Possible applications of Coherent X-ray Microscopy for Bio-Nanoconjugates Studies

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First demonstration (non-crystalline):

Coherent X-ray imaging of electron-density distribution in non-crystalline materials was first demonstrated by Miao, J. et al., Nature 1999:

“We believe that (with coherent X-ray imaging) resolutions of 10–20 nm should be achievable; this would provide an imaging resolution about 100 times lower than that attainable with conventional X-ray crystallography, but our method is applicable to structures roughly 100 times larger. This latter feature may facilitate the imaging of small whole cells or large subcellular structures in cell biology.”

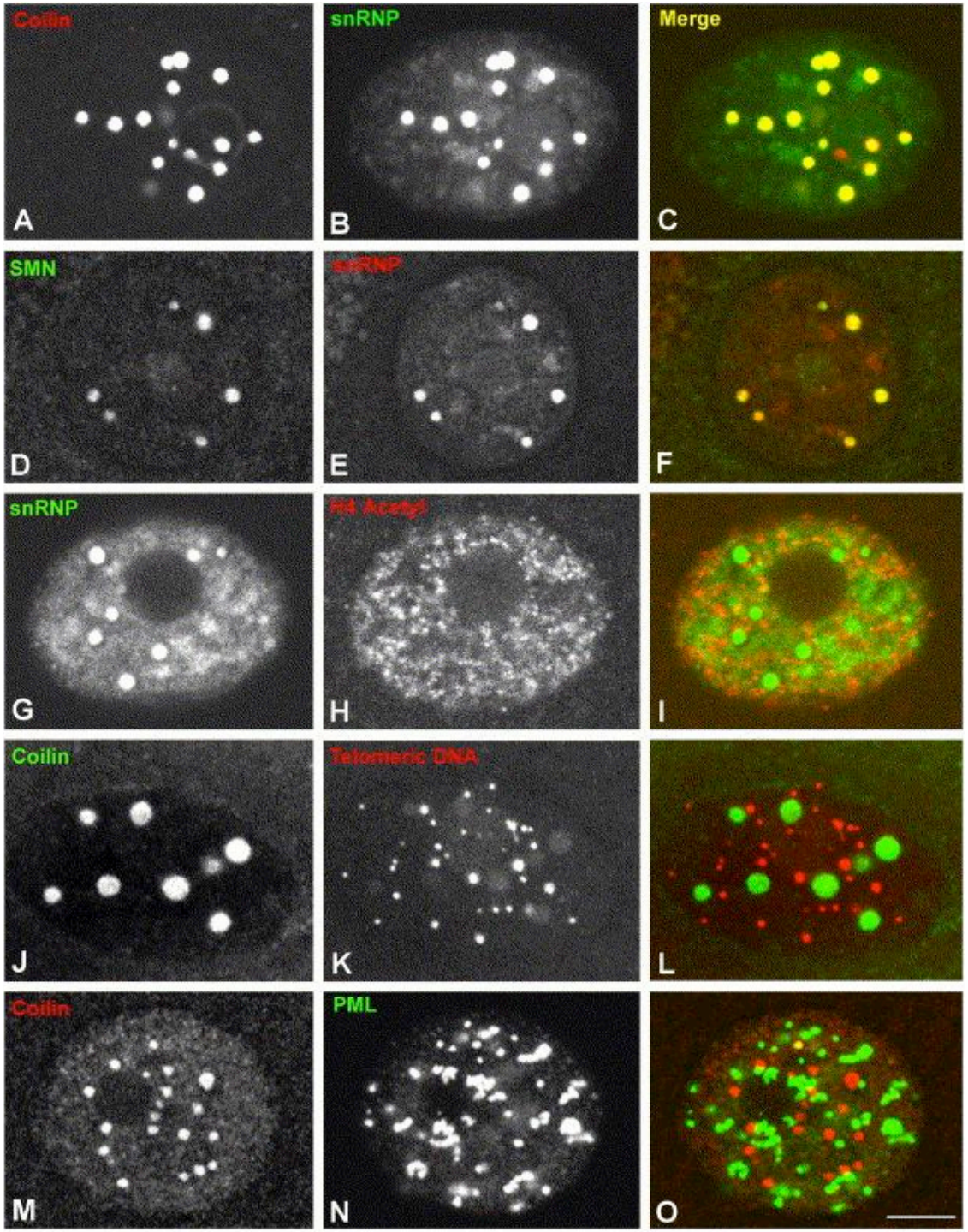
This opens possibilities to study subcellular protein assemblies *in situ*, for example:
compartmentalization inside nucleus:

A) **interchromatin compartment** with subcompartments:

- (i) **the nucleolus**, where rRNA synthesis and processing takes place
  - a) **fibrillar centers** (FCs), which store components of the transcription machinery of ribosomal genes such as RNA-polymerase I and upstream binding factor (UBF)
  - b) **the dense fibrillar component** surrounding the FCs, where transcription of rRNA genes and early rRNA processing take place
  - c) **the granular component**, the site of assembly of pre-ribosomal particles
- (ii) **nuclear speckles** of pre-mRNA splicing factors
- (iii) **Cajal bodies** (CBs), nuclear structures involved in the biogenesis of both small nuclear and nucleolar ribonucleoproteins (snRNPs and snoRNPs) required for the pre-mRNA and pre-rRNA processing, respectively
- (iv) **PML bodies**, which are implicated in several cellular processes such as transcriptional regulation, apoptosis and DNA repair

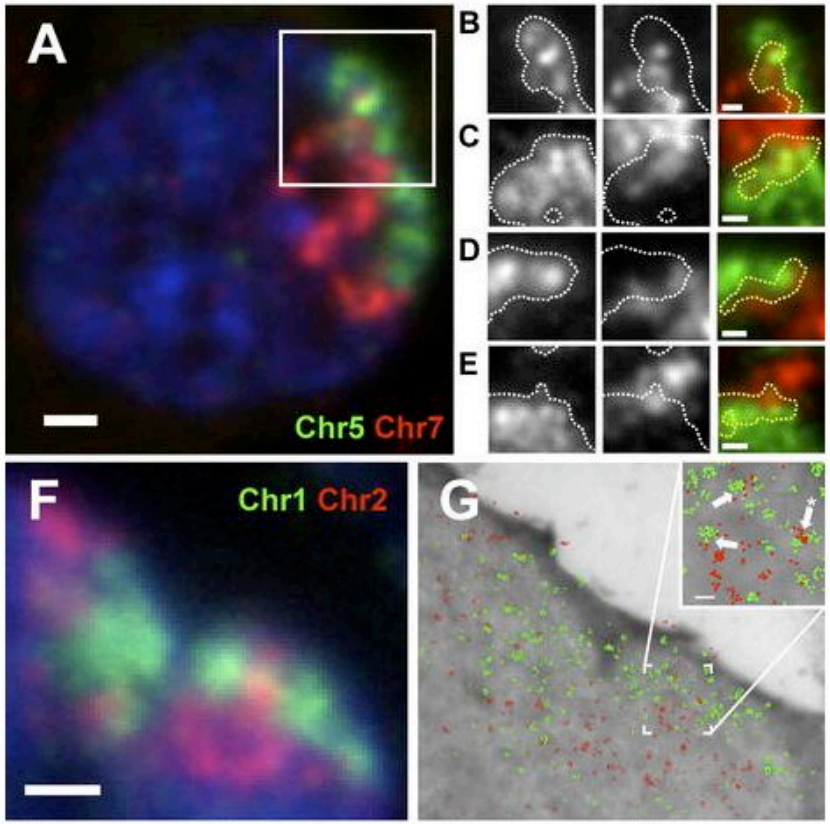
B) **chromosome territories**

None of these structures can be studied by protein crystallography.



Interchromatin compartments Confocal laser microscopy images of nuclei from human DRG neurons double stained for CBs and other nuclear compartments. (A–C) Colocalization of coilin and spliceosomal snRNPs, immunostained with an antibody that recognizes the 2,2,7-trimethylguanosine cap of the spliceosomal snRNAs, in numerous CBs. (D–F) Colocalization of the SMN factor and spliceosomal snRNPs in CBs. (G–I) Small nuclear foci immunoreactive for the histone H4 acetylated frequently appear closely associated with the periphery of CBs immunostained for snRNPs. (J–L) Combination of coilin immunostaining and *in situ* hybridization for telomeric DNA. Telomeres appear as numerous nuclear spots of different size distributed throughout the nucleus. Note the absence of a direct spatial association between CBs and telomeres. Co-staining for CBs (coilin) and PML bodies (PML) reveals the great abundance of both nuclear organelles in a large neuron. CBs and PML bodies frequently appear in direct contact. Scale bar: 5 μm.

Berciano et al., J. Struct. Biol., 2007



Chromosome territories

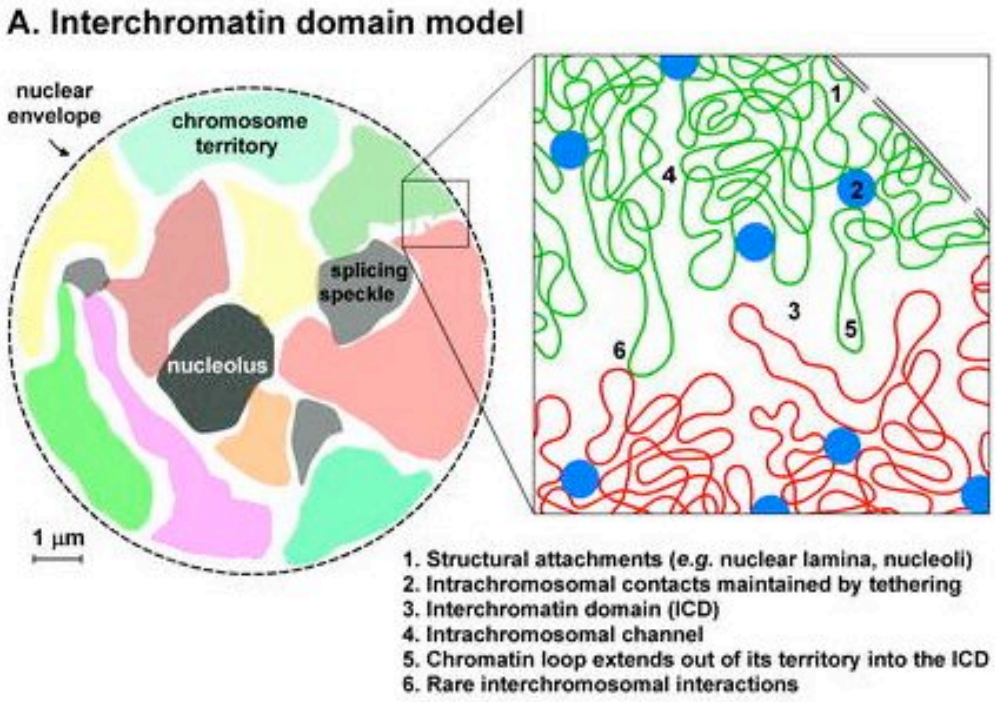
Branco and Pombo, *PLoS Biol.* 4 (2006)

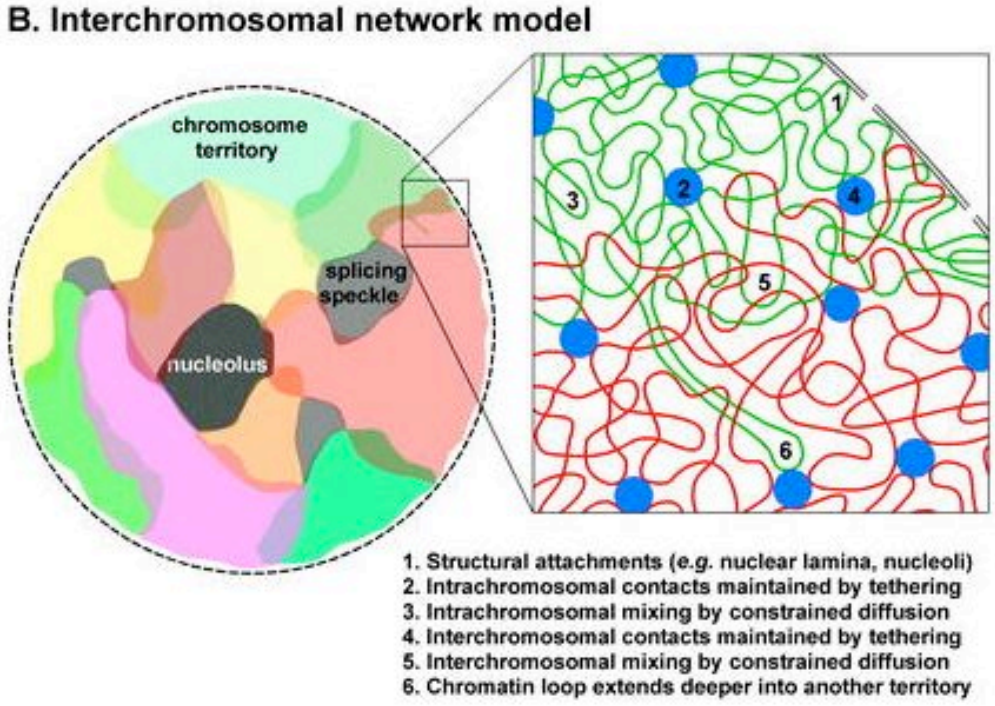
Chromosomes occupy discrete territories during interphase.
(Fluorescent probes A-F, gold nanoparticles G)

Q: Which model best describes territorial distribution of chromosomes?

•Interchromatin domain model, chromatin from different chromosomes is separated by an ICD compartment rich in nuclear machinery.

•Interchromosomal network model: chromatin from different chromosomes is not separated by a compartment but is allowed to expand into the surrounding territories; the presence of adjacent chromosomes, the nuclear membrane, and larger nuclear compartments restricts the amount of intermingling.

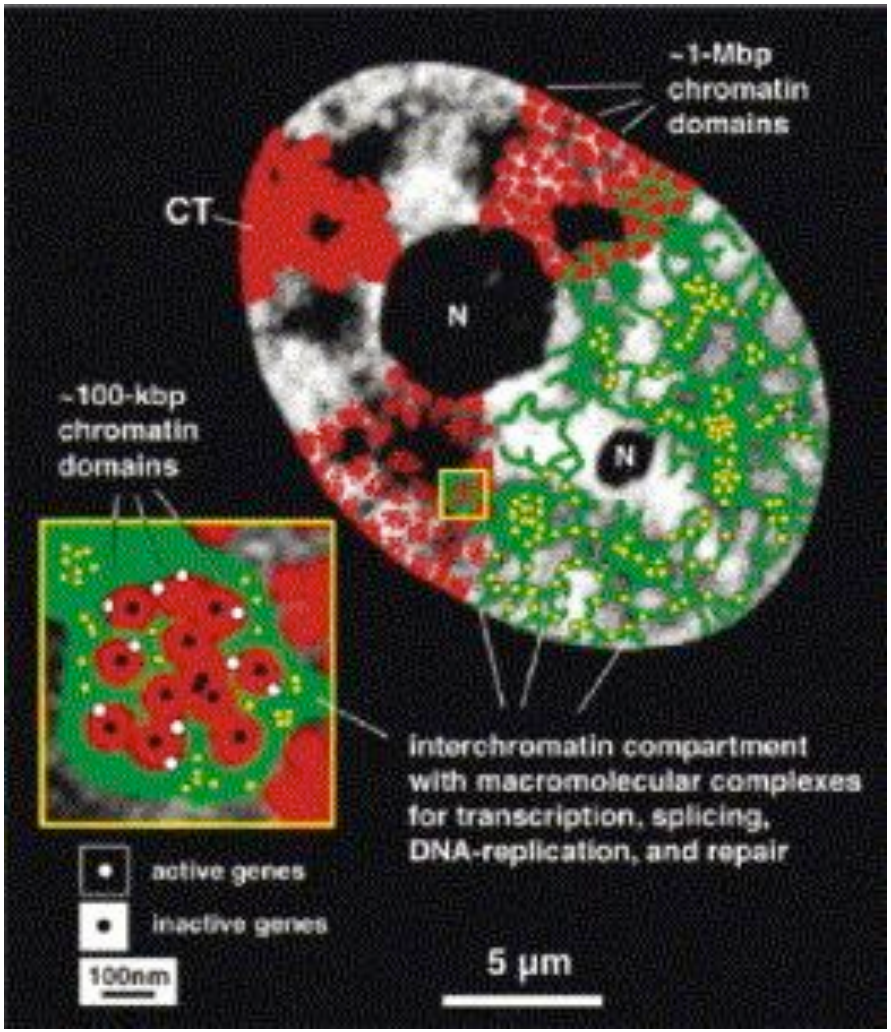




Chromosome territories-models

Branco and Pombo, *PLoS Biol.* 4 (2006)

Branco and Pombo, *PLoS Biol.* 4 (2006)



Chromosome territories-models

Cremer et al., Crit. Rev. Eukaryot. Gene Expr. 2000

Model #3: Chromosome territories (CTs) are composed of “1 Mbp chromatin domains”, which are in turn made up by “100 kbp chromatin loop domains”; the interchromatin compartment extends into the interior of CTs, contacting the surface of the 100 kbp domains, where active genes are located

Many more extremely important intracellular structures could be studied by coherent X-ray imaging:

- -membrane bilayer and embedded proteins
- -receptor assemblies above, inside and below cell membrane
- -fine structure of intracellular organelles
  - -mitochondria
  - -Golgi apparatus
  - -chloroplasts
  - -lysosomes
  - -…
- -protein assemblies in organelle membranes
- -…

The best that these structures have been studied was by EM…

None of these structures can be seen in 3D in their native state with resolution better than that of optical microscope.

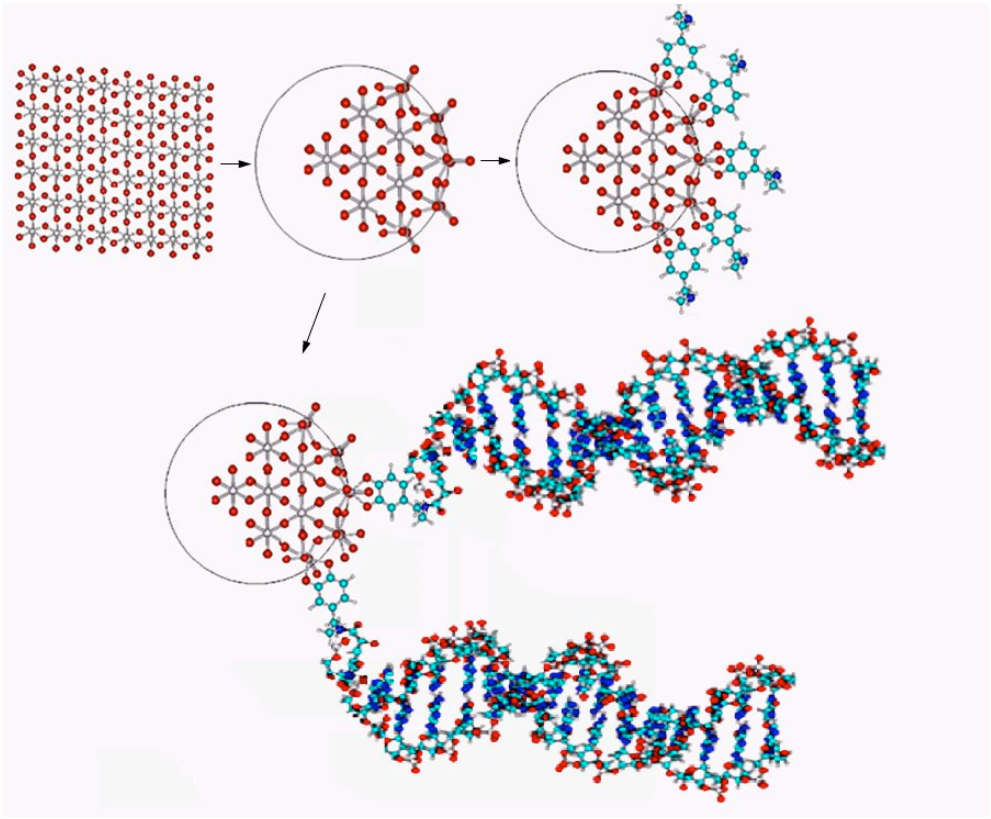
First demonstration (crystalline):

Coherent X-ray imaging of nanoparticles was first demonstrated by Robinson (Robinson et al. Phys. Rev. Lett. 2001), and the same group mapped a deformation field inside a nanocrystal (Pfeifer et al., Nature 2006).

The Future:

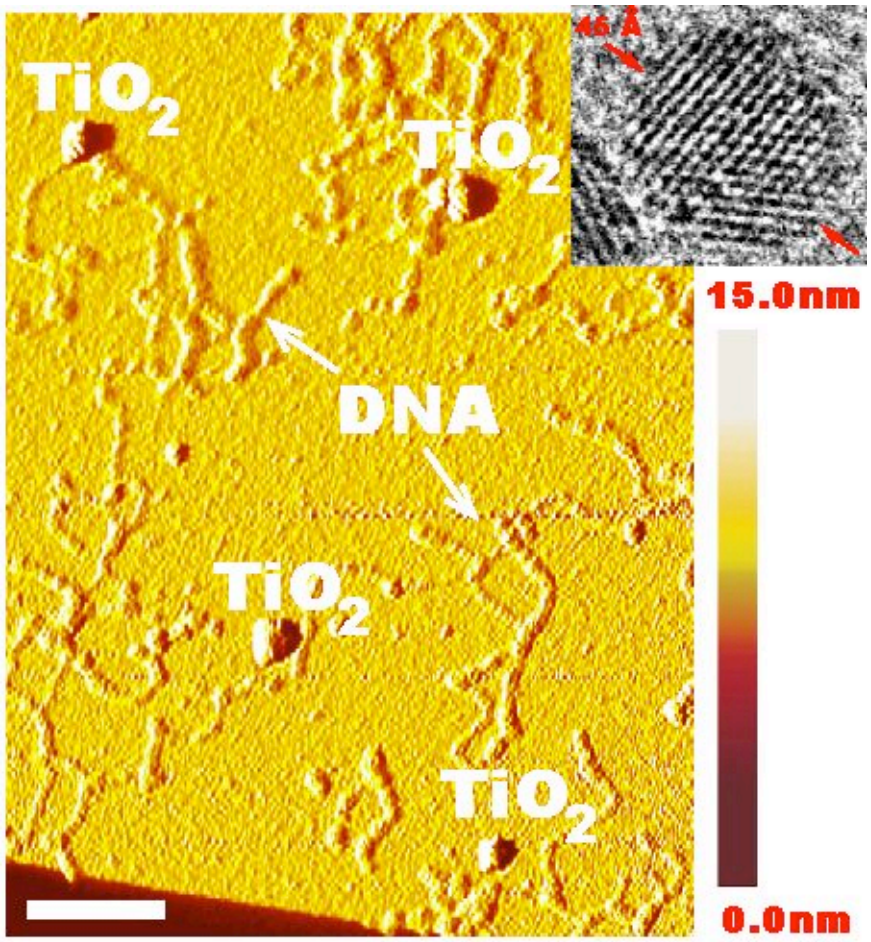
Future (for biology): simultaneous coherent X-ray imaging of both non-crystalline materials (cells) and nanocrystals used as fiducials, probes, intracellular assembly intitiation points, etc.

TiO_2-nucleic acid bio-nanoconjugates



Metal oxide (6nm nanoparticle) and nucleic acid components of TiO_2-DNA nanoconjugates are covalently bonded via dopamine molecules which also serve as an “electronic link” between these two nanoconjugate components.

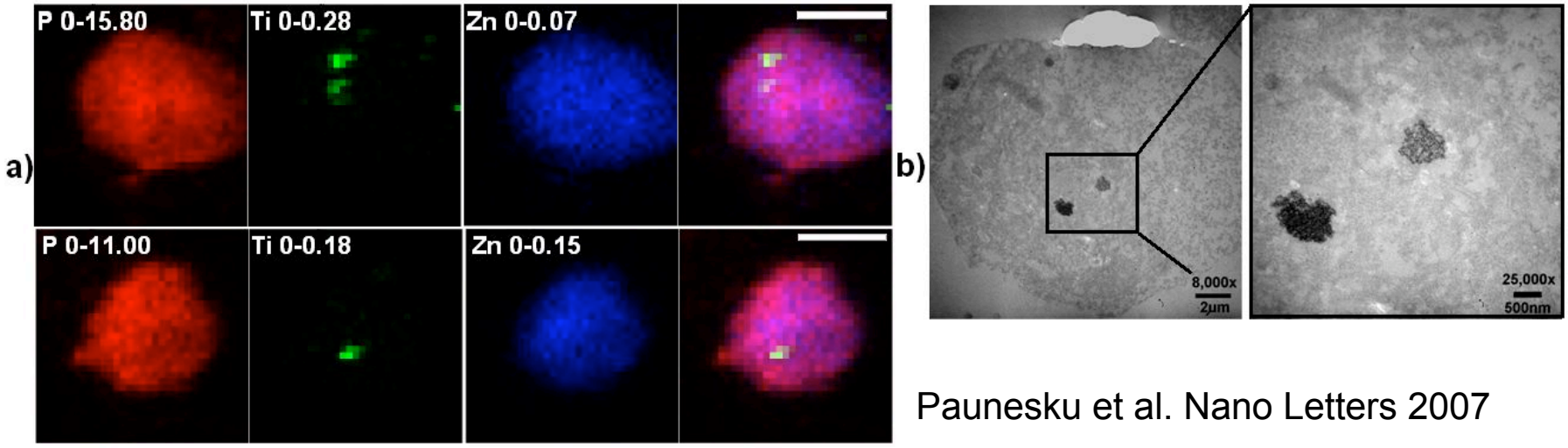
TiO_2-DNA bio-nanoconjugates hybridize to DNA *in vitro*



Atomic Force Microscopy image of TiO_2-oligonucleotide nanocomposites complementary to the lambda phage DNA following hybridization. In the upper right corner is a Transmission Electron Micrograph of a single TiO_2 particle showing its size (45 Å).

(Paunesku et al. Nature Materials (2003) 2:343-346)

TiO2-DNA bio-nanoconjugates hybridize to DNA in cells

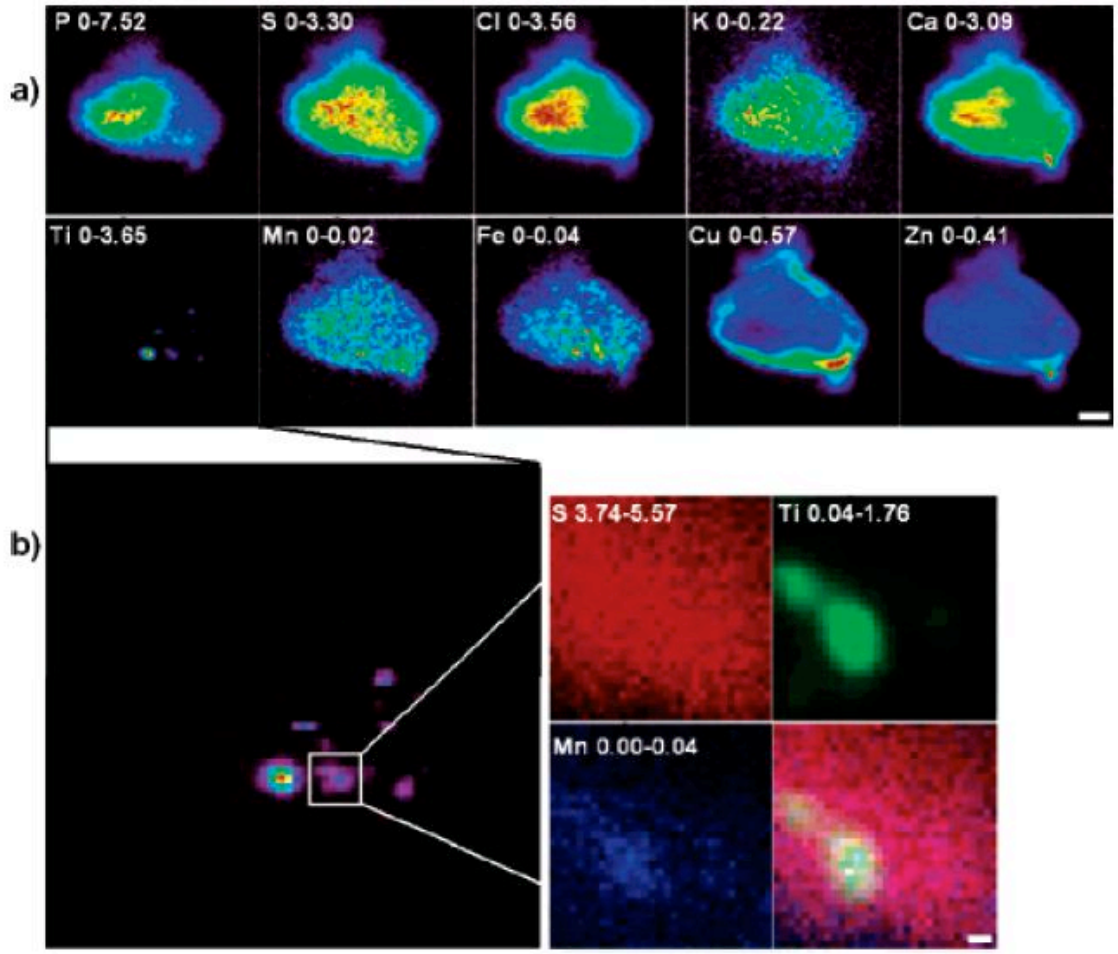


Paunesku et al. Nano Letters 2007

K alpha X-ray fluorescence microscopy and TEM imaging of breast cancer MCF-7 cells.

TiO_2-DNA oligonucleotide nanoconjugates (TiNCs) bind to appropriate target sequences in mammalian cells; TiNCs specific for the nucleolus are retained in the nucleolus; those specific for mitochondria are retained in mitochondria.

TiO2-DNA bio-nanoconjugates hybridize to DNA in cells



K alpha X-ray fluorescence microscopy of pheochromocytoma cancer PC12 cells.

TiO_2-DNA oligonucleotide nanoconjugates (TiNCs) bind to appropriate target sequences in mammalian cells; TiNCs specific for the nucleolus are retained in the nucleolus; those specific for mitochondria are retained in mitochondria.

Pauneskuet al. Nano Letters 2007

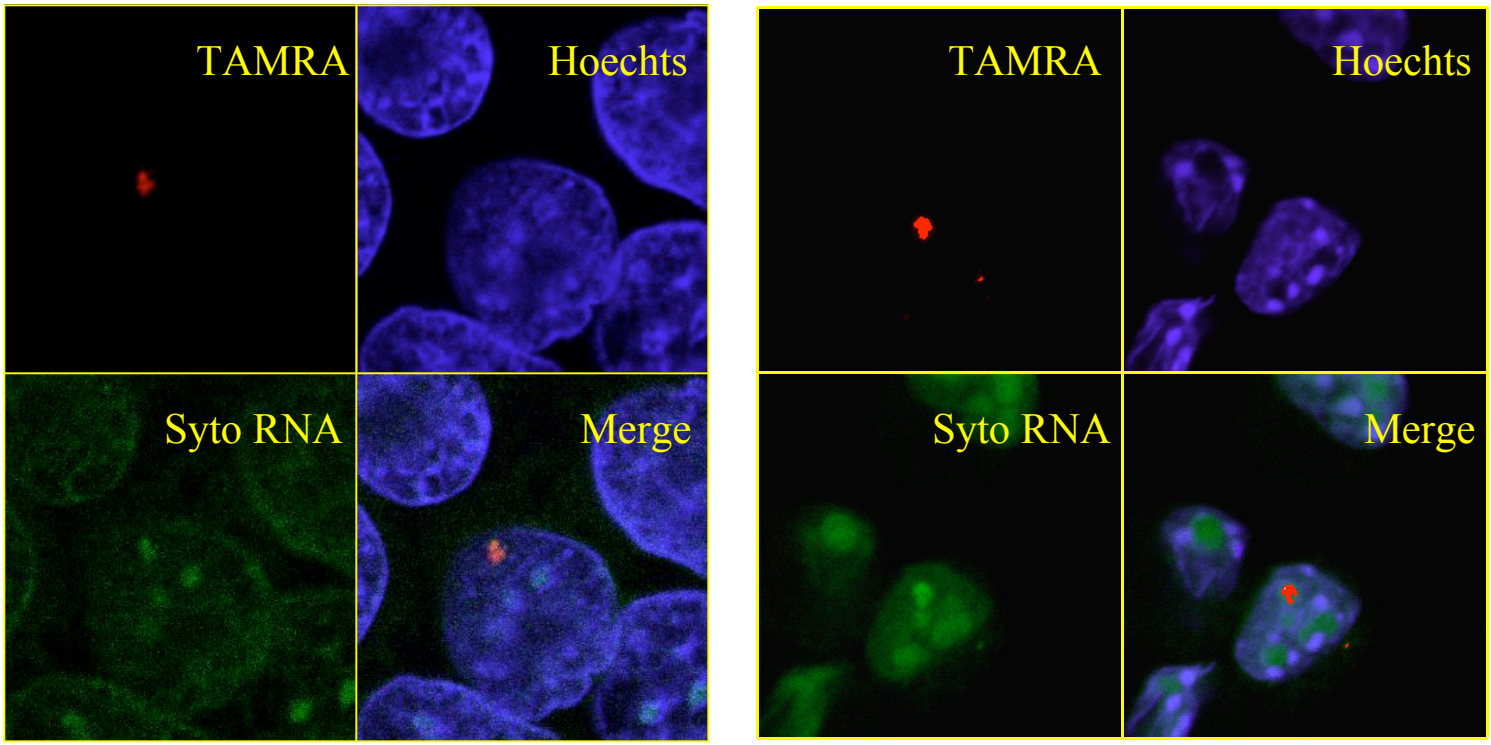
Parameters controlling cellular uptake of bio-nanoconjugates

- Transfection method
- Nanoconjugate coating
- Biomolecules attached to nanoconjugate
- Numbers of each biomolecule type attached to nanoconjugate
- Numbers and type of targets inside cells

Optical fluorescence can be used to track bio-nanoconjugates in cells

- Flow cytometry
- Confocal microscopy (resolution 200 nm)
- Integrity of nanoconjugates can be confirmed by K alpha X-ray fluorescence microscopy

Nucleolar subcellular localization of TiO2-DNA nanoconjugates

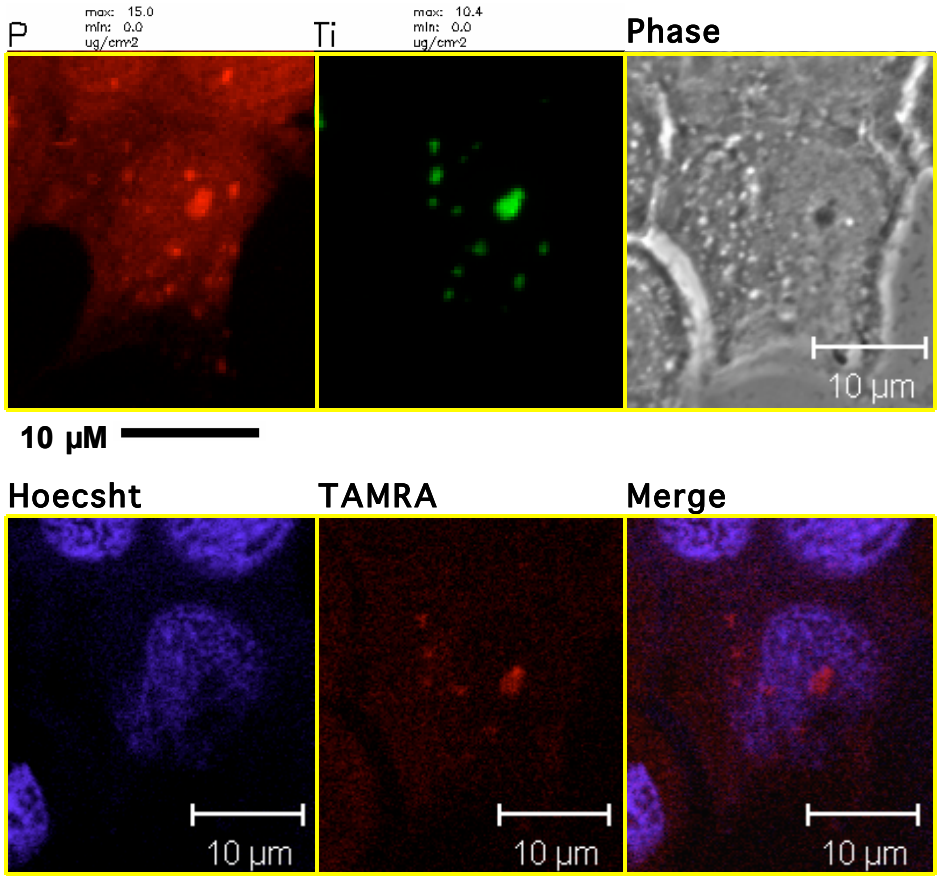


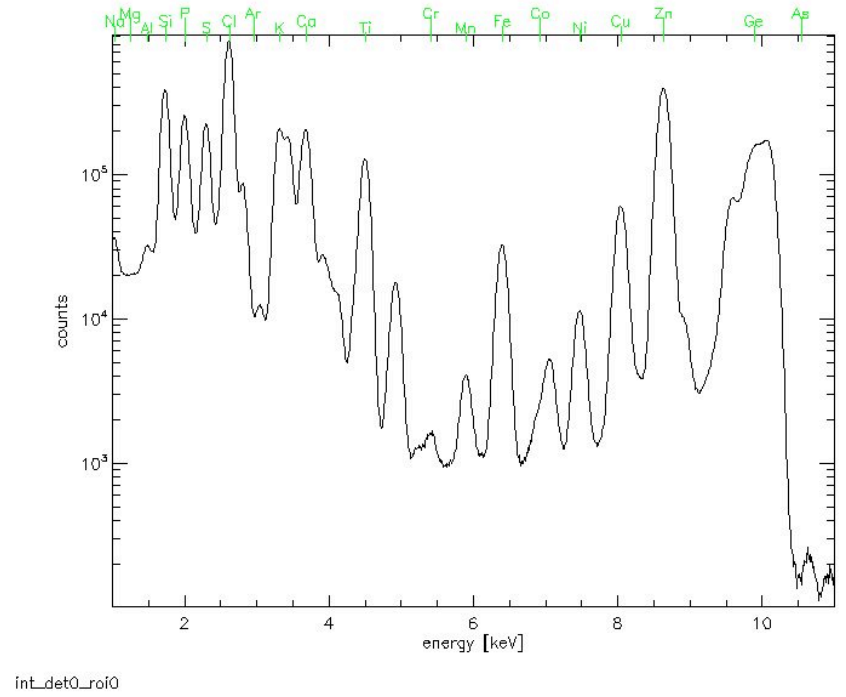
TAMRA-fluorescent label on oligonucleotide attached to nanoconjugate

Syto-RNA-dye marking intranuclear position of nucleolus

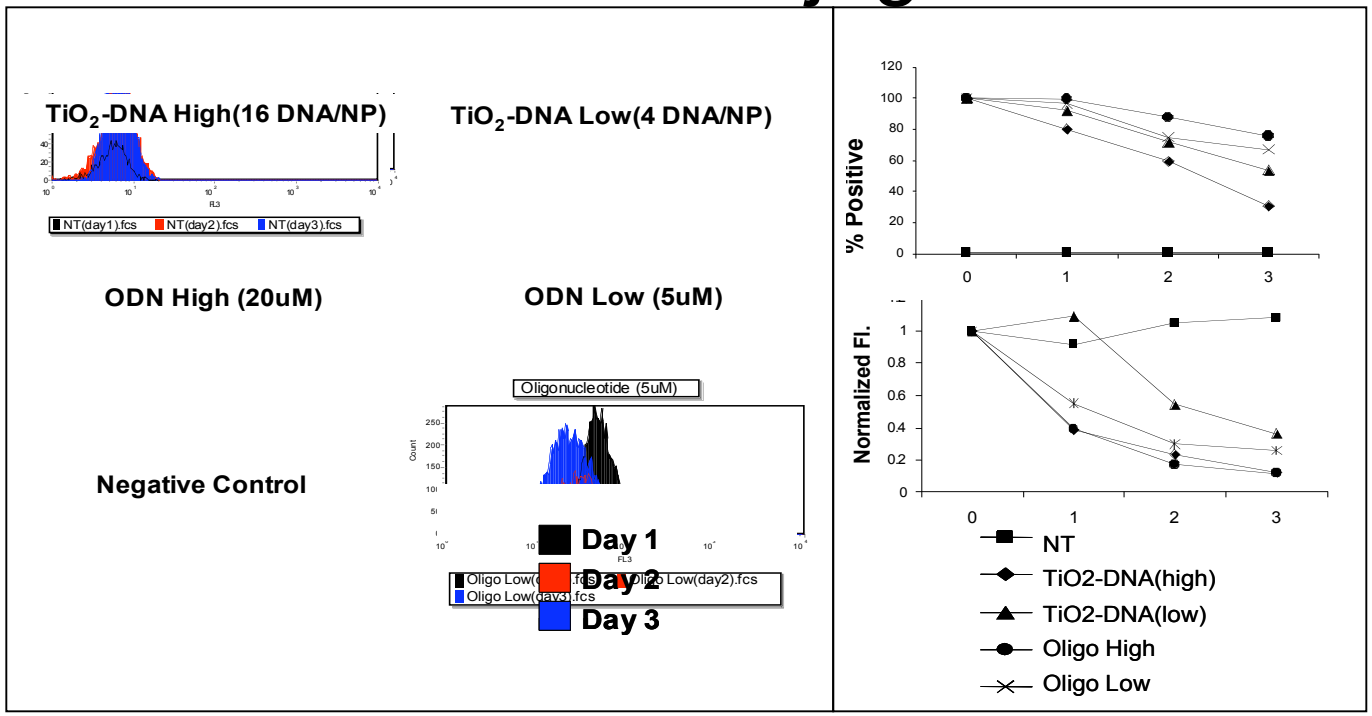
Hoechts-DNA dye labeling the position of the nucleus

Intracellular stability of nanoconjugates in MCF-7 cells transfected with TiO2-R18S-TAMRA





Influence of oligonucleotide:nanoparticle ratio on cellular uptake of TiO_2–DNA bio-nanoconjugates



The Flow cytometry data demonstrates that the DNA-TiO2 nanocomposites are taken up by cancer cells and retained. The avidity of the uptake varied with the change of ratio of DNA oligonucleotides to TiO2 nanoparticles. Cells took up more nanoparticles with lowed DNA concentration than those with high oligonucleotide concentration per nanoparticle. Retention of the low oligonucleotide nanoparticles was better as well at 3 days post transfection; finally, compared to retention of free oligonucleotides retention of nanoparticles with low-oligonucleotide coverage was once again superior.

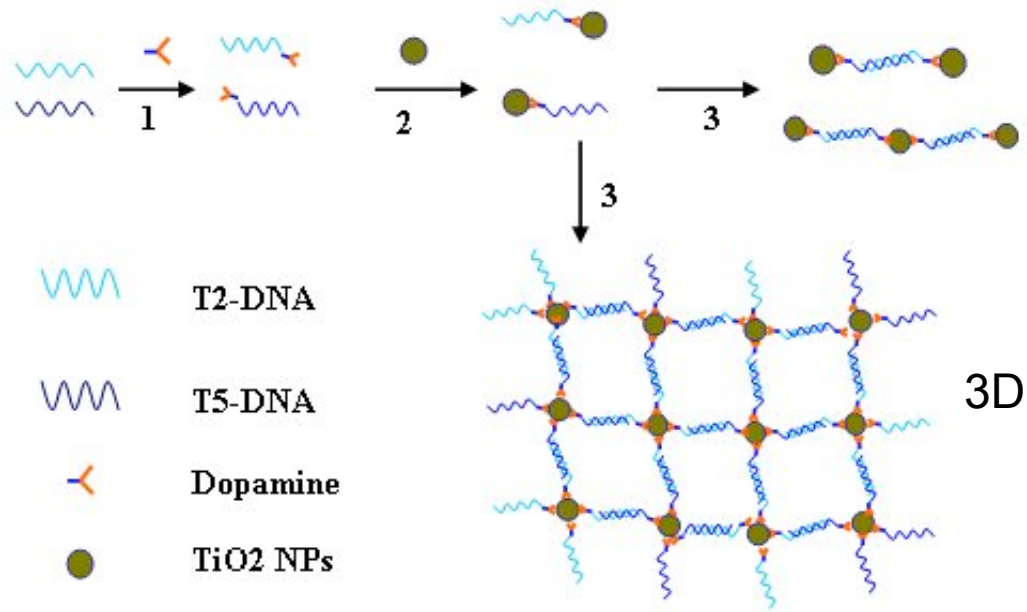
Approaches to Study Interactions between Bio-nanoconjugates

- Studies of bio-nanoconjugate interactions *in vitro*
- Studies of interactions between bio-nanoconjugates and cellular components *in vitro*

Numbers of Oligonucleotides use in studies impacts the outcome.

Interactions of TiO_2-DNA nanoconjugates *in vitro*

In vitro assemblies of TiO_2–DNA nanoconjugates



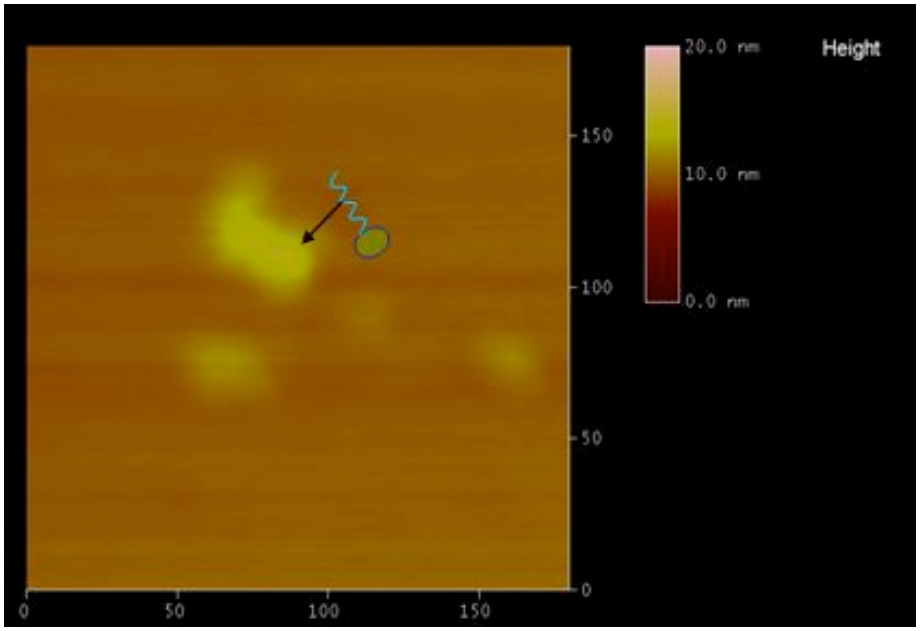
2D structure

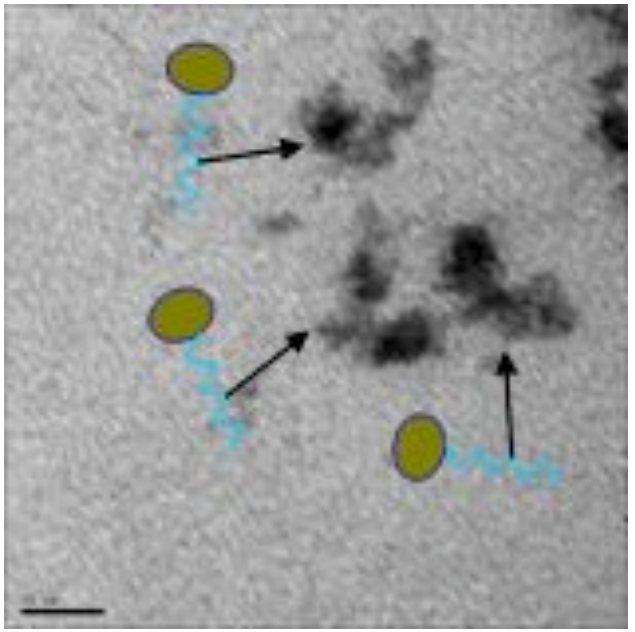
3D structure

A schematic diagram of TiO_2 nanoparticles assembled by DNA molecules:
Step 1. Combination of single strand DNA with a linker;
Step 2. Conjugation to TiO_2 NPs;
Step 3. annealing of the complementary-sequence TiO_2–oligonucleotide nanoconjugates to form

- 3D superstructures (high ratio of DNA/ TiO_2 NPs) and
- dumbbells or three-point rod of nanocomposites (low ratio of DNA/ TiO_2 NPs).

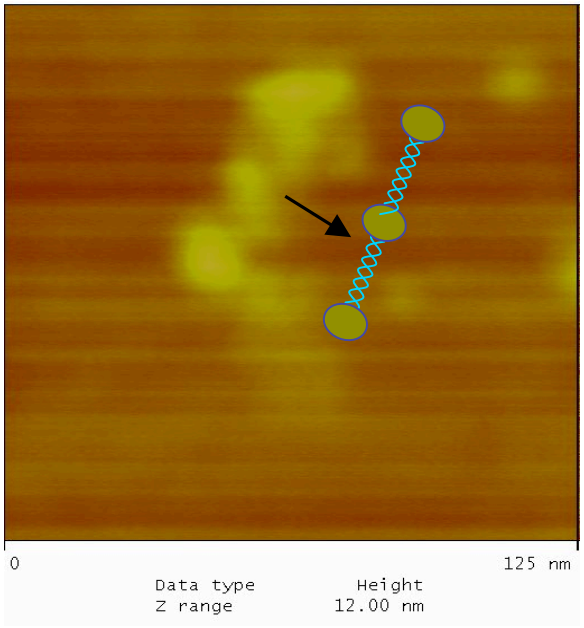
2D images of 2D dried nanoconjugates

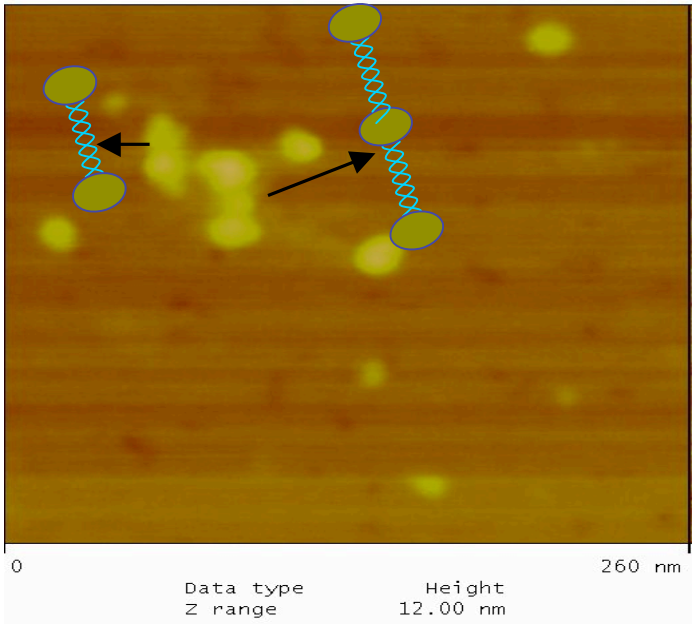




An AFM image of ssDNA-TiO2 nanocomposites without agarose and a TEM image of ssDNA-TiO2 in agarose (Low ratio of T5:TiO2, ~2 DNA per TiO2 nanoparticles (NPs).

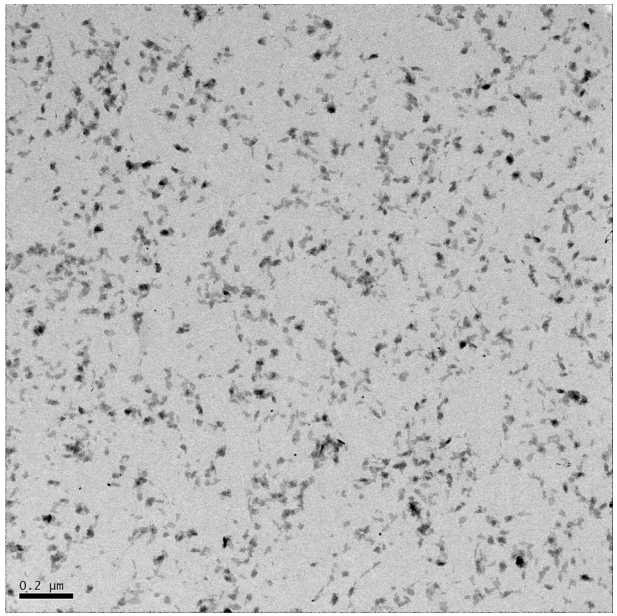
2D image of dried 2D dumbbells of nanoconjugates

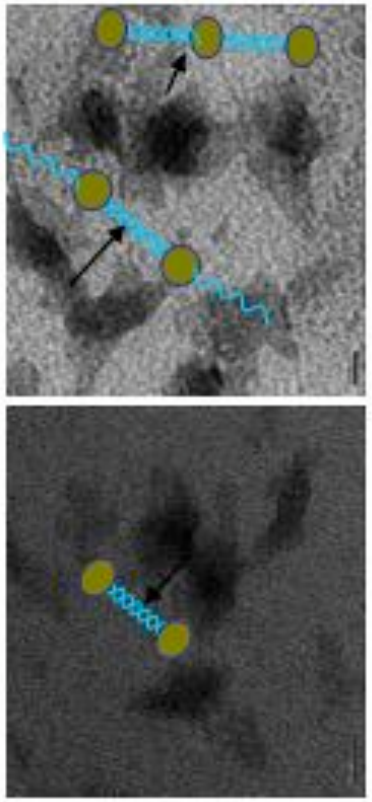




AFM images of dsDNA-TiO2 nanocomposites, i.e. TiO2 NPs assembled by DNA molecules (Low ratio of T2-T5 with TiO2 NPs: ~2 DNA per TiO2 NPs).

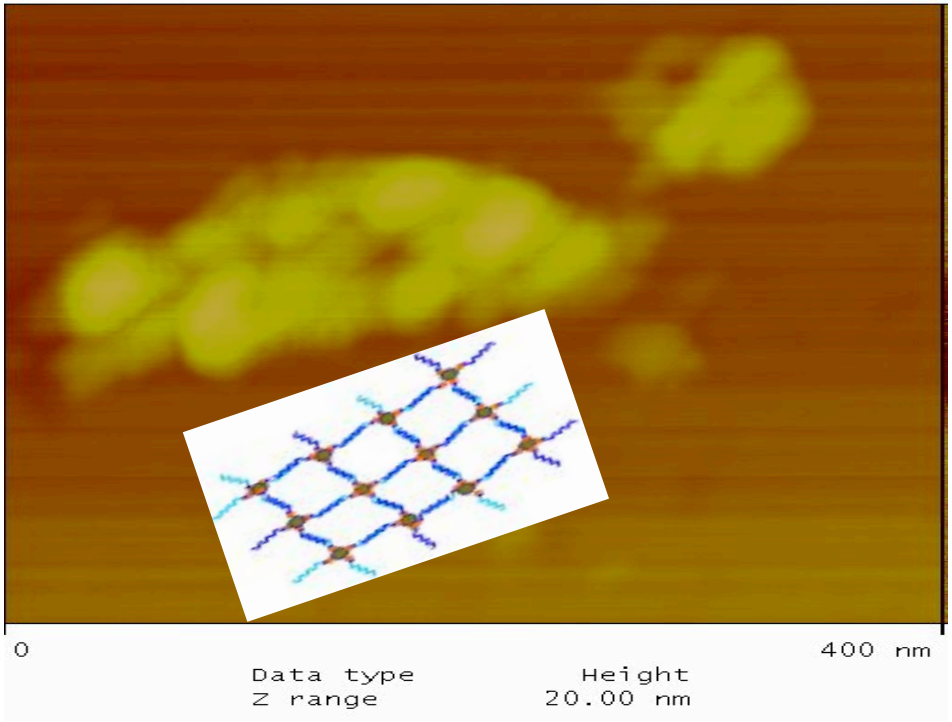
2D image of 2D dried dumbbells of nanoconjugates





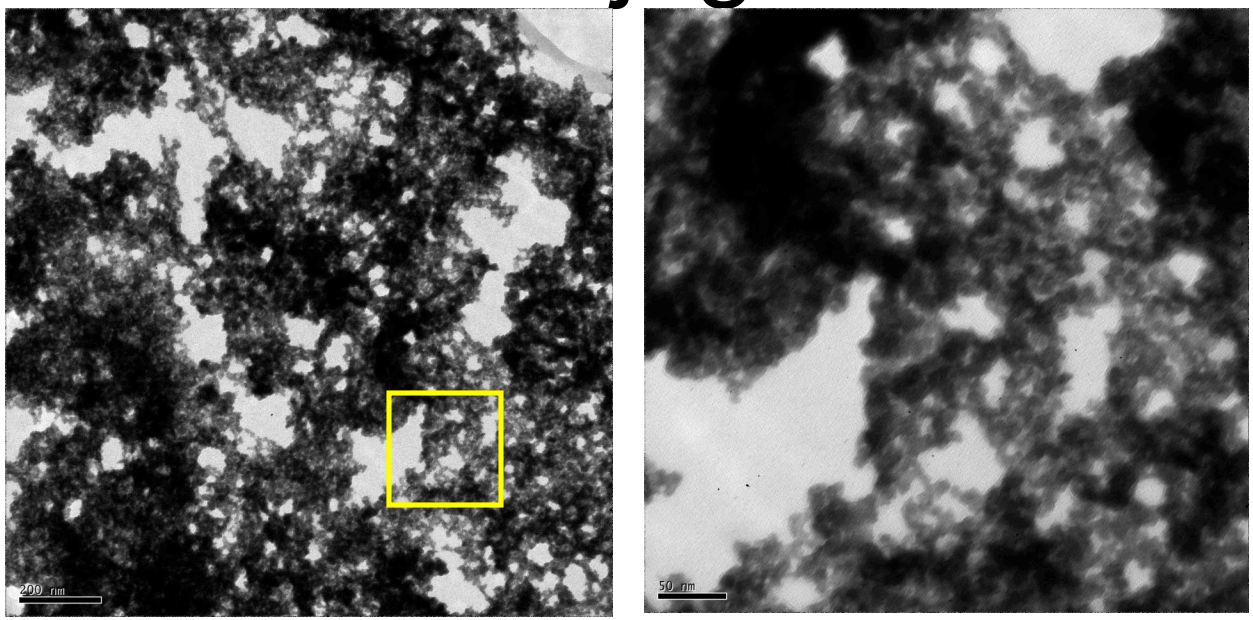
TEM images of dsDNA-TiO2 nanocomposites and some high-resolution images for individual nanocomposites, i.e. TiO2 NPs assembled by DNA molecules (Low ratio of T2-T5 with TiO2 NPs: ~2 DNA per TiO2 NPs). The scale bars are: Left, 200 nm; Right, 20 nm.

2D image of dried 3D networks of nanoconjugates



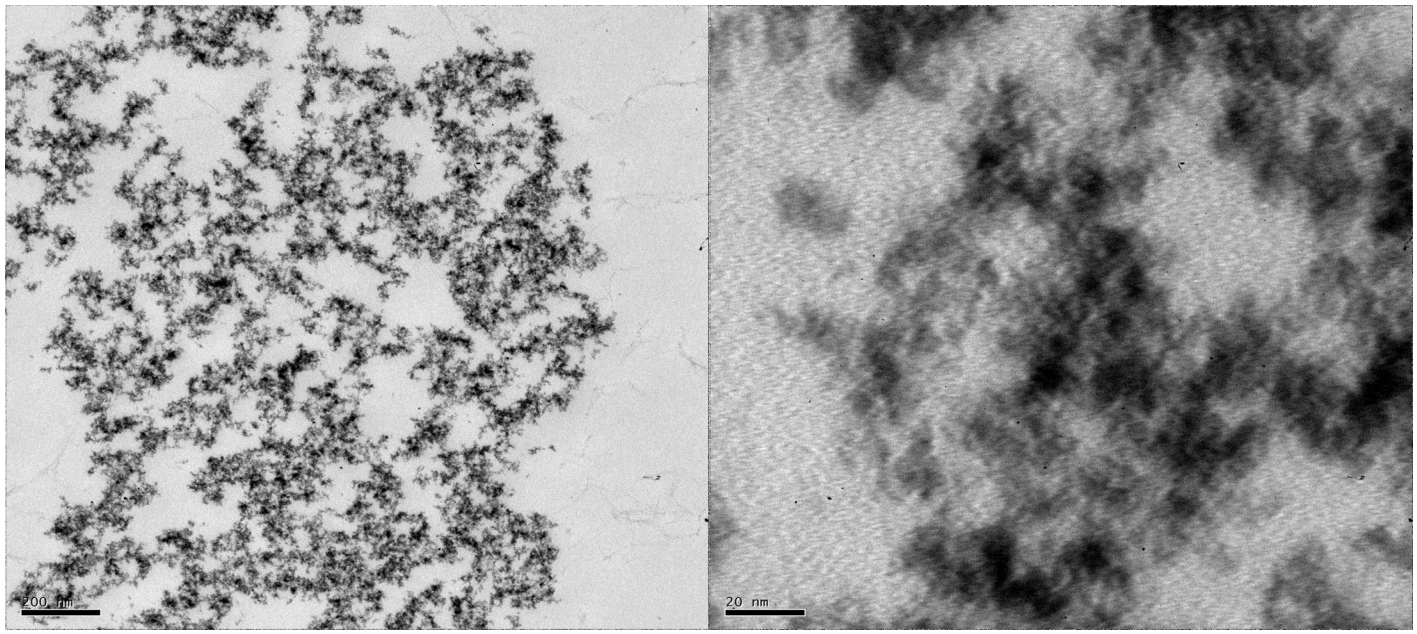
An AFM tophographical image of nanoparticulate superstructures of TiO2 nanoparticles assembled by DNA molecules, full scale size: 400 nm

2D image of dried 3D networks of nanoconjugates



A TEM image of nanoparticulate superstructures of TiO2 nanoparticles assembled by DNA molecules (Scale bar: 200 nm) and a zoomed area (Scale bar: 50 nm).

2D image of 3D network of nanoconjugates solidified in resin and sectioned

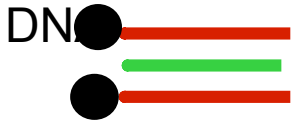


TEM images of nanoparticulate superstructures of TiO2 nanoparticles assembled by DNA molecules in solidified agarose (Scale bar: 200nm, Left) and a zoomed area (Scale bar: 20 nm, Right).

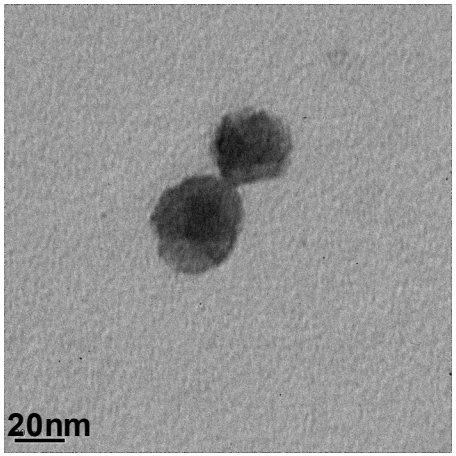
Interactions of TiO_2-PNA nanoconjugates *in vitro*

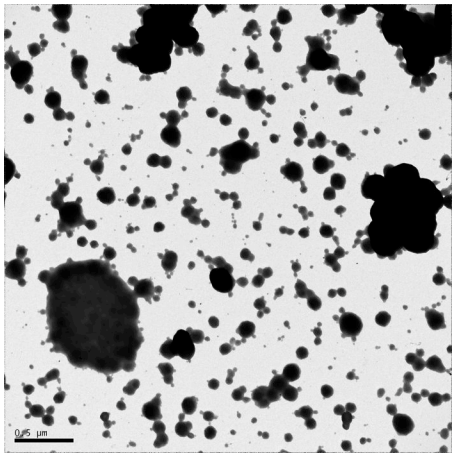
Molar Ratio of PNA-NC:DNA Affects Complexes Formed

↑ PNA-NC : DNA



Doublet NP More Prevalent

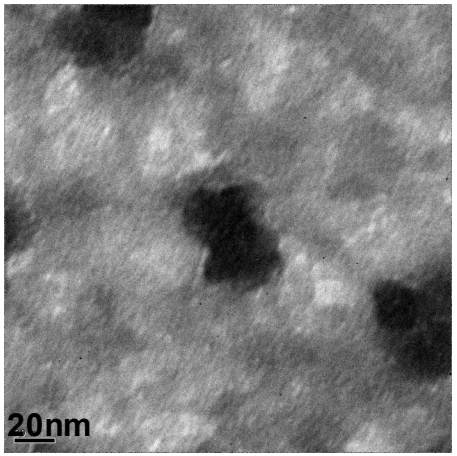


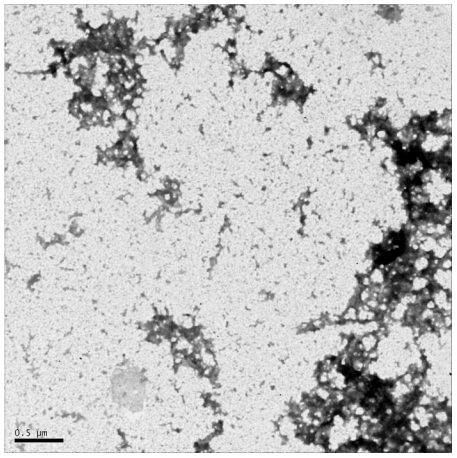


Background DNA Highly Sequestered by PNA-NC

↓ PNA-NC : DNA

Doublet NP Less Prevalent

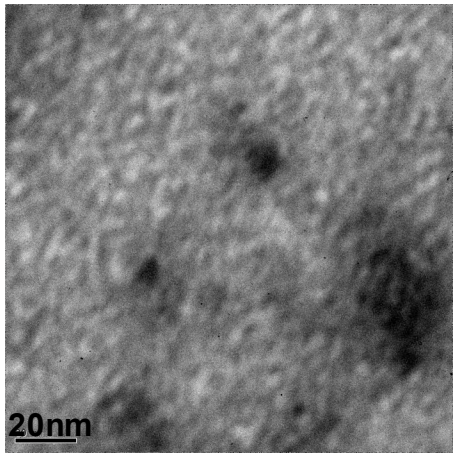


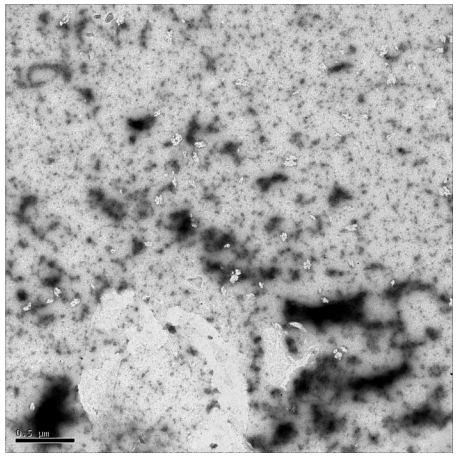


Background DNA Less Sequestered by PNA-NC

Nanoparticles +

Doublet NP Rarely Seen

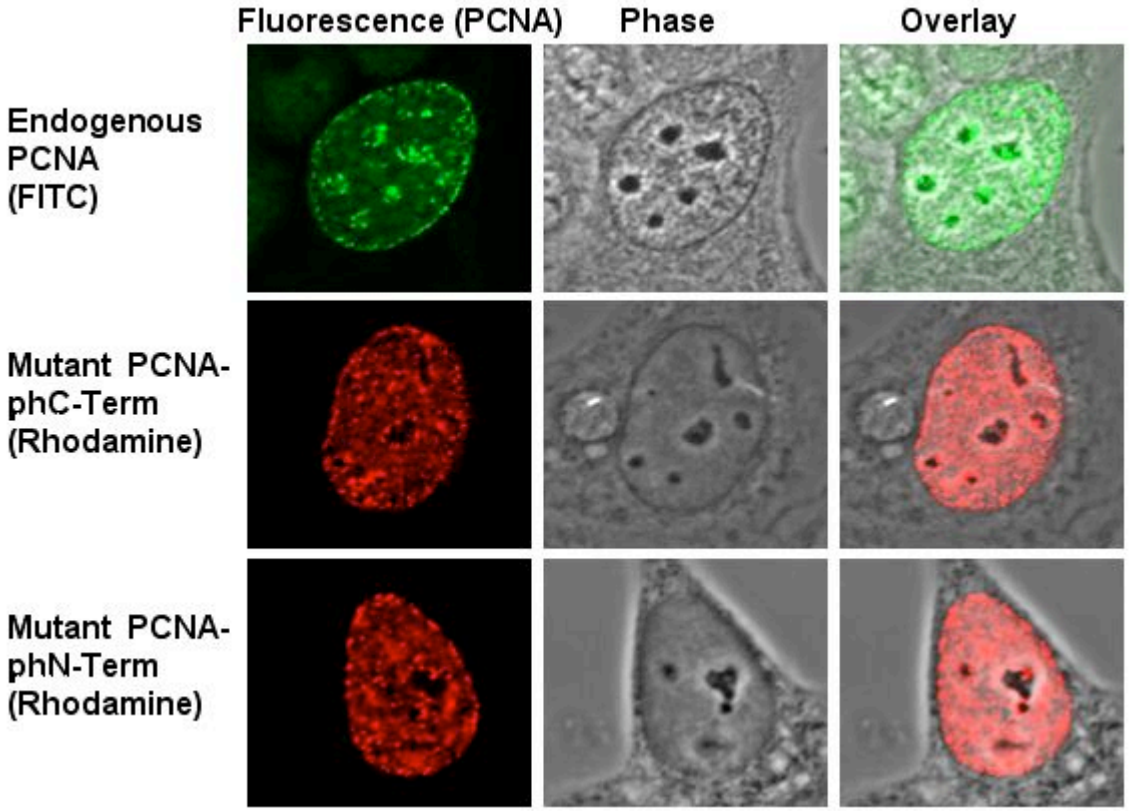




Background DNA Least Sequestered by NP

Interactions of TiO_2-DNA nanoconjugates and proteins *in vitro*

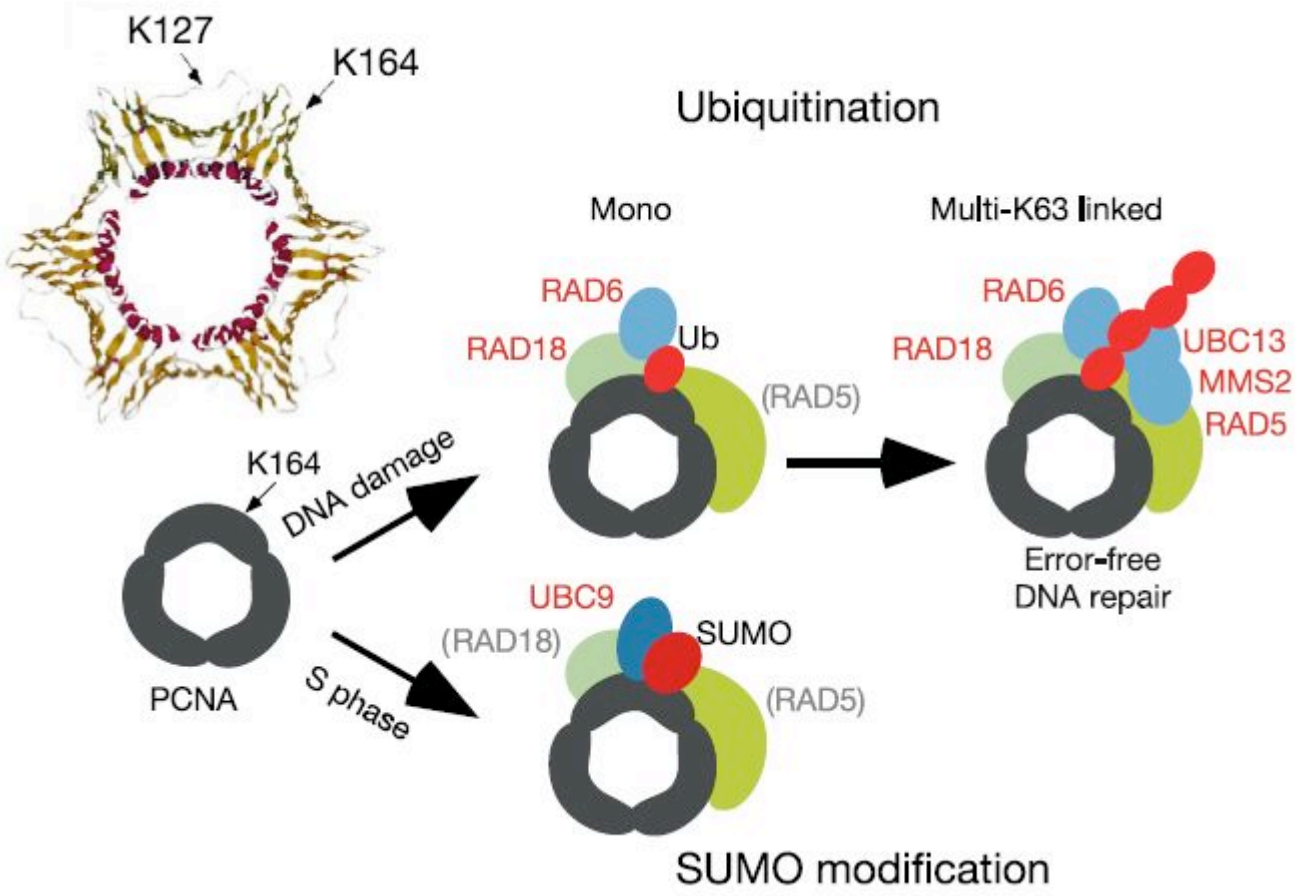
Cloned DNA binding protein proliferating cell nuclear antigen PCNA characterization



Fluorescent Confocal Microscope Images of PCNA of Mid-S-Phase Localization Patterns

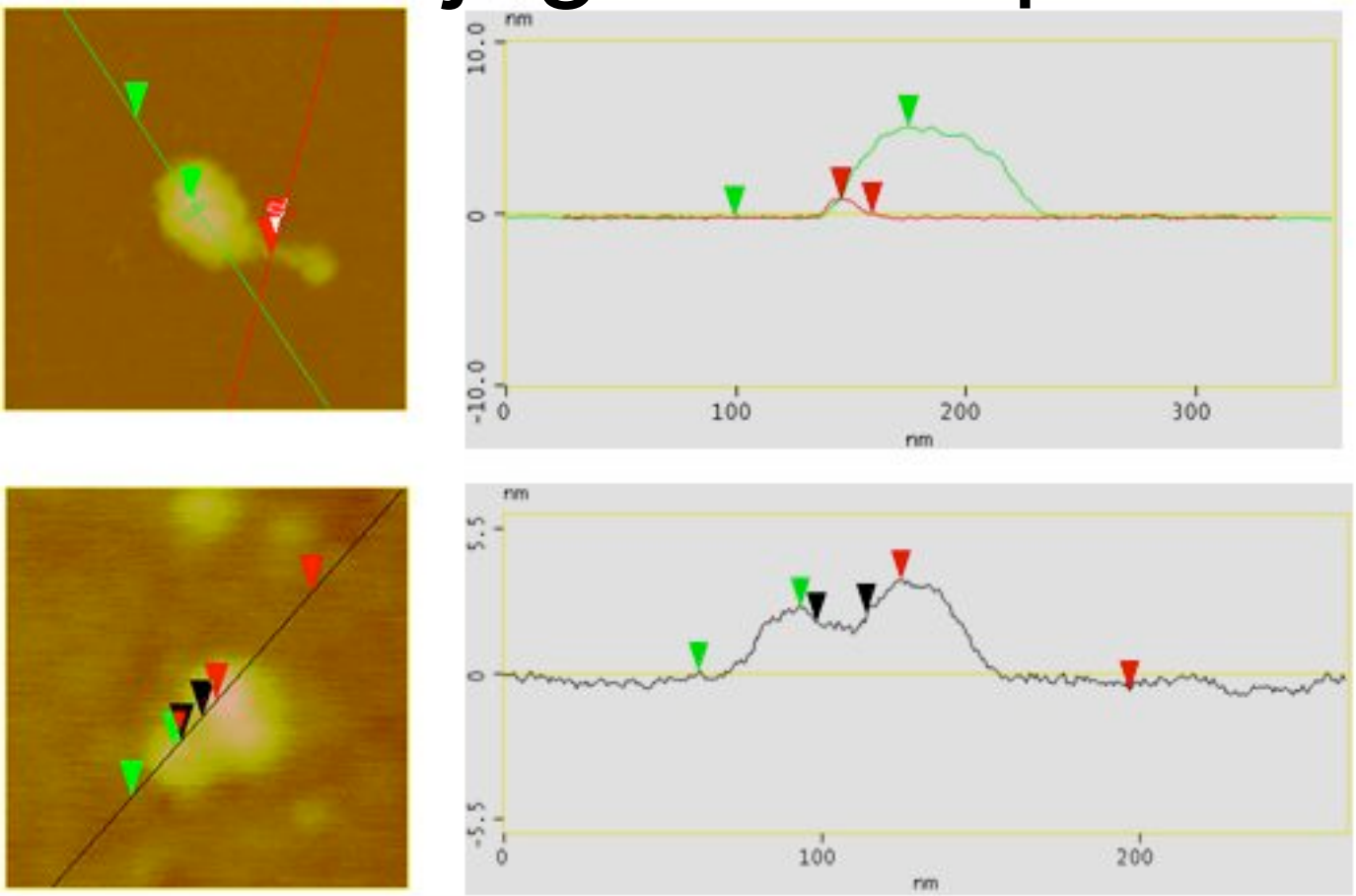
Mutant PCNAs are able to form the same type of mid-synthesis phase patterns as their endogenous counterparts.

Role of PCNA in cellular DNA maintenance: synthesis and repair



Hoege et al., 2002

2D image of dried protein-nanoconjugate complexes



Atomic Force Microscopy image of TiO_2-oligonucleotide nanocomposite on its own (top), or covered by the proliferating cell nuclear antigen (PCNA) protein trimer

How would Coherent X-ray Microscopy influence these studies?

- Nanoconjugate assemblies could be studied in 3D inside cells *in situ* [cryo capabilities would need to be implemented paired with inherent 3D high resolution imaging capability of CXM]
- Overlap of nanoconjugate assemblies and fluorescent images (obtained by confocal fluorescent microscopy) could be confirmed and imaged in more detail by Coherent X-ray Microscopy [resolution of optical microscopy is 200 nm while for CXM it can be 5 nm].

Lab picnic, July 2006