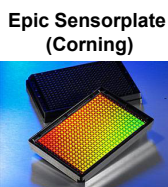


### Abstract

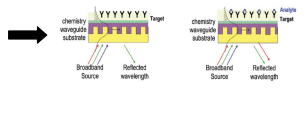
Drug discovery is a scientifically challenging field requiring constant development and optimization. One of the most important early drug discovery processes, HTS, has been established, optimized and automated over the last decade. Screening of >1.000.000 compounds is well feasible and yields 100s to 1000s of potential drug candidates. But follow-up activities show high attrition rates of the HTS hits and one limitation often is limited throughput of the decision relevant secondary assays. We are establishing processes to understand the biophysical mechanisms of binding and activity for our hit lists and drug candidates. Since years surface plasmon resonance (SPR) is used to characterize direct binding events of small molecule compounds on proteins (binding stoichiometry, KD, kon, koff, ...) and recently several related technologies with higher throughput capable to digest even larger hit numbers are evolving. Main focus is to assess the direct binding of hit compounds to target proteins and "SPR-like" compound binding assays do run as flow-channel systems or directly in microtiter plate-based systems. We have compared two very different "SPR-like" systems, the plate based Corning Epic system and the flow-channel based Bio-Rad ProteOn XPR36 system. A mini-screen and hit follow-up has been conducted to show strengths and weaknesses of both systems.

### The technologies: The Corning EPIC and the Bio-Rad ProteOn XPR36

#### Corning EPIC



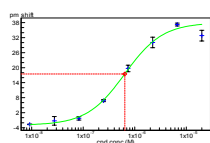
#### Principle



#### Plate Reader



"Yes/No" binding;  $K_D$

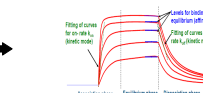
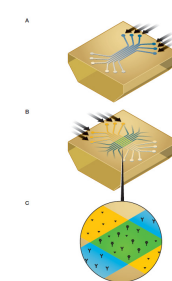


**Microplate**  
• 384-well format  
• Optical biosensor in each well (contains a signal and a reference area)  
• Surface chemistry

#### Bio-Rad ProteOn XPR36

#### SPR-Sensorchip (Bio-Rad)

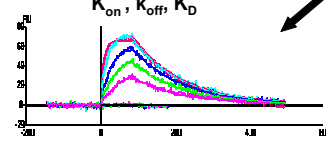
#### Principle



#### "Reader"



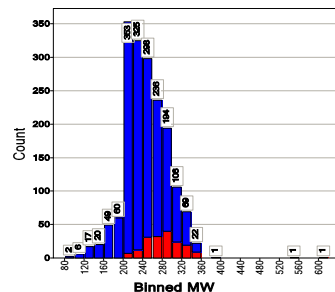
"Yes/No" binding;  
 $K_{on}$ ,  $K_{off}$ ,  $K_D$



Resonant Waveguide Grating Readout	
+	Fully compatible with HTS processes; 20x 384w assay plate in reader
-	The binding assay is prepared outside of the reader with 384w pipettors (performance risks, time consuming)
+	Each measurement is done in its own well (no analyte cross disturbance)
+/-	Reference zone does correct for some artifacts (DMSO effects, aggregation), but not all
+	Excellent screening throughput (immobilization, washing, soaking, analyte additions: 4-6 hours/assay, read time: 2 min per 384w plate; >>10000dp/day possible)
+/-	Sensitivity for low molecular weight binding detection lower than SPR, but sufficient
+/-	ca 1-3ug protein per data point (minimum 0.3mg per assay plate)
+	Data handling and evaluation can be easily automated and upscaled
-	Insoluble/Precipitating compounds lead to artifacts

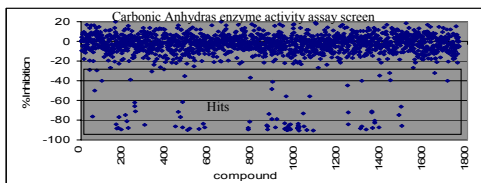
Surface Plasmon Resonance Readout	
-	2x 96w analyte plate in reader
+	The binding assay is performed inside the reader via injections from an analyte plate, all wash steps are done with a continuous flow system (easy to handle)
-	Consecutive injections on the same flow cell (analyte cross harming)
+	6 different analytes are measured on 6 different protein flow cells per injection (36 interactions at a time (very good to setup controls)
-	Decent throughput (immobilization ca 1 hour; analyte assay time 90-120 min per 96w plate; 500-600dp/day possible)
+	Sensitivity for low molecular weight binding detection is good
+	ca 0.2-1ug protein per data point (depends on number of injections; minimum 0.01-0.1mg per "assay")
-	Data handling and evaluation time consuming
+/-	Insoluble/Precipitating compounds can lead to artifacts

### "Mini" screen of a "fragment-derived" compound library on Carbonic Anhydrase II

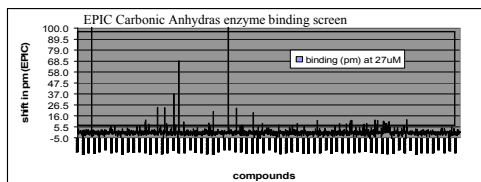


The tested compound library is a compilation of 1760 small molecules spiked with 67 sulfonamide-compounds, a well-known scaffold inhibitor class for Carbonic Anhydrase.

#### First "Round": Screening

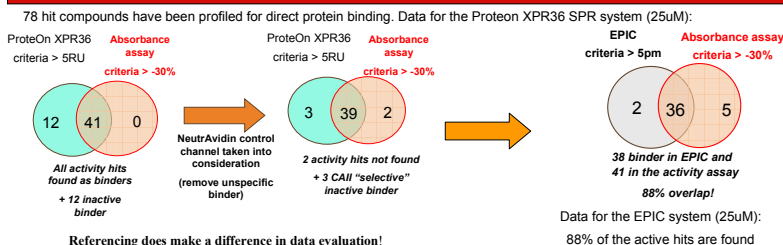


Set of 1760 compounds was tested in an enzyme activity assay as benchmark (27uM compound; 4-Nitrophenylacetate substrate; absorbance readout at 405nm incl baseline read)  
The "hit" criteria for the activity assay was set <-30% inhibition (>3Stdev): 89 primary hits

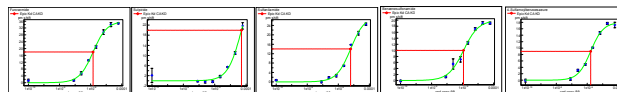


Set of 1760 compounds was tested on the Corning EPIC system at 27uM for binding.  
The "hit" criteria for the binding assay was set to >5.5pm shift (>3Stdev): 71 primary hits

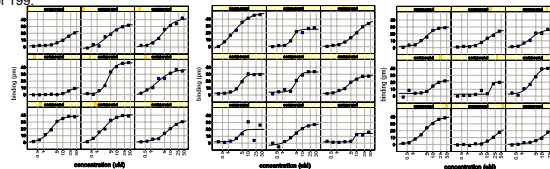
#### Second "Round": Technology comparison



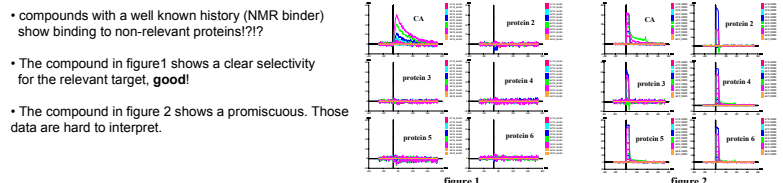
#### Third "Round": Dose response curves for KD determination



Published reference compounds do work reliable, even the weak inhibitor Sulpiride is found.  
35 compounds (27 active, 8 inactive) have been tested as dose response experiments up to 50 uM compound. All inactives show no binding, all actives show good dose response. The smallest and weakest binder identified has a KD of 50uM at a MW of 199.



#### Pitfalls, struggles and surprises...



### Summary

- Screening of higher compound numbers for direct compound to target binding is feasible with the EPIC system, but weak or very small hits on large proteins can get lost in the noise. The ProteOn XPR36 system has a better signal/noise ratio, but at far reduced throughput. Weaker compounds can be detected more reliably in the ProteOn system, on the other hand more replicates or dose response curve data can be handled in the EPIC system.
- Both technologies bare pitfalls, e.g. compound aggregation and insolubility can lead to misinterpretation of the data. Overloading of the surface with protein amount is not always helpful, it indeed can lead to pronounced unspecific compound binding.
- Both technologies are complementing each other in a project flow chart in terms of throughput, sensitivity, and application. The ProteOn can deliver kinetic data for binding, the EPIC system is suitable for label-free cellular screening and can handle even very large compound numbers.

### References

- [Tsafir Bravman, Vered Bronner, Kobi Lavie, Ariel Notovich, Giuseppe Papalia, David G. Myszkla] Exploring "one shot" kinetics and small molecules analysis using the ProteOn XPR36 array biosensor. Analytical Biochem 358 (2006), pages 281-288.
- [Fang J. Frutos A., Leder L., Ottl J.] Study of Small-Molecule/Protein Interactions using the Corning EPIC system, Scientific Poster for the MipTec Conference, May2007
- [Anthony M. Giannetti, Bruce D. Koch and Michelle F. Browner] Surface Plasmon Resonance Based Assay for the Detection and Characterization of Promiscuous Inhibitors. J.Med.Chem. 2008, 51, 574-580