

Ramifications of C-centering rather than N-centering of the active site FeMo-co of the enzyme nitrogenase.

Ian Dance

School of Chemistry, University of New South Wales, Sydney 2052, Australia.

E-mail: i.dance@unsw.edu.au

Supplementary Information

Table S1. Relative energies and relevant atomic spin densities for the more stable electronic states (ES) of N<sup>c</sup>-FeMo-co and C<sup>c</sup>-FeMo-co with total spin S=3/2 or S=1/2

| S=3/2                                | ES1 (3/2)      |                |       | ES2 (3/2)      |                |       | ES3 (3/2)      |                |       | ES4 (3/2)      |                |       | ES5 (3/2)      |                |       |
|--------------------------------------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|
|                                      | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  |
| rel energy<br>kcal mol <sup>-1</sup> | 0.00           | 0.00           |       | 0.42           | 0.55           |       | 1.75           | 1.49           |       | 5.32           | 7.23           |       | 5.39           | 7.19           |       |
| N <sup>c</sup> / C <sup>c</sup>      | 0.00           | 0.00           | 0.00  | 0.00           | 0.00           | 0.00  | 0.00           | 0.00           | 0.00  | 0.05           | 0.15           | 0.10  | 0.05           | 0.14           | 0.09  |
| Fe1                                  | 3.11           | 3.15           | 0.03  | 3.18           | 3.19           | 0.01  | 3.17           | 3.18           | 0.01  | 3.13           | 3.00           | -0.13 | 3.13           | 3.04           | -0.09 |
| Fe2                                  | -2.89          | -2.75          | -0.13 | -2.91          | -2.77          | -0.15 | 2.98           | 2.94           | -0.05 | -2.86          | -2.73          | -0.14 | -2.87          | -2.69          | -0.18 |
| Fe3                                  | 2.97           | 2.94           | -0.04 | -2.93          | -2.79          | -0.14 | -2.91          | -2.77          | -0.14 | -2.85          | -2.64          | -0.20 | -2.88          | -2.75          | -0.13 |
| Fe4                                  | -2.87          | -2.73          | -0.13 | 3.00           | 2.94           | -0.06 | -2.88          | -2.74          | -0.14 | -2.84          | -2.68          | -0.17 | -2.79          | -2.66          | -0.13 |
| Fe5                                  | 2.73           | 2.49           | -0.24 | -2.76          | -2.59          | -0.17 | 2.70           | 2.46           | -0.24 | 2.76           | 2.49           | -0.27 | -2.62          | -2.48          | -0.14 |
| Fe6                                  | 2.70           | 2.47           | -0.24 | 2.68           | 2.45           | -0.23 | -2.75          | -2.60          | -0.15 | 2.74           | 2.46           | -0.27 | 2.69           | 2.47           | -0.22 |
| Fe7                                  | -2.75          | -2.62          | -0.13 | 2.75           | 2.53           | -0.22 | 2.73           | 2.51           | -0.22 | -2.63          | -2.47          | -0.17 | 2.77           | 2.51           | -0.26 |
| Mo                                   | -0.39          | -0.29          | -0.09 | -0.34          | -0.29          | -0.05 | -0.31          | -0.28          | -0.03 | -0.44          | -0.39          | -0.05 | -0.40          | -0.39          | -0.02 |
| Scysteine                            | 0.14           | 0.10           | -0.05 | 0.14           | 0.09           | -0.05 | 0.14           | 0.09           | -0.05 | 0.16           | 0.09           | -0.07 | 0.16           | 0.10           | -0.06 |
| S1A                                  | -0.03          | 0.00           | -0.03 | 0.11           | 0.12           | 0.01  | 0.11           | 0.12           | 0.01  | -0.01          | -0.12          | 0.11  | -0.01          | -0.03          | 0.02  |
| S2A                                  | 0.08           | 0.10           | 0.02  | -0.05          | -0.02          | -0.04 | 0.09           | 0.11           | 0.02  | -0.06          | -0.07          | 0.02  | -0.05          | -0.11          | 0.06  |
| S3A                                  | -0.03          | -0.02          | -0.01 | -0.02          | -0.01          | -0.02 | -0.04          | -0.03          | -0.01 | 0.02           | 0.05           | 0.03  | -0.33          | -0.28          | -0.04 |
| S4A                                  | 0.08           | 0.11           | 0.02  | 0.10           | 0.11           | 0.01  | -0.05          | -0.01          | -0.04 | -0.05          | -0.06          | 0.01  | -0.06          | -0.06          | 0.01  |
| S1B                                  | 0.12           | 0.08           | -0.04 | 0.01           | 0.00           | -0.01 | -0.01          | -0.01          | 0.00  | 0.14           | 0.12           | -0.02 | 0.02           | 0.01           | -0.01 |
| S2B                                  | -0.01          | -0.01          | 0.00  | -0.02          | -0.02          | 0.00  | -0.05          | -0.03          | -0.01 | 0.05           | 0.07           | 0.02  | 0.04           | 0.05           | 0.02  |
| S3B                                  | 0.01           | 0.00           | 0.00  | 0.10           | 0.06           | -0.04 | 0.00           | -0.01          | 0.00  | 0.02           | 0.01           | -0.01 | 0.11           | 0.10           | -0.01 |
| S4B                                  | 0.00           | -0.01          | 0.01  | 0.01           | 0.00           | -0.01 | 0.13           | 0.09           | -0.04 | 0.01           | 0.00           | -0.01 | 0.03           | 0.01           | -0.02 |
| S5A                                  | -0.02          | -0.01          | -0.02 | -0.03          | -0.03          | 0.00  | -0.04          | -0.03          | 0.00  | -0.35          | -0.29          | -0.06 | 0.02           | 0.05           | 0.03  |

diff = |C<sup>c</sup> spin| – |N<sup>c</sup> spin|

Table S1 continued

| S=3/2                  | ES6 (3/2)      |                |       | ES7 (3/2)      |                |       | ES8 (3/2)      |                |       | ES9 (3/2)      |                |       |
|------------------------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|
|                        | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  |
| rel energy             | 5.73           | 7.51           |       | 7.56           | 9.69           |       | 7.57           | 9.18           |       | 8.14           | 9.15           |       |
| kcal mol <sup>-1</sup> |                |                |       |                |                |       |                |                |       |                |                |       |
| Nc / Cc                | 0.05           | 0.12           | 0.07  | -0.03          | 0.05           | 0.02  | -0.02          | 0.06           | 0.04  | 0.07           | 0.04           | -0.03 |
| Fe1                    | 3.12           | 3.08           | -0.05 | 3.14           | 3.16           | 0.02  | 3.08           | 3.12           | 0.04  | 3.09           | 3.12           | 0.04  |
| Fe2                    | -2.87          | -2.72          | -0.15 | -3.16          | -3.05          | -0.10 | -3.13          | -3.04          | -0.09 | -2.80          | -2.46          | -0.33 |
| Fe3                    | -2.89          | -2.76          | -0.13 | -2.95          | -2.89          | -0.06 | 2.94           | 2.87           | -0.08 | -2.80          | -2.51          | -0.29 |
| Fe4                    | -2.85          | -2.69          | -0.17 | 2.97           | 2.87           | -0.10 | -2.90          | -2.84          | -0.06 | -2.76          | -2.37          | -0.39 |
| Fe5                    | 2.79           | 2.49           | -0.30 | -2.76          | -2.65          | -0.10 | 2.78           | 2.40           | -0.38 | 2.97           | 2.47           | -0.50 |
| Fe6                    | -2.68          | -2.49          | -0.19 | -2.98          | -2.85          | -0.13 | -2.96          | -2.83          | -0.13 | 2.81           | 2.67           | -0.14 |
| Fe7                    | 2.83           | 2.53           | -0.31 | 2.77           | 2.42           | -0.35 | -2.79          | -2.69          | -0.11 | 2.94           | 2.68           | -0.26 |
| Mo                     | -0.41          | -0.43          | 0.03  | 0.34           | 0.35           | 0.01  | 0.29           | 0.34           | 0.05  | -0.98          | -1.03          | 0.05  |
| Scysteine              | 0.16           | 0.10           | -0.06 | 0.14           | 0.09           | -0.05 | 0.14           | 0.10           | -0.05 | 0.16           | 0.11           | -0.05 |
| S1A                    | -0.01          | -0.01          | 0.00  | 0.07           | 0.08           | 0.01  | -0.03          | -0.03          | -0.01 | -0.01          | 0.01           | 0.00  |
| S2A                    | -0.06          | -0.06          | 0.00  | -0.06          | -0.05          | -0.02 | 0.05           | 0.06           | 0.01  | -0.05          | -0.03          | -0.02 |
| S3A                    | 0.03           | 0.03           | 0.00  | 0.02           | 0.02           | 0.00  | 0.00           | -0.03          | 0.03  | -0.03          | -0.01          | -0.02 |
| S4A                    | -0.07          | -0.09          | 0.02  | 0.06           | 0.07           | 0.01  | 0.05           | 0.06           | 0.01  | -0.07          | -0.03          | -0.04 |
| S1B                    | 0.00           | 0.00           | 0.00  | -0.12          | -0.11          | -0.01 | -0.02          | -0.05          | 0.03  | 0.18           | 0.14           | -0.04 |
| S2B                    | -0.36          | -0.29          | -0.07 | -0.42          | -0.39          | -0.03 | -0.41          | -0.39          | -0.03 | -0.02          | -0.01          | -0.01 |
| S3B                    | 0.02           | 0.01           | -0.01 | -0.02          | -0.05          | 0.03  | -0.10          | -0.09          | -0.01 | 0.13           | 0.09           | -0.04 |
| S4B                    | 0.16           | 0.14           | -0.03 | -0.01          | -0.02          | 0.01  | -0.01          | -0.03          | 0.02  | 0.23           | 0.17           | -0.06 |
| S5A                    | 0.04           | 0.04           | 0.01  | 0.00           | -0.04          | 0.04  | 0.02           | 0.03           | 0.01  | -0.03          | -0.03          | 0.00  |

diff = |C<sup>c</sup> spin| – |N<sup>c</sup> spin|

Table S1 continued

| S=1/2      | ES1 (1/2)      |                |       | ES2 (1/2)      |                |       | ES3 (1/2)      |                |       |
|------------|----------------|----------------|-------|----------------|----------------|-------|----------------|----------------|-------|
|            | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  | N <sup>c</sup> | C <sup>c</sup> | diff  |
| rel energy | 5.44           | 8.26           |       | 6.06           | 8.44           |       | 7.35           | 7.06           |       |
| Nc / Cc    | 0.01           | 0.04           | 0.04  | 0.00           | 0.04           | 0.03  | 0.00           | 0.01           | 0.01  |
| Fe1        | 3.16           | 3.14           | -0.02 | 3.19           | 3.18           | 0.00  | 1.78           | 1.86           | 0.08  |
| Fe2        | -2.87          | -2.67          | -0.20 | -2.89          | -2.69          | -0.20 | 2.83           | 2.73           | -0.10 |
| Fe3        | 0.99           | 0.81           | -0.18 | -2.89          | -2.73          | -0.16 | -2.93          | -2.81          | -0.12 |
| Fe4        | -2.81          | -2.64          | -0.18 | 1.13           | 0.86           | -0.27 | -2.87          | -2.77          | -0.09 |
| Fe5        | 2.74           | 2.54           | -0.20 | -2.67          | -2.48          | -0.18 | 2.67           | 2.46           | -0.21 |
| Fe6        | 2.71           | 2.50           | -0.21 | 2.67           | 2.47           | -0.20 | -2.77          | -2.60          | -0.17 |
| Fe7        | -2.67          | -2.52          | -0.15 | 2.74           | 2.57           | -0.17 | 2.71           | 2.51           | -0.20 |
| Mo         | -0.38          | -0.32          | -0.06 | -0.31          | -0.31          | -0.01 | -0.30          | -0.29          | -0.01 |
| Scysteine  | 0.17           | 0.11           | -0.06 | 0.16           | 0.10           | -0.06 | 0.06           | 0.04           | -0.02 |
| S1A        | 0.00           | 0.04           | 0.03  | 0.03           | 0.06           | 0.02  | 0.01           | 0.01           | 0.00  |
| S2A        | 0.01           | 0.02           | 0.01  | -0.03          | 0.01           | -0.03 | 0.00           | 0.00           | 0.00  |
| S3A        | -0.04          | -0.01          | -0.03 | -0.16          | -0.16          | 0.00  | -0.01          | -0.02          | 0.01  |
| S4A        | 0.02           | 0.03           | 0.01  | 0.03           | 0.04           | 0.02  | -0.18          | -0.14          | -0.04 |
| S1B        | 0.10           | 0.07           | -0.03 | 0.01           | 0.00           | 0.00  | -0.01          | -0.01          | 0.00  |
| S2B        | -0.02          | 0.00           | -0.02 | -0.03          | 0.00           | -0.03 | -0.05          | -0.04          | -0.01 |
| S3B        | 0.01           | 0.00           | 0.00  | 0.07           | 0.04           | -0.03 | 0.01           | 0.00           | 0.00  |
| S4B        | 0.00           | 0.00           | 0.00  | 0.01           | 0.01           | 0.00  | 0.13           | 0.09           | -0.04 |
| S5A        | -0.15          | -0.16          | 0.00  | -0.05          | -0.02          | -0.03 | -0.05          | -0.04          | -0.01 |

diff = |C<sup>c</sup> spin| – |N<sup>c</sup> spin|

**Table S2.** Atom partial charges for models **1** to **4**.

|                       | <b>1</b>             |                      | <b>2</b>             |                      | <b>3</b>             |                      | <b>4</b>             |                      |
|-----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                       | <b>C<sup>c</sup></b> | <b>N<sup>c</sup></b> | <b>C<sup>c</sup></b> | <b>N<sup>c</sup></b> | <b>C<sup>c</sup></b> | <b>N<sup>c</sup></b> | <b>C<sup>c</sup></b> | <b>N<sup>c</sup></b> |
| C <sup>c</sup>        | -0.77                |                      | -0.65                |                      | -0.69                |                      | -0.68                |                      |
| N <sup>c</sup>        |                      | -0.93                |                      | -0.85                |                      | -0.85                |                      | -0.85                |
| Fe1                   | 0.66                 | 0.65                 | 0.65                 | 0.63                 | 0.65                 | 0.64                 | 0.65                 | 0.64                 |
| Fe2                   | 0.62                 | 0.64                 | 0.54                 | 0.52                 | 0.54                 | 0.52                 | 0.57                 | 0.57                 |
| Fe3                   | 0.63                 | 0.66                 | 0.61                 | 0.65                 | 0.60                 | 0.64                 | 0.61                 | 0.65                 |
| Fe4                   | 0.61                 | 0.64                 | 0.61                 | 0.65                 | 0.58                 | 0.62                 | 0.61                 | 0.64                 |
| Fe5                   | 0.60                 | 0.63                 | 0.59                 | 0.66                 | 0.60                 | 0.65                 | 0.57                 | 0.61                 |
| Fe6                   | 0.60                 | 0.63                 | 0.50                 | 0.51                 | 0.42                 | 0.45                 | 0.43                 | 0.41                 |
| Fe7                   | 0.61                 | 0.65                 | 0.61                 | 0.65                 | 0.59                 | 0.63                 | 0.61                 | 0.65                 |
| Mo                    | 0.95                 | 0.97                 | 0.94                 | 0.98                 | 1.02                 | 1.04                 | 1.00                 | 1.02                 |
| S1                    | -0.72                | -0.65                | -0.70                | -0.63                | -0.70                | -0.63                | -0.71                | -0.64                |
| S1A                   | -0.72                | -0.64                | -0.68                | -0.62                | -0.63                | -0.58                | -0.66                | -0.58                |
| S2A                   | -0.74                | -0.66                | -0.71                | -0.65                | -0.67                | -0.64                | -0.67                | -0.61                |
| S3A                   | -0.80                | -0.71                | -0.79                | -0.70                | -0.78                | -0.69                | -0.80                | -0.70                |
| S4A                   | -0.73                | -0.66                | -0.70                | -0.63                | -0.69                | -0.62                | -0.74                | -0.66                |
| S1B                   | -0.63                | -0.58                | -0.60                | -0.57                | -0.62                | -0.58                | -0.53                | -0.49                |
| S2B                   | -0.79                | -0.69                | -0.47                | -0.49                | -0.46                | -0.44                | -0.46                | -0.44                |
| S3B                   | -0.63                | -0.57                | -0.59                | -0.55                | -0.61                | -0.54                | -0.64                | -0.60                |
| S4B                   | -0.66                | -0.61                | -0.67                | -0.62                | -0.62                | -0.58                | -0.62                | -0.57                |
| S5A                   | -0.79                | -0.68                | -0.76                | -0.68                | -0.75                | -0.66                | -0.77                | -0.68                |
| S2B-H                 |                      |                      | 0.11                 | 0.14                 | 0.12                 | 0.14                 | 0.11                 | 0.13                 |
| Fe2-H                 |                      |                      | -0.29                | -0.21                | -0.21                | -0.18                | -0.22                | -0.16                |
| Fe6-H                 |                      |                      | -0.21                | -0.10                | -0.18                | -0.13                | -0.14                | -0.09                |
| N <sub>distal</sub>   |                      |                      |                      |                      | -0.21                | -0.14                | -0.10                | -0.09                |
| N <sub>proximal</sub> |                      |                      |                      |                      | 0.00                 | 0.02                 | -0.13                | -0.09                |