SESSION 2: MAXIMUM SAMPLING RATE FOR A SURFACE LIGAND BINDING ASSAY

Exercise 1.

This exercise intends to evaluate the possibility of sensing in real-time the change in concentration of a pollutant in water.

We make use of a real-time sensing technique (e.g. SPR or mass sensing). The sensor is placed at the bottom of a channel. The water is injected with a constant flow rate Q in the channel. Water samples are taken from the source at a given sampling rate f.

The molecules of pollutant are the analytes whose concentration must be determined.

Probe molecules having $K_A = 10^7\ M^{-1}$ affinity constant towards the analytes are immobilized on the sensor at a density $\Gamma_0 = 10^{12}$ molecules/cm^2.

The channel has the following dimensions:

L = 20 mm

W = 0.5 mm

H = 2 mm

Consider the following approximation of the exponential Langmuir kinetics:

- rate of target molecules captured on the sensor per unit surface in the association phase: 1.3×10^8 molecules/ (cm$^2 \cdot$ s)
- rate of target molecules released from the sensor per unit surface in the dissociation phase: 5.8×10^8 molecules/ (cm$^2 \cdot$ s)

1.a Draw the density of analyte molecules as a function of time as generated by subsequent injections of samples with the following concentrations, in order: 300 nM, 500nM, 200nM, 50nM, 1µM, 50nM.

1.b Determine the maximum sampling frequency, i.e. the inverse of the minimum time delay needed to reach equilibrium after the injection of a new sample with a different concentration. Consider samples of concentrations ranging between 50 nM and 1 µM.

Exercise 2.

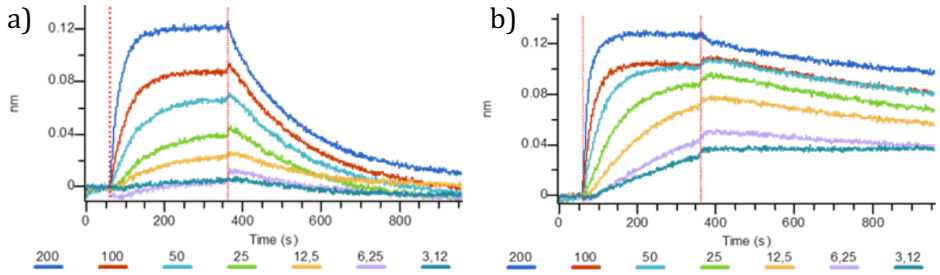

Figure 1 Profiles of association and dissociation of Cystatin C protein to anti-cystatin antibody a) clone A and b) clone B. *From https://www.caltagmedsystems.co.uk/information/whats-the-kd-of-my-antibody*

In order to identify in which contest to employ each monoclonal antibody, anti-cystatin clone A (anti-Cyst clone A) and clone B (anti-Cyst clone B), depending on their binding performances to Cystatin C, the human biomarker of kidney injury, the antibody manufacturer company performs assays on association and dissociation kinetics (Figure 1). The assessment of the kinetics is carried out by surface biosensors that measure the analytes surface density increase or decrease upon association or dissociation, respectively. Starting from these experimental data, the kinetics constants are calculated as follows:

	anti-Cyst clone A	*anti-Cyst clone B*
k_{on}	3.3 x 10^4 $M^{-1}s^{-1}$	35 x 10^4 $M^{-1}s^{-1}$
k_{off}	10^{-2} s^{-1}	7 x 10^{-5} s^{-1}
K_D	300 nM	0.2 nM

Considering a surface density of probe antibodies equal to Γ_0=10^{11} molecules/cm^2:

2.a For the two different antibodies calculate the equilibrium density of antibody-antigen complexes on the surface of the biosensor for a concentration of Cystatin C equal to 10 nM. Do they reach the condition of saturation coverage ($\Gamma_{eq} \cong \Gamma_0$) ?

2.b For a concentration of Cystatin C equal to 0.5 mM:

- Express the density of antibody-antigen complexes with time ($\Gamma(t)$) for both the association and the dissociation transient regimes and for both antibodies.

- Recalling that the exponential transient regimes contain the expression $e^{-t/\tau}$, with τ being the time constant, calculate numerically the dissociation time constants for the two antibodies under study.

- In the course of an ELISA assay, antibody-antigen complexes are formed on the surface of a multi-well plate. Afterwards, a 10 minutes washing step is performed in order to remove non-specific bindings before the final readout. At the end of this washing step, not more than 5% of the specific antibody-antigen complexes should be lost. Considering the two available antibodies (clone A and clone B), would they be suitable for an ELISA assay?