S K-edge XAS Studies of the Effect of DNA Binding on the $[Fe_4S_4]$ Site in EndoIII and MutY

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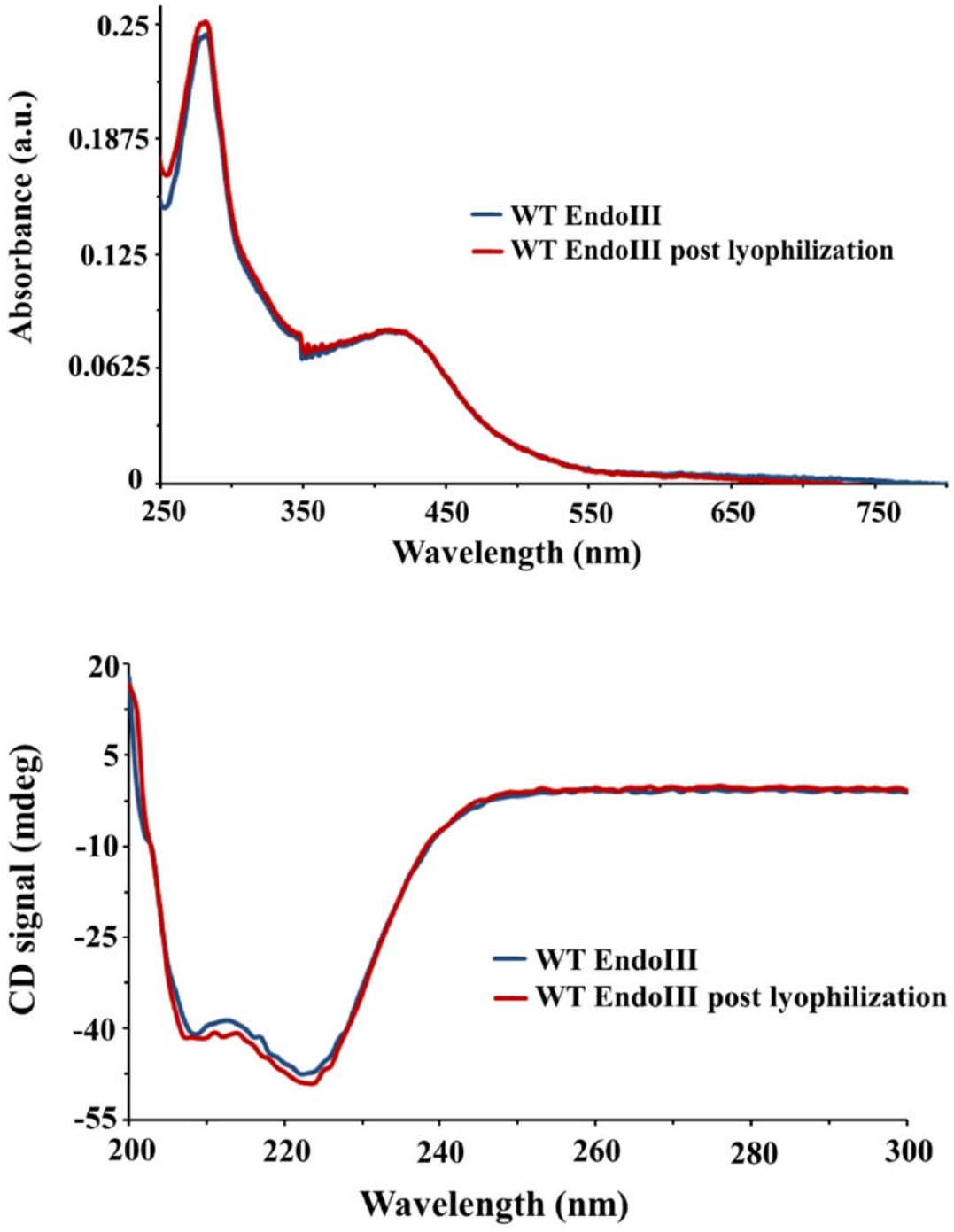
Supporting Info

Test of Re-dissolving Lyophilized Protein Samples

By UV-visible and circular dichroism spectroscopy, EndoIII, redissolved after lyphilization, is intact and indistinguishable from untreated samples. These results confirm that lyophilization does not affect overall protein structure or cluster loading, and supports the results obtained by XAS. Furthermore, EndoIII at concentrations similar to those used in XAS experiments shows no evidence of aggregation and exhibits no changes in UV-visible absorbance, and when diluted, remains active. The XAS spectrum of redissolved EndoIII is also the same.

In MutY, Active-site titrations using a gel-based glycosylase assay were performed using previously published methods ("Silvia L. Porello, Michelle J. Cannon and Sheila S. David, Biochemistry, 1998, 37 (18), pp 6465–6475). These assays entailed evaluating the adenine glycosylase activity of MBP-MutY with and without lyophilization pretreatment by incubating the enzyme with a 32P 5′-end labeled 30-bp duplex containing a centrally located OG:A mismatch (A-strand is labeled). Under conditions where enzyme concentration is less than that of the substrate, MutY displays burst kinetics due to rate-limiting product release. Under these conditions, the amplitude of the burst corresponds to the concentration of active enzyme. This assay revealed no significant differences in the percent active fraction between MBP-MutY

samples that been lyophilized for XAS experiments relative to those that had not been lyophilized (Figure S2).



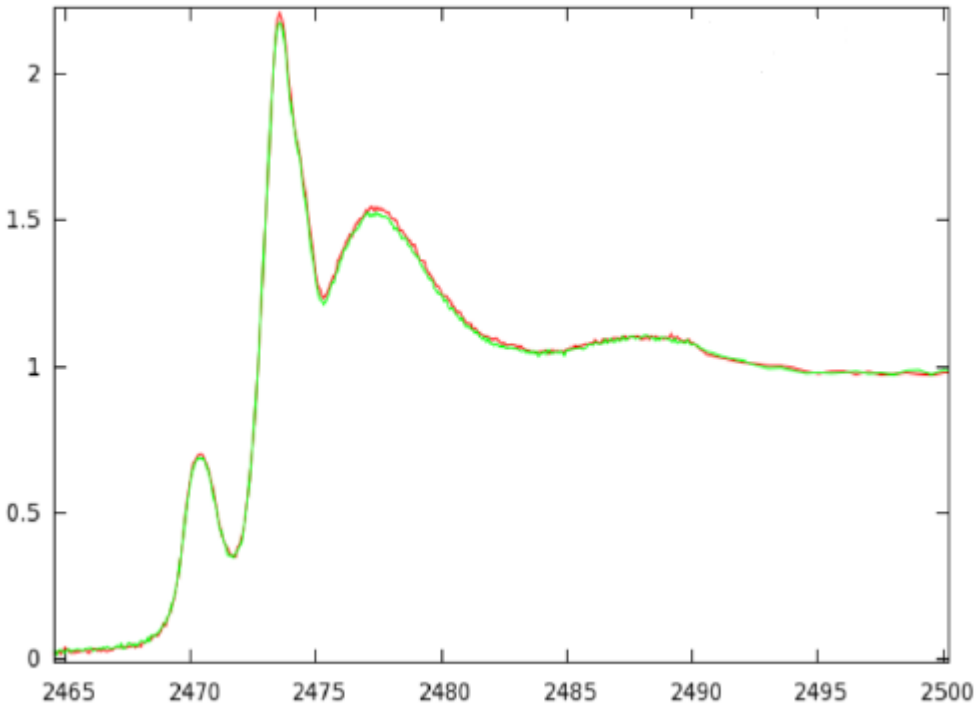
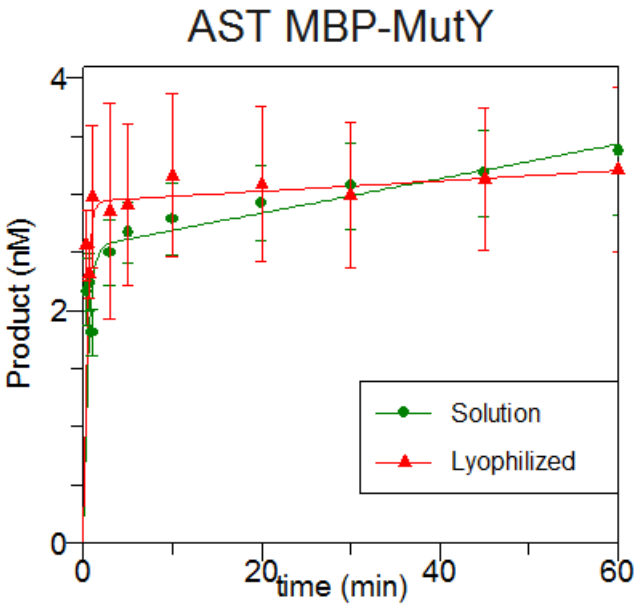
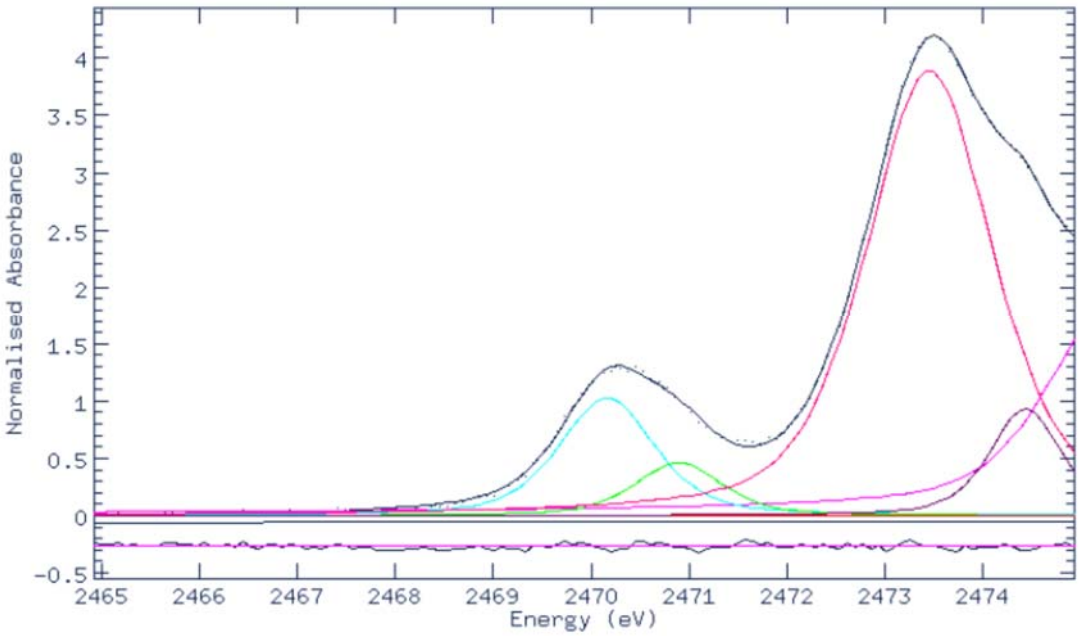


Figure S1 Spectroscopic comparisons of the EndoIII solution and re-dissolve after lyophilizaiotn. (A) UV-Vis, normalized at 280nm, in 20 mM sodium phosphate, 0.5 mM EDTA, 150 mM NaCl, 10% v/v glycerol at pH 7.5. (B) CD spectra. A222/A208 ratios were 1.17 from solution and 1.18 for re-dissolve, consistent with no major changes and fully intact protein. (C) S K-edge XAS of EndoIII solution (red) and after re-dissolve (green).

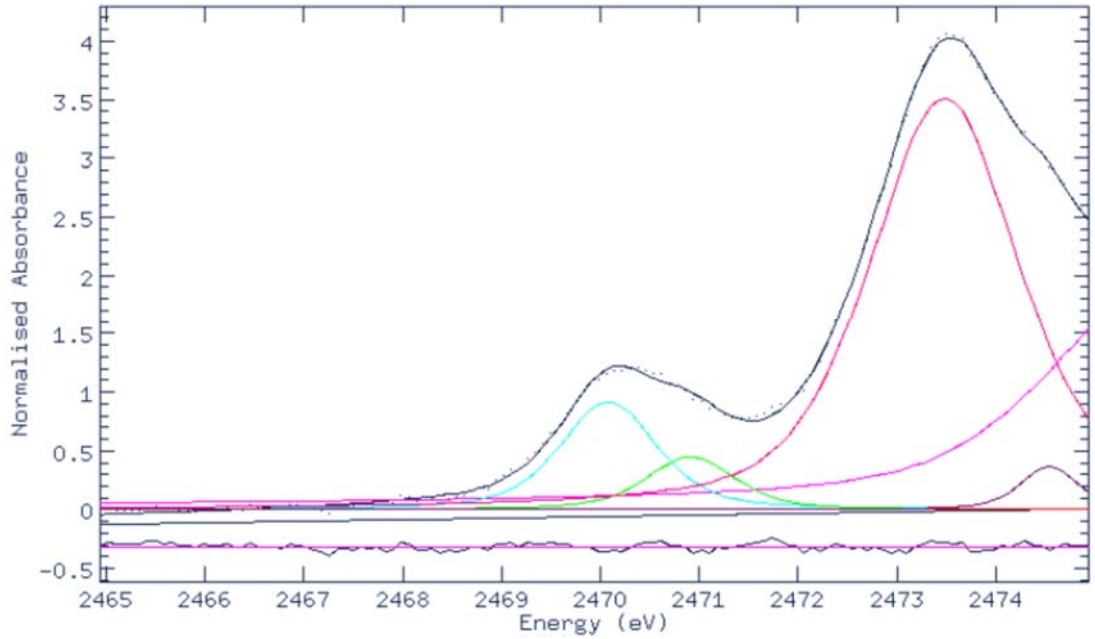


MBP-MutY Sample	% Active Fraction
Solution	51 ± 5
Lyophilized	59 ± 12

Figure S2. Average product curves and standard error bars of three separate aliquots each of solution and lyophilized MBP-MutY to determine the percent active fraction under MTO conditions.


Normalised Absorbance
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
2465
2466
2467
2468
2469
2470
2471
2472
2473
2474
Energy (eV)

A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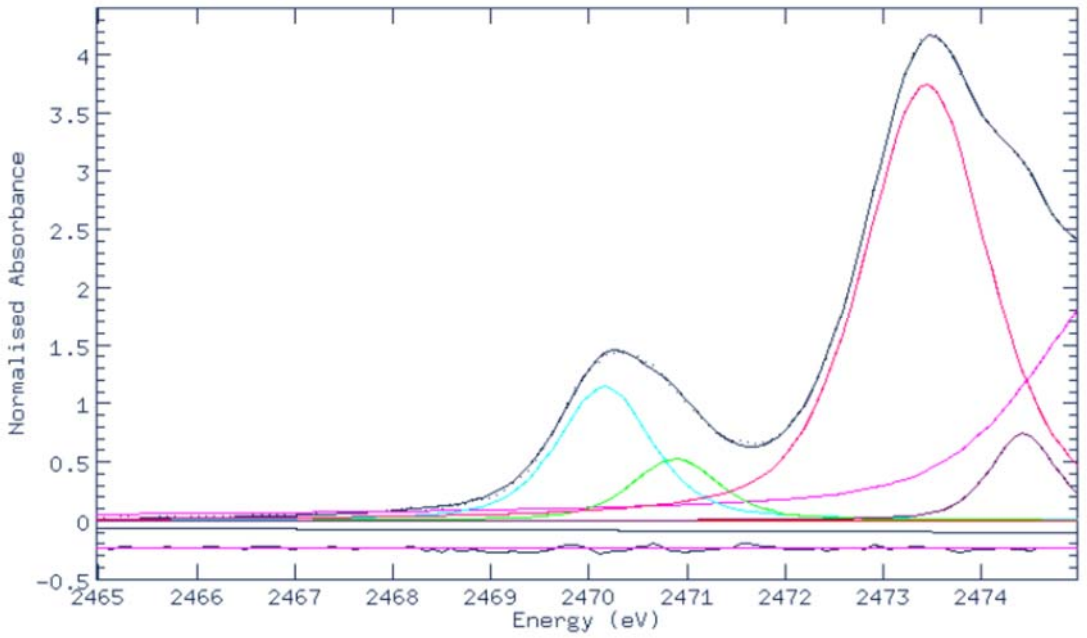
Energy (eV)

B



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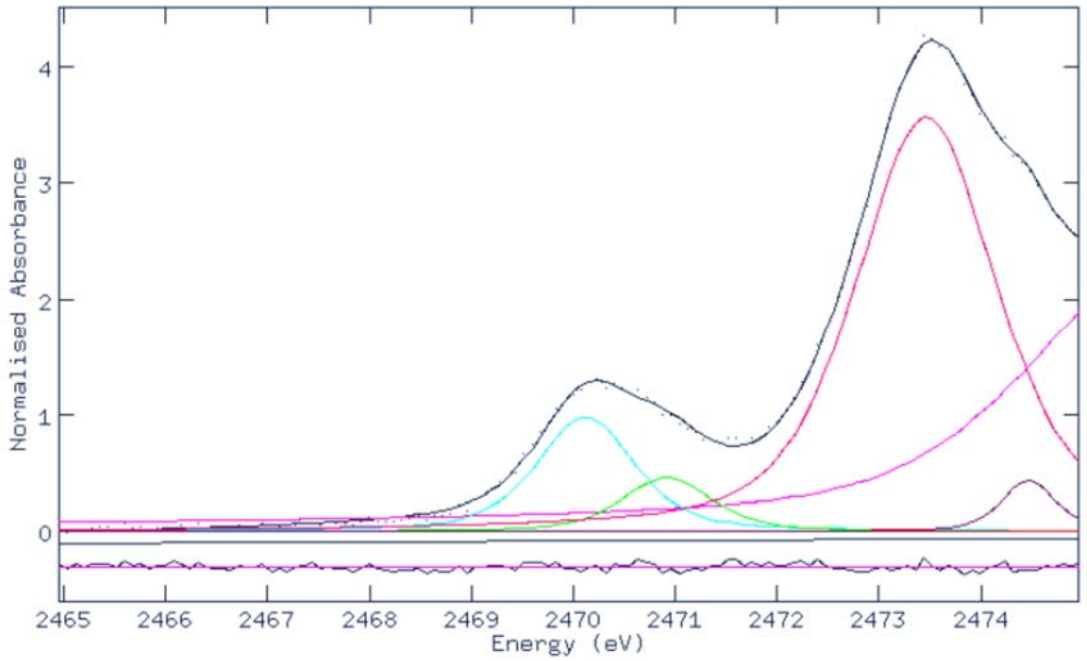


D

Figure S3 Fits for (A) EndoIII_Solution, (B) EndoIII_Lyo, (C) DNA+EndoIII_Solution, and (D) DNA+EndoIII_Lyo.

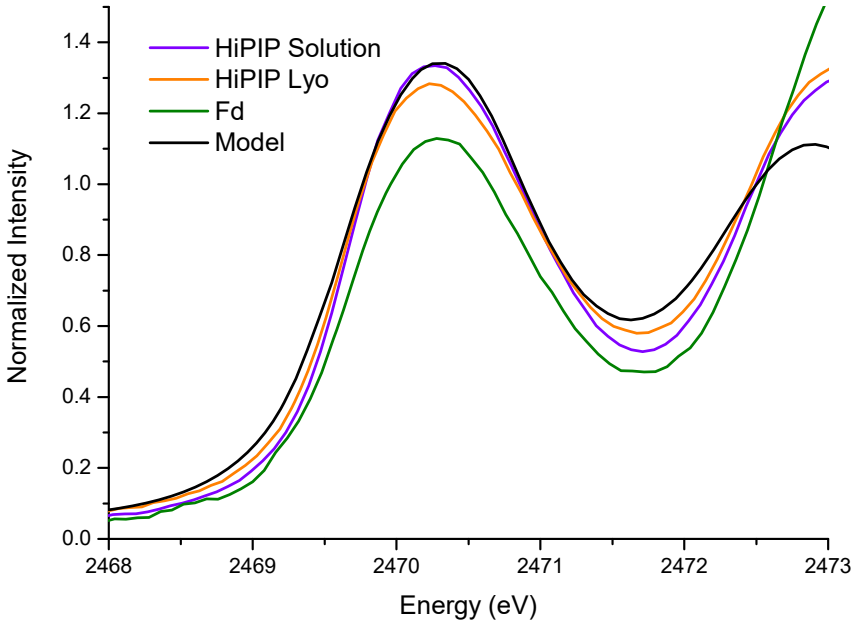


Figure S4: S-K XAS of HiPIP.

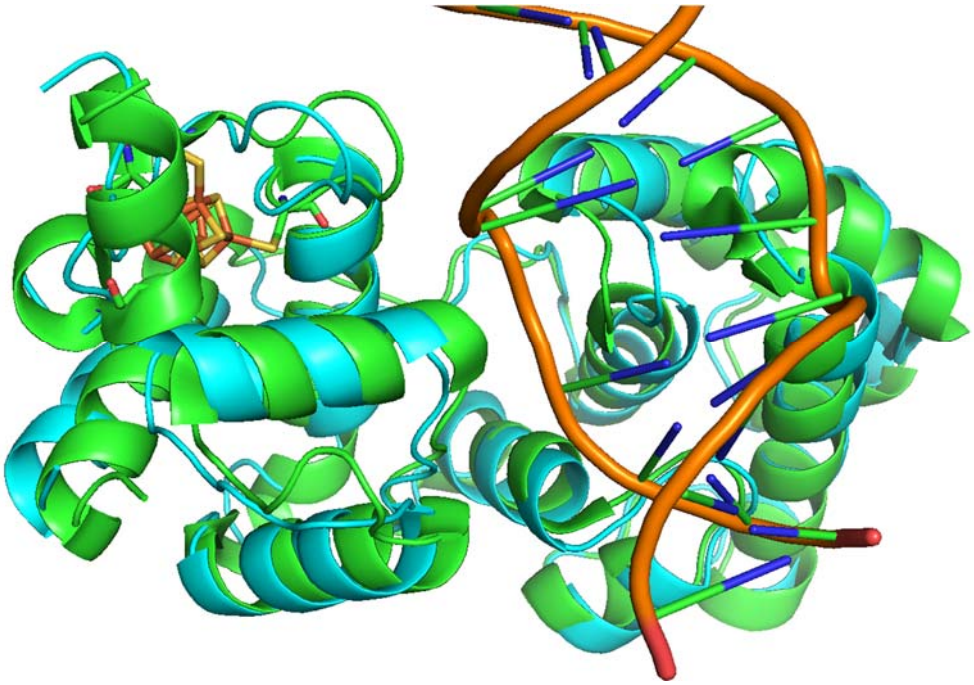
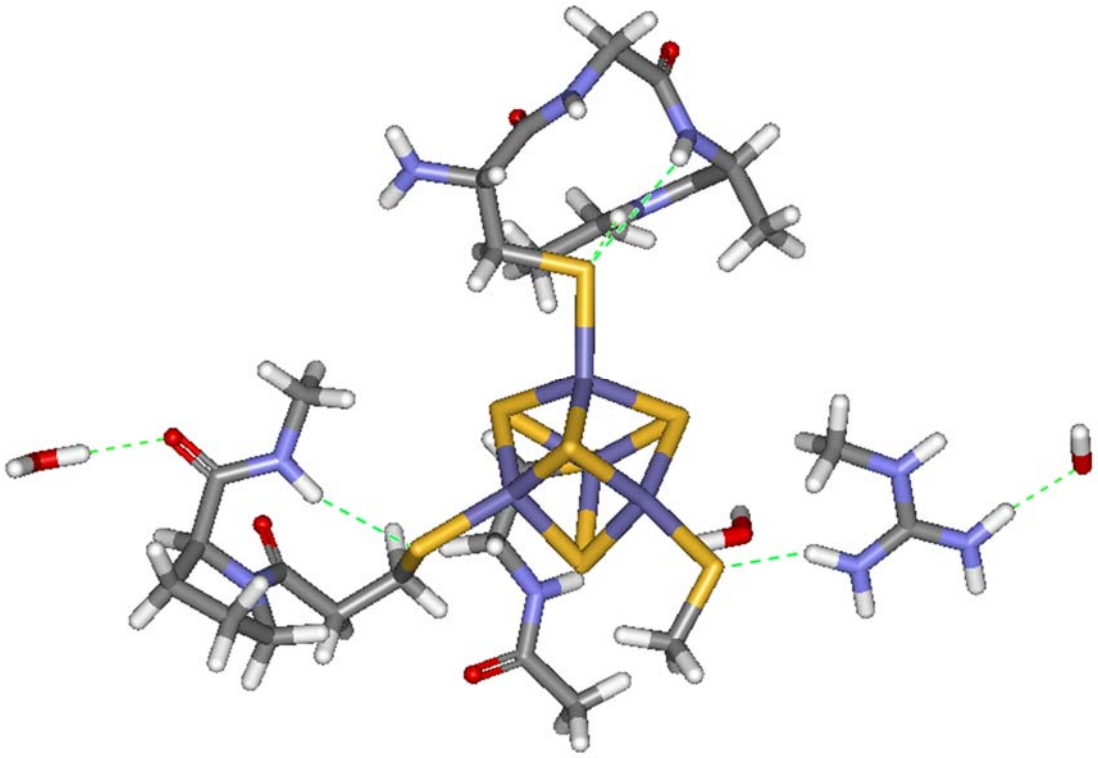
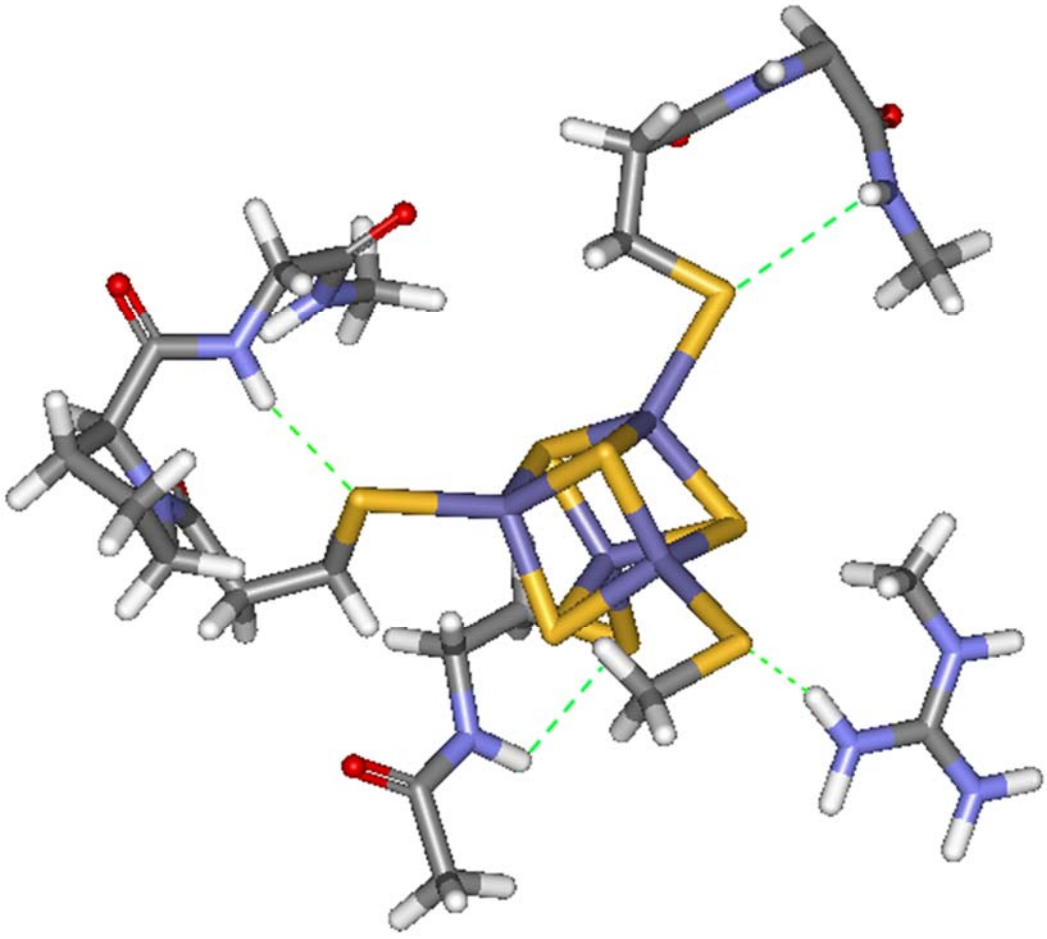


Figure S5 Structural alignment comparison of free (blue, pdb code 2ABK) and DNA bound (green, pdb code 1ORN) forms of EndoIII.



A



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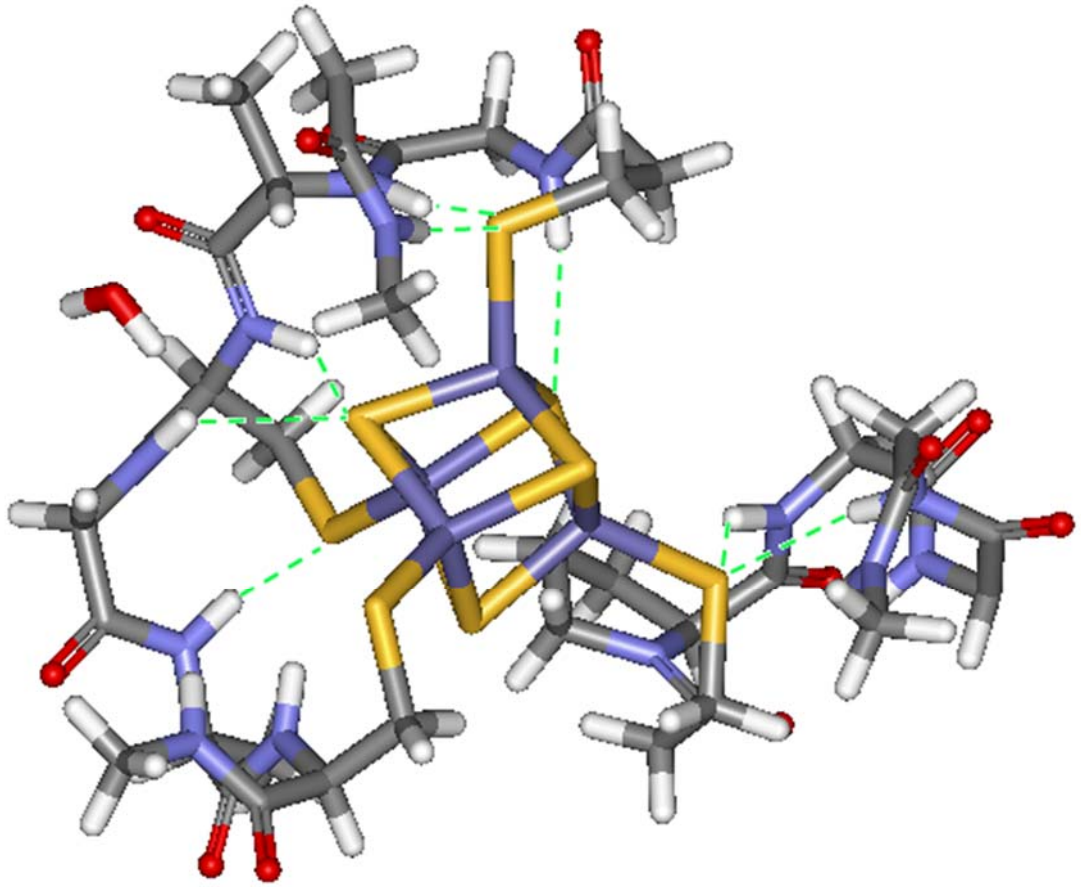

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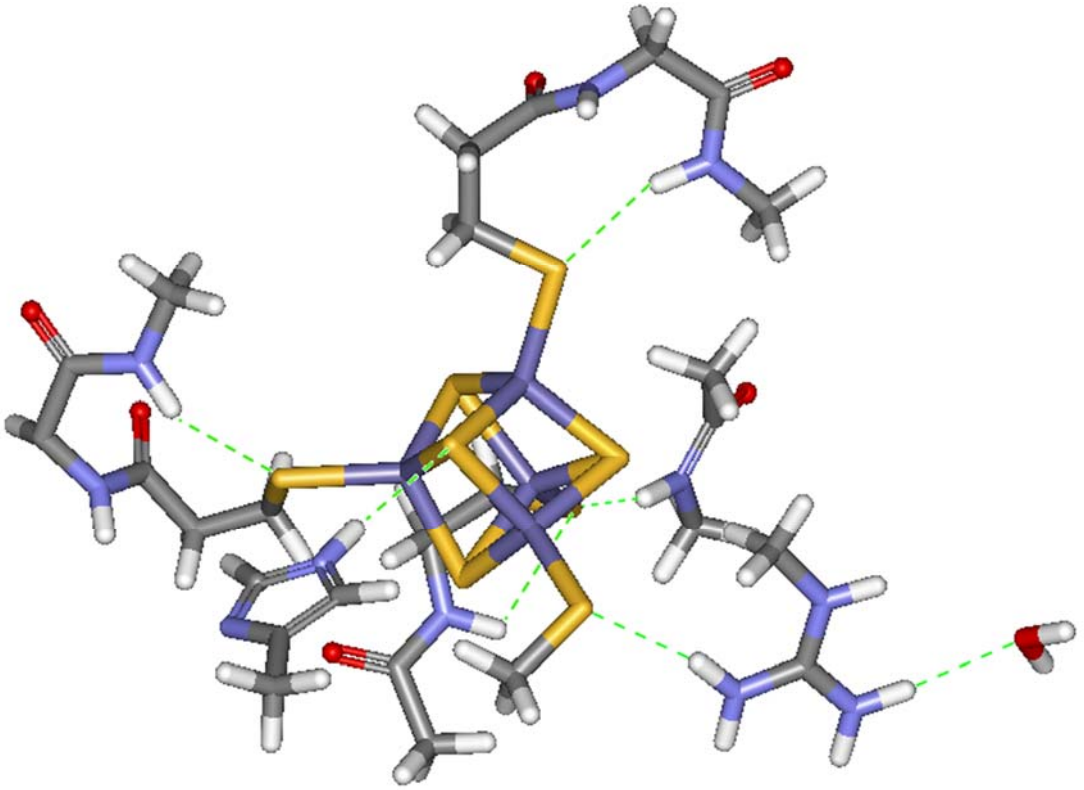


D

Figure S6. H-bond of (A) Ec MutY (unbound form, PDB code: 1KG2), (B) Gs MutY (DNA bound

form, PDB code: 1RRS) and (C) Fd (PDB code: 1IQZ, no crystal water is shown in the structure). (D) EndoIII without DNA (PDB code: 2ABK). In both MutY homologs, there is 1 H-bond from Arg, which is affected by solvent water, and 3 amide H-bonds. Also notice Fd both has more H-bonds from amide and importantly a solvent exposed cluster.

In the structure in D, the His H-bond is closer to the sulfide (3.5A) than the thiolate (3.8A). This is different than the structure in 1ORN and 4UNF, where His is H-bond to the thiolate (3.5A). This change in His H-bond does not correlate with the change in covalency, because in 2ABK, the His is close to thiolate (Figure S6D), and in MutY, there is no His H-bond to the cluster, yet we observe the same covalency change.

DFT calculations

DNA is rich in negative charge. To qualitatively test whether the presence of negative charge would increase total [Fe_4S_4] sulfur covalency and understand the nature of this effect, simple models were evaluated. The results are shown in table S1.

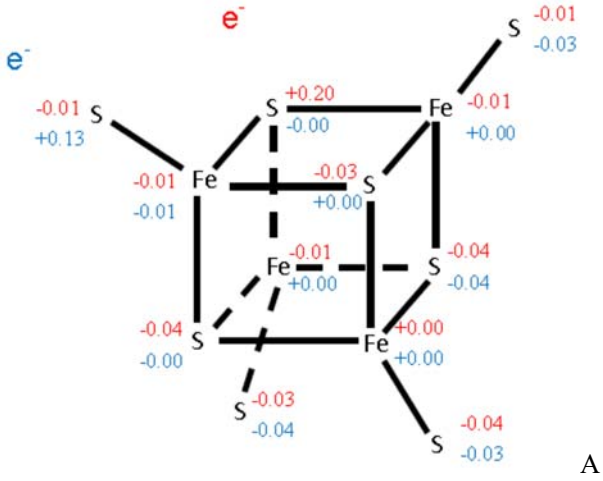
Placing a negative point charge 5Å away from one thiolate increases the total sulfide and thiolate contributions from 429.0% to 439.1% and from 139.3% to 144.8% respectively, which correspond to a total sulfur contribution increase from 568.4% to 583.9%. This is qualitatively similar to experiment but the effect is less than observed experimentally due to the simple model. Placing a negative point charge 5Å from one sulfide gave similar results (Table S1); the total sulfide and thiolate contributions increased to 438.3% and 143.9% respectively, and total sulfur contribution increased to 582.2%.

The DFT calculations in Table S1 show that the addition of the negative charge near the [Fe_4S_4] cluster can qualitatively model the increase in covalency observed with DNA binding and thus provide insight into the origin of this effect. Figure S7 gives the change in Mulliken charge distribution (A), Mayer bond orders (MBO) (B) and C^2 contributions (C, which sums the contributions of the valence atomic orbitals over the unoccupied valence molecular orbitals, for each Fe and S atom of the cluster) due to

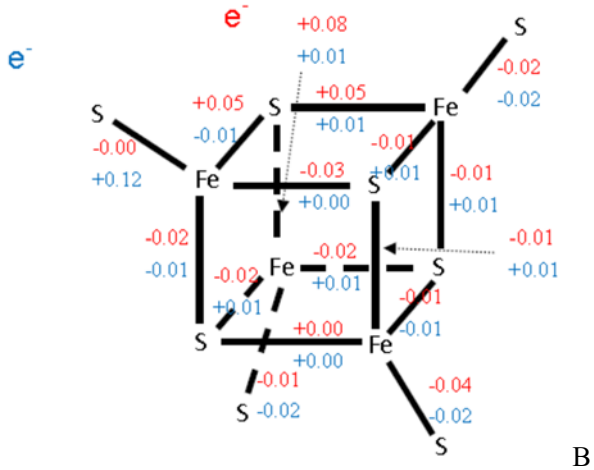
the addition of the negative point charge. The change in C^2 would directly relate to the change in S edge intensity observed experimentally. The changes calculated for placing the point charge next to a thiolate are given in blue, and next to a sulfide are given in red.

Adding a point charge near the thiolate (Figure S7, blue) results in a shift of the electron density toward the opposite side of the cluster (A). The thiolate next to the point charge (denoted S*) loses electron density (change in Mulliken charge +0.13), while the three thiolates and one sulfide on the opposite side of the cluster gain some negative charge (~ -0.03, A). Thus S* donates more charge density to the Fe it coordinates (denoted Fe*); this is reflected in its increase in MBO (+0.12 for Fe*-S* in B). The three sulfides that coordinate to Fe* then donate less to it to compensate (MBO ~-0.01 each in B), but donate more to the remaining Fe's (MBO ~+0.01). The other three thiolates have decreased MBO (-0.02 each in B) as they now donate less charge density to their coordinated Fe atoms, which is consistent with their increase in negative Mulliken charge (A). Although there are some compensation effects, the total MBO increases (+0.06). In terms of C^2 (Figure S7C), because the Fe*-S* bond becomes stronger (from the MBO), more S 3p character is mixed into the unoccupied Fe 3d orbitals, leading to the increase in S* C^2 contribution (+3.4) and decrease of the Fe* contribution (-4.9). Although the other three sulfide bonds to Fe* get slightly weaker (from the MBO in B), these sulfides have net higher C^2 (+4.6, -0.4, +3.4 in C). The negative point charge at S* destabilizes the occupied S p orbital energies (Scheme 2), which results in more S character mixed into the unoccupied valence molecular orbitals. The Fe d characters all decrease in response to the increase of S p, thus both sulfide and thiolate C^2 increase as observed experimentally.

Similar effects were observed when placing the point charge 5Å away from the sulfide at the corner of the cube (Figure S7, red). Note that except for the S atom and Fe-S bond(s) closest to the charge, there is only a limited change in Mulliken charge, MBO and C^2 contributions for the rest of the cluster. The net change is an increase in C^2 character, again qualitatively consistent with the increased intensity observed in the S K-edge XAS experiment upon DNA binding.



A



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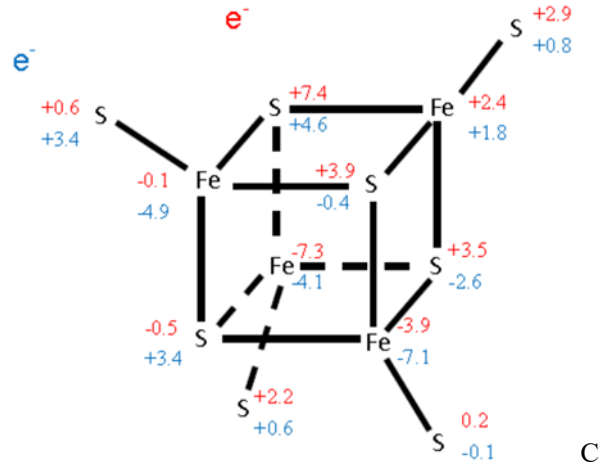

C

Figure S7, Difference in Mulliken charge distribution (A), Mayer bond orders (MBP) (B) and C^2 contributions (C) of each Fe and S atom of the cluster without and with a point charge at 5 Å distance to the cluster. Blue for point charge near a thiolate, and red for point charge near a sulfide.

Table S1 Results of DFT Calculation

Model	Total Fe %	Total Sulfide %	Total Thiolate %	Total S %
$[Fe_4S_4(SMe)_4]^{2-}$ model	958.4	429.0	139.3	568.4
$[Fe_4S_4(SMe)_4]^{2-}$ with $1e^-$ at 5Å from sulfide	944.1	438.3	143. 9	582.2
$[Fe_4S_4(SMe)_4]^{2-}$ with $1e^-$ at 5Å from thiolate	949.3	439.1	144.8	583.9