DynaPro Plate Reader to Examine Non-Specific Small Molecule Aggregation

Promiscuous non-stoichiometric inhibition can be a serious problem when screening libraries of compounds in a high-throughput format. According to one widely accepted model (McGovern *et al, J. Med. Chem.* 2003) promiscuous inhibition is connected to the formation of small micelle-like aggregates of the compound. A rapid method of detecting such compound behavior would greatly facilitate screening hits selection.

In order to develop such a method, we have utilized the DynaPro Plate Reader to examine the light scattering properties of a dilution series of known aggregating inhibitors.

We created a plate with a three-fold compound dilution series in duplicate along with two DMSO (non-compound) controls. We chose a 96-well format (Greiner Bio-One 96 SensoPlate) with a sample volume of 100μl.

Each well contained 99μl of 50 mM potassium phosphate buffer, pH 7 (filtered through a 0.2 μm filter) and 1 μl of either DMSO or the small molecule dilution series. The plate was subjected to a brief centrifugation prior to reading, to remove bubbles. Because of the heterogeneous nature of the samples, we used short reads with more replicates to allow for averaging of the population.

As expected, the general trend was that the compound dilution caused a reduction in the scattering signal intensity (Fig. 1). When examining the amplitude of the autocorrelation function, we observed an increase in the amplitude with increasing concentration of small molecules. As the concentration of a compound decreased below some threshold, signals reverted to buffer-like behavior (Fig. 2). An increase in normalized intensity was correlated with increasing small molecule concentration (Fig. 3).

In summary, we have successfully utilized the DynaPro Plate reader to examine the light scattering behavior of known promiscuous inhibitors. This approach can now be extended to a wider range of compounds and to study the effect of enzymes on aggregate formation.

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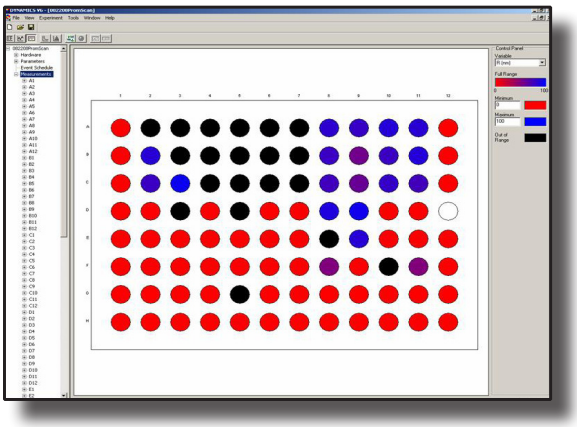

Figure 1 Plate layout and representative results. Lanes A-H are 3-fold serial dilutions of compounds starting at 100mM (row A). Lanes 1,12 DMSO controls; 2,3 Quercetin: 4, 5 Miconazole; 6,7 Clolrimazole, 8,9 Rottlerin; and 10, 11 I4PTH. Measurement of R, hydrodynamic radius in nm. Colors black, out of range; blue, fully saturated signal, graded to red, minimum signal; white, no sample/undetectable scattering signal.

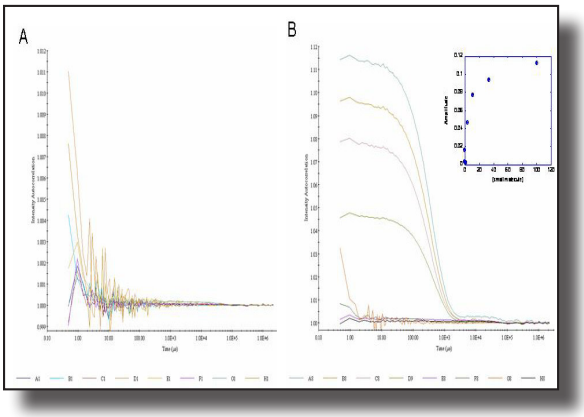

Figure 2 Comparison of Buffer to Rottlerin. A) Light scattering intensity autocorrelation for buffer with DMSO alone. B) Light scattering intensity autocorrelation for the dilution series of Rottlerin. The amplitude of the signal decreases with decreasing compound concentration (inset plot).

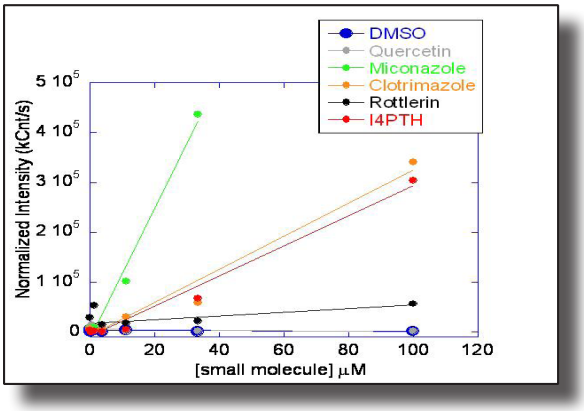

Figure 3 Correlation of Normalized Intensity and promiscuous inhibitor concentration.