Discover what makes your polymerase tick!

Activity Units
U/µL

Hot Start Properties
$T_{activate,}$ $time_{activate}$

Binding Kinetics
k_{ON}, k_{OFF}, K_D

Elongation Rate
k_{CAT}

Exonuclease Activity
k_{EXO}

Michaelis Constant
K_M

Inhibitor Screening
IC_{50}

Thermodynamics
ΔG, ΔH, ΔS

5 min time-to-result

Automated

Label-free

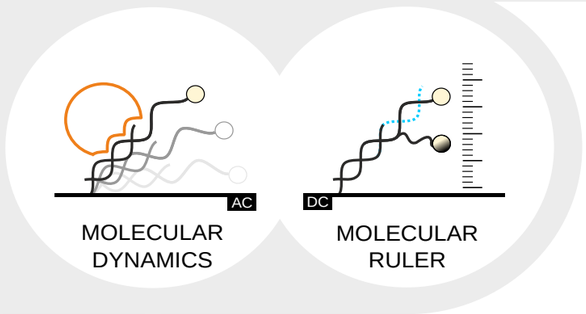
Research

Quality Control

APPROVED

switchSENSE® by DYNAMIC BIOSENSORS

Electro-switchable biochips for the characterization of polymerases: switchSENSE®

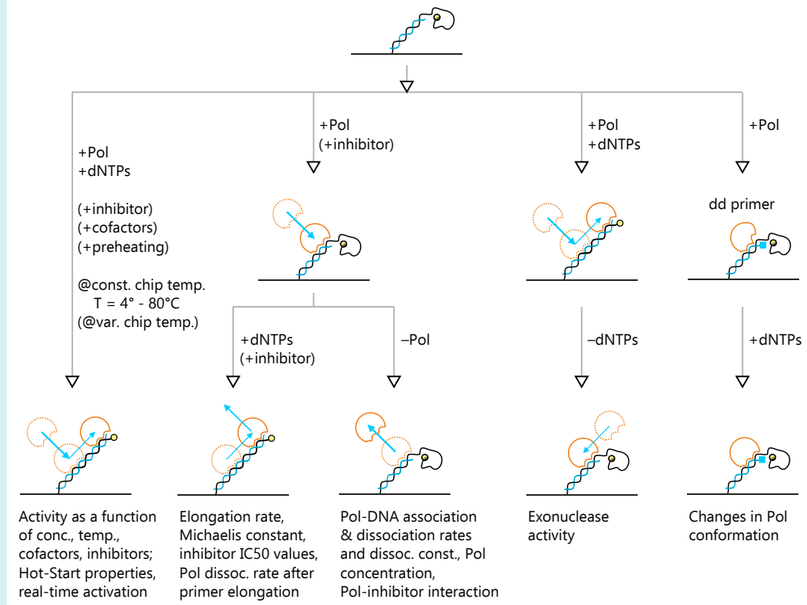


switchSENSE is the first and only chip-based biosensor that employs electro-switchable biosurfaces (ESB) for the biophysical analysis of molecular interactions. Two signals are analyzed simultaneously to follow interactions between proteins and nucleic acids and to monitor polymerisation or nuclease activity: Single stranded oligonucleotides are mounted on gold microelectrodes and are modified with fluorescent dyes at their top ends. The fluorophores report the height of the probes above the surface, because their emission is gradually quenched by the microelectrodes via non-radiative energy transfer when the dyes approach the gold surface.
This molecular ruler is used to gauge the extension of DNA/RNA primers in real-time during polymerisation or nuclease activity. Repulsive potentials that are applied to the electrodes ensure that the nucleic acids are aligned upright during enzymatic activity and quantitative signals are obtained.

A second, dynamic measurement mode is used to monitor the binding of proteins to nucleic acids. High frequency alternating potentials induce an oscillation of the nucleic acid nanolevers by repelling them from and attracting them to the microelectrodes 10 000 times per second. Again, the fluorescence signal is analyzed, but this time to follow the switching dynamics of the nanolevers on a sub-microsecond timescale. When proteins bind to the nucleic acid probes, hydrodynamic friction increases and the switching speed slows down. Hence, association and dissociation kinetics, affinities, the number of bound enzymes and even the location of the enzyme along the nucleic acid strand can be analyzed by monitoring the switching speed in real-time.

What would you like to analyze today?

Choose from a number of different workflows to quantitate the parameter of interest - or run multiple workflows for comprehensive characterization.



Workflow

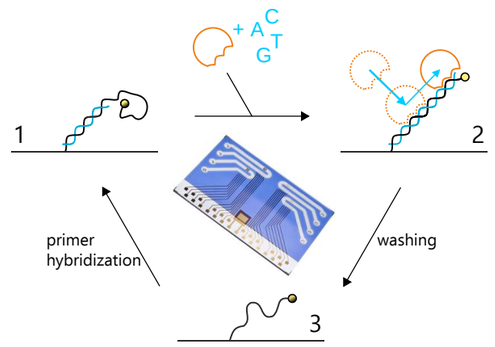
load samples into liquid handler

start program

auto-workflow:
- chip regeneration
- sample dilution & mixing
- sample injection 1
- measurement 1
- sample injection 2
- ...

analyze data with switchANALYSIS

Automatic chip regeneration

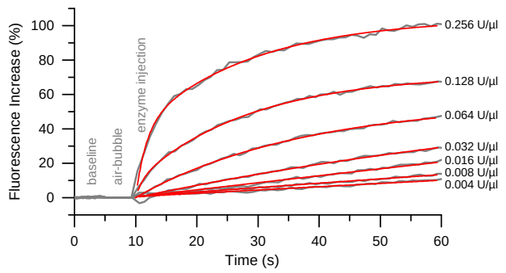


1 - 2: real-time monitoring of primer elongation during injection of polymerase and dNTPs;

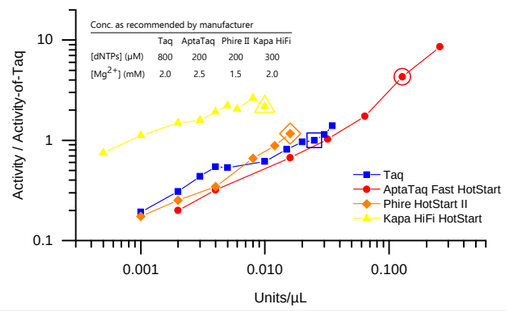
2 - 3: removal of elongated primer and residual enzyme by NaOH denaturation within seconds;

3 - 1: hybridization of fresh primer to surface-tethered single strand within 1 minute.

Activity quantitation in a minute



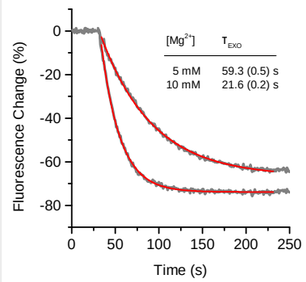
[Left] Real-time elongation assay of the Hot-Start polymerase AptaTaqFast (Roche) using the "molecular ruler" measurement mode (cf. top left). 5 s baseline measurement in running buffer, 5 s air bubble (for liquid plug separation), injection of enzyme/dNTP mix at t=10 s. Lines are exponential fits from which the enzymatic activity is analyzed.
switchSENSE chip 36/54, [dNTPs]=200 µM, [Mg^{2+}]=2.5 mM, T=65°C



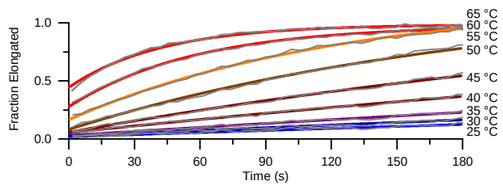
[Right] Comparison of the activity of three Hot-Start polymerases, Kapa HiFi (KAPA), Phire II (Thermo), AptaTaqFast (Roche) relative to Taq (Peqlab/VWR). Large open symbols indicate the enzyme concentrations recommended by the manufacturers for PCR applications. dNTP and Mg^{2+} concentrations were used according to manufacturer recommendation.
switchSENSE chip 36/54, T=65°C

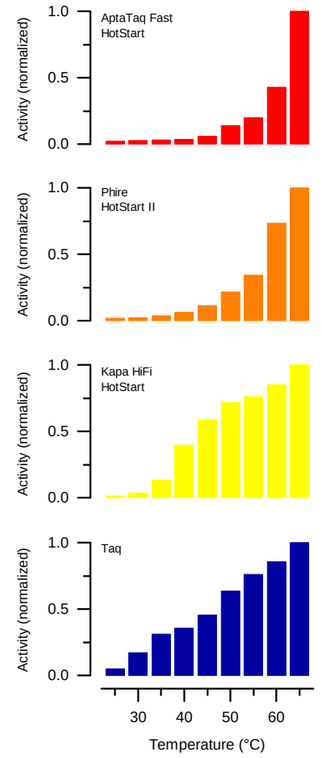
Exonuclease

Real-time measurement of T5 exonuclease (NEB) activity for different concentrations of Mg-ions. Red lines are single exponential fits from which the nucleotide removal time is analyzed. A standard switchSENSE chip (S2) with 48 bp DNA was used.



Hot-Start properties

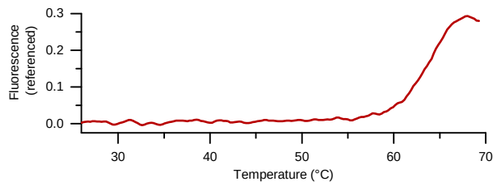




[Above] Temperature-dependent real-time elongation measurements of Phire HotStart II (Thermo). The chip temperature was adjusted prior to the enzyme injection and kept constant during the measurement. Gray lines are data, colored lines are exponential fits from which the elongation rate is analyzed.

[Right] Relative enzymatic activities of selected polymerases, analyzed from real-time elongation measurements at constant chip temperature. Values are normalized to activities at 65°C.

[Below] Real-time activation measurement in a temperature gradient. The enzyme/dNTP mix (AptaTaqFast, Roche) was injected into the flow channel at 25°C, afterward the chip temperature was ramped up at 10°C/min. The fluorescence increase at high temperatures indicates the onset of elongation. Total measurement time was 5 min.

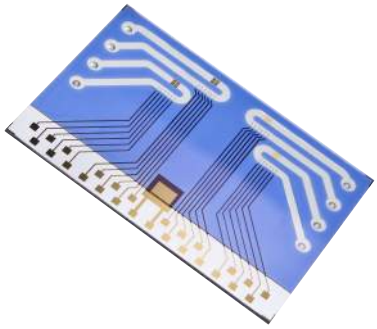


Comparison of interaction and enzymatic parameters analyzed with the switchSENSE biosensor and conventional chemical quench assays in solution using radioactive labels

		switchSENSE [i]	radioactive solution assay [ii]
Association rate Pol+DNA → P·DNA	k_{ON}	1.8 ± 0.2 × 10^7 $M^{-1}s^{-1}$	1.2 × 10^7 $M^{-1}s^{-1}$
Dissociation rate Pol·DNA → P + DNA	k_{OFF}	0.061 ± 0.005 s^{-1}	0.06 s^{-1}
Dissociation constant Pol ↔ DNA	K_D	3.2 ± 0.6 nM	5 - 8 nM
Dissociation constant after primer ext.	K_D	11.2 ± 1.5 µM	6 - 17 µM
Michaelis constant (primer extension)	K_M	0.58 ± 0.20 µM	0.41 ± 0.17 µM
Primer elongation rate DNA_n → DNA_{n+1}	k_{CAT}	2.0 s^{-1}	2.4 s^{-1}

(i) Sci. Rep. 5:12066 (2015)
(ii) Biochem. 26:8410 (1987), J. Biol. Chem. 267:8417 (1992), Nuc. Ac. Res. 37:3924 (2009)

Read more on the characterization of polymerases with switchSENSE:
Scientific Reports 5:12066 (2015), open access at
www.nature.com\srep\2015\150715\srep12066\full\srep12066.html



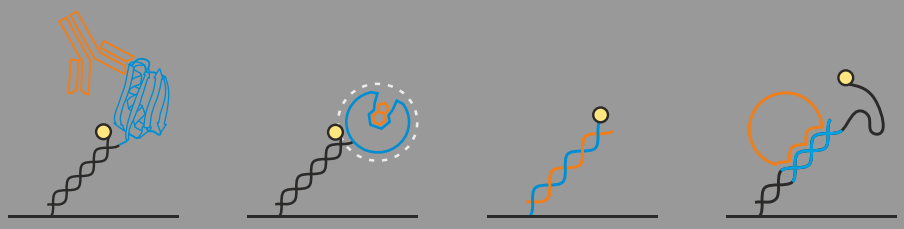
switchSENSE 4x6 chip
- 24 microelectrodes
- 4 integrated flow channels
- DNA encoded multiplexing

DRX switchSENSE analyzer
- 3 measurement modes
  - molecular switching dynamics
  - fluorescence proximity assay
  - molecular ruler
- 4° - 80°C
- integrated liquid handler
  96 well plate / 48 vials

Order Information

switchSENSE Analyzer Instrument DRX	A-01-24-DRX
Polymerase Activity Chip 54/36	C-P1-S-1
Polymerase Activity Chip 80/20	C-P2-S-1
RNA Research Chip	C-RB2-S-1
DNA Research Chip	C-DB2-S-1
Custom Chip	C-X

switchSENSE® proteins | nucleic acids | small molecules



Molecular interaction analysis and biophysical characterization with electro-switchable biosurfaces.

- Kinetics
- Affinity
- Hydrodynamic diameter
- Stoichiometry
- Conformational changes
- Enzymatic activity
- Melting analysis
- Thermodynamic energies

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