# A study of binding biotinylated nano-beads to the surface of (001) GaAs

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## ABSTRACT

We have investigated the deposition of biotinylated nano-beads on the surface of GaAs. The deposition procedure involved either direct coating of (001) GaAs with nano-beads, or binding the nano-beads with avidin immobilized on the surface of (001) GaAs through the interface of biotin and the $NH_2$ terminal group of 11-amino-1-undecanethiol ($HS(CH_2)_{11}NH_2$). The efficiency of binding was tested by washing the samples in a solution of a commercial detergent and by subjecting them to a deionized water ultrasonic bath. The results indicate that nano-beads deposited directly on the surface of (001) GaAs withstand the detergent washing test but they are easily removed by ultrasonic washing. In contrast, the nano-beads attached to (001) GaAs through the avidin-biotin-thiol interface survive the ultrasonic washing tests.

**Key words:** GaAs, photoluminescence, surface passivation, bio-functionalization, self-assembled monolayers, avidin, biotinylated nano-beads

## 1. INTRODUCTION

Efficient deposition of various biomolecules, including antibodies on semiconductor surfaces is of high interest for the development of quantum dot (QD) cellular imaging technology, novel bio-sensing devices and for studying inter-molecular interactions. In that context, GaAs plays an important role because of its direct bandgap structure and related strong photoluminescence (PL) [1]. It is also the material of choice for capping InAs QDs. Thus, the technology of GaAs surface passivation and bio-functionalization is crucial for innovative bio-diagnostic applications. A number of studies have demonstrated that alkanethiols, DNA and peptides could be covalently attached to the bare surface of GaAs *via* S-Ga and/or S-As bonding [2-8]. Such a modification to the surface of this semiconductor strongly influences its surface states and band bending, which translates into

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significant variations of its PL signal. The PL intensity from GaAs bandgap emission has been frequently used to monitor the physical and chemical state of its surface [5,9]. For instance, the strong sensitivity of the PL signal to the modifications taking place at the GaAs surface has been investigated by numerous laboratories for gas sensing applications [10]. The ability to immobilize probe biomolecules on the surface of GaAs is of paramount importance for the development of GaAs-based biosensors and GaAs-coated QD biosensors. To this day, many research activities have been focused on the immobilization of biomolecules on Au or Si substrates [8, 11-15]. Recently, we have reported on the influence of different thiols on the passivation performance of the (001) GaAs surface. We have established that self-assembled monolayers (SAMs) of hexadecanethiol (HDT: $HS(CH_2)_{15}CH_3$) on (001) GaAs provided the most efficient passivation among all the investigated thiols. Following these findings we succeeded in the immobilization of avidin on the surface of (001) GaAs [16,17]. Such a surface is expected to provide favourable conditions for the attachment of biotinylated antibodies. As a control step, we chose to investigate the robustness of the created interface by attempting to attach biotinylated nano-beads. In this paper, we report the preliminary results of the investigation of this new interface created on the surface of (001) GaAs that was functionalized with thiol, biotin and avidin.

## 2. EXPERIMENTAL DETAILS

The following chemicals were purchased from Aldrich: N-hydroxysuccinimidobiotin (NHS-Biotin), 10× Tris buffer solution (pH=7.4), dimethyl sulfoxide (DMSO, for molecular biology). The 11-amino-1-undecanethiol (T11: $HS(CH_2)_{11}NH_2$) was purchased from Dojindo Molecular Technologies Inc. Avidin (from egg white) and biotinylated nano-beads (*b*-NB, $\phi$ = 200nm) stained with an organic dye emitting green (~ 515 nm) fluorescence, were products from Molecular Probes. The nominally undoped (001) GaAs wafer (n-type at room temperature) was purchased from Atomergic Chemical Corp.

GaAs surface cleaning and functionalization process were reported in the previous publications [16, 17]. Both avidin functionalized and bare GaAs samples were immersed in a biotinylated nano-bead suspension for 2 hours and, consequently, subjected to rinse with water flow for 1 min..

The PL measurements were carried out to monitor the process of surface bio-functionalization. The PL response from (001) GaAs was measured in the band gap emission region at room temperature (868 nm) using a PL mapper (Philips PLM-150). The PL signal was excited with a laser operating at 532 nm and the signal was collected with an IR array of InGaAs detectors. A freshly etched GaAs wafer was always used as the reference. Fluorescence from the immobilized nano-beads was observed with a fluorescence microscope (Leica MZ FLIII Microsystems Digital Imaging with a DC300 camera) excited with a blue light source (450-490 nm) and viewed through a filter of 515 nm.

Tapping-mode atomic force microscope (AFM) measurements were performed with a Veeco Multimode Scanning Probe Microscope (Digital Instruments, Inc.). The applied resonant frequency and force constant were 300 kHz and 40 N/m, respectively. The scanning rate was around 2 Hz.

Only one "flattening" procedure was applied to raw images before roughness analysis. Root-mean-square (RMS) roughness, $\sigma_{RMS}$, was measured over 5 μm × 5 μm areas.

## 3. RESULTS AND DISCUSSION

The immobilization architecture for trapping *b*-NB on (001) GaAs is shown in Fig. 1. Although many other approaches are available to immobilize biomolecules on solid substrates, this architecture is expected to provide maximum probe biomolecule uptake and will consequently capture more target moieties for detection [8, 11, 13]. $-NH_2$ terminated alkanethiol attached to the (001) GaAs surface through S-As and/or S-Ga bonding were left to bind NHS activated biotin. Considering the relatively larger cross-sectional area of the biotin headgroup compared with that of the alkanethiol chain, the surface density of biotin is expected to be lower than that of the $-NH_2$ groups. XPS measurements on biotin exposed samples showed an increase of the N1s signal by approximately 20% in comparison to that observed after alkanethiol deposition [18]. This indicates that only 10% of the $-NH_2$ groups on the surface reacted and connected with biotin. Avidin is deposited on the biotinylated surface *via* the biotin-avidin specific interaction. However, it could also be physically (weakly) adsorbed at the uncovered surface of GaAs. Therefore, in addition to the *b*-NB material attached to the GaAs surface through the empty pockets of the immobilized avidin, some *b*-NB could be attached to the avidin molecules which were physically adsorbed on the free surface of GaAs.

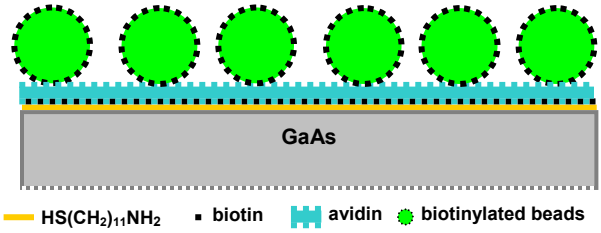


Fig. 1. The architecture of *b*-NB immobilization on (001) GaAs surface *via* avidin, biotin and alkanethiol.

The results of PL measurements have been summarized in Table 1. The deposition of $-NH_2$ terminated alkanethiols resulted in up to a 2.8-fold enhancement of the PL signal compared with bare GaAs. Due to the van der Waals interaction between the alkyl chains, the alkanethiol molecules tend to be highly packed. This results in a significant reduction of the non-radiative recombination centers and, consequently, an enhancement of the PL signal. Spectroscopic ellipsometry measurements have indicated that the alkanethiol layer is about 0.55 nm thick. This would correspond to a T11 chain tilt of approximately 70° from the surface normal, which exceeds the 57° reported for the octadecylthiol deposited on (001) GaAs [4]. A possible reason for this difference could be related to the more hydrophilic nature of the amino terminal group in this alkanethiol [16]. The deposition of biotin in effect, did not change the intensity of the PL signal, which is understandable considering a small quantity of biotin intake as judged by the XPS analysis. The attachment of avidin, however, has resulted in a 3.18 times increase of the PL signal. A similar increase had been reported by us earlier [10] and it was suggested that this could be due to the modification of the depletion width near the GaAs surface by the positively charged avidin. *b*-NB

Table 1. GaAs PL intensity following various surface modification processes. The signal is normalized to the freshly cleaned and HCl treated GaAs. T: $HS(CH_2)_{11}NH_2$, B: biotin, A: avidin, *b*-NB: biotinylated nano-beads.

| Sample | GaAs | GaAs-T | GaAs-T-B | GaAs-T-B-A | GaAs-T-B-A-*b*-NB |
|---|---|---|---|---|---|
| PL Intensity | 1 | 2.78 | 2.86 | 3.18 | 3.60 |

deposition further increased the PL signal to the final 3.6-fold enhancement in comparison to the freshly etched GaAs.

A fluorescence microscopic image of the (001) GaAs surface coated with *b*-NB is shown in Fig. 2. It is interesting to note that, qualitatively, the same fluorescence images were obtained for both bare and avidin functionalized surfaces exposed to the *b*-NB suspension. For comparison, an image of the bare surface of (001) GaAs that is shown in the inset of Fig. 2 was obtained under the same

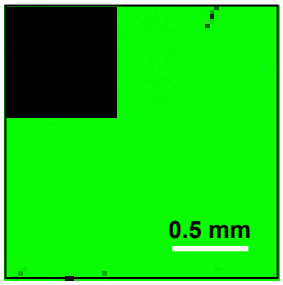


Fig. 2 A fluorescence microscopy image of the biotinylated and green-stained *b*-NB nano-beads on the surface of avidin functionalized (001) GaAs. The inset shows the surface image of the substrate obtained under the same collection conditions.

collection conditions. The color contrast is clearly seen in this picture. AFM images taken from bare and avidin functionalized surfaces, following their exposure to the *b*-NB suspension are shown in Fig. 3. In both cases, the density of coverage with nano-beads is comparable ($\rho \approx 1.4 \times 10^7$ mm$^{-2}$), which corresponds to the surface coverage of approximately 44%.

To test the strength of *b*-NB immobilization on GaAs surface *via* avidin, biotin and thiol, the immobilized *b*-NB was treated with detergent washing and ultrasonic cleaning, which is a routine treatment applied in clinical studies of specimens. A sample with *b*-NB directly deposited on GaAs was used as a control. On both samples, the *b*-NB stuck to the GaAs surface after detergent washing, as confirmed by both fluorescence and AFM measurements. However, no fluorescence could be observed from the sample covered with physically adsorbed *b*-NB following the 1-min ultrasonic treatment. The surface roughness in that case was reduced to $\sigma_{RMS} = 2.27$ nm. This likely indicates the presence of fragmented biomolecules and nano-bead material. In contrast, the *b*-NB immobilized on GaAs surface *via* avidin, biotin and alkanethiol survived the ultrasonic cleaning treatment. The AFM measurements indicated no significant change in the density of *b*-NB on the (001) GaAs surface following such treatment. The roughness of this new surface $\sigma_{RMS} = 84$ nm compares closely with that of the as-fabricated surface $\sigma_{RMS} = 82$ nm. These results suggest that the immobilization of *b*-NB on (001) GaAs *via* the avidin-biotin-alkanethiol interface is very stable.

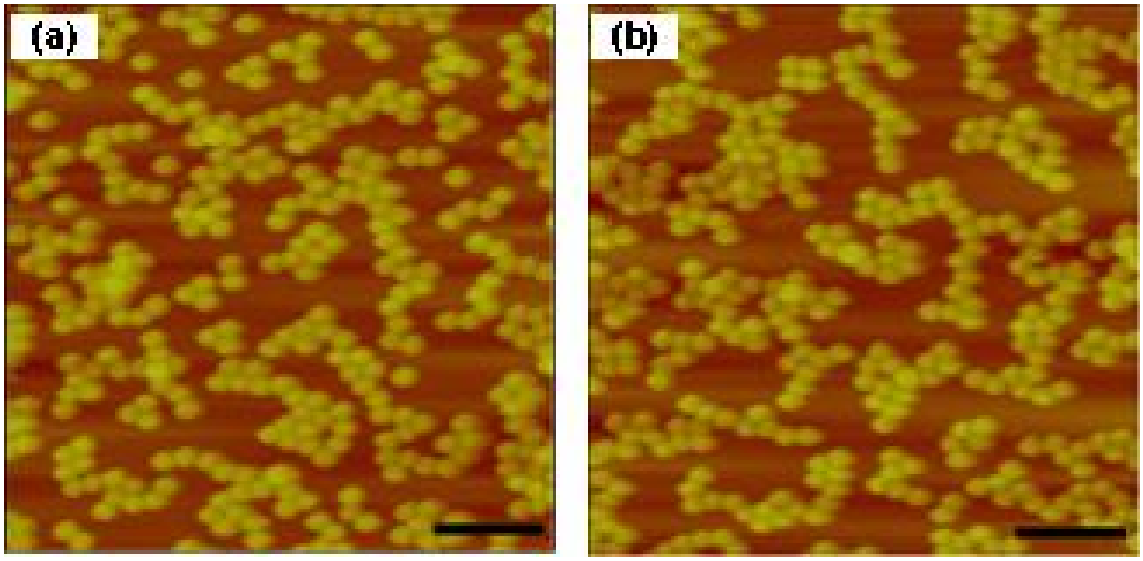


Fig. 3. Tapping mode AFM images of the *b*-NB on bare surface (a) and avidin functionalized surface (b) of (001) GaAs. The scale marker corresponds to 1 μm.

In our previous study [17], we reported that avidin immobilized on GaAs surface *via* a similar biotin-alkanethiol interface could not survive the detergent washing procedure as judged by the fluorescence microscopy observations. Current results demonstrate that such an interface provides an efficient link for the immobilization of *b*-NB on the surface of (001) GaAs. This apparent discrepancy is likely related to the large difference in the efficiency of binding a relatively small molecule of stained avidin (5.6 nm × 5 nm × 4 nm) to biotin available at the surface of GaAs in comparison to that of the significantly larger biotinylated nano-bead (200 nm diameter). Avidin would bind by one or two biotin-avidin bonds, whereas the nano-bead might be retained by tens of such bonds available at the bio-functionalized surface of GaAs. As it is illustrated by Fig. 2 and 3, uniform fluorescence intensity has been observed from the GaAs sample that was only partially covered by *b*-NB. A thin layer of stained avidin immobilized on GaAs is expected to produce much weaker fluorescence; this would be further reduced following the removal of the physically adsorbed stained avidin by the detergent washing procedure.

A preliminary study of laser-induced ablation of the avidin immobilized *b*-NB has indicated that this approach is efficient in selected area patterning without significant modification (deterioration) of the GaAs surface [19].

## 4. CONCLUSIONS

We have investigated the process of the immobilization of biotinylated nano-beads (*b*-NB, 200 nm diameter) on the surface of (001) GaAs. Directly deposited *b*-NB on GaAs survive a standard detergent washing procedure, but they could be easily removed by ultrasonic cleaning in DI water bath. The bio-functionalized surface of (001) GaAs that comprises the 11-amino-1-undecanethiol ($HS(CH_2)_{11}NH_2$)-biotin-avidin interface provides favourable conditions for the attachment of *b*-NB.

Fluorescence microscopy and AFM measurements have confirmed the formation of a robust interface that withstands a 1-min ultrasonic treatment. Since numerous bio-moieties of interest (e.g., viruses) are of comparable dimensions with the investigated *b*-NB, it is reasonable to expect that this approach will provide suitable conditions for the attachment and the study of selected pathogenic molecules.

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## REFERENCES

[1] Properties of Gallium Arsenide (3rd Edition), Eds. Brozel, M.R.; Stillman, G.E., Institution of Electrical Engineers, 1996.

[2] S. R. Lunt, G.N. Ryba, P. G. Santangelo, N. S. Lewis, ″Chemical studies of the passivation of GaAs surface recombination using surfides and thiols″, J. Appl. Phys. **70(12**), 7449(1991).

[3] C. W. Sheen, J. X. Shi, J. Mirtensson, A. N. Parikh, D. L. Allara, ″A New Class of Organized Self-Assembled Monolayers: Alkane Thiols on GaAs (100)″, J. Am. Chem. Soc. **114(4)**, 1514(1992).

[4] C. D. Bain, ″A New Class of Self-Assembled Monolayers: Organic Thiols on Gallium Arsenide″, Adv. Mater. **4(9)**, 591(1992).

[5] T. Hou, C.M.Greenlief, S. W. Keller, L. Nelen, J. F. Kauffman, ″Passivation of GaAs (100) with an Adhesion Promoting Self-Assembled Monolayer″, Chem. Mater. **9(12),** 3181**(**1997).

[6] L. Mohaddes-Ardabili, L.J.Martinez.-Miranda., J. Silverman, A. Christou, ″Attachment of DNA probes on gallium arsenide surface″, Appl. Phys. Lett. **83(1),** 192(2003).

[7] Y. Cho, A. Ivanisevic, ″Covalent Attachment of TAT Peptides and Thiolated Alkyl Molecules on GaAs Surfaces″, J. Phys. Chem. B, **109(26)**, 12731(2005).

[8] T.T. Huang, J.Sturgis, R. Gomez, T. Geng, R. Bashir, A. K. Bhunia, J. P. Robinson, M. R. Ladisch, ″Composite Surface for Blocking Bacterial Adsorption on Protein Biochips″, Biotech. Bioeng. **81(5)**, 618(2003).

[9] Z. S. Li, W. Z. Cai, R. Z. Su, G. S. Dong, D. M. Huang, X. M. Ding, X. Y. Hou, X. Wang, ″S2Cl2 treatment, a new sulfur passivation method of GaAs surface″, Appl. Phys. Lett. **64(25)**, 3425(1994).

[10] F. Seker, K.Meeker, T. F. Kuech, A. B. Ellis, ″Surface Chemistry of Prototypical Bulk II-VI and III-V Semiconductors and Implications for Chemical Sensing″, Chem. Rev. **100(7)**, 2505(2000), and references therein.

[11] R. N. Orth, T.G. Clark, H. G. Craighead, ″Avidin-Biotin Micropatterning Methods for Biosensor Applications. Biomedical Microdevices″, **5(1)**, 29(2003).

[12] J. Spinke, M.Liley, F. J. Schmitt, H. J. Guder, L. Angermaier, ″Molecular recognition at self-assembled monolayers: Optimization of surface functionalization″, J. Chem. Phys. **99(9)**, 7012(1993).

[13] R. Bashir, R.Gomez, A. Sarikaya, M. R. Ladisch, J. Sturgis, J. P. Robinson, ″Adsorption of Avidin on Microfabricated Surfaces for Protein Biochip Applications″, Biotech. Bioeng. **73(4),** 324(2001).

[14] K. E. Nelson, L.Gamble, L. S. Jung, M. S. Boeckl, E. Naeemi, S. L. Golledge, T. Sasaki, D. G. Castner, . T. Campbell, P. S. Stayton, ″Surface Characterization of Mixed Self-Assembled Monolayers Designed for Streptavidin Immobilization″, Langmuir, **17(9)**, 2807(2001).

[15] M. Riepl, K.Enander, B. Liedberg, M. Schaferling, M. Kruschina, F. Ortigao, ″Functionalized Surfaces of Mixed Alkanethiols on Gold as a Platform for Oligonucleotide Microarrays″, Langmuir, **18(18)**, 7016(2002).
[16] X. Ding, Kh. Moumanis, J.J. Dubowski, L. Tay, N. Rowell, ″FTIR and photoluminescence spectroscopy of self-assembled monolayers of long-chain thiols on (001) GaAs″, J. Appl. Phys. (in press).
[17] X. Ding, Kh. Moumanis, J.J. Dubowski, E. Frost, E. Esher, ″Immobilization of Avidin on (001) GaAs surface″, Appl. Phys. A (in press).
[18] G. Marshall, unpublished results.
[19] J.J. Dubowski, unpublished results.