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## Human Serum Albumin in the presence of Small Platinum Nanoparticles

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► **To cite this version:**

Xiaomin Yang, Erika Porcel, Laurent Marichal, Cesar Gonzalez Vargas, Amine Khitous, et al.. Human Serum Albumin in the presence of Small Platinum Nanoparticles. *Journal of Pharmaceutical Sciences*, 2024, 10.1016/j.xphs.2024.02.002 . hal-04476809

**HAL Id: hal-04476809**

**<https://hal.science/hal-04476809>**

Submitted on 25 Feb 2024

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1 **Title**

2 Platinum Nanoparticles Stabilize Human Serum Albumin Structure

3 Xiaomin Yang<sup>1</sup>, Erika Porcel<sup>1</sup>, Laurent Marichal<sup>2</sup>, Cesar Gonzalez Vargas<sup>1</sup>, Amine  
4 Khitous<sup>1</sup>, Daniela Salado-Leza<sup>1,3</sup>, Xue Li<sup>1</sup>, Jean-Philippe Renault<sup>2</sup>, Serge Pin<sup>2</sup>, Hynd  
5 Remita<sup>4</sup>, Frank Wien<sup>5#</sup>, Sandrine Lacombe<sup>1#</sup>

6 <sup>1</sup>Université Paris-Saclay, CNRS, Institut des Sciences Moléculaires d'Orsay, 91405  
7 Orsay, France

8 <sup>2</sup>Université Paris-Saclay, CEA, CNRS, NIMBE, 91191, Gif-sur-Yvette, France.

9 <sup>3</sup>CONACyT, Faculty of Chemical Sciences, Autonomous University of San Luis Potosi,  
10 78210 San Luis Potosi, Mexico

11 <sup>4</sup>Université Paris-Saclay, CNRS, Institut de Chimie Physique, UMR 8000, 91405 Orsay,  
12 France

13 <sup>5</sup>Université Paris-Saclay, Synchrotron Soleil, 91190, Saint-Aubin, France

14 **Corresponding Author:**

15 Prof. Sandrine Lacombe

16 Université Paris-Saclay, CNRS, Institut des Sciences Moléculaires d'Orsay, 91405,  
17 Orsay, France; Tel: +33 (1) 6915 8263 ; E-mail: [sandrine.lacombe@u-psud.fr](mailto:sandrine.lacombe@u-psud.fr).

18 Prof. Frank Wien

19 Université Paris-Saclay, Synchrotron Soleil, 91190, Saint-Aubin, France; Tel: +33 (1)  
20 6935 9665 ; E-mail: [frank.wien@synchrotron-soleil.fr](mailto:frank.wien@synchrotron-soleil.fr).

21

22 **Abstract**

23 Noble metals, especially platinum nanoparticles (Pt NPs) have immense potential in  
24 nanomedicine as therapeutic agents on account of their high surface area, intrinsic

25 catalytic properties, etc. However, our understanding of the interaction of nanoparticles  
26 with biological entities, especially proteins, is far behind the explosive development of  
27 nanotechnology. In this study, we measure the structural and stability changes of  
28 proteins at nanomolar concentrations upon interaction with Pt NPs using various  
29 techniques. A large increase of 18°C in the thermal unfolding of human serum albumin  
30 (HSA) with Pt NPs at the molar ratio 1:1 is observed using synchrotron radiation  
31 circular dichroism (SRCD), due to Pt NPs increased thermal stability of HSA through  
32 preferential hydration processes. The use of SRCD allows measuring critical parameters  
33 on NPs-protein interactions, provides additional information for understanding the  
34 influential role of PEG coating in NPs-cell interaction and has implications for  
35 nanomedicine and nanotoxicology.

36 **Key words:** Pt NPs, HSA, synchrotron radiation circular dichroism, thermal stability,  
37 thermodynamic properties

## 38 **1. Introduction**

39 After the pioneering work of Rosenberg in 1965, cis-platin have been widely used in  
40 cancer therapy (Rosenberg et al., 1969). From then on, numerous new Pt compounds  
41 have been discovered with better therapeutic performances than cis-platin (Dasari and  
42 Tchounwou, 2014). Despite of the clinical success of the Pt complex, it suffers from a  
43 lack of tumor tissue selectivity leading to some severe side effects (Medhat et al., 2017).  
44 Advances in nanotechnology have provided new and powerful tools in the development  
45 of multifunctional Pt NPs for effective detection and treatment of cancer, overcoming  
46 limitations associated with conventional cancer diagnosis and therapy (Srinivasan et al.,  
47 2015). Radiolytic synthesis (by gamma rays, X-rays, electron beams or other types of  
48 ionizing radiation) provides a major advantage for a large-scale production of  
49 monodisperse particles as it generates a uniform distribution of reducing agents in the

50 entire solution during the irradiation process (Abidi et al., 2010; Belloni et al., 1998;  
51 Holade et al., 2017; Uttayarat et al., 2015). Another advantage is that these  
52 nanoparticles are freshly prepared in water without using potentially toxic solvents. The  
53 functionalization of the NPs can be achieved during the radiolytic synthesis by adding  
54 an appropriate substance to the reaction (Freitas de Freitas et al., 2018). The  
55 functionalization with ligands or polymers such as poly(ethylene) glycol PEG enhances  
56 the stability and control of their size (Wang et al., 2011).

57 Pt NPs have received considerable attention in nanomedicine due to their tunable,  
58 highly sensitive optical, catalytic, and therapeutic properties. A promising area of  
59 research involves the amplification of radiotherapy in the presence of Pt NPs at the  
60 tumor site (Lacombe et al., 2017). The properties of radioenhancement were  
61 demonstrated for Pt NPs at nanoscale (Porcel et al., 2010; Schlathölter et al., 2016) and  
62 at cellular level (KA et al., 2018; Salado-Leza et al., 2020; Yang et al., 2020c). For this  
63 use, intravenous administration of NPs is preferable than intra tumoral, to avoid pain to  
64 the patient and ensure reproducible distribution via enhanced permeability and retention  
65 effect (EPR). Thus, further exploration of the interaction of NPs with biological systems  
66 and blood proteins in particular is an important issue.

67 Proteins participate in most of the biological functions of the living matter and play a  
68 crucial role in its maintenance. Their biologic activity highly depends on their three-  
69 dimensional secondary structure (Takano et al., 2016), while undesirable interactions  
70 with NPs, may lead to protein aggregation or dysfunction (Saptarshi et al., 2013). The  
71 present study is focused on the interaction of Pt NPs with human serum albumin (HSA),  
72 since serum albumins are major circulatory proteins, which play crucial roles in many  
73 important physiological functions like maintaining plasma oncotic pressure,  
74 transporting cargo, antioxidant and enzymatic activities (Lee and Wu, 2015). Once NPs

75 enter systemic circulation, particle-protein interaction is the first phenomenon taking  
76 place before distribution into various organs (Mu et al., 2014). The impact of NPs, when  
77 they interact with blood proteins, is thus a major question to answer. In particular, a  
78 modification of the protein structure is expected to be dramatic, by changing  
79 physiological functions (Prasanth et al., 2018).

80 The analysis of the structure and stability of proteins in protein-Pt NPs is a key to  
81 investigate and understand the change of the proteins' properties after their interaction  
82 with Pt NPs. The structure analysis can be performed with various techniques. Among  
83 them, Circular Dichroism (CD) allows to characterize the secondary structure, and thus  
84 folding properties of proteins, and has recently been used to detect structure changes of  
85 proteins interacting with NPs (Micsonai et al., 2015). Synchrotron Radiation Circular  
86 Dichroism (SRCD) spectroscopy is superior to the conventional CD spectroscopy,  
87 thanks to the high intensity of photon emission, and the higher signal-to-noise levels of  
88 the CD spectra. This allows to use lower amounts of proteins, and to observe subtle  
89 conformational changes as shown by Wallace and Janes (Wallace and Janes, 2010).  
90 Additionally, the spectral range in SRCD extends down to 175 nm (compared to 190 nm  
91 in CD). This enables elucidating finer details in the secondary structure of proteins  
92 (Gobeaux and Wien, 2018), especially in the presence of nanoparticles (Sanchez-  
93 Guzman et al., 2020). SRCD allowed Laera et al. to discover that the thermal unfolding  
94 temperature of HSA decreased by 6°C upon interaction with silver NPs. This indicates  
95 that the HSA is significantly destabilized by the NPs, possibly due to a more flexible  
96 folded structure formed in the presence of the silver NPs (Laera et al., 2011). On the  
97 contrary, Robin Capomaccio et al finds a melting temperature ( $T_m$ ) of 69°C for free  
98 HSA and of 75.4 °C for the AuNP-HSA complexes using SRCD, suggesting that Au  
99 NPs stabilize HSA, both by increasing its thermal stability, and by reducing its

100 propensity to aggregate (Capomaccio et al., 2015). Recently, our group has shown that  
101 Gd NPs (AGuIX) can modify the stability of HSA due to an indirect interaction (Yang  
102 et al., 2020a).

103 In this study, Pt NPs were synthesized, characterized and their potential impact on  
104 HSA was investigated. Our aim was to understand the interaction between Pt NPs and  
105 serum blood proteins by complementary techniques, an area in which the number of  
106 available reports is limited. In particular, we measured the secondary structure of  
107 proteins in low nanomolar concentrations, and in particular, the structural and thermal  
108 stability changes associated with proteins to Pt NPs up to 1:1 particle ratios using the  
109 SRCD technique at DISCO, Synchrotron SOLEIL. This extremely sensitive technique  
110 has allowed us to show that Pt NPs have significantly increased the thermal stability of  
111 HSA, and has also allowed accessing new detailed information about NPs-protein  
112 interactions.

## 113 **2. Experimental**

### 114 **2.1. Materials and chemicals**

115 Tetraammine platinum (II) chloride salt  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2 \cdot \text{H}_2\text{O}$  (Sigma-Aldrich, USA),  
116 poly(ethylene glycol)  $(\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH})$ ,  $M_w = 1000 \text{ g}\cdot\text{mol}^{-1}$ , Fluka), potassium  
117 phosphate monobasic  $(\text{KH}_2\text{PO}_4)$ , sodium phosphate dibasic  $(\text{Na}_2\text{HPO}_4)$ , HSA was  
118 purchased from Sigma-Aldrich, USA. HSA samples were prepared by dissolving HSA  
119 powder in 10 mM phosphate buffer at pH 7.0. All chemicals were of analytical grade.  
120 Pure water used in all experiments was filtered (18.4  $\text{M}\Omega \text{ cm}$ ) by a Milli-Q system  
121 (Millipore, Milford, MA, USA).

### 122 **2.2. Synthesis of Pt NPs**

123 Pt NPs were synthesized by facile and ecofriendly one-step radiolysis. The procedure  
124 of synthesis and characteristics of these NPs are detailed in the French Patent

125 Application: Nanoparticules et procédé de préparation, FR1900008 (Salado-Leza et al.,  
126 2020; Yang et al., 2020b). Briefly, the reduction was achieved in aqueous solution using  
127  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$  ( $10^{-2}$  mol/L) as precursor and polyethylene glycol (PEG-OH 1000, 1  
128 mol/L) as radical scavenger, stabilizer and biocompatible capping agent. After thorough  
129 deaeration with flushing nitrogen gas, the solutions were irradiated by a  $\gamma$ -source  
130 provided by a panoramic  $^{60}\text{Co}$   $\gamma$ -facility ( $E= 1.33$  MeV,  $\text{LET}= 0.2$  keV. $\mu\text{m}^{-1}$ ) with dose  
131 rate of  $68.2$  Gy. $\text{min}^{-1}$ .  $\text{Pt}^{\text{II}}$  was completely reduced to  $\text{Pt}^0$  at a dose of 1,000 Gy (Porcel  
132 et al., 2012; Porcel et al., 2010). The obtained Pt NPs were lyophilized for storage at 4  
133 °C for months.

### 134 **2.3. Characterization of Pt NPs**

135 **Morphological characterization** was conducted by a TEM-STEM JEOL1400  
136 (Ernest) transmission electron microscopy (TEM) with an accelerating voltage of  
137 120 kV. For TEM observations, a drop of the Pt NPs solution was deposited onto  
138 copper grids (200 mesh, Agar Scientific Ltd). The grids were dried under a flow of  
139  $\text{N}_2$  under ambient conditions before observations. Statistical analysis of Pt NPs diameter  
140 was converted to cumulative number-based distributions with 'Image J' program.

141 **Electrokinetic properties ( $\zeta$ -potential)** of Pt NPs were measured with  $\zeta$ -potential  
142 analyzer model Zeta Potential WALLIS (Cordouan Technologies, France). Pt NPs were  
143 diluted in Mili-Q water to reach 0.1 mM, and measured at 25°C in a disposable folded  
144 capillary cell.

145 **The optical absorption measurements** were performed on a CARY 300 Scan UV-  
146 Visible Spectrophotometer (Int. Agilent) in the frequency range of 200 nm-800 nm at  
147 room temperature before and after irradiation. The as-synthesized Pt NPs were diluted  
148 with Milli-Q water to a final concentration of Pt [1 mM] for UV-visible absorption  
149 measurements.

## 150 **2.4. Preparation of the samples**

151 The HSA mother solution (20 mg/mL) was prepared by dissolving HSA powder in 10  
152 mM phosphate buffer at pH 7.4 (Shaw and Pal, 2008). The concentration of HSA was  
153 determined by absorbance measurements using molar extinction coefficients of 0,58  
154 (g/L)<sup>-1</sup>cm<sup>-1</sup> at 280 nm (Dockal et al., 1999).

155 Pt NPs-HSA samples at different Pt NPs/HSA molar ratios (1:30 to 1:1) were  
156 prepared by mixing a 15 μM HSA solution with different concentrations of Pt NPs in  
157 phosphate buffer at pH 7.0. The samples were incubated at room temperature for 40  
158 minutes before measurements.

## 159 **2.5. Dynamic light scattering**

160 The hydrodynamic diameter of Pt NPs in the absence and presence of proteins (15 μM)  
161 (with molar ratios of Pt NPs/HSA from 1:30 to 1:1) was measured by dynamic light  
162 scattering (DLS) using a Cordouan VASCO-2 particle size analyzer (Cordouan  
163 Technologies, France). The Pt NPs were diluted in Mili-Q water to a final concentration  
164 of 0.5 mM, and the measurements were conducted at 25°C in a semi-micro polystyrene  
165 (PS) cuvette.

## 166 **2.6. Isothermal Titration Calorimetry (ITC)**

167 Adsorption isotherms of HSA on Pt NPs were performed by calorimetry using a  
168 MicroCal PEAQ-ITC (Malvern). All the solutions were thoroughly degassed prior to the  
169 titrations to avoid the formation of bubbles in the calorimeter cell. The reaction cell  
170 (280 μL) was loaded with a HSA solution at 1 x 10<sup>-4</sup> mol/L. The syringe (75 μL) was  
171 filled with a Pt NPs solution at 2 x 10<sup>-3</sup> mol/L. The proteins and NPs were prepared in  
172 the same phosphate buffer (pH 7.4) to prevent any pH effect. The experiments were  
173 done in duplicate at 20 °C by adding 2.4 μL of the Pt NPs solution to the HSA solution  
174 with an equilibration interval of 200 s. Raw data were obtained as a plot of heat (μcal)



175 against the injection number, and featured a series of peaks for each injection. These  
176 raw data peaks were transformed using the instrument software to obtain a plot of the  
177 enthalpy change per mole of injectant ( $\Delta H^0$ , kcal·mol<sup>-1</sup>) against the molar ratio. Control  
178 experiments included the titration of the Pt NPs solution into buffer, buffer into HSA,  
179 and buffer into buffer, with the same used sample concentration. Corrected data refer to  
180 experimental data after subtraction of the control data.

## 181 **2.7. Synchrotron radiation circular dichroism (SRCD)**

### 182 **SRCD spectra acquisition**

183 The SRCD spectra of samples were measured on the DISCO beamline, SOLEIL  
184 synchrotron radiation facility, Saint-Aubin, France.

185 Prior to the measurement, the instrument was calibrated using an aqueous solution of  
186 (+) camphorsulphonic acid (CSA). To prepare the sample, 4  $\mu$ L Pt NPs–HSA solution  
187 was placed in CaF<sub>2</sub> cuvettes with a 50  $\mu$ m pathlength (Wien and Wallace, 2005).

188 The HSA spectra were collected between 170 and 261 nm with a step size of 1 nm  
189 and an integration time of 1200 ms/nm at 37°C. All the spectra were recorded in  
190 triplicate, averaged, baseline subtracted and zeroed using CDToolX software (Lees et al.,  
191 2004). Raw data ellipticity expressed in millidegrees (mdeg) was converted to molar  
192 circular dichroism values ( $\Delta\epsilon$ ). For the molar circular dichroism values ( $\Delta\epsilon$ , M<sup>-1</sup>·cm<sup>-1</sup> =  
193 L·mol<sup>-1</sup>·cm<sup>-1</sup>), the following Eq. 1 was applied:

$$194 \quad \Delta\epsilon = \theta \times \frac{(0.1 \times \text{MRW})}{(P \times C) \times 3298} \quad (1)$$

195 where the MRW (mean residue weight) of HSA is 113.8 Da (g·mol<sup>-1</sup>), P (pathlength)  
196 is 0.005 cm (measured by interferometry) and C (protein concentration) was ~1 mg/mL.

## 197 **Thermal denaturation**

198 Thermal denaturation studies were carried out by collecting SRCD spectra in a  
199 temperature range between 26 and 95°C with a step of 3°C. Three spectra were recorded  
200 with 5 minutes equilibration time for each temperature.

201 Ellipticity values were obtained from thermal denaturation curves for each of the  
202 three peaks (223 nm, 210 nm and 192 nm) at each temperature. They were normalized  
203 by setting the peak values at 26°C to 1.0. The decreasing of the relative intensity of  
204 protein with temperature has been fitted to a Boltzmann type equation Eq. 2 with the  
205 software OriginLab (Laera et al., 2011).

$$206 \quad y = A + \frac{B-A}{1+e^{-\frac{x-x_0}{dx}}}$$

207 (2)

208 (where X is the temperature, X<sub>0</sub> the melting temperature (T<sub>m</sub>), and dx the width of the  
209 thermal transition) for a simple two-state unfolding process. T<sub>m</sub> of the protein defined as  
210 the midpoint of the unfolding transition.

## 211 **Structure analysis**

212 The contents of  $\alpha$ -helix,  $\beta$ -sheet, turn and other structures of HSA were calculated  
213 from SRCD spectra using BeStSel, a web server used for accurate secondary structure  
214 components determination (Micsonai et al., 2018; Micsonai et al., 2015).

## 215 **3. Results and discussions**

### 216 **3.1. Synthesis and characterization of Pt NPs**

217 The Pt NPs synthesized in water by radiolysis were precisely characterized using  
218 DLS, TEM and the zeta potential technique (**Figure 1**).

219 The Pt NPs observed by TEM show semi-spherical morphologies (**Figure 1a**) and a  
220 uniform size distribution with an average metallic core diameter of  $3.0\pm 0.8$  nm (**Figure**  
221 **1b**). DLS measurements showed that Pt NPs have an average hydrodynamic diameter of  
222  $18\pm 2.6$  nm (**Figure 1c**). Significant differences between TEM analysis and DLS  
223 measurement indicate the Pt NPs PEG coating. The zeta potential value of Pt NPs in  
224 MiliQ water is  $-14.2\pm 4.1$  mV. This result is in good agreement with experimental results  
225 of other works (Ehi-Eromosele, 2016; Ibrahim et al., 2016), the PEG coated NPs have  
226 an average negative  $\zeta$ -potential of -14 mV and -17 mV, respectively, in water (pH 6~7),  
227 which leads to interparticle repulsion (Ehi-Eromosele, 2016).

### 228 [Figure 1]

229 The reduction of Pt complex and the formation of Pt NPs were monitored using UV-  
230 vis absorption spectroscopy. Metallic NPs display strong optical absorptions due to their  
231 surface plasmon resonance (SPR). Platinum nanoparticles have a plasmon in the UV  
232 range (Salado-Leza et al., 2019; Yang et al., 2020b). **Figure 2** shows the UV-vis spectra  
233 of the aqueous solutions containing  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$  and PEG-OH before and after  
234 irradiation.

235 The spectra reveal that, before irradiation, the aqueous solutions composed of  
236  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$  or  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$  in the presence of PEG-OH, have the absorbance of the  
237 main peak close to 240 nm and a small shoulder around 290 nm, which correspond to  
238 the ligand-to-metal charge transfer (LMCT) band between  $(\text{NH}_3)_4^{2+}$  and  $\text{Pt}^{\text{II}}$  ions  
239 (Gharibshahi and Saion, 2012). After irradiation, the two initial absorption peaks  
240 disappear due to the complete reduction of  $\text{Pt}^{\text{II}}$  into  $\text{Pt}^0$  leading to formation of Pt NPs.  
241 Thus, there was no further charge transfer possible from ligand to metal ions (Inwati et  
242 al., 2016). Moreover, the appearance of a surface plasmon absorption band with a single

243 broad peak centered at around 270 nm indicated the presence of Pt NPs (Moores and  
244 Goettmann, 2006; Yang et al., 2020b).

245 The inserted picture in **Figure 2** shows the color of the colloidal solution, which  
246 changed from colorless (before irradiation) to dark brown (after gamma irradiation).  
247 The colour change is due to the radiolytic reduction of  $[\text{Pt}(\text{NH}_3)_4]^{2+}$  ions to  $\text{Pt}^0$  atoms  
248 indicating the formation of colloidal Pt NPs (Gharibshahi and Saion, 2012; Salado-Leza  
249 et al., 2020) as mentioned before.

250 [Figure 2]

### 251 3.2. Dynamic light scattering analysis

252 In the present work, DLS was used to measure the hydrodynamic diameter of  
253 different concentrations of Pt NPs in the absence and presence of HSA at pH 7.4 in  
254 10 mmol/L phosphate buffer. No significant change in the NPs size distribution after  
255 exposure to HSA at room temperature. It demonstrated there was no complex formation,  
256 which is probably due to the grafting of hydrophilic PEG-OH on Pt NPs having a high  
257 capacity to prevent the adsorption of proteins (Pelaz et al., 2015; Wang et al., 2015).  
258 Furthermore, PEGylation might prevents the uptake of Pt NPs by Reticuloendothelial  
259 System (RES) and enhance the circulation lifetime of NPs inside the body (Suk et al.,  
260 2016).

261 [Figure 3]

### 262 3.3. Isothermal Titration Calorimetry

263 The binding properties of the Pt NPs with HSA were consecutively studied using ITC.  
264 **Figure 4a** and **Figure 4b** show the plot of enthalpy change ( $\Delta H^0$ ) against Pt NPs-HSA  
265 molar ratio. The amount of heat due to the controls (dilution) is measured and  
266 subtracted from the amount of the heat obtained for the Pt NPs-protein system. ITC  
267 results did not show any significant binding, and no thermodynamic parameters could

268 be obtained. This finding is consistent with previous studies on telodendrimer-based  
269 NPs having PEG in the surface (Li et al., 2012). Indeed, the functionalization of NPs by  
270 PEG prevents interactions with proteins (Gref et al., 2000).

271 [Figure 4]

272

### 273 **3.4. Synchrotron radiation circular dichroism (SRCD)**

#### 274 **SRCD spectra acquisition**

275 The adsorption of proteins on the surface of nanomaterials, may induce  
276 conformational changes for some proteins, which influence the functionality and  
277 biological activity of the proteins (Satzler et al., 2016; Shemetov et al., 2012). The  
278 SRCD spectroscopy is widely used to study the conformation of proteins in aqueous  
279 solution. The far UV SRCD spectra (170~261 nm) provide the variations in the  
280 secondary structure of proteins as a result of the molecular interactions with other  
281 materials (Prasanth et al., 2018). **Figure 5** shows the SRCD spectra of pure HSA and  
282 HSA in the presence of different concentrations of Pt NPs (0.5 mM, 1 mM, 2 mM, and  
283 15 mM), corresponding to Pt NPs-HSA ratios of 1:30, 1:15, 1:7.5, and 1:1. The  
284 spectrum exhibits two negative bands at 210 and 223 nm, one positive band at 192 nm,  
285 characteristic of the  $\alpha$ -helical structure. The negative peak at 223 nm is due to the  $n \rightarrow \pi^*$   
286 transition of the carbonyl group of peptide, while the parallel and perpendicular  
287 excitation of the peptide  $\pi \rightarrow \pi^*$  transition is responsible for the other negative peak at  
288 210 nm and the positive peak at 192 nm (Gobeaux and Wien, 2018).

289 With the addition of different amounts of Pt NPs to the HSA aqueous solution, the  
290 SRCD spectra of the Pt NPs-HSA mixture remained unchanged for three repeated scans

291 at 37°C (**Figure 5**). This clearly indicates that Pt NPs does not induce a significant  
292 conformation change of the protein at 37°C.

293 **[Figure 5]**

#### 294 **Thermal denaturation**

295 The thermal stability of proteins without or with Pt NPs was assessed by recording  
296 the intensity of SRCD signal at 192 nm as a function of temperature. SRCD spectra of  
297 pure HSA and Pt NPs-HSA at different ratios from 1:30 to 1:1 were recorded at  
298 temperatures in the range of 26~95°C. The temperature scans of Pt NPs:HSA=1:7.5 are  
299 presented in **Figure 6**. The spectra clearly indicate that the temperature increase causes  
300 a gradual decrease of the alpha helical profile.

301 **[Figure 6]**

302 The decreasing of the relative intensity of protein with temperature in **Figure 7**,  
303 showed that the thermal denaturation curves were shifted with the increasing ratio of Pt  
304 NPs-HSA towards higher  $T_m$ . The resulting  $T_m$  is reported in **Table 1**. From the results,  
305 we observe the  $T_m$  increases with increasing the Pt NPs concentration. The free HSA  
306 has a  $T_m$  of 56°C, which is in good agreement with reported results that HSA undergoes  
307 morphological changes at 56°C (Das et al., 2014). The  $T_m$  of Pt NPs-HSA=1:1 is 74°C,  
308 increased 18°C compared to the  $T_m$  of free HSA. The tremendous increase in  $T_m$   
309 suggests that Pt NPs increase the thermal stability of HSA. It was shown by other  
310 groups that silver NPs decrease the thermal stability of HSA, while gold NPs (using the  
311 same preparation method as silver NPs) did not significantly change it (Laera et al.,  
312 2011) even at same ratio of NPs-HSA. However, different experimental conditions must  
313 be taken into consideration.

#### 314 **Structure analysis**

315 A more detailed analysis of the two spectra using deconvolution software BeStSel  
316 allows estimating the percentage of secondary structure  $\alpha$ -helix,  $\beta$ -sheet, turn and  
317 random coil elements present in each SRCD spectrum.

318 **Figure 7** shows that the content of  $\alpha$ -helix conformation of HSA decreases from 61%  
319 to 29%, on contrary  $\beta$ -sheet and random coil increase from 1% to 20% and 30% to 40%  
320 respectively, with a subsequent increase of unfolded protein, as a function of the  
321 increasing temperature. The turn component of HSA remains practically constant for all  
322 the samples  $\sim$ 10% (in the interval 26~95°C). The same trends were found in the  
323 presence of Pt NPs, except the structuration in beta sheet was significantly lower at high  
324 temperature with the addition of Pt NPs (ratios of Pt NPs-HSA ranging from 1:30 to  
325 1:1).

326 Protein conformational compositions in pure HSA indicate an increase in  $\beta$ -sheet  
327 conformation from 0% to 20% at the expenses of the  $\alpha$ -helix content (which diminished  
328 from 57% to 28%) when the temperature rises from 38°C to 89°C. As the temperature  
329 increases, the cooperative hydrogen bonds that stabilized the helical structure weakened  
330 and expose hydrophobic groups to the solvent, partially unfolding the protein structure,  
331 which favours the formation of beta-sheet-rich amyloid fibrils (Juárez et al., 2009). It  
332 was shown that the presence of NPs can influence the protein fibrillation process  
333 (Mahmoudi et al., 2013). In this case, the alpha helix decreased less in the presence of  
334 Pt NPs than in pure HAS, and an increase in random coils substituted the  $\alpha$ -helix loss,  
335 indicating that Pt NPs prevent inter-protein interactions, and thus increase the  
336 conformational stability of proteins.

343 **[Figure 7]**

344 **Thermodynamic analysis**

345 The biological responses to NPs are influenced by the forces (such as electrostatic,  
346 hydrophobic or Van der Waals forces) present at the bio-nano interface (Mariam et al.,  
347 2017). Hence it is essential to have a complete understanding of the thermodynamics of  
348 Pt NPs-HSA interaction. The thermodynamic properties (enthalpy variation, entropy  
349 variation) obtained by fitting the temperature changes of local helix content to the Van't  
350 Hoff equation Eq. 3 (Greenfield, 2006):

$$351 \quad \ln K = \frac{-\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} \quad (3)$$

352 where K is the binding constant at the corresponding temperature, and R is universal gas  
353 constant.  $\Delta H^{\circ}$  and  $\Delta S^{\circ}$  are presented in **Table 1**. We found out a gradual increase of  
354 enthalpy variation and entropy variation in the presence of Pt NPs with increasing  $T_m$ . It  
355 can be seen that the enthalpy variation increases significantly from 87 kJ/mol of pure  
356 HSA to 116 kJ/mol of Pt NPs-HSA=1:1. The same phenomenon was observed by other  
357 groups. It was explained by Senske *et al*, that nanoparticles can stabilize the native  
358 conformation of proteins compared to the unfolded state under external stresses such as  
359 temperatures (Senske et al., 2014). This stabilization is explained by an unfavourable  
360 interaction of the protein and the nanoparticles leading to a preferential exclusion of the  
361 nanoparticles from the protein surface and a preferential hydration of the protein. Since  
362 the solvent accessible surface area is usually increased for the unfolded state, in the  
363 presence of Pt NPs, this would increase the thermodynamically unfavourable situation  
364 and require more energy for unfolding (unfolding enthalpy variation would increase  
365 with the Pt NPs concentration) than in water. As a result, the folding equilibrium should  
366 tend to shifted toward the more folded, native state.

367 Finally, the assumption of the increase of the HSA stability with Pt NPs was  
368 supported by good agreement with the thermodynamic data and structure analysis for  
369 the thermal unfolding transition determined by far-UV SRCD.



370 [Figure 8]

371 **4. Conclusion**

372 In summary, we have successfully synthesized Pt NPs with homogeneous size  
373 using radiolytic reduction method by gamma irradiation. The DLS and TEM images  
374 indicate that the synthesized Pt NPs are semi-spherical in shape and uniform in size.  
375 UV-Vis spectra suggest the complete reduction of Pt<sup>II</sup> and the formation of Pt NPs. The  
376 coherent results of hydrodynamic diameter and ITC demonstrated that Pt NPs do not  
377 bind to HSA even at the high ratio of 1 Pt NP per HSA. SRCD secondary spectra show  
378 that even the high concentrations of Pt NPs do not induce significant change of the  
379 protein conformation. The thermal stability of protein is assessed by SRCD by  
380 monitoring the spectral changes in the spectra with increasing temperature. More  
381 importantly, an increase of 18°C in the thermal unfolding of HSA upon interaction with  
382 Pt NPs was found, which support the fact that Pt NPs stabilize the HSA structure. This  
383 structure analysis showed that, as the temperature rises, the loss of  $\alpha$ -helical content of  
384 HSA was decreased whereas a significant reduction in  $\beta$ -sheet was observed. This is due  
385 to increased stability of HSA in the presence of Pt NPs. The modification of the protein  
386 stability can be attributed to the preferential hydration of the protein. This stabilizing  
387 effect renders Pt NPs highly promising and applicable to be used as biocompatible  
388 stabilizing agents for proteins.

389 **Author contribution**

390 X.Y. synthesized and characterized Pt NPs, performed and analysed the DLS, UV-Vis  
391 spectroscopy, carried out the SRCD spectra analysis, data interpretation and drafted the  
392 manuscript. E.P. supervised the experimental protocols. L.M., S.P., J.P.R., A.K.  
393 conceived and performed the experiments of ITC. D.S.L, C. G., A. K. and X.L.  
394 contributed to the paper modifications. F.W., X. Y., E. P., A.K., C. G. optimized and

395 performed the SRCD experiments. H.R., and D.S.L were engaged in nanoparticles  
396 design, synthesis and characterization. S.L. conceived the entire project, reviewing and  
397 editing the manuscript. All authors revised the manuscript.

### 398 **Acknowledgements**

399 X.Y. acknowledges support from China scholarship council (CSC, N° 201607040068).  
400 We acknowledge support from the Université Paris Saclay for the "Initiative de  
401 Recherche Stratégique" IRS NanoTheRad project. This work also benefited from the  
402 RESPORE grant. SRCD measurements on DISCO beamline at SOLEIL synchrotron  
403 light source were performed under the proposal 20171494. We thank Ruxandra Gref  
404 (Institut des Sciences Moléculaires d'Orsay, Université Paris Saclay) for providing kind  
405 help on ITC experiments.

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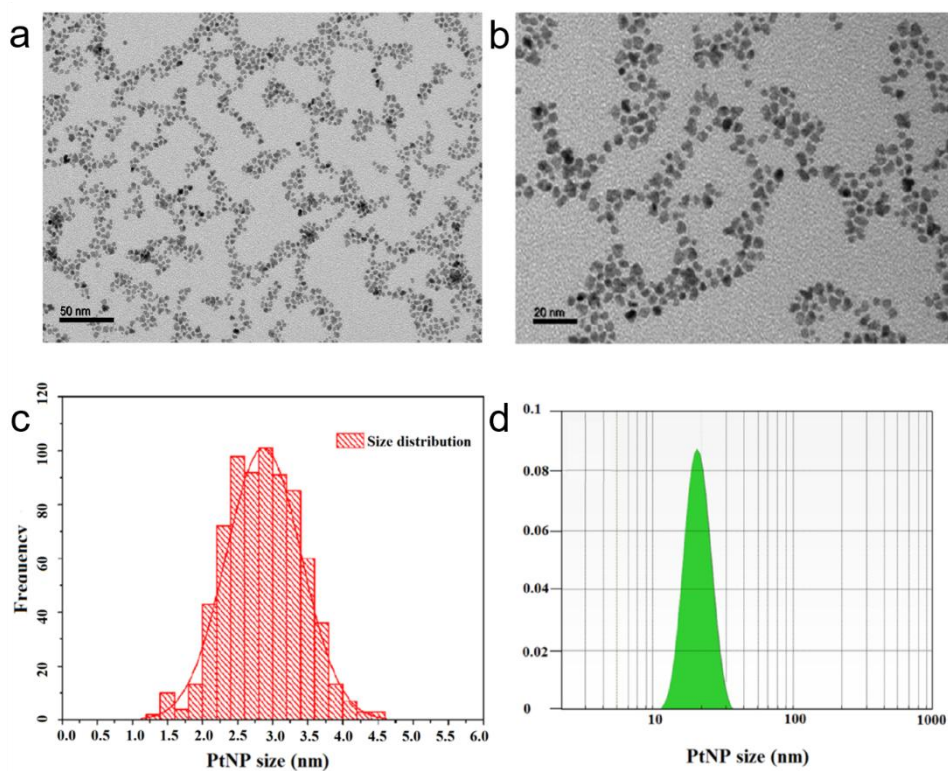
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**Captions to Figures**

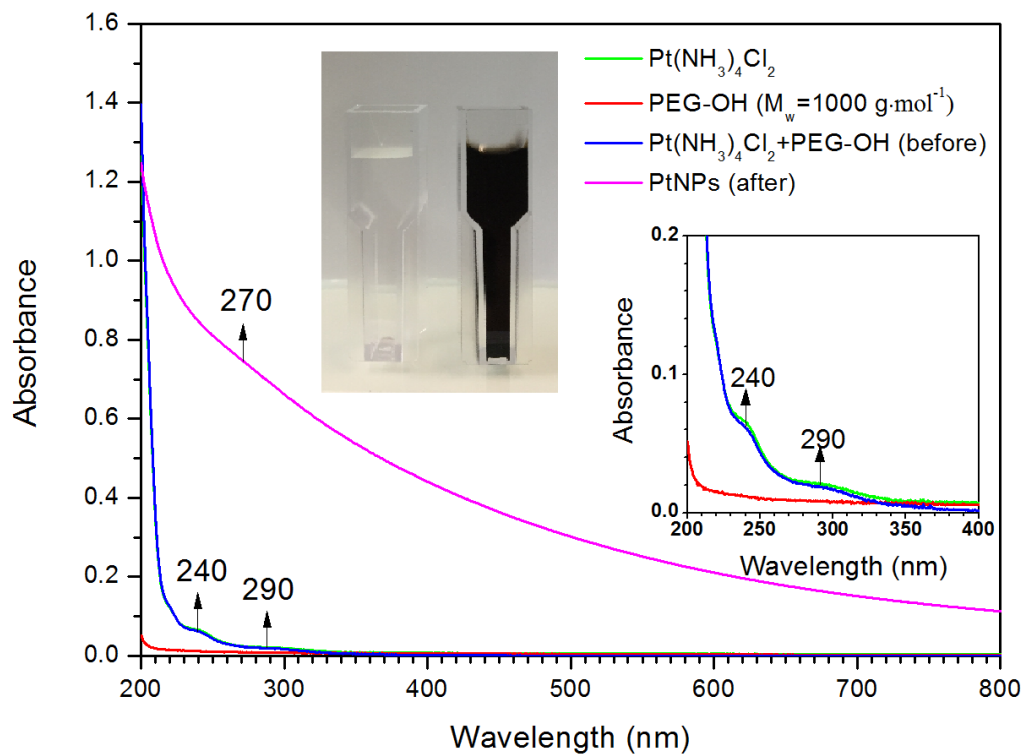


583

584 **Figure 1** (a) TEM image and (b) HRTEM images of Pt NPs; (c) TEM size distribution

585 histogram of Pt NPs; (d) DLS size distribution by intensity of Pt NPs;

586

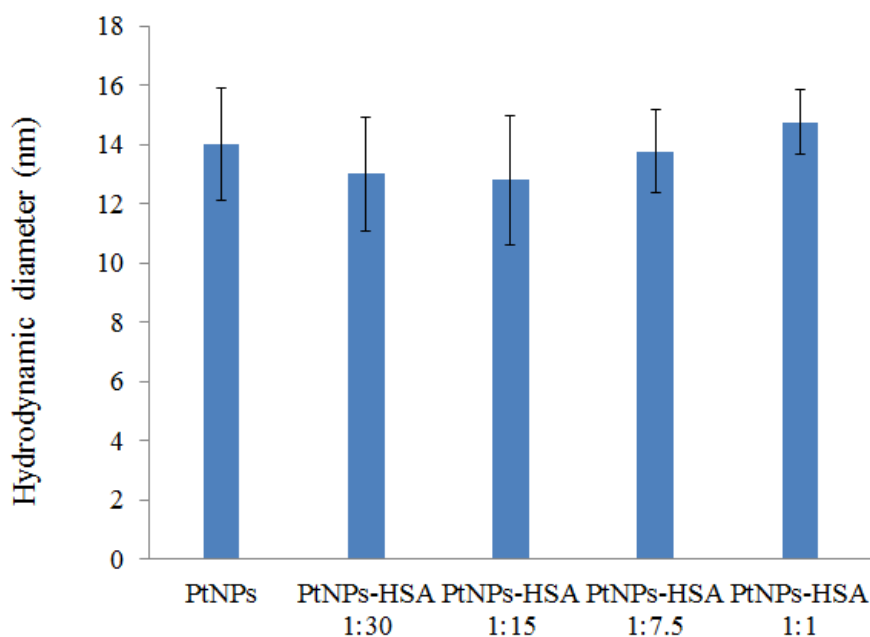


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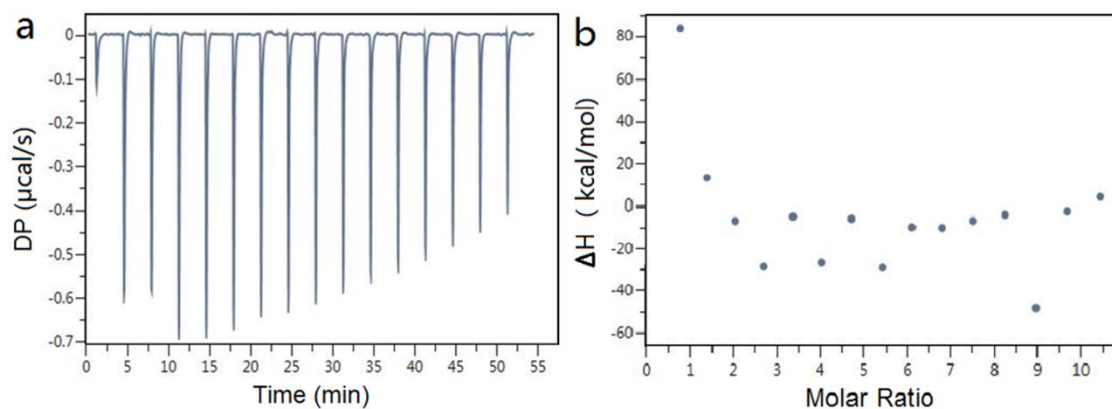
588 **Figure 2** UV-vis absorption spectra of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$  (green line) solution ( $10^{-3} \text{ mol.L}^{-1}$ ),  
 589 PEG-OH (red line) solution ( $10^{-1} \text{ mol.L}^{-1}$ ), and diluted Pt containing solutions ( $10^{-3}$   
 590  $\text{ mol.L}^{-1}$ ) before (blue line) and after (pink line) irradiation (optical path = 2 mm). Inset  
 591 is the absorption spectra of samples in a wavelength range from 200 to 400 nm.

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593

594 **Figure 3** Hydrodynamic diameter of Pt NPs and Pt NPs at different Pt NPs-HSA ratios  
 595 (from 1:30 to 1:1) at pH 7.4, 37°C.

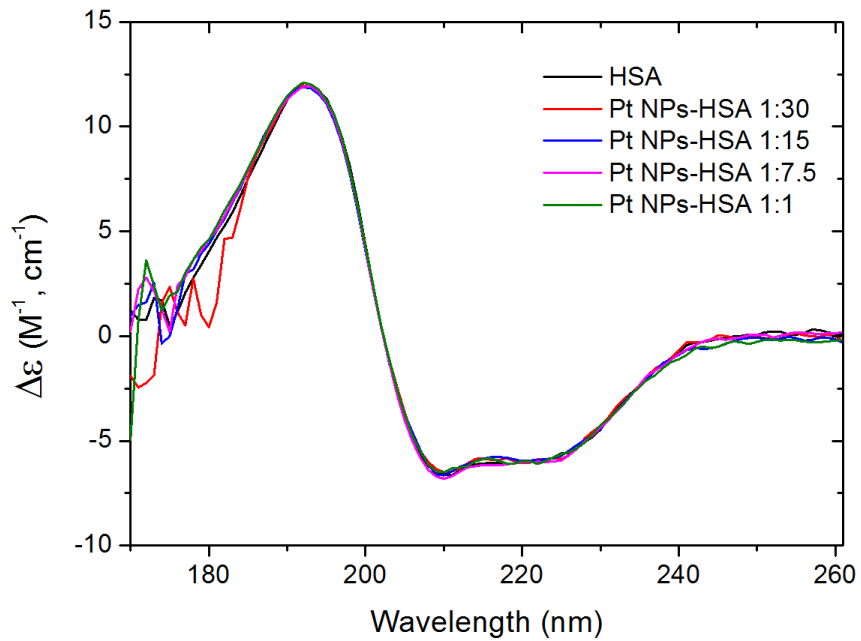


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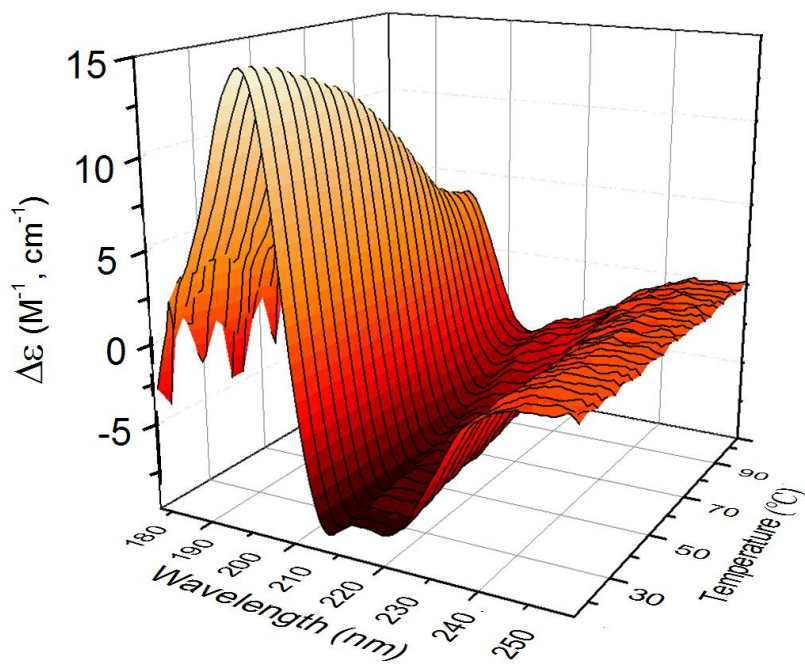
598 **Figure 4.** Isothermal titration calorimetry (ITC) plots of differential power throughout  
599 the titration (a), integrated heat as a function of the mole ratio of Pt NPs-HSA (b).

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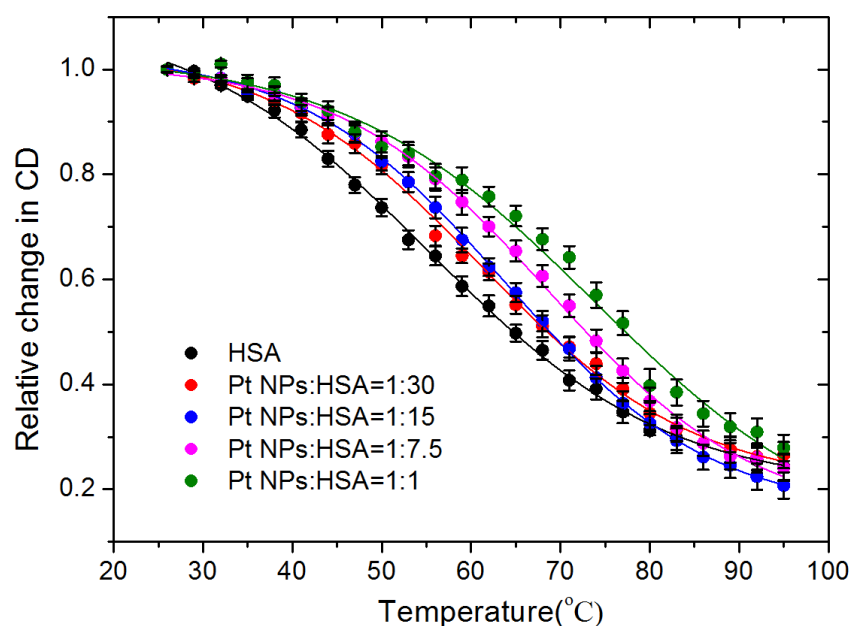
601

602 **Figure 5** SRCD spectra of HSA alone and Pt NPs-HSA at NPs:HSA molar ratios of  
603 1:30, 1:15 and 1:7.5 and 1:1, respectively at pH=7.4, 37°C.



604

605 **Figure 6** Temperature scans of Pt NPs:HSA=1:7.5 with steps of 3°C (from 26°C to  
 606 95°C).



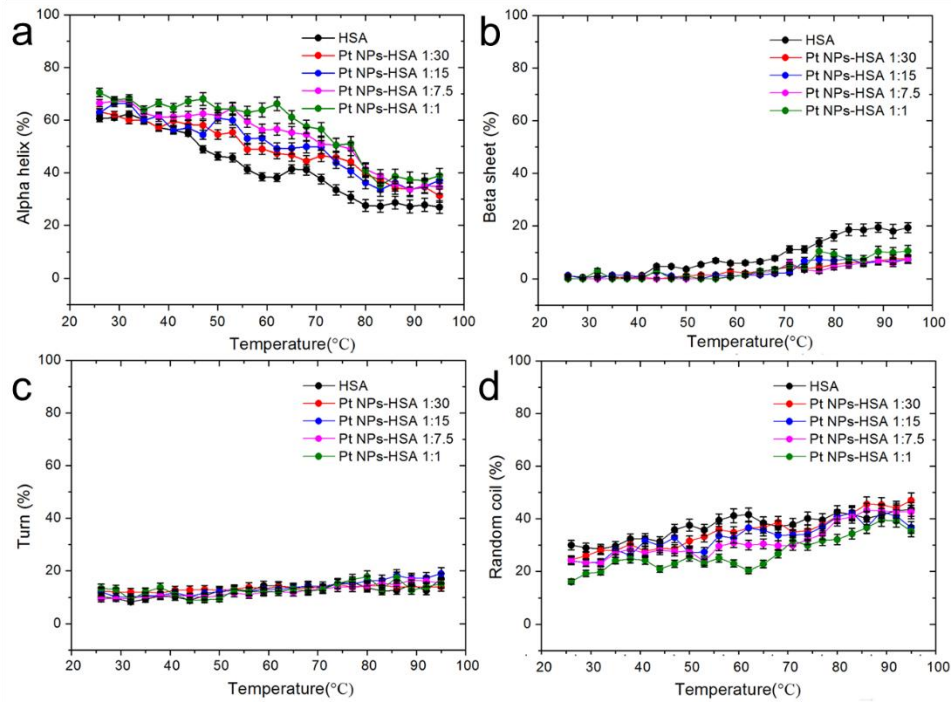
607

608 **Figure 7** Thermal denaturation curves of pure HSA and the Pt NPs-HSA complex at  
 609 different ratios (1:30, 1:15, 1:7.5, and 1:1) monitored at 192nm.

610

611 **Table 1. Values of the melting temperatures  $T_m$ , enthalpy variations  $\Delta H^\circ$  and**  
 612 **entropy variations  $\Delta S^\circ$  for HSA and Pt NPs-HSA at different concentrations**  
 613 **(ratios from 1:30 to 1:1) monitored at 192 nm.**

Sample	$T_m, ^\circ\text{C}$	$\Delta H^\circ, \text{kJ}\cdot\text{mol}^{-1}$	$\Delta S^\circ, \text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
HSA	$56 \pm 0.3$	$87.3 \pm 1.9$	$0.26 \pm 0.006$
Pt NPs-HSA 1:30	$61 \pm 0.3$	$89.5 \pm 1.3$	$0.27 \pm 0.004$
Pt NPs-HSA 1:15	$64 \pm 0.4$	$92.8 \pm 2.0$	$0.27 \pm 0.006$
Pt NPs-HSA 1:7.5	$69 \pm 0.4$	$101.3 \pm 4.2$	$0.28 \pm 0.010$
Pt NPs-HSA 1:1	$74 \pm 0.4$	$116.4 \pm 4.5$	$0.35 \pm 0.014$



614

615 **Figure 8** Contribution of the protein conformations: alpha-helix (a), beta sheet (b), turns  
 616 (c), and random coil (d), in the structure of HSA at different temperatures (from 26°C to  
 617 95°C), in the case of pure HSA and Pt NPs-HSA complex at different ratios (1:30, 1:15,  
 618 1:7.5, 1:1).

619