The KinExA® Advantage

A comparison of biosensors for K_d analysis

KinExA is a technique for measuring unmodified molecules with either both molecules in solution or with one in solution and the other expressed on a cell surface. This document will describe the advantages KinExA has over other biosensor technologies.

Technologies Considered

KinExA – Kinetic Exclusion Assay – Sapidyne Instruments
SPR – Surface Plasmon Resonance – Biacore, Carterra, many others
BLI – Biolayer Interferometry – Octet
FACS – Fluorescence Activated Cell Sorting – various
QCM – Quartz Crystal Microbalance – Attana
Ligand Tracer – Ridgeview Instruments
ELISA – Enzyme-Linked ImmuoSorbent Assay - various

Solution-Phase

A significant percentage of drugs are directed at soluble biomolecules rather than cell membrane proteins. Here KinExA has several advantages over other biosensors.

KinExA: KinExA measures binding of the unmodified drug to the unmodified target in solution. The solute can be anything in any kind of buffer, serum, cell lysate, etc. KinExA has been demonstrated to be effective for many single digit pM K_d measurements and has measured K_d's as low as 12 fM[14].

SPR, BLI, QCM: These surface techniques require immobilization of one binding partner on the surface. The immobilization can cause conformational changes in the molecule leading to a change in K_d[7,13], or the surface itself can cause artifactual errors in the measured K_d[8]. Examples using SPR to measure single digit pM K_d's can be found in the literature but most users don't trust it for K_d's below 100 pM. Many do not trust it below 1 nM. BLI and QCM tend to be used for nM and weaker binders.

FACS, Ligand Tracer: These techniques are for cells only and don't have the capability to measure soluble molecules.

ELISA: This is a surface technique and suffers from the weaknesses mentioned above for SPR, BLI, and QCM. In addition, what is usually reported for results is the IC50, the concentration of the enzyme labeled ligand that gives half the maximum signal obtained from saturating ligand. In most cases the coating density on the plate is as high as possible leading to a situation analogous to a concentration controlled curve. This means the IC50 observed in ELISA is virtually never equal to the K_d.

Cells

Although cell therapies (in which cells are administered as the therapeutic agent) are becoming more prevelant, most of existing drugs are soluble molecules that target a cell membrane protein[15]. Cell membranes are composed of lipids that have a hydrophilic (water loving) and hydrophobic (water hating) end. The complete membrane of every cell is a double layer of these molecules oriented with their hydrophobic ends touching and their hydrophilic ends facing the cell exterior or the cell membrane inner surface. Proteins are chains of amino acids whose function and activity are largely determined by their folded structure. Membrane proteins may span the membrane once (bitopic proteins), several times (polytopic proteins), or not at all (monotopic proteins) which attached to only one side of the membrane. Membrane protein structure is partially dependent on the membrane so anytime the protein is isolated and purified there is a risk that the binding epitope is modified, hidden, or simply eliminated.

KinExA: Measures binding of an unlabeled molecule to intact cells in any liquid matrix. KinExA has been successfully demonstrated on both engineered cell lines[1,2] and on native cells expressing endogenous proteins (unpublished). Measuring directly on native cells is as good as it can get in terms of biological relevance of affinity and activity measurements.

SPR: As of now, there are no publications that have highlighted the use of commercial SPR instruments with cells, however there is literature on a specific apparatus that could make this possible. One detects morphological changes in cells grown on a gold substrate[10]. This looks similar to DMR (see BLI below) and no binding constant is reported. The second reference to this used surface plasmon resonance microscopy to measure binding of wheat germ agglutinin to a single cell and report a K_d of 0.32uM[11].

They also show a very noisy sensorgram for an antibody binding to a membrane protein where no K_d is given.
Note: Researchers have said that because many targets are on cell surfaces, surface based measurements are superior to solution measurements. After purification and immobilization the membrane protein is far from it's natural state, which is why measuring to the cell is much preferred.

BLI: There is one reference with the use of cells on BLI[9]. However, they are not calculating K_d or detecting binding directly, instead they are measuring "dynamic mass redistribution"(DMR) . The signals they show are specific to living cells (at least unfixed) and can be inhibited by antagonist mAb. It's interesting and may have value in understanding cell biology but it is not directly measuring binding to cell membrane receptors.

FACS: In this technique, the amount of fluorescently labeled molecule bound to intact cells is measured. The main drawbacks to this technique are that the molecule must be labeled (which has the potential to change its binding) and the sensitivity of the technique limits it to K_d's of approximately 1nM and weaker[3,4].

QCM: Attana provides a special surface to grow adherent cells directly on the microbalance. They also support capturing suspension cells on the QCM surface. Either way, protein binding to the cells is monitored by the change in mass. For adherent cells this is arguably closer to nature than what KinExA does which lifts adherent cells off the surface and measures them in suspension. There is not much published on this, one potential problem may be getting the cells to grow on a special chip which would put extra development time constraints on the cell culture team. Sensitivity appears to be limited to nM K_d's[5,6].

Ligand Tracer: Adherent cells are grown on a Petri dish then radiolabelled (preferred) or fluorescently labeled molecules are introduced and the binding is measured over time as the tilted petri dish is rotated. K_d 's are reported based on direct on and off rate measurement of labeled molecule binding to cells[12]. Off rates are monitored for up to 8 hours. This is a dedicated cell measuring machine and does not offer solution binding analysis.

ELISA: This technique is not suitable for cells.

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