

1 Revisiting Protein Protected Gold and Silver Nanoclusters: Excited State 2 Dynamics and Long-Lived Emission

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9 **Abstract**

10 Owing to their intriguing photophysical properties, protein-functionalized metal nanoclusters
11 (MNCs) have become benchmark nanomaterials in various fields including electronics, optics,
12 energy, sensing, and biomedicine. However, the excited state dynamics of these MNCs,
13 especially the long-lived emission, remain a debatable topic. Here, we investigated the excited-
14 state dynamics of bovine serum albumin (BSA) functionalized Ag and Au nanoclusters (BSA-
15 Ag NCs and BSA-Au NCs) and provided new insights into their long-lived emission. Our
16 results showed completely different excited state dynamics in these MNCs despite their closely
17 similar number of atoms in the BSA-caged metal core. BSA-Au NCs showed short-lived
18 emission followed by a long-lived excited state at room temperature, originating from the core
19 and surface states, respectively. In contrast, the BSA-Ag NCs showed short-lived emission
20 originating only from the core states. The long-lived emission of BSA-Au NCs was attributed
21 to ligand-to-metal charge transfer (LMCT) facilitated by the presence of strong metal-sulfur
22 (M-S) bonds. The time-resolved luminescence imaging (TRLI) microscopy of both MNCs in
23 HeLa cells indeed confirmed the long-lived emission characteristics in BSA-Au NCs but not
24 in BSA-Ag NCs.

25
26 **Keywords:** Nanoclusters, Bovine serum albumin, long-lived emission, Room temperature
27 phosphorescence, Time-resolved luminescence imaging, TRANES

29 The metal nanoclusters (MNCs) have witnessed significant progress in recent years owing to
30 their intriguing photophysical properties such as near-infrared emission, large Stokes shift, low
31 cytotoxicity, and long photoluminescence lifetimes.¹⁻⁴ Among various ligand-protected MNCs,
32 protein-functionalized MNCs are particularly interesting because of their facile synthesis, low
33 toxicity and excellent biocompatibility.⁵⁻⁸ This makes them highly versatile for application in
34 multidisciplinary fields, including electronics, optics, energy, sensing, and biomedicine.⁹⁻¹¹
35 However, the excited state dynamics of these protein-functionalized MNCs remain a debatable
36 topic, largely due to the presence of multiple emissive centers within the nanoclusters.^{12,13}

37 Structurally, protein surface functionalized MNCs consist of tens of metal atoms encapsulated
38 within the protein cages or binding units, which prevent it from aggregation and provide
39 structural stability.^{5,14,15} The metal atoms are typically linked to the protein layer via the sulphur
40 atom of the cysteine residue of the protein (**Figure 1a**).⁸ Since the discovery of the
41 biom mineralization approach for the synthesis of bovine serum albumin (BSA) protected gold
42 and silver nanoclusters, there has been a growing number of studies on these clusters.^{6,16-20}
43 Time-resolved luminescence studies have been employed to elucidate the deexcitation
44 mechanism of the photoluminescence in these MNCs.^{21,22} Considering the decay
45 characteristics, it is proposed that the nanosecond lifetime mainly originates from the metal-
46 metal (M-M) core emission of the MNCs. A lifetime of sub-microseconds ranging from 100-
47 200 ns is associated with the emission from the ligand to metal charge transfer (LMCT) states,
48 originating from the intramolecular interactions involving metal core and surface motifs.²³
49 Most importantly, the long-lived lifetime from microseconds to milliseconds results from the
50 thermally activated delayed fluorescence (TADF) or room temperature phosphorescence (RTP)
51 (**Figure 1a**).²⁴⁻²⁶

52 The Jablonski diagram presented in **Figure 1b** depicts the above three types of deexcitation
53 mechanisms encountered in MNCs. The fluorescence emission is observed when the electron
54 relaxation occurs from the excited singlet state. The emission has also been occurring from the
55 electronic transition from LMCT states. These LMCT states arise from the metalophillic (M(I)-
56 M(I)) interactions and the charge transfer between the metal core and surface motifs.^{27,28} Both
57 TADF and RTP mechanisms involve triplet state harvesting, with a distinct difference in the
58 emission pathways. For TADF emitters, long-lived emission arises from the excited singlet
59 state following the reverse intersystem crossing (RISC) from the triplet state, whereas RTP
60 involves deexcitation directly from the triplet state. The dominance of the TADF or RTP

61 depends on the rates of intersystem crossing (K_{ISC}), rate of phosphorescence (K_{Ph}) and the rate
62 of reverse intersystem crossing (K_{rISC}).^{29,30}

63 Considering the massive size of BSA, with many electron donors, all the BSA nanoclusters
64 with nearly similar numbers of core metal atoms are expected to exhibit similar excited state
65 photophysical properties. Indeed, the reported emission spectra originating in the BSA
66 functionalized Au and Ag nanoclusters showed similar type of emission spectra in the red
67 region with a peak maximum spanning from 650 to 670 nm (**Figure 1c**). Here, we aim to revisit
68 the excited-state dynamics of BSA-protected Ag and Au nanoclusters (BSA-Au NCs and BSA-
69 Ag NCs) and to provide new insights into these well-studied MNCs through comprehensive
70 and systematic investigation. We show completely different excited state dynamics, especially
71 the long-lived emission characteristics in these MNCs. While BSA-Au NCs showed a short-
72 lived excited state followed by a long-lived emission originating from the core and surface
73 states, the BSA-Ag NCs showed predominant short-lived emission, involving the core states.
74 The strong interaction between the metal core and surface motifs via the M-S bond supports
75 LMCT states in BSA-Au NCs, whereas such interactions are weak in BSA-Ag NCs. The time-
76 resolved emission spectra (TRES), the time-resolved area normalization emission spectra
77 (TRANES) and the temperature-dependent luminescence measurements confirmed the RTP
78 mechanism in BSA-Au NCs for the long-lived emission. In contrast, BSA-Ag NCs exhibit only
79 fluorescence as the deexcitation mechanism at room temperature (RT). However, it is
80 transitioned to phosphorescence at 77 K which is due to the suppressed solvent and vibrational
81 relaxations in a rigid environment. The time-resolved luminescence imaging (TRLI)
82 microscopy of both MNCs in HeLa cells indeed confirmed the long-lived emission
83 characteristics in BSA-Au NCs but not in BSA-Ag NCs.

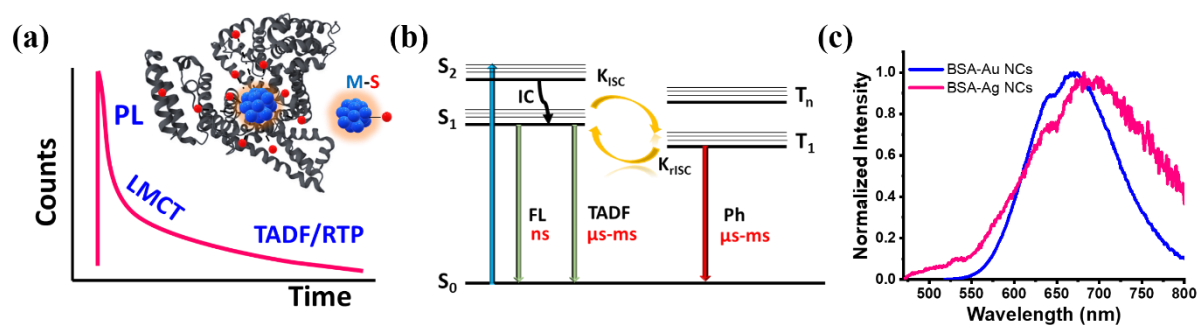


Figure 1. (a) Schematic representation of the possible structure and the typical lifetime decay curve of protein-conjugated MNCs. (b) The energy level diagram illustrating the mechanisms for the varied lifetime range, helped in understanding the excited state dynamics of (c) red-emissive BSA-Ag NCs and BSA-Au NCs.

84 The synthesis of BSA-Ag NCs and BSA-Au NCs was carried out by the previously reported
85 protocol.^{31,32} The transmission electron microscopy (TEM) data of about 100 particles
86 suggested that BSA-Ag NCs and BSA-Au NCs have closely similar diameters of 3.0 nm and
87 3.1 nm, respectively (**Figure S1a and S2a**). This was further well corroborated with the
88 hydrodynamic diameter of the nanoclusters calculated using fluorescence correlation
89 spectroscopy (FCS, **Figure S1b, S2b** details in supporting information SI). The parameters
90 obtained from the fitting analysis, namely diffusion times (τ_D), diffusion coefficient (D_t),
91 number of molecules (N), hydrodynamic radius (r_h), and diameter of the BSA-Ag NCs and
92 BSA-Au NCs are presented in **Table S1 and Table S2 respectively**. The matrix-assisted laser
93 desorption/ionization-time of flight (MALDI-TOF) mass spectrometry data showed that BSA-
94 Ag NCs consist of 30 Ag atoms (**Figure S3**). The number of Au atoms in BSA-Au NCs is 25,
95 corroborated by the reported literature.^{31,33,34} The XPS spectra of Ag 3d provide two distinct
96 components. The peaks with binding energy (BE) of 368.2 eV (Ag 3d_{5/2}) and 374.2 eV (Ag
97 3d_{3/2}) are designated for Ag (0), and 368.8 eV (Ag 3d_{5/2}) and 374.8 eV (Ag 3d_{3/2}) are assigned

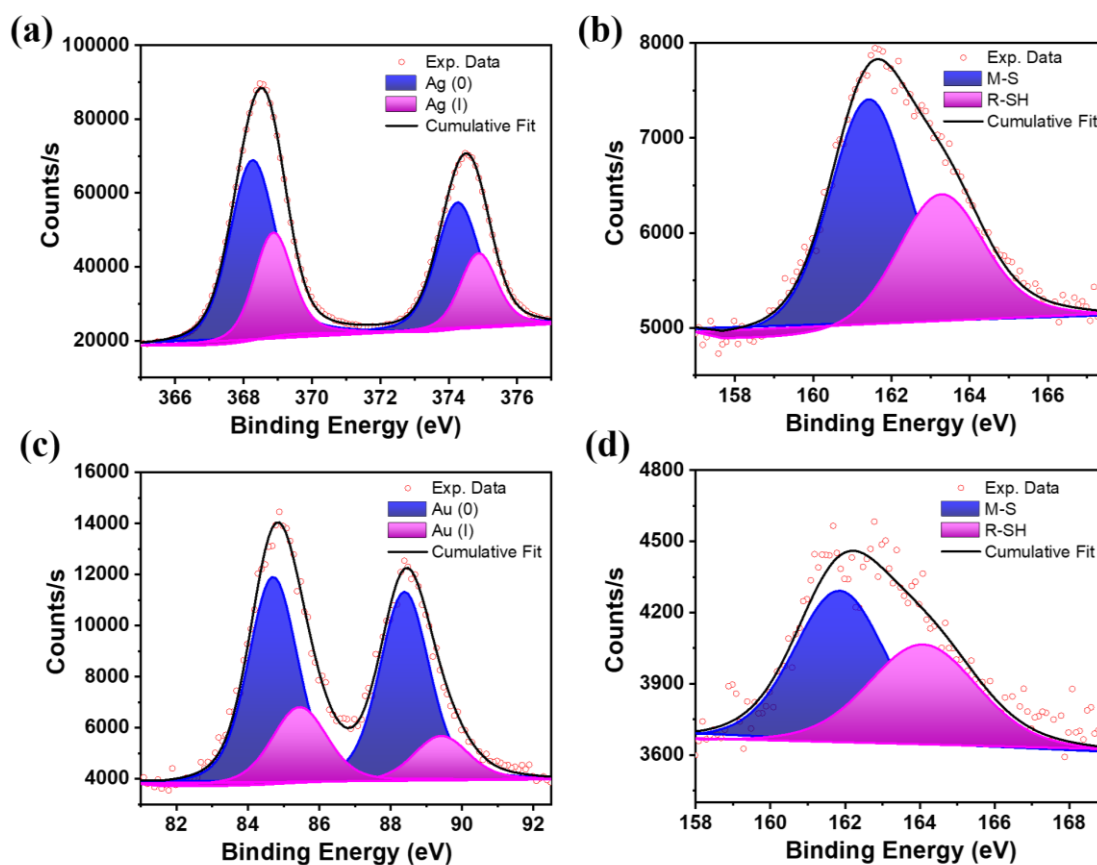


Figure 2. Deconvoluted X-ray photoelectron spectroscopy (XPS) profiles of (a) Ag 3d scan and (b) S 2p scan of BSA-Ag NCs. (c) Au 4f scan and (d) S 2p scan of BSA-Au NCs after etching time of 540.783 s.

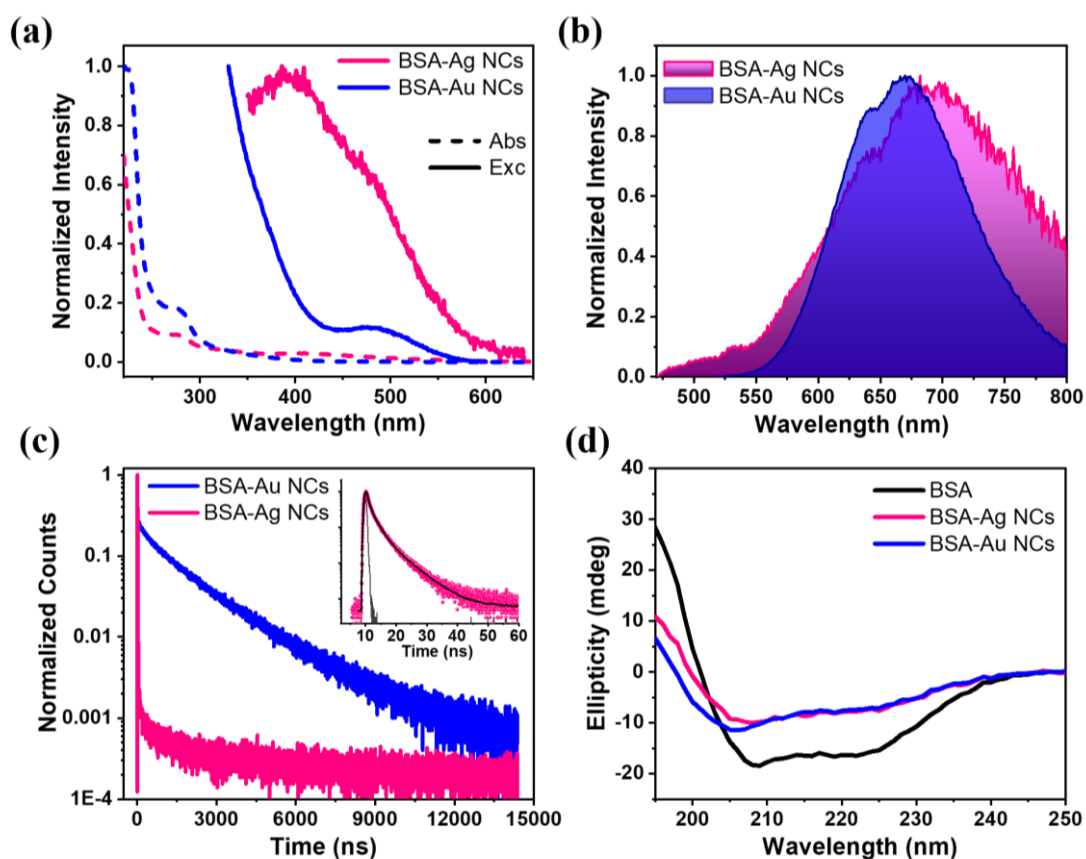


Figure 3. (a) The absorption, excitation and (b) the emission spectra of BSA-Ag NCs and BSA-Au NCs. (c) The lifetime decay curve of BSA-Ag NCs and BSA-Au NCs using measurement range of 13 μ s, inset: the lifetime decay curve of BSA-Ag NCs using measurement range of 100 ns. (d) The CD spectra of native BSA, BSA-Ag NCs and BSA-Au NCs all at the concentration of 0.1 mg mL⁻¹.

98 for Ag (I).³⁵ The contributions of Ag (0) and Ag (I) were 67% and 33%, respectively (**Figure**
 99 **2a**). These results indicate that the core of the BSA-Ag NCs is stabilized both by zerovalent
 100 and univalent silver atoms. The peak at 161.4 eV in the high-resolution deconvolution data of
 101 S2p of BSA-Ag NCs (**Figure 2b**) is assigned to the metal-sulphur bond and the peak at 163.4
 102 eV signified the unbound S of the BSA ligand.³⁶ The XPS data of BSA-Au NCs showed two
 103 peaks at 84.8 eV and 88.4 eV, which were attributed to 4f_{7/2} and 4f_{5/2} for Au respectively
 104 (**Figure 2c**) The former peak was further deconvoluted into two distinct components at 84.6
 105 eV and 85.4 eV which were assigned to Au (0) (75%) and Au (I) (25%) respectively.¹⁹ The S2p
 106 XPS spectrum of BSA-Au NCs shows two broad peaks at 161.8 eV and 164 eV, which is
 107 ascribed to sulphur bound to gold and the unbound sulphur of BSA, respectively (**Figure 2d**).³⁷
 108 It is important to note that the similar percentages of Ag (0) and Au (0) suggest a resemblance
 109 in the metallic core structure, while the similar percentages of Ag (I) and Au (I) indicate
 110 similarities in the surface ligand structure of both MNCs.

111 To understand the optical properties of BSA-Au NCs and BSA-Ag NCs, UV–vis absorption,
 112 excitation, and emission spectra were recorded. The absorption spectra of both the MNCs
 113 showed broad featureless absorption with a peak maximum at 280 nm, corresponding to the
 114 absorption originating from the tryptophan residues in BSA (**Figure 3a**).³⁸ The emission
 115 spectra in both cases showed a red emission with a peak maxima at around 670 nm for BSA-
 116 Au NCs and 680 nm for BSA-Ag NCs when excited at 450 nm (**Figure 3b**). Surprisingly, the
 117 excitation spectra of both the MNCs are distinctly different (**Figure 3a**). The BSA-Ag NCs
 118 show broad excitation with the excitation maximum at 400 nm, whereas the BSA-Au NCs
 119 showed progressively decreased excitation spectra with a hump at around 490 nm. This
 120 suggests that the different excited states are responsible for the emission in respective
 121 nanoclusters. The above results prompted us to understand the detailed mechanism and
 122 pathways of the excited state dynamics in both MNCs.

123 To investigate the excited state behaviour of red emissive MNCs, we employed luminescence
 124 lifetime measurements. BSA-Ag NCs showed the nanosecond lifetime as the dominant lifetime
 125 (**inset of Figure 3c**). The average lifetime of BSA-Ag NCs is 2.3 ns. The individual lifetime
 126 components with their amplitudes are presented in **Table 1**. Additionally, a negligibly small
 127 sub-microsecond component is observed from the decay curve of BSA-Ag NCs presented in
 128 **Figure 3c**. The short and long lifetimes presented in the lifetime decay curve are tail-fitted
 129 separately (**Figure S4**) with the fitting parameters mentioned in **Table S3**. On the contrary, the
 130 BSA-Au NCs showed a microsecond lifetime as the dominant one (**Figure 3c**). The average
 131 lifetime of BSA-Au NCs is found to be $\sim 1 \mu\text{s}$ (0.97 μs) using the triexponential function for
 132 fitting (**Table 1**). The fraction of microsecond lifetime (A_3) is much more in comparison to the
 133 nanosecond lifetime (A_1) in BSA-Au NCs. This distinct variation in the excited state lifetime
 134 in the MNCs led us to think if any conformational or secondary structural changes occurred in
 135 BSA during the MNCs formation. Hence, we performed circular dichroism (CD)
 136 measurements of the BSA and BSA conjugated MNCs. Typically, BSA showed two peaks in

Table 1. Represents the lifetime (ns) fitting parameters of BSA-Ag NCs and BSA-Au NCs including individual lifetime components with their amplitudes.

S. No.	τ_1	τ_2	τ_3	A_1	A_2	A_3	τ_{av}
BSA-Ag NCs	1.8	0.4	6.1	41.4	34.4	24.0	2.3
BSA-Au NCs	80	579	1700	20.8	34.6	44.4	972

137 the CD spectrum at 209 nm and 222 nm, which are the characteristics of the α -helical
138 secondary structure of native BSA.³⁹ The data presented in **Figure 3d** showed an obvious
139 change in the helical structure when both the BSA-MNCs were formed. However, the change
140 in ellipticity was similar in extent for both types of BSA-MNCs. We calculated the ellipticity
141 percentage changes for BSA and BSA conjugated MNCs.⁴⁰ For instance, the alpha helicity in
142 BSA is 56.5 %, while it is found to be 25.1 % and 21.4 % for BSA-Au NCs and BSA-Ag NCs,
143 respectively. This suggests similar conformational changes in BSA during the nanocluster
144 formation for both BSA-MNCs.⁴¹

145 The observed results ruled out the conformational or secondary structural changes, responsible
146 for the distinct excited state lifetimes in BSA-MNCs. Therefore, to further understand the
147 differences in their excited state decay profile at RT, TRES was employed to elucidate the
148 distribution of the emissive states that evolved with time.⁴² For the TRES acquisition of BSA-
149 Ag NCs, an excitation wavelength of 454 nm was used (details of the TRES acquisition are
150 provided in SI). **Figure 4a** displays the TRES of BSA-Ag NCs, which shows the evolution of
151 the emission spectra over the nanosecond timescale (at certain time delays). The data showed
152 spectral migration from 600 nm to 630 nm with the time evolution from 0.78 ns to 6.5 ns. The
153 TRES data aligned well with the time-resolved area normalized spectra (TRANES) showing
154 similar spectral shifting (**Figure 4b**). This spectral shifting seen in the TRES and TRANES of
155 BSA-Ag NCs might be from the heterogeneity of the excited states arising due to
156 intermolecular solvent relaxation.⁴³ To get more information, TRES and TRANES were also
157 recorded at 389 nm excitation, where no such spectral migration was seen, and the emission
158 originated from a single state (**Figure 4c and 4d**). Like BSA-Ag NCs, the TRES and TRANES
159 of BSA-Au NCs were also recorded at 454 nm and 389 nm excitation. A distinct excited state
160 dynamics was observed. The TRES data recorded at 454 nm excitation revealed the presence
161 of two excited states responsible for emission in BSA-Au NCs. However, the spectral
162 information of the short-lived state was inconclusive (**Figure 4e**). The TRANES plotted for the
163 TRES also failed to provide complete details about the short-lived state, despite the presence
164 of an iso-emissive point shown in **Figure 4f**. To fully characterize both the emissive states
165 involved in the deexcitation, TRES was acquired at 389 nm excitation. The data illustrate that
166 the short nanosecond lifetime originates from the singlet state at 450 nm, while the long-
167 lifetime emission is associated with the triplet state at 650 nm (**Figure 4g**). The short-lived
168 emissive state exists for 54 ns, after which the long-lived state dominates. Also, the iso-

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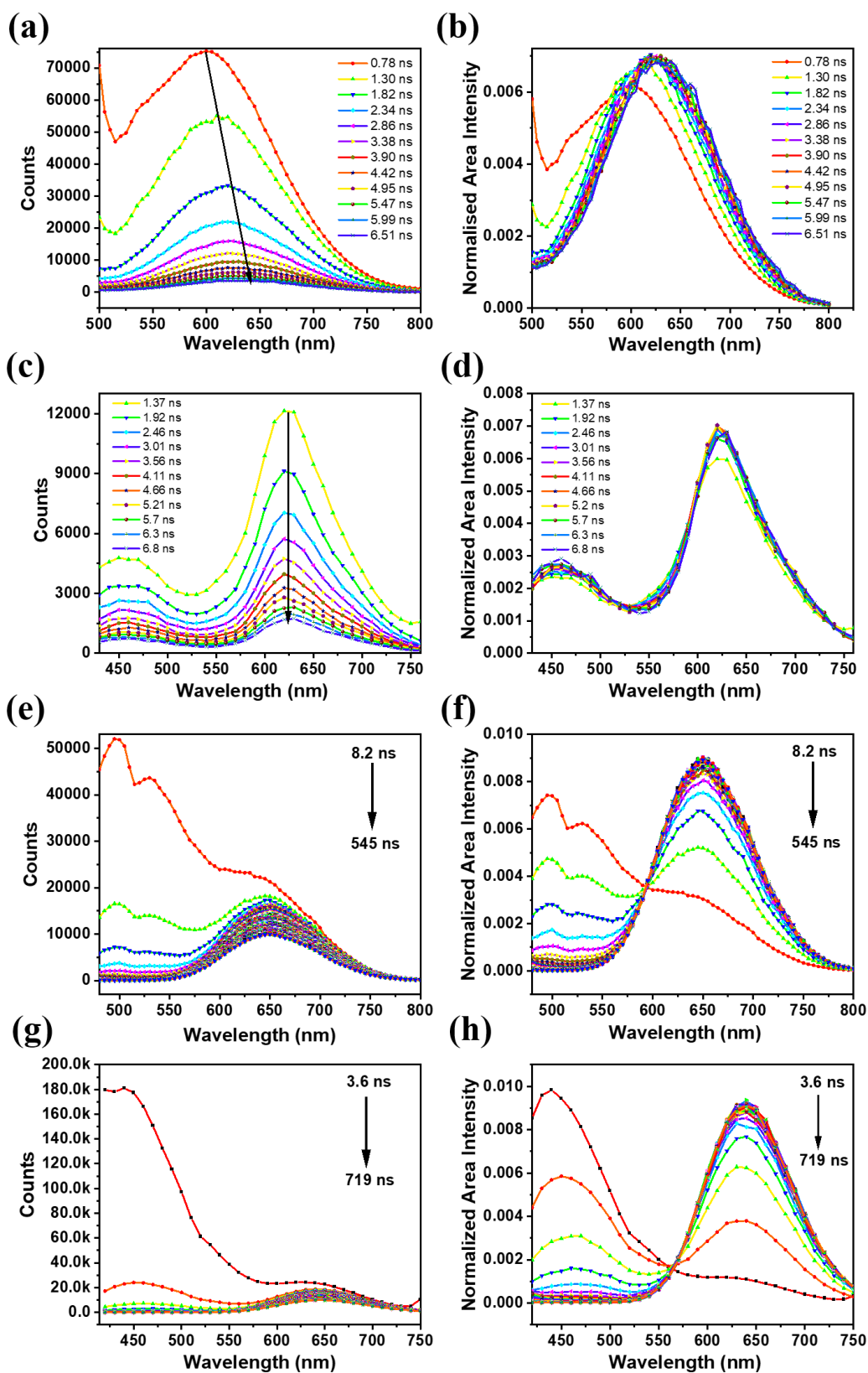


Figure 4. (a, c) The TRES and the (b, d) TRANES of BSA-Ag NCs [(a, b) - $\lambda_{\text{ex}} = 454$ nm; (c, d) - $\lambda_{\text{ex}} = 389$ nm (measurement range 100 ns)]. (e, g) The TRES and the (f, h) TRANES of BSA-Au NCs [(e, f) - $\lambda_{\text{ex}} = 454$ nm; (g, h) - $\lambda_{\text{ex}} = 389$ nm (measurement range 800 ns)].

170 emissive point in the TRANES spectra suggested that BSA-Au NCs showed dual emission

171 (Figure 4h).

172 Summarizing the above observations, both BSA-MNCs displayed heterogeneity in their
173 excited states. However, the origin of such heterogeneity is different in both the nanoclusters.
174 The TRES and TRANES studies of BSA-MNCs indicated that the high energy excitation (389
175 nm) is attributed to the core state excitation, while low energy excitation (454 nm) was
176 associated with surface state excitation.⁴⁴ For instance, the TRES data acquired at 454 nm,
177 failed to provide the core state information for BSA-Au NCs, whereas at 389 nm excitation,
178 spectral information of both the short and long-lived emissive states could be achieved. For
179 BSA-Ag NCs, as there is a lack of core and surface state interactions, only the core state
180 emission was found at 389 nm excitation. The spectral shifting due to solvent-induced
181 intermolecular interactions, occurring only at the surface motifs, was observed at 454 nm
182 excitation.

183 The difference in the core and surface state interaction might arise due to the variations in the
184 charge transfer from the surface ligands to the metal core via the sulphur-metal bond,
185 influenced by the specific metal involved in the interaction. The role of charge transfer states
186 in the emission of BSA-conjugated Au and Ag nanoclusters was demonstrated by observing
187 the change in the emission in both clusters using a well-known electron acceptor methyl
188 viologen (MV). To this endeavour, BSA-MNCs were incubated with the 1 mM solution of MV.
189 To our surprise, a complete quenching of fluorescence intensity was observed from steady-
190 state luminescence spectra of BSA-Au NCs, while the fluorescence intensity is unchanged with
191 the addition of MV in BSA-Ag NCs (Figure S5a and S5b). This result indicated that MV aids
192 the formation of the dark state by decreasing the potential energy barrier of the electron transfer
193 process in BSA-Au NCs. Also, the charge transfer states formed due to electron donation via
194 the S-metal bond have been quenched by the involvement of electron acceptor MV. This is due
195 to the electron donation from the N and O-rich groups in the BSA ligand to MV.⁴⁵ The complete
196 quenching of fluorescence intensity is supposed to be due to the disruption of charge transfer
197 states which is responsible for the origin of phosphorescence in BSA-Au NCs.

198 To gain greater details about the ambiguous results in both the MNCs, the stability of the BSA-
199 MNCs is understood by observing the changes in their chemical composition with aging,
200 through XPS analysis. For that, both the as-synthesized and aged BSA-MNCs (1 week old)
201 were subjected to XPS analysis. As evident from Figure S6 and Table S4, the contribution of
202 Ag (0) and Ag (I) within the BSA-Ag NCs, has changed from the as synthesized sample to the

203 aged sample (1 week old). Owing to the oxidation, the Ag (0) percentage decreased from 67
 204 % to 25 %, whereas the binding energies remained the same. The results highlight the intrinsic
 205 instability of BSA-Ag nanoclusters, which influences the harvesting of triplet states in the
 206 clusters. In contrast, when a similar analysis was performed on BSA-Au NCs, no significant
 207 changes in chemical composition were observed with aging; the contribution of Au (0) and Au
 208 (I) remained consistent (**Figure S6 and Table S5**).

209 For a deeper understanding of the triplet state dynamics, phosphorescence measurements of
 210 BSA-Ag NCs were done in cryogenic conditions. The phosphorescence spectrum was
 211 measured at 77 K, with a time delay of 100 μ s such that all the contributions from the
 212 fluorescence and scatterings could be eliminated. **Figure 5a** represents the normalized plot of
 213 fluorescence at room temperature (RT) and phosphorescence spectra at 77 K. It is believed that
 214 the emission is originating from the triplet state, which can be visualized from the red shift in
 215 the phosphorescence spectra of BSA-Ag NCs. Additionally, as we move from RT to 77 K, the
 216 spectral broadening decreases. This reduction may be due to rigidity induced at low
 217 temperatures, which restricts solvent-induced intermolecular interactions, which are the main
 218 cause for triplet state instability at RT. Due to the rigidity induced at 77 K, the lifetime decay
 219 curve of BSA-Ag NCs showed a sub-millisecond lifetime (**Figure 5c**).

220 We also unveiled the relaxation pathway of the long-lived emission in BSA-Au nanoclusters.
 221 For that, BSA-Au NCs is analyzed using TRES, temperature-dependent steady-state
 222 measurements, and time-resolved luminescence studies, with a focus on the delayed part of
 223 emission. As the long-lived emission (microsecond) is dominating in BSA-Au NCs, a time
 224 delay of 1 μ s is chosen for the temperature-dependent measurements. The emission intensity

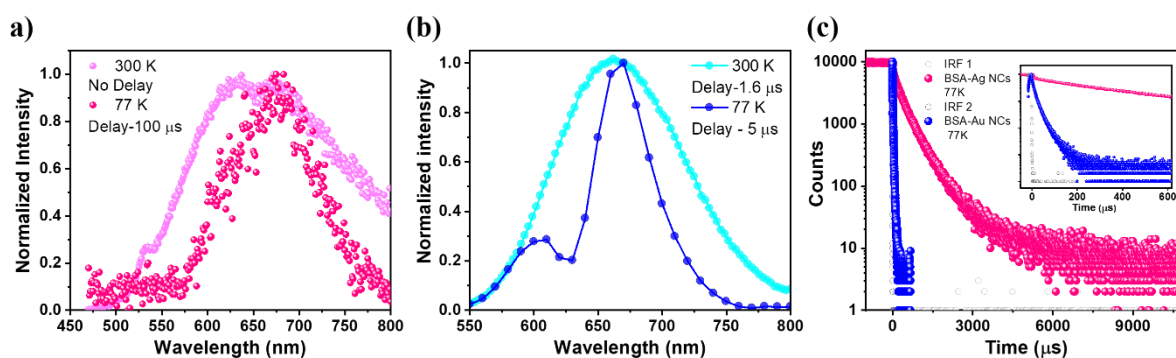


Figure 5. (a) Normalized emission spectra of BSA-Ag NCs recorded under ambient conditions ($T = 298$ K, $\lambda_{\text{ex}} = 454$ nm, no time delay) and 77 K ($\lambda_{\text{ex}} = 454$ nm, delay of 100 μ s), respectively. (b) Time-resolved emission spectra (TRES) profiles of BSA-Au NCs at 300 K (time delay: 1.6 μ s) and 77 K (time delay: 5.0 μ s). ($\lambda_{\text{ex}} = 417$ nm, $\lambda_{\text{em}} = 660$ nm). (c) The lifetime decay profile of BSA-Ag NCs ($\tau_{\text{av}} = 0.48$ ms) and BSA-Au NCs ($\tau_{\text{av}} = 18$ μ s) at 77 K; inset shows the zoomed view of the decay curves.

225 decreases with the increase in temperature from 283 K to 323 K (**Figure S7a**). As the rISC
226 become prominent at high temperatures, the decrement in the intensity rules out the TADF
227 process in BSA-Au NCs. To support this observation, the lifetime measurements were
228 performed, which showed the obvious decrement in the lifetime values with the increment in
229 the temperature from 283 K to 323 K (**Figure S7b**). The temperature-dependent measurements
230 exclude the possibility of the TADF mechanism in BSA-Au NCs. Similarly, TRANES data,
231 which distinguish the long-lived emissive state from the short-lived state, further rule out the
232 possibility of a TADF pathway for deexcitation. This suggests that room-temperature
233 phosphorescence is likely the dominant pathway for emission in BSA-Au NCs. To further
234 validate whether the luminescence occurred from the triplet state at room temperature, we
235 compared the room-temperature and low-temperature time-resolved emission spectra. For that,
236 time-resolved emission spectra are recorded at 300 K (time delay of 1.6 μ s) and 77 K (time
237 delay of 5 μ s), shown in **Figure 5b**. TRES at 300 K and 77 K show an overlap with the
238 emergence of two peaks at low temperatures. The deconvoluted peaks for 77 K are shown in
239 **Figure S8**. The spectral overlap at two different temperatures with a microsecond time delay
240 signifies that the dominating pathway of emission in BSA-Au NCs is RTP. Further, no lower
241 energy spectral shift at 77 K rules out the TADF process in BSA-Au NCs. The lifetime of BSA-
242 Au NCs measured at 77 K comes out to be 18 μ s (**Figure 5c**).

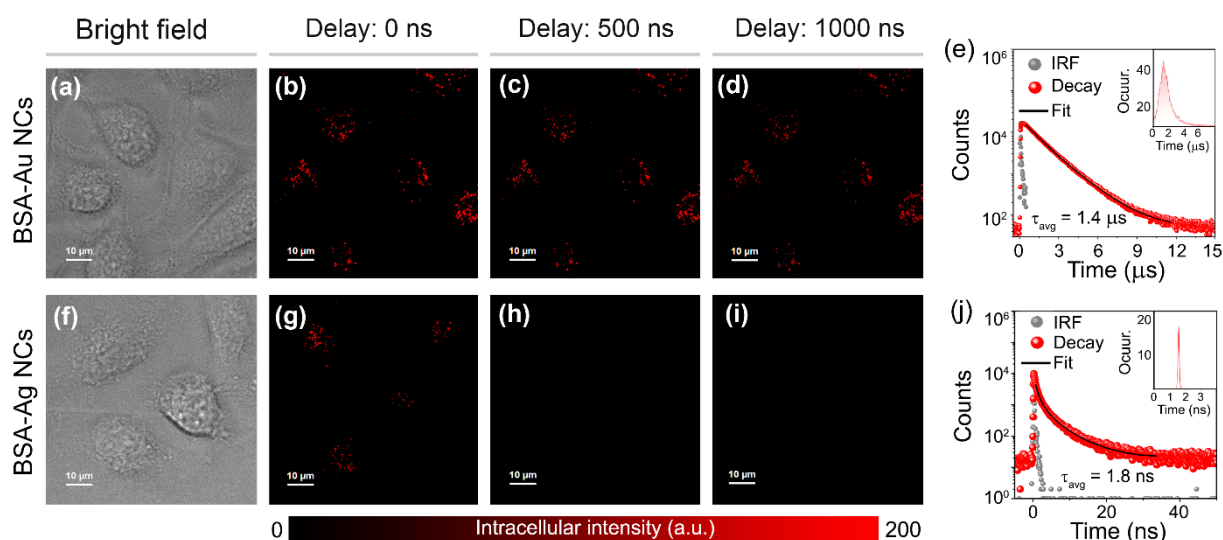


Figure 6: Time-resolved imaging of HeLa cells stained with an aqueous dispersion of (a-e) BSA-Au NCs ($\lambda_{\text{ex}} = 470$ nm, and $\lambda_{\text{em}} = 488\text{-}800$ nm) and (f-j) BSA-Ag NCs ($\lambda_{\text{ex}} = 470$ nm, and $\lambda_{\text{em}} = 488\text{-}800$ nm): (a, f) bright field images, (b, g) confocal laser scanning microscopy (CLSM) images with no time delay, time-gated images after a time delay of (c, h) 500 ns and (d, i) 1000 ns; scale = 10 μm . A common luminescence intensity scale is also indicated. Intracellular decay profile of (e) BSA-Au NCs and (j) BSA-Ag NCs incubated in HeLa cells ($\lambda_{\text{ex}} = 470$ nm, and $\lambda_{\text{em}} = 488\text{-}800$ nm, repetition rate = 50 kHz); inset: intracellular lifetime histograms are depicted.

243 The long-lived emission in BSA-MNCs prompts us to investigate the applicability of such
 244 probes in time-resolved luminescence imaging (TRLI).^{46,47} The confocal laser scanning
 245 microscopy (CLSM) images of BSA-Au NCs (1 mg mL⁻¹) stained HeLa cells depicted bright
 246 red fluorescence signals from the cytoplasmic region of the cells (**Figure S9a, 6b**). Previous
 247 studies have shown that BSA-conjugated NCs are selectively transported to lysosomes.^{38,48,49}
 248 Colocalization studies using LysoTracker™ Green (LTG) confirmed that BSA-Au NCs
 249 specifically localize into the lysosomes (**Figure S9b**). Next, we acquired TRLI images of BSA-
 250 Au NCs (1 mg mL⁻¹) stained HeLa cells by applying different delay times (**Figure 6a - 6e**).
 251 The corresponding time-gated images obtained from cells demonstrated noticeable
 252 luminescence even with a time delay of 1 μs (**Figure 6d, S10**). In contrast, upon applying a
 253 time delay of 1 μs , any noticeable luminescence was not observed from HeLa cells stained with
 254 BSA-Ag NCs (1 mg mL⁻¹), again suggesting its weak triplet harvesting nature under aqueous
 255 conditions (**Figure 6f - 6j, S10**).

256

257 In summary, we have shown the distinct excited state dynamics of BSA-Au NCs and BSA-Ag
258 NCs. It is found that the deexcitation pathways were significantly influenced by charge transfer
259 states formed via M-S bonds, the stability, and the rigidity of the protein coated nanoclusters.
260 From TRES and TRANES analysis, we concluded that the emission in BSA-Ag NCs comes
261 from the core states only, while BSA-Au NCs exhibits a dual emission consisting of short- and
262 long-lived states, originating due to core and surface state interactions. Quenching studies with
263 methyl viologen confirmed the presence of core and surface state interactions via LMCT states
264 in BSA-Au NCs, leading to facile triplet harvesting at room temperature. In contrast, the BSA-
265 Ag NCs exhibit fluorescence as the deexcitation mechanism at room temperature, transitioning
266 to phosphorescence with sub-millisecond lifetimes under cryogenic conditions. Conversely,
267 LMCT states in BSA-Au NCs facilitate triplet-state harvesting, resulting in RTP as the
268 dominant mechanism with a microsecond lifetime. The time-resolved luminescence imaging
269 of both MNCs in HeLa cells ascertained the long-lived emission characteristics in BSA-Au
270 NCs but not in BSA-Ag NCs.

271 **Author Contributions**

272 S.S. conceptualized and designed all the experiments. S.S. and S.D. conducted various steady
273 state and time-resolved luminescence measurements and analyzed the data with the help of
274 C.K.N. and A.P. S.D. also performed time-resolved luminescence imaging for cell-related
275 study. K.K. wrote the MATLAB code to automate the analysis of TRANES. C.K.N. and A.P.
276 guided the complete project thoroughly and wrote the manuscript with the help of S.S. and S.D.

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286

287 **Supporting Information**

288 **Sections I, II and III** providing the description of the materials and the synthesis procedures;
289 **Instrumentation section (IV. a-j)** including brief explanation of the characterization methods,
290 sample preparation, equations, and calculation parameters. **Supplementary section (V)** and
291 **(VI)** containing figures and tables of material characterization, photophysical and intracellular
292 studies.

293

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Graphical Abstract-Table of Content

Revisiting Protein Protected Gold and Silver Nanoclusters: Excited State Dynamics and Long-Lived Emission

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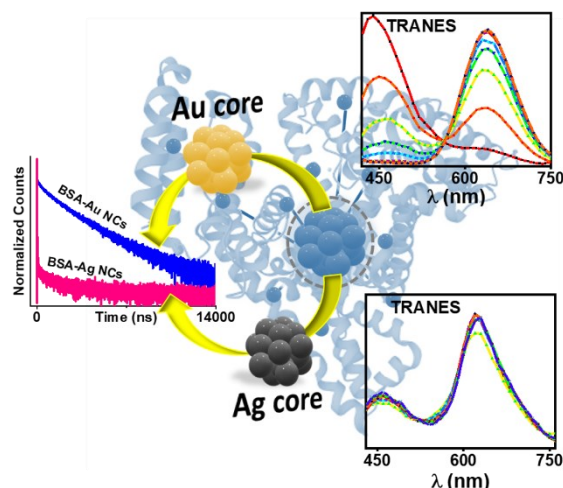
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Time-Resolved Luminescence Insights into the Distinct Excited State Dynamics of Protein-Conjugated Gold and Silver Nanoclusters.