Biological studies with the Bionanoprobe

Science Opportunity presented by
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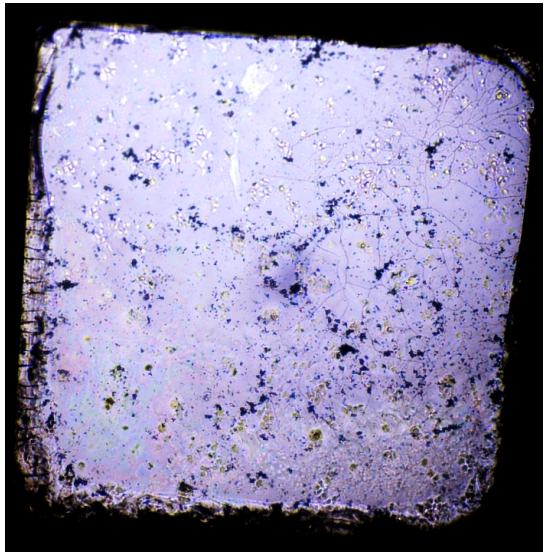
The Bionanoprobe

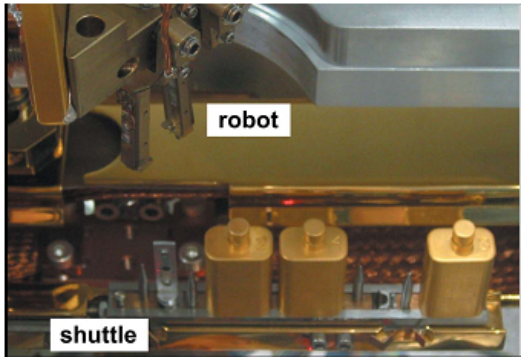


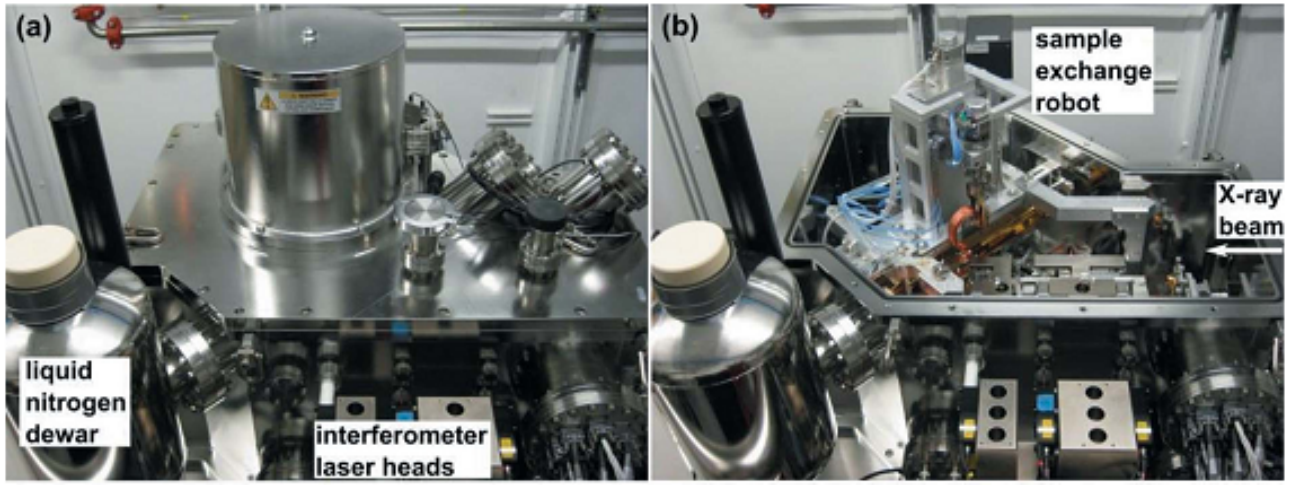
- Frozen hydrated samples = preservation of subcellular structure. Prevents diffusion of small molecules

-Tomography

-High resolution

-In vacuum





Bionanoprobe at present...

Research conducted with a variety of biological samples – similar to work with other X-ray microprobes but with frozen samples, high resolution done often as multiple projections for tomography, attempts at ptychography

Challenges:

- low signal intensity (much time is consumed for imaging of a single 10x10 micron cell with 200nm steps)
- computational demands are much greater than support

APS-U and Bionanoprobe

The APS Upgrade will provide

- order-of-magnitude increases in flux
- improved focused spot sizes, that will

Even with the use of equipment that is currently available this would lead to entirely new biological information

Bionanoprobe at present...

Woloschak lab research: focused on radiation biology in every form:

- effects of different doses and regimens of radiation and radionuclides on cells and whole organisms
- ways to prevent unwanted effects of radiation
- ways to use radiation as curative treatment for cancer
- development of nanomaterials to modulate effects of radiation

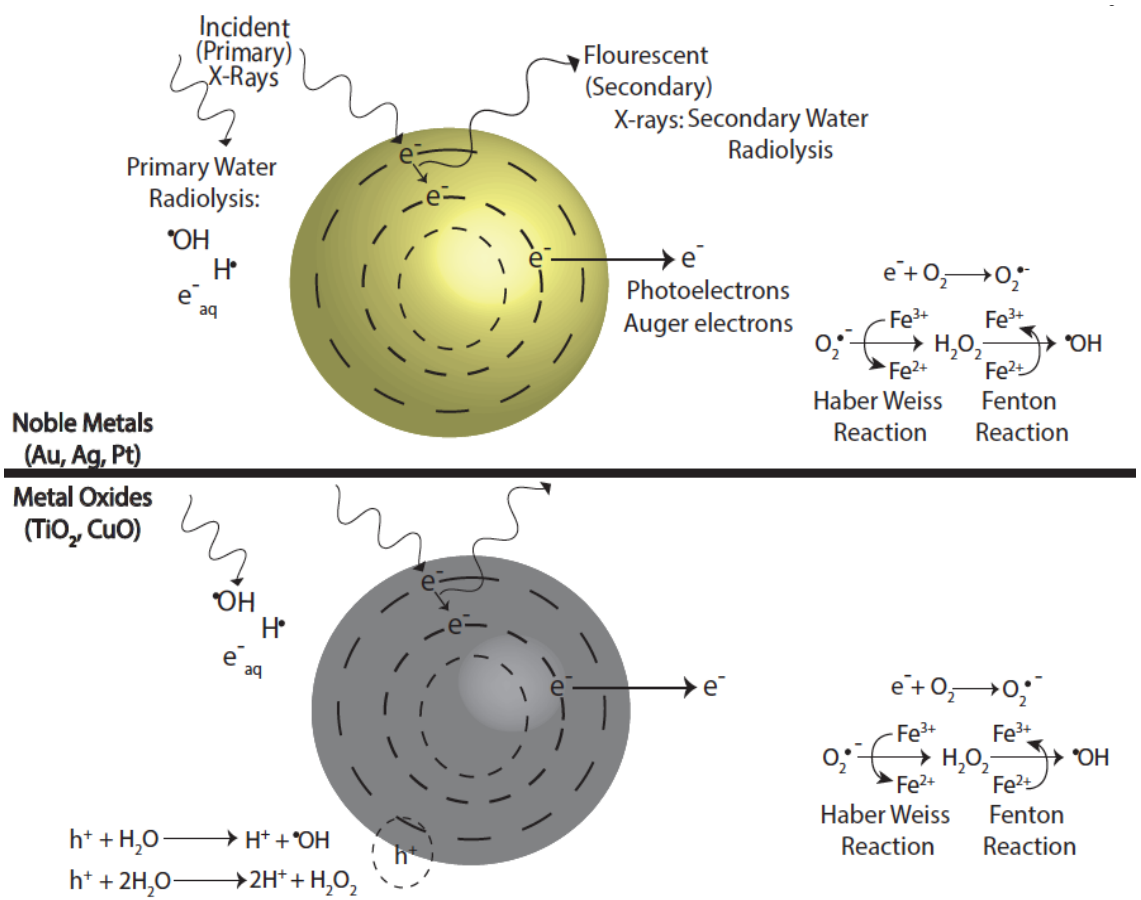
Only biological techniques and “standard optical imaging” are used for different mechanistic studies e.g. exosomal and cellular microRNAs are studied to investigate radiation responses of whole organisms

WHY: use of X-ray fluorescence imaging for this purpose is limited because of need for greatest possible resolution and relatively low elemental content of native biological samples

X-ray fluorescence microscopy at the Bionanoprobe is used (by our lab) mostly for nanoparticle related work

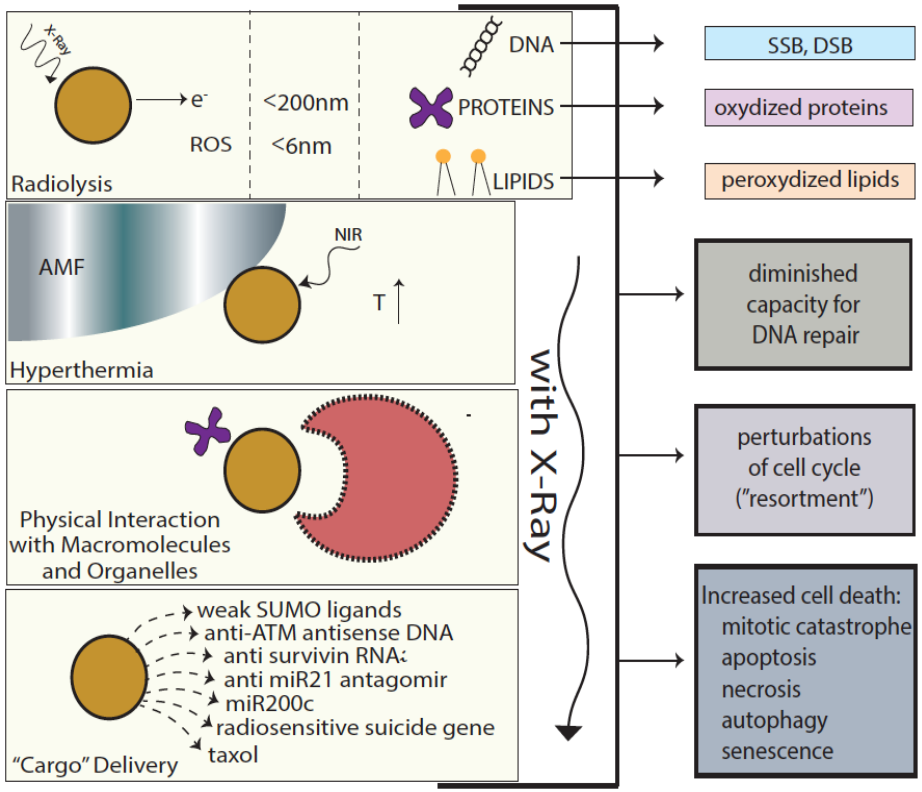
WHY: elemental concentration in nanoparticles is high enough to allow identification of particles to aid reconstruction in tomography

Developing nanomaterials to modulate effects of radiation



Exposure of nanoparticles to ionizing radiation (e.g. X-rays) causes Compton and Photoelectric effects – photoelectrons and Auger electrons (e^-) are ejected from the nanoparticles as well as photons of different energies, including secondary fluorescent X-rays. In, addition, in semiconductor nanoparticles release of electrons leads to formation of reactive electropositive holes (h^+) on nanoparticle surface. Free electrons and electropositive holes on nanoparticle surface can damage cellular components directly or lead to production of reactive oxygen species (superoxide $O_2^{\bullet -}$, hydroxyl radical $^{\bullet}OH$, hydrogen peroxide H_2O_2) which are also formed by radiolysis of water (Paunesku et al., 2015).

Nanomaterials in cellular milieu: Radiosensitization



Cellular effects of radiosensitizing nanomaterials. Several different "pathways" to radiosensitization by nanoparticles exist. In the first place, free electrons (released by nanoparticle ionization are absorbed by surrounding molecules within 15-200 nm from particle surface and ROS (which on average traverse 6nm distances in cytosol) produced by nanoparticles damage cellular components in addition to incident ionizing radiation and products of water radiolysis. Free electrons and ROS oxidize proteins, peroxidize lipid membranes (cell and organelle membranes both), and produce SSBs and DSBs in DNA. Secondly, nanomaterials may be used to develop hyperthermia under the influence of alternating magnetic field or near infrared. Thirdly, physical interactions between nanomaterials and cellular components, biomolecules, molecular assemblies and organelles modulate behavior of these cellular elements in different ways leading to such outcomes as perturbations in cell cycle or DNA repair. Fourthly, nanomaterials can be made of components (or carrying a cargo) specifically designed to make the recipient cells more sensitive to radiation, such as e.g. delivery of anisense oligonucleotides for DNA repair regulating genes, miRs or delivery of weak ligands for UPS machinery (Paunesku et al.2015)

Nanoparticles and Cells: functional endpoints

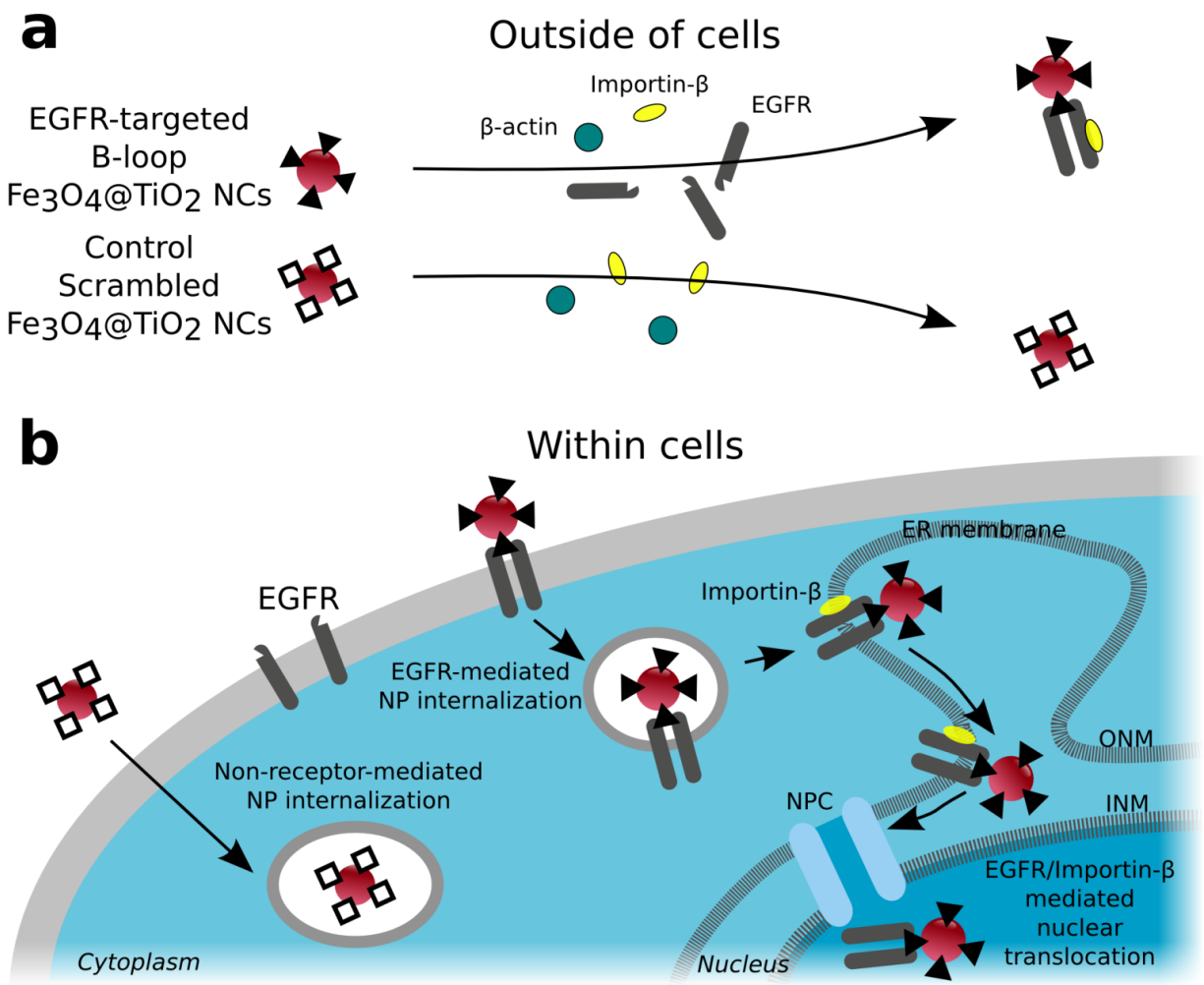
Semiconductor behavior of TiO_2 can be used to modulate DNA damage:

- white light & TiO_2nanoparticles / nanocomposites cause DNA dsbs when they would not normally occur, leading to cell killing
- UV light & TiO_2nanoparticles / nanocomposites lead to DNA dsbs, leading to increased cell killing
- gamma-rays & TiO_2nanoparticles / nanocomposites amplify DNA dsbs from gamma irradiation and increase cell killing

Nanoparticle surface reactivity can be used to modulate cell damage by nanomaterial:

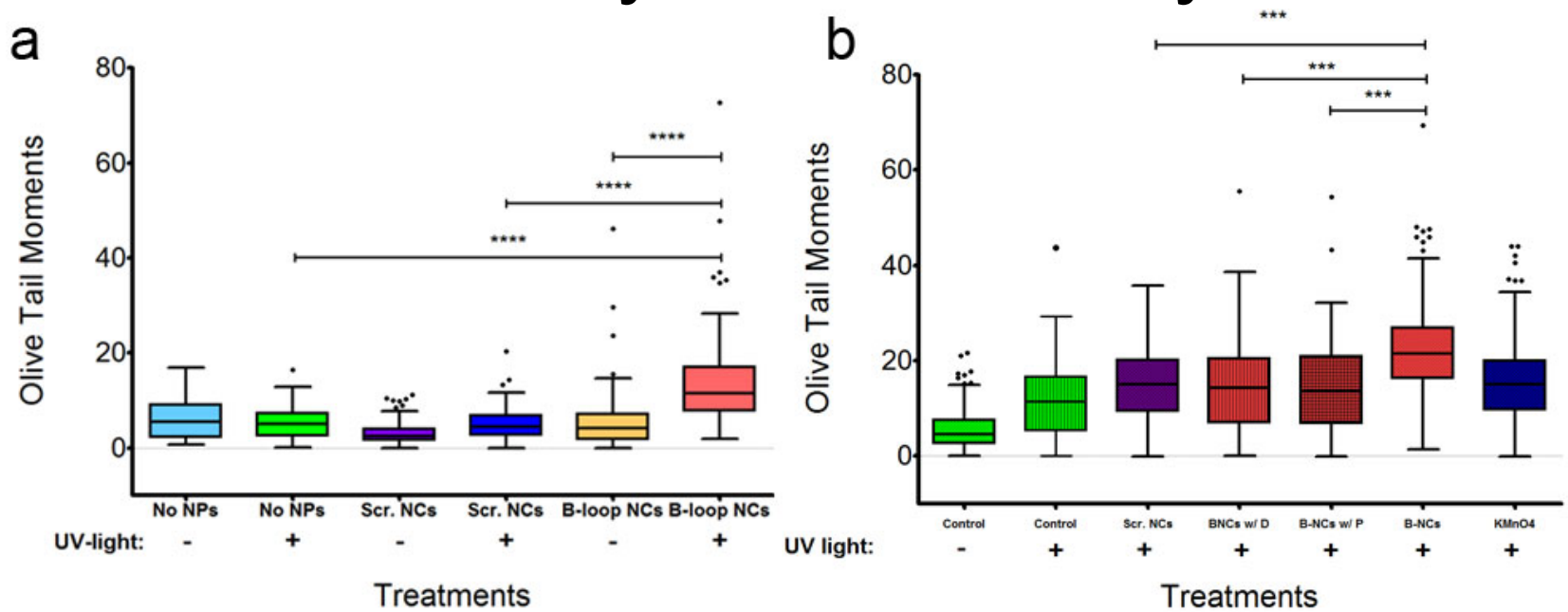
- Delivery of chemotherapeutic drugs
- Peptides and nucleic acids drive delivery to specific cell types and subcellular locations

Schematic interaction between EGFR, importin (karyopherin) and B-loop (EGF mimicking) peptide decorated nanoparticles

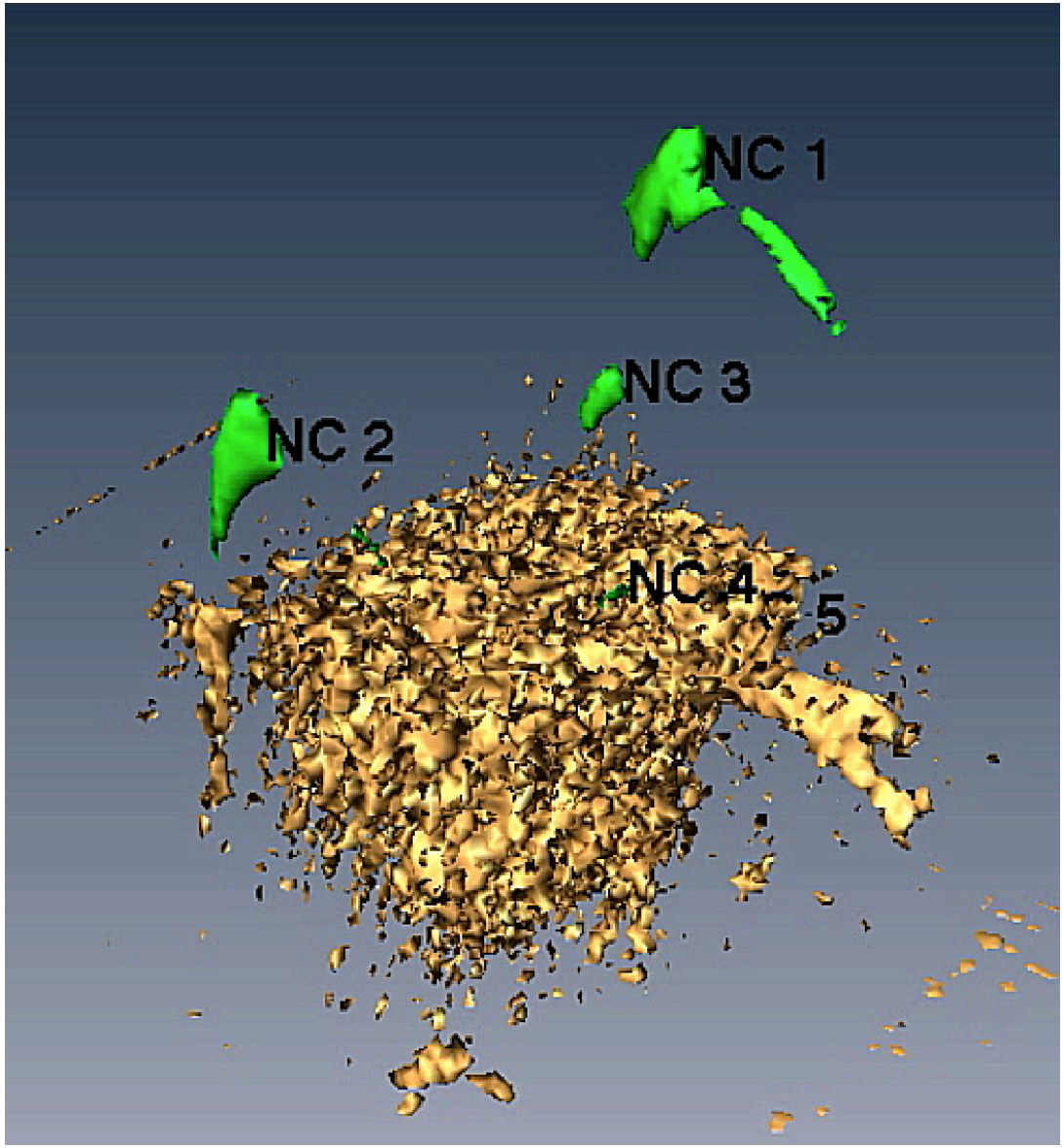


Yuan et. al., 2013 ACS Nano

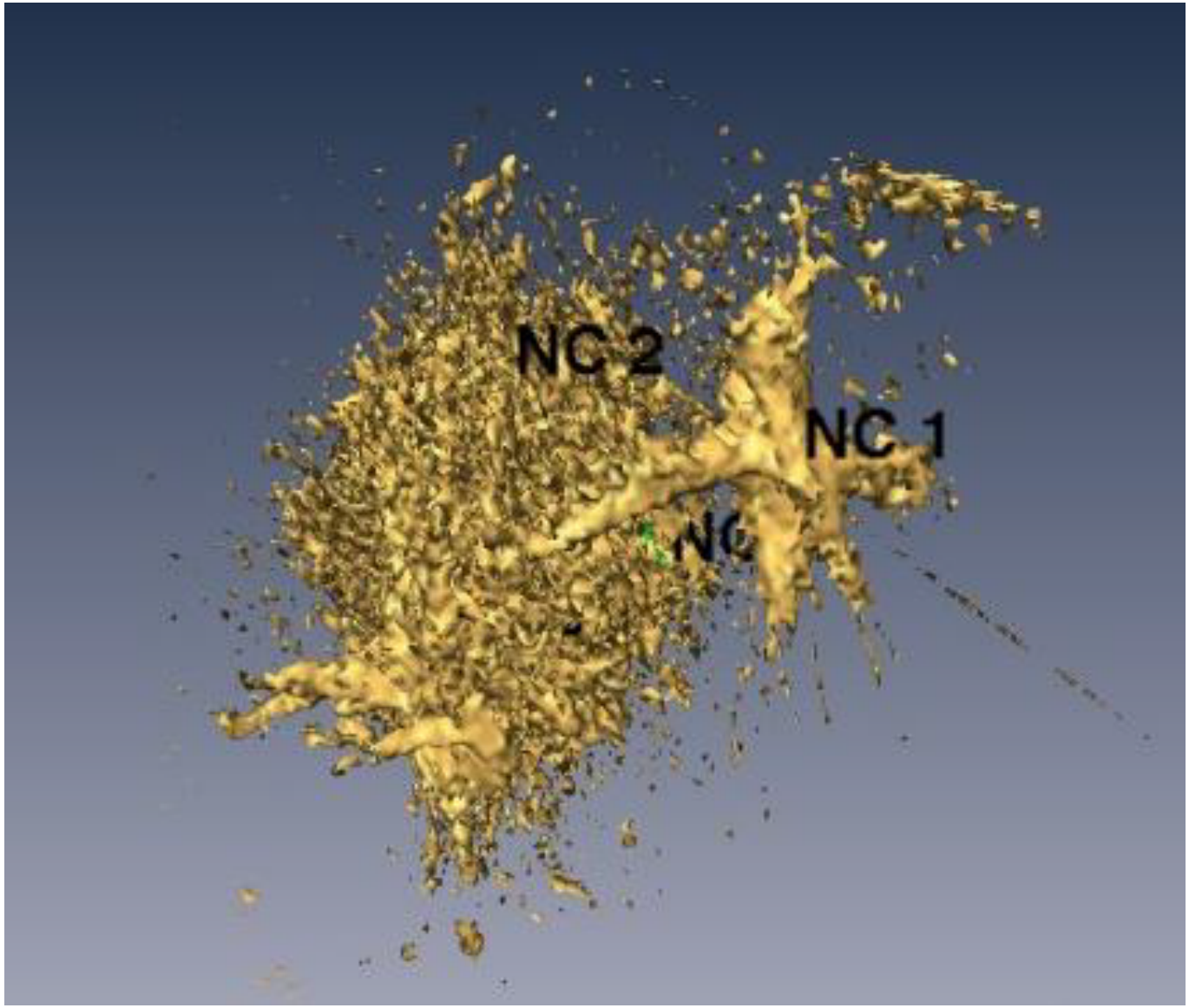
Nucleus targeted, light activated B-Loop nanoconjugates generate increased DNA dsbs by Comet assay



A, B) Comet assay, BNC = B-loop nanoconjugate, Scr. NCs = Scrambled nanoconjugate. D = dasatinib (EGFR nuclear transport inhibitor), P= EGFR nuclear transport competitor peptide



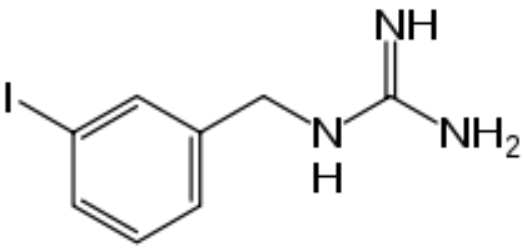
Yuan et al. PMC3919441
Still image from a video where nanoparticle (titanium) is green and nucleus (as zinc) is gold.



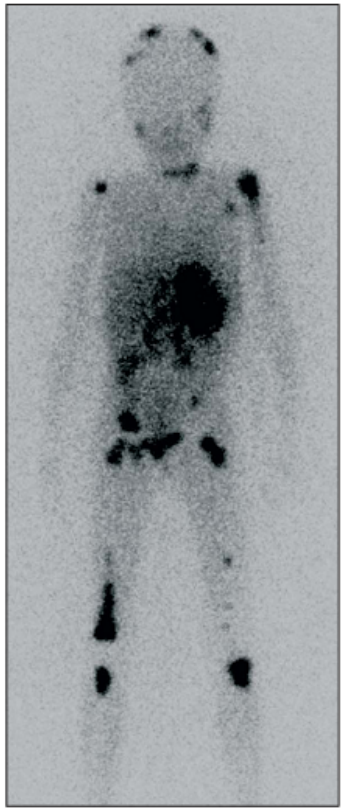
Yuan et. al., 2013
ACS NANO

Metaiodobenzylguanidine (MIBG), a common neuroblastoma imaging agent and therapy

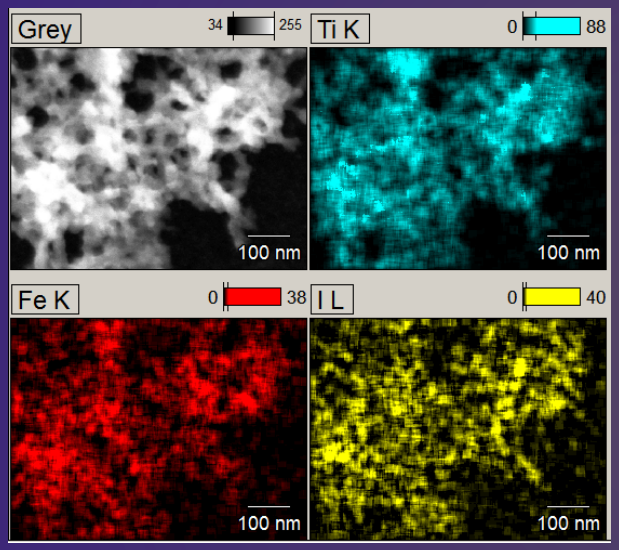
- MIBG is an imaging tool and radiotherapeutic which is selectively concentrated in over 90% of neuroblastomas
- Highly specific to neural crest tumors
- Two different iodine isotopes used:
  - I-123: used for diagnostic purposes
  - I-131: diagnostic and treatment purposes

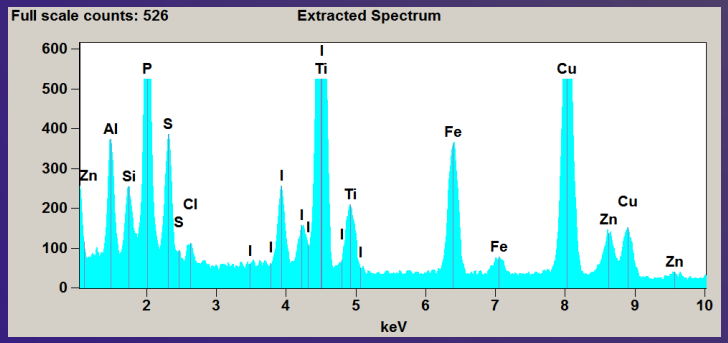


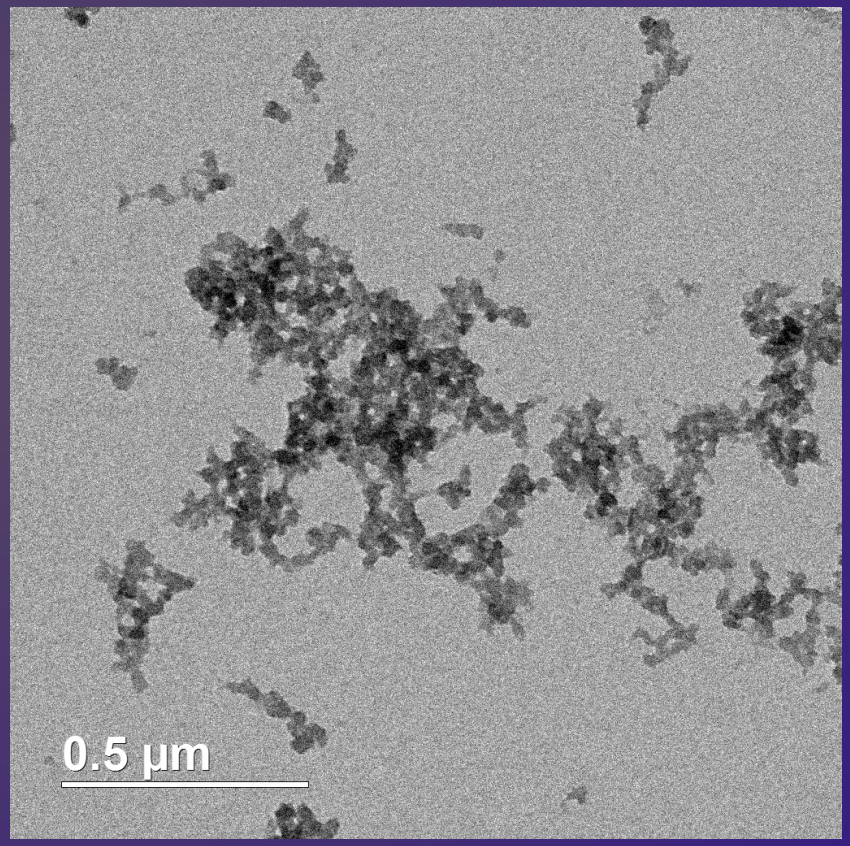
- Evidence that MIBG localizes to the mitochondria (?), but not the nucleus. Single study, <u>unconfirmed</u>
- Conjugation to Fe_3O_4@TiO_2 NPs for neuroblastoma targeting? Targeting of the nucleus via NP-NLS nanoconjugates?



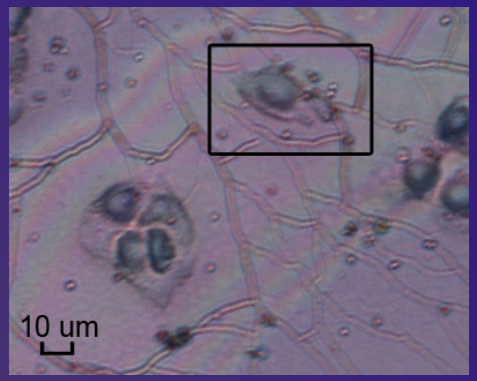
MIBG can be conjugated to Fe3O4@TiO2 nanocomposites

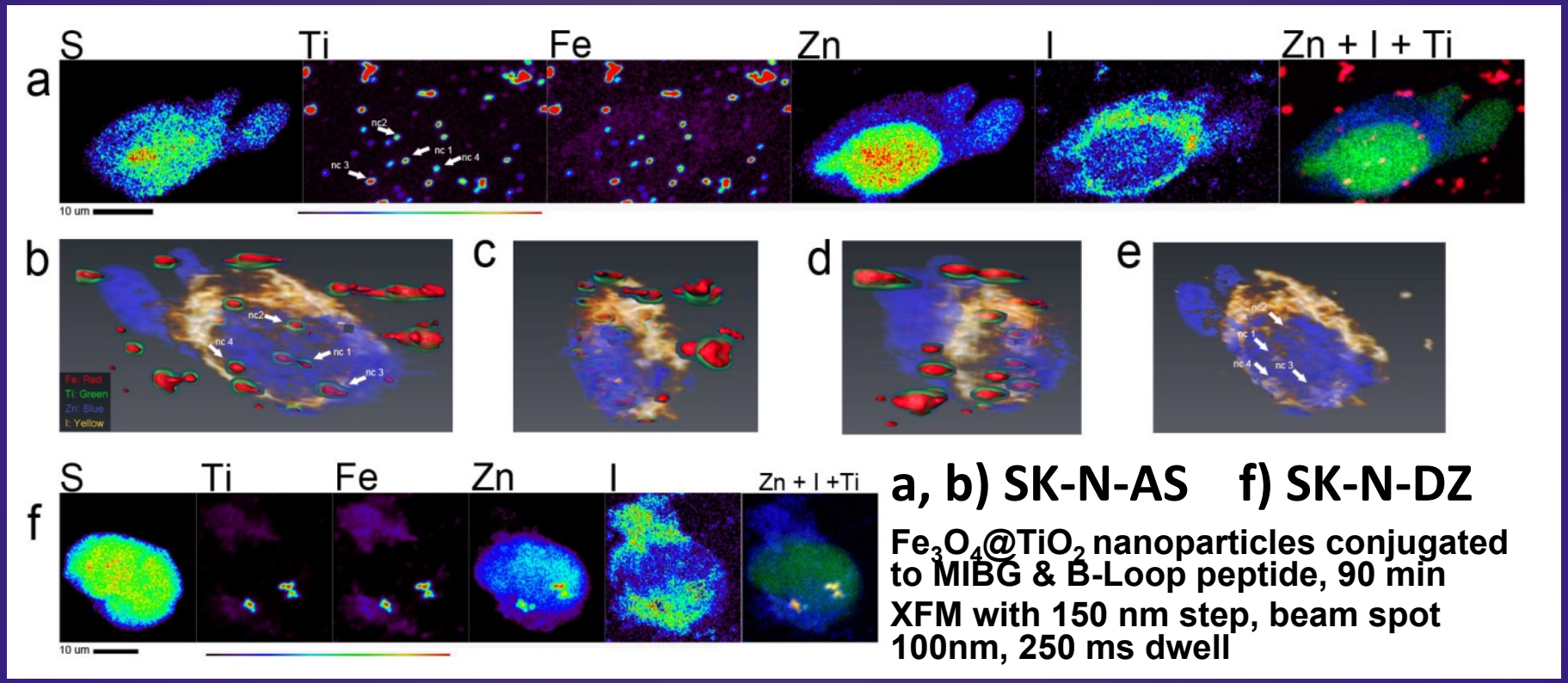




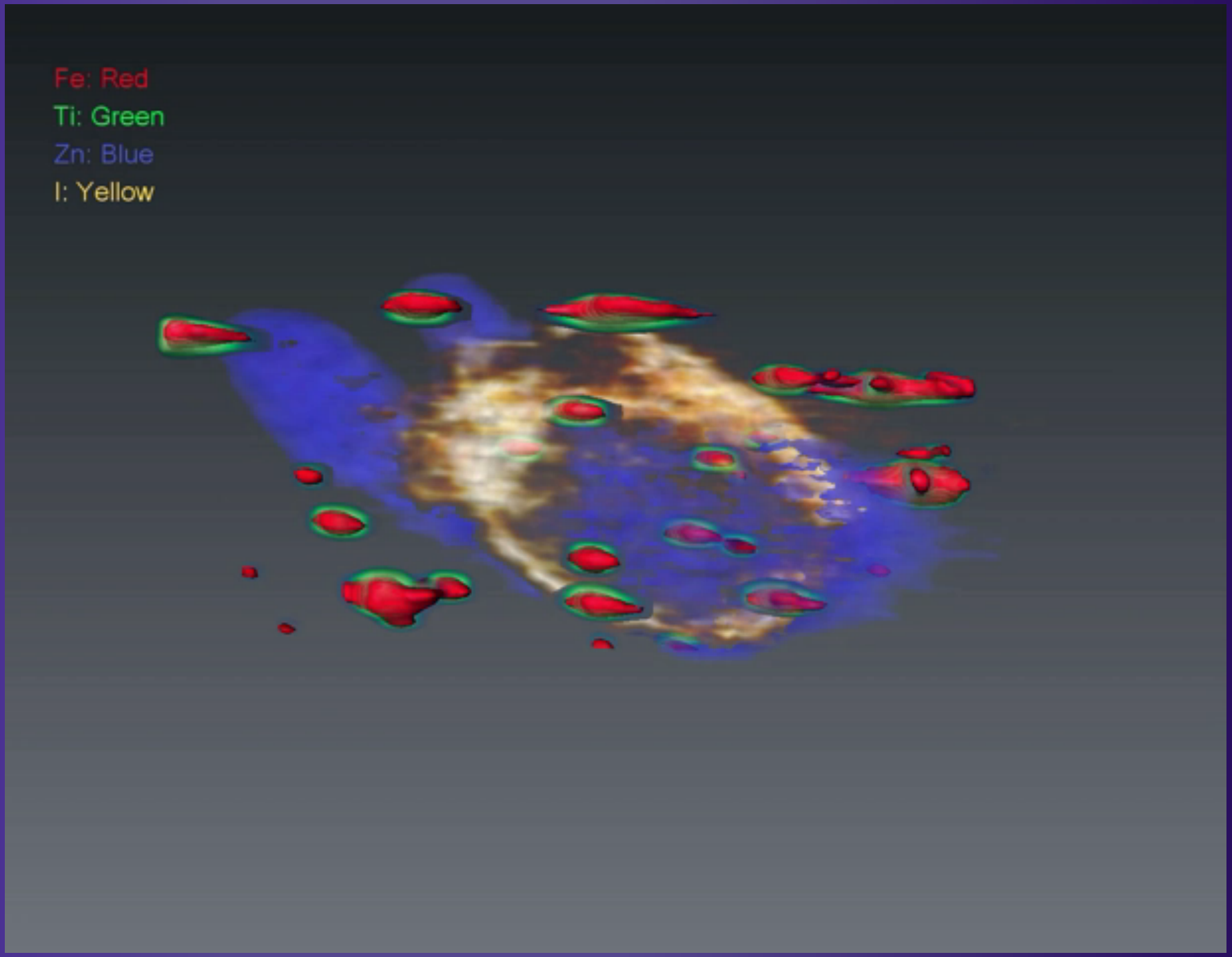


MIBG-Fe_3O_4@TiO_2 were additionally decorated with a peptide targeting EGFR called B-Loop – such nanoconstructs reach cell nuclei

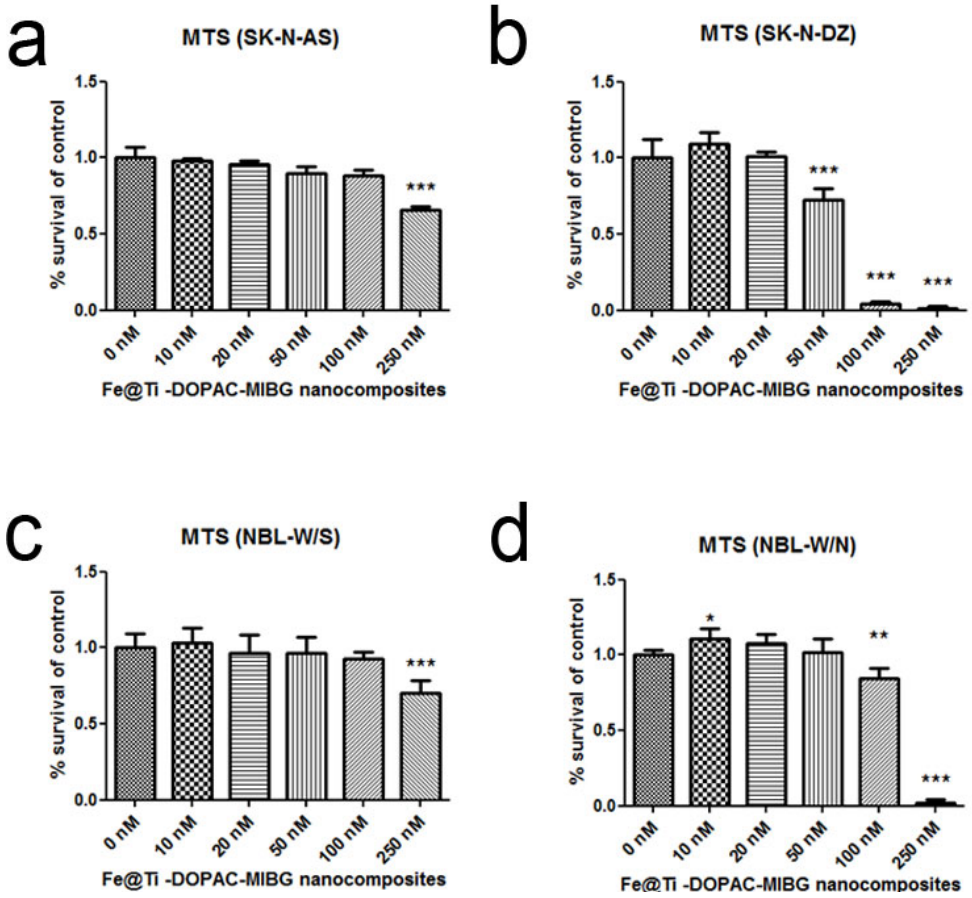




Considering cytoplasmic and nuclear localization of these nanoconstructs, several possible routes for cell injury are possible


Fe: Red
Ti: Green
Zn: Blue
I: Yellow

MIBG-Fe_3O_4@TiO_2 nanocomposites decrease neuroblastoma cell viability



- MIBG-Fe_3O_4@TiO_2 nanoconjugates significantly affect cell viability, particularly in SK-N-DZ cells and NBL-W/N cell lines (two of the “neural type” cell lines)

MIBG Nanoparticles Conclusions

Same nanomaterial may be differentially toxic to different cell types

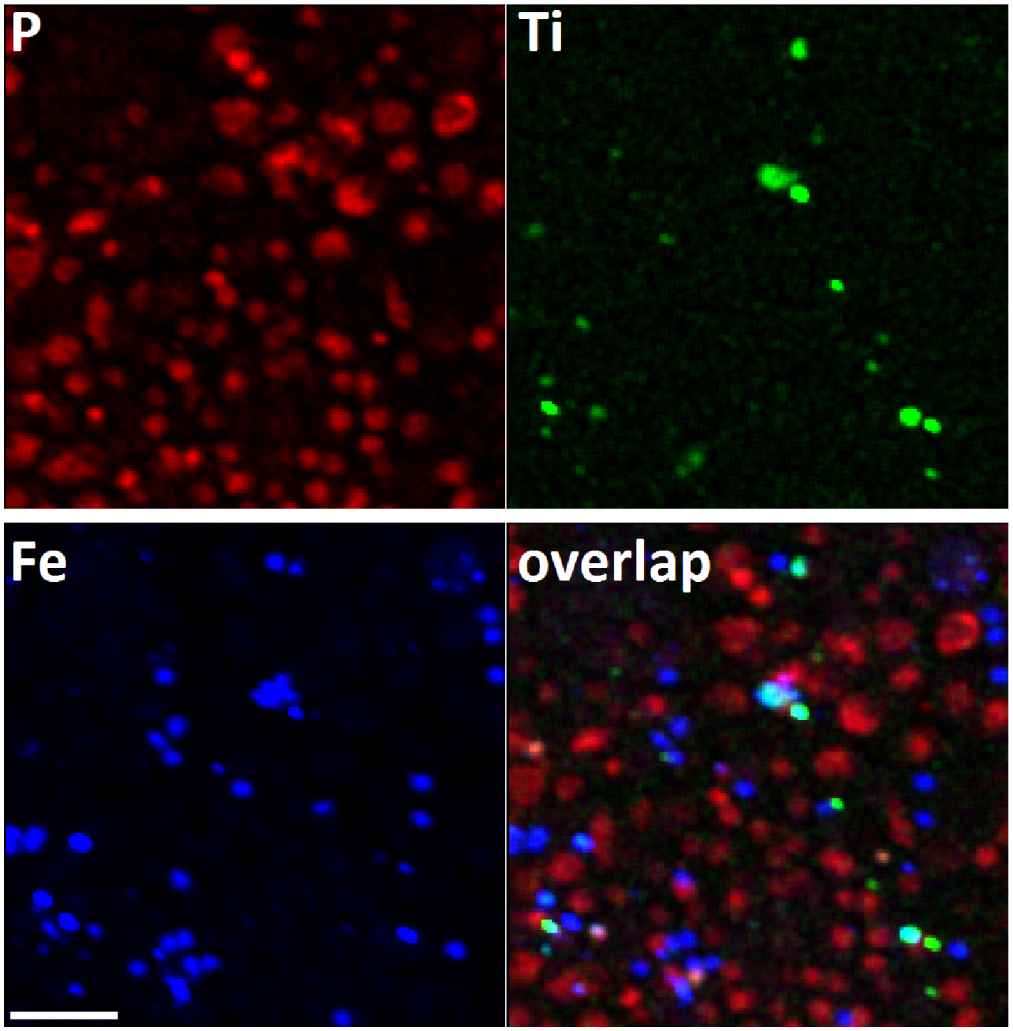
TiO_2 based nanomaterials can serve as radiosensitizers

Nanoconstructs can be targeted using peptides

X-ray fluorescence imaging at low resolution

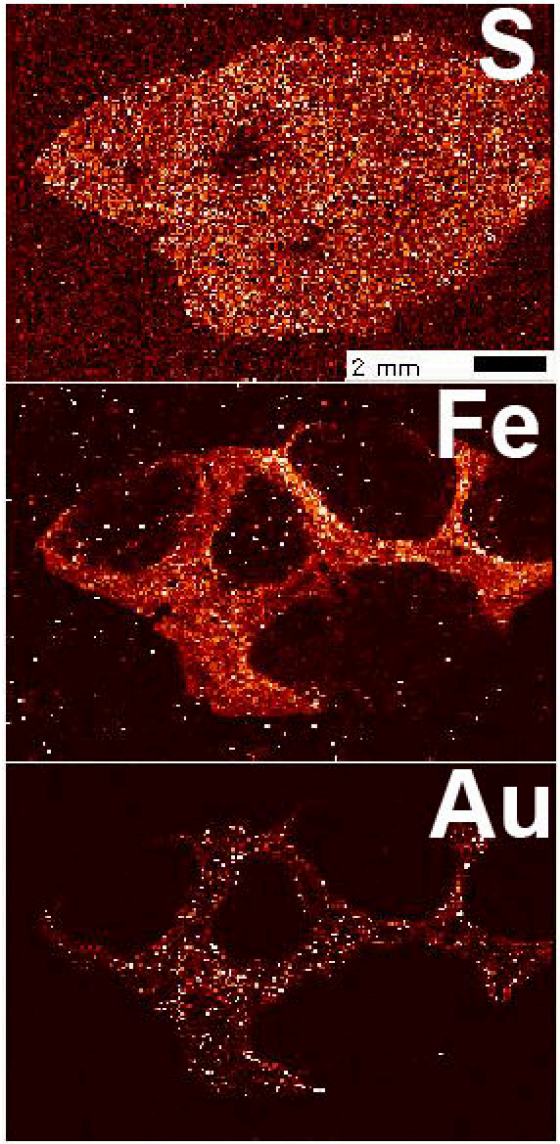
- work with tissue samples from animals is as important as work with individual cells – both are essential for a complete picture of biological events
- tissues from nanoparticle treated mice and rabbits were imaged at APS

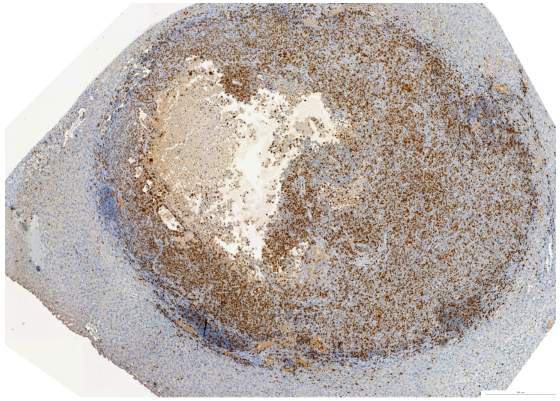
Distribution of Ti based nanoparticles in tissues



- Low resolution-high throughput tissue section imaging (paraffin embedded tissue, 4 micron thick section)
- cell nuclei (P)
- erythrocytes (Fe)
- nanoparticles (Ti)

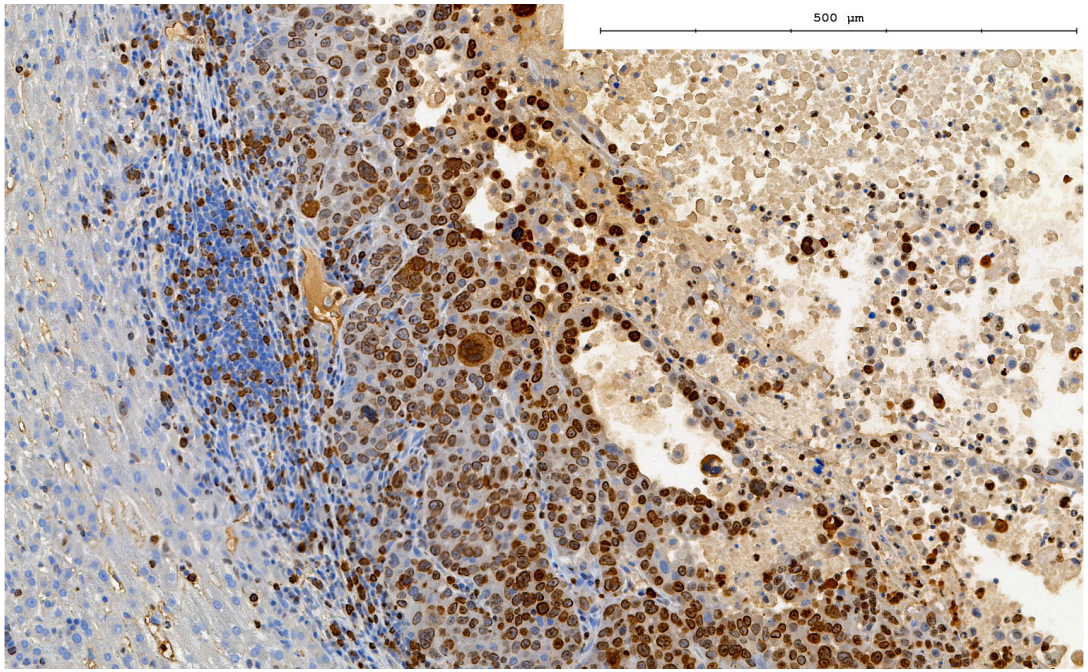
Liver, VX2 Bearing Rabbit, Nanoshells Distribute in Viable Tumor Area – “tumor EPR effect”

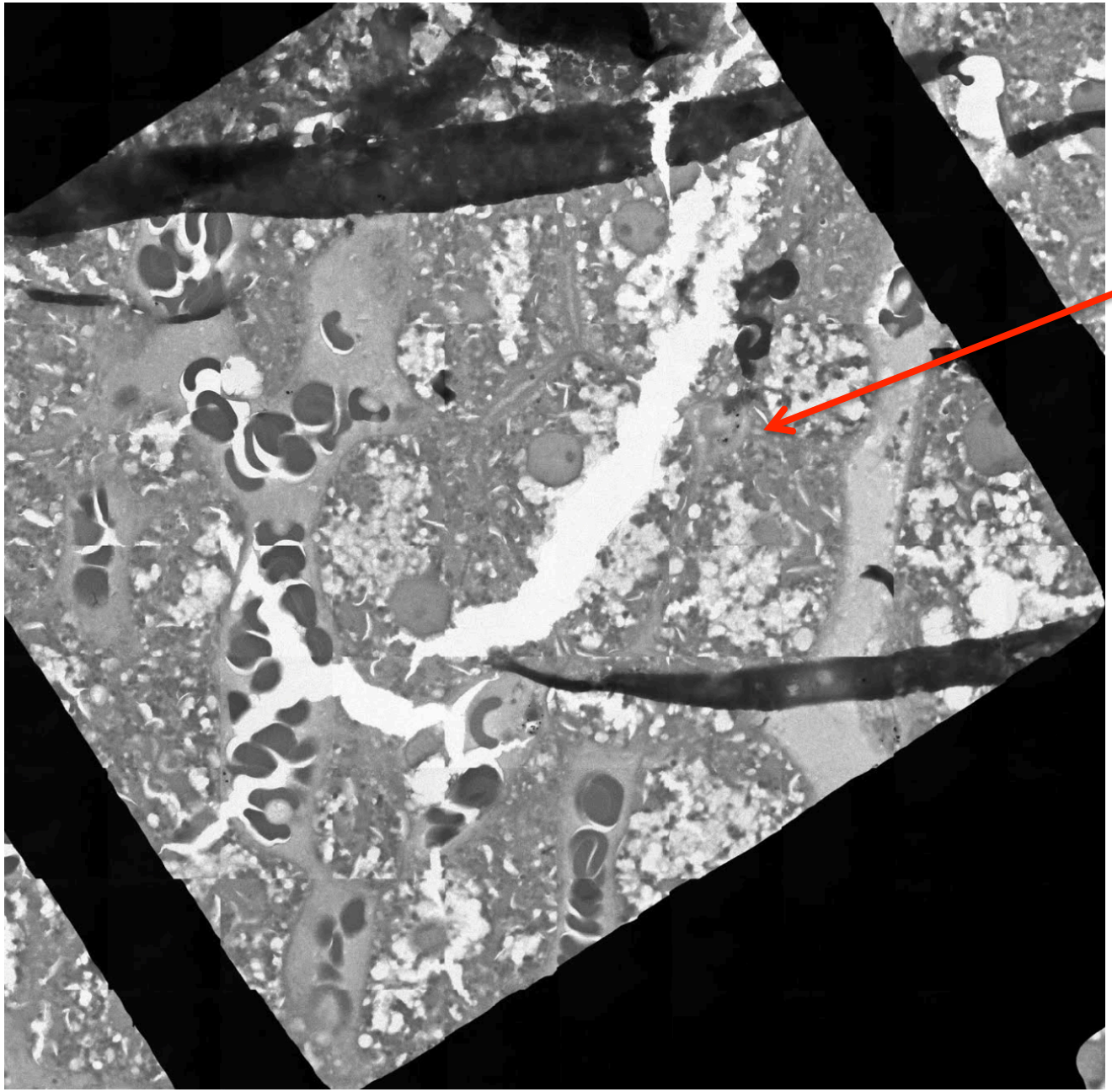




Left: Au-Si nanoparticles
Mean Au in viable tumor area: 718.8 μg/cm^2
in necrotic area: 34.1 μg/cm^2

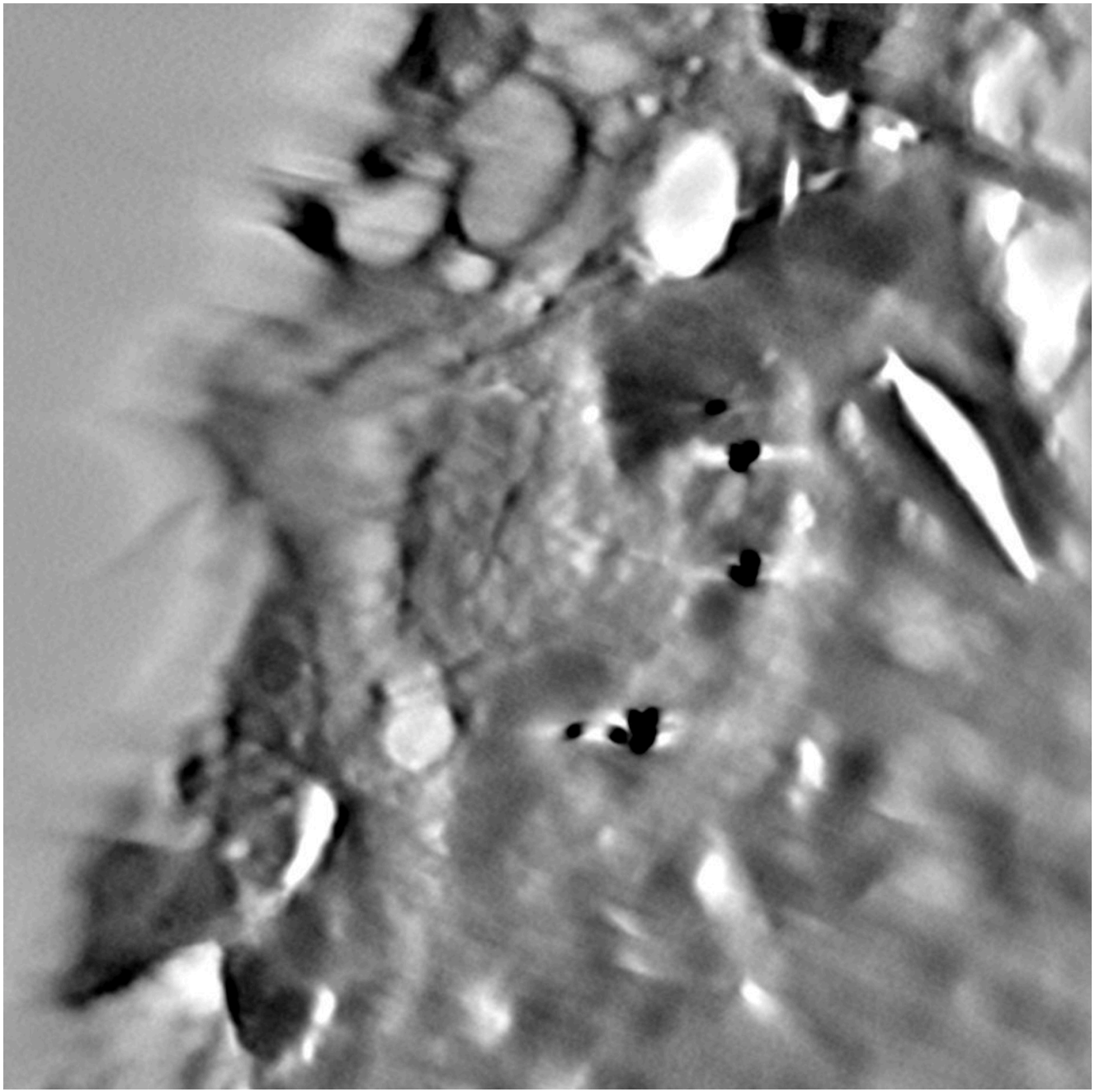
Right: Ki67 staining of the same sample.





Liver, VX2 Bearing Rabbit, nanoshells accumulate in macrophages

Soft x-ray microscopy done at ALBA



Liver, VX2 Bearing Rabbit, nanoshells accumulate in macrophages:

soft X-ray tomography of a macrophage inside tissue

Biological research with nanoparticles

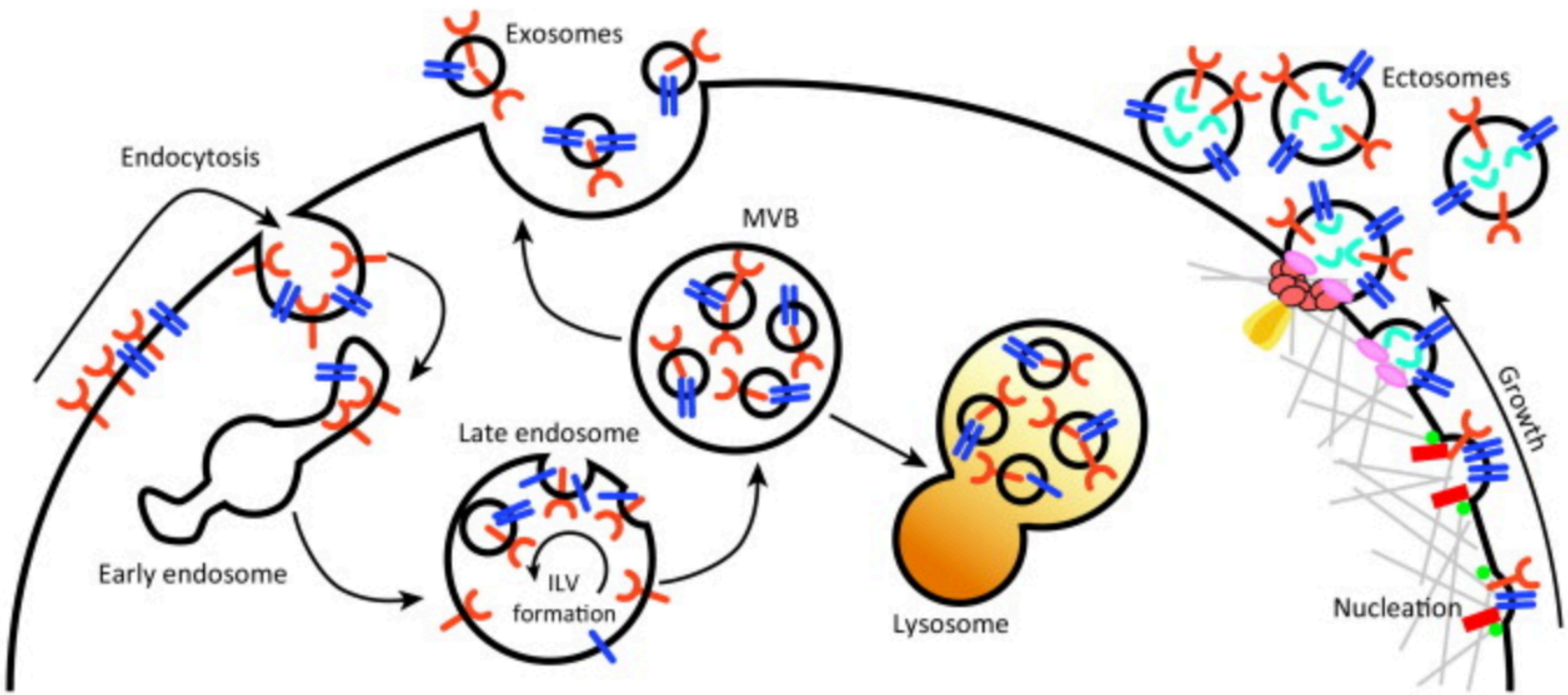
- Current x-ray imaging techniques provide information that is limited.
- APS-U will provide better resolution and higher flux
  - samples can be imaged more comprehensively (single cells and tissues)
  - information about each sample will increase with possibility to study it with high resolution and in 3D

Investigation of “purely” biological samples using x-rays

In depth studies of “low contrast/low elemental content” biological samples could revolutionize understanding of biological systems at different size scales.

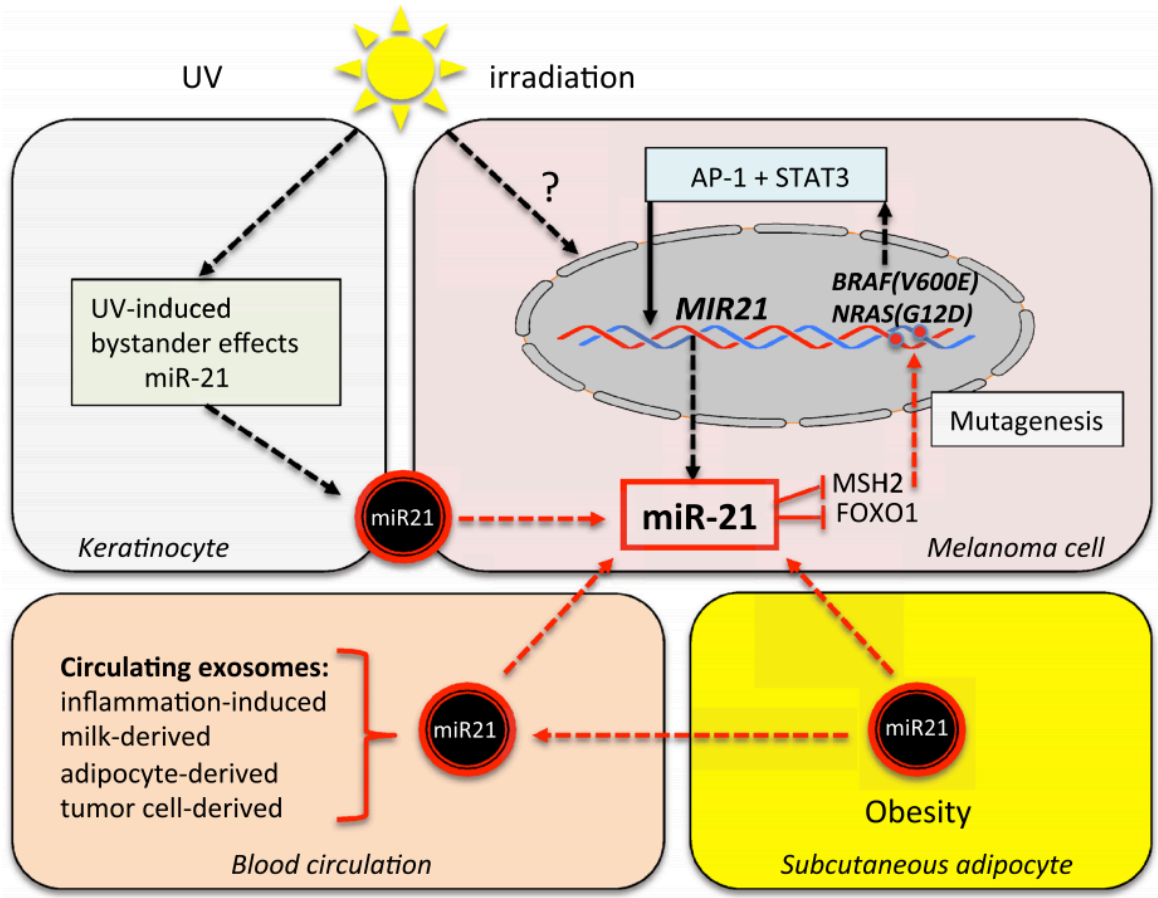
Cutting edge research in radiation biology and cancer biology is focused on microvesicles such as exosomes, especially those produced by immune system cells such as macrophages.

Exosomes and microvesicles



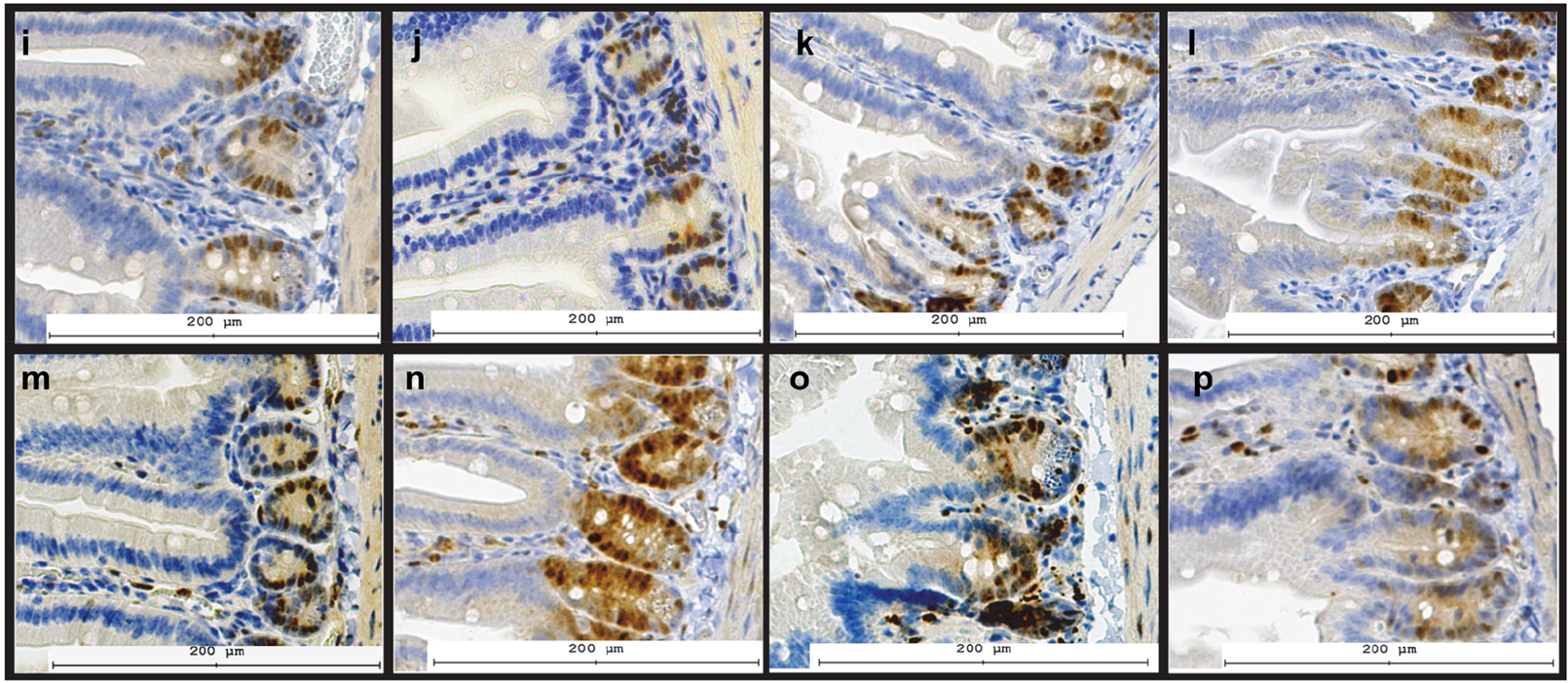
Cocucci E, Meldolesi J. (2015) **Ectosomes and exosomes: shedding the confusion between extracellular vesicles**. Trends Cell Biol

Exosomes in cancer



Working model of exogenous and endogeneous factors that increase exosomal miR-21 signaling in malignant melanoma. The common BRAF(V600E) and NRAS(G12D) mutations in cutaneous melanoma increase miR-21 expression by upregulating AP-1 and STAT3. **Keratinocyte derived exosomal miR-21 induced by UV-irradiation, exosomal miR-21 derived from the systemic circulation induced by dietary and environmental factors as well as obesity- and inflammation-associated exosomal miR-21 may enhance the total burden of miR-21 signaling of melanocytes promoting the transition to the malignant phenotype**. Melnik. J Transl Med (2015) 13:202

Exosomes in radiation biology

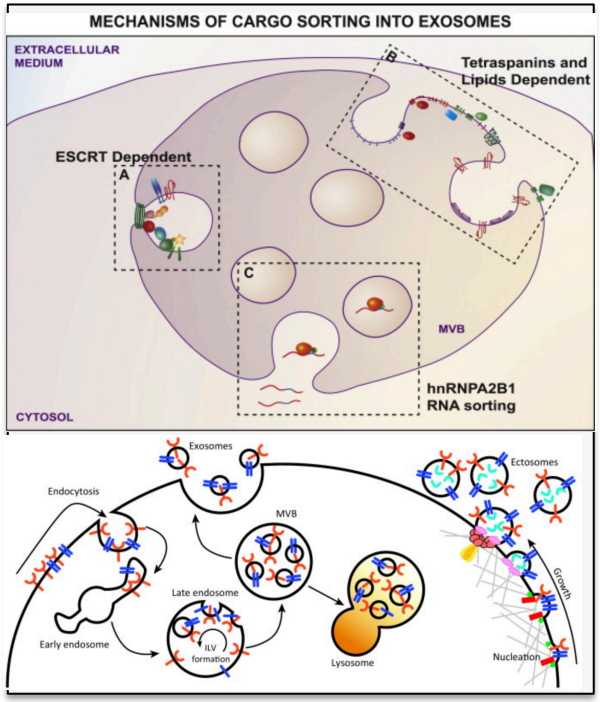


Radiation damages intestinal architecture (k, o) but modulation of immune system behavior through the use of the pe12 peptide (l, p) allows recovery of stem cells within jejunal tissue (Mirzoeva et al., 2014). EXOSOMES involved in these effects are not visible at current resolution but x-ray fluorescence imaging could allow their visualization as well as imaging of tissue cross sections.

Biological studies with the bionanoprobe

Science Opportunity presented by Tatjana Paunesku (Woloschak lab)

- **Science Challenge/Opportunity**
  - Understanding the role of exosomes, ectosomes and microvesicles in whole organism studies
  - Progress is impeded by lack of rapid and high resolution (sub 100nm) elemental and structural imaging
- **Significance & Impact**
  - Whole body functions rely on information networks – these include information exchange via exosomes
  - This research is timely because exploration of extracellular vesicles is on the rise in all fields of medicine
  - New approaches to solving cancer, diabetes and similar diseases, opening doors to novel ways in cell manipulation
- **Diffraction-limited storage ring strengths & challenges**
  - A hard x-ray diffraction-limited storage ring light source will provide brighter and better diffracting x-rays necessary for rapid high resolution imaging
  - Individual vesicles will be identifiable with high resolution and their content evaluated to study their production and fate

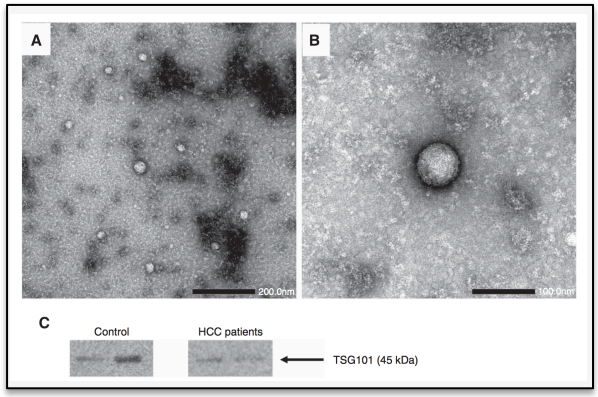


Top: Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M. (2014) **Sorting it out: Regulation of exosome loading**. Semin Cancer Biol

Bottom: Cocucci E, Meldolesi J. (2015) **Ectosomes and exosomes: shedding the confusion between extracellular vesicles**. Trends Cell Biol

High throughput high resolution elemental imaging Experimental Approach Opportunity presented by Tatjana Paunesku (Woloschak lab)

- **Techniques**
  - High resolution structure and elemental information
  - Development of new procedures for exosome work will open new complementary molecular approaches
- **Relevance**
  - Elemental and structural properties of extracellular vesicles will be studied
- **Tools**
  - Many optics and detectors developments match the needs of these studies
  - The most significant challenge will be R&D needed for computational data analysis
- **Diffraction-limited storage ring parameters**
  - Diffraction capabilities of the APS-U will provide entirely novel approaches for cellular substructure studies
  - High intensity of x-rays will allow detailed elemental information about different vesicular structures in 2D and 3D



Exosomes contain exosomal marker protein TSG101 that is used to isolate them from serum. However, using high flux elemental imaging coupled with diffraction we could investigate exosomes in tissues in situ.

Sugimachi et al., *British Journal of Cancer* (2015) **112**, 532–538.