Electronic Supplementary Information for Dalton Transactions

Electronic Supplementary Information

Formation of a homocitrate-free iron-molybdenum cluster on NifEN: Implications for the role of homocitrate in nitrogenase assembly

Aaron Wolfe Fay[a,‡], Michael Aaron Blank[b,‡], Janice Mariko Yoshizawa[a,‡], Chi Chung Lee[a], Jared Andrew Wiig[a], Yilin Hu*[a], Keith Owen Hodgson*[b,c], Britt Hedman*[c], Markus Walter Ribbe*[a]

[a] *Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697, USA. Fax: 949-824-8551; Tel: 949-824-9509; E-mail: mribbe@uci.edu or yilinh@uci.edu*

[b] *Department of Chemistry, Stanford University, Stanford, CA 94305, USA. Fax: 650-926-4100; Tel: 650-926-3153; E-mail: hodgson@ssrl.slac.stanford.edu*

[c] *Radiation Lightsource, SLAC, Stanford University, Menlo Park, CA 94025, USA. Fax: 650-926-4100; Tel: 650-926-3052; E-mail: hedman@ssrl.slac.stanford.edu*

‡ A.W.F., M.A.B. and J.M.Y. contributed equally to this work.

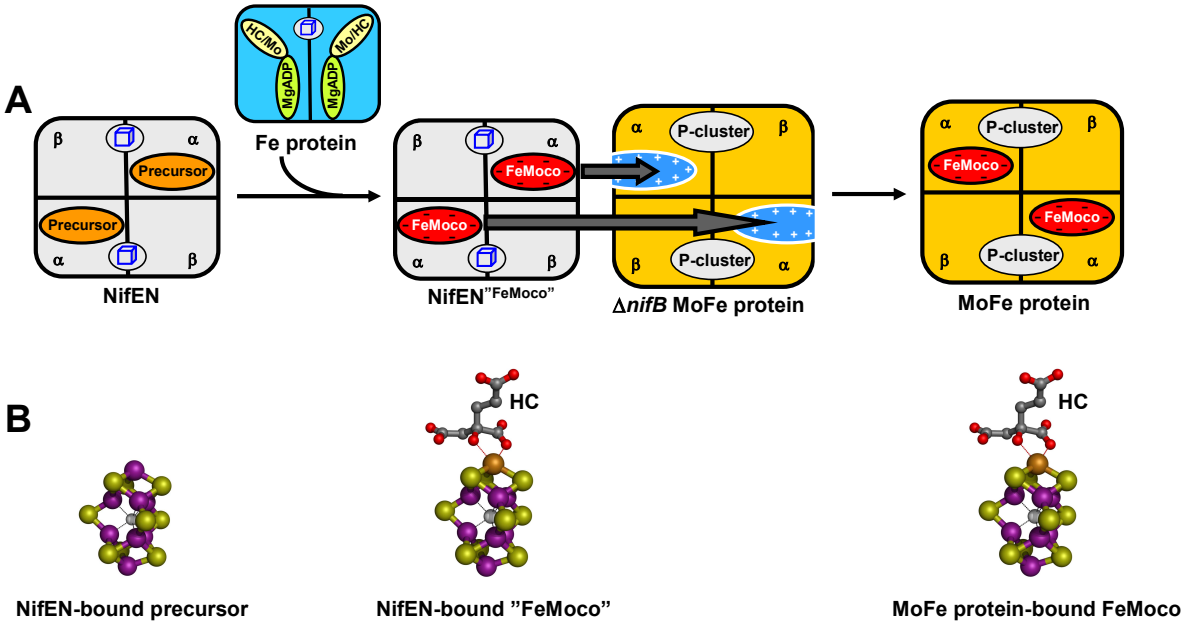

Fig. S1. Sequence of events (A) and structures of intermediates (B) during the process of FeMoco maturation and incorporation. (A) Fe protein loaded with molybdenum (Mo) and homocitrate (HC) can serve as a direct Mo/HC source for the maturation of NifEN-bound precursor. Incorporation of Mo and HC into the precursor completes the FeMoco maturation on NifEN. Subsequently, the fully matured FeMoco is transferred from NifEN to its target location within the MoFe protein upon direct protein-protein interactions. Charge-based interactions likely play an important role in the transfer of FeMoco between NifEN and MoFe protein, during which process the negatively charged FeMoco is inserted into the MoFe protein via a positively charged insertion funnel. The [Fe_4S_4] clusters of Fe protein and NifEN are represented by blue cubes. (B) Structures of NifEN-bound precursor, NifEN-bound "FeMoco" and MoFe protein-bound FeMoco. The atoms of the metal centers are colored as follows: Mo, orange; Fe, purple; S, lime; X (C, N or O), light gray; O, red; C, dark gray.

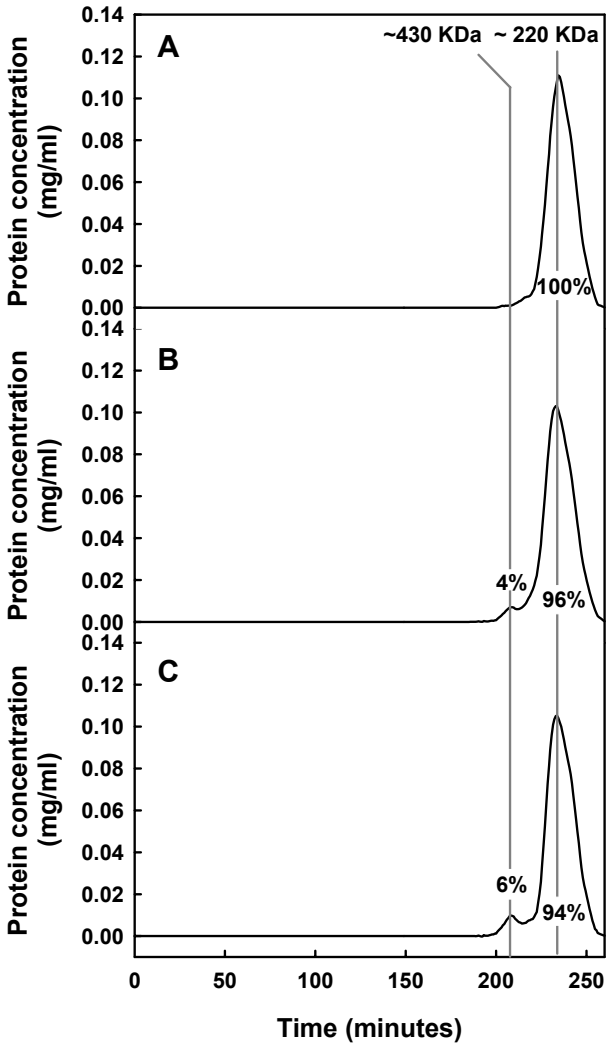

Fig. S2. Elution profiles of equimolar mixtures of NifEN^{Precursor}/Δ*nifB* MoFe protein (A), NifEN$^{"FeMoco"}$/Δ*nifB* MoFe protein (B), and NifEN$^{"FeMo"}$/Δ*nifB* MoFe protein (C) from an anaerobic Superdex 200 prep grade column [GE Healthcare (Piscataway, NJ); diameter: 1.5 cm; length: 100 cm; flow rate: 0.5 ml/min; buffer: 25 mM Tris-HCl (pH 8.0), 500 mM NaCl, 2 mM $Na_2S_2O_4$]. The protein concentrations were determined for all fractions from the gel filtration column by Bradford method (1). The molecular weights of proteins/complexes were determined on the basis of the following standards: Fe protein (60 kDa), conalbumin (75 kDa), aldolase (158 kDa), MoFe protein (230 kDa), ferritin (440 kDa) and blue dextran (2000 kDa). Apparently, portions of the NifEN$^{"FeMoco"}$/Δ*nifB* MoFe protein complex (B, the first peak) and the NifEN$^{"FeMo"}$/Δ*nifB* MoFe protein complex (C, the first peak) "survived" the lengthy gel filtration procedure; whereas no complex peak could be observed in the case of the NifEN/Δ*nifB* MoFe protein mixture (A). The molecular weight and percentage distribution of protein species are indicated.

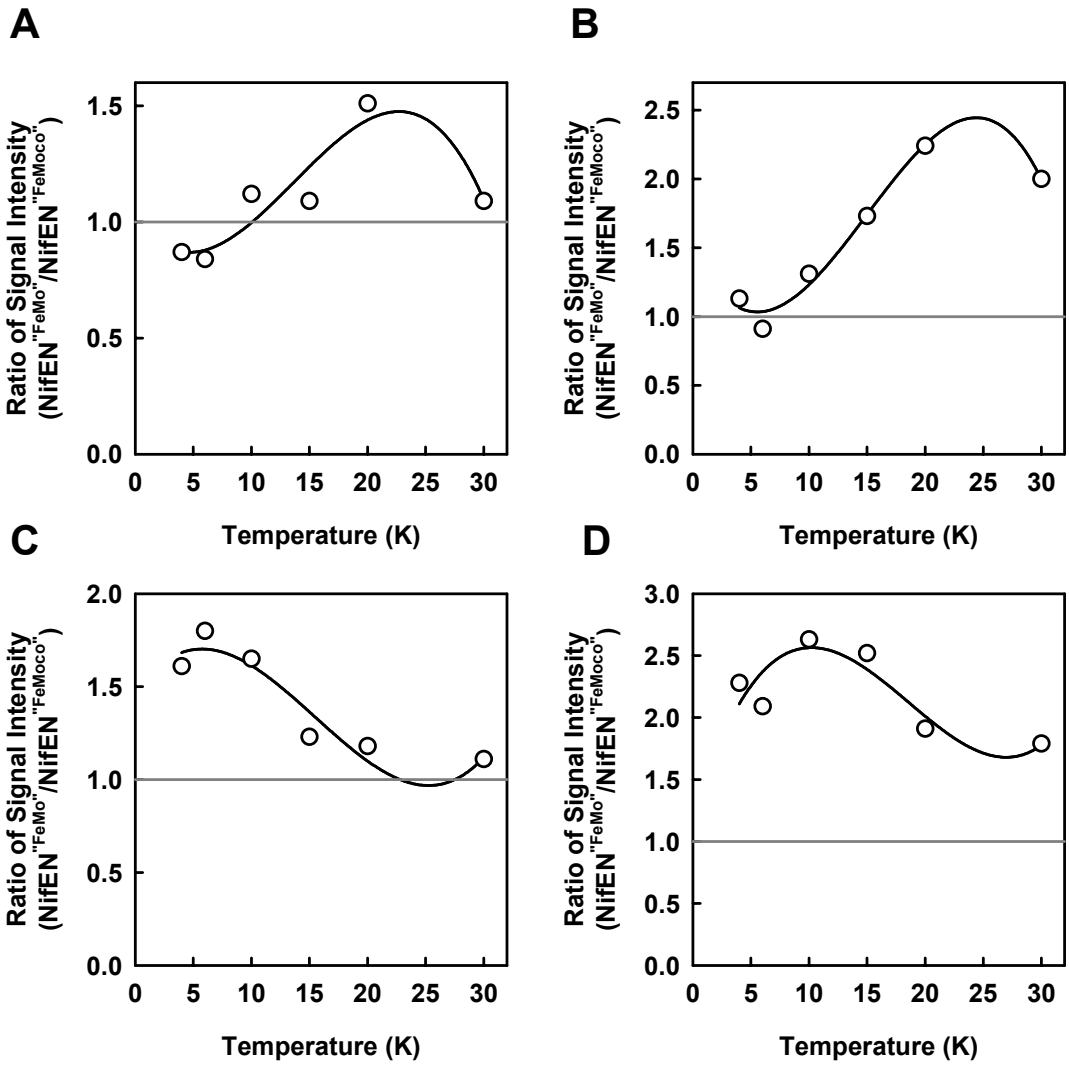

Fig. S3. Variation of ratios between the EPR signal intensities of NifEN$^{\text{“FeMo”}}$ and NifEN$^{\text{“FeMoco”}}$ upon a change in temperature. Shown are the ratios between the S = 3/2 (A, C) and S = 1/2 regions (B, D) of NifEN$^{\text{“FeMo”}}$ and NifEN$^{\text{“FeMoco”}}$ in the dithionite-reduced (A, B) and IDS-oxidized (C, D) states. A reference line indicating a 1/1 ratio between the EPR signal intensities of NifEN$^{\text{“FeMo”}}$ and NifEN$^{\text{“FeMoco”}}$ is included in A-D.

Supplementary references

1 M. M. Bradford, *Anal. Biochem,* 1976, **72,** 248.