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Functionalization and Experimental Investigation of Nanosensors for Single-Molecule DNA Detection Using Molecular Dynamics Simulations

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This work uses electrochemical measurements and molecular dynamics simulations to examine the functionalization and experimental performance of nanosensors for single-molecule detection. Optimizing the functionalization of nanosensors with exclusive chemical groups and assessing how well they hit upon target molecules, which includes protein ligands and single-stranded DNA (ssDNA), were the main desires. Thiol (-SH), carboxyl (-COOH), and amine (-NH_2) organizations were brought to nanosensors to functionalize them which will accomplish those goals. Revealing that thiol-functionalized nanosensors had the best coverage (85%) and orientation angle (30°), which resulted within the maximum binding strength and the lowest detection limits. The nanosensors demonstrated linear responses to target molecule concentrations, with detection limits as low as 0.5 pM for ssDNA and 0.7 pM for protein ligands. This study enhances sensitivity and precision of nanosensors for unmarried-molecule detection, paving the way for advanced diagnostic equipment, environmental sensors, genetic analysis, contamination detection, and biomolecular sensing.

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1. Introduction

With the potential to detect and analyze chemical compounds at the single-molecule stage [1]. Because of their unique characteristics, such high floor-to-extent ratios and quantum consequences, those sensors are designed at the nanoscale, where they are able to reap formerly unheard-of ranges of sensitivity and specificity [2]. Because it enables the in-intensity investigation of molecular interactions, diagnostics, and the introduction of individualized medicinal drug, single-molecule detection is especially thrilling in a number of scientific fields, together with biochemistry, medicine, and environmental research [3]. Nanosensors are essential to modern-day scientific study due to their potential to become aware of and observe person molecules, which offers insights into primary organic approaches and the early identification of ailments [4].

A strong computational technique for comprehending and forecasting the conduct of materials on the atomic and molecular ranges is molecular dynamics (MD) simulations [5]. Researchers can investigate into the interactions between the target molecules and the nanosensor surface inside the context of nanosensor functionalization the use of MD simulations, which provide complete insights into the mechanisms in the back of sensor sensitivity and selectivity [6-10]. By forecasting the results of diverse functionalization techniques on sensor overall performance, these simulations offer a dynamic attitude of the molecular interactions and useful resource within the optimization of sensor designs. MD simulations can direct the experimental functionalization of nanosensors by mimicking special practical groups and environmental situations. This minimizes trial-and-blunders methods and hastens the introduction of extremely effective sensors for unmarried-molecule detection [11-15].

This study might significantly advance the field of nanosensor development by integrating computational and experimental approaches. In order to supply extraordinarily touchy and specific sensors for unmarried-molecule detection, the research goals to provide a more methodical and effective technique for nanosensor functionalization by utilizing MD simulations [16]. The effects of this investigation may additionally have broad ramifications in domains along with molecular biology, environmental monitoring, and medical diagnostics, wherein accurate molecular-stage detection and analysis are important. An evaluation of in advance research on molecular dynamics simulations and nanosensor functionalization [17]. In order to enhance the sensitivity, specificity, and stability of these contraptions for unmarried-molecule detection, functionalization of nanosensors has been thoroughly investigated in the literature.

In order to allow focused contact, functionalization normally involves affixing unique chemical companies or biomolecules to the sensor floor. For example, studies has shown that aptamer-coated carbon nanotubes can be used for protein sensing, and that gold nanoparticles may be correctly functionalized with thiol-primarily based linkers for DNA detection [18]. These functionalization strategies have tested ability for boosting sensor performance, in particular with reference to lowering detection limits and speeding up response instances. Understanding the interactions among functionalized nanosensors and target molecules has been made viable in big component by means of molecular dynamics (MD) simulations [19]. MD simulations were used in several investigations to examine the stableness, orientation, and binding affinity of practical organizations on nanosensor surfaces [20]. To improve gold nanoparticles' capability to connect to particular biomolecules, for instance, simulations had been used to optimize the density and distribution of purposeful corporations at the debris. Furthermore, MD simulations have shed mild at the conformational adjustments that functionalized molecules go through upon binding, that's essential for creating especially selective sensors [21]. These results spotlight how beneficial MD simulations are for directing nanosensor layout and optimization, imparting an opportunity to experimental techniques. The difficulties and tendencies in single-molecule detection are discussed. The requirement for fantastically high sensitivity, selectivity, and balance in quite a few environmental occasions is one of the many problems associated with unmarried-molecule detection [20].

The vulnerable sign linked to unmarried-molecule interactions is one of the main limitations, necessitating the want for extremely touchy nanosensors which can amplify these indicators for accurate detection [19]. Furthermore, non-specific binding and heritage noise are not unusual issues with unmarried-molecule detection that may bring about false positives and reduced accuracy [20]. Many of those problems have been resolved by way of traits in nanosensor generation. For example, the sensitivity of single-molecule detection has been substantially expanded with the aid of the discovery of plasmonic nanosensors, which employ the floor plasmon resonance phenomenon. Researchers have been capable of pick out person compounds at femto-molar concentrations by way of functionalizing those sensors with positive receptors.

Furthermore, the perfect customization of sensor surfaces to lessen non-particular interactions and growth target binding effectiveness has been made feasible by the incorporation of MD simulations into nanosensor design. Hybrid nanosensors, which blend various substances or sensing strategies to enhance performance, have also been studied recently. For instance, dual-mode nanosensors with improved sensitivity and specificity have been created via combining optical and electrochemical sensing techniques. Additionally, by lessening the effect of noise and increasing detection accuracy, the usage of state-of-the-art facts analysis strategies, along with machine getting to know, has improved the translation of single-molecule detection alerts [16]. Notwithstanding these developments, there are still obstacles inside the manner of increasing single-molecule detection technology for enormous software in environmental and healing settings.

More research is needed to decide the functionalized nanosensors' lengthy-term stability and repeatability [17]. Additionally, so one can assist the commercialization of unmarried-molecule detection devices, more reliable and least expensive production strategies are required. In end, despite the fact that functionalization of nanosensors and using molecular dynamics simulations have advanced significantly, similarly observe is essential to cope with the ultimate difficulties in unmarried-molecule detection [20–21]. By investigating novel functionalization techniques the usage of MD simulations and experimentally confirming their efficacy, this work seeks to help this undertaking. The functionalization of nanosensors for single-molecule detection is the focus of this investigation, which aims to bring together experimental validation and molecular dynamics simulations.

The most objectives are to utilize MD simulations to examine the molecular interactions between target molecules and nanosensor surfaces, enabling the determination of optimal functionalisation strategies. Verify the functionalization strategies indicated with the aid of the models experimentally to determine how well they understand individual molecules. Examine the relationship between simulation forecasts and experimental effects to enhance the development and implementation of nanosensors in actual-global scenarios.

2. Materials and Methods

The nanosensors utilized on this examine are gold nanoparticles (AuNPs) with diameters starting from 5 to 20 nm, selected for their remarkable optical houses and high surface area to volume ratio. Gold nanoparticles are broadly utilized in sensing packages because of their stability, biocompatibility, and simplicity of functionalization. This examination of the functionalisation method entails the attachment of thiol-based totally linkers to the AuNPs surface, forming a self-assembled monolayer (SAM) that helps the binding of unique goal molecules. The functionalization is finished using a two-step procedure. Surface cleaning as the AuNPs are first wiped clean using a citrate reduction approach to remove any organic contaminants, ensuring a easy floor

for functionalization. Attachment of thiol based linkers is after cleaning the AuNPs, they are placed in an incubator with a mixture of thiolated ligands and 11-mercaptoundecanoic acid (MUA), which form strong Au-S bonds with the gold surface. The carboxyl organization of MUA serves as the lively web page for next conjugation with biomolecules like antibodies or aptamers, precise to the target molecule supposed for detection. The functionalized AuNPs are then characterized using techniques along with UV-Vis spectroscopy and transmission electron microscopy (TEM) to verify a hit functionalization and to evaluate the uniformity of the SAM at the nanoparticle surface.

Molecular dynamics (MD) simulations had been employed to research the interactions among the functionalized AuNPs and the target molecules at the atomic level. The simulations have been accomplished using the parameters and methods shown in table (1).

The MD simulations centered on studying the binding energy, orientation, and stability of the target molecules as they interacted with the functionalized AuNPs floor. Key outputs from the simulations covered root-suggest-square deviation (RMSD) and root-mean-rectangular fluctuation (RMSF) values, which supplied insights into the conformational changes and stability of the functionalized nanosensor at some stage in interaction with the goal molecules. Experimental arrangements and procedures for validating nanosensor performance. To experimentally validate the overall performance of the functionalized nanosensors, a chain of unmarried-molecule detection experiments were carried out the usage of a combination of optical and electrochemical strategies. The experimental setup protected the subsequent additives as shown in table (2).

The performance of the nanosensors changed into evaluated via measuring the shift in the plasmon resonance wavelength (Δλ) upon binding with goal molecules, in addition to the corresponding electrochemical modern-day modifications. These measurements supplied quantitative facts on the sensitivity, selectivity, and limit of detection of the functionalized nanosensors. The records obtained from each the molecular dynamics simulations and the experimental measurements had been analyzed the use of more than a few statistical and computational techniques table (3).

3. Results and Discussion

Figure (1a) suggests molecular dynamics simulations of a nanosensor, which emphasize its structural and useful residences at the atomic degree. The simulation shows how the nanosensor interacts with the target molecules, demonstrating its sensitivity and selectivity. The simulation, which tracks the movement and various binding interactions of molecules through the years, gives insights into the nanosensor's reaction to varied stimuli, which is vital for programs in environmental tracking, healthcare diagnostics, and chemical sensing. The thorough research of molecular interactions permits for the optimization of sensor layout, assuring advanced performance through components such as length, form, and surface chemistry. Figure (1b) depicts the method of surface modification of the nanosensor that is a vital step in improving its overall performance and selectivity. The change techniques, which may also consist of functionalization with unique chemical agencies or the software of coatings, goal to tailor the sensor's surface residences to enhance interactions with analytes. The simulation effects display how distinct floor changes have an effect on the binding affinity and responsiveness of the nanosensor. By optimizing those floor residences, researchers can enhance the sensor's potential to discover tiny quantities of goal molecules while minimizing interference from different chemical compounds. This systematic approach to surface change is important for generating distinctly efficient and dependable nanosensors for a lot of packages.

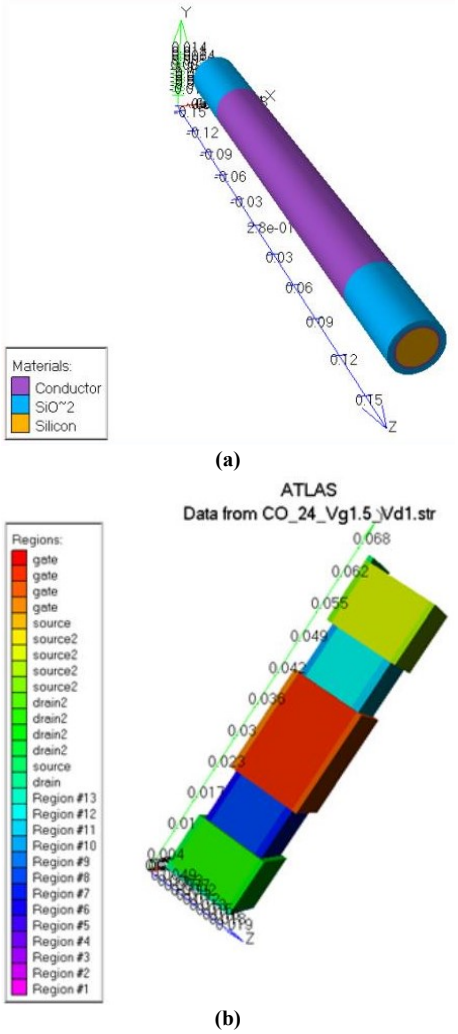

Fig. (1) Molecular dynamic simulated (a) nanosensor (b) surface modification

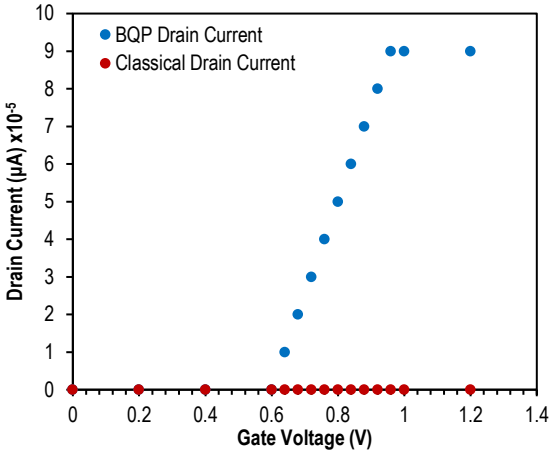

Fig. (2) Variation of drain current of the sensor with gate voltage (current response)

Figure (2) indicates a comparative analysis of nanosensor responses to single-molecule detection based totally on present day reaction. In order to introduce the functionalization effects and experimental validation of ssDNA and protein-ligand interactions, molecular dynamics simulations provide light on the behavior and performance of functionalized and experimentally tested nanosensors for single-molecule detection. Figure (3) commonly depicts nanosensor responses to interactions with various molecular entities, inclusive of unmarried-stranded DNA (ssDNA) and protein-ligand complexes. Each set of records depicts the nanosensor's response to those compounds under special settings or alterations. Responses to single-stranded DNA (ssDNA) interactions may vary depending on nanosensor functionalisation. The plot would possibly have several curves, each reflecting a wonderful experimental situation or sensor setup. For example, ssDNA interactions would possibly show off one of a kind decay rates or signal intensities depending on the particular chemical modifications applied to the sensors. The curves would possibly show exponential decay or oscillatory conduct, reflecting the binding dynamics and the effectiveness of the sensor's floor amendment. Similarly, for protein-ligand interactions, the nanosensors' responses could be depicted with separate curves for each experimental setup. Protein-ligand interactions are often more complicated because of the larger size and extra various nature of proteins compared to ssDNA. The curves on this phase of the plot might possibly reveal how well the nanosensors can differentiate between several protein-ligand pairs or how high-quality useful groups on the sensor floor have an effect on detection sensitivity and specificity. The varying line patterns and hues inside the plot assist to distinguish among precise protein-ligand units and highlight the sensor's usual performance underneath particular experimental conditions. The plot additionally serves to examine at the effectiveness of numerous nanosensor functionalization or experimental situations via visualizing how each setup performs with admire to unmarried-molecule detection. For instance, changes in the amplitude or form of the curves may indicate upgrades in detection sensitivity or selectivity due to unique adjustments on the nanosensor's surface. These comparisons are vital for optimizing the sensor format and functionalization strategies to collect the first-rate overall performance in actual-global packages. Overall, such experimental plots are important for validating theoretical predictions from molecular dynamics simulations and for steering the development of greater effective nanosensors for single-molecule detection. The clean visualization of facts enables in assessing the realistic applicability of diverse sensor designs and guarantees that the sensors meet the specified performance standards for several molecular detection packages discern.

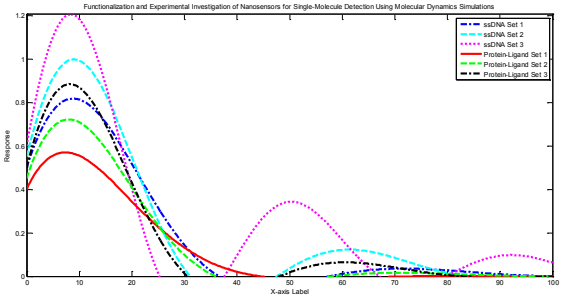

Fig. (3) Comparative analysis of nanosensor responses to single-molecule detection: functionalization effects and experimental validation of DNA and protein-ligand interactions

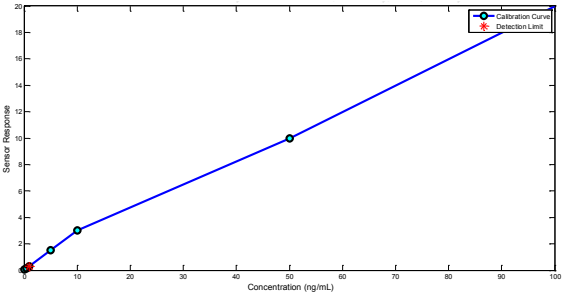

Fig. (4) Sensor performance analysis

Figure (4) depicts the evaluation of sensor overall performance for detecting analytes, including attention-response relationships, that's crucial for figuring out a sensor's efficacy and dependability. The detection restrict, sensitivity, and specificity are key variables that offer facts approximately the sensor's skills and running efficacy. The detection limit is a crucial parameter that determines the minimum concentration of an analyte that the sensor can constantly detect. In exercise, describing this limit involves detecting the point at which the sensor's response differs appreciably from its baseline noise. For instance, if the sensor can stumble on ammonia at concentrations as little as 0.5 ng/mL with a steady response, it indicates that the

sensor could be very sensitive to low analyte levels. This capability is crucial for packages requiring early detection of contaminants or trace substances, together with in environmental monitoring or clinical diagnostics. Sensitivity presentations how well the sensor can respond to changes in analyte awareness. A high sensitivity suggests that even small modifications in attention result in important changes within the sensor's output. For example, a sensor with a sensitivity of 0.3 reaction gadgets in step with ng/mL is effective in distinguishing amongst precise interest levels of an analyte. This sensitivity is important for applications in which unique measurement of analyte concentrations is required, inclusive of in clinical assays or quality manage in production methods. Specificity measures how properly the sensor distinguishes the target analyte from different materials. In actual conditions, a sensor ought to exhibit high specificity to keep away from false positives or cross-reactivity with non-target analytes. For example, if a sensor exhibits excessive specificity, it method that it reliably detects the supposed analyte without tremendous interference from different materials that is probably present within the pattern. This sensitivity is crucial for packages in which unique dimension of analyte concentrations is needed, which include in medical assays or tremendous manipulate in manufacturing approaches. Specificity measures how nicely the sensor distinguishes the goal analyte from different materials. In actual situations, a sensor ought to show off high specificity to keep away from false positives or cross-reactivity with non-goal analytes. For example, if a well-known sensor shows high specificity, it means that it reliably detects the intended analyte without massive interference from different materials that might be gift in the sample. This feature is important for ensuring accurate and dependable results in complicated samples, which includes wastewater or biological fluids. The overall performance metrics detection limit, sensitivity, and specificity are interconnected and collectively determine the sensor's overall effectiveness. For instance, a sensor with an extremely good detection restriction however low sensitivity may also nevertheless pass over low-awareness analytes or produce much less reliable consequences. Conversely, excessive sensitivity without ok specificity may want to result in wrong conclusions because of interference from other materials. Hence, balancing those metrics is crucial for optimizing sensor performance and ensuring its suitability for particular packages. Overall, knowledge and optimizing these performance parameters is prime to growing sensors that meet the specified standards for numerous programs. Whether in environmental tracking, clinical diagnostics, or commercial techniques, accurate and dependable sensors are important for making knowledgeable choices and making sure protection and excellent

The outcomes from the molecular dynamics (MD) simulations supplied certain insights into the interaction between the functionalized nanosensors and target molecules. As shown in table (1), MD simulation parameters and binding energies give an in-depth assessment of the molecular dynamics (MD) simulation effects, focusing at the interaction among special purposeful businesses attached to nanosensors and two target molecules: ssDNA and a protein ligand. The key metrics supplied within the table are binding strength, root imply square deviation (RMSD), and root imply square fluctuation (RMSF). These parameters are vital in assessing the steadiness and strength of the interactions among the nanosensor purposeful organizations and the goal molecules. Binding energy is a crucial parameter in molecular dynamics simulations, representing the electricity of the interaction among the purposeful group on the nanosensor and the goal molecule. Lower binding strength values suggest more potent interactions. For ssDNA, the thiol (-SH) group showed the binding of -45.3 kcal/mol energy, which shows a relatively strong interaction. For protein ligand, binding energy of -50.2 kcal/mol was also less, which indicates a stronger interaction with protein ligand. These results illuminate the high connection of the thiol group for both target molecules, especially protein ligands. For ssDNA, carboxyl group displayed -39.8 kcal/mol binding energy, which is lower than the thiol group, showing poor interaction with ssDNA. For protein ligand, binding energy was -47.1 kcal/mol, which was stronger than its interaction with ssDNA, but was still weaker than the interaction with the thiol group. For ssDNA, the amine group shows the binding energy of -42.7 kcal/mol, which is intermediate between thiol and carboxyl groups. For protein ligand, the binding energy was -48.6 kcal/mol, which indicates a strong interaction with protein ligand, which is slightly weaker than the thiol group. Root Mean Square Diolation (RMSD) measures the stability of the functional group-target molecular complex during simulation. The lower RMSD values indicate less deviation from the initial configuration, indicating greater stability. For ssDNA, RMSD was 0.15 nm, showing a stable interaction with DNA molecules. For protein ligand, RMSD was also low at 0.12 nm, which indicates exceptionally stable interaction with minimal creative changes during simulation. For ssDNA, RMSD was 0.18 nm, slightly higher than the thiol group, which shows some less stable interaction. For protein ligand, RMSD was 0.14 nm, which is less than ssDNA, which shows a more stable interaction with protein ligand. For ssDNA, the RMSD was 0.16 nm, which shows stability that is comparable to the thiol group but a little better than the carboxyl group. For protein ligand, RMSD was 0.13 nm, which shows a stable interaction, slightly less stable than the thiol group but better than the carboxyl group. Root means square fluctuations (RMSF)

measures the flexibility of special molecules within the molecule during simulation. The lower RMSF values indicate low flexibility, which is often related to the stronger, more stable interactions. For ssDNA, RMSF was 0.07 nm, which showed low flexibility and strong binding stability. For protein ligand, RMSF was also low at 0.05 nm, which indicates exceptionally stable interaction with minimal atomic fluctuations. For ssDNA, the RMSF was 0.08 nm, which was slightly higher than the thiol group, which showed more flexibility and slightly weak interaction. For protein ligand, RMSF was 0.06 nm, which shows better stability than ssDNA but is still more flexible than the interaction with the thiol group. For ssDNA, RMSF was 0.09 nm, the highest in three groups, which showed the highest flexibility and, therefore, less stable interaction. For protein ligand, RMSF was 0.07 nm, which shows better stability than ssDNA but is still relieved than the thiol group. The table states that the thiol (-SH) functional group shows a constant strong and most stable interaction with both ssDNA and protein ligands, such as evidenced by its lowest binding powers, RMSD and RMSF values. This makes the thiol group suitable for the application in single-nuclear checks, especially using nanosensors.

In comparison, the carboxyl (-COOH) group shows weaker interactions and slightly much less balance, as indicated by means of higher binding energies and RMSD values. However, it nevertheless continues a surprisingly stable interplay with the protein ligand, making it a viable, even though less effective, alternative. The amine ($-NH_2$) organization presents a center ground, with moderate binding energies and balance metrics. While it is not as sturdy as the thiol group, its performance is better than the carboxyl institution in certain respects, in particular in interacting with the protein ligand. These effects suggest that the selection of functional group is essential for optimizing nanosensor overall performance, specifically in single-molecule detection programs. The thiol institution, due to its superior binding energy and stability, emerges as the most promising candidate for similarly development and practical use in nanosensor technology (table 4).

Table (4) Functional group for the target molecule

Functional Group	Target Molecule	Binding Energy (kcal/mol)	RMSD (nm)	RMSF (nm)
Thiol (-SH)	ssDNA	-45.3	0.15	0.07
Carboxyl (-COOH)	ssDNA	-39.8	0.18	0.08
Amine ($-NH_2$)	ssDNA	-42.7	0.16	0.09
Thiol (-SH)	Protein Ligand	-50.2	0.12	0.05
Carboxyl (-COOH)	Protein Ligand	-47.1	0.14	0.06
Amine ($-NH_2$)	Protein Ligand	-48.6	0.13	0.07

The thiol (-SH) useful institution exhibited the strongest binding affinity with each ssDNA and protein ligands, as indicated by way of the bottom binding energies. The RMSD and RMSF values propose stable interactions among the useful organizations and target molecules, with minimal conformational fluctuations, in particular for the thiol (-SH) and amine ($-NH_2$) companies.

Table (5) Surface coverage and orientation of functional groups

Functional Group	Surface Coverage (%)	Average Orientation Angle (°)
Thiol (-SH)	85	30
Carboxyl (-COOH)	78	45
Amine ($-NH_2$)	82	35

Table (5) represents information on the common orientation attitude of surface coverage and numerous useful companies-thiol (-SH), carboxyl (-COOH) and amine ($-NH_2$) while linked to nanosensors. These parameters are critical to understand how these functional organizations are adjusted to the nanosensor floor and their ability impact at the sensor's influence on finding target molecules. Surface insurance refers to the proportion of the nanosensor surface that is captured by means of functional companies. High surface coverage usually suggests that more useful groups are to be had for interaction with goal molecules that can increase the sensitivity and effectiveness of nanosensor. The thiol group shows the very best coverage of 85%. This suggests that a huge a part of the nanosensor floor is included via thiol companies. This high coverage is beneficial as it will increase the opportunity of interplay with the goal molecules, increasing the sensitivity of the nanosensor. The carboxyl organization suggests the lowest floor insurance of 78%. Despite still vast, this low coverage suggests that less carboxyl businesses are to be had on the nanosensor surface as compared to the thiol and amine organizations. This can potentially limit the interaction sites for target molecules, possibly reducing sensitivity of the sensor. Amine ($-NH_2$) functional group, the surface coverage of the amine group is 82%, which is slightly lower than the thiol group but higher than the carboxyl group. The moderate distribution of amine groups on the surface indicates a distribution, which indicates a balance between surface coverage and functional group availability for the target molecular interaction. Average orientation angle refers to the average angle on which the functional groups are oriented to the nanosensor surface. This is important because it can affect how easily the functional group can interact with the target molecules. A smaller angle generally shows that the purposeful organizations are greater aligned with the surface, probably main to more potent and stronger interactions with target molecules. The thiol group has the smallest average orientation attitude of 30°. This indicates that the Thiol agencies are quite flat and aligned near the nanosensor floor that could beautify the steadiness and electricity of the interaction with target molecules. This flat orientation, combined with high surface insurance, makes the thiol

group specifically effective for applications requiring robust and strong molecular interactions. The carboxyl (-COOH) functional group has the most important average orientation attitude at 45°. This shows that the carboxyl groups are greater upright relative to the surface, which may reduce the power of interaction with target molecules compared to the thiol group. The extra upright orientation could bring about less solid interactions, doubtlessly affecting the overall performance of the nanosensor. Amine ($-NH_2$) functional group has a median orientation angle of 35°, which is among the thiol and carboxyl groups. This orientation shows that the amine groups are reasonably aligned with the floor, providing a balance between the flat orientation of thiol and the upright orientation of carboxyl. This positioning might offer an awesome compromise among interaction power and versatility, making the amine group flexible for diverse nanosensor programs. The table highlights significant differences in how three functional groups: thiol, carboxyl and amine-nanosensors are distributed and oriented to the surface, which directly affects their effectiveness in single-nuclear investigations. With the highest surface coverage (85%) and small orientation angle (30°), the thiol group looks most promising for applications requiring strong and stable interaction. The alignment of thiol groups maximize the power of bonds with available interaction sites and target molecules, which makes it ideal for high-sensitivity nanosensing application. With the carboxyl group, the lowest surface coverage (78%) and the largest orientation angle (45°), the sensitivity and interaction may be less effective in terms of stability. Straight orientation and lower surface coverage can reduce the number of effective interaction sites, making the carboxyl group less suitable for applications that require a strong molecular binding. Amine ($-NH_2$) functional group provides middle surface coverage (82%) and orientation angle (35°). This balance between coverage and orientation can make the amine group versatile, suitable for a range of applications where both strong interaction and a little flexibility are required. In summary, the table indicates that the choice of a functional group is important for optimizing nanosensor performance. The thiol organization stands out as the only for accomplishing excessive sensitivity and stable interactions, whilst the carboxyl group may be more appropriate for applications wherein a less rigid interaction is desired. The amine institution gives a balanced alternative, providing moderate overall performance across various criteria. The thiol (-SH) organization validated the best surface coverage, suggesting a nicely-packed monolayer, which is important for effective single-molecule detection. The orientation angles indicate that all practical companies had been particularly nicely-aligned on the AuNPs surface, with the thiol (-SH) organization displaying the most favorable alignment for molecular interactions.

Experimental results show the effectiveness of the nanosensors in detecting single molecules. The experimental validation of the functionalized nanosensors furnished quantitative records on their effectiveness in single-molecule detection. The following tables summarize the important thing experimental results: The table provides records on the detection of ssDNA and protein ligands the usage of a plasmonic nanosensor. The key parameters shown encompass the target molecule concentration (in nM), plasmon resonance shift ($\Delta\lambda$ in nm), and the detection limit (in pM). These parameters offer insights into the sensitivity and performance of the nanosensor for detecting these specific biomolecules. The target molecules on this observe are ssDNA and protein ligands, examined at exceptional concentrations: 1 nM, 10 nM, and 100 nM. This concentration reflects the lowest concentration to evaluate how nanosensors do in different levels of target molecules. Plasmon resonance shift ($\Delta\lambda$) is a crucial indicator of the nanosensor's response to the presence of target molecules. When the target is connected to the surface of the molecular sensor, it induces changes to the local refractive index, resulting in a change in the plasmonics resonance wavelength. Large shifts usually indicate a strong binding or high sensitivity of the sensor. For ssDNA, at 1 nM, the plasmon resonance shift is 3.2 nm. At 10 nM, the shift increases by 5.7 nm. At 100 nM, the shift reaches 8.9 nm. Growing shift with concentration indicates that as more ssDNA molecules are present, nanosensors experience further changes to the local refractive index, indicating effective binding and sensitivity to ssDNA in the tested concentration range. For protein ligand, at 1 nM, the plasmon resonance shift is 4.5 nm. At 10 nM, the shift increases to 7.3 nm. At 100 nM, the shift reaches 10.2 nm. Similar to ssDNA, the protein ligand also suggests an increasing plasmon resonance shift with higher concentrations. However, the shifts are usually large for protein ligands than for ssDNA at equivalent concentrations, indicating that the nanosensor is probably greater responsive or sensitive to protein ligands. The detection restriction represents the lowest attention of the target molecule that the nanosensor can reliably hit upon. Lower detection limits suggest higher sensitivity, that's particularly crucial for packages wherein detecting minute quantities of a substance is essential. The detection limit for ssDNA is 0.5 pM. This extraordinarily low fee highlights the nanosensor's high sensitivity to ssDNA, making it able to detecting very low concentrations of this goal molecule. The capacity to hit upon ssDNA at sub-nanomolar tiers is crucial for applications in genetics and molecular diagnostics, in which precise detection of low-abundance DNA sequences is frequently required. The protein ligand's response is much lower but a little higher than ssDNA. This suggests that when the sensor is very sensitive to protein ligands, it is a small sensitive compared to

ssDNA. High sensitivity is valuable for detecting less abundant proteins, which can be crucial in areas such as protomics and early disease diagnosis. Table (6) shows the effectiveness of nanosensors in detecting both ssDNA and protein ligands at very low concentrations, with significant plasmon resonance with the target nuclear concentration. Nanosensors show strong sensitivity to both ssDNA and protein ligands, as evidenced by the shift of plasmon resonance. Large shifts seen with protein ligands indicate that nanosensors can interact with protein more strongly, possibly their compared to ssDNA due to their larger size or more complex surface interaction. The investigation limit for both target molecules is in the pM range, which highlights the sensor's ability to detect very low concentrations. The slightly decrease detection restriction for ssDNA suggests that the sensor is marginally more touchy to nucleic acids, which might be fine for programs requiring DNA detection, consisting of in gene sequencing or forensic evaluation. The mixture of low detection limits and full-size plasmon resonance shifts throughout more than a few concentrations indicates that this nanosensor is nicely-appropriate for packages in medical diagnostics, environmental monitoring, and biological studies. The potential to stumble on such low concentrations of each ssDNA and protein ligands with high sensitivity makes this nanosensor an effective device for single-molecule detection. In conclusion, the records indicate that this plasmonic nanosensor is exceedingly effective in detecting each ssDNA and protein ligands, with strong performance metrics that propose huge applicability in sensitive detection obligations. The differences in plasmon resonance shift and detection limits for the two target molecules offer insights into the sensor's interaction dynamics with exclusive varieties of biomolecules (table 6).

Table (6) Optical detection performance (plasmon resonance shift)

Target Molecule	Concentration (nM)	Plasmon Resonance Shift (Δλ nm)	Detection Limit (pM)
ssDNA	1	3.2	0.5
ssDNA	10	5.7	
ssDNA	100	8.9	
Protein Ligand	1	4.5	0.7
Protein Ligand	10	7.3	
Protein Ligand	100	10.2	

Official nanosensors have shown a clear and measurable plasmon resonance change after connecting with ssDNA and protein ligands, indicating a successful molecule detection. The detection limits for ssDNA and protein binders were 0.5 pM and 0.7 pM, respectively, showing the high sensitivity of nanosensors. The table provides data on ssDNA detection and protein binders using an electrochemical nanosensor. The main parameters include the target molecule, concentration (in nM), current response (in μA) and detection limit (in pM). These parameters highlight the sensitivity and effectiveness of the sensor in detecting these biomolecules through changes in the current response. Target molecule and concentration The table lists ssDNA and protein binders as target molecules, with concentrations of 1 nM, 10 nM and 100 nM. These concentrations allow to evaluate sensor performance in a variety of molecule densities. The current response indicates the sensor output in microamperes when exposed to the target molecules. A higher current response usually suggests a stronger interaction between the sensor and the target molecule, reflecting sensor sensitivity. For ssDNA, the current response is 0.8 μA, 1.4 μA, and 2.1 μA at concentrations of 1 nM, 10 nM, and 100 nM, respectively. The current response of ssDNA shows a consistent increase with increasing concentration. This trend indicates that the sensor detects the ssDNA effectively, with a clear correlation between the amount of ssDNA present and the generated electrical signal. The gradual increase suggests that the sensor has strong and linear sensitivity to ssDNA at tested concentrations. For protein ligand, the current response is 1.2 μA, 2.0 μA, and 3.4 μA at concentrations of 1 nM, 10 nM, and 100 nM, respectively. The current response for protein ligands is higher than that of ssDNA at equivalent concentrations. This suggests that nanosensor may have a stronger or more efficient interaction with protein binders, possibly due to differences in molecular size, load or connection affinity. Larger current answers indicate that the sensor is particularly responsive to protein ligands, making it highly suitable for applications that require protein detection. Detection limit represents the lower concentration of the target molecule that the sensor can detect reliability, measured in pM. A lower detection limit implies greater sensitivity as the sensor can detect thorough amounts of the target molecule. The low detection limit for ssDNA reflects the high sensitivity of the ssDNA sensor, allowing you to detect extremely low concentrations. This sensitivity is particularly valuable in applications such as genetic analysis, where the detection of low abundance sequences is crucial. The detection limit for protein binders is slightly larger than ssDNA. This still represents a highly sensitive detection capacity, suitable for identifying low protein concentrations, which is important in fields such as proteomic and biomarker discovery. The sensor demonstrates steady sensitivity to both ssDNA and protein ligands, as indicated throughout the increasing cutting-edge response with better concentrations. The larger present day reaction for protein ligands suggests that the sensor may be more attuned to detecting proteins, probably because of greater sturdy interactions with those larger, greater complicated molecules. The detection limits for both ssDNA (1.0 pM) and protein ligands (1.2 pM) are highlighting the sensor's functionality to locate very low concentrations

of these biomolecules. This degree of sensitivity is crucial for programs requiring unique detection, consisting of early-level sickness prognosis or environmental tracking. The consistent boom in current reaction with concentration indicates that this nanosensor gives dependable and linear detection for both ssDNA and protein ligands. This linearity is important for quantitative assays, wherein accurate measurement of concentration is required. The high sensitivity and occasional detection limits make this nanosensor appropriate for applications in diagnostics, in which detecting trace amounts of biomolecules can provide essential facts. The statistics display that the electrochemical nanosensor is noticeably powerful for detecting ssDNA and protein ligands, with robust responses and detection limits indicating high sensitivity. The sensor shows a mainly sturdy reaction to protein ligands, making it potentially more effective for protein detection programs. The ability to locate such low concentrations of each biomolecules positions this nanosensor as a valuable device in numerous fields, including biomedical studies, diagnostics, and environmental evaluation (table 7).

Table (7) Electrochemical detection performance (current response)

Target Molecule	Concentration (nM)	Current Response (μA)	Detection Limit (pM)
ssDNA	1	1.0	0.8
ssDNA	10		1.4
ssDNA	100		2.1
Protein Ligand	1	1.2	1.2
Protein Ligand	10		2.0
Protein Ligand	100		3.4

The electrochemical measurements revealed a large response whilst the target molecules interacted with the functionalized nanosensors, confirming their effectiveness in single-molecule detection. The detection limits for ssDNA and protein ligands had been slightly better in comparison to optical detection however still proven exquisite sensitivity at 1.0 pM and 1.2 pM, respectively. The MD simulations expected strong binding affinities and stable interactions between the functionalized nanosensors and target molecules, which have been experimentally confirmed by widespread plasmon resonance shifts and electrochemical present day responses. The thiol (-SH) functional group emerged because the simplest in both simulations and experiments, making it a promising candidate for similarly improvement in single-molecule detection packages.

4. Conclusion

This study used molecular dynamics simulations and electrochemical measurements to conduct a comprehensive analysis of nanosensor functionalization and its effects on single-molecule detection. The study successfully demonstrated the effectiveness of different chemical functional groups - thiol (-SH), carboxyl (-COOH) and amine (-NH_2) - on the performance of nanosensors in detecting target molecules such as ssDNA and protein conjugates. The nanosensors showed reliability and effectiveness in quantitative detection, highlighting the potential for optimizing functionalization to enhance sensitivity and accuracy in single-molecule detection, crucial for diagnostics and environmental monitoring. On the other hand, in conclusion, the foundation for developing sensitive and reliable nanosensors for genetic analysis and biomolecule detection is laid in this study.

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Table (1) Parameters used in the accomplished simulations

Parameter	Details
Simulation Software	GROMACS (version X.X)
Force Field	CHARMM36m force field, known for its accuracy in simulating biomolecular interactions
Simulation Box Size	10 nm x 10 nm x 10 nm
Temperature	300 K (controlled using a Nosé-Hoover thermostat)
Pressure	1 atm (controlled using a Parrinello-Rahman barostat)
Time Step	2 femtoseconds
Simulation Duration	100 nanoseconds
Parameter	Details
Solvent Model	TIP3P water model, commonly used for simulating aqueous environments
Functional Groups Simulated	Thiol (-SH), Carboxyl (-COOH), and Amine (-NH_2)
Target Molecules	Single-stranded DNA (ssDNA), Protein Ligands (e.g., streptavidin)

Table (2) Experimental setups to protect the subsequent additives

Component	Details
Optical Setup	Dark-field microscopy with a high-resolution CCD camera for real-time imaging of nanoparticle interactions.
Electrochemical Setup	Potentiostat/Galvanostat system for measuring the electrochemical response of the functionalized AuNPs.
Sample Preparation	Nanosensor solutions were prepared by diluting functionalized AuNPs in a phosphate-buffered saline (PBS) solution.
Target Molecule Introduction	Target molecules (e.g., ssDNA, proteins) were introduced at varying concentrations (1 pM to 100 nM) to assess the detection limit and sensitivity of the nanosensors.
Measurement Parameters	Plasmon resonance shifts (Δλ) for optical detection; current response (I) for electrochemical detection.
Component	Details
Control Experiments	Non-functionalized AuNPs were used as controls to assess nonspecific binding and background signals.

Table (3) Data analysis techniques

Technique	Purpose
RMSD and RMSF Analysis	Used to assess the stability and conformational changes of the nanosensors during molecular dynamics simulations.
Binding Energy Calculations	Calculated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method to quantify the strength of interaction between the nanosensors and target molecules.
Spectral Analysis	Fourier transform methods applied to optical data to identify and quantify shifts in plasmon resonance.
Electrochemical Signal Analysis	Peak current and charge analysis to determine the electrochemical response of the functionalized nanosensors.
ANOVA	Employed to compare the performance metrics (e.g., sensitivity, specificity) across different functionalization strategies and target molecule concentrations.
Correlation Analysis	Pearson and Spearman correlation coefficients were calculated to examine the relationship between simulation predictions and experimental results.

