

Supporting Information

1. Enzyme fouling

Some loss in sensitivity is seen upon addition of enzymes into the flow stream, as shown in Figure S11. This was found to be due to the enzyme fouling the working electrode over time. We have found that this loss in sensitivity can be reduced by coating the working electrode with a protective layer such as poly(phenol), Figure S11. Although some sensitivity loss is still seen for the coated electrode this is minimal and the system still operated well for over 35 hours.

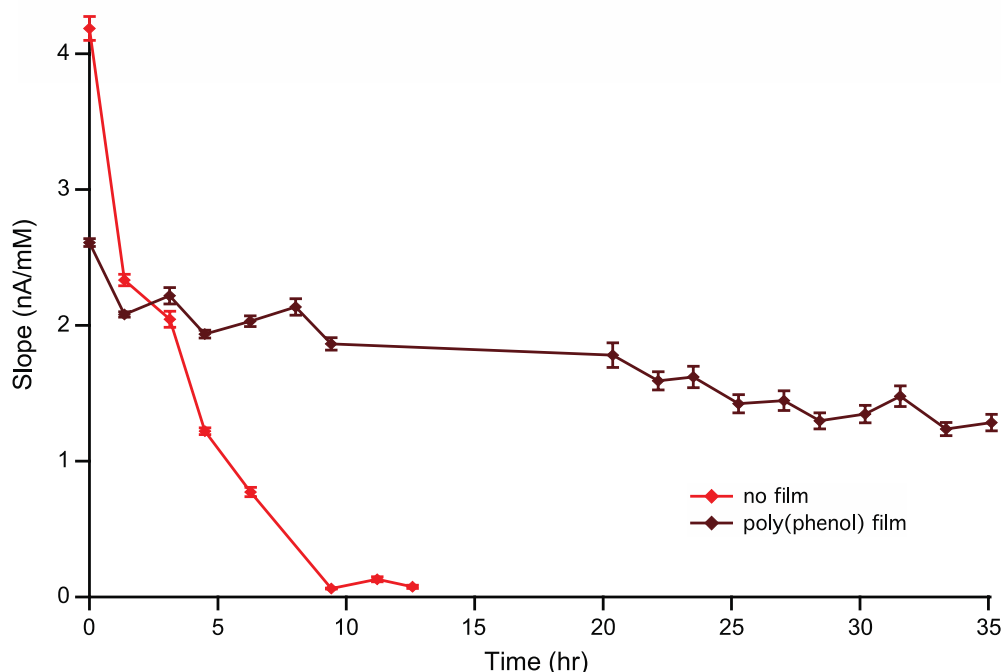
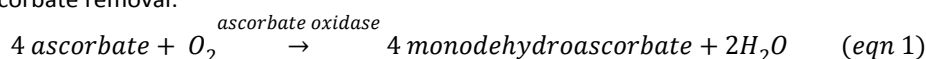


Figure S11

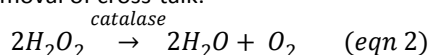
Slope of repeated glucose calibrations over time with and without a poly(phenol) blocking layer on the working electrode. A 5-point calibration was carried out by the addition of glucose oxidase (8.7 mg/ml, at 0.5 μ l/min) into a calibration flow stream (0-5 mM glucose step changes, at 2 μ l/min). Following a 1-hour pause, this was then repeated. Calibrations were carried out using a bare (red) and poly(phenol) coated (burgundy) 50 μ m platinum disc electrode, held at 0.7 V versus a Ag|AgCl reference electrode. Mean \pm standard deviation of measurement given.

2. Enzyme addition reaction mechanisms

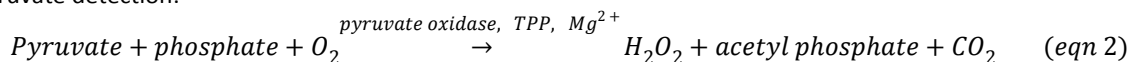
Ascorbate removal:



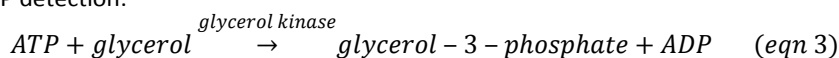
Removal of cross-talk:

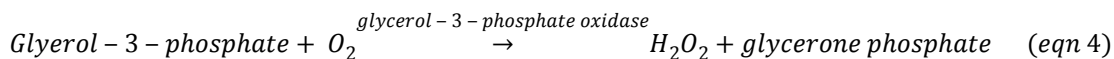


Pyruvate detection:



ATP detection:





3. Reagent addition

Clinical microdialysis samples are complex solutions that contain a representative concentration of all molecules in the tissue that can pass through the microdialysis membrane. Ascorbate is a commonly known interferent during electrochemical detection due to itself being electroactive. This modular microfluidic platform can be used to precisely dose reagents into a sample stream to solve common analytical problems such as removal of interferent species from the sample. If these interferents are not removed, their contribution to the signal can overwhelm the signal from the analyte of interest and lead to inaccurate measurements. For example, ascorbate oxidase can be added to the sample stream to react with and hence remove ascorbate (SI eqn 1) before it can be detected at the electrode. The setup is shown in Figure SI2a. Figure SI2b demonstrates how this platform can be used to remove the signal caused by ascorbate. Using this method, the interferent signal is reduced by $92.6 \pm 1.3\%$ ($n=4$). With the same layout configuration, the issue of cross-talk between different biosensors can also be addressed. Cross-talk can be a serious problem for macroelectrodes due to their larger surface area and hence the larger concentration of product in the flow stream, Figure SI2c. As an example, for oxidase-based enzyme biosensors, catalase can be added to the sample stream to react with the reaction product hydrogen peroxide in solution before it reaches the downstream electrode (SI eqn 2), eliminating cross-talk. In our example, a glucose biosensor is positioned upstream followed by a lactate biosensor downstream. Figure 5d shows the response at each biosensor; the green trace indicates the response to a solution with varying concentrations of lactate only and the red trace to a solution with varying concentrations of glucose only. As the downstream lactate biosensor should selectively respond only to lactate, the response seen to glucose infusions indicates that hydrogen peroxide formed at the upstream glucose biosensor has travelled downstream and has been detected at the lactate electrode. The pink trace shows the downstream lactate biosensor response to a glucose infusion when catalase is dosed in. Clearly, the addition of catalase has successfully solved the problem of cross-talk between the upstream and downstream electrodes, as demonstrated by the lack of response to the glucose-only solution at the lactate biosensor in the presence of catalase. Unsurprisingly, a decrease in current is also seen at the glucose sensor in the presence of catalase (left hand data). For demonstration purposes only, we engineered macroelectrode-based biosensors to deliberately create cross-talk, however the methodology of precisely dosing in enzymes has many potential uses for complex clinical samples. Microfluidics is ideally placed as only small amounts of enzymes are required, minimising analysis costs.

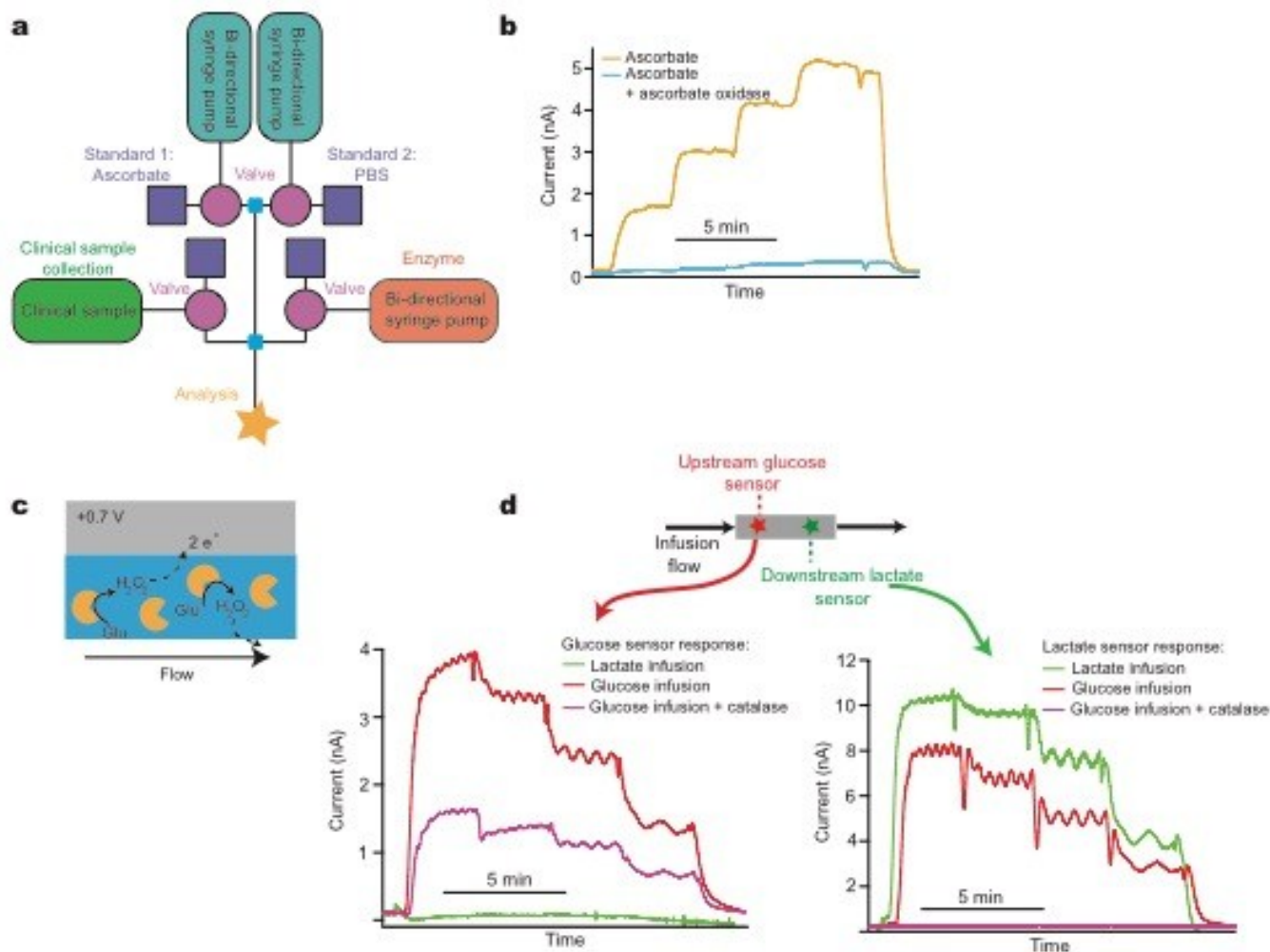


Figure S12

a: Schematic of the layout used to deliver enzyme to the sample. b: Yellow trace shows the response at a multiwall carbon nanotube-epoxy composite electrode¹ to varying levels of ascorbate (0.0, 0.0625, 0.125, 0.188 and 0.250 mM ascorbate) at 2 μ l/min. Blue trace shows the response to the same ascorbate concentration changes but with the addition of 6 mg/ml ascorbate oxidase at 0.5 μ l/min. c: Schematic of how the issue of cross-talk arises. Hydrogen peroxide is produced in the reaction of glucose (Glu) with glucose oxidase (yellow symbol). This product can either diffuse to the electrode surface where it is oxidised at +0.7 V to give a signal or can diffuse out of the hydrogel back into the fluid stream flowing past the downstream sensor tip. d: Two macroelectrodes in a microfluidic chip. The upstream electrode is a glucose biosensor (left hand data set) and the downstream electrode is a lactate biosensor (right hand data set). The green trace shows the response to infusions of different concentrations of lactate only (0–1 mM, steps: 1.00, 0.75, 0.50, 0.25 and 0.00 mM). The red trace shows the response to different concentrations of glucose only (0–2 mM, steps: 2.0, 1.5, 1.0, 0.5 and 0.0 mM). The response seen at the downstream lactate biosensor during a glucose-only calibration is caused by cross-talk from the upstream biosensor. The pink trace shows the biosensor response to a glucose-only calibration with an infusion of catalase (0.6 mg/ml, at 0.5 μ l/min), successfully eliminating the cross-talk effect.

4. Free flap surgery

Free flap surgery involves the transplantation of healthy tissue, together with its blood vessels, from one site of the body to another. The procedure is often carried out to rebuild large defects in the recipient site, for example in breast reconstruction or for head and neck reconstruction after tumour removal. During the surgery the donor site is prepared and the blood vessels are clamped and cut. Simultaneously, the recipient site is prepared for reconstruction. Once the free flap is in place, the blood supply is restored at the recipient site using microsurgery to re-join the blood vessels (anastomosis). The main risk is thrombosis in the flap artery or the vein following re-vascularisation. This leads to flap ischaemia and if undetected, flap failure, after which emergency surgery is required to re-establish blood supply to the flap pedicle. Not only is this at a huge monetary cost but the patient is also at risk of septicaemia and the likelihood of a successful flap reconstruction falls dramatically. Current clinical assessment of flap success is the colour, turgidity and temperature of the flap at the recipient site and these measurements are all subjective to the reviewer. We have shown that online microdialysis measuring levels of glucose and lactate can provide useful information about the health of the flap tissue during and after reconstruction, aiding clinicians in making time-critical decisions².

Here, we have used a microdialysis probe connected to an online biosensing system to monitor the health of the free flap in real time.² Free flap monitoring commenced prior to flap detachment so that baseline levels of glucose and lactate were established in the individuals'

healthy tissue. The blood vessels were clamped and cut creating the free flap and the tissue was moved to the recipient site; the microdialysis probe remained inserted so that monitoring continued throughout. Microsurgery was used to reconstruct the recipient site as well as reconnect the artery. Arterial blood flow was confirmed after which the vessels were re-clamped and the venous anastomosis was carried out. During ischaemia, glucose levels decrease and can reach very low concentrations, while lactate levels increase and can reach relatively high concentrations. In contrast, upon successful anastomosis, glucose levels increase and lactate levels decrease.² Therefore, for this application, a wide detection range is necessary for both glucose and lactate biosensors. In addition, relatively fast response times are required to detect these changes in real-time so that the information can be utilised by the clinical staff.

5. Custom-written scripts

The scripts listed below show the commands necessary to carry out the key actions in the examples described in the manuscript. These can easily be extended and adapted for different or more complex tasks. The script details are specific to the particular board configuration. The particular details that may vary from experiment to experiment are given at the start of each example script. These scripts are written in uProcess software (LabSmith) but can also be programmed in C++.

I. Calibration

In this case we have a board with two 20 μ l syringe pumps and 2 valves, see Figure 1.

```
; Pump 1: PBS
; Pump 1 volume: 20  $\mu$ l
; Pump 2: 1 mM glucose
; Pump 2 volume: 20  $\mu$ l
; Total flow rate 2  $\mu$ l/min
; Pump1 reservoir position 3
; Pump2 reservoir position 1
```

```
Step1 ; filling
      Manifold:      SetValves 3 1 3 0
      Pump1: SetFlowRate 100.000  $\mu$ l/min
      Pump1: MoveTo 20.000  $\mu$ l
      Pump2: SetFlowRate 100.000  $\mu$ l/min
      Pump2: MoveTo 20.000  $\mu$ l
      <WaitAllDone>
```

```
Step3; 100% glucose
      Manifold:      SetValves 3 3 2 0
      Pump2: SetFlowRate 2  $\mu$ l/min
      Pump2: MoveTo 0.1  $\mu$ l
      <Pause> 180s
```

```
Step4; 75%
      Manifold:      SetValves 1 3 2 0
      Pump2: SetFlowRate 1.5  $\mu$ l/min
      Pump2: MoveTo 0.1  $\mu$ l
      Pump1: SetFlowRate 0.5  $\mu$ l/min
      Pump1: MoveTo 0.1  $\mu$ l
      <Pause> 180s
```

```
Step5; 50%
      Manifold:      SetValves 1 3 2 0
      Pump2: SetFlowRate 1.0  $\mu$ l/min
      Pump2: MoveTo 0.1  $\mu$ l
      Pump1: SetFlowRate 1.0  $\mu$ l/min
      Pump1: MoveTo 0.1  $\mu$ l
      <Pause> 180s
```

```
Step6; 25%
      Manifold:      SetValves 1 3 2 0
      Pump2: SetFlowRate 0.5  $\mu$ l/min
      Pump2: MoveTo 0.1  $\mu$ l
      Pump1: SetFlowRate 1.5  $\mu$ l/min
      Pump1: MoveTo 0.1  $\mu$ l
      <Pause> 180s
      Pump2: Stop
```

```

Step7; 0%
    Manifold:      SetValves 1 1 2 0
    Pump1: SetFlowRate 2 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s

    Pump1: Stop

Step14;
    <Goto> Step1

```

II. Standard addition

In this case we have a board with two 20 μ l syringe pumps and 3 valves, see Figure 3.

```

; Pump 1: 1 mM glucose and lactate
; Pump 1 volume: 20  $\mu$ l
; Pump 2: PBS
; Pump 2 volume: 20  $\mu$ l
; Total flow rate 2  $\mu$ l/min
; Pump1 reservoir position 3
; Pump2 reservoir position 1
; Sample stream to chip position 3
; Sample for 1 hr before addition

Step1 ; filling
    Manifold:      SetValves 3 1 3 0
    Pump1: SetFlowRate 100.000 ul/min
    Pump1: MoveTo 20.000 ul
    Pump2: SetFlowRate 100.000 ul/min
    Pump2: MoveTo 20.000 ul
    <WaitAllDone>

Step3; 0% glucose/lactate
    Manifold:      SetValves 2 3 1 0
    Pump2: SetFlowRate 2.5 ul/min
    Pump2: MoveTo 0.1 ul
    <Pause> 180s

Step4; 25%
    Manifold:      SetValves 1 3 1 0
    Pump2: SetFlowRate 1.875 ul/min
    Pump2: MoveTo 0.1 ul
    Pump1: SetFlowRate 0.625 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s

Step5; 50%
    Manifold:      SetValves 1 3 1 0
    Pump2: SetFlowRate 1.25 ul/min
    Pump2: MoveTo 0.1 ul
    Pump1: SetFlowRate 1.25 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s

Step6; 75%
    Manifold:      SetValves 1 3 1 0
    Pump2: SetFlowRate 0.625 ul/min
    Pump2: MoveTo 0.1 ul
    Pump1: SetFlowRate 1.875 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s
    Pump2: Stop

Step7; 100%
    Manifold:      SetValves 1 2 1 0
    Pump1: SetFlowRate 2.5 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s

    Pump1: Stop

Step14; Sample stream

```

```

    Manifold: SetValves 3 1 3 0
    Pump1: SetFlowRate 100.000 ul/min
    Pump1: MoveTo 20.000 ul
    Pump2: SetFlowRate 100.000 ul/min
    Pump2: MoveTo 20.000 ul
    <Pause> 3600s

Step15; Addition 100%
    Manifold: SetValves 1 2 1 0
    Pump1: SetFlowRate 0.5 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s

Step16; Addition 50%
    Manifold: SetValves 1 3 1 0
    Pump2: SetFlowRate 0.25 ul/min
    Pump2: MoveTo 0.1 ul
    Pump1: SetFlowRate 0.25 ul/min
    Pump1: MoveTo 0.1 ul
    <Pause> 180s
    Pump1: Stop

Step17; Addition 0%
    Manifold: SetValves 2 3 1 0
    Pump2: SetFlowRate 0.5 ul/min
    Pump2: MoveTo 0.1 ul
    <Pause> 180s
    Pump2: Stop

Step18;
    <Goto> Step14

```

III. Enzyme addition

In this case we have a board with three 20 µl syringe pumps and 3 valves, see Figure SI2.

```

; Pump 1: 0.25 mM ascorbate
; Pump 2: PBS
; Pump3: 6 mg/ml ascorbate oxidase
; Total calibration flow rate 2 µl/min
; Enzyme flow rate 0.5 µl/min
; Pump1 reservoir position 3
; Pump1 volume: 20 µl
; Pump2 reservoir position 3
; Pump2 volume: 20 µl
; Pump3 reservoir position 1
; Pump3 volume: 20 µl

Step1 ; filling
    Manifold: SetValves 3 3 1 0
    Pump1: SetFlowRate 100.000 ul/min
    Pump1: MoveTo 20.000 ul
    Pump2: SetFlowRate 100.000 ul/min
    Pump2: MoveTo 20.000 ul
    Pump3: SetFlowRate 100.000 ul/min
    Pump3: MoveTo 20.000 ul
    <WaitAllDone>

Step2; 0% ascorbate
    Manifold: SetValves 3 1 3 0
    Pump2: SetFlowRate 2.0 ul/min
    Pump2: MoveTo 0.1 ul
    Pump3: SetFlowRate 0.5 ul/min
    Pump3: MoveTo 0.1 ul
    <Pause> 180s

Step3; ; 25%
    Manifold: SetValves 1 1 3 0
    Pump2: SetFlowRate 1.5 ul/min
    Pump2: MoveTo 0.1 ul
    Pump1: SetFlowRate 0.5 ul/min
    Pump1: MoveTo 0.1 ul
    Pump3: SetFlowRate 0.5 ul/min
    Pump3: MoveTo 0.1 ul
    <Pause> 180s

Step4; 50%
    Manifold: SetValves 1 1 3 0

```

```
Pump2: SetFlowRate 1.0 ul/min
Pump2: MoveTo 0.1 ul
Pump1: SetFlowRate 1.0 ul/min
Pump1: MoveTo 0.1 ul
Pump3: SetFlowRate 0.5 ul/min
Pump3: MoveTo 0.1 ul
<Pause> 180s
```

```
Step5; 75%
Manifold: SetValves 1 1 3 0
Pump2: SetFlowRate 0.5 ul/min
Pump2: MoveTo 0.1 ul
Pump1: SetFlowRate 1.5 ul/min
Pump1: MoveTo 0.1 ul
Pump3: SetFlowRate 0.5 ul/min
Pump3: MoveTo 0.1 ul
<Pause> 180s
Pump2: Stop
```

```
Step6; 100%
Manifold: SetValves 1 3 3 0
Pump1: SetFlowRate 2.0 ul/min
Pump1: MoveTo 0.1 ul
Pump3: SetFlowRate 0.5 ul/min
Pump3: MoveTo 0.1 ul
<Pause> 240s
```

```
Pump1: Stop
Pump3: Stop
```

```
Step7;
<Loop> Step1 0
```

References

1. Phairatana, T., Leong, C. L., Gowers, S. A. N., Patel, B. A. & Boutelle, M. G. Real-time detection of carboplatin using a microfluidic system. *Analyst* **141**, 6270–6277 (2016).
2. Rogers, M. L. *et al.* Online rapid sampling microdialysis (rsMD) using enzyme-based electroanalysis for dynamic detection of ischaemia during free flap reconstructive surgery. *Anal. Bioanal. Chem.* **405**, 3881–3888 (2013).