

SynBio in pharmaceuticals and update on Nanomot project

Synthetic Biology Workshop: From Science to
Governance

Brussels, 18.-19.3. 2010

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Science and Engineering, ETH Zurich @ Basel

20 μm



ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich



Biopharmaceutical manufacturing – Problems (selection)

Proteins –
posttranslational
processes

Microheterogeneity
(glycosylation,
phosphorylation,
sulfation, PEGylation)

Secretion (yield,
predictability)

Folding (eg disulfide
bonds)

Complex „small“
molecules

Impractical long
(bio)synthetic routes

Process development
times, predictability

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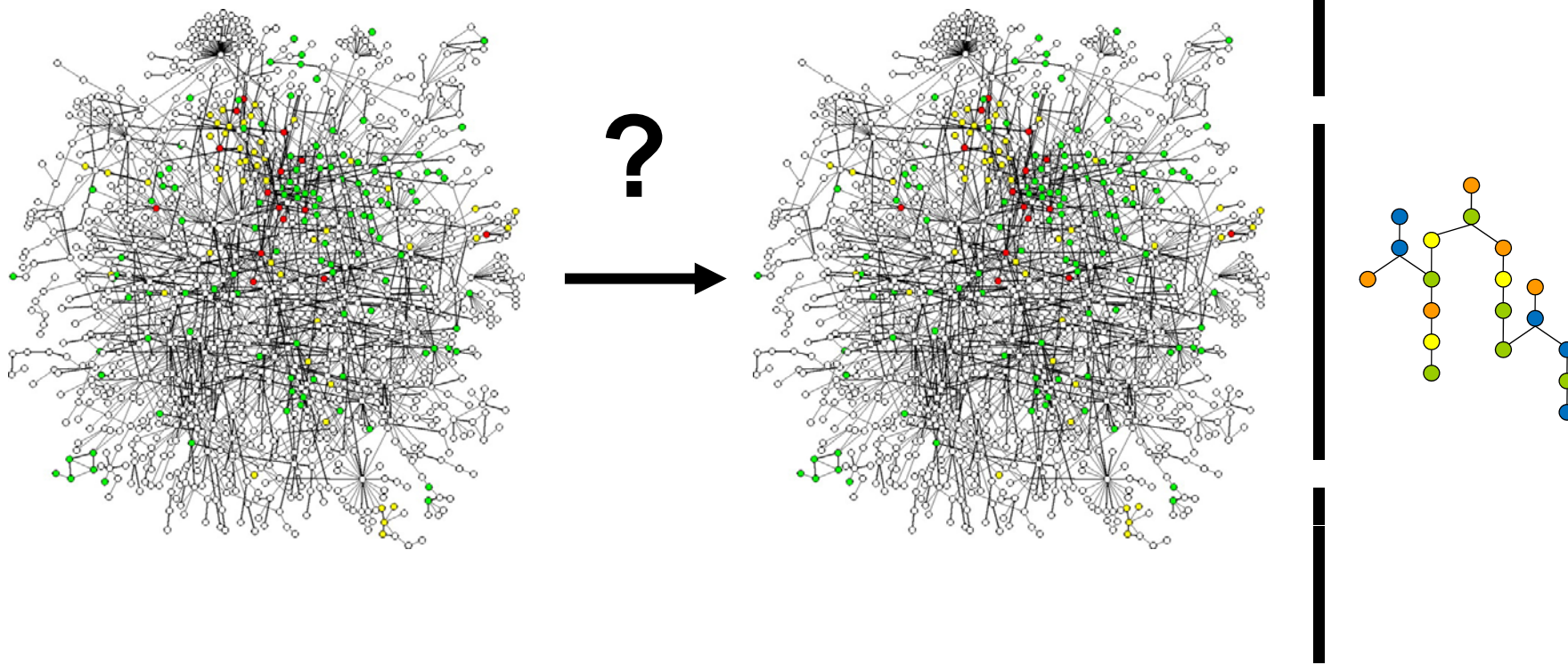
Process development
times, predictability

Parallel metabolisms

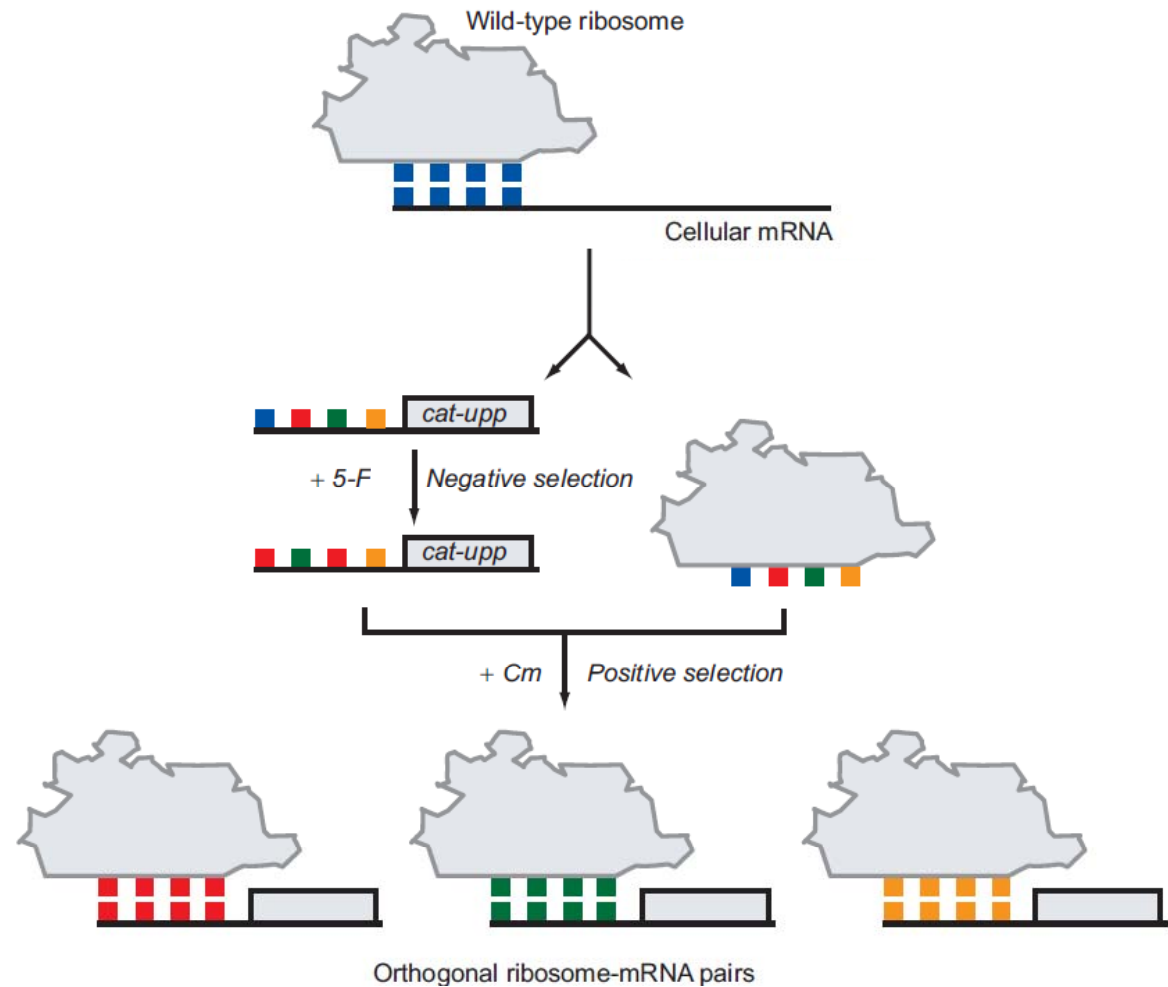
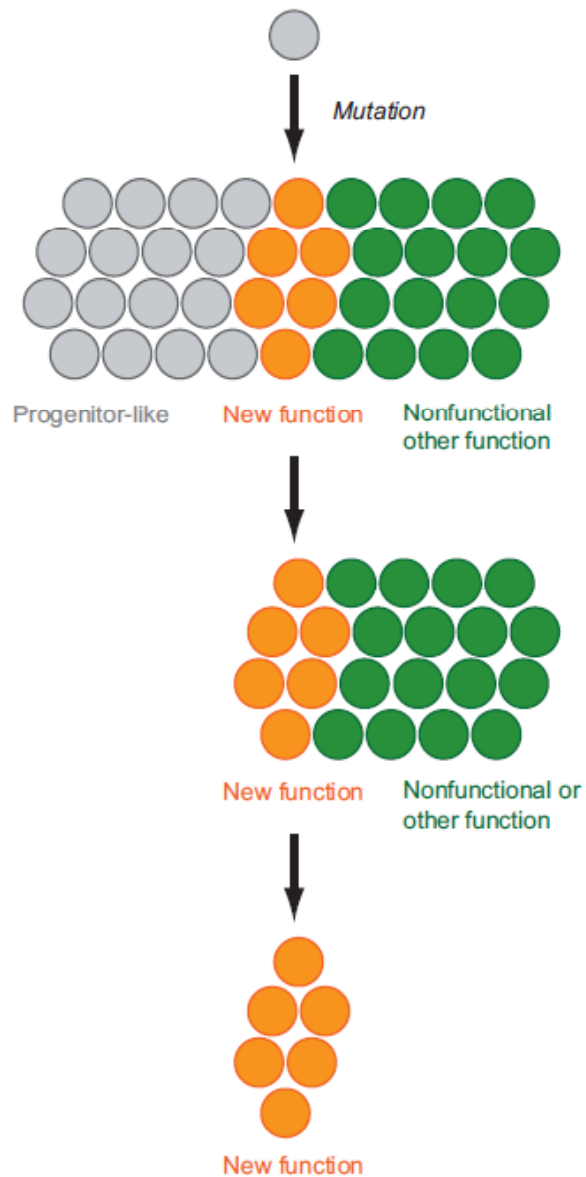
Massive increases in
design power
Simplified chassis

Re-defining chemical interfaces - orthogonal systems

The fundamental problem of implementing
orthogonality – unintended interactions

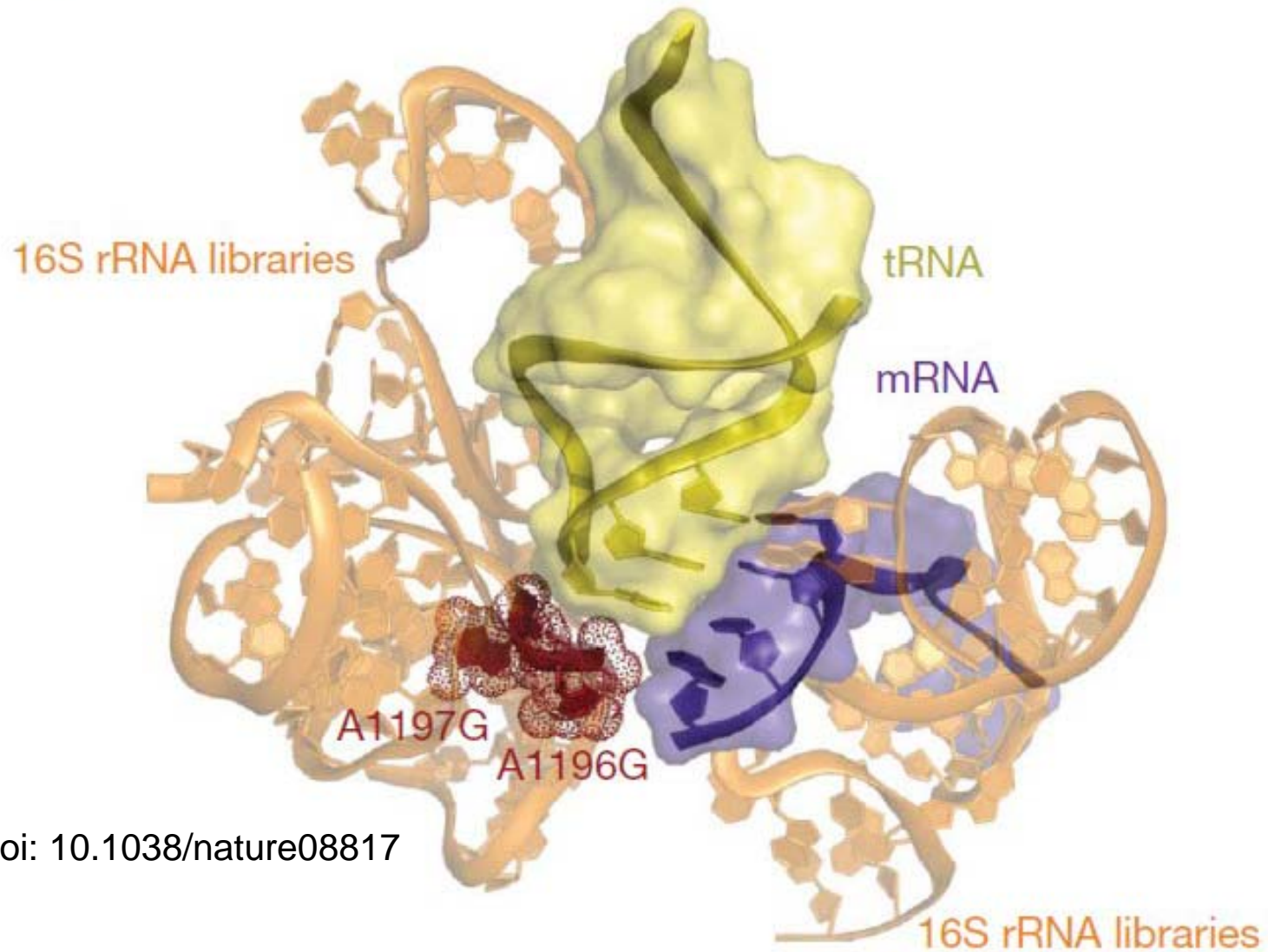


One option: Selecting for orthogonal interactions



Orthogonal ribosomes for orthogonal mRNAs

Ribosomes that recognize 4–nucleotide codons

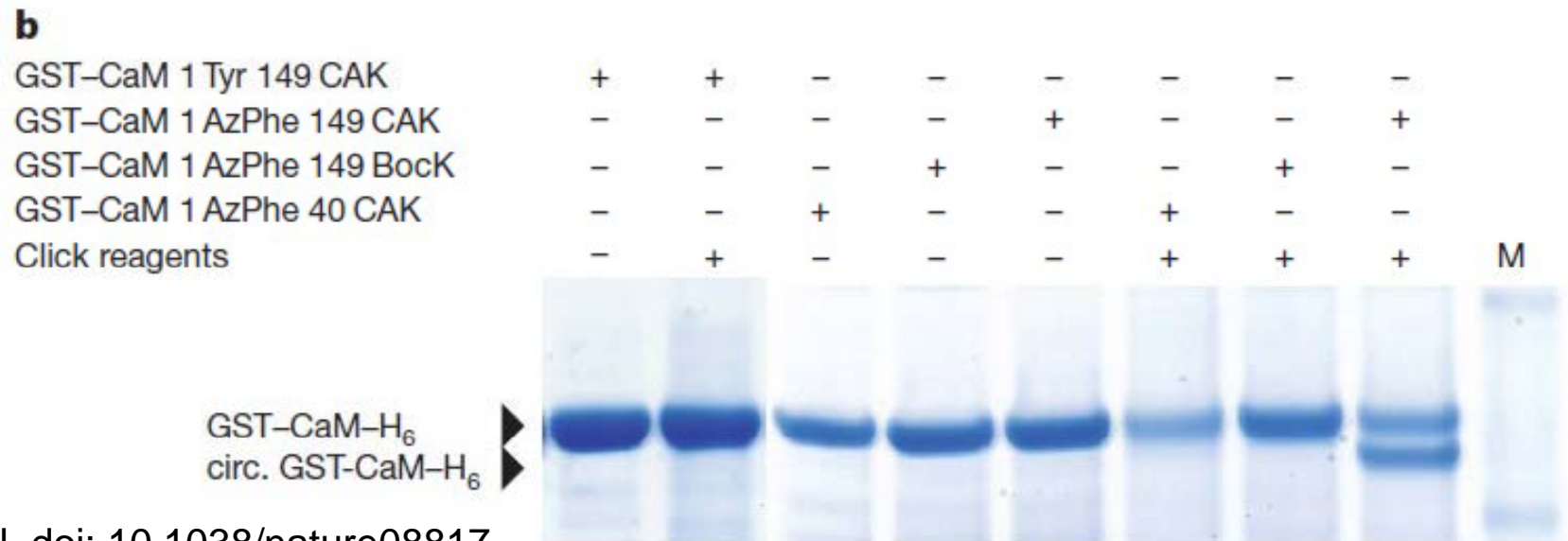
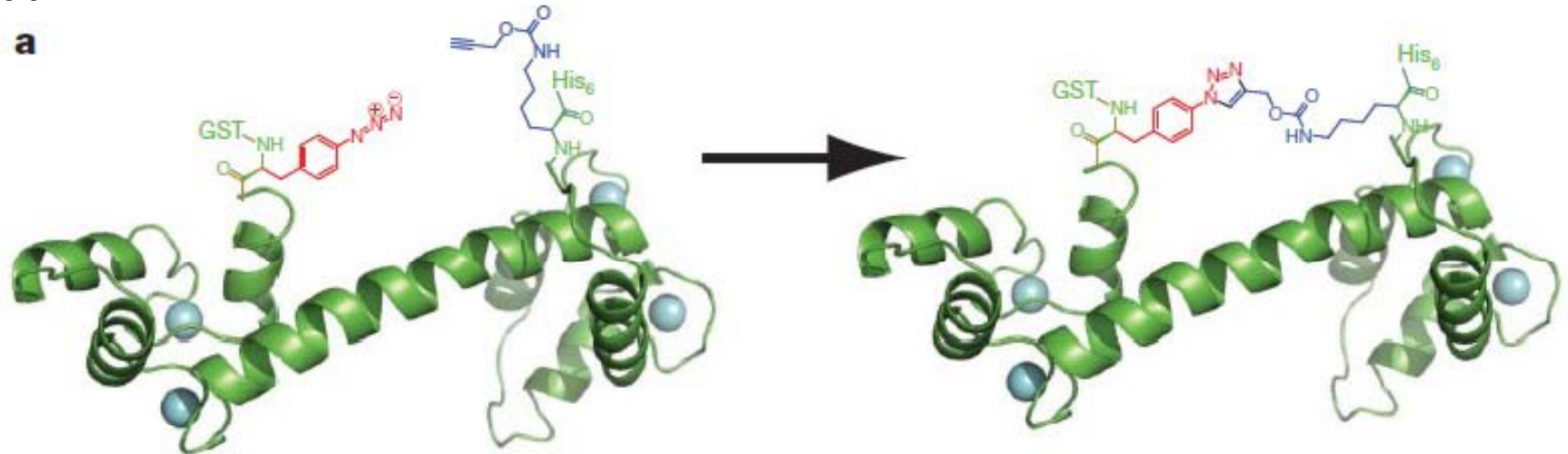


Chin et al, doi: 10.1038/nature08817

A ribosome that efficiently accepts tRNAs carrying p-azidophenylalanine (AzPhe) and N6-[(2-propynyloxy)carbonyl]-L-lysine (CAK) into a nascent polypeptide chain – click chemistry

Application: In vitro click chemistry

Calmodulin



Potential consequences:

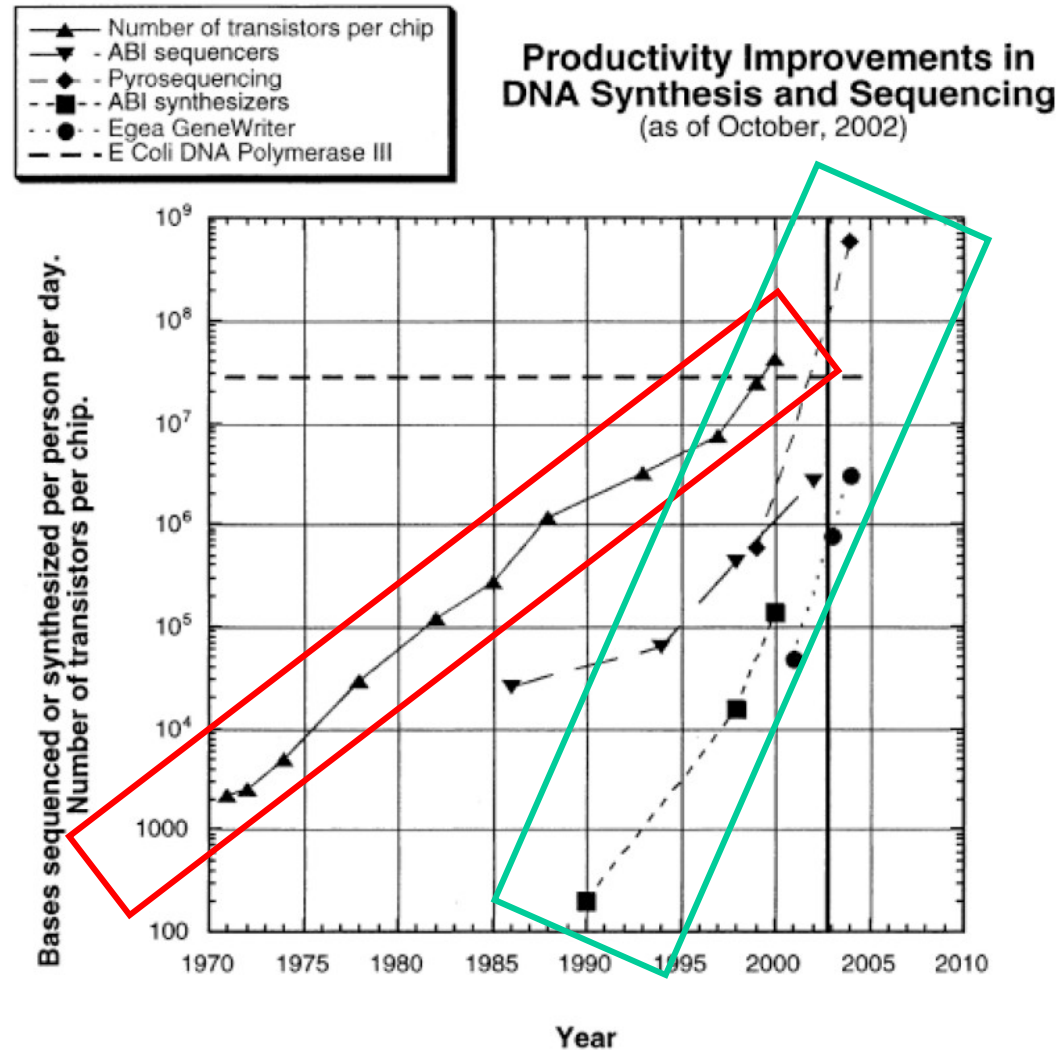
The implementation of effective parallel metabolisms would allow exploiting a much larger diversity of chemical reactions.

Example: non-canonical amino acids for protein synthesis.

One obvious application would be to produce proteins with novel, highly reactive amino acids that are orthogonal to cellular biochemistry, so that the non-canonical amino acids can be quantitatively posttranslationally modified. This would open completely novel ways of addressing the problem of microheterogeneity in its many facets.

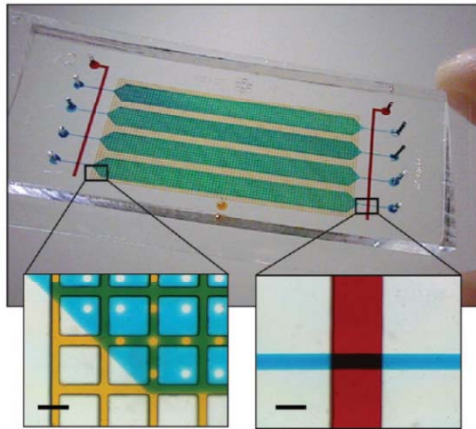
(If successful, it might even eliminate the „the process is the product“ problem of biopharmaceutical production).

Massive increases in design power – 1: Massively increased *de novo* DNA synthesis capacity



- Massive increases in design power –
- 2: Computer aided design tools
- 3: Improved assembly and analysis capacity

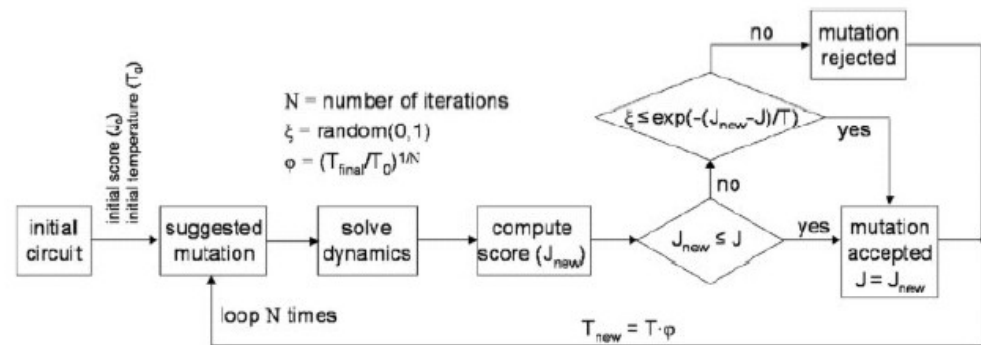
DNA/parts assembly
Parts characterization



Kong et al, NAR 35 e61
Zhong et al, Lab on a Chip 8:68

Microfluidics solutions

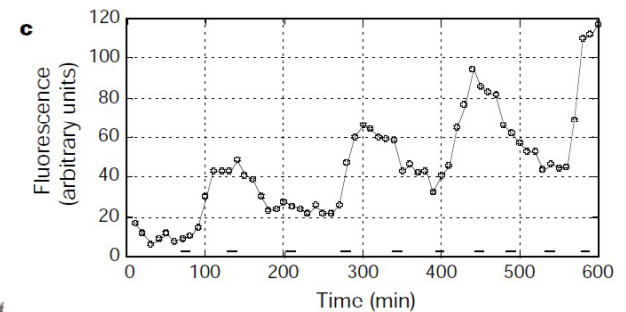
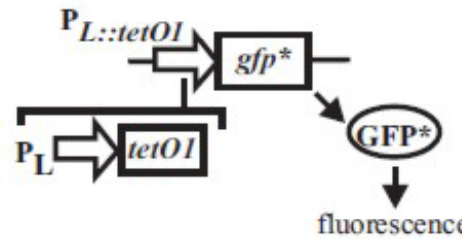
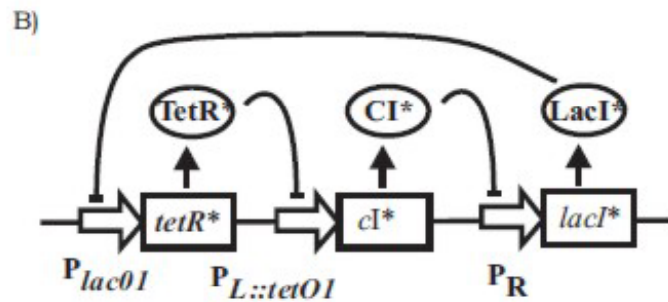
Computer-aided network design (Emergence)



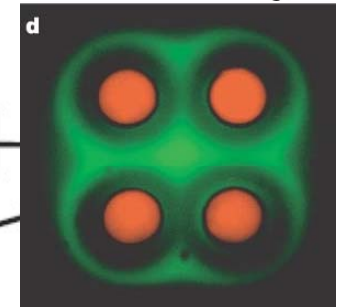
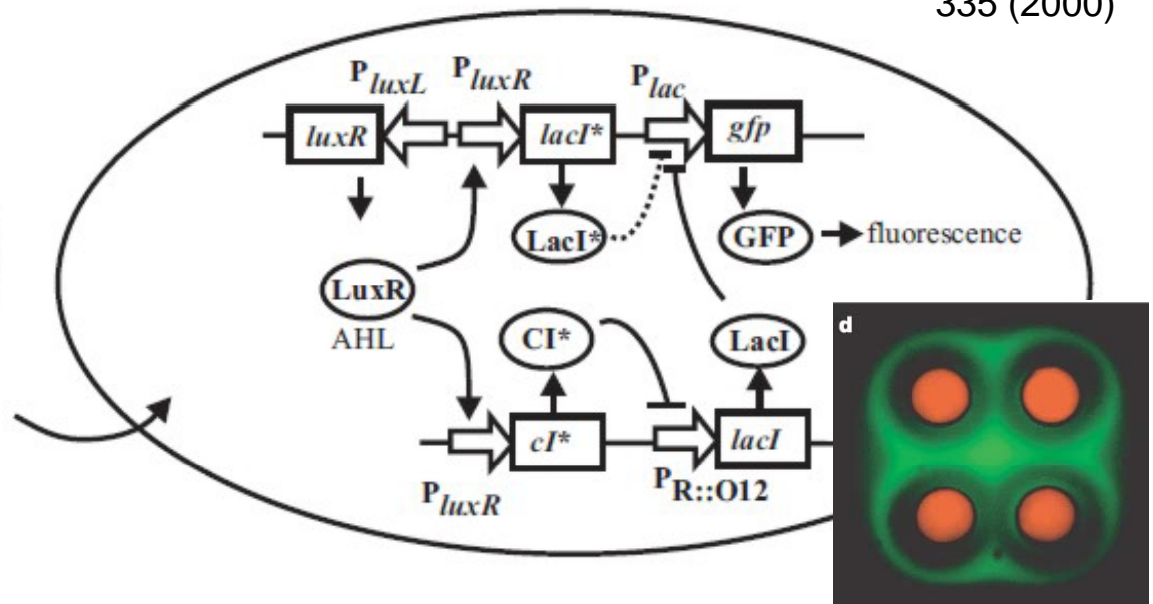
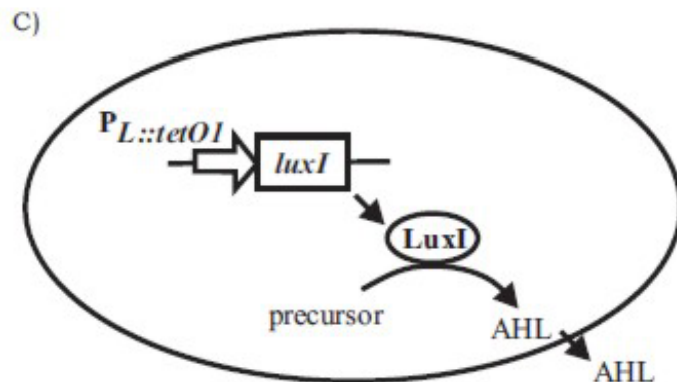
...AAGCTTCCAGAATT...

Rodrigo et al, Bioinformatics 23:14
Marchisio & Stelling, Bioinformatics 24:1903

Increases in design power allow expanding our design scope – genetic circuits to program complex behavior...

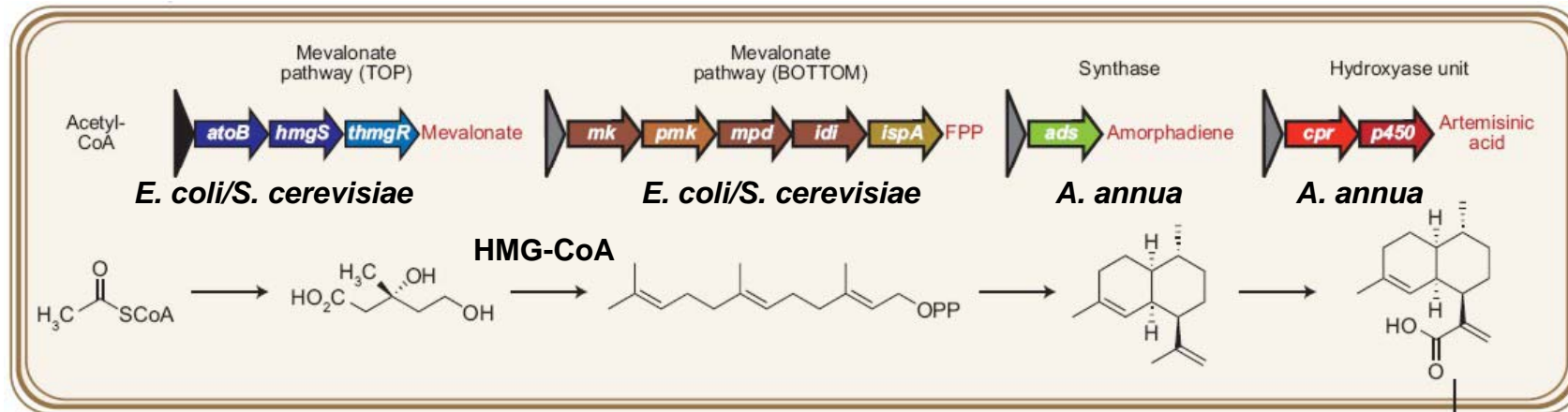


Elowitz & Leibler, Nature 403: 335 (2000)



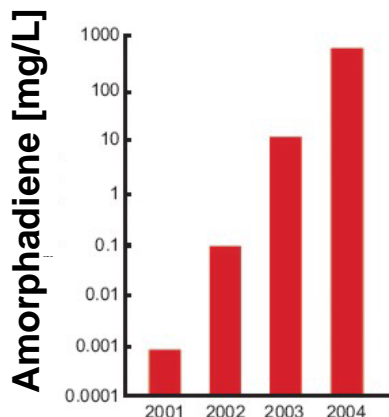
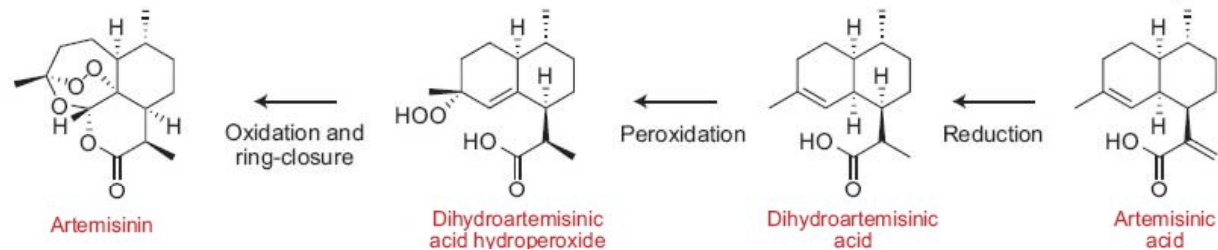
Basu et al., Nature 434:1130 (2005)

... or complex synthetic biochemical routes



E. coli

Chemical conversions



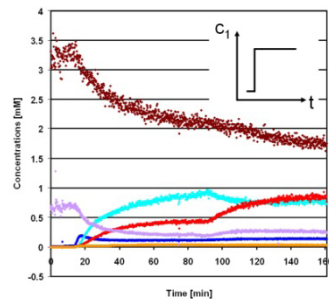
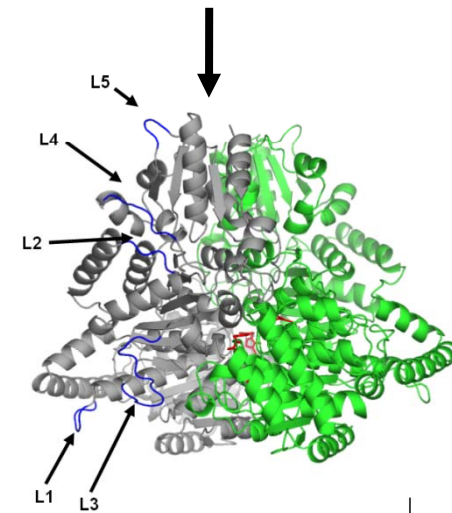
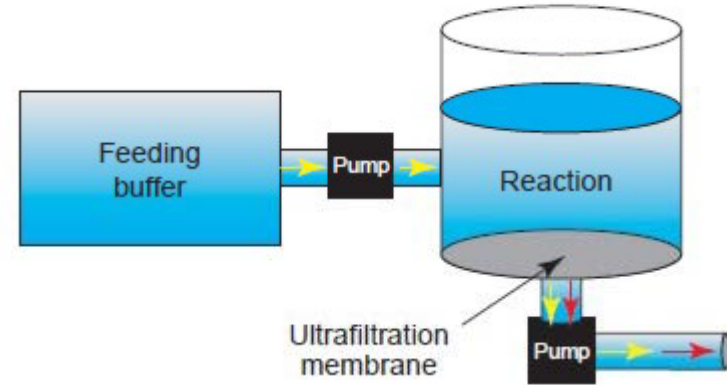
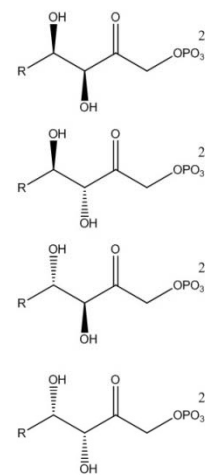
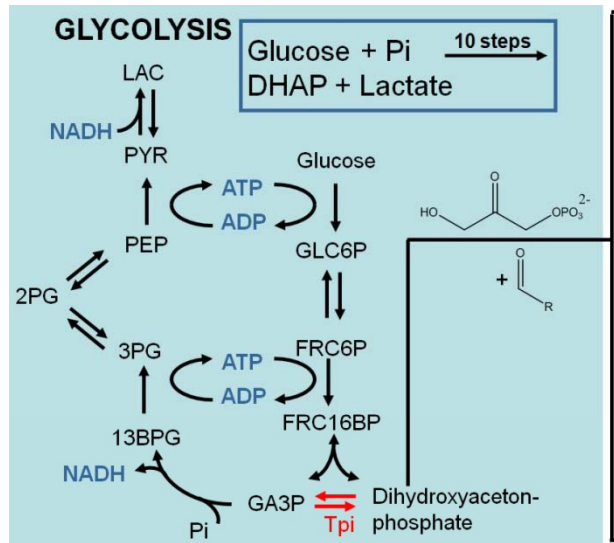
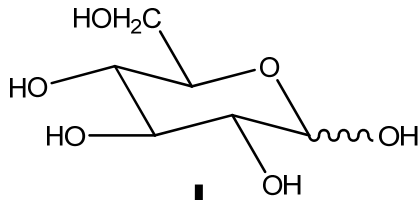
Combination of genes from 3 host in *E. coli*
– production of a precursor for an antimalaria drug

J. Keasling – Lab
UC Berkeley



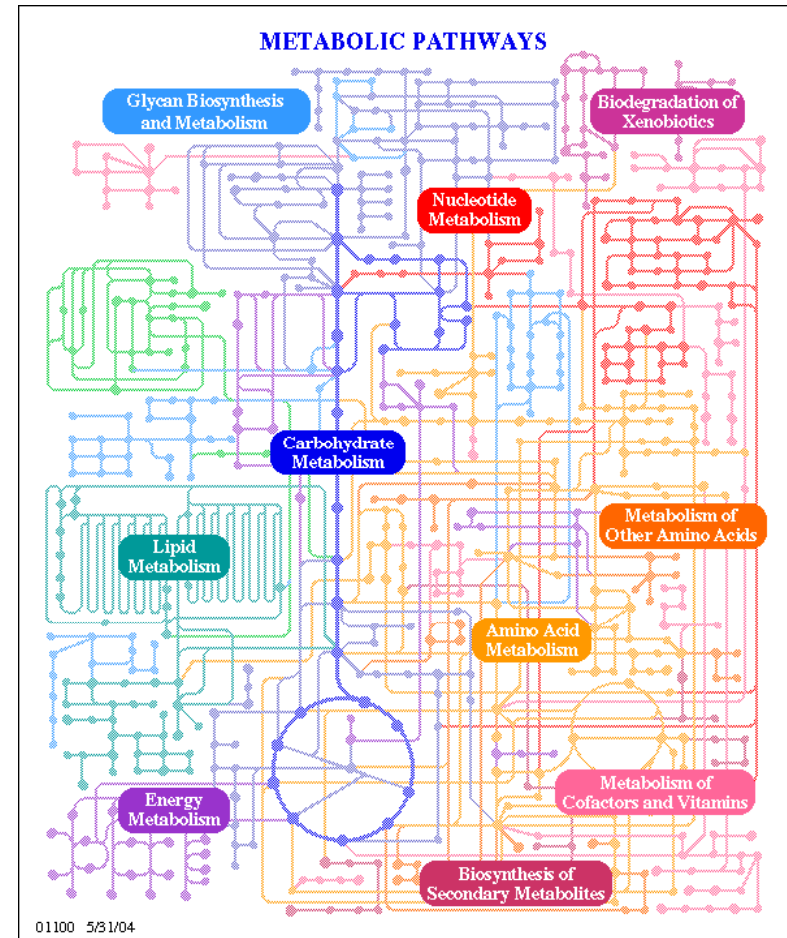
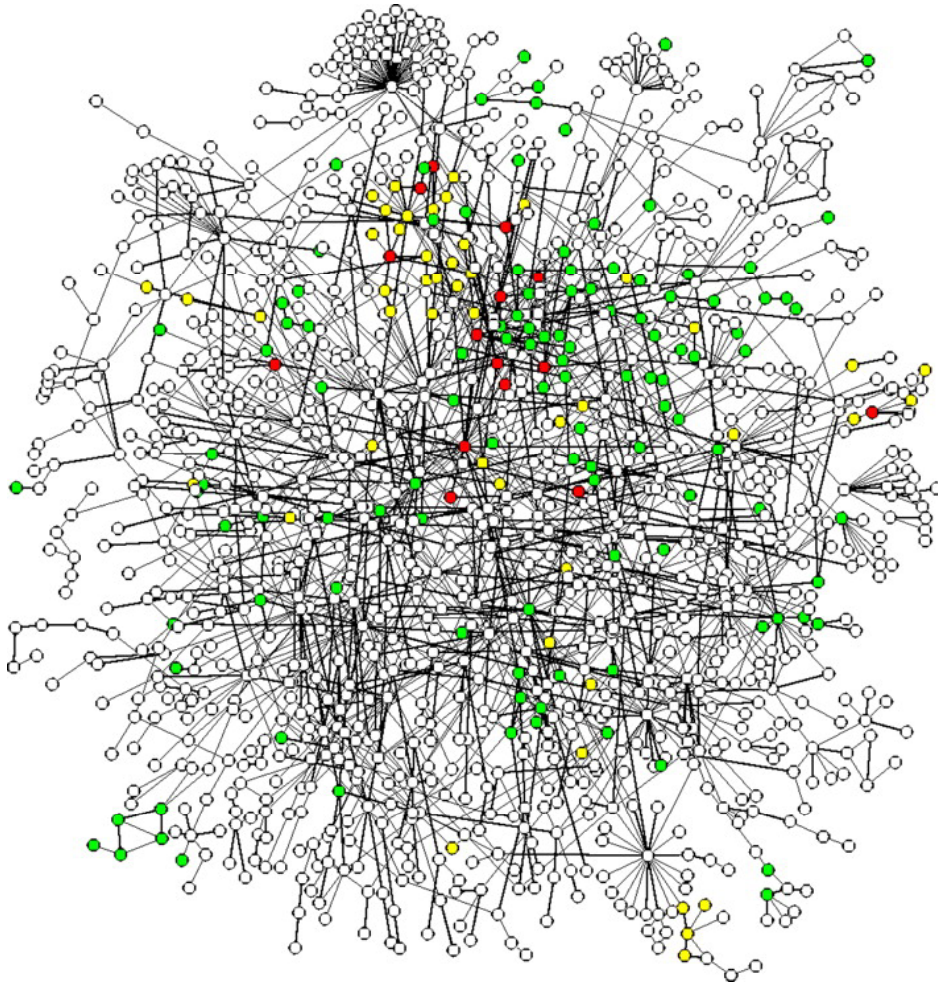
Simplified chassis

Reduced complexity – cell free systems



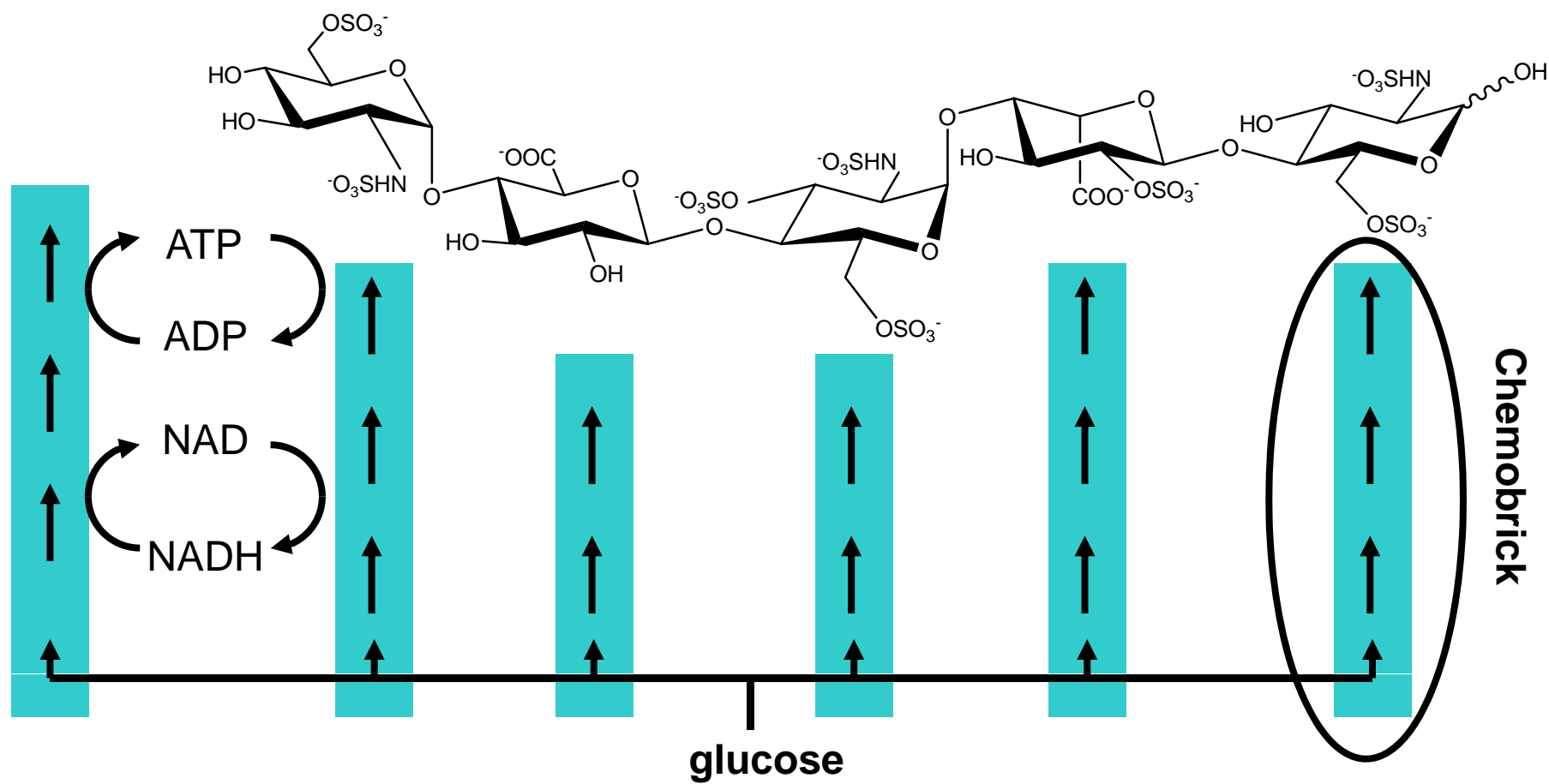
Systems of biotransformations, Panke-lab, ETH Zurich
 Cell-free protein synthesis, Swartz-lab, Stanford

Starting point:
Simplicity from complexity?

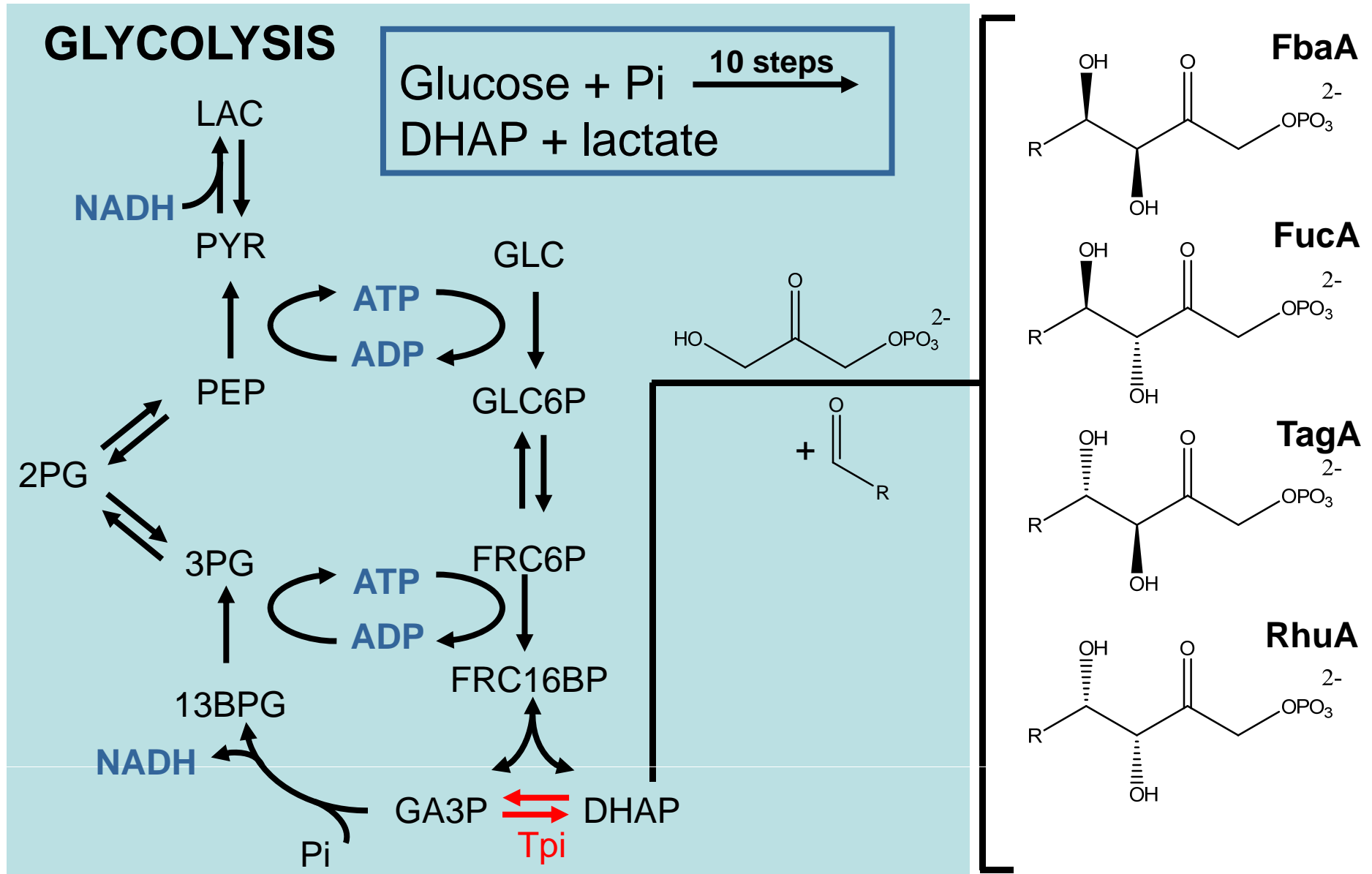


Forgacs et al., J. Cell Science 117: 2769

(Future) End point



A balanced reaction network for the production of artificial sugars (EuroBioSyn)

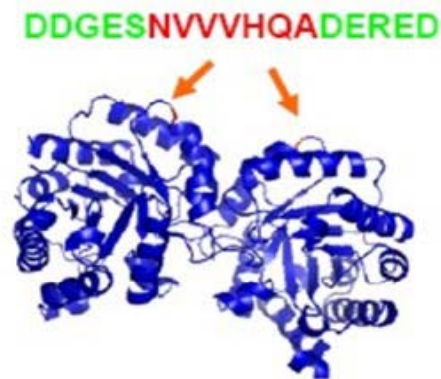


Enforcing orthogonality - Protein switches

euro biosyn
a modular platform for biosynthesis of complex molecules

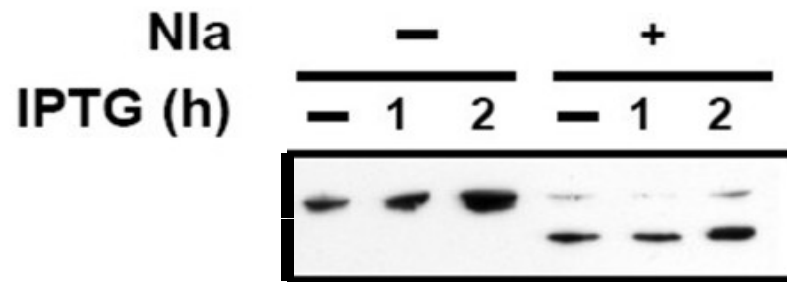
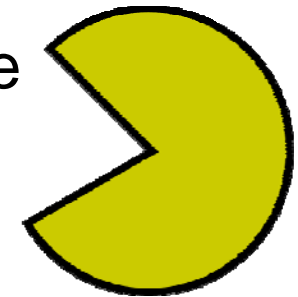


Tpi with proteolytic site inserted



Nla protease

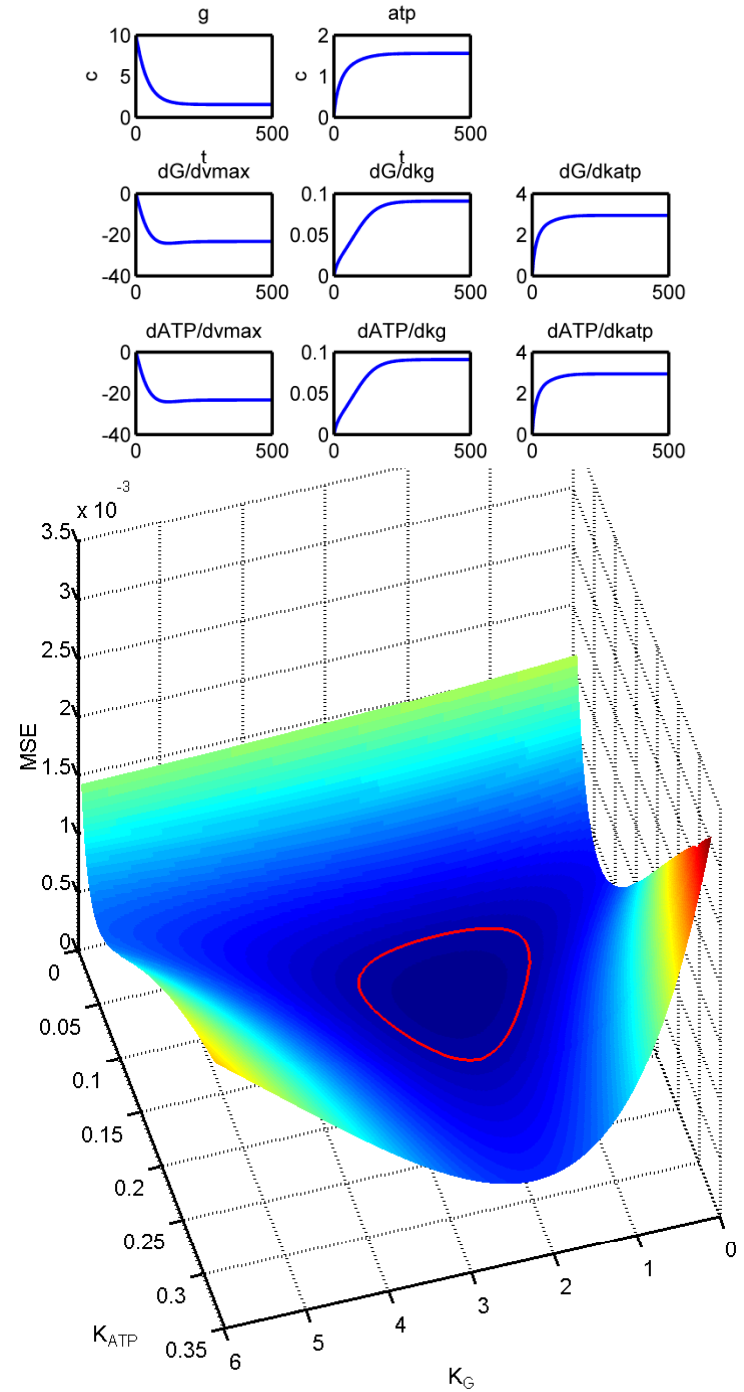
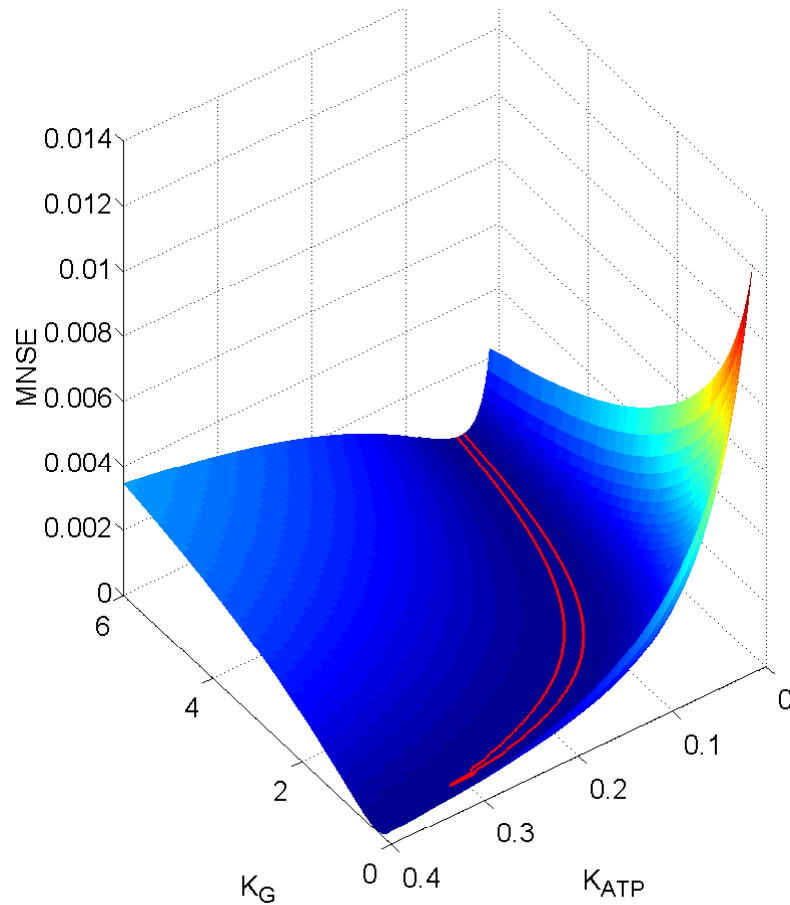
NVVVHQA



Etag-detection

Design of experiments – batch vs continuous for parameterization of glucokinase

$$v_{GK} = \frac{V_{GK}[G][ATP]}{K_G K_{ATP} + [G][ATP] + K_{ATP}[G]}$$



... to enable true network engineering

$$\begin{aligned}
 \frac{dc_G}{dt} &= -r_{GK} + D(c_G^{in} - c_G) \\
 \frac{dc_{G6P}}{dt} &= r_{GK} - r_{PGI} - Dc_{G6P} \\
 \frac{dc_{F6P}}{dt} &= r_{PGI} - r_{PFK1} - Dc_{F6P} \\
 \frac{dc_{FBP}}{dt} &= r_{PFK1} - r_{ALD2} - Dc_{FBP} \\
 \frac{dc_{DHAP}}{dt} &= r_{ALD2} - Dc_{DHAP} \\
 \frac{dc_{GAP}}{dt} &= r_{ALD2} - r_{GDH} - Dc_{GAP} \\
 \frac{dc_{BPG}}{dt} &= r_{GDH} - r_{PGK} - Dc_{BPG} \\
 \frac{dc_{3PG}}{dt} &= r_{PGK} - r_{PGM} - Dc_{3PG} \\
 \frac{dc_{2PG}}{dt} &= r_{PGM} - r_{ENO} - Dc_{2PG} \\
 \frac{dc_{PEP}}{dt} &= r_{ENO} - r_{PYK1} - Dc_{PEP} \\
 \frac{dc_{PYR}}{dt} &= r_{PYK1} + r_{LDH} - Dc_{PYR} \\
 \frac{dc_{LAC}}{dt} &= r_{LDH} - Dc_{LAC} \\
 \frac{dc_{ATP}}{dt} &= r_{PFK1} + r_{PGK} + r_{PYK1} + r_{PYK2} \\
 &\quad - r_{ADK} + D(c_{ATP}^{in} - c_{ATP}) \\
 \frac{dc_{ADP}}{dt} &= r_{GK} + r_{PFK1} - r_{PGK} - r_{PYK1} - r_{PYK2} \\
 &\quad + 2r_{ADK} - Dc_{ADP} \\
 \frac{dc_{AMP}}{dt} &= -r_{ADK} - Dc_{AMP} \\
 \frac{dc_{P_i}}{dt} &= -r_{GDH} + D(c_{P_i}^{in} - c_{P_i}) \\
 \frac{dc_{NADH_2}}{dt} &= r_{GDH} - r_{LDH} - Dc_{NADH_2} \\
 \frac{dc_{NAD}}{dt} &= -r_{GDH} + r_{LDH} - D(c_{NAD}^{in} - c_{NAD})
 \end{aligned}$$

18 balanced metabolites

$$\begin{aligned}
 v_{GK} &= \frac{V_{GK}[G][ATP]}{K_G K_{ATP} + [G][ATP] + K_{ATP}[G]} & v_{PGI} &= \frac{V_{PGI} \left([G6P] - \frac{[F6P]}{K_{PGI}} \right)}{K_{G6P} + [G6P] + \frac{K_{G6P}}{K_{F6P}} [F6P]} \\
 v_{PFK1} &= V_{PFK1} \bar{Y}_{MM} \bar{Y}_{MWC} \\
 \bar{Y}_{MWC} &= \frac{\alpha (1 + \alpha)^{n-1} + L' \alpha c}{L' + (1 + \alpha)^n} \\
 \bar{Y}_{MM} &= \frac{[ATP]}{K_{ATP,PFK1} (1 + [ADP]/K_{ADP,PFK1}) + [ATP]} \\
 v_{ALD2} &= \frac{V_{ALD2} \left([FBP] - \frac{[GAP][DHAP]}{K_A} \right)}{K_{FBP,ALD2} + [FBP] + \frac{K_{FBP,ALD2}}{K_{DHAP,ALD2}} [DHAP] + \frac{K_{FBP,ALD2}}{K_{ALD2}} [GAP][DHAP]} \\
 v_{GDH} &= V \frac{[NAD]^n [GAP][P_i] - \frac{[NADH]^n [BPG]}{K_{NADH} K_{BPG}}}{K_{NAD}^n K_{GAP} K_{P_i} \left(1 + \frac{[P_i]}{K_{P_i}} + \frac{[NAD]^n [GAP]}{K_{NAD}^n K_{GAP}} + \frac{[NAD]^n [P_i]}{K_{NAD}^n K_{P_i}} + \frac{[NAD]^n [GAP][P_i]}{K_{NAD}^n K_{GAP} K_{P_i}} + \frac{[NAD]^n [BPG]}{K_{NADH}^n K_{BPG}} \right)} \\
 v_{PGK} &= \frac{V \left([3PG] - \frac{[BPG][ATP]}{K_{BPG} K_{ATP}} \right)}{K_{BPG} K_{ADP} \left(1 + \frac{[BPG]}{K_{BPG}} + \frac{[3PG]}{K_{3PG}} + \frac{[ATP]}{K_{ATP}} + \frac{[BPG][ADP]}{K_{BPG} K_{ADP}} + \frac{[3PG][ATP]}{K_{3PG} K_{ATP}} \right)} \\
 v_{PGM} &= \frac{V_{PGM} ([3PG] - \frac{[2PG]}{K_{2PG}})}{K_{3PG} + [3PG]} & v_{ENO} &= \frac{V_{ENO} \left([2PG] - \frac{[PEP]}{K_{ENO}} \right)}{K_{2PG} + [2PG] + \frac{K_{2PG}}{K_{PEP}} [PEP]} \\
 v_{PYK1} &= \frac{\alpha (1 + \alpha)^2}{L' + (1 + \alpha)^n} & L' &= L_0 \frac{(1 + \beta)^4}{(1 + \gamma)^4} & \alpha &= \frac{[PEP]}{K_{R,PEP}} & \beta &= \frac{[ATP]}{K_{R,ATP}} \\
 v_{PYK2} &= V_P \frac{ADP}{K_{ADP} + ADP} & L_0 &= \frac{L}{(1 + \gamma)^n} & \gamma &= \frac{[AMP]}{K_{T,AMP}} & \gamma &= \frac{[FBP]}{K_{T,FBP}} \\
 v_{LDH} &= \frac{V_{LDH} \left([NADH][PYR] - \frac{[PEP][NAD]}{K_{LDH}} \right)}{K_{NADH,LDH} K_{PYR,LDH} + [NADH] K_{PYR,LDH} + \frac{K_{NADH,LDH} K_{PYR,LDH}}{K_{NAD}} [NAD]} \\
 v_{ADK} &= \frac{V_{ADK} \left([ATP][AMP] - \frac{[ADP]^2}{K_{ADK}} \right)}{K_{ATP} K_{AMP} \left(1 + \frac{[ATP]}{K_{ATP}} + \frac{[AMP]}{K_{AMP}} + 2 \frac{[ADP]}{K_{ADP}} + \frac{[ATP][AMP]}{K_{ATP} K_{AMP}} + \frac{[ADP]^2}{K_{ADP}^2} \right)}
 \end{aligned}$$

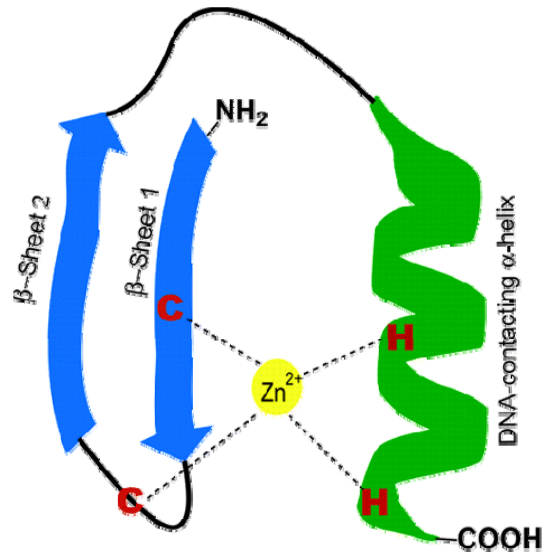
12 reaction laws for Michaelis-Menten type and cooperative/allosteric enzymes

Design power (2) – molecular modular building blocks

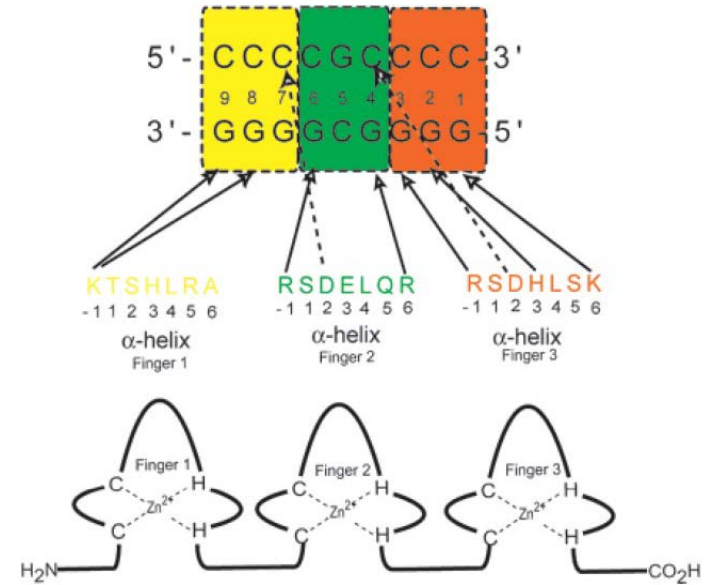
Can we use intramolecular protein domains as modular building blocks to assemble „molecular systems“?

(Rather than assembling e.g. gene circuits from existing parts?)

Scope – collections of parts



A ZF domain responsible for specific protein-DNA interactions. 4 conserved amino acids (2His, 2Cys) allow coordination of a Zn ion. The latter is important for maintaining conformation.

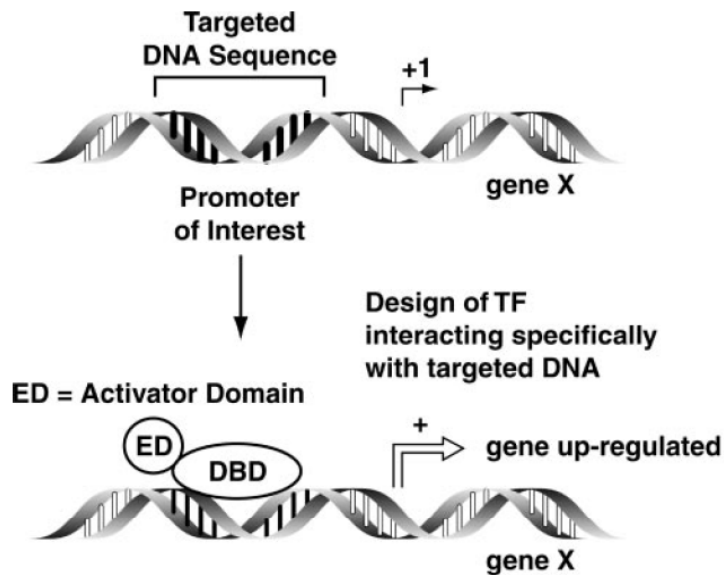


One ZF recognizes one specific DNA triplet. It is possible to generate ZFs for essentially any DNA triplet. By combining ZFs linearly, the DNA recognition sequence of the engineered proteins can be extended – up to 24, which is sufficient to address unique DNA sequences in the human genome.

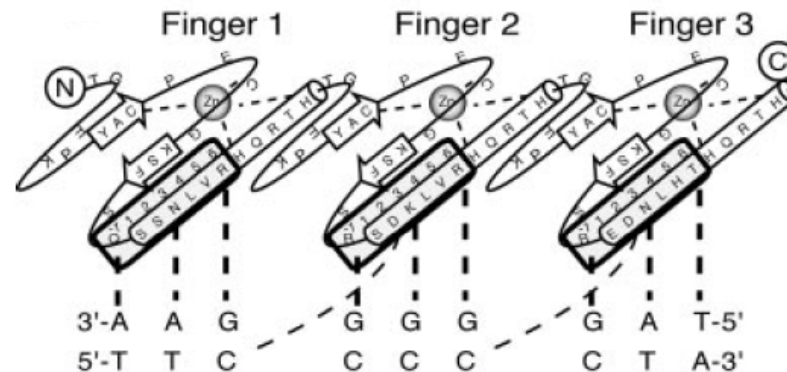
Designer transcription factors based on zinc finger domains

Barbas-lab, Blancafort et al., *Molec. Pharmacol.* 66: 1361 (2004)

Modular zinc finger domains are recombined to define novel DNA-sequence specificity

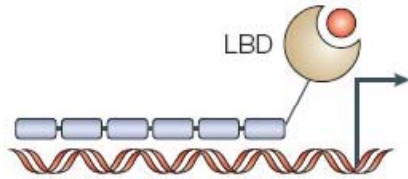


AAA	-1123456 Q R A N L R A	GAA	-1123456 Q S S N L V R
AAC	D S G N L R V	GAC	D P G N L V R
AAGk	R K D N L K N	GAGk	R S D N L V R
AAT	T T G N L T V	GAT	T S G N L V R
ACA	S P A D L T R	GCA	Q S G D L R R
ACC	D K K D L T R	GCC	D C R D L A R
ACGk	R T D T L R D	GCGk	R S D D L V R
ACT	T H L D L I R	GCT	T S G E L V R
AGA	Q L A H L R A	GGA	Q R A H L E R
AGC	N/A	GGC	D P G H L V R
AGGk	R S D H L T N	GGGk	R S D K L V R
AGT	H R T T L T N	GGT	T S G H L V R
ATA	Q K S S L I A	GTA	Q S S S L V R
ATC	N/A	GTC	D P G A L V R
ATGk	R R D E L N V	GTGk	R S D E L V R
ATT	H K N A L Q N	GTT	T S G S L V R
TAGk	R E D N L H T	TGGk	R S D H L T T

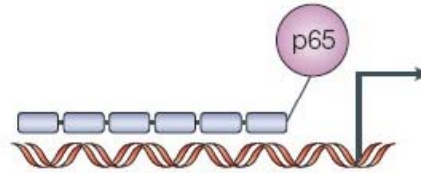


ED: Effector domain for regulation
 DBD: DNA-binding domain, designed to recognize target DNA sequence

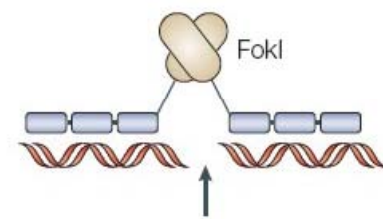
a High-throughput screening



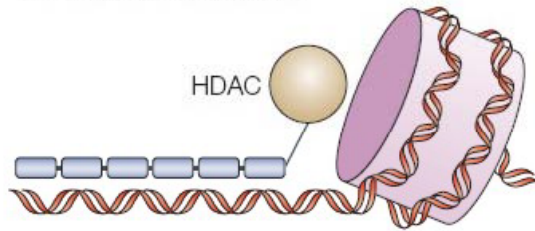
b Gene activation



c Targeted DNA cleavage

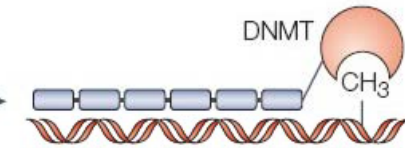


h Chromatin modification

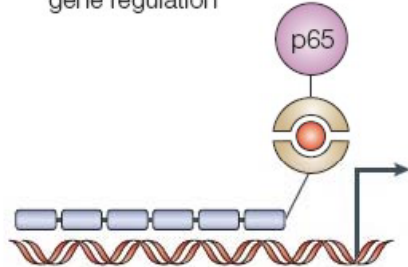


Adding functional domains to ZFPs enables diverse applications

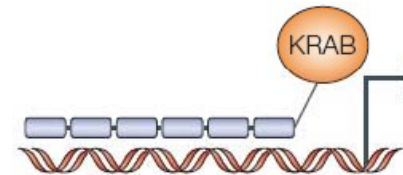
d DNA modification



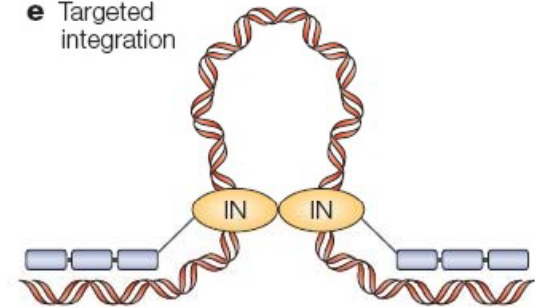
g Chemically controlled gene regulation



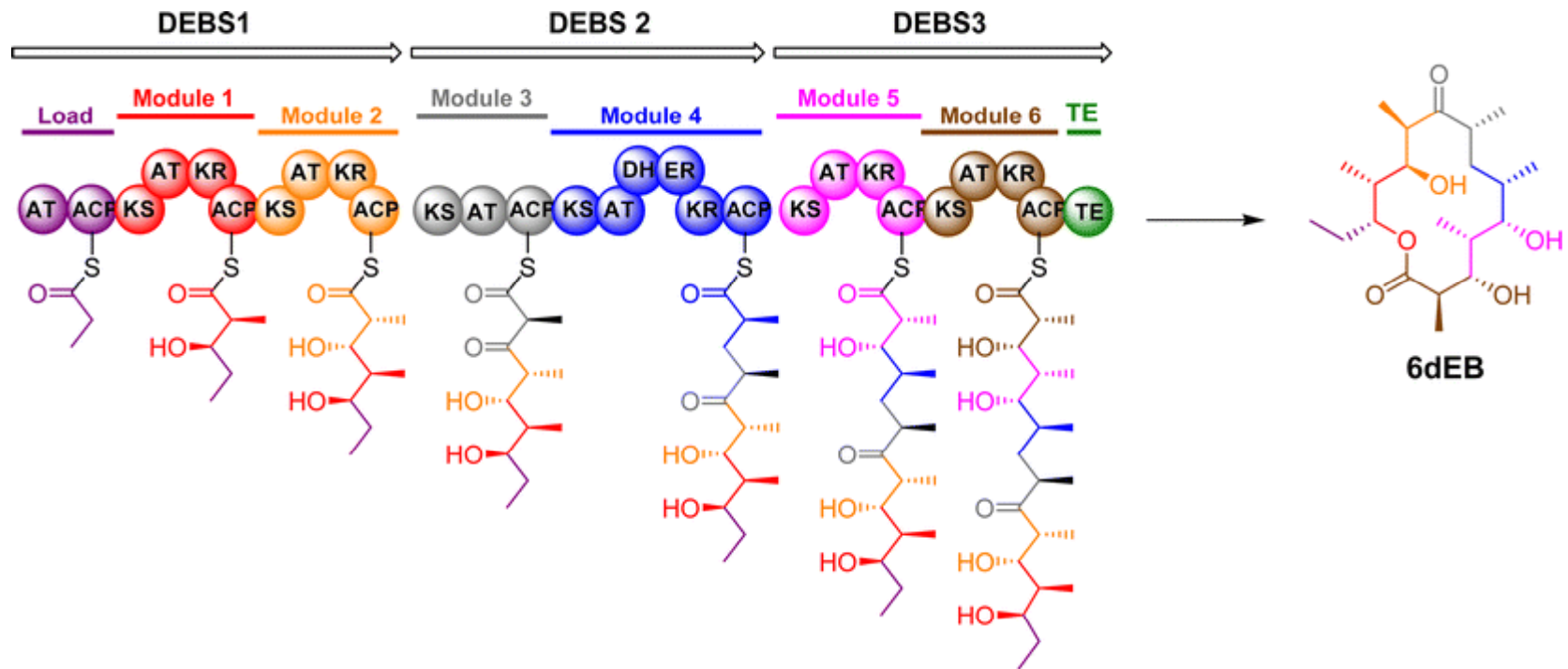
f Gene repression



e Targeted integration



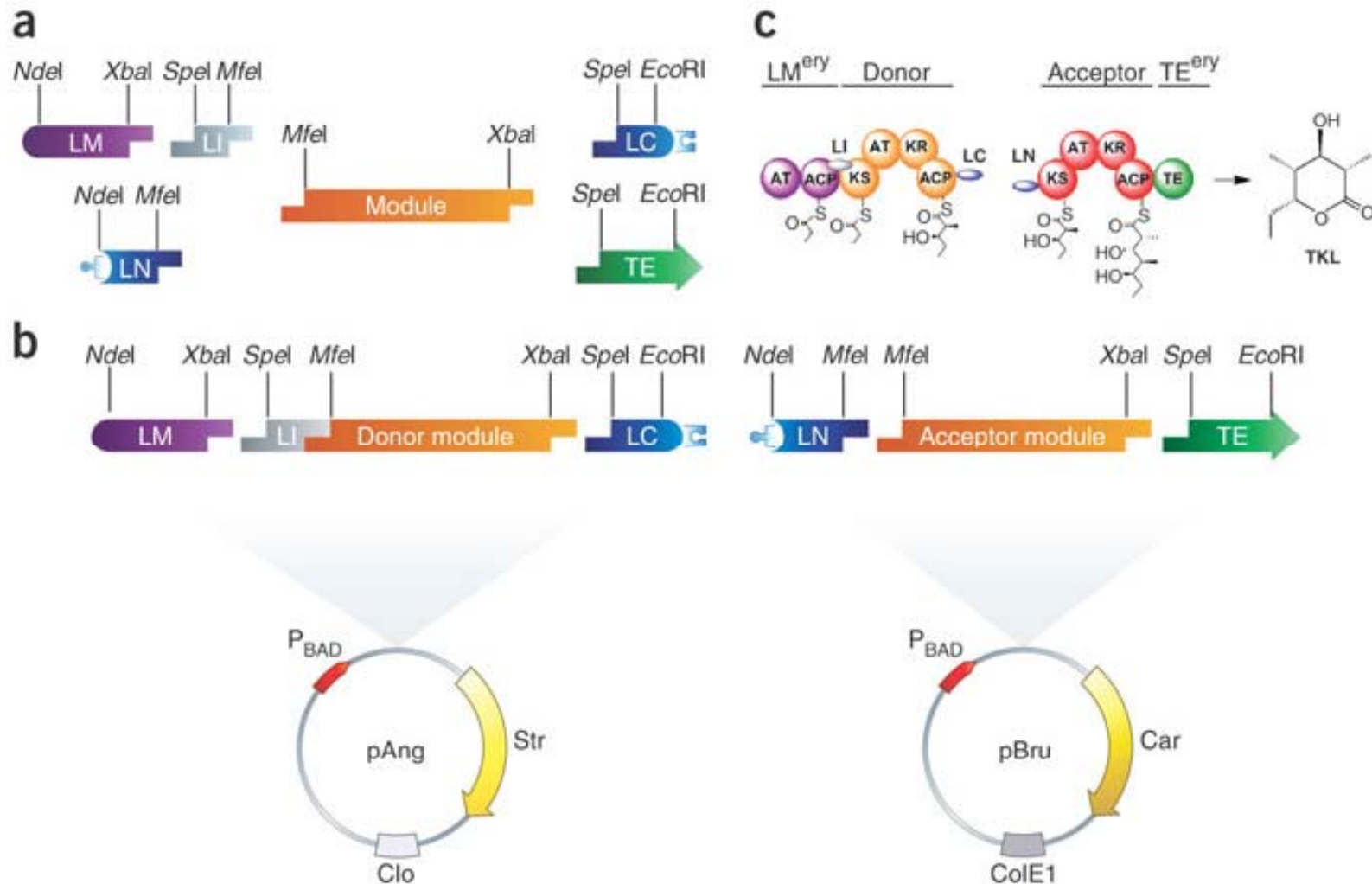
Novel antibiotics and cytostatics from reengineering polyketides



Menzella *et al.*, Nature Biotechnology 23:1171

This modularity can be exploited for easy recombination of modules leading to novel antibiotics:

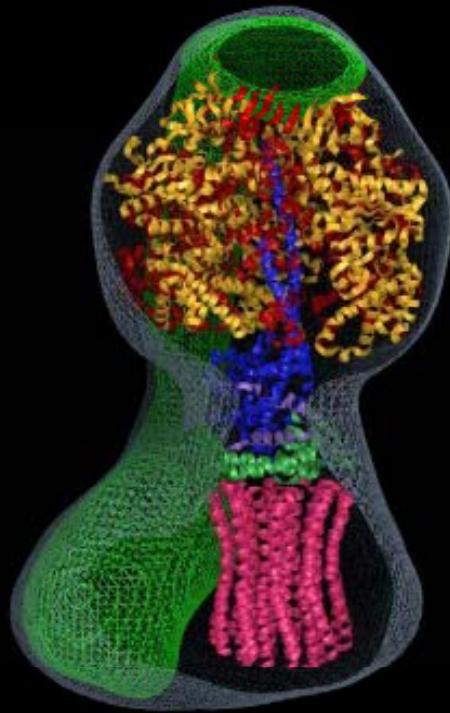
- De novo* DNA synthesis of polyketide synthase clusters for synthesis in *E. coli*
- Reorganization of cluster DNA (modularization/restriction enzymes)
- Exchanging of blocks – novel molecules



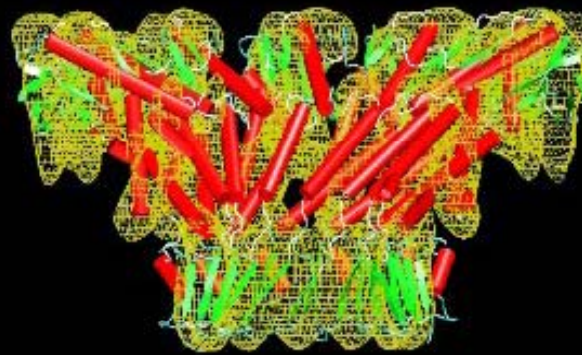


NANOMOT

Synthetic Biomimetic Nanoengines: A Modular Platform for Engineering of Nanomechanical Actuator Building Blocks



F-ATPase



Bacteriophage $\phi 29$
head/tail connector



Flagellar Rotor

Nanomot highlights

- Novel synthetic copolymer membranes for controlled application of membrane proteins in artificial environments
- Magnetic control of flagellar motors
- Linear (viral) motors in synthetic membranes
- Novel tools to control membrane traffic
- Comprehensive molecular models from ATPase and linear motors

Major lines of NANOMOT will continue in „NANOCELL“ (ESF)

Summary

Synthetic biology offers unique opportunities to address major bottlenecks in current biopharmaceutical processing.

The key strategies introduced by SynBio are:

- (1) Parallel, orthogonal metabolisms
- (2) (Drastically) Increased design power on all levels of the design process
- (3) Simplified, reduced chassis
- (4) Molecular building blocks

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