

Adenylate Kinase (1985-96). Several major controversies in the structure-function relationship of adenylate kinase, including a major disagreement in the substrate binding sites deduced from X-ray and NMR studies, have been resolved by the interplay of site-directed mutagenesis, structural analysis, and conventional biochemical and chemical experiments. His insistence of analyzing every mutant protein by NMR has also set a good standard in the field. The quantitative structure-function analysis has also led to the demonstration of complete reversal (by R44M mutation) and enhancement (by R97M mutation) of phosphorus stereoselectivity by single mutations, which has never been achieved for any enzyme before. A critical review published as "Perspectives in Biochemistry", entitled "Site-Directed Mutagenesis Versus X-ray and NMR", (publication #72) has been cited 87 times. Publications: #37, 47, 48, 52, 57, 60, 64, 67, 70, 71, 72, 74, 78, 84, 85, 87, 88, 95, 96, 102, 104, 118, 136.

1. "Mechanism of Adenylate Kinase. 1. Use of ^{17}O NMR to Study the Binding Properties of Substrates," D. A. Wisner, C. Steginsky, Y.-J. Shyy and M.-D. Tsai, *J. Am. Chem. Soc.* *107*, 2814-2815 (1985).
2. "Mechanism of Adenylate Kinase. 2. Does ATP bind to the AMP Site?" Y. J. Shyy, G. Tian, and M.-D. Tsai, *Biochemistry*, *26*, 6411-6415 (1987).
3. "Mechanism of Adenylate Kinase. 3. Use of Deuterium NMR to Show Lack of Correlation Between Local Substrate Dynamics and Local Binding Energy." C. R. Sanders II and M.-D. Tsai, *J. Am. Chem. Soc.* *110*, 3323-3324 (1988).
4. "Mechanism of Adenylate Kinase. 4. Histidine-36 Is Not Directly Involved in Catalysis, But Protects Cysteine-25 and Stabilizes the Tertiary Structure." G. Tian, C. R. Sanders II, F. Kishi, A. Nakazawa, and M.-D. Tsai, *Biochemistry*, *27*, 5544-5552 (1988).
5. "Mechanism of Adenylate Kinase. 4. Histidine-36 Is Not Directly Involved in Catalysis, But Protects Cysteine-25 and Stabilizes the Tertiary Structure." G. Tian, C. R. Sanders II, F. Kishi, A. Nakazawa, and M.-D. Tsai, *Biochemistry*, *27*, 5544-5552 (1988).
6. "Mechanism of Adenylate Kinase. 6. Are the Essential Lysines Essential?" G. Tian, H. Yan, R.-T. Jiang, F. Kishi, A. Nakazawa, and M.-D. Tsai, *Biochemistry* *29*, 4296-4304 (1990).
7. "Mechanism of Adenylate Kinase. 7. Structural and Functional Demonstration of Arginine-138 as a Key Catalytic Residue Which Cannot be Replaced by Lysine". H. Yan, Z. Shi, and M.-D. Tsai, *Biochemistry* *29*, 6385-6392 (1990).
8. "Mechanism of Adenylate Kinase. 8. Critical Evaluation of the X-Ray Model and Assignment of the AMP Site." H. Yan, T. Dahnke, B. Zhou, A. Nakazawa, and M.-D. Tsai, *Biochemistry* *29*, 10956-10964 (1990).
9. "Mechanism of Adenylate Kinase. 9. Demonstration of Functional Relationship Between Aspartate-93 and Mg^{2+} by Site-Directed Mutagenesis and ^1H , ^{31}P , and ^{25}Mg NMR." H. Yan and M.-D. Tsai, *Biochemistry* *30*, 5539-5546 (1991).
10. "Mechanism of Adenylate Kinase. 10. Reversing Phosphorus Stereospecificity of an Enzyme by Site-Directed Mutagenesis". R.-T. Jiang, T. Dahnke, and M.-D. Tsai, *J. Am. Chem. Soc.* *113*, 5485-5486 (1991).

11. "Mechanism of Adenylate Kinase. 11. Site-Directed Mutagenesis Versus X-Ray and NMR." M.-D. Tsai and H. Yan, *Biochemistry* 30, 6806-6818 (1991). [This is a *Perspectives in Biochemistry* article.]
12. "Mechanism of Adenylate Kinase. 12. Prediction and Demonstration of Enhancement of Phosphorus Stereospecificity by Site-Directed Mutagenesis". T. Dahnke, R.-T. Jiang, and M.-D. Tsai, *J. Am. Chem. Soc.* 113, 9388-9389 (1991).
13. "Mechanism of Adenylate Kinase. 13. Structural and Functional Roles of Arginine-97 and Arginine-132." T. Dahnke, Z. Shi, H. Yan, R.-T. Jiang, and M.-D. Tsai, *Biochemistry* 31, 6318-6328 (1992).
14. "Modification of a Bruker AM-600 Spectrometer for Double and Triple Resonance Three Dimensional and Four Dimensional Experiments Illustrated with Chicken Adenylate Kinase Resonance Assignments." E. S. Mooberry, A. S. Edison, F. Abildgaard, J. L. Markley, I.-J. L. Byeon, and M.-D. Tsai, *Proceedings of the International Symposium on Spectroscopy and Structure of Molecules and Nuclei*, N. R. Johnson, W. N. Shelton, and M. El-Sayed, Eds., World Scientific, pp. 375-380 (1992).
15. "Mechanism of Adenylate Kinase. 14. What Can be Learned from a Mutant with Minor Perturbation in Kinetic Parameters?" Z.-T. Shi, I.-J. L. Byeon, R.-T. Jiang, and M.-D. Tsai, *Biochemistry* 32, 6450-6458 (1993).
16. "Use of Unnatural Amino Acids to Probe the Importance of the Aromaticity in Tyrosine-95 and the Ring Size in Proline-17 in Adenylate Kinase." Z. Zhao, X. Liu, Z. Shi, B. Huang, R.-T. Jiang, and M.-D. Tsai, *Youji Huaxue* 17, 20-23 (1997).
17. "Nucleoside Monophosphate Kinases: Structure, Mechanism, and Substrate Specificity." Honggao Yan and Ming-Daw Tsai, *Adv. Enzymol. And Related Area of Mol. Biol.* 73: *Mechanism of Enzyme Action*, D. L. Purich, Ed., pp. 103-134 (1999).*
18. "Mechanism of Adenylate Kinase. 15. ¹H, ¹³C, and ¹⁵N NMR Assignments, Secondary Structures, and Substrate Binding Sites." I.-J. L. Byeon, H. Yan, A. S. Edison, E. S. Mooberry, F. Abildgaard, J. L. Markley, and M.-D. Tsai, *Biochemistry* 32, 12508-12521 (1993).
19. "Mechanism of Adenylate Kinase. 16. The Conserved Aspartates 140 and 141 Are Important for Transition State Stabilization Instead of Substrate-Induced Conformational Changes." T. Dahnke and M.-D. Tsai, *J. Biol. Chem.* 269, 8075-8081 (1994).
20. "Mechanism of Adenylate Kinase. 17. Manipulating the Phosphorus Stereospecificity of Adenylate Kinase by Site-Directed Mutagenesis." M.-D. Tsai, R.-T. Jiang, T. Dahnke, and Z. Shi, *Methods Enzymol.* 249, 425-443 (1995).
21. "Mechanism of Adenylate Kinase. 18. The Essential Lysine Helps Orient the Phosphates and the Active Site Residues to Proper Conformations." I.-J. L. Byeon, Z. Shi, and M.-D. Tsai, *Biochemistry* 34, 3172-3182 (1995).
22. "Structure-Function Relationship of Adenylate Kinase. 19. Glutamine-101 in AMP Specificity." S. Beichner, I.-J. L. Byeon, and M.-D. Tsai, *PEPTIDES: Chemistry, Structure, and Biology*, Kaumaya, P. T. P. and Hodges, R. S. (Eds.), Mayflower Scientific Ltd. (1996), Chapter 303, pp. 721-723.
23. "Mechanism of Adenylate Kinase. 20. Probing the Importance of the Aromaticity in Tyrosine-95 and the Ring Size in Proline-17 with Unnatural Amino Acids." Z. Zhao, X.

Liu, Z. Shi, L. Danley, B. Huang, R.-T. Jiang, and M.-D. Tsai, *J. Am. Chem. Soc.* *118*, 3535-3536 (1996).