Supporting Information for:

Electron Microscopy Imaging of Proteins on Gallium Phosphide Semiconductor Nanowires

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Protein solutions

HSA: Before use, HSA (Sigma) was purified from dimers, oligomers and contaminating proteins as previously described.¹ Fractions containing HSA monomer were pooled, lyophilized and desalted by gel filtration on a G25 Sephadex superfine column in Millipore water. In experiments with only HSA the concentration was 1 mg/ml in 10 mM Tris-HCl, pH 7.5.

Laminin: For the 80 nm diameter NWs 1.27 mg/ml laminin (Sigma) in 50 mM Tris-HCl, 150 mM NaCl, pH 7.5 was used. This laminin purification is mainly laminin 1. For the 20 nm NWs, the laminin solution was diluted 1:1 in 10 mM Tris-HCl, pH 7.5, resulting in a final buffer of 30 mM Tris-HCl, 75 mM NaCl, pH 7.5.

HSA+Laminin: In the experiments with a mixture of HSA and laminin the protein concentrations were 0.5 mg/ml HSA and 0.5 mg/ml Laminin in 10 mM Tris-HCl, pH 7.5.

Human blood plasma: Aliquots of defrosted citrated human blood plasma was centrifuged 3 times before use. The supernatant was carefully collected minding not to get any of the fat or precipitates
**Laminin adsorption on NWs of varying sizes**

Figure S1 depicts cryo-TEM images obtained from NWs incubated with either laminin, Figure S1(a), or laminin and HSA, Figure S1(b). For the NWs incubated with laminin only, it can be seen that the thicker NW has a less well-defined crystal structure as seen by the darker bands along its axial direction. This difference in crystal structure did not give any variations in the observed protein coverage. For the NWs incubated with laminin and HSA, we found a few kinked NWs, see Figure S1(b). These kinks did not give any observable effect on the protein corona, corroborating our belief that the protein adsorption is more related to NW material, than to specific crystallographic directions.

![Figure S1. Cryo-TEM images depicting laminin adsorbed on NWs (a), and laminin and HSA adsorbed on NWs (b).](image)

**Additional images showing the laminin corona in MEM**

When imaging the laminin 1 incubated NWs in MEM, a darker corona extending around the NWs could be observed. Figure S2(a) shows this darker area around the NWs for 20nm GaP NWs incubated with laminin 1. Figure S2(b) shows C1s imaging of the same area where it can be observed that the area between the NWs (dark in S2(a)) has a higher carbon content. Hence, it is most likely covered in protein.

![Figure S2. (a) MEM image depicting laminin 1 incubated 20 nm GaP NWs. (b) shows the same area but imaged using C1s photoelectrons. hν =330eV, photoelectron kinetic energy =41eV.](image)
**PEEM carbon imaging: references**

As a reference for our PEEM measurements, we studied NWs which were incubated in Tris buffer without any protein. In Figure S3(a), we show 20nm GaP NWs which have been incubated with Tris (without protein) as imaged using secondary photoelectrons in the PEEM. In Figure S3(b), we show the same area, but imaged using carbon 1s-photoelectrons. For the NWs that hadn’t been incubated with protein, it was not possible to see any traces of the NWs in the carbon images.

![PEEM images](image)

*Figure S3. (a) PEEM image obtained using seconday photoelectrons depicting 20 nm GaP NWs that have not been incubated with proteins. \( h\nu = 133\text{eV} \). (b) shows the same area but imaged using C1s photoelectrons. \( h\nu = 330\text{eV} \), photoelectron kinetic energy = 41eV. The scalebar in (b) is also valid for (a).*

**TEM imaging: references**

As a reference for our cryo-TEM measurements, we studied NWs which were incubated in Tris buffer without any protein. For the NWs that hadn’t been incubated with protein, it was not possible to see any traces of the proteins.

![TEM images](image)

*Figure S4. Cryo-TEM images showing GaP NWs that have not been incubated with proteins. The contrast has been adjusted in order to more clearly highlight the areas outside the NWs.*

**Laminin degradation upon annealing**

Upon annealing, the proteins are expected to degrade. We performed an in-vacuum anneal at 150°C in order to study the effect on the adsorbed carbon inside the SPELEEM instrument. More specifically, we studied the C1s peak shape for laminin covered NWs using micro-X-ray photoelectron spectroscopy (µXPS), *i.e.* studying the energy dispersion of the photoelectrons escaping from the C1s core level from a selected area on the sample. In Figure S5(a), the C1s core level spectrum from NWs with laminin before the anneal step is seen. It was possible to fit the measured data using two peaks which we attribute to be related to C-C and C=C bonding, and C-N and C=O bonding, respectively. Upon annealing, Figure S5(b), it was seen that the peak
corresponding to C-N/C=O bonding was reduced in intensity by more than 70% indicating large chemical changes in the adsorbed protein. In addition, the C-C peak becomes more narrow upon annealing indicating a more homogenous sample, presumably reducing the number of C=C bonds.

Figure S5. µXPS obtained from GaP NWs incubated with laminin before (a) and after (b) an in-vacuum anneal step at approximately 150°C. After the anneal, the peak associated with C-N bonding (green) is reduced in intensity. The figure is color coded according to measured data (black), fitted data (red), background (gray), fitted C-C/C=C peak (blue), and fitted C-N/C=O peak (green).

References