Research Summary

Viachaslau Bernat

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My research interests are centered around application of organic synthesis to designing molecules with useful properties for biomedical applications. This broad definition led me to pursue research in the range of subjects from medicinal chemistry to molecular pharmacology and chemical biology of membrane proteins and non-coding RNA, and development of diagnostic fluorescent sensors.

Biased modulators of GPCRs

Biased μ-opioid receptor agonists. As a part of multi-center collaboration I synthesized newly designed biased agonists of μ-opioid receptor. My main contribution was synthesis and preparative separation of individual enantiomers of the optimized hits. The stereochemistry turned out to be the key parameter in SAR that defined signaling bias and affinity. The most promising molecule – PZM21 – did not recruit β-2-arrestin, while being single-digit nanomolar activator of G_i protein signaling. Animal testing demonstrated strong analgesia, lack of side effects, and acceptable therapeutic index[1].

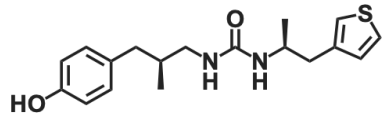

Figure 1: Structure of PZM21

[1] A. Manglik *et al.* *Nature*, 537(7619): 185–190, 2016

Biased negative allosteric modulators of CXCR3. Using homology modeling I constructed the structural model of CXCR3 based on X-ray structure of CXCR4 and docked known allosteric modulators (ChEMBL) into the model. Thus we identified amino acid residues that regulate small molecule binding and downstream signaling. Attempts to engage a nucleophilic subpocket via reversible covalent interaction with strategically placed electrophilic boronic acid led to discovery of the first biased negative allosteric modulator or CXCR3 (**14**, Fig. 3).[2]

Optimization of physico-chemical properties (cLogP and PSA) via introduction of flexible side chain led to identificaiton of the negative allosteric modulator with the opposite signaling bias (**1b**, Fig. 3). These biased modulators exhibited opposite probe dependence – they preferentially downregulated activation of CXCR3 by CXCL10 or CXCL11 chemokines.[3] Affinities of these small-molecule allosteric modulators were similar (double-digit nM) as measured in radioligand RAMX3 displacement assay.[4]

Structural model of CXCR3 that I prepared was subsequently used *as is* in virtual screening for identification of completely novel chemotypes of specific and dual CXCR3 and CXCR4 allosteric modulators.[5]

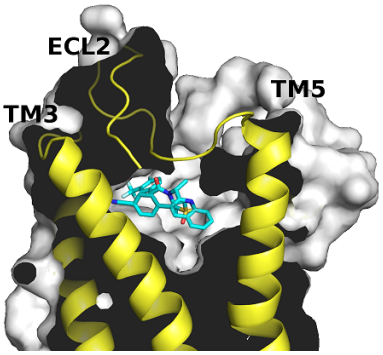

Figure 2: Homology model of CXCR3 with docked small molecule allosteric modulator.

[2] V. Bernat *et al.* *ACS Chem. Biol.*, 9(11): 2664–2677, 2014

[3] V. Bernat *et al.* *ChemMedChem*, 10(3): 566–574, 2015

[4] V. Bernat *et al.* *ChemMedChem*, 7(8): 1481–1489, 2012

[5] D. Schmidt *et al.* *ACS Chem. Biol.*, 10 (3):715–724, 2015

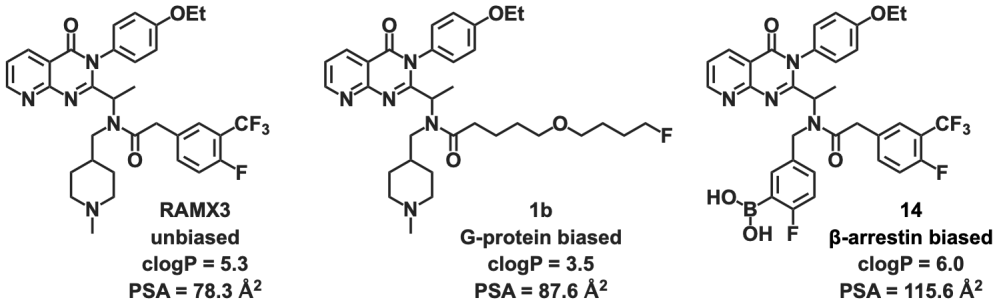

Figure 3: Small molecule allosteric modulators of CXCR3

Chemical biology of non-coding RNA

Non-coding RNA folds into 3D-structures that can be targeted by small molecules.[6] My research in chemical biology of RNA was about identification of new chemotypes targeting pathogenic RNA structures and development of chemical tools for studying their role in cellular markers of disease.

[6] V. Bernat and M. D. Disney. *Neuron*, 87 (1):28–46, 2015

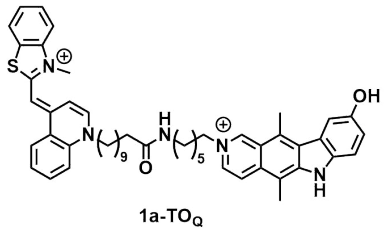

Figure 4: Fluorescent reporter for r(G4C2)^{exp} binding assay

[7] Z. F. Wang *et al*. *Cell Chem. Biol.*, 26(2): 179–190.e12, 2019

Ellipticines as ligands for $r(G4C2)^{ext}$. Building upon the previous work in the Disney lab, I developed fluorescent reporter 1a-TO_Q which served as a tool for quick SAR interrogation *in vitro* and led to discovery of better RNA binders from the same chemotype[7]. Key insight was that permanent positive charge was not required for potent binding to RNA and activity in cellular assays. This dramatically improved blood-brain barrier penetration in mice.

Chemical biology of $r(CUG)^{exp}$ and $r(CCUG)^{exp}$. To study the role of pathogenic non-coding RNAs in myotonic dystrophy I developed series of conjugates that disrupted interaction of r(CUG)^{exp} with proteins (GC-2H-CG, Fig. 6A) or targeted the pathogenic r(CCUG)^{exp} for degradation by latent cellular nuclease RNAse L (Fig. 6B). Further research in Disney lab built upon this work to develop microRNA degrading tool compounds.

NIR fluorescent glucose sensors

At Profusa, I developed near-infrared (NIR) fluorescent sensors for continuous sensing of glucose *in vivo*. The design of glucose sensors was based on known bis-boronic anthracene module that was incorporated into biocompatible hydrogel matrix. From a library of 120 dyes that I prepared[8], I could explore structure-activity relationships. Based on that I was able to rationally improve the performance of the company's prototype dye up to 5-fold. Finally, I scaled the synthesis of the lead dye to produce 1.8 g of material for clinical trials.

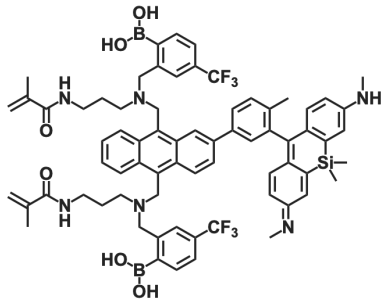

Figure 5: Example of glucose-sensing polymerizable NIR dye.

[8] S. Gamsey *et al*. US Pat. Appl. 16038657, 2018

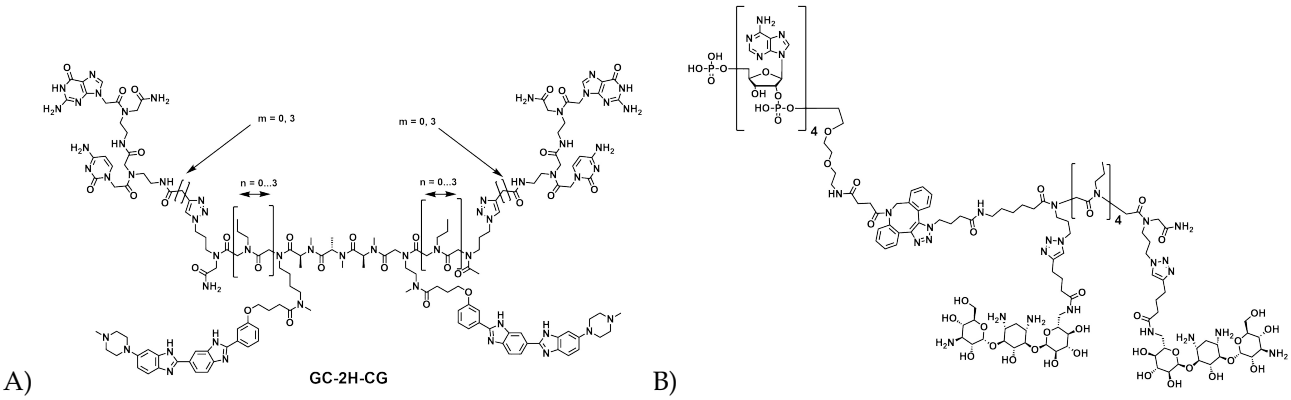

Figure 6: A) Structure of peptoid-PNA-Hoechst conjugate for disruption of r(CUG)^{exp} - MBNL1 interaction; B) Structure of peptoid-[2-5]A$_4$-Kanamycin conjugate for targeting r(CCUG)^{exp} for degradation by RNAse L

References

V. Bernat and M. D. Disney. *Neuron*, 87(1):28–46, 2015.

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