Electronic Supplementary Material (ESI) for Analytical Methods.

Towards Simultaneous Quantification of Protease Inhibitors and Inflammatory Biomarkers in Serum for People Living with HIV

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Supplementary Information

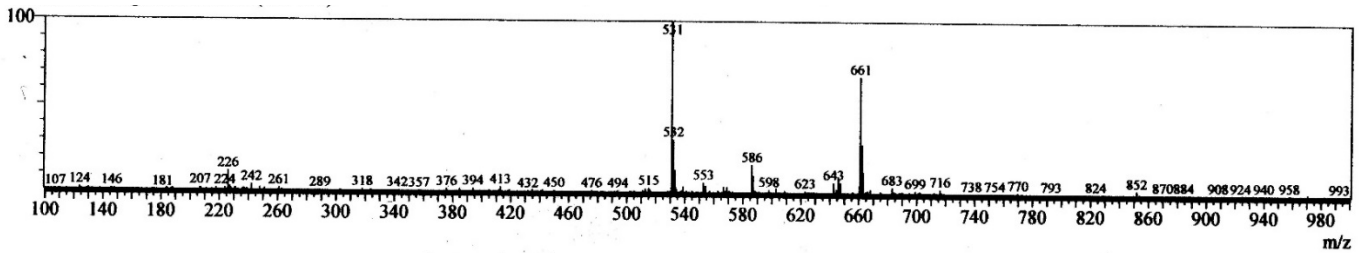
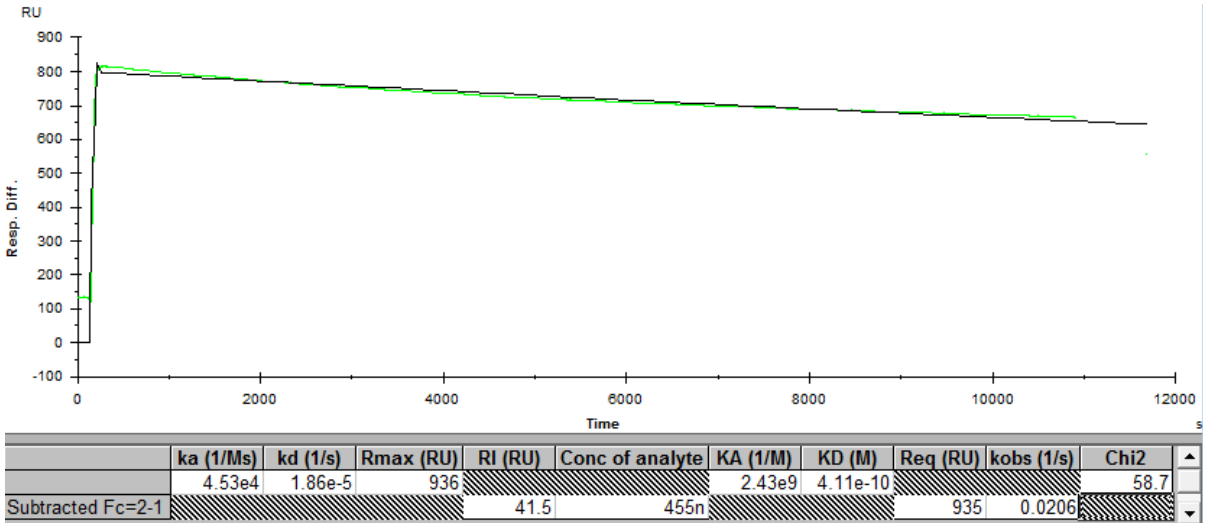


Fig S1. Atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) in positive mode of high-performance liquid chromatography (HPLC) purified DRV-linker product. 661 and 531-532 m/z are the peak of DRV-linker and its fraction from ionization.



	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	4.53e4	1.86e-5	936			2.43e9	4.11e-10			58.7
Subtracted Fc=2-1				41.5	455n			935	0.0206	

Fig S2. SPR trace (active cell - reference cell) of the affinity test of DRV-BSA to PR gives K_D = 0.41 nM.

Stability test of AIR Arrays:

To test the stability of DRV-BSA, AIR microarrays printed with DRV-BSA were immersed in 20% FBS mPBS-ET, pH 7.4 for 1 hr, 12 hr, 24 hr and 48 hr before being moved to 1 µg/mL PR or negative control samples. Fig S3 shows that no change in response was observed for arrays exposed to 20% FBS mPBS-ET, pH 7.4 for at least 12 hours, with a decrease in response at 48 hours. This confirms DRV-BSA and its attachment to the chip are stable long enough to cover the AIR experiment time frame.

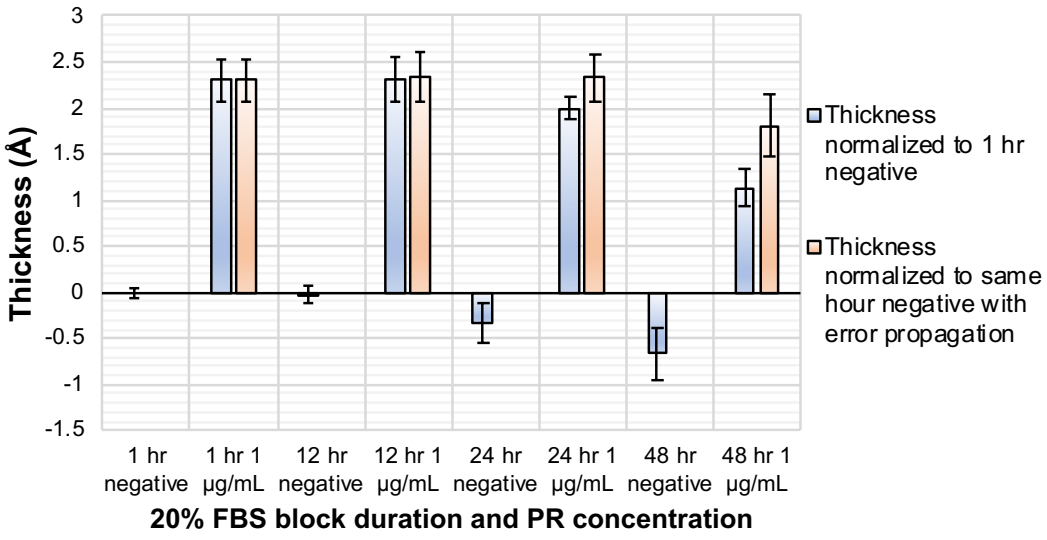


Fig S3. Stability test of DRV-BSA. The chips were printed with 1 mg/mL DRV-BSA in pH=5.8 mPBS and stayed in 20% FBS in pH=7.4 mPBS-ET for 1 hr, 12 hr, 24 hr and 48 hr before immersing in 20% FBS in pH=7.4 mPBS-ET with or without 1 µg/mL PR. The sample with PR labels "1 µg/mL"; without PR labels "negative". The DRV-BSA probe is stable up to at least 12 hours' stay in 20% FBS in pH=7.4 mPBS-ET.

Principle of AIR:

AIR relies on the creation and the target molecule-induced perturbation of an antireflective surface on a SiO_2-coated silicon chip. When operated with S-polarized HeNe laser light (632.8 nm wavelength), the reflected light at the air/SiO_2 interface and the reflected light at the SiO_2/Si interface interfere with each other (Fig. 4a). If the reflections are of equal magnitude and opposite phase, complete destructive interference and zero reflectivity is achieved. dThis complete destructive interference condition relies on the incident angle θ_1, and the SiO_2 thickness. Computational mapping of the overall reflectance as a function of θ_1 SiO_2 thickness revealed that the minimum reflectance point appears at a 70.6° incident angle and 1418.41 Å

SiO_2 thickness [1]. The incident angle is thus fixed at 70.6° for all AIR experiments. AIR chips are tailored to have a SiO_2 layer slightly thinner than 1418.41 Å, because the thickness will build up when the chip surface is functionalized and attached with probes. As the surface-functionalizing chemical (coated on the chip surface by chemical vapor depositing) and the attached probes (printed on the chip surface a micro-arrayer) have similar refractive indices as SiO_2, they together with SiO_2 are treated as one layer which is intended to have the thickness of 1418.41 Å. Later during detection, when analytes bind to the probes, the thickness increases. This causes it to deviate from the minimum reflectance point, and the increase of light intensity detected by the camera is then a quantitative indicator of each analyte's presence (Fig. 4b).

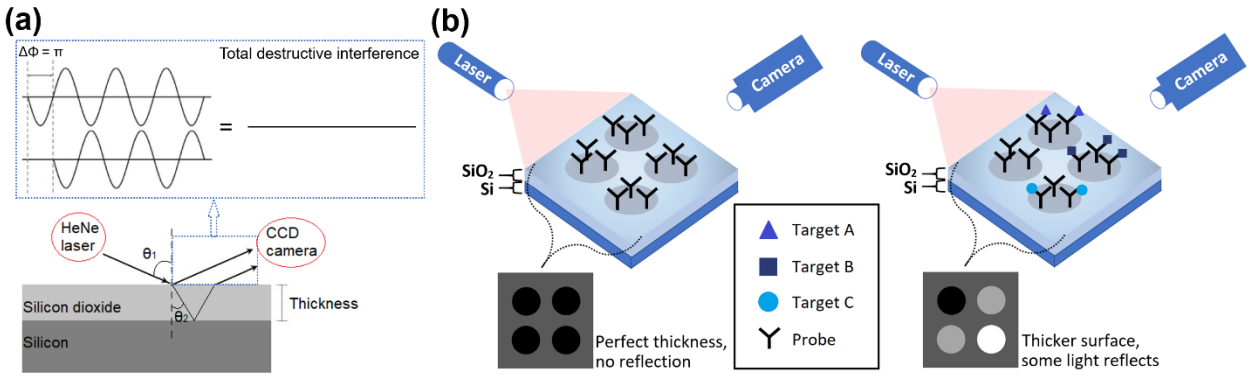


Fig S4. **(a)** Principle of AIR. The light detected by the camera depends on the phases of all reflected light. Total destructive interference can be achieved with certain incident angle and SiO2 thickness. **(b)** Illustration of an AIR chip with capturing antibody probes attached. The SiO_2 thickness is tailored to achieve a total destructive interference condition at the probe spots when there is no analyte. The capturing of analytes on the chip surface changes the thickness at the probe spots and disrupts the total destructive interference condition, resulting in the increase of brightness in chip images.

Examination of the detection range for DRV as a function of DRV-BSA / PR K_D and amount of DRV-BSA immobilized.

$$K_{D_{DRV\_BSA}} = \frac{[DRV\_BSA][PR]}{[DRV\_BSA\_PR]} = 0.41 \times 10^{-9}\ M \quad \text{Eq. 1}$$

$$K_{D_{DRV}} = \frac{[DRV][PR]}{[DRV\_PR]} = 0.41 \times 10^{-12}\ M \quad \text{Eq. 2}$$

From Eq. 1 & 2,

$$\frac{1000[DRV\_BSA\_PR]}{[DRV\_BSA]} = \frac{[DRV\_PR]}{[DRV]} \quad \text{Eq. 3}$$

$$[DRV\_BSA]_0 = [DRV\_BSA] + [DRV\_BSA\_PR] \quad \text{Eq. 4}$$

$$[DRV]_0 = [DRV] + [DRV\_PR] \quad \text{Eq. 5}$$

From Eq. 4 & 5, Eq. 3 can be rewritten as

$$\frac{1000[DRV\_BSA\_PR]}{[DRV\_BSA]_0 - [DRV\_BSA\_PR]} = \frac{[DRV\_PR]}{[DRV]_0 - [DRV\_PR]} \quad \text{Eq. 6}$$

From Eq. 1 & 4,

$$[PR] = \frac{0.41\times10^{-9}\ M \times [DRV\_BSA\_PR]}{[DRV\_BSA]} = \frac{0.41\times10^{-9}\ M \times [DRV\_BSA\_PR]}{[DRV\_BSA]_0 - [DRV\_BSA\_PR]} \quad \text{Eq.7}$$

$$[DRV\_PR] = [PR]_0 - [DRV\_BSA\_PR] - [PR] \quad \text{Eq. 8}$$

From Eq. 7 & 8, Eq. 6 can be rewritten as

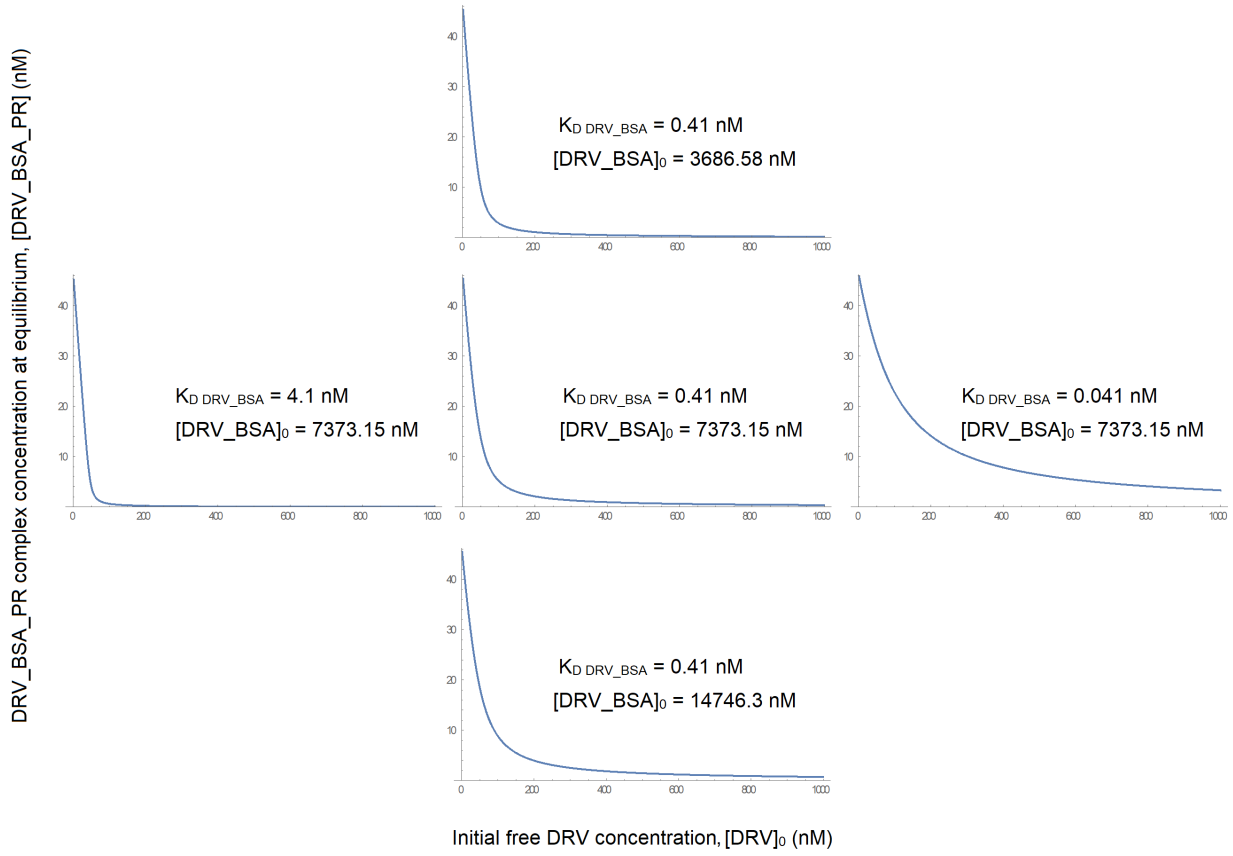
$$\frac{1000[DRV\_BSA\_PR]}{[DRV\_BSA]_0 - [DRV\_BSA\_PR]} = \frac{[PR]_0 - [DRV\_BSA\_PR] - \frac{0.41\times10^{-9}\ M \times [DRV\_BSA\_PR]}{[DRV\_BSA]_0 - [DRV\_BSA\_PR]}}{[DRV]_0 - [PR]_0 + [DRV\_BSA\_PR] + \frac{0.41\times10^{-9}\ M \times [DRV\_BSA\_PR]}{[DRV\_BSA]_0 - [DRV\_BSA\_PR]}} \quad \text{Eq. 9}$$

$[PR]_0$ is known to be 46.3 nM; and estimated from the calibration curve, $[DRV\_BSA]_0 = 7373.15$ nM

Let $[DRV]_0 = x$ nM, $[DRV\_BSA\_PR] = y$ nM, Eq. 9 becomes

$$\frac{1000y}{7373.15-y} = \frac{46.3-y-\frac{0.41y}{7373.15-y}}{x-46.3+y+\frac{0.41y}{7373.15-y}}$$ Eq. 10

Plot y vs. x with different $K_{D_{DRV\_BSA}}$ and $[DRV\_BSA]_0$ values:



Therefore the narrow range is due to (1) DRV-BSA is less competitive compaired with free DRV, and (2) only a small amount of DRV-BSA is immobilized on the chip.

[1] C. R. Mace, C. C. Striemer, and B. L. Miller, *Anal. Chem.*, 2006, **78**, 5578-5583.