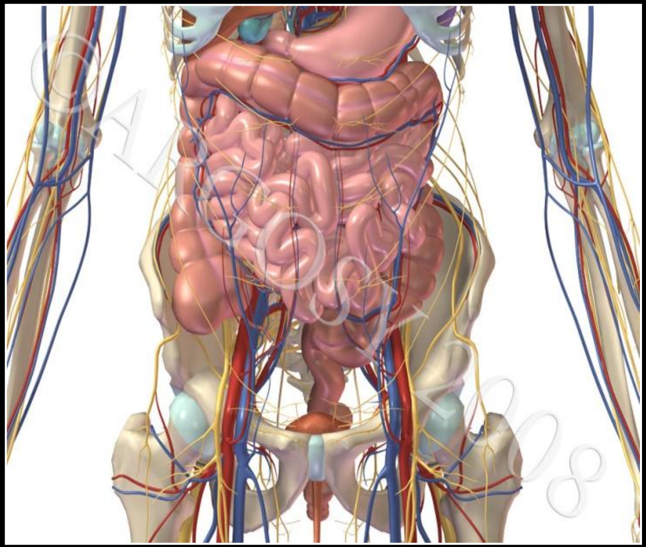


Protocells and Artificial (Chemical) Cells

- 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



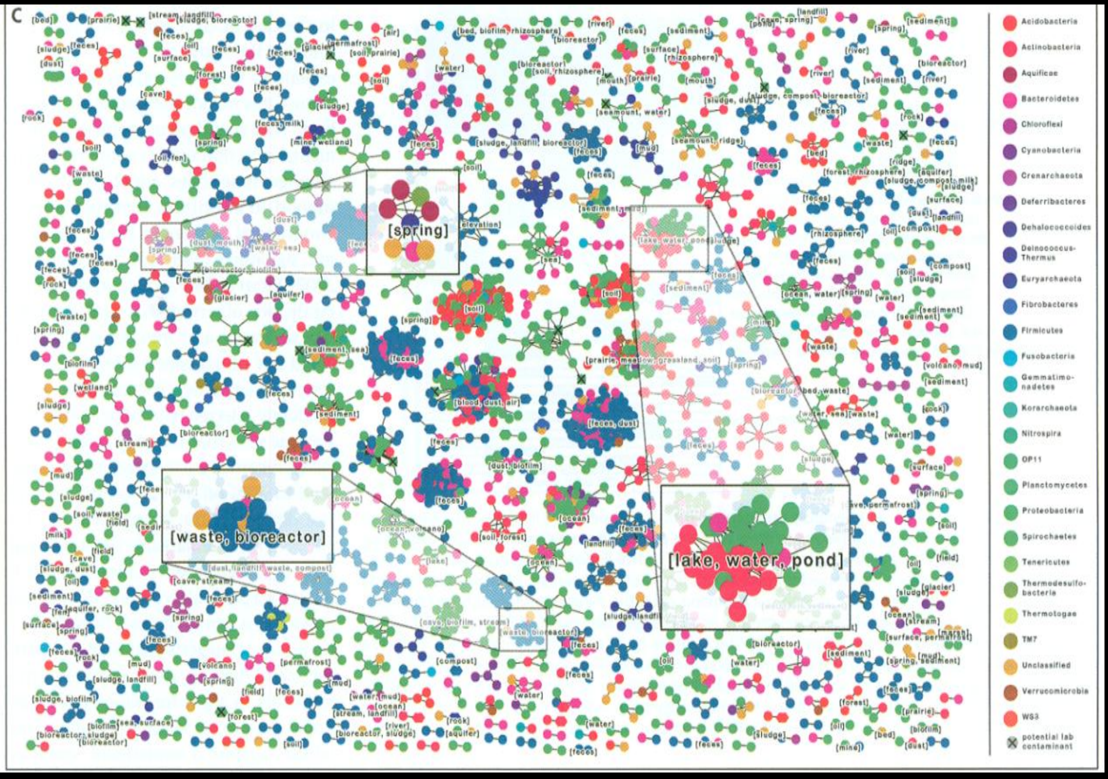


Metagenomics: Sampling the Extravagant Functional Diversity of Microorganisms

- 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”

earth microbiome project

Home About EMP EMP Projects Publications Meetings

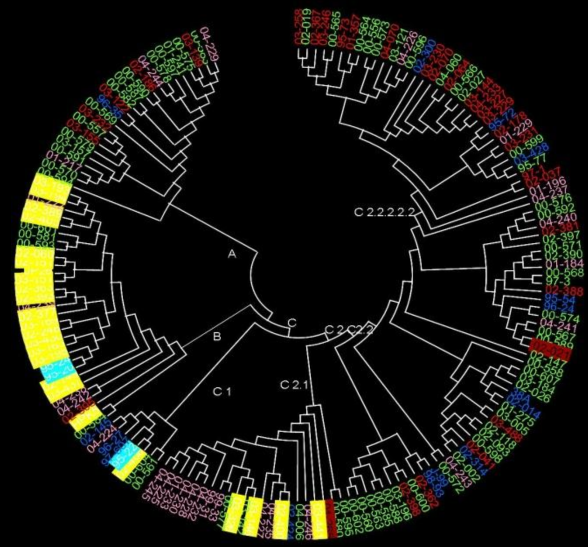


Global Network of Coexisting Microbial Lineages Profiled by 16S RNA Sequences

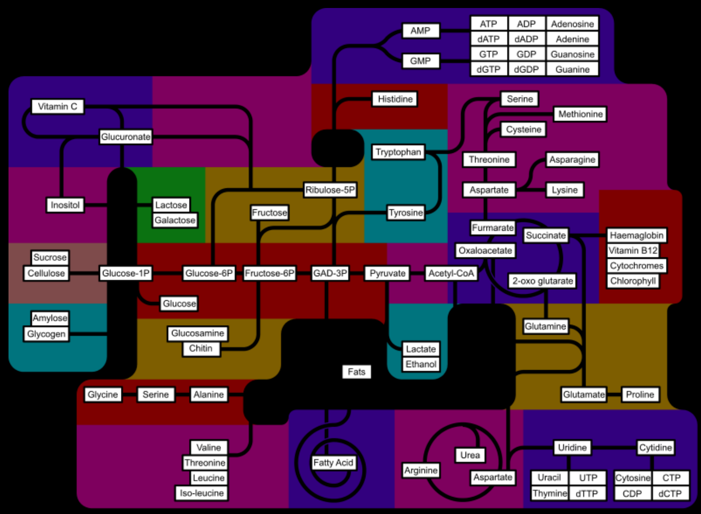
From: S. Chaffron et al. (2010) 20, 947
Sampling Sites: N. America, Caribbean, Africa and Middle East

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**



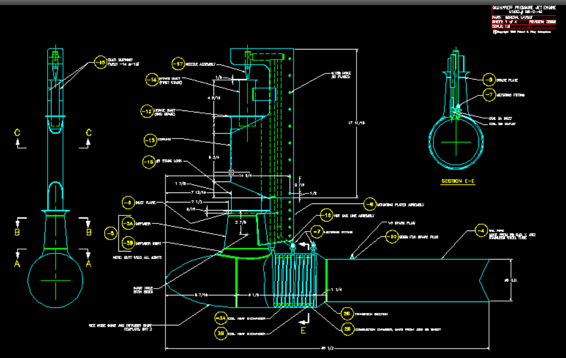
Informatics and High Performance Computing

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

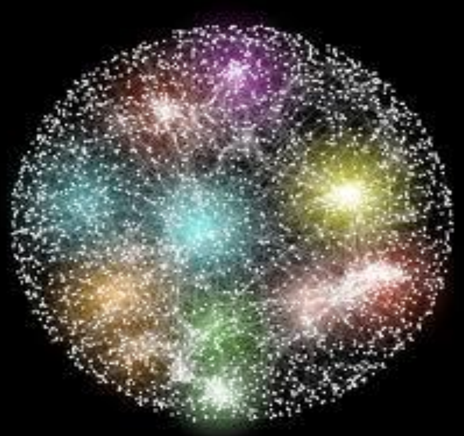
- computational infrastructure for anticipated rapid expansion to peta-/exa-byte scale datasets and databanks

Informatics and High Performance Computing

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



- modeling and simulation of biological networks of escalating complexity



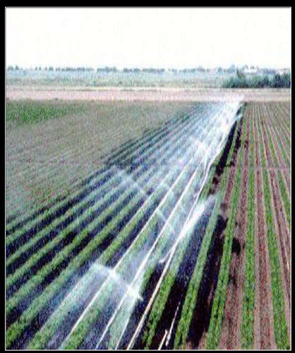
- development of new mathematical, statistical and computing tools for analysis and modeling of non-linear phenomena

Synthetic Biology:
An emerging technology with myriad applications in diverse industrial sectors

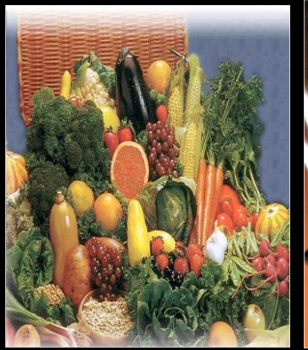
Healthcare

Public Health

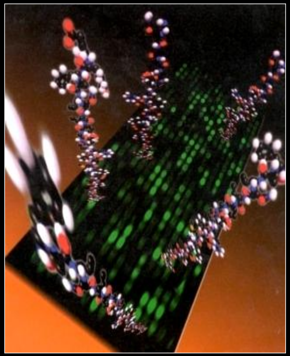
Agriculture



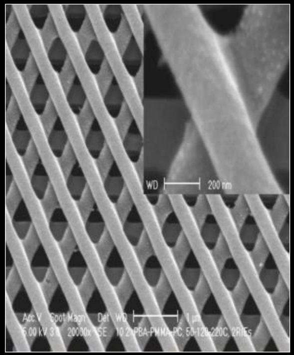
Improved Foods



Novel Materials

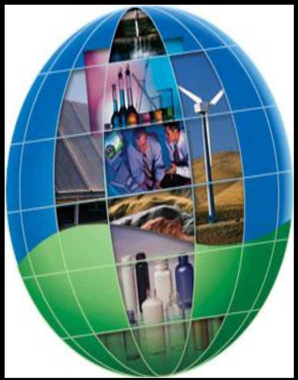


Textiles



Bioenergy and Biofuels

Industrial Enzymes



'Green' Mfg

Bio-remediation

Clean Water

Devices and Sensors

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

MASCOMA

VALERO ENERGY CORPORATION

BR PETROBRAS

VIRENT

genomatica

ExxonMobil

SYNTHETIC GENOMICS

PetroAlgae™ thinking outside the barrel™

cleantech BIOFUELS

gevo™

Mendel

KiOR Redefining Oil

AMYRIS

INEOS Bio

LSG Lab Services Group

jbei Joint BioEnergy Institute

GREAT LAKES BIOENERGY RESEARCH CENTER

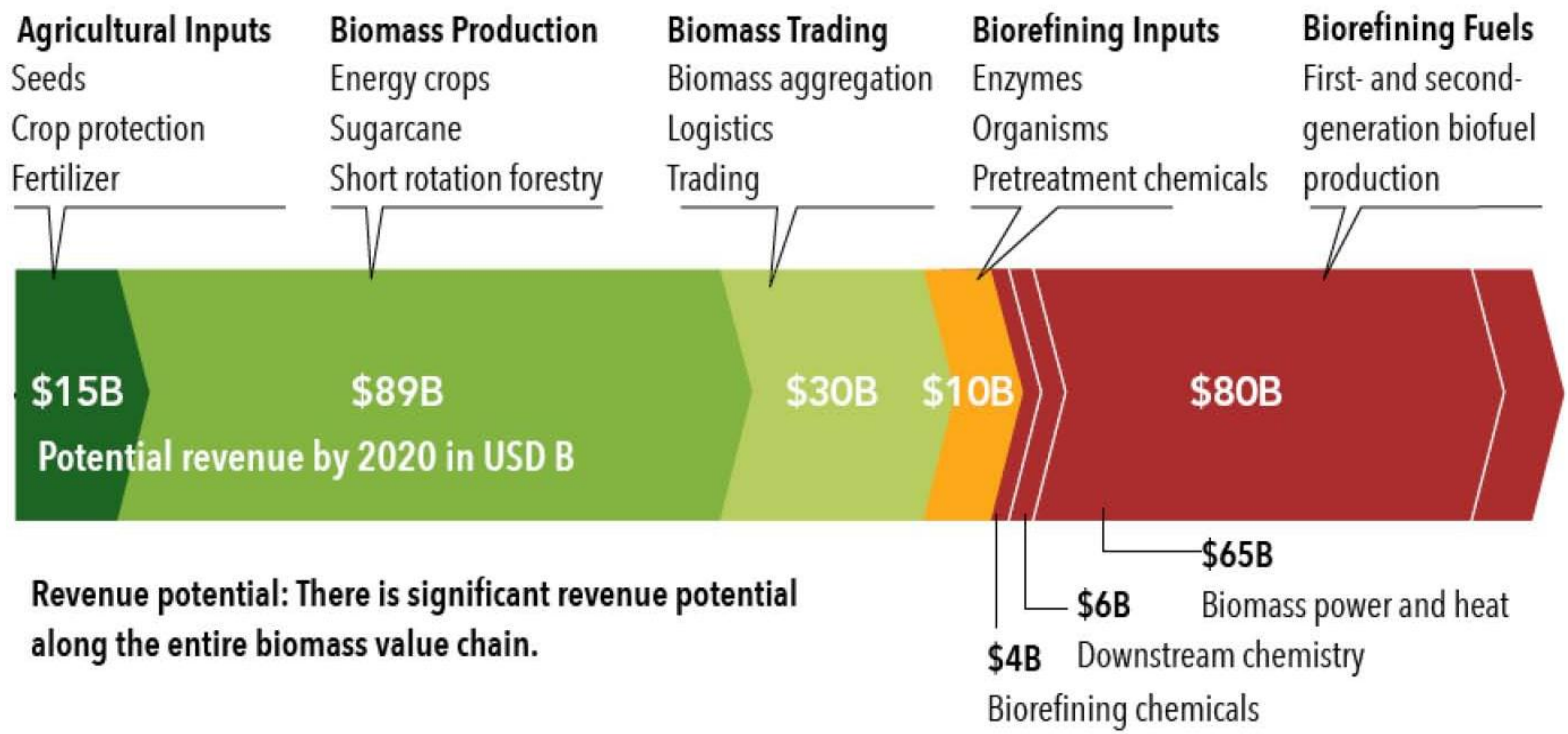
SUNKAT DEVELOPMENT CORPORATION

bp

Chevron Human Energy

DUPONT The miracles of science

The Quest for Sustainable and Stable Alternative Energy Sources

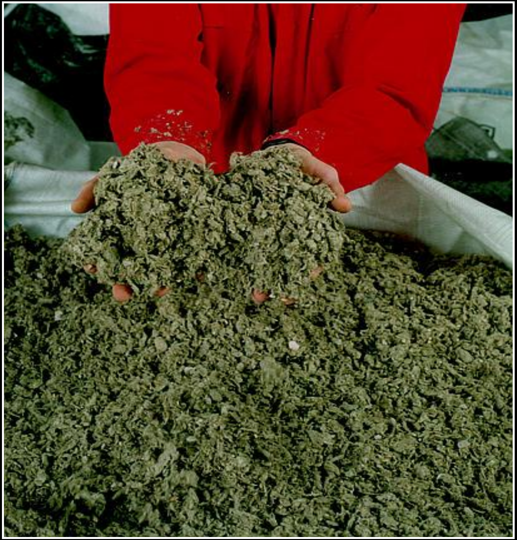


Source: World Economic Forum and Burrill and Co.

Abundant and Renewable Biomass:
An Under-Utilized Substrate for Bio-Based Industrial Feedstocks and Energy





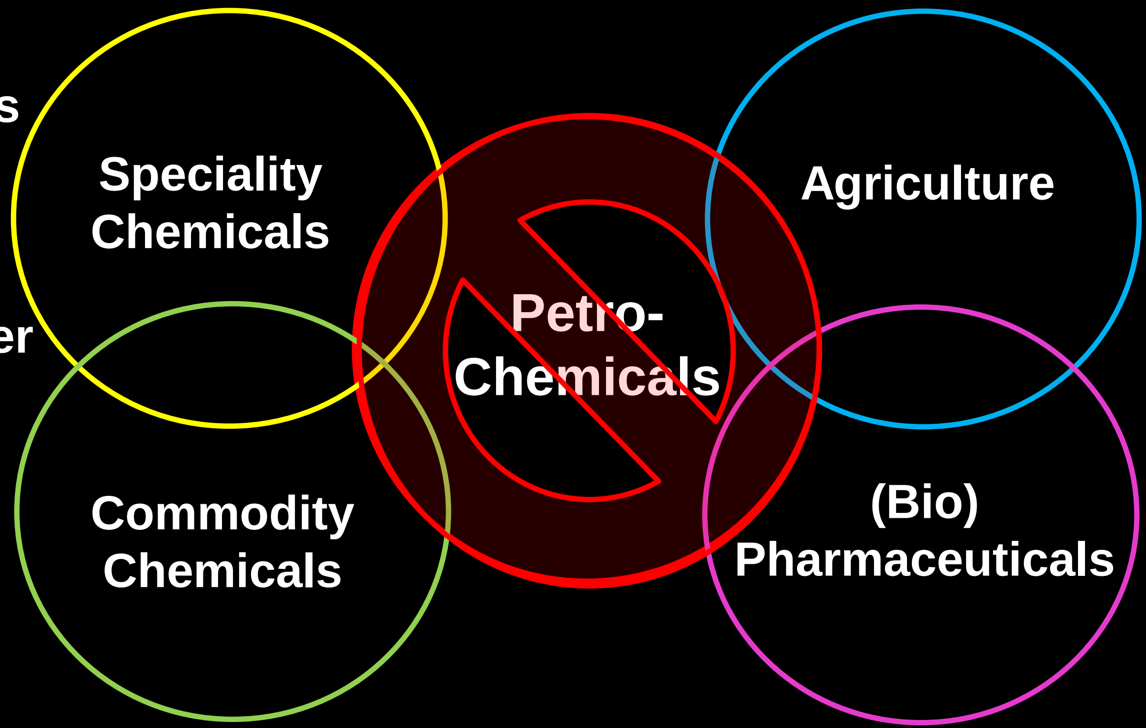



600 million wet tons/year (US)

Synthetic Biology and Bio-Based Manufacturing With Non-Petrochemical Feedstocks

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

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



- crop yield, protection and tolerance
- bioremediation

- complex macrocyclics/ intermediates
- vaccines

$1.5 trillion global industry: 70,000 products

Synthetic Biology and Engineering Enhanced Traits in Food, Feed and Fiber Products





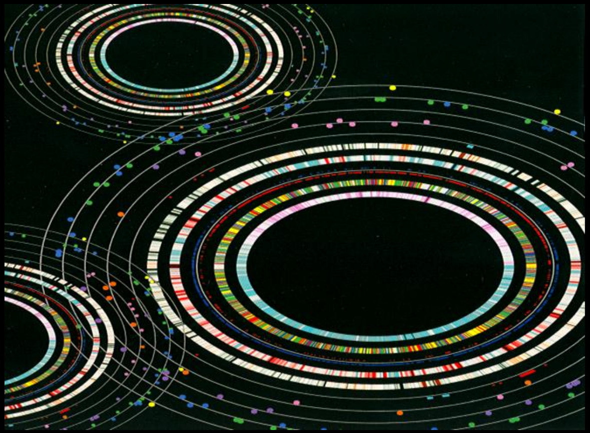


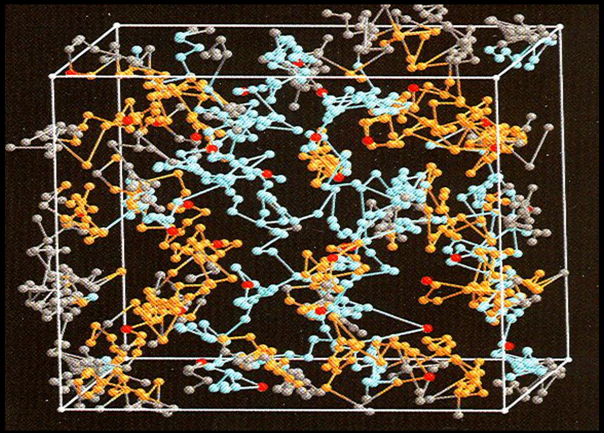

Not Immediately Available!

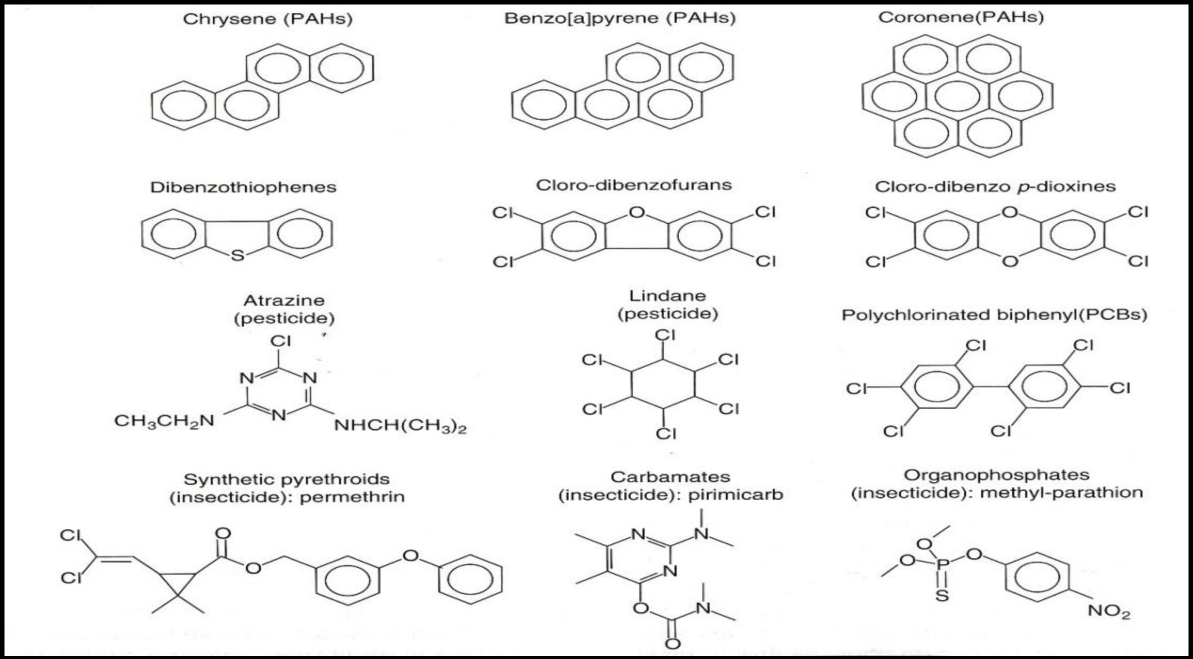
Site: geekologie.com

Source: Wired 10-10

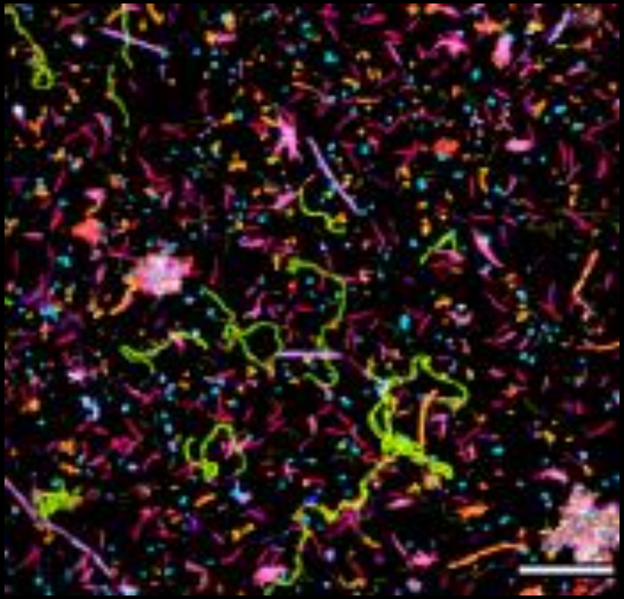
Microbial Genomics and Synthetic Biology: New Technology Platforms for Bioremediation and Improved Efficiency of Wastestream Management





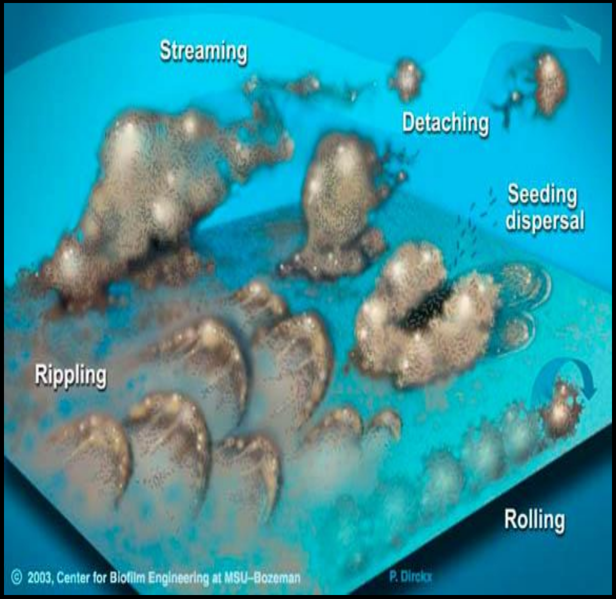


Synthetic Biology and Integration With New Insights Into Microbial Community Dynamics



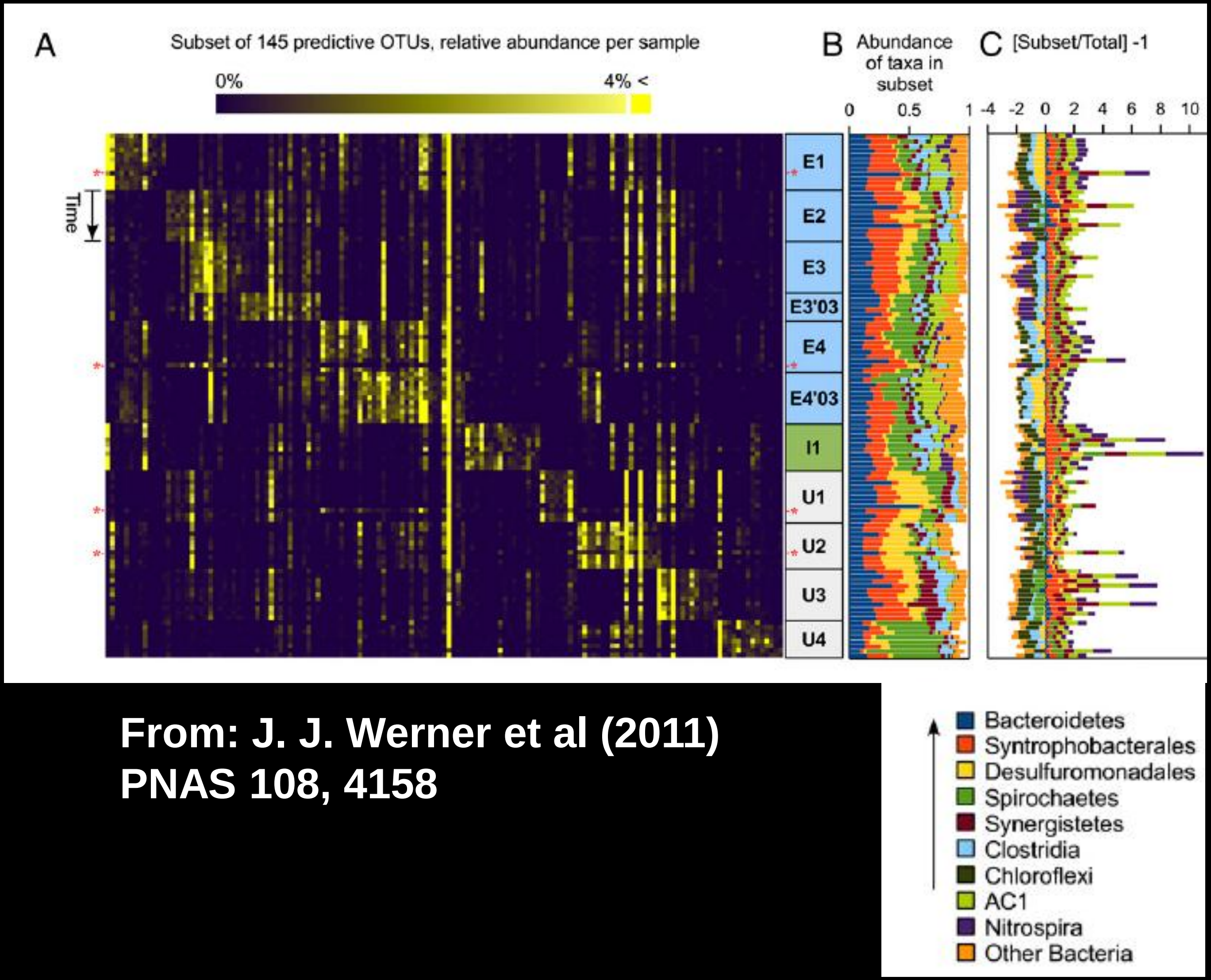
Role of Mixed Communities in Bioprocesses and Bioremediation

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



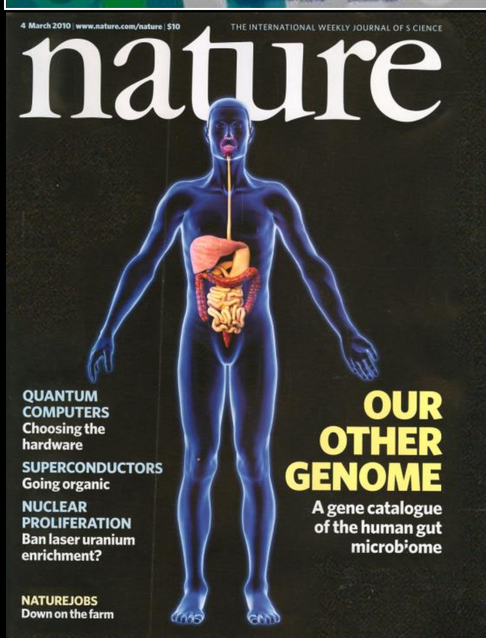
From: J. J. Werner et al (2011) PNAS 108, 4158

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	Value of Biofilm Disruption and Prevention
• bioremediation of water sources and wastestreams • bioreactor performance optimization with mixed microbial species • microbial fuel cells • biosensors and Bio-MEMS	• 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

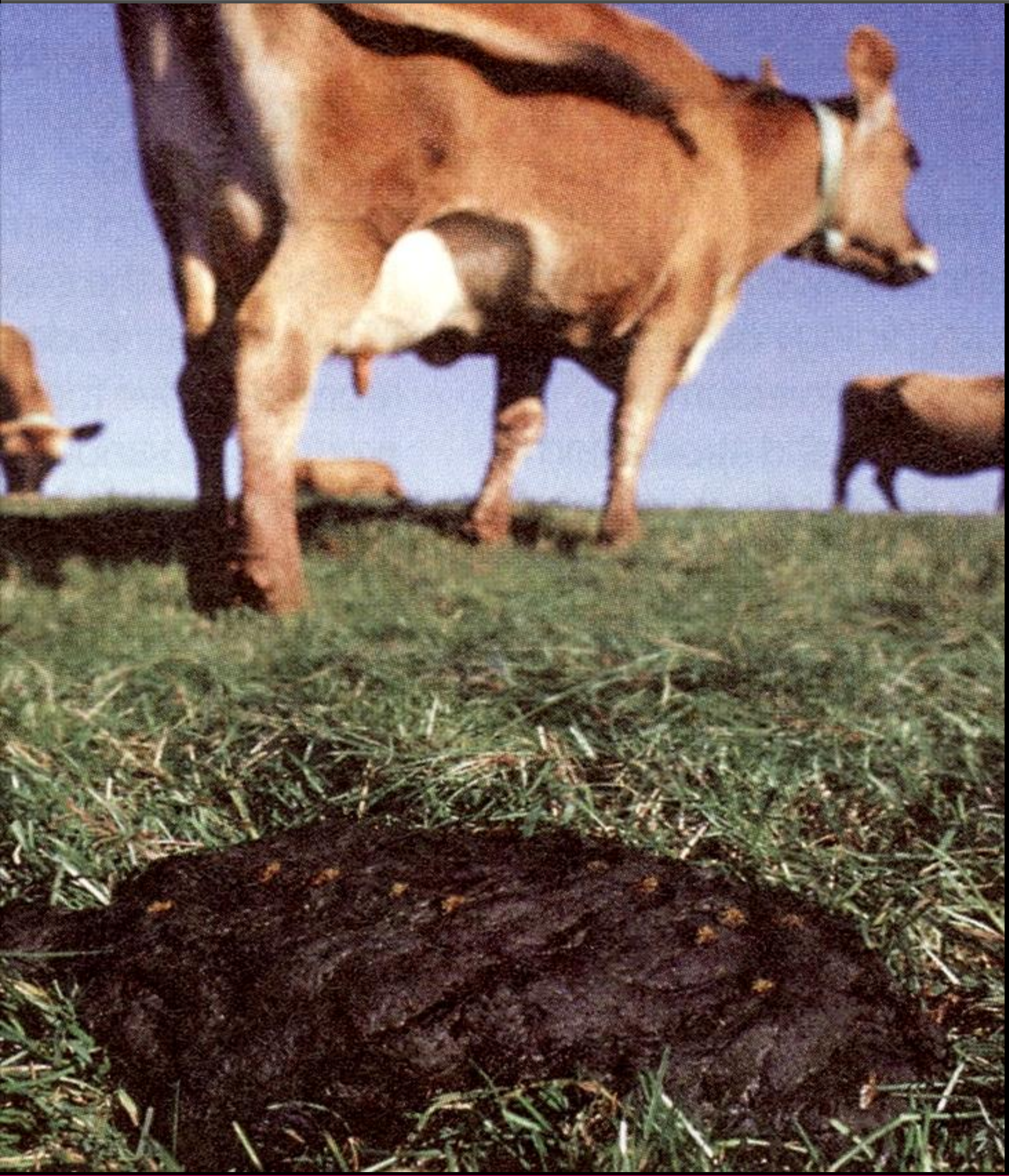
We Are Not Alone: The Human Microbiome – A Barely Understood Factor in Human Health and Disease



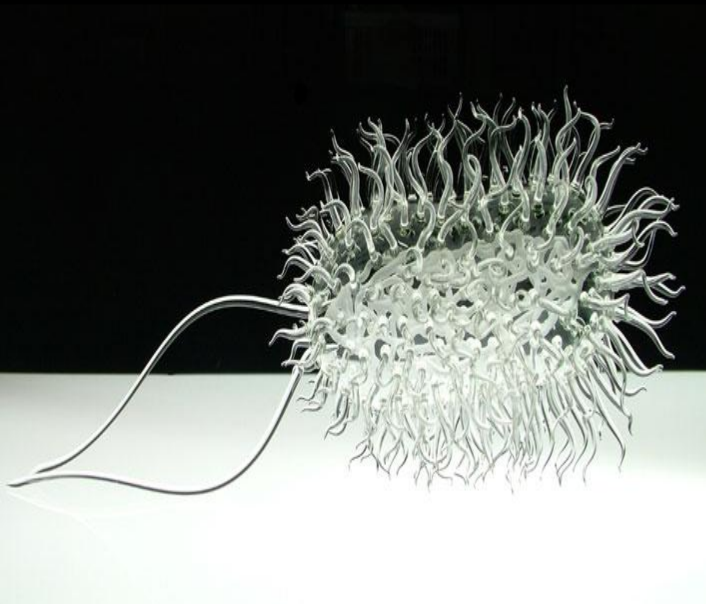
- human body contains 10x more bacterial cells than human cells
- complex meta-system
  - host, microbes, viruses, other organisms, metabolites, xenobiotics
  - is there a core microbiome?
  - how do perturbations affect disease and vice-versa?
  - modulating the microbiome for improved health/disease Rx

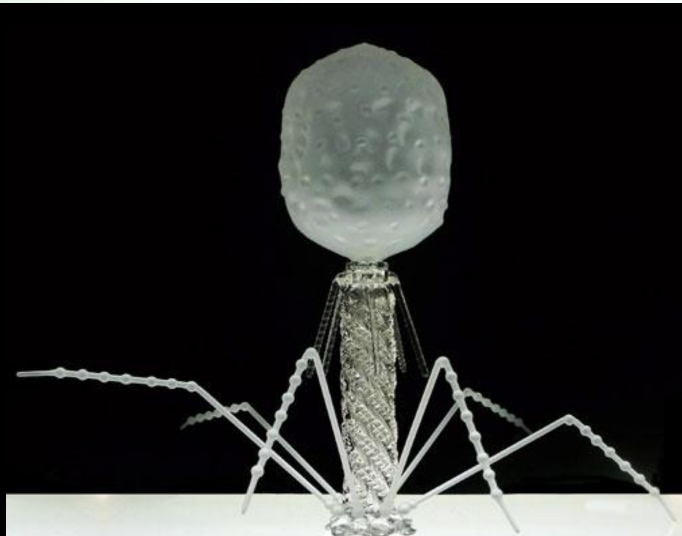

Supplement to PNAS
March 15, 2011 | vol. 108 | suppl. 1 | pp. 4513–4696
Proceedings of the National Academy of Sciences of the United States of America
www.pnas.org
Microbes and Health
Arthur M. Sackler Colloquium
of the National Academy of Sciences

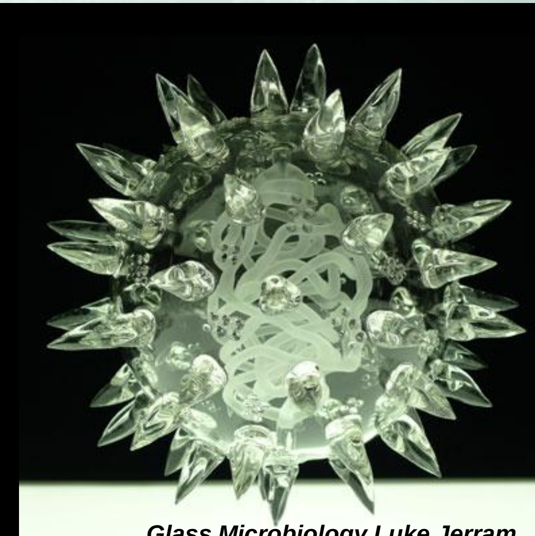
Modification of GI Microbiota for Enhanced Food Conversion Efficiency in Livestock



Engineered Microorganisms as Health Status Sentinels and Therapeutic Delivery Systems







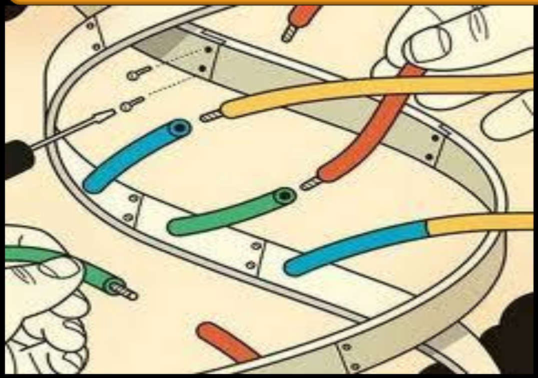
Glass Microbiology Luke Jerram

Decorating Microbes: Engineered Surface Display of Proteins

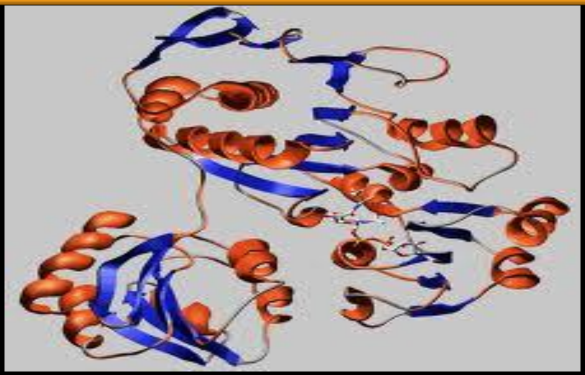
- optimized performance of bioreactor/ bioremediation microbes
- programmable systems for targeted drug delivery
- membrane insertion or C-or N- terminal domain coupling to OMPs, adhesins, virulence factors, autotransporters, ice nucleation proteins
- scaffolds for enzymes/antigens/other proteins that 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



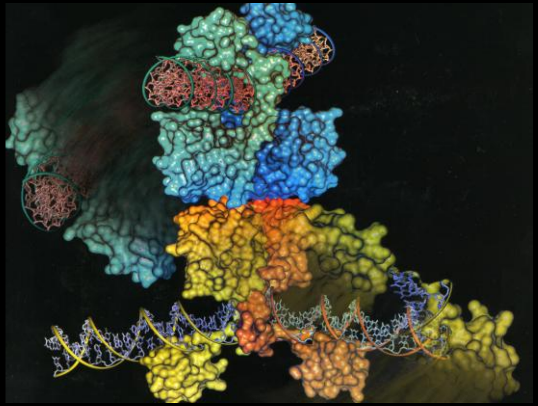
nucleotides



D-amino acids

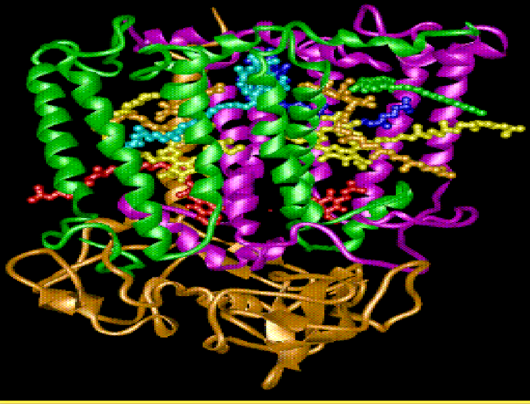
tRNA/ribosome engineering

Directed Evolution



novel genetic sequences and transcription factors

RNA/protein/peptide variants

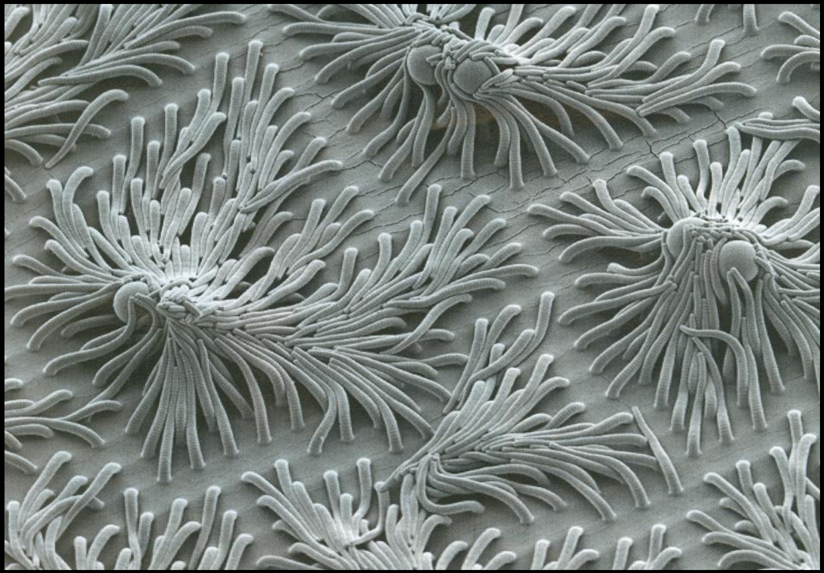


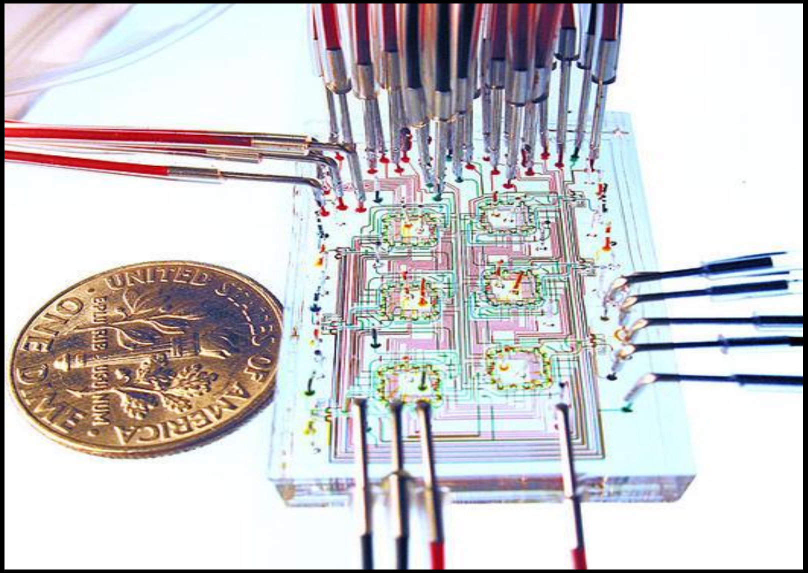
novel tertiary structures/ folds/motifs

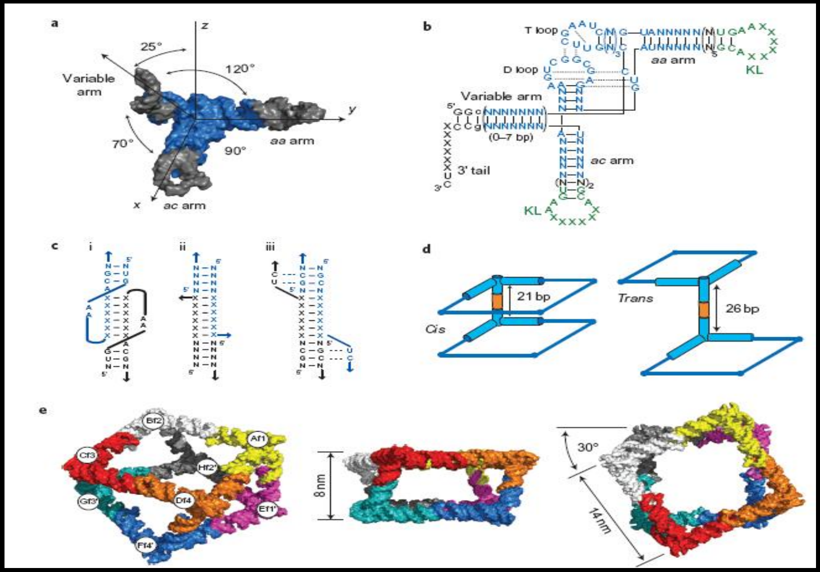
Synthetic Biology: Expanding Molecular Structure:Function "Space"

- 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





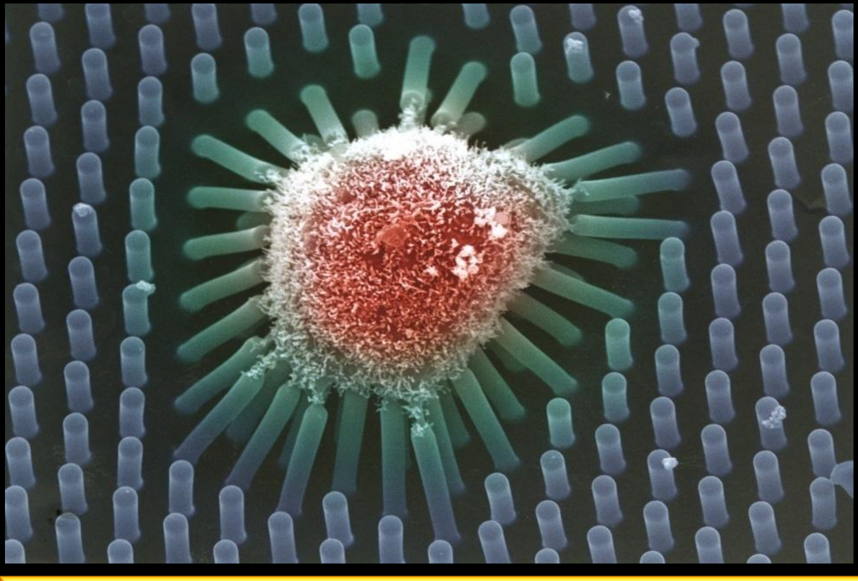


Directed Molecular Assembly and Construction of Novel Materials

- 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**

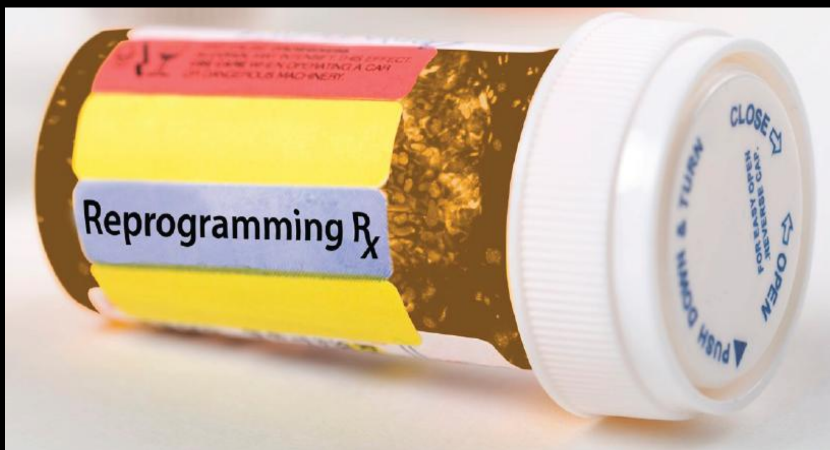


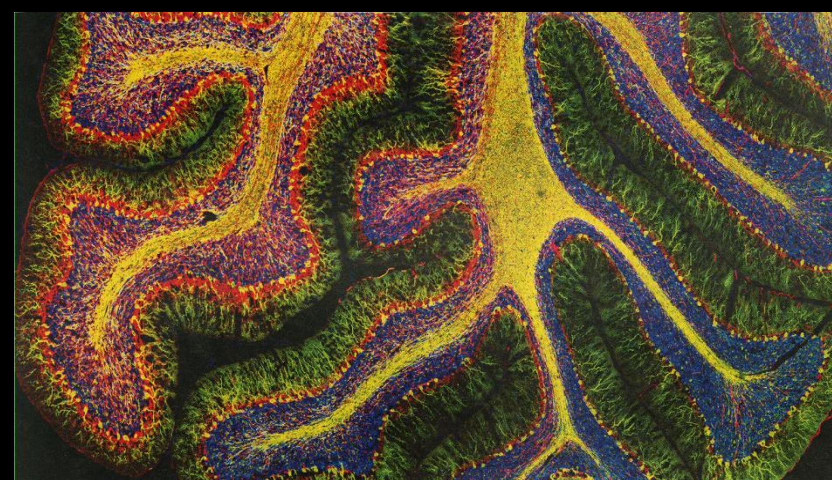
Modulated Elastomeric Substrates
J. Fu et. al. (2010) Nature Methods 7, 733

Directed Cell Lineage Pathways and Cell Fate Decision Engineering in ESCs or iPSCs

Cell Therapies and Regenerative Medicine: Replacement, Repair and Regeneration

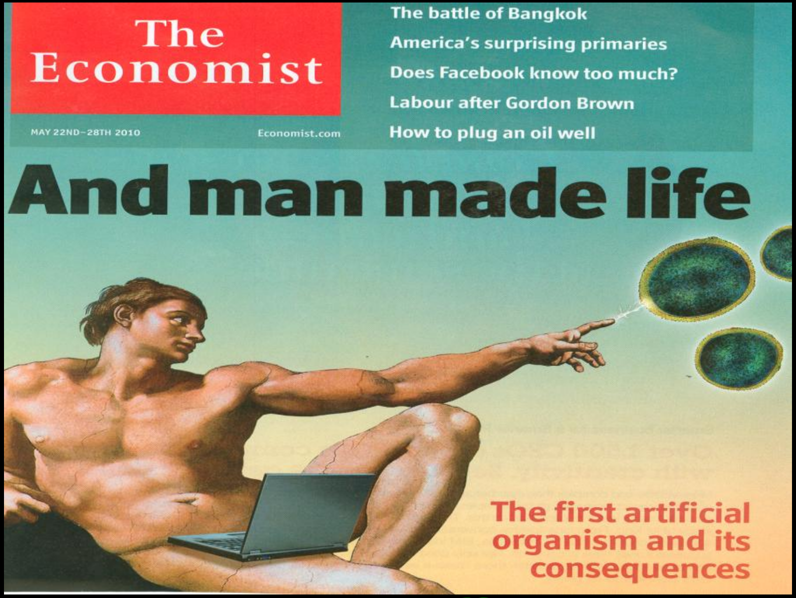
Reprogramming Rx with ESCs/iPSCs to Generate Specific Cell Lineages as Committed Precursors or End-Stage Differentiated Cells

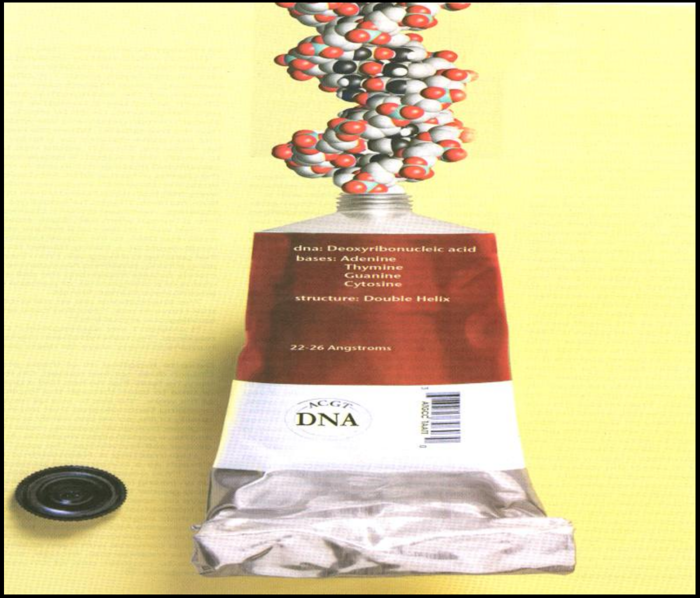


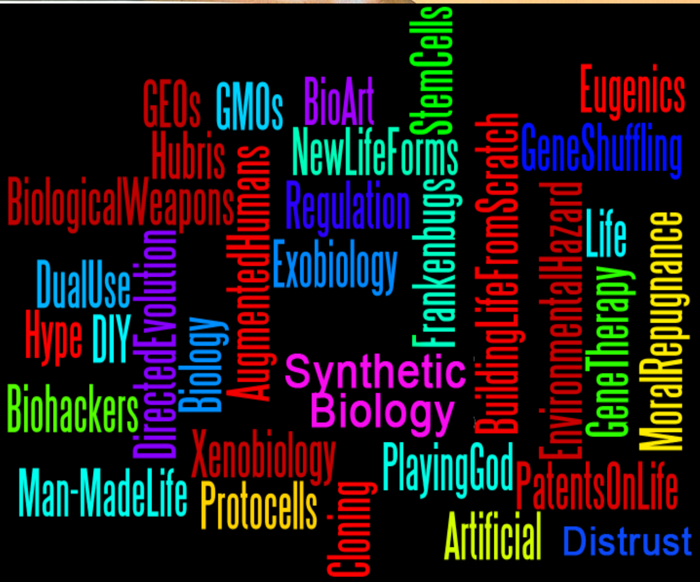


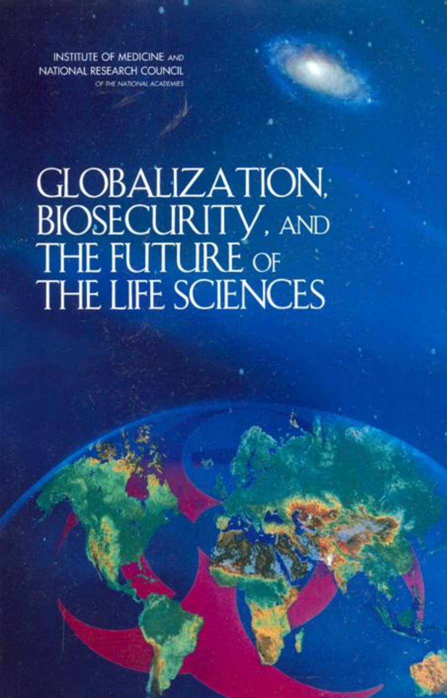
(Re)Building Complex Histiotypic Structures with Full Homeostatic Controls

Synthetic Biology: Policy Issues

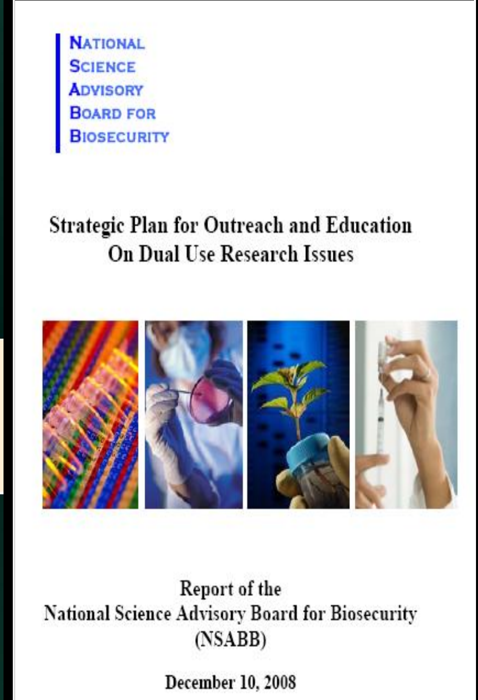


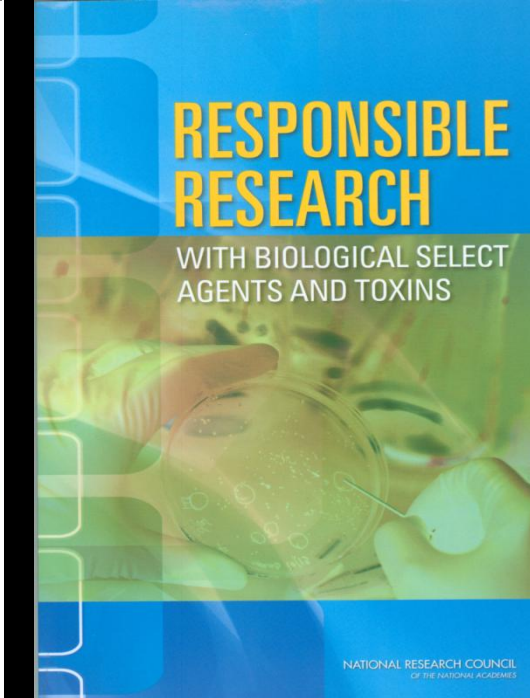




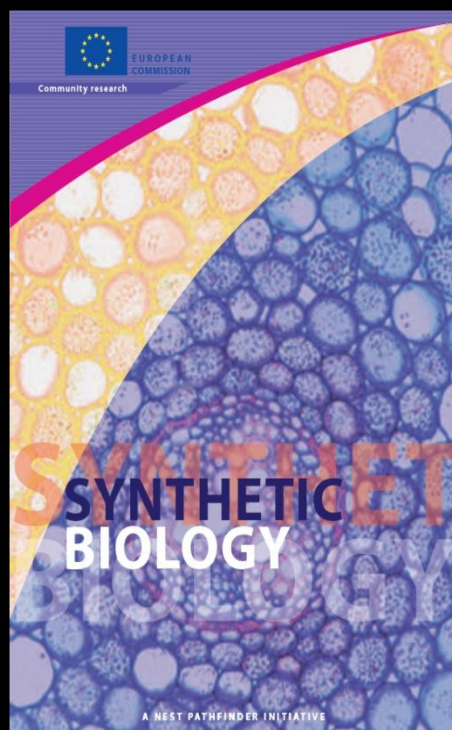

INSTITUTE OF MEDICINE AND
NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES
GLOBALIZATION, BIOSECURITY, AND THE FUTURE OF THE LIFE SCIENCES


THE ROYAL SOCIETY
CELEBRATING 350 YEARS
New approaches to biological risk assessment
Science Policy Centre
INTERNATIONAL WORKSHOP
web royalsociety.org/policy
twenty ten and beyond | 350 years of excellence in science

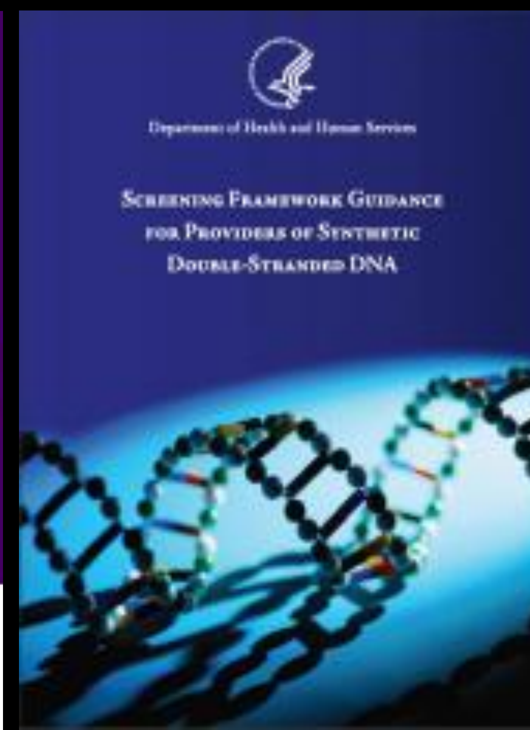

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY
Strategic Plan for Outreach and Education On Dual Use Research Issues
Report of the National Science Advisory Board for Biosecurity (NSABB)
December 10, 2008


RESPONSIBLE RESEARCH
WITH BIOLOGICAL SELECT AGENTS AND TOXINS
NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES


THE ROYAL SOCIETY
CELEBRATING 350 YEARS
Synthetic biology
2 and 3 June 2008
scientific
DISCUSSION MEETING SUMMARY
web royalsociety.org
twenty ten and beyond | 350 years of excellence in science


EUROPEAN COMMISSION
Community research
SYNTHETIC BIOLOGY
A NEST PATHFINDER INITIATIVE


Parliamentary Office of Science and Technology
postnote
July 2009 Number 340
THE DUAL-USE DILEMMA


Department of Health and Human Services
Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA

EUROPEAN PARLIAMENT

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STOA

Science and Technology Options Assessment

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

MAKING PERFECT LIFE

European Academies
easac
Science Advisory Council

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

EASAC policy report 13

December 2010

ISBN: 978-3-8047-2866-0

This report can be found at www.easac.eu

building science into EU policy

NUFFIELD COUNCIL ON BIOETHICS

Emerging biotechnologies

CONSULTATION PAPER

April 2011

Oversight of Synthetic Biology: Risk, Regulation and Responsibility

Biosafety: Risk from Legitimate R&D/Industrialization

Biosecurity: Deliberate Use to Cause Harm

Biohackers and Democratization of New Technology

IGSC international gene synthesis consortium

IASB International Association Synthetic Biology

Screening of Purchases/ Supply Transactions

Regulation, Legislation and Codes of Conduct

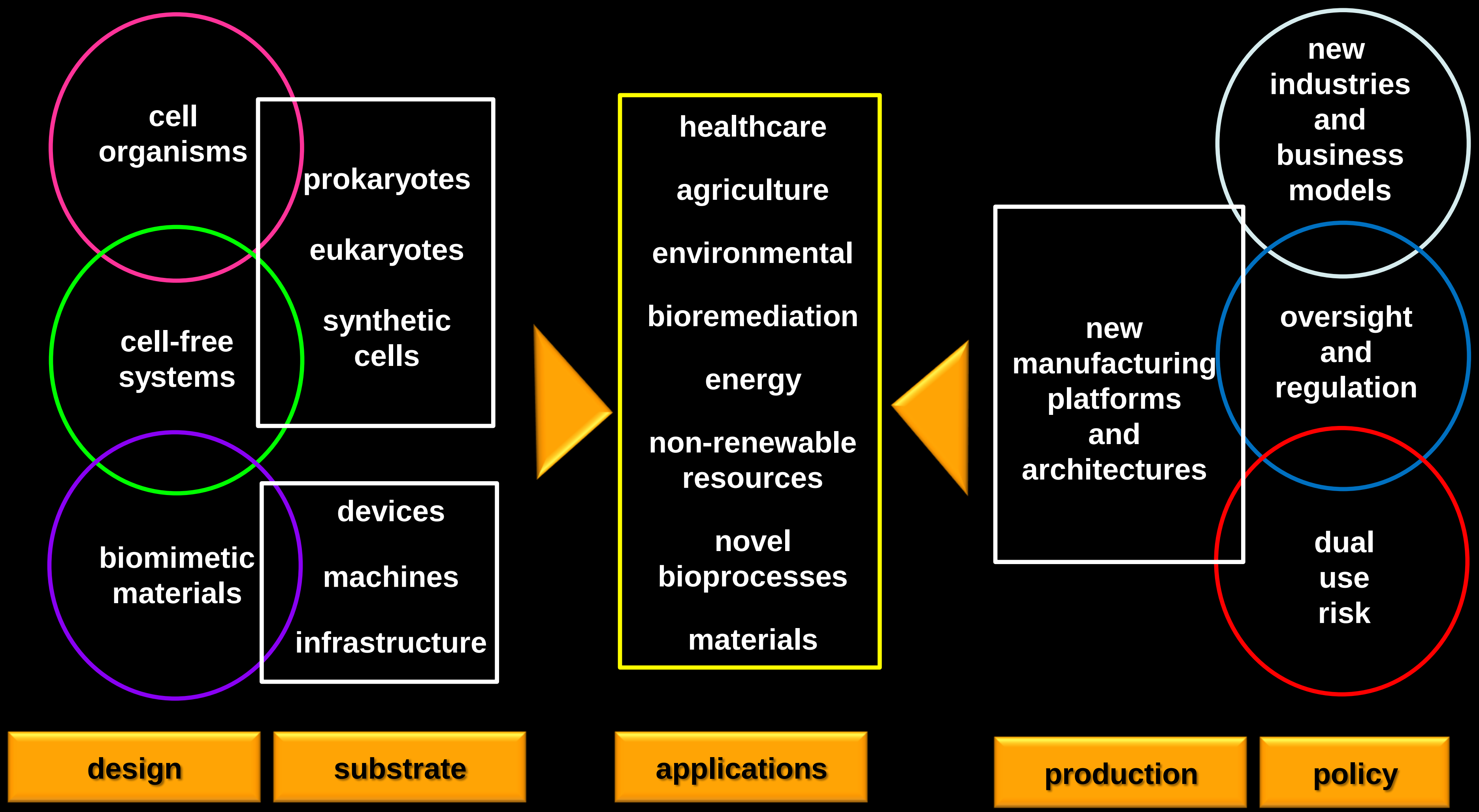
International Harmonization

Synthetic Biology

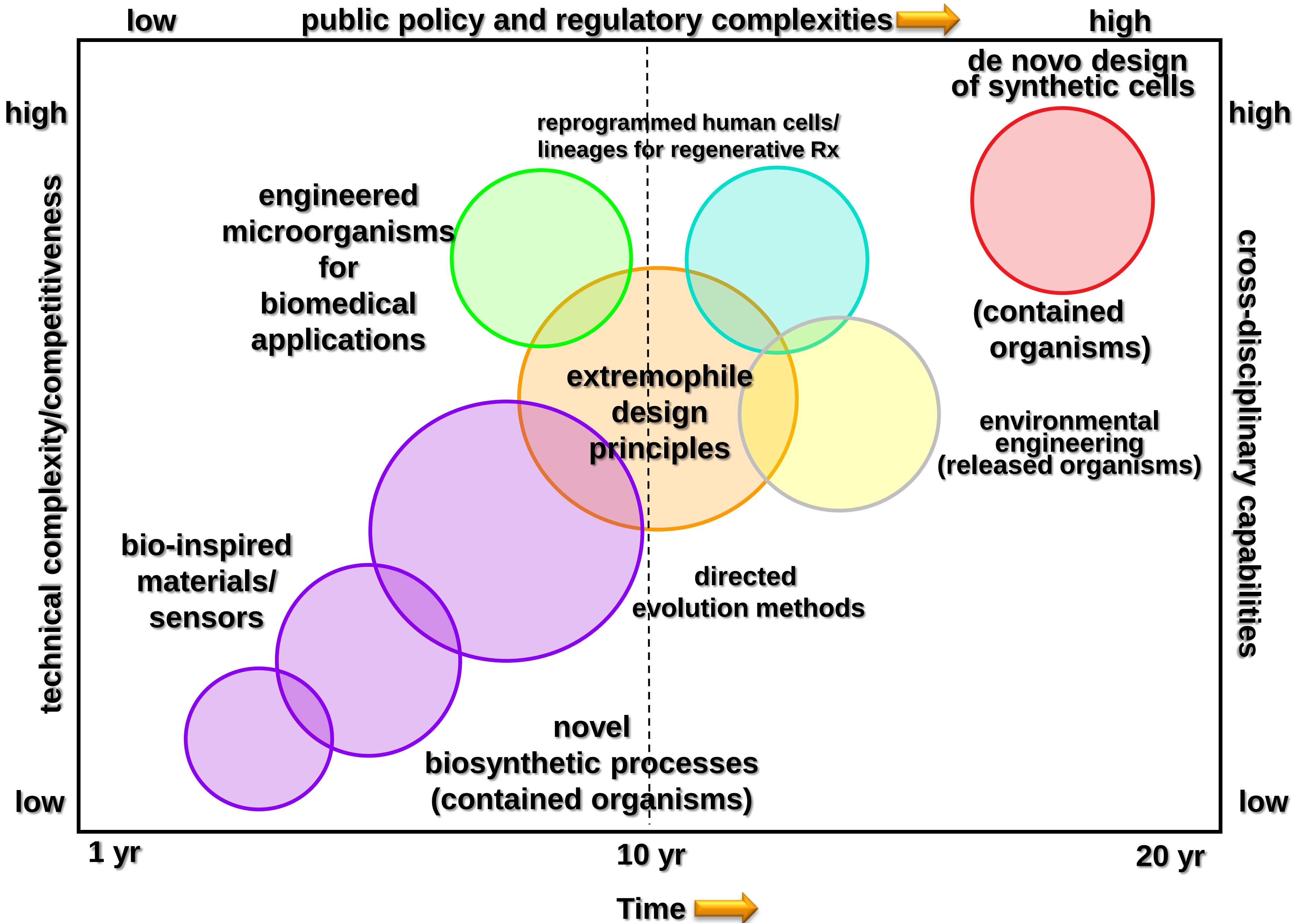
Next Era in the Intellectual Transition of the Life Sciences from Phenomenology to the Elucidation of the Design Principles for Complex Biological Systems

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



Projected Trajectories for Biosystems Engineering Using Synthetic Biology



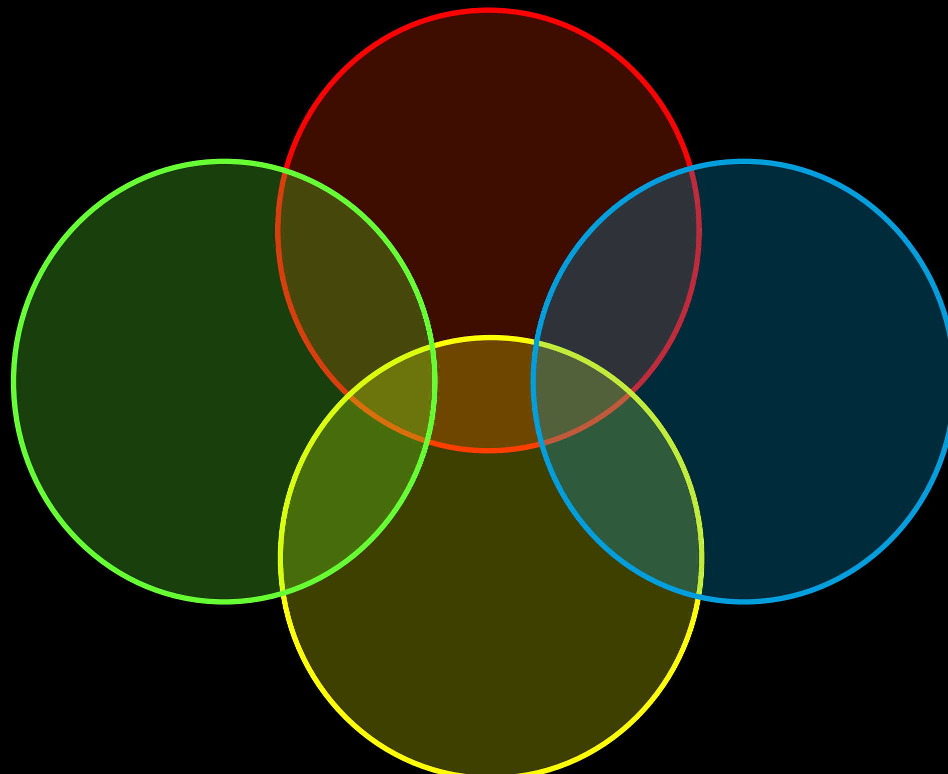
Mapping the Information Content and Design Rules for Biological Organization and Future Designer ‘Biospace’

Design Space

systems and synthetic biology

metagenomics
phylogenomics

Coding Space



ecosystems
biogeochemistry
astrobiology/
exobiology

Diversity Space

chemical biology
orthogonal components
xenosystems

Unexplored Biospace

Reframing the Challenge of "Managing the (Global) Commons"
The Imperative to Understand Complex Adaptive Systems

	Distribution/Control of Resources	Finite/Contracting Resources	Expanding Resources
Resources	• access • allocation • utilization	• biodiversity • non-renewable resources • unconstrained costs	• 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)

Synthetic Biology

Progress in Systems and Synthetic Biology Will Depend on New Trans-Disciplinary Knowledge Networks

The Rise of Synthetic Biology and Bio-inspired Engineering Will Require Major Changes in the Organization and Funding of Academic Research, Education and Training and Catalyze New Disruptive Business Models