487g High Throughput Approach to Drug Discovery: Sars Coronavirus - a Case Study

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Chemical compounds within individual nanoliter droplets of glycerol were microarrayed onto glass slides at 400 spots/cm2. Using aerosol deposition, subsequent reagents and water were metered into each reaction center in order to rapidly assemble diverse multicomponent reactions without cross-contamination or the need for surface linkage. This proteomics technique allowed the kinetic profiling of protease mixtures, protease-substrate interactions, and high throughput screening reactions. A library of pharmacologically active compounds was microarrayed in triplicates on 100 slides and screened against 40 different proteases. Novel inhibitors were detected for a protease target implicated in the mechanism of action for entry of SARS-CoV (Severe Acute Respiratory Syndrome-associated Coronavirus), for which there is currently no effective treatment. The best protease inhibitor had an IC50 of 2.5 nM in solution. Inhibition of infection mediated by SARS-CoV Spike glycoprotein was quantified using single round lentiviral-based pseudotype entry assays into 293T target cells with luciferase reporter gene as a read-out. The best inhibitor had an IC95 of 2 mM. Similar results were seen on Vero E6 cells. Our method shows that from one printing run that consumes <1 nanomole of compound, large combinatorial libraries can be subjected to numerous separation-free, homogeneous assays at volumes 103 to 104 smaller than current high throughput methods.