Surface Dynamics and Ligand-Core Interactions of Quantum Sized Photoluminescent Gold Nanoclusters

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ABSTRACT:
Quantum-sized metallic clusters protected by biological ligands represent a new class of luminescent materials; yet the understanding of structural information and photoluminescence origin of these ultra-small clusters remains a challenge. Herein we systematically study the surface ligand dynamics and and ligand-metal core interactions of peptide-protected gold nanoclusters (AuNCs) with combined experimental characterizations and theoretical molecular simulations. We propose that the emission brightness of the resultant nanoclusters is determined by the surface peptide structuring, interfacial water dynamics and ligand-Au core interaction, which can be tailored by controlling peptide acetylation, constituent amino acid electron donating/withdrawing capacity, aromaticity/hydrophobicity and by adjusting environmental pH. Specifically, emission enhancement is achieved through increasing the electron density of surface ligands in proximity to the Au core, discouraging photo-induced quenching, and by reducing the amount of surface-bound water molecules. These findings provide key design principles for maximizing the photoluminescence of metallic clusters through the exploitation of biologically relevant ligand properties.

Keywords: Gold quantum clusters, surface dynamics, ligand-metal interactions, molecular dynamics, peptide
INTRODUCTION

Ultra-small gold nanoclusters (AuNCs) in the quantum size regime have recently attracted tremendous interests due to their optical, chiral, magnetic and catalytic properties \(^1\)-\(^4\). Because their sizes are comparable to the de Broglie wavelength of electrons near the Fermi energy of metallic gold, the few-atom AuNCs experience molecule-like interactions with incident light to produce intense emission \(^5\)-\(^7\). AuNCs exhibit broad excitation ranges, size-dependent emission color, red/near-infrared (NIR) emission and less toxicity, making them ideal for cellular and subcellular imaging \(^8\)-\(^9\). Much effort has been devoted to the design of biocompatible gold clusters \(^10\). For instance, some of the first highly luminescent AuNCs to be synthesized using “green chemistry” routes involved exploiting the reduction capacity of bovine serum albumin \(^11\). Since then, a series of biomacromolecules \(^12\)-\(^15\) including oligonucleotide, peptide and protein have all been used to template AuNC nucleation, paving the way toward applications in biosensing, imaging and therapeutics. Understanding the surface behavior of biological ligands and their effects on the photoluminescence of AuNCs is therefore highly important yet remains challenging. The most stable and well characterized atomically precise gold–thiolate (SR) nanocluster is Au\(_{25}\)(SR)\(_{18}\), whose structure and optical properties have been a topic of intense research\(^16\). The Au\(_{25}\)(SR)\(_{18}\) nanocluster has a highly symmetric Au\(_{25}\)S\(_{18}\) framework that is composed of an icosahedral Au\(_{13}\) kernel/core protected by six Au\(_3\)(SR)\(_3\) complexes or “staples” \(^17\). From an electronic perspective, Au\(_{25}\)(SR)\(_{18}\) is often viewed as a “superatom”, \(^18\) with frontier orbitals almost exclusively distributed on the 13 Au atoms of the icosahedral kernel\(^19\). This core–shell geometric and electronic structuring is a feature of many AuNCs and therefore studies frequently (and conflictingly) advocate that AuNC excitation and emission originate from either the inner Au kernel or the surface/staple Au atoms that interact with the stabilizing ligands. Over the past few decades many hypotheses have emerged regarding the nature of AuNC photoluminescence \(^20\),\(^21\). For example, solid-state models suggest that the red/NIR fluorescence of Au\(_{25}\)(SG)\(_{18}\) (SG = glutathione) arises from intra-band (\(sp–sp\)) and inter-band (\(d–s\)) electronic transitions in the gold core \(^15\),\(^22\). Ligand exchange reactions of Au\(_{25}\)(SG)\(_{18}\) with functionalized-glutathione and 3-mercaptop-2-butanol indicated that “emission is an inherent property of the core, and the same electronic transitions can be accessed for a variety of ligands” \(^23\). Likewise, very recent work has highlighted that despite the fact that structural isomers of Au\(_{38}\)(SC\(_2\)H\(_4\)Ph)\(_{24}\) (Ph = phenyl) display varied absorbance spectra and electronic relaxation pathways, core-to-core transitions are still the underlying source of Au\(_{38}\)’s photoluminescence \(^24\). Contrasting theories propose that AuNC emission is independent of core-based electronic transitions and core size, but rather results from localized electronic surface states related to ligand atoms \(^25\). There have also been suggestions that AuNC fluorescence is correlated to ligand-to-metal charge transfer (LMCT) \(^26\)-\(^28\) which strongly depends on the type of ligands \(^29\), while others have found that luminescence of AuNCs originates from ligand-to-metal-metal charge transfer (LMMCT) which is associated with the presence of aurophilic (Au–Au) interactions \(^30\)-\(^32\). Additionally, the interplay between surface ligand and gold is reported to influence the emission intensity of AuNCs \(^33\)-\(^35\). The exchange of nonpolar ligands with more
polar species has been shown to increase emission intensity with a linear dependence on the number of substituted polar ligands \(^{36}\). Moreover, ligands containing electron-rich atoms (e.g., N, O) or groups (e.g., −COOH, −CONH\(_2\)) significantly enhance the photoluminescence quantum yield of \(\text{Au}_{25}(\text{SR})_{18}\) via a suggested ligand-to-gold direct donation of delocalized electron density \(^{27}\).

In complement to experimental findings, theoretical approaches are able to provide non-intuitive insight into the electronic, structural and dynamic behavior of the Au–bio interface that is not achievable through any other technique \(^{37}\). Time-dependent density functional theory (TDDFT) investigations into \([\text{Au}_{25}(\text{SR})_{18}]\) (R = H, CH\(_3\), CH\(_2\)CH\(_3\), CH\(_3\)CH\(_2\)CH\(_3\)) before and after photoexcitation reveal that all excited states arise from core-based orbitals, which indicates that ligands primarily affect photoluminescence via their interactions with the AuNC core \(^{21}\). DFT has also shown that electron-withdrawing ligands distort the \(\text{Au}_{25}\) framework, and that these geometric changes are strongly correlated to a reduction in the energy difference between the highest occupied and lowest unoccupied molecular orbitals, i.e. the HOMO–LUMO gap \(^{38}\). Others have used a quantum mechanics/molecular mechanics (QM/MM) approach to demonstrate that the HOMO–LUMO gap of \([\text{Au}_{25}(\text{SG})_{18}]\) and \([\text{Au}_{25}(\text{SCH}_3)_{18}]\) depends sensitively on both the ligands and the solvent \(^{39}\).

Despite considerable theoretical and experimental efforts, fundamental understanding into the photoluminescence origin of ligand-coated AuNCs remains incomplete. This is especially true for biological ligands such as proteins and oligopeptides due to the complex chemistries and conformational space that the biomolecules can explore, making it difficult to separate the effects of primary and secondary structure on the luminescence mechanisms of AuNCs. Here we demonstrate that the emission intensity of newly designed oligopeptide-protected AuNCs is closely related to local chemical environment as manifested via the peptide layer structuring and dynamics near the gold surface. Utilizing a combination of carefully controlled experimental parameters and atomistic simulations, factors that influence the emission of AuNCs are established through varying peptide-ligand constituent amino acid aromaticity/hydrophobicity and electron donating/drawing capacity, N-terminal acetylation, and solution pH. The outcomes of this work contribute to the design of biological ligand-capped AuNCs with modulated emission brightness for broad applications in the field of bioimaging and biosensing.

**RESULTS AND DISCUSSION**

**Sequence design for peptide-protected nanoclusters.** We synthesized a library of 23 different hexapeptides (SI Appendix, Table S1) via solid phase peptide synthesis (SPPS) and used them to produce quantum sized AuNCs. We studied the effect of peptide structure alteration (e.g., N-terminal acetylation, primary sequence and aromatic/hydrophobic character) and the solution pH on the AuNCs’ photoluminescence. Cysteine (C) residues at the N-terminus are introduced to anchor the peptides to the Au surface via covalent Au-sulfur bonding, and aspartic acids (D) are appended to the C-termini of the peptides to provide negative charges for enhanced solubility of the AuNC systems. The amino acids
between cysteine and aspartic acid are systematically varied to investigate their roles in determining the photoluminescence performance of the AuNCs.

The rationale behind the chosen sequences can be illustrated by examining the CHYGDD peptide sequence (Fig. 1a and 1b), which contains histidine (H), tyrosine (Y), and glycine (G), as an example. Since tyrosine is known to display both reducing and antioxidant capabilities, and histidine is electron-rich with a high affinity towards metallic surfaces, this sequence is expected to be a good candidate to promote in situ AuNC nucleation. Indeed, as shown in Fig. 1c, the resultant AuNC solution displays a brown appearance which signifies the formation of small metallic NCs. Furthermore, unlike the UV-Vis absorption spectra of larger Au nanoparticles which display a strong surface plasmon resonance, the as-synthesized AuNCs do not exhibit a strong absorbance peak over the range of 350–700 nm (Fig. 1c). Intense emission from the clusters is recorded to display an emission peak located at 690 nm (Fig. 1d) with the quantum yield of 2.16%, similar to the spectra of peptide-protected Au25 clusters. The measurement of fluorescence decay of AuNCs using a time-correlated single photon counting (TCSPC) technique showed a long fluorescence lifetime (~ 769 nS, Fig. 1e), which is a feature of ligand-protected AuNCs. This property makes them suitable for fluorescence lifetime imaging, a technique that can reduce the auto-fluorescence or other background signal using gated detection. X-ray photoelectron spectroscopy (XPS) confirms the binding energy (BE) of Au 4f5/2 and Au 4f7/2 at 88.1 eV and 84.2 eV, respectively (Fig. 1f). It is noted that the binding energy of Au 4f7/2 falls between the Au(0) BE (84 eV) of a metallic gold film and the Au(I) BE (86 eV) of gold thiolate, suggesting the coexistence of Au(0) and Au(I) in the clusters as had been previously reported for similar small AuNCs. Dynamic light scattering (DLS) suggests a hydrodynamic diameter (Dh) of ~3.1 nm which is slight larger than the physical size of Au core due to the surface coating of a peptide layer (Fig. S1a). Polyacrylamide gel electrophoresis (PAGE) confirms that there is only one main product (Fig. S1b). Transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS) confirmed the formation of ultrasmall AuNCs (Fig. S2 and S3). Moreover, inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis indicates that the Au/S ratio of AuNCs was close to 25:18. Following the same protocol, photoluminescence AuNCs protected with other peptide sequences were prepared in a similar method (Fig. S4).
Fig. 1. (a) Example of the peptide sequence CHYGDD used to synthesize AuNCs. (b) The corresponding Au$_{25}$(SP)$_{18}$ (P = peptide) starting structure used for MD simulations with ligands extended, amino acid side-chain atoms colored as per (a), gold atoms in orange, sulfur atoms in yellow and, the solvent hidden for clarity. (c) UV-Vis spectrum, (d) fluorescence excitation (left, $\lambda_{\text{em}} = 700$ nm) and emission (right, $\lambda_{\text{ex}} = 400$ nm) spectra, (e) fluorescence lifetime decay ($\lambda_{\text{ex}} = 404$ nm, $\lambda_{\text{em}} = 700$ nm), (f) X-ray photoelectron spectrum (XPS) of CHYGDD-protected AuNCs. A photo of AuNC solution is shown in inset of 1c.

To further characterize and explore the ligand structure, surface dynamics and interaction mechanisms of the peptide-coated AuNCs and water, we performed extensive all-atom MD simulations in explicit solvent. The ensemble of atomistic structures predicted from our MD simulations show an average radius of gyration ($R_g$) between 1.2–1.5 nm and a corresponding $D_h$ of 3.2–3.9 nm (SI Appendix, Fig. S5) in good agreement with experiment. Below we systematically explore peptide-functionalized AuNC design through experimental and modelling approaches to reveal design principles for controlling photoluminescence performance. Specifically, the role of terminal amine in proximity to the Au core, the effects of the aromatic/hydrophobic pattern, and the protonation state of the titratable groups (pH) in the peptide sequences are examined.

**Modulating photoluminescence by N-terminal acetylation.** Surprisingly, the photoluminescence intensity of AuNCs prepared from N-terminal capped peptides are higher than those of peptides without acetyl capping (Fig. 2). Emission enhancements were observed of 1.38, 3.95, 3.43 and 2.57-fold increases for CGGGDD/Ac-CGGGDD, CHGGDD/Ac-CHGGDD, CHYGDD/Ac-CHYGDD, and
CYHGDD/Ac-CYHGDD, respectively, while the emission peak wavelengths do not significantly shift (SI Appendix, Fig. S4). Since the photoluminescence properties of Au$_{25}$ are primarily dictated by ligand interactions with the AuNC core, MD is used to assess how N-terminal acetylation influences amino acid locality to the central Au$_{25}$S$_{18}$ atoms of the Au$_{25}$(SP)$_{18}$ (P = peptide) NCs. In particular, electron-rich groups such as phenol (tyrosine), imidazole (histidine), and carboxyl (aspartate) are expected to influence PL intensity when in close ($r < 0.5$ nm) proximity to Au$_{25}$S$_{18}$.

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For peptides containing tyrosine and histidine, our simulations reveal that the phenol and imidazole groups are predominantly located further than 0.5 nm from the nearest gold/sulfur atom (AuS$_{nearest}$) but acetylation subtly encourages their proximity to Au$_{25}$S$_{18}$ (SI Appendix, Fig. S6 and Table S2). In contrast, the core co-locality of C-terminal aspartate carboxyl groups is strongly discouraged by N-terminal acetyl capping (SI Appendix, Fig. S6). For example, AuNCs coated with peptide sequences CGGGDD, CHGGDD, CHYGDD and CYHGDD feature an average of 6–7 negatively charged ASP residues within 0.5 nm of Au$_{25}$S$_{18}$, whereas in counterpart acetyl-capped systems the closest ASP residue is an average of 0.7–1.0 nm away from Au$_{25}$S$_{18}$ (SI Appendix, Table S2). N-terminal acetylation considerably increases the number of electron-rich atoms near Au$_{25}$S$_{18}$ (SI Appendix, Fig. S7) and also leads to water exclusion from the peptide layers (discussed further below).

![Fig. 2. Effect of N-terminal acetyl capping on the photoluminescence of AuNCs. (a) Structures of CHYGDD and Ac-CHYGDD to show the subtle difference of peptide structure at the N-terminus. (b) Maximum emission of AuNCs prepared from capped and uncapped peptides ($\lambda_{ex}= 400$ nm, $\lambda_{em}= 700$ nm, n = 3).](image)

The proximity of ASP residues to Au$_{25}$S$_{18}$ for non-acetyl capped peptide sequences results from the electrostatic interaction between positively charged NH$_3^+$ groups on N-terminal CYS residues and negatively charged COO$^-$ groups on C-terminal ASP residues. The strong interactions between these groups drive the C-terminal region of the peptide chains to embed close to the Au$_{25}$S$_{18}$ core (SI Appendix, Fig. S8) and leads to more compressed ligand layers and a decrease in peptide configurational entropy (Fig. 3a, 3b and SI Appendix, Fig. S9). The replacement of N-terminal amine (NH$_3^+$) with acetyl (COCH$_3$) increases the configurational entropy of the ligands as well as the overall
hydrodynamic volume of the NCs (Fig. 3). Two-dimensional density plots of peptide C-terminal to AuSt nearest distances (x) as a function of peptide turn-like character, which is measured as a peptide backbone angle (θ), are used to quantify the distribution of peptide conformations exhibited on the various AuNCs (Fig. 3a, 3b and SI Appendix, Fig. S9). For AuNCs with amine terminated ligands, e.g., CGGGDD, CHGGDD, CHYGDD and CYHGDD, most peptides have small C-terminal distances to gold or sulfur (x = 0.5–1.0 nm) and rigid turn-like conformations (θ = 40–60°). The acetylated ligands such as Ac-CGGGDD, Ac-CHGGDD, Ac-CHYGDD and Ac-CYHGDD, are more conformationally flexible (fewer “hot spots” at low backbone angle and short C-terminal to Au or S distance) and display elongated structures (x = 1.0–1.8 nm and θ = 100–160°). These different peptide behaviors are reflected in an overall volume increase of 24–42% for the Au25(SP)18 nanoclusters when peptides are N-terminal acetyl capped (Fig. 3c) and demonstrate a correlation between cluster volume and emission intensity.

These size differences have been explored by small angle X-ray scattering (SAXS), where Kratky plots (SI Appendix, Fig. S10i) generated for the SAXS profiles of nanocluster structures suggest the peptide corona adopts an extended configuration. In such a case, parallels can be drawn between the clusters and flexible polymers, arranged around a solid core. The scattering functions of the clusters can be approximated using the unified model, a generalized model that can describe systems with multiple-levels of structurally related features such as polymers that present both a scattering Rg and a polymer persistence length. The flexibility in conformations of the peptide around the gold core will act to smear out the features of the scattering function expected from a monodispersed AuNC core, as will any variation in scattering length density of the peptide shell which would result from differing degrees of peptide solvation and packing. SI Appendix, Fig. S10 (and associated discussion) shows plots of the unified power law function fitted to the scattered intensity data. We observe that the AuNC Rg follows a comparable trend with peptide sequence as observed by MD (SI Appendix, Fig. S710), indeed we observe a 16% larger scattering Rg for Ac-CHYGDD (~1.04 nm) versus the uncapped CGGGDD (~0.90 nm), where a more extended configuration of the peptide appears to be present.
**Fig. 3.** Effect of N-terminal acetylation on peptide conformation and hydrodynamic nanocluster volume. (a, b) Density maps of peptide backbone angle as a function of the distance between the peptide C-terminus and the nearest Au or S atom. The lower left and upper right corners represent the most folded and extended peptide conformations, respectively. Peptide conformations that are frequently and rarely visited are colored red/orange and white/blue, respectively. The inset figures illustrate highly populated ligand conformations where only the central nanocluster framework ($\text{Au}_{25}\text{S}_{18}$) and peptide backbone of interest are shown for clarity. Inset structures are colored as follows: orange = Au, yellow = S, green = Cα, black = backbone, pink = acetyl, blue-dotted lines = C-terminal to Au$_{\text{Snearest}}$ distance. (c) Average $\text{Au}_{25}(\text{SP})_{18}$ (P = peptide) volumes with error bars representing standard deviation.

Despite a smaller overall volume, water has a greater propensity to penetrate into the AuNCs’ peptide layers in the absence of acetyl termination. To further explore solvent structuring in the MD simulations, water molecules are categorized into 4 mutually exclusive groups: bulk water (outside of the 1st hydration layer), interfacial water (present at the peptide hydration layer interface), embedded water (contained inside the peptide layer) and surface-bound water (within 0.4 nm of any $\text{Au}_{25}\text{S}_{18}$ atom). **Fig. 4a, b** (and SI Appendix, Fig. S11) present the time-averaged distribution of the water selections relative to the central gold atom (Au$_{\text{center}}$) of the nanoclusters’ Au$_{13}$ kernel. These pair correlation
functions reveal that acetyl capping significantly diminishes the amount of water (especially surface-bound water) internalized within the peptide layers (SI Appendix, Fig. S12).

The decrease in water uptake (and decline in surface-bound water retention, see SI Appendix, Fig. S13) in the capped systems also leads to a reduction in hydrogen bond (H-bond) formation. Surface-bound water molecules are involved in approximately two-times more H-bonds in uncapped (34.4 ± 18.0) versus acetyl-capped (17.1 ± 8.7) systems, where the weighting is such that H-bonds are multiplied by their occupancy (e.g., 2 × 20% H-bonds and 1 × 40% H-bond both contribute 0.4 to the weighted H-bond count) and all H-bonds with an occupancy less than 20% are rejected from the count. Through this type of H-bond weighting, populations shown in Fig. 4c reflect both the number and occupancy of significant bonds between donor and acceptor groups. In uncapped systems, ~40% ± 8% of all surface-bound H$_2$O H-bonds are between water (donor) and aspartate residues’ carboxylate oxygen atoms (acceptor), ~20% ± 2% have water acting as an acceptor from NH$_3^+$ groups on cysteine residues, ~12% ± 2% have water donating bonds to a backbone cysteine oxygen atom, and ~13% ± 8% are from water molecules establishing a solvent–solvent network near the gold core. In contrast, for Au$_{25}$ (SP)$_{18}$ where P = Ac-CGGGD, Ac-CHGGD, Ac-CHYGD and Ac-CYHGDD, H-bonds between solvent and cysteine make up ~45% ± 4% with the majority of these having H$_2$O as a donor (38% ± 2%), another ~34% ± 4% are from interactions with carbonyl oxygen atoms on acetyl, and both solvent–aspartate and solvent–solvent H-bonds are negligible.

While it should be noted that both amine and acetyl groups on N-terminal cysteine residues largely obstruct water molecules from coming in very close proximity to the gold core (SI Appendix, Fig. S7), surface-bound and embedded water molecules are still expected to influence the electron density distribution around the gold core and it is therefore suggested that a lower presence of internalized water in acetyl systems may be directly related to these systems’ enhanced photoluminescence. For example, peptide-coated AuNCs that are solvent exposed are more sensitive to O$_2$-mediated quenching, whereas AuNCs with dense, hydrophobic ligand shells are more efficient at minimizing the number of internal non-radiative relaxation pathways and collisional quenching from solvent. Other studies have also shown that the electronic (and optical) properties of quantum dot fluorophores are strongly influenced by the circumjacent surface-bound molecules. More detailed electronic structure investigations are required to fully understand the effects of solvent in proximity to Au$_{25}$S$_{18}$. 
Fig. 4. MD obtained water structuring around Au\textsubscript{25}(SP)\textsubscript{18}. (a) and (b) Average radial distributions of selected atomic components relative to the central Au\textsubscript{25}(SP)\textsubscript{18} gold atom (Au\textsubscript{centre}). (c) Average number of H-bonds (weighted by occupancy) formed between surface-bound water molecules (SOL\textsubscript{x}) and other solvent, residue (RES\textsubscript{1,6}), or N-terminal acetyl (ACE\textsubscript{0}) atoms. (d) and (e) Representative images of exemplar Au\textsubscript{25}(SP)\textsubscript{18} systems where P = CYHGDD and P = Ac-CYHGDD, respectively. H\textsubscript{2}O molecules are colored as per the legends of (a) and (b), the peptide van der Waals surface and individual chains are drawn transparently with backbones in black. Bulk water molecules and counter ions are not shown for clarity. Shaded regions and error bars in the plots represent standard deviation.

An additional effect of acetyl capping is that of providing electron-rich groups that can donate delocalized electron density to the gold core. Quantum mechanics (QM) calculations of cysteine functionalized Au\textsubscript{25}, i.e. Au\textsubscript{25}Cys\textsubscript{18}, reveal that there is an increase in electron density on the Au\textsubscript{13} kernel when the N-terminal group is changed from deprotonated amine (\textendash NH\textsubscript{2}) to protonated amine (\textendash NH\textsubscript{3}\textsuperscript{+}) to acetyl (\textendash COCH\textsubscript{3}, Table 1). This is in line with the experimental findings of Wu et al. that suggest the donation of delocalized electron density from the ligands to the gold should increase fluorescence\textsuperscript{27,35}. An analysis of atomic orbital contributions to molecular orbitals shows that the highest occupied molecular orbital (HOMO) is mostly on the central Au atom, while the lowest unoccupied molecular orbital (LUMO) is primarily distributed across the Au\textsubscript{13} kernel. Similarly, TDDFT has previously shown that photoexcited states of Au\textsubscript{25} arise from Au\textsubscript{13} kernel-based orbitals\textsuperscript{21}. QM calculations have also shown that asymmetry in ligand orientation can give rise to a net dipole moment on Au\textsubscript{13}.\textsuperscript{52} Here, we show that ligands with the same orientation but different N-terminal capping can induce considerably different dipole moments on the Au\textsubscript{13} kernel (Table 1, SI Appendix Fig. S14, Table S3). While the
The interplay between atomic coordinates, electron distribution and luminescence intensity is complicated and difficult to unravel, these results suggest that the majority of the increase in electron density on the Au_{25}S_{18} atoms in the acetyl-capped systems relative to the primary amine systems is on the Au_{13} kernel, and there is a corresponding reduction in the kernel dipole moment, which is correlated to an increase in PL intensity.

Table 1. Net partial atomic charges (in units of |e|) for different subsections of Au_{25}Cys_{18} with varying cysteine N-termination.

<table>
<thead>
<tr>
<th>N-terminus</th>
<th>Au_{13} kernel*</th>
<th>S_{12} core</th>
<th>Au_{12} staple</th>
<th>S_{6} staple</th>
<th>Ligands</th>
<th>Net</th>
</tr>
</thead>
<tbody>
<tr>
<td>−NH_{2}</td>
<td>+3.41 (19.38 D)</td>
<td>-4.91</td>
<td>-0.32</td>
<td>-0.86</td>
<td>+2.67</td>
<td>0.00</td>
</tr>
<tr>
<td>−NH_{3}+</td>
<td>+2.45 (6.05 D)</td>
<td>-3.66</td>
<td>+0.30</td>
<td>-1.06</td>
<td>+19.97</td>
<td>+18.00</td>
</tr>
<tr>
<td>−COCH_{3}</td>
<td>+1.37 (0.65 D)</td>
<td>-3.30</td>
<td>+0.26</td>
<td>-0.87</td>
<td>+2.54</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Magnitude of Au_{13} dipole moment in Debye given in parentheses.

Enhancing photoluminescence by increasing ligand hydrophobicity/aromaticity in proximity to Au core. We further studied the effects of residue hydrophobicity on the photoluminescence of AuNCs prepared from 15 peptide sequences (Fig. 5a). The peptide hydrophobicity is determined by summing the hydrophobicity indices of amino acid residues 2, 3 and 4. It is noted that the overall hydrophobicity of the middle amino acids predominantly influences the emission intensity of the AuNCs, with a higher hydrophobicity score correlating to stronger emission (Fig. 5b). This can be explained by the fact that hydrophobic amino acids, or their side groups, have a higher capability to push electron density to the Au core via the S–Au bond. According to the work from Jin and co-workers, surface ligands can influence AuNC photoluminescence through donation of delocalized electron density of electron-rich atoms or groups to the metal core. In the present work, electron-donating capability is adjusted by engineering peptide sequences to incorporate different hydrophobic side groups. In particular, tyrosine and phenylalanine that have even stronger electron-rich and donating groups (i.e., phenyl and phenol) are shown to enhance the photoluminescence more efficiently than alkyl groups. Furthermore, the distance between electron-donating groups and the Au surface affects the emission intensity of the AuNCs, with the close proximity of tyrosine to cysteine promoting a stronger photoluminescence (CYGGDD > CGYGDD, Fig. 5c). This is reasonable since the donation of delocalized electrons to the Au core is more likely for a shorter distance. Lastly, increasing the number of aromatic groups will promote the photoluminescence since it will provide a higher density of delocalized electrons, e.g., CYYGDD > CYGGDD (Fig. 5c).
Correlation of the hydrophobicity/aromaticity of constituent amino acids to the photoluminescence intensity of peptide-protected AuNCs.

(a) Hydrophobicity index of peptide sequences. (b) Correlation of peptides hydrophobicity with the emission intensity of AuNCs. The hydrophobicity index was calculated as the sum of the individual hydrophobicity indices of the three amino acids close to cysteine. (c) Effect of aromatic residues (i.e., tyrosine) on photoluminescence.

Our MD investigations show that as hydrophobicity/aromaticity of the peptide sequences increases, so too does the AuNC volume and $R_g$ (SI Appendix, Fig. S15) due to the larger number of bulky groups close to the gold core. For example, the AuNC $D_h$ is calculated to be 3.21 nm, 3.48 nm, 3.60 nm, and 3.68 nm for CGGGDD, CVVGDD, CHYGDD and CYYGDD, showing that $D_h$ increases as amino acid side groups become more hydrophobic and aromatic. This effect is further confirmed by SAXS. While the effect of Au-core polydispersity and peptide-shell valency will likely influence the apparent configurations of the peptide shell in the experimental systems, the $R_g$ trends support the conclusions drawn from the simulation results indicating a more expanded nature for hydrophobic/aromatic sequences of CVVGDD (0.97 nm), CYHGDD (1.01 nm) and CYYGDD (0.99 nm) compared to that of CGGGDD (0.90 nm). The expanded peptide structures on the Au surface indicates the longer distance from the C-terminal region of the peptide chains to the $Au_{25}S_{18}$ core. However, it is still unclear as to what roles the cluster sizes play in determining their emission brightness.

**Photoluminescence enhancement at lower pH.** Furthermore, we found that the solution pH plays an important role in the emission intensity of AuNCs prepared from CGGGDD, CVGGDD, CVVGDD, CHGGDD, CYGGDD and CHYGDD. As seen from Fig. 6, the photoluminescence significantly increased when the solution pH decreased from 7.5 to 5.5. Since histidine replacement does not affect
the pH sensitivity, this suggests that the observed effect is due to a change in the protonation state of the N-terminal amine groups (which have pKₐ values that range as high as 9.1 and as low as 6.8, with an average value of $\sim 7.7 \pm 0.5$) and in turn this modulates the electrostatic potential around the AuNC core (Table 1). To clarify the origin of the pH sensitivity, we studied the fluorescence of Au clusters prepared from Ac-CGGGDD, Ac-CHGGDD and Ac-CHYGDD, where the N-termini were capped with acetylation. Interestingly, no pH-responsive fluorescence behavior was observed in the capped systems confirming the role of the pH driven protonation state of the amine group in the observed pH sensitivity of the AuNCs. These results suggest the possibility of fluorescence quenching of AuNCs by the deprotonated N-terminal amine at higher pH. This effect is similar to the reported observation that fluorescence from CdSe nanocrystals was quenched by surface amine (e.g., cysteine and n-butylamine) via different mechanisms.

**Fig. 6.** Photoluminescence intensity ratio ($I_{5.5}/I_{7.5}$) at pH 5.5 and pH 7.5 of AuNCs. A ratio $\sim 1$ indicates a pH independent luminescence response while a ratio $> 1$ signifies intensified emission in acidic solution.

**Sequence-dependent cellular internalization and imaging of peptide-protected AuNCs.** The findings of dominating parameters that control the cluster fluorescence enabled us to design AuNCs that display bioactive epitopes without harming the fluorescence strength. As an example, we were able to synthesize AuNCs by using tyrosine-containing peptides CYYGDD and CYYGRR, which show similar fluorescence properties but different cell-nanocluster interactions. The cell surface is typically negatively charged at physiological pH due to the presence of anionic phospholipids, glycolipids and proteins in the membrane. Therefore, the positive charges (RR) on AuNCs were used to increase the cell-Au interactions and promote the cellular entry of photoluminescent AuNCs. We utilized fluorescence microscopy to image the uptake of peptide-protected AuNCs and observed significant cellular uptake of CYYGRR protected AuNCs (Fig. 7a). On the contrary, AuNCs from CYYGDD were not able to penetrate into the cell. This gives proof of the potential applicability of our rational design.
Fig. 7. Confocal images of HeLa cells internalizing peptide-protected AuNCs. Cellular internalization is significantly enhanced when peptide sequence charge is changed from negative to positive by replacing aspartic acid with arginine: (a) CYYGRR and (b) CYYGDD. The nucleus is stained with DAPI (4',6-diamidino-2-phenylindole, dihydrochloride); plasma membrane is stained with the probe WGA (wheat germ agglutinin). Scale bar: 10 μm for the bigger images and 2.5 μm for the inserts.

CONCLUSIONS

Through the observed correlations between the photoluminescence performance of peptide-protected AuNCs and the structure of the surface ligand layers, we propose three key design principles for the enhancement of the AuNC luminescence. (1) Acetyl capping of N-terminal amine groups synergistically enhances luminescence performance via a variety of mechanisms including: replacement of electron-withdrawing groups with partial electron density donating moieties in the vicinity of the Au core; removal of the electrostatic attraction between the peptide termini, which increases the thickness of the peptide layer and distances the negative C-terminus from the proximity of the Au core; and diminishing the number of surface-bound water molecules within the peptide layers; (2) introduction of hydrophobic/aromatic residues in the peptide sequences provides delocalized electron density from the
surface ligands to the Au core; (3) lowering of solution pH can affect the protonation state of the N-terminal amine and electrostatic potential around the AuNC core.

These findings not only contribute to the understanding of the molecular mechanisms behind the photoluminescence of AuNCs, they also help facilitate a rational approach for designing AuNCs for broad biomedical applications. Furthermore, the present methodology permits the tuning of the charges on the complex, opening the way for tailored charge engineering allowing, for example, an enhancement of AuNC uptake or of their persistence in the blood stream, depending on the peptide sequence of choice.

In contrast to gold nanoparticles that are protein-templated or coated with other biocompatible molecules such as PEG, synthetically engineered peptide-functionalized AuNCs allow for the fine tuning of charge, size, hydrophobicity and pH sensitivity while actively controlling photoluminescence intensity through the knowledge of how amino acid composition affects these characteristics. In particular, this opens up possibilities for the bottom-up engineering of peptide coatings with biocompatibility and biologically specific functionalities while tailoring nanoparticulate properties and consciously modulating their inherent photoluminescence. The outcomes of this work should stimulate further experimental and theoretical research into the use of peptide-coated AuNCs as molecular probes and therapeutic agents.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website. Experimental procedures, synthesis description, computational simulation and supporting figures.

The authors declare no competing financial interest

Data availability. Raw data will be made available upon acceptance.

ACKNOWLEDGMENTS

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number (e87) and Melbourne Bioinformatics, Australia. The Ganesha X-ray scattering apparatus used for this research was purchased under EPSRC Grant “Atoms to Applications” Grant ref. EP/K035746/1). The authors acknowledge use of the Facility for Imaging by Light Microscopy (FILM) at Imperial College London.

References
Supporting Information for

Surface Dynamics and Ligand-Core Interactions of Quantum Sized Photoluminescent Gold Nanoclusters

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Methods

Reagents and Materials. All Fmoc-protected amino acids, Rink Amide resin, and O-benzotriazole-N,N,N′,N′-tetramethyluronium-hexafluoro-phosphate (HBTU) were purchased from Anaspec. Inc. Gold(III) chloride trihydrate and sodium borohydride were purchased from Sigma-Aldrich (UK). All the other reagents were used as received.

Peptide design and synthesis. Peptides were synthesized using standard FMOC solid state synthesis using Rink amide resin. Protected amino acids were added to the growing peptide chain with the activating reagent 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). Upon the addition of the N-terminal amino acid, the FMOC group was removed under standard 20% piperidine in DMF deprotection conditions. The peptides were cleaved by trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5, v/v/v). The crude peptide was precipitated by cold ether several times and applied to reverse phase high performance liquid chromatography (HPLC, Shimadzu) to purify the target peptide. The Phenomenex C18 Gemini NX column was 150 x 21.2 mm and had a 5 µm particle size and 110 Å pore size. Matrix-assisted laser desorption spectroscopy (MALDI; Waters) was used to confirm the expected m/z ratio and α-Cyano-4-hydroxycinnamic acid was used as the MALDI matrix substance.
Synthesis of photoluminescent AuNCs. To synthesize peptide protected AuNCs, 0.5 mM HAuCl₄ were added into an aqueous solution of 2.0 mM peptide followed by vortexing for 10 sec. After 30 min, freshly prepared sodium cyanoborohydride was introduced to reduce gold ions at room temperature. The solution was stored in the dark overnight before measurements.

Characterization of AuNCs. UV-Vis absorbance was measured on Shimadzu (UV-1800) UV-visible spectrometer, deuterium lamp, scanning speed: 20 nm/sec, repeated 3 times. Fluorescence excitation and emission measurements were performed on Fluorolog®-3 spectrofluorometer. Quantum yield of AuNCs was obtained using the comparative method with Rhodamine 6G ethanolic solution (QY = 95%, λₑₓ = 488 nm) as a standard fluorophore. Dynamic light scattering (DLS) and ξ-potential was measured on a Malvern Zetasizer Nano ZS (Malvern, UK) with a backscattering detection at 173° equipped with a He-Ne laser (λ = 632.8 nm). XPS spectra were obtained on a Thermo Fisher K-Alpha spectrophotometer utilizing a monochromatic Al-Kα X-ray source (energy = 1486.71 eV). TEM was performed on a JEOL 2100F with an acceleration voltage of 200 kV. Elemental compositional analysis of gold clusters was determined by energy-dispersive X-ray spectroscopy (EDS). The time-resolved fluorescence measurements were acquired with a Horiba Deltamex system, using a 404 nm diode laser with a pulse duration of <100 ps as the light excitation source (λ = 700 nm). The IRF was collected at the excitation wavelength using a diluted Ludox® solution in water. Polyacrylamide gel electrophoresis (PAGE) experiment was performed by using a slab gel electrophoresis unit following the reported method.² The separating and stacking gels were prepared by acrylamide monomers with the total contents of 25 and 3 wt % (acrylamide/bis-acrylamide, 19:1), respectively. The eluting buffer contains 25 mM tris(hydroxymethylamine) and 192 mM glycine. The sample solution (20 μL) was loaded onto the stacking gel and eluted at a constant voltage mode (200 V).

Cell culture. All products from Life Technologies (UK) unless otherwise stated. HeLa cells were obtained from DSMZ (Brunswick, Germany) and maintained under standard mammalian cell culture conditions in DMEM supplemented with fetal bovine serum (10%, v:v) and penicillin-streptomycin (1%, v:v). For the particle uptake experiments, cells were plated at 10⁵ cells per well in 8 well chamber slides (Ibidi, UK). On the next day, the particle solution in sterile PBS was added to each well in serial dilutions, supplemented with an equal volume of culture medium and incubated for 24 hours, after which the samples were fixed in paraformaldehyde (4%, w:v) for 15 minutes and washed extensively in PBS.

Cell staining and confocal imaging. To visualize the cell membrane and nucleus, the samples were stained with WGA-488 and DAPI for 15 min following manufacturer’s instructions. After washing in PBS, images were taken using a Leica SP5 MP/FLIM inverted confocal microscope. Laser reflection and infrared emission were both used to localize the particles.

Computational simulation details. The AuNC structure used in the molecular dynamics (MD) simulations contains 25 gold atoms and a morphology consistent with that of highly stable Au25(SR)₁₈.
clusters, which can conceptually be divided into an icosahedral Au$_{13}$ core protected by six –[SR–(Au-SR)$_2$]– “staple” motifs. The peptide-coated AuNCs modeled (indicated by asterisks in Table S1) were constructed by attaching, via the N-terminal cysteine, 18 extended peptide ligands equidistantly onto the X-ray crystal structure of Au$_{25}$(SR)$_{18}$ (Fig. 1b). All peptides have been NH$_2$ capped on the C-terminus and N-terminated with either a NH$_3^+$ moiety or a CH$_3$CO acetyl group (denoted by an “Ac-” prefix). In total, 14 different Au$_{25}$(SP)$_{18}$ (P = peptide) simulation models were constructed with the following peptide sequences: CGGGDD as a control; CVGGDD, CHGGDD, CYGGDD, CGYGDD, CHYGDD, CYHGDD, CYYGDD, and CVVGDD to explore hydrophobicity and individual amino acid location; Ac-CGGGDD, Ac-CHGGDD, Ac-CHYGDD and Ac-CYHGDD to study N-terminal acetyl capping; as well as an additional positively charged peptide with arginine residues, CYYGRR, to investigate a cellular internalizing sequence. The chosen Au$_{25}$(SP)$_{18}$ models represent diverse and distinct experimental systems which display a distribution of PL intensities.

Explicit solvent MD simulations were conducted using the GROMACS 4.6.5 software. Peptide interatomic interactions were modelled using the all-atom AMBER99SB-ILDN force field (FF), with CYX parameters used for charge neutral (unprotonated) cysteine residues. The TIP3P model was used for water. Bonded parameters and Lennard-Jones potentials between Au and peptide atoms (S, C, and H) were adopted from a FF parameterized for similar monolayer-protected AuNCs assuming no explicit partial charges on gold atoms. Additional parameters for the Au-S-C-C dihedral were obtained from a quantum mechanics dihedral scan at the B3LYP/6-31G* level (with the LanL2DZ basis set for Au) in Gaussian09. For non-bonded interactions, long-range electrostatics were evaluated using the Particle Mesh Ewald (PME) method with a real space cutoff of 10 Å and a 1.2 Å fast Fourier transform (FFT) grid spacing, while van der Waals interactions were truncated at 10 Å. To preserve the core crystal structure of Au$_{25}$(SP)$_{18}$ during MD, distance restraints of 1000 kJ/mol/nm$^2$ were applied between Au-Au atoms. Each Au$_{25}$(SP)$_{18}$ was placed in a periodic cubic box of side length ~8.4 nm, solvated with ~19000 water molecules (water density of ~1 g/cm$^3$) and Na$^+$ or Cl$^-$ counter ions were added to ensure a neutral simulation cell. Energy minimization was carried out using the steepest descent algorithm to remove any steric clashes and 1 ns of position restrained MD was performed using the Berendsen thermostat and barostat to equilibrate the solvent around the AuNCs at 300 K temperature and 1 atm pressure. Position restraints were then removed and 100 ns of NPT (constant pressure and temperature ensemble) MD was performed with the Nosé-Hoover thermostat and Parrinello-Rahman barostat to maintain temperature and pressure at 300 K and 1 atm. The LINCS algorithm was applied to constrain all bonds to their equilibrium lengths, which enabled a time-step of 2 fs to be used for each simulation, and frames were outputted to a trajectory file every 2 ps. To enhance conformational sampling, each system was simulated ten times starting from different initial atomic velocities, resulting in a total of 1 µs of data for each Au$_{25}$(SP)$_{18}$. Statistical analysis and visualization of the data was performed using the GROMACS 4.6.5 suite analysis tools and the VMD 1.9.2 package. The properties and structures presented are ensemble averaged over the 10 independent trajectories for each Au$_{25}$(SP)$_{18}$ system and,
unless stated otherwise, analysis has been performed on the thermally equilibrated stage of the simulations covering the final 20 ns of each trajectory (200 ns of production data per system), as verified by monitoring energy trends, convergence of $R_g$ and root-mean-square deviations. Hydrodynamic diameter ($D_h$) estimates are obtained for $2 \times 10^4$ configurations per system (1 frame every 10 ps) using the path-integration program ZENO$^{15}$ with $1.5 \times 10^5$ random paths at each configuration to achieve low uncertainty in $D_h$.

Quantum mechanics (QM) calculations were performed in a manner similar to the approach of Fihey $et al.$$^{16}$ Initial geometries of the Au$_{25}$S$_{18}$Cys AuNCs with acetyl, protonated amine, and deprotonated amine N-termini were taken from equilibrated MD simulation snapshots. A geometry optimization of the cysteine ligands with N-methylamine C-termini was performed using the Dmol$^3$ program ($DMol3$. Dassault Systèmes Biovia Corp., San Diego, CA 92121 USA)$^{17}$ with the PBE DFT exchange correlation functional$^{18}$ and double-numeric polarized (DNP) basis set$^{19}$ with all electron relativistic core treatment. Solvent effects were treated using the conductor-like screening model (COSMO).$^{20}$ Electrostatic potential (ESP) atomic partial charge calculations of the optimized structures were performed using Gaussian09,$^{8}$ with the PBE DFT functional and the relativistic double-$\zeta$ LANL2DZ basis set and effective core potential (ECP) for Au atoms, and the 6-31G(d,p) basis set for other atoms. Solvent effects were treated using the Polarizable Continuum Model (PCM).$^{21}$

Table S1. Engineered sequences used to prepare peptide-protected AuNCs.

<table>
<thead>
<tr>
<th>CXXGDD</th>
<th>Ac-CXXGDD</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGGGDD*</td>
<td>Ac-CGGGDD*</td>
<td>CDGGGD</td>
</tr>
<tr>
<td>CSGGDD</td>
<td>Ac-CHGGDD*</td>
<td>DGYCGD</td>
</tr>
<tr>
<td>CVGGDD*</td>
<td>Ac-CHYGDD*</td>
<td>CYYGRR*</td>
</tr>
<tr>
<td>CTGGGD</td>
<td>Ac-CYHGDD*</td>
<td></td>
</tr>
<tr>
<td>CHGGGD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKGGDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFGGDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CQGGDD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNGGDD</td>
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<td></td>
</tr>
<tr>
<td>CYGGDD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGYGDD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVGGDD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVVGDD*</td>
<td></td>
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</tr>
<tr>
<td>CHYGDD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHYHGD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYYGDD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFFGDD</td>
<td></td>
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</tr>
</tbody>
</table>

*Au$_{25}$(SP)$_{18}$ (P = peptide) structures also investigated with molecular modelling.
**Figure S1.** (a) Dynamic light scattering of CHYGDD-protected AuNCs showing the hydrodynamic size of ~ 3.1 nm. This number is slightly larger than the physical size of Au core due to the surface coating of a peptide layer. (b) PAGE results for the CHYGDD-protected AuNCs.

**Figure S2.** Representative TEM image of CHYGDD-protected AuNCs.
Figure S3. Energy-dispersive X-ray spectroscopy (EDS) of CHYGDD-protected AuNCs showing the existence of Au elements in the nanoclusters.

Figure S4. UV-vis absorbance and fluorescence emission spectra of Au clusters protected by different peptide molecules: (a) CGGGDD, (b) CFGGDD, (c) CHGGDD, (d) CYGGDD, (e) CSGGDD, (f) CVGGDD, (g) CDGGGD, (h) CKGGDD, (i) CTGGDD, (j) CGYGDD, (k) CVVGDD, (l) CNGGDD, (m) CYYGDD, (n) CHYGDD, (o) CYHGDD, (p) CFFGDD, (q) CQGGDD, (r) Ac-CGGGDD, (s) Ac-
CHGGDD, (t) Ac-CHYGDD, (u) Ac-CYGHD, and (v) DGYCGD. The excitation wavelength was 400 nm.
Figure S5. Left panels: Hydrodynamic diameter ($D_h$) computed for $2 \times 10^4$ configurations using the ZENO$^{15}$ path-integration program. It should be noted that configurations within consecutive blocks of
$2 \times 10^3$ are correlated since they are taken from independent equilibrated trajectories and only every $50^{th}$ data point is plotted for clarity. **Right panels:** Probability density function (P.D.F.) of the ensemble shown with the modal $D_h$ and one standard deviation (shaded region).
Figure S6. Pairwise distribution functions, $g(r)$, for each sequence showing the minimum distances from ASP (red), TYR (green), HIS (blue), and ARG (orange) residues to the closest Au$_{25}$S$_{18}$ atom. The
insets in each plot show a zoomed in region to better illustrate the probability of residues being within close proximity to the gold core. The distributions of minimum distances are measured between the centers-of-mass of side-chain heavy-atoms (TYR: phenol, ASP: carboxyl oxygen, and HIS: imidazole) relative to the nearest gold or sulfur atom (AuS\textsubscript{nearest}).

Table S2. Average number of residues (ASP, TYR, HIS, ARG) within 0.5 nm of Au\textsubscript{25}S\textsubscript{18}.*

<table>
<thead>
<tr>
<th>System</th>
<th>ASP</th>
<th>TYR</th>
<th>HIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGGGDD</td>
<td>6.74</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CVGGDD</td>
<td>6.65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CVVGDD</td>
<td>6.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CHGGDD</td>
<td>6.85</td>
<td>0.15</td>
<td>(0.65 nm)</td>
</tr>
<tr>
<td>CHYGDD</td>
<td>6.58</td>
<td>0.21</td>
<td>(0.80 nm)</td>
</tr>
<tr>
<td>CYHGDD</td>
<td>6.31</td>
<td>0.50</td>
<td>(0.72 nm)</td>
</tr>
<tr>
<td>CGYGDD</td>
<td>6.52</td>
<td>0.01</td>
<td>(0.81 nm)</td>
</tr>
<tr>
<td>CYGGDD</td>
<td>7.14</td>
<td>0.44</td>
<td>(0.70 nm)</td>
</tr>
<tr>
<td>CYYGDD</td>
<td>6.57</td>
<td>0.71</td>
<td>(0.55 nm)</td>
</tr>
<tr>
<td>Ac-CGGGDD</td>
<td>0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ac-CHGGDD</td>
<td>0.05</td>
<td>-</td>
<td>1.44</td>
</tr>
<tr>
<td>Ac-CHYGDD</td>
<td>0.00</td>
<td>0.44</td>
<td>(0.58 nm)</td>
</tr>
<tr>
<td>Ac-CYHGDD</td>
<td>0.00</td>
<td>0.91</td>
<td>(0.51 nm)</td>
</tr>
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<table>
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</thead>
<tbody>
<tr>
<td>CYYGRR</td>
<td>0.00</td>
<td>0.45</td>
<td>(0.61 nm)</td>
</tr>
</tbody>
</table>

*Values in parentheses are distances between Au\textsubscript{25}S\textsubscript{18} atoms and the nearest (integer) residue.
Figure S7. Pairwise distribution functions, $g(r)$, of electron-rich (red), electron-deficient (green) and water molecules (blue) relative to the closest $\text{Au}_{25}\text{S}_{18}$ atom ($\text{AuS}_{\text{nearest}}$). Distributions for atoms in uncapped systems are shown as solid lines while those for the acetyl-capped are dashed. Electron-donating groups include (backbone/acetyl amine nitrogens, backbone/acetyl carbonyl oxygens, tyrosine phenol oxygen, aspartate carboxyl oxygens, histidine deprotonated imidazole nitrogen). Electron-withdrawing groups are (cysteine protonated amine nitrogen). Water molecules are taken as their oxygen atom location.

a)

b)
Figure S8. Representative structures of (a) CGGGDD and (b) Ac-CGGGDD showing how the presence of N-terminal NH$_3^+$ groups encourage more compact peptide structures due to the electrostatic attraction with C-terminal COO$^-$ groups. Peptide backbones are shown as black lines, gold atoms are orange, sulfur atoms are yellow, ASP carbonyl oxygen atoms and CYS amine nitrogen atoms are displayed in red and dark blue, respectively. Bulk water molecules and counter ions are not shown for clarity. Peptides with COO$^-$ groups not within close proximity of Au$_{25}$S$_{18}$ are drawn transparently.
Figure S9. Density maps of peptide backbone angles formed throughout the MD simulations as a function of C-terminal to AuS\textsubscript{nearest} distances. Angle $\theta$ is measured between backbone alpha carbon
atoms 1, 3 and 6 (Cα₁–Cα₃–Cα₆, where numbering starts from the N-terminus) and peptide C-terminal locations are taken as Cα₆ atom positions. Contour lines are drawn around density regions in increments of 1000 frequency.

**Figure S10.** (a-f) Plots of scattered X-ray intensity with Q (blue) as well as Hammouda’s Guinier-Porod model simulation fits (red traces). (g) Plot of emission peak maxima as a function of peptide sequence (Figure S1) showing the minimal variation in peak position indicative of minimal variation in the Au core size. (h) Plot of the value of scattering $R_g$ obtained for MD simulations (blue) and from the Hammouda model fits to the scattering data (pink) as a function of peptide sequence. (i) Kratky plots of scattered intensity highlighting the plateau formation of these systems which indicates flexible,
extended peptide configurations. (j) Tabulated fit data for $R_g$ and the Porod exponent $P$ along with the MD simulation scattering $R_g$ values obtained using Crysol.

The scattering data were measured using a Ganesha 300XL SAXS apparatus from SAXLAB (Denmark). The incident wavelength was 1.54 Å and the position of the Dectris Pilatus 300 area detector and diameter of the collimating pinholes were adjusted to give four overlapping ranges of scattering vector ($0.003$-$0.018$, $0.007$-$0.25$, $0.015$-$0.65$, $0.7$-$2.8$ Å$^{-1}$). The samples were sealed in 1.5 mm fused quartz capillary tubes (Capillary Tube Supplies Ltd, Bodmin) and the all the beam paths were evacuated. The sample scattering was regrouped to a 1D dataset of intensity vs scattering vector, $Q$, then corrected for transmission and thickness using SAXSGUI. The scattering from the capillary tubes was measured from an empty part and subtracted. The pure solvent data was treated in the same way and subtracted from the solution data before further analysis.

Data were analysed using the NIST SANS data reduction package in Igor Pro (Wavemetrics, OH) software.$^{22}$ The specific model used to fit the data was the Guinier-Porod model, developed by Hammouda,$^{23}$ which is a generalized empirical model for structures having Guinier and Porod components (Figure S10a-f). The structures were assumed to be globular, allowing a generalized power law and scattering radius of gyration ($R_g$) to be obtained from the experimental data. The $R_g$ obtained using this method will be susceptible to variation in the electron dense Au core size, which will contribute significantly to the scattered X-ray intensity. The fluorescence emission maxima of the clusters is known to vary as a function of cluster size$^{24}$ and we have used this indicator (Figure S10g) to suggest that there is minimal variation in core size where the emission peak maxima remains within the range of 680 to 705 nm. Indeed, any variation in peak maxima position does not follow the trend of increasing core size with increasing sequence hydrophobicity suggesting that the $R_g$ values obtained in SAXS measurements and fitting relate to the contribution of the peptide shell. Figure S10h highlights the tendency for the SAXS derived $R_g$ values to follow the same trend as the scattering $R_g$ obtained for MD simulation trajectories (generated in Crysol for multiple trajectory frames and averaged) where an increase in sequence hydrophobicity tends to relate to an increase in $R_g$. However, for a fixed number of peptides per cluster, the role of scattering length density of the shells will be linked to the variation in shell thickness. In such a case the mixing ratio of peptide to solvent will vary and influence the $R_g$ obtained experimentally and the presence of a complex interface between the electron dense gold core and organic shell may explain why the experimental $R_g$ values tend to be lower than the generated MD simulation values.
Figure S11. Radial distribution functions (RDF) of selected complex components relative to the central gold atom ($\text{Au}_{\text{center}}$) of the $\text{Au}_{25}(\text{SP})_{18}$ to highlight water structuring between the different peptide sequences. $g(r)$ has been normalized by volume in each radial shell ($dr = 0.05$ nm) by the density of “bulk” water far from the AuNCs ($\rho_{\text{bulk-water}} = 0.100573$). Explicitly, $g(r) = (N_{\text{atoms in } V_{\text{shell}}} / V_{\text{shell}}) / (\rho_{\text{bulk-water}}) = [N_{(r+dr)} - N_r] / [(4\pi/3)(r+dr)^3 - r^3)(\rho_{\text{bulk-water}})]$. Note that standard deviation is shown as the shaded region around each line.
Figure S12. Superimposed histograms of the average number of H$_2$O molecules that are internalized (surface-bound and embedded) and present at the peptide–solvent hydration layer (interfacial) of each simulated Au$_{25}$(SP)$_{18}$ system. Error bars represent standard deviation.
Figure S13. Water decay profiles (bulk, interfacial, embedded, or surface-bound) showing how internalized water has a much shorter retention time for acetylated Au25(SP)18 systems. Atom indices are monitored for each water selection (e.g. bulk, interfacial, etc.) in blocks of 100 ps at a frequency of 2 ps. At t = 0 ps, an initial selection of water atoms is made based on a distance criteria, then a count is maintained for molecules that consecutively remain in the selection over the 100 ps. To avoid correlated data, every 2nd trajectory block of 100 ps is not analyzed. At the end of data collection, all decays for a given system are averaged and presented with their standard deviation (shaded region). Bulk water profiles are obtained by using a 0.6 nm radial shell from interfacial water.

Table S3. Average bond distances in angstroms for the PBE/DNP optimized clusters with –NH2, –NH3+, and –COCH3 N-termini.

<table>
<thead>
<tr>
<th>Bond type*</th>
<th>Experimental a</th>
<th>–NH2</th>
<th>–NH3+</th>
<th>–COCH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au_center–Au_shell (12)</td>
<td>2.793±0.006</td>
<td>2.787±0.033</td>
<td>2.789±0.030</td>
<td>2.789±0.037</td>
</tr>
<tr>
<td>Au_shell–Au_shell (30)</td>
<td>2.94±0.09</td>
<td>2.935±0.149</td>
<td>2.939±0.156</td>
<td>2.938±0.157</td>
</tr>
<tr>
<td>Au_shell–S_terminal (12)</td>
<td>2.372±0.020</td>
<td>2.369±0.017</td>
<td>2.373±0.019</td>
<td>2.370±0.016</td>
</tr>
<tr>
<td>Au_staple–S_terminal (12)</td>
<td>2.301±0.008</td>
<td>2.290±0.006</td>
<td>2.290±0.004</td>
<td>2.290±0.009</td>
</tr>
<tr>
<td>Au_staple–S_central (12)</td>
<td>2.299±0.013</td>
<td>2.294±0.005</td>
<td>2.291±0.005</td>
<td>2.294±0.005</td>
</tr>
</tbody>
</table>
*Atom notation is taken from Weerawardene et. al \(^{25}\) and Vanzan et. al \(^{26}\) and number of bonds is given in parentheses.

\(^{a}\) See reference \(^{3}\)

**Figure S14.** Overlay of the QM optimized Au\(_{25}\)Cys\(_{18}\) structures, where cysteine N-termination is deprotonated amine (–NH\(_2\), red), amide (–NH\(^{+}\), blue), and acetyl (–COCH\(_3\), green). The Au\(_{25}\) RMSD is ~0.1 Å between the three systems.

**Moment of Inertia Tensor to Determine Nanocluster Size and Shape**

The best fitting ellipsoid for each Au\(_{25}(SP)_{18}\) is found using the moment of inertia tensor \(I\), defined as:

\[
I = \begin{bmatrix}
I_{xx} & I_{xy} & I_{xz} \\
I_{yx} & I_{yy} & I_{yz} \\
I_{zx} & I_{zy} & I_{zz}
\end{bmatrix} = \sum_{i=1}^{N} m_i \begin{bmatrix}
(y_i^2 + z_i^2) & -x_i y_i & -x_i z_i \\
-y_i x_i & (x_i^2 + z_i^2) & -y_i z_i \\
-z_i x_i & -z_i y_i & (x_i^2 + y_i^2)
\end{bmatrix}
\]

where \(m_i\) is the mass of particle \(i\) at position \((x_i, y_i, z_i)\) from the axis of rotation. The eigenvalues of the tensor are the principal moments of inertia \(I_1, I_2\) and \(I_3\) which can be obtained by the diagonalised tensor \(I\):

\[
I = \begin{bmatrix}
I_1 & 0 & 0 \\
0 & I_2 & 0 \\
0 & 0 & I_3
\end{bmatrix}
\]

For an ellipsoid, the eigenvalues are:

\[
I_1 = \frac{M}{5}(b^2 + c^2), \quad I_2 = \frac{M}{5}(a^2 + c^2), \quad I_3 = \frac{M}{5}(a^2 + b^2)
\]

where \(M\) is the total mass and \(a\), \(b\) and \(c\) are the major, intermediate and minor semi-axes lengths of the ellipsoid (i.e. Au\(_{25}(SP)_{18}\), inset of Figure S12). AuNC volume is then calculated using \(V = \frac{4}{3}\pi abc\).
**Figure S15.** MD obtained average $\text{Au}_{25}(\text{SP})_{18}$ volumes. The inset shows an example structure and the corresponding volume “ellipsoid” (green) displaying the major, intermediate and minor axes of the $\text{Au}_{25}(\text{SP})_{18}$.


