TiO_2 –Oligonucleotide Nanocomposites for Sequence-specific Cleavage of Nucleic Acids

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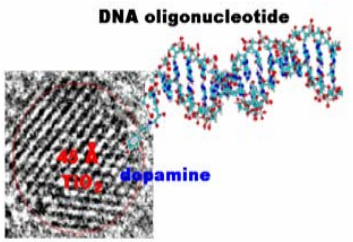
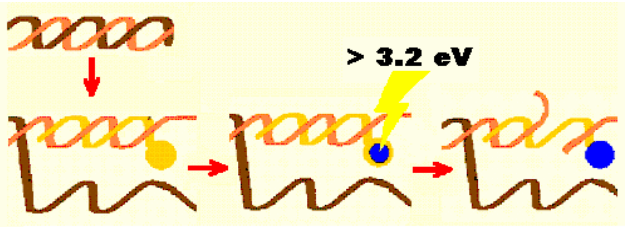
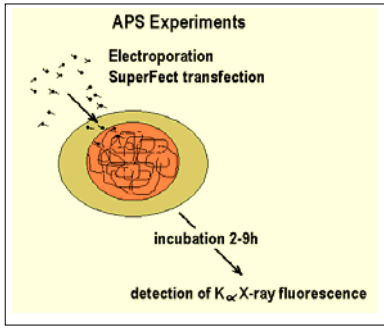


DNA oligonucleotides are stably attached to TiO2 nanoparticles via dopamine.

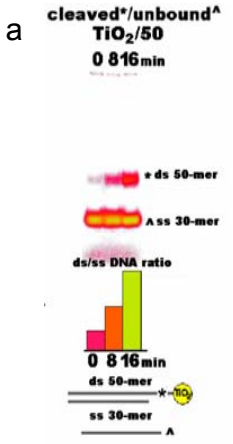


Excited TiO2 (upon accepting energy greater than 3.2 eV) generates electropositive holes that migrate onto the attached DNA. Accumulation of electropositive holes results in cleavage of the annealed nucleic acid.

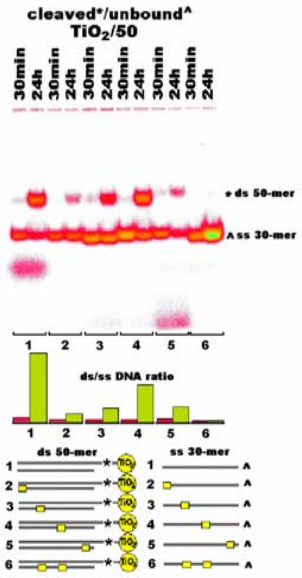


The possibility of using TiO2-oligonucleotide nanocomposites for sequence-specific nucleic acid cleavage inside cells may advance genetic engineering/gene therapy and other intracellular manipulations into a new era. In order to accomplish this we need to study conditions of entry and retention of TiO2-oligonucleotide nanocomposites inside cells. Detection of K alpha X-ray fluorescence at 2ID-E beamline of XOR CAT provides the most sensitive and speedy approach to intracellular Ti detection.

In vitro cleavage of nucleic acids by nanocomposites follows both illumination and exposure to radiation, and it is sequence specific



TiO_2-DNA oligonucleotides do not enter the gel during PAG electrophoresis due to the presence of the TiO_2 nanoparticle. It is only upon cleavage of DNA away from the nanoparticle that oligonucleotides that were part of the nanocomposite can enter the gel. Therefore, the amount of double-stranded DNA on the gel represents the amount of cleavage of the DNA induced by excitation of TiO_2.

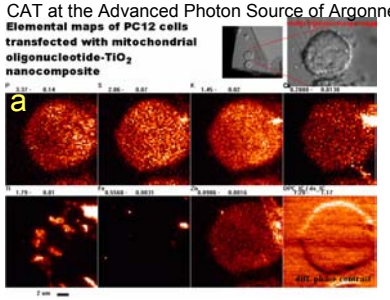


a) A nanocomposite 50 nucleotides in length was annealed with complementary oligonucleotide and illuminated with 300 W white light Xenon lamp for 0, 8 or 16 minutes.

b) A single 50 nucleotides long TiO_2-DNA nanocomposite was annealed with a series of complementary oligonucleotides containing either no mismatched nucleotides or a four nucleotide long stretch of mismatched oligonucleotides positioned at different locations along the complementary oligonucleotide.

Intracellular annealing of nanocomposites

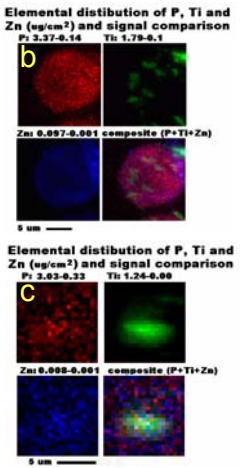
- Mitochondrial DNA oligonucleotide (5' carboxy dT-CACGACACTTAGCACCAACTTAC) was attached to nanoparticles as described in Paunesku et al., 2003.
- Transfection by SuperFect reagent or electroporation introduced nanocomposites into PC12 (rat pheochromocytoma) cells
- Kα X-ray fluorescence in these cells was detected using x-ray microprobe at the 2-ID-E beamline of the XOR-CAT at the Advanced Photon Source of Argonne National Laboratory.



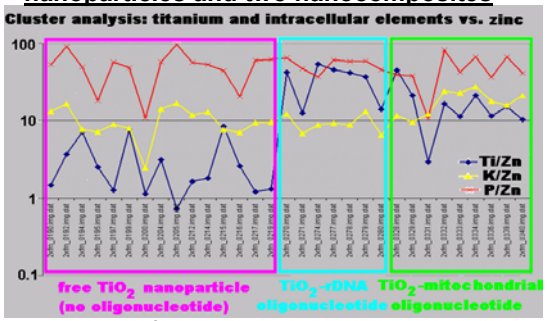
a) PC12 cells were located using a visible light microscope and their coordinates saved. Scanning of a 20 x 20 micron area was done, simultaneously providing complete X-ray fluorescence spectra for each pixel scanned. This data can be used to reconstruct elemental maps of P, S, K, Ca, Ti, Fe, Zn, as shown in figure a).

b) PC12 cell X-ray fluorescence maps of P, Ti and Zn and the overlap map showing the presence of Ti in the cell.

c) X-ray fluorescence maps of P, Ti and Zn and the overlapping map showing Ti signal in a single isolated mitochondria.



Ti content in cells transfected with "free" nanoparticles and two nanocomposites



There are no significant variations in P and K signal in a either group of cells—these elements are "natural" content of cells, while Ti signal highly depends on association of TiO_2 nanoparticles with DNA oligonucleotides (comprising nanocomposites).

Conclusions

TiO_2-DNA oligonucleotide nanocomposites
a)hybridize with complementary DNA with high sequence specificity
b)cleave DNA upon excitation of TiO_2 by light or radiation.
c)can enter cells and organelles

References

Paunesku, T., Rajh, T., Maser, J., Vogt, S., Stojićević, N., Protić, M., Lai, B., Oryhon, J., Wiederrecht, G., Thurnauer, M., and Woloschak, G. Biology of TiO_2 -oligonucleotide nanocomposites. Nat. Mater. 2003 May;2(5):343-6.

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