LIPIDIC CUBIC-PHASE NANOPARTICLES (CUBOSOMES) AS CARRIERS FOR DOXORUBICIN AND SHORT-LIVED RADIONUCLIDE FOR COMBINATION CANCER TREATMENT

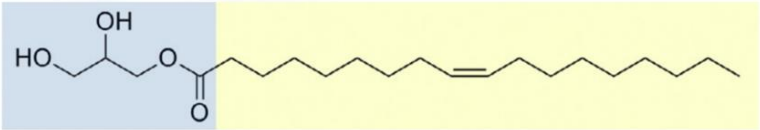
Adrianna Cytryniak,[a] Ewa Nazaruk,[a] Agnieszka Majkowska-Pilip,[b] Aleksander Bilewicz,[b] Renata Bilewicz [a]

[a]University of Warsaw, Faculty of Chemistry, Warsaw, Poland
[b]Institute of Nuclear Chemistry and Technology, Warsaw,

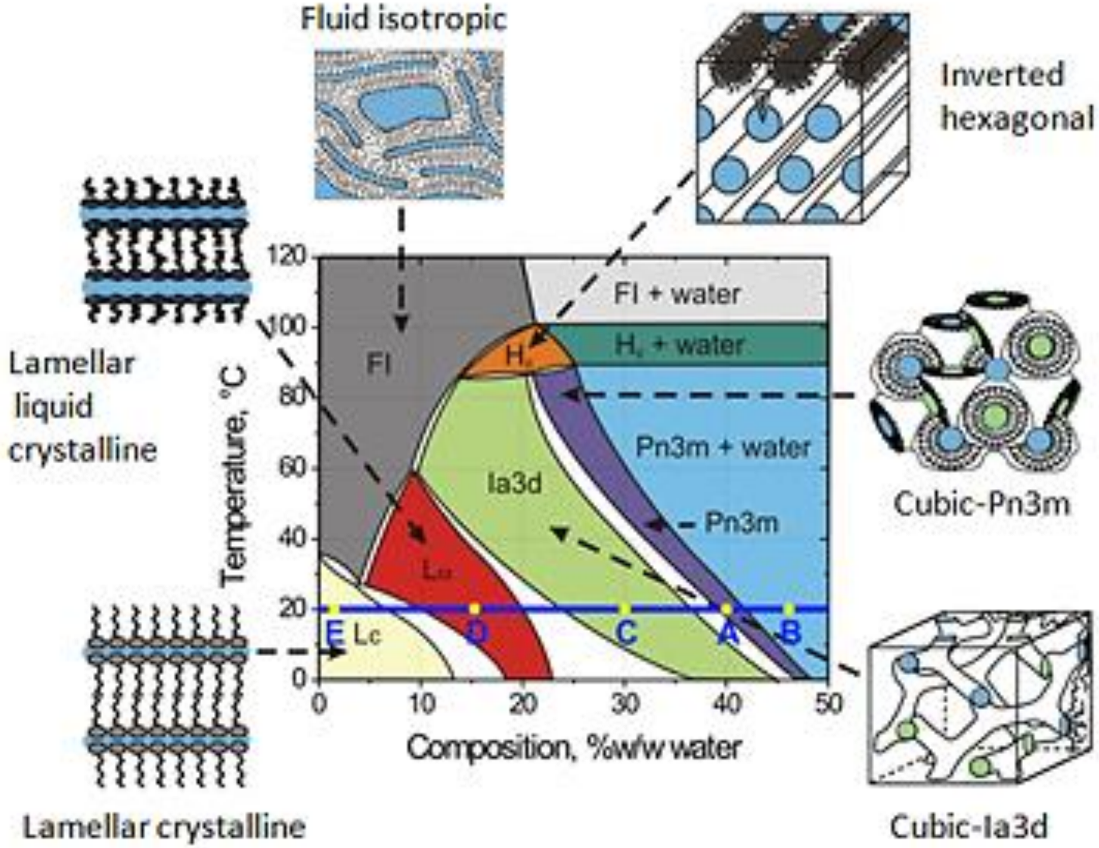
acytryniak@chem.uw.edu.pl

Fundusze Europejskie Wiedza Edukacja Rozwój
Rzeczpospolita Polska
Unia Europejska Europejski Fundusz Społeczny
Projekt realizowany w ramach Programu Operacyjnego Wiedza Edukacja Rozwój 2014-2020 współfinansowanego ze środków Europejskiego Funduszu Społecznego

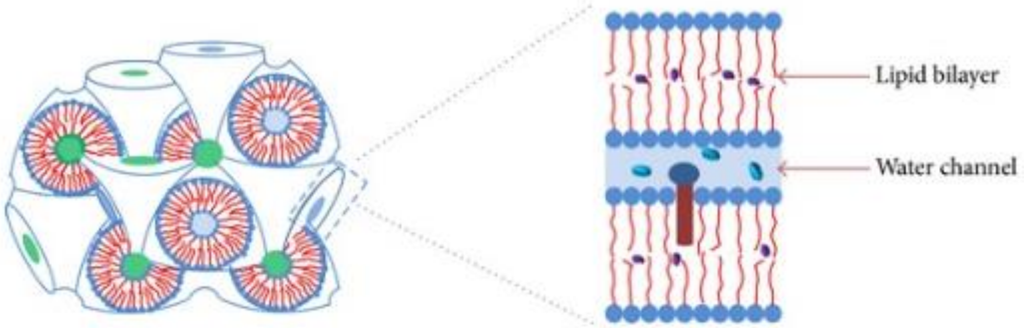
Cubic phases



Structure of monoolein (GMO)



Monoolein-water phase diagram



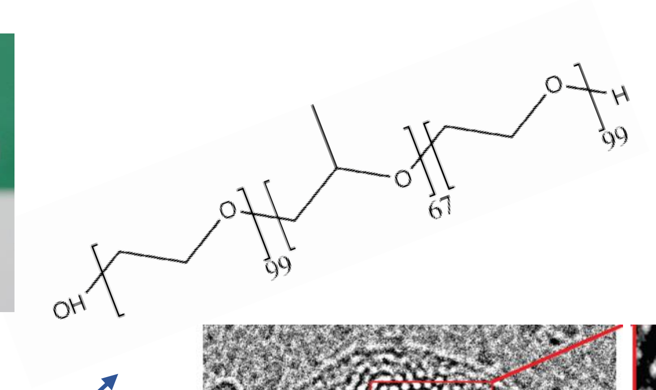
Properties of the lipid cubic mesophases (LCPs)

- ✓ Thermodynamic stability in excess of water (Pn3m)
- ✓ High internal surface area ($400 m^2/g$)
- ✓ Possibility to control water channel dimensions
- ✓ Ability to incorporate both hydrophobic and hydrophilic drug molecules
- ✓ Ability to control drug release

Caffrey, M. *Biochemical Society Transactions*, 39, 2011, 725-732

Cubosomes

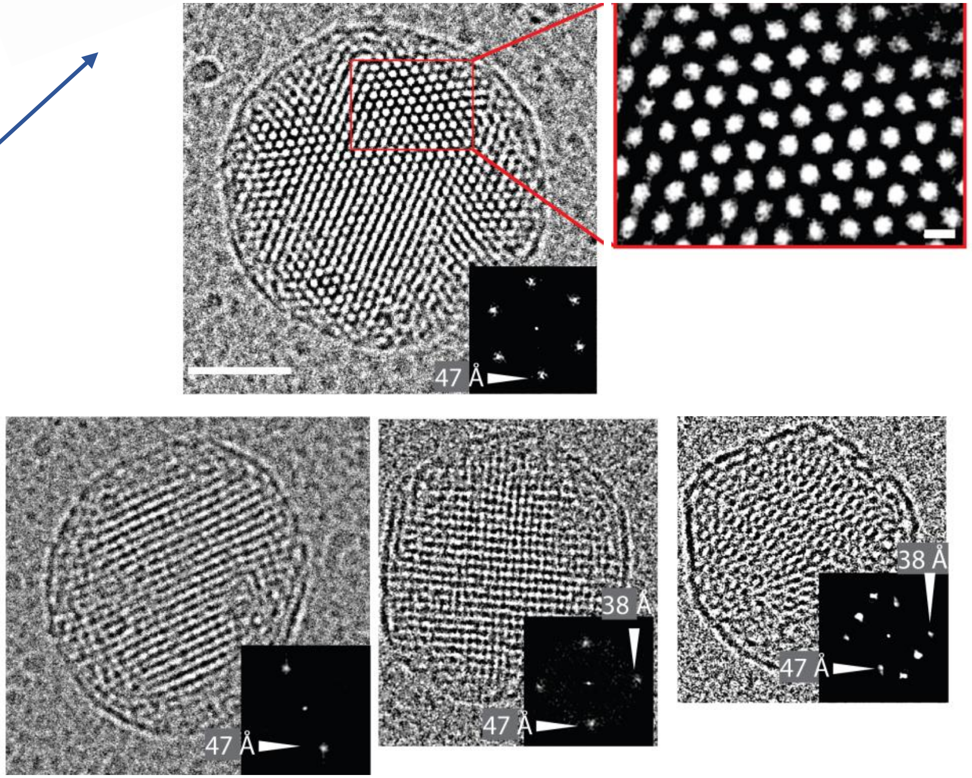
Cubic phase



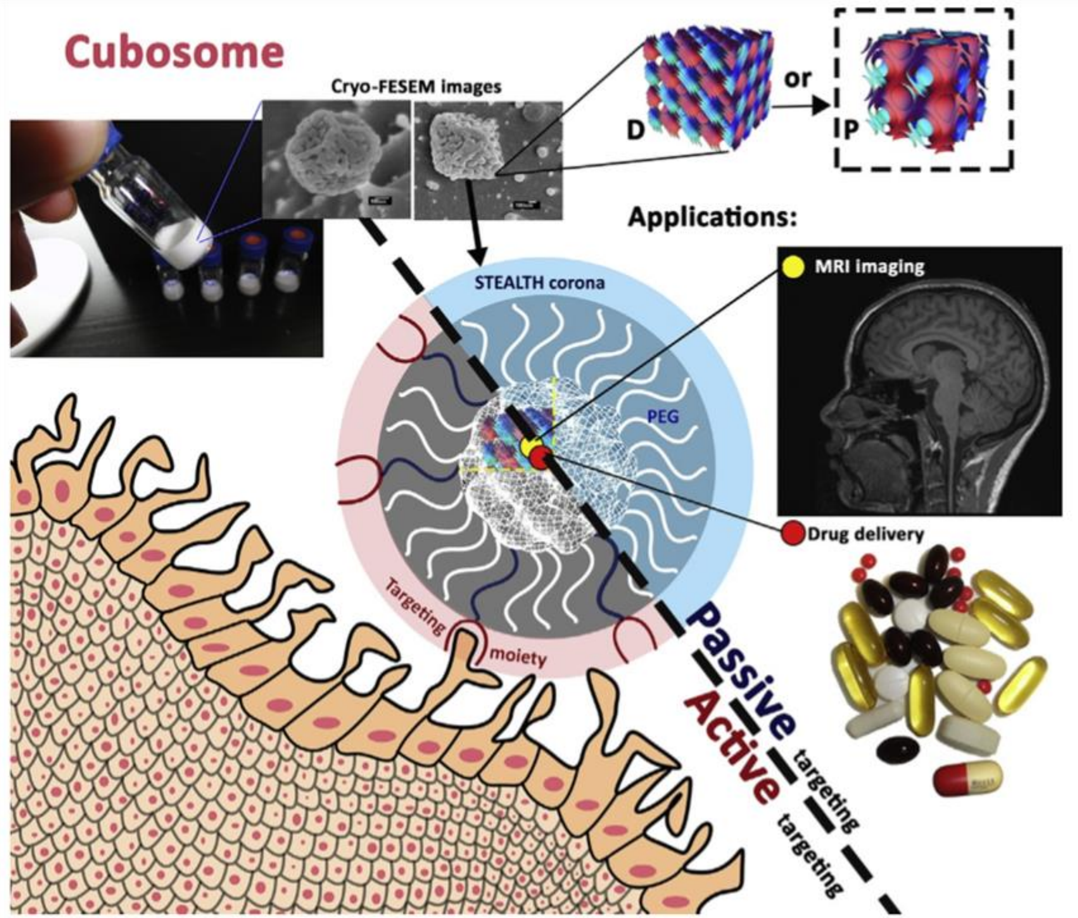
Homogenization

Pluronic F-127

Cubosomes



cryo-TEM images of cubosomes



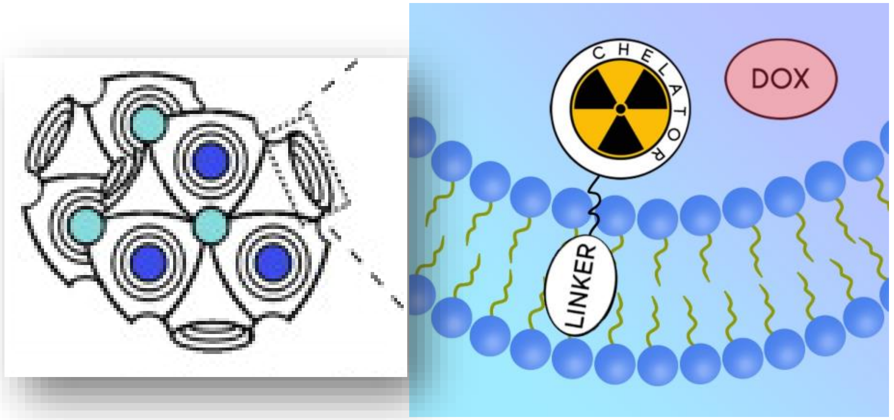
Conceptualised advanced drug delivery carrier

Alvarez-Malmagro, J.; Matyszewska, D.; Nazaruk, E.; Szwedziak, P.; Bilewicz, R. *Langmuir, 35*, 2019, 16650-16660

Mulet, X.; Boyd, B.; Drummond C. *Journal of Colloid and Interface Science*, 393, 2013, 1-20

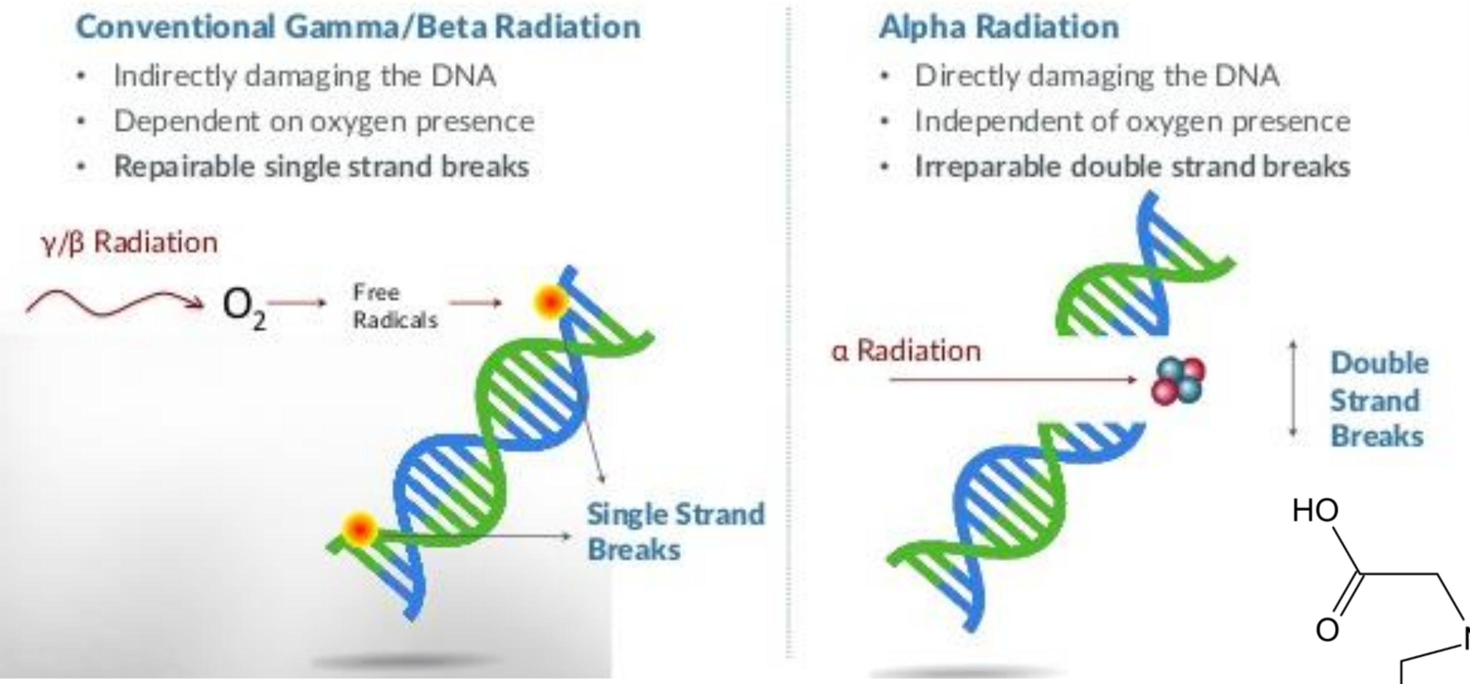
Aim of the research

- **Design and development of bimodal lipidic nanocarriers doped with chemotherapeutic and radionuclide for combined cancer treatment**

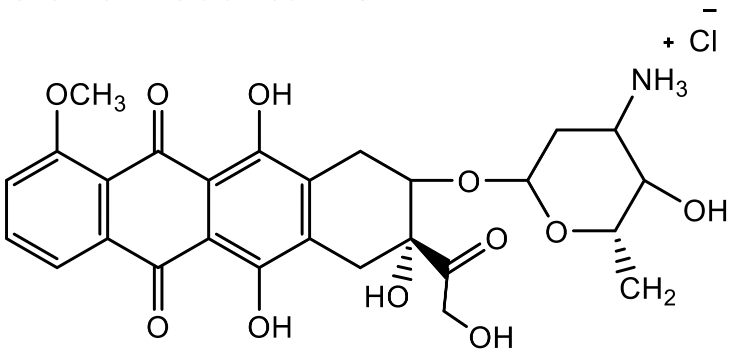


Scheme of bimodal carrier

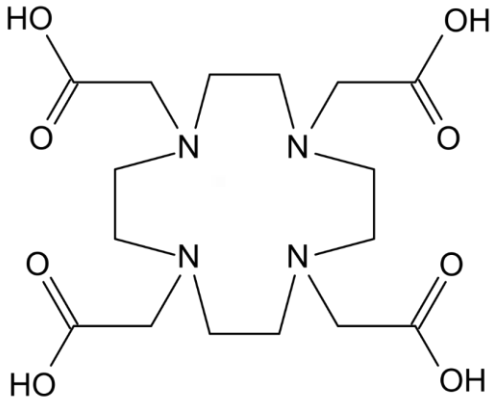
Targeted radionuclide therapy



Radiation interaction with DNA



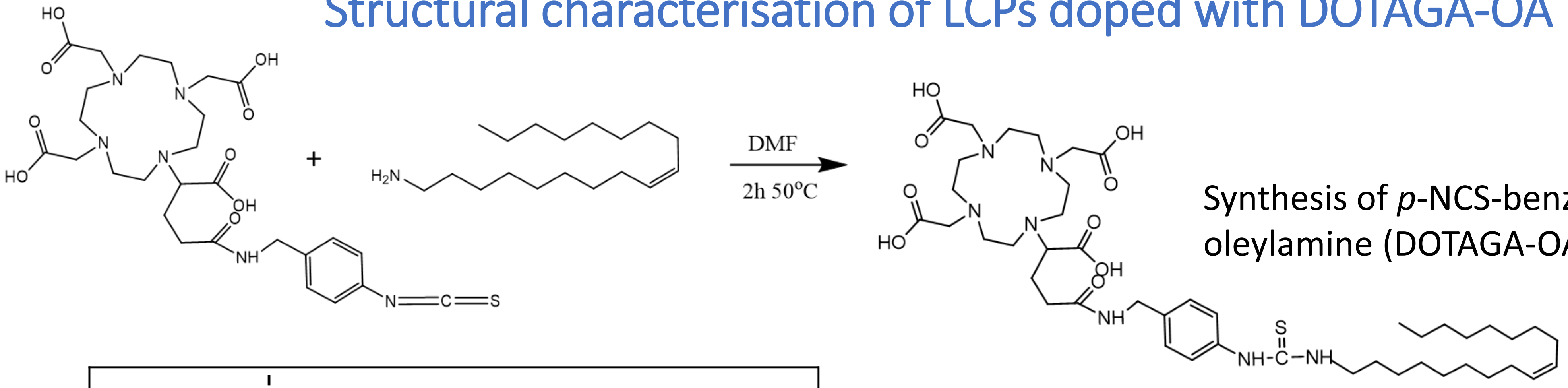
Structure of doxorubicin



Structure of DOTA

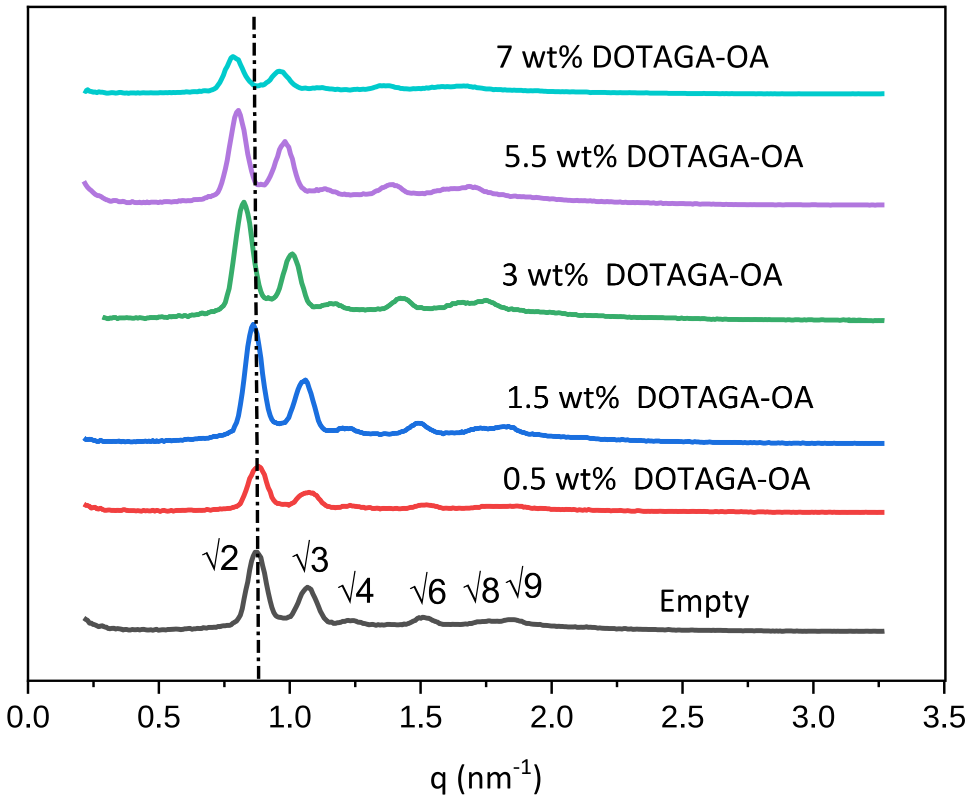
Advancing Nuclear Medicine Through Innovation, Committee on State of the Science of Nuclear Medicine, National Research Council, 2007

Structural characterisation of LCPs doped with DOTAGA-OA

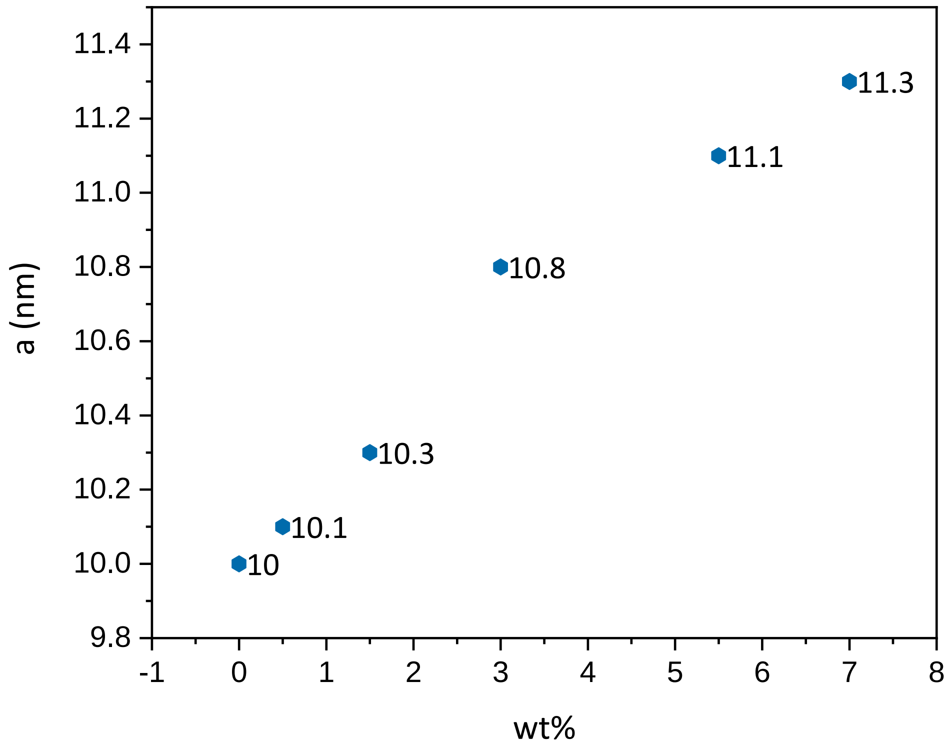


Synthesis of *p*-NCS-benzyl-DOTA-GA-oleylamine (DOTAGA-OA)

Synthesis: dr Adam Mames, ICHF

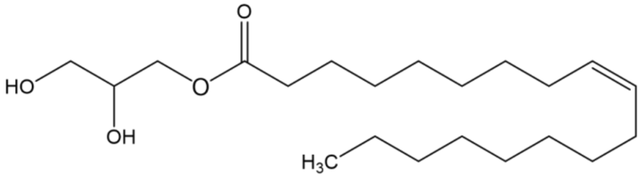


SAXS diffraction patterns obtained for LCPs doped with different amounts of DOTAGA-OA



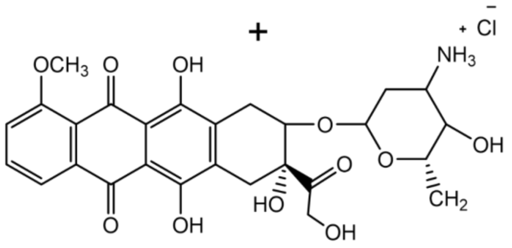
Unit cell (a) dependence of weight percent of DOTAGA-OA in LCPs

Preparation and structural characterization of cubic phases doped with DOX and DOTAGA-OA



Monoolein (58.1 wt%)

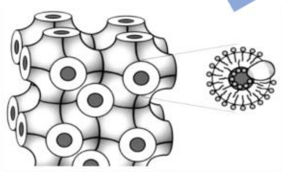
+



Doxorubicin (0.2 wt%)

+ DOTAGA-OA (1.9 wt%)
+ Buffer (39.8 wt%)

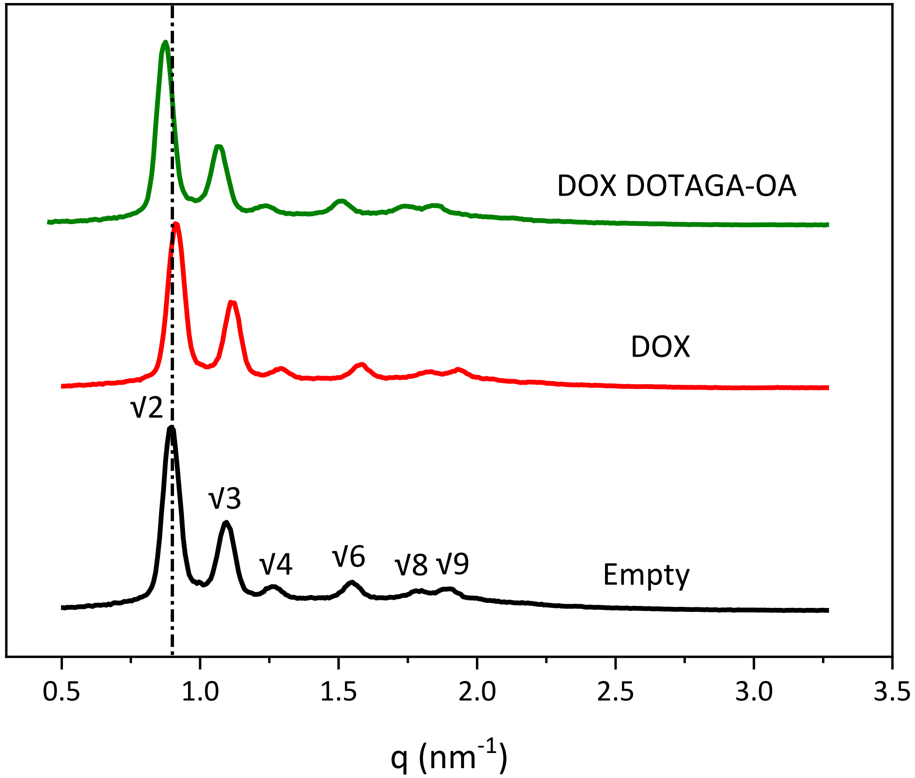
Self-assembly



Cubic phase doped with DOX and DOTAGA-OA

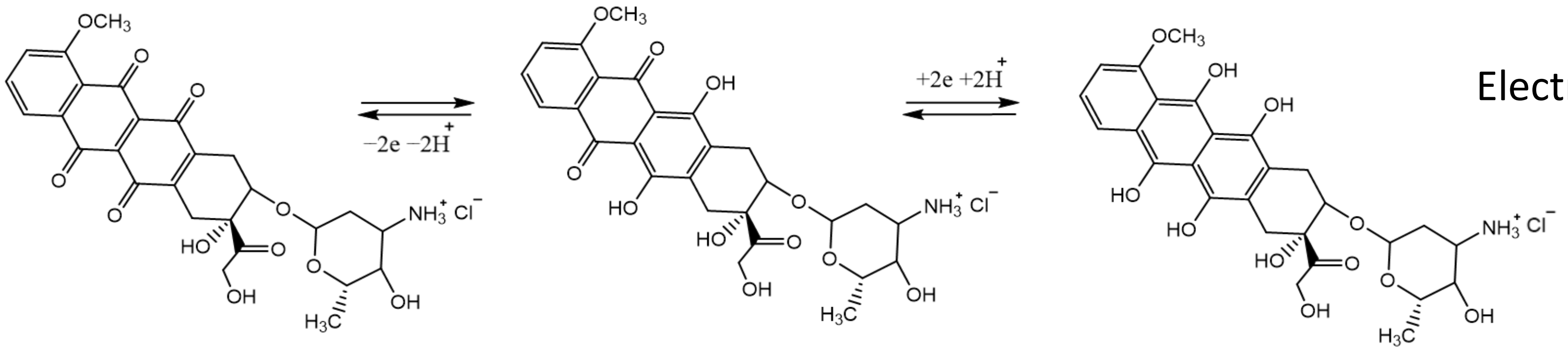
Phase	Symmetry	a (nm)	d_w (nm)
Empty	Pn3m	10.0	4.5
DOX	Pn3m	9.8	4.4
DOX DOTAGA-OA	Pn3m	10.1	4.7

Results of SAXS measurements for the LCPs: phase symmetry, lattice parameter a, water channel diameter d_w

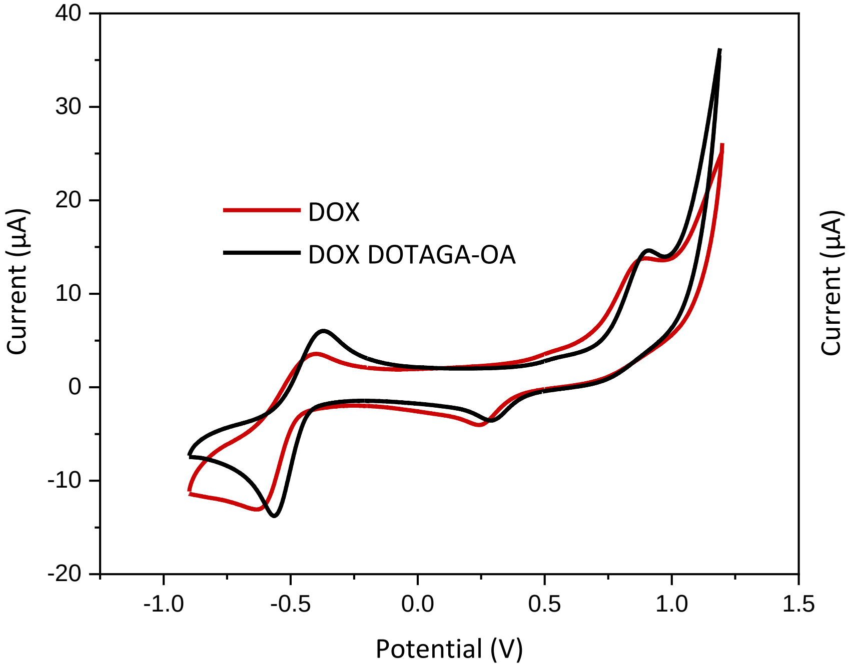


SAXS diffraction patterns obtained for the LCPs

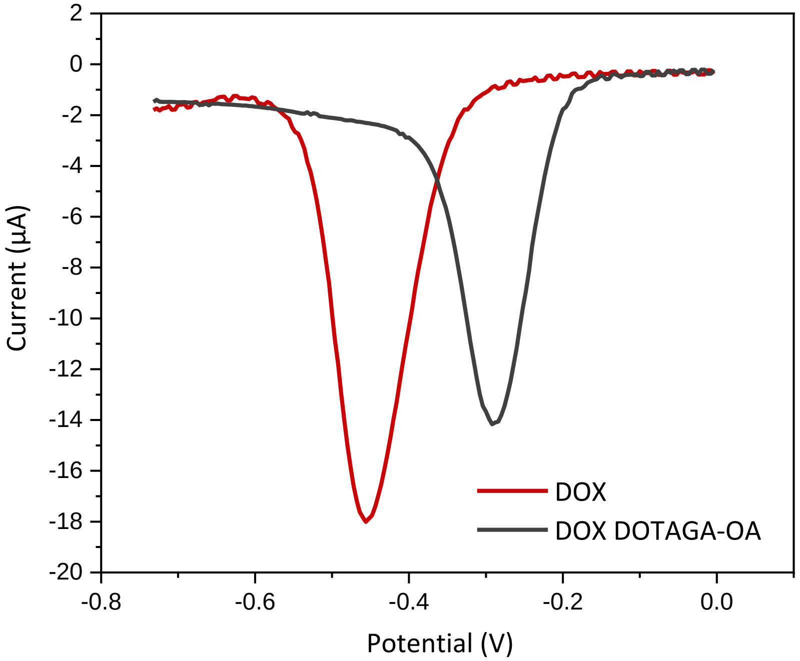
Electrochemical characterisation of DOX



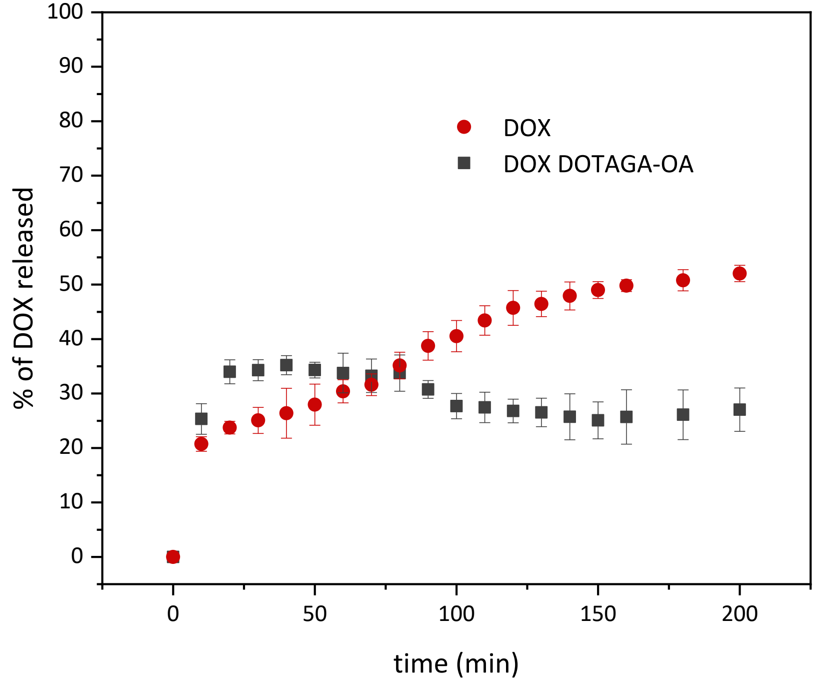
Electrode process of DOX



CV for DOX incorporated into cubic phases with or without DOTAGA-OA dopant. Scan rate: 100 mVs −1 , pH 5.5



DPV for DOX incorporated into cubic phases. Amplitude: ΔE=50 mV, pulse time: tp=50 ms



Release profiles of DOX-containing mesophases

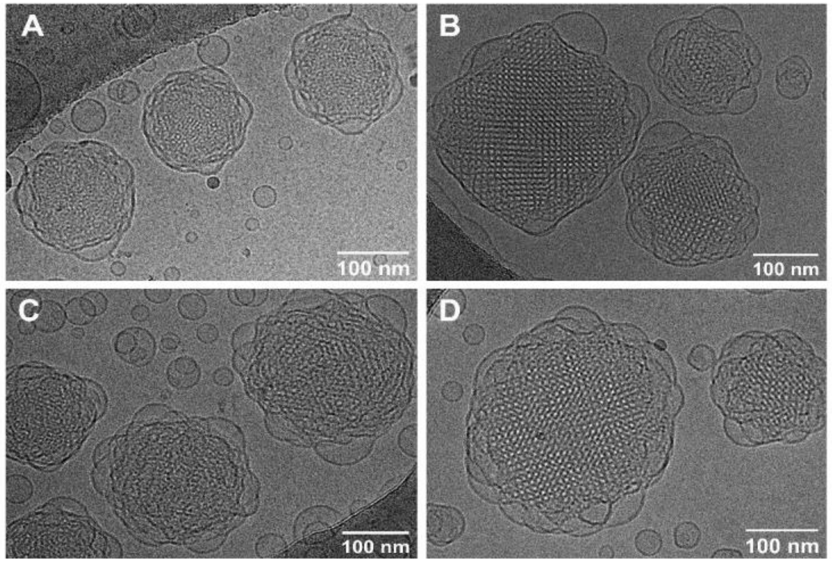
Formation and structural characterization of cubosomes doped with DOX and DOTAGA-OA

Cubic phase doped with DOX and DOTAGA-OA

Pluronic F-127

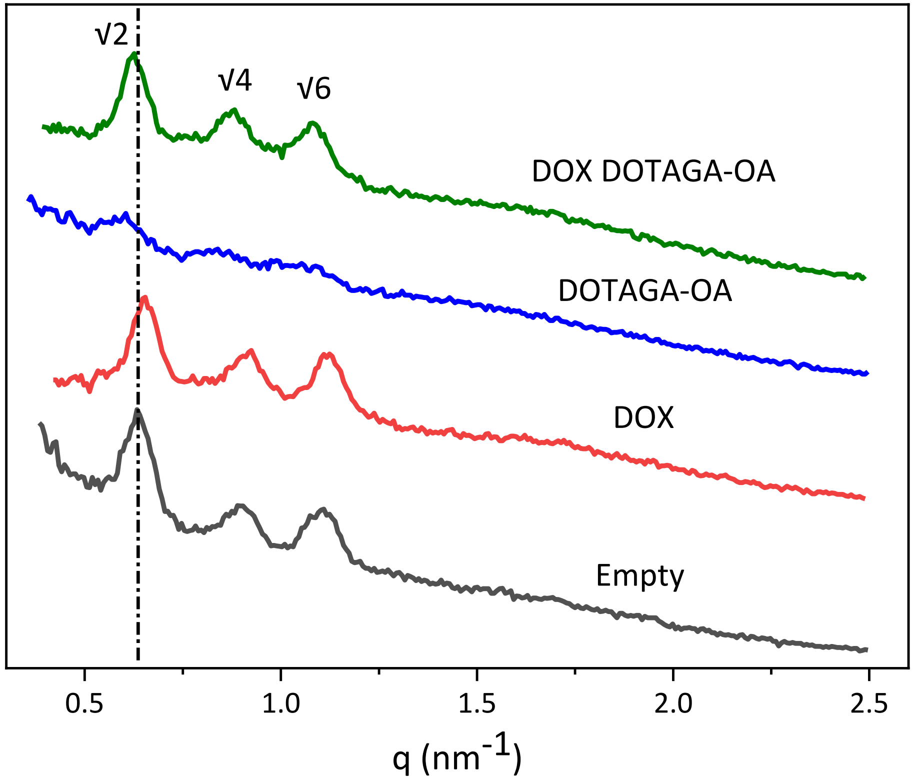
Homogenization

Cubosomes doped with DOX and DOTAGA-OA



cryo-TEM images of (A) empty cubosomes, (B) cubosomes doped with DOX, (C) DOTAGA-OA and (D) DOX and DOTAGA-OA.

The cryo-TEM imaging was conducted by dr Tomasz Góral at the Center of New Technologies, University of Warsaw, Poland.

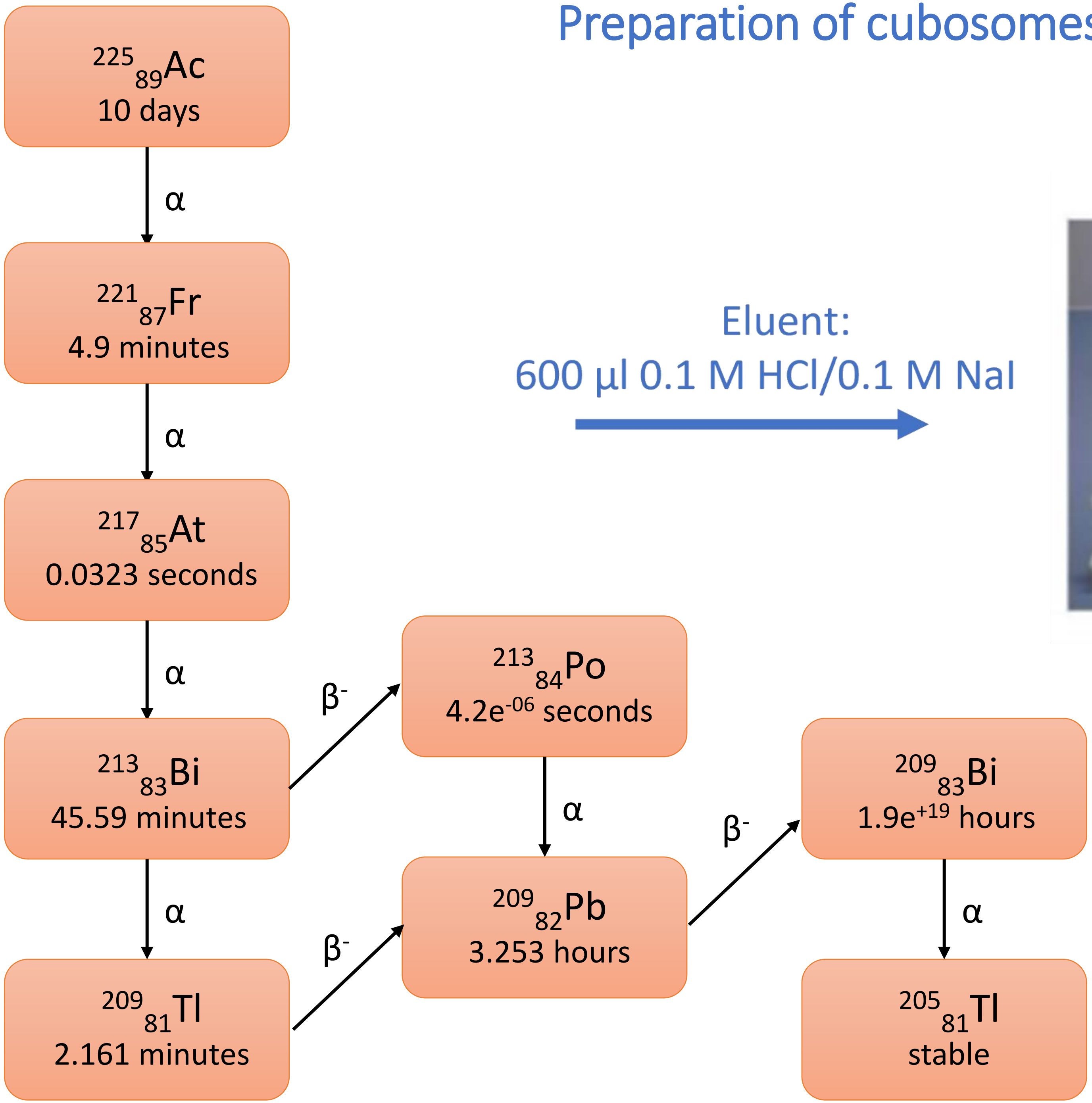


Diffraction patterns of cubosomes formulations

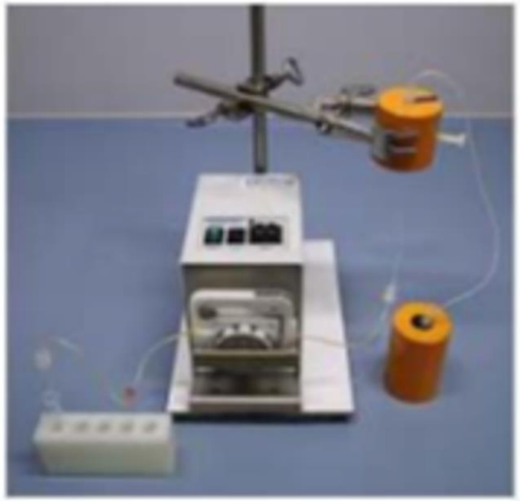
Cubosomes	Symmetry	a (nm)	Size (nm)	PDI	Zeta Potential (mV)
Empty **buffer/GMO/F-127** **94.62/4.84/0.54 wt%**	Im3m	14.0	140 ± 5	0.18 ± 0.02	-29 ± 0.9
DOX **buffer/GMO/DOX/F-127** **94.58/4.86/0.02/0.54 wt%**	Im3m	13.7	160 ± 10	0.19 ± 0.01	-24 ± 0.4
DOTAGA-OA **buffer/GMO/DOTAGA-OA/F-127** **94.45/4.85/0.16/0.54 wt%**	Im3m	14.7	130 ± 15	0.12 ± 0.02	-20 ± 0.6
DOX DOTAGA-OA **buffer/GMO/DOX/DOTAGA-OA/F-127** **94.44/4.84/0.02/0.16/0.54 wt%**	Im3m	14.2	150 ± 12	0.13 ± 0.03	-17 ± 0.8

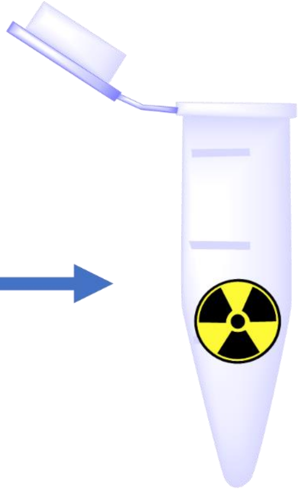
Properties of cubosomes formulations determined using SAXS and DLS

Preparation of cubosomes labeled with ^{213}Bi



Eluent:
600 µl 0.1 M HCl/0.1 M NaI

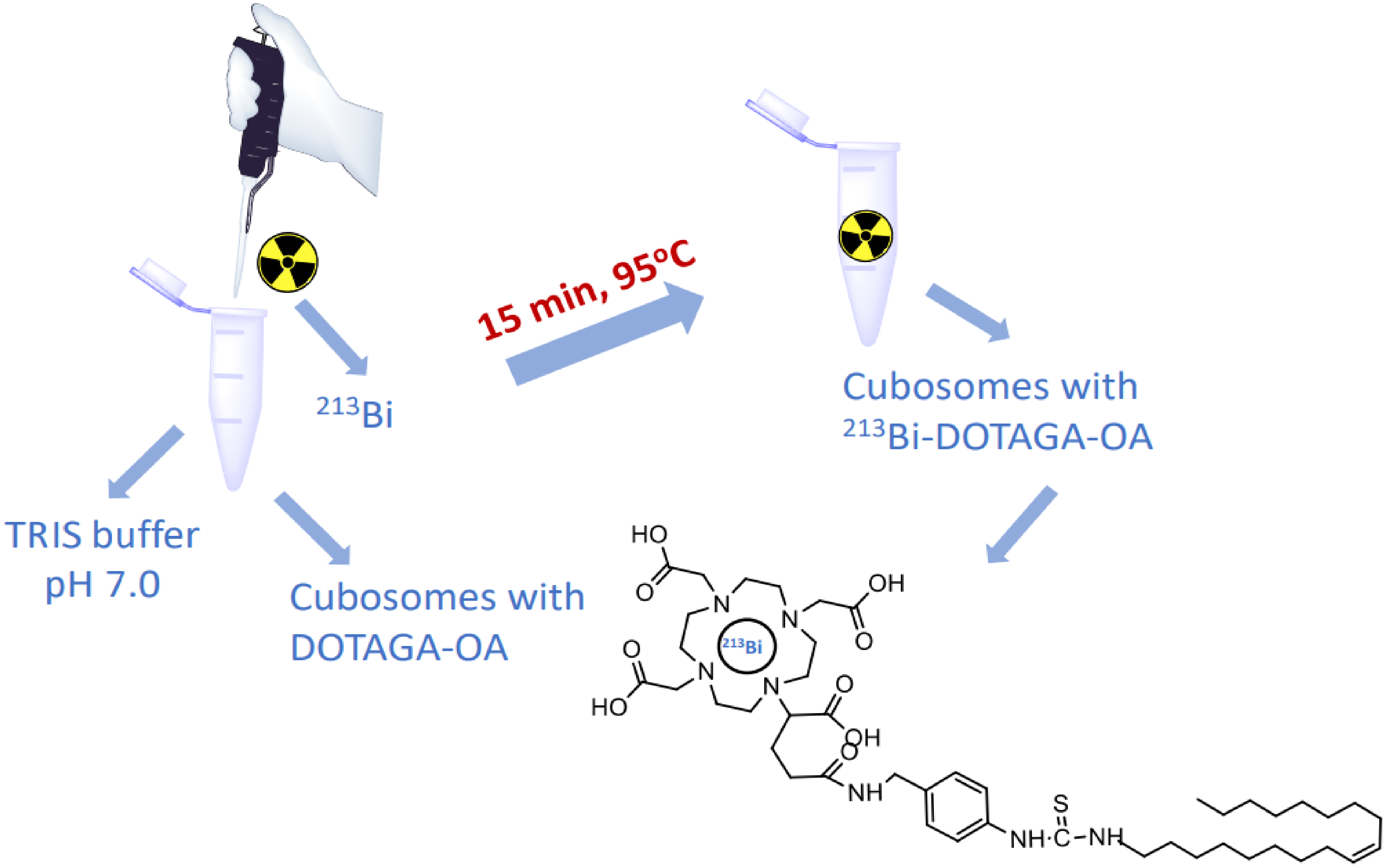




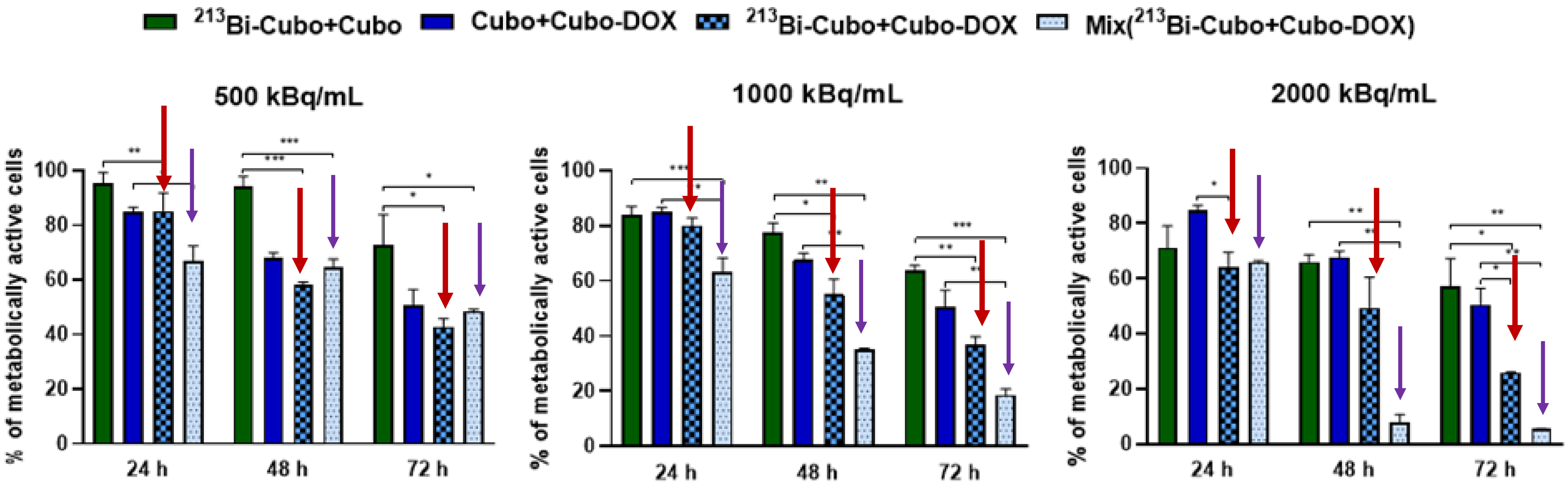
BiI_4^- / BiI_5^{2-}

$^{225}Ac/^{213}Bi$ Radionuclide Generator

Preparation of cubosomes radiolabeled with ^{213}Bi



Cytotoxicity studies



Viability of HeLa cells treated with ^{213}Bi-Cubo+Cubo, Cubo+Cubo-DOX, ^{213}Bi-Cubo+Cubo-DOX, Mix after 24 h, 48 h and 72 h of incubation. ^{213}Bi-Cubo+Cubo and Cubo+Cubo-DOX were used as a control.

- ✓ The MTS assay was used to evaluate the in vitro cytotoxicity of the cubosomes doped with ^{213}Bi and with DOX on HeLa cells
- ✓ The best procedure involved first irradiation of the cells and next exposure to the chemotherapeutic
- ✓ The enhancement of cytotoxicity achieved by combining doxorubicin and complexed ^{213}Bi treatments was observed

Summary

- ✓We prepared a new dopant: *p*-NCS-benzyl-DOTA-GA-oleylamine (DOTAGA-OA) which forms an inert complex with ^{213}Bi and can be accommodated in the cubosome in a stable way
- ✓We prepared cubic phases and cubosomes with DOX and DOTAGA-OA and characterized their structure by SAXS, DLS and cryo-TEM
- ✓The release of DOX from the carrier was monitored by electrochemical methods. We found that the presence of DOTAGA-OA ligand in the cubic phase leads to the decrease of the rate of DOX release from the mesophase
- ✓ The MTS assay shows significant decrease of viability of HeLa cancer cells using the sequential cell exposure: first to the radiolabeled cubosomes containing ^{213}Bi complex and next to DOX-doped cubosomes (Cubo-DOX) on HeLa cancer cells. However, we find favorable to deliver both drugs simultaneusly but encapsulated in separate cubosomes

Conferences 2020/2021

- A. Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, "Kubosomy jako nośniki leków przeciwnowotworowych oraz radionuklidów", XVII Konferencja "Elektroanaliza w teorii i praktyce", 19.11-20.11.2020, short communication
- A.Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, "Bimodal cubosomes as carriers of chemotherapeutics and radionuclides", Online 10th International Colloids Conference (COLL2020), 07.12-09.12.2020, poster
- A. Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, "Cubosomes as nanocarriers for doxorubicin and short-lived radionuclide for cancer treatment", II Wirtualna Konferencja Naukowa Kampusu Ochota (WKNKO2), 20.09-21.09.2020, poster
- A. Cytryniak, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, E. Nazaruk, „Lipid liquid crystalline nanocarriers for chemotherapeutic and short-lived radionuclide for combination cancer therapy", 35th Conference of the European Colloid and Interface Society (ECIS), Ahens, 05.09–10.09.2021, poster
- A. Cytryniak, E. Nazaruk, A. Majkowska-Pilip, A. Bilewicz, R. Bilewicz, „Lipidic cubic-phase nanoparticles (cubosomes) as carriers for doxorubicin and short-lived radionuclide for combination cancer treatment", 10th International Workshop on Surface Modification for Chemical and Biochemical Sensing, 5.11- 9.11 2021, short communication

Publications 2020/2021

- **A. Cytryniak**, E. Nazaruk, R. Bilewicz, E. Górzyńska, K. Żelechowska-Matysiak, R. Walczak, A. Mames, A. Bilewicz, A. Majkowska-Pilip; Lipidic Cubic-Phase Nanoparticles (Cubosomes) Loaded with Doxorubicin and Labeled with 177Lu as a Potential Tool for Combined Chemo and Internal Radiotherapy for Cancers. Nanomaterials, 2020, 10, 2272 (RadFarm)

- M. Jakubec , D. Novák, M. Zatloukalová, I. Císařová, R. Cibulka, L. Favereau, J. Crassous, **A. Cytryniak**, R. Bilewicz, J. Hrbáč, J. Storch, J. Žádný, J. Vacek; Flavin-Helicene Amphiphilic Hybrids: Synthesis, Characterization, and Preparation of Surface-Supported Films, ChemPlusChem, 2021, 86, 982

- M. Zatloukalová, L. Jedinák, D. Riman, J. Franková, D. Novák, **A. Cytryniak**, E. Nazaruk, R. Bilewicz, J. Vrba, B. Papoušková, M. Kabeláč, J. Vacek, Cubosomal lipid formulation of nitroalkene fatty acids: Preparation, stability and biological effects, Redox Biology, 2021, 46.

- *Another in progress*

Internship

- Deutsches Krebsforschungszentrum (DKFZ), Junior Research Group Molecular Biology of Systemic Radiotherapy (group leader Dr Martina Benešová), Heidelberg, Germany (1 month, 15.01.2022 – 14.02.2022)