

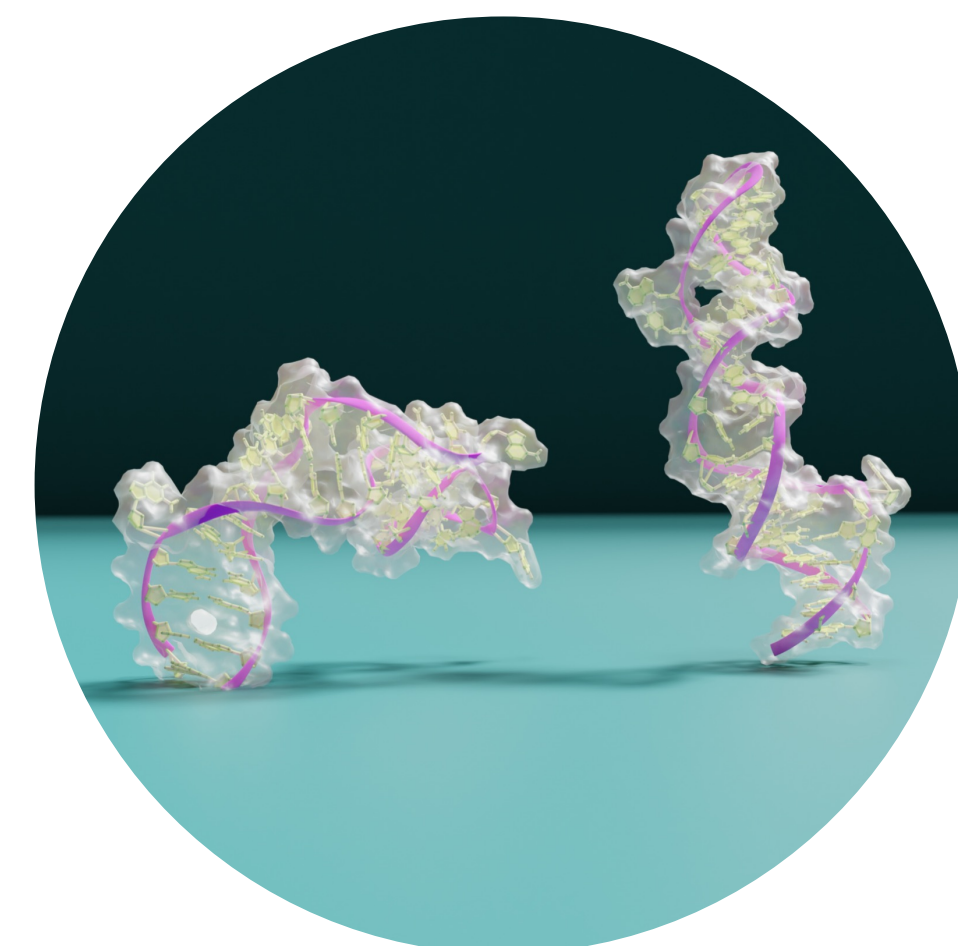
Applications Lesson 12

MSE 304

Nako Nakatsuka

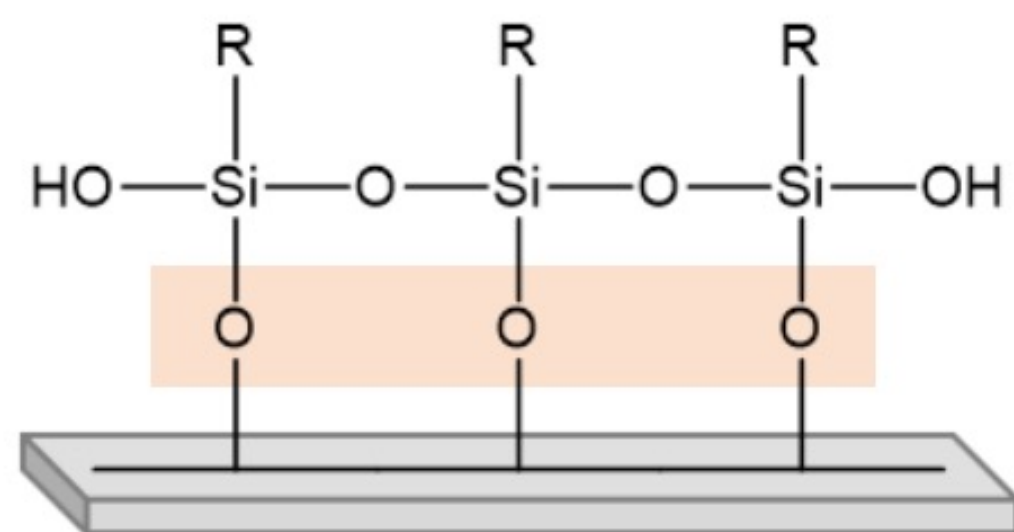
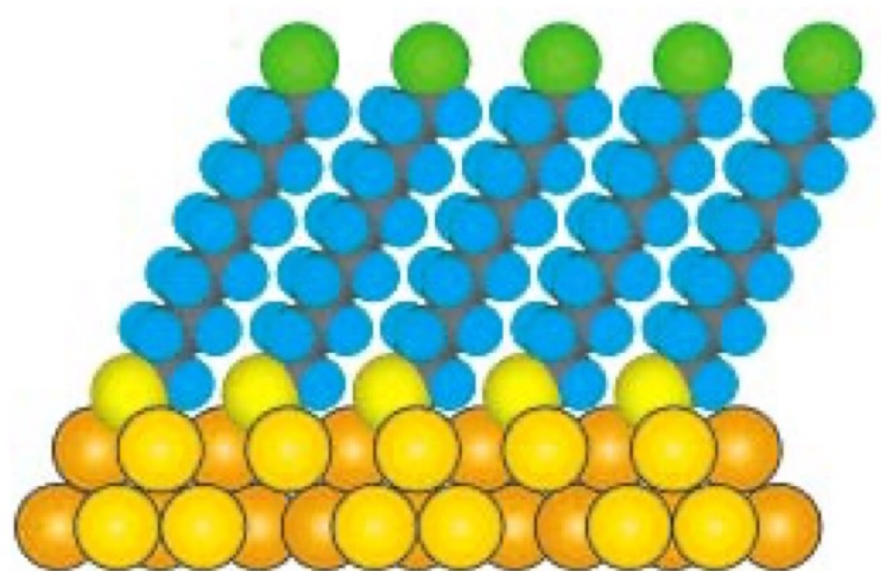
Nako.Nakatsuka@epfl.ch

December 6th 2024

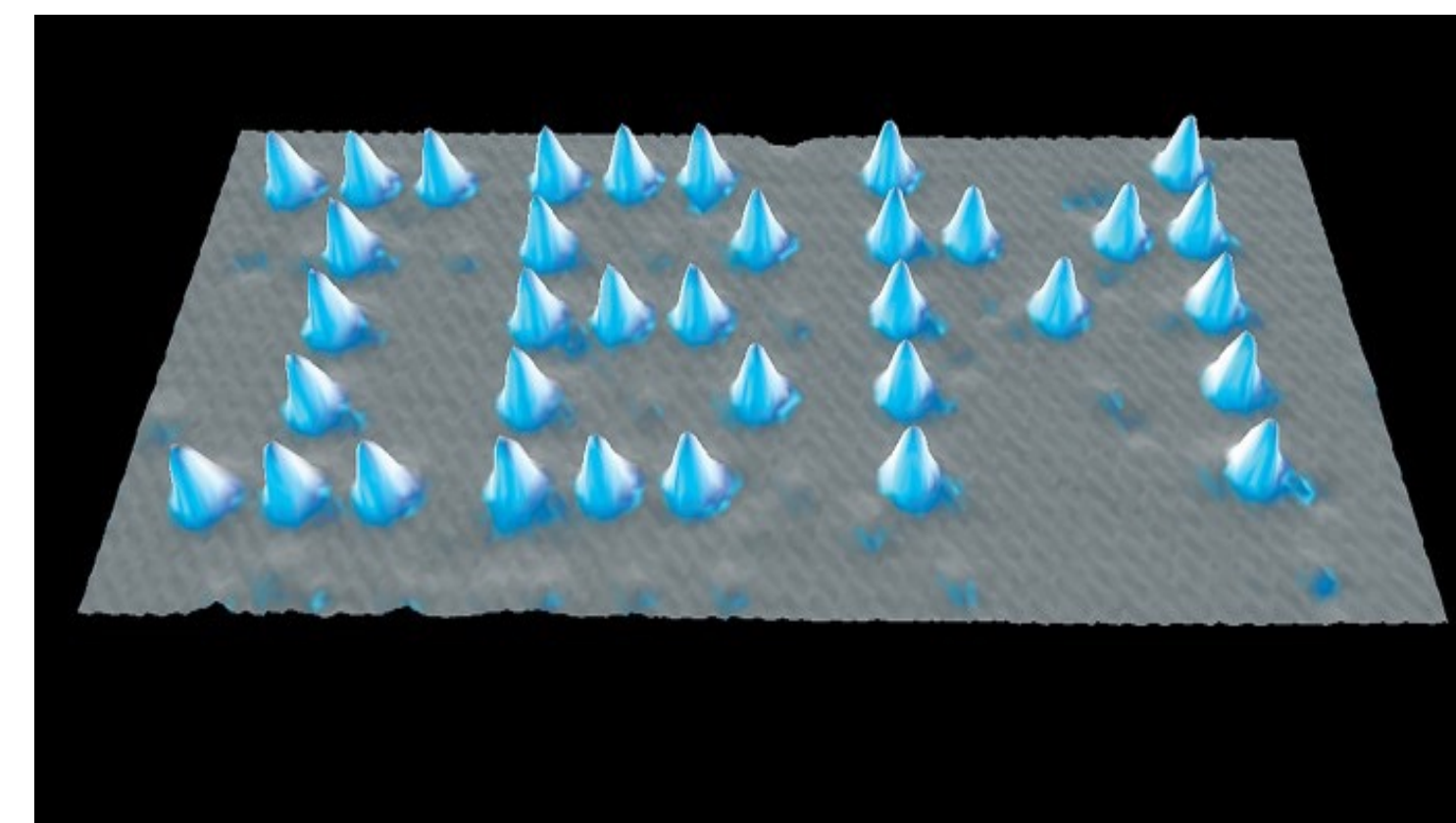


Recap of Last Week's Class on Applications

- **Surface Chemistry** – alkanethiols, alkylsilanes, polymers for nonspecific binding
- **Nanoscience** – interactions of nanoparticles with light (plasmon resonance)
- **AFM and STM to Visualize Surfaces**



Gold nanoparticles

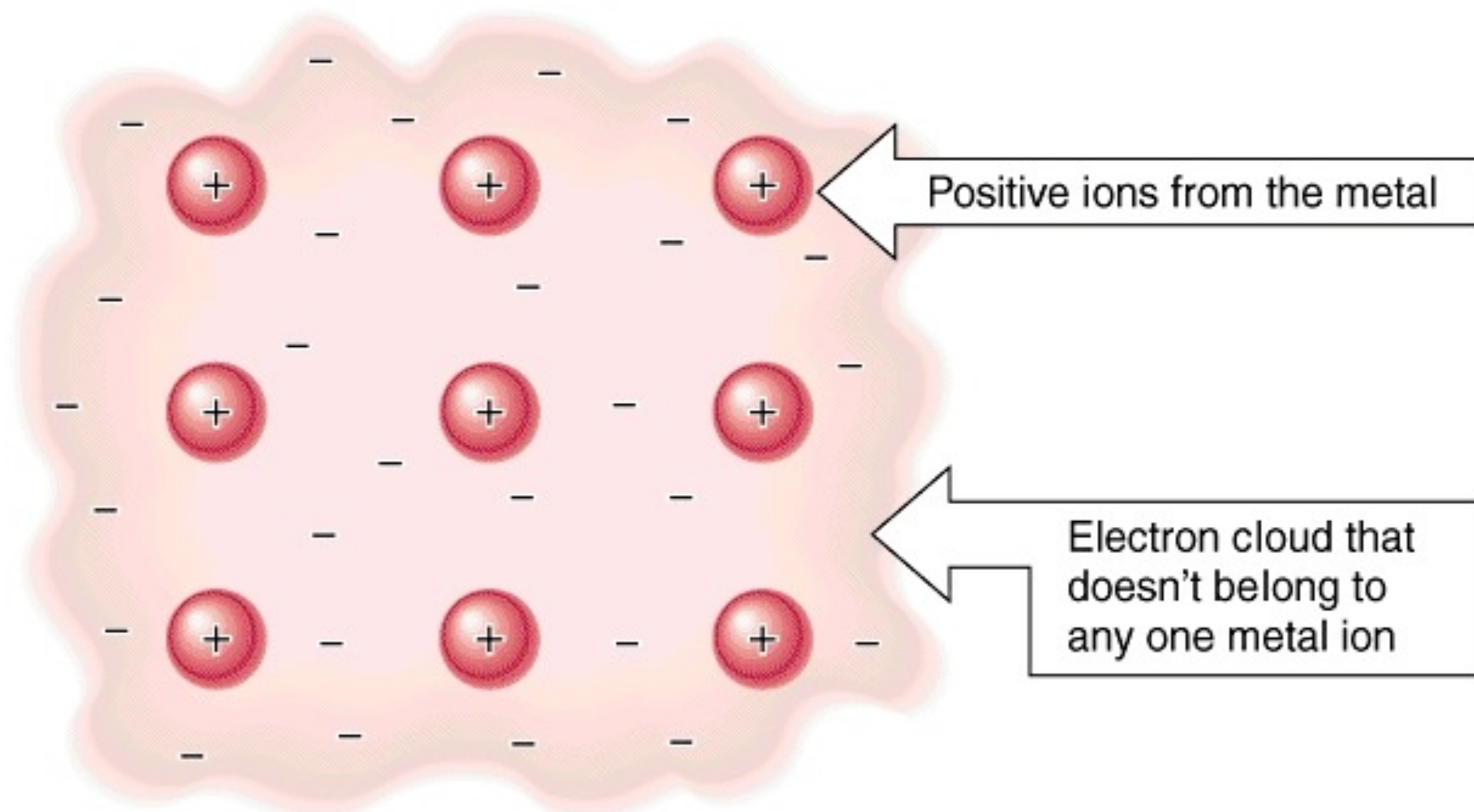


What We Cover in Today's Class

- **Plasmonic nanoparticles** – short recap on plasmon resonance used for rapid tests!
- **What is a Biosensor** – molecular recognition and performance parameters
- **History of Biosensors** – Clark electrode and recap of electrochemistry
- **State-of-the-art Biosensor: Glucose Sensor** – recap of electrolysis
- **Remaining challenges of Biosensors** – now let's take a look at the COVID-19 test

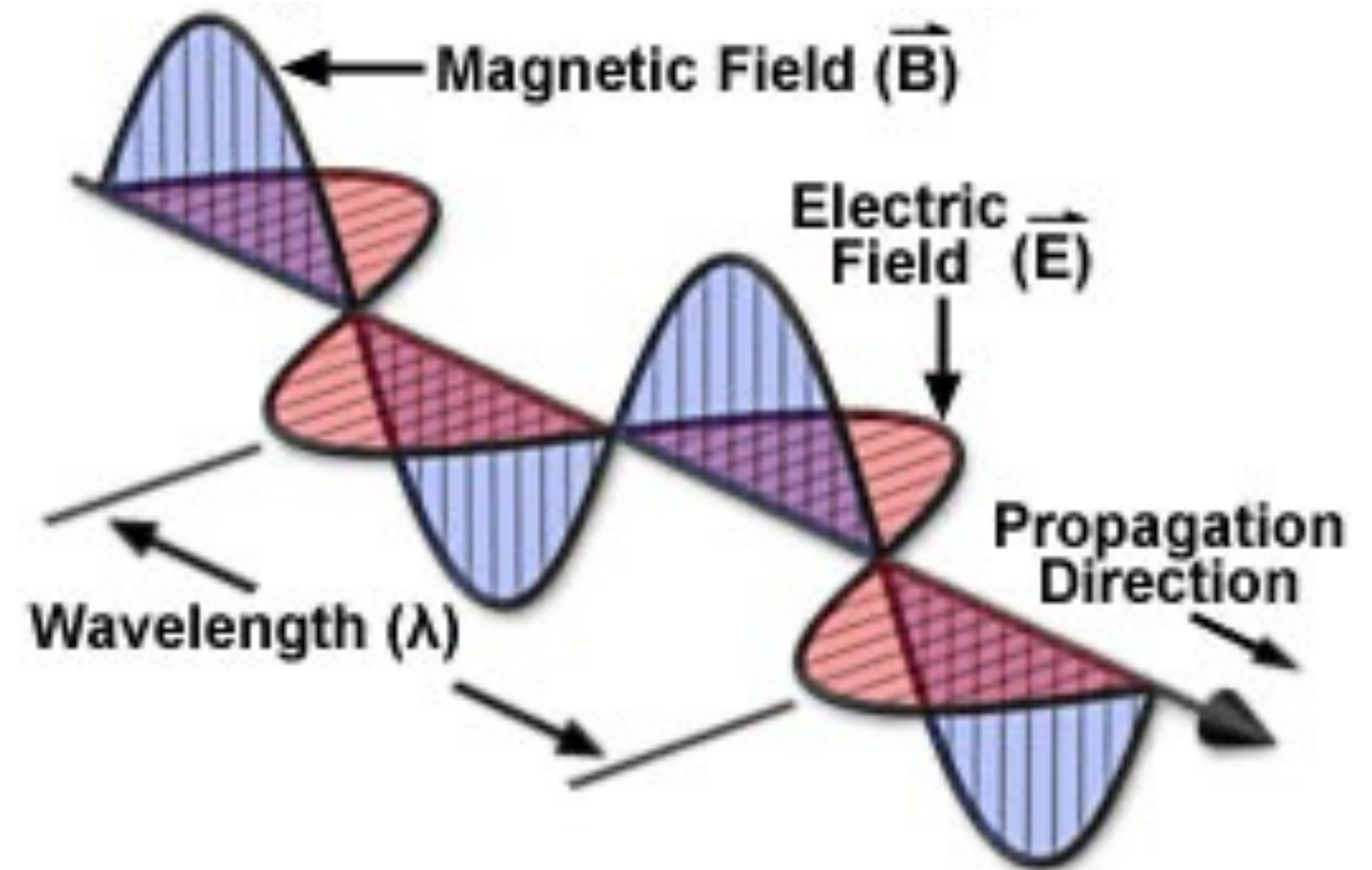
Influencing the Sea of Electrons on Surfaces Using Light

The Free Electron Sea



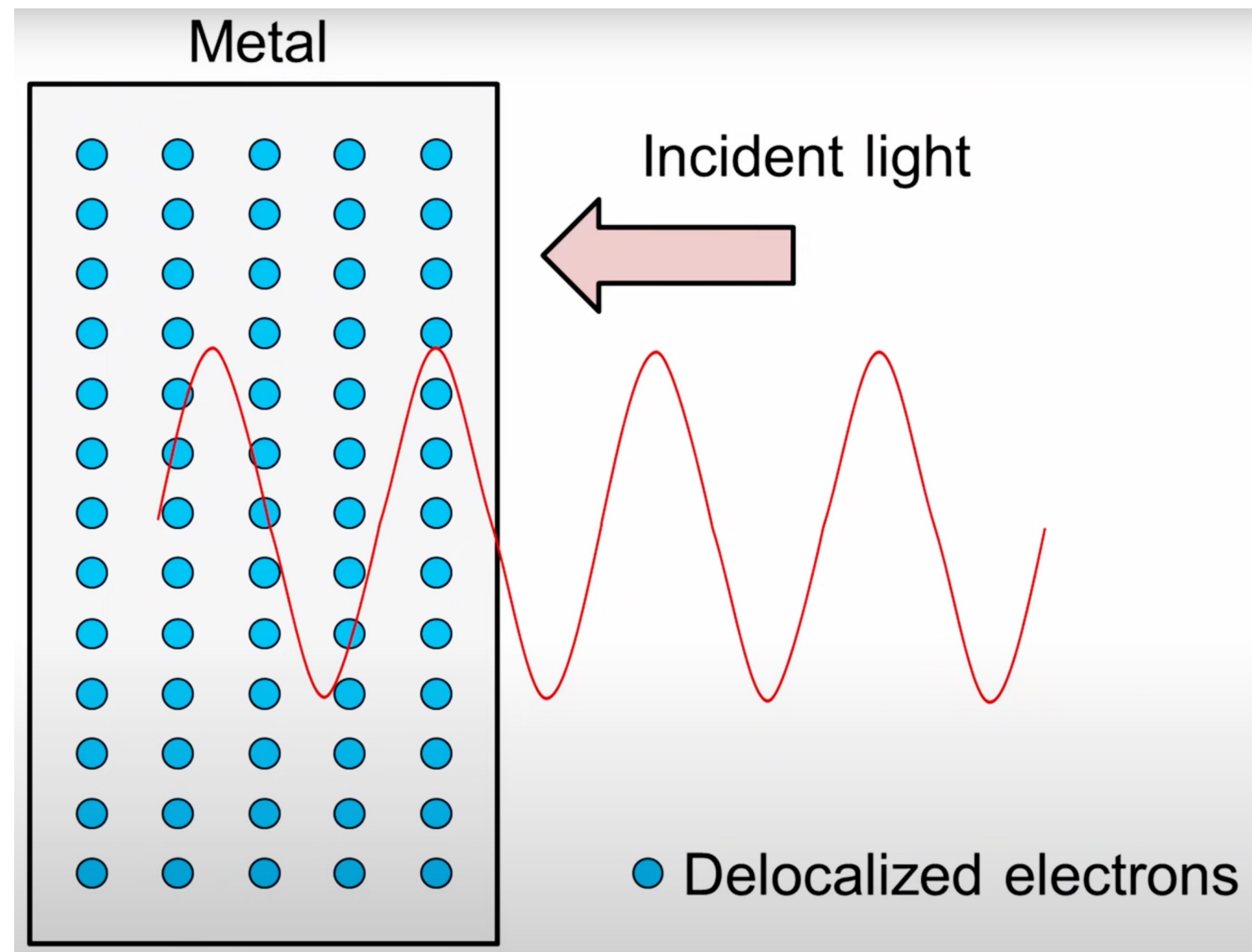
Copyright 1998 by John Wiley and Sons, Inc. All rights reserved.

Light as an oscillating electromagnetic field



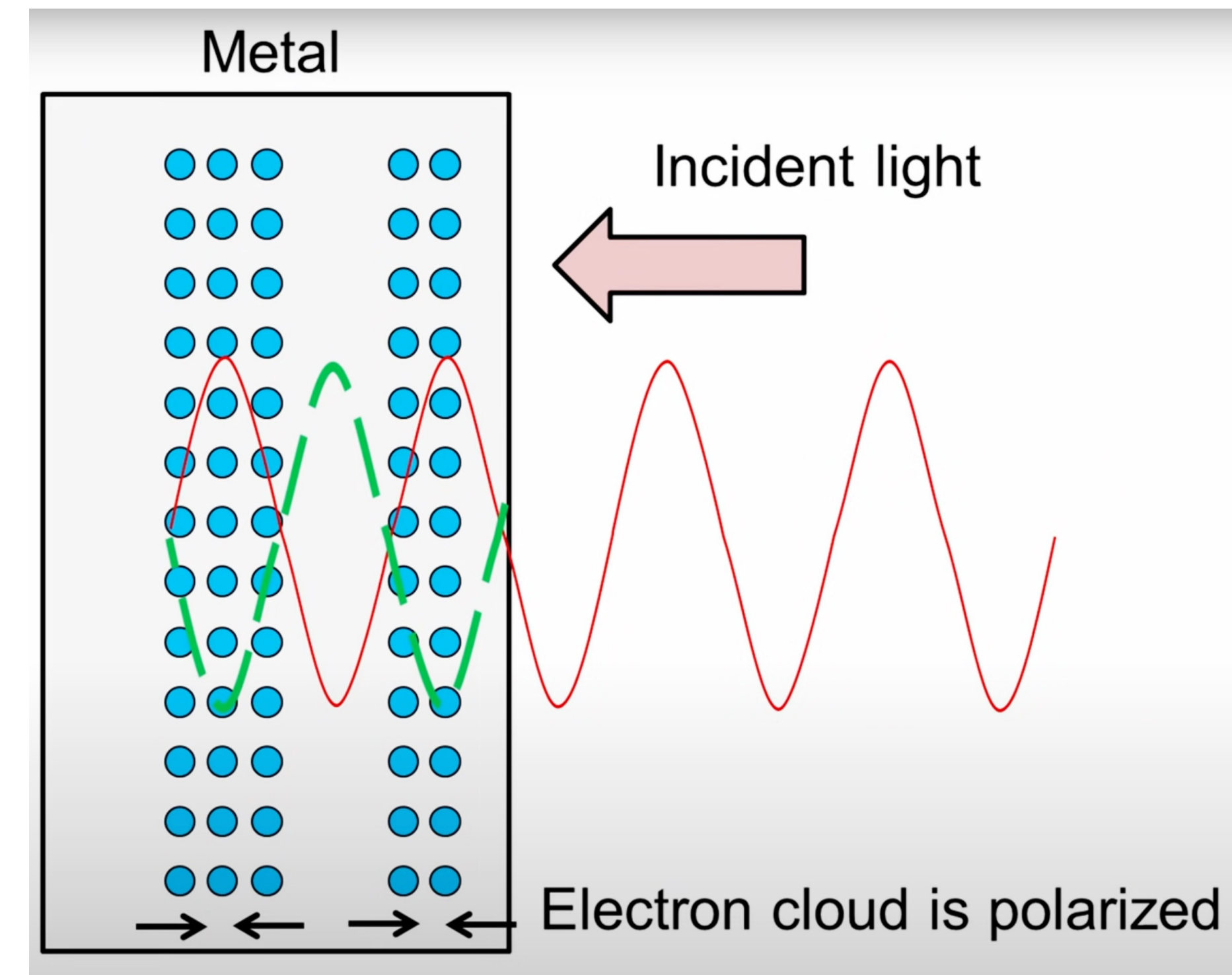
Electrons in metal are affected by the electromagnetic field
from light incident to surface

Effect of Plasmon Resonance



Incident light excites electrons on metal surface

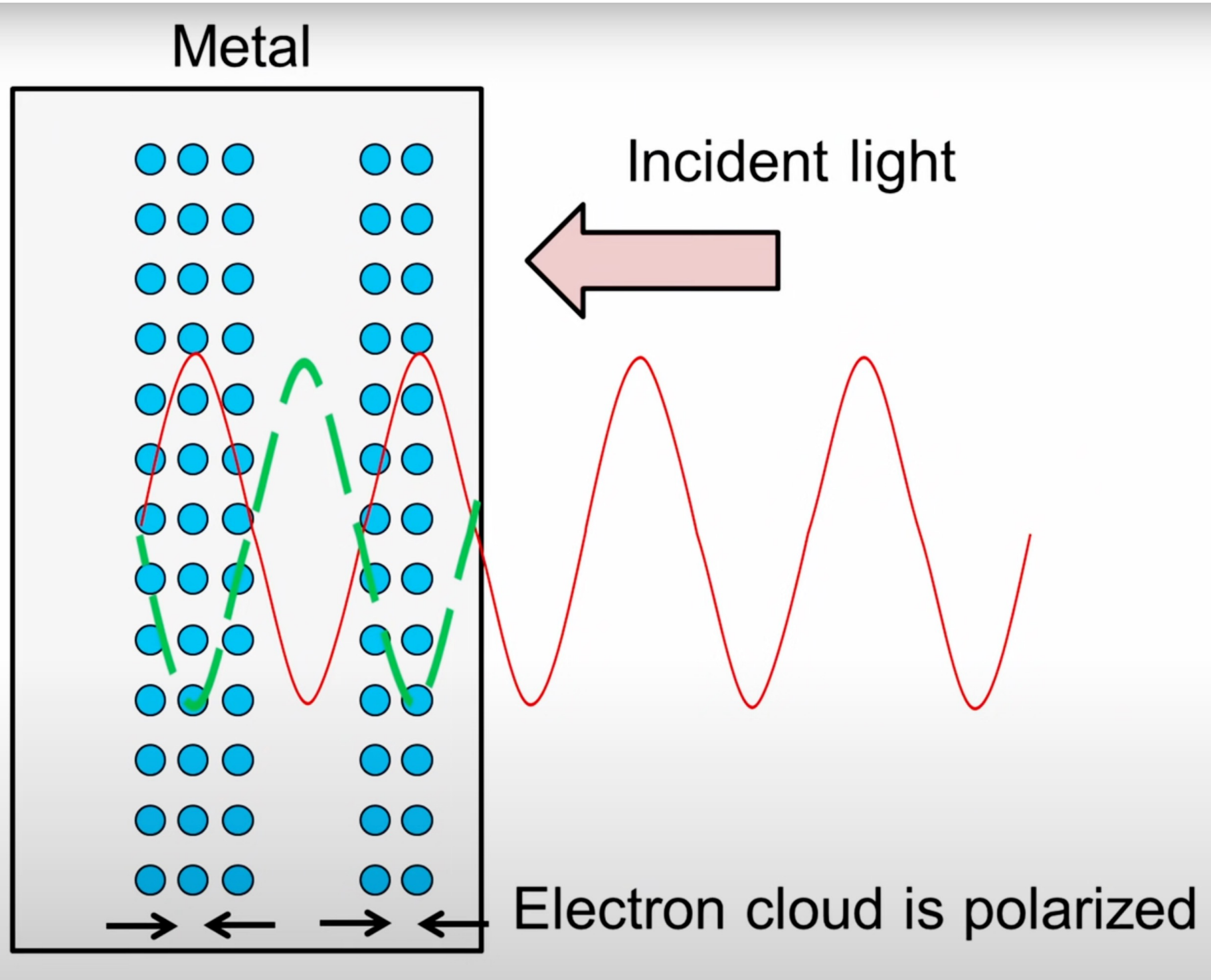
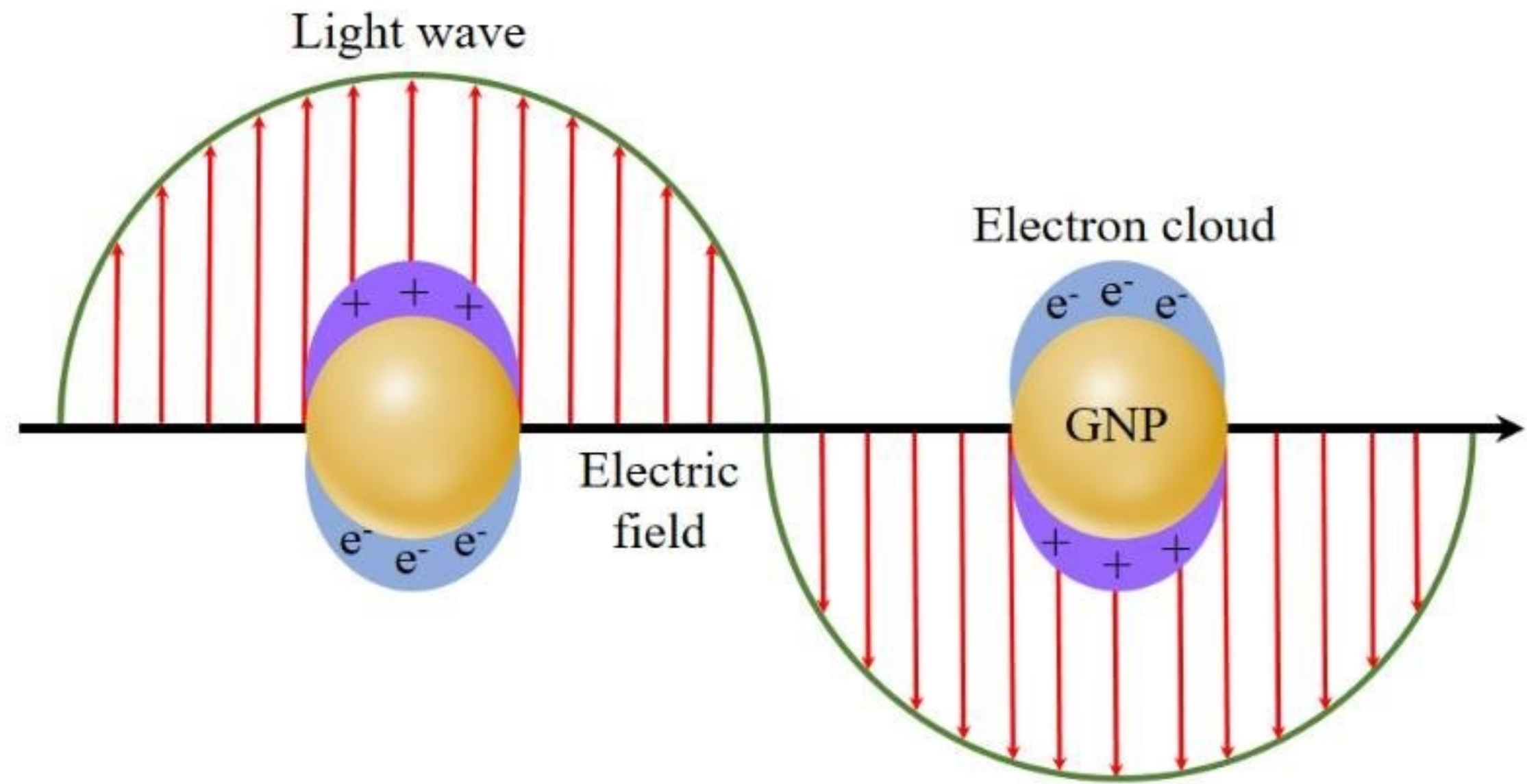
Oscillating field of light interacts with
free electrons



Electron cloud is perturbed

Electrons begin to oscillate collectively as a
polarized cloud (plasmon)

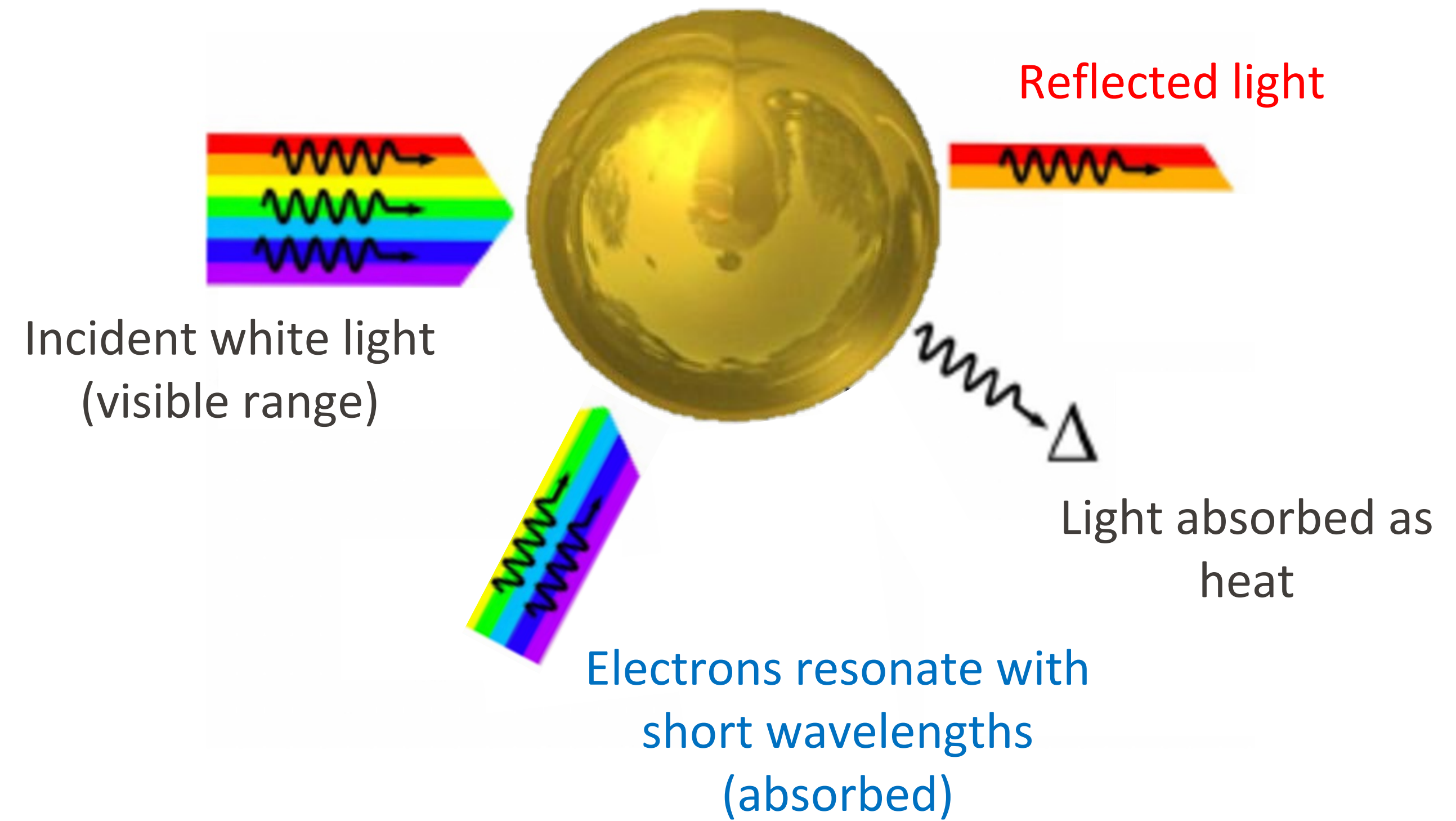
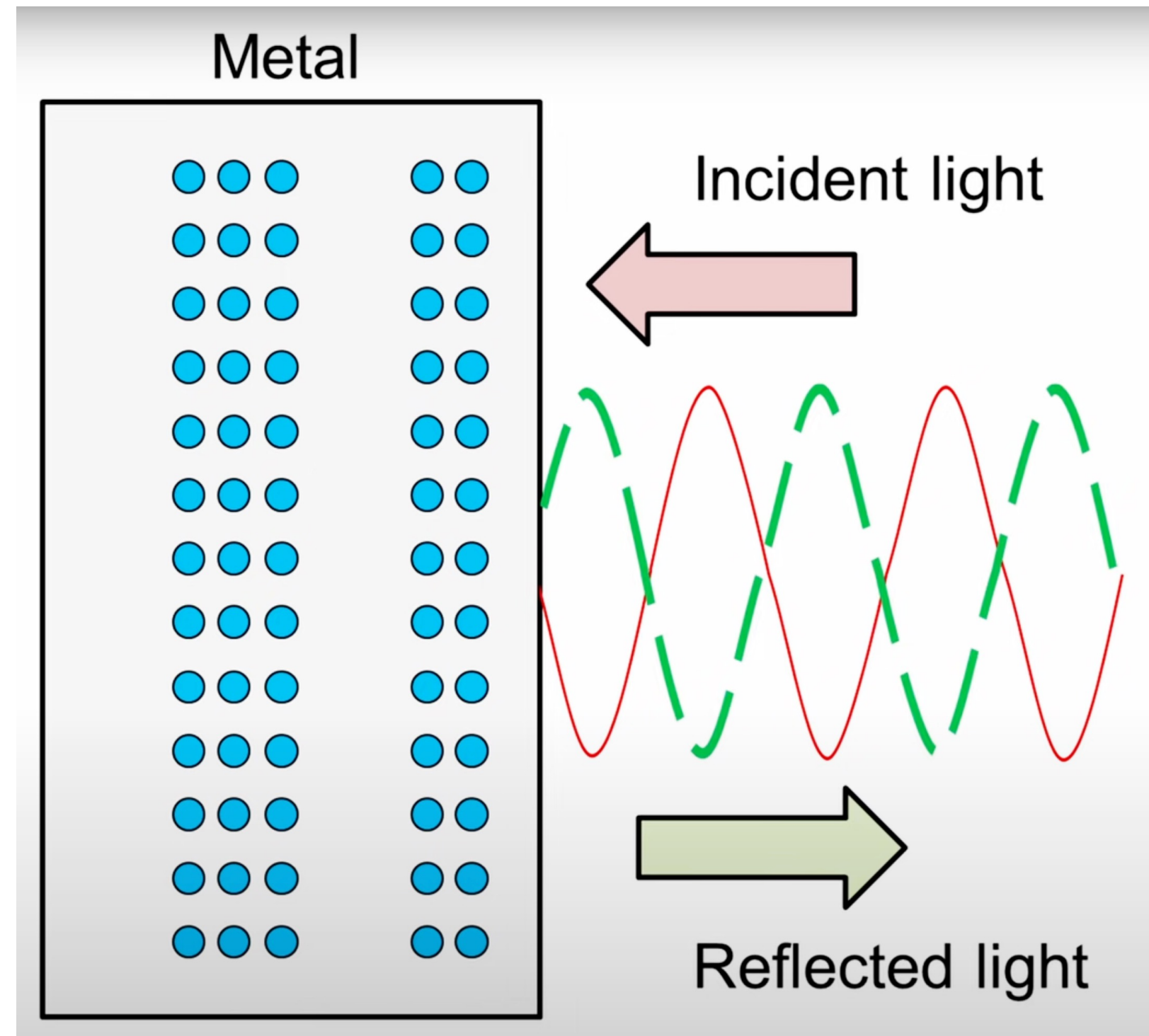
Effect of Plasmon Resonance



Electron cloud is perturbed

Electrons begin to oscillate collectively as a polarized cloud (plasmon)

Effect of Plasmon Resonance



At resonance frequency, incident light energy transferred to oscillating electron cloud (plasmon excitation)

Only specific wavelengths of light are reflected back while others are absorbed or scattered

Relative contributions of absorption and scattering determined by size and shape of plasmonic particles

Going Back Many Centuries (4th Century): Lycurgus Cup

The glass contains gold and silver nanoparticles 10–100 nm in size

Resonance scatters
green wavelengths



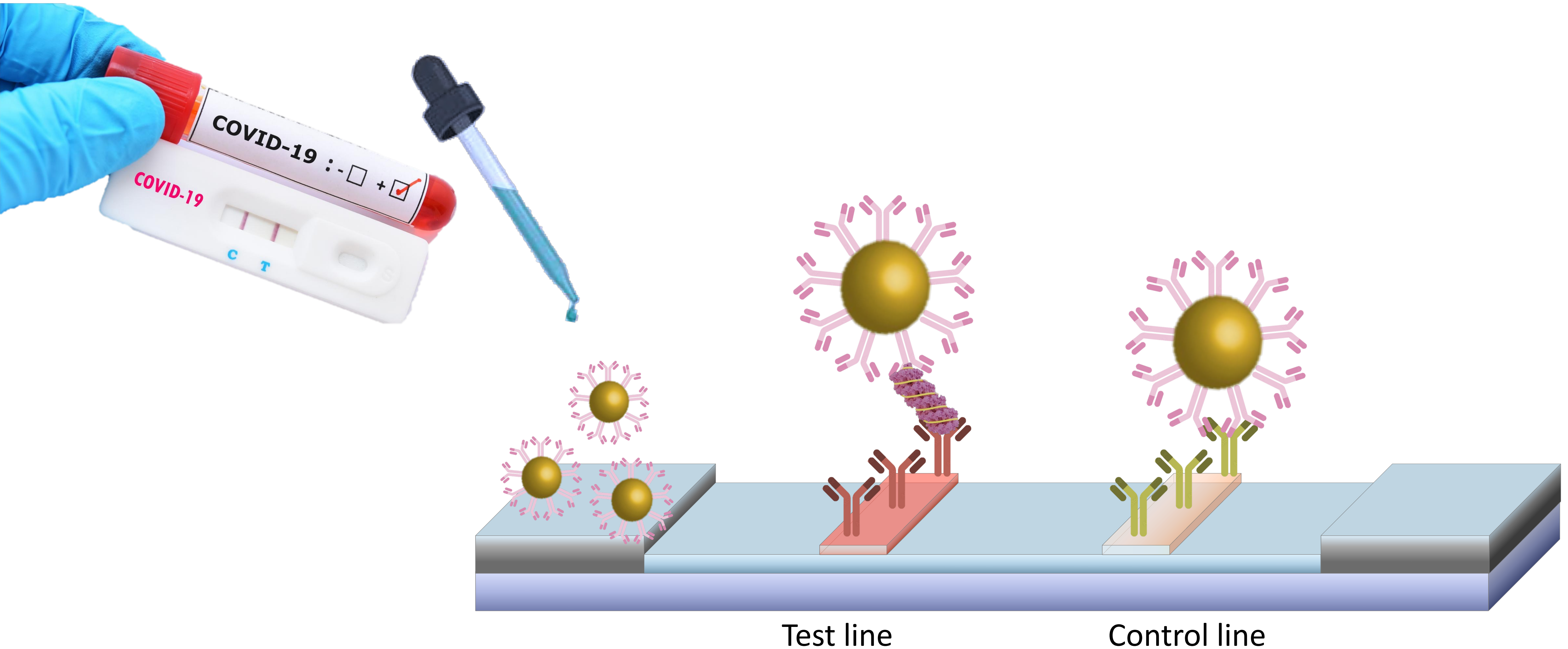
Lit from front
(reflection)

Nanoparticles absorb
shorter wavelengths
(blue and green) and
remaining wavelengths
pass through and is
transmitted



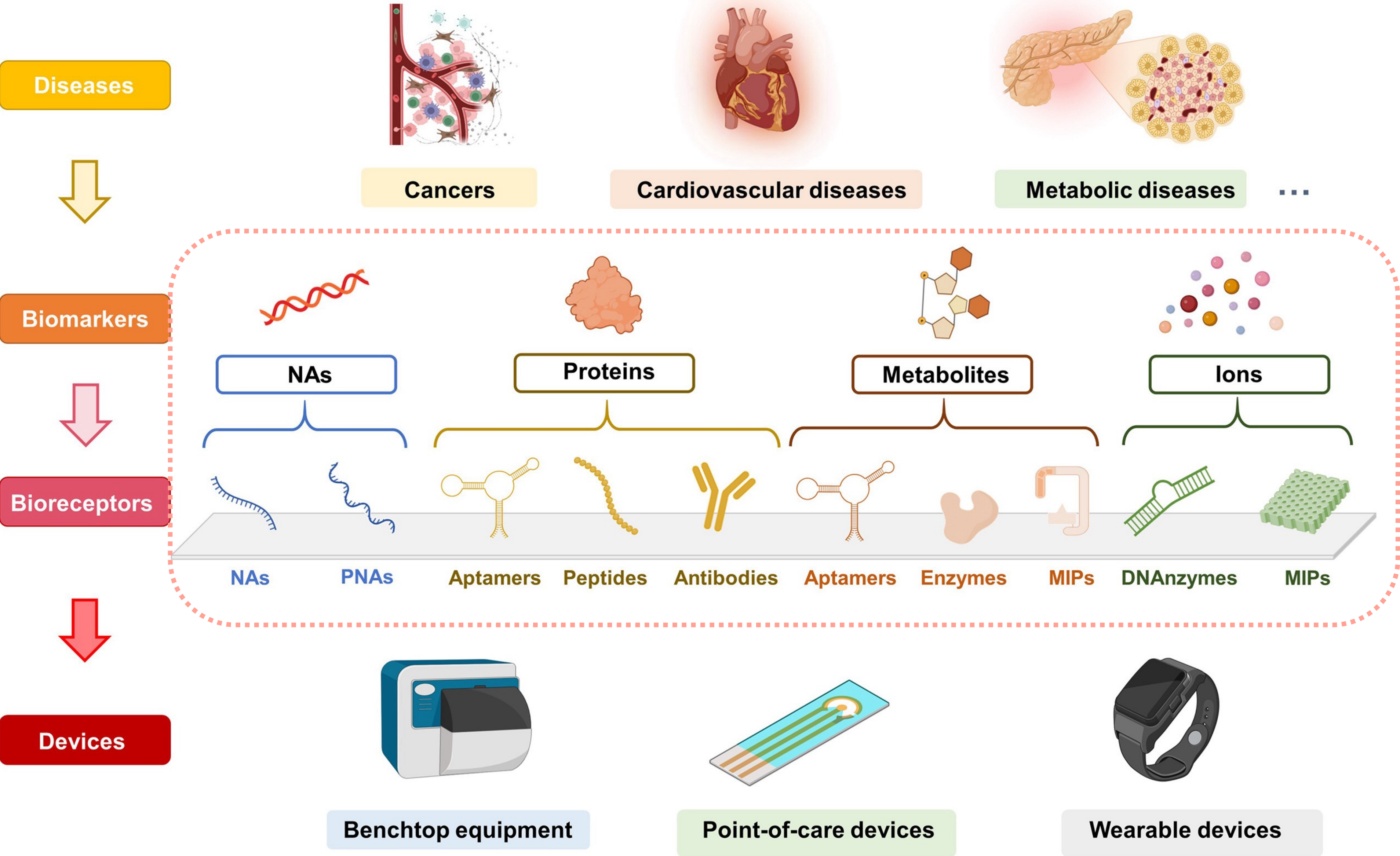
Lit from behind
(transmission)

COVID Rapid Tests – Use of Gold Nanoparticles



First, we need to understand how biosensors work!

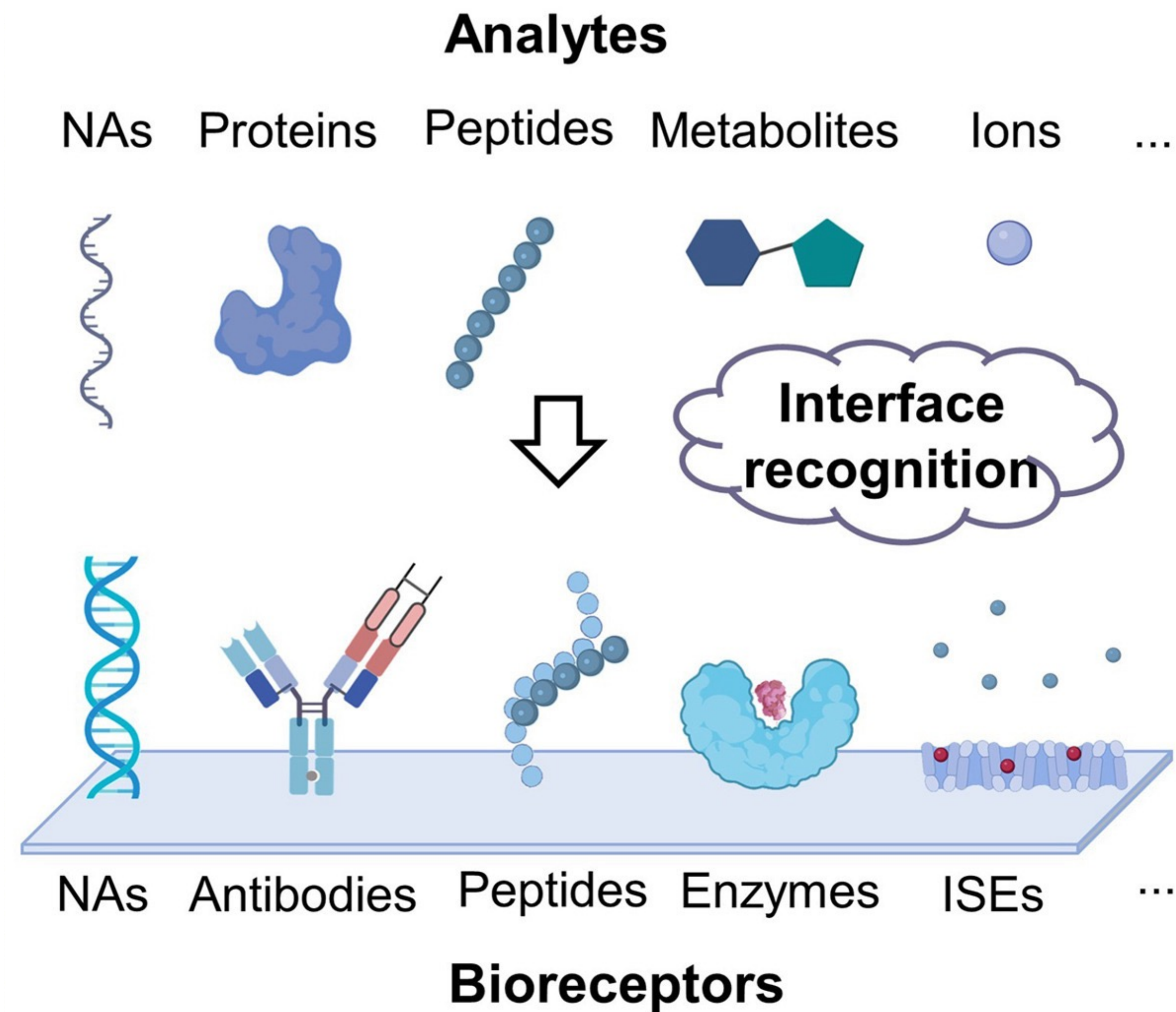
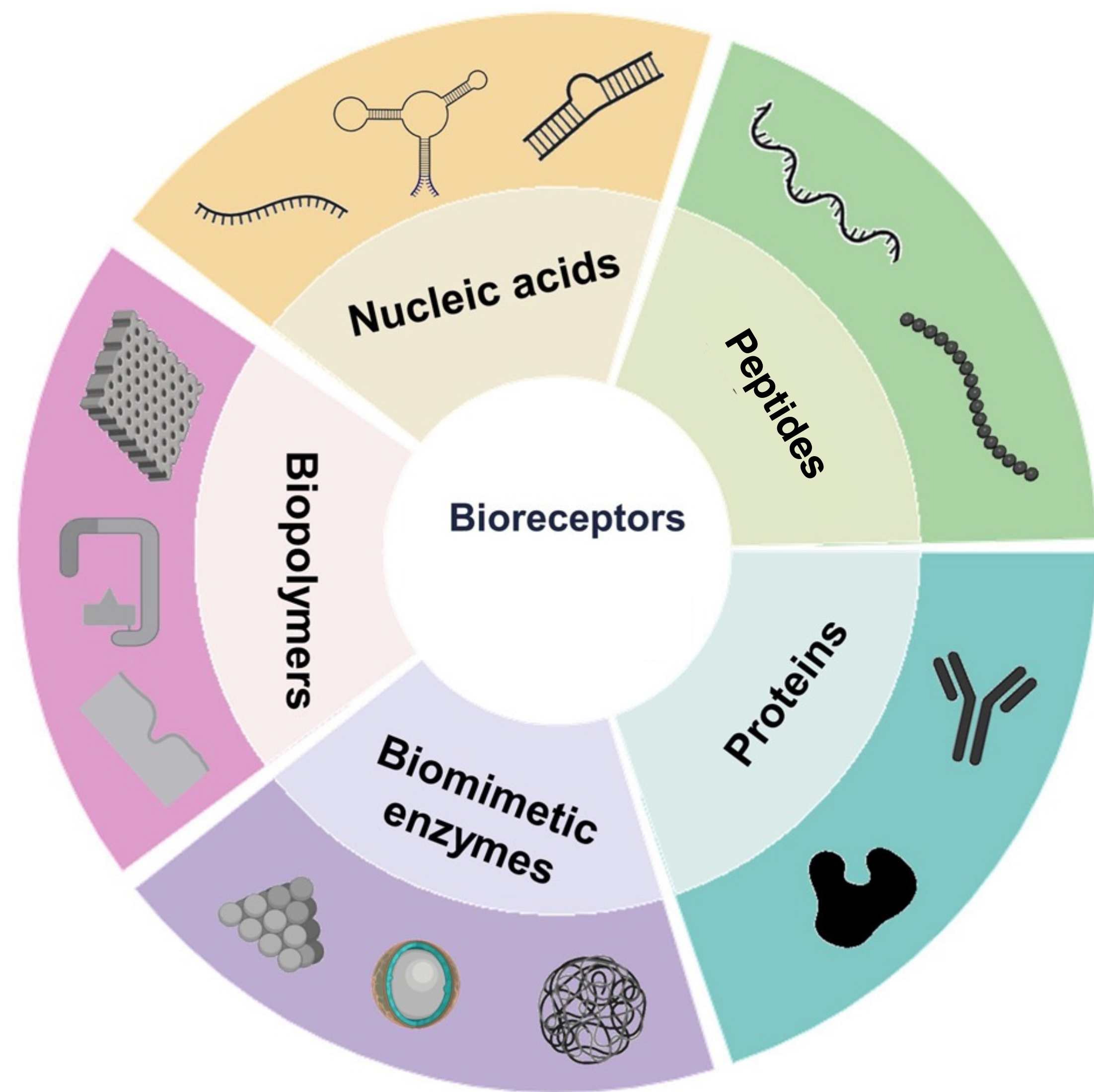
Biosensors For Human Health



What are Bioreceptors?

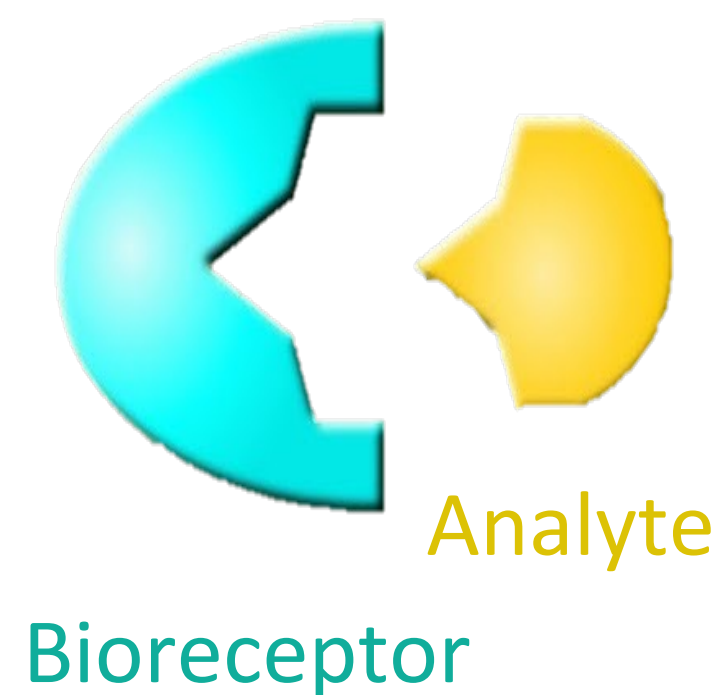
How does molecular recognition work?

Molecular Recognition Elements/Biological Receptors



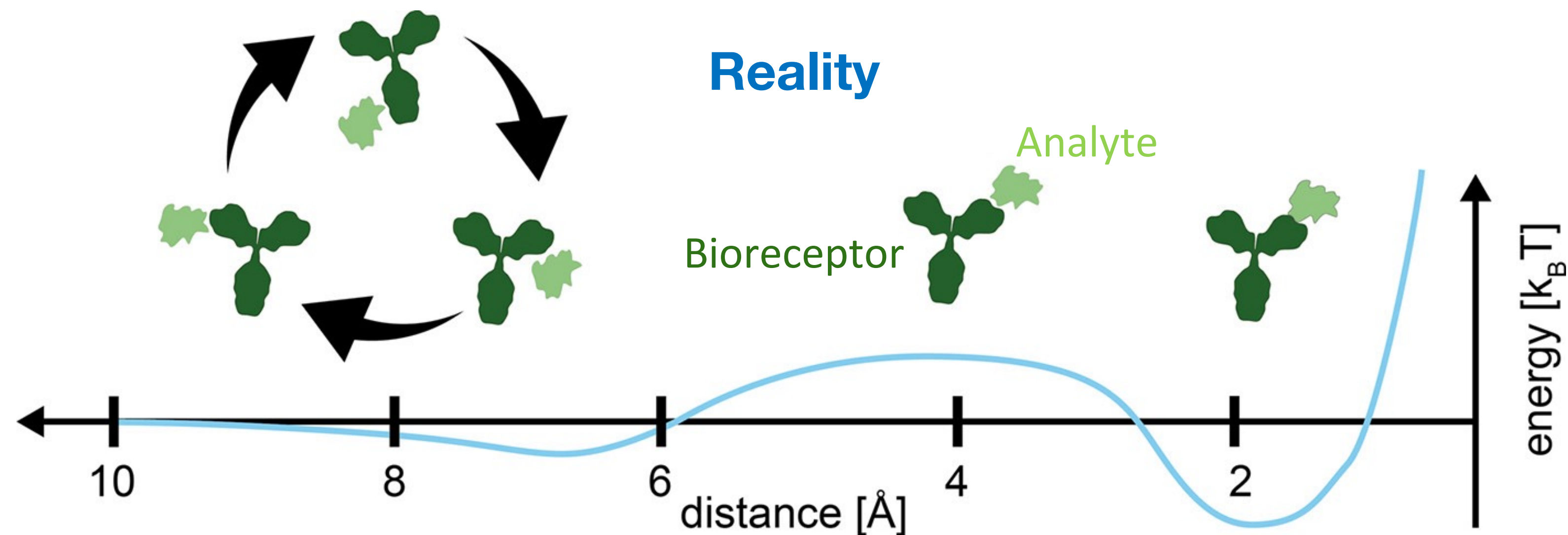
How do Bioreceptors Interact with their Analytes?

Expectation

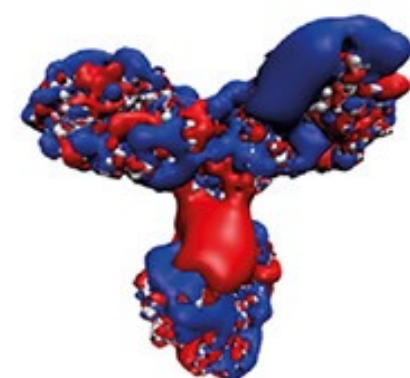


Molecular recognition of target by bioreceptor

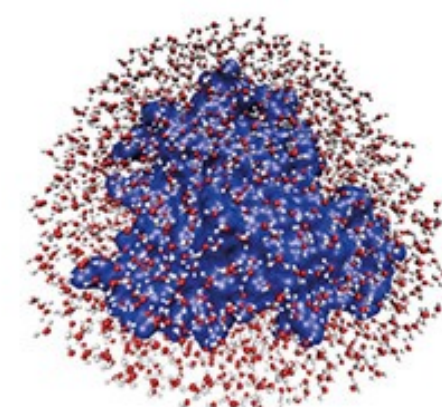
Reality



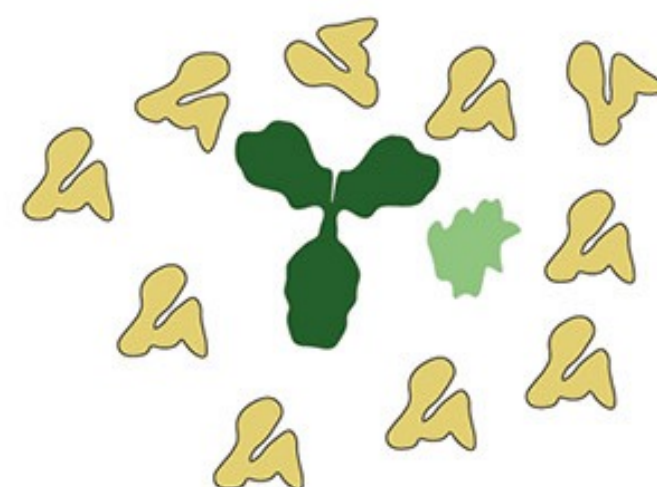
inhomogeneous surface potential
- electrostatic steering



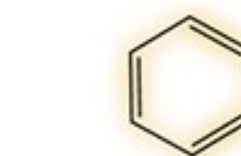
solvent effects
- hydrophobic attraction
- hydrophilic repulsion



molecular crowding



hydrophobic



π - π



cation- π

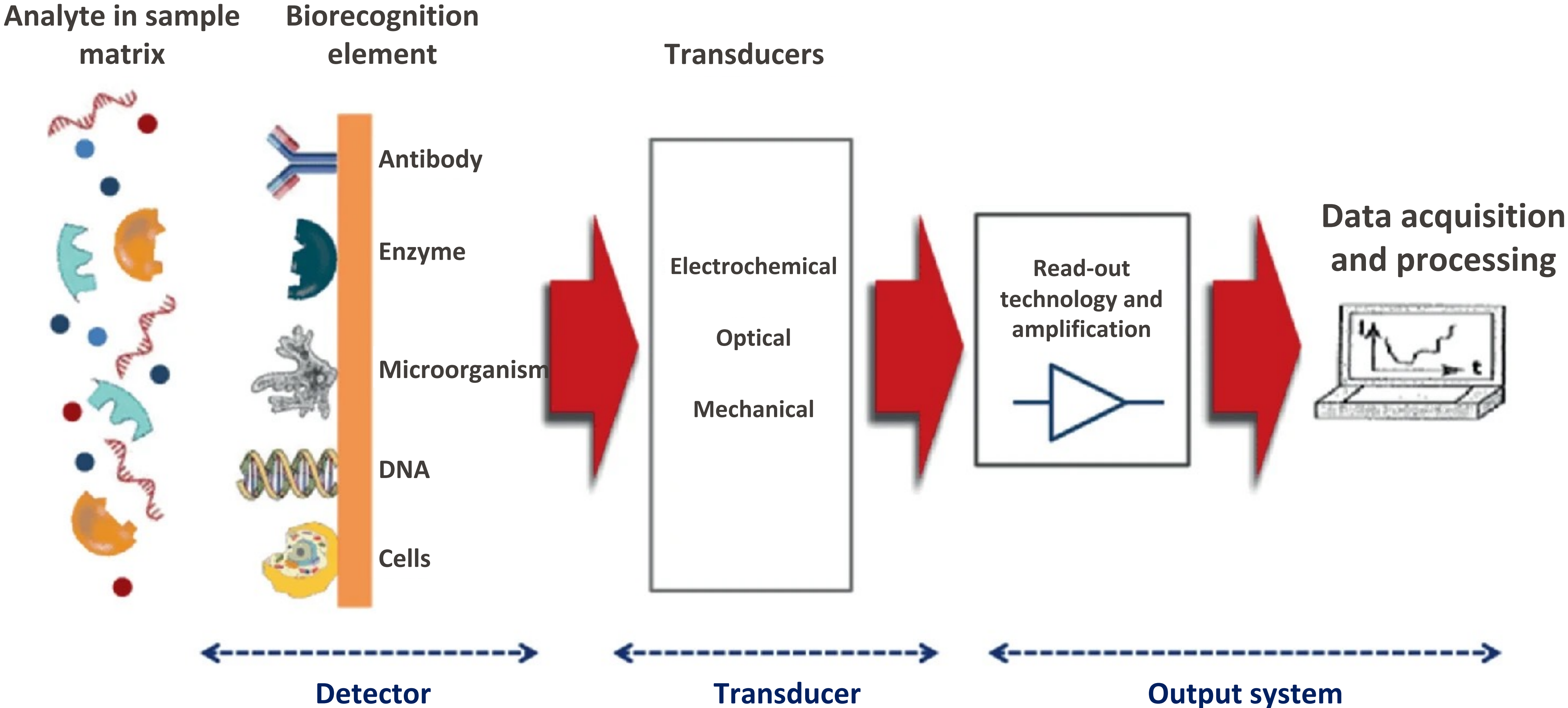


salt bridge



hydrogen bond

Biosensor: Transduces Molecular Binding Event to a Signal



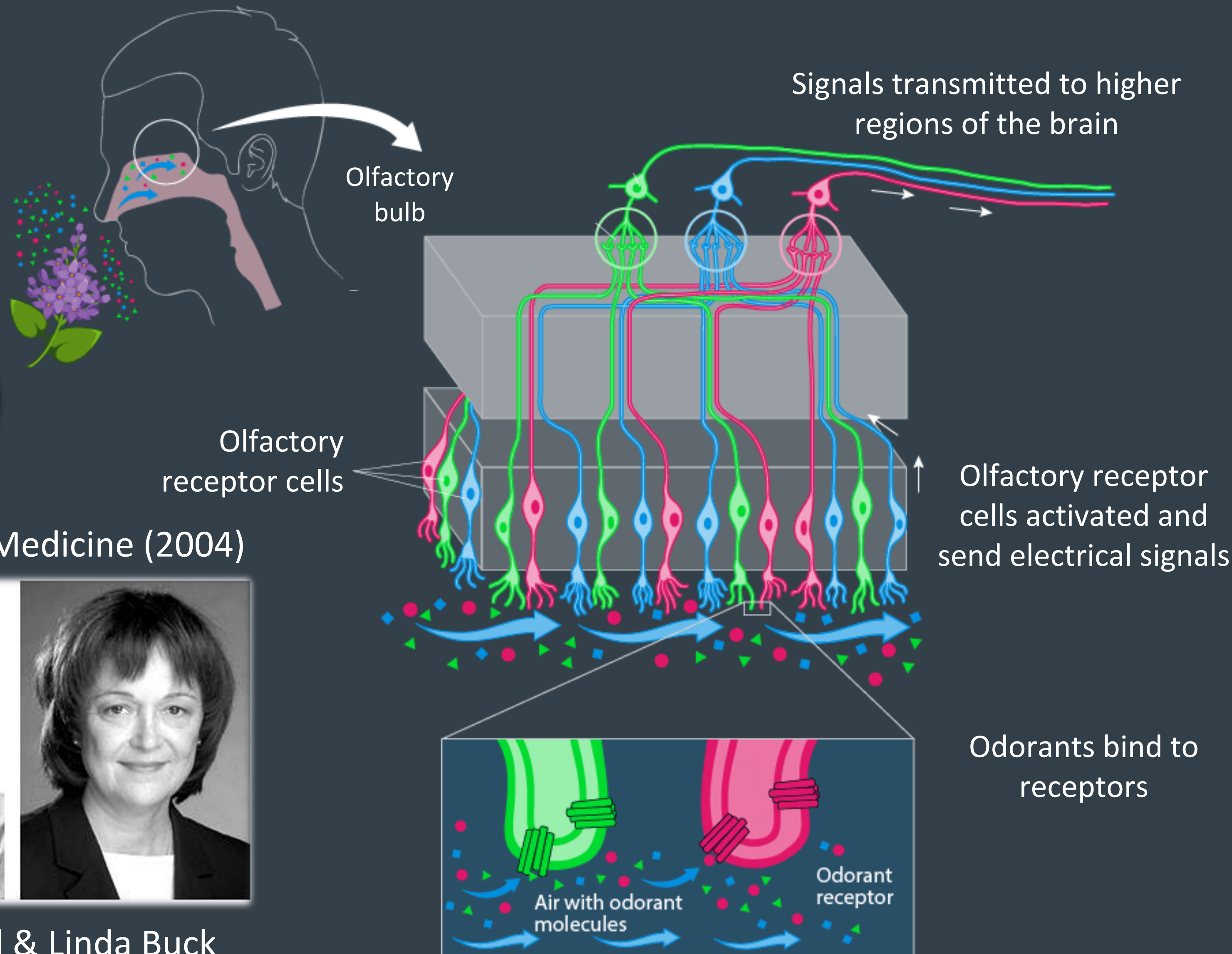
How Do We Smell Things?



Nobel Prize in Medicine (2004)



Richard Axel & Linda Buck



The World's Best "Biosensor"

Olfactory epithelium

Back of nasal passage that houses sensory receptors

Humans
5-10 cm²

Dogs
60-170 cm²

Olfactory bulb

Brain region responsible for processing smells

Dogs have **40x** larger olfactory bulbs than humans relative to total brain size



Olfactory receptors

Back of nasal passage that houses sensory receptors

Humans
5-6 million

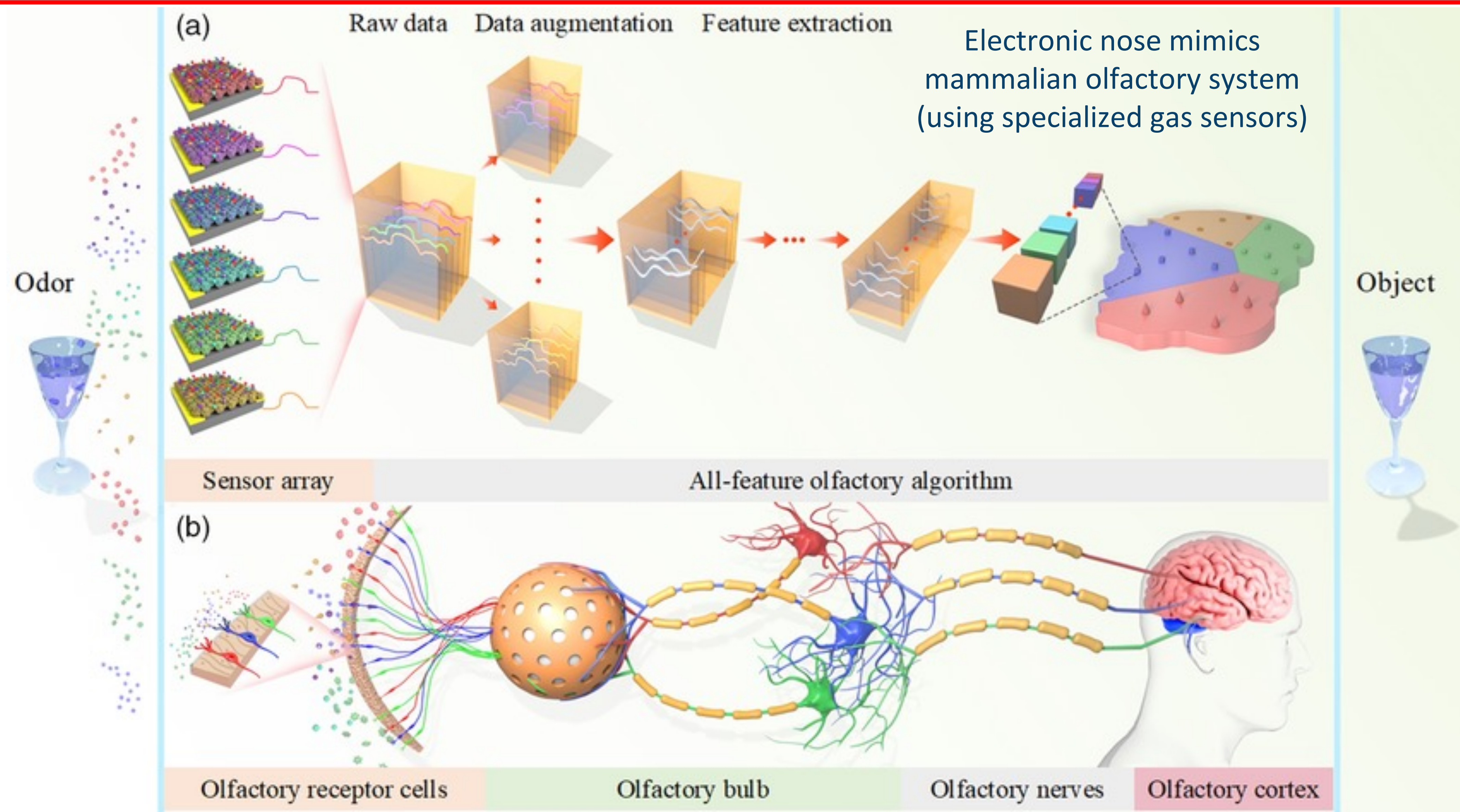
400 different types of receptor proteins

Dogs
220-300 million

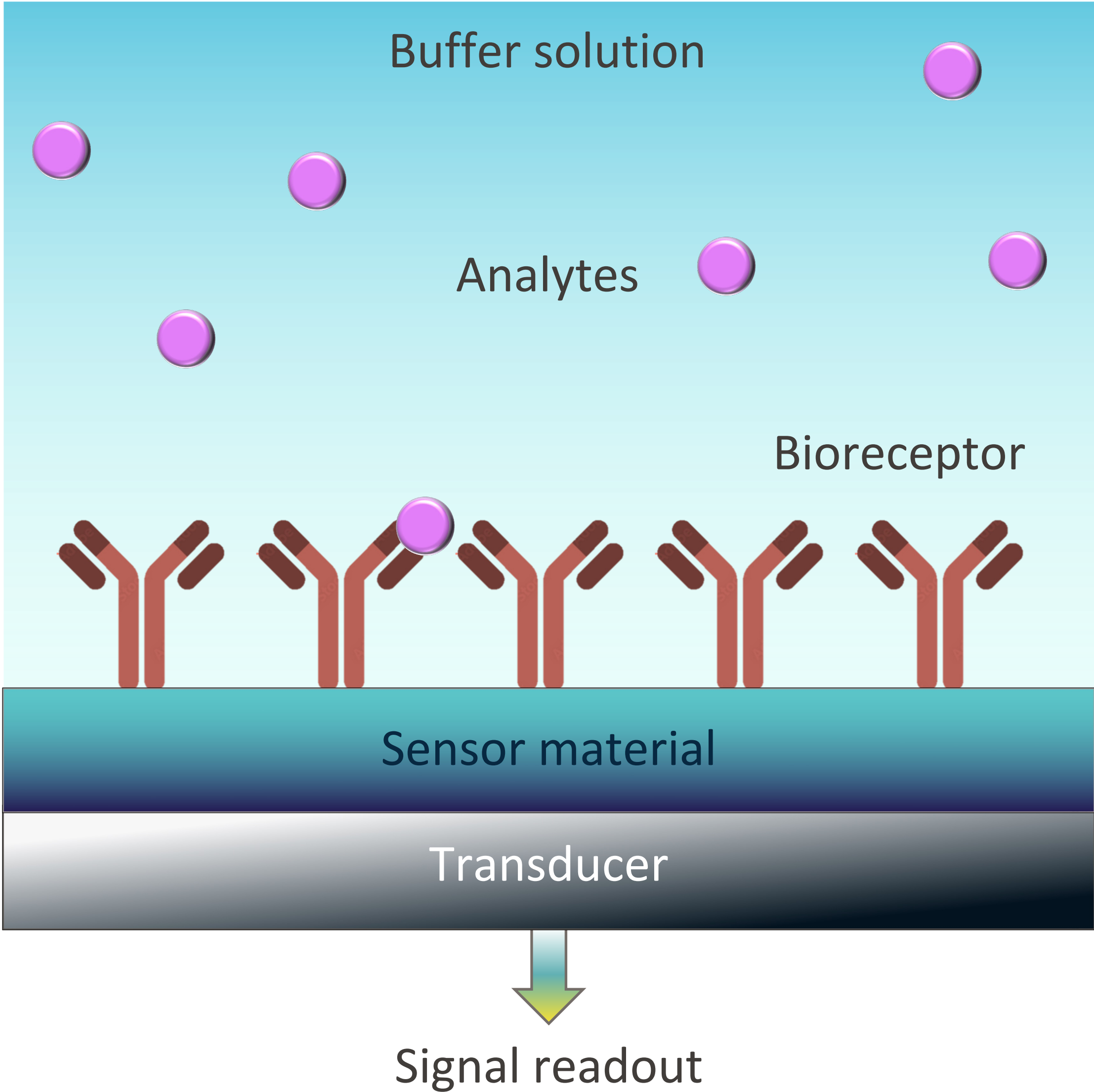
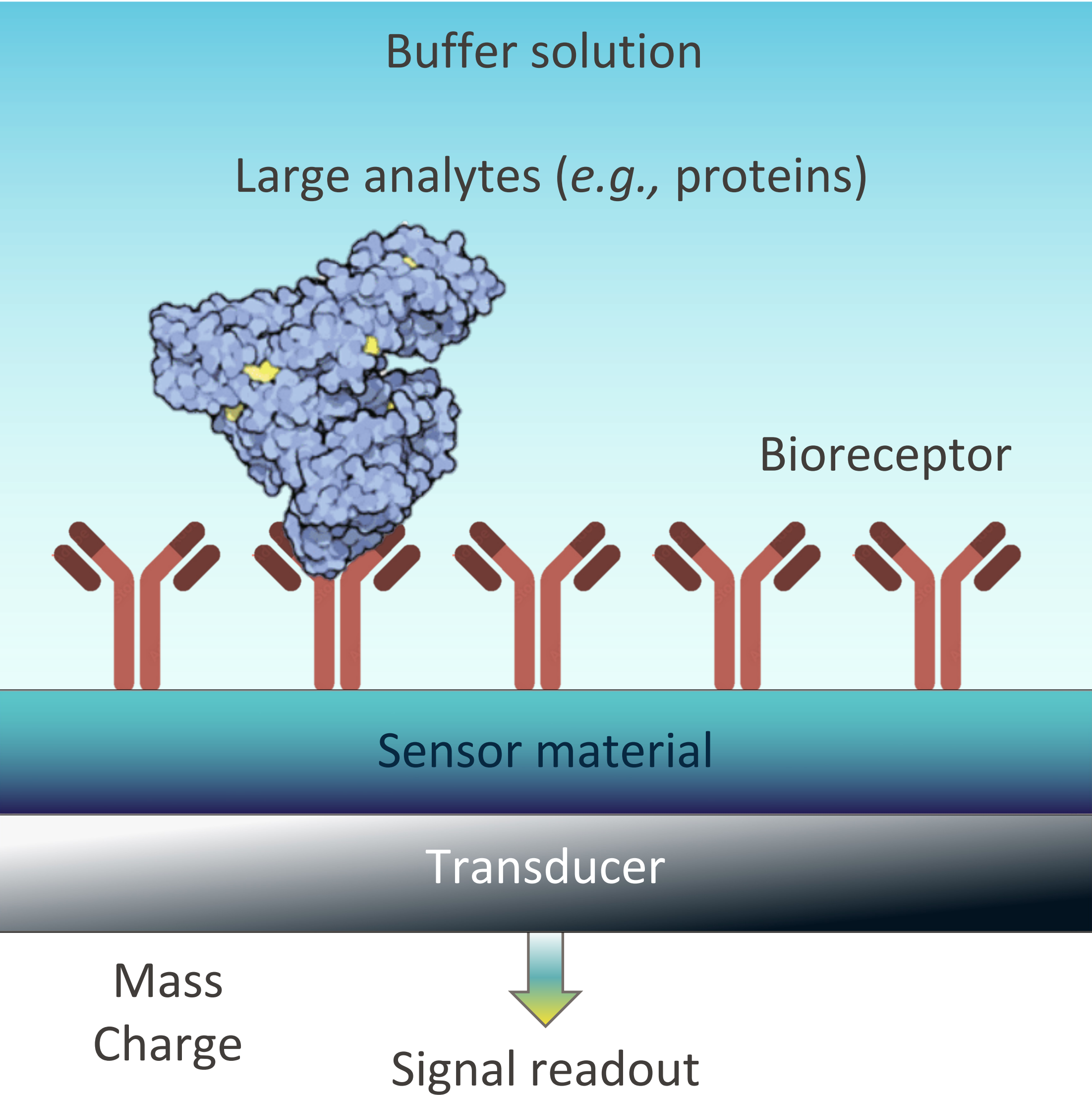
800-1200 different types pf receptor proteins

Dogs are estimated to be 10,000 – 100,000 x more sensitive to certain odors than humans

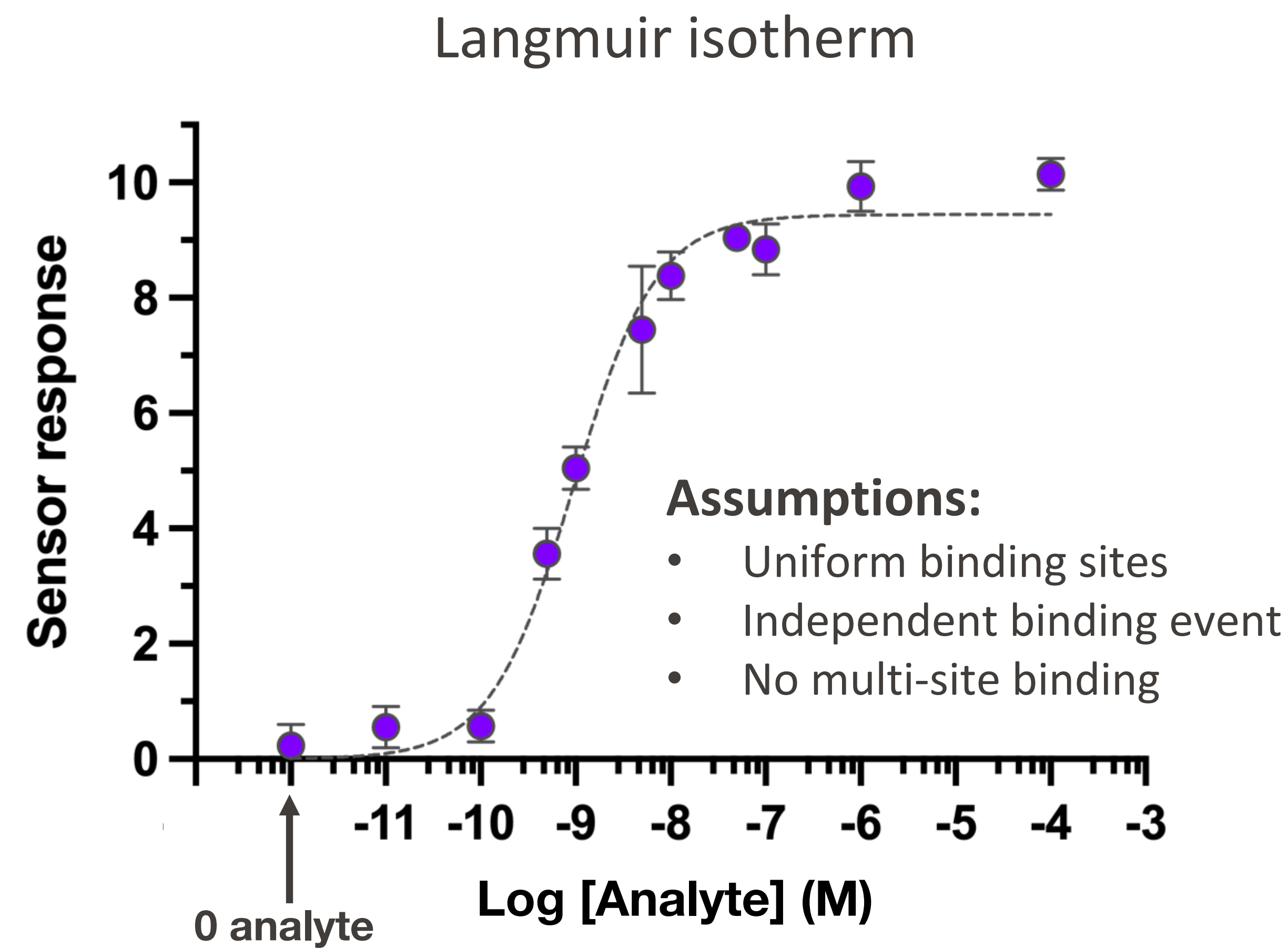
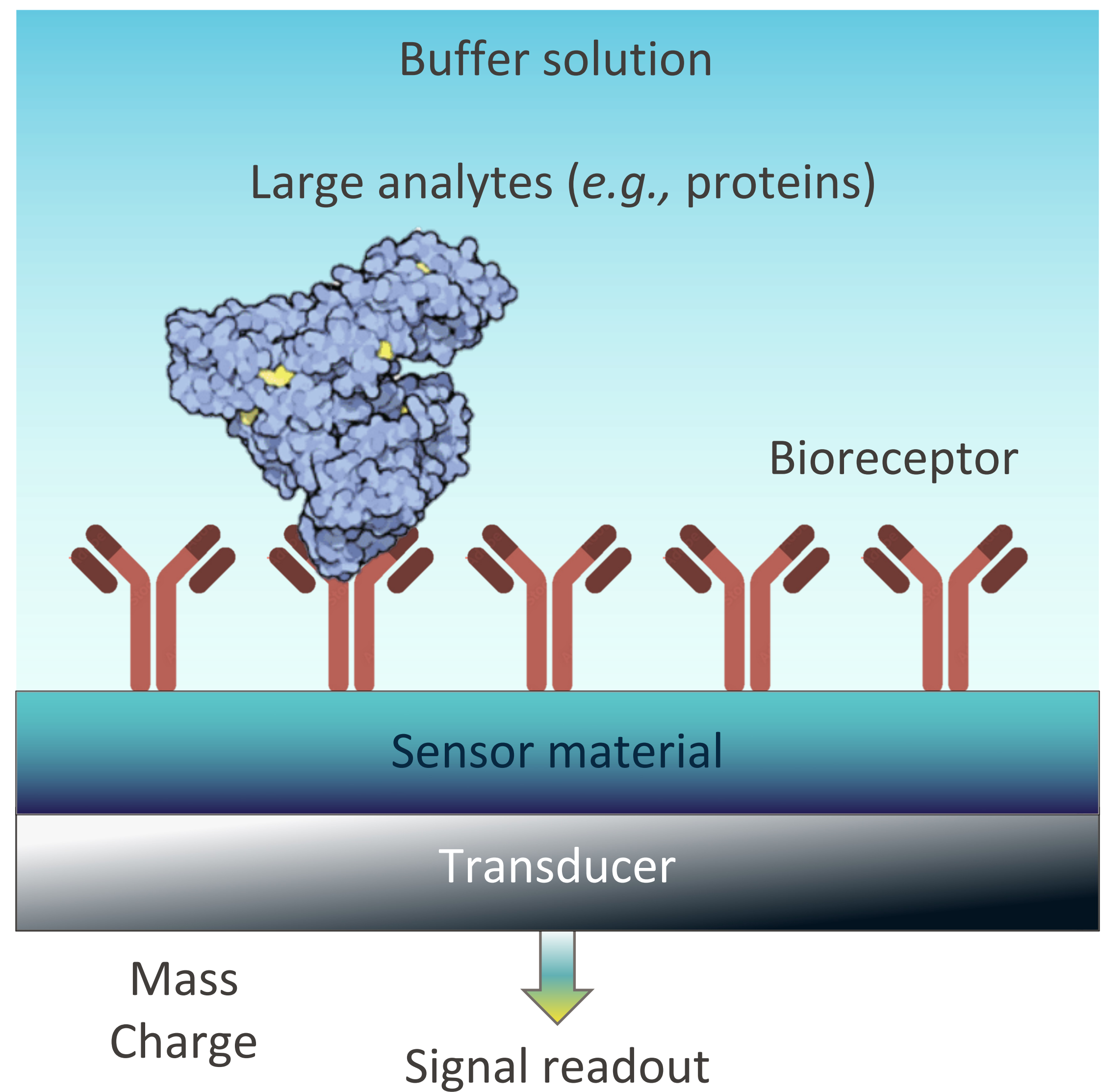
Mimicking Nature: Deep-Learning Inspired Electronic Nose



How to Read Out the Signal of a Biosensor



How to Read Out the Signal of a Biosensor

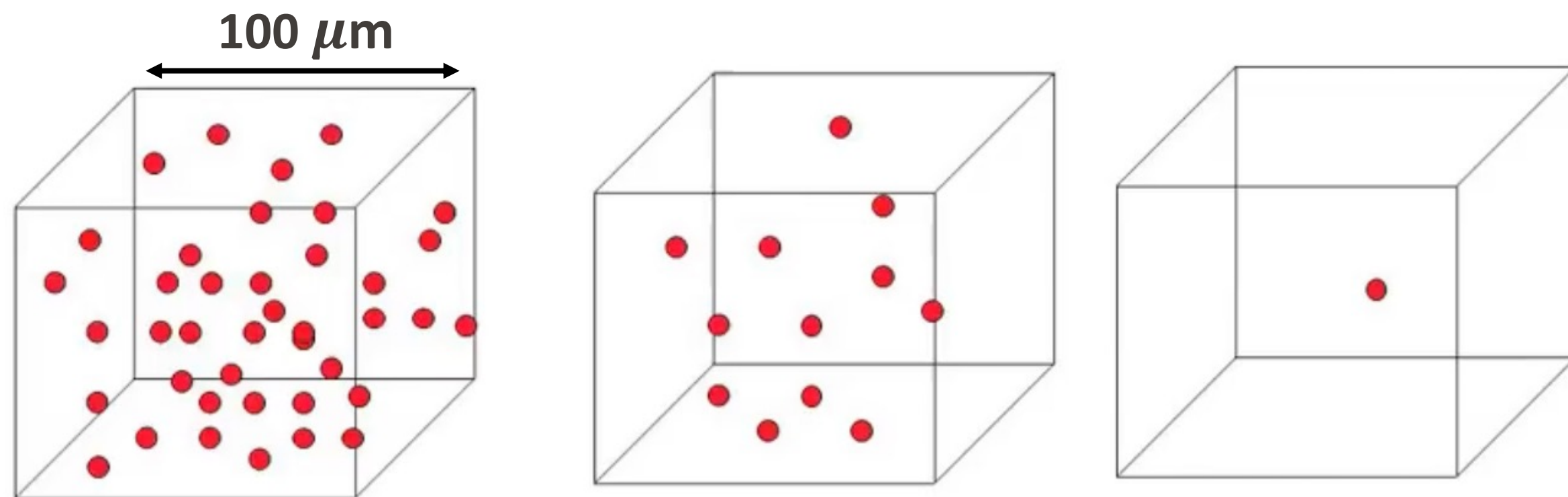


Concentration – What Do these Values Mean?

$$1 \text{ M} = 1 \text{ mol/L} = 6 \times 10^{23} \text{ molecules/L}$$

Note: Avogadro's number: 6×10^{23} molecules/mol

$$1 \text{ M} \sim 1 \times 10^{15} \text{ molecules}/100 \mu\text{m}^3$$

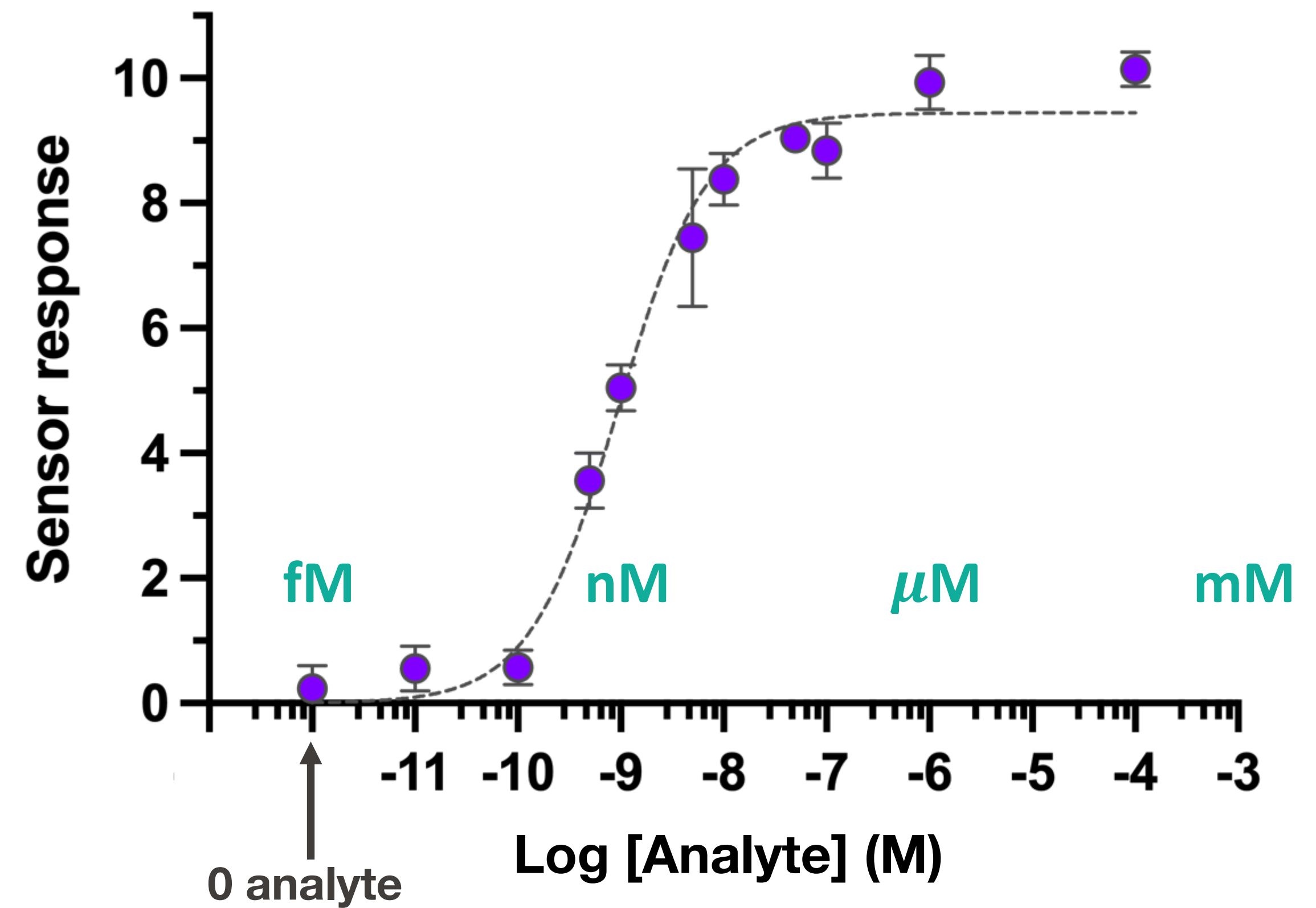


1 μM ~1 billion

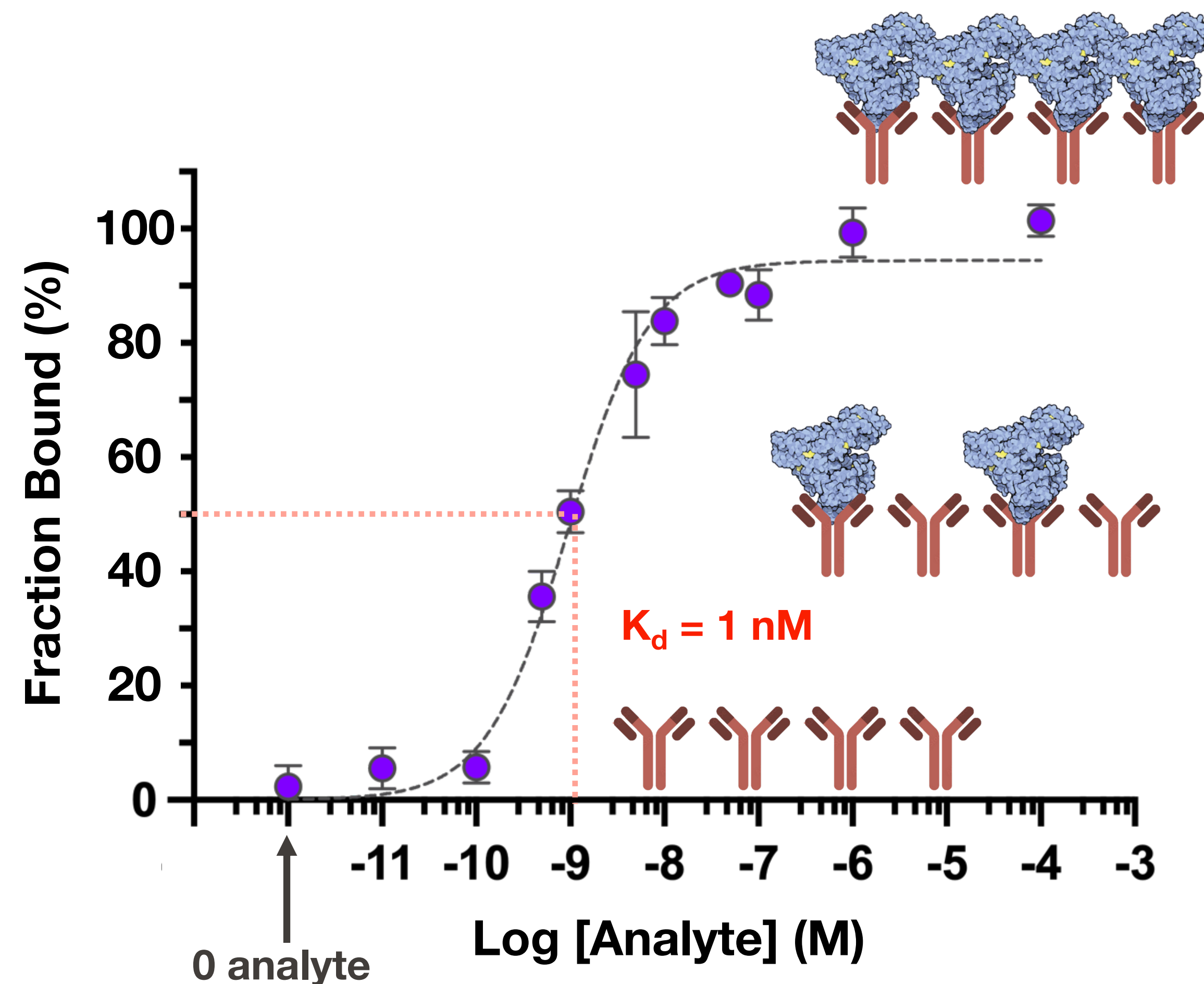
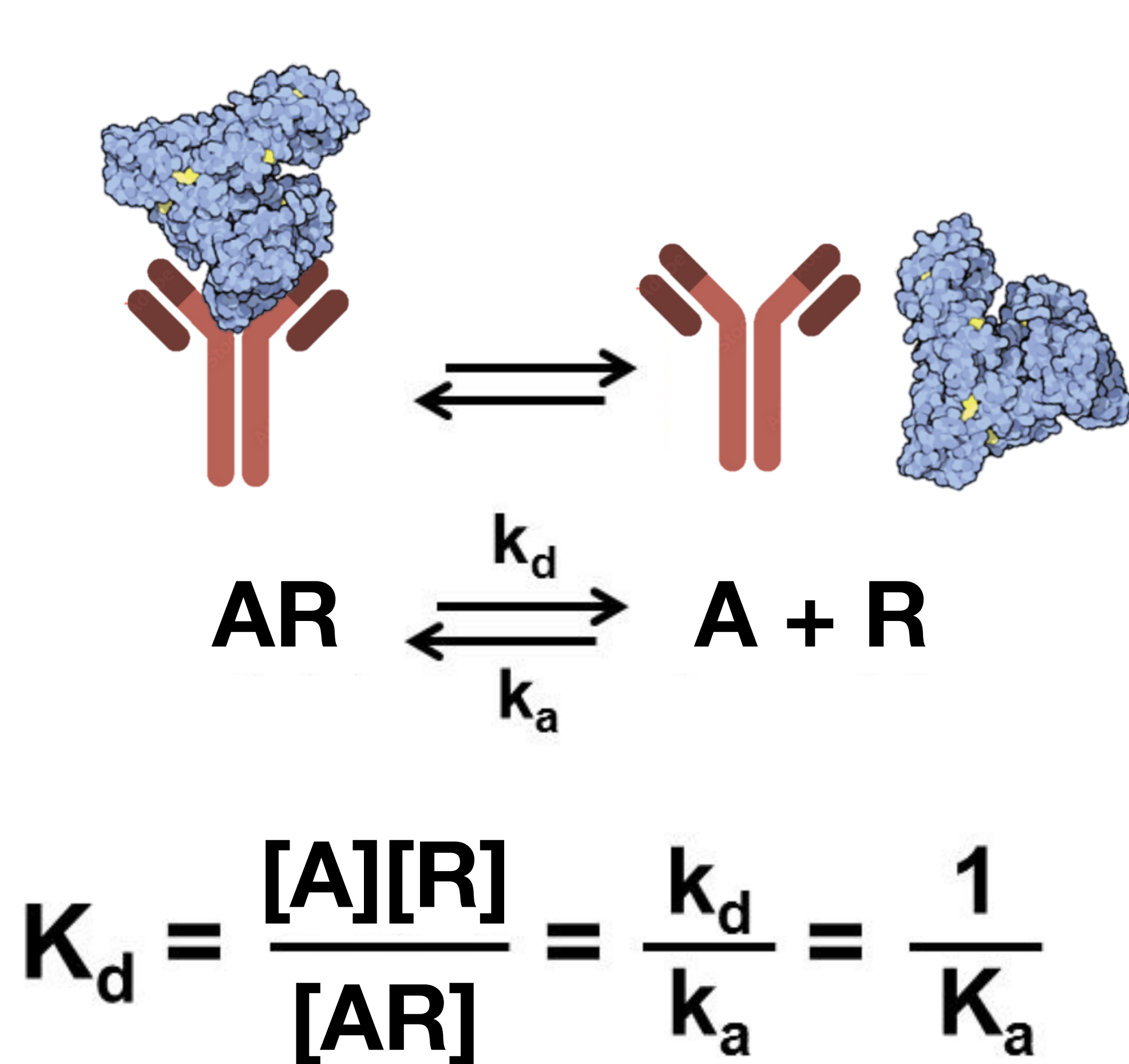
1 pM ~1000

1 fM ~1

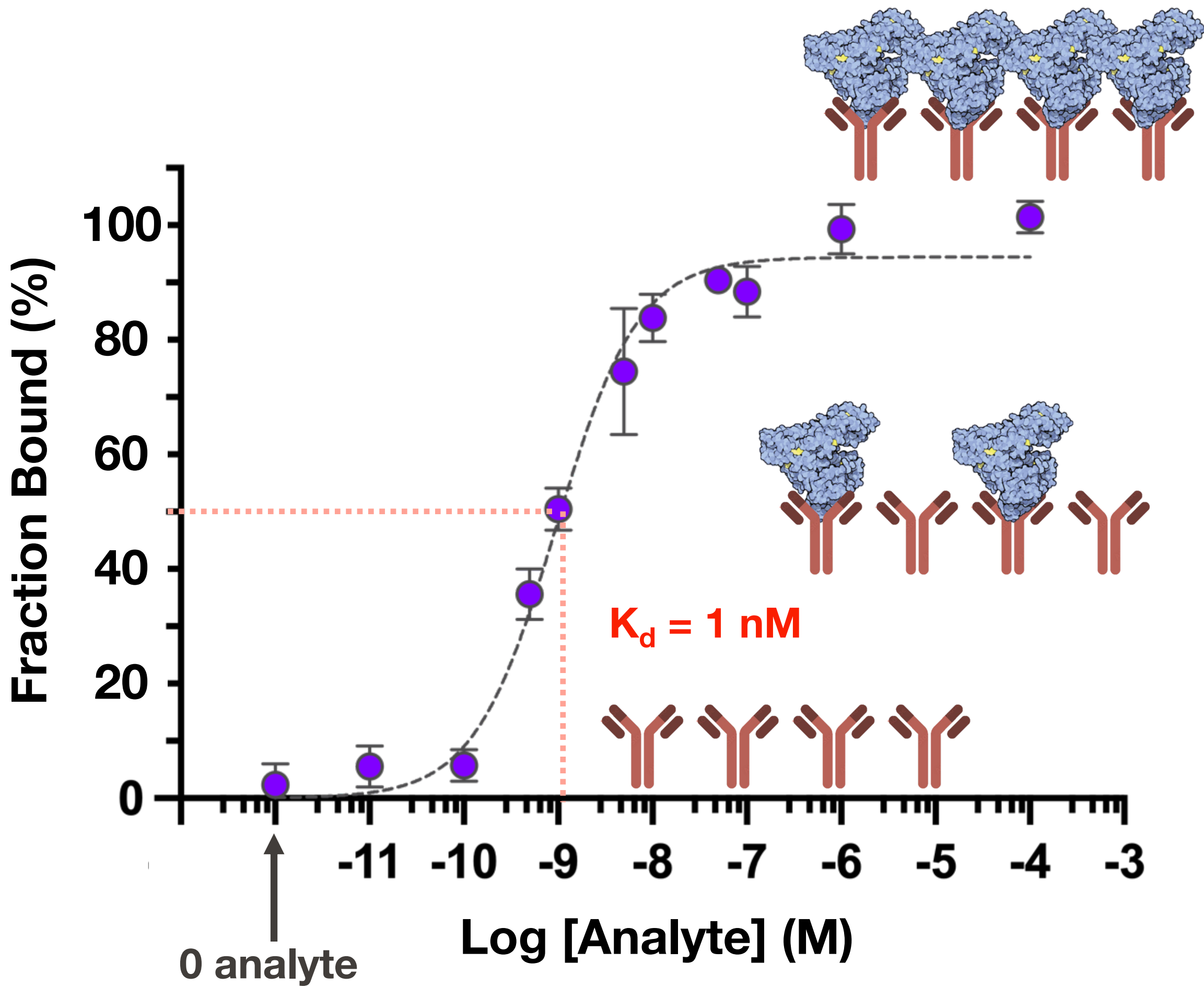
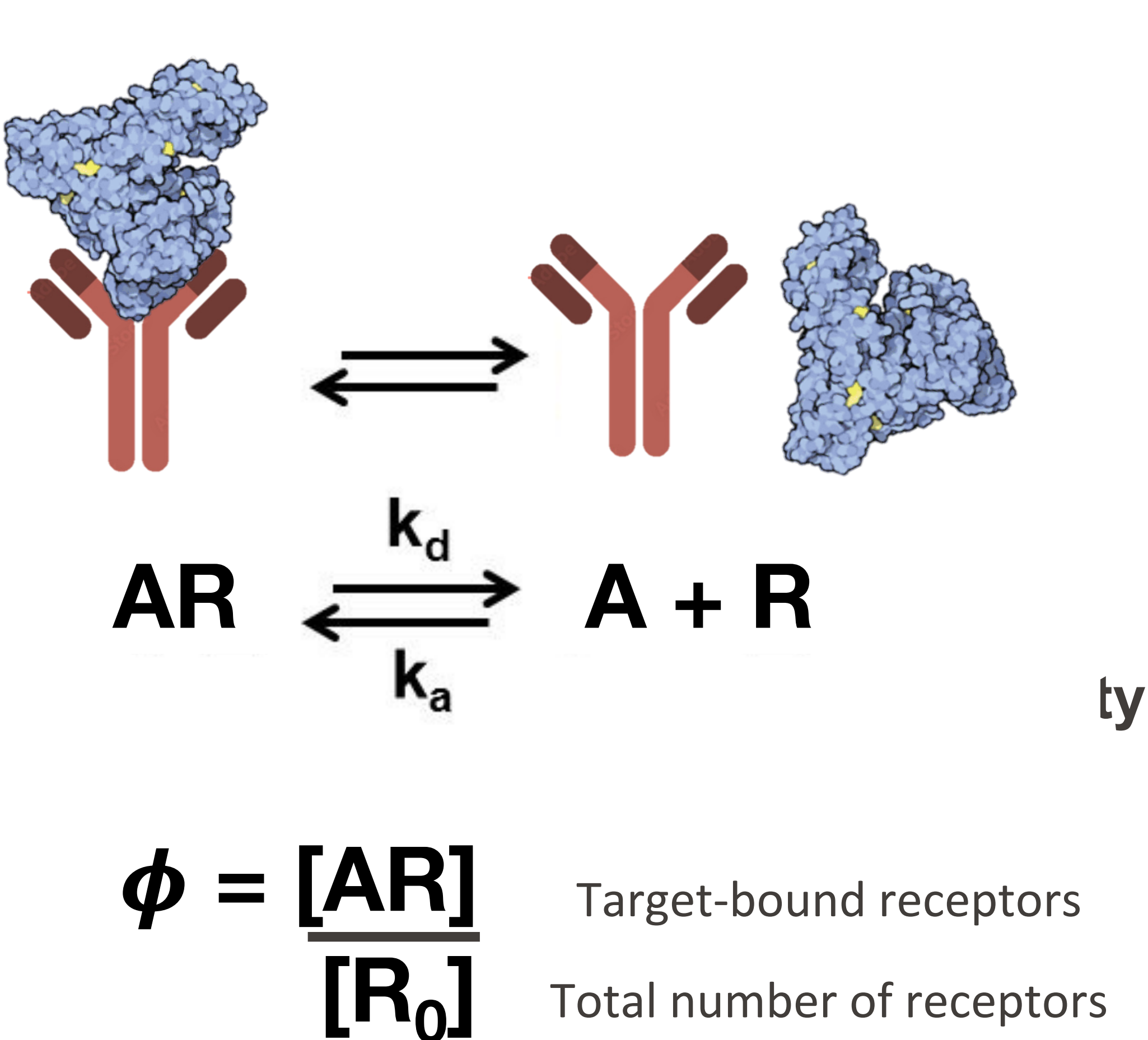
Single molecule of salt in
several Olympic-sized
swimming pools!



How to Read Out the Signal of a Biosensor



How to Calculate the Equilibrium Binding Affinity (K_d)

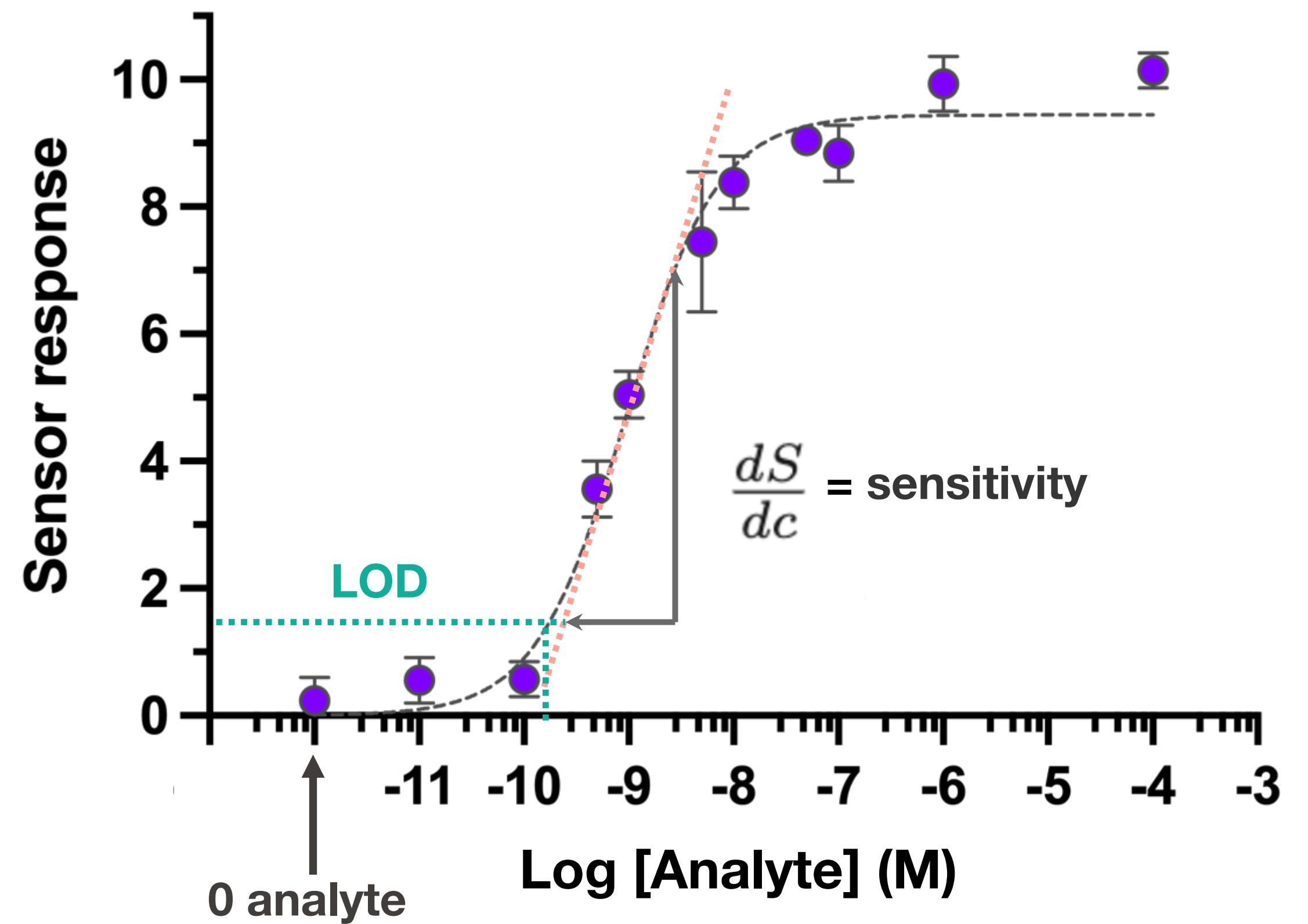
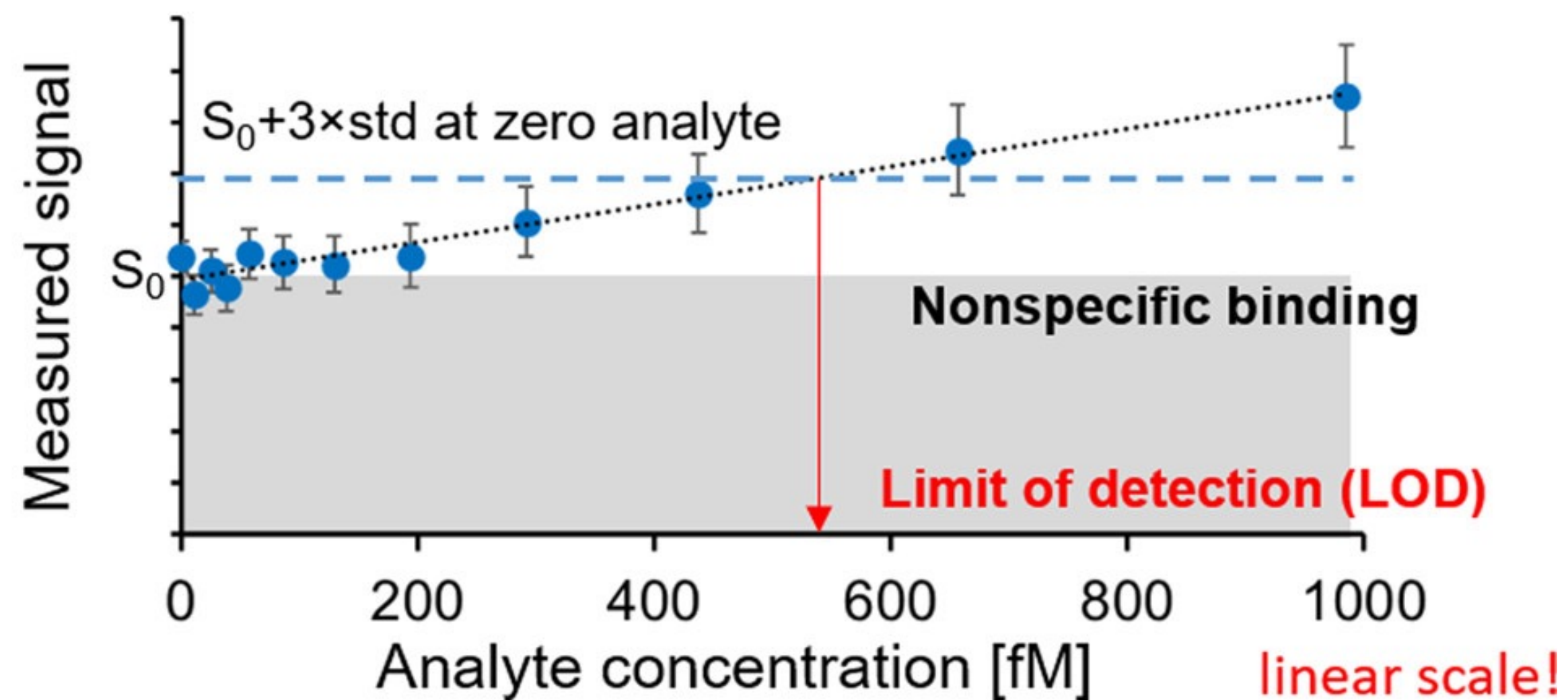


How to Calculate the Sensitivity and Limit of Detection (LOD)

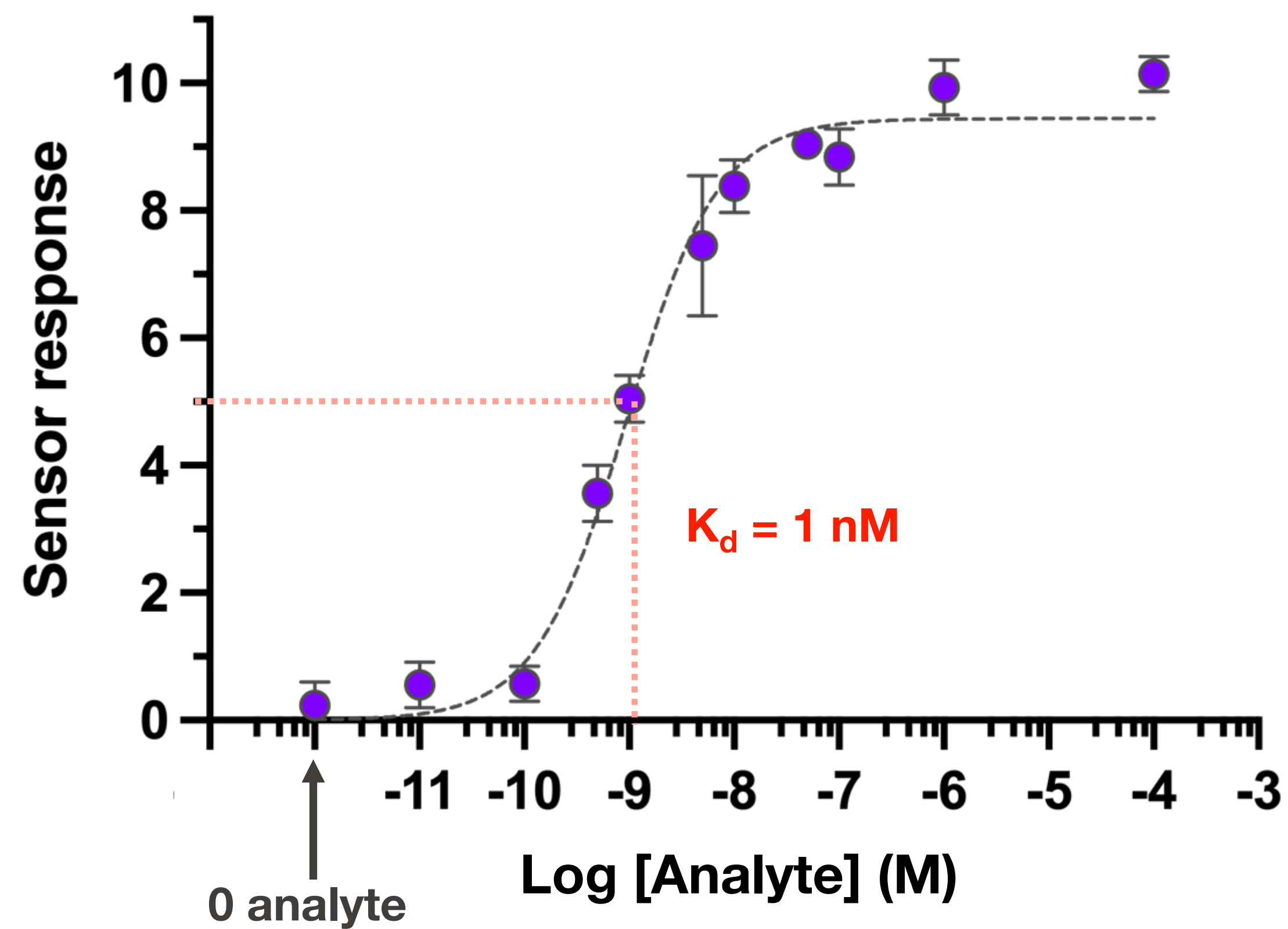
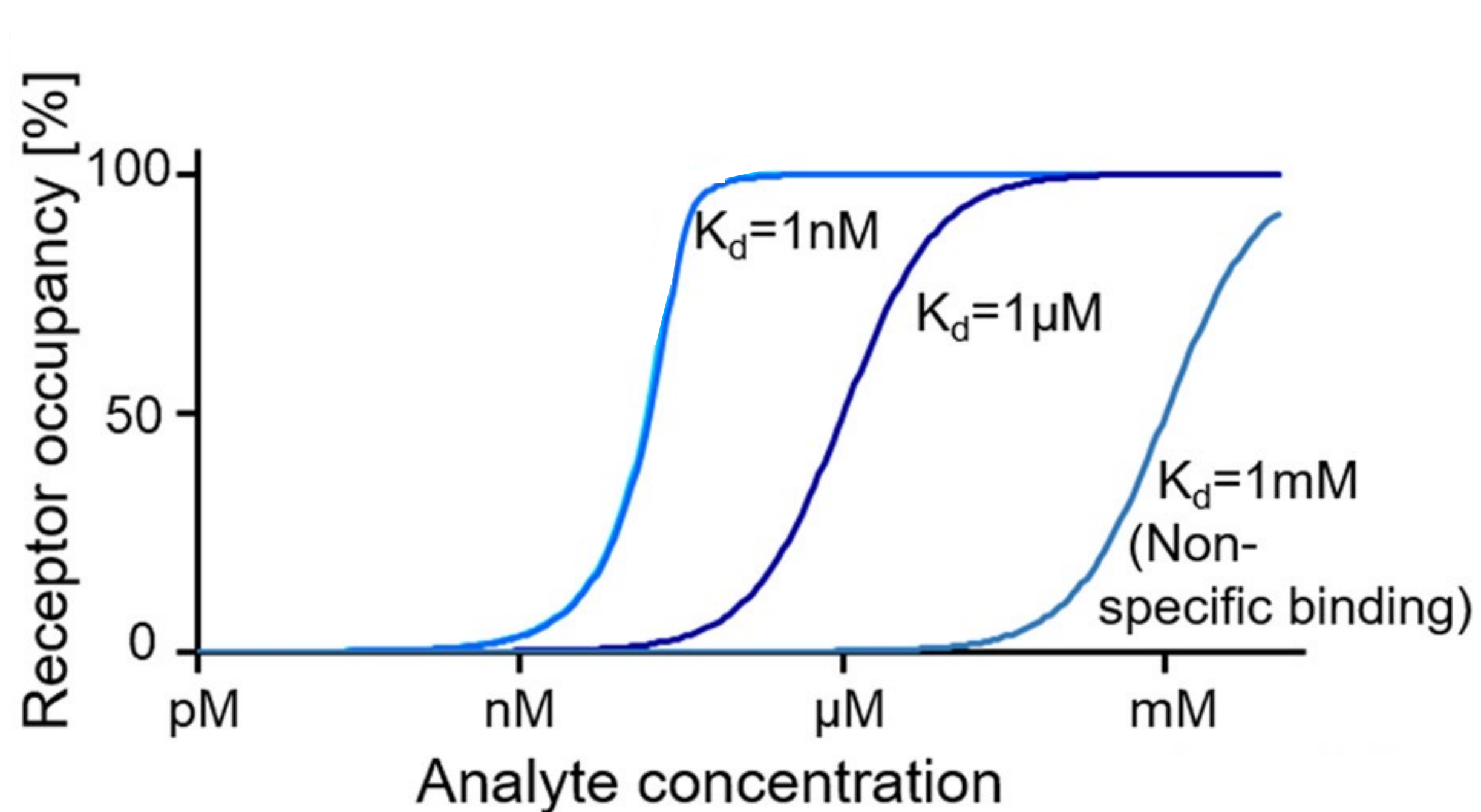
$$\text{LOD} = \frac{3\sigma_{S_0}}{\frac{dS}{dc}}$$

3 x standard deviation of zero analyte signal

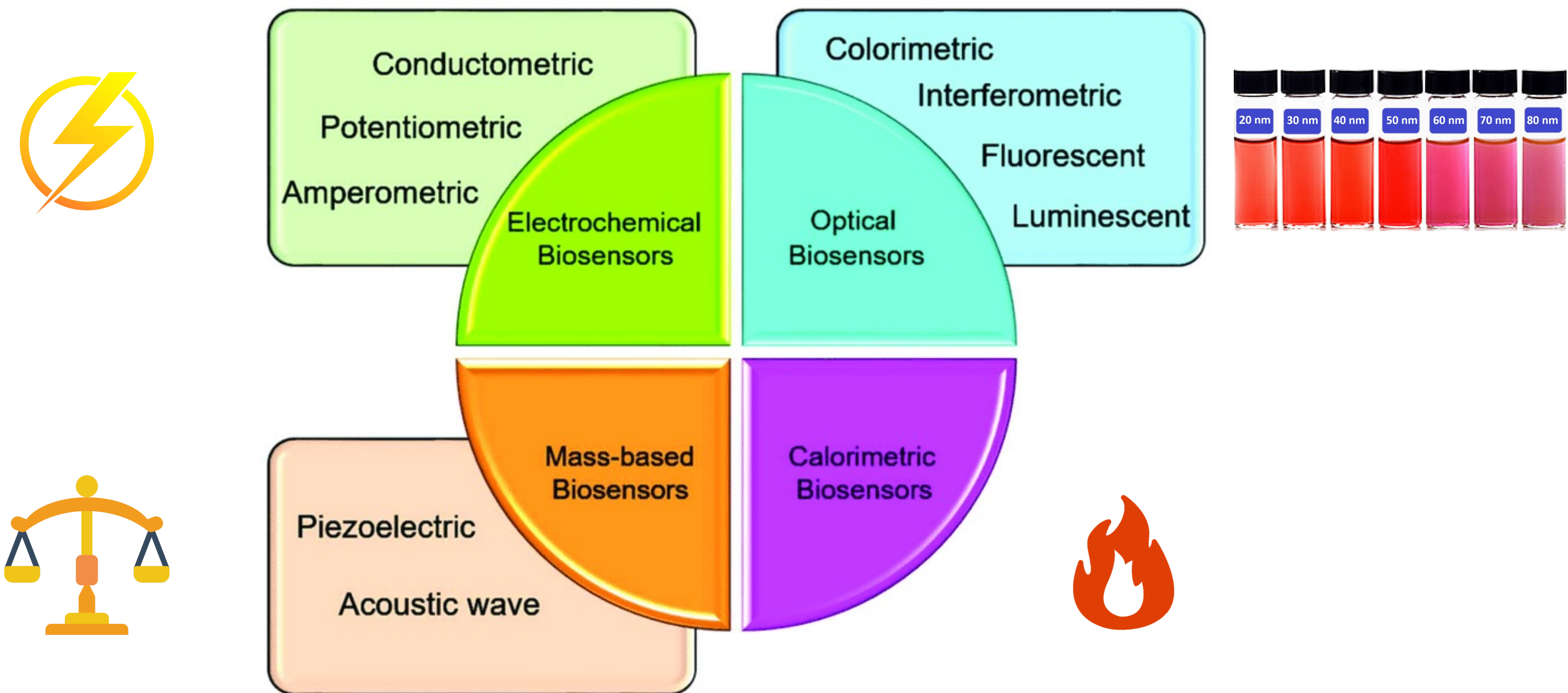
Sensitivity = Slope of the signal



The Detection Range is Dependent on the K_d of the Bioreceptor



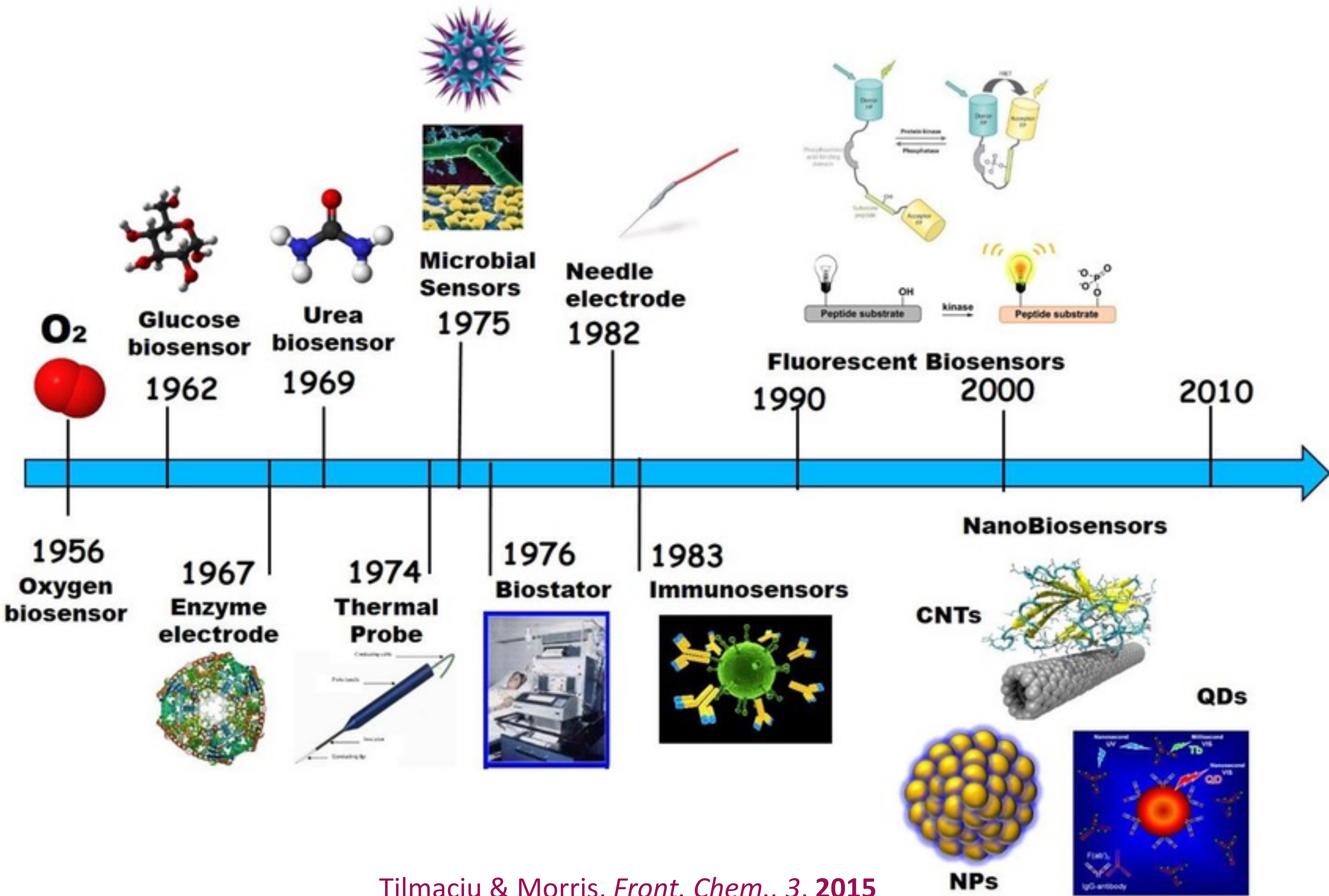
Different Signal Transduction Mechanisms of Biosensors



History of Biosensors



Leland Clark
The “Father of Biosensors”

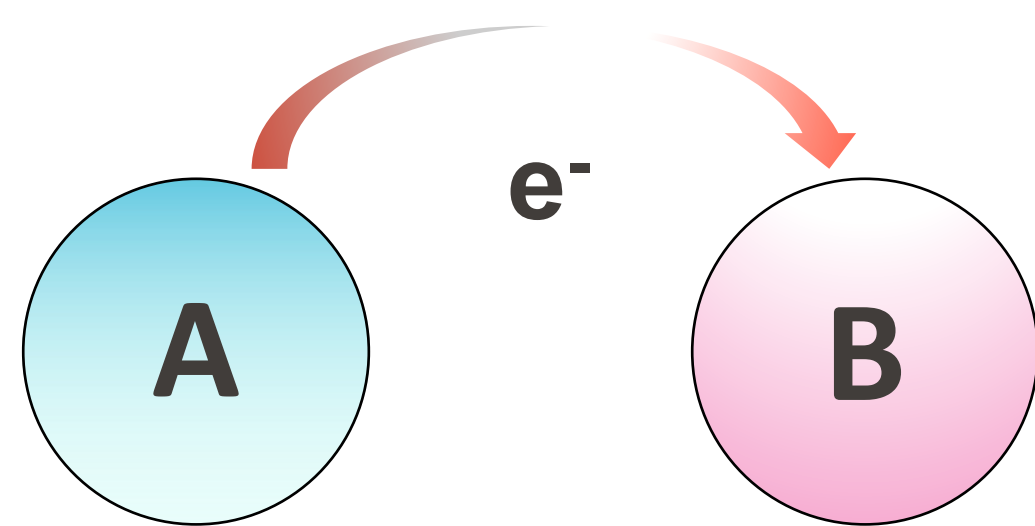


Tilmaciu & Morris, *Front. Chem.*, 3, 2015

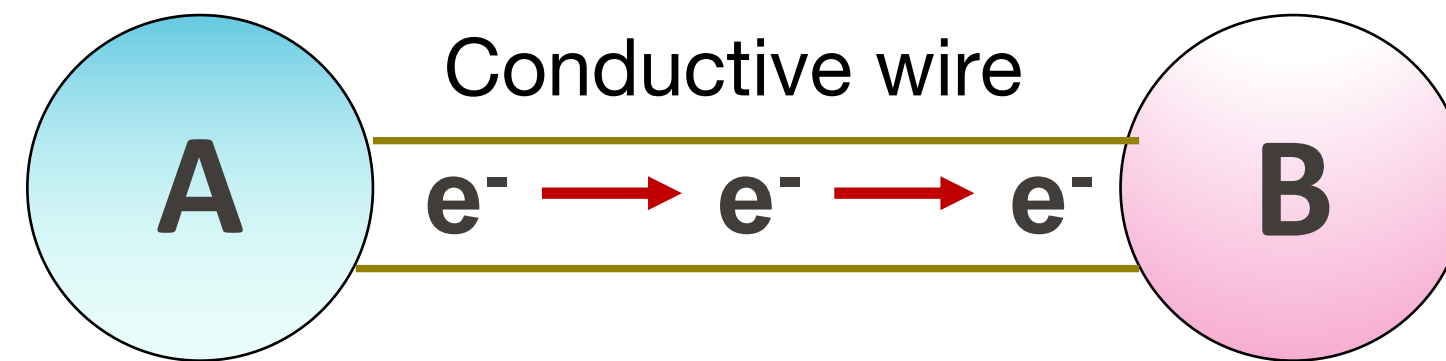
Electrochemistry: Relationship between Chemical Reactions + Electricity

Electricity: movement of electrons $e^- \rightarrow e^- \rightarrow e^-$

Certain chemical reactions $\rightarrow$ create electricity

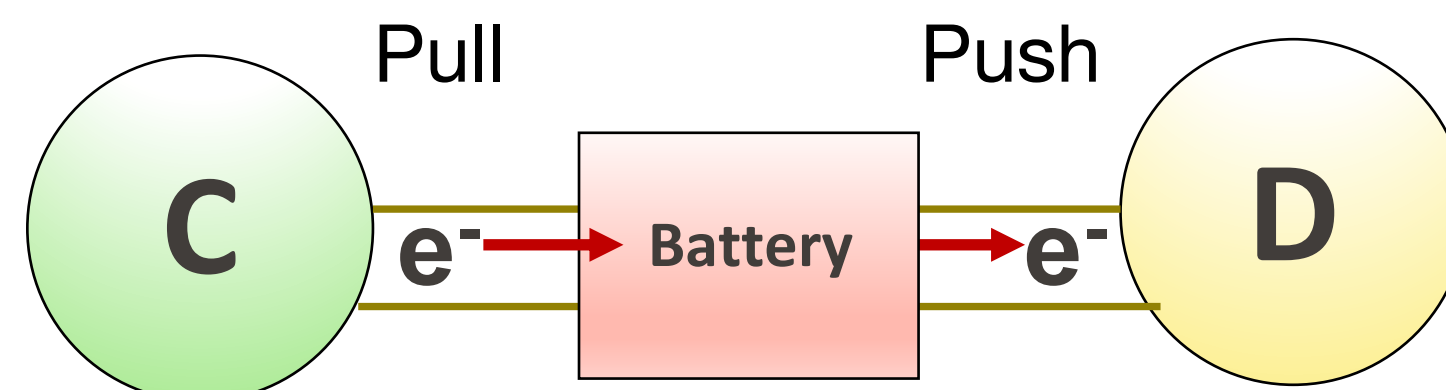
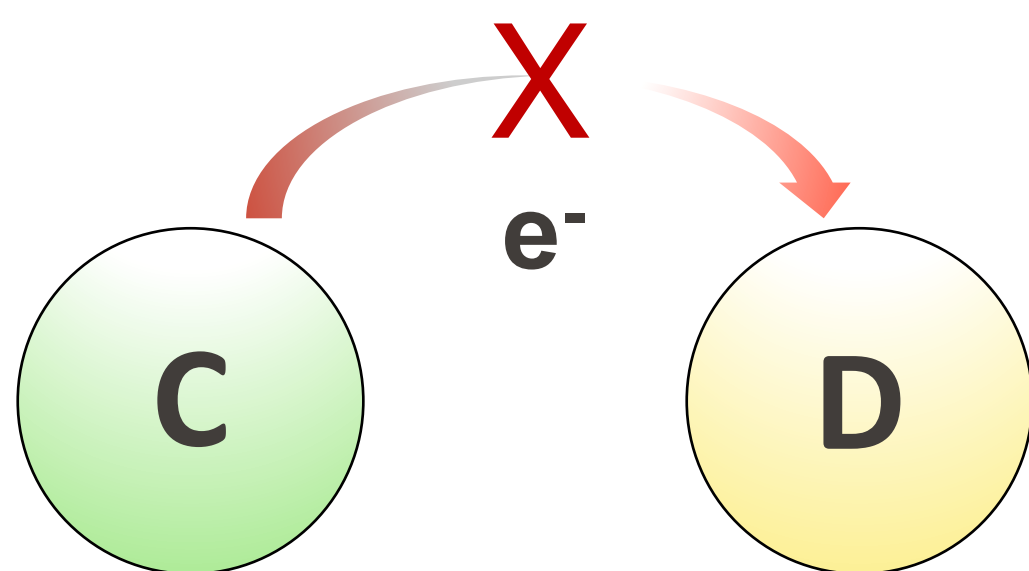


Oxidation & Reduction Reactions

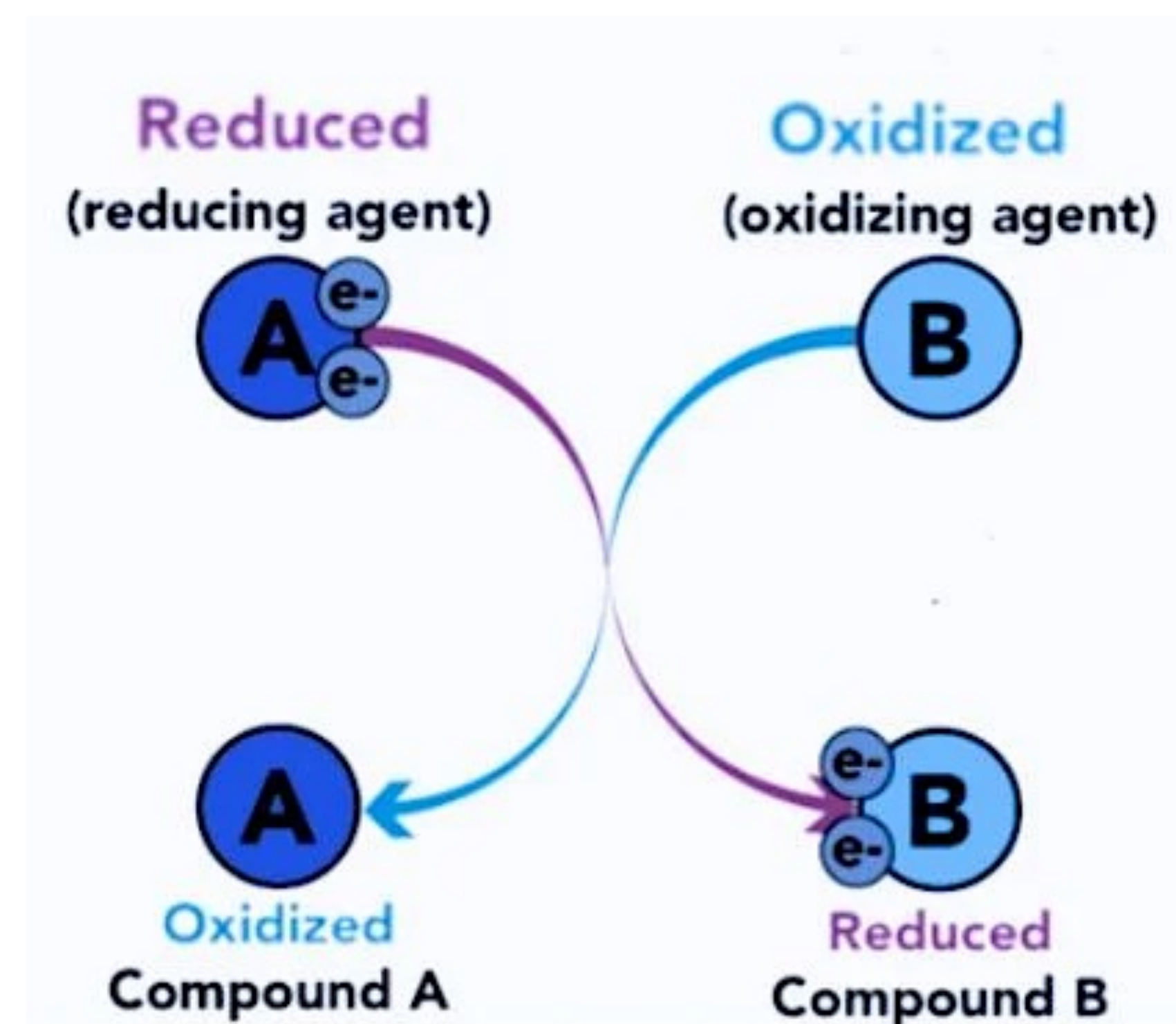


Spontaneous reaction

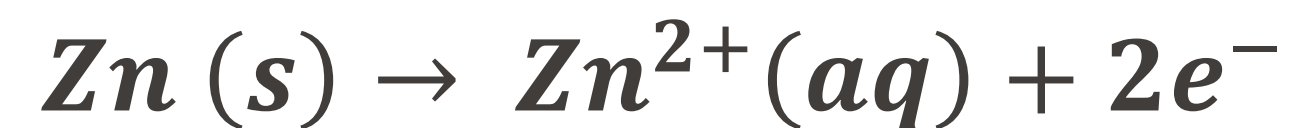
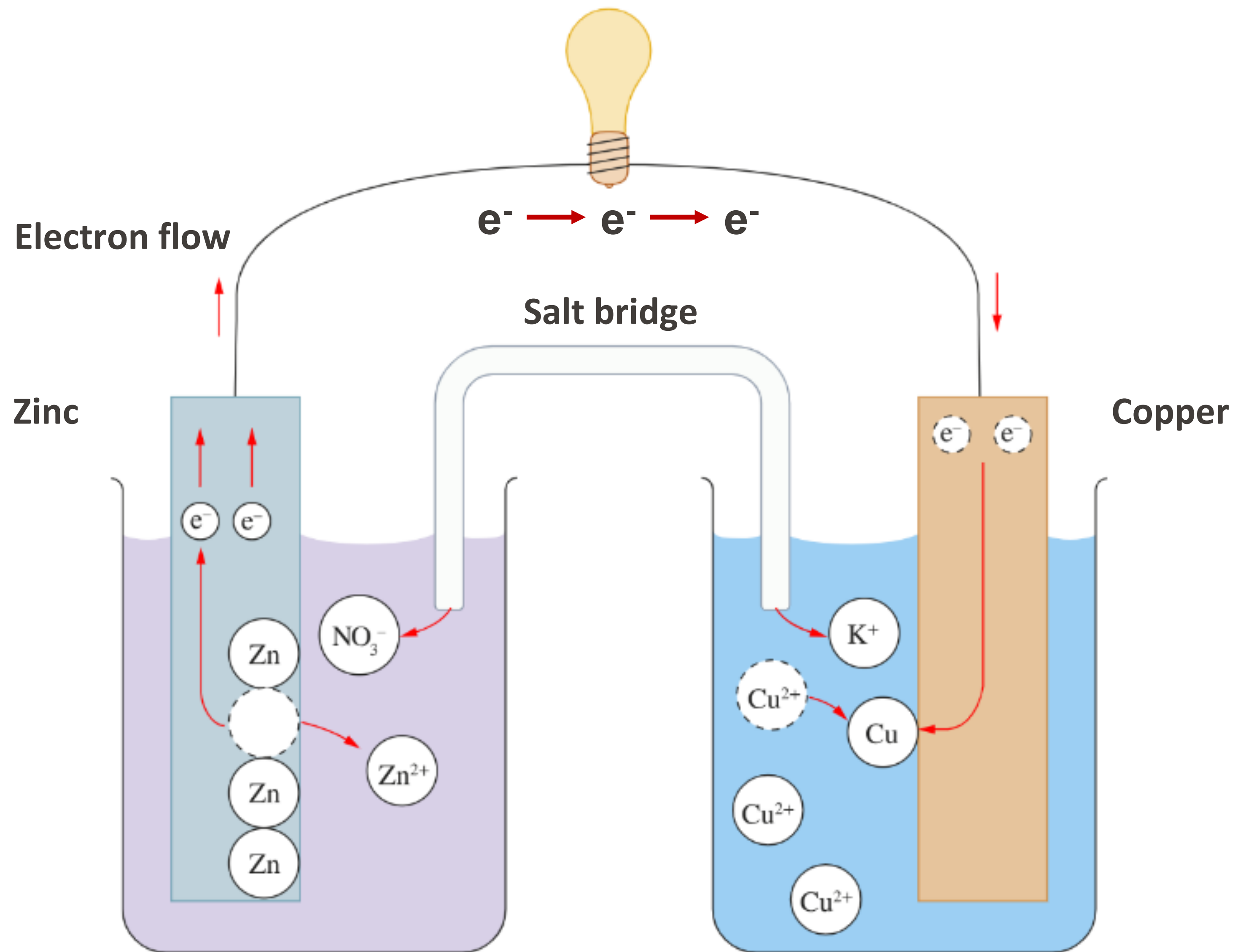
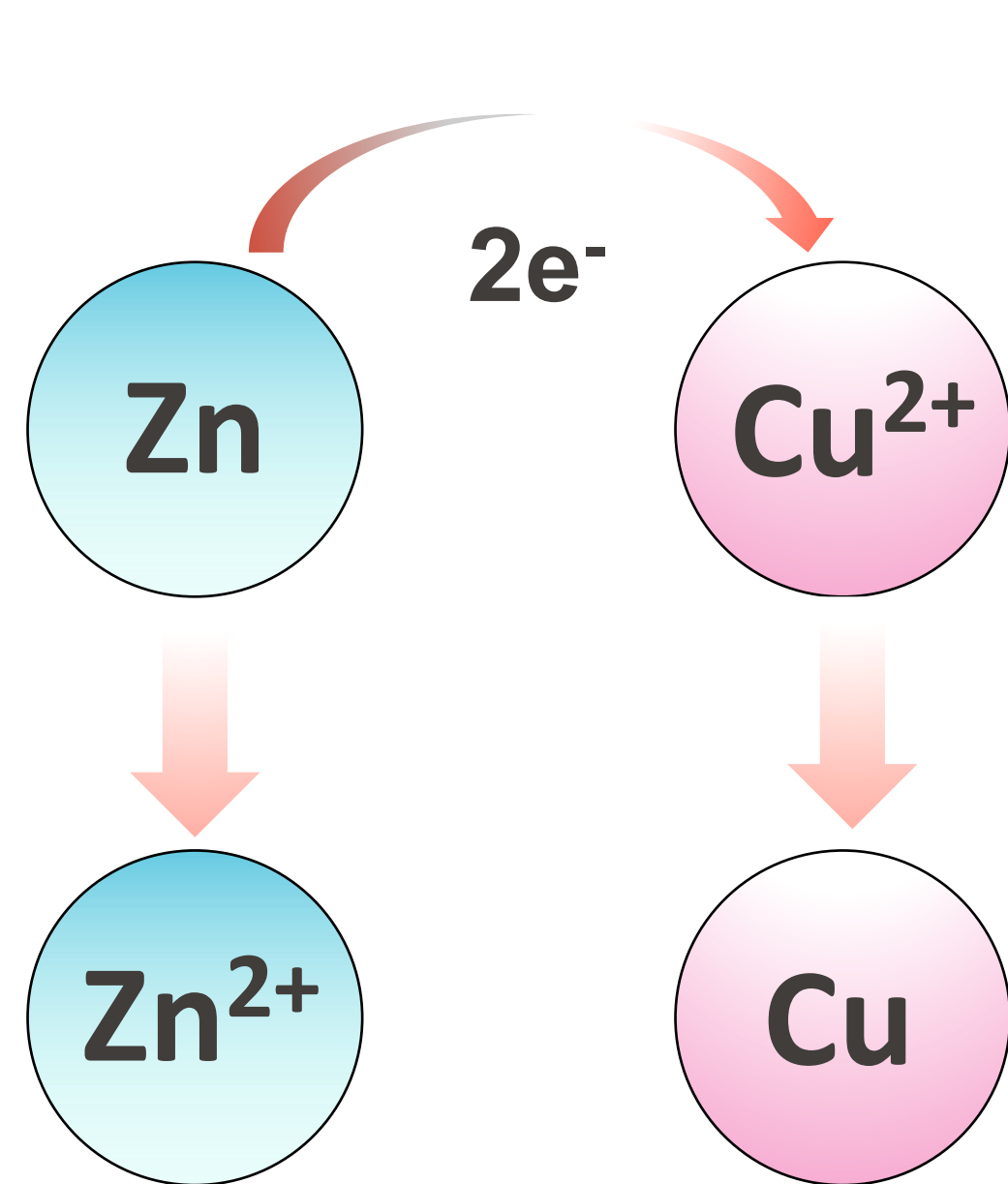
Electricity $\rightarrow$ make certain chemical reactions happen



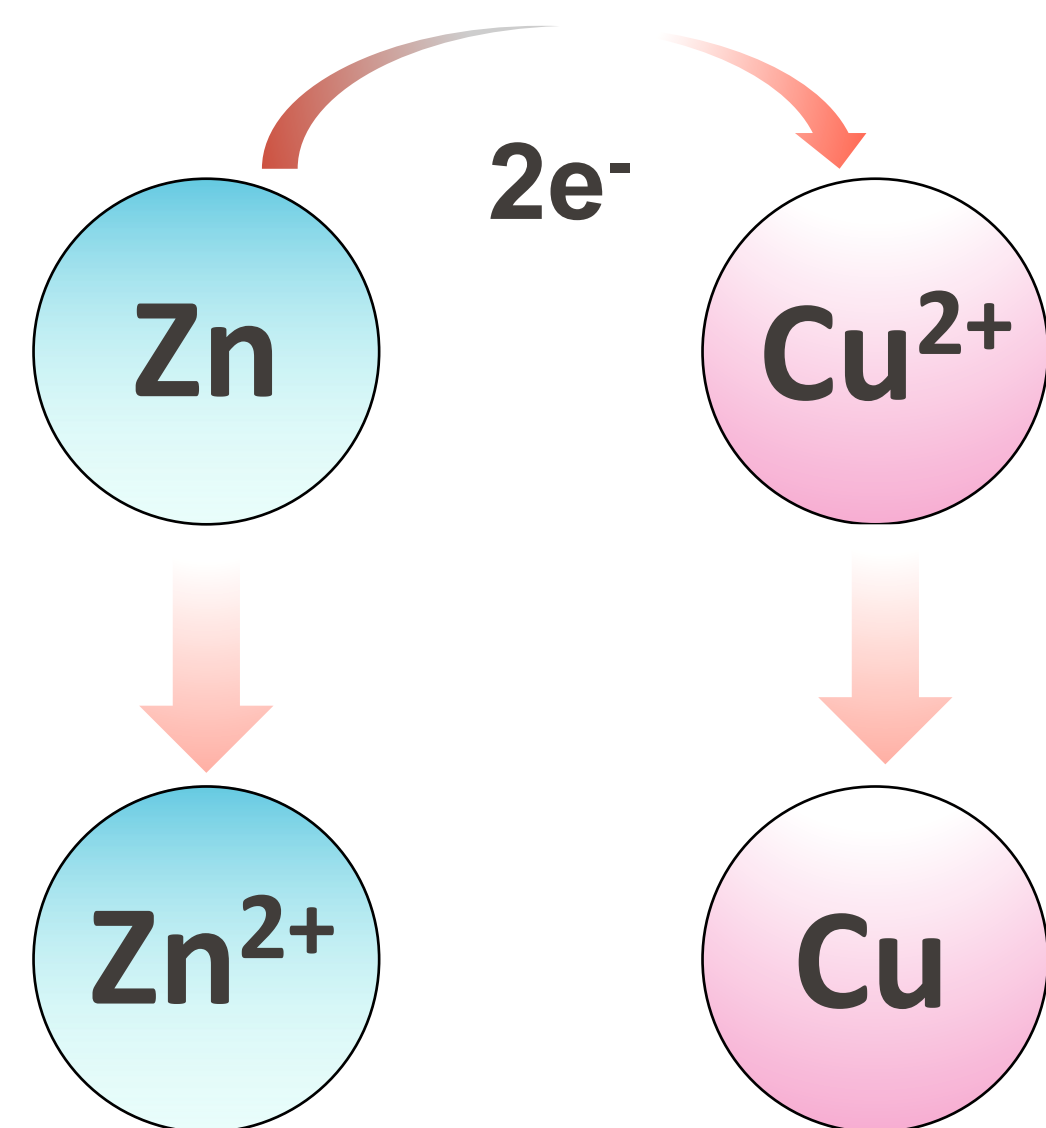
Non spontaneous reaction



Galvanic/Voltaic Cells for Electrochemistry



Standard Reduction Potential of Different Metals



Stronger pull for electrons

Under standard conditions:

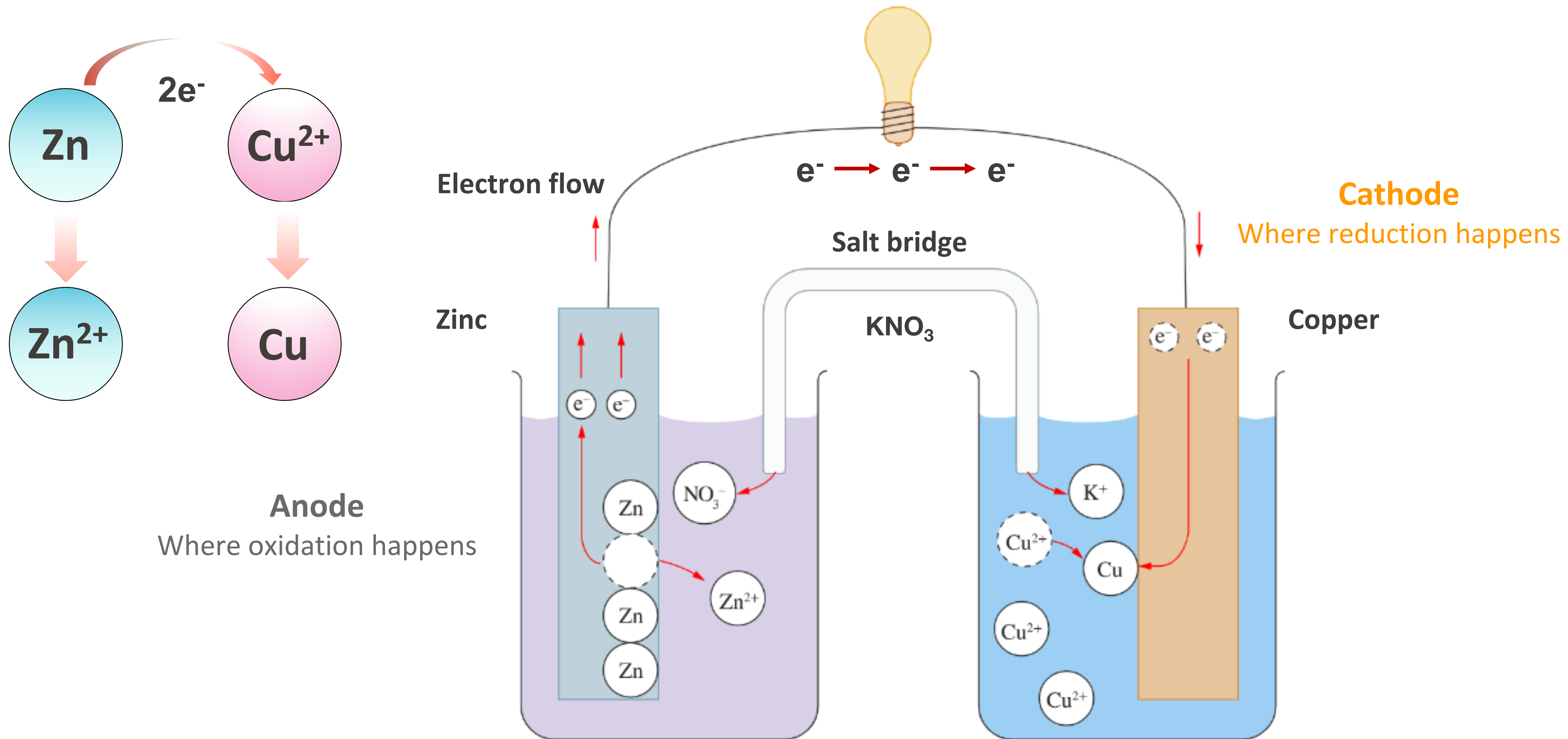
- 25 °C (298 K)
- 1 M concentration for reactants/products
- Relative to the standard hydrogen electrode (0.00 V)



Half Reaction					potential
F₂	+	2e ⁻	⇌	2F ⁻	+2.87 V
Pb⁴⁺	+	2e ⁻	⇌	Pb ²⁺	+1.67 V
Cl₂	+	2e ⁻	⇌	2Cl ⁻	+1.36 V
Ag⁺	+	1e ⁻	⇌	Ag	+0.80 V
Fe³⁺	+	1e ⁻	⇌	Fe ²⁺	+0.77 V
Cu²⁺	+	2e ⁻	⇌	Cu	+0.34 V
2H⁺	+	2e⁻	⇌	H₂	0.00 V
Fe³⁺	+	3e ⁻	⇌	Fe	-0.04 V
Pb²⁺	+	2e ⁻	⇌	Pb	-0.13 V
Fe²⁺	+	2e ⁻	⇌	Fe	-0.44 V
Zn²⁺	+	2e ⁻	⇌	Zn	-0.76 V
Al³⁺	+	3e ⁻	⇌	Al	-1.66 V
Mg²⁺	+	2e ⁻	⇌	Mg	-2.36 V
Li⁺	+	1e ⁻	⇌	Li	-3.05 V

Cu will pull electrons from Zn

Galvanic/Voltaic Cells for Electrochemistry

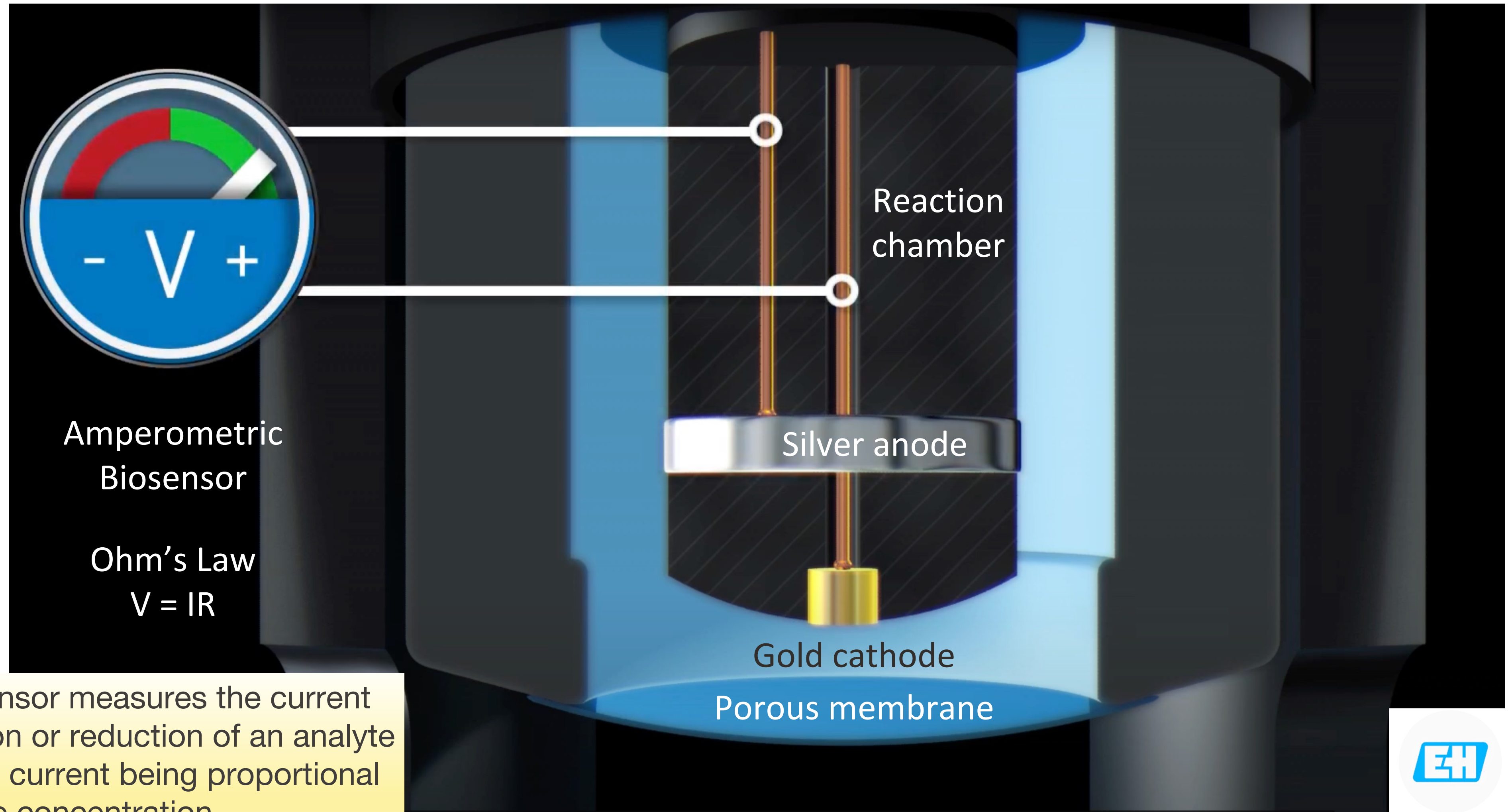


Clark Electrode Measures Ambient Oxygen Partial Pressure

Clark Electrode was originally designed to measure oxygen concentration in blood



Leland Clark
The “Father of Biosensors”



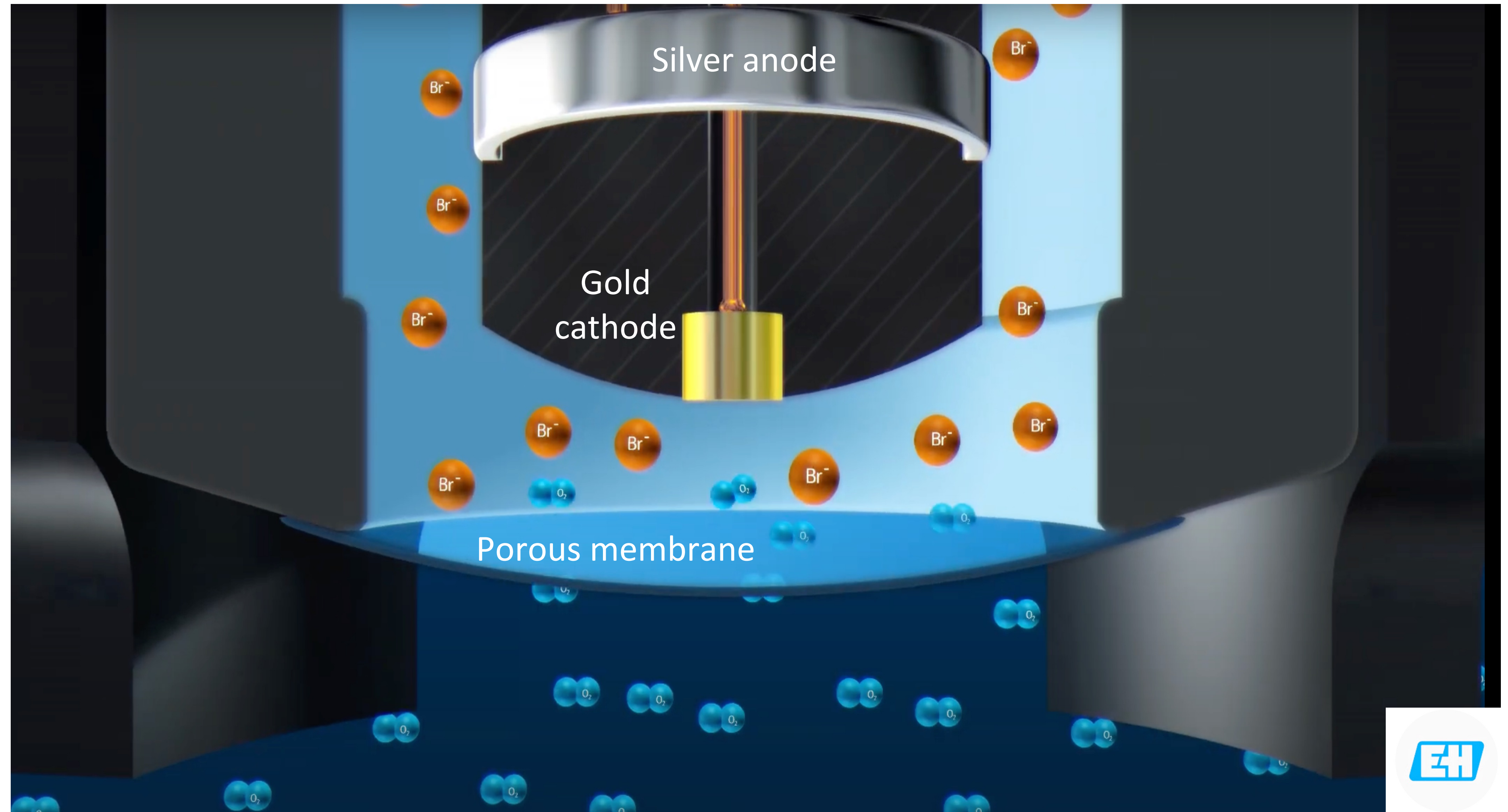
An amperometric biosensor measures the current generated by the oxidation or reduction of an analyte at an electrode, with the current being proportional to the analyte concentration.



Clark Electrode Measures Ambient Oxygen Partial Pressure



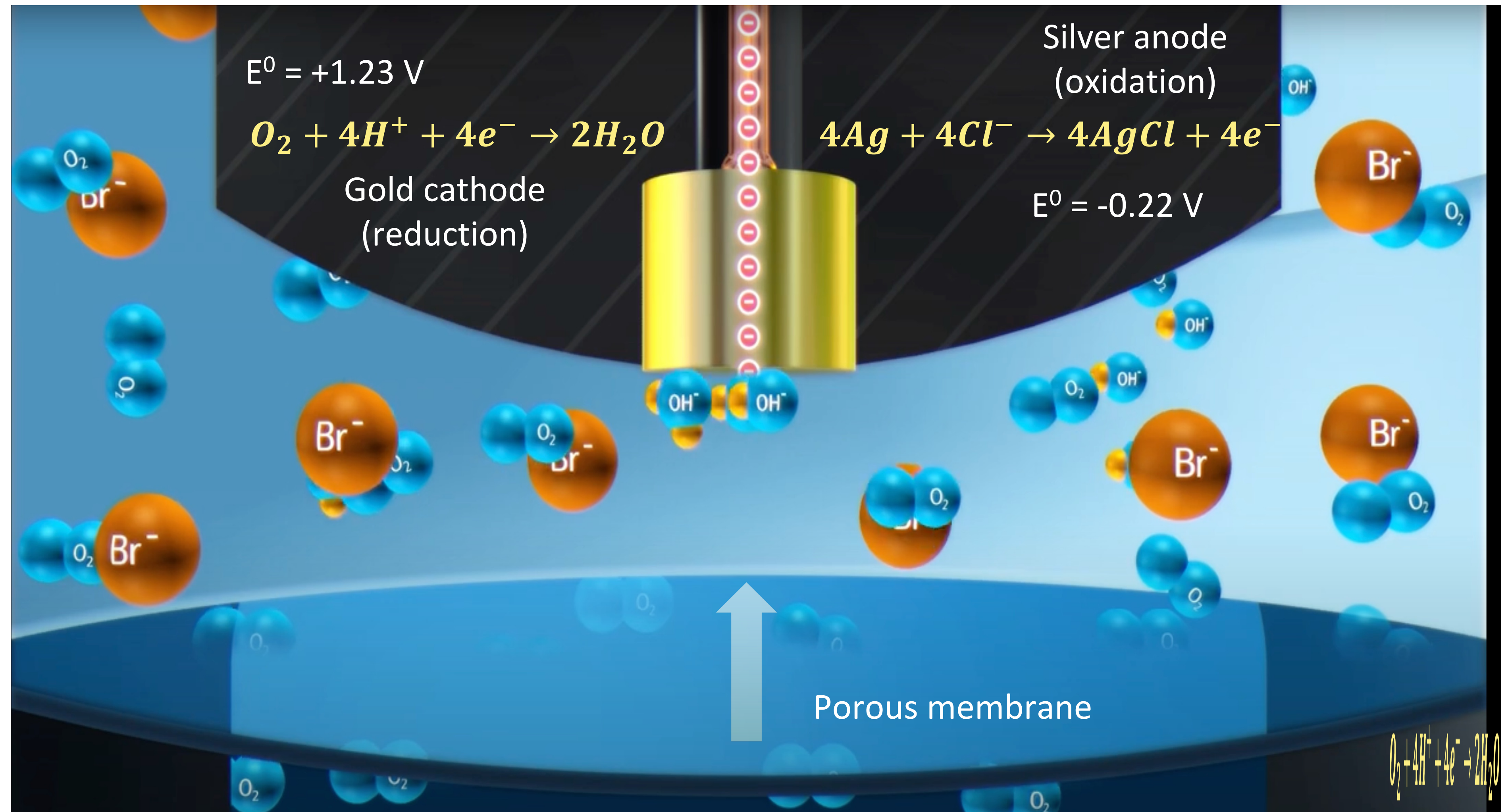
Leland Clark
The “Father of Biosensors”



Clark Electrode Measures Ambient Oxygen Partial Pressure

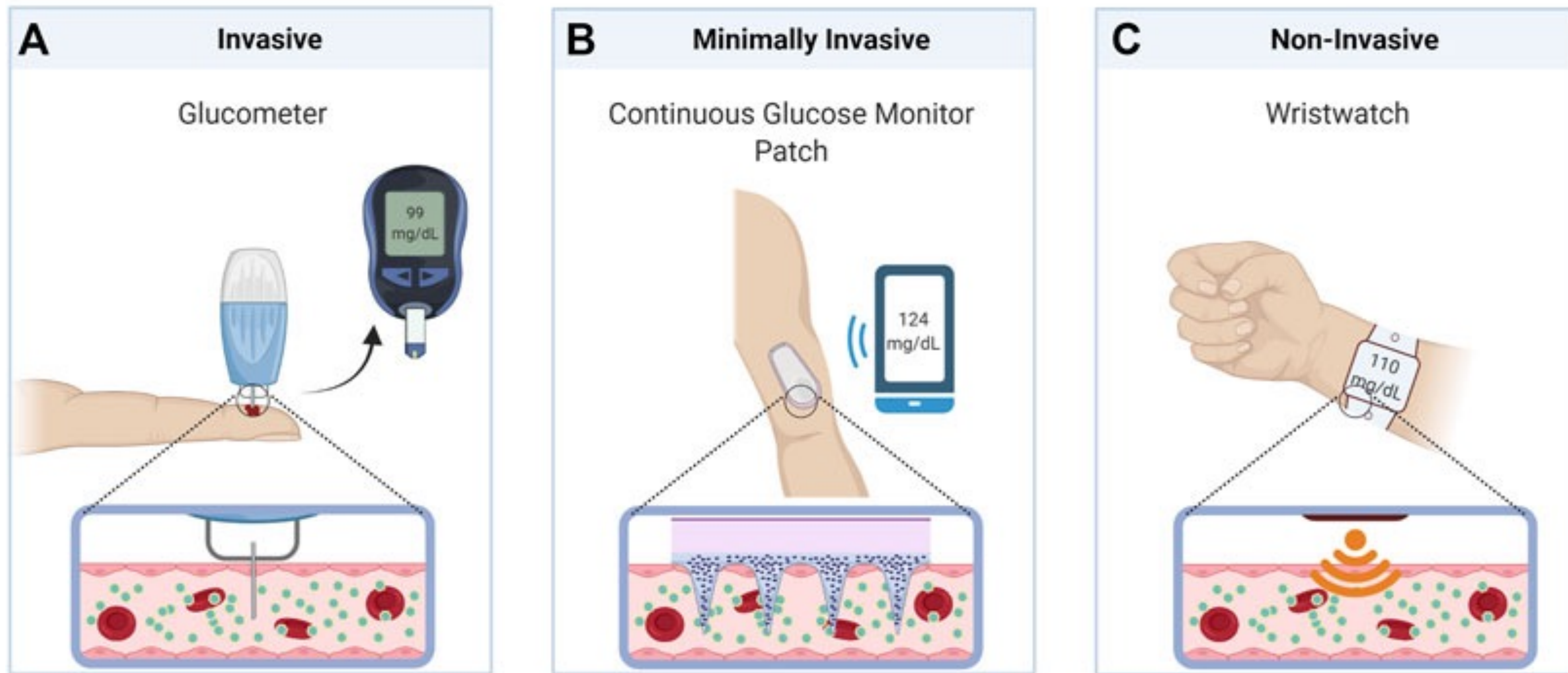
Redox processes are highly favorable so **spontaneous process**

Electron flow between electrodes generates current directly proportional to the partial pressure of oxygen in the sample



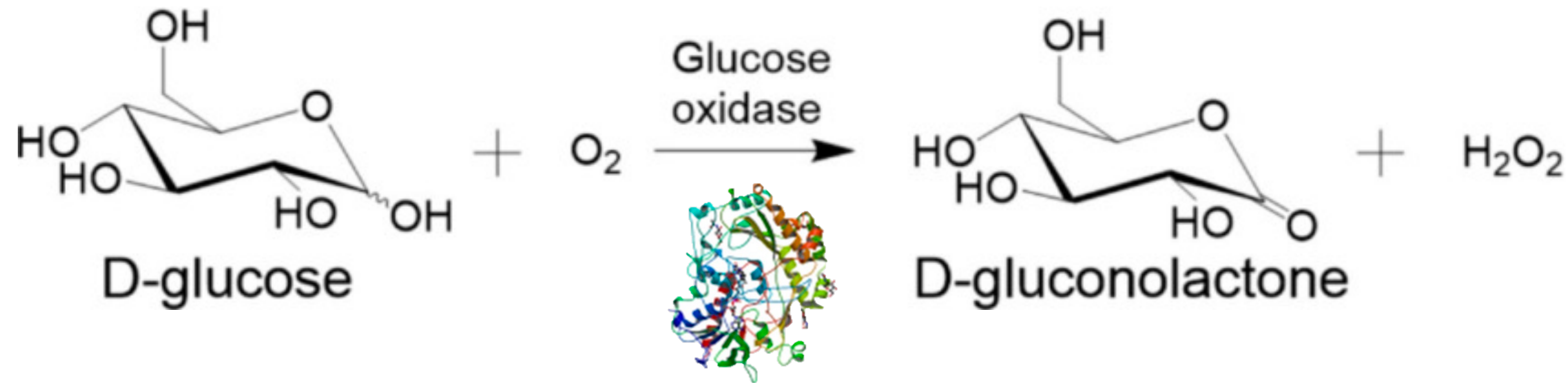
This work became the basis for the first glucose sensor

Glucose Biosensor – Transformative for Human Health



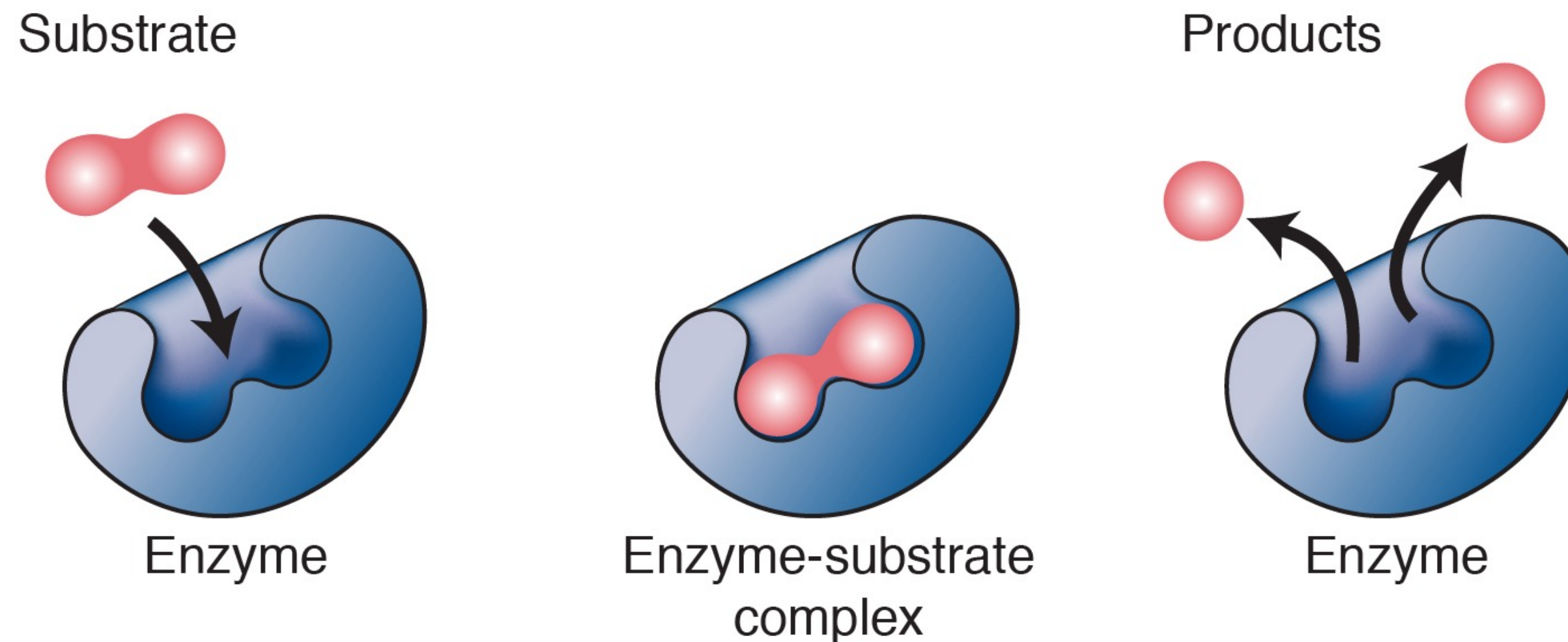
Todaro *et al.*, *Front. Chem.*, 10, 2022

Glucose Sensor – Incorporating an Enzyme (Biological Catalyst)

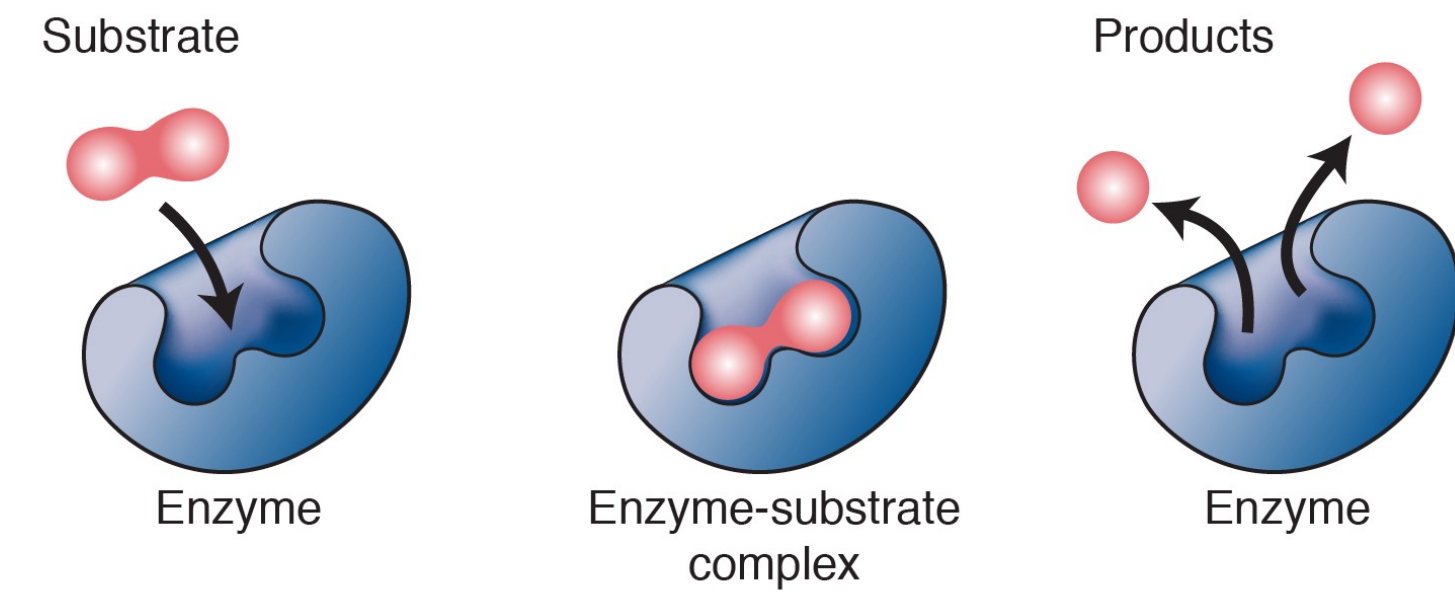
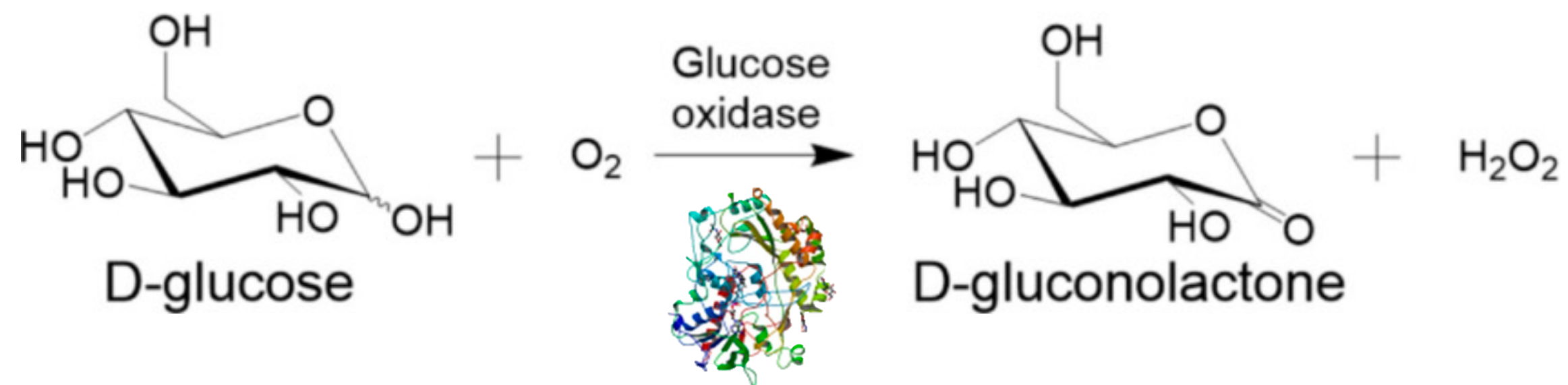


Catalyst: substance that accelerates the reaction rate without being consumed in the process

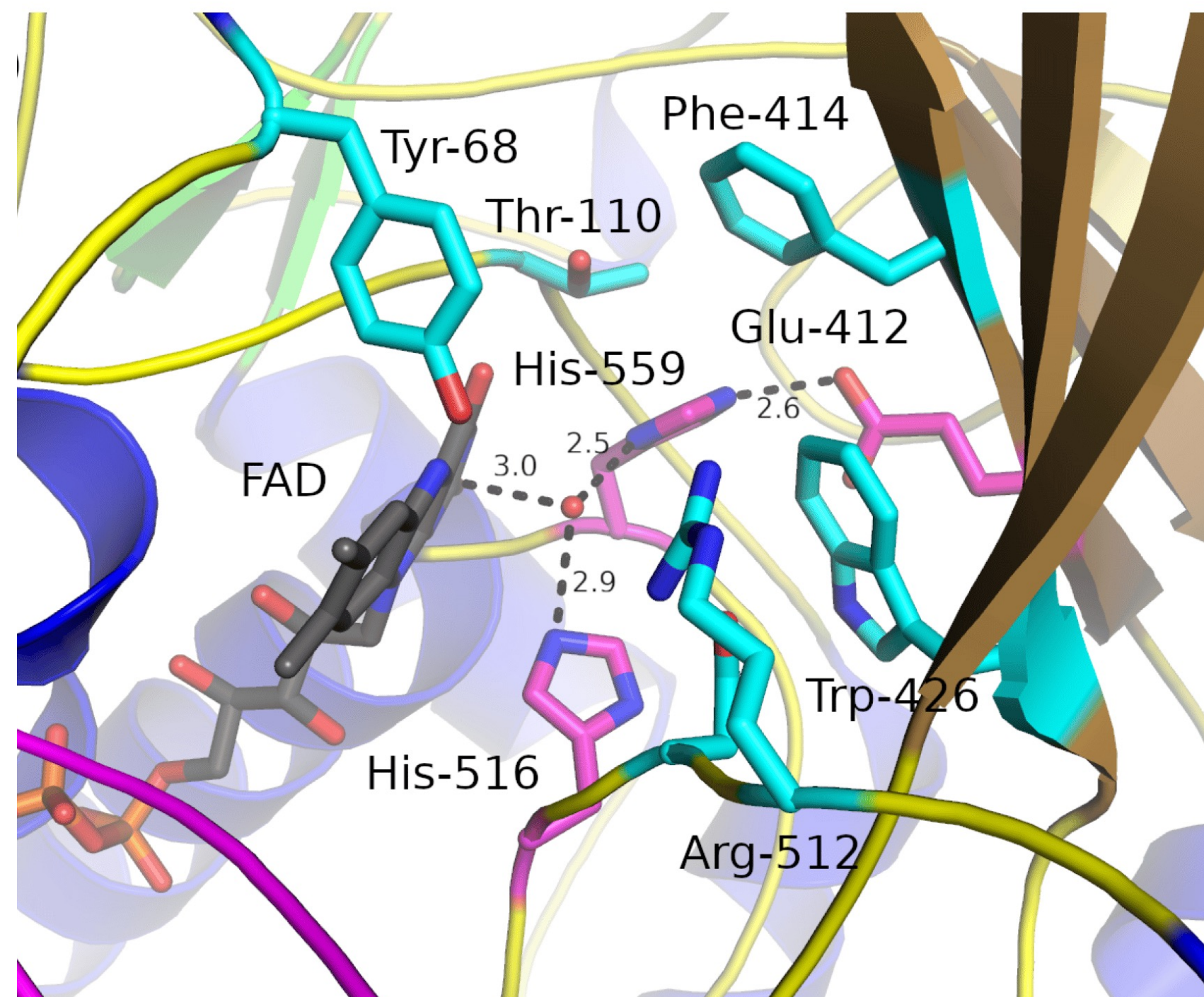
Catalysis: affects kinetics and not thermodynamics (it makes kinetics faster by decreasing activation energy)



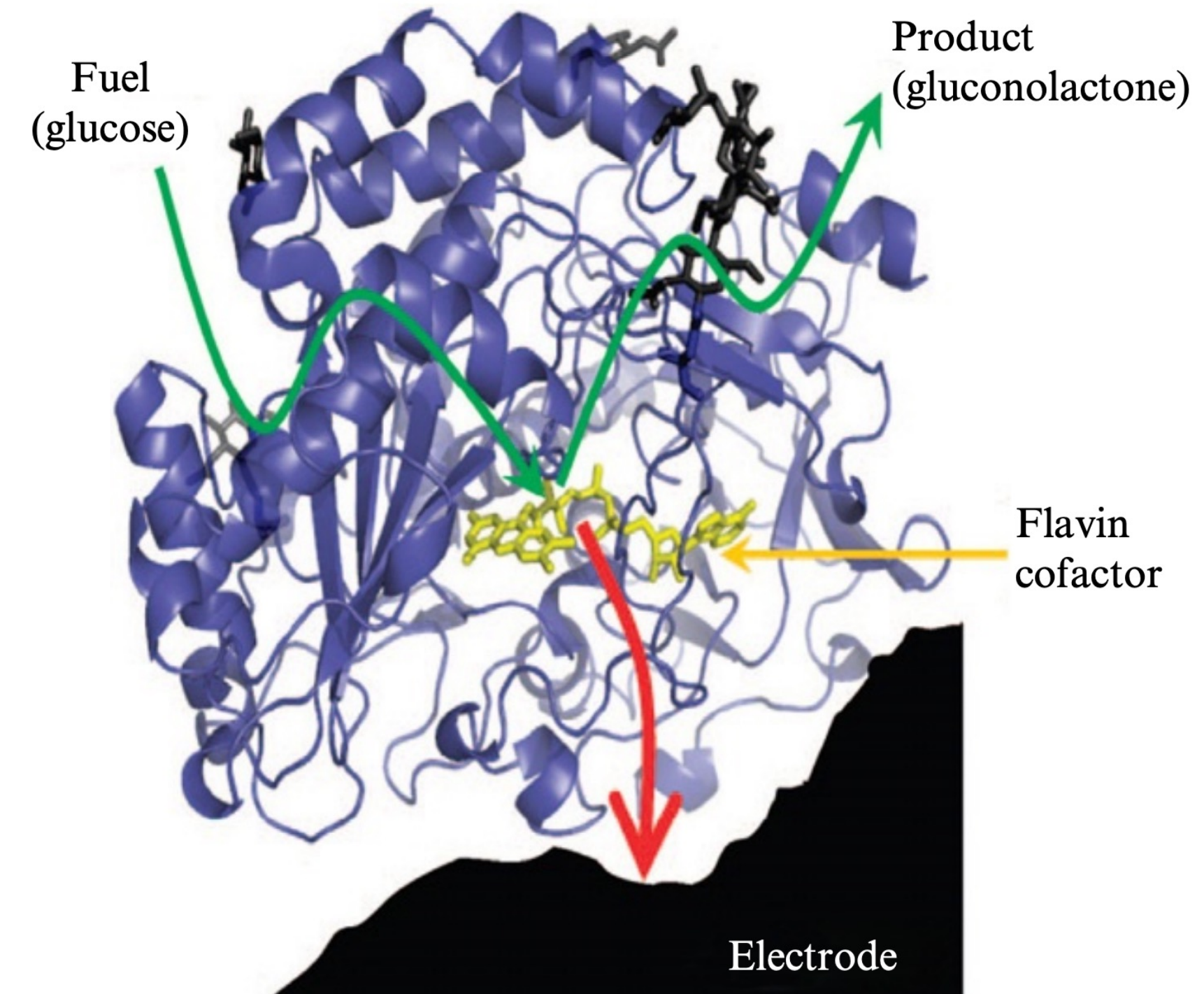
Intermolecular Interactions Between Enzyme and Substrate



Active site of glucose oxidase

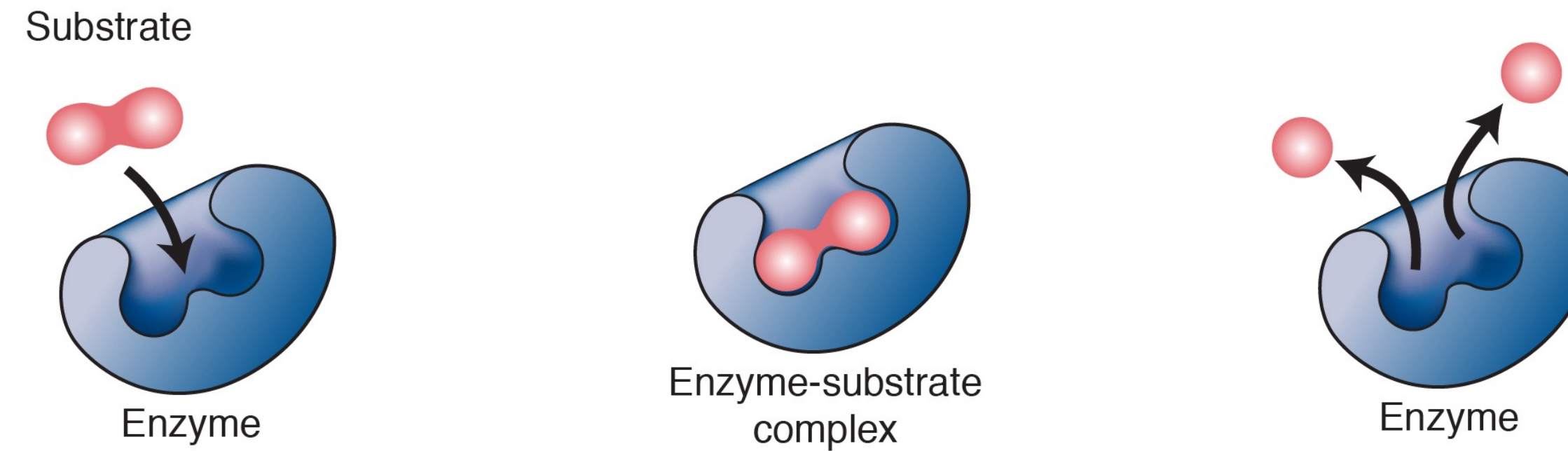


Bauer, *Biomolecules*, 12, 3, 2022

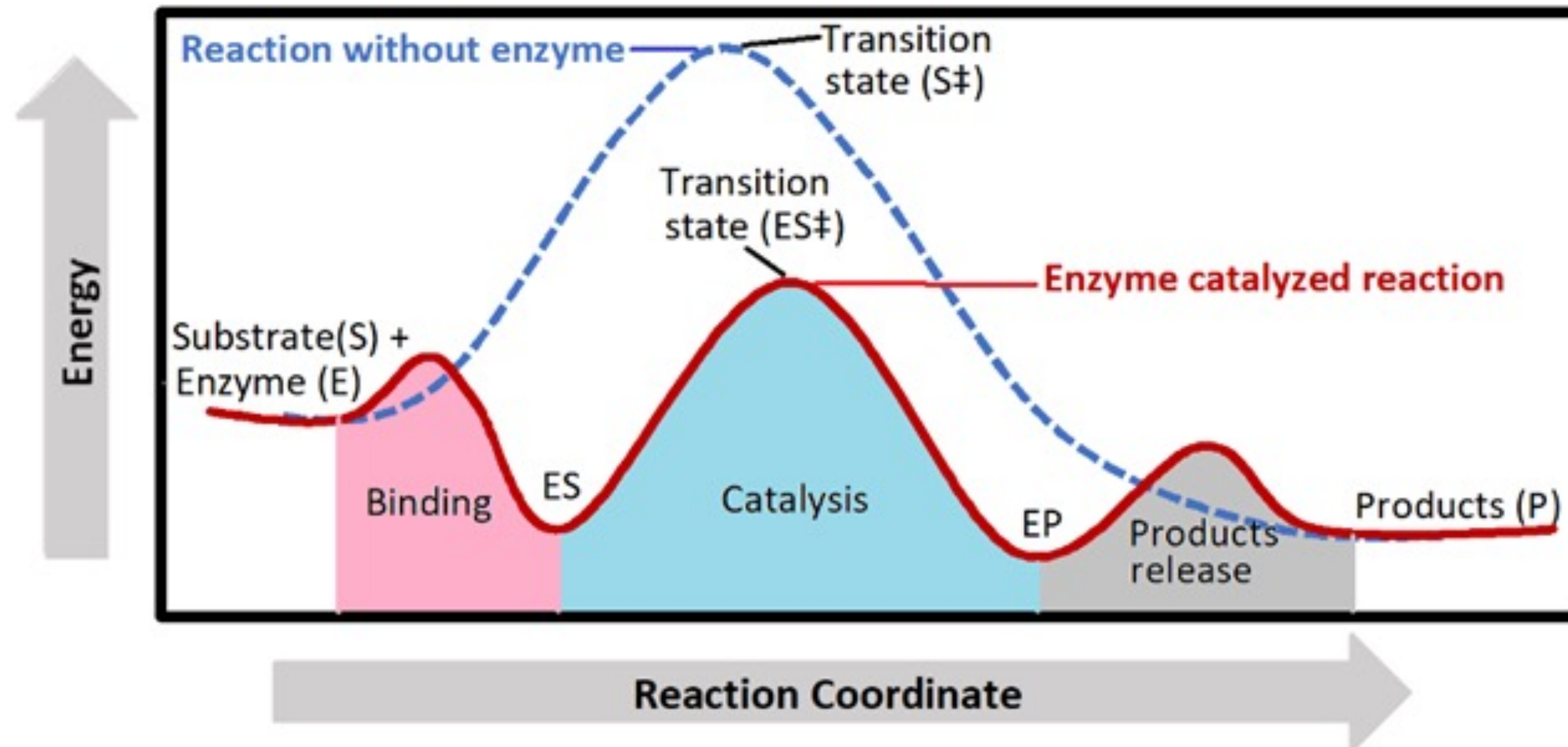


Viswanathan, *Methods Enzym.*, 509, 165, 2012

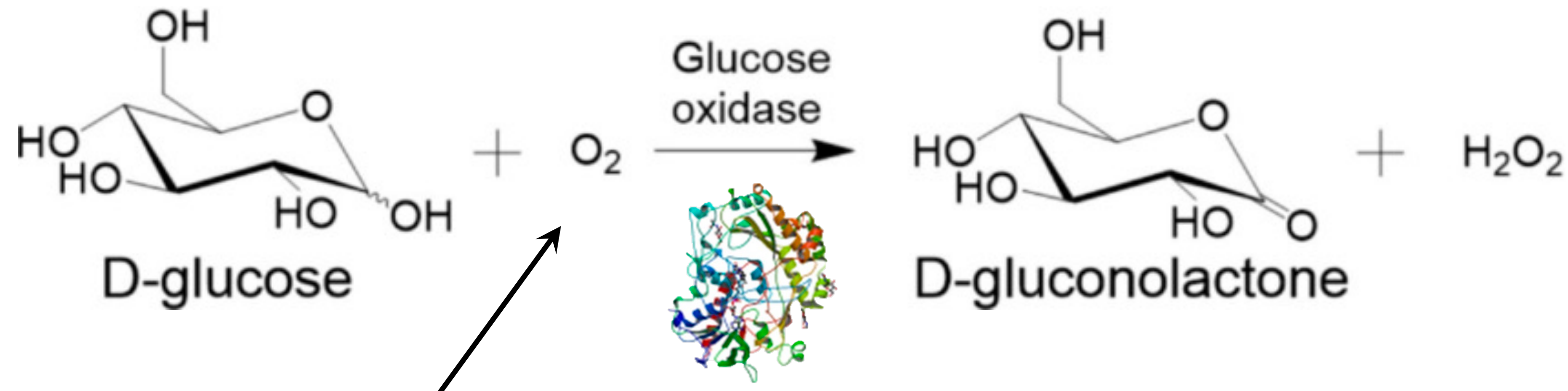
How Can Enzymes Catalyze Reactions?



Enzymes lower the activation energy of chemical reactions, allowing reactions to occur faster at the temperatures and conditions found in biological systems.



Glucose Sensor – Detection of Hydrogen Peroxide as Readout



Monitor decrease in O_2 as proxy for glucose

Lack of **sensitivity** – small signal change since O_2 levels high in biofluids

Lack of **specificity** – O_2 sensors influenced by changes unrelated to glucose (respiratory, temperature)

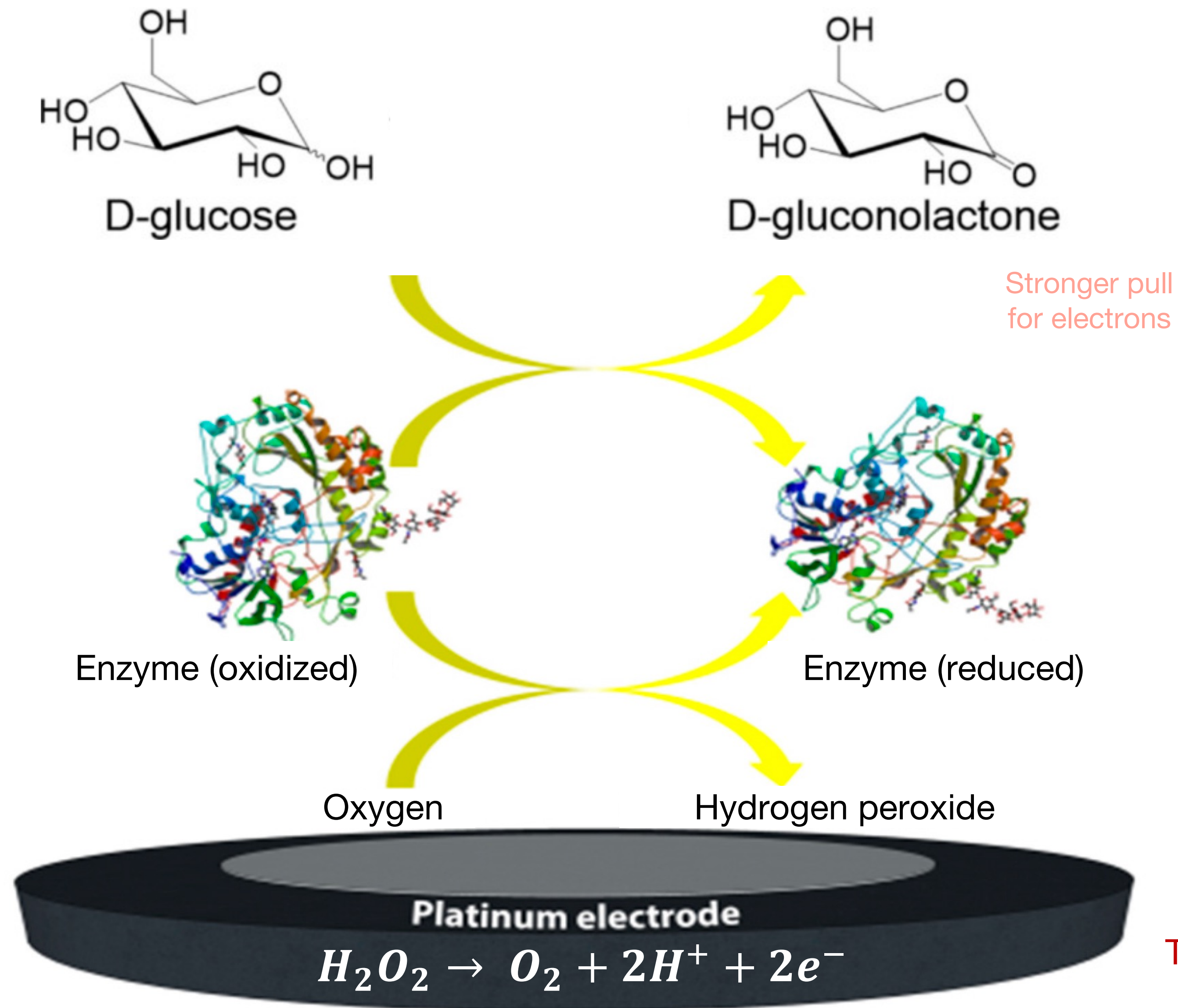
Monitor increase in H_2O_2 as proxy for glucose

Higher **sensitivity** – H_2O_2 not abundant in the sample prior to enzymatic reaction

Higher **specificity** – H_2O_2 is direct product of enzymatic reaction

$$S = \frac{\Delta \text{Signal}}{\Delta \text{Concentration}}$$

Oxidation of H₂O₂ on the Surface of Platinum Electrodes



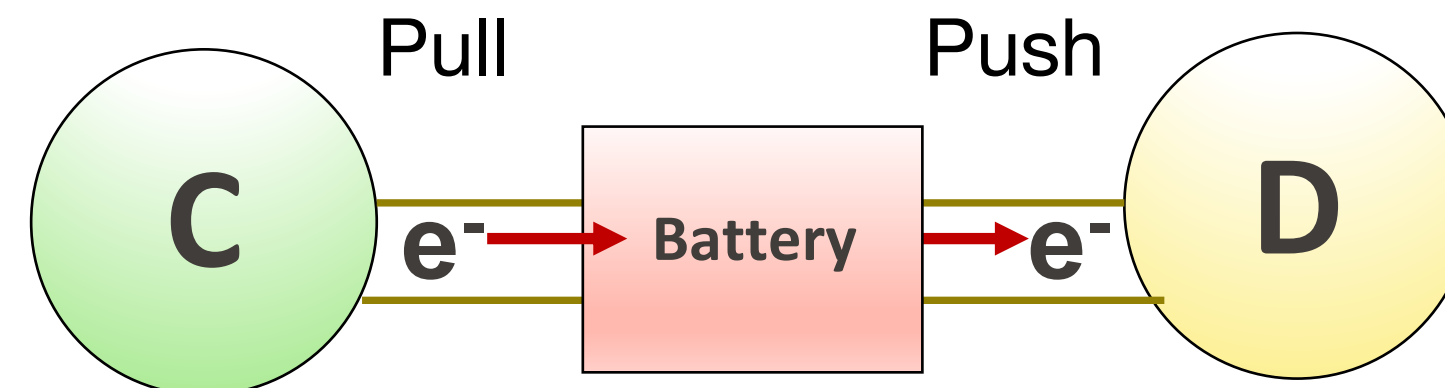
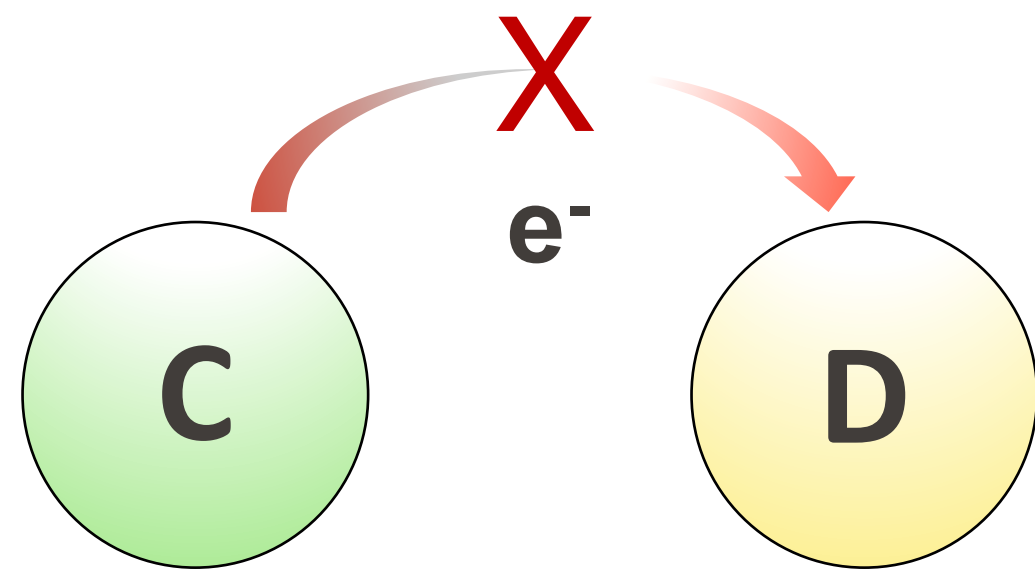
Half Reaction					potential
F₂	+	2e ⁻	⇌	2F ⁻	+2.87 V
Pb⁴⁺	+	2e ⁻	⇌	Pb ²⁺	+1.67 V
Cl₂	+	2e ⁻	⇌	2Cl ⁻	+1.36 V
Ag⁺	+	1e ⁻	⇌	Ag	+0.80 V
Fe ³⁺	+	1e ⁻	⇌	Fe ²⁺	+0.77 V
Cu ²⁺	+	2e ⁻	⇌	Cu	+0.34 V
2H⁺	+	2e ⁻	⇌	H₂	0.00 V

Standard reduction potential for
H₂O₂ : E⁰ = +0.695 V

So E_{oxidation} = -0.695 V

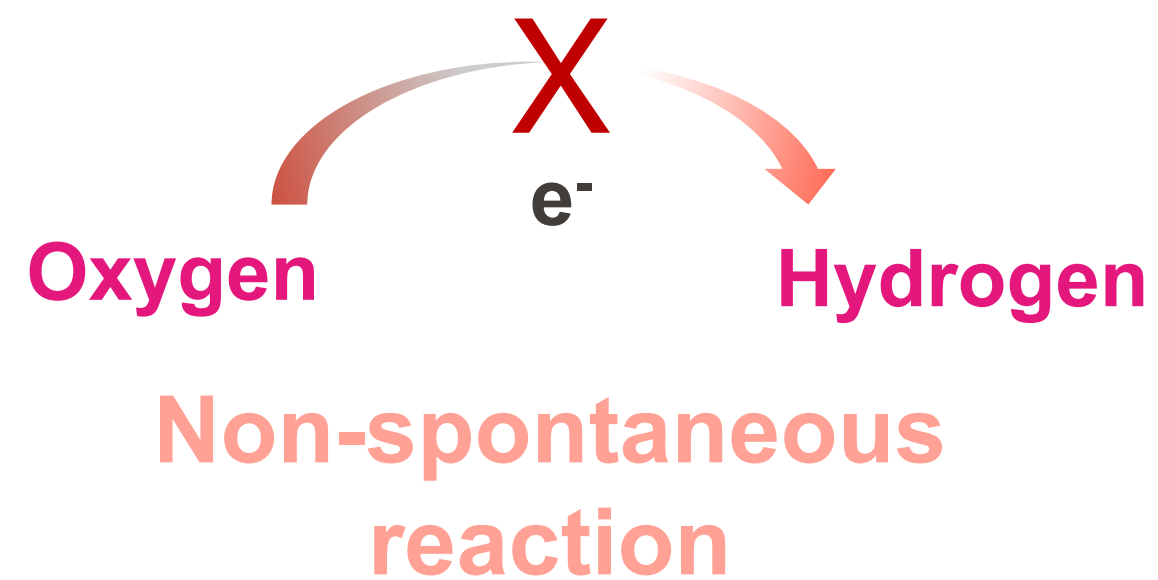
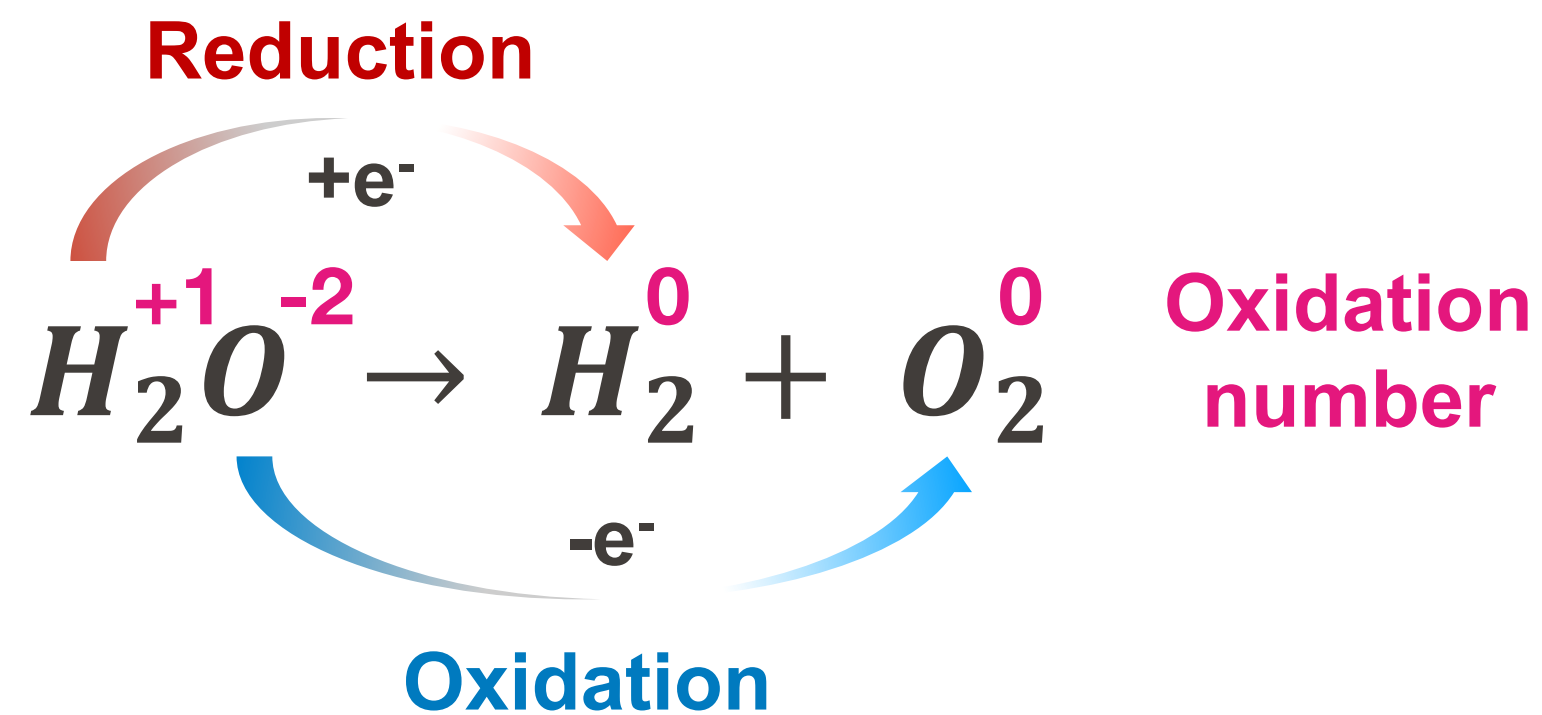
The oxidation process is non-spontaneous!

Electrolysis – Driving Chemical Reactions with Electricity



Non-spontaneous reaction

Water splitting

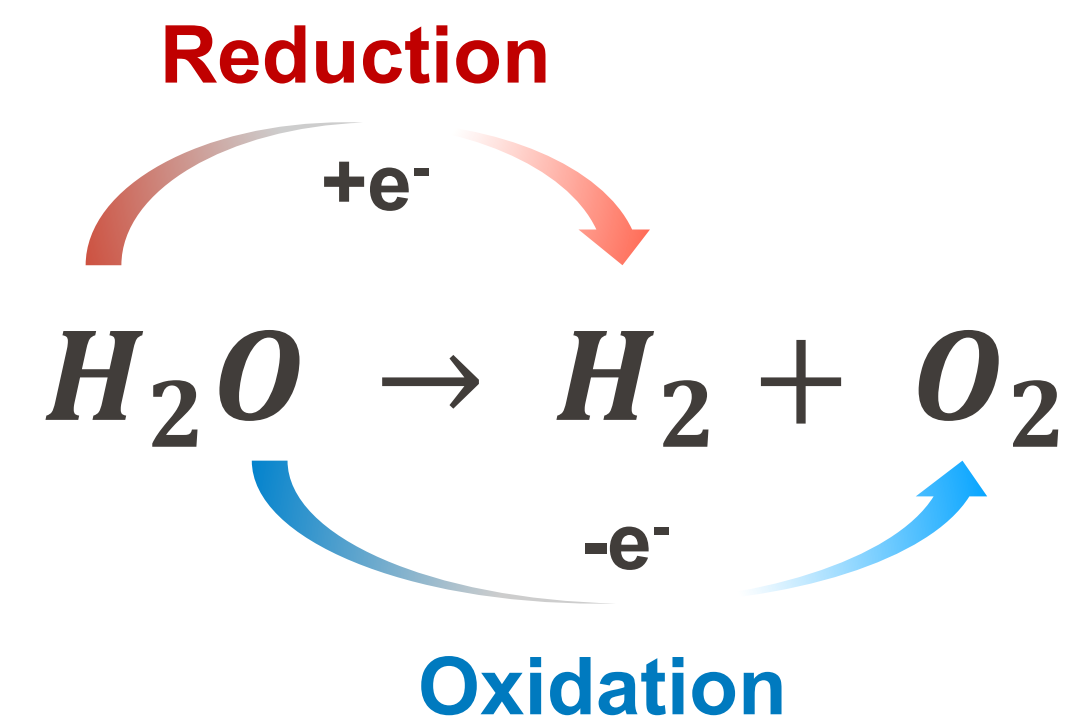
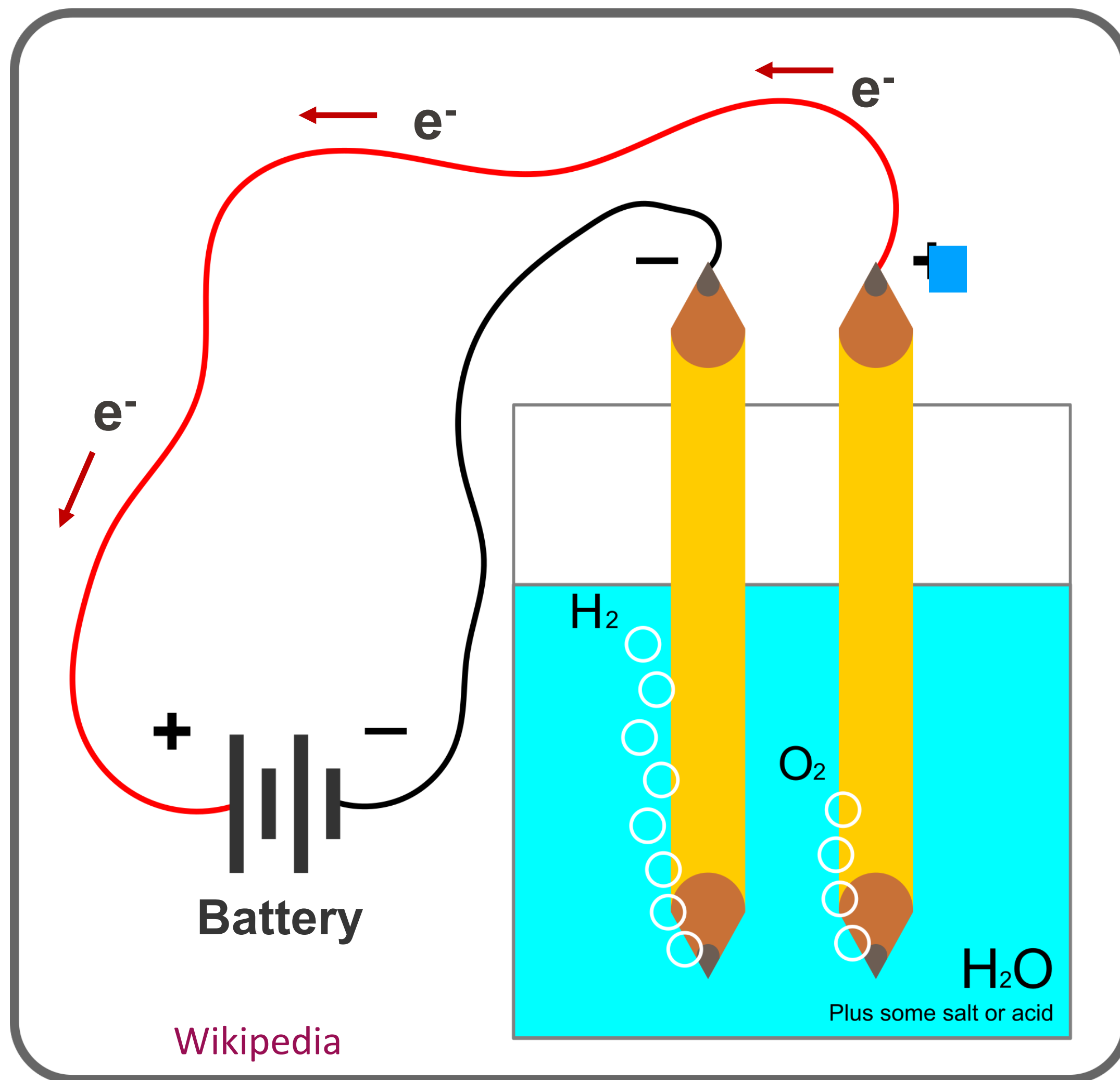


Half Reaction					potential
F_2	+	$2e^-$	$\rightleftharpoons$	$2F^-$	+2.87 V
Pb^{4+}	+	$2e^-$	$\rightleftharpoons$	Pb^{2+}	+1.67 V
O_2	+	$2e^- + 4H^+$	$\rightleftharpoons$	$2H_2O$	+1.23 V
Ag^+	+	$1e^-$	$\rightleftharpoons$	Ag	+0.80 V
Fe^{3+}	+	$1e^-$	$\rightleftharpoons$	Fe^{2+}	+0.77 V
Cu^{2+}	+	$2e^-$	$\rightleftharpoons$	Cu	+0.34 V
$2H^+$	+	$2e^-$	$\rightleftharpoons$	H_2	0.00 V

Stronger pull for electrons

We can force this reaction to happen using electricity

Electrolytic Cell – Driving Chemical Reactions with Electricity

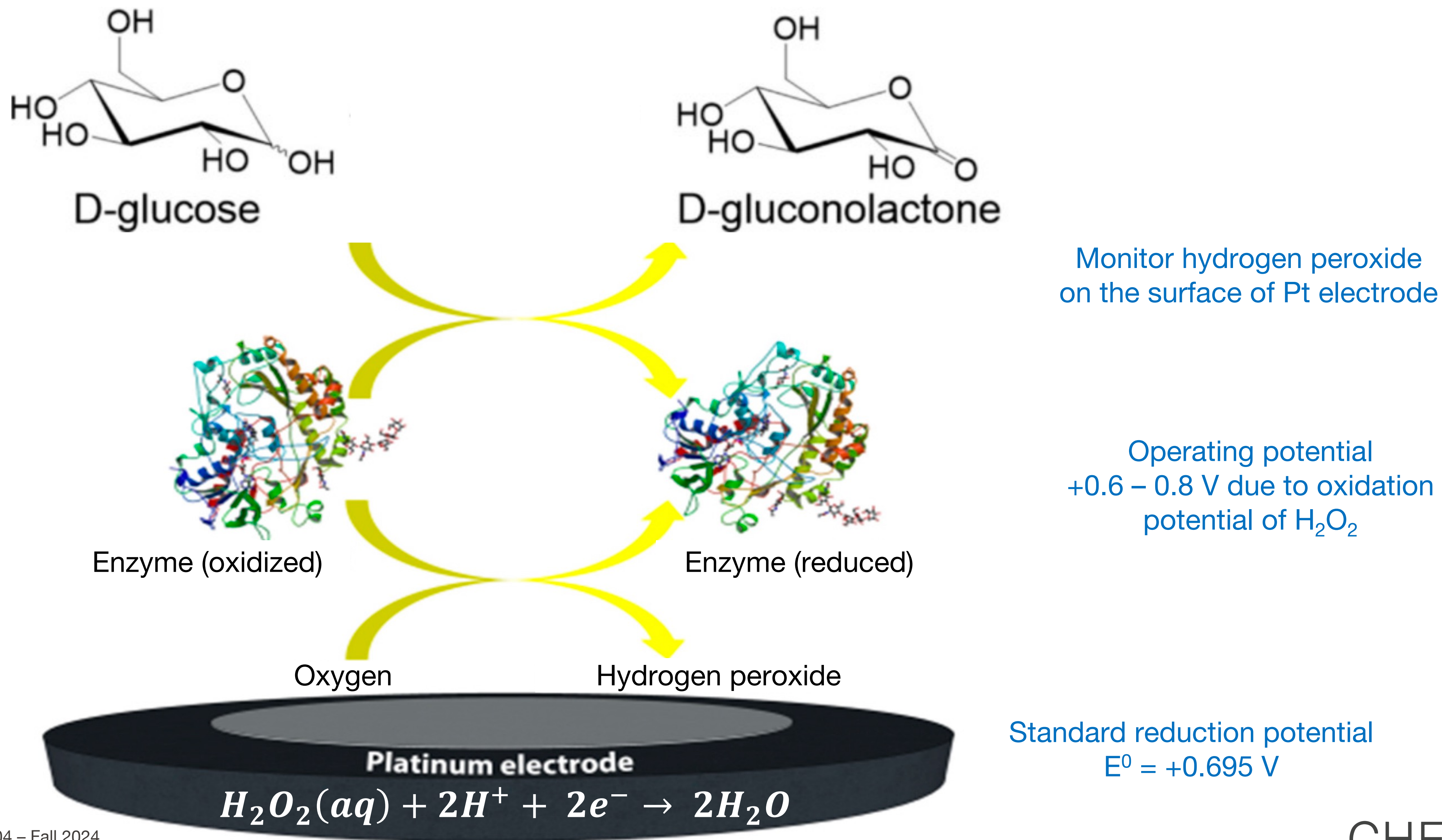


Applying a potential drives non-spontaneous redox reaction

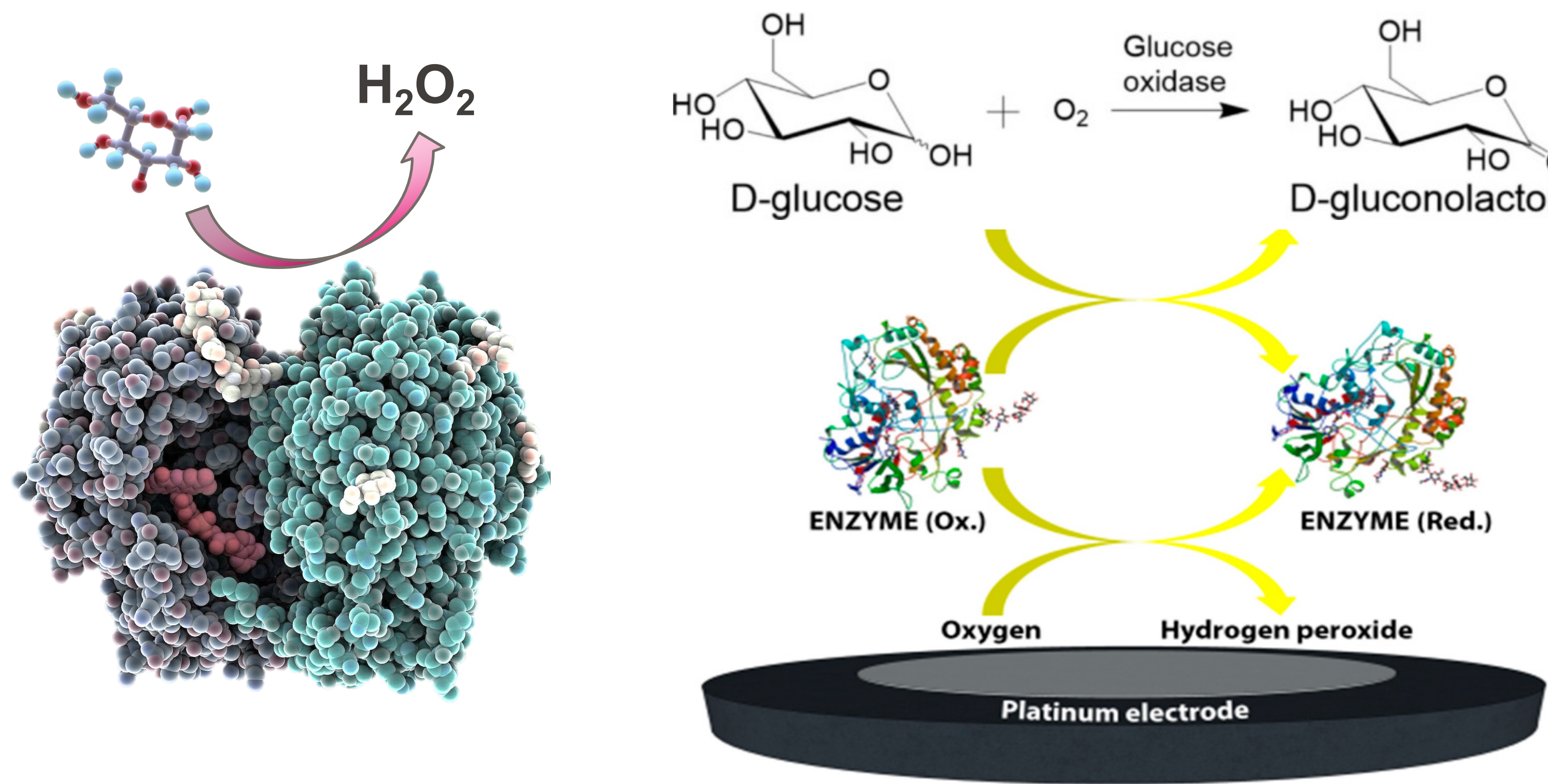
Operating potential applied to overcome activation energy barrier to drive reaction

We can force this reaction to happen using electricity

Glucose Sensor Requires an Operating Potential



Success of the Glucose Biosensor – Robust System



BLOOD GLUCOSE LEVELS CHART

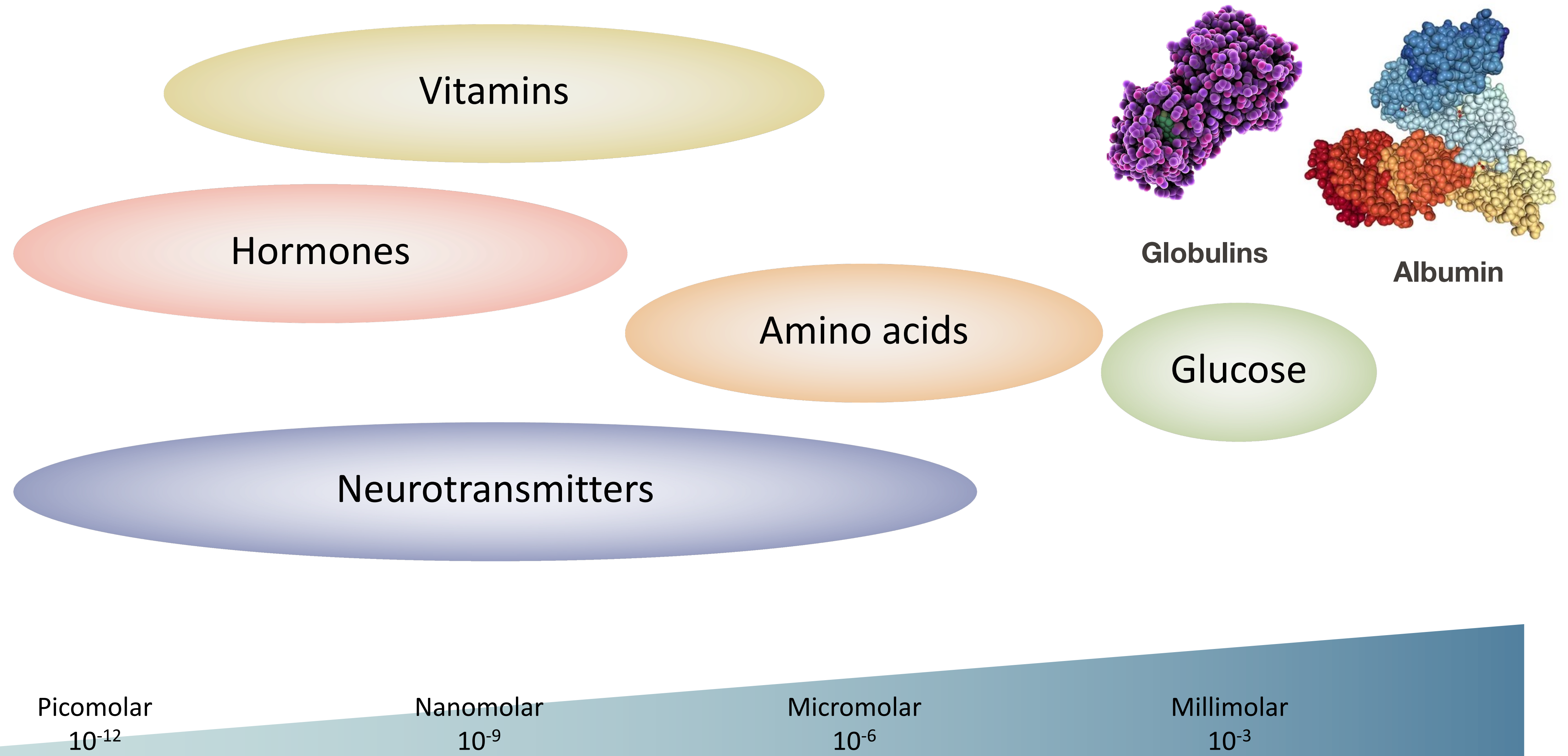
LEVEL	mg/dl	mmol/L	RISK	SUGGESTED ACTION
DANGER - HIGH	315+	17.4	VERY HIGH	MEDICAL ATTENTION
HIGH	280	15.6	HIGH	MEDICAL ATTENTION
HIGH	250	13.7	HIGH	MEDICAL ATTENTION
HIGH	215	11	HIGH	MEDICAL ATTENTION
BORDERLINE	180	10	MEDIUM	CONSULT DOCTOR
BORDERLINE	150	8.2	MEDIUM	CONSULT DOCTOR
BORDERLINE	120	7	MEDIUM	CONSULT DOCTOR
NORMAL	108	6	NO RISK	NO ACTION NEEDED
NORMAL	72	4	NO RISK	NO ACTION NEEDED
LOW	70	3.9	MEDIUM	CONSULT DOCTOR
DANGER - LOW	50	2.8	HIGH	MEDICAL ATTENTION

Highly specific and robust enzyme

Detection window for H_2O_2 doesn't overlap with other species in blood

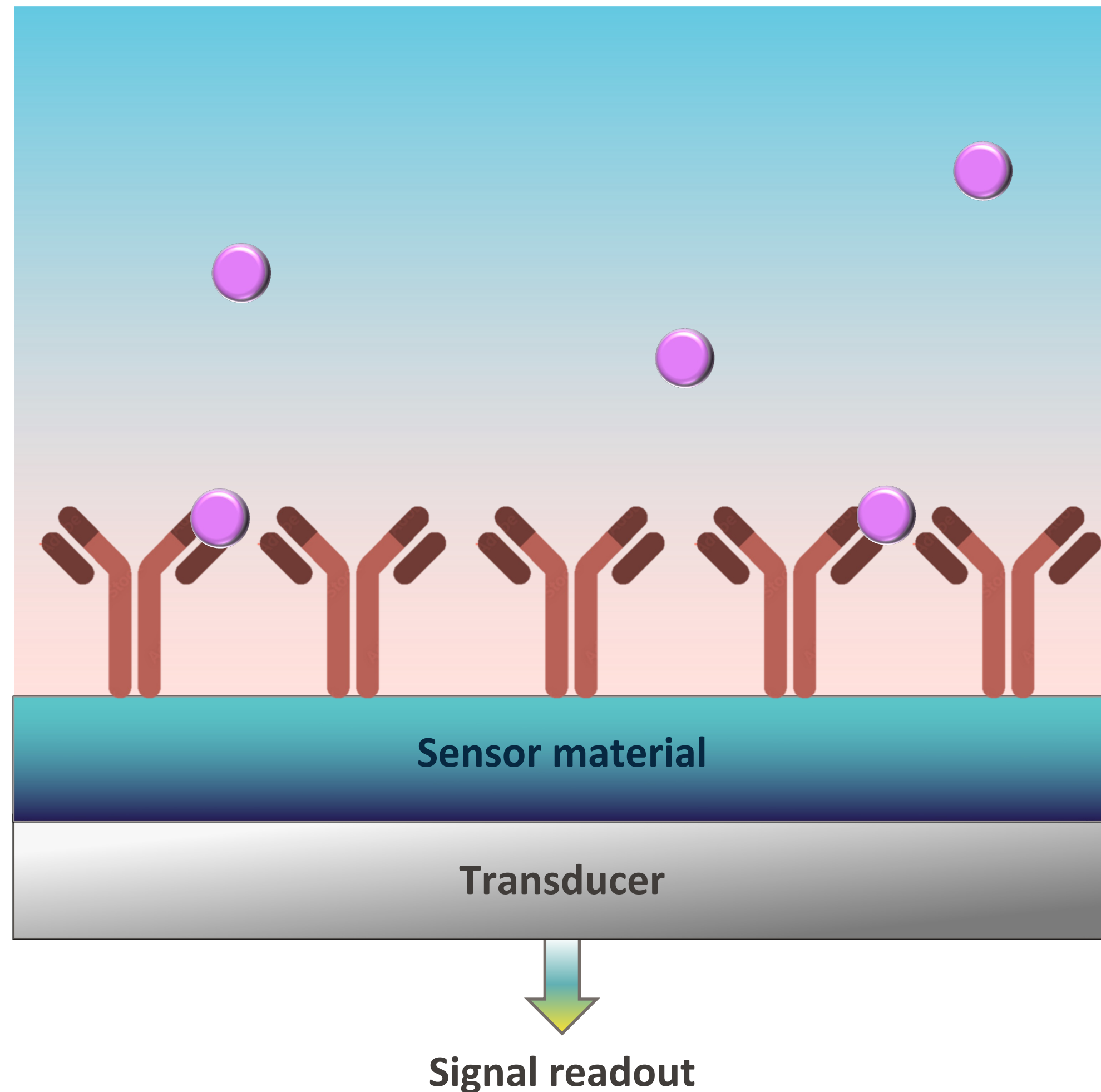
Clear values associated with healthy and disease states (diabetes)

Success of the Glucose Biosensor – Concentration Range

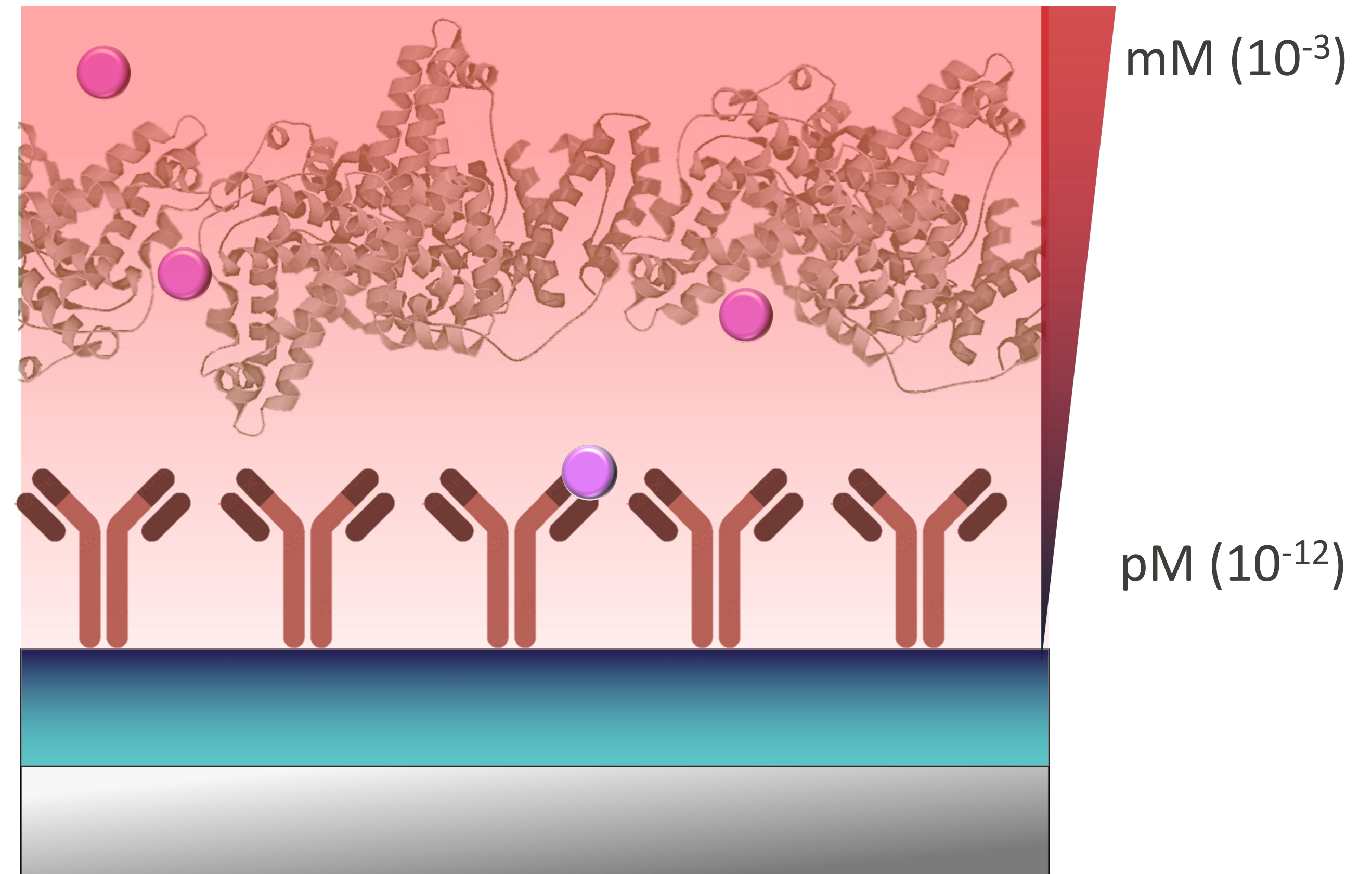


1. Nonspecific Binding (NSB) in Complex Environments

Expectation (phosphate buffered saline)

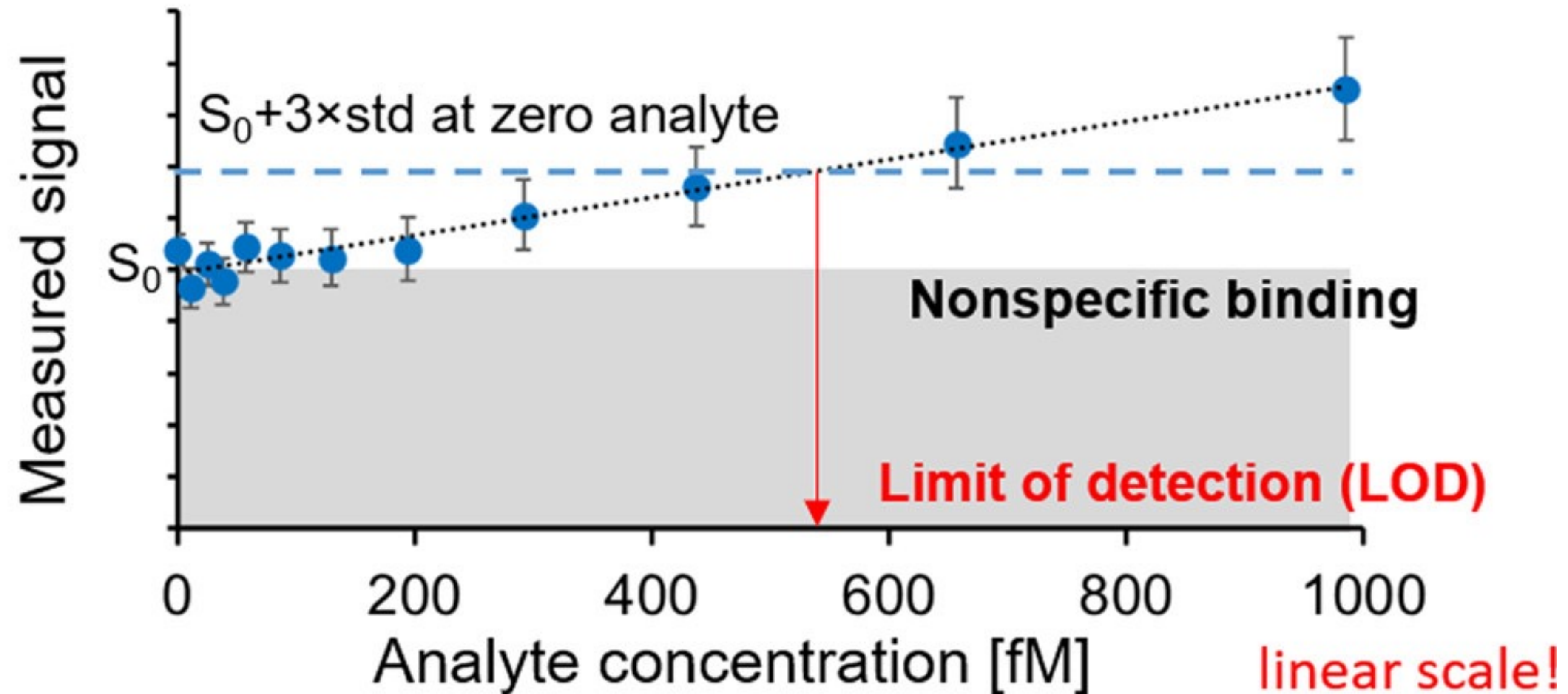


Reality (biofluids such as blood)



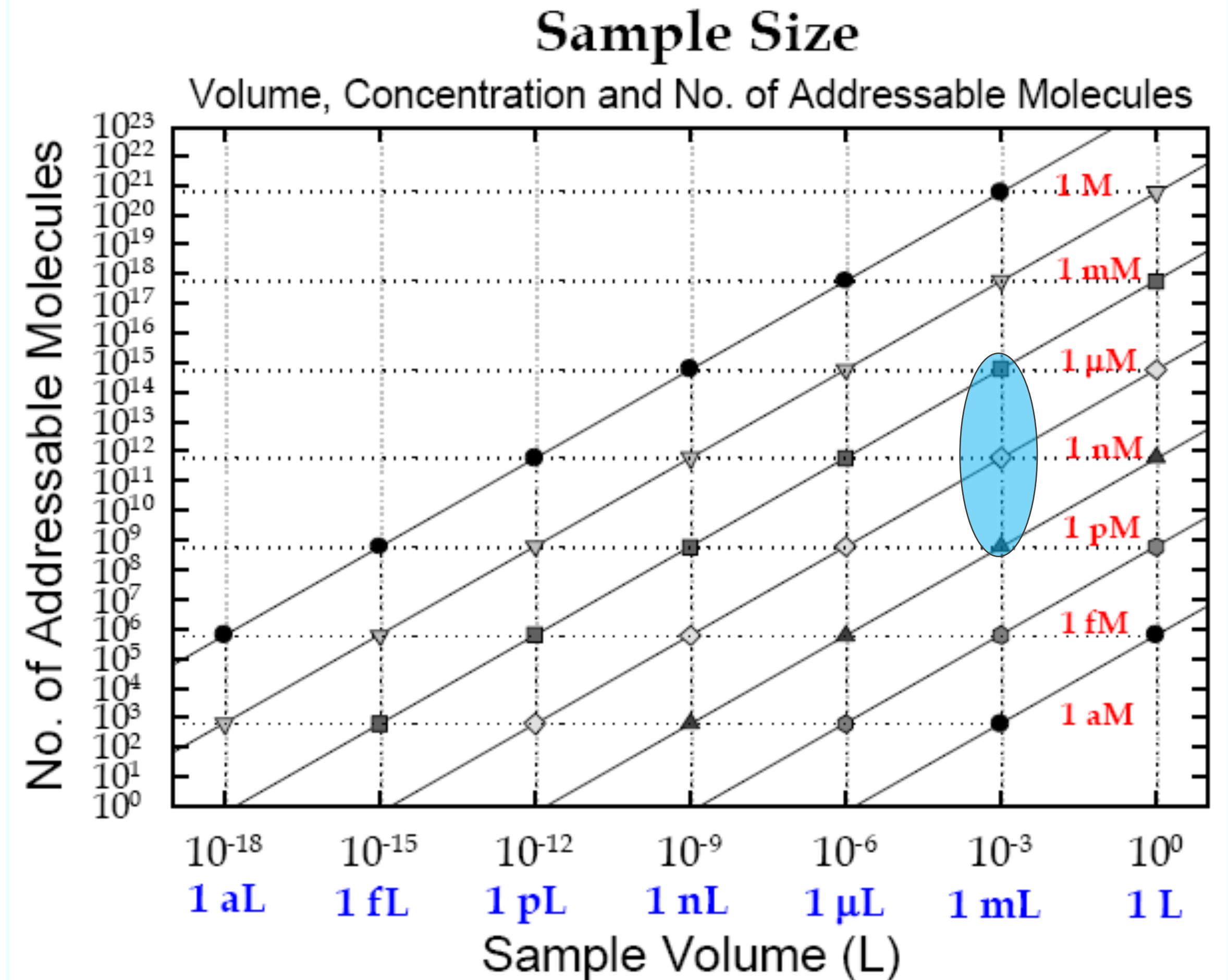
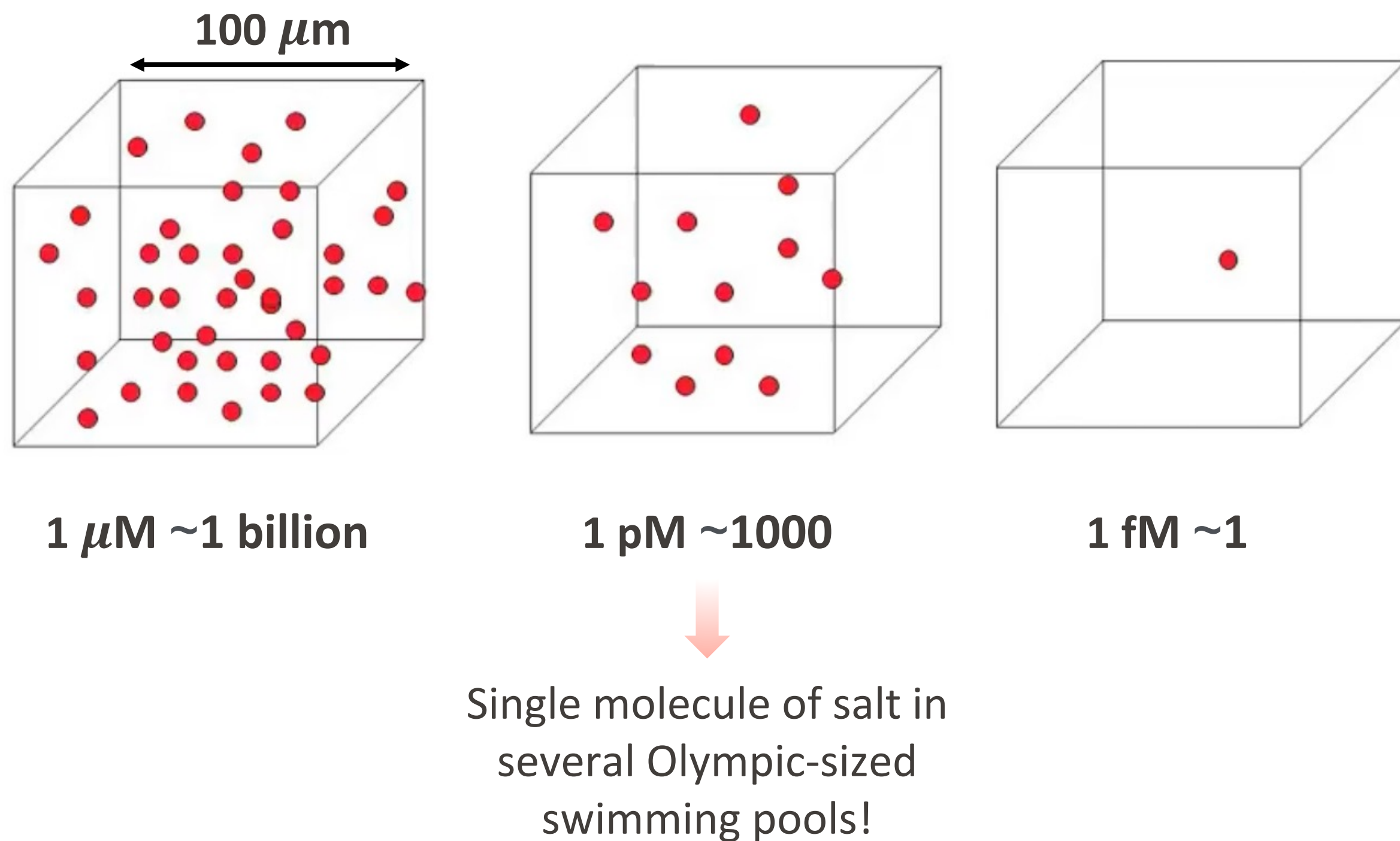
Most Existing Diagnostics are Limited by Nonspecific Binding

Non-specific binding induced noise at low concentrations limits diagnostic biosensors



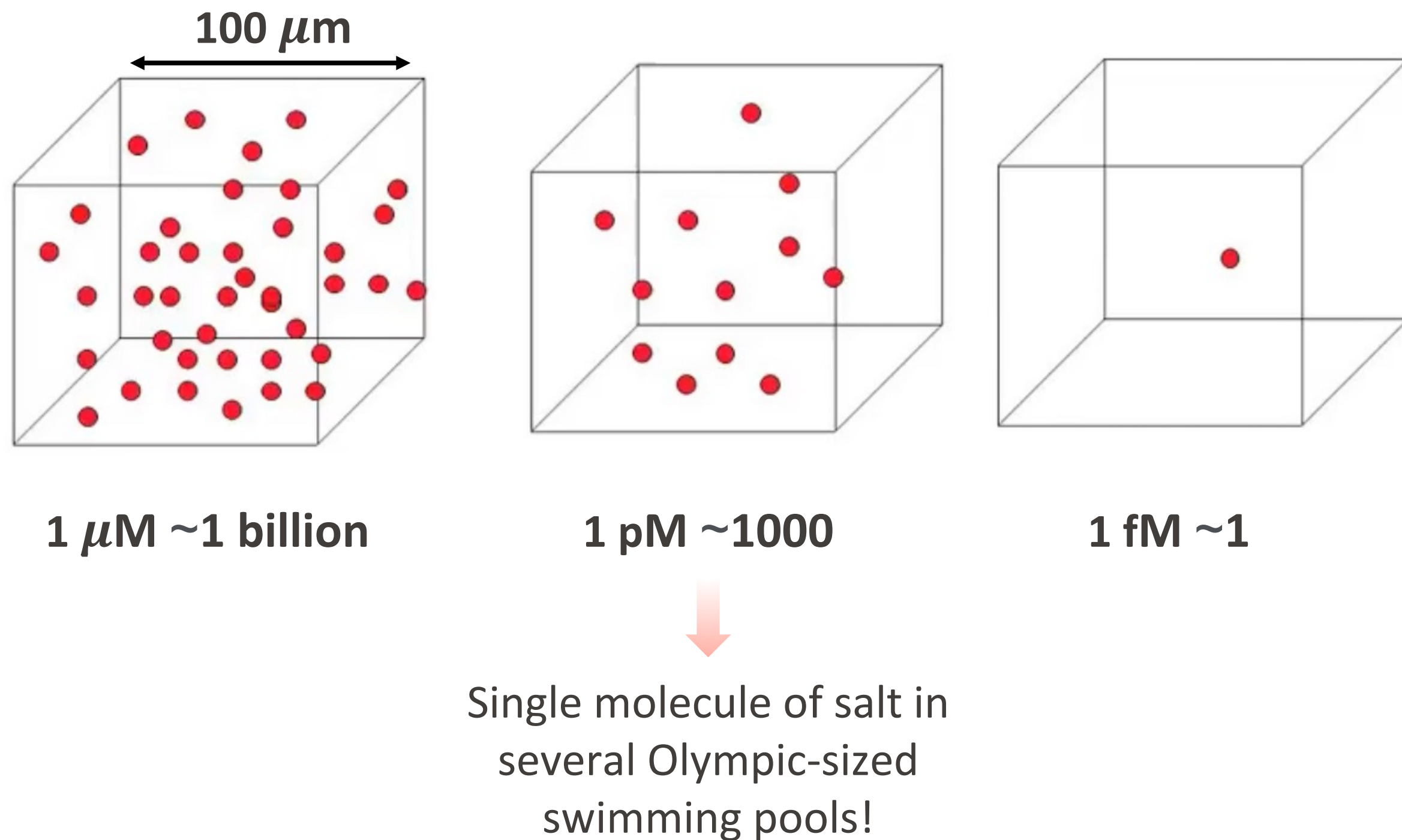
2. Very Few Number of Molecules Within Sample Size

Stochastic process – probability of the biosensor seeing such a low concentration of target diffusing to the surface?



Few Molecules in Large Volume – Diffusion to the Sensor Surface

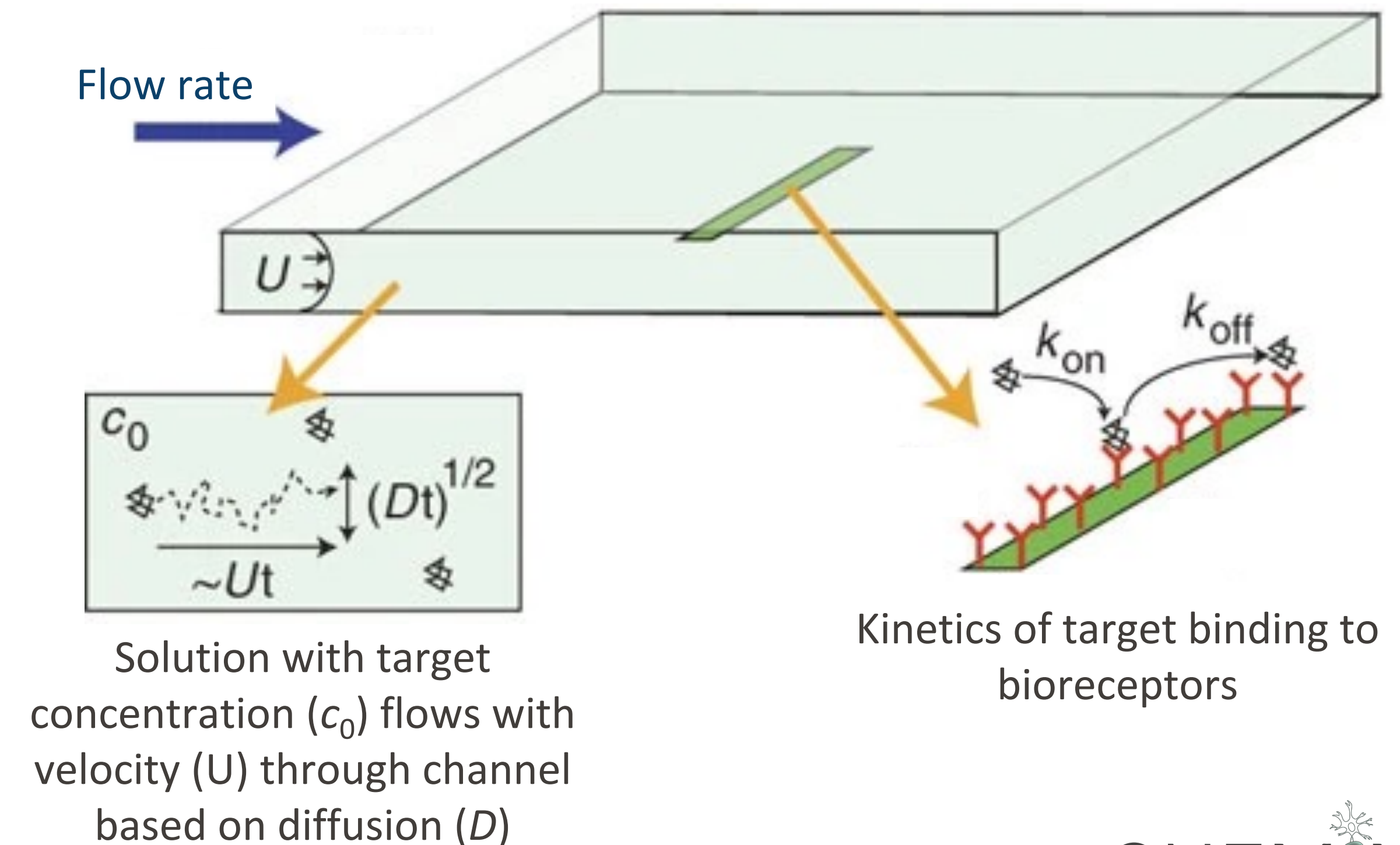
Stochastic process – probability of the biosensor seeing such a low concentration of target diffusing to the surface?



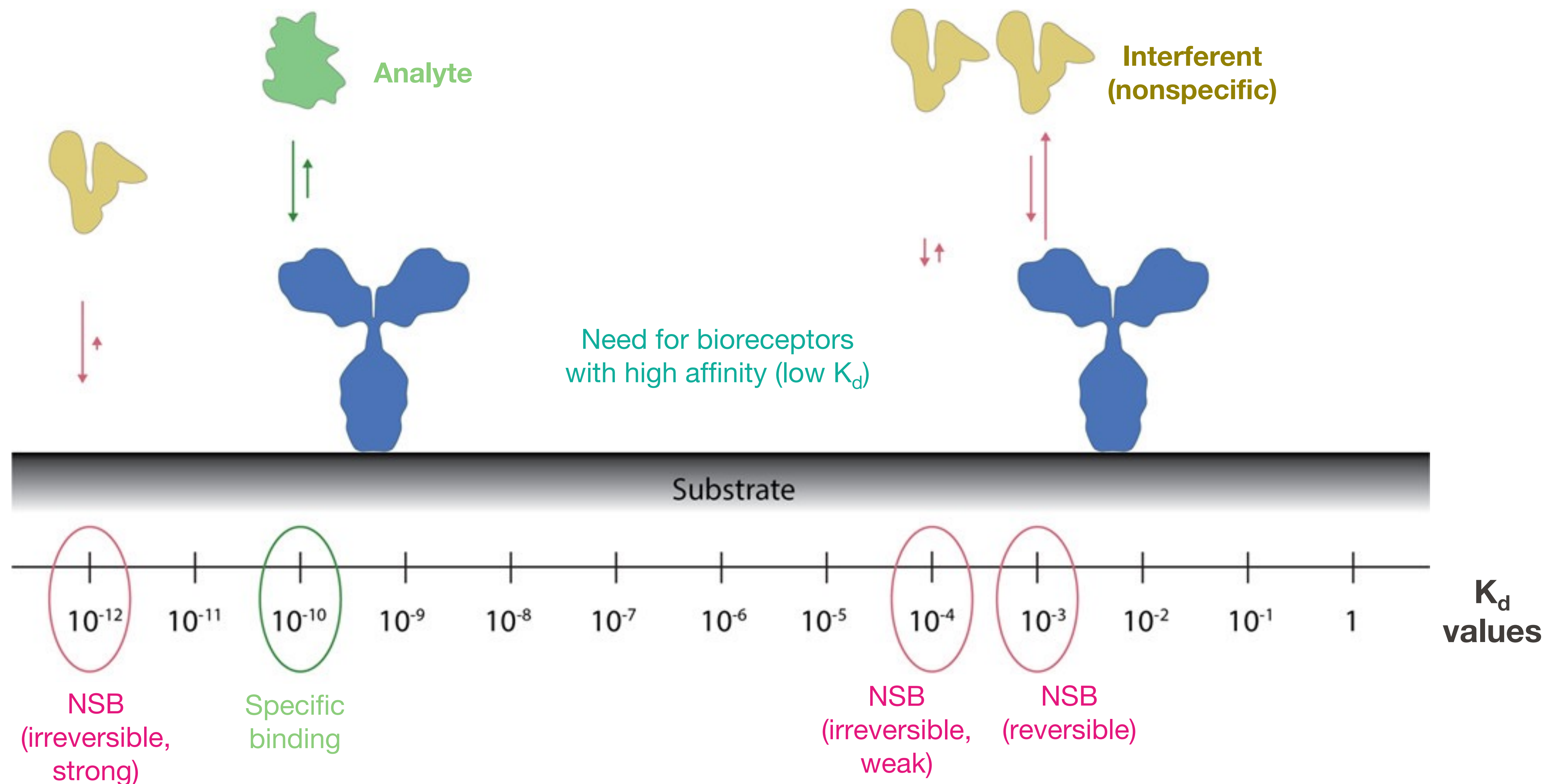
Making it stick: convection, reaction and diffusion in surface-based biosensors

[Todd M Squires](#) , [Robert J Messinger](#) & [Scott R Manalis](#)

[Nature Biotechnology](#) **26**, 417–426 (2008) | [Cite this article](#)



3. Challenges of Bioreceptor Specificity in Addition to NSB

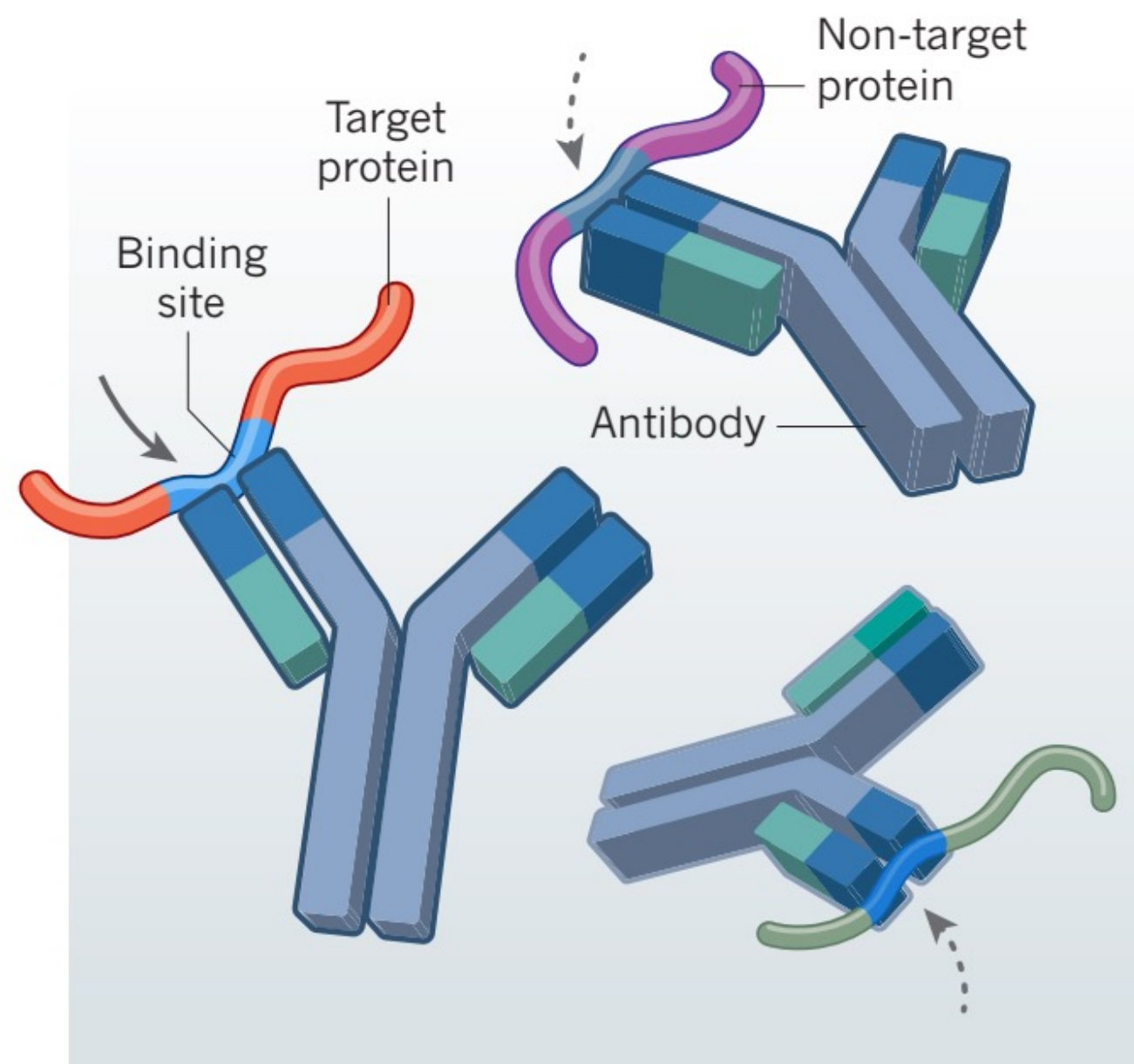


Bioreceptors Can be Cross-Reactive to Nonspecific Molecules

Reproducibility crisis: Blame it on the antib

[Monya Baker](#)

[Nature](#) **521**, 274–276 (2015) | [Cite this article](#)



Cross-reactivity: binding to other non-targets

Batch-to-batch variability

Sensitivity to environment

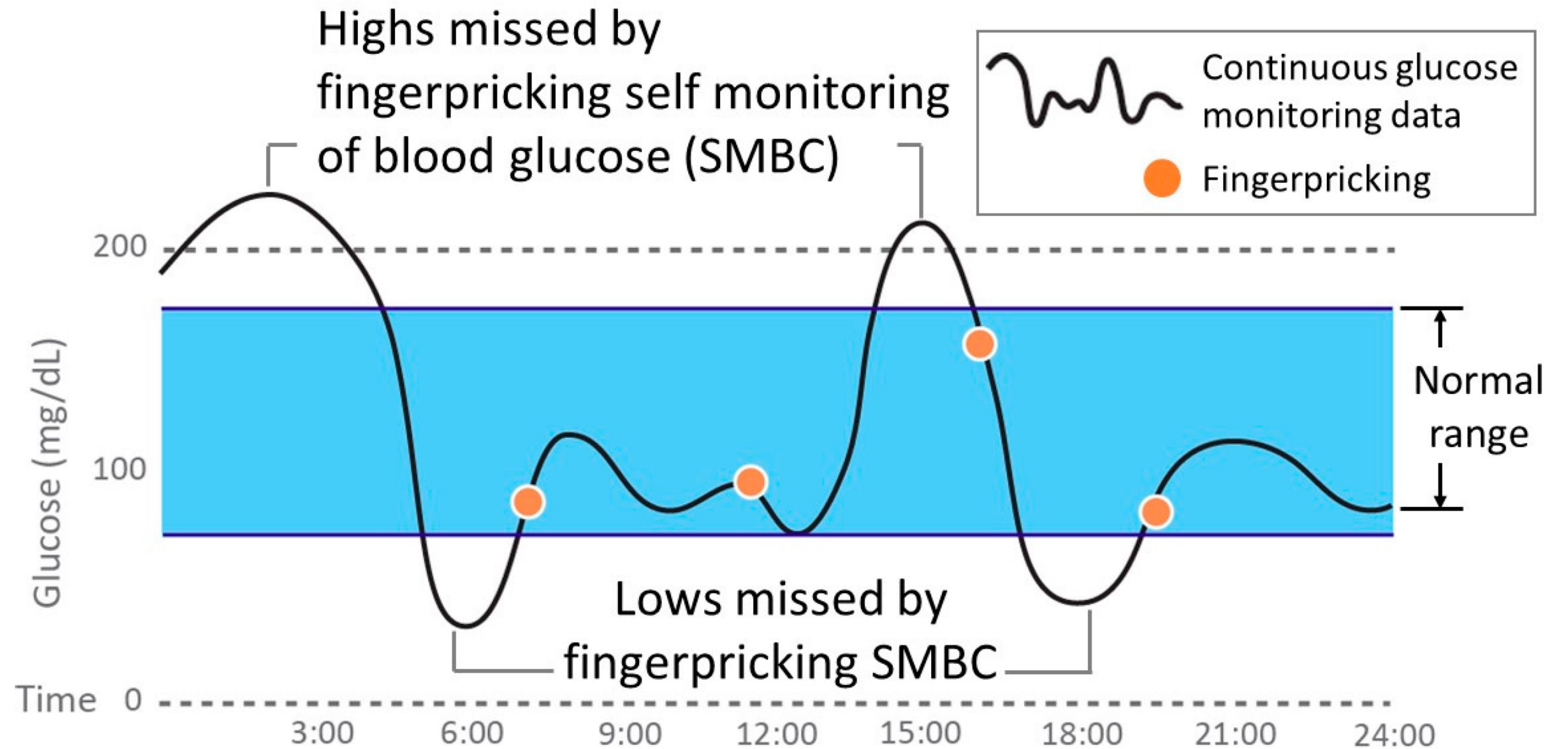
BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

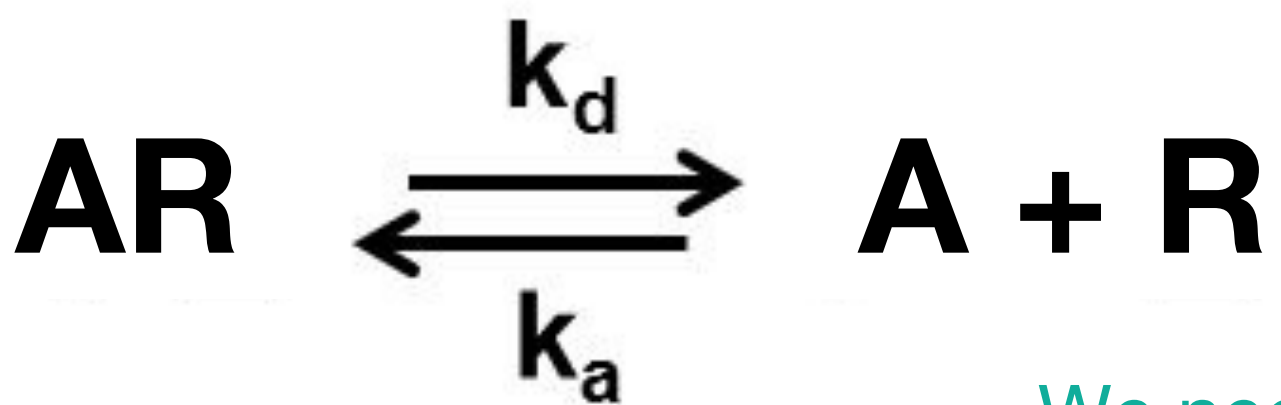
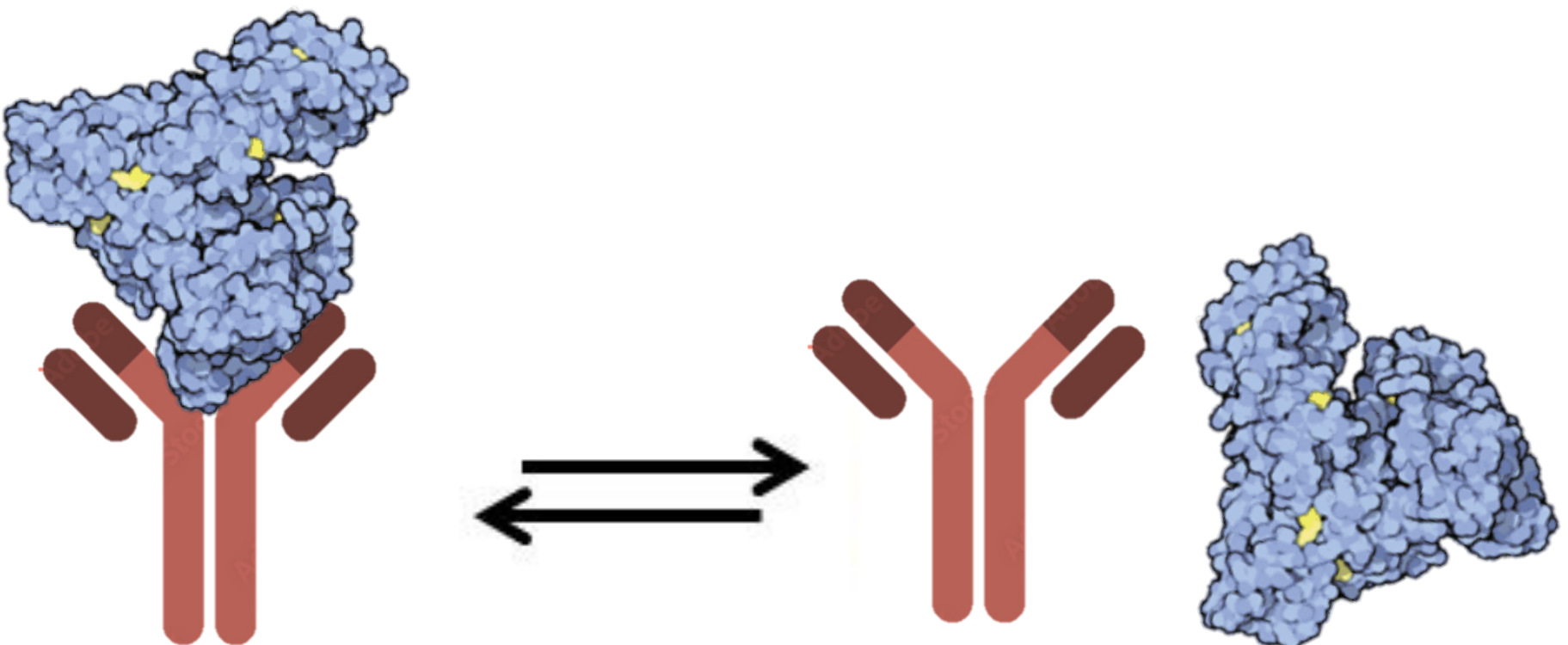
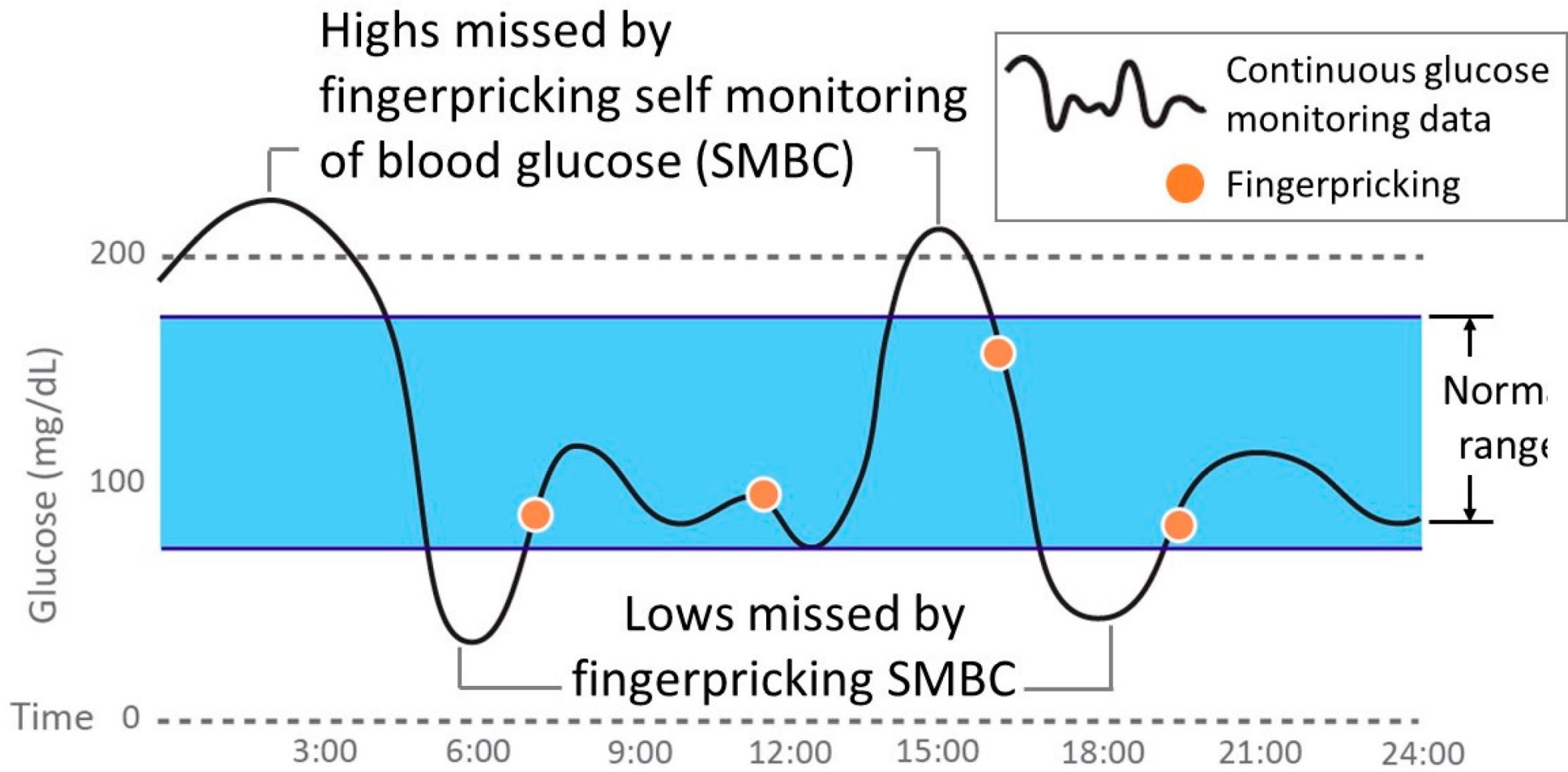
BY MONYA BAKER



4. Challenges of Continuous Monitoring



Continuous Measurements: Reversible Binding to Bioreceptors



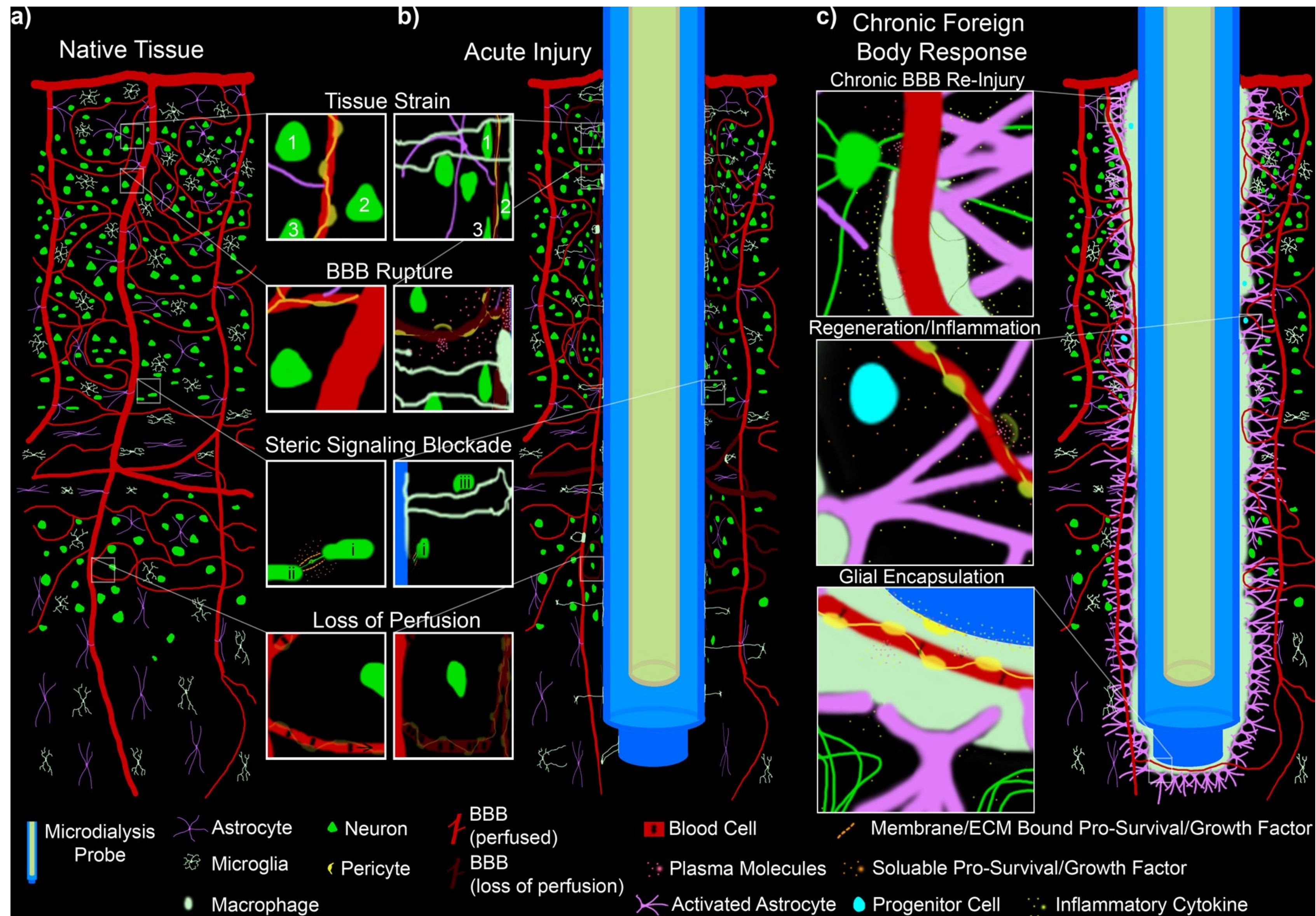
We need strong binding (low K_d)
To detect analytes at low concentrations

We need fast kinetics (high k_d)

$$K_d = \frac{[A][R]}{[AR]} = \frac{k_d}{k_a} = \frac{1}{K_a}$$

We need to engineer/redesign bioreceptors to overcome this mismatch

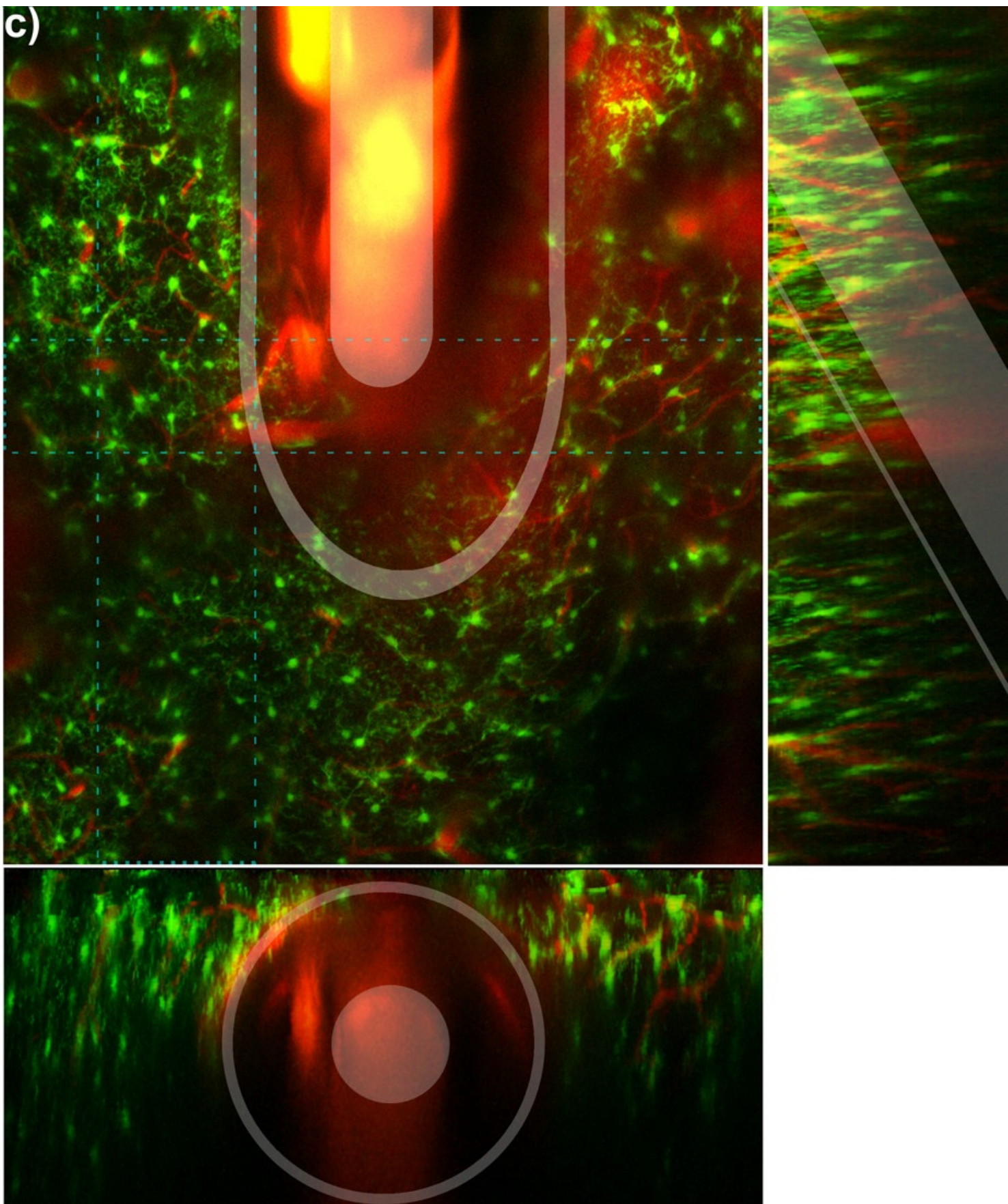
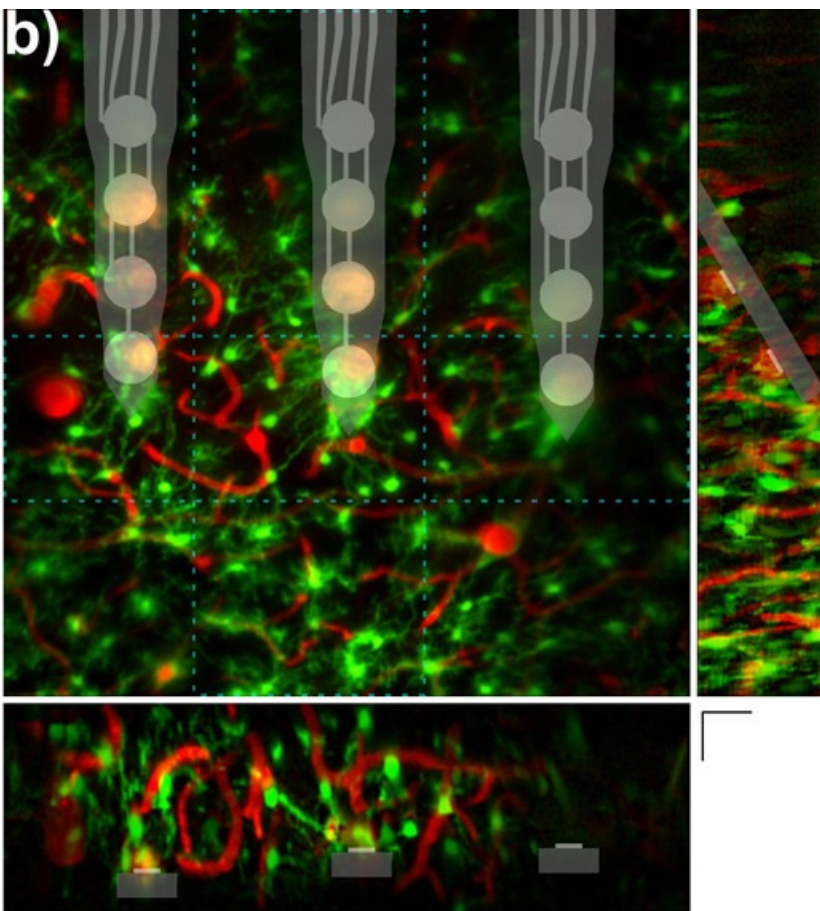
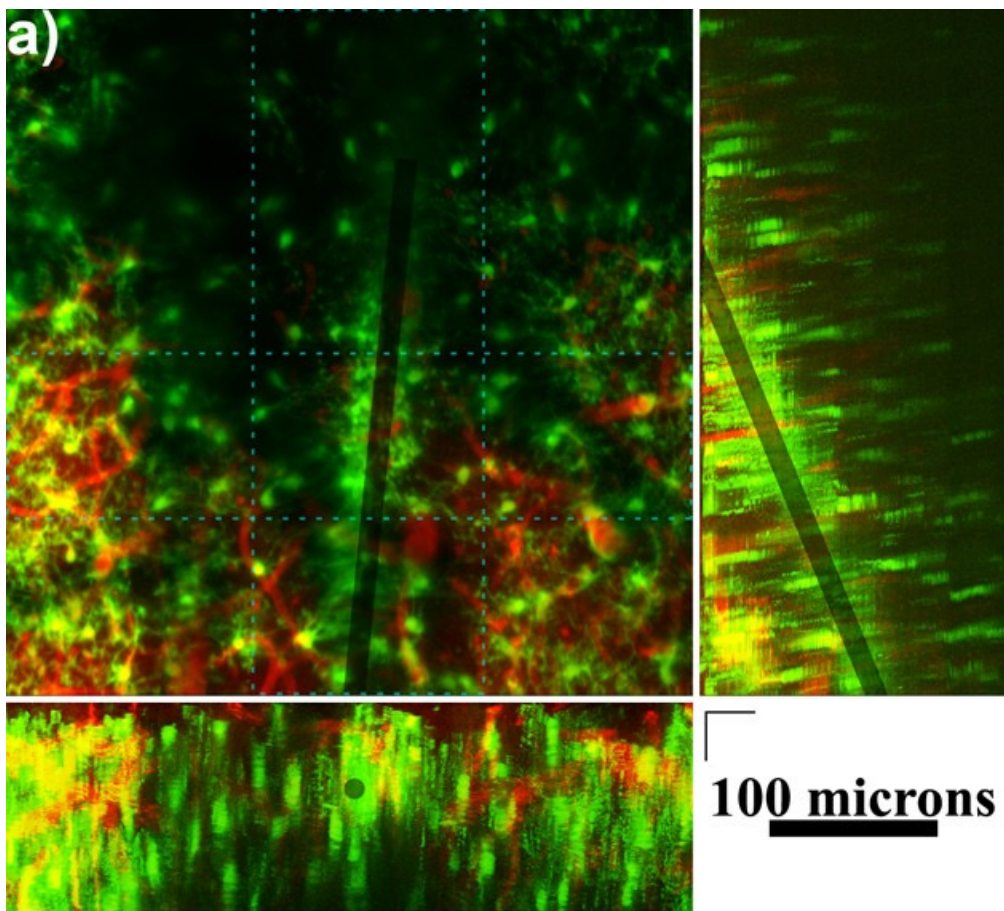
5. Tissue Response to Implanted Sensors Prevents Long-Term Use



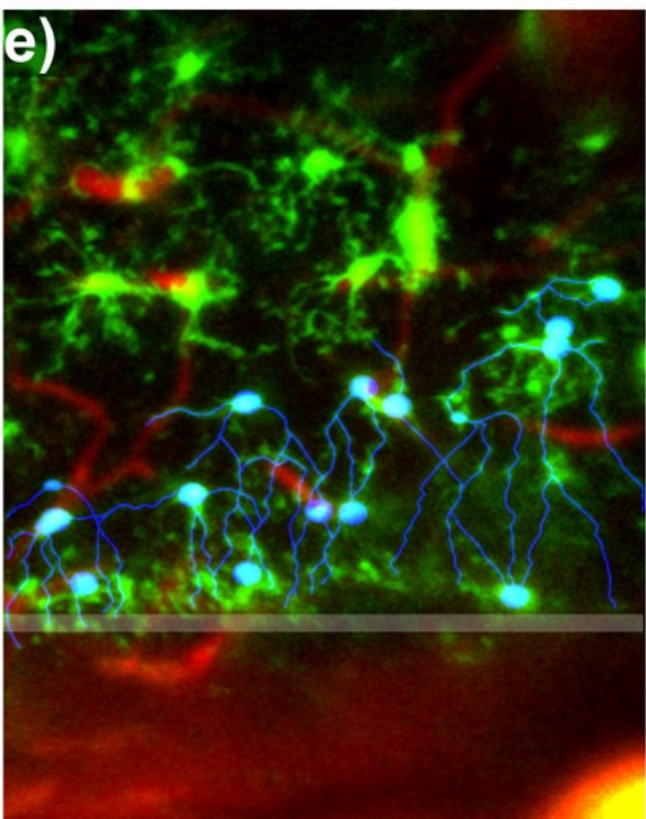
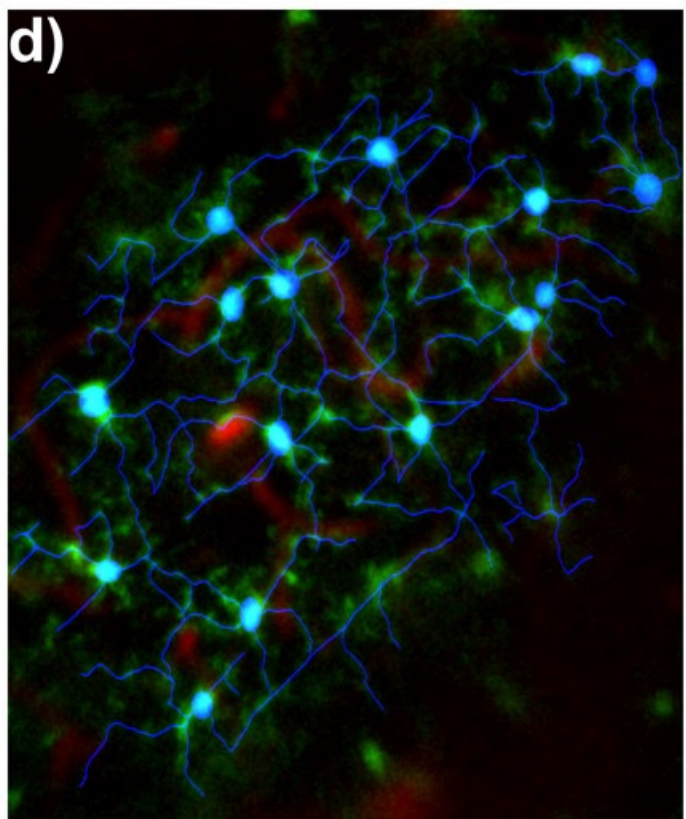
Size Matters: Miniaturization of Sensors

Microglia activation hours after implantation

Carbon fiber
electrodes
(20-60 μm^2)

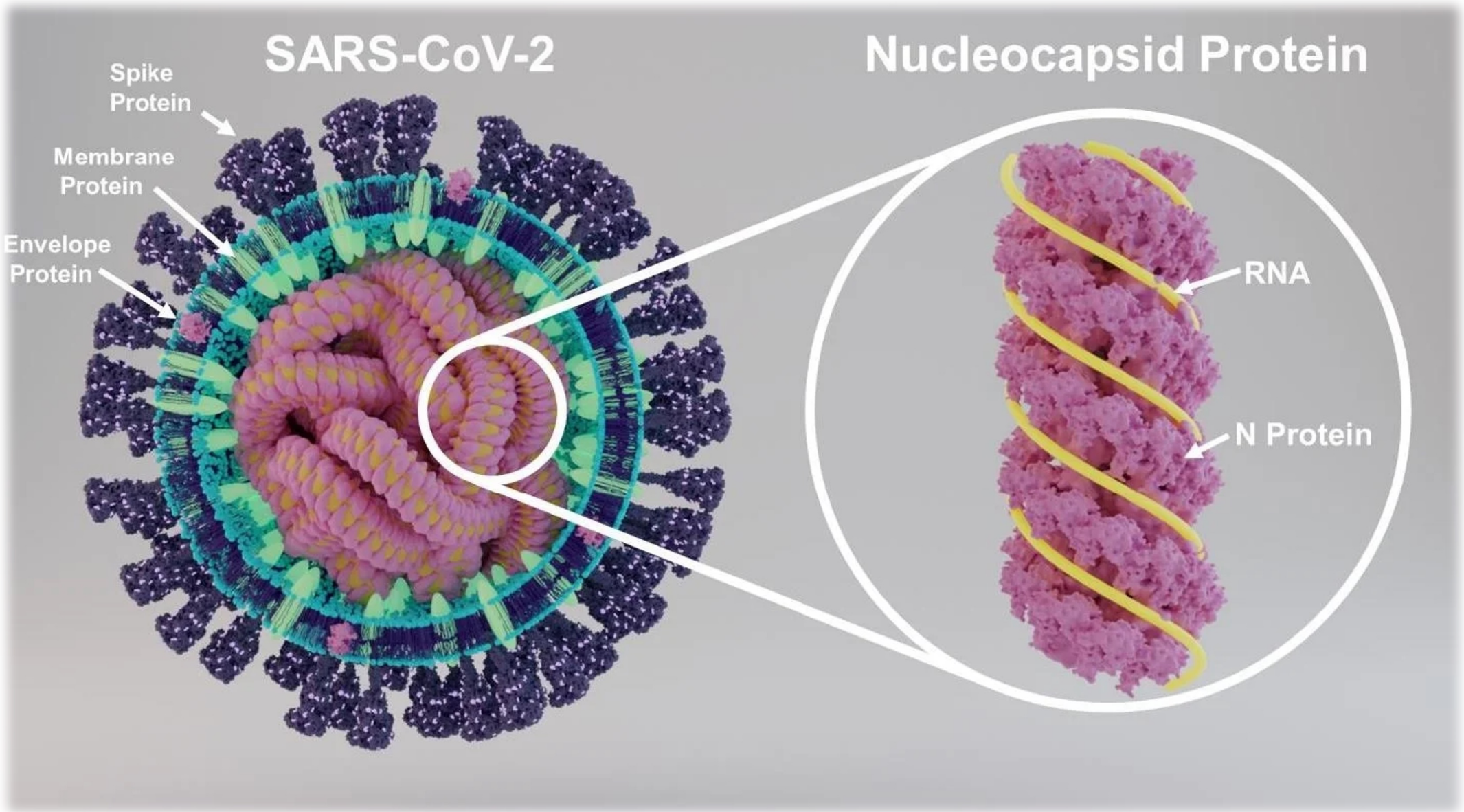
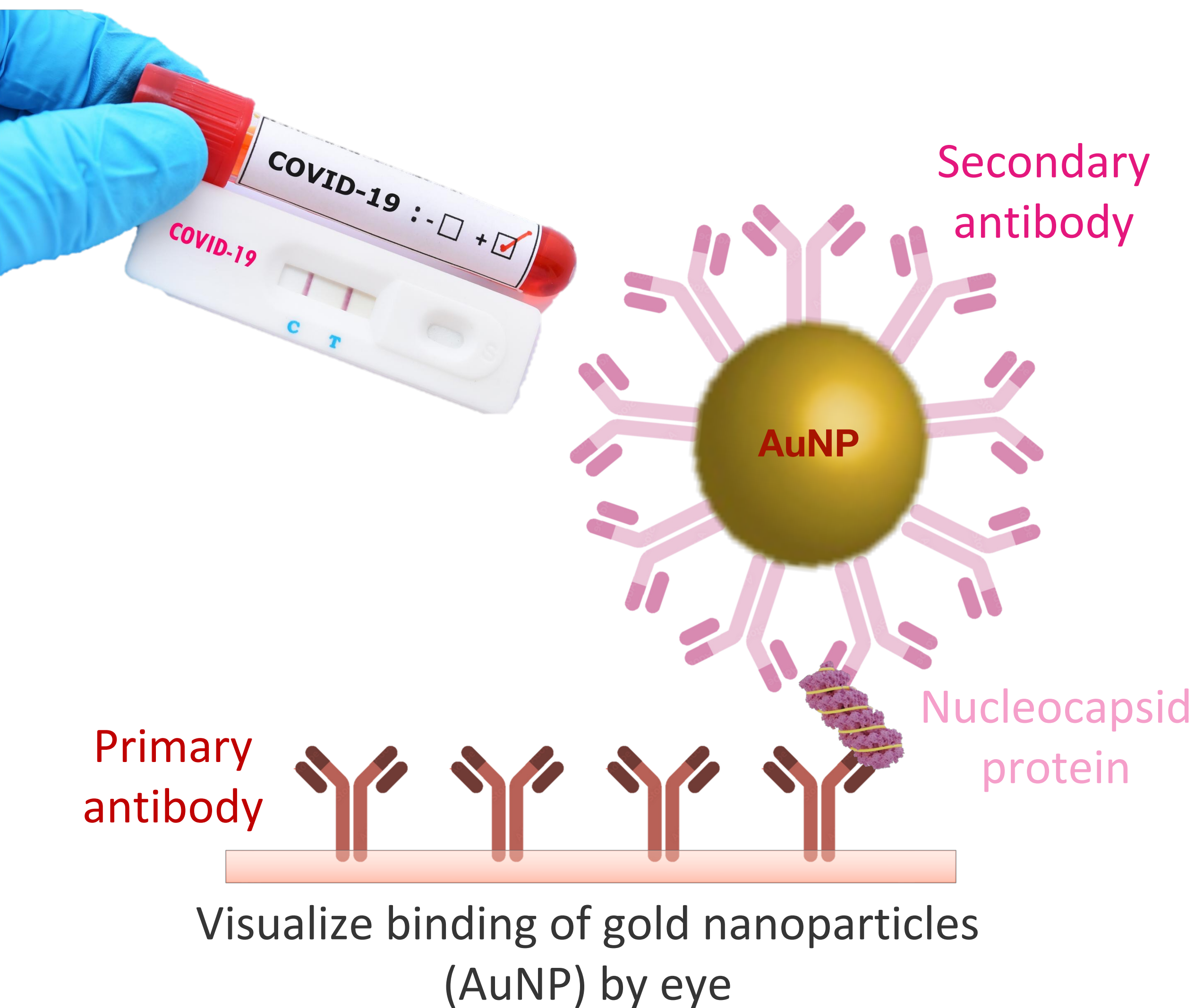


Microdialysis
probes
(30,000-70,000 μm^2)



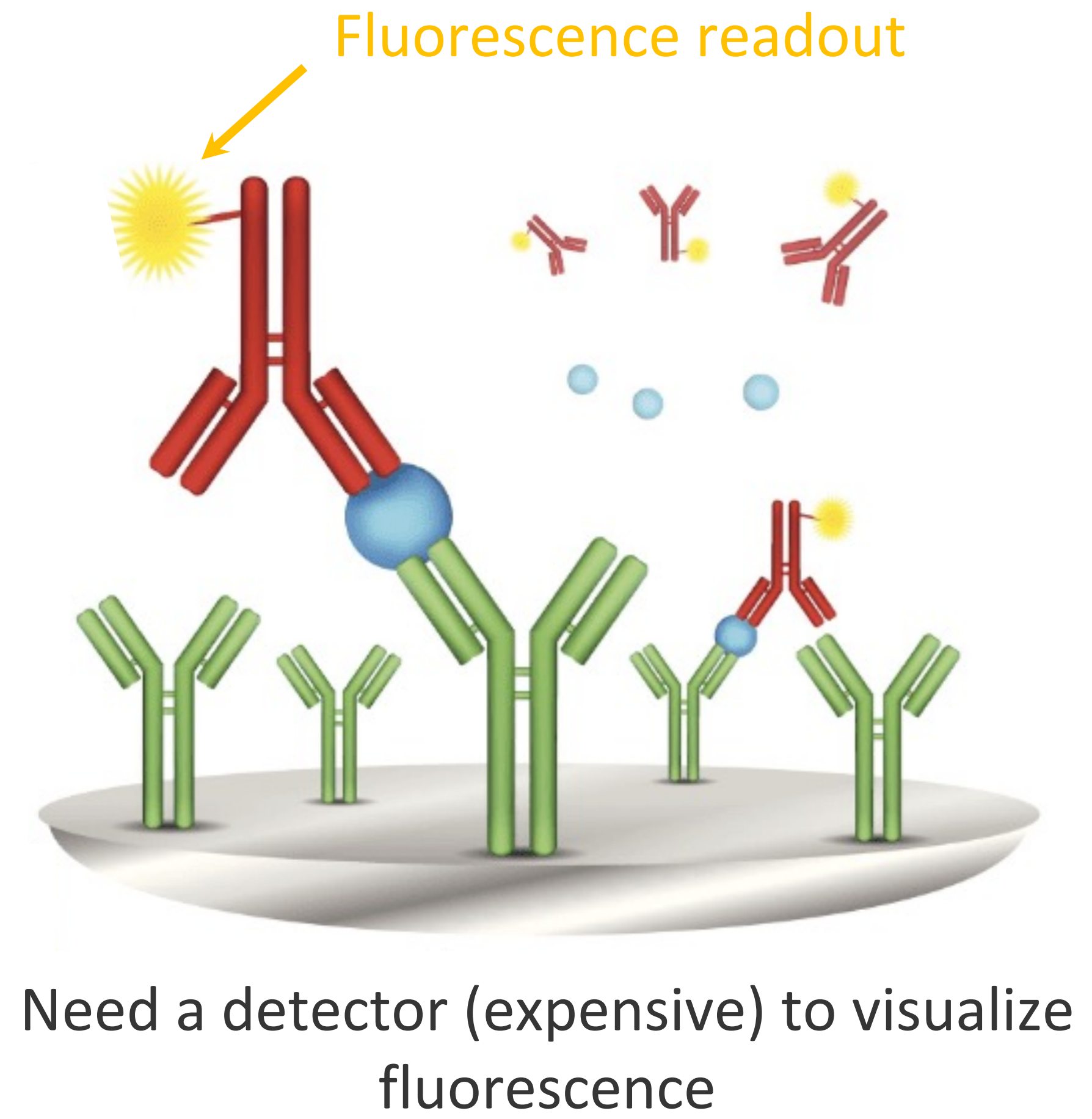
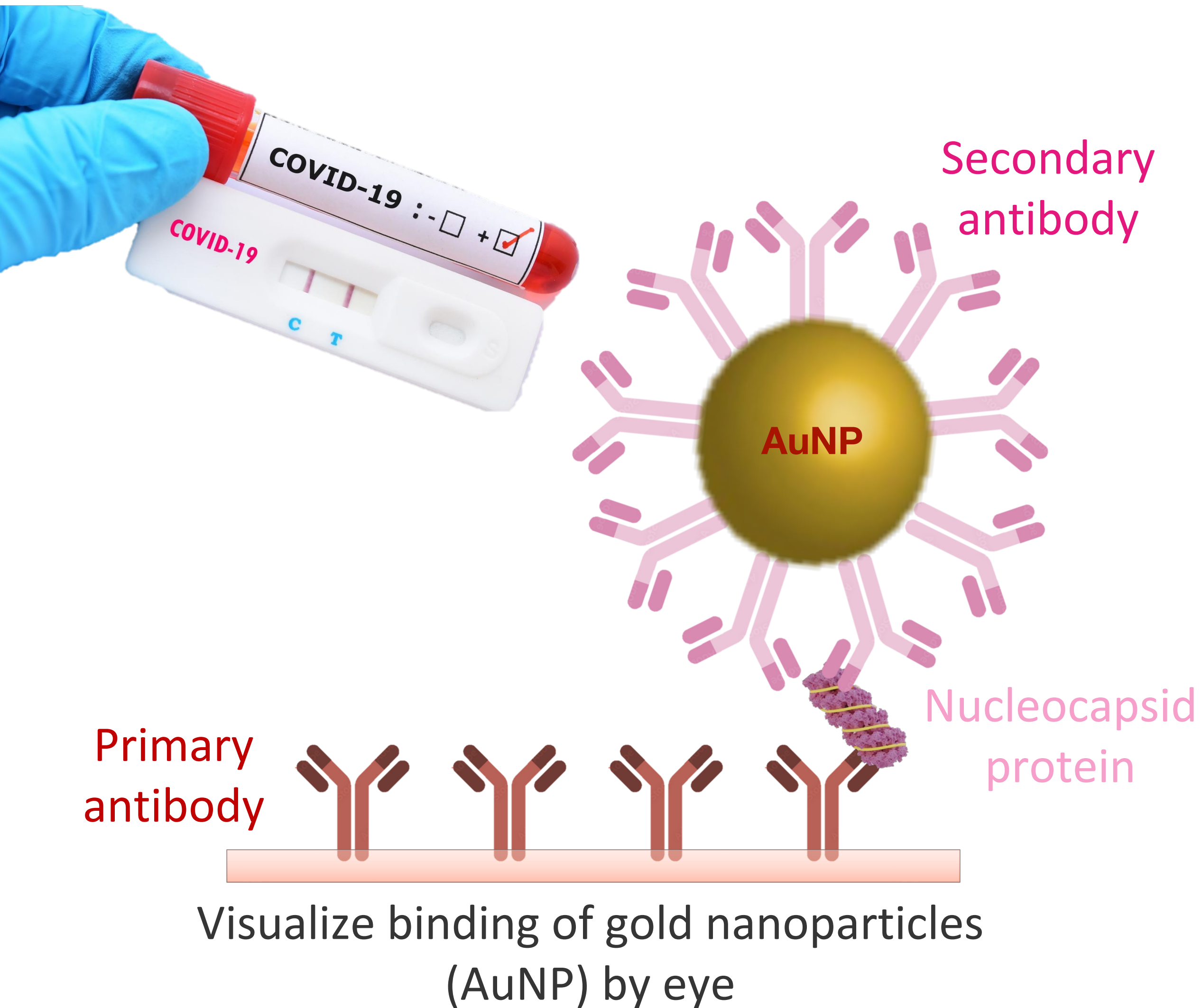
Planar silicon
electrodes
(3000-15,000 μm^2)

Let's Go Back to The COVID-19 Test: Antibody Sandwich Assays

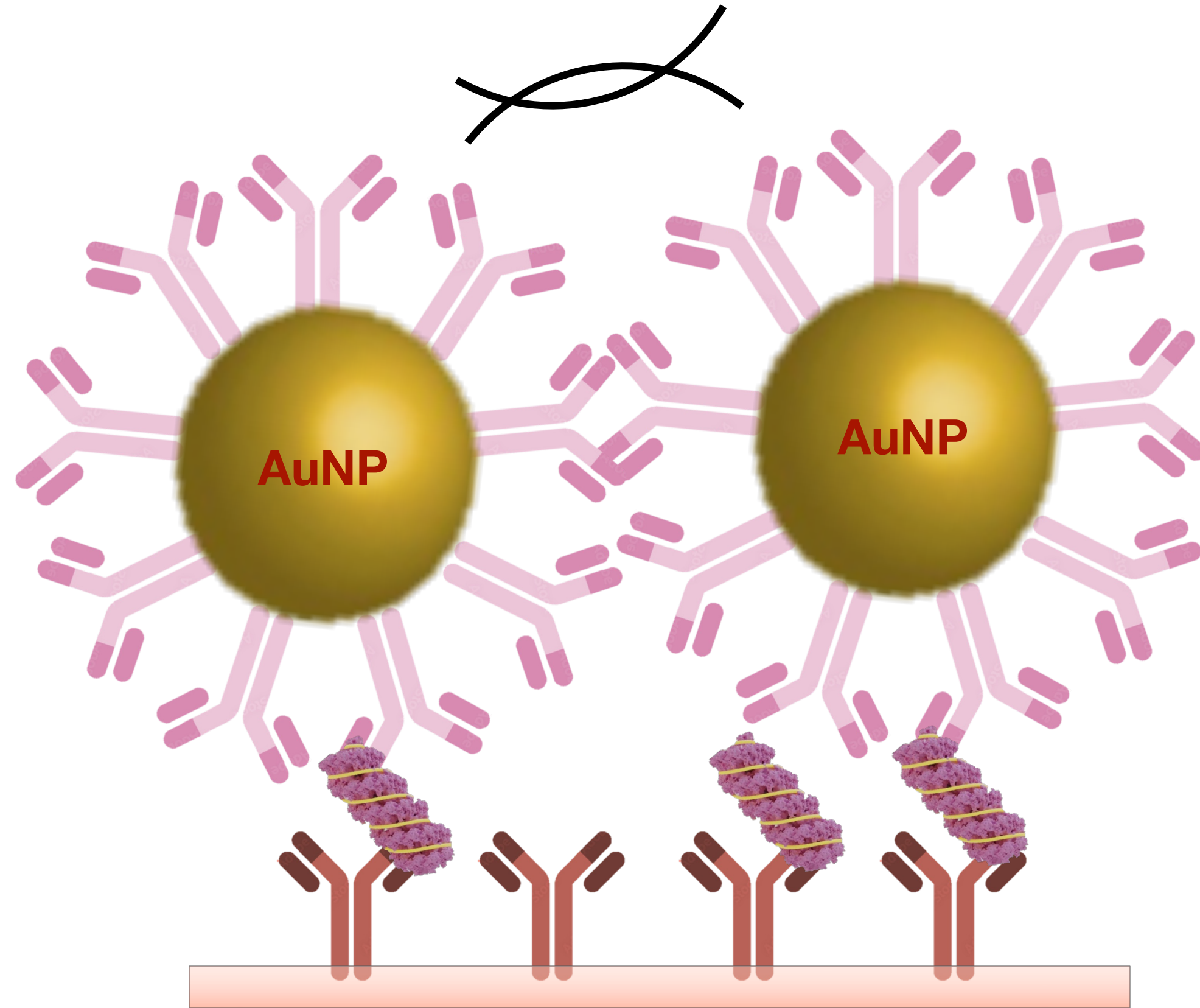


Nucleocapsid protein in high abundance and stable in nasal swabs

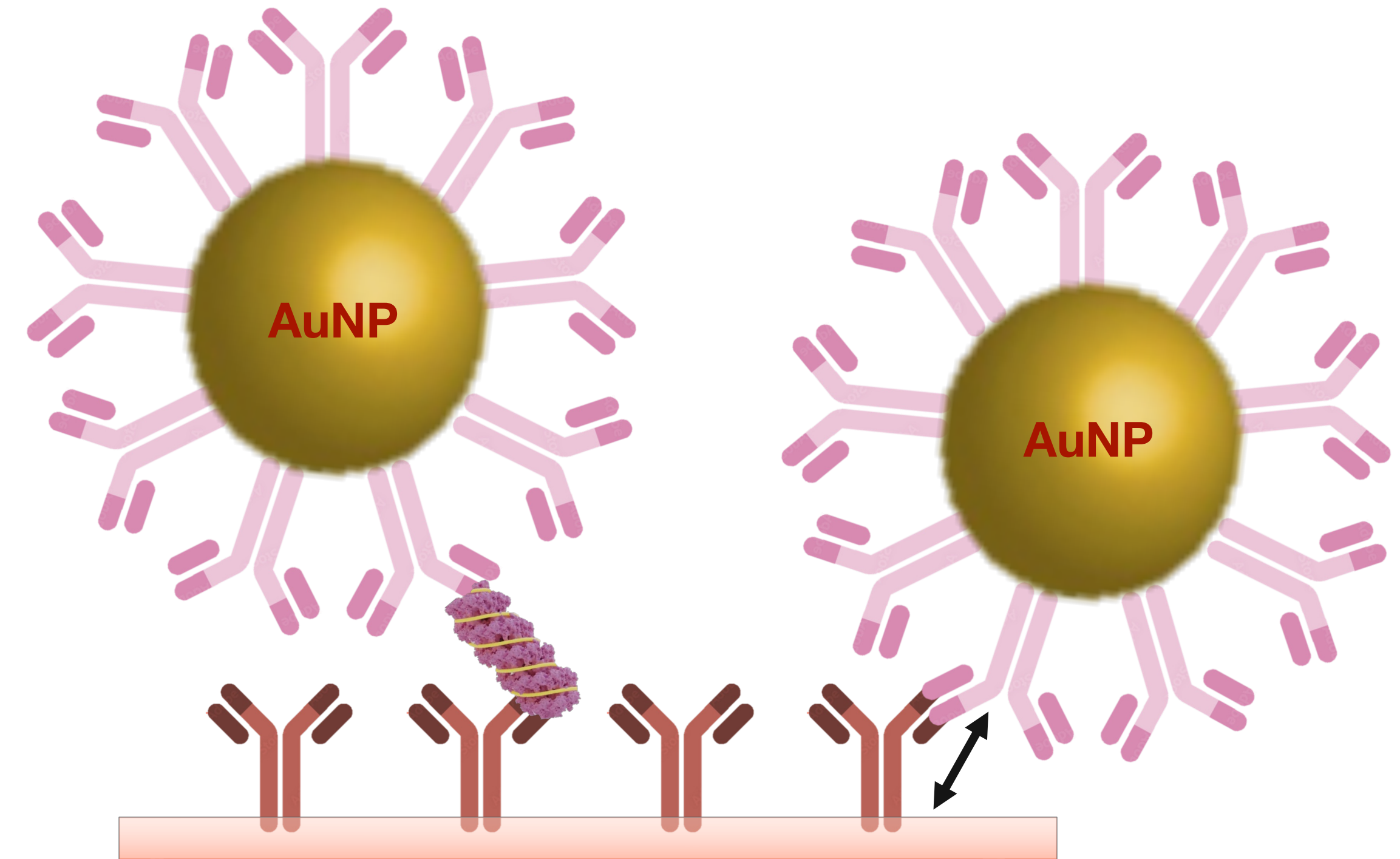
Antibody Sandwich Assays are Commonly Used in Biosensors



Challenges of Antibody Sandwich Assays

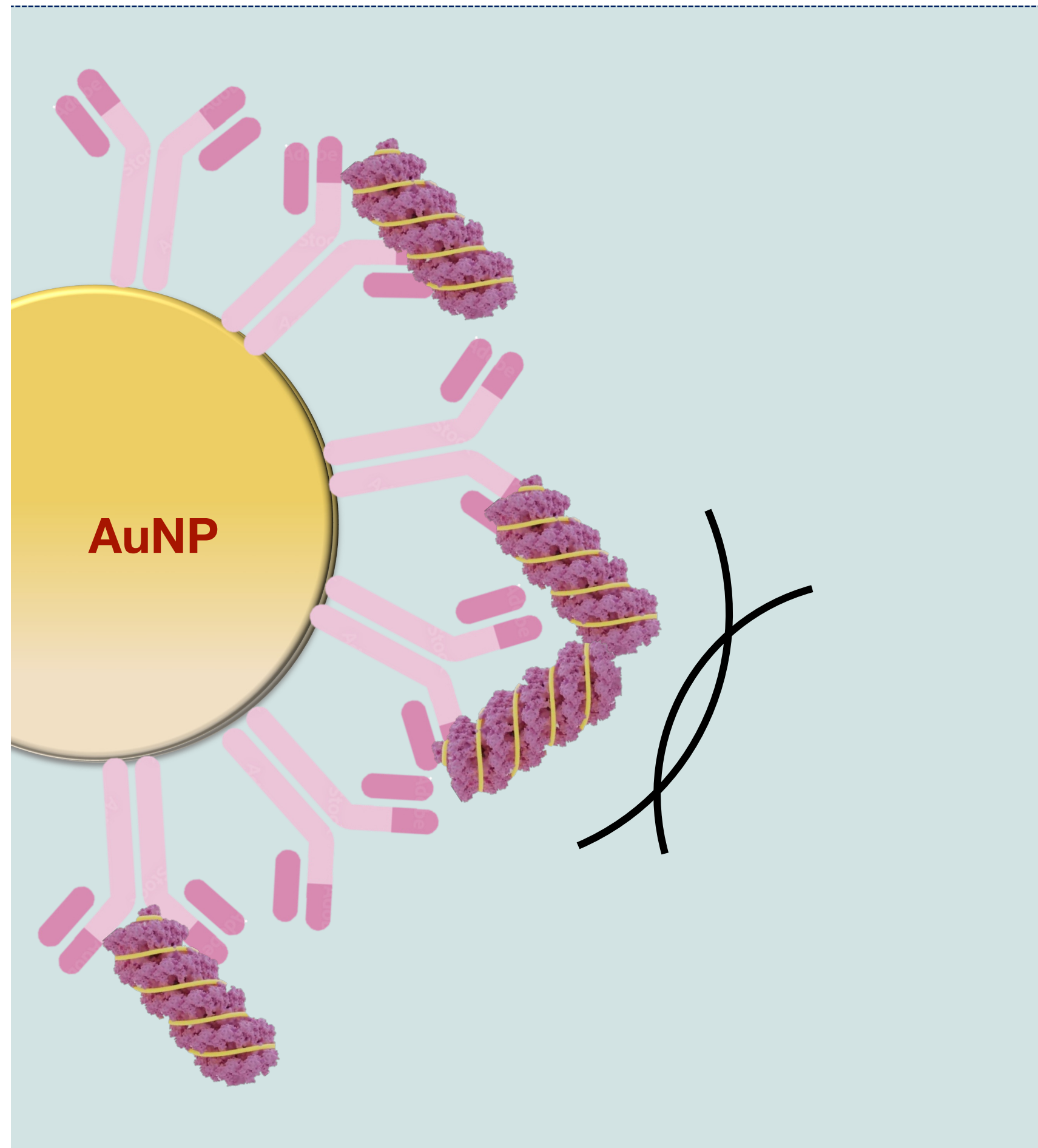


Steric hindrance at the surface
Density of bioreceptors need to be optimized
False negatives (not enough AuNPs bound)

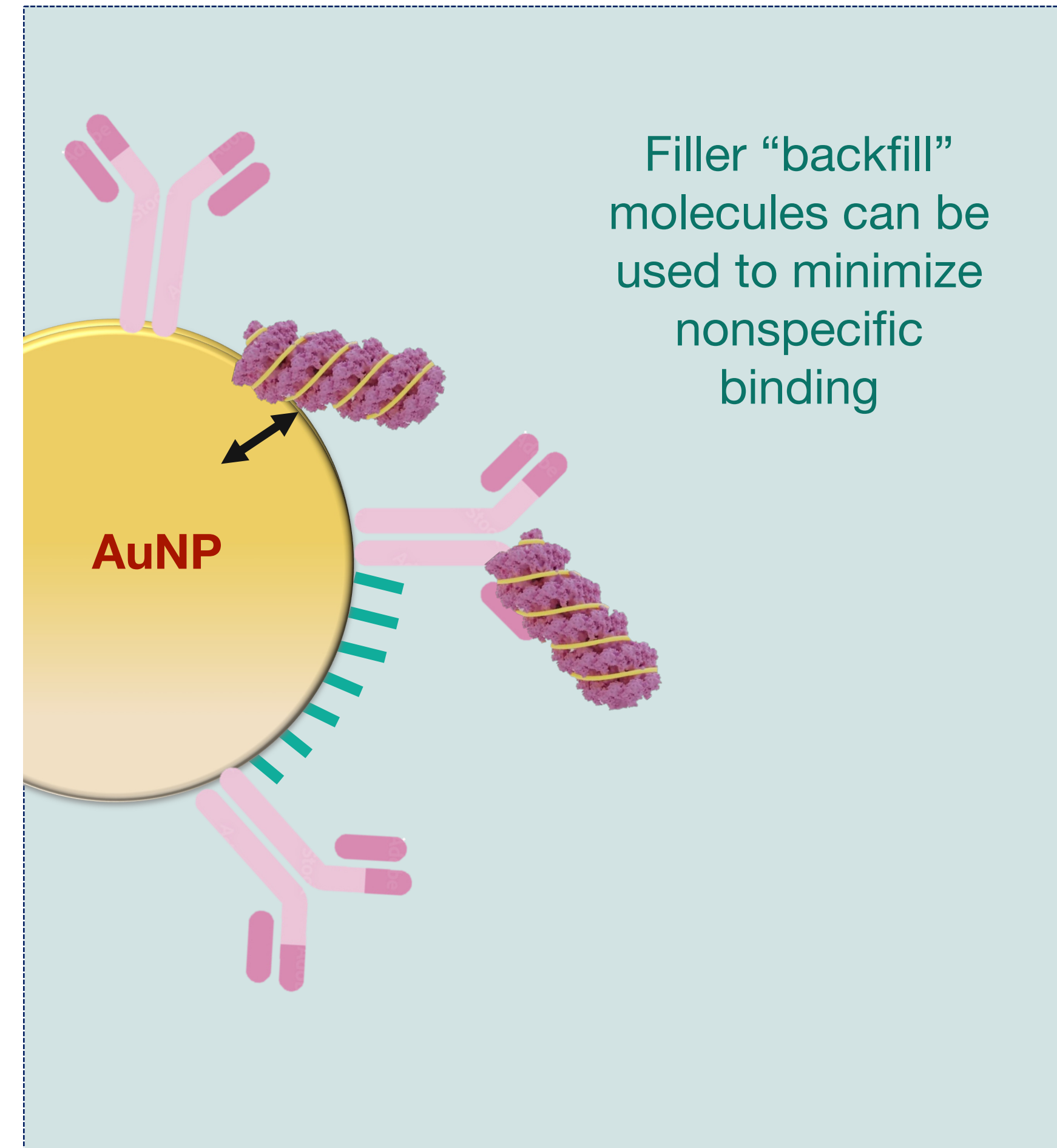


Nonspecific sticking of the gold nanoparticles
To the surface, to primary antibodies, *etc.*
False positives (too many AuNPs bound)

The COVID-19 Test Uses and Antibody Sandwich Assay

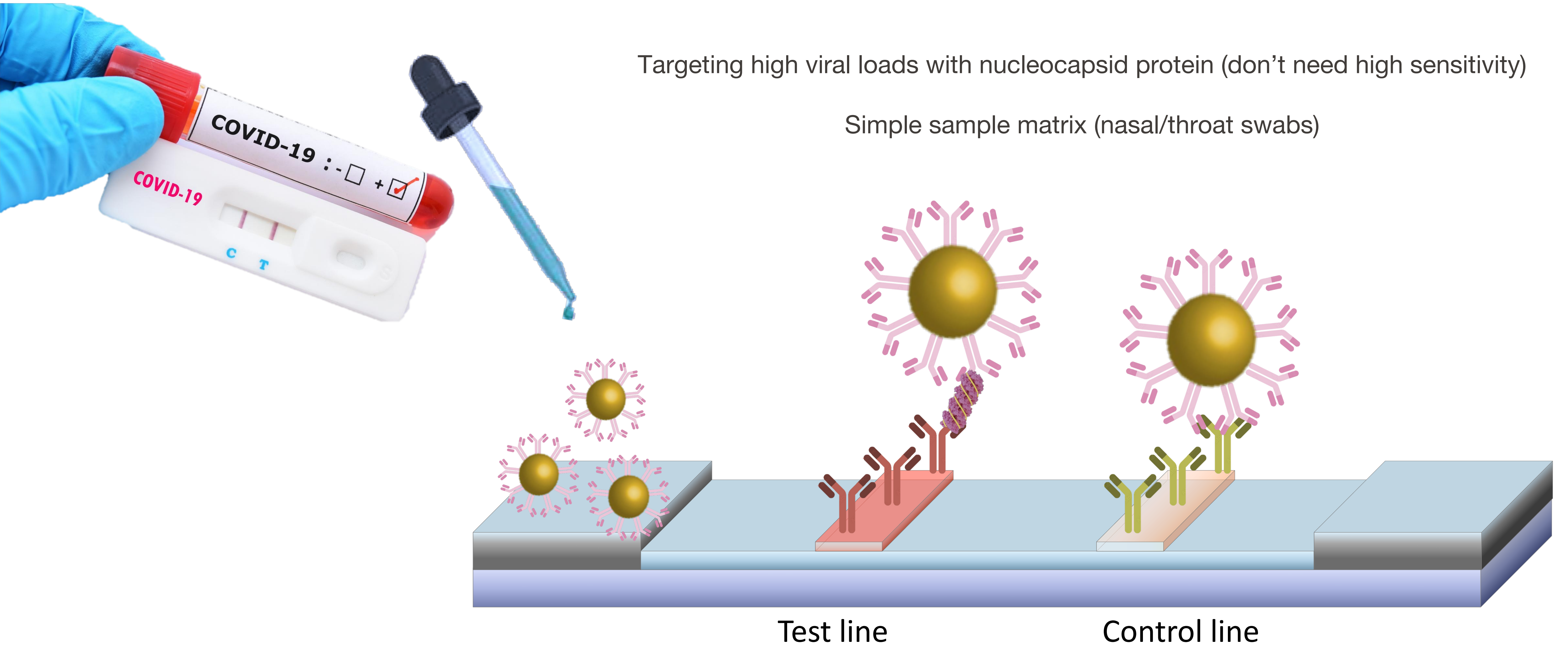


Steric hindrance at the surface



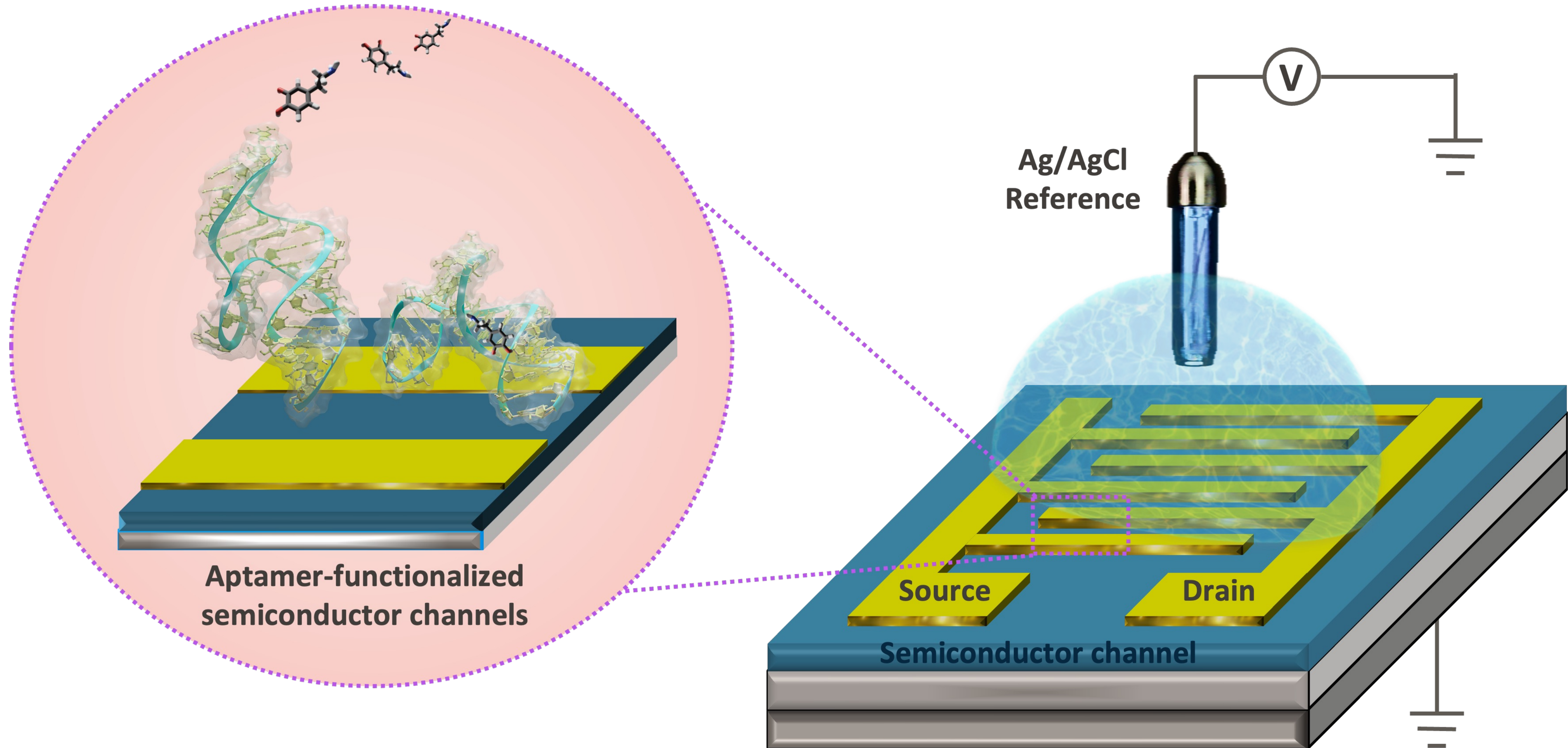
Nonspecific sticking to gold nanoparticles

Lateral Flow Assay Optimized to Detect COVID-19



Yes/No test – not quantitative!

Next Week: DNA Aptamers for Biosensing on Semiconductors



Nakatsuka *et al.*, *Science*, 362, 319, 2018

Nakatsuka, *Aptamer Technology Edition, Methods Mol. Biol.*, 2570, 187, 2022

Summary of Today's Class

- **Plasmonic nanoparticles** – short recap on plasmon resonance used for rapid tests!
- **What is a Biosensor** – molecular recognition and performance parameters
- **History of Biosensors** – Clark electrode and recap of electrochemistry
- **State-of-the-art Biosensor: Glucose Sensor** – recap of electrolysis
- **Remaining challenges of Biosensors**
- **How the COVID-19 rapid test works**

Exercise Session: Real-world applications of biosensors for human health (glucose and neurotransmitters)