BD Biosciences - Discovery Labware

LRIG Mid Atlantic Chapter

Poor Aqueous Solubility and Compound Aggregation: Detection, Differences, and impact on In-Vitro Screens

Feb 2nd, 2006

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Presentation Outline

- Propose Measurement of Particle Formation as an Effective Method for Evaluating compound availability in In-Vitro Screens.
- Challenges Presented by Poor Aqueous Solubility & Compound Aggregation
- Rational for Simultaneous Measurement of Aggregates and Precipitation
- Particle Detection Method Overview
- Sample Data showing the impact of particle formation on In-Vitro activity data.
- False positives are caused by compound aggregates
- Need for higher throughput solubility and aggregation detection methods
    - Available Technologies, strengths and weaknesses

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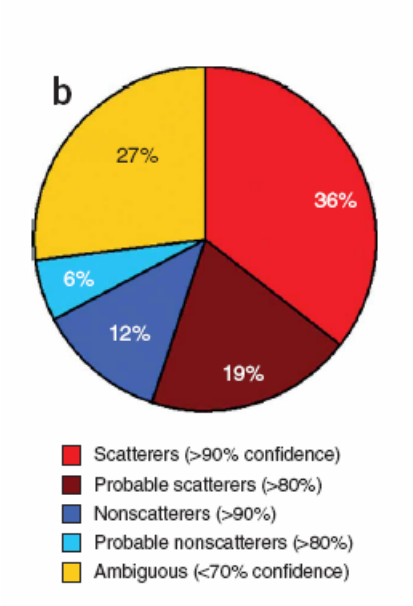
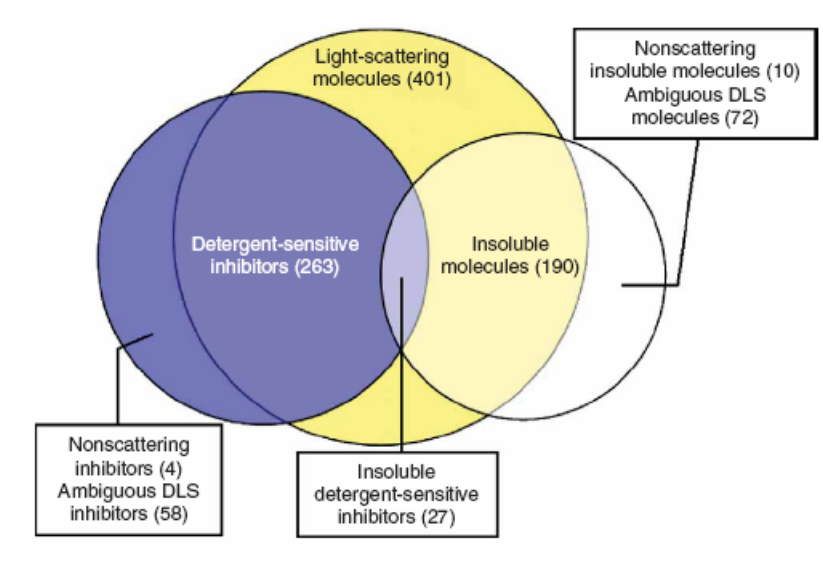
Prevalence of Particle Formation in Aqueous Assays

In a recent publication, Brian Feng (from Shoichet's group) investigated the frequency of promiscuous inhibitors in HTS libraries…. [Nature Chem Bio, volume 1, number 3, Aug 2005]



55% of compounds form particles in the randomly selected group (about 300 compounds) > 80% confidence Compounds @ 30 µM



Comparison of "light scattering" and "detergent sensitive" screens. Precipitation accounts for a significant percentage of "scattering" compounds

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Challenges Presented by Poor Aqueous Solubility & Compound Aggregation:

- **Poor aqueous solubility is a major issue for new drug candidates**
  - **40-50% of compounds in typical libraries have an aqueous solubility < 100uM**
  - **>50 % of compounds form Particles @ 30 µM (a large fraction of these are aggregating compounds)**
  - **Compound Aggregation and early Precipitation are kinetically driven and not well reflected by Thermodynamic Solubility assays.**
- **An enormous amount of time and money is spent in secondary screening to qualify the potency, activity, and properties of drug candidates**
  - **IC50 curves to estimate inhibition kinetics**
  - **Enzyme kinetics for metabolic screening**
  - **Absorption assays**
  - **Toxicity screens (HERG)**
- **The accuracy of the data coming from these assays depends on adequate compound solubility**
  - **Poor solubility results in inaccurate activity data**
  - **Poor solubility confuses ADME and Tox Screening results (false negatives and positives can result)**

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Solubility and Aggregation: Rational for Simultaneous Measurement

- **Both Solubility and Aggregation result in Decreased Effective Compound Concentrations In Vitro.**
  - **Precipitation (even early precipitation) results in decreased effective compound concentration**
  - **Formation of compound aggregates also limits the effective compound concentration (even if the compound does not “fall out of solution”).**
- **Both particle formation phenomenon can be measured by direct methods**
  - **TEM**
  - **Dynamic Light Scatter**
  - **Flow light Scatter**
  - **others**
- **It would be advantageous to distinguish Aggregation from Precipitation because of the added negative consequences of aggregates**
  - **Aggregates can directly cause False Positives, but aggregation itself does not rule out specific activity (this is true for detergent sensitivity as well).**
  - **Detection methods that do not use additional compound and have adequate throughput would be favored**

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Method Overview:
Particle Detection and Characterization using the BD Gentest™ Solubility Scanner:

- **High-Throughput Flow Particle Analyzer (8 seconds per well)**
- **Microplate-based, 96- and 384-well**
- **Laser excitation: 635nm (3mW)**
- **High Sensitivity: < 0.2 µM**

Applications include…

- **Aqueous solubility for *in vitro* assay concentration qualification**
- **Detection and confirmation of compound aggregation to reduce false positives**

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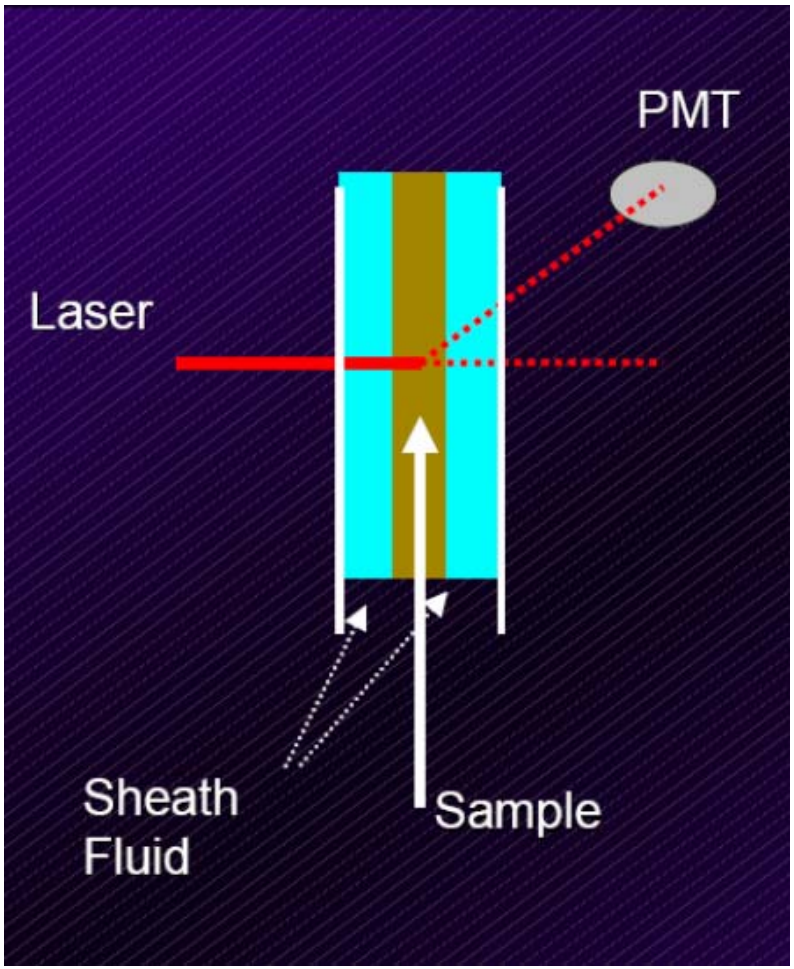
Particle Isolation using Flow Dynamics



- **The sample is mixed and then injected through a flow cell.**
- **The sample is carried through the flow cell by a secondary “carrier” liquid (sheath fluid).**
- **The pressurized sheath fluid narrows the sample stream (to less than 6 µm) so that *individual particles pass through the laser beam one at a time*.**
- **PhotoMultiplier tube collects photon signatures for each particle (90 degree scatter). The amount of light scattered is proportional to the size of the particle.**

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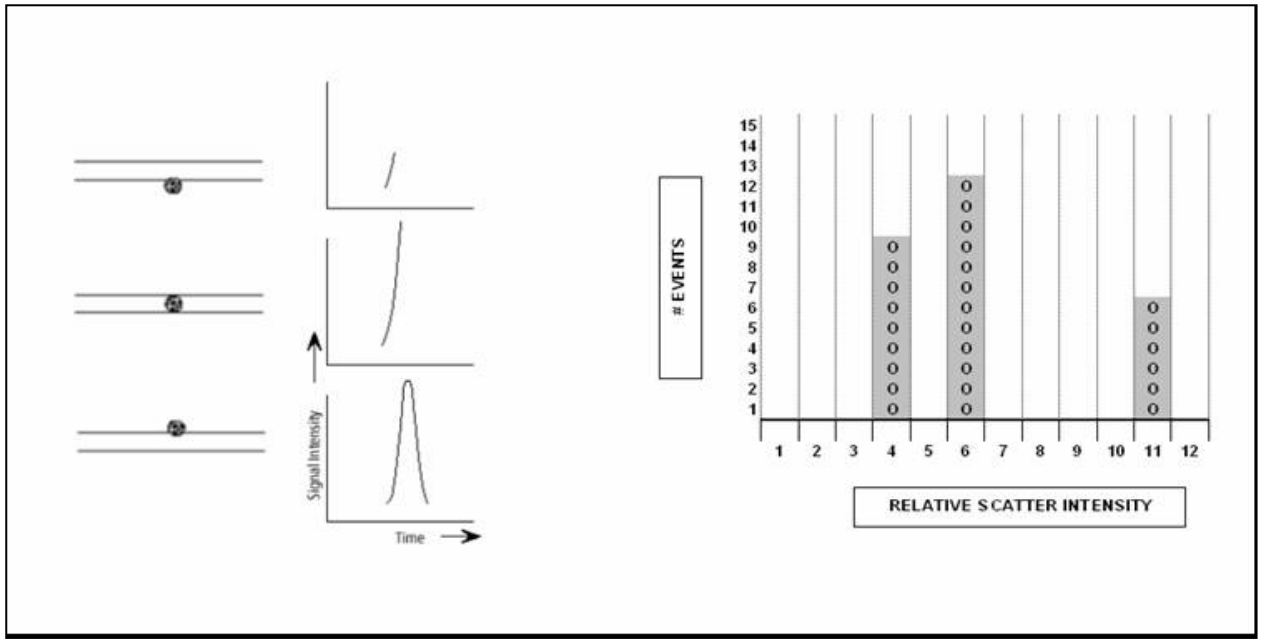
Data Processing Overview



The photon signatures are processed and saved in electronic (histogram) data files for each sample. This mock histogram shows 12 channels of increasing intensity. The BD Gentest™ Solubility Scanner saves 256 channels per sample. The resulting particle intensity distribution reflects both the number of particles detected and the particle size.

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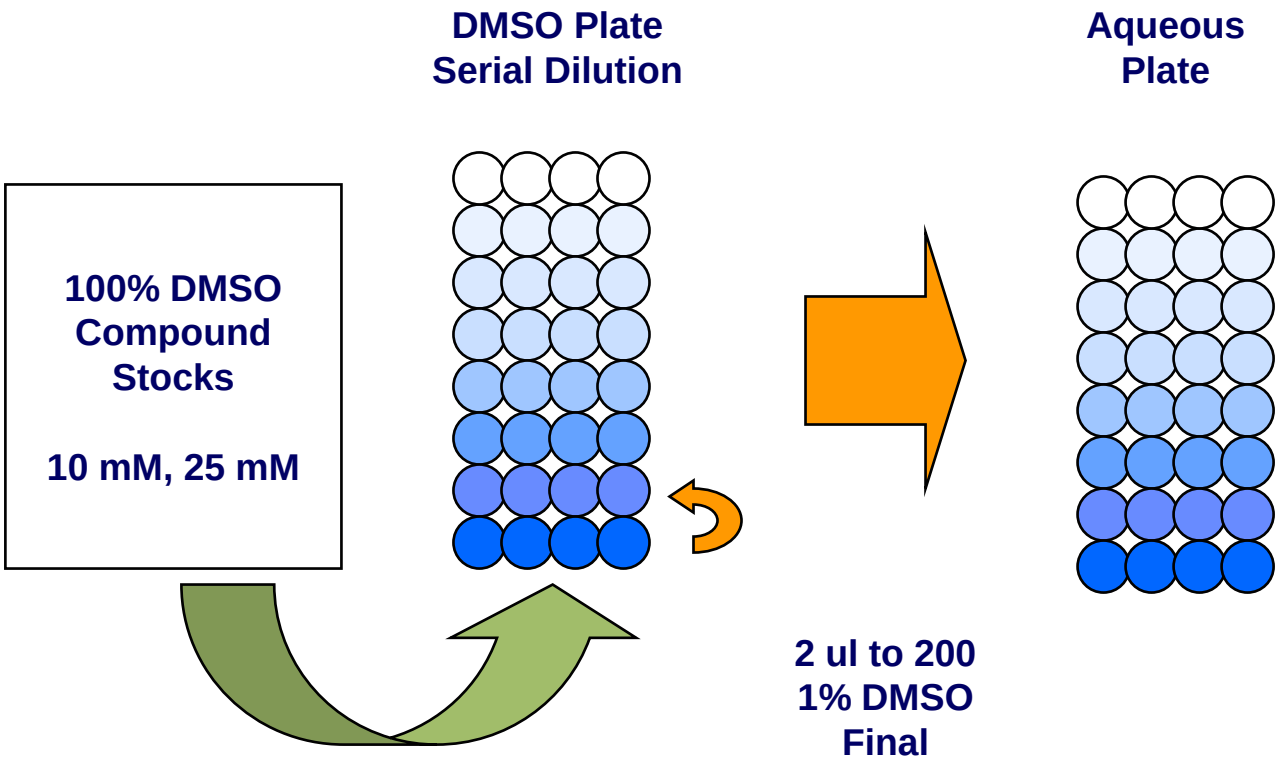
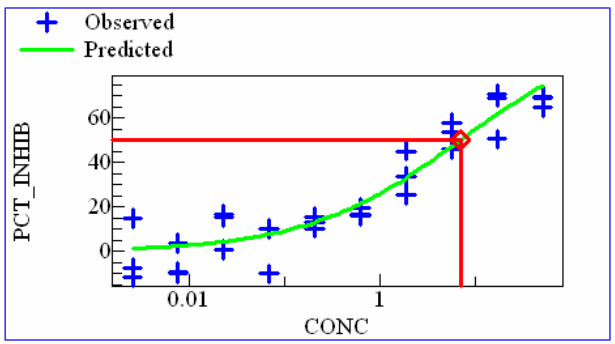
Typical Sample Preparation Protocol for Assay Qualification – Mimic Assay Prep Protocol



Impact of Particle Formation on IC_{50} curves
Sample Data using BD Gentest™ Solubility Scanner:

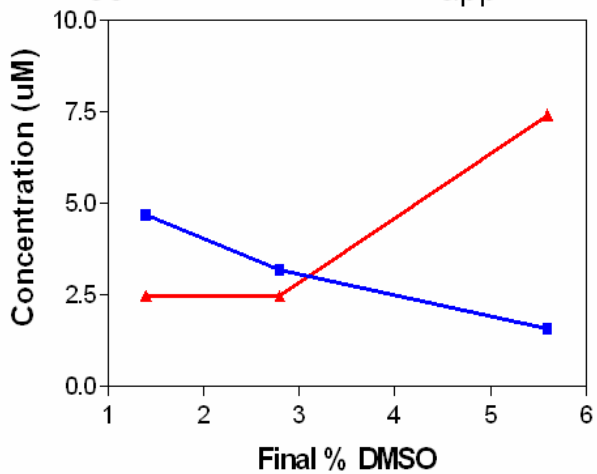
% DMSO	IC50 (uM)	Apparent Solubility (uM)
5.6	1.56	7.41
2.8	3.18	2.47
1.4	4.67	2.47

Assay at 2% DMSO



Low Maximum % inhibition

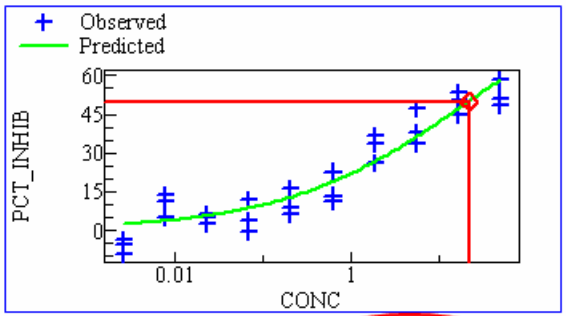
IC_{50} (■) versus S_{app} (▲)



Impact of Particle Formation on IC_{50} curves Sample Data using BD Gentest™ Solubility Scanner:

% DMSO	IC50 (uM)	Apparent Solubility (uM)
5.6	8.72	7.41
2.8	19.08	2.47
1.4	20.26	0.82

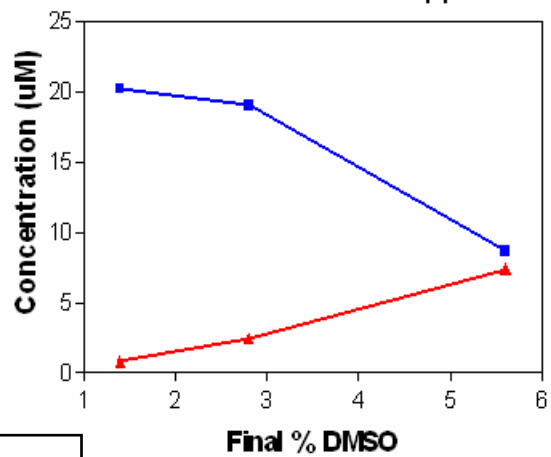
Assay at 2% DMSO



IC_{50} = 21.91 uM

Hill = 0.41

IC_{50} (■) versus S_{app} (▲)

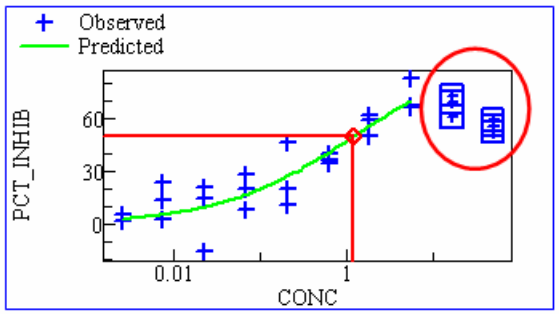


Low Max % inhibition & Low Hill

Impact of Particle Formation on IC_{50} curves Sample Data using BD Gentest™ Solubility Scanner:

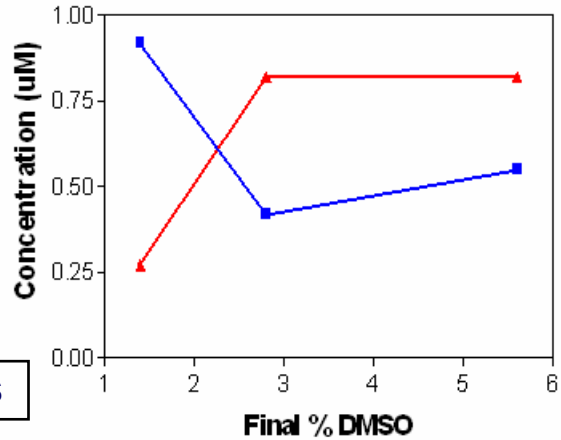
% DMSO	IC50 (uM)	Apparent Solubility (uM)
5.6	0.55	0.82
2.8	0.42	0.82
1.4	0.92	0.27

Assay at 2% DMSO



Outliers @ high concentrations

IC_{50} (■) versus S_{app} (▲)



Verification of CYP inhibition w/ HT Solubility

Substance	Apparent Solubility		Substance	Apparent Solubility	
	Plate 1	Plate 2		Plate 1	Plate 2
BMS-xxx256 Aq	1.41	1.41	BMS-646256 PBS	0.94	0.94
			BMS-xxx256 Bz	0.94	0.94
			BMS-xxx256 BFC	0.94	0.94
BMS-xxx652 Aq	11.25	11.25	BMS-xxx652 PBS	3.75	3.75
			BMS-xxx652 Bz	3.75	3.75
			BMS-xxx652 BFC	3.75	3.75
BMS-xxx523 Aq.	2.8	2.8	BMS-xxx523 PBS	**0.47**	**0.47**
			BMS-xxx523 Bz	**0.94**	**0.94**
			BMS-xxx523 BFC	**0.47**	**0.47**

Background: BMS-xxx523 demonstrated no CYP inhibition activity while related cmpds such as BMS-xxx256 and BMS-xxx652 showed significant CYP inhibition (sub uM activity in 2C9 and 2C19 and mid uM (3-5uM) in 2C9, 2C19 and 3A4-BFC respectively).

Question: Is '523 free of CYP inhibition or is solubility causing a false negative?

Conclusion: Results indicate that potent CYP inhibitors (<0.5uM) would be able to be detected with little to no interference from solubility issues.

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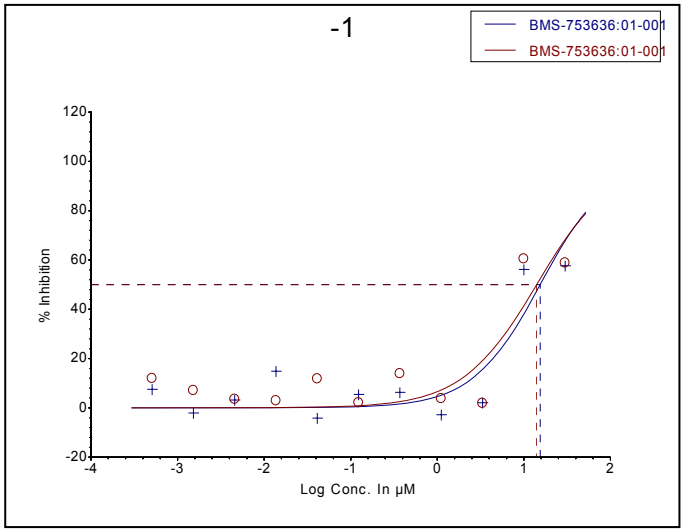
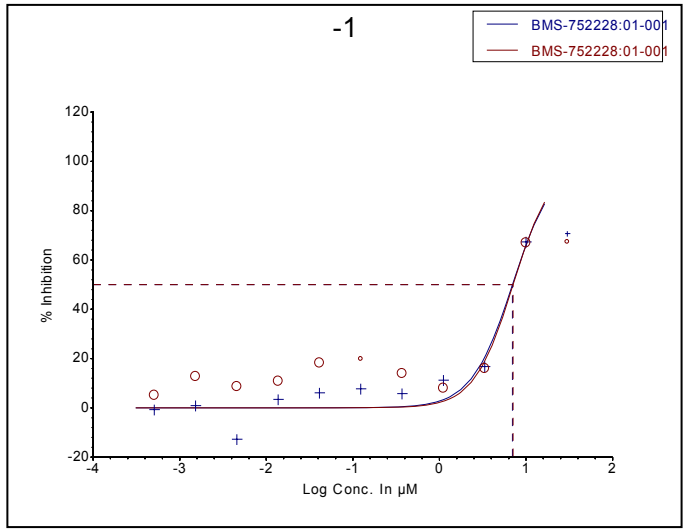
Biological and Solubility Data of Compounds in a DWG Assay

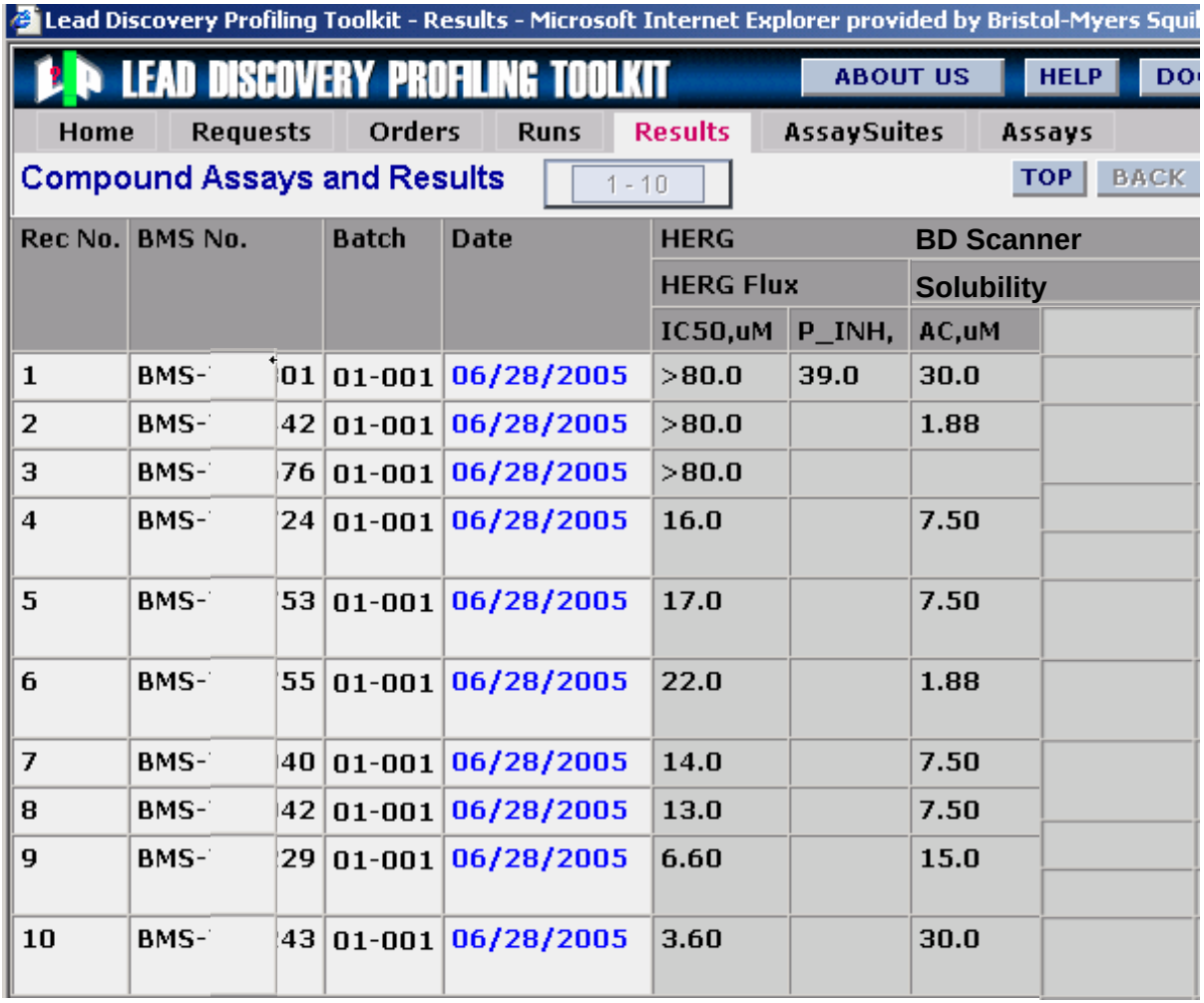




	Ki (µM)	IC_{50} (µM)	Hill	Observed Max. at 10 and 30 µM	Solubility (µM)
BMS-1	~4.4	~14.7	1.1	59%	6.6
BMS-2	~2.1	~7.0	0.9	67%	6.6

Low Ymax observed.
Poor Aqueous Solubility (BD Scanner) is contributing to the Low Ymax.

Qualification of Tox (HERG) Data with HT Flow Solubility Measurements



Lead Discovery Profiling Toolkit - Results - Microsoft Internet Explorer provided by Bristol-Myers Squi

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Compound Assays and Results 1 - 10 TOP BACK

Rec No.	BMS No.	Batch	Date	HERG		BD Scanner
				HERG Flux		Solubility
				IC50,uM	P_INH,	AC,uM
1	BMS- 01	01-001	06/28/2005	>80.0	39.0	30.0
2	BMS- 42	01-001	06/28/2005	>80.0		1.88
3	BMS- 76	01-001	06/28/2005	>80.0		
4	BMS- 24	01-001	06/28/2005	16.0		7.50
5	BMS- 53	01-001	06/28/2005	17.0		7.50
6	BMS- 55	01-001	06/28/2005	22.0		1.88
7	BMS- 40	01-001	06/28/2005	14.0		7.50
8	BMS- 42	01-001	06/28/2005	13.0		7.50
9	BMS- 29	01-001	06/28/2005	6.60		15.0
10	BMS- 43	01-001	06/28/2005	3.60		30.0

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Aggregation Overview: Particle Formation in Aqueous Solutions Can Cause False Positives

Compound aggregates were first characterized in a academic publication out of Northwestern University in Chicago. The paper was published in 2002….

"A Common Mechanism Underlying Promiscuous Inhibitors from Virtual and High-Throughput Screening"

Susan L. McGovern,‡ Emilia Caselli,‡,§ Nikolaus Grigorieff,⊥ and Brian K. Shoichet*,‡
J. Med. Chem., 45, 1712 (2002)

"High-throughput and virtual screening are widely used to discover novel leads for drug design. On examination, many screening hits appear non-drug-like: they act noncompetitively, show little relationship between structure and activity, and have poor selectivity. Attempts to develop these peculiar molecules into viable leads are often futile, and much time can be wasted on the characterization of these "phony" hits."

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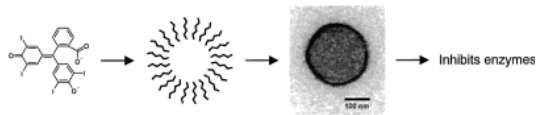
Additional Negative Effects of Compound Aggregates

- **Aggregates show activity (appear to be hits) in high-throughput screens initially.**
- **Follow-up testing confirms that although there was apparent activity against the target (enzyme) in the initial screening assay, the activity was not specific for the enzyme.**
- **Proposed Mechanism:**



Bottom line is that FALSE POSITIVES cost drug developers weeks and sometimes months in development time and, therefore, significantly increase the overall cost of the development process.

If aggregation is causing the activity in the screen (inhibition) then the compound has no specific activity against the target and should not be selected as a drug candidate.

Low solubility compounds, on the other hand, may have specific activity for the intended target, and may be valuable drug candidates.

Therefore, the earlier aggregate-based false positives can be detected and eliminated the better.

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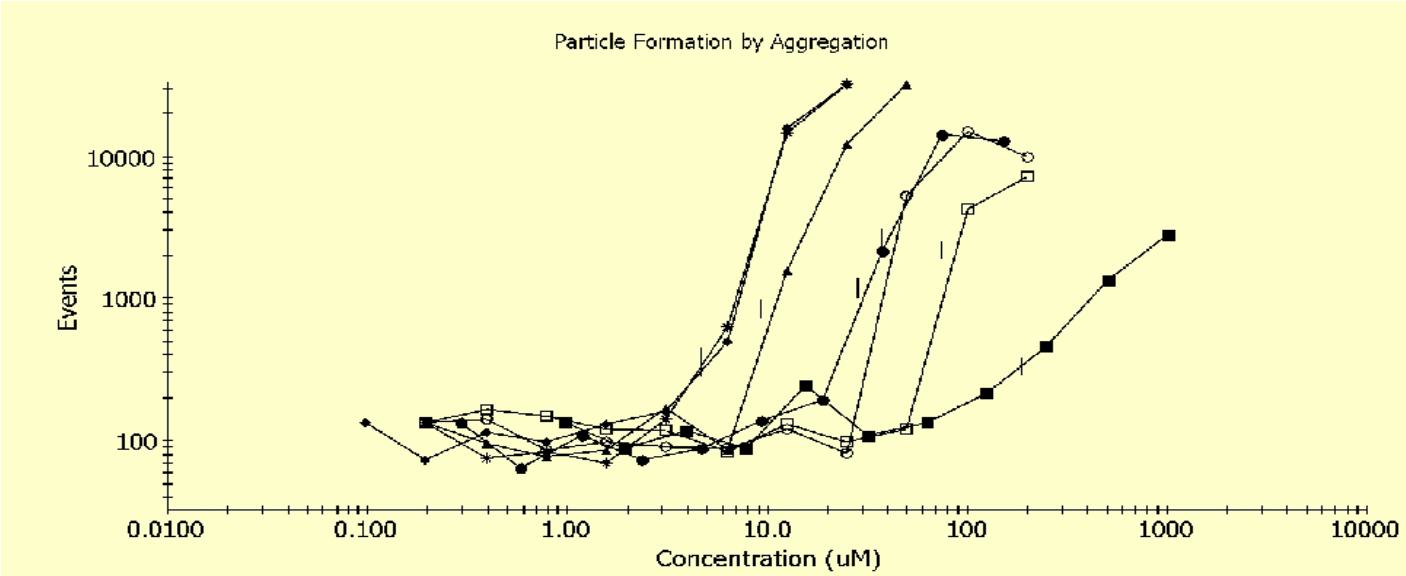
Aggregates are particles and can be detected by the BD Gentest™ Solubility Scanner

- **Compound aggregates form at micro-molar concentrations and can be similar in size to compound precipitate.**



		Compound	Particle For...	Region	pH	Assay Detail	Time
■	——	Mefenamic ...	188.0	Total	7.2	NA	01:00:00
○	——	Econazole	37.5	Total	7.2	NA	01:00:00
◆	——	Miconazole	4.69	Total	7.2	NA	01:00:00
●	——	Nicardipine	28.1	Total	7.2	NA	01:00:00
□	——	Quercetin	75.0	Total	7.2	NA	01:00:00
✳	——	Sulconazole	4.69	Total	7.2	NA	01:00:00
▲	——	TIPP	9.38	Total	7.2	NA	01:00:00

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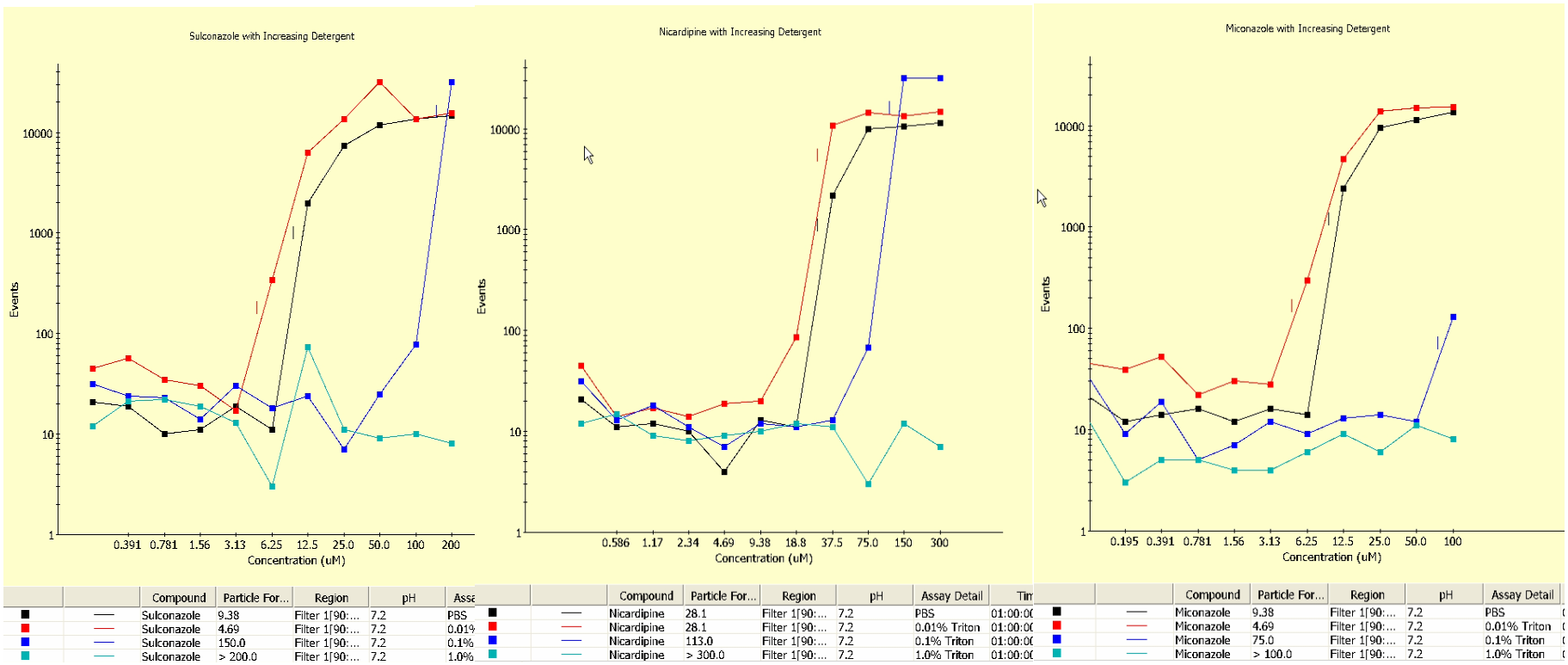
Aggregate Confirmation Assays on BD Gentest™ Solubility Scanner: Aggregate Disruption by Detergents

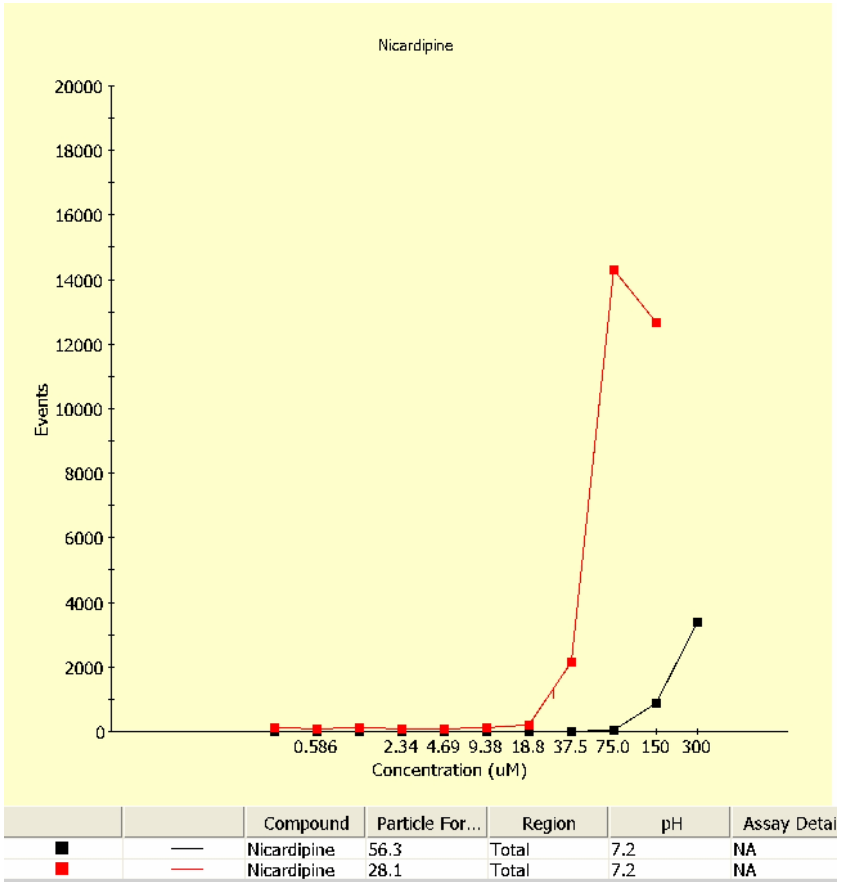


		Compound	Particle For...	Region	pH	Assa
■	—	Sulconazole	9.38	Filter 1[90:...	7.2	PBS
■	—	Sulconazole	4.69	Filter 1[90:...	7.2	0.01%
■	—	Sulconazole	150.0	Filter 1[90:...	7.2	0.1%
■	—	Sulconazole	> 200.0	Filter 1[90:...	7.2	1.0%

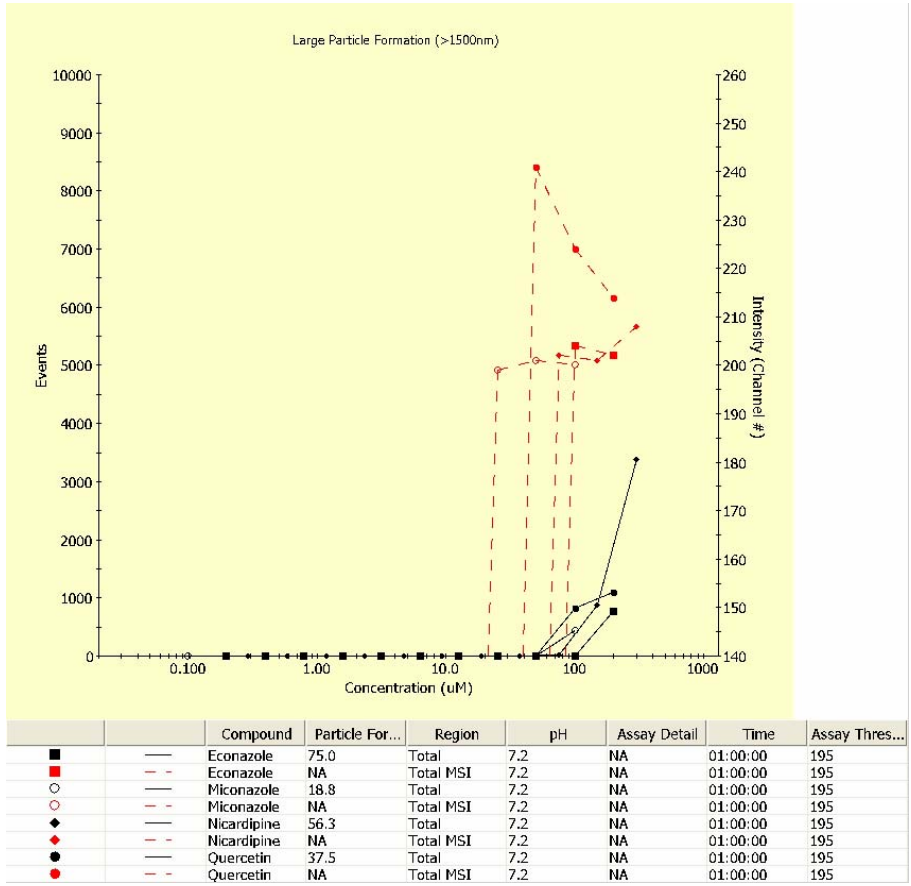
		Compound	Particle For...	Region	pH	Assay Detail	Tim
■	—	Nicardipine	28.1	Filter 1[90:...	7.2	PBS	01:00:0(
■	—	Nicardipine	28.1	Filter 1[90:...	7.2	0.01% Triton	01:00:0(
■	—	Nicardipine	113.0	Filter 1[90:...	7.2	0.1% Triton	01:00:0(
■	—	Nicardipine	> 300.0	Filter 1[90:...	7.2	1.0% Triton	01:00:0(

		Compound	Particle For...	Region	pH	Assay Detail
■	—	Miconazole	9.38	Filter 1[90:...	7.2	PBS
■	—	Miconazole	4.69	Filter 1[90:...	7.2	0.01% Triton
■	—	Miconazole	75.0	Filter 1[90:...	7.2	0.1% Triton
■	—	Miconazole	> 100.0	Filter 1[90:...	7.2	1.0% Triton

Aggregate Confirmation Assays on BD Gentest™ Solubility Scanner: Large Particle Intensity Distribution

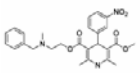


		Compound	Particle For...	Region	pH	Assay Detail
■	——	Nicardipine	56.3	Total	7.2	NA
■	——	Nicardipine	28.1	Total	7.2	NA

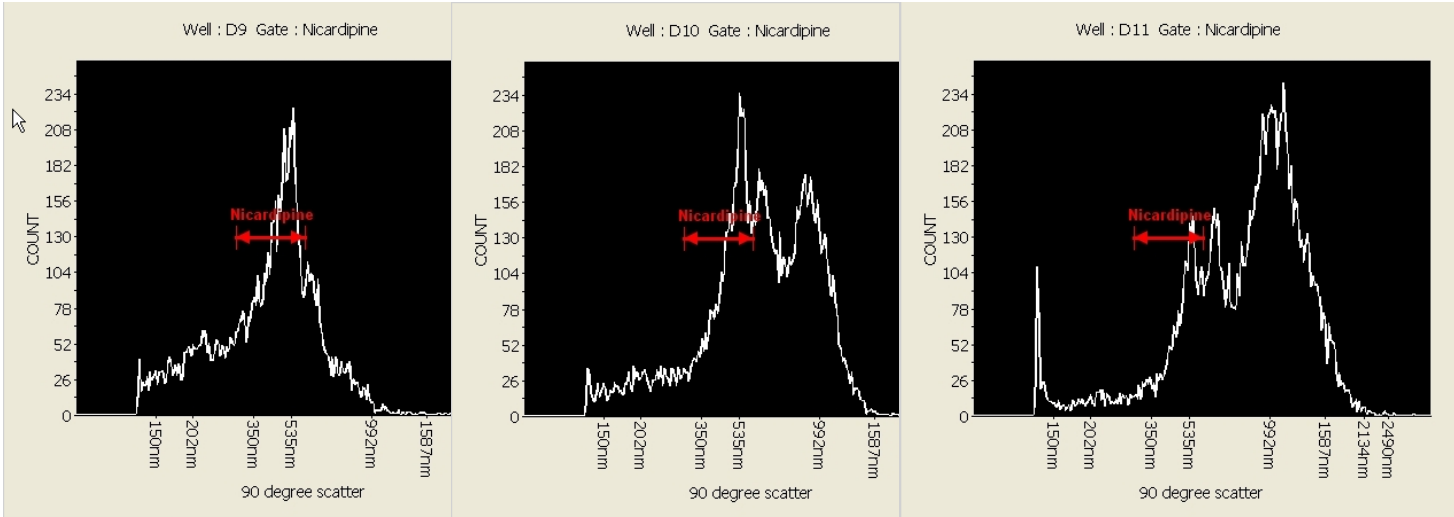


		Compound	Particle For...	Region	pH	Assay Detail	Time	Assay Thres...
■	——	Econazole	75.0	Total	7.2	NA	01:00:00	195
■	– –	Econazole	NA	Total MSI	7.2	NA	01:00:00	195
○	——	Miconazole	18.8	Total	7.2	NA	01:00:00	195
○	– –	Miconazole	NA	Total MSI	7.2	NA	01:00:00	195
◆	——	Nicardipine	56.3	Total	7.2	NA	01:00:00	195
◆	– –	Nicardipine	NA	Total MSI	7.2	NA	01:00:00	195
●	——	Quercetin	37.5	Total	7.2	NA	01:00:00	195
●	– –	Quercetin	NA	Total MSI	7.2	NA	01:00:00	195

Aggregate Characterization on BD Gentest™ Solubility Scanner: Particle Intensity Distribution



Nicardipine



Low	High	Events	Mean	% Total	CV	Peak	Pea
89	127	4673	111.711	54.87%	9.271	121.0	223

Low	High	Events	Mean	% Total	CV	Peak	Pea
89	127	4140	114.656	34.09%	8.53	120.0	235

Low	High	Events	Mean	% Total	CV	Peak	Peak Value
89	127	2308	115.658	17.67%	8.264	122.0	144

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Key Distinctions for Aggregate Detection Methods

1. **Dynamic light scatter, classical light scatter and plate nephelometry all measure particles in bulk solutions.**
2. **Only the flow scanner method creates an individual photon signature for each particle evaluated, resulting in…**
    - **Much faster measurement**
    - **Increased sensitivity to lower particle concentrations**
    - **Ability to restrict measurement to distinct particle size ranges**
    - **Handle more complex buffers and solutions**
    - **Quickly analyze large and small particle formation for aggregate confirmation**
    - **Rapid visualization of complex particle population distributions**

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Higher Throughput Methods Needed for Solubility and Aggregate Detection and Differentiation

- **Traditional Thermodynamic Solubility Measurements (longer duration, equilibrium methods)**
  - **Do not have adequate throughput to handle the number of compounds that undergo early ADMET or biological screens**
  - **Do not detect compound aggregates**
  - **Are not designed to provide relevant short-term solubility data for assay qualification**

Nephelometry (microplate, traditional)

- **Cannot measure particle size**
- **Cannot effectively distinguish aggregates from precipitates**
- **Cannot measure solubility using consistent particle size cut-off**

High Throughput Flow Particle Analysis (BD Gentest™ Solubility Scanner)

- **8 Seconds per well, 10 min read time for 96-well plate**
- **Uses particle size to measure solubility under assay-specific conditions**
- **Can detect compound aggregates and be used to distinguish aggregation and precipitation**

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Conclusions

1. Poor Solubility and compound Aggregation are significant problems in drug discovery and development (starting with DMSO dissolved compound stocks).
2. Solubility and Aggregation are both particle formation phenomenon.
3. Poor aqueous solubility and compound aggregation can both impact the amount of compound available in assays. Particle formation data can greatly help the interpretation and accuracy of *in vitro* screens.
4. Compound aggregates can also directly interact with ligand-enzyme binding interactions and cause false positives.
5. The BD Gentest™ Solubility Scanner can be used to quickly and definitively measure compound solubility and aggregation.
6. More detailed particle characterization experiments can be run on the BD Gentest™ Solubility Scanner to distinguish aggregates from compound precipitation.
7. Particle detection and characterization studies can be run at relatively high rates to meet the needs of assay qualification and secondary screens and can often be run on the same plates used in the initial binding experiments.

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BD Gentest™ Solubility Scanner

BD Gentest™ Solubility Scanner Specifications

- **Plate format** 96- and 384-well
- **635 nm diode laser** 3 mW
- **PMT** true 90 degree light scatter
- **Sensitivity** <0.2 microMolar
- **Throughput** 7 seconds/well (<10 min 96-well plate)
- **Compound usage** 0.35 µl or less of 10 mM stock
- **Sample volume** 25 µl/read
- **Detection method** Light scatter of individual precipitation events
- **Processing method** 256 channels light scatter
- **Automated Mixing** Individual well mixing
- **Analysis** Real-time scatter processing
- **Warranty** 1 year parts and labor
- **QC** Automatic sample stream alignment monitoring

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For More Information on Today's Webinar or the BD Gentest™ Solubility Scanner

Please e-mail your questions to:

solubility_scanner@bd.com