Specific DNA Duplex Formation at an Artificial Lipid Bilayer

Ionovation Application Note 08

Introduction

The analysis of genes or gene segments is currently based on DNA chips. These chips or DNA microarrays are made up of a solid carrier (usually a glass object plate) on which single-stranded DNA molecules with a known sequence are attached in a regular and dense pattern.
Here, we present an alternative to the DNA chip technology which renounces any chemistry on solid supports by using duplex formation of target DNA with lipid-oligonucleotide conjugates (nucleolipids) at a lipid-bilayer-water interface.

This method bypasses numerous issues that come with traditional chip technologies. In particular, these are:

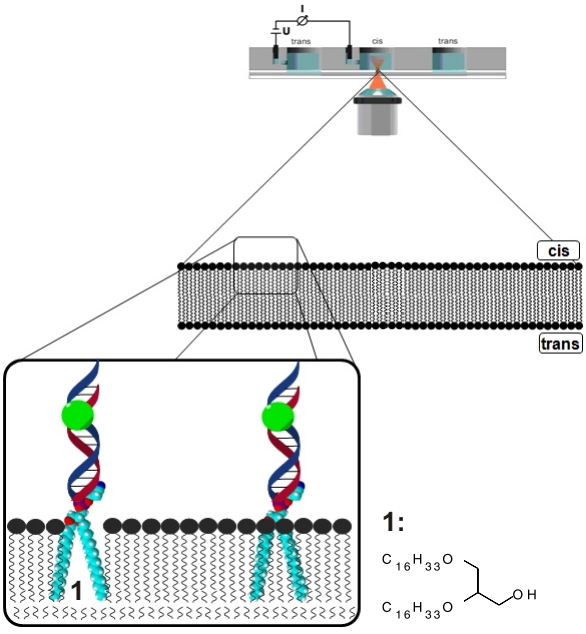
- synthesis of oligonucleotides directly on the carrier
- comprehensive amount of deprotection reactions and washing steps
- activation of a glass plate with functional groups in a complicated manner (spotting)
- chemical reaction between the ready-made functionalised nucleic acids and the activated functional groups on the surface of the array in order to achieve a covalent bond between the array and the nucleic acid

In order to test for specific DNA duplex formation, modified oligonucleotides with 5'-hexadecyl side chains were synthesized. These nucleolipids were incorporated into artificial lipid bilayers.

Three oligonucleotide constructs were generated:

- Two different probe nucleolipids carrying both the double-tailed lipid at the 5'-terminus,
  P1: 5'-d(**1**-p-TAG GTC AAT ACT)-3' and
  P2: 5'-d(**1**-p-ATC CAG TTA TGA)-3'
- the target oligonucleotide 5'-d-AGT ATT GAC CTA-3'

The sequence of P1 is complementary to the target sequence in an antiparallel strand orientation, whereas the sequence of P2 is the same.
P1 or P2 were incorporated into a lipid bilayer, which was generated automatically using an **Ionovation Explorer**.
After addition of target oligonucleotides and perfusion, DNA duplex formation was confirmed by a SybrGreen fluorescence assay as described in Werz et al.*



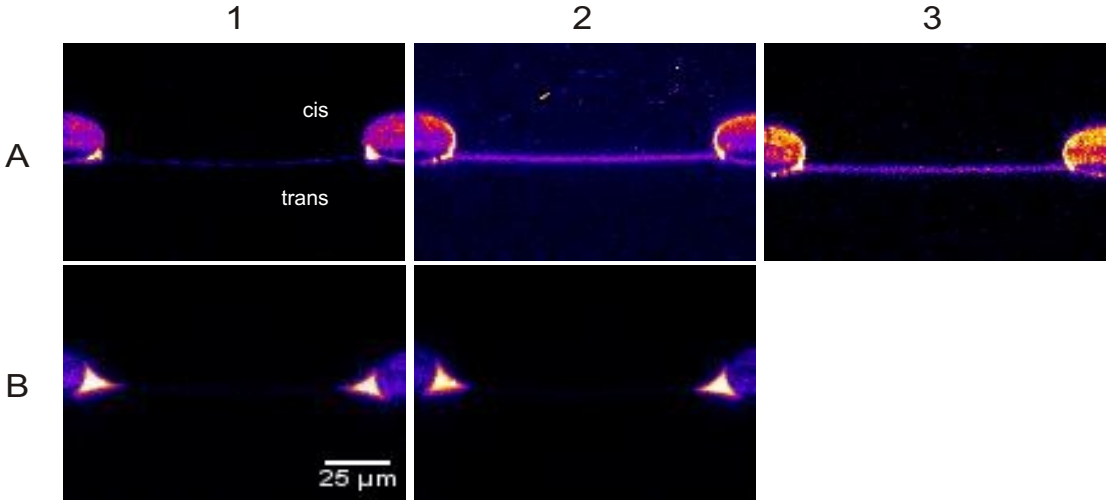
Schematic drawing of the lipid bilayer with incorporated double-tailed nucleolipids and the oligonucleotide target with incorporated SybrGreen.

Ionovation GmbH • Westerbreite 7 • 49084 Osnabrück, Germany Phone +49 (541) 9778-660 • Fax +49 (541) 9778-666
www.ionovation.com • contact@ionovation.com

Specific DNA Duplex Formation at an Artificial Lipid Bilayer

Fluorescence intensity and diffusion time results

Images perpendicular to the lipid bilayer plane (z-scans) clearly show that the target oligonucleotides specifically bind to the incorporated complementary strands but neither to the non-matching strand nor unspecifically to the bilayer.



z-scans of a lipid bilayer
1) after the addition of the nucleolipids to the cis compartment, followed by an incubation for 30 min and perfusion, **2)** after the addition of the oligonucleotide and SybrGreen to the cis compartment,
3) after perfusion of the cis compartment
In row **A** the sequence of the oligonucleotide conjugated to the lipid anchor was complementary to the added oligonucleotide, in row **B** the sequences were identical.

Diffusion time measurements (τ_D) using fluorescence correlation spectroscopy (FCS) show the immobilisation of target oligonucleotides due to specific binding to membrane-integrated probe nucleolipids.

τ_D in solution without bilayer **[ms]**	0,19 ± 0,01	
τ_D in the bilayer **[ms]**	**after perfusion**	
	1.	**2.**
	1,30 ± 0,28	1,56 ± 0,22

Conclusion

- The experiments confirm that the nucleolipids are immobilized at the bilayer and that specific base pairing at a lipid bilayer – water interface occurs.
- The DNA duplex formation is resistant to perfusion.
- The **Ionovation Explorer** is a powerful tool enabling this new DNA analysing technique and offers even more options when used on a laser scanning microscope.
- The new technique is a promising tool for simplified detection of oligonucleotides in biological samples.

* Werz et al., Chem Biodivers 2012, 9: 272-281, Specific DNA Duplex Formation at an Artificial Lipid Bilayer: Towards a New DNA Biosensor Technology

Ionovation GmbH • Westerbreite 7 • 49084 Osnabrück, Germany Phone +49 (541) 9778-660 • Fax +49 (541) 9778-666
www.ionovation.com • contact@ionovation.com

Ionovation Application Note 08