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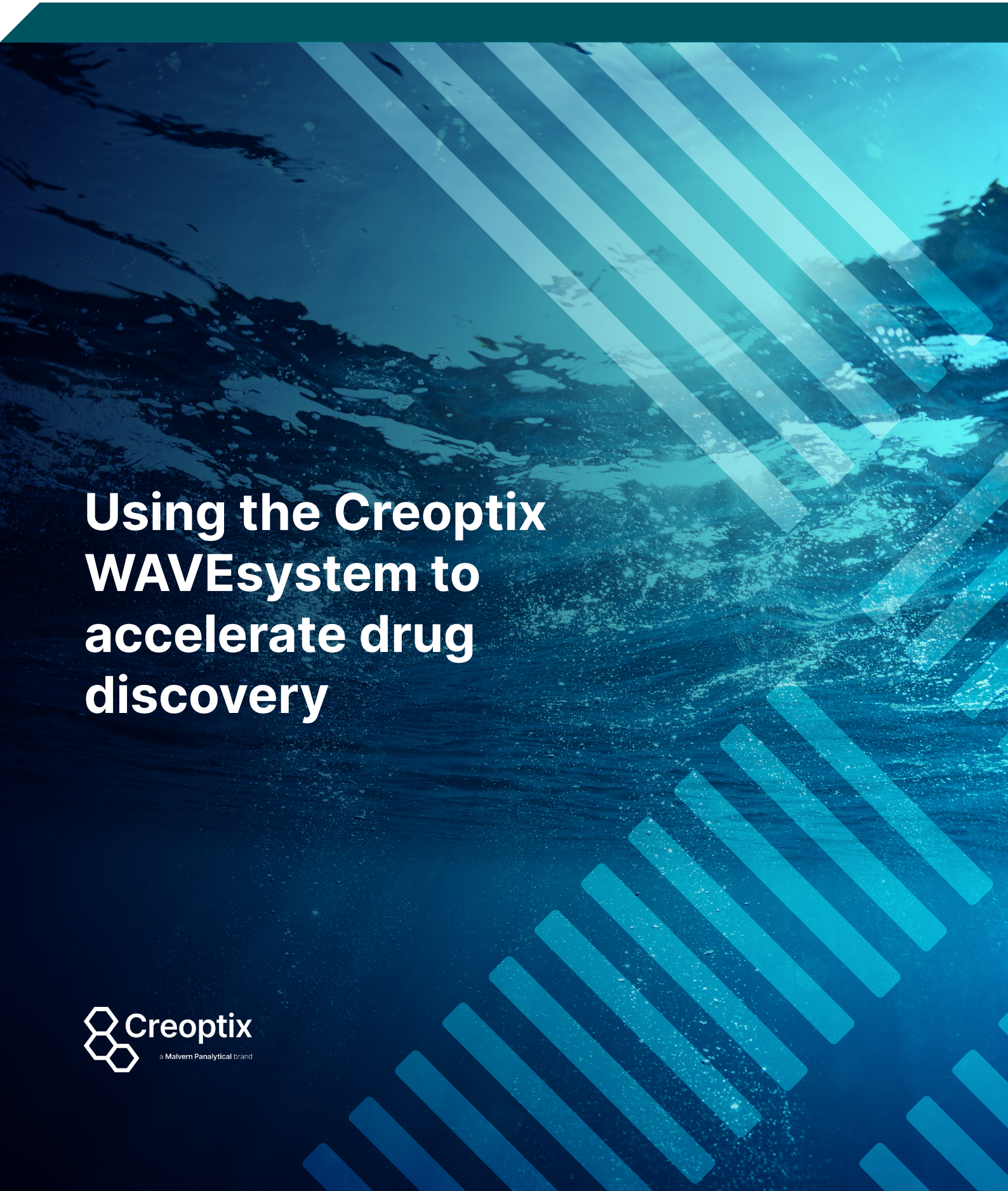

Using the Creoptix WAVEsystem to accelerate drug discovery

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How the Creoptix WAVEsystem drove real-time kinetic and affinity analysis for faster and better characterization of candidates – from fragments to biologics

Impact

- Hit screening campaigns cut from weeks to days
- Faster hit-to-lead progression with superior resolution of off-rates above 1 s^{-1}
- Six promising Sybody® binders to Spike RBD identified and rapidly characterized
- Antibodies characterized directly from clinical blood plasma samples

Introduction

Bioassays, such as the enzyme-linked bioabsorbant assay (ELISA), are standard techniques in the laboratory that require enzyme or fluorescent labels for the detection of target analytes. While these label-based studies give important information about a sample, such as binding affinity, they can only measure the interaction of an analyte at a given concentration and time. This means that such assays are unable to gather kinetic analysis, for example the speed or length of binding. Yet kinetic analysis is fundamental to many stages of the drug discovery and development pipeline, from lead development to complex biologics investigation. Without this data, scientists are forced to make less informed decisions, causing delays to drug discovery and development.

To improve decision-making in drug research and development, researchers need instruments that allow them to obtain high-quality kinetics data. **The Creoptix WAVEsystem** provides label-free interaction analysis allowing you to obtain kinetic and affinity data, alongside evaluation of binding specificity, in real-time. The system is ideal for analyzing samples based on two key patented developments: **Grating-Coupled Interferometry (GCI) technology**, and no-clog **WAVEchip** microfluidics systems.

The first development, GCI, measures changes in the refractive index within the evanescent field, near the biosensor surface. By measuring the interaction between your analytes with the ligand and the consequent changes in refractive index, you can determine kinetic rate constants, analyte concentration and affinity constants. Compared to surface plasmon resonance (SPR) techniques, GCI gives much more sensitive measurements as the evanescent wave spans across the entire biosensor surface.

The second development the Creoptix WAVEsystem benefits from is its disposable, no-clog microfluidics cartridge, WAVEchip. This cartridge allows the system to analyze crude samples, harsh solvents, and large particles up to 1000 nm, normally only possible with plate-based assays. Further, very complex matrices can also be analyzed, thanks to the no-clog microfluidics enabling analysis of analytes. The chip design also allows for the determination of off-rates up to 10 s^{-1}, meaning the kinetics of weakly binding fragments can be studied.

The combination of GCI and no-clog microfluidics makes the Creoptix WAVEsystem perfect for the delivery of high-quality data from the most challenging sample types. Here, we look at four different examples where the Creoptix WAVEsystem enabled laboratories to perform faster and more sensitive analysis, and kinetic analysis, to accelerate drug discovery and development campaigns.

Kinetic characterization of drug hit compounds

High-throughput screening provides vital information to drug discovery programs, by producing hits showing potential interaction with drug targets. However, the further development of any hit requires a full characterization of their binding kinetics. Due to the large numbers of hit compounds, this process is extremely time- and material-consuming, particularly as suitable dilution series from hundreds of hits must be prepared.

Creoptix waveRAPID on the Creoptix WAVEdelta with four parallel flow channels can streamline projects such as these by enabling **robust kinetic characterization from a single well in one injection**. In a recent example, the HTS drug discovery group at Idorsia Pharmaceuticals Ltd ran a campaign to **determine the binding kinetics of 146 drug hit compounds using the waveRAPID method**. Here, samples at a single concentration were injected in pulses of varying duration to create a concentration gradient directly in the sensor area.

The method not only expedited the project but also gave high-quality results. **Characterization time for each compound was mere minutes, which can cut large campaigns down from weeks to days** — saving valuable time and materials (Table 1). In addition, comparing the data to that obtained by traditional multi-cycle kinetic measurements showed strong correlation for calculated dissociation constants (k_D) with minimal outliers.

The waveRAPID technology could also **generate data for more difficult compounds**, including those with large bulk refractive index contribution or slow dissociation. Traditionally, these would require surface regeneration and re-immobilization of the ligand protein, which can be disruptive to workflows.

Overall, the WAVEsystem brought massive benefits to the HTS drug discovery group at Idorsia Pharmaceuticals Ltd, by enabling a **much faster kinetic characterization of drug hit compounds** compared to traditional multi-cycle kinetic assays.

Off-rate screening of crude reaction mixtures

The screening of fragments — that is, small molecules of low molecular weight — for binding to a target is another extremely powerful approach to aid early drug discovery by identifying initial hit compounds. While their small size makes them more likely to bind, it is generally with weak affinity.

Creoptix sensors can be used to help identify fragments that bind to most binding sites on most targets but growing the fragments to larger hit compounds with higher affinity remains a significant challenge. Conventionally, individual reactions are performed to synthesize each

	waveRAPID on WAVEdelta	4-channel SPR	8-channel SPR
Number of Targets	2	2	2
Number of Samples	384	384	384
Type of Assay	waveRAPID	Kinetics	Kinetics
Affinity Range	nM	nM	nM
Captured Steps per Sample	0	0	0
Reagent Steps per Sample	0	0	0
Runtime (h)	58	490	122
Samples / 24h	159	19	75

Table 1: Excellent assay run times and sample throughput comparing the waveRAPID method on the WAVEdelta to multi-cycle kinetics methods run on state-of-the-art SPR technologies, for full kinetic characterization of 384 samples on 2 targets.

desired larger compound. To then measure the compound's affinity requires purification and making up the samples at a defined concentration, which is extremely time-consuming and costly.

Vernalis has demonstrated that crude-reaction mixtures (CRMs) can be screened for affinity, significantly improving speed and cost of synthesis. To grow the fragments to larger hit compounds, reactions are undertaken in parallel, usually in a plate-based format, to incorporate different substituents. Only **minimal sample purification is required**, and the crude reaction mixtures can then be investigated for changes in the off-rate for binding to a target.

The change in off-rate is normally detected by SPR, and this indicates whether the compound has improved affinity. However, the GCI technology of the Creoptix WAVE gives the approach superior sensitivity over SPR-based methods. Furthermore, the **no-clog microfluidics mean that many solvents can be used for the reactions**, thereby increasing the versatility of chemistries tolerated.

To demonstrate the effectiveness of WAVEdelta compared to SPR, off-rate screening (ORS) studies to kinetically sample the hit-to-lead chemical space[1] were performed. Selected compounds were studied, and results obtained from Creoptix WAVEdelta agreed with those from a competitor 4-channel SPR instrument. Additionally, **WAVEdelta could more reliably solve very fast dissociation constants (koff) for compounds with off-rates above 1 s^{-1}**.

Through this study, a Pyruvate Dehydrogenase Kinase 2 (PDHK-2)-selective hit was identified, which was characterized as purified compound (VER235377) on the

	k_{on} ($M^{-1}.s^{-1}$)	k_{off} (s^{-1})	Rmax (pg/mm^2)	K_D (nM)
HSP90 4-channel SPR	$1.92x10^5$	0.130	24.1	679
HSP90 WAVEdelta	$1.82x10^5$	0.122	25.2	669
PDHK2 4-channel SPR	$6.10x10^5$	0.116	18.3	191
PDHK2 WAVEdelta	$3.16x10^5$	0.052	14.5	166

Table 2: Kinetic data for Tpurified compound VER235377 over HSP90 and PDHK2.

WAVEdelta. The kinetic data for this is shown in Table 2.

This study demonstrates that the Creoptix WAVEsystem is ideal for off-rate screening and fragment-based drug design. The GCI technology means similar kinetic data is obtained to that from SPR instruments, while giving **superior resolution of off-rates above 1 s^{-1}**. Therefore, incorporating the Creoptix WAVE into a drug discovery program can lead to **faster hit-to-lead progression**.

Investigation of Sybody® candidates binding onto SARS-CoV-2 Spike RBD

Beyond small molecules, complex biologics such as antibodies and nanobodies are now being investigated in drug discovery programs and require extensive characterization. One such example is synthetic single-domain antibodies, known as Nanobodies® or Sybody, which could help with the development of novel Coronavirus disease (COVID-19) preventative treatments. These antibodies are ideal to be delivered in the form of inhaled nanobody formulations to reach the site of action quicker[2].

Yet analysis of Sybody molecules is challenging, particularly as biologic drugs are influenced by their manufacturing process and so can exist as multiple variants. Binding affinity is one of the most widely studied properties of these candidates but needs to be evaluated alongside other qualities of the drug to effectively predict clinical accuracy. Therefore, real-time analysis of binding kinetics should also be included, through accurately measuring the association and dissociation rates for the antibody-target interaction.

The Creoptix WAVE can drive biologics research, as demonstrated through **the investigation of binding affinities and selectivity of potential Sybody candidates to the receptor-binding domain (RBD) region of the SARS-CoV-2 Spike protein**. The research was enabled by the **no-clog microfluidics and superior sensitivity compared to competitor SPR technologies**.

In the above study, 57 unique Sybody candidates were created[3] and analyzed with ORS experiments on the Creoptix wave, with **six strong binders to Spike RBD being identified**. These binders were further characterized and investigated for their ability to compete for ACE2 binding on Spike RBD. GCI data obtained is in line with

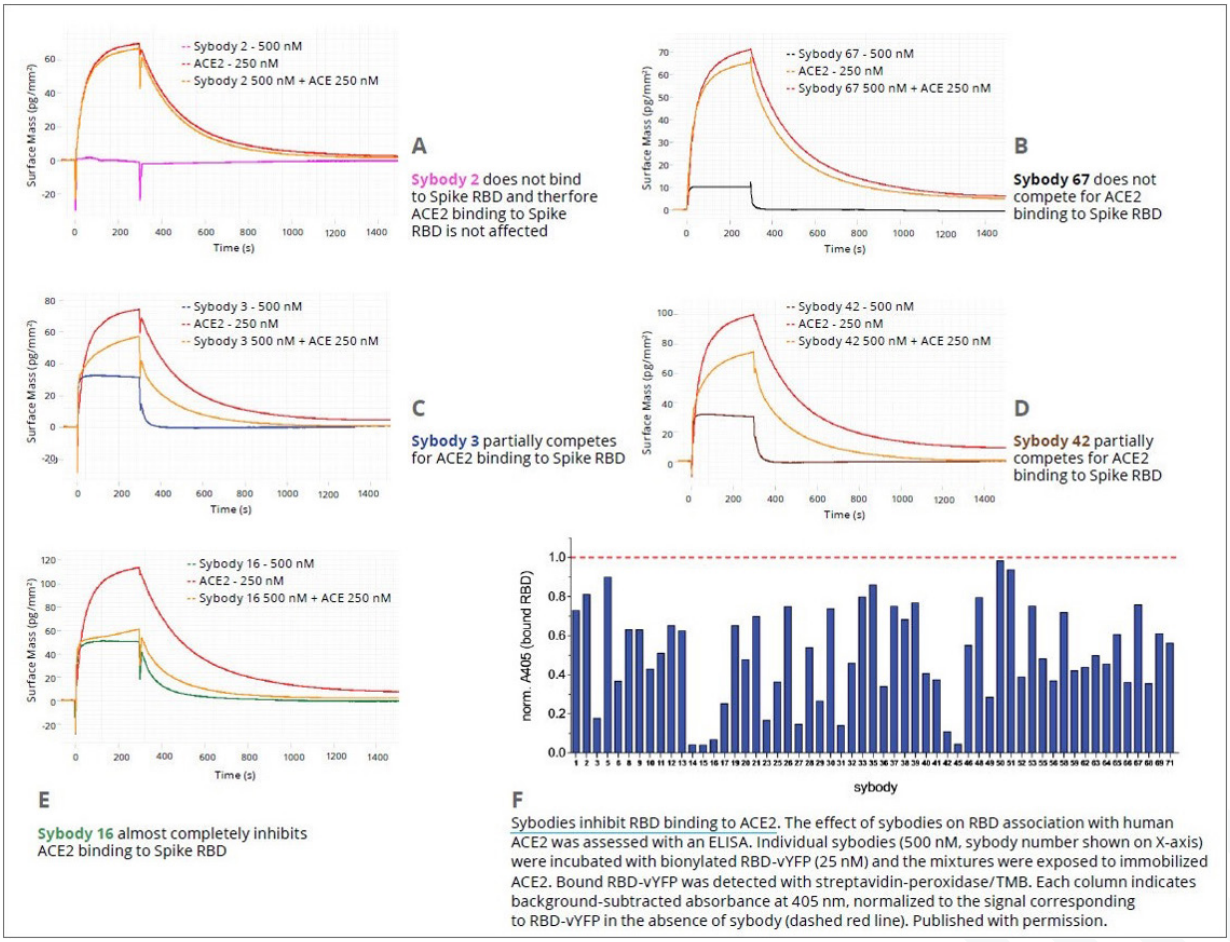

Figure 1: Sybody candidates compete for ACE2 binding onto spike RBD.

previously reported ELISA results (Figure 1), but with **much faster characterization of this inhibition assay**.

The Creoptix WAVE, therefore, is ideal for rapid characterization of Sybody candidates. **Faster characterization of binding and understanding of competition dynamics** in this way is essential to accelerate development of therapeutics for new and emerging threats such as COVID-19.

Antibody characterization from COVID-19 patient plasma

The Creoptix WAVEsystem not only enables rapid characterization of Sybody candidates for COVID-19, but also helps researchers further comprehend the underlying biology of COVID-19, too. While scientists relied on tools and knowledge on hand from other infectious diseases like Dengue fever, Zika virus and Ebola to try and understand COVID-19, these technologies were inappropriate for the Coronavirus reported in 2020. In particular, complex sample matrices caused clogging and potential damage of microfluidics systems. Additionally, matrix components

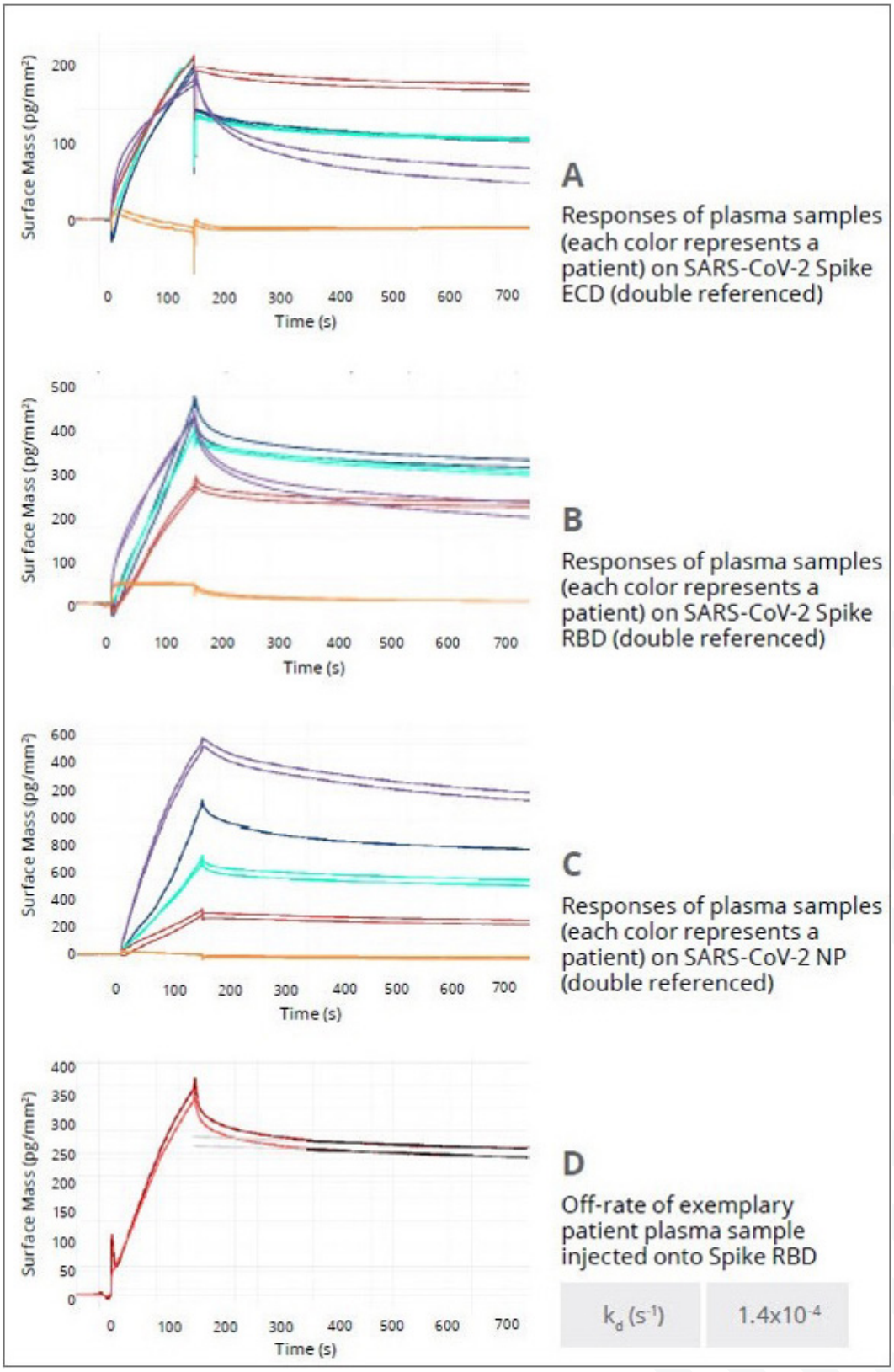

Figure 2: Binding responses of polyclonal antiSARS-CoV-2 antibodies in patient plasma samples

such as serum albumin were incompatible with label-free surface-based biosensors, thanks to high non-specific binding.

With its innovative disposable, no-clog microfluidics, Creoptix WAVE can overcome these limitations as it is highly tolerant to crude matrices. **The proprietary GCI technology allows Creoptix WAVE to obtain highly sensitive results**, and enables characterization of antibody kinetics directly from clinical blood plasma samples.

In this case study, standard amine coupling chemistry was used to immobilize three SARS-CoV-2 antigens (Spike ECD, Spike RBD and Nucleocapsid Protein) on a 4PCP **WAVEchip**. The binding responses of polyclonal anti-SARS-CoV-2 antibodies from patient plasma samples binding to the three viral antigens were determined (Figure 2).

The Creoptix WAVEsystem allows similar measurements at higher plasma (and serum) concentrations, which means **antibodies can be quantified without dilution**. The system therefore **offers better limits of detection**, as dilution is often responsible for the perceived limit of detection of traditional label-free technologies.

In summary, this study shows that the system was highly effective at bringing detailed insight into early immune response in current infection (IgM) and is **ideal for characterization directly from clinical blood plasma samples**.

Deeper insights for faster progress

The four studies highlighted clearly demonstrate that Creoptix WAVEsystem brings unprecedented benefits to numerous areas of the research and analytical laboratory through detailed kinetics studies. Whether screening crude mixtures, or characterizing drug hit compounds, antibodies and other biologics, the Creoptix WAVEsystem provides you with sensitive and rapid label-free analysis to unlock deeper insight in your studies.

To learn more about how the WAVEsystem can help you with label-free interaction analysis, **contact our expert team** or **book a demo today**.

1 Murray, J.B. et al. 2014. Off-Rate Screening (ORS) By Surface Plasmon Resonance. An efficient method to kinetically sample hit to lead chemical space in unpurified reaction products. J. Med. Chem. 57, 2845-1850 doi: /10.1021/jm401848a

2 van Heeke, G. et. al. 2017. Nanobodies as inhaled biotherapeutics for lung diseases. Pharmacol Ther. 169, 47-56. doi: 10.1016/j.pharmthera.2016.06.012

3 Zimmermann, I. et al. 2018. Synthetic single domain antibodies for the conformational trapping of membrane proteins. eLife 7:e34317. doi: 10.7554/eLife.34317

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