SPR imaging and the use of antibody and protein microarrays for diagnostics and drug profiling

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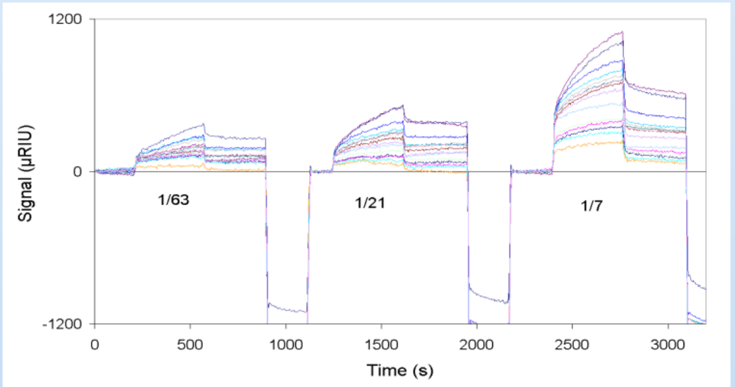
Abstract

We have used a label free imaging SPR system (Plexera PlexArray™) to analyze antibody microarrays for quantitative proteomics. Our goal was to detect several proteins in complex mixes such as serum. Samples from patients with liver cancer or non-liver cancer were tested for a panels of 396 proteins. At least 39 of these proteins show significant changes. The small amount of sample required by this technology can expand this application to diseases where the biological samples are difficult to obtain in large quantities. Our findings clearly show the advantages of using label free multiplexed SPR imaging and antibody microarrays to discover and quantify biomarkers as well as better antibodies in the diagnostic field. More recently, protein microarrays have been tested to look at antibody and small molecule profiling. This will dramatically increase the quality of these therapeutic molecules, reducing attrition rates due to off target hits and increasing efficacy. As a proof of concept, we show here that a small molecule inhibitor of the HIV-Integrase can be detected in a microarray format. New surface chemistry and immobilization strategies are being developed in order to increase sensitivity and functionality of these functional protein arrays. We believe that SPRi will significantly lower the cost of drug profiling and expand the binding assays to thousands of molecular targets.

Imaging, binding, and regeneration of an SPRi antibody array

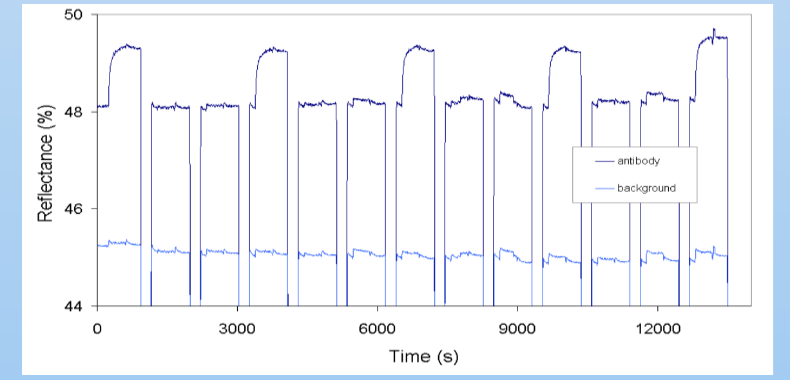
Raw SPR image of the792-feature microarray under PBS buffer

Eighteen rows and 22 columns are printed in duplicate on a gold-coated, high-index glass slide. Antibodies were diluted to 0.2-0.5mg/ml for printing.



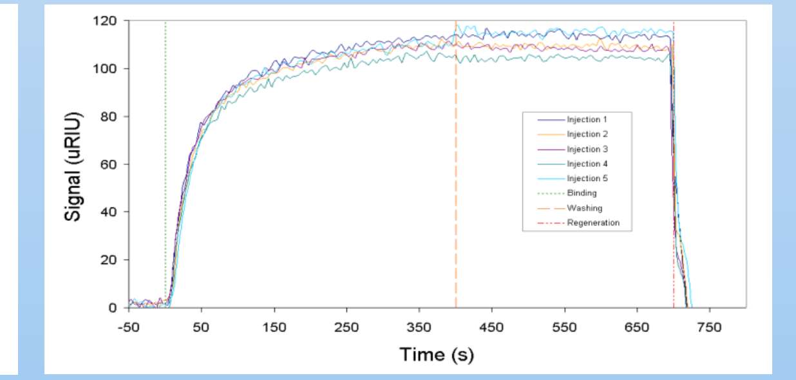
Three dilutions of serum analyzed on one surface

Binding curves showing three injections of the same sample at dilution factors 1:7, 1:21, and 1:63. Each injection cycle consists of binding, washing, and surface regeneration.



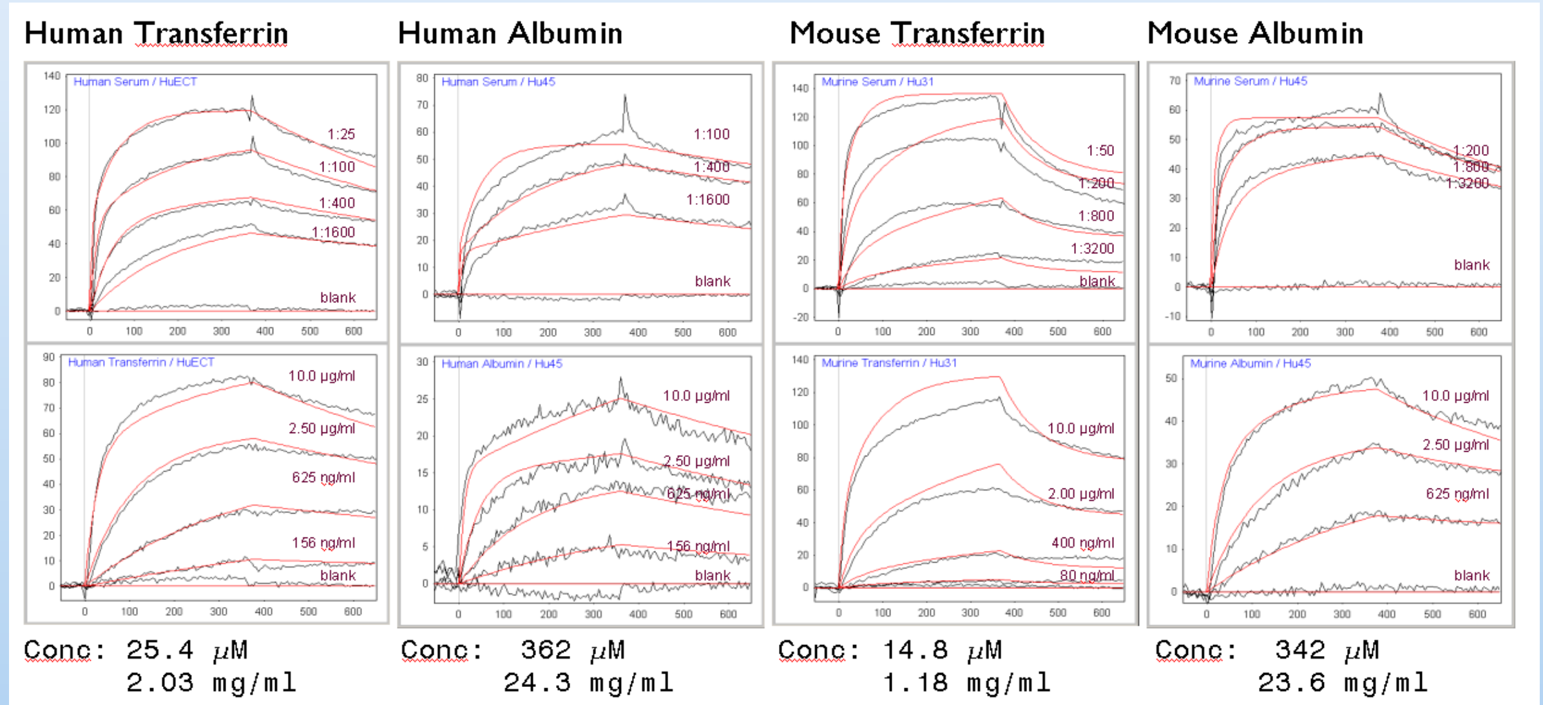
Regeneration of the antibody microarray surface (1)

The microarray was subject to 13 cycles of binding, washing, and regeneration over the course of four hours. Transferrin (66 nM in PBS) was injected once followed by two injections of blank sample (PBS). Raw reflectance data is plotted from an anti-transferrin feature and its local background.



Regeneration of the antibody microarray surface. (2)

Sensorgrams of binding from the five protein injections are overlaid. The signal is highly reproducible and no degradation in antibody performance is detectable. The binding signal is 109.0 +2.7 µRIU and the average pairwise correlation coefficient for the curves is 0.994.



Quantification of endogenous protein concentration in serum

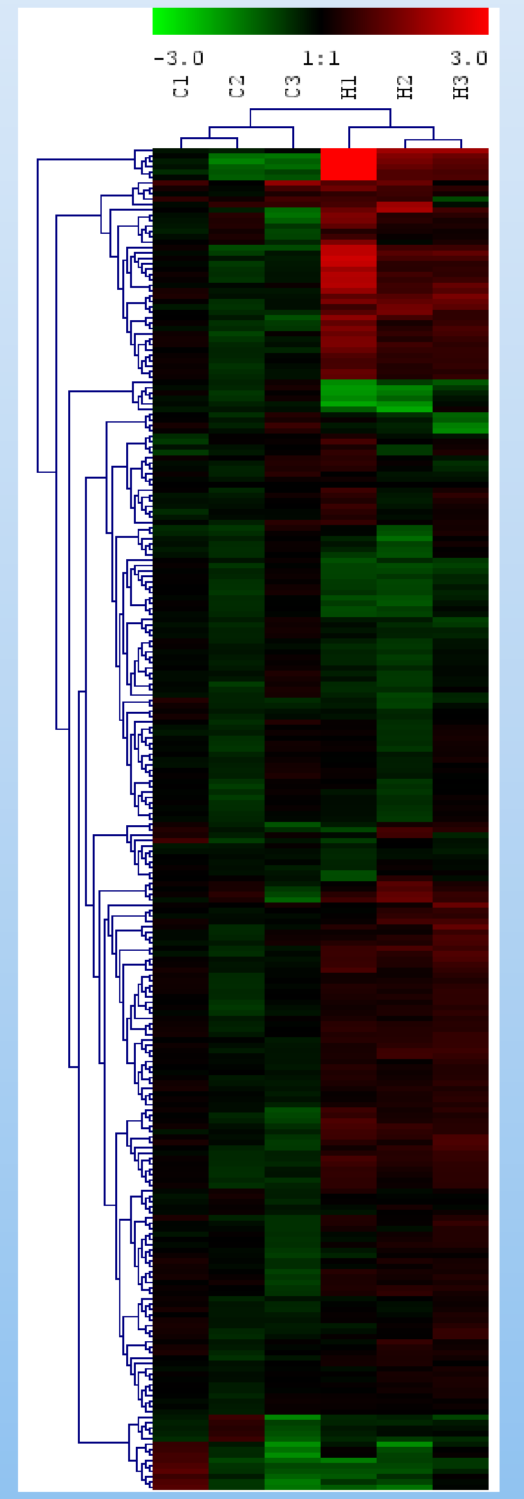
Kinetic analysis was used to determine concentrations for two human and two mouse proteins in their respective sera. Purified proteins and sera were serially applied to an antibody microarray over a range of dilution factors. The purified proteins were used as references. The sensorgrams were fit to a two-component, heterogenous ligand model to produce kinetic parameters and serum protein concentrations.

				Component 1				Component 2							
Organism	Protein	M.W. kD	Antibody	k_{fwd} 1/Ms	k_{rev} 1/s	k_D nM	Response µRIU	k_{fwd} 1/Ms	k_{rev} 1/s	k_D nM	Response µRIU	LOD ng/mL	LOD pM	Concentration µg/mL	Concentration µM
Human	transferrin	80	HuECT	3.2×10^5	5.4×10^{-4}	1.72	53.7	1.4×10^4	1.8×10^{-3}	125	74.0	14	175	2.06	25.7
Human	albumin	67	Hu45	5.9×10^5	1.0×10^{-4}	1.72	15.6	5.4×10^3	4.3×10^{-4}	79.4	41.9	50	750	24.3	362
Mouse	transferrin	80	Hu31	9.0×10^4	$< 10^{-4}$	< 1.11	81.8	5.4×10^5	1.2×10^{-2}	22.9	59.1	58	725	1.18	14.8
Mouse	albumin	69	Hu45	2.8×10^5	$< 10^{-4}$	< 0.36	24.9	4.1×10^4	2.5×10^{-3}	60.7	33.6	52	755	23.6	342

Kinetic parameters and limits of detection for four arrayed antibodies

A series of dilutions of four serum proteins in PBS was applied to the high density array. Kinetic parameters for a two-compartment model were determined by curve-fitting.

Hierarchical clustering of human liver cancer serum samples



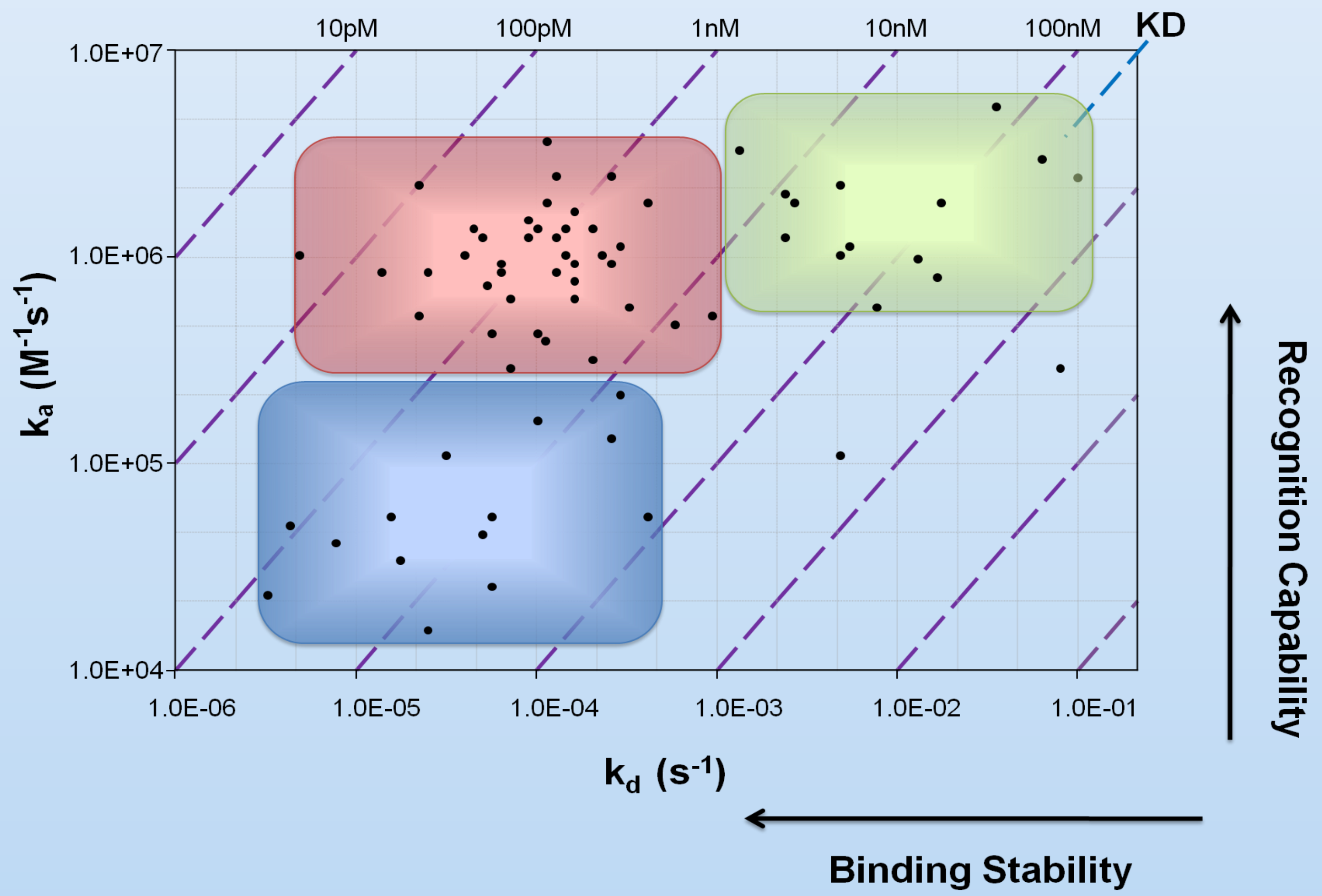
Sera from liver cancer (H1, H2, H3) and non-liver cancer (C1, C2, C3) subjects were analyzed with a Plexera HT SPRi system. The protein binding patterns from the six subjects cluster into two distinct groups. Red color represents increased protein relative to normal healthy serum, while green color represents decreased measurement. Measurements from 39 array features differed significantly between the two groups. Alpha fetoprotein, a known marker of liver cancer, was one such measurement.

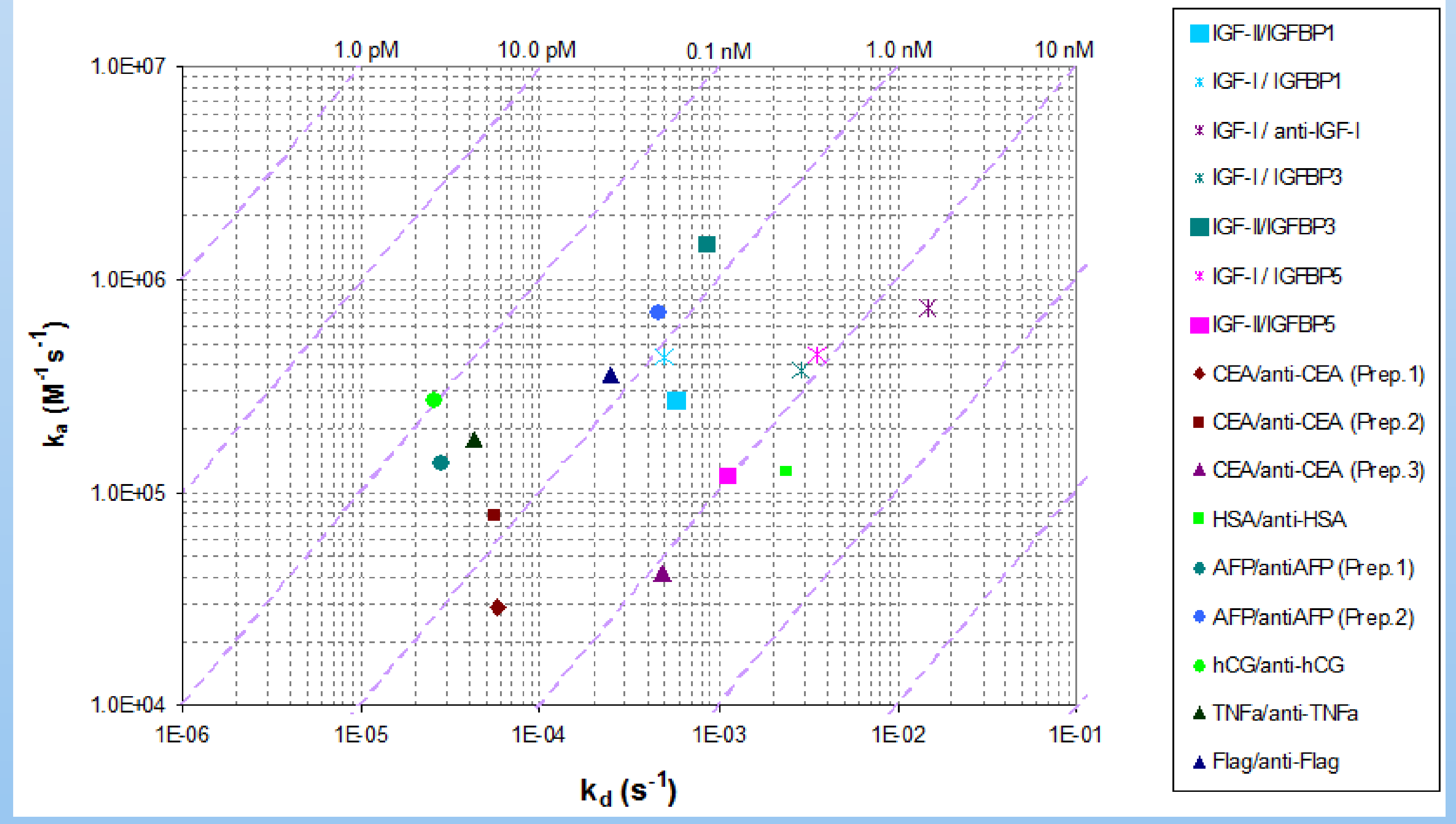
Antibody target	SPR Log2 Change	T-test	HCC Serum Literature	HCC Related
Ubiquitin	0.821	0.002		Yes
AMOG, sodium/potassium-transporting A	0.989	0.005		Yes
T-cell surface antigen T3/Leu-4 epsilon cl	0.639	0.009		Yes
Nanog	0.934	0.009		Yes
Carbamoyl-phosphate synthetase 1	0.667	0.010	Yes	Yes
Plasma retinol-binding protein precursor	0.610	0.012		Yes
alpha fetoprotein	***0.465***	***0.013***	***Yes***	***Yes***
Adenylate kinase 2	0.643	0.013		Yes
Death-associated protein kinase 3	0.369	0.014		
Beta-1B-glycoprotein 3hemopexin	0.713	0.017		Yes
dipeptidyl-peptidase 4 or CD26	1.772	0.018	Yes	Yes
Protein tyrosine phosphatase, receptor-ty	-0.682	0.018		Yes
JE	0.407	0.018		
CCL5	1.318	0.019		
CD16/CD32	0.789	0.019		
Transferrin	0.398	0.021	Yes	Yes
Proto-oncogene tyrosine-protein kinase F	0.835	0.024		Yes
IL-12	0.867	0.025		
Fructose-bisphosphate aldolase	-1.054	0.027	Yes	Yes
G protein-coupled receptor 38?	1.129	0.028		
CD16/CD32	0.543	0.028		
CD8a	1.367	0.029		
CD36 antigen-like 2	-0.913	0.031		
RBPJ, Renal carcinoma antigen NY-REN	0.492	0.031		
Complement factor H	-0.651	0.032		Yes
Catalase	0.559	0.033	Yes	Yes
Complement C4	0.554	0.033		Yes
Aldehyde dehydrogenase 4A1	0.574	0.035		Yes
ATP2B1, Plasma membrane calcium ATF	0.510	0.036		Yes
CD45	0.732	0.038		
Tumor Necrosis Factor	0.856	0.038	Yes	Yes
TNF receptor superfamily member 5	1.033	0.038		Yes
Hepatocyte-derived fibrinogen- related pr	0.412	0.038		Yes
IL-4	0.683	0.039	Yes	Yes
tumor necrosis factor receptor superfamily	0.802	0.040		
IL-10	0.667	0.041	Yes	Yes
IL-6	0.794	0.043	Yes	Yes
fibroblast growth factor 4	0.722	0.047		
beta-catenin	3.198	0.049		Yes

Targets found to differ significantly between liver and non-liver cancer samples

39 significant changes were observed (t-test P<0.05) including alpha fetoprotein (AFP), a bonafide marker of liver cancer. Ten targets have been previously observed to change in serum. Most genes have already been related to Hepatic Cell Carcinoma (HCC) by microarray expression analysis.

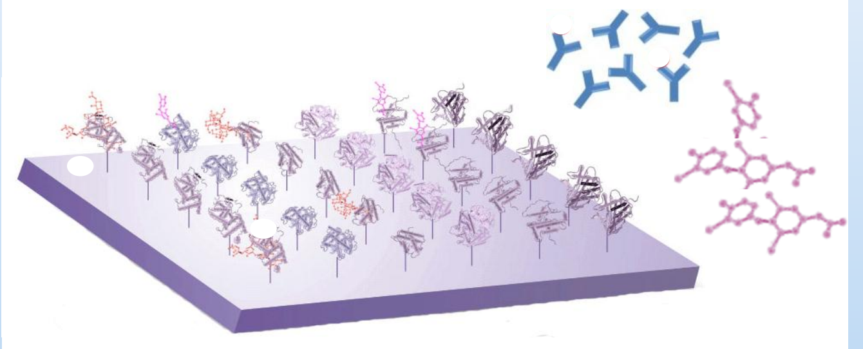
Importance of kinetics in Drug discovery



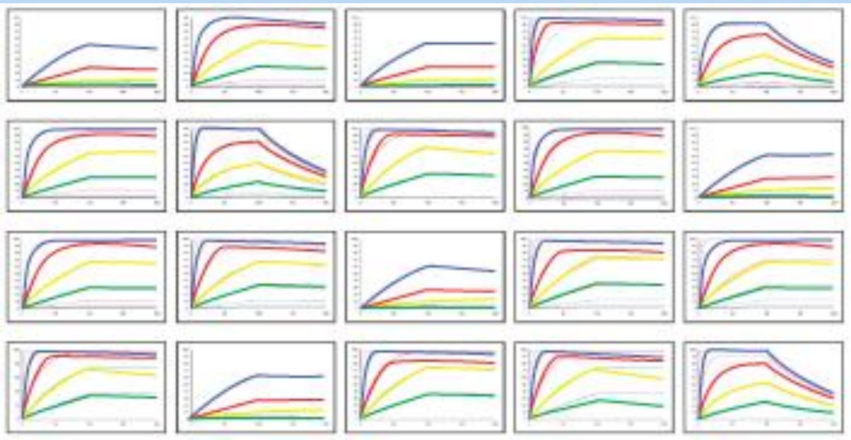


Isoaffinity plot of Kinetic parameters. Binding data from several biomolecular interactions. Diagonal dashed lines represent the pairs of K_a and K_d that yield the same affinity constant for dissociation (K_D). In the legend, analytes are on the left and spotted ligands (Abs) on the right. The ideal parameters for a good therapeutic antibody is to have a fast K_a and a slow K_d but many antibodies do not fall in this category despite the fact that they have good overall affinity (K_D). SPRi is emerging as a new technology to help screening for better antibodies in a high throughput and cost effective manner.

SPRi and Protein microarrays in antibody or drug profiling



Potentially (5000-12,000 full length human proteins)

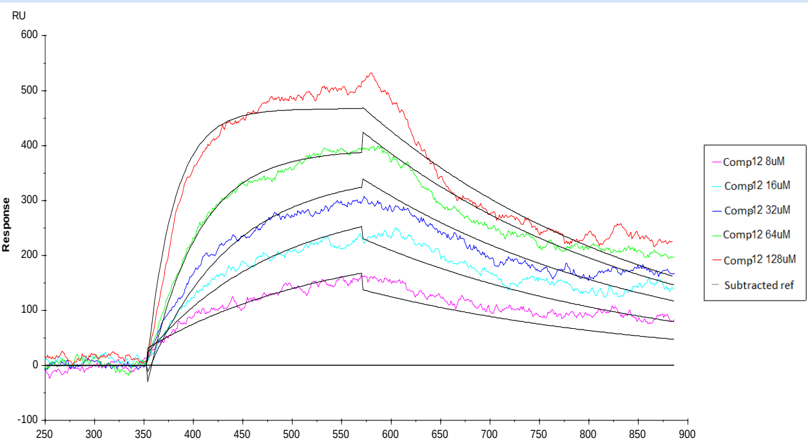


100-150 micron spots
at densities of 0.1-50pg/mm^2

SPRi system: PlexArray HT™

Sensorgrams
for up to 5000 spots.
Microfluidics to minimize
sample requirements
(30µl nM range)
See it at booth 1238

Immobilization of several proteins including HIV-Integrase and detection of specific binding with a new compound.



Random immobilization was performed on 3D SIP chips (Surface Initiated Polymerization) using the well described EDC/NHS chemistry. HIV-Integrase as well as other protein controls were immobilized in acetate buffer at 3 different pH (4.0, 4,5 and 5.0). Taking advantage of the high throughput PlexArray HT system, we were able to obtain data from various immobilization conditions (concentration, pH, % of DMSO of the compound solution). This data shows that the sensitivity of this multiplex microarray system is good enough to detect at least a 300Da small molecule binding to an immobilized 32 kDa protein.

We are currently improving the 3D surface chemistry and the immobilization strategies using commercial as well as proprietary tags to increase functionality and standardize immobilization conditions for thousands of protein targets. This is particularly important for small molecule detection rather than Antibody profiling.

Conclusions

- ❖ SPR imaging provides label-free microarray analysis that supports quantitative proteomics.
- ❖ These antibody microarrays can detect changes in concentration of serum protein biomarkers for hepatocellular carcinoma as well as other diseases or conditions.
- ❖ Antibody arrays can also be utilized to select for better antibodies with improved kinetic parameters that can have a significant impact in the therapeutic antibody as well as in the diagnostic field.
- ❖ The ability to immobilize large panels of proteins (circa 5000 spots per chip), can expand the application of this SPRi technology to Antibody and small molecule profiling. The ability to use small amount of samples is a great advantage to lower costs and improve throughput.

Acknowledgments

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