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Meeting-report

Direct Evidence of Phosphate Binding on Ferritin Based on Quantitative Elemental Analysis at the Single-Particle Level

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Field-effect transistor (FET) sensors are at the forefront of revolutionizing detection and analytical technologies, which hinges on the modulation of electrical conductivity through a semiconductor channel by the field effect of charged analytes, enabling the direct transduction of molecular interactions into measurable electrical signals. This unique capability allows for the real-time, label-free detection of a vast array of targets, from ions and small molecules to proteins and nucleic acids, making FET sensors attractive in various critical applications [1]. A key component of a FET sensor is the capturing probe material on the surface of a gate electrode or a semiconductor channel, which plays a role to interact and bind with the target analyte (molecules or ions) to enable specific detection [1, 2]. For instance, ferritin, an iron-storage protein that is usually found in the liver and spleen of mammals and functions in transporting oxygen [3] and involves in synthesizing phosphorus-containing molecules [4], can be uniquely harnessed as a probe material of FET sensors for highly sensitive detection of phosphate ions [5]. Beyond the detection, the highly selective interaction between ferritin and phosphates also opens an avenue for phosphorus removal and recovery from wastewater [6]. However, the underlying mechanism for interactions between ferritin and phosphate ions has been rarely explored, especially at the level of single ferritin particle.

The ultrahigh signal collection efficiency of an X-ray Perimeter Array Detector (XPAD) equipped in the Analytical PicoProbe Scanning Transmission Electron Microscope (STEM), the prototype of the ThermoFisher Spectra Ultra X, makes it a powerful tool to measure dynamical evolutions of the chemical composition of specific nanoparticles [7-9]. Herein, we conducted quantitative elemental analysis on monodispersed ferritins loaded on single-layer graphene before and after being exposed to phosphate ions. For the sample preparation, as shown in Fig.1, the aqueous dispersion of single layer graphene was dropped onto the surface of a lacey-carbon-coated copper grid. The copper grid loaded with graphene was then annealed at 400 °C in 100 mL/min N_2 environment for 1 hour to make the graphene stable. After that, ferritin particles were dispersed on the stabilized graphene and dried at 80 °C. After collecting the elemental signals on the pristine sample (referred to as Ferritin/graphene), the sample was immersed into 100 ppm K_2HPO_4 aqueous solution for 1 hour for the phosphate adsorption/binding and the resulting sample was referred to as Ferritin/graphene-HPO_4^{2-}. All results were obtained under 300 kV. For quantitative elemental analysis, empirical mode was used for background deduction and Brown-Powell mode was used for the spectra fitting.

According to the High Angle Annular Dark Field (HAADF) image (Fig. 2a) and the elemental analysis results (Fig. 2b), the pristine ferritin features an iron oxide (FeO_x) core with a diameter of 7.8 nm, surrounded by a protein shell. This shell contains carbon (C), nitrogen (N), oxygen (O), sulfur (S), and phosphorus (P) with a thickness of approximately 4 nm measured from the distribution of N. Near the ferritin particle, we identified a smaller protein particle enriched with S, suggesting that it might be peeled off from the shell of certain ferritin particles, as evidenced by Fig. 2a and 2b. Notably, the pristine ferritin contains a small amount of phosphorus, which is primarily distributed on the surface of the FeO_x core (Fig. 2b). The quantitative analysis for the Ferritin/graphene sample, as shown in Fig. 2c and 2d, reveals that the atomic ratio between P and Fe is ~7.9%. In contrast, for the sample Ferritin/graphene-HPO_4^{2-} (Fig. 2g and 2h), the atomic ratio between P and Fe increases to ~23.2%, indicating an almost threefold increase. The elemental distribution images in Fig. 2f and 2g demonstrate that the P is still located on the surface of FeO_x core, which implies the possibility that the interaction occurs mostly between the ferritin core and HPO_4^{2-}.

Based on the quantitative elemental analysis for individual particles, we have provided direct evidence of ferritin-phosphate binding that could be useful toward ferritin-based FET sensor for the detection of phosphates. Further characterizations are underway to confirm this potential interaction and to identify the binding sites and types between ferritin and phosphates, which are crucial for understanding the underlying mechanism. This work may offer a general methodology for the characterization of similar processes and shed light into the material design for novel phosphate sensors [10].

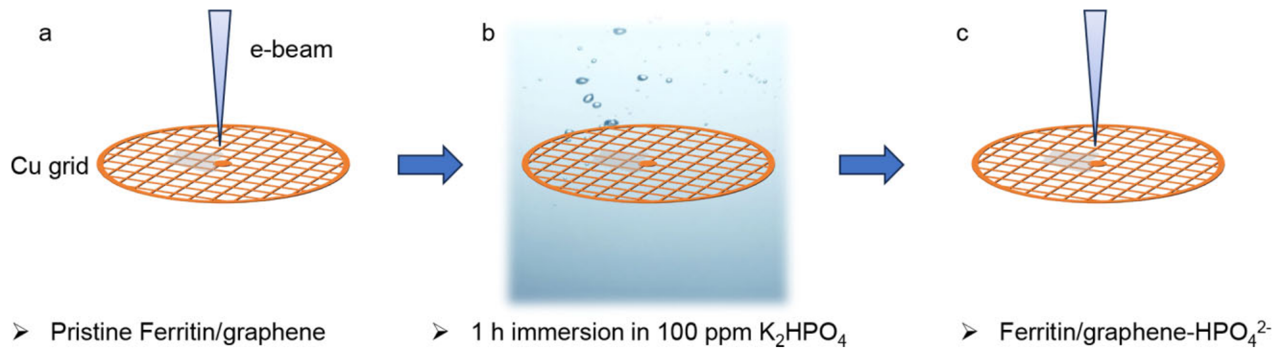

Fig. 1. STEM experimental procedure for evaluating the phosphate adsorption/binding on ferritins. (a) Observation of pristine sample Ferritin/graphene. (b) Immersion of the pristine sample in 100 ppm K_2HPO_4 aqueous solution for 1 hour. (c) Observation of the resulting sample Ferritin/graphene- HPO_4^{2-} after the interaction with HPO_4^{2-}.

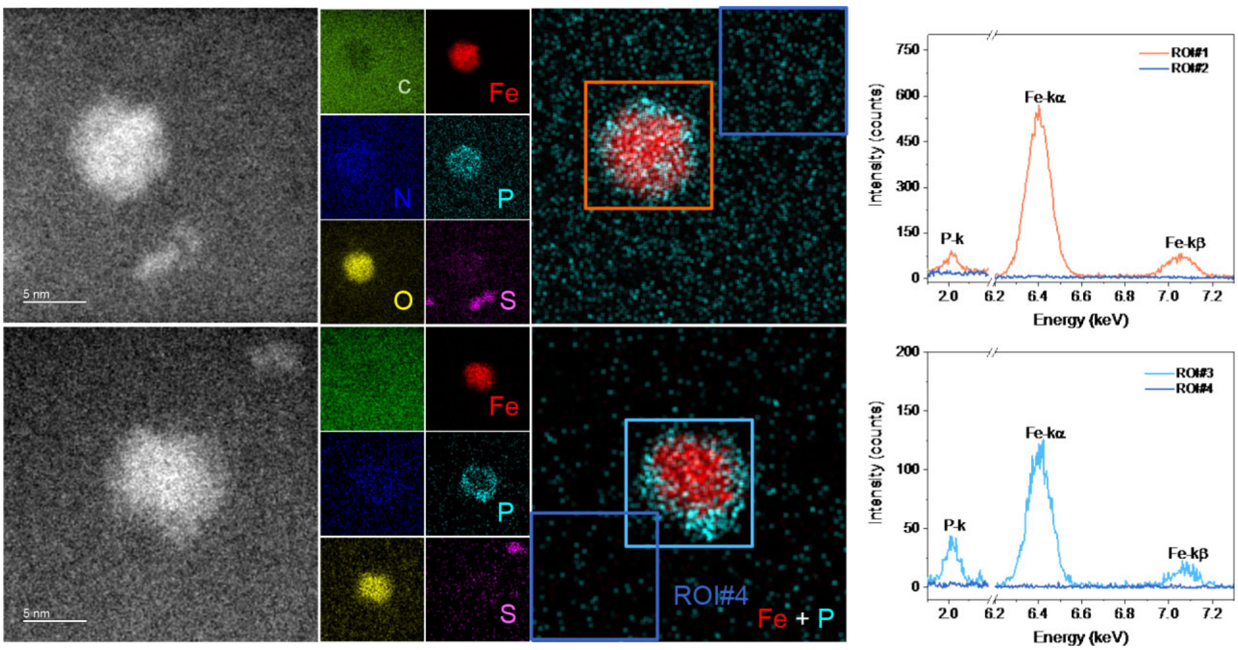

Fig. 2. STEM observation and elemental analysis of Ferritin/graphene (a-d) and Ferritin/graphene-HPO_4^{2-} (e-h). (a and e) HAADF images of individual ferritin nanoparticles. (b and f) The corresponding elemental distribution (C, Fe, N, P, O and S). (c) The overlapping distribution of Fe and P. (d and h) The spectra collected from the Region of Interest (ROI) #1 and #2, #3 and #4 from the corresponding boxes in 2c and 2g, respectively. Scale bar = 5 nm.

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