

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

My research interests are centered on understanding gene expression control. First, we are interested in the mechanisms of the RNA-associated gene expression control involving drug-like small molecules and proteins on the levels of transcription and RNA stability. Second, we are focusing on the biological role of gaseous molecules and development of novel antibacterials targeting enzymes involved in the biosynthesis of gases.

Both research directions are natural progression of my prior studies on non-coding RNA termed riboswitches that control gene expression via specific binding to small cellular metabolites. Since riboswitches bind to drug-like molecules and control genes vital for bacteria, riboswitches were deemed promising drug targets and major research efforts were made to understand molecular principles underlying their high selectivity to small molecules. I contributed to the three-dimensional structure determination of 13 riboswitch types, both in the Dinshaw Patel (MSKCC) and my own laboratory at the NYUGSoM. We explained how thiamine pyrophosphate and lysine riboswitches can specifically bind conventional antibiotics pyrithiamine (*Nature*, 2006) and oxalysine (*Nature*, 2008). We showed that antibiotic roseoflavin binds flavin mononucleotide-specific (FMN) riboswitch (*Nature*, 2009). This study was instrumental for identification of the FMN-riboswitch-targeting antibiotic ribocil (*Nature*, 2015) by Merck.

In 2011 (Shatalin *et al.*, *Science*), the Nudler Laboratory discovered that bacteria produce hydrogen sulfide (H₂S) and that this gas protects bacteria from the antibiotic-induced stress. My laboratory infused biochemical and structural expertise into the project, resulting in determining the X-ray crystal structure of the H₂S-producing enzyme cystathionine γ -lyase (CSE) from *Staphylococcus aureus*. This structure was used for virtual screening and identification of novel inhibitors of CSE (Shatalin *et al.*, *Science*, 2021). We showed by structural studies that these compounds inhibit catalytic activity allosterically, unlike other CSE inhibitors that target the active site. Importantly, the leading inhibitors potentiate the action of bactericidal antibiotics and significantly reduce antibiotic tolerance.

We have noticed that human CSE contains a similar inhibitor-binding site and demonstrated that some of our inhibitors against bacterial CSE suppress activity of the human enzyme that produces H₂S in many tissues and organs. Remarkably, elevated H₂S levels have been reported in the brain and appear to be associated with pathogenic mechanisms in adult glioblastomas. Therefore, suppressing endogenous H₂S production could be a viable anti-cancer therapy. In our pilot study, supported by the award from the NYU Cancer Center, we showed that some of our inhibitors substantially decreased H₂S levels and arrested proliferation of the glioblastoma cell lines. In his grant application, we propose to develop potent CSE inhibitors that have the strongest effect on adult glioblastoma cell lines and test these inhibitors in the animal models of glioblastoma.

Recently awarded and completed grants that I would like to highlight include:

PR220077 Department of Defense Serganov/Nudler (MPI) 08/15/23 – 08/14/27
Development of Innovative Therapeutics Against Pressure Ulcer-Associated Bacteria

The goal of this project is to develop more potent inhibitors of H₂S biogenesis to enhance antibactericidal properties of antibiotics for treatment of infections associated with pressure injuries.

Developmental Project Program (P30CA016087) award NYU Laura & Isaac Perlmutter Cancer Center
Serganov/ Lechpammer/Nudler (MPI) 12/01/22 – 11/30/23

Towards the Novel Approaches to Treat Adult Glioblastomas

The goal of this project was to demonstrate feasibility of developing a new type of inhibitor of H₂S biogenesis as a stand-alone or companion therapeutic for treatment of adult glioblastoma.

PR171734 Department of Defense Nudler/Serganov (MPI) 09/01/2018-08/31/2021
Development of Innovative Combination Therapy Against Multidrug-Resistant Bacteria

The goal of this project was to conduct a proof-of-concept study demonstrating feasibility of a conceptually novel therapy associated with inhibition of H₂S biogenesis to treat multidrug-resistant bacterial infections.

Citations

1. 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.
2. Gao, A. & Serganov, A. (2014). Structural insights into recognition of c-di-AMP by the *ydaO* riboswitch. *Nat. Chem. Biol.* 2014; 10: 787-792. (Included in the "Greatest Hits of Decade" by *Nat. Chem. Biol.* 2015, 11: 364-367) PMID:PMC4294798
3. Peselis, A. & Serganov, A. (2018) *ykkC* riboswitches employ an add-on helix to adjust specificity for polyanionic ligands. *Nat. Chem. Biol.* 14: 887-894.
4. Shatalin, K., Nuthanakanti, A., Kaushik, A., Shishov, D., Peselis, A., Shamovsky, I., Pani, B., Lechpammer, M., Vasilyev, N., Shatalina, E., Rebatchouk, D., Mironov, A., Fedichev, P., Serganov, A. and Nudler, E. (2021) Inhibitors of bacterial H₂S biogenesis targeting antibiotic resistance and tolerance. *Science*, 372, 1169-1175. (Perspective in *Science* 2021; 372, 1153; Research Highlights in *Nature Reviews Drug Disc.* 2021, 20: 585 and *Nature Chemical Biology* 2021, 17: 839; Recommended by *Faculty of 1000*).

B. Positions and Honors

Positions and Employment

2021- Vice-chair, Department of Biochemistry and Molecular Pharmacology, NYU Grossman School of Medicine (NYUGSOM).

2020- Tenured Associate Professor of Biochemistry and Molecular Pharmacology, NYUGSOM.

2017-2020 Associate Professor of Biochemistry and Molecular Pharmacology, NYUGSOM.

2015- Member of Board of Advisors (not compensated), OmniCyte.

2011-2017 Assistant Professor of Biochemistry and Molecular Pharmacology, NYUGSOM.

2003-2011 Senior Research Scientist, Memorial Sloan-Kettering Cancer Center, New York

Other Experience and Professional Memberships

Grant applications review:

International

2021, 2022 French Alliance for Life Sciences and Health/INSERM

2020 Houska Award, Austria

2018 Biotechnology and Biological Sciences Research Council, United Kingdom

2017, 2018 German Research Foundation

2014, 2015 National Science Centre, Poland

2013 Royal Society University Research Fellowship Program

2013 European Research Council

National

| | |
|-----------|---|
| 2021 | ESI-MIRA Study Section, NIH |
| 2019-2020 | Biochemistry and Biophysics of Biological Macromolecules Fellowship Applications Study Section, NIH |
| 2019- | Member, National Center for CryoEM Access and Training User Review Committee, NIH |
| 2018 | Department of Defense Congressionally Directed Medical Research Programs |
| 2016 | NIH, Prokaryotic Cell and Molecular Biology Study Section |
| 2015 | Department of Defense Congressionally Directed Medical Research Programs |
| 2014 | M.J. Murdock Charitable Trust, USA |
| 2009 | Louisiana Board of Regents, Experimental Program to Stimulate Competitive Research |
| 2008 | USA Defense Threat Reduction Agency Basic Research Program |

Editorial Positions

| | |
|-----------|---|
| 2020- | Editorial Board, <i>ncRNA</i> |
| 2015-2017 | Ad hoc Editor, <i>Proc Natl Acad Sci USA</i> |
| 2012- | Editorial Board, <i>Journal of Proteome Science and Computational Biology</i> |
| 2005-2022 | Editor, Open Life Sciences (former <i>Central European Journal of Biology</i>) |

Reviewer for: *Acta Crystallographica Section D and F*; *ACS Chemical Biology*; *Biochimica et Biophysica Acta*; *Biological Chemistry*; *Biochemistry*; *BMC Structural Biology*; *Biotechnology Journal*; *Bioorganic & Biotechnology Journal*; *Bioorganic & Medicinal Chemistry*; *Cell Chemical Biology*; *Cell Reports*; *Cell Research*; *ChemBioChem*; *eLife*; *FEBS Letters*; *Future Medicine*; *Gene*; *Journal of the American Chemical Society*; *Journal of Bacteriology*; *Journal of Biological Chemistry*; *Journal of Bacteriology*; *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*; *Nature Structural & Molecular Biology*; *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.

Honors

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

C. Contributions 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. 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, followed by determining structures of nine classes of riboswitches in the D.J. Patel laboratory and four riboswitch classes in my own laboratory. These studies explained the molecular principles of metabolite recognition by a natural RNA and provided insights on various riboswitch mechanisms.

- 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. (Recommended by *Faculty of 1000*).
- 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 (News & Views in *Nature* 2006; 441: 1054-1055; preview in *Cell* 2006; 126: 19-22; Point of View in *ACS Chem. Biol.* 2006; 1: 341-345; recommended by *Faculty of 1000*).

- c. Serganov, A., Huang, L. & Patel, D.J. (2008) Structural insights into amino acid binding and gene control by a lysine riboswitch. *Nature*, 455: 11263-11267 (Point of View in *ACS Chem. Biol.* 2008; 3: 660–665).
- d. Serganov, A., Huang, L. & Patel, D.J. (2009) Coenzyme recognition and gene regulation by a flavin mononucleotide riboswitch. *Nature*, 458: 233-237 (Recommended by *Faculty of 1000*).

2. Structure and mechanism of ribozymes.

Catalytic RNAs are among the most exciting discoveries in the RNA world. However, most 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. (Recommended by *Faculty of 1000*).
- 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 the 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. Protein structures commonly phased by using anomalous scattering of selenium atoms after substitution of methionines by selenium-labeled amino acid during bacterial growth. 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 and were first to successfully use 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. (Recommended by *Faculty of 1000*). 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 that revealed the molecular basis for RNA selectivity and catalysis. 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.
- d. Gao, A., Vasilyev, N., Kaushik, A., Duan, W. & Serganov, A. (2020) Principles of RNA and nucleotide discrimination by the RNA processing enzyme RppH. *Nucleic Acids Res.* 48: 3776-3788 (April cover).

Complete list of publications in My Bibliography

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

BIOGRAPHICAL SKETCH

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NAME: Abhishek Kaushik

eRA COMMONS USER NAME (credential, e.g., agency login): kausha03

POSITION TITLE: Postdoctoral Fellow, NYU School Of Medicine

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 |
|--|---------------------------|-------------------------------|-----------------------------|
| Inst. Of Applied Medicine and Research (C.C.S University in Meerut), Ghaziabad (UP) India | BSc. | 07/2007 | Biotechnology (Honors) |
| School Of Biological Sciences, Madurai Kamaraj University, Madurai, (TN) India | MSc. | 07/2010 | Microbial Technology |
| Jointly, CSIR-Inst. Of Microbial Technology, Chandigarh & Jawaharlal Nehru University, New Delhi | PhD. | 11/2017 | Biochemistry and Biophysics |

A. Personal Statement

My current research interests are focused in two main area. First, it is antimicrobial resistance, which has doomed the world with many drug-resistant strains. We are developing antimicrobial inhibitors against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Our recent studies have establish bacterial H₂S as a multifunctional defense factor and cystathionine γ -lyase (CSE) as the primary generator of H₂S in these two major human pathogens (*Science*, 2021). Second, we are studying the mechanism of RNA degradation in bacteria. We are primarily focused on the functional aspects i.e., catalytic mechanism and substrate specificity of Nudix hydrolases like RppH, (*Nucl. Acids Res.*, 2020), which trigger the 5'-end-dependent RNA degradation.

During PhD, I worked on biochemical and structural characterization of Cysteine Regulatory Complex (CRC). It is a multienzyme complex responsible for cysteine biosynthesis in bacteria and plant. CRC is formed by oligomerization of CysE (Serine-acetyl transferase (SAT)-Hexamer) and CysK (O-acetylserine sulfhydrylase (OASS)-Dimer). It is reported to form a ~ 310 kDa complex (one hexamer and two dimers). For the first time, we demonstrated that CRC can exist in equilibrium in more than one state with different masses (~450 kDa and ~310 kDa) and that the oligomeric states are sensitive to its natural substrates, OAS and Na₂S (*Biochemistry*, 2017). In another study, using hybrid enzyme-inhibitor complex, we demonstrated the mechanism of the low-affinity-substrate-induced dissociation of the high affinity enzyme-inhibitor complex. This study showed that CRC employs a novel competitive-allosteric mechanism to selectively recruit its substrate in the presence of a natural inhibitor (*Biochemistry*, 2017). In this study, we traced the molecular features that controls selective recruitment of substrate (*J. Biol. Chem.*, 2021).

Citations:

Shatalin, K., Nuthanakanti, A., Kaushik, A., Shishov, D., Peselis, A., Shamovsky, I., Pani, B., Lechpammer, M., Vasilyev, N., Shatalina, E., et al. (2021). Inhibitors of bacterial H₂S biogenesis targeting antibiotic resistance and tolerance. *Science* 372, 1169-1175. PMID: pending

Gao, A., Vasilyev, N., Kaushik, A., Duan, W., and Serganov, A. (2020). Principles of RNA and nucleotide discrimination by the RNA processing enzyme RppH. *Nucleic Acids Res* 48, 3776-3788. PMID: PMC7144940

Kaushik, A., Ekka, M.K., and Kumaran, S. (2017). Two Distinct Assembly States of the Cysteine Regulatory Complex of *Salmonella typhimurium* are Regulated by Enzyme-Substrate Cognate Pairs. *Biochemistry* 56, 2385-2399.

Kaushik, A., Rahisuddin, R., Saini, N., Singh, R.P., Kaur, R., Koul, S., and Kumaran, S. (2021). Molecular mechanism of selective substrate engagement and inhibitor disengagement of cysteine synthase. *J Biol Chem* 296, 100041. PMID: PMC7948407

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

2019- Postdoctoral Fellow, NYU School Of Medicine NY, New York, USA.
2017-2019 Scientist-Clinical Development Specialist, Cytiva (GE healthcare life sciences), Bangalore, India.
2016-2017 CSIR Senior Project Fellow, Inst. Microbial Technology, Chandigarh, India
2013-2016 CSIR-Senior Research Fellow, Inst. Microbial technology, Chandigarh, India.
2011-2013 CSIR-Junior Research Fellow, Inst. Microbial Technology, Chandigarh, India.
2010-2011 Lecturer, Inst. Of Applied Medicine and Research, Ghaziabad India.

Honors

2016 Travel Award, 1st BioStruct-X Mediterranean Macromolecular Crystallography Workshop, Israel.
2011-2016 Research fellowship for PhD studies (CSIR-Fellowship).
2010 Graduate Aptitude Test for Engineering (GATE- Biotechnology-2010), All India Rank 290.
Granted eligibility for Masters in Engineering and Technology and Graduate Studies.
2010 Council of Scientific and Industrial Research (CSIR-NET-Lifescience-2010) examination, all India Rank 199. Granted eligibility to Lectureship and fellowship for graduate studies.

C. Contributions to Science

1) Anti-Microbial Resistance

Bacteria have inherent property to defy antibiotic treatment. Some bacteria can persist under stresses by altering their metabolism. Such persisters outlast the antibiotic treatment and cause recurrent infections, which often result in developing biofilms that lead to chronic difficult-to-treat infections. We showed that genetic inactivation of CSE (Cystathionine-γ-Lyase) strongly suppressed occurrence of persisters and development of biofilms in two major human pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. CSE has been identified as the primary generator of H₂S and is believed to mitigate oxidative stress caused by bactericidal antibiotics. We have identified and validated a class of molecules that suppresses CSE activity and potentiates the killing effect of sub-lethal doses of antibiotics. These inhibitors also help killing persister cells and prevent formation of biofilms. Using structural methods, we have shown that inhibitors bind CSE in proximity to the catalytic site, allosterically inhibiting activity of the enzyme. Currently, we work on improving our lead inhibitors to potentiate the killing more effectively.

- a) Shatalin, K., Nuthanakanti, A., Kaushik, A., Shishov, D., Peselis, A., Shamovsky, I., Pani, B., Lechpammer, M., Vasilyev, N., Shatalina, E., et al. (2021). Inhibitors of bacterial H₂S biogenesis targeting antibiotic resistance and tolerance. *Science* 372, 1169-1175. PMID: pending

2) mRNA RNA degradation

mRNA degradation is important to regulate gene expression in all organisms and is essential for adapting to environmental conditions. Most bacteria use 3'-end-dependent exonucleases for RNA degradation; however, mRNA first has to be cleaved by endonucleases, such as *E. coli* RNase E, to provide exonucleases with access to the unobstructed 3' ends. Activity of RNase E is greatly accelerated on 5' monophosphorylated RNA ends.

Since primary transcripts are typically produced with triphosphates on the 5' ends, special enzymes, such as Nudix hydrolase RppH, convert triphosphorylated RNAs to monophosphorylated substrates for subsequent RNase E cleavage. We have shown how RppH recognizes its cognate and non-cognate substrates. We determined that 5' diphosphorylated RNA is favored over triphosphorylated RNA. We unraveled that nucleotides can also act as substrates, though less favorably, and can compete with RNA for RppH, thereby contributing to regulation of mRNA degradation.

- a) Gao, A., Vasilyev, N., Kaushik, A., Duan, W., and Serganov, A. (2020). Principles of RNA and nucleotide discrimination by the RNA processing enzyme RppH. *Nucleic Acids Res.* 48, 3776-3788. PMCID: PMC7144940

3) Cysteine Metabolism

Sulfur metabolism in bacteria is not well understood. Cysteine acts as precursor for other sulfur containing amino acids and therefore controls sulfur availability. The two enzymes that are involved in cysteine biosynthesis in bacteria form a large multi-enzyme complex, the Cysteine Regulatory Complex (CRC). CRC is formed by association of serine O-acetyltransferase (SAT) and O-acetylserine sulfhydrylase (OASS) and acts as a sensor and modulator of thiol metabolism by responding to changes in nutrient conditions. Given importance of sulfur metabolism, CRC represents an attractive drug target for pathogenic bacteria, with several inhibitors already identified. Recently, in contrary to previous reports, we have shown that CRC exists in two major assembly states, low-molecular weight (CRC₁; 1[SAT]_{hexamer} + 2[OASS]_{dimer}) and high-molecular weight (CRC₂; 1[SAT]_{hexamer} + 4[OASS]_{dimer}) states and the CRC₂ can be selectively dissociated in the presence of its substrates (OAS and Na₂S). We have also shown that OASS employs a novel competitive-allosteric mechanism to selectively recruit its substrate in the presence of a natural inhibitor and can dissociate this high affinity inhibitor upon substrate binding. These data provide important insights on the future development of CRS inhibitors.

- a) Kaushik, A., Rahisuddin, R., Saini, N., Singh, R.P., Kaur, R., Koul, S., and Kumaran, S. (2021). Molecular mechanism of selective substrate engagement and inhibitor disengagement of cysteine synthase. *J Biol Chem* 296, 100041. PMCID: PMC7948407
- b) Pratap Singh, R., Saini, N., Sharma, G., Rahisuddin, R., Patel, M., Kaushik, A., and Kumaran, S. (2021). Moonlighting Biochemistry of Cysteine Synthase: A Species-specific Global Regulator. *J Mol Biol*, 167255.
- c) Kaushik, A., Ekka, M.K., and Kumaran, S. (2017). Two Distinct Assembly States of the Cysteine Regulatory Complex of *Salmonella typhimurium* are Regulated by Enzyme-Substrate Cognate Pairs. *Biochemistry* 56, 2385-2399.
- d) Singh, A.K., Ekka, M.K., Kaushik, A., Pandya, V., Singh, R.P., Banerjee, S., Mittal, M., Singh, V., and Kumaran, S. (2017). Substrate-Induced Facilitated Dissociation of the Competitive Inhibitor from the Active Site of O-Acetyl Serine Sulfhydrylase Reveals a Competitive-Allostery Mechanism. *Biochemistry* 56, 5011-5025.

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