SUPPORTING INFORMATION

Janus plasmonic-magnetic gold-iron oxide nanoparticles as contrast agents for multimodal imaging

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1 TEM characterization

1.1 Asymmetric dumbbell nanoparticles



Figure S1: TEM images of nanodumbbells with 16 nm iron oxide cores.



Figure S2: TEM images of nanodumbbells with 20 nm iron oxide cores.

1.2 Janus magnetoplasmonic nanostars (JMNSs)

- JMNSs with 16 nm iron oxide parts















Figure S3: TEM images of JMNSs with 16 nm iron oxide parts.





Figure S4: TEM images of JMNSs with 20 nm iron oxide parts.

2 Nanoparticle sizes

To calculate the size of the particles TEM was used. Several images per nanoparticle type were analyzed with the use of the software package Image J. The images were analyzed based on the gray scale contrast, making use of the threshold tools and particle analysis.

For the asymmetric nanodumbbells the gold part was first measured using its higher contrast (darker), and the iron oxide was them measured by removing the gold part and selecting the iron oxide based on grey scale levels. Manual measurements were performed where the grey scale did not offer a good contrast. The sizes of gold and iron oxide were calculated from the areas assuming a spherical shape (equivalent average diameter).

Table S	1: Average	diameter,	measured by	TEM,	of the	asymmetric	nanodui	nbbells	used a	s seeds	in the
JMNS s	ynthesis.										

	Iron oxide diameter (nm)	Gold diameter (nm)
ND.16	16.2± 2.8	5.3±0.8
ND.20	20.5± 4.0	5.7±1.2

For the JMNSs, only the total size was measured. Two different values were acquired: an equivalent average diameter, considering the area and assuming an equivalent spherical shape, and the Feret's maximum diameter that corresponds to the maximum tip to tip distance of the 2D projected nanostars. Error bars correspond to \pm the standard deviation after the measurement of a minimum of 100 nanoparticles.

Average sizes are shown in the table and represented in the next graphs.

Sample	D equivalent (nm)	D Feret (nm)
JMNS.16.47	46.9±8.2	59.8±10.5
JMNS.16.43	43.0±6.8	55.3±9.2
JMNS.16.37	36.7±4.9	50.6±8.2
JMNS.16.28	28.5±2.9	37.5±4.5
JMNS.16.25	25.0±2.9	31.9±4.5
JMNS.16.19	19.4±3.2	24.3±4.2
JMNS.20.43	43.2±10.3	56.2±13.3
JMNS.20.41	41.4±5.9	54.4±7.6
JMNS.20.36	35.8±5.0	45.9±7.3

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JMNS.15.X 60 Diameter(nm) 50 40 30 20 5 . 10 25 15 20 [Au]/[Fe] (mol/mol) JMNS.20.X 60 Diameter (nm) 50 40 30 10 1 [Au]/[Fe] (mol/mol) 0 5 15 20

Figure S5: JMNS diameters, corresponding to nanoparticles with 16 nm and 20 nm iron oxide part, as a function of the gold to iron ratio used in the synthesis ($D_{equivalent}$ in red, D_{Feret} in red).

X-ray photoelectron spectroscopy (XPS) 3

XPS measurements were performed in a SPECS Sage HR 100 spectrometer with a non-monochromatic X-ray source (Aluminum Ka line of 1486.6 eV energy and 300 W), placed perpendicular to the analyzer axis and calibrated using the $3d_{5/2}$ line of

Table S2: Average diameter measured by TEM of the JMNSs. Equivalent diameter (assuming spherical nanoparticles) and Feret's (equivalent to a maximum tip to tip distance of the TEM image)

Ag with a full width at half maximum (FWHM) of 1.1 eV. The selected resolution for the spectra was 10 eV of pass energy and 0.15 eV/step. All measurements were made in an ultra high vacuum (UHV) chamber at a pressure around $5 \cdot 10$ -8 mbar. The data was fitted using asymmetric and Gaussian-Lorentzian functions (after a Shirley background correction), where the FWHM of all the peaks were constrained while the peak positions and areas were set free.



Figure S6: XPS spectra of ND.20 sample. **a**) Fe 2p spectrum. The absence of satellite or shake-up peaks indicates that the sample is mainly Fe_3O_4 . Both FeO and Fe_2O_3 materials can be easily identified since they present satellite peaks at around 716 and 730, and 719 and 733 eV, respectively.^{1,2} **b**) Fe 3p fitted spectrum. The Fe^{3+}/Fe^{2+} theoretical ratio for pure Fe_3O_4 is 2/1. The Fe^{3+}/Fe^{2+} ratio for the sample is around 2.2, which is in agreement with the shape of the Fe 2p spectrum.

4 Fitting of magnetic plots

The magnetic plots were analyzed by comparing the experimental results to several standard models.³ Below is the detailed description of the Langevin and non-interacting models used to fit the experimental results, and the plots after fitting the models

4.1 <u>Non-interacting Super-paramagnetic model: fit of M(H)</u> <u>measurements</u>

The standard Langevin approach to the superparamagnetism (ideal SPM model) provides quantitative information about the size of the particles.⁴

The magnetization M of a magnetic nanoparticle system as a function of external field

H and temperature *T* is defined as:

$$M = \frac{NM_o\nu}{V} (\operatorname{coth} x - 1/x) = M_s L(x) \quad x = M_o \nu \mu_o H / K_B T,$$
(1)

where N is the number of nanoparticles, v volume of a nanoparticle, V volume of the system, M_{\circ} bulk saturation magnetization, and Lx the Langevin function.^{5,6}

In the Langevin model the nanoparticle sizes are assumed to be Gauss distributed around the mean hydrodynamic diameter D_h , and hence, the total magnetization of the system is not just Nv, but an integral over the measured size distribution:

$$\int_{0}^{max} L\left(\frac{M_{o}\nu\mu_{o}H}{K_{B}T}\right)$$

$$M = M_{s} \int_{0}^{max} L\left(\frac{M_{o}\nu\mu_{o}H}{K_{B}T}\right) f(D)dD$$
(2)

The magnetization of saturation, $[M_s] = A \cdot m^2$ (kg), is considered as a variable independent of the nanoparticles or domain magnetization, [M] = kA (m^3) and both variables are related by the inorganic content of the sample and density. Often, domain magnetization initially M is fixed to the theoretical expected value, and with the fit a value for the mean size and polydispersity of the magnetic core is provided. Thus, the fit of these measurements at room temperature provides the calculation of the mean size and the standard deviation of the magnetic nanoparticles.

4.2 Determination of Anisotropy Constant

- Fit of ZFC/FC measurements

A non-interacting model was used to fit the obtained experimental measurements.⁷ The population of magnetic nanoparticles (given by a size distribution fD) is divided in two groups at each temperature, depending on their nanoparticle size: the fraction in an ideal superparamagnetic state that corresponds to nanoparticles below a certain critical volume and those, above such limit, whose super spin remains blocked:

$$M_{ZFC}(T) = \int_{0}^{V_{c}(K_{eff},T)} M_{s}L\left(\frac{MVH}{k_{B}T}\right) f(V)dV + \int_{V_{c}(K_{eff},T)}^{\infty} M_{s}\frac{MH}{3K_{u,c}} f(V)dV$$
(3)

In the first term, the low energy barrier approximation is used, where the energy barrier (defined as $K_{eff} V$, being V the nanoparticle volume) is much smaller than the thermal energy ($k_B T$ where k_B is the Boltzmann Constant), and thus can be omitted. As a consequence, the response of the magnetization to changes of magnetic field or temperature (H or T) follows a Langevin function, where M is the particle magnetization (A/m in S.I.) and M_s is the experimental saturation magnetization (including non-magnetic mass contribution, in general). The experimental magnetization and the nanoparticle magnetization are allowed to decrease with temperature following a spin wave-like behaviour⁸ "Bloch type law" as:

$$M(T) = M(0)e^{-BT^{3/2}}$$
(4)

the Bloch constant (*B*) is obtained from the magnetization measurements as a function of temperature under the maximum field of 7 T, being between 2 and 4×10^{-5} in all cases. It is important to note that the anisotropy constant K is dependent of the temperature and it has to be taken into account to calculate the value.⁹

All the mentioned fittings are represented in Figure S6 (black curves).



Figure S7: Magnetic characterization of nanodumbbells (ND.16 and ND.20) and JMNSs (JMNS.16.25 and JMNS.20.41) showing hysteresis loops and ZFC-FC. Fittings of both measurements are represented by black lines.

5 MRI characterization



5.1 MRI relaxation times vs. concentration

Figure S8: Relaxation constants (1/T2 and 1/T1) as a function of iron concentration for the different JMNSs with a 16 nm iron oxide part. The black lines show the linear fittings of the different plots. The slope (relaxivity) and the coefficient of determination are expressed for every fitting.



Figure S9: Relaxation constants (1/T2 and 1/T1) as a function of iron concentration of the different JMNSs with a 20 nm iron oxide part. The black lines show the linear fittings of the different plots. The slope (relaxivity) and the coefficient of determination are expressed for each fitting.



5.2 r1 and r2/r1 graph

Figure S10: r1 relaxivity of JMNSs with 16 nm (brown symbols) and 20 nm (blue symbols) iron oxide parts.



Figure S11: r2/r1 relaxivity of JMNSs with 16 nm (brown symbols) and 20 nm (blue symbols) iron oxide parts.

6 CT attenuation pots

The experiments were performed starting with a highly concentrated solution, which was then successively diluted. The contrast efficiency was obtained by the slope after a regression line to a y = ax function. The graphs below represent the attenuation plots for every sample at three different energies.



JMNS.16.28





JMNS.20.43



Figure S12: CT attenuation experiments using 16 nm and 20 nm iron oxide JMNSs at three different energies (70 KeV, red circles; 80 KeV, blue squares; 100 KeV, green triangles).

7 Optical imaging



Figure S14: Control experiments showing a lack of Prussian blue staining (top) or scattered light (bottom), imaged using bright-field and dark-field microscopy respectively, after treatment of A549 cells with media alone, and thereafter stained and imaged using the same methods as for cells incubated with JMNSs. The lack of Prussian blue staining and scattered light confirms the specificity of both techniques for the visualization of iron oxide and gold respectively.

8 References

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