A perspective on Synthetic Biology

Vincent Rouilly

MRes, December 2009

Next-Generation Sequencing

SOLiD 3 Plus – a Systems Biology Tool

Michael Rhodes, Ph.D., Senior Manager Sequencing Portfolio, Applied Biosystems

Friday December 4th 2009

13:00 – 14:00h

Imperial College, South Kensington Campus

Sir Alexander Fleming Building, 119 Seminar Room

Biotechnologies: a definition

- "Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."

  United Nations Convention on Biological Diversity

The Biotechnology Landscape

- History
- Area of applications
- Technology toolbox
- Economy
- IP / Patent System
- Ethics and Social implications

Biotechnology Chronology

- Ancient time
- Age of Science
- Molecular biology era
- Genetic engineering era
- Genomics era
- Post-Genomics era

Biotech Companies / Market

MONSANTO

$ 11 billions

Genentech

$ 9.2 billions

AMGEN

$ 14.2 billions

genzyme

$ 3.1 billions

2% of US GDP (+20% year) -
Rob Carlson

Biotechnologies: technologies

- **Enabling technologies**
  - Recombinant DNA
  - DNA sequencing
  - DNA synthesis
  - High throughput technologies
  - Computational analysis

Biotechnologies: applications

- **Applications and Successes**
  - Biofuels
  - Biomaterials
  - Biosensing
  - Therapeutics
  - Bioremediation
  - Plant engineering

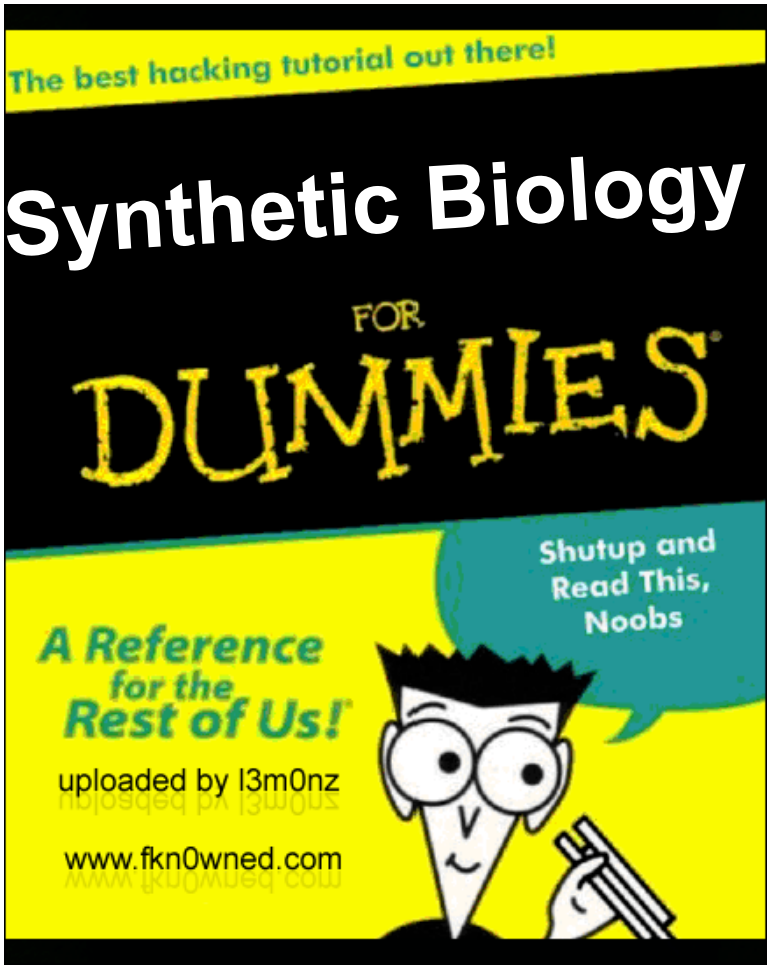
A Diverse Biotech Community

- Cellular Biologists
- Molecular Biologists
- Metabolic Engineers
- Protein Engineers
- Systems Biologists

Synthetic Biology

- How does Synthetic Biology fit into this **existing Biotech landscape** ?

Synthetic Biology Textbook



What would you expect to find inside ?

Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Foundations for Synthetic Biology

Vincent Rouilly
Bioengineering Department
Imperial College London

Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Standard for Physical DNA Composition

Vincent Rouilly
Bioengineering Department
Imperial College London

Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Standards for Functional Composition

Vincent Rouilly
Bioengineering Department
Imperial College London

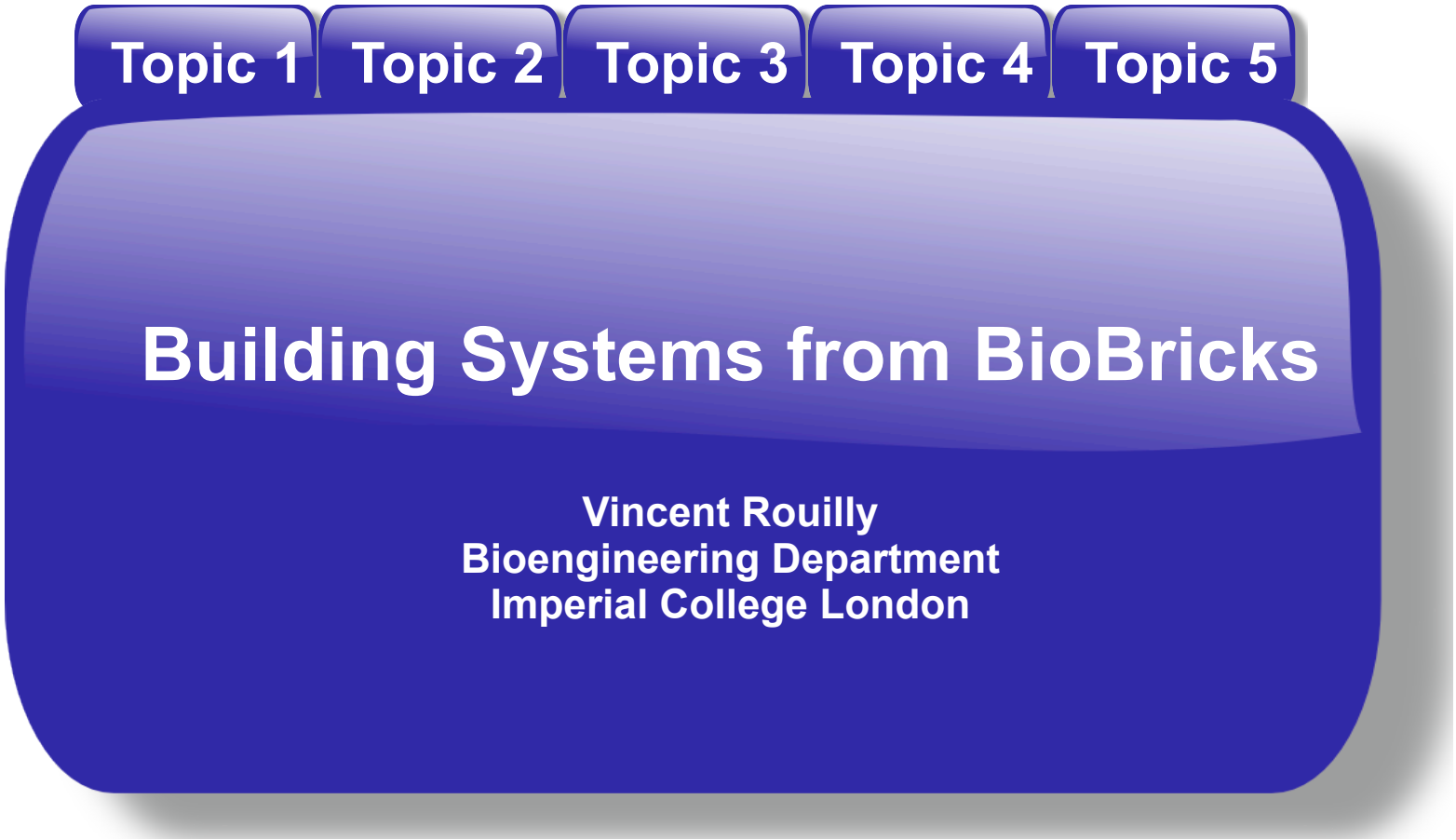
Introduction to Synthetic Biology

Topic 1 | Topic 2 | Topic 3 | Topic 4 | Topic 5

Characterising Biological Parts

Vincent Rouilly
Bioengineering Department
Imperial College London

Introduction to Synthetic Biology



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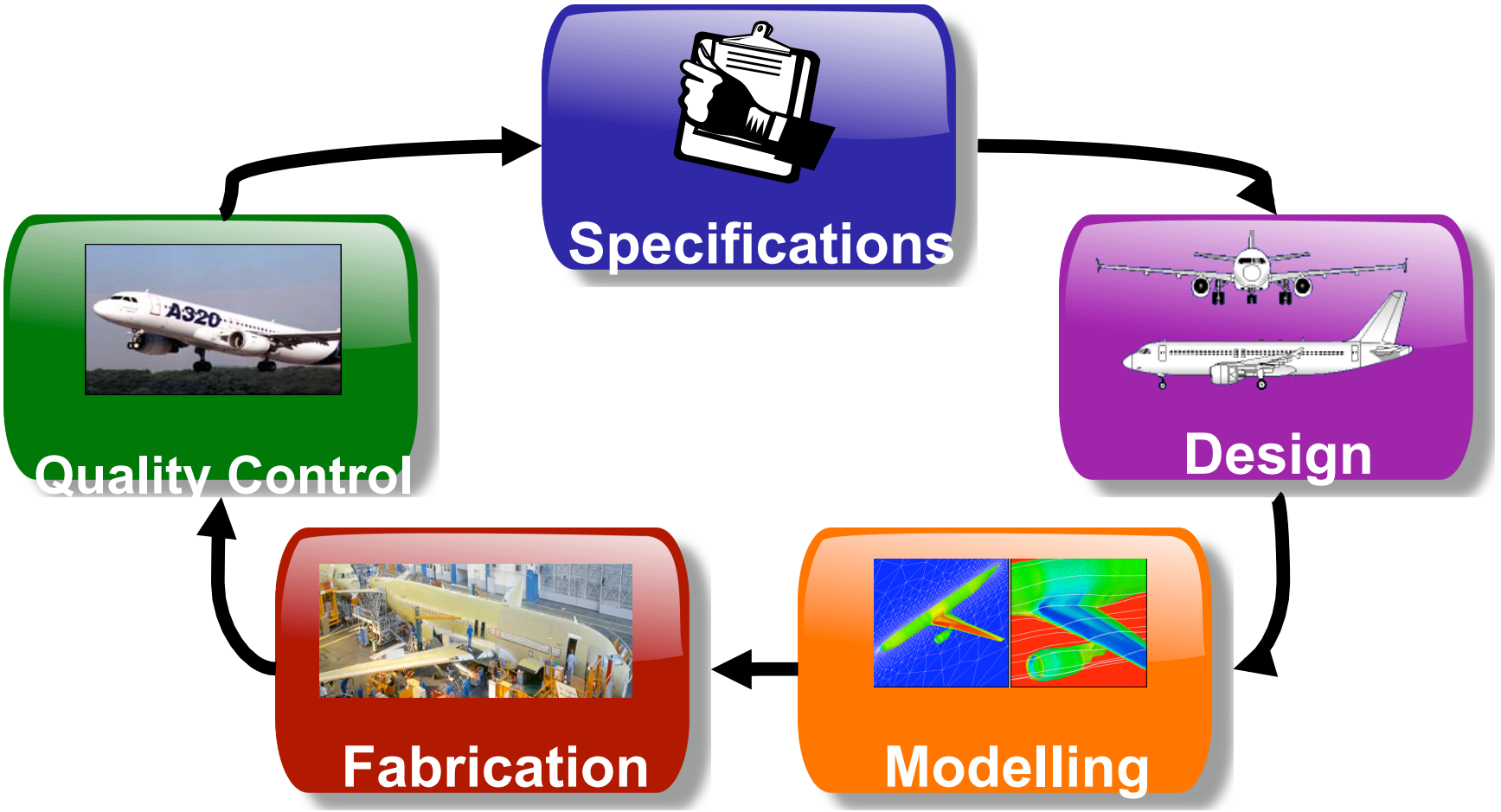
Biotech Innovation Bottlenecks

- Innovation pipeline is slow
- Mass production / scale-up can be an issue
- Similarities with pre-Industrial period
- What made engineering disciplines so successful ?

Synthetic biology success stories

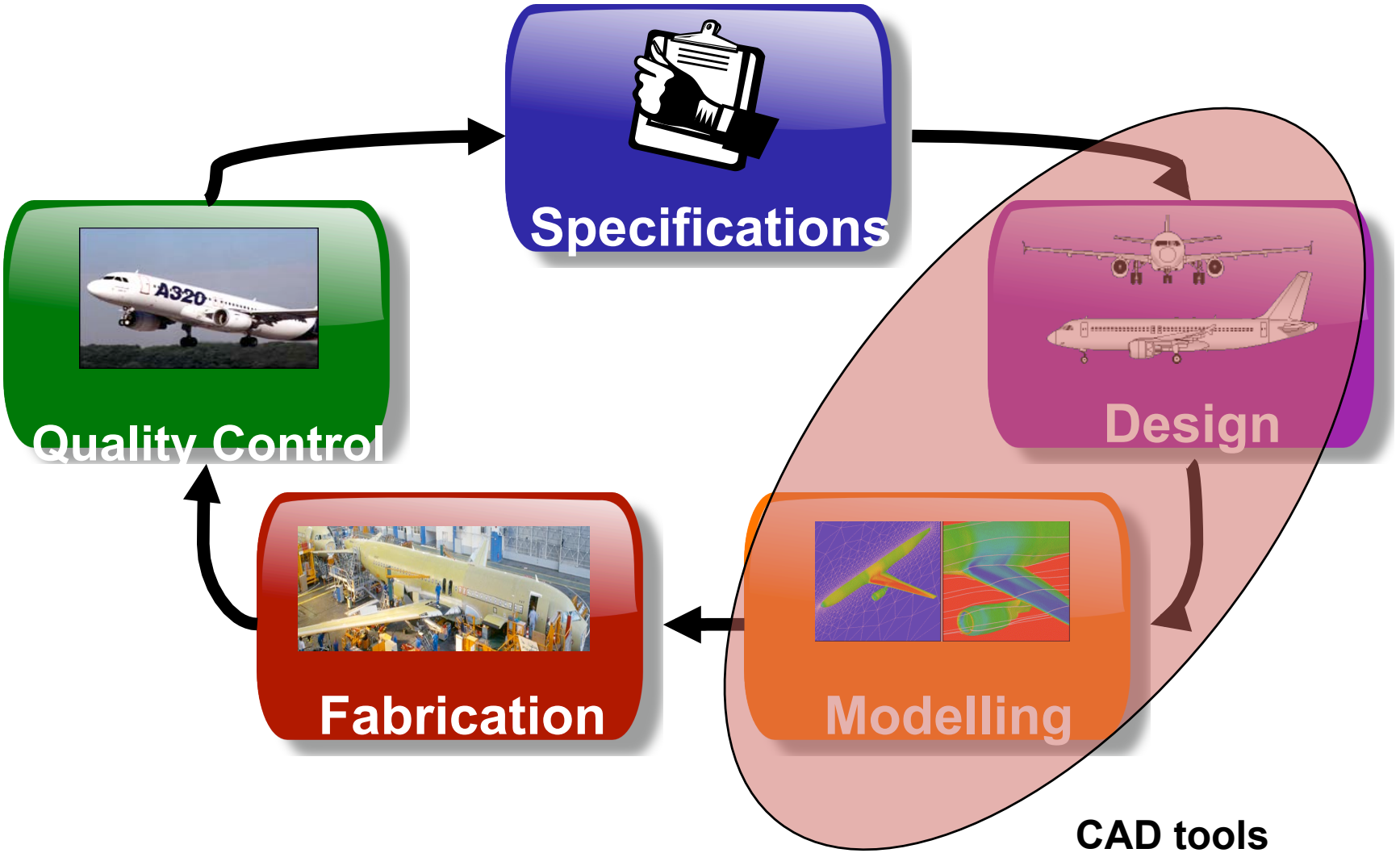
- Seminal papers (Toggle Switch, Repressilator)
- Band-pass detector
- Edge detector
- PoPS amplifier
- Others ?

Current Trends in Synthetic Biology



- Speeding-up the development cycle
  - Building supporting technologies

Current Trends in Synthetic Biology



SynBio CAD Systems

GenoCAD BETA

Clotho

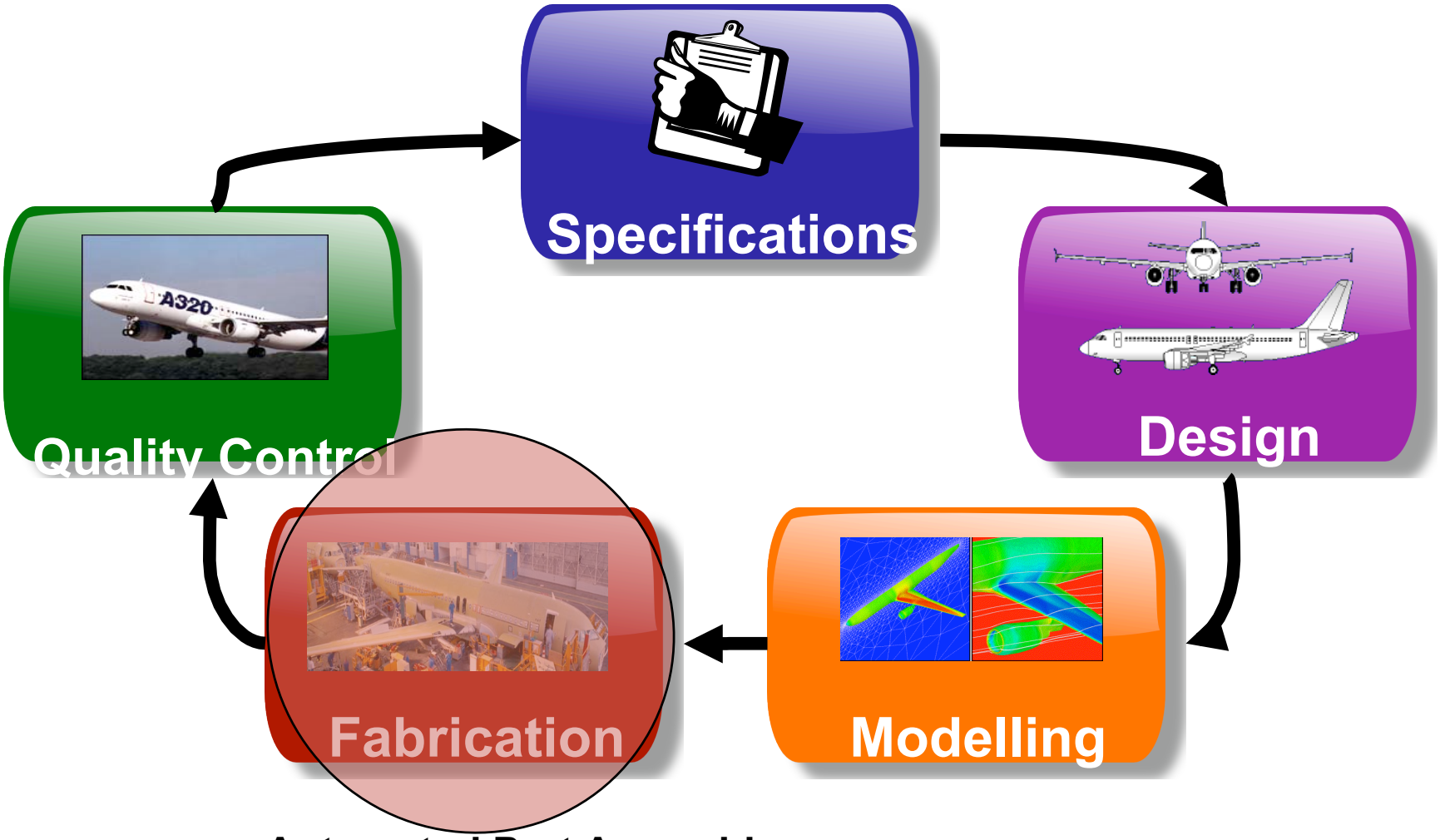
JBEIR

BioJade

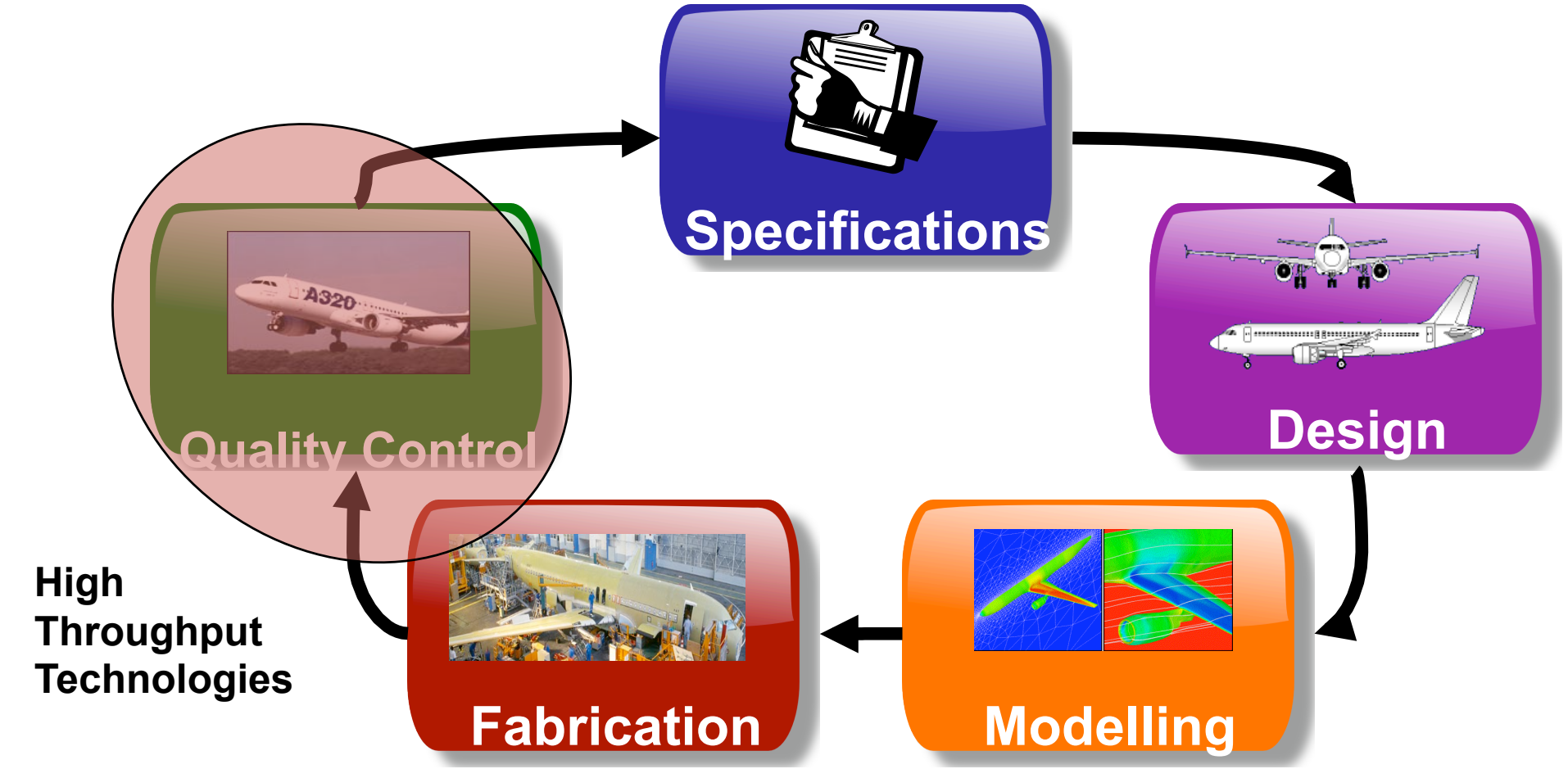
DNA 2.0

GeneDesigner

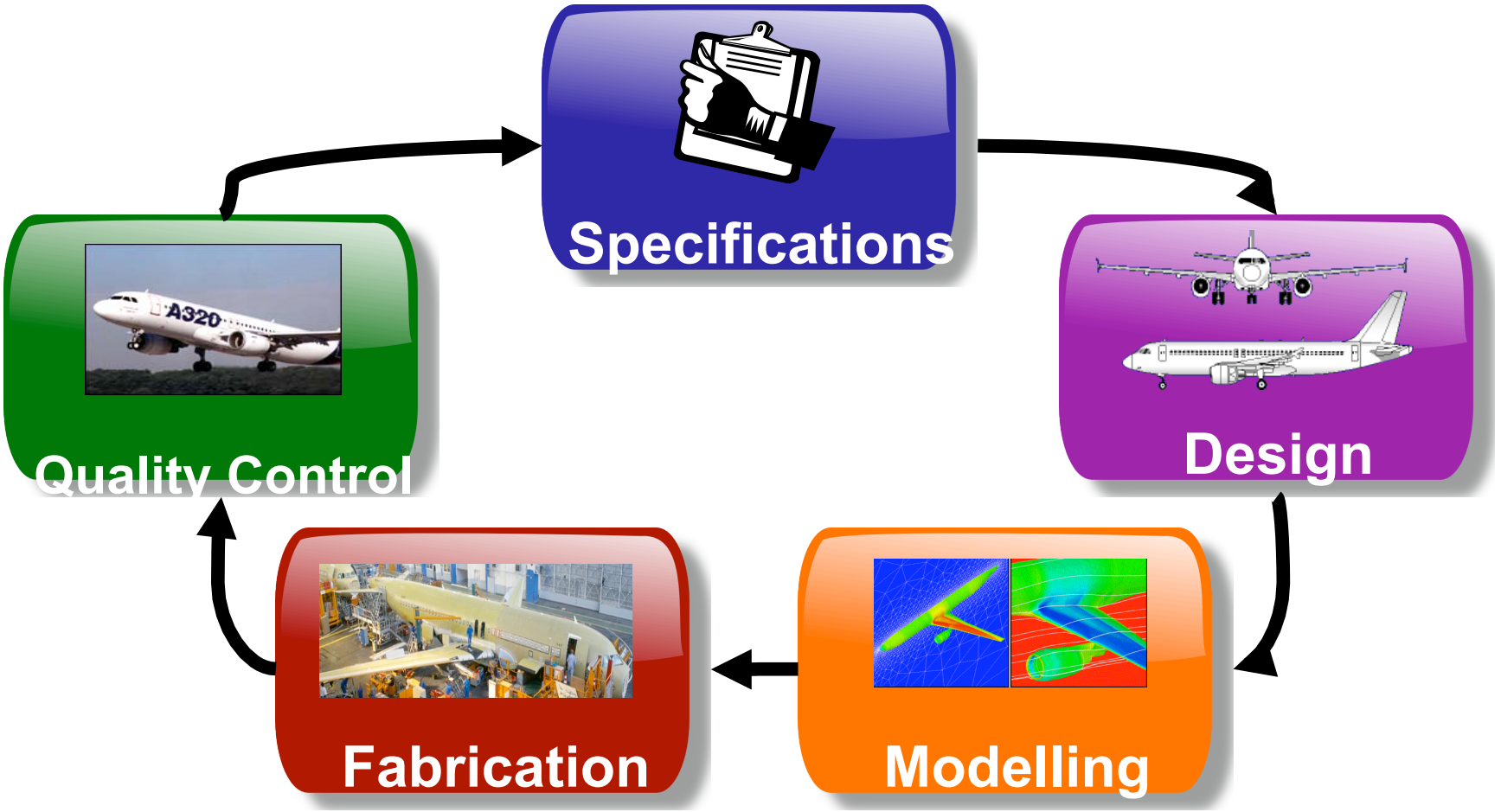
Current Trends in Synthetic Biology



Current Trends in Synthetic Biology



Current SynBio Challenges



No improvement if you can't reuse biological parts / experience
Need for modularity and Decoupling

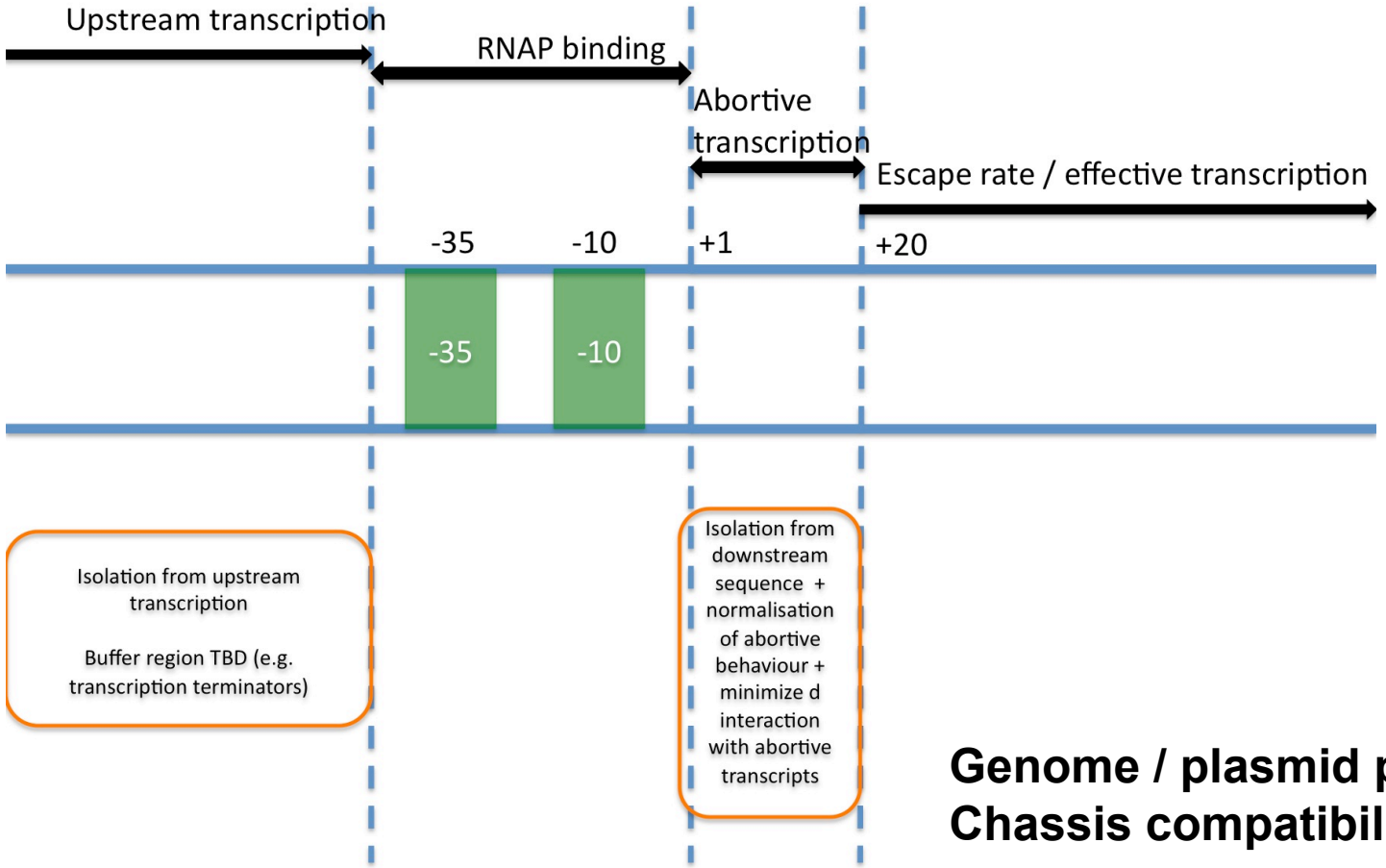
Current SynBio Challenges

- **Rules of composition (modularity)**
  - It is not because you have DNA **BioBricks** that you have a **Modular Biology**
  - Biology appears to be a very **context dependent language**:
    - DNA - RNA - Protein - Networks
    - See: RBS paper, promoter examples
  - Inverter characterisation example
  - Any other problematic composition ?

Composition rules: Promoters

Context dependent Promoter

Transcription initiation model



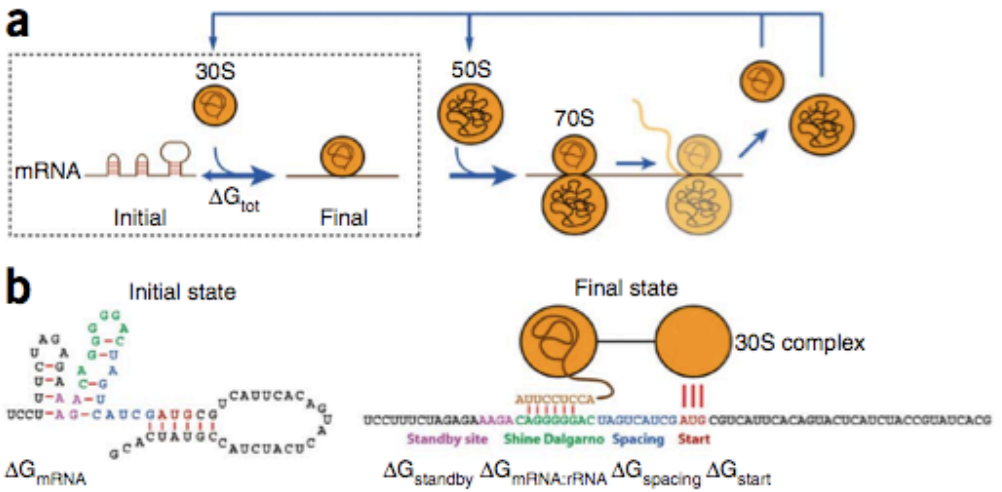
Genome / plasmid position
Chassis compatibility

Context dependent RBS

nature biotechnology

Automated design of synthetic ribosome binding sites to control protein expression

Howard M Salis[1], Ethan A Mirsky[2] & Christopher A Voigt[1]



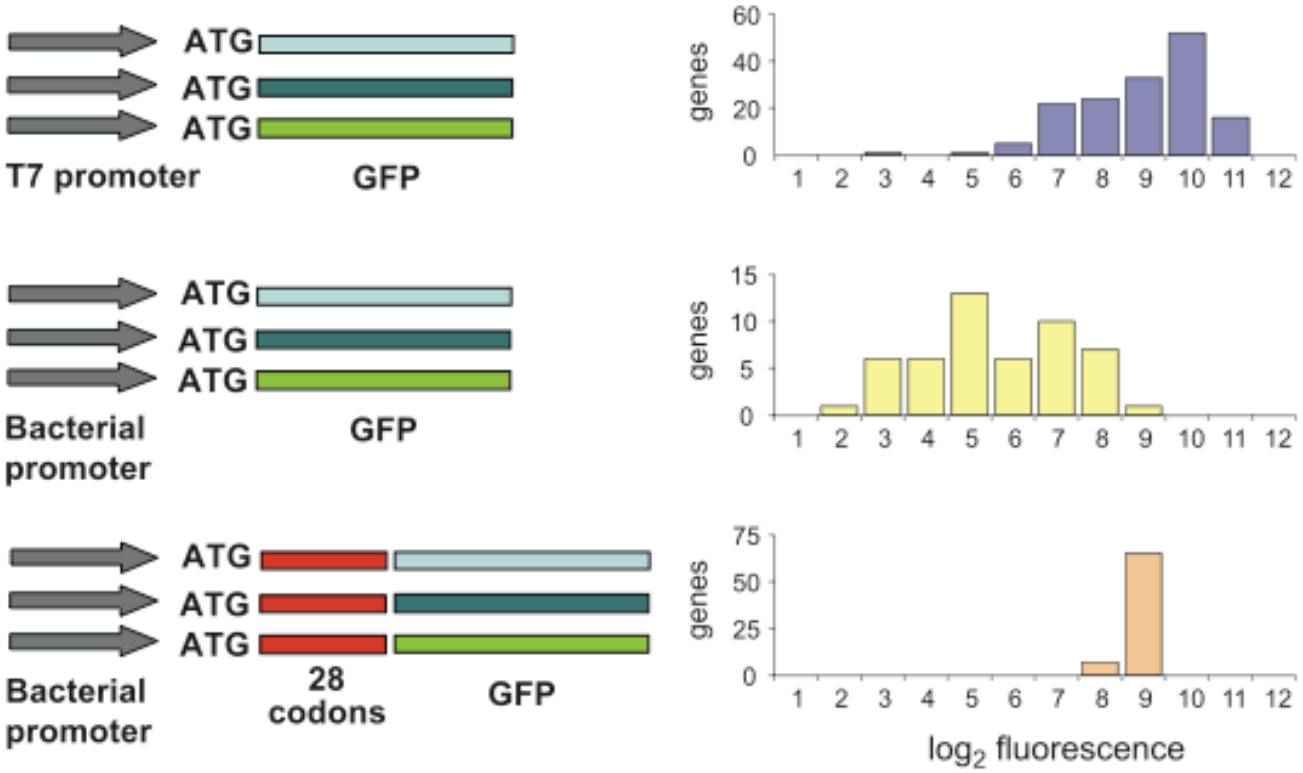
Given a specific mRNA sequence—called the sub-sequence—surrounding a start codon, ΔG_{tot} is predicted according to an energy model (equation (2)), where the reference state is a fully unfolded sub-sequence with G = 0.

$$\Delta G_{tot} = \Delta G_{mRNA:rRNA} + \Delta G_{start} + \Delta G_{spacing} - \Delta G_{standby} - \Delta G_{mRNA} \quad (2)$$

Context dependent CDS

Coding-Sequence Determinants of Gene Expression in *Escherichia coli*

Grzegorz Kudla,[1]* Andrew W. Murray,[2] David Tollervey,[3] Joshua B. Plotkin[1]†



Context dependent CDS (2)

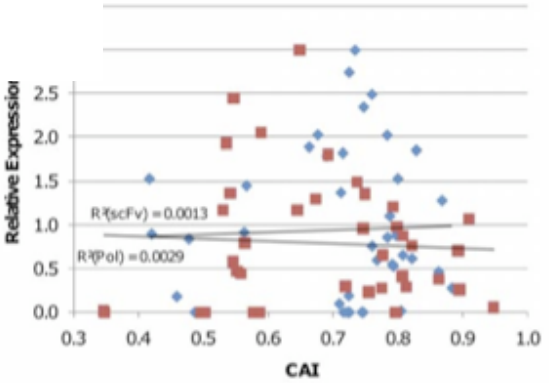
OPEN ACCESS Freely available online

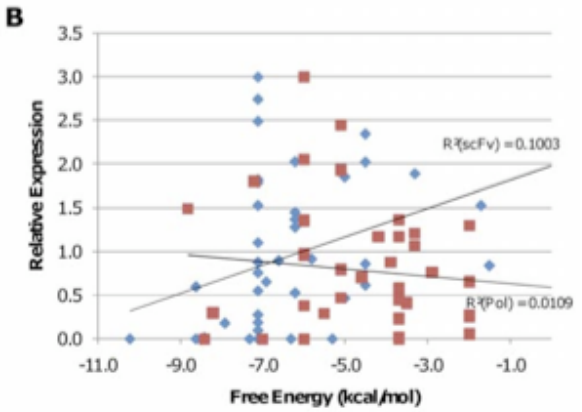
PLoS one

Design Parameters to Control Synthetic Gene Expression in *Escherichia coli*

Mark Welch[1]*, Sridhar Govindarajan[1], Jon E. Ness[1], Alan Villalobos[1], Austin Gurney[2], Jeremy Minshull[1], Claes Gustafsson[1]

1 DNA2.0, Menlo Park, California, United States of America, 2 OncoMed Pharmaceuticals, Inc., Redwood City, California, United States of America





Current SynBio Challenges

- **Measurements (Quality control)**
  - Reliable and reproducible
  - Quantitative Measurements on the Central dogma:
    - DNA - RNA - Protein - Network - Phenotype - Popu
  - **Minimum Information Required** initiative

Part Characterisation

Refinement and standardizatic biological parts and devices

Barry Canton[1,4] Anna Labno[2–4] & Drew Endy[1]

Why does it break at some point ?

BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 19 October 2007

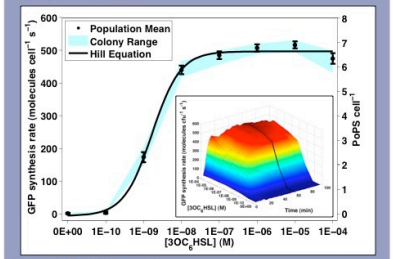
Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC6HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC6HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Parts

BBa_C0062: luxR ORF
BBa_R0040: LuxR-regulated operator
BBa_R0040: TetR-regulated operator

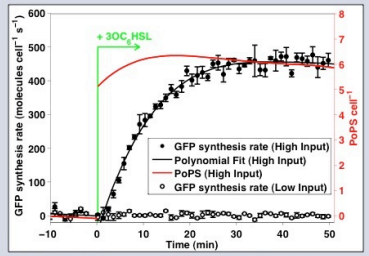
Transfer Function*



P_{max}: **6.7** PoPS cell^{-1}
K: **2E-09** M 3OC$_6$HSL
n: **1.2**

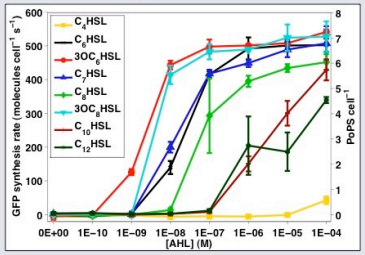
$$P_{out} = P_{max} \frac{[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

Response Time*



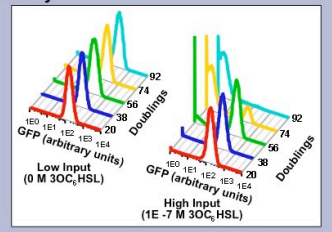
BBa_F2620 Response Time: **<1** *min*
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M 3OC$_6$HSL)

Input Compatibility*



Part Compatibility
Chassis: MC4100, *MG1655*, and *DH5α*
Plasmids: pSB3K3 and *pSB1A2*
Devices: E0240, *E0430* and *E0434*
Crosstalk with systems containing *C0040*

Stability**



Genetic: >**92/<74** replication events**
Performance: >**92/<74** replication events**
(low/high input)

Transcriptional Output Demand (low/high input)
Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
Polymerases: **4.4E-3xNt / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

Conditions (abridged)
Output: PoPS measured via *BBa_E0240*
Culture: Supplemented M9, 37°C
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

Signaling Devices

Registry of Standard Biological Parts
making life better, one part at a time

DNA Parts Descriptors

ELSEVIER

Available online at www.sciencedirect.com

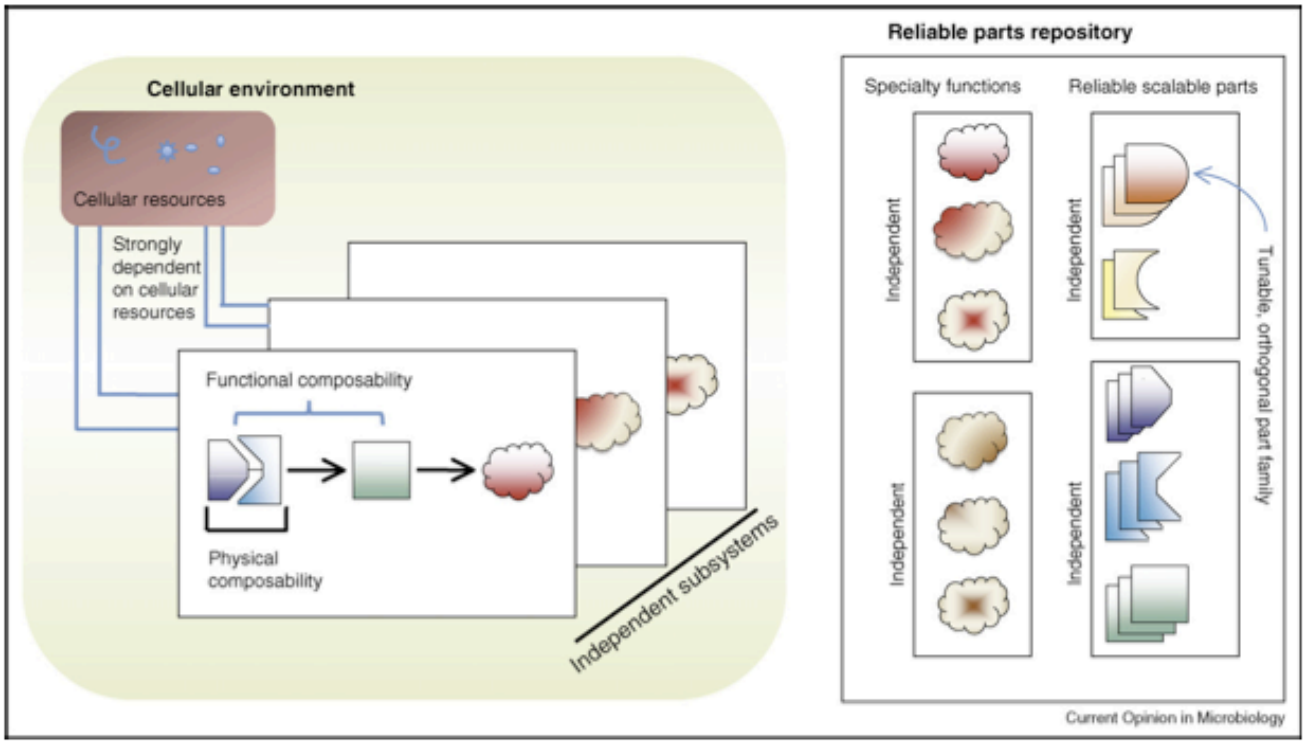
ScienceDirect

Current Opinion in Microbiology

Toward scalable parts families for predictable design of biological circuits

Julius B Lucks[1,2], Lei Qi[3], Weston R Whitaker[1] and Adam P Arkin[1,4]

Figure 1



Current SynBio Challenges

- **Standardisation**
  - DNA parts ?
  - Measurements ?
  - Reporting ?
  - **Request for Comments (RFC)**
    - examples

Active projects in SynBio

- BioBrick standard + RFCs
- Automated DNA Assembly
- Promoter characterisation
- Chassis of choice (minimal, yeast, B. sub, E. coli)
- “POBOL”: Description Language for BioBricks
- BioBrick Licensing Schema
- Ethical issues

Thank you

Any Further Questions ?