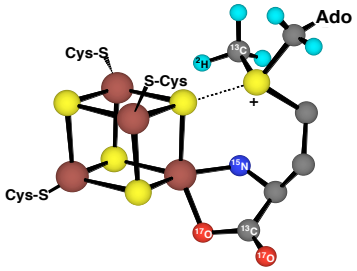
Iron-Sulfur Clusters in AdoMet-Mediated Radical Chemistry

Joan B. Broderick,[1,*] Jian Yang,[1] Danilo Ortillo,[1] William E. Broderick,[1] Jeffrey M. Buis,[1] Egidijus Zilinskas,[1] Sunil Naik,[2] Boi Hanh Huynh,[2] Charles J. Walsby,[3] and Brian M. Hoffman[3]

[1]Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA; [2]Department of Physics, Emory University, Atlanta, GA, 30322, USA; [3]Department of Chemistry, Northwestern University, Evanston, IL 60208, USA

Iron-sulfur clusters are central to the function of the characterized members of the radical-SAM protein superfamily, which catalyze a diverse range of reactions including sulfur insertions, rearrangement reactions, glycyl radical formation, and DNA repair. These enzymes utilize S-adenosylmethionine (AdoMet or SAM) and a [4Fe-4S] cluster to initiate radical chemistry, and it appears likely that all do so by a similar mechanism involving an intermediate 5'-deoxyadenosyl radical. A common feature of the radical-SAM enzymes is the presence of a three-cysteine motif, which suggests the presence of a conserved site-differentiated [4Fe-4S] cluster in these enzymes. We have used Mössbauer and ENDOR spectroscopies to demonstrate the presence of a site-differentiated [4Fe-4S] cluster in pyruvate formate-lyase activating enzyme (PFL-AE), and to show that the unique site is coordinated by AdoMet. Mechanistic implications will be discussed. PFL-AE generates the catalytically essential glycyl radical on pyruvate formate-lyase (PFL), an essential enzyme in anaerobic glucose metabolism in *E. coli*. PFL is activated *in vivo* only when cells experience anaerobic conditions, and yet PFL-AE is constitutively expressed; these observations led us to question the form of PFL-AE present under aerobic conditions, particularly given the oxygen sensitivity of the [4Fe-4S] form of PFL-AE. Recent whole-cell spectroscopic studies of *E. coli* overexpressing PFL-AE will be presented and discussed.



Spore photoproduct lyase (SPL) is a member of the radical-SAM superfamily that catalyzes the repair of UV-induced DNA damage in *Bacillus* and other spore-forming organisms. The iron-sulfur clusters present in purified SPL include [2Fe-2S] and [3Fe-4S] clusters, however these clusters are converted to [4Fe-4S] clusters upon reduction. We have demonstrated that the cluster is essential for DNA repair activity, and that the cluster affects the binding affinity for both AdoMet and DNA. A proposed mechanism of DNA repair involving C-6 H atom abstraction will be presented and discussed.