--UV absorbance (not labeled either fluorescently or radioactively):

Cannot add same product and measure that product formation rate simultaneously (e.g., not rigorously possible w single product)

Since UV abs of substrate and product differ, can carry out reverse MM kinetics in absence of substrate

Example b-lactamase: can do forward and reverse MM kinetics.

--Fluorescent or radiolabeled product:

Can add the same product (or slightly modified by fluorescent label) and measure that product formation rate simultaneously

So product inhibition assays are possible

Radiolabeling is almost always applicable to sysid of any enzyme (both forward and reverse reactions w product inhibition) but not suitable for ht

--Having two products can simplify things, while adding more parameters: the product that is added exogenously need not be the one measured

Example sirtuins: we are using a fluorescent assay on one product while adding the other one exogenously

--Continuous assays are best for ht automation:

Some fluorescence-based assays are not continuous; the ones that are may limit the substrate choice

UV assays are

Examples: we use a continuous UV assay for b-lactamase

We can at least use a continuous assay for preliminary screening of activity of sirtuins and basic MM kinetics. We can choose between sirtainty and the Pnc continuous assay here

We can use a continuous fluorescence assay for sirtuins with specific substrate, in order to do product inhibition assays in continuous format: there is this option:<https://shop.jpt.com/2-Enzyme-Substrates/63-Individual-Substrates/1000632-Universal-Continuous-Sirtuin-Assay-Kit.html> Which uses a specific fluorophore tagged substrate but is contnuous

With a discontinuous fluorescence assay for sirtuins (FdL), we can tailor label any desired substrate, but there will be some decrease in throughput and increase in cost

So far it seems fdl may be the only suitable general high throughput assay for this