Folic acid functionalized, condensed magnetic nanoparticles for the selective delivery of doxorubicin to cancer cells overexpressing the folic acid receptor

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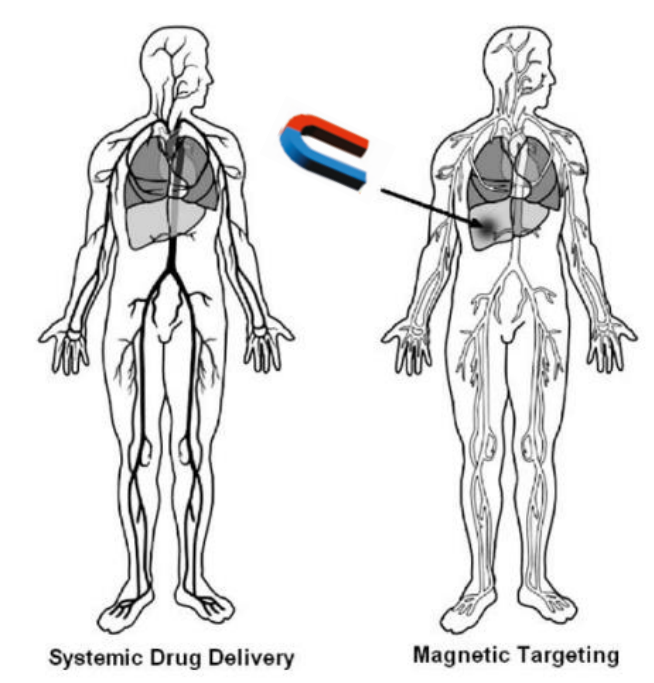
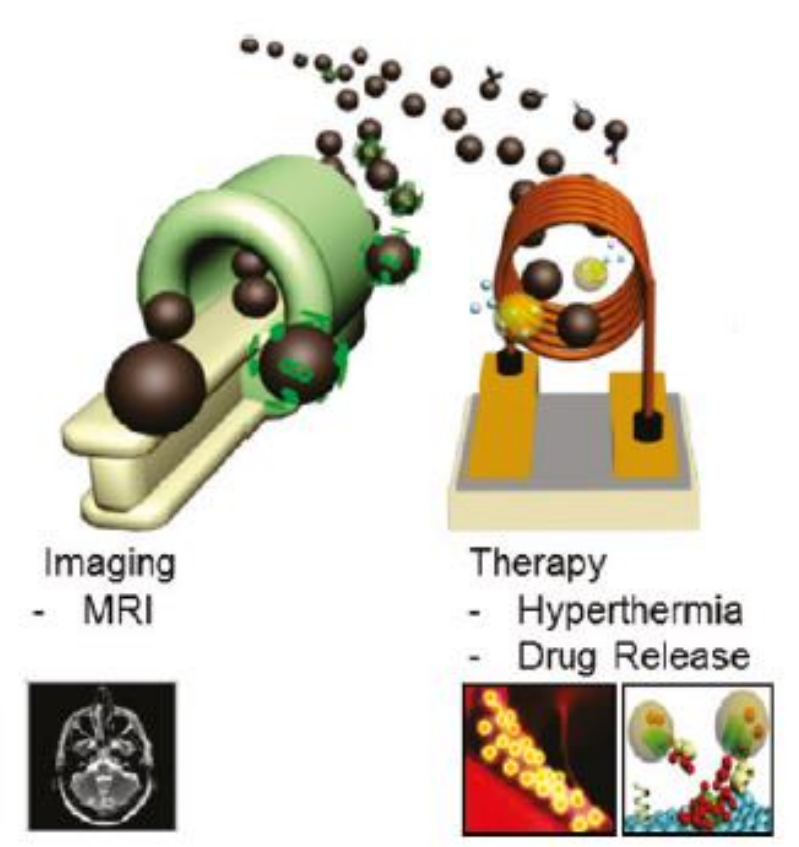
Magnetic Nanoparticles

- **Biocompatible**
- **Surface functionalization**
- **Multifunctional**





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Colloidal Nanocrystal Clusters (CNCs)

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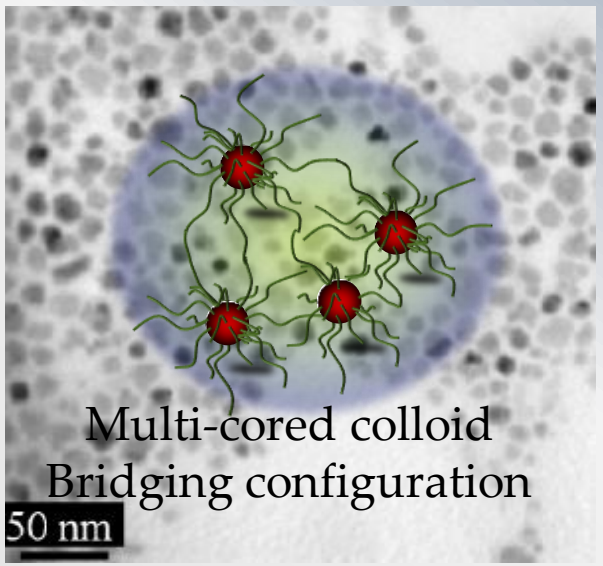
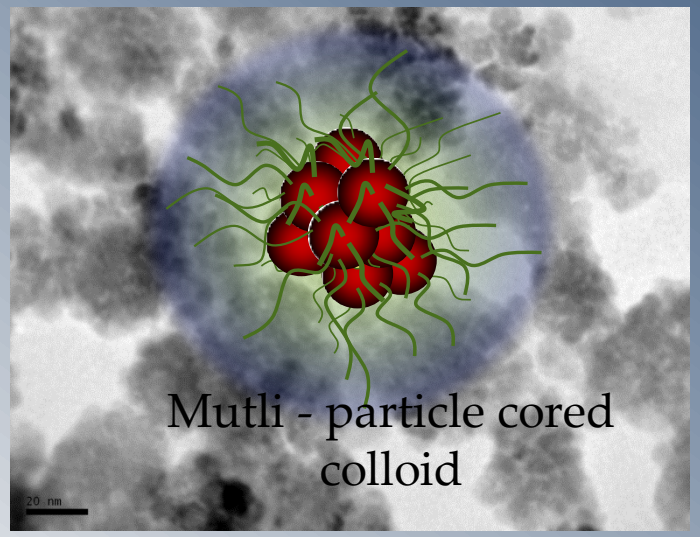
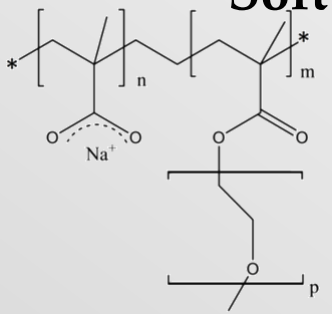


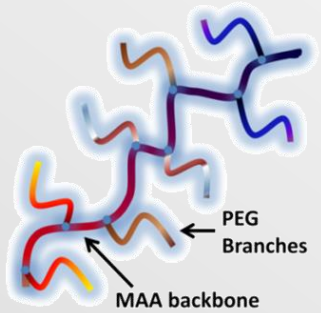
Multi-cored colloid
Bridging configuration



Mutli - particle cored colloid

Soft-CNCs

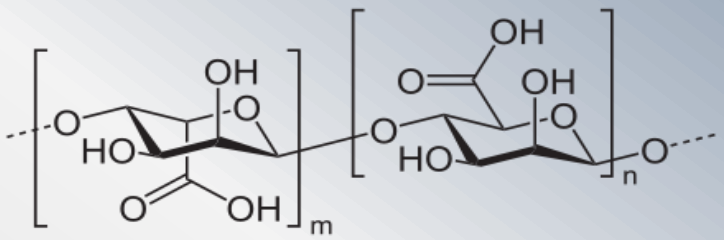




poly(methacrylic acid)-*graft*-poly(ethyleneglycol methacrylate)

A. Bakandritsos et al. Small **2012**, *8*, 2381.

Condensed-CNCs



Alginic Acid
Natural biopolymer

A. Bakandritsos et al., *Chem. Mater.*, DOI: 10.1021/cm404053v, (2014).

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Epitaxial Crystal Growth
Magnetic properties

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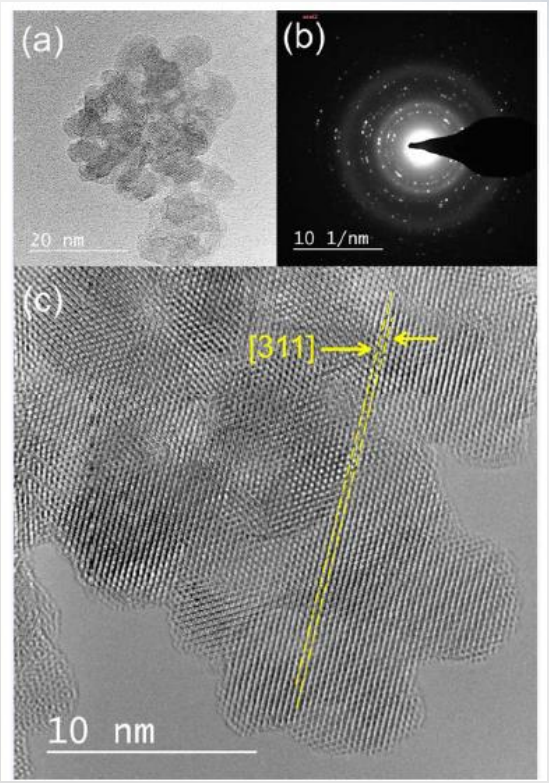
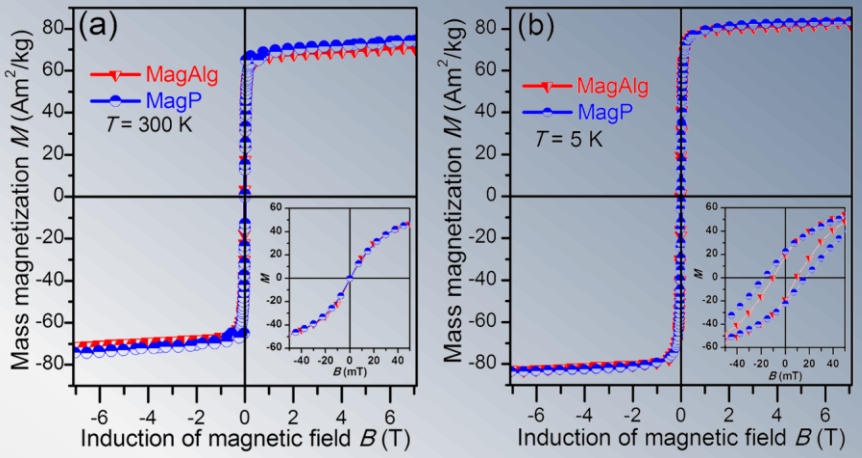
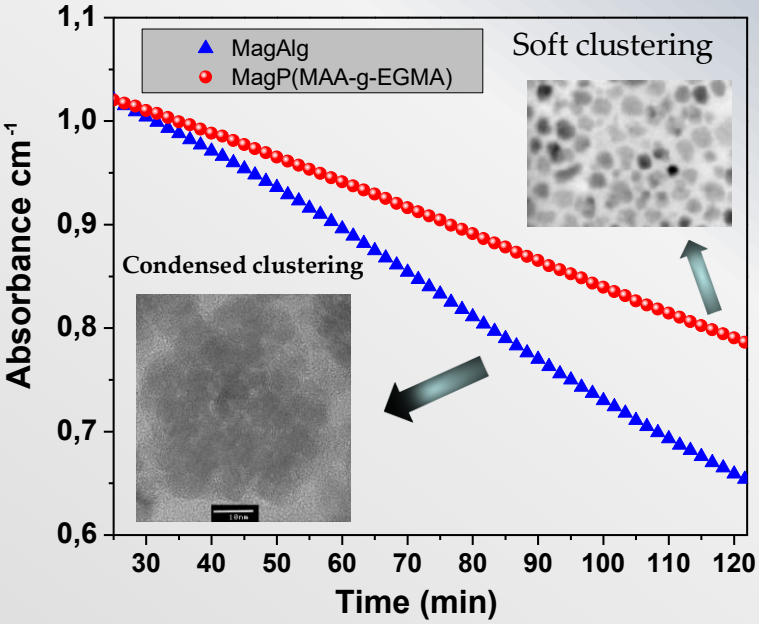


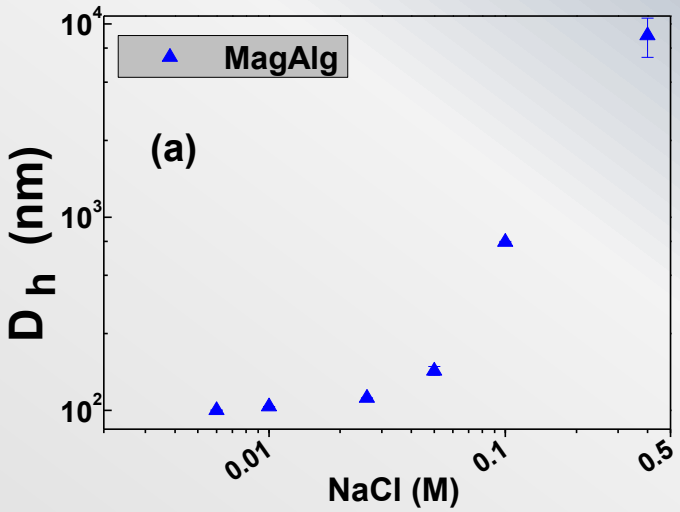
crystallographically aligned nanocrystallites

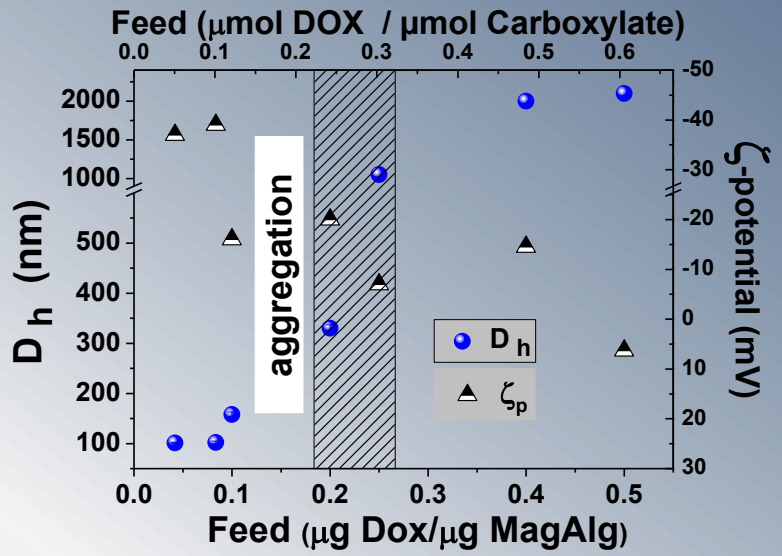




Sample	Polymer content (% wt)	T (K)	$M_{S\pm}$ (Am²/kg)	${}^{a}M_S^{Fe_2O_3}$ (Am²/kg)	B_C (mT)	M_R (Am²/kg)
MagP	11±3	5	83.5		17.0	22.4
		300	74.3	83.5	0	0
MagAlg	17±3	5	82.6		9.3	19.6
		300	71.1	85.6	0	0

Colloidal Stability





Medium	Size (nm)	PDI
RPMI 1640	400	0,431
Human Blood Plasma 50% v/v	147	0,453

Co-MIONs electrostatic stabilization quickly collapses even at low ionic strength solutions (<0.05M NaC) due to surface charge screening from free ions.

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Experimental Outline

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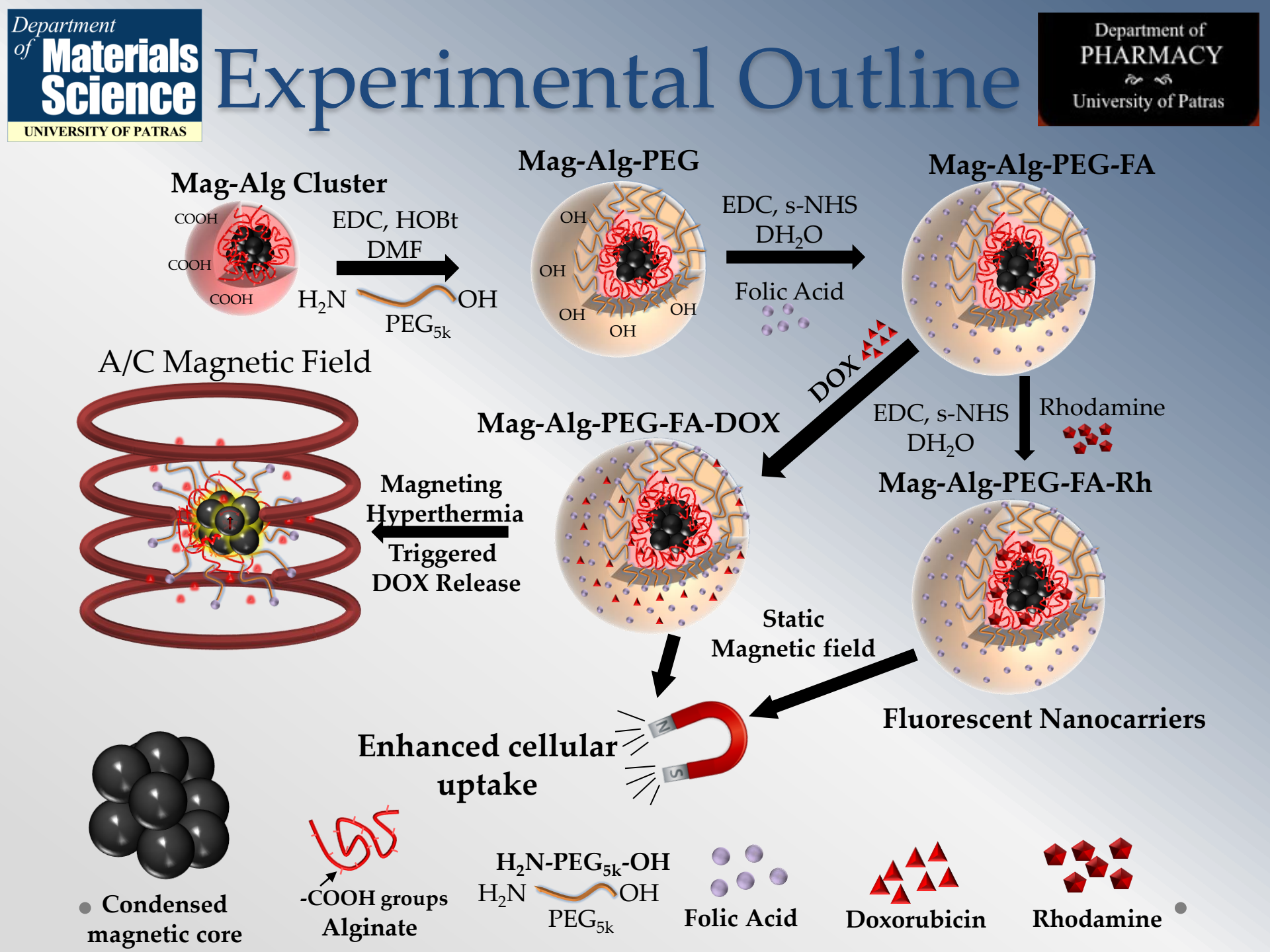
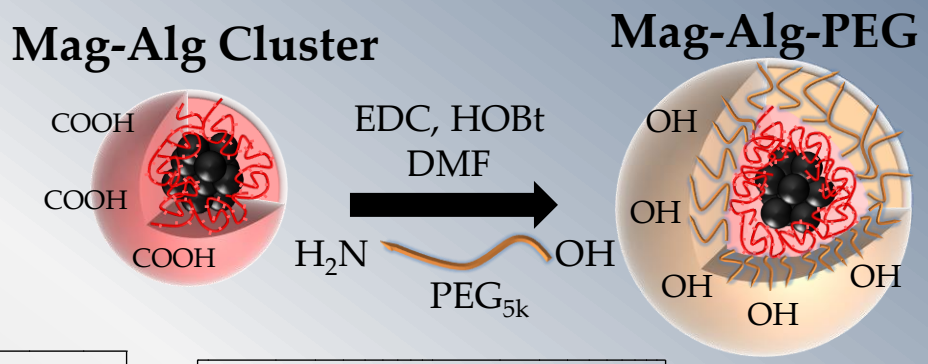
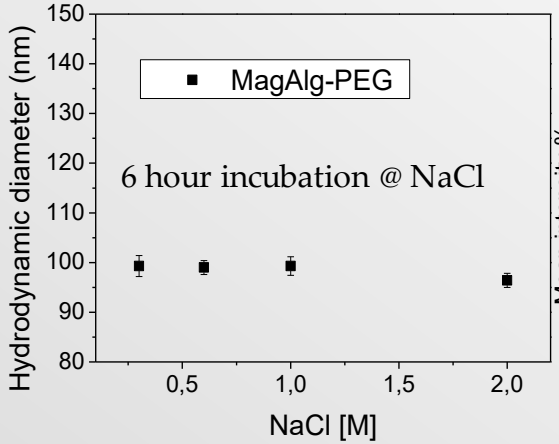
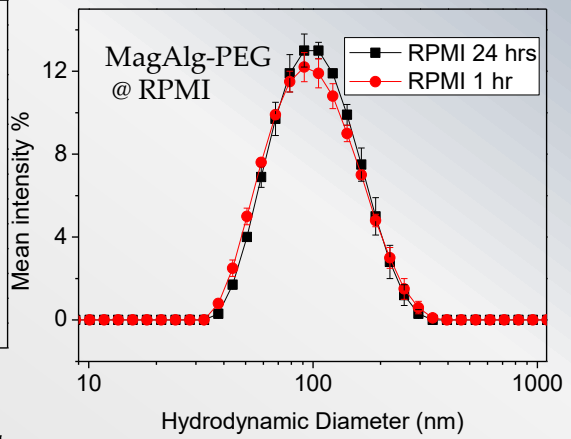
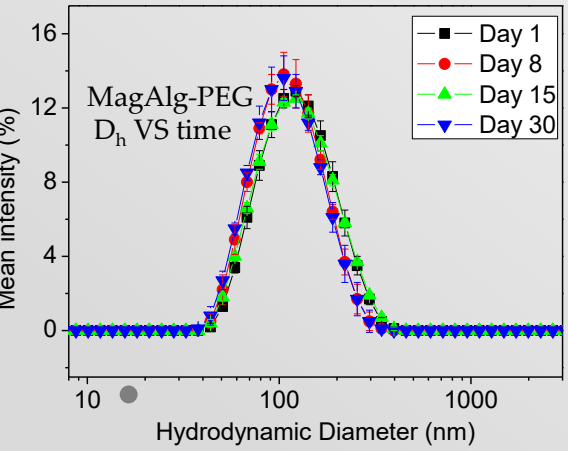


PEGylation of co-MIONs









co-MIONs PEGylation led to excellent colloidal stability in biorelevant media and in highly salted media up to 2M NaCl

Sample \ Medium	DH_2O (pH 7.4)			NaCl 2M		RPMI 1640		Blood Plasma	
	D_h (nm)	PDI	Zeta-p (mV)	D_h (nm)	PDI	D_h (nm)	PDI	D_h (nm)	PDI
MagAlg	**87**	0.14	- 43	-	-	**400**	0,43	**147**	0,45
MagAlg-PEG	**95**	0.12	- 2	**97**	0.18	**92**	0,21	**138**	0,27

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Folic acid functionalization

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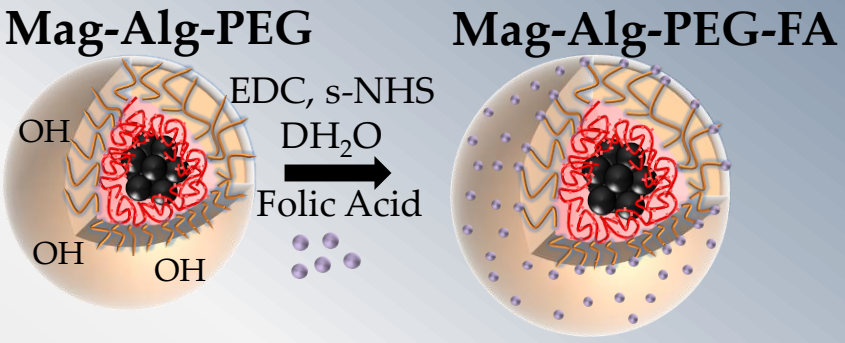
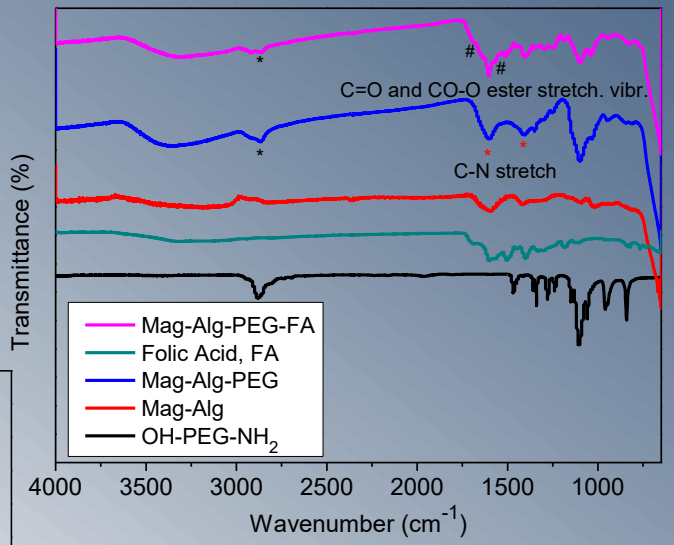
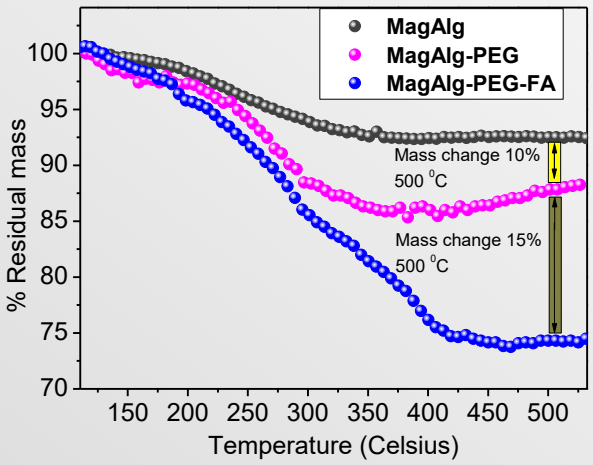
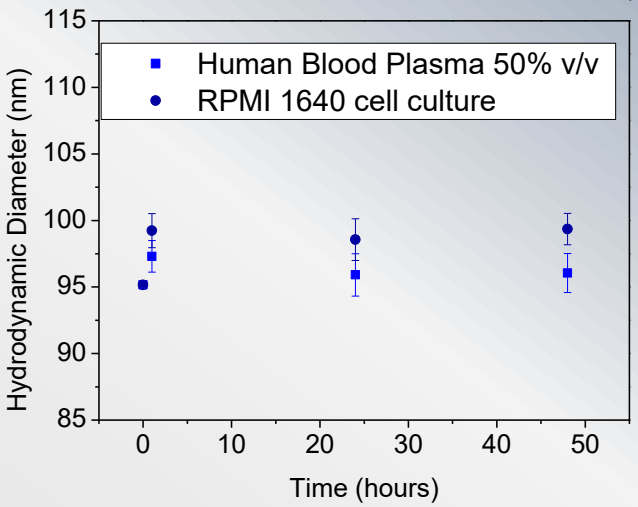








Sample \ Medium	DH_2O (pH 7.4)			NaCl 2M		RPMI 1640		Blood Plasma	
	D_h (nm)	PDI	Zeta-p (mV)	D_h (nm)	PDI	D_h (nm)	PDI	D_h (nm)	PDI
MagAlg	**87**	0.14	- 43	-	-	**400**	0,43	**147**	0,45
MagAlg-PEG	**95**	0.12	- 2	**97**	0.18	**92**	0,21	**138**	0,27
MagAlg-PEG-FA	**96**	0.12	-22	**105**	0.19	**96**	0.15	**98**	0.17

FA modified NPs demonstrated enhanced stability in blood plasma and cell medium

Doxorubicin Interactions

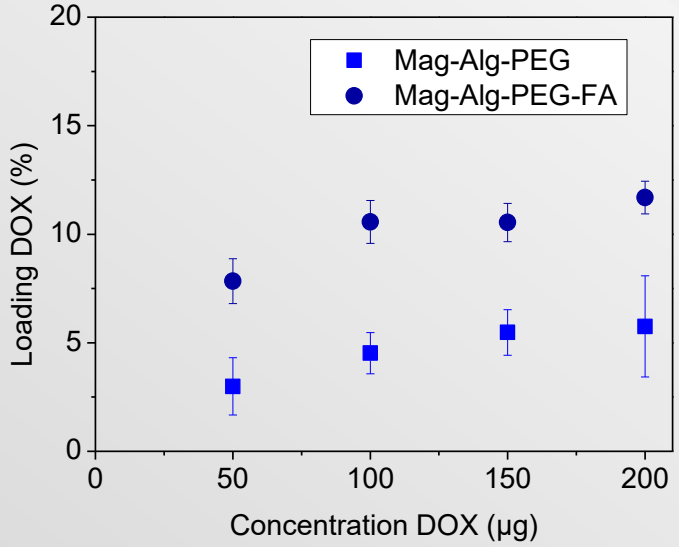
Mag-Alg-PEG-FA

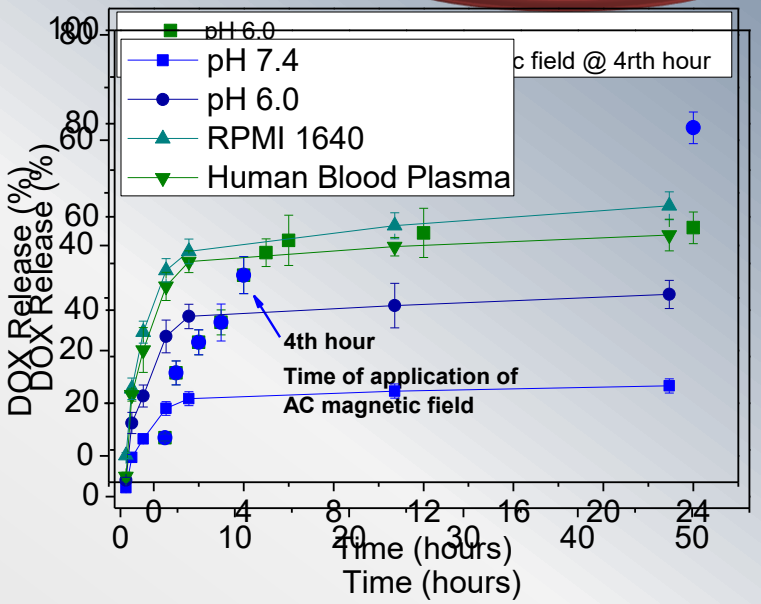
Mag-Alg-PEG-FA-DOX

DOX

Magneting Hyperthermia Triggered DOX Release

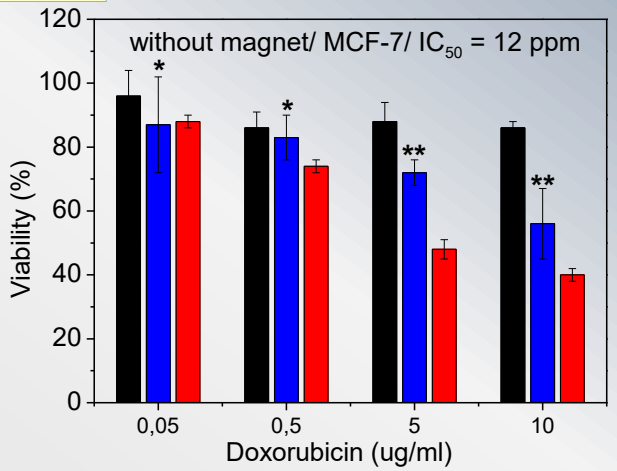
A/C Magnetic Field

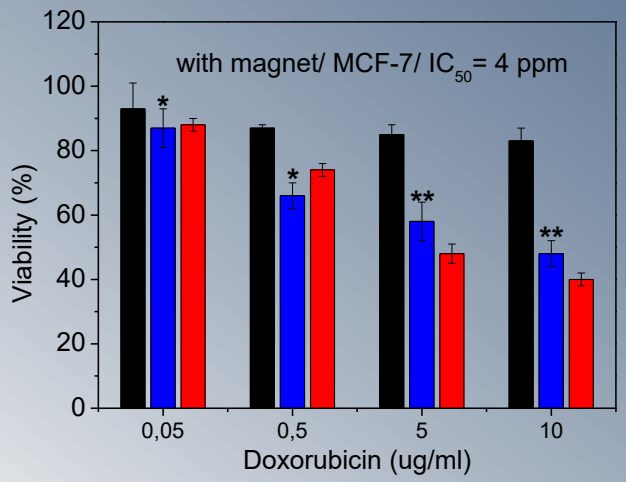


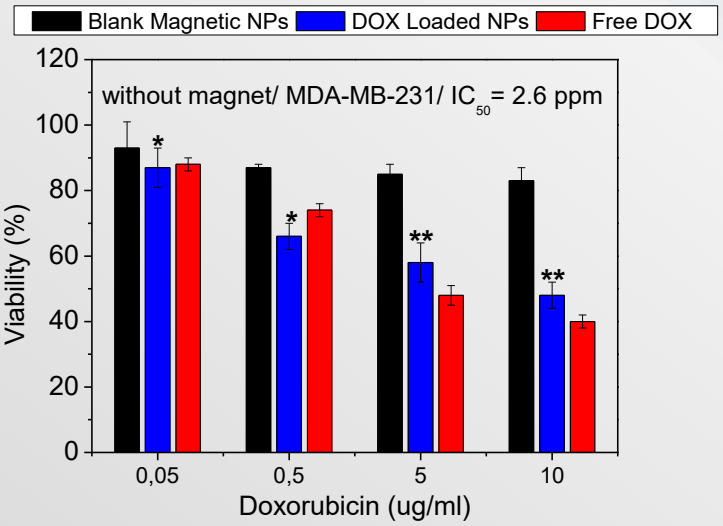


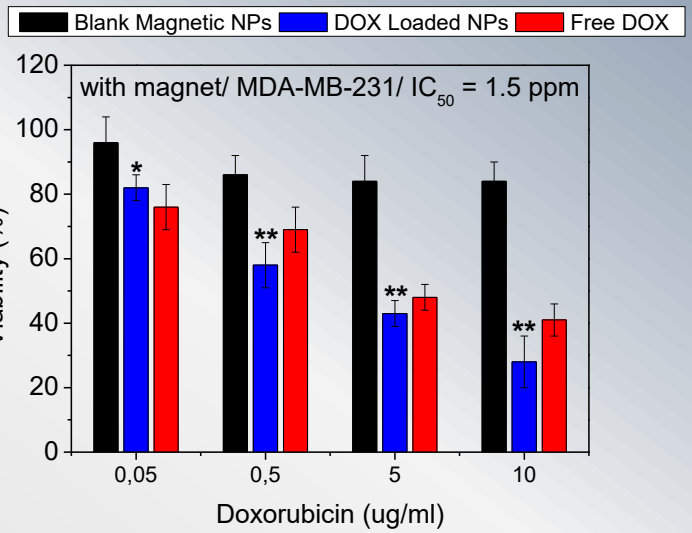
- Folic acid functionalization resulted to higher drug loading
- AMF application stimulated increased DOX release from the NCs

In vitro Cytotoxicity Assay



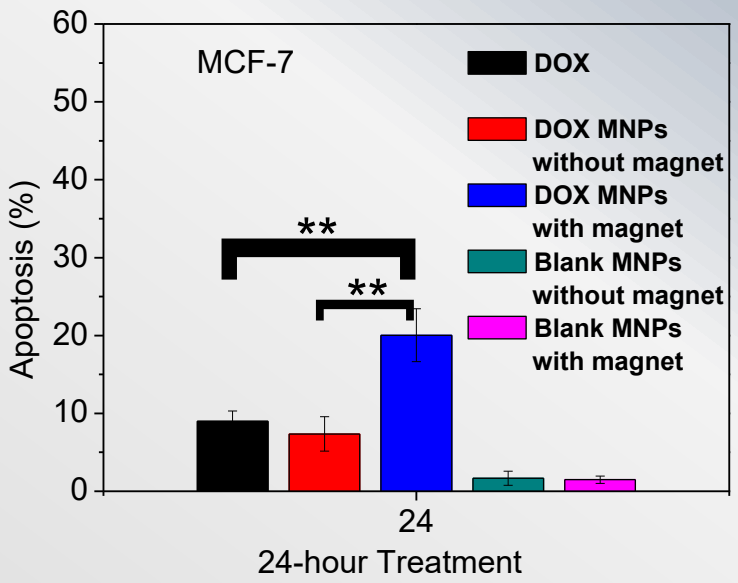


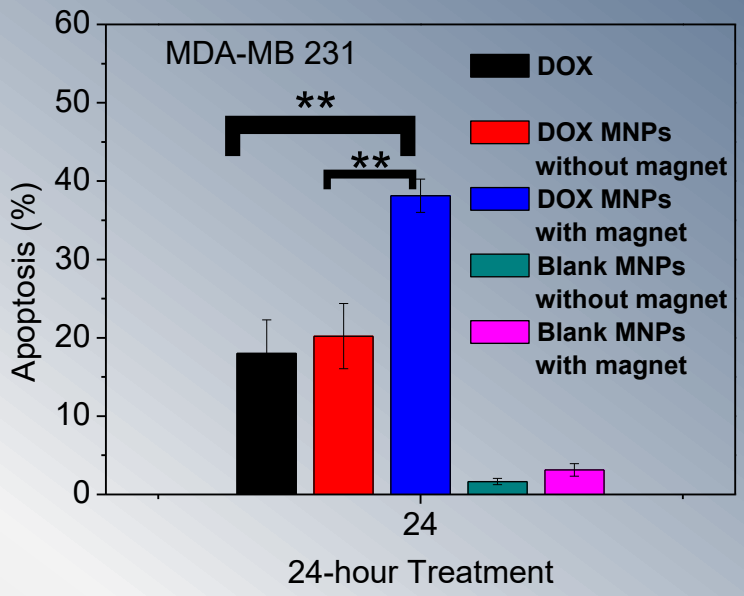




- co-MIONs exhibited high cell viability rates (>80%) for both cell lines.
- Static magnetic field application during incubation resulted in higher cytotoxicity for the MDA-MB 231 cell line compared to free DOX

Apoptosis evaluation

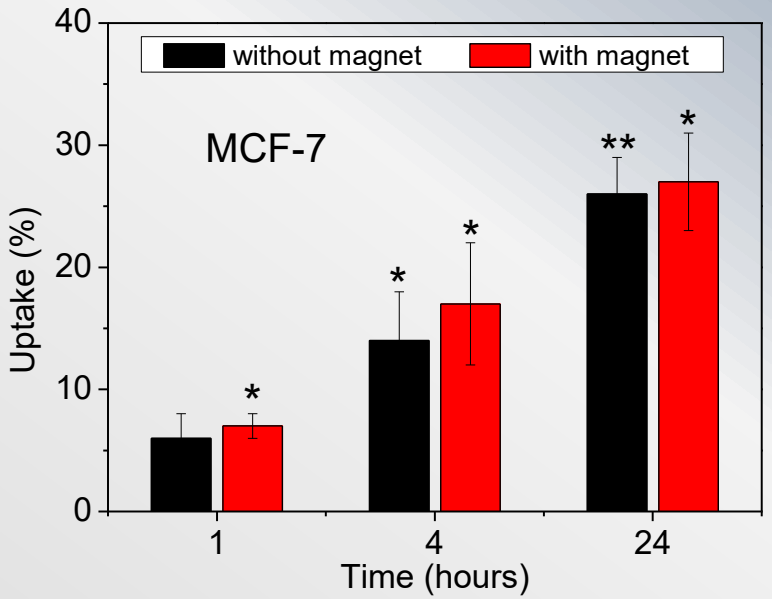


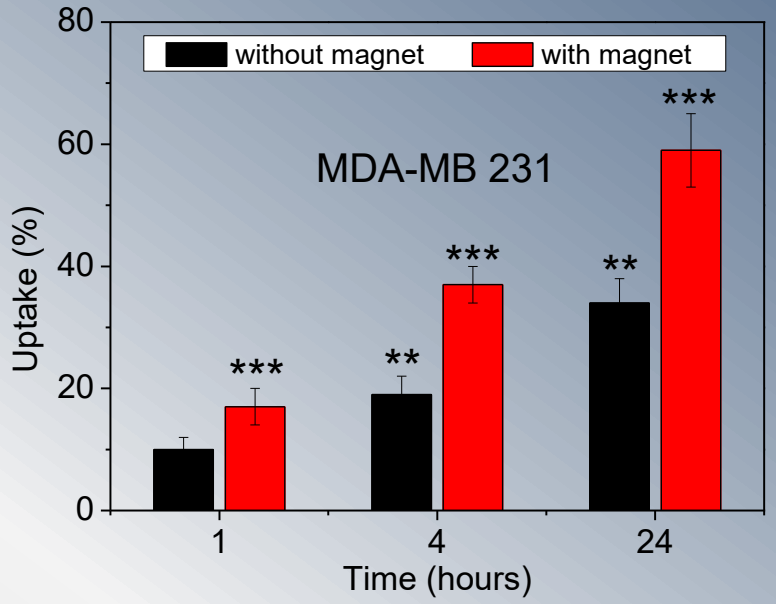


24-hour incubation time
* $p<0.05$
** $p<0.005$

DOX loaded NCs stimulated significantly higher degree of apoptosis upon the application of a magnetic field gradient compared to free DOX

Cellular uptake





- Mag-Alg-PEG-FA nanoparticles were labeled with Rhodamine in order to examine their cellular uptake by the MCF-7 and MDA-MB 231 cells
- NPs uptake increased over time for both cell lines
- Magnetic field application increased significantly the uptake only for the MDA-MB 231 cell line

* $p<0.05$
** $p<0.005$
*** $p<0.001$

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Cellular uptake MDA-MB 231

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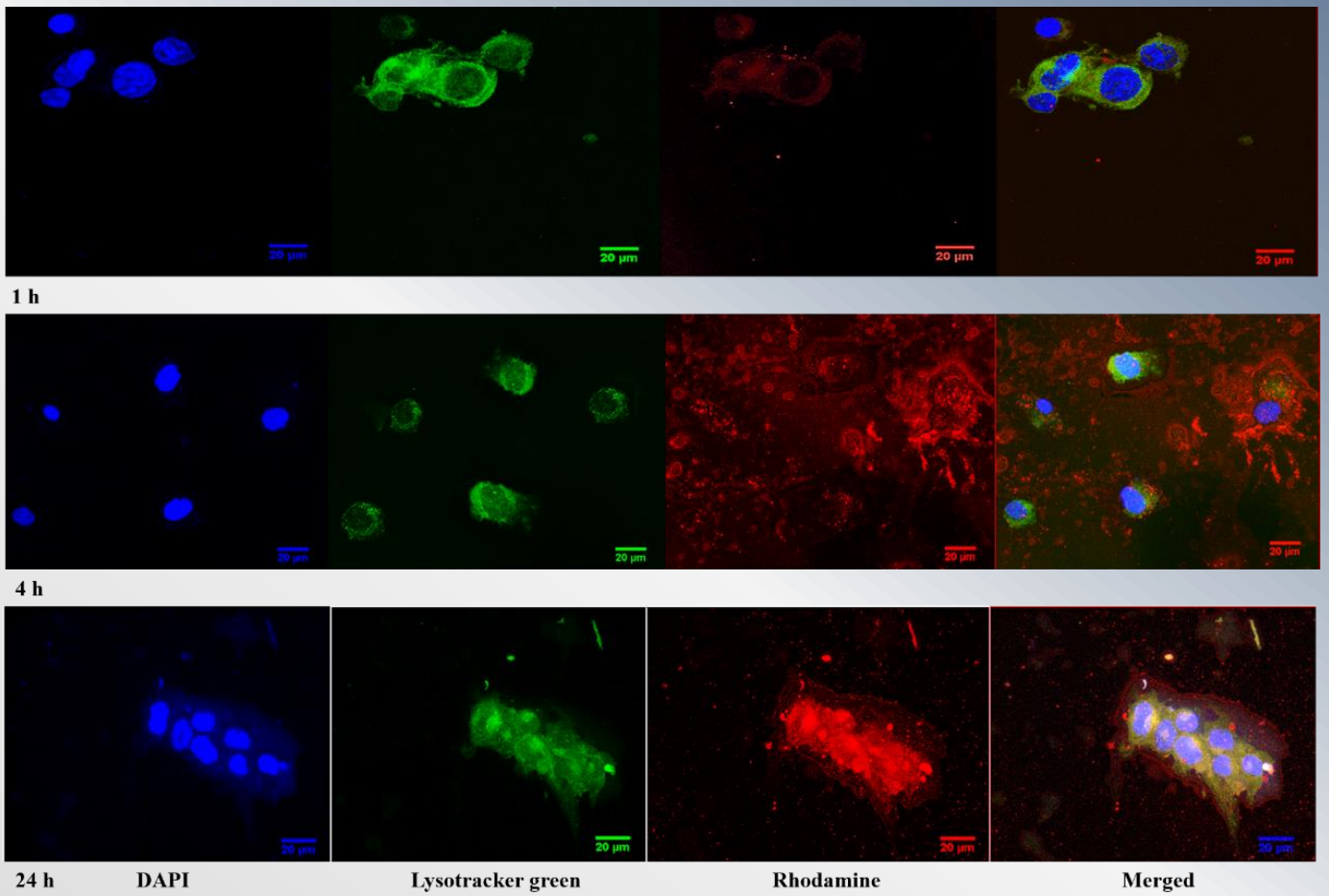
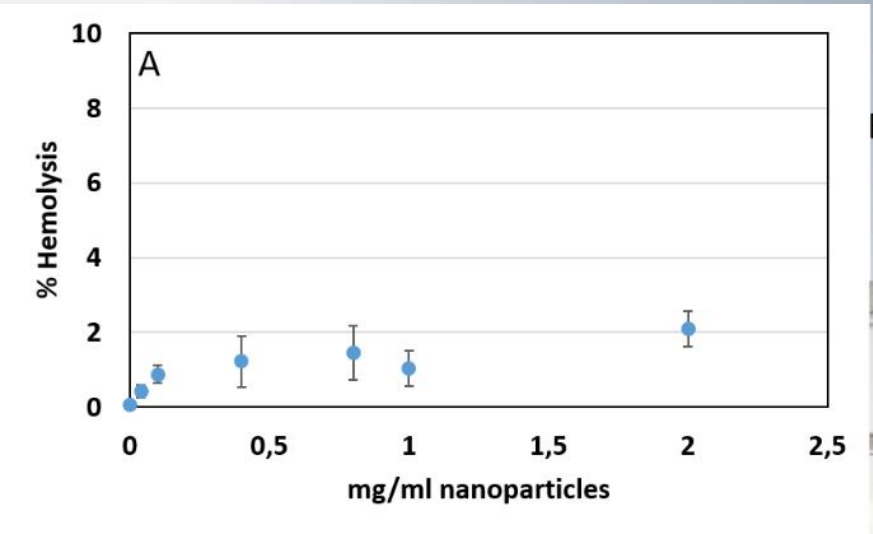
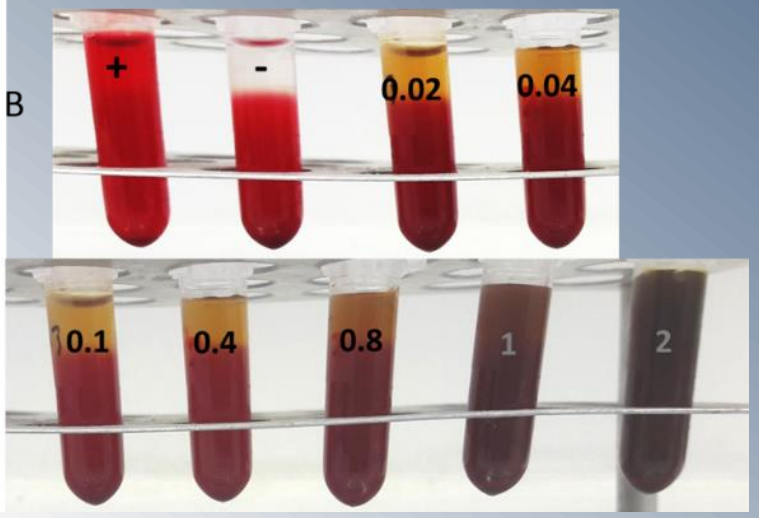


Confocal fluorescence microscopy images for MDA-MB 231 cell line upon application of a static magnetic field.

Hemolysis Assay





Biocompatibility of co-MIONs was further evaluated with hemolysis assay. Magnetic nanoparticles indicated no hemolytic activity (less than 2%) for the tested concentrations

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Conclusions

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Nanomedicines of epitaxially co-MIONs colloidal nanocrystal clusters appear to represent a highly promising structural motif and a tunable platform for magnetoresponsive and theranostic applications.

- The developed PEGylated co-CNCs were functionalized with molecular targeting units of folic acid.
- Attachment of functional PEG and FA units resulted to excellent colloidal stability.
- Increased nanoparticle uptake for cancer cells overexpressing folic acid receptors.
- Nanoparticles exhibited controlled DOX release.
- Acceleration of release can be triggered in acidic pH or in response to an Alternating Magnetic Field.
- Application of magnetic field gradient enhanced the cellular uptake and cytotoxicity of DOX-loaded NCs

Acknowledgements

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European Social Fund

Operational Programme
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