INTRACELLULAR SENSING BY A SURVIVIN MOLECULAR BEACON COUPLED TO PMMA NANOPARTICLES IN HUMAN CANCER CELLS

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BACKGROUND

Polymethylmethacrylate core-shell fluorescent nanoparticles (**PMMA-NPs**) promote the internalization of a **molecular beacon** (**MB**) specific for **survivin mRNA**, in human lung **A549 cancer cells** (Fig.1A-C).

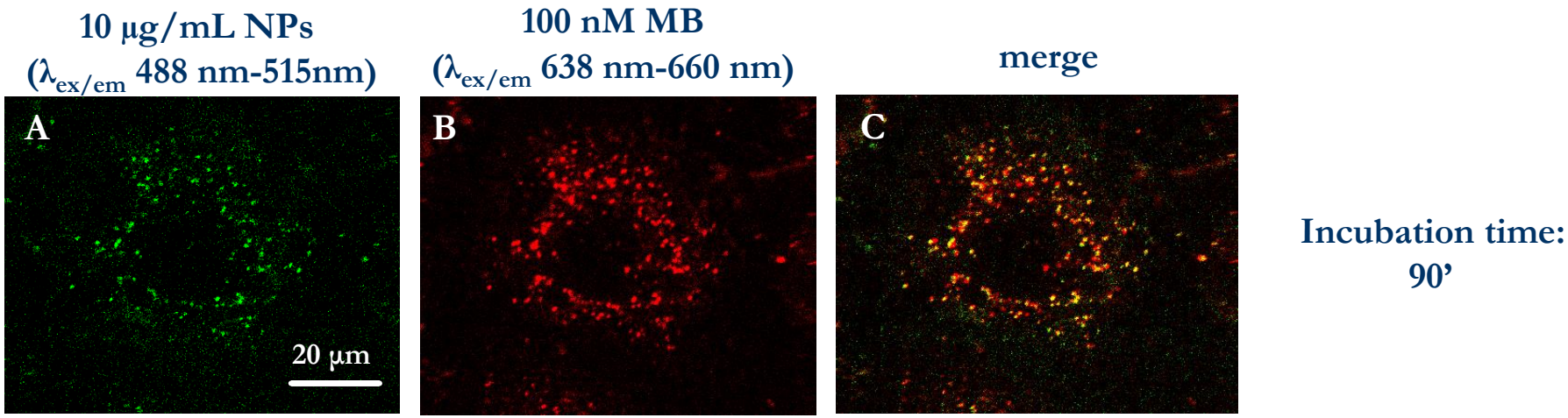
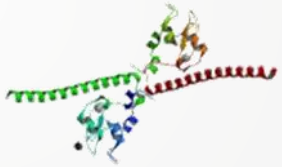


Fig.1

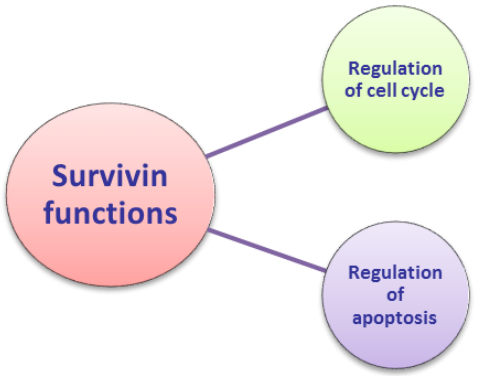
Adinolfi B. et al., Biosens Bioelectron., 88, 15-24 (2017)

To design an effective drug delivery system the knowledge of the uptake mechanism and the fate of nanoparticles and MB are required.



INTRACELLULAR TARGET: SURVIVIN *m*RNA

Survivin belongs to a family of proteins, known as **inhibitor of apoptosis** protein (**IAP**), which plays a key role in the **regulation** of *apoptosis* and *cell division*



Survivin expression is very high in **most cancer cells**. It is rarely present in healthy tissues

Curr Oncol Rep (2012) 14:120–128

Table 1 Frequency of survivin overexpression in solid tumors

Tumor type	Expression (%)
Breast cancer	70.7
NSCLC	85.5
Neuroblastoma	47
Glioma	NS
Colorectal	53.2
Melanoma	67
Ovarian	73.5
Pancreatic	88
Esophageal	80
Gastric	34.5

NSCLC non–small cell lung cancer

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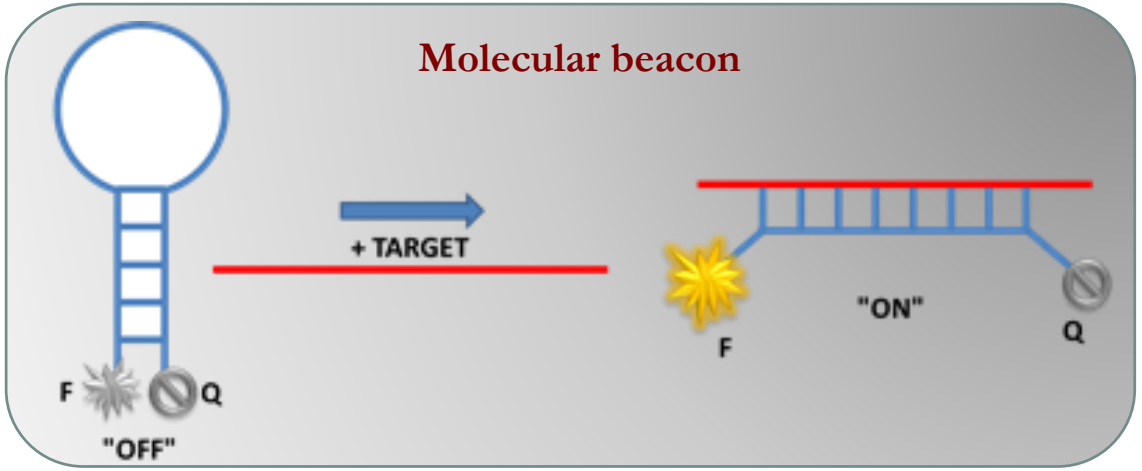
MOLECULAR BEACON-BASED STRATEGY

Oligonucleotide optical switches are among the **most promising *optical sensors*** proposed in the recent years.

They are suitable **molecules capable** of **turning on** or **modifying** their **light emission** after molecular **interaction** with **well-defined molecular targets**.

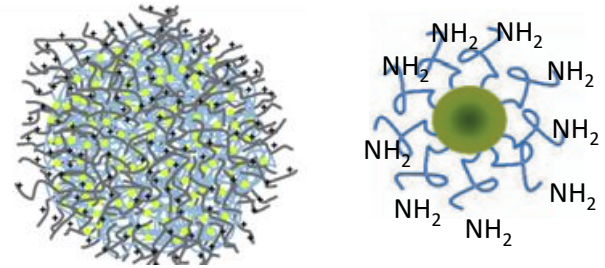


Molecular Beacon strategy may conjugate the *sensing properties* with the *silencing activity*

POLYMETHYLMETHACRYLATE (**PMMA**) **NANOPARTICLES**: CHARACTERIZATION **IN SOLUTION**

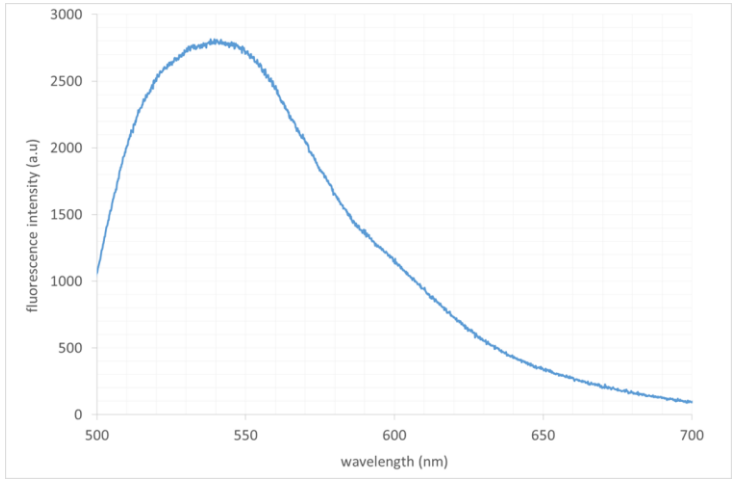
Nanoparticles consist of a ***core*** **of PMMA,** surrounded by a shell bearing ***cationic groups*** and ***amine groups***.

Fluoresceine is covalently immobilized inside the nanoparticles.



Size: 57.2 ± 1.3 nm (PCS)
Zeta potential: 74.4 ± 14.1 mV

Duchi S. *et al.*, Journal of Controlled Release, 2013, 168 (2), 225-237



Fluorescence spectra (λ_{ex}= 488 nm, pH=8, int. time 1 sec) (Fluoresceine)

AIMS

a) to evaluate the involvement of **endocytosis** as **a PMMA-NP uptake mechanism** by confocal microscopy, using the Alexa Fluor 647 Dextran™ (DXT);

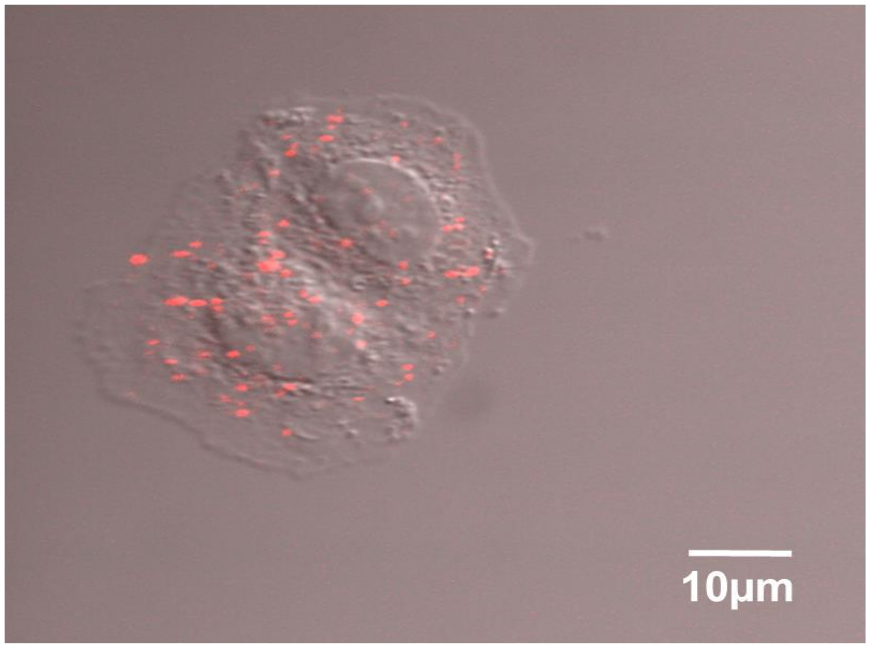
b) to evaluate the **fate** of the **PMMA-NPs** at different times of incubation with the cells by photometric measurements and to verify their **localization in lysosomes**, by confocal microscopy, using LysoTracker Deep Red™ (Lys);

c) to investigate, by using ER-Tracker Green™ (ER-T), the **localization of the MB** fluorescence signal with respect to the **Endoplasmic Reticulum (ER)** where presumably the target mRNA is located.

RESULTS:
PMMA-NPs increase endocytosis

A

400 µg/mL DXT ($\lambda_{ex/em}$ 638 nm-660) nm)



400 µg/mL DXT+10 µg/mL NPs

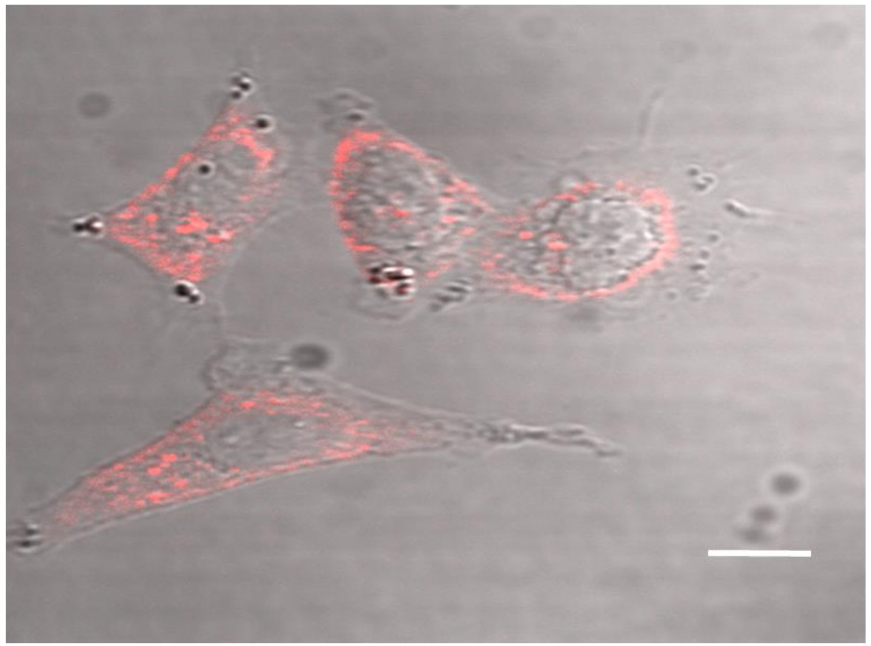


Fig.2

Incubation time: 30'

RESULTS:
PMMA-NPs colocalize with lysosomes

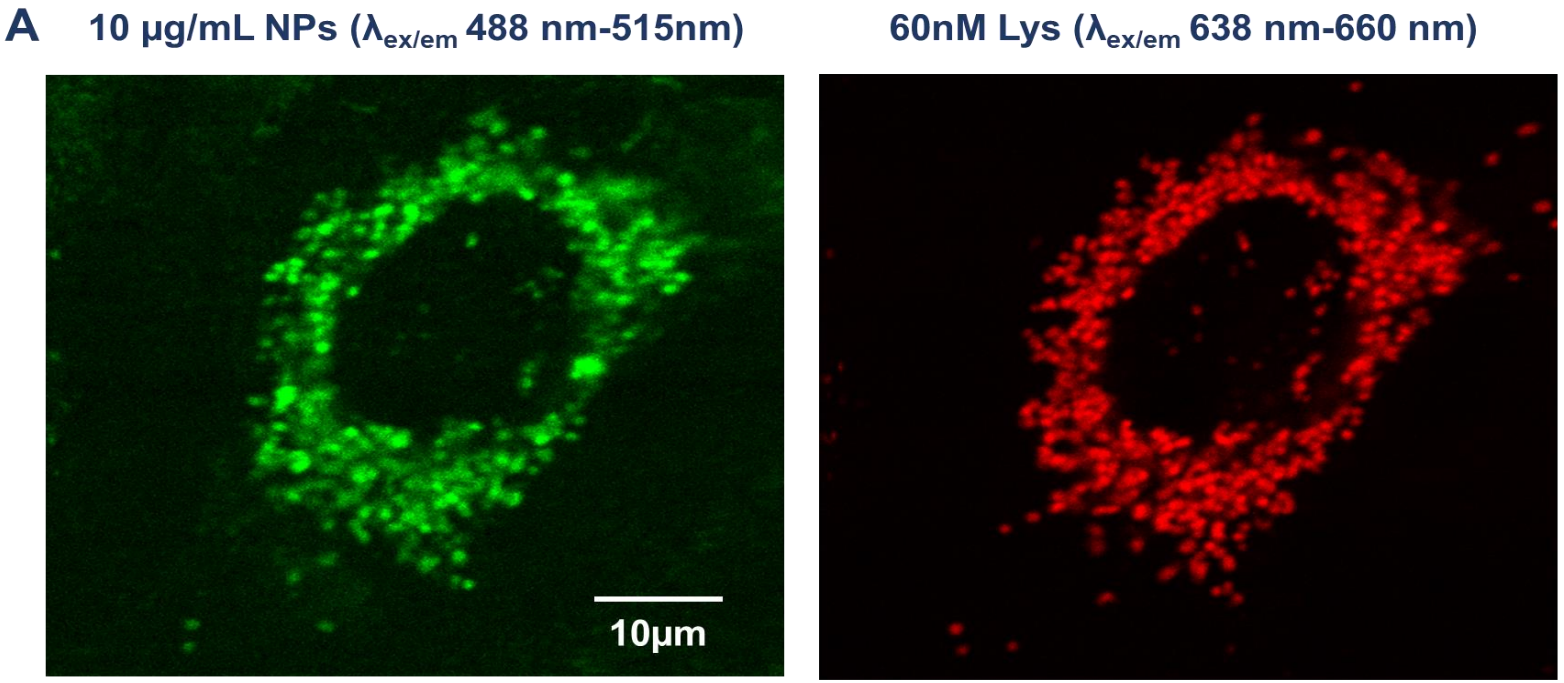


Fig.3 **Incubation time: 48h NPs+70' Lys**

The co-localization analysis was performed using the JACoP plugin of Image J. The fractional overlap of fluorescent signals was measured via the Manders' Colocalization Coefficients (MCC). The colocalization analysis indicated that fractions of about 65% of Green and Red fluorescence signals overlap (N=20 cells).

lysosomal exocytosis is probably involved in the PMMA-NP elimination

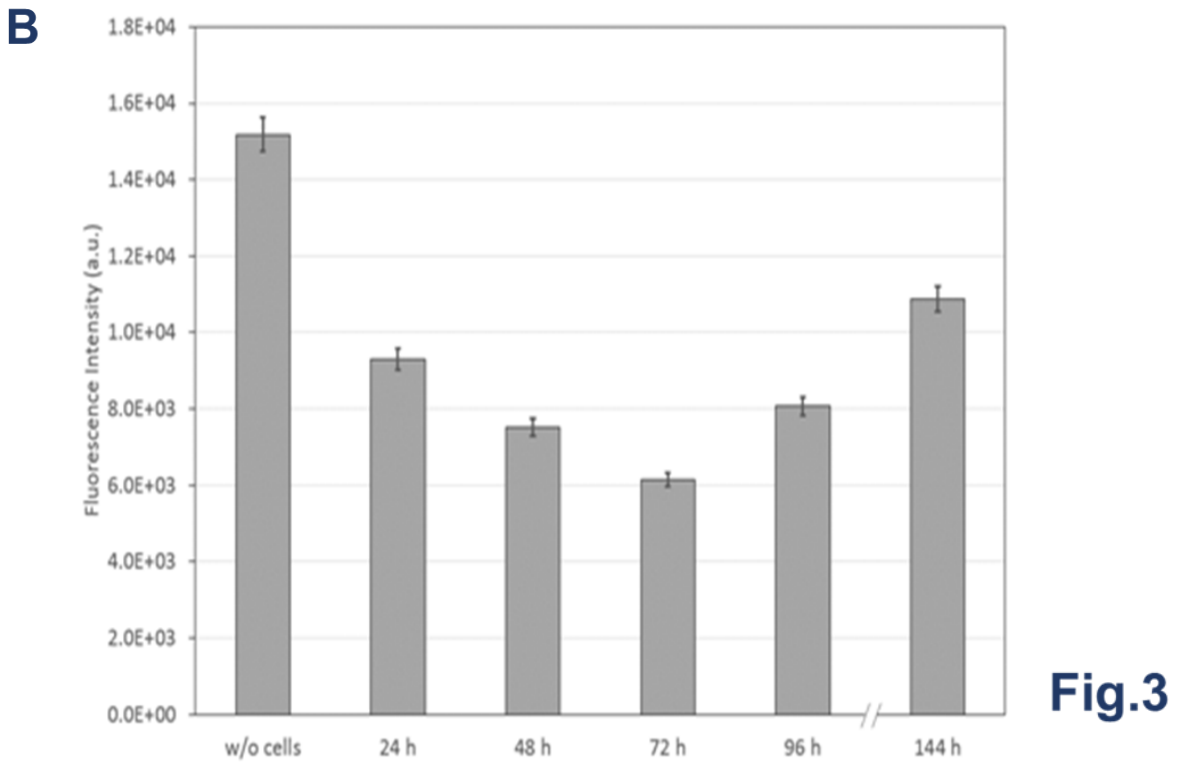


Fig.3

A cellular release of NPs detected in the culture medium suggested a role of lysosomal exocytosis in nanoparticle elimination.

RESULTS:
survivin MB opens up in proximity of the Endoplasmic Reticulum (ER)

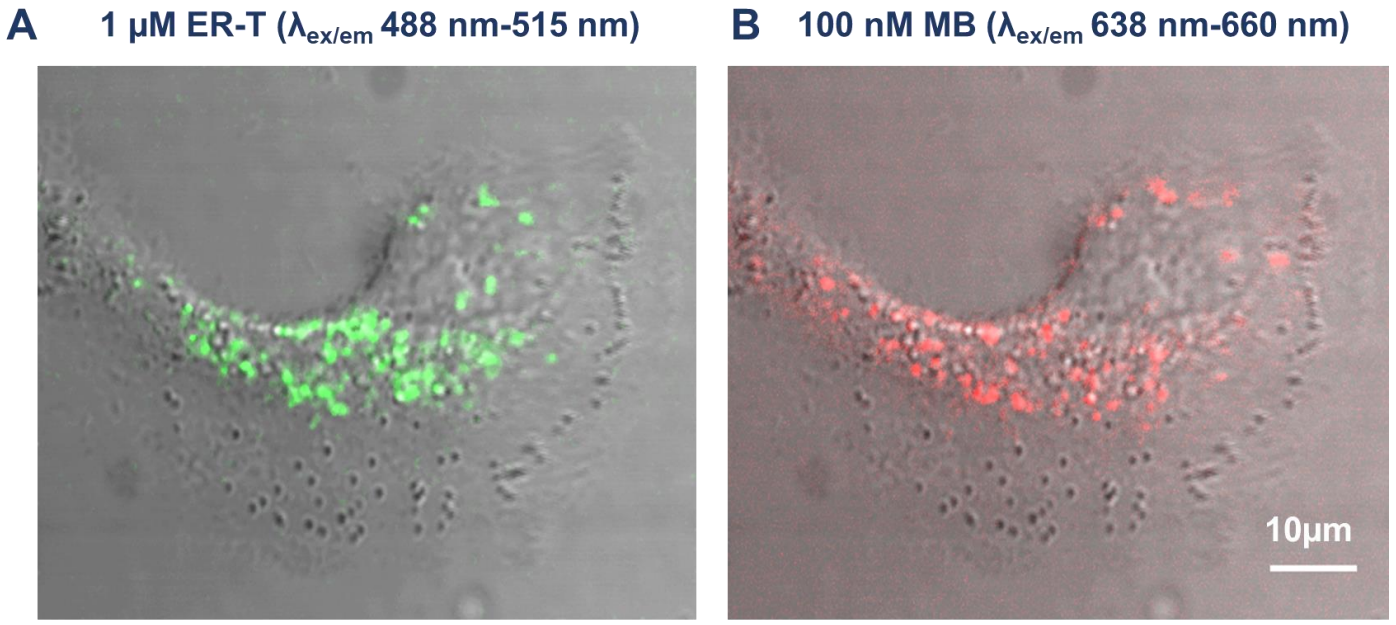


Fig.4 Incubation time: 30' ER-T+90' non fluorescent NPs-MB

Colocalization analysis indicated that fractions of about 25% of Green and Red fluorescence signals overlap (N=70 cells).

CONCLUSIONS

- The NPs increase endocytosis
- The NPs colocalize with Lysosomes and lysosomal exocytosis is probably involved in the NP elimination
- The opening of the MB occurs in proximity of the ER where its target mRNA is presumably located

ACKNOWLEDGMENTS

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Many thanks for your attention

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