Establishment of a High-throughput Screening System for the Genetic Engineering of Myoglobin Reconstituted with an Iron Corrole Complex

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Incorporation of a synthetic metal cofactor into a protein scaffold generates a new type of biocatalysts, designated as an artificial metalloenzyme. The catalytic performance of the artificial metalloenzymes can be improved by the design of a synthetic metal cofactor and the genetic engineering of a protein scaffold. Our group has previously constructed artificial peroxidase, in which myoglobin (Mb) is reconstituted with an iron corrole complex **1** (Figure 1a).[1] Since the trianionic character of the corrole ligand can stabilize the high-valent species of iron-oxo intermediates, the resulting artificial metalloenzyme (Mb-**1**) showed promising catalytic activity toward guaiacol oxidations.

To further improve its peroxidase activity based on a genetic engineering methodology such as directed evolution, we here developed a high-throughput screening (HTS) platform for the reconstituted Mb. Our group has recently reported an HTS platform for an artificial metalloenzyme which involves affinity purification system using a maltose binding protein (MBP)-tag.[2] According to this previous report, a fusion protein of Mb with MBP-tag (mbpMb) was prepared in this study. The apo-form of mbpMb was obtained in high expression level. The expressed mbpMb was subsequently purified by the affinity purification system in a 96 well format (Figure 1b). Furthermore, the reconstitution of the apo-mbpMb with an iron corrole complex **1** was also carried out. Herein, we will present development of the HTS platform for the genetic engineering of Mb-**1**.

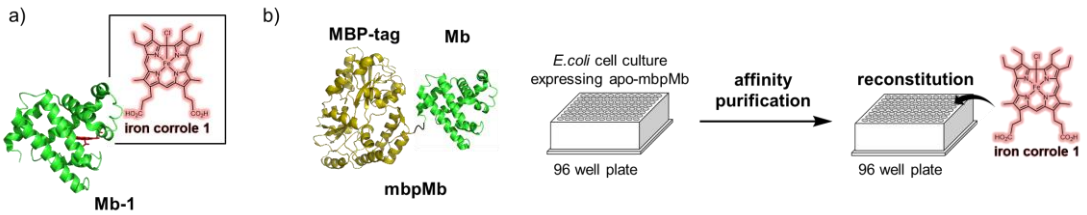

Figure 1. a) Reconstituted Mb with an iron corrole complex (Mb-**1**), b) HTS platform for directed evolution of Mb-**1**

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