

Introduction to Biosensing

Lesson 12

MSE 304

Nako Nakatsuka

Nako.Nakatsuka@epfl.ch

December 5th 2025



Plan of the Course: Fundamentals, Characterization, and Applications

1: Intro to Surfaces & Interfaces

2: Surfaces in the Real World - Adsorption

3: Surface Energetics & Interfacial Phenomena

4: Atomic Structure of Real Surfaces

5: Solid-Solid Interfaces

6: From Ideal Planes to Real Materials (Recap)

7: Characterization of Surfaces & Interfaces

8: Surface Chemistry

9: Surface Patterning and Polymer Chemistry

10: Probing Functional Interfaces

11: Surface Phenomena at the Nanoscale

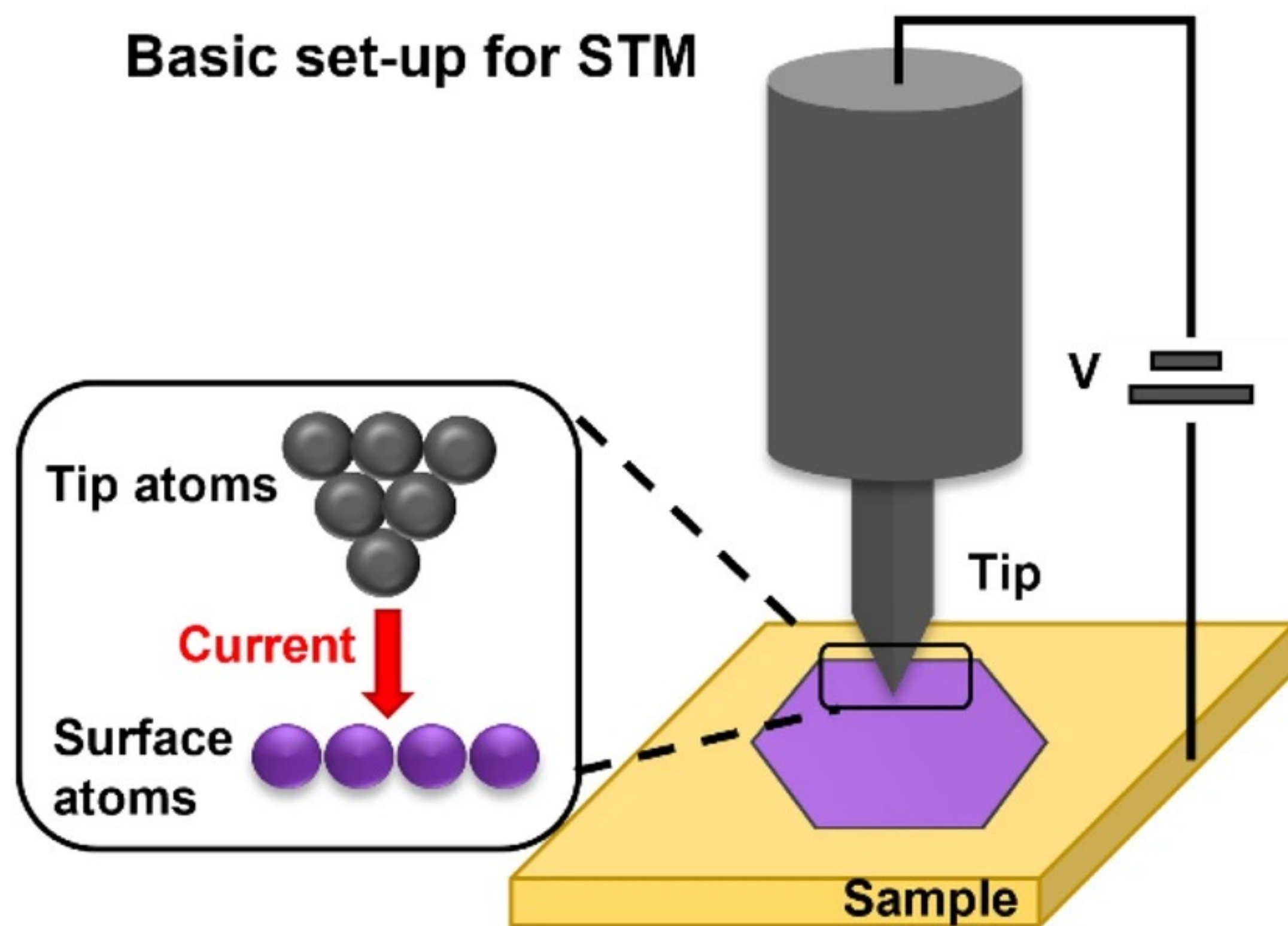
12: Introduction to Biosensing

13: Biosensing applications

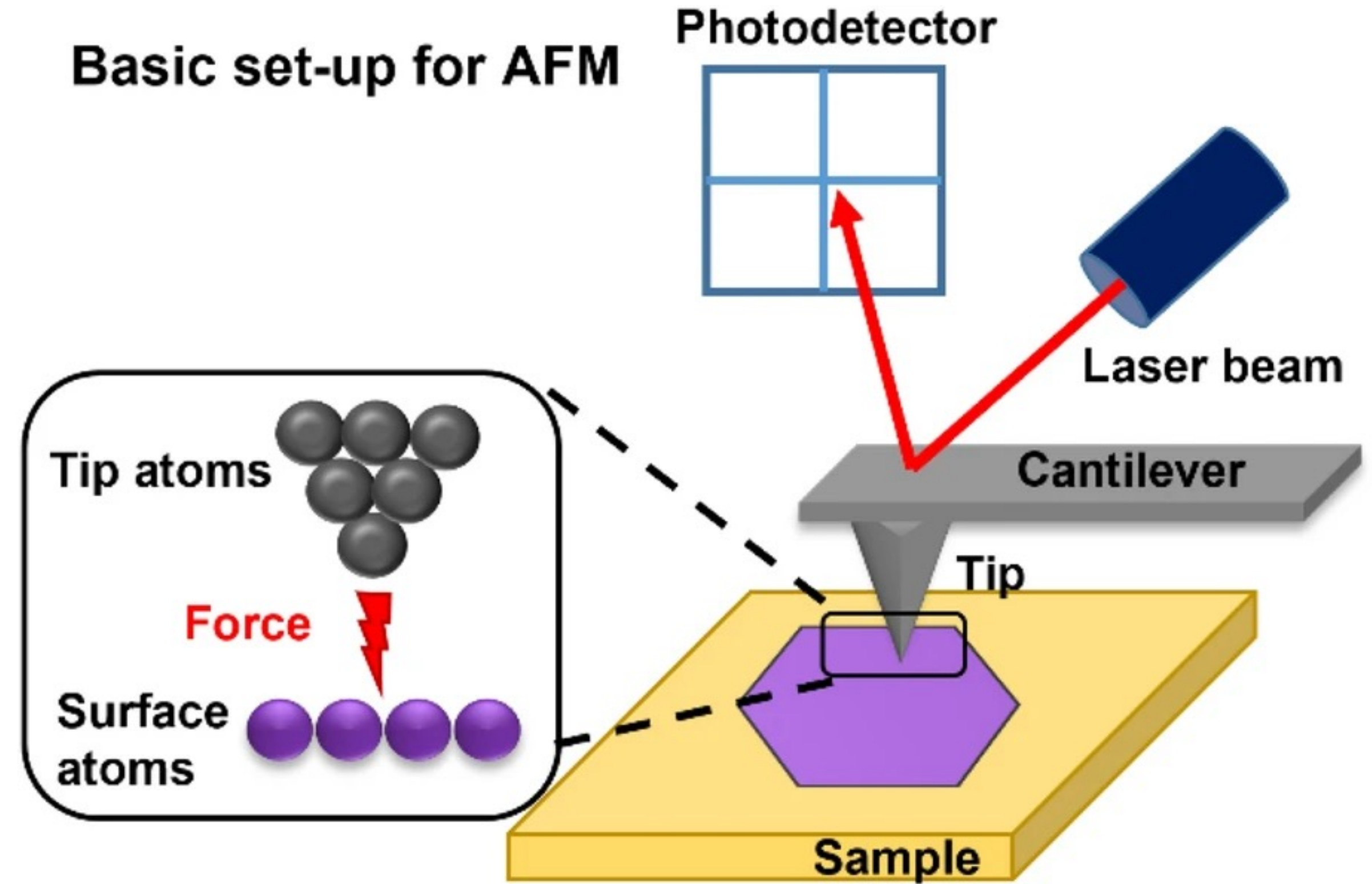
14: Chemistry of Semiconductor Surfaces & Beyond



Recap: STM vs. AFM for Nanoscale Topography

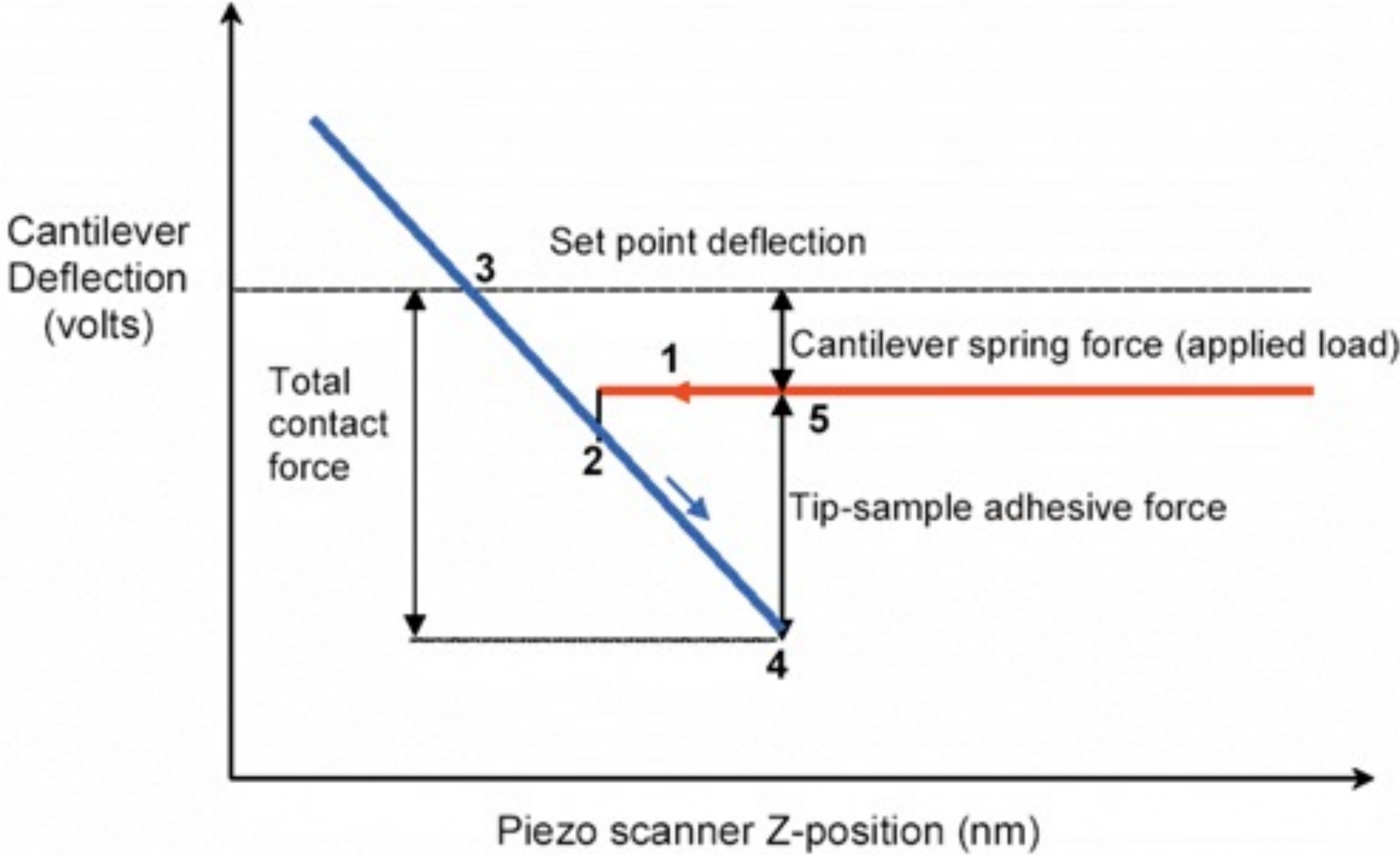
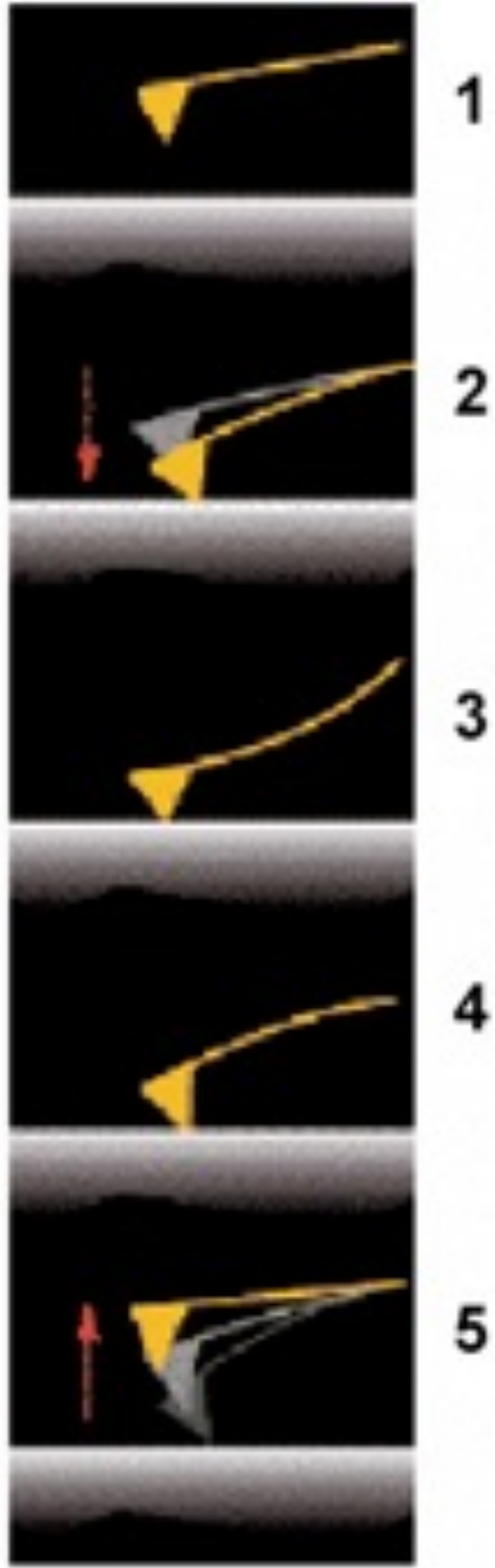


Electrons tunnel through ~ 1 nm gap between sharp metal tip and conductive surface

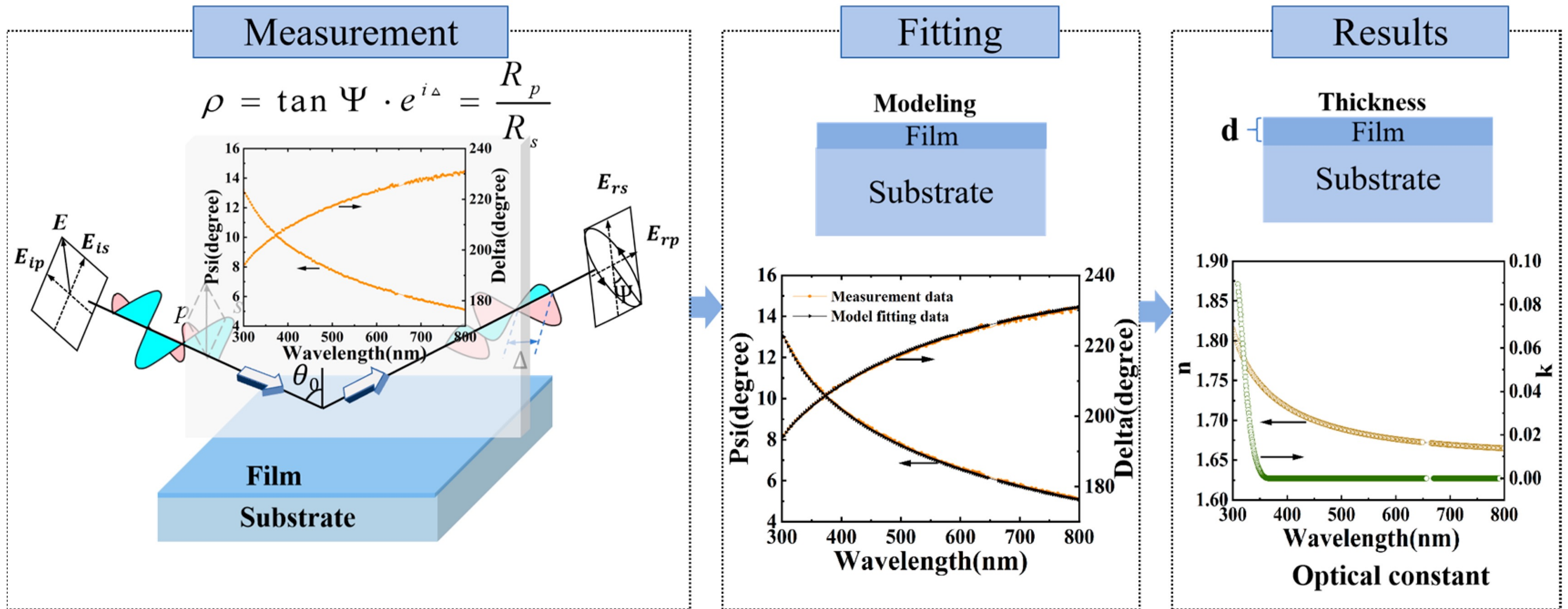


Sharp tip on a flexible cantilever senses attractive and repulsive forces as it scans the surface

Recap: Force-Distance Curve for Mechanical Measurements



Recap: Ellipsometry – From Measurement to Results



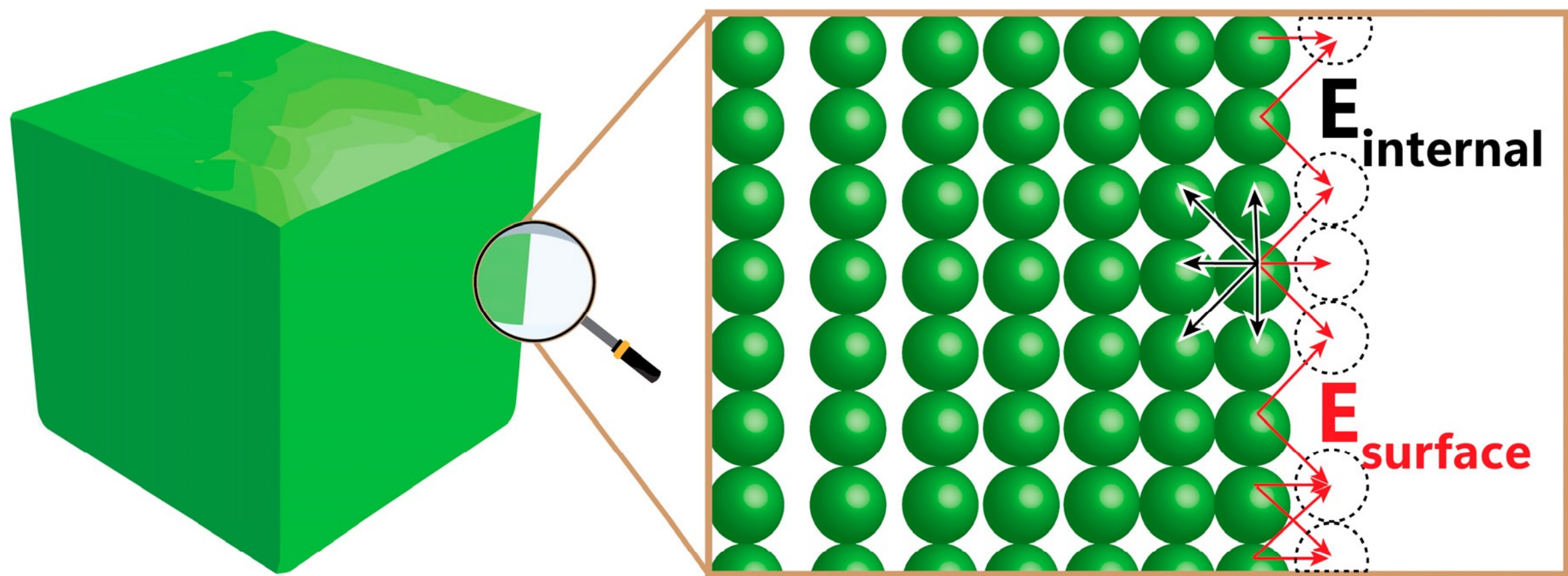
Measures how reflection changes the polarization to extract Ψ and Δ

Measured values Ψ and Δ compared to model

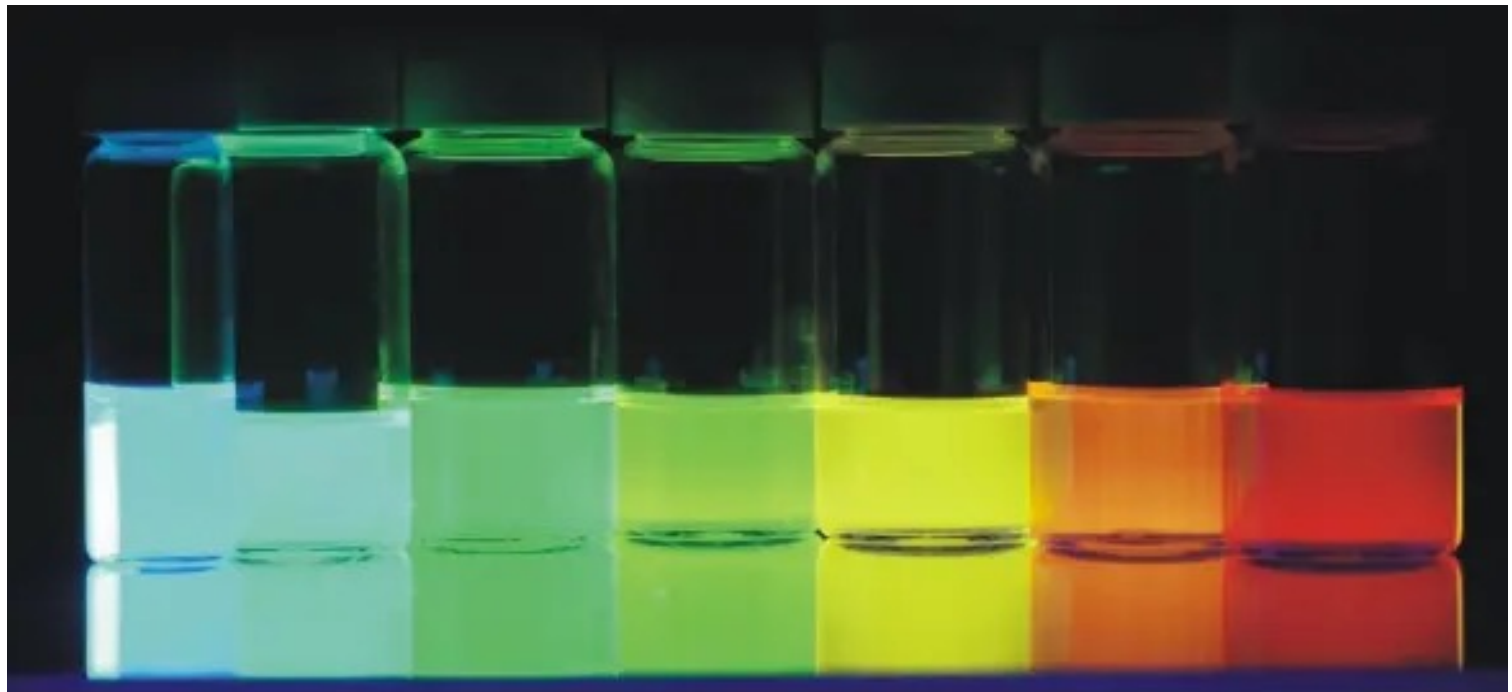
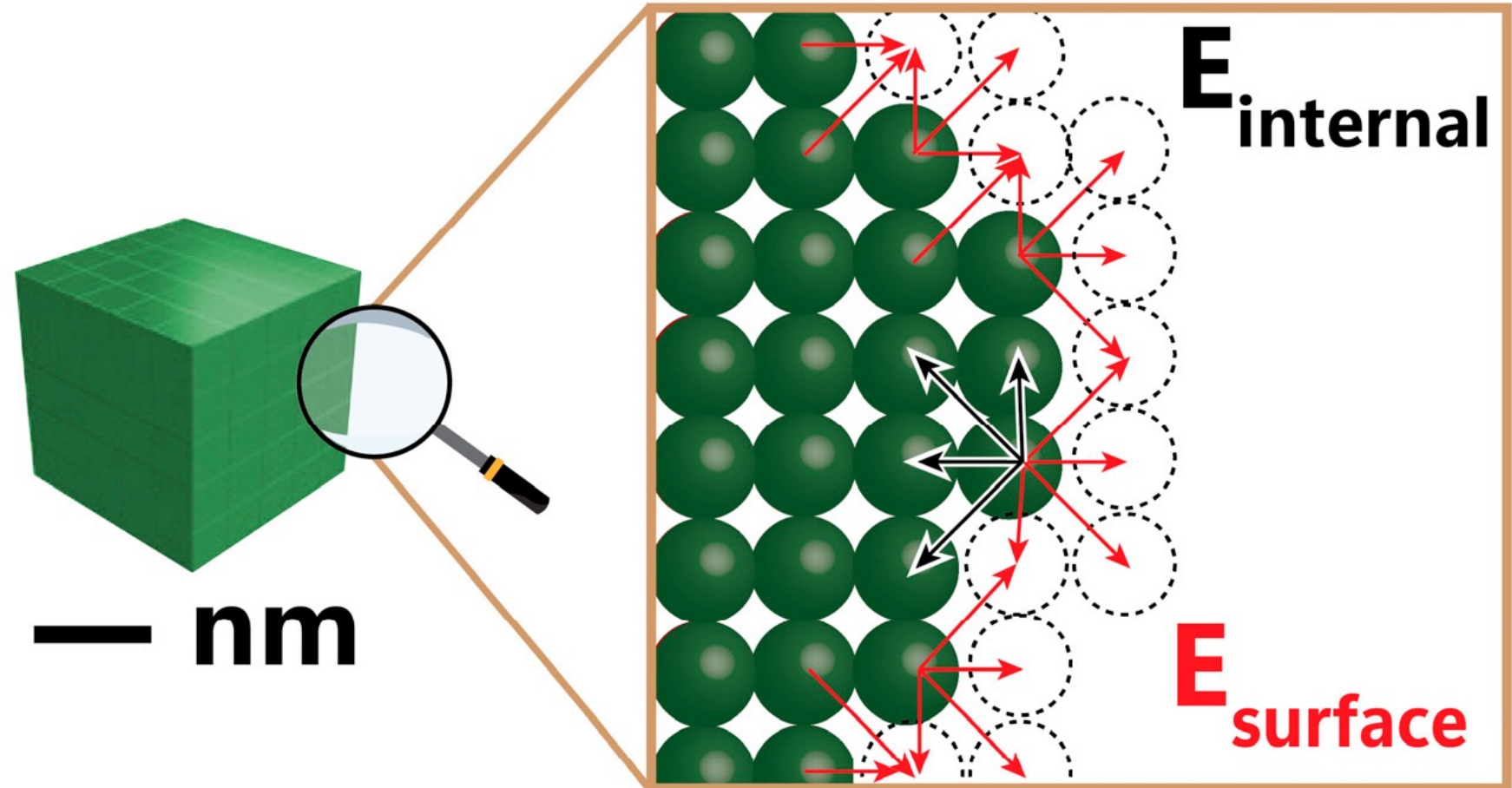
Film thickness and optical constants (n, k) extracted

Recap from Lesson 11: Nanoscale Phenomena

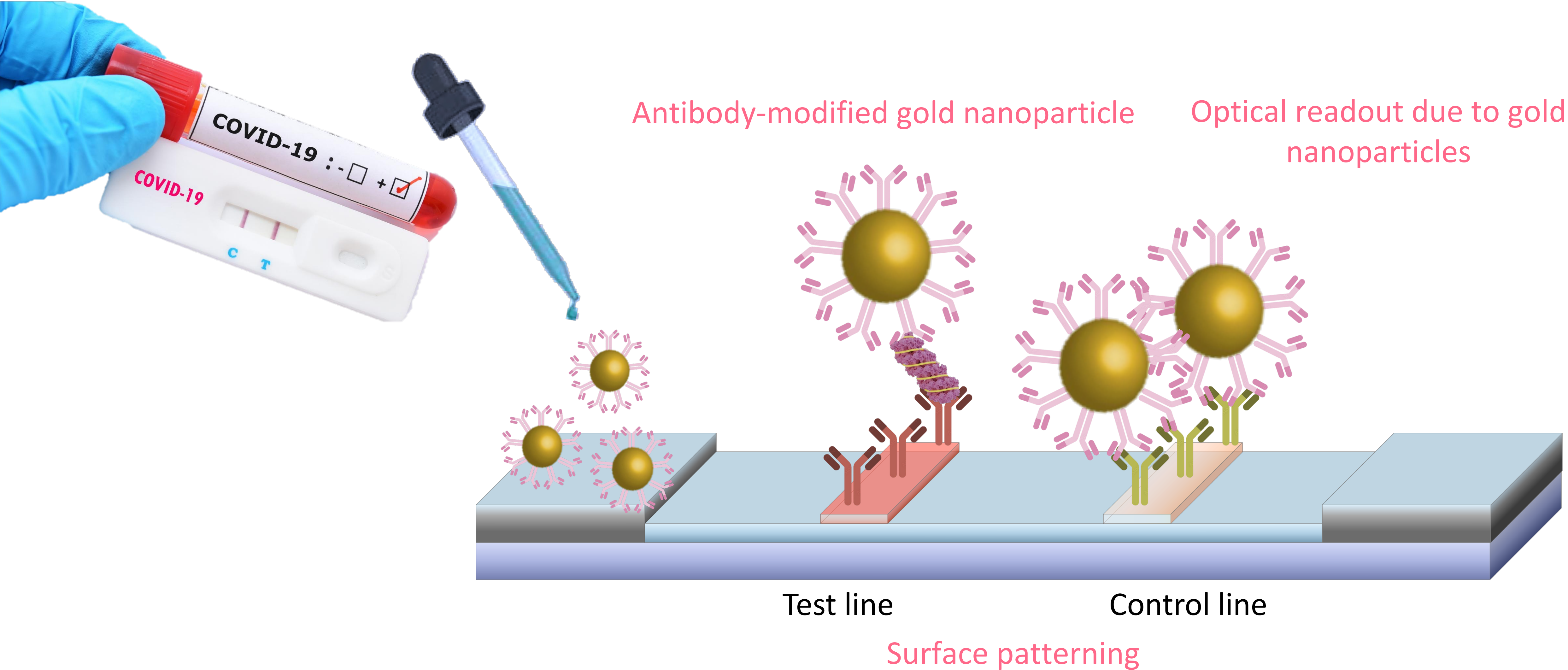
Macroscale



Nanoscale

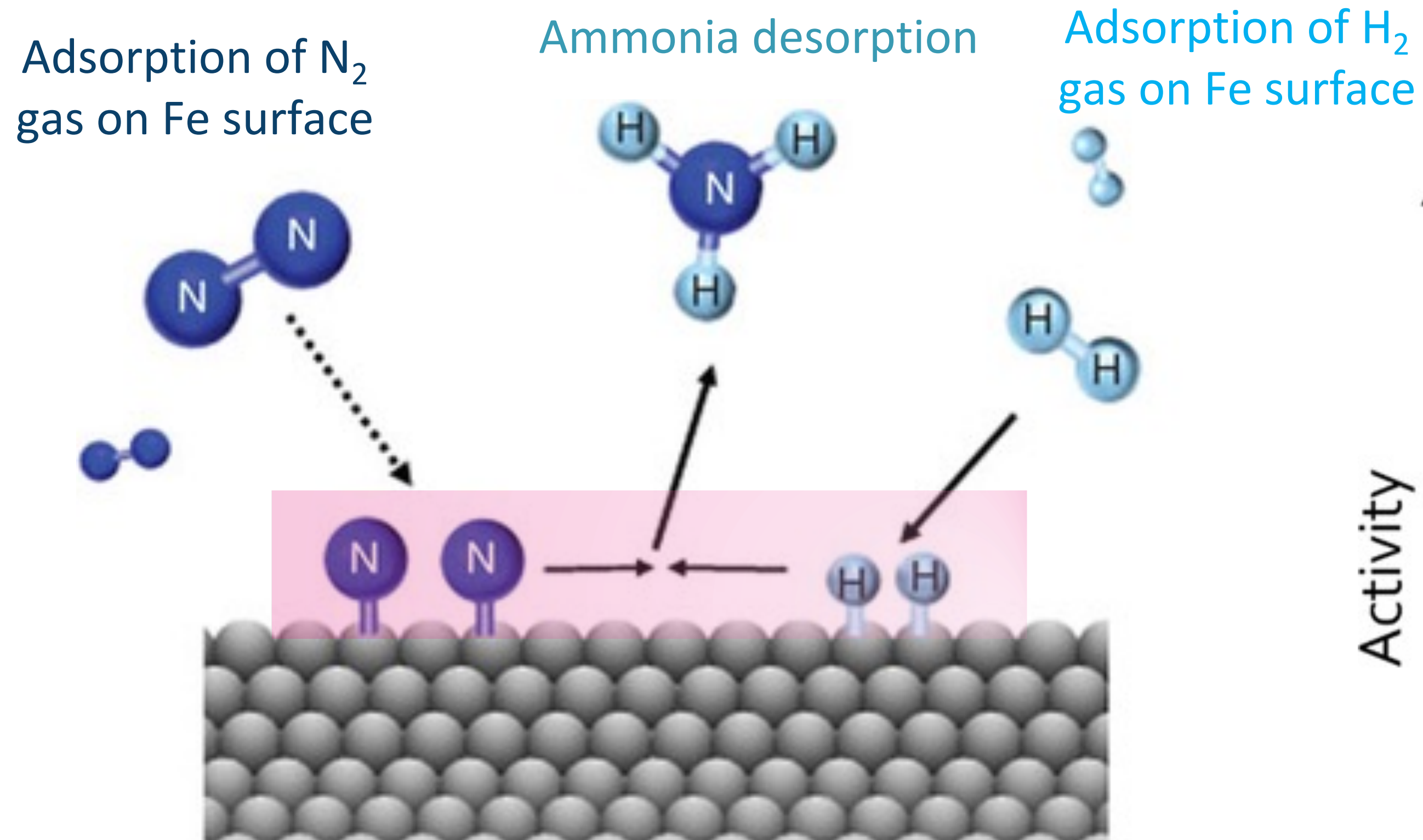


COVID Rapid Tests – Gold Nanoparticles + Surface Chemistry



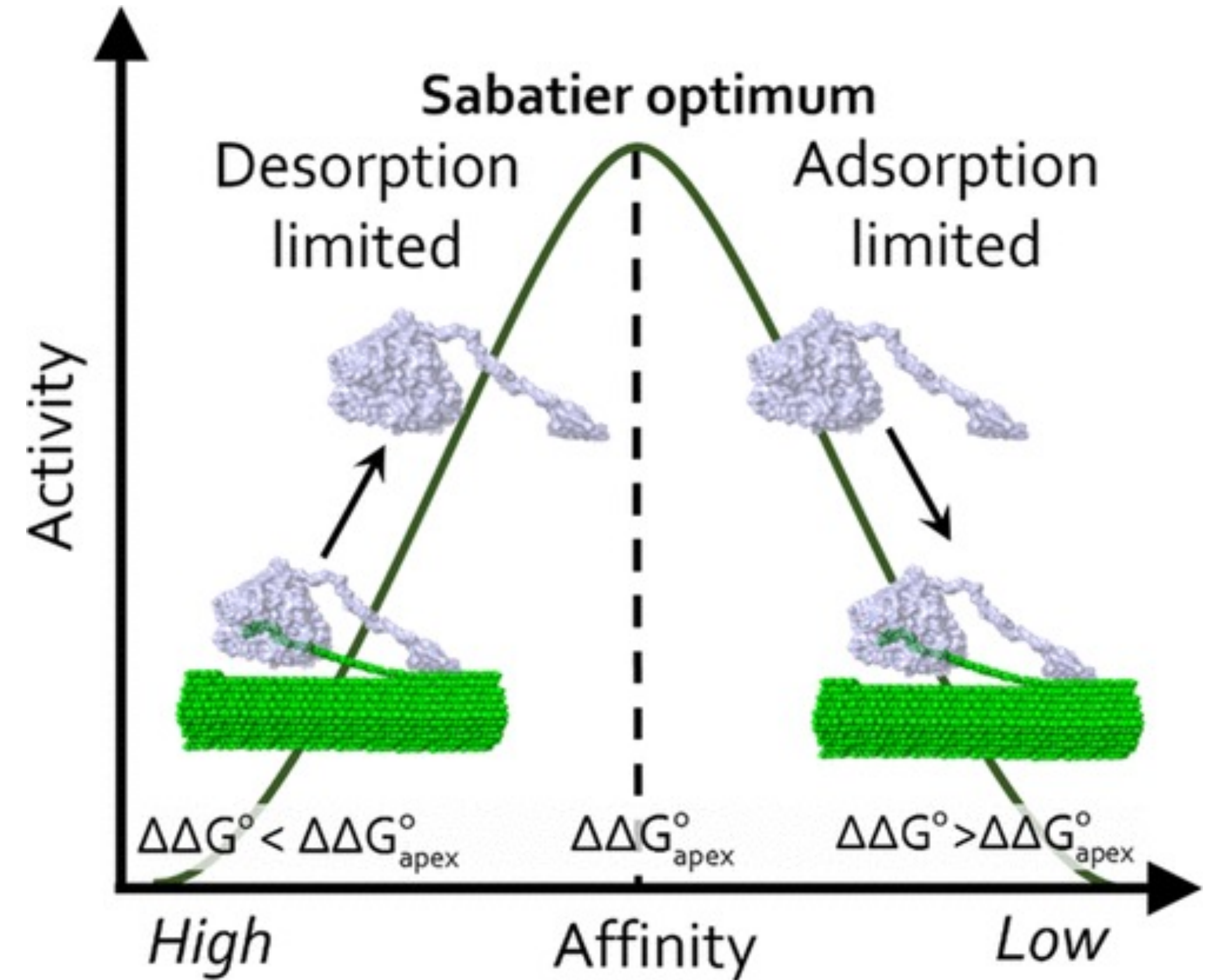
Bringing knowledge from different lectures together!

Recap from Lesson 11: Catalysis



Fe catalyst provides active surface that adsorbs molecules, weakening their bonds

Nitrogen atoms easier to dissociate from the surface

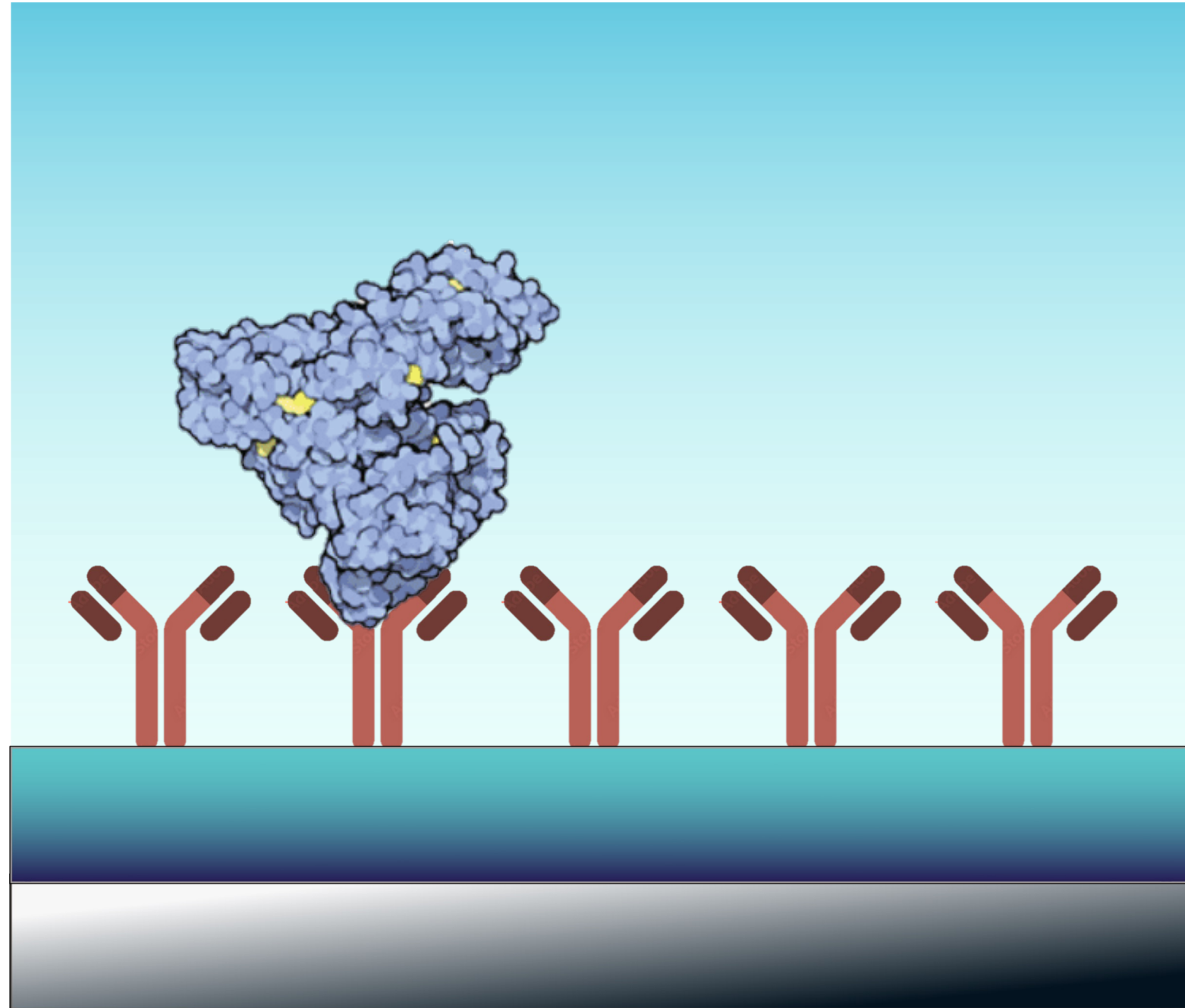


Outline of Lesson 12

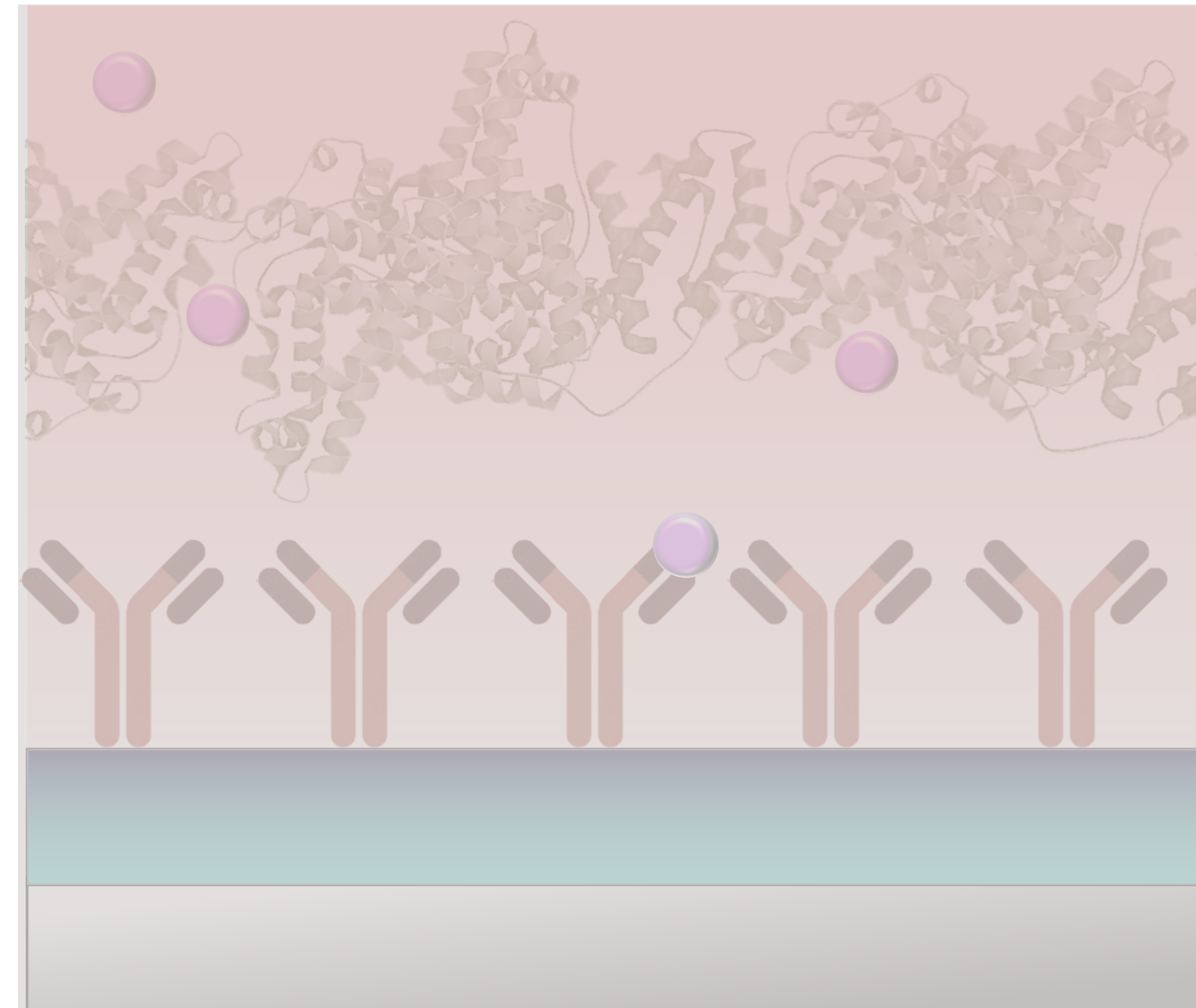
- Intro to biosensors – molecular recognition and bioreceptors
- How to transduce molecular recognition to measurable signals
- Glucose sensor (electrochemical and Raman spectroscopy)
- Understand how a COVID-19 rapid test works
- Recap of fundamentals: electrochemistry, electrolysis, electrolytic cell



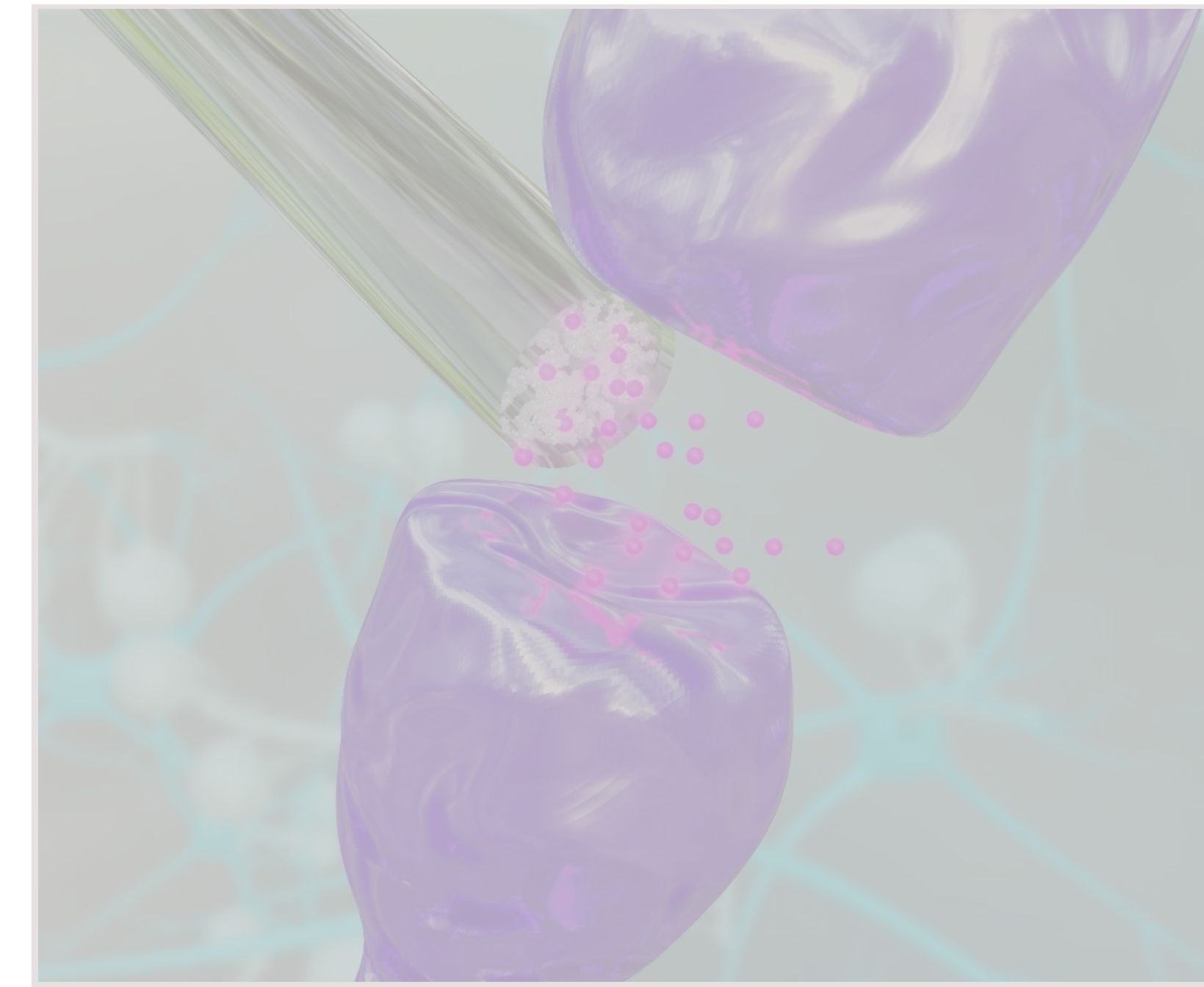
Topics We Cover in the Next Two Lectures on Biosensing



Fundamentals of Biosensors

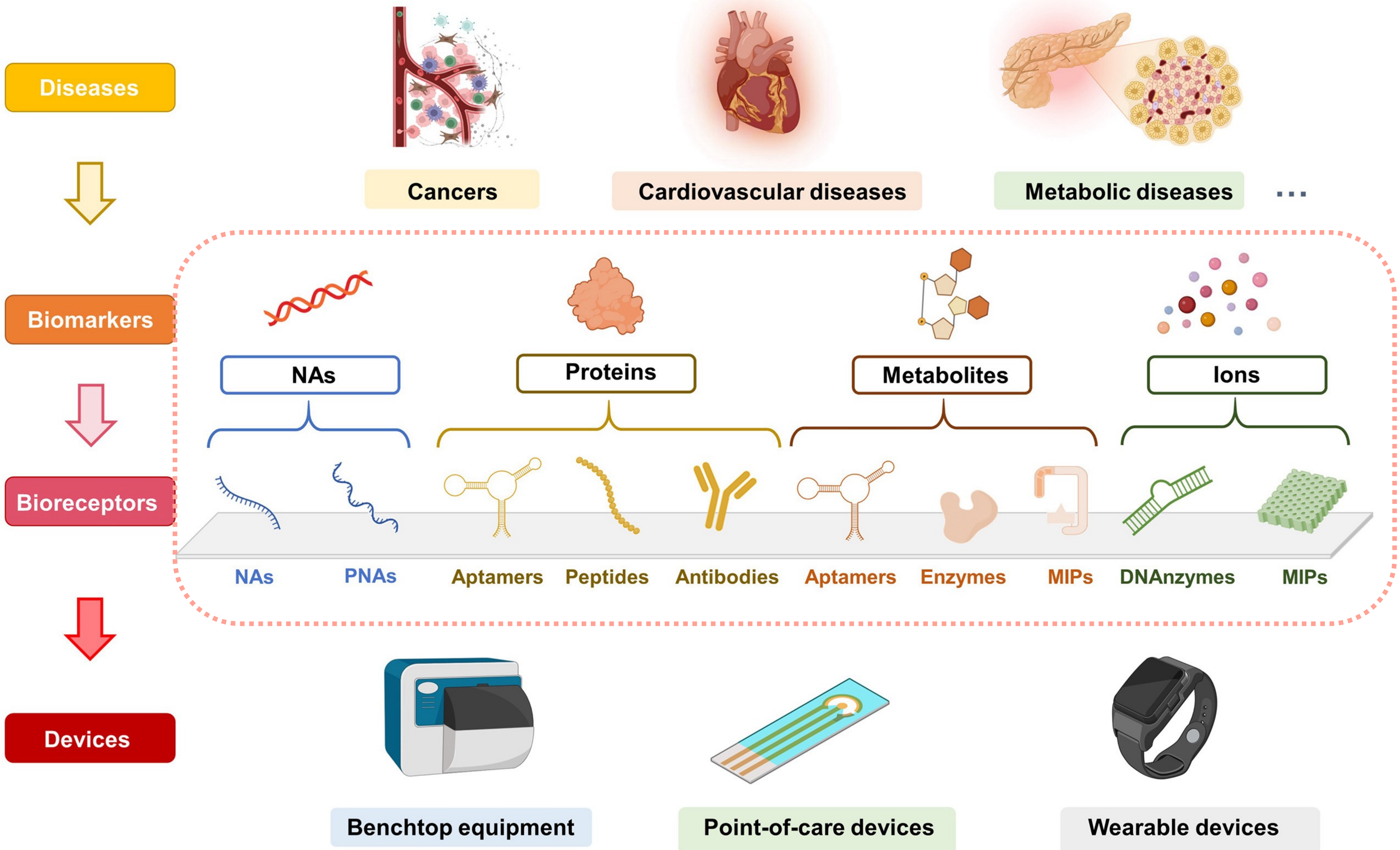


Challenges of Biosensing in Complex Environments



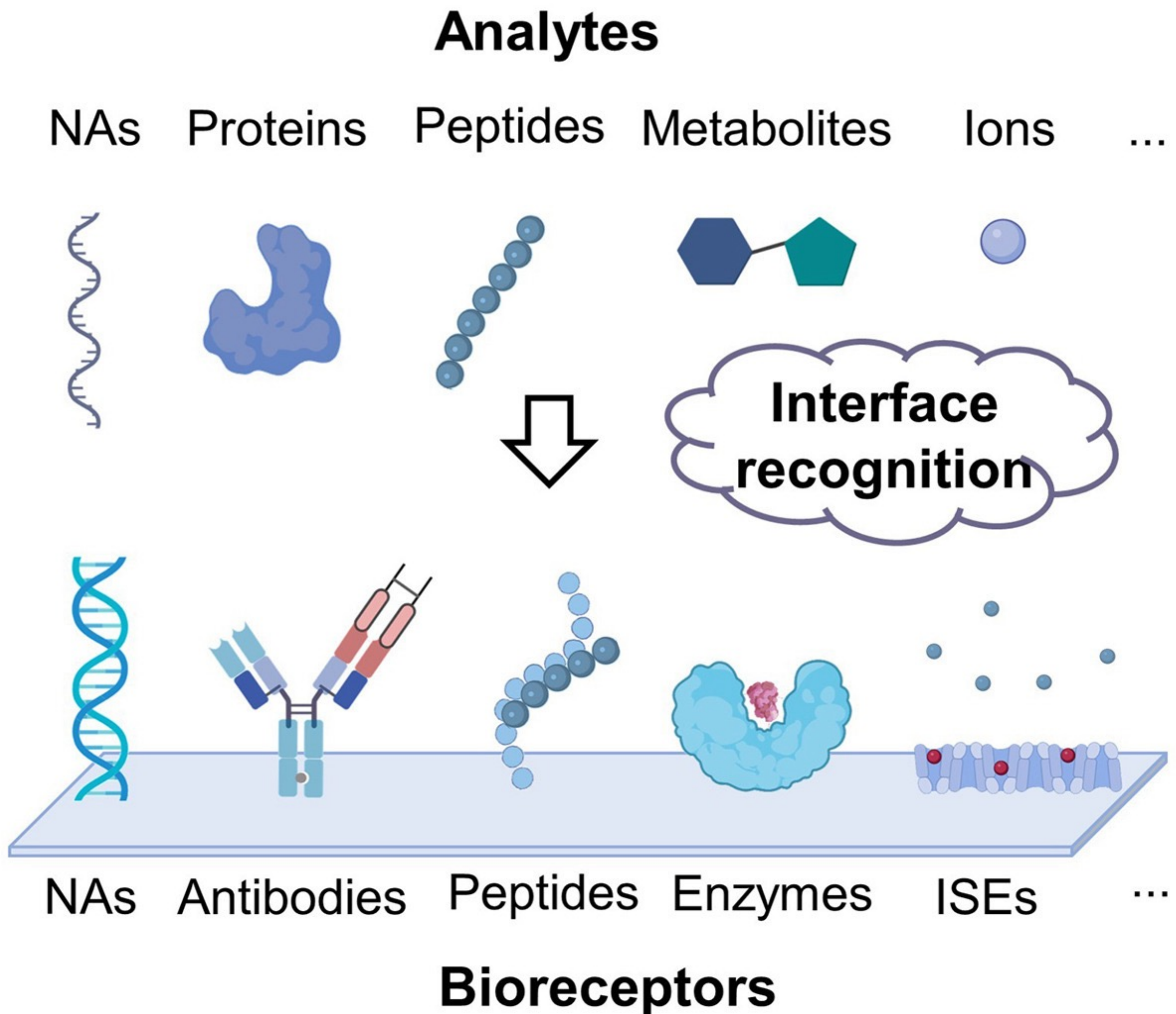
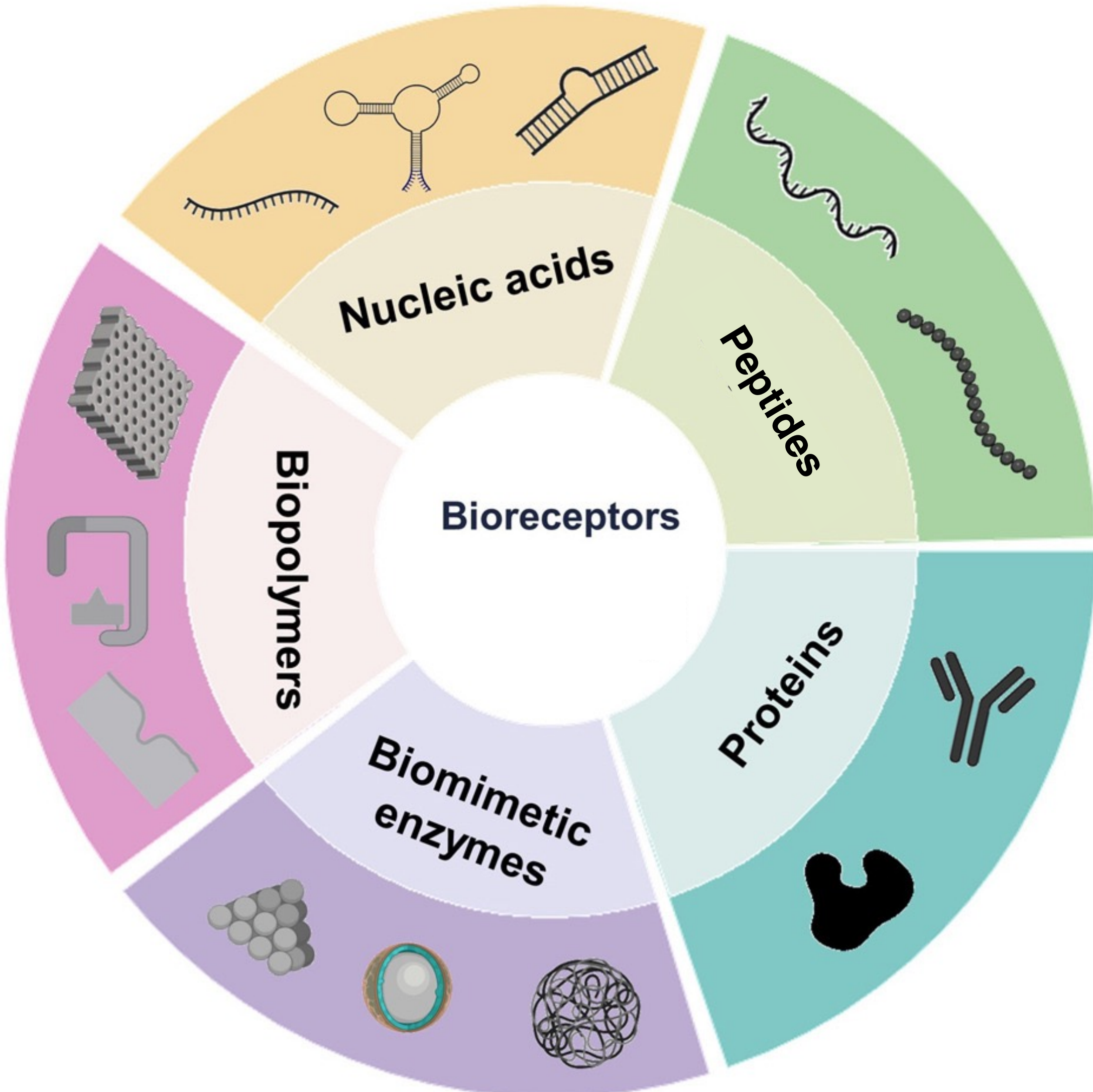
DNA-Based Biosensing that Tackle Challenges

Biosensors For Human Health



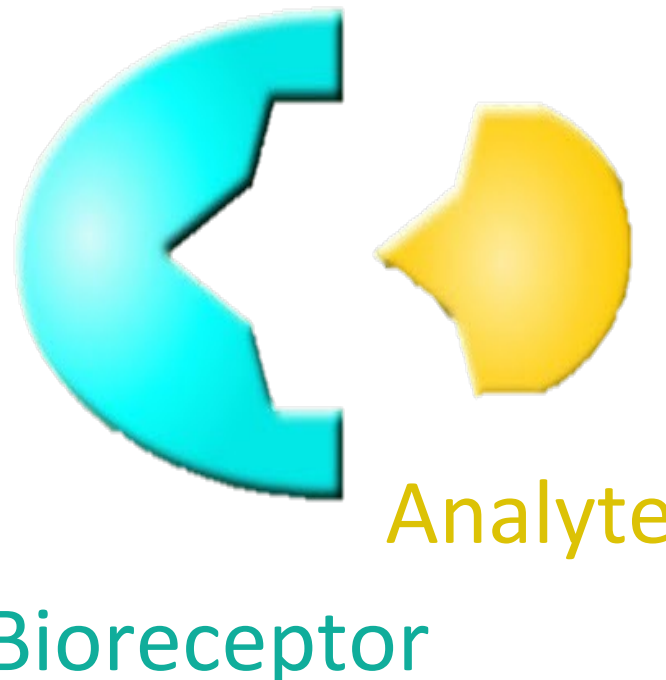
What are Bioreceptors?
How does molecular recognition work?

Molecular Recognition Elements/Biological Receptors



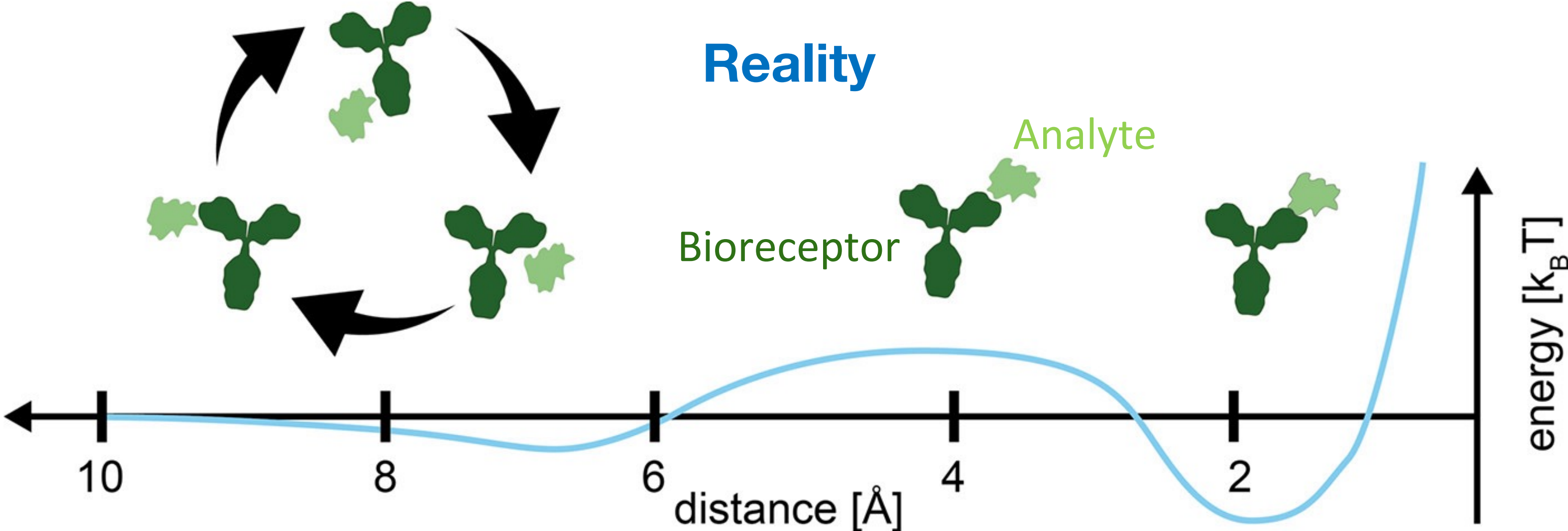
How do Bioreceptors Interact with their Analytes?

Expectation

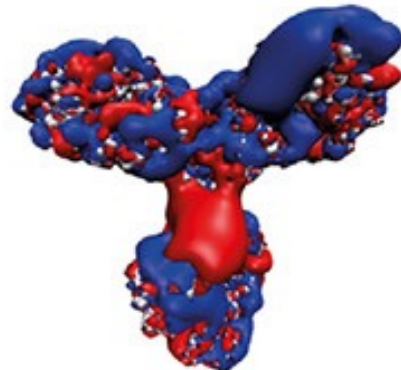


Molecular recognition of target by bioreceptor

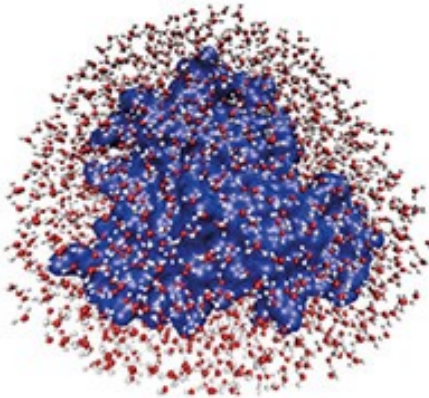
Reality



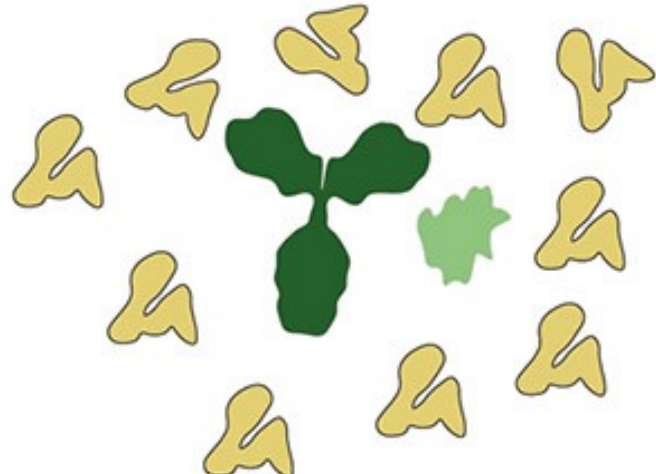
inhomogeneous surface potential
- electrostatic steering



solvent effects
- hydrophobic attraction
- hydrophilic repulsion

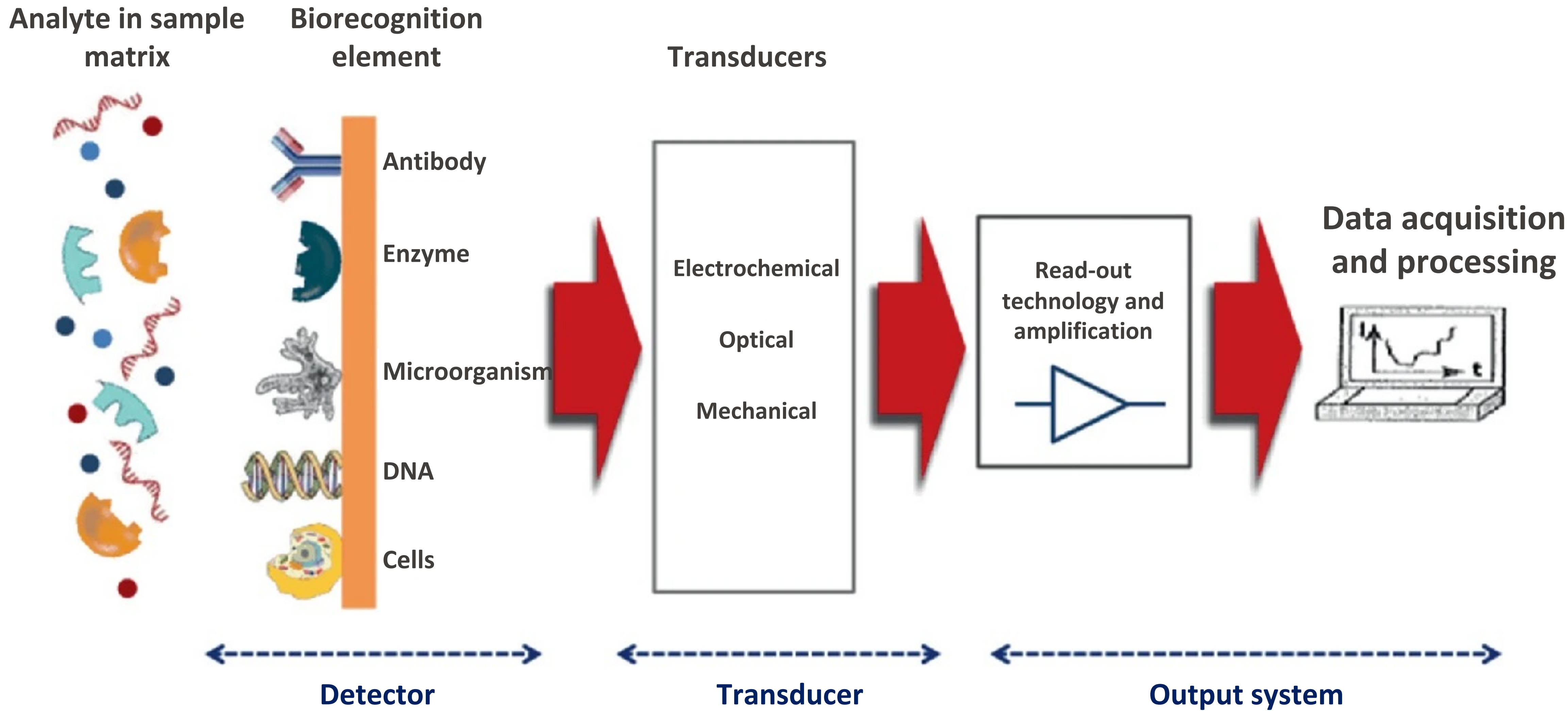


molecular crowding

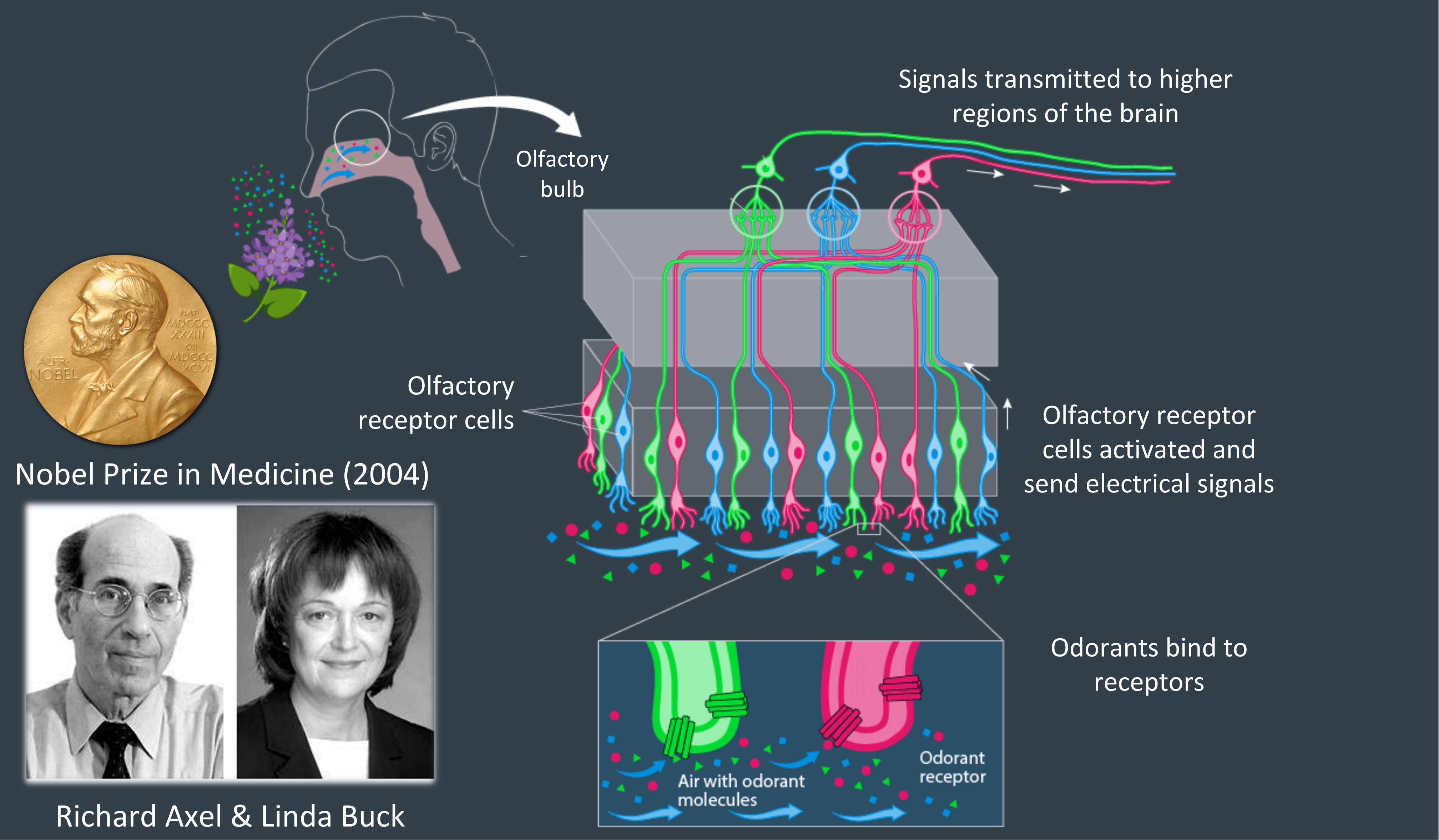


- hydrophobic
- π - π
- cation- π
- salt bridge
- hydrogen bond

Biosensor: Transduces Molecular Binding Event to a Signal



How Do We Smell Things?



Simple Science



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

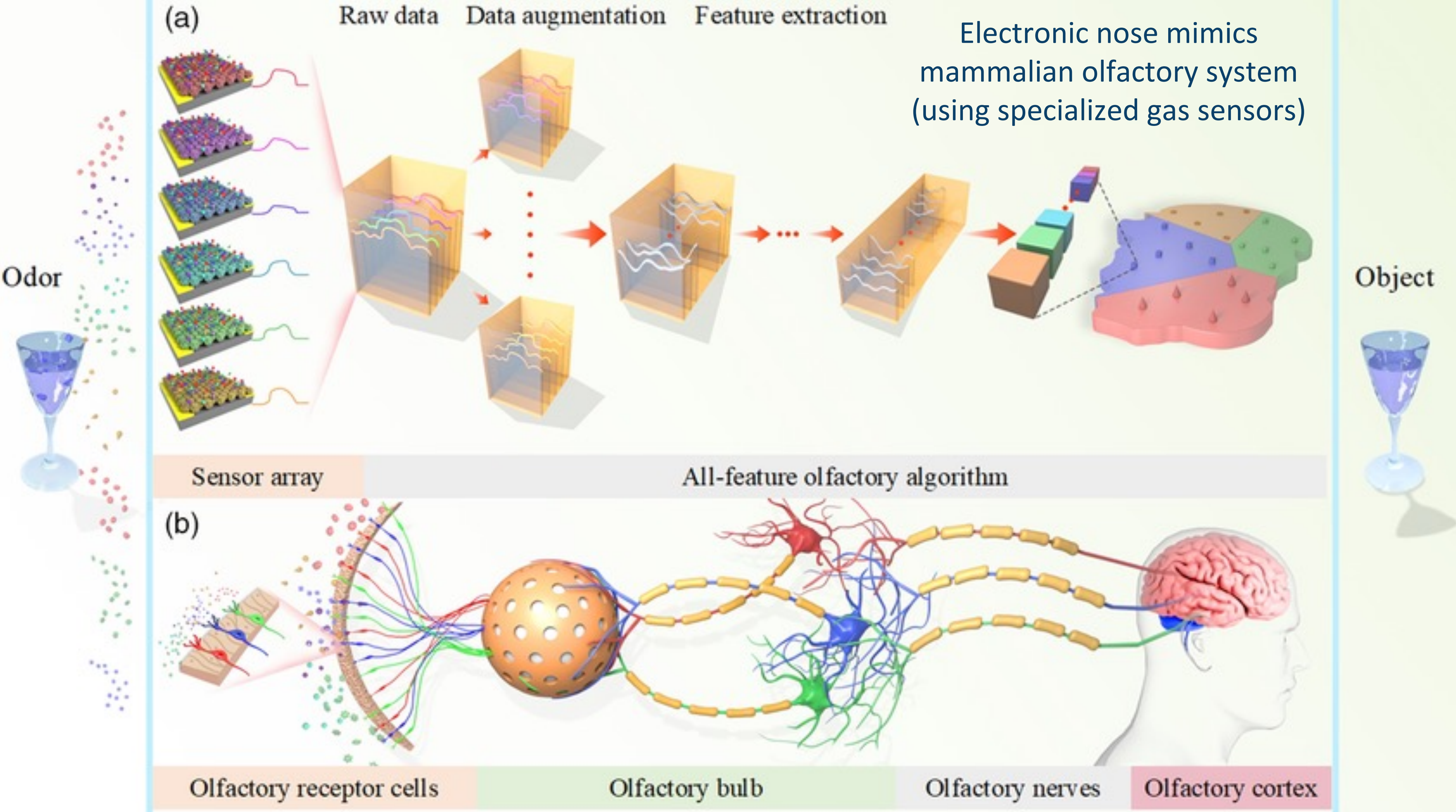
400 different types of receptor proteins

Dogs
220-300 million

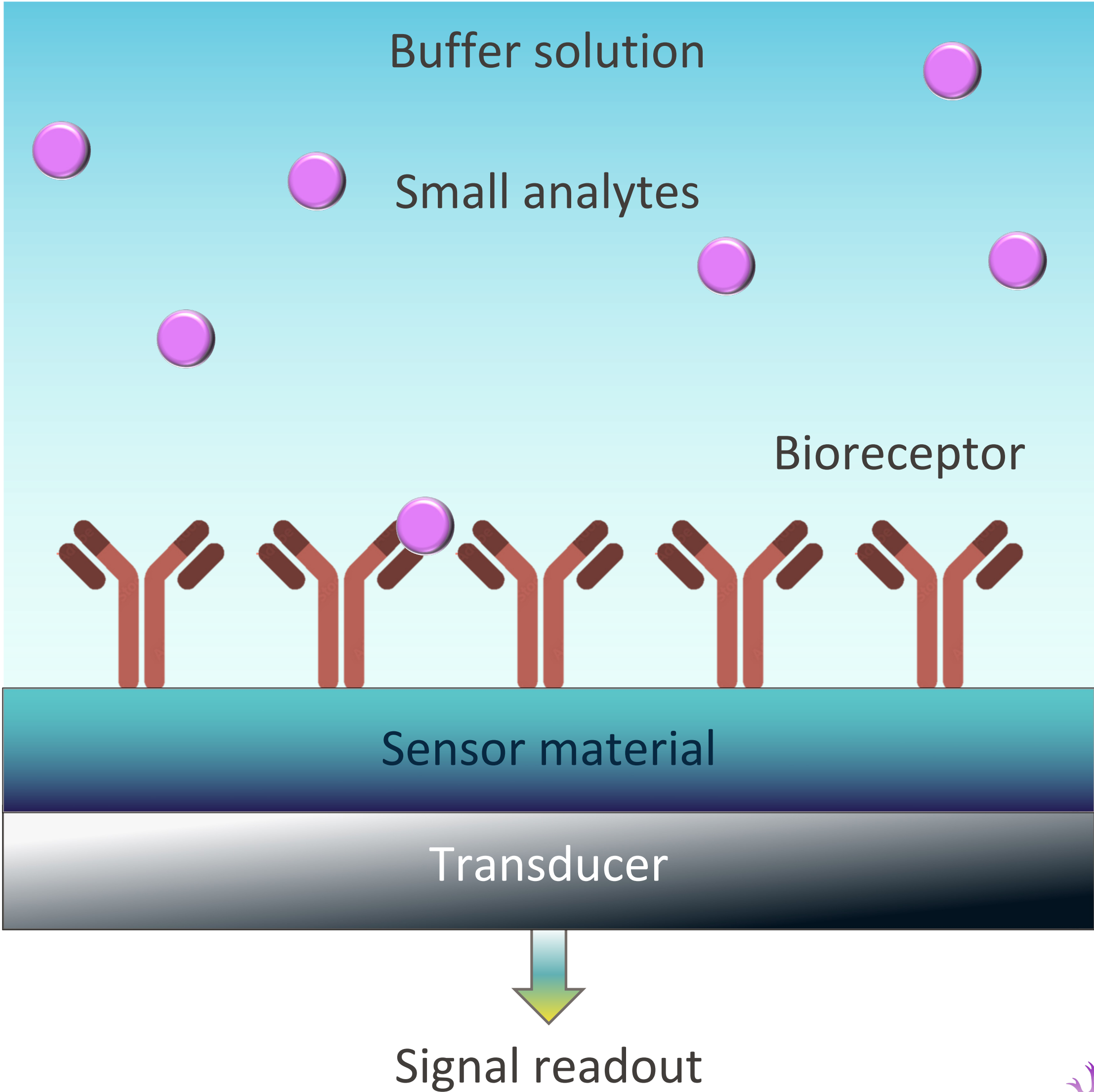
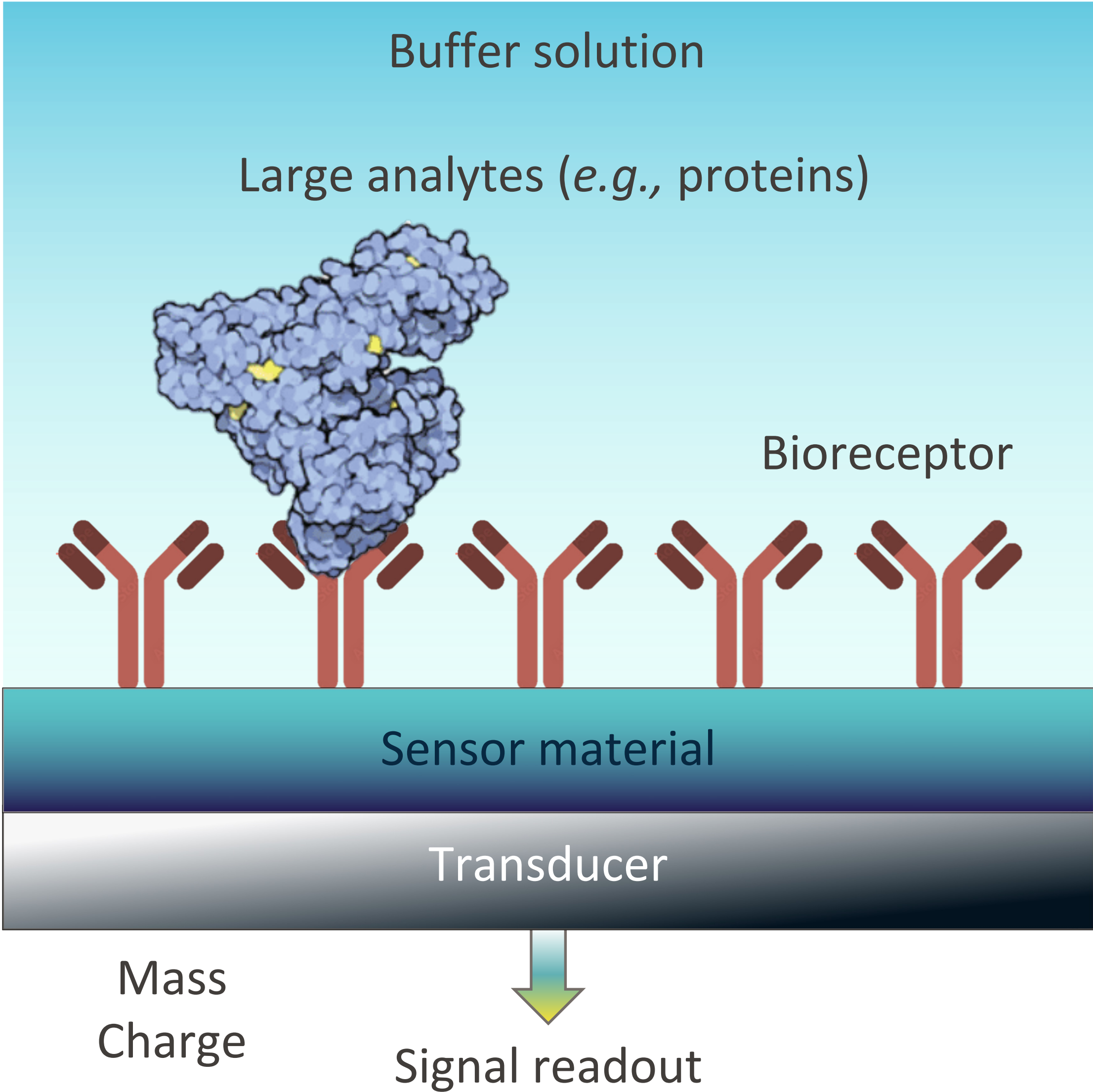
800-1200 different types of receptor proteins

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

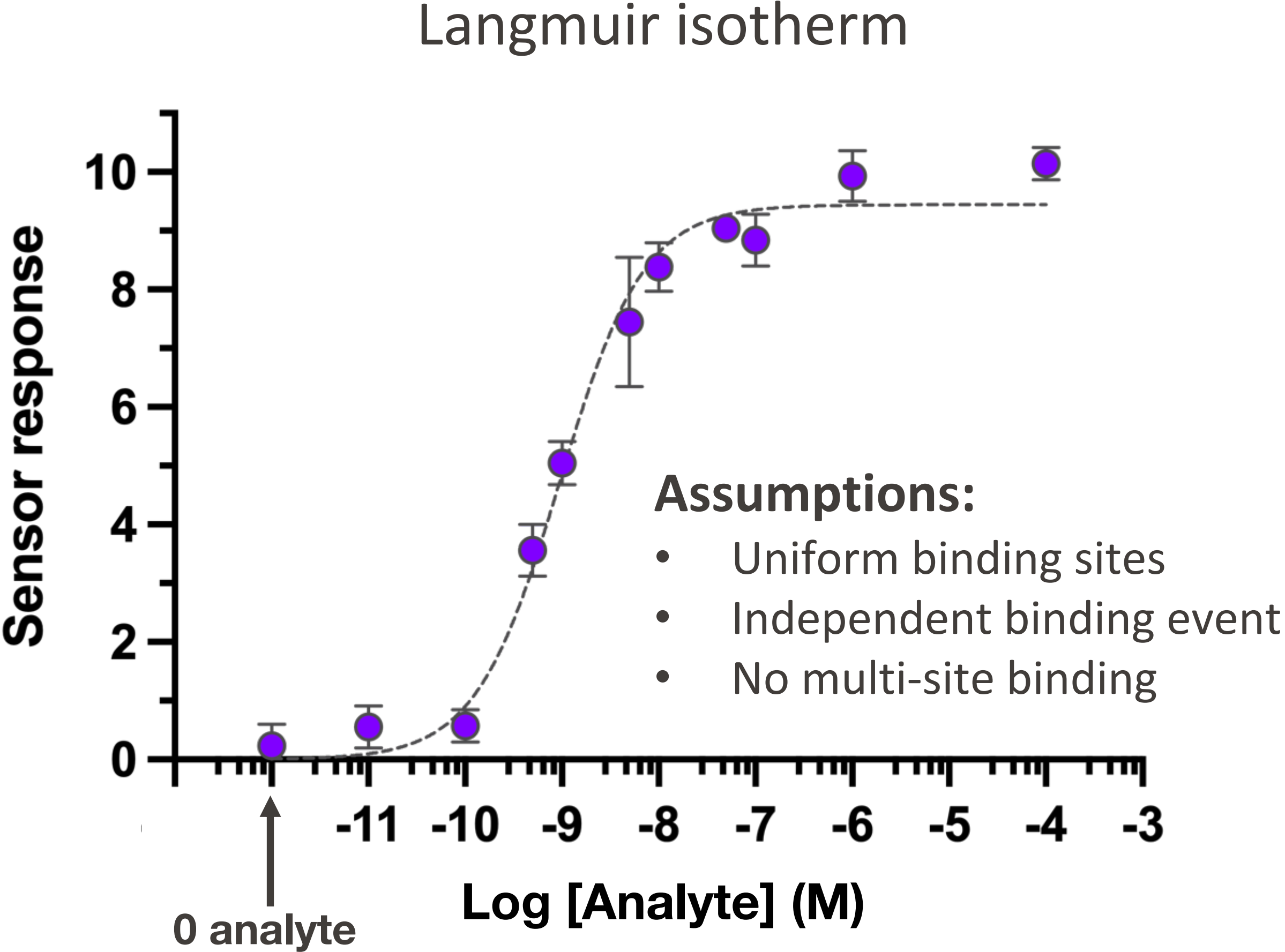
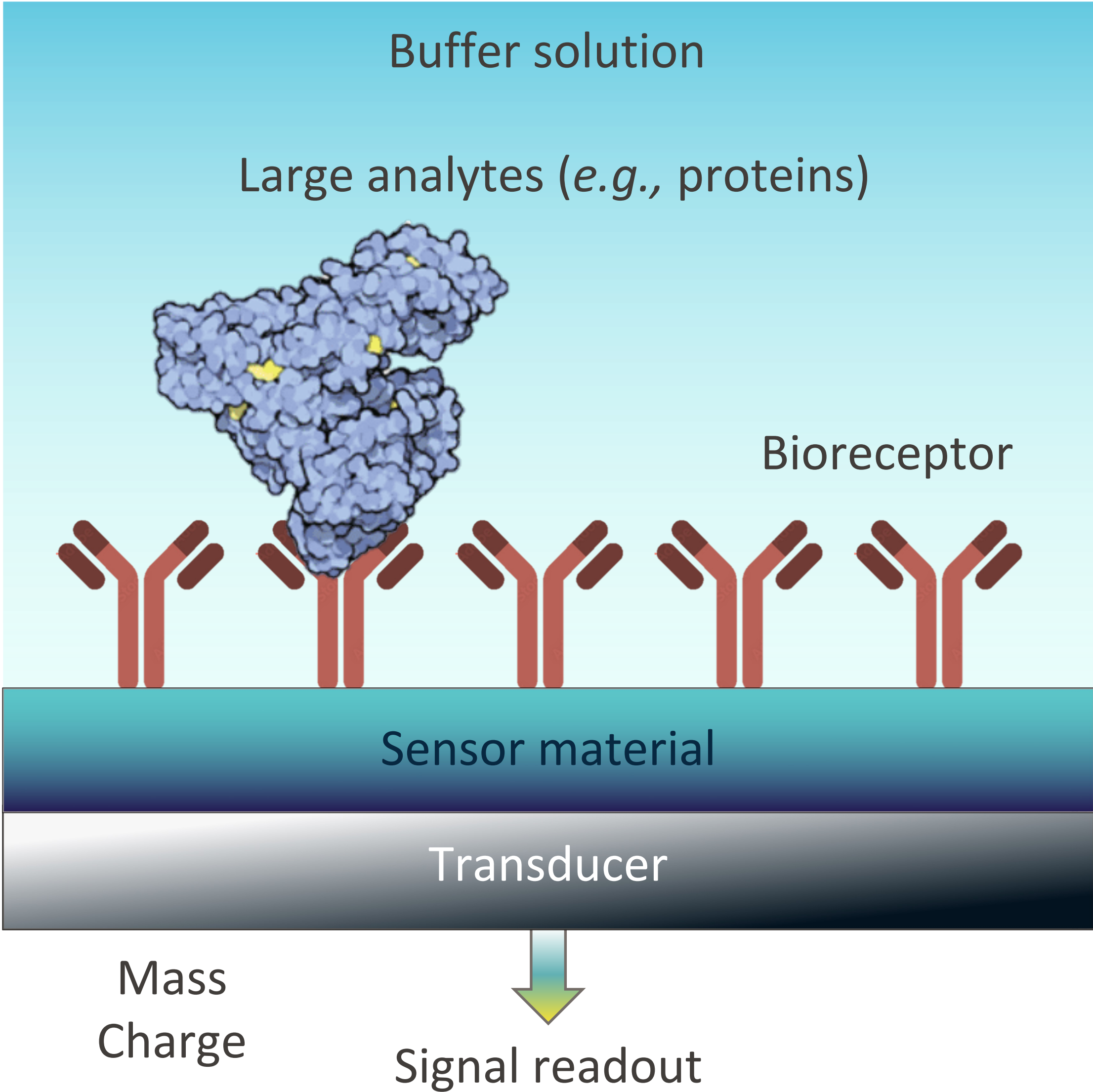
Mimicking Nature: Deep-Learning Inspired Electronic Nose



How to Read Out the Signal of a Biosensor



How to Read Out the Signal of a Biosensor

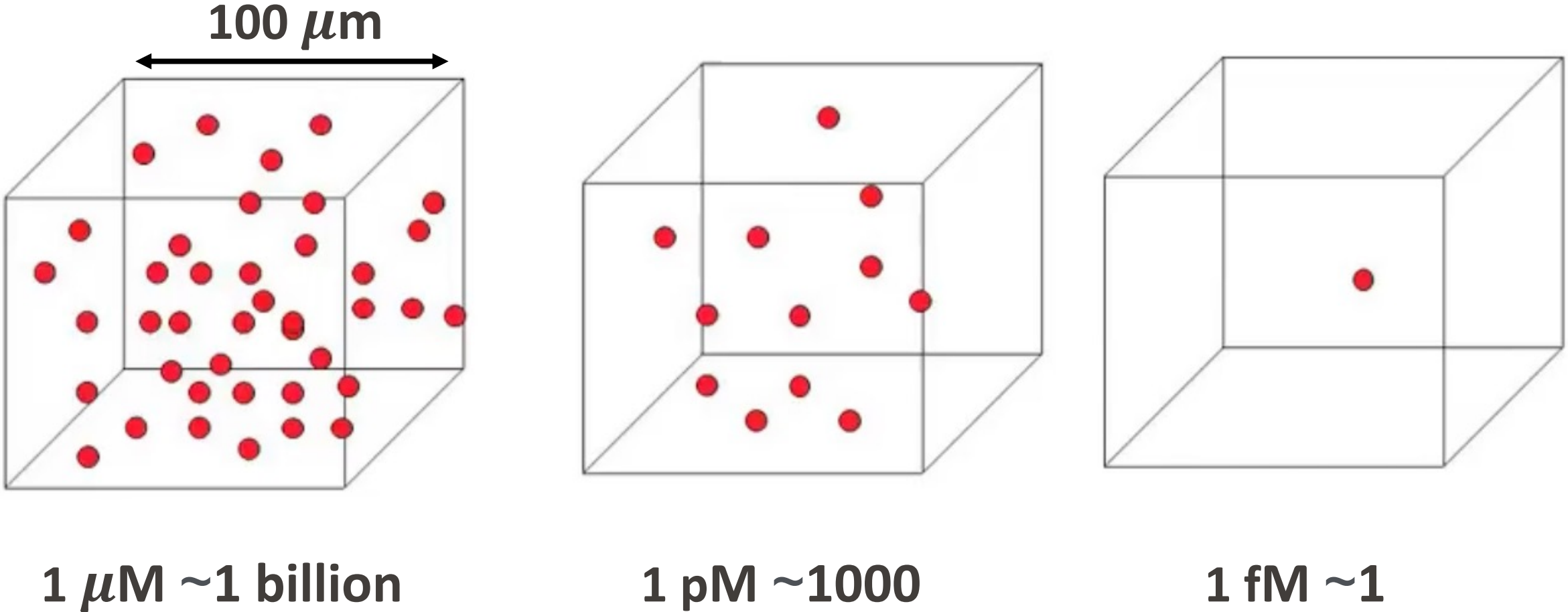


Concentration – What Do these Values Mean?

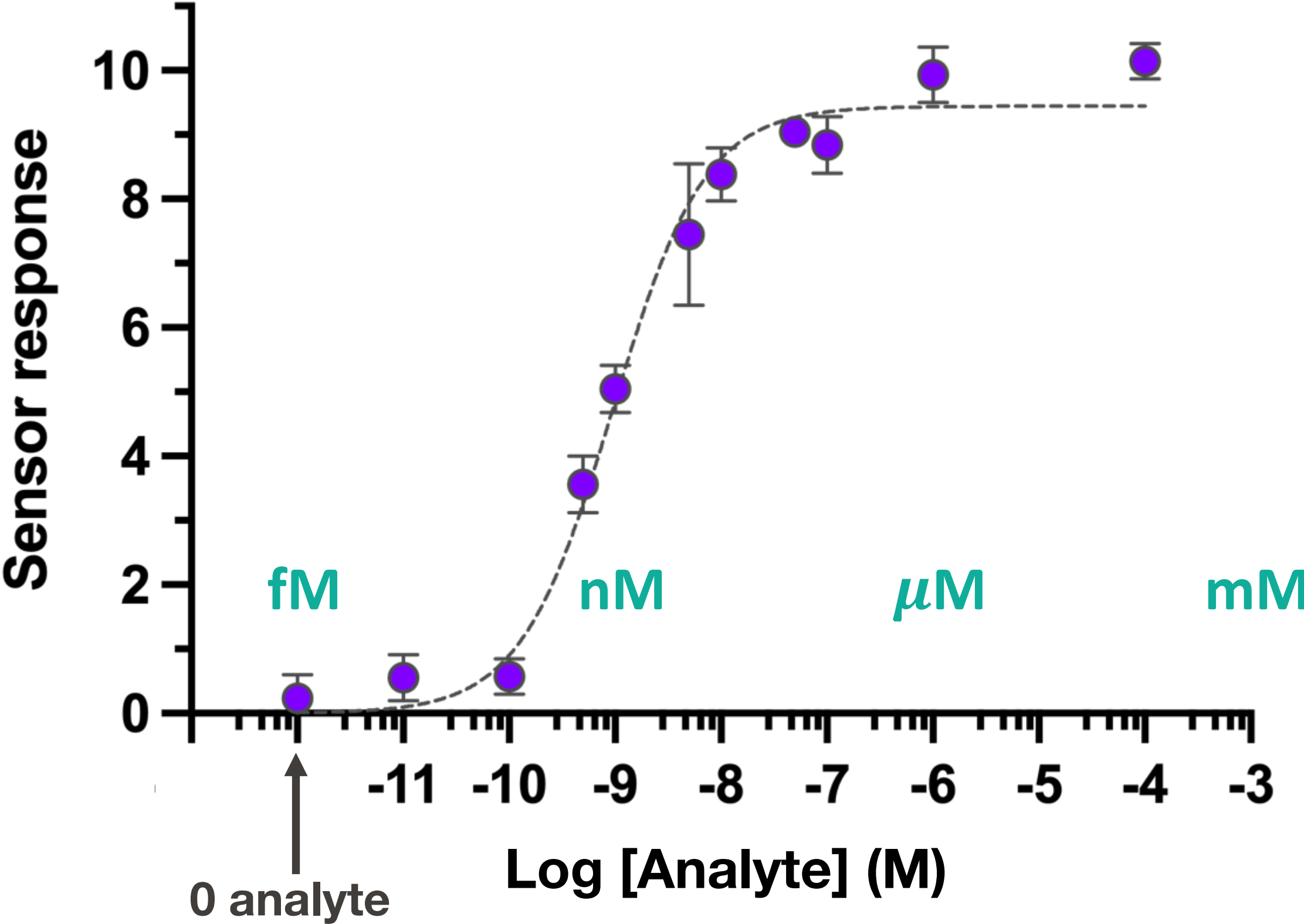
$1 \text{ M} = 1 \text{ mol/L} = 6 \times 10^{23} \text{ molecules/L}$

Note: Avogadro's number: $6 \times 10^{23} \text{ molecules/mol}$

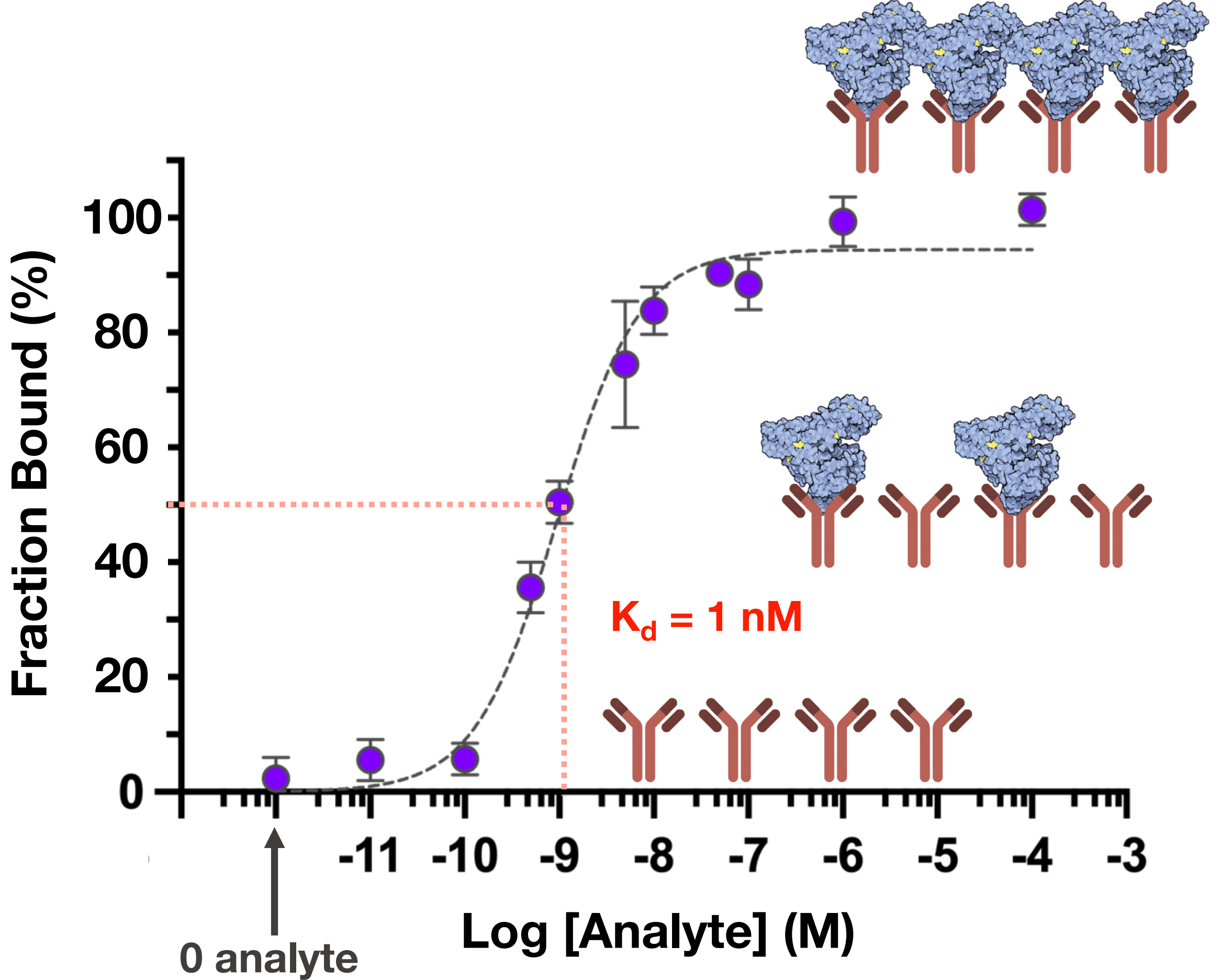
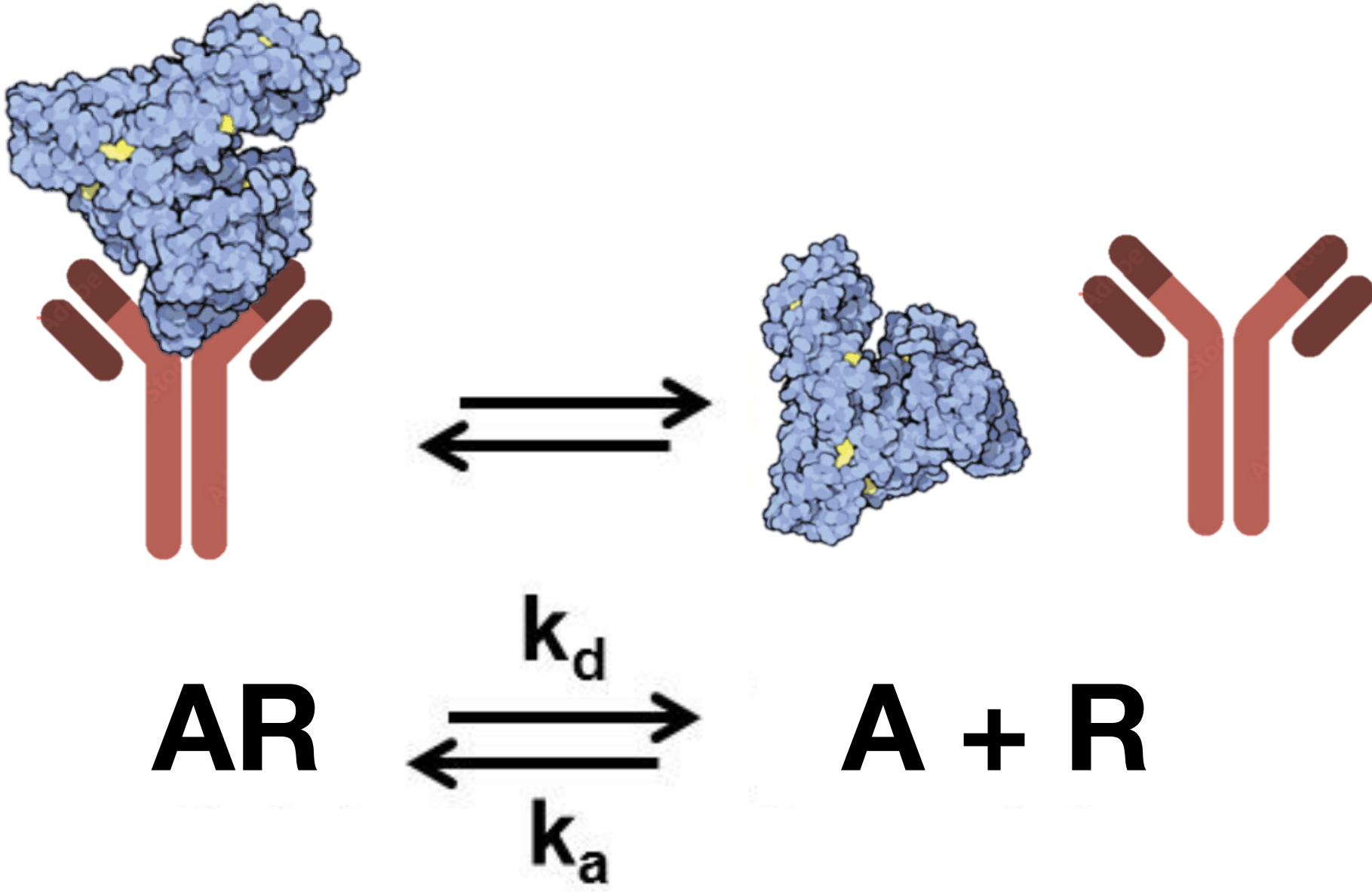
$1 \text{ M} \sim 1 \times 10^{15} \text{ molecules}/100 \mu\text{m}^3$



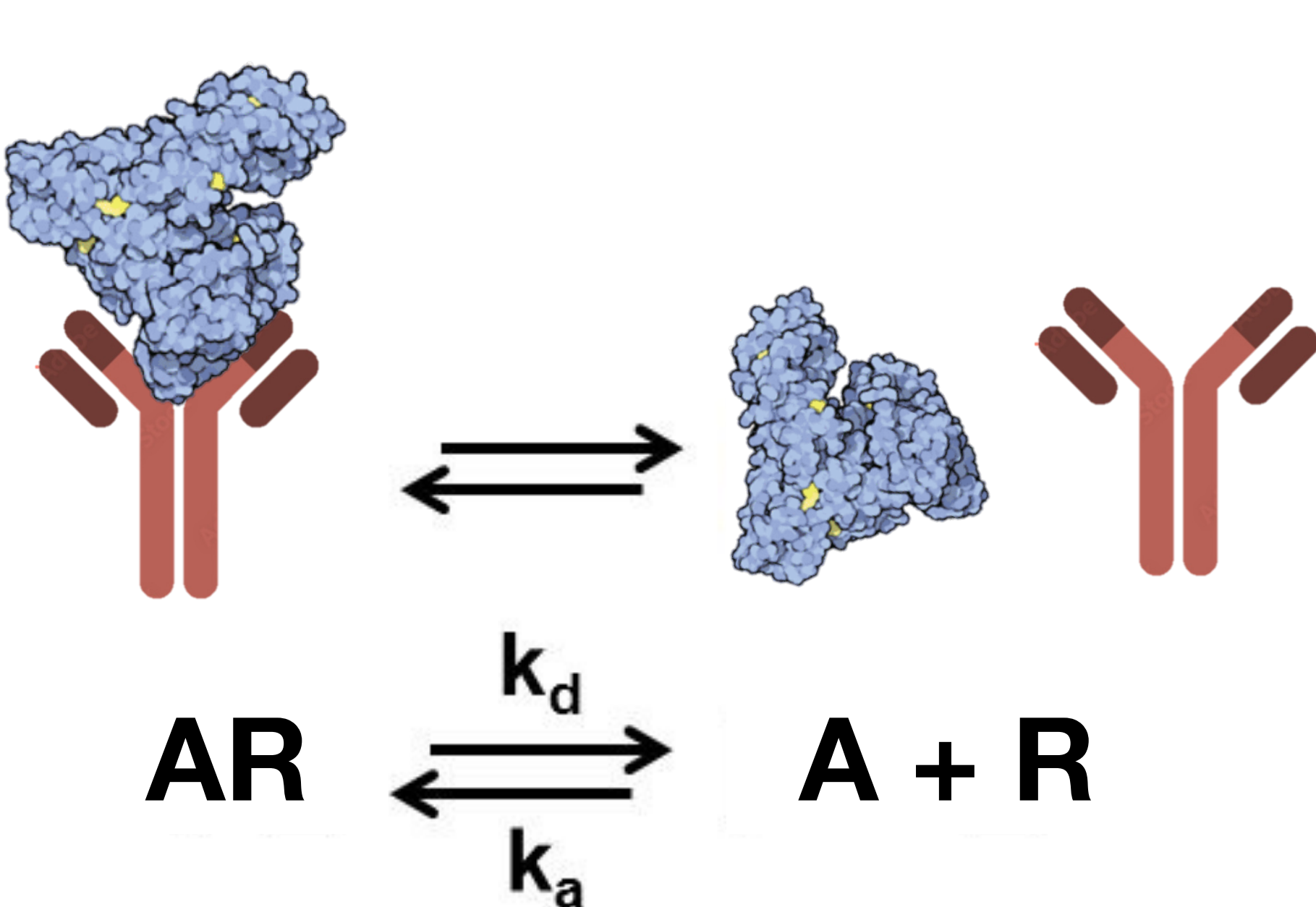
Single molecule of salt in several Olympic-sized swimming pools!



How to Read Out the Signal of a Biosensor



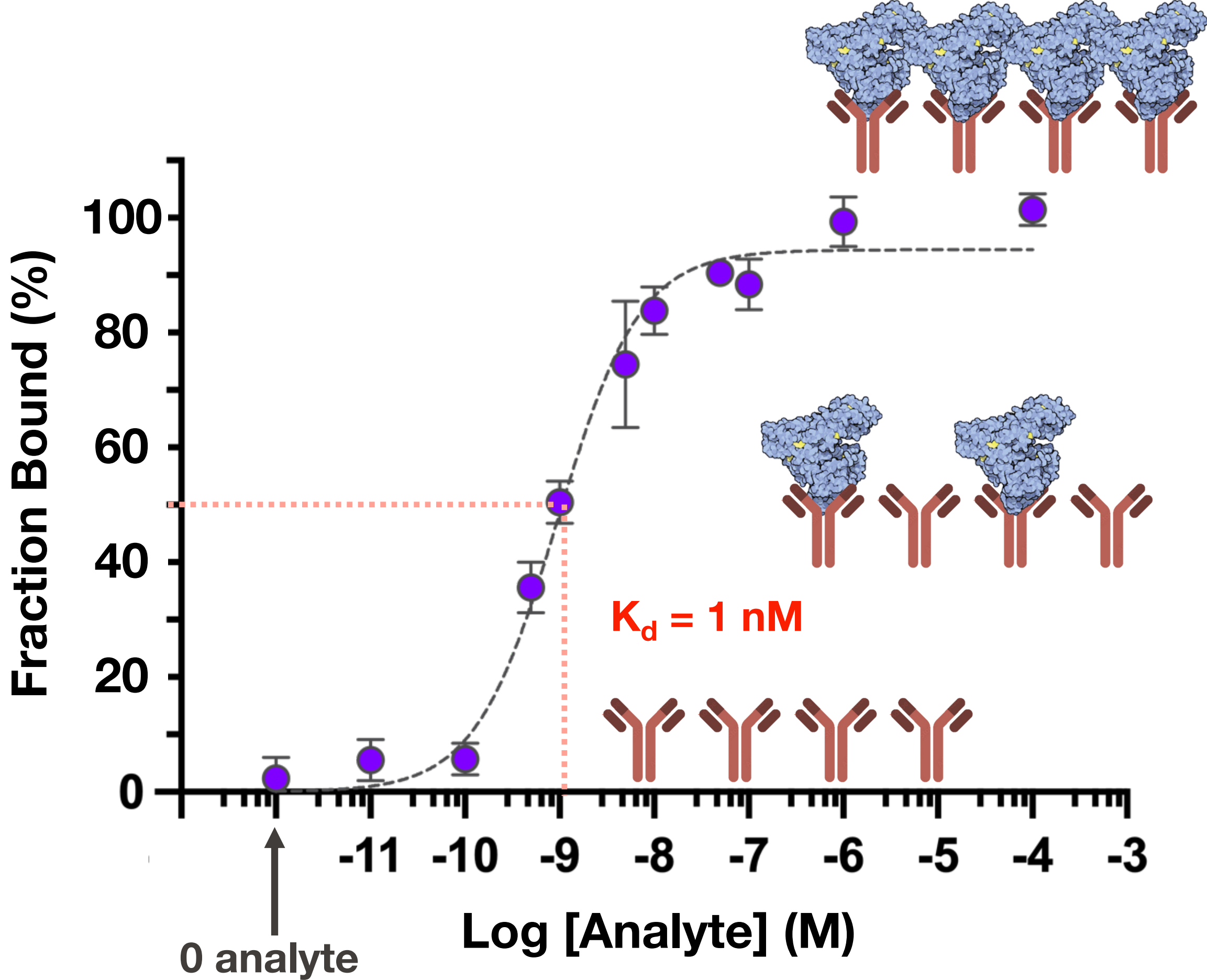
How to Calculate the Equilibrium Binding Affinity (K_d)



Fractional occupied receptor density

$$\phi = \frac{[\text{AR}]}{[\text{R}_0]}$$

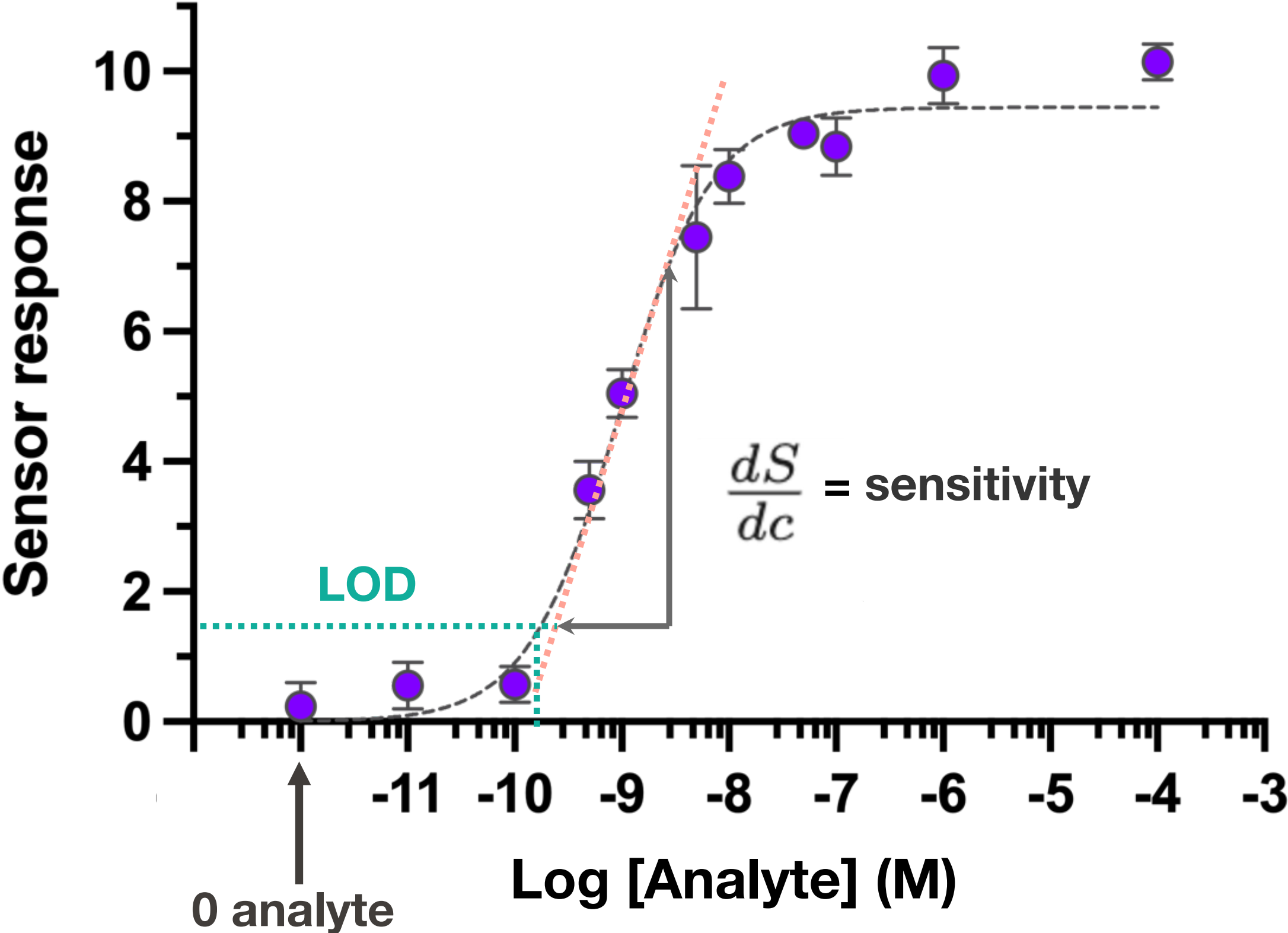
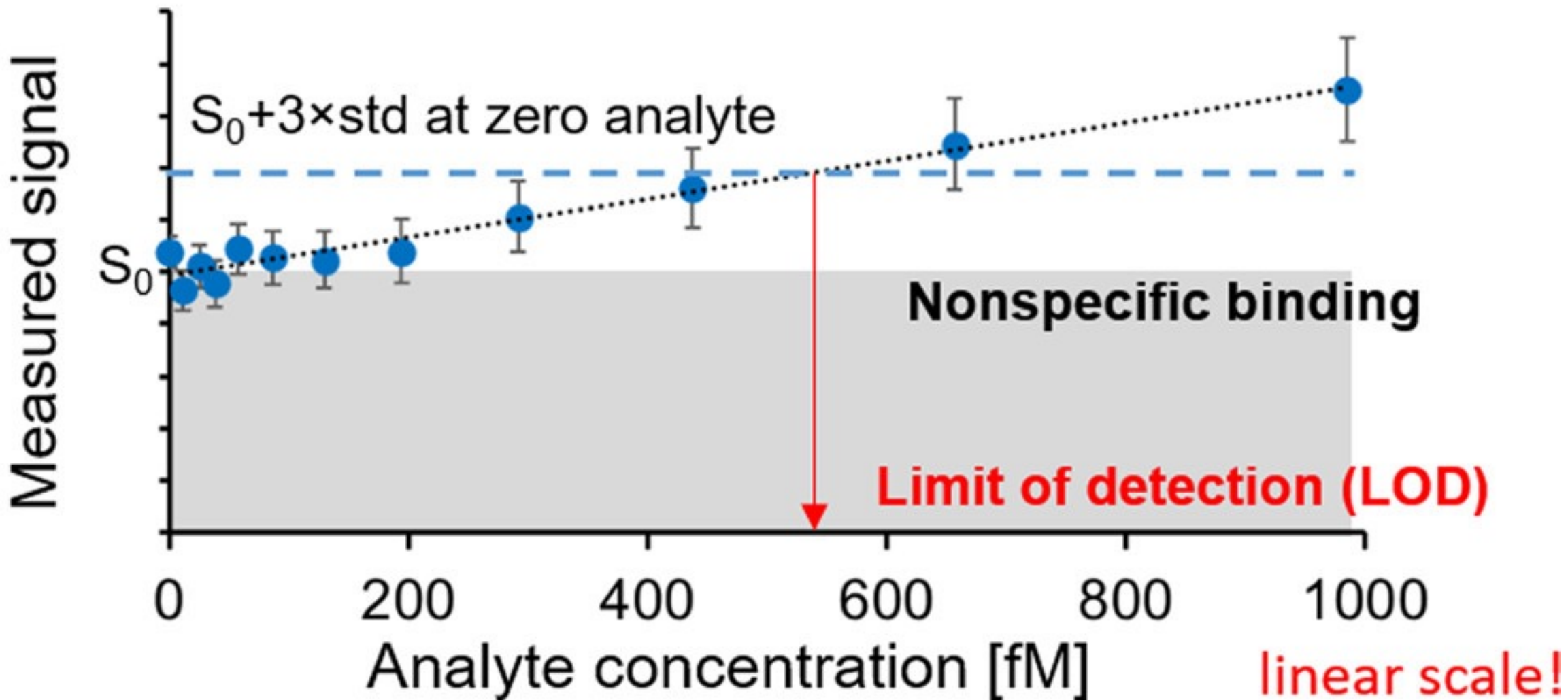
Target-bound receptors
Total number of receptors



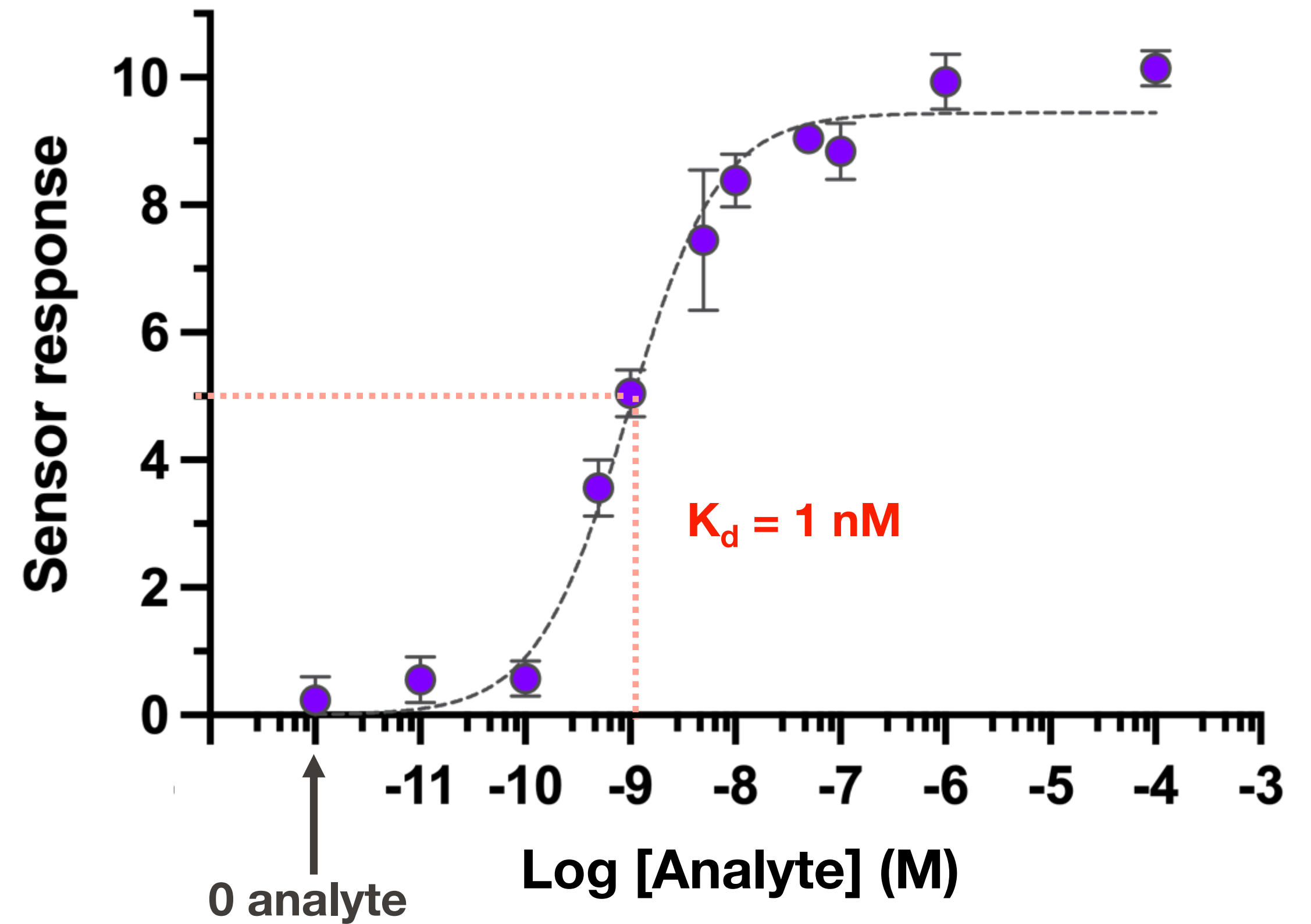
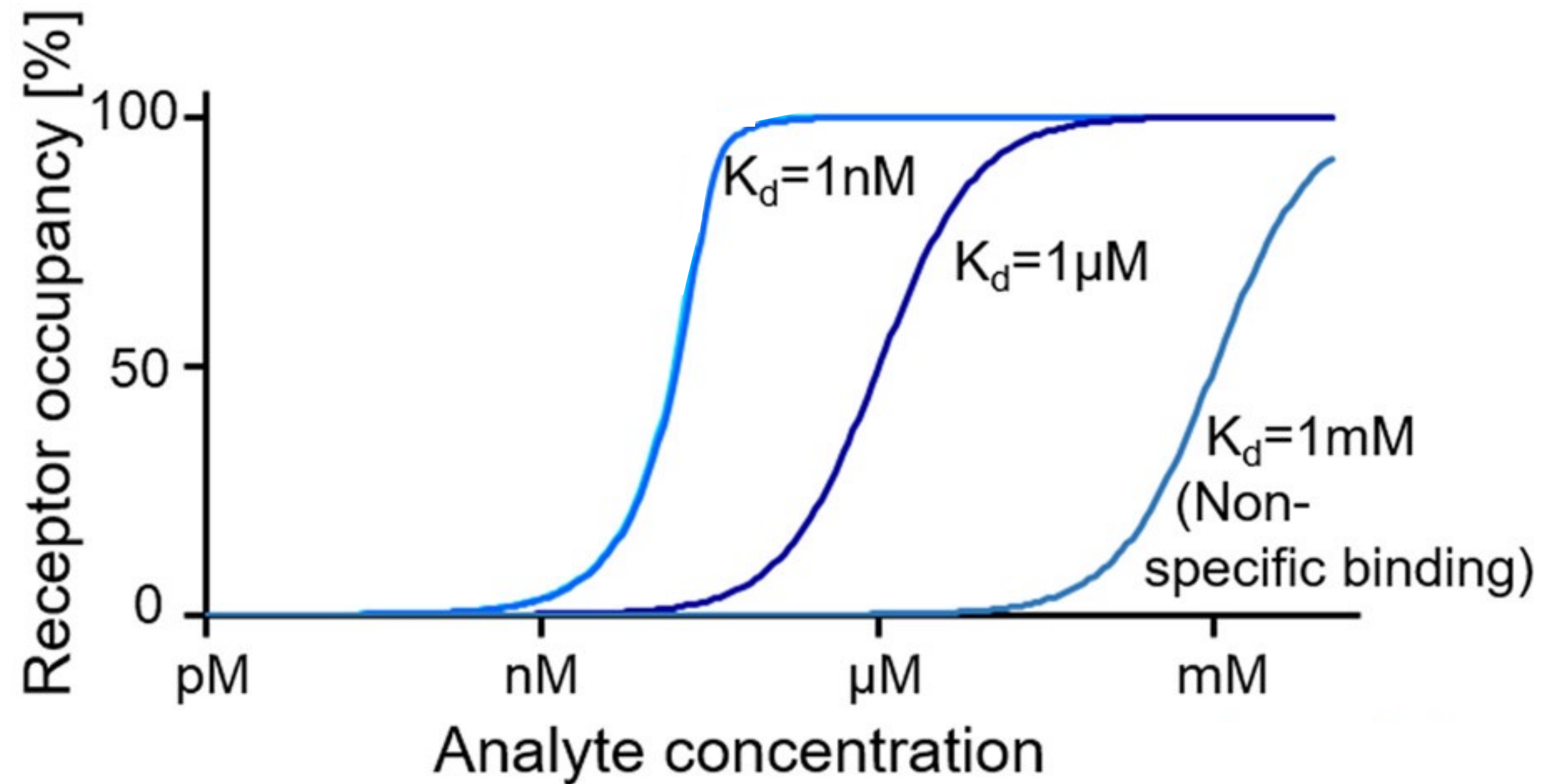
How to Calculate the Sensitivity and Limit of Detection (LOD)

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

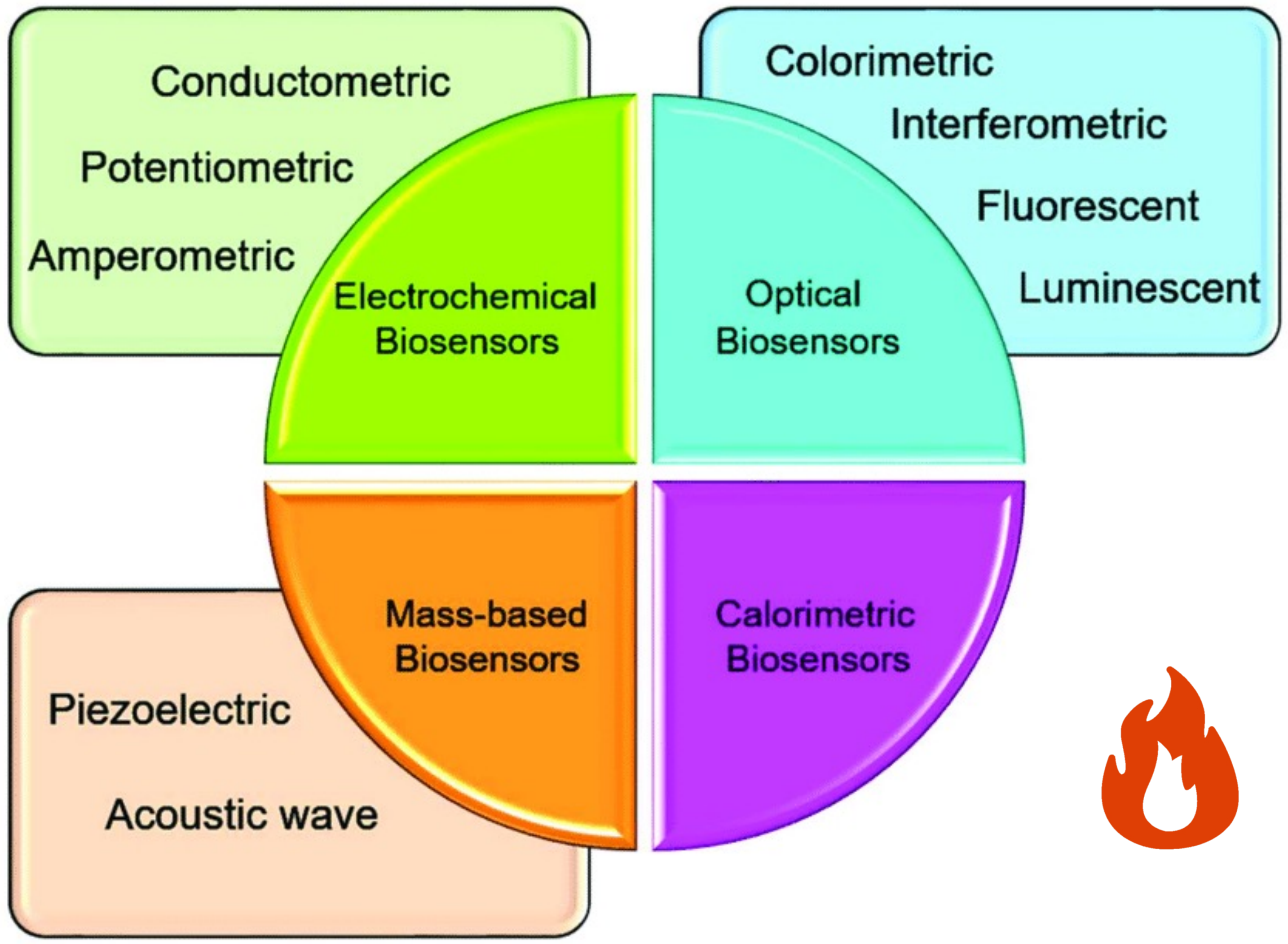
3 x standard deviation of zero analyte signal
Sensitivity = Slope of the signal



The Detection Range is Dependent on the K_d of the Bioreceptor



Different Signal Transduction Mechanisms of Biosensors



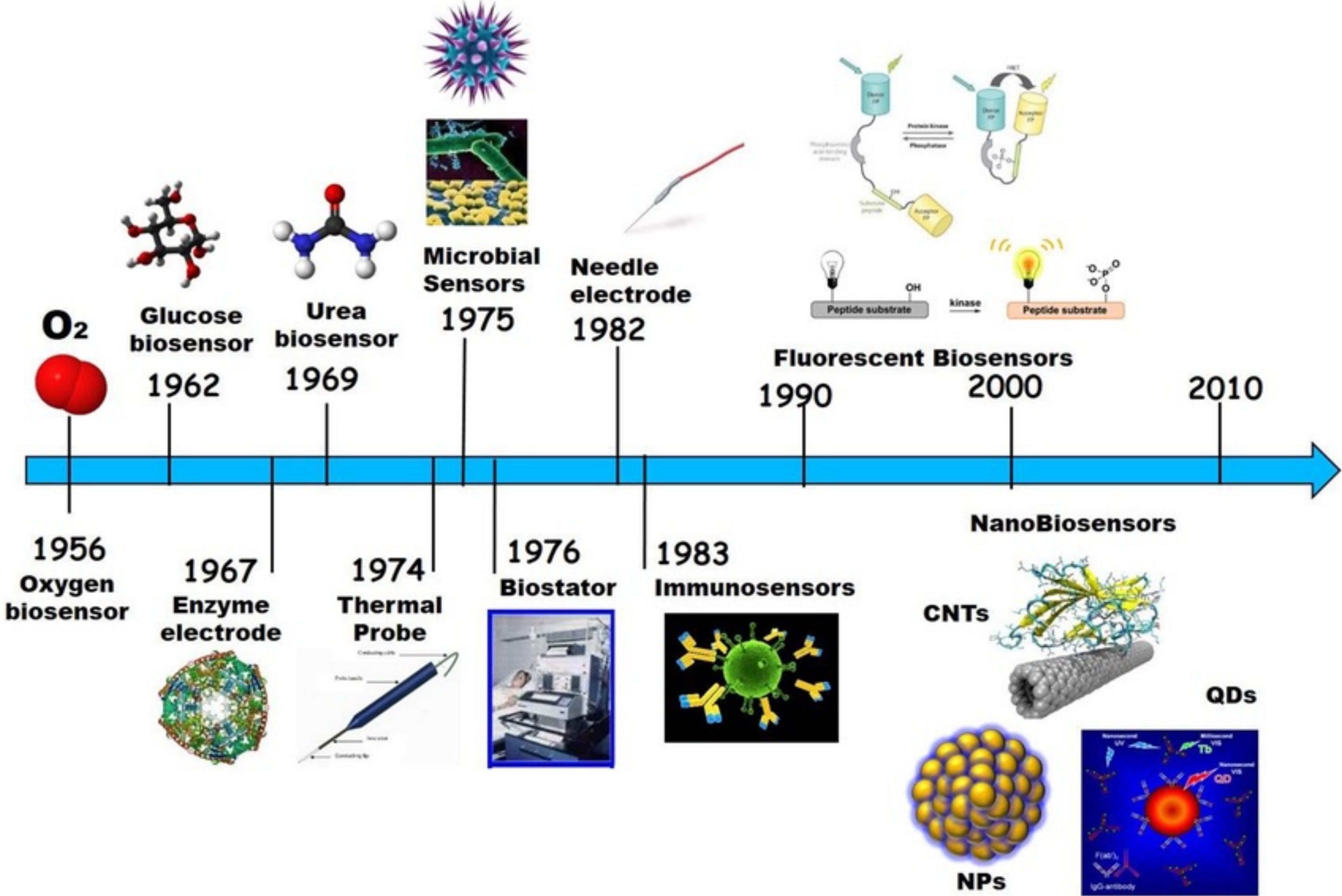
Key Takeaways

- Bioreceptors interact with their analytes through various mechanisms
 - Mimicking Nature – the dog's nose as the best biosensor
 - How to read out signals from biosensors
 - Binding affinity (K_d), limit of detection (LOD), sensitivity
 - Different transduction mechanisms of biosensors

History of Biosensors



Leland Clark
The "Father of Biosensors"

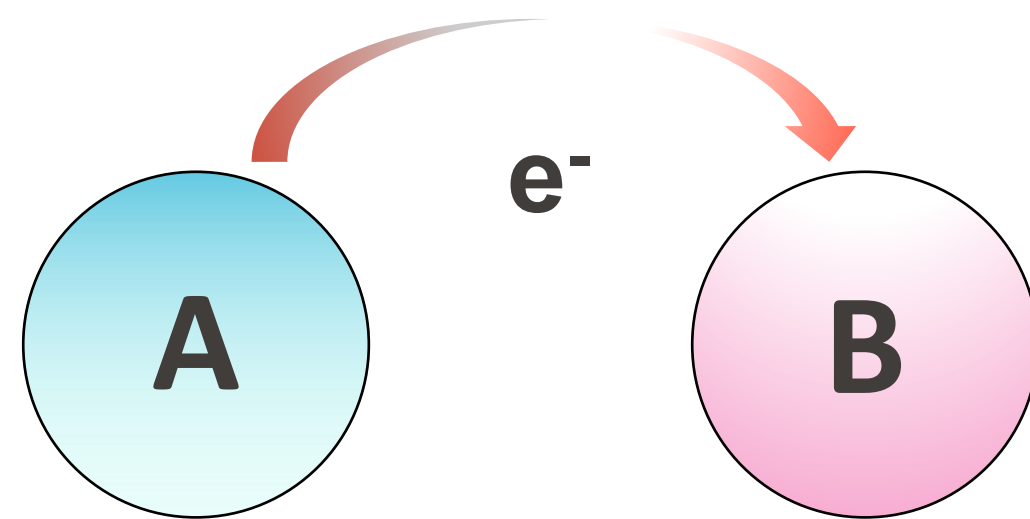


Tilmaciu & Morris | *Front. Chem.* | 2015

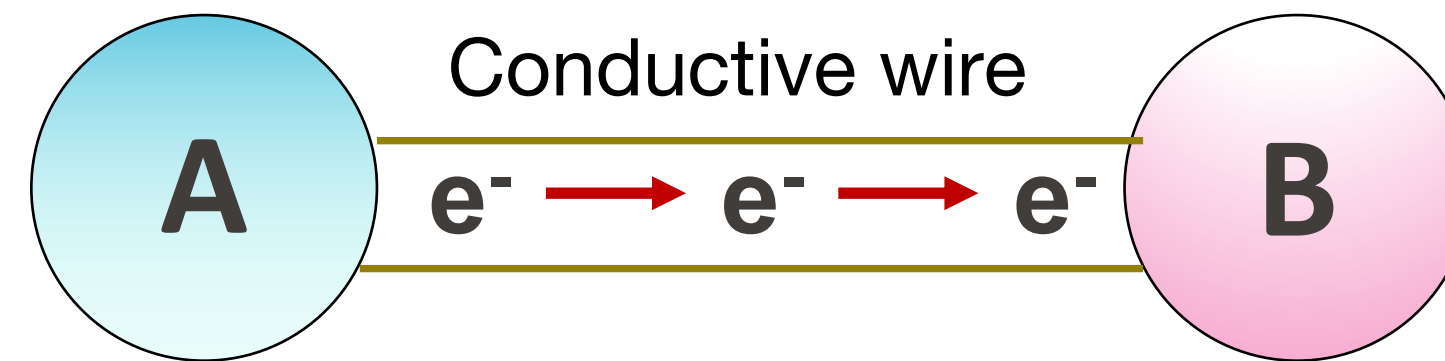
Electrochemistry: Relationship between Chemical Reactions + Electricity

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

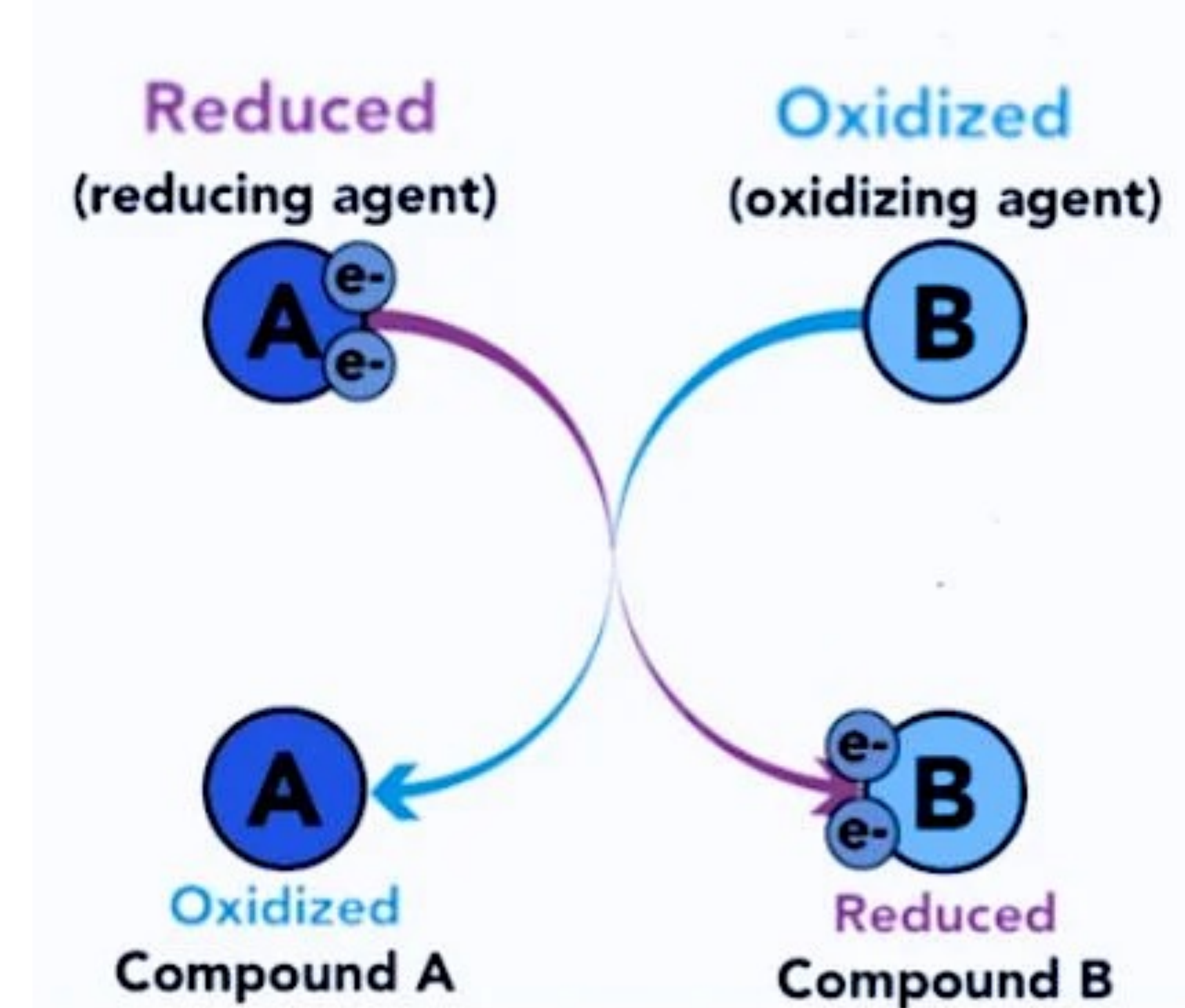
Certain chemical reactions \rightarrow create electricity



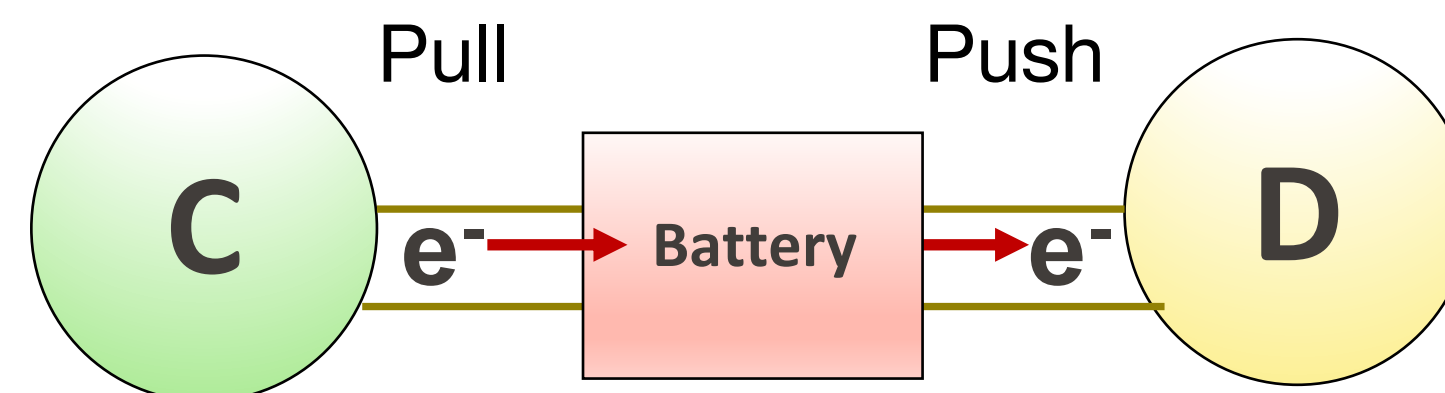
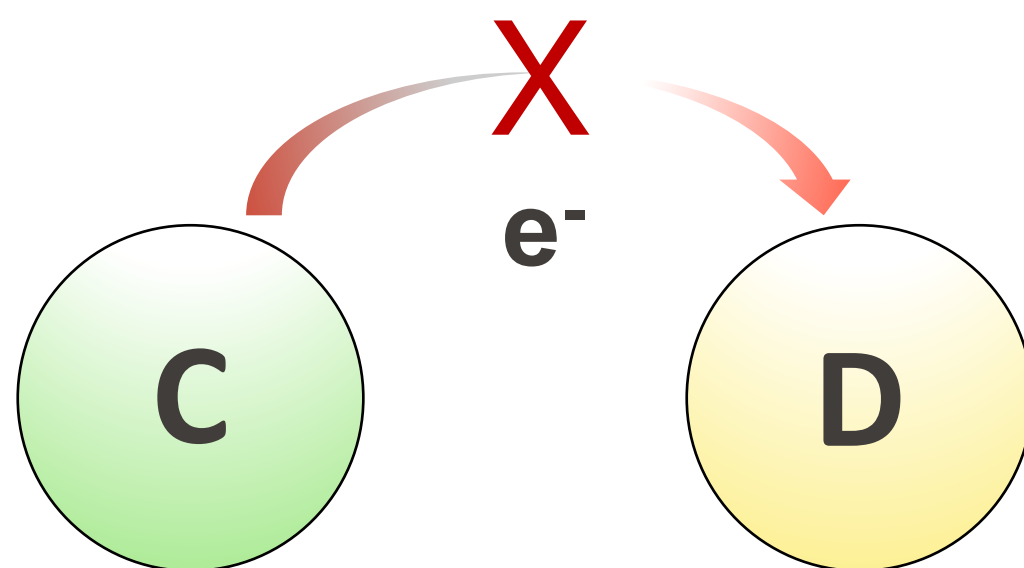
Oxidation & Reduction Reactions



Spontaneous reaction

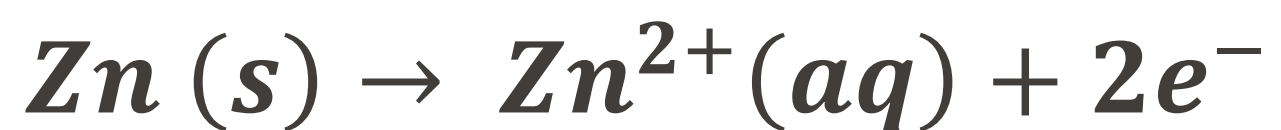
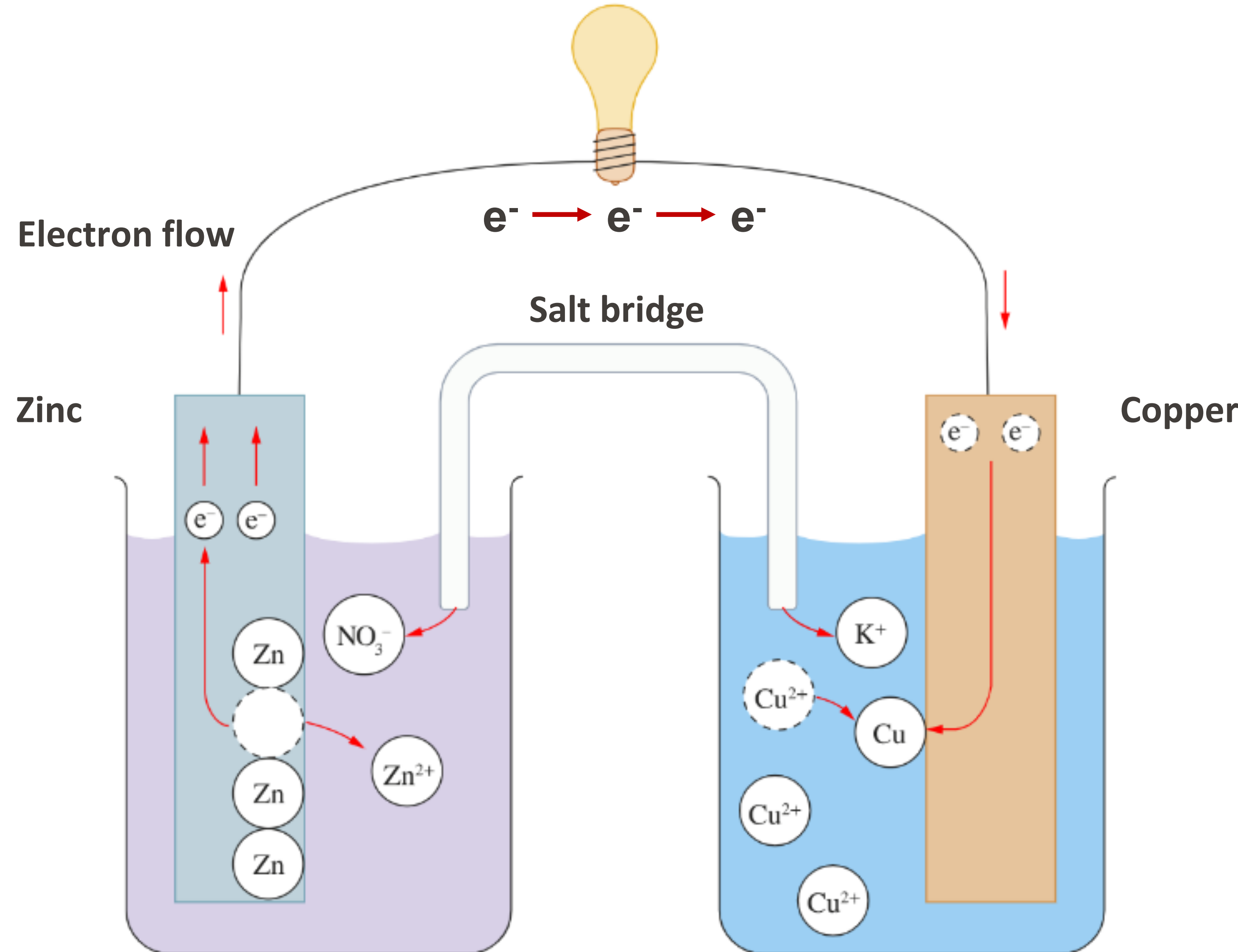
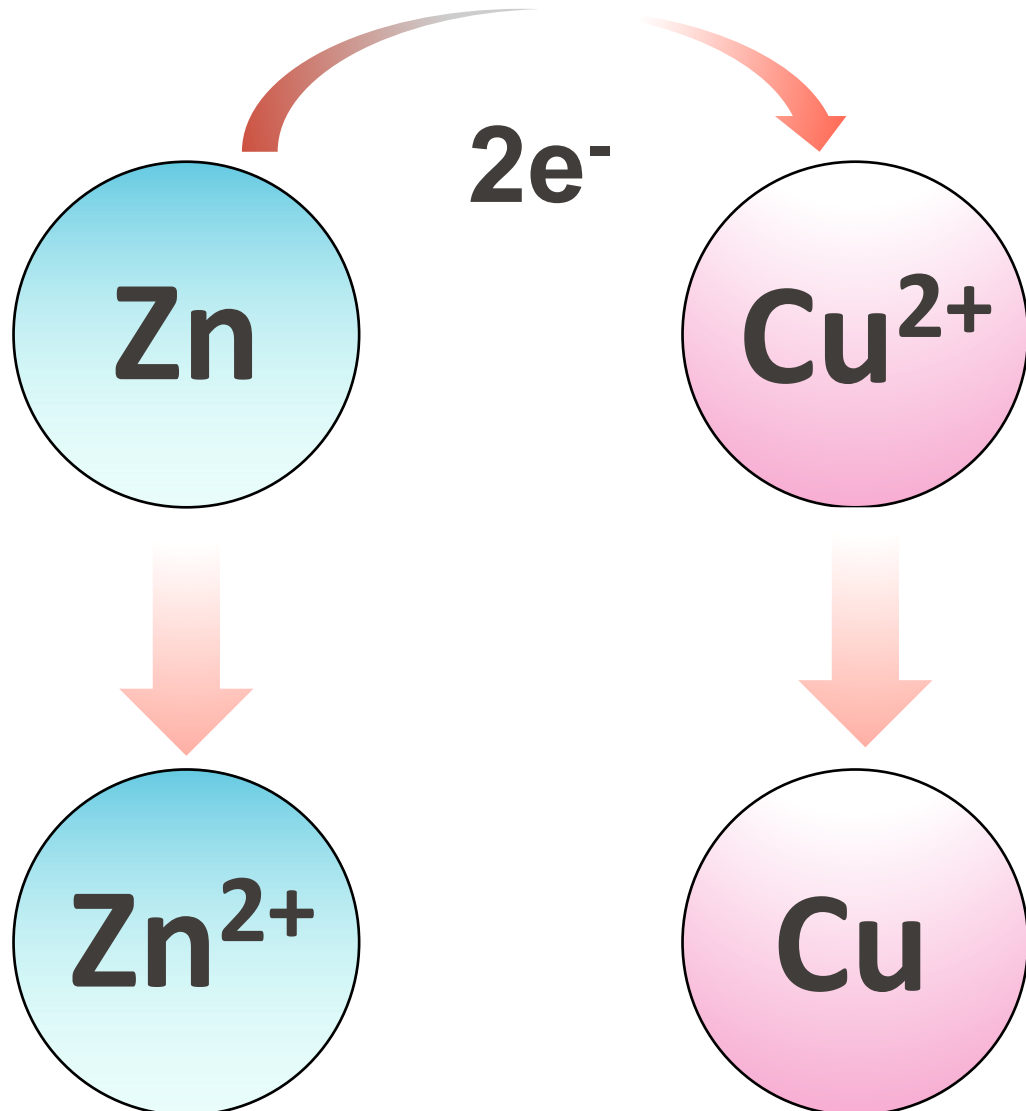


Electricity \rightarrow make certain chemical reactions happen

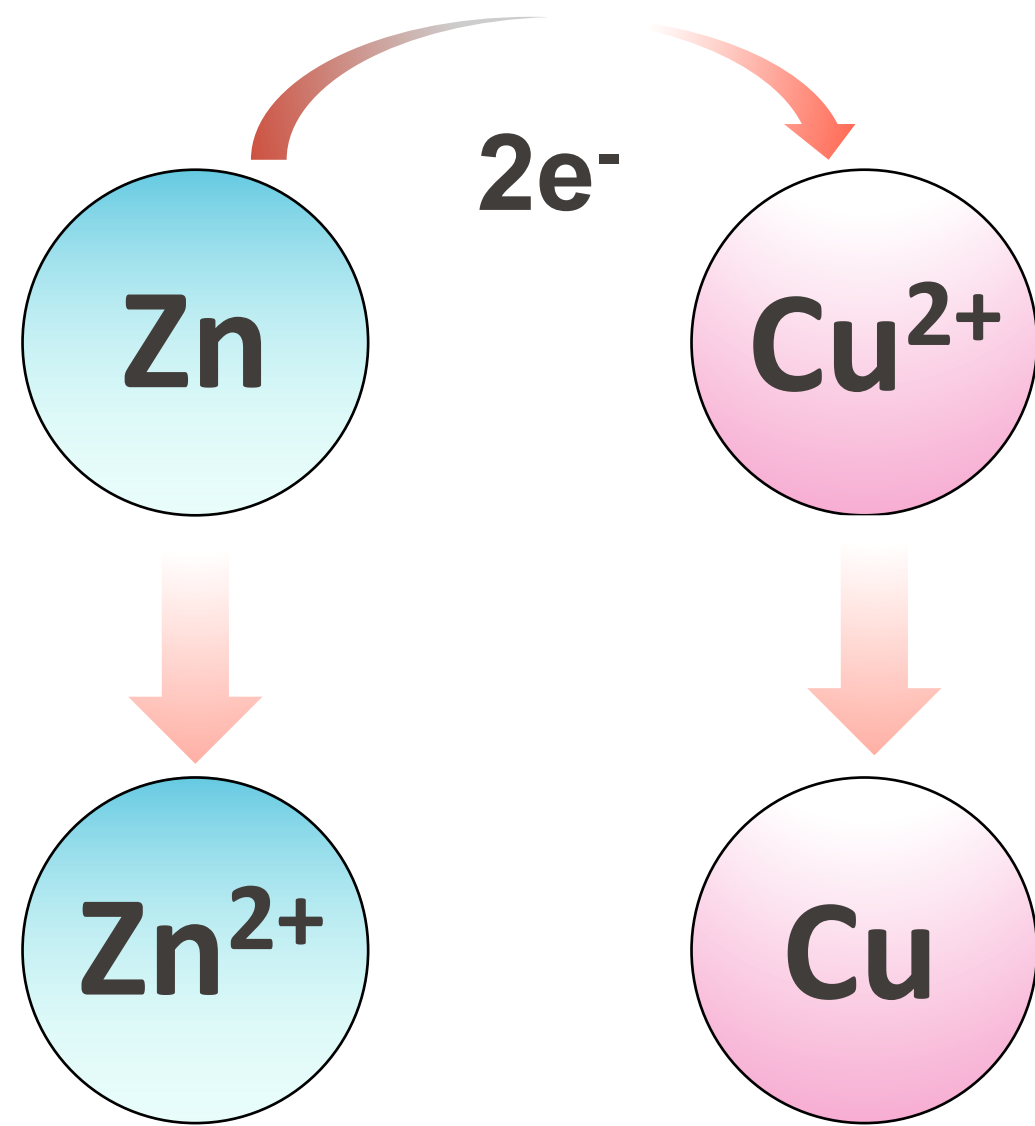


Non-spontaneous reaction

Galvanic/Voltaic Cells for Electrochemistry



Standard Reduction Potential of Different Metals



Stronger pull for electrons

Under standard conditions:

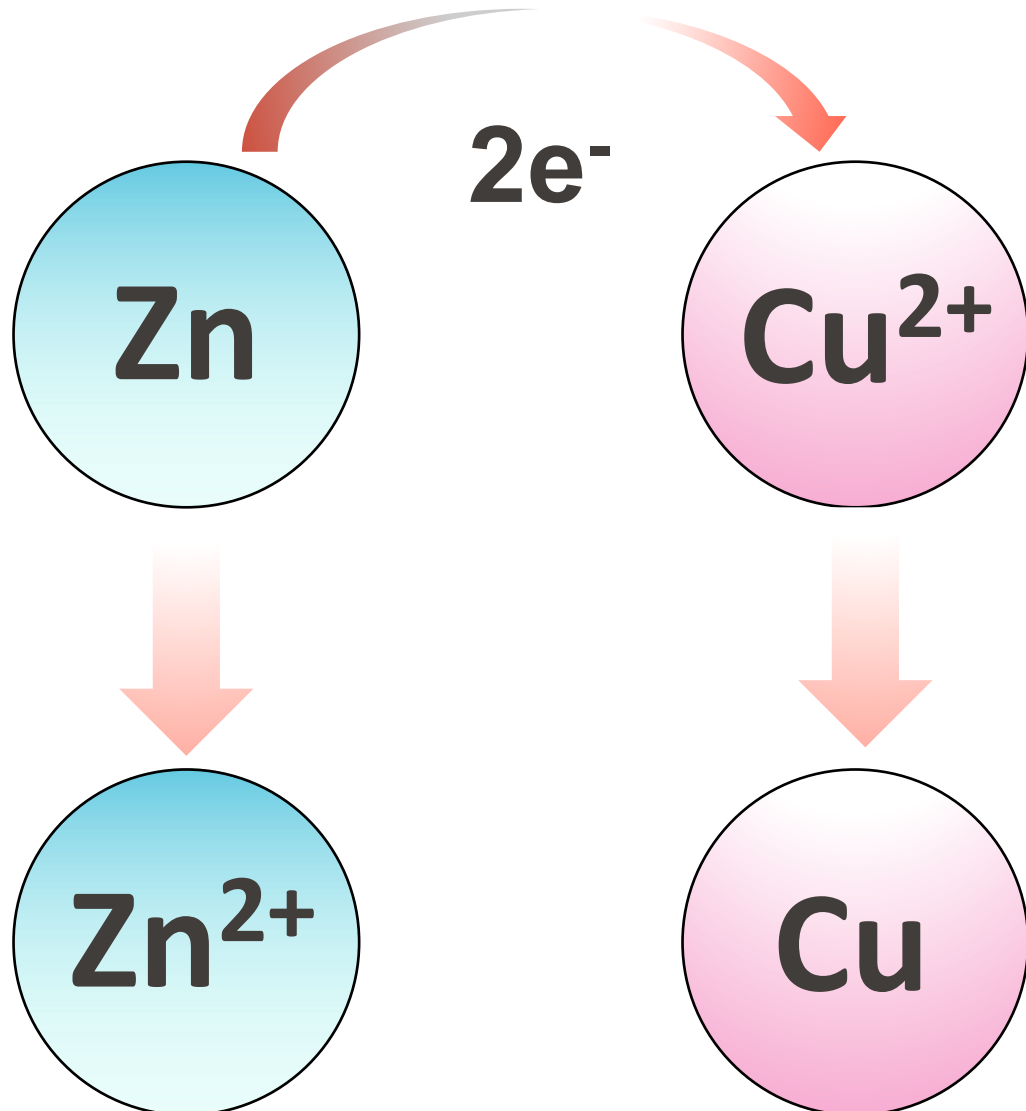
- 25 °C (298 K)
- 1 M concentration for reactants/products
- Relative to the standard hydrogen electrode (0.00 V)



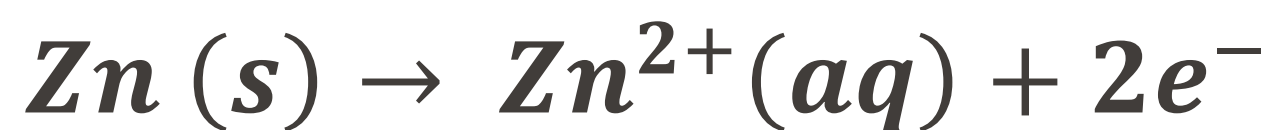
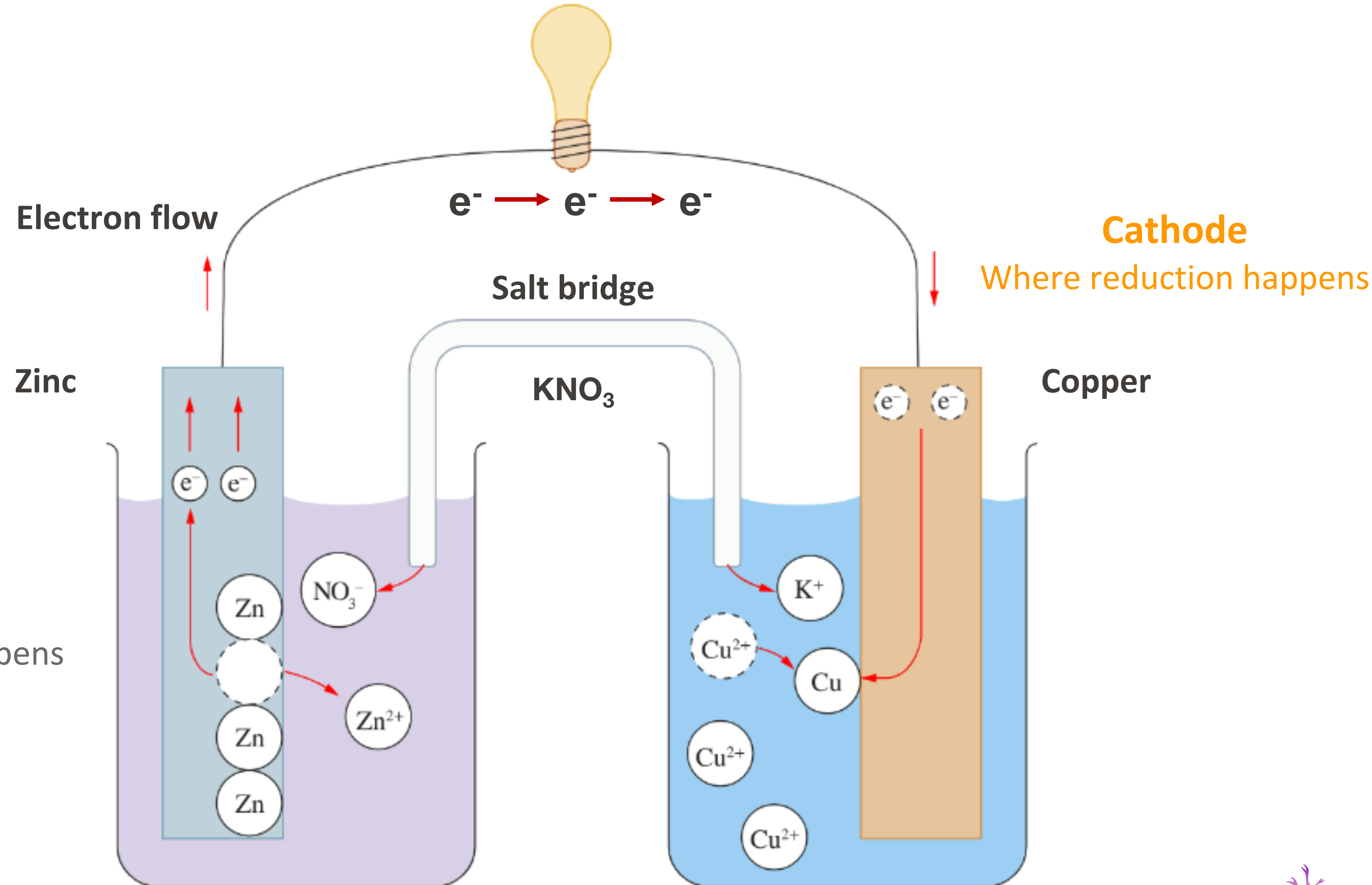
Half Reaction		potential
F₂	+ 2e ⁻ ⇌ 2F ⁻	+2.87 V
Pb⁴⁺	+ 2e ⁻ ⇌ Pb ²⁺	+1.67 V
Cl₂	+ 2e ⁻ ⇌ 2Cl ⁻	+1.36 V
Ag⁺	+ 1e ⁻ ⇌ Ag	+0.80 V
Fe³⁺	+ 1e ⁻ ⇌ Fe ²⁺	+0.77 V
Cu²⁺	+ 2e ⁻ ⇌ Cu	+0.34 V
2H⁺	+ 2e ⁻ ⇌ H ₂	0.00 V
Fe³⁺	+ 3e ⁻ ⇌ Fe	-0.04 V
Pb²⁺	+ 2e ⁻ ⇌ Pb	-0.13 V
Fe²⁺	+ 2e ⁻ ⇌ Fe	-0.44 V
Zn²⁺	+ 2e ⁻ ⇌ Zn	-0.76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



Anode
Where oxidation happens



Clark Electrode Measures Ambient Oxygen Partial Pressure

Clark Electrode was originally designed to measure oxygen concentration in blood



Leland Clark

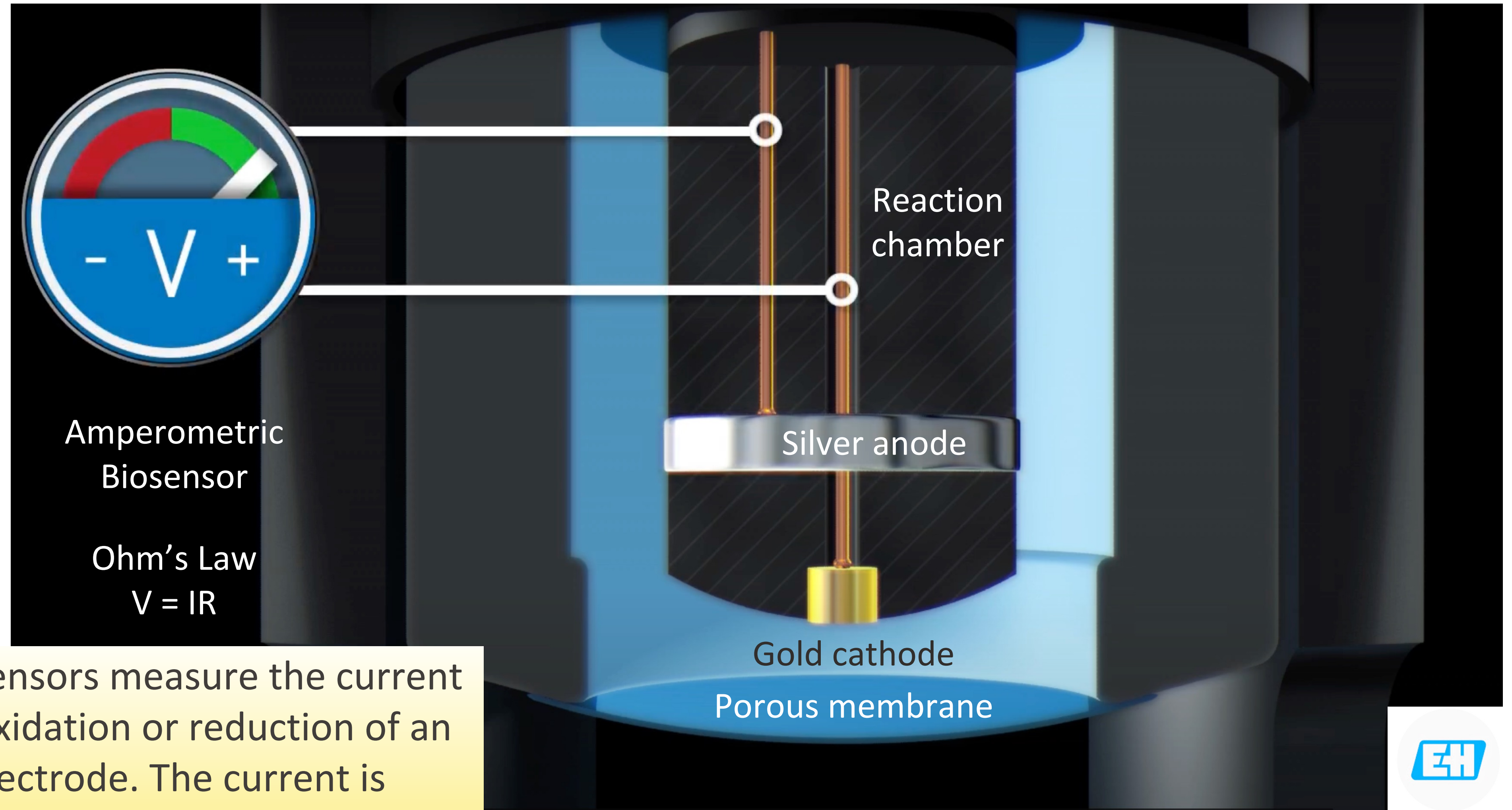
The "Father of Biosensors"



Amperometric
Biosensor

Ohm's Law
 $V = IR$

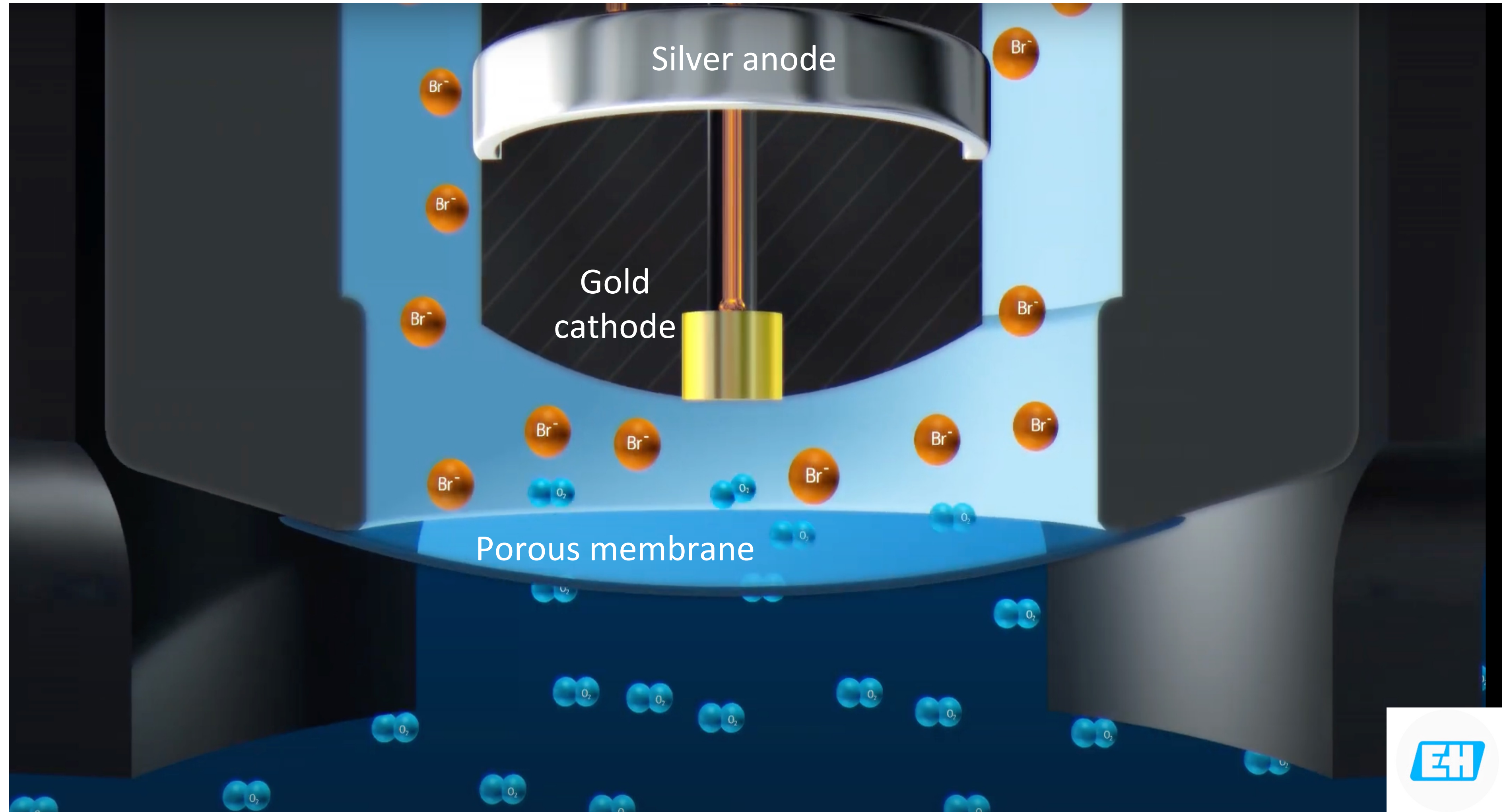
Amperometric biosensors measure the current generated by the oxidation or reduction of an analyte at an electrode. The current is proportional to the analyte concentration.



Clark Electrode Measures Ambient Oxygen Partial Pressure



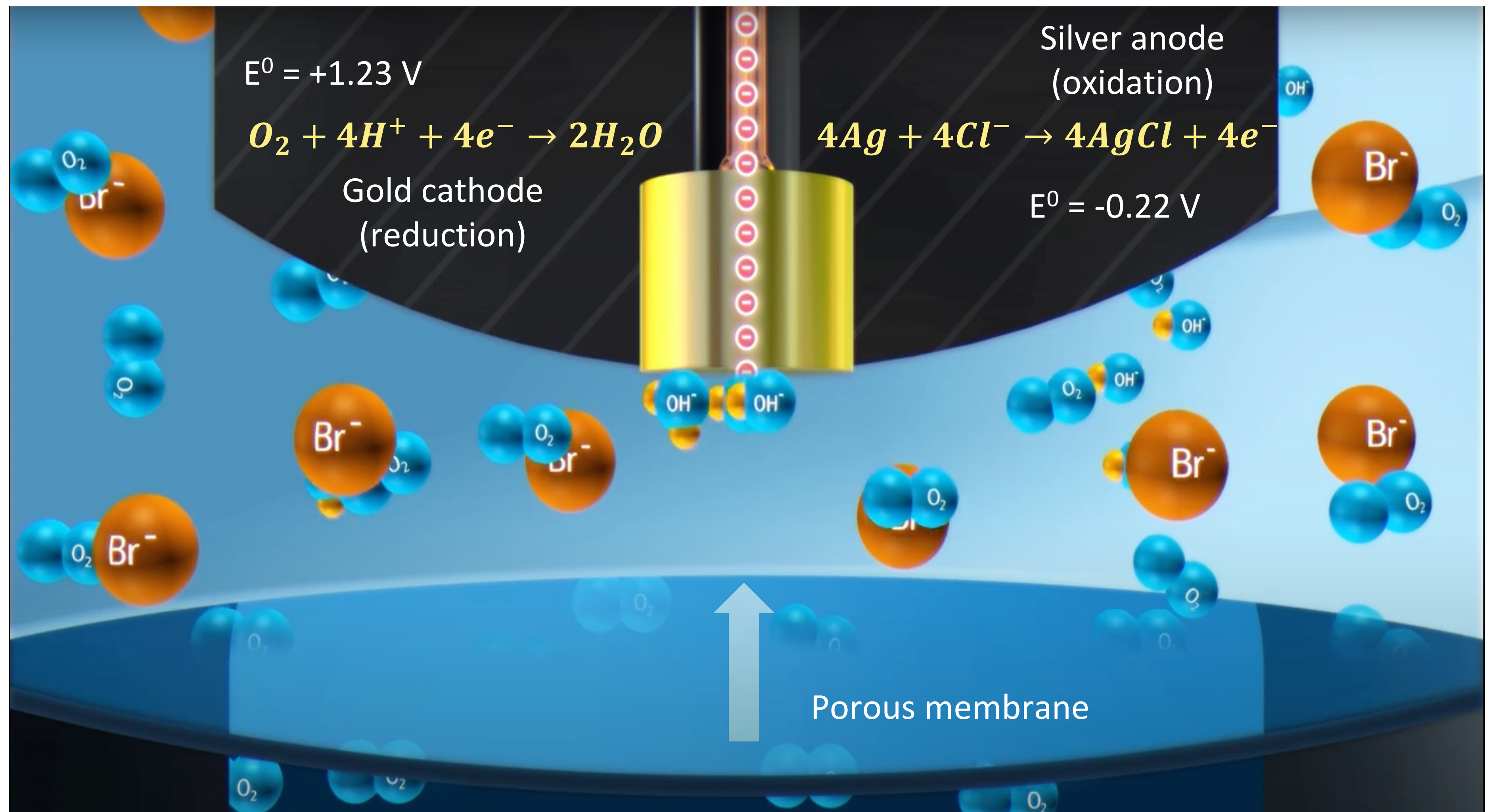
Leland Clark
The “Father of Biosensors”



Clark Electrode Measures Ambient Oxygen Partial Pressure

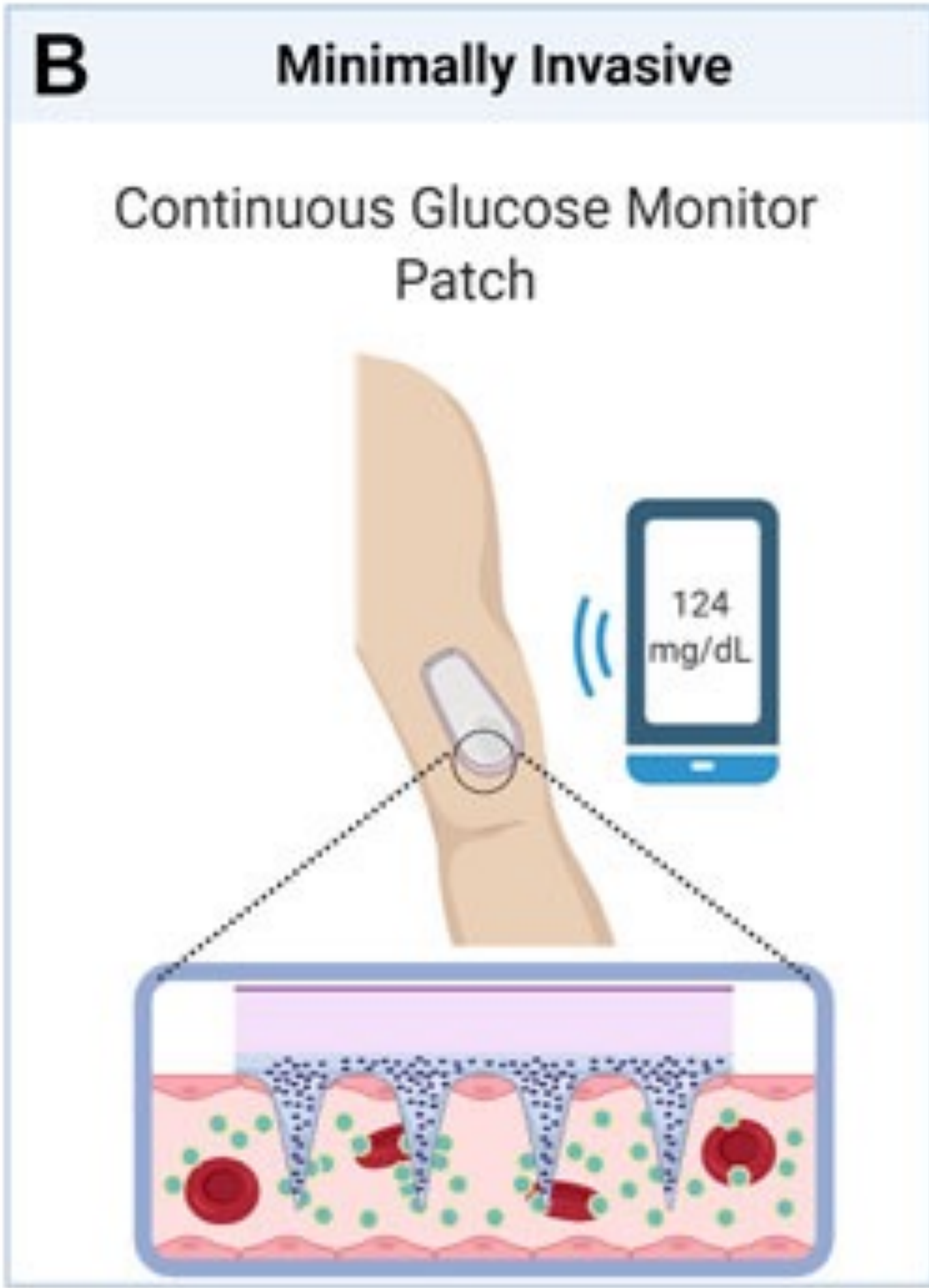
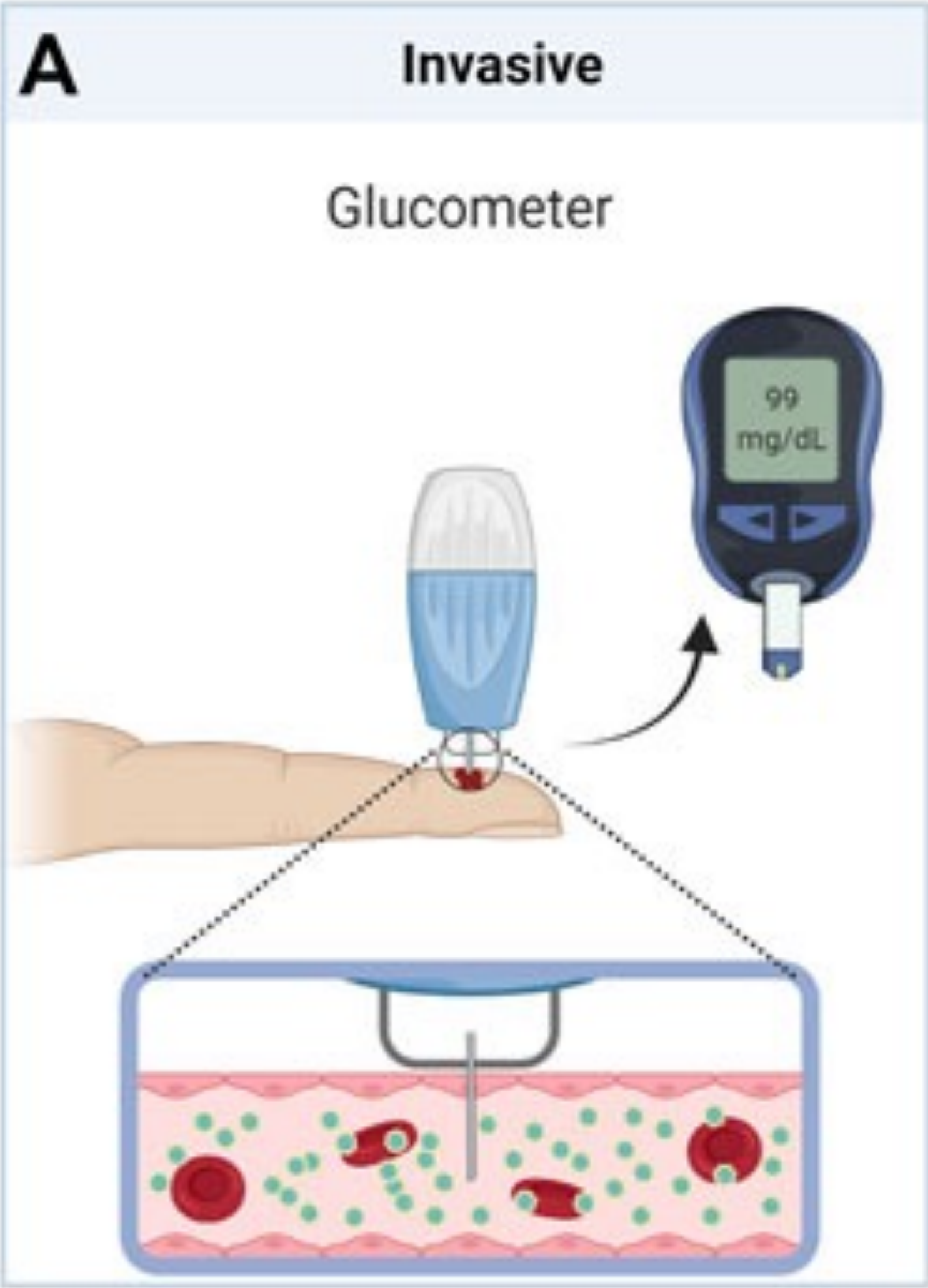
Redox processes are highly favorable so **spontaneous process**

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



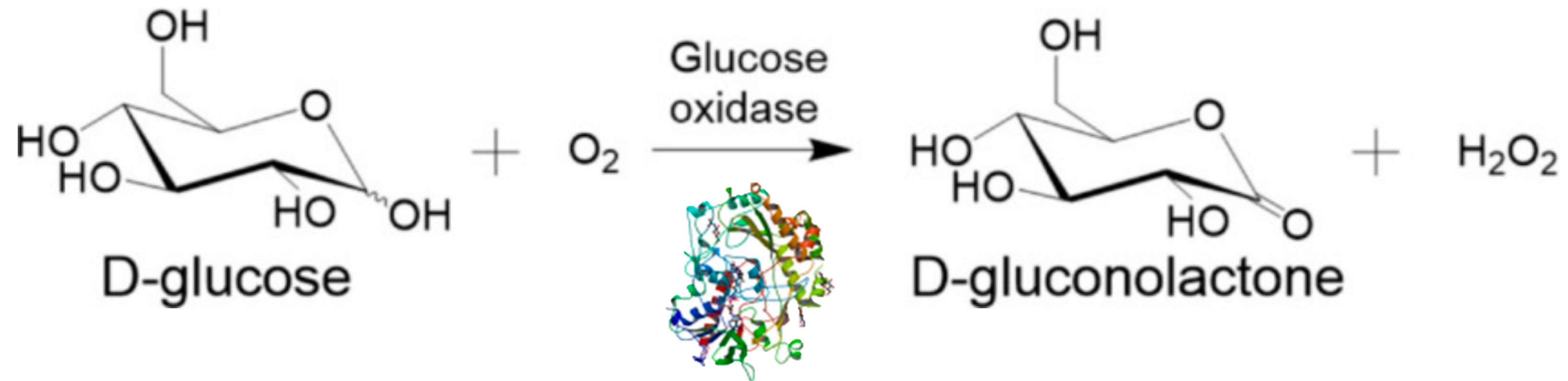
This work became the basis for the first glucose sensor

Glucose Biosensor – Transformative for Human Health



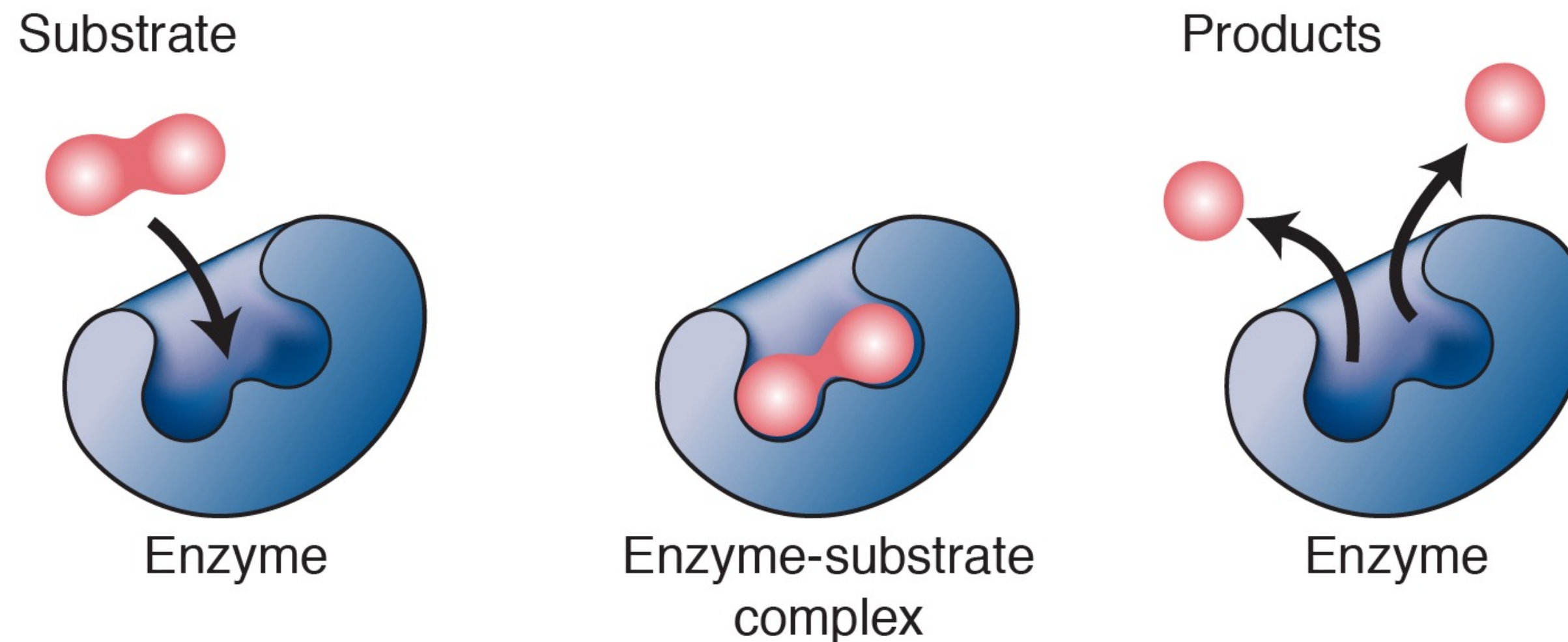
Todaro et al. | *Front. Chem.* | 2022

Glucose Sensor – Incorporating an Enzyme (Biological Catalyst)

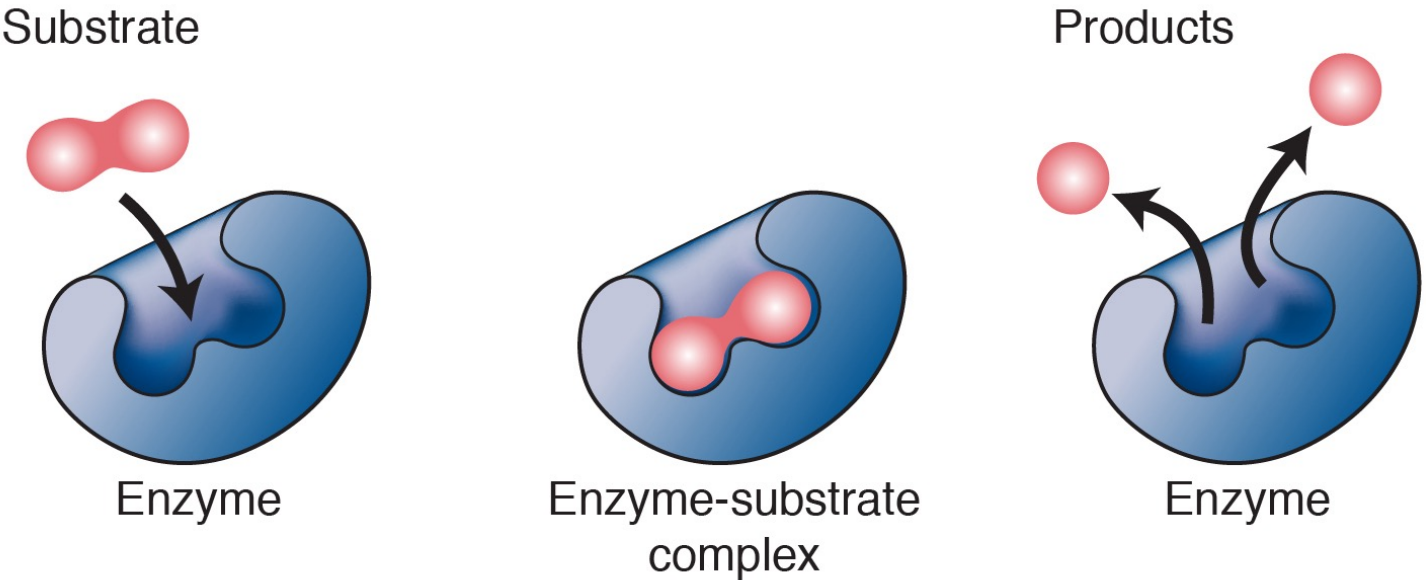
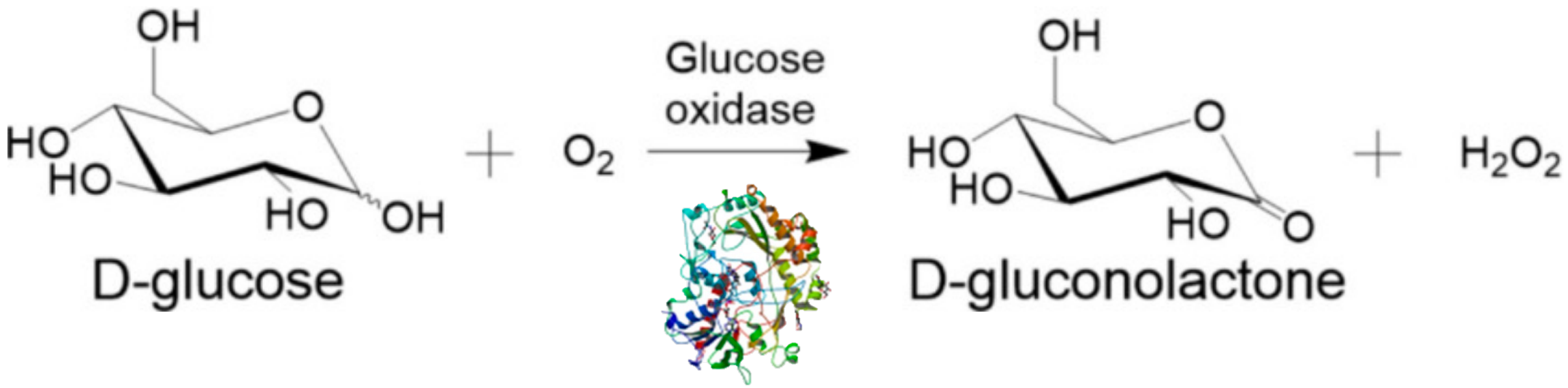


Catalyst: substance that accelerates the reaction rate without being consumed in the process

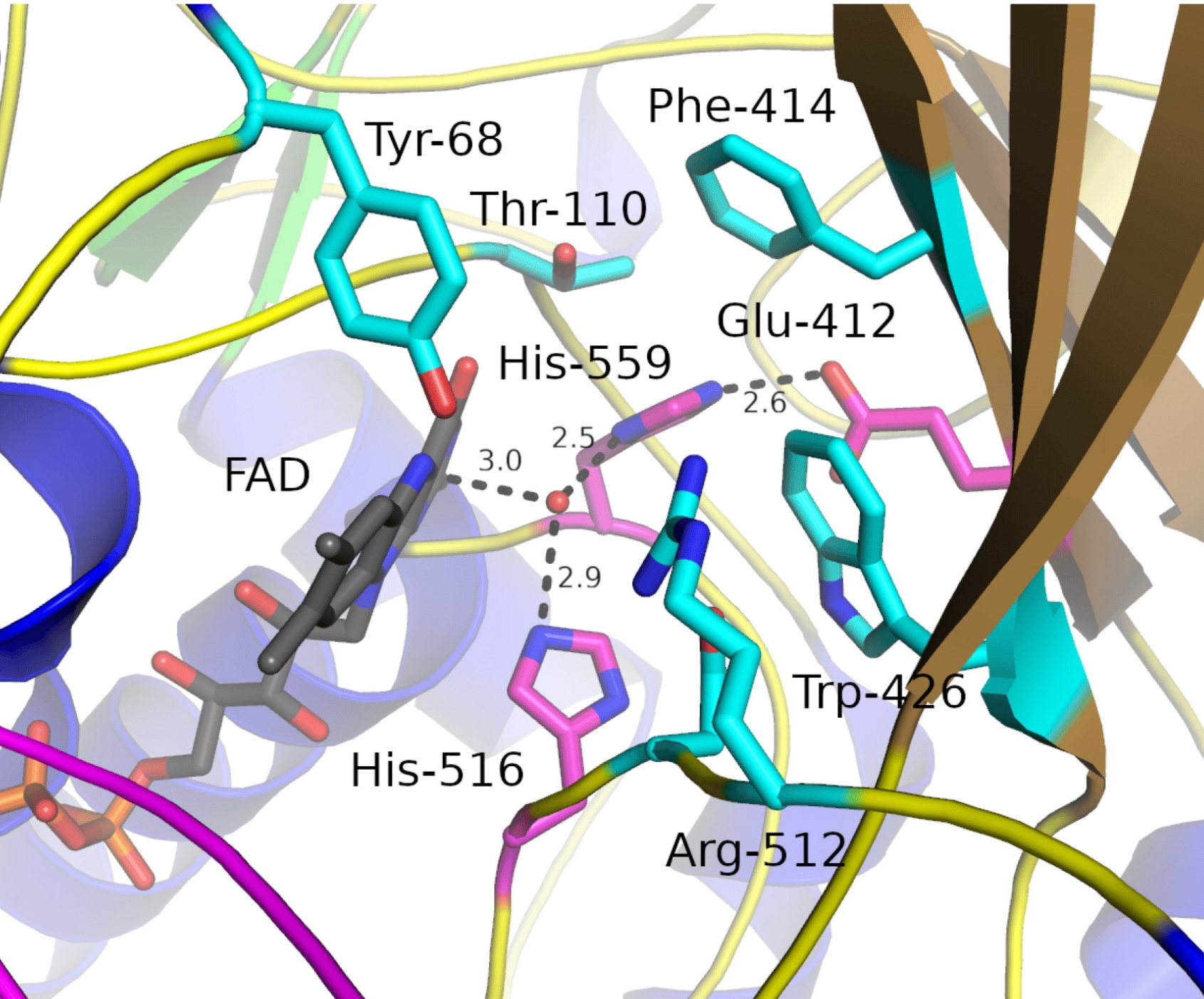
Catalysis: affects kinetics and not thermodynamics (it makes kinetics faster by decreasing activation energy)



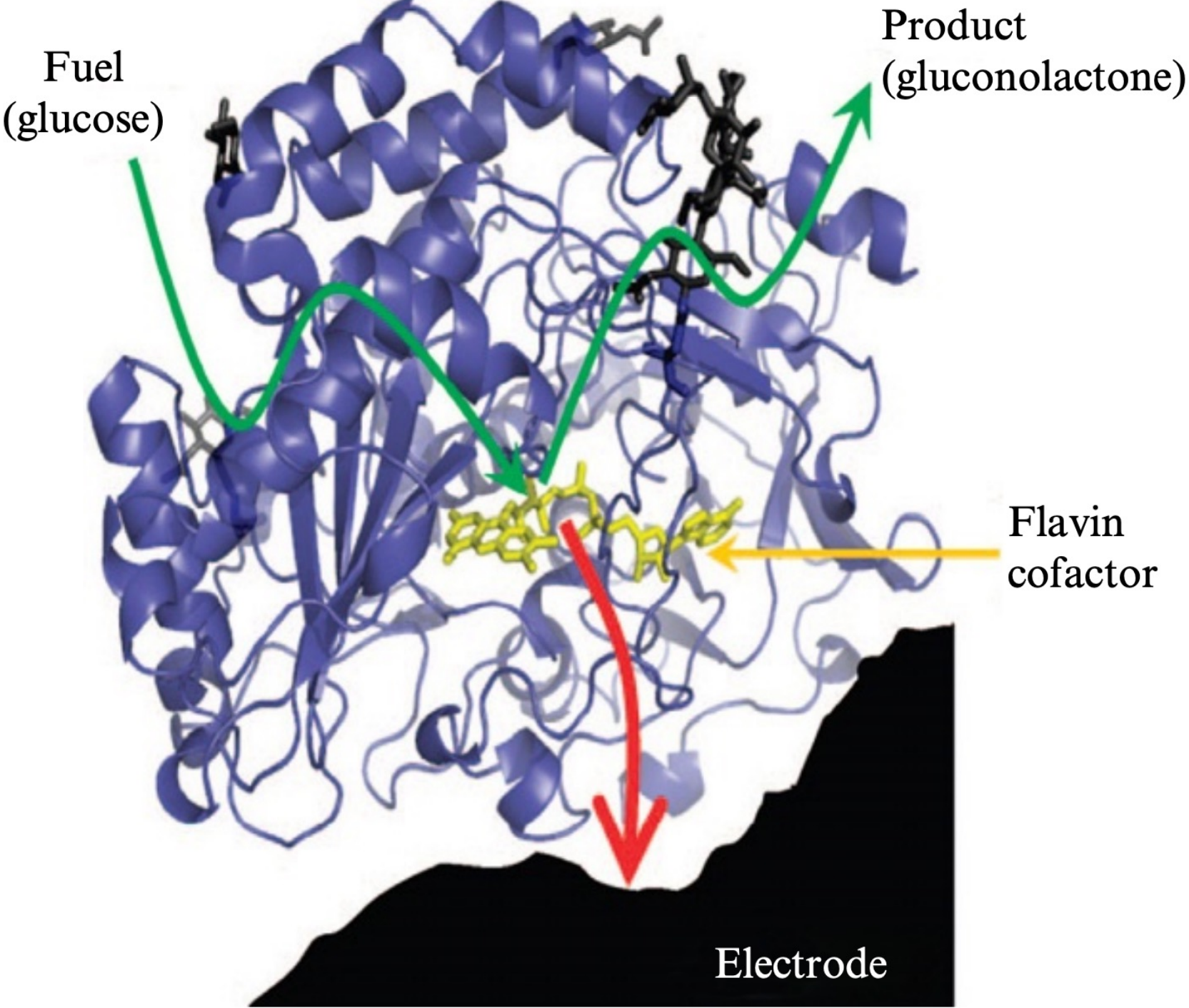
Intermolecular Interactions Between Enzyme and Substrate



Active site of glucose oxidase

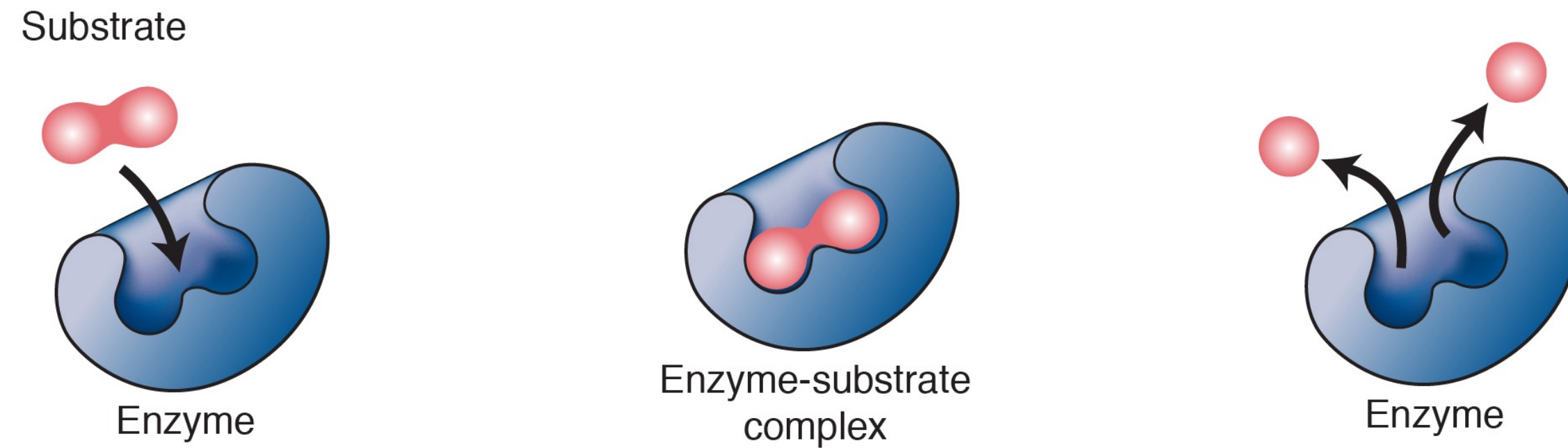


Bauer | *Biomolecules* | 2022

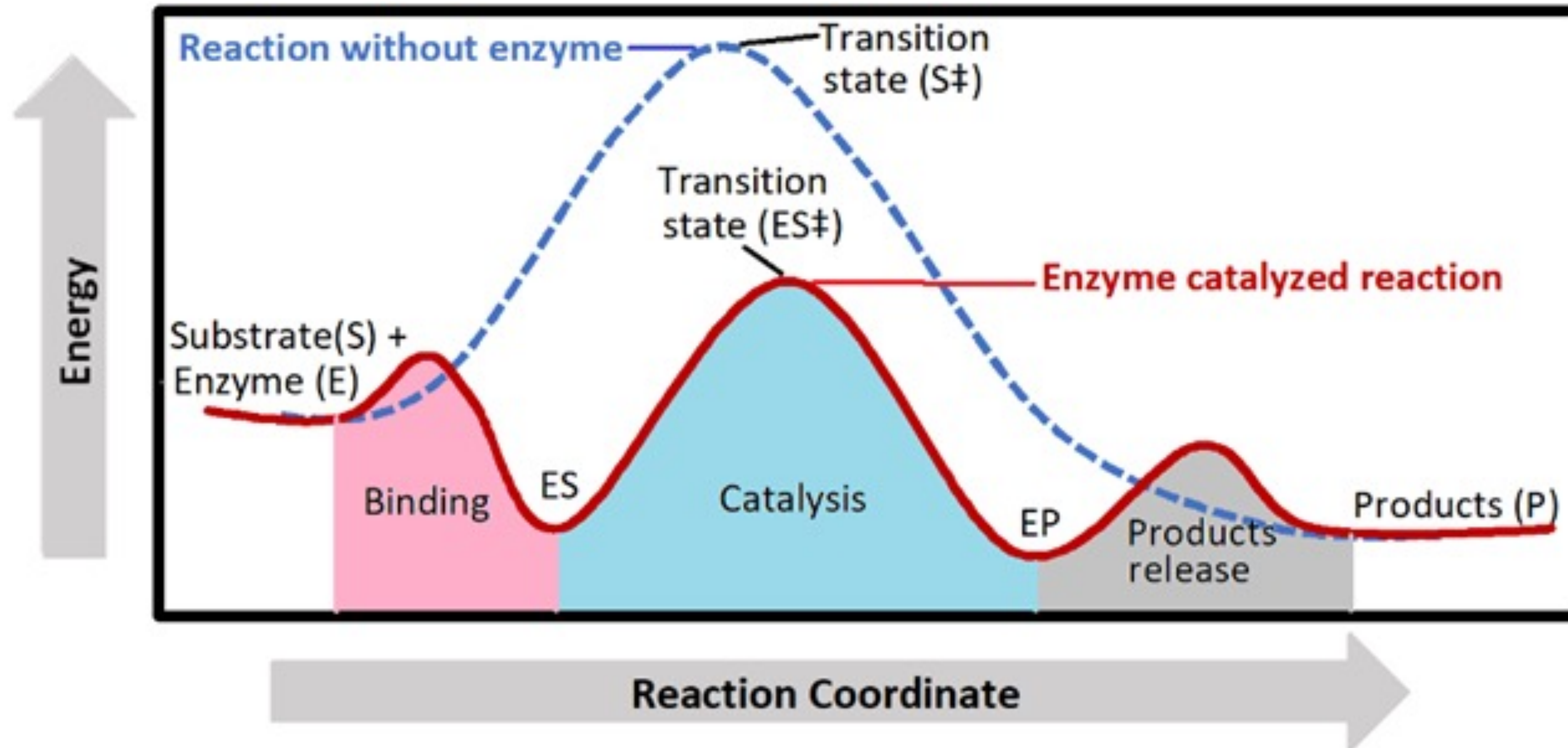


Viswanathan | *Methods Enzym.* | 2012

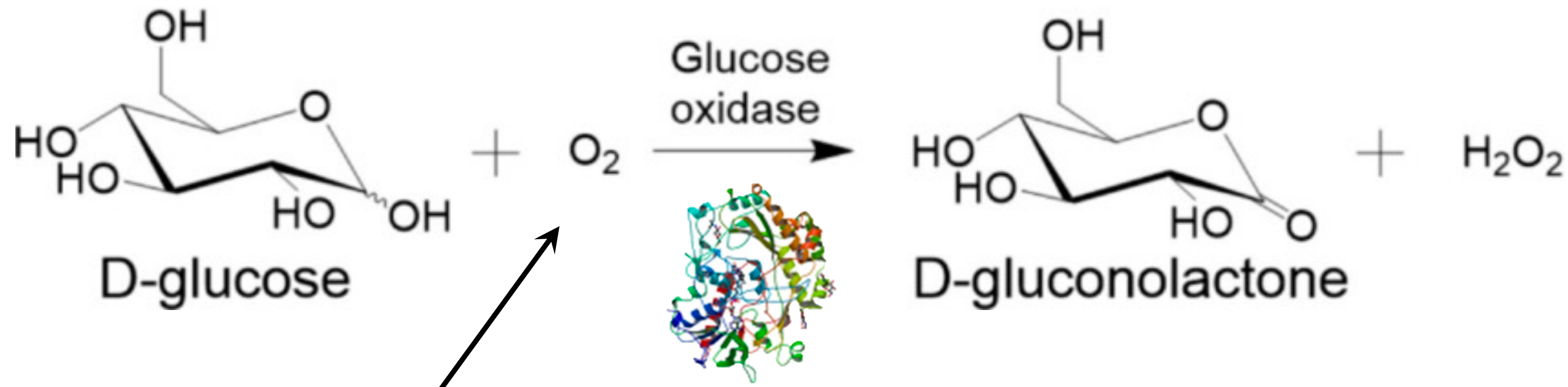
How Can Enzymes Catalyze Reactions?



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



Glucose Sensor – Detection of Hydrogen Peroxide as Readout



Monitor decrease in O₂
as proxy for glucose

Monitor increase in H₂O₂ as
proxy for glucose

Lack of **sensitivity** – small signal change since O₂ levels high in biofluids

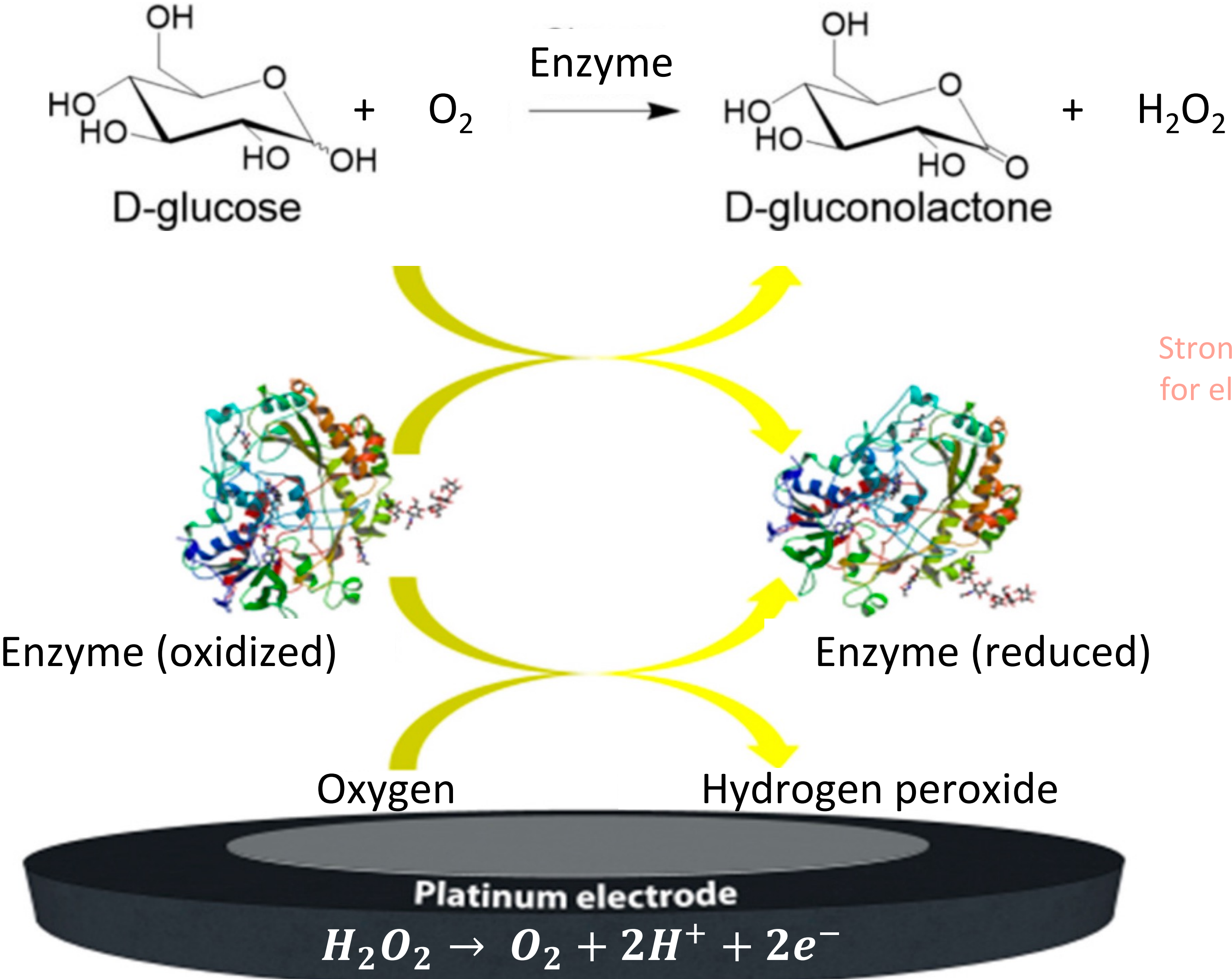
Higher **sensitivity** – H₂O₂ not abundant in the sample prior to enzymatic reaction

Lack of **specificity** – O₂ sensors influenced by changes unrelated to glucose (respiratory, temperature)

Higher **specificity** – H₂O₂ is direct product of enzymatic reaction

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

Oxidation of H₂O₂ on the Surface of Platinum Electrodes



↑ Stronger pull for electrons

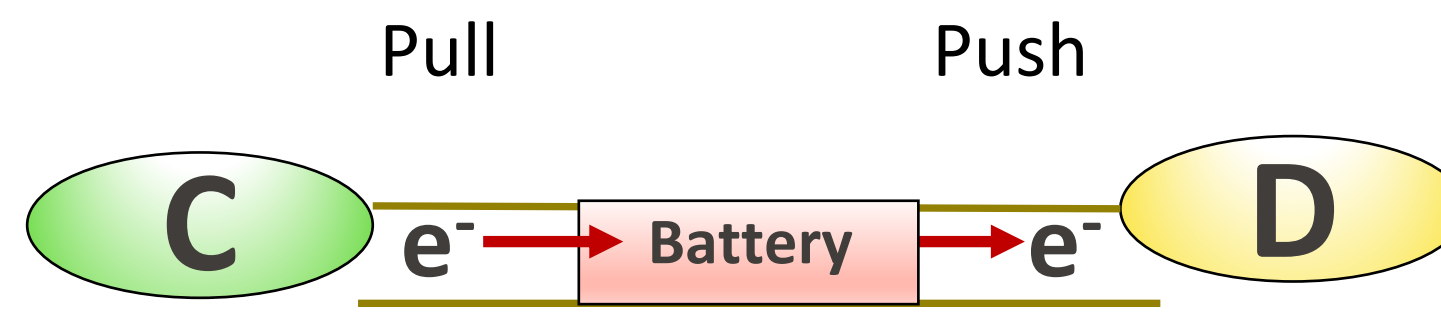
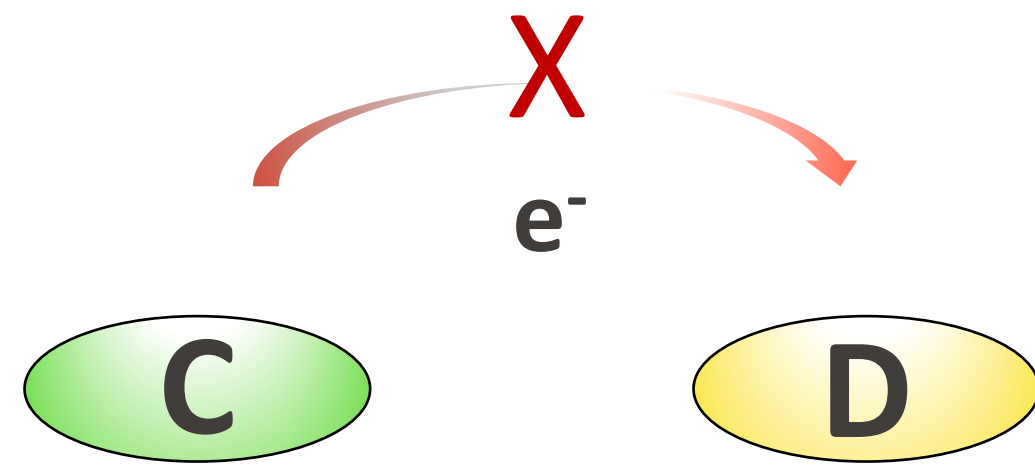
Half Reaction	potential
$\text{F}_2 + 2\text{e}^- \rightleftharpoons 2\text{F}^-$	+2.87 V
$\text{Pb}^{4+} + 2\text{e}^- \rightleftharpoons \text{Pb}^{2+}$	+1.67 V
$\text{Cl}_2 + 2\text{e}^- \rightleftharpoons 2\text{Cl}^-$	+1.36 V
$\text{Ag}^+ + 1\text{e}^- \rightleftharpoons \text{Ag}$	+0.80 V
$\text{Fe}^{3+} + 1\text{e}^- \rightleftharpoons \text{Fe}^{2+}$	+0.77 V
$\text{Cu}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cu}$	+0.34 V
$2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2$	0.00 V

Standard reduction potential for H₂O₂ : E⁰ = +0.695 V

So E_{oxidation} = -0.695 V

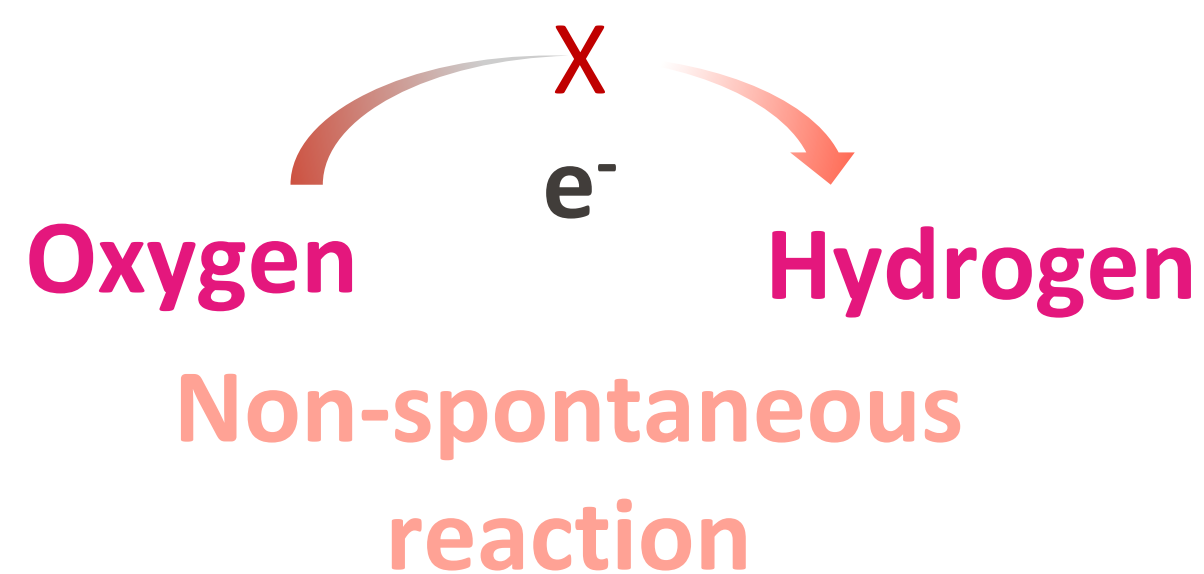
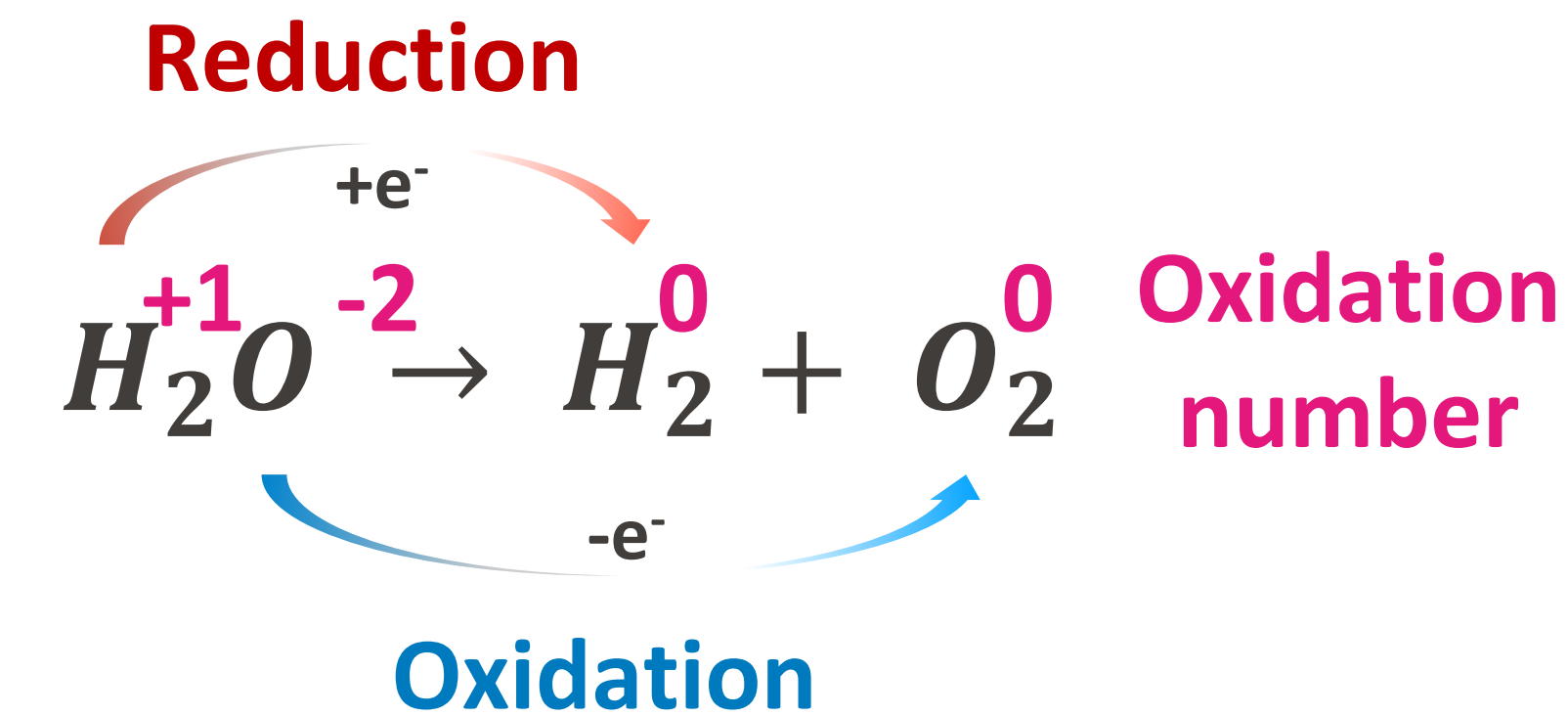
The oxidation process is non-spontaneous!

Electrolysis – Driving Chemical Reactions with Electricity



Non-spontaneous reaction

Water splitting



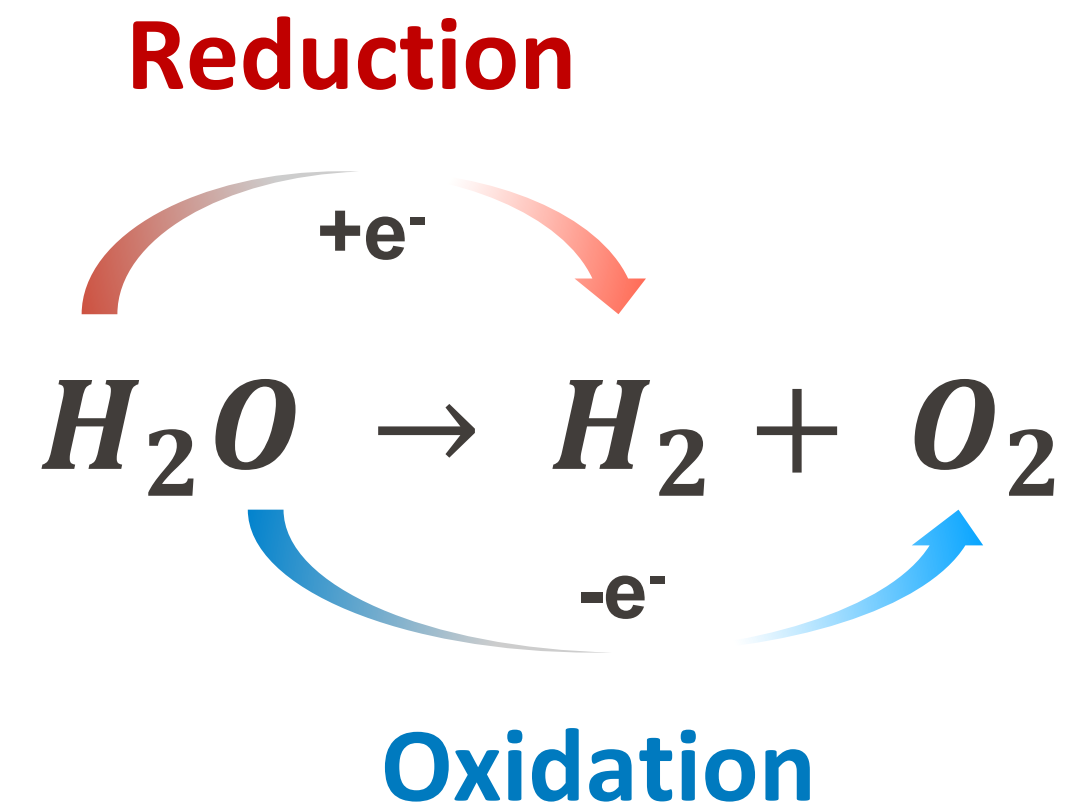
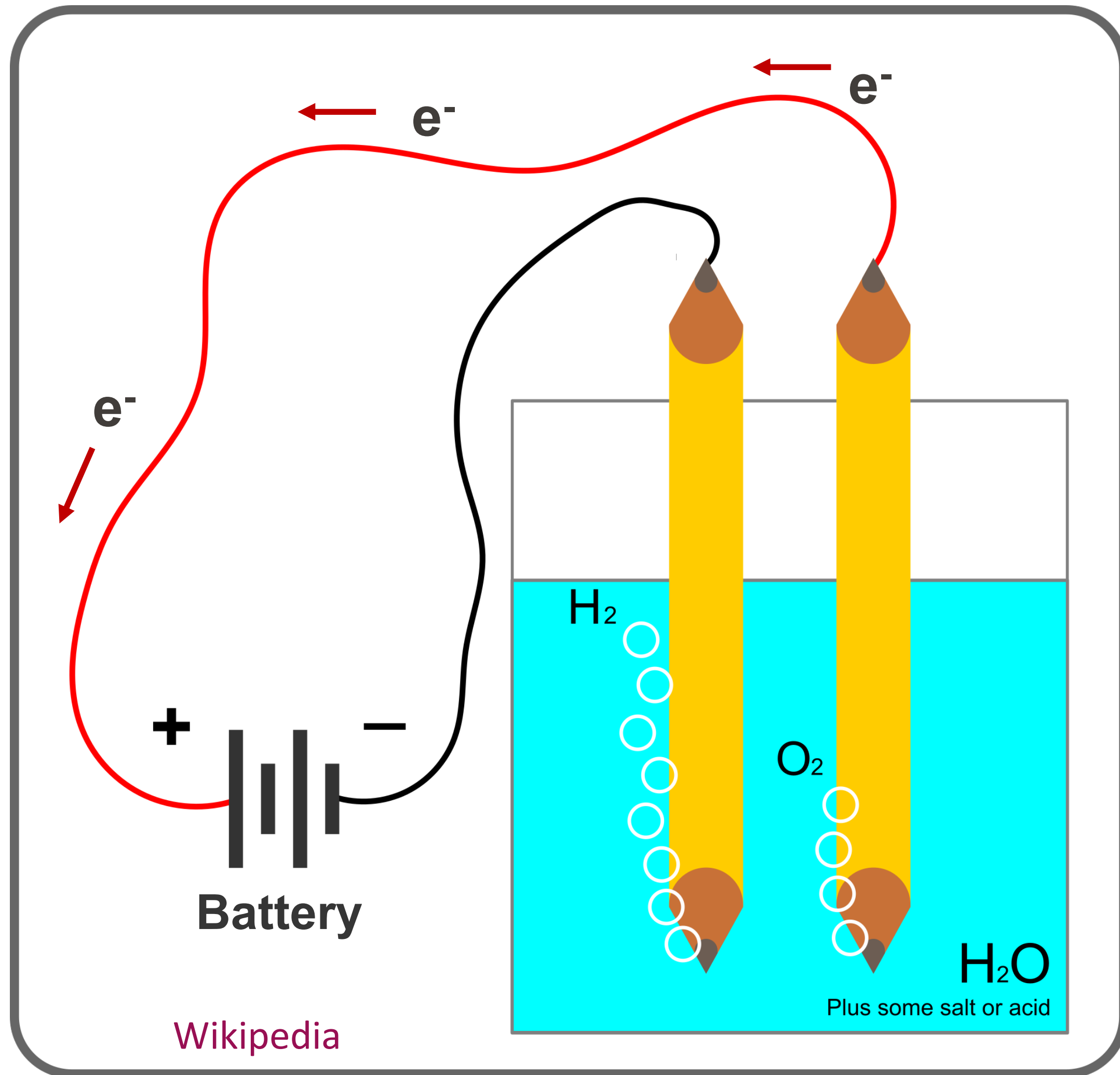
Half Reaction	potential
$F_2 + 2e^- \rightleftharpoons 2F^-$	+2.87 V
$Pb^{4+} + 2e^- \rightleftharpoons Pb^{2+}$	+1.67 V
$O_2 + 2e^- + 4H^+ \rightleftharpoons 2H_2O$	+1.23 V
$Ag^+ + 1e^- \rightleftharpoons Ag$	+0.80 V
$Fe^{3+} + 1e^- \rightleftharpoons Fe^{2+}$	+0.77 V
$Cu^{2+} + 2e^- \rightleftharpoons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

Stronger pull for electrons

Increasing

We can force this reaction to happen using electricity

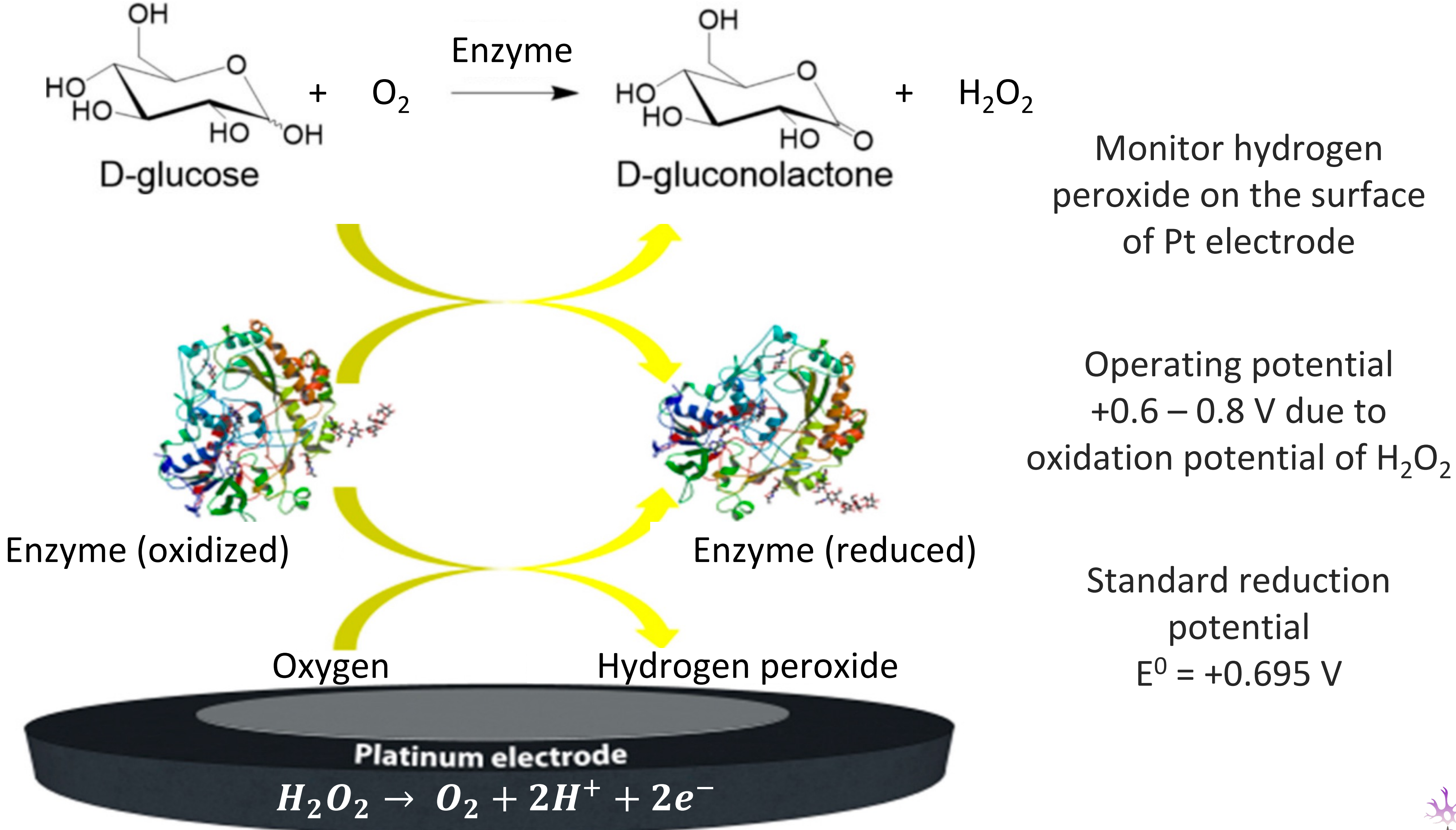
Electrolytic Cell – Driving Chemical Reactions with Electricity



Applying a potential drives non-spontaneous redox reaction

Operating potential applied to overcome activation energy barrier to drive reaction

Glucose Sensor Requires an Operating Potential

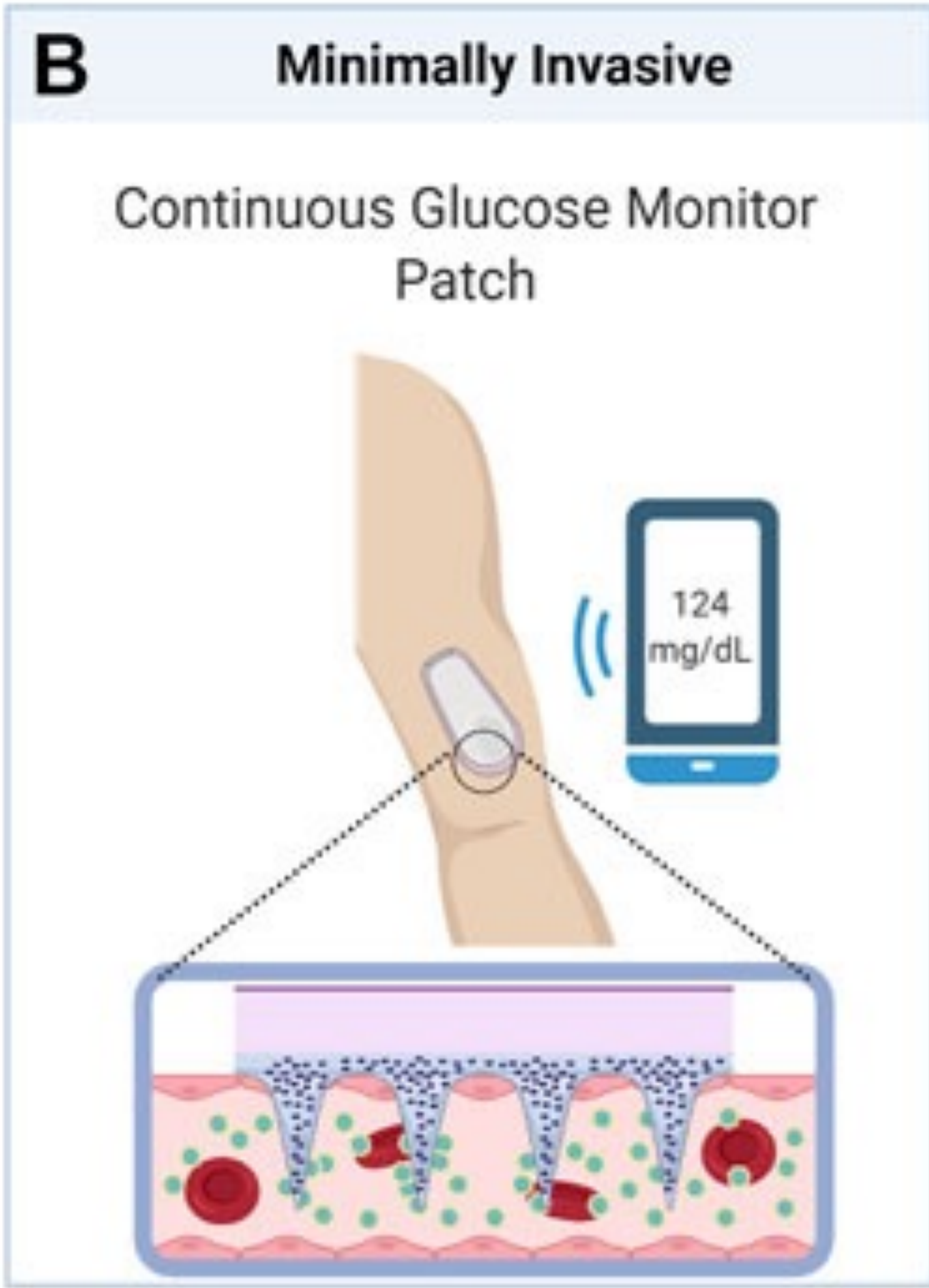
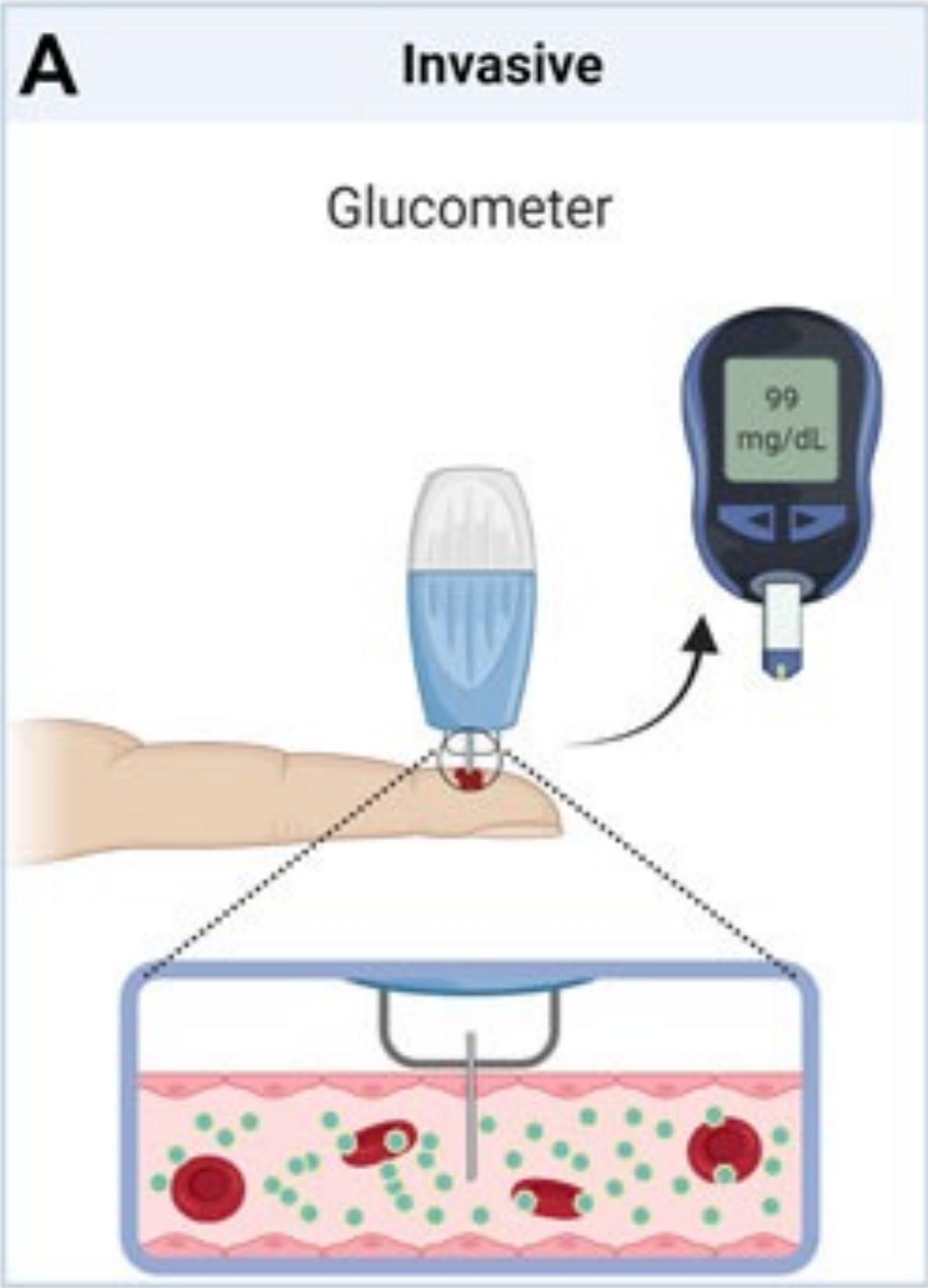


Monitor hydrogen peroxide on the surface of Pt electrode

Operating potential +0.6 – 0.8 V due to oxidation potential of H_2O_2

Standard reduction potential $E^0 = +0.695 \text{ V}$

Glucose Biosensor – Transformative for Human Health



Todaro et al. | *Front. Chem.* | 2022

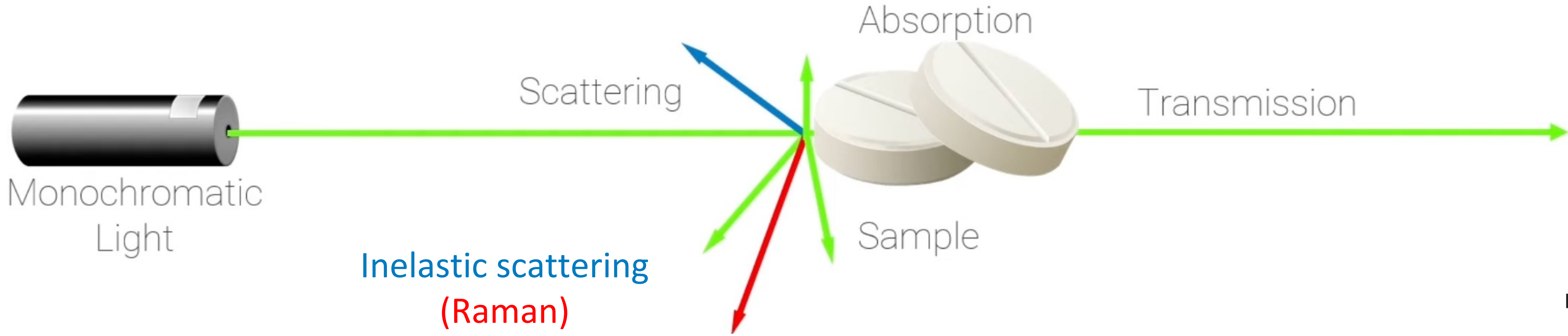
Raman Spectroscopy for Non-Invasive Glucose Detection



Chandrasekhara Venkata Raman



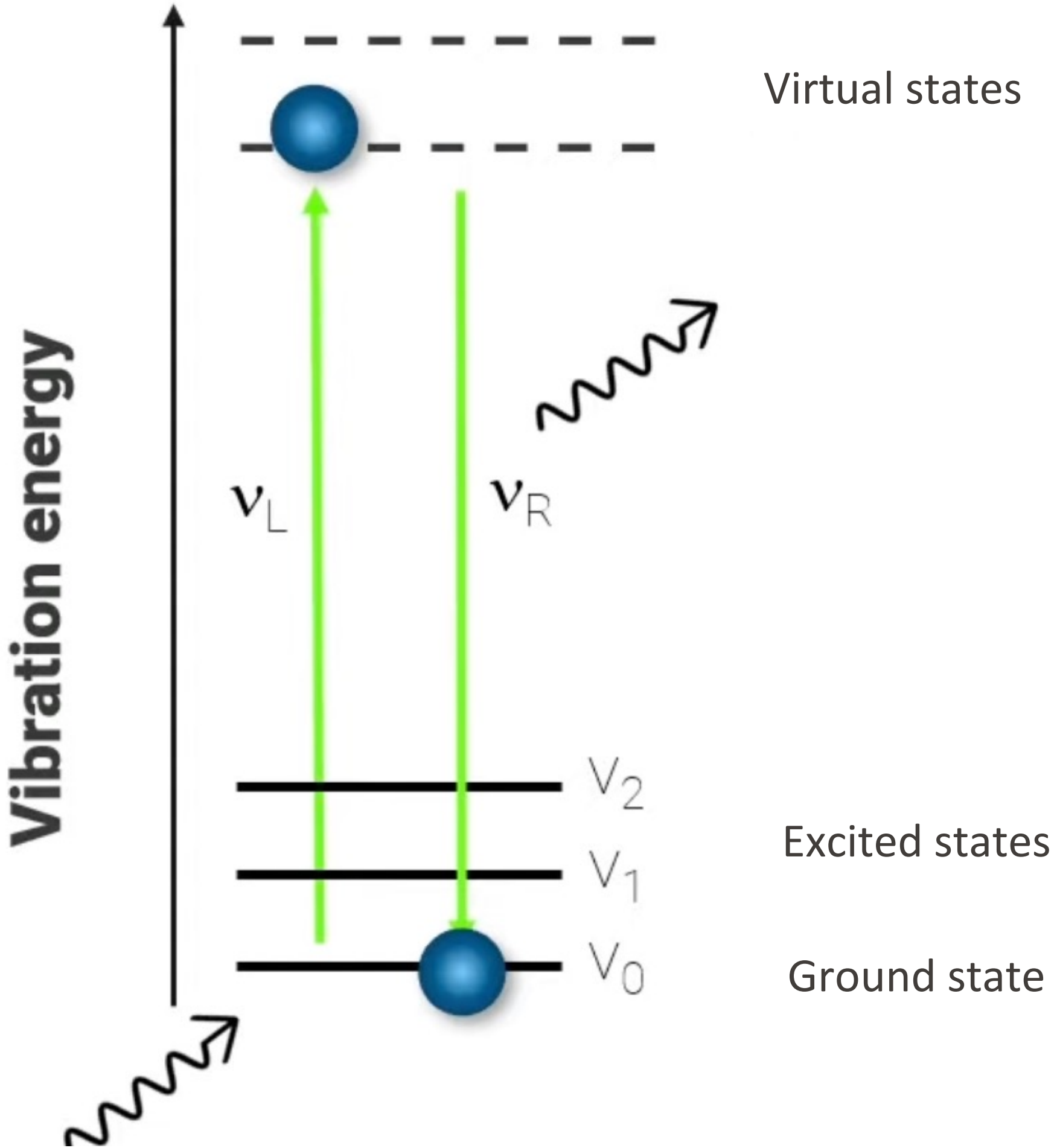
Nobel Prize in Physics (1930)



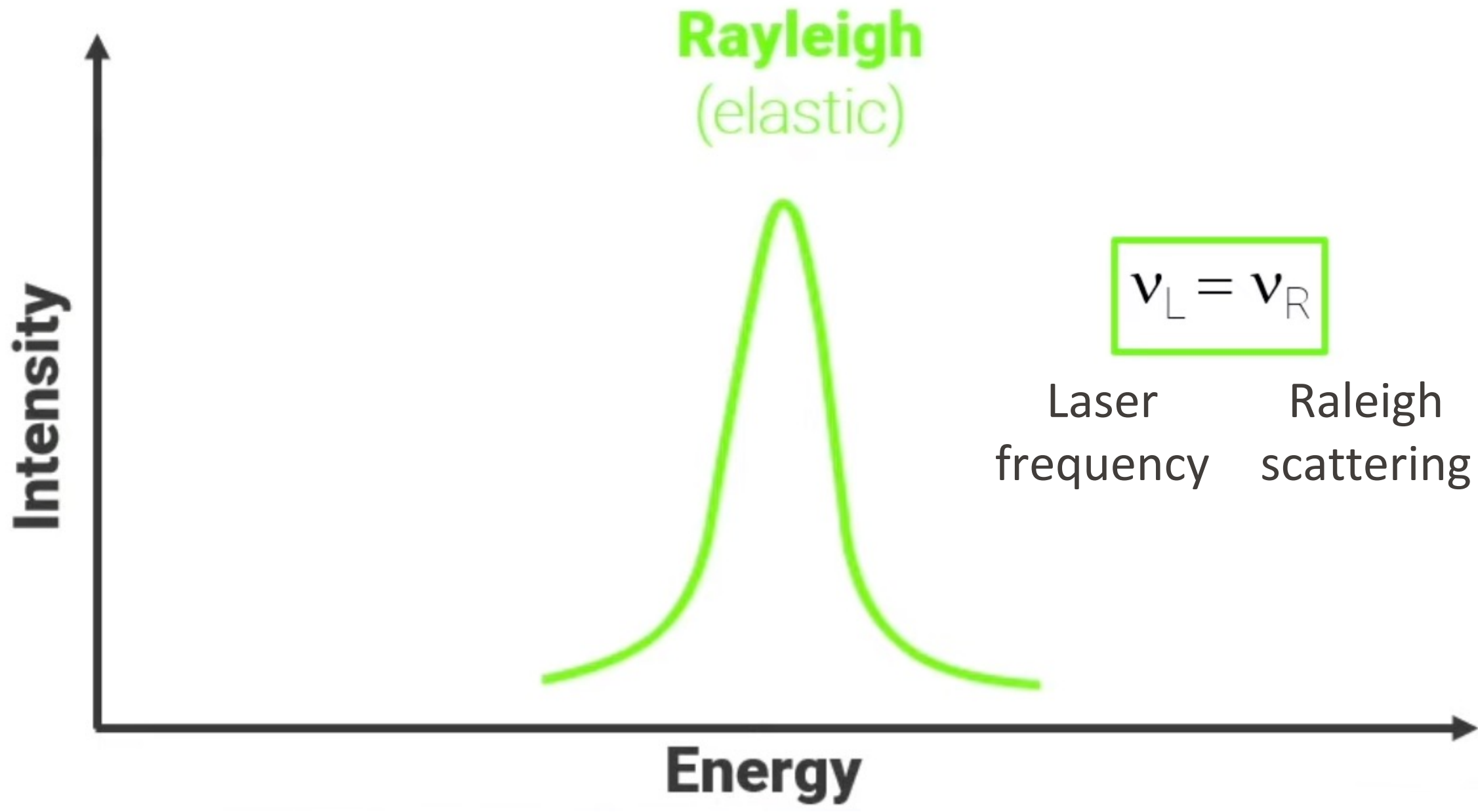
Todaro et al. | Front. Chem. | 2022

Raleigh Scattering: Molecule Falls to Ground State

Elastic scattering (Raleigh)

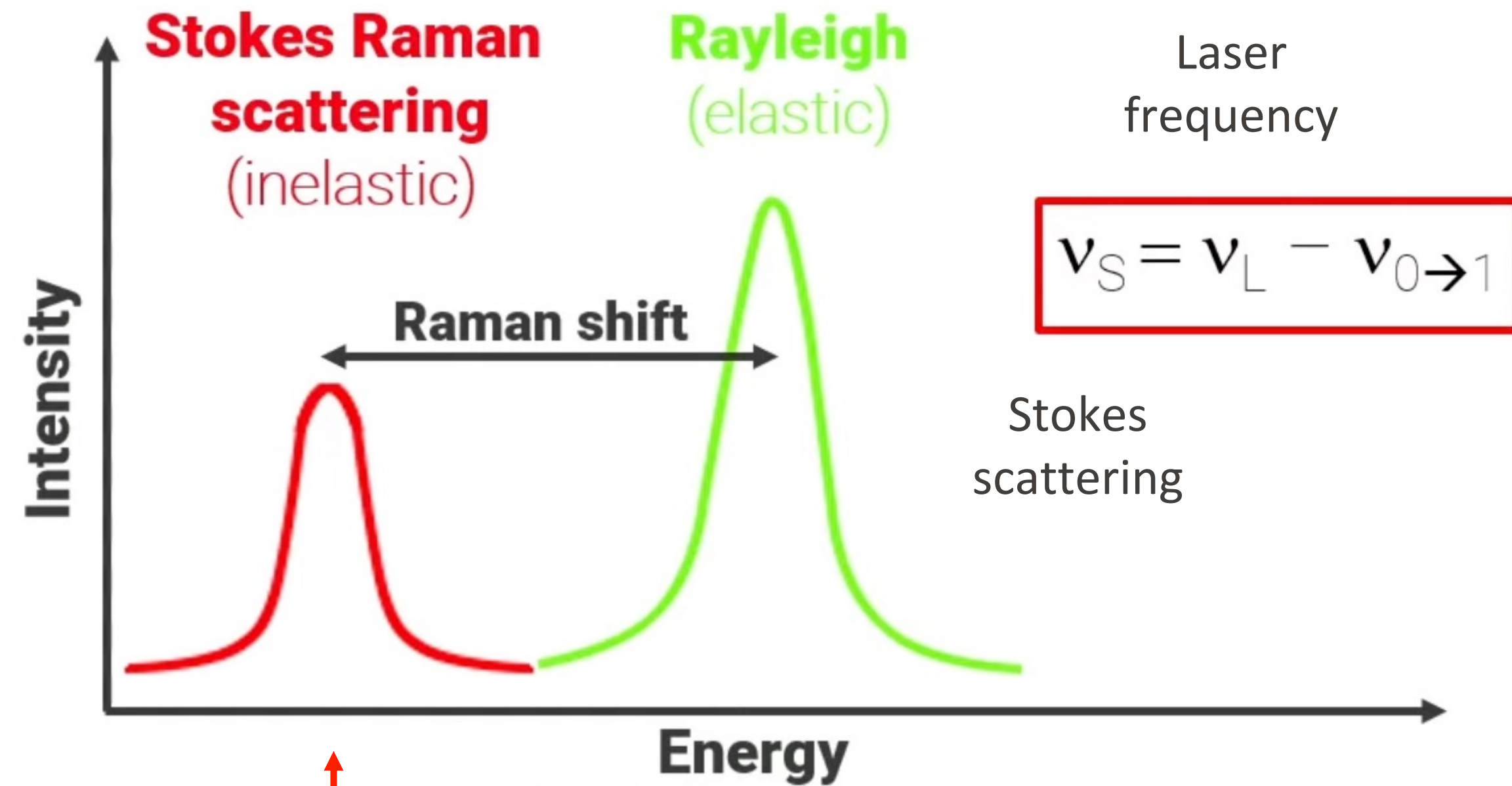
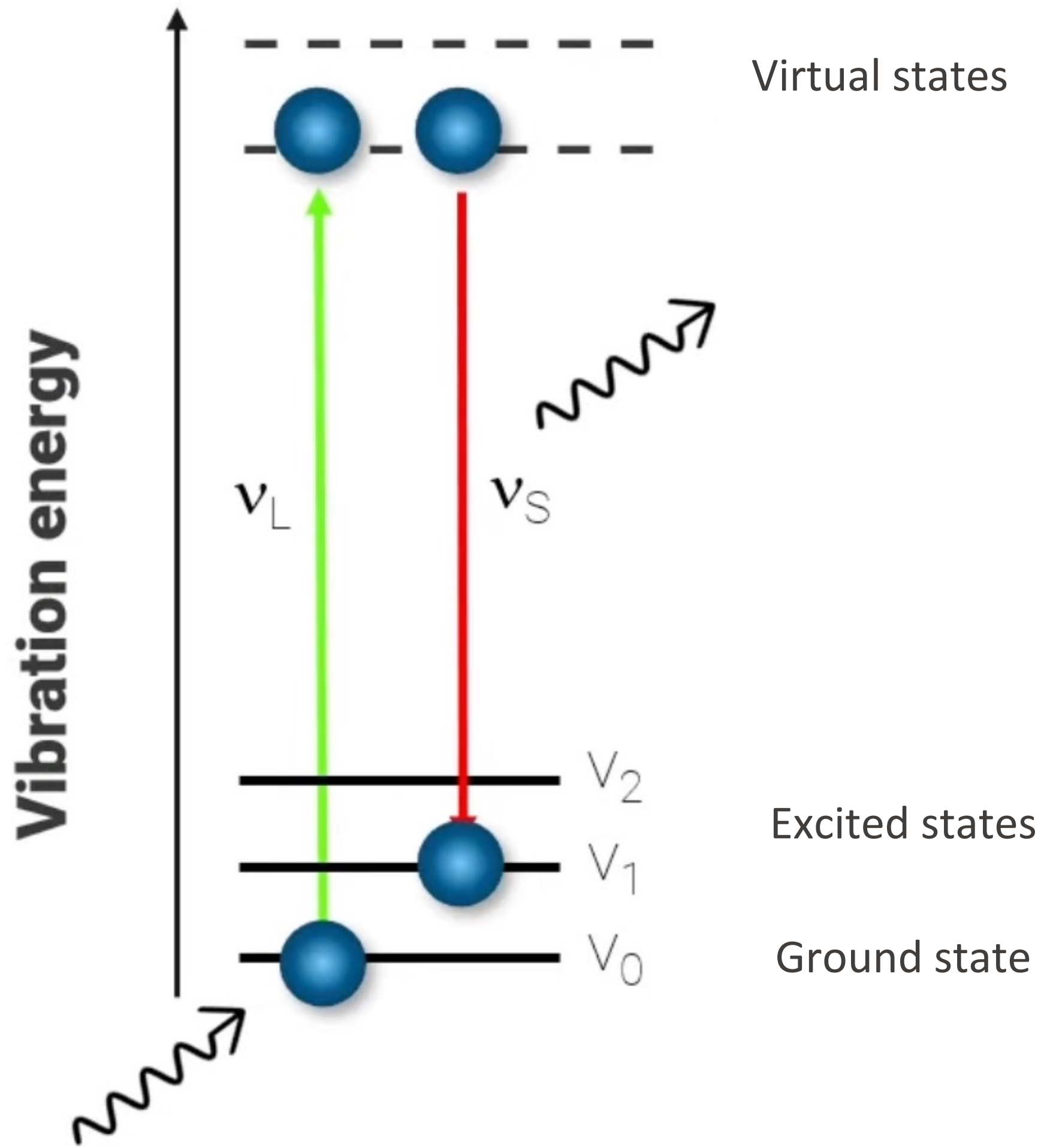


Molecules have different vibrational levels that are defined by specific energy differences



Raman Scattering = Molecule Falls to Excited State

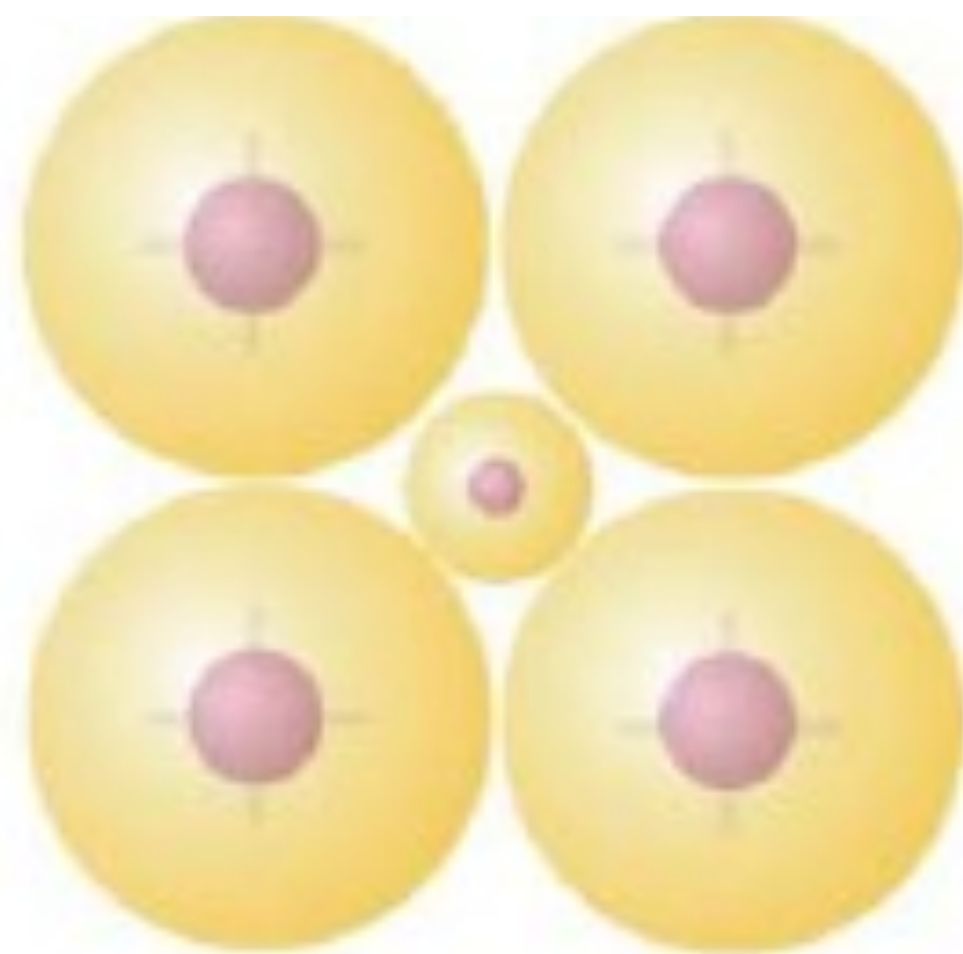
Inelastic scattering (Raman)



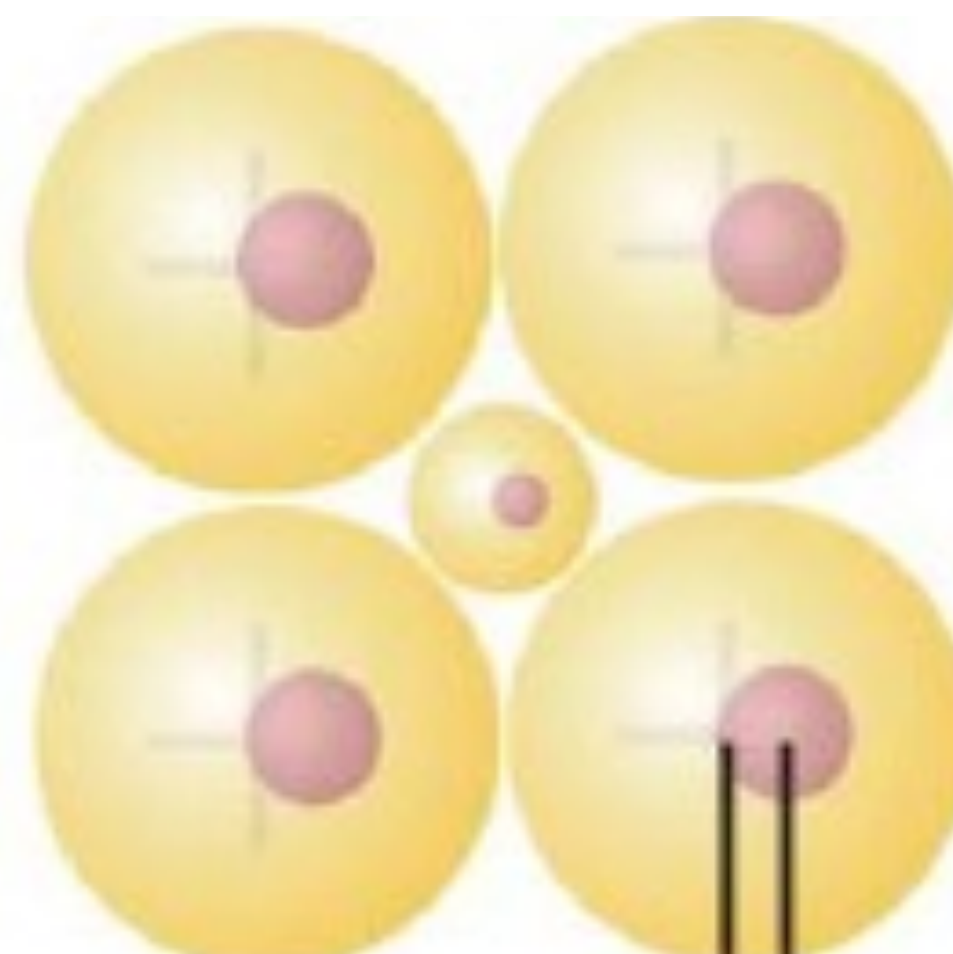
What is Polarizability?

Polarizability: The tendency of a substance to form a dipole moment when an electric field (light) is applied

Without electric field



With electric field



X : Induced dipole moment

α : Polarizability

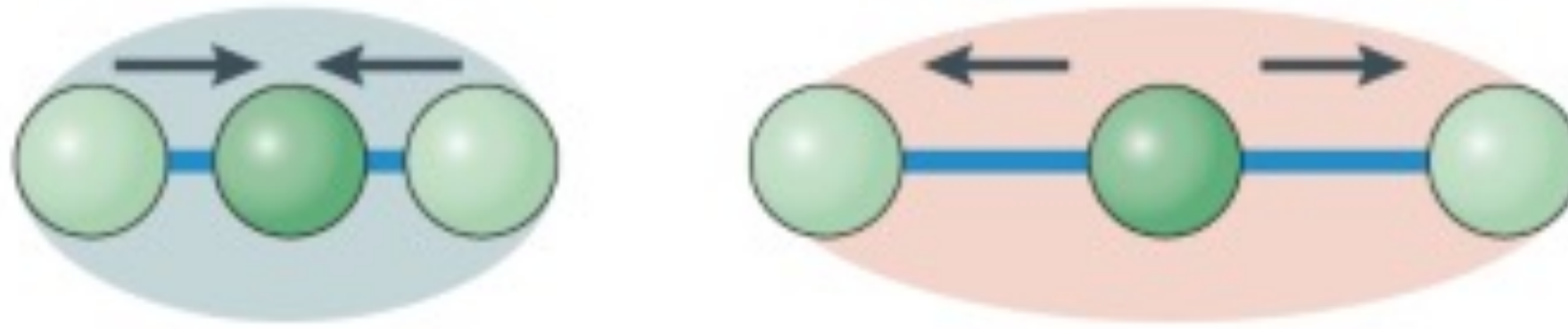
E : Electric field

$$X \sim \alpha \cdot E$$

What Exactly is the Raman Shift? Frequency of Vibrations

Polarizability changes as the volume occupied by electrons changes

Symmetrical stretch



- Polarizability change during vibration
- **Raman active**
- Infrared inactive

Raman active:
change in
polarizability

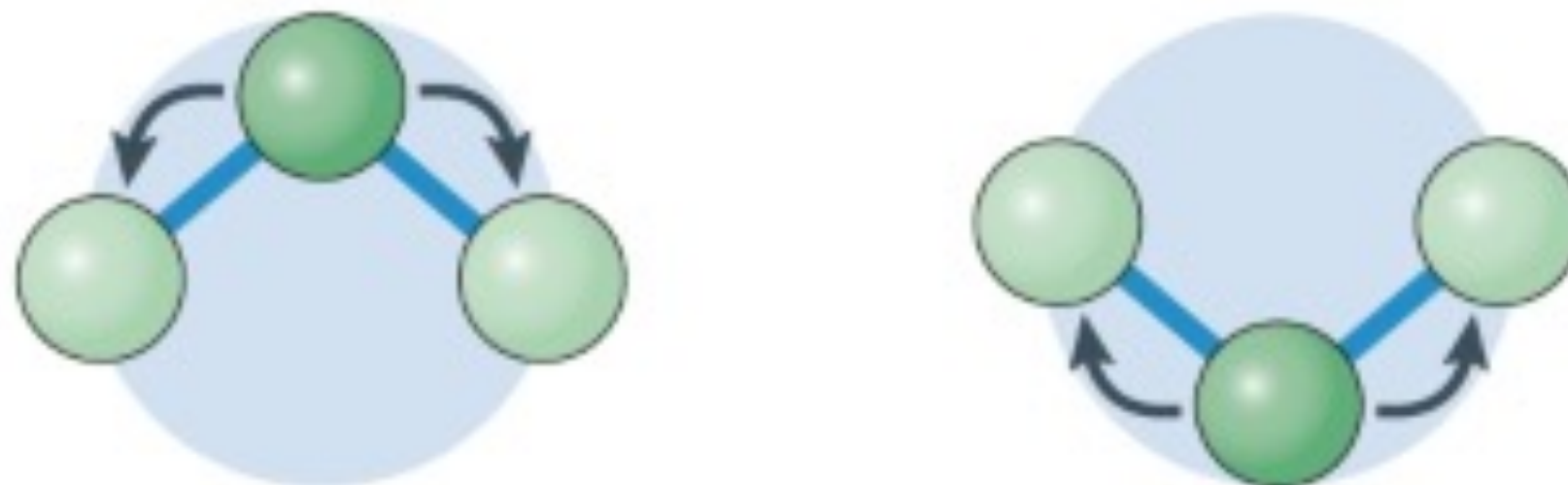
Asymmetrical stretch



- Polarizability unchanged during vibration
- **Raman inactive**
- Infrared inactive

Infrared (IR) active:
change in dipole
moment

Bending



- Polarizability unchanged during vibration
- **Raman inactive**
- Infrared active

What Exactly is the Raman Shift? Frequency of Vibrations

Raman shift depends on the vibrational frequencies of the molecule, which are determined by the mass of the atoms and the stiffness of the chemical bonds.

Vibrational frequency of molecule

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$$

Bond force constant (stiffness)

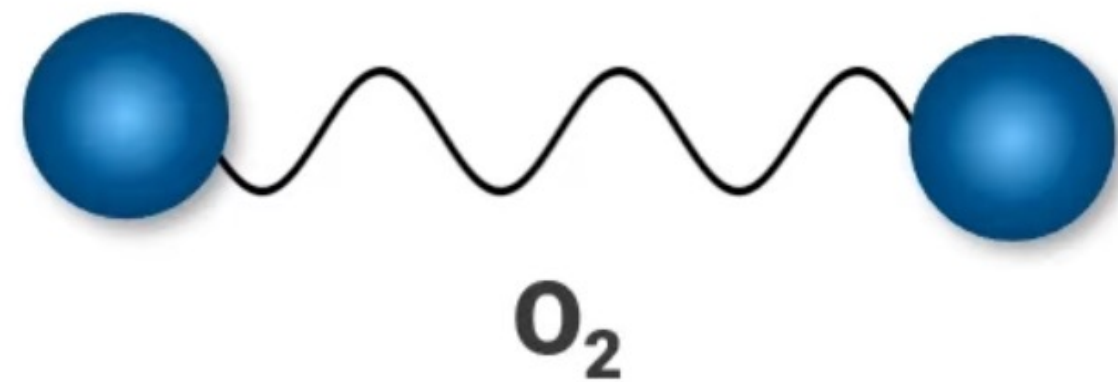
Reduced mass ($m_1 m_2 / (m_1 + m_2)$)

Raman shift

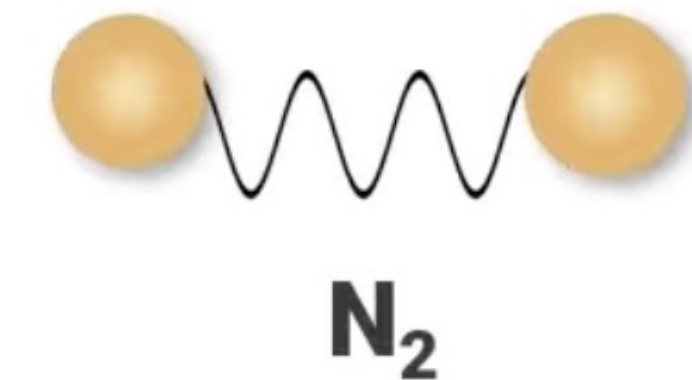
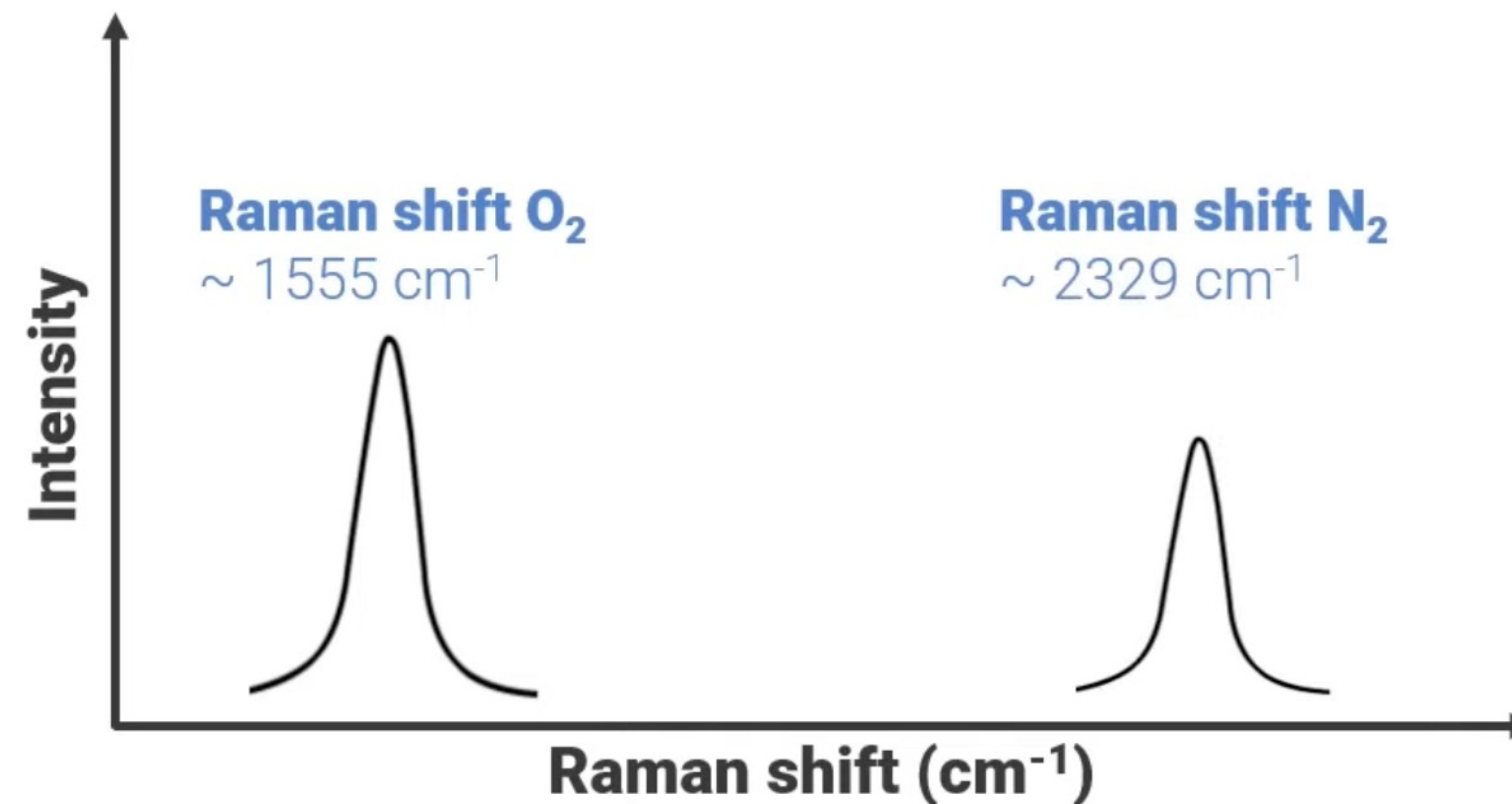
$$\Delta\nu = \nu_0 - \nu_v$$

Frequency of incident light

Vibrational frequency of molecule

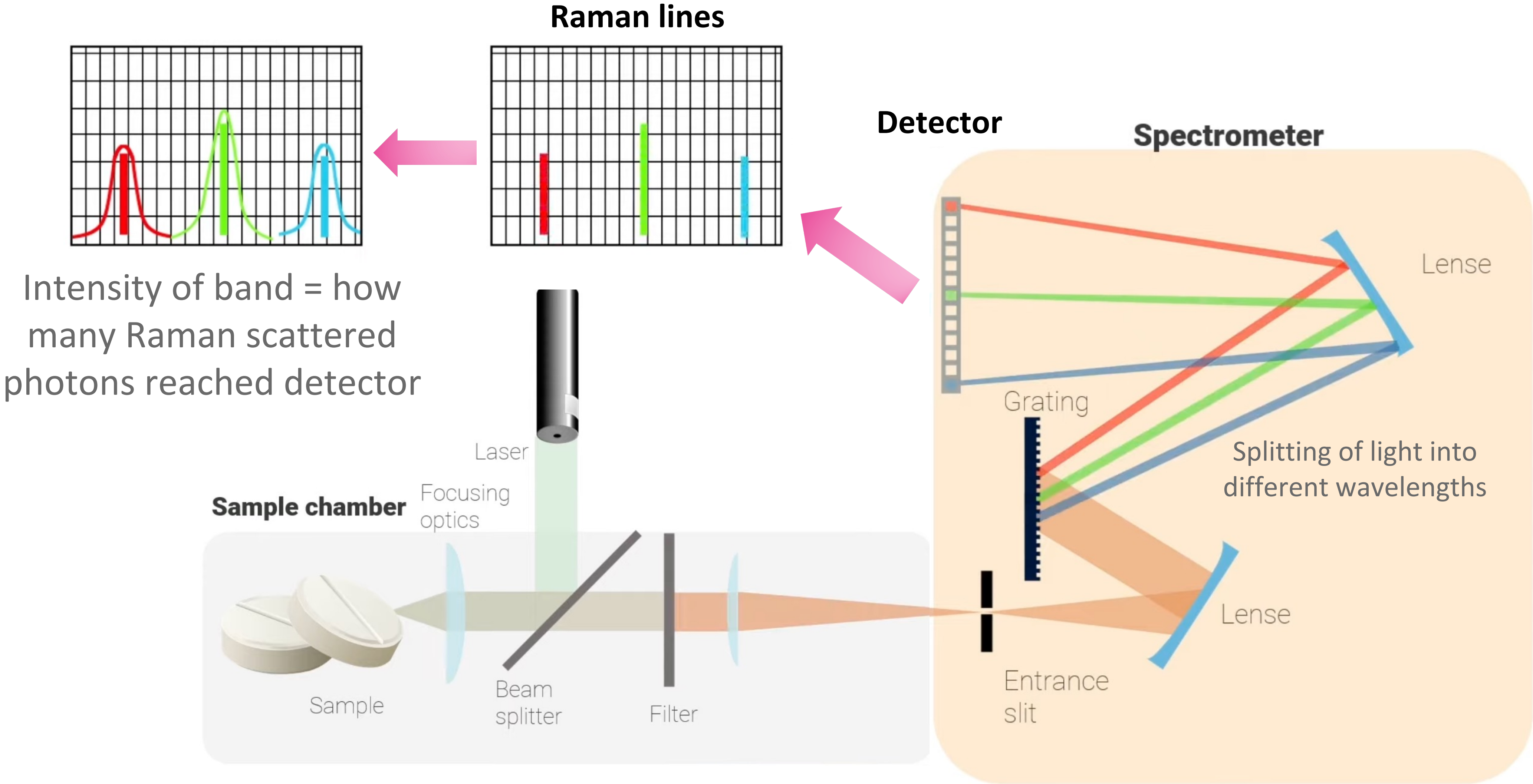


Heavier molecules have long wavelengths and low frequency

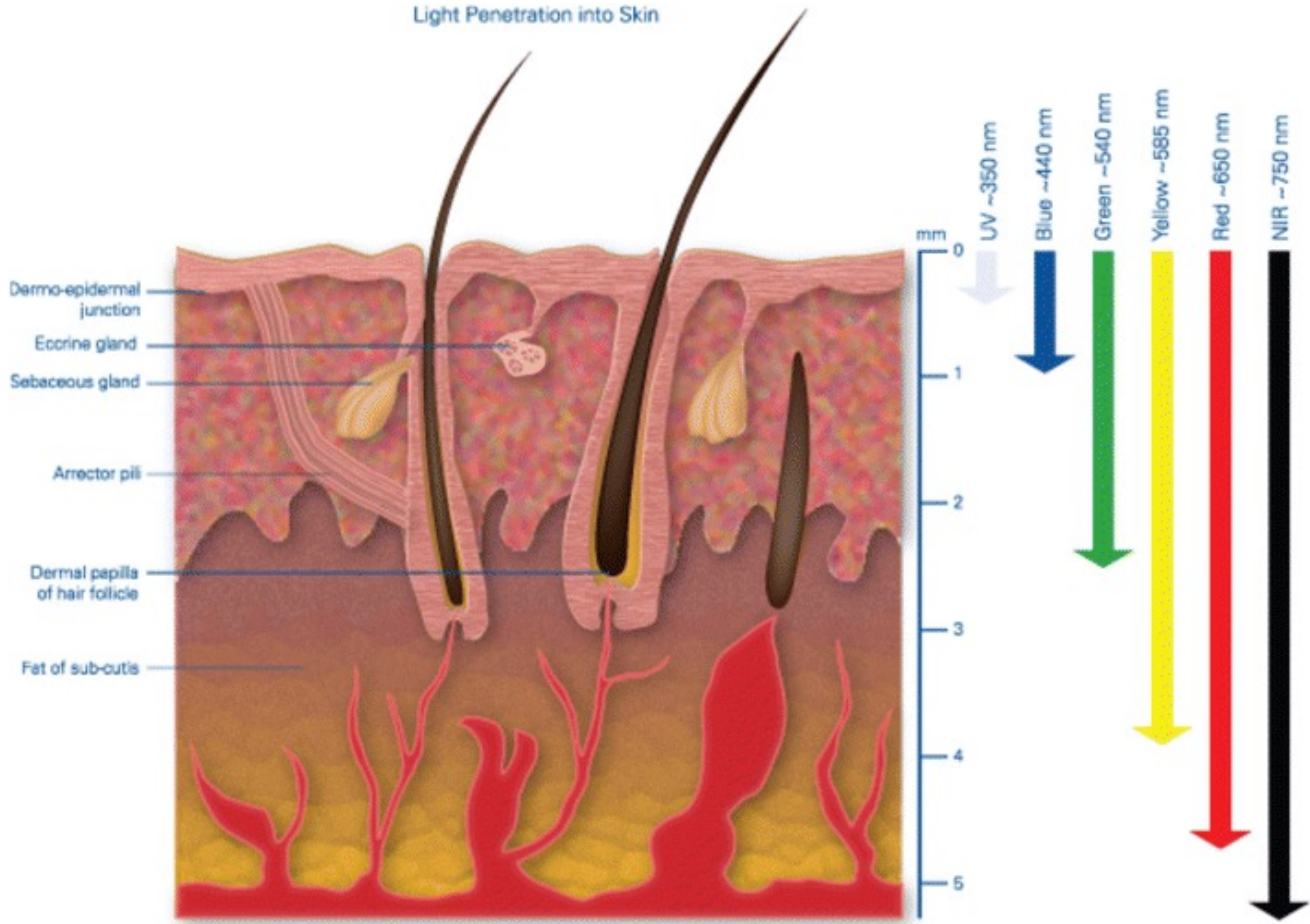


Lighter molecules have short wavelengths and high frequency

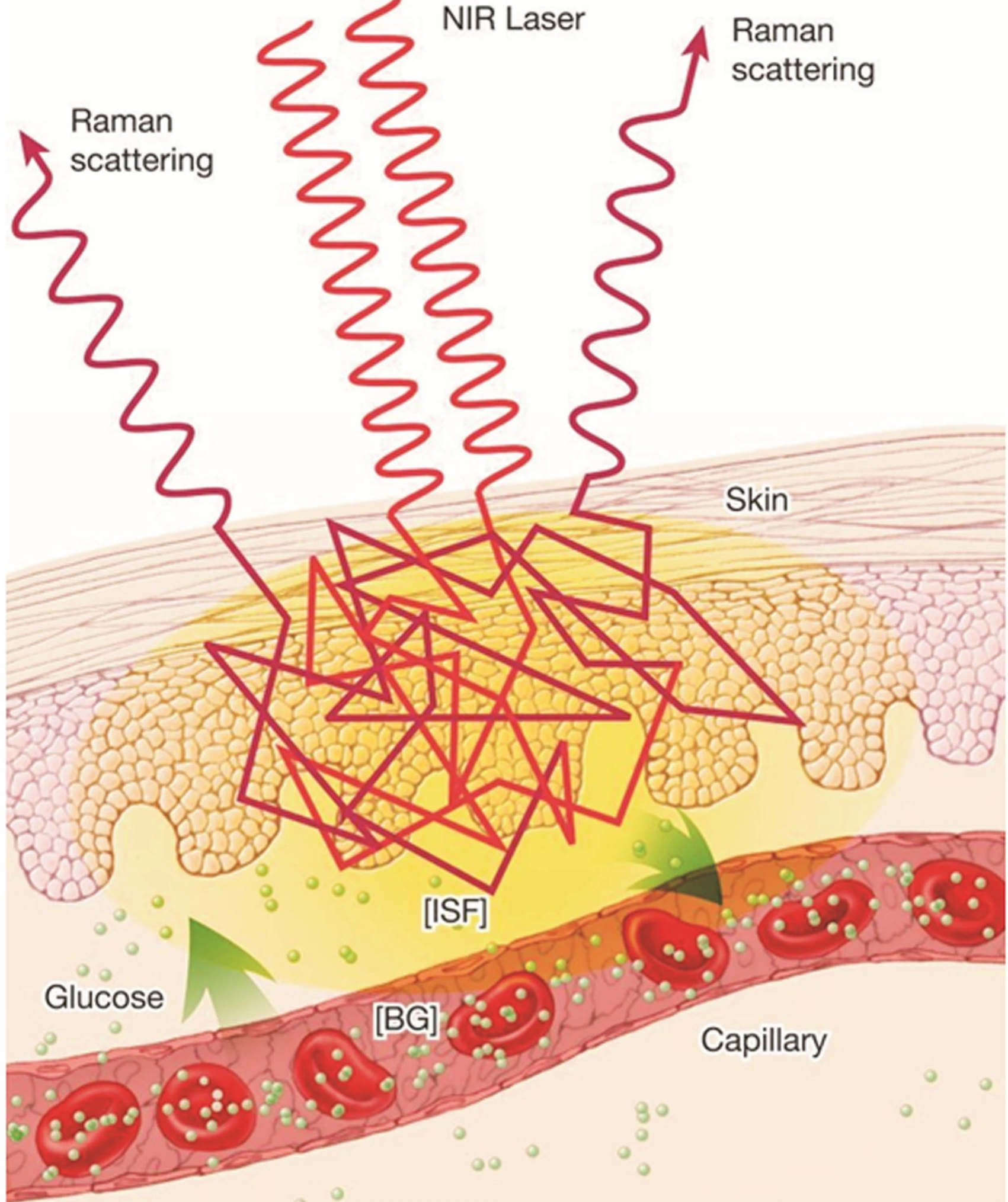
Raman Spectroscopy for Non-Invasive Glucose Detection



Tissue Penetration using Near-IR Laser



Ash et al. | *Las. Med. Sci.* | 2017

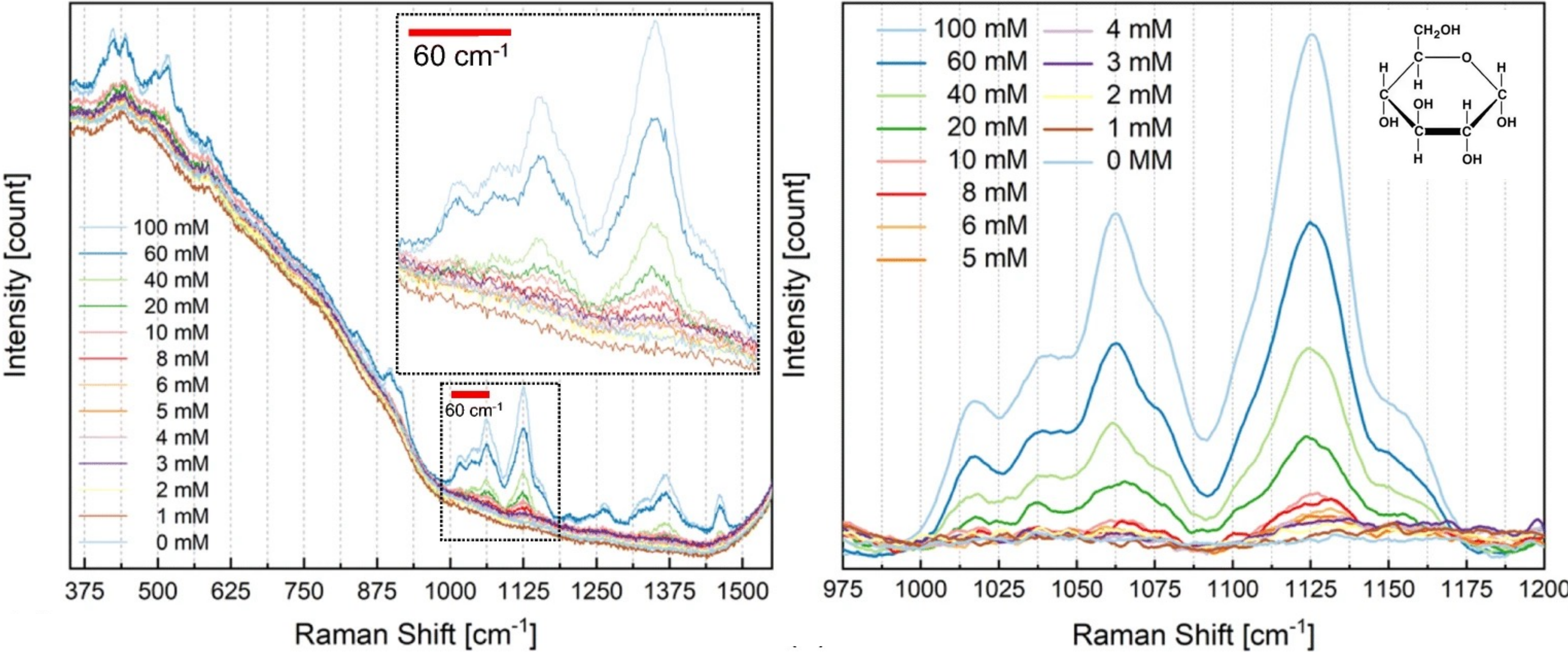


Spegazzini et al. | *Sci. Rep.* | 2014

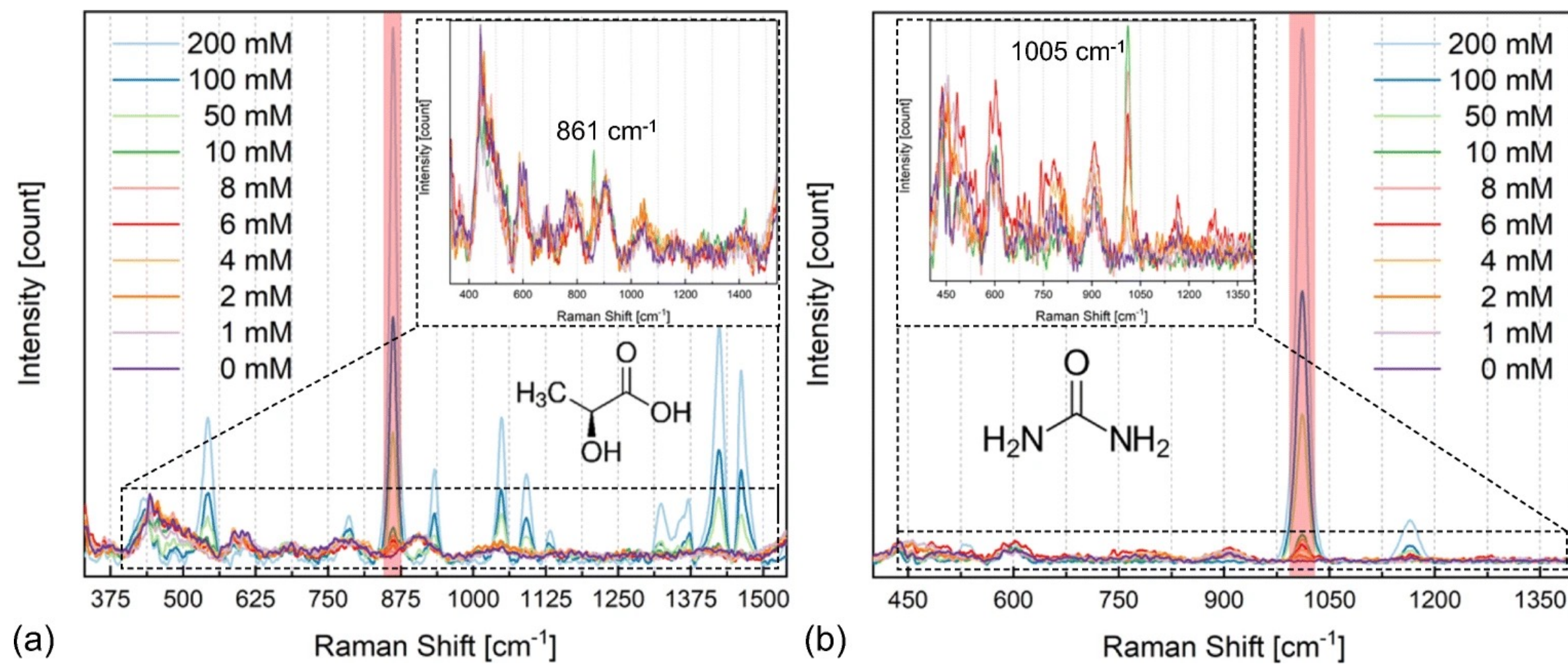
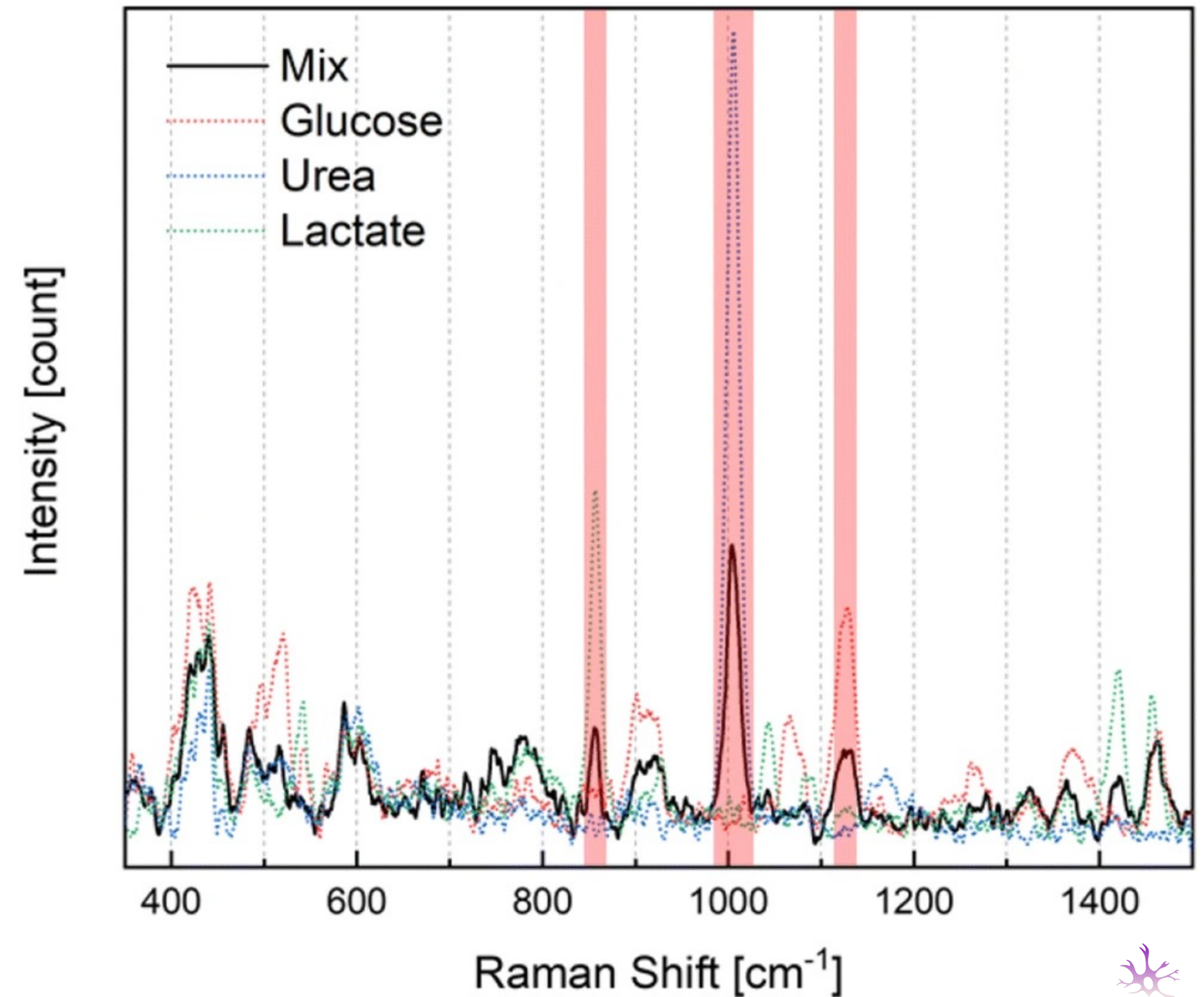
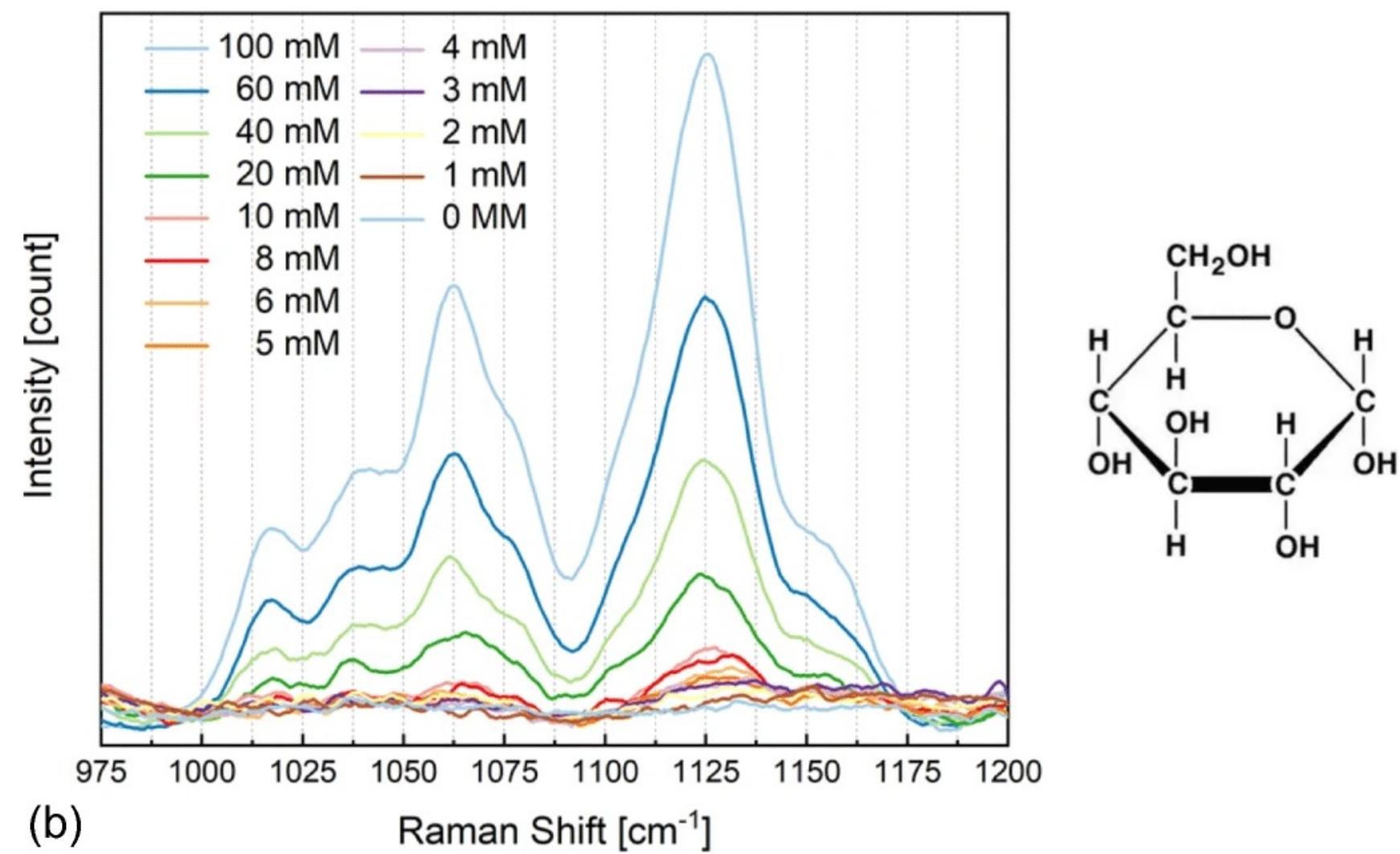
Raman Spectroscopy for Non-Invasive Glucose Detection

Complex molecules have more vibrational modes

Raman Fingerprint: Target of Raman is the material specific pattern of the spectrum



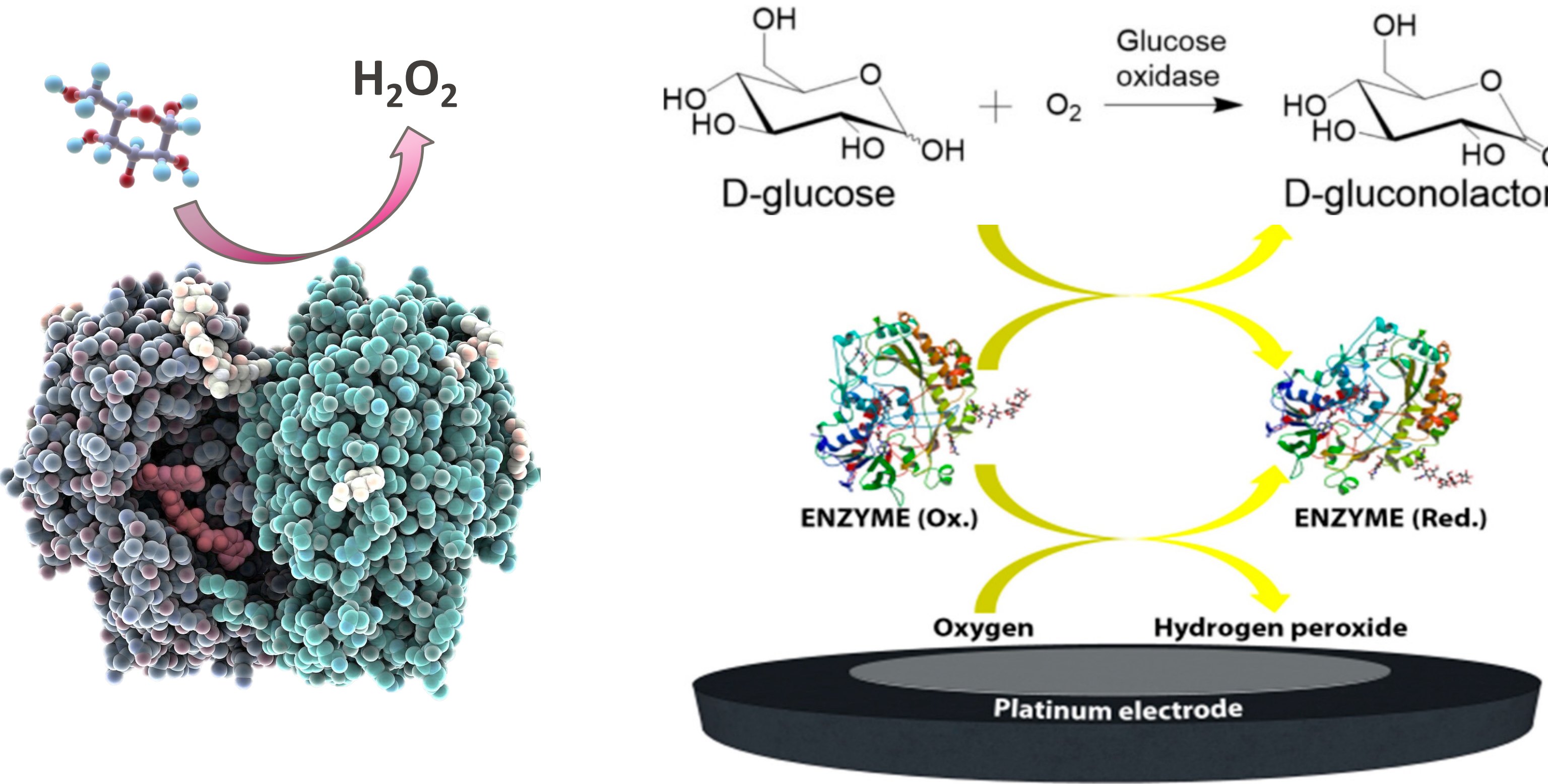
Raman Spectroscopy for Non-Invasive Glucose Detection



Key Takeaways

- Clark electrode – one of the first biosensors for oxygen
- Glucose detection through electrochemical reactions
- Use of catalysts (enzymes) enable glucose detection through byproducts
- Raman spectroscopy to monitor glucose non-invasively

Success of the Glucose Biosensor – Robust System



BLOOD GLUCOSE LEVELS CHART 

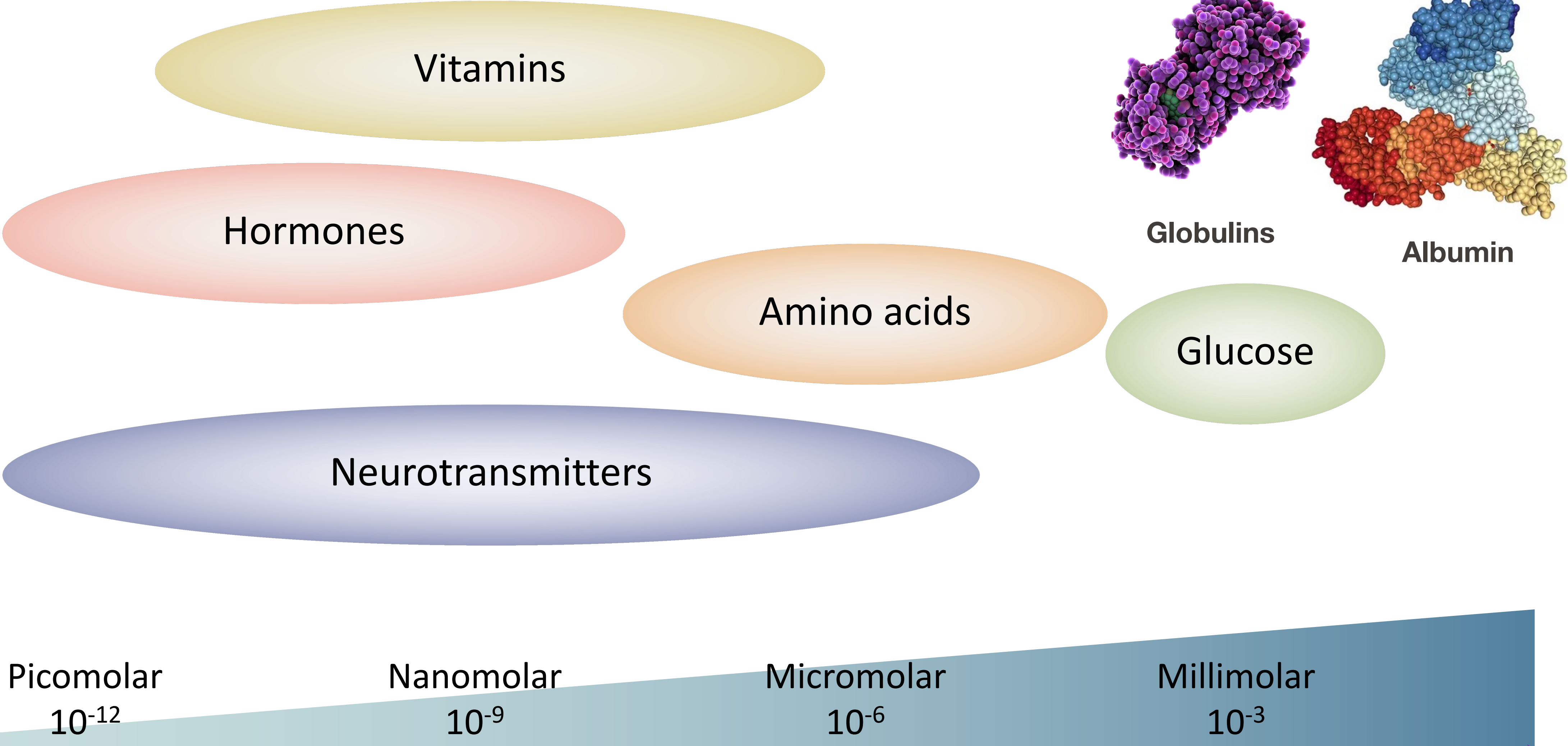
LEVEL	mg/dl	mmol/L	RISK	SUGGESTED ACTION
DANGER - HIGH	315+	17.4	VERY HIGH	MEDICAL ATTENTION
HIGH	280	15.6	HIGH	MEDICAL ATTENTION
HIGH	250	13.7	HIGH	MEDICAL ATTENTION
HIGH	215	11	HIGH	MEDICAL ATTENTION
BORDERLINE	180	10	MEDIUM	CONSULT DOCTOR
BORDERLINE	150	8.2	MEDIUM	CONSULT DOCTOR
BORDERLINE	120	7	MEDIUM	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

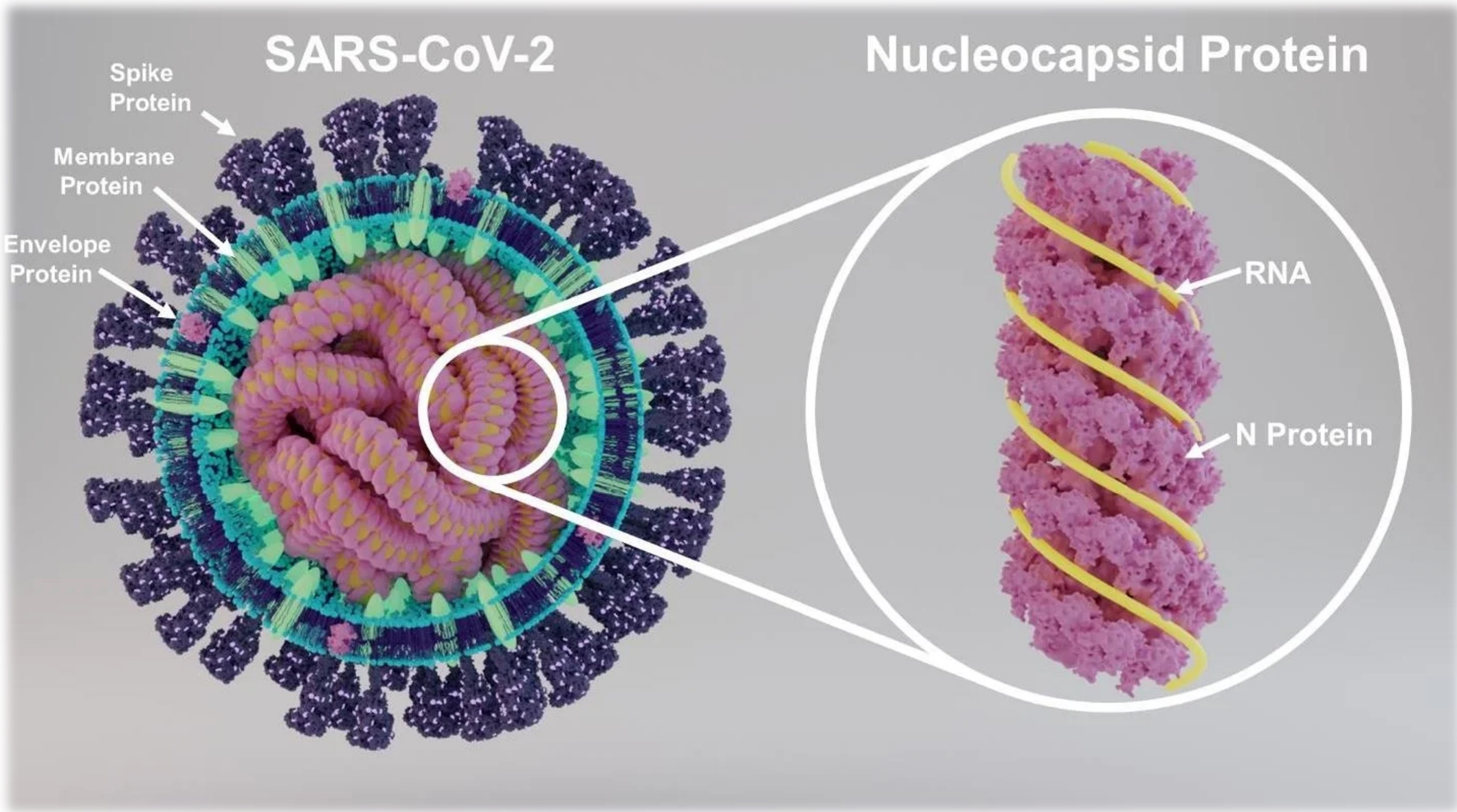
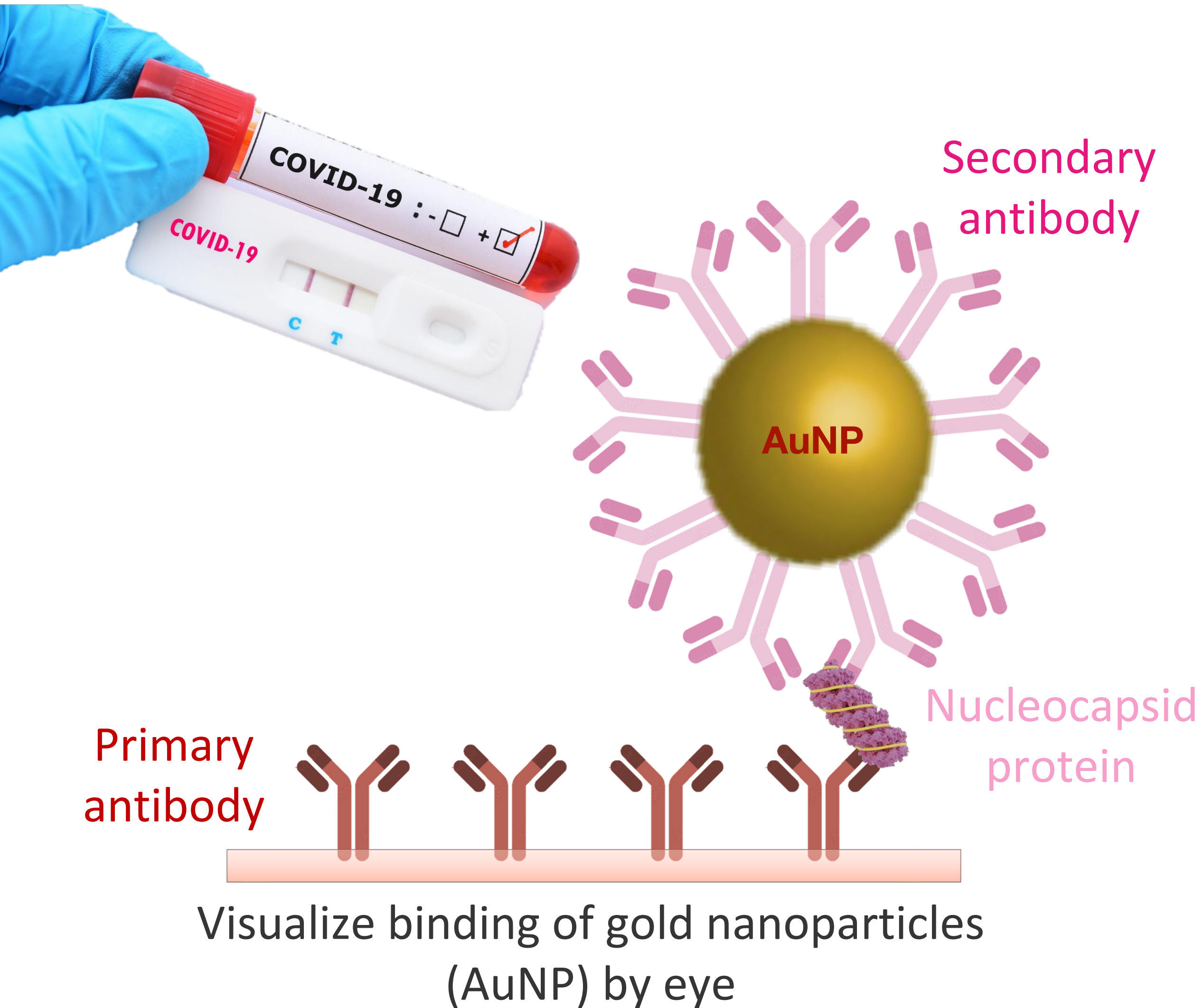
Detection window for H₂O₂ doesn't overlap with other species in blood

Clear values associated with healthy vs. disease states (diabetes)

Success of the Glucose Biosensor – Concentration Range



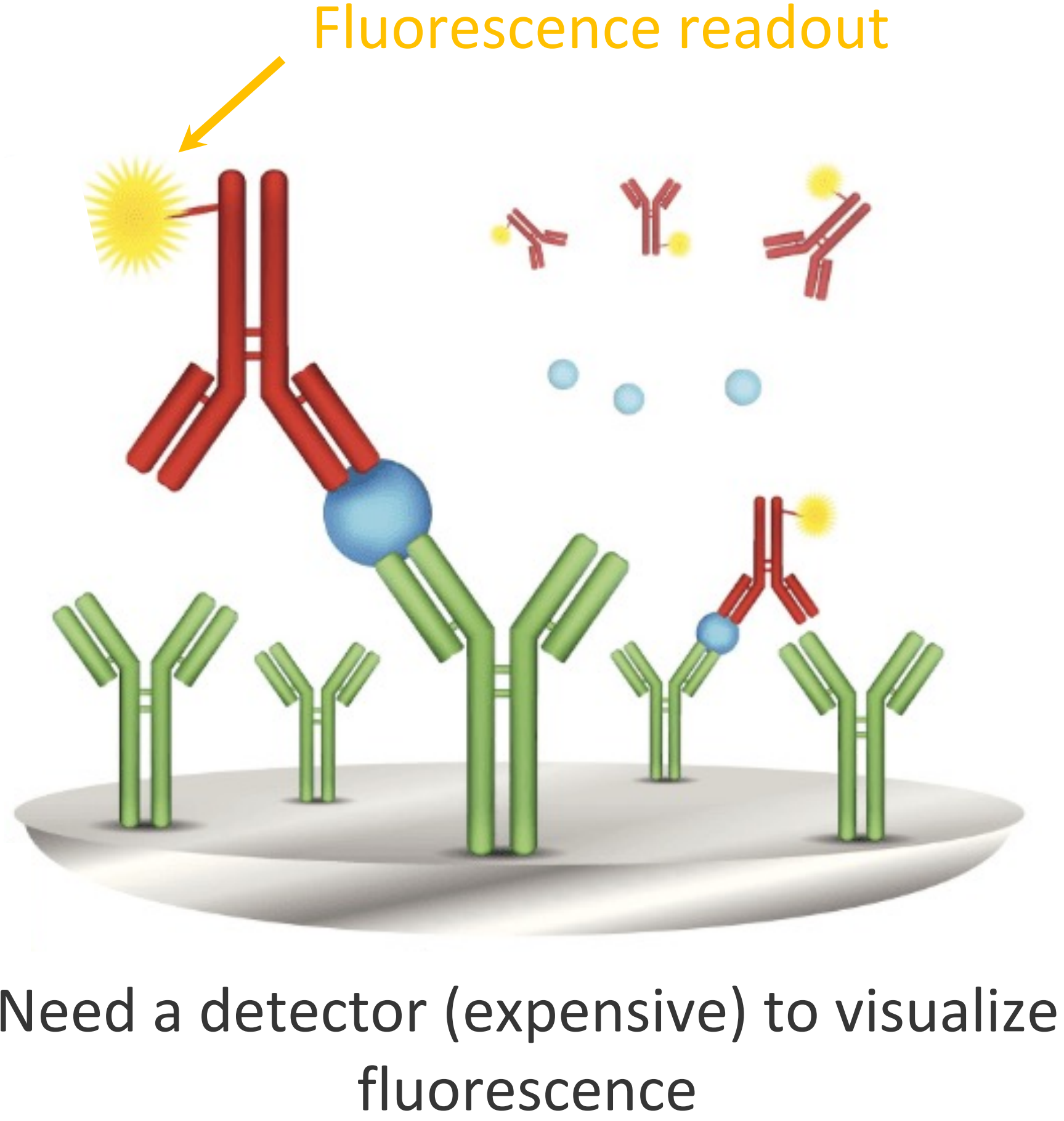
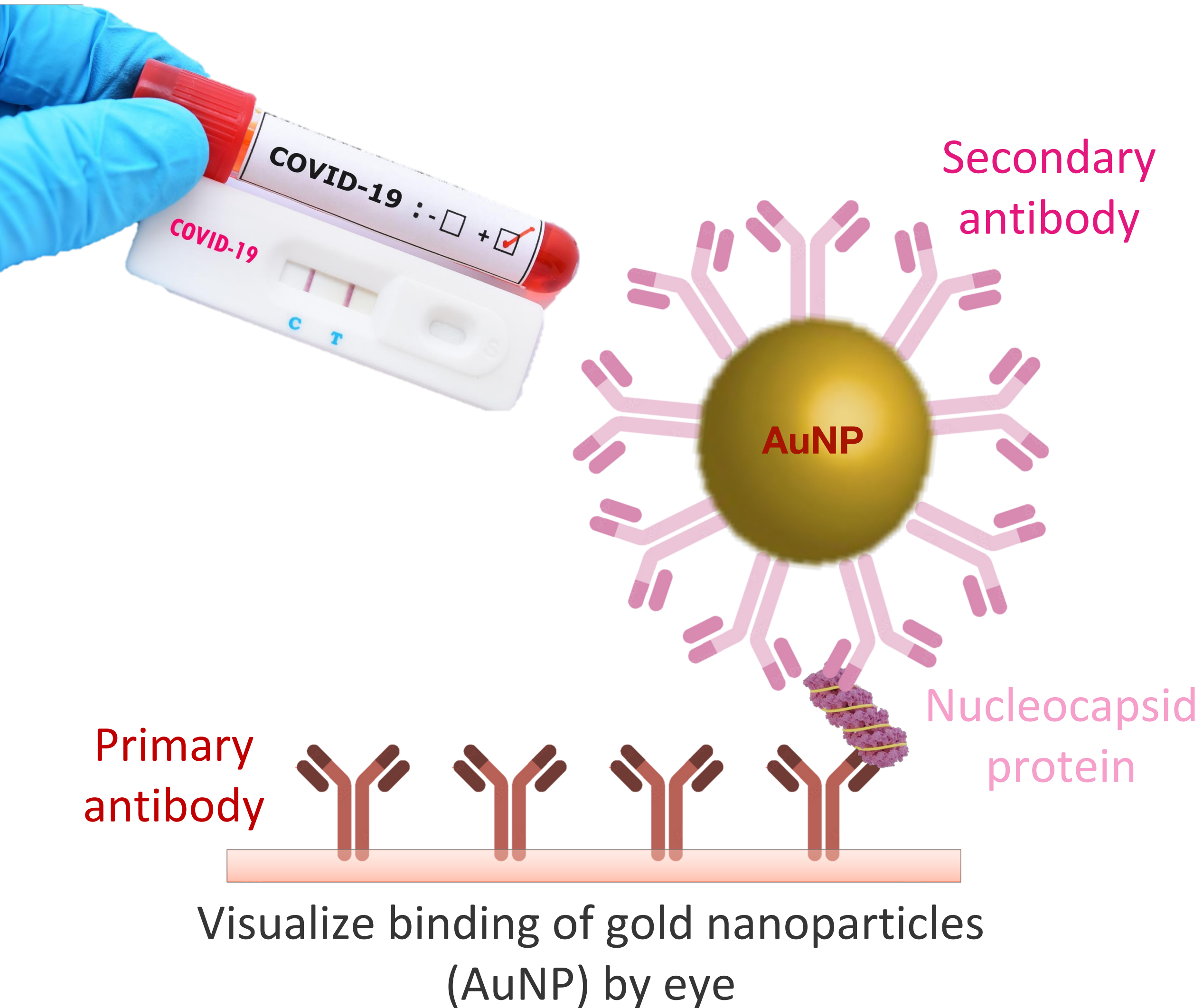
Let's Go Back to The COVID-19 Test: Antibody Sandwich Assays



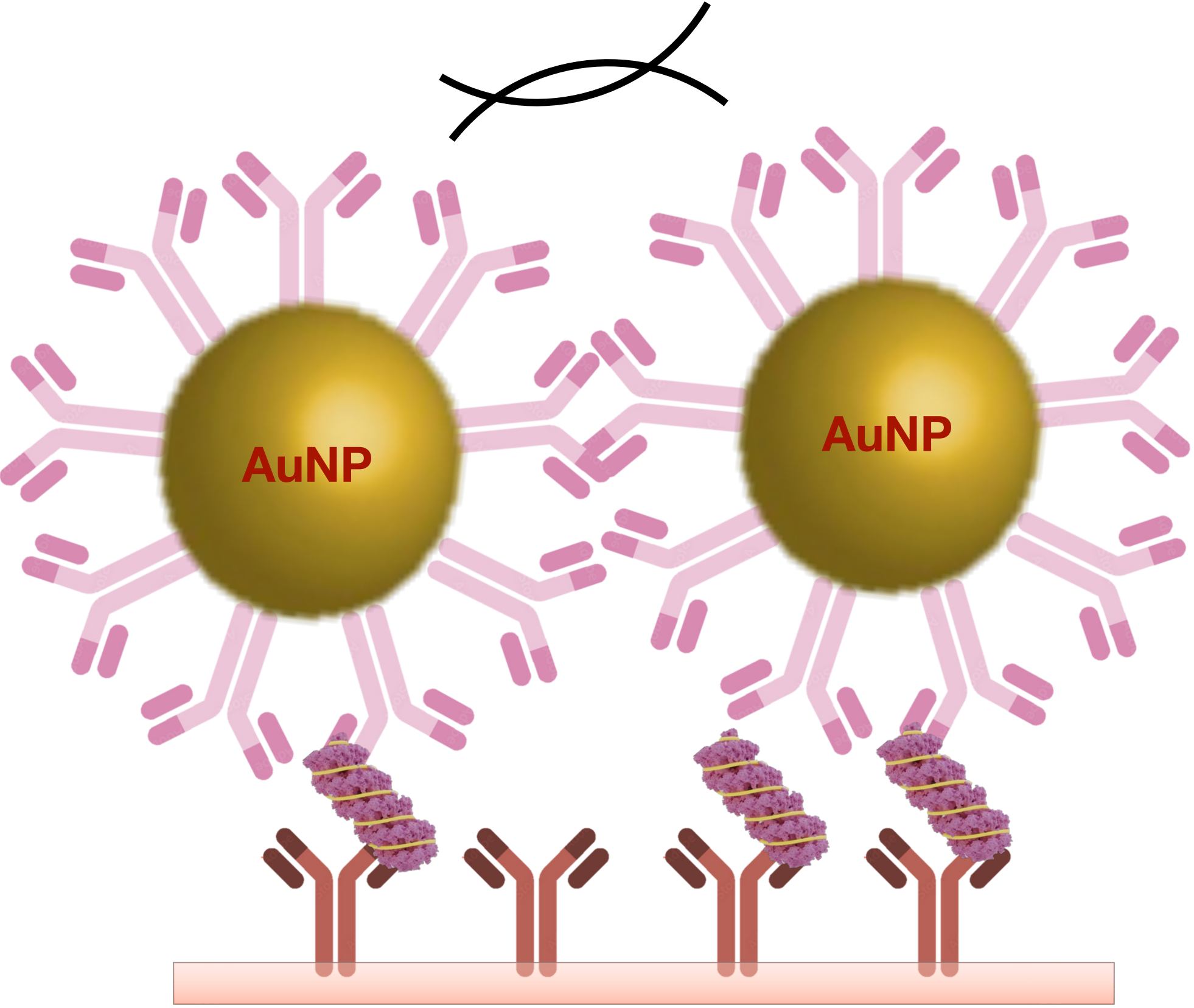
Nucleocapsid protein in high abundance and stable in nasal swabs



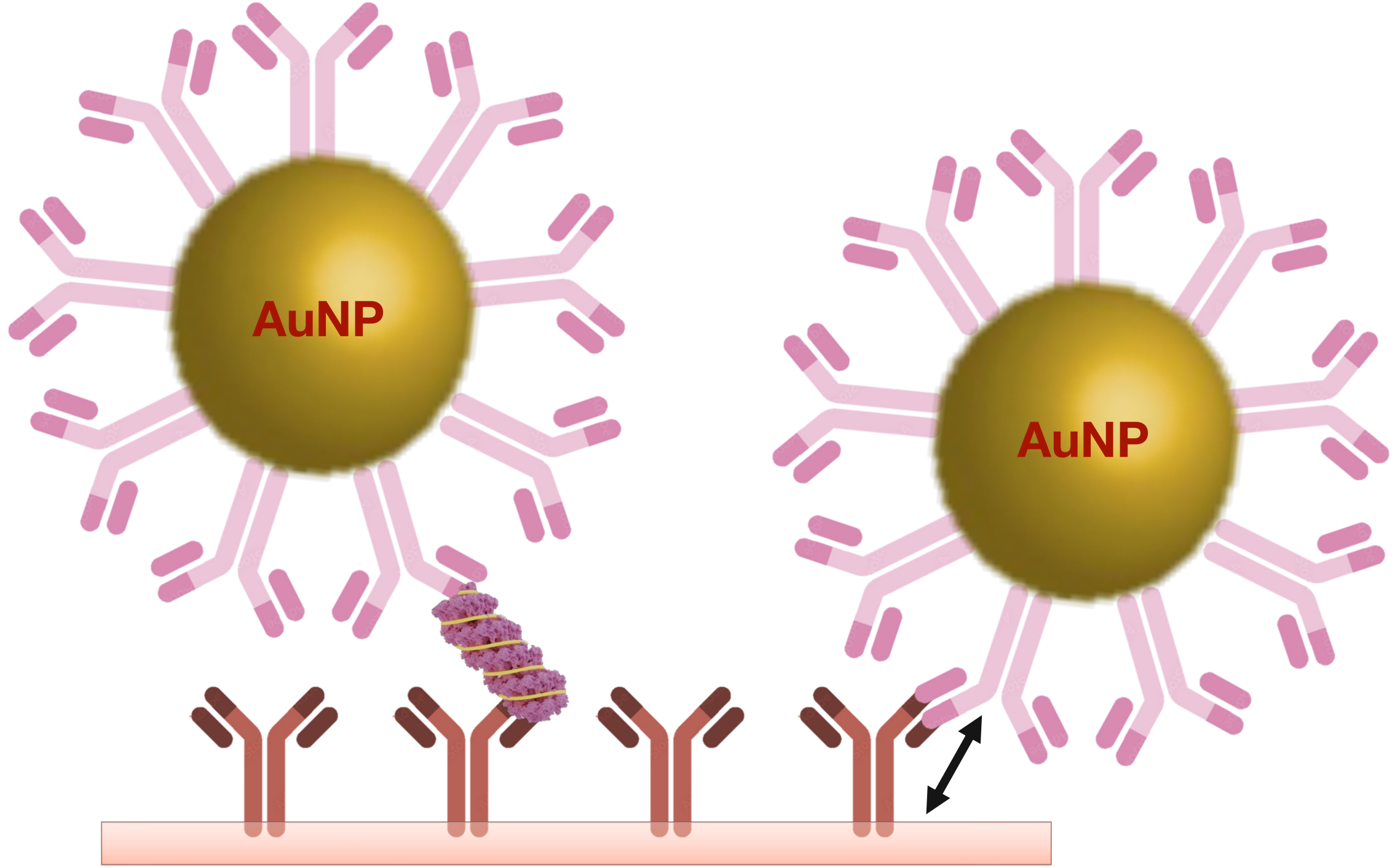
Antibody Sandwich Assays are Commonly Used in Biosensors



Challenges of Antibody Sandwich Assays

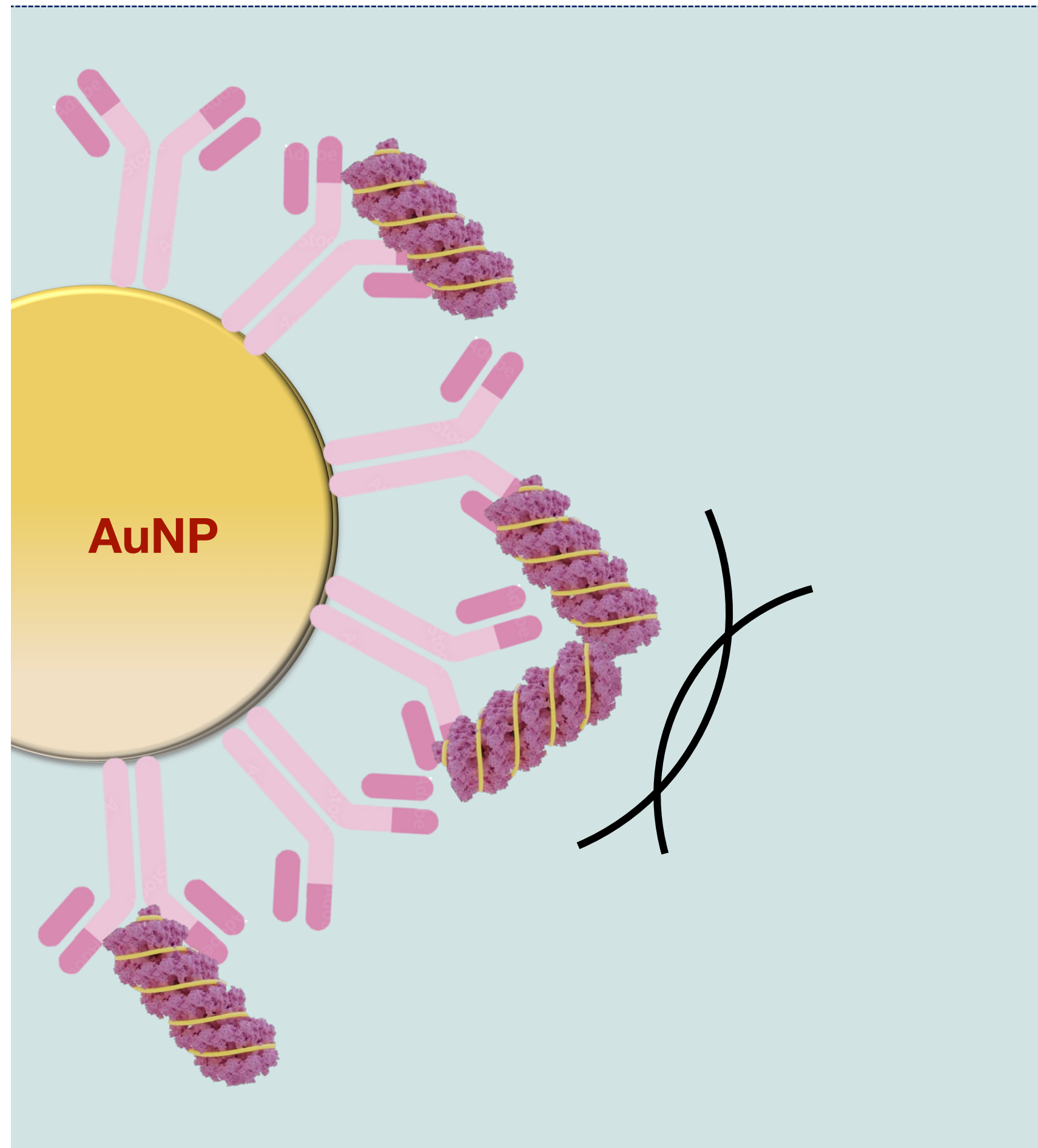


Steric hindrance at the surface
Density of bioreceptors need to be optimized
False negatives (not enough AuNPs bound)

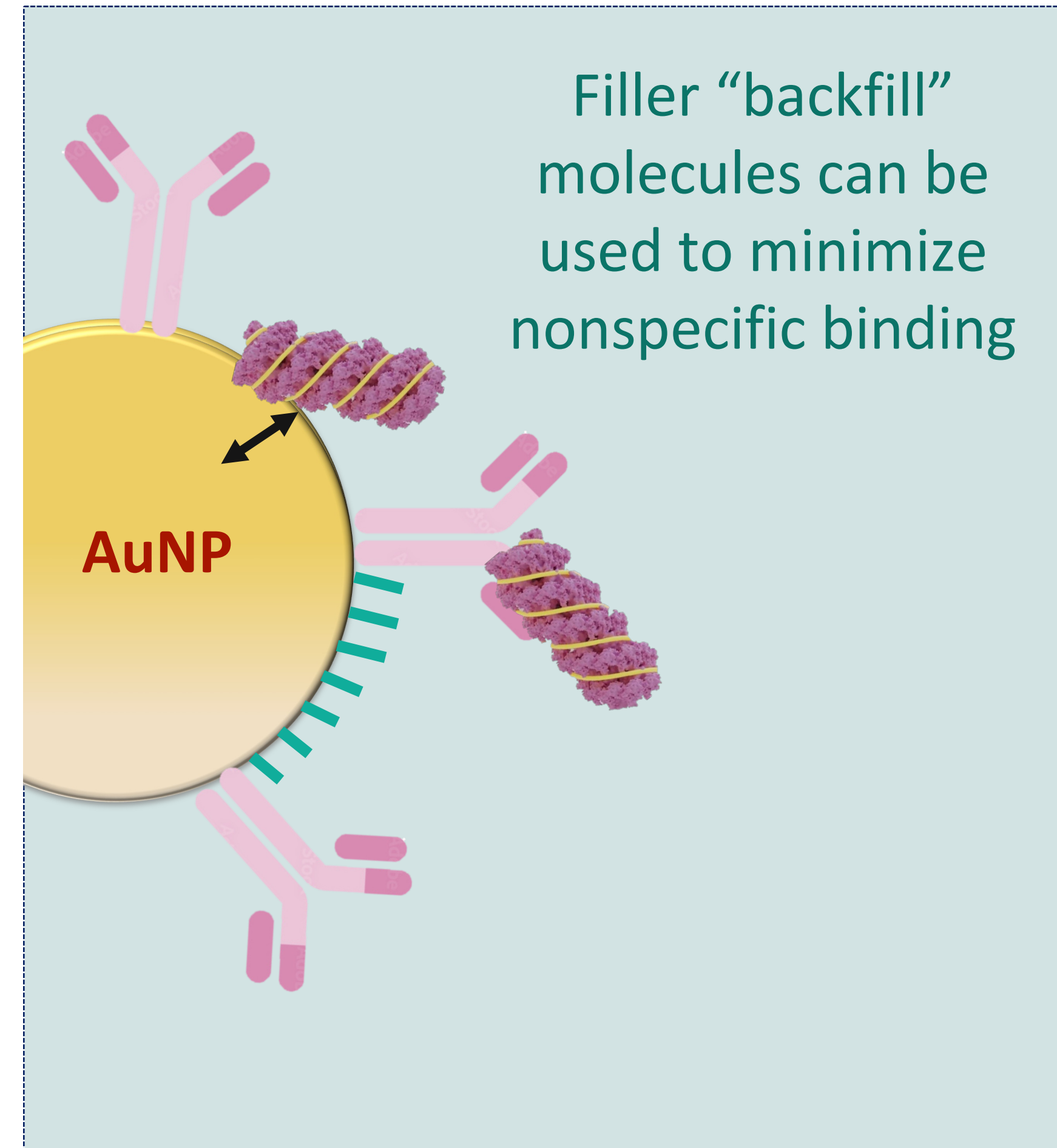


Nonspecific sticking of the gold nanoparticles
To the surface, to primary antibodies, *etc.*
False positives (too many AuNPs bound)

The COVID-19 Test Uses and Antibody Sandwich Assay



Steric hindrance at the surface



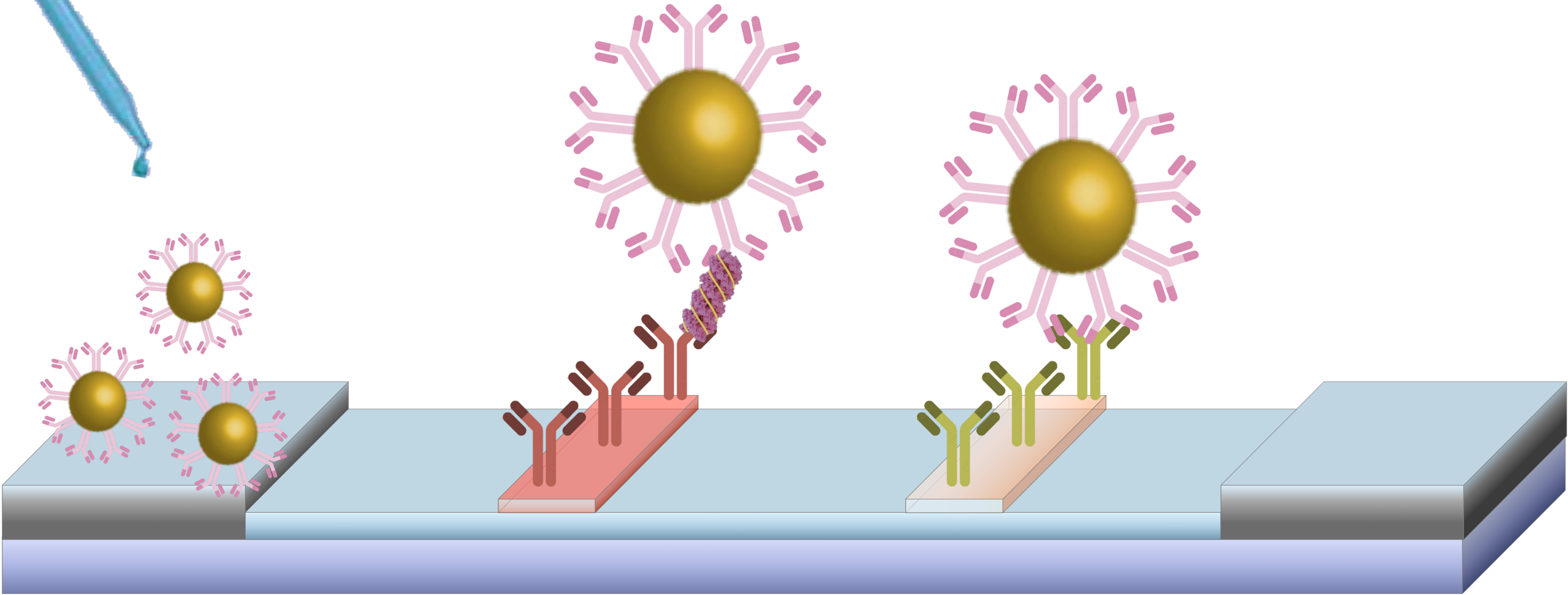
Nonspecific sticking to gold nanopartic

Lateral Flow Assay Optimized to Detect COVID-19



Targeting high viral loads with nucleocapsid protein
(don't need high sensitivity)

Simple sample matrix (nasal/throat swabs)



Test line

Control line

Yes/No test – not quantitative!

Summary of Today's Class

- Basic components of biosensors – biological receptors, transducers, and data readout
- Biosensor parameters: binding affinity, limit of detection , sensitivity
- Electrochemical biosensors – Clark electrode for oxygen sensing and glucose biosensor
- Non-invasive molecular monitoring using Raman spectroscopy
- Reason for success behind the glucose sensor
- Workings of the COVID-19 rapid test

Prof. Nako Nakatsuka
nako.nakatsuka@epfl.ch

EPFL
Laboratory of Chemical Nanotechnology
Campus Biotech
Chemin des Mines 9
1202, Geneva

<https://www.epfl.ch/labs/chemina/>

