Biophysical Analysis of Cas9-DNA Interactions and Enzymatic Activity with the switchSENSE® Biosensor Platform

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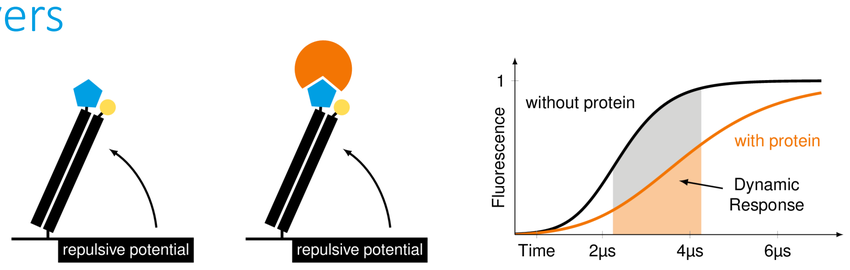
The switchSENSE® Principle: Electro-Switchable DNA Nanolevers

DNA nanolevers are electrically actuated at high-frequency on microelectrodes, while their orientation is monitored by time-resolved single photon counting. The binding of analyte molecules slows the switching dynamics in a characteristic way.

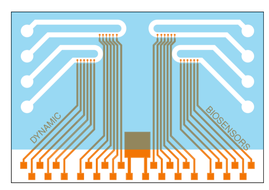
Nature Commun. 4:2099 (2013) | Bioanal. Rev. 4 (2) 97-114 (2012) | JACS 134, 15225 (2012) PNAS 107, 1397 (2010) | JACS 132, 7935 (2010)



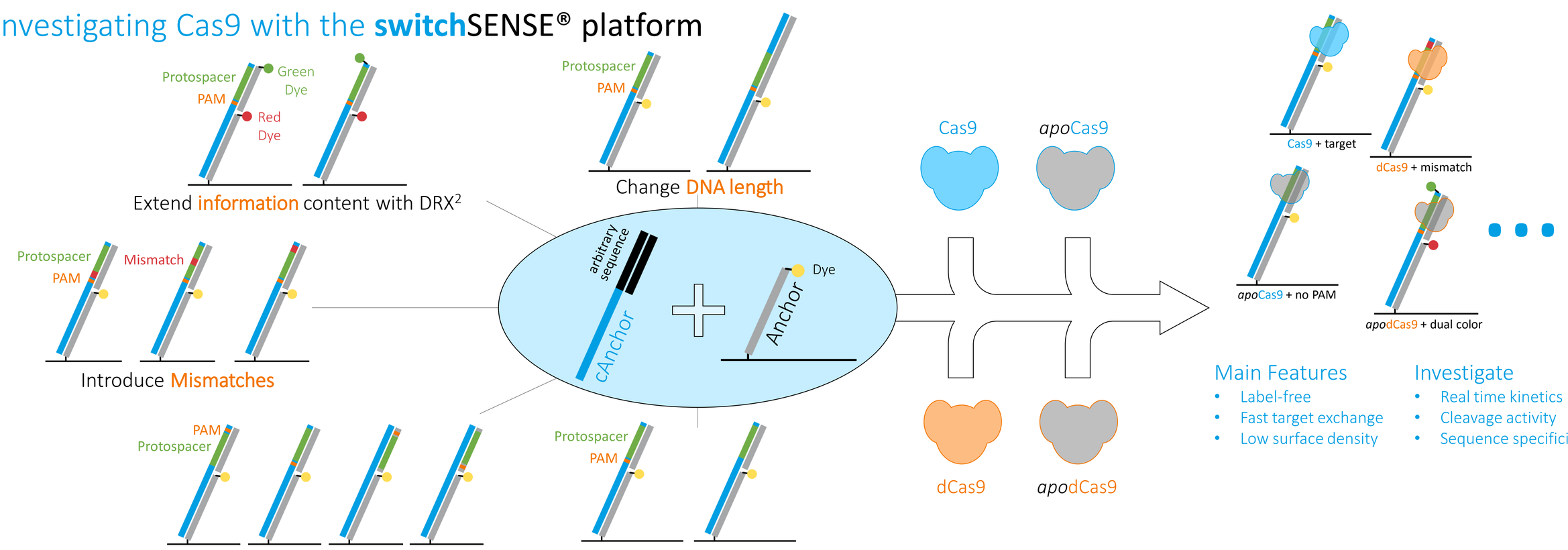
High Sensitivity | Kinetics and Affinity | Size and Conformation | Cooperativity and Avidity | Thermodynamics

DRX & DRX²
Limit of detection 10 fM
Dissociation constant 50 fM – 1 mM
Association rate constant 1E3 – 1E8 /Ms
Dissociation rate constant 1E-6 – 1E0 /s
Hydrodynamic diameter accuracy of 0.1 nm
Temperature 8° – 75°C

switchSENSE® biochip
4 flow channels
20 detection spots á 0.01 mm²
Multiplexing via DNA encoded addressing



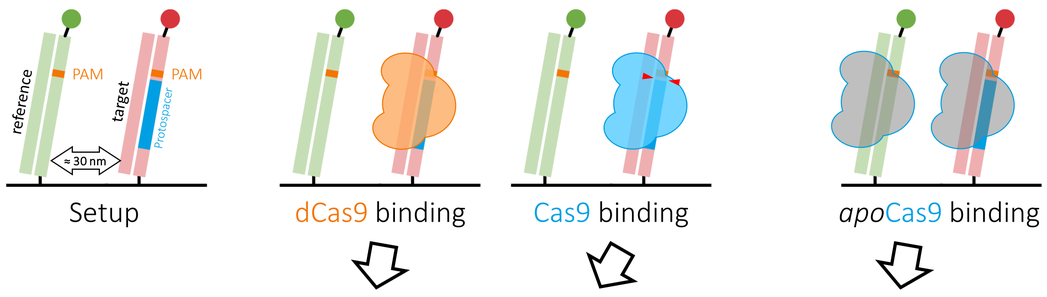
Investigating Cas9 with the switchSENSE® platform

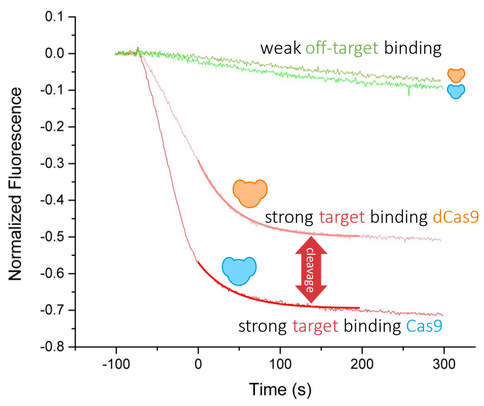


Main Features
- Label-free
- Fast target exchange
- Low surface density

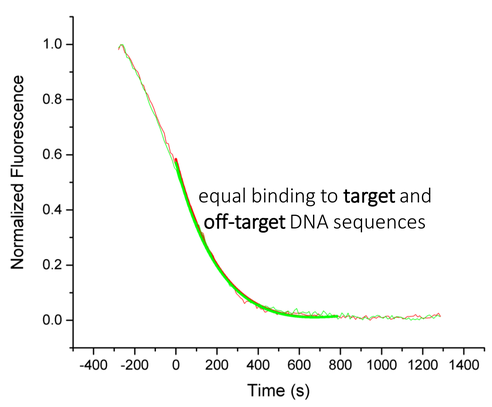
Investigate
- Real time kinetics
- Cleavage activity
- Sequence specificity

dCas9 vs. Cas9 vs. *apo*Cas9 kinetics





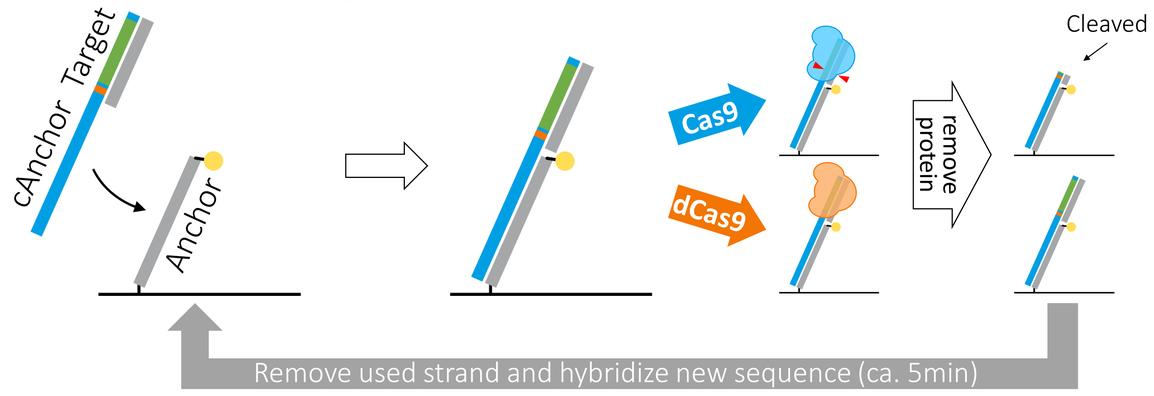
↑ **Binding** curves of dCas9 and Cas9 (c = 20 nM) show **weak off-target binding**. Their binding kinetics are monitored through fluorescent proximity sensing. Cas9 shows stronger fluorescence decrease than dCas9, indicating **DNA cleavage** due to **fluorophore removal**. We find an on-rate for dCas9 and Cas9 of $k_{on} \approx 1 \cdot 10^6$ $M^{-1}s^{-1}$.

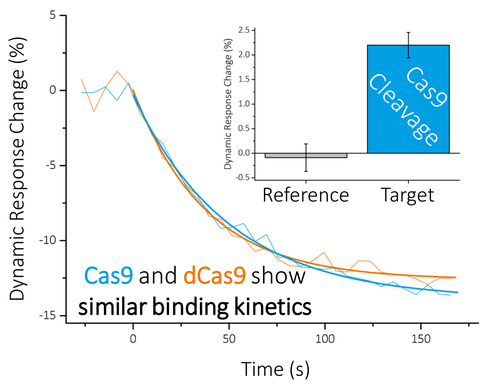


↑ *apo*Cas9 (c= 20 nM) binds DNA **sequence independent** as found by Doudna et al. (2014)[1]. We find **PAM independent** of *apo*Cas9 (data not shown), with an on-rate, k_{on} = **2.11±0.01·10⁵** $M^{-1}s^{-1}$. We hypothesize unspecific binding of the free gRNA binding site of *apo*Cas9 to NAs.

[1]Sternberg, S. H., Redding, S., Jinek, M., Greene, E. C., & Doudna, J. A. (2014) *Nature*, *507*(7490), 62–67. http://doi.org/10.1038/nature13011

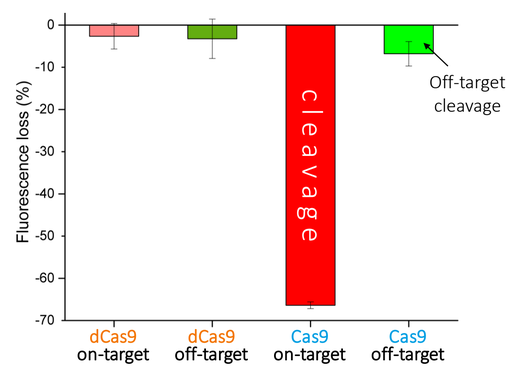
Exchangeable target sequence



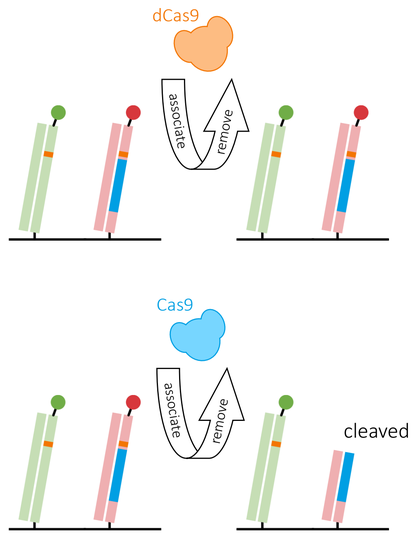


Positioning the target sequence above the fluorophore provides an **easy and fast** way for **exchanging the target sequence**. The binding of Cas9 and dCas9 is very well observable and the found **on-rates agree** with the above presented setup. The use of molecular dynamics measurements allows **detection of the cleavage** of DNA by Cas9. This presents an efficient setup for investigating **sequence dependent interactions** of any kind of nucleic acids with Cas9.

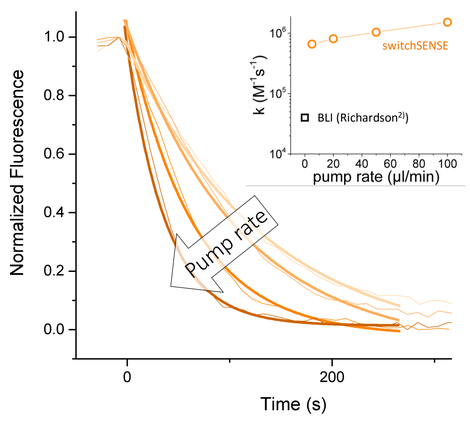
dCas9 vs. Cas9 cleavage activity



↑ **Cleavage efficiency** and **specificity** of dCas9 and Cas9. Comparing fluorescence levels before binding and after removal of dCas9/Cas9 reveals only **cleavage activity** of Cas9. Cas9 drastically reduces the fluorescence of the target strands which directly correlates to DNA cleavage. We also find weak off-target cleavage by Cas9 (**≈5%** of the total cleavage activity).



So far unidentified fast on-rates with switchSENSE®



← **Mass-transport limitation:** Conventional biosensors with high-ligand densities and non-optimal flow conditions are prone to measurement artifacts like mass-transport limitations and rebinding. switchSENSE® reveals **faster on-rates** than previously identified for dCas9/Cas9. High flow rates reveal faster on-rates indicating mass transport limitation. The found on-rate, k_{on} = **1.5·10⁶** $M^{-1}s^{-1}$ is about **50 times faster** than the on-rate found by Richardson et al. through BLI measurements.

[2]Richardson et al. *Nat Biotech* 34 (3) 339 (2016)