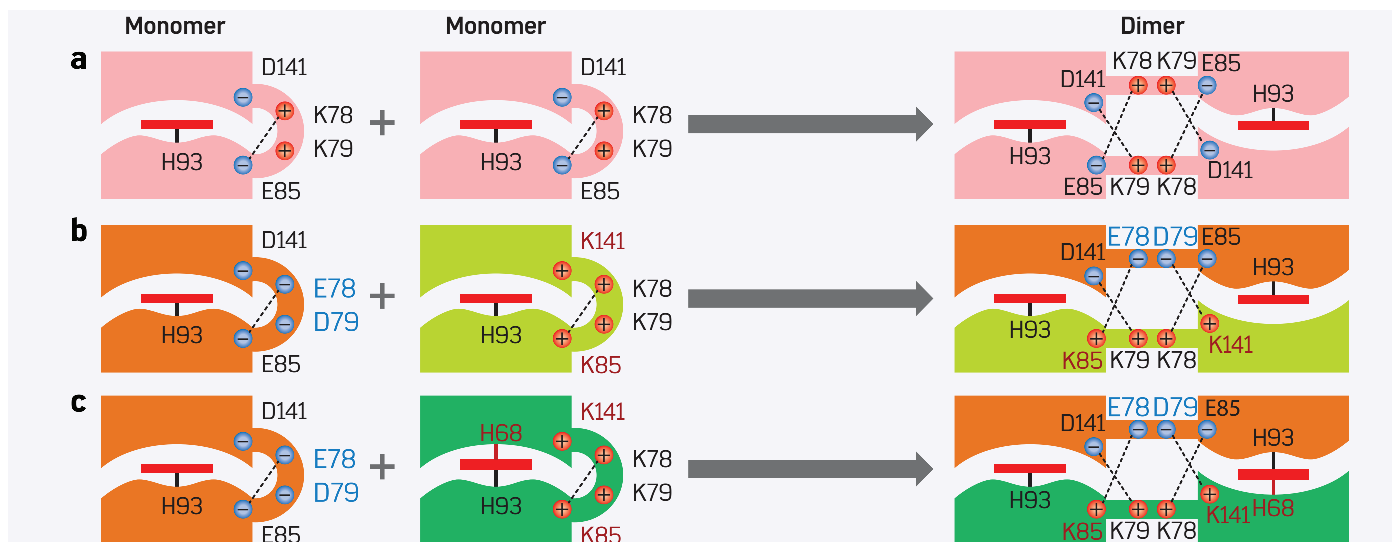
NAIST® Research Highlights



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Myoglobin dimer formation using (a) the natural protein monomer, (b) two mutant monomers obtained by switching the positions of the bridging residues (E78/K85 and D79/K141) and (c) two mutant monomers with switched bridging residues and different heme active sites (H93 and H68/H93).

Artificial proteins

Growing enzyme diversity using mutations

A targeted protein mutation strategy opens the door to multifunctional artificial enzymes

Site-specific modifications and pairing of natural proteins are expected to generate artificial enzymes capable of catalysing multiple reactions simultaneously, reveals new research by NAIST[1].

The work of enzymes relies on protein folding. Existing protein design methods have produced several artificial enzymes that promote oxidation, reduction, and hydrolysis reactions. These methods have rested on metal ions or other additional compounds called co-factors to control protein folding and achieve these functions. Other self-assembly approaches have led to similar results by forming protein pairs, or dimers. However, the variety of synthetic enzymes remains limited.

To expand this artificial enzyme library, Shun Hirota, from the Graduate School of Materials Science, and co-workers have developed a new strategy that produces various dimers by exchanging specific residues in natural proteins. As a proof-of-concept, they applied this domain swapping approach to iron-containing proteins responsible for oxygen transport and storage in the body, known as hemeproteins.

Having recently discovered that domain swapping governed the polymerization and oligomerization of hemeproteins, such as cytochrome *c* and myoglobin, Hirota's team decided to exploit this phenomenon to create new proteins containing a few monomer units, or oligomers. "Domain swapping with mutation allows us to construct artificial proteins possessing active sites with different structures," he adds.

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By examining the X-ray crystallographic structure of the natural myoglobin dimer in detail, Hirota's team identified four bridges between each protein subunit. These bridges resulted from electrostatic interactions between positively and negatively charged residues, holding the dimer together. Consequently, to control the dimerization, the researchers engineered two different myoglobin mutants by switching the positions of the charged residues in each mutant (see figure). Specifically, they replaced two positively charged residues by two negatively charged ones in one mutant, and two negatively charged residues by two positively charged residues in the second mutant. These two mutants gave a stable dimer upon pairing.

Moreover, to generate a dimer with two distinct active sites, Hirota and co-workers altered one of the heme sites in the second myoglobin mutant before coupling it with the first mutant. A spectrometric analysis of the resulting dimer demonstrated that the two sites exhibited different reactivities upon exposure to a mild reducing agent. These results suggested that the sites operated independently — evidence for the usefulness of domain swapping in the design of multi-heme proteins.

"We are currently designing other artificial hemeproteins comprising different active sites by domain swapping," says Hirota. By expanding their library, the researchers are also planning to produce multifunctional enzymes.

Reference

1. Lin, Y.-W., Nagao, S., Zhang, M., Shomura, Y., Higuchi, Y. & Hirota, S. Rational design of heterodimeric protein using domain swapping for myoglobin. *Angewandte Chemie International Edition* **54,** 511–515 (2015).

More information about the group's research can be found at the Laboratory for Supramolecular Science webpage: http://mswebs.naist.jp/LABs/hirota/member1_e.html