Improve the Integrity of Your Fragment Library for Fragment-Based Drug Discovery

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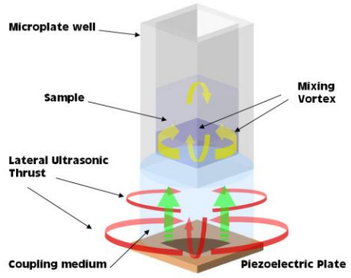
Abstract

Fragment-based drug discovery (FBDD) is gaining recognition for its many advantages over high-throughput screening including better hit-to-lead rates and broad chemical space for possible compound scaffolds. Due to the low affinity of fragments to the biological target, fragment libraries may contain weak hits and therefore scientists screen fragment libraries at concentrations as high as 200 mM. Even though many fragments have high solubilities in DMSO, environmental shocks introduced by DMSO hydration or repeat freeze/thaw cycles can cause compounds to crash out of solution, and consequently affect the accuracy of FBDD screening results. To address this issue, Microsonic Systems developed the HENDRIX SM100 Ultrasonic Fluid Processor specifically for solubilization, thawing, and mixing. Using the HENDRIX SM100, compound precipitates can now be solubilized across an entire microplate or tube rack simultaneously in just a few minutes. In this poster, we present the outcome of a solubilization study. We also look at how ultrasonic fluid processing improves assay results.

Technology Overview

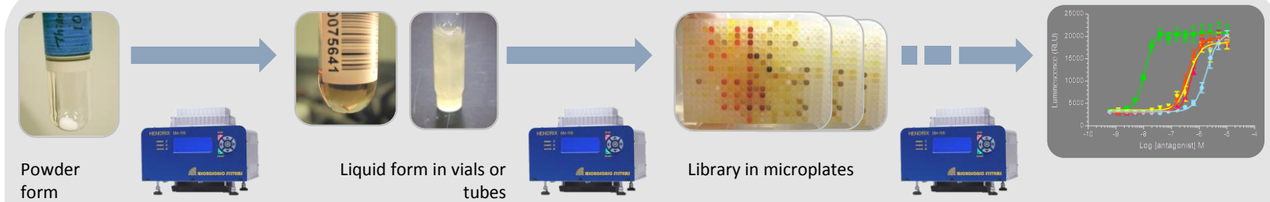
Microsonic Systems' patented Lateral Ultrasonic Thrust™ (LUT™) technology works by using a Micro-Electrical-Mechanical Systems (MEMS) based transducer, which when excited with RF power generates ultrasonic waves. These ultrasonic waves pass into the sample as broad beams of acoustic energy. The energy creates regions of strong Lateral Ultrasonic Thrust that in turn creates strong mixing in the form of a rapid vortex. LUT technology, unlike other ultrasonic methods, does not cause cavitation. At high power, LUT technology can be used for solubilization and thawing applications, and at low power, the same technology can be used for assay mixing or bead suspension.



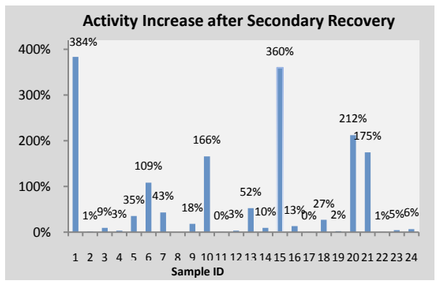
The **HENDRIX SM100 Ultrasonic Fluid Processor** utilizes LUT technology to solubilize compounds and recover precipitated samples. The same system is also used for HTS assay mixing, thawing frozen tubes and plates and bead suspension applications.

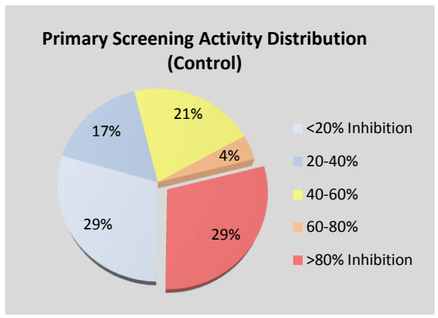
The HENDRIX SM100 comprises a Fluid Processor Unit (FPU) and a Base Control Unit (BCU). The FPU processes samples in various densities, from 24-vial & 96-tube racks to 3456-well formats. The BCU houses the main control system and a Peltier chiller for keeping the coupling fluid at a constant temperature.

Secondary Recovery: Maintain Compound/Fragment Concentration throughout the Process



Changes in Fragment Library Primary Screening Activity





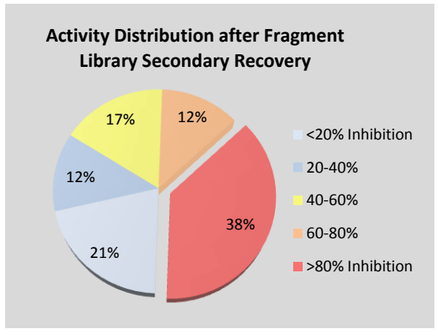


Figure 1. Secondary Recovery: Using the HENDRIX SM100, we re-solubilized precipitated samples back into solution in DMSO. Twenty-four samples were then sent to primary screening, and the % inhibition results were compared to the control group (no ultrasonic processing). As shown in the bar chart, fragments put through the HENDRIX resulted in activity up to 384% higher when compared to the control group.

Figure 2. In the control experiment, 29% of the screened fragments showed less than 20% inhibition (light blue), 17% of the group showed 20-40% inhibition (blue), 21% of the group showed 40-60% inhibition (yellow), 4% showed 60-80% inhibition (orange) and 29% showed high activity with greater than 80% inhibition (red).

Figure 3. In the secondary recovery experiment, we used the HENDRIX SM100 system to re-solubilize fragment samples. With precipitated samples recovered back into solution, the primary screening accuracy increased. This resulted in 38% of the screened fragments showing high activity with greater than 80% inhibition (red), 12% with 60-80% inhibition (orange), and 17% with 40-60% inhibition (yellow).

Reduced IC_{50} Values

Table 1. We compared the IC_{50} values of twelve samples before and after the secondary recovery ultrasonic process. As shown in the table below, the IC_{50} values of all the test fragments left-shifted to lower concentrations; those with more than two digits in % reduction are highlighted in yellow.

Sample #	Original IC_{50} Value (M)	IC50 Value (M) after Secondary Recovery	%Reduction in IC_{50} Value
1	2.45E-06	2.93E-07	88%
2	3.87E-07	4.64E-08	88%
3	7.60E-08	2.28E-09	97%
4	5.60E-08	5.60E-10	99%
5	1.00E-09	9.90E-10	1%
6	3.00E-09	1.68E-09	44%
7	3.45E-07	2.24E-07	35%
8	1.77E-06	1.73E-06	2%
9	8.76E-07	8.67E-07	1%
10	2.00E-09	1.24E-09	38%
11	2.55E-07	2.24E-07	12%
12	8.77E-06	2.80E-06	68%

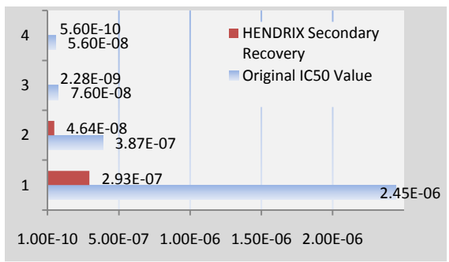


Figure 4. Fragment samples 1, 2, 3 and 4 showed significant changes in IC_{50} values after secondary recovery, in particular, the IC_{50} value of sample 4 shifted from 5.60E-08 M (56 nM) to 5.60E-10 M (0.56 nM). This indicated that the potencies of these fragment samples were much stronger than originally thought.

Summary

We used the HENDRIX SM100 ultrasonic fluid processor to re-solubilize precipitated samples back into solution before performing fragment library primary and secondary screening, in a process termed secondary recovery. The outcomes of the experiments are summarized here:

1. In primary screening, fragments put through the HENDRIX resulted in higher activity. Comparing to the control group, we observed up to a 384% activity increase.
2. Higher activity in primary screening also generated more leads at a higher quality for secondary screening; for example, the >80% inhibition group expanded from 29% to 38% of the screened fragments.
3. We observed a reduction in IC_{50} values, with many samples showing significant differences. One sample originally had an IC_{50} value of 56 nM, and after secondary recovery process, the IC_{50} value was found to be 0.56 nM.
4. This indicated that the potencies of these fragment samples were much stronger than originally thought.

Conclusion

Fragment libraries are screened at much higher concentrations than small molecule libraries due to their low affinity to biological targets. This makes fragment libraries more susceptible to precipitation, and consequently affects the accuracy of screening results. The data presented in this poster shows that the HENDRIX SM100 ultrasonic fluid processor can be used to recover the precipitated fragment samples back into solution, and thus increase both the sample concentration and the accuracy of the screening results. After putting the fragment library through secondary recovery using the HENDRIX system, we observed higher % inhibition in primary screening and lower IC_{50} values in secondary screening.

By adding secondary recovery into the drug discovery process, the quantity and quality of primary screening leads can be improved.

Acknowledgment

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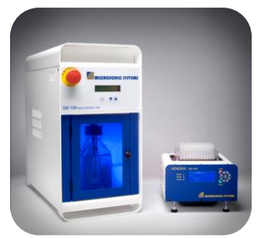

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