HTRF® cAMP assays: discussion of best practices for assay design and data analysis

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Abstract:
HTRF cAMP assays generate a ratiometric signal which can then be transformed to a concentration of cAMP by using a cAMP standard curve. When the x-axis of the standard curve is plotted on a logarithmic scale a sigmoidal curve is generated which can be fit to a 4-parameter curve fit using Ymin., Ymax., Slope, and EC_{50}. The ratiometric signal for the unknown sample can then be transformed to a concentration of cAMP by determining x from y using the standard curve generated equation. Ideally, the compound response should fall within what is often mistakenly termed the "linear range" of the standard curve for sensitive detection and transformation of raw data into a concentration of cAMP generated. This presentation will discuss best practices for assay design to obtain high quality data. The presentation will focus on the effect of data transformation of ratiometric data to cAMP data on concentration response curves for compounds which activate or inhibit adenylyl cyclase, with emphasis on EC_{50}/IC_{50} and Ymax. changes that occur. By understanding the effect that data transformation has on concentration response curves, a Gs-coupled allosteric potentiator assay will be described.

Resume:
I did my undergraduate degree, B.Sc. in Applied Biology, at Hatfield Polytechnic, Hertfordshire, England, UK. I then moved to the University of Leicester, England, UK. for my Ph.D. in Molecular Pharmacology. My thesis was entitled: "Cell Signaling in Chinese Hamster Ovary Cells Expressing Recombinant Muscarinic Receptors." and my supervisor was Prof. Stefan Nahorski. Post-doctoral work included follow-up work on muscarinic receptor signaling at Leicester, followed by work on opioid receptor signaling at the University of California San Francisco, CA, USA. where my Principle Investigator was Prof. Wolfgang Sadee. I worked briefly as a Research Scientist at Incyte Genomics, Palo Alto, CA, mining novel GPCR sequences from the human genome, before joining Bristol-Myers Squibb in Wallingford, CT in 2000. At Bristol-Myers Squibb I currently lead the GPCR Hit Identification Group within the Department of Lead Discovery, Profiling and Compound Management.