Project A5.7: Light Activable Nanoparticles and Biomoleculs as Structural Basis for Design of Funcional Photonic Nanodevices and Switchabel Cell Probes

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Introduction

The organic and synthetic worlds are merging at the interface between nanomaterials and biologic systems and for the field of nanobiotechnology to evolve, more insights into the interactions at the interface of the nanomaterial surface and biological components are needed. However, the design of novel biohybrids, which are to be used in medicine and in the design of clever, responsive materials requires deeper understanding of basic synthetic chemistry principles as well as physics and biochemistry behind the biomolecular interactions and bio self assembly. Although the synthesis of nanostructured materials based on nanoparticles and bioplymers such as DNA evolved rapidly in the past decade[1], the initial discoveries still failed to deliver the number of available hybrid functional devices such as biosensors or novel materials with diagnostics/drug delivery potential. Nevertheless, the growth of the knowledge base in the area is immense and new insights into nanoworld, together with the interdisciplinary approach in development of the concepts and experimental procedures, will help pushing its boundaries towards new limits. The particular areas to be further addressed are development of the novel synthetic procedures that enable the control of the surface interaction between nanomaterials and biomolecules and development of novel approaches to probe the interface as well as the *in vivo* effects of nanostructures. In particular, to enable the studies *in vivo*, inorganic nanomaterials need to be designed with particular optical characteristics or additionally modified both to give required reporting signal and to penetrate the cell.

However, the biofunctionlisation of nanoparticles (and nanorods), although very important for design of biosensors and *in vivo* studies (transport, labelling), still poses a significant challenge. Despite the advances achieved in attachment of different classes or proteins (antibodies, STV, peroxidases, fluorescent proteins) and DNA to the surface of metallic NPs such as Ag, Au and quantum dots, protein attachment suffers both from little control over stereochemistry and stoichiometry of attachment and protein deactivation.[2] The other issues, which arise when metal or metal oxide NP are used in bio applications, is first, their inherent lack of optical properties that would enable both quantification and tracking of such NP within cell, and second, their toxicity due to the radical production (i.e. TiO_2), which can lead to a protein and DNA damage. Although originally considered as an obstacle due to the toxicity issues, this ability of oxide and quantum dot NPs can be utilised for a controlled apoptosis of cells, the investigation of which is a topic of one of the subprojects described below.

We are trying to address several issues occurring on the nanoparticle-biomolecule interface and use light to control different properties of NPs. For example, we are investigating the chemical design of bi-functional and tri-functional bio-friendly linkers containing fluorophores, to stabilise and visualise NPs *in vivo.* In addition we are trying to explore the ability of photo induced radical production of certain NP species for programmed apoptosis of cancer cells.

Shortly, the aims of this interdisciplinary project is to harvest the potential of different classes of nanoprticles for design of biosensors, cell delivery systems and the study of signalling pathways within the cell as well as to investigate the nanostructuring potential of different classes of biomolecules (i.e. DNA), which can be used both in the development of new, bio based materials and catalysts.

Presented A5.7 project is divided into several interdisciplinary sub projects, which are based on NP synthesis, organisation and biofunctionalisation of NPs as well as the use of biomolecular anchors for different bio-nano hybrid design.

We started working on the concepts year and a half ago and the manuscripts are in preparation to be submitted for publications at the beginning of 2011 and first publications are expected in spring 2011 onwards.

1. ***DNA Modifications for Protein Conjugation and Immobilisation (Dr. Daniel Fritz, 9 months, Dr. Ishtiaq Ahmed, 3 months - ongoing)***

Short DNA strands have found an important application in nanotechnology, due to the inherently strong but reversible DNA base pairing and ease of their chemical synthesis. The programmable DNA strands can be used in design of complex DNA structures, DNA computing and DNA directed immoiblisation.[3] However, to enable the use of DNA as anchoring element, DNA usually needs to be attached to the biomolecule or nanoparticle of interest. Several methodologies are in use already to enable design of protein-DNA or protein–NP conjugates based on use of bifunctional linkers, thiols DNA or modification of cofactors with DNA.[4] However, there is a range of very important proteins which cannot be modified using chemical methods such as bifuntional linkers. Therefore we focused our efforts on design of novel protein tag binders based on Ni-NTA- His tag or tyrosine binding molecules which can be attached onto 5' of synthetic DNA. In particular, we are interested in preparation of *prion*- (collaboration with Dr. Loredana Casalis, Prof. Giacinto Scoles, SISSA laboratories, Trieste) and *proteasome*- (collaboration with Dr. Michal Sharon, Weizman Institute and Dr. Ester Segal, Technion, Haifa) – *DNA conjugates.*

Prions are highly infective, small proteins implicated in Alzheimer and Creutzfeldt Jakob diseases, which generally exist in two forms, harmless folded and infectious misfolded. To investigate the prion interactions with other proteins or potential drugs, infectious prions should be immobilized in a form of the chip to result in a prion nanoarray. To achieve that, His tagged prions should be attached onto the suitable surface using mild method of DNA directed immoilisation[3] where prion-DNA conjugate is made and hybridized to the complementary DNA strand attached to suitable surface. To prepare prion-DNA conjugates we have synthesized DNA containing NTA molecule (Figure 1) , which can coordinate His tagged prion in the presence of Ni^{2+} salt.

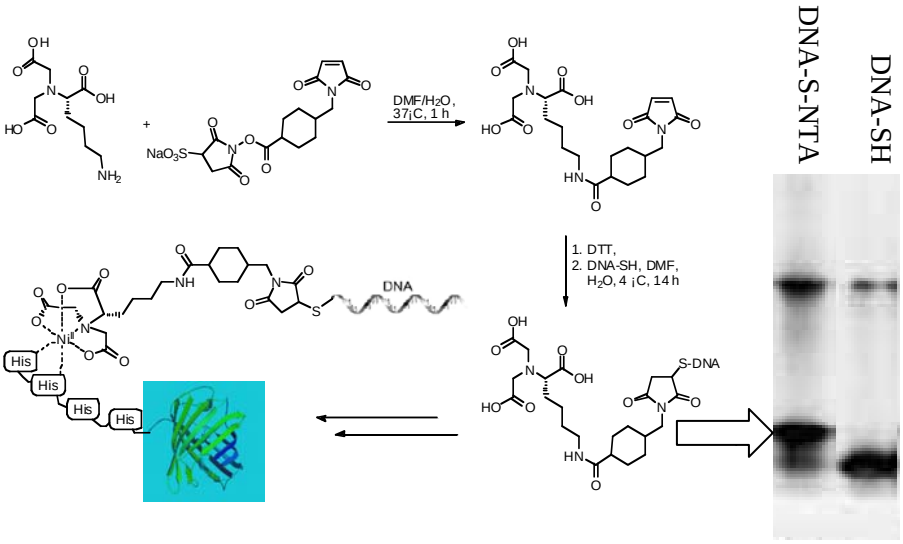


Figure 1: Synthesis of the bifunctional NTA linker, subsequent DNA modification and the principle of prion attachment. The NTA - DNA construct is marked with arrow on the gel shown on the right hand side.

To improve the binding constants of His tagged protein and NTA modified DNA, more than one interaction between NTA, Ni and His tag is needed.[5] We are currently synthesising new DNA modifier containing up to three NTA groups and maleimide group, which can be covalently attached to thiolated DNA under mild conditions. Complementary DNA is positioned to the gold surface using AFM nanografting[6] and the hybridasation of modified prions is achieved (Figure 2 and manuscript in preparation).

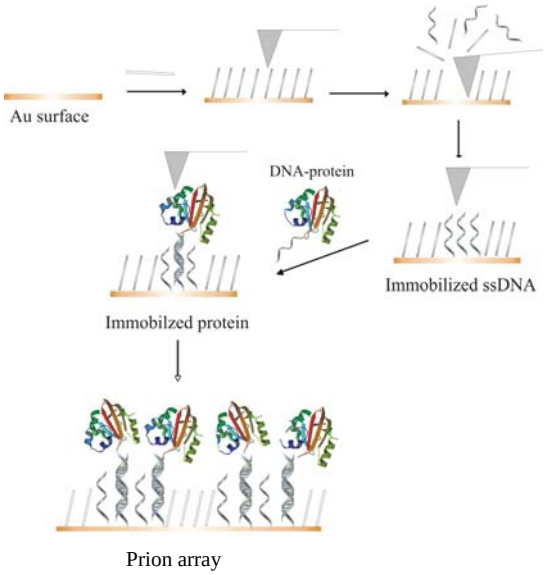


Figure 2: Principle of AFM nanografting and attachment of DNA modified prions.

In addition, Dr. Ahmed is working on utilising the property of certain classes of molecules to bind to tyrosin to enable mild modification strategy of proteasome-DNA conjugate. Such conjugates are used in design of proteasome activity sensor based on attachment of proteasome on porous silica substrate (Figure 3) and detection of degradation products using mass spectroscopy (collaboration with Dr. Sharon and Dr. Segal). Proteasome complex makes up the major degradation machinery of the cell which enables to degrade old and damaged proteins in specific manner.[7] One of the amazing properties of the cell is its ability to regenerate and renew itself. Old and damaged proteins are constantly being degraded and new proteins are continuously produced. The major degradation machinery is composed of the proteasome complex, a huge protein assembly with a mass of 2.5 MDa that breaks down proteins in a specific manner , details of which are still not understood. Therefore the design of novel proteasome sensors could shed light on the complex mechanism of this important cell protein pathway.

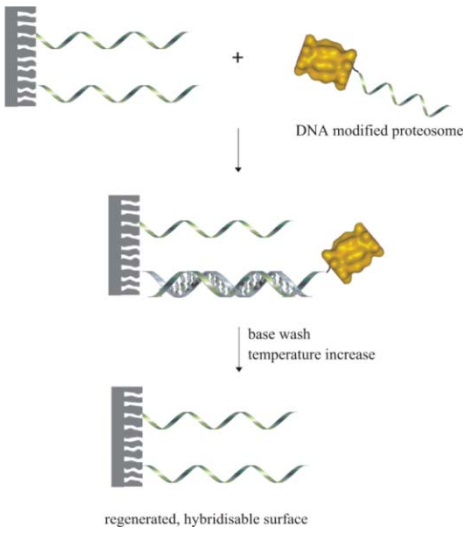


Figure 3: Mild attachment and removal of immoiblised proteasome from porous silicon substrate to enable design of proteasome activity sensor based on DNA directed immobilisation.

2. *Design of Protein/peptide Nanoparticle Hybrids (Dr. Martina Altemöller, 16 months; Dr. Andre Petershans, 2 months- ongoing, Dania Kenziora, 19 months- ongoing)*

Preparation of NP-protein hybrids is crucial for numerous applications such as labelling, protein interactions studies or design of biosensors. However, this still represents a huge challenge due to the different properties of both building blocks, in particular protein sensitivity to chemical modification. Thereofore we have focused our efforts to address problems of mild attachment and visualisation of hybrids by developing novel bio friendly linkers and chemical routes in hybrid design.

2a) Synthesis of Tri-functional Linkers for Nanoparticle Modification

Metal and metal oxide NPs posses a range of remarkable properties such as strong surface plasmons and the ability to be used in induced hyperthermia or photodynamic treatments.[8] However, limited success has been obtained with use of such nanoparticles for drug delivery or *in vivo* biosensing applications due to the lack of control over the surface modification and subsequent *in vivo* tracking.

Therefore, we have and continue to design bio-friendly NP surface modifying linkers, which enable stable (covalent) attachment of molecules of interests (proteins, DNA, drug candidate) and fluorophores easing the NP tracking within the cell (Figure 4).

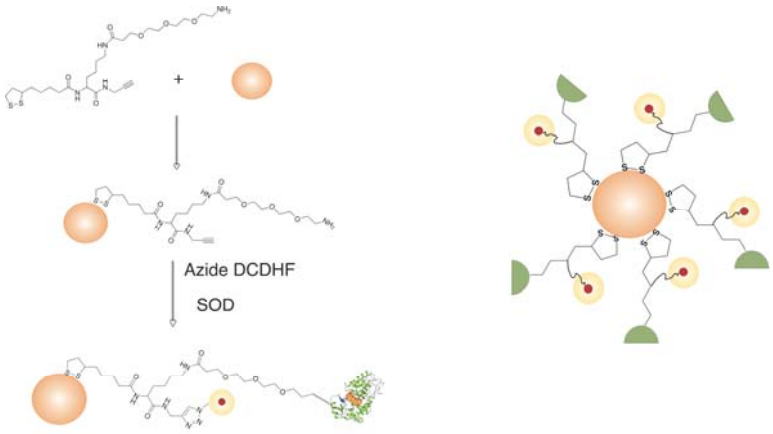


Figure 4 : Principle of tri-functional linker use for biomolecule attachment onto NP surface.

2b) Attachement of Bag1 peptide onto magnetic and gold NPs

Bag1 peptides are found in many cancer cells and are believed to play an important role in their growth inhibition.[9] We are attempting to utilise the cancer supression ability together with hyperthermic effect of certain classes of NPs (magnetic and Au NP) to induce controlled apoptosis of cancer cells. To achieve this, different approaches are investigated to attach Bag 1 to the surface of NPs in its functional form, such as use of HA tag modification (through tyrosine attachment chemistry) of protein with trifunctional linker containing functional groups for NP attachment (thiols for Au NP and amines for magnetic) and fluorophores (*via* click chemistry) (Figure 5). The Bag 1 peptides are expressed in Prof. Andrew Cato's group (Campus Nord, KIT) and all Bag 1-NP hybrid investiagtion is done in collaboration with Campus Nord.

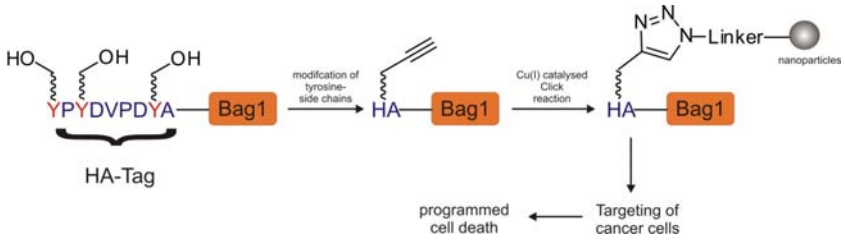


Figure 5: Principle of Bag1 modification and attachement onto the surface of NPs.

2c) Use of SNAP protein tag for attachment of proteins to quantum dots.

We are trying to develop the methodology based on SNAP tag technology to attach proteins modified with SNAP tag onto quantum dots to enable tracking of the proteins in cells. This project has shown promising results although currently in the initial phase and we are sure to be able to report more on it in the second reporting phase.

2d) Biotemplated synthesis of metal NP for direct attachment of proteins

Metalothionein (MT) proteins are known to bind metals though its cystein rich regions. We are trying to use this property and express MT-protein of interest (POI) fusion proteins to enable the growth of NP in proximity of proteins and under mild conditions. Standard procedures of metallic NP preparation use either harsh reduction conditions or heating, both of which can cause protein denaturation. Therefore, we have chosen a photochemical approach using a photoinitiator and light to reduce metal salts added to the fusion protein.[10] In this way Au, Ag, Cu and Pt NP could be formed (Figure 6), although the conditions are still not optimised. Until now, maltose binding protein (MBP)-MT protein was expressed, with future experiments focusing on more biologically relevant proteins such as fluorescent proteins or peroxidases.

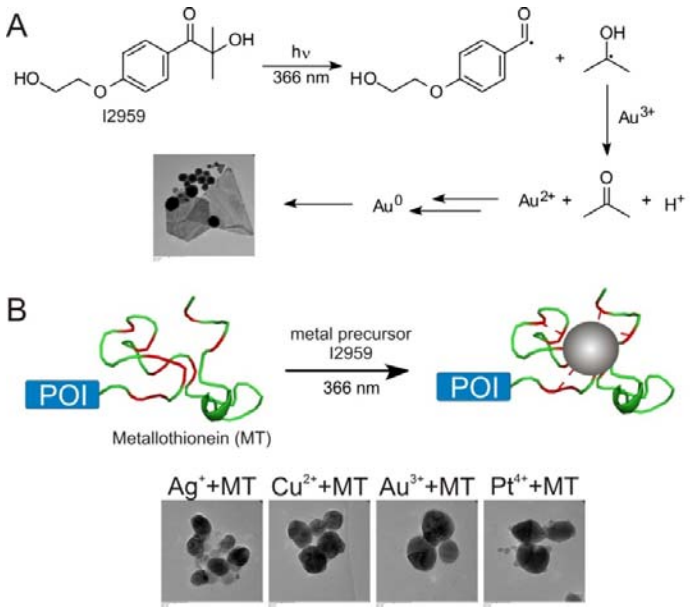


Figure 6: The principle of NP synthesis in presence of photoinitiator (A) and the principle of controlled growth of NPs in presence of fusion proteins (**B**).

3) Synthesis and Biofunctionalisation of TiO_2 NP (Bianca Geiseler, 19 months – ongoing)

TiO_2 NPs have a wide range of applications in solar cell technology, photocatalysis and medicinal biotechnology.[11]We are working on a design of well characterised bio-TiO_2 systems based on use of linkers that enable covalent attachment of biomolecules to TiO_2 NPs.

3a) Design of bifunctional TiO_2 modifiers

For the modification of the TiO_2 NP, a catechol moiety is very useful since OH groups are known to form a strong charge transfer complex with TiO_2 surface. We have used this property to design bifunctional linkers based on dopamine backbone. Such linkers contain both catechol moeity to attach onto TiO_2 surface and maleimdie group for thiol attachment. Cysteine containing molecules such as modified fluorescent proteins, short peptides or peptoids (collaboration with Prof. Braese) and thiol containing oligonucleotides of different length can be coupled directly onto the linker (Figure 7).

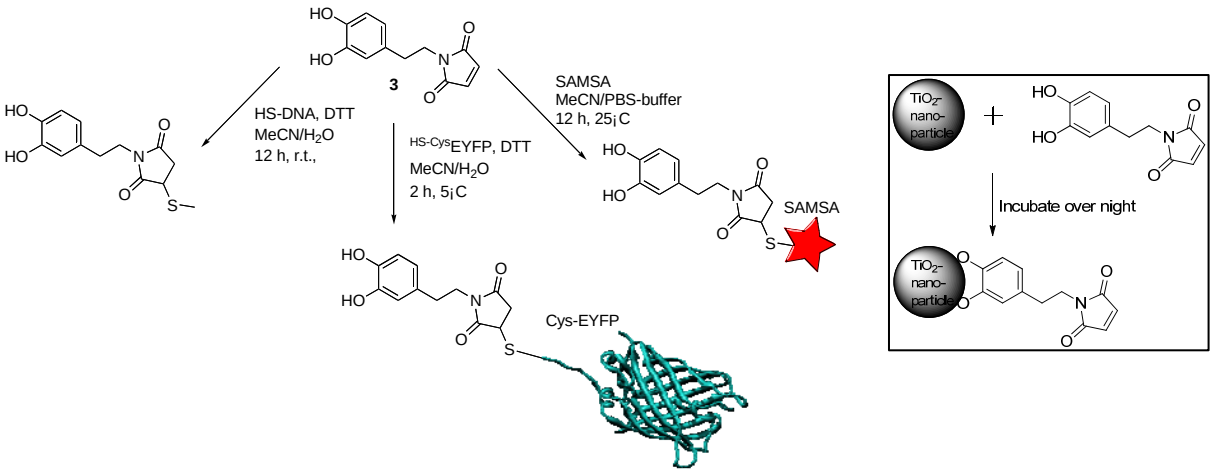


Figure 7: Attchment of different classes of thiol containing molecules to dopamine maleimide. Inset shows attachment of dopamine-maleimide to TiO_2 NPs.

Biomoleucle-TiO_2 hybrids are analysed using HR-TEM, gel electrophoresis, fluorescence correlation spectroscopy (FCS) (in collaboration with Prof. Nienhaus) and dynamic light scattering (DLS). Both modified and unmodified TiO_2 NPs are used to investigate the production of reactive oxygen species upon light irradiation. ROS are well known to take part in cell damage but have recently been shown to play an important role in control over signalling pathways. Therefore, we have investigated the production of radical species *in vitro* using methodology established by Fruk *et al*.[12] In addition, naked and dopamine modified NPs were used to investigate ROS production in BY-2 tobacco cells (collaboration with group of **Prof. P. Nick**). We have shown that there is a significant increase in ROS production when TiO_2 NPs are present and we are currently preparing a manuscript for publication. In addition, several undergraduate students were working on design of novel dopamine based linkers, which could give us a library of linkers for TiO_2 modification.

4) Use of Plasmonic NPs in Solar Cell Design

The alternative energy research is getting on top of the list of issues to be resolved in the next decade. The most promising, long-term energy sources are driven by the sun: light, wind, waves and biomass. Thin-film solar cells are of high interest due to good electrical properties and low material consumption. Traditional thin-film cells, however, have considerable transmission losses because of the reduced absorption volume which leads to lower efficiency. A promising new route to enhance

absorption in the active layer is the light-trapping by plasmonic nanostructures. Metallic nanoparticles have, in particular, shown large enhancement of the photocurrent in thin-film devices. In collaboration with **Dr. D. Schaadt** (CFN) we used Au, Ag and Pt nanoparticles prepared in different shapes by polyol and seed mediated methods to design of plasmonic solar cells. Polyol method typically uses ethylene glycol both as the solvent and reducing agent and in seed-mediated synthesis, small nanoparticle seeds are first prepared and then used to promote the growth of the different shapes of nanoparticles, among which we particularly focused on the use of nanocubes and nanospheres (Figure 8).

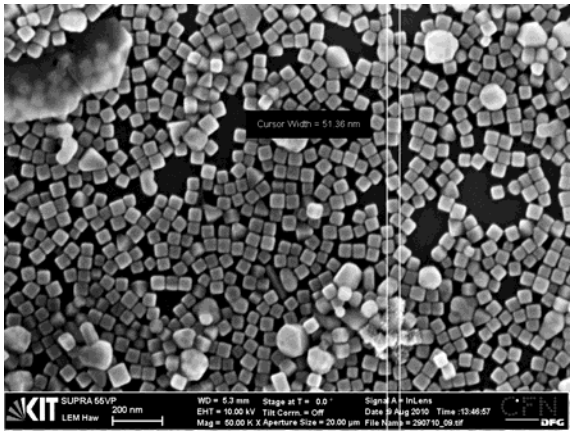
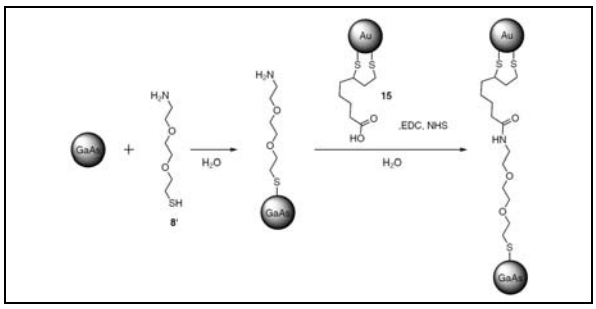


Figure 8: SEM image of Ag nanocubes prepared by polyol method. Avarage size of the cubes is 60 nm.

Following the nanoparticle preparation, a new method to immobilize particles on GaAs surfaces by use of bifucntional linkers (Figure 9) such as cysteinamine and lipoic acid have been developed and the use of linker prevented aggregation of NPs and allowed the control over the NP surface density.



A)

B)

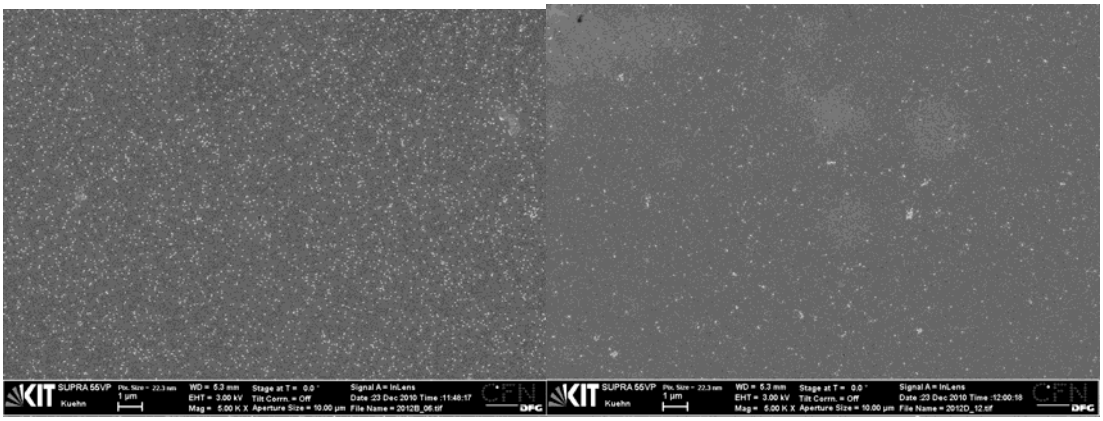


Figure 9: Immobilisation of Au NP onto GaAs surface using bi-fucntional linker and Au NPs on GaAs with (A) and without B) linker.

Photocurrent spectra of GaAs solar cells with and without particles have been recorded. These measurements show the dependence of the photocurrent enhancement on particle material, shape and density.

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