

Gold Nanoparticle-Assisted AFM Study of DNA Damage and Repair

E. Demir¹, H. Asimgil², H. Kavakli¹, S. Kizilel¹

¹Koc University, Chemical&Biological Engineering,
Istanbul, Turkey, 34450

²Koc University, Materials Science&Engineering, Istanbul, Turkey, 34450

ABSTRACT

The purpose of this research is to investigate gold nanoparticles as a potential scaffold for the characterization of DNA damage and repair using atomic force microscopy (AFM) techniques. The procedure consists of functionalizing the surface of gold nanoparticles with DNA which are then immobilized onto amine modified silicon or glass surfaces. Our objective is to examine various lesions in individual DNA molecules and follow in the AFM their direct reversal by DNA repair enzymes. In order to achieve this goal DNA repair proteins such as photolyase (repairs UV damaged thymine dimers in DNA using blue-light energy) is used as damage markers and imaged in complexes with DNA by AFM in order to locate and identify the damage sites. Force spectroscopy measurements will determine the mechanical fingerprints of various types of DNA damage caused by UV and gamma radiation and directly follow damage reversal in the presence of the repair activities. AFM images will be further compared with *in vitro* photoreactivation assay results.

Keywords: atomic force microscopy, colloidal gold, dna detection, photolyase

INTRODUCTION

Exposure of DNA to UV-light (~250-320 nm) can cause major UV-induced lesions such as cyclobutane-primidine dimers (CPDs) and pyrimidine-pyrimidone 6-4 lesions (6-4 lesions) [1]. The possible consequences of this excitement of DNA molecule by UV-light reach to serious diseases such as skin cancer in humans. As a purpose to survive the irradiation, living organisms have various abilities that counteract the damage done to their DNA. Particular repair proteins detect and repair specific lesions on the genome [1]. Especially photolyase represents specific type of protein functions through photoreactivation [2]. This process uses the energy of light (the energy comes from blue light photons) to reverse the damage done to DNA [3]. These studies on DNA damage and repair were generally performed *in vivo*. However, *in vitro* studies which have several uses in gene therapy and other clinical applications didn't have much evolvment except the

optical methods containing fluorescent dyes for DNA detection [4]. Detecting DNA damage with labeling methods has limited sensitivity which include the treatment of cells with radioactive compounds, isolation of labeled DNA, and characterization of DNA modifications by chemical methods.. [5]

In order to address these limitations, the emerging necessity of better signaling probes for DNA detection has met by metallic nanoparticles. These nanomaterials has been investigated for variety of applications in biological sciences during the last decade. Especially colloidal gold, with its desirable optical and magnetic properties, is used as chemical sensors and in detection of biomolecular structures [6]. Colloidal gold has been widely used for for biospecific interaction analysis as a sensing platform, for detection of DNA hybridization. [6] The approach that uses colloidal gold as probes for oligonucleotide detection was made by Mirkin *et al.* [7] who developed DNA/nanoparticle hybrids in order to get benefit of gold nanoparticles in optical measurements. DNA-nanoparticle hybrids has been formed using thiol adsorption chemistry in immobilization of DNA on nanoparticles where thiol capped (from 5' or 3' ends) DNAs are covalently bound to nanoparticle surface. Then the hybridization with other immobilized oligonucleotides results in particle aggregation.

For the quantitative as well as the qualitative analysis of oligonucleotides, atomic force microscopy represents a high resolution technique effective in even low concentrations (10 pM to 1 nM) of target DNA [8]. So it's quite sensitive to detect DNA concentrations and monitoring the nanoparticle size and aggregation in DNA hybrids by AFM. This is especially important in diagnostic methods which are commonly used for clinical purposes. Higher concentrations of DNA allow the UV-vis spectroscopy, however at lower concentrations, AFM has clear advantages to detect individually the nanoparticles and 50-mer oligonucleotides bound on the nanoparticles [8].

There are some studies done on AFM imaging single or double-stranded nucleic acids. Hansma and co-workers was able to see single stranded and double stranded DNA from 25 bases up to 100 bp immobilized on silicon surface with AFM [9]. Hamon *et al.* also investigated single stranded DNA-protein complexes at high resolution [10]. However the limitations of AFM technique in detection of soft biological molecules

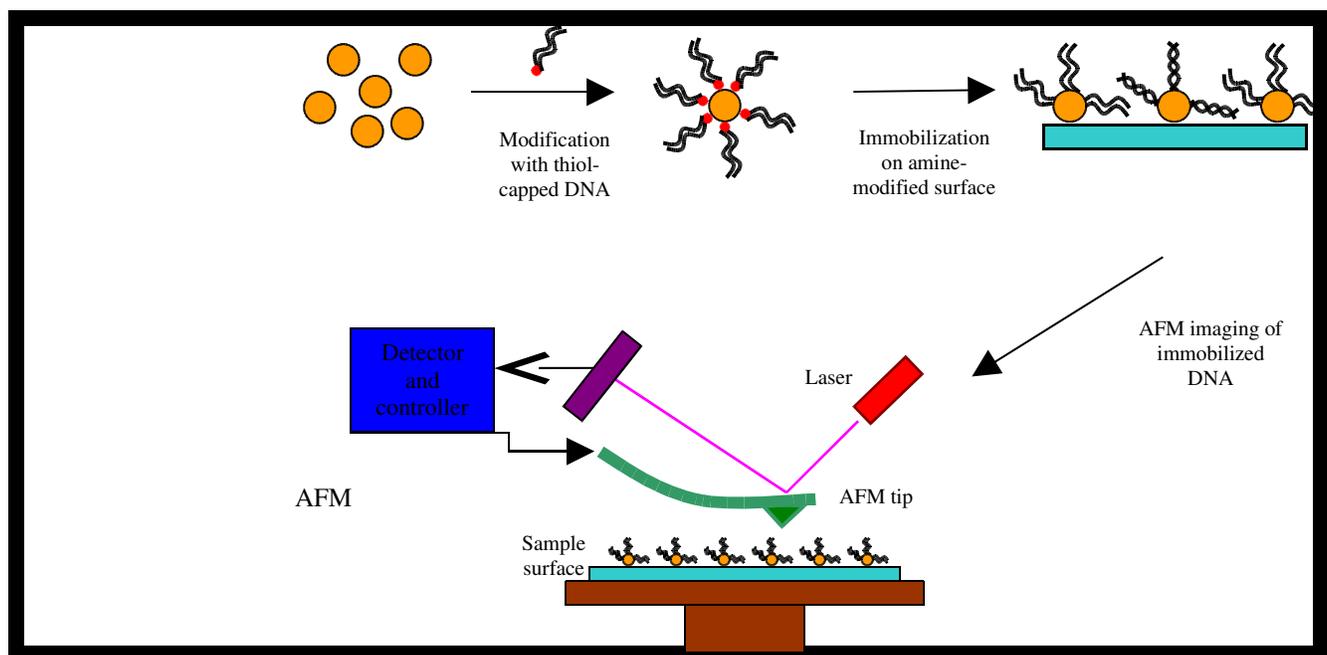


Figure 1. Schematic representation of the steps of modification of gold nanoparticles with DNA and AFM characterization.

prevents sensitive and effective imaging of DNA structure [11]. Monitoring DNA by AFM in air can be problematic due to the necessity of the stabilization of 3D structure. On the other hand, using buffer solution in imaging is also not as simple as imaging in air and gives lower resolution in images [12].

In vitro photoreactivation assay, modified photoreactivation assay, are biochemical techniques to identify CPD photolyase activity of a protein. However, repair activity of photolyase can only be seen indirectly. In this assay, plasmid DNA is exposed to UV light. UV damaged DNA lesions are then repaired *in vitro* by CPD photolyase in the presence of white or blue light. Following transformation of UV damaged and repaired plasmids into bacteria; Colonies are counted on selective solid medium and survival rate is calculated.

In this study, a healthy or damaged DNA will be immobilized on the surfaces of gold nanoparticles, which will then be immobilized on silanized glass surfaces for characterization of DNA by AFM (Figure 1). The effect of radiation dose on the degree of lesions in individual DNA molecules and their direct reversal by DNA repair enzymes will also be characterized by AFM in conjunction with photoreactivation assay.

MATERIALS AND METHODS

Preparation of Colloids

Gold nanoparticles were prepared according to Turkevich's method as described in previous reports [14-15]. 13 nm-sized nanoparticles were prepared by citrate reduction of H_{AuCl}₄. Next, the glassware used was

cleaned with aqua regia (3 HCl-1 HNO₃) and rinsed with distilled water. Stock solutions of 38.8 mM Sodium Citrate and 1 mM H_{AuCl}₄ were prepared and used whenever needed. First, 500 mL of 1 mM H_{AuCl}₄ in a 1L round bottom flask was brought to boiling with vigorous stirring. Then,, 50 mL of 38.8 mM sodium citrate was added and continued to boiling for 10 min. Finally, boiling was stopped, heating mantle was removed and stirring was continued for additional 15 min. After cooling to room temperature, the size of the nanoparticles were found to be 13±3 nm showing a absorbance maximum at 520 nm in UV-vis spectrophotometer(Fig. 2).

Modification of gold nanoparticles with thiolated oligonucleotide

50 µL of 10 µM ssDNA is added to 950 µL of AuNP solution (0.57nM). This solution is added 1 mL pf PBS buffer (10mM NaCl, 5 mM NaH₂PO₄/Na₂HPO₄, pH 7.2) and modification is progressed at RT for 24 h. The solution then is centrifuged at 14000 rpm for 20 min. The supernatant will be removed. We dispersed reddish pellet in 1 mL PBS buffer. After repeating this procedure twice, the dispersion is stored at 4 °C until use [8].

UV damage and repair of DNA

3 of the 4 aliquots are exposed to UV at 10, 30 and 50J/m². 4 aliquots of our ssDNA (one undamaged and 3 UV-damaged are dispersed into the colloidal gold solution that is previously divided to 4 test tubes. The aliquot #3 is treated with photolyase in the yellow light while #4 is treated in blue light. An in-depth photoreactivation assay protocol contains: 46-mer single-stranded oligomers (100ng/µl) are exposed to UV_{254nm} light with UV fluences

of 10,30 and 50 J/m² at fluence rate of 0.5 J/m² s. The UV damage will be analyzed by PAGE electrophoresis with AgNO₃ staining. Thiol conjugated UV treated ss-oligomers (100ng/μl) are incubated with gold nanoparticle solution in order to attach oligomers on to gold surface via thiol bonds. Unbound oligomers are washed. UV treated oligonucleotides that are attached to gold nanoparticles are suspended in photoreactivation buffer (17.5mM Tris-Cl pH=7.4, 52.5Mm NaCl, 0.35Mm EDTA, 5mM DTT, 0.1 mg/ml BSA, 17.5% glycerol).

Photolyase is incubated with UV exposed ss oligonucleotides attached to gold nanoparticles at various concentrations in the photoreactivation buffer under blue light at the rate of 3W/m² for 1 h at room temperature. Control photoreactivation is carried out at dark.

***In vitro* Photoreactivation Assay**

Ampicilin resistant gene containing plasmid ppr3-N (25ng) was used as substrate. The substrate DNA was irradiated at fluence of 30J/m² UV light at 254nm at fluence rate of 0.5 J/m²s (Sylvania G8W). Photoreactivation was carried out under 1-cm thick glass cover to filter out light below 300nm. UV-damaged substrate was incubated with 1.88 nM *Vc*-photolyase (*Vc*-PHR) in photoreactivation buffer (17.5mM Tris-Cl pH=7.4, 52.5Mm NaCl, 0.35Mm EDTA, 5mM DTT, 0.1 mg/ml BSA, 17.5% glycerol) under saturated blue light at 366nm for 1 hour. (Sanyko Denki F8T5/BLB black light.) . Dark control was kept in the dark during photoreactivation at room temperature. Regular chemical transformation protocol was applied to transform plasmids into *E.coli* strain of UNC 523 (*phr*;*UvrA*⁻). Then, the cells were plated onto LB-amp (100μg/ml) selective medium and incubated under dark at 37°C overnight. Colonies were counted and survival rate was calculated.

RESULTS

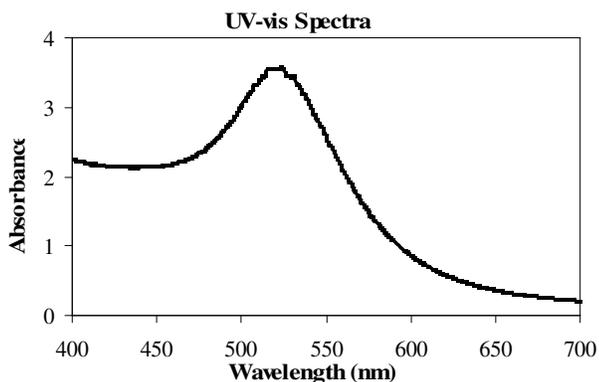


Figure 1. UV-vis spectrum of the bare gold nanoparticles

The absorbance maximum was reached at 520 nm (Fig. 2) which shows an average nanoparticle size of 13 nm as reported by Grabat *et al.* [13].

After the nanoparticle size was determined, the UV damaged dsDNA (double stranded) and photolyase repaired strands has been modified on gold nanoparticles. This immobilization process is in progress. The work in progress includes the UV-vis spectra of oligonucleotide-modified nanoparticles and their immobilization on amino-modified glass surface for force spectroscopy measurements.

AFM will help to characterize the damaged and repaired DNA qualitatively especially at low concentrations that UV-vis spectroscopy is unable to detect.

Photoreactivation assay

In vitro photoreactivation assay showed that plasmid DNA pPR3-N was effectively damaged by UV irradiation at 254 nm as the survival rate decreased around one log. Figure 3 indicates *Vc*-photolyase repaired UV lesions on plasmid DNA under blue light at 366nm.

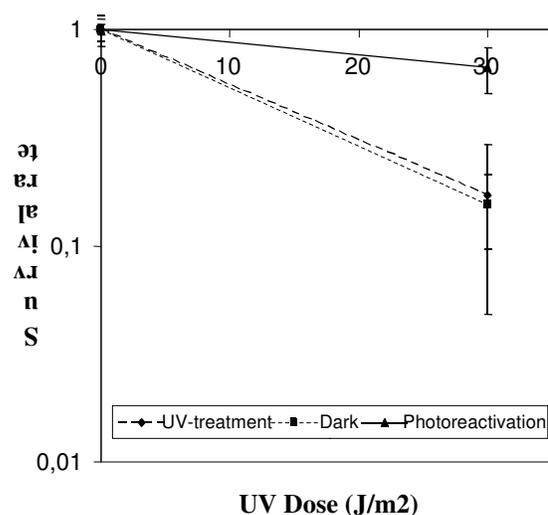


Figure 3. UV irradiation on plasmid and *in vitro* photoreactivation

CONCLUSION

In this study, we aim to demonstrate a diagnostic technique for improved DNA damage and repair detection using AFM imaging. AFM imaging platform for a sensitive detection of DNA damage will play a significant role supplementing traditional techniques for high-resolution measurements of UV-damage to DNA. Using gold nanoparticles as signaling probes can introduce promising results for detecting DNA damage and repair in several spectroscopic techniques. The AFM measurements of damaged, photolyase-bound and repaired DNA will show this possibility of a sensitive DNA detection proving that the gold nanoparticle modification improve the previous DNA damage-and-repair identification methods further in simplicity and sensitivity *in vitro*. This can help

to replace the less sensitive fluorescence or radioactive labeling techniques that proves to be also less selective but more expensive. So far, gold colloids at expected size has been carried and an in vitro photoreactivation assay was performed in order to support our incoming results of AFM images of damaged and repaired DNA modified on gold nanoparticles. It was shown that our 46-mer oligonucleotide was successfully UV-damaged and then repaired by *Vc photolyase*. We expect to see our AFM images show consistent data with the photoreactivation assay results. For future, SPR (surface plasmon resonance) imaging can also be included in the study to get further benefit of the optical and magnetic properties of gold colloids.

REFERENCES

- [1] Sinha R. P., Hader D-P., „UV-induced DNA damage and repair: a review,“ *Photochem. Photobiol. Sci.*, 1, 225-236, 2002.
- [2] Sancar A., “Structure and Function of DNA Photolyase and Cryptochrome Blue-light Photoreceptors,” *Chem. Rev.*, 103, 2203-2237, 2003.
- [3] Britt A. B., “DNA damage and repair in plants,” *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47, 75–100, 1996
- [4] Fang X., Liu x., Schuster S., Tan W., “Designing a Novel Molecular Beacon for Surface-Immobilized DNA Hybridization Studies,” *J. Am. Chem. Soc.*, 121, 2921-2922, 1999.
- [5] Friedberg E.C., Walker G.C., Siede W., “DNA repair and mutagenesis,” ASM Press, 1995.
- [6] N.L. Rosi and Chad A. Mirkin, "Nanostructures in Biodiagnostics," *Chem. Rev.*, **105** (4), 1547–1562, 2005.
- [7] C. Minard-Basquin, R. Kügler, N.N. Matsuzawa, A. Yasuda, “Gold-nanoparticle-assisted oligonucleotide immobilization for improved DNA detection,” *IEE Proc.-Nanobiotechnol.*, 152 (2), 2005.
- [8] Mirkin C. A., Letsinger R. L., Mucic R. C., Storhoff J. J., “A DNA-based method for rationally assembling nanoparticles into macroscopic materials,” *Nature*, 382, 607-609, 1996.
- [9] Ngoc Bui M-P., Baek J.B., Seong G. H., “Gold nanoparticle aggregation-based highly sensitive DNA detection using atomic force microscopy,” *Anal. Bioanal. Chem.*, 388, 1185-1190, 2007.
- [10] Hansma H. G., Revenko I., Kim K., Laney D. E., “Atomic force microscopy of long and short double-stranded, single-stranded and triple-stranded nucleic acids,” *Nucleic Acids Research*, 24 (4), 713-720, 1996.
- [11] Hamon L., Pastre D., Dupaigne P., Le Breton C., Le Cam E., Pietrement O., “High-resolution AFM imaging of single-stranded DNA-binding(SSB) protein-DNA complexes,” *Nucleic Acids Research*, 35 (8), 1-7, 2007.
- [12] Pope L. H., Davies M. C., Roberts C. J., Tendler S. J. B., Williams P. M., “DNA analysis with scanning probe microscopy,” *Analytical Comm.*, 35, 5H-7H, 1998.
- [13] Hegner M., Wagner P., Semenza G., “Immobilizing DNA on gold via thiol modification for atomic force microscopy imaging in buffer solutions,” *Fed. Of European Biochem. Soc.*, 336 (3), 452-456, 1993.
- [14] Grabar K. C., Freeman R. G., Hommer M. B., Natan M. J., “Preparation and Characterization of Au Colloid Monolayers,” *Anal Chem.*, 67, 735-743, 1995.
- [15] Tokonami S., shiigi H., Nagaoka t., “Open Bridge-Structured Gold Nanoparticle array for Label-Free DNA Detection,” *Anal Chem.*, 80, 8071-8075, 2008.