

Quantitative and Kinetics Assays using Gator[®] System



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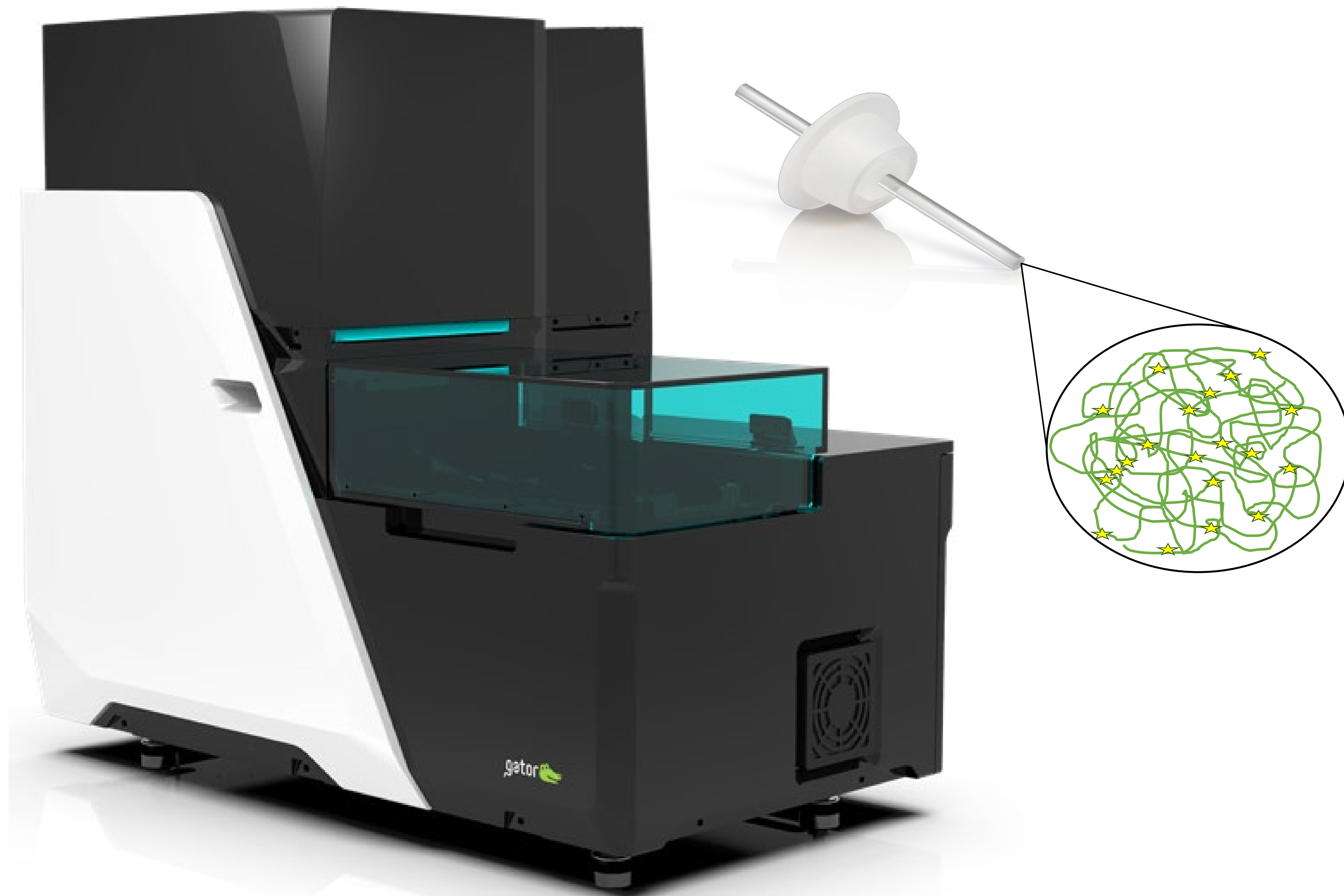
ABSTRACT

Traditional Bio-Layer Interferometry (BLI) has been used for the determination of kinetics, affinity, and antibody/protein quantitation, however, there are some limitations in sensitivity, dynamic range and sensor regeneration. For example, two different biosensors are needed for mouse IgG quantitation and kinetic screening separately, and furthermore, the Anti-Murine IgG (AMQ) biosensor is not regenerable. Using novel designed biosensor with the Gator BLI systems, both quantitation and kinetic assay can be performed with the same biosensor (e.g., MFC). In addition, significant cost saving is achieved by regeneration capability. An easy and rapid method to perform quantitative and kinetic assays will be demonstrated.

INTRODUCTION

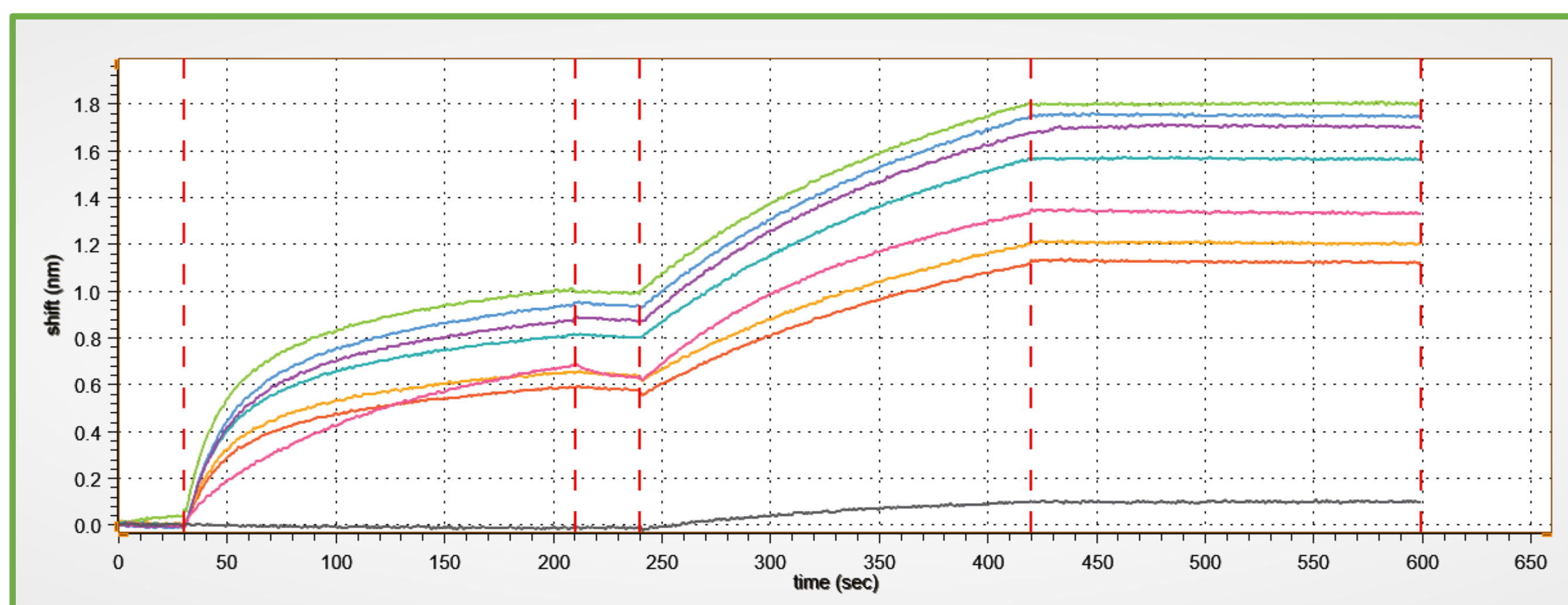
Gator Bio biolayer interferometry is a valuable asset to replace and/or expand your label free binding capabilities. Whether you are quantitating proteins or performing detailed kinetic characterization, the Gator systems are a perfect multi-tool for the analytical and biochemistry lab. We have an expanding range of increased sensitivity for small molecules, peptides, antibodies, nucleic acids, and more.

With 8 independent spectrometers capable of running 8 samples at once, this instrument allows for assay sizes from 1 sample to 168 samples in one run (Gator Prime) or from 1 sample to 456 samples in one run (Gator Plus). Flexible to accommodate various applications including quantitation, kinetics, and epitope binning. Easy to use and operate, this is an instrument that everyone in the laboratory can know how to run. This is truly a plug and play instrument, taking less than 30 minutes to set up and install.

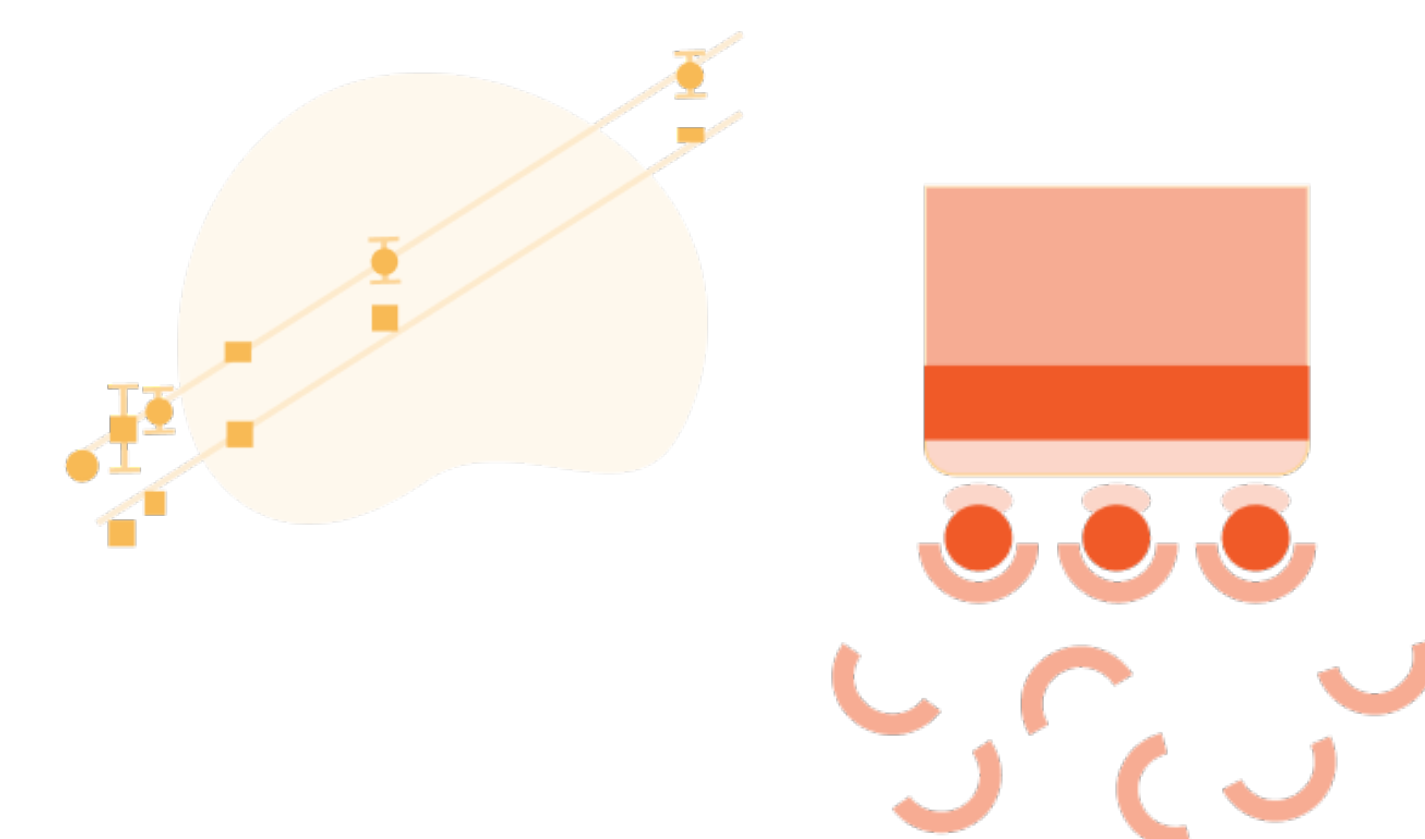


QKR

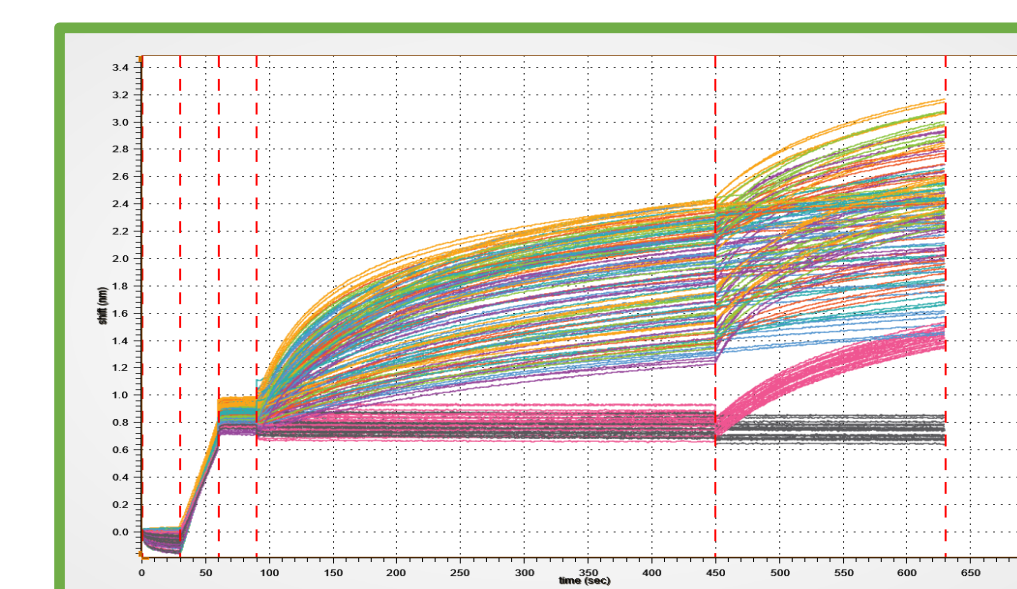
Quantitation and Kinetics with Regeneration. In the same assay.



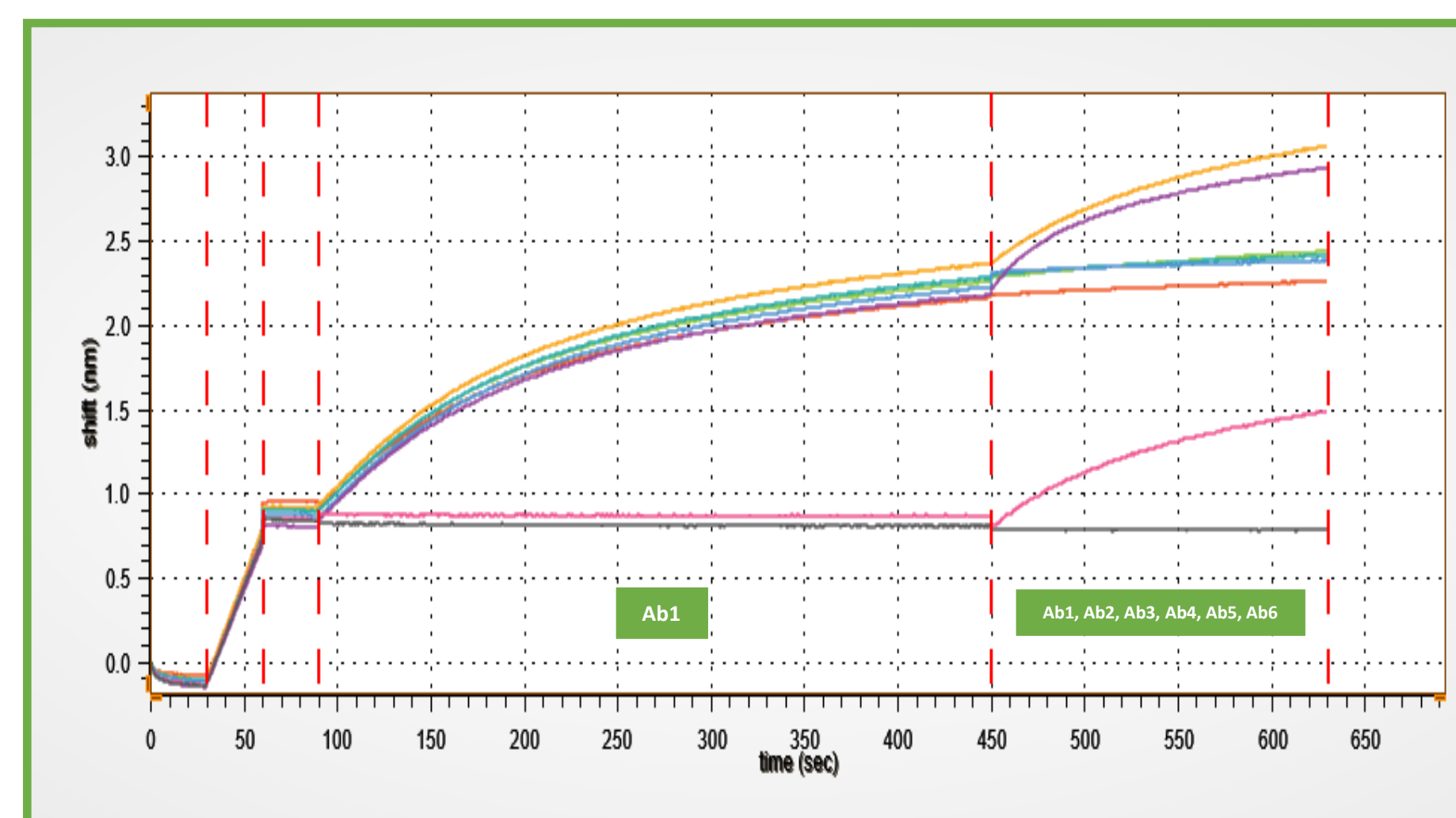
QKR give the ability to quantitate and get kinetic constants in the same assay. Using regeneration, the same probes can be used for an entire plate of samples.



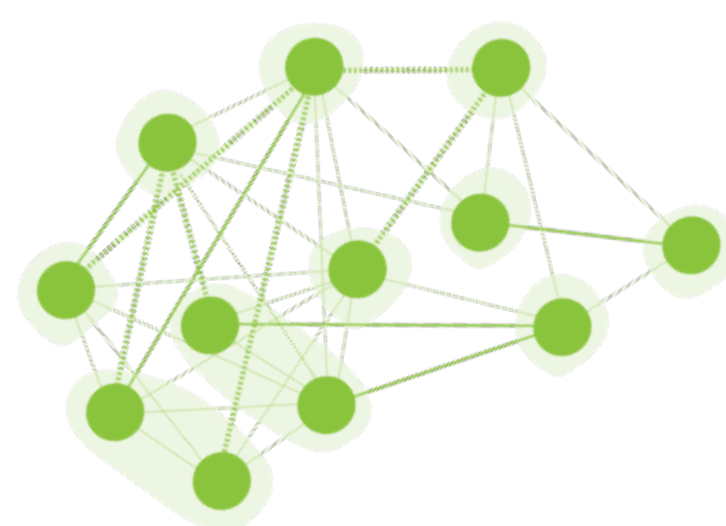
Epitope Binning



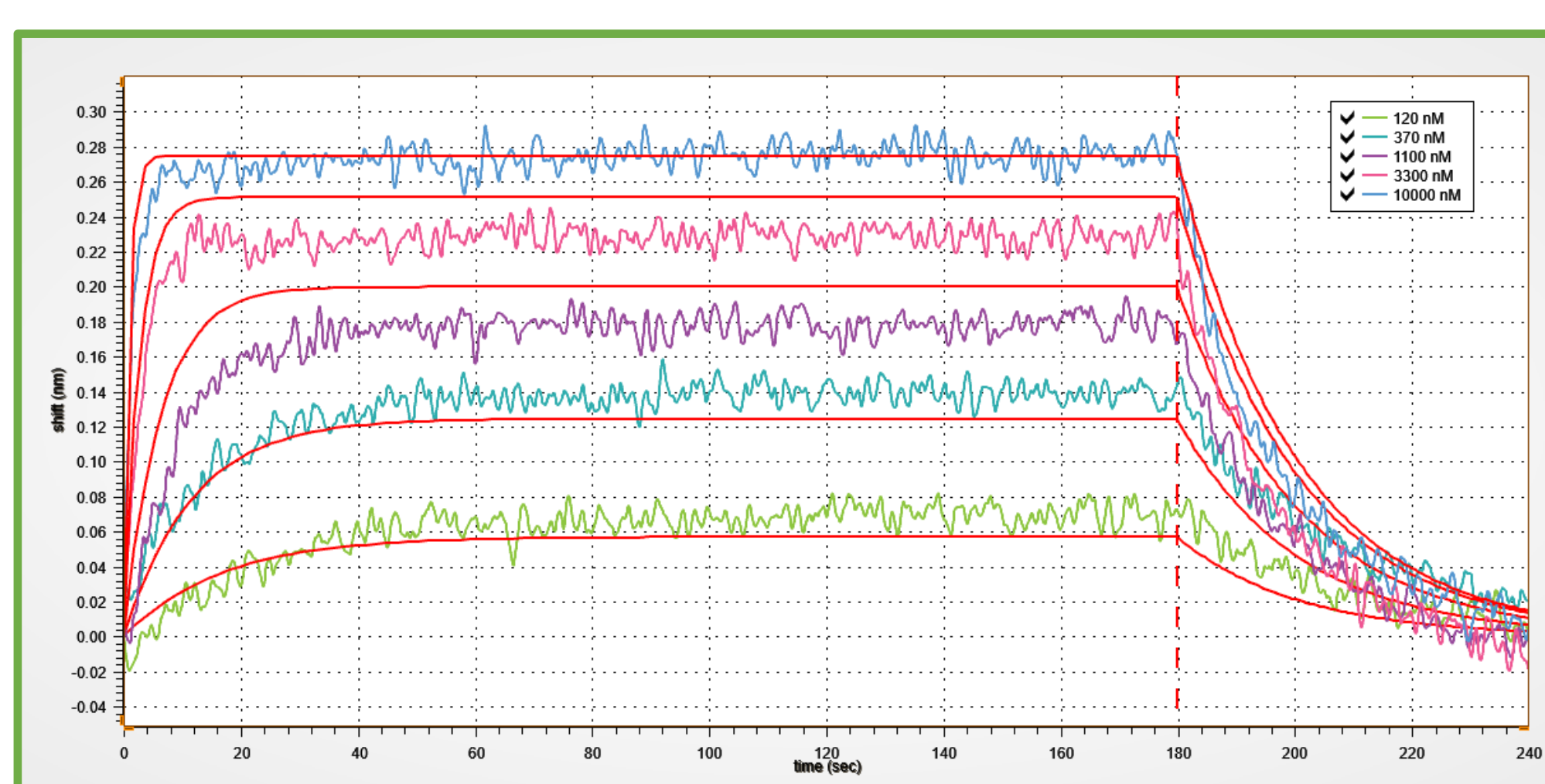
	AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
AB1	0.015	0.046	0.082	0.29	0.395	0.465	0.326	0.434	0.078	0.207	0.043	0.238
AB2	0.020	0.117	0.097	0.021	0.055	0.086	0.775	0.118	0.661	0.793	0.026	0.096
AB3	0.275	0.067	0.246	0.715	0.075	0.704	0.705	0.466	0.702	0.711	0.064	0.782
AB4	0.097	0.215	0.181	0.155	0.237	0.303	0.087	0.255	0.133	0.186	0.240	0.031
AB5	0.022	0.037	0.071	0.58	0.216	0.193	0.020	0.238	0.062	0.127	0.039	0.21
AB6	0.551	0.204	0.713	0.368	0.095	0.153	0.792	0.201	0.087	0.015	0.043	0.31
AB7	0.155	0.055	0.091	0.338	0.423	0.571	0.165	0.53	0.096	0.343	0.048	0.046
AB8	0.041	0.15	0.714	0.036	0.064	0.087	0.701	0.118	0.051	0.709	0.055	0.48
AB9	0.350	0.002	0.179	0.795	0.051	0.791	0.325	0.797	0.519	0.718	0.020	0.273
AB10	0.096	0.3	0.075	0.372	0.225	0.263	0.094	0.281	0.155	0.182	0.275	0.36
AB11	0.041	0.12	0.071	0.107	0.135	0.198	0.064	0.164	0.096	0.13	0.136	0.217
AB12	0.065	0.214	0.714	0.011	0.029	0.126	0.795	0.199	0.099	0.779	0.795	0.121



24 assays were performed using the same 8 anti-His biosensors. The sensorgram on the right shows the raw data. The table on the right shows the epitope matrix created in the Gator software.

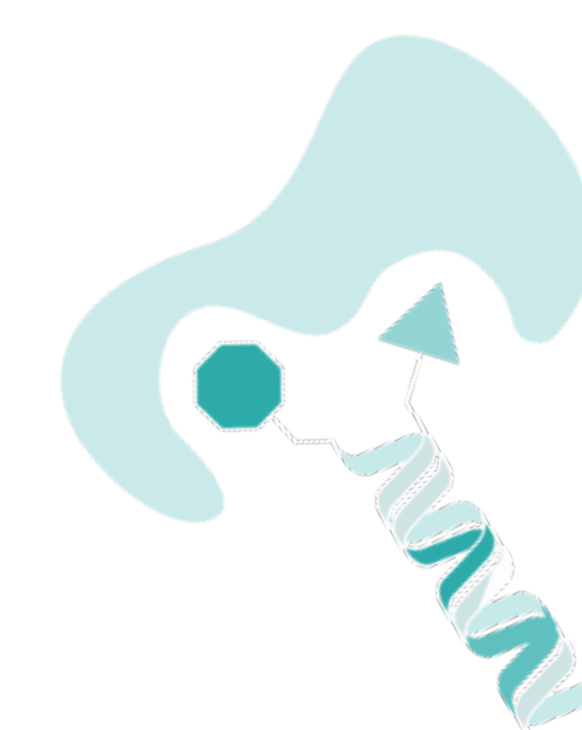


Small Molecule Analysis

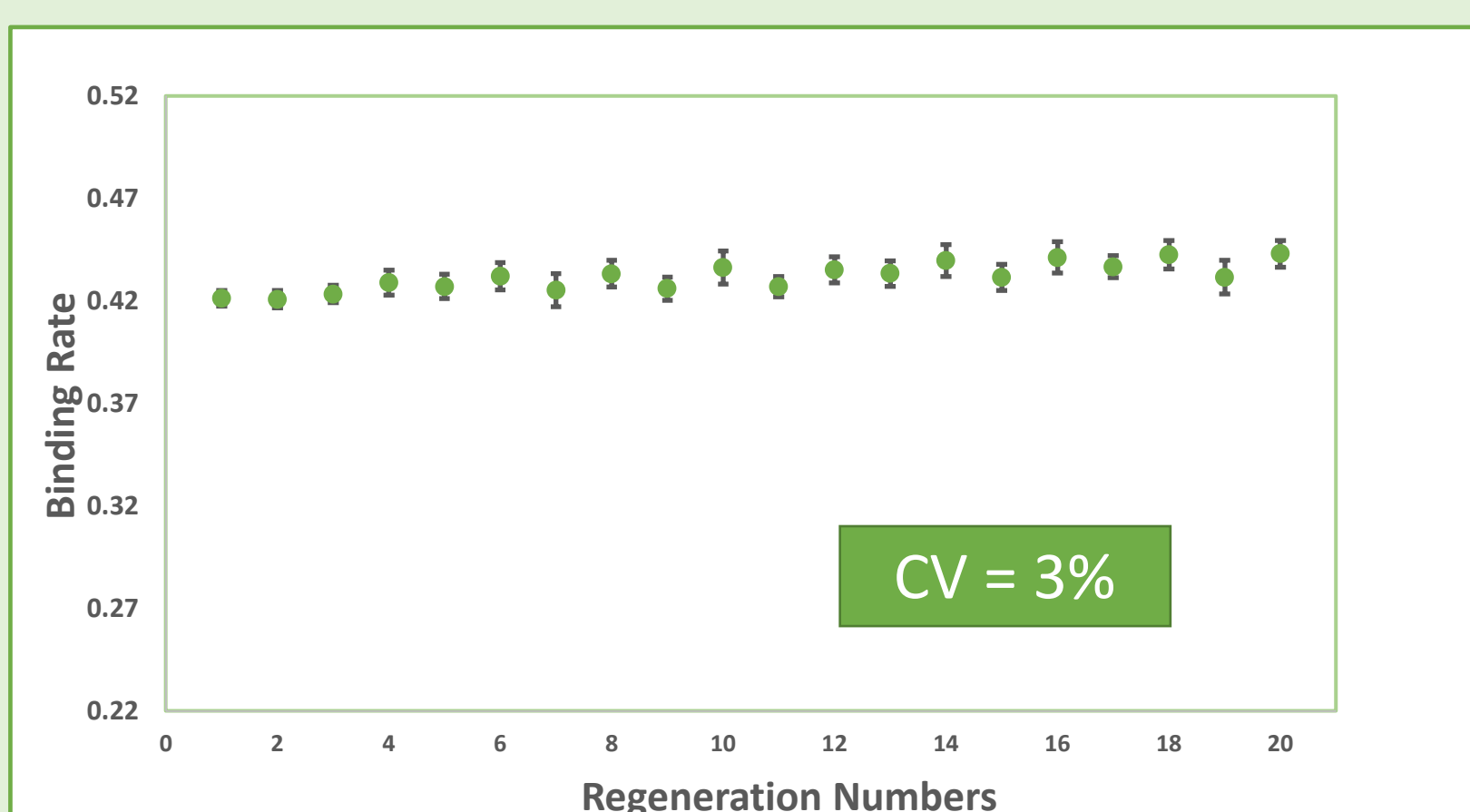


Our high-capacity streptavidin probes supplies the sensitivity to measure interactions of molecules well below 500 Da. Here we show kinetic constants between biotinylated carbonic anhydrase II Furosemide (330 Da).

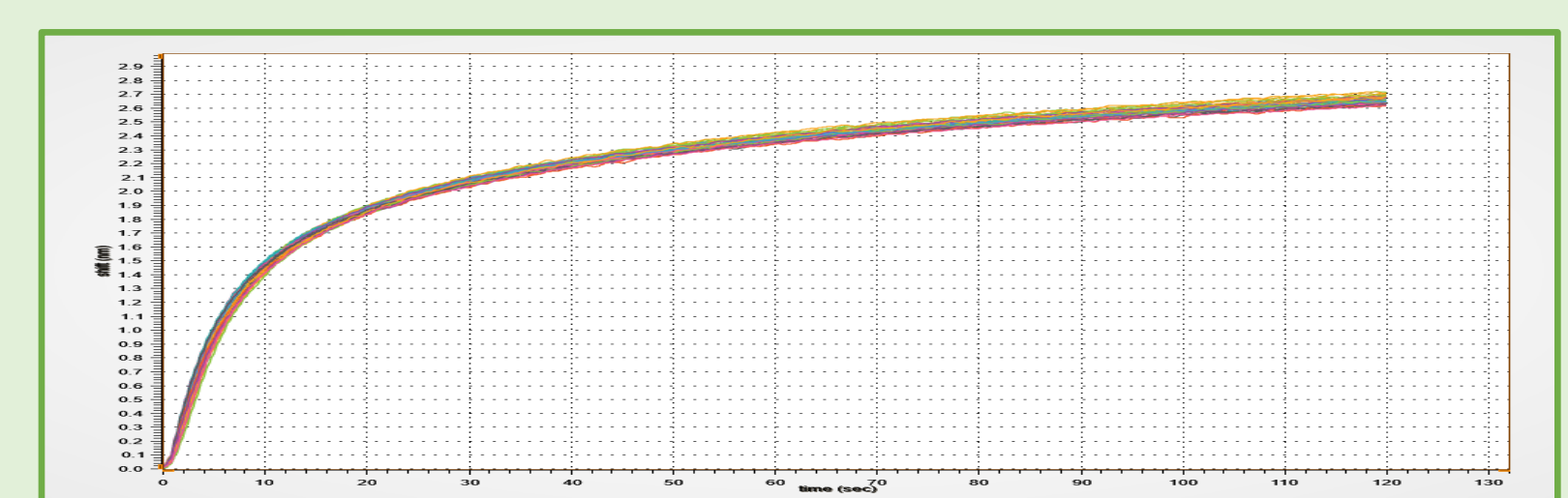
	Gator	SPR
k_{off} (1/s)	4.86 E-02	4.96 E-02
k_{on} (1/Ms)	1.00E+05	9.66E+04
K_D (M)	4.85E-07	5.13E-07



REGENERATION CAPABILITIES OF GATOR BIOSENSORS



Using 8 Protein-A biosensors, 160 samples of the same concentration were measured. CV = 3%



8 quantitation assays of 200 µg/mL were performed with the same MFC biosensors with regeneration. Binding rate CV = 6%.

Assay	k_{off} (1/s)	k_{on} (1/M·s)	K_D (nM)
Assay 1	9.95 e-4	5.14 e5	1.93
Assay 2	8.73 e-4	5.15 e5	1.70
Assay 3	7.90 e-4	4.95 e5	1.60
Assay 4	7.41 e-4	4.74 e5	1.57
Assay 5	7.90 e-4	4.58 e5	1.72
Assay 6	7.85 e-4	4.50 e5	1.74
Assay 7	7.51 e-4	4.45 e5	1.69
Assay 8	7.42 e-4	4.53 e5	1.64
Assay 9	7.35 e-4	4.32 e5	1.70
Assay 10	7.24 e-4	4.20 e5	1.73

The calculated k_{off} , k_{on} , and K_D values of mouse anti-fluorescein and fluorescein-BSA binding measured using ProG probes with regeneration in between assays.

REFERENCES

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3. Gator Bio Regeneration Studies-Regenerate Without Compromising Quality. https://mcusercontent.com/51459f48975b7d0defbd8ef5e/files/f6dd977d-0573-4dbd-8f3e-05656147496a/mFc_Regenerations.01.pdf