

Fluorescent magnetic nanoparticles for modulating the level of intracellular Ca²⁺ in motoneurons

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Experimental

Synthesis of iron oleate complex

Iron oleate complex was synthesized according to published method¹. Briefly, a certain mass of FeCl₃ dissolved in doubly distilled water was mixed with sodium oleate dissolved in a mixture of ethanol, hexane and doubly distilled water in a round-bottom reaction flask. Then, this mixture was heated to 70°C and refluxed at this temperature using oil bath for 4 h under Ar and vigorous stirring. Next, the hexane layer was separated and washed 5 times with doubly distilled water. After that, hexane was removed using rotary evaporator and the remaining iron oleate was dried under vacuum at 32°C for 24 hours.

Synthesis of 17 nm iron-oxide nanoparticles

Iron-oxide nanoparticles were synthesized according to well-known literature procedure^{1, 2}. Firstly, 3.3436 g of iron oleate (III) was dissolved in 12.32 mL of n-eicosane with 1.15 mL of oleic acid and stirred under argon at 69°C to dissolve the components. Next, the obtained

reaction mixture was heated up to 330°C (average heating rate 2.93°C/min) and kept at this temperature for 30 min under argon and vigorous stirring (800 rpm) in a reflux system. Then, the resulting black solution containing iron oxide crystals was cooled down to 43°C and a mixture of hexane and acetone (58 mL, volume ratio 1:4) was added to precipitate the crystals. Next, the nanoparticles were separated by centrifugation (20 min, 12500 rpm) and washed 7 times with 15 mL of hexane/acetone mixture to remove excess of oleic acid and organic solvents remaining in the sample. The obtained nanoparticles were dried in vacuum at 90°C for 130 min and characterized. Yield: 219.1 mg (76%).

Synthesis of Fe-Ru@SNs

0.01 g of the synthesized oleate-coated iron-oxide nanoparticles (17±1 nm) were dispersed in 3.125 mL of cyclohexane using ultrasound water bath for 60 minutes. Then, the obtained dispersion was added dropwise using a syringe pump (adding rate 1 mL min⁻¹) to the mixture of 1.49 g of Triton X-100, 1.43 mL n-heptanol, and 2.7 mL of cyclohexane. The mixture was stirred for 10 minutes followed by adding of 0.125 mL NH₃ (28-30%) and 0.69 mL [Ru(dipy)₃]Cl₂ in water (C=0.01 M). After the stirring of for 30 minutes the additionally prepared solution (1.49 g of Triton X-100, 1.43 mL of n-heptanol, 5.82 mL of cyclohexane and 0.125 mL of TEOS) was added to the mixture with the adding rate 1 mL min⁻¹. After the stirring (750 rpm) for 24 hours the nanoparticles were separated from the mixture by adding of acetone and was then washed with acetone/ethanol mixture (1:1), ethanol by 2X and water by 3X centrifugation/redispersion steps.

Synthesis of Fe-Ru@amino-SNs

0.01 g of the synthesized oleate-coated iron-oxide nanoparticles (17±1 nm) were dispersed in 3.125 mL of cyclohexane using ultrasound water bath for 60 minutes. Then, the obtained dispersion was added dropwise using a syringe pump (adding rate 1 mL min⁻¹) to the mixture of 2.98 g of Triton X-100, 2.8 mL n-heptanol, 8.515 mL of cyclohexane. After 15 min of stirring 0.7 mL [Ru(dipy)₃]Cl₂ in water (C=0.01 M) and 0.093 ml NH₃ (28-30%) were added. The mixture was stirred for 15 minutes followed by adding of 0.08 mL of TEOS. Then, the mixture was stirred for 24 hours before the adding of TEOS (0.08 mL) and APTES (0.016 mL) 30 minutes later than TEOS, followed by further stirring for 24 hours.

After the stirring (750 rpm) for 24 hours the nanoparticles were separated from the mixture by adding of acetone and were then washed with acetone/ethanol mixture (1:1), ethanol by 2X and water by 3X centrifugation/redispersion steps.

The fluorescence procedure with the use of fluorescamine (ESI, Fig. S3)^{3,4} was used for quantitative analysis of amino groups on the surface of the amino group-modified SNs. In order to approximately evaluate a number of NH₂ groups on the surface of Fe-Ru@amino-SNs

nanoparticles the knowledge of mass of a single nanoparticle is necessary. Since the density of Fe-Ru@amino-SNs nanoparticle was not possible to determine, we had calculated its mass as follows. Taking into consideration that density of silica is 1.96 g/cm^3 [5] and diameter is about 78 nm, the mass of a single spherical nanoparticle was calculated to be about $4.9 \cdot 10^{-16} \text{ g}$ (eq. 1).

$$m = 4/3\pi r^3 \cdot \rho \quad (1)$$

Given that magnetite density is 5.17 g/cm^3 [6], the mass of single spherical 17.08 nm iron-oxide nanoparticle is about $1.35 \cdot 10^{-17} \text{ g}$. Then we have subtracted the mass of single 17 nm silica sphere ($5.03 \cdot 10^{-18} \text{ g}$) from the mass of single 78 nm silica sphere ($4.9 \cdot 10^{-16} \text{ g}$). The obtained value was added to $1.35 \cdot 10^{-17}$ giving the resulting value of $4.985 \cdot 10^{-16} \text{ g}$ which is the approximate mass of single Fe-Ru@amino-SNs nanoparticle.

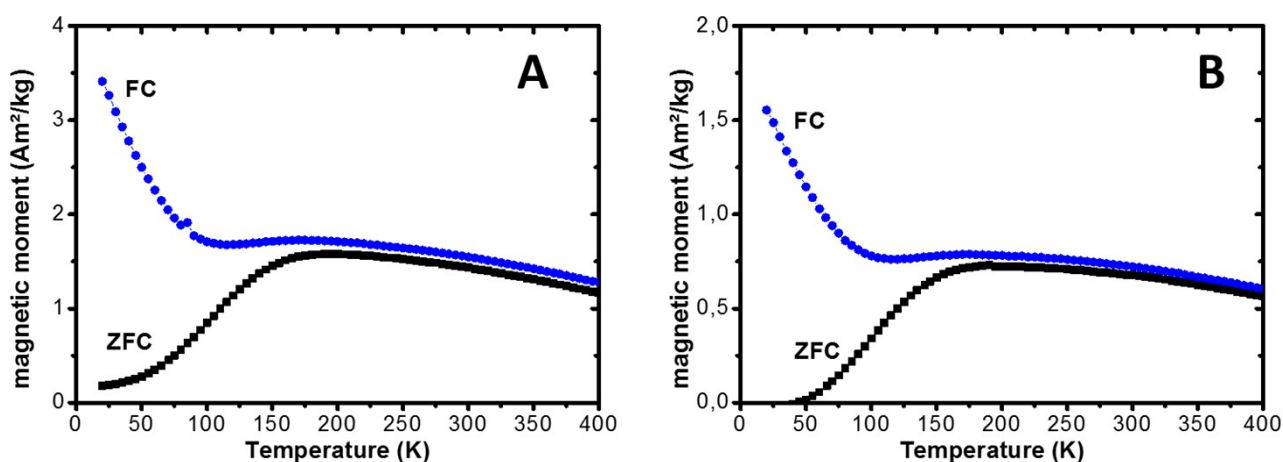


Fig. S1. FC/ZFC curves of Fe-Ru@SNs (panel A) and of Fe-Ru@amino-SNs (Panel B).

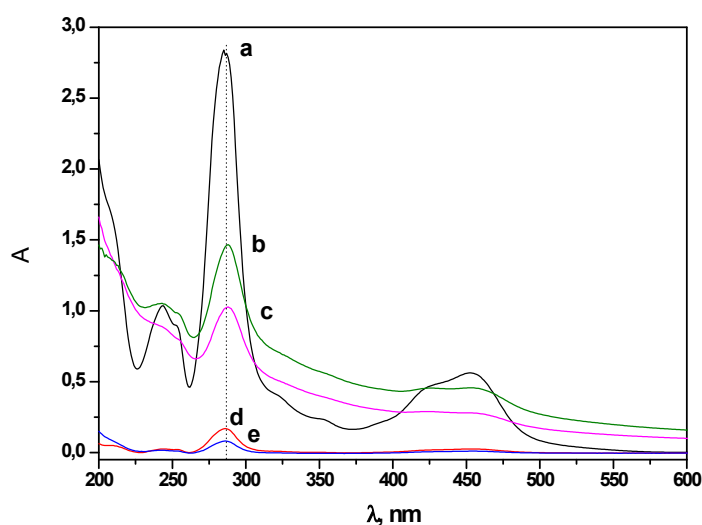


Fig. S2. UV-Vis spectra of (a) $[\text{Ru}(\text{dipy})_3]\text{Cl}_2$ ($C=0.01 \text{ mM}$) in water, (b) Fe-Ru@SNs, (c) Fe-Ru@amino-SNs in water ($C=0.15 \text{ g L}^{-1}$), (d) supernatant after 2X centrifugation of Fe-Ru@SNs and (e) supernatant after 2X centrifugation of Fe-Ru@amino-SNs.

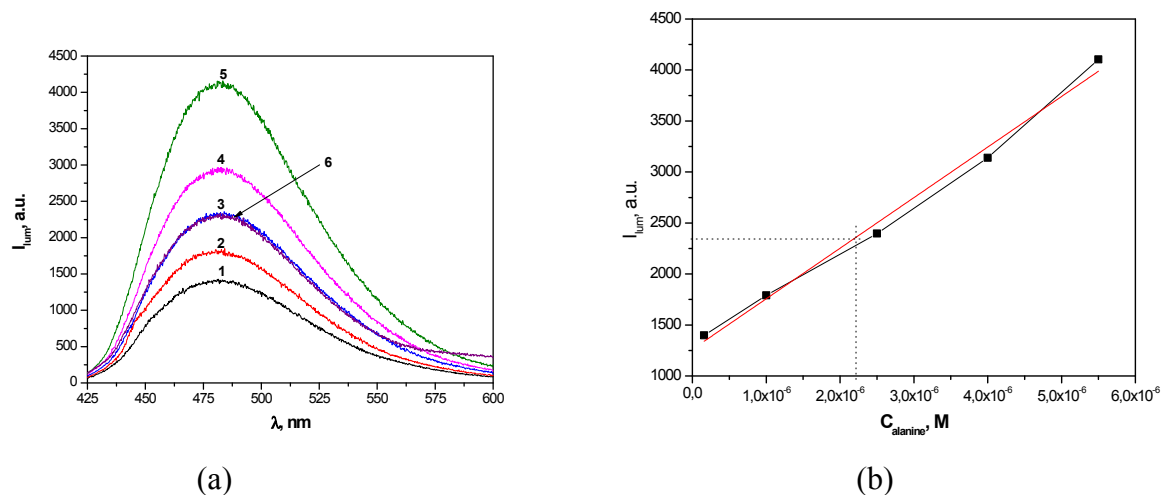


Fig. S3. (a) The emission spectra of fluorophore, obtained from interaction of fluorescamine (0.55 mM) with various concentrations of alanine (1 – $1.6 \cdot 10^{-7}$, 2 – $1 \cdot 10^{-6}$, 3 – $2.5 \cdot 10^{-6}$, 4 – $4 \cdot 10^{-6}$, 5 – $5.5 \cdot 10^{-2}$) and with Fe-Ru@amino-SNs (6) (0.0433 g L^{-1}) at 50 mM borate buffer pH=9; (b) The calibration graphic.

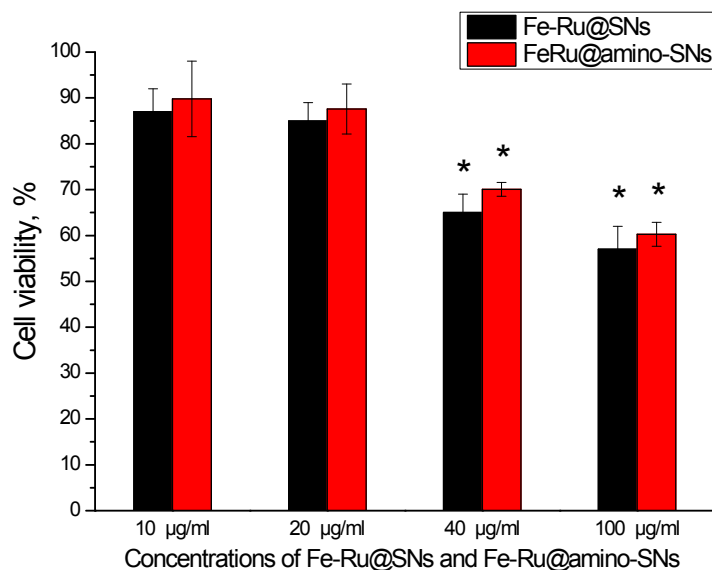


Fig. S4. Effect of Fe-Ru@SNs and Fe-Ru@amino-SNs on viability of motoneurons. Motoneurons were treated with vehicle or different concentrations of Fe-Ru@SNs and Fe-Ru@amino-SNs for 24 hours. Cell viability was analyzed using the MTT assay. Data represented as mean \pm SE of three independent experiments made in three replicates. * $P < 0.05$ versus vehicle control group.

Table S1. Coupled plasma optical emission spectrometry (ICP-OES) data for Fe-Ru@SNs and Fe-Ru@amino-SNs ($C=0.2 \text{ g L}^{-1}$)

Sample	Element content mg L^{-1} , $\pm 10\%$
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	Si (251.611 nm)	Fe (259.940 nm)	Ru (267.876 nm)
Fe-Ru@SNs	58.8	12.0	1.76
Fe-Ru@amino-SNs	52.7	4.94	0.794

Video S1. Magnetic stimulation of motoneurons culture loaded fluorescence calcium dye. Fluorescence video showing increase intracellular calcium levels with magnetic forces.

References

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