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Construction of ratiometric Si-Mn:ZnSe nanoparticles for the immunoassay of SARS-CoV-2 spike protein

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ABSTRACT

The continuing global spread of Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection, has led to an unprecedented global health crisis. Effective and affordable methods are needed to diagnose SARS-CoV-2 infection. In this work, a ratiometric fluorescence probe, Si-Mn:ZnSe nanoparticles, was constructed through the electrostatic interaction between Si dots and Mn:ZnSe QDs, and the fluorescence of Mn:ZnSe QDs has a specific response to H$_2$O$_2$. An immunocomplex was formed by the recognition of capture antibody/spike (S) protein/spike neutralizing antibody/biotinylated second antibody/streptavidin/biotinylated catalase (CAT). In the presence of S protein, CAT effectively catalyzed the decomposition of H$_2$O$_2$ in the system, and the fluorescence of Mn:ZnSe QDs was not specifically quenched. Based on this principle, a ratiometric immunoassay of SARS-CoV-2 S protein was established. The sensitivity of the proposed ELISA method was comparable to that of the commercial kit. In addition, this method can effectively distinguish the pseudo-SARS-CoV-2 virus and other pseudovirus. Therefore, this method provided a reliable and potential direction for diagnosing SARS-CoV-2 infection.

1. Introduction

Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly worldwide, which leads to an increase in infection death and seriously disrupts normal social activities [1]. A variety of methods for diagnosing SARS-CoV-2 infection have been implemented by the detection of viral nucleic acids, viral antigens, or host immune response (antibody) [2,3]. Each target detection has its own advantages and utilization window. For example, nucleic acid detection, which has become the gold standard for diagnosis and guiding clinical decisions in China [4], displays high sensitivity and specificity, containing RT-PCR [5], LAMP [3], SHERLOCK [6]. However, nucleic acid detection is still facing many inconveniences, such as time-consuming and professional technicians [7,8]. Antibody/antigen detection using immunoassay is simple and rapid, while antibody detection has the problems of long detection window period and low accuracy [8]. Antigen detection has been widely promoted for SARS-CoV-2 positive screening in Europe [9] and the United States [10].

SARS-CoV-2 spike glycoprotein (S protein), the crucial component in infection process, is composed of S1 and S2 subunits [11]. S1 subunit, containing receptor binding domain (RBD), can recognize and bind to the receptors on host cell surface, and S2 subunit promotes the membrane fusion of virus and host cell after the binding of RBD and host cell surface receptors [11,12]. Therefore, rapid detection of S protein is essential to control the spread of the epidemic [13,14].

Enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for viral antigen detection, and it is simple and sensitive [15,16]. Colorimetric and fluorescence methods are often used as the signal model of ELISA [17,18]. The sensitivity of fluorescence method is 10–100 times than that of colorimetry. Quantum dots (QDs) have wide absorption spectrum, large Stokes shift, high fluorescence intensity, and low biological toxicity [17,19,20]. They are ideal fluorescent probes and can be used in the field of bioanalytical chemistry [17]. Fluorescence detection mostly adopts single-emission peak probe, which is affected by environmental variation, probe concentration change, and instrumental
fluctuation [21,22]. Compared with single-emission fluorescence analysis, ratiometric fluorescence method adds internal standard calibration in the detection system, which has the advantages of high stability and low detection background [21,23,24].

In this work, Si dots and Mn:ZnSe QDs were prepared. Si dots were prepared by hydrothermal method with APTES as Si source and sodium citrate as reducing agent. Mn:ZnSe QDs were prepared by water-bath method through Mn-doping into ZnSe core. As shown in Scheme 1, Si-Mn:ZnSe nanoparticles (NPs) were constructed as ratiometric fluorescent probes, which were self-assembled by Si dots and Mn:ZnSe QDs through electrostatic interaction. H$_2$O$_2$ can damage -SH on surface of Mn:ZnSe QDs and quench their fluorescence, but it had no effect on the fluorescence of Si dots. The ratiometric fluorescence response of H$_2$O$_2$ to Si-Mn:ZnSe NPs was established. Catalase (CAT), which was labeled on the immunocomplex, can effectively decompose H$_2$O$_2$. The fluorescence intensity ratio (F$_{610}$/F$_{440}$) was positively correlated with the concentration of S protein. Therefore, the ratiometric fluorescence method can be applied for detecting SARS-CoV-2 S protein through ELISA.

2. Experiments

2.1. Reagents and instruments

MnAC$_2$$\cdot$2H$_2$O, ZnAC$_2$$\cdot$2H$_2$O, selenium powder, NaBH$_4$, 3-aminopropyl triethoxysilane (APTES), dimethyl sulfoxide (DMSO), NHS-dPEG$_4$-biotin, catalase (CAT) from bovine liver and L-glutathione were purchased from Sigma-Aldrich Co., Ltd. (USA). SARS-CoV-2 S protein detecting antibody pairs, biotinylated goat anti-mouse IgG, SARS-CoV-2 spike RBD recombinant protein, and SARS-CoV-2 S protein detection ELISA Kit (KIT40591) were obtained from Sino Biological Co., Ltd. (Beijing, China). Above biomaterials were used with the recommended concentrations in the manufacturer’s protocols.

TEM images of nanoparticles were obtained on the JEM 2100 microscope (JEOL Ltd.). XRD spectra and XPS were obtained using the Ultima IV XRD system (Rigaku) and Nexsa (Thermo Scientific), respectively. UV–vis absorption spectra were obtained using Cary 3500 (Agilent Technologies, Inc.). FT-IR spectra were recorded using the Nicolet iS20 (Thermo Scientific). Fluorescence spectra were recorded using the F-7100 (Hitachi High-Tech Co., Ltd.). Fluorescence lifetime were measured using FLS980 Fluorescence Spectrofluorometer (Edinburgh Instruments).
The Si dots were prepared according to the previous reference and some changes were made [25]. 1 mL APTES (Si source) and 0.184 g sodium citrate (reducing agent) were dissolved in 4 mL water, respectively. The above solution was stirred for 10 min and transferred to the tetrafluoroethylene reactor at 200 °C for 2 h. The obtained Si dots were purified with acetonitrile. The Si dots solution was mixed and purified with acetonitrile at a volume of 1:3 for 3 times, and vacuum-dried at 60 °C for 12 h. The Si dots solid was stored at 4 °C for further use.

The Mn:ZnSe QDs preparation is described below and some changes were made [26]. To prepare Zn-GSH solution, 85 mg ZnAC·2 H2O and 300 mg GSH were dissolved in 40 mL water and its pH was adjusted to 11.0 with 3 mol/L NaOH. The Se2- solution was obtained by dissolving 20 mg Se powder with 20 mg NaBH4 in 1 mL water and reacting until the solution was colorless and transparent. 29.3 mg MnAC·2 H2O was dissolved in 1 mL water to get 0.1 mol/L Mn2+ solution. 10 μL Mn2+ (0.1 mol/L), 10 μL Se2- (20 mg/mL) and 200 μL Zn-GSH were firstly mixed and heated at 95 °C for 10 min to prepare MnZnSe core, and 200 μL Zn-GSH was added and heated at 85 °C for 30 min for ZnSe shell growth to get Mn:ZnSe QDs.

### 2.2. Synthesis of nanoparticles

The Si dots were stirred for 10 min and transferred to the tetrafluoroethylene reactor at 200 °C for 2 h. The obtained Si dots were purified with acetonitrile. The Si dots solution was mixed and purified with acetonitrile at a volume of 1:3 for 3 times, and vacuum-dried at 60 °C for 12 h. The Si dots solid was stored at 4 °C for further use.

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### 2.3. Detection of S protein

First, SARS-CoV-2 spike protein antibody, namely capture antibody (100 ng/mL), was coated onto microporous plates with 100 μL/well at 4 °C overnight. Next, 2.5 % (w/t) skin milk with 300 μL/well was coated into the plate at 37 °C for 2 h to block the well. Then, 100 μL different concentrations of S protein was incubated at 37 °C for 1 h. Afterwards, SARS-CoV-2 S protein neutralized antibody, used as detecting antibody (100 μL/well) to bind with the captured S protein, was incubated at 37 °C for 1 h to form sandwich immune complex. The sandwich immune complex was bound with a biotinylated secondary antibody and incubated at 37 °C for 1 h. After washing the plate, SA (5 μg/mL) and biotinylated CAT were bound with biotinylated secondary antibody. 100 μmol/L H2O2 was added and incubated at 30 °C for 1.5 h. The immobilized CAT could fully decompose H2O2. Finally, the Si-Mn:ZnSe NPs were added and incubated at 30 °C for 1 h. The fluorescence spectra were collected with excitation wavelength of 350 nm. In this section, the washing buffer containing 0.01 mol/L PBS and 0.2 % Tween-20 was used to wash plate three times after each step. The specificity of SARS-CoV-2 S protein detection was investigated. The concentration of both SARS-CoV-2 nucleoprotein and BSA was 100 ng/mL, and the S protein concentration was 10 ng/mL.

### 2.4. Analysis of virus samples

First, the SARS-CoV-2 S plasmid (10 μg) and pNL4-3-KFS (ΔEnv) (10 μg) were co-transfected into 293 T cells to construct a SARS-CoV-2 pseudovirus. Further, pSV-G (10 μg) and pNL4-3-KFS (ΔEnv) (10 μg) were co-transfected into 293 T cells to construct VSV-G pseudovirus (negative control) [27]. A PEG20000 solution (20 %, w/t) and the pseudoviruses were mixed at a volume ratio of 1:1 and incubated at 4 °C overnight. The mixture was centrifuged at 9000 rpm for 20 min to purify pseudovirus, which was resuspended in 1 mL Opti-MEM for subsequent experiments. The SARS-CoV-2 S protein in the samples was detected by our newly developed method and compared with a commercial kit.

### 3. Results and discussion

#### 3.1. Characterisation of Si-Mn:ZnSe NPs

Si-Mn:ZnSe NPs were prepared by the self-assembly of Si dots and Mn:ZnSe QDs. The Si dots were prepared according to previous reports [25], and the Mn:ZnSe QDs were prepared through Mn-doping in ZnSe core. The Mn:ZnSe QDs preparation conditions were optimized, as shown in Fig. S1. When the concentration of Mn2+ and Se2- were 1 mmol/L, and the reaction temperature was 80 °C, the Mn:ZnSe QDs showed highest fluorescence intensity. FT-IR spectra were used to characterize the surface groups of Mn:ZnSe QDs. The elemental composition and crystal structure of Mn:ZnSe QDs were investigated by XPS and XRD, respectively. As shown in Fig. S3, Zn2p, Mn2p, S2p, and Sc3d peaks were observed, indicating the presence of Zn, Mn, S, and Se elements in Mn:ZnSe QDs and the relative contents are basically consistent with reactant. The XRD spectrum showed sharp characteristic diffraction peaks at 28, 45, and 53 degrees, attributing to the formation of Mn2S and MnSe. The elemental composition and crystal structure of Mn:ZnSe QDs were investigated by XPS and XRD, respectively.

The optical properties of the prepared Si-Mn:ZnSe NPs were examined based on UV–vis absorption and fluorescence spectrophotometer. As shown in Fig. 1A, Si-Mn:ZnSe NPs showed two absorbance peak at 270 nm and 370 nm, which were attributed to Mn:ZnSe QDs and Si dots, respectively. In Fig. 1B, the emission wavelengths of Si dots and Mn:ZnSe QDs were located at 440 nm and 610 nm, respectively. Si-Mn:ZnSe NPs owned these two emission peaks, however, the intensity of the emission peak at 610 nm was lower than that observed in Mn:ZnSe QDs alone, which may be due to the electrostatic interaction between Si dots and Mn:ZnSe QDs. From Fig. S5A, the zeta potential of Si dots and Mn:ZnSe QDs were +5.14 eV.
and – 35.2 eV, respectively, and the zeta potential of Si-Mn:ZnSe QDs were – 21.16 eV. The zeta potential decrease in Si dots and zeta potential increase in Mn:ZnSe QDs after interaction further indicated the electrostatic interaction between Si dots and Mn:ZnSe QDs.

The surface morphology and particle size distribution of Si dots, Mn:ZnSe QDs, and Si-Mn:ZnSe NPs were also characterized (Fig. 2). The Si dots and Mn:ZnSe QDs were found to be nearly spherical particles with a uniform distribution, and the sizes of Si dots and Mn:ZnSe QDs were 3.0 nm and 2.7 nm, respectively (Fig. 2A-2D). Compared with them, Si-Mn:ZnSe NPs were significantly larger, with an average size of 31.8 nm (Fig. 2E and 2F, n > 100), likely due to the interaction and aggregation of the two sets of particles. The purity of Si-Mn:ZnSe NPs was further demonstrated by the hydrodynamic size distribution, and the hydrodynamic size of Si-Mn:ZnSe NPs was about 67.49 nm with good dispersion, larger than that of Si dots (8.35 nm) and Mn:ZnSe QDs (10.69 nm) (Fig. S5B).

As shown in Fig. S6A, the fluorescence intensity of Mn:ZnSe QDs hardly changed at different pH values from 5.5 to 8.0, which might be due to the passivation and protection of ZnSe shells [26,28]. In Fig. S6B, with the increase of excitation duration, the fluorescence intensity of individual Si dots remained unchanged, and the fluorescence intensity of Mn:ZnSe QDs decreased slightly. These results indicated that the Mn:ZnSe QDs had good pH stability and photo-stability. The stability of Si-Mn:ZnSe NPs was also investigated under different pH and during continuous excitation. As shown in Fig. S7A, the fluorescence intensity ratio (F<sub>610</sub>/F<sub>440</sub>) of Si-Mn:ZnSe NPs remained stable in the range of pH 5.5–8.0. It may be due to the decrease of surface area with the increase of NPs size. Most of Si dots and Mn:ZnSe NPs located in the inside NPs and NPs were insensitive in pH of external environment, which suggesting that the probe can be applied under typical physiological conditions. From Fig. S7B, with the increase of excitation duration, the fluorescence intensity at 440 nm remained unchanged, and the fluorescence intensity at 610 nm decreased slightly. Together, the results showed that the prepared Si-Mn:ZnSe NPs had good stability after self-assembly and could be used for subsequent experiments.

![Fig. 2. TEM images and size distribution of Si dots (A and B), Mn:ZnSe QDs (C and D), and Si-Mn:ZnSe NPs (E and F), respectively.](image-url)
3.2. Ratiometric ELISA of SARS-CoV-2 S protein

The fluorescence response of H$_2$O$_2$ to Si-Mn:ZnSe NPs was investigated. The fluorescence of Si-Mn:ZnSe NPs at 610 nm was quenched significantly as the H$_2$O$_2$ concentration increased from 0 to 50 μmol/L, while the emission at 440 nm showed no obvious change (Fig. S8). Considering that ELISA was a type of heterogeneous reaction, 100 μmol/L was selected as the actual experimental concentration for H$_2$O$_2$. The mechanism via which H$_2$O$_2$ reacts with Si-Mn:ZnSe NPs was investigated. XPS was performed to analyze the Mn and S elements of Mn:ZnSe QDs before and after reaction with H$_2$O$_2$ (Fig. S9). The signals of Mn2p and S2p were weakened after the oxidation reaction. This result suggested that H$_2$O$_2$ can quench the Mn:ZnSe QDs fluorescence by oxidizing Mn$^{2+}$ and -SH. The fluorescence lifetime of Mn:ZnSe QDs hardly changed with different concentrations of H$_2$O$_2$ (Fig. S10). These results indicate that H$_2$O$_2$ causes static quenching of Mn:ZnSe QDs.

To further improve the detection sensitivity of the method, the concentration of the capture antibody and the duration time of H$_2$O$_2$ decomposition via immobilized CAT were optimized (Fig. S11). When the concentration of capture antibody increased, the signal ($F_{610}/F_{440}$) also increased, and 100 ng/mL of capture antibody was selected for subsequent experiments (Fig. S11A). When H$_2$O$_2$ was decomposed for 1 h, the fluorescence ratio was the highest (Fig. S11B). Therefore, 1 h was selected as the decomposition time for subsequent tests.

Under the optimum conditions, the linear relationship between the concentration of S protein and the fluorescence intensity ratio of Si-Mn:ZnSe NPs was obtained. As shown in Fig. 3A and 3B, the fluorescence emission of Si-Mn:ZnSe NPs at 610 nm increased with the increased S protein concentration from 0.05 ng/mL to 300 ng/mL. As shown in Fig. 3C, a good linear relationship ($R^2 = 0.9904$) between lg (S) and $\Delta (F_{610}/F_{440})$ appeared in the range of 0.05–10 ng/mL ($\gamma = 0.2318x + 0.3378$). Fig. 3D showed a good linear relationship ($R^2 = 0.9987$).
between the concentration of S protein from 5 to 50 ng/mL and $O_2$ with $y = 0.0116x + 0.4479$ with the detection limit of 0.032 ng/mL. This work provided a better sensitivity for SARS-CoV-2 S protein detection than most of previous reports, which listed in Table S1. The specificity was evaluated by detecting 100 ng/mL of N protein and BSA, the concentration of which was 10 times higher than S protein. This method had no obvious response to N protein and BSA, and had good selectivity to S protein (Fig. S12).

### 3.3. Virus sample test

The SARS-CoV-2 S protein was incorporated into a HIV pseudovirus production system to construct pseudo-SARS-CoV-2 virus. The pseudovirus were tested using the newly developed method to provide a proof-of-concept. Fig. 4 showed the comparison of the detection of S protein concentration in pseudo-SARS-CoV-2 by the proposed ELISA and the commercial kits. The concentration of S protein measured by the newly developed ELISA was similar to that obtained by the commercial kit, indicating that this method has a high accuracy. VSV-G pseudovirus was constructed to serve as the control virus. The proposed ratiometric ELISA method was used to detecte SARS-CoV-2 S protein in pseudo-SARS-CoV-2 and VSV-G pseudovirus. As shown in Fig. 5, 10 pseudo-SARS-CoV-2 samples and 10 VSV-G pseudovirus samples were examined and the results were accurate, indicating that the method has high selectivity for SARS-CoV-2 S protein detection. In addition, S protein in VSV-G pseudovirus samples was detected by commercial kit and this method respectively (Fig. S13), and the results showed that neither of the two methods could be detected. This further demonstrates that the proposed method has significant specificity for SARS-CoV-2 S protein.

### 4. Conclusion

In this work, Si dots and Mn:ZnSe QDs were prepared, and subsequently, Si-Mn:ZnSe NPs were prepared through the electrostatic interaction of Mn:ZnSe QDs and Si dots. The prepared Si-Mn:ZnSe NPs showed stability under a wide pH range and displayed good photo-stability. The fluorescence of Mn:ZnSe QDs at 610 nm was significantly quenched with the increase in $O_2$ concentration from 0 to 50 μM/L. In ELISA, CAT-linked secondary antibody/reporter antibody/S protein/capture antibody immunocomplexes were formed on microplate. The decomposition of $O_2$ by CAT caused the decrease in fluorescence quenching of Mn:ZnSe QDs. Thus, the Si-Mn:ZnSe NPs can be applied as a ratiometric fluorescent probe for immunoassay of S protein. Two linear relationships between S protein concentration $O_2$ and the fluorescence intensity ratio change were obtained in concentration ranges of 0.05–10 ng/mL and 5–50 ng/mL with the detection limit of 0.032 ng/mL. Pseudo-SARS-CoV-2 virus was constructed to serve as an actual viral sample. The results of detection with the proposed newly developed method were comparable to those of commercial kits. Therefore, this method had high accuracy and specificity, and could be a novel and useful tool for the simple diagnosis of SARS-CoV-2 infection.

### CRediT authorship contribution statement

Guobin Mao: Conceptualization, Writing – review & editing, Funding acquisition, Supervision. Yifang Li: Data curation, Formal analysis, Writing – original draft. Guoqiang Wu: Data curation. Silu Ye: Formal analysis. Shijie Cao: Investigation. Wei Zhao: Investigation. Junnan Lu: Data curation, Funding acquisition. Junbiao Dau: Funding acquisition, Supervision. Yingxin Ma: Conceptualization, Supervision, Funding acquisition, Resources, Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no competing interests.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2022.132306.

### References

[1] S.I. Mallah, O.K. Ghorab, S.A. Salmi, O.S. Abdellatif, T. Tharmaratnam, M. A. Iskandar, et al., COVID-19: breaking down a global health crisis, Ann. Clin. Microbiol Antimicrob. 20 (2021) 35, https://doi.org/10.1186/s12941-021-00438-7.

[2] E. Sheikhzadeh, S. Eissa, A. Ismail, M. Zourob, Diagnostic techniques for COVID-19 and new developments, Talanta 220 (2020), 121392, https://doi.org/10.1016/j.talanta.2020.121392.

[3] A. Eftekhari, M. Alipour, L. Chodari, S. Maleki Dizaj, M. Ardalan, M. Samiei, et al., A comprehensive review of detection methods for SARS-CoV-2, Microorganisms 9 (2021), https://doi.org/10.3390/microorganisms9020232.

[4] M. Shen, Y. Zhou, J. Ye, A.A. Abdullah Al-maskri, Y. Kang, S. Zeng, et al., Recent advances and perspectives of nucleic acid detection for coronavirus, J. Pharm. Biomed. 10 (2020) 97–101, https://doi.org/10.1016/j.jpb.2020.02.016.

[5] Y. Jiang, S. Zhang, H. Qin, S. Meng, X. Deng, H. Lin, et al., Establishment of a quantitative RT-PCR detection of SARS-CoV-2 virus, Eur. J. Med. Res. 26 (2021) 147, https://doi.org/10.1186/s40001-021-00608-5.

[6] J. Joung, A. Ladha, M. Saiot, N.-G. Kim, A.E. Woolley, M. Segel, et al., Detection of SARS-CoV-2 with SHERLOCK one-pot testing, N. Engl. J. Med. 383 (2020) 1492–1494, https://doi.org/10.1056/NEJMoa2006172.

[7] D. Li, J. Zhang, J. Li, Primer design for quantitative real-time PCR for the emerging Coronavirus COVID-19, Theranostics 10 (2020) 7150–7162, https://doi.org/10.7150/thno.47649.

[8] T. Ji, Z. Liu, G. Wang, X. Guo, S. Akbar Khan, C. Lai, et al., Detection of COVID-19: A review of the current literature and future perspectives, Biosens. Bioelectron. 166 (2020), 112455, https://doi.org/10.1016/j.bios.2020.112455.

[9] WHO/EU, Antigen-detection in the diagnosis of SARS-CoV-2 infection: interim guidance, 2021.

[10] S.M. Hahn, Coronavirus (COVID-19) Update: FDA Authorizes First Antigen Test to Help in the Rapid Detection of the Virus that Causes COVID-19 in Patients, 2020.

[11] Y. Huang, C. Yang, X.F. Xu, W. Xu, S.W. Liu, Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19, Acta Pharmacol. Sin. 41 (2020) 1141–1149, https://doi.org/10.1038/s41401-020-0485-4.

[12] L. Duan, Q. Zheng, H. Zhang, Y. Niu, Y. Lou, H. Wang, The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: implications for the design of spike-based vaccine immunogens, Front Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.576622.

[13] B.D. Kevadiya, J. Machhi, J. Herskovitz, M.D. Oleynikov, W.R. Blomberg, N. Bajwa, et al., Diagnostics for SARS-CoV-2 infections, Nat. Mater. 20 (2021) 593–605, https://doi.org/10.1038/s41563-020-00906-z.
Y. Feng, L. Liu, X. Wu, and W. Du, "Detection of trace level SARS-CoV-2 spike-protein for real-time, selective, and low-cost detection using AI and machine learning: Recent advances and future perspectives," Sensors and Actuators: B. Chemical 369 (2022), pp. 132306.

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