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

2017- Tenure-track Associated Professor, 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. PMCID: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. PMCID: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. PMCID: 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

Career Scientists Award Irma T. Hirschl/Monique Weill-Caulier Trust Serganov (PI) 01/01/15-12/31/19
Elucidating Physiological Roles of Proteins in Fragile X Syndrome
Role: PI
This project is focused on elucidation of proteins involved in Fragile X syndrome.

1 R01 GM112940-01A1 NIH Serganov (PI) 09/21/15 - 08/31/20
Molecular Basis for mRNA decay in bacteria
Role: PI
The goal of this project is to elucidate mechanisms of 5′-end-dependent mRNA degradation in bacteria.

1 R21 MH112165-01 NIH Serganov (PI) 09/26/16 - 08/31/19
RNA targets for Fragile X Mental Retardation Protein
Role: PI
The goal of this project is to identify RNA species capable of specific binding to FMRP *in vitro*.

PR171734P1 DoD Serganov (co-PI) 09/01/18 - 08/31/21
Development of Innovative Combination Therapy Against Multidrug-Resistant Bacteria
Role: co-PI
The goal of this project is to develop a new type of adjuvant to enhance antibactericidal properties of antibiotics.

Completed Research Support

Award Edward Mallinckrodt Jr. Foundation Serganov (PI) 10/01/14-09/30/17
Mechanism of Translational Repression by Fragile X Mental Retardation Protein
Role: PI
This project is focused on identification of protein factors impacting translational repression by Fragile X Mental Retardation Protein.

Sponsor Award, OmniCyte Inc. Serganov (PI) 01/01/15-03/30/15
Development of a Novel Therapeutic for Rgr Expressing T-Cell Malignancies
This award supplemented a salary component to the Leukemia and Lymphoma Society grant.

1 R21 MH103655-01 Serganov (PI) 09/26/13 - 09/25/15
NIH
RNA-protein interactions in Fragile X syndrome
Role: PI
The major goal of this project was to restore the pilot project, affected by the superstorm Sandy, aimed at identifying and characterizing the interactions between FMRP and its molecular targets.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Jacob Weaver

eRA COMMONS USER NAME (credential, e.g., agency login): JWW339

POSITION TITLE: Graduate Student

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
University of Rochester, Rochester, NY	B.S.	05/2011	Biochemistry
New York University, New York, NY	Ph.D.	In Progress	Structural Biology

A. Personal Statement

My Ph.D. project centers on understanding the mechanisms of RNA transcription and its contribution to other cellular processes. My first direction is to understand the molecular mechanism of transcription-coupled DNA repair. Previously, Dr. Nudler's laboratory showed that a well-known DNA repair factor UvrD binds bacterial RNA polymerase (RNAP) and exerts control over it by pulling the polymerase backwards, a mechanism known as backtracking. This finding unexpectedly links two most important cellular processes, RNA transcription and DNA repair. Since these processes are fundamental in all organisms, the close relationship between transcription and genome stability suggest new means for elucidating mechanisms and developing novel therapeutic approaches to treat human diseases. How UvrD is able to cause backtracking is yet unknown. I plan to extend the aim centered on understanding the structural and mechanistic bases of UvrD-mediated backtracking. I intend on determining the three-dimensional cryo-EM structures of the UvrD-RNAP complex alone as well as with DNA template and RNA transcript. By catching the UvrD-RNAP complex at various stages during backtracking, the exact mechanism can be elucidated and verified biochemically. My second research direction focuses on understanding how bacterial RNA polymerase initiates transcription from non-canonical nucleotides. Recently, the Dr. Joel Belasco laboratory has found that under stress conditions certain mRNAs in *E. coli* are initiated from non-canonical nucleotides, which extends the half-life of the mRNAs and affect protein biosynthesis. Most interestingly, initiation from the non-canonical nucleotides is more efficient than from the canonical nucleotides. We want to determine the cryo-EM structure of the RNAP initiation complex and understand the molecular details which favor non-canonical initiation.

Prior to enrollment at NYUMC, I spent a year under Dr. Harold Smith learning protein expression and purification. After graduating from the University of Rochester, I worked extensively with *Vibrio cholerae* and *Escherichia coli* where I gained skills in molecular cloning, site directed mutagenesis and bacterial genetics under Dr. Michelle Dziejman. Now, in the laboratories of Dr. Evgeny Nudler and Dr. Alexander Serganov, I am learning the full spectrum of biochemical and biophysical techniques including single particle cryoelectron microscopy, X-ray crystallography, and cross-linking mass-spectrometry. Altogether, my skillset is well suited to approach this project as a graduate student. Given the importance of my project for human health, I am excited to work diligently and anticipate making significant contributions to the field of genome stability and the scientific research community in general. During the course of my graduate study, I expect the research experience and scientific training to cement my foundation as an independent researcher on my path to one day overseeing my own research group. Graduate studies at NYUMC have afforded me opportunities I could only dream of during my youth. Now, with the possibility of advancing scientific understanding at a fundamental level with repercussions in human health, I am thrilled to be given a chance to contribute to a project that has such far reaching implications.

B. Positions and Honors

Positions and Employment

2009 – 2011 University tutor for General Chemistry, Organic Chemistry, and Mathematics, University of Rochester, Rochester, NY
2010 – 2011 Undergraduate Researcher with Dr. Harold Smith, Department of Biochemistry, University of Rochester Medical Center, Rochester, NY
2011 – 2013 Research Assistant with Dr. Michelle Dziejman, Department of Microbiology, University of Rochester Medical Center, Rochester, NY
2013 – Graduate Assistant with Dr. Evgeny Nudler and Dr. Alexander Serganov, Department of Biochemistry and Pharmacology, New York University School of Medicine, New York, NY

Professional Memberships

2013 – NY Academy of Sciences

Honors

2007 – 2011 Dean's List, University of Rochester, Rochester, NY

C. Contribution to Science

Regulatory control of pathogenesis in *V. cholerae*

Vibrio cholerae is a genetically diverse species and causative agent of cholera, a secretory diarrhea, which affects millions of people worldwide and causes thousands of deaths every year. It is of primary importance to understand the mechanisms of an infection that involves colonization of the small intestine. While epidemic *V. cholerae* O1 and O139 serogroup strains use a toxin coregulated pilus (TCP) for intestinal colonization, other clinically isolated strains largely do not encode TCP and instead utilize other means for host colonization. One such clinical isolate, AM-19226, uses a type 3 secretion system (T3SS) encoded on a horizontally-acquired pathogenicity island for infection. Two transmembrane transcriptional regulators, $VttR_A$ and $VttR_B$, are essential for T3SS expression, intestinal colonization, and effector protein translocation via T3SS. To examine how VttR proteins coordinate colonization and pathogenesis, I performed genetic epistasis experiments in regulator deletion strains pointing to a regulatory hierarchy converging on $VttR_B$. Additionally, I used molecular and genetic techniques to probe how external stimuli feed into these regulatory networks. My work has shown how a non-O1/non-O139 clinical strain of *V. cholerae* orchestrates a pathogenesis on a transcriptional level. This work suggests similar mechanisms may be used by other Gram-negative pathogenic intestinal bacteria.

1. Miller, K.A., Sofia, M.K., Weaver, J.W.A., Seward, C.H., Dziejman, M. (2016) Regulation by ToxR-like proteins converges on *vttRB* expression to control Type 3 secretion system-dependent Caco2-BBE cytotoxicity in *Vibrio cholerae*. *J Bacteriol*. 198: 1675-1682. PMCID: PMC495928.
2. Chaand M, Miller KA, Sofia MK, Schlesener C, Weaver JW, Sood V, Dziejman M. (2015) Type 3 secretion system island encoded proteins required for colonization by non-O1/non-O139 serogroup *V. cholerae*. *Infect Immun*. 83: 2862-2869. PMCID: PMC4468554.

D. Research Support

None