

1 Novel sensor-integrated proteome on chip (SPOC) platform with thousands of folded proteins on a 1.5
2 sq-cm biosensor chip to enable high-throughput real-time label-free screening for kinetic analysis.

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7 **Abstract**

8 An automated proteomic platform for producing and screening an array of functional proteins on
9 biosensor surfaces was developed to address the challenges of measuring proteomic interaction kinetics
10 in high throughput (HTP). This technology is termed Sensor-Integrated Proteome On Chip (SPOC®) which
11 involves *in-situ* cell-free protein expression in nano-liter volume wells (nanowells) directly from rapidly
12 customizable arrays of plasmid DNA, facilitating simultaneous capture-purification of up to 2400 unique
13 full-length folded proteins onto a 1.5 sq-cm surface of a single gold biosensor chip. Arrayed SPOC sensors
14 can then be screened by real-time label-free analysis, including surface plasmon resonance (SPR) to
15 generate kinetic affinity, avidity data. Fluorescent and SPR assays were used to demonstrate zero crosstalk
16 between protein spots. The functionality of the SPOC protein array was validated by antibody binding
17 assay, post-translational modification, mutation-mediated differential binding kinetics, and catalytic
18 activity screening on model SPOC protein arrays containing p53, Src, Jun, Fos, HIST1H3A, and SARS-CoV-2
19 receptor binding domain (RBD) protein variants of interest, among others. Monoclonal antibodies were
20 found to selectively bind their target proteins on the SPOC array. A commercial anti-RBD antibody was
21 used to demonstrate discriminatory binding to numerous SARS-CoV-2 RBD variants of concern with
22 comprehensive kinetic information. With advantages of HTP, flexibility, low-cost, quick turnaround time,
23 and real-time kinetic affinity profiling, the SPOC proteomic platform addresses the challenges of
24 interrogating protein interactions at scale and can be deployed in various research and clinical
25 applications.

26 **Introduction**

27 Protein interactions are core to innumerable biological functions that occur within the complex
28 milieu of a cell and determine many core processes such as DNA transcription and translation, metabolic
29 processing, regulation of enzyme turnover, intra- and inter-cellular signal transduction, and motor
30 functions and cytoskeletal rearrangement, among many others. These protein interactions are
31 characterized by unique binding affinities determined by a dynamic balance between association and
32 dissociation rates amongst free and bound complexes. As a result, measuring the kinetic parameters of
33 protein interactions with other proteins, DNA, RNA, metabolites and other biomolecules is critical to the
34 functional characterization of biological interactions, development of more effective drug molecules and
35 vaccines, and the discovery of new biomarkers and phenotypes. Therefore, to comprehensively
36 characterize dysregulated protein interaction networks for disease diagnostics, and to assess the potency
37 and safety of drug candidates during preclinical development, next-generation proteomic tools must be
38 capable of capturing the kinetics of numerous and diverse protein interactions.

39 Functional protein characterization through measurement of interaction kinetics is key in many
40 disease areas, including cancer, neurodegenerative diseases, and autoimmunity. In cancer, driver
41 mutations can have a variety of effects on protein activity commonly leading to gain or loss of function,
42 and acquisition of drug resistance. Across over 33 different cancer types and subtypes, missense
43 mutations have been observed to be disproportionately enriched at protein-protein interfaces, indicating
44 that mutations that disrupt interaction networks between proteins are common and would require kinetic
45 screening to unravel their impacts in detail (Cheng et al. 2021). In this context, high-throughput
46 proteomics technologies with the capability to characterize hundreds of cancer-associated mutations and
47 isoforms at the level of protein function would hasten the mechanistic understanding of the consequences
48 of these mutations, and further inform and improve the accuracy of advanced computational tools for
49 driver mutation identification. In regards to the immune system, binding kinetics play a big role in
50 evaluating important immune parameters such as infectivity of a new viral variant, efficacy of a new
51 drug/neutralizing antibody, and immune parameters related to the development of autoimmunity such
52 as the efficiency of central tolerance, efficiency of T-cell priming, and the efficiency of destroying a target
53 cell expressing an autoantigen (Koehli et al., 2014). Neurodegenerative diseases, such as Alzheimer's,
54 Parkinson's, and Huntington's, are characterized by the abnormal accumulation and aggregation of
55 specific proteins within the brain, leading to the formation of pathological structures. Understanding the
56 kinetics of protein interactions, including their rates of synthesis, folding, misfolding, aggregation, and
57 degradation, is crucial for unraveling the molecular mechanisms involved in neurodegenerative diseases.

58 Kinetic studies enable researchers to elucidate the temporal aspects of these processes, helping to
59 identify critical stages and potential therapeutic targets. In pharmacology and drug development, the
60 kinetics of protein-drug interactions are crucial for determining the pharmacodynamics of therapeutic
61 compounds. Likewise, in diagnostic applications, kinetic information can reveal additional information
62 that current endpoint protein assays are unable to resolve, such as early detection and disease sub-typing.
63 For example, in autoimmune disease diagnostics, autoantibodies (AAb) which have characteristic binding
64 strength for their target antigens (avidities/affinities) may be predictive of disease pathogenesis and
65 severity. For instance, in systemic autoimmune rheumatic diseases the presence of low affinity anti-
66 citrullinated protein antibodies (ACPAs) among patients with rheumatoid arthritis is linked to increased
67 presence of AAb synovial tissue penetration and more severe joint destruction (van der Woude et al.,
68 2010; Suwannalai et al., 2014). This kind of information is unavailable via endpoint protein assays.

69 Drug screening and vaccine development, infectious disease surveillance and diagnostics efforts
70 would benefit greatly from availability of technology platform for large-scale kinetic profiling of protein
71 interactions. Unfortunately, despite the significant progress in proteomics, there remains a notable deficit
72 in tools available for measuring protein binding kinetics in high throughput (HTP). Although fluorescence-
73 based protein microarrays are commonly used for protein research, they are primarily suited for
74 qualitative and semi-quantitative analysis where a relatively simple, low-medium-high scale of protein
75 binding is needed. These do not output quantitative data needed for kinetic analysis. Protein interactions
76 are transient equilibrium reactions and by leveraging label-free real-time biosensing approaches it
77 becomes possible to directly measure kinetic parameter such as rates of association (k_a) and dissociation
78 (k_d), dissociation half-life ($t_{1/2}$), maximal feasible signal generated by the interaction between a
79 ligand/analyte pair (R_{max}), etc. The equilibrium dissociation constant (K_D) or affinities, avidities can then
80 be computed from these kinetic parameters, which can otherwise only be approximated by fluorescence-
81 based approaches (Nagata et al., 2000). Various approaches exist for measuring protein kinetics, each
82 with distinct advantages and limitations. Isothermal titration calorimetry (ITC) offers detailed
83 thermodynamic information, such as binding constants, enthalpy, and entropy changes, but is low
84 throughput and requires large sample input, making it less suitable for weak or fast-binding systems
85 (Wang et al., 2020). Fluorescence Resonance Energy Transfer (FRET) allows for real-time monitoring of
86 conformational changes and interactions, particularly in live cells, but relies on fluorescent labeling, which
87 is prone to photobleaching and background fluorescence (Liao et al., 2020) and is not fully compatible
88 with HTP kinetic screening. Stopped-Flow spectroscopy is adept at studying rapid reactions on a

89 millisecond timescale but is limited to fast processes and may demand a substantial sample size (Zheng
90 et al., 2015). Another method, Electrophoretic Mobility Shift Assay (EMSA) is a simple and cost-effective
91 technique measuring changes in protein migration through a gel matrix in response to binding to specific
92 ligands like nucleic acids. However, it primarily applies to specific interactions, such as DNA-protein
93 binding, and may not provide kinetic data (Hellman and Fried, 2007). Nuclear Magnetic Resonance (NMR)
94 Spectroscopy detects changes in nuclear magnetic resonance signals during protein-ligand interactions,
95 offering structural, dynamic, and kinetic insights (Maity et al., 2019). However, NMR necessitates stable
96 isotopic labeling, may not suit all systems, and is often constrained to relatively small proteins or
97 complexes and is not an HTP method. Bio-layer interferometry (BLI) is another recent technique that
98 monitors changes in interference patterns due to binding interactions between immobilized ligands and
99 analyte proteins (Shah and Duncan, 2014). BLI is real-time and label-free and attractively available in high-
100 throughput (HTP) instrument formats capable of monitoring interactions from 96- and 384-well plates,
101 however the throughput can be at the cost of sensitivity and reproducibility compared to other, well-
102 established label-free formats (Yang et al. 2016). Surface plasmon resonance (SPR) stands at the forefront
103 of protein kinetics measurement, measuring changes in refractive index as molecules, such as proteins,
104 bind and dissociate from a biomolecule or ligand coated gold biosensor surface. This method offers real-
105 time, label-free, quantitative data, including kinetics and affinity information. However, this technique
106 demands specialized equipment, which can be costly. Current state-of-the-art SPR systems, like GE's
107 Biacore, excel in quantitative protein binding analysis but has limitations in terms of throughput (Maity et
108 al., 2019; Shah and Duncan, 2014).

109 The lack of accessible proteomic tools to produce an array of full-length proteins and measure
110 binding kinetics in HTP hinders the ability to comprehensively study and understand the dynamics of
111 protein interactions in complex biological systems. Importantly, the high cost of producing pure, fully
112 folded and functional protein microarrays remains a challenge. Current methods for producing protein
113 arrays rely on the laborious, time consuming and often cost-prohibitive method of expressing individual
114 recombinant proteins in a cell-based or cell-free system, followed by purification and manual or robotic
115 spotting of the affinity-purified recombinant proteins. Protein purification is time-consuming and
116 expensive, and with purified commercial proteins costing upwards of \$250 per protein, many labs lacking
117 the proper infrastructure or expertise are locked out of functional proteomic studies. In terms of stability,
118 traditional methods require low-temperature storage conditions to maintain the structural integrity and
119 activity of recombinant proteins throughout the entire process, increasing costs and often leading to loss

120 of materials and sample integrity. Consequently, cost can often add up significantly when scaled to
121 screening thousands of proteins. Therefore, traditional methods of expression and robotic immobilization
122 of individual proteins, either purified or from lysate, to produce functional protein arrays lack scalability,
123 flexibility, versatility and customizability and cannot feasibly address challenges of measuring proteomic
124 interaction kinetics in HTP. Promisingly, cell-free expression such as nucleic acid programmable protein
125 array technology (NAPPA; Takulapalli et al., 2012; Ramachandran et al., 2004; Link and LaBaer et al.,
126 2008a; Link and LaBaer et al., 2008b; Yu et al., 2017) and Isolated Protein Capture (IPC; Karthikeyan et al.,
127 2016) represent significant milestones in protein microarray production methods that have considerably
128 reduced the cost of protein microarray production while enabling facile array customization. However,
129 measuring kinetic analysis of thousands of protein interactions simultaneously remains a challenge as
130 most cell-free protein microarray platforms, including traditional NAPPA technology, are designed for
131 label-based end-point detection methods such as fluorescence or luminescence. The reliance on these
132 label-based end-point readout of analyte binding to arrayed proteins implies only a semi-quantitative and
133 indirect measure of any given interaction.

134 Given the relevance of kinetics in proteomics it has become increasingly important to develop
135 flexible, scalable proteomic tools with multiplex and HTP capabilities that combine scaled production of
136 proteins, real-time label-free biosensing and kinetic analysis. One way to achieve this important capability
137 is by integrating recent advances in SPR biosensing with *in situ* cell-free expression and IPC approaches
138 for making and screening functional protein arrays. Recently, researchers combined cell-free protein
139 expression and SPR to study protein interactions using mutants of p53 and oncoprotein MDM2 as a model
140 system (Fuentes et al., 2021). Their method circumvents IPC by directly printing plasmid DNAs for
141 expressing various p53 mutants on a gold SPR sensor as in traditional NAPPA on glass. Another SPR
142 method involves transcription and translation of RNA from surface-bound double-stranded DNA (dsDNA)
143 templates for the on-chip multiplexed biosynthesis of aptamer and protein microarrays in a microfluidic
144 format (Fasoli and Corn, 2015). However, the notable caveat with these methods is that the presence of
145 impurities (components of the print mix and DNA) can impair downstream kinetic analysis by SPR due to
146 introduction of components that crowd the evanescent field wave which can reduce sensitivity, block
147 access to the ligand by incoming analytes and lead to mass-transport effects, or introduce non-specific
148 binding in certain applications. The HuProt Human Proteome Microarray is a multiplexed platform that
149 requires robotic printing of all 21,000 proteins on its database from a pre-purified recombinant protein
150 stock; however, expressing and purifying thousands of protein stocks, followed by printing individual

151 21,000 proteins on an array is not only cumbersome and time-consuming but causes loss in functionality
152 in addition to being expensive and non-customizable. Importantly, HuProt arrays use end-point
153 fluorescence detection and hence are incompatible with kinetic analysis.

154 To address the challenges of making functional protein microarrays in HTP with low-cost, quick
155 turnaround, and which are readily customizable and allow for real-time, large-scale measurement of
156 interaction kinetics, SPOC Proteomics, Inc. has developed a novel Sensor-Integrated Proteome On Chip
157 technology (SPOC®) which automates the production and capture purification of *in situ* cell-free expressed
158 functional protein arrays for quantitative kinetic analysis. To the best of our knowledge, SPOC is the only
159 large-scale proteome array platform available today for large-scale study that overcomes the traditional
160 challenges of protein microarray technologies highlighted here.

161 **Results and Discussion**

162 **1. SPOC as a novel platform for high throughput production of proteins on biosensor chips**

163 SPOC is a first-of-its-kind proteomic biosensor system capable of delivering comprehensive
164 quantitative, qualitative, and kinetic data within a single large-scale assay. We have successfully
165 demonstrated *in situ* expression and simultaneous capture of proteins from immobilized plasmid DNAs
166 directly onto a functionalized biosensor chip yielding a functional protein array (or SPOC array) for label-
167 free analysis by SPR (**Fig. 1a**). The SPOC platform is scalable, versatile, and highly customizable. First, a
168 customized plasmid DNA array is created by printing the plasmids onto a 25 x 75 mm silicon nanowell
169 slide containing thousands of 2.0 nL volume nanowells, with center-to-center spacing of 225 to 375 μm
170 for 30,000 wells/slide (30k) and 10,000 wells/slide (10k), respectively (**Fig. 1b**). The assay is currently
171 configured such that proteins of interest are expressed from the printed plasmids as HaloTag fusion
172 proteins, i.e. proteins fused with Halo protein as a common tag, for later covalent capture on the array.
173 Next, the DNA-printed silicon nanowell slide is held in proximity to the surface of the biosensor capture
174 slide whose surface has been pre-functionalized with HaloTag chloro-alkane linker to covalently capture
175 the HaloTag protein, on the expressed fusion protein. A human HeLa-cell based, *in vitro* transcription and
176 translation (IVTT) lysate mix is then injected between the nanowell slide and biosensor slides which are
177 subsequently press-sealed against each other effectively isolating the nanowells to create nanoliter-
178 volume reaction chambers for isolated protein expression (IPC). The sandwich is then incubated at 30°C
179 for 2-4 hours during which the IVTT lysate expresses the protein encoded in the printed plasmid DNA

180 contained in each nanowell, which are simultaneously capture-purified onto the biosensor capture slide
181 forming the SPOC protein arrays. The entire process is facilitated by a custom AutoCap instrument
182 developed and manufactured in house. This instrument contains four independent expression channels
183 that enable four 25 mm x 75 mm nanowell slides to be expressed simultaneously, in a semi-automated
184 fashion. From each nanowell slide, 4 SPR biosensors containing SPOC arrays can be produced, yielding 16
185 SPOC chips from four channels within a single expression run. Importantly, the number of proteins that
186 can be arrayed on any capture slide is dependent on the size of the capture slide and density of the DNA-
187 printed wells in the silicon nanowell slides. For example, our 10k silicon nanowell slide design expresses
188 proteins in staggered pattern at a density of 716 protein spots/sq-cm which is equivalent to ~1000 spots
189 on the analysis area of the Carterra LSA^{XT} SPR instrument. However, our 30k nanowell slide design
190 produces up to 1,975 spots/sq-cm, or 2400 protein spots on the Carterra LSA^{XT} SPR analysis area. **Figure**
191 **1c** shows an actual protein array on a gold biosensor produced by SPOC, highlighting distinct spots where
192 individual proteins were immobilized. This innovative AutoCap instrument effectively reduces the cost by
193 an astounding 10-100 times compared to traditional workflows that involve recombinant protein
194 production and purification, making HTP proteomic screening more accessible.

195 **2. Functional validation of high-density SPOC array using Carterra LSA^{XT} SPR instrument**

196 SPOC technology is adaptable to any SPR instrument; however, for this publication, the Carterra
197 LSA^{XT} SPR and Horiba Scientific OpenPlex SPR imaging (SPRi) instruments were used for validation because
198 both instruments are amenable to HTP screening and require minimal effort to integrate with our SPOC
199 protein array platform. Proteins captured on SPR biosensor chips for kinetic assay are henceforth referred
200 to as protein-ligands. The functionality of the SPOC protein array is interrogated in this study using various
201 screens including: detection of IVTT expressed proteins at discrete spots using protein-specific antibodies,
202 differential binding kinetics of antibodies against variants of the same protein, demonstration of post
203 translational modification (PTM) capabilities of arrayed proteins, autophosphorylation activity of arrayed
204 proteins, and longitudinal stability of immobilized IVTT proteins after 10 days of slide storage.

205 We have demonstrated that SPOC arrays can be regenerated during SPR experiments, enabling
206 multiple sample injections and kinetic screening from a single SPOC protein biosensor chip (**Supplemental**
207 **Fig. S1**). The HaloTag-fusion proteins are covalently bound to the HaloTag chloro-alkane linker coated on
208 the SPR biosensor chips, and we have found that harsh, 10 mM Glycine-HCl (pH 2.4) regeneration buffer
209 conditions can be used for certain interactions without significant loss of biosensor integrity or interaction

210 detection, while a 2 M NaCl buffer formulation is preferable and as effective for more sensitive binding
211 interactions. SPOC is compatible with either condition depending on the analyte, and effectively strips
212 non-covalently bound analytes from the covalently bound HaloTag-fusion proteins that constitute the
213 SPOC array, enabling repeat sample injections for multiplexed applications on a single SPOC chip.

214 2.1 Antibody Reactivity and HTP-Deep Kinetic Analysis of SPOC Array: Protein-specific antibodies were
215 demonstrated to bind to their respective proteins on the SPOC array with high specificity. **Figure 2** shows
216 the result of antibody assays performed on SPOC protein chips screened via the Catterra LSA^{XT}, validating
217 the ability to detect multiple proteins on the SPOC array in HTP while providing primary kinetic
218 information. Locations of protein array spots on SPR biosensor chip are defined by region of interest (ROI)
219 selection, when viewing the optical image of the SPOC protein chip docked on the SPR instrument. To
220 enable mapping of protein array spots during ROI selection, a physical hockey-stick-shaped orientation
221 marker was created on the nanowell slide that is imprinted on the SPR slide during expression/capture of
222 the SPOC array and which is visually apparent on the SPR flowcell optical image (**Fig. 2a**). This is important
223 because the nanowell pattern/spots on the sensor slide can be mapped in reference to this marker to
224 identify the location of each protein on the SPOC array. The chip is probed with anti-HaloTag antibody
225 that binds to Halo protein at all expression spots, to ensure successful immobilization and capture of the
226 expressed HaloTag fused protein array. Due to software limitations, only 384 spots were initially selected
227 and analyzed at one time. **Figure 2b** shows representative sensorgrams from 384 ROI/spots on the SPOC
228 array, revealing successful capture of the HaloTag proteins expressed from the plasmids in the nanowell
229 slide. Varying signal intensity (R_{\max}) between ligand containing spots reflects differential expression and
230 capture levels between the SPOC array proteins. This is not unexpected as the parent plasmid DNA array
231 encodes genes of proteins with different sizes and characteristics, and therefore, varied levels of
232 expression. Different strategies are currently being explored to improve and normalize the protein
233 expression and capture levels, including (1) printing higher concentration of DNA or using linearized
234 plasmid DNA to increase expression levels and achieve self-normalization via capture surface saturation,
235 (2) maximizing the capture efficiency of the biosensor chip by optimizing self-assembled monolayer and
236 hydrogel surface conditions, and (3) encoding low-expressing genes on plasmids with high expression
237 promoters optimized for IVTT expression. Data normalization techniques are also being explored whereby
238 kinetic data obtained from reactive ligands are normalized to that of the ligand's respective HaloTag R_{\max}
239 signal. Low protein capture levels can limit sensitivity and analyte detection. However, protein capture
240 density is preferable in many cases to reduce mass transport and avidity effects. Overall, similar kinetic

241 rates and affinities can be estimated based on surfaces with varying ligand densities (Kamat and Rafique,
242 2017). Furthermore, within the current iteration of the SPOC array, bivalent analytes which can interact
243 with two protein-ligands simultaneously (such as the antibodies utilized in this study) can produce avidity
244 affects, when utilized as analytes in SPR assays which adds complexity to the measured kinetic data. It is
245 acknowledged that the kinetics of the observed antibody-analyte interactions reported are confounded
246 by the bivalency of the antibody analytes, and that the 1:1 binding modeling of the double-referenced
247 kinetic traces provide an imprecise estimate of the interaction kinetics. While the kinetics reported here
248 cannot be isolated from the analyte avidity, the kinetic dissociation constants are described as “affinity”
249 for simplicity. Methods to eliminate avidity effects by reducing and controlling the density of the captured
250 protein-ligands on the SPOC array such that only one of the two antibody paratopes can associate with a
251 single protein-ligand, favoring a 1:1 interaction, form part of future SPOC technology development
252 roadmap. This will be achieved by engineering and controlling the density of HaloTag chloro-alkane linker
253 coated on SPR biosensor chips, thereby controlling the proteins on biosensor surface to avoid avidity
254 effects.

255 **Figure 2c** emphasizes the highly specific detection of SPOC arrayed p53, Jun and Src when probed
256 with their respective protein-specific antibodies. For example, when the sensor was probed with anti-p53
257 (first column), only the p53-HaloTag protein spot was detected. There was no response from spots where
258 Jun and Src or other proteins were expressed and captured. Injection of anti-Jun (second column) showed
259 response for the location of the sensor where Jun was captured, but not p53 and Src. Similarly, anti-Src
260 could only detect Src proteins, highlighting the highly specific nature of SPOC assay. All spots showed
261 response with anti-HaloTag. The surface of the sensor was regenerated with 10 mM Glycine-HCl (pH 2.4)
262 between injections. Iso-affinity plot of the on- and off-rate measures estimated for the three commercial
263 antibodies against their respective protein targets demonstrate the kinetic rates measured across
264 multiple SPOC arrays are consistent (**Fig. 2d**). The iso-affinity plot clearly shows that the mouse anti-p53
265 antibody has a high affinity (avidity) with a $K_D = \sim 100$ pM while the mouse anti-Src spot replicates cluster
266 around a $K_D = \sim 1.0$ nM. Compared to the anti-p53 antibody, the iso-affinity plot reveals that this lower
267 affinity is driven by a slower on-rate, while the overall dissociation rate between the anti-p53 and anti-Src
268 antibodies is comparable. The mouse anti-Jun antibody had the lowest measured affinity clustering
269 around a K_D just under 10 nM with a similar on-rate to that of the anti-Src antibody, but a comparably
270 faster off-rate driving the lower affinity for the anti-Jun antibody. These results highlight the potential
271 utility of the SPOC platform in antibody drug development leveraged for biological target validation (both

272 on- and off-targets), and to screen artificial intelligence (AI) designed antibody clonal libraries to identify
273 those with desired specificity and affinity.

274 2.2 Ultra-High-Density SPOC arrays exhibit zero cross reactivity between adjacent protein spots: **Figure 3a**

275 shows a SPOC protein chip image produced from our ultra-HTP 30k array which can yield up to 2400
276 protein spots on the Carterra LSA^{XT} analysis area with zero crosstalk between neighbor spots. The sole
277 limitation of ultra-HTP SPOC analysis using the Carterra LSA^{XT} SPR instrument is the unavailability of
278 software to display and analyze kinetic information from 2400 proteins at once. The current Carterra LSA^{XT}
279 control and analysis software is capable of interrogating up to 384 spots, although a 1000 spot analysis
280 software is in development and is expected to be commercially available in near future.

281 Zero cross reactivity (CR) between ultra-HTP 2400 protein spots was demonstrated by probing the
282 30k SPOC chip containing multiple p53 (24 spots) and Src (12 spots) protein spots with mouse anti-p53
283 and anti-Src antibodies (**Fig. 3b**). A larger version of the 384 ROI sensorgrams from the 30k array after
284 anti-p53 and anti-Src antibody injection is reported in **Supplemental Figure S2** and **Supplemental Figure**
285 **S3**, respectively. Reproducibility was ensured by injecting a serial titration of each detection antibody (four
286 total injections each) followed by sensor regeneration with 10mM Glycine-HCl (pH = 2.37) between each
287 antibody injection (**Fig. 3c & d**). **Figure 3b** shows highly specific detections of p53 and Src protein spots,
288 indicated by an increase in their sensorgram response curves. Conversely, control spots around p53 and
289 Src showed no response, indicating that protein expressed in sealed nanowells does not diffuse and
290 capture at neighboring spots during the expression and capture step. This demonstrates close to zero
291 crosstalk between spots. The iso-affinity plot of anti-p53 and anti-Src antibodies shows clustering of
292 kinetic parameters from replicate spots, indicating minimal variations in on-rate estimates while the off-
293 rate estimates were more variable (**Fig. 3e**). The variability in the off-rate estimates appear specific to the
294 30k array as compared to the kinetics measured on the 10k array (**Fig. 2**) and may be due to the challenges
295 such small spots mount to precise ROI placement and could be improved with better reference ROI
296 selection and placement during sensor installation.

297 2.3 Analysis of SPOC Arrays using Orthogonal OpenPlex SPRi Instrument: **Figure 4** shows the result of SPRi

298 analysis using data obtained from a SPOC array on the lower throughput OpenPlex SPRi instrument
299 (Horiba Scientific). The OpenPlex analysis area is approximately 1.0 sq-cm and therefore using our 10k
300 silicon nanowell slide design we are able to interrogate up to 500 SPOC proteins (**Fig. 4a**). As expected,
301 the orientation marker was visible on the flowcell image (**Fig. 4a**). In 'SPR imaging' based configurations

302 like the OpenPlex instrument, the CCD camera which monitors the amount of reflected light from the
303 sensor is fixed at an angle close to the SPR reflectance dip or minimum (the point at which most of the
304 light is no longer reflected due to the surface-plasmon effect). Analyte binding to ligands on the sensor
305 surface leads to a refractive index change that shifts the minimum and thus the camera records a
306 concomitant increase in reflected light at the area of the sensor where binding is occurring, which is
307 expressed as percent reflectivity in the sensorgrams. Due to this unique SPRi configuration, a difference
308 image can be generated which shows the view from the CCD camera as analytes bind and produce a signal
309 in the form of increased reflected light. At baseline, the difference image is black as it is set near the
310 minimum and records little reflected light. In our case, as analytes bind to the ligand on the SPOC array,
311 the spots themselves become visible in the difference image and interactions at discrete locations of the
312 array can be visualized while the instrument records the magnitude of the percent reflectivity change
313 occurring at each spot on the array. The signal visualized from the difference image can be directly
314 correlated to the kinetic traces plotted in the sensorgrams generated from the OpenPlex. The SPOC array
315 becomes plainly visible in the OpenPlex difference image after injection of 133 nM anti-HaloTag antibody
316 which binds the HaloTag fusion proteins immobilized on the chip (**Fig. 4b**). The SPRi difference image
317 showed positive detection of many HaloTagged proteins on the array. The varying intensity signals is
318 consistent with our observation in **Fig. 2b**, highlighting differential expression and capture levels between
319 SPOC proteins at expected locations based on the printed DNA (array map for the OpenPlex sensor is
320 shown in **Supplemental Figure S4**). The OpenPlex SPRi instrument was also used to detect protein-specific
321 antibody interaction on the SPOC array. **Figures 4c** and **4d** shows the difference image and sensorgrams,
322 respectively, of two Jun spots on the SPOC array following interrogation with three concentrations of anti-
323 Jun antibody (50, 100, and 200 nM). **Figures 4e** and **4f** depict similar information for four SPOC p53 protein
324 spots after interrogation with 1.78 and 17.8 nM injections of anti-p53 antibody. These results show highly
325 specific detection of SPOC proteins, with zero cross reactivity. The OpenPlex iso-affinity plot reveals
326 minimal variation in computed kinetic parameters for the anti-Jun antibody, with higher variation for the
327 anti-p53 kinetic measures between replicates (**Table 3**). Comparison of the K_D values obtained from the
328 manual injection-based OpenPlex instrument versus the highly automated and modern Carterra LSA^{XT}
329 showed differences in measured kinetic parameters for the interactions and may be due to instrument
330 features. The control/analysis software on our OpenPlex was dated and imaging-based SPR is generally
331 less sensitive with a limit of detection $\sim 5\text{-}10\text{ pg/mm}^2$ compared to other configurations like angular-based
332 instruments such as the Carterra LSA^{XT} which can be as low as 0.1 pg/mm^2 (Homola et al., 1999; Wang et
333 al., 2019). Discrepancies between the kinetic rates may also be due to the fact that the OpenPlex data

334 were collected from bare gold sensors derivatized with Thiol-PEG-NHS self-assembled monolayers,
335 whereas the SPOC arrays analyzed on the Carterra LSA^{XT} were performed on hydrogel HC30M slides
336 (Xantec).

337 Detection of post-translational citrullination of four HIST1H3A protein spots on the SPOC array
338 was demonstrated with OpenPlex instrument. Prior to on-slide citrullination, lack of citrulline group on
339 the HIST1H3A protein spots was confirmed by probing a freshly prepared SPOC array with anti-cit-
340 HIST1H3A, which also contained few protein spots not known to be citrullinated. As expected, after the
341 citrullination reaction and injection of anti-cit-HIST1H3A and secondary antibody, the resulting SPRI
342 difference image and graph of reflectivity change showed marked increase in HIST1H3A spots contrast
343 and reflectivity signals (**Supplemental Fig. S5**; green boxes). Conversely, the control protein spots showed
344 no signals, underscoring the potential of the SPOC platform for use in studying certain PTMs. This assay is
345 currently being optimized to output primary kinetic information.

346 **3. Orthogonal immunofluorescence validation of SPOC protein arrays captured on glass slides**

347 The production of SPOC arrays is not limited to biosensor chips alone, but also possible on a
348 standard 25 x 75 mm microscope glass slide to yield high-density protein arrays of up to 30,000 proteins
349 for semi-quantitative analysis by immunofluorescence (**Fig. 5a-c**). The functionality of the SPOC protein
350 array on glass was validated via series of experiments using an array containing replicates spots of
351 HaloTagged p53, Fos, Jun, FGA, HIST1H3A, among many others. Protein expression/capture were
352 confirmed by HaloTag antibody immunofluorescence assay (**Fig. 6a&b**). The SPOC array exhibited minimal
353 cross reactivity (between adjacent protein spots (**Fig. 6c**). Cross reactivity (CR) was determined by dividing
354 the average anti-p53 antibody signal in wells surrounding each of the 184 total p53 spots with the average
355 anti-p53 signal in p53 spots. The variation (CV) was also measured by dividing the standard deviation of
356 the fluorescent intensity of all p53 spots by the average fluorescent intensity of the same spots and
357 multiplying by 100 and was calculated to be 31% for the anti-Halo-probed slide shown in **Fig. 6c**, and 28%
358 for all four dense areas shown enlarged on **Fig. 6d**, yet, spots are still very clearly observable via antibody
359 stain.

360 In terms of functionality, the SPOC protein array on glass was shown to bind protein specific
361 antibodies after probing with anti-p53, anti-Fos, anti-cit-FGA, and anti-cit-HIST1H3A antibodies. These
362 antibodies specifically bound to only their protein targets on the SPOC array and were detected with a

363 secondary fluorescently-tagged antibody. **Fig. 6c** shows a representative image of p53 fluorescence signals
364 after probing with anti-p53, emphasizing nearly zero background CR. Further, the catalytic activity
365 function of enzymes displayed on SPOC array was demonstrated using Src kinase as a model. This protein
366 is capable of self (auto) phosphorylation on several of its tyrosine residues. To show activity, the SPOC
367 array was first dephosphorylated and probed with anti-phosphotyrosine antibody (**Fig. 6e**, Slide 1). No
368 signal was observed, indicating all phosphate groups were removed. On a matching slide, the SPOC array
369 was dephosphorylated and then incubated in kinase buffer containing ATP. Phosphorylation of Src protein
370 on this SPOC glass slide (Slide 2) was confirmed after staining with anti-phosphotyrosine antibody, as
371 expected (**Fig. 6.**). By contrast, if the SPOC glass slide was treated once more with dephosphorylation
372 buffer after kinase buffer incubation, no signal was observed from the anti-phosphotyrosine antibody (**Fig.**
373 **6e**, Slide 3). Further, post-translational modification of SPOC proteins was demonstrated by *in vitro*
374 citrullination of HIST1H3A and FGA proteins on a SPOC glass slide array using PAD2 enzyme. The results
375 show distinct anti-cit-FGA and anti-cit-HIST1H3A in test slides which were absent in the control slides
376 incubated in the same conditions minus PAD2 (**Fig. 6f**). Finally, protein-protein interaction functionality of
377 SPOC proteins was demonstrated using Jun proteins arrayed on a SPOC glass slide array (**Fig. 6g**). The slide
378 was probed with or without a recombinant Fos protein in solution and then detected with anti-Fos
379 antibody. The result revealed highly specific Fos signals on the two Jun protein spots (**Fig. 6g** left panel) as
380 well as Fos protein spots (positive control), but no Fos signal was detected on the Jun protein spots in the
381 control slide that was not probed with recombinant Fos (**Fig. 6g** right panel). Furthermore, the above Fos-
382 Jun interactions, capacity to introduce citrulline PTM to HIST1H3A, in addition to monitoring of antibody-
383 antigen interactions were observed to be stable and readily performed successfully after the SPOC arrays
384 had been stored for 10 days under optimized conditions (**Supplemental Figure S6**). These results
385 underscore the SPOC platform as a potential tool to study protein interactions, PTM, and protein activity
386 assays in HTP.

387 **5. SPOC platform for drug screening: Kinetic profiling of differential antibody response to SARS-CoV-2** 388 **Spike RBD variants:**

389 Considering the rapidly mutating SARS-CoV-2 virus responsible for the COVID-19 pandemic and
390 the need to develop platform technologies for assessing the potency of neutralizing antibody drugs for
391 variants of concern (VoC), we sought to validate if SPOC can resolve differential kinetics of antibody drug
392 binding to variants of SARS-CoV-2 receptor binding domain (RBD) using the Carterra LSA^{XT} for SPR analysis.
393 In addition to the wildtype Wuhan RBD, nine variants - Alpha, Delta, and Omicron variants and subvariants

394 BA.1, BA.5, BA.2.75, BQ.1, XBB.1.16, XBB.1.5 and BQ.1.1 were arrayed on a SPOC chip and then screened
395 for strength of binding to a commercial anti-RBD antibody (Proteintech Group, cat#: 67758-1) from two
396 different manufacturer lots. Results of orthogonal qualitative anti-HaloTag fluorescence manual spotting
397 assay revealed comparable protein expression levels profiles among the RBD variants as well as qualitative
398 differential binding of the anti-RBD antibody. This antibody bound robustly to Wuhan, Alpha, and Delta
399 RBD variants, but exhibited significantly lower binding affinity to Omicron BA1 (**Fig. 7a**) and binding was
400 undetectable with other variants (data not shown).

401 As expected, label-free screening by SPOC SPR provided additional information that was lacking
402 in the end-point fluorescence assay, with real-time SPR capable of detecting binding of the anti-RBD
403 antibody to all the RBD variants revealing differential kinetics, including variants that were undetected
404 using fluorescence assay (e.g. BA1). In the first SPOC SPR assay, a SPOC chip containing five RBD variants
405 and wildtype Wuhan was probed with Proteintech anti-RBD from manufacturer Lot 1. Evaluation of K_D
406 values revealed that Wuhan, Delta, and Alpha RBDs exhibited the strongest affinities to this antibody (K_D
407 = 70, 74, and 80 nM, respectively). These affinities were ~2-fold higher than K_D of Omicron variants BA.1
408 (127 nM), BA.5 (137 nM) and BA.2.75 (105 nM) and characterized by faster off-rates (**Supplemental Fig.**
409 **S7** and **Fig. 7c**). The primary anti-RBD was highly specific, and did not bind to hundreds of other Halo-
410 fusion proteins captured on the SPOC array (not shown).

411 In another set of experiments, a SPOC array of all nine SARS-CoV-2 RBD variants and Wuhan
412 wildtype was probed with the Proteintech anti-RBD from manufacturer Lot 2. K_D values of the Wuhan,
413 Alpha and Delta RBD variants were K_D of 15, 14, and 22 nM, respectively. The Omicron variants BA.1, BA.5,
414 and BA.2.75 clustered together with values of 60, 66, and 75 nM, respectively. The K_D values of other
415 variants - BQ.1, XBB.1.16, XBB.1.5 were calculated as 71, 54, and 85 nM, respectively. The response curve
416 of anti-RBD to BQ.1.1 variant was too low in this experiment and not computed by the analysis software.
417 A repeat experiment was performed with the Lot 2 anti-RBD antibody using a replicate SPOC chip
418 produced and screened one week apart. The result remained consistent, with the antibody exhibiting the
419 highest affinity to Wuhan, Alpha and Delta RBD (mean $K_D \pm$ standard deviation from both experiment with
420 Lot 2; 14.5 ± 0.7 , 15 ± 1.4 , and 26 ± 5.7 nM, respectively). The mean K_D values for BA.1, BA.5, BA.2.75,
421 BQ.1, and XBB.1.16 variants, were found to be 81.5 ± 30.4 , 75 ± 12.7 , 64.5 ± 14.8 , 61 ± 14.1 , and $54.5 \pm$
422 0.7 , respectively (**Fig. 7b**). In this repeat experiment, binding of the antibody against the XBB.1.5 variant
423 was too low to extract kinetic information. The lowest anti-RBD binding affinity was obtained against the
424 BQ.1.1 variant ($K_D = 120$ nM). The minimal SD observed in these replicate SPOC screening of the Lot 2 anti-

425 RBD validates the reproducibility of the SPOC assay. An iso-affinity plot of the estimated anti-RBD kinetics
426 against the RBD VoC from the two antibody lots is depicted in **Fig. 7c**. The result shows the trend of
427 differential binding kinetics similar in both lots, albeit with variations in K_D values (**Fig. 7c**). In both
428 screening assays, Wuhan, Alpha, and Delta RBD, which had the highest affinities, clustered together, while
429 the other variants induced lower affinity binding (**Table 4**).

430 Overall, the Proteintech anti-RBD antibody from Lot 2 demonstrated notably greater affinity
431 compared to Lot 1, underscoring the SPOC platform's potential for evaluating batch-to-batch variation in
432 GMP-manufactured antibody products. Although the exact epitope of the Proteintech anti-RBD used in
433 this experiment is unknown, the fact that the mutations present in the Omicron variant RBD significantly
434 reduces antibody recognition may suggest that the epitope is closer to the N-terminus of the domain
435 where the mutations on Omicron least overlap with those present in the other variants. Therefore, these
436 findings also highlight the potential value of the SPOC platform in pandemic response situations,
437 particularly when there is a need to promptly monitor and characterize the impact of mutations at
438 immunogenic viral epitopes, or evaluate the potential risk of immune escape or diminished vaccine
439 efficacy associated with any emerging novel variant.

440 **Conclusion**

441 SPOC is a first-of-kind platform for high throughput proteomic kinetic profiling on biosensors,
442 providing real-time label-free quantitative, qualitative, and kinetic affinity data. SPOC protein chips are
443 rapidly customizable, contain folded full-length proteins, and enables 10-100 times reduced cost of assay
444 due to the lack of requirement for laborious and expensive individual protein expression, pre-purification,
445 and printing. The democratization of kinetic profiling will be facilitated through the provision of SPOC
446 protein chips to both research and industry users as catalog array and off-the-shelf protein chips,
447 expediting their proteomics kinetics research workflow. Further, we have expanded our capacity for
448 flexible sensor development by creating and applying self-assembled monolayers on bare gold slides
449 which allows us to produce gold sensor chips with custom surface chemistries and to tailor SPOC
450 technology according to user needs and applications. We have developed processes to fabricate gold SPRI
451 slides from scratch to help reduce the overall cost of technology development and the SPR chips offered
452 to customers in future. The SPOC platform will enable researchers to customize protein panels for HTP
453 applications such as monoclonal antibody characterization, AI-designed drug validation, and preclinical
454 drug development to screen libraries of drug candidates against thousands of on- and off-protein targets.

455 Other potential application areas include determination of basic protein-protein interaction kinetics,
456 vaccine efficacy monitoring, serum profiling, biomarker discovery, and diagnostics.

457 **Methods**

458 **1. DNA preparation, SARS-CoV-2 RBD mutants, Recombinant plasmids construction:** Genes of interest
459 were cloned into *in vitro* transcription and translation-compatible expression vectors, including
460 pJFT7_nHALO (or cHALO) from DNASU plasmid repository, Biodesign Institute, Arizona State University
461 and custom designed pT7CFE1_nHalo (or cHalo) vectors. These expression vectors ensure *in vitro*
462 expression of proteins of interest with an N- or C-terminal HaloTag for immobilization on HaloTag chloro-
463 alkane linker functionalized surfaces. Most of the recombinant plasmids expressing the genes of interest
464 were either procured from DNASU plasmid repository or outsourced to our commercial vendors –
465 Genscript and Twist Biosciences for custom syntheses. Dispense plates for DNA printing were prepared
466 with recombinant plasmids at a concentration of 100 ng/ μ L.

467 **2. Preparation of silicon nanowell substrate for DNA printing:** Silicon slides consisting of thousands of
468 nanowells were fabricated at a semiconductor foundry and prepared for DNA printing by vapor-phase
469 deposition of (3-Aminopropyl)-triethoxysilane (APTES) on the surface using an internally optimized
470 protocol. To clean the silicon nanowell slides prior to printing, slides were placed in 50 mL conical tubes
471 containing distilled water and then centrifuged at 1,400 $\times g$ for 2 minutes, inverted and repeated
472 (Premiere, Model XC-2450 Series Centrifuge). Afterwards, slides were dried with a nitrogen gun and then
473 subjected to oxygen plasma treatment at 50W for 2 mins using a Plasma Etch instrument (Plasma Etch
474 Inc., Model PE-100HF). The nanowell substrates were immediately placed in a vacuum desiccator
475 containing 2 mL APTES in a vial, subjected to vacuum/filling for a total of 15 min using low pressure
476 nitrogen, and eventually incubated for 1.5 h under vacuum. Afterwards, APTES-modified nanowell
477 substrates were removed, cured at 100°C for 1 h, and stored in a Bel-Art autodesiccator (catalog number
478 420740117).

479 **3. Immobilization of unique expression plasmids in nanowells:** DNA printing was outsourced to
480 Engineering Arts, LLC and performed on the RM3 system. The technique employs high-speed piezo
481 printing with an au302 piezo dispense system, featuring an integrated alignment system designed for
482 microwell, micrometer angular alignment fixture, look down camera, transfer arm, and a vacuum tray
483 (Karthikeyan et al., 2016). First, silicon nanowell slides were placed and aligned on the vacuum tray using

484 the robotic transfer arm, micrometer angular alignment fixture, and look down camera. Print mix was
485 then printed in picoliter quantities “on-the-fly” using a multi-dispense head into each nanowell, followed
486 immediately by printing of picoliter quantities of expression plasmid DNAs from the dispense plates. The
487 print mix contained HaloTag chloro-alkane linker (O4) amine, which was varied from 0 to 1 mM (Promega,
488 P6741), 1.46 mg/ml BS3 in DMSO, and 7.4 mg/mL BSA, in nuclease-free water. The addition of HaloTag
489 chloro-alkane linker into the print mix was used for limited capture of expressed HaloTagged proteins at
490 the bottom of nanowells for expression validation using anti-HaloTag antibody via immunofluorescence.
491 After the piezo printing process, the nanowell slides were stored in an autodesiccator until they were
492 ready for use.

493 **4. Bio-functionalization of SPOC slides with HaloTag chloro-alkane linker:** Bare gold-coated slides
494 compatible with SPRi biosensing were purchased commercially, in addition to hydrogel coated slides
495 purchased from Xantec (HC30M). Bare gold slides used in OpenPlex SPOC array analysis were derivatized
496 with a self-assembled monolayer (SAM) using a 2 kDa Thiol-PEG-NHS reagent (BioPharma PEG; HE003023-
497 2K) towards HaloTag chloro-alkane linker modification and Halo-protein capture. For SAM assembly, 20
498 μ l of 200 μ M Thiol-PEG-NHS solution in DMSO was pipetted onto a clean microscope slide and the bare
499 gold SPR slide was placed gold-side down onto the solution. After 2 h incubation at room-temperature,
500 the slide was rinsed briefly in 200 Proof Ethanol and distilled water and dried with compressed air. For
501 preparation of HC30M slides for Carterra LSA^{XT} SPOC array analysis, sensors were activated with a 1:1:1
502 mixture of 0.4M N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC), 0.1 M N-hydroxysuccinimide
503 (NHS), and 0.1M 2-(N-morpholino) ethanesulfonic acid, pH = 5.5 (MES) for 10 minutes at room-
504 temperature. For either Thiol-PEG-NHS derivatized sensors or HC30M hydrogel slides, after rinsing with
505 distilled water and drying, the slides were incubated for either 1 hour or overnight with 1 mg/mL of
506 HaloTag chloro-alkane linker (Iris Biotech; RL-3680). The sensors were then incubated for 30 min 0.5 M
507 Ethanolamine (pH 8.5) to quench any remaining free-NHS esters.

508 **5. Automated *in-situ* production of SPOC array:** SPOC arrays were produced via automated IPC using a
509 custom AutoCap instrument designed and built in-house. Nanowells printed with DNA plasmids of interest
510 were filled via centrifugation in 50 mL conical tubes containing water for 4 min on each end at 1,400 x *g*,
511 followed by incubation in Superblock (ThermoFisher Scientific) for 30 min with gentle rocking. SPOC arrays
512 were blocked for 30 min in 0.5 M ethanolamine, pH 8.5 to quench any free ester groups. After blocking,
513 both the nanowell and SPOC slides were rinsed at least 3 times with water and dried gently with
514 compressed air. 1-Step Human Coupled IVT mix was prepared and centrifuged for 20 min at 19,000 RCF

515 at 4°C to remove particulates. The supernatant was gently removed to a fresh tube and stored at 4°C until
516 injection into the instrument. The automated IPC instrument contains four stations, each one capable of
517 holding one 25 mm x 75 mm nanowell slide. Each station contains a hydraulic actuator-driven base that
518 holds a nanowell slide surrounded by a silicone gasket. A hinged lid designed to fit the SPOC arrays in a
519 precise location above the nanowell slide is fitted onto the base of each station. Automated software was
520 designed in-house using LabView to control all mechanical parts of the instrument. For protein expression
521 and capture, the nanowell slide was placed on the base and the SPOC slide was mounted to the lid. The
522 lid was then affixed firmly to the base using wingnuts and, using the automated software, low pressure
523 (20 psi) was applied via the actuator, creating an airtight chamber containing the two slides with the SPOC
524 array positioned immediately above the nanowell slide with a small gap in between. Following application
525 of low pressure, the chamber and fluidic lines were vacuumed for a total of 12 min, with the goal of
526 evacuating air from the nanowells and chamber. Using a primed 500 µL Hamilton syringe, the lysate was
527 aspirated into the chamber via vacuum following automated valve switching from vacuum line to the
528 injection line. This process results in the nanowells and the small gap between the slides being
529 immediately filled with the lysate mixture. Once the slide space is filled (less than 10 sec), high pressure
530 (220 psi) is applied via the actuator to tightly press-seal the two slides together. The instrument space was
531 closed and brought to 30°C for 2-4 h for *in vitro* translation and expression (IVTT), with the nanowells
532 serving as nanoreactors for IVTT and the resulting HaloTagged protein being concurrently captured onto
533 the SPOC array. Following IVTT, the pressure is removed, and the slides are immediately washed in cold
534 1X PBST 3 times prior to assay to remove any unbound IVTT components and incompletely expressed
535 protein.

536 **6. Protein analysis on surface plasmon resonance instruments:** SPR experiments were performed on
537 Carterra LSA^{XT} SPR and Horiba Scientific OpenPlex SPRI instruments. SPOC protein array chips from the
538 expression/capture process were washed with 1X PBST and deionized water and dried with compressed
539 air prior to prism mounting and instrument installation.

540 6.1 Sensor response assay protocol using Carterra LSA^{XT}: The sensor for SPOC Carterra LSA^{XT} analysis was
541 prepared by mounting the SPOC chip onto a custom research-use-only plain-prism Carterra LSA^{XT} cartridge
542 using 15.0 µL of a refractive index matching oil (Cargille. Cat# 19586) and then inserted into the
543 instrument. Once installed, the single flow cell was docked onto the sensor and the sensor was primed
544 with running buffer (1X PBS pH 7.3, 0.2% BSA, 0.05% Tween-20; filtered and degassed). Sensor
545 temperature was maintained at 15°C for the duration of the experiment. Regions of interest (ROI) were

546 manually assigned over spots of interest in reference to the spot array orientation mark (hockey stick
547 mark). Antibody titrations were performed by diluting antibodies into running buffer followed by
548 performing 3, 3-fold serial dilutions resulting in a total of 4 serially diluted analyte samples with 300 μ L
549 volumes each. Association and dissociation rates varied from 4 to 30 min and 8 to 45 min, respectively
550 and all screens were performed using the QuickStart experiment menu of the Catterra LSA^{XT} control
551 software.

552 6.2 Sensor response assay protocol using the OpenPlex SPRi: The SPOC chip was mounted onto a Horiba
553 glass prism using 0.5 μ L of high index oil (Serie B, Part# 1300007816, Horiba Scientific). The sensor-coupled
554 prism was installed into the instrument by manually pressing onto a gasketed flowcell. The SPR Running
555 Buffer for OpenPlex consisted of freshly prepared 1X PBS added with 0.2% BSA, 0.05% Tween-20, filtered
556 using a 0.22 μ m bottle top filter (Corning; CLS430626) and de-gassed for 20 min. SPR Running Buffer was
557 initially flushed over the SPOC protein chip at a high flow-rate of up to 2,000 μ L/min to wet the sensor
558 surface and remove bubbles. Afterwards, the buffer flow rate was reduced to 50 μ L/min and the flowcell
559 temperature was set at 25°C for the remainder of the experiment. After ROI selection and instrument
560 calibration with 1% glycerol in the running buffer, 200 μ L analyte in running buffer was flowed over the
561 sensor surface via a sample injection loop with the association phase performed for 3.5 min followed by
562 5 min dissociation phase for all blank and analyte injections. Protein expression/capture on the SPOC chip
563 was confirmed by flowing 200 μ L of mouse anti-HaloTag (133 nM) in running buffer. Binding to protein-
564 specific antibodies and cross reactivity was validated by injecting mouse anti-Jun (200 nM) and mouse
565 anti-p53 (17.8 nM). On-slide post translational citrullination of four spots of unmodified Halo-HIST1H3A
566 proteins on SPOC array was performed on the OpenPlex instrument. Prior to this reaction, the chip which
567 also contained two protein spots each of Halo-RBD BA.1 and Halo-CT45A3 as controls was probed with
568 anti-Halo and 200 μ L of 1:50 rabbit anti-citrullinated HIST1H3A in running buffer to confirm protein
569 expression and the absence of citrulline group on the native HIST1H3A proteins. The citrullination reaction
570 was performed on the chip while mounted on OpenPlex instrument by re-circulating for 2 h at RT a 1 mL
571 citrullination reaction mixture containing 0.3 U/mL Peptidyl Arginine Deiminase (Sigma P1584-25UN), 10
572 mM CaCl₂, 100 mM Tris pH 7.6, and 5 mM DTT. After buffer wash, the anti-cit-HIST1H3A was injected,
573 followed by secondary anti-rabbit antibody injection to detect citrullinated HIST1H3A. The difference
574 image was captured and graph of reflectivity change for the HIST1H3A, BA.1 RBD and CT45A3 spots was
575 plotted.

576 6.3 Extraction of HTP Kinetic Information: Raw data from the Catterra LSA^{XT} were analyzed in Kinetics

577 analysis software (Carterra, v1.9.0.4167), while data from the OpenPlex were processed using
578 ScrubberGen2 (BioLogic Software, v2.0g). In all cases, the data were y-aligned and double-referenced via
579 blank, running buffer alone injection subtraction and referencing of the data against control spots of the
580 array where no binding was expected and observed. Once pre-processed, all data were globally fit in the
581 software using a 1:1 Langmuir binding model to obtain kinetic parameters and equilibrium dissociation
582 constants. Since the analytes used in the assay are bivalent, the data is complicated by avidity effects and
583 therefore the 1:1 binding model is acknowledged to yield only an approximation of the affinity for each
584 antibody-antigen interaction interrogated.

585 6.4 Regeneration of SPOC Chips: Different sensor regeneration conditions and buffers were tested to
586 validate functionality of SPOC proteins, including 0.5-2 M NaCl in 5 mM EDTA, 10-100 mM HCl, and 10
587 mM glycine-HCl, pH 2.4. In this experiment, SPOC array of 132 HaloTagged protein spots was mounted on
588 Carterra LSA^{XT}, followed by injection of anti-HaloTag antibody. The sensor surface was regenerated using
589 the different buffers and then probed again with anti-HaloTag. The process was repeated three times. In
590 each case, average response units \pm standard deviation from all 132 Halo-protein spots on the array was
591 calculated. A bar graph of the average response units for the initial anti-Halo injection and all three
592 regenerations was plotted (**Supplemental Figure S1**).

593 **7. Determination of differential binding kinetics of a commercial anti-SARS-CoV-2 Receptor Binding**
594 **Domain (RBD) antibody against SARS-CoV-2 RBD using SPOC**: Recombinant plasmid DNAs encoding
595 SARS-CoV-2 RBD domain variants Alpha, Delta, Omicron variants and subvariants BA.1, BA.5, BA.2.75,
596 BQ.1, XBB.1.16, XBB.1.5 and BQ.1.1, and the wildtype Wuhan RBD as HaloTag genes were printed in silicon
597 nanowell slides. Using the SPOC protein array production platform, RBD variants were expressed *in situ*
598 and capture-purified onto replicate SPOC chips as Halo-fusion protein arrays. The SPOC chip was screened
599 on the Carterra LSA^{XT} SPR instrument. Protein expression/capture was validated by injecting 133 nM of
600 anti-HaloTag antibody, followed by surface regeneration using 2 M NaCl and buffer wash prior to probing
601 with anti-RBD antibody. SPOC RBD chips were screened with two different manufacturer lots of a
602 commercial mouse anti-RBD from Proteintech Group (catalog number: 67758-1) to determine the
603 differential binding kinetics to the RBD variants. To ensure reproducibility, assays were performed
604 multiple times using SPOC chips produced at separate protein expression/capture runs and screened
605 weeks apart. Also, serial concentrations 19.75, 59.26, 177.78, and 533.33 nM of each anti-RBD Lot was
606 injected in succession to confirm sensor response curves. In the first replicate SPOC assay, SPOC chip
607 containing the Wuhan wildtype RBD and five RBD variants – Alpha, Delta, Omicron BA.1, BA.5, BA2.75 was

608 probed with the mouse anti-RBD from manufacturer Lot 1. The second and third assays were similar and
609 performed with SPOC chips containing all nine variants and the Wuhan wildtype and probed with the
610 mouse anti-RBD from Lot 2. The association/on- rate (10 min) and dissociation/off- rates (15 min) kinetics
611 were monitored during injections, and sensor surface was regenerated using 2 M NaCl between injections.
612 The differential binding was validated orthogonally by immunofluorescence assay using the mouse anti-
613 RBD and secondary goat anti-mouse Cy3.

614 **8. Antibodies used for label-free and fluorescent SPOC assays:** Mouse anti-HaloTag antibody utilized for
615 confirming expression of IVTT expressed HaloTag-fusion proteins by SPR was purchased in glycerol-free
616 format from Chromotek (28a8), while a rabbit anti-HaloTag from Promega was used for fluorescent-based
617 expression validation (G9281). Protein specific antibodies used in this study include mouse anti-Jun
618 (ThermoFisher, 39-7500), mouse anti-p53 (Sigma-Aldrich, P6874), mouse anti-Src (ThermoFisher,
619 AHO1152), mouse anti-RBD (Proteintech, 67758-1), rabbit anti-Fos (14C10) rabbit anti-citHIST1H3A
620 (Abcam, ab5103), rabbit anti-citFGA (ImmunoPrecise, MQ13.102), and anti-phosphoTyrosine
621 (ThermoFisher, 03-7700). For fluorescent assays, secondary antibodies used include goat anti-Rabbit-Cy3
622 (Jackson ImmunoResearch, 111-165-003) and goat anti-Mouse-Cy3 (Jackson ImmunoResearch, 115-165-
623 062).

624 **9. Fluorescence assays to validate functionality of SPOC proteins captured on regular 25 mm x 75 mm**
625 **glass slide:** *Validation of Halo-protein capture and binding to protein-specific antibodies* on SPOC glass
626 slide was confirmed via immunofluorescence by incubating the whole slide in 5% milk in 1X PBST
627 containing 1:750 rabbit anti-HaloTag or protein-specific antibodies (anti-p53, Jun, or Fos). After 30 min –
628 1 h of incubation at RT and washing with PBST, the slide was again incubated for 30 min with 1:500 dilution
629 of the appropriate secondary antibody fluor-conjugate in 5% milk-PBST. After the secondary incubation,
630 the slide was washed with PBST, rinsed with water, dried with compressed air, and scanned using an
631 InnoScan 910 AL Microarray Scanner (Innopsys, Carbonne, France). *In vitro citrullination of SPOC proteins*
632 was performed by incubating SPOC glass slides containing Halo-FGA and Halo-HIST1H3A with the
633 citrullination reaction mixture containing 0.3 U/ml Peptidyl Arginine Deiminase (Sigma P1584-25UN), 10
634 mM CaCl₂, 100 mM Tris pH 7.6, and 5 mM DTT. After incubation for 2 h and buffer wash, citrullinated FGA
635 and HIST1H3A on the SPOC glass slide were probed by incubating for 1 h with 1:500 rabbit anti-
636 citrullinated HIST1H3A or FGA and detected with goat anti-rabbit Cy3 using the InnoScan 910 AL
637 Microarray Scanner. PAD2 was omitted from control slides. *Protein-Protein Interaction assay on glass*
638 *slides* were performed by probing a whole SPOC glass slide containing two Halo-Jun and six Halo-Fos

639 protein spots with buffer solution containing 10 ug/mL commercial recombinant Fos protein in 1% milk-
640 PBST for 1 h at RT. Binding of the recombinant Fos protein to the SPOC Jun was detected by incubating
641 the SPOC glass slide with 1:500 rabbit anti-Fos antibody in 5% milk PBST and then goat anti rabbit Cy3,
642 followed by fluorescence scanning with the InnoScan 910 AL. *Enzymatic activity assays on SPOC glass*
643 *slides* were performed using dephosphorylation and autophosphorylation of Src kinase on SPOC array. To
644 ensure that endogenous phosphorylation of Src from IVTT expression is not being detected, the slide was
645 first dephosphorylated by incubating for 30 min at 37°C with assay mixture containing 0.5 units of calf
646 intestinal alkaline phosphatase in 1X dephosphorylation buffer (Thermo Scientific, 18009019) and 1 mM
647 MgCl₂, followed by 3 washes with Tris-buffered saline. The removal of phospho groups from immobilized
648 Src kinase was confirmed by staining with anti-phosphotyrosine antibody. Next, auto-phosphorylation of
649 the Src was performed by incubating the SPOC glass slide for 45 min at RT in phosphorylation buffer
650 containing 4 mM ATP, 50 mM Tris pH 7.6, 5 mM MgCl₂, and 0.5 mM DTT. The phosphorylated Src was
651 then detected via anti-phosphotyrosine antibody.

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724 whose invaluable contributions were pivotal in the initial validation of the SPOC technology. Our gratitude
725 extends to the Carterra R&D team, particularly Dr. Rebecca Rich and Noah Ditto who supported the
726 integration of SPOC proteomic biosensor platform with the Carterra LSA^{XT} instrument.

727 **Author Contributions**

728 B.T conceived the experiments. W.M, C.V.A, and R.C performed the experiments, analyzed the data and
729 prepared the figures. M.M, L.G helped with project planning and joined the discussions. C.V.A drafted the
730 manuscript. All authors reviewed and approved the manuscript.

731 **Data availability statement**

732 The authors confirm that the data supporting the findings of this study are available within the article and
733 its supplementary materials.

734 **Figure legends**

735 **Figure 1 | Schematic of *in situ* protein production in nanowell slide and capture on SPOC biosensor chip.**
736 (a) By leveraging human cell-free *in vitro* transcription and translation (IVTT) lysate, SPOC arrays are

735 **Table 1 | 10k SPOC array kinetic parameters derived from three protein specific antibody analytes**
 736 **binding to their respective target ligands as measured on the Carterra LSA^{XT} instrument.** Reported values
 737 were utilized to generate the iso-affinity plot in Fig. 2d.

Analyte	SPOC Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
Mouse anti-p53	p53 - Spot 1	1.8E+06	2.1E-04	1.17E-10
	p53 - Spot 2	1.7E+06	1.7E-04	1.00E-10
	p53 - Spot 3	1.6E+06	2.8E-04	1.75E-10
	p53 - Spot 4	1.8E+06	1.8E-04	1.00E-10
	p53 - Spot 5	1.8E+06	1.6E-04	8.89E-11
	p53 - Spot 6	1.9E+06	1.6E-04	8.42E-11
	p53 - Spot 7	1.6E+06	1.3E-04	8.13E-11
	p53 - Spot 8	1.5E+06	2.1E-04	1.40E-10
	p53 - Spot 9	1.7E+06	1.6E-04	9.41E-11
	p53 - Spot 10	1.8E+06	1.7E-04	9.44E-11
	p53 - Spot 11	1.8E+06	2.2E-04	1.22E-10
	p53 - Spot 12	1.9E+06	2.3E-04	1.21E-10
	p53 - Spot 13	2.4E+06	3.1E-04	1.29E-10
	p53 - Spot 14	2.2E+06	1.7E-04	7.73E-11
	p53 - Spot 15	2.1E+06	5.2E-05	2.48E-11
	p53 - Spot 16	2.3E+06	2.3E-04	1.00E-10
	p53 - Spot 17	2.3E+06	1.9E-04	8.26E-11
	p53 - Spot 18	2.5E+06	1.8E-04	7.20E-11
	p53 - Spot 19	2.2E+06	1.7E-04	7.73E-11
	p53 - Spot 20	2.3E+06	1.6E-04	6.96E-11
Mouse anti-Jun	Jun - Spot 1	3.6E+05	1.4E-03	3.89E-09
	Jun - Spot 2	1.4E+05	1.2E-03	8.57E-09
	Jun - Spot 3	1.9E+05	9.4E-04	4.95E-09
Mouse anti-Src	Src - Spot 1	1.7E+05	2.7E-04	1.59E-09
	Src - Spot 2	2.2E+05	2.8E-04	1.27E-09
	Src - Spot 3	1.2E+05	2.1E-04	1.75E-09
	Src - Spot 4	1.4E+05	1.5E-04	1.07E-09
	Src - Spot 5	1.3E+05	2.2E-04	1.69E-09
	Src - Spot 6	1.3E+05	1.9E-04	1.46E-09
	Src - Spot 7	1.5E+05	1.5E-04	1.00E-09
	Src - Spot 8	1.5E+05	2.2E-04	1.47E-09
	Src - Spot 9	1.5E+05	1.2E-04	8.00E-10

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740 **Table 2 | 30k SPOC array kinetic parameters derived from two protein specific antibody analytes binding**
 741 **to their respective target ligands as measured on the Catterra LSA^{XT} instrument.** Reported values were
 742 utilized to generate the iso-affinity plot in **Fig. 3e.**

Analyte	SPOC Ligand	k_a (M ⁻¹ s ⁻¹)	k_d (s ⁻¹)	K_D (M)
Mouse anti-p53	p53 - Spot 1	8.4E+05	6.6E-04	7.86E-10
	p53 - Spot 2	1.0E+06	2.5E-05	2.50E-11
	p53 - Spot 3	7.7E+05	3.0E-05	3.90E-11
	p53 - Spot 4	8.7E+05	5.8E-05	6.67E-11
	p53 - Spot 5	5.5E+05	4.3E-05	7.82E-11
	p53 - Spot 6	8.5E+05	6.3E-05	7.41E-11
	p53 - Spot 7	8.5E+05	1.1E-04	1.29E-10
	p53 - Spot 8	8.3E+05	1.1E-05	1.33E-11
	p53 - Spot 9	8.0E+05	4.8E-05	6.00E-11
	p53 - Spot 10	9.3E+05	6.9E-05	7.42E-11
	p53 - Spot 11	8.8E+05	9.2E-05	1.05E-10
	p53 - Spot 12	1.1E+06	2.1E-05	1.91E-11
	p53 - Spot 13	9.6E+05	2.3E-05	2.40E-11
	p53 - Spot 14	7.9E+05	4.0E-05	5.06E-11
	p53 - Spot 15	8.3E+05	8.1E-05	9.76E-11
	p53 - Spot 16	8.5E+05	1.1E-04	1.29E-10
	p53 - Spot 17	8.7E+05	2.9E-05	3.33E-11
	p53 - Spot 18	8.1E+05	6.6E-05	8.15E-11
	p53 - Spot 19	1.0E+06	1.4E-04	1.40E-10
	p53 - Spot 20	8.8E+05	7.5E-05	8.52E-11
	p53 - Spot 21	8.9E+05	8.5E-05	9.55E-11
	p53 - Spot 22	9.1E+05	1.4E-05	1.54E-11
	p53 - Spot 23	9.0E+05	2.1E-04	2.33E-10
	p53 - Spot 24	8.4E+05	5.3E-05	6.31E-11
	p53 - Spot 25	8.1E+05	2.9E-05	3.58E-11
	p53 - Spot 26	8.5E+05	3.6E-05	4.24E-11
Mouse anti-Src	Srs - Spot 1	1.4E+05	1.8E-04	1.29E-09
	Srs - Spot 2	1.4E+05	1.3E-04	9.29E-10
	Srs - Spot 3	1.6E+05	8.9E-05	5.56E-10
	Srs - Spot 4	1.7E+05	1.4E-05	8.24E-11
	Srs - Spot 5	1.4E+05	8.3E-05	5.93E-10
	Srs - Spot 6	1.7E+05	2.9E-05	1.71E-10
	Srs - Spot 7	1.4E+05	4.6E-05	3.29E-10
	Srs - Spot 8	1.9E+05	2.1E-05	1.11E-10
	Srs - Spot 9	1.5E+05	1.3E-04	8.67E-10
	Srs - Spot 10	1.4E+05	3.7E-05	2.64E-10
	Srs - Spot 11	1.7E+05	5.0E-05	2.94E-10
	Srs - Spot 12	9.1E+04	7.5E-05	8.24E-10
	Srs - Spot 13	1.5E+05	2.4E-04	1.60E-09
	Srs - Spot 14	1.2E+05	2.3E-04	1.92E-09
	Srs - Spot 15	2.0E+05	1.2E-04	6.00E-10
	Srs - Spot 16	1.1E+05	1.4E-04	1.27E-09
	Srs - Spot 17	1.2E+05	1.6E-04	1.33E-09
	Srs - Spot 18	1.8E+05	5.9E-05	3.28E-10
	Srs - Spot 19	1.2E+05	9.4E-05	7.83E-10

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744

745 **Table 3 | 10k SPOC array kinetic parameters derived from two protein specific antibody analytes binding**
 746 **to their respective target ligands as measured on the OpenPlex instrument.** Reported values were
 747 utilized to generate the iso-affinity plot in **Fig. 4g.**

Analyte	SPOC Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
Mouse anti-p53	p53 - Spot 1	1.8E+06	5.3E-05	2.91E-11
	p53 - Spot 2	1.5E+06	5.0E-05	3.38E-11
	p53 - Spot 3	1.7E+06	5.0E-05	2.87E-11
	p53 - Spot 4	2.1E+06	5.6E-05	2.73E-11
	p53 - Spot 5	1.1E+06	2.1E-04	1.91E-10
	p53 - Spot 6	4.9E+05	1.8E-04	3.67E-10
	p53 - Spot 7	8.8E+05	2.7E-04	3.07E-10
	p53 - Spot 8	6.7E+05	1.2E-05	1.79E-11
	p53 - Spot 9	1.4E+06	1.5E-04	1.04E-10
	p53 - Spot 10	1.5E+06	1.5E-04	9.61E-11
Mouse anti-Jun	Jun - Spot 1	4.59E+04	8.30E-04	1.81E-08
	Jun - Spot 2	3.64E+04	8.10E-04	2.23E-08
	Jun - Spot 3	2.30E+04	9.50E-04	4.13E-08
	Jun - Spot 4	2.27E+04	8.40E-04	3.70E-08

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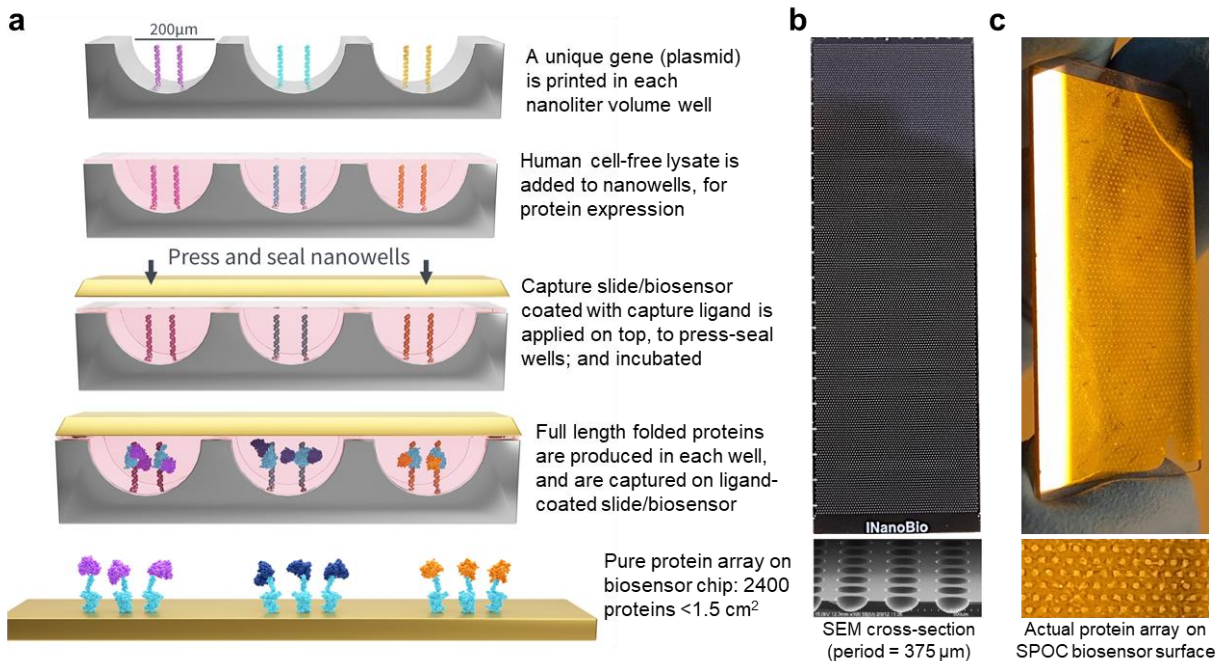
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750 **Table 4 | Kinetic parameters derived from the Proteintech mouse anti-SARS-CoV-2 RBD antibody**
 751 **analyte binding to Wuhan RBD and variants.** Reported values were utilized to generate the iso-affinity
 752 plot in **Fig. 7c**. Cells with dashes indicate interactions which were not measured, while cells with N/A
 753 indicate interactions where no kinetic parameters could be estimated due to low binding signal. The assay
 754 was performed on Carterra LSA^{XT}.

anti-RBD Ab Lot	Replicate	Kinetic Measure	Wuhan	Alpha	Delta	BA1	BA5	BA2.75	BQ.1	BQ.1.1	XBB.1.5	XBB.1.16
Lot 1	#1	k_a (M ⁻¹ s ⁻¹)	2.7E+03	2.60E+03	3.20E+03	2.90E+03	2.60E+03	2.30E+03	---	---	---	---
		k_d (s ⁻¹)	1.9E-04	2.10E-04	2.40E-04	3.70E-04	3.60E-04	2.40E-04	---	---	---	---
		K_D (M)	7.0E-08	8.1E-08	7.5E-08	1.3E-07	1.4E-07	1.0E-07	---	---	---	---
Lot 2	#1	k_a (M ⁻¹ s ⁻¹)	1.3E+04	1.20E+04	1.20E+04	8.00E+03	9.20E+03	7.00E+03	9.80E+03	N/A	1.20E+04	8.70E+03
		k_d (s ⁻¹)	2.0E-04	1.70E-04	2.70E-04	4.80E-04	6.10E-04	5.20E-04	7.00E-04	N/A	9.80E-04	4.70E-04
		K_D (M)	1.5E-08	1.4E-08	2.3E-08	6.0E-08	6.6E-08	7.4E-08	7.1E-08	N/A	8.2E-08	5.4E-08
	#2	k_a (M ⁻¹ s ⁻¹)	8.4E+03	7.70E+03	8.90E+03	7.10E+03	8.50E+03	7.20E+03	8.20E+03	7.80E+03	N/A	7.60E+03
		k_d (s ⁻¹)	1.1E-04	1.20E-04	2.70E-04	7.30E-04	7.20E-04	3.90E-04	4.20E-04	9.30E-04	N/A	4.20E-04
		K_D (M)	1.3E-08	1.6E-08	3.0E-08	1.0E-07	8.5E-08	5.4E-08	5.1E-08	1.2E-07	N/A	5.5E-08

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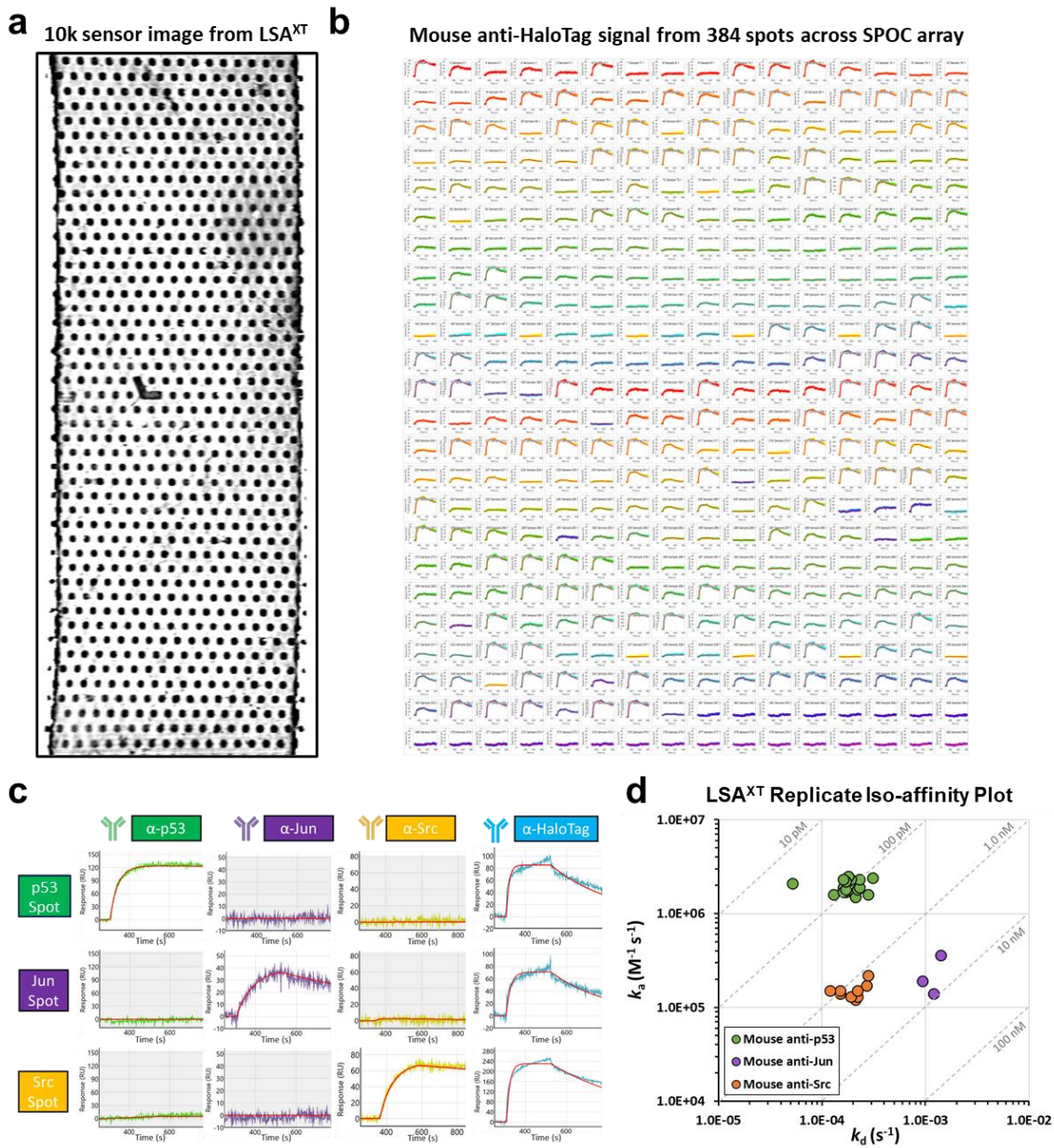


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758 **Figure 1 | Schematic of *in situ* protein production in nanowell slide and capture on SPOC biosensor chip.**

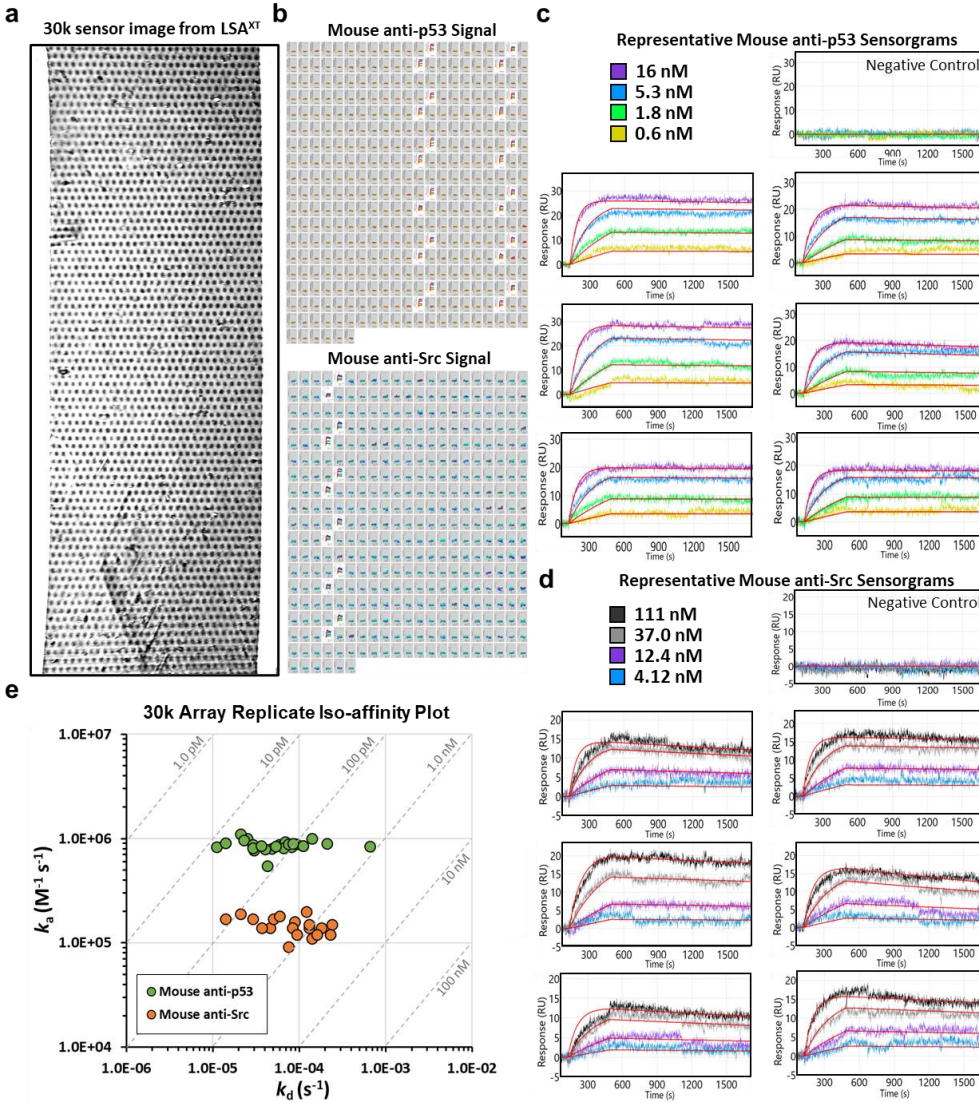
759 (a) By leveraging human cell-free *in vitro* transcription and translation (IVTT) lysate, SPOC arrays are
 760 produced via *in situ* expression of full-length folded proteins, which are encoded by recombinant plasmid
 761 DNAs printed in nanowell slides. Expressed proteins are simultaneously capture-purified as a monolayer
 762 of arrayed spots onto biosensor chips for label-free analysis by SPR. The entire process from protein array
 763 production on biosensor chip to label-free kinetic analysis is called SPOC and is facilitated by our custom
 764 SPOC protein array production instrument. This instrument can produce 4 SPOC biosensors from a single
 765 nanowell slide. The instrument has four channels in each of which can produce a nanowell slide in a semi-
 766 automated fashion; ultimately yielding up to sixteen SPOC chips in a single run. (b) Image of a 10k nanowell
 767 slide into which expression plasmid DNAs are printed. Protein arrays are expressed from the nanowell
 768 slide are captured on a variety of substrates, including glass microscope slides for standard fluorescent
 769 screens, or gold-coated SPOC biosensors for label-free kinetic screening via SPR. A scanning electron
 770 microscope (SEM) image of a nanowell slide cross-section is shown below the full top-down image of the
 771 nanowell slide (25 mm x 75 mm). (c) Image of a SPOC biosensor and the protein microarray pattern formed
 772 on the surface of the gold-coated biosensor slide after the protein expression/capture process outlined in
 773 a.

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775

776 **Figure 2 | Carterra LSA^{XT} data of antibody interactions on SPOC chip.** (a) Image of the SPOC sensor surface
 777 (10k array) as viewed from the flowcell of the LSA^{XT}. The hockey-stick shaped orientation mark which aids
 778 in orienting and mapping the array is clearly visible in the center of the array. (b) Sensorgrams reporting
 779 the mouse anti-HaloTag antibody binding responses from across 384 spots simultaneously on the SPOC
 780 slide are shown and validate capture of IVTT expressed Halo fusion proteins. (c) Sensorgrams
 781 corresponding to each of three spots on the SPOC array containing p53, Jun, and Src HaloTag fusion
 782 proteins are shown demonstrating the binding responses observed after injection of mouse anti-p53 (16
 783 nM), anti-Jun (67 nM), anti-Src (67 nM), and anti-HaloTag (133 nM) antibodies. The 1:1 interaction model
 784 is overlaid on the double-referenced data (red line). (d) Iso-affinity plot of the kinetics measured for the
 785 mouse anti-p53, anti-Jun, and anti-Src antibody injections (p53 = 20 spots across 3 sensors; Jun = 3 spots
 786 across 2 sensors; Src = 9 spots across 4 sensors).



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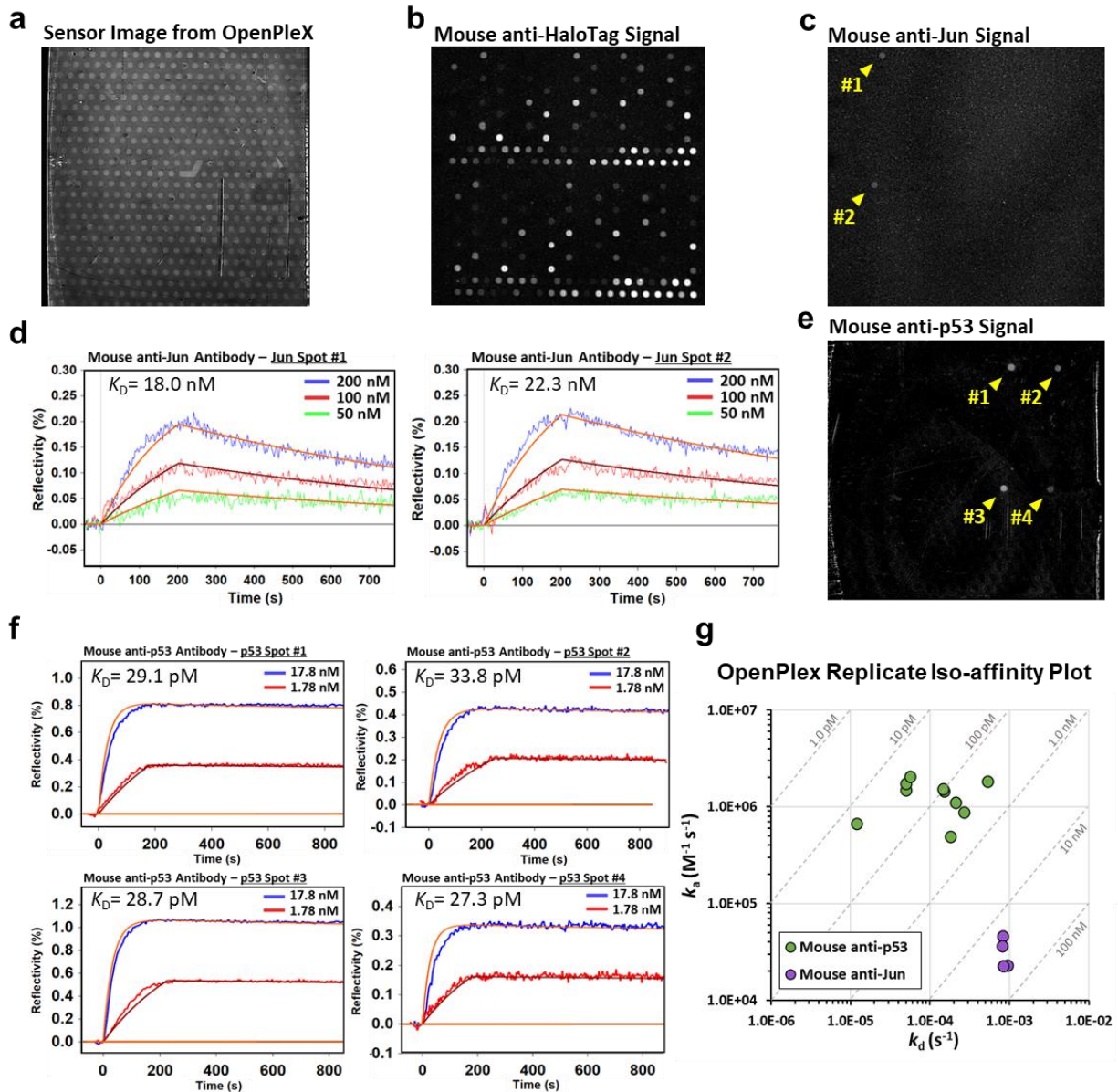
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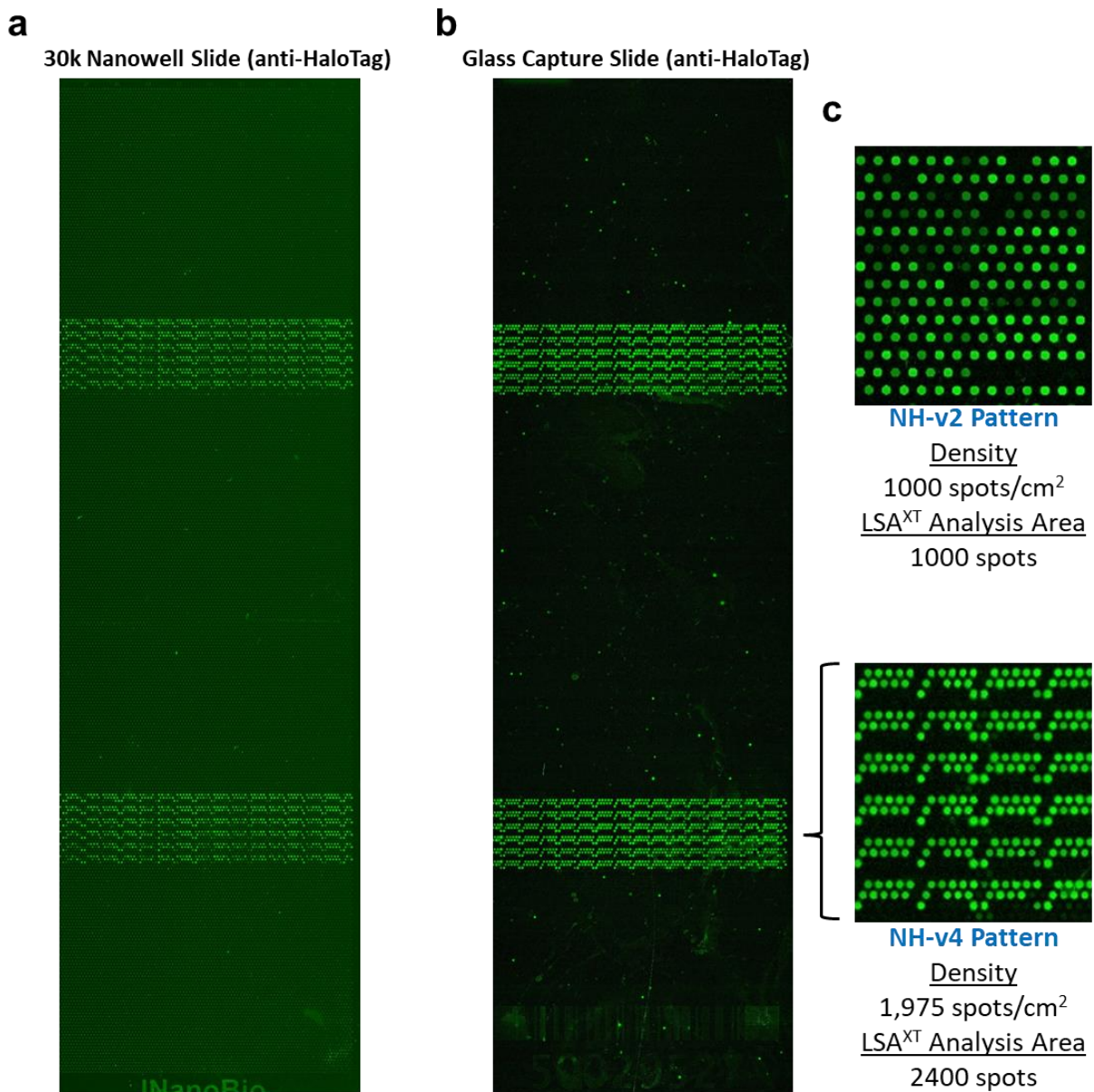
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Figure 3 | Carterra LSA^{XT} antibody kinetic data obtained from a SPOC chip produced from the high-density 30k nanowell slide. (a) Image of the SPOC sensor surface (30k array) as viewed from the flowcell of the LSA^{XT}. (b) Sensorgrams from the Mouse anti-p53 and anti-Src antibody injections across 384 spots of NHv4 30k array are shown with positive binding responses unshaded, and areas with no binding responses shaded grey. Representative sensorgrams of the protein-specific antibodies binding to their specific protein targets (p53 or Src) are shown enlarged in c and d. (c) Six representative sensorgrams from a total of twenty-four p53-HaloTag spots bound by the p53 antibody from b are shown and the binding responses after the mouse anti-p53 antibody injections are displayed, the 1:1 interaction model (red lines) is overlaid on the double-referenced data. Concentrations of antibody titration are shown in the key above the sensorgrams alongside a sensorgram showing binding responses observed on a representative control spot of the array. (d) Six representative sensorgrams from a total of twelve Src-HaloTag spots are shown and the binding responses after the mouse anti-Src antibody injections are displayed, the 1:1 interaction model (red lines) is overlaid on the double-referenced data. (e) Iso-affinity plot of the kinetics measured for the mouse anti-p53 and anti-Src antibody injections (p53 = 26 spots across 2 sensors; Src = 19 spots across 2 sensors).



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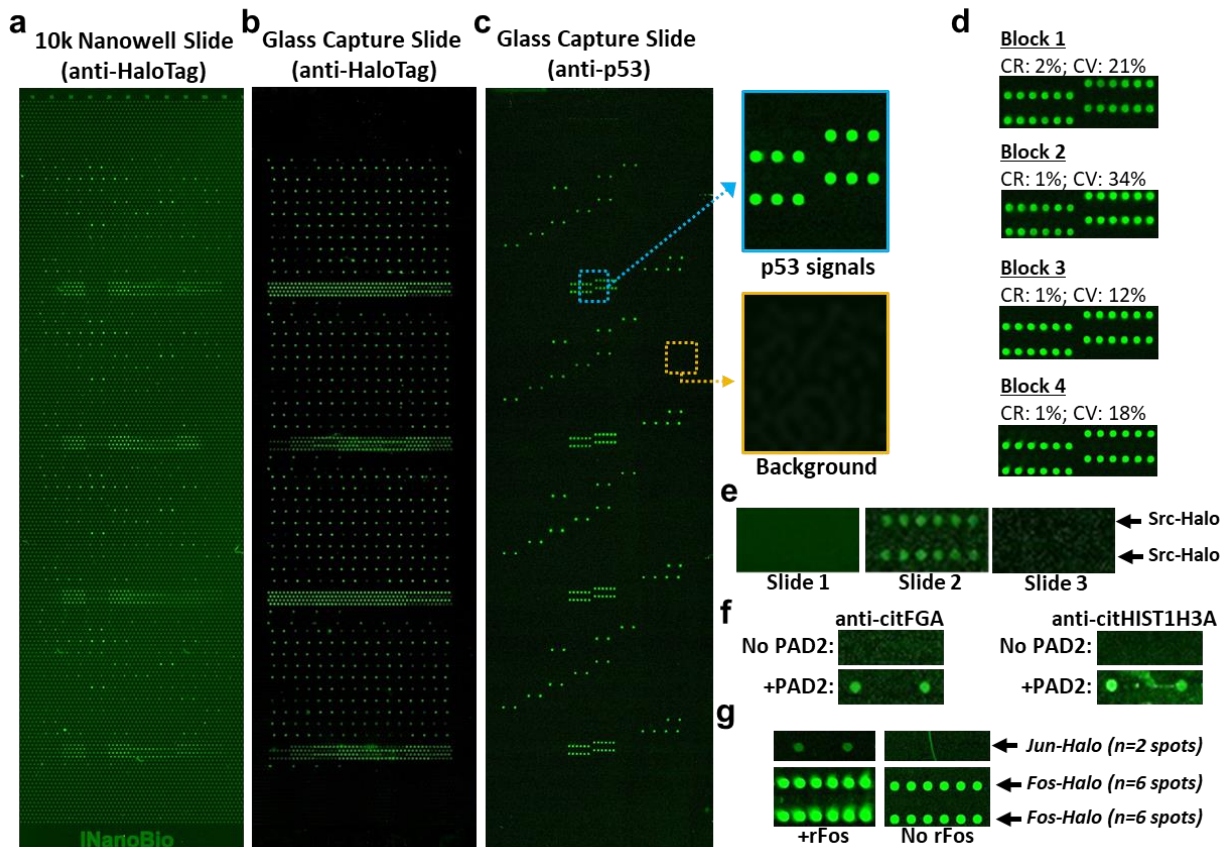
804 **Figure 4 | OpenPlex data of antibody interactions on SPOC chip.** (a) Image of the SPOC sensor surface
 805 (10k pattern) as viewed from the flowcell of the OpenPlex. The hockey-stick shaped orientation mark is
 806 visible in the center of the array. (b) Difference image showing the pattern of binding observed on the
 807 SPOC biosensor surface after injection of mouse anti-HaloTag antibody (133 nM). (c) Difference image
 808 showing the pattern of binding observed after the Mouse anti-Jun antibody injection (200 nM) which
 809 specifically detects two spots (yellow marks) where Jun-HaloTag was capture-purified as expected. (d) 1:1
 810 fit (orange line) OpenPlex data of the anti-Jun antibody binding to Jun Spots #1 and #2 from c show similar
 811 binding profiles and equilibrium dissociation constants. (e) Difference image shows the pattern of binding
 812 observed after the mouse anti-p53 antibody injection (17.8 nM) which specifically detects four spots
 813 (yellow marks) where p53-HaloTag was capture-purified as expected. (f) 1:1 fit (orange lines) OpenPlex
 814 data of the anti-p53 antibody binding to the four p53 spots detected in e show similar binding profiles and
 815 equilibrium dissociation constants. (g) Iso-affinity plot of the kinetics measured for the Mouse anti-p53
 816 and anti-Jun injections (p53 = 10 spots across 3 sensors; Jun = 4 spots across 2 sensors).



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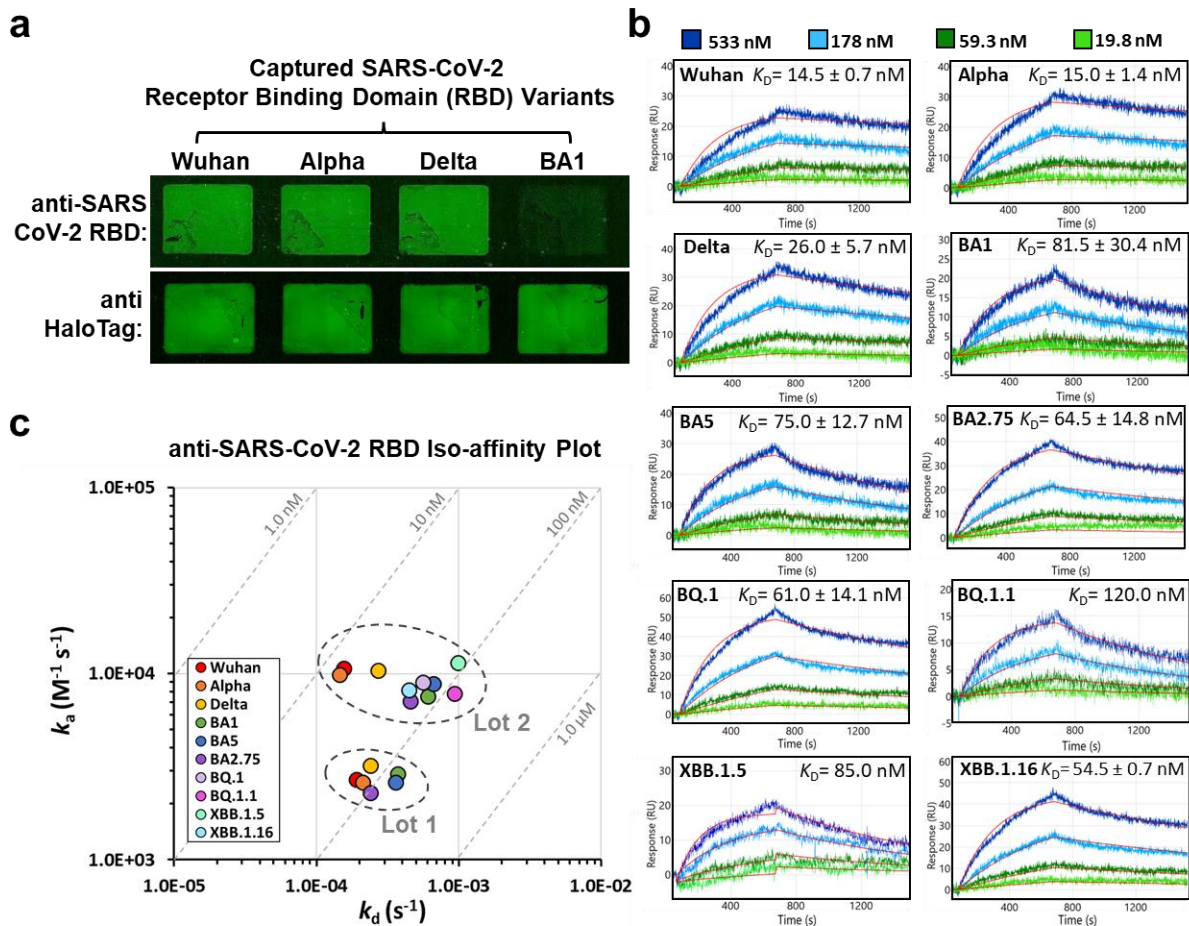
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819 **Figure 5 | Nanowell slide design facilitates high-density SPOC arrays capture-purification.** (a) Mouse
 820 anti-HaloTag fluorescence staining pattern observed on a high-density 30k nanowell slide design
 821 demonstrates expression of HaloTag fusion proteins in the nanowells of two subarrays containing various
 822 expression plasmids of interest. (b) Mouse anti-HaloTag fluorescence staining pattern observed on a glass
 823 capture slide functionalized with HaloTag chloro-alkane linker reveals diffusion-free capture of the high-
 824 density protein array generated from the 30k nanowell slide after capture-purification on the SPOC
 825 instrument (c) A comparison of the spot patterning achieved on the 10k versus the 30k nanowell slides,
 826 and the respective spot densities achieved is shown. The 30k design generates a >2-fold denser array
 827 than the 10k.



828

829 **Figure 6 | Automated production of pure in-situ protein arrays capture-purified on glass.** (a) Validation
 830 data showing fluorescence images of Halo-tagged proteins expressed in silicon nanowell, probed with
 831 anti-Halo, (b) captured onto HaloTag chloro-alkane linker-modified capture glass slides which were
 832 probed with anti-Halo, and (c) the same slide layout as shown in (b), but probed with anti-p53 antibody
 833 to detect p53 protein spots. These images show strong HaloTag and p53 signals on capture-purified
 834 protein arrays on capture glass slide, with <2% crosstalk between proteins in neighboring spots where
 835 protein was otherwise detected in (b). (d) Shows statistical analysis of cross reactivity (CR) and variation
 836 (CV) of p53 spot signals calculated from each block as a whole (sparse and dense areas). The enlarged
 837 images show the dense area only of each block to demonstrate lack of signal between spots and in
 838 neighboring non-p53 spots. Functional validation testing of protein arrays captured on glass slides. (e)
 839 demonstrates the enzymatic capability of bound Src kinase proteins via on-slide phosphorylation and
 840 dephosphorylation. Slide 1-3 were stained with anti-phosphotyrosine (n = 12 Src spots). Slide 1 consisting
 841 freshly expressed and captured Halo-SRC kinase protein spots was dephosphorylated by incubating with
 842 alkaline phosphatase and then stained. Slide 2 - Src spots were phosphorylated by incubating with kinase
 843 buffer to allow Src to autophosphorylate, followed by anti-phosphotyrosine staining. Slide 3 – Src was
 844 dephosphorylated again followed by staining. (f) Proteins can be altered with known PTMs associated
 845 with autoimmunity, such as citrullination. Here, Halo-fibrinogen (FGA) and Halo-Hist1H3A spots were
 846 treated with or without PAD2 enzyme to catalyze citrullination and then probed with citrullination-specific
 847 anti-cit-FGA and anti-cit-HIST1H3A. (g) Capture protein can bind to known protein interaction partners,
 848 such as Fos and Jun. Halo-Fos and Halo-Jun spots were incubated with (g) or without (h) recombinant Fos
 849 in solution. Slides were then probed with anti-Fos to determine if Fos bound to Halo-Jun. Fos was detected
 850 on Halo-Jun spots only if first incubated with rFos, and was detected at all spots known to contain Halo-
 851 Fos as a positive control – demonstration functionality of produced capture-purified protein arrays.



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853 **Figure 7 | Carterra LSA^{XT} screening of mouse anti-SARS-CoV-2 receptor binding domain (RBD) against**
 854 **RBD variants arrayed on SPOC chip. (a)** Orthogonal fluorescent glass-slide assay shows the staining
 855 pattern observed after capturing via manually spotting the indicated IVTT expressed SARS-CoV-2 RBD-
 856 HaloTag proteins and probing with mouse anti-RBD and HaloTag antibodies. **(b)** Sensorgrams reporting
 857 the binding responses observed after a serial titration of mouse anti-RBD antibody (Lot 2) was injected
 858 over a SPOC array containing 10 RBD VoC via the Carterra LSA^{XT}. 1:1 fits are overlaid on the data (red
 859 curves) and the equilibrium dissociation constants were determined from duplicate screens from Lot 2 of
 860 the antibody. **(c)** Iso-affinity plot of the various kinetics measured for the mouse anti-RBD antibody from
 861 two different lots against the RBD VoC are reported. The antibody from Lot 1 was only screened on a
 862 sensor with 6 of the RBD VoC while Lot 2 was screened across 2 sensors containing all 10 CoV-2 RBDs of
 863 interest.