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Chemical and Biological Characterizations of an Effective Bimodal Probe to Target Apoptosis

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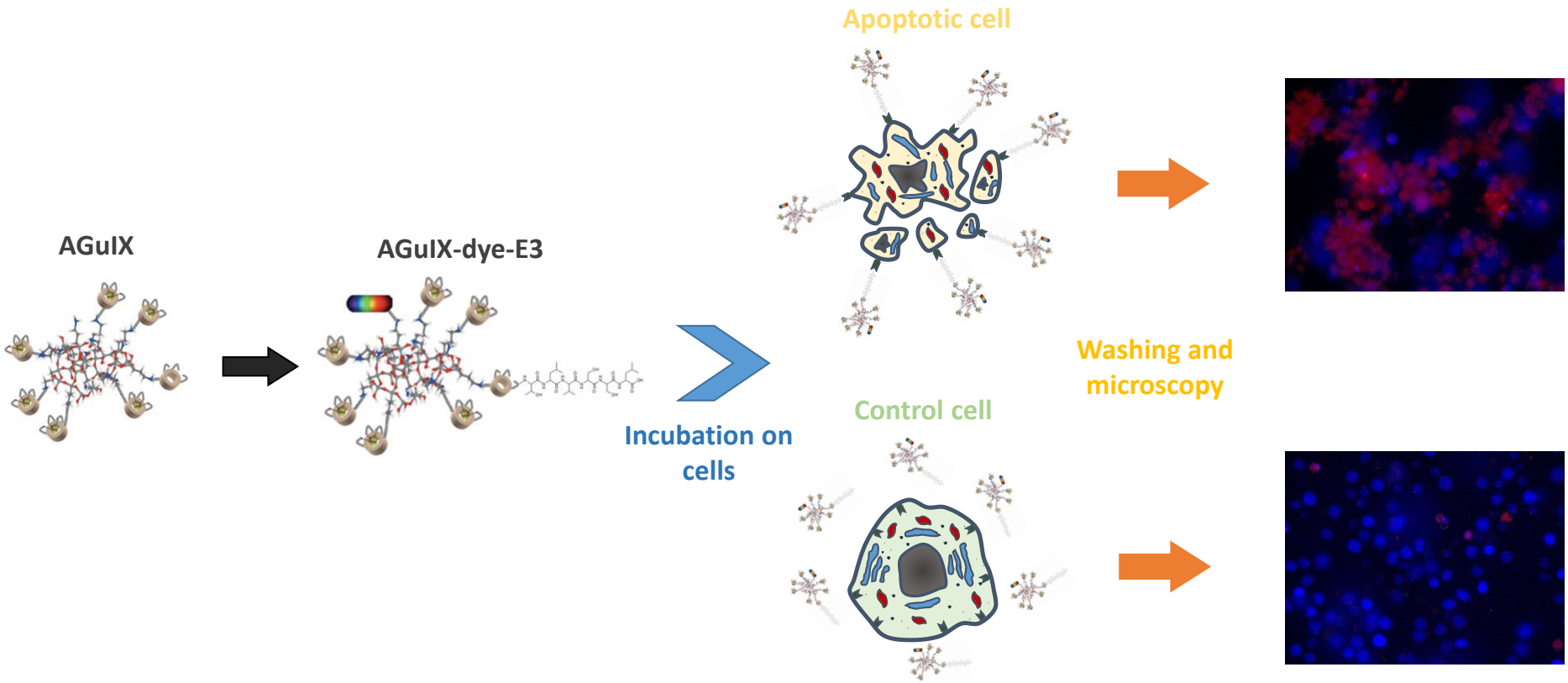
Graphical Abstract



Abstract

AGuIX® nanoparticles have been recently developed for applications in medical imaging. These nanoparticles are formed after the dissolution of core/shell Gd_2O_3 nanoparticles with a polysiloxane coating and Gd-DOTA complexes are grafted on surface. These small and paramagnetic platforms are easily eliminated after injection. Several studies have shown that they have multimodal and theragnostic properties.

Apoptosis is a process of cell death. Targeting of apoptotic cells is interesting for a forward diagnosis of atherosclerosis or Parkinson disease. Optical dyes and TLVSSL peptides have been grafted on nanoparticles to develop a bimodal probe that target effectively the overexpressed phosphatidylserine on apoptotic cells. These nanoobjects were charachterised by chemical and biological methods such as PCS, HPLC, relaxometry and fluorescence microscopy on targeted Jurkat cells.

Keywords: Bimodal; Nanoparticles; AGuIX; Apoptosis; MRI; Optical Imaging

Introduction

Apoptosis

Natural process of cell death
Mechanism involved in cell renewal

Apoptosis is characterized by a structural modification of the cell and an inversion of membrane phospholipids (flip-flop mechanism)

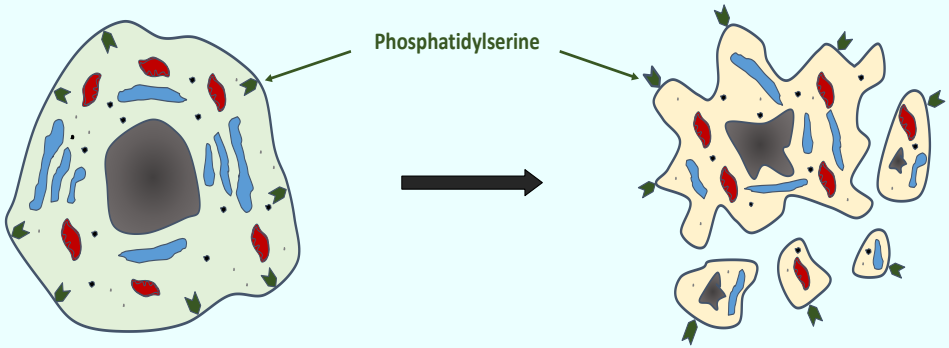


Fig 1 : Schematic illustration of cell modification in apoptosis process

Dysfunction of apoptosis process ?

Excess of apoptosis →neurodegenerative diseases
Lack of apoptosis → tumors, autoimmune diseases

Active targeting ?

- **Diagnosis of neurodegenerative diseases**
- **Follow-up of an anti-tumoral therapy**

Introduction

AGuIX® nanoparticles[1]

AGuIX® NP are small platforms of polysiloxane grafted with Gd-DOTA. The network of polysiloxane was synthesized by hydrolysis-condensation of a mixture of aminopropyltriethoxysilane (60%) and tetraethylorthosilicate (40%)

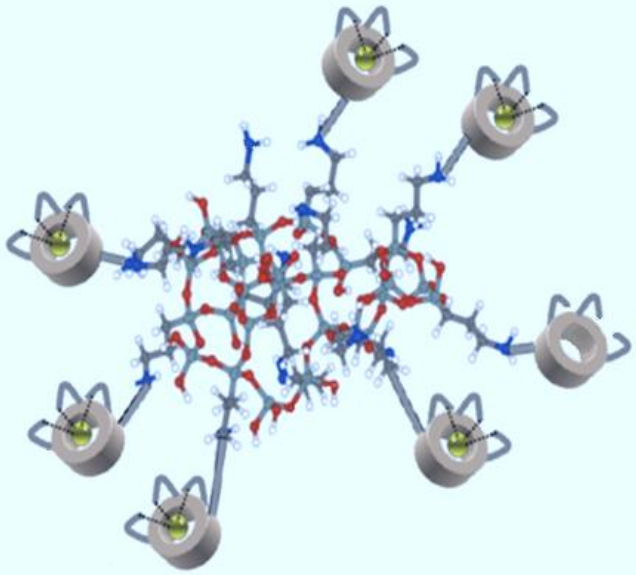


Fig 2 : AGuIX® nanoparticle

Physico-chemical characteristics[2]

- Mass = 8.5 kDa
- Diameter ≈ 2 nm
- 10 DOTA/nanoparticle , 7 are complexed with Gd^{3+} ions.
  - $\log\beta_{110}$=25.58
- Longitudinal relaxivity = 10 ± 1 $s^{-1}mM^{-1}$ (60 MHz, 37°C)

Introduction

AGuIX® Nanoparticle Grafting

AGuIX® were grafted with the peptide TLVSSL (E3) on DOTA free carboxylic sites *(fig 3)*. This peptide has a high affinity for phosphatidylserine, a phospholipid overexpressed by apoptotic cells. To maximize the vectorization yield, AGuIX nanoparticles were previously treated to increase the number of non complexed DOTA. AGuIX were also grafted with peptide with a small spacer (L-E3). Bimodal nanoparticles were also labeled with optical dyes (Cy5,5 or RITC) to allow applications in optical imaging *(fig 4)*. Fig. 5 illustrates the targeting of phosphatidylserine by grafted-AGuIX nanoparticles.

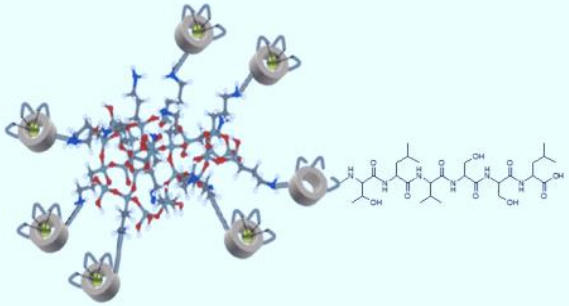


Fig 3 : AGuIX-E3 nanoparticle

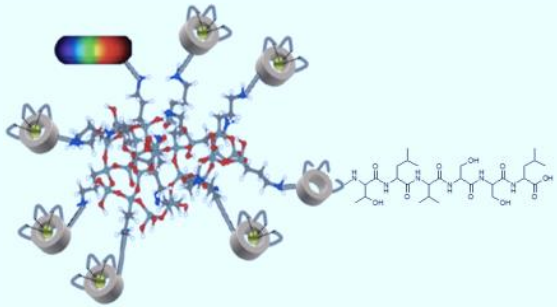


Fig 4 : AGuIX-dye-E3 nanoparticle

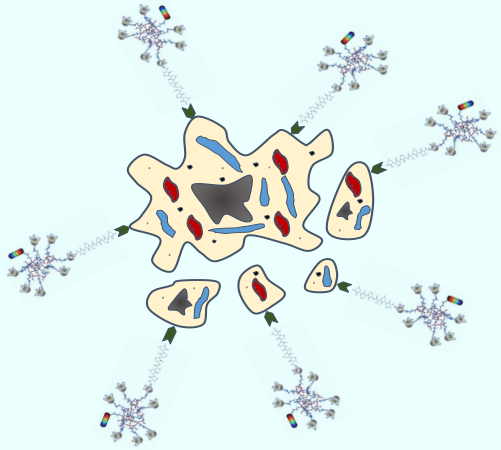
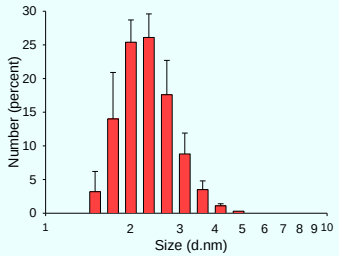


Fig 5 : Representation of apoptotic cell targeting by grafted-AGuIX

Results and discussion

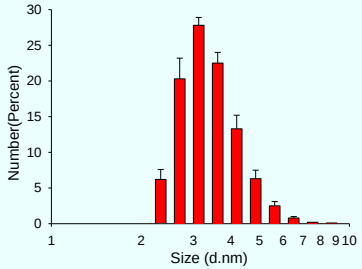
Photon Correlation Spectroscopy

AGuIX



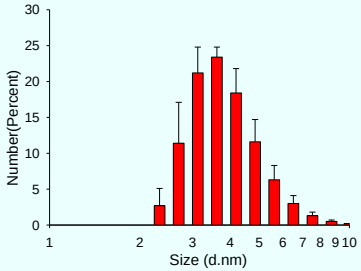
Hd ≈ 2.3 ± 0.7nm

AGuIX-RITC



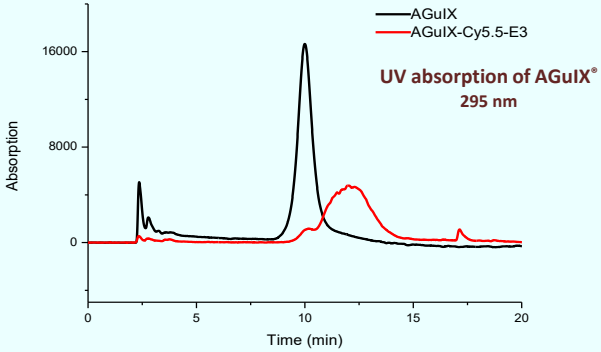
Hd ≈ 3.4 ± 0.8nm

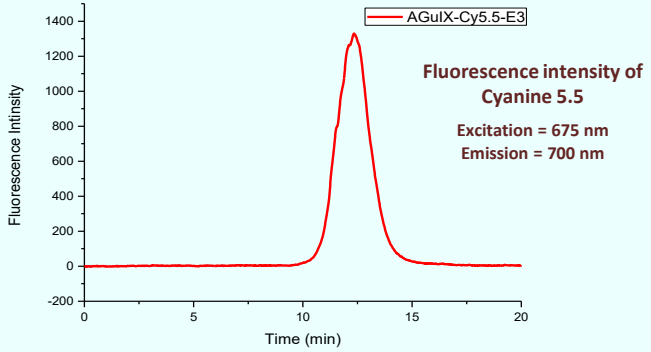
AGuIX-RITC-E3



Hd ≈ 4.0 ± 1.4nm

HPLC





Confirmation of the vectorization by an increase of nanoparticle hydrodynamic diameter and HPLC retention time

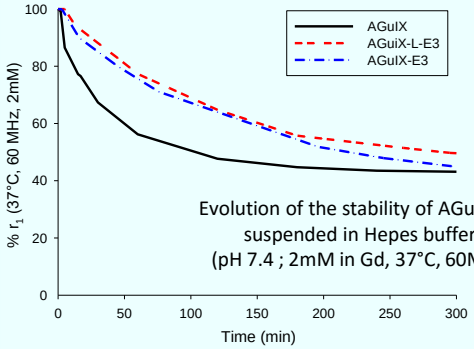
Results and discussion

Relaxometric analyses

Table 1. Relaxivity of AGuIX nanoparticles at 2mM in Gd measured 5 min after dilution

($s^{-1}mM^{-1}$) 37°C	AGuIX	AGuIX-E3	AGuIX-L-E3	AGuIX-Cy5.5	AGuIX-Cy5.5-E3	AGuIX-RITC	AGuIX-RITC-E3	AGuIX-RITC-L-E3
r_1 (20 MHz)	7.8	12.7	18.2	13	13.1	12.9	16.1	17.7
r_2 (20 MHz)	8.7	14.7	20.8	13.8	14	13.9	17.4	20.7
r_1 (60 MHz)	6.9	10.3	15.2	11.6	11.4	11.2	12.1	15.7
r_2 (60 MHz)	8.9	15.3	21.8	15.3	16	15.0	18.9	21.5

Degradation study in aqueous solution



NMRD profiles of AGuIX nanoparticles (4mM Gd in water, 37°C, 10 min after dilution)

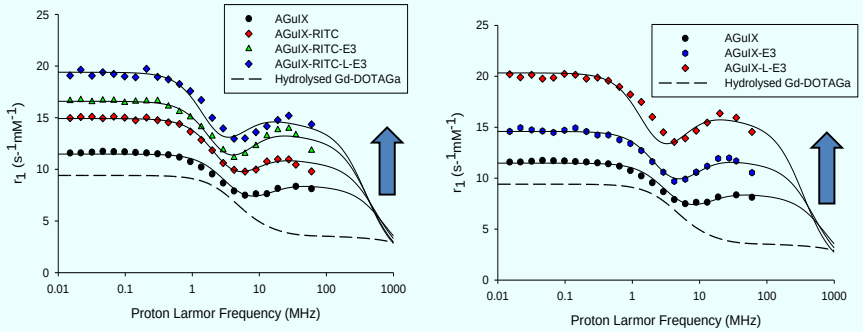


Table 2. Profile fitting parameter values obtained using Salomon-Bloembergen-Morgan model

	H. Gd-Dotaga	AGuIX	AGuIX-E3	AGuIX-L-E3	AGuIX-RITC	AGuIX-RITC-E3	AGuIX-RITC-L-E3
τ_M	116 ns	100 ns	100 ns	100 ns	100 ns	100 ns	100 ns
τ_R	69.0 ± 6.7 ps	223.0 ± 4.8 ps	330.0 ± 4.9 ps	475.0 ± 5.8 ps	300.0 ± 5.7 ps	386.0 ± 5.4 ps	427.0 ± 6.9 ps
τ_{SO}	301 ± 89 ps	122.0 ± 1.9 ps	164.0 ± 2.1 ps	265.0 ± 3.7 ps	177.0 ± 2.7 ps	196.0 ± 2.6 ps	255 ± 3.8 ps
τ_V	3.9 ± 1.4 ps	20.7 ± 1.0 ps	34.0 ± 1.3 ps	50.0 ± 3.5 ps	32.3 ± 1.6 ps	42.4 ± 1.8 ps	53.8 ± 3.2 ps

An increase of rotational correlation time (τ_R) after grafting was observed as shown in the NMRD profiles

Stabilization and proton relaxation enhancement

Grafted-AGuIX® NP are more efficient as paramagnetic contrast agents for MRI than non vectorised AGuIX® NP

Results and discussion

Biological Targeting Tests

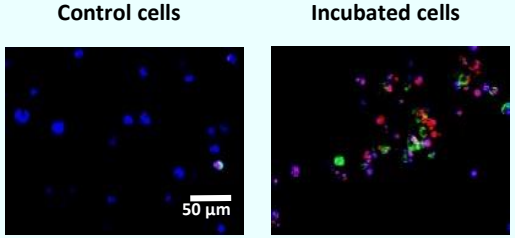
The specificity for apoptosis of grafted-AguIX NP has been checked by fluorescence microscopy analysis. Jurkat cells (lymphoblastic human T cell line) have been treated 24h with camptothecin to induce apoptosis.

Validation of the apoptosis induction protocol

Nuclei marked by Hoechst

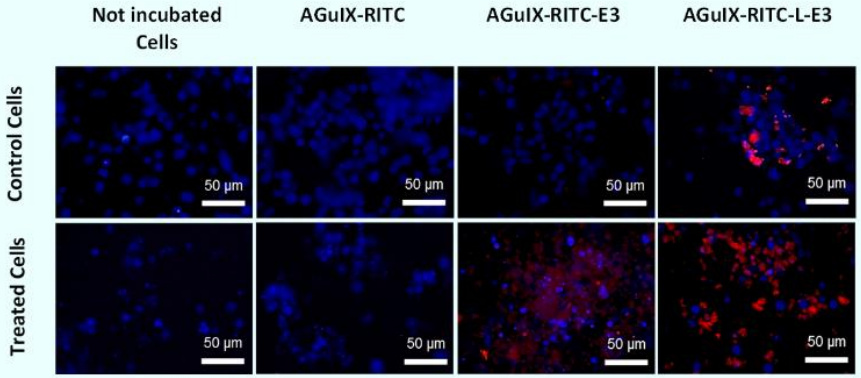
Apoptotic cells targeted by Annexin-FITC

Necrotic cells marked by Propidium iodide



Apoptosis is induced by camptothecin incubation

Specificity study of grafted-AGuIX-RITC for apoptotic cells (RITC dye : Rhodamine isothiocyanate)



Nuclei marked by DAPI

Apoptotic cells are specifically targeted by grafted AGuIX

Conclusion

AGuIX® nanoparticles are effective contrast agents for magnetic resonance imaging. Their grafting with apoptosis-specific peptide has been confirmed by several techniques and allows to create a new probe able to target effectively apoptotic cells. Various *in vitro* experiments allowed to confirm that E3 grafted-AGuIX NP are effective probes to target apoptotic cells.

References

[1] F.Lux et Al., *Angew. Chem. Int. Ed.* ***2011****, 50(51),* 12299– 12303
[2] A. Mignot et Al. , Chem. Eur. J.*,* **2013**, *19*, 6122–6136.

Acknowledgments

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