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Cyanoarylporphyrazines with high viscosity sensitivity in dosimetry-assisted photodynamic cancer treatment

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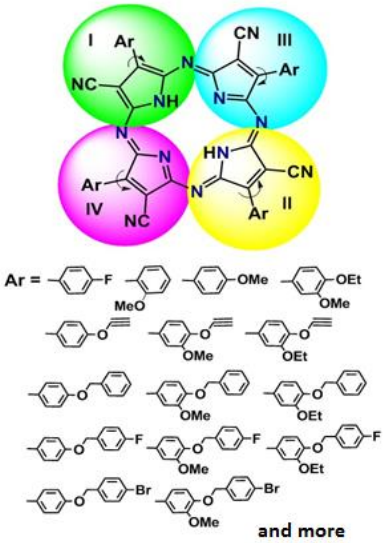
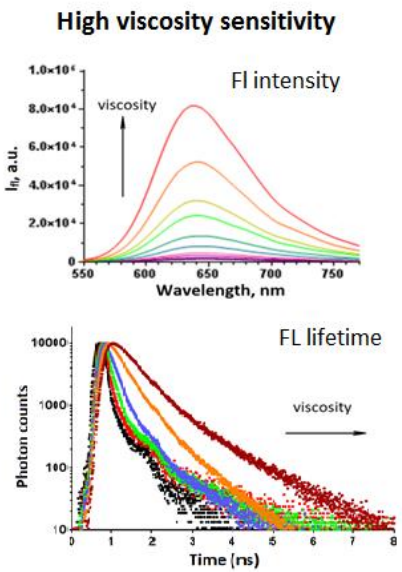
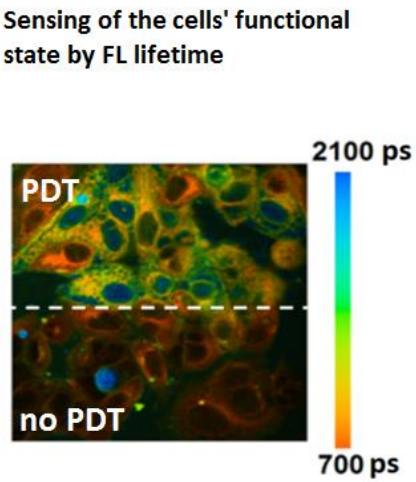
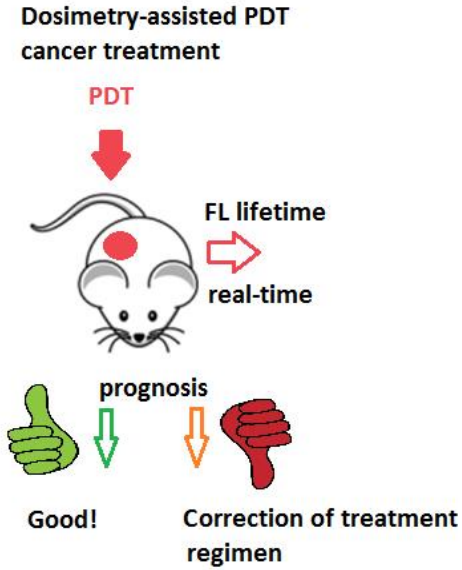
Cyanoarylporphyrazines with high viscosity sensitivity in dosimetry-assisted photodynamic cancer treatment

Graphical Abstract









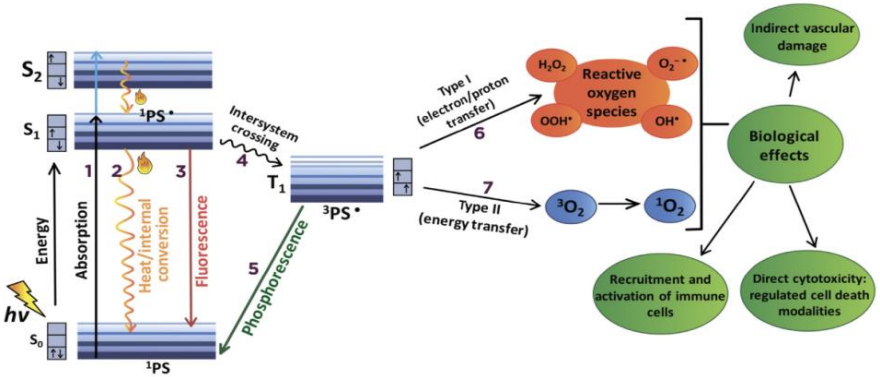
Abstract:

Despite the significant relevance of photodynamic therapy (PDT) as an efficient strategy for anticancer treatment, several challenges compromise its efficiency. Here we report the novel group of tetracyanotetra(aryl)porphyrazine dyes that enable real-time assessment of tissue response and thus predict treatment efficacy. The most remarkable and promising feature of the studied compounds is their belonging to the group of fluorescent molecular rotors. The quantum yield and fluorescence lifetime of the cyanoarylporphyrazines are strongly depend on the local viscosity, so they can be used as intracellular viscosity sensors. The cyanoarylporphyrazines demonstrate high photo-induced toxicity both in vitro and in vivo. Of note, complete recovery from cancer was observed for more than half of the treated animals without any signs of dark toxicity. It was shown that photoinduced cell damage is accompanied by a significant dose-dependent increase in cell viscosity. The viscosity changes under PDT treatment may result from denaturation of macromolecules, inter- and intramolecular crosslinking, membrane disorganization, etc., and reflect the severity of PDT-induced changes in cell physiology. We have proposed the approach for noninvasively measuring the tissue viscosity during the PDT procedure by registering the fluorescence lifetime of the cyanoarylporphyrazines. We believe that the unique properties of the compounds provide a tool for PDT dosimetry and tailoring the PDT treatment regimen to the individual characteristics of each patient.

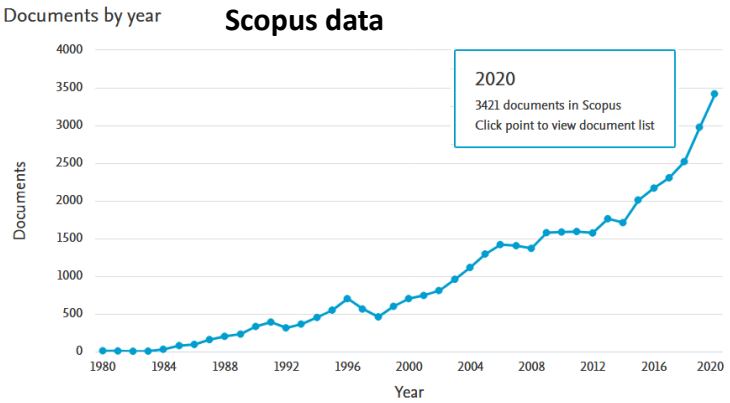
Keywords: cancer treatment; cyanoarylporphyrazines; fluorescent molecular rotors; photodynamic therapy; viscosity sensing

Introduction

Photodynamic therapy (PDT) is based on the production of the cytotoxic reactive oxygen species (ROS) under light excitation of a sensitizing drug (photosensitizer, PS) in the presence of molecular oxygen



Alzeibak R. et al. J Immunother Cancer, 2021



The main research trends in PDT:

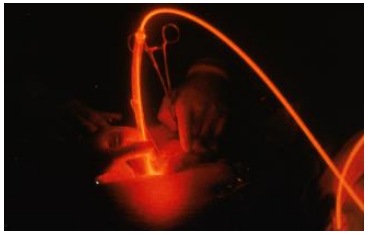
- Increasing selectivity (e.g., by using vehicles)
- Increasing depth of tissue penetration (NIR dyes, interstitial PDT)
- Increasing efficiency (relieving hypoxia, using combination therapy)
- New areas of application

Personalized/precision medicine is the main trend nowadays

Introduction

Is it possible to tailor the PDT treatment to the individual characteristics of each patient?

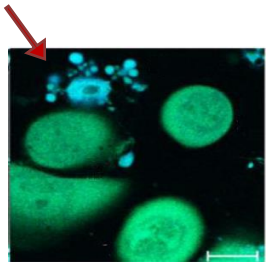
- PDT provides **diagnosis** based on PS fluorescence
- PDT **treatment** is based on ROS production and triggering direct and indirect tumor eradication mechanisms
- What about assessment of efficacy and **prediction of treatment outcome**?



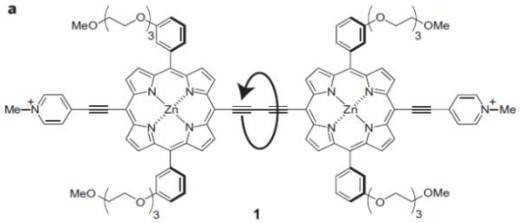
In clinical practice, tumor response to PDT is estimated **2 months** after treatment

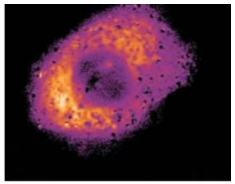
Possible solution:

PS with sensor properties



The first using of tetrapyrrolic photosensitizer exhibiting molecular rotor properties for recording intracellular viscosity during PDT treatment: M. Kuimova *et al*, **Nature Chemistry**, 2009



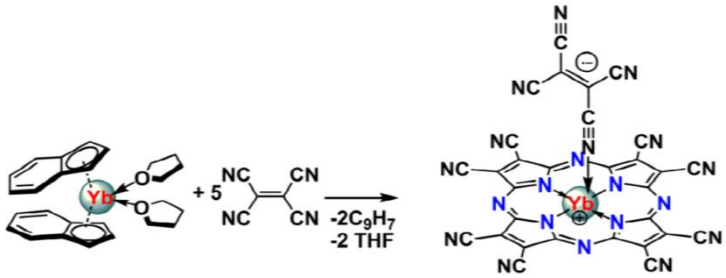


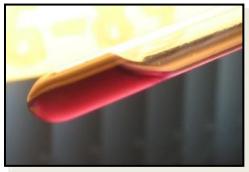
Results and discussion

Tetracyanotetra(aryl)porphyrazine dyes

The new synthetic approach to the template assembling of porphyrazine macrocycles containing CN-groups in the peripheral frame

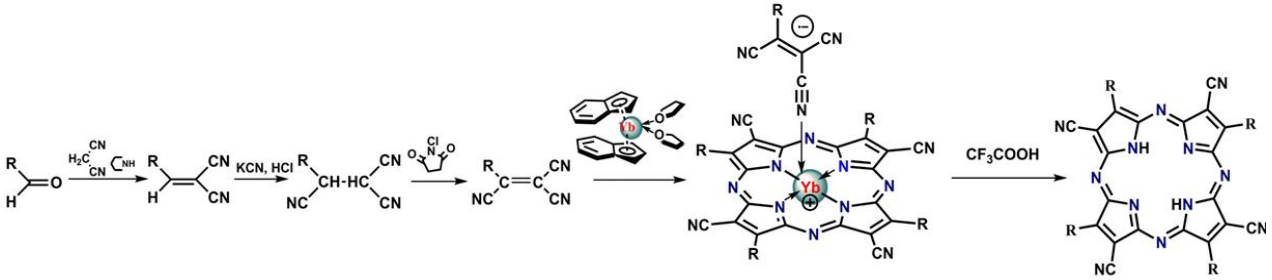
L.G. Klapshina, et al. Chem Comm, 2007





- ✓ Versatile method
- ✓ Proceeds smoothly at room temperature
- ✓ Fast (≤1h) synthesis
- ✓ High yield

Synthesis of tetracyanotetra(aryl)porphyrazine free bases



- synthesis of tricyanoethylenes
- template assembling of porphyrazine macrocycle on Yb^{3+}
- demetallation and formation of free bases

Results and discussion

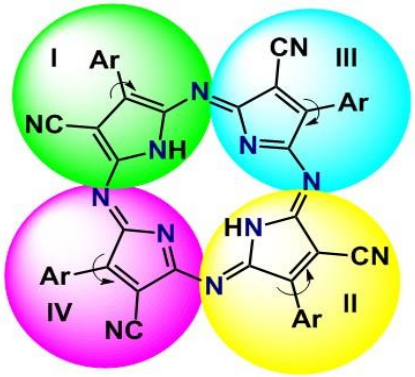
Tetracyanotetra(aryl)porphyrazine dyes

Photonic and optoelectronic applications

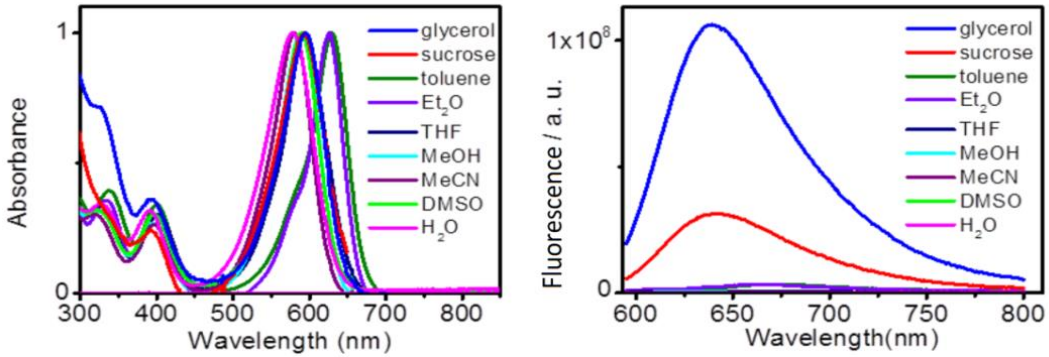
Klapshina et al. Journal of Material Chemistry, 2009
Klapshina et al., Functional Materials, 2010
Grigoryev et al. Nanotechnologies in Russia, 2012

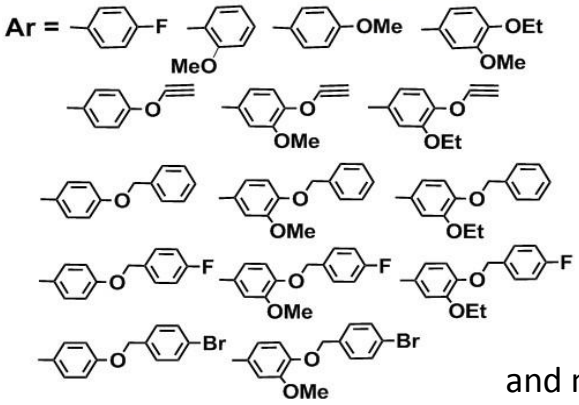
Synthesis of compounds for biophotonic applications

Klapshina et al., Chem Comm, 2010
Yakimansky et al., J Polymer Sci, Part A: Polymer Chemistry, 2013
Lermontova et al., Rus J General Chemistry, 2016, 2017, 2018, 2020



pz[FPh] is most studied for biomedical application

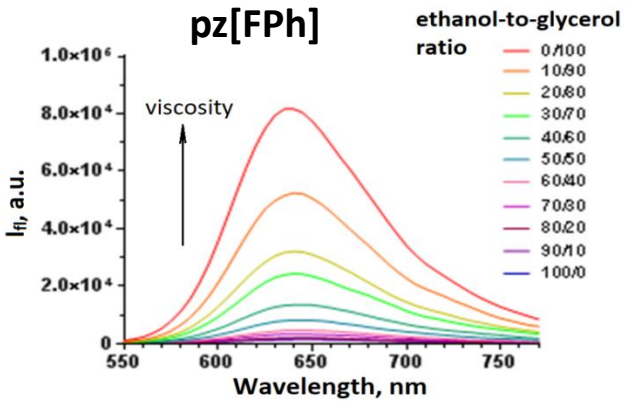


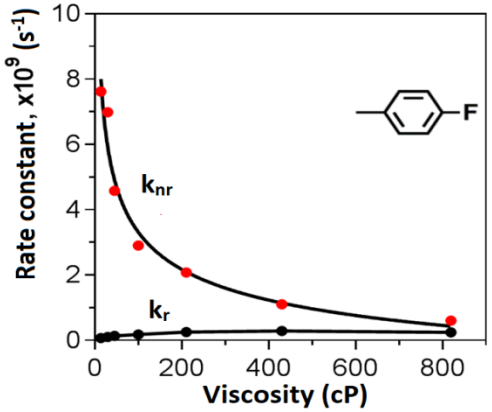


and more…

Results and discussion

The fluorescence quantum yield and lifetime strongly depend on viscosity of the medium

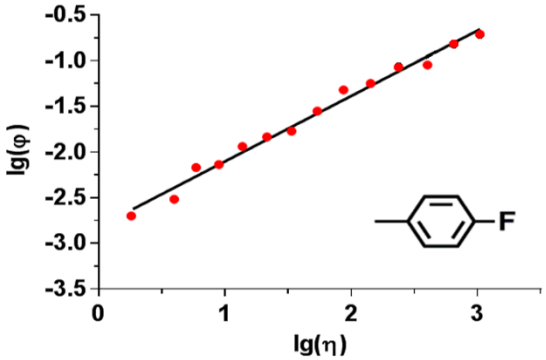




(In collaboration with Prof. M. Kuimova, Imperial College London)

Izquierdo et al., Journal of Materials Chemistry B, 2014
Balalaeva et al., Molecules, 2021

Quantum yield changes from 0.003 in water to 0.27 in glycerol at 25 °C



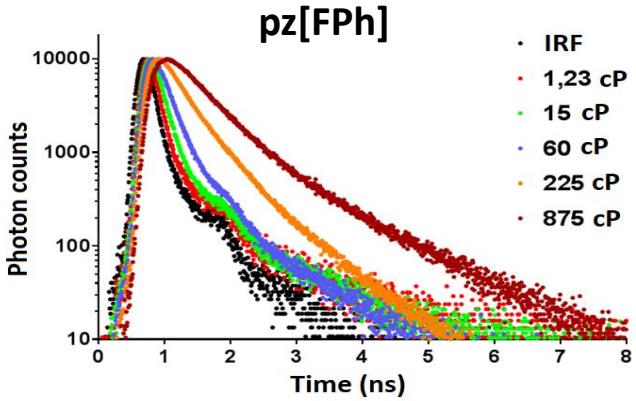
Förster–Hoffmann equation

$$\lg(\varphi) = z + \alpha\lg(\eta)$$

Coefficient α varies from 0.3 to 0.7 depending on aryl groups in the macrocycle periphery

Results and discussion

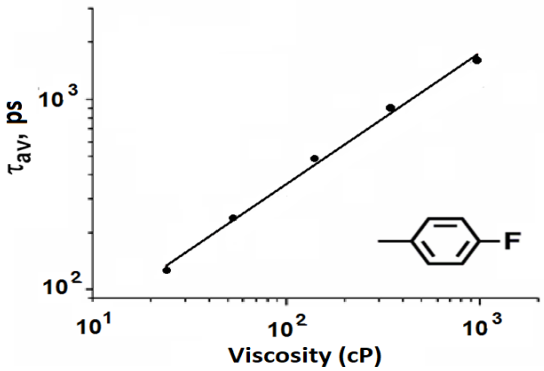
The fluorescence quantum yield and lifetime strongly depend on viscosity of the medium



Several twisting/rotating groups in a molecule lead to multiexponential fluorescence decay

$$f(t) = \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau 2},$$

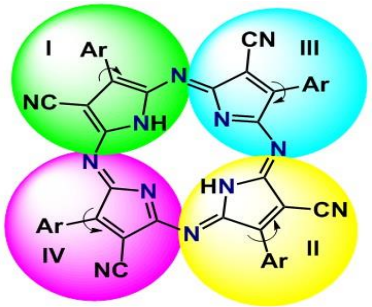
intensity weighted mean lifetime $\tau_{av} = \frac{\alpha_1 {\tau_1}^2 + \alpha_2 {\tau_2}^2}{\alpha_1 \tau_1 + \alpha_2 \tau_2},$



Förster–Hoffmann equation

$$\lg(\tau) = \lg\left({}^{z}/_{k_r}\right) + \alpha \lg(\eta),$$

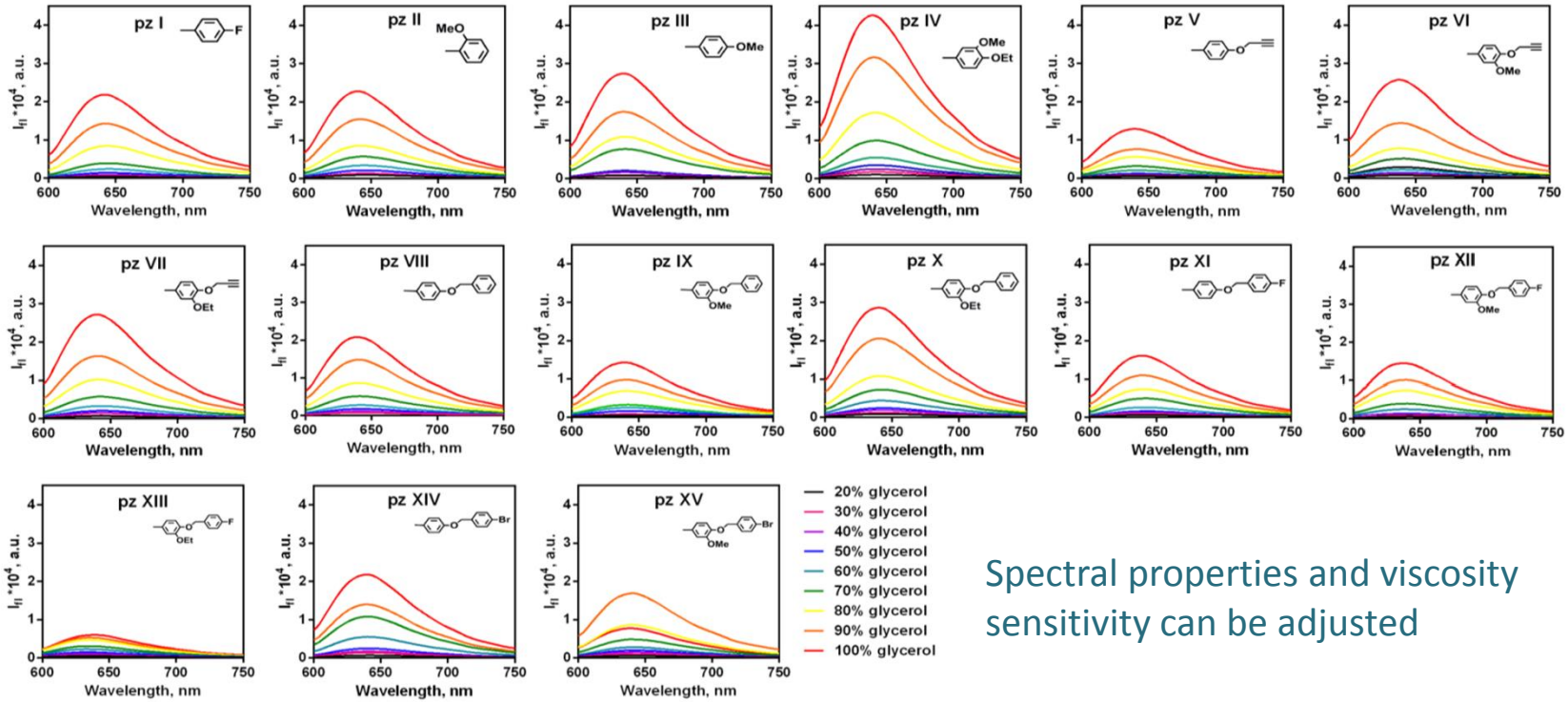
Coefficient α varies from 0.3 to 0.7 depending on aryl groups in the macrocycle periphery



Izquierdo et al., Journal of Materials Chemistry B, 2014
Balalaeva et al., Molecules, 2021

Results and discussion

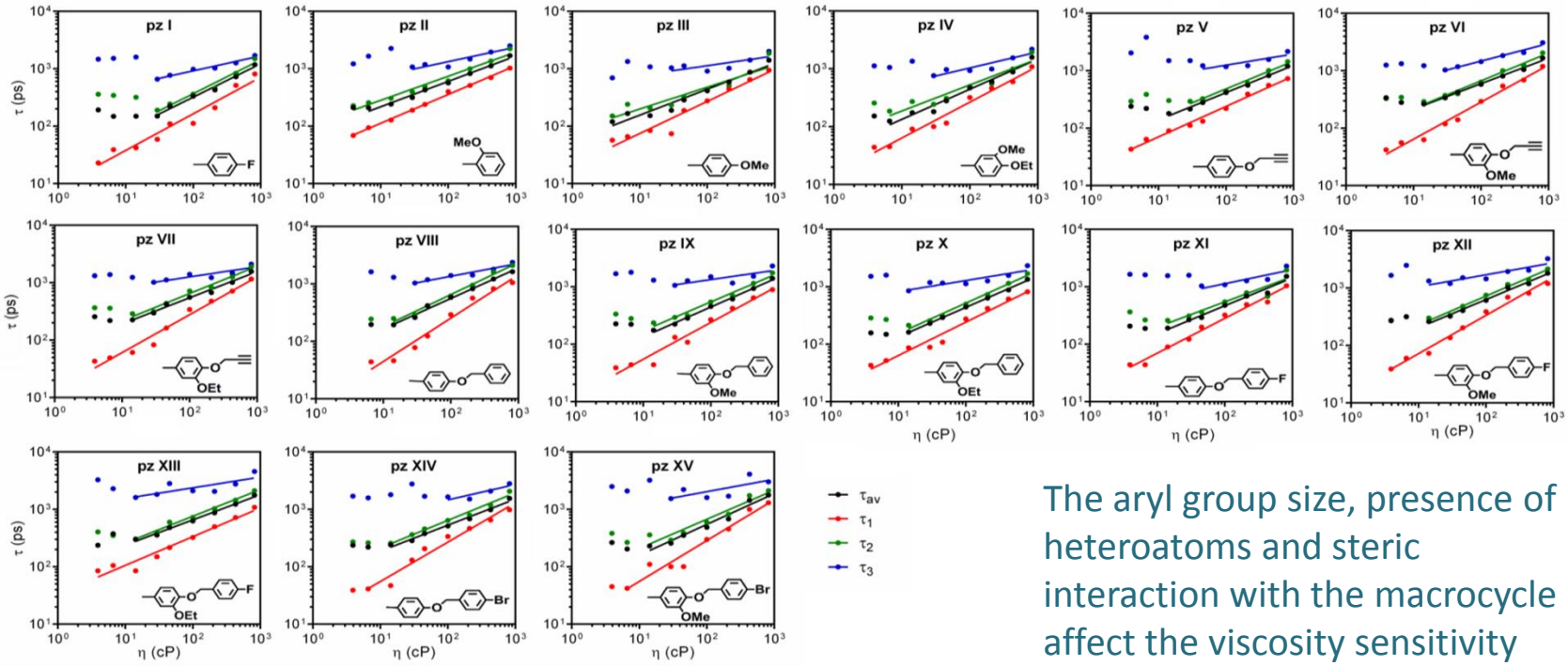
Tunability of Pz properties by modifying the macrocycle periphery



Spectral properties and viscosity sensitivity can be adjusted

Results and discussion

Tunability of Pz properties by modifying the macrocycle periphery



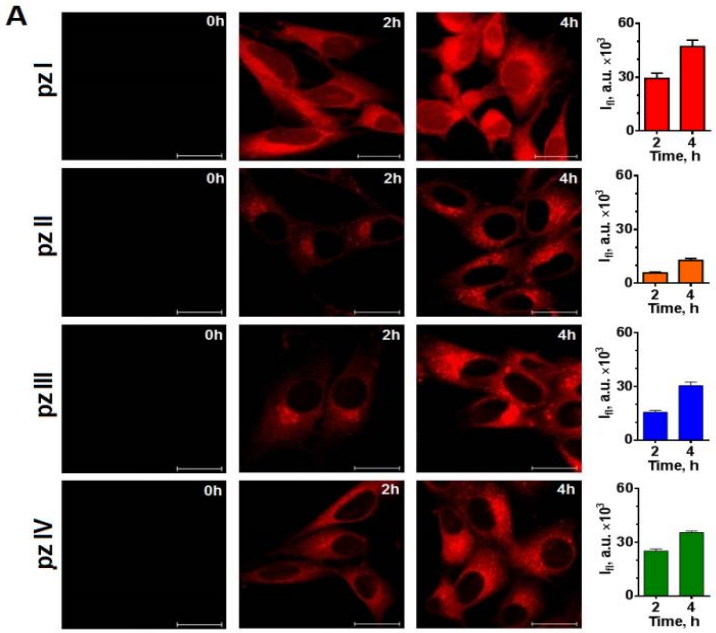
The aryl group size, presence of heteroatoms and steric interaction with the macrocycle affect the viscosity sensitivity

Results and discussion

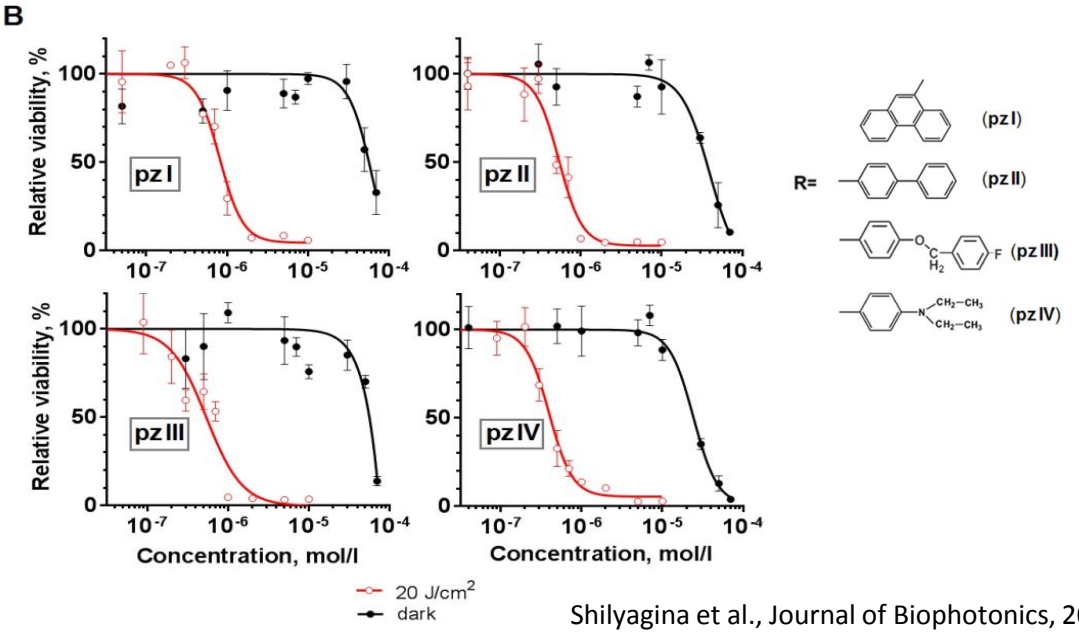
Pz are efficient photosensitizers against cancer cells of various origin

Skin, bladder, cervical human cancer, murine colon cancers, human and murine glioma, murine sarcoma

- Cellular uptake within 1-4 hrs
- IC_{50} (20 J/cm^2): $2 \cdot 10^{-7}$ – $5 \cdot 10^{-6}$ mol/l



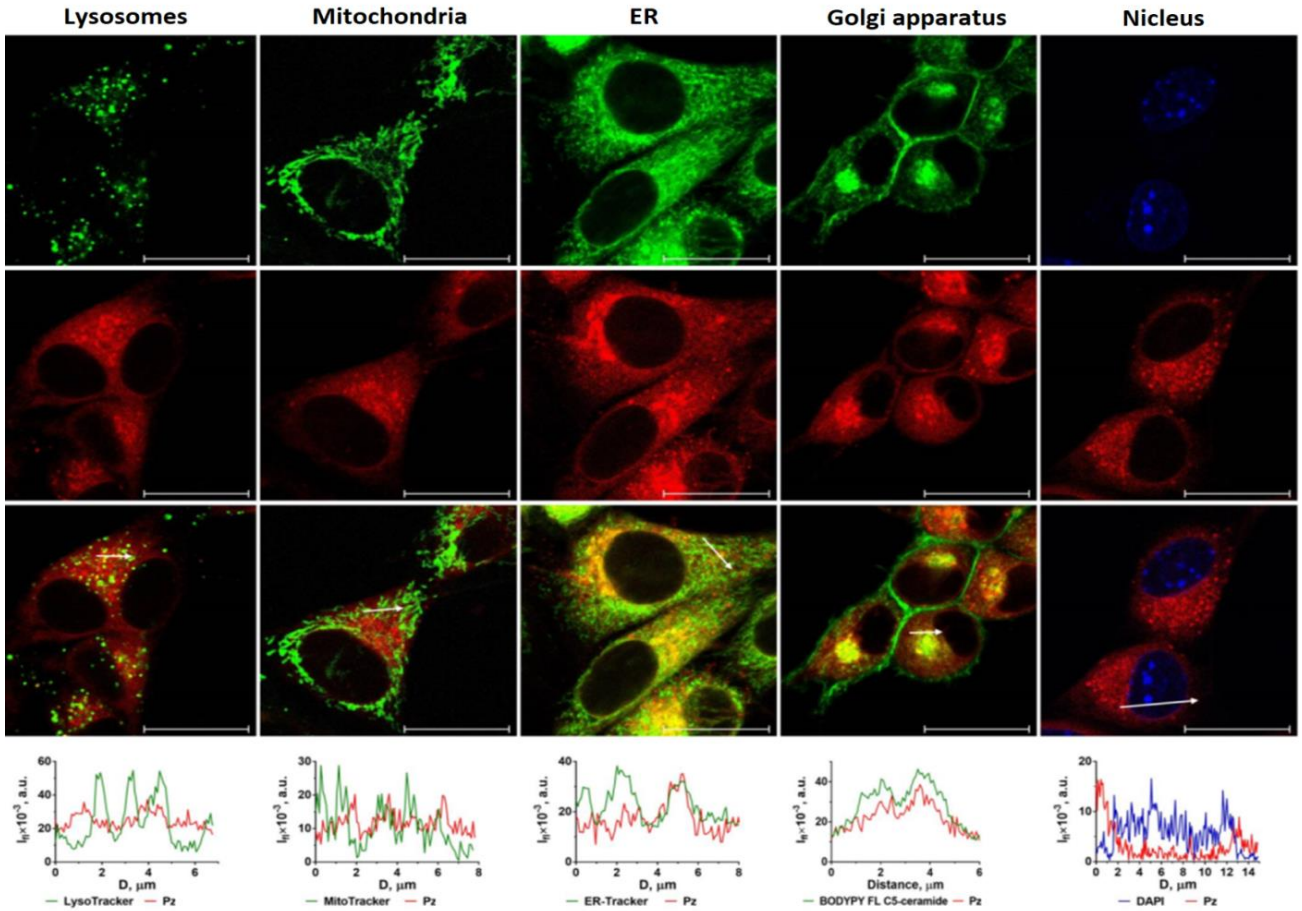
Murine glioma cells



Shilyagina et al., Journal of Biophotonics, 2017
Mischenko et al., Journal of Biophotonics, 2020
Turubanova et al., Sci Report, 2021
Balalaeva et al., Molecules, 2021

Results and discussion

Pz localize in intracellular membranes



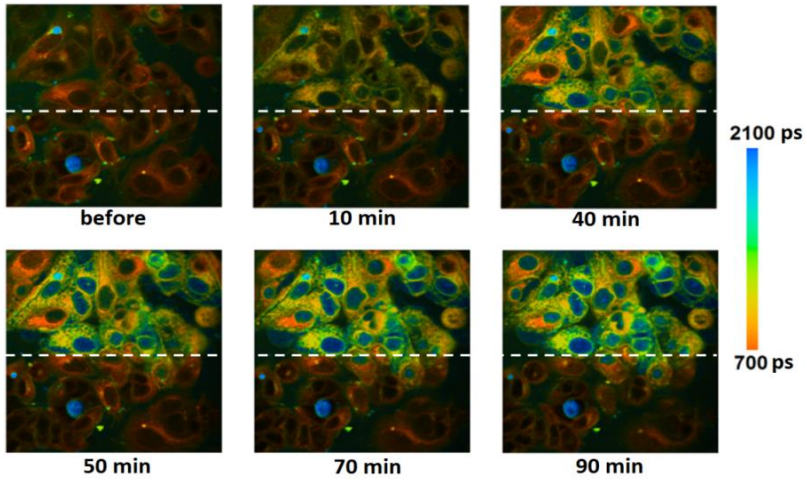
- Predominant Pz localization in ER and Golgi apparatus
- We assume the ER-stress to be the key step in cellular response to PDT

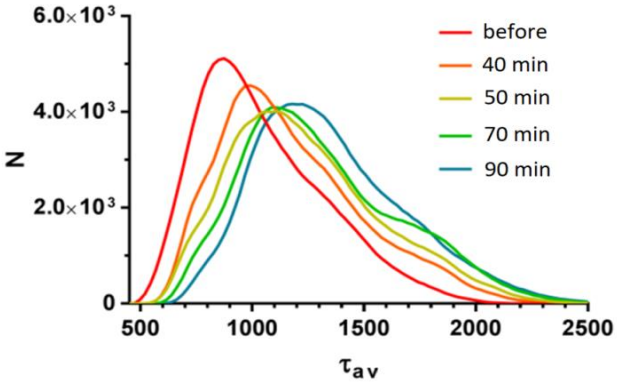
Results and discussion

Is it possible to sense the functional state of cells during PDT procedure?

pz[FPh]

Skin cancer cells (A431 cell line) pre-treated with 5μM pz[FPh] for 4 hrs





FLIM-images of the same field of view before and after PDT treatment (50 J/cm^2). Only the upper half of the field of view was irradiated with intensive light. Irradiated and non-irradiated regions are divided by dotted line. λex 800 nm, λem 600-750 nm.

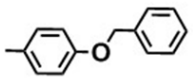
- Irradiation is accompanied by an increase of Pz fluorescence lifetime ("an increase in local viscosity")
- Viscosity changes intensify during 1.5 hrs, in parallel with morphological changes

Izquierdo et al., Journal of Materials Chemistry B, 2017

Results and discussion

Is it possible to sense the functional state of cells during PDT procedure?

pz[BnOPh]



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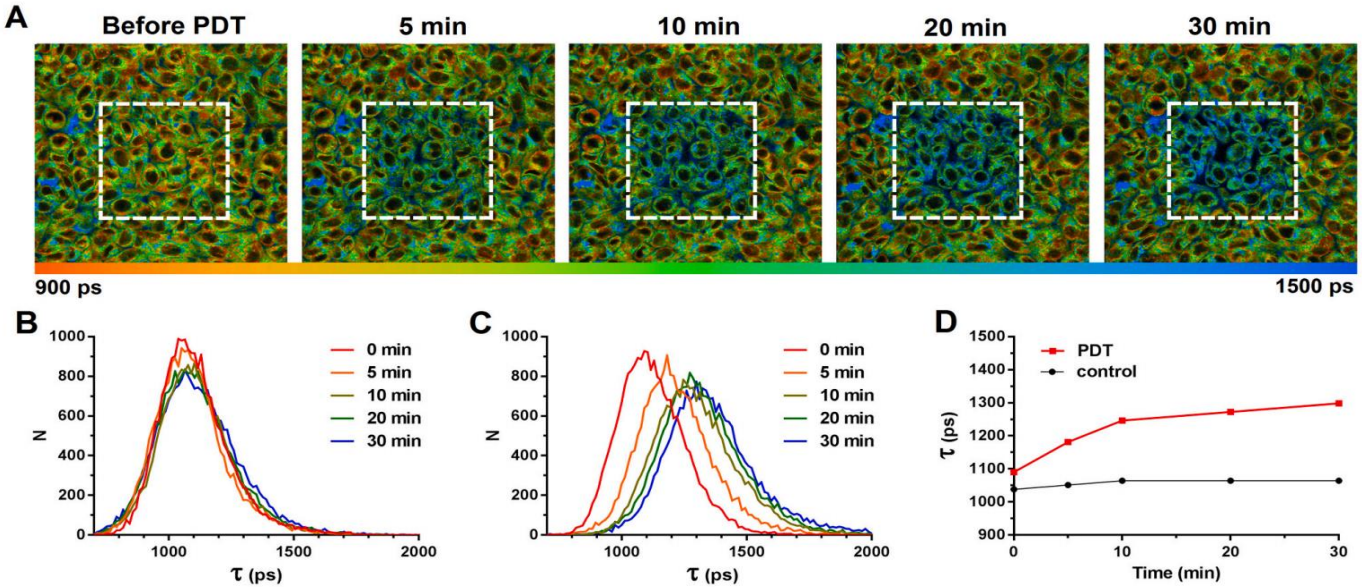


FLIM-images of the same field of view before and after PDT treatment (50 J/cm^2). The area inside the dotted line square only was irradiated with intensive light. λex 800 nm, λem 600-750 nm.

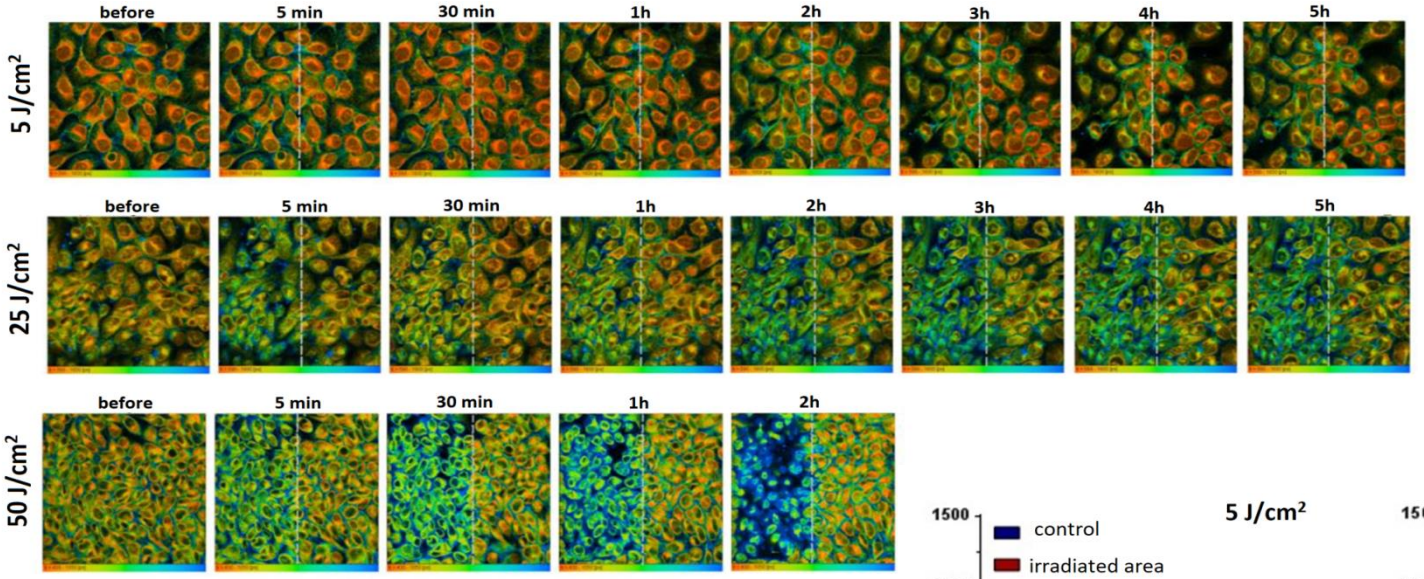
- Cellular response to PDT has been registered with several Pz with different viscosity sensitivity

Peskova et al., J Photochem Photobiol B Biology, 2021

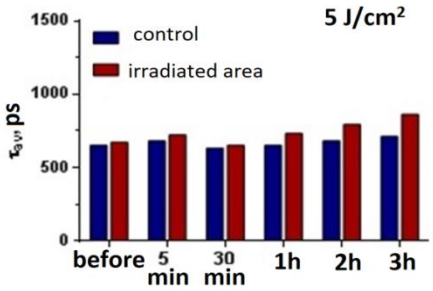
Balalaeva et al., Molecules, 2021 (pz[biPh]; pz[diEtPh])

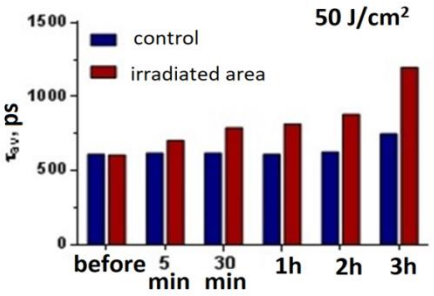
Results and discussion

Rate and amplitude of changes in photophysical properties of Pz are dose-dependent



pz[FPh]





In vitro:
The more intensive treatment, the more rapid and more manifested 'viscosity response'

Results and discussion

PDT treatment *in vivo* with monitoring of Pz fluorescence lifetime

Balb/c mice

Subcutaneous murine colon carcinoma CT26

9 days

i.v. administration of **pz[FPh]** solubilized in polymer brushes, 15 μg/kg

3 h to get the **pz[FPh]** accumulation in tumor tissue

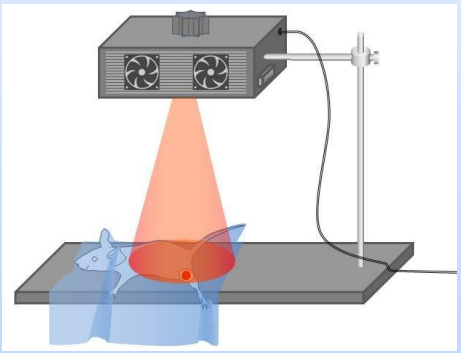
PDT

Monitoring for 3 h

Treatment outcome

Fluorescent imaging in vivo

Fluorescence lifetime imaging (FLI) in vivo



PDT device:

LED light source (UNN & IAP RAS)

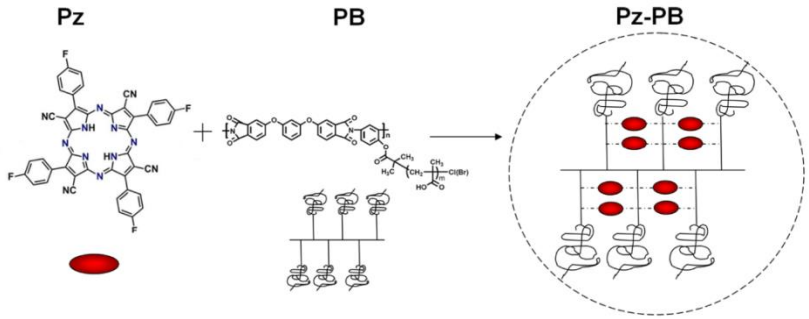
Wavelength, **620-655 nm**

Light intensity, **20 to 300 mW/cm²**

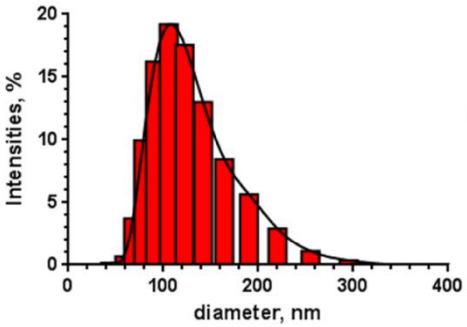
Uniformly irradiated area, **Ø 35 мм**

Results and discussion

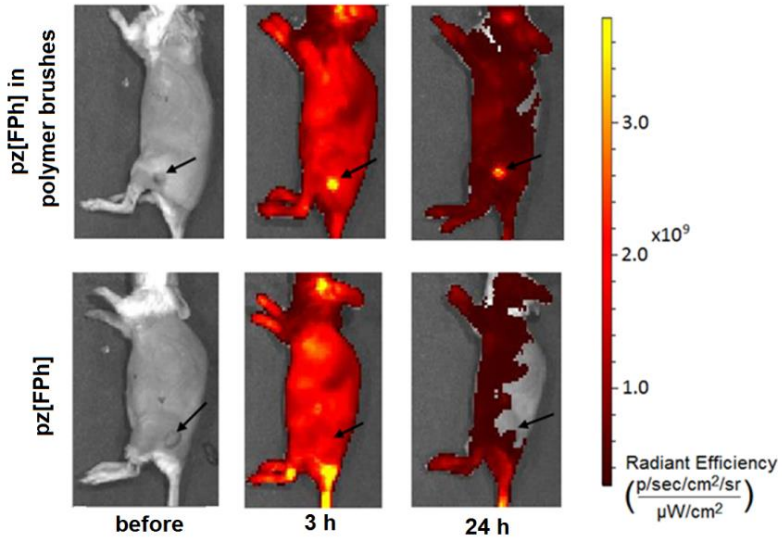
pz[FPh] selectively accumulates in tumor tissue

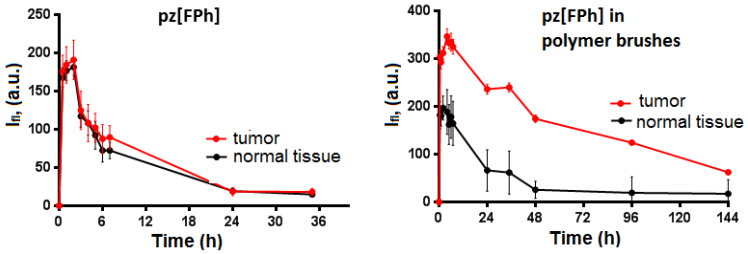


Polymer brushes (PB) form nanoparticles and provide selective accumulation of Pz in tumor due to EPR-effect (Enhanced Permeability and Retention)



Pz-PB nanoparticles, 100-150 nm





Yakimansky et al., J Polymer Sci, Part A: Polymer Chemistry, 2013
Shilyagina et al., Journal of Biophotonics, 2017
Krasnopeeva et al., Nanomaterials, 2021

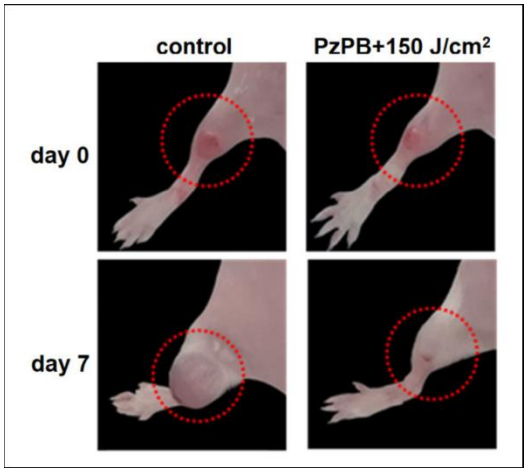
Results and discussion

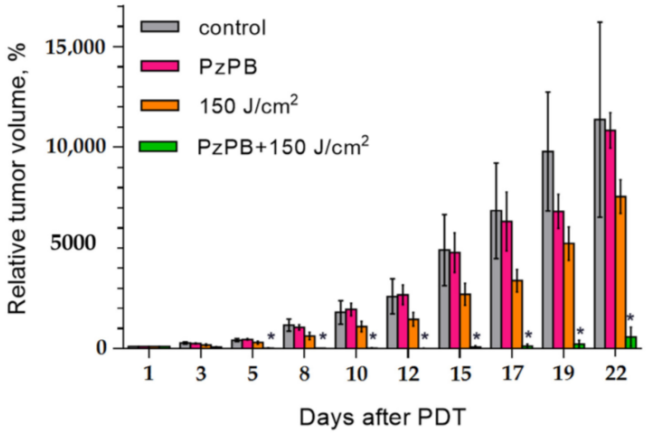
pz[FPh] is an efficient PDT agent

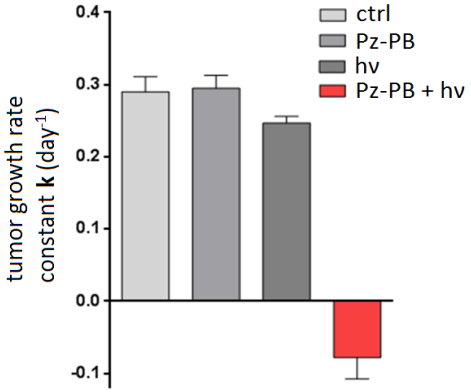
pz[FPh] 15 μg/kg
150 J/cm²

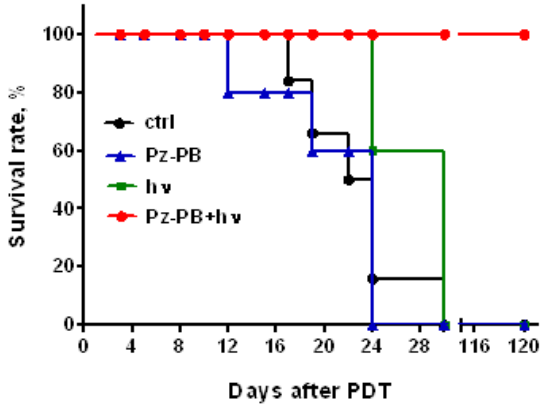
Relapse-free recovery – 5 of 7 animals

Tumor growth inhibition coefficient > 95%









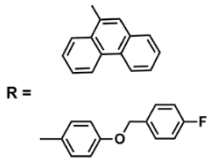
Krasnopeeva et al., Nanomaterials, 2021

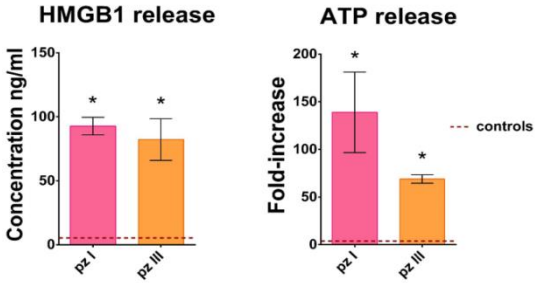
Results and discussion

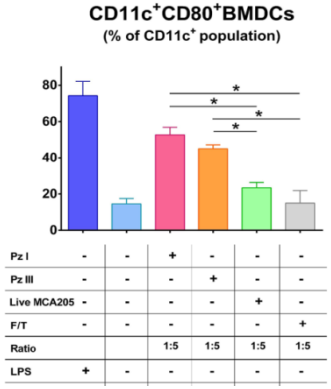
PDT can induce immunogenic cell death

(In collaboration with Prof. D. Krysko, Ghent University, Belgium)

Turubanova, Balalaeva et al., J ImmunoTherapy of Cancer, 2019
Turubanova et al., Sci Report, 2021

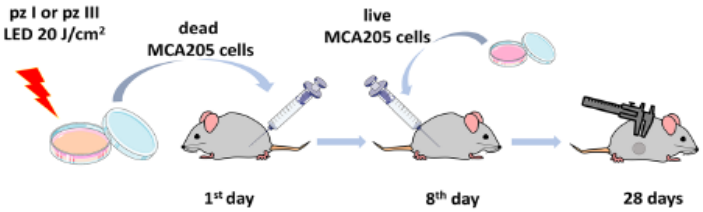


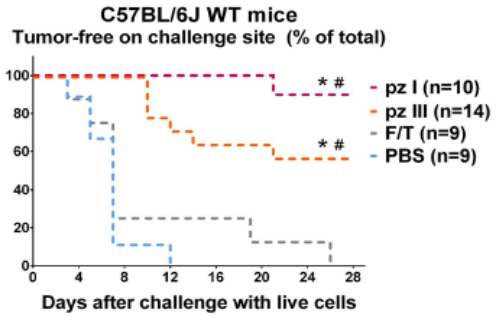


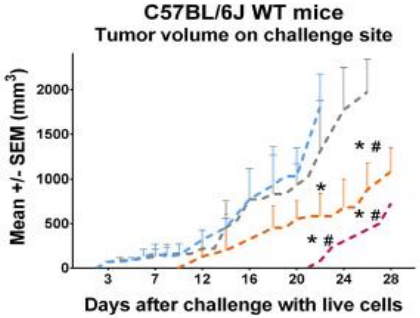


- Dying cell release DAMPs
- Cancer cells killed by Pz-based PDT are phagocytized and induce activation and maturation of dendritic cells
- Vaccination of mice with PDT-killed cells prevents them from tumor growth after re-challenge with cancer cells

Model of prophylactic vaccination

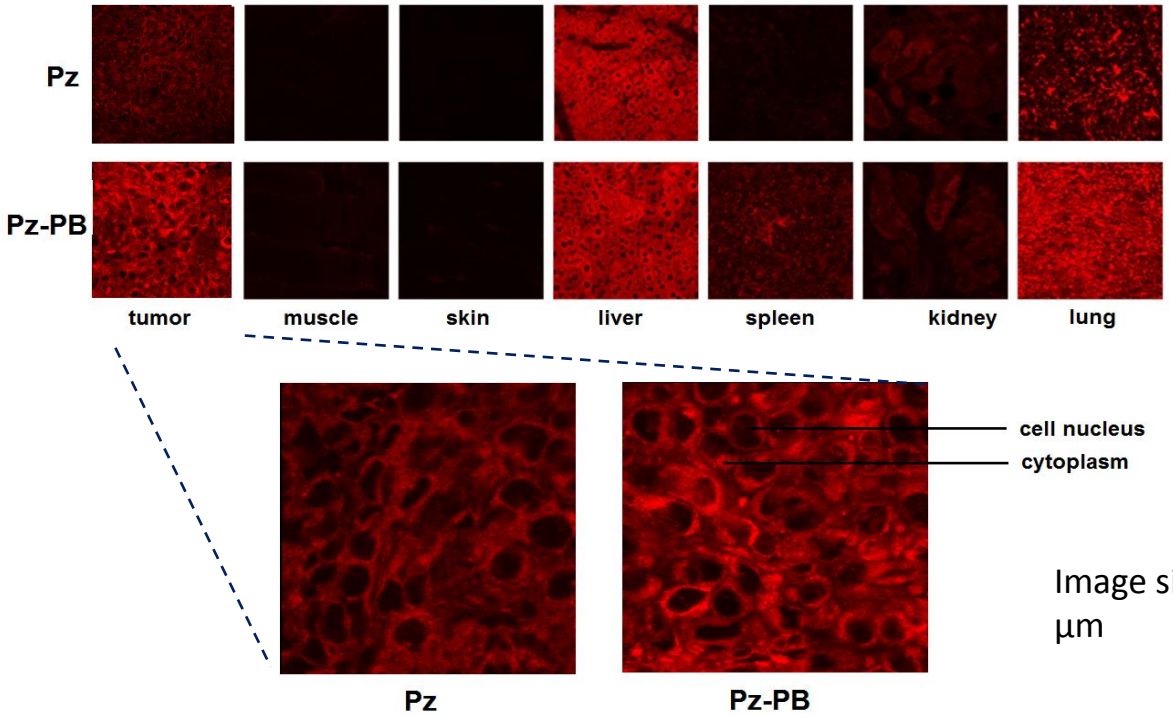






Results and discussion

pz[FPh] localizes in cytoplasm of tumor cells



λ_{ex} 633 nm,
λ_{em} 650-710 nm

Image size, 225×225 μm

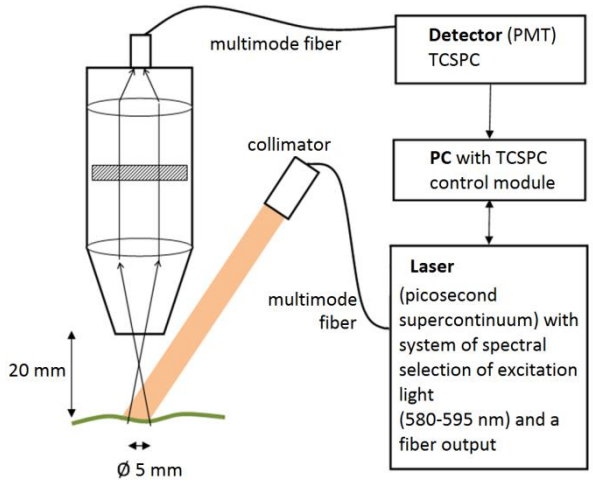
Image size, 75×75 μm

- Pz[FPh] localization in tumor cells gives a chance to analyze their viscosity properties during/after PDT

Results and discussion

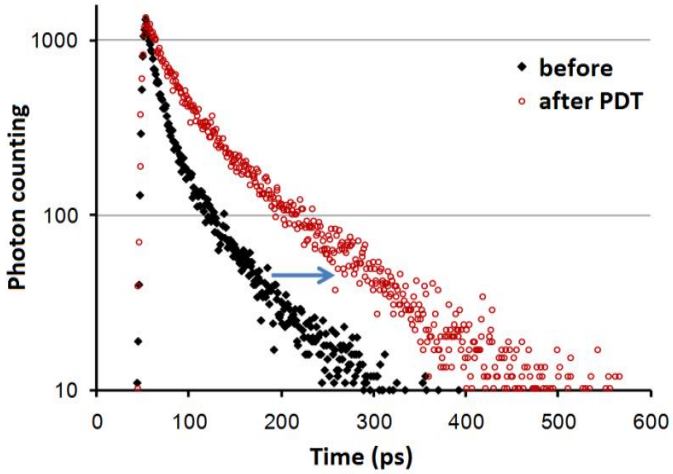
Registration of Pz fluorescence lifetime in vivo

Home-build setup for
Fluorescence lifetime imaging (FLI) in vivo (UNN & IAP RAS)



TCSPC- Time-Correlated Single Photon Counting

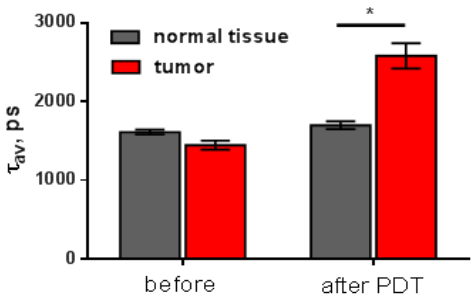
Integrated fluorescence from an area of Ø 5 mm
Measurement time, 2 s

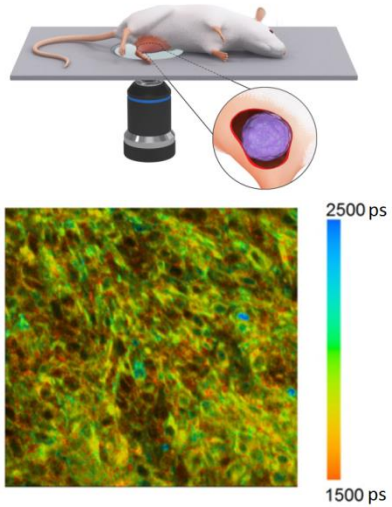


- Multiexponential decay similar to in vitro measurements
- Irradiation of tissue leads to drastic changes in Pz fluorescence lifetime

Results and discussion

Pz fluorescence lifetime changes under PDT procedure

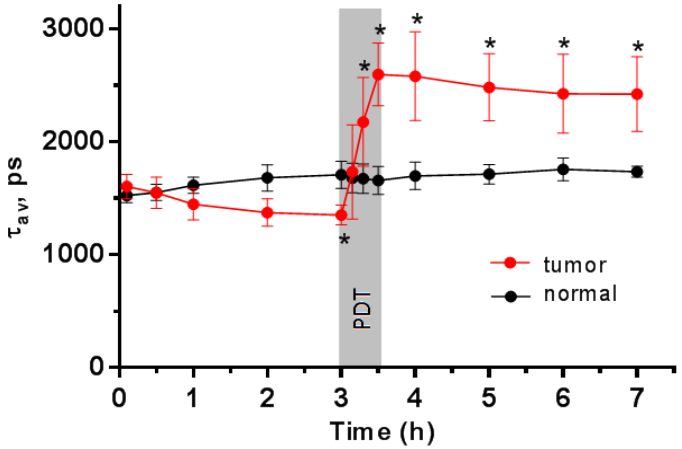




Intravital FLIM-image of tumor tissue

In vivo

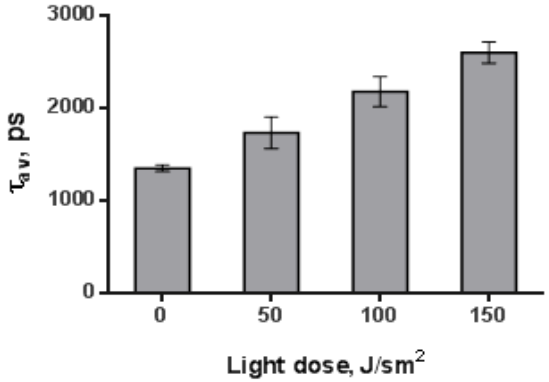
- **Tissue complexity:** Values of Pz fluorescence lifetime in tumor tissue measured *in vivo* (1.3-1.7 ns) is higher than in cells *in vitro* (0.8-1.0 ns)
- **Independence on measuring technique:** Values of Pz fluorescence lifetime are similar when measured with whole-body FLI setup and FLIM microscopy



- **Sensitivity to PDT treatment:** Pz fluorescence lifetime gradually rise during the PDT procedure

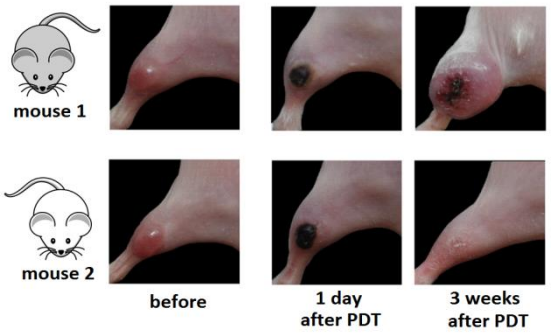
Results and discussion

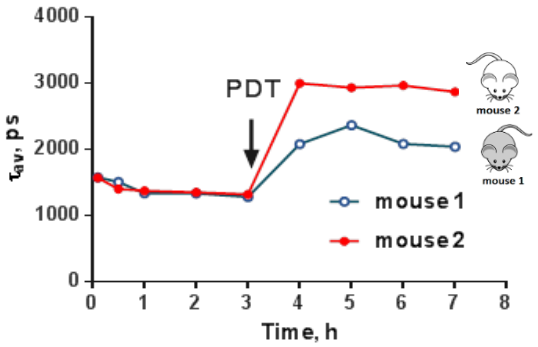
Can we predict the outcome of the PDT procedure?

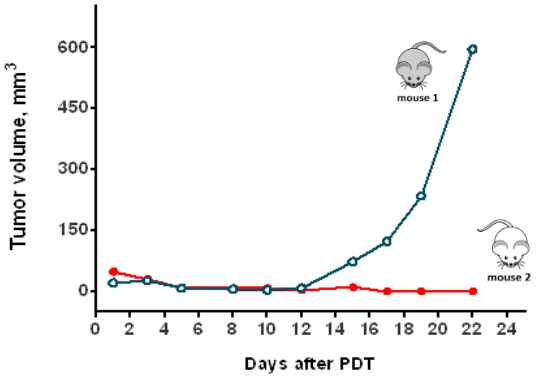


- **Dose-dependent response:** the higher the dose, the more manifested the changes
- "Weak" response of Pz fluorescence lifetime to PDT may be a predictor of a "poor" treatment outcome

An example: Two animals with different responsivity to PDT showed different treatment outcome







Conclusions

- Tetracyanotetra(aryl)porphyrazine dyes (Pz) with high viscosity sensitivity are the potent **agents for PDT** application.
- The real-time monitoring of the Pz fluorescence lifetime provides the rational basis for **dosimetry** during PDT.
- Pz fluorescence lifetime reflects the physiological state of the irradiated tissue and can be potentially used for **individualization** of the PDT procedure.

We are still on the way to obtain the “ideal ” photosensitizer…

The question “Can we predict the outcome of the PDT procedure?” is still open.

That are the precise mechanisms underlying the ‘viscosity response’?

Is the viscosity reaction universal for cellular stress response?

Translation to clinic is ahead, and it is extremely challenging.

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