Sensitivity Analysis

Run on trajectory CIACGACxrd, 50 ns

Input Variables:

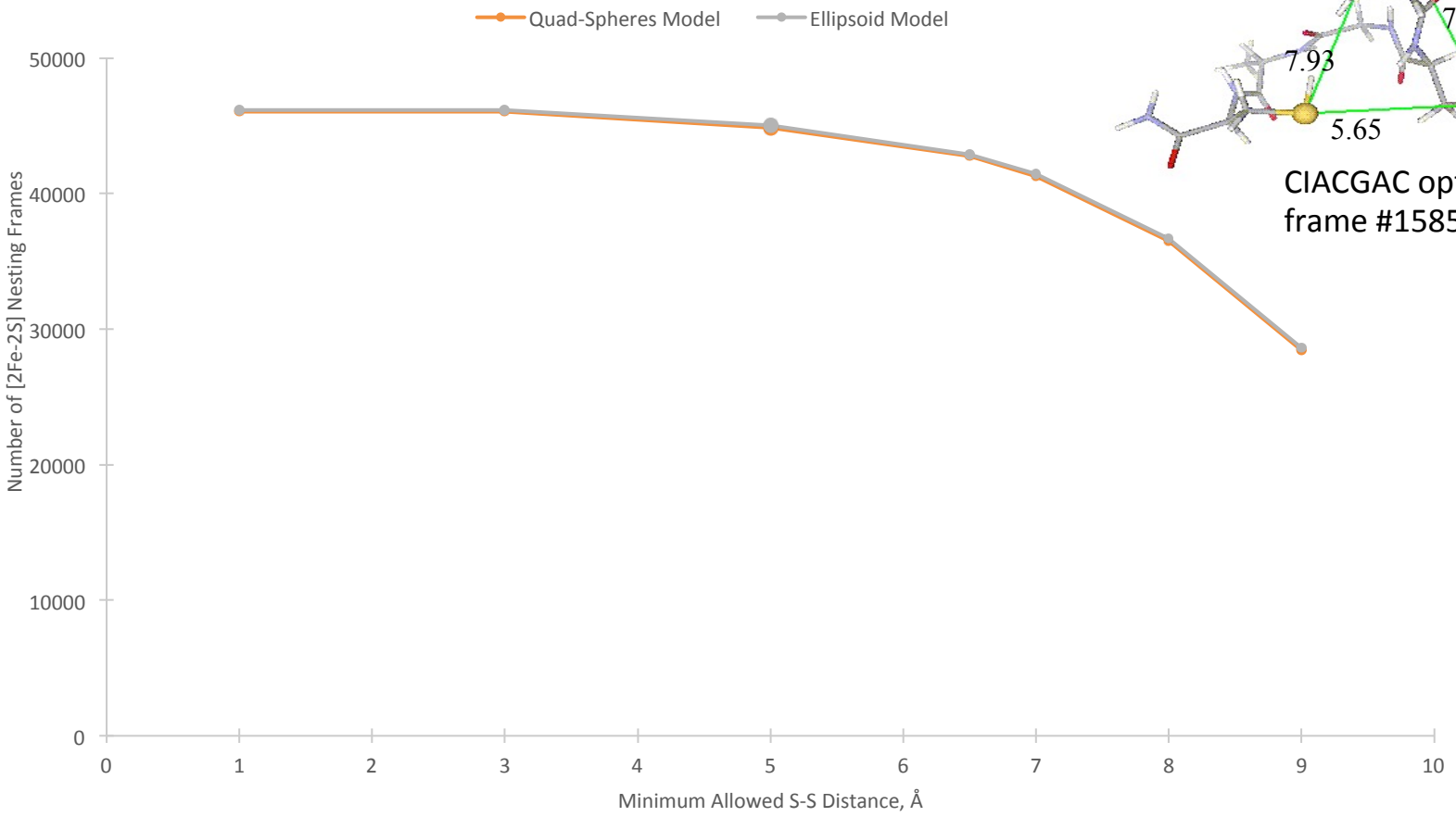
```
NESTING ANALYSIS INPUT PARAMETERS
                          Minimum distance allowed between cysteine sulfur atoms: 5.0 Å
  Maximum distance allowed between cysteine sulfur atoms (in threading analysis): 7.0 (8.0) Å
    Expansion of cluster radius to exclude ionic interactions hindering the nest: 1.50 Å
                      [4Fe-4S] defined distance between sulfur and cluster edge: 2.27 Å
            (average +/- 0.01 Angstroms determined from 5D8V crystal structure)
   [4Fe-4S] maximum distance allowed between cluster edge and tested sulfur atoms: 2.30 Å
      Radius of [4Fe-4S] cluster (from 5D8V crystal structure +/- 0.01 Angstroms): 1.95 Å
                                              [2Fe-2S] ellipsoid x, y, and z-radii: 2.8120, 2.8120, 1.8506 Å
                [2Fe-2S] sphere radius (determined from 3WCQ crystal structure): 1.76 Å
[2Fe-2S] sphere displacement from center (determined from 3WCQ crystal structure): 1.85 Å
```

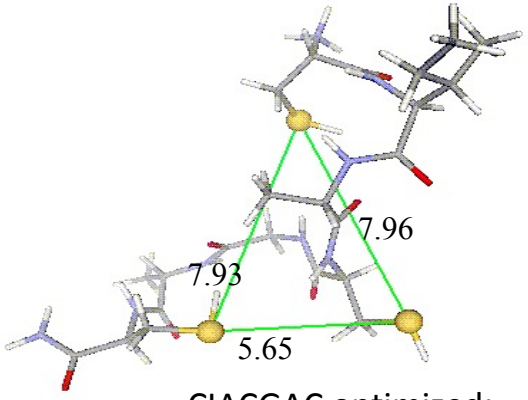
NESTING ANALYSIS INPUT PARAMETERS

	Input Value (Å)		% Difference in Nesting out of Total Trajectory	
Minimum distance allowed between cysteine sulfur atoms:	5	+10%	−4.04/−4.02	Filter used for [2Fe-2S] Ellipsoid/[2Fe-2S] Spheres
		−10%	+3.27/+3.25	
Maximum distance allowed between cysteine sulfur atoms in nesting analysis:	7	+10%	+13.18/+13.15/+0.03	[2Fe-2S] Ellipsoid/[2Fe-2S] Spheres/[4Fe-4S] opening or closing the criteria used to consider a frame (a pre-nesting filter) − does not reflect whether a cluster fit into the allotted space
		−10%	−14.27/−14.21/−0.01	
Maximum distance allowed between cysteine sulfur atoms in threading analysis:	8	+10%	+0.99	Non-Threading filter
		−10%	−1.00	
Expansion of cluster radius to exclude ionic interactions hindering the nest:	1.5	+10%	−0.05/−0.06/−0.00	[2Fe-2S] Ellipsoid/[2Fe-2S] Spheres/[4Fe-4S]
		−10%	+0.04/+0.06/+0.00	
[4Fe-4S] defined distance between sulfur and cluster edge (average +/- 0.01 Å from 5D8V crystal structure):	2.27	+10%	−0.01	[4Fe-4S]
		−10%	+0.00	
[4Fe-4S] maximum distance allowed between cluster edge and tested sulfur atoms:	2.3	+10%	+0.00	[4Fe-4S]
		−10%	−0.01	
Radius of [4Fe-4S] cluster (from 5D8V crystal structure +/- 0.01 Å):	1.95	+10%	−0.00	[4Fe-4S]
		−10%	+0.00	
[2Fe-2S] ellipsoid x, y, and z-radii:	2.8120, 2.8120, 1.8506	+10%*	−0.02	[2Fe-2S] Ellipsoid
		−10%*	+0.03	
[2Fe-2S] sphere radius (determined from 3WCQ crystal structure):	1.76	+10%	−0.07	[2Fe-2S] Spheres
		−10%	+0.07	
[2Fe-2S] sphere displacement from center (determined from 3WCQ crystal structure):	1.85	+10%	−0.05	[2Fe-2S] Spheres
		−10%	+0.04	

*based on ellipsoid volume

Sensitivity Analysis — S-S distance limits

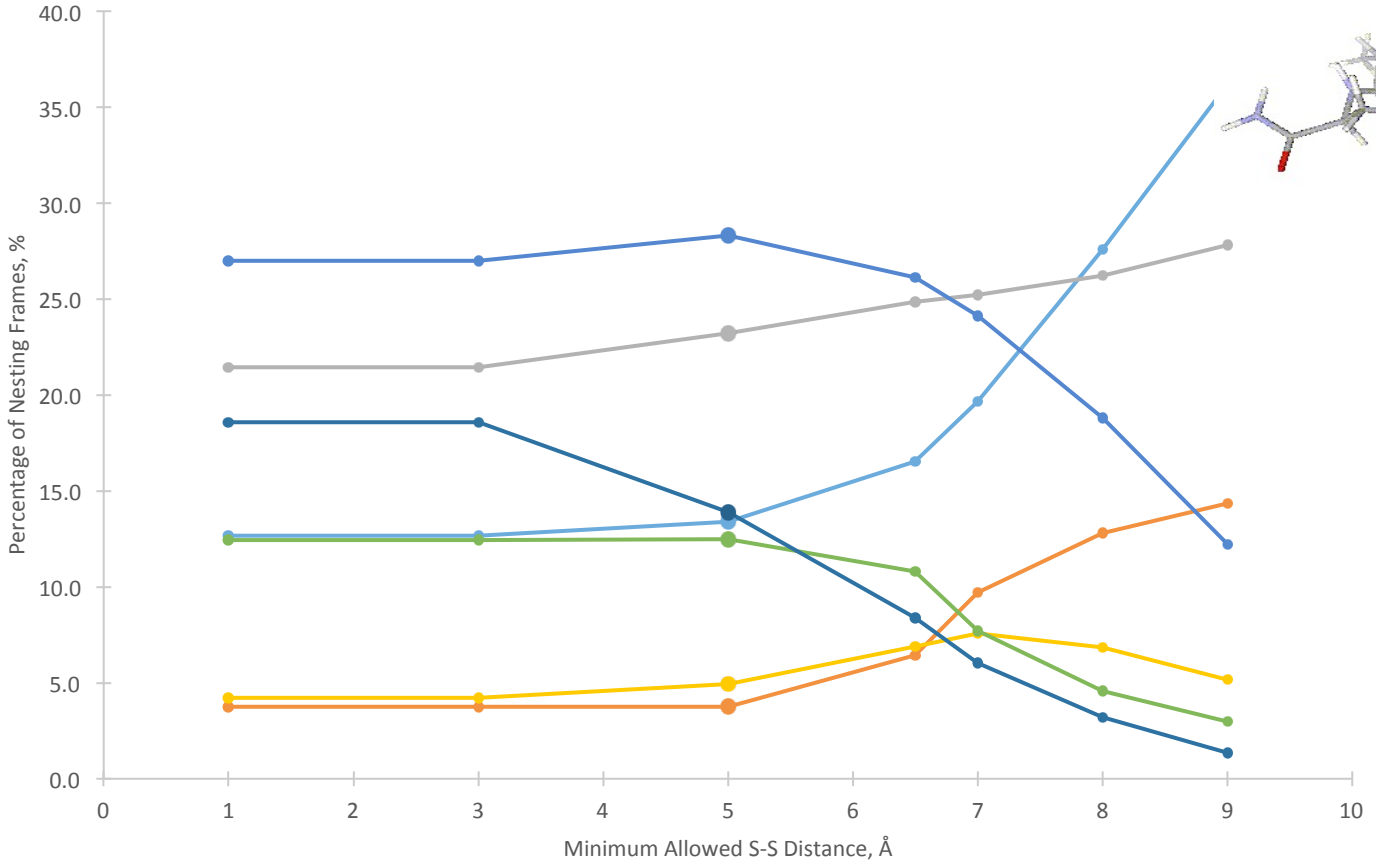


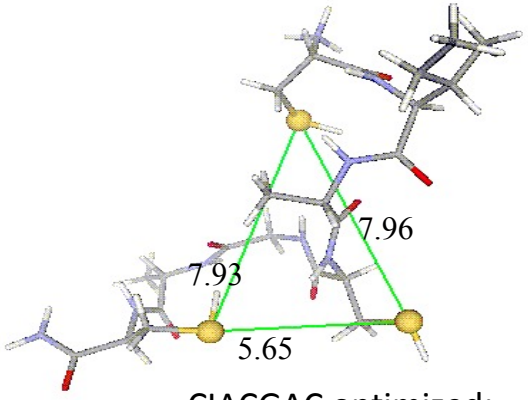


CIACGAC optimized:
frame #1585

Figure S1A. Plot of the minimum allowed distance between cysteine sulfur atoms versus the number of [2Fe-2S] nesting frames. As expected, as the minimum allowed distance gets larger than the nesting distances in the 1DUR crystal structure, the number of [2Fe-2S] nesting frames decreases. The results for the quad-spheres and ellipsoid models are consistent and the selected value of **5.0 Å** occurs prior to any significant decrease in nesting.

Sensitivity Analysis — S-S distance limits

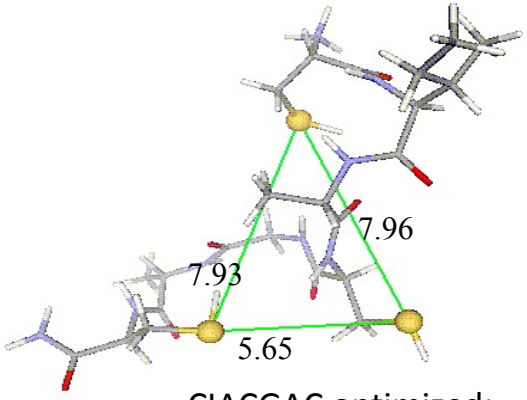




CIACGAC optimized: frame #1585

Figure S1B. Plot of the minimum allowed distance between cysteine sulfur atoms versus the percentage of [2Fe-2S] nesting frames for each coordination isomer using the ellipsoid model. As expected, as the minimum allowed distance gets larger than the experimental nesting distances in the 3WCQ crystal structure, the percentages of single coordination [2Fe-2S] nesting increases while double and triple coordination decreases. The selected minimum of **5.0 Å** occurs prior to any significant changes in the nesting percentages.

Sensitivity Analysis — S-S distance limits



CIACGAC optimized:
frame #1585

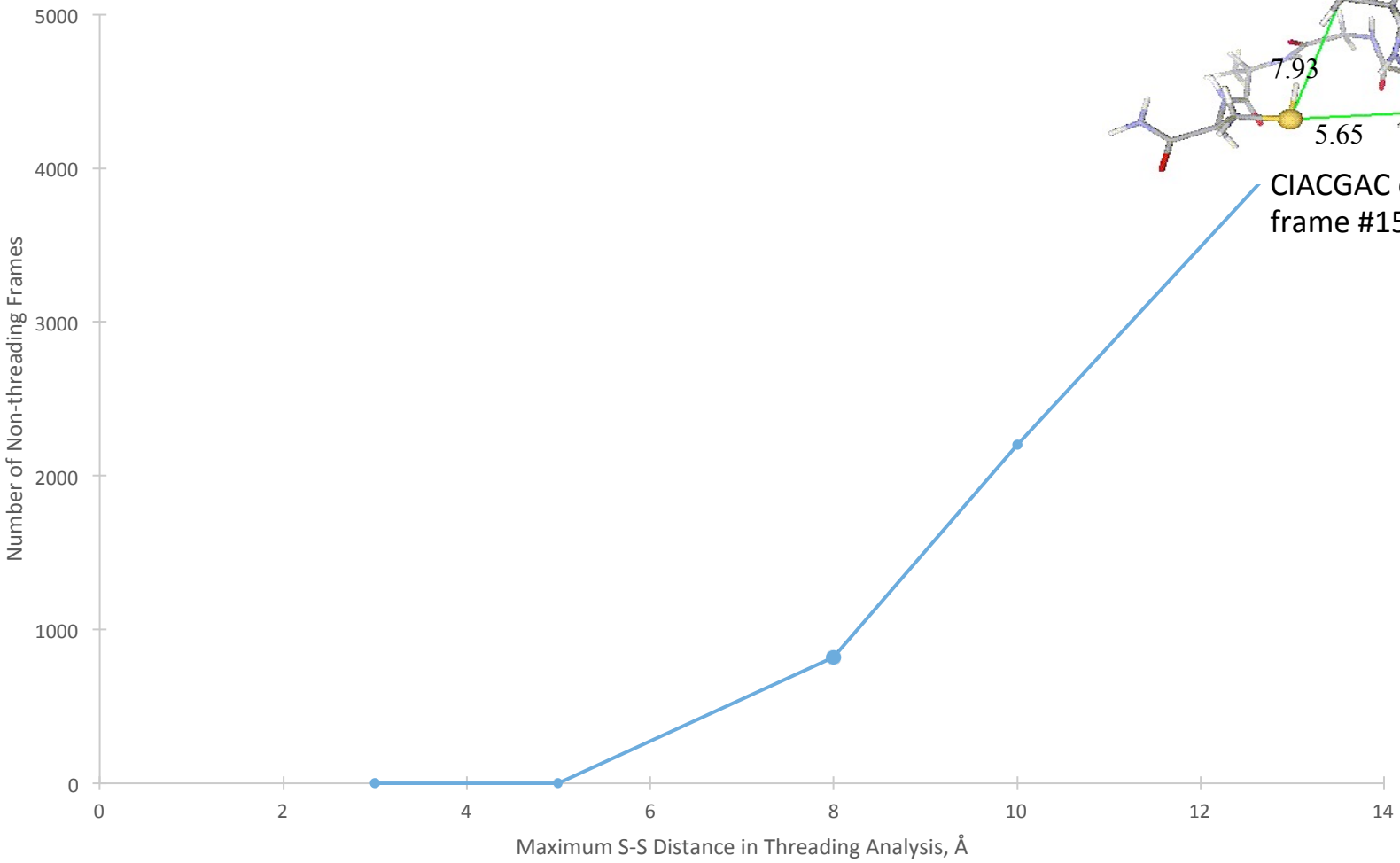
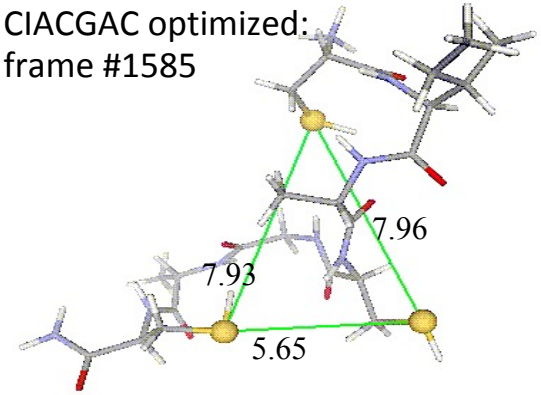
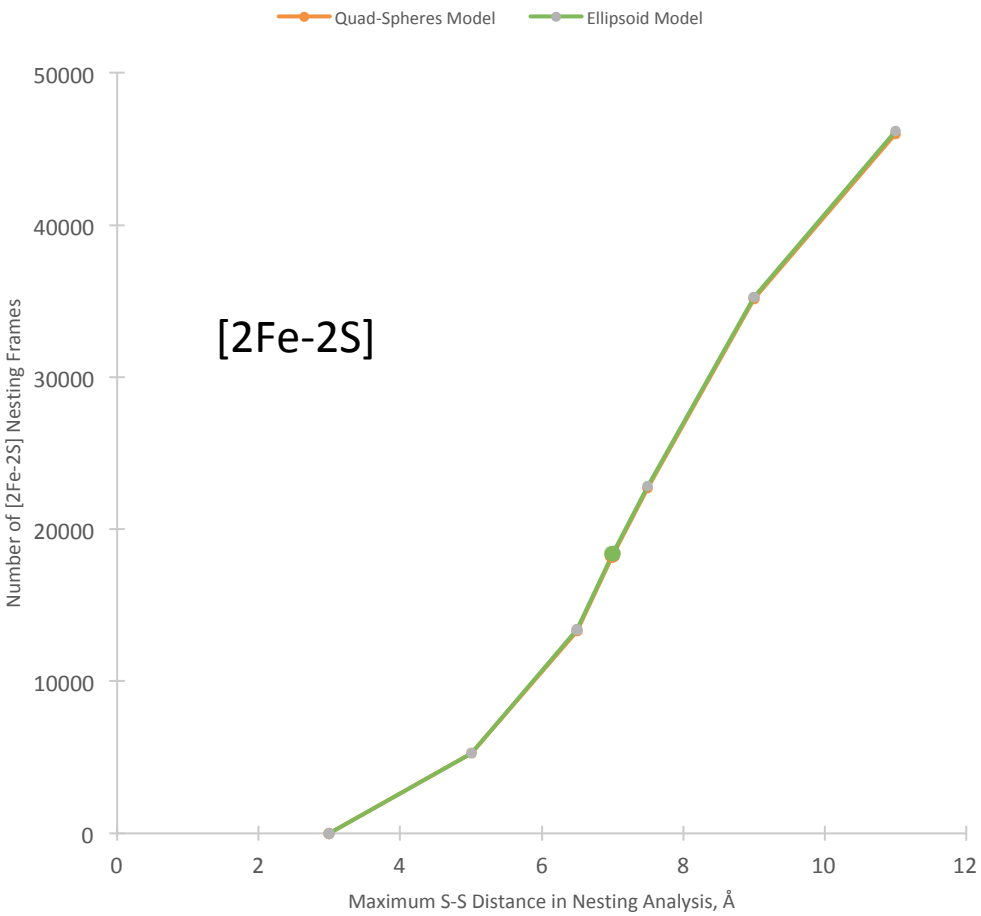


Figure S2. Plot of the maximum allowed distance between cysteine sulfur atoms versus the number of non-threading frames. As expected, as the maximum allowed distance gets larger than the nesting distances in the 1DUR crystal structure, the number of non-threading frames increases. The selected value is **8.0 Å**.

Sensitivity Analysis — S-S distance limits





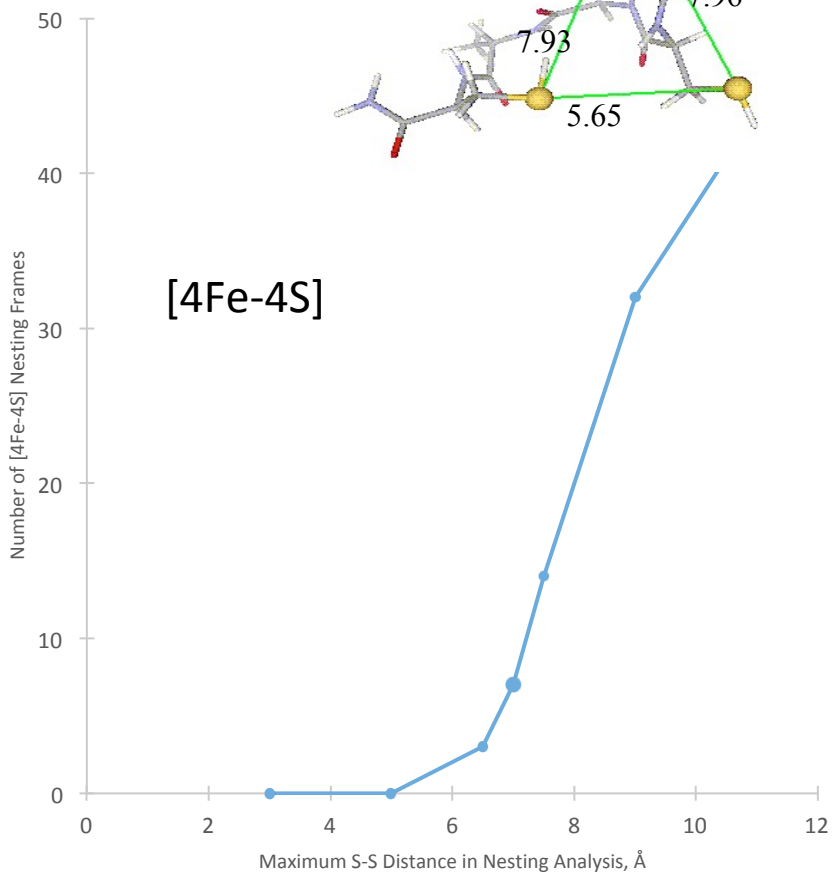
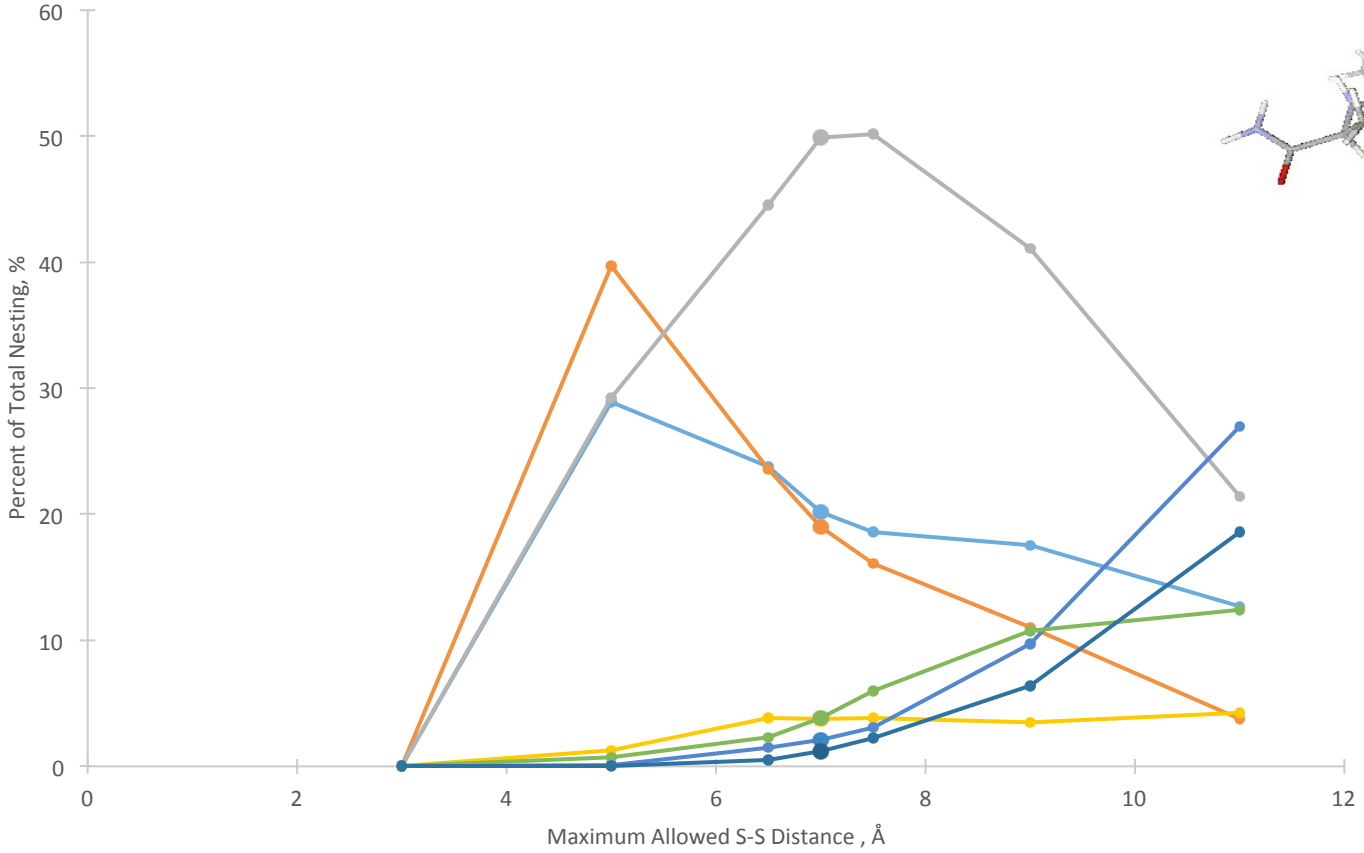
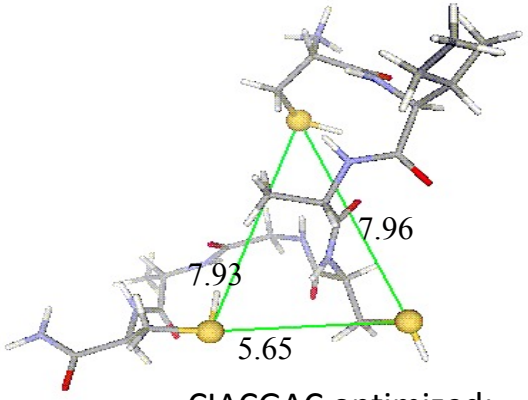


Figure S3A. Plot of the maximum allowed distance between cysteine sulfur atoms versus the number of [2Fe-2S] (left) and [4Fe-4S] (right) nesting frames. As expected, as the maximum allowed distance gets larger than the nesting distances in the 1DUR crystal structure, the number of both [4Fe-4S] and [2Fe-2S] nesting frames increases. The results for the quad-spheres and ellipsoid models for [2Fe-2S] are consistent. The selected value is **7.0 Å**.

Sensitivity Analysis — S-S distance limits

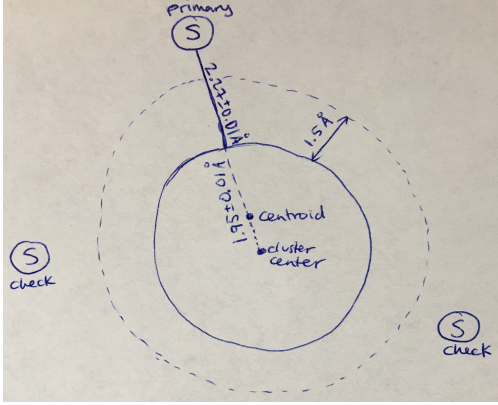


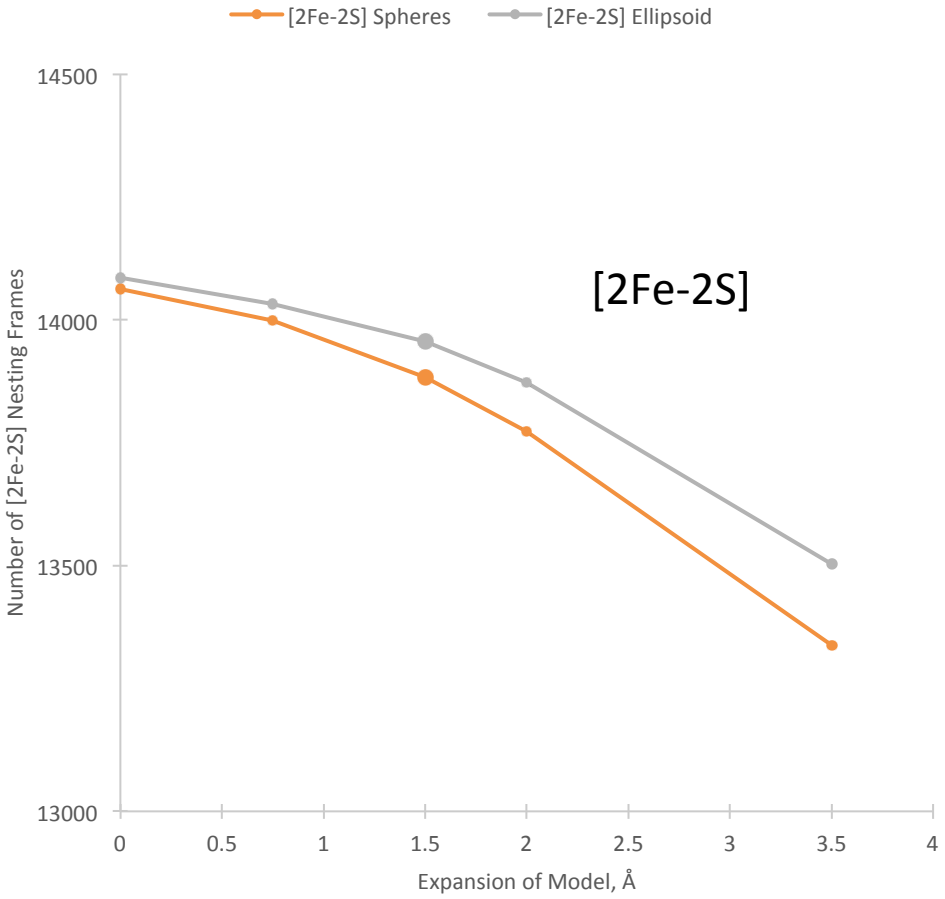


CIACGAC optimized: frame #1585

Figure S3B. Plot of the maximum allowed distance between cysteine sulfur atoms versus the percentage of [2Fe-2S] nesting frames for each coordination isomer using the ellipsoid model. As expected, as the maximum allowed distance approaches the nesting distances in the 3WCQ crystal structure, the nesting percentages increase overall. Beyond that point, the percentages of single coordination [2Fe-2S] nesting decrease while double and triple coordination increase. This trend is consistent with the results in Figure S1B looking at the minimum allowed sulfur-sulfur distance. The selected maximum distance value is **7.0 Å**.

Sensitivity Analysis — Cluster model size





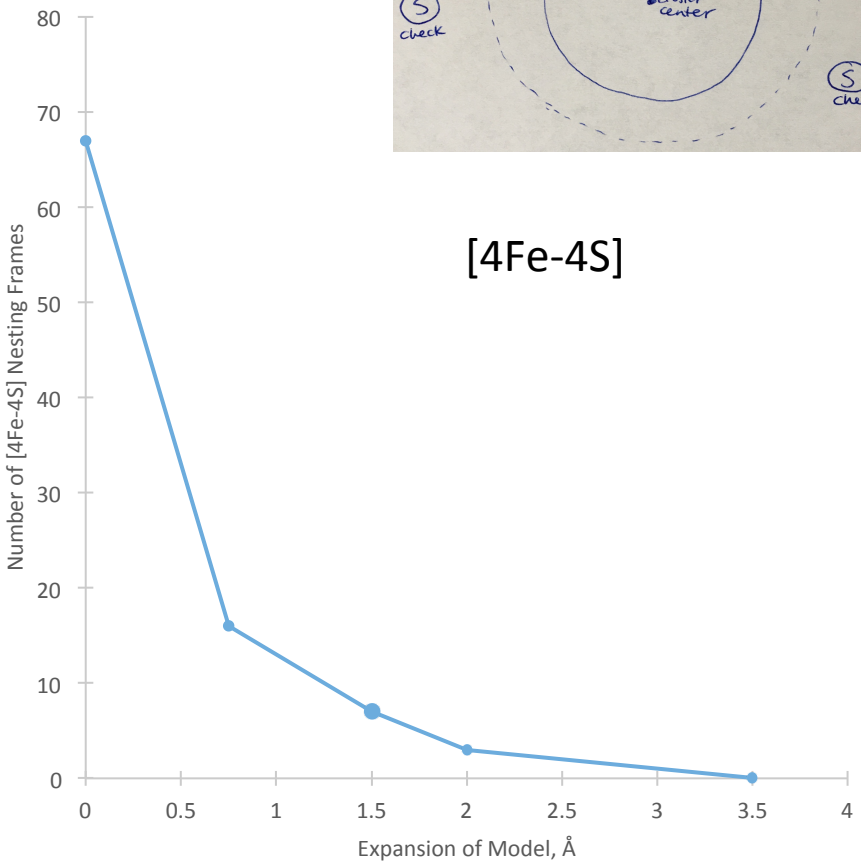
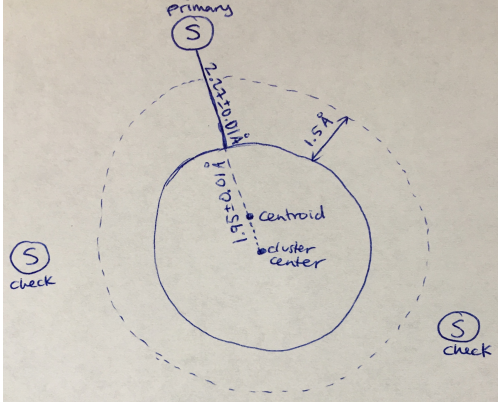


Figure S4A. Plot of the model expansion to exclude ionic interactions hindering the nest versus the number of [2Fe-2S] (left) and [4Fe-4S] (right) nesting frames. As expected, the farther the model is expanded, the smaller the numbers of [2Fe-2S] and [4Fe-4S] nesting frames. The results for the quad-spheres and ellipsoid models for [2Fe-2S] are consistent for the selected value of **1.50 Å** with significantly less than a 1% difference. However, the deviation between the models increases with further expansion of the model.

Sensitivity Analysis — Cluster model size



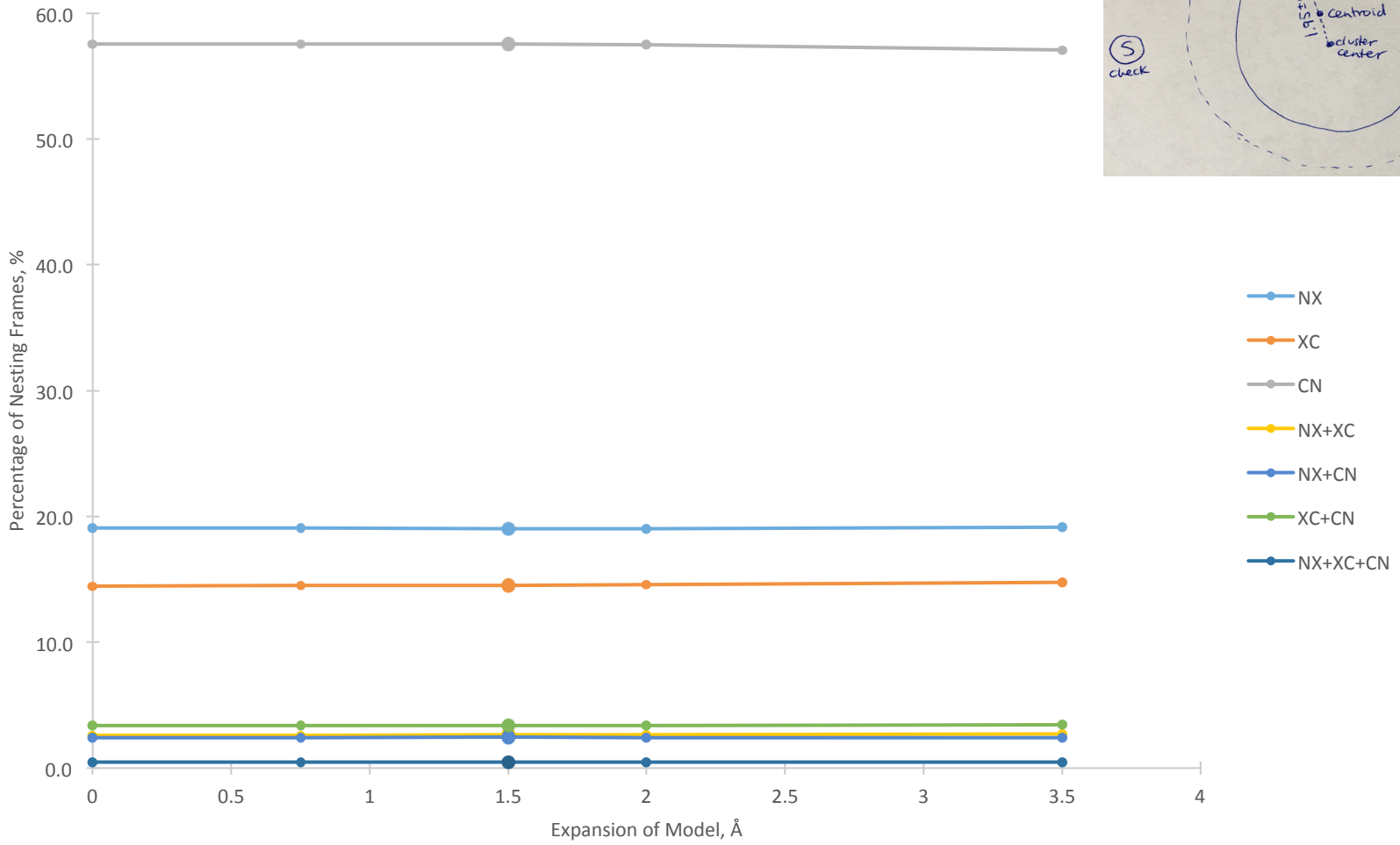
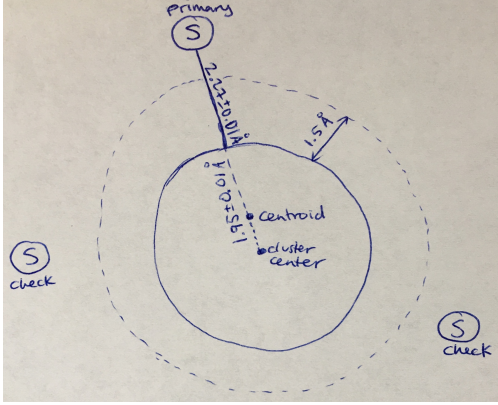


Figure S4B. Plot of the model expansion to exclude ionic interactions hindering the nest versus the percentage of [2Fe-2S] nesting frames for each coordination isomer using the ellipsoid model. As expected, the expansion of the model has relatively no impact on the overall nesting percentages. The selected value for expansion is **1.50 Å**.

Sensitivity Analysis — Cluster model size



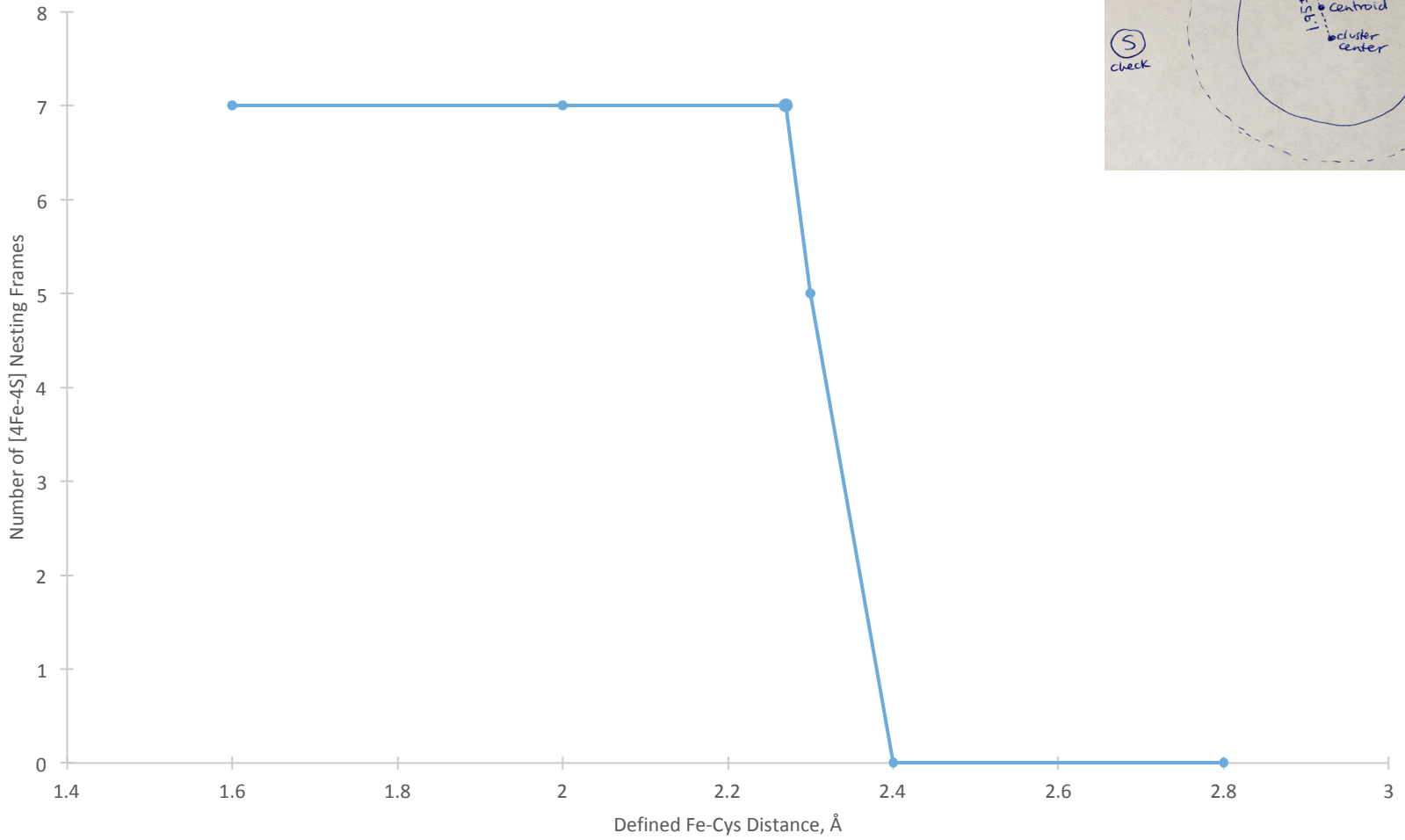
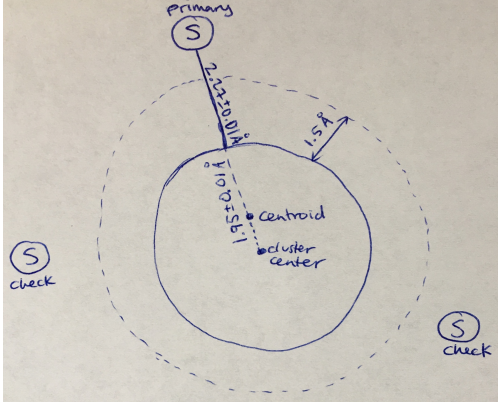


Figure S5. Plot of the defined distance between the cysteine sulfur and the [4Fe-4S] cluster edge versus the number of [4Fe-4S] nesting frames. As expected, as the iron-sulfur distance increases beyond the **2.27 ± 0.01 Å** measured in the 5D8V crystal structure, the model expands and the number of [4Fe-4S] nesting frames rapidly goes to zero.

Sensitivity Analysis — Cluster model size



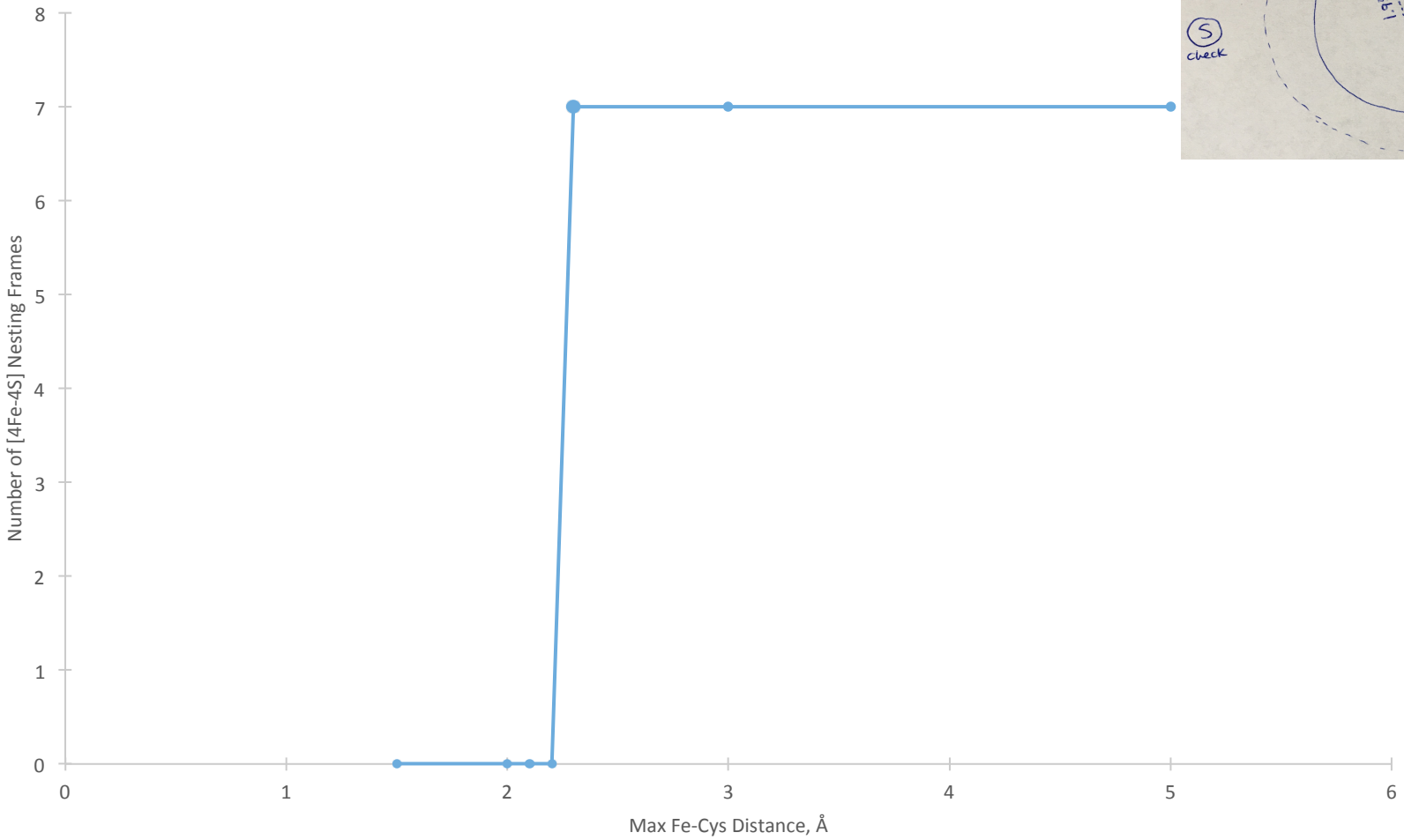
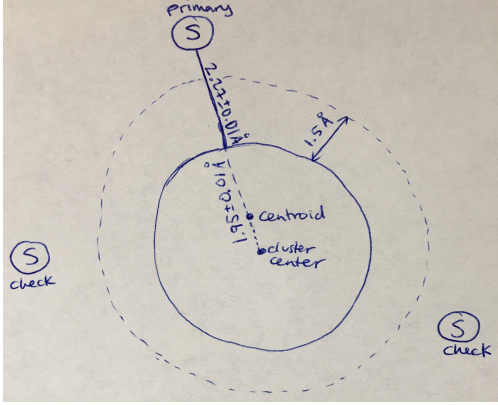


Figure S6. Plot of the allowed distance between the [4Fe-4S] cluster edge and the tested cysteine sulfur versus the number of [4Fe-4S] nesting frames. As expected, as the cluster-sulfur distance increases beyond the 2.27 ± 0.01 Å measured in the 5D8V crystal structure, the model becomes more lenient and the number of [4Fe-4S] nesting frames increase. The selected allowed distance is **2.30 Å**.

Sensitivity Analysis — Cluster model size



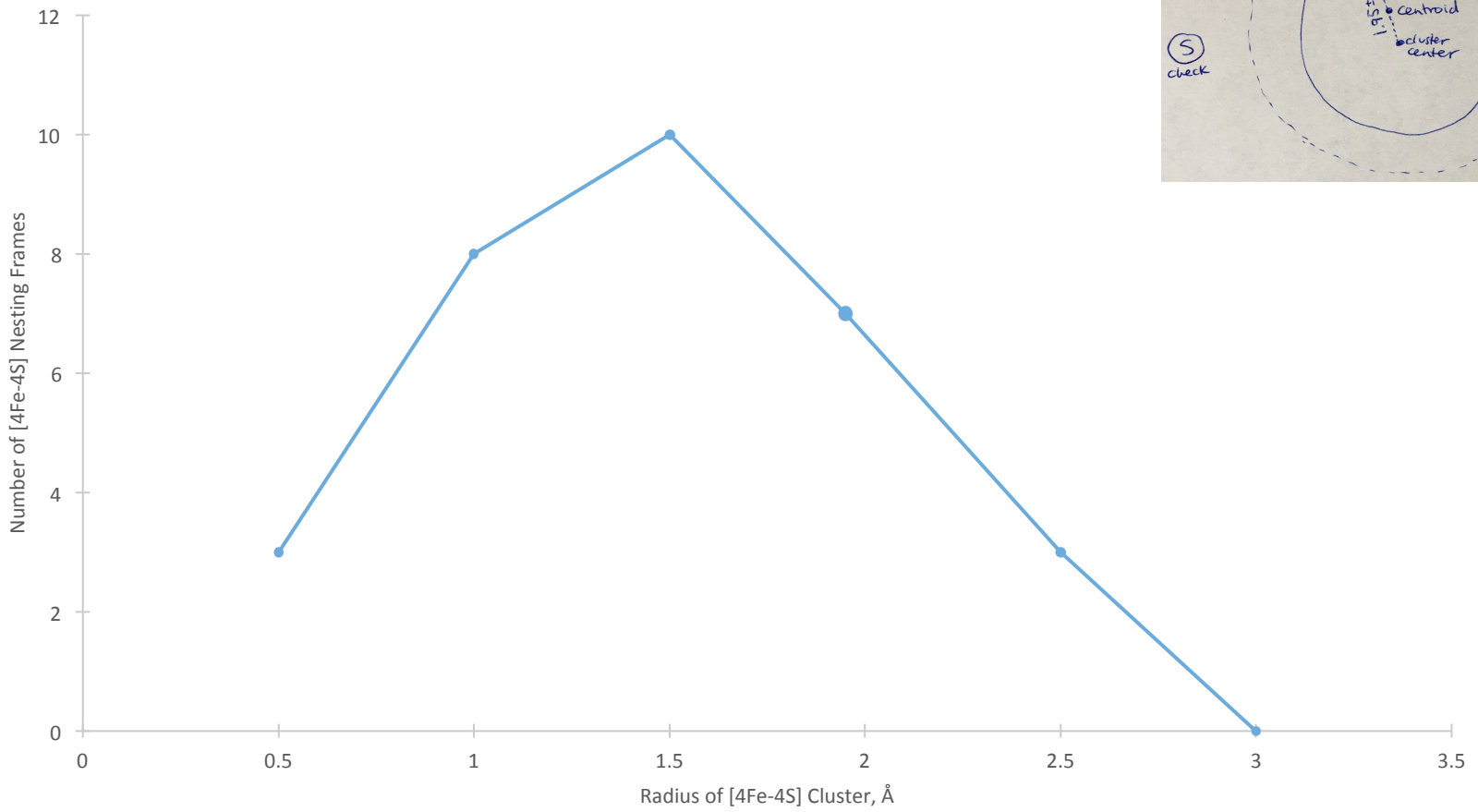
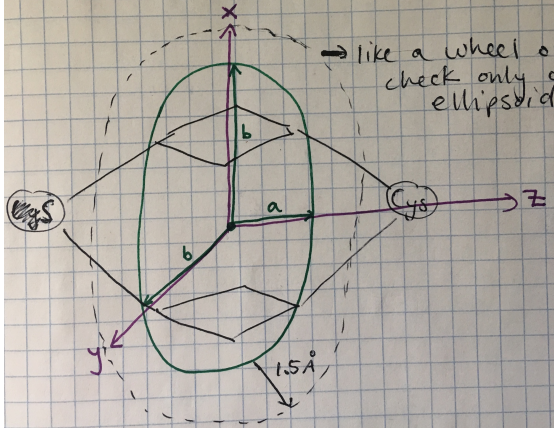


Figure S7. Plot of the radius of the [4Fe-4S] cluster spherical model versus the number of [4Fe-4S] nesting frames. As expected, the number of [4Fe-4S] nesting frames increases with the radius until the model is large enough to fit within the three cysteine sulfur atoms. That this point the size of the model becomes such that the likelihood of atoms penetrating the model increases, eliminating the possibility of nesting and causing the number of [4Fe-4S] nesting frames to decrease. The selected value of **1.95 Å** is defined using the 5D8V crystal structure.

Sensitivity Analysis — Cluster model size



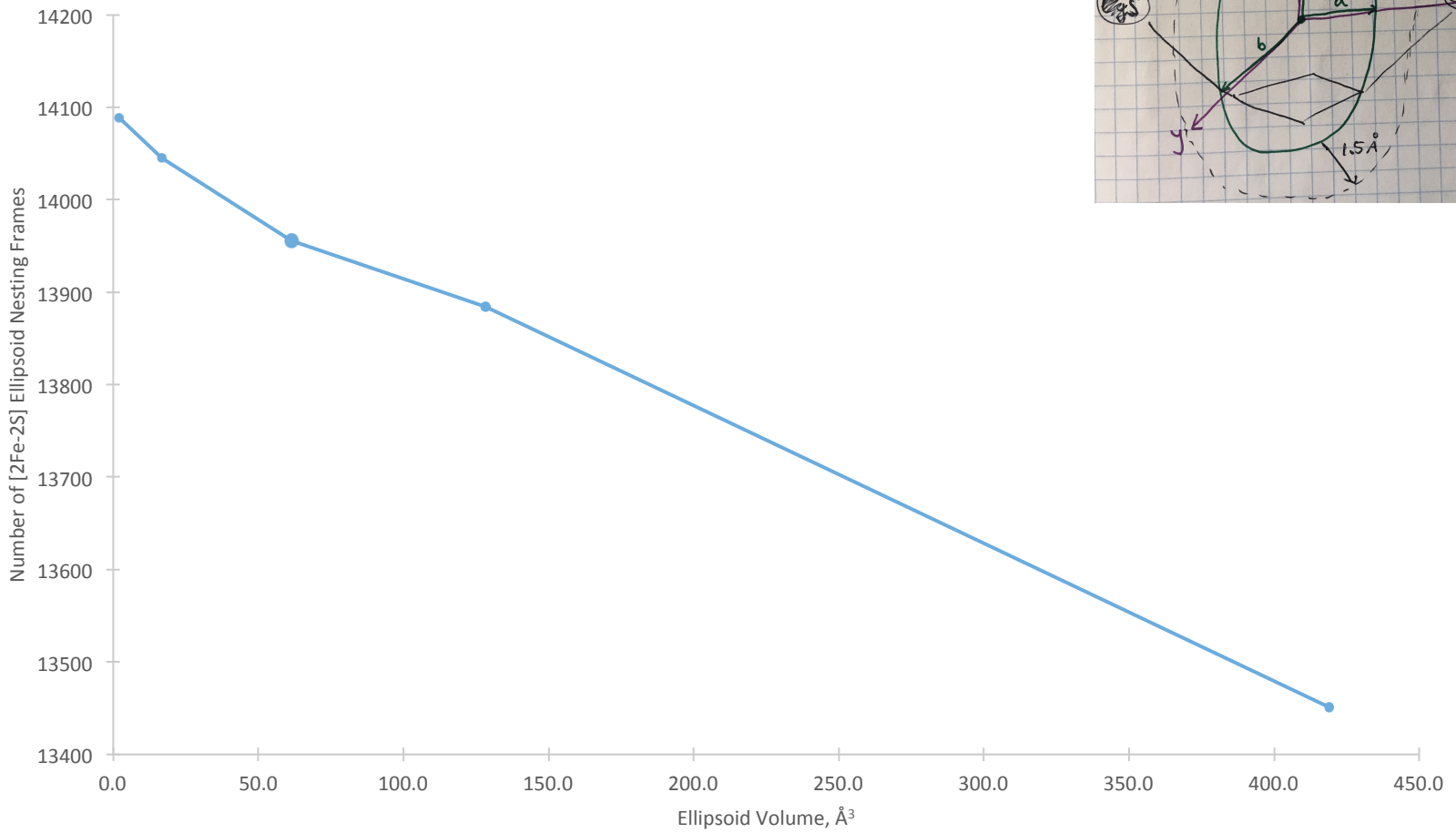
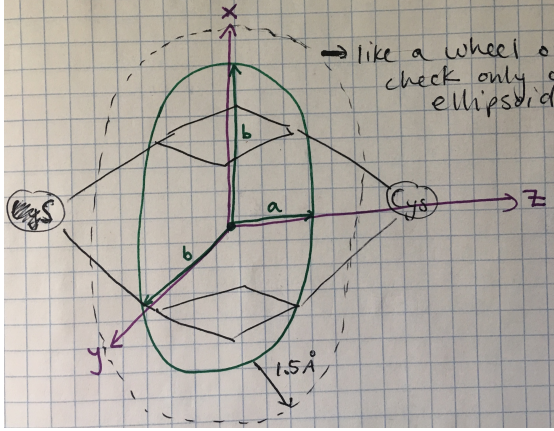


Figure S8A. Plot of the volume of the [2Fe-2S] cluster ellipsoid model versus the number of [2Fe-2S] nesting frames. As expected, the number of [2Fe-2S] nesting frames decrease as the ellipsoid volume increases. The trend seen in Figure S7 is not reflected here because the sulfur-sulfur distances are such that a contracted [2Fe-2S] cluster model still fits within the defined nest. The selected radii are **2.8120, 2.8120, 1.8506 Å** for a volume of 61.3 Å^3 are defined using the 3WCQ crystal structure.

Sensitivity Analysis — Cluster model size



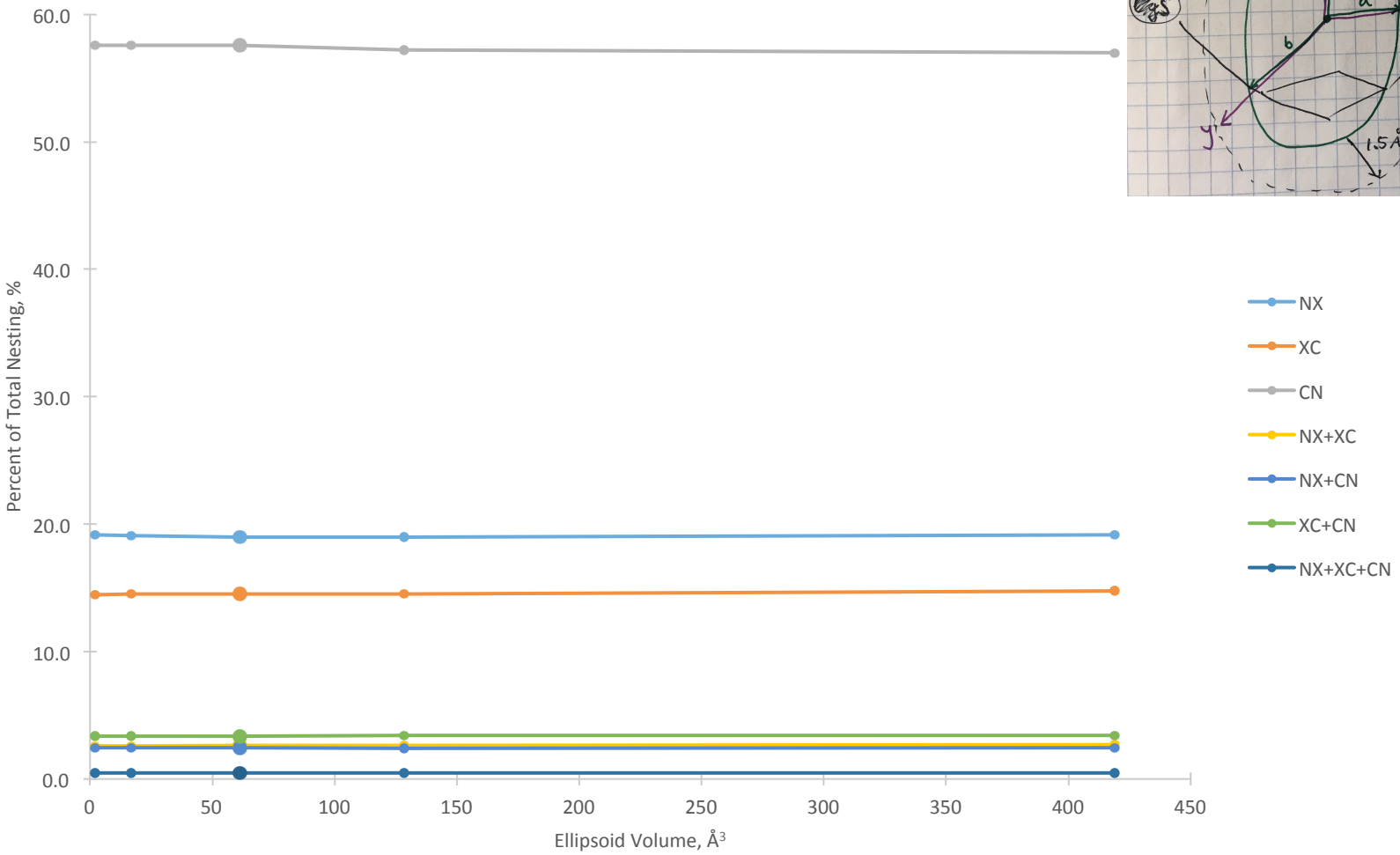
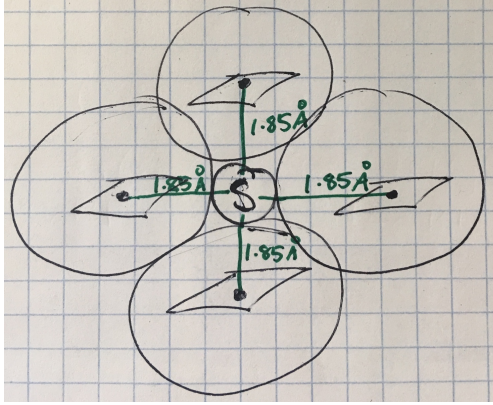


Figure S8B. Plot of the volume of the [2Fe-2S] cluster ellipsoid model versus the percentage of [2Fe-2S] nesting frames for each coordination isomer. As expected, the expansion of the model volume has relatively no impact on the overall nesting percentages. The selected radii are **2.8120, 2.8120, 1.8506 Å** for a volume of 61.3 Å^3 are defined using the 3WCQ crystal structure.

Sensitivity Analysis — Cluster model size



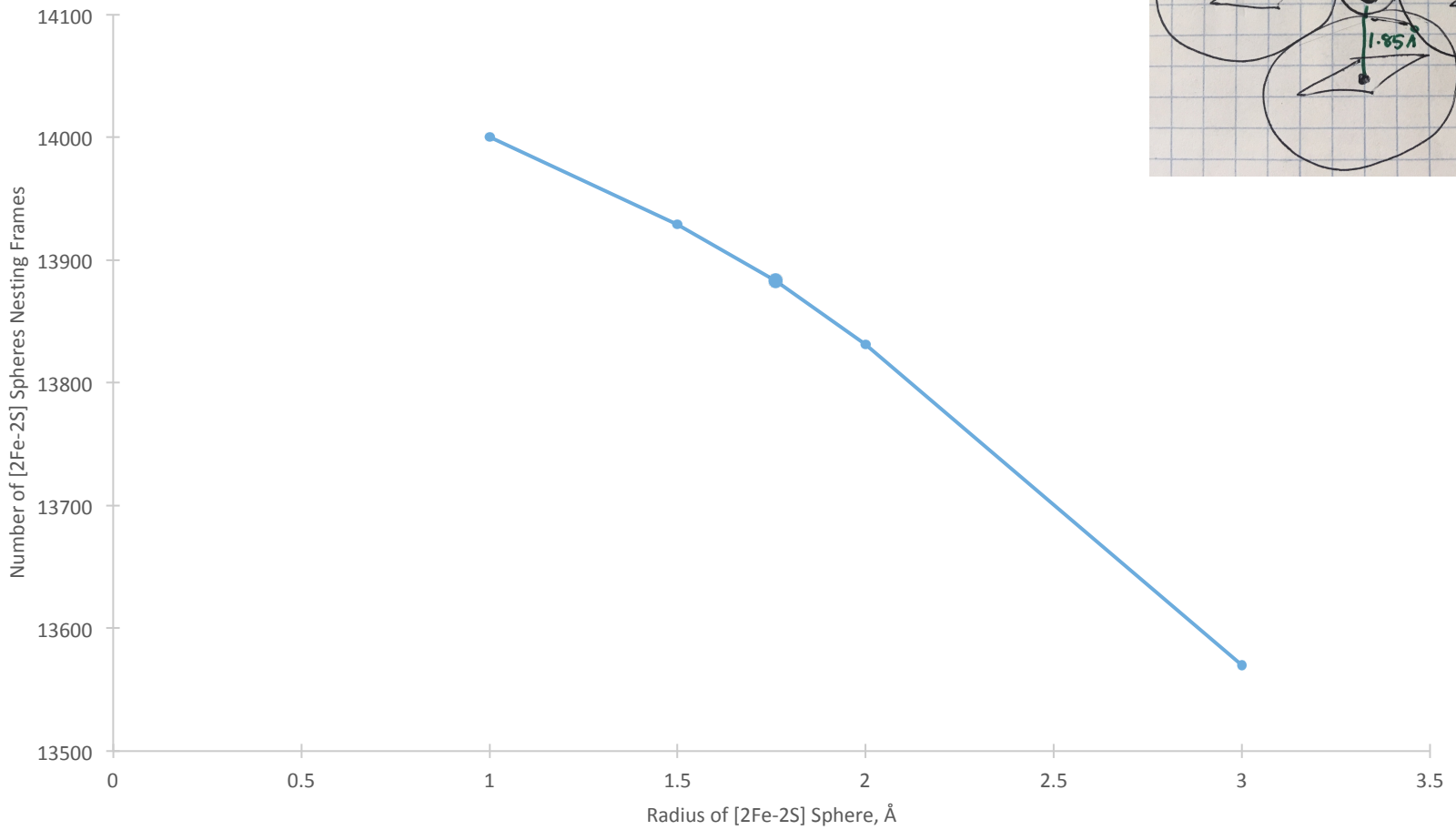
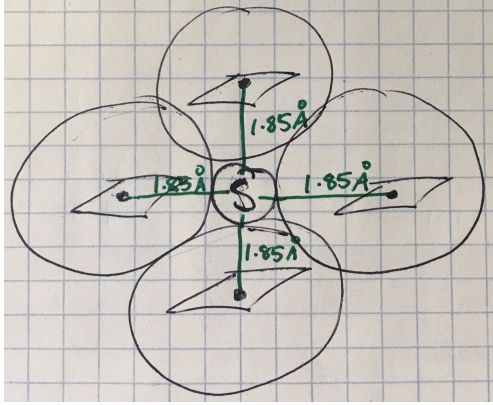


Figure S9A. Plot of the radius of a single sphere of the [2Fe-2S] cluster quad-sphere model versus the number of [2Fe-2S] nesting frames. As expected, the number of [2Fe-2S] nesting frames decrease as the sphere radius increases. The trend seen in Figure S7 is not reflected here because the sulfur-sulfur distances are such that a contracted [2Fe-2S] cluster model still fits within the defined nest. The selected radius of **1.76 Å** is defined using the 3WCQ crystal structure.

Sensitivity Analysis — Cluster model size



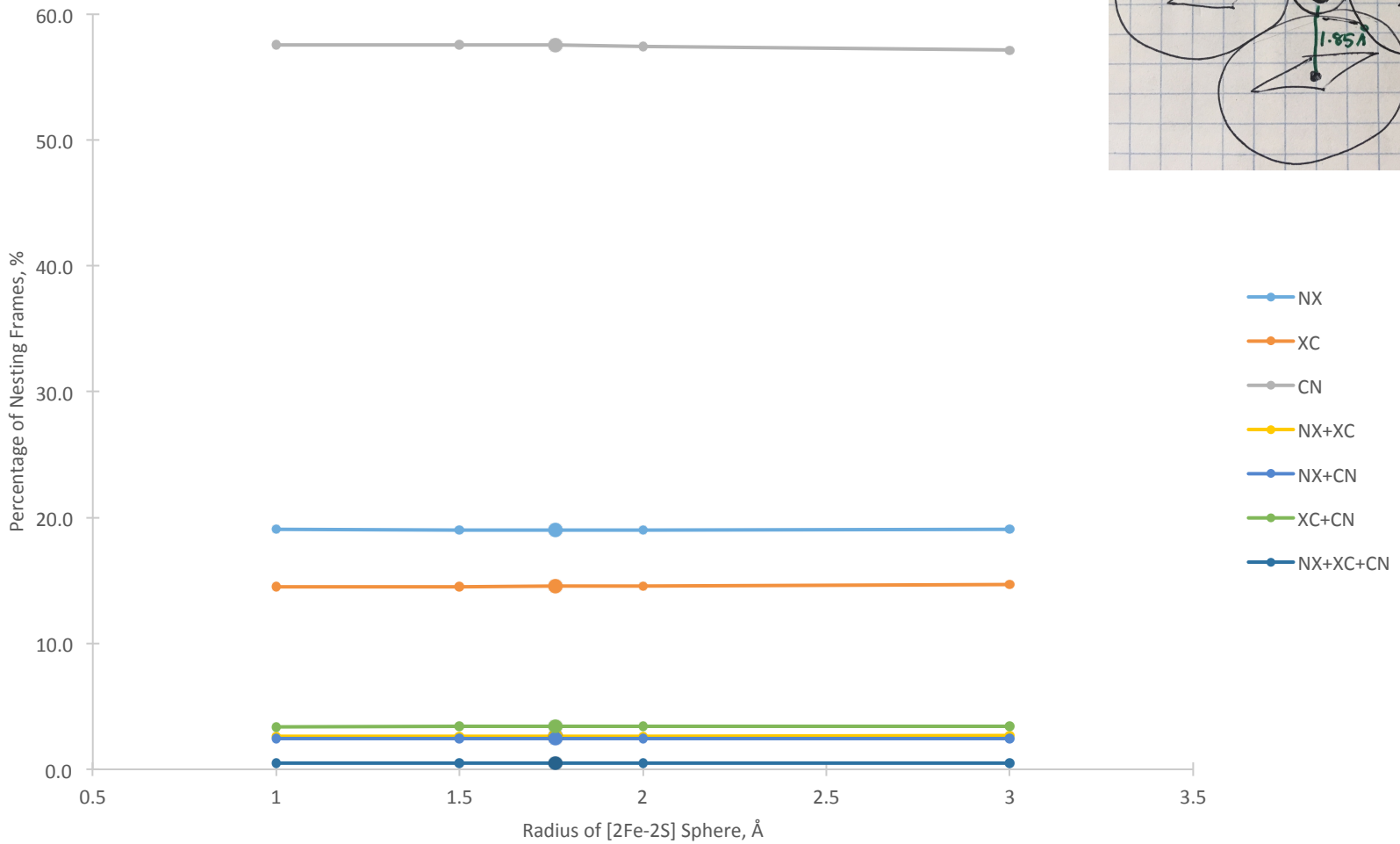
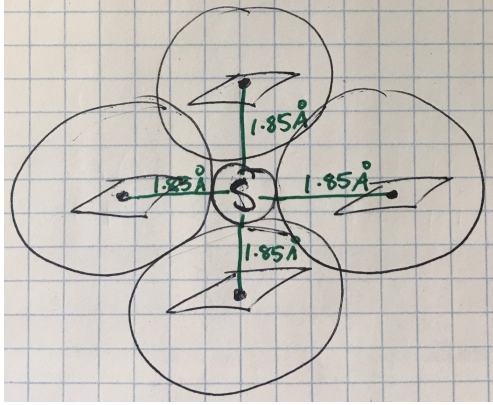


Figure S9B. Plot of the radius of a single sphere of the [2Fe-2S] cluster quad-sphere model versus the percentage of [2Fe-2S] nesting frames for each coordination isomer. As expected, the expansion of the model volume has relatively no impact on the overall nesting percentages. The selected radius of **1.76 Å** is defined using the 3WCQ crystal structure.

Sensitivity Analysis — Cluster model size



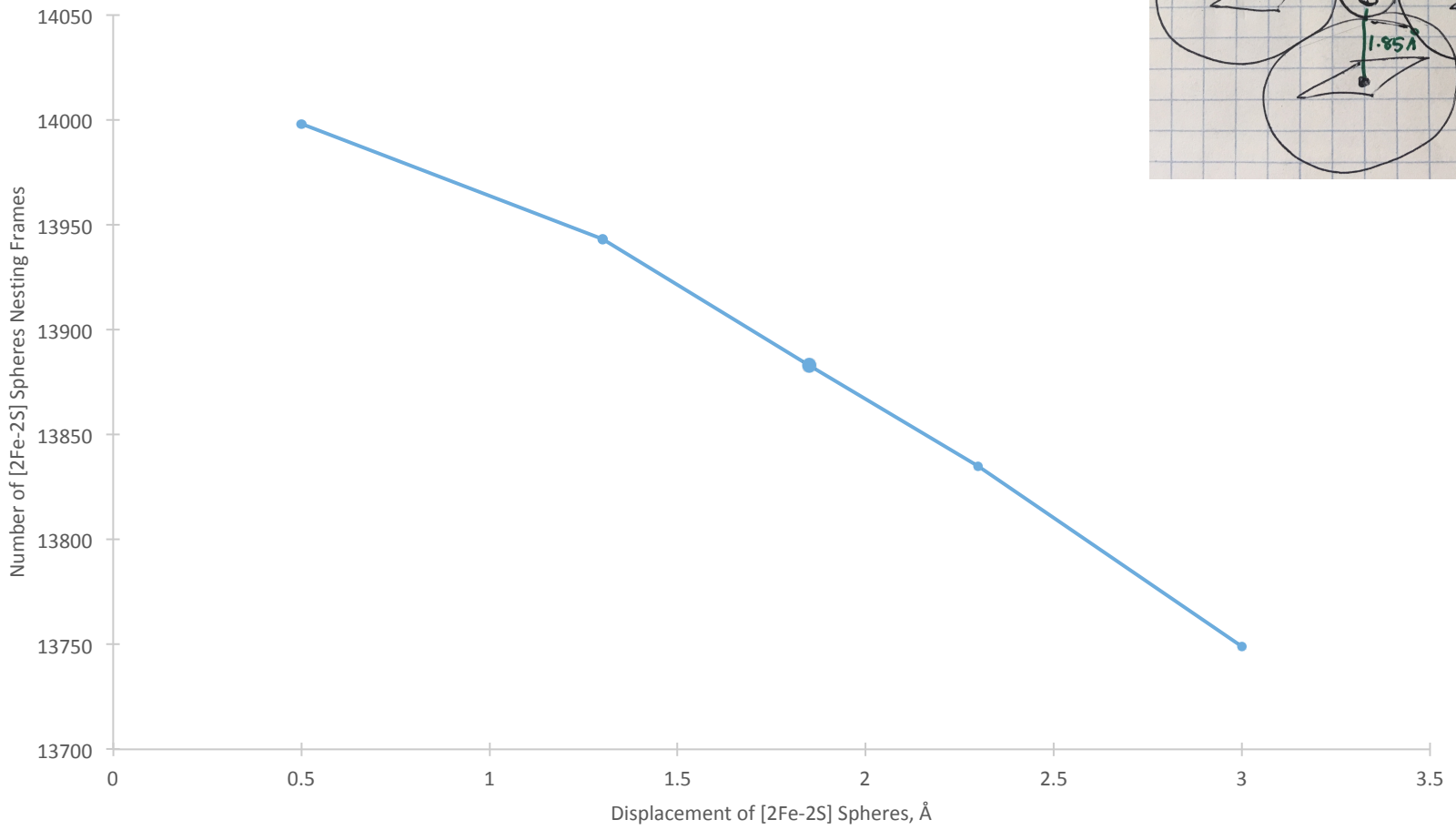
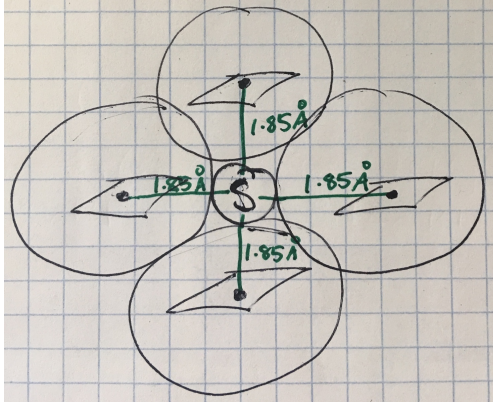


Figure S10A. Plot of the sphere displacement from the centroid in the [2Fe-2S] cluster quad-sphere model versus the number of [2Fe-2S] nesting frames. As expected, the number of [2Fe-2S] nesting frames decrease as the sphere displacement increases. The trend seen with model expansion in Figure S7 is not reflected here because the sulfur-sulfur distances are such that a contracted [2Fe-2S] cluster model still fits within the defined nest. The selected displacement value of **1.85 Å** is defined from the 3WCQ crystal structure.

Sensitivity Analysis — Cluster model size



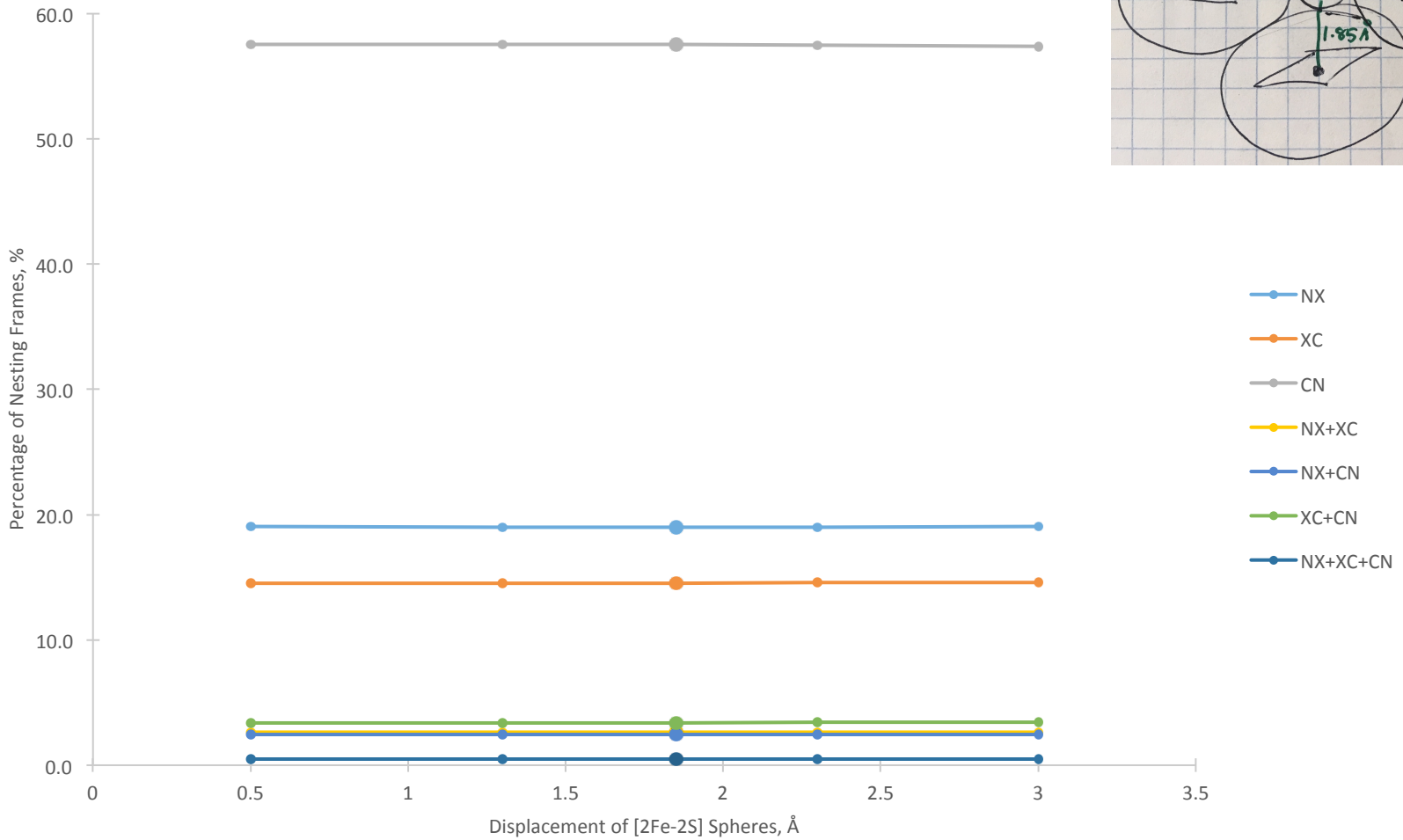


Figure S10B. Plot of the sphere displacement from the centroid in the [2Fe-2S] cluster quad-sphere model versus the percentage of [2Fe-2S] nesting frames for each coordination isomer. As expected, the expansion of the model has relatively no impact on the overall nesting percentages. The selected displacement value of **1.85 Å** is defined from the 3WCQ crystal structure.

Sensitivity Analysis — Crossover

Run on CGGCGGC fixed S-S distances, 30 ns
Self correlation analysis
Total possible unique frame combinations: 899,940,000

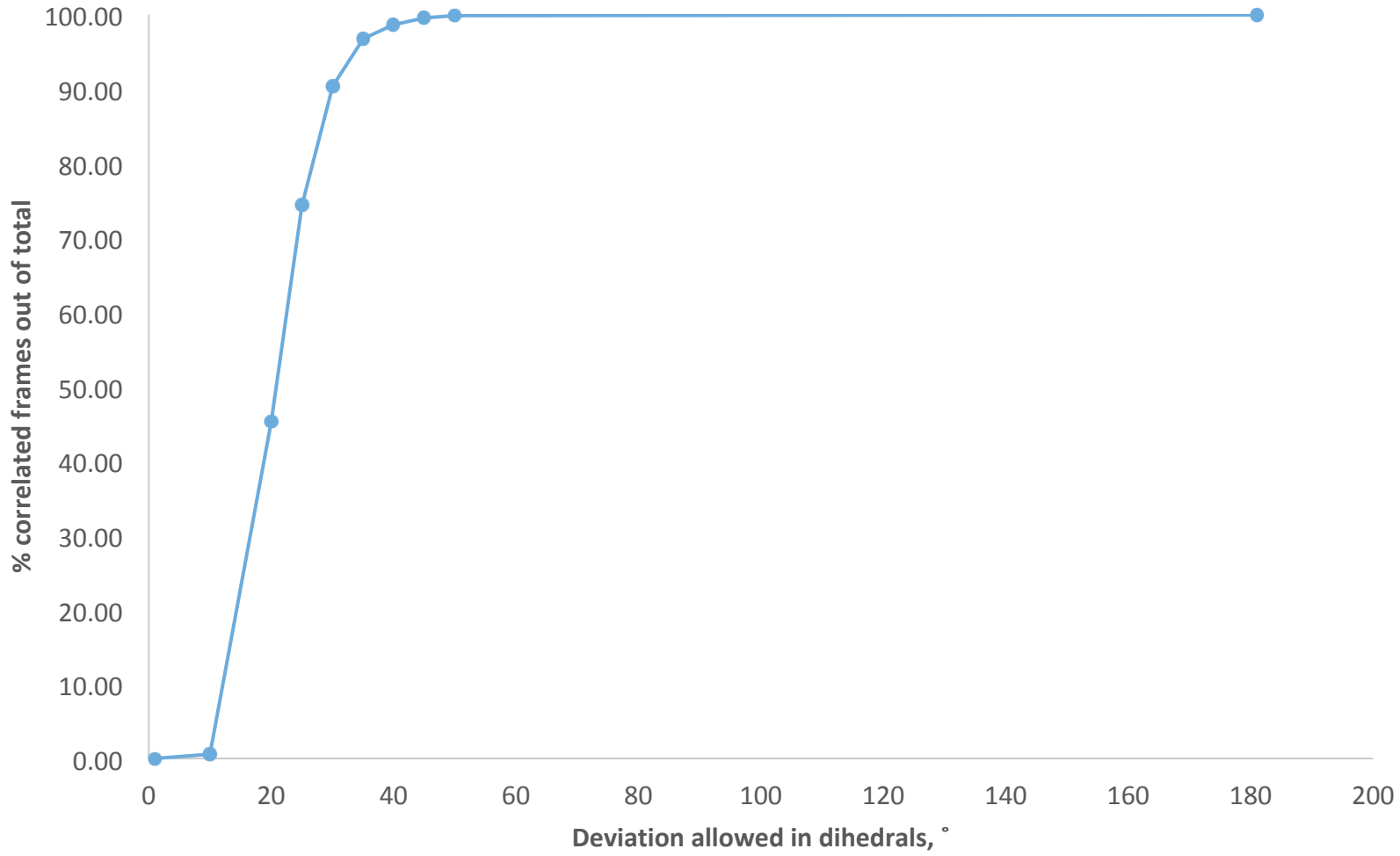
[2Fe-2S] nesting: 100%
[4Fe-4S] nesting ceiling: 100%
[4Fe-4S] nesting: 3%

Standard deviation in fixed S-S distances: 0.04 Å
Maximum deviation of fixed S-S distances: 0.41 Å

Sensitivity Analysis — Crossover

Allowed Deviation					
S-S Distance (Å)	Dihedral (˚)	# correlated combinations	% of possible combinations	# correlated frames	% of possible frames
1	1	0	**0.00**	1	**0.00**
1	10	151	**0.00**	169	**0.56**
1	20	54224	**0.01**	13600	**45.33**
1	25	306442	**0.03**	22314	**74.38**
1	30	1191531	**0.13**	27115	**90.38**
1	35	3510324	**0.39**	29021	**96.74**
1	40	5688935	**0.63**	29578	**98.59**
1	45	15488331	**1.72**	29881	**99.60**
1	50	32172470	**3.57**	29972	**99.91**
1	181	899,940,000	**100.00**	30000	**100.00**

Sensitivity Analysis — Crossover



Sensitivity Analysis — Crossover