Supporting Information for

Electrostatic Control of Chemistry in Terpene Cyclases

Dan T. Major

Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel

E-mail: majort@biu.ac.il

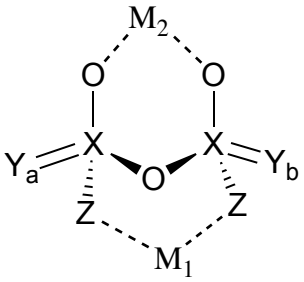
Effect of size of QM region. First, to validate the computational strategy, we test the sensitivity of the computed thermodynamics and kinetics on the size of the QM region, as this is a contentious point of much recent interest.[1-3] The standard treatment of the QM region in multiscale simulations of terpene synthases is to describe the carbocation, the PP moiety, and Mg^{2+} ions as QM atoms.[4] This allows the inclusion of any chemical effect exerted by the PP unit. However, one may also exclude the PP moiety and Mg^{2+} ions from the QM region if one is only interested in the electrostatic effect of these ions. In Table S1 and Figures S2 and S3 we show that this is a reasonable treatment for both monoterpene and sesquiterpene synthases. The barrier heights are nearly identical with the small and large QM regions. The differences in reaction free energies are somewhat larger, and this is ascribed to tumbling of the resulting carbocation intermediate, which does not form any hydrogen bonds with the enzyme matrix. Indeed, this effect is more pronounced for the smaller monoterpene, which tumbles more. We note that these findings regarding the required size of a QM region in QM/MM simulations is in agreement with those of a recent work of Jindal and Warshel,[3] but does not support the recent findings of Kulik and co-workers.[1-2]

Table S1. Thermodynamic Results (Kcal/Mol) For The Formation Of Terpinyl Cation in Bornyl Diphosphate Synthase and Bisabolyl Cation In Trichodiene Synthase Using Large and Small QM Regions.[a]

Enzyme	QM Region	$\Delta G^{\ddagger}$	ΔG_r
BPPS	Large	2.2	-4.9
	Small	2.8	-8.3
TDS	Large	4.1	3.0
	Small	3.1	2.0

[a] The large QM region includes the substrate hydrocarbon and the metal-pyrophosphate cluster PP-$(Mg^{2+})_3$. The small QM region includes the substrate hydrocarbon only.

Table S2. Mulliken charges of various phosphate-cation moieties showing how chemical modifications can change the charge distribution.[a]



	X=P $Y_{a/b}$=O Z=O M_1=Mg M_2=Mg	X=P $Y_{a/b}$=O Z=O M_1=Zn M_2=Zn	X=P $Y_{a/b}$=O Z=O M_1=Co M_2=Co	X=P $Y_{a/b}$=O Z=O M_1=Mn M_2=Mn	X=P $Y_{a/b}$=O Z=O M_1=Ni M_2=Ni	X=P $Y_{a/b}$=O Z=O M_1=Fe M_2=Fe	X=P $Y_{a/b}$=O Z=O M_1=Cu M_2=Cu	X=P $Y_{a/b}$=S Z=O M_1=Mg M_2=Mg	X=P $Y_{a/b}$=O Z=S M_1=Zn M_2=Mg	X=V $Y_{a/b}$=O Z=O M_1=Mg M_2=Mg	X=P Y_a=O Y_b=BH_3 Z=O M_1=Mg M_2=Mg
X	2.02	2.06	2.07	2.03	2.08	2.05	2.07	0.95	1.01	0.61	2.04
Y_a	-0.67	-0.65	-0.70	-0.66	-0.68	-0.65	-0.71	-0.11	-0.56	-0.37	-0.67
Z	-0.76	-0.77	-0.83	-0.84	-0.81	-0.83	-0.82	-0.59	0.02	-0.45	-0.77
O	-0.76	-0.77	-0.82	-0.84	-0.81	-0.83	-0.77	-0.59	-0.64	-0.45	-0.77
O	-1.18	-1.30	-1.16	-1.27	-1.20	-1.24	-1.13	-0.86	-0.60	-0.18	-1.04
X	2.02	2.06	2.07	2.03	2.08	2.05	2.07	0.95	1.01	0.61	1.12
O	-0.76	-0.77	-0.83	-0.84	-0.81	-0.83	-0.82	-0.59	-0.64	-0.45	-0.65
Z	-0.76	-0.77	-0.82	-0.84	-0.81	-0.83	-0.77	-0.59	0.02	-0.45	-0.65
Y_b	-0.67	-0.65	-0.70	-0.66	-0.68	-0.65	-0.71	-0.11	-0.56	-0.37	-0.19
M_1	0.76	0.79	0.86	0.94	0.83	0.88	0.79	0.78	0.00	0.76	0.78
M_2	0.76	0.79	0.86	0.94	0.83	0.88	0.79	0.78	0.94	0.76	0.78

[a] M06-2X/6-31+G(d,p)

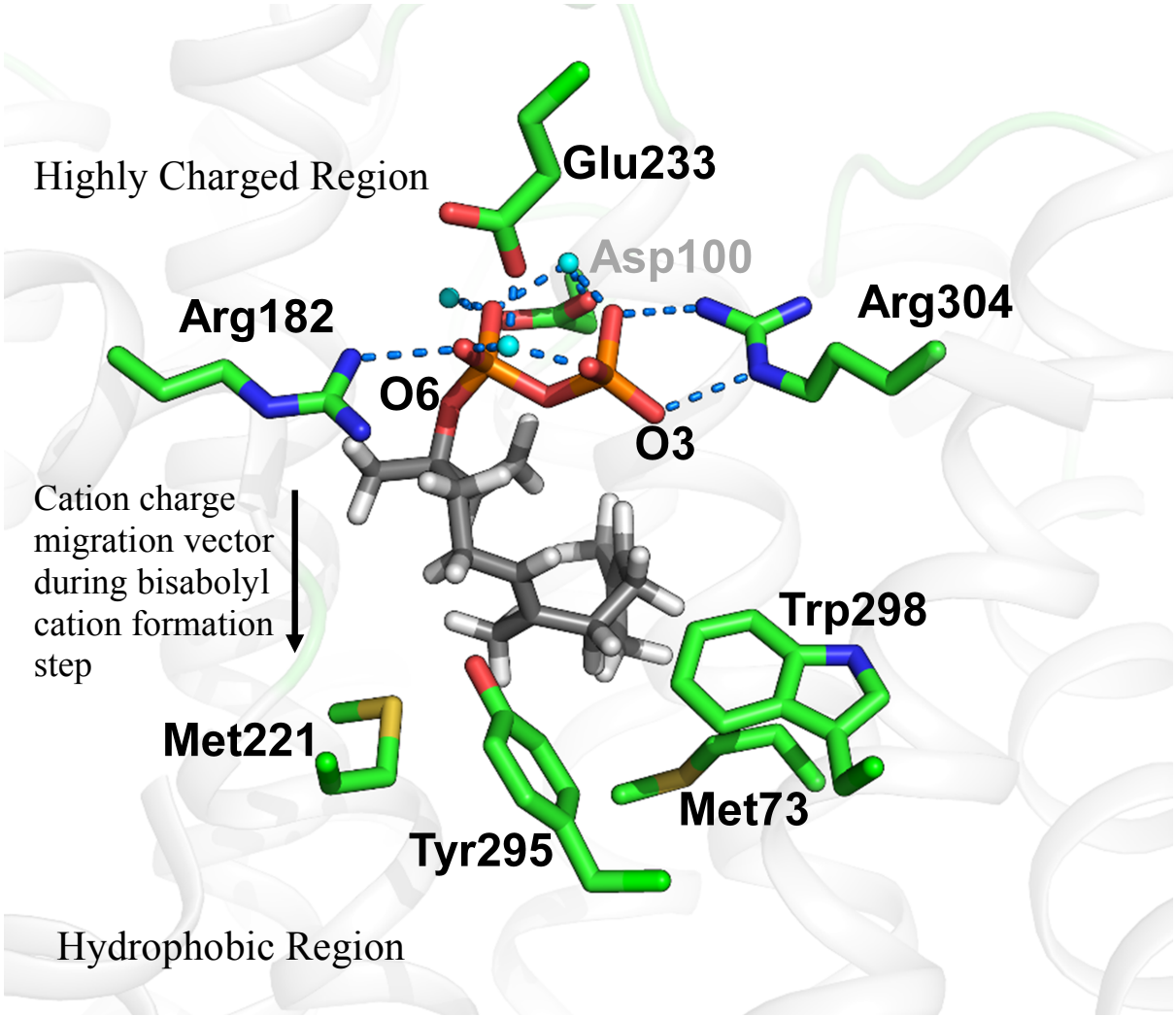


Figure S1. The Active Site in TDS Showing FPP bound in the Highly Charged and Hydrophobic Regions of the Active Site (only selected residues shown). This Binary Active Site Architecture is Typical in Terpene Synthase.

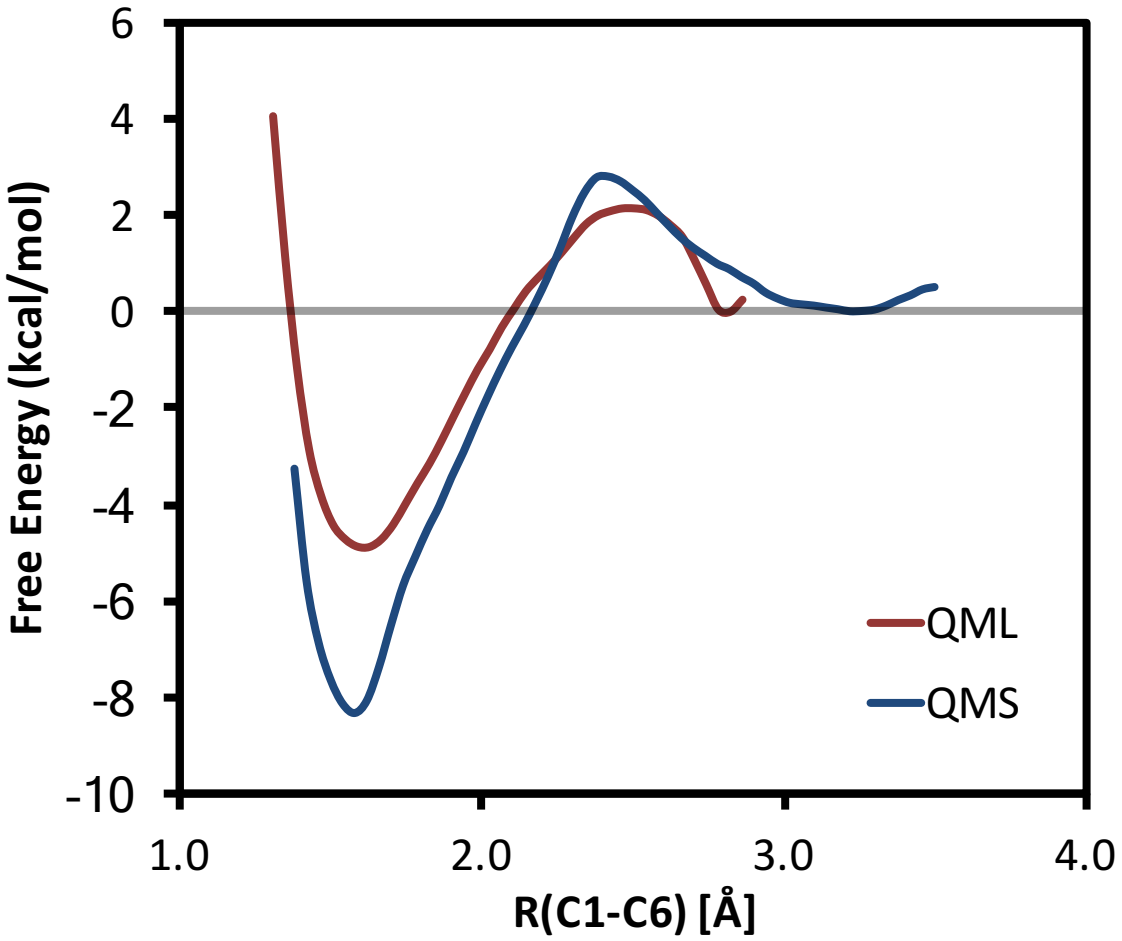


Figure S2. Free Energy Profiles for the Terpinyl Formation Step in Bornyl Diphosphate Synthase for the Fully Charged Enzyme Using Large and Small QM Regions.

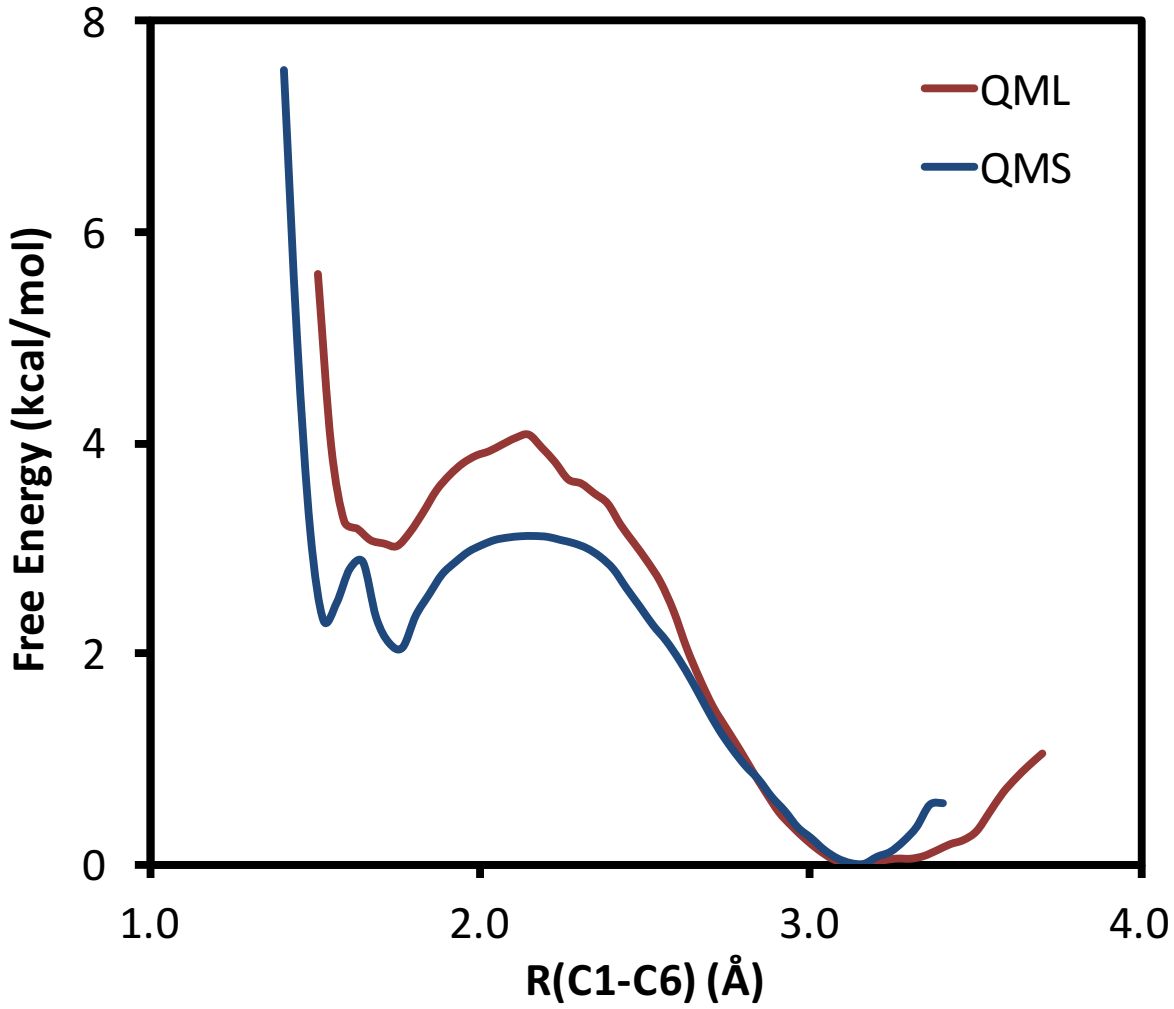
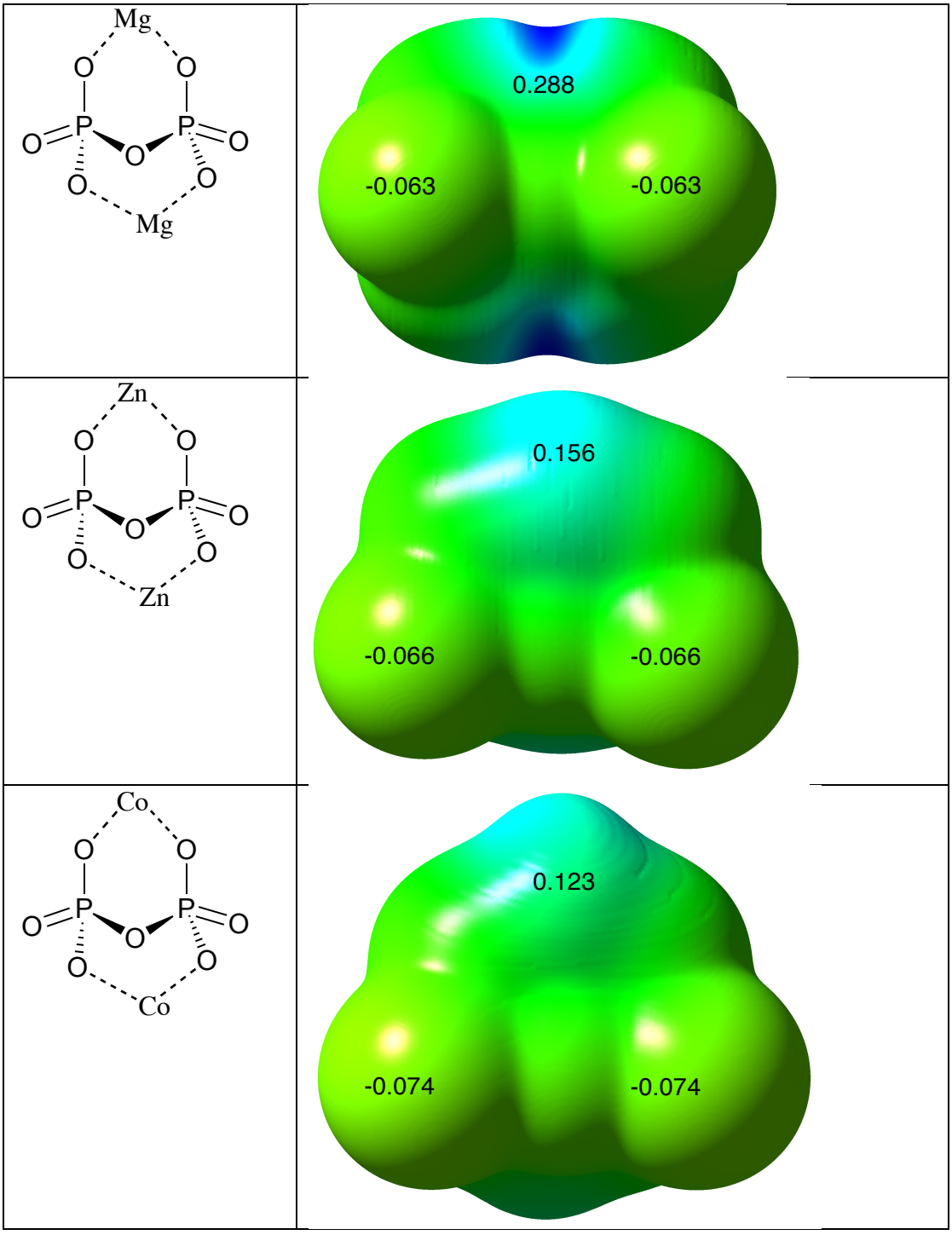


Figure S3. Free Energy Profiles for the Bisabolyl Formation Step in Trichodiene Synthase for the Fully Charged Enzyme Using Large and Small QM Regions.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Zn
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Co
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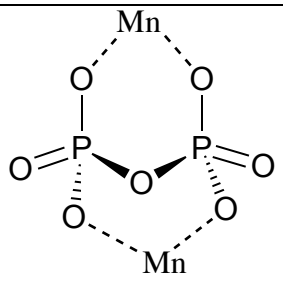
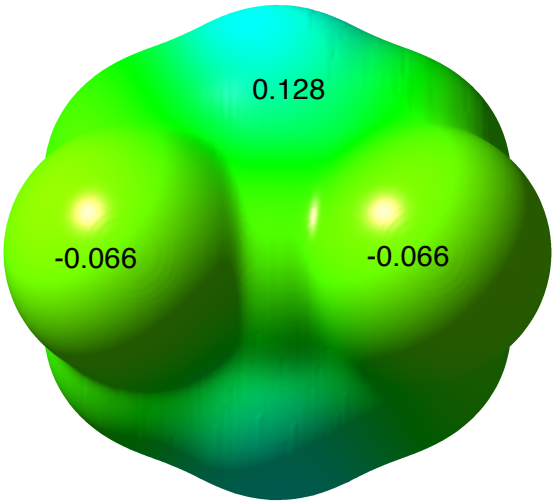
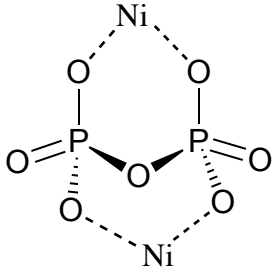
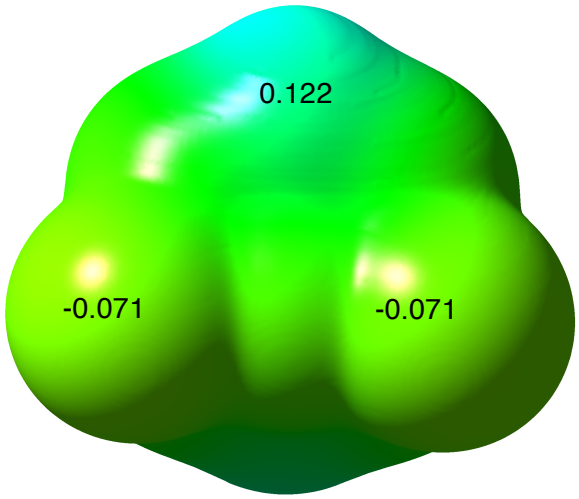
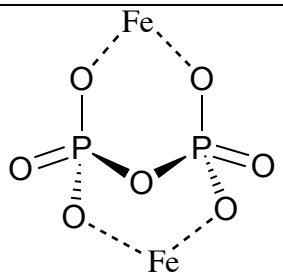
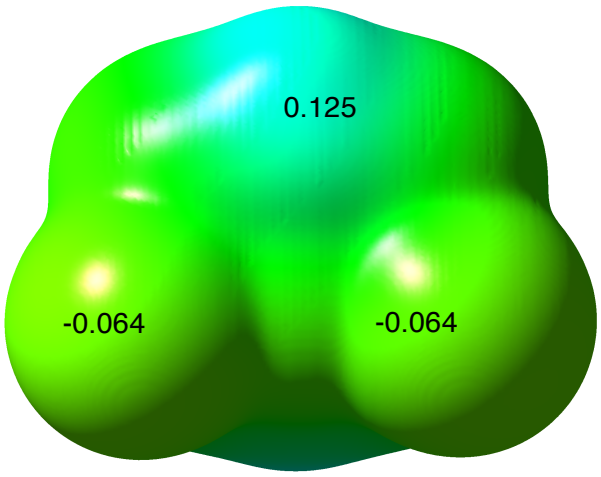
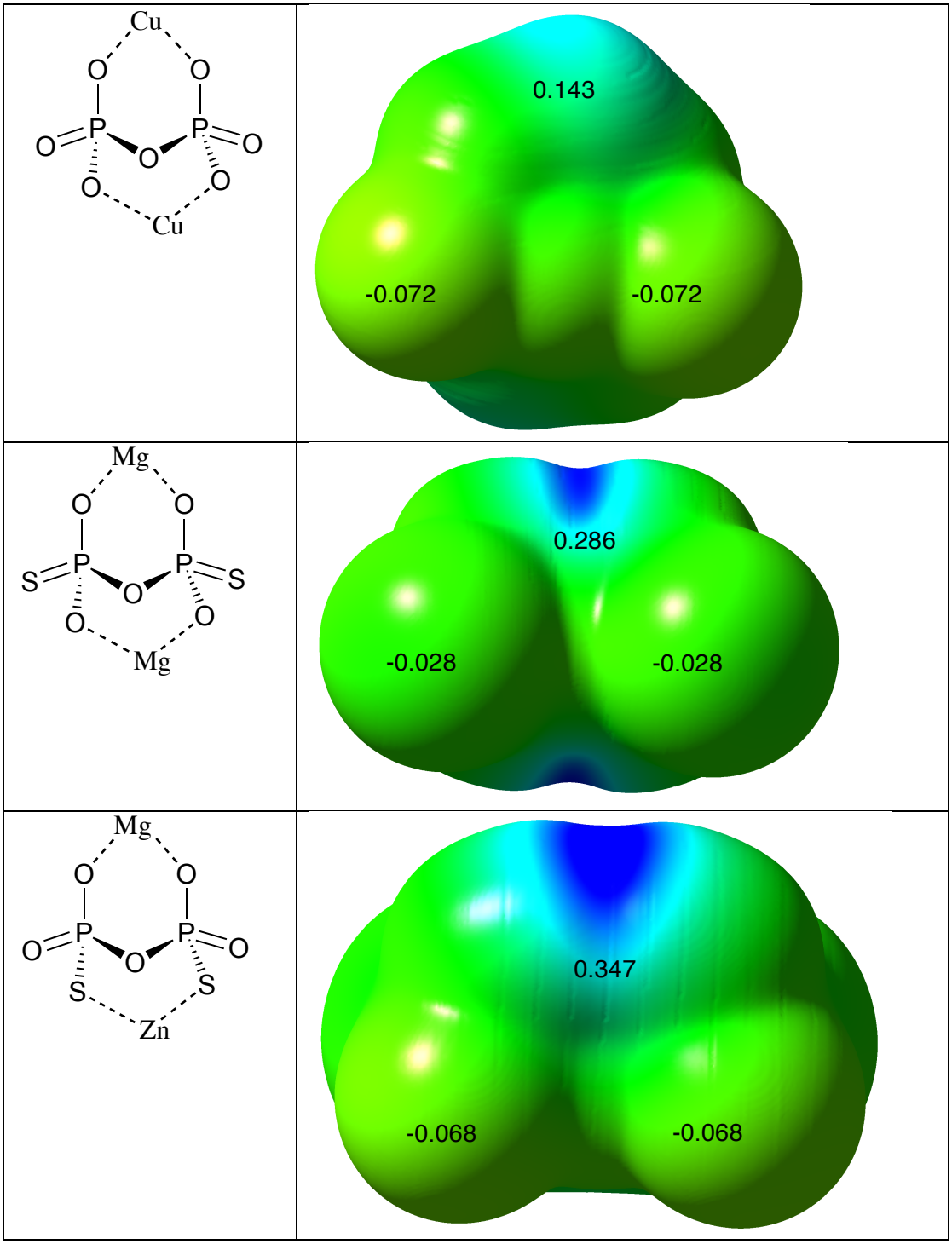
 Mn O O P P O O O O O Mn	 0.128 -0.066 -0.066
 Ni O O P P O O O O O Ni	 0.122 -0.071 -0.071
 Fe O O P P O O O O O Fe	 0.125 -0.064 -0.064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S
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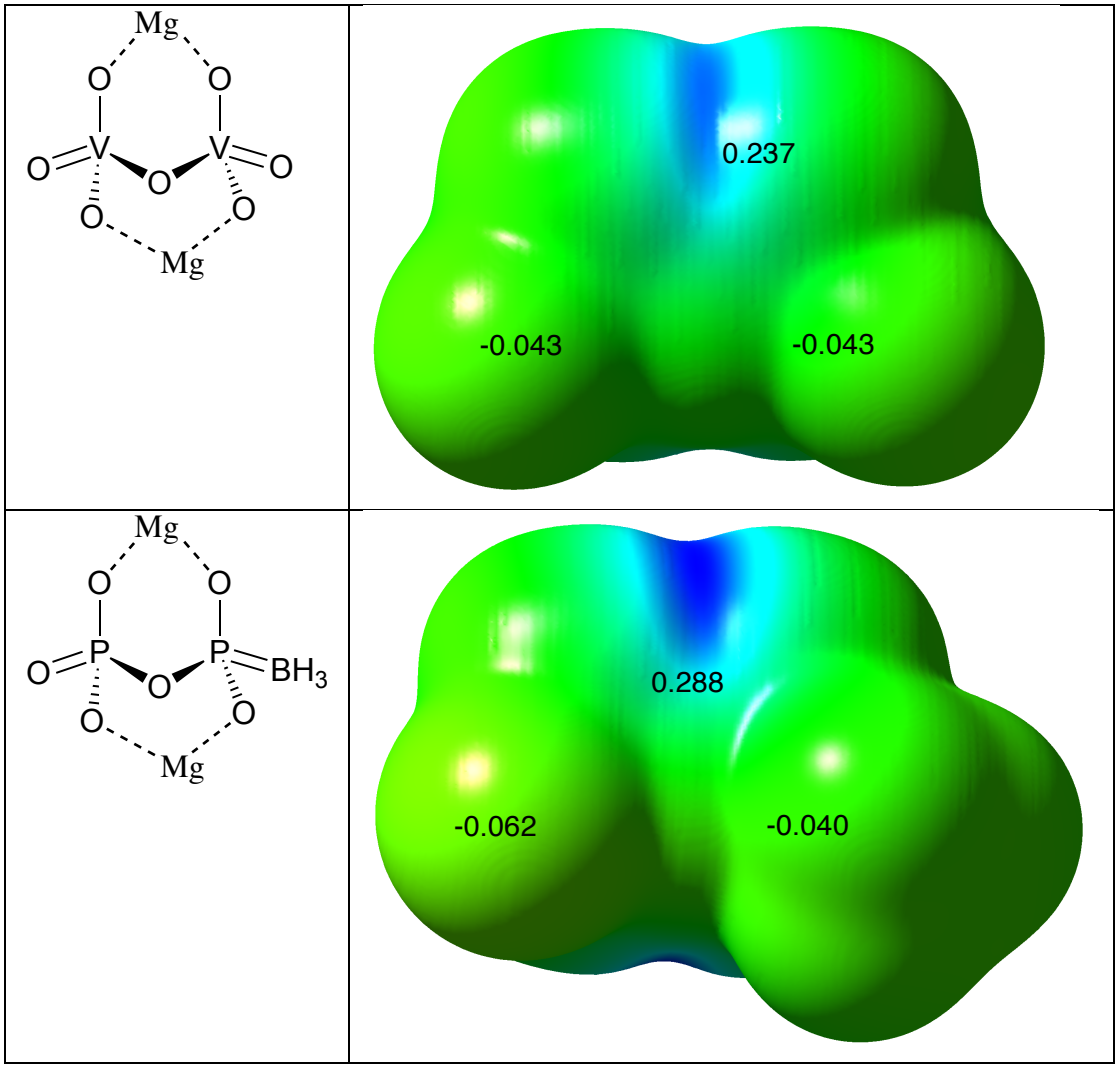

Figure S4. Molecular Electrostatic Potential (M06-2X/6-31+G**) for various phosphate-cation moieties showing how chemical modifications can change the charge distribution that is flanking the active site in terpene synthases. Selected extrema electrostatic potential values are shown mapped onto an isodensity surface of 0.0004 (a.u.). The color scale is normalized to be identical for all figures.

References:

1. Kulik, H. J.; Zhang, J.; Klinman, J. P.; Martínez, T. J. *J. Phys. Chem. B* **2016,** *120*, 11381-11394.
2. Karelina, M.; Kulik, H. J. *J. Chem. Theory Comput.* **2017,** *13*, 563-576.
3. Jindal, G.; Warshel, A. *J. Phys. Chem. B* **2016,** *120*, 9913-9921.
4. Major, D. T.; Freud, Y.; Weitman, M. *Curr. Opin. Struct. Biol.* **2014,** *21*, 25-33.