

# “Binding modes of zaragozic acid A to human squalene synthase and staphylococcal dehydrosqualene synthase”

Liu *et al.* 2012

## Supplemental data:

**Supplementary Table 1 – Data collection and refinement statistics**

Crystals	Human SQS (Native)				Human SQS/ZA-A	Y248A CrTM	Y248A CrTM/ZA-A
	[Form I]	[Form II]	[Form III]	[Form IV]	[Form IV]		
PDB ID code	3VJ8	3VJ9	3VJA	3VJB	3VJC	3VJD	3VJE
molecules/ ASU	1	1	2	6	6	1	2
Data Collection							
Radiation source	NSRRC-BL13B1	NSRRC BL13 B1	NSRRC BL13 B1	NSRRC BL13B1	NSRRC BL13B1	SPring-8 BL44XU	SPring-8 BL44XU
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_1$	$P2_1$	$P3_221$	$P3_221$
Unit cell $a, b, c$ (Å)	36.35, 93.87, 108.55	51.78, 76.66, 82.50	52.0, 82.34, 77.18	85.61, 153.86, 91.52	85.87, 153.15, 91.86	79.59, 79.59, 90.76	80.18, 80.18, 185.41
$\beta$ (°)			91.57	91.68	91.72		
Resolution (Å) <sup>a</sup>	30-1.52 (1.57-1.52)	30-1.52 (1.57-1.52)	30-1.77 (1.83-1.77)	30-2.05 (2.12-2.05)	30-1.89 (1.96-1.89)	30-1.48 (1.53-1.48)	30-2.12 (2.20-2.12)
No. of reflections	57879 (5662)	51144 (5041)	62838 (6177)	144874 (13406)	187887 (18760)	55684 (5523)	39844 (3907)
Completeness (%)	99.4 (98.4)	99.5 (99.3)	98.3 (97.8)	98.5 (91.4)	99.4 (99.2)	99.7 (100)	99.5 (99.9)
Redundancy	7.1 (6.7)	8.0 (8.1)	4.9 (4.8)	3.9 (3.7)	3.7 (3.6)	9.2 (9.3)	9.0 (9.0)
$R_{\text{merge}}$ (%)	3.3 (30.9)	4.5 (50.9)	6.1 (48.9)	7.7 (40.2)	6.0 (47.2)	4.9 (58.0)	7.1 (48.5)
$I/\sigma$ (I)	50.3 (5.4)	40.9 (4.1)	25.3 (3.8)	20.8 (3.6)	20.8 (2.3)	50.3 (4.2)	33.6 (4.8)
Refinement							
Reflections (work)	54872 (7789)	48484 (6971)	59604 (8490)	137577 (18416)	178388 (25845)	52719 (7632)	37663 (5423)
Reflections (free)	2933 (423)	2598 (356)	3180 (431)	7255 (967)	9431 (1376)	2820 (418)	1992 (280)
$R_{\text{work}}$ (%)	16.4 (17.9)	16.0 (15.6)	16.5 (17.4)	16.9 (19.8)	14.8 (18.3)	16.2 (14.9)	20.5 (21.0)
$R_{\text{free}}$ (%)	19.6 (21.8)	21.4 (21.9)	23.3 (28.2)	22.7 (25.8)	19.7 (25.5)	20.6 (20.2)	25.4 (30.5)
Bond lengths (Å)	0.007	0.007	0.007	0.007	0.007	0.007	0.007
Bond angles (°)	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Mean B-values (Å <sup>2</sup> ) / No.							
Protein atoms	24.3 / 2705	23.0 / 2705	30.0 / 5404	53.7 / 15962	37.6 / 16139	23.5 / 2428	47.7 / 4770
Compound atoms					34.9 / 294	32.7 / 10	50.1 / 98
Ions		39.3 / 2	35.8 / 2		36.4 / 32		
Water molecules	44.7 / 335	42.7 / 459	48.3 / 568	56.1 / 926	42.5 / 1390	40.4 / 335	59.3 / 167
Ramachandran plot (%)							
Favored	98.5	98.8	98.6	97.0	98.4	98.6	98
Allowed	1.5	1.2	1.4	3.0	1.6	1.4	2

**Supplementary Table 2 - Hydrogen bonds between ZA-A and human SQS or *S. aureus* CrtM residues**

ZA-A	human SQS	<i>S. aureus</i> CrtM Y248A
<b>bicyclic core</b>		
O-2		Arg <sup>45</sup> (NH2)
C-3 COOH	Arg <sup>52</sup> (NH1); Arg <sup>77</sup> (NH2); Lys <sup>117</sup> (NZ)	Arg <sup>45</sup> (NH1); Arg <sup>265</sup> (NH2)
C-4 OH	Thr <sup>50</sup> (O); Arg <sup>77</sup> (NH1)	His <sup>18</sup> (O)
C-4 COOH	Arg <sup>52</sup> (N; NE)	Lys <sup>20</sup> (N; NZ); Arg <sup>171</sup> (NH1)
C-5 COOH	Ser <sup>51</sup> (OG); Ser <sup>53</sup> (N); Tyr <sup>73</sup> (OH)	Ser <sup>19</sup> (OG); Ser <sup>21</sup> (N); Tyr <sup>41</sup> (OH)
C-7 OH		Asn <sup>168</sup> (ND2)
O-8		
<b>C-1 alkyl side chain</b>		
OAc	Val <sup>175</sup> (O)	
<b>C-6 acyl side chain</b>		
ester group		Arg <sup>171</sup> (NH2)

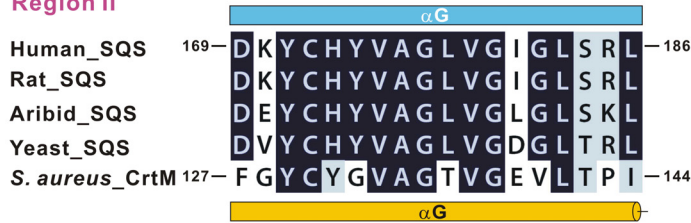
**Supplementary Table 3 - Hydrophobic interactions between ZA-A and human SQS or *S. aureus* CrtM residues**

ZA-A	human SQS	<i>S. aureus</i> CrtM Y248A
<b>C-1 alkyl side chain</b>		
C1'		
C2'		
C3'-exo-double bond	Asp <sup>80</sup> (CG; CB), Arg <sup>77</sup> (CD)	Asp <sup>48</sup> (CB; CG)
C4'-OAc group	Met <sup>150</sup> (SD); Met <sup>154</sup> (CE)	Leu <sup>107</sup> (CD1; CD2)
C5'	Tyr <sup>73</sup> (CD2; CE2)	Tyr <sup>41</sup> (CE1)
C5'-methyl group	Tyr <sup>73</sup> (CD2; CE2; CZ; CG)	Tyr <sup>41</sup> (CD1; CE1; CG; CZ); Cys <sup>44</sup> (CB)
C6'	Val <sup>179</sup> (CG1); Tyr <sup>73</sup> (CA)	Val <sup>137</sup> (CG1)
C6'-phenyl ring	Phe <sup>54</sup> (CG; CD1; CD2), Tyr <sup>73</sup> (CD2; CA; CB), Val <sup>179</sup> (CG1), Phe <sup>288</sup> (CZ)	Phe <sup>22</sup> (CE2); Phe <sup>26</sup> (CE2); Tyr <sup>41</sup> (CD1; CA; CB); Val <sup>137</sup> (CG1); Leu <sup>141</sup> (CD1);
<b>C-6 acyl side chain</b>		
C1'	Ser <sup>53</sup> (CB)	Phe <sup>22</sup> (CE2)
C2'	Ser <sup>53</sup> (CB), Phe <sup>54</sup> (CE1; CB)	Phe <sup>22</sup> (CE2; CD2; CZ); Ser <sup>21</sup> (CB)
C3'	Ser <sup>53</sup> (CB)	Ser <sup>21</sup> (CB; CZ)
C4'		Ser <sup>21</sup> (CB)
C4'-methyl group	Ser <sup>53</sup> (CB), Pro <sup>292</sup> (CB; CA), Leu <sup>211</sup> (CD2; CB); Met <sup>295</sup> (SD); Val <sup>322</sup> (CG2)	Leu <sup>164</sup> (CD2)
C5'	Met <sup>295</sup> (CB)	Phe <sup>22</sup> (CE1); Leu <sup>164</sup> (CD2); Ile <sup>251</sup> (CD1)
C6'		Ile <sup>247</sup> (CG2)
C6'-methyl group	Met <sup>295</sup> (CB); Leu <sup>211</sup> (CD2)	Phe <sup>22</sup> (CE1); Leu <sup>164</sup> (CD2); Ala <sup>244</sup> (C; CA); Ala <sup>248</sup> (CA; CB); Ile <sup>247</sup> (C; CB; CG2)
C7'	Met <sup>295</sup> (C), Ala <sup>296</sup> (CA; CB)	
C8'	Leu <sup>211</sup> (CD2; CB), Thr <sup>214</sup> (CG2; CB), Ala <sup>296</sup> (CA; CB); Asn <sup>215</sup> (CG)	Ala <sup>25</sup> (CB); Ala <sup>244</sup> (CA)

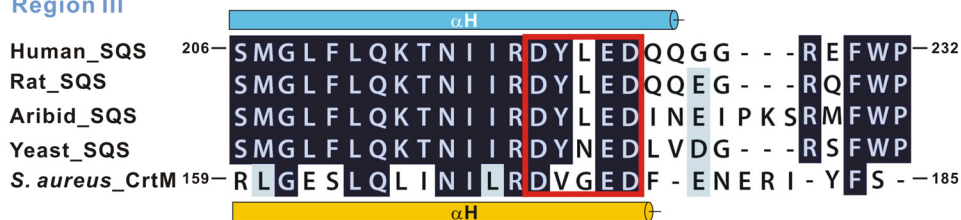
### Region I



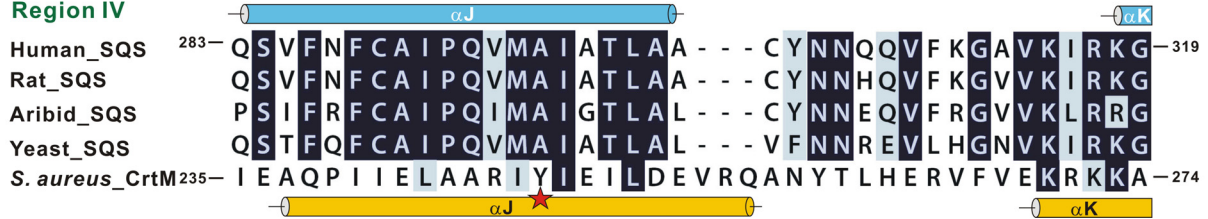
### Region II



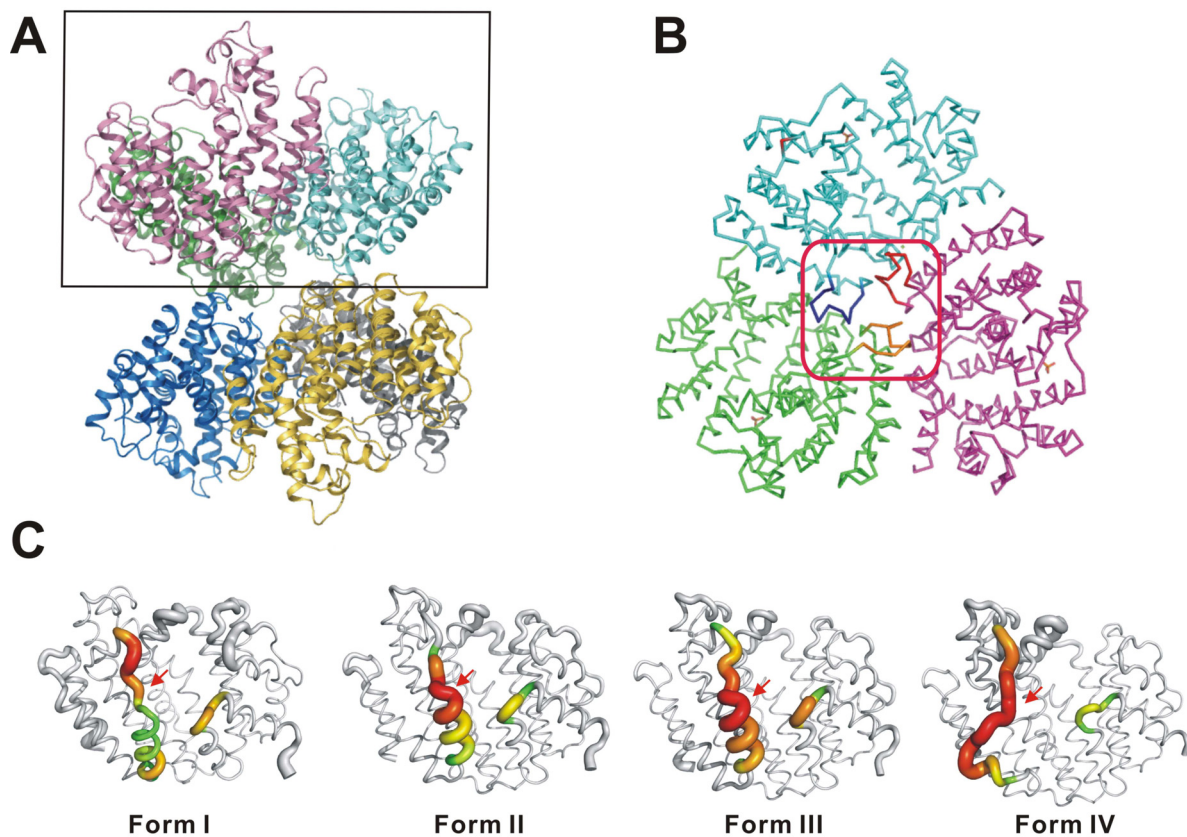
### Region III



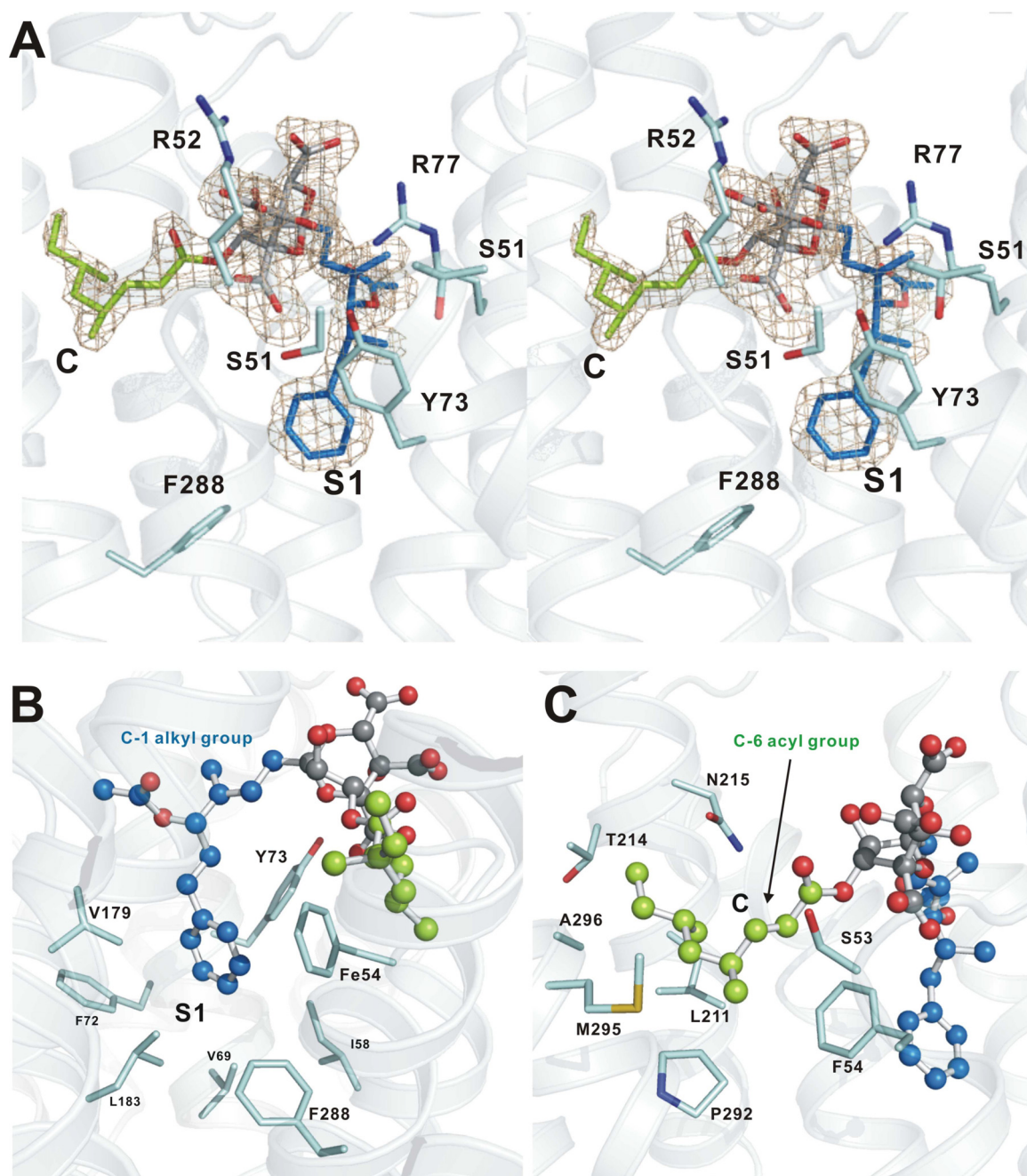
### Region IV



**Supplementary Figure 1 - Structure-based sequence alignment of conserved regions of SQS proteins with *S. aureus* CrtM.** The human, rat, *A. thaliana*, *S. cerevisiae* SQS and *S. aureus* CrtM sequences were aligned using PSI-Blast with manual adjustment. The secondary structural elements were assigned by the X-ray structures of human SQS and CrtM. Strictly conserved residues are highlighted in black, homologous amino acids are shaded in gray. Regions I, II, and III shows moderate similarities to the CrtM sequence. The two conserved aspartate-rich motifs located in regions I and III are boxed in red. Tyr<sup>248</sup> of CrtM is indicated by an asterisk.

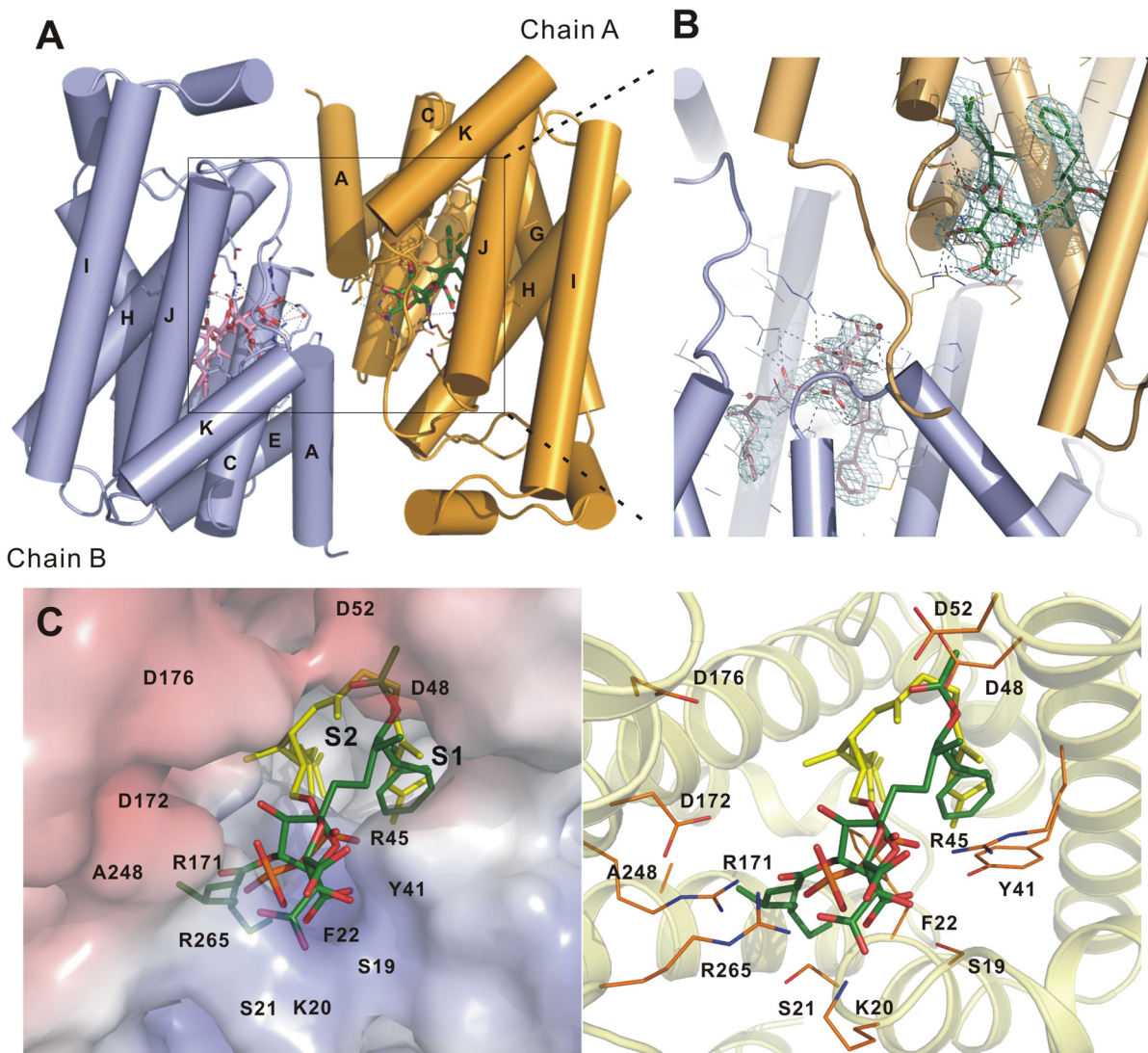


**Supplementary Figure 2** - (A) A ribbon model for the asymmetric unit of crystal form IV, which belongs to space group  $P2_1$  and contains two trimers of human SQS (31-370). The chains of the human SQS (31-370) hexamer are shown with different colors. (B) A close-up view of the trimeric packing unit. The various conformations of  $\alpha$ K due to the effects of crystal packing are highlighted in a box. (C) Human SQS displayed according to the B-factor putty using PyMOL (Red high B-factor to Blue: low B-factor). Two flexible regions in various crystal forms of human SQS are highlighted. The region <sup>315</sup>KIRKGGQAVTLMMD<sup>327</sup> (red arrow) in crystal form IV showing a diverse conformation from the others, has the highest B-factor.



**Supplementary Figure 3 - ZA-A binding mode.** (A) Stereoview of the ZA-A binding site. The ZA-A molecule (Chain A) is shown as a stick model superimposed on the corresponding  $2F_0 - F_c$  electron density maps, which are contoured at  $1.0 \sigma$  (gray mesh). The bicyclic core, C-1 alkyl side chain, and C-6 acyl side chain of ZA-A are shown in gray, marine, and green, respectively. Residues near the bicyclic core are shown as thin sticks (palecyan). Residues lining the S1 site for ZA-A C-1 alkyl side chain (blue) (B) and the C site for ZA-A C-6 acyl side (green) (C) binding are indicated. The ZA-A is shown as a ball-and-stick model.





**Supplementary Figure 4 - Overall structure of the Y248A CrtM/ZA-A complex.** (A) Two monomers in the Y248A CrtM asymmetric unit are shown in a cylinder diagram. (B) The densities in the  $2F_0-F_c$  maps (contoured at  $1.0 \sigma$ ) for the ZA-A in both monomers were well-defined. (C) Comparison of the crystal structures of Y248A CrtM/ZA-A and Y129A CrtM/PSPP. The bound conformation of ZA-A (green carbons) is overlaid on that of PSPP (yellow carbons). Residues lining the pocket are shown as wire models (orange carbons).