# Quantum mechanical/molecular mechanical and density functional theory studies of a prototypical zinc peptidase (carboxypeptidase A) suggest a general acid-general base mechanism

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**Support information:**

## 1. Stationary point structures along the promoted-water pathway for the peptide substrate

The structures of six stationary points along the promoted-water pathway for a peptide substrate identified by DFT full optimization at the B3LYP/6-31G(d) level of theory are displayed in Fig. S1.

## 2. Stationary point structures along the anhydride pathway for an ester substrate

Although no acyl-enzyme intermediate was found in both the QM/MM and DFT studies with a peptide substrate, we have identified a stable acyl-enzyme complex for the hydrolysis of an ester substrate with a truncated active-side model, which consists of the zinc ion, analogs of the three protein ligands, an acidic acid representing Glu270, an guanidinium group representing Arg127 and a substrate analog. The acyl-enzyme complex was identified by DFT full optimization at the B3LYP/6-31G(d,p) level of theory. Fig. S2 displays the energy profile for the formation of the acyl complex (AC) and the corresponding stationary points. The AC complex features a $sp^3$ hybridization for the central carbon ($C_6$). In addition, the carbonyl oxygen of the scissile carbonyl carries a fractional negative charge and is coordinated with Zn(II). The existence of a stable acyl-enzyme intermediate suggests that the anhydride mechanism might be operational for ester substrates.

## 3. Full citations of Refs 63 and 79.

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(79) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven Jr., T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; Gaussian, Inc.: Pittsburgh, PA, 2003.

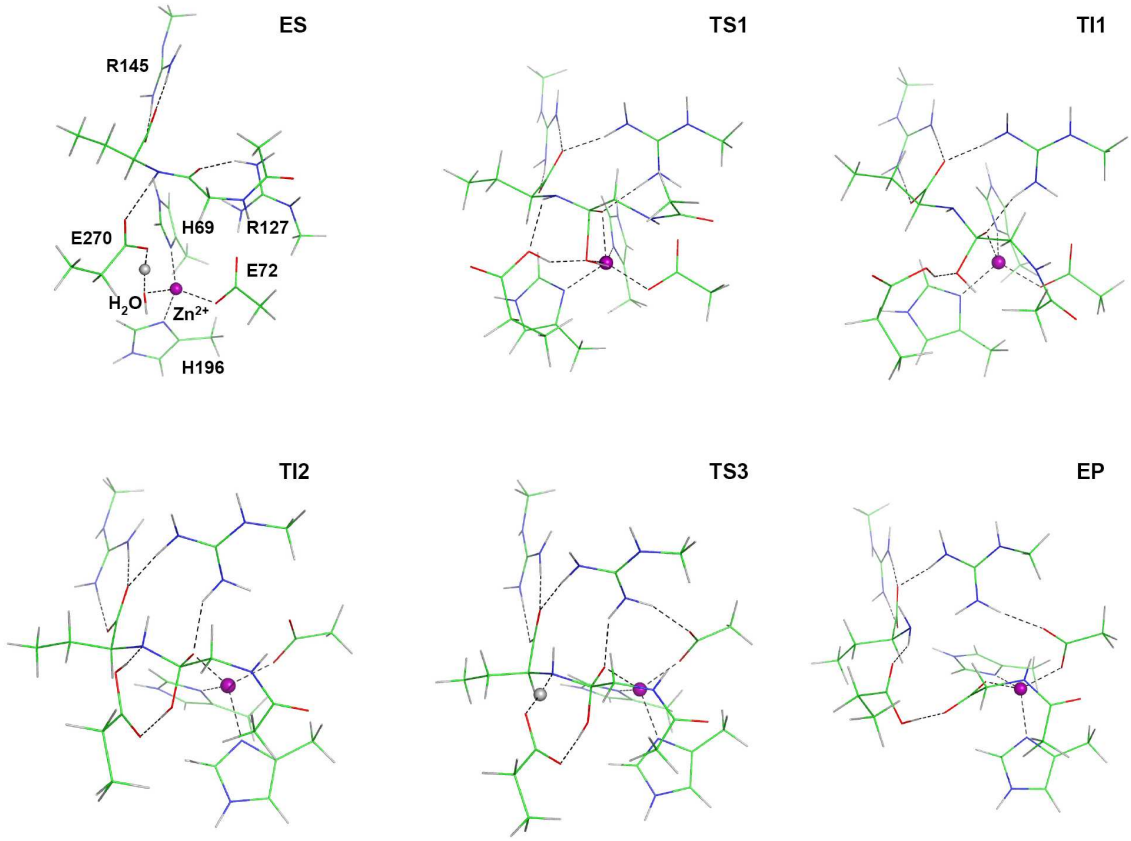


Fig. S1. Stationary point structures of the promoted-water pathway for a peptide substrate obtained at the B3LYP/6-31G(d) level of theory.

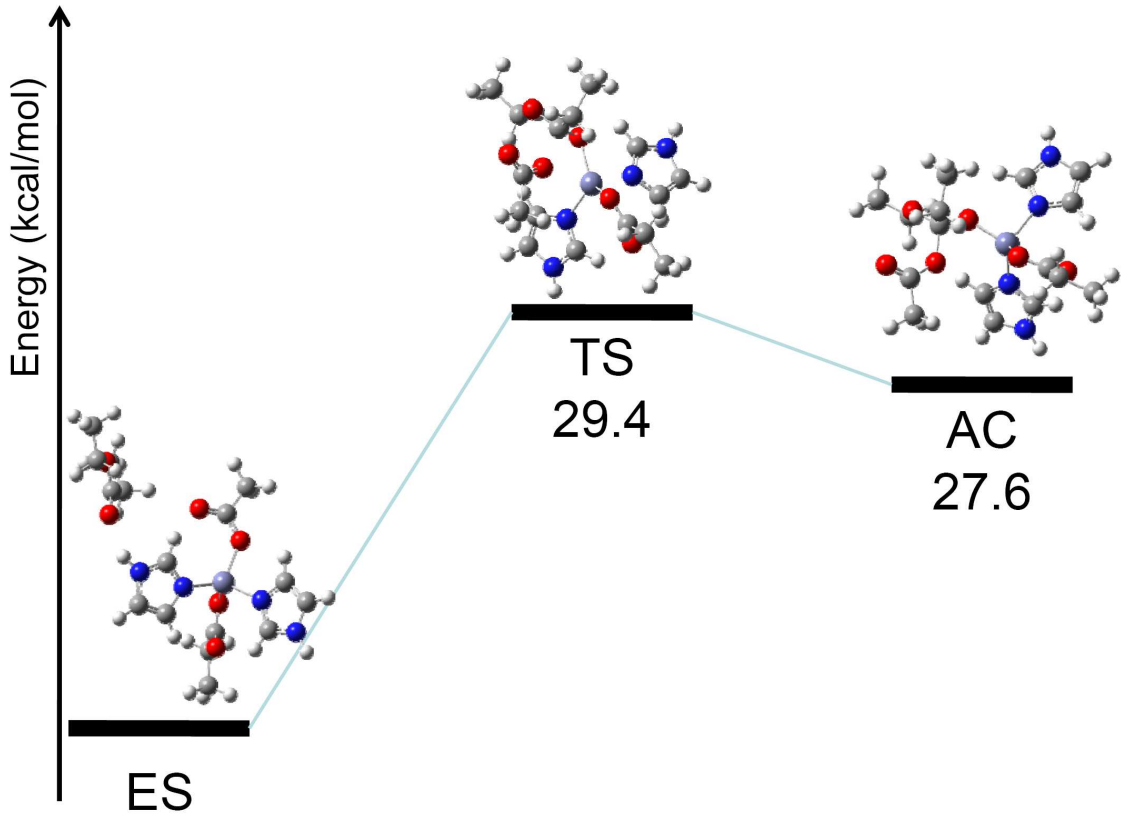


Fig. S2. Energy profile of the anhydride pathway for an ester substrate and the corresponding stationary point structures at the B3LYP/6-31G(d,p) level of theory.