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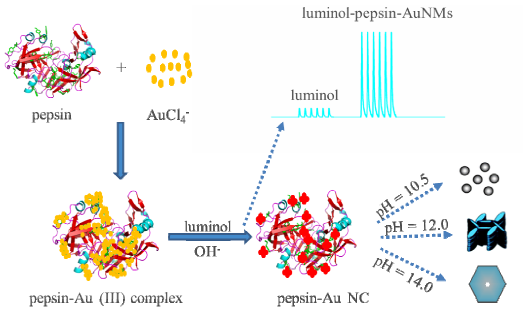
Graphical Abstract



Highlight: The different size and shape AuNMs were generated in the Pep-HAuCl$_4$ system based on photochemical induce effect of alkaline luminol.

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Photochemical induced formed Au nanomaterial with size and shape controlled by luminol-pepsin chemiluminescence reaction

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Size and shape controlled Au nanomaterial (AuNMs) were generated in different alkaline luminol-pepsin (Pep) chemiluminescence (CL) reaction solution based on the photochemical induced effect of luminol. These finding showed that luminol photochemical intensity could be used as a major control parameter for metals NMs growth.

Size and shape provides important and profound influence over many of the physical and chemical properties of nanomaterial (NMs), including luminescence[1-5], conductivity[6-8], and catalytic activity[9-11]. The preparation of the NMs with size and shape controlled is an attractive and paramount goal in developing and fabricating all sorts of biosensor and bioelectronics in the field of chemistry[12, 13], materials[14], biology[15], and medicine[16]. The method for both size and shape controlled simultaneously remains an extremely difficult task which is always complicated and time consuming, only partially characterized interplay of kinetics and thermodynamics[17]. Recently, tremendous efforts have been put toward to regulate the properties of the NMs by controlling quantum confinement phenomena (QCP) in the process of NMs growth[18-21]. The nature of QCP for photochemical reaction is mainly from the considerable active free radical, which was associated with electronic transitions from excited state to ground state[22-26]. Based on this, photo-induced technology for forming NMs with size and shape controlled could shift the apparent thermodynamic equilibrium of redox reaction to favor room temperature catalysis process with mild reaction condition[27-30]. However, the nature of time-consume for NMs formation is no completely changed due to shortage of special endogenous power in wide spectral range. Photochemical induced technology[31, 32] as one of photo-induced technology have the characterization of endogenous fluorescence and elimination of photo-bleaching, particularly, associated with strong QCP in the photochemical process. In view of the novel characterization for photochemical induced technology, it could explore the new way for forming NMs with size and shape controlled under room temperature with mild condition.

Luminol, as one of common organic photochemical induced agent (E_m = 425 nm), has the different quantum efficiency with alkaline medium-dependent nature in the luminol chemiluminescence (CL) reaction[33]. In the previous report, the $HAuCl_4$ could be effectively catalysed to form the Au nucleation and eventually grown nanoparticles (AuNPs) [34, 35], which could accelerate electronic transition rate and enhance the luminol CL intensity in the luminol-$HAuCl_4$ CL reaction[34, 36]. In our former work, we found that $HAuCl_4$ and pepsin (Pep) could sharply enhance the CL intensity under the alkaline condition (pH = 10.5) (Support Fig. 1), and formed the Au nanoclusters (AuNCs), then, the colour of reaction solution rapidly changed from light yellow to light red. The possible mechanism might be attributed as the joint action of luminol photochemical induced effect and micro-change of Pep confirmation in the CL system under alkaline condition.

With those thoughts in mind, here, we reported a native luminol photochemical induced method for forming mono-disperse Au nanomaterials (AuNMs) with size from 1 to 400 nm, like as spherical-like AuNCs, flower-like AuNPs and regular hexagonal-like Au nanosheets (AuNSs) in CL reactions solution under different pH (Fig. 1). All above size AuNMs were rapidly formed in CL reaction solution in 30 min based on the luminol photochemical induce at E_m = 425 nm. In this approach, according to the generation mechanism for protein protected AuNMs by Glomm's group[37], combined with instability conformation of Pepsin in alkaline solution[38] and the different quantum efficiency with alkaline medium-dependent nature in luminol CL reaction[33], the possible mechanism for size and shape controlled of AuNM in Pep-luminol alkaline solution with different pH might be attributed as following: First was the nucleation stage. Pep surface contain massive negative charges under the weak acid or neutral condition (above Pep's pKa), and charge density matching between Au^{3+} and negatively charged amino acid residues yields high metal concentrations, forming the Pep-Au^{3+} complex on the surface of Pep[39]. This step might provide the necessary microenvironment for triggering the Au nucleation growth with high redox potential. Second was initialization and growth stage. Based on the property of high quantum efficiency of luminol at alkaline solution, the electron transferring rate between Pep-Au^{3+} complex could be accelerated by excited 3-aminophthalate, which given rise to free radical with light emission at different pH luminol alkaline solution. It was worth noting that Pep as an acidic protein, have the instability conformation and construction at the alkaline solution. It was ascribed the interaction of Asp11 and Asp159 in Pep construction, which showed abnormally high pK_a values compared with other amino acid residues, like Glu4, Glu13 and Asp118 with an abnormally low pK_a value[39]. For the spherical-like AuNCs, The Au^{3+} on the surface of Pep were flowed into the vicinity of negatively charged amino acid residues on the principle of charge density matching, and eventually formed pepsin protected AuNCs with the bond of Au-S in the cavity of pepsin at the pH of 10.5. At the pH of 12.0, Pep had the instability conformation at the alkaline solution. The instability conformation and partial unfolding of the polypeptide chain of Pep resulted in close contact between adjacent Au nucleation and subsequent aggregation on the Pep surface, eventually formed AuNPs with irregular shape, named as flower-like AuNPs. While the pH reached to 14.0, it was the instability conformation of Pep increased and the collision between Peps further exacerbated that leaded to high contact probability between Peps exposed. Under this situation, the conformation flexibility of polypeptide chain, the charge density surrounding nucleation sites and the proximity of the nucleation to the surrounding aqueous environment could induce Au nucleation to orderly aggregate with luminol photochemistry, and eventually formed hexagonal-like AuNSs. The last was the termination stage. Termination could be occur *via* depletion of quantum efficiency of luminol produced by QCP, redox potential decreasing (dynamics) and Au^{3+} concentration equilibrium between the bulk phase and Pep surface (electrostatic and steric). At this stage, the colour of solution remained almost unchanged, the electrostatic barrier for bringing Au atom into close proximity becomes too large to overcome, thus preventing further growth. For above process, the micro-change of Pep confirmation acted as the template, which could provide the micro-environment for Au nucleation accumulation and aggregation, then ultimately accelerate the growth tendency of Au nanocrystals in CL reaction for forming different size and shape AuNMs in alkaline solution. With the joint action of the QCP by the photochemical induced and the micro-change of Pep conformation mediated by the nature of alkaline condition, the different AuNMs were eventually formed with different CL intensity. As a control experiment, there were only unified and mono-disperse quasi-spherical structure AuNPs formed with average diameters of 55 ± 4.2 nm for different pH at the absent of Pep (Support Fig. 2). Throughout the entire process, we could speculate that pH as the key factor, it was not only influenced the CL intensity, which promoted rapid reaction by photochemical induced mechanism, but also induce the instability conformation or construction of Pep in alkaline solution, which could provide the effective template or microenvironment for Au atom accumulating and aggregating, form with the different size and shape controlled AuNMs in alkaline solution.

(Fig.1)

The different size and shape AuNMs could be controlled by the sideway of different CL intensity with the different pH value. Interesting, with the pH increasing, the CL intensity showed the decreasing tendency, on the contrary, the average particle size was the increasing tendency. For example (Fig. 2a), injecting luminol solution with pH of 10.5 obtained 1-2 nm spherical-like AuNCs associated with CL intensity of 1150, with 12.0 yielded 35 ± 5.6 nm flower-like AuNPs for CL of 125, whereas with 14.0 produced 400 ± 25 nm regular hexagonal-like AuNSs for CL of 32. The above results fitted the hypothesis that alkaline luminol solution not only influenced the QCP, but also induce the instability conformation of Pep. In addition, with the pH increasing of alkaline solution, the CL intensity measured by PMT showed the exponential decay trends. This trends would be partly relative to the pH influenced to the CL intensity, but mainly subject to reducing the quantum effect and enhancing scatter effect, while the AuNMs particle size increasing. The stability of AuNMs colloid solution formed in the luminol-Pep CL reaction was investigated too. With time elapsed, protein protected AuNCs have the good stability for 48 days, the flower-like AuNPs have the moderate stability for 15 days and hexagonal-like AuNSs have the relative poor stability only for 7 days. The stability of AuNMs in colloid solution mainly depended on factor of particle size, adulterating degree and surface ligand[40, 41]. The XRD spectrum (Fig.2b) showed that AuNMs prepared in luminol-Pep CL reaction had virtually no adulterating element only with Au, Na, C, O and Cl. From the point of particle size and surface ligand, different size and shape AuNMs had the different stability in alkaline solution. For spherical-like AuNCs, it was capped into the Pep construction with the bond of Au-S with hydrophilic surface. This characterization of AuNCs endowed it had good stability in alkaline solution. For flower-like AuNPs, it had larger specific surface area due to the conformation instability and partial unfolding of the polypeptide chain, which could provide the site for Au nucleation aggregating on the surface of Pep. It was the particle size increasing and hydrophilic group decreasing that made AuNPs had the moderate stability in alkaline solution compared with the long stability of AuNCs. For the regular hexagonal-like AuNSs, its shape and size mainly depended on the collisions of inter-Peps and polypeptide chain folding. The relative poor stability was ascribed to the maximum of particle size and surface tension of strong alkaline solution.

(Fig.2)

This reported herein indicated that luminol as the photochemical induced agent could rapidly induce Au^{3+} to form Au nucleation, and eventually grown of different size and shape AuNMs. The UV-Vis results (Fig.3a) showed, as expected, the absorption peak of Pep and $HAuCl_4$ were 265 nm and 280nm, respectively, which was smothering by the characterization absorption peak of luminol at 285 nm and 325 nm. The characteristic absorption peak of luminol took on the decreasing tendency, while the pH increased from 10.5 to 14.0. A produced absorption peak in the 525-560 nm was the characteristic absorption peak of Au with the tendency of blue shift in the pH of 10.5-14.0, which would be attributed to that more luminol derived, 3-AP absorbed on the Au surface with the bond of Au-N. But interesting, the colour of AuNCs solution was light red, while the pH set at 10.5. If pH set at 12.0, the solution was red, then, turned to dark red when it was 14.0 (Fig. 3b), this results of solution colour corresponded with the UV-Vis results.

(Fig.3)

In conclusion, we found that, with the joint action of the QCP by the photochemical induced and the micro-change of Pep conformation mediated alkaline luminol solution, the different AuNMs with size and shape controlled could be formed in the CL reaction solution. It demonstrated that alkaline luminol solution not only influence the QCP of CL reaction, but also act as the micro-environment of Pep confirmation under room temperature. Further study this unusual and intriguing feature would help to clarify some the photo-induced mechanism of AuNMs formation in different CL reaction, which remained obscure at present. For practical purpose, this procedure opened a simple and fast way to tailor the properties of photochemical prepared metal NMs to the need of a specific use, by their size and shape in the fields of biosensor and bioelectronics.

Notes and references

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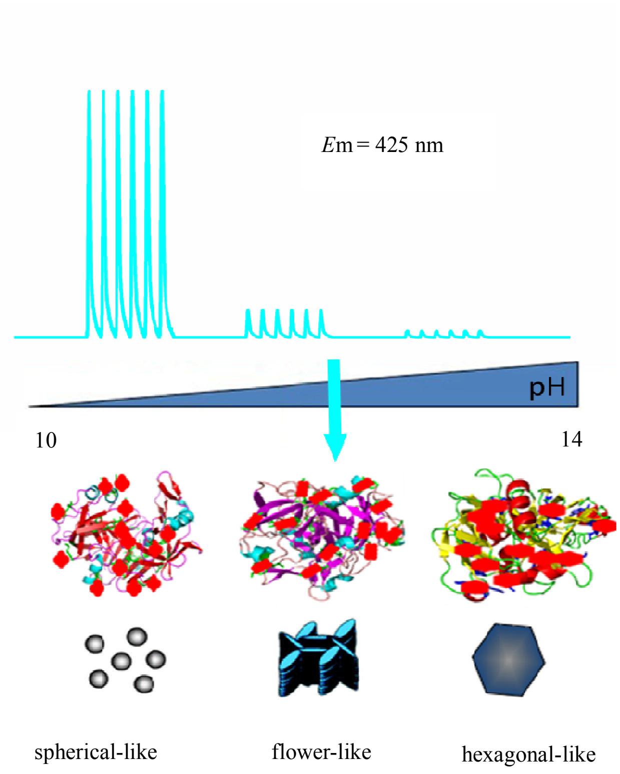

Fig.1 The scheme of different size and shape AuNMs formed in luminol-Pep CL system with photo-chemical induced with *E*m = 425 nm.

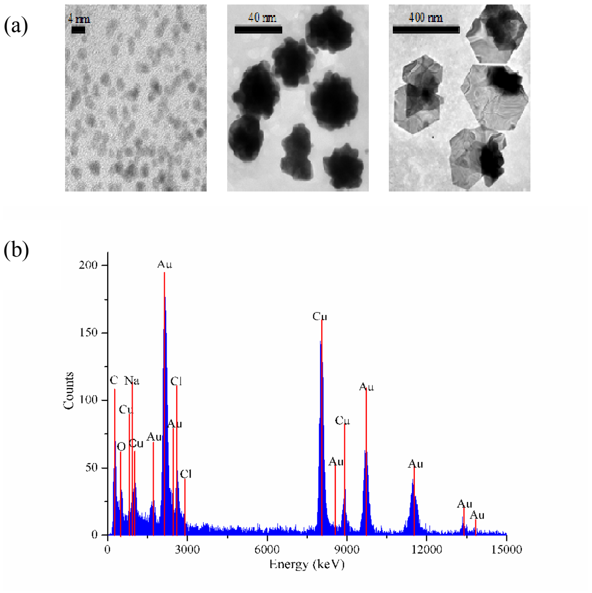

Fig.2 (a) The TEM imagine of AuCNs (1-2 nm), AuNPs (35 ± 5.6 nm) and AuNSs (400 ± 25 nm). (b) The EDX energy

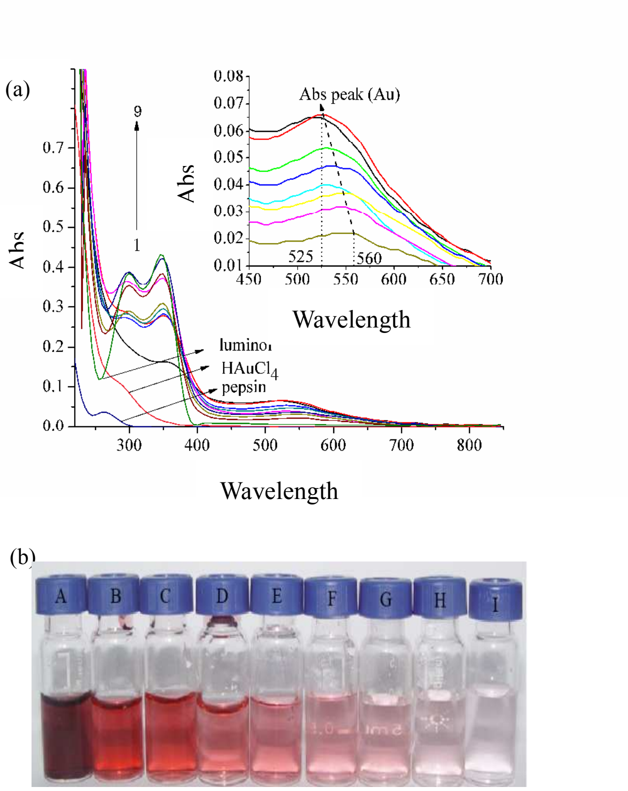

Fig.3 (a) The UV-Vis spectrum of luminol-$HAuCl_4$-pepsin reaction with pH from 10.0 to 14.0. The corresponding concentrations of pepsin (1), $HAuCl_4$ (2) and luminol (3) were 1.0×10^{-5}, 2.5×10^{-5} and 2.5×10^{-4} $mol\cdot L^{-1}$, (4-9) were the different pH of 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5 and 14.0, respectively. (b) The different color of AuNMs produced with different pH from14.0 to 9.0 (A-I) in 30 min.