Amino-acid conjugated protein–Au nanoclusters with tuneable fluorescence properties

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Abstract

Au-based protein nanoclusters (PNCs) represent an emerging class of fluorescence probes that are inherently biocompatible and combine the functionality of proteins and optical properties of Au nanoclusters. Here we report on a methodology to create conjugated Au PNCs using amino acid coupling strategies from a series of common laboratory proteins. We discover that the host protein and the specific conjugation chemistry has a profound influence on the resulting fluorescence properties. Synchrotron analyses showcase local Au NC aggeration upon PNC conjugation, which causes local environment changes to invoke differences in fluorescence properties. The observed aggeration does not give rise to plasmonic properties nor significant fluorescence quenching, strongly indicating the PNCs are still in a near-native cluster state. Our methodology and findings here could open new pathways for tuning PNC fluorescence properties in a rational fashion, having a potential impact in host of biomedical and sensing applications.

1. Introduction

Metal nanoclusters (NCs) are engineered at dimensions approaching the Fermi wavelength of electrons, allowing for the formation of discrete energy states that result in interesting fluorescence properties that differ to those observed in larger metal nanoparticles with plasmonic properties [1–4]. While avoiding the toxic components of traditional fluorescent quantum dots (QDs), metal NC have demonstrated high quantum yield, tuneable photonic emission, highly efficient two-photon absorption, and tailorable hybridization pathways. For biomedical applications in particular, the tailoring of NC properties and biocompatibility through biomediated synthesis approaches has proven instrumental [5–9]. Not surprisingly, protein-stabilized nanoclusters (PNC) have emerged as versatile hybrid platforms that can leverage functional properties of both the NC and protein components [10–14].

In traditional metal NCs, ligand parameterization and corresponding electronic structure is very well established [15–17]. In PNCs, however, identification of the stabilization region and specific ligand interactions of NCs within a protein scaffold remains elusive and represents a major deficiency towards controllable PNC synthesis and optimized functionality. If cluster location and protein interaction could be better understood, one can learn how NCs are stabilized and what reaction mechanisms allow for growth of the NC to their targeted size, electromagnetic properties, and responsive functionality. With this knowledge, the rational identification and engineering of new proteins could be accomplished to grow NCs with pre-determined properties. As Au–thiol interactions available through metal-binding residues within the protein present likely target regions for NC stabilization, recent studies have targeted these through both simulation and designer protein approaches [18]. While such studies provide secondary support for likely stabilization regions, more direct characterization is required to definitively identify NC–ligand interactions in a protein host.

Direct examination of the cluster region with a protein structure requires the utilization of x-ray and crystallographic techniques. For the latter, highly crystalline materials are required which have yet to be
achieved with PNC hybrids. One avenue to create highly-ordered PNC structures is through their incorporation into metal–organic framework (MOF) scaffolds, however, solvent incompatibility has proven a challenging issue for the integration of NCs into ordered MOFs [19, 20]. One method to overcome this barrier involves the precipitation of nanoclusters with a metal ion solution, such as Zn$^{2+}$, to form aggregates that are amenable to methanol dissolution. With proper precipitation, the NCs have been demonstrated compatible for processing into zeolitic imidazole framework (ZIF-8) MOFs [20, 21]. While PNCs have, to date, not been reported in composite form with MOF structures, proteins incorporation into ZIF-8 has been shown [22]. In addition to ordering through MOFs, researchers have recently begun to examine large-pore protein crystals (LPC) as scaffolds for NC incorporation. With LPC pore diameters up to 8 nm and aqueous processing conditions, glutathione capped Au25 nanoclusters can be readily adsorbed into the patterned crystal pores [23]. The ability to utilize purely organic frameworks reduces the processing requirements in addition to providing a platform to enable precise patterning of the embedded materials within the LPC.

More recently, a new methodology to directly conjugate PNCs and form crystal-like structures has been developed [24]. By synthesizing PNCs in the presence of select amino acids (AA), protein conjugation enabled the formation of supramolecular structures. Interestingly, through simple variations in pH and AA composition, Ding et al demonstrated the ability to controllably tune NC fluorescence emission wavelength across the visible spectrum for BSA-stabilized gold nanoclusters (AuNCs) conjugates [24]. As this approach enables formation crystal-like structures composed nearly entirely of the PNC of interest with limited additional materials, subsequent analysis of the gold-ligand stoichiometry can be more readily studied without extraneous contaminating material signals. Additionally, it is hypothesized that the crystal-like structure could generate PNCs of consistent orientation in a close-packed cluster; enabling more precise x-ray analysis to resolve protein–Au NC interactions.

Motivated by the aforementioned study, we report on the conjugation of PNCs using a series of protein hosts and amino acid conjugation conditions. We found that the conjugated PNCs exhibit fluorescence characteristics that depend on the nature of conjugation chemistry and host protein using BSA, trypsin, pepsin, and lysozyme. To study the atomic-scale origins of these phenomena, we further study the conjugated PNCs using a x-ray absorption spectroscopy (XAS) and small angle x-ray scattering (SAXS). With these methods, we observe unexpected clustering effects in the conjugated PNCs that are dependent on the protein host. These findings should help guide and refine our ability to make Au PNCs with optimized properties.

2. Methods

2.1. Chemicals
All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and solutions prepared were aqueous, unless otherwise noted: gold (III) chloride hydrate (HAuCl$_4$), BSA, trypsin, lysozyme, pepsin, L-histidine, glycine, hydrochloric acid (HCl), and sodium hydroxide (NaOH).

2.2. Synthesis of PNC’s and AA-conjugated PNCs
The synthesis of AA-conjugated PNC (AA-PNC) was performed with slight modifications of previously reported methods [24]. In brief, hybrids were prepared with varied composition of AAs, including L-histidine (L-His) and glycine (Gly), and protein hosts to include BSA, trypsin, lysozyme, and pepsin. Stock solutions were prepared of the selected proteins (50 mg ml$^{-1}$), L-His (31 mg ml$^{-1}$), Gly (15 mg ml$^{-1}$), and HAuCl$_4$ (16 mg ml$^{-1}$). Samples were prepared according to table 1 by sequentially adding the protein stock (1 ml), HAuCl$_4$ (1 ml), AA (5 ml), and DI water (3 ml) to a glass vial. The pH was adjusted as outlined in table 1 with 1 M solutions of NaOH or HCl. This procedure results in a final reaction solution of 5 mg ml$^{-1}$ protein, 1.6 mg ml$^{-1}$ HAuCl$_4$, and 7.5 mg ml$^{-1}$ Gly (or 15.5 mg ml$^{-1}$ L-His depending on amino acid selection). Samples were stirred at 37$^\circ$C for 12 h. The final solution is purified through centrifugation at 10 000 g for 30 min, with collection of the pellet to target formed conjugates. The pellet was resuspended in DI water and centrifugal purification repeated three times. The final pellet resuspended in 5 ml of DI water. Control protein nanoclusters were made for each protein host utilizing the same procedure outlined above without the addition of amino acids. Controls were synthesized at pH 12 and stirred for 12 h at 37$^\circ$C. Samples were purified through a 10 kDa molecular weight cut off (MWCO) centrifugal filter to remove unreacted metal ions.

2.3. Characterization
Fluorescent emission of the protein-nanocluster hybrids was measured on a Horiba Fluorolog-3 with excitation at 350 nm and emission capture range of 400–700 nm. All measurements were made in aqueous solutions in a quartz cuvette with a path length of 1 cm. XAS measurements were performed at the 12-BM beamline of the Advanced Photon Source (APS), Argonne National Laboratory (ANL). A sample from each
Table 1. Synthesis parameters for each sample.

| Protein       | BSA  | Trypsin | Lysozyme | Pepsin |
|---------------|------|---------|----------|--------|
| Sample        | B1   | B2      | B3       | B4     |
| pH            | 5.5  | 1.5     | 3.5      | 12.5   |
| AA            | L-His Gly Gly Gly | L-His Gly Gly Gly | L-His Gly Gly Gly | L-His Gly Gly Gly |

3. Results and discussion

Motivated by the ability to better understand NC location within a protein host, AA conjugated PNCs were synthesized using a series of proteins. While we were unable to get quality materials for single-crystal XRD, we did observe interesting synthesis-dependent properties that warranted further investigation. Utilizing the methodology reported by Ding et al [24], AA conjugated PNC growth was successfully performed using four different protein models: BSA, trypsin, lysozyme, and pepsin. To the authors’ knowledge, this is the first reported AA-conjugated PNCs created with trypsin, lysozyme, and pepsin protein hosts. As shown in figure 1, this approach provides tunable fluorescent emission wavelengths that can be obtained across the visible spectrum. The control PNCs formed for each protein host yielded red emissions, which corresponds to previous studies of pH 12 synthesis of BSA [5, 28], trypsin [29–31], pepsin [32], and lysozyme [33, 34].

While pepsin has been shown to readily create smaller clusters (i.e. blue-shifted emission) through modulation of reaction pH [32], this tunability of BSA, lysozyme, and trypsin to the blue spectrum has been limited. As can be seen in figure 2, the ability to tune each protein model to the blue spectrum has been achieved through select AA integration into the synthesis process.

Although AA conjugated PNCs were successfully formed with each protein model, fluorescent PNCs were not formed with every sample composition outlined in table 1. For non-fluorescent AA-conjugated PNCs, plasmonic responses were observed, strongly suggesting the possibility of Au NC destabilization under certain conjugation conditions. For the remaining PNCs, the fluorescent emission spectrum are shown in figure 2. In all cases, the non-conjugated NCs exhibit fluorescence at lower energy (i.e. red-shifted) wavelengths than the AA-conjugated PNCs. This suggests that the process promotes the formation of smaller cluster sizes, as Au NC emission wavelengths directly correlate to the number of Au atoms in the cluster [32].

Previous studies with BSA have demonstrated the ability for Gly and L-His to migrate into the protein during the unfolding/refolding process [24]. As the protein contains the residues required to reduce the metal salts, the additional Gly or L-His can assist in the stabilization and capping of the cluster during formation. Both amino acids, Gly and L-His, have previously shown the preferential capping of smaller metal cluster structures, shifting the emission towards the blue spectrum [35, 36]. With respect to the formation of conjugated PNC structures, the presence of additional AA capping residues within the protein structure could inhibit proper protein refolding and induce aggregation. Although certain amino acids have shown the ability to inhibit protein aggregation during the unfolding/folding process, this is not the case for glycine and L-histidine [37].

In the case of BSA, lysozyme, and pepsin; conjugated PNCs were formed with both L-His and Gly approaches. Interestingly, the L-His conjugation of BSA stabilized PNCs yielded a spectral blue-shift in the cluster emission, which is supported by reported literature (figure 2(A)); however yield a green cluster emission in the lysozyme and pepsin models along with a blue emission of the trypsin AA-PNCs (figures 2(B)–(D)). As the incorporation of L-His into the synthesis mechanism shifts the traditional spectral response of each protein model, it is likely that L-His plays a direct role in NC formation and stabilization.
beyond simple protein conjugation and supports direct capping/stabilization of the cluster as it is reduced/stabilized within the protein scaffold.

To better understand the effect of host protein and conjugation chemistry on the local structure of the Au clusters, XAS was used to as an element specific probe. The x-ray absorption near edge spectra (XANES) for both as-synthesized Au PNC and the AA-conjugated PNCs are shown in figure 3(A). Overall, all probed PNCs exhibit a ~+1–2 eV shift in $E_0$ from the Au reference, indicating a partial increase in oxidation state. The biggest shift in $E_0$ is observed for PNCs stabilized within BSA, followed by trypsin, lysozyme, and pepsin stabilized PNCs. This shift in $E_0$ is expected given the small cluster size and corresponding surface atoms interactions with host protein moieties. Moreover, the XANES features observed in the as-synthesized PNCs are significantly broad in comparison to the bulk Au reference as expected [38, 39], given the small cluster size not replicating the bulk fcc lattice of Au. Upon conjugation, all PNCs exhibit a reduced $E_0$ compared to their as-synthesized counterparts that is more akin to the Au reference. Additionally, XANES features better resemble that of the Au reference, albeit with an increase in white line intensity. These findings were unexpected and indicate that the protein conjugation must force the Au atoms more into a larger aggregated state, yielding XANES features more resemble of fcc Au. The increase in white line intensity as compared to the reference Au additionally suggest sufficient Au–ligand interactions, indicating electron transfer from the Au to the protein is still present.

After edge-step normalization, $k^2$-weighting, and Fourier transform, the extended x-ray absorption fine structure spectra (EXAFS) reveal interesting local structural changes in the Au NCs (figure 3(B)). Note the EXAFS is not corrected for phase shift, wherein peaks in the EXAFS are shifts ~0.3–0.4 Å lower from actual distances. The as-synthesized PNCs all exhibit a prominent feature centered below 2.0 Å and smaller features that somewhat align with those observed in from the bulk Au. This first distance is likely Au–thiol bonding through interactions with the protein that are commonly observed in thiolated AuNC complexes with organic ligands [38]. It is important to note that the pepsin PNC has a significantly shifted distance for the Au–biotic interaction, perhaps indicating that different cluster binding motif may be present.

Upon conjugate formation, notable structural differences can be observed between the AA-conjugated PNCs vs their non-conjugated counterparts. Features more resemblance of fcc Au become more evident, albeit at slightly shifted distances. Qualitatively, this indicates that protein conjugation condenses the clusters
together in an aggregated cluster arrangement that is likely perturbed significantly from the bulk lattice due to the likely imperfect nature of cluster assembly during protein conjugation. The majority of the PNCs also exhibit a feature <~1.5 Å. This feature is shorter than the ligand–Au distances observed for the as-synthesized Au NCs, strongly indicating that protein–Au interactions are changing during AA conjugation.

The EXAFS data was then modelled as described in the experimental section with results presented in table 2. The modeled data produces nearest neighbor distances (NNDs) and coordination numbers (CNs) of the specified scattering pairs. For the as-synthesized Au NCs, small Au–Au CNs were achieved and are reflective of the small size of the clusters with high surface area. Au–Au NNDs are consistent with those observed in previous clusters [38, 40], with a slightly longer distance of 2.94 ± 0.02 Å for NCs grown within BSA. This suggests that the structural influence of the protein may be used to alter the NC’s properties using BSA. For Au–S contributions to the EXAFS, the Au–S CNs for all the as-synthesized NCs are ~2.0 (within fitting error), suggesting strong surface coordination within the cluster and the protein. NNDs for Au–S reflect those qualitatively observed in the EXAFS, with the Au-NC synthesized with pepsin exhibiting a slightly shorter feature. Upon conjugation, all Au–Au CNs increase to 3.45 ± 0.61, 5.00 ± 0.51, 5.18 ± 0.13, and 8.84 ± 1.02 for PNCs implementing trypsin, pepsin, BSA, and lysozyme respectively. Using idealized nanocrystal models, which are a rough estimation given the observed nature of cluster aggregation, these CNs correspond to those with sizes of ~0.75 nm, ~1.0 nm, and ~1.5 nm respectively [41]. NNDs for Au–Au are all similar to those found in nanoscale Au as well. The shorter-scale features from Au–ligand interactions exhibit sufficient changes upon conjugation to PNCs as well [41]. Au–S CNs are reduced by over 50% for all samples, while shorter low-Z contributions become more evident in the remaining samples. It is important to emphasized that Au nanoparticle formation is likely not occurring at an appreciable amount given that visible fluorescence is reminescence of Au NCs and not plasmonic Au nanoparticles.

Morphological differences between as-synthesized and conjugated PNCs were probed using SAXS as shown in figure 3(C). PNCs synthesized with BSA and trypsin are shown in the black and green dotted scattering patterns respectively and showcase typical form factor scattering for metallic NCs in the immediate q-range along with some clear aggregation at lower q-range. Conversely, the AA-conjugated PNCs

![Figure 2. Emission spectra of methodologies that yield nanocluster fluorescence for (A) BSA, (B) lysozyme, (C) pepsin, and (D) trypsin protein hosts. Non conjugated Au PNCs are provide for reference.](image-url)
| Sample      | Au-S CN | Au-S NND (Å) | Au-S $\sigma^2$ | Au-Au (cluster) CN | Au-Au (cluster) NND (Å) | Au-Au $\sigma^2$ | Au-Z CN | Au-Z NND (Å) | Au-Z $\sigma^2$ |
|-------------|---------|---------------|-----------------|-------------------|------------------------|-----------------|---------|---------------|-----------------|
| BSA         | 2.00 ± 0.10 | 2.324 ± 0.002 Å | 0.002 ± 0.001 | 0.68 ± 0.24 | 2.69 ± 0.02 Å | 0.002 ± 0.003 | –       | –             | –               |
| Pepsin      | 2.47 ± 0.21 | 2.25 ± 0.02 Å   | 0.007 ± 0.003  | 1.57 ± 0.62 | 2.88 ± 0.04 Å | 0.010 ± 0.001 | –       | –             | –               |
| Trypsin     | 1.90 ± 0.11 | 2.30 ± 0.01 Å   | 0.002 ± 0.002  | 0.68 ± 0.27 | 2.84 ± 0.03 Å | 0.002 ± 0.001 | –       | –             | –               |
| Lyso        | 1.91 ± 0.08 | 2.30 ± 0.01 Å   | 0.002 ± 0.001  | 0.46 ± 0.11 | 2.84 ± 0.03 Å | 0.002 ± 0.002 | –       | –             | –               |
| BSA AA-PNC  | 0.75 ± 0.04 | 2.288 ± 0.004 Å | 0.004 ± 0.002 | –       | –             | –               | –       | –             | –               |
| Pepsin      | 0.15 ± 0.23 | 2.36 ± 0.03 Å   | 0.002 ± 0.001  | –       | –             | –               | –       | –             | –               |
| Trypsin-PPC | 0.76 ± 0.21 | 2.31 ± 0.03 Å   | 0.002 ± 0.001  | –       | –             | –               | –       | –             | –               |
| Lyso-PPC    | 0.81 ± 0.16 | 2.18 ± 0.02 Å   | 0.002 ± 0.001  | –       | –             | –               | –       | –             | –               |
Figure 3. Synchrotron characterization for selected PNCs; Au L$_3$-edge (A) XANES and (B) EXAFS along with (C) SAXS and (D) corresponding P(r) fitting.

exhibit markedly different SAXS profiles. The form factor scattering is largely absent, which is coupled to a highly linear region at lower q-range. This strongly suggests that protein-protein aggregation within the sample is not prevalent and that PNCs are beginning to order in solution through the conjugation process. Note that the ordering is not in a highly ordered periodic fashion to result in Bragg features, such as those observed in DNA-programmed superlattices of Au nanoparticles [42, 43]. To further probe the effect of conjugation on morphology, SAXS profiles were fitted to pair distance distribution functions, P(r) (red lines, figure 3(C)), using known methods. The resulting P(r) functions are shown in figure 3(D) and showcase a large difference in size and morphology for between the as-synthesized and crystallized materials. PNCs synthesized with BSA and trypsin exhibit a main feature ~20 Å, and $d_{\text{max}}$ values for 84 Å and 108 Å respectively. The AA-conjugated PNCs, on the other hand, yield P(r) maxima at values >60 Å for all materials and $d_{\text{max}}$ values >170 Å over similar q-range values. This strongly indicates and organization of PNCs into supramolecular structures caused by conjugation processes within the proteins. Taken together, the synchrotron characterization demonstrate that AA-conjugation creates an aggregation of Au NCs resembling some more similar to Au nanoparticle. However, the visible light fluorescence properties are strongly suggest non-plasmonic behavior. This finding illustrates that cluster aggregation rates and the appropriate protein host could be used to control fluorescence properties without inducing a plasmonic response, and may provide insights in the generation of new PNCs for various applications.

4. Conclusions

In summary, AA-conjugated PNCs were created in multiple protein models, including BSA, trypsin, pepsin, and lysozyme PNCs. Fluorescence properties were shown to be dependent on Au NC–protein interaction and the aggregation of Au NCs that occurs during conjugation as revealed using synchrotron characterization methods. Our finding suggest that choice of host protein and the degree of NC aggregation allows for the possible tunability of fluorescence properties, proving new pathways to create new NC fluorescence materials with user defined properties.
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References

[1] Zheng J, Nicovich P R and Dickson R M 2007 Highly fluorescent noble-metal quantum dots Angew. Chem. Int. Ed. 46 7908–10
[2] Lu Y and Chen W 2012 Sub-nanometre sized metal clusters: from synthetic challenges to the unique property discoveries Acc. Chem. Res. 45 439–48
[3] Zhang L and Wang E 2014 Metal nanoclusters: new fluorescent probes for sensors and bioimaging ACS Appl. Mater. Interfaces 6 21 31322–8
[4] Mathew A and Pradeep T 2014 Noble metal clusters: applications in energy, environment, and biology Prog. Part. Part. Syst. Charact. 31 1017–53
[5] Xie J, Zheng Y and Ying J Y 2009 Protein-directed synthesis of highly fluorescent gold nanoclusters J. Am. Chem. Soc. 131 888–9
[6] Zuber G, Weiss E and Chiper M 2019 Biocompatible gold nanoclusters: synthetic strategies and biomedical prospects Acc. Chem. Res. 52 3078–87
[7] Zhang Y, Zhang C, Xu G, Wang X, Liu C, Waterhouse G I N, Wang Y and Yin H 2019 Ultrasmall Au nanoclusters for biomedical and biosensing applications: a mini-review Nano Today 9 205–35
[8] Knoblauch E, Grieb M and Friedrich C 2017 Recent advances in the field of bionanotechnology: an insight into optoelectric bacteriorhodopsin, quantum dots, and noble metal nanoclusters Sensors 14 19731–66
[9] Griep M H, West A L, Sellers M S P, Karnam, M, Zhao E and Houque N 2015 Biocompatible metal nanoclusters: synthesis and theory Handbook of Nanoparticles, ed M Ailiofkhazraei (Cham: Springer International Publishing) pp 1–24
[10] West A L, Griep M H, Cole D P and Karnam S P 2014 DNase 1 retains endodeoxyribonuclease activity following gold nanocluster synthesis Anal. Chem. 86 7377–82
[11] Sahu D K, Sarkar P, Singh D and Sahu K 2017 Protein-activated formation of silver nanoclusters into blue and red-emitting nanoclusters ACS Appl. Mater. Interfaces 9 19864–72
[12] Wang B, Zhao M, Mehdi M, Wang G, Gao P and Zhang K-Q 2019 Biomolecule-assisted synthesis and functionality of metal nanoclusters for biological sensing: a review Mater. Chem. Front. 3 1722–35
[13] Meng X, Zare I, Yan X and Fan K 2020 Protein-protected metal nanoclusters: an emerging ultra-small nanomachine WIREs Nanomed. Nanobiotechnol. 12 e16057
[14] West A L, Schaeublin N M, Griep M H, Maurer-Gardner E I, Cole D P, Fakner A M, Hussain S M and Karnam S P 2016 In situ synthesis of fluorescent gold nanoclusters by nontumorigenic microglial cells ACS Appl. Mater. Interfaces 8 21221–7
[15] Jin B 2015 Atomically precise metal nanoclusters: stable sizes and optical properties Nanoscale 7 1549–65
[16] Jin B, Zeng C, Zhou M and Chen Y 2016 Atomically precise colloidal metal nanoclusters and nanoparticles: fundamentals and opportunities Chem. Rev. 116 10346–115
[17] Åkens C M 2017 Electronic and geometric structure, optical properties, and excited state behavior in atomically precise thiolate-stabilized noble metal nanoclusters Acc. Chem. Res. 51 3065–73
[18] Aires A, Llarena I, Moller M, Castro-Smirnov J, Cabanillas-Gonzalez J and Cortajarena A L 2019 A simple approach to design proteins for the sustainable synthesis of metal nanoclusters Angew. Chem. Int. Ed. 58 6214–9
[19] Gao Q, Xu S, Guo C, Chen Y and Wang L 2018 Embedding nanocluster in MOF via crystalline ion-triggered growth strategy for improved emission and selective sensing ACS Appl. Mater. Interfaces 10 16059–65
[20] Li Y, Hu X, Zhang X, Cao H and Huang Y 2018 Unconventional application of gold nanoclusters/Zn-MOF composite for fluorescence turn-on sensitive detection of zinc ion Anal. Chem. Acta 1024 145–52
[21] Fan C, Lu X, Liu F, Feng L, Liu M, Cai Y, Liu H, Wang J, Yang Y and Wang H 2018 Silver nanoclusters encapsulated into metal–organic frameworks with enhanced fluorescence and specific ion accumulation toward the microdot array-based fluorimetric analysis of copper in blood ACS Sensors 3 441–50
[22] Lyu F, Zhang Y, Zare R N, Ge J and Liu Z 2014 One-pot synthesis of protein-embedded metal–organic frameworks with enhanced biological activities Nano Lett. 14 5761–5
[23] Hartje L F, Ackerson C J and Snow C D 2017 Adsorption-coupled diffusion of gold nanoclusters within a large-pore protein crystal scaffold J. Phys. Chem. B 121 7652–9
[24] Ding H, Li H, Wang X, Zhou Y, Li Z, Hiltunen J K, Shen J and Chen Z 2017 Expanding toolbox of imageable protein-gold hybrid materials Chem. Mater. 29 8440–8
[25] Ravel B and Nevill M 2005 ATHENA, ARTEMIS, HEPHAESTUS: data analysis for x-ray absorption spectroscopy using FEFF FIT J. Synchrotron Radiat. 12 537–41
[26] Yang H, Wang Y, Edwards A J, Yan J and Zheng N 2014 High-yield synthesis and crystal structure of a green Au30 cluster co-capped by thiolate and sulfide Chem. Commun. 50 14325–7
[27] Hura G L et al 2009 Robust, high-throughput solution structural analyses by small angle x-ray scattering (SAXS) Nat. Methods 6 606
[28] Griep M H, Domarest J D, Cole D P, Henry T C and Karnam S P 2020 Protein-mediated synthesis of Au nanocluster decorated reduced graphene oxide: a multifunctional hybrid nano-bio platform Plasmonics 15 897–903
[29] Kawasaki H, Yoshimura K, Hamaguchi K and Arakawa R 2011 Trypsin-stabilized fluorescent gold nanocluster for sensitive and selective Hg2+ detection Anal. Sci. 27 591
[30] Fan J, Li R, Xu P, Di J, Tu Y and Yan J 2014 Sensitive sulfide sensor with a trypsin-stabilized gold nanocluster Anal. Sci. 30 457–62
[31] Liu J-M, Chen J-T and Yan X-P 2013 Near infrared fluorescent trypsin stabilized gold nanoclusters as surface plasmon enhanced energy transfer biosensor and in vivo cancer imaging bioprobe Anal. Chem. 85 3238–45
[32] Kawasaki H, Hamaguchi K, Osaka I and Arakawa R 2011 pH-dependent synthesis of pepsin-mediated gold nanoclusters with blue green and red fluorescent emission Adv. Funct. Mater. 21 3508–15
[33] Lin Y-H and Tseng W-L 2010 Ultrasensitive sensing of Hg²⁺ and CH₃Hg⁺ based on the fluorescence quenching of lysozyme type VI-stabilized gold nanoclusters Anal. Chem. 82 9194–200
[34] Lu D, Liu L, Li F, Shuang S, Li Y, Choi M M F and Dong C 2014 Lysozyme-stabilized gold nanoclusters as a novel fluorescence probe for cyanide recognition Spectrochim. Acta A 121 77–80
[35] Zhang X, Wu F-G, Liu P, Gu N and Chen Z 2014 Enhanced fluorescence of gold nanoclusters composed of HAuCl₄ and histidine by glutathione: glutathione detection and selective cancer cell imaging Small 10 5170–7
[36] Kravets V, Culhane K, Dmitruk I and Pinchuk A 2012 Glycine-Coated Photoluminescent Silver Nanoclusters SPIE Proceedings Vol. 8232, Colloidal Nanocrystals for Biomedical Applications VII 2012
[37] Shiraki K, Kudou M, Fujiwara S, Imanaka T and Takagi M 2002 Biophysical effect of amino acids on the prevention of protein aggregation J. Biochem. 132 591–5
[38] MacDonald M A, Zhang P, Qian H and Jin R 2010 Site-specific and size-dependent bonding of compositionally precise gold–thiolate nanoparticles from x-ray spectroscopy J. Phys. Chem. Lett. 1 1821–5
[39] Simms G A, Padmos J D and Zhang P 2009 Structural and electronic properties of protein/thiolate-protected gold nanocluster with “staple” motif: a XAS, L-DOS, and XPS study J. Chem. Phys. 131 214703
[40] MacDonald M A, Chevrier D M, Zhang P, Qian H and Jin R 2011 The structure and bonding of Au₂₅(SR)₁₈ nanoclusters from EXAFS: the interplay of metallic and molecular behavior J. Phys. Chem. C 115 15282–7
[41] Frenkel A I, Yeivick A, Cooper C and Vasic R 2011 Modeling the structure and composition of nanoparticles by extended x-ray absorption fine-structure spectroscopy Annu. Rev. Anal. Chem. 4 23–39
[42] Macfarlane R J, Lee B, Jones M R, Harris N, Schatz G C and Mirkin C A 2011 Nanoparticle superlattice engineering with DNA Science 334 204
[43] Jones M R, Macfarlane R J, Lee B, Zhang J, Young K L, Senesi A J and Mirkin C A 2010 DNA-nanoparticle superlattices formed from anisotropic building blocks Nat. Mater. 9 913–7