UNIVERSITÄTSMEDIZIN GÖTTINGEN UMG

Application of Nanomedicine in Cancer Imaging: where we are and what needs to be done

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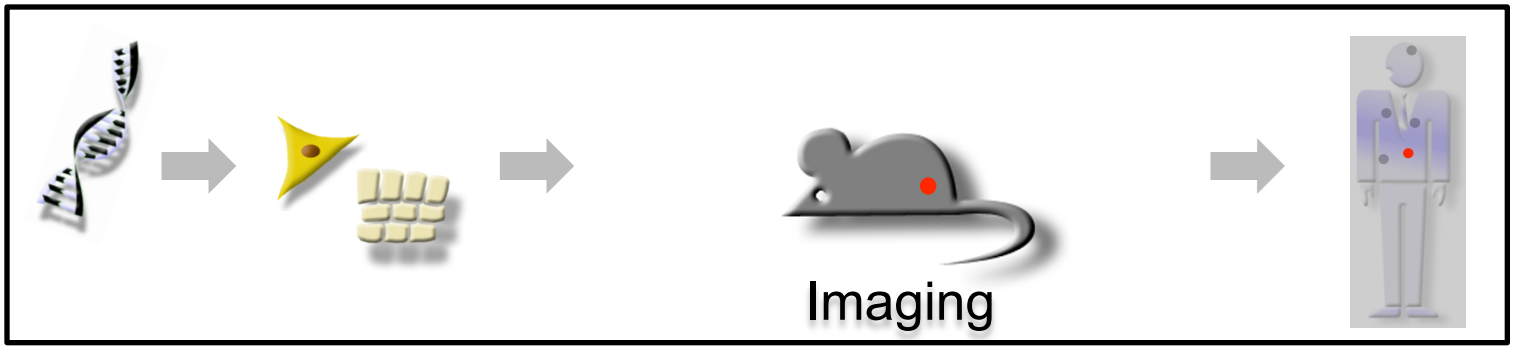
Dept. Molecular Biology of Neuronal Signals

Dept. Hematology and Medical Oncology
Institute of Diagnostic and Interventional Radiology

Multimodal non-invasive imaging in preclinical cancer research



Functional imaging

SPECT*

Optical imaging

Fluorescence

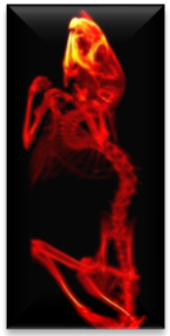
Bioluminescence

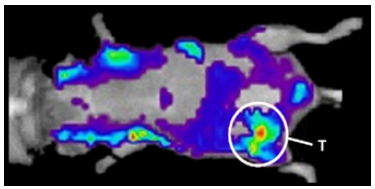
Anatomical imaging

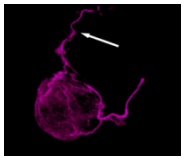
CT**

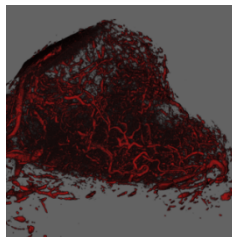
VCT***

µCT



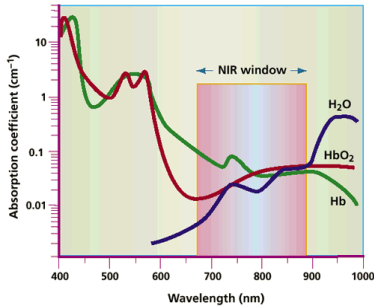






Near infrared fluorescence(NIRF) imaging in oncology to analyse molecular events in deep tissue in vivo

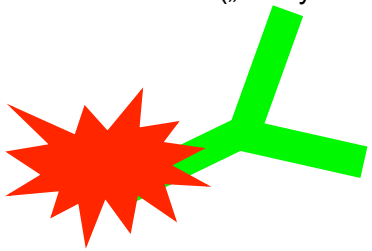
NIR fluorescence dye



Active targeting

Targeting specific markers

(„always-on“ probes)



Antibody, Peptide, Small molecule

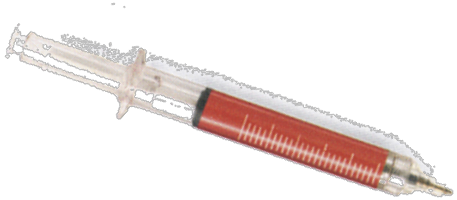
equipped with 4 Laser diodes:

635, 670, 730, 785 nm

measurement of

fluorescence intensity and

fluorescence lifetime



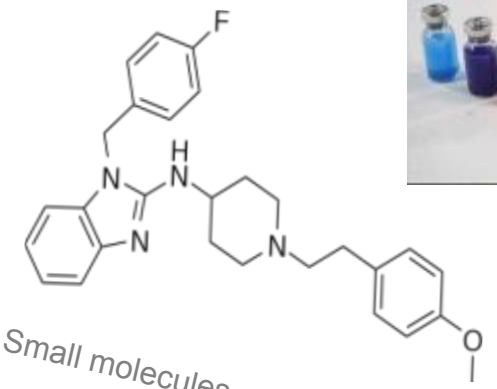
probe application

measure fluorescence

Fluorescence probes in the near infrared range

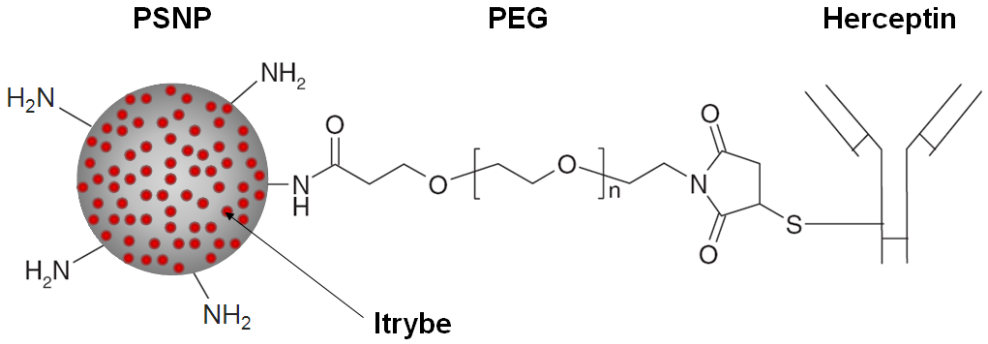
Antibody IgG

Antibody fragments

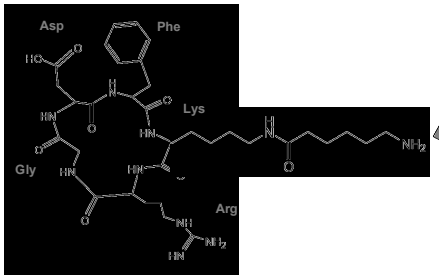


Small molecules

Nanoparticle based probes

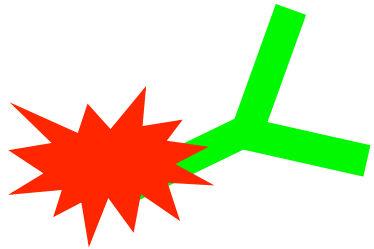


Peptide



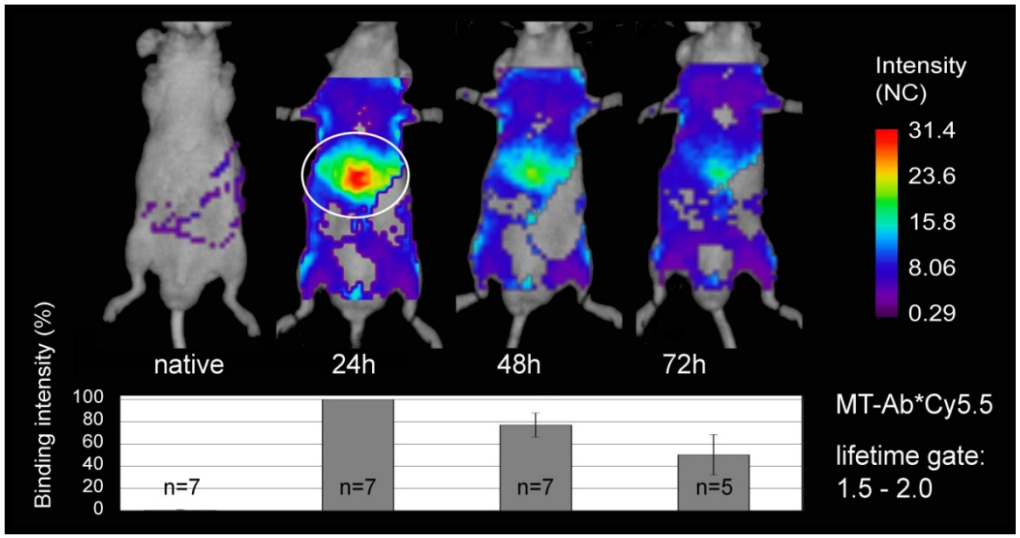
Evaluation of novel therapeutic concepts in oncology by NIRF imaging

- Matriptase - based tumor therapy



  - Assessing expression of biomarkers in cancer
  - Targeting molecular events (activatable probes)

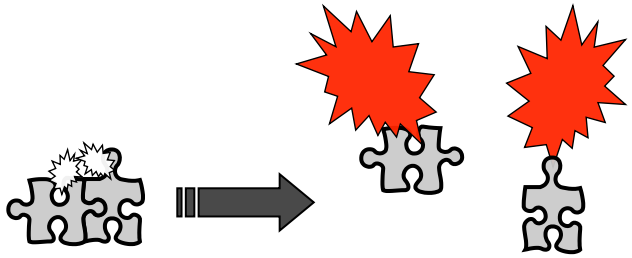
MT-Ab*Cy5.5

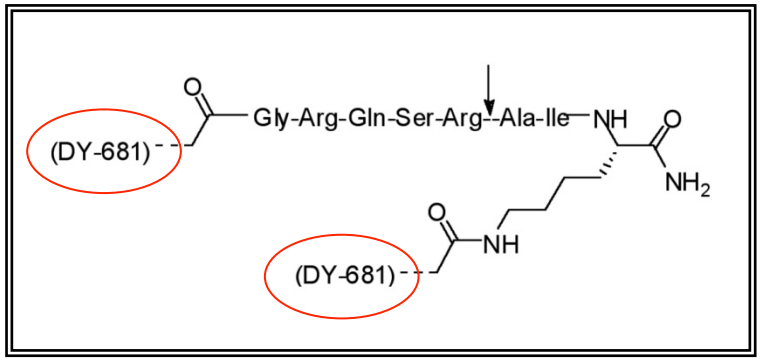


Monitoring of matriptase activity *in vivo* by activatable probes

- Targeting molecular events

  (activatable probes)

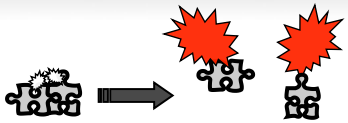


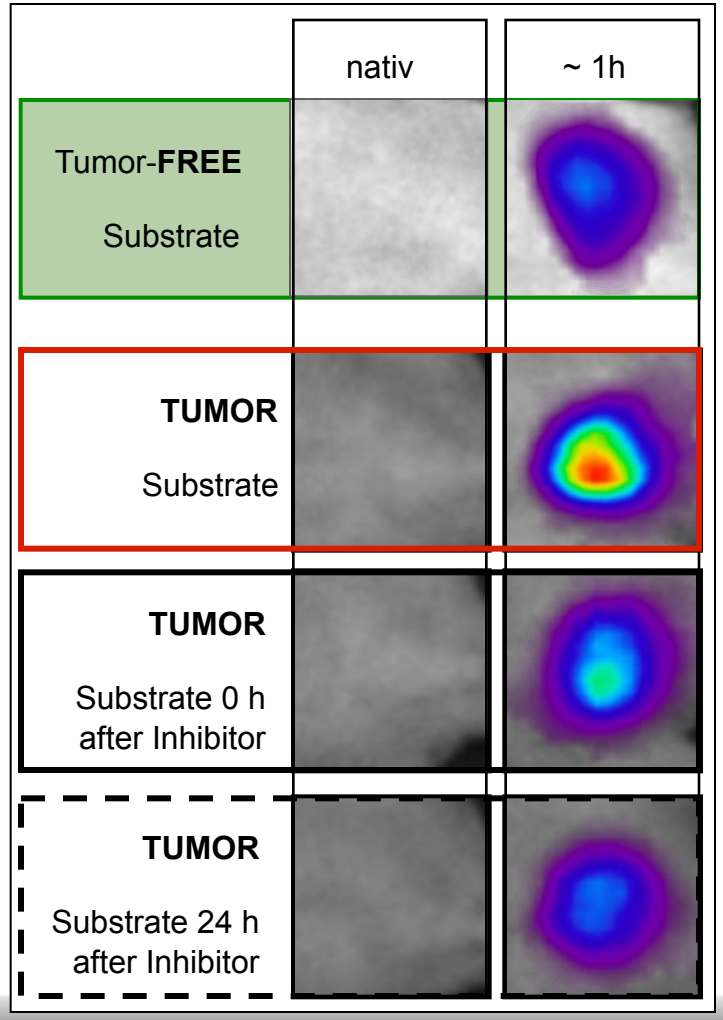


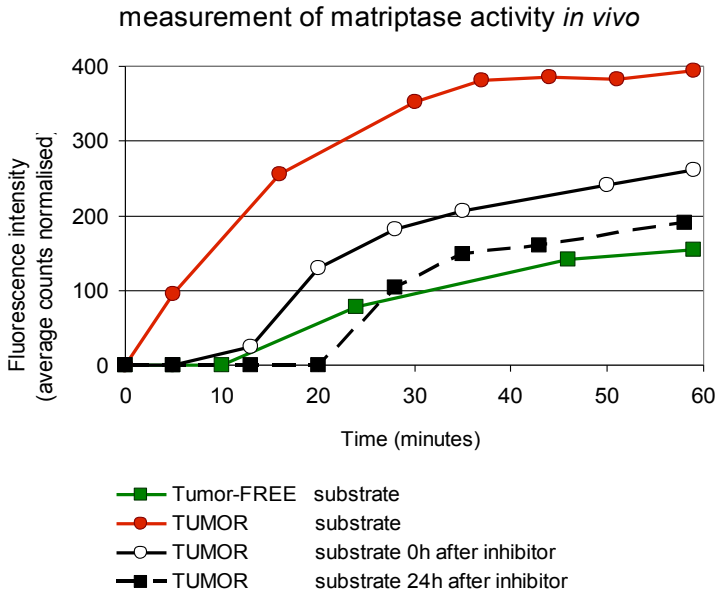
S*DY-681

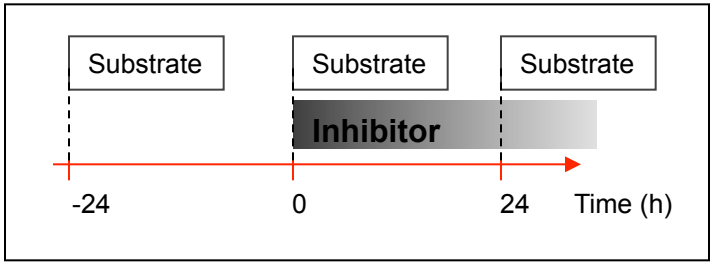
Napp et al. *Int J Cancer* 2010

Matriptase activity – *in vivo*





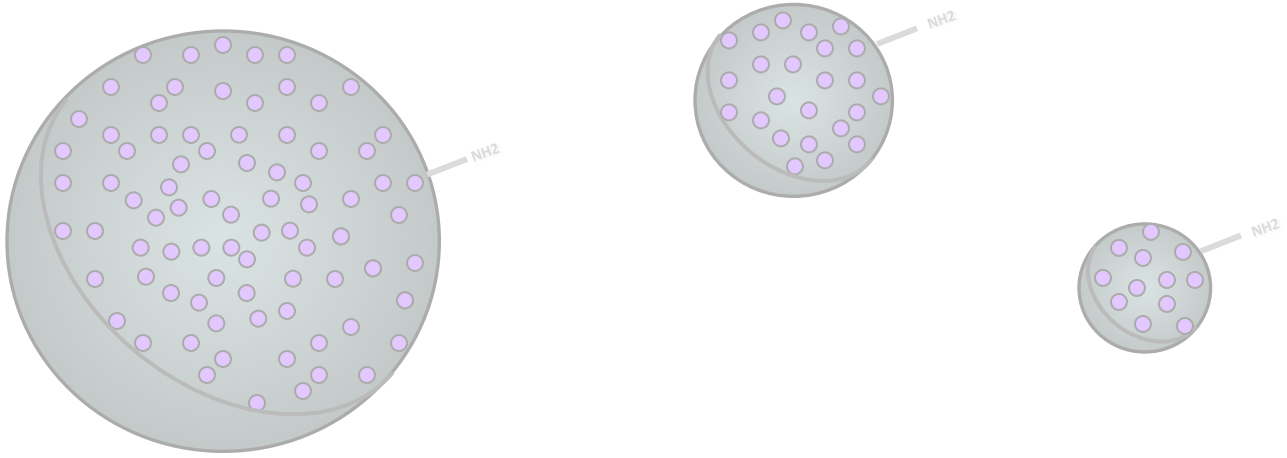




Napp et al. *Int J Cancer* 2010

Improving imaging probes by the use of nanoparticle

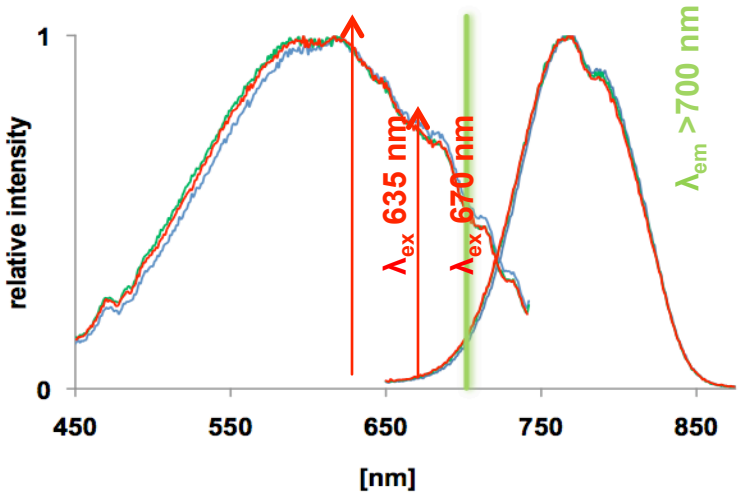
- Itrybe-loaded polystyrene nanoparticles



Itrybe-loaded and surface-modified NPs were generated by T. Behnke, BAM I.5, Berlin

Brighter fluorescence probes: Itrybe-loaded polystyrene nanoparticles

Broad spectra of Itrybe



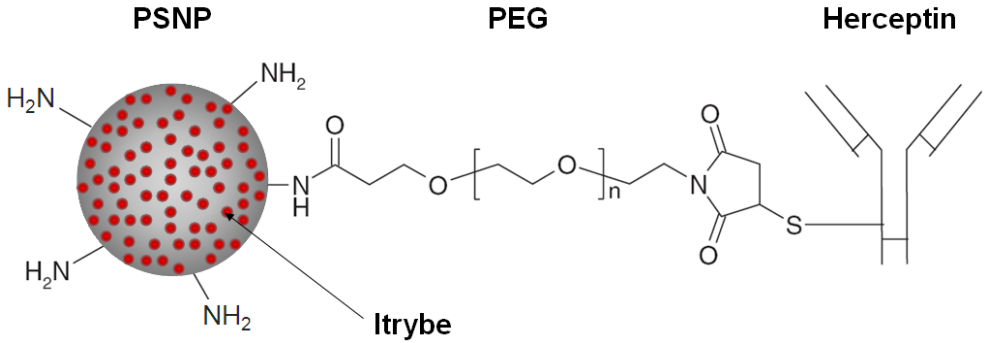
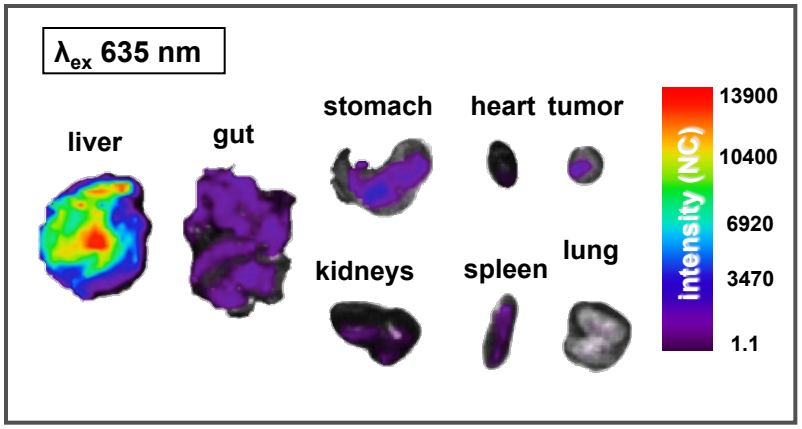


Figure modified, Steinhauser et al., 2006, Biomaterials

The problem: Bringing the systemic administered NPs to the tumor site!



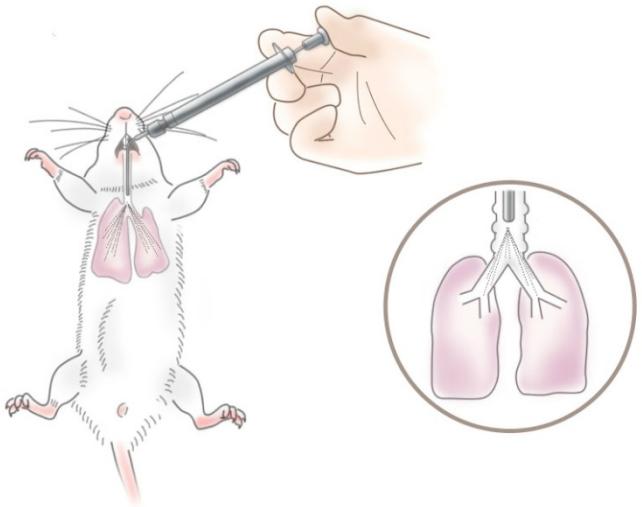
400 µg 100 nm-PEG1.5 kDa-Her NPs, 24 h after *i.v.* injection in KPL-4 tumor-bearing mice $n = 2$

Use of Itrybe Nanoparticles in other imaging settings

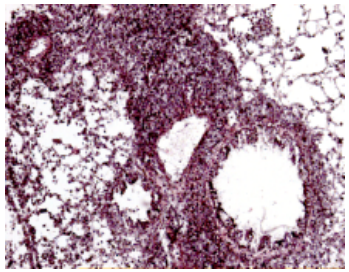
Behnke, Mathejczyk et al., Biomaterials 2012

Cell tracking by the use of nanoparticle: optical imaging

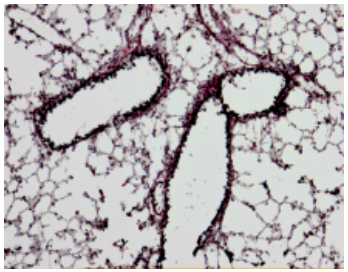
in an OVA induced allergic asthma mouse model

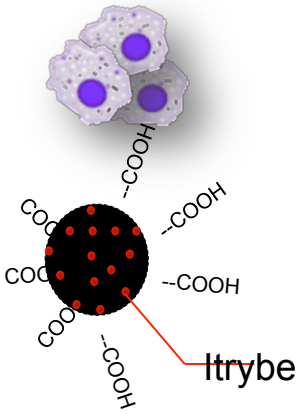


OVA induced asthma



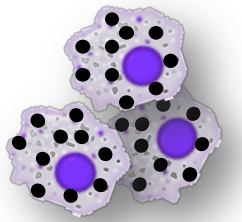
Control





immortalized alveolar macrophages

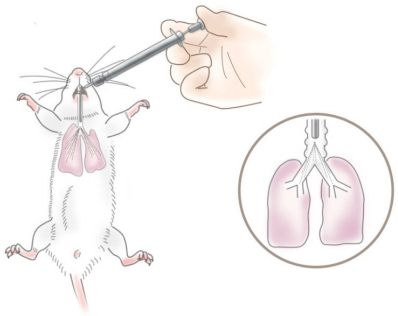
NIRF containing Nanoparticle

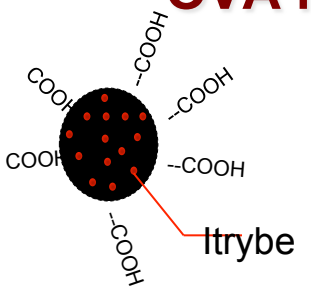


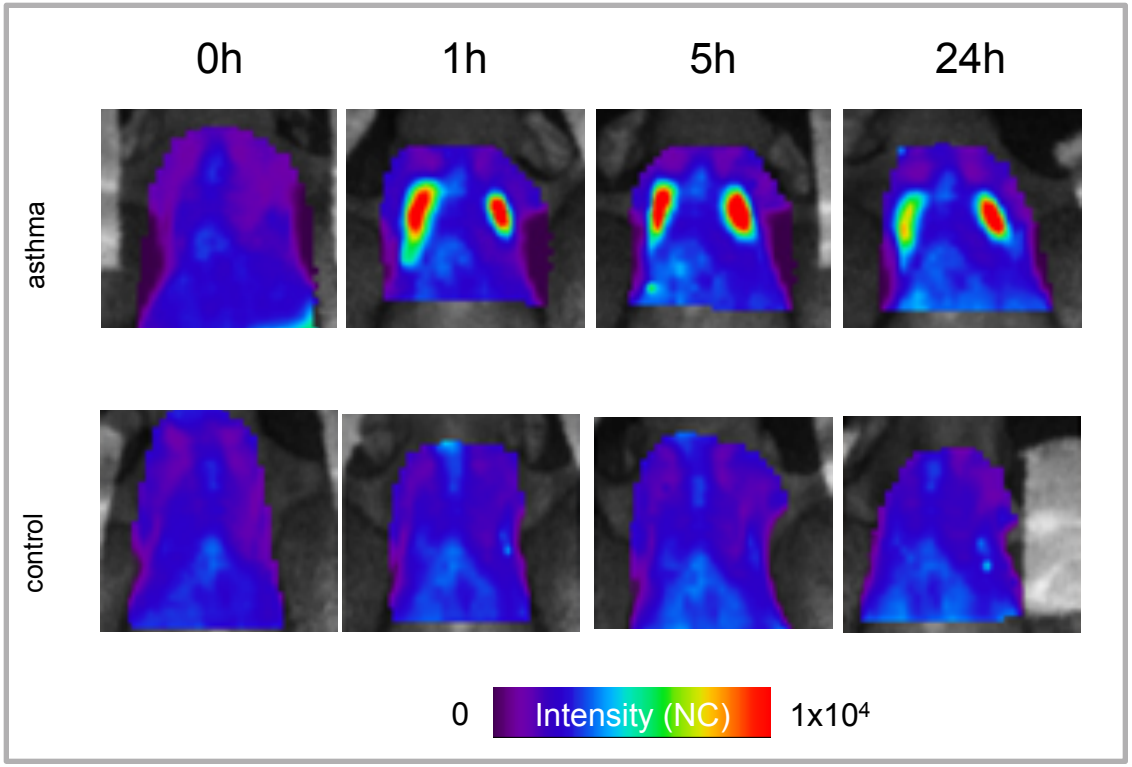
NIRF imaging

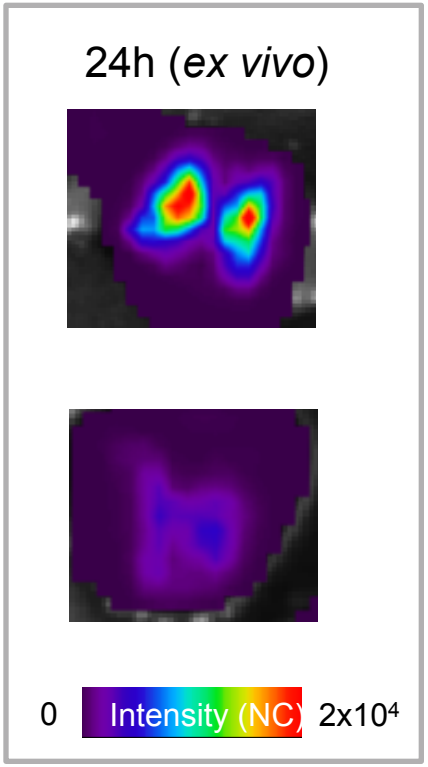
In vivo and *ex vivo* lung imaging

OVA induced allergic asthma mouse model





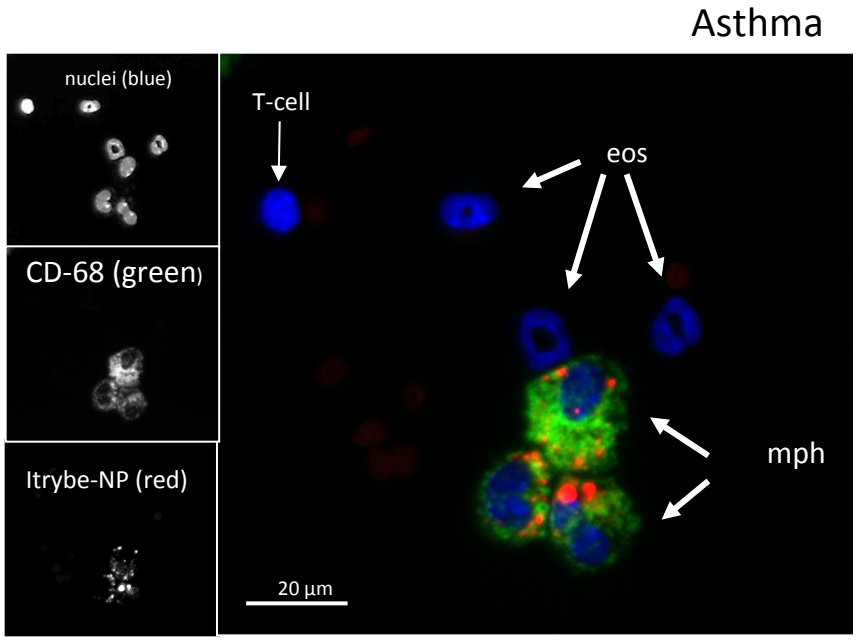




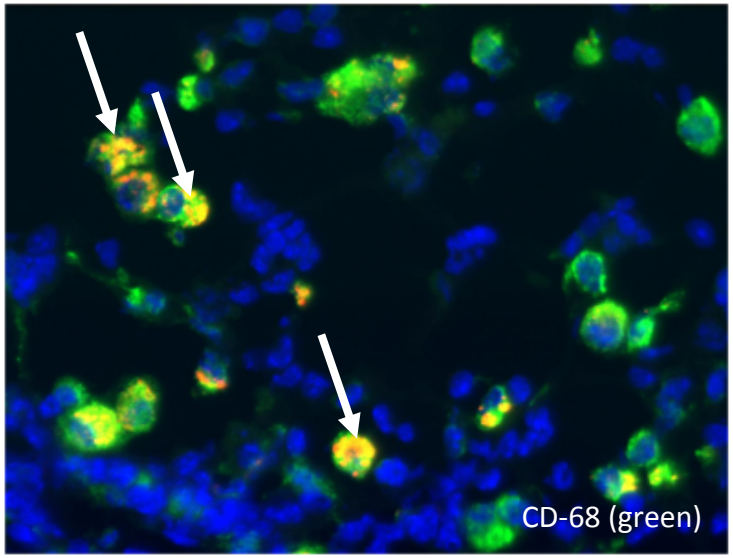
i.n. application; 160 µg PSNPs Itrybe

Markus A, Napp J et al., ACS Nano, Nov 2015

In vivo optical imaging to track macrophages within the lung using Itrybe NIRF nanoparticles



Bronchial alveolar lavage (BAL)



Lung tissue cryosections

Markus A, Napp J et al., ACS Nano, Nov 2015

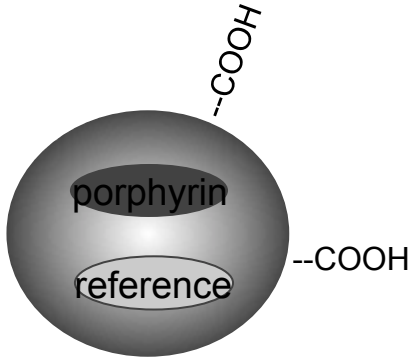
Translation of concepts to in vivo cancer imaging

- Functionalizing Nanoparticles to reach the tumor site
- Improving the pharmakokinetic of Nanoparticles
- Optimizing specificity, stability and non-toxicity of Nanoparticles
- **Visualization of immune cells within the tumor site**
- **Use of Nanoparticles for sensing probes**

Use of Nanoparticles to assess the metabolic state of tumors by non invasive NIRF imaging

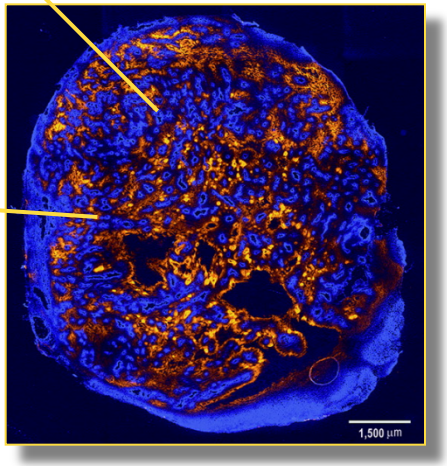
- Hypoxia sensing

Analyte sensing probes

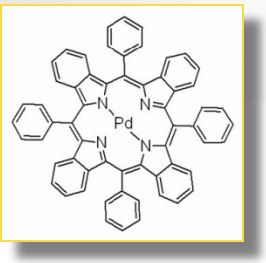


50–60% of solid tumors exhibit hypoxic areas

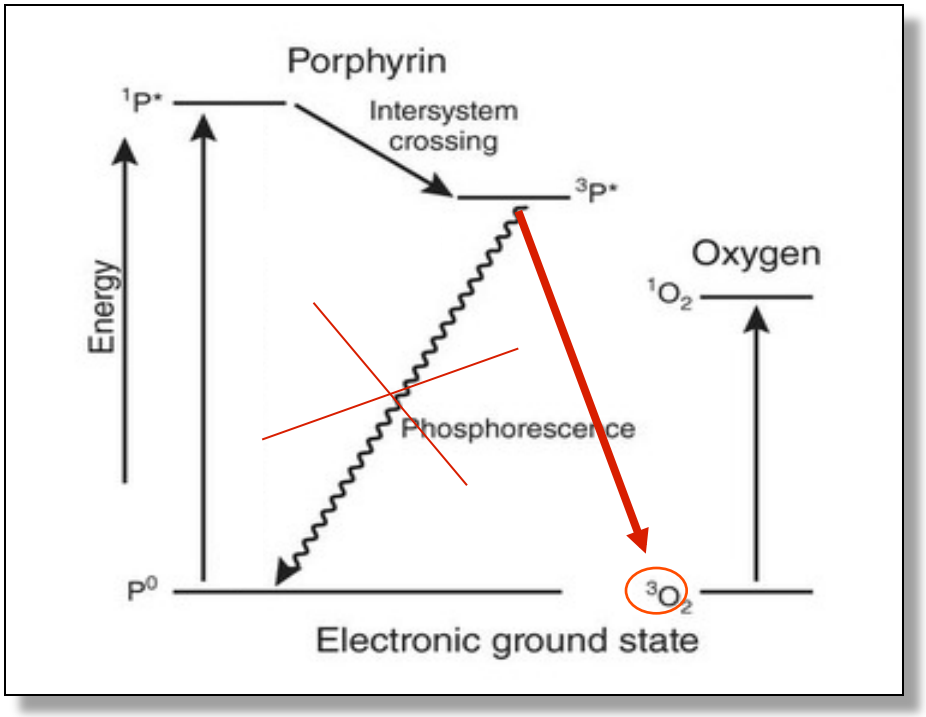
Independent of clinical size, stage, histology, grade, nodal status and a series of other tumor characteristics



Oxygen-dependent phosphorescence quenching

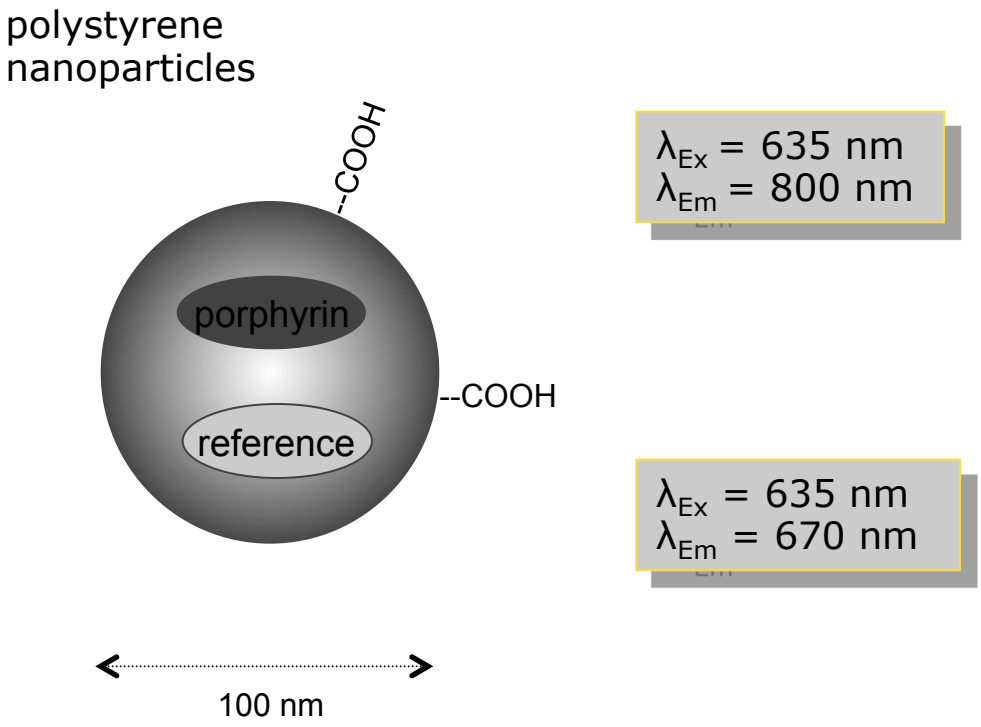


NIR oxygen sensing dye
Palladium(II)-tetaphenyl-tetrabenzoporphyrin



Some fluorophores can transfer the excitation energy from their triplet state to other molecules. Thereby the acceptor molecule will be transformed to the excited state and the fluorophore will return to the ground state (**intermolecular energy transfer**).

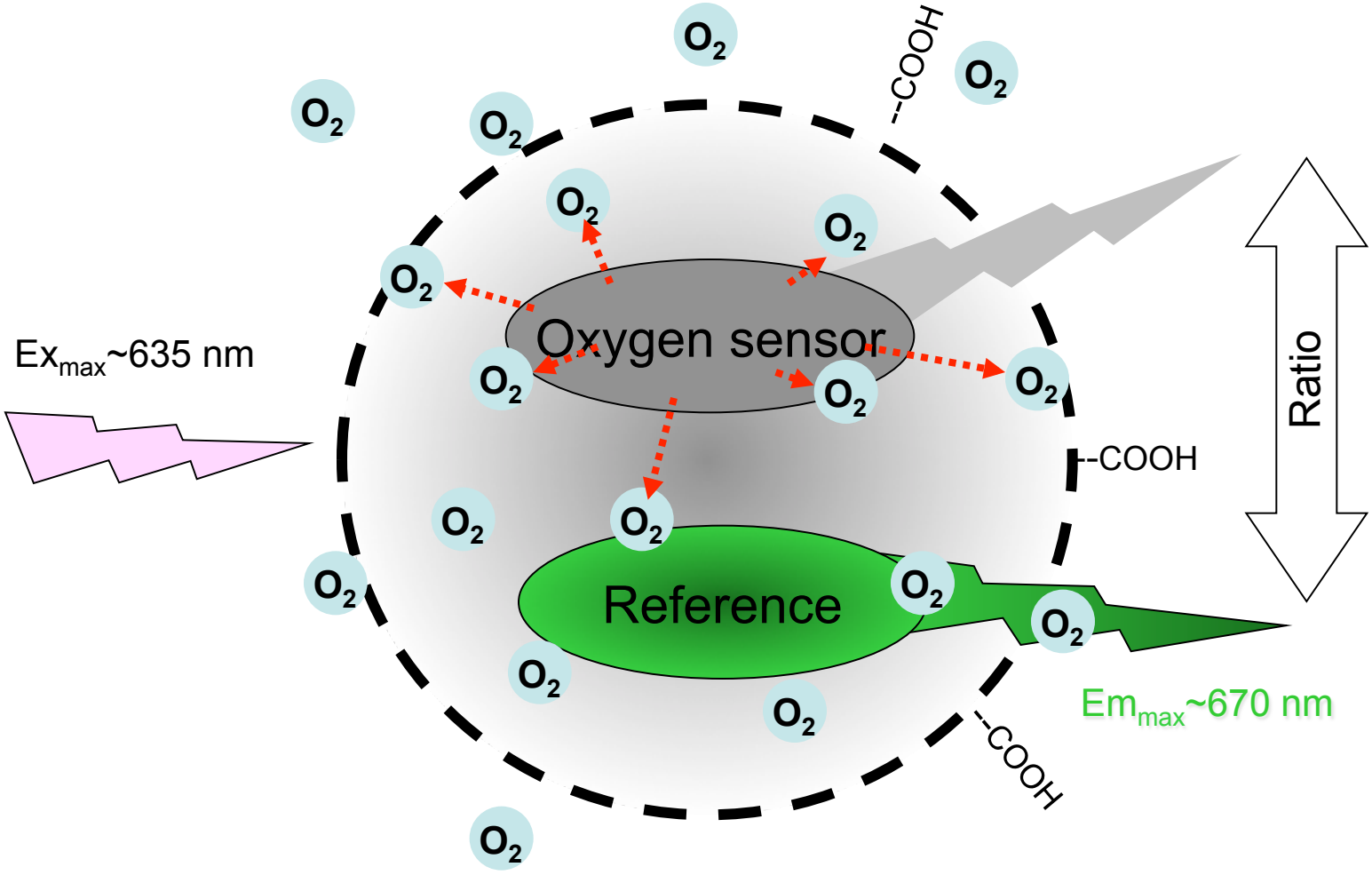
Referenced OX-NPs



In cooperation with U. Resch-Genger (BAM) and M. Schäferling (Uni. Regensburg)

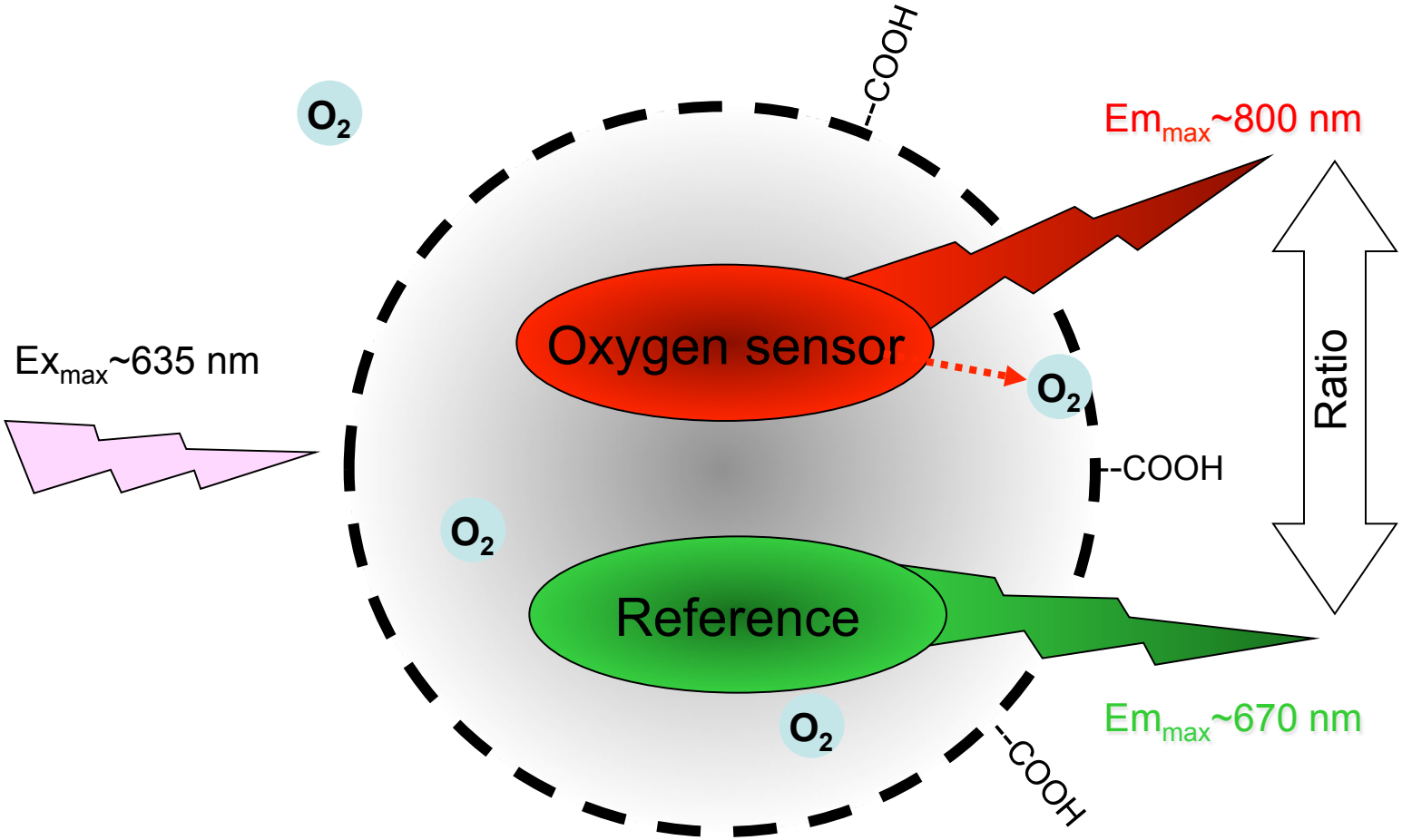
Napp et al. *Anal Chem* 2011

Referenced system for oxygen sensing



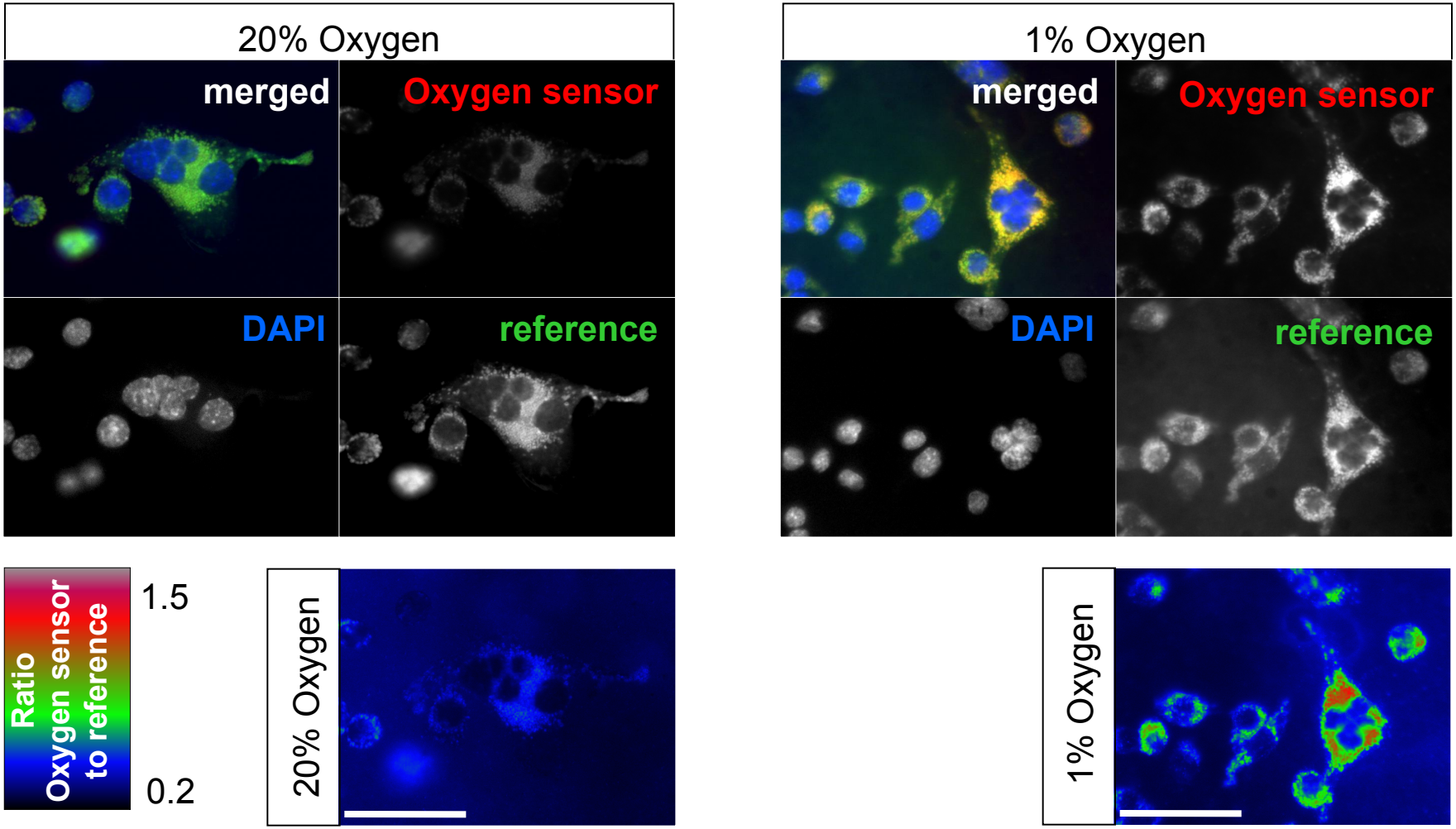
Napp et al. *Anal Chem* 2011

Referenced system for oxygen sensing

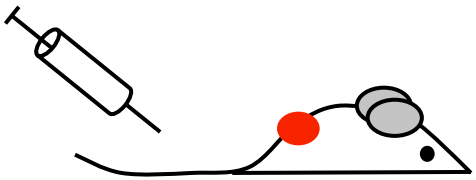


Napp et al. *Anal Chem* 2011

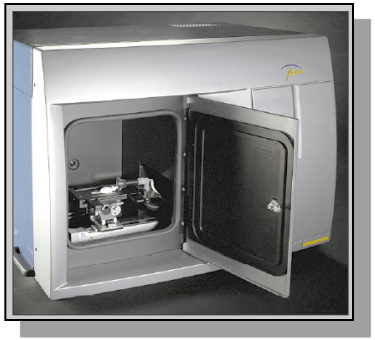
Oxygen sensing *in vitro*

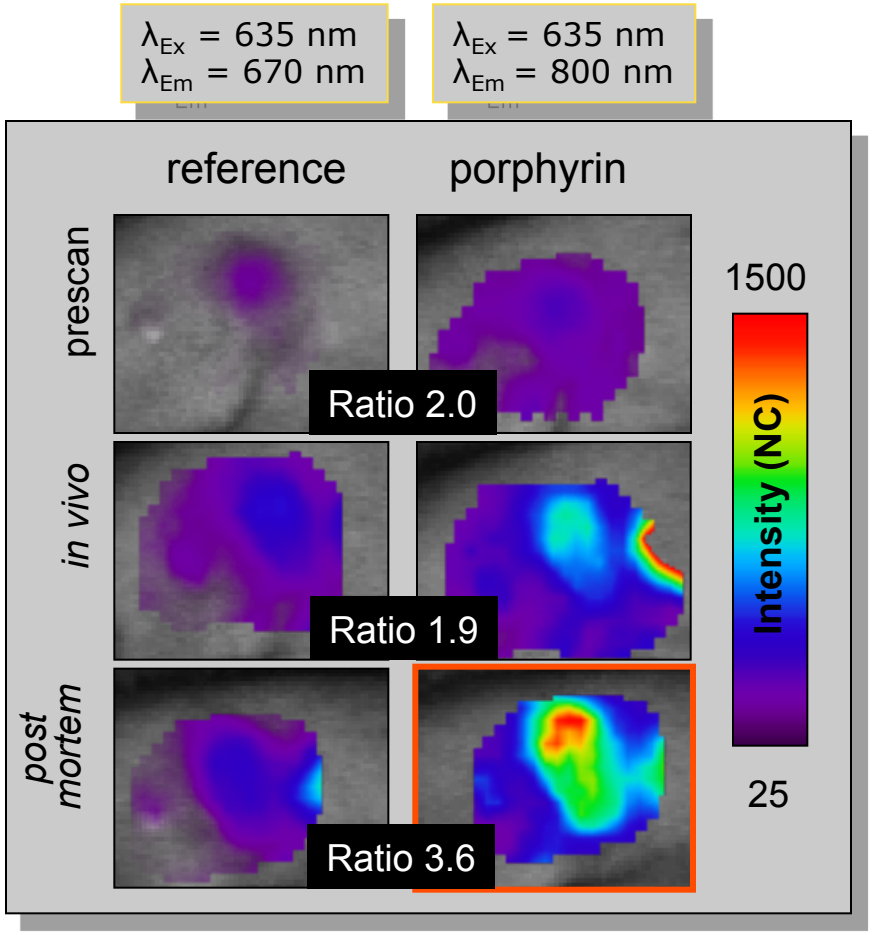


Proof of concept for *in vivo* imaging



Optix MX2



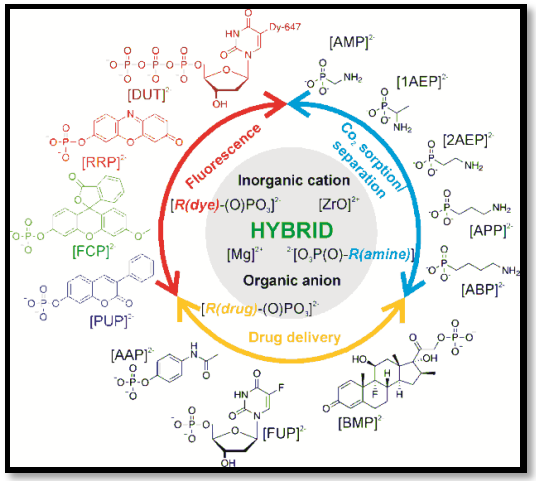


Napp et al. *Anal Chem* 2011

Novel probes: Inorganic-organic hybrid nanoparticles for imaging and drug delivery

In cooperation with Prof. Feldmann and Joachim Heck
Institute of Inorganic Chemistry; Karlsruhe Institute of Technology

Expected clinical advantages: prolonged action due to the prolonged drug release, less side effects, simultaneous monitoring of NPs



$[M]^{2+}[R_{function}(O)PO_3]^{2-}$

M = ZrO, Mg_2O
R = functional organic group

$[M]^{2+}[R_{dye}(O)PO_3]^{2-}$

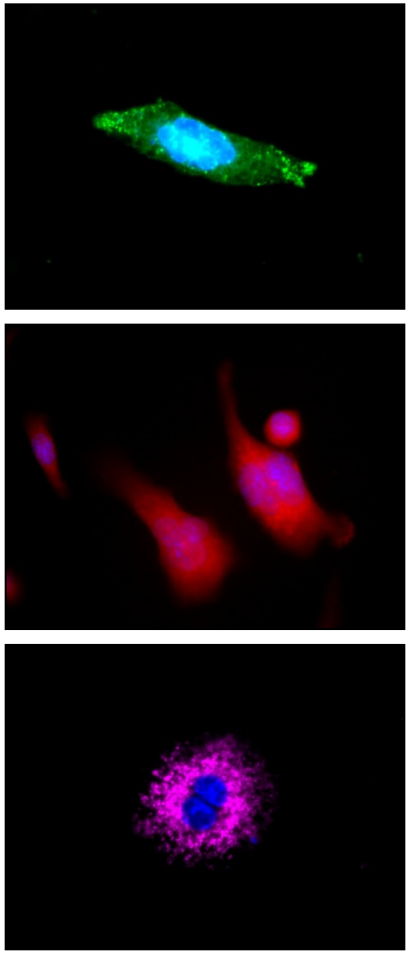
$[M]^{2+}[R_{drug}(O)PO_3]^{2-}$

„**Anti-inflammatory" NPs:** Betamethasonephosphate (BMP)
„**Anti-tumor" NPs:** 5-Fluoruracil (5FU)

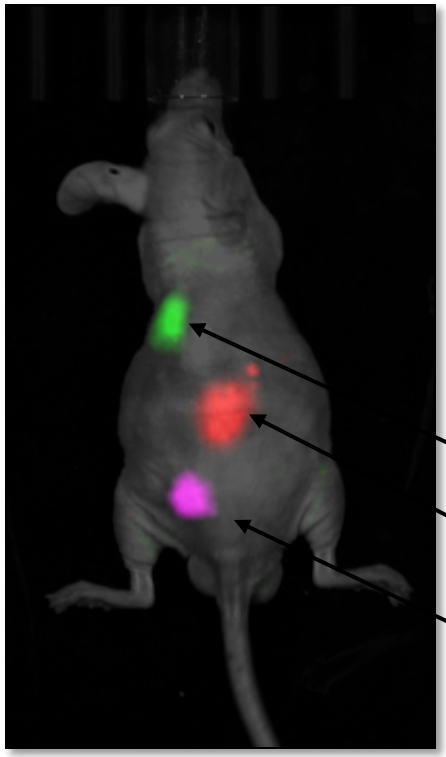
Heck et al., 2015, J Am Chem Soc. 137: 7329-36. ; Patent DE 10 2014 004 512.9;

Nanoparticles for prolonged drug release and silmultaneous imaging

Imaging



Heck et al., 2015, J Am Chem Soc. 137: 7329-36.



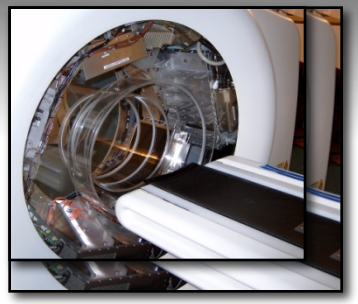
$[ZrO]^{2+}[MFP]^{2-}$

$[ZrO]^{2+}[RRP]^{2-}$

$[ZrO]^{2+}[BMP]^{2-}_{0.996}[DUT]^{2-}_{0.004}$

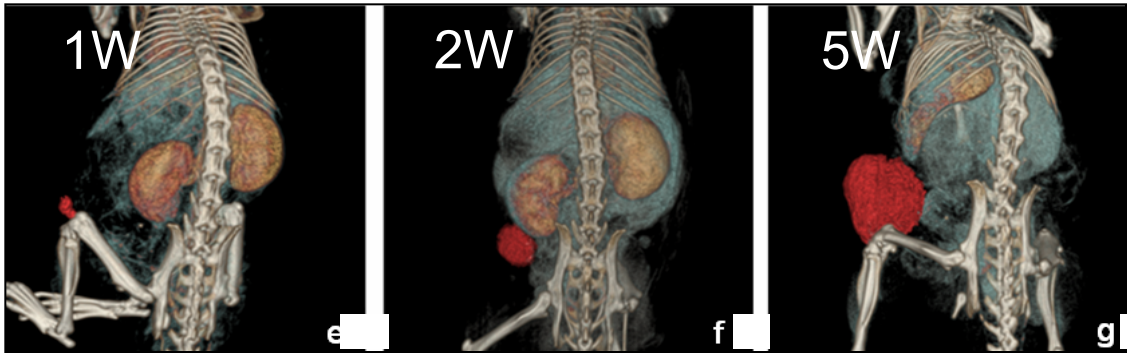
MH-S cells 50 µg in 1 ml; 24h 10 µg in 50 µl PBS subcutaneous

Joanna Napp



Assessing preclinically the efficacy of therapeutic effects in oncology by anatomical Imaging by CT

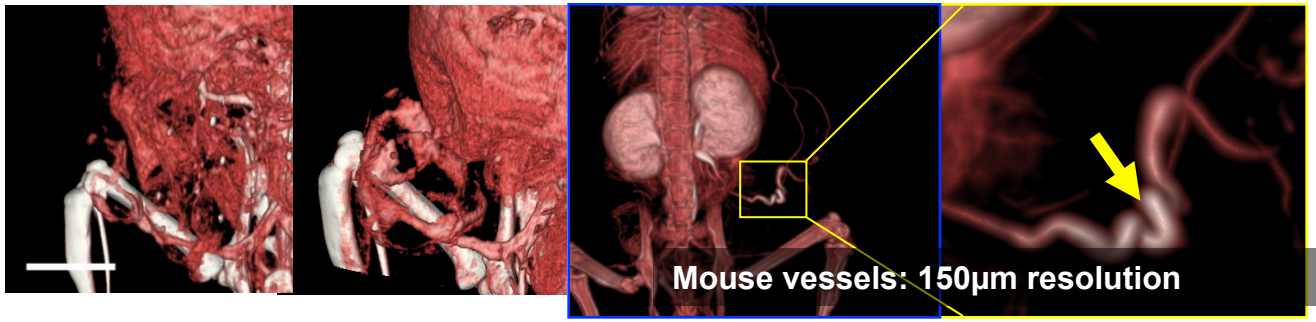
Monitoring of tumor growth rates



J. Mißbach-Güntner et al., *Neoplasia*, 2008

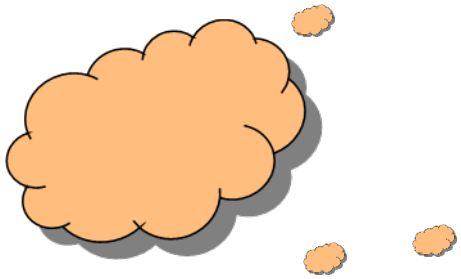
K. Jannasch et al., *Int J Cancer,* 2009

Analysis of tumor vascularization

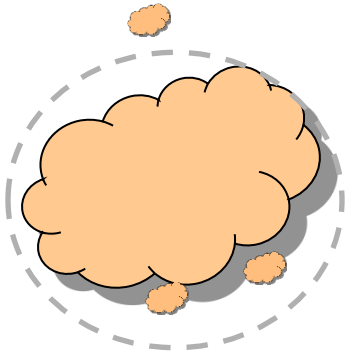


Missbach et al., Neoplasia, 2007

The clinical problem to be solved



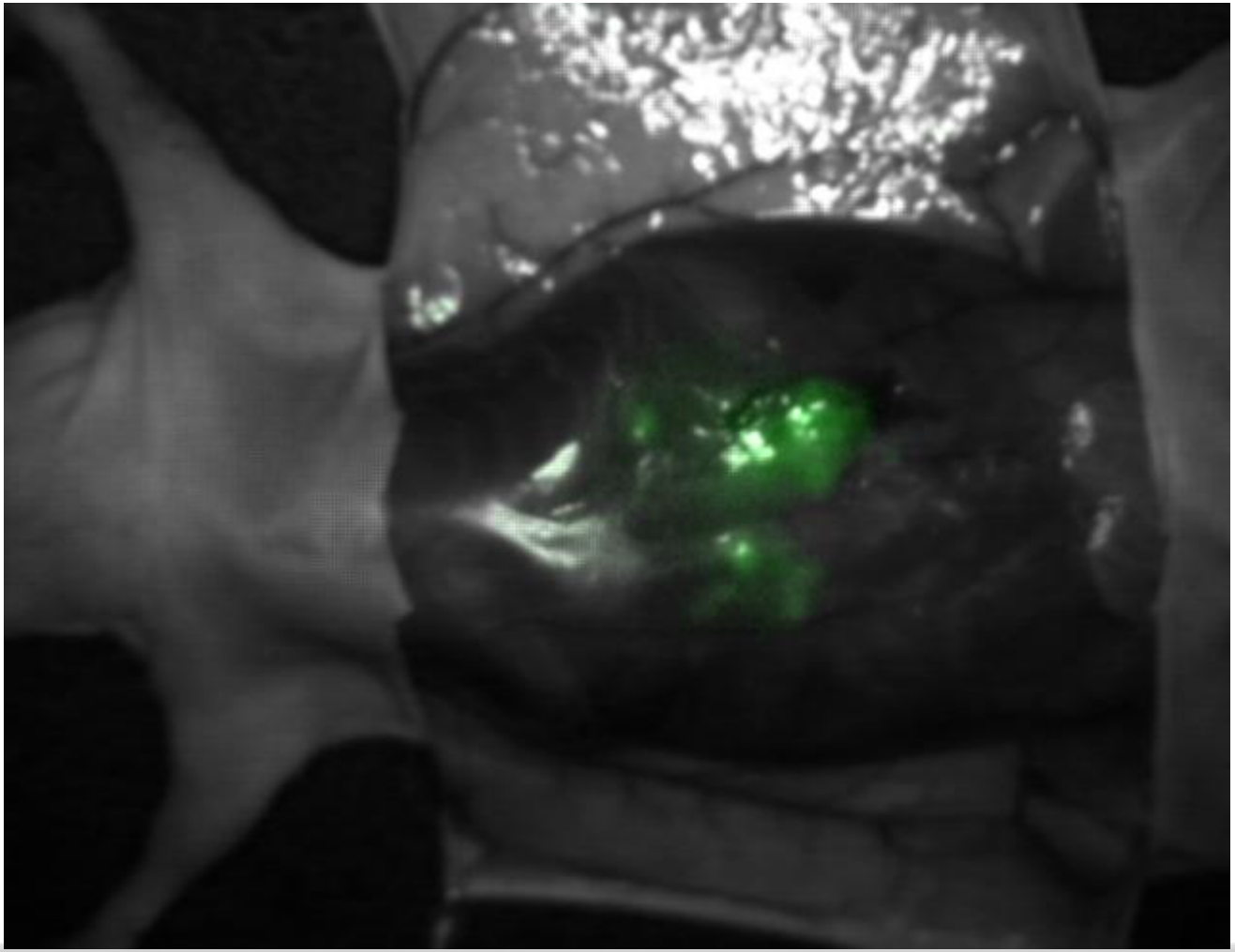
Tumour



Cut is too close to the tumor

Too much healthy tissue being cut out together with the tumour

Image guided surgery: preclinical validation of CW800-Cetuximab in an ASPC-1 mouse model



Mara Saccamano, Joanna Napp

Clinical application of optical imaging techniques

Developments needed from chemists, physicists and medical disciplines :

- Handhold camera systems
- Improvement of specific, stable and nontoxic probes including NPs
- Clinical approval
- Improvement of probes and optics to detect signals in deeper tissues

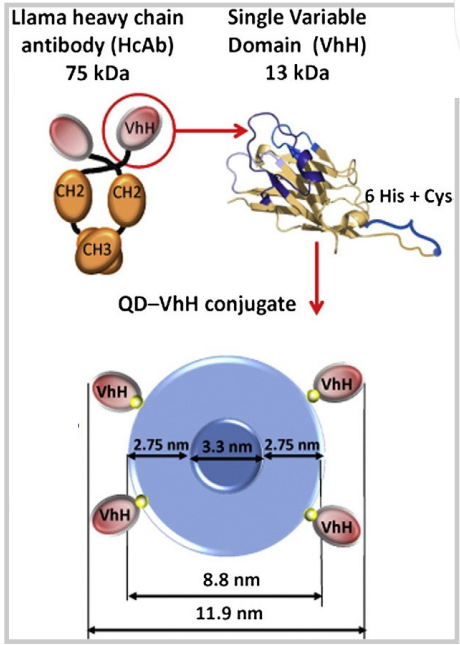
NOVEL *in vitro* diagnostic probes to detect metastases and cancer at an early stage

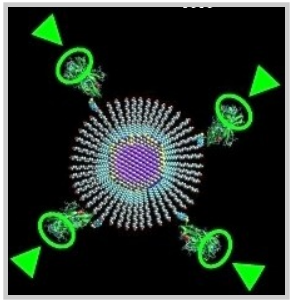
Highly sensitive and tumour-specific photoluminescent QDs

NAMDIATREAM
NANOTECHNOLOGICAL TOOLKITS FOR MULTI-MODAL DISEASE DIAGNOSTICS AND TREATMENT MONITORING

CdSe-ZnS QDs

photostable, functionalised with PEG, water soluble, stable in aqueous solution, quantum yield of 50%, optimised for minimal unspecific binding





sdAb-QDs- QDs

single C-terminal free cysteine residue for specific site-directed and oriented conjugation with the QDs for specific detection of tumor cells

⇒ anti-HER2; anti-EGFR; anti-CEA

Namdiatream EU grant.

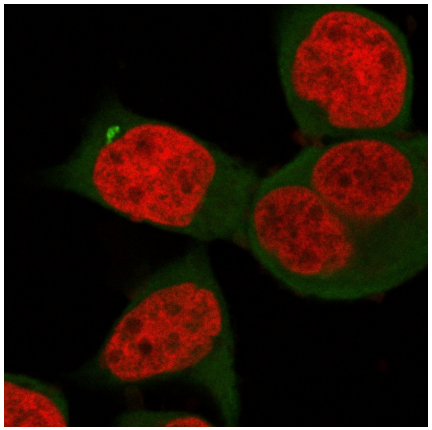
A. Sukhanova et al., Nanomedicine, 2011

Novel diagnostic tool:
to detect metastases and cancer at an early stage

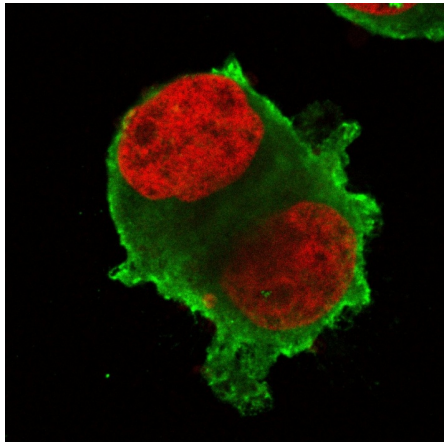
NAMDIATREAM: HER2 positive SK-BR3 cells

staining of HER2 expressing SK-BR3 **breast cancer** cells by anti-HER2 sdAb-QDs

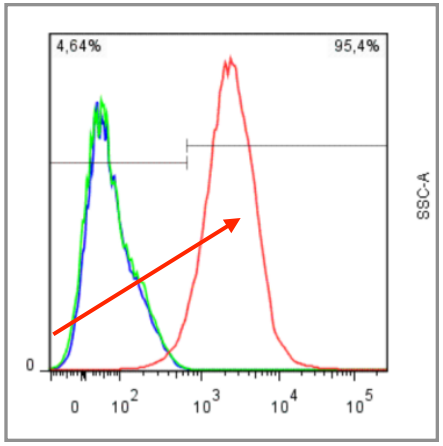
QD



QD-HER2



staining of HER2 expressing **SKBR3 breast cancer** cells by anti-HER2 sdAb-QDs (FACS)



Summary

Nanoparticles are promising tools for

In vivo:
Sensing of hypoxia
Tracking of cells
For drug delivery and simultaneous imaging

In vitro:
Novel high sensitive diagnostic tools to detect cancer cells

However they have to be improved to

- reach the tumor site specifically
- be stable, non toxic, biocompatible and biodegradable
- combine imaging and drug delivery

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