In vitro Screening Platforms for the Discovery of Novel Epigenetic Probes

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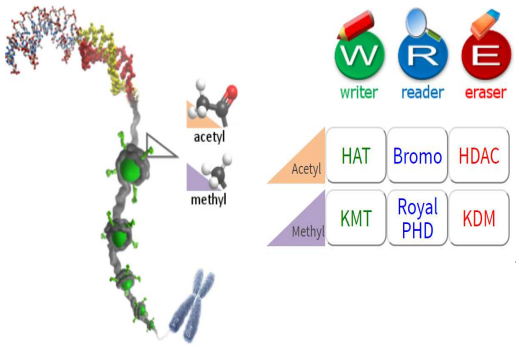
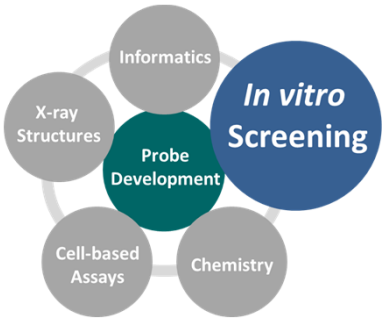
Epigenetics Biology

- Epigenetics involves the regulation of genomic functions, including gene expression.
- Epigenetic modifications are partly inherited, but unlike the genome itself, are cell specific, plastic, and responsive to environmental influences.
- The proteins involved in epigenetic modifications can be divided into:
  - those which introduce the changes – writers (e.g. methyl transferases)
  - those which read the changes – readers (e.g. bromodomains)
  - and those which remove the changes – erasers (e.g. demethylases)
- The SGC's interest is mainly in histone modifications (acetylation, methylation, phosphorylation, etc.).
- The SGC develops chemical probes to study the roles the various domains play in genomic functions.



Chemical Probes

- Chemical probes are potent, selective cell permeable inhibitors of protein function:
  - *In vitro* potency of <100 nM
  - >30-fold selectivity against other subfamilies
  - On target effect in cellular assay at <1 µM
- Valuable tools for early stage drug discovery allowing target validation.
- Made available to all researchers with no restrictions.



- Chemical probes are developed using a multi-disciplinary drug discovery style model.
- *In vitro* testing is conducted by the Screening Group on compounds from internal chemistry as well as from academic and pharma partners.

Screening at the SGC

- A wide range of assay formats and technologies are deployed at the SGC in order to screen compounds and determine accurate binding affinities.
- A selection of these methodologies is outlined here.
- These assays are also used to determine selectivity of binding to other protein family members.
- Each technique has strengths and weaknesses (see table below) and it is good practice to obtain data in multiple formats prior to further compound characterisation.

	ALPHAScreen	DSF	BLI	ITC	MST
Throughput (data points/day)	10000	1000	Low	Low	Low
Cost	High	Low	Moderate	Low*	Low
Protein requirement	Low	Moderate	Low	High	Low
False positives	Yes	Yes	Yes	No	No
Accurate Kd	No	No	Yes	Yes	Yes

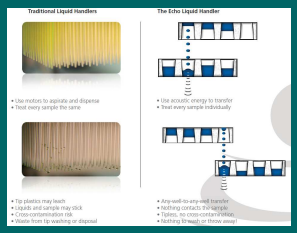
* If the protein is made in-house.

- To date, 24 chemical probes have been released to the research community as well as a number of broader specificity "tool" compounds.
- These have been used to assess the epigenetic mechanisms of various diseases and speed up drug discovery in those therapeutic areas.

1. Labcyte Inc.
2. PerkinElmer Inc.
3. Frank H Niesen et al. Nature Protocols. 2007; 2, 2212 - 2221
4. Panagis Filippakopoulos et al. Nature. 2010 December 23; 468 (7327)
5. Pall ForteBio Corp.
6. NanoTemper Technologies GmbH

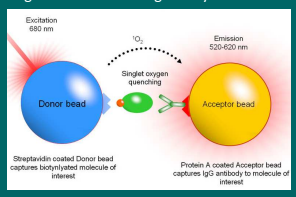
Echo® Liquid Handler (1)

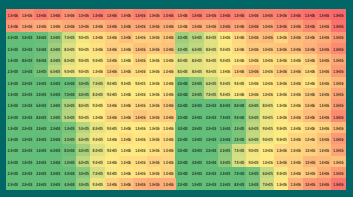
- The Echo liquid handler is used to prepare compounds for screening, using acoustic energy.
- Sound waves eject precisely sized droplets from a source onto a microplate, slide or other surface suspended above the source.
- The ECHO therefore, does not use tips, pin tools or nozzles—completely eliminating contact between the instrument and the samples.
- Fluids are transferred in nanoliter increments hence enabling assay miniaturization (accuracy within 10 % at 2.5 nl).
- Larger volumes are transferred at the rate of hundreds of droplets per second.

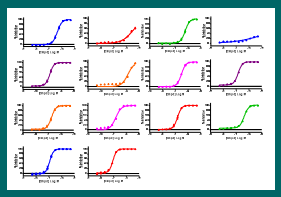


Amplified Luminescent Proximity Homogeneous Assay- ALPHAScreen (2)

- The AlphaScreen assay is used to identify inhibitors for many of our epigenetic compound screening programmes.
- Donor beads converts ambient oxygen to singlet oxygen upon illumination at 680 nm which has a 4 µsec half-life and can diffuse 200 nm in solution.
- Protein-ligand interactions bring Acceptor beads into close proximity and energy is transferred from the singlet oxygen to Acceptor beads culminating in light production at 520–620 nm. In the absence of close proximity Acceptor beads, singlet oxygen falls to ground state and no signal is produced.
- The assay allows the determination of the IC50s of moderately to highly potent compounds (IC50 range 500 µM – 5 nM) with high signal to background ratios and high assay Z' values.

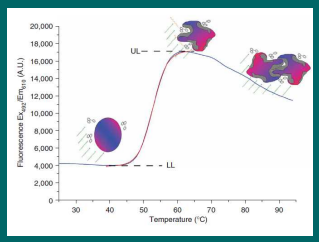


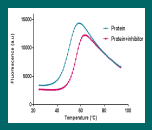




Differential Scanning Fluorimetry – DSF (3)

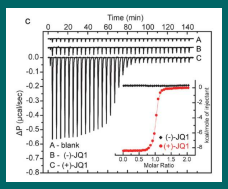
- DSF also known as the Thermal Shift Assay, is used to identify ligands that bind and stabilize target proteins and is performed using a conventional real-time PCR instrument.
- The temperature at which a protein unfolds is measured by an increase in the fluorescence of a dye with affinity for internal hydrophobic areas of the protein, which are exposed as the protein unfolds.
- A simple fitting procedure allows quick calculation of the transition midpoint.
- The difference in the temperature of this midpoint in the presence and absence of ligand is related to the binding affinity of the small molecule.





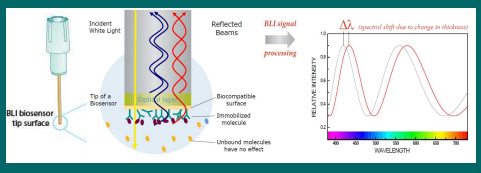
Isothermal Titration Calorimetry – ITC (4)

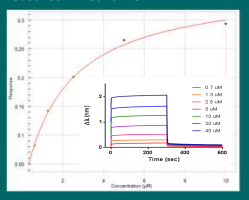
- ITC is used to determine ligand binding constants in solution by measuring binding heats that are either released (enthalpic) or consumed (entropic).
- ITC is a direct method which can be applied to diverse ligand- receptor systems.
- The figure shows data for three titration experiments using the bromodomain of BRD4.
- A is Bank titration (ligand into buffer), B is the Inactive (-)-JQ1 stereoisomer. And C is the active (+)-JQ1 showing exothermic strong binding.



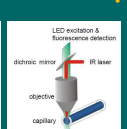
Biolayer Interferometry – BLI (5)

- BLI is performed using the Octet Red 384 machine which uses immobilised protein on biosensors to obtain binding data for bio-molecular interactions.
- Increasing binding increases the optical layer thickness and causes a shift in the interference pattern which can be measured in real time.
- Association and dissociation rates can be determined and used to calculate a Kd.





MicroScale Thermophoresis – MST (6)



- MST uses fluorescence to monitor movement of molecules (protein) in a temperature gradient within narrow capillaries.
- Ligand binding alters the movement by changes in hydration shell, molecular weight and charge.
- The change in fluorescence signal is proportional to the degree of binding allowing generation of IC50 and Kd values.
- MST requires as little as 4 µl of sample and no immobilisation.