INTEGRATED DNA TECHNOLOGIES

Strategies for Attaching Oligonucleotides to Solid Supports
—Quick Look—

This is a modified, "Quick Look" version of the full Technical Report "Strategies for Attaching Oligonucleotides to Solid Supports." Please see the full version for a more comprehensive explanation and detailed chemical reactions.

Many important molecular applications, such as DNA oligonucleotide arrays, utilize synthetic oligonucleotides attached to solid supports. The most accessible approach for producing an oligonucleotide microarray is to synthesize individual oligonucleotides and subsequently immobilize them to a solid surface. For this immobilization to take place, the oligonucleotides must be modified with a functional group in order to have attachment to a reactive group on a solid surface.

Surface Modification

Oligonucleotides can be attached to flat two-dimensional surfaces, such as glass slides, as well as to three-dimensional surfaces such as micro-beads and micro-spheres. Construction of arrays involves a number of parameters each of which must be optimized for efficient and effective experimental design.

Two-dimensional Surfaces (Microarray slides)

Substrates for arrays are usually silicon chips or glass microscope slides. Most attachment protocols involve chemically modifying the glass surface to facilitate attachment of the oligo. Silianized oligonucleotides can also be covalently linked to an unmodified glass surface [1].

Different modifications allow immobilization onto different surfaces:

Modification	Surface treatment
NH2-modified oligos	Epoxy silane or Isothiocyanate coated glass slide
Succinylated oligos	Aminophenyl or Aminopropyl-derivatized glass slide
Disulfide modified oligos	Mercaptosilanized glass support
Hydrazide (I-Linker™)	Aldehyde or Epoxide

Issues to consider when choosing and appropriate support and attachment chemistry:

- Level of scattering and fluorescence background in the support material and added chemical groups
- Chemical stability and complexity of the construct
- Amenability to chemical modification or derivatization
- Surface area
- Loading capacity and the degree of non-specific binding of the final product [2]

Surface Treatment

The two-dimensional surface is typically prepared by treating the glass or silicon surface with an amino silane which results in a uniform layer of primary amines or epoxides [2, 3]. A low surface coverage of the oligonucleotide probe will yield a correspondingly low hybridization signal and will decrease the hybridization rate. Conversely, high surface densities may result in steric interference between the covalently immobilized oligonucleotides which may impede access to the target DNA strand [2]. In addition, the planar surface structure of glass slides or silicon chips can limit the loading capacity of oligonucleotides. To address this limitation, acrylamide gels can be applied to glass slides to construct a three-dimensional surface which will greatly increase the surface area per spot [4, 5].

Three-dimensional Surfaces (Micro-spheres)

In these micro-sphere-based assays, each oligonucleotide is attached to a micro-sphere. The micro-spheres can be individually assayed, usually with a flow cytometer, or isolated based on the physical characteristics of the bead.

Attachment:

Nucleic acids can be covalently attached to micro-spheres with any of several methods. Carboxyl and amino groups are the most common reactive groups for attaching ligands to surfaces. Attaching an amino group to the 5’ or 3’ end of an oligonucleotide or a PCR primer is straightforward and inexpensive. The amine-modified oligos can then be reacted with carboxylate-modified micro-spheres with carbodiimide chemistry in a one-step process at pH 6-8 (Figure 1).

A number of reactive groups can be used for coupling to micro-spheres such as Carboxylic acid (-COOH), Hydrazide (-$CONHNH_2$), Primary aliphatic amine (-RNH_2), Aldehyde (-CHO), Aromatic amine (-$ArCH_2Cl$), Hydroxyl (-OH), Chloromethyl (vinyl benzyl chloride) (-$ArCH_2Cl$), Thiol (-SH), Amide (-$CONH_2$), and Epoxy (-COC-).

Oligonucleotide modifications (Available at IDT)

- I-Linker™
- Amine-modified oligos covalently linked to an activated carboxylate group or succinimidyl ester
- Thiol-modified oligos covalently linked via an alkylating reagent such as an iodoacetamide or maleimide
- Acrydite™-modified oligos covalently linked through a thioether
- Digoxigenin NHS Ester
- Cholesterol-TEG
- Biotin-modified oligos captured by immobilized Streptavidin

I-Linker™

I-Linker is a hydrazide attachment chemistry for oligonucleotides that was developed at IDT. The modifier is attached to the 5'-end of the oligo. I-Linker can be substituted for amino modifications in many applications. In addition, I-Linker expands the range of reactive groups that can be used for conjugation, including aldehyde and ketone-modified ligands or surfaces.

Amino-Modified Oligonucleotides

The attachment of an amino-modified oligonucleotide to a surface or another molecule requires an acylating reagent. Depending on which acylating reagent is used, carboxamides, sulfonamides, ureas, or thioureas are formed upon reaction with the amine moiety. The kinetics of the reaction depends on the reactivity and concentration of both the acylating reagent and the amine. When using any amine-reactive reagent, avoid buffers that contain free amines, such as Tris and glycine.

Attachment chemistries currently in use for amino modified oligonucleotides for linkage to molecule or surface:

Acylating Agent	Linkage	Features
Carbodiimide	Carbonyl amide	Most common method, stable attachment
Isothiocyanate	Thiourea	Stable covalent attachments
Sulfonyl chloride	sulfonamide	Sulfonyl chloride is unstable in water, but once conjugated to the oligo the sulfonamide bond is very stable
Succinimidyl esters (NHS-ester)	carboxamide	Carboxamide bond formed is very stable

For detailed chemical reactions, please refer to the full length technical report.

Attachment of amine-modified oligos to Surfaces:

- An epoxide-opening reaction will generate a covalent linkage between a 5'-amino-modified oligonucleotide and an epoxy silane-derivatized glass surface [3, 6].
- Reacting the surface bound amino groups with excess p-phenylene 1,4 diisothiocyanate (PDC) will convert the support's bound primary amines to amino-reactive phenylisothiocyanate groups. This is followed by a reaction which couples the 5'amino-modified oligos to the phenylisothiocyanate and resulting in the covalent attachment of the oligonucleotide [2].
  Modifications include using homobifunctional crosslinking agents to convert glass bound amino groups:
  - (DCS) disuccinimidylcarbonate– converts to reactive isothiocyanates
  - (DSO) disuccinimidyloxalte– converts to reactive N-hydroxysuccimimidy-esters (NHS-esters)
  - (DMS) dimethylsuberimidate– converts to reactive imidoesters
  - EDC (1-ethyl=3-(3-dimethylaminopropl)-carbodiimide hydrochloride) is a heterobifunctional cross linker that has been employed with numerous supports such as amino controlled-pore glass [7], latex beads [8, 9], dextran supports [10], and polystyrene [11]. The use of EDC to bind oligonucleotides onto glass surfaces has been widespread [12, 13].

Thiol-Modified Oligonucleotides

The thiol SH modifier can be placed either at the 5' end or 3' end of an oligo and enables covalent attachment of an oligo to a variety of ligands. Maleimide, bromide, iodide, or sulphonyl derivatives are suitable for tagging thiol-linked oligonucleotides with a variety of groups such as fluorescent dyes [14], biotin [15], and alkaline phosphates [16]. The thiol modification also enables attachment to solid surfaces via a disulphide bond [17] or malemide linkages.

To ensure full reactivity, thiol-modified oligos should be reduced immediately before use. In general, the oligo is treated with a reducing agent (like DTT) and this agent is fully removed prior to coupling. Please refer to the full length technical report for specific protocols for this treatment.

Cross-linkers used for attachment of thiol-modified oligos:

The cross-linkers used to attach thiol-modified oligonucleotides to solid supports are heterobifunctional, meaning that they possess functional groups capable of undergoing a reaction with two chemically distinct function groups, amines and thiols. The linkers serve two purposes: to covalently bind two distinct chemical entities which otherwise would remain un-reactive toward each other and as a physical spacer which provides greater accessibility and or freedom to each of the linked biomolecules [12]. One such cross linker, succinimidyl 4-maleimidophenylbutyrate (SMPB) can be used to link a thiol-modified oligo to an amine derivatized solid support.

Acrydite™-Modified Oligonucleotides

Proprietary small molecule attachment chemistry, developed by Apogent Discoveries (no longer in business), enables covalent attachment of macromolecules to surfaces via acrylic linkages. An acrylic acid group can be directly attached to the 5'-end of an oligonucleotide (with a 6-carbon

linker arm) at the time of synthesis using Acrydite™, an acrylic-phosphoramidite developed by Mosaic Technologies. The Acrydite™ chemistry is stable prior to coupling and will remain stable in aqueous solutions over a wide range of temperature and pH. In addition, it is versatile and can be immobilized on glass, polymer, or chromatography media.

Attachment of Acrydite™-Modified Oligonucleotides

The Acrydite™ acrylamide group has been used to immobilize oligonucleotides, which are fully available for hybridization, to thiol-modified glass slides. Immobilization can be accomplished with standard inexpensive gel polymerization techniques that are already widely used in molecular biology laboratories. When an aqueous solution with Acrydite™ modified material is copolymerized with another monomer, such as acrylamide, and with a crosslinker, such as bis, the resulting product is a crosslinked gel with the concentration of nucleic acids in the polymer determined by input concentrations and the ratio of Acrydite™-modified material to monomer. The attachment to solid phases occurs with the formation of high stable carbon-carbon or thioether bonds which are stable in all conditions routinely encountered in standard molecular biology protocols.

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