P-2.28

Biochemical And Structural Insights of Natural and Synthetic Molecules Selectively Binding to Hcn4 Pacemaker Channel

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are the molecular correlate of the Ih (or If) current, which plays a key role in controlling rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. HCN channels are activated by voltage and modulated by the direct binding of cAMP. HCN channels are further regulated by cyclic di-GMP (c-di-GMP), an emerging class of second messengers in mammals. C-di-GMP binds the pacemaker HCN4 channels and antagonizes cAMP regulation of the channel (1). To date, this is the only example of an isotype-specific drug for HCN channels. We used a fluorescence size exclusion chromatography-based thermostability assay (FSEC-TS) to monitor the binding of c-di-GMP to purify GFP-tagged HCN4 protein. Overall, this is the first biochemical evidence of c-di-GMP binding to HCN4 pacemaker channels. Based on these promising results, we collected single particle cryo-EM data of HCN4 bound to c-di-GMP and we are currently analyzing the dataset to obtain the high-resolution EM density map of the complex. Moreover, the finding of an allosteric binding site in HCN4 led to the identification of a synthetic molecule, termed C11, that selectively binds to HCN4 and fully prevents cAMP modulation (1). We are also analyzing HCN4 – C11 complex via cryo-EM.