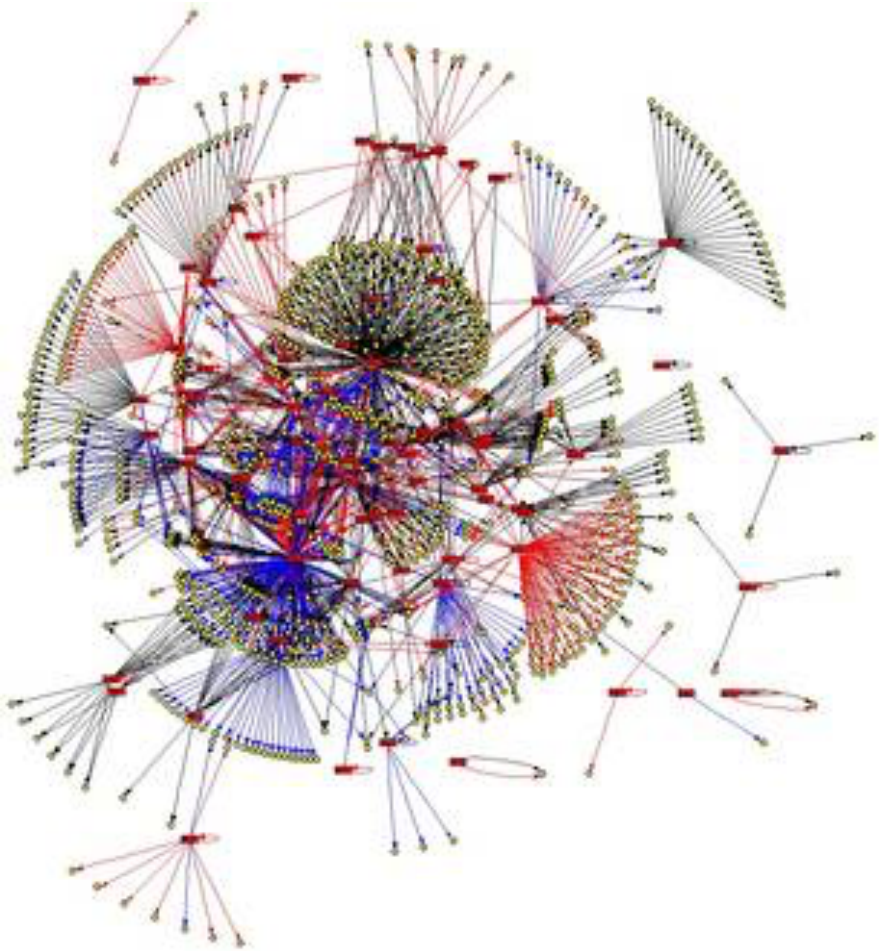
Synthetic and Systems Biology

Andrew Griffiths
Laboratoire de Biochimie
Room B 231
Tel: 01 40 79 45 39
Email: andrew.griffiths@espci.fr

Philippe Nghe
Laboratoire de Biochimie
Room B 229
Tel: 01 40 79 45 87
Email: philippe.nghe@espci.fr



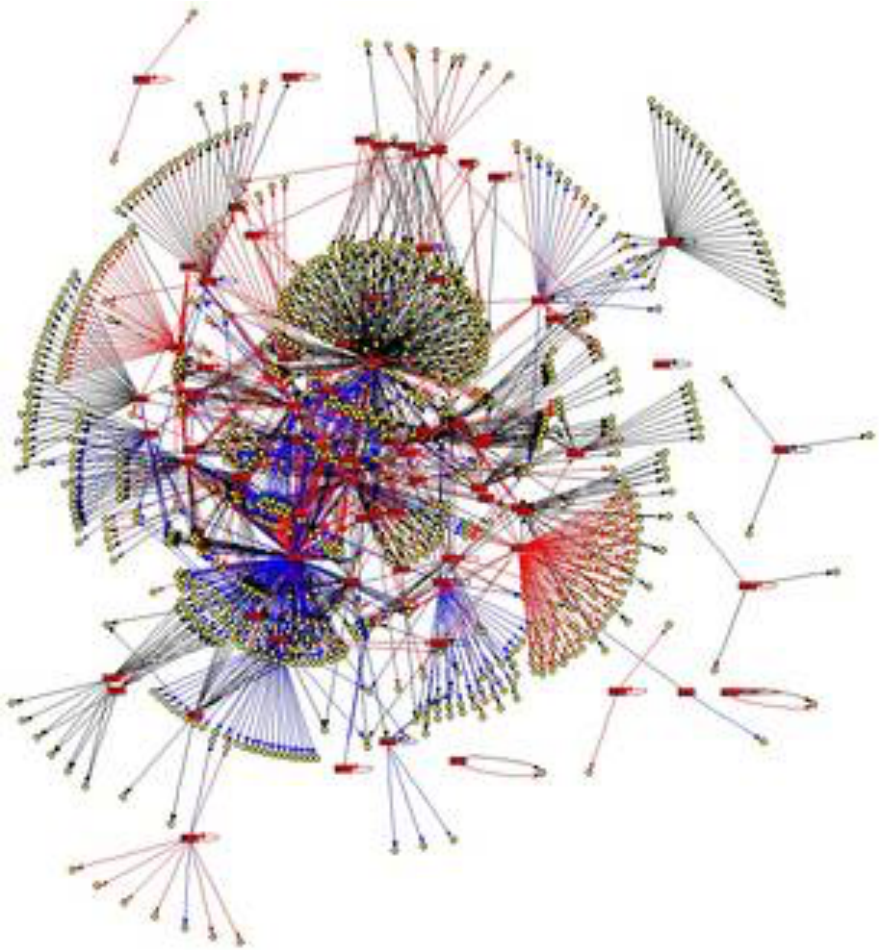
Synthetic and Systems Biology

Supporting material

All Powerpoint presentations and other supporting material (animations/movies/papers) available on the intranet:

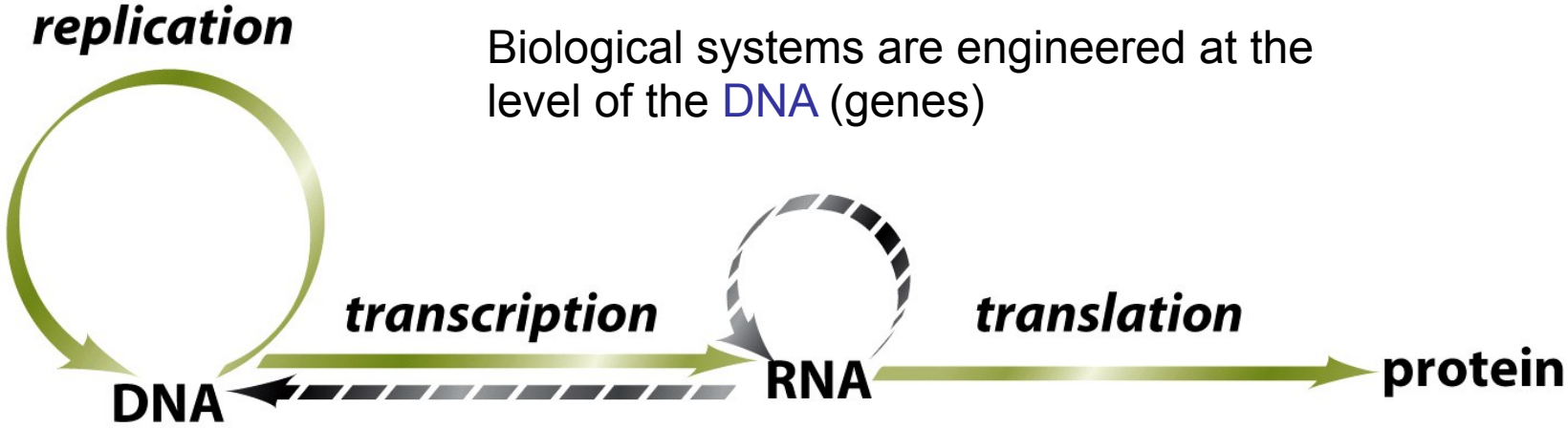
https://cours.espci.fr/

Use your normal intranet username and password



Synthetic Biology
Definition and enabling technologies

Definition
- The design and construction of new biological parts, devices, and systems
- The re-design of existing, natural biological systems for useful purposes.

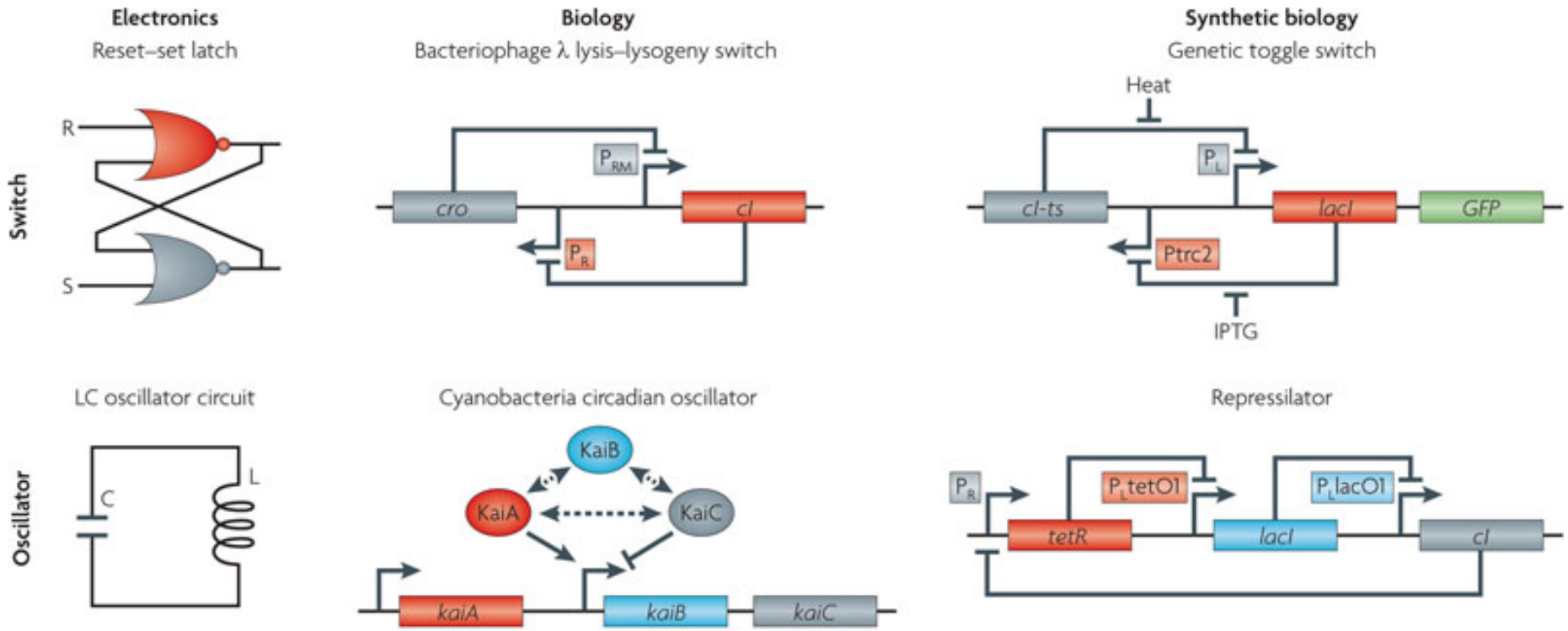


Biological systems are engineered at the level of the DNA (genes)

Key enabling technologies
- Standardized DNA parts
- DNA synthesis
- DNA sequencing
- Modeling

Synthetic Biology

Engineering of biological systems

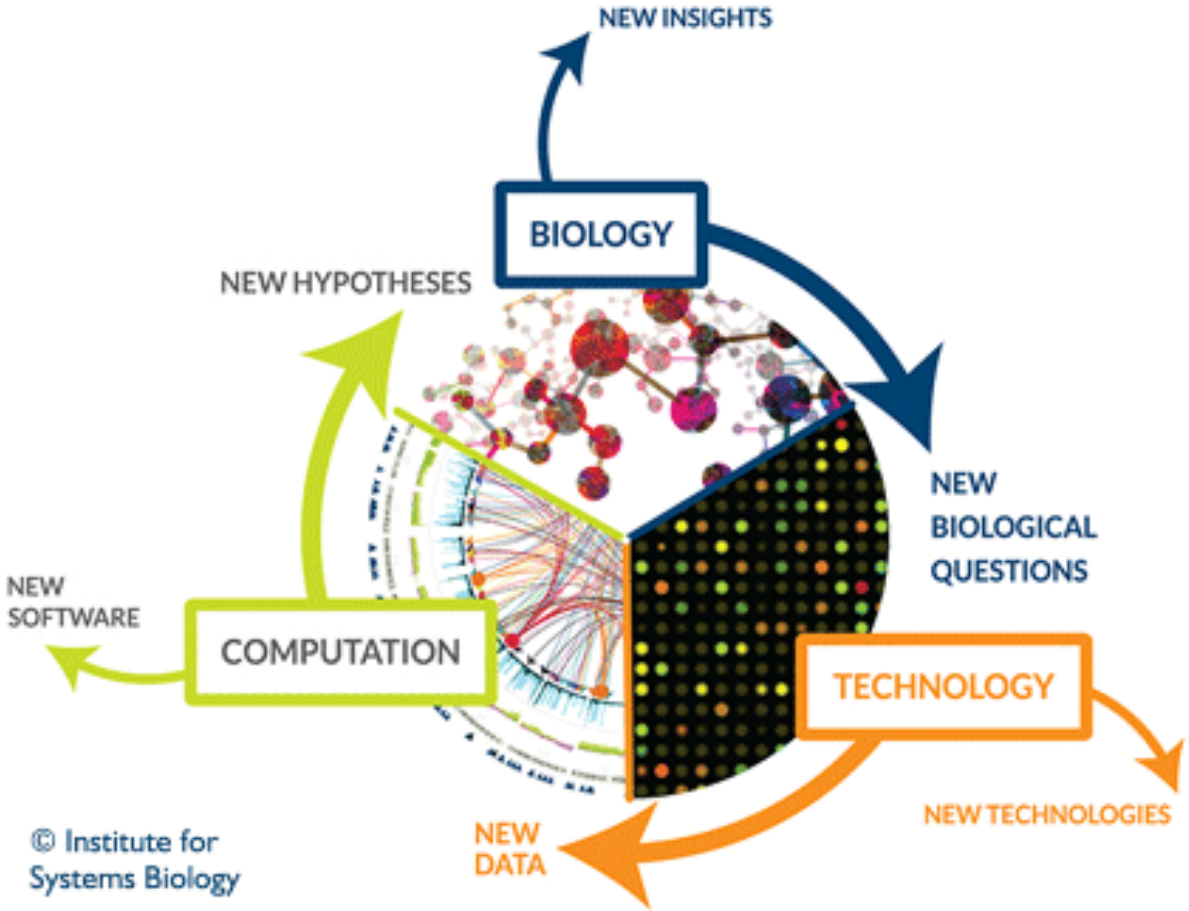


Switches and oscillators that occur in electronic systems are also seen in biology and have been engineered into synthetic biological systems.

Systems Biology
Definition

Definition

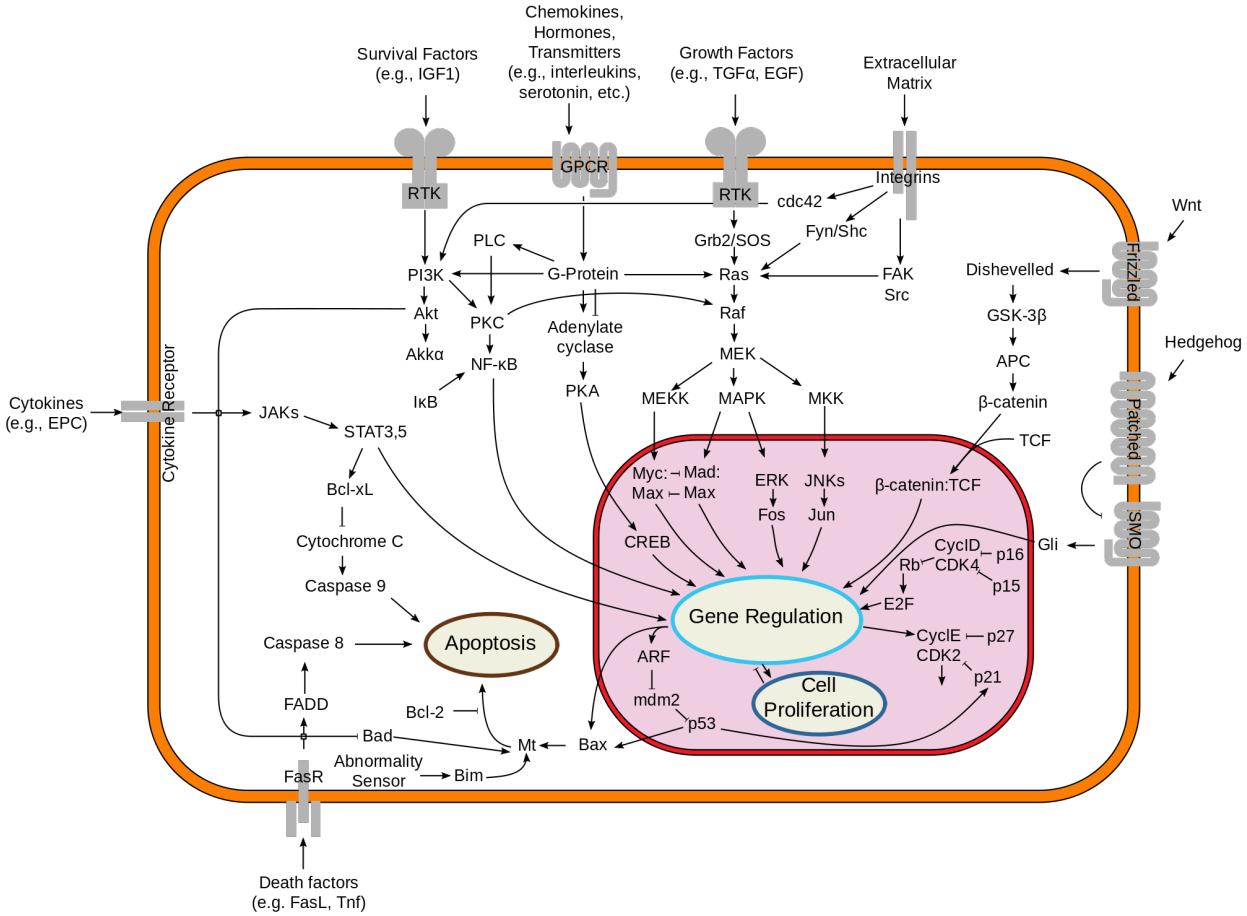
- The computational and mathematical modeling of complex biological systems.



Synthetic and Systems Biology

How are they related

- Biological systems are complex systems



Overview of signal transduction pathways in eukaryotic cell

- Engineering such systems therefore requires systems biology

Synthetic and Systems Biology

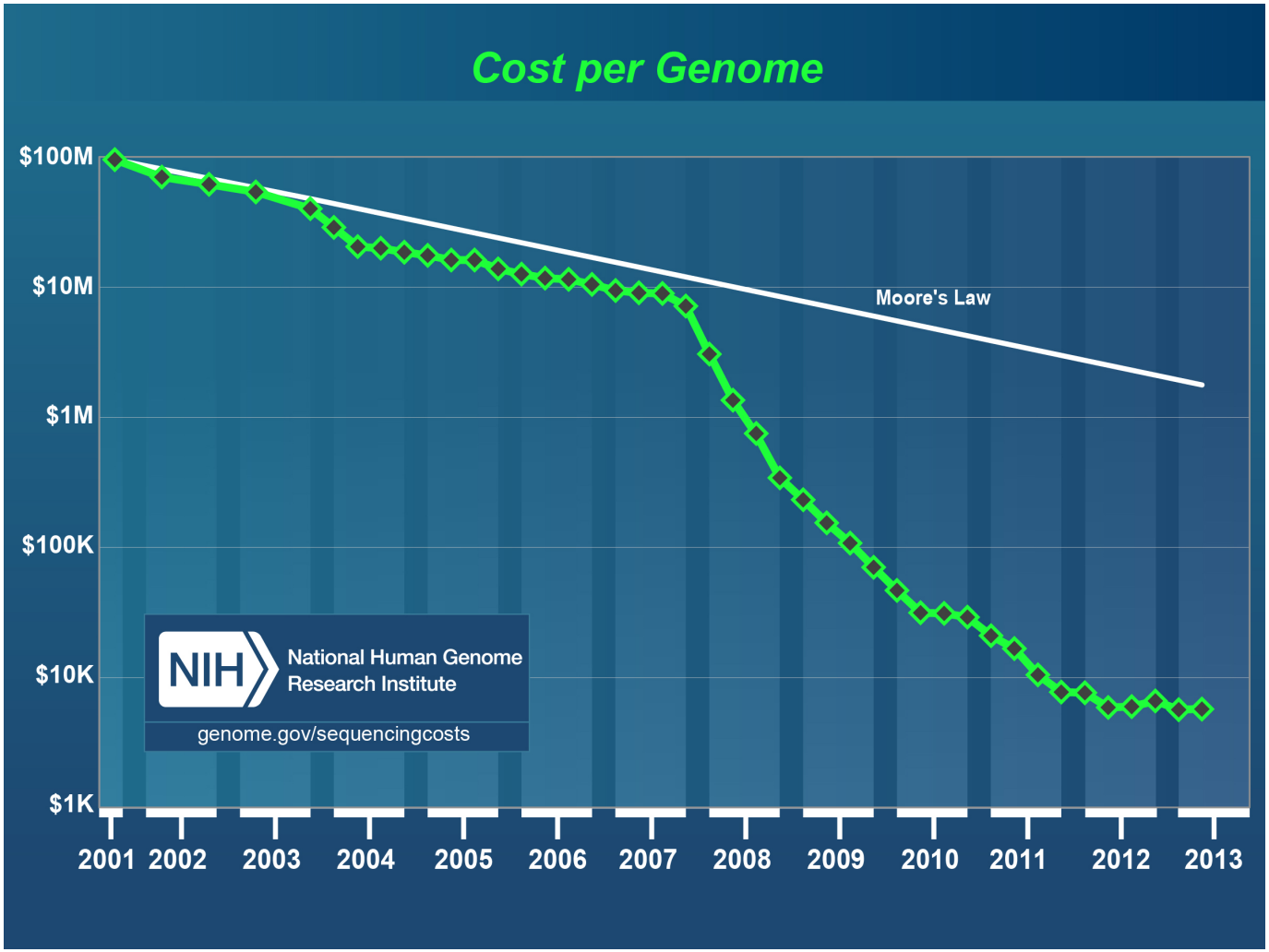
Who, what and when

Date	Day	Time	Duration (h)	Who	What
5 January	Tuesday	14:00-15:00	1	AG/PN	Introduction + project presentation
5 January	Tuesday	15:10-17:30	2	RR	Chemistry and systems biology for drug discovery 1
12 January	Tuesday	14:00-17:30	3	RR	Chemistry and systems biology for drug discovery 2
19 January	Tuesday	14:00-17:30	3	MD	Practical on biological databases 1
26 January	Tuesday	14:00-17:30	3	RR	Chemistry and systems biology for drug discovery 3
2 February	Tuesday	14:00-17:30	3	CN	Physics inspired models of biological systems 1
9 February	Tuesday	14:00-17:30	3	CN	Physics inspired models of biological systems 2
16 February	Tuesday	8:30-12:00	3	JMF	Microbial metabolic pathway engineering and applications 1
16 February	Tuesday	14:00-15:00	1	MD	Flux Balance Analysis software
16 February	Tuesday	15:10-17:30	2	JMF	Microbial metabolic pathway engineering and applications 2
19 February	Friday	13:00-15:00	2	SA	Numerical simulation of gene networks
1 March	Tuesday	14:00-17:30	3	RN	Reading and writing genes 1
4 March	Friday	13:00-15:00	3	RN	Reading and writing genes 2
8 March	Tuesday	14:00-17:30	3	CN	Physics inspired models of biological systems
11 March	Friday	9:40-12:00	2	PN/MD	Group Projects
???			1/2 day		Exam week: presentation of the Group Projects

Andrew	GRIFFITHS	ESPCI
Philippe	NGHE	ESPCI
Clément	NIZAK	ESPCI
Matt	DEYELL	ESPCI
Simon	ARSENE	ESPCI
Rob	NICOL	Broad Institute of Harvard and MIT, Cambridge MA
Jean-Marie	FRANCOIS	Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), Toulouse
Raphaël	RODRIGUEZ	Institut Curie

Reading Genes

Next-generation sequencing systems are revolutionizing modern biology



Human Genome Project's reference genome:

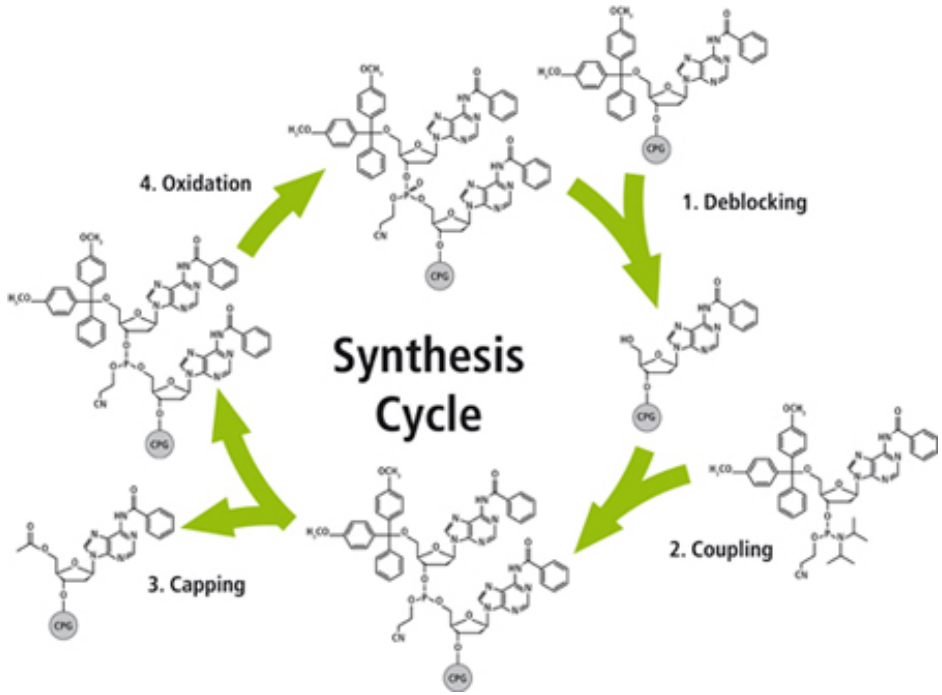
- $3 billion >10 years

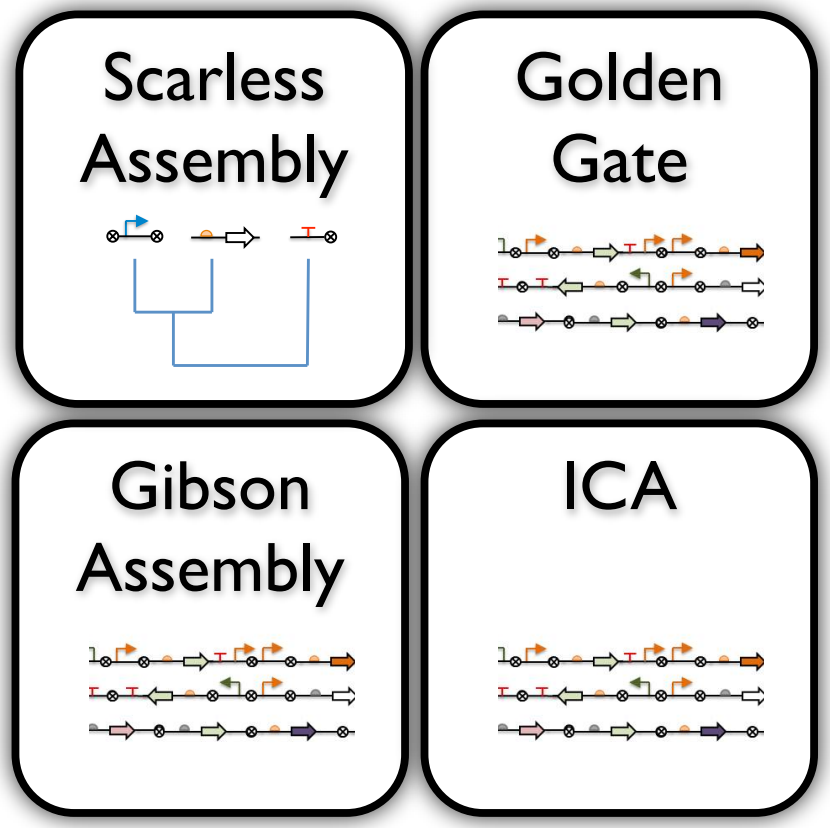
A human genome can now be sequenced for:

- ~$8,000 in ~2 days

Writing Genes

DNA synthesis and assembly



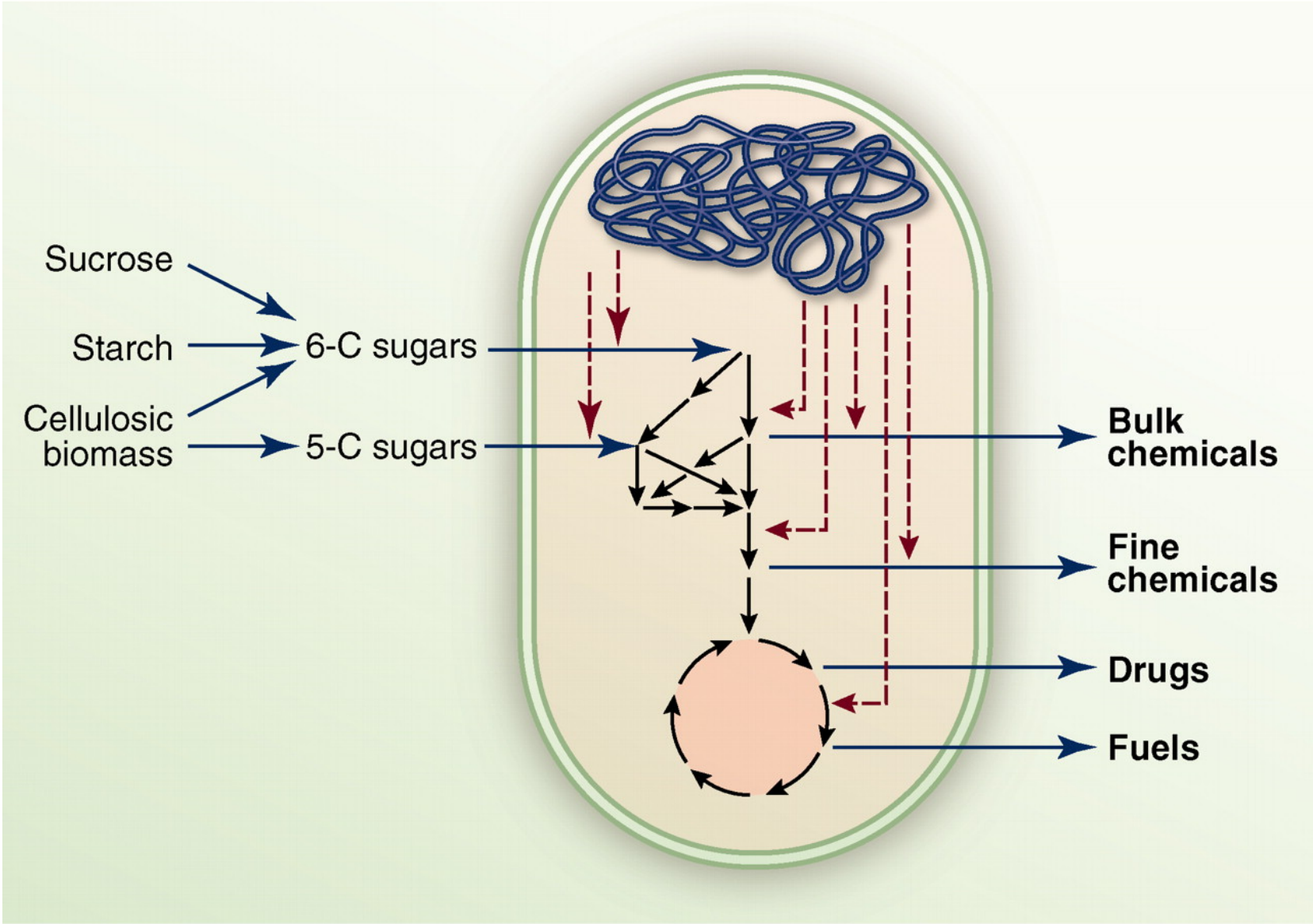


Chemical synthesis of DNA

Assembly of genes and gene clusters

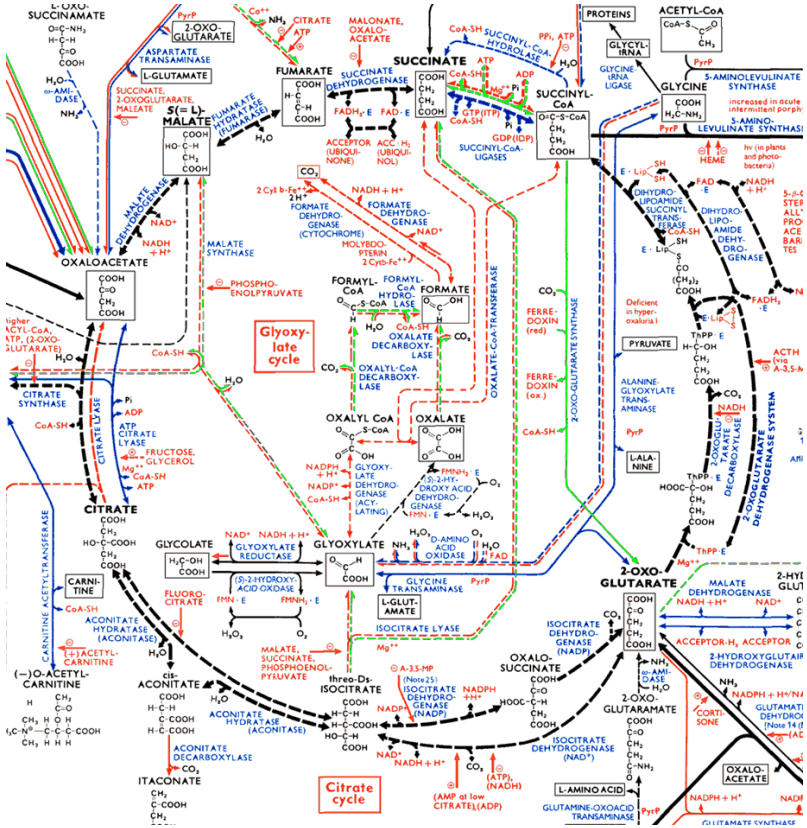
Metabolic Engineering with Microbes

From systems biology to synthetic biology

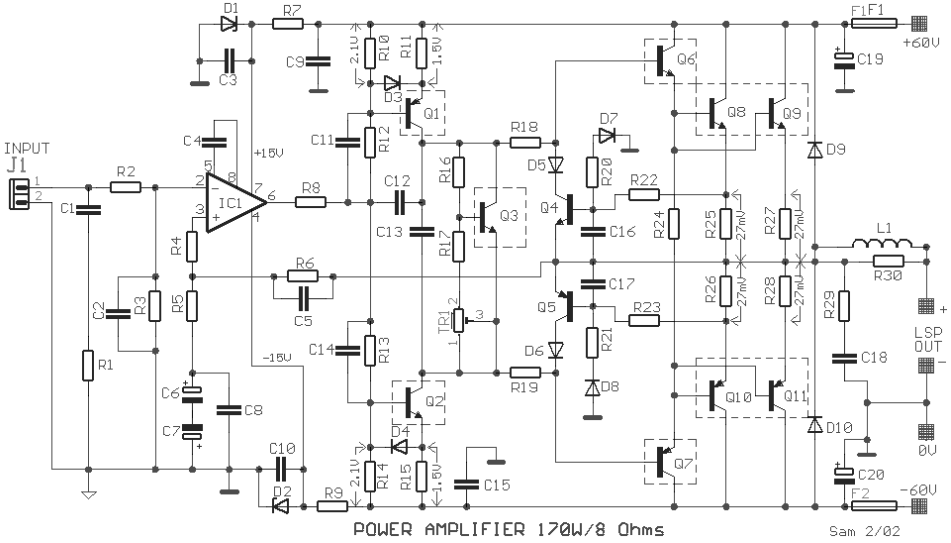


Biological Networks

Applying principles from engineering and physics



Biological (metabolic) system

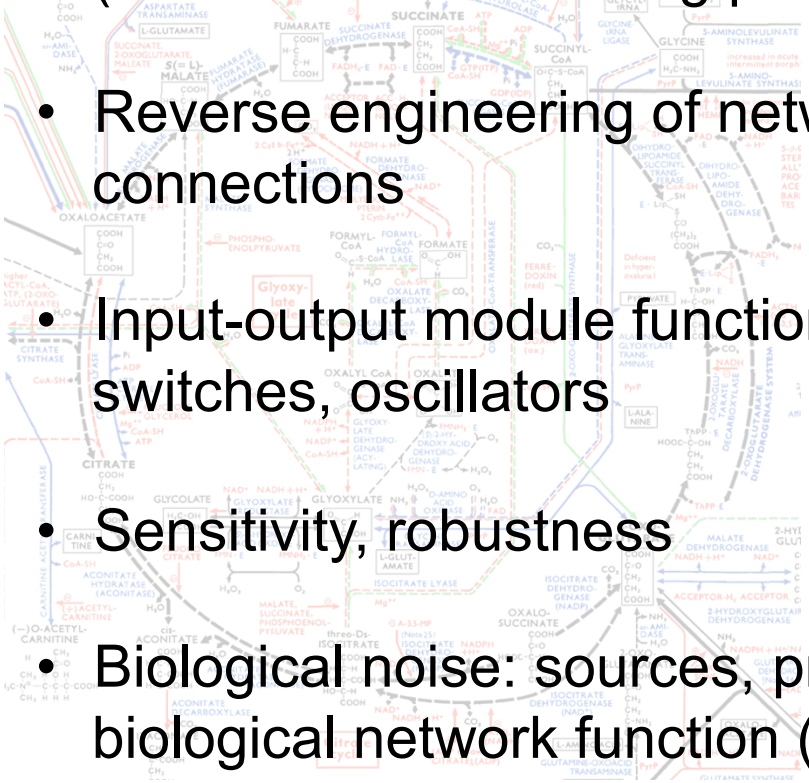


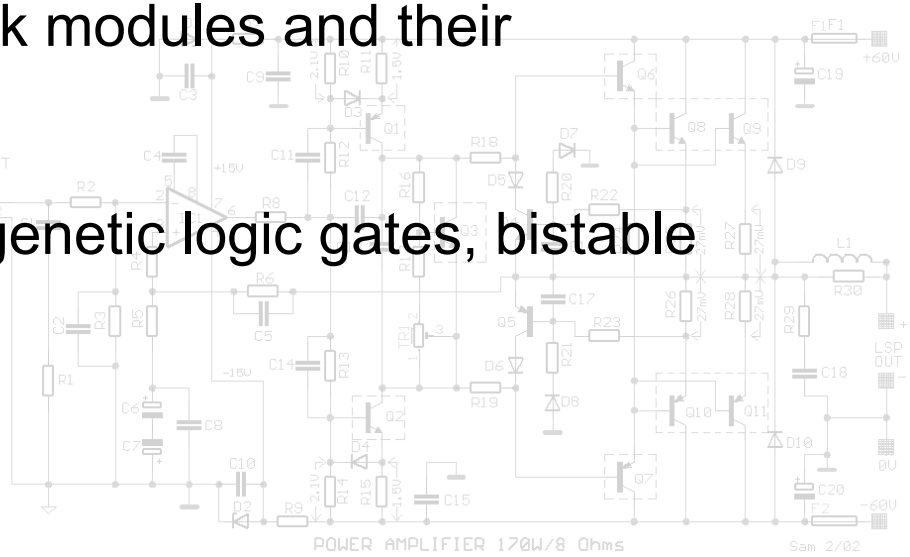
Electronic system

Biological Networks

Applying principles from engineering and physics

- Engineering & physics perspective on genetic networks (evolution as a tinkering process)
- Reverse engineering of network modules and their connections
- Input-output module function: genetic logic gates, bistable switches, oscillators
- Sensitivity, robustness
- Biological noise: sources, propagation, filtering, its role in biological network function (differentiation)



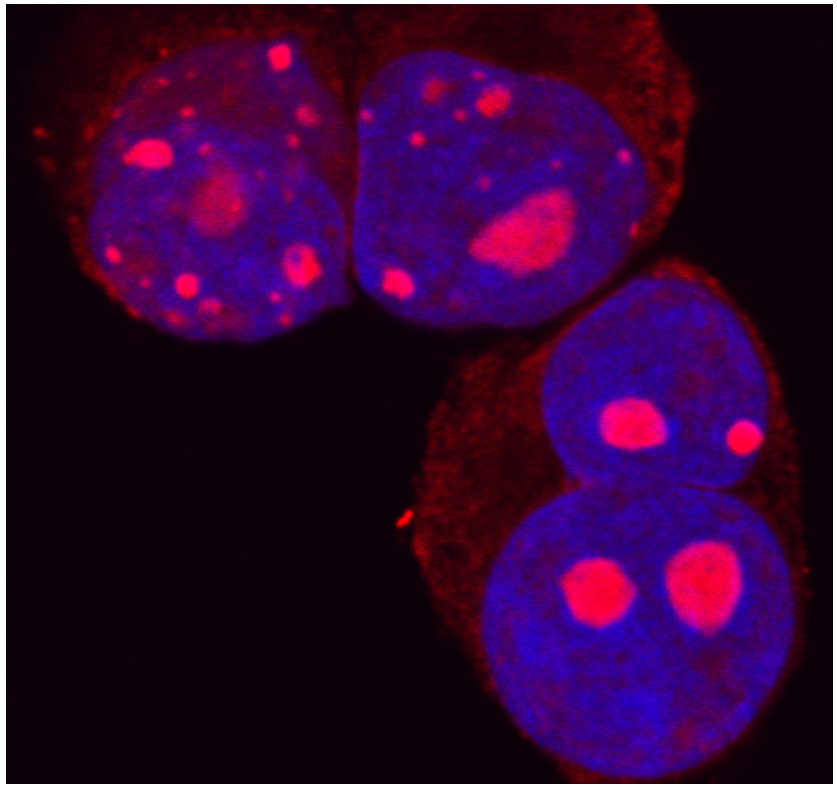


Chemical and Systems Biology for Drug Discovery

Using small molecules to decipher biological processes

Chemical biology spans chemistry, biology and physics

- It uses chemical tools, often compounds produced by synthetic chemistry to study and manipulate biological systems.
- Probing or perturbing using small molecules is a powerful tool to study biological systems.
- It is particularly useful when used in combination with other systems biology techniques.
- It is a very powerful tool for drug discovery.



Biological Practicals

Simon Arsene and Matt Deyell

(Salle Multimédia)

Biological Matlab Practical

Training with Matlab

Genetic Networks

- Numerical simulations of gene networks

Flux Balance Analysis

- Cobra Toolbox
- Optimize gene expression in E. coli

Biological Databases Practical

Training with databases

Databases

- NCBI- Blast
- Genbank
- Registry of Biological Parts
- RBS Calculator
- Codon Optimization
- Ecocyc/Biocyc
- KEGG
- Cloning Software (Serial Cloner)

Biological Databases Practical

Tasks using databases

- Reverse Engineer Given Sequence
  - Identify Promoter, RBS, CDS, Terminator, Restriction sites
- Design a Simple Genetic Circuit
  - Use Registry to find new promoters
  - Use RBS Calculator to optimize expression
  - Codon Optimized CDS

Group Projects

Philippe Nghe & Matt Deyell

Group Projects
Schedule

- You have until the 19th January to form binomes and choose a subject
- First come, first served, but more subjects than groups!
- If you have decided before, send an email to philippe.nghe@espci.fr, otherwise communicate your choice during the practical with Matt Deyell on Monday 19th January (Salle Multimédia) (14:00-17:30).
- You will have a session to help with the practicals Friday 11th March, 9:40-12:00 (Salle Multimédia)
- You will be scored during the exam week (18-22nd of April) on your presentation (~30 min) and your participation to questions on other group's presentations.

Group Projects
What is expected

A. A pedagogical synthesis on the subject aimed for the other students (~50 % of the presentation):
   - What is the general problem?
   - What is the state of the art?
   - What are the implications?
   - Explain the concepts that have been developed for this problem.

B. Illustrate one approach theoretically (analytical or numerical) (OR detail statistical tools if relevant).

C. Formulate a research question and propose an experimental design to study this question.

Group Projects
Categories of projects

There are 2 types of subjects:

- Systems biology. In this case you are expected to treat both objectives B (theory) and C (experimental design).
- Synthetic or chemical biology. In this case, objective B is not mandatory but objective C should be more developed, in particular justify your choices from the use of biological databases.

A full list of projects will be posted on the server https://cours.espci.fr/, together with the associated references

Alternatively to the list proposed, you can propose a subject based on a recent article, to be validated by Philippe Nghe before the deadline (19 January).

Potential Projects

Further projects may be added to the list on the server over the next few days

Group Projects
Systems biology projects

Regulating metabolism

- [Scott2010] What is optimal way to invest in the production of the different proteins to optimize growth?
- [Vilar2003] How to coordinate sensing, import and use of a source of carbon?
- [Doucette2011] How to implement the balance between carbon and nitrogen intake at a molecular level?

Group Projects
Systems biology projects

Cell Adaption

- [Locke2011] How do cells decide to respond to stress?
- [Kussell2005] How is the rate of phenotypic switching influenced by the environment?
- [Ceroni2015] How can we minimize the burden of synthetic circuits in a cell?

Group Projects
Systems biology projects

Organizing the cell

- [Meinhart2001] How do bacteria localize their mid-line to divide correctly?
- [Chambers2015] Q-quadruplex nucleic acids in genomes: what is the evidence and how would one experimentally approach the question?
- [Gibson2010] What is the minimal genome required for a synthetic cell?

Group Projects
Systems biology projects

Shaping an organism

- [Gomez2008] How to determine the number of segments along the body of a vertebrate?
- [Eldar2002] How to localize correctly segments along the body of an insect?
- [Sprinzak2010] How to create sharp boundaries between different cell types?
- [He2012] How to make sure an organ has the good proportions of different cell types?

Group Projects
Systems biology projects

Treating Disease

- [Gupta2009] How can we selectively target cancer stem cells?

Group Projects
Synthetic biology projects

Genetic Oscillator

Objective: Create a genetic circuit that acts as an oscillating system.

- Find inducible promoters and the genes that encode their transcription factors.
- Create RBS and find terminators to construct expression cassettes.
- Find a reporter for the state of the system.
- Insert into an E. coli expression vector.

*Challenge – Set up a system to tune the frequency of your system so that you can adjust your ‘genetic clock’.

Reference:

Elowitz M, Leibler S. A synthetic Oscillatory Network of Transcriptional Regulators. Nature. 2000.

Stricker J et al. A fast, robust and tuneable synthetic gene oscillator. Nature. 2008.

Group Projects

Synthetic biology projects

Biosensor

Objective: Engineer E. coli to produce a detectable signal in the presence of a specific compound.

- Find a promoter that activates in the presence of your compound.
- Find a suitable indicator gene, a terminator and Create a RBS to construct your expression cassette.
- Insert into a expression vector for E. coli

*Challenge - Create a tuning device that allows you to differentiate between different concentrations of your compound.

Reference: iGEM teams Edinburgh (2006) Arsenic, Cambridge (2009) Arsenic, Groningen (2012) Rotten Meat, UC Davis (2014) Olive Oil

French CE, de Mora K, Joshi N, et al. SYNTHETIC BIOLOGY AND THE ART OF BIOSENSOR DESIGN. In: Institute of Medicine (US) Forum on Microbial Threats. The Science and Applications of Synthetic and Systems Biology: Workshop Summary. Washington (DC): National Academies Press (US); 2011. A5.

Group Projects

Synthetic biology projects

Cure Trimethylaminuria

Objective: Engineer *E. coli* to degrade trimethylamine and prevent fish odor syndrome (Trimethylaminuria)

- Find gene that degrades Trimethylamine.
- Codon optimize the gene for expression in E. coli
- Find a promoter, RBS, and Terminator to construct a suitable expression cassette.
- Insert into a expression vector for E. coli

*Challenge – Design a system to express a pleasant odor of your choosing in addition to eliminating fish odor.

Reference: Paris Bettencourt iGEM 2014

Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. PNAS. 2012.

Chen Y, et al. Bacterial Flavin-containing monooxygenase is trimethylamine monooxygenase. PNAS. 2011.

Group Projects
Synthetic biology projects

Sulphite reduction

Objective: Create Synthetic sulphite reduction pathway in E. coli that mimics the pathway in Mycobacteria.

- Find genes for sulphite reduction in E. coli and Mycobacteria
- Codon optimize Mycobacteria genes for E. coli
- Find promoter, RBS, and Terminator
- Construct Cassettes
- Insert into E. coli expression vector.

*Challenge - Set up your synthetic pathway to only be expressed when H_2S levels are low.

Reference: Paris Bettencourt 2012 iGEM Team

Barstow B, Agapakis CM, Boyle PM, Grandl G, Silver PA, Wintermute EH. A synthetic system links FeFe-hydrogenases to essential E. coli sulfur metabolism. Journal of Biological Engineering 2011;5:7. doi: 10.1186/1754-1611-5-7.

Group Projects
Synthetic biology projects

Refractory metabolism to assimilate or reduce loss of CO_2

This exercise will be a critical analysis of two paper recently published, allowing either

- Assimilation of CO_2 into a chemical synthon, Li & Lia (2013)
- Engineering a cyanobacterium as a catalyst for the photosynthetic assimilation of $C0_2$ into 1,2 propanediol. Microbial Cell fact 12, 4.

The second paper is about refactoring glycolysis to avoid loss of $C0_2$ - see Bogorad et al (2013) Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature, 502, 693-697.

The exercise will be to present their approaches and to critically discuss these approaches . The objective, to avoid loss of $C0_2$ was relevant, but the final metabolite generated had a low technological value.

How might the strategy be improved?

Group Projects

Synthetic biology projects

Propose a rapid low-cost sequencing based RNA/DNA pathogen diagnostic method

One of the key challenges in fighting emerging infectious disease (Ebola, Tuberculosis) is a method to rapidly detect these pathogens at their site of origin. The challenge is to do this with a low-cost field-deployable system that can be used in third world countries. Reagents must be able to survive without refrigeration and computing must be limited to a smart phone.

References: Yager et al., (2006) Nature, 442; 412-418;

Chin et al., (2012) Lab Chip, 12; 2118-2134

Foudeh et al., (2012) Lab Chip, 12, 3249-3266

Gubala et al., (2012) Analytical Chemistry, 84, 487-515

Group Projects

Synthetic biology projects

Propose a metabolic engineering strategy for optimal production of the production of Succinate Acid by E. coli

Succinic acid is a precursor to some plastics and polyesters. 1,4 –butanediol derived from succinic acid had a market size of 4.72 billion in 2013. It is also used as a food additive, primarily as an acidity regulator with global production estimated at 16,000 to 30,000 tonnes a year with an annual growth rate of 10%.

References: [Yin2015, Zhang2010]

Group Projects

Synthetic biology projects

Propose a metabolic engineering strategy for optimal production of the production of Glycolic Acid by E. coli

Glycolic acid is found in many skin care products, the textile and food processing industries, and in adhesives and plastics. Due to its moisturizing and exfoliating properties, glycolic acid is mostly used in the manufacturing of hair shampoos, face creams and lotion, and other similar products. With a total market value at 93.3 Million USD in 2011 it has an annual growth rate of 11.8%

References: [Dischert2010]

Group Projects

Synthetic biology projects

Identify a high value natural product and compare potential in-vitro and in-vivo production methods

Natural products have yielded most of our current set of antibiotics in addition to many high-value drugs. Encoded in organisms throughout our planet are many more high-value molecules that could be produced either synthetically or via engineered organisms using synthetic biology. Identify a high-value molecule produced naturally and compare the technical and economic feasibility of producing it using traditional chemical synthesis and new biological engineering methods.

References: Li and Vederas, (2009) Science, Vol 325, 5937; 161-165;
Walsh and Fischbach, (2010) J. Am. Chem. Soc., 132(8); 2469-2493
Winter and Tang, (2012) Curr. Op. Biotech., Vol 23, 5, 736-743
Zakeri and Lu, (2013) ACS Synth. Biol., 2 (7), 358-372