Electronic Supplementary Information

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Plastic-based lateral flow immunoassay device for electrochemical detection of NT-proBNP

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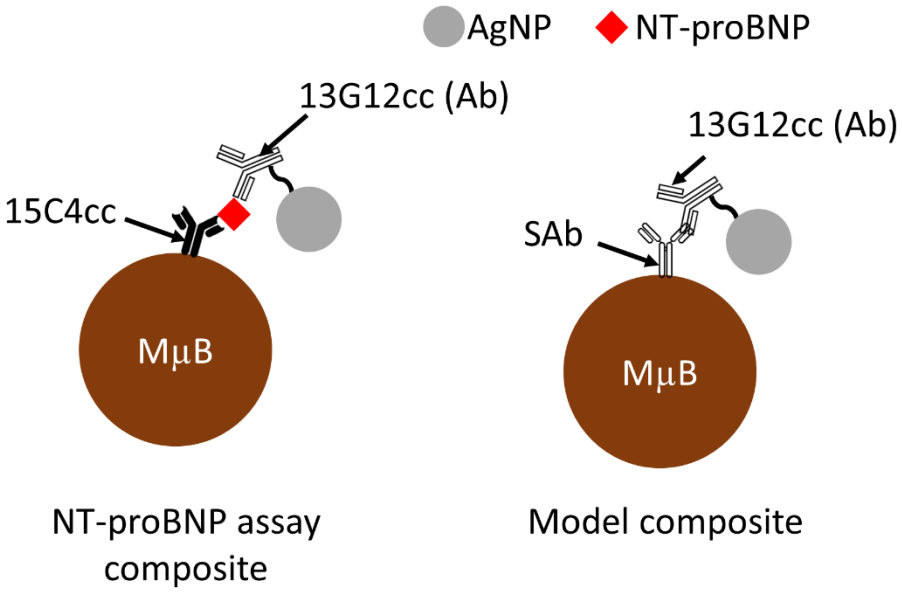

Figure S1. Schematic illustration showing the antigen-antibody immunosandwich used for NT-proBNP detection and the antibody-antibody model composite (MC) immunoassay.

Experimental Methods

Instrumentation. A Sorvall Legend Micro 21R centrifuge from Thermo Scientific (Grand Island, NY) was used for washing and separation steps during bioconjugation. A tube revolver (cat. no. 88881001), also from Thermo Scientific, was used for incubation steps during the conjugation of Abs to MµBs. A Mini Vortexer (945300) from VWR International (Radnor, PA) was used to briefly mix solutions while a BioShake iQ from Quantifoil Instruments GmbH (Jena, Germany) was used for incubation steps during bioconjugation. Epilog Laser Zing 16 was used to cut hydrophobic adhesive sheets and stencils for electrode printing. HP laser printer (HP LaserJet 400 color M451dn) was used to print hydrophobic ink onto the transparency sheets.

Preparation of the AgNP-Ab conjugates. 13G12cc Ab and AgNPs were conjugated by adding 4.0 µL of 5.0 mg/mL 13G12cc Ab with 500 µL of AgNPs (4.9×10^{11} AgNPs/mL) for 1 h in a bioshake at room temperature (RT, 22 ± 3 °C). Following incubation, the AgNP surface was back-filled with 50.0 µL of 10.0 µM mPEG-SH in DI water for 20 min at 600 rpm in a bioshake at RT. The conjugate was then centrifuged for 30 min at 16,600 g at 4°C to remove any excess material. Finally, the remaining bioconjugate was resuspended either in 300 µL or 40 µL of SBB, depending on the objective of the experiment. This conjugate will be referred to as the AgNP–Ab conjugate for this study.

Preparation of the MµB-SAb conjugates. For the MC, the biotinylated SAb was conjugated to streptavidin-coated Mµ Bs using the protocol provided by the manufacturer.[1] Specifically, 100 µL of Mµ Bs (~$7–10 \times 10^9$ Mµ Bs/mL) were aliquoted and washed using magnetic separation wherein the Mµ Bs were collected on the wall of a microcentrifuge tube with a neodymium magnet, the supernatant was removed, and the conjugate was resuspended in PBS and washed again. This process was carried out three times.

Next, 40.0 μL of 6.67 μM SAb were added to the tube and the resulting solution was incubated for 30 min at 30 rpm at RT using the tube revolver. Following conjugation, the MμBs were washed using magnetic separation five times with 100 μL of PBS and then resuspended in a final volume of 100 μL of either 1% BSA w/v in PBS or sugar solution depending on the objective of the experiment. The resulting conjugate will be referred to as MμB-SAb.

For the NT-proBNP assay, the 15C4cc capture Ab was biotinylated using a kit (ThermoFischer, Cat. No. 90407) and the protocol provided by the manufacturer.[2] Next, a similar procedure as described for the MμB-SAb was used to conjugate modified 15C4cc to the streptavidin-coated MμBs. Specifically, 20.0 μL of the 6.67 μM biotinylated 15C4cc capture Ab were incubated with 50.0 μL of the streptavidin-coated MμBs for 1.0 h at 30.0 rpm at RT on the tube revolver followed by washing using magnetic separation. Finally, the conjugate was resuspended in either 50 μL of 1% BSA w/v in PBS or sugar solution depending on the objective of the experiment. The resulting product is referred to as the MμB-15C4cc conjugate.

Fabrication of the *oc*Flow device

The steps for the *oc*Flow device fabrication are shown in Figure S2(a). First, an HP laser printer was used to print hydrophobic ink on the hydrophilic transparency sheet to define three different features of the device as shown in Figure S2(b): (i) a circular hydrophobic drying zone (diameter = 3.7 mm), (ii) a stopper line (l = 2.6 mm, w ~ 100 μm), and (iii) guidelines to print carbon-paste electrodes (red lines) and to align laser-cut adhesive sheet with the respective components of the device (black lines). Next, a CAD drawing of the stencil for defining the 3.0 mm-diameter disk-shaped working electrode (WE), hook-shaped carbon quasi-reference electrode (CQRE), and counter electrode (CE) was created using CorelDRAW (Ottawa, ON). The stencil was cut into a thin sheet of plastic using an Epilog laser engraving system (Zing 16). Finally, following red guidelines, the stencil was placed over the transparency sheet and the electrodes were printed through the stencil using the conductive carbon paste. The transparency sheet was left to dry in the air for 14 h.

To define hydrophobic regions, a self-adhesive sheet was sprayed with superhydrophobic spray from NeverWet and left to dry for ~30 min. Following this, Zing 16 laser cutter was used to cut the hydrophobic adhesive sheet according to the CAD drawing shown in Figure S2(c). Next, the transparency and the adhesive sheets were joined together with the help of black guidelines to generate hydrophilic/hydrophobic contrasting patterns defining the inlet, the flow channel, and the detection zone. Finally, the device was cut at the edges to yield its final form (Figure S3).

The WE was further modified by electrodepositing Au onto the carbon paste WE. This step was performed on-chip and was carried out by placing a 30.0 μL droplet of a solution consisting

of 6.0 mM Au^{3+} and 0.10 M KNO_3 onto the detection zone. Next, the potential of the working electrode was stepped from 0 to −0.60 V (unless otherwise stated, all potentials are vs. CQRE) for 2.0 s, and the electrode was then rinsed twice with DI water and dried with a Kimwipe. This final device was then stored in a cool, dark place.

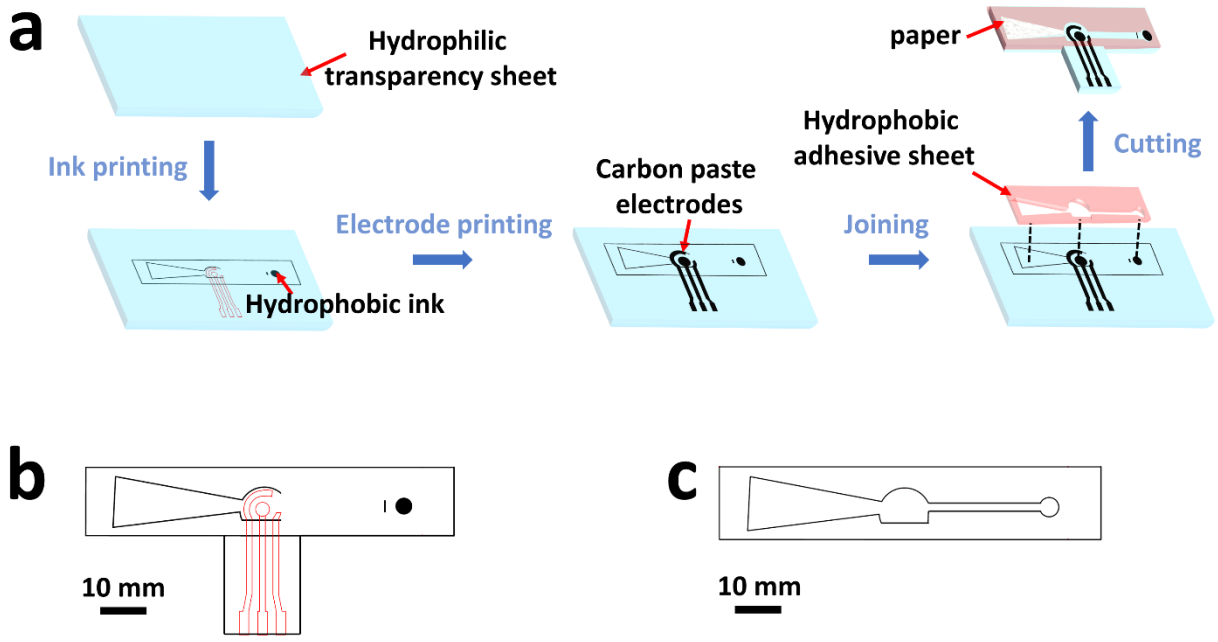

Figure S2. (a) Fabrication steps of the *oc*Flow device. (b) Drawing for the printing of hydrophobic ink on the transparency sheets to define the drying zone, stopper line, and guidelines. (c) CAD drawing used to cut the hydrophobic self-adhesive sheet using a laser cutter.

Image of the *oc*Flow device

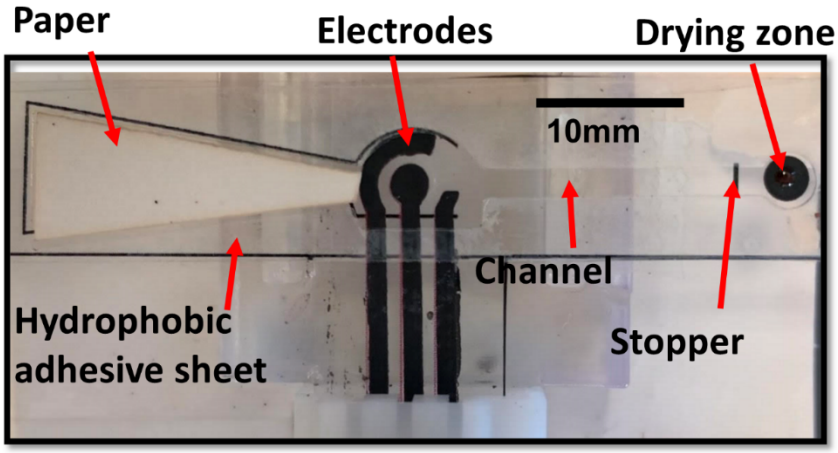

Figure S3. An image of *oc*Flow showing the individual components. The image also shows reagents dried at the drying zone.

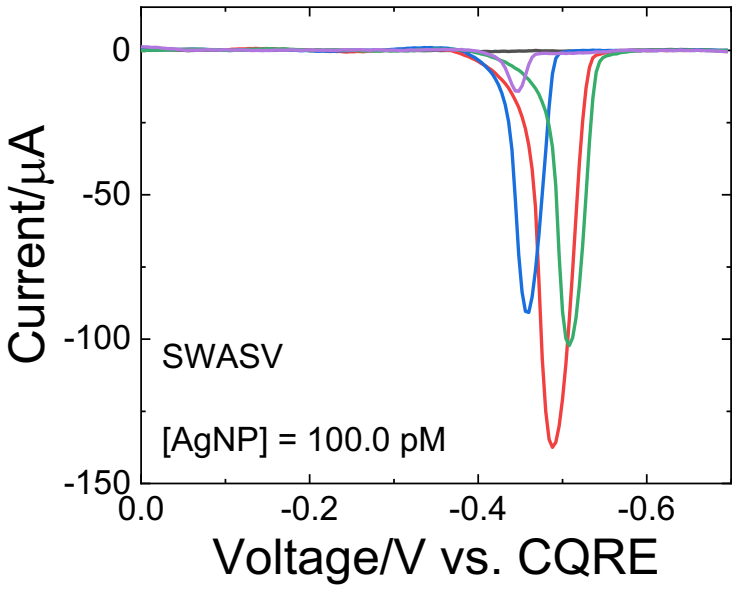

Figure S4 Baseline-corrected SWASV voltammograms for five independently fabricated *oc*Flow devices. These voltammograms were obtained by conducting 'wet' assay experiments for the MC ([AgNP] = 100.0 pM) suspended in a 100% serum sample when only a single 500.0 µL PBS wash step was employed. The position of the SWASV peaks varies slightly because of the use of a CQRE. The detailed experimental procedure for this experiment is discussed in the main text in the context of Figure 2.

Effect of passive resuspension on the assay performance

The poor assay performance in the passive resuspension technique (Figure 3, main text) is hypothesized to be due to the improper resuspension of MμB-SAb in the sample solution. Specifically, because of their large mass, MμBs could not be homogeneously resuspended in the solution *via* passive diffusion and thus mainly remain near the surface of the device. This has two main consequences. First, the MμB-SAb is inaccessible for the interaction with AgNP-Ab in the solution causing a less-than-optimal amount of MC formation. Second, the as-formed MC is unfavorably distributed on the WE.

To further investigate the second cause, the effect of incomplete immunoreaction was eliminated by forming the MC off-chip and then drying the pre-formed MC onto the device. We call this a 'dry' assay (see the Experimental Section in the main text). Specifically, the MC (100 pM AgNPs) was prepared by conjugating MμB-SAb and AgNP-Ab in PBS. Next, 2.0 μL of this MC was dried overnight onto the device in a sugar solution. Subsequently, the dried MC was resuspended using 20.0 μL of PBS solution using the passive resuspension technique. Following resuspension, the content at the inlet was transferred to the detection zone for the electrochemical analysis following an identical procedure as the 'instant mix-1' experiment (see main text). Additionally, an identical experiment, but using the active resuspension method was also conducted for comparison with the passive mixing method.

The current signals (average SWASV peak height) obtained from these experiments are shown in Figure S5a. These results indicate that the active resuspension technique yields a peak height that is slightly (~1.3 times) better than the passive resuspension technique. As discussed at the start of this section, we attribute this result to the unfavorable

distribution of MC onto the WE caused by the passive resuspension method. Specifically, in passive resuspension, the MC tends to cluster to the leading edge of the WE compared to the more uniform distribution observed with active resuspension (Figure S5b).

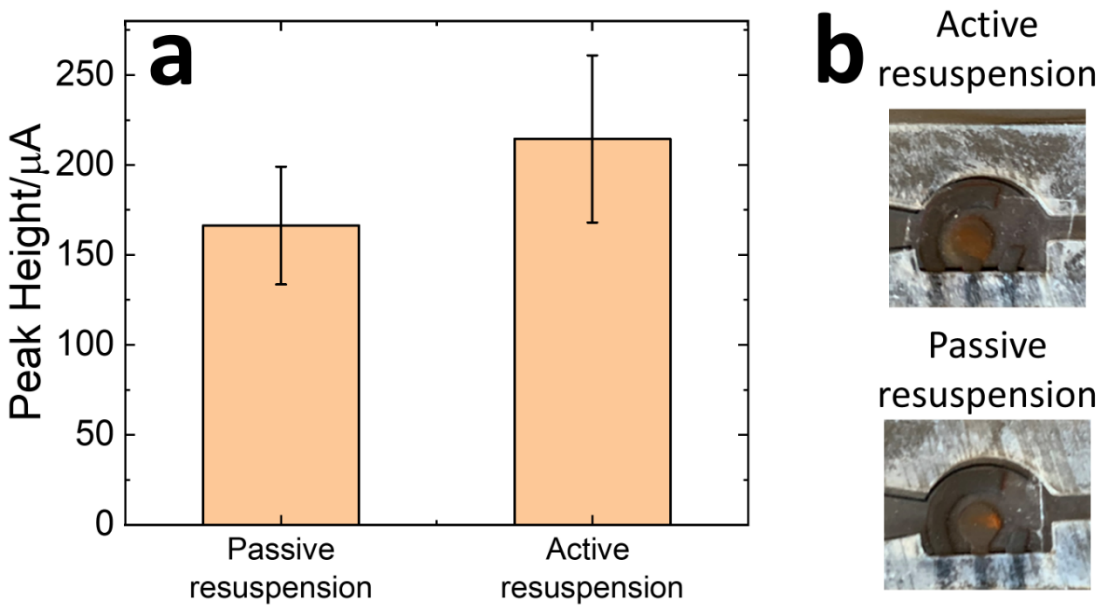

Figure S5 (a) Histogram showing the effect of passive and active resuspension on the analysis of the MC while conducting a 'dry' assay. (b) Distribution of the MC on the WE for both active and passive resuspension experiments. All the experiments were conducted on the MC (100 pM of AgNPs). The detailed experimental procedure is provided in the Experimental Section and in the context of Figure 3 in the main text. Error bars represent the standard deviation from the mean obtained using 3-4 independently fabricated *oc*Flow devices.

Fit equation and parameters for calibration curves

$$Peak\ height\ (\mu A) = a(1 - e^{-b \times [AgNP]}) \quad \text{S1}$$

Table S1. Fit parameter values of the calibration curve for MC detection. See Figure 4 in the main text for details.

Parameters	Values
a	260.1 ± 11.7
b	0.019 ± 0.003
R^2	0.99

$$Peak\ height\ (\mu A) = a + b \times [NT - proBNP] \quad \text{S2}$$

Table S2. Fit parameter values for the calibration curve for NT-proBNP detection. See Figure 5 in the main text for details.

Parameters	Values
a	-0.97 ± 8.83
b	0.057 ± 0.007
R^2	0.95

References

1. Thermofisher, Dynabeads MyOne Streptavidin T1 Protocol.

2. Thermofisher, Pierce Antibody Biotinylation Kit for IP.