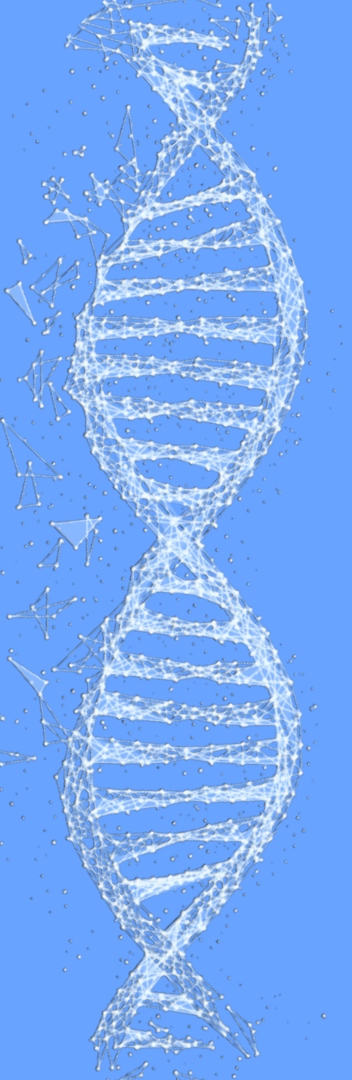


Lomonosov Moscow State University

Faculty of Biology

От редактирования геномов к программированию функциональных генетических схем

Алексей К. Шайтан

д.ф.-м.н, чл.-корр. РАН

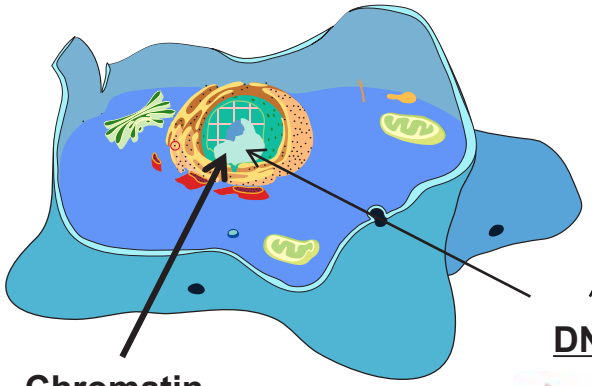
кафедра биоинженерии, кафедра синтетической биологии

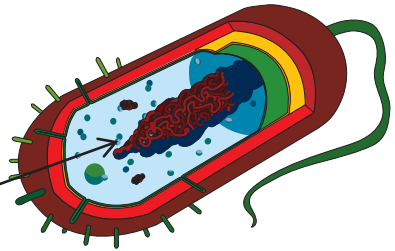
«Редактирование генома: теория и практика», 21 ноября 2023

Eukaryotic cell

Virus

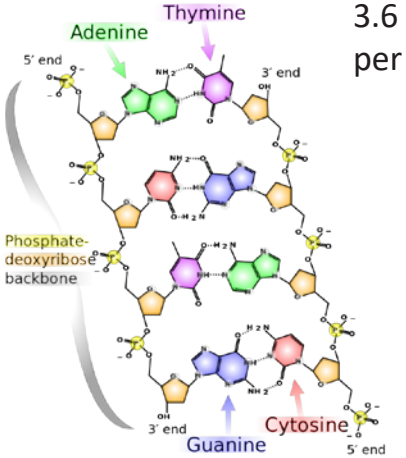
Bacterial cell



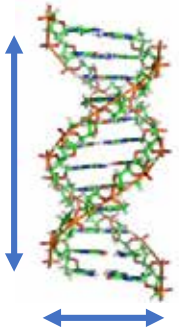


Chromatin

DNA

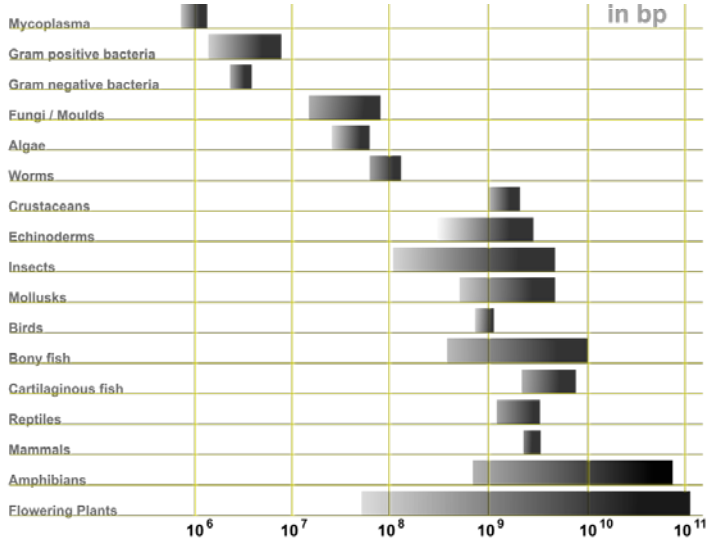


3.6 nm per turn



2 nm

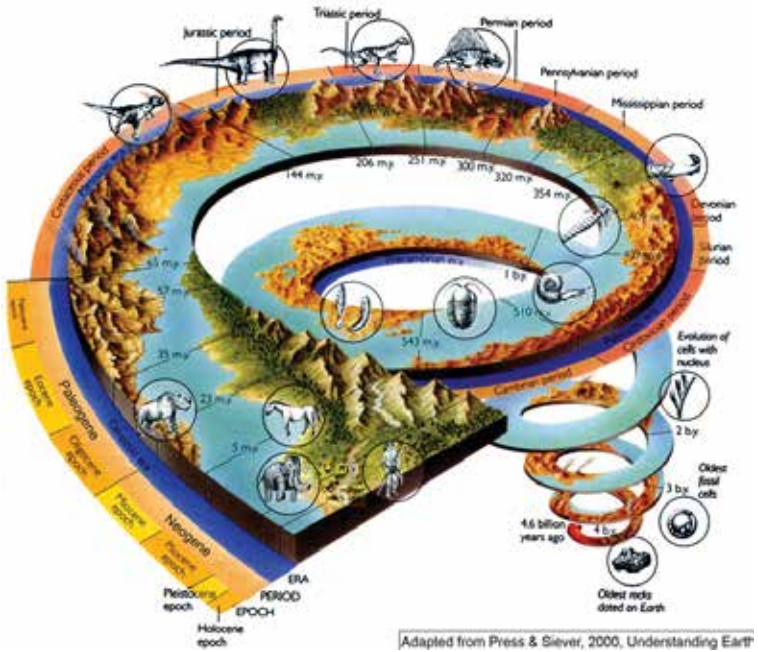
Persistent length ~50 nm, ~147 bp

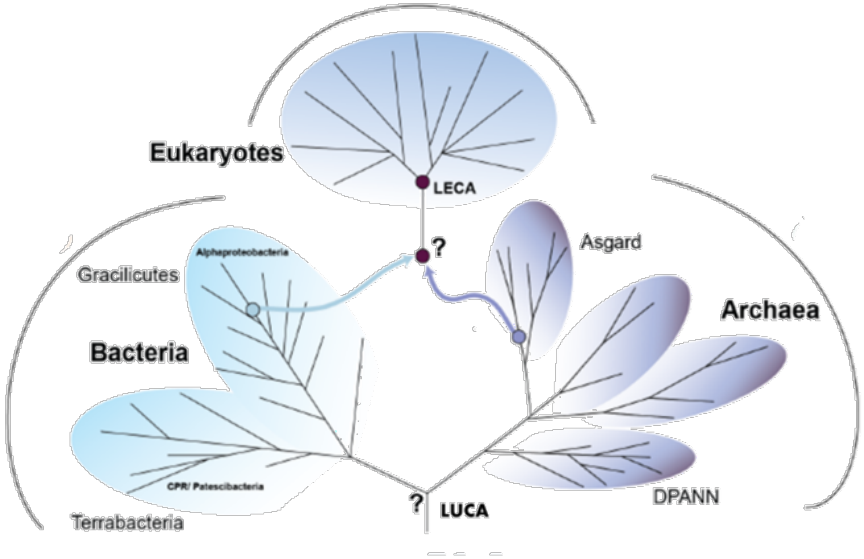


Genome size, bp

“Nothing in Biology Makes Sense Except in the Light of Evolution”

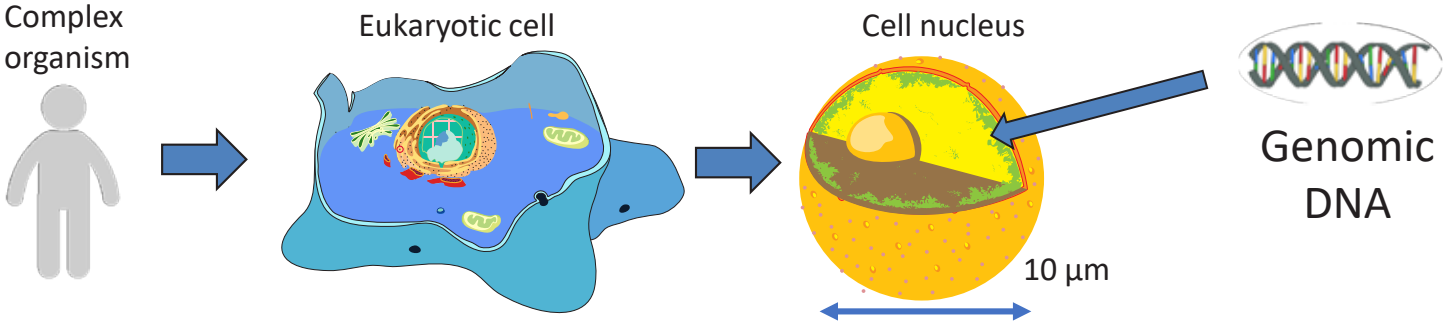
Феодосий Григорьевич Добржанский



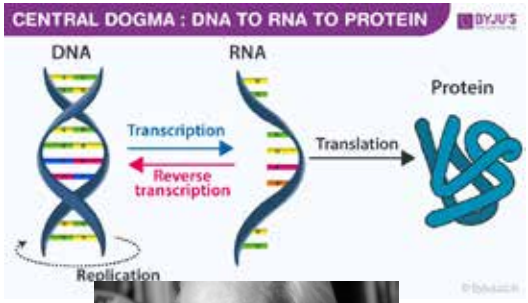


ДНК – дискретный цифровой код. Живые системы – информационные системы

Challenges in understanding (human) genomes

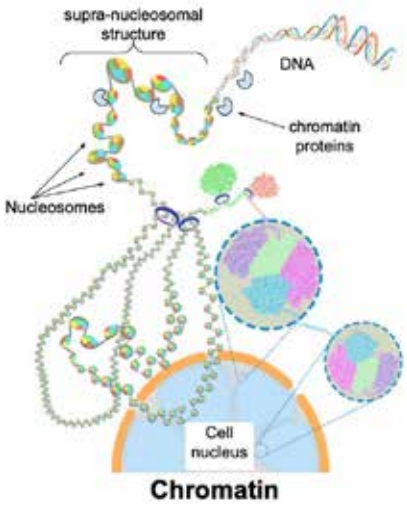


“Digital” information processing



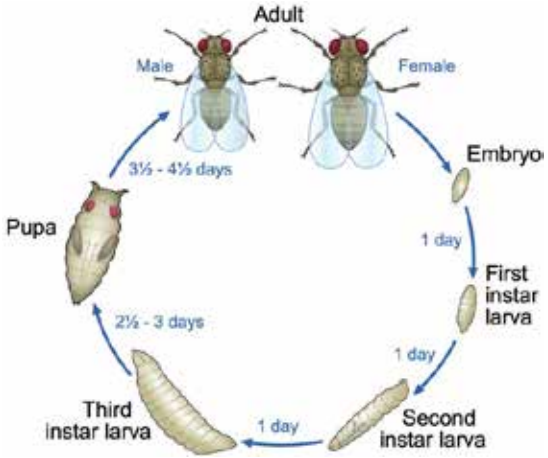
Francis Crick formulated central dogma 1957

“Analog” information processing

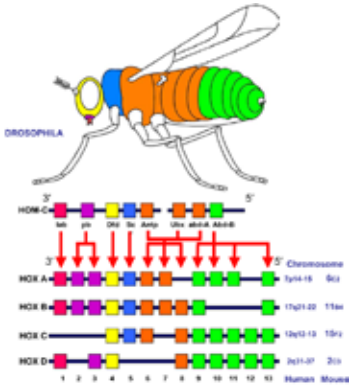


Physical 3D interactions of biomolecules regulate what genes work and how they work

Nature invented first (genetic) programs



Ong et al. 10.3109/17435390.2014.940405

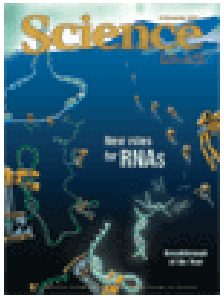


Single mutations may change body development programs

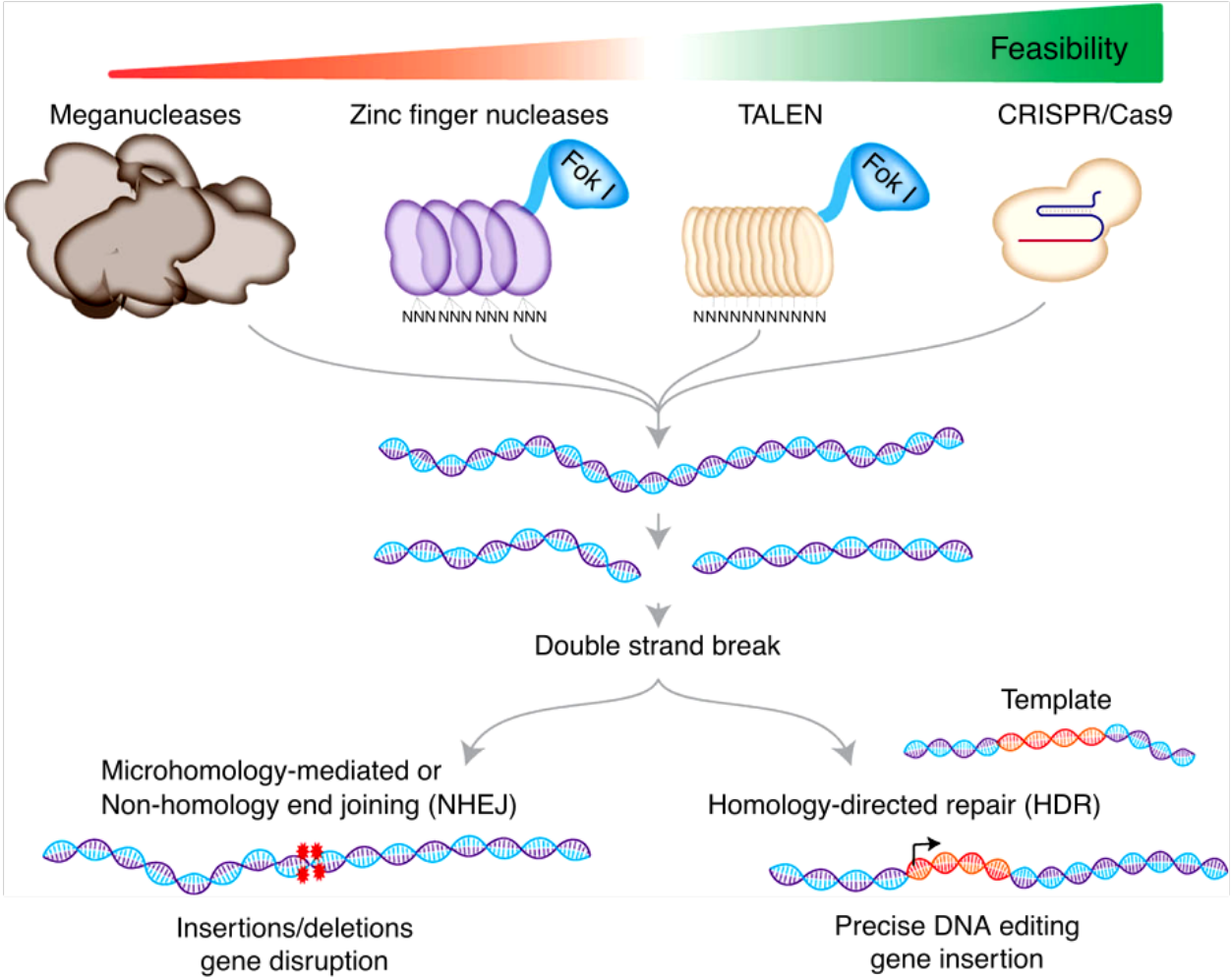
14.14A: © Eye of Science/ Science Source. 14.14B: © Science VU/ Dr. F. Rudolph Turner/Visuals Unlimited, Inc.

Scientific breakthroughs in the 21 century

- 2000: **Full genome sequencing**
- 2001: Nanocircuits or Molecular circuit
- 2002: **RNA interference**
- 2003: Dark energy
- 2004: Spirit rover landed on Mars
- 2005: **Evolution in action**
- 2006: Proof of the Poincaré conjecture
- 2007: **Human genetic variation**
- 2008: **Cellular reprogramming**
- 2009: **Ardipithecus ramidus**
- 2010: The first quantum machine
- 2011: **HIV treatment as prevention**
- 2012: Discovery of the Higgs boson
- 2013: **Cancer immunotherapy**
- 2014: Rosetta comet mission
- 2015: **CRISPR genome-editing method**
- 2016: First observation of gravitational waves
- 2017: Neutron star merger (GW170817)
- 2018: **Single-cell sequencing**
- 2019: A black hole made visible
- 2020: **COVID-19 vaccine**
- 2021: **An AI brings protein structures to all**
- 2022: James Webb Space Telescope debut

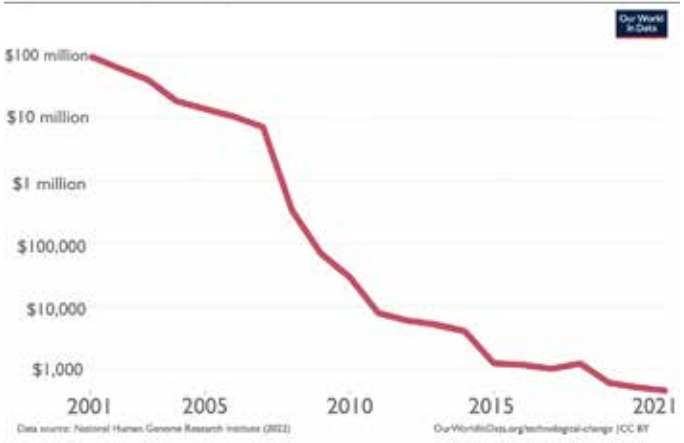


https://www.science.org/topic/tags/breakthrough-year


Feasibility
Meganucleases
Zinc finger nucleases
TALEN
CRISPR/Cas9
Fok I
Fok I
NNN NNN NNN NNN
NNNNNNNNNNN
Double strand break
Template
Microhomology-mediated or
Non-homology end joining (NHEJ)
Homology-directed repair (HDR)
Insertions/deletions
gene disruption
Precise DNA editing
gene insertion

DNA sequencing/synthesis and computer/data analysis performance

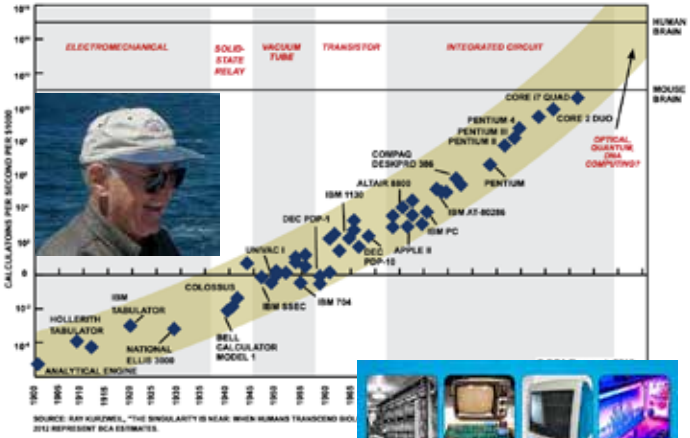
DNA sequencing costs



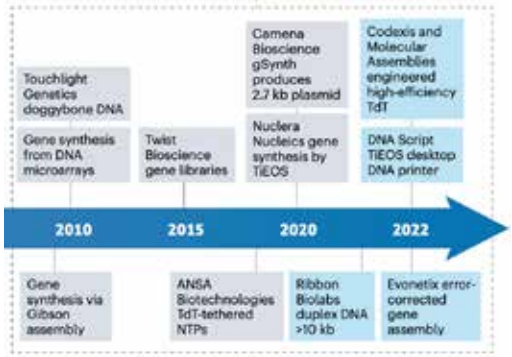
Nanopore sequencing

MGI DNBSEQ-T7
60 human genomes per day

Moore's law

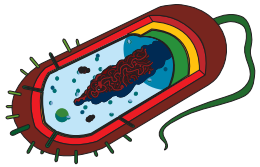


DNA synthesis

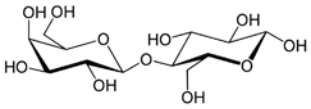


Hoose et al. 10.1038/s41570-022-00456-9

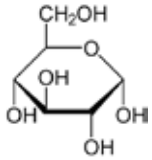
We need to understand how genes are regulated at molecular level



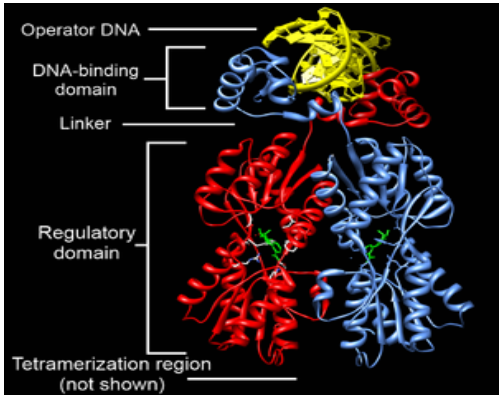
Bacteria



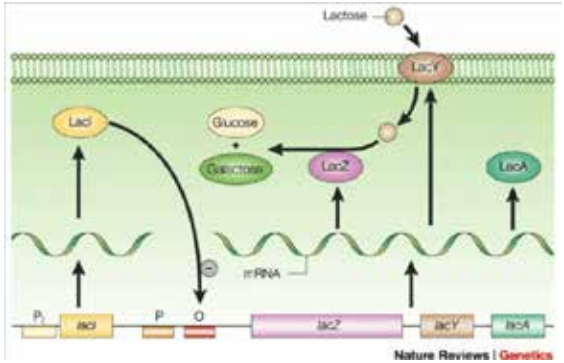
Lactose



Glucose



LacI protein bind DNA in the absence of lactose

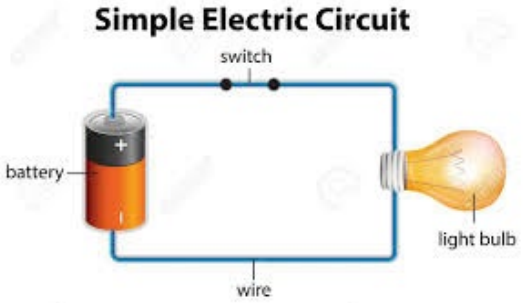


Lactose operon

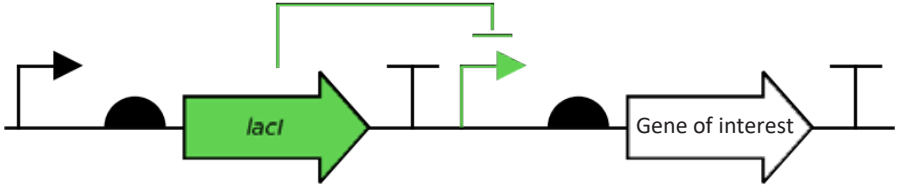
Nobel prize 1965

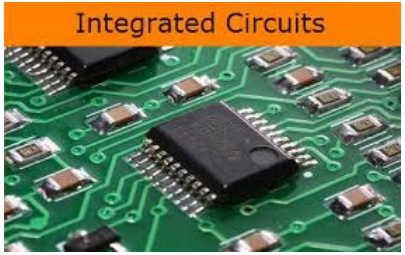
https://en.wikipedia.org/wiki/File:Binding_and_unbinding_mechanism_of_LacI.webm

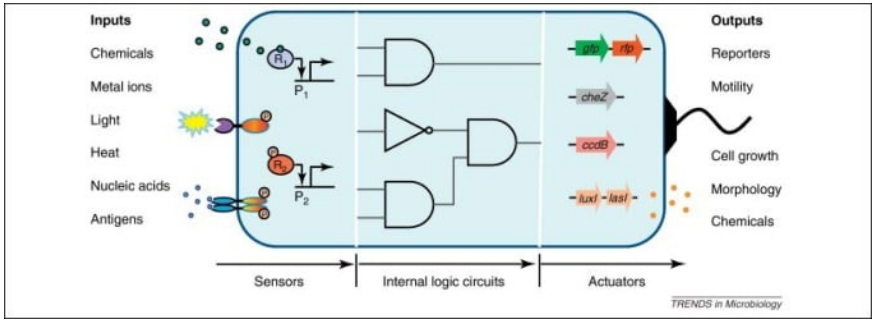
From electric circuits to genetic circuits



Simple genetic circuit

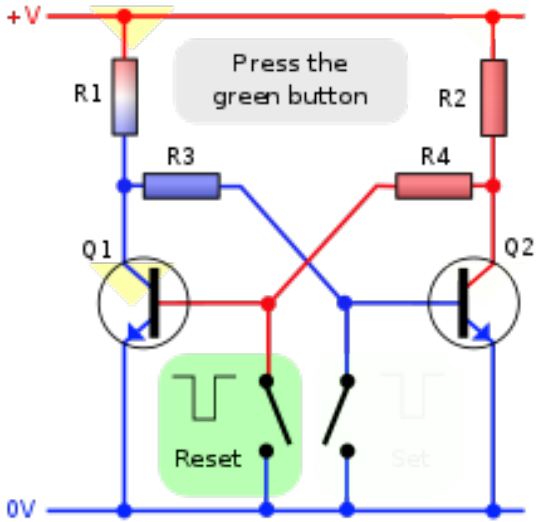






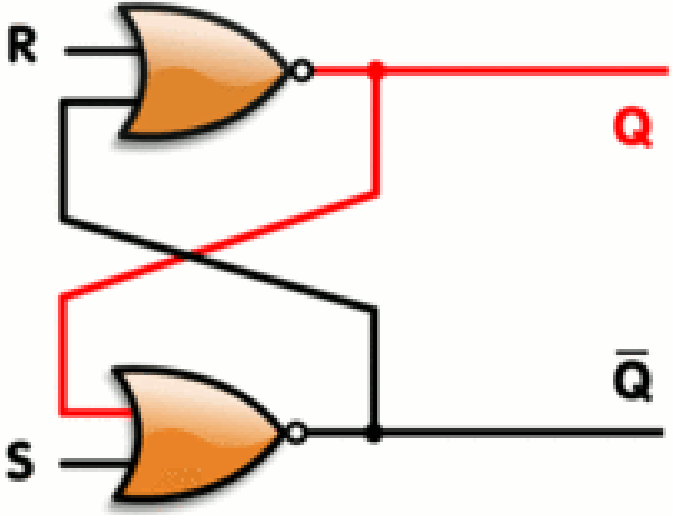
https://www.cell.com/trends/microbiology/fulltext/S0966-842X(12)00082-0

Flip-flop switch (toggle) – a memory element



Electrical scheme

Abstraction level: wiring diagram



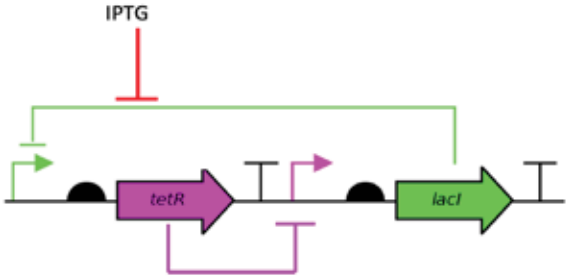
Logical scheme

Abstraction level: logic gates

First artificial genetic circuits

P. Zaytsev, J. Collins, M. Bokov at iGEM 2019

Flip-flop switch



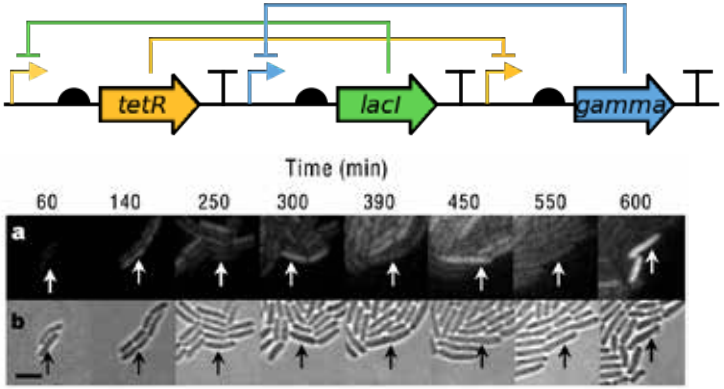
Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

2000

Repressilator

Oscillations of protein expression in time



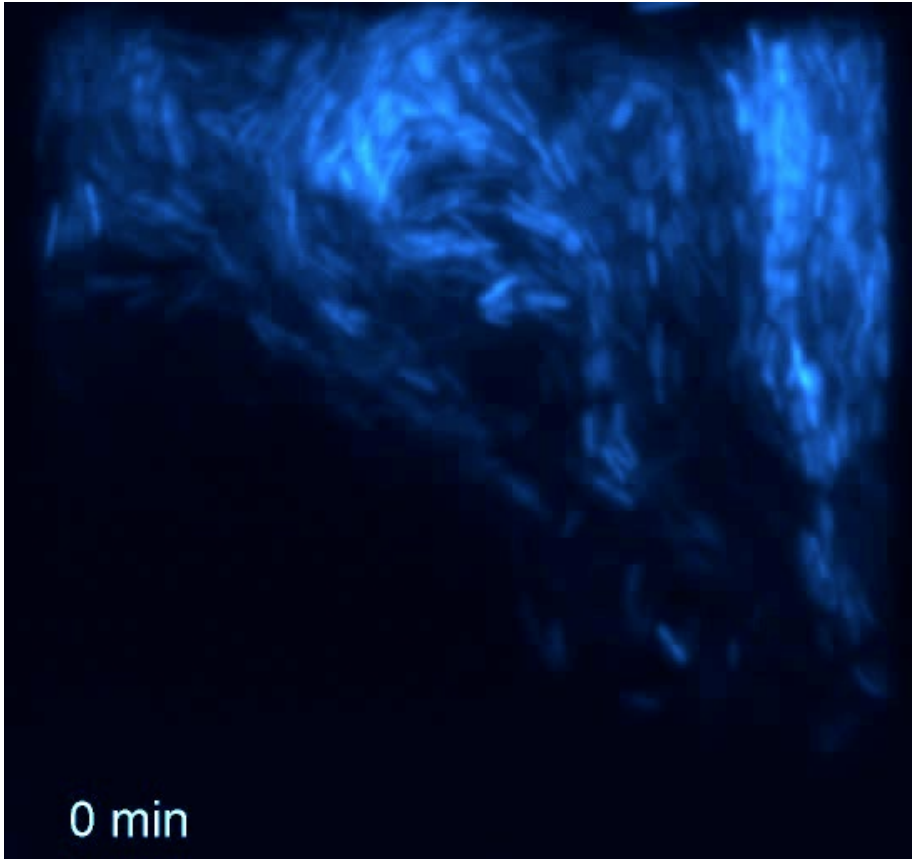
A synthetic oscillatory network of transcriptional regulators

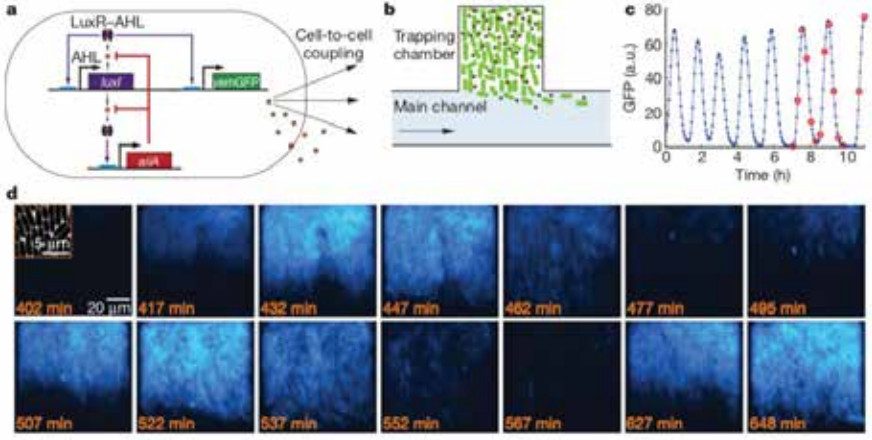
Michael B. Elowitz & Stanislas Leibler

Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA

2000

Synchronized oscillatory genetic circuit





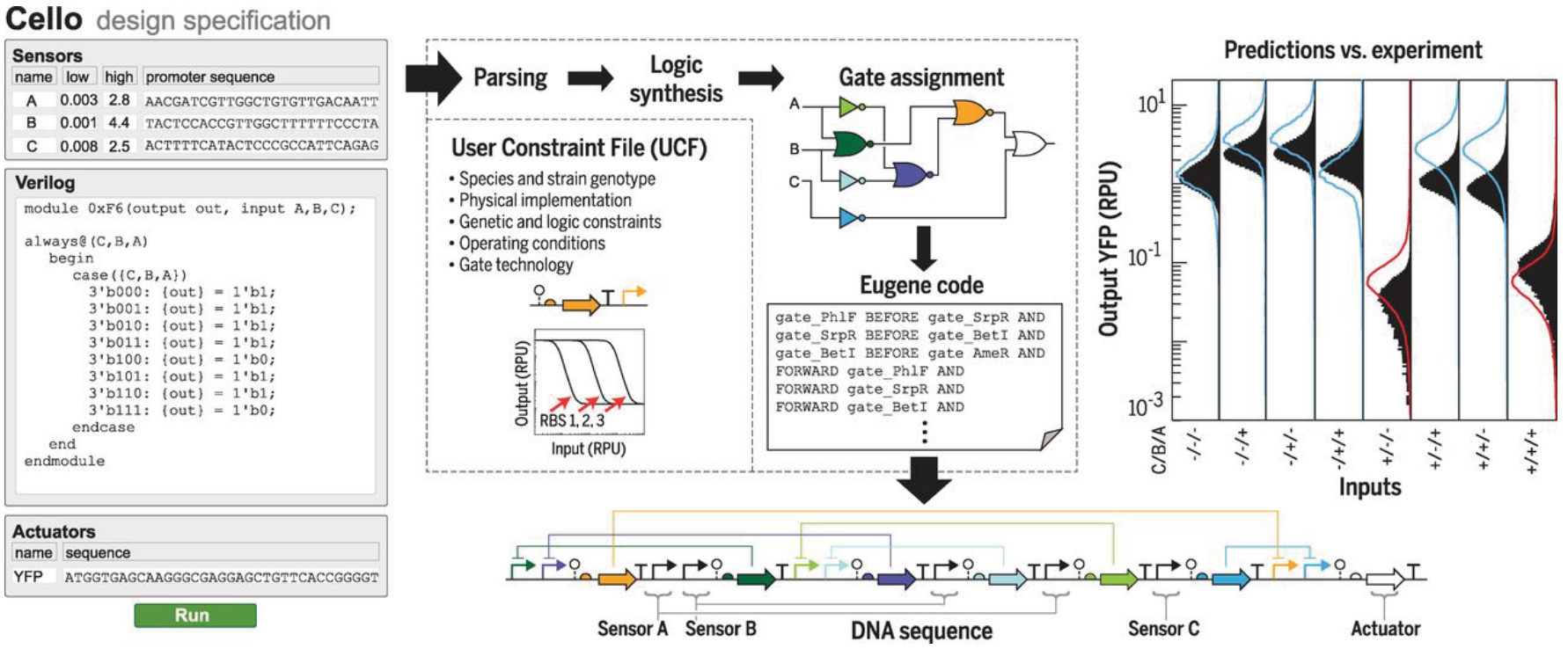
A synchronized quorum of genetic clocks

Tal Danino[1]*, Octavio Mondragón-Palomino[1]*, Lev Tsimring[2] & Jeff Hasty[1,2,3]

Nature **463**, 326–330 (2010)

2010

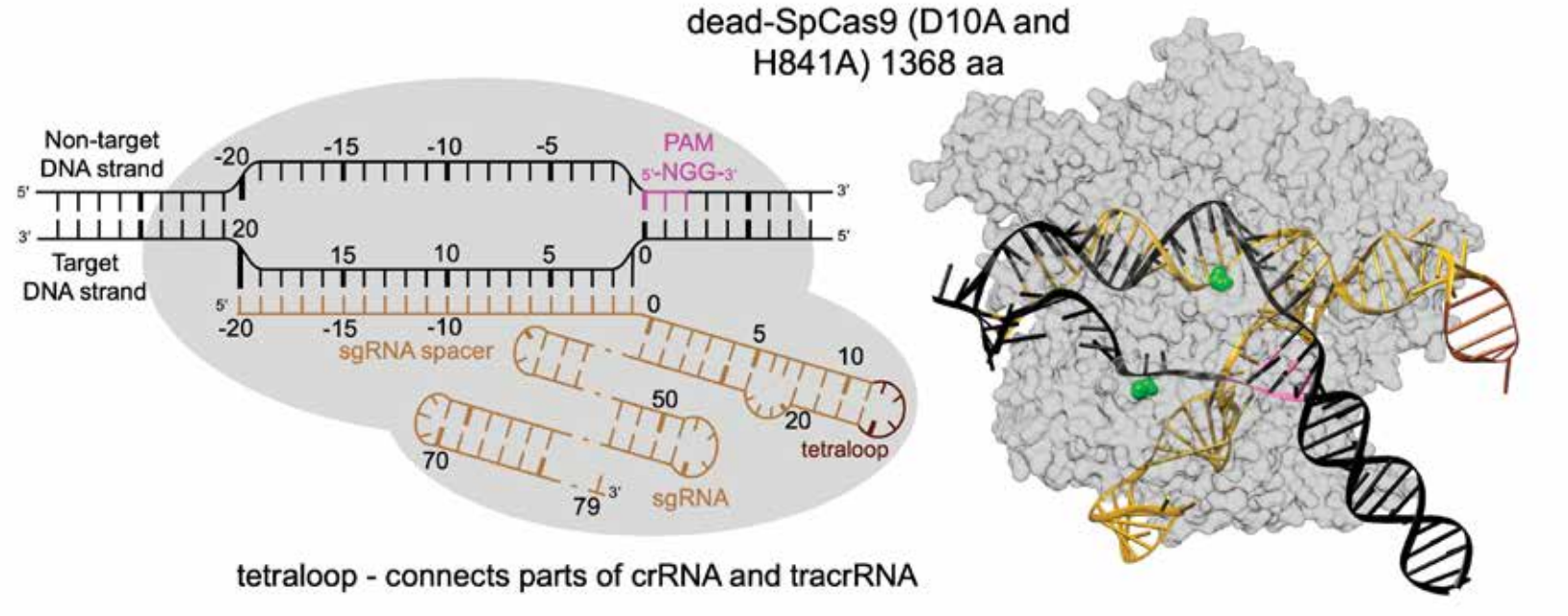
Пример программы дизайна генетических сетей



Cello, http://www.cellocad.org

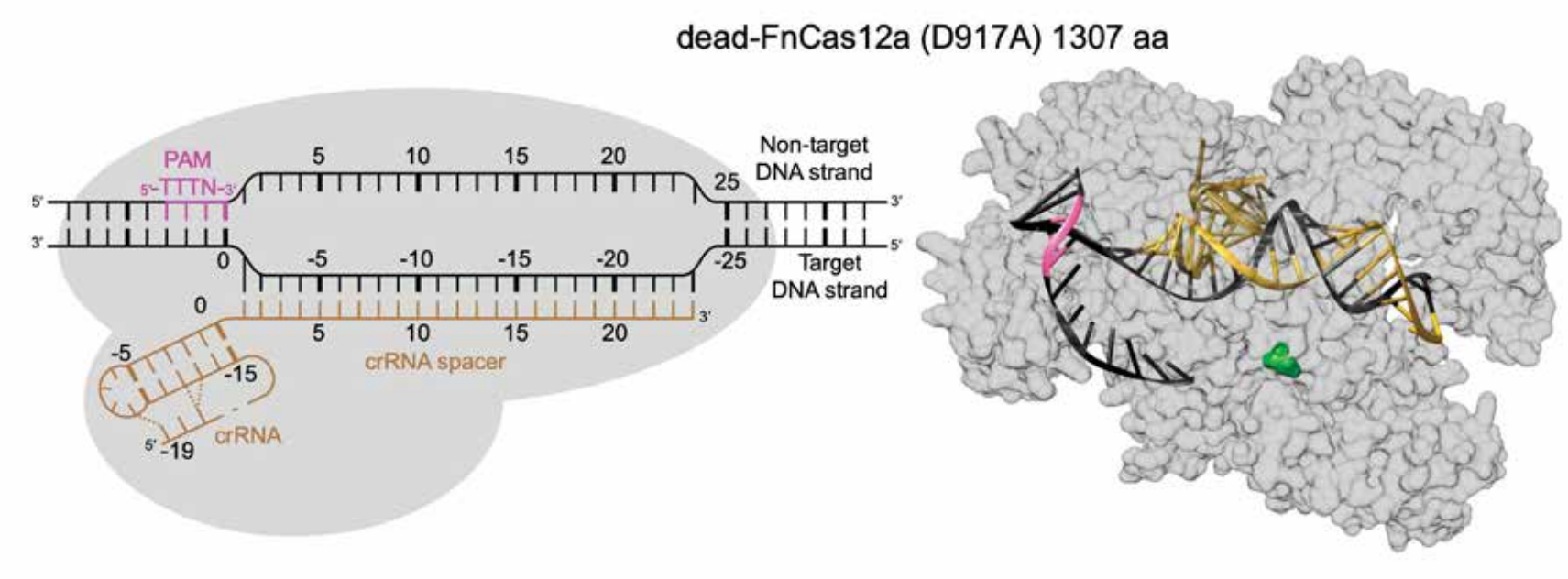
- https://youtu.be/SLn_SkL7vkQ
- Nielsen, A. A. K., Der, B. S., Shin, J., Vaidyanathan, P., Paralanov, V., Strychalski, E. A., … Voigt, C. A. (2016). *Genetic circuit design automation. Science, 352(6281), aac7341–aac7341.*

dCas9



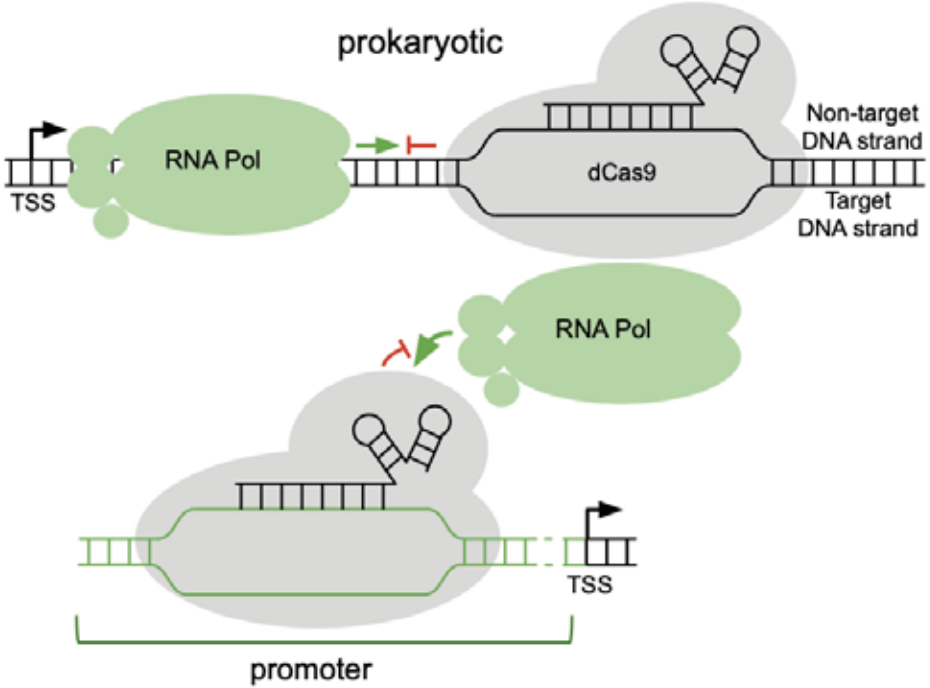
Shaytan, A.K.; Novikov, R.V.; Vinnikov, R.S.; Gribkova, A.K.; Glukhov, G.S. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526, doi:10.3389/fmolb.2022.1070526.

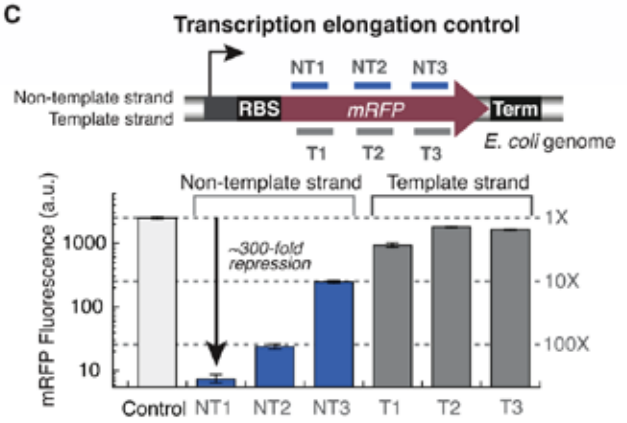
FnCas12a

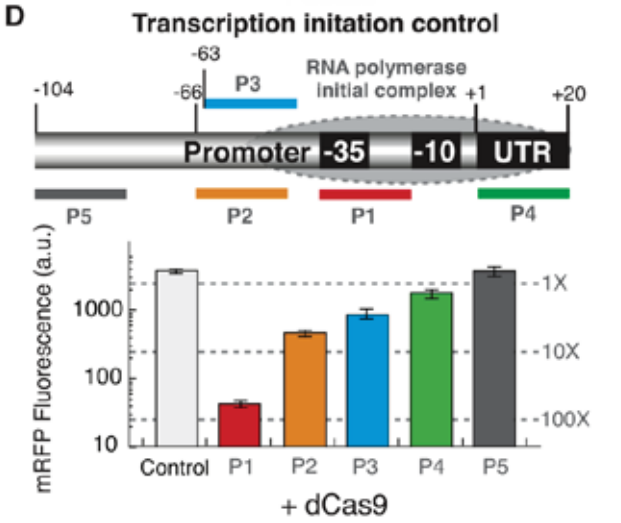


Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526

CRISPRi

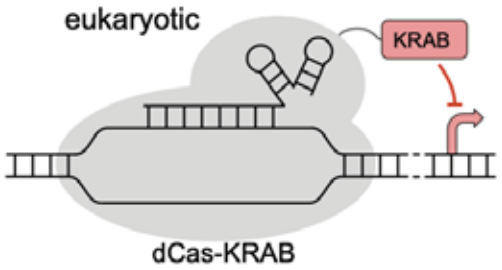




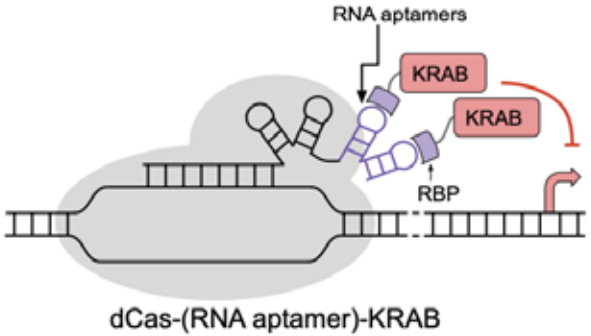


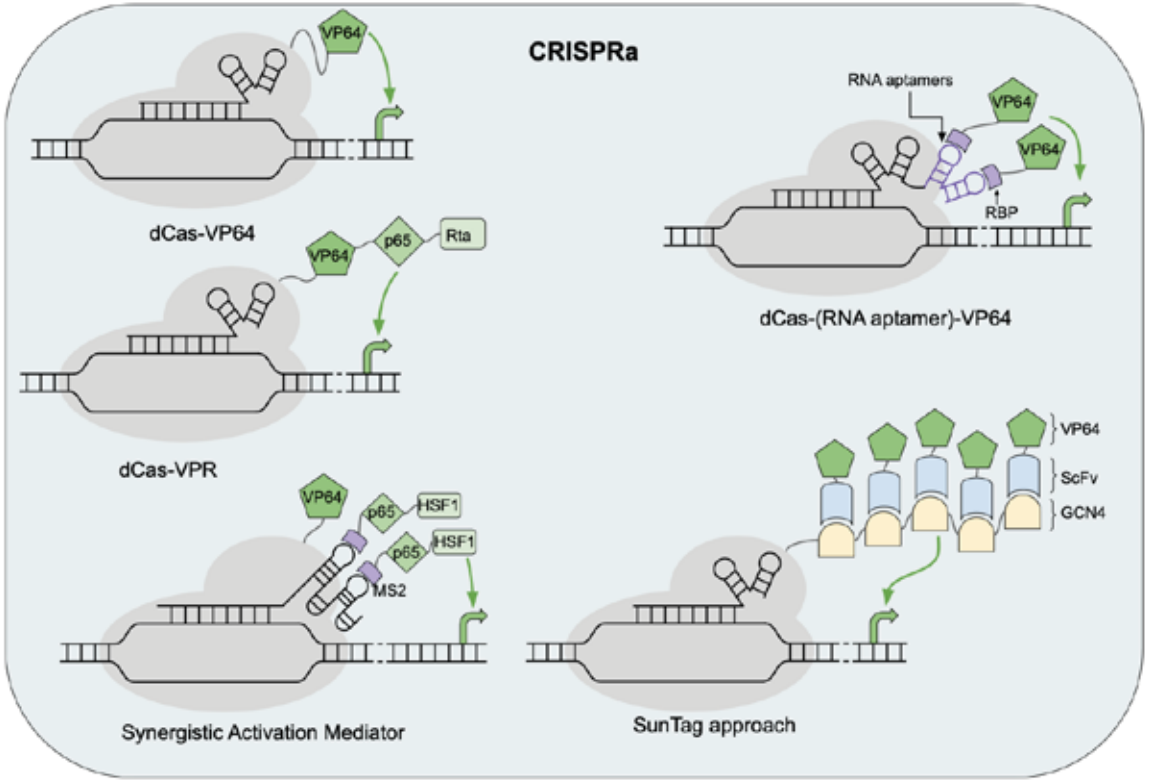
Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526
Qi, L.S.; et al. Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. *Cell* **2013**, *152*, 1173–1183

CRISPRi/CRISPRa in eukarotes



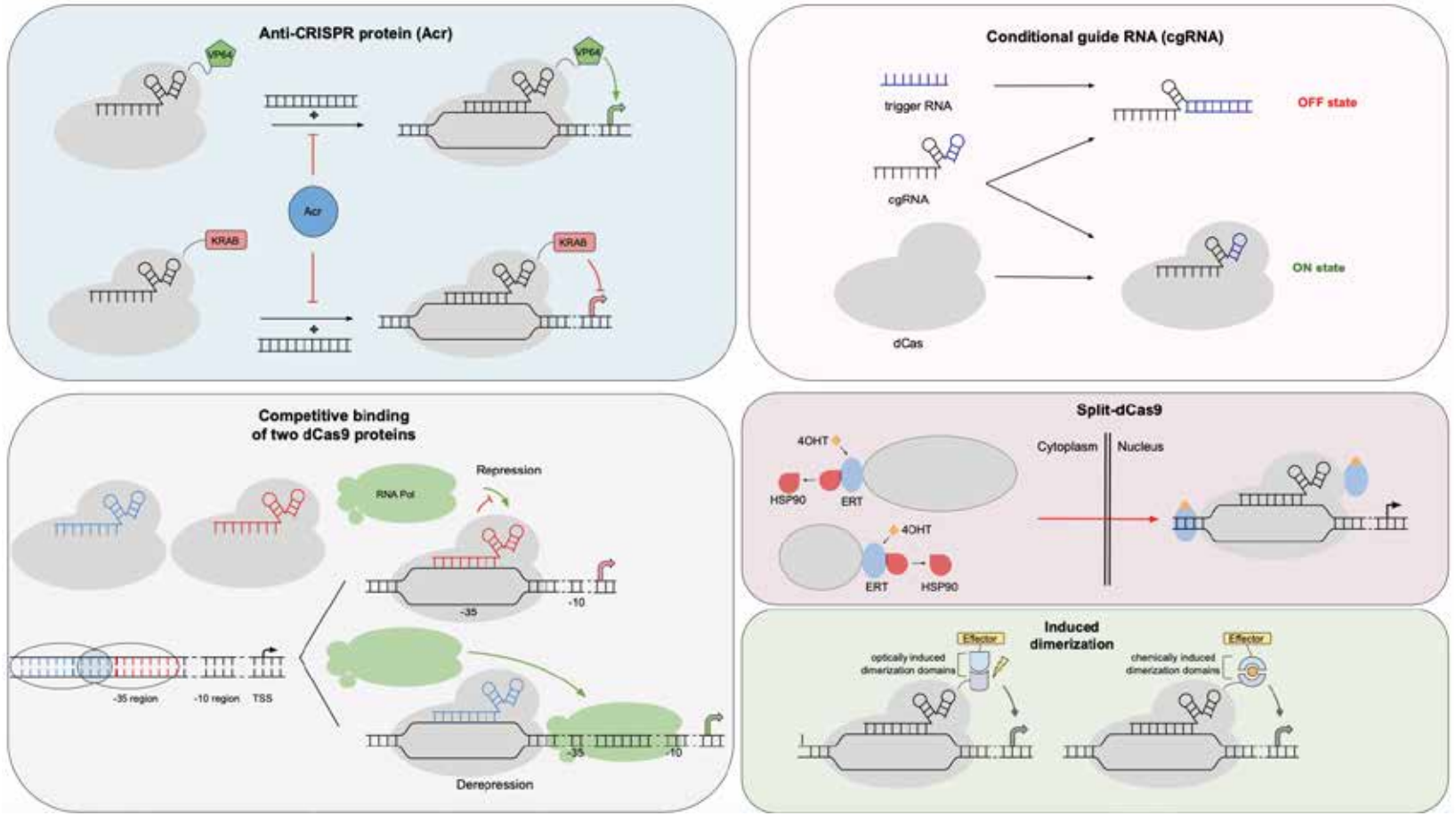
RNA aptamer	RNA-binding protein (RBP)
MS2	MCP
com	Com
PP7	PCP





Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526

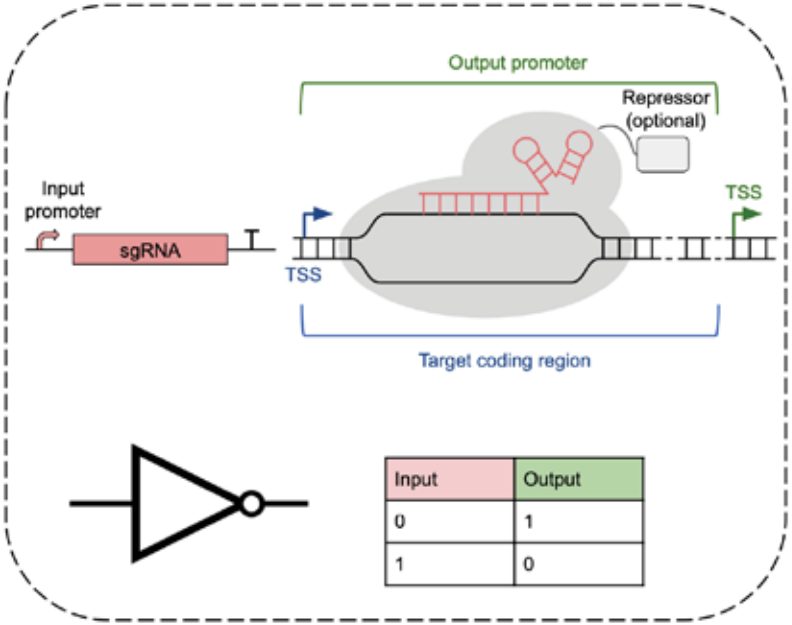
Andvanced CRISPR-based devices



Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526

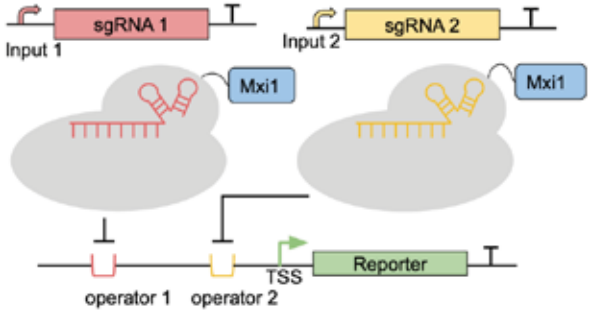
Логические вентили на CRISPR/dCas9

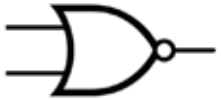
NOT gate (prokaryotic, eukaryotic)



Input	Output
0	1
1	0

NOR gate (eukaryotic, yeast)

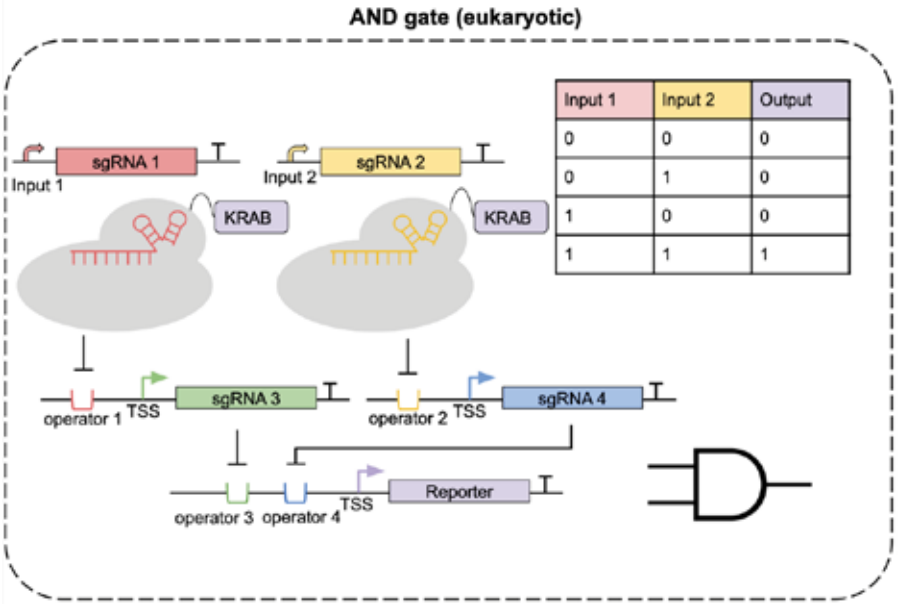




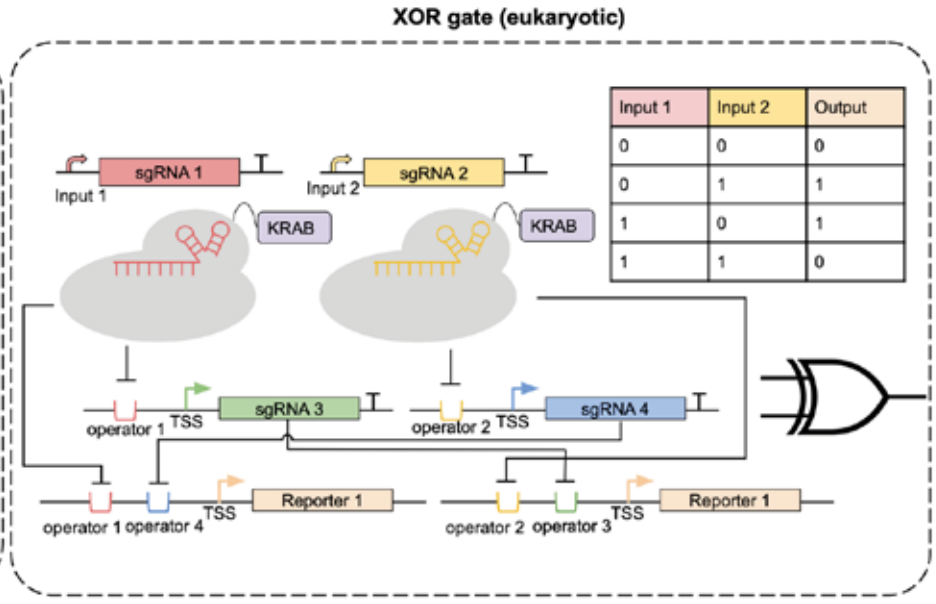
Input 1	Input 2	Output
0	0	1
0	1	0
1	0	0
1	1	0

Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526

Логические вентили на CRISPR/dCas9



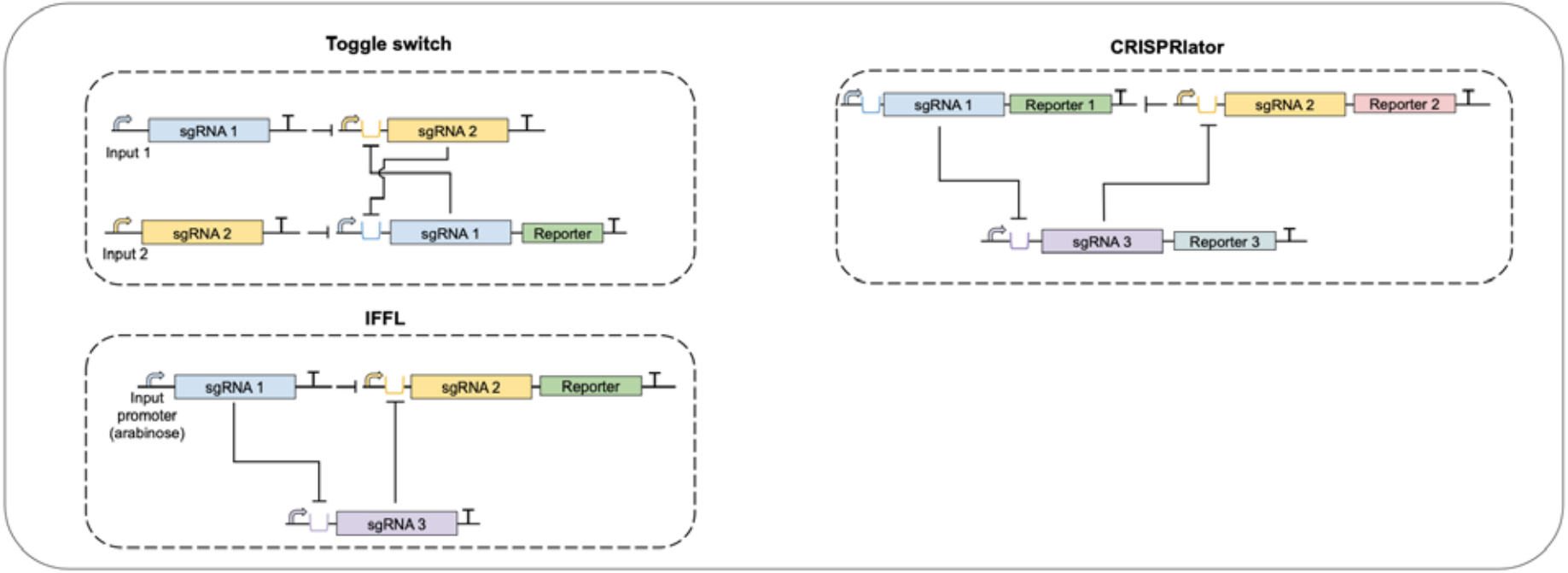
Input 1	Input 2	Output
0	0	0
0	1	0
1	0	0
1	1	1



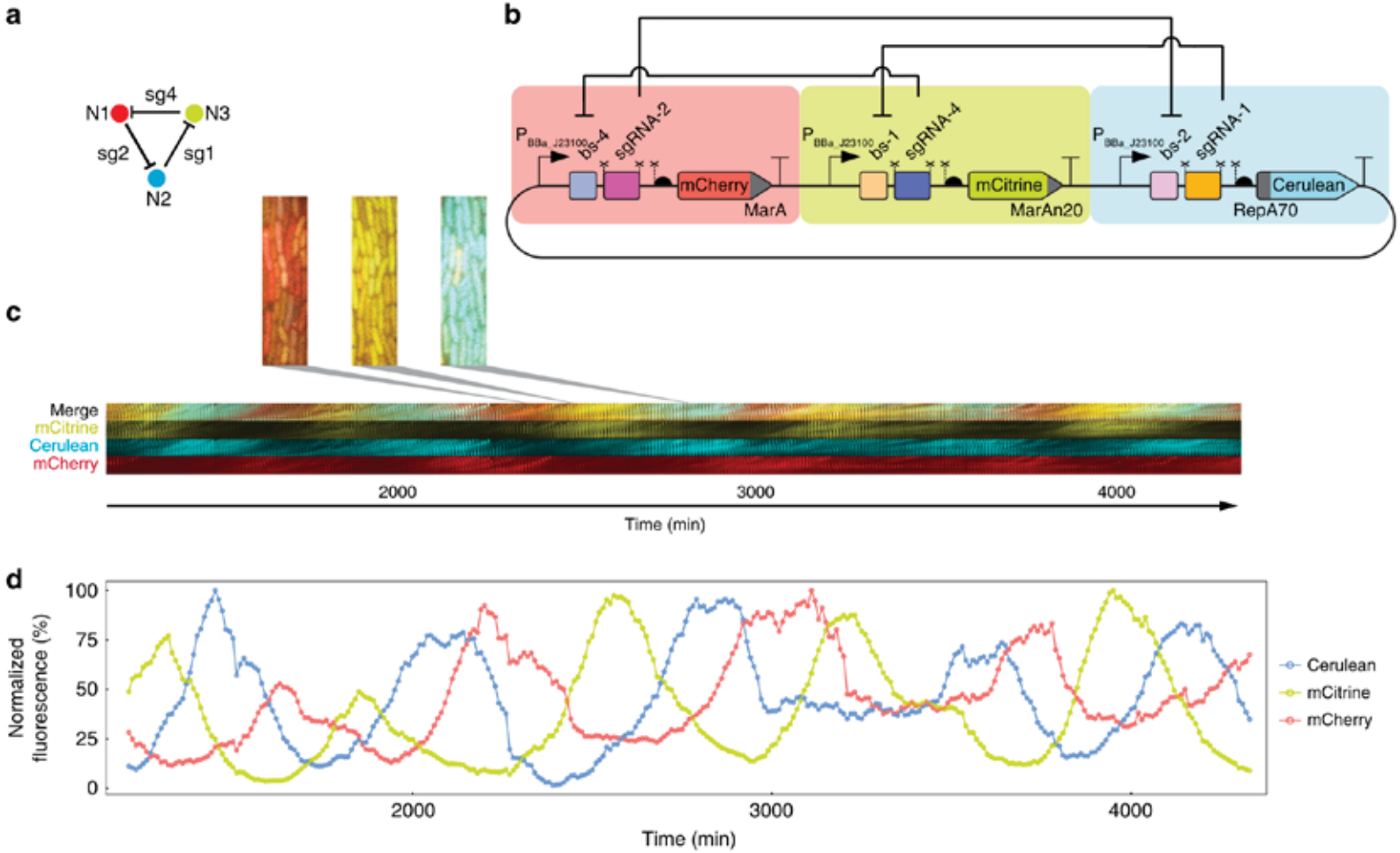
Input 1	Input 2	Output
0	0	0
0	1	1
1	0	1
1	1	0

Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526

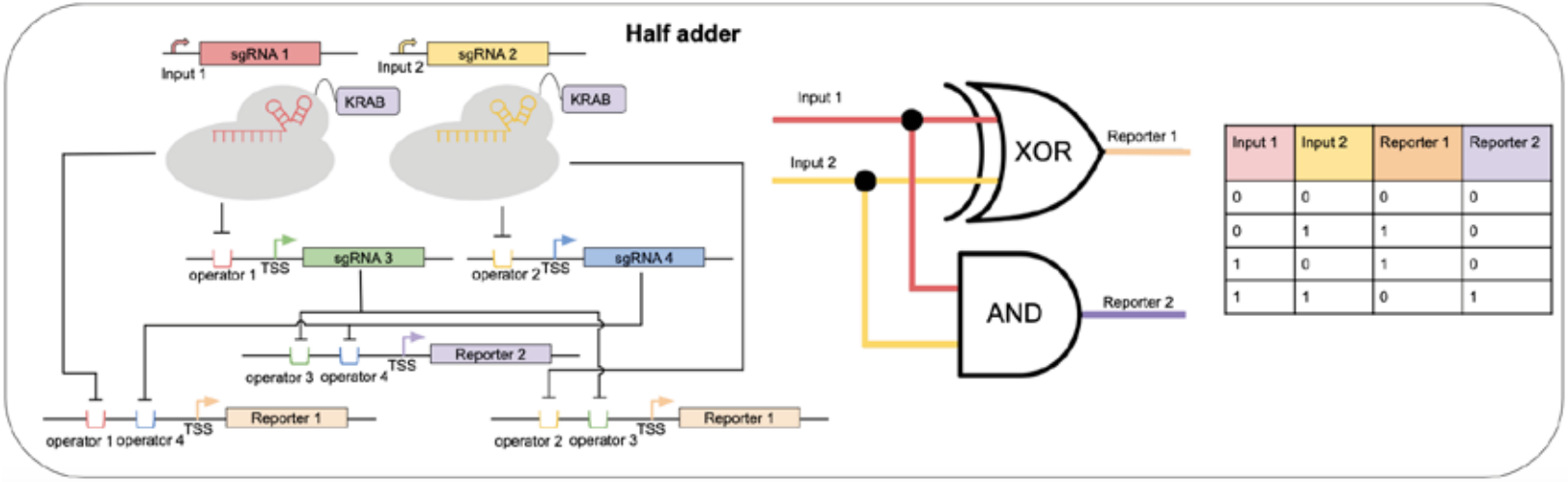
Простейшие схемы



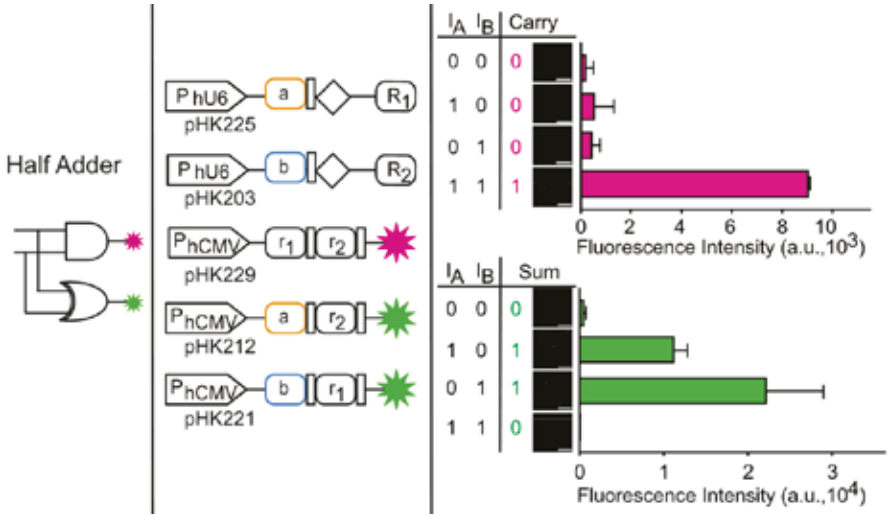
Shaytan, A.K. et al.. From DNA-Protein Interactions to the Genetic Circuit Design Using CRISPR-dCas Systems. *Front. Mol. Biosci.* **2022**, *9*, 1070526



Santos-Moreno, J.; Tasiudi, E.; Stelling, J.; Schaerli, Y. Multistable and Dynamic CRISPRi-Based Synthetic Circuits. *Nat Commun* **2020**, *11*, 2746, doi:10.1038/s41467-020-16574-1.



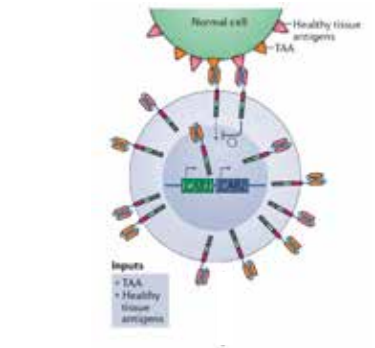
Input 1	Input 2	Reporter 1	Reporter 2
0	0	0	0
0	1	1	0
1	0	1	0
1	1	0	1



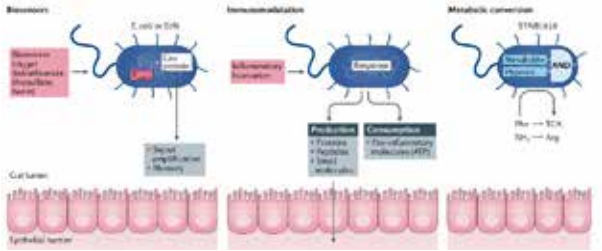
Kim, H.; Bojar, D.; Fussenegger, M. A CRISPR/Cas9-Based Central Processing Unit to Program Complex Logic Computation in Human Cells. *PNAS* **2019**, *116*, 7214–7219, doi:10.1073/pnas.1821740116.

Зачем нужны генетические схемы?

Smart living theraputics



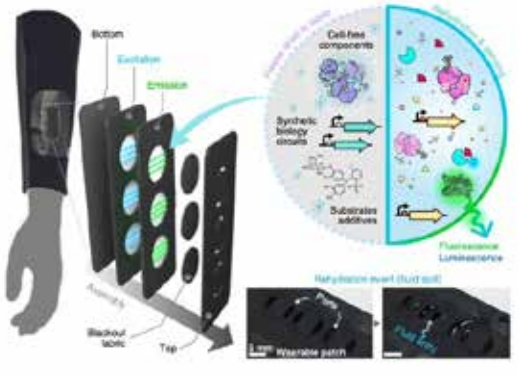
Programming smart immune cells to fight cancer



Smart bacterial therapies for the microbiome

Cubillos-Ruiz,A., et al. *Nat Rev Drug Discov*, **20**, 941–960, 2021

Biosensors



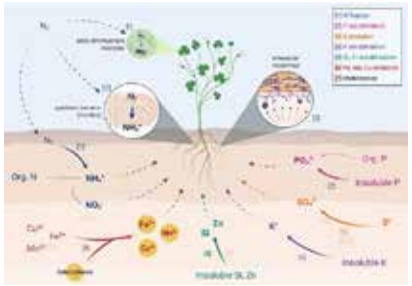
Wearable sensors that detect physiological status, disease states and exposure to pathogens or toxins

Nguyen,P.Q. et al. *Nat Biotechnol*, **39**, 1366–1374. 2021

Plant synthetic biology

Programming plant root structure

Brophy,J.A.N. (2022). *Science*, **377**, 747–751.

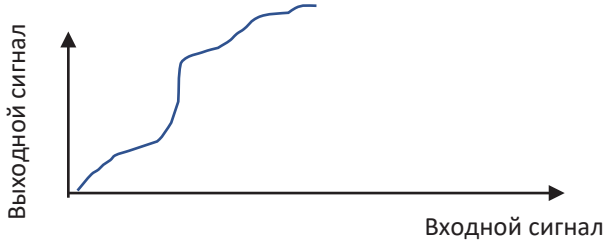


Biofertilizers

Mitter et al. 10.3389/fsufs.2021.606815

Ограничения dCas-систем

- Токсичность dCas-белков (E. coli – 500 белков на клетку) => мутации в PAM-связывающей области уменьшают токсичность
- Кооперативность отсутствует
- Иммуногенность



Transfer function (функция передачи)

Спасибо за внимание!

“What I cannot create, I do not understand”

R. Feynman
(1918-1988)