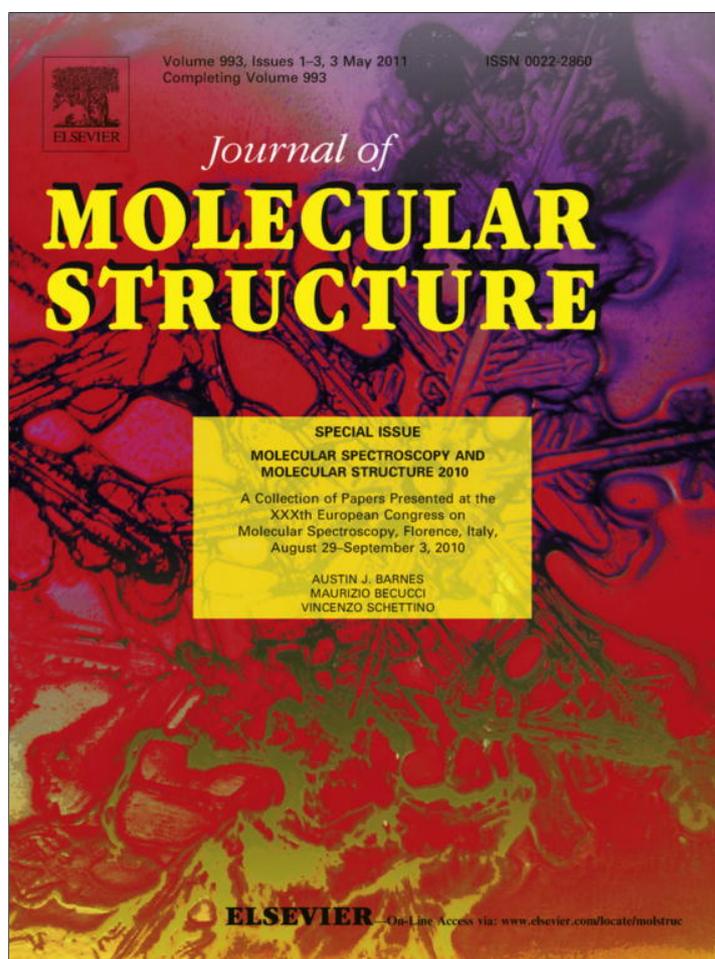


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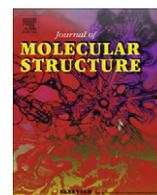
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Journal of Molecular Structure

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Fluorescent monolayer protected gold nanoparticles – Preparation and structure elucidation

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ARTICLE INFO

Article history:

Available online 14 December 2010

Keywords:

1,8-Naphthalimide derivatives
Monolayer Protected Clusters
Gold nanoparticles
Chemical reduction
Organic fluorophores

ABSTRACT

A novel N-substituted 4-methoxy-1,8-naphthalimide (NAFTA 8) especially designed for fluorescent labeling of gold nanoparticles has been synthesized. NAFTA 8 bears a long methylene chain at the imide N atom and has a terminal SH group, which enables its chemical binding to gold nanostructures. The longest wavelength absorption maximum of NAFTA 8 in chloroform is at 370 nm, the fluorescent maximum is at 430 nm and the fluorescent quantum yield is 0.95. The newly synthesized fluorophore is applied for functionalization of gold nanoparticles with diameter 1.5 ± 0.5 nm prepared through chemical reduction. The obtained Monolayer Protected Clusters are characterized by elemental analysis, TEM, XPS, FT-IR, absorption and fluorescence spectroscopy. The performed investigations provide evidence for the formation of chemical bond between the thiol ligand and the gold surface. They also show that the obtained metal/dielectric 3D structures are highly fluorescent.

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1. Introduction

Over the past few years, an increasing interest has been directed toward the preparation and the characterization of noble metal nanoparticles. This interest is due to their specific size-dependent optical, electronic, and chemical properties, which make them applicable in different fields of nanotechnology [1].

Once prepared, noble metal nanoparticles are not stable and tend to aggregate. The main procedure for their stabilization is modification of the surface with organic compounds such as thiols, amines, disulfides, nitriles, carboxylic acids and silanes [2,3]. The application of organosulfur compounds as modifying agents is one of the predominantly developed methods since their derivatives in thio-form (R-S-S-R or R-SH) are able to form spontaneously chemical bonds with noble metal surfaces [2,3]. As a result, Monolayer Protected Clusters (MPCs) also called core-shell structures are obtained, where the core is a noble metal nanoparticle and the shell (or the monolayer) consists of organic ligands [2].

Noble metal nanoparticles modified with organic fluorophores are of particular importance for fabrication of new fluorescent materials for bio- and chemical sensing [1,4,5]. For a long period, it was accepted that gold and silver nanoparticles strongly quench the emission of fluorophores attached to their surface through an energy-transfer mechanism [6–9]. However, recent studies on the photophysical properties of linked gold nanoparticles (AuNPs)

with fluorescent compounds like porphyrine [10], pyrene [3,11] and fluorescein [12] show a strong suppression in the emission quenching.

The nature of the interactions between noble metal nanoparticles and organic fluorophores is still not totally understood. These interactions, which depend on various factors, including the size of the nanoparticles, the distance between the metal surface and the fluorophore moiety and the spectral overlap between the fluorophore's emission and the nanoparticle's surface plasmon absorption band, often result in an enhancement or a quenching of the fluorescence [13–16]. Additional investigations are needed in order to obtain new effective organic fluorescent markers for AuNPs and to shed light on the factors affecting the fluorescent ability of the obtained MPCs.

The investigations described in the present paper comprise design, synthesis, structure elucidation and photophysical study of MPCs of AuNPs modified with new organic fluorophore aiming at the preparation of highly fluorescent metal/dielectric 3D structures.

2. Experimental

2.1. General

Thin layer chromatography (TLC) used aluminum sheets pre-coated with silica gel 60 F₂₅₄ (Merck). Flash column chromatography was carried out using silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, Merck). Commercially available solvents for TLC and column chromatography were used after distillation – hexane, diethyl ether

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(Et₂O), dichloromethane (DCM). Melting points were determined in capillary tubes on an Electrothermal MEL-TEMP 1102D-230 VAC apparatus without corrections. FT-IR spectra of NAFTA 8, hexadecanethiol and the MPCs were measured pressed into a KBr disk. The spectra were recorded on Bruker IFS 113v, in cm⁻¹ at 64 scans. The NMR spectra were recorded at 300 K on a Bruker Avance DRX-250 (250.13 for ¹H and 62.90 MHz for ¹³C) and at 293 K on a Bruker Avance II+ 600 (600.13 for ¹H and 150.92 MHz and for ¹³C NMR) spectrometers with TMS as internal standard for chemical shifts (δ , ppm). ¹H spectra were calibrated to the signal of TMS ($\delta = 0.0000$). ¹³C spectra were calibrated to the signal of the solvent (CDCl₃, $\delta = 77.0000$). The following additional NMR techniques were used: DEPT 135, COSY, HSQC and HMBC. ¹H and ¹³C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, identification, and coupling constants (in Hz). For numbering of the atoms see Scheme 1. Mass spectra (MS) were measured with a Thermo Scientific High Resolution Magnetic Sector MS DFS by chemical ionization (CI), and were reported as fragmentation in *m/z* with relative intensities (%). Elemental analyses were performed by the Microanalytical Laboratory for Elemental Analysis of the Institute of Organic Chemistry, Bulgarian Academy of Sciences. The following starting chemicals were commercially available (from Sigma–Aldrich or Fluka): 4-Bromo-1,8-naphthalic anhydride, 8-aminooctanoic acid, sodium methoxyde, triphenylphosphine (PPh₃), iodine (I₂), hexamethyldisilathiane (Me₃Si)₂S, tetrabutylammonium fluoride trihydrate (TBAF·3H₂O), 1-hexadecanethiol (HDT), gold(III) chloride trihydrate (HAuCl₄·3H₂O), sodium borohydride NaBH₄, tetraoctylammonium bromide (TOAB), methanol (MeOH), ethanol (EtOH).

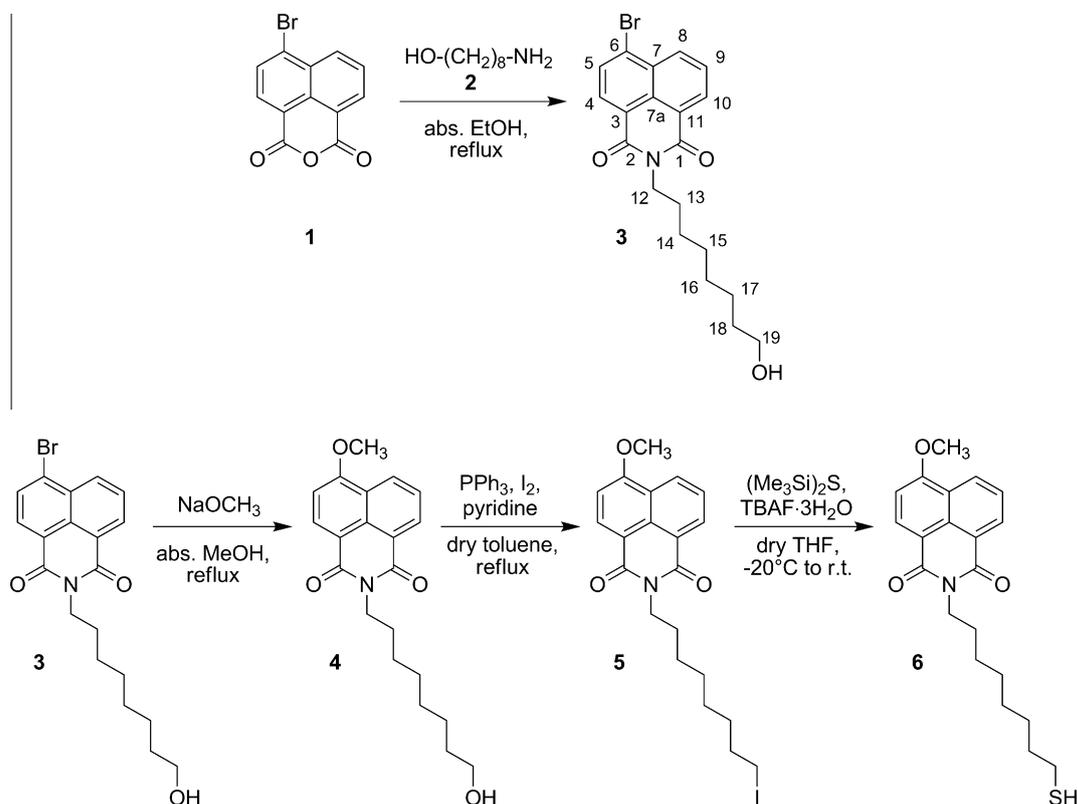
The TEM investigations were performed by TEM JEOL 2100 with an accelerating voltage of 200 kV. The specimens were prepared by grinding and dispersing them in n-heptane by ultrasonic treatment for 2 min. The suspensions were dropped on standard carbon films/Cu grids and drying in air for at least 45 min.

The XPS measurements were carried out in the UHV chamber of the electron spectrometer ESCALAB-MkII (VG Scientific) with a base pressure of about 1.10⁻¹⁰ mbar (during the measurement 1 × 10⁻⁹ mbar). The photoelectron spectra were obtained using unmonochromatized AlK α ($h\nu = 1486.6$ eV) radiation. Passing through 6 mm slit (entrance/exit) of a hemispherical analyzer, electrons with energy 20 eV were detected by a channeltron. The instrumental resolution measured as the full width at a half-maximum (FWHM) of the Ag3d_{5/2}, photoelectron peak is about 1 eV. The energy scale was corrected to the C1s – peak maximum at 285 eV for electrostatic sample charging. The fitting of the recorded XPS spectra was performed, using a symmetrical Gauss–Lorentzian curve fitting after Shirley-type subtraction of the background.

The absorption spectra were scanned on a Perkin Elmer Lambda 25 UV–Vis spectrophotometer and the corrected fluorescence spectra – on a Perkin Elmer LS 55 spectrofluorimeter. The fluorescent quantum yield (*Q_F*) was determined relative to that of 3-*p*-methoxyphenylmethylene-1(3*H*)-isobenzofuranone (*Q_F* = 0.12 in ethanol) [17]. Fluorescence decay measurements were performed using the time-correlated single photon counting (TCSPC) technique. Excitation was carried out using <90 ps pulses generated with a laser diode at 395 nm (PicoQuant model LDH-PC-400B) and fluorescence was detected at magic angle. The full width at half-maximum (FWHM) of the instrument response function (IRF) was around 200 ps [18]. The solvents used were of fluorescent grade.

2.2. Synthesis of 1,8-naphthalimide derivatives

The new fluorescent naphthalimide thiol **6** containing eight membered aliphatic chain was synthesized in four steps (Scheme 1). The first step included condensation of 4-Bromo-1,8-naphthalic anhydride (**1**) with 8-aminooctan-1-ol (**2**) (preliminary prepared through reduction of methyl ester of 8-aminooctanoic acid [19]).



Scheme 1. Four-step synthesis of thiol ligand NAFTA 8.

The reaction was carried out selectively in refluxing absolute EtOH, avoiding participation of the aromatic bromine [20,21]. After column chromatography the pure 1,8-naphthalimide **3** was isolated and in the second step refluxed with NaOMe in absolute methanol to substitute the aromatic bromine atom of **3** with methoxy group [22], without catalyst. This reaction is possible when at least one electron withdrawing substituent is attached to the aromatic system (in case of **3** – two carbonyl groups are attached), causing decreasing of Ar–Br bond energy. The product **4** was obtained in excellent yield after column chromatography. Conversion of the aliphatic hydroxy group of **4** to halogenide **5** was performed successfully under the conditions of the Mukaiyama reaction (with some modifications) [23,24]. At the last step, iodide **5** was converted to the desired thiol **6** using hexamethyldisilathiane in mild and high yielded procedure [25]. The product **6** was purified by column chromatography. It is very important to obtain **6** without any impurities (especially fluorescent), which can compromise the further application of **6** as a ligand for derivatization of gold nanoparticles and the photophysical investigations.

To the best of our knowledge, all compounds **3–6** are new. They were purified by column chromatography and fully characterized by melting points, ¹H and ¹³C NMR, elemental analysis. For compounds **5** and **6** MS spectra (CI) were also recorded.

2.2.1. Synthesis of 6-bromo-2-(8-hydroxyoctyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3**)

To a suspension of 4-Bromo-1,8-naphthalic anhydride (**1**) (1.00 g, 3.61 mmol) in 60 ml abs. EtOH 8-aminoctan-1-ol (**2**) (0.57 g, 4.33 mmol) was added. The mixture was refluxed for 8 h (reaction monitoring by TLC – hexane:Et₂O = 1:4). The solvent was evaporated *in vacuo* and crude product was purified by column chromatography (80 g silica gel, phase DCM:Et₂O = 10:1) to obtain 1.04 g (71%) of **3** as white crystals. m.p. 98–99 °C. ¹H NMR (600.13 MHz, CDCl₃, 293 K): δ = 8.63 (d, 1H, 10-H, J = 7.3 Hz), 8.53 (d, 1H, 8-H, J = 8.5 Hz), 8.38 (d, 1H, 4-H, J = 7.8 Hz), 8.01 (d, 1H, 5-H, J = 7.8 Hz), 7.83 (t, 1H, 9-H, J = 7.8 Hz), 4.15 (m, 2H, 12-H), 3.64 (t, 2H, 19-H, J = 6.7 Hz), 1.72 (td, 2H, 13-H, J = 15.3, 7.6 Hz), 1.57 (m, 2H, 18-H), 1.48 (br s, 1H, OH), 1.30–1.45 (m, 8H, 14-H, 15-H, 16-H, 17-H). ¹³C NMR (150.92 MHz, CDCl₃, 293 K): δ = 163.54 (1C, C=O), 163.52 (1C, C=O), 133.14 (1C, 8-C), 131.95 (1C, 10-C), 131.14 (1C, 4-C), 131.02 (1C, 5-C), 130.50 (1C, 7-C), 130.14 (1C, 6-C), 128.87 (1C, 7a-C), 128.01 (1C, 9-C), 123.02 (1C, 11-C), 122.16 (1C, 3-C), 62.95 (1C, 19-C), 40.52 (1C, 12-C), 32.68 (1C, 18-C), 29.19 (1C, CH₂), 29.18 (1C, CH₂), 27.93 (1C, 13-C), 26.94 (1C, 15-C), 25.60 (1C, 16-C). Anal. calcd. for C₂₀H₂₂BrNO₃ (404.30): C, 59.42; H, 5.48; Br, 19.76; N, 3.46. Found: C, 59.47; H, 5.45; Br, 19.71; N, 3.48%.

2.2.2. Synthesis of 2-(8-hydroxyoctyl)-6-methoxy-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4**)

To a solution of **3** (3.50 g, 8.66 mmol) in 80 ml abs. methanol NaOMe (1.40 g, 25.97 mmol) was added. The mixture was refluxed for 22 h (reaction monitoring by TLC – DCM:Et₂O = 5:1). The solvent was evaporated *in vacuo* and aqueous citric acid (70 ml) was slowly added. The water phase was extracted with DCM. The organic phase was separated, dried over anhydrous Na₂SO₄ and evaporated *in vacuo*. The crude product was purified by column chromatography (85 g silica gel, phase DCM:Et₂O = 8:1, then 4:1) to obtain 2.81 g (91%) of **4** as light yellow crystals. m.p. 99–100 °C. ¹H NMR (600.13 MHz, CDCl₃, 293 K): δ = 8.60 (dd, 1H, 10-H, J = 7.3, 1.2 Hz), 8.55 (br d, 2H, 4-H, 8-H, J = 8.3 Hz), 7.70 (dd, 1H, 9-H, J = 8.3, 7.3 Hz), 7.04 (d, 1H, 5-H, J = 8.3 Hz), 4.15 (m, 2H, 12-H), 4.13 (s, 3H, OCH₃), 3.63 (t, 2H, 19-H, J = 6.7 Hz), 1.72 (td, 2H, 13-H, J = 15.3, 7.6 Hz), 1.56 (m, 2H, 18-H), 1.30–1.46 (m, 8H, 14-H, 15-H, 16-H, 17-H). ¹³C NMR (150.92 MHz, CDCl₃, 293 K): δ = 164.53 (1C, 1-C), 163.97 (1C, 2-C), 160.75 (1C, 6-C), 133.41

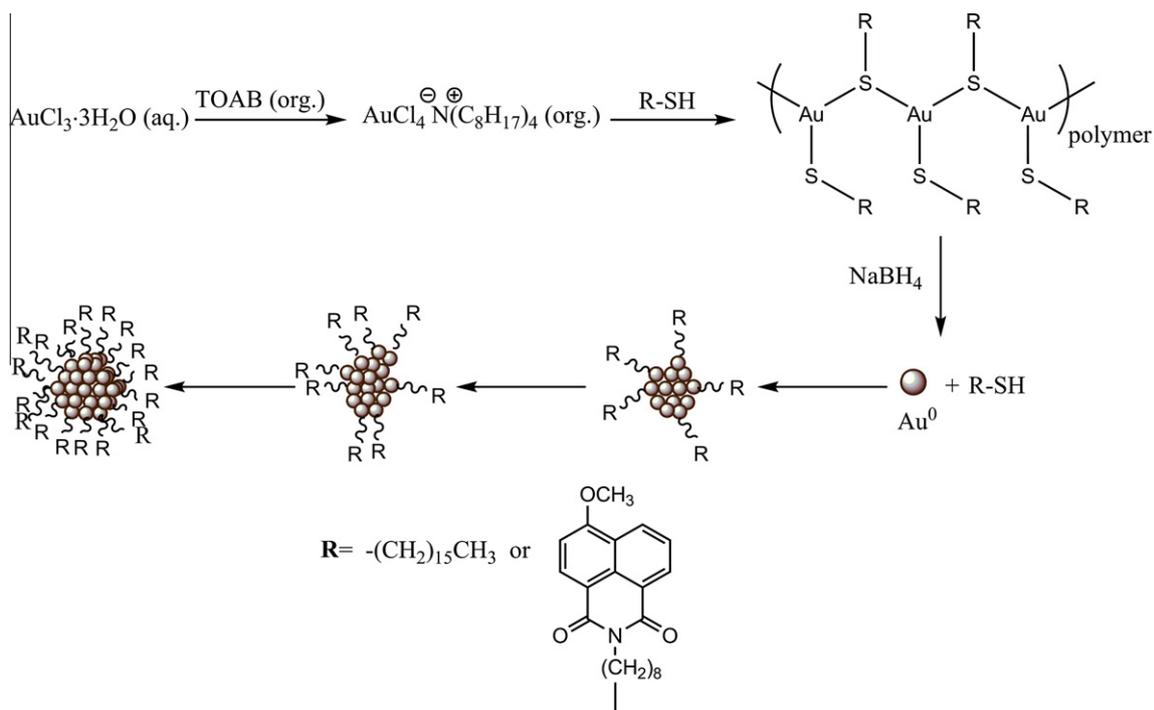
(1C, 4-C), 131.51 (1C, 10-C), 129.33 (1C, 7a-C), 128.58 (1C, 8-C), 125.91 (1C, 9-C), 123.45 (1C, 7-C), 122.41 (1C, 11-C), 115.11 (1C, 3-C), 105.15 (1C, 5-C), 63.02 (1C, 19-C), 56.17 (1C, OCH₃), 40.26 (1C, 12-C), 32.72 (1C, 18-C), 29.23 (1C, CH₂), 29.21 (1C, CH₂), 28.04 (1C, 13-C), 26.99 (1C, 15-C), 25.61 (1C, 16-C). Anal. calcd. for C₂₁H₂₅NO₄ (355.43): C, 70.96; H, 7.09; N, 3.94. Found: C, 70.99; H, 7.10; N, 3.92%.

2.2.3. Synthesis of 2-(8-iodooctyl)-6-methoxy-1H-benzo[de]isoquinoline-1,3(2H)-dione (**5**)

To a solution of **4** (2.83 g, 7.96 mmol) in 50 ml dry toluene were added consequently iodine (2.42 g, 9.55 mmol), Ph₃P (2.62 g, 9.95 mmol) and dry pyridine (1.60 ml, 19.91 mmol). The resulting mixture was refluxed under inert atmosphere for 1.5 h (reaction monitoring by TLC–DCM). After cooling the reaction mixture was filtered and the solid residue was washed with toluene. The combined filtrate was washed with aqueous Na₂S₂O₃ and brine, dried over anhydrous Na₂SO₄ and evaporated *in vacuo*. The crude product was purified by column chromatography (150 g silica gel, phase DCM:cyclohexane = 1:3, then 1:1) to obtain 2.96 g (80%) of **5** as light yellow crystals. m.p. 96–97 °C. ¹H NMR (250.1 MHz, CDCl₃, 300 K): δ = 8.56 (dd, 1H, 10-H, J = 7.3, 1.2 Hz), 8.55 (m, 2H, 4-H, 8-H), 7.69 (dd, 1H, 9-H, J = 8.4, 7.3 Hz), 7.04 (d, 1H, 5-H, J = 8.3 Hz), 4.15 (m, 2H, 12-H), 4.12 (s, 3H, OCH₃), 3.17 (t, 2H, 19-H, J = 7.1 Hz), 1.65–1.88 (m, 4H, 13-H, 18-H), 1.24–1.50 (m, 8H, 14-H, 15-H, 16-H, 17-H). ¹³C NMR (62.9 MHz, CDCl₃, 300 K): δ = 164.51 (1C, 1-C), 163.95 (1C, 2-C), 160.77 (1C, 6-C), 133.39 (1C, 4-C), 131.51 (1C, 10-C), 129.39 (1C, 7a-C), 128.56 (1C, 8-C), 125.92 (1C, 9-C), 123.51 (1C, 7-C), 122.49 (1C, 11-C), 115.19 (1C, 3-C), 105.17 (1C, 5-C), 56.18 (1C, OCH₃), 40.24 (1C, 12-C), 33.51 (1C, CH₂), 30.43 (1C, CH₂), 29.10 (1C, CH₂), 28.39 (1C, CH₂), 28.07 (1C, 13-C), 26.99 (1C, CH₂), 7.19 (1C, 19-C). MS (CI) *m/z* (rel. int.): for [M+1]⁺ 467 (21) and 466 (100), 465 (12, M⁺), 338 (25, [M-HI]⁺). Anal. calcd. for C₂₁H₂₄INO₃ (465.32): C, 54.20; H, 5.20; N, 3.01. Found: C, 54.24; H, 5.18; N, 3.03%.

2.2.4. Synthesis of 2-(8-mercaptooctyl)-6-methoxy-1H-benzo[de]isoquinoline-1,3(2H)-dione (**6**) (NAFTA 8)

To a solution of **5** (1.51 g, 3.25 mmol) in 30 ml dry THF (inert atmosphere) were added at –20 °C consequently (SiMe₃)₂S (0.82 ml, 3.89 mmol) and TBAF·3H₂O (1.13 g, 3.58 mmol). The mixture was stirred overnight (16 h) at r.t. and concentrated *in vacuo*. Water and DCM were added and extracted. The organic phase was separated, dried over anhydrous Na₂SO₄ and evaporated *in vacuo*. The crude product was purified by column chromatography (80 g silica gel, phase DCM:cyclohexane = 1:1, then 2:1, then DCM) to obtain 0.98 g (81%) of **6** as light yellow crystals. m.p. 99–100 °C. IR (KBr): 2925 (m, ν_{as}CH₂), 2848 (m, ν_sCH₂), 2555 (w, νSH), 1690 (s, νC=O), 1655 (vs, νC=O), 1595 (s, Ar), 1582 (s, Ar), 1514 (m, Ar), 1463 (m, δ CH₂), 1426 (w, δ CH₂–N), 1268 and 1246 (doublet, νAr–OCH₃) cm^{–1}. ¹H NMR (600.13 MHz, CDCl₃, 293 K): δ = 8.60 (dd, 1H, 10-H, J = 7.3, 1.2 Hz), 8.55 (m, 2H, 4-H, 8-H), 7.70 (dd, 1H, 9-H, J = 8.3, 7.3 Hz), 7.04 (d, 1H, 5-H, J = 8.3 Hz), 4.15 (m, 2H, 12-H), 4.13 (s, 3H, OCH₃), 2.51 (dd, 2H, 19-H, J = 14.8, 7.7 Hz), 1.72 (m, 2H, 13-H), 1.60 (dt, 2H, 18-H, J = 14.8, 7.2 Hz), 1.41 (m, 2H, 14-H), 1.33–1.39 (m, 4H, 15-H, 17-H), 1.28–1.35 (m, 2H, 16-H), 1.32 (t, 1H, SH, J = 7.7 Hz). ¹³C NMR (150.92 MHz, CDCl₃, 293 K): δ = 164.50 (1C, 1-C), 163.94 (1C, 2-C), 160.74 (1C, 6-C), 133.39 (1C, 4-C), 131.50 (1C, 10-C), 129.33 (1C, 7a-C), 128.56 (1C, 8-C), 125.90 (1C, 9-C), 123.45 (1C, 7-C), 122.42 (1C, 11-C), 115.11 (1C, 3-C), 105.14 (1C, 5-C), 56.17 (1C, OCH₃), 40.25 (1C, 12-C), 34.00 (1C, 18-C), 29.19 (1C, CH₂), 28.91 (1C, 16-C), 28.29 (1C, CH₂), 28.07 (1C, 13-C), 27.02 (1C, 14-C), 24.62 (1C, 19-C). MS (CI) *m/z* (rel. int.): for [M+1]⁺ 373 (25) and 372 (100), 371 (20, M⁺), 370 (19). Anal. calcd. for C₂₁H₂₅NO₃S (371.49): C, 67.89; H, 6.78; N, 3.77; S, 8.63. Found: C, 67.92; H, 6.75; N, 3.79; S, 8.61%.



Scheme 2. Preparation of covalently functionalized AuNPs with desired size through chemical reduction.

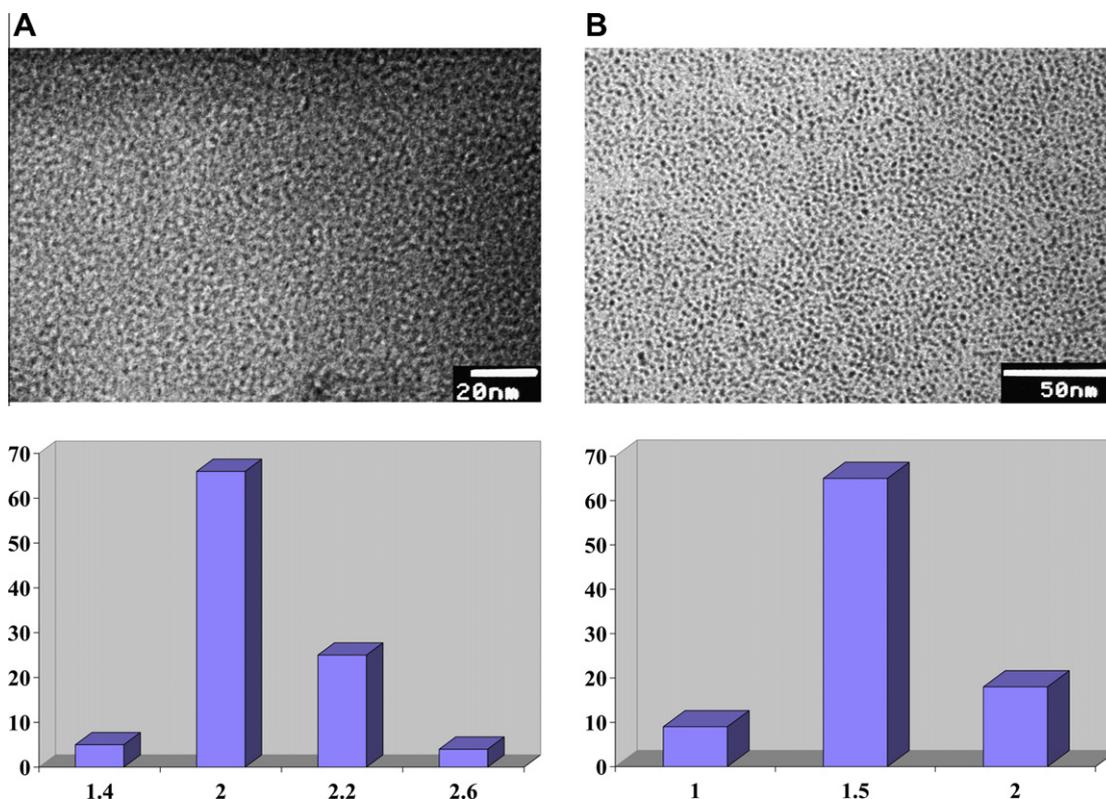


Fig. 1. Bright field electron micrographs and histograms of: (A) AuNPs MPCs modified with NAFTA 8; (B) AuNPs MPCs modified with HDT.

2.2.5. Preparation of MPCs of AuNPs modified with NAFTA 8 and 1-hexadecanethiol (HDT)

The MPCs of AuNPs modified with NAFTA 8 and HDT were synthesized based on a standard procedure [26] through a reduction of

the gold salt by sodium borohydride in the presence of the corresponding thiol [27–29] (Scheme 2).

In a round-bottom flask 2 eq. (0.55 mmol) TOAB acting as capping and phase-transfer agent was dissolved in toluene (30 ml).

Table 1

Diameters of the gold cores (D) and the corresponding number of Au atoms in the bulk (N_{Au}) and on the surface (N_{out}) of AuNP, and of the molecules in the organic shell (N_{thiol}); Δ – size dispersion of the diameter (nm).

Au MPCs	D (nm)	$\pm \Delta$ (nm)	N_{Au}^a	N_{thiol}^a	N_{out}^a	Coverage (%) $N_{\text{thiol}}/N_{\text{out}}$	Molar ratio Au/S ^b
HDT	2.0	± 0.6	246	59	137	43	1.4:1
NAFTA 8	1.5	± 0.5	104	33	71	46	2.29:1

^a Applying Leff's formalism [26]; $N_{\text{Au}} = 4\pi R^3/3v_g$; $N_{\text{out}} = 4\pi/3v_g[R^3 - (R - 2.38)^3]$; $N_{\text{thiol}} = 4\pi R^2/R_{\text{vw}}$, where v_g is the constant volume of one Au atom in a bulk state; R is the radius of the Au core in Å; R_{vw} is the van der Waals radius of the ligand.

^b Determined from elemental analysis.

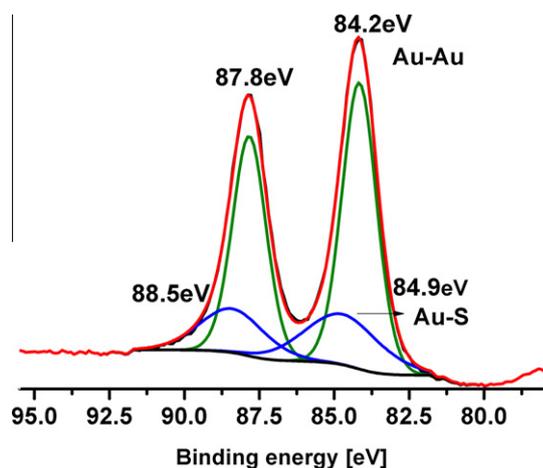


Fig. 2. XPS Au_{4f} spectrum for NAFTA 8 adsorbed on AuNPs surfaces.

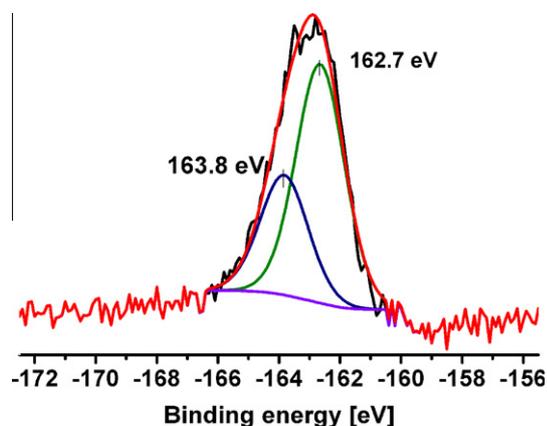


Fig. 3. XPS S_{2p} spectrum for NAFTA 8 adsorbed on AuNPs surfaces.

An aqueous solution (25 ml) of 1 eq. (0.28 mmol) H₂AuCl₄·3H₂O was added and stirred for 10 min at room temperature (the aqueous phase turns from orange to colorless). The aqueous solution was separated and a toluene solution (15 ml) of 0.5 eq. (0.14 mmol NAFTA 8 or HDT) was added to the flask. The reaction mixture was stirred for another 10 min and then an aqueous solution (10 ml) of 10 eq. reduction agent NaBH₄ (2.80 mmol) was added slowly on portions. The mixture was stirred overnight at room temperature. The organic phase was collected and the solvent was removed under vacuum. The precipitated powder was washed by the following procedure: (i) dispersion of the MPCs in abs. C₂H₅OH; (ii) sonication for 2 min; (iii) centrifugation. The cycle was repeated five times. The final ethanol dispersion was filtered and dried in air. The purity of the MPCs was monitored by ¹H NMR. Anal. found for NAFTA 8 modified MPCs: Au, 62.14%; C, 26.87%; H, 2.92%; S, 4.44%; N, 3.63%. Anal. found for HDT modified MPCs: Au, 61.83%; C, 27.64%; H, 4.30%; S, 6.23%.

3. Results and discussion

The newly synthesized fluorophore NAFTA 8 is especially designed for modification of AuNPs. It bears long methylene chain at the imide N atom and has a terminal SH group, which allows its chemical binding to the gold surface. The longest wavelength absorption maximum of NAFTA 8 in chloroform is at 370 nm, the fluorescent maximum is at 435 nm and the fluorescent quantum yield is 0.95. The fluorescent lifetime is 6.7 ns (Fig. 8). The position of the fluorescent maximum of NAFTA 8 makes it appropriate for fluorescent labeling of AuNPs as its emission band does not overlap the surface plasmon absorption of AuNPs around 500–520 nm.

The MPCs of AuNPs modified with NAFTA 8 and hexadecanethiol were synthesized as described above through a reduction of the gold salt by sodium borohydride in the presence of the corresponding thiol (Scheme 2). The chosen thiol: H₂AuCl₄·3H₂O ratio 1:2 enables the preparation of AuNPs with the desired average diameter of ≤ 2 nm as described in [27]. The HDT modified MPCs were synthesized in order to follow the influence of the ligands' structure on the size of the AuNPs and to estimate the contribution of the AuNPs to the absorption spectrum of the MPCs modified with NAFTA 8 in the region 300–400 nm.

The prepared MPCs were characterized by TEM, elemental analysis, XPS, FT-IR, absorption and fluorescence spectroscopy.

Determination of Au core diameters was performed based on a combination of analytical method (TEM) and theoretical approach. The TEM images of the MPCs modified with NAFTA8 and HDT dispersed in heptane are presented on Fig. 1A and B. As it can be seen from the figures, the dispersity of the core size in both cases is rather low. The prevalent diameter of the HDT modified AuNPs is 2.0 ± 0.6 nm, while this of NAFTA 8 modified ones is 1.5 ± 0.5 nm. The difference between the diameters of AuNPs is a strong evidence that their mean size depends not only on the ratio thiol: H₂AuCl₄·3H₂O as described in [27], but on the chemical structure of the ligands as well. The latter could be explained by the mechanism of MPCs formation. The established mechanism [30] includes a reduction of gold cations from the auric acid salt to zero valence gold atoms. They collide in solution with each other, which triggers nucleation and growing of the gold "seeds". The agglomeration process is impeded by the presence of a stabilizing agent which coordinates onto the metallic surface. Hence the molecular structure of the protective agent plays a decisive role in determining the size of the prepared clusters. In this respect, the smaller average diameter of NAFTA 8 capped MPCs compared to the respective ones for HDT capped Au clusters is assigned to the presence of the bulky fluorophore substituent introduced at the end of the chain. It could be concluded that this substituent induces a sterical hindrance at the surface of the formed colloids and ceases earlier the agglomeration process.

The quantitative characterization (Table 1) of the obtained fluorophore modified AuNPs MPCs was performed based on the measured diameter of the particles following the formalism of Leff [31]. According to this formalism, the colloidal solution of AuNPs is assumed to be ideal (i.e. the nanocrystals do not interact with each other) and the gold nanocrystals are spherical drops, which

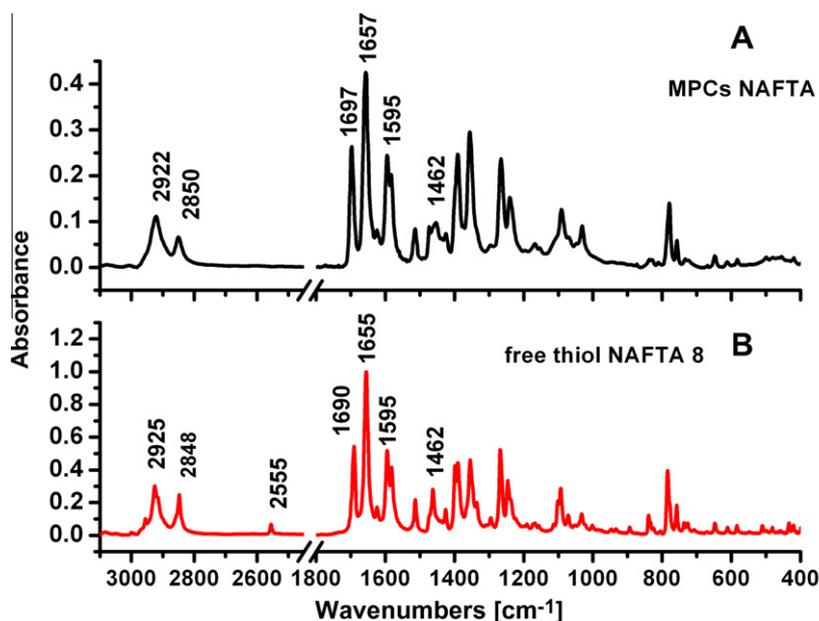


Fig. 4. FT-IR spectra of: (A) MPCs of AuNPs modified with NAFTA 8; (B) free thiol NAFTA 8.

contain N_{Au} gold atoms, stabilized by N_{thiol} thiol molecules. For NAFTA 8 modified AuNPs with diameter 1.5 nm the calculated total number of Au atoms in one AuNP is 104, the number of gold atoms at the interface of the nanocrystal and thus adjacent to the thiol molecules is 71 and the number of the ligands chemically bonded to one AuNP is 33 (46% coverage).

The XPS spectra of the MPCs modified with NAFTA 8 are presented on Figs. 2 and 3. The spectra showed signals from C, N, S and Au elements and indicated the presence of both AuNP (Fig. 2) and alkanethiol (Fig. 3) in the composite material.

The spectrum of the Au_{4f} band serves two gold bands, $4f_{7/2}$ and $4f_{5/2}$, which are identified at 84.2 and 87.8 eV, respectively. The positions and the difference between the two peaks (3.6 eV) observed here are very close to the values reported in the literature for the zero valence gold [32,33]. The width of the corresponding

peak is larger than the observed for the 2D polycrystalline Au^0 . This broadening is inferred by the presence of another doublet peak at 84.7 and 88.3 eV which is characteristic for first valence Au [32]. The area ratio of these two double bands indicates that approximately 1/2 of the Au atoms at the nanoparticle's surface are chemically bonded to the fluorescent ligands NAFTA 8 [34]. This experimental result is in good agreement with the data obtained based on the Leff's model (71 Au atoms on the surface of the AuNP and 33 thiol ligands bonded to them).

The S2p spectrum gave a weak signal due to the small atomic percentage of sulfur atoms. The signal consists of a doublet, $S2p_{3/2}$ and $S2p_{1/2}$, at 162.6 and 163.8 eV, respectively (Fig. 3). This result is very consistent with the spectra of the alkanethiol self-assembled monolayers on polycrystalline gold [35] and confirms the presence of a chemical bond between the AuNPs and the thiol moi-

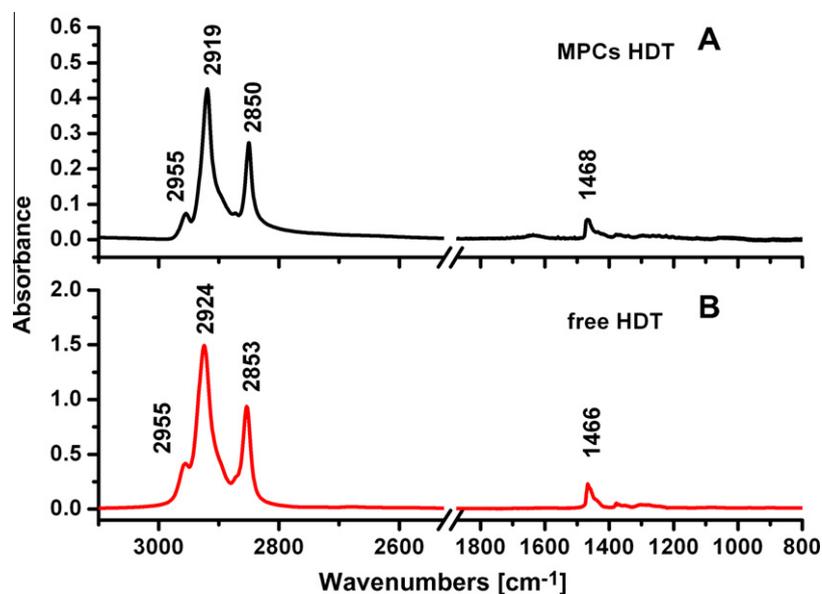


Fig. 5. FT-IR spectra of: (A) MPCs of AuNPs modified with HDT; (B) free HDT.

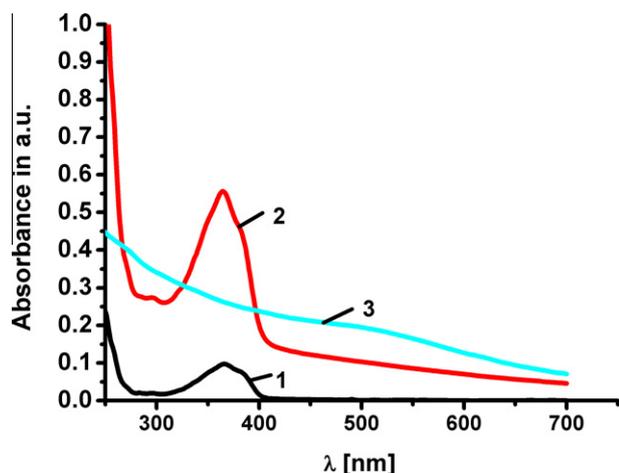


Fig. 6. UV-Vis absorption spectra in chloroform ($c = 2.10^{-7}$ M): curve 1 – NAFTA 8; curve 2 – MPCs modified with NAFTA 8; curve 3 – MPCs modified with HDT.

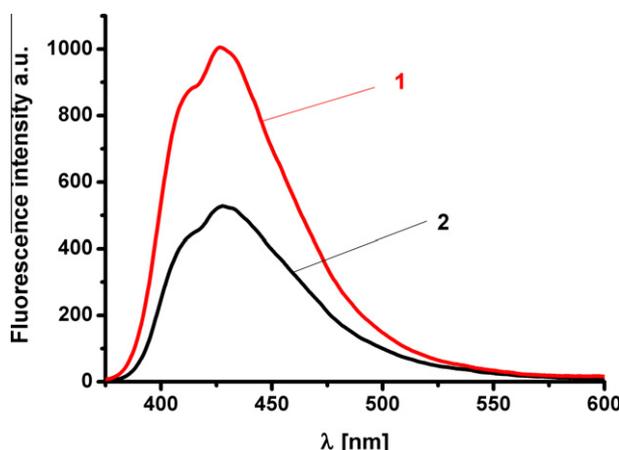


Fig. 7. Fluorescence spectra in chloroform ($c = 2 \times 10^{-7}$ M): curve 1 – NAFTA 8; curve 2 – MPCs modified with NAFTA 8.

eties. We did not observe any signal in the S2p region of non-bonded sulfur (physisorbed thiol molecules) which is at ~ 163.7 eV for S2p_{3/2} [33]. Signals from oxidized sulfur species, which are expected in the interval 167–169 eV were also not observed, even though the clusters were stored under ambient conditions for up to 7 weeks before the XPS experiment was conducted. This unambiguously proved the air stability of the prepared NAFTA 8 modified MPCs of AuNPs.

The IR spectra of the unbound thiol (NAFTA 8) (bottom) and of the corresponding cluster (top) are depicted in Fig. 4. Both spectra display very similar features which confirm that thiol molecules are present at the surface.

The SH stretching vibration band of the free ligand is found at 2555 cm^{-1} (Fig. 4B). The relative absorbance peak disappeared in the spectrum of the MPCs (Fig. 4A). This result indicates the disruption of the SH bond and proves the chemisorption of NAFTA 8 on the gold nanoparticles surface. The band corresponding to the anti-symmetric methylene stretching $\nu_{\text{as}}(\text{CH}_2)$ in the MPCs, is located at 2922 cm^{-1} . This frequency is lower than the relative absorption in the bulk spectrum of the pure thiol (2925 cm^{-1}), which is attributed to the existence of large number of gauche conformations typical for a short carbon chain. The observed frequencies for the CH₂ stretching modes are higher in comparison to the established values characteristic for *trans zig-zag* crystalline conformation

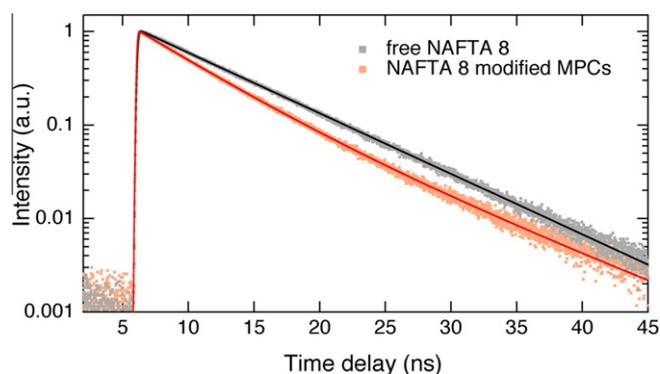


Fig. 8. Time profiles of the fluorescence intensity measured at the emission maxima upon 395 nm excitation of free NAFTA 8 and NAFTA 8 modified MPCs.

($2916\text{--}2919\text{ cm}^{-1}$) [36] but the reduced frequency in the MPCs spectrum implies that the alkyl chains in the 3D-SAM phase adopt lower amount of gauche conformations and are getting closer to the crystalline-like packing.

The same dependence was observed for the HDT (Fig. 5).

In the bulk spectrum, the frequency of the antisymmetric methylene band appeared at 2924 cm^{-1} , whereas in the 3D-SAM phase the position of this band was shifted to 2919 cm^{-1} . This result also shows a change in the order conformation of the long chains that corresponds to a transition from the liquid to an ordered crystalline-like phase.

The UV-Vis absorption and fluorescence spectra were measured using equimolar solutions of NAFTA 8 and the corresponding MPCs. The concentration was calculated based on the data from the elemental analysis. According to these data, the mass percentage of the organic component in the MPCs modified with NAFTA 8 is 37.86%. Therefore the molar concentration of the respective thiol 6 in the prepared cluster solutions was calculated regarding this value.

The MPCs modified with NAFTA 8 have an absorption band with maximum at 370 nm, which is assigned to the absorption of the fluorophore moiety (Fig. 6, curve 2). As it can be seen from the UV-Vis spectrum of HDT modified AuNPs (Fig. 6, curve 3), the absorption of the fluorophore around 370 nm is also accompanied by an intense signal in the 300–400 nm region due to the absorption of the Au core [36], which limits the determination of the optical density in the absorption maximum at 370 nm and thus the estimation of the fluorescent quantum yield of the MPCs. Additionally, a broad surface plasmon absorption band with low intensity, attributed to the AuNPs is observed at 500–520 nm [37]. The fluorescent intensity of the MPCs modified with NAFTA 8 is approximately twice as low as that of the corresponding free fluorophore in equimolar solutions (Fig. 7). Taking into account the high fluorescent quantum yield of NAFTA 8, it can be concluded that the prepared fluorophore modified AuNPs are strongly fluorescent.

The decay of the fluorescence intensity is dominated – more than 90% at the fluorescence maximum, by a 4.6 ns component. This lifetime shortening compared to free NAFTA 8 is most probably due to the occurrence of energy transfer [13] (Fig. 8).

4. Conclusion

A new fluorophore NAFTA 8 bearing long methylene chain and SH terminal group especially designed for fluorescent labeling of AuNPs has been synthesized. The MPCs of AuNPs modified with NAFTA 8 and HDT were prepared through chemical reduction. It is shown that the size of the nanoparticles depends not only on the preparation protocol but on the structure of the ligand as well. The experimental results provide evidence that the ligands are

chemically bonded to the AuNPs, which leads to the formation of stable and highly fluorescent 3D structures with possible application for bio- and chemical sensing.

Acknowledgements

The authors thank the National Science Fund of Bulgaria (Project DTK 02/25 – 17.12.2009) and the Swiss National Science Foundation – Programme SCOPES (Project No. 127864) for the financial support.

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