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Synthesis and Characterization of a Bifunctional Platform Based on Magnetite-Gold Nanoparticles for Theranostics of Cancer [†]

I. V. Chudosai [1,2], M. A. Sorokina [2], M. A. Abakumov [2,3] and N. L. Klyachko [1]

[1] Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russia; chudosay@gmail.com (I.V.C.); nlklyachko@gmail.com (N.L.K.)
[2] National University of Science and Technology (MISIS), Moscow, Russia; sorokina.marina.a98@gmail.com (M.A.S.); abakumov1988@gmail.com (M.A.A.)
[3] Pirogov Russian National Research Medical University, Department of Medical Nanobiotechnology, Moscow, Russia

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Abstract: One of the most interesting objects in terms of use in biomedicine are the hybrid structures based on magnetic nanoparticles (NPs) and NPs of noble metals which make it possible to simultaneously introduce two types of ligands onto the surface of NPs for their further use for photodynamic cancer therapy (PDT) (a combination of a photosensitizer (PS) for therapy and a fluorophore (FP) for platform detection). Synthesis and the research NPs Fe_3O_4-Au with "dumbbell" structure as the bifunctional platform for therapy of oncological diseases was the purpose of this work.

Keywords: nanomaterials; targeted therapy; magnetite; PDT; photosensitizer; fluorophore; FRET; theranostics

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1. Introduction

Recently, the synthesis and characterization of dimeric nanoparticles (NPs) as a bifunctional platform has attracted much research attention [1–4]. This structure provides two functional surfaces for the attachment of different types of molecules, which makes these nanoparticles particularly attractive as multifunctional probes for diagnostic and therapeutic purposes [5,6]. Dumbbell type NPs Fe_3O_4-Au are one such multifunctional system. They contain both gold and magnetite nanoparticles, which are biocompatible and widely used in biomedicine for optical and magnetic applications [7–10]. Compared to conventional single-component Au or Fe_3O_4 nanoparticles, dimeric NPs Fe_3O_4-Au systems have several advantages: (1) this structure contains both magnetic (Fe_3O_4) and optically active (Au) parts and is suitable for optical and magnetic monitoring simultaneously; (2) the presence of two surfaces in NPs Fe_3O_4-Au enables the attachment of different chemical functional groups; (3) both magnetite and gold dimensions can be controlled to optimize magnetic and optical properties.

All the above advantages of NPs Fe_3O_4-Au enable their use as a bifunctional platform. In a number of publications [1,7,9] they are proposed to be used for the treatment of cancer by hyperthermia, transportation and targeted drug delivery, enhancement of image contrast in MR imaging, photodynamic therapy (PDT), etc.

The advantage of nanoparticles is determined by their size, restored with the size of intracellular biological objects. A high ratio of surface area to volume of nanoparticles is immediately detected on their basis of complex nanoplatforms, is registered in therapeutic and diagnostic areas. One of such platforms is the magnetite-gold bifunctional platform. Due to their magnetic properties, magnetic nanoparticles can be targeted and held at the site of action of an external magnetic field, which is used for magnetic hyperthermia and targeted drug delivery. Magnetite nanoparticles measure significant superparamagnetic properties in significant quantities and can be used as an indicator of a contrast agent in magnetic resonance imaging (MRI). Gold nanoparticles are special

Relevant for diagnostics is the detection of a pronounced plasmon resonance. Processing gold nanoparticles is easy to functionalize sulfur-containing ligands-thiols and disulfides due to the formation of a strong sulfur-gold covalent bond. Combining magnetite and gold nanoparticles into one the system certainly uses each of the types of nanoparticles. The bifunctional magnetite-gold system due to external modifications of two types of ligands can be used simultaneously for photodynamic therapy of cancer (PDT) and fluorescent diagnostics.

Therefore, the aim of this work is to synthesize and characterize dumbbell-shaped modified dimeric NPs Fe_3O_4-Au containing a photosensitizer (PS) on magnetite surface and fluorophore (FP) on gold surface to enable the combined use of photodynamic therapy (PDT) and fluorescent diagnosis (FD) for cancer therapy.

2. Materials and Methods

A scheme of stepwise modification of NPs Fe_3O_4-Au bifunctional platform is shown in Figure 1.

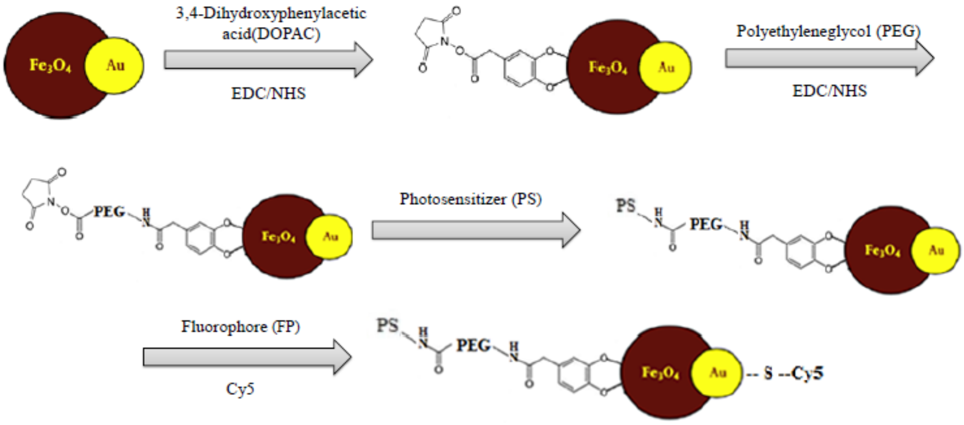

Figure 1. Systematic synthesis diagram of a bifunctional platform.

In the first stage the synthesis of dimeric NPs Fe_3O_4-Au was carried out by joint thermal decomposition of $Fe(CO)_5$ and $HAuCl_4$ (with in situ formed Au particles) following the methodology taken from [11] with some modifications. Magnetite nanoparticles epitaxially grown on gold nanoparticles can be produced by heating to 120 °C in an inert gas atmosphere while stirring a mixture of 1-octadocene, oleic acid, oleylamine and 1,2-hexadecanediol, introduction of iron pentacarbonyl into the mixture, incubation of the resulting mixture followed by introduction of a solution containing a mixture of gold chloride-hydrate and oleylamine in 1-octadocene, preheated in an inert gas atmosphere, reheating to a temperature of 300–310 °C, keeping the heated mixture at 300–310 °C for 30–45 min, its subsequent cooling to room temperature, held in an inert gas atmosphere, keeping the mixture in the presence of air, adding monatomic alcohol to the mixture and separation of nanoparticles magnetite-gold by centrifugation.

A further synthesis step to obtain stable aqueous solutions of magnetite-gold nanoparticles involves stabilization carried out in several steps: The magnetite surface is covalently bonded to DOPAC (3,4-dihydroxyphenylacetic acid) with further activation by carbodiimide (EDC/NHS) method further stabilized by PEG (polyethylene glycol with

COOH group) with further activation of the carboxyl group by carbodiimide (EDC/NHS) method.

To functionalize the surface of NPs Fe_3O_4-Au, a FRET pair of chromophores was selected: a bacteriophosphorbide series photosensitizer (Figure 2, left) and a cyanine series fluorophore—(Cy5) (Figure 2, right). The photosensitizer is immobilized by chemical conjugation to the stabilized magnetite surface. Derivative of fluorescent dye Cy5 containing disulfide bond is immobilized on gold surface by thiol group, namely, by using NHS ester of fluorescent dye Cy5 with cystamine dihydrochloride with disulfide bond formation and further «cross-linking» to gold surface by thiol group.

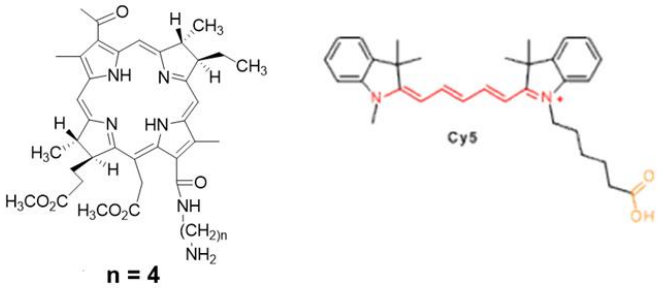

Figure 2. (**a**) photosensitizer (**b**) fluorophore (right).

3. Results

As a result of the synthesis dimeric dumbbell-shaped NPs Fe_3O_4-Au were obtained. The study of the crystal structure of dimeric NPs Fe_3O_4-Au by X-ray phase analysis yielded the following results. As can be seen from a diffraction picture (Figure 3) the relative intensity of all peaks as well as their positions in the diffraction patterns are in complete agreement with the structure of Fe_3O_4 magnetite (ICDD PDF-2 №00–019–0629), the same is true for the structure of Au gold (ICDD PDF-2 №03–065-8601) (yellow spheres). It should be noted that 100% correspondence of the spectrum «magnetic» component of all samples of low frequency Fe_3O_4-Au to a type of cubic structure of spinel Fd-3m (magnetite type structure) is observed. Lattice parameters, crystallite sizes and volume fraction were calculated for each phase from X-ray spectra (Table 1).

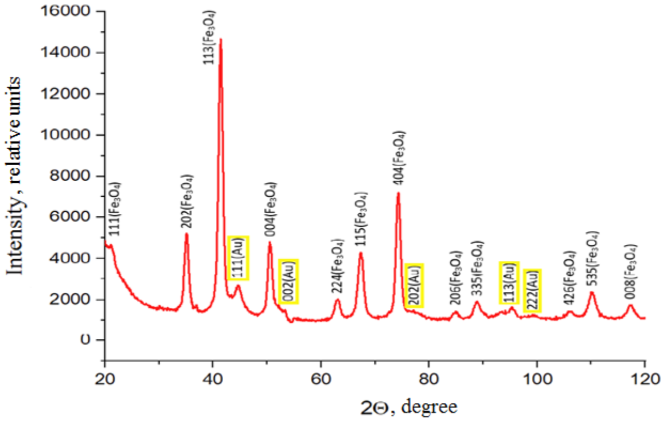

Figure 3. X-ray diffraction of Fe_3O_4-Au.

Table 1. Size, morphology and structure of NPs Fe_3O_4-Au characterized by X-ray phase analysis and TEM.

TEM			X-ray Phase Analysis					
d, nm		**Particle Shape**	**Volume Fraction,%**		**Crystallite Size, nm**		**Crystal Lattice Period,nm**	
Fe_3O_4	**Au**		**Fe_3O_4**	**Au**	**Fe_3O_4**	**Au**	**Fe_3O_4**	**Au**
11	4	Spherical	99.3 ± 2.0	0.7 ± 0.2	12 ± 2.0	5 ± 1.0	0.8387 ± 0.0004	0.4064 ± 0.0004

The microphotographs (TEM) (Figure 4) show gold nanoparticles (darker) and magnetite nanoparticles (lighter) in pairs. In addition, it can be seen that the obtained sample does not contain aggregates of nanoparticles, as well as free nanoparticles of magnetite and gold. Based on the data presented, we can conclude that the synthesis and subsequent purification resulted in the production of dimeric dumbbell-like NPs Fe_3O_4-Au with «double» chemistry, which, in turn, indicates the possibility of further obtaining nanoparticles with heterobifunctional surface properties.

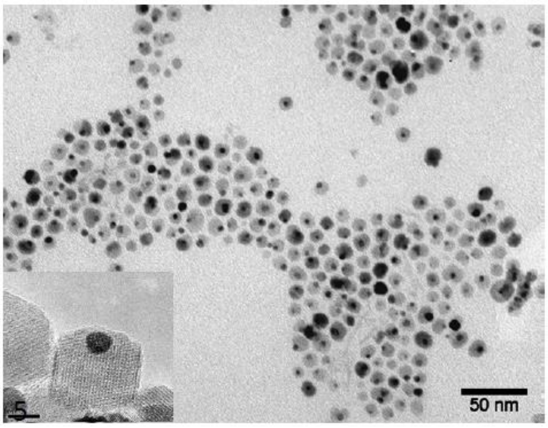

Figure 4. TEM micrograph of dimeric NPs Fe_3O_4-Au and high-resolution micrograph.

According to the results of calculations, the average diameter of magnetite and gold particles was 11 ± 1.5 nm and 4 ± 1 nm, respectively (Figure 5).

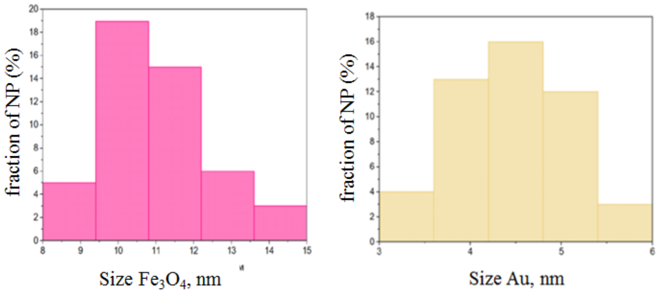

Figure 5. Fe_3O_4 and Au diameter size distribution for dimeric Fe_3O_4-Au nanoparticles.

The investigation of the structure of NPs Fe_3O_4-Au by high-resolution TEM and X-ray analysis revealed that the particles have defect-free single crystalline structure.

The investigation of the magnetostatic properties of Fe_3O_4-Au particles was carried out by hysteresis loop measurements at 300 K (Figure 6). The obtained values of saturation magnetization and coercive force were 62.3 ± 2.0 and 14 ± 2 mTl, respectively.

The value of coercive force based on the data in Figure 4 was less than 15 mT (14 mT), hence, the synthesized NPs Fe_3O_4-Au lowers can be considered as superparamagnetic. It is known that the saturation magnetization of nanosized magnetite [10] is 70–80 e.m.u./g, while that of magnetite is between 40 e.m.u./g. Thus, it can be assumed that the main phase in the studied sample is magnetite and not maghemite.

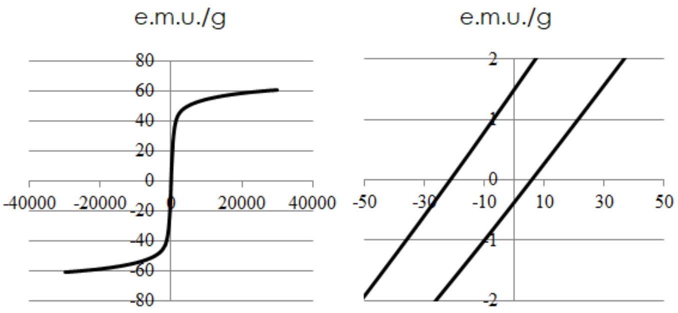

Figure 6. Magnetic properties of hybrid NP Fe_3O_4-Au: (**a**) hysteresis loops of ensamples at MF ± 2 Tesla at T = 300 K and (**b**) their magnified near-zero regions.

At each stage of the synthesis and modification of NPs Fe_3O_4-Au by dynamic light scattering, the particle size distribution was investigated, the values of which are given in Table 2.

Table 2. Hydrodynamic diameter.

Sample	**Fe_3O_4-Au/ DOPAC**	**Fe_3O_4-Au/ DOPAC/PEG**	**Fe_3O_4-Au/ DOPAC/PEG/PS**	**Fe_3O_4-Au/ DOPAC/PEG/FP**	**Fe_3O_4-Au/ DOPAC/PEG/PS/FP**
Size, nm	23.36	24.47	25.76	26.01	25.99
PDI	0.239	0.233	0.329	0.275	0.299

After the steps of conjugation with PS and FP, the absorption spectra of the Fe_3O_4-Au/ DOPAC/PEG/PS and Fe_3O_4-Au/ DOPAC/PEG/FP platforms and the spectra of pure solutions with PS and FP (DMSO solvent) were recorded. Using the Fe_3O_4-Au/ DOPAC/PEG/PS system as an example, the absorption spectrum of this system with respect to the spectrum of pure PS/DMSO (Figure 7) is presented.

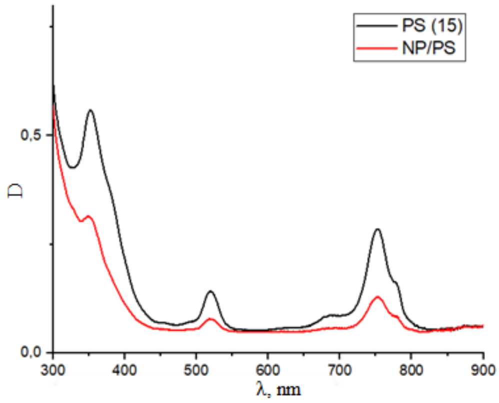

Figure 7. Absorption spectrum of the Fe_3O_4-Au/ DOPAC/PEG/PS system with respect to the spectrum of pure PS/DMSO.

The values of the maxima of the pure PS absorption spectra correlate with the Fe_3O_4-Au/ DOPAC/PEG/PS values (360 nm, 530 nm, 750 nm respectively). From which we can conclude about the effective covalent conjugation of PS to the magnetic surface of NPs.

In vitro studies were performed on CT26 colon cancer cells (Figure8) with an exposure time of up to 120 min, which showed that the Fe_3O_4-Au/PS and Fe_3O_4-Au/FP systems exhibit localized emission of fluorescence in their respective wavelength range in the time interval up to 120 min.

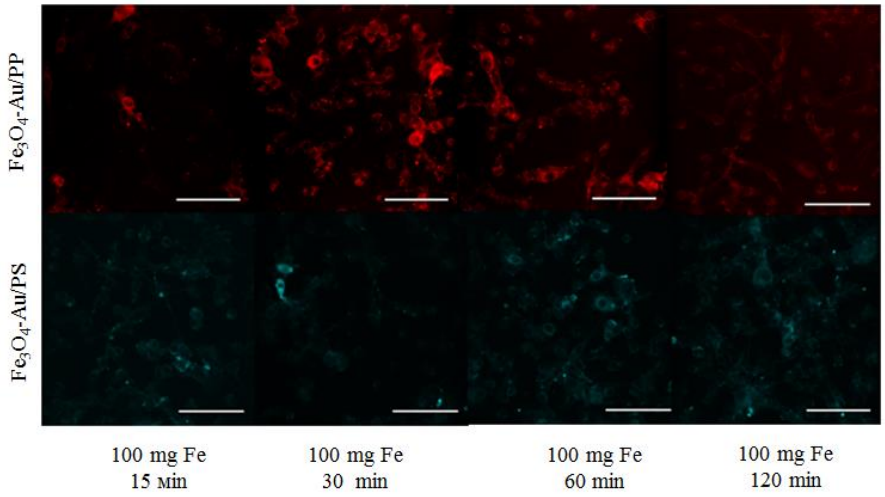

Figure 8. In vitro studies (CT26).

4. Discussion

Depending on the temperature and size, ferro- and ferrimagnetic particles can be in a multi-domain or single-domain ferromagnetic state, and at d = 10–20 nm in a superparamagnetic state. The latter, the most important for magnetic hyperthermia, is characterized by the magnetic ordering of atomic magnetic moments inside each magnetic nanoparticle. In this study, we synthesized a platform based on hybrid magnetite-gold nanoparticles with a size of about 15 nm. Such hybrid NPs have many advantages due to biocompatibility, magnetic properties and the presence of two surfaces that can be modified. Previously, it was shown that such NPs have proven themselves as contrast agents for MRI [13]. The presence of two surfaces, which increases the variability of various modifications, makes these NPs ideal candidates for use as targeted drug delivery platforms, and is also capable of providing a synergistic effect of magnetic hyperthermia and photothermal/photodynamic therapy due to the potential combination of their magnetic and plasmonic properties, provided that the size ratio Fe_3O_4 and Au components are matched to the hybrid structure.

The study of the magnetic properties of the synthesized Fe_3O_4-Au NPs at room temperature showed that NPs could be considered superparamagnetic. According to the magnetization curves (Figure 6), in a field of 7 mT, the magnetization of NPs is 7 $Am^2\ kg^{-1}$. Given that NPs form conglomerates in an aqueous solution or culture medium, the total magnetization per conglomerate is higher. Thus, NP-FPs respond to various types of low frequency (rotating/alternating) and constant magnetic fields in a magneto-optical system.

The main problem in the development of (bis)chromophore systems is the occurrence of a process of resonant energy transfer between the components of the conjugates, depending on the direction of which either the photodynamic or fluorescent properties of the systems deteriorate. Therefore, today the task is to find ways to minimize energy transfer.

The essence of the FRET (Fluorescence Resonance Energy Transfer) effect is that when two objects (donor and acceptor) approach each other and the fluorescence spectrum of the first one overlaps with the absorption spectrum of the second, energy is transferred nonradiatively, without emission of photons [11].

Energy transfer is possible not for any, but for strictly defined pairs of donors and acceptors. The main requirement for a donor-acceptor pair is the overlapping of the spectra. The donor must emit light of those wavelengths that the acceptor can absorb.

Another important criterion is the distance between the donor and the acceptor - the efficiency of energy transfer depends on the distance inversely proportional to the sixth power of the distance between them. The optimal distance between the donor and the acceptor can be determined by calculating the value of the Foerster radius, which is the maximum distance between the donor and acceptor molecules at which the FRET energy transfer will occur at the level of 50%.

To implement PDT, the photosensitizer and fluorophore must be selected in such a way that the pair is exchanged minimally via the donor-acceptor mechanism, otherwise, the fluorescent characteristics of the system deteriorate. Therefore, it is necessary to choose a pair of PS and FP for which the critical Foerster radius would be minimal. For this, it is necessary to calculate the quantum yield of generation of singlet oxygen by the photosensitizer, the quantum yield of fluorescence by the fluorophore, and the efficiency of energy transfer.

In order to select a FRET pair of PS and FP, the following parameters must be taken into account: determine the quantum yield of PS singlet oxygen generation, determine the FF fluorescence quantum yield, and calculate the energy transfer efficiency.

At this stage of the research, the minimum radius of Ferster was calculated with the formula [12]:

$$R_0^6 = \frac{9000 \ln 10 k^2 \varphi_{D,0}^{fl}}{128\pi^2 N_A n^4} \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \tag{1}$$

The calculated minimum Forster radius was 14.64 Å. The size of the synthesized particles was 15 nm, which is a satisfactory parameter to level out the possibility of energy transfer from PS to FP.

For further research, it is planned to carry out experiments and calculate:

- fluorescence quantum yields of PS and PP using the formula

$$\varphi^{fl} = \varphi_R^{fl} \cdot \frac{S \cdot (1-10^{-AR}) \cdot n^2}{S_R \cdot (1-10^{-A}) \cdot n_R^2} \tag{2}$$

- by the chemical trap method, the singlet oxygen generation for the selected PS
- FRET energy transfer efficiency using the formula

$$k_{FRET} = \frac{1}{\tau} \cdot \left(\frac{R_0}{r} \right)^6 \tag{3}$$

Further investigation and characterization of the Fe_3O_4-Au/ DOPAC/PEG/PS/FP system and the Fe_3O_4-Au/ DOPAC/PEG/PS and Fe_3O_4-Au/ DOPAC/PEG/FP conjugates in vivo are also planned.

5. Conclusions

1. The method of joint thermal decomposition of iron pentacarbonyl and gold tetrachloraurate allows to obtain hybrid NP magnetite-gold of size 11 ± 1.5 nm Fe_3O_4 and 4 ± 1 nm, stabilized by oleic acid;

2. A FRET pair of PP/PS (PS, n = 4/Cy5) is selected and the optical properties are studied;

3. The modification of the NPs with DOPAC and PEG followed by EDC/NHS activation allows for an efficient bonding of the PS to the magnetic surface of the NCs in a two-phase system (water-DMSO);

4. The immobilization of the FP disulphide derivative on the gold surface of the NPs is done by the thiol group;

5. The efficiency of the Fe_3O_4-Au/PS and Fe_3O_4-Au/FP systems has been demonstrated by in vitro studies.

References

1. Yu, H.; Chen, M.; Rice, P.M.; Wang, S.X. Dumbbell-like bifunctional Au-Fe_3O_4 nanoparticles. *Nano Lett.* **2005**, *5*, 379–382.
2. Teranishi, T.; Inoue, Y.; Nakaya, M.; Oumi, Y.; Sano, T. Nanoacorns: Anisotropically Phase-Segregated CoPd Sulfide Nanoparticles. *J. Am. Chem. Soc.* **2004**, *126*, 9914–9915.
3. Shi, W.L.; Zeng, H.; Sahoo, Y.; Ohulchanskyy, T.Y.; Ding, Y. A general approach to binary and ternary hybrid nanocrystals. *Nano Lett.* **2006**, *6*, 875–881.
4. Glasser, N.; Adams, D.J.; Krausch, G. Janus particles at liquid-liquid interfaces. *Langmuir* **2006**, *22*, 5227–5229.
5. Gu, H.W.; Yang, Z.M.; Gao, J.H.; Chang, C.K.; Xu, B. Heterodimers of nanoparticles: formation at a liquid-liquid interface and particle-specific surface modification by functional molecules. *J. Am. Chem. Soc.* **2005**, *127*, 34–35.
6. Choi, J.S.; Jun, Y.W.; Yeon, S.I.; Kim, H.C. Biocompatible Heterostructured Nanoparticles for Multimodal Biological Detection. *J. Am. Chem. Soc.* **2006**, *128*, 15983.
7. Sokolov, K.; Follen, M.; Aaron, J.; Pavlova, I.; Malpica, A. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res*. **2003**, *63*, 1999–2004.
8. Schultz, D.A. Plasmon resonant particles for biological detection. *Curr. Opin. Biotechnol.* **2003**, *14*, 13–22.
9. El-Sayed, I.H.; Huang, X.; El-Sayed, M.A. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Lett.* **2005**, *5*, 829–834.
10. Gupta, A.K.; Naregalkar, R.R.; Vaidya, V.D.; Gupta, M. Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine* **2007**, *2*, 23–39.
11. Ushakova, E.V. Energy transfer of photoexcitation in the systems of quantum dots. *Sci. Tech. Bull. Inf. Technol. Mech. Opt.* **2008**, *51*, 283-288.
12. Yu, H.; Chen, M.; Rice, P.M.; Wang, S.X.; White, R.L.; Sun, S. Dumbbell-like Bifunctional Au Fe_3O_4 Nanoparticles. *Nano Lett.* **2005**, *5*, 379–382.
13. Efremova, M.V.; Naumenko, V.A.; Spasova, M.; Garanina, A.S.; Abakumov, M.A.; Blokhina, A.D.; Melnikov, P.A.; Prelovskaya, A.O.; Heidelmann, M.; Li, Z.-A.; et al. Magnetite-Gold nanohybrids as ideal all-in-one platforms for theranostics. *Sci. Rep.* **2018**, *8*, 11295.