Ten best research publications with highlights. (listed as per order of importance)

1. Nayak, S.R., Joesph, D., Hoefner, G., Dakua, A., Athreya, A., Wanner, K.T., Kanner, B.,& Penmatsa, A.* CryoEM structure of GABA transporter 1 reveals substrate recognition and transport mechanism. *Nat. Struct. Mol. Biol.* (2023).

Highlight: The study resolves the cryoEM structure of the GABA transporter that is a major player in Using a novel approach of epitope transfer to transfer a Fab binding site from dDAT to rGAT1.the control of the synaptic GABA levels and thereby inhibitory neurotransmission mediated by GABA. This allowed us to study the three dimensional architecture of GAT1 bound to a molecule of GABA that is ready to exit the transporter binding site and study a transport state that allows us to understand the mechanism of GABA uptake.

2. Hussain, N., Apotikar, A., Pidathala, S., Mukherjee, S., Burada, A.P., Sikdar, S.K., Vinothkumar, K. R. & Penmatsa, A.*. CryoEM structures of Pannexin 1 and 3 reveal differences among Pannexin isoforms, *Nat. Commun*, (2024) 15(1):2942.

Highlight: The study delves into the mechanistic aspects of large pore ion channels, Pannexins, to understand ATP and carbenoxolone (gout medication) interactions with Pannexin isoforms and pore mutants. The cryoEM structures of pannexins 1 and 3 were resolved and the pore residues were analysed to understand the effect on channel properties. A congenital mutant of pannexin1 was studied that severely hampers channel conductance properties and blocks the pore through an allosteric effect on the pore-lining residue.

3. Joseph, D., Nayak, S.R., & Penmatsa, A.* Structural insights into GABA transport inhibition using an engineered neurotransmitter transporter. *EMBO J.* (2022) 41(15):e110735.

Highlight: This study employs a strategy to engineer the binding pocket of a neurotransmitter transporter and shift the specificity from antidepressants to antiepileptics in the binding pocket. This work done primarily by my graduate students displays the reorganization of the binding pocket within GAT1 to display a kidney bean shape instead of a trilobed architecture. The study therefore defines the specificity of drug interactions among antiepileptic drugs that target GAT1.

4. Shabareesh, P., Mallela, A.K., Joseph, D. & Penmatsa, A.* Structural basis of norepinephrine recognition and transport inhibition in neurotransmitter transporters. *Nat. Communs*. (2021) 12:2199.

Highlight: The manuscript is focused on understand the basis of noradrenaline interactions with the fruitfly catecholamine transporter and allows insights into the role of subsites in the interactions with chronic pain medications involved in treatment of fibromyalgia, chronic pain and neuropathy. The binding site is organized as three subsites (A, B,C) that can have an influence on the interaction propensities of inhibitors. The subsite C is implicated in specificity and subsite B interactions allowed increased affinity towards blocking the transporter. The work was performed by my postdoc and students and was done entirely in my laboratory.

5. Majumder, P., Ahmed, S., Ahuja, P., Athreya, A., Ranjan, R., Penmatsa, A.* Cryo-EM structure of antibacterial efflux transporter QacA from *Staphylococcus aureus* reveals a novel extracellular loop with allosteric role. *EMBO J.* (2023) 42: e113418.

Highlight: The study delves into the structure and mechanism of a major antibacterial efflux pump, QacA, from a superbug *S. aureus*. The efflux transporter is a highly promiscuous antibacterial efflux pump capable of effluxing numerous antibacterial compounds. My graduate students and I were involved in the isolation of camelid antibodies against QacA and using them as fiducial markers to study the structure of QacA through single particle cryoEM. The study resolves the high resolution cryoEM structure of QacA and allows mapping the positions of the protonation sites meant for

antibacterial recognition and efflux. A unique extracellular hairpin was observed in the structure that is vital for gating and efflux of antibacterial compounds.

6. Majumder, P., Khare, S., Athreya, A., Hussain, N., Gulati, A. & Penmatsa, A.* Dissection of protonation sites for antibacterial recognition and transport in QacA, a multidrug efflux transporter. *J. Mol. Biol*. (2019). 431, 2163-2179.

Highlight: The study delves into the promiscuous behaviour of QacA towards its monovalent and divalent cationic substrates. We demonstrated the activity of QacA in reconstituted liposomes and also optimized everted vesicle and proton exchange experiments with most of the work done by graduate students within the lab. We could observe the roles of multiple acidic residues as protonation sites in the vestibule of QacA.

7. **Kumar, S., Athreya, A., Gulati, A. Nair, R.M. Mahendran, Mahendran, I., Ranjan, R. & Penmatsa, A.* Structural basis of inhibition of a putative drug efflux transporter NorC, through a single-domain camelid antibody. *Commun Biol.* (2021) 4:836.**

Highlight: The study provides insights into the structure of NorC complexed to a single-domain camelid antibody isolated in my lab that would interact with NorC in an outward-open conformation and prevent the interaction of antibacterial compounds to the vestibule of NorC. This was the first X-ray structure of a membrane protein performed in my lab with my postdocs and graduate students working on it.

8. Kumar, S., Mahendran, I., Athreya, A., Ranjan, R. & Penmatsa, A.* Isolation and structural characterization of a Zn^{2+}-bound single-domain antibody against NorC, a multi-drug efflux transporter in bacteria. *J. Biol. Chem.* (2020). 295(1), 55-68.

Highlight: First study detailing the identification of a unique zinc-bound single domain camelid antibody targeting NorC. Work was done primarily at IISc and immunization of camels was carried out at the NRCC, Bikaner. The novel antibody has a Zn coordination in its CDR3 which is usually observed with a disulphide bond. The presence of this metal ion stabilization was never previously observed in this class of antibodies. Disruption of the Zn site severly hampers the interactions of this antibody with the antigen.

9. Wang, K. H.[1] Penmatsa, A.[1] & Gouaux, E. Neurotransmitter and psychostimulant recognition by the dopamine transporter. *Nature.* (2015). 521, 322-327.

Highlight: An extensive study that was part of my postdoctoral work exploring the interactions of dopamine and psychostimulants like cocaine and amphetamines with monoamine neurotransmitter transporters. The study explored the binding site interactions of cocaine, RTI-55, methamphetamine and amphetamine with Drosophila dopamine transporter to understand the structural basis of substrate and psychostimulant recognition and transport.

10. Penmatsa, A.[1], Wang, K. H.[1] & Gouaux, E. X-ray structure of dopamine transporter elucidates antidepressant mechanism. *Nature.* (2013). 503, 85-90.
Highlight: The first structural study on a eukaryotic neurotransmitter transporter done as part of my postdoctoral work. The study employed extensive thermostabilization of the dopamine transporter to stabilize the molecule and induce the stabilization of an outward-open inhibitor bound state. The structure in complex with a tricyclic antidepressant allowed the characterization of the pharmacology of antidepressant interactions with neurotransmitter transporters.

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