Relay-type sensing mode: A strategy to push the limit on nanomechanical sensor sensitivity based on the magneto lever

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ABSTRACT

Ultrasensitive molecular detection and quantization are crucial for many applications including clinical diagnostics, functional proteomics, and drug discovery; however, conventional biochemical sensors cannot satisfy the stringent requirements, and this has resulted in a long-standing dilemma regarding sensitivity improvement. To this end, we have developed an ultrasensitive relay-type nanomechanical sensor based on a magneto lever. By establishing the link between very weak molecular interaction and five orders of magnitude larger magnetic force, analytes at ultratrace level can produce a clearly observable mechanical response. Initially, proof-of-concept studies showed an improved detection limit up to five orders of magnitude when employing the magneto lever, as compared with direct detection using probe alone. In this study, we subsequently demonstrated that the relay-type sensing mode was universal in application ranging from micromolecule to macromolecule detection, which can be easily extended to detect enzymes, DNA, proteins, cells, viruses, bacteria, chemicals, etc. Importantly, we found that, sensitivity was no longer subject to probe affinity when the magneto lever was sufficiently high, theoretically, even reaching single-molecule resolution.

KEYWORDS
relay-type sensing mode, magneto lever, nanomechanical sensor, ultrasensitivity

1 Introduction

Ultrasensitive detection of molecular interactions is essential for widespread practice ranging from environmental monitoring, drug discovery to medical diagnostics and fundamental research such as cell signaling, cellular metabolism and growth, DNA replication and repair, and regulation of gene expression [1–5]. Nanomechanical sensors can convert a weak force into a displacement or resonant frequency drift that is measurable by electrical or optical means, and have become valuable tools for the understanding and recognition of molecular interactions in microcosms [6–10]. Hence, in terms of the nanomechanical sensor, the key to the sensitive molecular recognition is the ability to measure very small forces, such as those induced by antibody (Ab) and antigen binding. Since the 1990s, physicists and engineers have exclusively focused on enhancing the force sensitivity of cantilevers [11]. These efforts include microfabrication of ultrasoft microcantilevers and improving the displacement detection [12–15]. Despite intensive efforts over the last decade, progress in force sensitivity has been modest due to material and quantum limits [16, 17]. Moreover, if this is the case, environmental perturbations can also generate conspicuous mechanical responses [18–20], thus restricting the application of nanomechanical sensor.

Therefore, the fundamental workaround for increased sensitivity appears to improve signal-to-noise by rational and innovative design, which has driven the pursuit of new approaches in maximizing the generation and further amplification of specific signals. For a conventional nanomechanical sensor, the signal generation is mainly derived from the changes in molecular conformation or steric hindrance, and thus is heavily affected by analyte delivery in solution and effective probe loci at the interface [21–24]. On the one hand, researchers have developed techniques such as electrokinetics-assisted binding that accelerates the delivery of analytes towards the sensing interface by electrostatic fields in solution; on the other hand, various attempts have been made to develop efficient modification methodologies for interface construction [25–29], including using secondary Abs or G-quadruplexes to achieve directed orientation, and using Ab fragments such as half Ab fragment, single-chain Fv Ab fragment, and nanobody to achieve dense probe layers. However, improvements in these methods are all based on intermolecular force limited by probe–analyte binding efficiency. As a result, despite substantial efforts to guarantee the adequate reaction between analytes and probes over the past decade, the difficulty in stepping over the affinity barrier eventually restricts the signal generation. Additionally, it is well documented that high-affinity probe screening is still technically difficult, time-consuming, and expensive. Hence, the amplification of the weak signal appeared to be the last viable option for further improving the detection limit.
of the nanomechanical sensor, especially represented by DNA amplification in polymerase chain reaction (PCR) and enzymatic reaction in enzyme-linked immunosorbent assay (ELISA) [30–33]. In terms of the nanomechanical sensor, examples include using gold nanoparticles (AuNPs) [34] or magnetic nanoparticles (MNPs) as signal amplifiers in sandwich assays [12, 35–37]. Nevertheless, due to the requirement of at least two identifiable sites on the target, this strategy is applicable to macromolecule detection only. More importantly, these efforts are not outside the realm of molecular forces. Thus, despite these advances, researches still have not achieved transformative enhancements in sensitivity.

In order to address the issue of weak intermolecular interactions, a sensing mode urgently needs redesign from the fundamental perspective of force generation. To this end, we enlarged our view to electronic, mechanical, optical, and other basic disciplines, which can serve as sources of inspiration. And we found that the working mode of lever and relay fulfilled our requirements. As relay uses small currents to control large currents, establishing the link between ultralow-concentrated analytes and other large interaction forces is a promising strategy. Superparamagnetic MNPs have good biocompatibility and synthetic nanoparticles can carry desired functional groups enabling immobilization on the sensor surface. Under an applied neodymium magnet magnetic field (longitudinal section, Fig. 1(a)), a force up to 10^10 pN (z = 24 mm, Fig. 1(b)) can be exerted on every single Fe₃O₄ nanoparticle (diameter ~ 10 nm, Figs. 2(a) and 2(b)), which can be five orders of magnitude higher than long-range intermolecular force [38–41], and can then cause a mechanical signal such as bending a microcantilever based on interface vertical effect, or MNP-induced out-of-plane pulling force. The applicability of the MNPs and magnetic field is here expanded beyond the common applications for sample dissociation and enrichment [42]. Upon establishment of magnetic-to-mechanical conversion, the dilemma of ultrasensitivity has changed dramatically for nanomechanical sensor. Therefore, improving the generated response to target molecules at ultralow concentration is no longer an intractable problem. The key question, then, becomes “How do target molecules influence the binding of MNPs on the sensor surface?” Fortunately, replacement structure is common in aptasensors [43, 44]. Here we present that, for the first time, the ultrasensitive detection can be achieved conveniently by replacement of the

![Figure 1](image-url)

**Figure 1.** Schematic representation of the overall relay-type sensing mode based on a magnetic-force switch. (a) The magnetic field distribution (magnetic flux density and magnetic field lines) of the laboratory-made permanent magnet. (b) The magnetic force along the x-direction exerted on a single MNP under the magnetic field shown in (a) via MATLAB simulations. (c) The preparation and reaction mechanism of functionalized MNPs. The significantly enlarged mechanical response of (d) the magneto lever method compared to (e) a conventional aptasensor.
MNPs with target molecules under magnetic field (Fig. 1(d)), although direct measurement can be submerged in environmental noises. This sensing mode is called relay-type sensing mode as it can use target molecules at exceptionally low concentrations to lever much larger forces, thus fundamentally breaking intermolecular-force limitations. In addition, this on-chip designed nanomechanical system is compatible with molecule, cell, bacterium, virus, and microbe detection, thus achieving a real universal molecular lever and sensing system.

In this work, we have developed a creative relay-type sensing strategy on the basis of a magneto lever, indicating a possible direction and providing a foundation for further development of the next-generation nanomechanical sensor, with ultrahigh sensitivity. Benefitting from the successful leverage of substantially larger magnetic force with weak intermolecular force, we achieved considerable improvement in detection sensitivity up to five orders of magnitude for small-molecule dopamine (DA) detection, compared with a direct detection scheme based on an aptamer-functionalized microcantilever. Simultaneously, to further demonstrate its technical adaptability and utility, another magnetic-enhancement system was constructed for carcino-embryonic antigen (CEA) detection which served as a representative macromolecule.

2 Experimental section
All the experimental details including materials, apparatus, the synthesis process of S1@MNPs (S1 represents the complementary DNA oligonucleotide of probe aptamer S2, which can bind directly to the target molecules), work flow of detection, characterizations instrumentation, and the numerical simulation methods are in Electronic Supplementary Material (ESM).

3 Results and discussion

3.1 Relay-type sensing mode principle
The synthesis processes of S1@MNP are provided in detail in the ESM. Then, S1@MNP can bind to aptamer S2 through partial complementary sequence between oligonucleotide S1 and S2 (Fig. 1(c), step 3). And S2 can be displaced from S2@S1@MNP by its target molecule, owing to the higher affinity of target molecule for S2 compared to S1 (Fig. 1(c), step 4).

The general equation for tracking the force-bearing states of MNPs under a magnetic field [45], \( F \), on the basis of physical properties of the nanoparticle and distribution of the magnetic field is as follows

\[
F = \mu_0 \kappa V H \text{grad} H
\]

where \( \kappa \) is the volume magnetization rate of MNPs that can be calculated by \( M/H \), \( M \) is the magnetization strength of MNPs, \( H \) is the magnetic field strength, \( \text{grad} H \) is the magnetic field gradient, \( \mu_0 \) is the vacuum permeability, and \( V \) is the MNPs volume. Based on the magnetic field distribution of the laboratory-made neodymium magnet (Fig. 1(a)) and the characterization of the prepared MNPs (Figs. 2(a) and 2(b)), we calculated that the magnetic force exerted on a single MNPs can reach \( 10^{-16} \) nN (Fig. 1(b)), which is vertically at the microcantilever surface, indicating a five orders of magnitude increase versus long-range intermolecular force at \( 10^{-6} \) nN level (see Van der Waals force calculation in the ESM) [40,41,46]. Fortunately, based on the replacement mechanism of MNP (Fig. 1(c), step 4), we could interconnect these two forces with each other, thus theoretically priming both ends of the lever with a leverage over 10 million times. However, to truly break the constraint of the affinity-based intermolecular force, the last unsolved issue is the fulcrum, which needs a mechanical signal transducer.

Hence, the design of the relay-type sensing strategy is enabled through using the microcantilever sensor as a fulcrum (Fig. 1(d)). After the S2 was immobilized on the gold surface of the microcantilever by Au–S covalent bonds, the conventional aptasensor was ready. Normally, the microcantilever deflection should be almost negligible for a trace target relying on S2-target binding alone, as shown in Fig 1(e). However, the primed relay-type method also required that the S1@MNPs should be associated with the microcantilever, which was dependent on the partially complementary strand between oligonucleotides S1 and S2. Then magnetized MNPs under an inhomogeneous magnetic field exerted a magnetic force on the Au side of the microcantilever serving as an out-of-plane pulling force. Naturally, it caused a significant degree of microcantilever bending. Due to the higher affinity than S1 towards S2, target molecules easily replaced S1@MNPs (Fig. 1(d), right panel). Thereby, with the release of magnetically induced tension, this accordingly generated a comparatively large upward deformation recovery of the microcantilever, which was a clear contrast to the inappreciable signal of the conventional aptasensor. This strategy is similar to electromagnetic relay, as it can be implemented to utilize a weak intermolecular force to lever a much larger magnetic force. Obviously, the sensing sensitivity was dramatically improved by the application of the relay-type sensing mode based on the magnetic-force switch. Moreover, it has long been established that affinity is critically important in biochemical detection, since affinity is inherently limited by the maximum number of target molecules bound to probe molecules. Hence, there is still deeper significance existing for high-affinity probe screening, because our method only requires aptamers to have a slightly higher binding affinity towards target molecules than its complementary oligonucleotides.

3.2 Surface functionalization and characterization of MNPs
To simulate magnetic force of MNPs under external magnetic field, we must include the intrinsic parameters of the MNPs in the theoretical calculations. Hence, we characterized the MNPs by transmission electron microscopy (TEM) and vibrating sample magnetometer (VSM). The TEM results are shown in Fig. 2(a), and indicated that the average particle diameter of the synthesized MNPs was ~ 10 nm. And the VSM results are shown in Fig. 2(b), suggesting a saturation \( M \) of ~ 40 emu·g⁻¹ for the prepared MNPs.

In order to verify whether S1 was successfully coupled to the MNPs surface, dynamic light scattering (DLS), zeta potential experiments, and ultraviolet–visible (UV–vis) absorption spectroscopy were performed before and after the coupling. DLS results exhibit an increase in the average hydrodynamic diameter from 23.54 (Fig. 2(c), green line) to 28.04 (Fig. 2(c), orange line) nm with a basically unchanged particle dispersion, which was comparable to the length of S1 (~ 4.5 nm). The zeta potential experiments show that the average zeta potential of MNPs changed from ~ 39.5 (Fig. 2(d), green line) to ~ 45.6 (Fig. 2(d), orange line) mV after S1, intrinsically negatively charged, coupling. As shown in Fig. 2(e), S1 solution was incubated with MNPs. After the MNPs were locked at the bottom of the tube, a significant intensity decrease from 0.996 (S1 solution, Fig. 2(e), green line) to 0.091 (supernatant, Fig. 2(e), orange line) a.u. was observed in UV–vis spectra at 260 nm. Together these observations adequately address a scenario: S1 has been successfully conjugated to the MNPs surface.

The premise behind the magneto lever method is that S1@MNPs can be correctly replaced by target molecules. To test
this, we analyzed the reactivity between S2@S1@MNP and next detection object, DA. As seen from Fig. 2(f), DA was incubated with S2@S1@MNP and the supernatants were collected after MNP elution. The UV–vis absorption spectrum of the supernatant revealed an obvious intensity increase from 0.628 (DA solution, Fig. 2(f), green line) to 0.716 (Fig. 2(f), orange line) a.u. at 260 nm after DA-S2 binding, which demonstrated the successful replacement of S2 and good activity between S2@S1@MNP and DA. More importantly, this result provides a molecular basis for establishing the link between intermolecular force and magnetic force.

3.3 Construction and characterization of the high-performance sensing interface

After the confirmation of successful replacement of S1@MNP by DA, another crucial point for the development of the magneto lever method is the construction of S2@S1@MNP sensing layer on the sensor surface. To ascertain S2 was correctly immobilized on the sensor surface, we used X-ray photoelectron spectroscopy (XPS) to directly characterize the surface of bare (Fig. 3(a), blue line) and S2-modified (Fig. 3(a), green line) microcantilevers, respectively. When compared with the bare microcantilever, we observed an obvious absorption peak at 134.43 eV and 162.26 eV in the binding energy (BE) of S2-modified microcantilever for P 2p (Fig. 3(b), green line) and S 2p (Fig. 3(c), green line), respectively, which are the characteristic peaks of phosphate in the DNA backbone and sulphydryl group. However, for the bare microcantilever, no characteristic peak of nitrogen or phosphorus was observed (Figs. 3(b) and 3(c), blue line). Likewise, we also observed an obvious increase for both P and S atomic concentration on the sensor surface upon S2 conjugation. The above XPS results confirmed that the S2 layer had been successfully immobilized on the gold surface.

To confirm that the MNP s were successfully immobilized on the microcantilever surface, we performed scanning electron microscopy (SEM). With technical difficulty in achieving a spatial resolution of 10 nm, we turn instead to use 100-nm MNP s to modify the microcantilever. After MNP immobilization, the resulting functionalized microcantilever had a slightly sparse spatial distribution of nanoparticles with an average diameter of 100 nm (Fig. 3(d), bottom left, white dashed box) and the red dashed box area was enlarged. The observation provided evidence that the MNP s were spatially fixed on the sensor surface.

After the successful functionalization of S2@S1@MNP at interface, the holding activity of S2@S1@MNP also merits consideration. To this end, we monitored the assembly of S2@S1@MNP and the replacement of S1@MNP in real-time, using surface plasmon resonance (SPR). As seen in Fig. 3(e), 5 ng·mL⁻¹ S1@MNP was injected to S2-modified chip (orange line, step 1), producing a SPR response of 6.12 RU (orange line, step 2). Followed by a dissociation time of 10 min, a decrease of 2.35 RU in the SPR response was observed (orange line, step 3). After 1 µM DA injection, we observed a reduction of 1.07 RU in the SPR response (orange line, step 4), which demonstrates the successful replacements of S1@MNP by DA. What is more, this result indicates that S2@S1@MNP complexes still retain potent activity when constrained on the sensor surface.

To further verify the activity of S2@S1@MNP on the sensor
surface, atomic force microscopy (AFM) was carried out to study the surface topography or morphology and surface roughness of the sensor surfaces before and after target molecule binding. As shown in Fig. 3(f), the root-mean-square roughness ($R_q$) value of the MNP-immobilized gold-coated surface was 5.83 nm (equivalent to step 3 of orange line, Fig. 3(e)). Upon DA binding, we observed that the $R_q$ value of the gold surface conjugated with MNPs reduced to 3.13 nm (equivalent to step 4 of orange line, Fig. 3(e)). This latter decrease in $R_q$ proved that DA was able to displace S1@MNP from the interface, suggesting a holding DA-binding capacity of the S2@S1@MNP complex on the microcantilever surface.

Taken as a whole, based on the above XPS, SEM, SPR, and AFM observations, we can testify the successful formation of a S2@S1@MNP sensing layer in the sensing interface. More importantly, it is active, thus laying the foundation for the next ultratrace detection using magneto lever method.

### 3.4 Feasibility of the relay-type sensing mode based on the magneto lever

Since we have shown the sensing mechanistic principle, we analyzed the feasibility of the relay-type sensing mode based on a magnetic-force switch and chose DA, a micromolecule marker for Alzheimer’s disease and Parkinson’s disease, as the detection target. At the outset, we applied an excitation coil (see insert in Fig. S7(b) in the ESM) to produce the magnetic field. Due to the existing thermal noise that was difficult to remove completely, we discontinued use of electromagnetic field and instead utilized a neodymium magnet (see insert in Fig. S7(d) in the ESM). Owing to the low gradient of the generated magnetic field (longitudinal section, see simulation result in Fig. S7(c) in the ESM), we designed a new magnetic field generator to achieve a boosted magnetic field gradient. As shown by the insert in Fig. 1(b), the apparatus consisted of two tiers: a magnet pair in the upper tier, which was composed of a middle cylindrical neodymium magnet (M1) and a reversed annular neodymium magnet (M2), and an alloy with high magnetic permeability in the bottom tier. Since the magnetic field lines accumulated in the upper surface of M2 (Fig. 1(a), $H < 0$, positive magnetic field direction is defined to be in the $+z$ direction) rather than diverging towards infinity ($H = 0$), the $\text{grad}H$ was significantly increased.

When the home-made magnetic field device was in place, to verify the signal-enhancement ability of the magneto lever, contrasting experiments were performed and the curve of deflection versus time in a buffer flowing system was recorded as shown in Fig. 4(a). After a consistent drift was achieved, the deflection of the microcantilever functionalized by S2 (curve (a) in Fig. 4(a)) and S2@S1@MNP (curve (b) in Fig. 4(a), diameter ~ 10 nm) was 5.1 and 10.8 nm (bending towards the silicon side is defined as positive) at the equilibrium under 1 $\mu$M and 100 nM DA injection, respectively. And the MCH-blocked microcantilever only bent ~ 1 nm following the injection of 100 nM DA (control in Fig. 4(a)), serving as a control to help discriminate the bending signals from nonspecific interactions. For S2 alone on the microcantilever, the surface stress induced by the specific interaction was insufficient to produce a large deflection. When
MNP conjugates were used as the signal amplifier without a magnetic field, the sensing ability of the microcantilever for DA detection achieved decent improvement (at least 2 times), probably due to the lateral force changes between neighboring S2 upon MNP replacement and conformational transitions from the double helix hybridization structure of S1-S2 pairing to the globular chain structure by S2 capped DA (Fig. 4(a), middle right) [47]. Meanwhile, without mechanical effects, the SPR response originating from MNP replacing (Fig. 3(e), orange line) was comparable to the one based on direct DA binding (Fig. 3(e), green line) following 1 μM DA injection, suggesting limited improvement in sensing signal.

However, when an external magnetic field was applied, the microcantilever bent sharply along with MNPs replaced by 100 nM DA and reached a stable plateau up to 62.1 nm within 60 min (curve (c) in Fig. 4(a)), implying at least an enhancement of 12 times for the microcantilever deflection response on the basis of alternative intermolecular force by the magnetic force. Simultaneously, unlike conventional deflection difference technique (i.e., reference beam technique) used in static-mode of microcantilever [48, 49], this is the first-time for the inverse deflection difference technique. It is implicit that, the relay-type sensing mode provides not only the possibility of adjusting in-plane and out-of-plane interaction forces simultaneously, but also a negative bending control. Taken together, the use of MNPs in combination with the intervention of magnetic field, greatly increases the adsorption-induced bending moment [50–52], which should be the origin of ultrahigh sensitivity and promising superior detection limit. Apparently, the relay-type sensing mode based on the magnetic-force switch produced an even greater degree of bending. In order to verify that enhanced microcantilever deflection was mainly caused by magnetic force load, we also conducted a finite element simulation study. Simulations indicated that the intervention of magnetic field and MNP replacement can cause a deformation recovery above 60 nm. The relevant theoretical predictions quantitatively or qualitatively agree well with the relevant magneto experiments on the microcantilever deflections. Arguably, therefore, our magneto lever method is effective for biochemical detection.

3.5 Nanomechanical detection using magneto lever

To further assess the detection limit using magneto lever, time courses of the mechanical responses at different DA concentrations (0.01–100 nM) were recorded (Fig. 4(b)),
quantifying the relationship between the microcantilever deflection and DA concentration, simultaneously. When the baseline deflection was stable in a steady continuous flow using phosphate buffered saline (PBS), sample (DA) solution at various concentrations was circulated in the cell sequentially. The deflection was monitored in situ for approximately 60 min. Equilibrium-bending signals of 62.1, 33.7, 20.3, 10.8, and 5.5 nm were obtained for 100, 10, 1, 0.1, and 0.01 nM DA, respectively. With decreasing concentration of DA, the deflection signal of the microcantilever decreased, indicating a significant positive correlation between the signal and DA concentration. To investigate the effect of nonspecific interactions, in addition to using the MCH-blocked microcantilever as a negative control (see control in Fig. 4(a)), 1 μM chlorosulfuron (a nonbinder molecule, C₉H₈Cl₂N₂O₅S, MW = 357.8 Da) in PBS was injected as another reference and the MNP-functionalized microcantilever subsequently only showed little deflection (see control in Fig. 4(b)). These two contrasting experimental results indicate that considerable bending was caused by the specific MNP replacement, stemming from the specific binding between S2 and DA. Moreover, when DA concentration was as low as 100 nM, we noted that noise was non-negligible in the conventional aptamer assay, whereas using the novel magneto lever method, noise was vanishingly low. Bending towards the Si side was caused by the release of magnetic force imposed on the Au surface when MNPs were replaced by DA owing to a higher affinity for S2 (Fig. 1(d)). These data demonstrated that our method enables real-time, rapid, ultrasensitive, and quantitative DA detection, as a representative example for small-molecule detection.

Furthermore, Fig. 4(c) illustrates the variations in equilibrium deflections in microcantilevers functionalized with S2@S1@MNP (purple points) and S2 (orange points), respectively, versus DA concentrations. Experiments performed in triplicate are represented by each dot. By defining limit of detection (LOD) as three times the standard deviation of the background, the LOD of DA was only 1 μM via the aptasensor (orange fit line) with a background noise of ~ 1 nm. However, with introduction of relay-type nanomechanical sensing mode, 100 nM DA can still cause a considerable mechanical response, though the deflection degree was below the environmental perturbation level under same DA concentration for aptamer-functionalized microcantilever. In particular, benefitting from the magneto lever as high as 100,000×, DA concentrations as low as 10 pM were detectable on the basis of MNP replacement. Compared with a conventional microcantilever-based aptasensor, this was an incredible improvement in sensitivity up to five orders of magnitude. Arguably, our proposed relay-type sensing mode here shows great superiority and reliability in terms of sensitivity and LOD, indicating invaluable potentials in the ultrasensitive detection and quantification of target molecules (e.g., cellular metabolites, disease biomarkers, environmental pollutants). Moreover, since affinity limitation has often been cited as the ultimate barrier for further increasing the sensitivity of conventional nanomechanical sensors, the greater significance of our findings is pointing the way towards future construction of next-generation nanomechanical sensor for ultrasensitive detection, even reaching the single-molecule resolution free from the existing affinity dependence.

Inspired by sandwich assay enhancement, some scientists have shown that a nanomechanical sensor can exploit the magnetization of MNPs to exert additional forces for further improving mechanical responses [53–55]. This design is meaningful progress in the sensitivity improvement. Nevertheless, there is an implicit non-negligible constraint in sandwich assay applications, that is, the requirement of at least two identifiable sites on the target. Hence, it does not work for micromolecule detection, whereas our magneto lever methodology is applicable. Besides, the sandwich assay method can be inefficient due to multiple point-to-point conjugations. As consequence, previous studies chose to use micrometer-large MNPs, which led to poor reproducibility for trace analyte analysis, and thus, ultimately affected the detection reliability and sensitivity. On the contrary, since detection procedure requires only one DNA strand displacement reaction, 10-nm MNPs were selected for our method based on the high reaction efficiency, which effectively avoided that randomness.

Having demonstrated that the relay-type sensing mode can significantly improve the detection limit of target molecules, we next examined the impact of MNP size on sensitivity using 100-nm and 1-μm MNPs. At the initiation of this trial, we were surprised to find that the detected response signal was markedly reduced compared with the 10-nm MNP trial for 100 nM DA (Fig. 4(e)). Similar results were observed under 5 μM and 10 nM DA. This result is somewhat counterintuitive. Thus, there may be potential issues in the self-assembly of MNPs on the sensor surface or binding of DA to S2@S1@MNP complexes. And the magnetic force is proportional to the 3rd power of the MNP diameter. Hence, we conjectured that, fast diameter growth could allow the magnetic force to exceed the cutoff of hydrogen bond strength of the complementary part between S2 and S1.

To validate this conjecture, we next characterized the MNP distribution on the microcantilever surface using SEM. As shown in Fig.S5 in the ESM, 100-nm MNPs were distributed in a relatively intensive average density without magnetic treatment. However, following magnetic treatment, the MNPs were sparsely distributed. It is clear that many MNPs were shed or dislodged from the gold surface in the presence of the magnetic field. The result was in accordance with the hypothesis mentioned above, indicating force-accelerated rupture of the S2-S1 interaction that disfavored the subsequent binding events. Our findings strengthen our understanding of the regulatory mechanism governing the interaction between external forces and biomolecular structures. Moreover, this study may pave the way for the construction of high-performance biospired surfaces or films, which serve as the cornerstone and prerequisite of any ultrasensitive biosensing system.

3.6 Selectivity, adaptability, and generalizability of the presented sensor

To analyze the selectivity of the proposed method, the microcantilever functionalized by MNPs was exposed to three structural and functional DA analogues including ascorbic acid (AA), lysine (Lys), and L-3,4-dihydroxyphenylalanine (L-DOPA) at the same concentration (100 nM), and the curve of deflection versus time was recorded as shown by the left panel in Fig. 4(d), and the average deflections of three experiments are shown in the right panel. Under the same experimental conditions, it was found that only the DA solution caused a significant deflection of the microcantilever, while the analogues had a negligible effect. This indicates that the magneto lever method has excellent selectivity and can specifically recognize DA from other congeners.

Finally, we would like to test the universality of our method, and therefore a magneto lever sensor for macromolecule CEA [9], a representative biomarker for colorectal cancer diagnosis, detection was also developed. The solid lines in Fig. 4(f) show the time courses of microcantilever-deflection responses under various CEA concentrations. Microcantilever deflection signals exhibited significant correlations with CEA concentrations, indicating the successful establishment of a real-time, rapid, highly sensitive and quantitative CEA detection method. With a background noise of ~ 1.5 nm, the LOD for CEA detection was
considerably lower than 100 pg·mL⁻¹ estimated from 3-fold noise, meeting the criteria for clinical settings [56]. The presented sensor also achieved an approximately three orders of magnitude lower LOD in detecting CEA, compared with CEA-aptamer-functionalized microcantilevers (60 ng·mL⁻¹, dotted lines in Fig. 4(f)). These data indicated the adaptability and generalizability of our technique. Hence, we can, indeed, argue that our methodology is generalized to a variety of targets, including small molecules (e.g., cytokines, pheromones, and ions), macromolecules (e.g., proteins, nucleic acids, poly saccharides, and enzymes), and even viruses, bacteria, exosomes, and cells.

4 Conclusions

In summary, we developed a novel relay-type sensing mode that can resolve the detection dilemma of ultratrace levels of various molecules due to affinity, material, and quantum limitations, whose implementation included but not limited to a magneto-mechanical switch. In our study, we established the link between weak intermolecular forces and much larger magnetic forces by replacing MNPs with target molecules, which enabled ultralow concentrations of molecules to lever large forces, thus causing a marked mechanical response. Using magneto lever, there was a five orders of magnitude improvement in the sensitivity compared to the microcantilever-based aptasensor. Our results provide a potentially transformative approach for novel, ultrasensitive, real-time, and universal biochemical sensing. Importantly, even single-molecule resolution can theoretically be achieved under sufficiently high lever. Hence, we argue that this magneto lever method can be a uniquely powerful tool for disease diagnosis, drug development, food safety, and basic biochemical research.

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