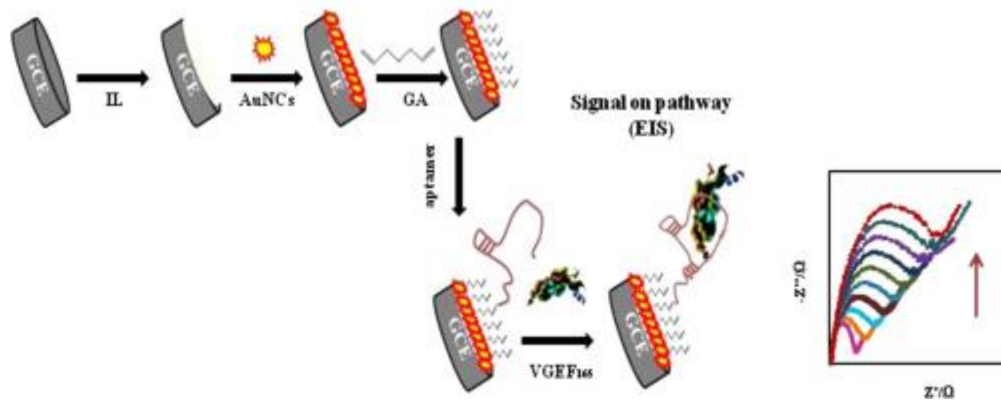
Saptasense Protocol

Depositing Nanocomposite

Background

In this project, we are building an electrochemical aptasensor. In short, an aptamer is attached to an electrode. When the target molecule (sarcosine) interacts with the aptamer, a change in impedance is detectable. In order to amplify this change in impedance, the surface of the electrode is typically modified with additional molecules that increase the exposed surface area. This protocol will detail the modification of the electrode as well as the attachment mechanism of the aptamer.



*NOTE: This protocol is primarily based on an article utilizing GCEs. Based on sources, you can transfer the protocols to SPEs regardless, however the deposition methods will likely be dependent on dropping solutions onto the electrode itself (since the sensors are only a few mm in dimension). We can also test the protocol on GCEs as a control, however they are much more expensive (albeit, reusable/rewashable)

Materials

Alumina Slurry* (typically 0.05-1micron)
Synthetic Cloths*
Ultrapure Water

BSA-AuNC soln
PBS
Glutaraldehyde (GA)
Aminated Aptamer (NH2 group @5' end)

Instruments/Equipment

Ultrasonic Bath/Sonicator
Hairdryer/air heating mechanism
Thermometer
Flat glass plate (ie petri dish)
IL (EMIMPF6)

*All samples prepared with 0.1M phosphate buffer solution (PBS pH 7.4)

Modifying WE

- Prepare glutaraldehyde (GA) solution

    - GA, 25% in water
- Prepare 2.5% GA in Phosphate Buffer Solution (PBS)
- Prepare 0.25% Bovine Serum Albumin (BSA) solution
- Prepare aminated aptamer solution (10uL at 0.6uM for aptamer, 0.1M of PBS of pH 7.4
- Prepare 1:1 solution of 5mM [Fe(CN)6)]^(3-/4-) and 0.1M PBS (pH=7.4)
- Place 0.2g of IL (EMIMPF6) on theWE
- Using a hair dryer or heat source, heat the WE to above IL melting point (58-62C) in order to cover up the electrode
- Coat WE by dropping 10uL of the BSA-AuNCs on the modified surface, heat at 50 degrees C using hair dryer for 10 min
- Drop-cast 2.5% GA/PBS solution and let it incubate for 1 hour
    - Rinse WE with ultrapure water
- Drop 10uL of aptamer solution (10 μL, 0.6 μM, 0.1 M PBS of pH 7.4) and incubate for 12 hrs at room temperature
- Drop enough PBS onto WE to cover it entirely, let sit for 10 minutes (removes unbound DNA)
    - Rinse with ultrapure water
- Drop enough BSA solution onto WE to cover completely, let sit for 30 min (decrease non-specific binding)
    - Rinse again with ultrapure water
- Drop 20uL of sarcosine at fixed concentrations onto WE, incubated at room temp for 40 min
    - Wash WE with PBS to remove unbound sarcosine
- Record the EIS responses with the WE covered in or submerged in the FeCN/PBS solution

*Acceptable ILs include ones that are imidazolium based

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1) https://www.sciencedirect.com/science/article/pii/S0956566315302414
2) https://pubs.rsc.org/en/content/articlehtml/2016/an/c6an00167j
3) file:///Users/mackenziedillenbeck/Downloads/biosensors-10-00009.pdf