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To Bind or not to Bind: Understanding how CooA Activates

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To Bind or not to Bind: Understanding how CooA Activates

CooA, a gas-sensing heme protein found in several organisms, binds to DNA in the presence of carbon monoxide (CO) to regulate important metabolic functions. CO binding to a heme, an iron-containing entity of the protein, initiates a series of structural changes of CooA that enables it to bind a specific DNA target. The broader goal of this research is to understand the key steps that take place as CooA is activated for DNA binding. Previously and surprisingly, data suggested CooA appeared to bind DNA under non-native conditions: without CO and at low pH values. This observation was potentially important because it suggested the breaking of a CooA salt-bridge (a pair of oppositely-charged amino acids that are attracted to one another) may be a key step that allows the protein to change shape into its “active” form. Current research definitely refuted one key aspect of this hypothesis as experiments revealed this apparent low pH DNA binding is not an attribute unique to CooA; specifically results of fluorescence anisotropy assays showed that several proteins without hemes and having a range of properties also showed apparent binding activity at low pH to a DNA target specifically tailored for CooA. Although low pH binding is not supported by these experiments, this does not rule out the importance of the breaking of the salt bridge as a critical part of CooA activation. To further investigate this idea, variants of CooA with mutated salt-bridge amino acids were made and purified. Analysis of these CooA variant proteins are underway and experimental results will be presented in light of the proposed CooA activation mechanism.