The application of Design of Experiment and the dragonfly® discovery to miniaturise, automate and accelerate assay optimization

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Introduction

Traditional, iterative assay development, though employed and respected throughout the pharmaceutical industry, can be a long, sometimes challenging and cumulatively expensive process. Due to these long and often challenging assay development efforts there is renewed interest in employing methodologies, such as Design of Experiments (DoE) using JMP software, to apply a more statistical, streamlined approach to reduce timelines.

Automation to carry out complex DoE experiments has always been a limiting factor, both in terms of speed and cost. The dragonfly® discovery from SPT Labtech, currently in early development, is a rapid and reliable low volume liquid handler that employs true positive displacement technology to enable the dispensing of all aqueous solutions and solvents, from 200nL through to 4mL. The dragonfly® discovery's plate-map file format made it simple to convert and import JMP DoE files and subsequently execute these experimental designs in all microplate types, including 1,536 well plates.

Here we present fluorescence polarization (FP) and time-resolved fluorescence resonance energy transfer (TR-FRET) assay development and screening data for a bromodomain (Brd) binding interaction. DoE and a prototype of the dragonfly® discovery were employed to develop these assays in 1,536 well microplates in rapid time compared to traditional methods. This was achieved without the use of any manual pipetting and 384 well microplates, in addition to a significant reduction in reagent requirements due to low liquid handler dead volumes. A further advantage of using the dragonfly® discovery for low volume, 1,536 well microplate assay development was that we were able to utilise the same liquid handling technology to then screen in excess of 40,000 compounds.

This combination of Design of Experiments and dragonfly® discovery demonstrates that it is possible to reduce assay development cycles times, in addition to removing well known assay bottlenecks associated with the transfer of subsequent assays into high throughput screening.

Design of Experiment

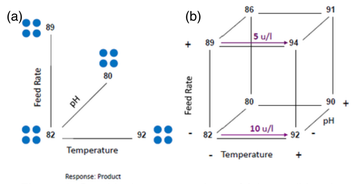


Figure 1. Graphical representations of (a) a traditional assay development and (b) a DoE three-factor full factorial design.

- Traditional assay development
  - Change one experimental factor at a time and keep all others fixed
  - Information on the factor you are changing but no interaction effects between factors measured
- Design of Experiment (DoE) assay development
  - Measures effects of individual experimental factors and alsothe interaction between factors using statistical models

There is now renewed interest in DoE for assay development as a tool for reducing assay cycle times and aiding the investigation of multifaceted problems of increasingly complex systems.

jmp Statistical Discovery.™ From SAS.

SAS JMP software utilised for all Do Estudies.

Assay Technology

Fluorescence polarisation (FP) utilises the tumbling of a fluoro- ligand and polarised light to detect protein-ligand interactions. When a fluoro-ligand is bound to the target protein there is a decrease in tumbling velocity resulting in an increased in polarised light emission.

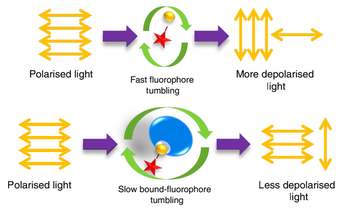


Figure 2. A schematic explaining how interactions between protein and ligand are detected using polarised light in FP.

Time-resolved fluorescence resonance energy transfer (TR-FRET) relies on the excitation of a lanthanide donor which subsequently excites an acceptor fluorophore, when in close proximity, by Förster resonance energy transfer.

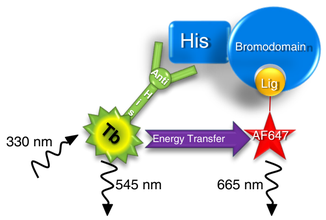
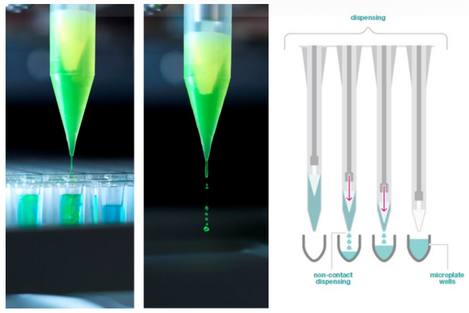


Figure 3. A schematic of the mechanism of detection for TR-FRET, using examples specific to these experiments.

dragonfly® discovery

Key Features of the dragonfly discovery (DFD):

- True positive displacement technology
- Able to dispense a wide range of fluids and volumes, upwards from 200nL, with no changes to the instrument settings
- Accurate and reliable, ~5% CV for the majority of liquid classes in all microplate densities
- Non-contact dispensing
- Minimal dead volume reducing reagent wastage
- Disposable tips and reservoirs
- 96-, 384- and 1536-well microplate capability
- Simple file format allows third party software integration



Manual vs. dragonfly®

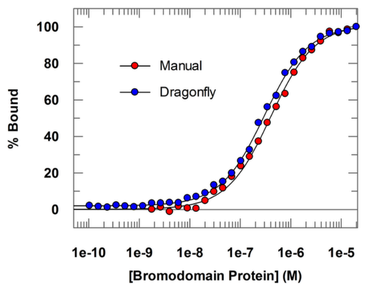


Figure 4. Protein titration binding curves using two differentmethods;manual pipetting and the dragonfly® discovery.

Method	Capacity	Kd (nM)	Background
Manual	100 ± 1	400 ± 20	0.1 ± 1
DFD	99 ± 0.5	300 ± 10	2 ± 0.3

Feature	Manual	dragonfly
Microplate density	384-well	1536-well
No. of test conditions	24	32
No. of replicates	3	8
Experiment preparation	Serial dilution	Discrete gradient
Experiment time	~1 hour ~1mL	~20 minutes
Reagent dead volume		280uL

Ligand Competition

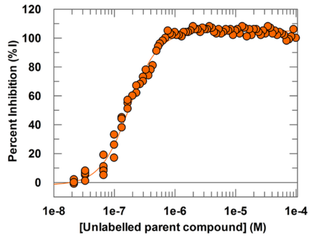


Figure 5. An IC50 curve for labelled and unlabelled ligands in direct competition for the protein target.

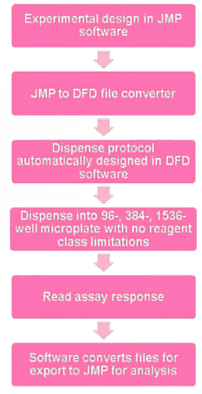
Adirect competition experiment was performed using the optimized FP assay and the unlabelled parent of the fluoro-ligand.
The dragonfly® discovery was used to dispense the protein and fluoro-ligand solutions and an HP digital dispenser was used to dispense the parent molecule.

Parameter	Y Range	IC 50	Slope Factor	Background
Value	105 ± 2	180 ± 5	-2 ± 0.1	-2 ± 2

Based on existing data, the expected IC50 was between 150 and 300nM.

dragonfly® discovery- Design of Experiment Integration

The application of Design of Experiment (DoE) and the dragonfly discovery (DFD) to the development of this TR-FRET assay allowed for the optimization of the components of the buffer to be used in experiments, a process that is not usually performed due to of its challenging complexity.



Complex, randomised, multi-factorial experiment designed in 1536-well microplate format

jmp Statistical Discovery.™ From SAS.

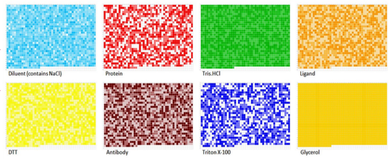


Figure 6. Colourized plate dispense protocols for an 8 component buffer optimization, screenshots taken from DFD protocol design software.

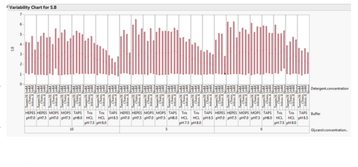


Figure 7. The JMP data analysis output from an experiment to optimize buffer salts based on signal to background ratio. Peaks in the graphs denote those combinations that worked best according to the test parameters.

FP Assay Screening Data

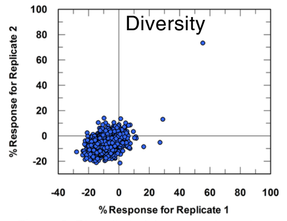


Figure 8. Diversity training screening set replicate correlation (1,408 compounds).

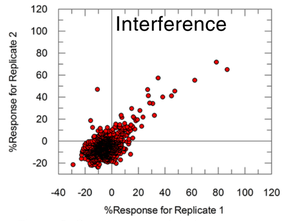


Figure 9. Common assay interference set replicate correlation (~900 compounds).

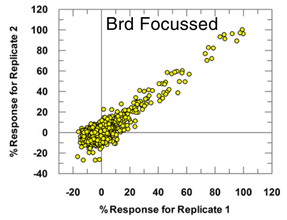


Figure 10. Focused bromodomain target set replicate correlation (~900compounds).

Data Statistic	Diversity	Interference	Brd Focussed
Mean (% I)	- 6	- 5	- 0.5
Standard deviation (%I)	5	7	6.6
% Inhibition cut-off	10	16	19.5
Hit rate	0.5	2.4	7.3
Mean Z'	0.7	0.65	0.76

Excellent assay performance, low interference and identification of known Brd inhibitors

Conclusions

Successful implementation of Design of Experiment and the dragonfly® discovery into a newmode of assay development and validation:

- Expansion of high throughput technology – reduction in assay time, labour and reagent requirements through full 1536-well microplate development
- Increased efficiency – maximising and prioritising data collection and reducing cycle times by combining Design of Experiment and the dragonfly® discovery
- Increased reliability – the ability of the dragonfly® discovery to dispense a wide range of solutions and volumes rapidly and accurately
- Decreased assay transfer "bottlenecks" – utilisation of the same liquid handling technology throughout the assay development and screening process
- High quality screening data – Brd FP assay data demonstrates good assay quality and reproducibility