MSD™ FOR HIGH-THROUGHPUT INTEGRIN INHIBITOR SCREENING

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INTRODUCTION

Several different technologies have been previously described that measure integrin:ligand binding in an isolated protein:protein assay format. These include ELISA, ORIGEN®, fluorescence polarization, and scintillation proximity assays. Each of these assay platforms has particular advantages, such as genericity in the case of ELISA and ORIGEN®, and throughput in the case of fluorescence polarization and scintillation proximity for example.

We have developed integrin:ligand binding assays using a new technology, the MesoScale™ Discovery (MSD™) platform. This technology is based on the properties of ruthenium (TAG™) electrochemiluminescence, as previously used in ORIGEN®, however the MSD™ technology is plate based rather than bead based. The significant advantage of the MSD™ platform is greatly increased throughput (>40 fold), placing MSD™ as a true HTS compatible technology. We describe the development, robustness, and reproducibility of this new platform for integrin:ligand binding assays.

MATERIALS AND METHODS

Proteins: Ligands used were expressed as GST fusion proteins in XL1-Blue *E.coli* cells according to standard procedures. Integrin proteins were either purchased from external suppliers or made recombinantly within GSK. TAG™-labelled streptavidin was purchased from MesoScale™ Discovery.

Plate Coating and Protein Labelling: Integrin proteins were directly absorbed to the carbon electrode that is the base of each well of MSD™ assay plate, according to supplied protocols. GST-Ligand was biotinylated using NHS-Biotin according to standard methods.

MSD™ Assays: Buffer used contained 25mM HEPES pH7.4, 150mM NaCl, 1mM $MnCl_2$, 1mM $MgCl_2$, 0.1mM $CaCl_2$, 0.5% BSA, 0.1% Tween-20, unless otherwise indicated, in a 25µL well volume. After 4 hours incubation, when optimal signal to noise is reached, 10µL MSD™ read buffer containing tripropylamine is added before reading.

ASSAY FORMAT

Our studies using MSD™ technology have concentrated on establishing generic assay formats applicable to multiple integrins. GST-tagged ligands are made in large quantities and have been used in the solution phase in a biotinylated form (bGST-Ligand). Integrin proteins are immobilised directly to MSD™ 384-well assay plates. The addition of TAG™-labelled streptavidin generates the readout for biotinylated GST-Ligand bound to integrin. A schematic of this assay format is shown in Figure 1 and is applicable to multiple integrins.

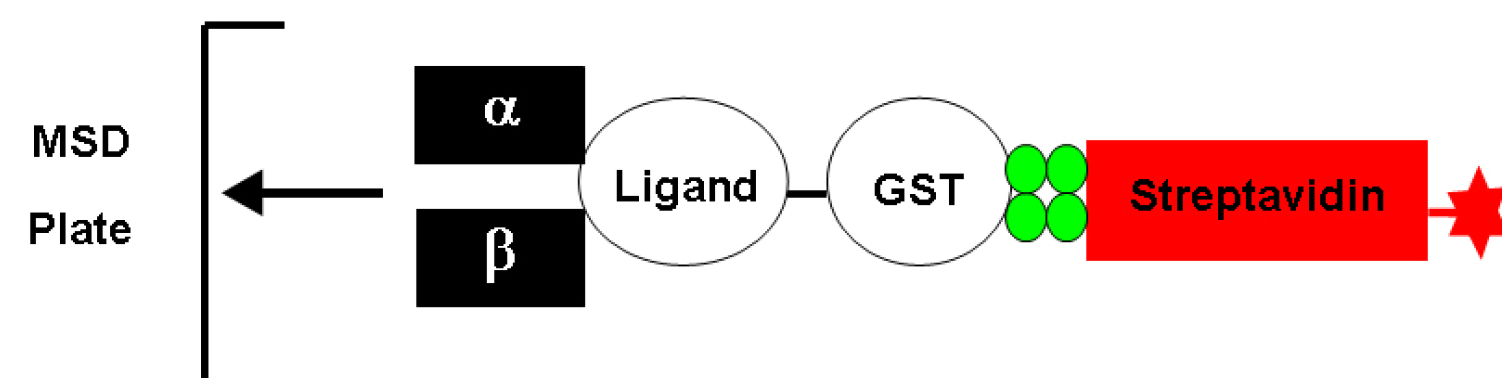

Figure 1 : MSD™ Assay Format

BASIC ASSAY PARAMETERS

Using the assay format shown in Figure 1, saturation binding experiments were performed by varying the concentration of ligand at fixed [Integrin]. Figure 2A shows data obtained from this type of experiment, where the assay generated a large signal with an average K_D of 22.5nM for 1.25,2.5, 5 and 10ng/well integrin protein. Subsequently assays were operated at fixed [bGST-Ligand] and [Integrin] and the properties of standard inhibitors investigated. For the integrin shown, the standard IC_{50} data were highly reproducible with a pIC_{50} of 7.89±0.10 over 32 determinations (Figure 2B). Similar data were obtained in other integrin assays.

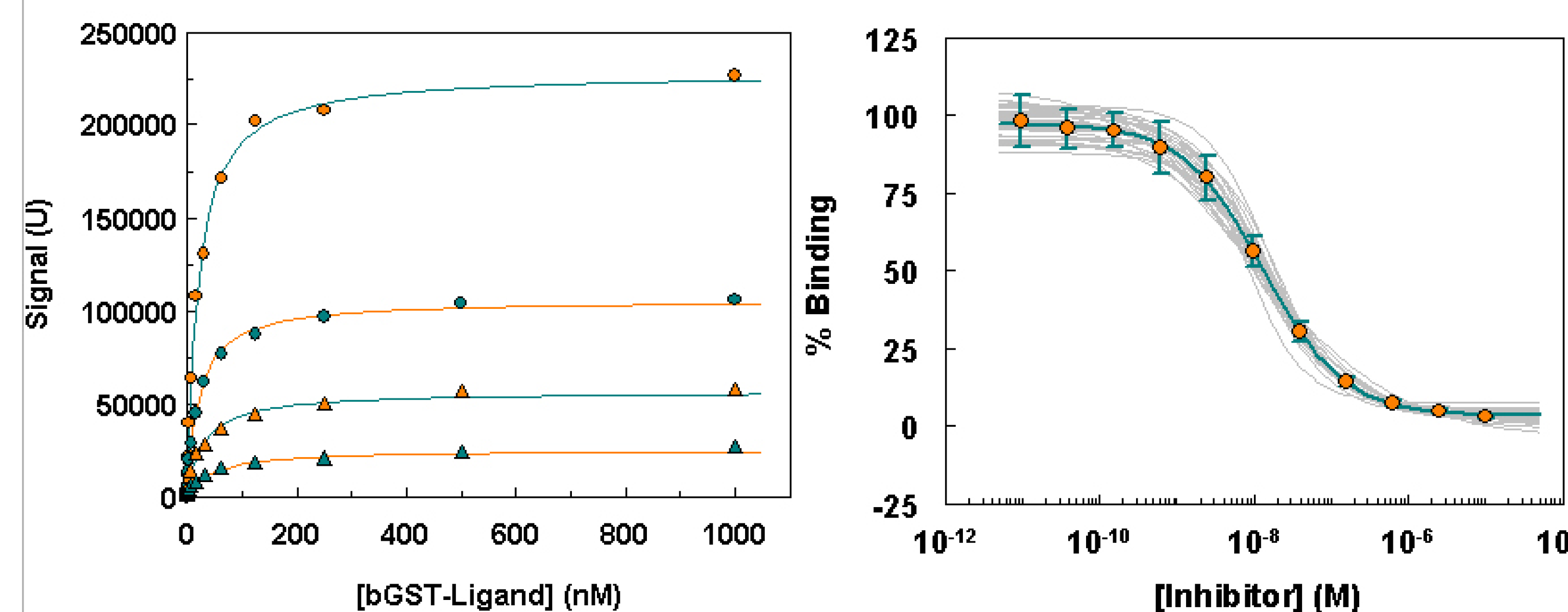

Figure 2A :

Saturation binding experiment of a Ligand to immobilised Integrin

Figure 2B :

Standard Inhibitor Reproducibility in an Integrin Assay

COMPOUND INTERFERENCE STUDIES

The susceptibility of various Integrin:Ligand assays to compound interference were assessed by testing the hit rate of an internal GSK compound set. The set was tested twice at 10µM and the data analysed (Figure 3). Plate Z' values were between 0.4-0.9 and all assays only detected (>50% inhibition) compounds known to inhibit the specific binding site of these integrins. Hence these assays are not hypersensitive to compound interference. The wavelength of the emitted chemiluminescence (~620nm) and the short detection distance between the carbon electrode and the TAG™ is presumed to remove many of the potential problems.

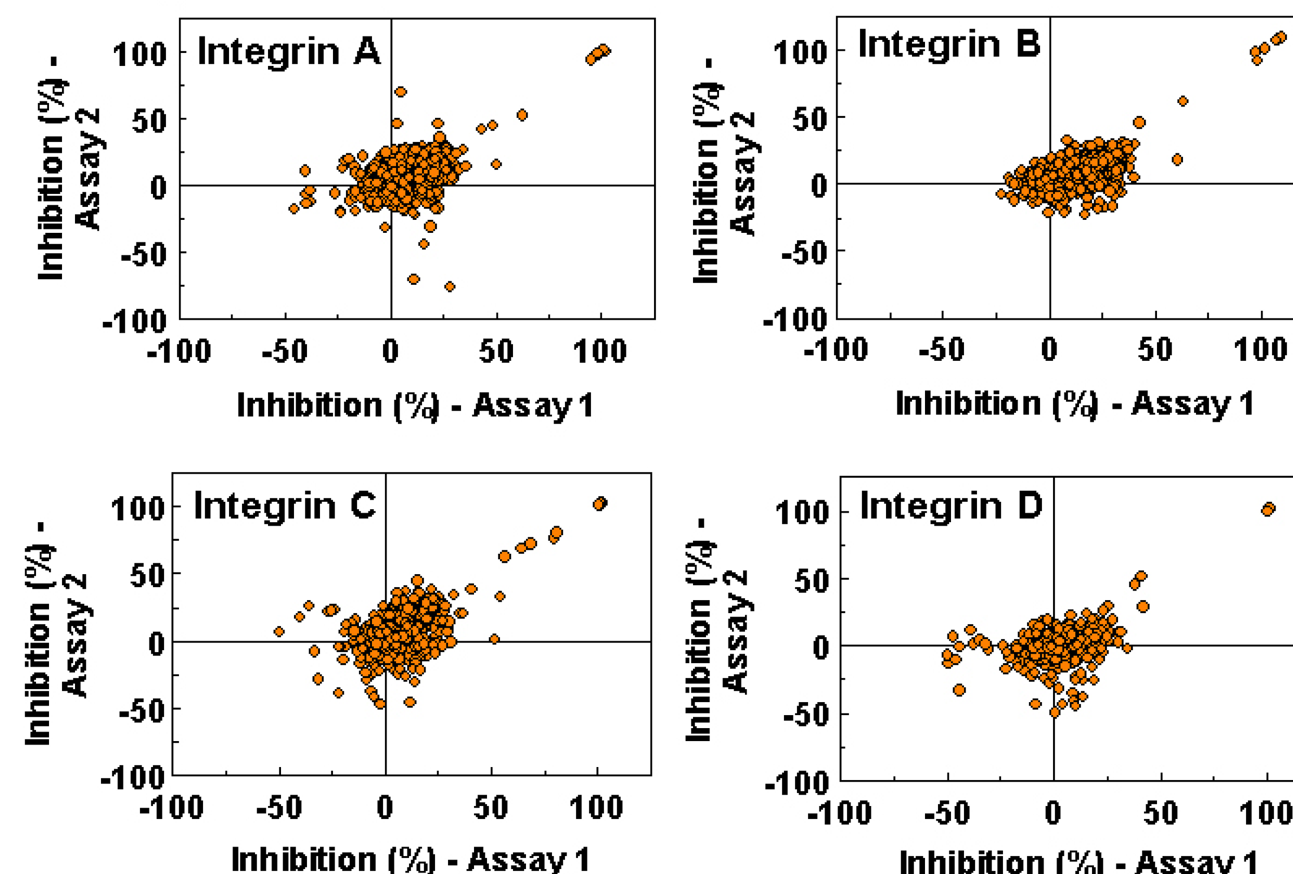

Figure 3 : Susceptibility of Integrin Assays to Compound Interference

FULL CURVE SCREENING

Compounds identified as active from single concentration screening in integrin assays are further investigated by screening in full curve mode to establish absolute potencies. The result of screening a set of compounds directed at integrin pharmacophores is shown (Figure 4). Duplicate assays correlated excellently across this entire pK_i range (4.7 - 10), with gradients of between 0.94 and 1.00 and correlation coefficients > than 0.96, demonstrating good reproducibility and agreeing well with data previously generated using other technologies.

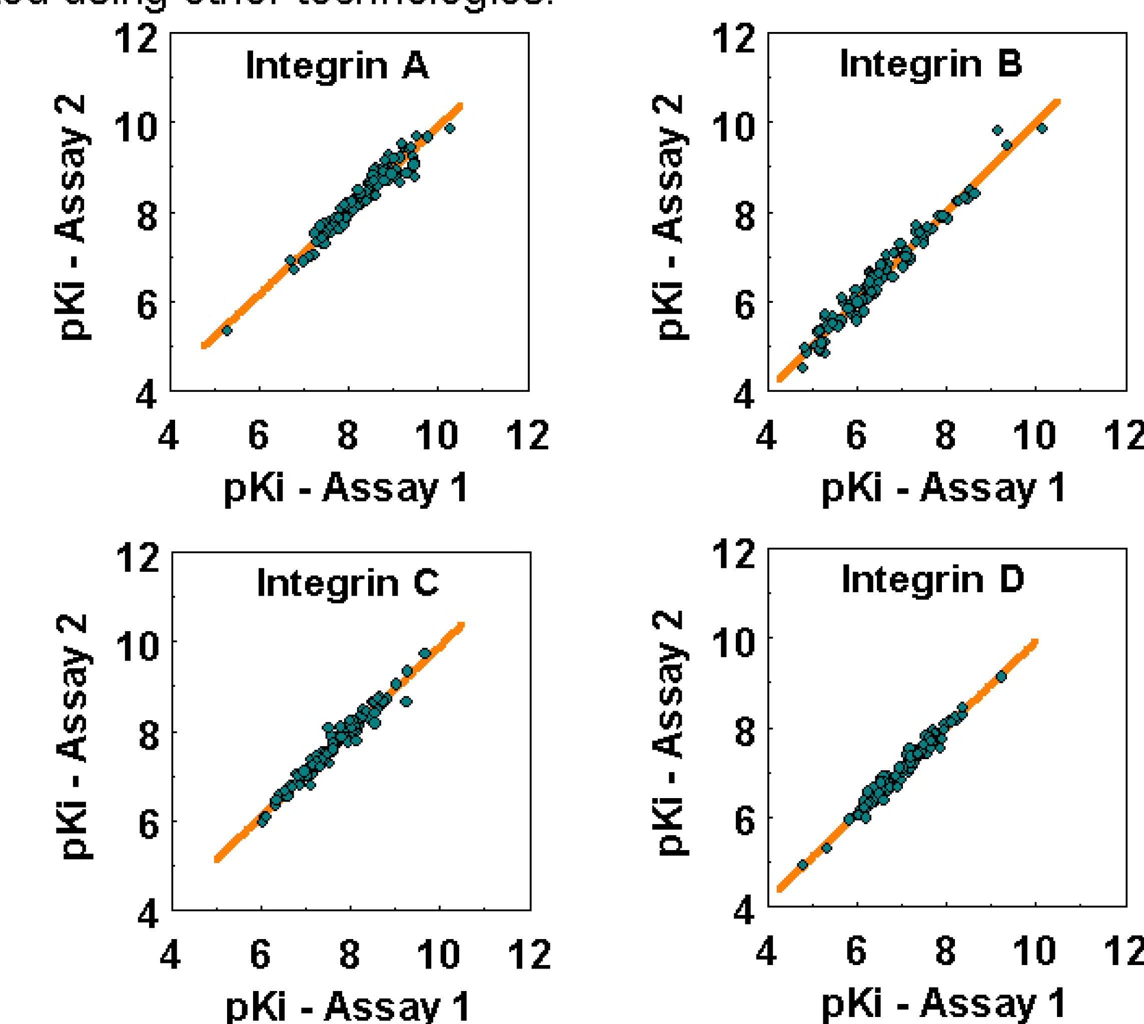

Figure 4 : Determination of Active Compound Potencies

DISCUSSION

MSD™ technology provides a superior platform for high-throughput identification of inhibitors of integrin:ligand interactions

- MSD™ allows HTS-scale throughput, enabled by an automated two-step assay procedure using the Biomek FX for compound handling and the Thermo Labsystems Multidrop for assay reagent addition
- MSD™ assay plates directly coated with protein eliminates the requirement for plate shaking whilst incubating and the plate reader being free of fluidics avoids the need to monitor system buffer and waste levels, therefore maximising the number of plates processed at any one time
- Assay reproducibility and reliability is high as shown by plate Z' values up to 0.9 and by excellent correlation between compound pIC_{50} values measured on separate occasions
- Assay demonstrates low susceptibility to compound interference
- MSD™ 384-well format assay and sensitivity permits conservation of valuable protein reserves
- MSD™ platform uses natural ligands

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