

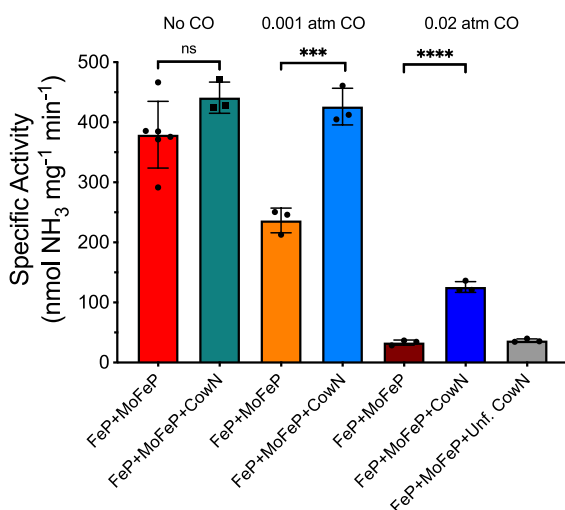
Supporting information for:

Mutational analysis of the nitrogenase carbon monoxide protective protein CowN reveals that a conserved C-terminal glutamic acid residue is necessary for its activity

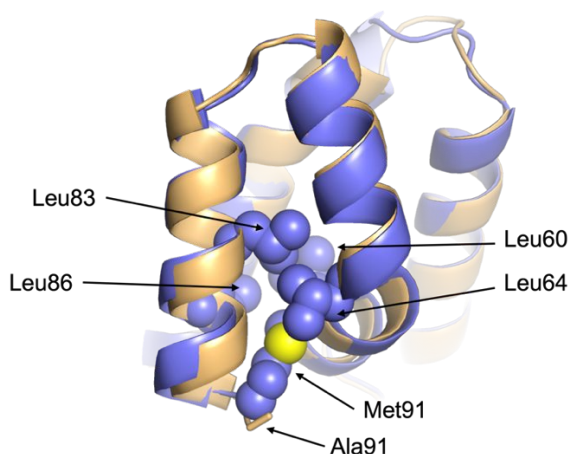
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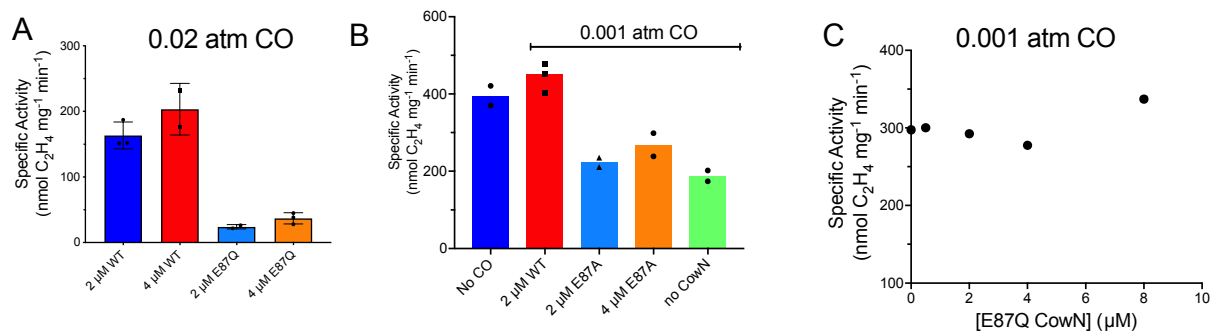
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**Figure S1.** N<sub>2</sub> reduction by nitrogenase under CO, with and without CowN. The data in this figure represents the raw data that was converted into percent activity in Figure 1 in the main text. The stars represent results from an unpaired t-test comparing means, with \*\*\* meaning  $p < 0.01$  and \*\*\*\* meaning  $p < 0.001$ .



**Figure S2.** Structural model of M91A CowN (orange) aligned to a model of WT CowN (blue), showing that M91A CowN is predicted to be identical to WT CowN. Both models were generated using Robetta.<sup>1</sup> In WT CowN, Met91 may be involved in forming a hydrophobic core with three leucine residues, Leu60, Leu64, Leu83, and Leu86. Ala91 would not be able to take part in such hydrophobic packing.



**Figure S3.** (A) C<sub>2</sub>H<sub>2</sub> reduction by nitrogenase under 0.02 CO, protected by either E87Q CowN or WT CowN, indicating that raising the CowN concentration from 2 μM to 4 μM has insignificant effect on activity restoration for E87Q CowN. Similar results are obtained when measuring C<sub>2</sub>H<sub>2</sub> reduction by nitrogenase under 0.001 CO with (B) E87A CowN and (C) E87Q CowN. In (C), the concentration of CowN is increased as high as 8 μM, suggesting that there is little to no E87Q CowN concentration-dependent activity restoration.

**Table S1. List of WT CowN peptides found in crosslinked gel bands and sequence coverage.**

GSHMTEQIDR VIAHAQGLDDLRL
20% sequence coverage MGSSHHHHHSSGLVPR <b>GSHMTEQIDR</b> YVSFRNVEWERRTAEVFALLQPHFDGSTSP FWDYFLRQR <b>VIAHAQGLDDLRL</b> VLHNFLPTLKDILLEELDDGRTLRLSRLEEVLVLCM

**Table S2. List of MoFeP α subunit peptides found in crosslinked gel bands and sequence coverage.**

TIVPVRCEGFR LNLIHCYR KVMLYVGGRLRPR VMLYVGGRLRPR HAAFETTPYDVNVIGDYNIGGDAWSSRILLEEMGLR ILLEEMGLR AKLNLIHCYR MSLDEDK HLNVAGQAEAEAQDAGEEGVMLSECDVK DWVFDGEDKHAAFETTPYDVNVIGDYNIGGDAWSSR YQPLVDAVIEK
28% sequence coverage <b>MSLDEDK</b> TNSAFHARLIAEVLEAYPDKARKRRQK <b>HLNVAGQAEAEAQDAGEEGV</b> <b>MLSECDVK</b> SNVKSVPVGMVIRGCAAYAGSKGVVWGPVKDMVHISHGPGVCGQYSWS QRRNYYIGNTGVDSFVTMVFQTSDFQEKDIVFGGDKKLEKIIDEIDELFPLAKGISVQSE

CPIGLIGDDIEAVSRKKKKEIGK**TIVPVRCEGFR**GVSQSLGHHIANDAIR**DWVFDGEDK**  
**HAAFETTPYDVNVIGDYNIGGDAWSSRILLEEMGLR**VVGNWSGDATLAEIERAPKAK  
**LNLIH**CYRSMNYICRHMEEKYNIPWTEYNFFGPSQIAASLRKIAALFDEKIQEGAERVI  
**AKYQPLVDAVIEK**FRPRLAGK**KVMLYVGGLRPR**HVVNAYNLDLGMIEIVGTGYEFGHN  
DDYQRTGHYVREGTLIYDDVTGYELEKFIEGIRPDLVGSIGIKEKYPVQKMGIPFRQMH  
SWDYSGPYHGYDGF AIFARDMDLAINNPVWSMFKAPWKNA

**Table S3. List of MoFeP  $\beta$  subunit peptides found in crosslinked gel bands and sequence coverage.**

GILEHFWK  
MYDGGTKIEAAR  
FILETGGEPK  
AWGGKDLWHMR  
LMFPIFDR  
FPVWGYQGALR  
YLERD TD TPLIR  
LENMPPADK  
VVEIADWTK  
HFKEPSSAVSSSMTEDAAVFGGLNNMVDGLANTYK  
TASFHYPLGIGATDDLQKLSEISGKVPVQELEMER

32% sequence coverage

MPQNVDKILDHAPLREPEYQEMLAGKAK**LENMPPADK****VVEIADWTK**SWEYREKNF  
ARESLSVNPACQPLGAVFVASGFERTMSFVHGSQGCVAAYRSHLSR**HFKEPSSAV**  
**SSSMTEDAAVFGGLNNMVDGLANTYK**LYDPKMIAVSTTCMAEVIGDDLHAFIQTAK  
GKGSVPEEFDVFPFAHTPAFVGSHTGYDNMLK**GILEHFWK**GRTPVPNRSVNIIPGFDG  
FAVGNNRELKRILGMMGVQYTILSDVSDQFDTPSDGEYR**MYDGGTKIEAAR**DAVNA  
DYTISLQEYCTPKTLEYCQSFQK**TASFHYPLGIGATDDLQKLSEISGKVPVQELEME**  
**RGRLVDALADSQAYLHGKTYAIYGDPDFVYGMAR****FILETGGEPK**HCLATNGSKAWE  
AQMQLFDSSPFGVCK**AWGGKDLWHMR**SLLATEKV D LLIGNSYGK**YLERD TD TPL**  
**IRLMFPIFDR**HHHHR**FPVWGYQGALR**VLV T LLDKIFDKLDDDTIQAGVTDYSFDLTR

### Supplemental References:

- [1] Baek, M., DiMaio, F., Anishchenko, I., Dauparas, J., Ovchinnikov, S., Lee, G. R., Wang, J., Cong, Q., Kinch, L. N., Schaeffer, R. D., Millan, C., Park, H., Adams, C., Glassman, C. R., DeGiovanni, A., Pereira, J. H., Rodrigues, A. V., van Dijk, A. A., Ebrecht, A. C., Opperman, D. J., Sagmeister, T., Buhlheller, C., Pavkov-Keller, T., Rathinaswamy, M. K., Dalwadi, U., Yip, C. K., Burke, J. E., Garcia, K. C., Grishin, N. V., Adams, P. D., Read, R. J., and Baker, D. (2021) Accurate prediction of protein structures and interactions using a three-track neural network, *Science* 373, 871-876.