

## Adsorption and electrochemical study of cage-shape protein with GBP aptamer

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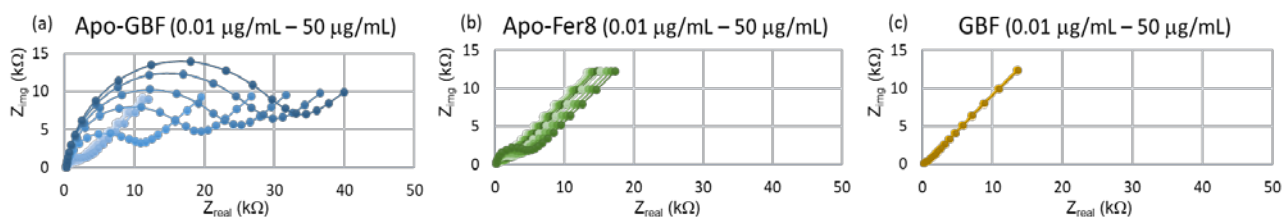
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Aptamers are the bio-material which could bind to a specific target, and attracting researchers attention with the high affinity and specificity. Some peptide aptamers can adsorb inorganic materials which have great potential to connect the organic molecules and inorganic materials. In our previous work, a small cage-shaped protein was genetically modified to have two kinds of peptide aptamers on its outer surface.<sup>1,2</sup> In this study, we expand this design to realized a biosensor with EIS. We plan to modify a cage-shaped protein which has electrode binding aptamers and one target bio-marker aptamers. It is expected that immersing an electrode into such recombinant protein solution would cover the electrode surface by the protein. As a result, the sensor electrode surface displays target-specific aptamers. To investigate the feasibility of this concept, we produced a mutant cage-shaped protein, ferritin<sup>3</sup>, which presented gold binding aptamer on its outer surface and investigated their binding affinity to the gold electrode of Electrochemical Impedance Spectroscopy (EIS). Au working electrode and Pt counter electrode were used for EIS measurement. The frequency scan width was set from 1kHz to 0.5Hz, and the applied ac voltage amplitude (Vac) was 5mV. The buffer solution was PBS pH7.4 with 1 mM K<sub>4</sub>[Fe(CN)<sub>6</sub>] and 1 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>]. The Nyquist plot shows the typical semicircle and Warburg resistance originated from the mediators' diffusion. The Rct of fer8 increased from 0.4k to 4 k Ohm in proportion to the fer8 concentration up to 50μg/mL. On the other hand, the Rct of apo-GBF increased much from 1.5k to 31k Ohm. It was shown that the GBF adsorbed on the gold surface very effectively, comparing the non-specific adsorption of fer8. We also studied how the iron core changes the Rct, and the results were very different from apo-fer8 and apo-GBF. The Rct was drastically decreasing, raging from 816 ohms to 696 ohms when GBF with the iron core. GBF with ferrihydrite core made the electrons transfer easily, where Rct increase could be detected with ease

The GBP attached to the N-terminal of subunit, which was displayed on the outer surface of the protein shows high adsorption ability. The peptides aptamer have enough force to anchor 450 kDa ferritin, nevertheless apoferritin or ferritin. Rct decreases much by iron oxide core in the GBF shell. This result indicates that the GBF adsorbed EIS electrode is suitable for biosensor because the charge transfer is highly effective and Rct is small. This work was supported by JST CREST Grant Number JPMJCR18I3, Japan.



### References:

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