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Supporting Information for

Water-Soluble Noncovalent Adducts of the Heterometallic Copper Subgroup Complexes and Human Serum Albumin with Remarkable Luminescent Properties

P. S. Chelushkin,[a] D. V. Krupenya,[b] Yu-JuiTseng,[c] Ting-Yi Kuo,[c] Pi-Tai Chou,[c] I. O. Koshevoy,[d] S. V. Burov[a] and S. P. Tunik[b]*

[a] *Institute of Macromolecular Compounds, Russian Academy of Sciences, Bolshoi pr. V.O., 31, 199004, St.-Petersburg, Russia.*
[b] *St.Petersburg State University, Department of Chemistry, Universitetskii pr. 26, 198504, St.-Petersburg, Russia.*
[c] *Department of Chemistry, National Taiwan University, Taiwan.*
[d] *Department of Chemistry, University of Eastern Finland, Finland*
[*] *E-mail: stunik@inbox.ru; Tel/Fax: +78123241258.*

1. Experimental details

All reagents and chemicals were used as received. Human serum albumin (HSA, 98 % purity, crystalline) was purchased from “Sigma-Aldrich” (USA). All the solutions and all dialyses were performed using twice distilled water. Dialysis tubes (32 mm diameter) with 12.5 kDa cut-off and 50 mm closures were purchased from “Orange Scientific” (Belgium). Dialyses were performed using manufacturer’s recommendations: water was changed at least 5 times and at least 3 hours per each equilibration. Lyophilization of dialyzed solutions was performed on the “Thermo Savant ModulyoD” Freeze Dryer (“Thermo Savant”, USA) equipped with VLP200 Vacuum Pump (“Savant Instruments”, USA). The sample aqueous solutions to be lyophilized were freezed to -25 °C and dried overnight at -50 °C and 100 mBar vacuum. PPN[Au(acac)$_2$][1] and 4-ethenylbenzoic[2] acid were prepared according to literature.

Table S1. Summary on synthesis and properties of organometallic clusters.

	M.W., g/mole	Molecular formula	Reference[a]

1	3542	**[{$Au_3Cu_2(C_2C_6H_5)_6$}$Au_3(PPh_2C_6H_4PPh_2)_3$](PF$_6$)$_2$**	[3]
2	3808	**[{$Au_3Cu_2(C_2C_6H_4COOH)_6$}$Au_3(PPh_2C_6H_4PPh_2)_3$](PF$_6$)$_2$**	see below
3	3098	**[$Au_8(C_2Bu^t)_6(PPh_2C_2PPh_2)_2$](PF$_6$)$_2$**	[4]
4	7661	**[$Au_{14}Ag_4(C_2Ph)_{12}(PPh_2C_6H_4PPh_2)_6$](PF$_6$)$_4$**	[5]
5	3495	**[$Au_6Cu_2(Ph_2PC_6H_4PPh_2)_2(C_2C(OH)iBu_2)_6$](PF$_6$)$_2$**	[6]

[a]References contain information on synthesis and photophysical characterization of clusters

2. Preparation of [{$Au_3Cu_2(C_2C_6H_4COOH)_6$}$Au_3(PPh_2C_6H_4PPh_2)_3$][$PF_6$]$_2$ (2)

A solution of PPN[$Au(acac)_2$] (214 mg, 0.229 mmol) in acetone (15 ml) was added to a solution of 4-ethynylbenzoic acid (76 mg, 0.497 mmol) in acetone (4 ml). The reaction mixture became turbid and orange. After stirring the reaction mixture for one hour a solution of [$Au_2(Ph_2PC_6H_4PPh_2)_2$](PF6)$_2$ (181 mg, 0.115 mmol) and [$Cu(NCMe)_4$]PF_6 (57 mg, 0.153 mmol) in CH_2Cl_2 (5 ml) was added. The reaction mixture was stirred for 2 hours, filtered and evaporated to dryness. The pure compound was obtained as an orange powder after recrystallization of residue by gas phase diffusion of ether into acetone solution (150 mg, 51%). 31P{1H} NMR ([D_6]-acetone, 25 °C): δ = 44.1 (s, 6P), -144.8 (sept, J = 707 Hz, 2P, PF6). **^{1}H NMR** ([D_6]-acetone, 25 °C) δ = **diphosphine**: 8.02 (dm(ABXX'), $^3J_{H,H}$ = 7.2 Hz, $^3J_{P,H}$ = 13.6 Hz, 24H, ortho-H), 7.84 (m(A_2X_2) , 12H, {P-C_6H_4-P}), 7.67 (t, $^3J_{H,H}$ = 7.4 Hz, 12H, para-H), 7.49 (dd, $^3J_{H,H}$ = 7.4 and 7.2 Hz, 24H, meta-H), **{Au($C_2C_6H_4COOH$)$_2$** 7.45 (d, $^3J_{H,H}$ = 8.0 Hz, 12H, ortho-H), 6.89 (d, $^3J_{H,H}$ = 8.0 Hz, 12H, meta-H). **ESI-MS**: *m/z* = 1759.12 $[M]^{2+}$ (calc. 1759.12). $C_{144}H_{102}Au_6Cu_2F_{12}O_{12}P_8$: calc. C 45.41%, H 2.70%, found C 45.36%, H 2.70%.

General procedure for adduct formation between compounds 1–5 and HSA adducts using the dialysis technique. The solution of 34.6 mg (0.52 mmole) HSA in 1.6 ml of water:DMSO (25 : 75 vol%) mixture was added to the solution of 0.52 mmole of complex in 780 ml of the same solvent mixture. Mixing of the above transparent solutions resulted in turbid solution, which became transparent upon further dilution with two volumes (4.8 ml) of water. The resulting solution was dialyzed against double distilled water. Dialysis tubes with 12.5 kDa cut-off were chosen. Precipitation was observed occasionally during the dialysis but the precipitate was easily dissolved upon addition of a small amount of HCl. After dialysis the adducts were lyophilized. Lyophilized samples appeared as colored solids easily soluble in water and phosphate buffer solution (PBS). Lyophilized samples were protected from light to prevent label photobleaching and stored at 4^{o}C to prevent HSA degradation.

3. Photophysical (Fluorescence) measurements

Steady-state absorption and emission spectra were recorded by a Hitachi (U-3310) spectrophotometer and an Edinburgh (FS920) fluorimeter, respectively. Nanosecond lifetime studies were performed with an Edinburgh FL 900 photon-counting system with a hydrogen-filled lamp as the excitation source.

4. Confocal microscopy studies

For confocal microscopic measurement, HeLa cells, derived from human cervical cancer, were seeded onto 22 mm coverslips in a 6-well plate with 5×10^4 cell/well density in serum-free culture medium to promote the uptake of HAS-comples adducts. After 4 hours incubation time with albumine complexes at a concentration of 300 ppm (red), cells were washed twice with PBS and then fixed with 10% paraformaldehyde in PBS. For the confocal fluorescence imaging, 4',6-diamidino-2-phenylindole (DAPI, "Molecular Probes", USA) was chosen to stain the nucleus (blue) and fluorescein isothiocyanate (FITC, "Sigma-Aldrich", USA) was chosen to stain the cell membrane. The cells were observed by a Zeiss LSM710 NLO confocal spectral microscope equipped with 63X (P-APO, 1.40 oil immersion) objective. A combination of 405 nm diode laser, 458 nm argon laser and 488 nm diode laser was used as the excitation source for nucleus, albumin complexes and cell membrane, respectively.

5. Time-gated confocal imaging

The time-gated confocal imaging of the complex and DAPI in the HeLa cells with various delay time inverted is measured by microscope (IX81, Olympus) with an ICCD camera(PI-MAX 3, Princeton Instruments) in conjunction with an Imaging Spectrograph and mirror mode of Monochromator(Acton SP-2358). The gate channel of the ICCD was open at 80 ns and synchronized with the firing time of the excitation pulse and the gate width is 1μs. The third harmonic (355 nm, FWHM ~8 ns) of an Nd:YAG laser (Continuum Surelite) was used as the excitation pulse.

Steady-state absorption and emission spectra were recorded by a Hitachi (U-3310) spectrophotometer and an Edinburgh (FS920) fluorimeter, respectively. Nanosecond lifetime studies were performed with an Edinburgh FL 900 photon-counting system with a hydrogen-filled lamp as the excitation source.

6. References

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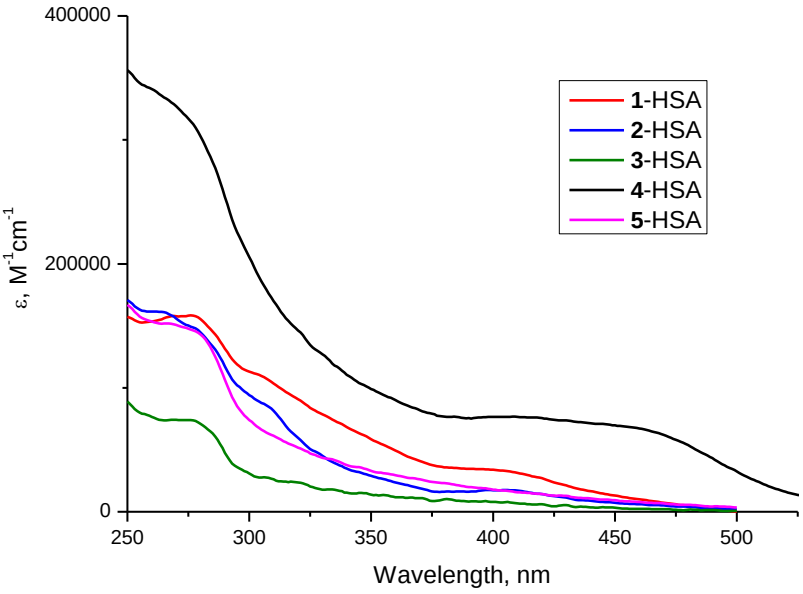

Fig. S1. Absorption spectra of HSA adducts in water solution.

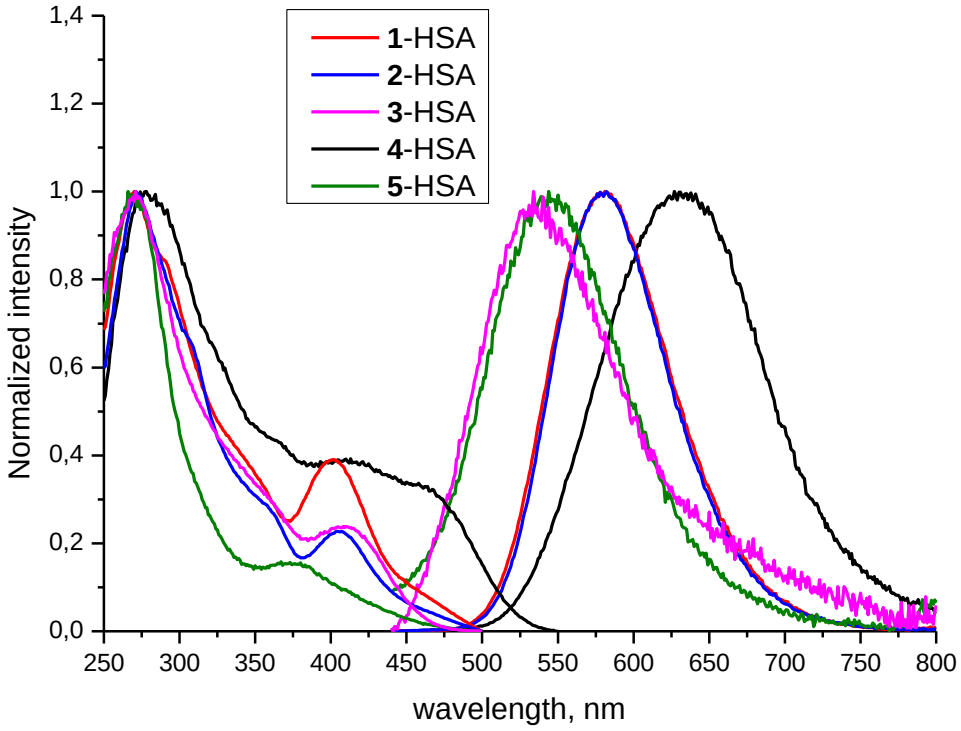

Fig. S2. Excitation and emission spectra of HSA adducts in water solution. λ_{ex}=380 nm.

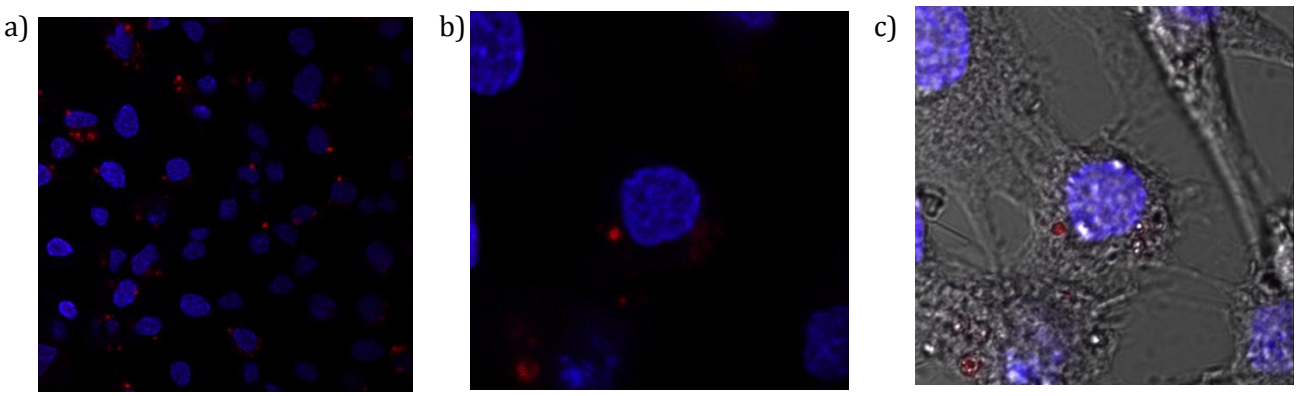

Fig. S3. Confocal fluorescence and overlaid fluorescence/bright-field images of HeLa cells stained by **1**-HSA adduct (red) and DAPI (blue). Confocal fluorescence images (a,b); overlaid fluorescence/bright-field image (c).

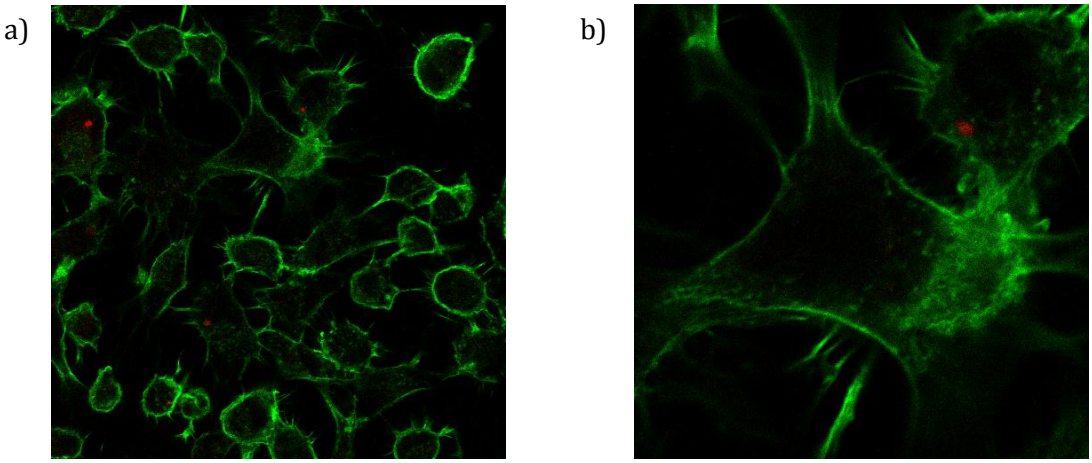

Fig. S4. Confocal images of HeLa cells stained by **2**-HSA adduct (red) and FITC (green).

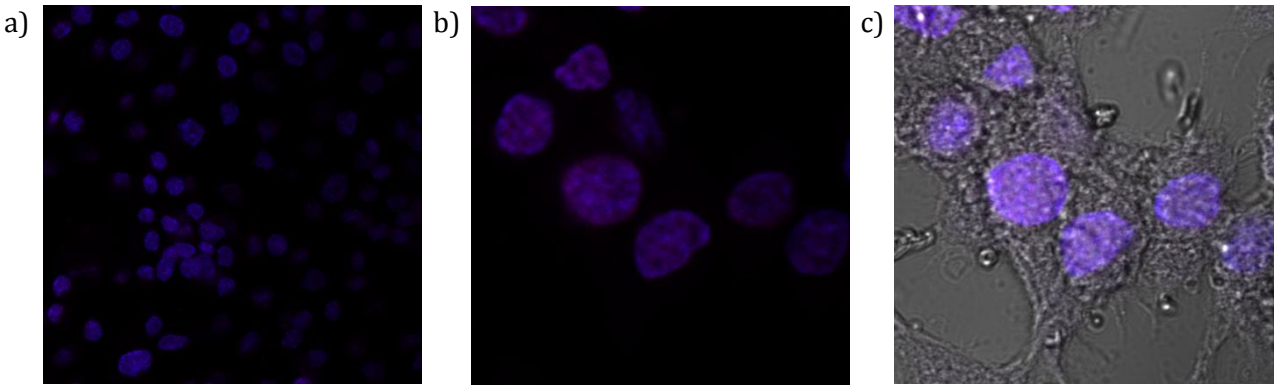

Fig. S5. Confocal fluorescence and overlaid fluorescence/bright-field images of HeLa cells stained by **3**-HSA adduct (red) and DAPI (blue). Confocal fluorescence images (a,b); overlaid fluorescence/bright-field image (c).

a) b)

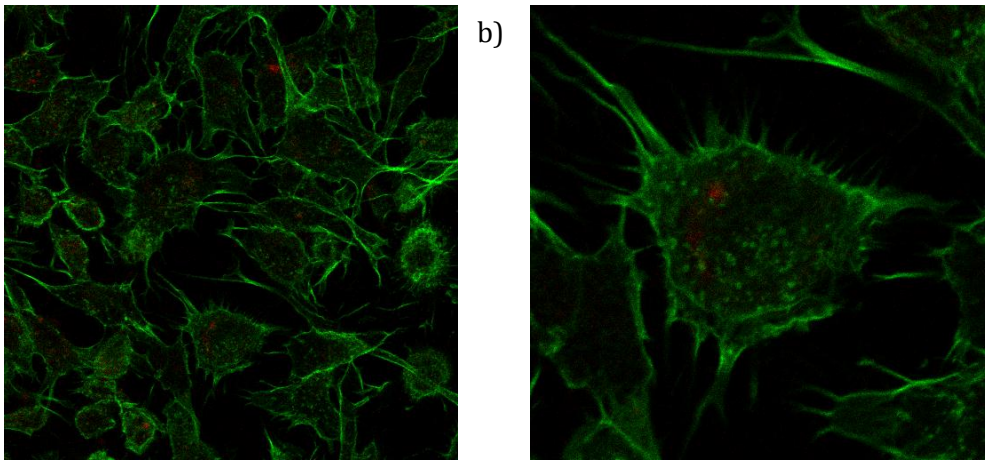

Fig. S6. Confocal images of HeLa cells stained by **5**-HSA adduct (red) and FITC (green).