

Before selecting the best crosslinker, each crosslinker was individually studied to determine the optimal enzyme immobilization conditions for use. Optimal concentrations of EDC, BS3, and EGS were determined to be ~6, ~100, and ~100 mg/ml, respectively. GAH is applied as a vapor rather than solution, so the optimal exposure time to 5% GAH was determined. Since increased GAH exposure leads to enzyme denaturation, GAH exposure time was minimized to 60 s (60 s was observed to be the minimum exposure time needed to prevent enzyme from being washed off the microelectrode surface *in vitro*).

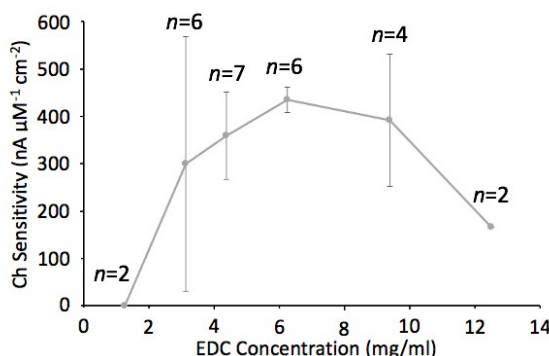


Figure S1. Effect of EDC concentration on Ch sensor sensitivity in 37 °C PBS buffer. In all cases, the mass ratio of ChOx to BSA was 2:3 and the enzyme layers were 3-4 μm thick. Enzyme layer thicknesses were observed to be thinner at low EDC concentrations due to poor crosslinking.

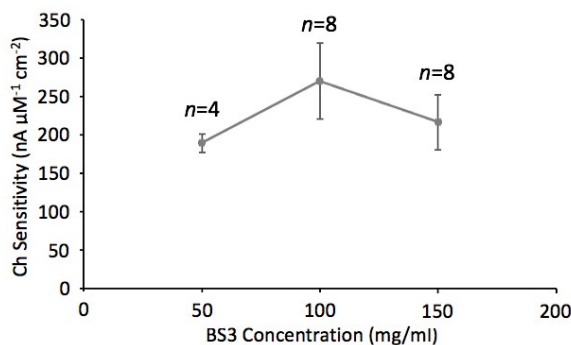


Figure S2. Effect of BS3 concentration on the Ch sensor sensitivity in 25 °C PBS buffer. In all cases, the mass ratio of ChOx to BSA was 2:3 and the enzyme layers were 3-4 μm thick. Enzyme layer thicknesses were observed to be thinner at low BS3 concentrations due to poor crosslinking. BS3 concentrations >100 mg/ml were difficult to solubilize.