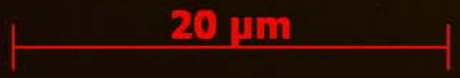
SynBio in pharmaceuticals and update on Nanomot project

Synthetic Biology Workshop: From Science to Governance
Brussels, 18.-19.3. 2010

Sven Panke
Bioprocess Laboratory, Department of Biosystems Science and Engineering, ETH Zurich @ Basel



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

Biopharmaceutical manufacturing – Problems (selection)

Proteins – posttranslational processes

Microheterogeneity (glycosylation, phosphorylation, sulfation, PEGylation)

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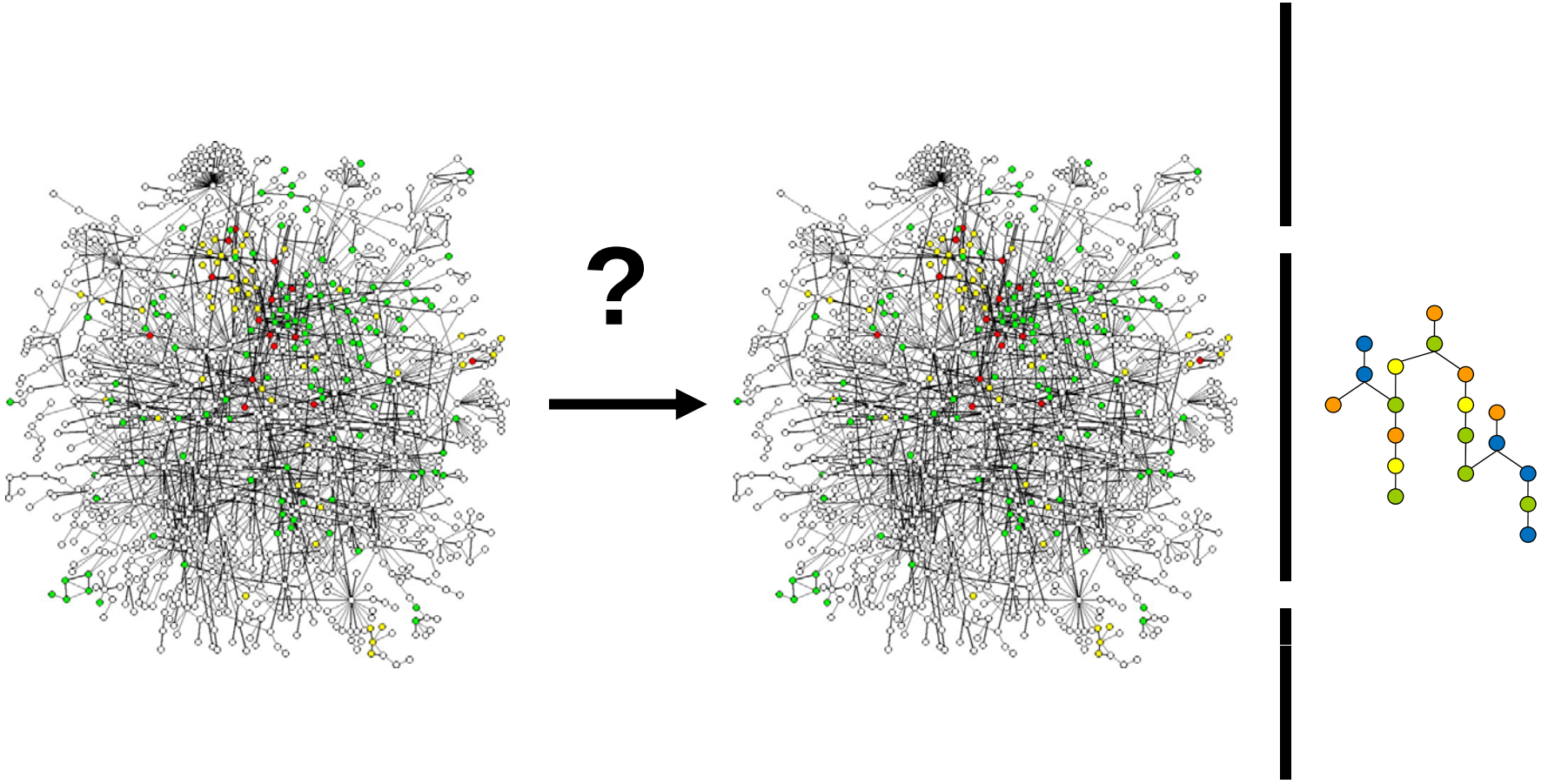
Process development times, predictability

Parallel metabolisms

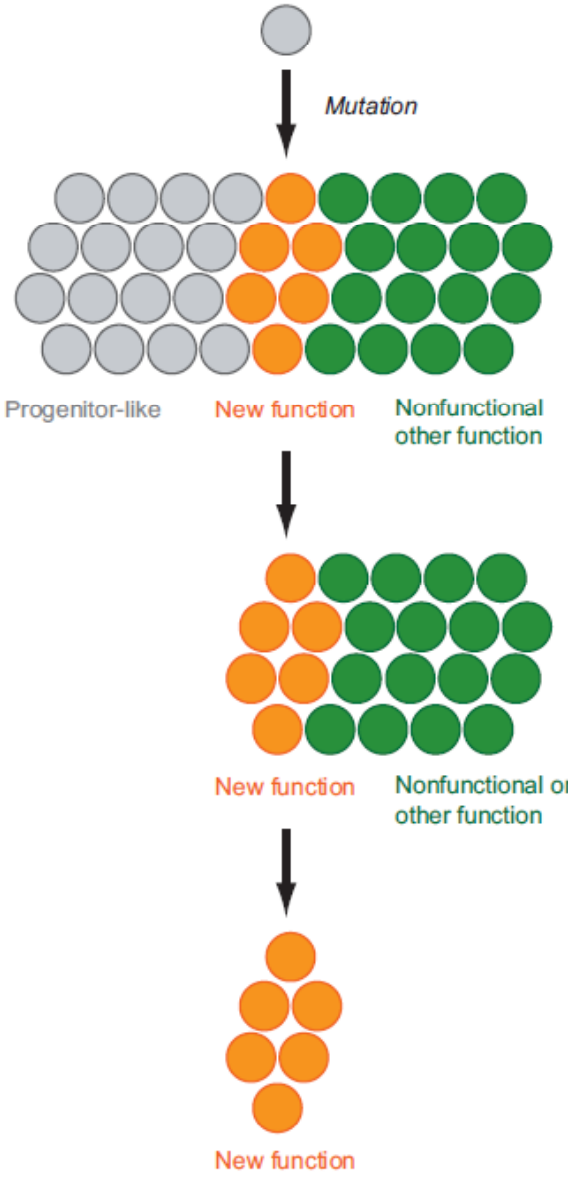
Massive increases in design power
Simplified chassis

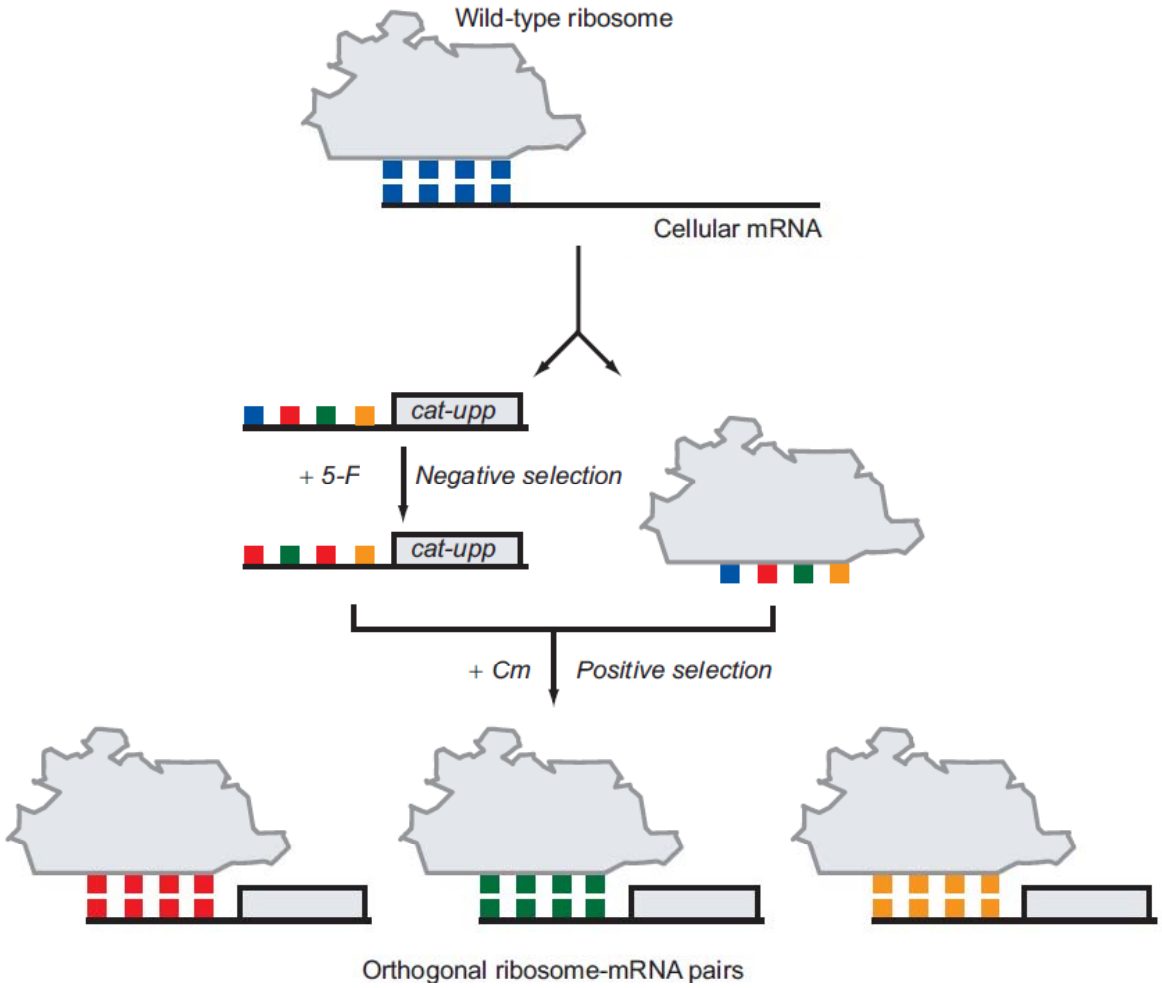
Re-defining chemical interfaces - orthogonal systems

The fundamental problem of implementing orthogonality – unintended interactions



One option: Selecting for orthogonal interactions



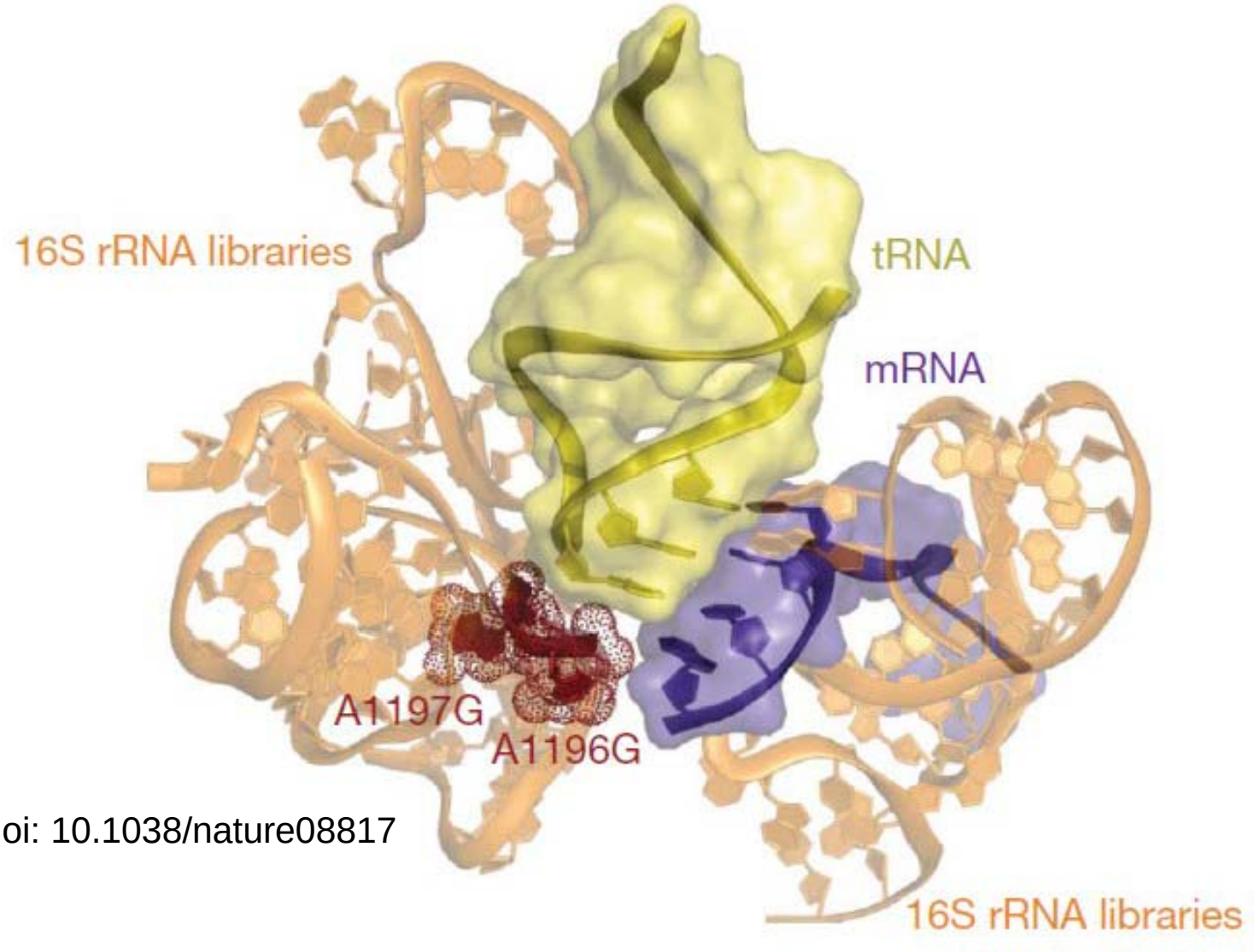


Orthogonal ribosomes for orthogonal mRNAs

Filipovska & Rackham, ACS Chem Biol 3:51

Chin – Lab (MRC Cambridge)

Ribosomes that recognize 4–nucleotide codons

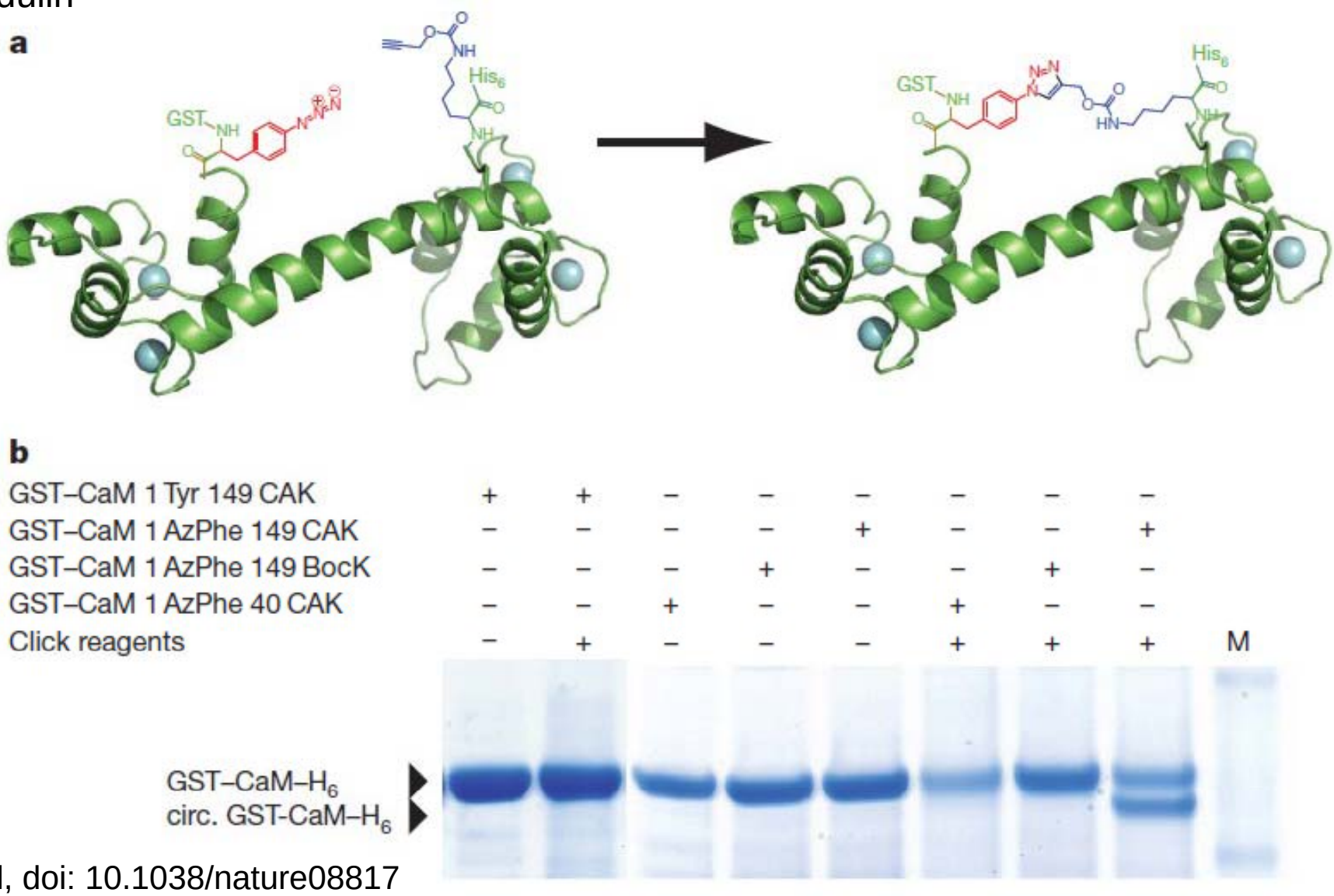


Chin et al, doi: 10.1038/nature08817

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



Chin et al, doi: 10.1038/nature08817

Potential consequences:

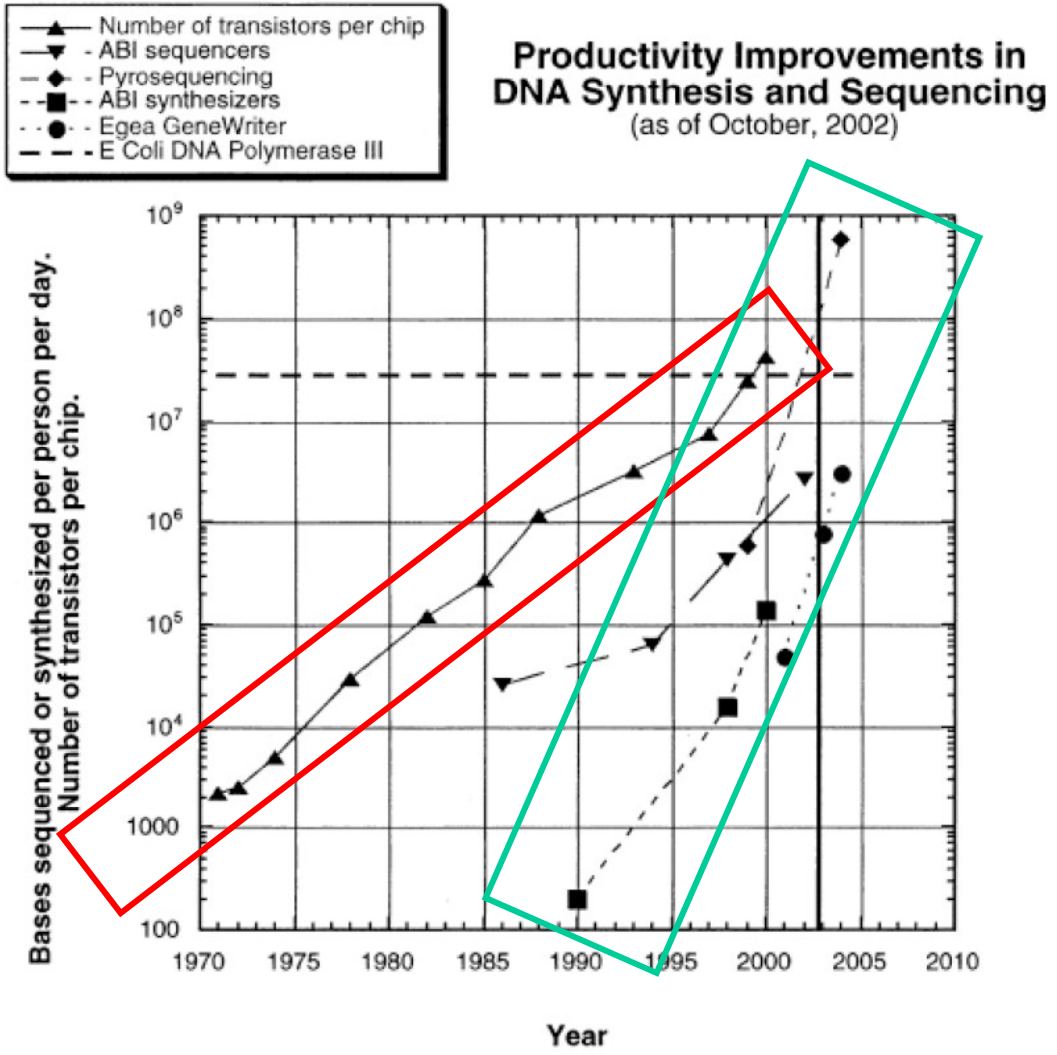
The implementation of effective parallel metabolisms would allow exploiting a much larger diversity of chemical reactions.

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).

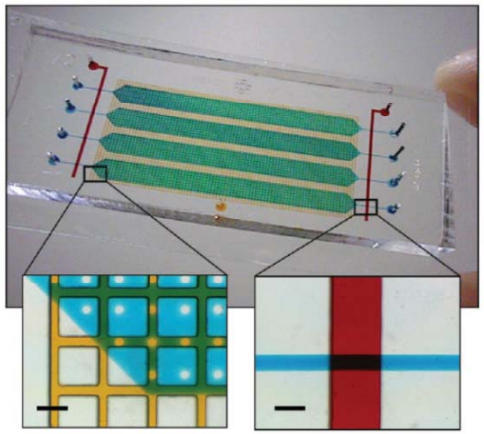
Massive increases in design power – 1: Massively increased *de novo* DNA synthesis capacity



Carlson, Pace & Proliferation of Biological Technologies, Biosec. & Bioterror. 1(3):1 (2003)

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

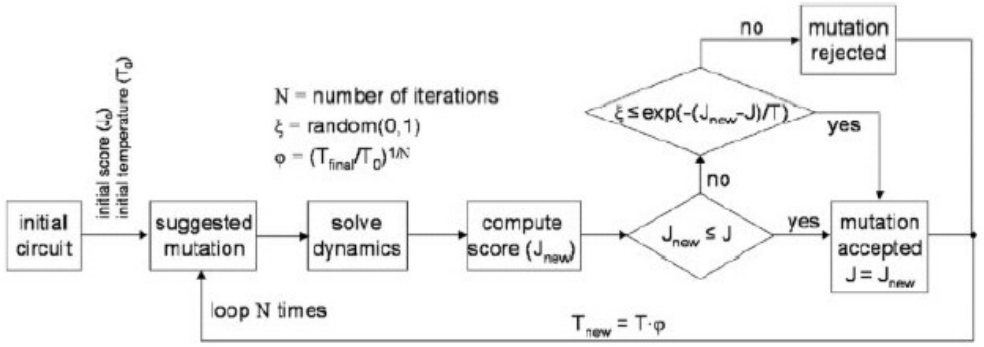
DNA/parts assembly
Parts characterization



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

Microfluidics solutions

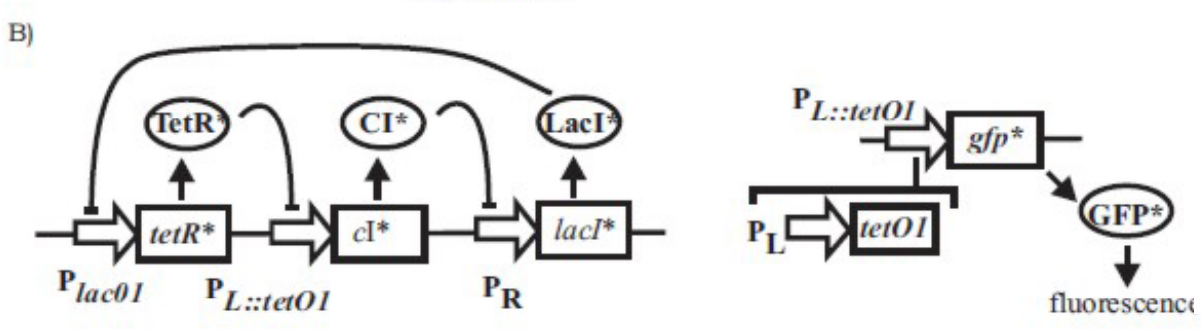
Computer-aided network design
(Emergence)

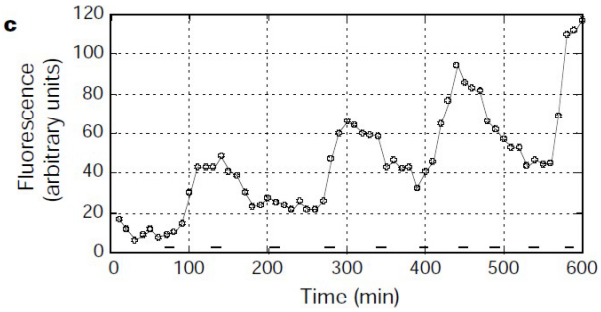


. . . AAGCTTCCAGAATT . . .

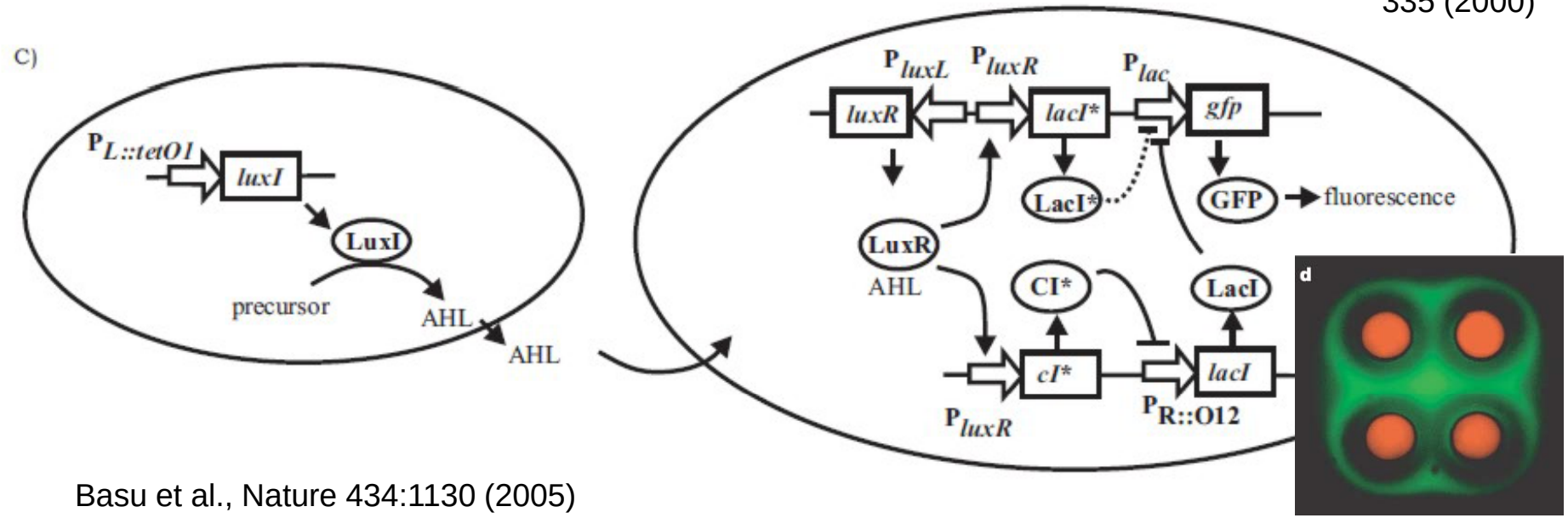
Rodrigo *et al*, Bioinformatics 23:14
Marchisio & Stelling, Bioinformatics 24:1903

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



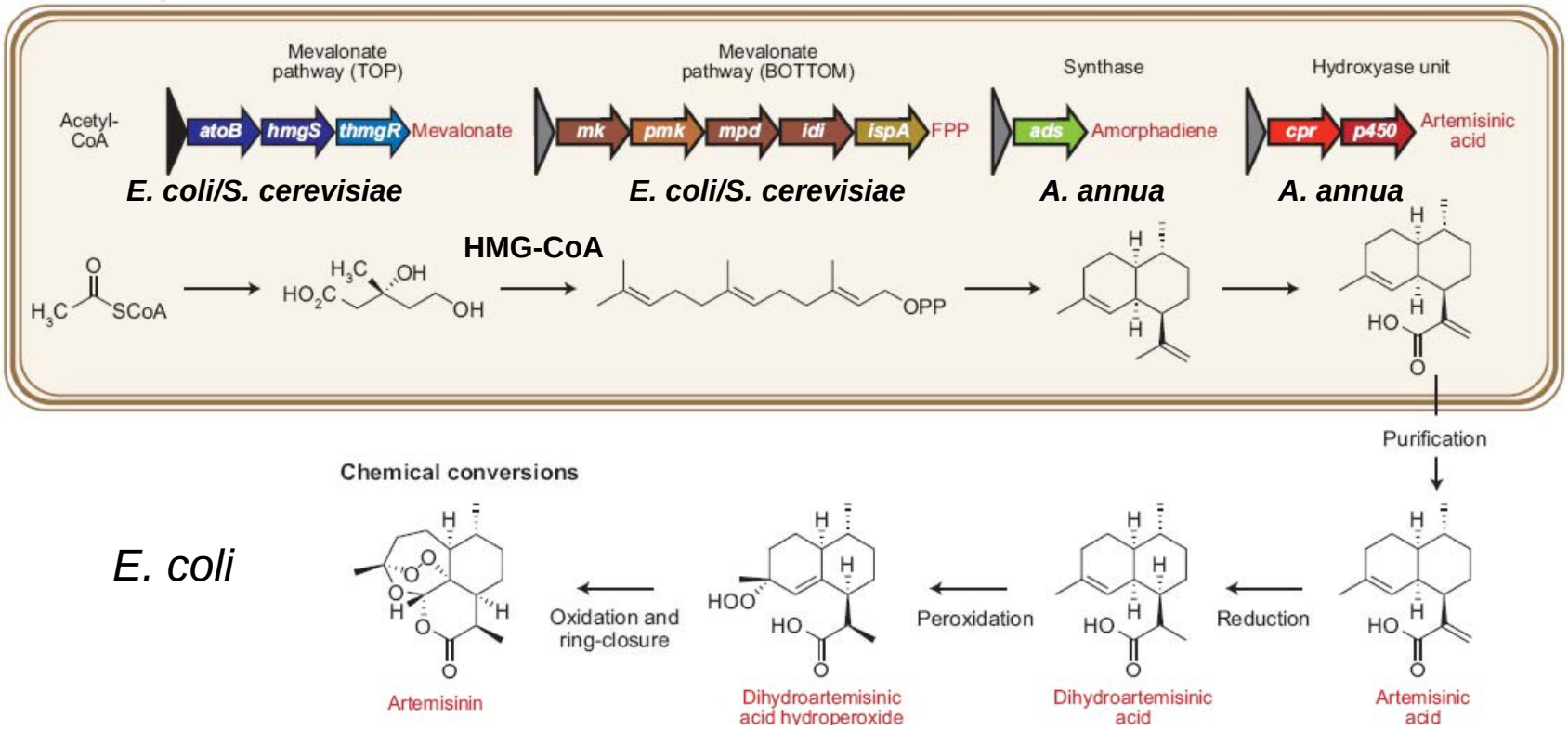


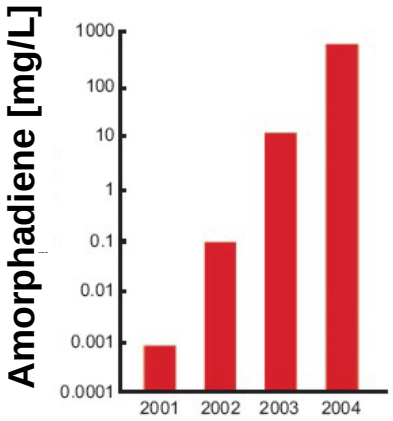
Elowitz & Leibler, Nature 403: 335 (2000)



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

... or complex synthetic biochemical routes





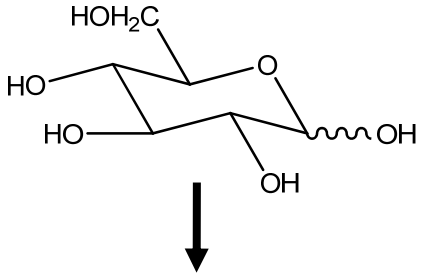
Combination of genes from 3 host in *E. coli* – production of a precursor for an antimalaria drug

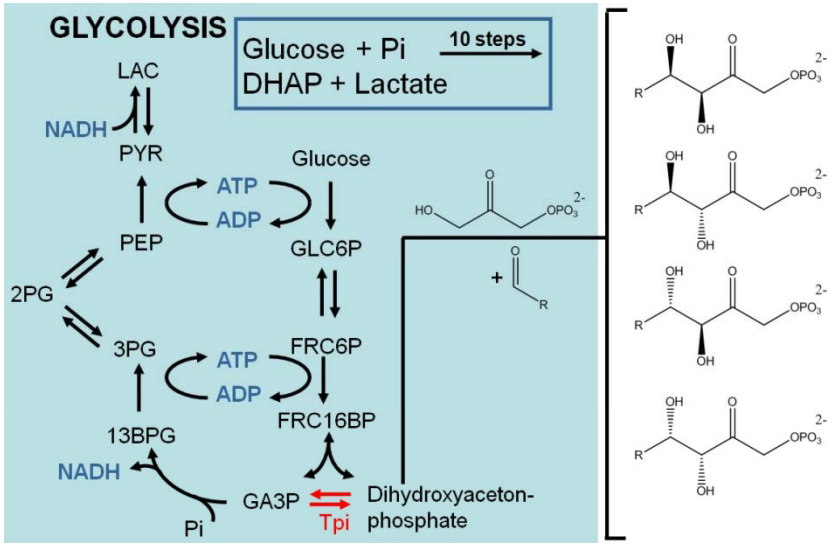
J. Keasling – Lab
UC Berkeley

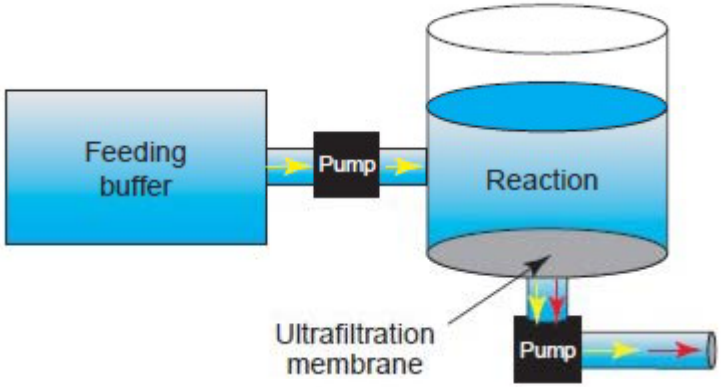
AMYRIS BIOTECHNOLOGIES

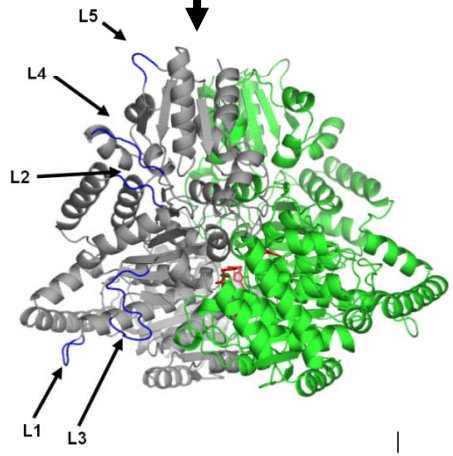
sanofi aventis

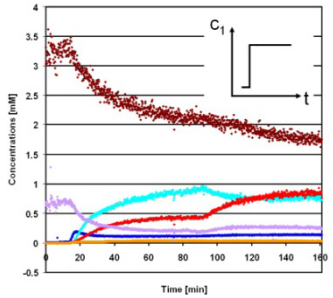
Simplified chassis
Reduced complexity – cell free systems





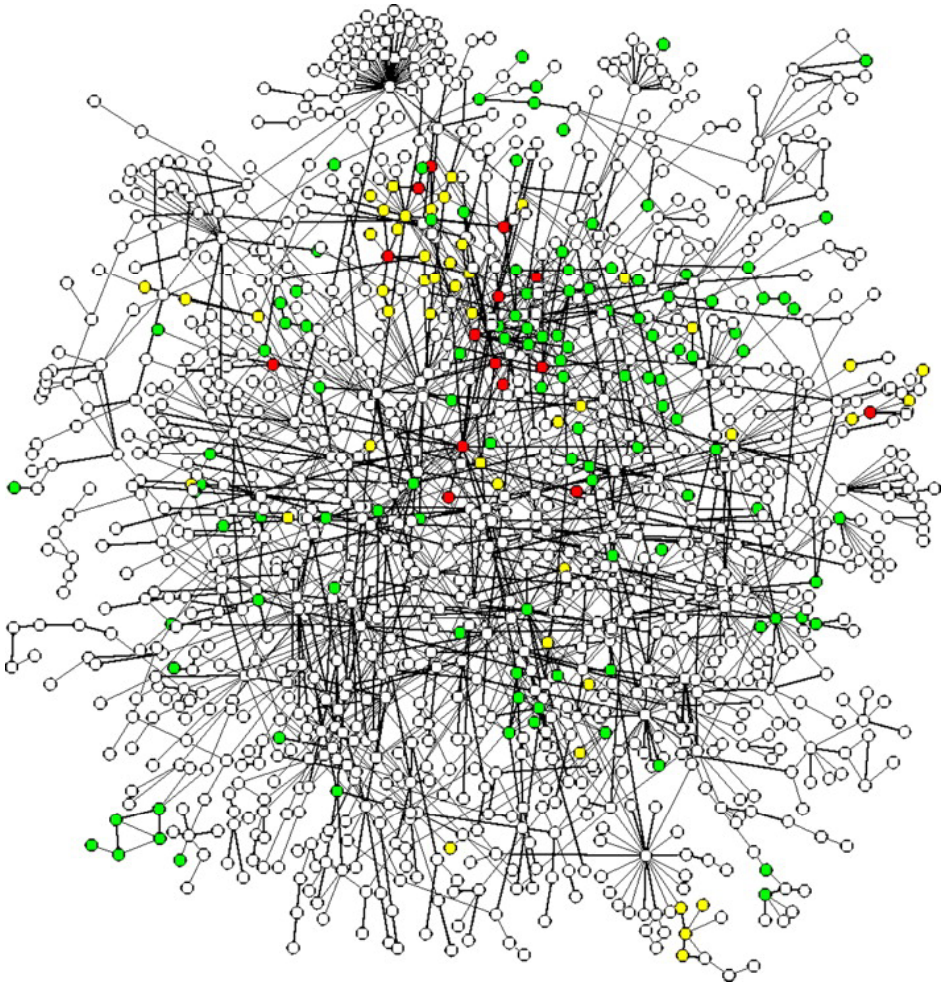


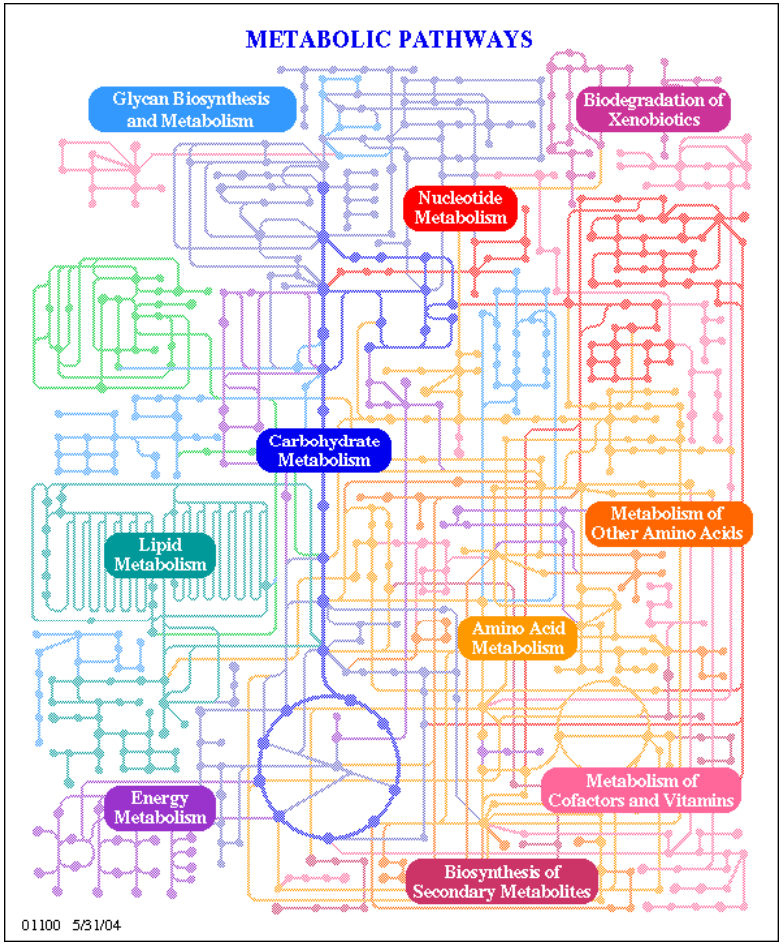




Systems of biotransformations, Panke-lab, ETH Zurich
Cell-free protein synthesis, Swartz-lab, Stanford

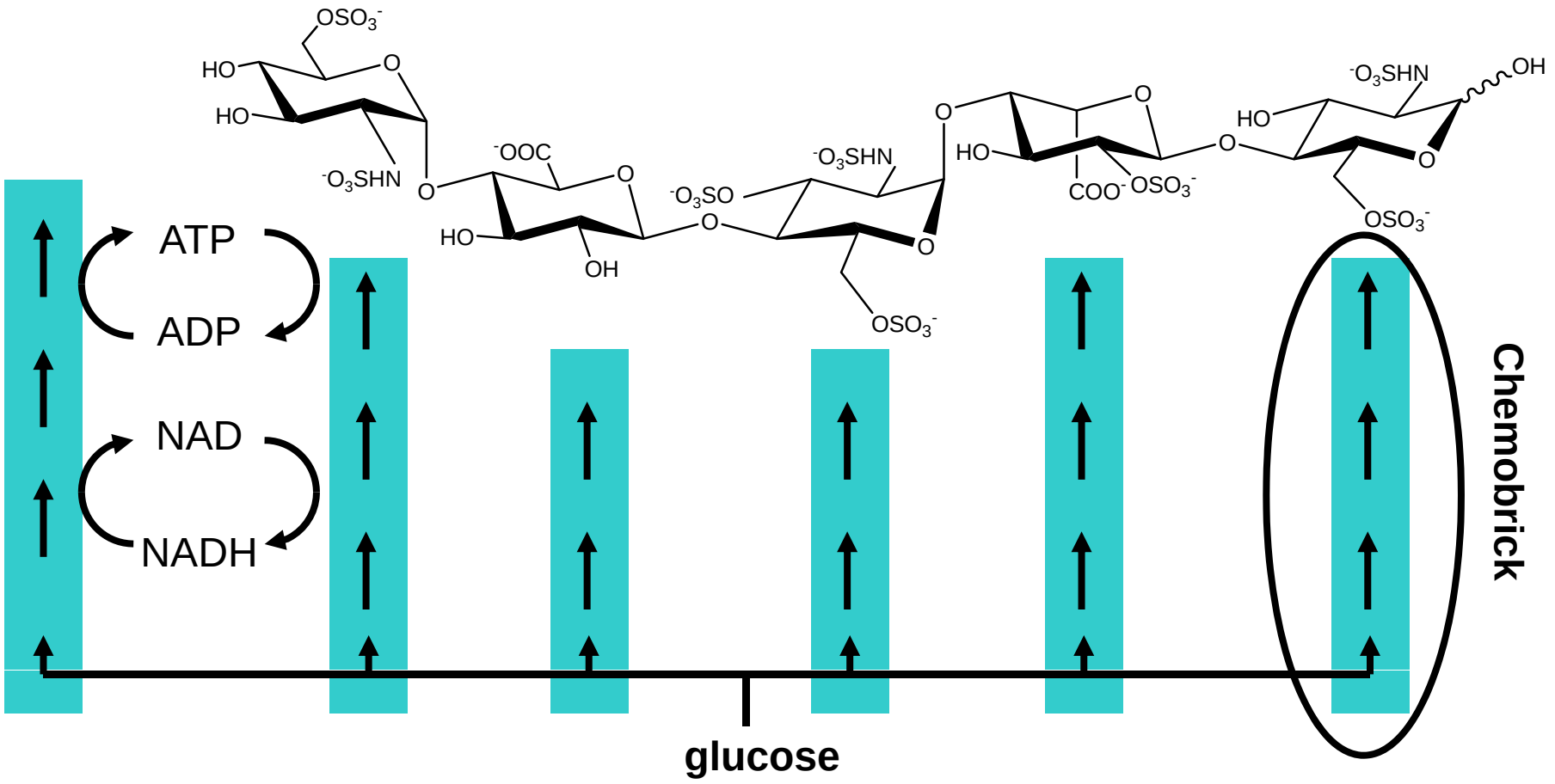
Starting point:
Simplicity from complexity?



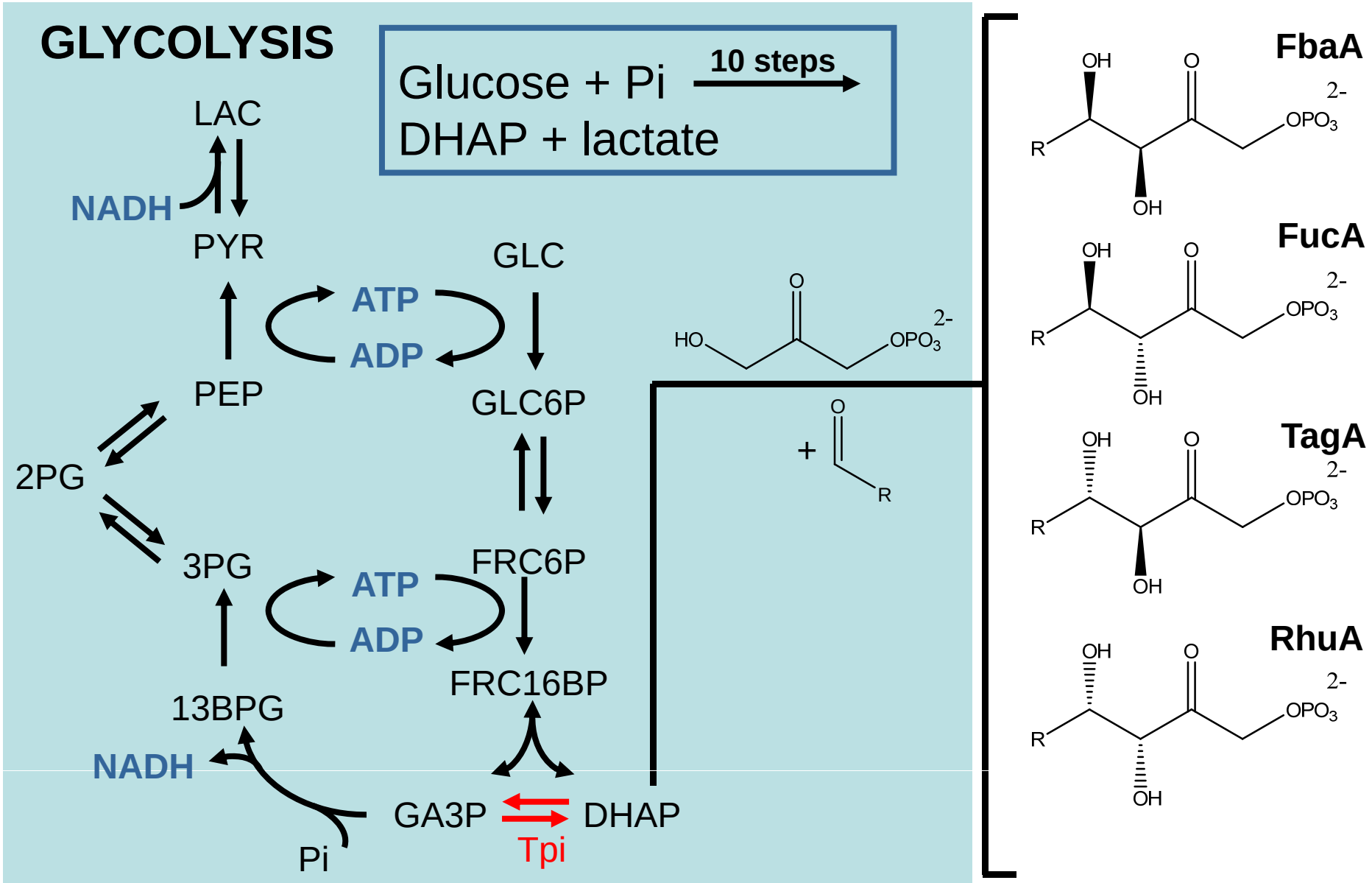


Forgacs et al., J. Cell Science 117: 2769

(Future) End point



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



Enforcing orthogonality - Protein switches

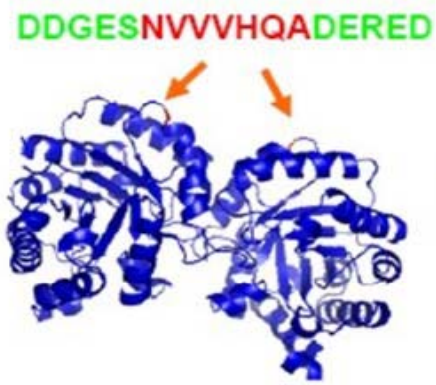
eurobiosyn
a modular platform for biosynthesis of complex molecules

ITB

CSIC

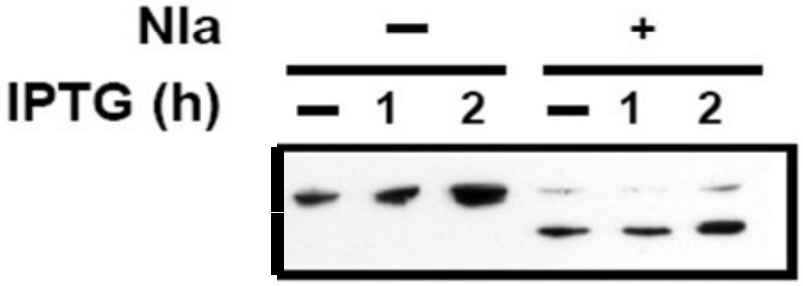
CNB
CENTRO NACIONAL DE BIOTECNOLOGIA

Tpi with proteolytic site inserted



NIa protease

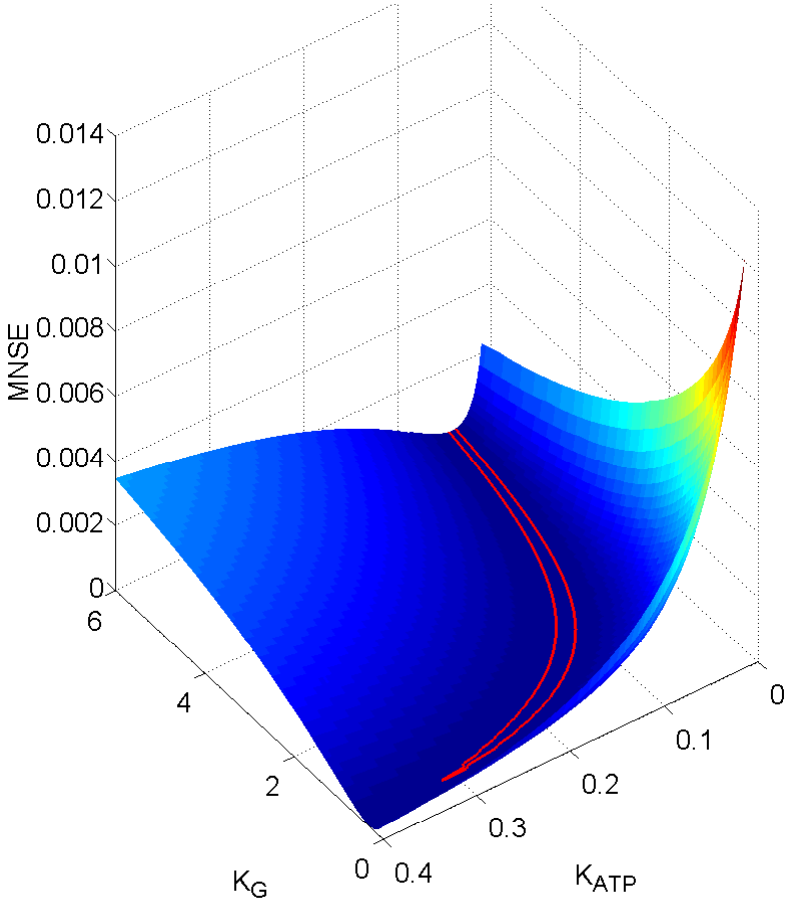
NVVVHQA

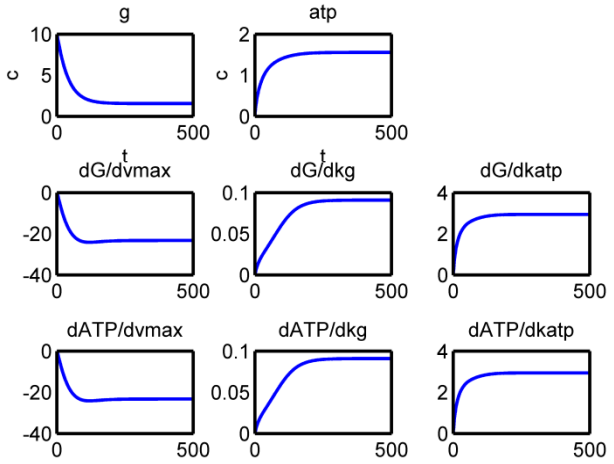


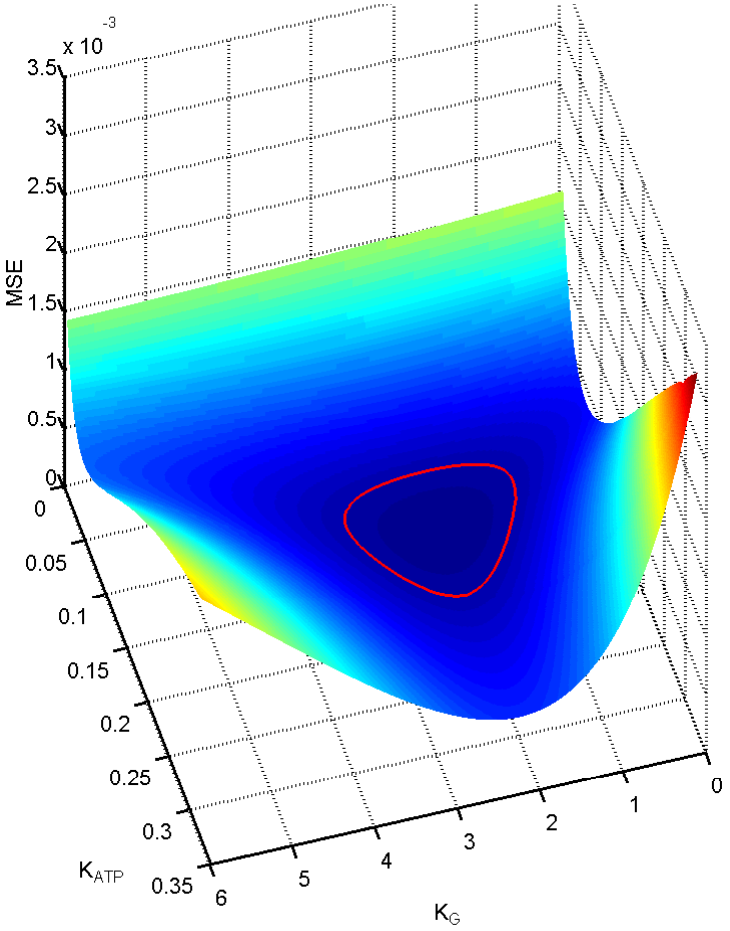
Etag-detection

Design of experiments – batch vs continuous for parameterization of glucokinase

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







... to eanble true network engineering

$$\frac{dc_G}{dt} = -r_{GK} + D(c_G^{ein} - c_G)$$
$$\frac{dc_{G6P}}{dt} = r_{GK} - r_{PGI} - Dc_{G6P}$$
$$\frac{dc_{F6P}}{dt} = r_{PGI} - r_{PFK1} - Dc_{F6P}$$
$$\frac{dc_{FBP}}{dt} = r_{PFK1} - r_{ALD2} - Dc_{FBP}$$
$$\frac{dc_{DHAP}}{dt} = r_{ALD2} - Dc_{DHAP}$$
$$\frac{dc_{GAP}}{dt} = r_{ALD2} - r_{GDH} - Dc_{GAP}$$
$$\frac{dc_{BPG}}{dt} = r_{GDH} - r_{PGK} - Dc_{BPG}$$
$$\frac{dc_{3PG}}{dt} = r_{PGK} - r_{PGM} - Dc_{3}$$
$$\frac{dc_{2PG}}{dt} = r_{PGM} - r_{ENO} -$$
$$\frac{dc_{PEP}}{dt} = r_{ENO} - r_{PY} \quad K2 - Dc_{PEP}$$
$$\frac{dc_{PYR}}{dt} = r_{PYK1} + \quad - r_{LDH} - Dc_{PYR}$$
$$\frac{dc_{LAC}}{dt} = r_{LD} \quad C$$
$$\frac{dc_{ATP}}{dt} = \quad PFK1 + r_{PGK} + r_{PYK1} + r_{PYK2}$$
$$K + D(c_{ATP}^{ein} - c_{ATP})$$
$$\frac{dc_{ADP}}{dt} = r_{GK} + r_{PFK1} - r_{PGK} - r_{PYK1} - r_{PYK2}$$
$$+2r_{ADK} - Dc_{ADP}$$
$$\frac{dc_{AMP}}{dt} = -r_{ADK} - Dc_{AMP}$$
$$\frac{dc_{Pi}}{dt} = -r_{GDH} + D(c_{Pi}^{ein} - c_{Pi})$$
$$\frac{dc_{NADH2}}{dt} = r_{GDH} - r_{LDH} - Dc_{NADH2}$$
$$\frac{dc_{NAD}}{dt} = -r_{GDH} + r_{LDH} - D(c_{NAD}^{ein} - c_{NAD})$$

18 balanced metabolites

$$v_{GK} = \frac{V_{GK}[\mathrm{G}][\mathrm{ATP}]}{K_G K_{ATP} + [\mathrm{G}][\mathrm{ATP}] + K_{ATP}[\mathrm{G}]} \qquad v_{PGI} = \frac{V_{PGI}\left([\mathrm{G6P}] - \frac{[\mathrm{F6P}]}{K_{PGI}^{eq}}\right)}{K_{G6P} + [\mathrm{G6P}] + \frac{K_{G6P}}{K_{F6P}}[\mathrm{F6P}]}$$

$$v_{PFK1} = V_{PFK1}\bar{Y}_{MM}\bar{Y}_{MWC}$$

$$\bar{Y}_{MWC} = \frac{\alpha(1+\alpha)^{n-1} + L'\alpha c}{L' + (1+\alpha)^n}$$

$$\bar{Y}_{MM} = \frac{[\mathrm{ATP}]}{K_{ATP,PFK1}(1 + [\mathrm{ADP}]/K_{ADP,PFK1}) + [\mathrm{ATP}]}$$

$$v_{ALD2} = \frac{V_{ALD2}\left([\mathrm{FBP}] - \frac{[\mathrm{GAP}][\mathrm{D}}{K_{A}^{eq}}\right)}{K_{FBP,ALD2} + [\mathrm{FBP}] + \frac{K_{FBP,ALD2}}{K_{DHAP,ALD2}}[\mathrm{DHAP}] + \quad \overline{\quad}[\mathrm{GAP}][\mathrm{DHAP}]}$$

$$v_{GDH} = V \frac{[\mathrm{NAD}]^n[\mathrm{GAP}][\mathrm{P}_i] - \frac{[\mathrm{NADH}}{}}{K_{NAD}^n K_{GAP} K_{Pi}(1 + \quad \frac{}{AP} + \frac{[\mathrm{P}_i]}{K_{Pi}}}$$
$$+ \frac{[\mathrm{NAD}]^n[\mathrm{GAP}]}{K_{NAD}^n K_{G}} + \frac{[\mathrm{NAD}]^n[\mathrm{P}_i]}{K_{NAD}^n K_{Pi}}$$
$$+ \frac{[\mathrm{NAD}]^n \mathrm{GAP}[\mathrm{P}_i]}{K_{NAD}^n K_{GAP} K_{Pi}} + \frac{[\mathrm{NAD}}{K_{}^n} \quad \frac{[\mathrm{NADH}]^n[\mathrm{BPG}]}{K_{NADH}^n K_{BPG}})$$

$$v_{PGK} = \frac{V \quad \mathrm{DP}] - \frac{[\mathrm{3PG}][\mathrm{ATP}]}{K_{PGK}^{eq}}\right)}{K_{BPG}K_{ADP}\left(1 + \frac{[\mathrm{BP}}{K} \quad \frac{3\mathrm{PG}]}{K_{3PG}} + \frac{[\mathrm{ATP}]}{K_{ATP}} + \frac{[\mathrm{BPG}][\mathrm{ADP}]}{K_{BPG}K_{ADP}} + \frac{[\mathrm{3PG}][\mathrm{ATP}]}{K_{3PG}K_{ATP}}\right)}$$

$$v_{PGM} = \frac{V_{PGM}\left([\mathrm{3PG}] - \right.}{K_{3PG} + [\mathrm{3PG}]} \qquad v_{ENO} = \frac{V_{ENO}\left([\mathrm{2PG}] - \frac{[\mathrm{PEP}]}{K_{ENO}^{eq}}\right)}{K_{2PG} + [\mathrm{2PG}] + \frac{K_{2PG}}{K_{PEP}}[\mathrm{PEP}]}$$

$$v_{PYK1} = \frac{\alpha(1+\alpha)^{} \quad L' = L_0\frac{(1+\beta)^4}{(1+\gamma)^4} \quad \alpha = \frac{[\mathrm{PEP}]}{K_{R,PEP}} \quad \beta = \frac{[\mathrm{ATP}]}{K_{R,ATP}}}{L' + (1 \quad \overline{\mathrm{DP}}}$$

$$v_{PYK2} = V_P \quad \frac{\mathrm{ADP}}{K_{ADP} + \mathrm{ADP}} \quad L_0 = \frac{L}{(1+\gamma)^n} \quad \gamma = \frac{[\mathrm{AMP}]}{K_{T,AMP}} \quad \gamma = \frac{[\mathrm{FBP}]}{K_{T,FBP}}$$

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

$$v_{LDH} = \frac{V_{LDH}\left([\mathrm{NADH}][\mathrm{PYR}] - \frac{[\mathrm{PEP}][\mathrm{NAD}]}{K_{LDH}^{eq}}\right)}{K_{NADH,LDH}K_{PYR,LDH} + [\mathrm{NADH}]K_{PYR,LDH} + \frac{K_{NADH,LDH}K_{PYR,LDH}}{K_{NAD}}[\mathrm{NAD}]}$$

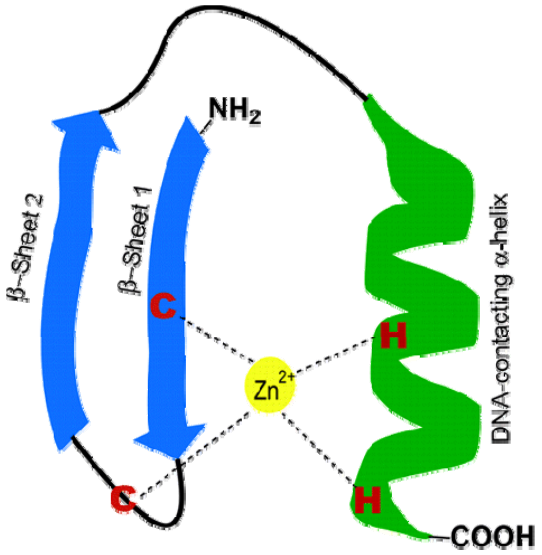
$$v_{ADK} = \frac{V_{ADK}\left([\mathrm{ATP}][\mathrm{AMP}] - \frac{[\mathrm{ADP}]^2}{K_{ADK}^{eq}}\right)}{K_{ATP}K_{AMP}\left(1 + \frac{[\mathrm{ATP}]}{K_{ATP}} + \frac{[\mathrm{AMP}]}{K_{AMP}} + 2\frac{[\mathrm{ADP}]}{K_{ADP}} + \frac{[\mathrm{ATP}][\mathrm{AMP}]}{K_{ATP}K_{AMP}} + \frac{[\mathrm{ADP}]^2}{K_{ADP}^2}\right)}$$

Design power (2) – molecular modular building blocks

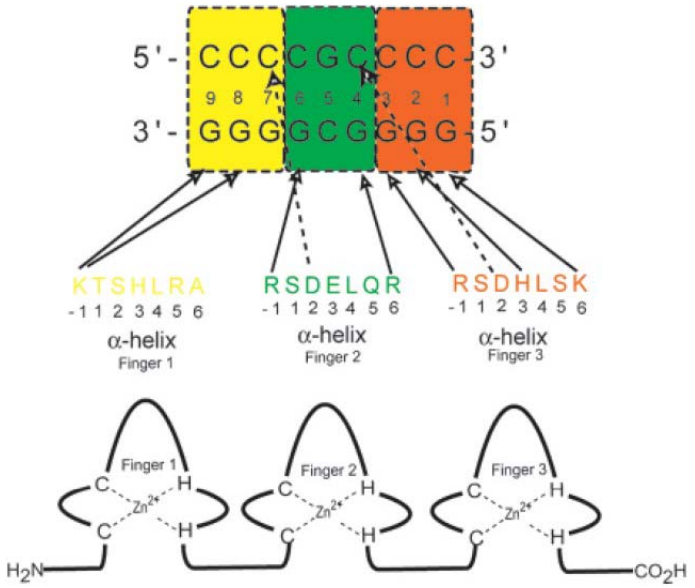
Can we use intramolecular protein domains as modular building blocks to assemble „molecular systems“?

(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.

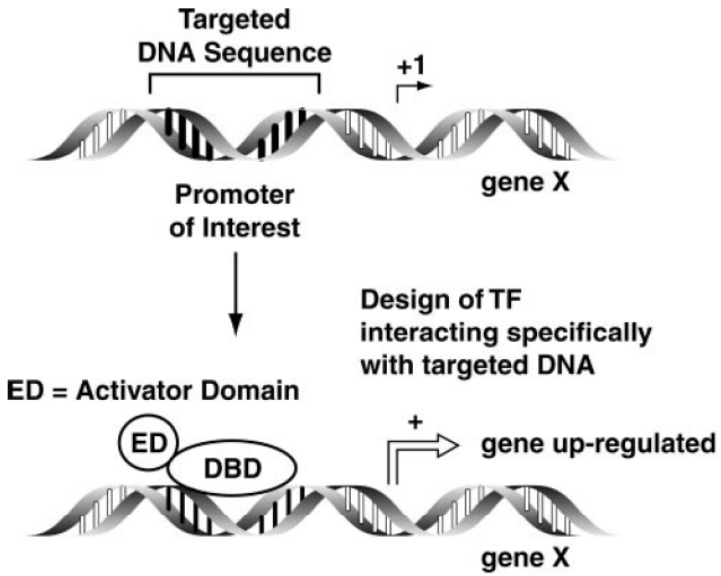


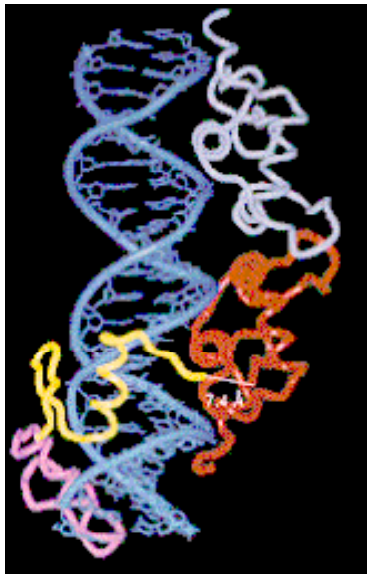
One ZF recognizes one specific DNA triplet. It is possible to generate ZFs for essentially any DNA triplet. By combining ZFs linearly, the DNA recognition sequence of the engineered proteins can be extended – up to 24, which is sufficient to address unique DNA sequences in the human genome.

Designer transcription factors based on zinc finger domains

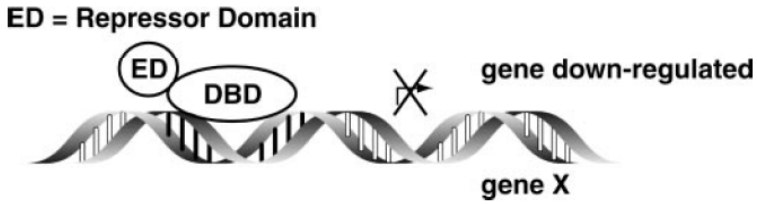
Barbas-lab, Blancafort et al., *Molec. Pharmacol.* 66: 1361 (2004)

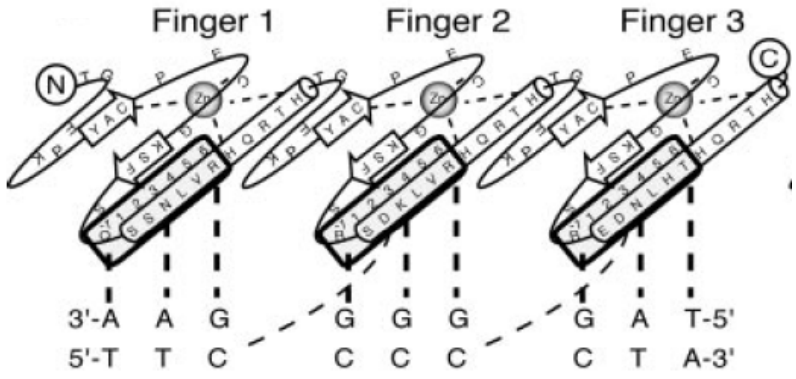
Modular zinc finger domains are recombined to define novel DNA-sequence specificity



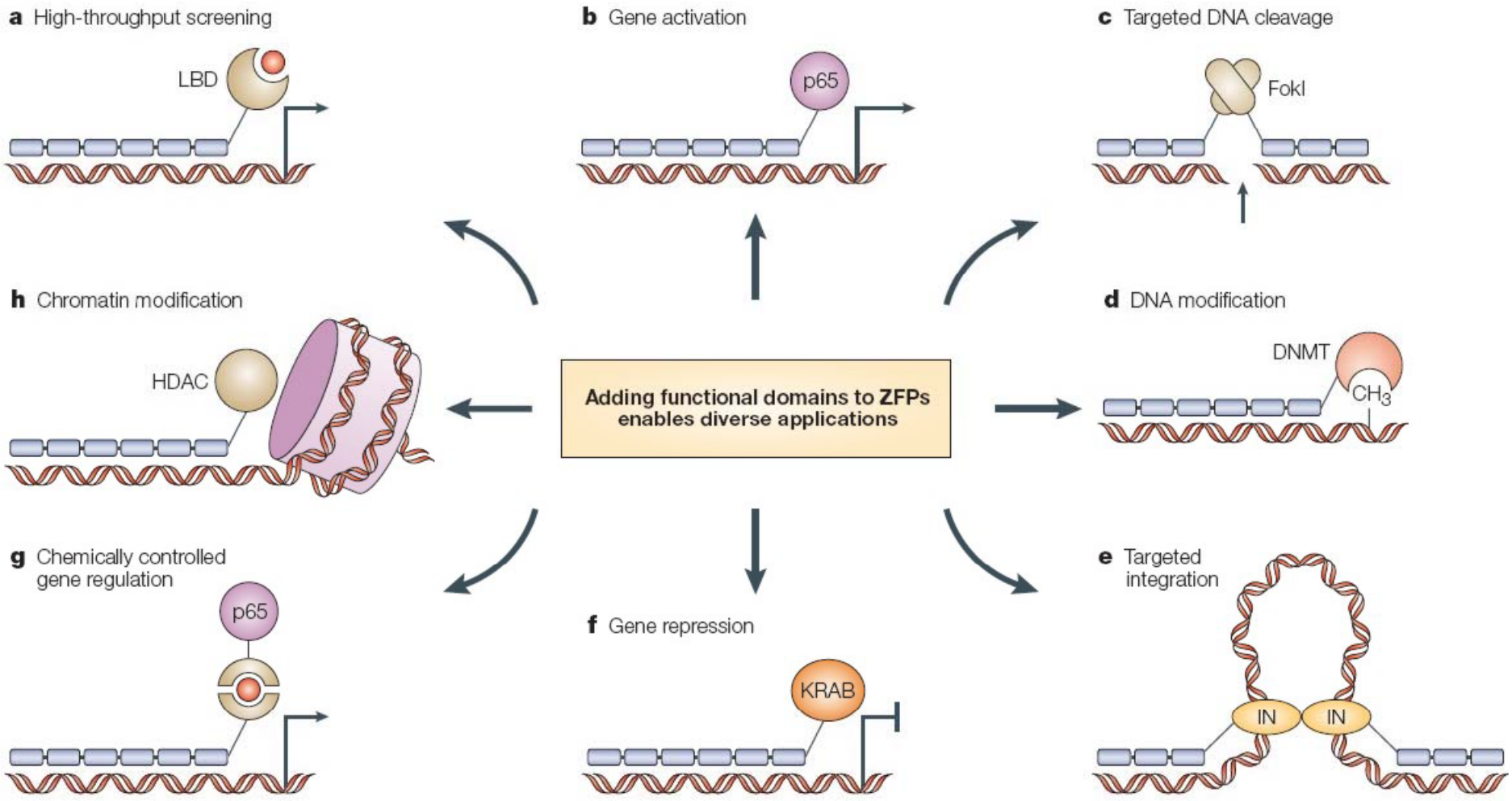


	-1 1 2 3 4 5 6		-1 1 2 3 4 5 6
AAA	QRANLRA	GAA	QSSNLVR
AAC	DSGNLRV	GAC	DPGNLVR
AAGk	RKDNLKN	GAGk	RSDNLVR
AAT	TTGNLTV	GAT	TSGNLVR
ACA	SPADLTR	GCA	QSGDLRR
ACC	DKKDLTR	GCC	DCRDLAR
ACGk	RTDTLRD	GCGk	RSDDLVR
ACT	THLDLIR	GCT	TSGELVR
AGA	QLAHLRA	GGA	QRAHLER
AGC	N/A	GGC	DPGHLVR
AGGk	RSDHLTN	GGGk	RSDKLVR
AGT	HRTTLTN	GGT	TSGHLVR
ATA	QKSSLIA	GTA	QSSSLVR
ATC	N/A	GTC	DPGALVR
ATGk	RRDELNV	GTGk	RSDELVR
ATT	HKNALQN	GTT	TSGSLVR
TAGk	REDNLHT	TGGk	RSDHLTT

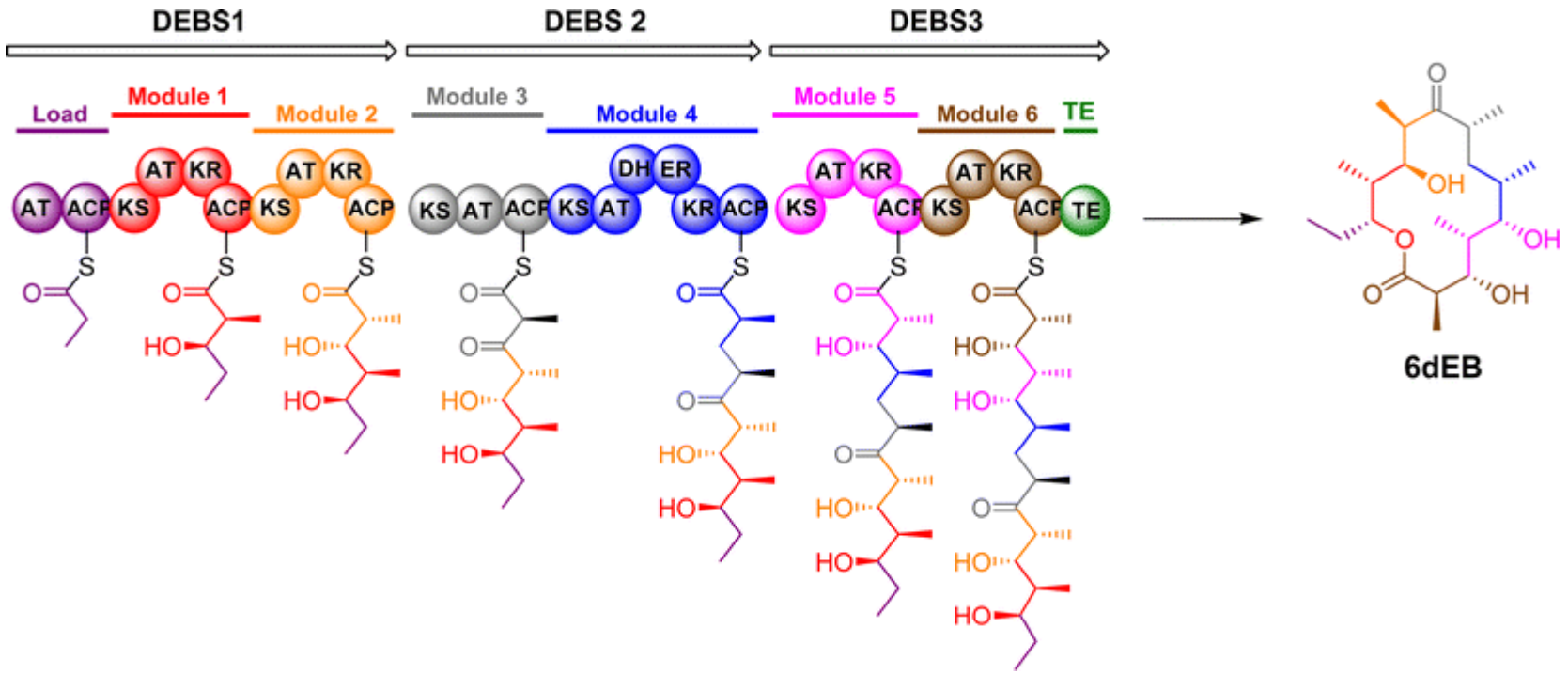




ED: Effector domain for regulation
DBD: DNA-binding domain, designed to recognize target DNA sequence


a High-throughput screening
LBD
b Gene activation
p65
c Targeted DNA cleavage
FokI
h Chromatin modification
HDAC
Adding functional domains to ZFPs enables diverse applications
d DNA modification
DNMT
CH3
g Chemically controlled gene regulation
p65
f Gene repression
KRAB
e Targeted integration
IN
IN

Novel antibiotics and cytostatics from reengineering polyketides



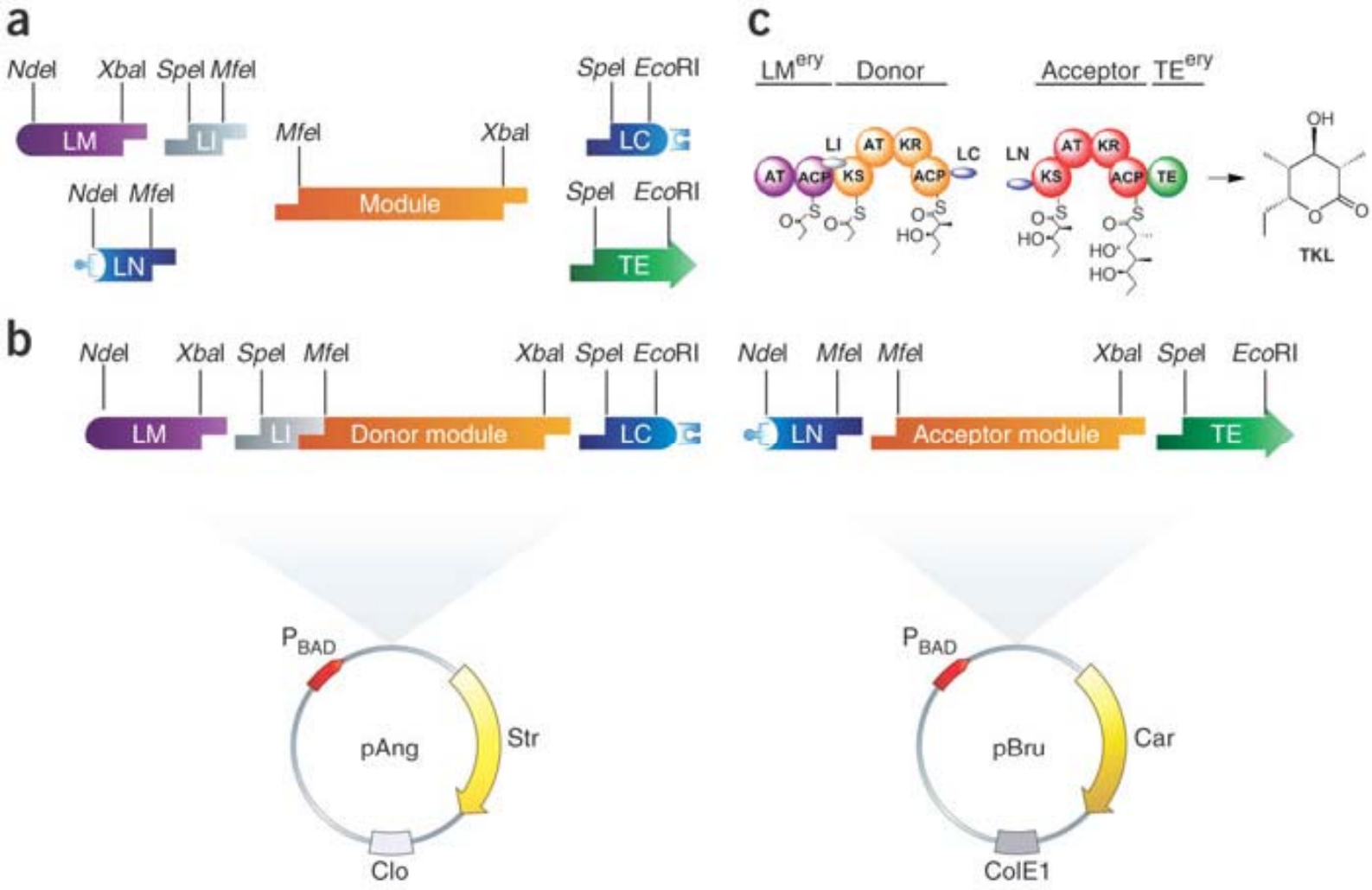
Menzella *et al.*, Nature Biotechnology 23:1171

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

a) *De novo* DNA synthesis of polyketide synthase clusters for synthesis in *E. coli*

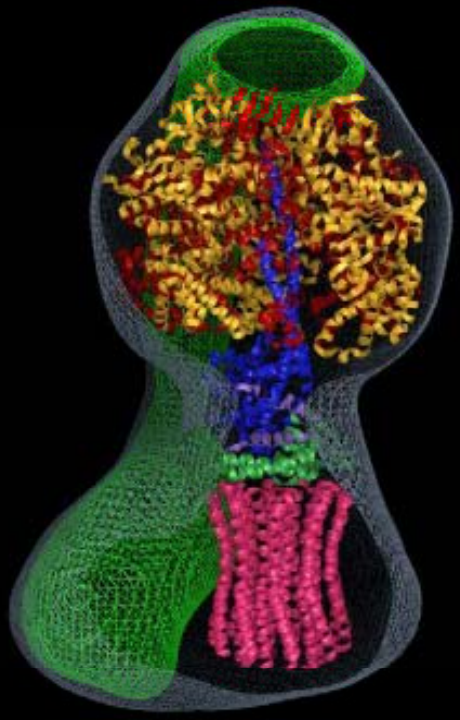
b) Reorganization of cluster DNA (modularization/restriction enzymes)

c) Exchanging of blocks – novel molecules



NANOMOT

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



F-ATPase

Bacteriophage ϕ29
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