FACILE SOLID-PHASE SYNTHESIS OF BIOTINYLATED ALKYL THIOL

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Abstract—Biotinylated alkyl thiols with the capacity to graft avidin proteins are in increasing demand for the development of self-assembled
monolayers on gold. Here we propose 2-Chlorotrityl Chloride solid-phase resin as a new platform to produce these functionalized alkyl thiols.
Biotinylated alkyl thiols of non-obvious solution synthesis were obtained rapidly using this method and without previous purification steps.

Self-assembled monolayers (SAMs) are a popular tool for tailoring the reactive properties of surfaces. To produce these monolayers, molecules may be physisorbed from solution or more tightly grafted by covalent bond formation, as with gold substrates and alkyl thiols. Because of the features of the functional group of alkyl thiols, surface properties can be easily modified by simply changing the chemical nature of the terminal groups. Alkyl thiols are widely used, mainly in biological applications.[1] Gold surfaces can be derivatized to bind proteins[2] carbohydrates[3], peptides DNA,[4] haptenes[5] or to produce new surfaces for cell culture studies such as cell attachment, differentiation or proliferation.[6] For all of these purposes, rapid and efficient access to a diversity of functional alkyl thiols is required. In an attempt to minimize the difficulty of access to these molecules, researchers introduce the desired functionalities (biotines, haptenes, polyglycols) by reaction over amino- or acid-terminating SAMs. Reactions over a previously formed SAM do not ensure perfect derivatization because the processes between surfaces and solution are not kinetically well afforded. This problem is exacerbated when the gold surface to be derivatized is a fragile gold AFM tip or a micro(nano)electrode because the manipulation and the solvent rinsing procedures required to remove the undesired reagents may cause damage. Consequently, a thiol with the desired functionality is required. Given that Dip Pen nanolithography, the AFM-based soft-lithography developed by Mirkin et al. in 1999[7], involves the direct deposition of thiols in nanometer scale using an AFM probe, the development of functional alkyl thiols is crucial for this technique. Here we propose a novel solid-phase strategy for the development of biotin alkyl thiol (BAT) derivates. BAT structures are formed by the thiol, the aliphatic chain (n= 11, 16), a PEG linker and the biotin group (**Fig. 1**).

BATs are useful for the development of biosensors as they allow the production of well-defined biotinylated surfaces (**Fig 2.a**). Biotin surfaces are one of the most used tools to immobilize antibodies (i.e. antigens, enzymes or DNA) onto surfaces through the biotin-streptavidin (i.e. neutravidin, avidin) pair ($K_d \sim 10^{-13}$ M) as building block. Streptavidin has four equivalent sites for biotin (two on one side and two on the opposite). Streptavidin vacancies for biotin can be used to link the protein almost irreversibly to the surface and to create well-oriented free biotin sites that are exposed to the surface (**Fig. 2.b**). These exposed sites allow the grafting of biotinylated biomolecules for the preparation of biosensors and have minimal impact on biological activity (**Fig. 2.c**). BAT structures must include PEG groups in order to avoid the non-specific adsorption of streptavidin and other proteins onto surfaces, and to allow a good orientation of the streptavidin molecule.

Here we describe a new solid-phase approach, based on the 2-Chlorotrityl Chloride® (CTC) resin, for the development of BATs. CTC resin is a polymer support functionalized with chlorotrityl groups that graft nucleophiles, such as thiols,[11] amines[12] or carboxylates,[13] hereby allowing cleavage of the final product under acidic conditions. One of the advantages of this resin is that it can be regenerated several times[14]. Moreover, it offers a new platform to obtain alkyl thiols of interest[15]. The solid-phase method reported here allows the synthesis of high purity BATs in only a few days. The number of carbons of the alkyl chain, the presence or number of glycols, in the case of BAT with PEG linkers, and the functional group can be chosen freely. In solution-phase synthesis, modifications in molecule design change the purification procedures, which must be optimized for any new molecule. We obtained two BATs, with and without PEG, using the same solid-phase synthetic procedure, which indicates the robustness of the

new method. The development of this method allows the production of custom-designed BATs or other functionalized alkyl thiols.(Scheme 1)

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Figures:

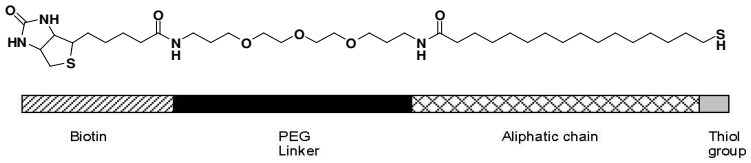


Figure 1. BAT structure.

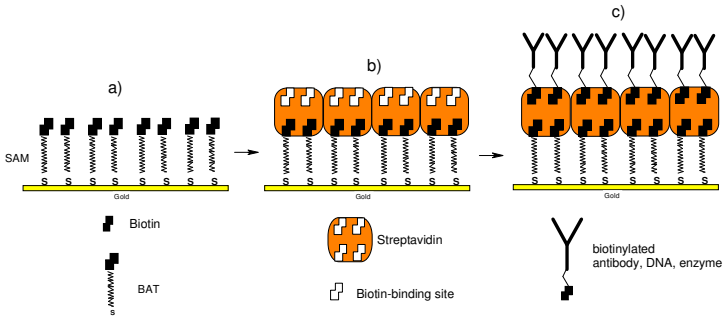
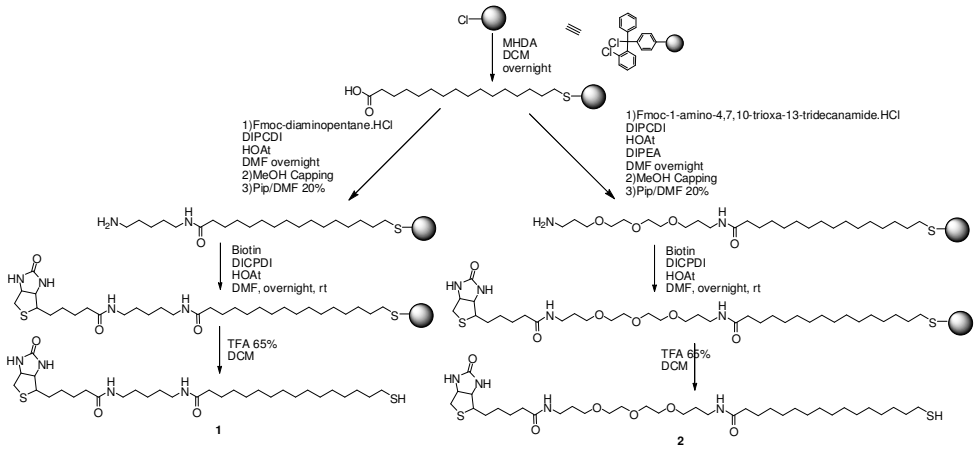


Figure 2. Streptavidin–biotin procedure to immobilize biomolecules: (a) biotinylated gold surface, (b) streptavidin-grafted surface through biotin groups and
formation of biotin vacancies on the surface, (c) grafting of biotinylated biomolecules over the surface.



Scheme 1. Solid-phase of 1 and 2. (a) MHDA (2 equiv), DCM, overnight, rt; (b) Fmoc-diaminopentane hydrochloride (3 equiv), DIPCDI (3 equiv), HOAt(3 equiv), DIPEA (3 equiv), DMF, overnight, rt; (c) MeOH (2 mL/g resin), DIPEA (0.3 mL/g resin), DCM, 10 min, rt; (d) piperidine–DMF (1:4, v/v),1_1 min, 2_10 min, rt; (e) Fmoc-1-amino-4,7,10-trioxa-13-tridecanamine hydrochloride (3 equiv), DIPCDI (3 equiv), HOAt (3 equiv), DIPEA (3 equiv),DMF, overnight, rt; (f) Biotin (5 equiv), DICPDI (5 equiv), HOAt (5 equiv), DMF, overnight, rt; (g) TFA 65% in DCM/TES (95:5, v/v).