

S001 Engineering function in synthetic protein maquettes
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Artificial proteins with no sequence similarity to any natural protein offer a clean slate on which to understand engineering and assembly principles underlying natural functions. We illustrate this approach by assembling an oxygen transport protein, akin to human neuroglobin. Beginning with a helix forming sequence comprising just three different amino acids, we assemble a four helix bundle position histidines in the interior to bis-his ligate hemes, and exploit a helical rotation and glutamate burial on heme binding to introduce strain on the distal histidine. This facilitates histidine exchange for oxygen and carbon monoxide. Stable oxygen binding without oxidation of the reduced heme requires exclusion of water from the bundle interior, which is accomplished through simple packing of the protein interior and construction of loops to reduce the mobility of the helical interface without stopping helical rotation. The protein self-assembles around heme to form a singular structure. It reproduces O_2 affinities and exchange timescales typical of neuroglobin, myoglobin, and related hemoglobins with the notable exception that it displays a 10-fold binding preference of O_2 over CO, the opposite of any natural heme protein.