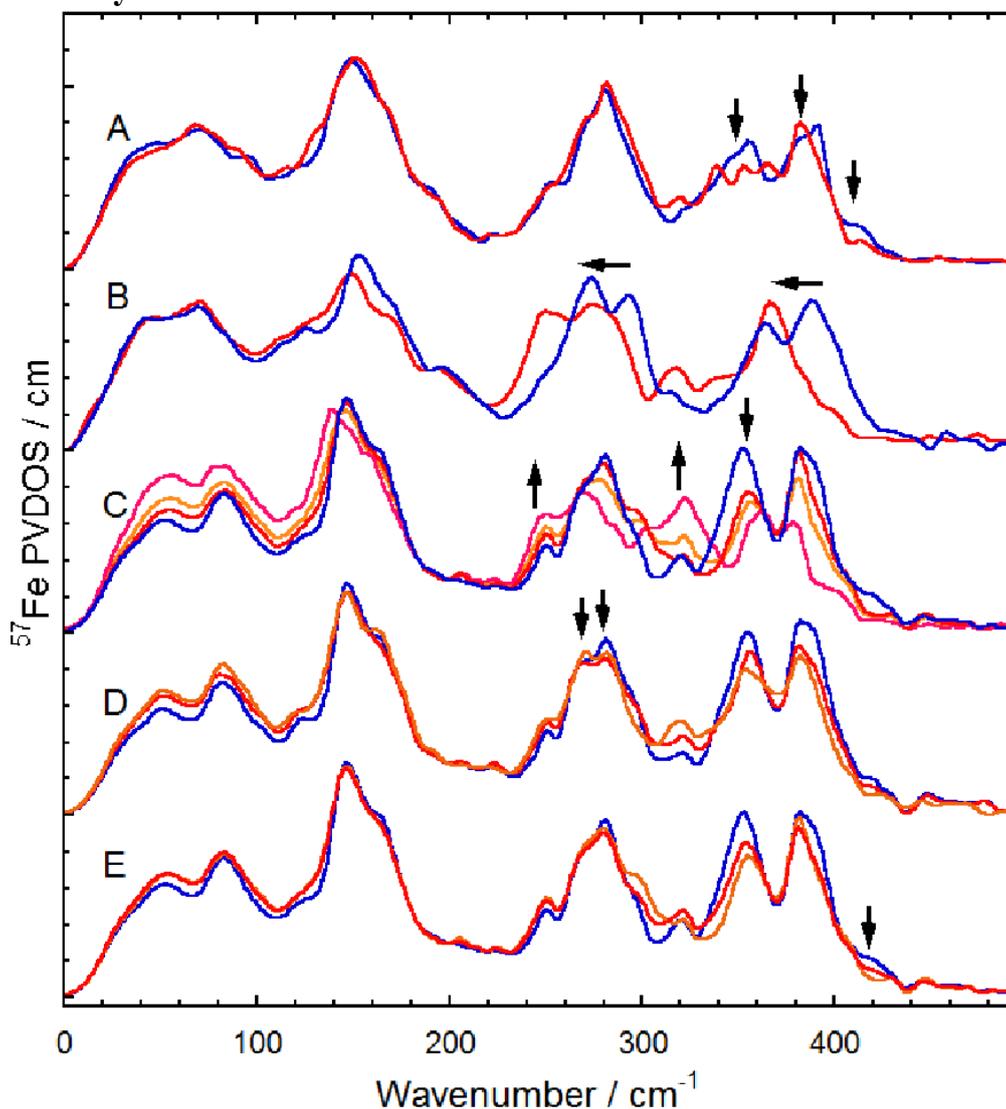


1 **Nuclear resonance vibrational spectroscopy reveals the FeS cluster**
2 **composition and active site vibrational properties of an O₂-tolerant**
3 **NAD⁺-reducing [NiFe] hydrogenase**

4
5 Lars Lauterbach,^{1,2,*} Hongxin Wang,^{2,3,‡} Marius Horch,^{1,‡} Leland B. Gee,² Yoshitaka Yoda,⁴ Yoshihito Tanaka,⁵
6 Ingo Zebger,¹ Oliver Lenz,¹ Stephen P. Cramer^{2,3,*}

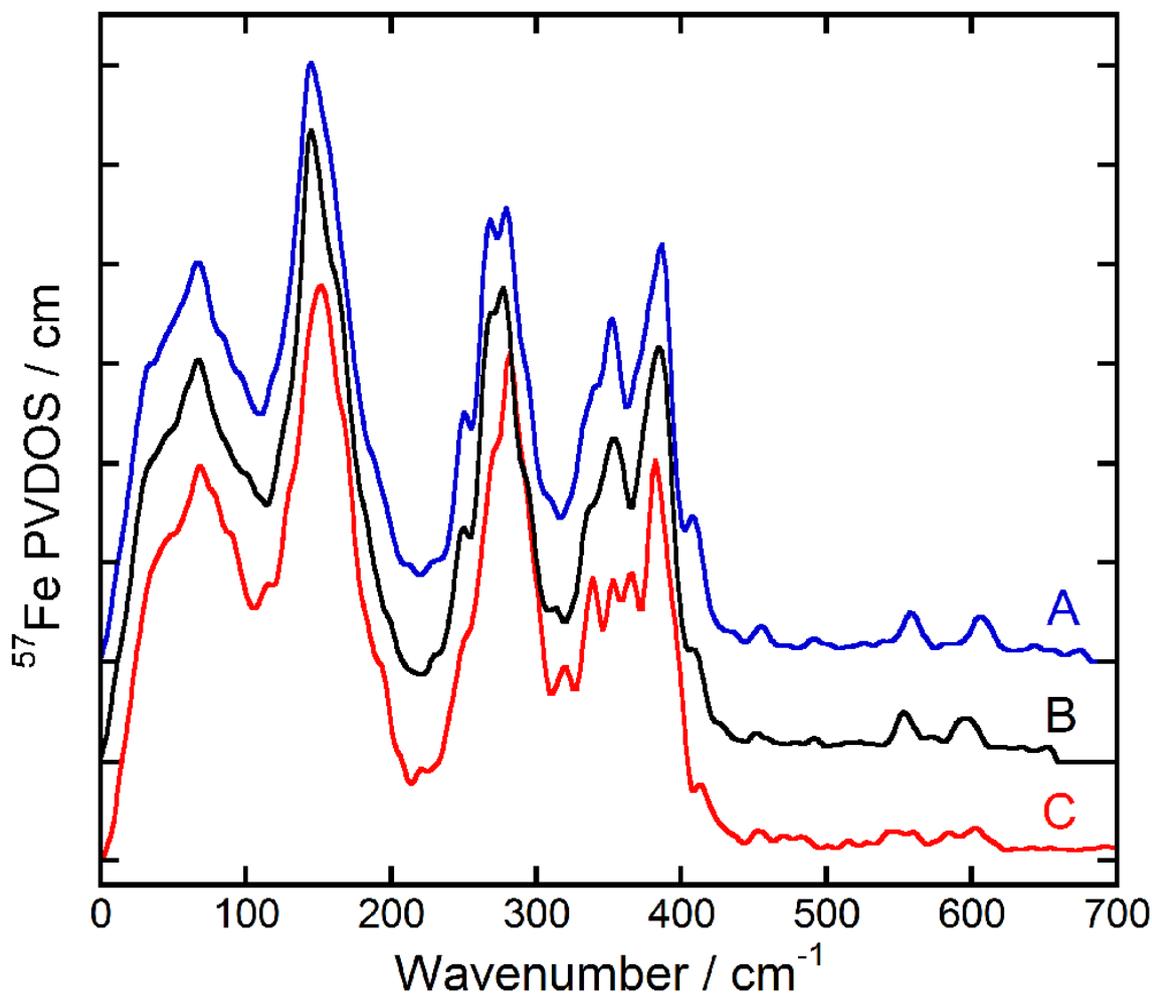
7 ¹ Institute of Chemistry, Technische Universität Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany;²
8 Department of Chemistry, University of California, One Shields Ave, Davis CA 95616, USA;³ Physical Biosciences
9 Division, Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley CA 94720, USA; ⁴ JASRI, SPring-
10 8, 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5198, Japan; ⁵ RIKEN, SPring-8, 1-1-1 Kouto, Mikazuki-cho,
11 Sayo-gun, Hyogo 679-5198, Japan

12 **Supplementary Data**



13
14
15 Figure S1. Comparison of weighted sum spectra calculated from *Rhodobacter capsulatus* (*Rc*)
16 [2Fe2S] ferredoxin, *Pyrococcus furiosus* (*Pf*) [4Fe4S] ferredoxin, and *Pyrococcus furiosus*
17 [3Fe4S] ferredoxin (*Pf*) with the NRVS of the NAD⁺-reducing hydrogenase (SH) and
18 *Desulfovibrio vulgaris* (*Dv*) Miyazaki F hydrogenase .

19 A: Oxidized (blue) and NADH-reduced (red) SH.
 20 B: Oxidized (blue) and reduced (red) *Dv* hydrogenase.
 21 C: Influence of the number of reduced [4Fe4S] clusters on the weighted sum spectrum: (Blue)
 22 One oxidized [2Fe2S] *Rc* ferredoxin plus four oxidized [4Fe4S] *Pf* ferredoxin. (Red) One
 23 reduced [2Fe2S] *Rc* ferredoxin plus three oxidized and one reduced [4Fe4S] *Pf* ferredoxin.
 24 (Orange) One reduced [2Fe2S] *Rc* ferredoxin plus two oxidized and two reduced [4Fe4S] of *Pf*
 25 ferredoxin. (Purple) One reduced [2Fe2S] *Rc ferredoxin* plus four reduced [4Fe4S] *Pf* ferredoxin.
 26 D: Influence of a [3Fe4S] cluster on the weighted sum spectrum: (Blue) One oxidized [2Fe2S]
 27 *Rc* ferredoxin plus three oxidized [4Fe4S] ferredoxin and one oxidized [3Fe4S] *Pf ferredoxin*.
 28 (Red) One reduced [2Fe2S] *Rc* ferredoxin plus two oxidized and one reduced [4Fe4S] ferredoxin
 29 plus one oxidized [3Fe4S] *Pf* ferredoxin. (Orange) One reduced [2Fe2S] *Rc* ferredoxin plus two
 30 oxidized and one reduced [4Fe4S] *Pf* ferredoxin plus one reduced [3Fe4S] *Pf ferredoxin*.
 31 E: Influence of the redox state of [2Fe2S] *Rc* ferredoxin on the weighted sum spectrum: (Blue)
 32 one oxidized [2Fe2S] *Rc* ferredoxin plus four oxidized [4Fe4S] *Pf* ferredoxin. (Red) 1/2 reduced
 33 [2Fe2S] *Rc* ferredoxin plus 1/2 oxidized [2Fe2S] *Rc* ferredoxin plus four oxidized [4Fe4S] *Pf*
 34 ferredoxin. (Orange) One reduced [2Fe2S] *Rc* ferredoxin plus three oxidized and one reduced
 35 [4Fe4S] *Pf* ferredoxin. Arrows indicate changes between spectra of the same set.
 36
 37



38 Figure S2. Comparison of NRVS for A: as isolated (blue), B: ¹³CO-labelled as isolated (black),
 39 and C: NADH-reduced (red) SH.