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Screening Protein Conformational Responses by Molecular Friction Sensing

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Abstract

Small-molecule drugs often act by stabilizing specific protein conformations, yet most biophysical screening platforms report binding affinity or kinetics without directly capturing structural response. Here we introduce molecular friction sensing (MFS), a screening-compatible approach that detects ligand-induced changes in protein conformation through changes in hydrodynamic drag exerted on electrically switchable DNA origami nanolevers. Across a diverse protein panel and under physiological conditions, the method quantitatively reports protein size and resolves ligand-dependent conformational shifts with sub-Angström sensitivity. In maltodextrin-binding protein, cognate and non-cognate maltodextrins produced distinct friction signatures that mirrored their biological roles. In a carbonic anhydrase screen, molecular friction sensing identified compound-dependent conformational responses among SPR-validated binders, revealing that affinity and kinetics do not predict structural outcome. Molecular friction sensing therefore adds a structural dimension to interaction analysis and provides a route to prioritize drug candidates by the conformations they induce.

Proteins are typically depicted as static three-dimensional structures with well-defined folds. In reality, however, they often populate an ensemble of energetically accessible conformational states [1,2,3,4]. The ability to undergo conformational changes – from the large-scale rearrangement of entire domains to subtle shifts of individual loops – enables proteins to perform a wide range of molecular functions. These functions include enzymatic catalysis, signal transduction, allosteric regulation, molecular recognition, and transport [5,6,7].

The conformational plasticity of proteins offers unique opportunities for therapeutic intervention. Many FDA-approved drugs act by allosteric modulation or by stabilizing specific protein conformations, thereby shifting conformational equilibria to activate or inhibit function rather than directly blocking active sites. Examples include the kinase inhibitors imatinib[8] and trametinib[9]; the GPCR and ion-channel modulators cinacalcet[10], maraviroc[11], ivacaftor[12], and benzodiazepines[13]; the nuclear-receptor ligands tamoxifen[14] and rosiglitazone[15]; the allosteric enzyme inhibitor efavirenz[16]; and the conformational modulators venetoclax[17] and lenalidomide[18], all of which act by shifting protein conformational equilibria rather than solely competing at orthosteric sites. Allosteric binding and the subsequent induction of conformational changes represent a promising strategy to enhance specificity and reduce off-target effects, particularly in protein families with highly conserved active sites – such as kinases or ion channels – where orthosteric targeting is often associated with poor selectivity [19,20].

Conventional high-throughput screening platforms typically identify hits based on binding affinity or kinetic parameters. Although these are important prerequisites for target engagement, they do not necessarily predict the structural or functional consequences of binding of allosteric or conformationally selective compounds. Conversely, high-resolution structural and conformational methods such as Cryo-EM, X-ray crystallography, NMR and HDX-MS provide detailed mechanistic information, but are typically less scalable across a larger compound library. A critical bottleneck therefore exists between high-throughput biochemical binding assays and more complex, low-throughput structural biology and cell-based studies. Biophysical screening technologies capable of detecting and quantifying ligand-induced conformational responses can help bridge this technological gap by identifying compounds that induce or stabilize specific conformational states and those that merely bind without structural consequences. Integrating such measurements into early screening can improve prediction of hit quality, increase hit-to-lead conversion rates, and ultimately accelerate development timelines (Figure 1).

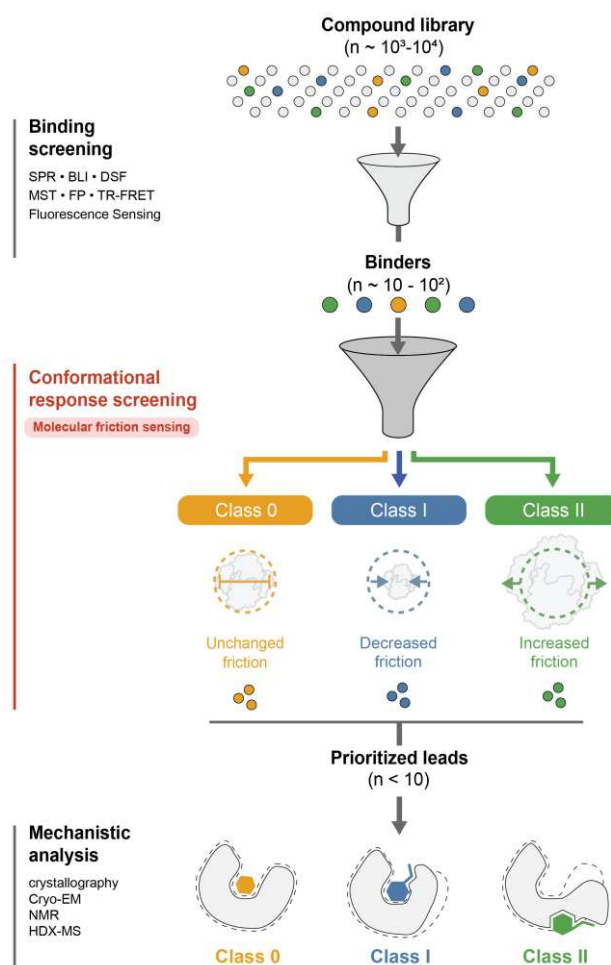


Fig. 1 | Molecular friction sensing introduces conformational response as an orthogonal screening parameter in drug discovery. Conventional high-throughput screening methods identify compounds based on affinity and kinetic parameters, informing on whether and how strongly they bind to their target protein. Molecular friction sensing provides a complementary, screening-compatible workflow that reports on binding-induced conformational changes. By classifying selected binders according to their ability to induce a conformational response, this screening approach reveals mechanistic differences between compounds that may not be apparent from their respective binding strengths or kinetic parameters. Therefore, molecular friction sensing enables prioritization of hits and leads based on conformational responses, supporting downstream high-resolution structural analysis and mechanistic characterization.

Here, we present a biophysical method that enables the identification and classification of small molecules that modulate protein conformation. The approach is grounded in the premise that ligand binding-induced structural rearrangements, if they appreciably alter a protein's external geometry, will translate into changes in hydrodynamic drag. Such changes in drag are, in principle, measurable. For spherical particles, for example, the friction coefficient ζ scales with the hydrodynamic radius R_h and the solution viscosity η through Stokes' law, $\zeta = 6\pi\eta R_h$.

Conventional techniques for assessing protein friction or diffusivity, such as dynamic light scattering, offer limited sensitivity, require substantial sample quantities, and are therefore not readily adaptable to high-

throughput screening. Here, we hypothesized that tethering proteins to dynamically actuated DNA origami nanolevers on a biosensor surface would enable detection of changes in hydrodynamic friction and facilitate integration with established microfluidic screening platforms. This configuration allows exposure to large libraries of candidate small-molecule binders within a screening-compatible workflow.

Electrical actuation of DNA origami nanolevers

To enable a fully automated, screening-compatible biosensor workflow, we developed a method in which proteins are covalently tethered to the distal end of a DNA origami nanolever that is anchored to a microelectrode surface (Figure 2a). Upon application of an alternating voltage, the negatively charged DNA origami nanolever undergoes periodic oscillations between *lying* and *standing* orientations (switchSENSE method) [21,22]. These oscillations are synchronized across the nanolever ensemble, allowing coherent, population-level measurements. Nanolever motion is monitored in real time using electrically triggered time-correlated single photon counting (E-TCSPC). A fluorophore integrated within the DNA origami nanolever reports its position relative to the electrode surface via distance-dependent surface energy transfer, allowing fluorescence intensity to serve as a proxy for nanolever orientation [23]. A typical diffusion-synchronization cycle is illustrated in Figure 2b. In the initial state (at positive potential) all nanolevers are in the surface-attracted *lying* position. Upon voltage reversal, the nanolevers are released from the surface and diffuse into the *standing* position, which is a dynamic equilibrium of orientations due to thermal fluctuations and entropic contributions [24]. Reversing the voltage again restores the initial state by trapping the nanolevers in the *lying* configuration as they stochastically diffuse into proximity of the evanescent electric field. Due to the well-defined and synchronized nature of its initial state, the diffusion-dominated upward trajectory is used for further quantitative analysis.

To meet the advanced performance requirements of hydrodynamic friction-based measurements, the DNA origami nanolever was specifically engineered to extend the capabilities of conventional switchSENSE adapter nanolevers. While standard dsDNA nanolevers have proven successful in a wide range of applications [25,26], the 4-helix bundle (4HB) DNA origami-based design introduces key enhancements. Most notably, its increased length (4HB: ~75 nm, vs 48bp dsDNA: ~16 nm) and structural rigidity (persistence length, 4HB: ~740 nm, vs dsDNA: 50 nm) allow attachment of large proteins without introducing steric hindrance that would otherwise compromise friction-dominated oscillation dynamics [22,27,28]. The higher bare charge (-1200 e) and increased line charge density of the DNA origami nanolever improve performance across a broad range of buffer conditions by mitigating electrostatic screening effects from counterions acting on both the nanolever and the applied evanescent electric field, thereby enabling operation under physiological buffer conditions [23]. Furthermore, the increased hydrodynamic drag imposed by the DNA origami nanolever itself ensures that its oscillation dynamics are temporally decoupled from electrode charging processes [29]. Consequently, the upwards trajectory constitutes a diffusion-dominated motion that is inherently insensitive to electrostatic actuation artefacts. This

architecture maximizes sensitivity to binding-induced structural changes through alterations in friction coefficient, while at the same time suppressing electrostatic effects typically encountered in electrically actuated DNA monolayers.

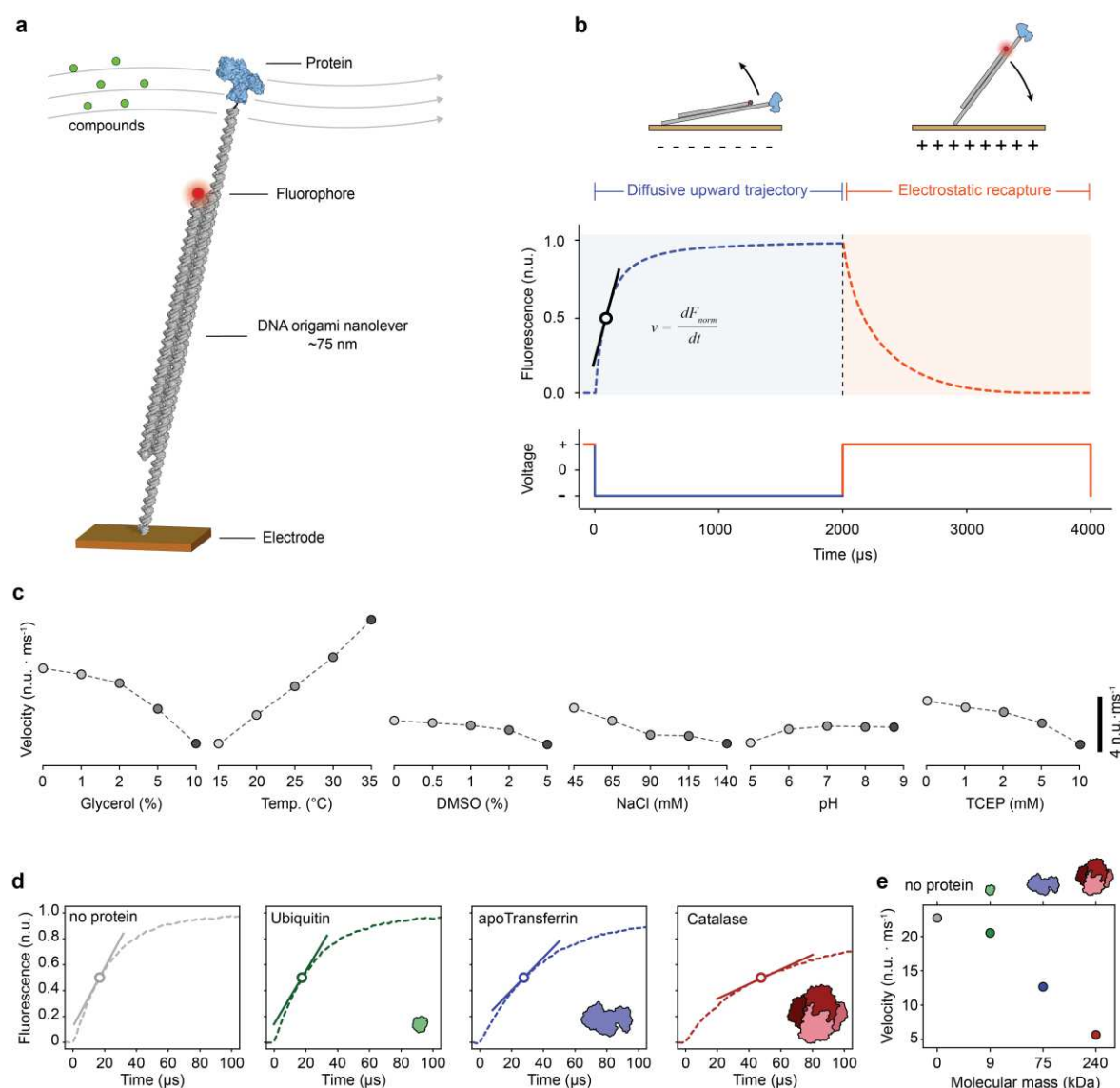


Fig. 2 | Electrically driven oscillation of (protein-functionalized) DNA origami nanolevers. **a.** DNA origami nanolever covalently functionalized at its distal end with a protein of interest is tethered to a microelectrode. Conformational changes induced by binding of small molecule ligands (green spheres) to the protein are translated into changes in hydrodynamic friction. **b.** Nanolever oscillation trajectory under alternating voltage potential. The nanolever oscillates between a surface-attracted *lying* state (at positive potential) and a surface-released *standing* state (at negative potential). A fluorophore integrated in the nanolever enables motion tracking via Electrically triggered Time-Correlated Single Photon Counting (E-TCSPC). A characteristic nanolever velocity is defined as the slope of the upwards trajectory at half maximum fluorescence. **c.** Velocity response of the bare nanolever to a range of measurement conditions. Scalebar represents 4 n.u. · ms⁻¹. **d.** Initial 100 μs of the upwards trajectories of the bare nanolever and nanolevers functionalized with three proteins of increasing size. Solid lines indicate the tangents at half maximum fluorescence and reveal a decreasing velocity with increasing protein size. **e.** Velocity of the DNA origami nanolever functionalized with proteins of increasing size.

Hydrodynamic friction modulates nanolever oscillation dynamics

To quantify the upwards trajectory of nanolever oscillations, the first derivative (i.e., slope) of each fluorescence trace was computed at the point of half-maximum fluorescence (0.5 n.u., Figure 2b, Supplementary Note 1). The resulting “velocity” parameter v (n.u. $\cdot$ ms⁻¹) captures the instantaneous rate of signal increase and serves as a proxy for the rotational diffusive mobility of the tethered nanolever.

First, we tested whether nanolever velocity responded to changes in environmental parameters associated with Brownian mobility (Figure 2c). As expected from the Stokes-Einstein relation, increasing solution viscosity substantially reduced nanolever velocity. Similarly, nanolever velocity increased with increasing temperature, consistent with a combined effect of thermal agitation and decreasing water viscosity. Next, we evaluated compatibility with solution conditions and additives commonly encountered in biophysical assays. Changes in DMSO concentration, ionic strength, pH and the presence of reducing agents have minor effect on baseline nanolever velocity, thus confirming the robustness of molecular friction sensing across a broad range of solution conditions.

Having established the baseline response of the unmodified DNA origami nanolever to alternating voltage potentials under various experimental conditions, we next evaluated the effect of protein tethering on its dynamic behavior. Figure 2d shows upward trajectories of the unmodified DNA origami nanolever alongside those functionalized with three proteins of distinct molecular weights (~9 kDa, ~75 kDa and ~240 kDa). A clear, protein size-dependent effect on the upwards trajectory was observed, with larger proteins inducing a more pronounced decrease in the rate of fluorescence recovery. Quantitative analysis confirmed a systematic decrease in velocity with increasing molecular weight, consistent with the principle that tethered proteins impose additional hydrodynamic drag, and thus effectively act as a ‘friction brake’ on the nanolever’s mobility (Figure 2e).

Nanolever oscillation velocity scales with protein hydrodynamic radius

To investigate how protein physicochemical properties influence nanolever oscillation dynamics, we next analyzed a panel of 12 proteins spanning molecular weights from 9 to 240 kDa and corresponding hydrodynamic radii from 16 to 45 Å (Table 1). This panel was assembled to cover the range in protein sizes commonly encountered in high throughput small molecule biophysical screening [32]. Each protein was covalently conjugated to a 48-nt oligonucleotide and hybridized to the distal end of the DNA origami nanolever (Supplementary Note 1). Switching trajectories of the protein-functionalized nanolever were recorded at spot 1, alongside a real-time reference nanolever lacking the respective protein on a second measurement spot (spot 2). To isolate the protein-specific contribution to hydrodynamic friction, the differential velocity ($v_{\text{diff}} = v_2 - v_1$) was computed, where v_1 and v_2 denote the velocities of the protein-functionalized and reference nanolever, respectively. Hence, v_{diff} quantifies the lag of the protein-modified nanolever relative to the unmodified nanolever. When v_{diff} was plotted against structure-derived hydrodynamic radii calculated using HYDROPRO (Supplementary Note 2), a strong positive linear correlation emerged (Pearson's $r = 0.992$). The predictive accuracy of this linear model was further assessed via leave-one-out cross-validation, yielding excellent agreement between MFS-measured and structure-derived hydrodynamic radii, with a root mean square error (RMSE) of 1.5 Å and a maximum deviation of 2.4 Å (Figure 3a, Table 1, Figure S2-S4).

Table 1 | Protein panel used to establish v_{diff} vs. R_h , v_{diff} vs. MW and v_{diff} vs. μ_e correlation

Protein	Identifier	MW (kDa)	R_h calc. (Å) ^a	R_h meas. (Å)	R_h deviation (R_h meas. - R_h calc.) (Å)	R_h deviation (%)
Ubiquitin	1UBI	8.6	15.9	17.0 ± 0.5	+1.1	+6.9
Cytochrome C	2B4Z	11.6	16.9	19.1 ± 0.5	+2.2	+12.7
β-Lactoglobulin	1QG5	18.3	19.7	20.3 ± 0.2	+0.6	+2.9
Trypsin Inhibitor	1BA7	20.1	19.8	19.2 ± 0.3	-0.6	-3.0
Trypsinogen	1TGN	23.3	21.1	22.5 ± 0.9	+1.4	+6.5
Carbonic Anhydrase	1V9E	28.8	23.0	21.3 ± 0.2	-1.7	-7.3
Myokinase	4AKE	23.2	23.3	22.4 ± 0.9	-0.9	-3.7
Tetra-Ubiquitin ^b	Q96C32-F1-v4	34.2	27.8	25.6 ± 0.3	-2.1	-7.6
apoTransferrin	2HAU	75.2	34.2	32.0 ± 0.1	-2.2	-6.6
GAPDH	1J0X	143.1	40.3	40.2 ± 0.5	-0.1	-0.2
Aldolase	1ADO	156.9	43.7	46.1 ± 0.3	+2.4	+5.4
Catalase	4BLC	239.1	45.0	44.7 ± 0.6	-0.3	-0.6

^a Calculated using HYDROPRO [33,34]

^b Structure obtained from AlphaFold Protein Structure Database [35]

In contrast, plotting v_{diff} against molecular mass calculated from primary amino acid sequences revealed a distinctly non-linear relationship (Figure 3b). A cube-root scaling model – based on the approximation of proteins as uniformly and equally dense spheres (mass $\propto$ radius³) – yielded a good correlation. Nevertheless, deviations from the model were notably larger than those observed for hydrodynamic radius, likely reflecting the inherently anisotropic nature of real proteins which deviate from ideal spherical geometry (Figure S5). Collectively, these results indicate that nanolever velocity is sensitive to subtle variations in protein size and shape and further identify molecular friction as a reliable descriptor of hydrodynamic radius.

To further rationalize these findings, we simulated the nanolever's upward trajectory using stochastic thermodynamics, integrating the overdamped Langevin equation with the rotational diffusion coefficient $D_{\text{rot}} = k_B T / \zeta_{\text{rot}}$ (Supplementary Note 3). The rotational friction coefficient ζ_{rot} was computed for a composite object comprising a rigid, slender rod (length L , non-uniform diameter d) representing the DNA origami nanolever, and the tethered protein modelled as a sphere with radius R (Figure 3c). The sphere moves along an arc of radius $L + R$, reflecting the geometric constraint imposed by the nanolever. The velocity of a simulated nanolever with $R = 0$ closely matched experimental values for the reference nanolever (Figure S7). Similarly, the effect of environmental factors such as temperature and solvent viscosity on the movement of the reference nanolever were accurately captured by the model (Figure S8). Simulated trajectories for nanolevers with tethered spheres with radii ranging from 0 to 50 Å reveal a progressively slower fluorescence increase with larger sphere size (Figure 3d) and the simulated data follows a near linear relation between velocity and R (Figure 3e). Deviations in both slope and intercept between the simulated and measured data, however, highlight that modelling proteins as idealized inert spheres does not fully capture their intricate hydrodynamic properties.

To benchmark nanolever-based molecular friction sensing against established methods for assessing protein size, we first performed native polyacrylamide gel electrophoresis (PAGE) on ligand-strand conjugated proteins hybridized to an excess of a complementary, fluorescently labeled oligonucleotide (Figure 3f). Most protein-DNA hybrids migrated as single, well-defined bands, while trypsinogen and apo-transferrin displayed two distinct bands, and GAPDH and Aldolase appeared as less defined bands, possibly due to dissociation of their native tetrameric assemblies under electrophoretic conditions. Electrophoretic mobilities (μ_e) were determined relative to excess fluorescent oligonucleotide mobility. Figure 3g shows a clear, positive linear correlation between v_{diff} and μ_e , albeit with greater deviations compared to v_{diff} vs. R_h . These deviations likely reflect protein instability during electrophoresis and charge-dependent migration effects (e.g., $z_{\text{catalase}} = -11.6$, $z_{\text{aldolase}} = +9.4$, Table S4). In contrast, protein charge did not measurably influence nanolever oscillation dynamics, consistent with the protein being positioned beyond the screened electric field range and the dominant nanolever charge ($z_{\text{lever}} = -1,200$; Debye length, $\lambda_D \approx 0.8$ nm at 140 mM NaCl). Consistent with this, no significant correlation was observed between protein net charge and deviations from the v_{diff} vs. R_h correlation (Figure S9).

We next compared molecular friction sensing with dynamic light scattering (DLS), a standard ensemble technique for molecular size characterization. Across the protein panel, v_{diff} correlated strongly with DLS-derived R_h (Figure 3h, Table S5), confirming robustness of the nanolever-based friction approach. Notably, the DLS derived radius of trypsin inhibitor deviated significantly, revealing a substantially larger hydrodynamic radius than expected from crystal structures or obtained with molecular friction sensing. This may reflect concentration-dependent association or aggregation under conditions required for DLS. Collectively, these results establish nanolever velocity as a sensitive, quantitative proxy for protein hydrodynamic radius, compatible across a broad range of protein sizes.

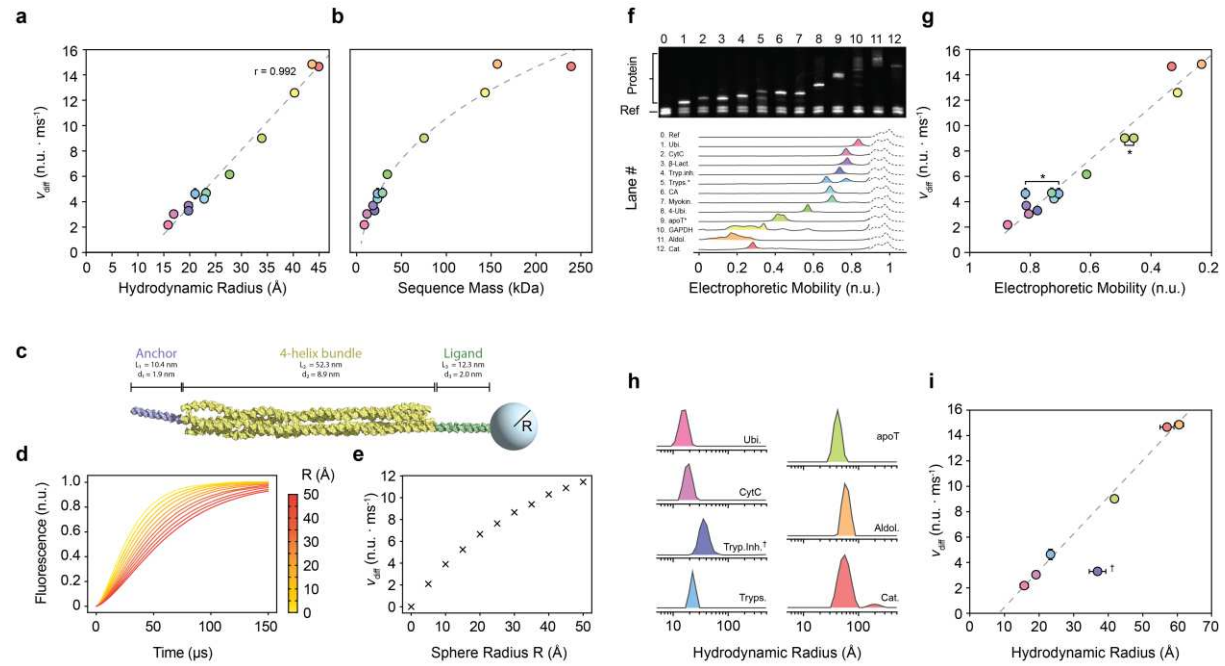


Fig. 3 | Molecular friction sensing benchmarked against protein size measurements. **a.** Differential velocity (v_{diff}) versus hydrodynamic radius (R_h) derived from crystal structures (Table 1). Dashed line: linear regression of experimental data. Pearson correlation coefficient ($r = 0.992$) indicates a near perfect positive linear relation between v_{diff} and R_h . **b.** v_{diff} versus calculated sequence mass. Dashed line: cubic-root model fitted to experimental data. **c.** Stochastic thermodynamics simulations. The nanolever-protein complex was modeled as a composite of a rigid slender rod with non-uniform diameter connected to a sphere of radius R . **d,e.** Upward trajectories and corresponding v_{diff} simulated for $R = 0 - 50 \text{ \AA}$. **f.** Non-denaturing electrophoretic mobility shift assay. Protein-oligonucleotide conjugates were hybridized to a two-fold excess of a complementary fluorescently labeled oligonucleotide and electrophorized on a non-denaturing 5 % PAGE gel. **g.** Gaussian models were fitted to the protein-DNA hybrid band and excess fluorescent oligonucleotide in the 1D intensity lane profiles. Electrophoretic mobility of the protein-DNA hybrid was derived relative to gel pocket (0) and excess fluorescent oligonucleotide (1). * indicates proteins that migrate as 2 clearly defined bands **h.** Dynamic Light Scattering (DLS) validation. v_{diff} for a subset of proteins plotted against R_h measured by DLS. Dashed line: linear regression of experimental data. $\dagger$ Indicates protein excluded from linear regression (Trypsin Inhibitor). Error bars represent s.d. of triplicate technical replicates.

Ligand binding-induced conformational changes affect nanolever oscillation velocity

Having established a robust correlation between nanolever-based molecular friction sensing and protein hydrodynamic radius, we applied this approach to a curated set of proteins reported to undergo ligand binding-induced structural rearrangements (Figure 4a-c). Instead of deriving absolute R_h values from v_{diff} , protein conformational changes were quantified relative to an internal blank ($v_{diff,blank}$) measured under identical conditions in the absence of ligand. This minimizes the influence of experimental factors and eliminates regression-associated uncertainty. Under saturating ligand concentrations ($[ligand] \gg K_d$), all protein-ligand pairs exhibited significant shifts in v_{diff} relative to their corresponding blank measurements. Myokinase and calmodulin showed decreased v_{diff} upon their respective inhibitors binding, corresponding to reduced hydrodynamic friction consistent with the compaction observed in the respective structural models. In contrast, HiBiT peptide binding to LgBiT resulted in an increase in v_{diff} , indicating greater friction associated with structural expansion. These observations closely match the R_h changes inferred from published crystal structures, where myokinase and calmodulin compact by -1.52 Å and -1.72 Å respectively, whereas LgBiT expands by +0.15 Å [36,37,38,39].

Using these structure-derived hydrodynamic radii as reference values, together with the measured means and variances of v_{diff} in the presence and absence of ligand, we computed the minimally detectable difference (MDD) for each protein under its respective measurement conditions. At a one-sided significance level of $\alpha = 0.01$ the MDDs were 0.3 Å for Myokinase, 0.4 Å for Calmodulin and 0.1 Å for LgBiT, defining the sensitivity limits of molecular friction sensing for this set of proteins (Supplementary Note 4).

We next verified whether molecular friction sensing can discriminate between functionally distinct ligand-bound conformations using maltodextrin-binding protein (MalE). MalE interacts with a broad range of (hydrogenated) malto-oligosaccharides, yet only native maltodextrin ligands yield a complex with MalE that is compatible with productive docking to the ABC transporter MalFGK₂ (Figure 4d) [40,41]. To probe this ligand specificity, we analyzed a representative panel of ligands (lactose, hydrogenated maltodextrins and maltodextrins) and quantified friction response as $\Delta v_{diff} = (v_{diff,ligand} - v_{diff,blank})$. The resulting Δv_{diff} values clearly separated the malto-oligosaccharides according to their known biological roles (Figure 4e): the nonbinding control (lactose) produced no detectable conformational change ($\Delta v_{diff} \sim 0$), hydrogenated maltodextrins (maltotriitol, maltotetraitol) induced a small but significant friction decrease ($\Delta v_{diff} < 0$), and the cognate maltodextrins (maltose, maltotriose, maltotetraose) produced a pronounced friction increase ($\Delta v_{diff} > 0$). Next, v_{diff} was measured over a range of maltose concentrations (Figure 4f). A clear concentration dependence was observed, consistent with a ligand-binding induced conformational transition. Nonlinear regression of the Hill equation to the measured v_{diff} series yielded an EC₅₀ of 426 nM, corresponding to the concentration at which the apo and ligand-bound conformations are equally populated. This value agrees well with the reported equilibrium dissociation constant of ~1100 nM, supporting the interpretation that the observed friction changes arise from ligand-driven population shifts between conformational states [40]. Together, these results demonstrate that molecular friction sensing with dynamically actuated DNA

nanolevers can resolve functionally relevant, ligand-dependent conformational changes and underscores the link between protein conformation and biological function.

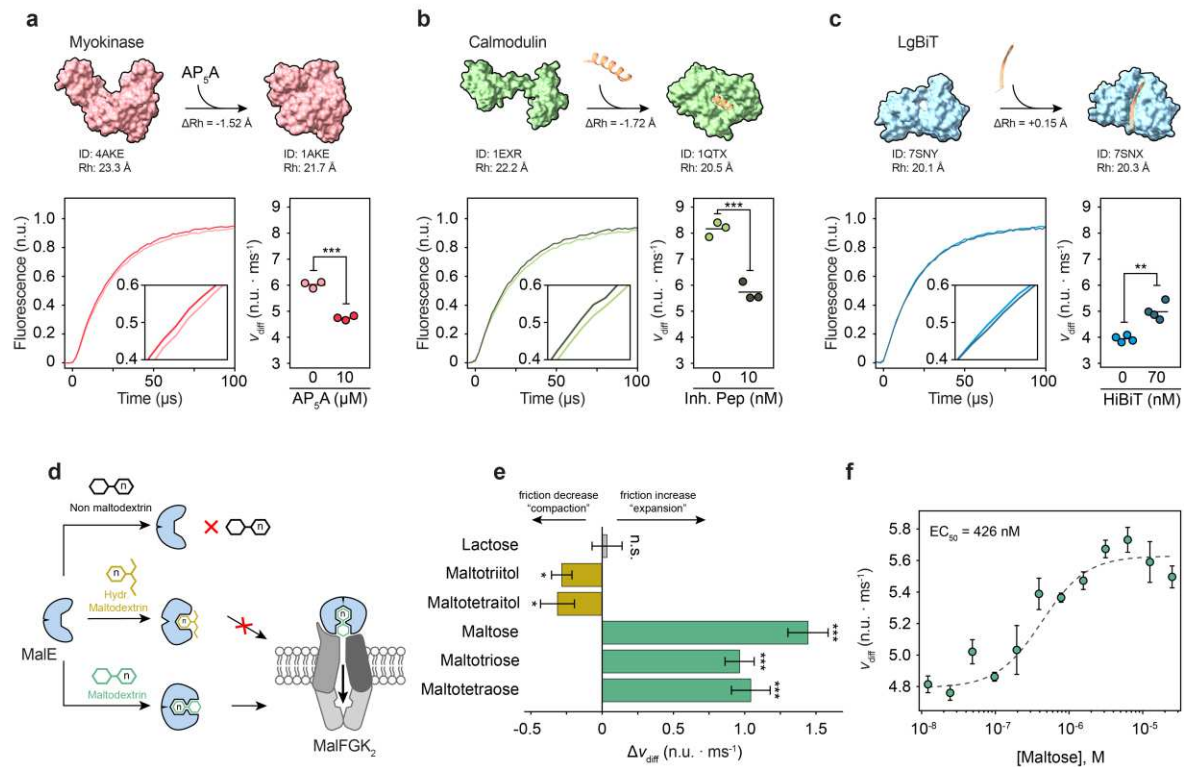


Fig. 4 | Ligand binding-induced conformational changes alter nanolever oscillation velocity. Changes in referenced velocity reflect ligand-binding induced alterations in hydrodynamic friction resulting from conformational rearrangements. Averaged upwards trajectories of blank and ligand-bound states, and the associated differential velocities are shown for **a**. Myokinase binding to Ap5A. **b**. Calmodulin binding to Calmodulin Inhibitory Peptide (CIP). **c**. NanoLuc luciferase reconstitution by binding of HiBiT to LgBiT. **d**. Schematic of the functional behavior of a panel of ligands binding to Maltodextrin-binding protein (MalE) and subsequent docking on the ABC transporter complex MalFGK₂. **e**. Differential velocity change (ΔV_{diff}) of MalE measured for the ligand panel including negative control (lactose), hydrogenated maltodextrins (maltotriitol, maltotetraitol) and cognate maltodextrins (maltose, maltotriose, maltotetraose). **f**. V_{diff} measured across a range of maltose concentrations. Nonlinear least-squares optimization of the Hill equation (dashed line) yields an apparent EC_{50} of 426 ± 77 nM. Error bars represent SEM of triplicate repeats. Statistical significance was evaluated by a Welch's t-test (n.s. $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$).

Conformational response of carbonic anhydrase resolved by molecular friction sensing

Finally, we applied molecular friction sensing as a screening tool to detect binding-induced conformational rearrangements using bovine carbonic anhydrase II (CA) as a model system. CA is a thoroughly characterized zinc metalloenzyme that interacts with numerous small molecule inhibitors [42,43]. CA is typically considered as comparatively rigid, with inhibitor binding described by a lock-and-key type mechanism, consistent with the absence of pronounced conformational rearrangements in multiple co-crystallized CA-inhibitor structures [44,45,46]. However, orthogonal technologies such as molecular

dynamics (MD) simulations, ultraviolet photodissociation (UVPD) mass spectrometry and (solid-state) nuclear magnetic resonance (NMR) have suggested subtle structural changes in CA can occur upon inhibitor binding [47,48,49].

Given this debate, CA provides a challenging test case to benchmark the sensitivity of molecular friction sensing in a realistic screening workflow. First, we evaluated a curated set of 48 small molecule compounds spanning a wide array of chemical features in a surface plasmon resonance (SPR) binding screen (Supplementary Note 5, Figure S10, Table S7). As expected, primary sulfonamides were markedly more potent binders than secondary/tertiary sulfonamides or non-sulfonamide analogues [50,51]. From this initial hit screen, 22 compounds were confirmed binders and were further kinetically characterized by either Multi Injection Cycle Kinetics (MICK) or Titration Cycle Kinetics (TCK), depending on dissociation rates estimated from the initial hit screen (Table S8, Figure S11-12). The resulting association and dissociation rate constants (k_{on} , k_{off}) spanned over two orders of magnitude, yielding sub-nanomolar to micromolar K_d values (Figure 5a).

Next, all SPR-validated hits were measured by molecular friction sensing at fixed ligand concentrations ($10 \times K_d$) to ensure uniform, near-saturating occupancy of the CA active site across the small-molecule panel (Table S2). Anticipating only subtle ligand binding-induced structural reconfigurations of CA, we performed extensive randomized replicates to suppress potential stochastic experimental variability (Figure 5b). Across the 22 SPR-validated binders, the measured differential velocity change did not cluster into discrete classes but rather revealed a continuous bidirectional distribution. Some binders increased apparent friction ($\Delta v_{diff} > 0$), some decreased apparent friction ($\Delta v_{diff} < 0$), and others elicited no significant response at all ($\Delta v_{diff} \approx 0$). Although absolute responses were small ($< 1 \text{ n.u.} \cdot \text{ms}^{-1}$), responses with $|\Delta v_{diff}| \gtrsim 0.2 \text{ n.u.} \cdot \text{ms}^{-1}$ were reproducibly resolved with statistical significance, indicating subtle conformational changes in carbonic anhydrase (10 out of 22 binders, Figure S13). Ranking binders by their induced Δv_{diff} reveals that chemically related binders (e.g., ETS/BZ1, AZM/MZM and TRU/H CZ, Table S9) elicited similar Δv_{diff} responses (Figure 5b), supporting a relationship between chemotype and the elicited conformational response of CA. Importantly, within this compound panel, affinity and kinetic rate constants did not predict the direction or magnitude of the molecular friction response (Figure 5c-e). Molecular friction sensing therefore provides a biophysical dimension that is experimentally orthogonal to binding interaction parameters.

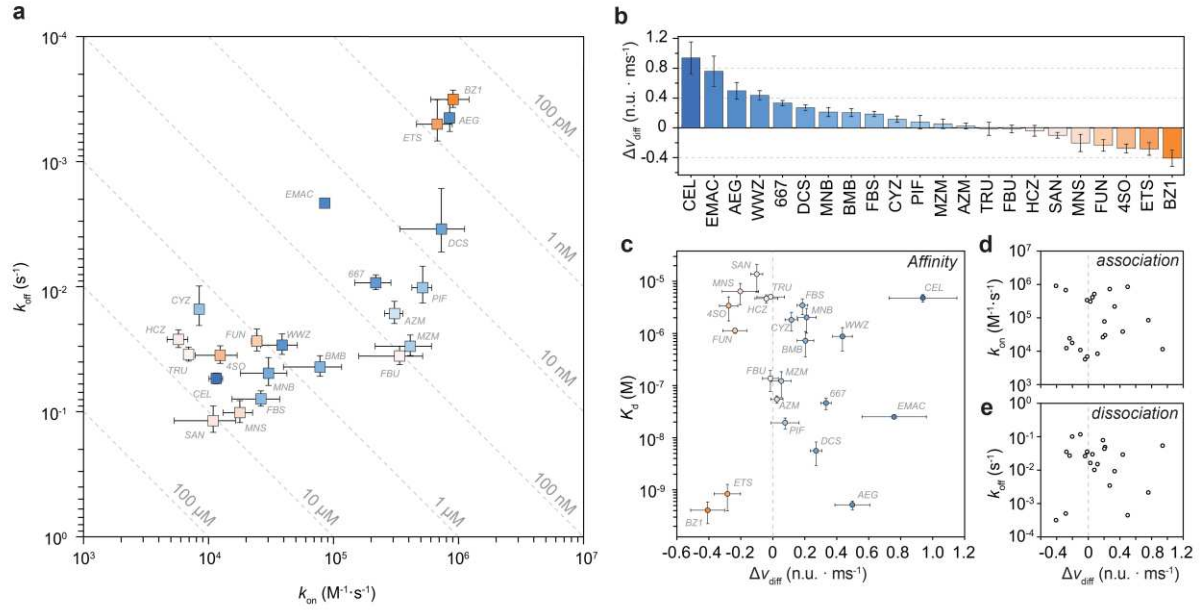


Fig. 5 | Screening carbonic anhydrase binders for ligand-induced conformational changes. **a.** Surface plasmon resonance (SPR) characterization. Kinetic rate constants of 22 confirmed carbonic anhydrase binders, obtained by either Multi Injection Cycle Kinetics (MICK) or Titration Cycle Kinetics (TCK), depending on dissociation rate estimated from the initial hit screen. Kinetic parameters were extracted by fitting a 1:1 Langmuir model (MICK) or Kinetic Titration Model (TCK) to the sensorgrams. Error bars indicate standard deviation of a minimum of triplicate measurements. **b.** Bar chart showing small molecule carbonic anhydrase binders ranked by their induced differential velocity change (Δv_{diff}). Error bars represent SEM from five independent measurements. **c.** Plotting measured equilibrium dissociation constants (K_d) versus measured Δv_{diff} reveals no clear correlating between affinity and induced conformational change. **d.** Measured association rate constants (k_{on}) plotted against measured Δv_{diff} values. **e.** Measured dissociation rate constants (k_{off}) plotted against measured Δv_{diff} values.

Conclusion

In this work, we established molecular friction sensing as a screening-compatible workflow for measuring relative protein size and ligand-induced conformational changes. Central to this technology is the use of a slender, rigid nanolever constructed by sequence-programmable DNA origami. This DNA origami nanolever overcomes critical limitations of conventional electrically actuated DNA monolayers: (i) it operates robustly at a wide range of experimental conditions, including physiological ionic strengths, rather than being restricted to low-salt buffers; and (ii) its upward trajectory is diffusion-dominated, thereby decoupling nanolever motion from electrode charging and active electrostatic actuation. In this setup, the alternating potential thus merely repeatedly synchronizes the initial (out-of-equilibrium) nanolever orientation, after which diffusion-dominated relaxation is recorded by E-TCSPC. Together, these properties enable the detection and screening of ligand-dependent protein conformational changes with sub-Angstrom sensitivity under biologically relevant conditions.

Despite these advantages, the current implementation comes with inherent limitations. First, proteins with intrinsic DNA binding characteristics or DNA-binding/intercalating small molecules may non-specifically

interact with the nanolever, affecting nanolever velocity and potentially obscuring specific binding-induced friction changes. Second, proteins are conjugated to the distal end of the DNA origami nanolever via a 48-nt oligonucleotide through non-selective amine-directed chemistry (i.e., targeting Lys/N-terminus). This might destabilize sensitive proteins, perturb their native conformation or interfere with ligand binding. Third, while the Stokes-Einstein framework for protein diffusivity and friction assumes rapid tumbling of the protein, tethering of the protein to the nanolever inherently introduces an orientation bias. This makes this type of molecular friction sensing sensitive to effective hydrodynamic radius as well as orientation of the protein relative to the nanolever. Conformational changes that substantially shift the protein's center of mass relative to the attachment site alter the effective length of the nanolever-protein complex and can therefore give rise to nonlinear changes in velocity.

As demonstrated for maltose-binding protein and screening of carbonic anhydrase binding small-molecules, steady state binding affinity as well as kinetic parameters do not necessarily correlate with the small molecule's effect on protein conformation. High-affinity ligands can produce minimal structural rearrangements, whereas weaker binders may elicit pronounced structural responses. This decoupling underscores the value of identifying functional behavior of small molecule-protein interactions at an early stage in the drug development pipeline. Potential applications include allosteric modulators, molecular glues, PROTAC ternary complexes, cryptic pocket stabilizers, kinase activation-state classifiers, and GPCR ligands associated with biased agonism. In these contexts, molecular friction sensing may complement affinity- and kinetics-based assays by helping prioritize compounds according to the structural responses they induce. With a consumption of ~2 pmol protein and a total time-per-datapoint of ~30 minutes per protein-ligand interaction, molecular friction sensing is well suited to refining large libraries of candidate small molecule binders and prioritizing compounds that induce relevant conformational states.

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Author contributions

W.E. and U.R. designed and supervised the research. P.P.P. and W.E. developed the DNA origami nanolever and switchSENSE measurement routines. L.A. designed and implemented hardware in heliX® instrument. P.P.P. and W.E. designed and implemented the analysis pipeline for molecular friction sensing data interpretation. P.P.P., A.K., T.J., V.H., and W.E. obtained and analyzed experimental data. P.P.P., W.E., and U.R. interpreted the data. P.P.P. and W.E. prepared the figures. P.P.P., U.R. and W.E. wrote the manuscript. T.J. critically revised the manuscript. L.A., A.K., and V.H. proofread the manuscript.

Conflict of Interest

P.P.P., L.A., T.J., V.H., U.R. and W.E. are employed by Bruker Dynamic Biosensors – Branch of Bruker Daltonics GmbH & Co. KG which commercializes the heliX® biosensor used in this research. The remaining author(s) declare no competing interests.

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