dynamic
BIOSENSORS

because **structure**

means **function**

switchSENSE®

analysis of **PROTEIN DIAMETERS**
and **CONFORMATIONAL CHANGES**

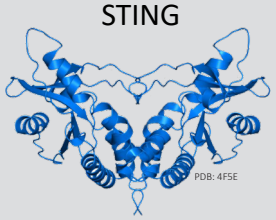
k_{ON} | k_{OFF} | K_D | IC_{50} | D_H | ΔD_H | T_M | ΔG | ΔH | ΔS | k_{CAT} | K_M | U

Structure = Function

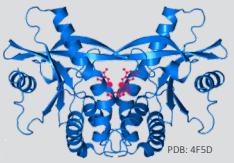
Function, activity, and interactions of proteins are controlled by their three-dimensional structures and the relative arrangement of individual protein domains.

switchSENSE® is the first biosensor that analyzes protein conformation and analyte binding kinetics, in the same measurement, under native conditions, and with minimal sample amounts.

STING



open



closed

switchSENSE® Applications

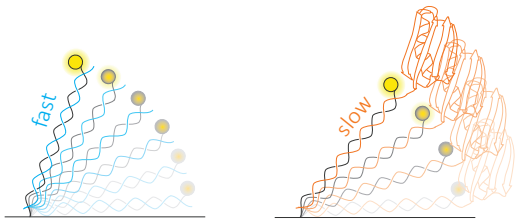
- Conformation screening, hit validation and characterization in drug development.
- Self interaction and aggregation.
- Protein quality and stability.
- Mode-of-action investigations in structural biology, often complementary to NMR and crystallography.

switchSENSE® next generation **biosensor**

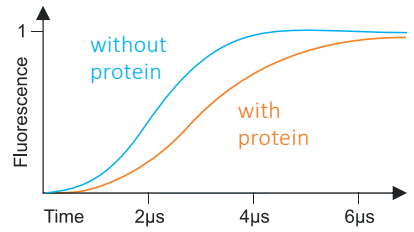
switchSENSE® analyzes proteins attached to DNA nanolevers that are driven to oscillate at high frequencies on a biochip. The nanolever motion is measured with microsecond time resolution by fluorescence energy transfer using patented single-photon counting technology.

The nanolever motion depends on the hydrodynamic drag of the attached protein. Analyzing the nanolever switching speed yields a protein's effective hydrodynamic diameter D_H, in like manner to a light scattering experiment, but with much higher accuracy.

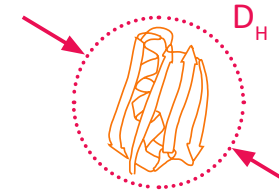
switchSENSE® experiment



Molecular dynamics



Diameter in nm

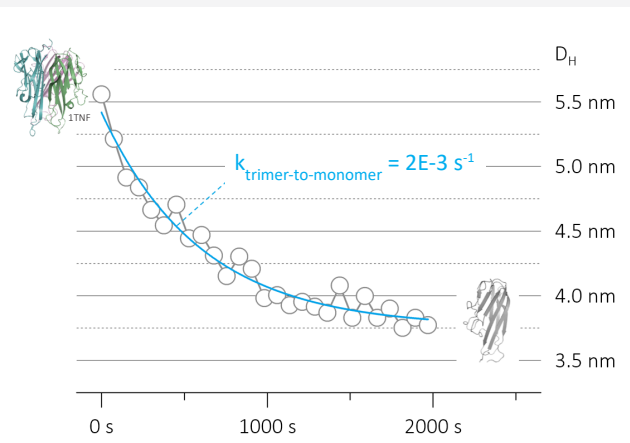


Characterization of **multimerization** and **self-interaction processes**

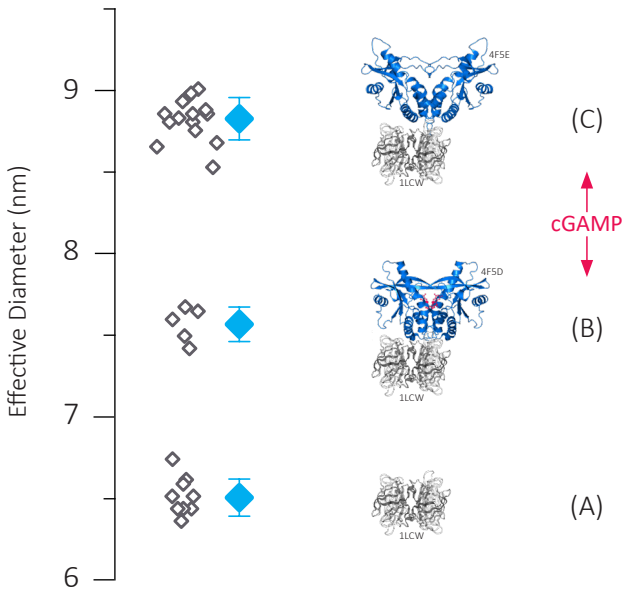
Decay of the TNFα trimer

Tumor necrosis factor α (TNFα) is a cell signaling protein involved in inflammation and the regulation of immune cells. TNFα is a homotrimer, which loses its bioactivity when it decays to a monomer. As a major drug target in inflammatory diseases like rheumatoid arthritis, it is crucial to understand the nature of the interaction of antibody drugs with TNFα trimers vs. TNFα monomers. In turn, this makes it necessary to characterize the multimerization state of TNFα when performing experiments.

RIGHT: Decay of TNFα trimer to monomer, followed in real-time in a switchSENSE sizing measurement. D_H is the average hydrodynamic diameter of app. 100 000 molecules on the sensor surface. TNFα was coupled to the surface-functionalized DNA nanolevers, and solute TNFα was removed from the flow channel at t=0.



Conformational changes, induced by small molecule binding, ions, or post-translational modifications



STING conformational change

STING (stimulator of interferon genes) protein is an important activator of the innate immune response to intracellular pathogens like viruses and bacteria. STING activation involves a conformational change upon binding cyclic dinucleotides, such as cGAMP: the butterfly-like conformation of the STING dimer switches between open and closed conformations upon cGAMP binding.

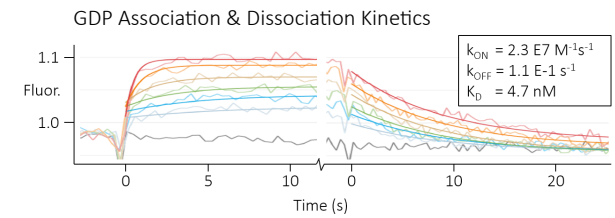
cGAMP-induced closing of the STING protein can clearly be measured by **switch**SENSE® with high reproducibility. Standard deviations of diameter measurements are 0.11 nm.

LEFT: cGAMP mediated conformational changes in STING. Biotinylated dimeric STING was immobilized on the biosensor using streptavidin capture. Small open diamonds show independent measurements, large full diamonds are mean values ± standard deviations. Diameter of (A) streptavidin before STING capture (B) the STING-streptavidin complex after cGAMP addition (C) the STING-streptavidin complex before cGAMP addition

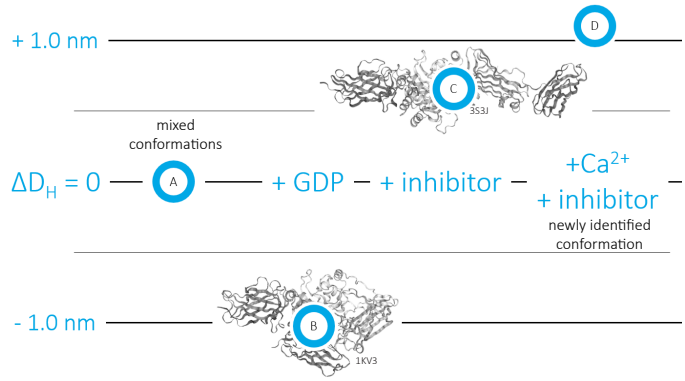
Transglutaminase 2 conformations

TG2 is a multifunctional enzyme, catalyzing the cross-linking of proteins, which is involved in autoimmune and neurodegenerative diseases. Its activity is controlled by distinct conformational changes that depend on GDP and $Ca2^{+}$ binding.

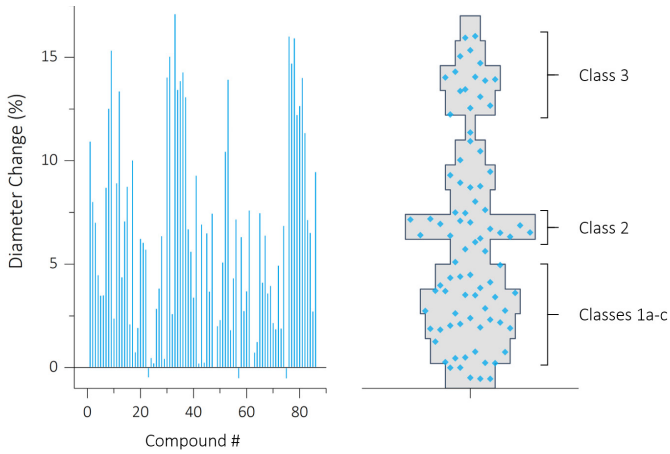
BELOW: Real-time association and dissociation kinetics of GDP binding TG2. Measurements were performed in switchSENSE fluorescence proximity sensing mode, [GDP] = 0, 2.5, 5.0, 10, 20, 40, 80 nM.



BELOW: Transglutaminase 2 (TG2) conformations, observed as changes in the effective protein diameter ΔD_H: (A) initial state of mixed conformations, (B) GDP induced compacted state, (C) inhibitor bound extended conformation (D) newly discovered conformation of inhibitor-bound TG2 with $Ca2^{+}$, which to date could not be crystallized.



Conformation screening in drug development and hit validation



Key to identifying suitable drug candidates is analyzing the mode-of-action of small molecule compound binders in the early stages of the drug discovery pipeline. Therefore, establishing a functional assay setup to measure compound binding kinetics and binding-induced changes in the target protein conformation boost the overall success rate of identifying suitable drug candidates.

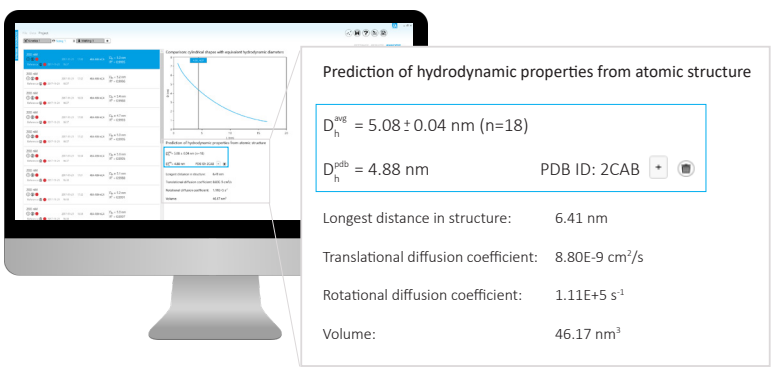
switchSENSE® features a temperature-controlled autosampler for 96 well plates, enabling automated measurement workflows to screen up to 192 compounds per day.

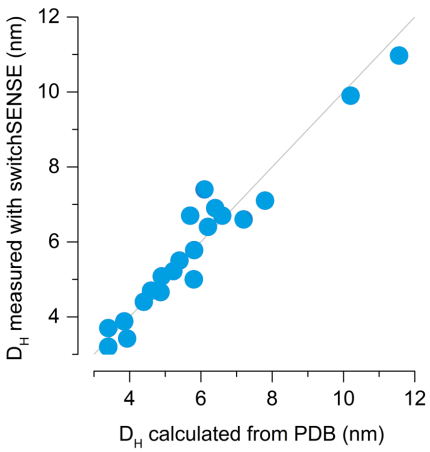
TOP: Conformation screening of a cancer-related target protein against 86 small molecule compounds. Distribution analysis: column scatter plot of measured diameter changes, overlaid by a symmetrical bar plot with binned data. Class 1 binders induced weak diameter changes <5%, Class 2 binders induced intermediate conformational changes, and Class 3 binders induced large changes in the protein conformation, corresponding to more than 10% or 0.8 nm change in the hydrodynamic protein diameter.

Sizing made easy

The **switch**ANALYSIS software compares the measured time-resolved nanolever switching dynamics to a library of solutions for different protein diameters and identifies the protein diameter based on the best match [J. Phys. Chem. B 118:597 (2014)].

Furthermore by simply entering the PDB identifier, the **switch**ANALYSIS software automatically accesses the PDB server and calculates the hydrodynamic protein diameter based on available crystal or NMR structures.





Analyzable Parameters

Conformation changes, induced by small molecules, PTMs, ions

Determination of binding stoichiometry and multimerization state

Binding kinetics, affinity and avidity

Thermal stability and temperature-dependent kinetics

low sample consumption
≥ 0.1 µg protein per measurement

fully automated high throughput
2 x 96 well plates / day

protein diameter accuracy
0.1 nm

switchSENSE® Instruments DRX & DRX²

Dual-color technology for the simultaneous detection of two interactions on the same sensor spot. Reusable biochips. Temperature-controlled chip and samples. Automated liquid handling for 96-well plates and vials.

Limit of detection	10 fM
Dissociation constant	50 fM- 1 mM
Association rate constant	10^3 - 10^8 $M^{-1}s^{-1}$
Dissociation rate constant	10^{-6} - 1 s^{-1}
Hydrodynamic diameter accuracy	0.1 nm
Temperature	8°- 75°C

For further information, please visit our website
www.dynamic-biosensors.com

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