Research Article

Multiscale Simulation of Adsorption Based Microcantilever Biosensors for Radiation Exposure Effects

Fouad Mollaei [1,2], Peiman Aliparast [1*], Abolghasem Naghash [2]

[1] Aerospace Research Institute, Ministry of Science and Research and Technology, Tehran, Iran
[2] Aerospace Engineering Department, Amirkabir University of Technology, Tehran, Iran

Corresponding author: Peiman Aliparast, Aerospace Research Institute, Ministry of Science and Research and Technology, Tehran, Iran. Tel: +982188366030, 283, Fax: +982188362011, E-mail: aliparast@ari.ac.ir, PO Box:TR- 1591634311

Background: This article is focused on biological measurements based on molecular interactions. The specific biomarker implemented for radiation biosensor is FLT3, which bears changes in the body regarding radiation exposure. Experimental results of sensing vancomycin verify the overall results of two steps of numerical methods for different scales.

Objectives: The aim is to provide adequate modeling procedures to predict sensory data. Multiscale modeling is implemented to simulate molecular interaction and its consequent micro mechanical effects. The method is implemented to calculate surface traction of microcantilever biosensor.

Materials and Methods: The method consists of molecular dynamics simulation of adsorption process by implementing classical mechanics theory to calculate the final response of the sensor as tip deflection. The sequential information transaction is assumed between the physical parameters of two governing scales. The numerical method consists of the location of particles providing for a nano-metric periodic boundary conditioned functionalized surface implemented, and the numerical thermodynamic formula is, in turn, use energy parameters to acquire macro-mechanical deflection of a specific microcantilever. Also, novel sensitivity analysis of the results as the adsorption process moves toward more saturated substrate provided.

Results: Verification of the simulation method for Vancomycin sensing results enjoys less than 20 percent of deviation regarding the experimental data. The standard deviation of 0.054 in the final expected response of the sensor is calculated as the accuracy of the radiation biosensor based on FLT3.

Conclusions: The method is still to reach a correlation between the concentration of target molecules in solution and the number of adsorbed molecules per area of the sensor. A scaled correlation between sensor's response and the amount of biomarker is found using tip deflection of a sample designed microcantilever. Around one micrometer deflection that can be read out using various conventional methods was observed at saturation of adsorption surface. The analyses provide adequate data to design a sensor capable of measuring the effect of cosmic radiation to the human body.

Keywords: Multi-Scale, Adsorption, Molecular Dynamics, Micro-Cantilever

1. Background

Detection and measurement of biological agents are assumed as transduction of so-called data into circuit-based measurable parameters. The process is known as biosensing thought; the clinical and academic significance is needless to mention further (1). Therefore, providing facts about transduction mechanisms would have positive incomes scientifically and clinically. The transaction can be classified into three different disciplines, that transfer biological signals into optical, electrical or mechanical signals (2).

In the current article, mechanical transduction (changing biological signal to mechanical) is only taken into account. The aim is to simulate the process of changing biological signal as a specific molecular concentration, changing to surface traction i.e., a mechanically significant parameter that can be measured as the deflection of a flexible part. Regarding that, the amount of loading is off the scales of the flexibility of a normal macro-scale mechanical part, and to enable in-vivo sensing, a specific microstructure is chosen (3). Although larger i.e. و non-micro structures can also provide such a deformation analytically, providing an appropriate read-out device puts constraints technologically and, or financially; as the response magnitude shrinks. Further discussion around challenges in measurement of induced deflection is beyond the scope of the current text.

Microfabricated cantilever beam (microcantilever or abbreviated as MC) biosensor is chosen due to its

capability of dual-mode sensing i.e., static and dynamic modes (4). The latter is considered to enhance precision.

2 Objectives

The aim is to provide adequate modeling procedures to predict sensory data. Multiscale modeling is implemented to simulate molecular interaction and its consequent micromechanical effects. The method is implemented to calculate surface traction of microcantilever biosensor. Multiscale simulation is implemented to calculate the final deflection of the beam; because the dimensions of sensing principle i.e., changes in surface traction due to molecular binding are in the nano-metric scale, and cantilever is considered to have micro-metric dimensions. The implemented method is sequential multiscale modeling. The method provides micro-scale calculations by one-way input data provided from nano-scale molecular dynamic simulations.

As the biological signal may cover a wide range, only a bio-molecular protein signal is considered in advance. The main objective is to provide adequate data to design a biosensor which is capable of measuring the effect of radiation to human body. So-called effects are considered by tracing concentration of a specific molecule. First, the method is verified using another biosensor that implements the same platform. The latter is used because data on such a specific sensor for radiation biomarker is not available. Therefore two target molecules are studied. The first studied case is Vancomycin sensing (5) which is an antibiotic. The second case is the FLT3 molecule (6). The former is to verify the model, and the latter is to provide necessary data to design desired biosensor for radiation effects (7).

3. Materials and Methods

3.1. Multiscale Modeling Method

In order to provide an adequate understanding of the model, the working environment and the principles are described in advance. As illustrated in **Figure 1**, the MC is used as-built and by adding a very thin layer of Gold on one side, by which non-symmetric (only on top surface) functionalization is enabled. Then by a particular chemical process, receptor molecules that selectively adsorb target molecules are added (functionalization). In the current text, more details of functionalization were put out of scope; the latter is due to referring to experimental works done previously. Then the MC gets encapsulated inside a microfluidic channel that delivers samples in aquatic media. If the sample has a specific amount of target molecules, so-called molecules are getting adsorbed to the functionalized surface; which changes the surface

traction on the surface. The differential deflection induced by adsorption is then calibrated using other methods capable of finding target molecules concentration. The MCs are working as an array of sensors consisting of reference beams and functionalized ones, which together measure the differential deflections.

Two major surfaces (top and bottom) can act as adsorption substrates as the cross-section of the beam usually has a high aspect ratio, as shown in **Figure 1 (A)**. When the MC is functionalized asymmetrically, changes in surface traction lead to beam bending toward one side that leads to maximum deflection at the tip of MC. The governing mathematical model of MC deflection is calculated in continuum mechanical principles in the micro-scale. Changes in surface traction duo to adsorption process lie within molecular principles i.e., discreet physics in nano-scale; each scale has comprehended theoretical background so far.

3.2. Computational Theory

The tip deflection of the cantilever (x), due to change in surface traction (ϭ) is calculated using Stoney's formula as brought in equation (1) (8).

$$x = \left(\frac{l}{t}\right)^2 \frac{(1-\nu)}{E} \Delta\sigma \qquad (1)$$

Where t and l are the thickness and length of the cantilever, respectively, E and ν are Young's modulus and Poisson ratio. The model provides a macro and micro-mechanical model to calculate final adsorption induced beam deflection.

Therefore, MC's material and geometrical properties should be assumed. As described before, experimental work is selected to verify the calculation method for Vancomycin sensing (9). The implemented beam has 750 by 100 micrometers of sensing surface and thickness of 1 micrometer. The top surface is Gold coated to host adsorption substrate. The Young's modulus of ceramic (silicon) MC is assumed as 130 Giga Pascal and the Poisson's ratio to be 0.06 (10). For Gold layer, the Young's modulus is assumed 79 Giga Pascal and Poisson's ratio to be 0.42 (11). By using classical mechanics, two compositions are combined to an equivalent beam of the same thickness. In the macro-scale i.e., continuum, the only theoretical calculation is carried out and no numerical solution is carried.

The surface traction at discreet scale i.e., nano-scale is calculated by using molecular dynamics. This calculation is the bridge between nano and continuum scales. As brought in equation (2), surface tension difference related to the difference in Gibbs free energy, ΔG, target molecule mass, m, simulated element area,
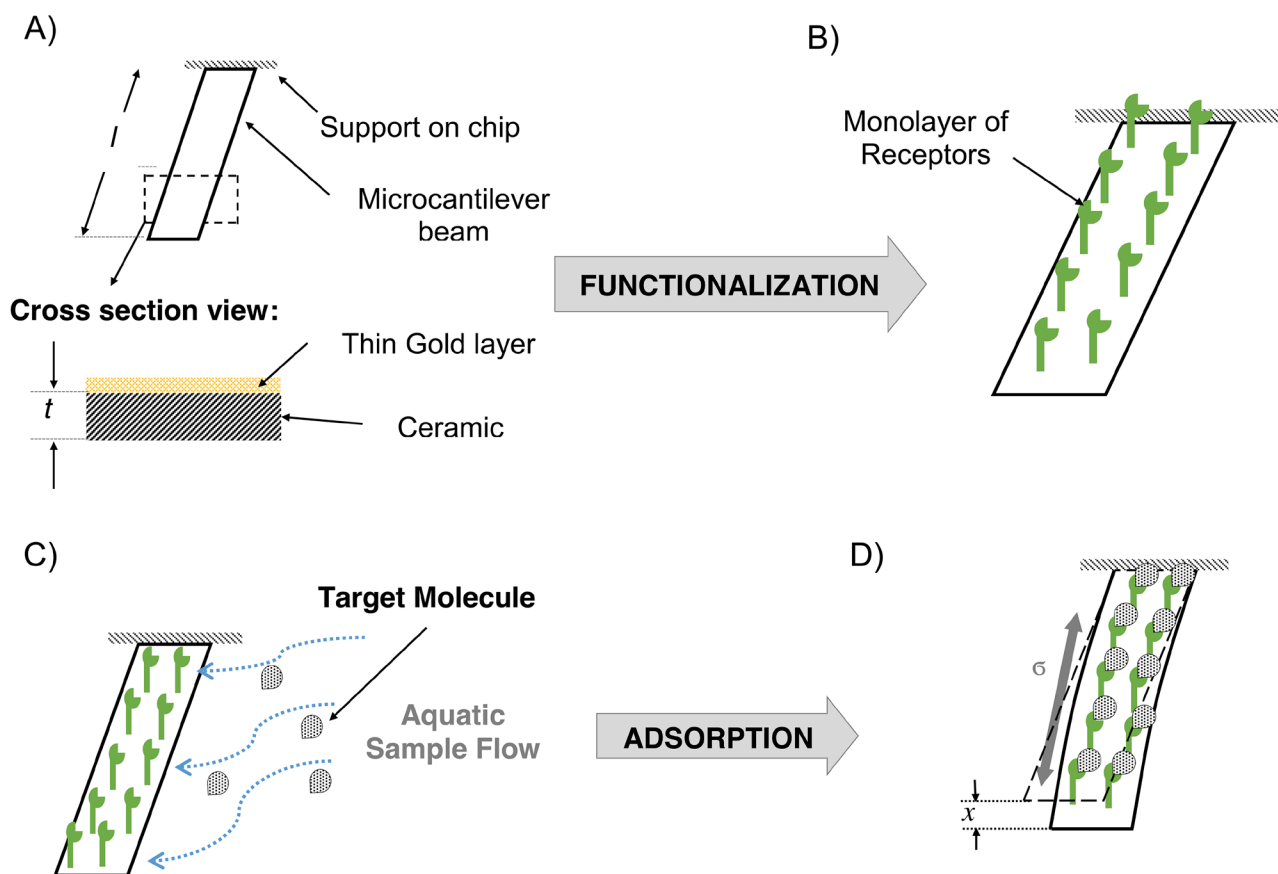
Fig. 1. Overall of biosensing using microcantilever beam, **(A)** bare microcantilever beam details, **(B)** functionalized MC hosting array of receptors (SAM) on top layer, **(C)** sample delivery in aquatic media, **(D)** induced beam deflection after target molecule adsorption on sensor

A, and molecular weight of target molecule, M, (12).

$$\Delta\sigma = \frac{(\Delta G \times m/A)}{M} \qquad (2)$$

The relationship is describing Gibbs free energy in terms of classical thermodynamics as the energy representation is assumed as equation (3).

$G = U - TS + PV$ (3)

In above equation (3), U is internal energy, T is temperature, S is entropy, and P and V are pressure and volume. The free energy perturbation study is used previously and sought to have finite error (13). Here Gibbs free energy is studied to find changes in surface traction. Changes in entropy i.e., second term in right-hand side of equation 3, there is usually considered to have variations (14). In current study, the latter has been neglected due to implementing experimentally validated molecular models, low deviation in atomic positions, and lack of any conformational change during the simulations.

To calculate energy differences of the system during adsorption, the process is modeled using molecular dynamics (MD). The calculation requires an atomic model of substrate and target molecules and aquatic media. The NAMD computational platform has been implemented to solve the dynamic motion of the model and extract the desired terms of statistical thermodynamics (15).

The sequential multiscale modeling consists of first solving MD for adsorption, finding terms of energy, bridging the Gibbs energy to surface traction, and finally using Stoney's formula to calculate the deformation of MC, respectively.

Mentioned dimensions and material properties have a standard deviation practically, and the results in Gibbs energy calculations have also scattered. Therefore, six-sigma analysis taken into account to find final data deviation using a normal distribution assumption and curve fitting. The deviation was used to calculate accuracy and precision expected for the biosensor and also to choose appropriate read-out strategy and MC dimension.

3.3. Molecular Models and Ensembles

The overall sensory setup consists of multiple modules of which only the close vicinity of the MC's surface is taken into account in MD simulations. Due to miniature

dimensions of the MC, the flow of samples is assumed to be homogeneous with a mean velocity that is less than the Brownian movement of particles by orders of magnitude. The top surface of MC is functionalized with specific receptors of one target molecule. The receptors thought to form a single assembly monolayer (SAM) on top of the Gold layer. The formation of a functionalized surface is not studied explicitly, but the corresponding experimental sensing value suggests its success generally (9).

The first aim of the study is to find a molecular ensemble that has specific adsorption affinity to vancomycin. The receptor that forms SAM is Vancomycin-binding peptide (Cys-Gly-Gly-Gly-Gly-L-Lys-D-Ala-D-Ala). The molecular model created using Vega software in standard form to be used by computational code (16). Vancomycin molecular structure is studied relying on X-ray experimental scan data and is accessible in the form of protein data base (pdb code 1HGH) with adding missing atoms; the experimental data suggests two D-Ala amino acids at the end of the chain are to bind vancomycin electrostatically (17, 18). The bottom end of the receptor molecule has a Sulfur atom, which is assumed to bond to the Gold substrate; the bonding in models is made manually. The interaction between substrate and biomolecules is considered negligible in comparison to receptor-target molecules specific binding.

The electrostatic charge distribution of a single receptor is calculated using CHARMM parameters and illustrated in **Figure 2 (A)** from side view (i.e., along the substrate) (19). By assuming electrostatic forces to govern the most, a repeating geometrical pattern is considered to form a stable formation for receptors in SAM despite unordered formations. The longitudinal and the lateral distances between neighboring receptors is considered to vary as the dimensions of the receptors vary from the top view, as shown in **Figure 2 (B)**. Regarding experimental works (20), the mean distance is accounted for similar to cysteine SAM on Gold, and the pattern is then built asymmetrically in two different directions along the surface. The rotational degrees of freedom for receptors are kept inactive to maximize the same charge particle distance. To ensure the possibility of placement of receptor molecules in the suggested distance, the incremental increase of distance between two target molecules in vacuum also studied. Thought rotation is not considered, the cut-off distance in various directions complied with the mean distance. Therefore the cut-off distance in the different directions are suggested to be proportionate with the size of molecules i.e., made of charged particles.

The minimum size of the repeating pattern is four receptors, as were shown in **Figure 2 (B)**. The repeating pattern has in-plane dimensions of 110 by 89 angstroms for vancomycin. The so-called dimensions are calculated for FLT3 sensing by finding relaxation distance of two receptors using MD total energy calculations. The size of the smallest pattern calculated as 250 by 180 angstroms. Periodic boundary condition (abbreviated as PBC) is applied to the faces of the repeating pattern. The first case of sensor simulation consists of a model of 36 receptors. Choosing a nine-fold pattern of minimum size (for Vancomycin case) helps to study fine increments of adsorption. By transferring target molecules onto the adsorption site from far-field, the changes of related energy terms were studied. The algorithm of selecting each site in advance is based on adding maximum squared distance (accumulated) by the next increment. Therefore, after the first site selection, the sum of squared distance between an arbitrary site and adsorbed site(s) is calculated. The next site is conditioned to have a maximum value of the accumulated distance between not adsorbed sites. The distance is calculated by taking a periodic boundary effect into account. To make sure far-field target molecules would not interact, two free-standing target molecules were also modeled with the absence of aquatic media in different distances. In the latter case, no PBC was implemented. For the next simulation cases, only minimum pattern i.e., four receptors are taken into account.

The models also consist of 40 angstroms of Gold substrate and enough water molecules to reach the desired density. The corresponding number of target molecules i.e., 36 vancomycin molecules, are also placed at far enough distance to ensemble not-adsorbed condition. The distances between target-molecules were also studied to ensure they are not interacting when placed at the image location corresponding to receptors. Gold atoms kept fixed exceptionally.

The solution which carries the sample toward MC in a corresponding experimental survey is reported to have ions that are not included in the current study; Such elimination is justified here as the ionic concentration is small (0.1 M PBS) regarding model boundary size.

The same manner of minimum pattern incremental study is also applied to FLT3 case; in which the molecular structure of target molecule and its receptor also considered (pdb code 1RJB) (21), although no experimental background is available for SAM formation of the FLT3 ligand on Gold surface (pdb code 1ETE) (22). All simulations performed assuming one million steps, each a half femtosecond length.

In order to minimize the cost of the read-out system and sensitivity of the radiation's effects sensor, softer
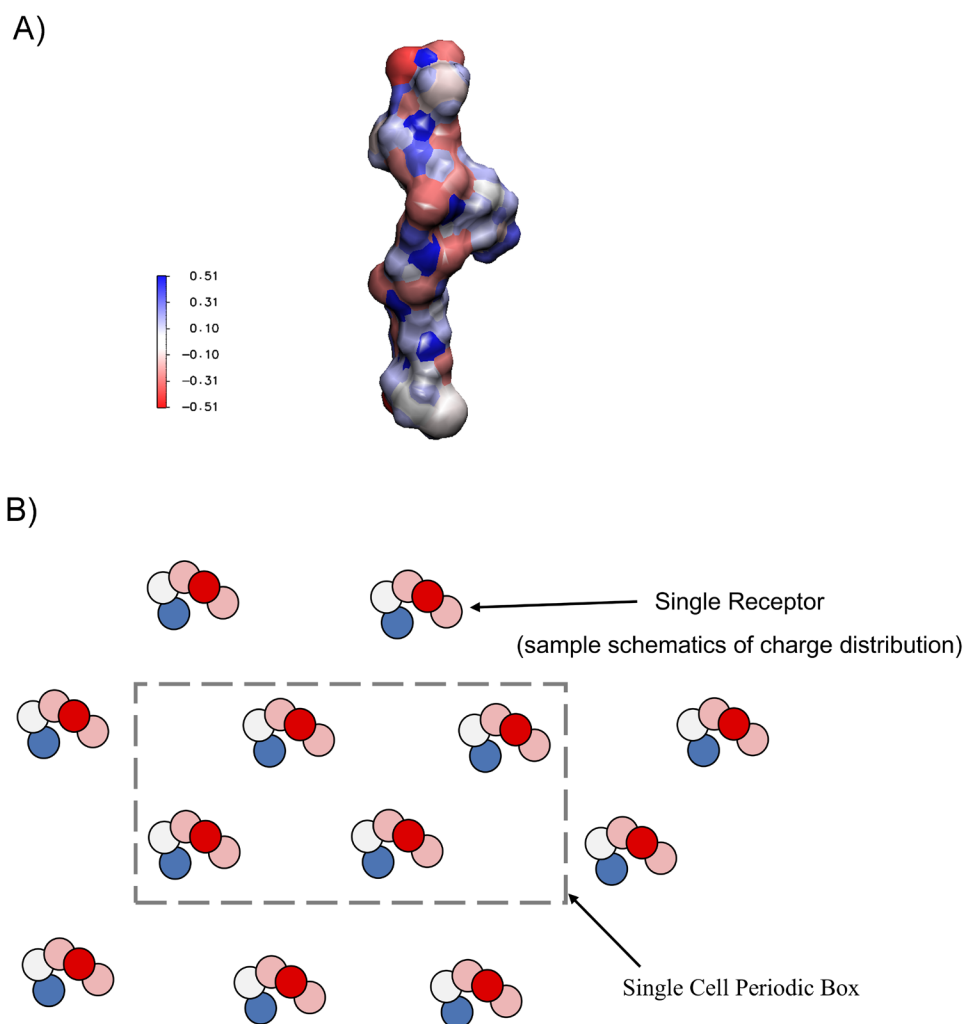
A)

B)

Single Receptor

(sample schematics of charge distribution)

Single Cell Periodic Box

Fig. 2. Charge density of Vancomycin receptor, **(A)** Charge Distribution of a single receptor; **(B)** PBC boundaries of ordered SAM formation

material will provide more significant displacement; therefore, a polymeric material is chosen for MC having Young modulus equal to 3.5 Giga Pascal with 0.5 Giga Pascal deviation and the Poisson ratio of 0.22. Duo to presumed limitations of devices of reading tip deflection of the current study, length of MC calculated as 350 micrometers by 100 micrometers width. Each supposed to have 120 nanometers deviation using available manufacturing techniques. The thickness is assumed 3.5 micrometers with 20 nanometers deviation as observed using the atomic force microscope study. The novelty of current work is using patterned substrate receptor formation in order to find sequential adsorption effects on energy analysis. Also, considering FLT3 for the measurement of radiation using MC has not been implemented yet. As the spatial location of the molecules acquired from experimental data, the interaction mechanism of so-called adsorption is not taken into account. Although finding exact values of mutation energy consist of various steps of MD simulation (23),

here only the energy difference between adsorption and far distance placement of target molecules studied. It is assumed that the latter would not affect the results, as the distance of target molecule placement is far enough that no electrostatic interaction is supposed to happen; the so-called independence is studied numerically, but the results are not included as a matter of compendium. As the substrate atoms are considered to have no electrical charge, and regarding the spatial rigidity of the biomolecules implemented in the simulation, it is assumed no interaction between molecules and substrate occurs except anchoring the last Sulfur atom of each receptor on the surface.

4. Results
The results of the calculation are presented in the same order of implementation. Incremental adsorption verification calculation is performed first using relaxation analysis results of two individual receptor molecules; Vacuum media with no periodic boundary

and only two receptors with incrementally increasing distance studied and total energy terms tracked that lead to 5nm optimal mean distance between receptors. Adsorption study of FLT3 and vancomycin biosensors then carried out taken data uncertainty into account, and finally, reliability of biosensor is presented by mean of a brief study on data deviations. In order to prove presumed negligible entropy change, RMSD of modeled protein throughout the simulations estimated below 0.14 angstrom and no conformational change were observed during minimization as well as molecular dynamics simulations.

4.1. Results of Adsorption Versus Concentration

The relationship between the concentration of target molecules and adsorption sites is unknown in current cases. In the present studies, it is assumed that two concepts correlate. As described before, an algorithm is used to decide which adsorption site to transport the target molecule onto at each step. The results of the algorithm were presented as **Figure 3 (A)**. The result of simulations is presented as MC's tip deflection presented in **Figure 3 (B)** by considering so-called dimensions and material properties.

A)

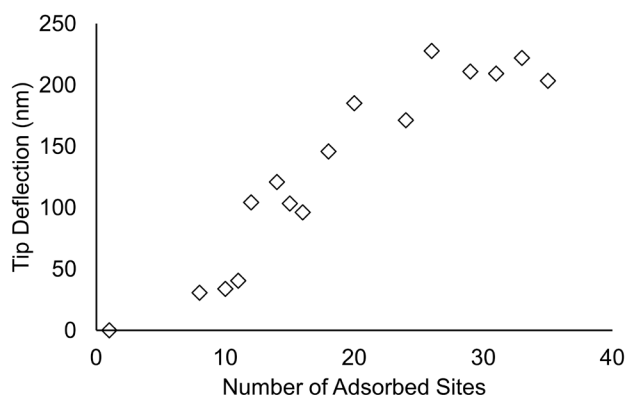| 10	4	21	22	13	29
26	32	12	7	18	34
6	9	19	20	24	14
30	33	25	1	8	35
2	5	15	17	28	27
16	36	31	11	3	23

B)

Fig. 3. Incremental adsorption results, **(A)** calculated incremental location of adsorbed site based on minimum RMS of distance from top view of the model, **(B)** Calculated incremental adsorption response of MC for Vancomycin biosensor by 36 MD calculations

The results of surface traction for FLT3 adsorption are calculated in the same manner and by shrinking the model to a ninth of the area and shown in **Figure 4**. The expected deviation around average values also illustrated.

4.2. Reliability Analysis of Biosensor

The distribution of output data is also presented in **Figure 5**. The grey lines illustrate the expected 20 percent error between calculations and experiments observed during vancomycin biosensor simulation case. The latter is not expected to happen during experiments as it can be omitted by calibrations. The black error lines show uncertainty regarding numerical analysis and MC uncertainties.
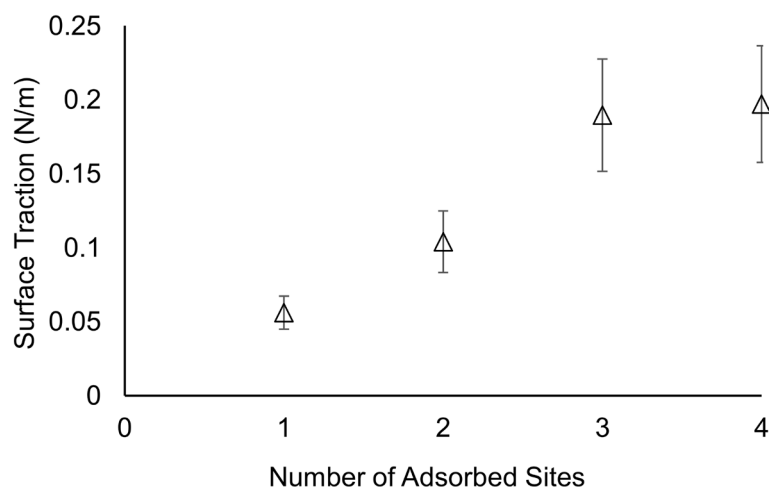
Fig. 4. Calculated surface traction by incremental adsorption of FLT3 to corresponding Kinase receptors
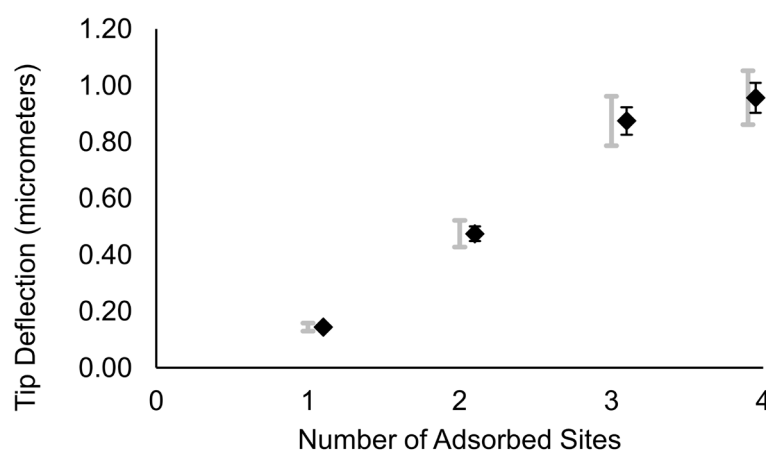

Fig. 5. Final response of the biosensor and data deviations in terms of MC's tip deflection versus number of sites participates in active adsorption.

5. Discussion

The molecular mechanics (MM) energy method used in the current manuscript is chosen as a procedure to determine docking energy of protein ensemble alike previously implementations (24, 25). Currently methods are available to find the docking energy i.e., called adsorption energy difference in here. Some investigations are focused on quantum mechanics (QM) of the adsorption procedure and are applied for a single receptor/target molecule configuration (26, 27), but in the current manuscript, the main concern is SAM adsorption calculations. The so-called method is not suitable for SAM adsorption because of the enormous computational effort needed for QM calculations. The latter is mainly emboldened as considering lots of molecular configurations for the selected biosensor. Therefore MM energy method is implemented here to find energy changes in each stage of adsorption on a periodic SAM to predict the response of the biosensor.

The first set of results in **Figure 3** shows a flatness trend near concentration i.e., full adsorption. This may corresponding to a similar trend near saturation in the experiment, as discussed by Bai et al. (9).

For the case of Vancomycin sensing simulations, the maximum deflection of the present study is calculated as 227 nanometers, which are expected not to happen practically due to expected instability resulted by the decline of total energy toward either side of the curve in **Figure 3 (B)**. By taking deviation of output data into account, it is expected that maximum active adsorption sites from 18 to 24 sites. Considering the latter, the final results were shown coincidence to experimental results by less than 10 percent error. Thought instable, the maximum calculated tip deflection lies within 20 percent error with experimental results; that was assumed as a maximum possible bias, which is omissible using experimental calibrations.

Also, the roots of observed difference may lay on

experimental uncertainties e.g., uneven coverage of SAM, and, or any possible simulation error. Therefore, a maximum of 20 percent error is expected between the results of implicating multiscale simulation results of radiation biosensor. Another source of the observed difference in final results may be due to stiffness of MC's support on the chip that depends on the fabrication process. The calculated surface traction in the FLT3 sensing case is higher than Vancomycin biosensor, which leads more deflection to the same MC; that would have the most impact on read-out required system specifications. It is expected that such a design creates the possibility of a lab-on-the chip radiation device capable of in-vivo sensory application.

By considering the linear relationship between the concentration of target molecule and adsorbed molecules, deviations of tip deflections can be calculated using a mathematical calculation. The maximum tip deflection is 0.96 micrometers with 0.0554 percent standard deviation, respectively. The standard deviation could be accounted for accuracy of the sensor, and slope of change in surface traction shows the expected value for accuracy of the biosensing system.

6. Conclusion

It can be concluded that the trend of MC biosensor response to the presence of the target molecule can be found thoroughly by means of presented sequential multiscale modeling.

The calculation can also be implemented to find the final deviation of sensory output.

An adsorption based microcantilever biosensor has been assumed and simulated that can account for the effect of radiation to the human body by sensing the amount of FLT3 in an aquatic sample. A deflection of 0.96 micrometers is expected that can be read out using various conventional methods.

The method is still to reach an experimentally validated correlation between the concentration of target molecules in solution and the number of adsorbed molecules per area of the biosensor.

The standard deviation of 0.054 in the final expected response of the sensor is calculated as the accuracy of the biosensor. Also, the precision can be found using experimental calibration.

Conflict of Interests

Computing servers have been used by contract ISI-DCE-DOD-Cloud-700101-2440 with HPCRC.

Acknowledgements

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