Direct visualization of DNA damage at single - molecule level by atomic force microscopy after photodynamic therapy

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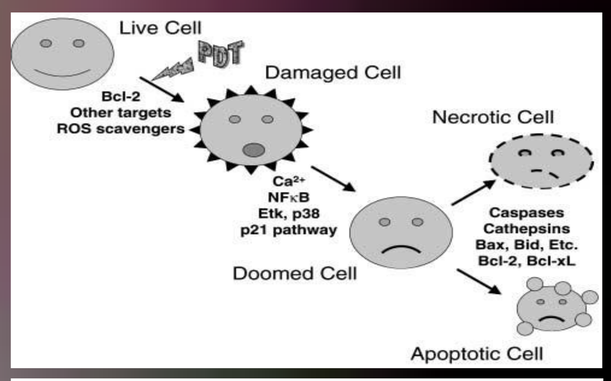

Fig. 1 Scheme of photodynamic treatment
(addopted from Oleinick et all 2001)

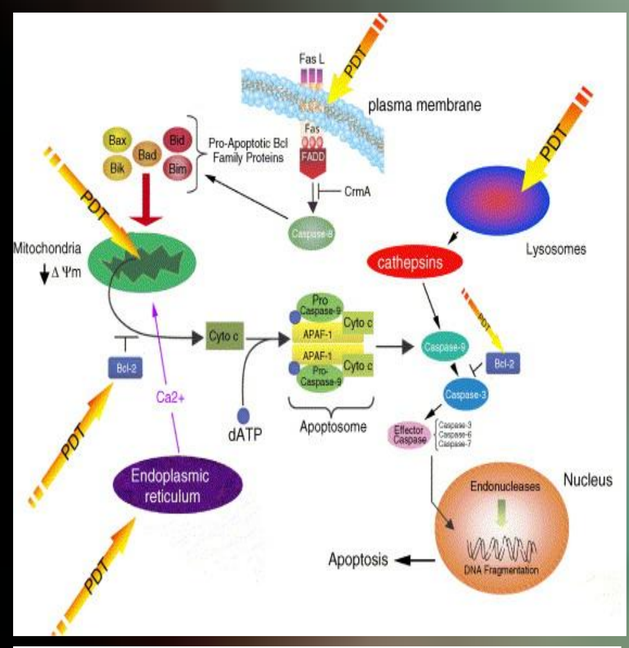

Fig. 2 Scheme of photodynamic treatment with respect to DNA damage **(addopted from Castano et all 2005)**

Summary

Photodynamic therapy (PDT) is a promising modality of cancer treatment based on the selective properties of the tumor tissue and the phototoxic effect of the photosensitizing dyes. To investigate the mechanisms underlying apoptosis in breast cancer cells, photodynamic therapy was used as an apoptotic stimulus in the human breast cancer cell lines MCF-7[1]. The detection and quantification of damage to DNA at a single-molecule level by atomic force microscopy (AFM) is reported.

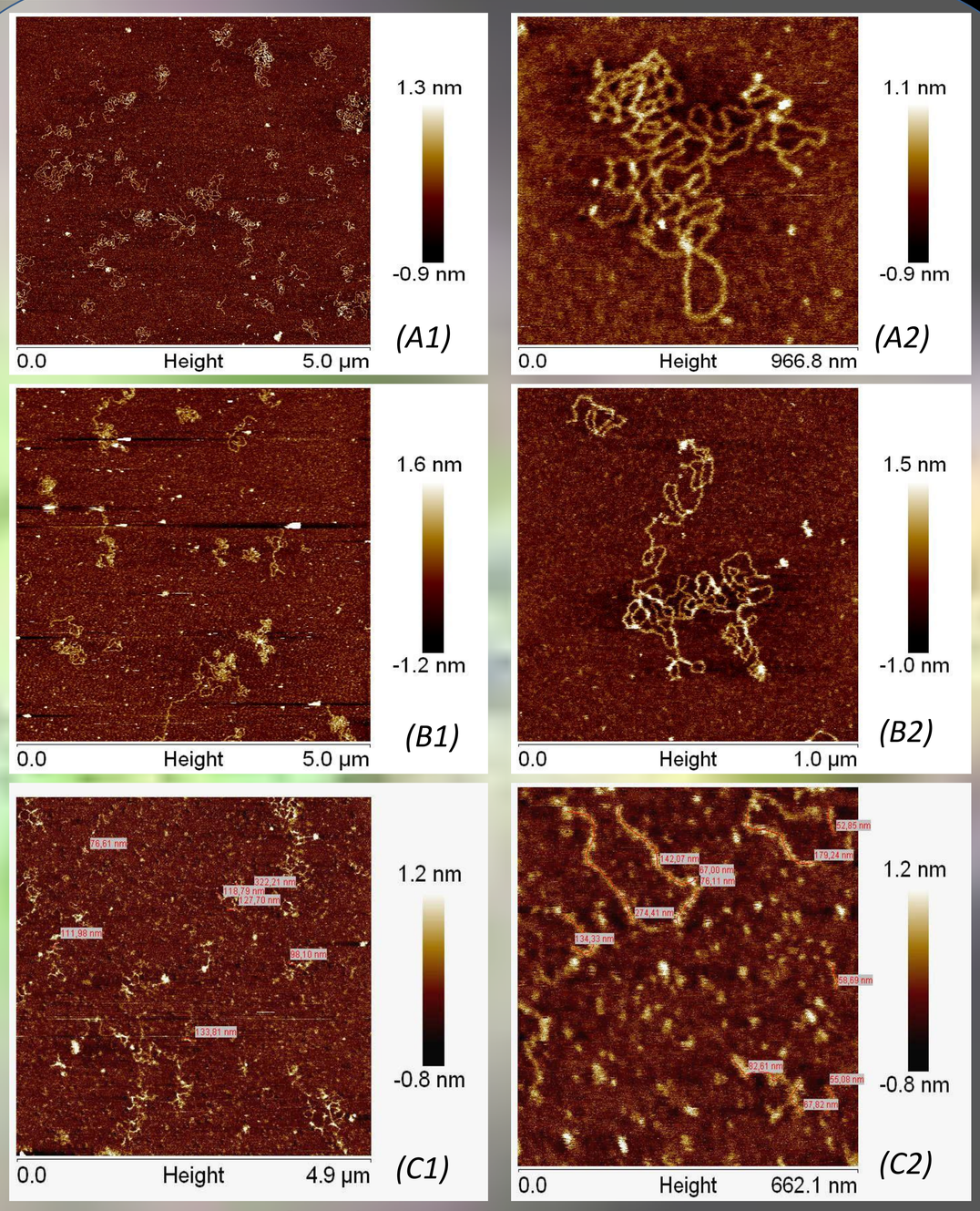

***Fig. 3** TP-AFM images of single-molecule DNA after photodynamic treatment*
***(A) TMPyP (1µM),** supercoiled linear DNA, 5µm²(A1), zoom 1µm²(A2)*
***(B) TMPyP (5µM),** less supercoiled linear DNA, 5µm²(B1), zoom 1µm²(B2)*
***(C) TMPyP (50µM),** fragmented DNA, 5µm²(C1), zoom 0,66µm²(C2)*

Methods

PDT treatment: different photosensitiser concentration (TMPyP, 1µm; 5µm; 50µm) and low light dose 1J/cm² (LED irradiator, λ_{ex}=414nm) were used in our experiment. Light dose and TMPyP concentration were determined by MTT test of cell viability, LC_{50} corresponds to 5µmTMPyP at 1J/cm², data not shown).

DNA isolation: DNA was isolated from control samples and PDT treated MCF-7 cells by standard kit for genomic DNA isolation (NORGEN). DNA aliquots in HEPES buffer were stored at -20°C until next use. Stored DNA concentration: 5µg/ml (40mM HEPES, 10mM $MgCl_2$, pH7,6) .

DNA deposition: Bivalent cathion deposition method[2] was used for DNA immobilization to mica substrate. Briefly: 2µm $NiCl_2$ was added to DNA to final concentration 0,5µg/ml and deposited on freshly cleaved mica strips. After 15 min incubation (RT) were mica strips gently washed, dried and used for AFM imaging. For fragmented DNA APS modified mica[3] was used as a suitable substrate for AFM imaging.

AFM imaging: AFM images were taken in tapping mode (TP) in air. RTESPA tip with resonant frequency at 300kHZ and k ~ 40N/m were used for imaging. Scan area of 5µm² (zoom area of 1µm² and app. 600nm) were taken to visualize DNA length and overall DNA fragmentation.

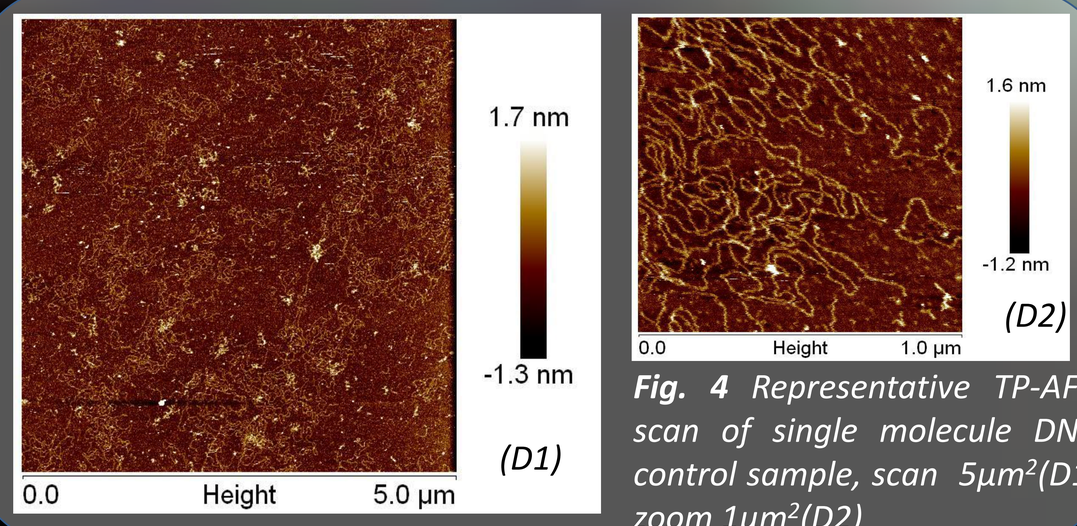

***Fig. 4** Representative TP-AFM scan of single molecule DNA, control sample, scan 5µm²(D1), zoom 1µm²(D2)*

Conclusion

AFM scans of control DNA samples (no treatment, light alone, or TMPyP alone) of MCF-7 cells clearly show supercoiled DNA molecules with DNA length of thousands base pairs (bp) (Fig. 4). In contrary AFM scans of PDT treated samples show dose-response relationship of DNA damage and clearly visualize DNA fragmentation during apoptotic process. Image analysis of fragmented DNA (Fig. 3 C1, C2) shows cleavage of chromatin DNA into nucleosomal fragments of roughly 180bp/ ~ 60nm and multiples thereof (360 bp/ ~ 122nm, 540bp/ ~ 183nm etc.), induced by 50µMTMPyP, light dose 1J/cm². The shortest DNA fragments (180bp/ ~ 60nm and shorter) are tightly packed and visualized mainly as globular shape whereas longer fragments (360bp /~120nm and longer) take linear form. **Based on our AFM results we showed that AFM is a potential tool for DNA damage examination on single molecule level.**

Acknowledgement: LF_2013_006 CZ.1.05/2.1.00/01.0030

[1]Mooney et all 2002, *Apoptotic mechanisms in T47D and MCF7 human brest cells* [2]Hansma et all, 1995, *Applications for AFM of DNA* [3]Shlyakhtenko et all, 2003, *Silatrane based surface chemistry*