

# IUCrJ

**Volume 10 (2023)**

**Supporting information for article:**

**Structural and biochemical insights into Zn<sup>2+</sup>-bound EF-hand proteins, EFhd1 and EFhd2**

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**Table S1.**Distances between ligands within EF-hand motifs and metals (Ca<sup>2+</sup> or Zn<sup>2+</sup>)

<b>EFhd1</b>						
	<b>EF1</b>			<b>EF2</b>		
	AA <sup>atom</sup>	Distance (Å)		AA <sup>atom</sup>	Distance (Å)	
		Ca <sub>1</sub>	Zn <sub>1</sub>		Ca <sub>2</sub>	Zn <sub>2</sub>
<b>1 (X)</b>	D104 <sup>OD1</sup>	2.2	2.2	D140 <sup>OD1</sup>	2.3	2.1
<b>3 (Y)</b>	H <sub>2</sub> O	2.3	2.4	D142 <sup>OD1</sup>	2.3	2.3
<b>5 (Z)</b>	D108 <sup>OD1</sup>	2.5	2.3	D144 <sup>OD1</sup>	2.4	2.3
<b>7 (-Y)</b>	F110 <sup>O</sup>	2.5	2.2	K146 <sup>O</sup>	2.5	2.3
<b>9 (-X)</b>	H <sub>2</sub> O	2.3	2.4	H <sub>2</sub> O	2.2	2.4
<b>12 (-Z)</b>	E115 <sup>OE1</sup>	2.4	2.4	E151 <sup>OE1</sup>	2.5	2.4
	E115 <sup>OE2</sup>	2.5	2.6	E151 <sup>OE2</sup>	2.7	2.7
	average	2.4	2.4	average	2.4	2.4

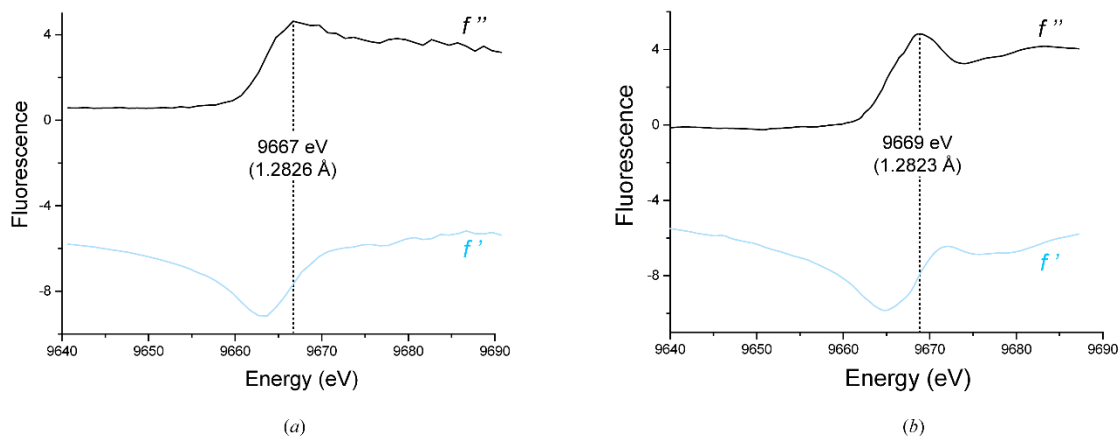
<b>EFhd2</b>						
	<b>EF1</b>			<b>EF2</b>		
	AA <sup>atom</sup>	Distance (Å)		AA <sup>atom</sup>	Distance (Å)	
		Ca <sub>1</sub>	Zn <sub>1</sub>		Ca <sub>2</sub>	Zn <sub>2</sub>
<b>1 (X)</b>	D105 <sup>OD1</sup>	2.6	2.2	D141 <sup>OD1</sup>	2.6	2.3
<b>3 (Y)</b>	H <sub>2</sub> O	2.5	2.0	D143 <sup>OD1</sup>	2.6	2.4
<b>5 (Z)</b>	D109 <sup>OD1</sup>	2.5	2.4	D145 <sup>OD1</sup>	2.6	2.1
<b>7 (-Y)</b>	F111 <sup>O</sup>	2.5	2.1	K147 <sup>O</sup>	2.6	2.5
<b>9 (-X)</b>	H <sub>2</sub> O	2.5	2.0	H <sub>2</sub> O	2.6	2.1
<b>12 (-Z)</b>	E116 <sup>OE1</sup>	2.6	2.0	E152 <sup>OE1</sup>	2.6	2.5
	E116 <sup>OE2</sup>	2.7	2.8	E152 <sup>OE2</sup>	2.7	2.5
	average	2.6	2.2	average	2.6	2.3

<b>Tse3</b>		
<b>EF-hand-like motif</b>		
<b>AA<sup>atom</sup></b>	<b>Distance (Å)</b>	
	<b>Ca<sub>1</sub></b>	<b>Zn<sub>1</sub></b>
<b>E375<sup>OE1</sup></b>	2.6	2.6
<b>E375<sup>OE2</sup></b>	2.6	2.7
<b>S378<sup>O</sup></b>	2.7	2.6
<b>R379<sup>O</sup></b>	2.4	2.3
<b>D382<sup>OD1</sup></b>	2.5	2.6
<b>N384<sup>O</sup></b>	2.5	2.4
<b>H<sub>2</sub>O</b>	2.5	2.6
<b>average</b>	2.5	2.5

<b>CaM</b>						
	<b>EF2</b>			<b>EF3</b>		
	<b>AA<sup>atom</sup></b>	<b>Distance (Å)</b>		<b>AA<sup>atom</sup></b>	<b>Distance (Å)</b>	
		<b>Ca<sub>1</sub></b>	<b>Zn<sub>1</sub></b>		<b>Ca<sub>2</sub></b>	<b>Zn<sub>2</sub></b>
<b>1 (X)</b>	D56 <sup>OD1</sup> (D57 <sup>OD1</sup> )	2.1	2.0	D93 <sup>OD1</sup> (D94 <sup>OD1</sup> )	2.2	2.2
<b>3 (Y)</b>	D58 <sup>OD1</sup> (D59 <sup>OD1</sup> )	2.3	2.5	D95 <sup>OD1</sup> (D96 <sup>OD1</sup> )	2.5	2.6
<b>5 (Z)</b>	N60 <sup>OD1</sup> (N61 <sup>OD1</sup> )	2.4	2.3	N97 <sup>OD1</sup> (N98 <sup>OD1</sup> )	2.3	2.6
<b>7 (-Y)</b>	T62 <sup>O</sup> (T63 <sup>O</sup> )	2.3	2.4	Y99 <sup>O</sup> (Y100 <sup>O</sup> )	2.2	2.3
<b>9 (-X)</b>	H <sub>2</sub> O	2.3	-	H <sub>2</sub> O	2.6	-
<b>12 (-Z)</b>	E67 <sup>OE1</sup> (E68 <sup>OE1</sup> )	2.6	2.6	E104 <sup>OE1</sup> (E105 <sup>OE1</sup> )	2.4	2.4
	E67 <sup>OE2</sup> (E68 <sup>OE2</sup> )	2.4	2.6	E104 <sup>OE2</sup> (E105 <sup>OE2</sup> )	2.6	2.5
	<b>average</b>	2.3	2.4	<b>average</b>	2.4	2.4

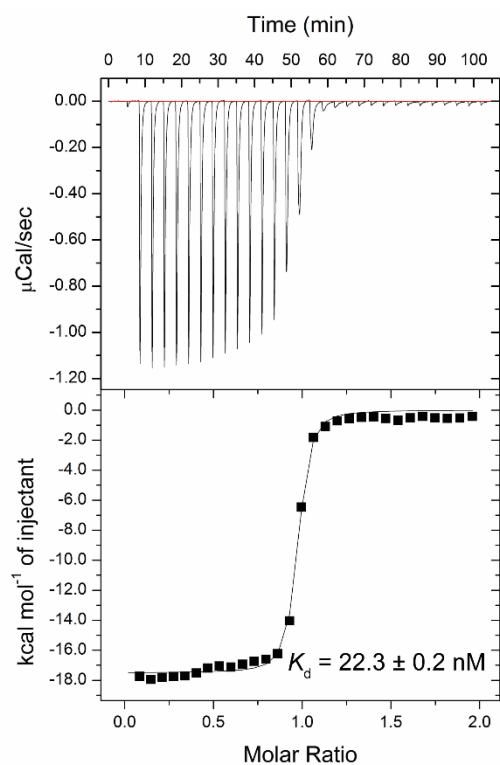
<b>CaM</b>			
<b>EF4</b>			
	AA <sup>atom</sup>	Distance (Å)	
		Ca <sub>3</sub>	Zn <sub>3</sub>
<b>1 (X)</b>	D129 <sup>OD1</sup> (D130 <sup>OD1</sup> )	2.3	2.2
<b>3 (Y)</b>	D131 <sup>OD1</sup> (D132 <sup>OD1</sup> )	2.4	2.6
<b>5 (Z)</b>	D133 <sup>OD1</sup> (D134 <sup>OD1</sup> )	2.3	2.3
<b>7 (-Y)</b>	Q135 <sup>O</sup> (Q136 <sup>O</sup> )	2.3	2.3
<b>9 (-X)</b>	H <sub>2</sub> O	2.6	2.1
<b>12 (-Z)</b>	E140 <sup>OE1</sup> (E141 <sup>OE1</sup> )	2.6	2.4
	E140 <sup>OE2</sup> (E141 <sup>OE2</sup> )	2.6	2.5
	average	2.4	2.3

<b>ALG-2</b>						
	<b>EF1</b>			<b>EF3</b>		
	AA <sup>atom</sup>	Distance (Å)		AA <sup>atom</sup>	Distance (Å)	
		Ca <sub>1</sub>	Zn <sub>1</sub>		Ca <sub>2</sub>	Zn <sub>2</sub>
<b>1 (X)</b>	D36 <sup>OD1</sup>	2.1	2.2	D103 <sup>OD1</sup>	2.3	2.3
<b>3 (Y)</b>	D38 <sup>OD1</sup>	2.3	2.3	D105 <sup>OD1</sup>	2.6	2.4
<b>5 (Z)</b>	S40 <sup>OD1</sup>	-	2.4	S107 <sup>OD1</sup>	2.7	2.8
<b>7 (-Y)</b>	V42 <sup>O</sup>	2.5	2.1	M109 <sup>O</sup>	2.3	2.5
<b>9 (-X)</b>	H <sub>2</sub> O	2.3	-	-	-	-
<b>12 (-Z)</b>	E47 <sup>OE1</sup>	2.9	2.5	E114 <sup>OE1</sup>	2.4	2.4
	E47 <sup>OE2</sup>	2.5	2.8	E114 <sup>OE2</sup>	2.4	2.8
	average	2.4	2.4	average	2.5	2.5



**Figure S1. X-ray absorption scan of EFhd1<sup>Zn</sup> and EFhd2<sup>Zn</sup>(P) at the Zn K-edge region**

(a,b) The plots show X-ray absorption scan of EFhd1<sup>Zn</sup> or EFhd2<sup>Zn</sup>(P) at the Zn K-edge. The energy and wavelengths of the peak positions are indicated on the dashed line in each plot.



**Figure S2. Measurement of the Ca<sup>2+</sup> binding affinity for wild-type EFhd1 determined with ITC**

ITC profile for wild-type EFhd1 with Ca<sup>2+</sup>: 20 μM EFhd1 (residues 69–200) was titrated with 30 injections of 0.3 mM CaCl<sub>2</sub> at 20°C. The experiment was repeated in triplicate.