Biosensing for Human Health

Today we learned about how biosensors work where molecular recognition between a bioreceptor and a target allows detection. The following questions will help you to interpret the data that you get out of a biosensing experiment.

1. Let's first do a problem involving the classical glucose electrochemical biosensor. A glucose biosensor operates by detecting hydrogen peroxide H_2O_2 produced during the enzymatic oxidation of glucose. The generation of H_2O_2 is measured at the Platinum electrode, which has a standard reduction potential of +0.695 V for the reaction: $\mathbf{H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-}$

   a) Based on this reduction potential, is this reaction spontaneous? Why or why not?

   b) The glucose sensor generates a current of 10 µA during the measurement. Calculate how many moles of H_2O_2 are oxidized per second.

   Here is **Faraday's law of electrolysis** for you to use: $n = \frac{I}{zF}$

   c) What factors could influence the efficiency of the platinum electrode in oxidizing H_2O_2?

   d) Blood contains other electroactive molecules like ascorbic acid and uric acid. How could these interfere with the sensor? Propose one strategy to minimize these interferences.

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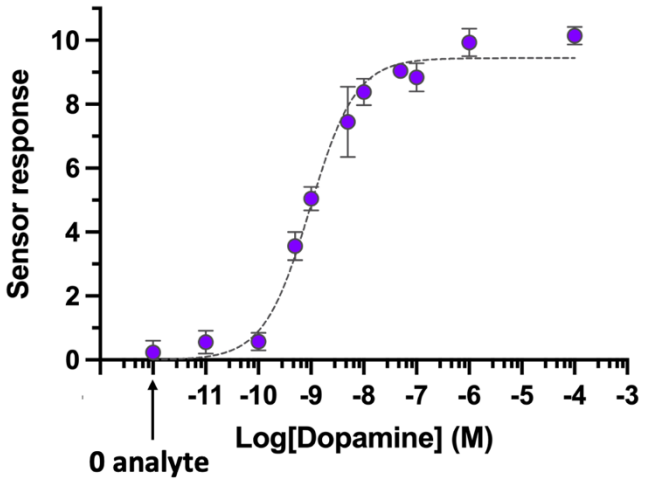
2. To monitor concentration flux of neurochemicals such as dopamine in the brain, the physiologically relevant range is 10 nM (1 x 10^{-8} M) to 1 µM (1 x 10^{-6} M). We want to see whether a newly isolated dopamine antibody is an optimal bioreceptor to monitor the change in dopamine concentrations in the brain. To test this new dopamine antibody, we functionalized these receptors to the sensing substrate of a device (*e.g.,* surface plasmon resonance). When dopamine was added to the solution, the signal change was plotted as a function of dopamine concentration (see table, SD = standard deviation, error bar) and shown in Figure 1.



Concentration (M)	Mean	SD
0	0.234	0.363
1 x 10^{-11}	0.557	0.361
1 x 10^{-10}	0.576	0.279
5 x 10^{-10}	3.559	0.439
1 x 10^{-9}	5.046	0.369
5 x 10^{-9}	7.452	1.105
1 x 10^{-8}	8.379	0.412
5 x 10^{-8}	9.039	0.191
1 x 10^{-7}	8.838	0.439
1 x 10^{-6}	9.933	0.430
1 x 10^{-4}	10.142	0.276

Figure 1. *Biosensor signal change dependence on dopamine (target) concentration when dopamine-specific antibodies (bioreceptors) are functionalized on the surface.*

a) What is the limit of detection (LOD, lowest detectable concentration) of this dopamine sensor? Note the equation for the LOD:

$$LOD = \frac{3\sigma_{S_0}}{\frac{dS}{dc}}$$

You can approximate the slope as 5 x 10^9 (you can check this by plotting the curve linearly rather than logarithmically)

b) What is the sensitive detection regime for this sensor for dopamine? In other words, what concentration range of dopamine can we differentiate with this sensor?

c) What is the approximate dissociation constant (K_d value) of the interaction between dopamine and the dopamine antibodies based on this sensing curve?

d) Now that you know the K_d of the dopamine aptamer (1 nM), we conduct an experiment where we add 600 pM of dopamine in buffer to the sensor surface. Using the law of mass action, calculate the expected fractional occupied receptor density ($\boldsymbol{\phi}$) in equilibrium.You can assume the free dopamine concentration is unchanged due to the reaction ([A] = [A_0]).

e) Would this biosensor be useful for monitoring dopamine flux in the brain microenvironment? The physiologically relevant range in humans is 1 nM (10^{-9} M) to 1µM (10^{-6} M).

Key Takeaways from this Exercise:

- Seeing the real-world applications of biosensors for human health (glucose for diabetes, neurotransmitters for brain chemical dynamics).
- Understanding how amperometric biosensors like the glucose sensor work by monitoring the generation of H_2O_2 based on enzymatic activity.
- Getting a grasp on reduction potentials and predicting the spontaneity of a reaction (whether an operating potential is necessary or if the reaction is spontaneous).
- Understanding the key metrics for evaluating biosensor performance such as the LOD, sensitivity, dissociation constant, fractional receptor occupancy

Solution:

Problem 1:

Let's first do a problem involving the classical glucose electrochemical biosensor. A glucose biosensor operates by detecting hydrogen peroxide H_2O_2 produced during the enzymatic oxidation of glucose. The generation of H_2O_2 is measured at the Platinum electrode, which has a standard reduction potential of +0.695 V for the reaction: $H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$

a) Based on this reduction potential, is this reaction spontaneous? Why or why not?

The standard reduction potential for the oxidation of H_2O_2 at the platinum electrode is $E^\circ = +0.695$ V. A reaction is spontaneous when the electrode potential is positive relative to the reduction potential of the coupled reaction. Since this is an oxidation reaction (reversed reduction), the negative potential means it is **not spontaneous**. Thus, external energy (*via* operating potential) is required to drive the reaction.

b) The glucose sensor generates a current of 10 µA during the measurement. Calculate how many moles of H_2O_2 are oxidized per second.

Faraday's Law of Electrolysis:

$$n = \frac{I}{zF}$$

where n is the number of moles oxidized per second
I is the current = 10 µA = 1 x 10^{-5} A = 1 x 10^{-5} C/s
z is the number of electrons transferred
F is Faraday's constant: 96485 C/mol.

Substitute the values:

$$n = \frac{1 \times 10^{-5}\ C/s}{2 \times 96485\ C/mol}$$

$$\boldsymbol{n = 5.18 \text{ x } 10^{-11} \text{ mol/s}}$$

Which is the number of moles of H_2O_2 oxidized per second.

c) What factors could influence the efficiency of the platinum electrode in oxidizing H_2O_2?

- **Surface area** – the larger the surface area, the higher the reaction rates with H_2O_2.
- **Operating potential** – an insufficient operating potential may not overcome the activation energy to oxidize H_2O_2.
- **pH and temperature** can both affect the reaction kinetics and electrode performance

d) Blood contains other electroactive molecules like ascorbic acid and uric acid. How could these interfere with the sensor? Propose one strategy to minimize these interferences.

Other electroactive molecules can also be oxidized at the electrode which produces nonspecific current and reducing selectivity for H_2O_2. Minimization of this nonspecific current generation includes coating the electrode surface (surface chemistry) to make the surface permeable to specific molecules (glucose) but not others (e.g., Nafion coating).

Problem 2:

To monitor concentration flux of neurochemicals such as dopamine in the brain, the physiologically relevant range is 10 nM (1×10^{-8} M) to 1 µM (1×10^{-6} M). We want to see whether a newly isolated dopamine antibody is an optimal bioreceptor to monitor the change in dopamine concentrations in the brain. To test this new dopamine antibody, we functionalized these receptors to the sensing substrate of a device (*e.g.,* surface plasmon resonance). When dopamine was added to the solution, the signal change was plotted as a function of dopamine concentration (see table) and shown in Figure 1.

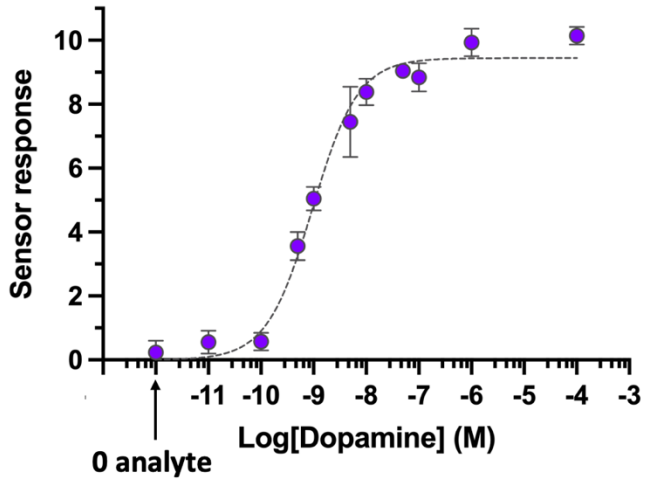


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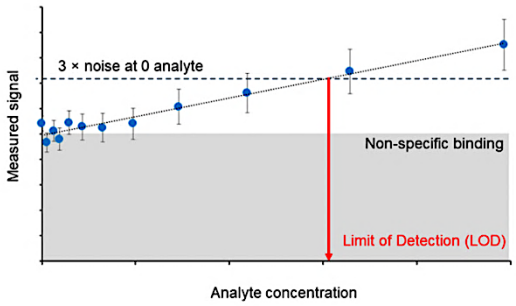
Figure 1. *Biosensor signal change dependence on dopamine (target) concentration when dopamine-specific antibodies (bioreceptors) are functionalized on the surface.*

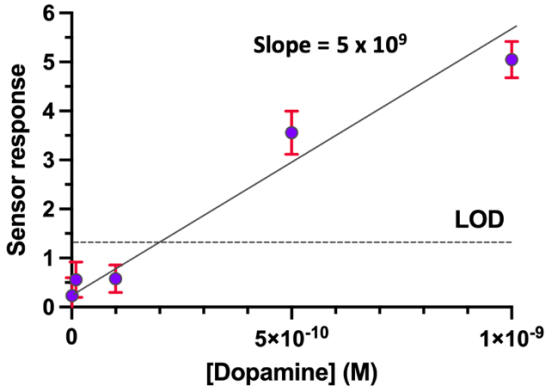
a) What is the limit of detection (LOD, lowest detectable concentration) of this dopamine sensor? Note the equation for the LOD:

$$LOD = \frac{3\sigma_{S_0}}{\frac{dS}{dc}}$$

Recall this diagram for calculating the LOD:

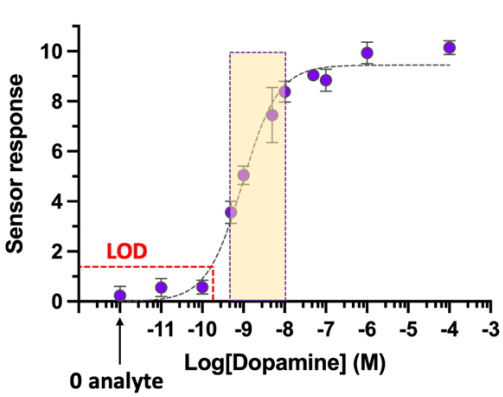


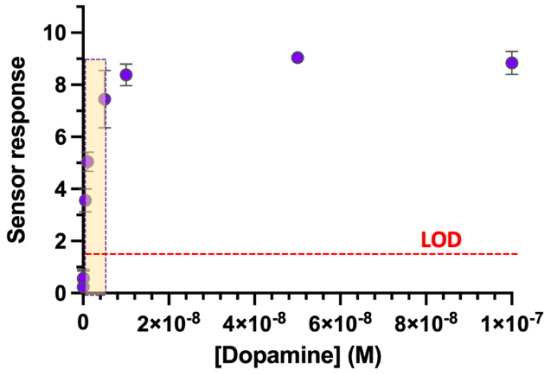
By using the standard deviation given in the table at zero analyte concentration (0.363, now highlighted in red) and using the value of the slope in the linear regime, we can calculate the LOD. If you plot the data with linear dopamine concentration on the x-axis (not logarithmic) you would see this:



$$\text{LOD} = \frac{3 \text{ x } 0.363}{5 \text{ x } 10^9} = 2 \text{ x } 10^{-10} \text{ M} = 0.2 \text{ nM}$$

b) What is the sensitive detection regime for this sensor for dopamine? In other words, what concentration range of dopamine can we differentiate with this sensor?

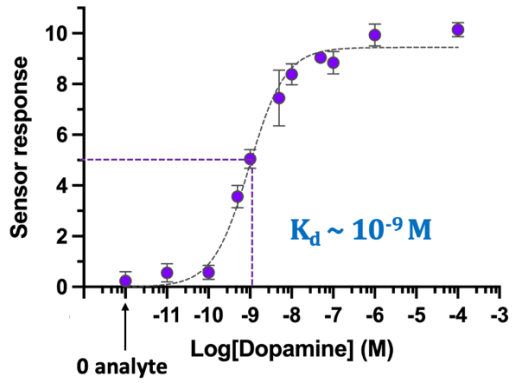




In the range of **5 x 10^{-10} to 10^{-8} M**. Remember, once the sensor saturates at higher concentrations, you can no longer detect changes.

c) What is the approximate dissociation constant (K_d value) of the interaction between dopamine and the dopamine antibodies based on this sensing curve?

The dissociation constant (K_d) is simply the **analyte concentration at which half the DNA aptamer receptors are bound.** If we take the half maximal binding on our sensing plot, we get a **K_d value of ~10^{-9} M (1 nM).**



d) Now that you know the K_d of the dopamine aptamer (1 nM), we conduct an experiment where we add 600 pM of dopamine in buffer to the sensor surface. Using the law of mass action, calculate the expected fractional occupied receptor density ($\boldsymbol{\phi}$) in equilibrium. You can assume the free dopamine concentration is unchanged due to the reaction ([A] = [A_0]).

The fractional occupied receptor density can be defined as the ratio of receptor-target complexes to the total receptor concentration. Using the law of mass action, we can express this as:

$$\phi = \frac{[RT]}{[R_{\text{total}}]} = \frac{[A]}{[A] + K_d}$$

Where [A] is the free ligand (dopamine) concentration = 600 pM = 6 x 10^{-10} M

K_d is the dissociation constant = 1 nM = 1 x 10^{-9} M

Now substitute in these values:

$$\phi = \frac{6 \times 10^{-10}}{6 \times 10^{-10} + 1 \times 10^{-9}}$$

$$\phi = \frac{6 \times 10^{-10}}{1.6 \times 10^{-9}}$$

$$\phi = 0.375$$

The fractional occupied receptor density is **0.375 or 37.5 %** at equilibrium – *i.e.*, the number of bound receptors on the sensor surface would be 37.5 %.

Here is the derivation to get to the right side of this equation:

$$\phi = \frac{[RT]}{[R_{\text{total}}]} = \frac{[A]}{[A] + K_d}$$

Step 1: At equilibrium, the dissociation constant, K_d is defined as:

$$K_d = \frac{[R][A]}{[RA]}$$

Where [R] = concentration of free bioreceptors; [A] = concentration of free ligands; [RA] = concentration of receptor-target complexes.

Step 2: Express [R] in terms of $[R_{total}]$, where the total receptor concentration includes both the bound and unbound receptors:

$$[R_{\text{total}}] = [R] + [RA]$$

Rearrange for [R]:

$$[R] = [R_{\text{total}}] - [RA]$$

Step 3: Substitute [R] into the K_d expression:

Using the K_d definition:

$$K_d = \frac{([R_{\text{total}}] - [RA])[A]}{[RA]}$$

Rearrange to isolate $[RA]$:

$$K_d[RA] = ([R_{\text{total}}] - [RA])[A]$$

Distribute $[A]$:

$$K_d[RA] = [R_{\text{total}}][A] - [RA][A]$$

Rearrange terms to group $[RA]$:

$$[RA](K_d + [A]) = [R_{\text{total}}][A]$$

Solve for $[RA]$:

$$[RA] = \frac{[R_{\text{total}}][A]}{K_d + [A]}$$

Step 4: Define the fractional occupancy and solve:

$$\phi = \frac{[RA]}{[R_{\text{total}}]}$$

Substitute $[RA]$ from the equation above:

$$\phi = \frac{\frac{[R_{\text{total}}][A]}{K_d+[A]}}{[R_{\text{total}}]}$$

Cancel $[R_{\text{total}}]$:

$$\phi = \frac{[A]}{K_d + [A]}$$

e) Would this biosensor be useful for monitoring dopamine flux in the brain microenvironment? The physiologically relevant range in humans is 1 nM (10^{-9} M) to 1 µM (10^{-6} M).

Partially yes! The dynamic range of the sensor is somewhat overlapping with the physiologically relevant range for dopamine monitoring in humans. However, for concentrations higher than the detection range, we would have to shift the sensitivity regime through strategies like altering the surface density of the bioreceptors on the surface of the biosensor.