Overview of High-Throughput Screening Methods in Small-Molecule Drug Discovery - Emily Ricq - 11/20/12

Two major high-throughput screening approaches:

- Target-based: searching for a compound that interacts with a molecular target, typically a protein or protein complex. Sometimes referred to as 'reverse' chemical genetics.
    - OVERLAPS WITH: biochemistry, biophysics, chemical biology, computer science
    - PROS: lower cost, may be poised for medicinal chemistry
    - MAJOR CHALLENGES: formulation of hypothesis, translation from *in vitro* to *in vivo*, biomarker discovery
- Phenotype-based: searching for a compound that has a desired biological outcome, independent of its molecular target. Sometimes referred to as 'forward' chemical genetics.
    - OVERLAPS WITH: cell biology, molecular biology, genetics, proteomics
    - PROS: hypothesis generating, physiological conditions
    - MAJOR CHALLENGES: target identification, generality of model system

Examples of <u>target-based</u> screening measurements, and their major outcomes:

- Activity-based:
    - Enzymatic assays measuring substrate conversion
    - Requires bulk source of recombinant protein, typically expressed and purified in bacteria, which may or may not recapitulate normal biochemistry
    - Assay kinetics may allow for determination of mechanism of inhibition
    - Promiscuous inhibitors and aggregators are common false-positives
    - Often relies on a 'coupled' enzyme, such as trypsin, luciferase, or HRP, to convert product into a fluorescencent or luminescent species for detection. Orthogonal and counter-screens required.
- Affinity-based:
    - Change of mass or T_M upon ligand binding, such as surface plasmon resonance or calorimetry
    - Detection of antibodies, bound to antigen, broadly referred to as immunostaining.
    - Detection of antibodies, bound to target/small-molecule complex. The small-molecules will be tethered to a chip by a linker, which may limit structural diversity.
    - Sequencing of DNA-tethered small-molecules
    - Computed binding enthalpy of ligands docked to a regulatory or active site
    - None of the affinity-based methods guarantee inhibition or activation of your target.
- Outcomes of target-based screens:
    - What is the consequence of inhibiting/activating your enzyme of interest on the phenotype?
    - Need to identify downstream biomarker(s), selectivity with related enzymes

Examples of <u>phenotype-based</u> screening measurements, and their major considerations:

- Pathway-based
    - Reporter gene stably transfected/transformed into model cell system. Generally luciferase or eGFP driven by upstream regulatory element (promoters/enhancers) of a gene of interest, or a consensus binding sequence for a transcription factor. Readout is downstream of signaling pathway.
    - Generally requires co-transfection with a selectable mark, such as antibiotic resistance, and a constituently active construct for normalization.
    - Requires validation with endogenous model system, usually by RT-PCR
- Morphology-based
    - High-throughput imaging and automated image analysis software allows for the measurement of many cellular morphologies, including cell division, neurite outgrowth, and apoptosis/cell death.
    - Simple measurements such as cell division (BrdU) and apoptosis (TUNEL) can be quickly screened. More complex measurements are more computationally demanding.
    - Highly dependent on antibody quality/availability. Each plate should contain sufficient positive and negative controls to be independently normalized to account for day-to-day variability.
- Behavior-based
    - Zebrafish motility!
- Outcomes of phenotype-based screens
    - What is the molecular target of the compound?
    - Affinity purification and proteomics, such as MALDI-TOF MS/MS, common starting points.

Next steps: cluster hits and begin lead optimization, *ie.* medicinal chemistry, metabolic profiling, pharmacology…