The Effect of Cellular Context on Intracellular Measurements of Drug-Target Binding Kinetics

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The determination of drug-target binding kinetics is incredibly desirable during drug discovery. Kinetic quantification of binding provides additional opportunity for optimisation of compound efficacy and therapeutic window over IC_{50} values. The effects of cellular context on intracellular measurements of binding kinetics is poorly understood. Given the benefits of investigating binding kinetics, along with the fact that many current drug discovery projects concern intracellular targets, this presents a significant issue. To isolate the effect of cellular context on kinetic measurements, we compared the k_{on} and k_{off} values of nineteen inhibitors of a receptor tyrosine kinase (RTK) measured by NanoBRET in whole cells with those from membranes preparations. The differences were found to be significant and followed noticeable trends, potential causes of which are discussed. On-rate measurements were found to be distorted by cell permeability and ATP competition. Overall this study highlights the need to consider cellular context when interpreting data from intracellular kinetic assays.