Докторантура в областта на ДНК чипове и ДНК компютри в Schloss Birlinghoven, FhG, near Bonn, Germany

Доктор по генетика от Cologne University, Germany

ПостДок в област на РНК синтетичната биология и откриването на нови антибиотици

Kline Biology Tower,
MCDB,
Graduate School of Arts and Sciences,
Yale University, New Haven, CT

д-р Роберт Пенчовски,
доц. по генетика, биоинформатика и синтетична биология,
Биологически Факултет, СУ

Съдържание

1. Създаване на нови антибиотици чрез инхибиране растежа на човешки патогенни бактерии с използване на антисенс олигонуклеотиди, които се свързват с бактериални рибопревключватели

2. Създаване на нови универсални стратегии за лечение на рака с използването на алоетсрични рибозими

Литература:

1.Penchovsky R. & Traykovska M., *Designing drugs that overcome antibacterial resistance: where do we stand and what should we do?* Expert Opinion on Drug Discovery, 10(6), 631-650. (2015). (IF:3.46)

2. Penchovsky, R Computational design of allosteric ribozymes as Molecular Biosensors, Biotechnology Advances, 32, 1015-1027 (2014). – Impact factor (IF:11.08)

3. Penchovsky, R & Kostova, G. Computational selection and experimental validation of allosteric ribozymes that sense a specific sequence of human telomerase reverse transcriptase mRNAs as universal anticancer therapy agents. Nucleic Acid Therapeutics, 23, 408-431 (2013). - (IF:2.92)

4. Penchovsky, R. Present and Future RNA-based Approaches to Medical Genomics, International Journal of Genomic Medicine, 10.4172/2332-0672.1000110, 1(2), 1-7 (2013).

5. Penchovsky R. Engineering integrated digital circuits with allosteric ribozymes for scaling up molecular computation and diagnostics. ACS Synthetic Biology, 1, 471-482 (2012). – (IF: 3.95).

6. Penchovsky, R. Computational design and biosensor applications of small molecule-sensing allosteric ribozymes. ACS Biomacromolecules, 14, 1240–1249 (2013). – (IF:5.87)

7. Penchovsky R. & Stoilova C.C. Riboswitch-based antibacterial drug discovery using high-throughput screening methods. Expert Opinion on Drug Discovery, 8, 65-82. (2013). - (IF:3.46)

9. Penchovsky R. Chapter 5: Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future, in the book "Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine: Technologies and Processes", Publisher IGI Global, DOI: 10.4018/978-1-120-7, 71-96 (2012).

10. Blount, K., Puskarz, I., Penchovsky, R. & Breaker, R.R. Development and application of a high-throughput assay for glmS Riboswitch Activators. RNA Biology, 3, 77-81 (2006). – (IF: 5.6)

11. Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nature Biotechnology, 31, 1424-143 (2005) (IF: 39.2)

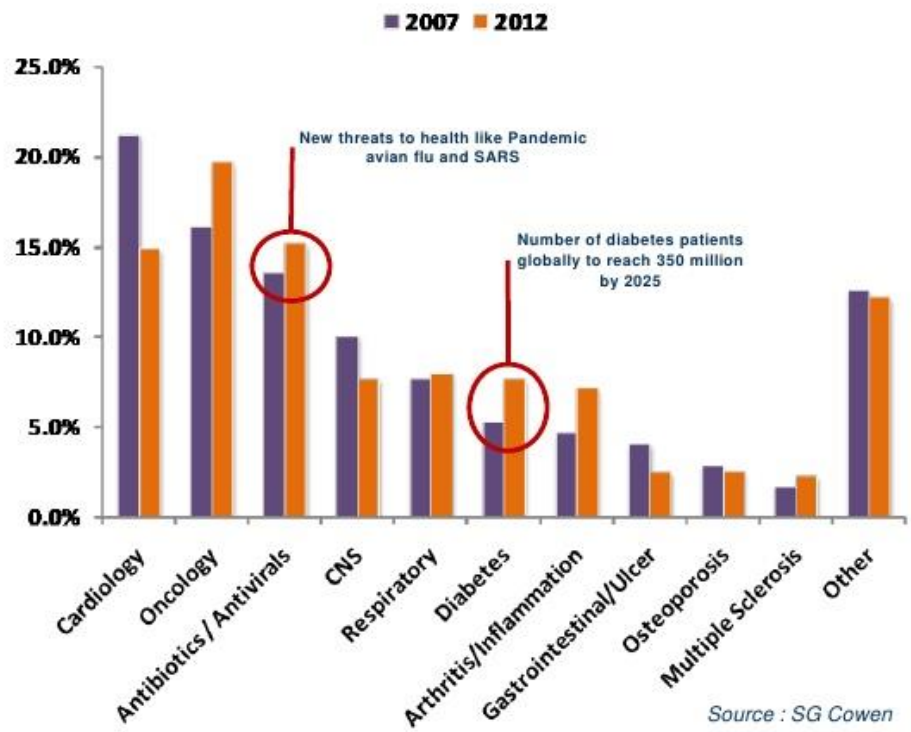
12. Breaker, R.R. & Penchovsky, R. *Computational Design of Ribozymes*. Patent: US20110288826

Глобалния пазар на лекарства

FROST & SULLIVAN

Oncology / Hematology, Antibiotics / Antivirals and Cardiovascular Lead the way in the Global Pharmaceutical Market in 2012 in terms of Market Size

New markets will continue to emerge in obesity, aging. Targeted therapies will drive the well-established cancer and cardiovascular indications.

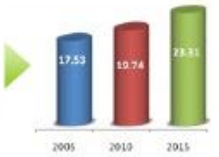


Others: Include Transplant, Sleep Disorders, Alzheimer's, Incontinence, Orphan Diseases, Sex Dysfunction, Ophthalmology, Obesity, Pain

Global Mortality

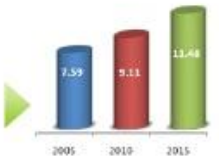
Cardiovascular Disease

Secondary prevention measures and new therapies are shifting treatment from inpatient to ambulatory care setting.



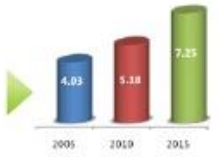
Cancer

The treatment model is changing from acute to chronic disease management as mortality rates fall. New treatments likely to have significant costs. Demand likely to grow in primary care settings.



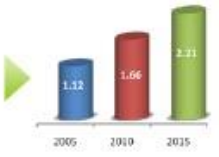
Respiratory Disease

No major treatment improvements on the horizon. Unmet needs exist.

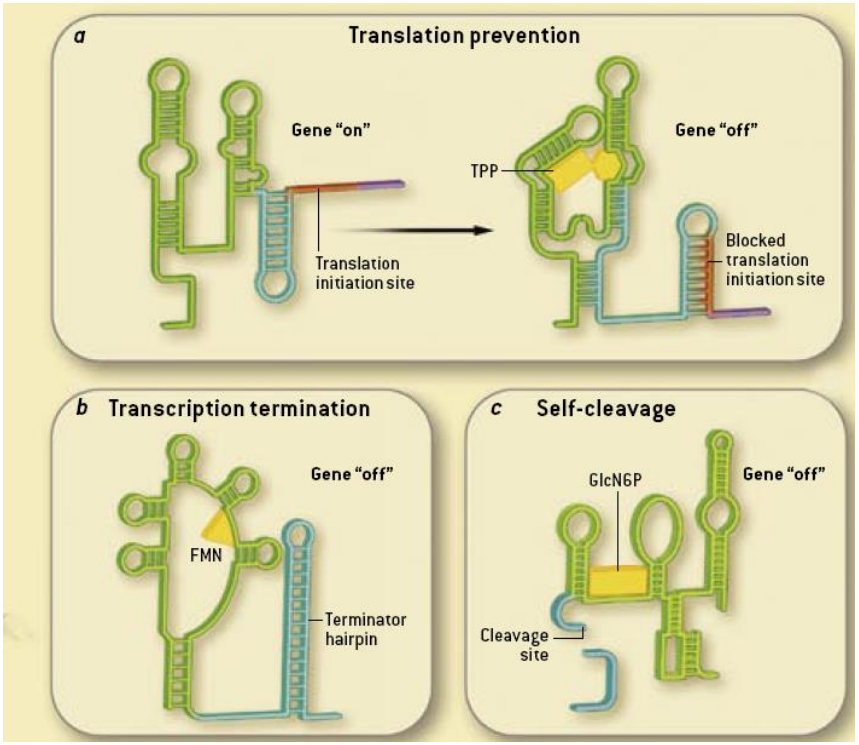


Diabetes

Cell therapy, better monitoring and new pharmacological treatments should reduce mortality in the long term.



Бактериални рибоплевлкючвтели – контрол на генна експресия в много патогенни бактерии

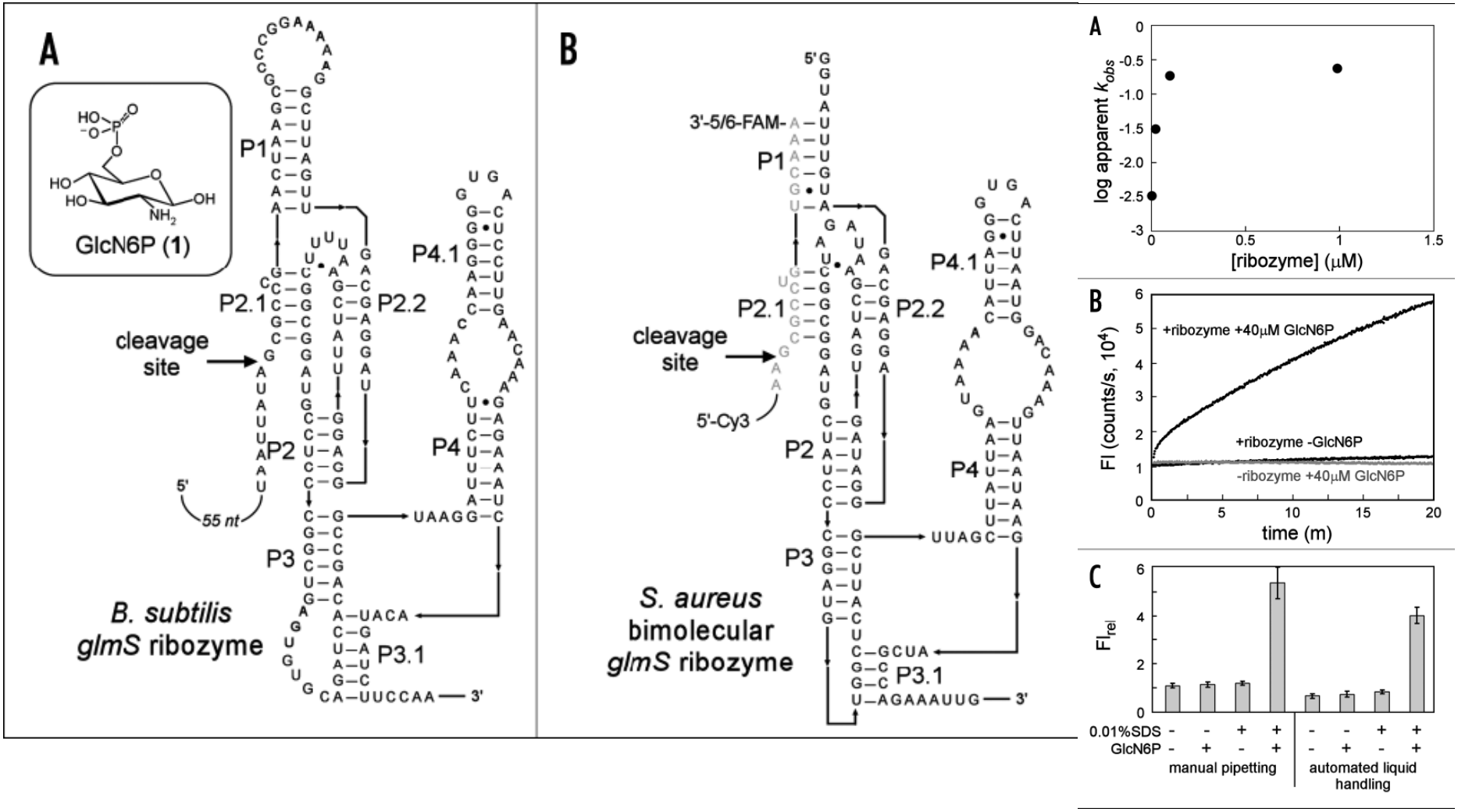


Human Bacterial Pathogen	Riboswitch Classes	Genes Regulated
Acinetobacter baumannii	4	6
Bacillus anthracis	9	82
Brucella melitensis	5	21*
Enterococcus faecalis	7	17
Escherichia coli	4	15*
Francisella tularensis	4	8
Hemophilius influenzae	5	15*
Helicobacter pylori	1	2
Listeria monocytogenes	9	49
Mycobacterium tuberculosis	3	13
Pseudomonas aeruginosa	3	27
Salmonella enterica	3	34*
Staphylococcus aureus	8	30*
Streptococcus pneumoniae	5	19
Vibrio cholerae	5	13
Yersinia pestis	3	11

17 различни класа рибоплевлкючвтели са открити в 34 патогенни за човека бактерии. Те могат да се използват като нови мишени за създаване на антибиотици.

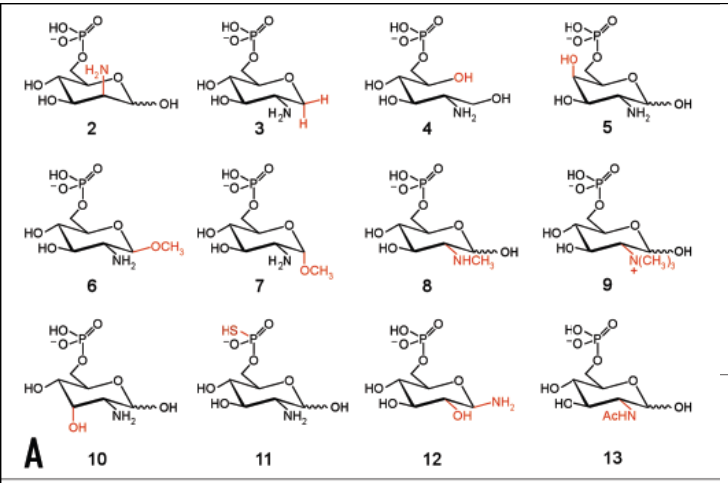
Penchovsky R. & Stoilova C.C. Riboswitch-based antibacterial drug discovery using high-throughput screening methods. Expert Opinion on Drug Discovery, 8, 65-82. (2013).

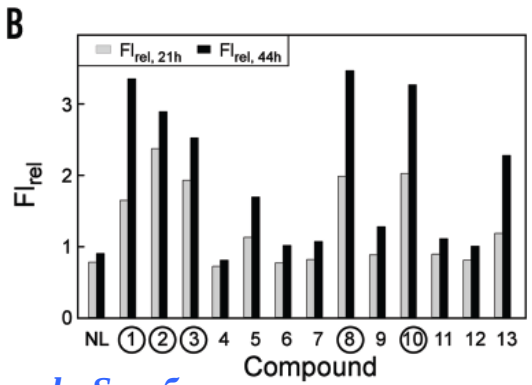
Създаване на високо производителен метод за откриване на малки молекули, които активират *glmS* рибопревключвателя

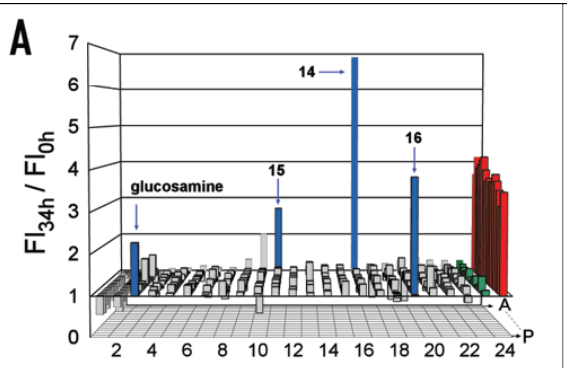


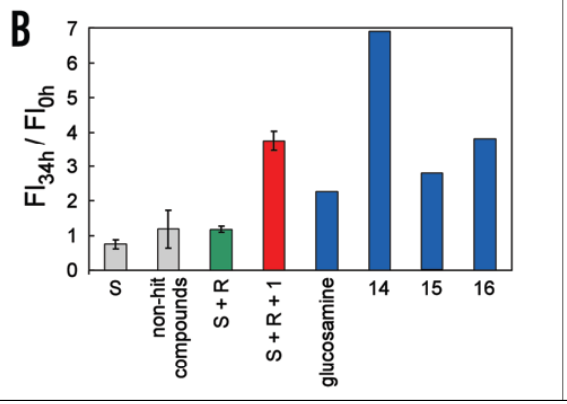
10. Blount, K., Puskarz, I., Penchovsky, R. & Breaker, R.R. Development and application of a high-throughput assay for glmS Riboswitch Activators. RNA Biology, 3, 77-81 (2006).

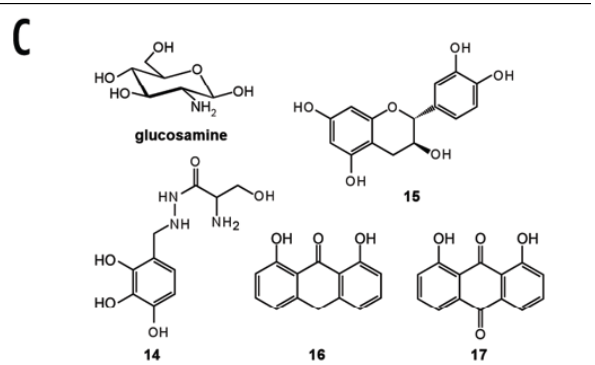
Създаване на високо производителен метод за откриване на малки молекули, които активират *glmS* рибопревключвателя

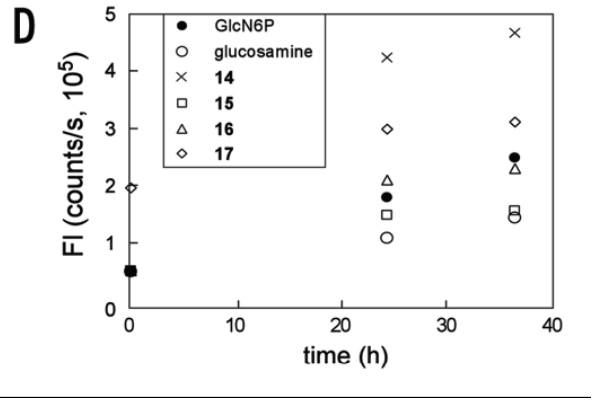










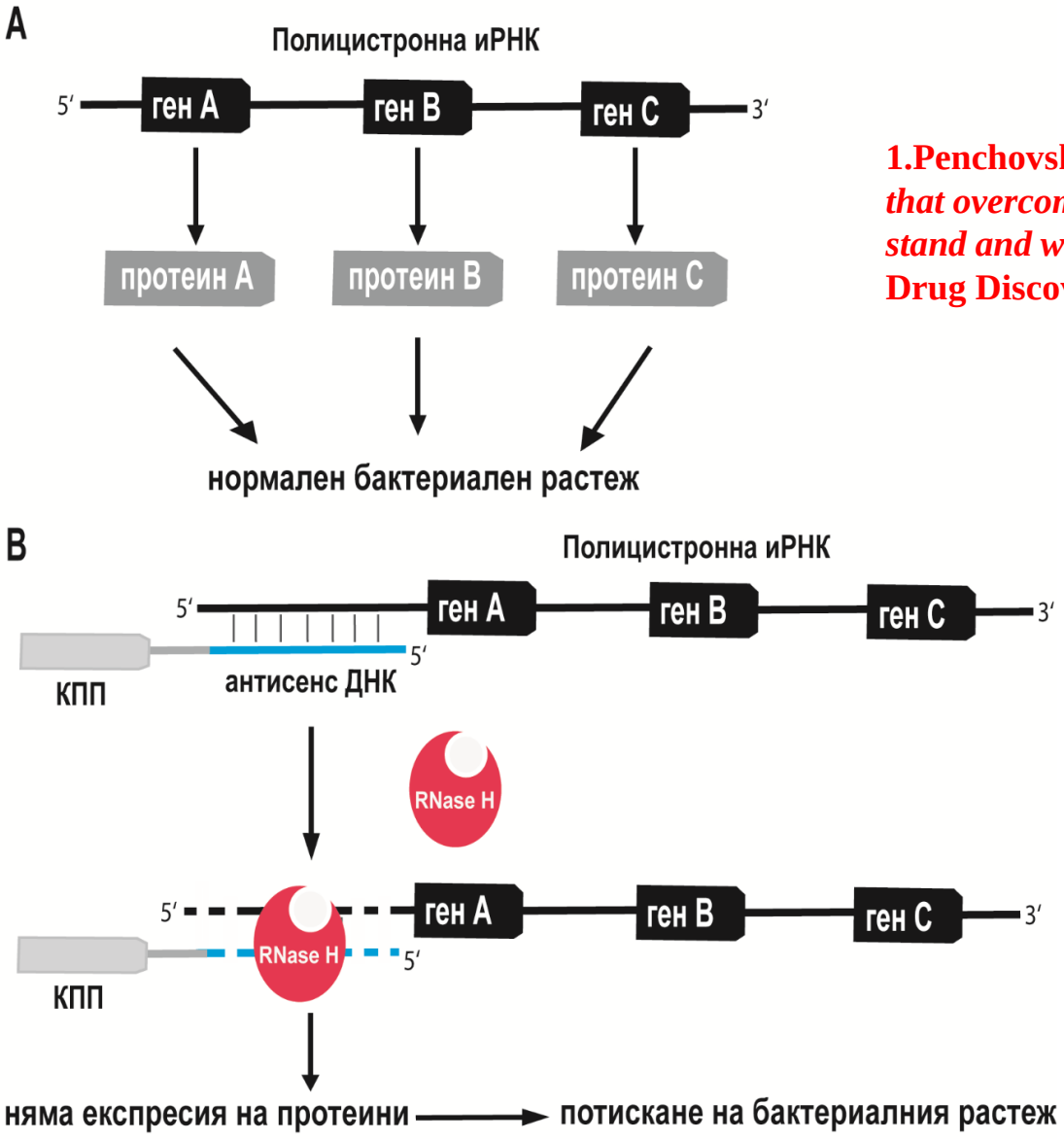


***glmS* рибопревключвателя се среща в няколко човешки патогенна включително:**

1. **Enterococcus faecalis**
2. **Bacillus anthracis**
3. **Listeria monocytogenes**
4. **Staphylococcus aureus**

10. Blount, K., Puskarz, I., Penchovsky, R. & Breaker, R.R. Development and application of a high-throughput assay for glmS Riboswitch Activators. RNA Biology, 3, 77-81 (2006).

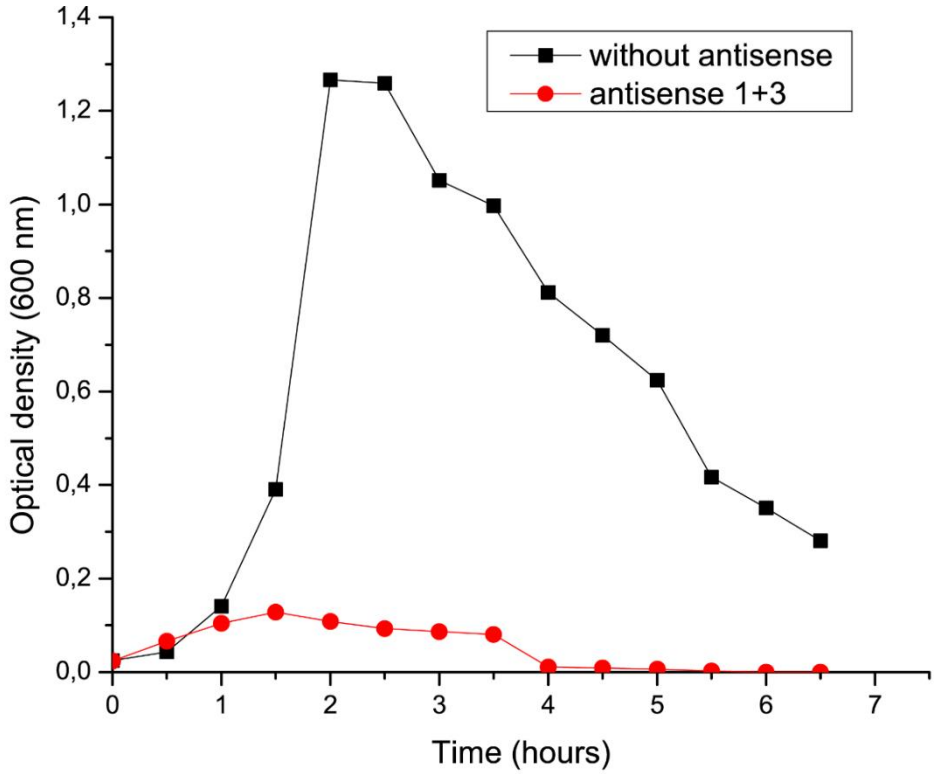
Инхибиране на специфични РНКи чрез антисенс олигонуклеотиди



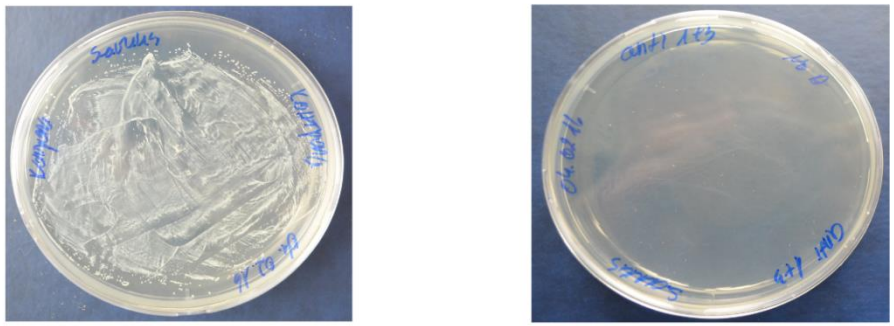
1.Penchovsky R. & Traykovska M., *Designing drugs that overcome antibacterial resistance: where do we stand and what should we do?* Expert Opinion on Drug Discovery, 10(6), 631-650. (2015).

Инхибиране растежа на Staphylococcus aureus чрез антисенс олигонуклеотиди

A



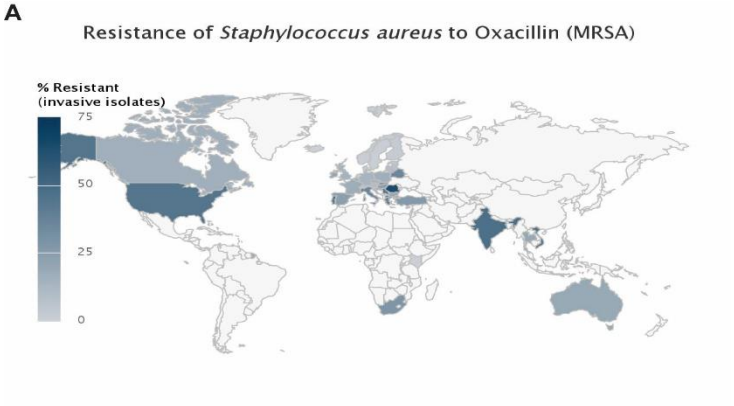
B

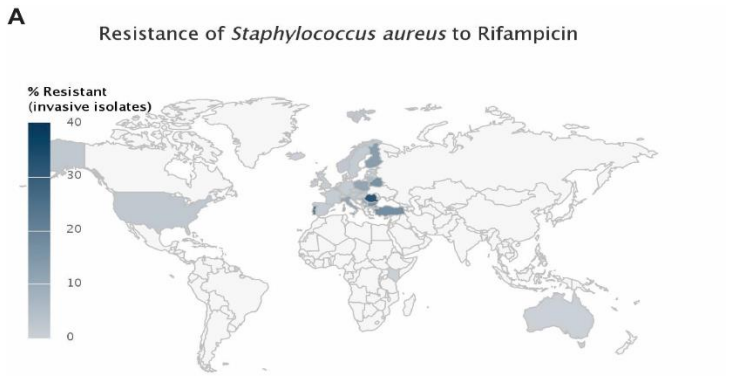


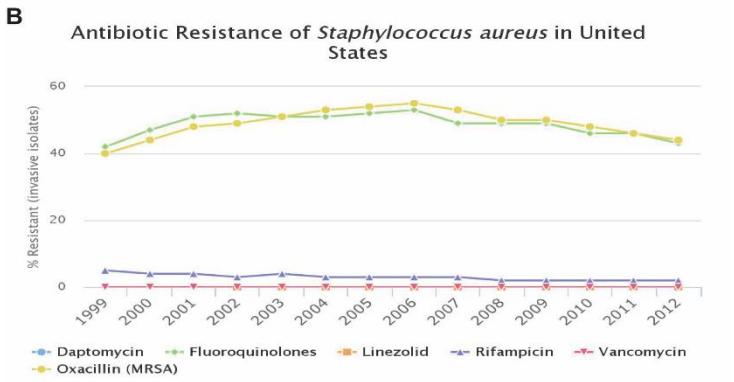
without antisense

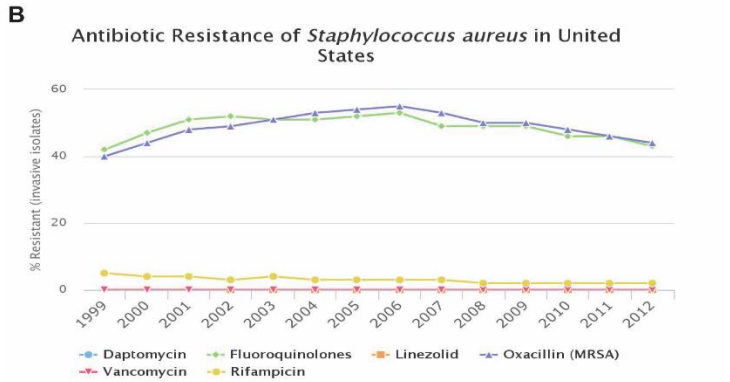
antisense 1+3

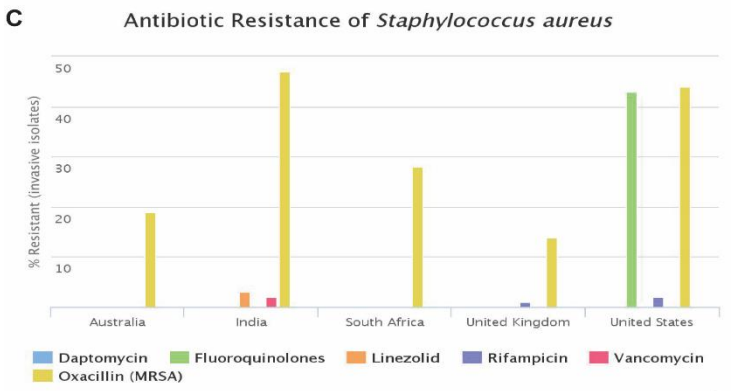
Резистентни щамове на Staphylococcus aureus по света

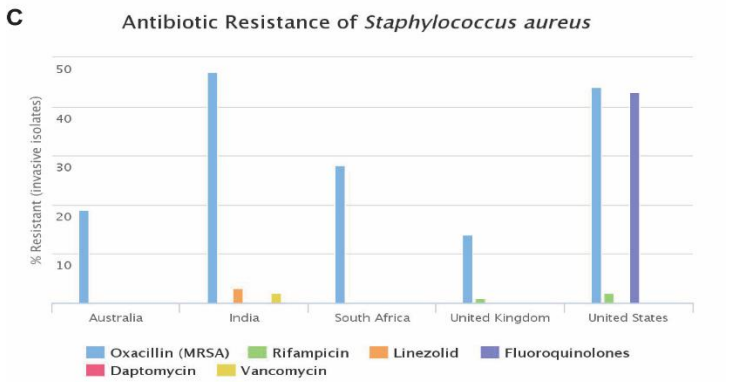








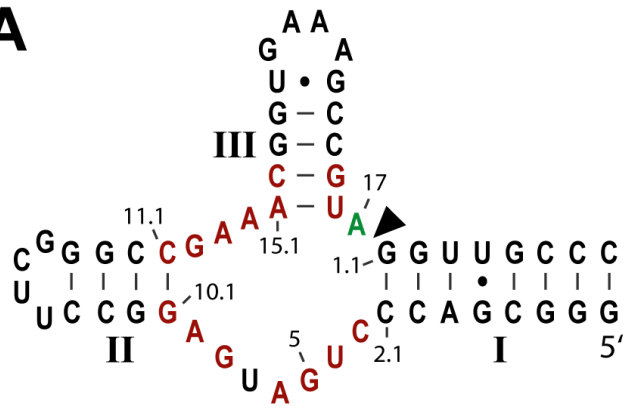




Вторични структури на Hammerhead Ribozymes

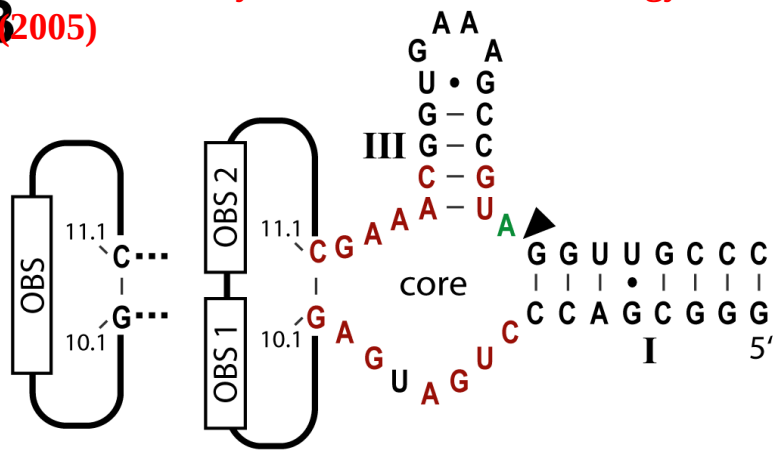
11. Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nature Biotechnology, 31, 1424-143 (2005)

A



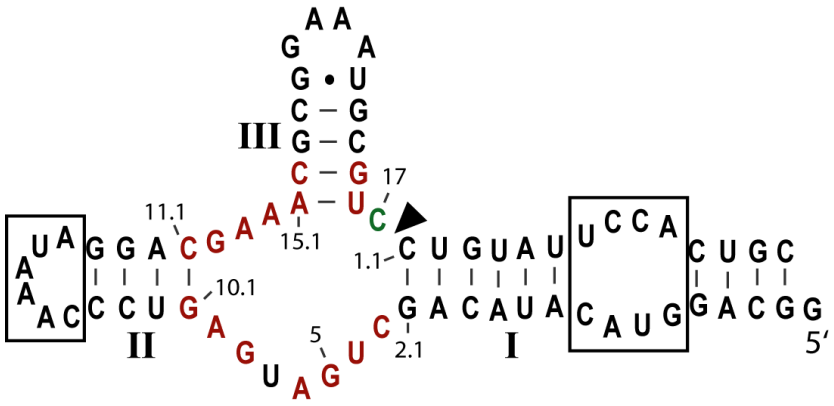
minimal hammerhead ribozyme

B



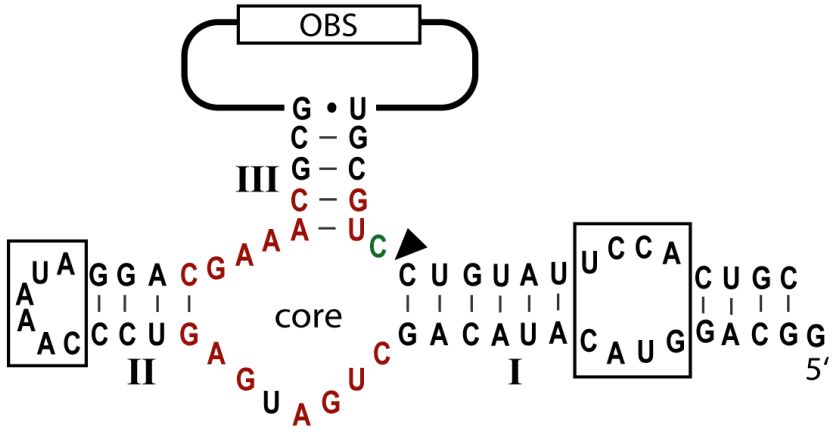
allosteric ribozyme architecture based on the minimal version

C



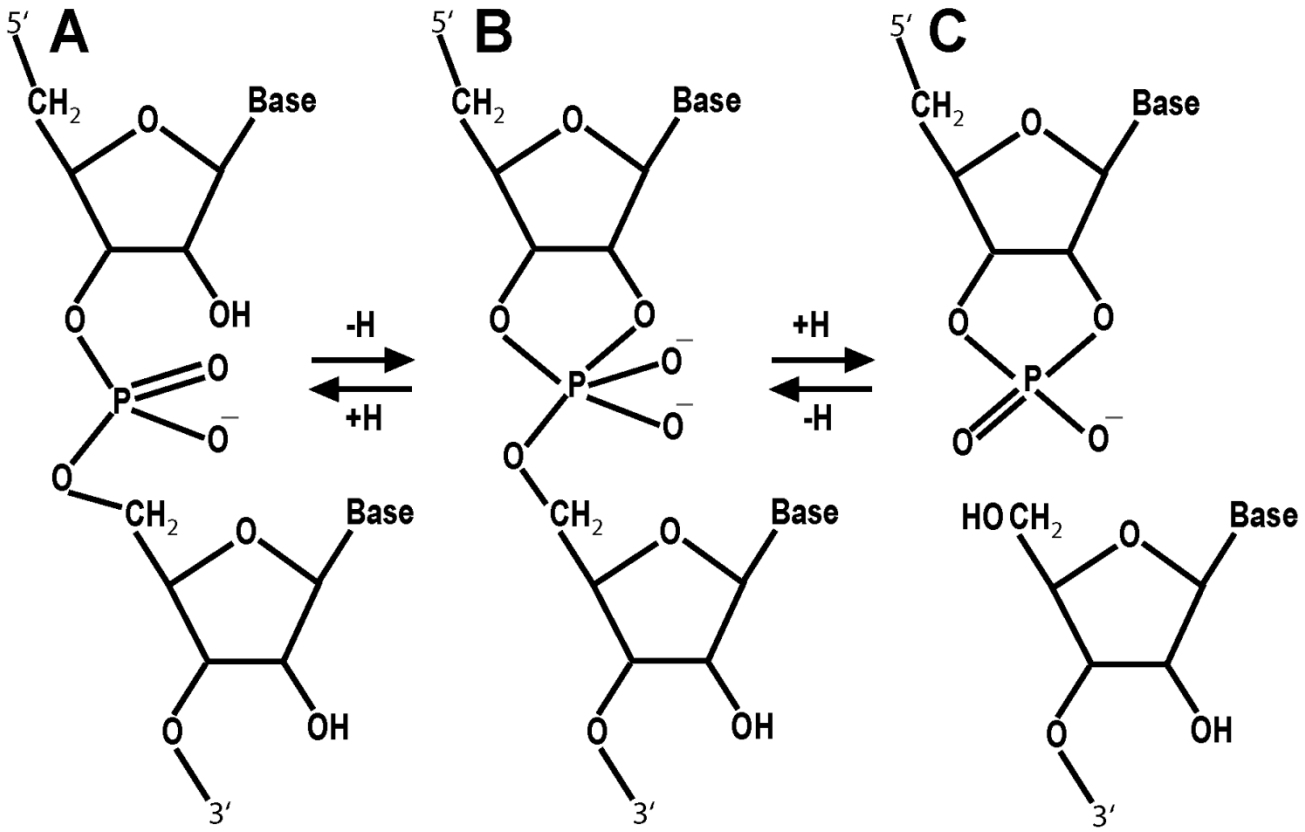
extended version of the hammerhead ribozyme from Schistosoma mansoni

D



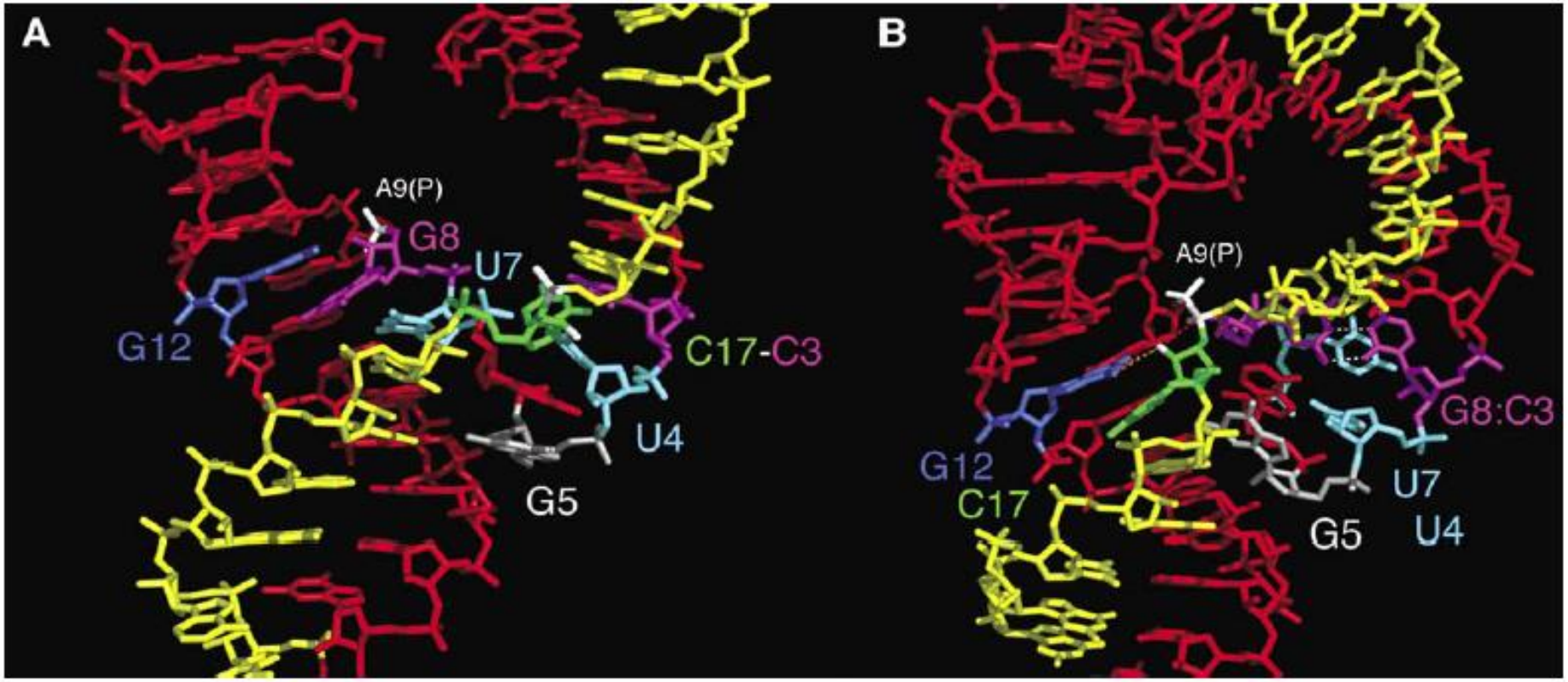
allosteric ribozyme architecture based on the extended version

Механизъм на каталитичната функция на хамърхед рибозимата



Penchovsky, R., Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future, in the book "Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine: Technologies and Processes", Publisher IGI Global, in press (2010)

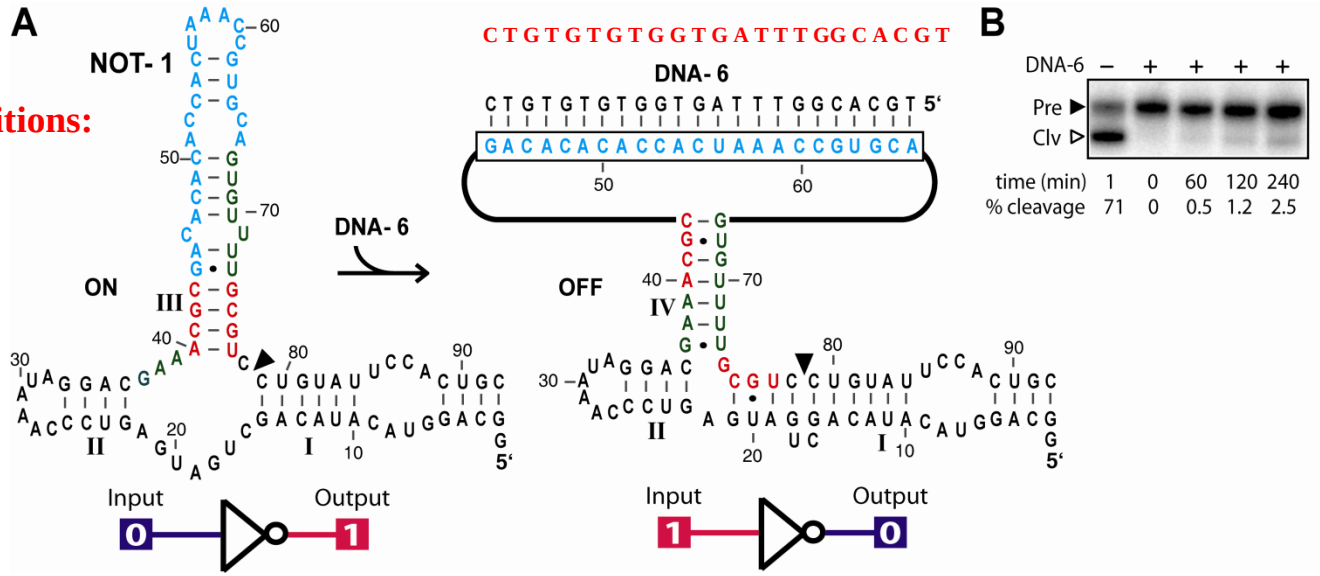
Третични структури на хамърхед рибозимата

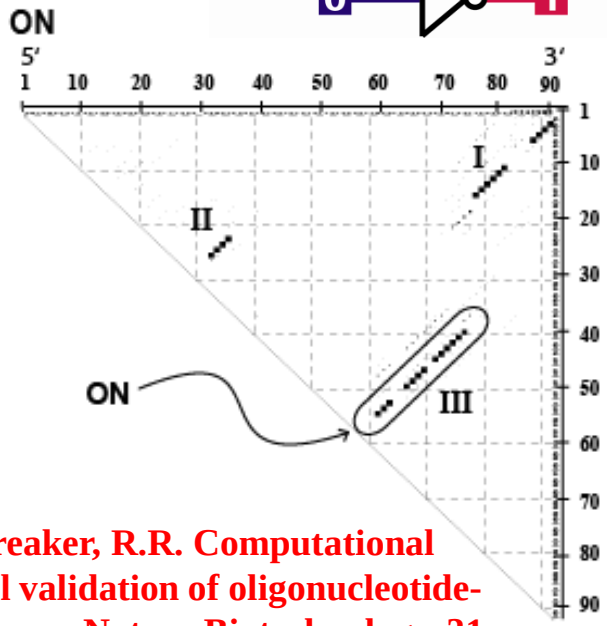


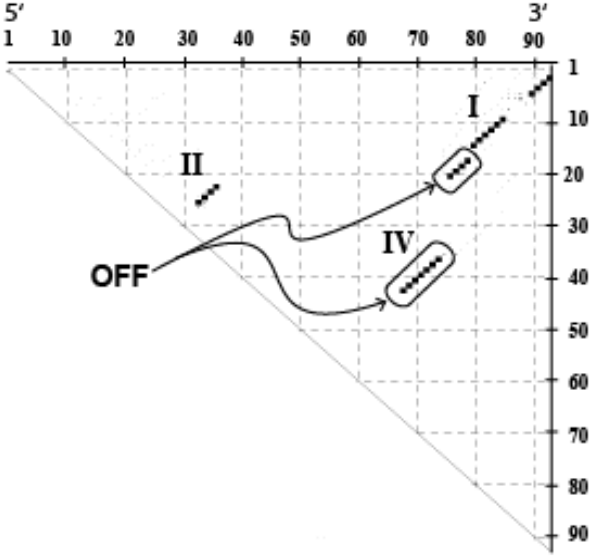
Martick, M. et al., Cell, 2006

Компютърен дизайн на алостерична хемърхед рибозима с НЕ логическа функция

Under physiologically relevant conditions:
1 mM MgCl2
100 mM KCl
25 mM NaCl
50 mM HEPES pH 7.5 at 37 oC







11. Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nature Biotechnology, 31, 1424-143 (2005)

penchovsky_nat_biotech_review.pdf (SECURED) - Adobe Acrobat Reader DC

File Edit View Window Help

Home Tools penchovsky_nat_bio... Sign In

1 / 3 61.9%

at a ratio of 1:1, two days before radical dissection of regional lymph nodes. The cells were successfully tracked *in vivo* using a noninvasive 3T MRI system. In 4 out of 8 patients, MRI demonstrated that the dendritic cells were actually delivered into the perinodular fat (and not into the lymph node), whereas scintigraphy detected a single spot at the injection site. MRI could detect up to 1.5×10^5 migrated cells and image up to 5 individual lymph node sites (whereas scintigraphy detected two sites in most cases). By co-injecting equal numbers of dendritic cells labeled with ^{111}In or with SPIO particles, they demonstrated that MRI is as sensitive as scintigraphic imaging for the detection of dendritic cells *in vivo*.

The major advantage of MRI over scintigraphic imaging is the presence of high-resolution anatomical background contrast, allowing for a precise anatomical localization of SPIO-labeled cells, first at the injection site and then at distant sites. Thus, MRI proved valuable for monitoring the migratory capacity of dendritic cells. Remote lymph nodes containing SPIO-labeled cells could be visualized individually in patients *in vivo*, and this result was confirmed by histological analysis of lymph node biopsies. The high spatial resolution of MRI and lack of saturation of images represent significant advantages over scintigraphy. However, according to de Vries *et al.*, scintigraphic imaging is superior to MRI for quantifying the number of cells that have migrated to lymph nodes. Therefore, the authors argue that both techniques should be combined to achieve accurate quantitative and qualitative information on the fate of adoptively transferred dendritic cells.

The current work is of biological and medical significance. Proper inoculation of dendritic cell vaccines should improve our knowledge of dendritic cell biology and, more importantly, should boost the clinical response to antigen-loaded dendritic cells,

Boolean calculations made easy (for ribozymes)

Adam A Margolin & Milan N Stojanovic

A new algorithm facilitates the design of ribozymes that encode logical operations.

The basic electronic devices that perform Boolean calculations are called logic gates. Elementary logic gates, such as AND, NOT and OR, are organized into more complex electronic circuits that, together with other devices, are used to build computers. Molecules can function as logic gates in that their behavior (outputs) is influenced by the presence of other molecules (inputs). The construction of a reliable set of molecular-scale logic gates that can communicate with each other would allow the engineering of molecular circuits capable of performing complex logical operations in solution. From this perspective, the contribution by Penchovsky and Breaker[2] in this issue is groundbreaking; they describe a precise protocol to construct and test a complete set of ribozyme-based logic gates whose self-cleavage activity is modulated by the presence of oligonucleotide inputs. This work also provides us with a tantalizing glance into how these elementary units can be organized into more complex circuits.

To construct molecules that perform basic Boolean operations in solution, Penchovsky and Breaker generate allosteric ribozymes using the hammerhead ribozyme secondary structure motif (**Fig. 1a**). For each logic gate, a computer program searches millions of potential sequences, and a partition function algorithm identifies those sequences that are most likely to form a dominant active or inactive secondary structure and robustly switch to the alternate state in the presence of one or more oligonucleotides.

For a sensor (YES) gate, the ribozyme must assume an inactive conformation except in the presence of a particular oligonucleotide, which triggers activity. For a NOT gate, the effect of the oligonucleotide is reversed; that is, it deactivates the ribozyme.

For the two-input logical operations AND and OR, the partition function must consider four possible states corresponding to the presence or absence of two inputs.

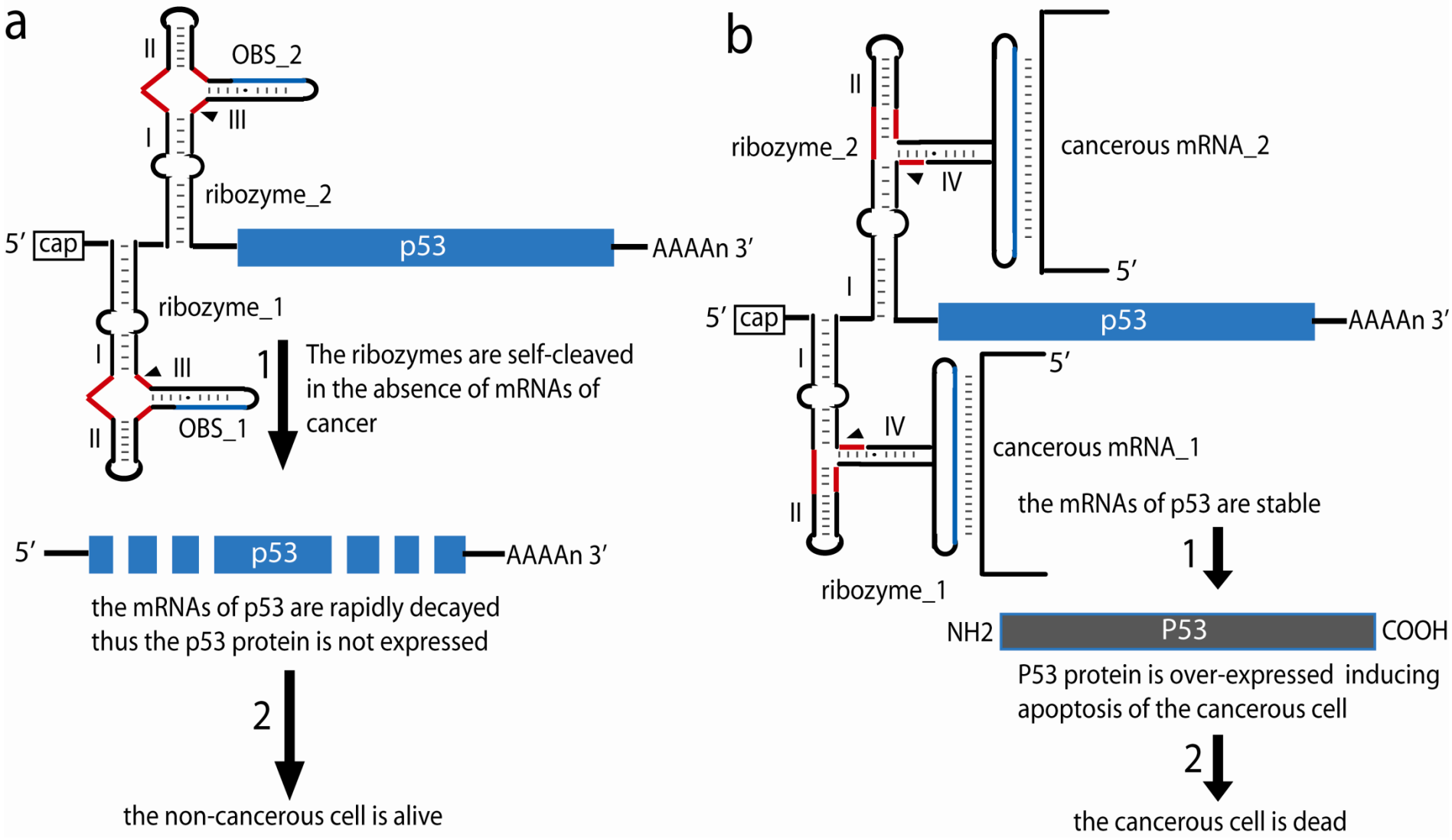
All gates designed by Penchovsky and Breaker consist of a catalytic module that undergoes self-cleavage if the gate is in the active form, and a recognition module that binds one or more input oligonucleotides (**Fig. 1a**). Recognition modules can be altered to exhibit different input specificities with a high likelihood that they will maintain robust switching

Adam A. Margolin is in the Department of Biomedical Informatics and the Joint Centers for Systems Biology, Columbia University, 1130 St. Nicholas Ave. and Milan N. Stojanovic is at the NSF Center for Molecular Cybernetics and the Division of Experimental Therapeutics, Department of Medicine, Columbia University, 650 W. 168th St., New York, New York 10032, USA.
e-mail: mns18@columbia.edu

11. Penchovsky, R. & Breaker, R.R. Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nature Biotechnology, 31, 1424-143 (2005) (IF: 39.2)

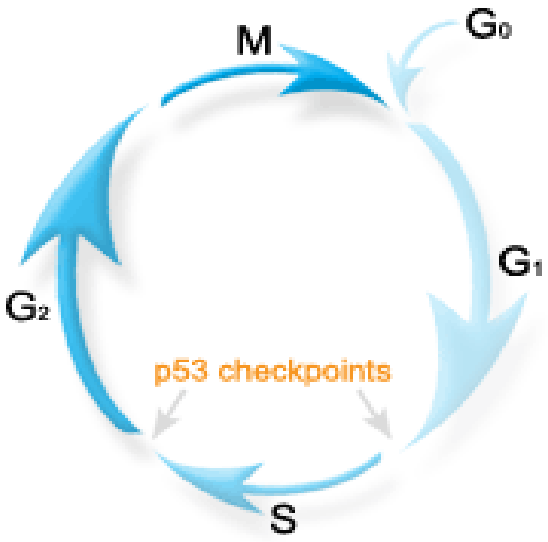
12. Breaker, R.R. & Penchovsky, R. *Computational Design of Ribozymes*. Patent: US20110288826

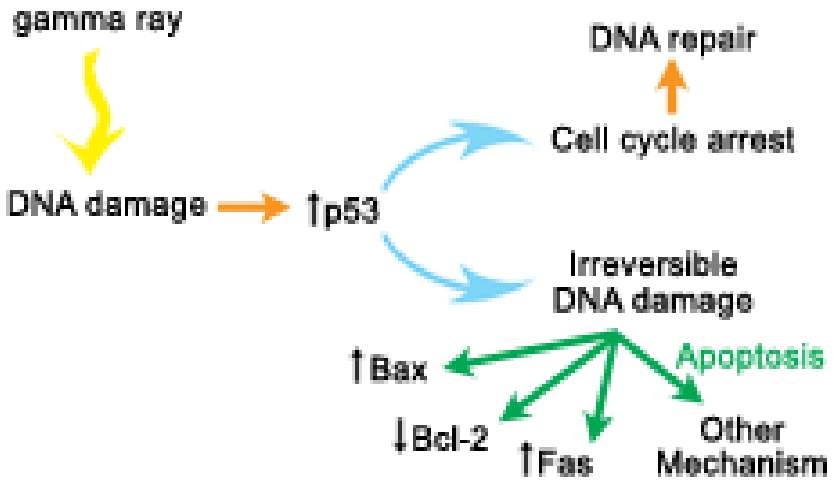
Ре-програмиране на клетъчната смърт с алостерична хемърхед рибозима с НЕ логическа функция

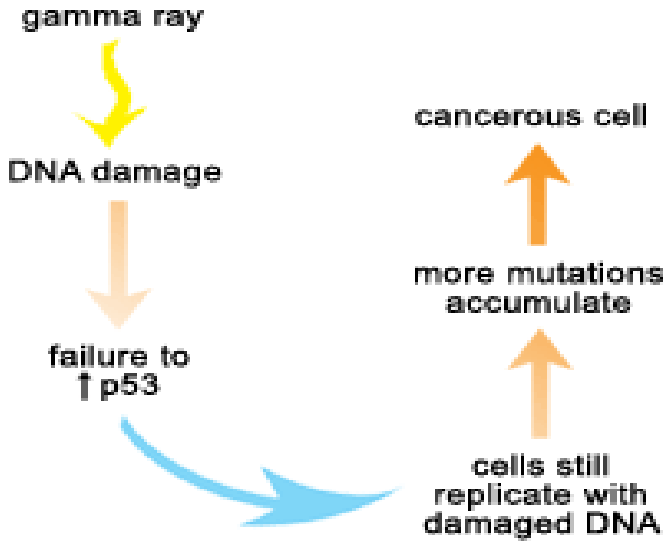


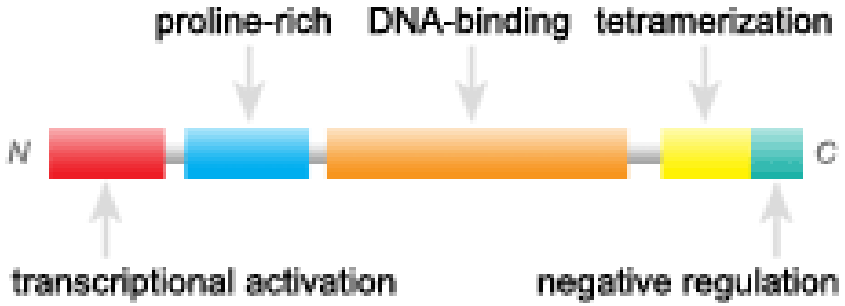
Penchovsky, R., **Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future,** in the book "Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine: Technologies and Processes", Publisher IGI Global, in press (2010)

Ролята на p53 програмираната смърт на клетката

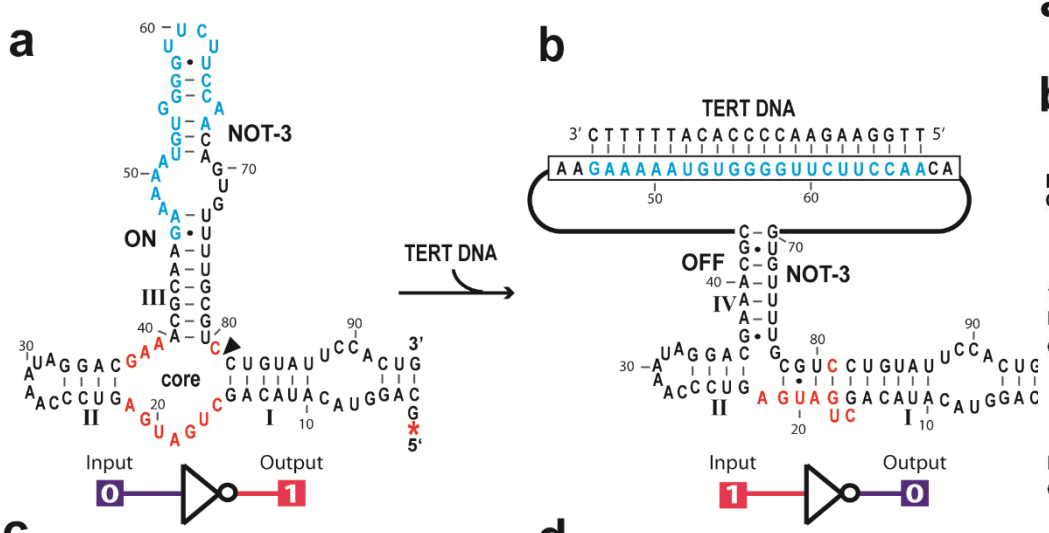


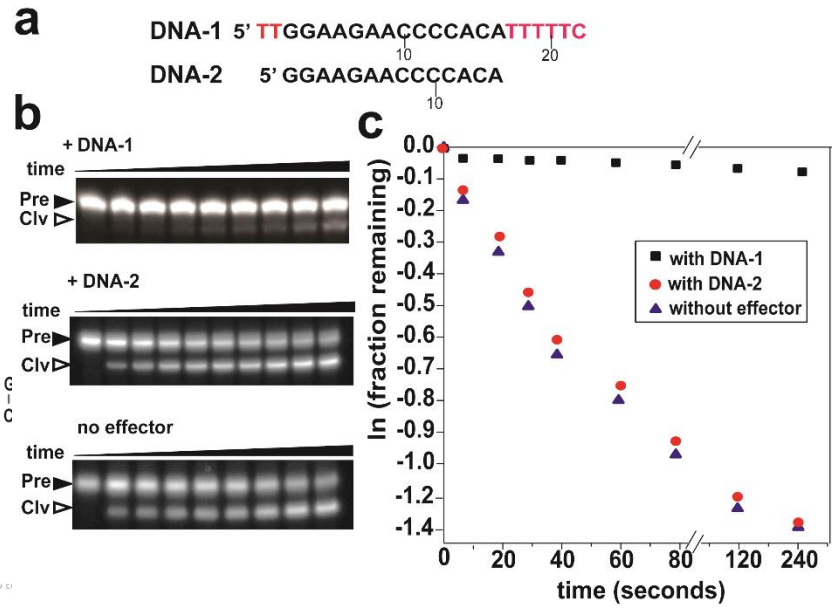


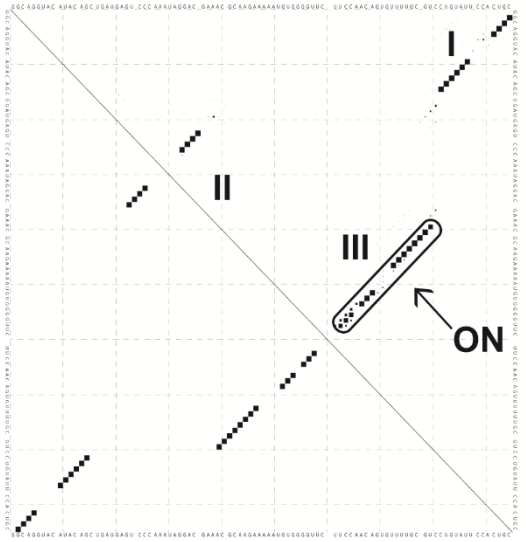


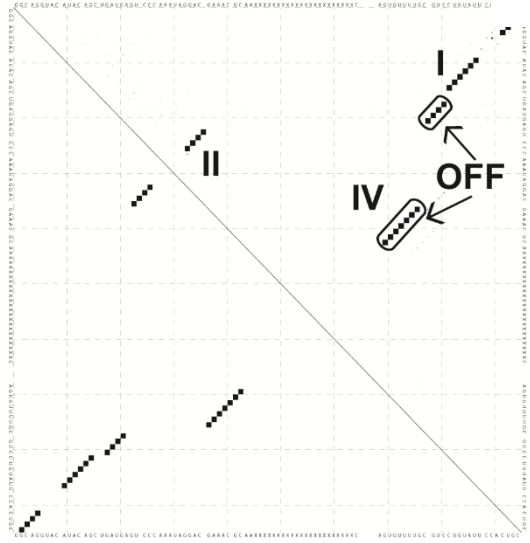


Ре-програмиране на клетъчната смърт с алостерична хемърхед рибозима с НЕ логическа функция









Penchovsky, R, & Kostova, G.T. Computational Selection and Experimental Validation of Allosteric Ribozymes that Sense a Specific Sequence of Human Telomerase Reverse Transcriptase mRNAs as Universal Anticancer Therapy Agents, Nucleic Acid Therapeutics, (2013).

Result.pdf - Adobe Acrobat Reader DC

Home Tools Result.pdf Sign In

FEATURE
RIBOZYMES

altering the nucleotides in the arms.

Several groups are using this technique to design ribozymes to recognise and cleave specific RNA sequences, both to help elucidate molecular mechanisms in vivo and for biotechnological applications. Matsuura Fukuda and his group at Fukuoka University in Japan are using it to understand some of the mechanisms involved in RNA editing. This name is given to processes that change the chemistry of the bases in RNA during or after their transcription from DNA. Adenosine-to-inosine (A-to-I) RNA editing, as the name implies, involves the deamination of adenine to form another purine, hypoxanthine (inosine is the hypoxanthine nucleoside). Since adenosine is decoded as guanine, it can alter the protein sequence when it occurs in protein-coding regions, and it regulates a variety of biological processes.

Fukuda and his co-workers used the generic fact that hammerhead ribozymes can cleave the base sequence UAA but not UGA (U: uracil; A: adenine; G: guanine) and the similarity between guanosine and inosine to engineer a ribozyme structure that cleaves an RNA editing target site only if the A-to-I substitution has not been made. They then created a library of ribozymes containing this central motif extended on each side with random sequences, and selected the most stable and therefore most active one for further testing. 'This ribozyme cleaved unedited RNA sequences but not edited ones, but it was fully active only at higher magnesium concentrations than those that occur in cells,' says Fukuda. 'Creating a ribozyme that works at physiological salt concentrations will require further modification.' Aberrations in A-to-I RNA editing have been observed in several diseases, and Fukuda hopes that novel nucleic acid-targeted therapies may one day be created using similar techniques. However, it is not yet clear whether these aberrations are a cause or an effect of disease. 'The first applications of these techniques are likely to be novel tools for molecular biology research,' adds Fukuda.

Robert Penchovsky of Sofia University in Bulgaria is using a similar approach to design ribozymes that detect and respond to the presence of specific small molecules. An aptamer is a short nucleic acid sequence or a small protein that binds to a given molecular target. Most aptamers are synthetic, and allosteric ribozymes – ones that are active only with a ligand bound outside the catalytic domain – can be designed by fusing an RNA aptamer containing the binding site with, for example, a catalytic hammerhead ribozyme.

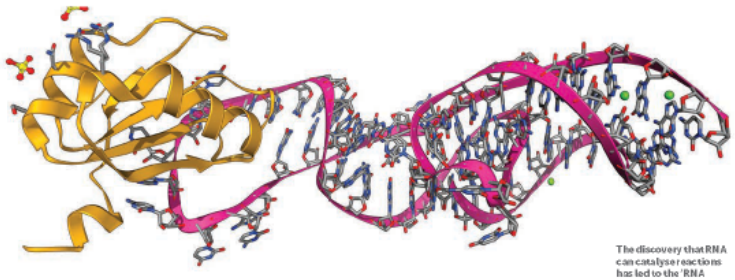
> The first applications are likely to be tools for molecular biology

Penchovsky used computer modelling to design molecules of this type in which the ribozyme changes conformation between active and inactive states in response to theophylline, a purine that is chemically similar to caffeine. 'These simple ribozymes can act as molecular logic gates with the Boolean logic YES function if theophylline binding activates the ribozyme and the NOT function if binding deactivates it,' says Penchovsky. 'I can also design oligonucleotide-sensing ribozymes that act as AND and as OR gates, and similar biosensor systems have already been used in high throughput screening arrays for antibacterial drug discovery.' There are other potential applications of this technology in molecular computing and antibiotic design, and Penchovsky is seeking industrial collaborators to develop these further.

The ribosome

Hammerhead ribozymes are fairly small, tractable molecules that can be synthesised relatively easily. There are other biologically important ribozymes, however, that are right at the other end of the macromolecular spectrum. The ribosome, the 'molecular machine' that catalyses protein synthesis and is found in all living cells, has two subunits that only come together during protein synthesis, and each subunit includes many protein and RNA molecules. When the first high resolution structure of the large subunit was published, the researchers involved, Tom Steitz and his group at Yale, commented that there were proteins everywhere on the subunit's surface 'except in the active site where peptide bond formation occurs and where it contacts the small subunit'. Steitz and his co-workers concluded that, very unexpectedly, it was the RNA molecules that were principally, if not solely, involved in peptide synthesis, with the proteins acting as 'staples' to hold them in place. They had established that the ribosome is a ribozyme.

Venki Ramakrishnan from the MRC Laboratory of Molecular Biology in Cambridge, UK, shared the 2009 Nobel prize in chemistry for studies of the ribosome with Steitz and with Ada Yonath of the Weizmann Institute in Israel. He has devoted much recent research to understanding the mechanism through which the ribosome recognises the three nucleotides that form a 'stop codon', terminates protein synthesis and releases the newly formed protein. The standard genetic code uses three stop codons, UAA, UGA and UAG, and Ramakrishnan's



The discovery that RNA can catalyse reactions has led to the 'RNA world' hypothesis

1. Благодаря за Вашето вниманието!

2. Благодаря на моя докторат Мартина Трайковска и на всичките 10 дипломанта защитили в моята лаборатория от 2010г насам - http://penchovsky.atwebpages.com/teaching.php?page=14

3. Благодаря на г-н Аврамов и акад. Съботинов за поканата да изнеса тази лекция.

Имате ли въпроси?