
(3) SURFACE LIGAND BINDING SENSORS

Miniaturization Of The Sensing Area

Array of surface sensors

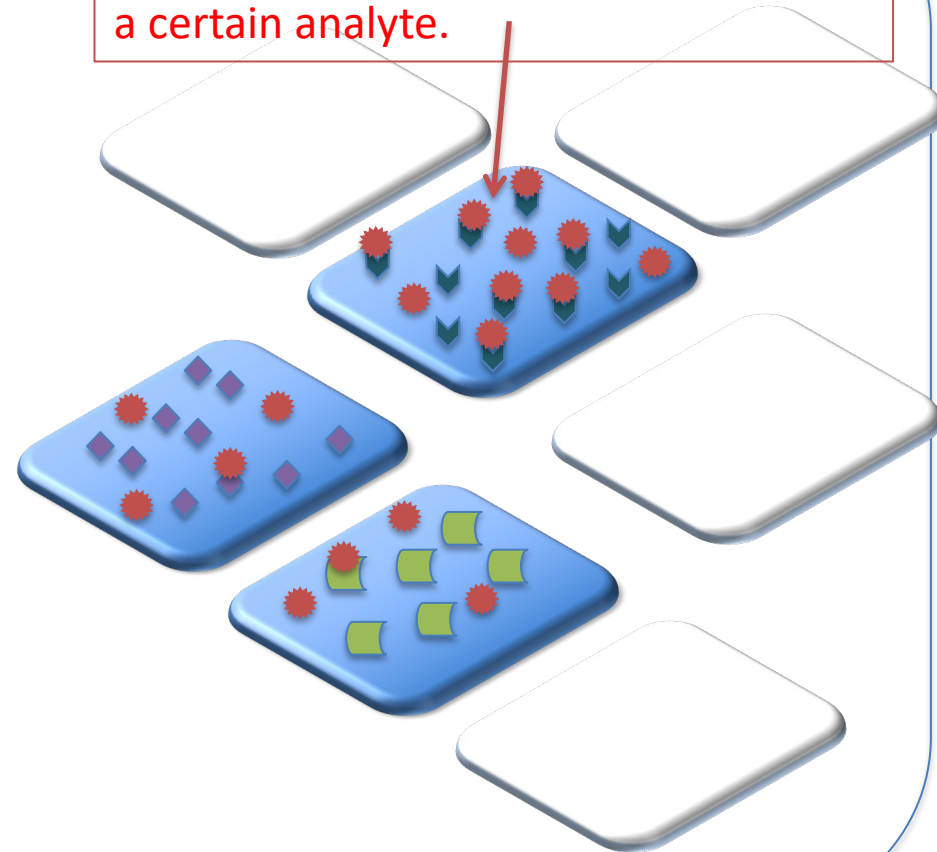
Main features:

Surface-sensing of molecules
Multiple probes (high-throughput)
Multiple targets (high-throughput)
Small sample volume

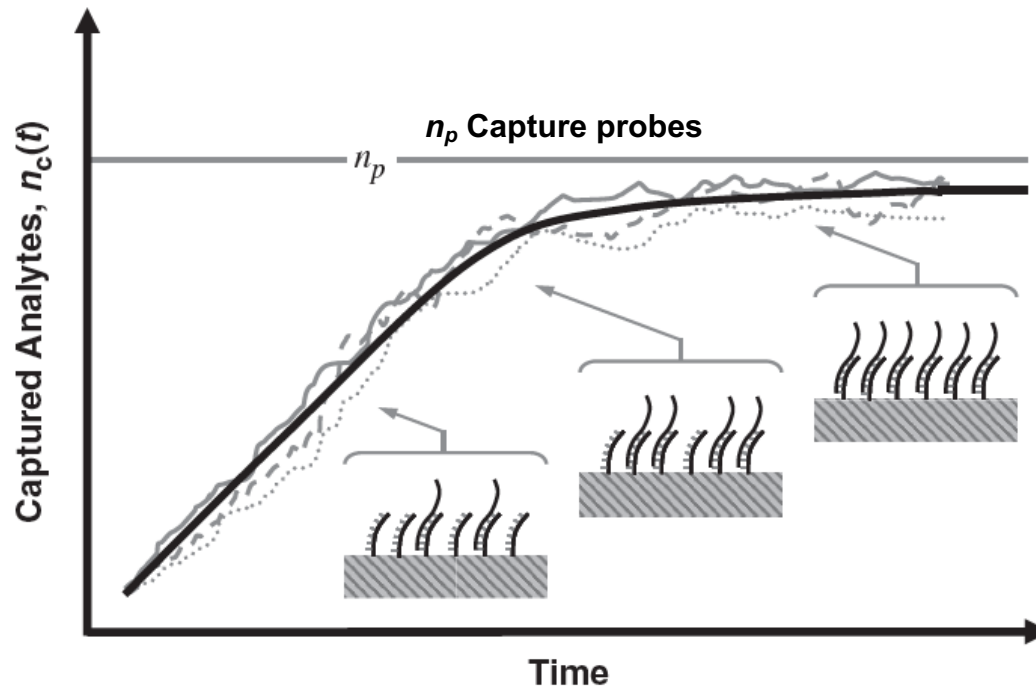
Main issues:

- Non specific adsorptions
- Smallest feature-size compromise
- Surface-related binding issues like steric hindrance and repulsive forces

Feature where specific ligands are bound. Feature: region featuring ligand molecules which are specific to a certain analyte.



E.g.: Binding kinetics oligonucleotide-oligonucleotide



If the concentration of target DNA is sufficiently high, the total amount of captured analytes increases until there are no more available DNA capturing probes on the surface (or it is not possible to increase further the target density), i.e. saturation coverage.

Expected behavior (thick solid line); Experiments (thin solid line, dashed line, dotted line).

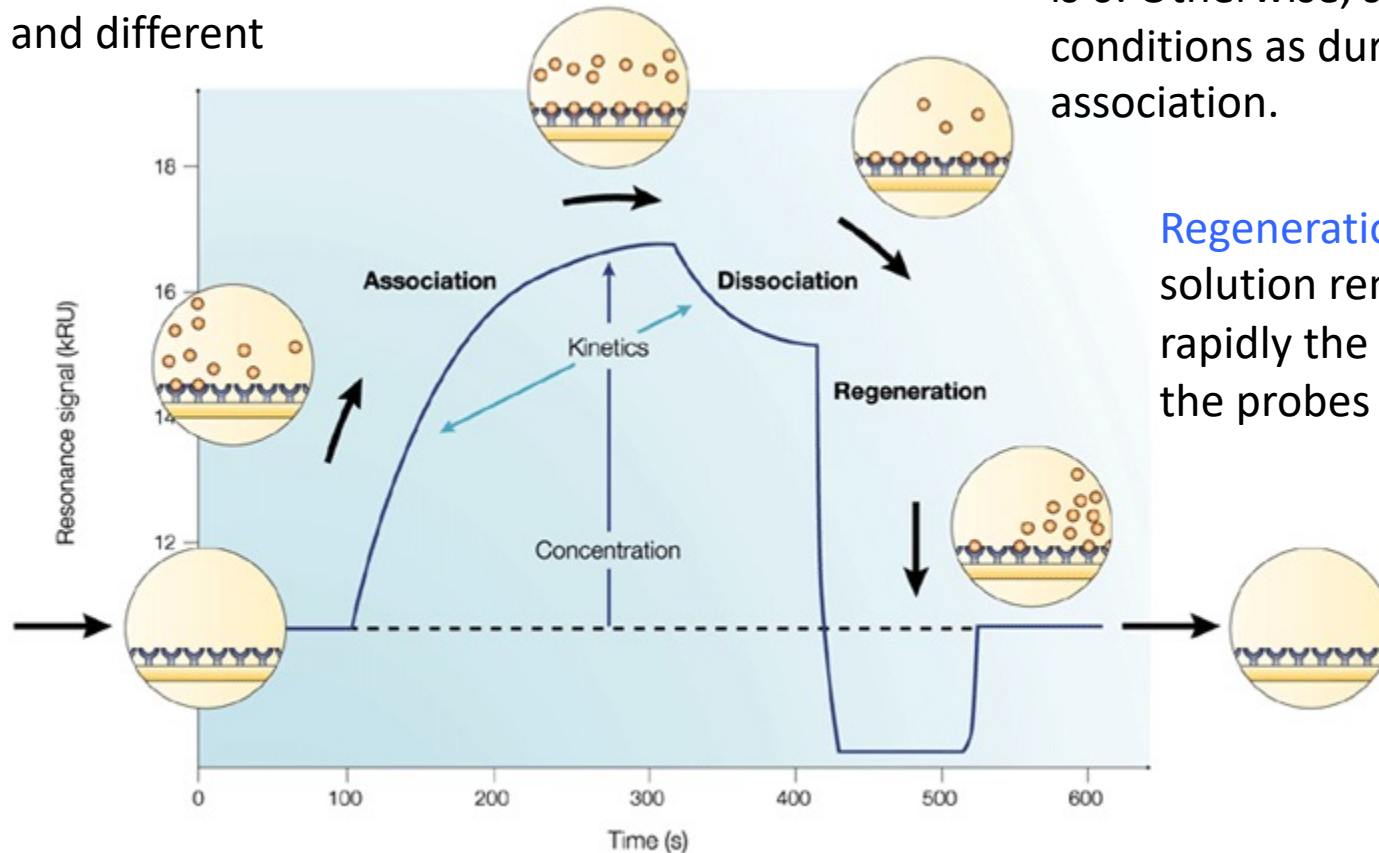
Binding and dissociation kinetics on surface.

Association:

Concentration of targets molecules in bulk is constant and different from 0

Dissociation: Concentration of targets molecules in bulk is 0. Otherwise, same conditions as during association.

Regeneration: Harsh solution removes rapidly the targets from the probes



Binding kinetics on surface.

1. Receptor (probe) molecules are immobilized on the sensor surface.
2. At $t = 0$ s, the surface is in contact with a buffer solution, the same that will be used later for the association and dissociation phases.
4. At $t = 100$ s, a solution of analyte (target) in the running buffer is passed over the receptor and the density of the analyte on the surface increases. If the concentration of the analyte is known, then the association rate constant of the interaction (k_{ass}) can be determined. At equilibrium, by definition, the amount of analyte that is associating and dissociating with the receptor is equal. The response level at equilibrium is related to the concentration of active analyte in the sample. At $t = 320$ s, the analyte solution is replaced by buffer, and the receptor–analyte complex is allowed to dissociate. Analysis of these data gives the dissociation rate constant (k_{diss}) for the interaction.
5. A pulse of a regeneration solution (for example, high salt or low pH) is used at $t = 420$ s to disrupt binding and regenerate free receptors.
6. The entire binding cycle is normally repeated several times at varying concentrations of analyte to generate a robust data set for global fitting to an appropriate binding algorithm. The affinity of the interaction can be calculated from the ratio of the rate constants ($K_D = k_{\text{diss}}/k_{\text{ass}}$) or by a linear or nonlinear fitting of the response at equilibrium at varying concentrations of analyte. In addition to determining the interaction affinities and kinetics, a thermodynamic analysis of a biomolecular interaction is also possible.

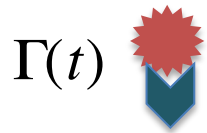
Langmuir binding isotherm



C Concentration of target molecules in bulk



Γ_0 Density (per unit surface) of binding sites (or density of probe molecules)



$\Gamma(t)$ Density (per unit surface) of target molecules captured on surface at time t

Γ_{Eq} Density of target molecules on surface at equilibrium

$$\Gamma_{Eq} = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C}$$

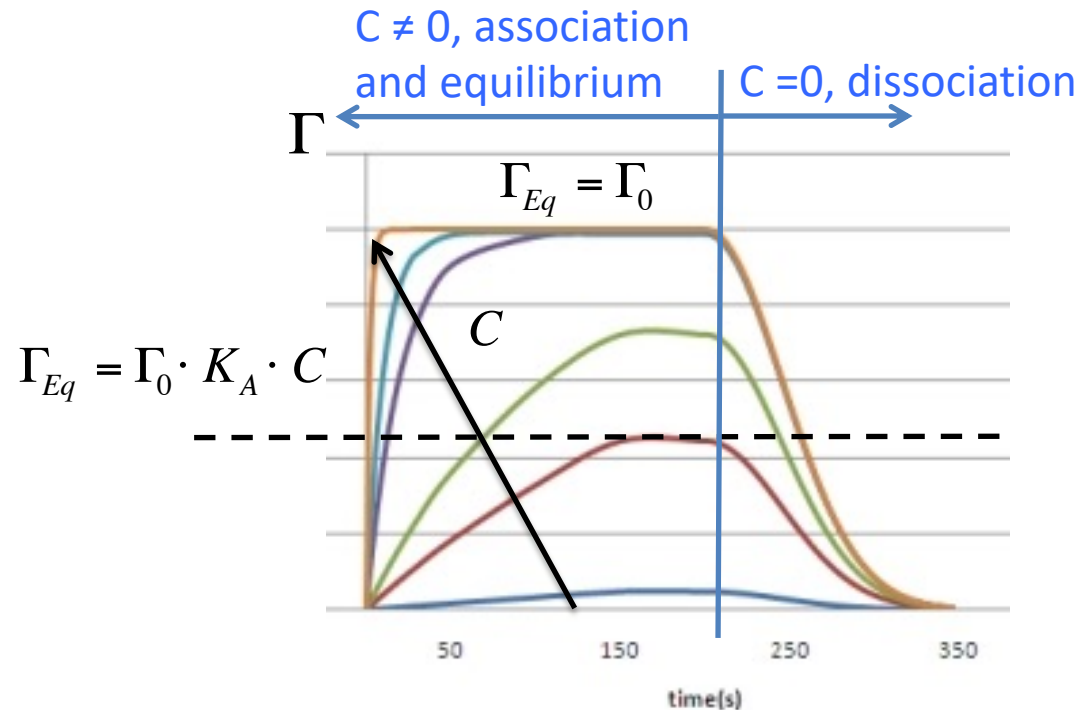
k_{on} Association-rate [$M^{-1} s^{-1}$]
(second order reaction)

k_{off} Dissociation-rate [s^{-1}]
(first order reaction)

$$K_A = \frac{k_{on}}{k_{off}} \quad \text{Affinity constant } [M^{-1}]$$

$$K_D = 1 / K_A \quad \text{Dissociation constant } [M]$$

Langmuir binding isotherm



association

$$\Gamma(t) = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C} \left(1 - e^{-(k_{off} + k_{on} C)t} \right)$$

equilibrium

$$\Gamma_{Eq} = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C} = \frac{C \Gamma_0}{C + K_D}$$

dissociation

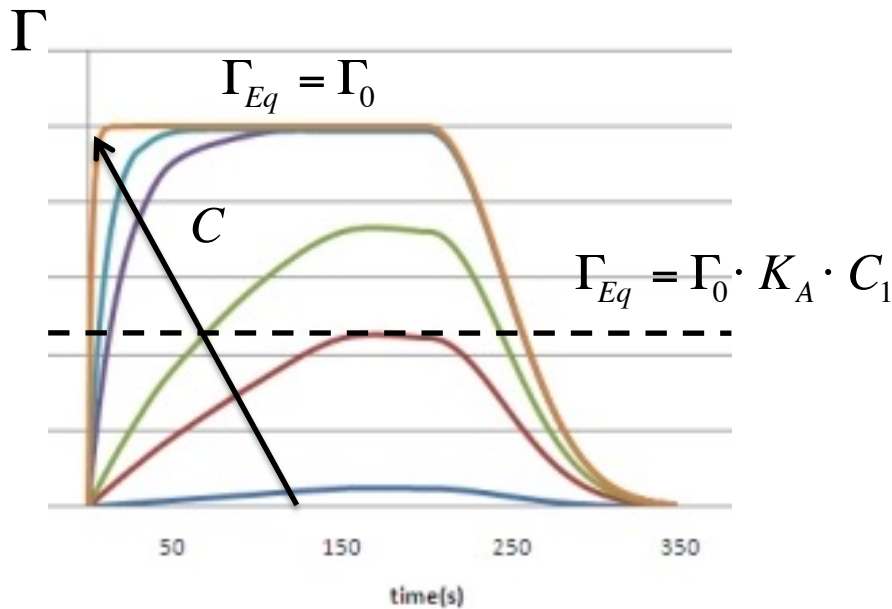
$$\Gamma(t) = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C} \cdot e^{-k_{off} t}$$

Adsorption kinetics



$$\Gamma(t) = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C} \left(1 - e^{-(k_{off} + k_{on} C)t}\right)$$

$$\Gamma_{Eq} = \frac{\Gamma_0 \cdot k_{on} \cdot C}{k_{off} + k_{on} \cdot C} = \frac{C \cdot \Gamma_0}{C + K_D}$$



eg: $K_A = 10^8 M^{-1}$

$$C_1 = 1 pM = 10^{-12} M$$

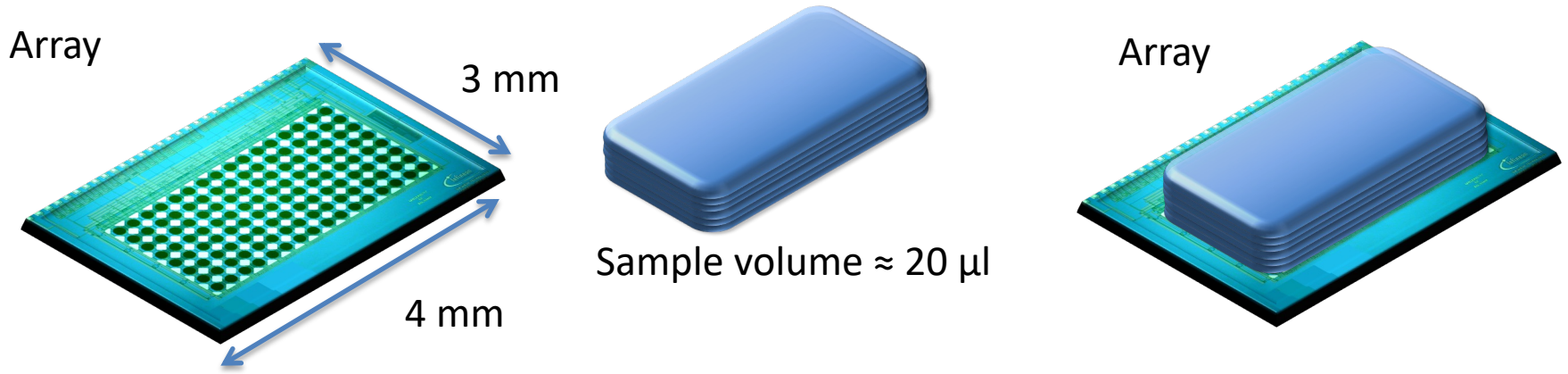
$$\frac{\Gamma_{Eq1}}{\Gamma_0} \approx K_A C = 10^{-4}$$

$$C_2 = 1 \mu M = 10^{-6} M$$

$$\frac{\Gamma_{Eq2}}{\Gamma_0} = \frac{10^2}{1 + 10^2} \approx 1$$

Binding reactions in incubation chambers

Concentration of analyte and total amount of analyte



Target concentration in the sample:

(Avogadro constant = $6.022137 \times 10^{23} \text{ mol}^{-1}$)

1 mM	→ 1 nmol/ μl	→ 6.022×10^{14} molecules/ μl
1 μM	→ 1 pmol/ μl	→ 6.022×10^{11} molecules/ μl
1 nM	→ 1 fmol/ μl	→ 6.022×10^8 molecules/ μl
1 pM	→ 1 amol/ μl	→ 6.022×10^5 molecules/ μl
1 fM	→ 1 zmol/ μl	→ 6.022×10^2 molecules/ μl
1 aM	→ 1 ymol/ μl	→ 0.6022 molecules/ μl

Number of sample molecules in 20 μl :

1 mM	→ 10^{16} molecules
1 μM	→ 10^{13} molecules
1 nM	→ 10^{10} molecules
1 pM	→ 10^7 molecules
1 fM	→ 10^4 molecules
1 aM	→ 10^1 molecules

Number of analytes captured on the surface at equilibrium

▼ Probe density: 10^4 molecules/ μm^2



Feature size: $10^4 \mu\text{m}^2$

features: = 100

Total surface = $10^6 \mu\text{m}^2$

probes on one feature: = 10^8 molecules

probes on the ensemble of all features = 10^{10} molecules

$$K_A = 10^8 \text{ M}^{-1}$$

Large concentrations: 1nM concentration

⇒ according to the Langmuir kinetics the number target molecules at equilibrium on the surface of the chip would be $\approx 10^9$ molecules. (n.b.: available molecules in solution : 10^{10})

Small concentrations 1fM concentration

⇒ number target molecules at equilibrium on the surface of the chip would be $\approx 10^3$ molecules. (n.b.: available molecules in solution : 10^4)

To overcome this issue: Role of the probe density and volume of incubation chamber

Decreasing the **surface density**

helps keeping the concentration constant

Nevertheless, It would also mean to reduce the absolute number of captured analytes at equilibrium (this might challenge the limits of the transducer for lower concentrations)

Increasing the **sample volume** (height of the chamber)

would increase the number of available analytes in solution.

Nevertheless, the farther the molecules are from the surface, the more it takes to reach it by diffusion.