

BIOGRAPHICAL SKETCH

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NAME: Alexander Serganov

eRA COMMONS USER NAME (credential, e.g., agency login): SERGAA02

POSITION TITLE: Associate Professor of Biochemistry and Molecular Pharmacology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Lomonosov Moscow State University, Moscow	B.S.	05/1992	Biochemistry
Inst. Of Protein Research, Russian Acad. Sciences	Ph.D.	05/1997	Molecular Biology
Inst. Molecular Cellular Biology, CNRS, Strasbourg	Postdoctoral	08/1997	Molecular Biology
Memorial Sloan-Kettering Cancer Center, New York	Postdoctoral	09/1999	Structural Biology

A. Personal Statement

I was trained as a biochemist and crystallographer and led structural and biochemical research on various macromolecules involved in the regulation of gene expression. I joined the Department of Biochemistry and Molecular Pharmacology at the New York University School of Medicine as a tenure-track Assistant Professor in 2011 and was promoted to the tenure-track Associate Professor rank in 2017.

My research interests are centered on using various approaches to understand the structure and function of proteins and RNAs. First, we are interested in the mechanisms of RNA-mediated gene expression control and the recognition between RNA and their partners, including drug-like small molecules and proteins. Second, we are focusing on interactions between proteins and small molecules to understand the catalytic mechanisms of enzymes and identify novel antibacterials. Lastly, we study the mechanisms of RNA transcription and degradation.

Our last direction includes elucidation of the 5'-end-dependent RNA degradation pathway. Bacterial mRNAs are transcribed with a 5' triphosphate, which serves as a cap to protect mRNAs from degradation by 5'-to-3' exonucleases and 5'-end-dependent endonucleases. Cellular enzymes, such as RNA pyrophosphydrolase RppH, contributes to removing the triphosphate and triggering the RNA degradation. RppH was discovered by Joel Belasco's laboratory as an enzyme that accelerates mRNA degradation by cleaving off a terminal pyrophosphate, yielding monophosphorylated mRNAs that are more susceptible to cleavage by RNase E. However, some bacterial RNAs are modified on the 5'-end and these modifications decrease the degradation rate. We have teamed up with the Belasco laboratory to combine structural and genetic expertise in order to understand the molecular mechanisms of the 5' end modifications and 5'-end-dependent RNA degradation.

I have over 15 years of experience in protein and RNA crystallography and other techniques relevant to this application. I am confident that my research experience and biochemical expertise will ensure the success of the proposed project.

B. Positions and Honors

Positions and Employment

2003-2011	Senior Research Scientist, Memorial Sloan-Kettering Cancer Center, New York, NY
2011	Tenure-track Assistant Professor, New York University School of Medicine, New York, NY
2012-	Graduate Student Advisor, Molecular Biophysics Program, the Sackler Institute of Graduate Biomedical Sciences, New York University School of Medicine, New York, NY

Other Experience and Professional Memberships

Grant applications reviewer: NIH, DoD, European Research Council, Biotechnology and Biological Sciences Research Council (United Kingdom), German Research Foundation, Royal Society University Research Fellowship Program (United Kingdom), National Science Centre (Poland), M.J. Murdock Charitable Trust (USA), Louisiana Experimental Program to Stimulate Competitive Research (USA).

Editor: *Central European Journal of Biology*, *Proc Natl Acad Sci USA* (*ad hock*)

Editorial board: *Journal of Proteome Science and Computational Biology*

Reviewer: *Acta Crystallographica Section D*; *ACS Chemical Biology*; *Biochimica et Biophysica Acta*; *Biological Chemistry*; *Biochemistry*; *BMC Structural Biology*; *Bioorganic & Biotechnology Journal*; *Bioorganic & Medicinal Chemistry*; *Cell Chemical Biology*; *Cell Reports*; *Cell Research*; *Central European Journal of Biology*; *ChemBioChem*; *eLife*; *FEBS Letters*; *Future Medicine*; *Gene*; *Journal of the American Chemical Society*; *Journal of Biological Chemistry*; *Journal of Microbial & Biochemical Technology*; *Journal of Molecular Graphics and Modelling*; *Journal of Molecular Biology*; *International Journal of Molecular Sciences*; *Molecular Biotechnology*; *Molecular Cell*; *Nature Biotechnology*; *Nature Chemical Biology*; *Nature Communications*; *Nucleic Acids Research*; *PLoS One*; *Proc. Natl. Acad. Sci. USA*; *Process Biochemistry*; *Proteins: Structure, Function, and Bioinformatics*; *Structure*; *Trends in Pharmacological Sciences*; *RNA*; *RNA Biology*, *WIREs RNA* and other journals.

Membership: New York Academy of Sciences, American Chemical Society, RNA society

Honors

1992	Graduated with Honors, M.V. Lomonsov Moscow State University
1992	FEBS fellowship, FEBS advanced course
1993	FEBS fellowship, FEBS/EMBO summer school
1994	Doctoral Fellowship, International Soros Science Education Program
1995	Doctoral Fellowship, International Soros Science Education Program
1996	Doctoral Fellowship, International Soros Science Education Program
1997	Travel Award, IUBMB Congress
1997	Fellowship, NATO/EMBO, NATO Advanced Institute "Biomolecular Recognition"
1997	Travel Award, Netherlands Society for Biochemistry and Molecular Biology
1998	Postdoctoral Fellowship, Foundation for Medical Research, France
2012	The RNA Society Travel Award
2013	Whitehead Fellowship for Junior Faculty in Biomedical and Biological Sciences, New York University, USA
2014	Edward Mallinckrodt Jr. Foundation Award
2015	Irma T. Hirschl Career Scientists Award

C. Contribution to Science

1. Structural and functional insights on riboswitches.

For many years, it had been assumed that bacteria control expression of metabolic and transport genes through sensing of cellular metabolites by protein molecules. Over a decade ago, this view had been overturned by the discovery of riboswitches. Riboswitches are mRNA elements that directly sense cellular metabolites without protein participation and modulate expression of genes via metabolite-dependent conformational changes. To understand the molecular basis for riboswitch function, we determined (concurrently with the R. Batey laboratory) the first structures of riboswitches. These structures provided the first glance at the molecular principles of metabolite recognition by a natural RNA and the riboswitch mechanisms. Over years, I have played an instrumental role in determining structures of nine classes of riboswitches in the D.J. Patel laboratory and three riboswitch classes in my own laboratory. These studies uncovered many interesting features of riboswitches and made a significant contribution to our understanding of these exciting regulatory RNAs.

- a. Serganov, A., Yuan, Y.R., Pikovskaya, O., Polonskaia, A., Malinina, L., Phan, A.T., Hobartner, C., Micura, R., Breaker, R.R., Patel, D.J. (2004). Structural basis for discriminative regulation of gene expression by adenine- and guanine-sensing mRNAs. *Chem. Biol.*, 11, 1729-1741
- b. Serganov, A., Polonskaia, A., Phan, A.T., Breaker, R.R., Patel, D.J. (2006). Structural basis for gene regulation by a thiamine pyrophosphate-sensing riboswitch. *Nature*, 441, 1167-1171.
- c. Peselis, A. & Serganov, A. (2012). Structural insights into ligand binding and gene expression control by an adenosylcobalamin riboswitch. *Nat. Struct. Mol. Biol.*, 19: 1182-1184.
- d. Gao, A. & Serganov, A. (2014). Structural insights into recognition of c-di-AMP by the *ydaO* riboswitch. *Nat. Chem. Biol.* 2014; 10: 787-792. PMID:PMC4294798

2. Structure and mechanism of ribozymes.

Catalytic RNAs represent one of the most exciting discoveries in the RNA world. However, the majority of natural ribozymes are limited by the chemistry of the reaction they perform. Typically, they cleave and ligate the RNA backbone using a phosphodiester transfer reaction. The chemical repertoire of ribozymes can be greatly expanded by *in vitro* selection, which resulted in finding ribozymes capable of performing different reactions. I have obtained the first three-dimensional structure of a catalytic RNA that performs a chemical reaction other than a phosphodiester transfer. This ribozyme catalyzes the stereospecific formation of carbon-carbon bonds via Diels-Alder reaction. Our structure of the Diels-Alder ribozyme provided the first mechanistic insights on the ribozyme catalysis involving small organic molecules and revealed several novel principles of an RNA-mediated chemical reaction.

- a. Serganov, A., Keiper, S., Malinina, L., Tereshko, V., Skripkin, E., Hobartner, C., Polonskaia, A., Phan, A.T., Wombacher, R., Micura, R., Dauter, Z., Jaschke, A., Patel, D.J. (2005). Structural basis for Diels-Alder ribozyme-catalyzed carbon-carbon bond formation. *Nat. Struct. Mol. Biol.*, 12, 218-224.
- b. Wombacher, R., Keiper, S., Suhm, S., Serganov, A., Patel, D.J. & Jaschke, A. (2006). Control of stereoselectivity in an enzymatic reaction by backdoor access. *Angew. Chem. Int. Ed. Engl.*, 45: 2469-2472.

3. Gene expression control of ribosomal proteins.

In bacteria, biosynthesis of ribosome components is a coordinated process. If ribosomal proteins are synthesized in excess over rRNA, several ribosomal proteins bind to multicistronic mRNAs encoding for ribosomal proteins and repress their translation. To understand this feedback regulation, I characterized ribosomal protein S15 from mesophilic and thermophilic bacteria and mapped their binding sites on mRNA and rRNA. As a result of these efforts, we determined the first three-dimensional structure of an α -helical RNA-binding protein and the first high-resolution structure of an RNA-protein complex from the small ribosomal subunit. These structures were later found instrumental in validating the X-ray structure of the 30S ribosomal subunit. In addition, we put forth a hypothesis of the structural mimicry between mRNA and rRNA targets, which explains RNA-binding properties of many proteins whose RNA targets do not bear similarity on the sequence level. I also obtained the first experimental evidence for regulation on the level of translation initiation in extreme thermophiles. Lastly, we revealed the molecular basis for the entrapment mechanism of translation initiation control by determining the cryo-EM structures of *E. coli* S15/mRNA/ribosome complexes. Together these findings deciphered the entrapment and competition mechanisms of the S15-mediated autoregulation and provided valuable insights on the specificity of RNA-protein interactions.

- a. Serganov, A., Masquida, B., Westhof, E., Cachia, C., Portier, C., Garber, M., Ehresmann, B. & Ehresmann C. (1996) The 16S rRNA binding site of *Thermus thermophilus* ribosomal protein S15: comparison with *Escherichia coli*, minimum site and structure. *RNA*, 2: 1124-1138.
- b. Serganov, A., Ennifar, E., Portier, C., Ehresmann, B. & Ehresmann, C. (2002) Do mRNA and rRNA binding sites of *E. coli* ribosomal protein S15 share common structural determinants? *J. Mol. Biol.*, 320: 963-978.
- c. Serganov, A., Polonskaia, A., Ehresmann, B., Ehresmann, C. & Patel, D.J. (2003) Ribosomal protein S15 represses its own translation via adaptation of an rRNA-like fold within its mRNA. *EMBO J.*; 22: 1898-1908.
- d. Marzi, S., Myasnikov, A.G., Serganov, A., Ehresmann, C., Romby, P., Yusupov, M. & Klaholz, B.P. (2007), Structured mRNAs regulate translation initiation by binding to a dedicated site on the ribosome. *Cell*, 130: 1019-1031.

4. Development of novel approaches for RNA structure phasing.

X-ray structure phasing is a step required for each structure determination effort. Several methods were developed for phasing protein structures. One of the most common methods includes substitution of selenium atoms for sulfur atoms in proteins by growing protein-producing bacteria with selenium-labeled amino acids. Selenium is then used for phasing. Phasing of X-ray RNA structures is more problematic since RNAs cannot be prepared in the same way as proteins. Heavy-atom soaking procedures with RNA crystals are also difficult because of the negative charge of RNA and the lack of hydrophobic surfaces for binding of heavy-atom compounds. Based on the prior idea of incorporating Se atoms to DNA, in collaboration with the R. Micura laboratory, we have developed a robust methodology for chemical synthesis of Se-modified RNAs. We were the first to show feasibility of this approach and successfully used this methodology for phasing a sizable RNA molecule (Diels-Alder ribozyme).

- a. Serganov, A., Keiper, S., Malinina, L., Tereshko, V., Skripkin, E., Hobartner, C., Polonskaia, A., Phan, A.T., Wombacher, R., Micura, R., Dauter, Z., Jaschke, A., Patel, D.J. (2005). Structural basis for Diels-Alder ribozyme-catalyzed carbon-carbon bond formation. *Nat. Struct. Mol. Biol.*, 12, 218-224.
- b. Hobartner, C., Rieder, R., Kreutz, C., Puffer, B., Lang, K., Polonskaia, A., Serganov, A. & Micura, R. (2005). Syntheses of RNAs with up to 100 nucleotides containing site-specific 2'-methylseleno labels for use in X-ray crystallography. *J. Am. Chem. Soc.*, 127: 12035-12045.
- c. Moroder, H., Kreutz, C., Lang, K., Serganov, A. & Micura, R. (2006). Synthesis, oxidation behavior, crystallization and structure of 2'-methylseleno guanosine containing RNAs. *J. Am. Chem. Soc.*, 128: 9909-9918.
- d. Olieric, V., Rieder, U., Lang, K., Serganov, A., Schulze-Briese, C., Micura, R., Dumas, P. & Ennifar, E. (2009). A fast selenium derivatization strategy for crystallization and phasing of RNA structures. *RNA*, 15: 707-715. PMID:PMC2661828

5. mRNA degradation in bacterial cells.

mRNA decay enables rapid changes in protein biosynthesis and critically affects the adaptability of all organisms to changing environmental conditions. In bacteria, hundreds of mRNAs are degraded by the 5'-end-dependent pathway that involves a key conversion of the 5' terminus from a triphosphate to a monophosphate followed by endonucleolytic cleavage with 5'-monophosphate-dependent RNase E and degradation of resulting RNA pieces by 3'-end-dependent exoribonucleases. RppH, a Nudix hydrolase, participates in the conversion of 5' ends to monophosphates. We determined the X-ray structures of the most abundant type of RppH in the apo and RNA-bound forms that revealed the molecular basis for RNA selectivity and catalysis of RppHs. We found a stable intermediate in the pathway, a diphosphorylated mRNA species, and overturned the view that mRNAs are predominantly triphosphorylated in bacterial cells. We identified diphosphorylated mRNAs as major natural substrates of RppH and proved involvement of additional enzyme(s) in the initial modification of the 5' mRNA ends. Most recently, we determined the structure of RppH bound to its activator, a metabolic enzyme DapF, which is the first structure of the modulated "decapping" bacterial complex, and identified two mechanisms of RppH stimulation on different substrates. Our work provided insights on the 5'-end-dependent mRNA degradation pathway and revealed striking similarities and differences with the eukaryotic mRNA decapping system.

- a. Vasilyev, N. & Serganov, A. (2015). Structures of RNA complexes with the *Escherichia coli* RNA pyrophosphohydrolase RppH unveil the basis for specific 5'-end-dependent mRNA decay. *J. Biol. Chem.* 290: 9487-9499. PMID: PMC4436661
- b. Luciano, D.J., Vasilyev, N., Richards, J., Serganov, A. & Belasco J.G. (2017). A novel RNA phosphorylation state enables 5'-end-dependent degradation in *Escherichia coli*. *Mol. Cell.* 67: 44-54.e6
- c. Gao, A., Vasilyev, N., Marsiglia, W.M., Traaseth, N. J., Belasco J.G. and Serganov, A. (2018) Structural and kinetic insights into stimulation of RppH-dependent RNA degradation by the metabolic enzyme DapF. *Nucleic Acids Res.* 46: 6841-6856.

Complete list of publications in My Bibliography

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1jWVf66w7ozkp/bibliography/43568851/public/?sort=date&direction=descending>

D. Research Support

Ongoing Research Support

NIH 5R01GM112940-07	Serganov (PI) \$210,000	09/21/15-08/31/24 3.6 cal months
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MOLECULAR BASIS FOR MRNA DECAY IN BACTERIA

The goal of the project is to provide insights into the molecular details of mRNA capping and decapping and the molecular mechanisms at the foundation of regulatory networks that affect the adaptability and virulence of pathogenic bacteria.

No overlap

Department of Defense W81XWH1810626	Nudler/Serganov (MPI) \$251,185	09/01/2018-08/31/2021 2.4 cal months
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DEVELOPMENT OF INNOVATIVE COMBINATION THERAPY AGAINST MULTIDRUG-RESISTANT BACTERIA

The aim of the proposal is to develop a conceptually novel therapy to treat multidrug-resistant bacterial infections and manage combat-related and other drug-resistant infections

No overlap

Completed Research Support

Award, Edward Mallinckrodt Jr. Foundation	Serganov (PI) \$60,000	10/01/14-09/30/17 1.2 cal months
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MECHANISM OF TRANSLATIONAL REPRESSION BY FRAGILE X MENTAL RETARDATION PROTEIN

This project focuses on identification of factors impacting translational repression by Fragile X Mental Retardation Protein.

No overlap

NIH 1R21MH112165-01	Serganov (PI) \$150,000	09/26/16 - 08/31/19 2.4 cal months
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RNA TARGETS FOR FRAGILE X MENTAL RETARDATION PROTEIN

The goal of this project is to identify RNA species capable of specific binding to FMRP *in vitro*.

No overlap

Career Scientists Award, The Irma T. Hirschl/Monique Weill-Caulier Trust	Serganov (PI) \$35,000	01/01/15-12/30/20 0.6 cal months
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ELUCIDATING PHYSIOLOGICAL ROLES OF PROTEINS IN FRAGILE X SYNDROME

The main goal of this project is a study focused on elucidating the function of FMRP and defining the molecular features leading to the development of FXS.

No overlap