Enzymes are important drug targets. Many marketed drugs today function through modulation (inhibition and activation) of enzymes mediating disease phenotypes. Therefore, how to accurately measure the changes of enzyme activity affected by modulators becomes essential and important. There are many different approaches, which generally be classified as following

UV absorbance

In these assays, the UV absorbance can distinguish between substrate and product without either fluorescent or radioactive label. It can carry out reverse Michaelis-Menten kinetics in the absence of substrate. It cannot add same product and measure that product formation rate simultaneously (e.g., not rigorously possible w single product). -Lactamase is an ideal example. We measured the loss of absorption of the substrate and the loss is very significant even after 10 min of the reaction. This is not possible for Sirtuins because of high NAD concentration and less efficient product formation.

Fluorescent or radiolabeled product:

In these assays, the product can be added or slightly modified by fluorescent label. The product formation rate can be measured simultaneously. Therefore the product inhibition assays are possible. Radiolabeling is applicable to any enzyme system (both forward and reverse reactions with product inhibition) but not suitable for high throughput. For enzyme reactions having two or more products, one product can be added exogenously without influence the readout since the other product can be used for quantification. By doing so, more parameters can be studied. For example, product inhibitor was found in sirtuins reaction, who has multiple products. A fluorescent assay is suitable to be applied to study the product inhibition while adding product inhibitor exogenously.

Continuous assays

In continuous assay, the progress of the reaction is followed as it occurs. Continuous assays are much more convenient. Some fluorescence-based assays are not continuous. The FdL assay is not a continuous assay, and may limit the substrate choice. The aforementioned -lactamase assay is a continuous UV assay.

For preliminary screening of activity of sirtuins and basic MM kinetics, Pnc continuous assay and Sirtainty assay (need to confirm with EMD) can be used. For those two assays, specific substrates can be studied and the amounts of NAM formed are measured. In order to do product inhibition assays in continuous format, the “Universal Substrate for Sirtuin 1-6 Activity assay” can be performed. A fluorophore tagged universal substrate for Sirtuin 1-6 is used.

In order to study the product inhibition on other substrates except FdL substrates, one can tailor label any desired substrates in a discontinuous fluorescence assay for sirtuins (FdL). However, there will be some decrease in throughput and increase in cost. Also previous reports mentioned the FdL produced false positive results due to the use of FdL-substrate. So far it seems FdL may be the only suitable general high throughput assay to serve our purpose.