List of nominee's ten most significant publications

Name of all the authors as they appear in each paper	Title of the Paper	Name of Journal	Year of Publication	Volume	Page No
Singh, J., Raina, R., Vinothkumar, KR., **Anand, R*.**	Decoding the Mechanism of Specific RNA Targeting by Ribosomal Methyltransferases.	ACS Chemical Biology **(Impact factor:4.63)**	2022	17, 4	829-839
	Methylation of specific nucleotides is integral for ribosomal biogenesis and also serves as a common mechanism to confer antibiotic resistance by pathogenic bacteria. Here, by determining the high-resolution structure of the 30S−KsgA complex by cryo-electron microscopy, a state was captured, where KsgA juxtaposes between helices h44 and h45 of the 30S ribosome, separating them, thereby enabling remodeling of the surrounded rRNA and allowing the cognate site to enter the methylation pocket. With the structure as a guide, several mutant versions of the ribosomes, where interacting bases in the catalytic helix h45 and surrounding helices h44, h24, and h27, were mutated and evaluated for their methylation efficiency revealing factors that direct the enzyme to its cognate site with high fidelity. The biochemical studies show that the three-dimensional environment of the ribosome enables the interaction of select loop regions in KsgA with the ribosome helices paramount to maintain selectivity.				
Sharma, N., Singh, S., Tanwar, A.S, Mondal, J., **Anand, R***.	Mechanism of Coordinated Gating and Signal Transduction in Purine Biosynthetic Enzyme Formylglycinamidine Synthetase.	ACS Catalysis **(Impact factor:13.08)**	2022	12, 3	1930-1944
	Enzymes that harbor transient tunnels have a complex interplay of allostery that links their assembly/disassembly with the catalytic cycle. Here, by employing PurL, a purine biosynthetic enzyme, as a model system, we decipher the mechanism of catalytic coupling, precise orchestration of signal transduction, associated conformational changes, and their link with formation of the transient ammonia tunnel. We show that ammonia passage in PurL is controlled by two gates “mouth-gate” and “end-gate”, with the seed of the allosteric cycle residing at the “end-gate”. Overall, we believe that the complex interaction relay system between two coupled reaction centers, presented here, where a dynamic allosteric switch gives rise to a pulsating ammonia tunnel, regulated by a dual gating network is an example of a biological system that has been fine-tuned to achieve perfection.				
Roy R., Ray S., Chowdhury* A., **Anand R*.**	Tunable Multiplexed Whole-Cell Biosensors as Environmental Diagnostics for ppb-Level Detection of Aromatic Pollutants.	ACS Sensors **(Impact factor:9.6)**	2021	Vol 6, 5	1933–1939
	Aromatics such as phenols, benzene, and toluene are carcinogenic xenobiotics which are known to pollute water resources. By employing synthetic biology approaches combined with a structure-guided design, we created a tunable array of whole-cell biosensors (WCBs). The MopR genetic system that has the natural ability to sense and degrade phenol was adapted to detect phenol down to ∼1 ppb, making this sensor capable of directly detecting phenol in permissible limits in drinking water. Importantly, by using a single WCB design, we engineered mutations into the MopR gene that enabled generation of a battery of sensors for a wide array of pollutants. The engineered WCBs were able to sense inert compounds like benzene and xylene which lack active functional groups, without any loss in sensitivity. Overall, this universal programmable biosensor platform can be used to create WCBs that can be deployed on field for rapid testing and screening of suitable drinking water sources.				
Sharma, N., Ahalawat, N., Sandhu, P., Strauss, E., Mondal, J., **Anand, R.***	Role of Allosteric Switches and Adaptor Domains in Long Distance Cross-Talk and Transient Tunnel Formation.	Science Advances **(Impact factor:14.14)**	2020	Vol. 6, no. 14	eaay7919

	Transient tunnels that assemble and disassemble to facilitate passage of unstable intermediates in enzymes containing multiple reaction centers are controlled by allosteric cues. Using the 140-kDa purine biosynthetic enzyme PurL as a model system and a combination of biochemical and x-ray crystallographic studies, we show that long-distance communication between ~25-Å distal active sites is initiated by an allosteric switch, residing in a conserved catalytic loop, adjacent to the synthetase active site. This study asserts that substrate channeling is modulated by allosteric hotspots that alter protein energy landscape, thereby allowing the protein to adopt transient conformations paramount to function.				
Bhujbalrao, R., **Anand, R*.**	Deciphering Determinants in Ribosomal Methyltransferases that Confer Antimicrobial Resistance.	J. Am. Chem. Soc. **(Impact factor:16.38)**	2019	141(4)	1425-1429
	Post-translational methylation of rRNA at select positions is a prevalent resistance mechanism adopted by pathogens. In this work, KsgA, a housekeeping ribosomal methyltransferase (rMtase) involved in ribosome biogenesis, was exploited as a model system to delineate the specific targeting determinants that impart substrate specificity to rMtases. With a combination of evolutionary and structure-guided approaches, a set of chimeras were created that altered the targeting specificity of KsgA such that it acted similarly to erythromycin-resistant methyltransferases (Erms), rMtases found in multidrug-resistant pathogens. Moreover, in vivo studies confirmed that chimeric constructs are competent in imparting macrolide resistance. This work explores the factors that govern the emergence of resistance and paves the way for the design of specific inhibitors useful in reversing antibiotic resistance.				
Gaded, V., **Anand, R.***	Selective Deamination of Mutagens by a Mycobacterial Enzyme.	J. Am. Chem. Soc. **(Impact factor:16.38)**	2017	139(31)	10762-10768
	Structure-based methods are powerful tools that are being exploited to unravel new functions with therapeutic advantage. Here, we report the discovery of a new class of deaminases, predominantly found in mycobacterial species that act on the commercially important s-triazine class of compounds. The enzyme Msd from *Mycobacterium smegmatis* was taken as a representative candidate from an evolutionarily conserved subgroup that possesses high density of Mycobacterium deaminases. Biochemical investigation reveals that Msd specifically acts on mutagenic nucleobases such as 5-azacytosine and isoguanine and does not accept natural bases as substrates. Determination of the X-ray structure of Msd to a resolution of 1.9 Å shows that Msd has fine-tuned its active site such that it is a hybrid of a cytosine as well as a guanine deaminase, thereby conferring Msd the ability to expand its repertoire to both purine and pyrimidine-like mutagens.				
Ray S., Senapati, T., Sahu, S; Bandyopadhyaya, **R., Anand, R*.**	Design of Ultrasensitive Protein Biosensor Strips for Selective Detection of Aromatic Contaminants in Environmental Wastewater.	Analytical Chemistry **(Impact factor: 8.8)**	2018	90 (15)	8960-8968
	Phenol and its derivatives constitute a class of highly toxic xenobiotics that pollute both river and groundwater. Here, we use a highly stable enzyme-based in vitro biosensing scaffold to develop a chip-based environmental diagnostic for in situ accurate, direct detection of phenol with selectively down to 10 ppb. Mesoporous silica nanoparticles (MCM41) having a pore diameter of 6.5 nm was screened and found to be the optimal solid support for creation of a robust immobilized protein based sensor, which retains stability, enzyme activity, sensitivity, and selectivity at par with solution format. The sensor strip exhibits minimal cross reactivity in simulated wastewater, crowded with several common pollutants. Moreover, this design is competent towards detection of phenol content with 95% accuracy in real-time environmental samples collected from local surroundings, making it a viable candidate for commercialization.				
Ray, S., Panjikar, S., **Anand, R*.**	Design of Protein based Biosensors for Selective Detection of Benzene Group of Pollutants.	ACS Sensors 2018. **(Impact factor: 9.6)**	2018	3(9)	1632–1638
	Benzene and its derivatives form a class of priority pollutants whose exposure poses grave risk to human health. Since benzene lacks active functional groups, devising specific sensors for its direct detection from a milieu of aromatics has remained a daunting task. Here, we report three engineered protein-based biosensors that exclusively and specifically detect benzene and its derivatives up to a detection limit of 0.3 ppm. Further, the biosensor design has been engineered to create templates that possess the ability to specifically discriminate between alkyl substituted benzene derivatives; such as toluene, m-xylene, and mesitylene. This work demonstrates the potential of structure guided protein engineering as a competent strategy toward design of selective biosensors for direct detection of benzene group of pollutants from real time environmental samples.				

Ray, S.,Gunzburg, M., Wilce, M., Panjikar, S., **Anand, R.***	Structural Basis of Selective Aromatic Pollutant Sensing by the Effector Binding Domain of MopR, an NtrC Family Transcriptional Regulator.	ACS Chemical Biology. **(Impact factor: 4.63)**	2016	11	2357−2365.
	Phenol and its derivatives are common pollutants that are present in industrial discharge and are major xenobiotics that lead to water pollution. To monitor as well as improve water quality, attempts have been made in the past to engineer bacterial in vivo biosensors. Here, we present the crystal structure of the sensor domain of MopR (MopRAB) from *Acinetobacter calcoaceticus* in complex with phenol and its derivatives to a maximum resolution of 2.5 Å. Furthermore, comparative analysis of MopR with XylR and DmpR sensor domains enabled the design of a MopR binding pocket that is competent in binding DmpR-specific ligands. Collectively, these findings pave way towards development of specific/broad based biosensors, which can act as useful tools for detection of this class of pollutants.				
Bhukya, H., Bhujbalrao, R., Bitra, A., **Anand, R.***	Structural and functional basis of transcriptional regulation by TetR family protein CprB from S. coelicolor A3(2).	Nucleic Acids Res. **(Impact factor: 19.16)**	2014	42 (15)	10122-33.
	Antibiotic production and resistance pathways in Streptomyces are dictated by the interplay of transcriptional regulatory proteins that trigger downstream responses via binding to small diffusible molecules. To decipher the mode of DNA binding and the associated allosteric mechanism in the sub-class of transcription factors that are induced by γ-butyrolactones, we present the crystal structure of CprB in complex with the consensus DNA element to a resolution of 3.25 Å. It was demonstrated that the CprB displays a cooperative mode of DNA binding, following a clamp and click model. Apart from serving as an autoregulator, CprB is potentially a part of a network of proteins that modulates the γ-butyrolactone synthesis and antibiotic regulation pathways in *S. coelicolor A3(2)*.				