Supporting Information

Binding modes and metabolism of caffeine

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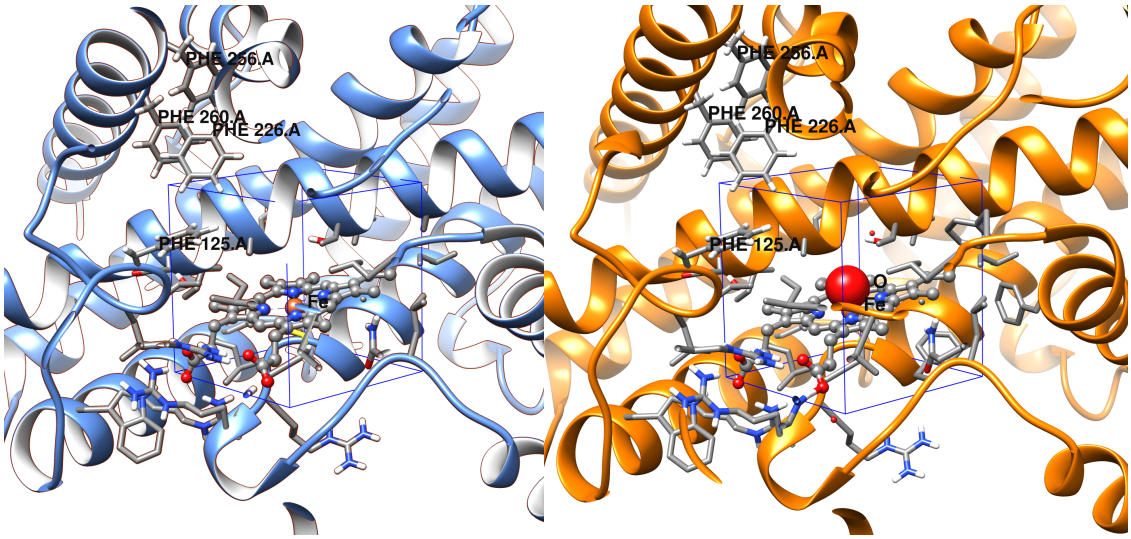

Figure S1: Visualization of the docking protocol in AutoDockVina through UCSF Chimera.[1] The blue cube represents a defined cavity into which caffeine molecule was docked. In blue is the receptor with heme and in orange is the receptor with CPDI.

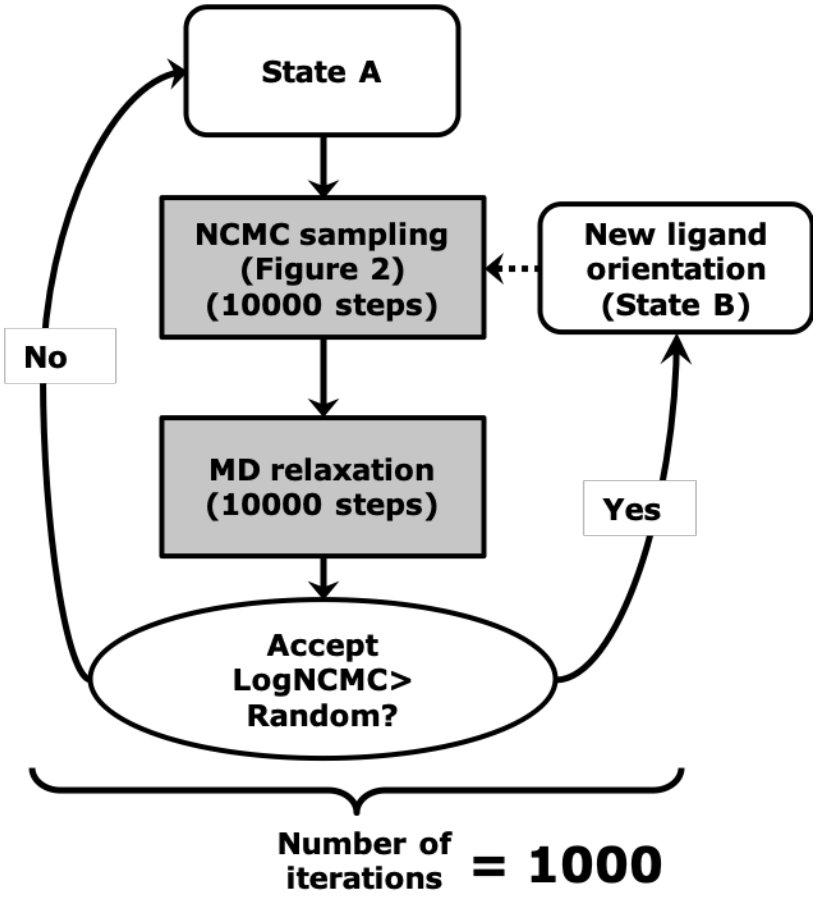

Figure S 2: Scheme of BLUES workflow

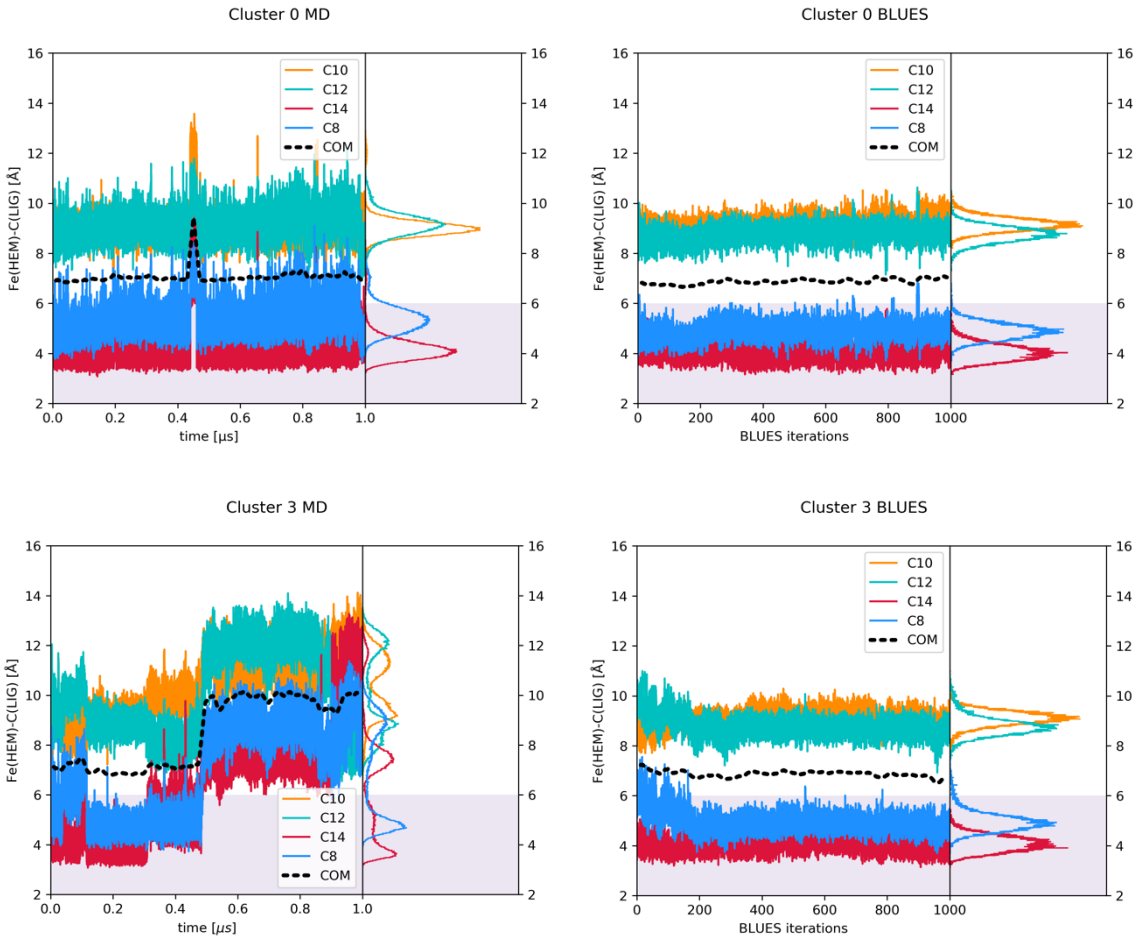

Figure S3: Time series of the distance from the heme iron (ferric state) to indicated atoms in caffeine and its center of mass (COM) from simulations of clusters 0 and 3. The right hand-side panels show the corresponding histograms.

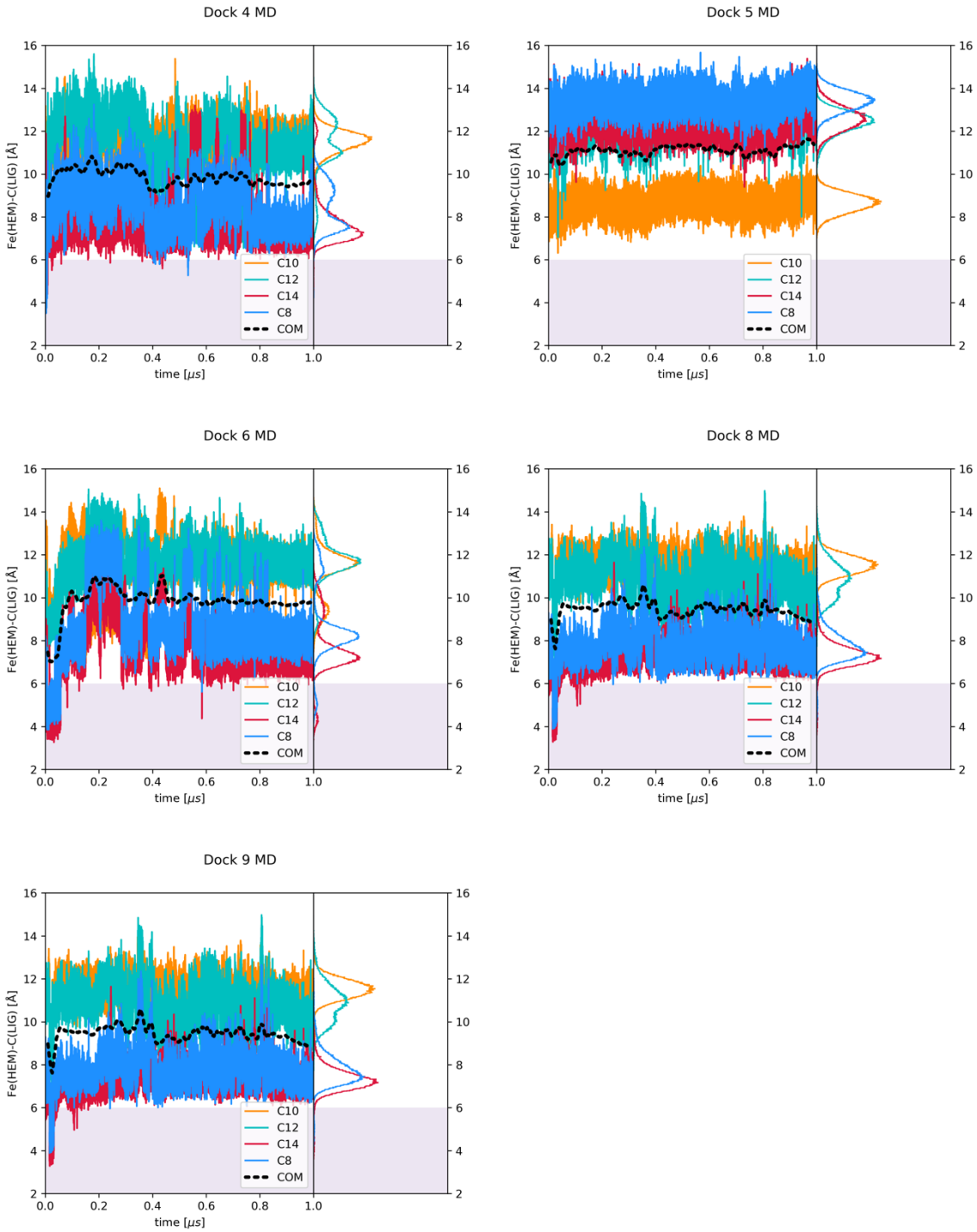

Figure S4: Time series of the distance from the heme iron (ferric state) to indicated atoms in caffeine and its center of mass (COM) from simulations of additional docked modes. The right hand-side panels show the corresponding histograms.

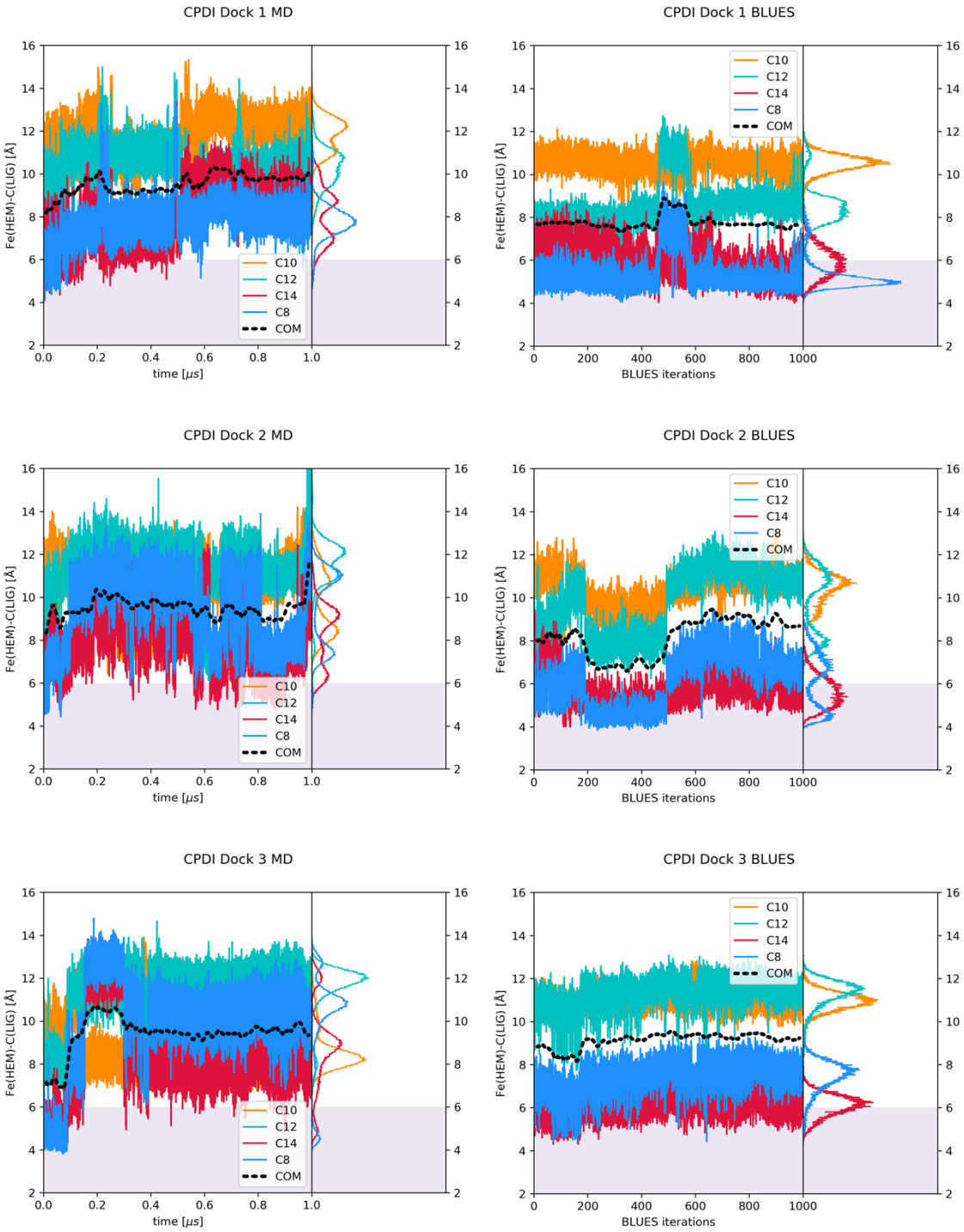

Figure S5: Time series of the distance from the heme iron (ferric state) to indicated atoms in caffeine and its center of mass (COM) from simulations with compound I. The right hand-side panels show the corresponding histograms.

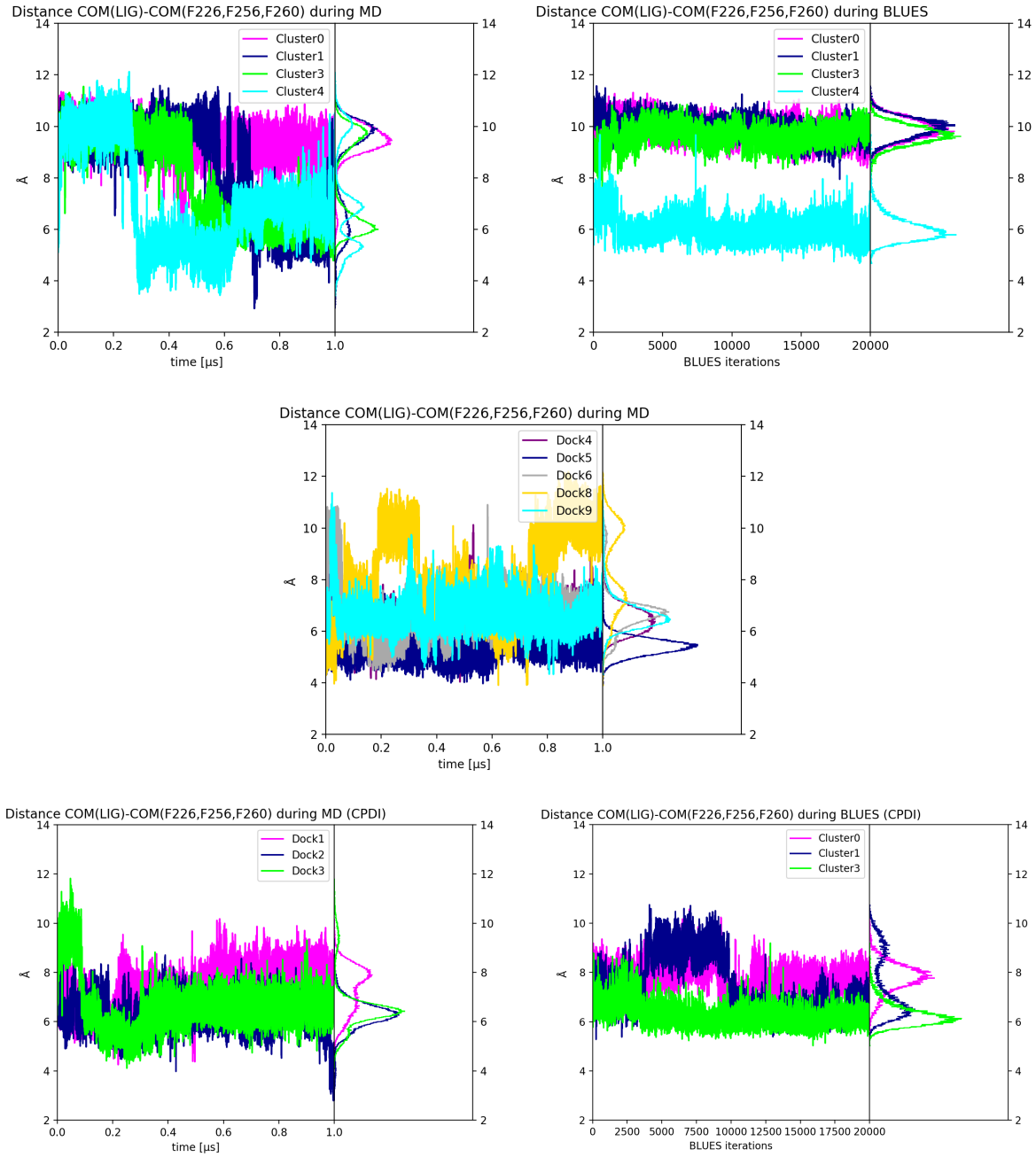

Figure S6: Time series of the distance from the center of mass of three phenylalanines (Phe226, Phe256 and Phe260) to the center of mass of caffeine (COM) from MD and BLUES simulations with heme (first row) from MD simulations of remaining docking poses with heme (second row) and MD and BLUES simulations with compound I (third row). The right hand-side panels show the corresponding histograms.

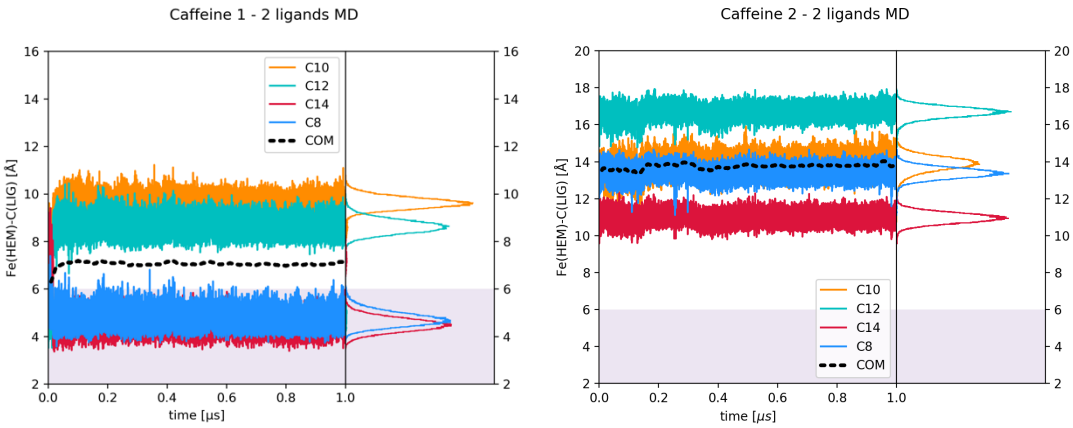

Figure S7: Time series of the distance from the heme iron (ferric state) to indicated atoms in caffeine and its center of mass (COM) from simulations with two molecules of caffeine bound to the active site. The right hand-side panels show the corresponding histograms.

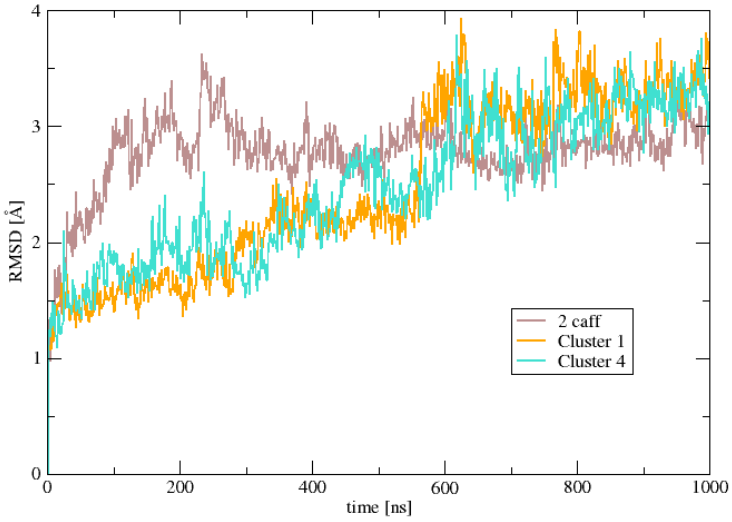

Figure S8: RMSD time series of protein backbone atoms in simulations of the enzyme in the ferric form, with a single caffeine molecule bound, starting from cluster 1 and cluster 4 and with two caffeine molecules bound (2 caff).

		Cluster 0		Cluster 1		Cluster 3		Cluster 4		MD 2 cafs		100% HS*	100% LS*	72%HS +28% LS*
SOM		**MD**	**BLUES**	**MD**	**BLUES**	**MD**	**BLUES**	**MD**	**BLUES**	**caf1**	**caf2**	**(5 mM)**		**(25 mM)**
C8	$\langle r \rangle$	5.4	4.9	6.8	4.9	7.0	5.0	10.0	10.0	4.8	13.4			
	$\langle r^{-6} \rangle^{-1/6}$	5.2	4.8	5.3	4.8	5.5	4.9	7.4	8.9	4.7	13.4			
N1-C10	$\langle r \rangle$	9.0	9.2	9.7	9.4	10.4	9.1	10.2	11.1	9.6	13.9			
	$\langle r^{-6} \rangle^{-1/6}$	9.0	9.1	9.5	9.3	9.8	9.1	9.6	9.8	9.6	13.8	7.4	4.9	6.7
N3-C12	$\langle r \rangle$	9.2	8.8	10.0	8.5	10.1	8.8	8.7	10.0	8.6	16.7			
	$\langle r^{-6} \rangle^{-1/6}$	9.1	8.7	9.2	7.2	9.2	8.7	8.1	8.9	8.0	16.7	7.2	4.8	6.6
N7-C14	$\langle r \rangle$	4.2	4.0	5.9	4.4	6.3	4.1	10.9	10.6	4.5	11.0			
	$\langle r^{-6} \rangle^{-1/6}$	4.1	4.0	4.7	4.2	4.5	4.0	5.7	9.5	4.4	10.9	7.4	4.9	6.7
COM	$\langle r \rangle$	7.1	6.9	8.1	7.0	8.5	6.9	9.6	10.2	7.0	13.7			
	$\langle r^{-6} \rangle^{-1/6}$	7.0	6.9	7.5	6.9	7.7	6.9	8.7	10.1	7.0	13.7			

Table S1: Average distances $\langle \boldsymbol{r} \rangle$ and estimated relaxation times distances $\boldsymbol{\langle r^{-6} \rangle^{-1/6}}$ between the SOM in simulations with ferric heme. * average and normalized distances taken from Regal et al. [2].

SOM	Cluster 0		Cluster 1		Cluster 3		Cluster 4		MD 2 cafs	
	MD	BLUES	MD	BLUES	MD	BLUES	MD	BLUES	caf1	caf2
C8	88.8	99.7	59.6	99.7	41.4	97.0	10.5	0.3	99.8	0.0
N1-C10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N3-C12	0.0	0.0	0.0	4.3	0.0	0.0	1.3	0.0	1.6	0.0
N7-C14	97.9	100.0	63.0	95.7	47.0	100.0	22.5	0.2	98.4	0.0

Table S2: Percentage of frames where $r_{(SOM-FE)} < 6$ Å in simulations with ferric heme.

SOM	Cluster 0		Cluster 1		Cluster 3		Cluster 4		MD 2 cafs	
	MD	BLUES	MD	BLUES	MD	BLUES	MD	BLUES	caf1	caf2
C8	3.5	3.3	10.2	8.9	17.5	2.6	2.0	11.7	25.0	0.0
N1-C10	0.0	0.0	6.7	0.0	0.0	0.0	2.9	30.2	0.0	0.0
N3-C12	0.0	0.0	0.7	3.9	7.2	0.0	69.4	50.3	1.3	0.0
N7-C14	96.5	96.7	82.4	87.3	75.3	97.4	25.6	7.8	73.7	100.0

Table S3: Percentages of simulation time when the SOM is the closest atom of caffeine to the heme iron in simulations with ferric heme.

SOM	Distance average in Å	Dock 1		Dock 2		Dock 3	
		MD	BLUES	MD	BLUES	MD	BLUES
C8	$\langle r \rangle$	7.8	5.3	9.5	6.2	10.2	7.0
	$\langle r^{-6} \rangle^{-1/6}$	7.4	5.1	8.1	5.3	6.8	7.0
N1-C10	$\langle r \rangle$	11.7	10.5	9.7	10.4	8.6	11.0
	$\langle r^{-6} \rangle^{-1/6}$	11.2	10.5	9.0	10.2	8.4	10.9
N3-C12	$\langle r \rangle$	10.2	8.6	11.6	9.8	11.6	11.3
	$\langle r^{-6} \rangle^{-1/6}$	9.9	8.3	10.7	9.0	10.7	11.1
N7-C14	$\langle r \rangle$	8.1	5.9	8.2	5.6	8.8	6.1
	$\langle r^{-6} \rangle^{-1/6}$	7.4	5.6	7.3	5.4	7.0	5.9
COM	$\langle r \rangle$	9.5	7.8	9.5	8.2	9.4	9.1
	$\langle r^{-6} \rangle^{-1/6}$	9.4	7.7	9.4	7.7	8.9	9.0

Table S4: Average distances ⟨r⟩ and estimated relaxation times distances $\langle \boldsymbol{r^{-6}} \rangle^{-1/6}$ between the SOM of caffeine and iron in simulations with compound I.

SOM	Dock 1		Dock 2		Dock 3	
	MD	BLUES	MD	BLUES	MD	BLUES
C8	3.4	87.4	1.8	40.8	8.6	3.9
N1-C10	0.0	0.0	0.0	0.0	0.0	0.0
N3-C12	0.0	0.0	0.0	0.0	0.0	0.0
N7-C14	3.4	56.7	5.9	71.8	8.3	39.0

Table S5: Percentage of frames where $r_{(SOM\text{-}FE)}$ < 6 Å in simulations with compound I.

SOM	Dock 1		Dock 2		Dock 3	
	MD	BLUES	MD	BLUES	MD	BLUES
C8	60.7	83.1	6.7	39.7	8.9	1.3
N1-C10	2.3	0.0	43.0	0.0	66.1	0.0
N3-C12	0.4	0.0	2.6	0.0	0.0	0.0
N7-C14	36.7	16.9	47.6	60.3	25.1	98.7

Table S6: Percentages of simulation time when the SOM is the closest atom of caffeine to the compound I iron.

Heme

	BLUES								MD							
	Cluster 0		Cluster 1		Cluster 3		Cluster 4		Cluster 0		Cluster 1		Cluster 3		Cluster 4	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
C8	3.31	0.13	8.86	0.2	2.62	0.11	11.74	0.23	3.46	0.06	10.16	0.1	17.46	0.12	2.04	0.04
C10	0	0	0	0	0	0	30.16	0.33	0	0	6.72	0.08	0	0	2.94	0.05
C12	0	0	3.87	0.14	0	0	50.25	0.36	0	0	0.68	0.03	7.23	0.08	69.38	0.15
C14	96.69	0.13	87.27	0.24	97.39	0.11	7.84	0.19	96.54	0.06	82.45	0.12	75.31	0.14	25.63	0.14

CPDI

	BLUES						MD					
	Dock 1		Dock 2		Dock 3		Dock 1		Dock 2		Dock 3	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
C8	83.06	0.27	39.68	0.35	1.31	0.08	60.68	0.15	6.73	0.08	8.85	0.09
C10	0	0	0	0	0	0	2.27	0.05	43.04	0.16	66.09	0.15
C12	0	0	0	0	0	0	0.39	0.02	2.61	0.05	0	0
C14	16.94	0.27	60.32	0.35	98.69	0.08	36.66	0.15	47.63	0.16	25.06	0.14

Heme

	MD									
	Dock 4		Dock 5		Dock 6		Dock 8		Dock 9	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
C8	9.79	0.09	0	0	8.6	0.09	5.78	0.07	21.67	0.13
C10	0.01	0	99.99	0	15.27	0.11	0.26	0.02	0.01	0
C12	5.24	0.07	0.01	0	0.02	0	12.37	0.1	0.01	0
C14	84.97	0.11	0	0	76.1	0.13	81.59	0.12	78.31	0.13

Table S7: Percentage of the closest SOMs to the heme iron in all simulations – detailed values of the data in Figure 5 with standard error of the mean (SEM) estimated by 10000 bootstrap replicates.

References

1. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E., UCSF chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry* **2004,** *25* (13), 1605-1612.
2. Regal, K. A.; Nelson, S. D., Orientation of caffeine within the active site of human cytochrome P450 1A2 based on NMR longitudinal (T-1) relaxation measurements. *Archives of Biochemistry and Biophysics* **2000,** *384* (1), 47-58.