R&D funding:

Quality Management

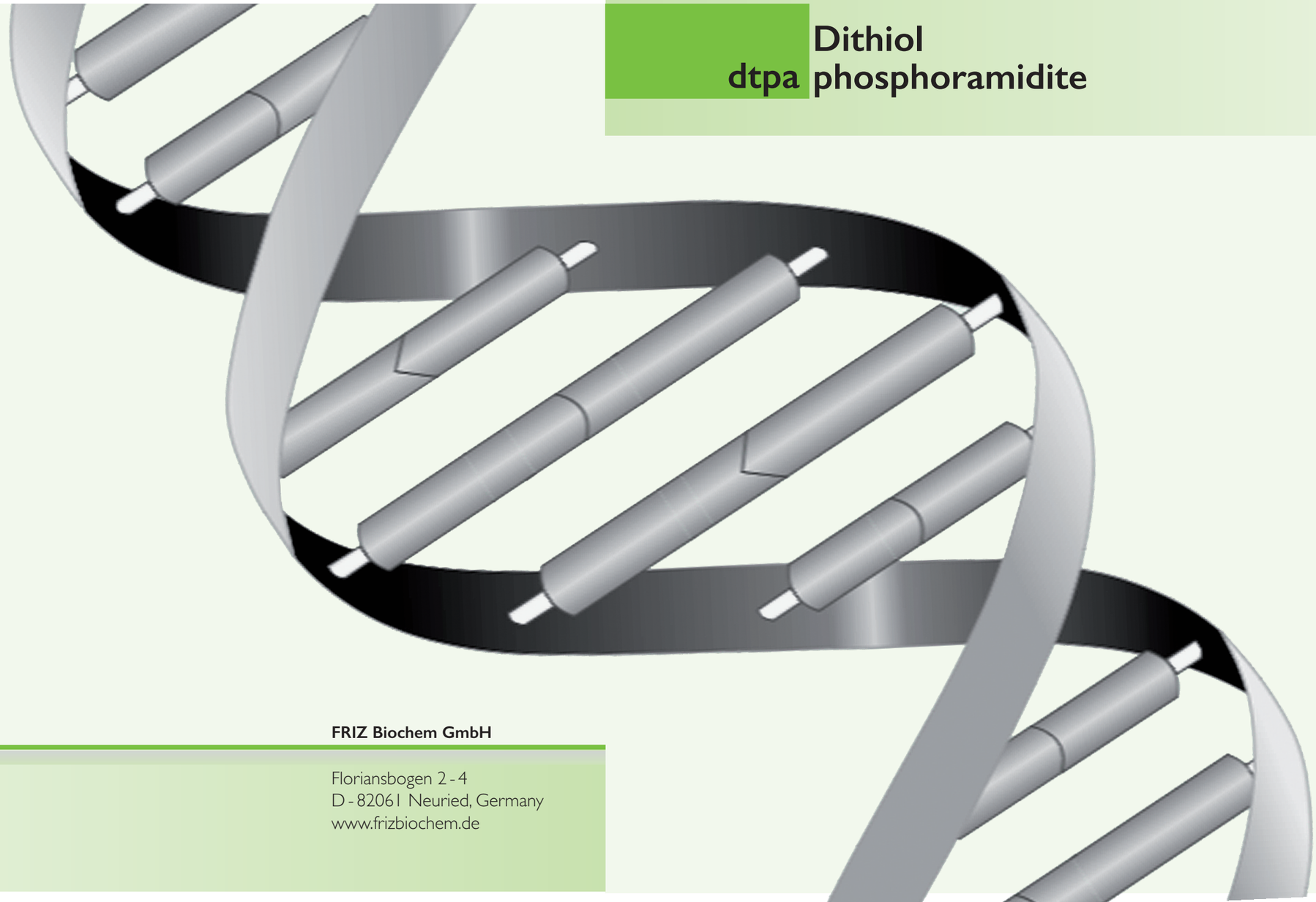
DTPA_02.pdf

molecular CYCLE® diagnostics

dna Custom oligonucleotides

dtpa Dithiol phosphoramidite

functional Nano particles



dtpa Dithiol phosphoramidite

FRIZ Biochem GmbH

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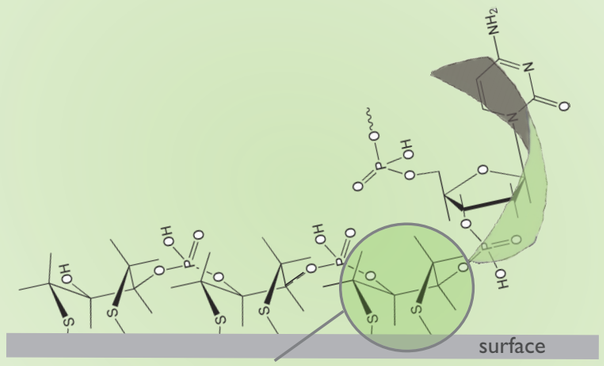
FRIZ Biochem GmbH, founded in 2004, is a privately held biotech company located in Neuried near Munich. Our team, focused on advanced electronic biochips and molecular diagnostics, is knowledge driven and dedicated to provide superior solutions. Direct electrical detection of molecular recognition processes forced us to establish a proprietary anchoring of molecular probes on metal surfaces via DTPA (dithiolphosphoramidite, US7601848, EP1626952), a cyclic disulfide.

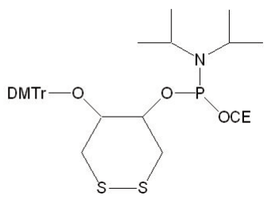
Poor stability of the monothiol-to-gold bonds is a major issue for chemical sensors based on thiol-capped gold nanoparticle (Au-NP), silver nanoparticle (Ag-NP) or planar gold or silver surfaces. Polythiol anchoring, however, results in an unrivalled stability of the derivatised surface.



DTPA

Technical information

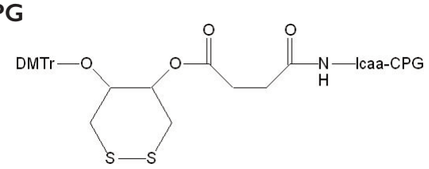
DTPA



1,2-Diathiane-4-O-dimethoxytrityl 5-[(2-cyano-ethyl)-N, N-diisopropyl)]-phosphoramidite

Diluent: Acetonitrile ≤ 10 ppm water
Coupling: A prolonged coupling time (6 min) is recommended. To avoid oxidative cleavage of the disulfide linkage, all oxidation steps should use 0.02 M Iodine solution.
Deprotection: no changes needed from standard method recommended by synthesizer manufacturer for the other nucleobases used in the oligonucleotide.
Storage: freeze at -10 to -30 °C, dry.
Stability: 0.1 M solution on synthesizer: approx. 24 h.

DTPA-CPG



1,2-Diathiane-4-O-dimethoxytrityl-5-succinoyl-long-chain-aminoalkyl-CPG

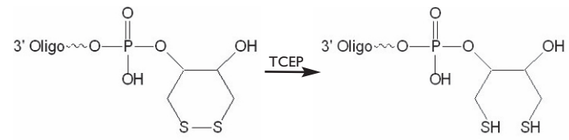
Diluent: n.a.
Coupling: To avoid oxidative cleavage of the disulfide linkage, all oxidation steps should use 0.02 M Iodine solution.
Deprotection: no changes needed from standard method recommended by synthesizer manufacturer for the other nucleobases used in the oligonucleotide.
Storage: freeze at -10 to -30 °C, dry.
Stability: n.a.

Versatile Surface Modification

DTPA and DTPA-CPG have been developed for the use in automated solid phase synthesis of DNA and RNA oligonucleotides to improve the attachment of these oligonucleotides to thiol-reactive surfaces but also to proteins or other molecules like maleimides, halogens, iodacetamides, pyridyldisulfides or proteins. DTPA modified oligos are already applied in numerous areas such as gold or silver electrodes and nanostructures. [1-10]

Superior Stability

DTPA can be inserted into an oligonucleotide at the 5' position, internally or via DTPA-CPG at the 3' position. After reduction with TCEP or DTT each insertion results in 2 SH groups for coupling with ligands or surfaces. DTPA can be inserted in series (two, three or more dithiol-groups) to increase efficiency of ligand/surface interaction.



Stability to chemical stress

In a physiological environment, the gold surfaces functionalised via thiol anchoring will be subjected to stress by other thiol-containing molecules, which may/will displace the original thiol anchored loading of the surface. Though this displacement is common for mono-thiol anchored functionalisation, it is significantly reduced by the use of DTPA.

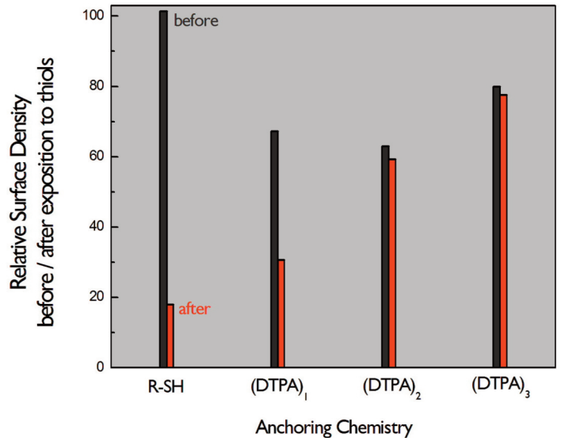


Fig. 1: Au-NP with ds oligo: one strand anchored via thiol moieties and hybridised with a perfect matching second strand that is terminally modified with fluorescein. During hybridised state, fluorescence is quenched by Au-NP. In a thiol environment functional ds-oligo is removed from the surface, depending on actual anchoring chemistry. The bars indicate decrease as well as surface coverage before and after chemical stress due to treatment with 1mM 6-mercaptohexanole for one hour. To assess relative measures of functional hybrids, fluorescence was recorded by temperature induced dehybridisation before and after chemical stress induction.

Temperature stability

Temperature stability of various thiol anchoring strategies: The use of 2 or 3 DTPA units increases the temperature stability dramatically as compared to mono-thiol anchoring. Surprisingly, anchoring with one DTPA is less stable than a mono-thiol anchoring and can be used to realise temperature induced desorption of the surface modification in hyperthermy-experiments.

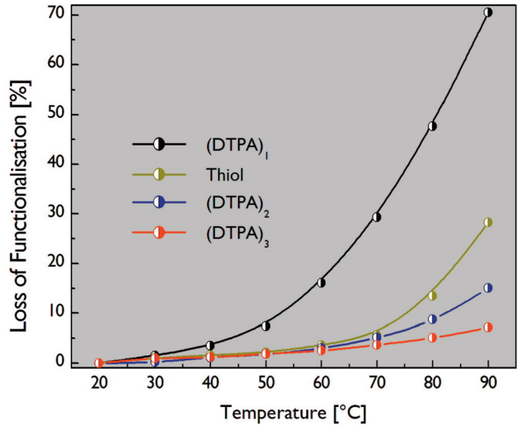


Fig. 2: Au-NP loaded with 5' Fluorescein- CCT CCT TTA CCG TGA TT-X capped with MeO-PEG-SH (X = monothiol, one DTPA, two DTPA or three DTPA). Temperature was raised incrementally in 10 °C steps and kept for 5 min, then temperature induced desorption of immobilised oligos and concomittand increase of fluorescence was recorded and normlised to the number of immobilised oligonucleotides. Oligonucleotide loading of Au-NP was determined by immobilising fluorescein modified oligonucleotides and completely dissolving the gold-core of a known amount of nanoparticles in 0.05 M KCN. The amount of released fluorescein-oligonucleotide was determined by quantitative measurement of fluorescence in KCN.

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