A magnetic internal heating single-precursor approach was exploited to fabricate higher quality Prussian blue nanoparticles (PBNPs) with excellent crystallinity, dispersibility and uniformity. Furthermore, the magnetic properties and MRI contrast effect were improved. Subsequently, significantly increased nanoenzyme activity has been demonstrated.

Prussian blue nanoparticles (PBNPs) have been widely used for biomedical imaging, biosensors, catalysis, photothermal therapy and drug delivery.1–3 Therefore, PBNPs have become a hot material for researchers. In particular, the discovery of the nanoenzyme-like activity of PBNPs, such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), further attracts extensive research interest.4,5 However, to date, there have been few reports on improving the nanozyme activity of PBNPs. Besides, PBNPs can be used as a T₁ weighted contrast agent for MRI diagnosis in vivo because of the presence of iron (iii) with a high-spin electron configuration.6,7

It is well-known that the nanoparticles’ structural parameters, such as size, crystallinity, dispersion, and uniformity, are crucial for their function, which is significantly related with the preparation methods. Generally, the formation of nanoparticles mainly includes two stages: nucleation and growth. The thermal energy of the reaction system and supersaturation of the reactants determine the nucleation and growth rate during the formation of the nanoparticles, which subsequently affects their degree of crystallization, dispersion and uniformity.8 Therefore, controlling the temperature of the reaction process and the concentration of the reactants may significantly affect the performance of the nanoparticles. However, the traditional aqueous synthesis of PBNPs mainly utilizes an exogenous heating method. This may cause the uneven heating of the reaction system during the nucleation stage, which further results in the nonuniform supersaturation of the reactants.9,10 Exogenous heating may also cause poor crystallization and uniformity by influencing the crystal growth rate of PBNPs. To acquire higher quality PBNPs, it is critical to solve the uneven thermal effect.

Inspired by the external field-assisted preparation methods, such as microwaves and ultrasound, in recent years,11 we have attempted to explore an alternating-current magnetic field (AMF)-assisted single precursor synthetic technique to fabricate PBNPs in this study (Scheme 1). During the process, the single precursor [Fe(CN)₆]³⁻ can slowly release Fe³⁺ in acidic solution, which will be further reduced to Fe²⁺ by polyvinylpyrrolidone (PVP). The formed Fe²⁺ can then immediately react with the precursor to generate PBNPs in situ.12 These initially formed nanoparticles are capable of uniformly heating the area around the nanoparticles under the action of an AMF, which promotes further slow growth of the initial nanoparticles or the generation of new nanoparticles. Under these countless microdomain-reactions, the finally obtained PBNPs exhibit good dispersion, uniformity and crystallinity.

Scheme 1 A schematic illustration of the fabrication of PBNPs assisted by an alternating-current magnetic field.
Compared to the traditional external heating synthesis method, PBNPs prepared by this method exhibit outstanding catalytic performance and MRI contrast efficiency.

Generally, the size and crystallinity are critical factors to the properties of the nanomaterial. TEM images (Fig. 1a) indicated that both the PBNPs prepared by the magnetic internal heating synthesis strategy (PBNPs-IH) and PBNPs synthesized by the conventional external heating method (PBNPs-EH) were cubic nanoparticles with an average diameter of 241 ± 12 nm and 231 ± 10 nm, respectively. There was no significant difference in size and morphology between the two samples. However, high-resolution transmission electron microscopy (HRTEM) images (Fig. 1b) show that it seems that the lattice fringes and crystalline boundaries of the PBNPs-IH are clearer than those of the PBNPs-EH. Moreover, the selected area electron diffraction (SAED) of the PBNPs-IH displayed higher intensity diffraction spots than those of the PBNPs-EH. According to these results, it is speculated that the crystallization of the PBNPs-IH is improved. X-ray diffraction (XRD) was used to further verify the crystal structure and degree of crystallization of the PBNPs. According to Fig. 1c, the two samples displayed similar diffraction 2θ angles, which could be indexed as the PB cubic space group Fm3m. Interestingly, the diffraction peak half width of the PBNPs-IH (0.23°) was obviously less than that of the PBNPs-EH (0.33°), which further implied that the PBNPs-IH had a higher degree of crystallization compared to the PBNPs-EH.

In addition to the crystallization, the dispersibility and uniformity can also significantly impact the performance of the nanomaterial. According to the statistical analysis of the nanoparticles’ size and distribution (Fig. 1d), the dispersion uniformity of the PBNPs-IH (209 ± 32 nm) might be better than that of the PBNPs-EH (199 ± 43 nm). The polydispersity index (PDI) of the PBNPs-IH and PBNPs-EH obtained by dynamic light scattering (DLS) (Fig. S1a, ESI†) were 0.015 and 0.045. The sizes of the PBNPs-IH and PBNPs-EH measured by DLS were 283 ± 5 nm and 281 ± 7 nm, respectively. These results demonstrated that the magnetic internal heating approach could improve the dispersion uniformity of the PBNPs. This might be attributed to the uniform thermal effect generated by the magnetic nanoparticles under an AMF. Detailed information for both samples was provided by thermogravimetric analysis (Fig. S1b, ESI†), Fourier transform infrared spectra (FTIR) (Fig. S1c, ESI†) and ultraviolet-visible (UV-Vis) spectra (Fig. S1d, ESI†). All of these characterizations confirm that the dimensions, morphology, structure, composition and content of PVP coated on the surface of the PBNPs-IH and PBNPs-EH show no great difference.

Magnetism is an important parameter of PBNPs in biomedical applications such as magnetic resonance imaging (MRI) and magnetic separation. The results indicated that both samples displayed ferromagnetic behavior, rather than superparamagnetic properties (Fig. 2a). It is noteworthy that

![Fig. 1](image1.png)

*Fig. 1*  (a) TEM images of the PBNPs; (b) HRTEM images and the corresponding selected-area electron diffraction (SAED) patterns of the PBNPs; (c) XRD profiles of the PBNPs; (d) SEM images of the PBNPs and the statistical distribution map of the nanoparticles’ diameter.

![Fig. 2](image2.png)

*Fig. 2*  (a) Field dependence of the magnetization of the PBNPs-IH and PBNPs-EH; (b) The T₁ relaxation rates of the PBNPs-IH and PBNPs-EH; (c) T₁-Weighted MR images of the PBNPs. The absorption changes with time under the catalysis of PBNPs-IH and PBNPs-EH: (d) POD-like and (e) CAT-like activity of the PBNPs-IH and PBNPs-EH.
the magnetization process was obviously different between the PBNPs-IH and PBNPs-EH. The saturation magnetization intensity of the PBNPs-IH was 64.2 emu g$^{-1}$ PB, which was slightly higher than that of the PBNPs-EH (60.1 emu g$^{-1}$ PB). The enhancement of the magnetic performance could be attributed to the improved crystallization. Furthermore, the hysteresis shapes of both samples were different. The residual magnetization ($M_r$) of the PBNPs-IH (2.5 emu g$^{-1}$ PB) was much lower than that of the PBNPs-EH (6.2 emu g$^{-1}$ PB). It has been reported that the presence of an interior hollow cavity could increase the $M_r$ value of PBNPs because of the incomplete alignment of local magnetic moments (spin) along the external magnetic field.$^{15}$ According to this principle, we could further speculate that PBNPs with higher crystallinity exhibited lower $M_r$ value than PBNPs with more crystal defects. This could be used to explain the above phenomenon, where the $M_r$ value of the PBNPs-IH was smaller than that of the PBNPs-EH. The longitudinal relativities ($r_l$) were determined under serial dilutions in vitro (Fig. 2b and c). The $r_l$ values of the PBNPs-IH and PBNPs-EH were calculated to be 1.47 mM$^{-1}$ s$^{-1}$ and 1.25 mM$^{-1}$ s$^{-1}$, respectively. These results illustrated that the PBNPs-IH maintained a better $T_1$-weighted MRI contrast effect than the PBNPs-EH.

As shown in our previous research,$^7$ PBNPs can exhibit a similar activity to natural enzymes. In this study, we mainly evaluated the POD-like and CAT-like activities of both samples due to their wide applications in the biomedical field. The catalytic curve (Fig. 2d) showed that the reaction rate of the PBNPs-IH [(4.76 ± 0.29) × 10$^{-8}$ M s$^{-1}$] was much larger than that of the PBNPs-EH [(3.09 ± 0.24) × 10$^{-8}$ M s$^{-1}$] within 15 min, which implied that the PBNPs-IH with improved crystallinity, dispersibility and uniformity had a higher POD-like catalytic activity. Statistical analysis results based on six batch samples showed that the difference in reaction rates between the PBNPs-IH and PBNPs-EH was very significant ($P < 0.05$). Generally, the crystallinity gradually increases with the prolongation of the ripening time. To further confirm the effect of crystallinity on the catalytic activity, the POD-like catalytic activity of three samples with a ripening time of 3.5, 10 and 20 h was investigated (Fig. S2, ESI†). The results showed that the sample with an aging time of 20 h exhibited a higher catalytic rate than the other two samples. For CAT-like catalytic activity (Fig. 2e), the rate of oxygen generated by the PBNPs-IH-catalyzed system [(3.44 ± 0.11) × 10$^{-5}$ M s$^{-1}$] was higher than that of the PBNPs-EH [(2.66 ± 0.21) × 10$^{-5}$ M s$^{-1}$] within 1 min, which indicated that the PBNPs-IH had an improved CAT-like catalytic performance compared to the PBNPs-EH. Statistical analysis results showed that the difference in reaction rates between the PBNPs-IH and PBNPs-EH was very significant ($P < 0.05$). According to reports, the nanoenzyme activities of PBNPs are likely attributed to efficient electron transporters. Therefore, we conjectured that PBNPs with good crystallinity might have more efficient electron transport efficiency, thus exhibiting higher catalytic performance.

To reveal the growth process of the PBNPs under an AMF, time-dependent TEM images were obtained. Fig. 3a and b show the images of the PBNPs prepared using 6.5 A and 11.5 A output current intensity under an AMF, respectively. In Fig. 3a, the cube-shaped nanoparticles exhibited a relatively smooth surface and clear edges after 1.5 h of reaction. It could be found that these nanoparticles had a good crystal structure, which contained clear lattice fringes from HRTEM. The corresponding SAED pattern showed that these nanoparticles had a single crystal-like structure of clear diffraction spots. When the reaction time was prolonged to 5 h, the formed nanoparticles showed no significant change in morphology. However, the size distribution of the products was wider compared to the reaction for 1.5 h. A large number of smaller PBNPs were present according to the TEM images. The HRTEM images and SAED pattern showed that these smaller nanoparticles were crystalline, which was consistent with the crystal structure of the larger nanoparticles. Based on the above experimental results, we speculated that the growth process might undergo a classical crystallization process containing nucleation and growth stages for the PBNPs-IH at 6.5 A. The size of the nanoparticles was increased continuously with the reaction time.

In Fig. 3b, the TEM images show that there were a large number of extremely small PBNPs with diameters around 5 nm after 0.5 h reaction. These nanoparticles could further assemble to form the cube-shaped nanoparticles according to the images. When the reaction time increases to 5 h, the morphology and size of the nanoparticles are diverse. The smaller nanoparticles tended to be spherical, while the larger nanoparticles were cubic with clear edges. The crystallinity of the
nanoparticles with different sizes was also investigated by SAED. The results demonstrated that there were almost no diffraction spots for the smaller nanoparticles, which implied a weaker degree of crystallization. For the medium-sized nanoparticles, the numbers of diffraction spots were slightly increased, which suggested an improved crystallinity. The larger nanoparticles with single crystal-like periodic patterns exhibited a higher degree of crystallization. However, these nanoparticles still had a rough surface and a large number of lattice defects, which intimated that the crystal structure could be referred to as a mesocrystal. Thus, it could be concluded that the formation mechanism of the PBNPs followed a typical non-classical crystallization process under these conditions. At the early stage of the reaction, explosive nucleation is preferred, leading to easy formation of small nanocrystals. Then, large nanoparticles emerged on the oriented assembly of the small nanoparticles. Finally, nanoparticles with good crystallinity are formed by the self-driving fusion process at the interior of the nanoparticles with the extension of the reaction time (Fig. S3, ESI†).

On the basis of the above time-dependent TEM images, a growth mechanism of PBNPs-IH was proposed as displayed in Fig. 3c. At the initial stage, the nuclei were easily formed through early nucleation and growth processes because of the extremely low solubility product \(K_{sp} = 1 \times 10^{-41}\) of PB. When the current intensity was 6.5 A, these small nanocrystals could continuously enlarge through the growth of the corresponding ions or molecules on the nuclei surfaces. Such a crystallization process usually resulted in nanoparticles with a good crystallinity and smooth surface in all stages of crystallization. When the current intensity was increased to 11.5 A, these small nanoparticles were more susceptible to directional aggregation into a superstructure with more crystal defects, a rough surface and a single crystal-like SAED pattern. Nanoparticles formed by this crystallization process are generally referred to as mesocrystals, and subsequently transformed into real single crystals by fusion at the interior of the nanoparticles. At this moment, the ragged surfaces became glossy, and the porous nanocrystals converted into dense nanocrystals.\(^{16,17}\)

In fact, the nucleation rate played a major and direct role in the crystallization process.\(^{18}\) When the current intensity was 6.5 A, the temperature of the reaction system was 55 °C (Fig. S4, ESI†), leading to the slow degradation of the [Fe(CN)\(_6\)]\(^{3−}\) ions, which strongly influenced the nucleation rate at commencement. On the other hand, the weaker magnetic field strength produced by the lower current intensity might be detrimental to the directional assembly of the small nuclei. When the current intensity was 11.5 A, the temperature of the reaction system was increased to 80 °C, which accelerated the generation of Fe\(^{2+}\) to result in an explosive nucleation. The generated large numbers of nuclei implied that their growth consumed more Fe\(^{3+}\), which could discourage the further growth of nuclei. In order to decrease the high surface energy, these small nuclei could undergo a mesoscale self-assembly in a highly ordered way to form a superstructure.\(^{19,20}\) Moreover, due to the inherent magnetic properties of PB, the assembly process could be further ordered with the assistance of a higher magnetic field strength generated by the enhancement of the current intensity. This was also one of the reasons why the PBNPs-IH had higher crystallinity than the PBNPs-EH.

**Conclusions**

In summary, a novel magnetic internal heating synthetic strategy was developed, which improved the performance of PBNPs. The prepared PBNPs not only maintain good crystallinity, but also exhibit improved dispersion and uniformity. More importantly, a higher performance of magnetism and nanocatalysis of the PBNPs was obtained compared to the ambient heating preparation method. The results also indicated that an AMF might play a key role in the crystallization process. The electric field induced by the AMF possibly ordered the mesoscale self-assembly process of the PBNPs, which could improve the crystallinity. We believe that the work to explore the magnetic internal heating synthesis strategy to improve the quality and performance of PBNPs is highly important, and could promote the clinical application of PBNPs.

**Conflicts of interest**

There are no conflicts to declare.

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**Notes and references**


