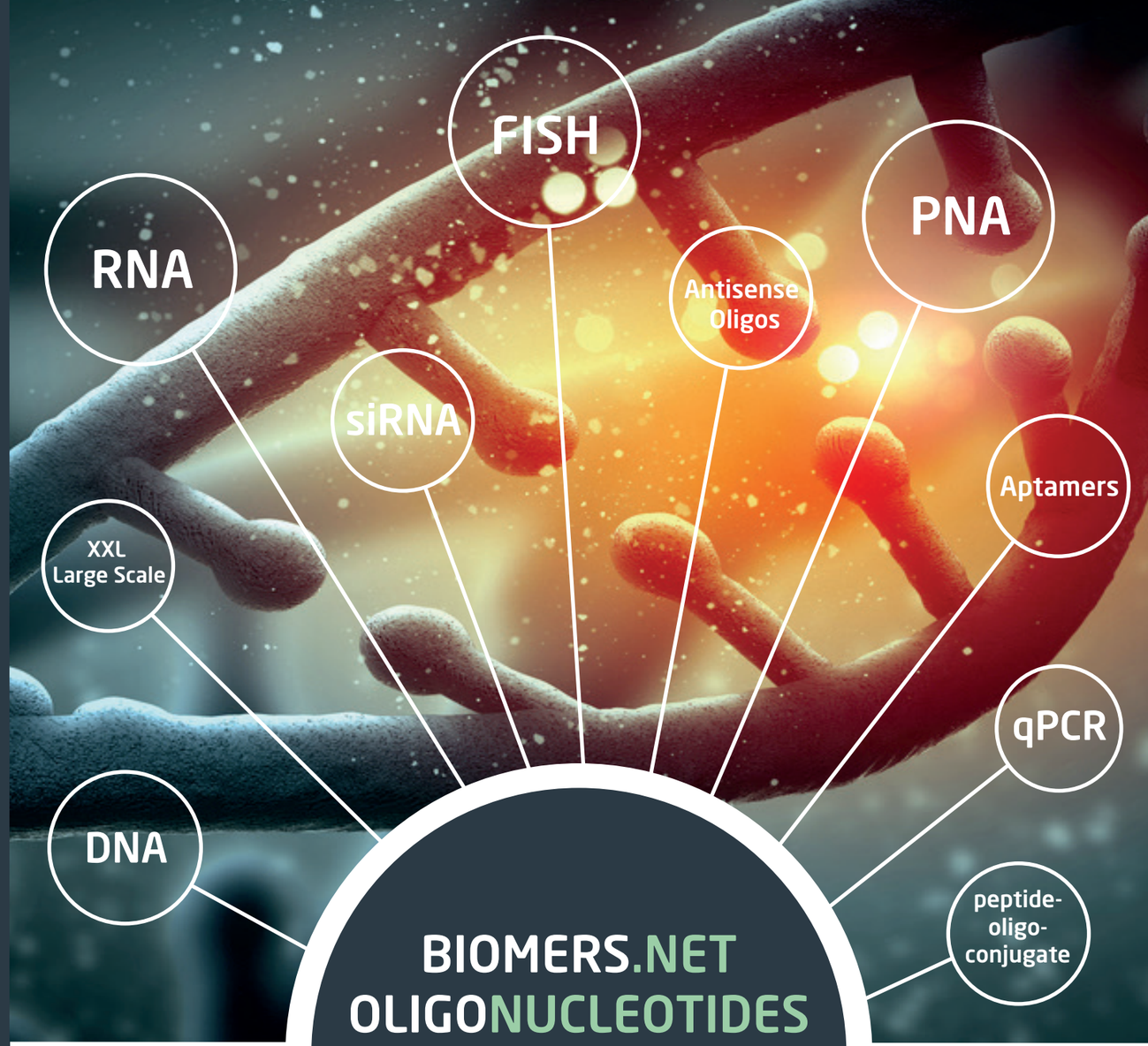

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Reactive linkers: Aminolink

An aminolink adds a terminal amino group (NH2) to an oligonucleotide and can be used to couple additional molecules. The aminolink is used as well to bind oligos to adequately prepared surfaces. Furthermore, if attached at the 3´-terminus, the oligonucleotide is more resistant against exonuclease digestion.
Sometimes it can be useful to link an amino group inside the oligonucleotide. For this purpose, a thymidine nucleotide's C5 methyl group can be easily replaced by a C6 linker with an amino group at its end. Thus the interaction between the amino group and the DNA is reduced as far as possible so that the modified oligonucleotide behaves in hybridisation comparable to a respective unmodified one.

Redox-reactive reporters - Ferrocene

Oligonucleotide-based electrochemical biosensors, so-called E-sensors, are based on the interaction between a target molecule in solution, a respective target-binding probe, which is generally an oligo, and a solid electrode surface.

The oligonucleotide is labelled at one end with a redox modification and linked via the other terminus to a conductive surface (e.g. electrode). The attachment of the redox-active oligo probes to conductive surfaces such as gold electrodes is achieved via a terminal thiol or thioctic acid modification.

In the absence of a complementary target strand, redox-active oligo probes take a specific conformation with a defined distance between probe and electrode.
The hybridisation of a complementary target molecule to the surface-bound oligo leads to a change in conformation, thus influencing the electron transfer between the redox unit and the electrode. This potential change can be detected by suitable measuring methods.

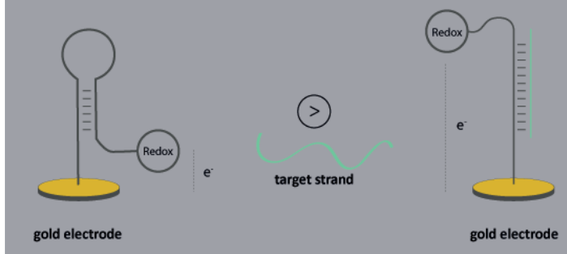
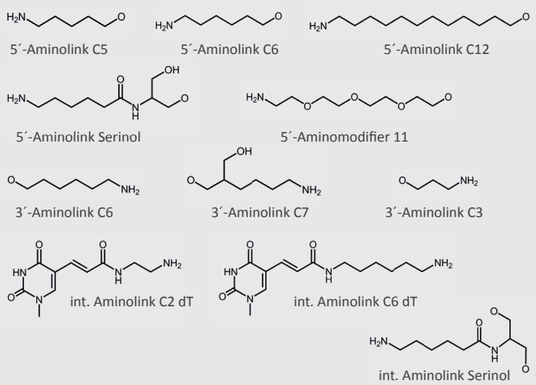
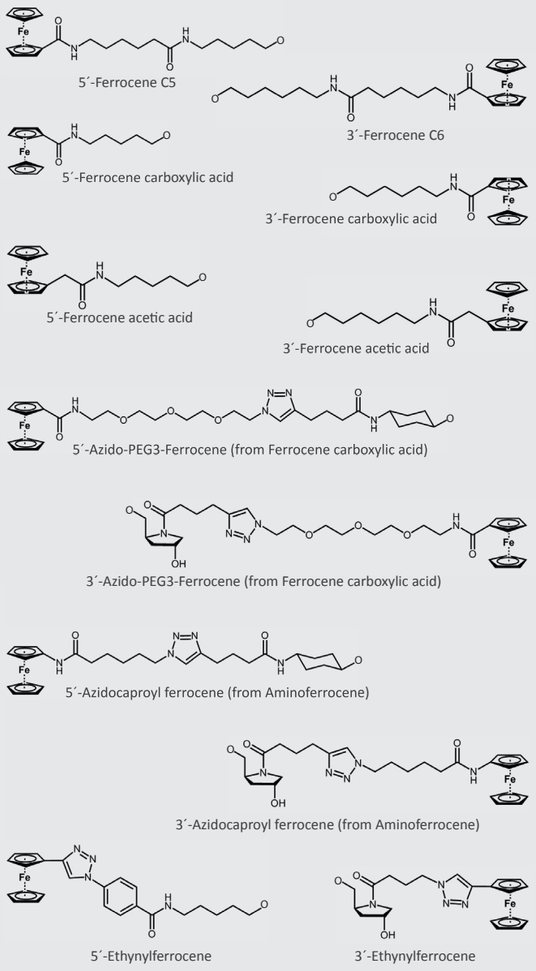


Figure 1: Conformational change of an oligo probe with redox modification before and after hybridisation of a complementary target strand. The change in electrode potential can be finally detected.

By forming an amide bond, the amino group (NH_2) can bind further molecules (dyes, proteins, etc.). Linkers of variable length are available for use in different applications. Aminolinks can be coupled to both the 5´- and the 3´-end of the oligonucleotide and internally.



For effective detection, several redox-active ferrocene reporters with different linkers are available:



Reactive linkers: Hydrazide-Aldehyde

An efficient way to link oligos to other biomolecules is the hydrazide-aldehyde coupling. Hydrazide, as well as hydrazine or aminooxy compounds (e.g. aminooxyacetic acid, AOA) are well suited reaction partners for aldehydes. Aldehydes represent highly reacted carbonyl compounds that can react with nucleophiles like amino groups or thiol compounds.

Aldehyde-reaction partners:

- Hydrazide
- Hydrazine
- Aminooxy compounds (e.g. aminooxyacetic acid)

Distearoyl Lipid

Fatty acids like the distearoyl lipid with its two long fatty acid tails can be attached to oligonucleotides in order to impart an amphiphilic character to the conjugate. This allows conjugates to be bound to lipid phases, bilayers or vesicles with the oligo-moiety being presented to the aqueous phase while the fatty acid part „dips“ into the lipid phase.

Different fatty acids are available with biomers.net:

- Distearoyl lipid
- Palmitate
- Stearate

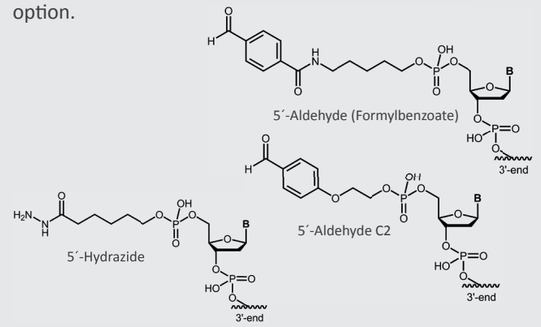
SiR fluorescent dye

The fluorescent dye silicon-rhodamine (SiR) which is structurally related to the rhodamine fluorophores (e.g. Texas Red), is a relatively small and bright fluorescent dye with a spectral range in the far-red fluorescent spectrum.

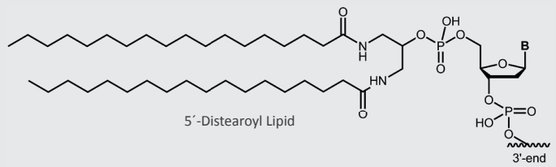
ABS 652 nm
EM 674 nm

The red-shifted excitation wavelength can minimise phototoxicity and because of its excellent photostability SiR is particularly suited for high-resolution microscopy (e.g. STORM, STED) in living cells.
Due to the long wavelength of red excitation, also a higher penetration depth and thus a more intensive imaging could be observed.

Hydrazide modified oligonucleotides are able to react with aldehydes under neutral or slightly acidic conditions to form stable hydrazone conjugates in a fast and efficient way. Thus, a conjugation of oligonucleotides to other (bio)molecules can be achieved easily under physiological conditions.
Hydrazides react with N-hydroxy succinimidyl esters at neutral pH in contrast to standard amino functions (e.g. 5‘-Aminolink). Especially for reactions with alkaline labile molecules, this coupling strategy is an interesting option.



Distearoyl lipid-oligonucleotide conjugates consist of a negatively charged DNA and two diamine-linked long hydrocarbon chains (stearic acid). Thus, they are relatively similar to a natural phospholipid structure and can form micelles.
Distearoyl lipids interact with bilayers and can facilitate anchoring of oligonucleotides on the cell surface.



In contrast to other fluorescent dyes in this spectral range, the dye molecule SiR shows high membrane permeability in living cells, so that the use of fixed cells or the permeabilisation of cells, which is always associated with disruption of the cellular environment, can be dispensed with.
SiR is compatible with most microscopes as it can be used with standard Cyanine 5 filter settings.