

^a Université de Lorraine, CNRS, L2CM UMR 7053, F-54506 Vandoeuvre-lès-Nancy, France

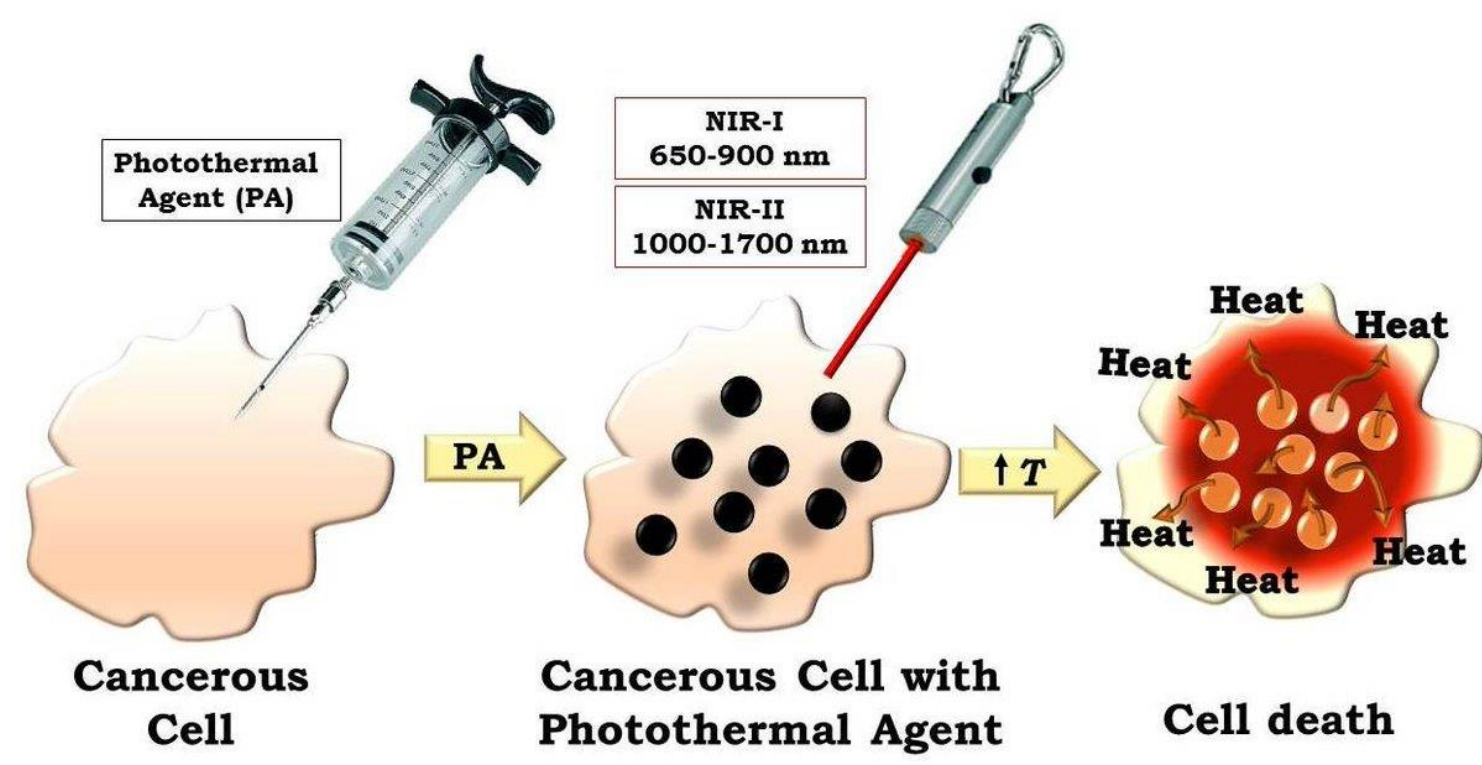
^b Université de Lorraine, CNRS UMR 7039, CRAN, F-54000 Nancy, France

^c Institut de Cancérologie de Lorraine, Unité de Recherche Translationnelle, F-54000 Nancy, France

Introduction

Photothermal therapy (PTT)

Extension of photodynamic therapy using **photothermal agent** (PA) and **light** to induce temperature rising inside cancer cells/tissues.



Main features:

- High spatiotemporal selectivity
- Oxygen independency
- Low invasive effect
- The use of a near infrared (NIR) LASER allows deep penetration of light into biological tissues

(Liu et al, 2019, Chem Soc. Rev.)

Indocyanine green (ICG) / ICG J-aggregates (ICG-J)

Advantages of ICG:

- Clinically approved molecule
- Amphiphilic cyanine
- Absorption peak in NIR at 780 nm

(Dash et al, 2021, Int J Mol Sci.)

Limitations for PTT application:

- Poor aqueous stability & photostability
- Rapid clearance from the body (2-4min)

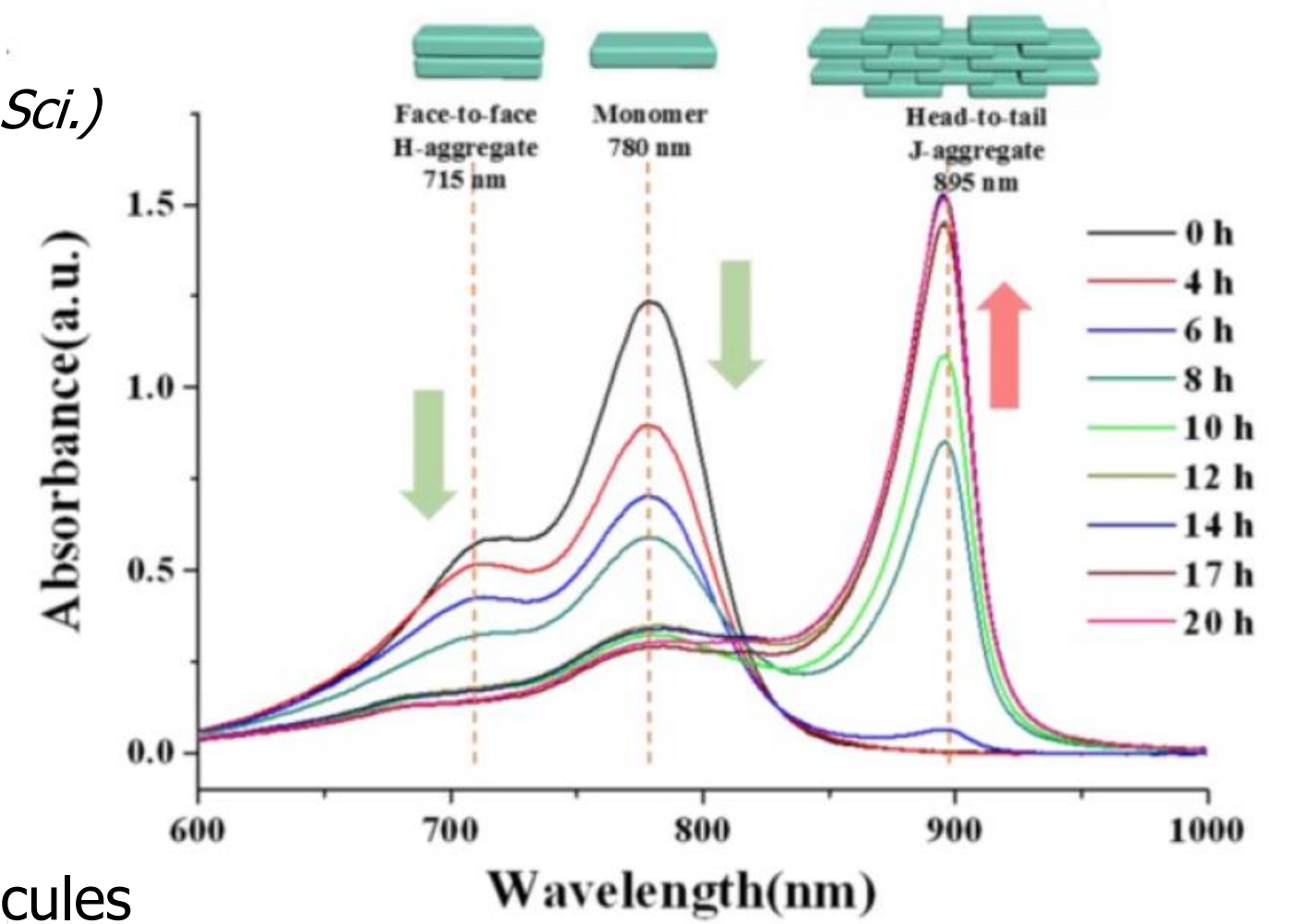
ICG-J aggregates as PA:

- Characterized by a redshift in absorption peak (780 to 890 nm)
- Better photothermal efficiency compared to ICG

(Liu et al, 2017, Nanotheranostics)

- Instable in biological solutions: **need for formulation**

Stabilization by cationic **Calix[4]arene (CX)** molecules

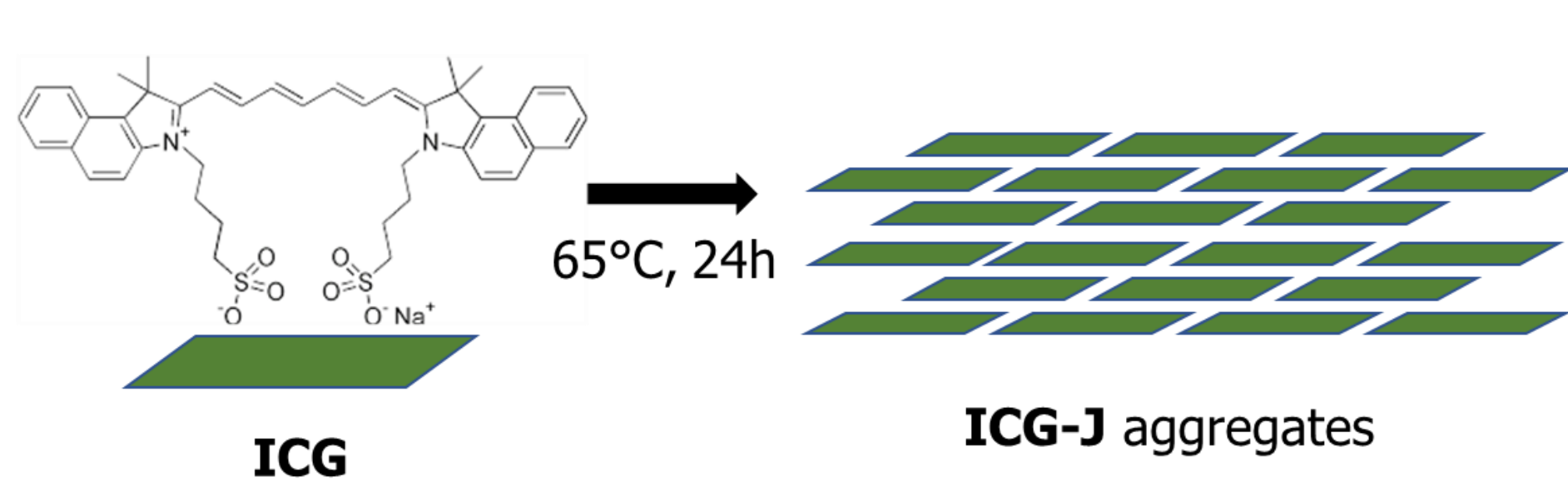


Objective

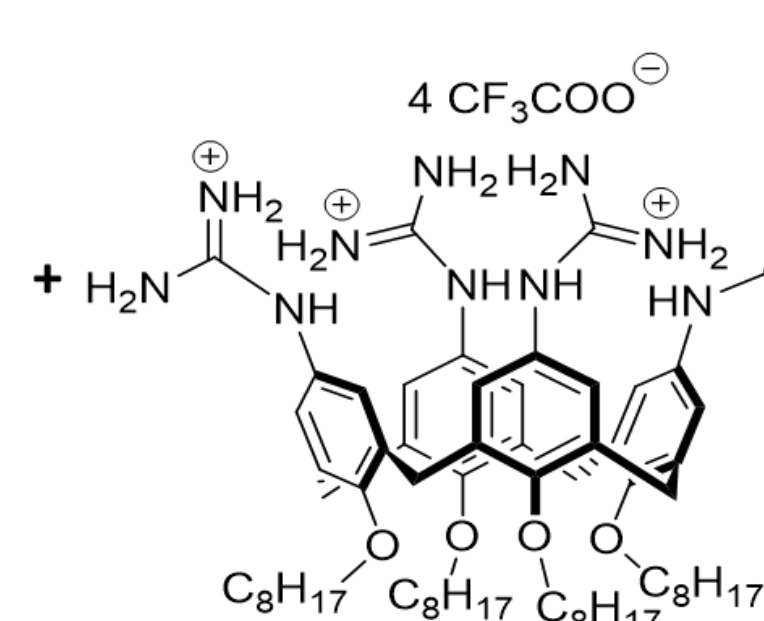
Characterization of our novel ICG-J/Calix[4]arene nanoformulation to evaluate its physicochemical properties, photostability, accumulation and photothermal efficiency in a head and neck adenocarcinoma cell line.

Materials & Methods

Nanoformulation of ICG-J aggregates and Calix[4]arene:



Calix[4]arene



ICG-J/CX precipitation

Nanoprecipitation

Cell culture:

FaDu human head and neck squamous carcinoma cells were maintained in RPMI-1640 medium supplemented with 9% foetal bovine serum and 1% glutamine (37°C, 5% CO₂ in humidified atmosphere).

Results

Photophysical characteristics

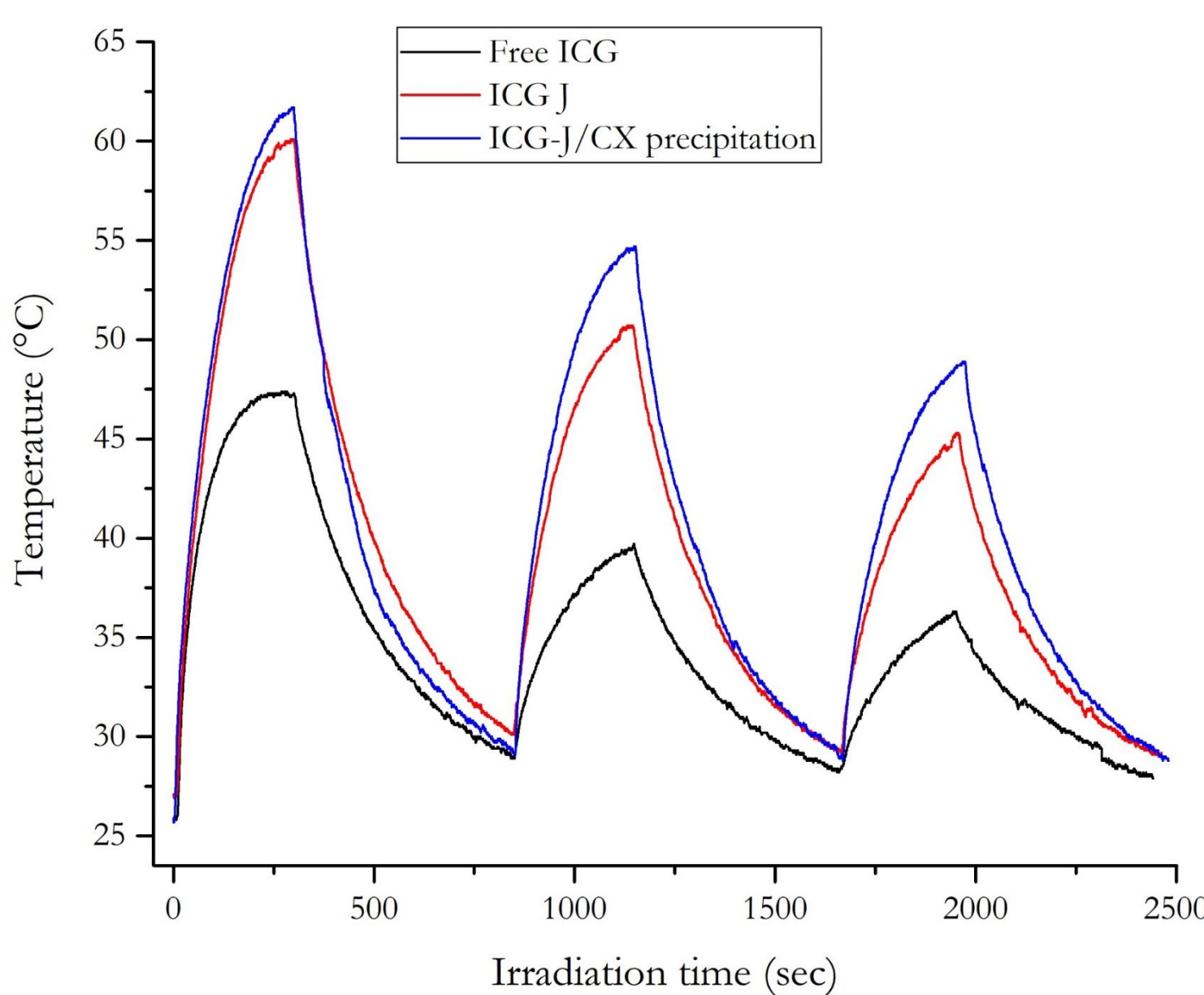
	Free ICG	ICG-J	ICG-J/CX precipitation
Hydrodynamic diameter (nm)	N.A	106 ± 8	135 ± 8
Zeta potential (mV)	N.A	-24 ± 6	-2 ± 5
Stability in time	< 7 days	< 30 days	> 30 days
λ_{max} (nm)	780	890	885

Table 1: Physical characterization of ICG formulations.

The hydrodynamic diameter and zeta potential were determined using a Zetasizer ultra instrument (Malvern Instruments). The stability and λ_{max} were obtained from UV-vis-NIR spectra using a Lambda 1050 spectrophotometer (Perkin Elmer).

ICG-J/CX precipitation: **Increased stability** and particles with a **neutral zeta potential**

Photothermal properties in solution



- 1st cycle T_{max} attained (°C): **ICG-J/CX > ICG-J > free ICG**
- 3rd cycle: T_{max} remains **higher** compared to free ICG and ICG-J

ICG-J/CX precipitation: **Improved PTT properties**

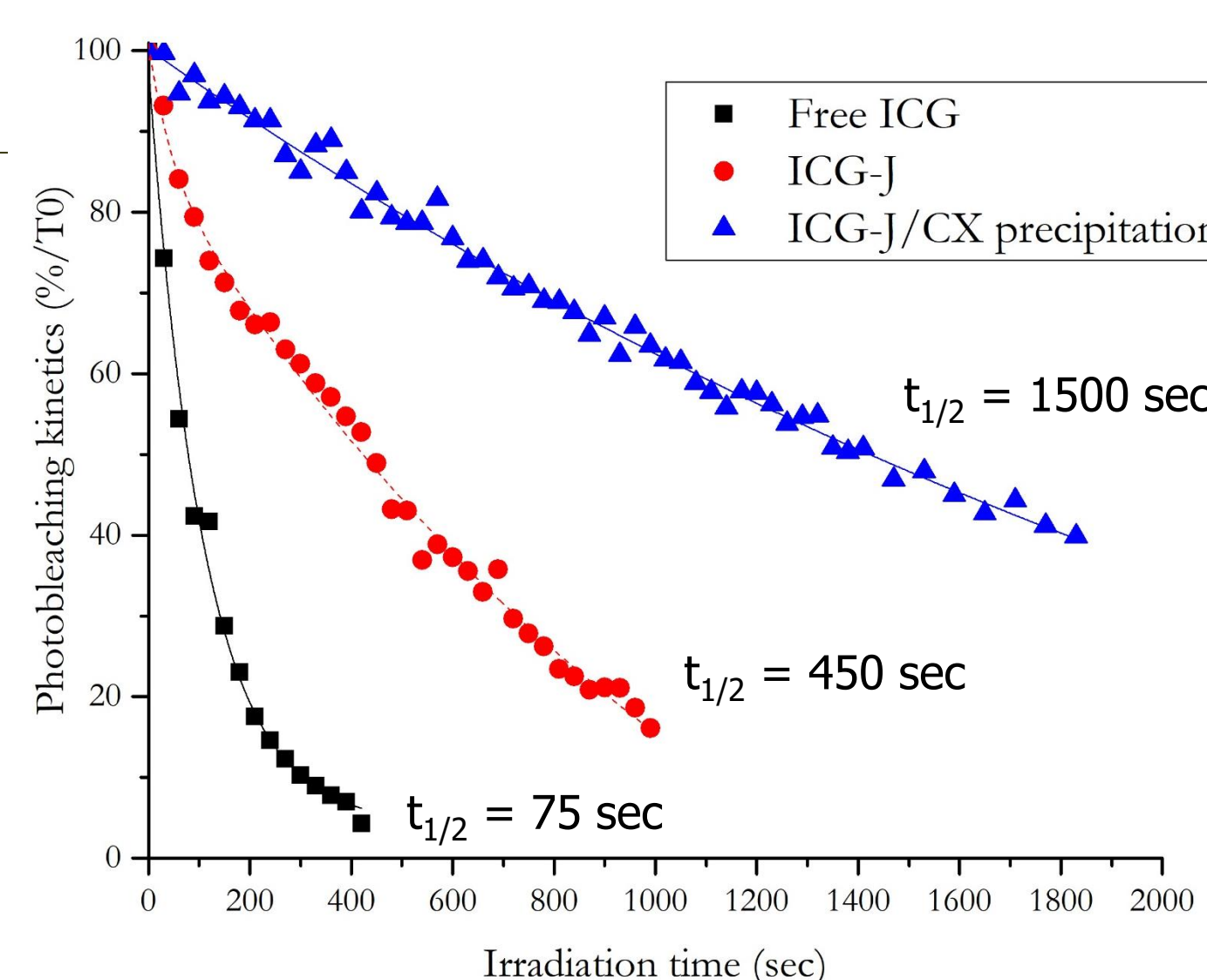
Figure 1: Photothermal production experiments. The solutions were prepared at 10μg/mL and irradiated at 808 nm - 1W/cm². The temperature was monitored in real-time. Three successive irradiation cycles were realized (300s laser ON - 600s laser OFF).

Photobleaching kinetics

Time to obtain 50% of photodegradation (t_{1/2}) with ICG-J/CX precipitation: **6 and 20-fold increase** compared to ICG-J and free ICG

ICG-J/CX precipitation: **Reduced photobleaching**

Figure 2: Photobleaching kinetics after irradiation. The solutions were prepared at 10μg/mL and irradiated at 808 nm - 1W/cm². Absorbance decay was recorded every 30 sec and the area under the curve was calculated between 550 and 1000 nm. Results were normalized to the spectrum obtained at T₀.



Dark cytotoxicity

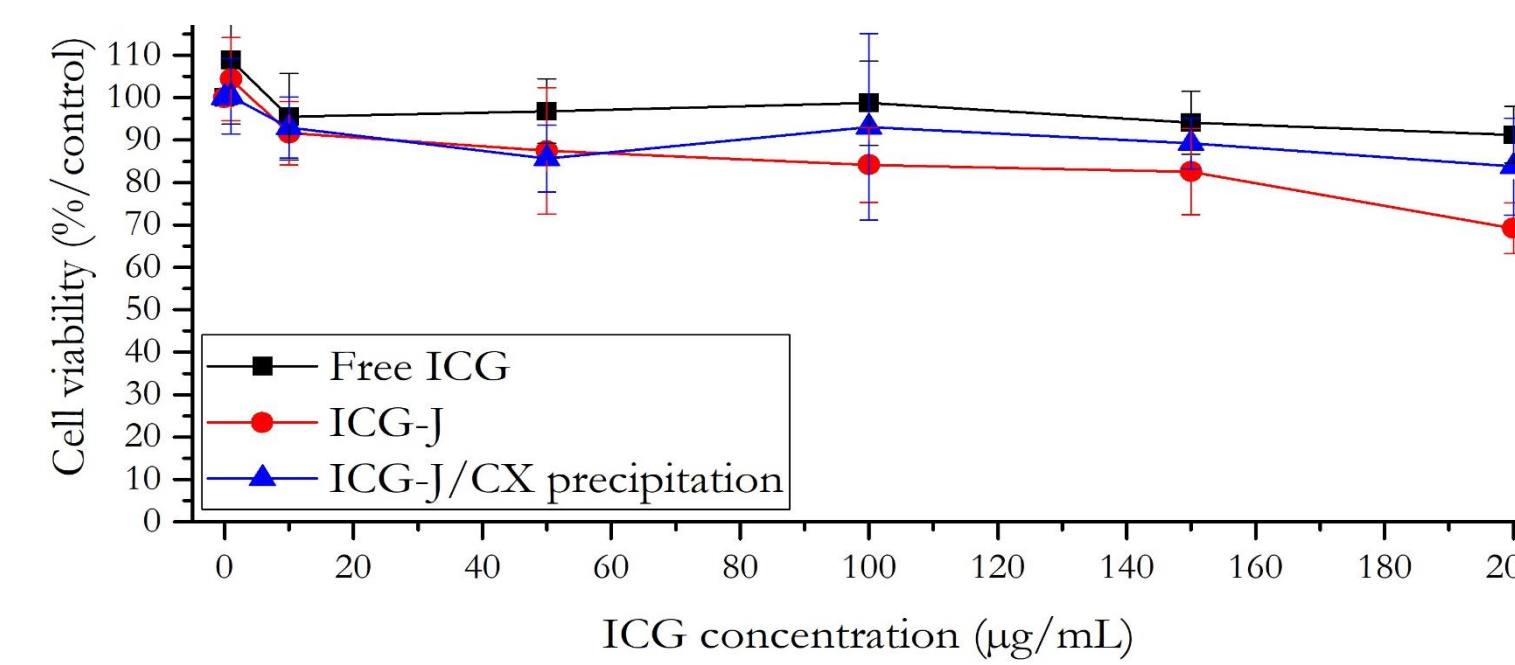
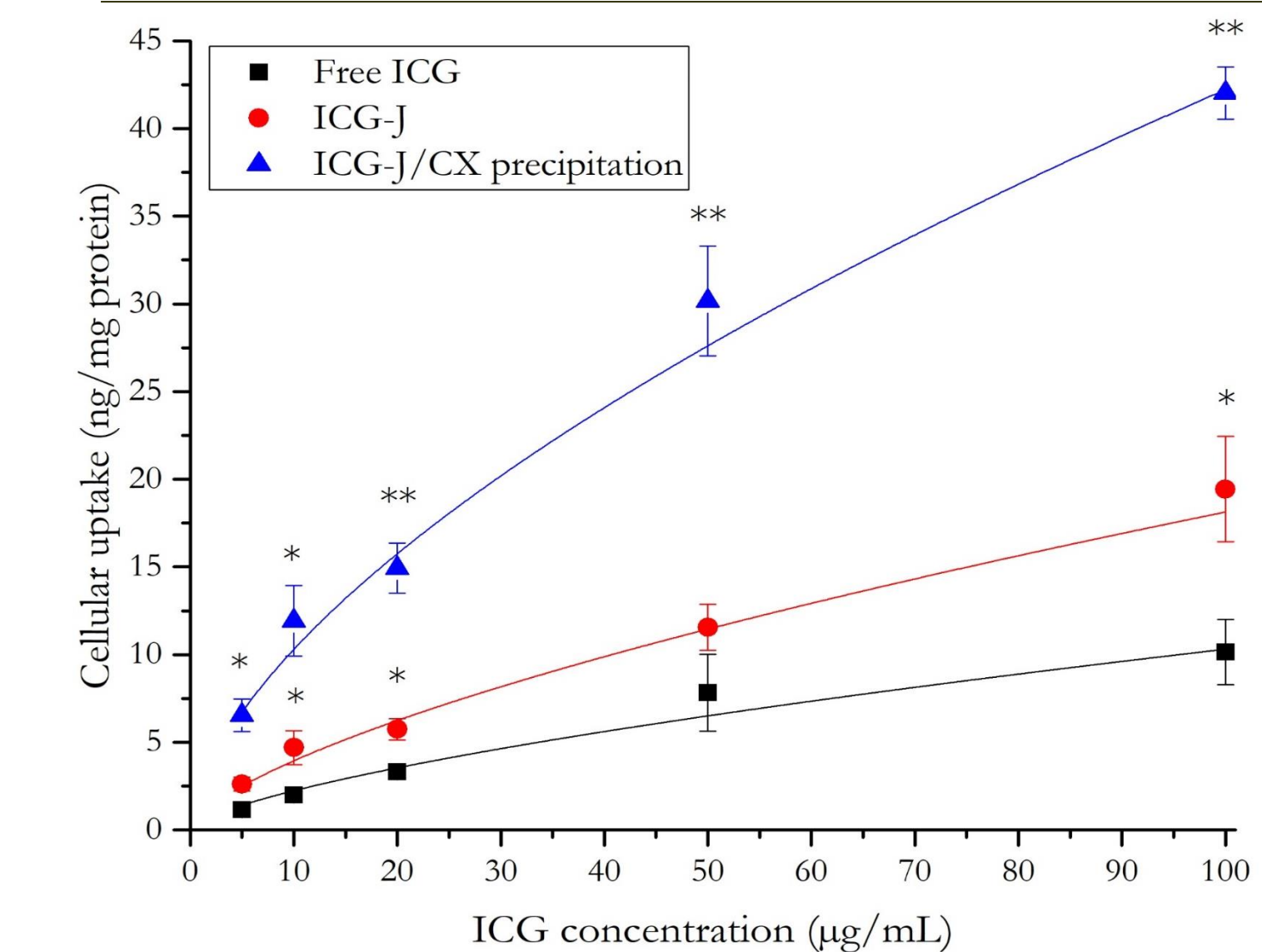


Figure 3: Toxicity in dark in FaDu cells. The cells were incubated 24h with various concentration of ICG formulations (0 to 200 μg/mL). Crystal violet assays were performed to determined cell viability. Results are expressed as mean ± S.D.

Up to 200 μg/mL, **less than 10% toxicity** was observed after incubation with free ICG and ICG-J/CX precipitation compared to ICG-J which reaches 30% toxicity at this concentration

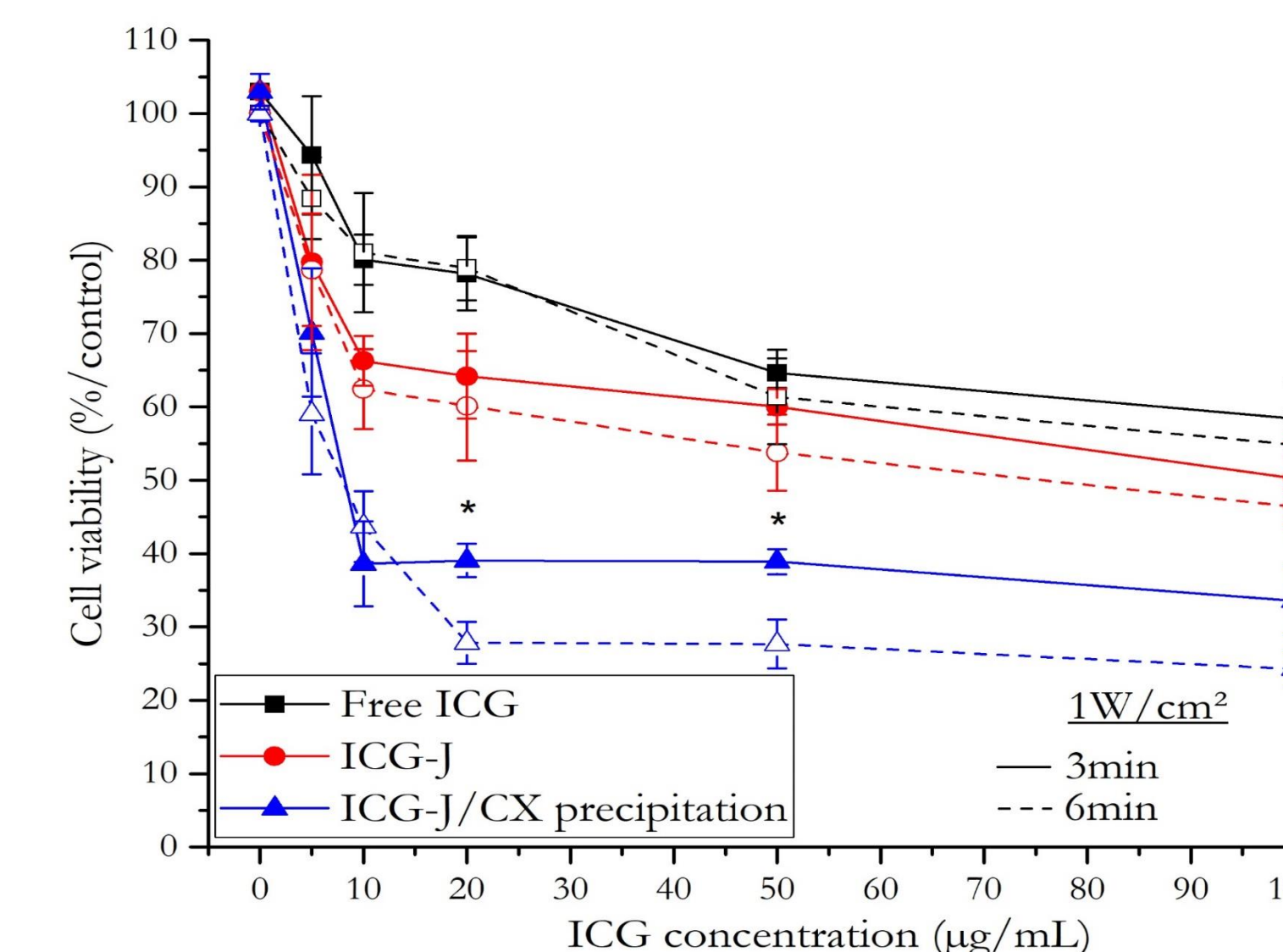
ICG uptake



Uptake of ICG-J/CX precipitation: **2.3-fold increase** compared to ICG-J and **5-fold increase** compared to free ICG

Figure 4: Cellular uptake in FaDu cells. The cells were treated 24h with ICG formulations (0 to 100 μg/mL). Absorbance was measured at 780 nm from cells extracts and ICG concentration was evaluated by absorbance calibration curves. Results were normalized to protein quantity and expressed as mean ± S.D. *: p < 0.05; **: p < 0.01.

Photothermal efficiency



Similar **PTT efficiency** were obtained for free ICG and ICG-J after 3 and 6min of irradiation

PTT efficiency was significantly **increased** for ICG-J/CX precipitation. IC50 was up to **10 times less** than ICG-J

Figure 5: Cellular viability after PTT in FaDu cells. The cells were treated 24h with formulations (0 to 100μg/mL). PTT was delivered at 1 W/cm² for 3 or 6 min. After 24h, the cellular viability was determined by crystal violet assay. Results are normalized to those obtained from control groups (no drug, no light) as mean ± S.D. *: p < 0.05.

Conclusions & Perspectives

Our new ICG-J/CX precipitation showed improved stability, uptake & photothermal efficiency. These results can be explained by the decreased photobleaching and the stabilization by serum proteins.

The promising results obtained in monolayer cells with our new ICG-J/CX construct need to be consolidated in more clinically relevant models (multicellular tumor spheroids and preclinical models).