Directed Evolution of Myoglobin Reconstituted with an Iron Corrole Complex Using a New High-throughput Screening Platform Based on an Affinity Purification System

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Artificial metalloenzymes (ArMs), where a synthetic metal cofactor is incorporated into a protein scaffold, have emerged as a new class of biocatalysts. ArMs hold a great potential to combine attractive features of natural enzymes and artificial transition metal catalysts. For example, the non-natural catalytic activities of ArMs can be tuned by a series of genetic engineering techniques, such as directed evolution technology. Our group has previously constructed an ArM, termed rMb-**1**, in which myoglobin (Mb) is reconstituted with an iron corrole complex **1** (Fig 1).[1] Notably, rMb-**1** was found to show higher H_2O_2-dependent peroxidase activity compared to the native Mb. Based on this promising result, we here set out to perform the directed evolution of rMb-**1** to further improve its peroxidase activity.

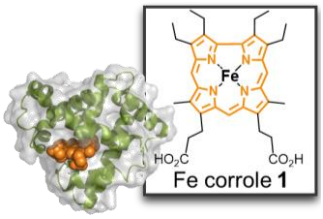

Fig 1. ArM (rMb-**1**)

To realize the directed evolution campaign of rMb-**1**, we first developed a new high-throughput screening (HTS) platform based on an affinity purification system using maltose binding protein (MBP) tag (Fig 2).[2] This HTS platform enabled us to quickly purify the recombinant protein scaffolds in a 96-well format and allowed to assemble the target ArMs without any influence of the host cellular contaminants. Furthermore, we have screened a site-saturation mutagenesis (SSM) library of rMb-**1** using the HTS platform to accomplish the directed evolution campaign. In this presentation, we will present the details of the HTS platform and the results of the screening for the directed evolution of rMb-**1**.

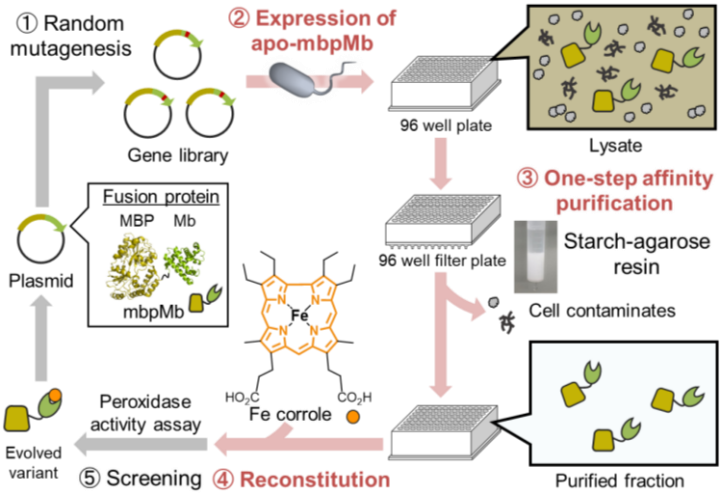

Fig 2. HTS platform that involves an affinity purification step

1) T. Matsuo, A. Hayashi, M. Abe, T. Matsuda, Y. Hisaeda, T. Hayashi, *J. Am. Chem. Soc.* **2009**, *131*, 15124. 2) S. Kato, A. Onoda, N. Taniguchi, U. Schwaneberg, T. Hayashi, *ChemBioChem*, **2021**, *22*, 679.