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# Antibody Molecules Discriminate between Crystalline Facets of a Gallium Arsenide Semiconductor

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**ABSTRACT**

**Seamless integration of biomolecules with manmade materials will most likely rely on molecular recognition and specific binding. In the following we show that combinatorial antibody libraries, based on the vast repertoire of the human immune system, can be harnessed to generate such binders. As a demonstration, we isolate antibody fragments that discriminate and bind selectively GaAs (111A) facets as opposed to GaAs (100). The isolated antibodies are utilized for exclusive localization of a fluorescent dye on (111A) surfaces in a structure comprising a mixture of (100) and (111A) surfaces. The potential importance of structure rigidity to facet recognition is suggested vis-a-vis published experiments with short and longer peptides.**

The mammalian immune system offers a vast repertoire of antibody molecules capable of binding selectively an immense number of molecules presented to the body by invading pathogens such as bacteria, viruses, and parasites. Albeit the fact that this repertoire evolved to target mostly biomolecules, it may potentially contain selective binders to other targets or be expanded to include such binders. Indeed, injection of cholesterol and 1,4-dinitrobenzene[1,2] microscopic crystals as well as $C_{60}$ conjugated to bovine thyroglobulin in mice[3] have resulted in generation of antibodies against these materials by the immune system of the injected animal. Here, we expand the scope and show that human antibody libraries (specifically, scFv, single chain Fv[4] which are the antibody variable binding domains) contain specific binders, capable of discriminating between different crystalline facets of a GaAs semiconductor crystal, an almost flat target, unfamiliar to the immune system. This selectivity is remarkable given the very simple structure of semiconductors compared with biomolecules.

Using phage display technology we demonstrate in vitro isolation of scFv that bind GaAs (111A) facets almost one hundred times better than GaAs (100). More generally, our finding implies that antibody molecules may find application in the assembly of nanoelectronics,[5−7] in producing templates for localizing nanoparticles,[8] or for biosensors.[9]

Isolation of short peptides that bind inorganic materials has been demonstrated for gold,[10,11] silver,[12] silica,[13] metal oxides,[14,15] minerals,[16] carbon nanotubes,[17] and various semiconductors.[18−20] Reference 20 is particularly relevant to our paper. In that reference the authors report isolation of peptides (by phage display) that bind GaAs (100) preferentially to GaAs (111A) and (111B). However, all assays in that reference probed the peptides displayed on the phages rather than free peptides. Indeed, when one of these peptides was later synthesized and applied to GaAs,[21] no selectivity was found between the (100) and (110) facets (Figure 5 in ref 21 and the discussion following it). Puzzled by this discrepancy we turned to study nonspecific binding of M13 phages, **carrying no peptides or antibodies,** to GaAs (100), GaAs (111A), and GaAs (111B) and found that M13 binds preferentially to the (100) facet through its coat protein (Figure 1S, Supporting Information). Since those phages are identical to the library phages used in ref 20, and given the lack of selectivity displayed by the only free peptide tested thus far,[21] it seems that further experiments with free peptides are needed in order to confirm or disprove semiconductor facet recognition by short peptides. In contrast to ref 20, our antibody was also tested and found selective to crystal orientation when detached from the phage.

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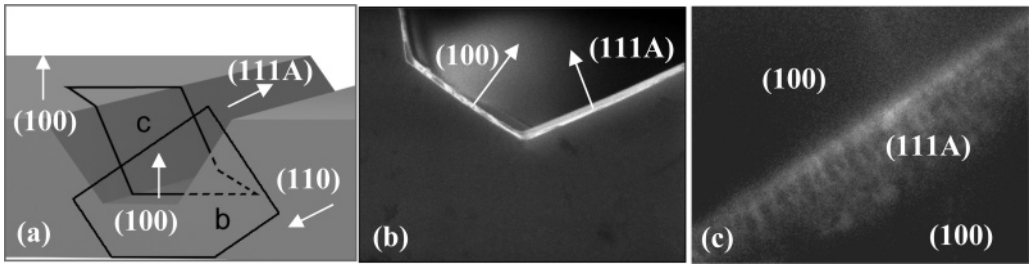


**Figure 1.** (a) Diagram of the etched trench labeled with the various crystalline facets. Black frames correspond to the views depicted in panels b and c. (b) SEM image of a cut across the trench. (c) Fluorescence image of the trench viewed from the top. Fluorescence is confined to the (111A) slopes proving selective binding of the scFv fragments to that facet. Note the negligible binding of antibody molecules to the (100) facets.

The 7- and 12-mer peptides used in most in vitro selection of binders to inorganic crystals are typically too short to assume a stable structure. Antibodies on the other hand, display a rigid three-dimensional (3D) structure which is potentially essential for high affinity selective binding.[1,2] Moreover, the recognition site in the latter case involves six amino acid sequences grouped into three complementarity determining regions (CDR). All together these CDRs form a large, structured binding site spanning up to 3 × 3 nm. The critical role of the antibody 3D structure for the recognition of organic crystal facets is well established.[1,2]

Another hint to the importance of rigidity for facet recognition is provided by the rigid structure characterizing antifreeze peptides that target specific ice facets.[22] It has also been shown that the stable helical structure of a 31-mer peptide catalyzing calcite crystallization is essential for inducing directed crystal growth along a preferred axis,[23] possibly due to its differential binding to the various facets. Structure rigidity may thus turn central to facet recognition by biomolecules, underscoring the importance of antibody libraries as a promising source for selective binders.

Selective binding to specific crystalline facets can be directly utilized for numerous micro- and nanotechnological applications including positioning of nanocrystals at a well-defined orientation, governing crystal growth and forcing it to certain directions,[23] and positioning nanometer scale objects at specific sites on a substrate marked by certain crystalline facets. An application of one of our soluble antibodies to the latter task is demonstrated in Figure 1. Using conventional photolithography and $H_3PO_4$:$H_2O_2$:$H_2O$ etch, a long trench has been defined on a GaAs (100) substrate in the (110) direction (Figure 1a). Due to the slow etching rate of phosphoric acid in the (111A) direction, the process leads to slanted (111A) side walls and a flat (100) trench floor (Figure 1a). Figure 1b depicts a scanning electron microscopy (SEM) image of a cut across the trench, proving the slanted walls are indeed tilted in the (111A) direction (54.7° relative to the (100) direction). When the isolated scFv antibodies are applied to the GaAs substrate, they attach themselves selectively to the (111A) slopes.

To image the bound antibody molecules, they were targeted with anti-human secondary antibodies conjugated to a fluorescent dye, Alexa Fluor. As seen in Figure 1c, fluorescence is limited solely to the (111A) slopes with practically no background signal coming from the (100) surfaces. Control experiments depleted of the scFv fragments exclude possible artifacts such as natural fluorescence of the (111A) facets, selective binding of the fluorescent dye, or secondary antibodies to that facet, etc.

Figure 1 already proves that the selected scFv antibody molecules recognize and bind selectively GaAs (111A) as opposed to GaAs (100). As such, they can be used to localize practically any microscopic object on (111A) surfaces with negligible attachment to other crystalline facets. The rest of the paper describes the isolation of such binders using phage display technology, quantification of their selectivity, and analysis of their amino acid sequences.

The Ronit1 scFv antibody phage library[24] used in the present study, is a phagemid library[25] comprising $2 \times 10^9$ different human semisynthetic single chain Fv fragments, where in vivo formed CDR loops were shuffled combinatorially onto germline-derived human variable region framework regions of the heavy ($V_H$) and light ($V_L$) domains.

To select scFv binders to GaAs (111A), about $10^{11}$ phages (≈100 copies of each library clone) were applied to the semiconductor crystal (panning step). After the unbound phages were washed, the bound ones were recovered by rinsing the sample in an alkaline solution. The recovered viruses were then quantified by infecting bacteria and plating dilution series on Petri dishes. The amplified sublibrary was applied again to the target crystal facet and so on. It typically took three to four panning rounds to isolate excellent binders to the target. As evident from Figure 2, the number of bound phages retrieved from the semiconductor grew 300-fold when panning was repeated three times. For comparison, the nonspecific binding of identical phages (M13) carrying no scFv fragments remained low throughout the selection process. It was experimentally found that blocking with milk (see Methods) was essential for preventing nonspecific binding of the phages to the GaAs targets. Interestingly, as shown in the Supporting Information, in the absence of blocking against nonspecific binding (a step missing in ref 20), the nonspecific binding of phages through their coat protein to GaAs (100) was larger than to GaAs (111A). Figure 2 proves selection of increasingly better binders to GaAs (111A), yet it provides no indication of selectivity with respect to GaAs (100). Indeed, as indicated by the left two columns of Figure 3, application of the polyclonal population of binders selected on GaAs (111A) to GaAs (100) shows

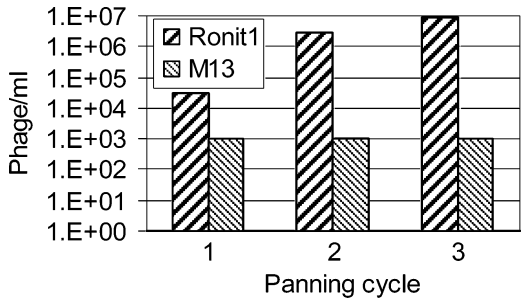


**Figure 2.** Enrichment of anti-GaAs (111A) phages carrying scFv fragments vs panning cycle. Phage concentration has been deduced by counting colonies of *E. coli* bacteria infected with different dilutions of the phages recovered after each cycle. The monotonic increase in binding of phages carrying scFv (Ronit1) is contrasted with the much weaker, nonspecific binding of similar phages lacking the scFv antibody. The value, 1000 phages/mL, of the latter sets an experimental upper limit on their binding. The actual values are likely to be smaller.

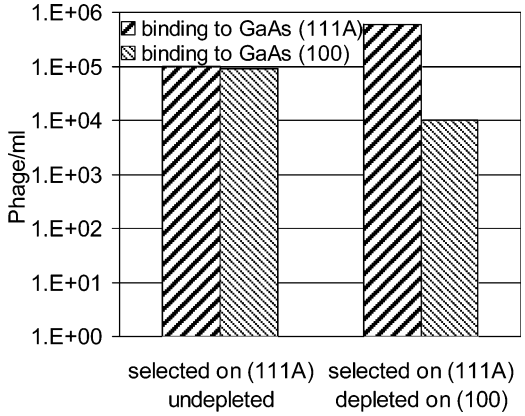


**Figure 3.** Density of recovered binders to GaAs (111A) after three panning cycles. The two right (left) columns correspond to selection on GaAs (111A) with (without) depletion on GaAs (100).

similar binding to the latter crystalline facet. The process described above thus produced good but nonselective binders.

Preferential binding to a given crystalline facet was achieved by a slight modification of the process. The phages recovered from the first panning on GaAs (111A) were amplified in *E. coli* bacteria and then applied to GaAs (100). This time the **unbound** phages were collected and applied in a second panning step to GaAs (111A). As evident from the two right columns of Figure 3, the "depletion" step on GaAs (100) enriched for specific phage clones that both bind GaAs (111A) and lack binding to GaAs (100). Binding of the selected phages to the (111A) facet was this time almost 100 times higher than to the (100) facet. This depletion step, which was crucial in our case, is missing in ref 20.

The polyclonal population of selected phages contains different scFv fragments, each characterized by different affinity and selectivity to the two crystalline facets. To correlate specificity with sequence, we next analyzed binding selectivity of individual clones. Monoclonal binders were isolated by infecting *E.* coli bacteria with the sublibrary and plating them on solid agar. Since each bacterium can be infected by a single phage, all bacteria in a given colony carry DNA coding for the same scFv fragment. Infection of the colony with helper phages resulted in release of phages displaying the same scFv on their PIII coat proteins. The isolated monoclonal phages were then analyzed by ELISA against GaAs (111A) and (100). The sequences of the light ($V_L$) and heavy ($V_H$) CDR3 of 10 monoclonal binders that were identified by the ELISA assay are listed in Table 1. The sequences of CDR1 and 2 can be found in the Supporting Information. Clones b7, e11, d11, and a3 bind GaAs (111A) and (100) equally well. Clones e1, f1, and EB bind preferentially GaAs (111A). Clones c7 and d3 bind GaAs (100) better than GaAs (111A). Colors label the nature of the amino acid side group according to the classification in the bottom row.

The 10th clone, marked EB, was found in another scFv pool. The sequence of its $V_H$ CDR3 shares similarities with the same region in the Ronit1 library clones. The sequence of its $V_L$ CDR3 is identical, except a single amino acid, to the $V_L$ of b7. Inspection of the sequences reveals significant similarities between the different clones, some of which can be traced to conserved amino acids in the library. However, other similarities are attributed to the selection process itself, most notably; the abundance of positively charged amino acids in $V_H$ CDR3 is twice as high compared with the frequency of such residues at those positions in a random sample of library clones. Out of 47 clones sampled randomly from the library, only one $V_H$ contained a positively charged amino acid in the first position and only 11 displayed such an acid in the second position. The 10 selected binders, on the other hand, contained three positively charged amino acids in the first position and five in the second position. We have also compared the sequences of Table 1 to those of scFv selected on gold[26] (not shown). The abundance of positively charged amino acids in $V_H$ CDR3 of the latter was less than half compared with the former, and no negatively charged amino acid was found in the first six positions of CDR3 $V_L$. Those were replaced by polar uncharged amino acids, predominantly serine. The comparison with the library and anti-gold scFv sequences thus indicates the importance of positively charged amino acids in positions 1−3 of CDR3 $V_H$ and the negatively charged amino acids in CDR3 $V_L$ for binding GaAs. Higher content of positively charged amino acids was also found in both chains of CDR1.

Figures 2 and 3 correspond to scFv fragments displayed on phage particles. For practical applications, such as the one demonstrated in Figure 1, it is desired to have soluble monoclonal scFv fragments detached from the phage coat proteins. ELISA assays of the EB scFv fragment of Table 1, in its soluble form, are presented in Figure 4.

Bars 1−6 correspond to six ELISA assays on GaAs (111A) and GaAs (100) pieces, 4 × 4 mm each. After the substrates were washed, the bound antibodies were reacted with anti-human horseradish peroxidase (HRP) and binding was quantified by adding tetramethylbenzidine (TMB) colorimetric substrate, and reading the resulting optical density at 450 nm. Bars 7−9 provided the following controls. Bar 7 quantified nonspecific binding of the secondary anti-human HRP to the ELISA plate in the absence of the EB scFv and

**Table 1.** CDR3 of the Light and Heavy Chains of 10 Clones[a]

| Clone | $V_H$ CDR3 | $V_L$ CDR3 |
|---|---|---|
| c7 | G G L G K R G A D C P D Y | Q Q Y G N S P H T |
| b7 | D R T A G Y F D Y | N S R D S S G N R V V |
| e1 | D E G T F | Q T S D S R L D A |
| f1 | R E K I G C G G D C L D L | Q S Y D S R L S A |
| f10 | R R R G A T A F D Y | Q A W D S D T |
| e11 | D R A N S G G W F G G D S | Q S Y D S N D Y V F |
| d11 | G T S G W Y G I D Y | Q Q S Y S T P W |
| a3 | A D D L W I D N H P P N H Y S F D S | Q Q S Y S A P P |
| d3 | P R E M N A T Y P F D S | Q Q Y G R S P T G G |
| EB | R R Y A L D Y | N S R D S S G N H V V |
| Positively Charge | Negatively Charge | Non polar Aliphatic | Polar Uncharged | Aromatic | Cyclic | |

[a] Colors depict the nature of the amino acid according to the legend presented in the bottom row.

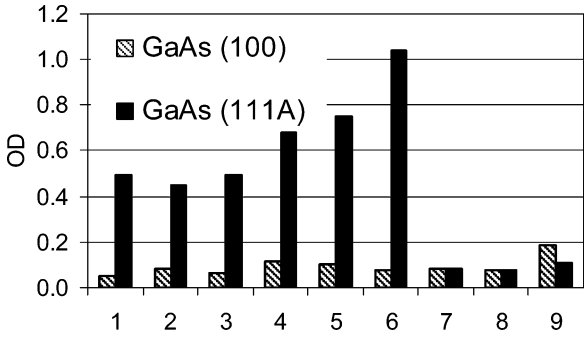


**Figure 4.** Bars 1−6 display the results of six comparative ELISA assays of the EB scFv molecule (detached from the phage) on GaAs (111A) and GaAs (100) substrates. The optical density (OD) reflects the number of bound molecules in arbitrary units. Bars 7−9 display the results of three control experiments (see text) and can be used to estimate the background signal, about 0.1 OD, coming from sources other than selective binding of the scFv to the semiconductor substrates.

semiconductor substrates. Bar 8 corresponded to nonspecific binding of the scFv to the plate and bar 9 to nonspecific binding of the secondary antibodies to the semiconductor substrates.

The background ELISA signal, depicted by bars 7−9, accounts for most of the GaAs (100) signal in columns 1−6. Subtracting this background from columns 1−6, we find remarkable preference to GaAs (111A) compared with (100). Interestingly, the binding of the secondary antibody to GaAs (100) is almost twice as large compared with its binding to GaAs (111A), just opposite to the selectivity of the isolated scFv fragments. Overall, Figure 4 proves that the isolated scFv preserves its selectivity also when detached from the phage.

Little is known on the interaction between biomolecules and inorganic surfaces, let alone recognition of such surfaces by antibody molecules. The GaAs surface is modified by surface reconstruction, oxidation, and possibly other chemical reactions. Moreover, it displays atomic steps and possibly surface defects. It is therefore difficult to estimate how much of the underlying crystalline order manifests itself in the recognition process. Unfortunately, no experimental tools capable of determining these parameters with atomic resolution exist for the moment. The recognition mechanism is hence unclear except the accumulating indications for the importance of structural rigidity discussed in the Introduction. The discrimination between the two crystalline facets may reflect the different underlying crystalline structure, may stem from the different surface chemistry of the two facets, or may result from global properties such as atom density and different electronegativity. The latter factor has been found to be important for the differential binding of short peptides to different semiconductors.[21] The abundance of positively charged amino acids in the heavy chain of CDR1 and CDR3 and the light chain of CDR1 may indicate affinity to the exposed gallium atoms. The negatively charged amino acid in CDR3 $V_L$ (missing in anti-gold scFv isolated from the same library) combined with the positively charged CDR3 $V_H$ may match the polar nature of GaAs.

In summary, the remarkable variety of binding sequences produced by the immune system, as represented in a semisynthetic antibody library displayed on the surface of phages, was shown to include single chain Fv discriminating between different facets of the same semiconductor. Beyond applications to the positioning of nanometer scale objects at desired sites or direction of crystal growth, the effect of semiconductor binding on the antibody may be harnessed to trigger biological processes, similar to those resulting from binding of biological antigens. In that respect, antibody binding is biologically more accessible than peptide binding. Imagine for instance the task of localizing a semiconductor nanocrystal at a given site. In a free running process confirmation of such assembly is practically impossible. However, the triggering of a certain biological process by the binding of the nanocrystal to the antibody may be engineered to signal successful assembly and even trigger the next assembly step. Recognition of manmade materials by antibodies thus opens new opportunities for a **functional** interface between biology and nanotechnology, far beyond what was exercised up until now.

On a more fundamental level we note that the remarkable selectivity reported here, combined with previous studies of

structured peptides, hint to the importance of a rigid 3D structure for crystal facet recognition.

**Methods. (a) Antibody Selection. (1) Blocking.** The semiconductor substrates were incubated for 1 h at room temperature in Tris-buffered saline (TBS) and 2% milk and then rinsed six times in TBS to wash excess milk.

**(2) Panning.** The semiconductor substrates were exposed to the Ronit1 library in TBS, 1% milk. After the samples were rocked for 1 h at room temperature, the surfaces were washed 10 times with TBS, 0.1% TWEEN 20.

**(3) Elution.** The phages were eluted from the surfaces by adding triethylamine (pH 12, 0.1 M) for 10 min, transferred to a fresh tube, and then neutralized with Tris (HCl) (pH 7).

**(4) Quantification.** The eluted phages were titrated in dilution series on solid agar, and colonies were counted to estimate the number of adsorbed phages. Nonspecific binding was estimated by titrating the Kanamycin resistant M13KO7 helper phages on Kanamycin plates.

**(b) ELISA Assay.** Squares (4 × 4 mm) of GaAs (111A) and GaAs (100) were placed in a 96-well ELISA plate with 4% milk for 60 min and then washed six times with PBS. The soluble scFv were then added in PBS and 2% milk and allowed to complex with the surface. Unbound scFv were washed away, and labeled secondary anti-human HRP were added to the wells. Binding of the scFv to the substrates was quantified by reaction with TMB colorimetric substrate.

**(c) GaAs Etching.** GaAs (100) was etched in $H_3PO_4$:$H_2O_2$:$H_2O$ 1:13.8:13.2 mixture in a −12 °C bath for 30 min.

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**Supporting Information Available:** Figures showing the M13KO7 phage binding to the various substrates and the light and heavy chains of CDR1 and -2 for 10 clones. This material is available free of charge via the Internet at http://pubs.acs.org.

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