Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Au-thymine, thymidine and thymidine 5'-monophosphate nanoparticles: chemical characterisation and cellular uptake studies into U87 cancer cells.

Svetlana Avvakumova[a], Giorgio Scari[b] and Francesca Porta*[a]

[a]*Dipartimento di Chimica Inorganica Metallorganica Analitica "L. Malatesta", Milan University, Milan, Italy*
[b]*Dipartimento di Biologia, Milan University, Milan, Italy*

e-mail: francesca.porta@unimi.it

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I. General information.

Commercially available reagents were used as received without further purification. Reactions were carried out in a glass beaker equipped with a stirring bar, at air atmosphere and room temperature. Reactions were monitored by UV-vis spectrometry on a Jasco V-530 UV-vis spectrophotometer in the 800 to 400 nm wavelength range.

^{1}H and ^{31}P NMR spectra were recorded on a 400 Hz Brucker Avance instrument in D_2O. The samples were prepared by lyophilizing the colloidal gold solutions and re-dispersing them in D_2O. NMR spectra were referenced to internal standards [^{1}H signals to tetramethylsilane, (TMS, 0 ppm) and ^{31}P signals to 85% H_3PO_4.(0 ppm). The peak assignments are reported in section IV, as follows: chemical shift, number of protons and multiplicity (s = singlet, d = doublet, t=triplet, m=multiplet).

ATR-FTIR analysis was performed on Biorad FTS-40 spectrometer equipped with a Specac Golden Gate ATR platform with diamond crystal. The spectra of free ligands were recorded in the solid state, while the Au nanoconjugates were analysed throughout the formation of a colloidal thin film structured on the ATR crystal surface. The band assignments are reported in section VIII where the band intensity is indicated as s=strong, m=moderate, w=weak, vw=very weak.

Confocal microscopy studies on the cells were performed by a Leica TCS-NT instrument using reflected-light optics at a magnification of 63× (1.53 NA Plan-Apochromat). The samples were illuminated with a 488 nm Argon/Krypton laser, using an intensity of AOTF filter by 10%. A neutral filter RT 30/70 was used as beam splitter and placed at a 45° angle in the path of beam.

II. General procedure for the preparation of gold sols.

Au- Thymine

To 60 mL of mQ water, a $NaAuCl_4$ H_2O aqueous solution (1.76 mL, $3.41 \cdot 10^{-2}$ M) and a thymine aqueous solution (92.3 μL, 6.5 mM) were added. After 5 min, a $NaBH_4$ aqueous solution (1.2 mL, 0.1 M) was added obtaining a red sol that was stirred for further 2 hours.

Au- Thymidine

To 60 mL of mQ water, a $NaAuCl_4$ H_2O aqueous solution (1.76 mL, $3.41 \cdot 10^{-2}$ M) and a thymidine aqueous solution (182 μL, 2.3 mM) were added. After 5 min, a $NaBH_4$ aqueous solution (1.2 mL, 0.1 M) was added obtaining a red sol that was stirred for further 2 hours.

Au- 5'- TMP

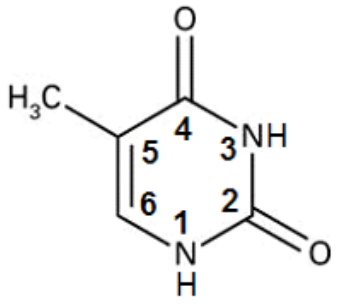
To 60 mL of mQ water, a $NaAuCl_4$ H_2O aqueous solution (1.76 mL, $3.41 \cdot 10^{-2}$ M) and a thymidine 5'-monophosphate aqueous solution (461 μL, 1.3·mM) were added. After 5 min, a $NaBH_4$ aqueous solution (1.2 mL, 0.1 M) was added obtaining a red sol that was stirred for further 2 hours.

Table 1. Molar ratios between reagents.

Capping agent	n, mmol	Ratio Au:capping agent:BH4-
Thymine	6·10-6	1:0.003:2
Thymidine	$1.8 \cdot 10^{-5}$	1:0.009:2
Thymidine 5'-monophosphate	$2 \cdot 10^{-5}$	1:0.01:2

III. Structure of thymine, thymidine and 5'-TMP.

ESI-Figure 1. Structure of 1) thymine, 2) thymidine, 3) thymidine 5'-monophosphate (disodium salt).



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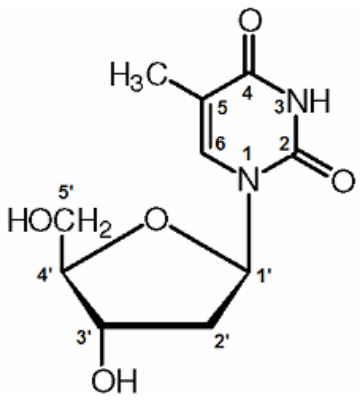

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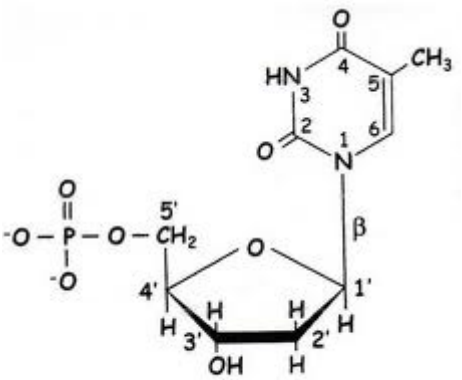

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IV. ^{1}H and ^{31}P NMR data.

Thymine:

^{1}H NMR [400 Hz , D_2O] δ(ppm): 1.79 (d, 3H, **CH_3**), 7.31 (q, 1H, **6**).

Au-Thymine:

^{1}H NMR [400 Hz , D_2O] δ(ppm): 1.81 (d, 3H, **CH_3**), 7.31 (q, 1H, **6**).

Thymidine:

^{1}H NMR [400 Hz , D_2O] δ(ppm): 1.83 (s, 3H, **CH_3**), 7.59 (d, 1H, **6**), 6.22 (t, 1H, **1'**), 4.41 (d, 1H, **3'**), 3.96 (dd, 1H, **4'**), 3.74 (ddd, 1H, **5'**), 2.31 (t, 2H, **2'**).

Au-Thymidine:

^{1}H NMR [400 Hz , D_2O] δ(ppm):1.86 (s, 3H, **CH_3**), 7.60 (d, 1H, **6**), 6.25 (t, 1H, **1'**), 4.43 (d, 1H, **3'**), 3.98 (dd, 1H, **4'**), 3.76 (ddd, 1H, **5'**), 2.34 (t, 2H, **2'**).

Thymidine 5'-monophosphate:

^{1}H NMR [400 Hz , D_2O] δ(ppm): 1.87 (d, 3H, **CH_3**), 7.76 (d, 1H, **6**), 6.28 (t, 1H, **1'**), 4.52 (dt, 1H, **3'**), 4.08 (q, 1H, **4'**), 3.89 (m, 1H, **5'**), 2.31 (m, 1H, **2'**);

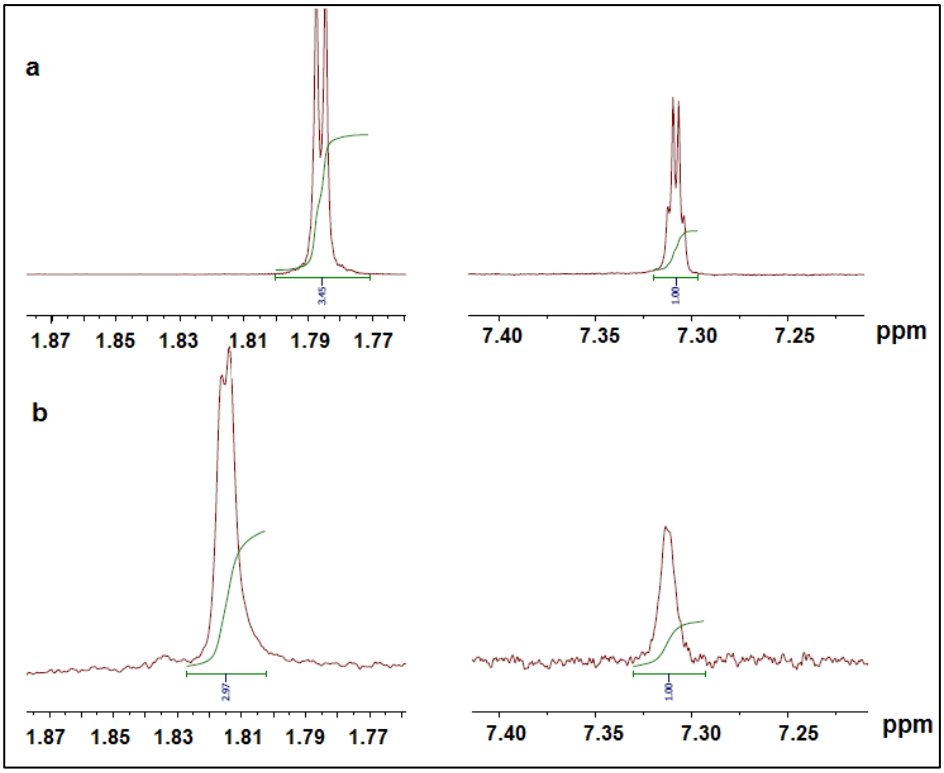
^{31}P NMR [161.9 MHz, D_2O] δ(ppm): 3.79 (s, 1P, -O-P(O) O_2^-).

Au-Thymidine 5'-monophosphate:

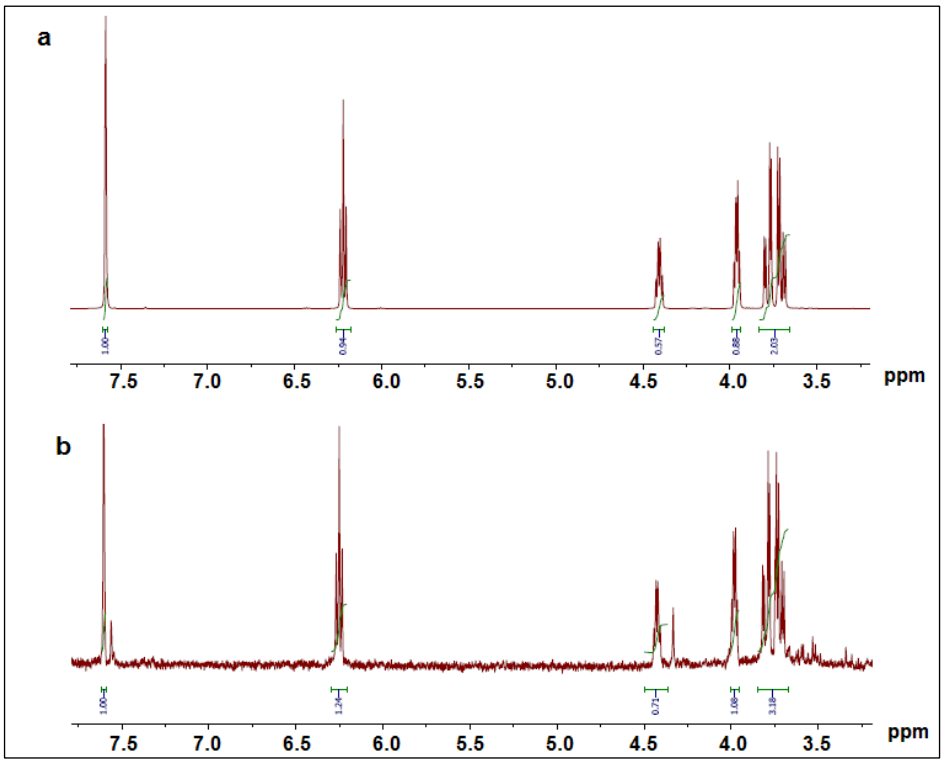
^{1}H NMR [400 Hz , D_2O] δ(ppm): 1.87 (d, 3H, **CH_3**), 7.71 (d, 1H, **6**), 6.31 (t, 1H, **1'**), 4.53 (m, 1H, **3'**), 4.14 (m, 1H, **4'**), 4.03 (m, 1H, **5'**), 2.33 (m, 1H, **2'**);

^{31}P NMR [161.9 MHz, D_2O] δ(ppm): 0.27 (s, 1P, -O-P(O) O_2^-).

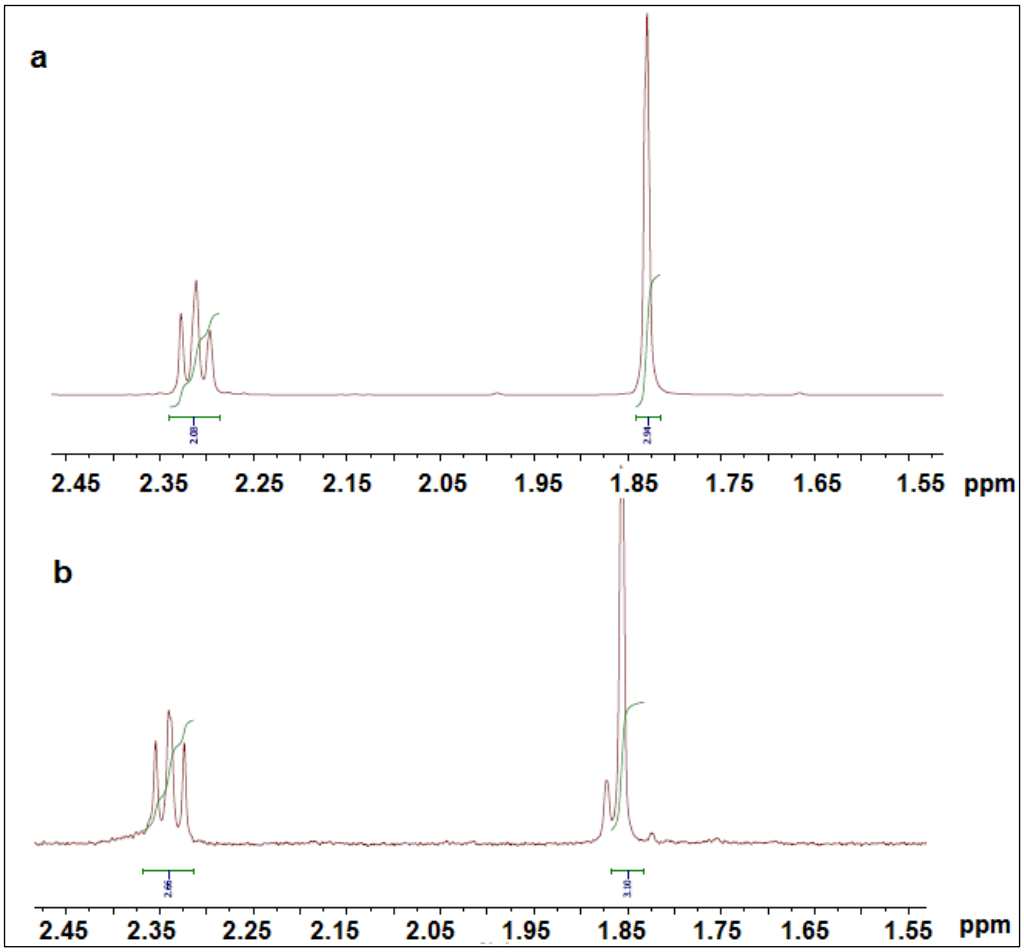
V. Copies of ^{1}H and ^{31}P NMR spectra of (1)-(3) and Au-(1)-(3) NPs.



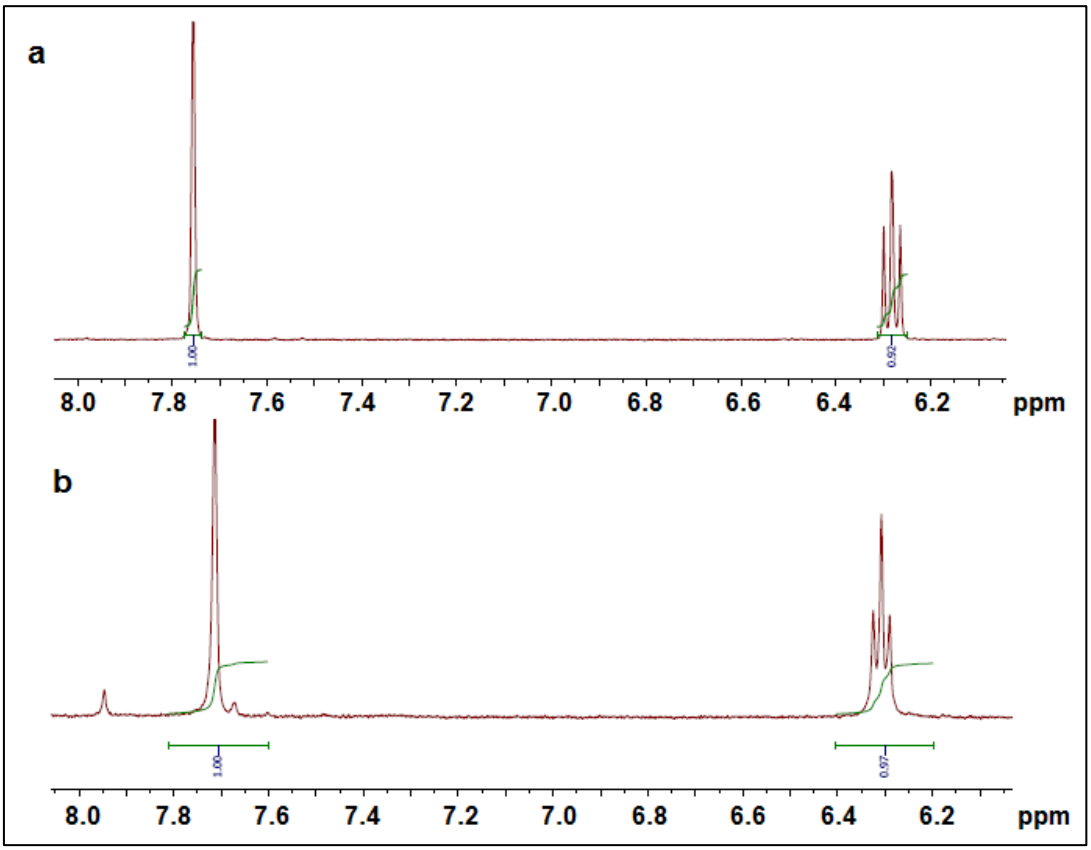
ESI – Figure 2. ^{1}H NMR spectra of thymine (a) and Au-thymine NPs (b) in D_2O in the range from 1.9 to 1.75 ppm and from 7.4 to 7.2 ppm.



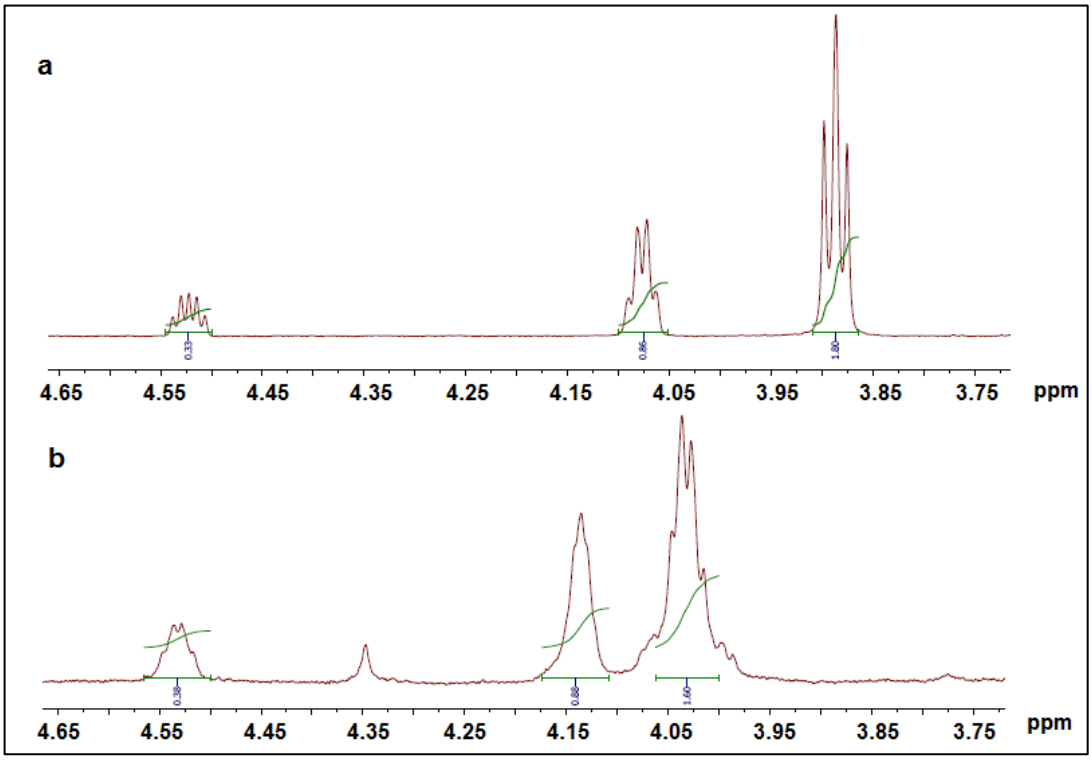
ESI – Figure 3. ^{1}H NMR spectra of thymidine (a) and Au-thymidine NPs (b) in the 7.5 to 3.5 ppm range.



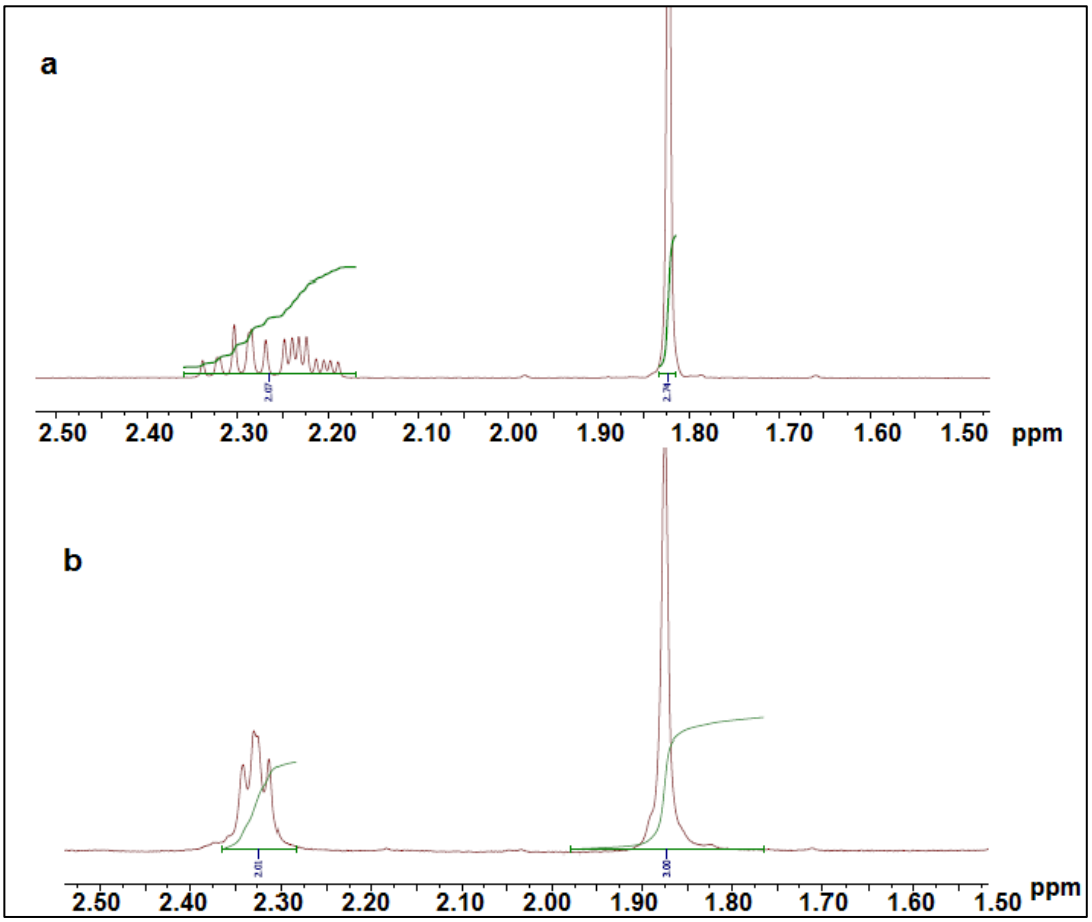
ESI - Figure 4. 1H NMR spectra of thymidine (a) and Au-thymidine NPs (b) in D_2O in the 2.5 to 1.5 ppm range.



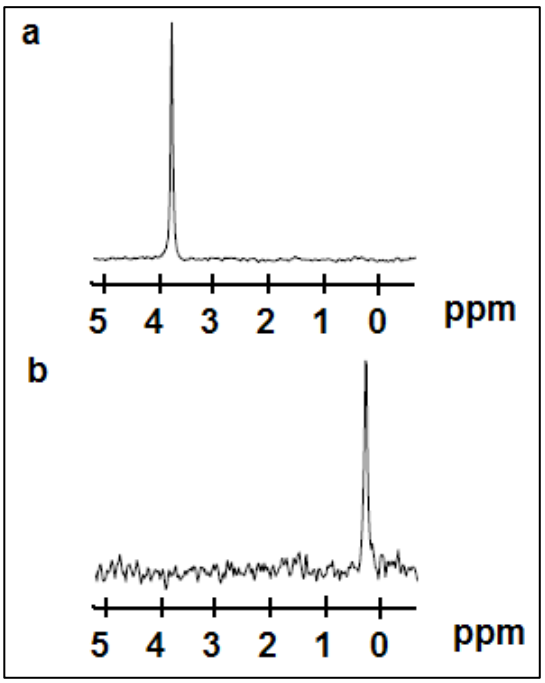
ESI – Figure 5. ^{1}H NMR spectra of thymidine 5'-monophosphate (a) and Au-thymidine 5'-monophosphate NPs (b) in D_2O in the 8 to 6.2 ppm range.



ESI – Figure 6. ^{1}H NMR spectra of thymidine 5'-monophosphate (a) and Au-thymidine 5'-monophosphate NPs (b) in D_2O in the 4.7 to 3.7 ppm range.

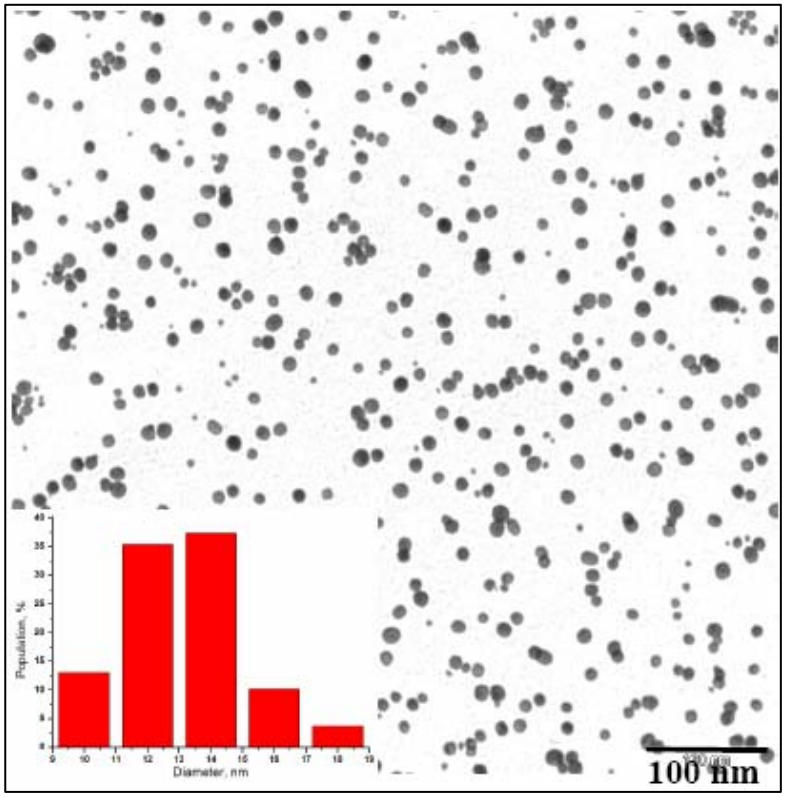


ESI – Figure 7. ^{1}H NMR spectra of thymidine 5'-monophosphate (a) and Au-thymidine 5'-monophosphate NPs (b) in D_2O in the 2.5 to 1.5 ppm range.

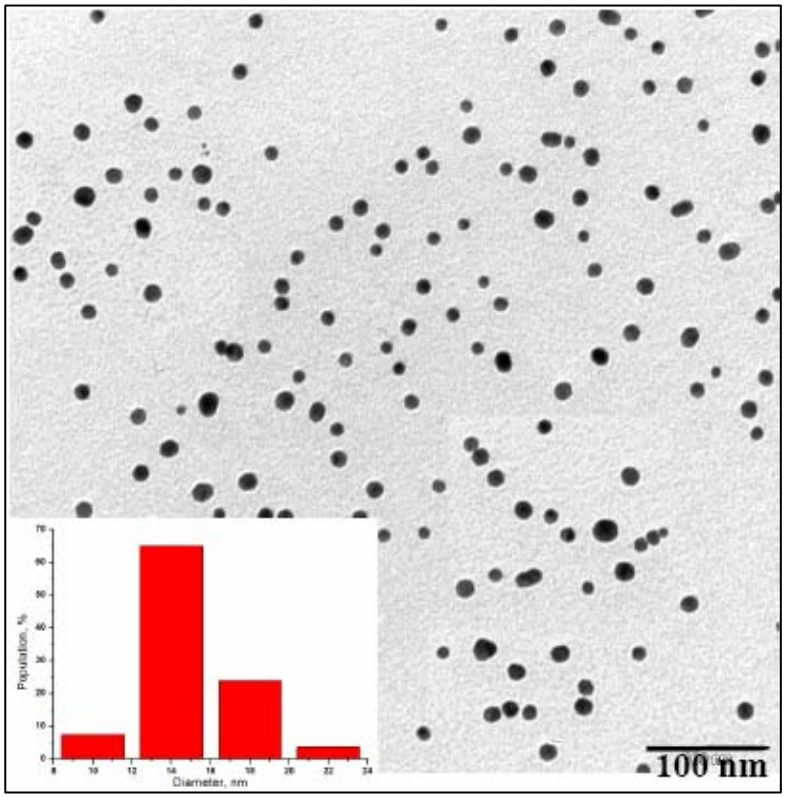


ESI – Figure 8. ^{31}P NMR spectra of thymidine 5'-monophosphate (a) and Au-thymidine 5'-monophosphate NPs (b) in D_2O in the 5 to 0 ppm range.

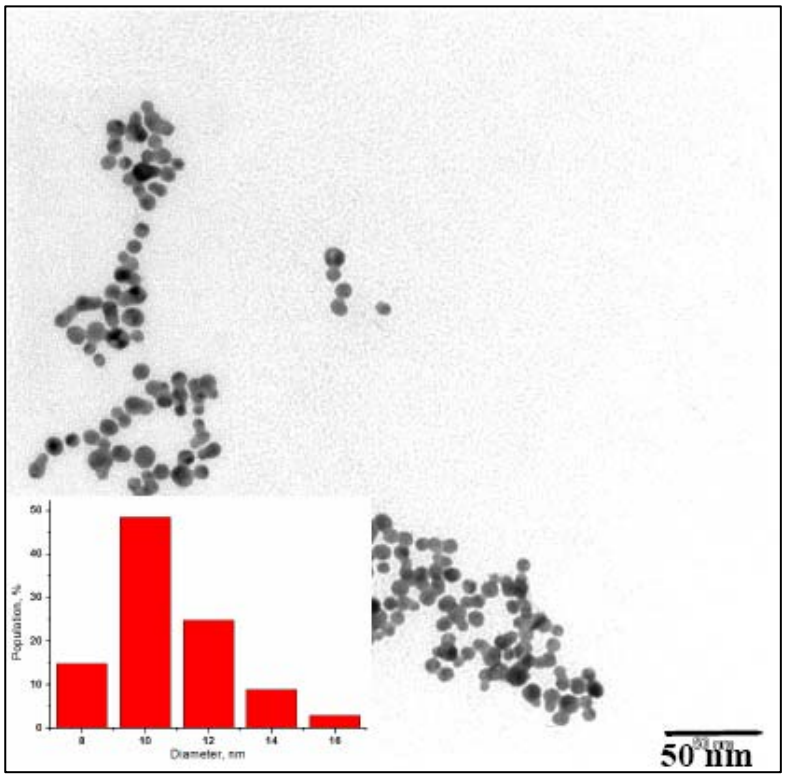
VI. TEM micrographs and UV-vis spectra of Au-(1)-(3) NPs.



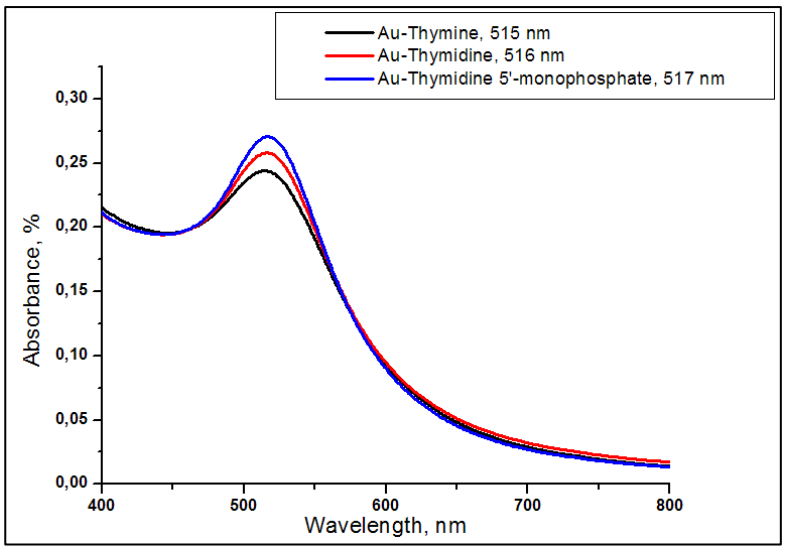
ESI- Figure 9. TEM micrograph and histogram of size distribution of Au-thymine NPs (mean diameter 11 nm).



ESI- Figure 10. TEM micrograph and histogram of size distribution of Au-thymidine NPs (mean diameter 13 nm).



ESI- Figure 11. TEM micrograph and histogram of size distribution of Au-thymidine 5'-monophosphate NPs (mean diameter 10 nm).



ESI- Figure 12. UV-vis spectra of Au-thymine (black line, 515 nm), Au-thymidine (red line, 516 nm) and Au-thymidine 5'-monophosphate (blu line, 517 nm)NPs in the 400-800 nm range.

VII. Dynamic Light Scattering and Zeta-potential data.

Dynamic Light Scattering (DLS) measurements were performed at 90° with a 90 Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY) working at 15 mW of a solid-state laser (λ = 661 nm). Measurements were carried out at 25°C in acqueous media. The Zeta-potential was determined with the same instrument equipped with AQ- 809 electrode and data were processed by ZetaPlus Software. The Zeta potential was automatically calculated from electrophoretic mobility based on the Smoluchowski theory.

Table 2. Plasmon bands, mean particle diameters vy DLS and TEM and zeta potential values of Au-(1)-(3) NPs.

Capping agent	Plasmon band, nm	Diameter (DLS), nm	Diameter (TEM), nm	Zeta Potential, mV
Au-thymine	517	13	11	-40.63
Au-thymidine	516	32	16	-49.17
Au-thymidine 5'-monophosphate	517	12 (83)	10	-51.71

VIII. ATR-FTIR data.

Modes	Free, cm-1	Bound, cm-1
ν (NH)	3158	3204 (s)
ν (CH)	3023 2907	-- 2913 (vw)
ν (C=O)	1651	1661 (s)
δ (NH)	1431	1512 (s) 1415 (s)
δ (C-O)	1194 1030 933	1169 (vw) 1091 (vw) 950 (s)
δ (C-H(ar) oop)	744	765 (m)

ESI - Table 3. Main vibrational modes of free and bound thymine.

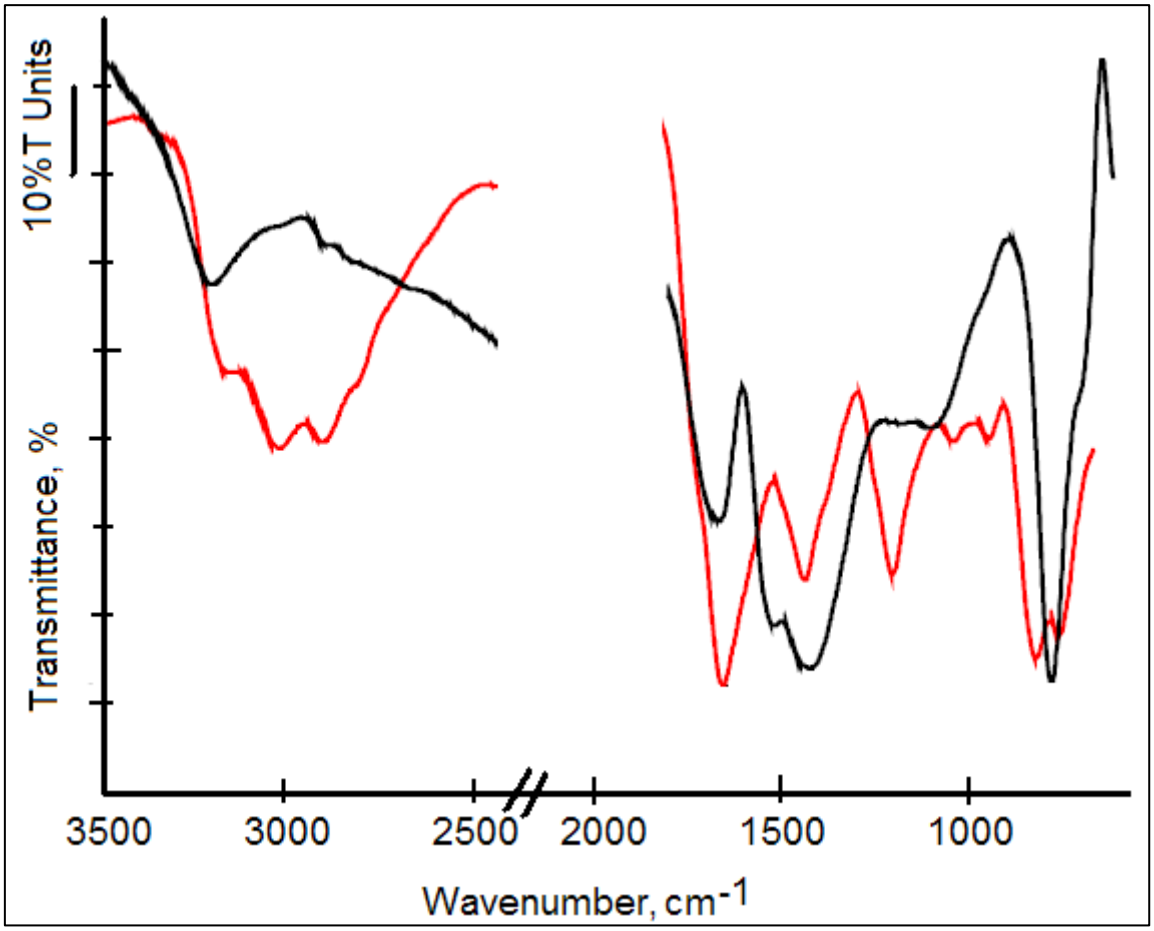
Modes	Free, cm-1	Bound, cm-1
ν (NH)	3288	3204 (s)
ν (CH)	3011 2829	2893 (w) 2829 (w)
ν (C=O)	1655	1693 (w) 1651 (w)
δ (NH)	1443	1384 (s)
δ (C-O)	1265 1061 968	1220 (m) 1036 950
δ (C-H(ar) oop)	756	778

ESI - Table 4. Main vibrational modes of free and bound thymidine.

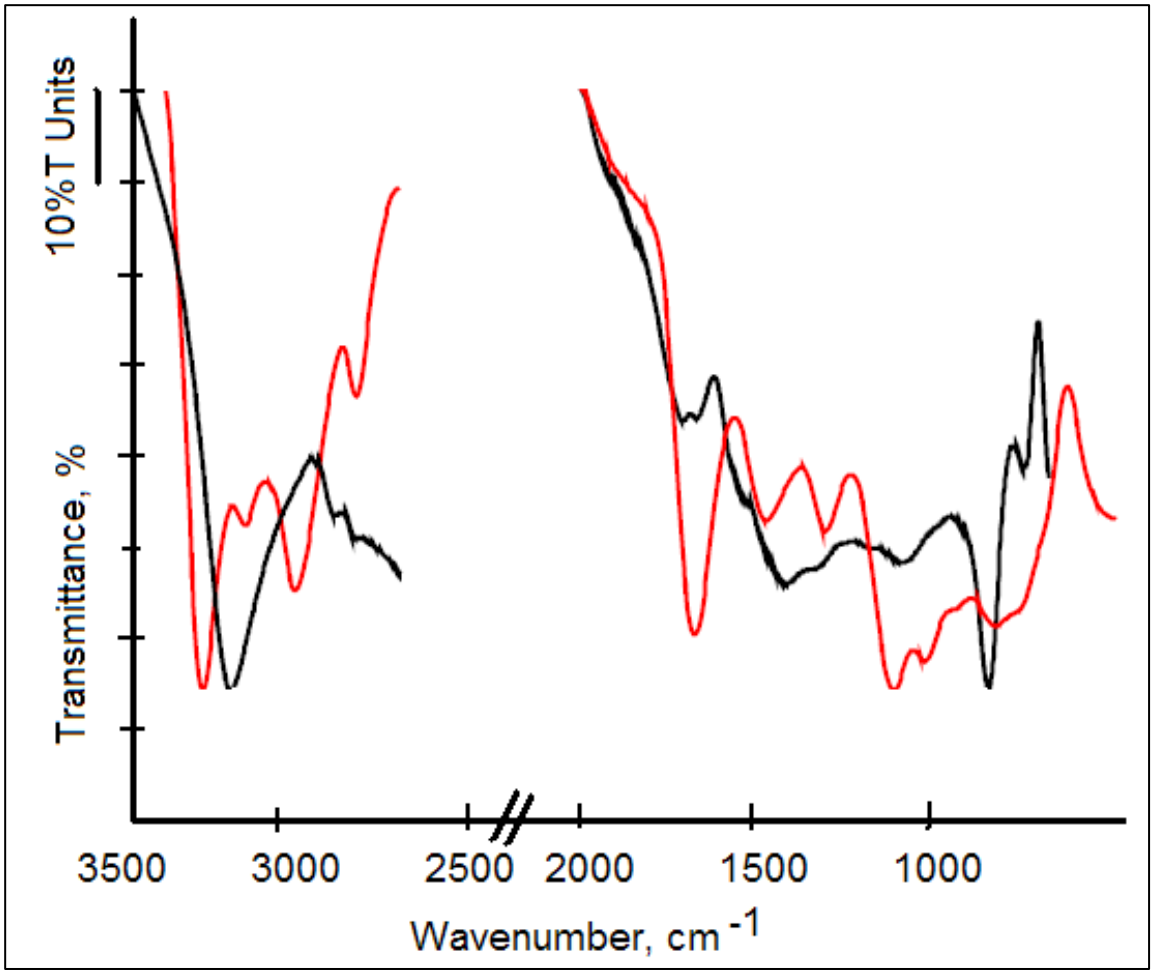
Modes	Free, cm-1	Bound, cm-1
ν (NH)	3167	3161 (s)
ν (C=O)	1663	1680 (s)
δ (NH)	1447	1390 (s)
δ (C-O)	1271	1220 (vw)
δ (C-O, P=O)	1064	---
ν (P-O)	961	950 (vw)
δ (C-H(ar) oop)	756	849 (m)

ESI - Table 5. Main vibrational modes of free and bound thymidine 5'-monophosphate.

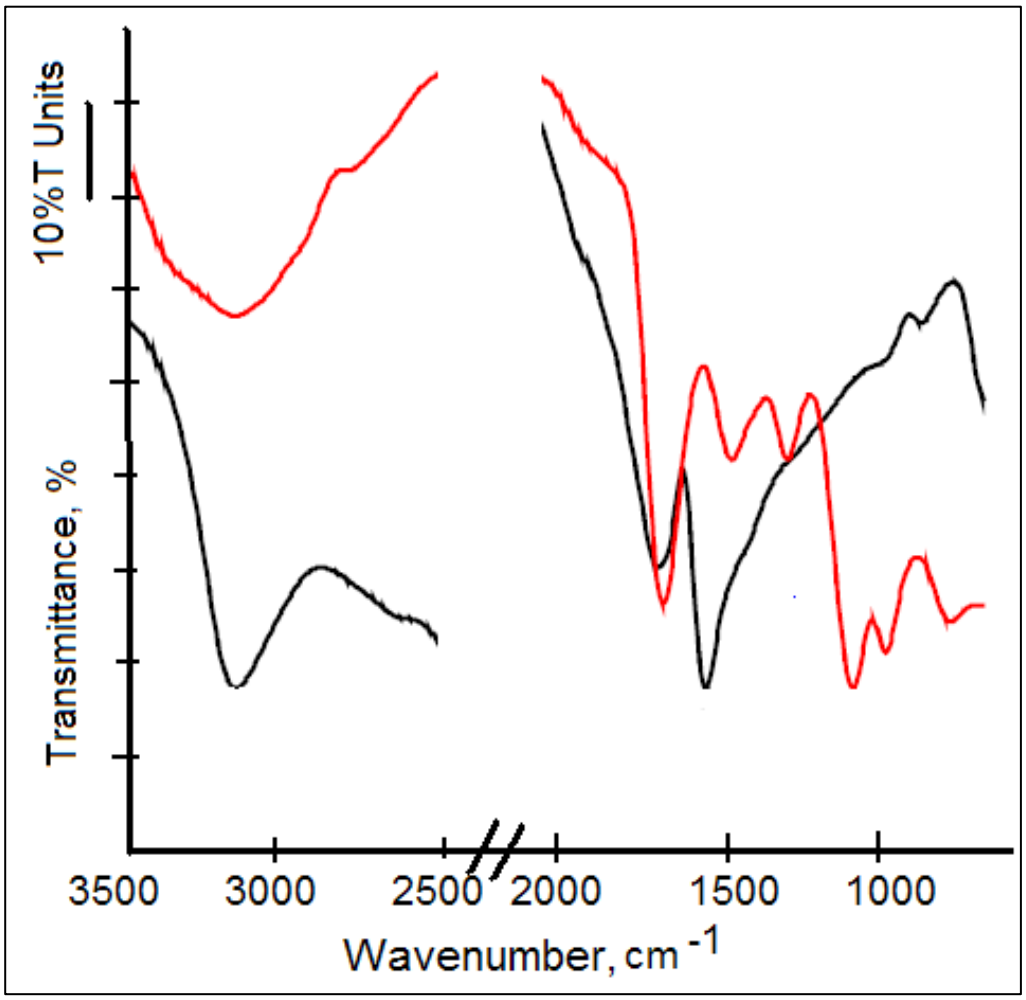
IX. ATR-FTIR spectra of (1)-(3) and Au-(1)-(3) NPs.



ESI Figure 13. ATR-FTIR spectra of thymine (red line) and Au-thymine NPs (black line).



ESI - Figure 14. ATR-FTIR spectra of thymidine (red line) and Au-thymidine NPs (black line).



ESI - Figure 15. ATR-FTIR spectra of thymidine 5'-monophosphate (red line) and Au-thymidine 5'-monophosphate NPs (black line).

X. Cell preparation.

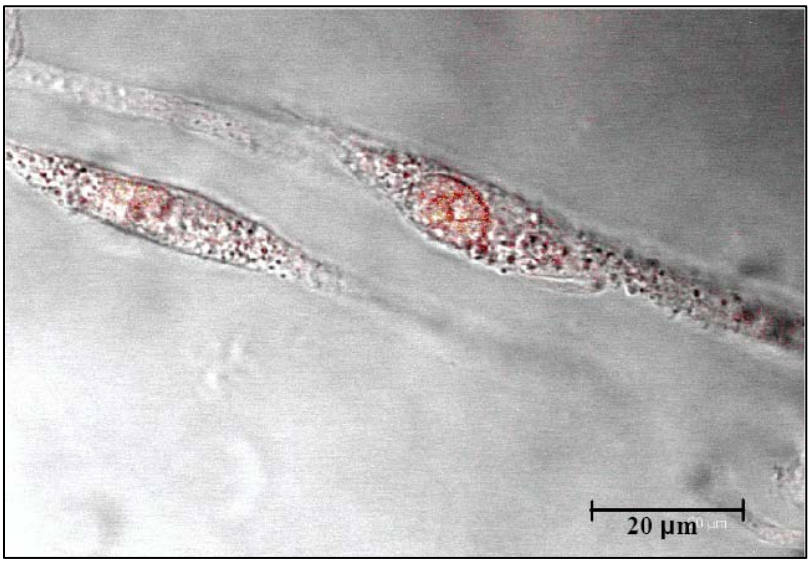
The U87 cells (human glioblastoma cell line) were cultured in Dulbecco Minimal Essential Medium, supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine and 1% (v/v) antibiotic mixture (Sigma). At confluence, the cells were splitted on sterile coverslips in 3 mL of the above described medium.

For cytotoxicity experiments, cells were treated with an appropriate amount of 0.4 mM NPs solutions in medium for 2 hours, followed by wash in PBS and the addition of 0.4% trypan blue solution. The cells were counted observing at least 20 different fields, using optical microscopy technique.

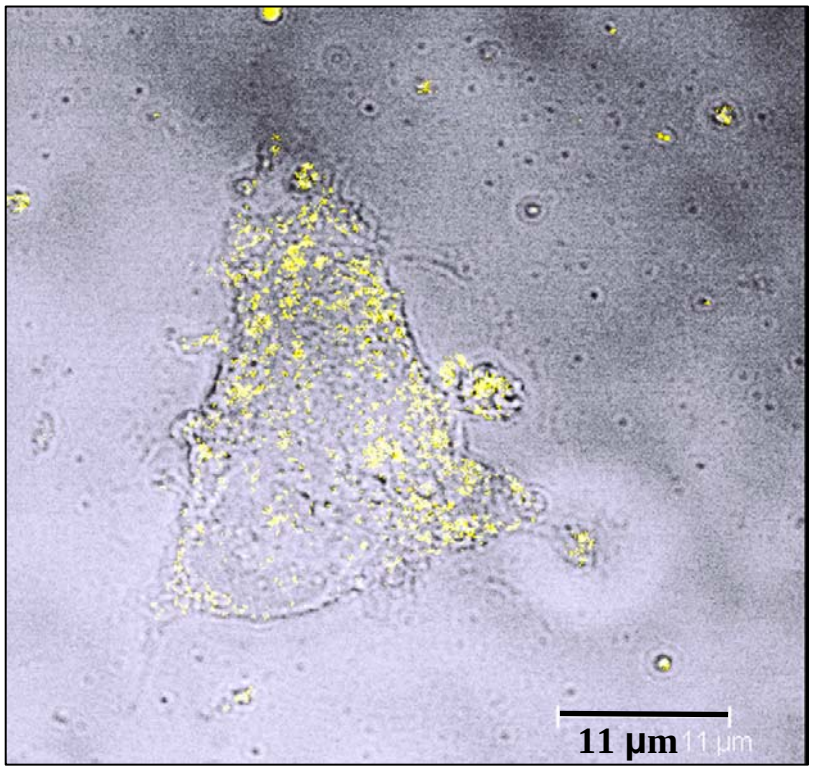
For cell uptake imaging experiments, U87 cells were plated into a petri dish at $2x10^6$ cells per dish and treated with 200 μL of a 0.4 mM solution of NPs in medium for 1 hour.

For TEM imaging cells were first fixed by using a 2% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, at pH 7.2 for 15 min, and subsequently washed with 3 portions of a 0.2 M sodium cacodylate buffer solution at pH 7.2. Afterwards, the cells were postfixed for 10 min with a solution containing 1% (v/v) OsO_4 buffered with 0.1 M sodium cacodylate at pH 7.4. Finally, the cells were washed three times with the 0.2 M sodium cacodylate buffer solution and dehydrated in an ascending ethanol series. Propylene oxide was used as solvent. The cells were infiltrated for 2h in a 1:1 mixture of Epon-Araldite and propylene oxide, then the mixture was removed and replaced with 100% Epon-Araldite-resin. The specimens were polymerized for 24 h at 60 °C. The resin embedded specimens were thinly sectioned (30 nm) by ultramicrotome (Ultracut Reichert FC4), and then mounted on 300 mesh copper grid. The specimens were observed using a EFTEM LEO 912AB transmission electron microscope operating at 100 kV. Digital images were obtained by a CCD Camera System and Leo Image software.

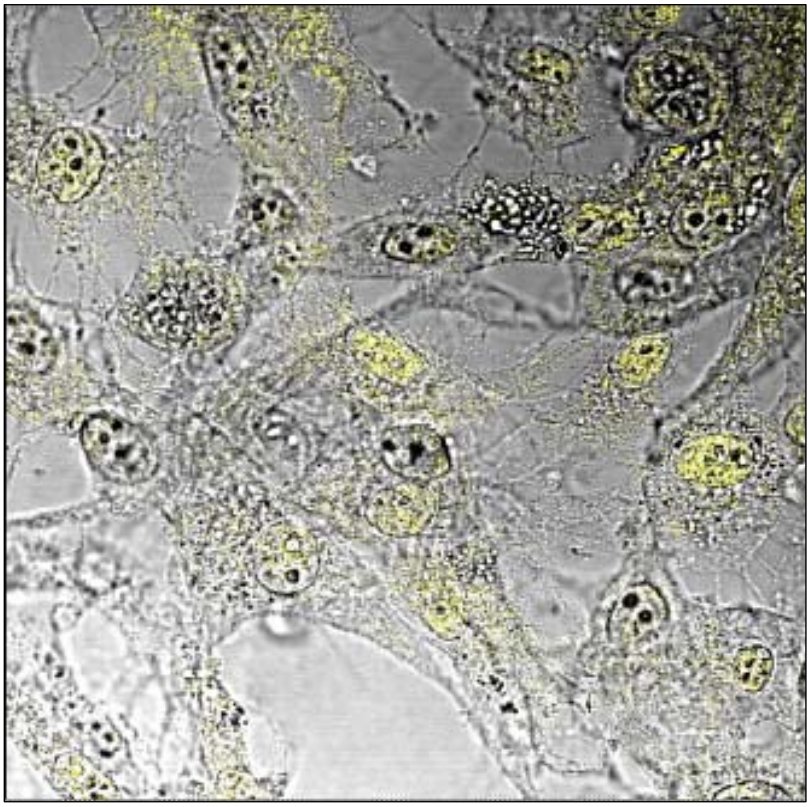
XI. Confocal microscopy images of the U87 cells incubated with Au-(1)-(3) NPs.



ESI - Figure 16. Confocal microscopy image of Au-thymine NPs into U87 cancer cells (overlaid in both reflection and transmission modes).

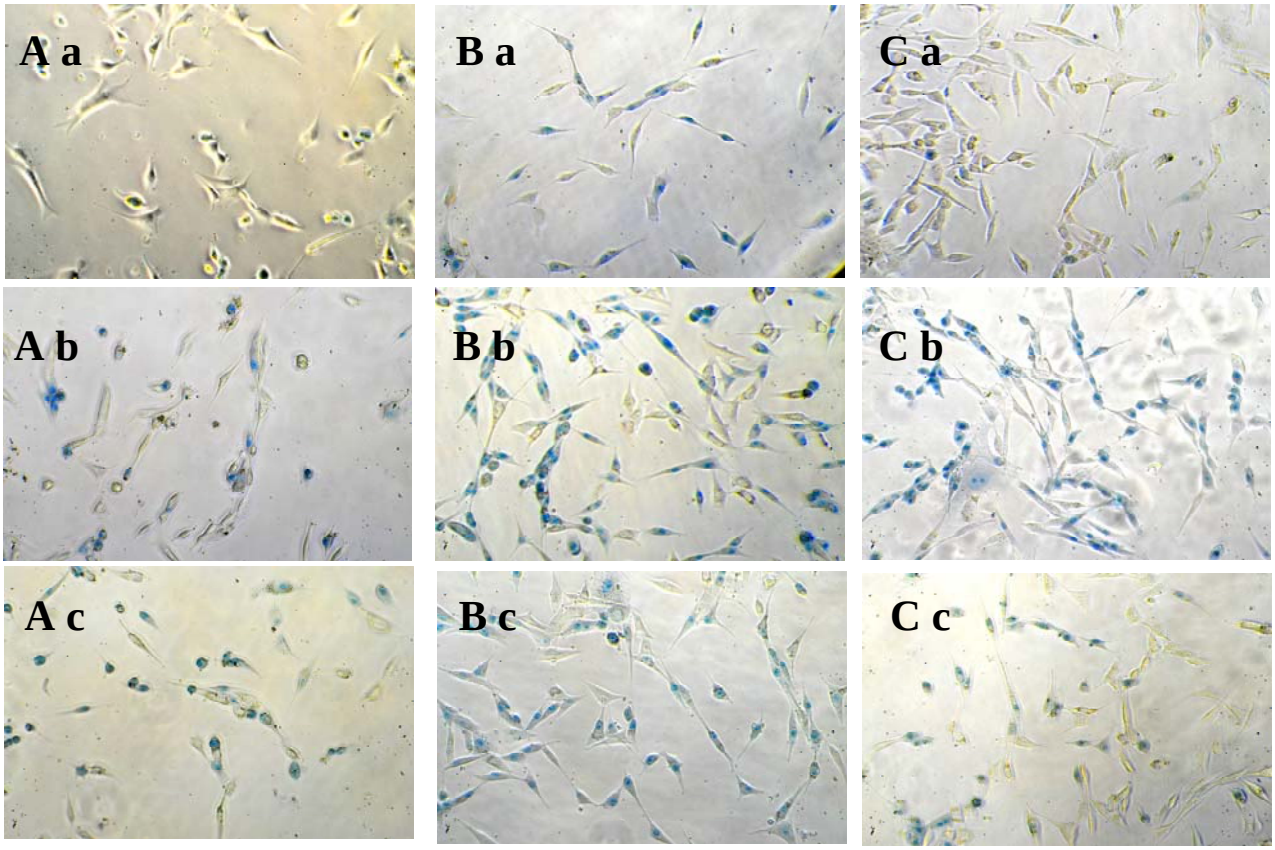


ESI - Figure 17. Confocal microscopy image of Au-thymidine NPs into U87 cancer cells (overlaid in both transmission and reflection mode).

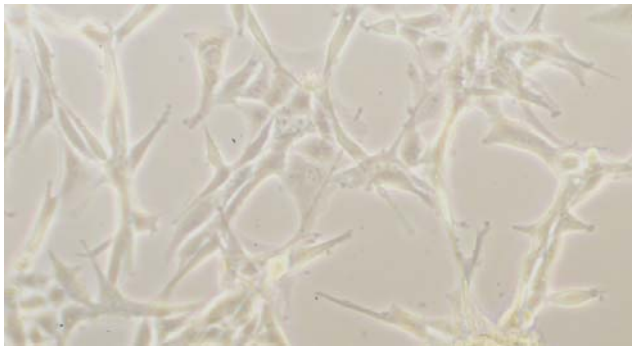


ESI - Figure 18. Confocal microscopy image of Au-thymidine 5’-monophosphate NPs into U87 cancer cells (overlaid in both transmission and reflection mode).

XII. Trypan blue exclusion assay for cytotoxisity studies.



ESI – Figure 19. Optical miscroscopy images of the U87 cells treated with: 19 μM Au-T NPs (A, a); 36 μM Au-T NPs (A, b); 52 μM Au-T NPs (A, c); 19 μM Au-dT NPs (B, a); 36 μM Au-dT NPs (B, b); 52 μM Au-dT NPs (B, c); 19 μM Au-5'-TMP NPs (C, a); 36 μM Au-5'-TMP NPs (C, b); 52 μM Au-5'-TMP NPs (C, c).



ESI – Figure 20. Optical miscroscopy images of the U87 cells treated with 36 μM Au-Citrate NPs.