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Whitepaper

The Throughput Booster for Binding Interaction Screening –

the waveRAPID® Kinetics Assay



The accurate and efficient measurement of intermolecular interactions and binding events is a critical element for all manner of basic research and an indispensable component of drug discovery programs. Ligand binding assays can be performed using labeled molecules (radiolabels, fluorescent labels, etc.), but appropriate, non-disruptive labeling and often elaborate washing and purification steps are required. Additionally, the kinetics of interactions are not easily acquired or understood using these methods. Surface plasmon resonance (SPR) changed the game with its ability to monitor and measure interactions in real time using unlabeled species, but challenges remain. Measurements usually require significant time investment because they involve repeated introductions of increasing concentrations of analyte, and they are sensitive to bulk refractive index changes in buffers; these issues limit the utility of SPR in screening programs. Creoptix previously developed and patented the grating-coupled interferometry (GCI) technology as the most sensitive optical sensing-based label-free analysis standard, and we are now excited to introduce a revolutionary detection method—waveRAPID®—that will drastically reduce assay time and reagent consumption while improving readout to identify leads in the drug discovery process faster and with more confidence.

Traditional Kinetics Measurements

The state of the art for optical detection of molecular interactions usually involves SPR or GCI. In a typical experiment, after ligand immobilization on the sensor surface, an analyte of interest is repeatedly injected at increasing steady concentrations, each of which is applied for the same duration. Both SPR and GCI measure small changes in the refractive index near a surface to which a ligand of interest is immobilized; as the analyte interacts, the refractive index changes proportionally to the mass of the interacting analyte. After each injection, the signal is often allowed to return to baseline in the absence of analyte (sometimes forcibly with a regeneration conditions that disrupt binding). Alternatively, the analyte concentration can be increased steadily in a regeneration-free experiment. Binding affinity (K_D) and kinetics (k_a, k_d) are obtained from the resulting response signal (Figure 1), but several concentrations of analyte are needed to obtain a robust fit, limiting throughput.

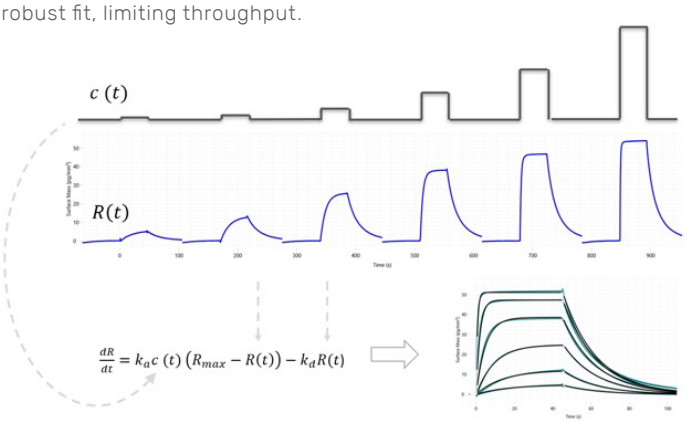


Figure 1. Traditional kinetics analysis. In a typical ligand binding assay, the analyte is introduced at increasing concentrations, with each injection being of uniform duration. At top, c(t) shows the concentration of analyte introduced as a function of time. R(t) in the middle portion is the sensorgram response, proportional to the mass of interacting analyte. The time-dependent known concentration of analyte and associated response are applied to the equation shown at bottom to infer k_a and k_d. Black lines in graph at lower right show the resulting mathematical fit using inferred values overlaid with the recorded response shown in blue lines.

The New waveRAPID Method: Kinetic Characterization from One Well

As a new alternative approach, **R**epeated **A**nalyte **P**ulses of **I**ncreasing **D**uration (waveRAPID) uses pulses of analyte at a single concentration, but each pulse is applied for increasing duration. As in the case of regeneration-free kinetics, the response does not need to return to baseline before a subsequent analyte pulse is introduced. Much like traditional kinetics measurement, with waveRAPID, the observed binding curve is a response to the time-dependent concentration input of the injected analyte. The new method, however, does not rely on the injection of different concentrations from multiple wells to create a time-dependent concentration function. Instead, a sophisticated arrangement of injection durations of the same concentration is coordinated through Creoptix WAVEcontrol software. While the result is the same—a robust determination of kinetic parameters—the data are obtained from a single well within a fraction of the time (Fig. 2).

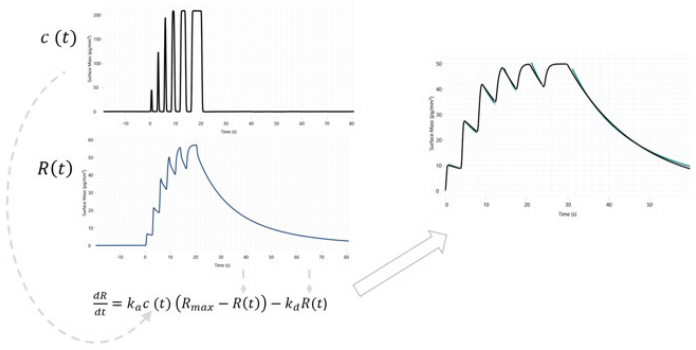


Figure 2. waveRAPID. Each analyte pulse is applied for increasing duration. The time-dependent concentration function is shown at the top left, and the sensorgram response is shown at the lower left. As with a traditional kinetics approach, the association and dissociation constants can be obtained by applying c(t) and R(t) values to the equation at bottom. waveRAPID assays can be interpreted using only the dissociation portions of a sensorgram (blue lines at right).

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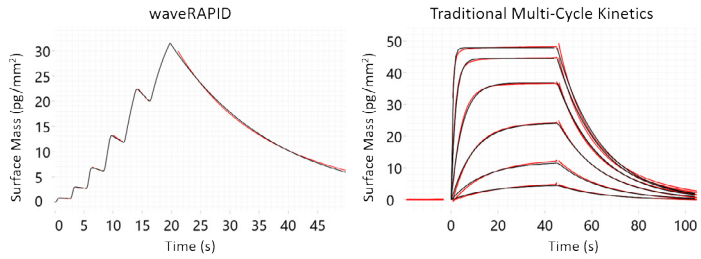
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A waveRAPID experiment can be completed in significantly less time compared with a traditional assay. No dilution series is necessary. The decreased assay time will produce snowballing effects, as it increases throughput, making screening for hit compounds much more feasible and reducing complications associated with unstable proteins or other species. Furthermore, the full kinetic parameters of each analyte can be obtained during the screening experiment.

A major advantage of waveRAPID is that the response can be interpreted by analyzing only the dissociation portions of the measurement curve, as represented in Figure 2 (right side). This analysis method overcomes one of the greatest fundamental drawbacks of refractive index-based sensors—typical measurements are confounded by refractive index disturbances in buffer. By only considering the dissociation regimes of the response curves, refractive index disturbances have no impact. With this method, you can wave goodbye to DMSO correction. Importantly, both the association rate and the dissociation rate can still be extracted from acquired data using the same differential equation as for traditional kinetics. Simply put, this is possible because each individual dissociation window begins at a level that is dependent on the association rate.

Importantly, comparing the highly reproducible results of waveRAPID measurements for binding of the small molecular analyte furosemide to carbonic anhydrase II (CAII) against traditional multi-cycle kinetic measurements confirms that both methods deliver highly consistent results (Figure 3).



RAPID kinetics parameters		
Rmax	57.00	pg/mm²
ka	2.93±0.07E4	$M^{-1}s^{-1}$
kd	5.59±0.08E-2	s^{-1}
Kd	1.904	µM

Traditional kinetics parameters		
Rmax	49.67	pg/mm²
ka	2.79±0.02E4	$M^{-1}s^{-1}$
kd	5.41±0.03E-2	s^{-1}
Kd	1.937	µM

Figure 3. Comparison of kinetic data for the small molecular compound furosemide binding to carbonic anhydrase II (CAII) generated with either waveRAPID (left panel) or with traditional multi-cycle kinetics (right panel). The double-referenced response data (red) are fit with a 1:1 binding model (black lines) using the direct kinetics engine of the WAVEsystem. Kinetic parameters including statistical errors for the kinetic rate estimation are shown in the corresponding tables.

High Sensitivity and Robust Microfluidics to Accommodate the Toughest Samples

The GCI technology developed by Creoptix is inherently better suited for kinetics and binding experiments compared with traditional SPR. Traditional SPR uses polarized light, introduced at a certain resonance angle, to excite electrons in an electrically conducting surface and induce an evanescent field beyond the surface. Refractive index changes created by binding interactions at the surface cause a shift of the resonance angle, allowing direct measurement of binding events. However, these surface plasmons are quickly attenuated in the direction of travel. Waveguide interferometry similarly relies on refractive index changes within an evanescent field near a surface, but instead of relying on surface plasmons, the light travels through a waveguide parallel to the binding surface. Because the light travels along the entire length of sample, more binding events are captured, increasing sensitivity and the signal-to-noise ratio. Additionally, the resulting evanescent field does not penetrate as deeply into the sample, reducing complications from bulk solution refractive index changes. With GCI, this setup is improved further by using gratings to couple both a measurement arm and a reference beam into the waveguide. This setup makes GCI much more tolerant to misalignments, temperature changes, and vibrations compared with previous waveguide interferometers.

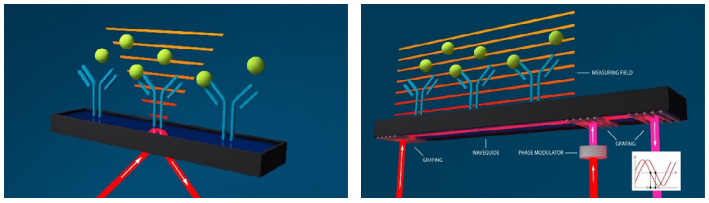


Figure 4. SPR and GCI Assay Schematics. With SPR, left, molecular interactions are detected as refractive index changes within an evanescent field (orange) of the surface plasmon, appearing as energy dips at a specific incidence angle. With grating-coupled interferometry (GCI), right, the reference beam is coupled into the waveguide. Consequently, interference occurs within the waveguide, and a high-resolution, time-dependent, and robust phase shift signal is created.

The microfluidics of the WAVEsystem make it the best choice for waveRAPID assays. A unique design is employed in disposable cartridges with parallel flow channels that enable ultra-fast transition times of 150 ms and prevent clogs. The no-clog microfluidics make the direct study of biofluids and crude reaction mixtures possible, and the system is compatible with or tolerant to harsh, uncommon solvents such as acetonitrile or high DMSO concentrations.

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Competing technologies introduce a single time-dependent concentration gradient of analyte to the SPR flow cell to reduce assay time. However, this approach depends on natural diffusion, and molecules with slightly different diffusion constants will behave very differently. The waveRAPID format, alternatively, allows controlled distribution using valves and will thus produce more reliable data for lower analyte concentrations and be more tolerant to imperfect calibration.

waveRAPID is Accurate and Reproducible

To demonstrate the accuracy and reproducibility of the waveRAPID method, Creoptix performed kinetic analysis of the small molecular analyte furosemide binding to carbonic anhydrase II (CAII) over a range of different conditions. CAII was immobilized at 3 different densities, resulting in R_{max} values of 1.6, 2.4, and 50.3 pg/mm^2. Furosemide was then introduced at 5, 17, or 50 µM with injection times of 20 or 30 seconds. All measurements were completed within 20 minutes, and the double-referenced response data were fit with the Creoptix direct kinetics engine to a 1:1 binding model on the WAVEcontrol software. The calculated kinetic parameters revealed excellent reproducibility, showing standard deviations for k_a, k_d, and K_D of 8.6, 4.8, and 8.6%, respectively (Figure 5).

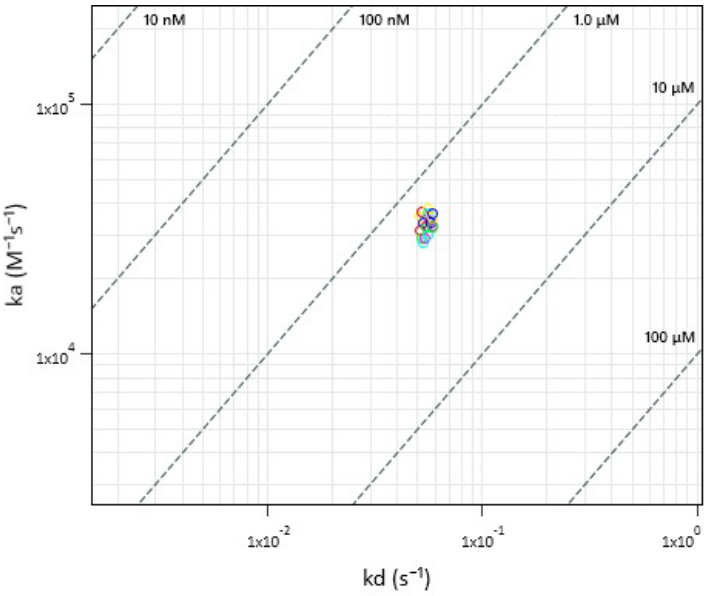


Figure 5. Tightly grouped kinetics values for furosemide interaction with Carbonic Anhydrase II (CAII) measured with varied ligand density, analyte concentration, and injection duration.

Measure Kinetics in Hours, Rather than Days

The waveRAPID technology is exquisitely suited for accelerating kinetic measurements, thus allowing high-throughput kinetic determination of many analytes in a screening setup. Because assays can be completed in significantly less time, inherently unstable species can more easily be studied. Creoptix performed kinetic characterization of small molecule drug hits on the WAVEdelta system for an undisclosed drug target using 90 different analytes, each applied for 25 seconds of total injection duration prior to 300 seconds of dissociation. In just 18 hours of total assay time, several hits were successfully identified with KD values ranging from nM to µM concentrations (Figure 6). With its ability to detect weak binding interactions more reliably, the WAVEdelta system combined with waveRAPID methodology is optimally suited for small molecule compound screens and even fragment-based screens.

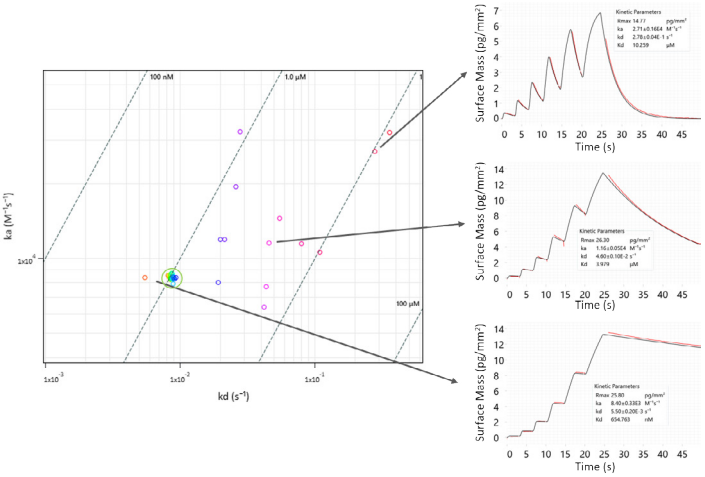


Figure 6. waveRAPID kinetic measurements of small molecular drug hit candidates. Ninety molecules were analyzed, producing kinetic data in 18 hours. The results shown in the rate map were filtered to include only molecules with low statistical errors in calculated rate constants. waveRAPID enables high-throughput kinetic analysis in a screening setting. Circled in green are 16 replicates of a control compound run throughout the assay.

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Conclusion

waveRAPID is the new standard for measuring kinetics. Simple sample preparation with no more serial dilutions, no more DMSO correction curves, and kinetics results from a single concentration from a single well. These add up to more room in your microtiter plate. Using the waveRAPID detection method on the WAVEdelta system from Creoptix, assay throughput is increased dramatically to provide unparalleled speed. Binding affinity and kinetic parameters can all be determined in a primary screen to accelerate the drug discovery process. With sensitivity that is superior to SPR, weak binders and fast-dissociating compounds can be reliably detected. The GCI technology with its improved optics is poised to maximally leverage the waveRAPID assay format and requires no new equipment for existing users. The fluidics are compatible with complex primary fluids such as serum, plasma, or cerebrospinal fluid or with crude reaction mixtures containing various additives and high concentrations of DMSO or other organic solvents. By obtaining kinetics from a single well in one injection, reagent and sample consumption are reduced. Suddenly, a week's worth of work is complete in a day. Time, money, and mental energy can all be redirected to other stages of drug discovery.

About Creoptix

Creoptix is a private company headquartered in Wädenswil, at the lake of Zurich in Switzerland, and with US offices in the Boston area. Creoptix is leading the advancement of breakthroughs in life science research by developing and commercializing next-generation bioanalytical instruments for drug discovery and life sciences for both industry and academic research. Based on its innovative proprietary biosensor, microfluidics, and software technologies, the Creoptix WAVEsystem provides exceptionally high sensitivity and resolution to study real-time biological interactions involving fragments, small molecules, peptides, membrane proteins, biologics, and other molecules even in crude reaction mixtures and biofluids such as undiluted serum and plasma.

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