# BioSensorLab

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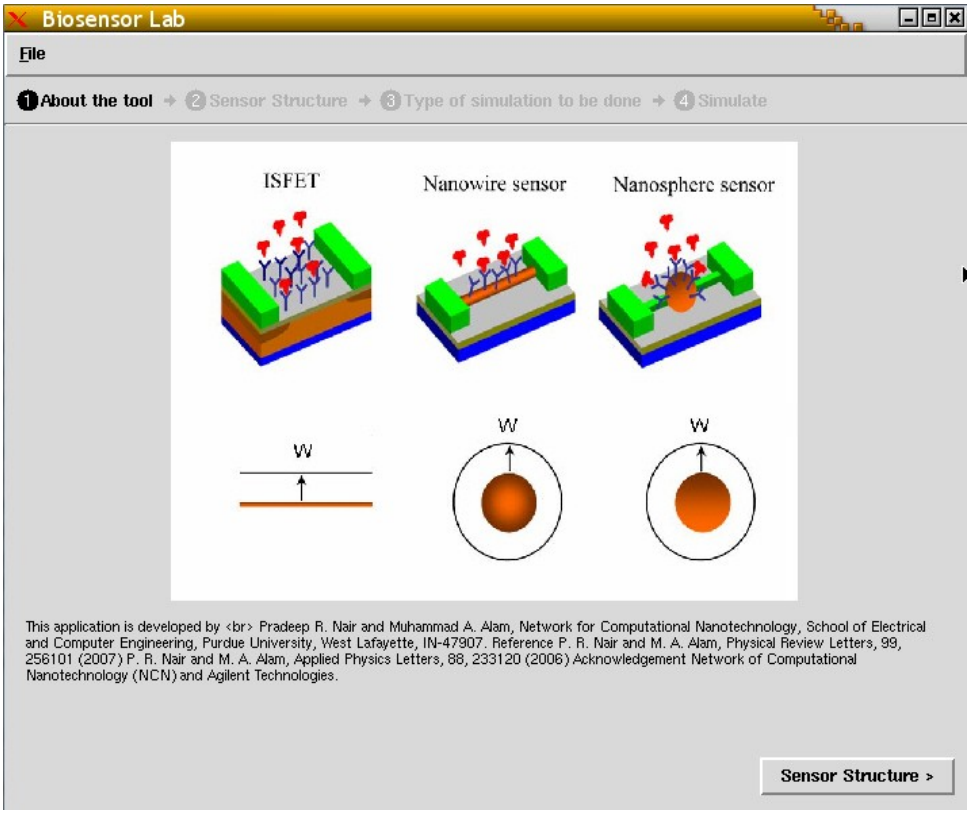
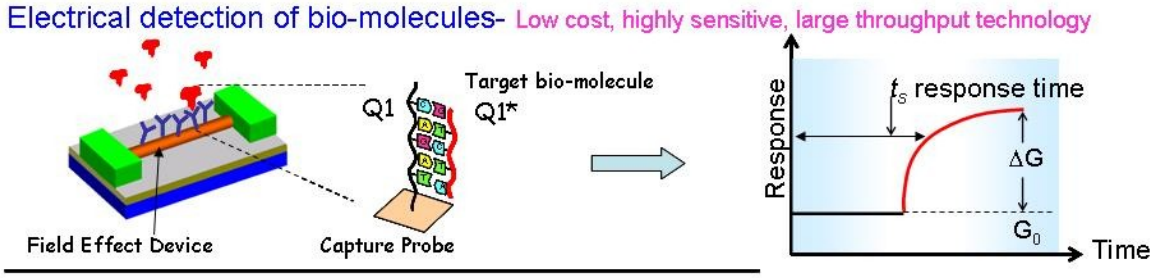
## 1. Introduction.

BioSensorLab is a numerical simulator to predict the performance metrics for various types of label-free, electronic biosensors (see figure below). At present, the BioSensorLab focuses only on those sensors that can detect the presence of charged biomolecules near the sensor surface by electrostatic interaction. To avoid parasitic response, the surface of electronic biosensors like the planar Insulated Gate FET or ISFET, are first functionalized with receptor molecules (blue Y in the Figure below) of known identity. When unknown target molecules (marked red) are introduced to the sensor volume, they diffuse throughout the sensor volume These molecules will be 'captured' by the receptors only if the target is a specific and exclusive complement to the receptor ('lock and key' principle). Bio-molecules like DNA carry negative charge under normal physiological conditions, while the net charge of a protein molecule depends on the pH of the solution. The excess charge of the receptor-bound target bio-molecule modulates the conductivity of FET channel electrons via coulomb interaction. And this change in conductivity signals the presence of complementary target molecules in the solution. In addition to electrostatic nanobiosensing, in the future, other types of biosensors based on optical detection, magnetic focusing, surface Plasmon response will be also become a part of this toolset. Stay tuned for updates!




## 2. Basics of Biosensing.




The response of a sensor is characterized in terms of its ***Settling time***, ***Sensitivity*** and ***Selectivity***. The time taken by the sensor to produce a stable signal change defines the ***Settling time***. It is determined by the concentration of the analyte bio-molecules (it takes longer to detect lower concentration analyte, for obvious reasons), their diffusion coefficients in water, and their conjugation affinity (ability to bind) to the receptor molecules .

***Sensitivity*** corresponds to the relative change in sensor characteristics upon attachment of target molecules on the sensor surface. This is determined mainly by the geometry of the sensor (e.g., oxide thickness, doping density of the sensor) as well as characteristics of the fluidic environment (e.g., salt concentration needed to stabilize

the conjugated molecules, pH concentration, etc.) When using BiosensorLab, make sure that the parameters are appropriately defined.

Finally, ***Selectivity*** denotes the ability of receptors to bind with the desired target in the presence of various other (possibly similar) biomolecules and is entirely determined by the functionalization schemes. For example, to detect target DNA, receptors based on Peptide Nucleic Acid (PNA) are shown to be more selective than their DNA counterparts. The relevant parameters involve geometry of the surface (planar, cylindrical, etc.), footprint of the molecules, as well as types of passivation schemes used to obviate parasitic binding.

The main purpose of BioSensorLab is provide rough theoretical estimate of the performance parameters of nanobiosensors (Settling time, Sensitivity and Selectivity). The theoretical model is based on self-consistent solutions of Diffusion-Capture model (for the Settling time response), Poisson-Boltzmann and Drift-Diffusion Equations (for electrolyte screening and conductance modulation) and the statistical properties of bio-molecule adsorption (Selectivity). However, the Selectivity module is not released for public domain right now and will be made available shortly. Although the models may appear relatively simple (see the discussion below), the implications of the models are actually profound and if you understand the basics and familiarize yourself with the predictions of the tool, you may actually have a very sophisticated understanding of the electronic biosensing.

## 3.1 Transient capture of target molecules: Diffusion-Capture Model

Time dynamics of molecule capture on a sensor surface is essentially a two step process: transport of the target molecules to the sensor surface and the subsequent conjugation with the receptor molecules. The Diffusion-Capture (D-C) model is widely used to describe this process. The model assumes that the molecule transport is diffusion-limited and the target-receptor conjugation is treated as a first-order chemical reaction. The model equations are:

$$\frac{d\rho}{dt} = D\nabla^2\rho, \tag{1a}$$

$$\frac{dN}{dt} = k_F(N_0 - N)\rho_s - k_R N. \tag{1b}$$

Eq. (1a) represents the diffusion of target molecules to the sensor surface where D and $\rho$ are the diffusion coefficient and concentration of target biomolecules (analyte) in solution, respectively. Eq. (1b) represents the capture of biomolecules by the receptors on sensor surface, where $N$ is the density of conjugated receptors, $N_0$ is the total density of receptors on the sensor surface, $k_F$ and $k_R$ are the capture and dissociation constants, and $\rho_s$ is the concentration of target analyte particles at the sensor surface. BioSensorLab solves Eq. (1) both numerically as well as analytically. For details, consult

the reference [1-4] as well as nanohub.org lecture by Prof. Alam (resource: http://nanohub.org/resources/2048).

## 3.2 Conductance modulation of sensor: Electrostatics

The full charge of the captured bio-molecules is not effective in modulating the conductance of sensors due to the electrostatic screening of ions present in the electrolyte. To account for screening, one must solve the non-linear Poisson-Boltzmann equation

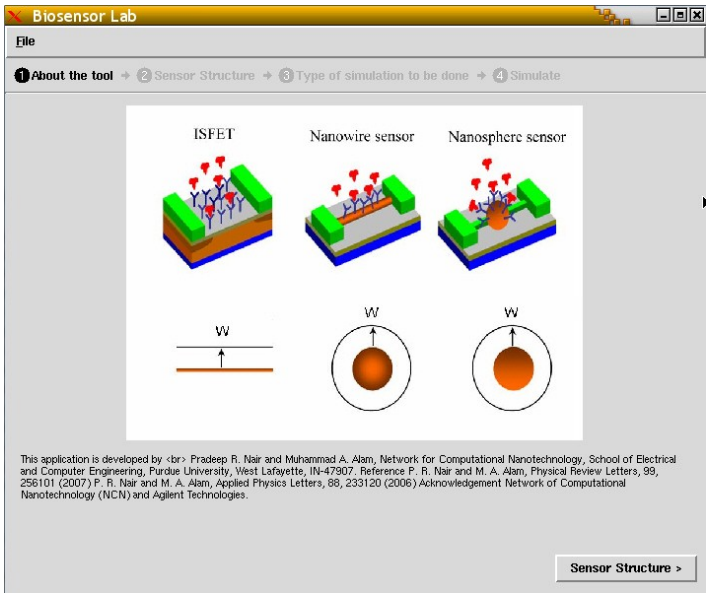
$$-\nabla^2 \Psi(r) = \frac{\kappa^2}{\beta} \sinh(\beta\Psi) + \frac{\sigma}{\varepsilon_W} N_t \delta(r) \quad (2)$$

where $\kappa$ is Debye-Huckel screening length and r is the spatial coordinate. $\kappa^2 = 2q^2 I_0 N_{avo} (\varepsilon_W k_B T)^{-1}$, where $I_0$ is the ion concentration in molar units, and $\varepsilon_W$ is the dielectric constant of electrolyte. The *sinh* term denotes the contribution due to a 1-1 electrolyte (e.g., $Na^+$-$Cl^-$), whose ions are assumed to follow Boltzmann distribution. The right hand side denotes the charge due to the conjugated target molecules on sensor surface (e.g., the net charge due to the phosphate ions in the backbone of a DNA strand). Please see Ref. [3, 5] for details of the model. For now, we will assume non-Faradic reference electrode and will not consider more sophisticate concepts like Stern layers as well as finite size of the biomolecules, etc. These improvements will be considered in future releases. The implications of the limitations are discussed in [6, 7].

# 4. How to use the Tool

BioSensorLab can be used to study the time dynamics of biosensors. In this tool, we solve the simultaneous system of D-C equation and P-B equations as discussed in Sec. 3.

Once you launch the BioSensorLab, you will find near the top of the opening page four tabs marked as **'About the tool'**, '**Sensor Structure'**, **'Type of Simulations',** and **'Simulate'**. We will first need to define input parameters by sequentially going through these tabs (see below) and once the definitions are complete, run the final tab called 'Simulate' to execute the program.

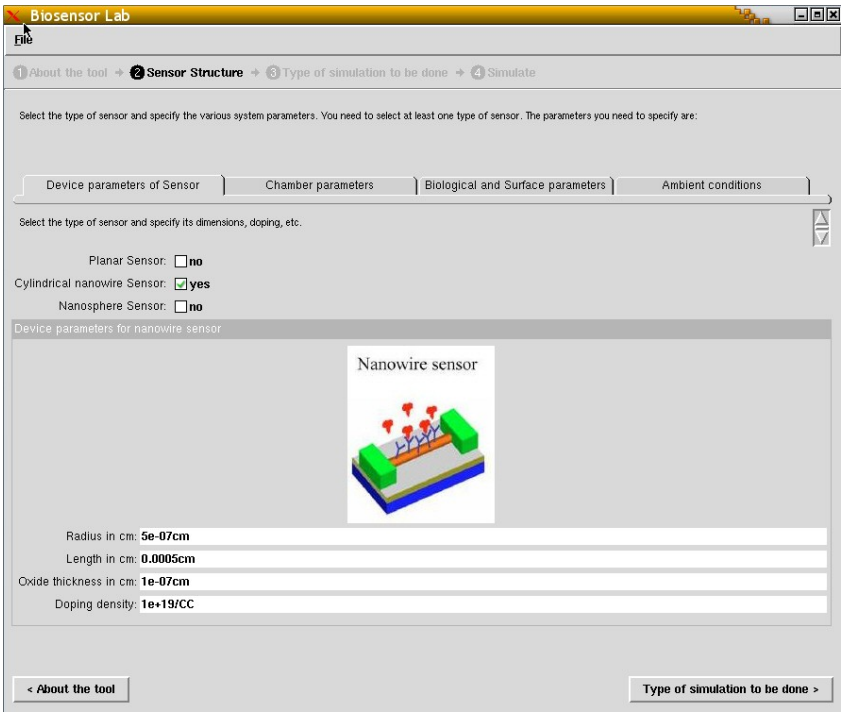



The basic simulation steps are:

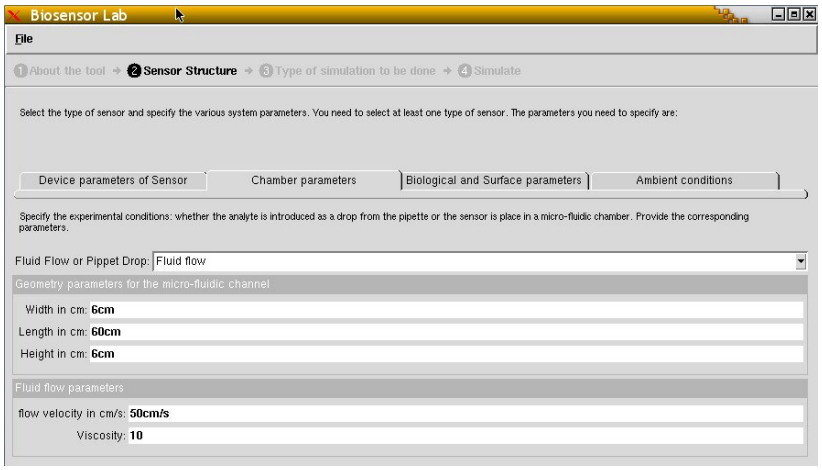
**Tab 1: Sensor Structure**

Once you click 'Sensor Structure' tab either at the top or the bottom of the opening page, you will be asked to define the geometry of the biosensors as well as the fluidic environment around the sensor that is used to funnel the biomolecules to the sensor. If you move your cursor over various options, you will find a short description of the parameter. Pay particular attention to the units – researchers from different field are comfortable with different units and those may not be the same as the ones used in this simulator.

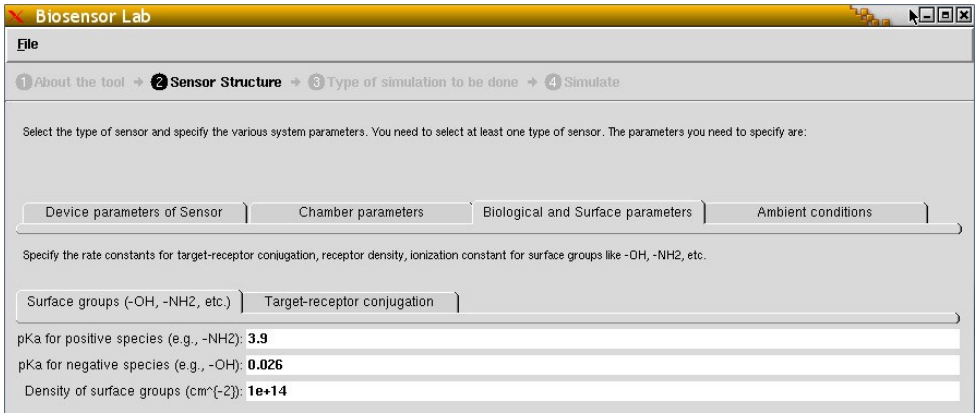
1. ***Device parameters of sensor***: First, choose the type of sensor (You need to choose at least one) by clicking the square box. The options are: (i) Planar ISFET, (ii) Cylindrical Nanowire sensor, and (iii) Spherical Nanosphere. For each of the selected options, provide the various input parameters like oxide thickness, doping density, device dimensions, etc. If you do not see the list, scroll down below the figures.



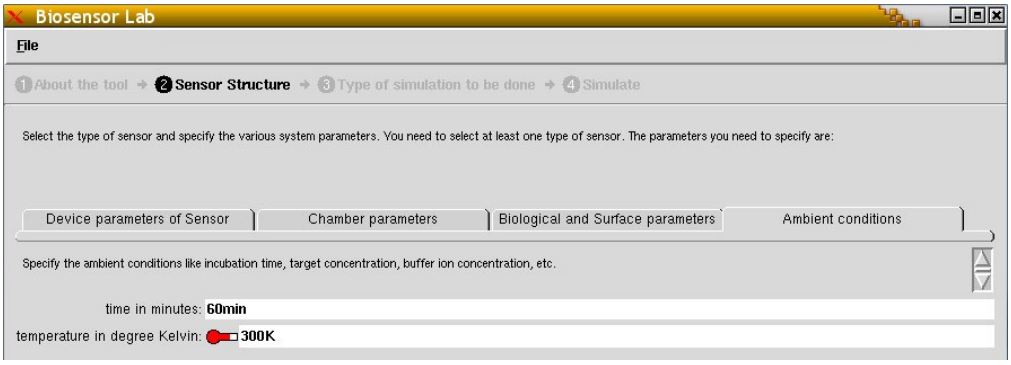

2. ***Microfluidic Channels Parameters***: Now we can move to the next tab (see in the middle of the page) called microfluidic Channels parameters. Specify the experimental conditions like the dimensions of microfluidic channels, volume of the analyte introduced, etc. If you do not know these parameters, keep the default values.




3. ***Biological and Surface Parameters***: Specify the reaction constants for target-receptor conjugation, receptor density on sensor surface, the surface parameters like the type of terminations (-OH, -NH2), their ionization constants, etc. These set of parameters are used to estimate the equilibrium concentration of captured target molecules on the surface and their response to pH variations.



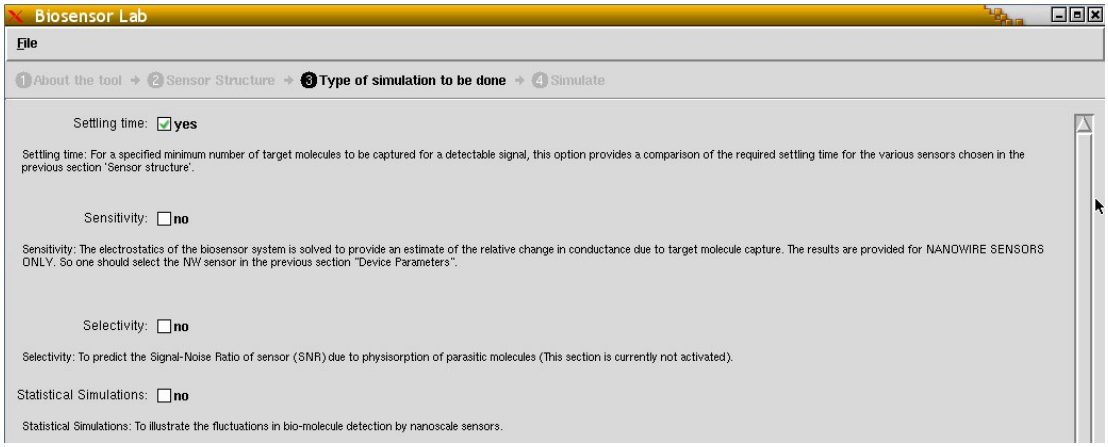

4. ***Ambient conditions***: Temperature at which your experiments are being carried out (typically room temperature) and the maximum amount of time you are willing to wait before making a measurement (mins, hours or even possibly overnight).




Now you are done defining the system, you can now click on the tab marked 'Type of Simulation to be done' at the bottom of the page to move to the next step.

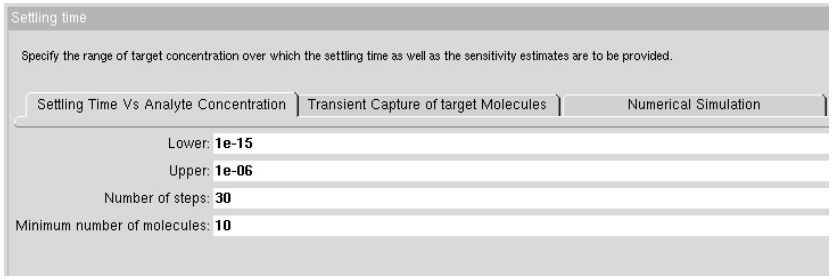
## Panel 2: Type of simulations

Here the user needs to specify the type of simulations:

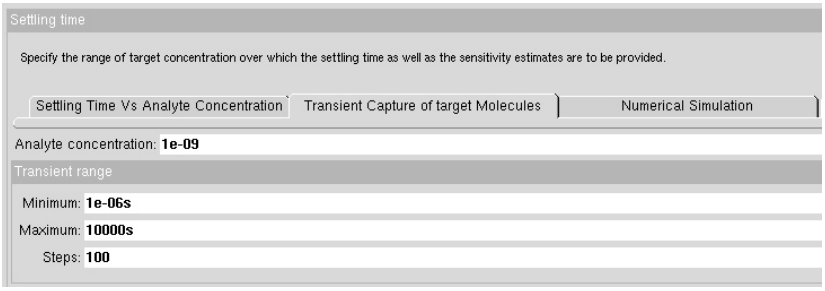



(i) ***Settling time:*** For a given range of target analyte concentration, this option will provide you the *average or mean* settling time required to capture a specified density of target molecules on the sensor surface. For example, if you started with 100 sensors each with separate reaction volume or if you made the same measurement 100 times, average settling time means that 50% of the sensors would have responded by this time. Once you select this option, the user can specify the various output options as shown

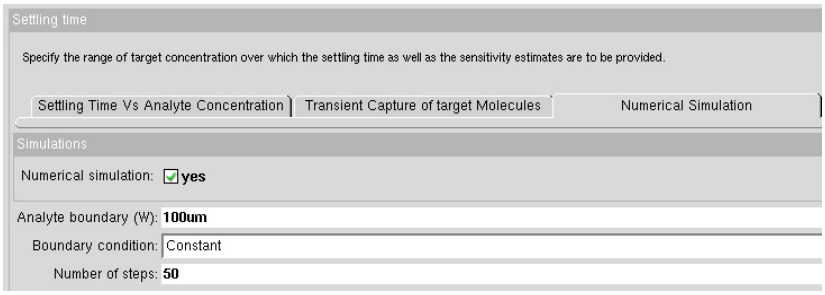
- **Settling time vs. Analyte Concentration:** This option provides the user the Settling time required to capture a given density of target molecules on sensor surface.




- **Transient capture of target molecules:** The user will be able to study the Diffusion limited capture of bio-molecule capture on sensor surface for a given analyte density. The reaction constants from the section "Surface and Biological parameters" are used for this set of simulations.




- **Numerical Simulations:** The user can avail the option of performing numerical simulations to check the consistency of the analytical results.




(ii) ***Sensitivity:*** The electrostatics of the system is considered in this case. For the range of analyte concentrations, the option predicts the relative change in conductance of the NW sensor (at a particular buffer ion concentration). For a given target molecule density, the variation with buffer ion concentration is also shown. Similarly, the response of the sensor to variations in pH is illustrated. **Note that the input 'Cylindrical NW' needs to be selected in 'Device Parameters' to perform Sensitivity simulations.** The various options are:

- **Conductance Modulation vs. Analyte concentration:** Assuming equilibrium conditions (i.e., the surface conjugation of target molecules have reached steady state), this option provides an estimate for the steady-state conductance modulation of NW biosensors, at a particular buffer ion concentration

- **Conductance Modulation vs. Buffer Ion concentration:** Assuming equilibrium conditions, this option provides an estimate for the dependence of conductance modulation on buffer ion concentration, for a specific bulk target density.

- **Conductance Modulation vs. pH:** Variation in the [H+] concentration of the buffer results in protonation/de-

protonation of surface groups like –OH, -NH2. Using the pKa values provided in section 'Surface and Biological Parameters', this option predicts the variation of NW surface potential as a function of pH at a particular buffer ion concentration.

(iii) ***Selectivity:*** For a given incubation time, this option predicts the surface coverage due to receptor molecules and the Signal-Noise Ratio due to the physisorption of parasitic molecules on the sensor surface. **This option is currently not activated.**

(iv) ***Statistical Fluctuations***: As the target density reaches femto-molar limit, statistical fluctuations in the arrival time of analyte molecules on sensor surface becomes increasingly important: statistically some sensors find the target molecule long before others do. This range of arrival time distribution has significant practical implications, because if one needs to design for biosensors chip so that 99.99% of the sensors can complete the conjugation before the test is terminated. Mean arrival time discussed above will provide an optimistic limit of this time. This option allows the user to explore how the minimum detection time varies from the average detection time.

## Panel 3: Simulate

Once the specifications are made, you can now go the final tab called 'Simulate' to obtain the results of the analysis. Clicking on the tab invokes the program and within a couple of minutes the results will be returned. Depending on the input parameters, the output section illustrates the trade-off involved between various parameters like settling time, analyte density, buffer ion concentration, pH, etc.on the response of a biosensor. The user may wish to change the input conditions to further explore the key features of electrical detection of bio-molecules.

**Example Problem:**

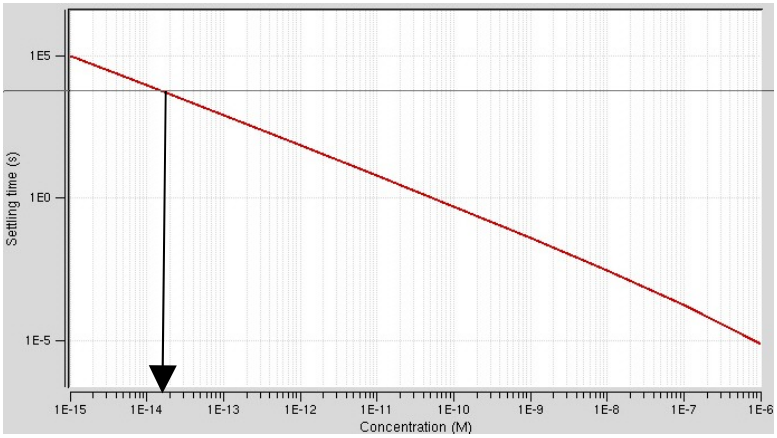
*Here is an example problem that you should try to get comfortable with the simulator.*

Assume that you want to design a cylindrical nanowire (NW) sensor of 50 nm radius for the purpose of capturing 20-bp (base pair) DNA molecules. The given device's stability time is limited to 10,000 seconds (about 3 hours). The surface conjugation parameters are not known exactly, but assume for the time being typical values of $k_f = 3\times10^6\ M^{-1}s^{-1}$ and $k_r = 1\ s^{-1}$. The test fluid of 6 cc is injected to the sensor via pipette drop rather than continuous external flow. Now see if you can answer the following questions.

*a) What is the limit of detection?*

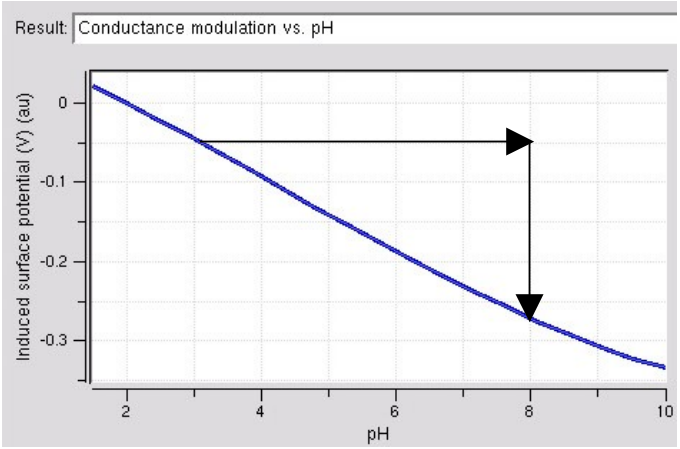
Answer: In '2. Sensor Structure' tab and 'Device Parameter' subtab, choose NW sensor, set radius of 50 nm. Keep length, oxide thickness, and doping density the same for the time being because these values were not specified. For Chamber Parameters, choose "pipette drop". The $k_f$ and $k_r$ values are now specified in subtab 'Biological and Surface parameters/Target-receptor conjugation". In the same subtab change DNA strand length (bp) to 20 and Diffusion parameters to DNA diffusion model. We will not change the 'Surface group (-OH, -NH2, etc)' parameters for the time being. We will assume room temperature in 'Ambient conditions' tab.

For this problem, we are interesting in 'Settling time', so let us mark this option in the '3. Type of Simulation to be done' tab. In the sub-tab of "Settling time vs. analyte concentration", we will keep the parameters unchanged for the time-being. If everything is properly set, you will get a curve like the one shown below. And you will be able to see that with 3hr incubation or settling time (horizontal line), the minimum analyte density you can detect (intersection point between red line and black horizontal line) is $1.5 \times 10^{-14}$ M.



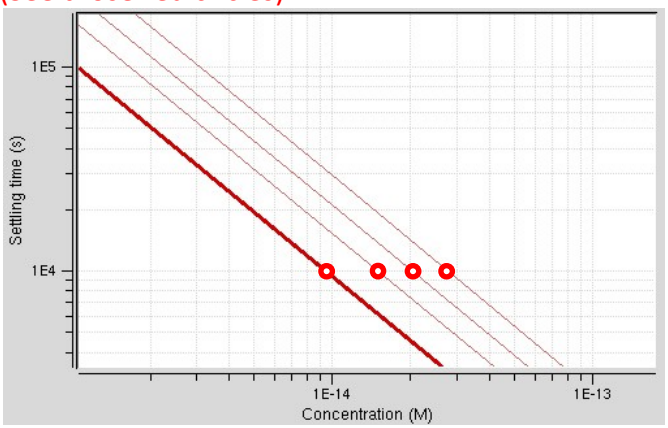

*b) If pH of the test fluid changes from pH 3 to pH 8, how does the surface potential change?*

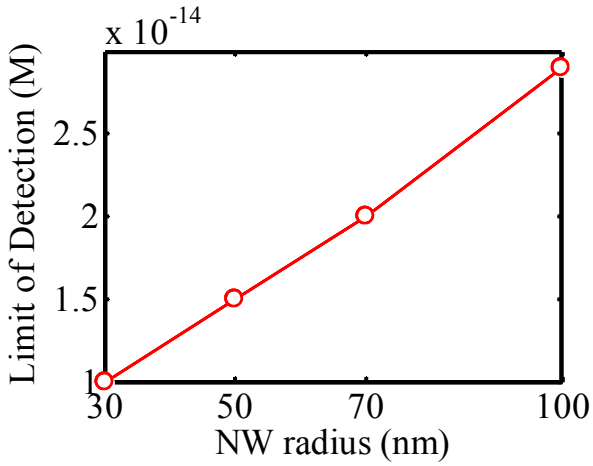
Answer : This question is related to Sensitivity of a biosensors, therefore choose Sensitivity in the Type of Simulation to be done'. You should also select the 'Conductance modulation vs. pH' plot and specify the range of pH in the specification. The plot below shows that the change in surface potential is 0.2 V.




*c) Due to the statistical variation of NW radius during device fabrication process, the corresponding sensor response would change as well. If the known range of NW radius is between 30 nm and 100 nm, what is the corresponding fluctuation in the limit of detection? Plot the limit of detection versus NW radius.*

Answer. You need to do a series of simulation for different radii between 30 nm and 100 nm one by one. Do not change the parameters other than the NW radius. Choose 30 nm, 50 nm, 70 nm, and 100 nm as the radii of NW for example. To get the results for different NW radii, you must go back to the '2. Sensor structure' tab, change NW radius and re-run your simulation. After all simulations are done one by one, press the 'All' button in the bottom left of the simulation result window for 'Settling time vs. analyte concentration'. This will show you all the simulation results together. Then magnify the plot by dragging cursor on the plot and measure the corresponding changes of detection limit, as shown below (See those red circles).






**References**


[1] P. Nair and M. Alam, "Performance limits of nanobiosensors," *Applied Physics Letters,* vol. 88, p. 233120, 2006.

[2] P. Nair and M. Alam, "Dimensionally frustrated diffusion towards fractal adsorbers," *Physical Review Letters,* vol. 99, p. 256101, 2007.

[3] P. Nair and M. Alam, "Screening-Limited Response of Nanobiosensors," *Nano Letters,* vol. 8, pp. 1281-1285, 2008.

[4] J. Go and M. Alam, "Statistical interpretation of "femtomolar" detection," *APPLIED PHYSICS LETTERS,* vol. 95, pp. -, JUL 20 2009 2009.

[5] P. Nair and M. Alam, "Design Considerations of Silicon Nanowire Biosensors," *IEEE Transactions on Electron Devices,* vol. 54, pp. 3400-3408, 2007.

[6] D. Landheer*, et al.*, "Calculation of the response of field-effect transistors to charged biological molecules," *IEEE SENSORS JOURNAL,* vol. 7, pp. 1233-1242, SEP-OCT 2007 2007.

[7] Y. Liu and R. Dutton, "Effects of charge screening and surface properties on signal transduction in field effect nanowire biosensors," *JOURNAL OF APPLIED PHYSICS,* vol. 106, pp. -, JUL 1 2009 2009.


Happy Computing !!!