Supplementary information

AuNPs@PMo_{12} nanozyme: highly oxidase mimetic activity for sensitive and specific colorimetric detection of acetaminophen

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**Table S1:** Physicochemical properties of acetaminophen [32].

| Drug          | Chemical name and formula | Structure | $\text{So H}_2\text{O}$ (25°C mg.mL$^{-1}$) | pKa |
|---------------|---------------------------|-----------|----------------------------------------|-----|
| Acetaminophen (AP) | 4-hydroxyacetanilide | ![Structure Image] C$_8$H$_9$NO$_2$ | 14 | 9.5 |
**Sonochemical synthesis of AuNPs@PMo\(_{12}\) nano-hybrid [32]**

The sono-reduction process of PMo\(_{12}\) and Au ions was carried out using ultrasonic irradiation (US) which is shown in scheme S1. Before US irradiation of PMo\(_{12}\) solution, no significant absorption band was observed in the visible region (curve (a) in scheme S1). After sonication of the PMo\(_{12}\) solution, a broad absorption band centered at 750 nm was obtained and the solution became pale green (curve (b) in scheme 1). The observation of a new metal-to-metal intervalence charge transfer (IVCT) band confirmed the electron transfer between the organic substrate (1-propanol) and heteropolyanions (PMo\(_{12}\)O\(_{40}\)\(^{-}\)) which produced the reduced form of PMo\(_{12}\) (PMo\(_{12}\)O\(_{40}\)\(^{4-}\)) (pale green solution). Furthermore, the sonication of PMo\(_{12}\) solution in the presence of Au ions led to the synthesis of AuNPs@PMo\(_{12}\) (pink solution, curve c in scheme S1). In water media, PMo\(_{12}\) dissociated as the following equation (eq.1):

\[
H_3PMo_{12}O_{40} \xrightarrow{H_2O} PMo_{12}O_{40}^{\text{\textminus}} + 3H^+ \quad (1)
\]

Before US irradiation the pH of the reactants in water media was measured at pH 3.5\(\pm\)0.5. It is suggested that an association between the PMo\(_{12}\)O\(_{40}\)\(^{3-}\) and 1-propanol (S) occurred under US irradiation in aqueous media. The sono-reduction of the PMo\(_{12}\) is shown as follows (eqs. 2-4):

\[
PMo_{12}O_{40}^{\text{\textminus}} + S \xrightarrow{} PMo_{12}O_{40}^{\text{\textminus}} \cdots S \quad (2)
\]

\[
PMo_{12}O_{40}^{\text{\textminus}} \cdots S \xrightarrow{} PMo_{12}O_{40}^{\text{\textminus}} \cdots \text{S} \quad (3)
\]

\[
PMo_{12}O_{40}^{\text{\textminus}} \cdots \text{S} \xrightarrow{} PMo_{12}O_{40}^{\text{\textplus}} + S_{\text{ox}} \quad (4)
\]
Electrons accumulated on the $\text{PMo}_{12}\text{O}_{40}^{4+}$ can be withdrawn by different electron acceptors (eq. 5):

$$\text{PMo}_{12}\text{O}_{40}^{4+} + \text{Oxidant} \xrightarrow{\text{sonic}} \text{PMo}_{12}\text{O}_{40}^{3-}, \text{etc.} \quad (5)$$

In eq. (5), etc. refers to reducing form of oxidant, $\text{O}_2^-$ and $\text{H}_2\text{O}$ due to reduction of $\text{O}_2$ and $\text{H}^+$. The reduced form of $\text{PMo}_{12}$ is a strong reducing agent that leads to the reduction of metal ions into their corresponding metal nanoparticles. In the case of gold ion, the following reaction occurs (eq. 6):

$$3\text{PMo}_{12}\text{O}_{40}^{4+} + \text{Au}^{3+} \xrightarrow{\text{sonic}} 3\text{PMo}_{12}\text{O}_{40}^{3-}......\text{Au}^{0} + 3\text{H}^+ \quad (6)$$

According to Okitsu et al. under ultrasonic waves, localized hotspots with a high temperature are produced due to the rapid collapse of cavitation bubbles. Under such conditions, the homolysis of water molecules gives rise to highly reactive $\cdot\text{H}$ and $\cdot\text{OH}$ radicals. The sono-reduction of Au(III) in the presence of an organic additive (1-propanol) can occur via the following reactions (eqs 7-9):

$$\text{RH} + \cdot\text{OH} (\text{H}^+) \rightarrow \text{R}^* + \text{H}_2\text{O} (\text{H}_2) \quad (7)$$

$$\text{RH} \rightarrow \text{Pyrolysis radicals and unstable products} \quad (8)$$

$$\text{Au (III)} + \text{reducing species (} \cdot\text{H, } \cdot\text{R, etc.}) \rightarrow \text{Au (0)} \quad (9)$$

where RH is an organic additive. Reactions (7) and (8) show the sonochemical formation of the reducing radicals and reductants: (1) $\cdot\text{H}$ is created by sonolysis of water, (2) $\cdot\text{R}$ and $\text{H}_2$ are created from the abstraction reaction of RH with $\cdot\text{OH}$ or $\cdot\text{H}$, and (3) pyrolysis of RH and water led to the pyrolysis radicals and unstable products. Finally, the generated organic radicals can reduce the target substrate in solution, which in this instance are Au (III) metal nanoparticles.
ions, through further complex reaction steps. Consequently, the reduction of PMo$_{12}$ and gold ions are also accelerated under such ultrasonic irradiation.

According to the reactions (7)-(9) the rate of Au(III) reduction strongly depends on the concentration of their primary and secondary reducing radicals. However, the acoustic intensity also affects other reaction parameters, such as temperature and pressure inside the collapsing cavitation bubbles, the size, number and distribution of bubbles, and the effect of 1-propanol on bubble temperature, secondary radical formation, and so forth.

Scheme S1. The synthetic strategy for AuNPs@PMo$_{12}$ showing the preparation and reduction of PMo$_{12}$ under ultrasonic irradiation (a: PMo$_{12}$O$_{40}^{3-}$ solution, b: reduced PMo$_{12}$O$_{40}^{4+}$, c: AuNP@PMo$_{12}$) (synthesis condition: intensity= 26 Wcm$^{-2}$, T=37±1°C, Au:POM ratio (1:2)).
Characterization of AuNPs@PMo₁₂ nano-hybrid[32]

The XRD pattern of AuNPs@PMo₁₂ nano-hybrids is shown in Figure S1a. It indicates the face-centered cubic (fcc) structure of the Au crystals with the peaks in 38.1°, 44.3°, 64.5° and 77.5° correspond to the (111), (200), (220) and (311) crystal faces of metallic gold nanoparticle according to the JCPDS-04-0784.

FTIR spectroscopy was conducted on the surface study of AuNPs@PMo₁₂ nano-hybrids. Figure S1b indicates the infrared transmission spectra of phosphomolybdic acid PMo₁₂ and AuNPs@PMo₁₂. The peaks of PMo₁₂ at 1060 cm⁻¹ and 966 cm⁻¹ related to ν_as (P-O) and ν_as (Mo-O₄), which appear in AuNPs@PMo₁₂ at 1080 cm⁻¹ with a shoulder in 1033 cm⁻¹. This blue shift (shorter wavelength) confirms the interaction between PMo₁₂ and the surface of Au. The peak at 876 cm⁻¹ is attributed to the vibration of (Mo–O₆–Mo) which disappears in the AuNPs@PMo₁₂ sample. The peak around 796 cm⁻¹ is attributed to the vibration of (Mo–O₅–Mo) and shows a red-shift (increase wavelength) in the AuNPs@PMo₁₂ at 619 cm⁻¹. These results proposed interaction between PMo₁₂ and Au via (Mo–O₅–Mo) group. Table S1 shows the most important IR vibrations for PMo₁₂ and AuNPs@PMo₁₂ nano-hybrid.

DLS analysis was performed to measure the size distribution and hydrodynamic diameter of AuNPs@PMo₁₂ in water. The average size of AuNPs@PMo₁₂ is found to be 19.8 ± 1.7 nm at 37±1°C. The polydispersity index (PDI) was also measured, and it was obtained about 0.17 indicating monodispersed AuNPs. Zeta potential analysis was recorded to find the surface charge of an as-prepared AuNPs@PMo₁₂ colloidal solution. The ζ-potential analysis is crucial for the investigation of the dispersion behavior of the nanoparticles in liquid media. The higher the ζ potential leads to a more stable suspension. The average ζ potential of suspensions was obtained -2 mV. According to these results, the surface of nanoparticles negatively charged due to the
coating of PMo$_{12}$ polyanions on the Au surface. Also, the results show high colloidal stability (for several months), and no precipitation was observed during this time.

The electrostatic repulsions between charged particles cause more dispersed particles and less coagulation. In this study, the ζ-potential of the as-prepared nanohybrid surface is not sufficiently high to obtain high colloidal stability of nanoparticles in the medium. It is assumed that the hydrogen bonding between OH groups of water molecules (in water media) and adsorbed PMo$_{12}$ molecules onto the surface of AuNPs is responsible for the observed stability. Thus, the higher stability of dispersion (AuNPs @PMo$_{12}$) might be due to the solvation of adsorbed PMo$_{12}$ molecules onto the surface of AuNPs.

The presence of PMo$_{12}$ onto the surface of AuNPs was further confirmed by electrochemical measurements. It should be mentioned that PMo$_{12}$ can undergo a multi-electron reversible redox process without losing its integrity. The cyclic voltammograms (CVs) of PMo$_{12}$ and AuNPs@PMo$_{12}$ in 50% v/v water: DMF solution containing 1 M H$_2$SO$_4$ as an electrolyte are shown in Figure S1c. By comparing the CVs of PMo$_{12}$ and AuNPs@PMo$_{12}$ (Figure S1c), it can be deduced that voltammetric peaks at ca. -0.02, 0.18 and 0.4 V (vs. Ag/AgCl) in AuNPs@PMo$_{12}$ electrode should be originated from the characteristic redox behavior of PMo$_{12}$ and verified the adsorbed PMo$_{12}$ onto AuNPs surface during the synthesis of the AuNPs@PMo$_{12}$ (Figure S1c).

Scanning electron microscopy (SEM) and high angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images were performed to study the morphology of AuNPs@PMo$_{12}$ (Figure S1d). Results exhibit truncated tetrahedral and hexagonal shapes with rod-like structure. The average size of AuNPs@PMo$_{12}$ nanoparticles was measured by about 24 nm. Also, the average length of the rod-like structure was measured around 85 nm.
It is proposed that ultrasonic waves enhance the mass transfer and diffusion of precursors from the bulk into the diffusion sphere, so the facets along the more active axis should be rapidly growing and led to the generation of the faceted and 1D structure under the acoustic wave. Another key factor is the diffusion sphere thickness, which is decreased by ultrasonic irradiation, and this phenomenon led to an increase in flux diffusion and rapid growth along the active axis.

The chemical composition analysis of AuNPs@PMo$_{12}$ has been carried out using EDS analysis (Figures S1e). A cross-sectional EDS line scan profiles extracted from the HAADF-STEM image confirm the coverage of Mo elements in the immediate area surrounding the AuNPs, suggesting they are from the PMo$_{12}$ structure. The Cu peaks are the signal detected from the SEM grid.
Figure S1. a) XRD pattern of AuNPs@PMo$_{12}$, b) FT-IR spectra of PMo$_{12}$ and AuNPs@PMo$_{12}$, c) Cyclic voltammograms (CVs) of AuNP@PMo$_{12}$ and pure PMo$_{12}$ in 50% (v/v) water–DMF solution containing 1 M H$_2$SO$_4$, d) HAADF-STEM images (average diameter of 24 nm ± 9 nm from about ~190 NPs), and e) EDS analysis and corresponding HAADF-STEM image (yellow part) of AuNP@ PMo$_{12}$ nano-hybrid (synthesis condition: T; 37±1°C).
**Table S2.** The most important IR vibrations for PMo$_{12}$ and AuNPs@PMo$_{12}$ nano-hybrid.

| Band assignment | Observed band (cm$^{-1}$) AuNPs@PMo$_{12}$ | Observed band (cm$^{-1}$) PMo$_{12}$ |
|-----------------|------------------------------------------|---------------------------------------|
| $\nu$ (P-O)     | 1080                                     | 1060                                  |
| $\nu$ (Mo-Od)   | 1033                                     | 966                                   |
| $\nu$ (Mo–Ob–Mo)| ---                                      | 876                                   |
| $\nu$ (Mo–Oc–Mo)| 619                                      | 796                                   |
| $\delta$ H2O    | 1660                                     | 1600                                  |
| $\nu$ OH        | 3430                                     | 3400                                  |
Fig. S2. Oxidation pathway of 3,5,3',5'-tetramethylbenzidine (TMB) [49]
**Fig. S3:** DLS characterization of a) AuNPs@PMo$_{12}$ nanohybrids, b) AuNPs@PMo$_{12}$ nanohybrids + AP (150 mg/L), and c) AuNPs@PMo$_{12}$ nanohybrids + AP (150 mg/L) + TMB (0.7 mM).