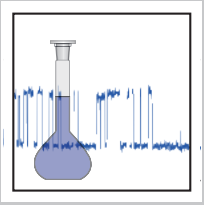
doi:10.2533/chimia.2021.210 Chimia 75 (2021) 210 © Swiss Chemical Society



Highlights of Analytical Sciences in Switzerland

Division of Analytical Sciences

A Division of the Swiss Chemical Society

Single-Molecule Analytics of Reaction Products of Guanine Oxidation in Oligonucleotides

Jens Sobek*and Ralph Schlapbach

*Correspondence: Dr. J. Sobek, E-mail: jens.sobek@fgcz.ethz.ch, Functional Genomics Center Zurich, ETH Zurich/University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

Keywords: CY3 · Dyes · Electron transfer · Fluorescence · Guanine · Oligonucleotides · Oxidation · Single molecules

We are using a modified RSII+ DNA sequencer to study molecular interactions and chemical reactions at the single-molecule level. Molecules are immobilised on a chip containing 150'000 nanostructures of about 150 nm in diameter and well depth, respectively. The small dimensions create a zero-mode waveguide through which light cannot pass but becomes evanescent. For this reason, only fluorescent molecules near the surface can be excited and thus perfectly discriminated from bulk solution.[1] Typically, some 10'000 single molecules can be monitored in parallel with a time resolution of 13 ms resulting in a large number of single-molecule fluorescence traces to give an excellent statistics for determination of binding kinetics.

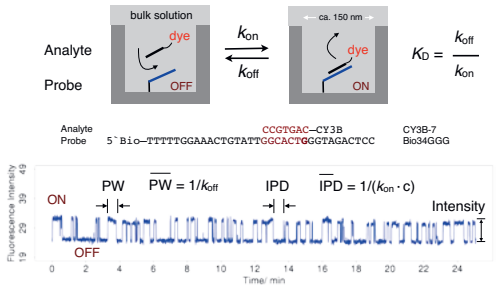


Fig. 1. Experimental setup and a resulting pulse sequence.

We investigated the hybridisation of immobilised probes (7–93 nucleotides, nt) with short oligonucleotides labelled with CY3 dye derivatives whose fluorescence is very sensitive to the stacking base pair.[2,3] Hybridisation reveals a sequence of pulses which can be monitored over a long period. The pulse sequence creates a pattern characterised by pulse width (PW), interpulse duration (IPD), and fluorescence intensity, from which kinetic constants can be calculated.[2] In many fluorescence traces, pulse pattern changes were observed that indicate chemical reactions take place at the immobilised probe which are initiated by a photoinduced electron transfer from a guanine (G) donor to the dye (Fig. 2).[4] The G radical cation can react with water, oxygen, and reactive oxygen species, respectively, leading to a large number of oxidation products, of which we so far identified 8-oxoG, and products of secondary reactions. Product formation was confirmed by SPR measurements using oligonucleotides modified with the respective oxidation product of G, and static fluorescence spectra since CY3 fluorescence strongly depends on the local environment, *i.e.* the stacking base pair.[3]

After hybridisation to a cyclic 93 nt oligonucleotide, the RSII+ allows the chemically converted molecules to be sequenced. The nature of lesions can be determined by the delay of polymerase activity (IPD ratio) that is specific for a base modification.[5]

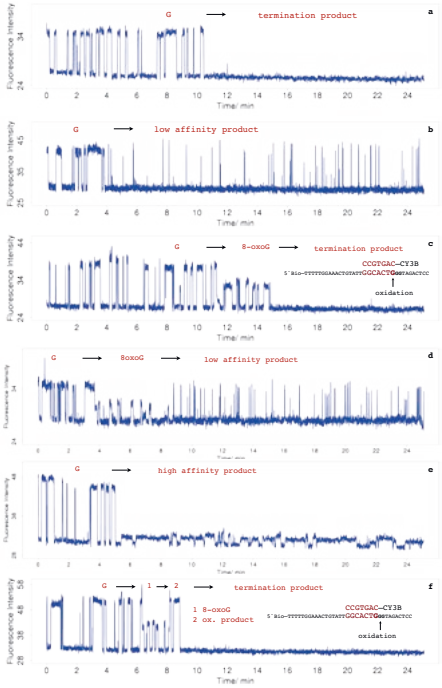


Fig. 2. Hybridisation of a 34 nt probe with CY3B-7 in the presence of oxygen. a) Formation of a product characterised by a loss of affinity (termination), b) formation of a low affinity product (short pulses), c) formation of short-lived 8-oxoG followed by termination, d) formation of 8-oxoG followed by creation of a low affinity product, e) formation of a product that is strongly quenching CY3 fluorescence, f) formation of 8-oxoG in the guanine *adjacent* to the hybrid region, further oxidation does not influence hybridisation kinetics. c) and d) feature different reaction pathways of 8-oxoG oxidation.

Monitoring the fate of single molecules over a long period enables us to follow sequences of chemical reactions, to analyse reaction pathways, to detect short-lived intermediates, and to determine product yields.

Received: January 20, 2021

[1] J. Korlach *et al.*, *Methods Enzymol.* **2010**, *472*, 431.
[2] J. Sobek, H. Rehrauer, S. Schauer, D. Fischer, A. Patrignani, S. Landgraf, J. Korlach, R. Schlapbach, *Methods Appl. Fluoresc.* **2016**, *4*, 015002.
[3] J. Sobek, R. Schlapbach, *Molecules* **2020**, *25*, 5369.
[4] J. Sobek, M. Schmidt, J. Grossmann, H. Rehrauer, L. Schmidt, R. Schlapbach, *Methods Appl. Fluoresc.* **2020**, *8*, 035010.
[5] J. Korlach, S. Turner, *Curr. Opin. Struct. Biol.* **2012**, *3*, 251.

Can you show us your analytical highlight?
Please contact: Dr. Veronika R. Meyer, Unterstrasse 58, CH-9000 St. Gallen
Tel.: +41 71 222 16 81, E-mail: VRMeyer@bluewin.ch