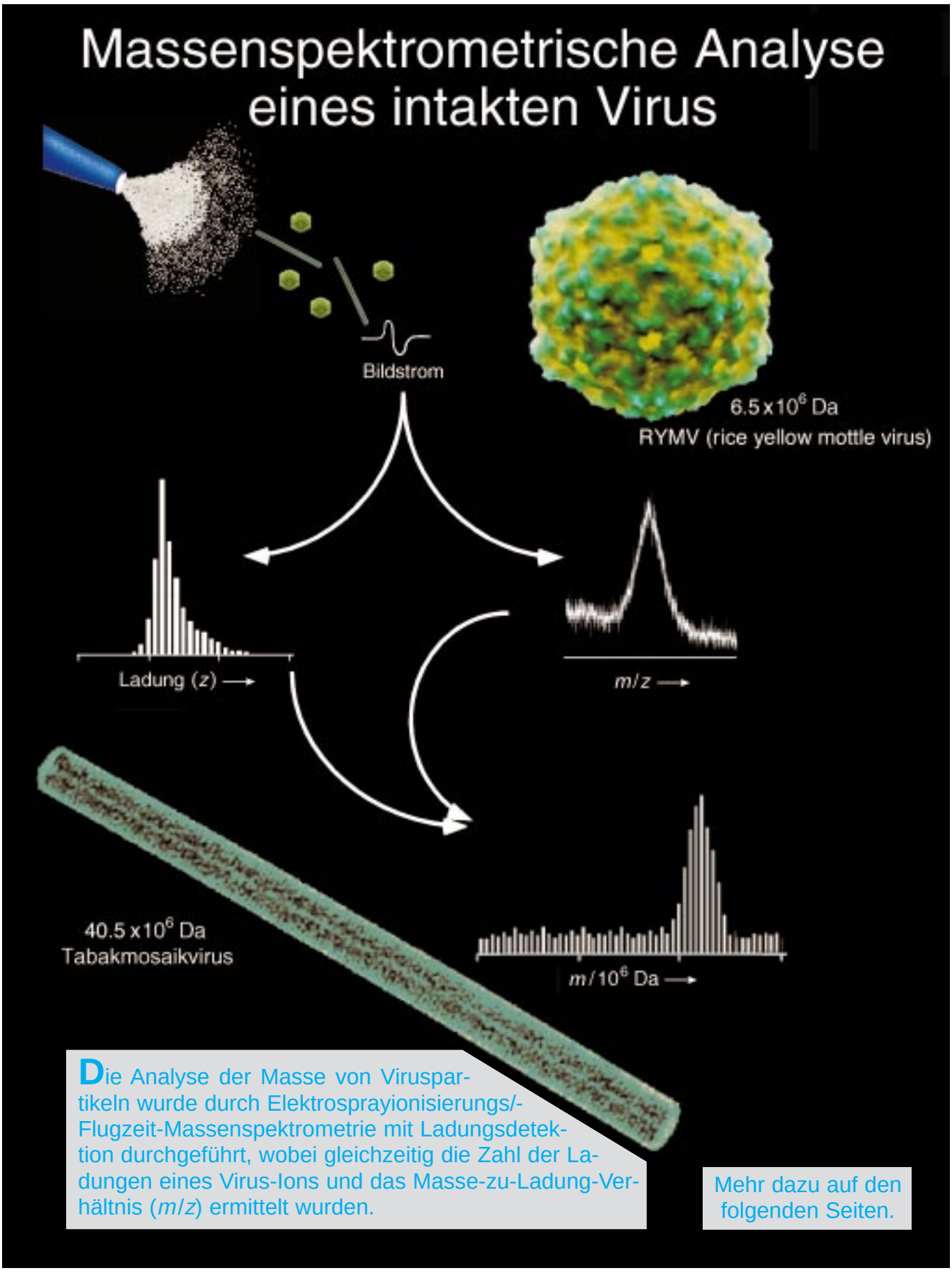
Massenspektrometrische Analyse eines intakten Virus



Die Analyse der Masse von Viruspartikeln wurde durch Elektrosprayionisierungs/-Flugzeit-Massenspektrometrie mit Ladungsdetektion durchgeführt, wobei gleichzeitig die Zahl der Ladungen eines Virus-Ions und das Masse-zu-Ladung-Verhältnis (m/z) ermittelt wurden.

Mehr dazu auf den folgenden Seiten.

  0044-8249/01/11303-0559 $ 17.50+.50/0

Mass Spectrometry of an Intact Virus**

Stephen D. Fuerstenau, W. Henry Benner,*
John J. Thomas, Christophe Brugidou, Brian Bothner, and Gary Siuzdak*

The development of electrospray ionization (ESI) for biomolecular analysis marks one of the historically significant events in mass spectrometry (MS).[1] As a consequence largely of electrospray, the utility of mass spectrometry has been extended far beyond routine determinations of molecular weight to applications in such diverse fields as chemistry, immunology, and structural biology.[2] For example, ESI allows for the analysis of noncovalent interactions[3, 4] such as those that form the basis of supramolecular chemistry,[5] protein folding,[3, 6] and viral protein–protein interactions.[7, 8] In fact, experiments performed on whole viruses[8] demonstrate that viruses can be observed with mass spectrometry and that noncovalent supramolecular structures can successfully withstand the rigors of vaporization, ionization, and the vacuum of a mass spectrometer. In the initial experiments[8] viral ions to be analyzed by ESI were nondestructively introduced into the vacuum of a mass spectrometer and electrostatically focused onto a collector plate within the instrument. Interestingly, the virus particle ions were found to retain both their native structure and infectivity; however, mass measurements could not be obtained with the available mass spectrometers. We have circumvented the problems associated with detecting large ions by using charge-detection mass spectrometry to make a simultaneous measurement of charge (z) and mass-to-charge (m/z) ratio for individual particles.

In previous experiments it was found that tobacco mosaic virus (TMV) could be passed through a quadrupole mass analyzer in radio frequency-only mode and weakly detected with an electron multiplier.[8] Yet in those studies we were unable to perform mass analysis on intact virus ions because their mass-to-charge (m/z) values were too large to yield peaks in our quadrupole mass spectrometer which had a m/z range of 2300. Furthermore, even if our instrument had had sufficient mass range, the limited resolution as well as formation of adducts during electrospray ionization would have made traditional determination of the mass by assignment of the charge state of adjacent peaks extremely difficult or impossible.[2] The impressive mass measurements by Robinson and co-workers[9] of viral capsid ions up to 2.5×10^6 Da and with over 100 charges suggest that traditional ESI-MS methodology will face significant challenges when applied to the measurement of ions with a mean charge state of several hundred. An alternative method based on the direct detection of charge number[10–14] was explored to overcome these limitations for the analysis of whole viruses.

Charge-detection mass spectrometry is a relatively new technique that circumvents limitations associated with detecting large, highly charged ions by making a simultaneous measurement of charge (z) and mass-to-charge (m/z) ratio for individual ions. The detection technique permits the mass analysis of electrospray ions with virtually unlimited mass[10–14] and has been performed on both Fourier-transform ion-cyclotron resonance[14] and time-of-flight[11–13] mass analyzers. The electrospray ionization source used in these studies[11] produced an aerosol of highly-charged virus particles in the ion-evaporation region of the electrospray ion source at atmospheric pressure. Virus ions pass one at a time through a small metal flight tube attached to a charge-sensitive preamplifier that captures their image current, the magnitude of which is proportional to the ion's charge. In addition, by measuring the time-of-flight[15] of each ion through the flight tube the m/z value can be determined. The mass of each ion is obtained from a combination of both the charge and m/z value, where the ion charge can typically be determined with a precision of better than $\pm 75\ z$ (as demonstrated in previous experiments with microparticles). A more complete description of the experiments and the system is published elsewhere.[11]

Two viruses were examined in these studies: rice yellow mottle virus (RYMV) and tobacco mosaic virus (TMV). RYMV is an icosahedral virus (29 nm in diameter) consisting of a single-stranded RNA surrounded by a homogenuous protein shell made up of 180 copies of a single coat protein.[16] RYMV has a calculated molecular weight of 6.5×10^6 Da and was collected and purified as previously described.[17, 18] TMV is rod-shaped and is formed from approximately 2140 identical protein subunits wound in a 300-nm long helix with a diameter of 17 nm. A central hollow cylindrical core holds the viral genome—a 6395-nucleotide strand of RNA. TMV has a calculated molecular weight of 40.5×10^6 daltons.[8] The purified RYMV or TMV were typically diluted in unbuffered water to a concentration of approximately 3×10^{12} particles per mL (5 pM).

The results of these analyses were typically obtained from measuring thousands of individual ions and required approximately 30 minutes for collection. The charge-state distribution of large particles results from factors related to particle shape (where more extended or open conformations often lead to more charges), competition for charge between large particles and easily desorbed low-mass ions, as well as electrospray conditions such as solvent composition and pH value. The most highly charged species we have generated are

[*] Prof. Dr. G. Siuzdak, Dr. J. J. Thomas, Dr. C. Brugidou, B. Bothner
The Scripps Research Institute
Center for Mass Spectrometry
BCC157, 10550 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-9496
E-mail: siuzdak@scripps.edu
Dr. W. H. Benner
Lawrence Berkeley Laboratory
MS 70A-3363, 1 Cyclotron Road, Berkeley, CA 94720 (USA)
Fax: (+1) 510-486-5857
E-mail: whbenner@lbl.gov
Dr. S. D. Fuerstenau
Jet Propulsion Laboratory
California Institute of Technology
4800 Oak Grove Drive, Pasadena, CA 91109 (USA)

[**] The authors gratefully acknowledge Jennifer Boydston for her helpful comments and suggestions. G.S. is grateful for support from the NIH (GM55775). The work at LBL was supported by the Director, Office of Energy Research, Office of Health and Environmental Research, Human Genome Program, U.S. Department of Energy under contract number DE-AC03-76SF00098.

  0044-8249/01/11303-0560 $ 17.50+.50/0

linear molecules of single-stranded DNA since these ions are likely to be elongated in the gas phase, thereby stabilizing or distributing many charged sites along the polymer without significant Coulombic repulsion. Coulombic repulsion limits the maximum number of charges that can reside on the surface of the virus particle. Accordingly, we observed less charging for RYMV and TMV than for significantly smaller DNA biopolymers.[12] The charge-state distribution observed for TMV (Figure 1) was similar to that previously observed for DNA.[19] The RYMV and TMV ions possessed a charge distribution of between 300 and 1000 positive unit charges with higher charge states observed for TMV.

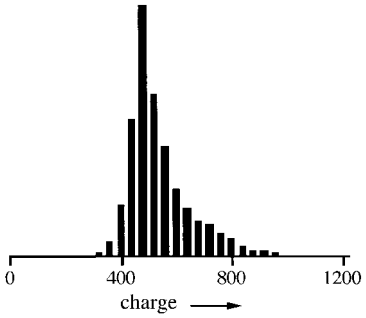


Figure 1. Histogram showing the charge spectrum obtained for tobacco mosaic virus (TMV) using a time-of-flight mass analyzer with charge detection.

The mass spectrum obtained on RYMV (Figure 2) showed a maximum signal centered between 6×10^6 and 7×10^6 Da with tailing on the high mass end. This data corresponds well

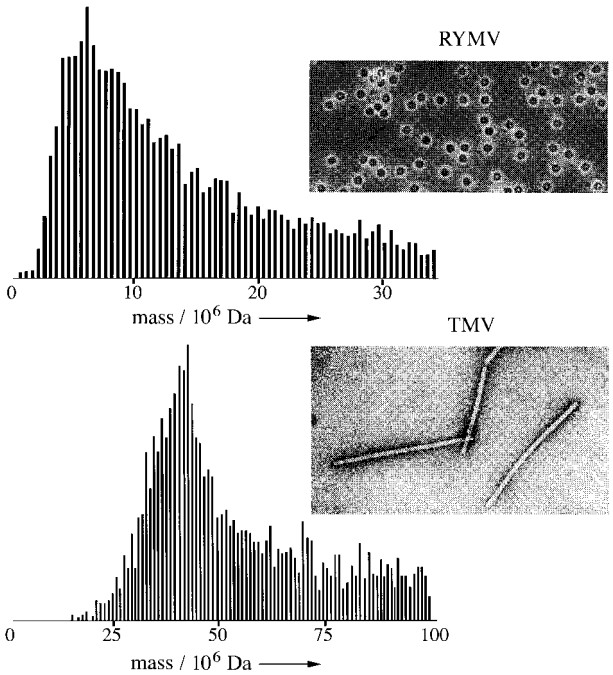


Figure 2. Mass spectra of rice yellow mottle virus (RYMV, top) and tobacco mosaic virus (TMV, bottom) particles analyzed with an electrospray-ionization charge-detection time-of-flight mass spectrometer. Insets: electron micrographs of the icosahedral RYMV (diameter 28.8 nm; top) and the cylindrical TMV (approximately 300 nm long and 17 nm in diameter; bottom). The known molecular weights of RYMV and TMV are 6.5×10^6 and 40.5×10^6 Da, respectively.

to the theoretical mass of a single intact RYMV particle (6.5×10^6 Da) within the error of the instrument ($\pm 15\,\%$). The mass spectrum on the TMV particles (Figure 2) generated a mass centered between 39×10^6 and 42×10^6 Da, which is consistent with the calculated mass of 40.5×10^6 Da and also within the error of the instrument. While the mass measurements were quite accurate, the relatively large error in the mass determination is derived from both the charge detection and m/z time-of-flight measurements. To improve the accuracy of these measurements we are developing a more stable charge-detection approach to reduce the variation in the signals from ion to ion. In addition, by increasing the length of the mass spectrometer's flight tube we also hope to further reduce the error in the m/z value through more accurate time-of-flight mass measurements. It should also be noted that the mass spectral data show a broadening commonly observed with electrospray time-of-flight analysis of porous protein assemblies with irregular surfaces.[20–22] While the soft ionization conditions of electrospray help to maintain the macromolecular structure, these conditions also allow for the formation of adducts and incomplete desolvation of large porous structures, thereby contributing to the broad peak shape of the gas-phase particle.

Mass spectrometry has made significant strides into the realm of clinical applications,[23] and this approach could present another diagnostic method for pathogenic diseases. Currently, mass spectrometric measurements provide valuable insight into virus identification, structure, and function,[24] and mass measurements of whole viruses would be advantageous. For example, examination of the charge-state distribution on viruses could further reveal different dynamic conformers. Charge detection could also be used to monitor biologically important interactions such as DNA–protein, virus–antibody, and virus–cell receptor complexes since the flexibility, and thus the charge state of macromolecules, can change with intermolecular interactions. Additionally, the mass measurement of viral subgroups within a population would allow virologists to better understand the diversity of viruses or to measure members of a population that have been labeled, for example, with antibodies. Far from being merely a routine mass-measurement tool, mass spectrometry continues to offer new perspectives on the solution and gas-phase properties of biomolecular complexes, including whole viruses.

Experimental Section

Virus preparation and purification: Plants were infected from seed stocks of RYMV (ATCC PV-515) and TMV (ATCC 135P-PV) which had been purchased from American Type Culture Collection. The purification was performed by the protocol of Fauquet and Thouvenel.[25] Briefly, infected leaves were ground and homogenized in 0.1M phosphate buffer with 0.2 % β-mercaptoethanol (pH 5.0), and filtered through cheesecloth (Fisher Scientific). The homogenate was centrifuged with an equal volume of water and the aqueous phase was collected and stored overnight at 4 °C with 6 % polyethylene glycol. Samples were then pelleted and the virus was collected through a 20–30 % sucrose gradient. Samples were stored at 4 °C, and dialyzed prior to mass spectrometric analysis.

The RYMV virus solutions were infused into the heated capillary at 0.5 μL min^{-1}. The solutions containing TMV were infused at 0.05 μL min^{-1}. A counter-flow gas of nitrogen assisted the desolvation and direction of the

  0044-8249/01/11303-0561 $ 17.50+.50/0

electrospray ions. A voltage of −2500 V was applied to the electrospray needle, which was held 1–3 cm away from the inlet to the vacuum.

Received: September 1, 2000 [Z15749]

[1] M. Yamashita, J. B. Fenn, *J. Phys. Chem.* **1984**, *88*, 4451–4454.
[2] G. Siuzdak, *Mass Spectrometry for Biotechnology*, Academic Press, San Diego, **1996**, p. 162.
[3] V. Katta, B. T. Chait, *Rapid Commun. Mass Spectrom.* **1991**, *5*, 214–217.
[4] B. Ganem, Y.-T. Li, J. D. Henion, *J. Am. Chem. Soc.* **1991**, *113*, 7818–7819.
[5] C. A. Schalley, *Int. J. Mass Spectrom.* **2000**, *194*, 11–39.
[6] A. Miranker, C. V. Robinson, S. E. Radford, R. T. Aplin, C. M. Dobson, *Science* **1993**, *262*, 896–900.
[7] B. Bothner, R. Chavez, J. Wei, C. Strupp, A. Schneeman, G. Siuzdak, *J. Biol. Chem.* **2000**, *275*, 13455–13459.
[8] G. Siuzdak, B. Bothner, M. Yeager, C. Brugidou, C. M. Fauget, K. Hoey, C.-M. Chang, *Chem. Biol.* **1996**, *3*, 45–48.
[9] M. A. Tito, K. Tars, K. Valegard, J. Hajdu, C. V. Robinson, *J. Am. Chem. Soc.* **2000**, *122*, 3550–3551.
[10] W. H. Benner, *Anal. Chem.* **1997**, *69*, 4162–4168.
[11] S. D. Fuerstenau, W. H. Benner, *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1528–1538.
[12] J. C. Schultz, C. A. Hack, W. H. Benner, *Rapid Comm. Mass Spectrom.* **1999**, *13*, 15–20.
[13] J. C. Schultz, C. A. Hack, W. H. Benner, *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 305–313.
[14] R. D. Smith, X. Cheng, J. E. Bruce, S. A. Hofstadler, G. A. Anderson, *Nature* **1994**, *369*, 137–139.
[15] R. J. Cotter, *Time-Of-Flight Mass Spectrometry: Instrumentation and Applications in Biological Research*, American Chemical Society, Washington, **1997**, p. 432.
[16] M. N. A. Yassi, C. Ritzenthaler, C. Brugidou, C. Fauquet, R. N. Beachy, *J. Gen. Virol.* **1994**, *75*, 249–256.
[17] C. Fauquet, J. C. Thouvenel, *Plant Dis. Rep.* **1977**, *61*, 443–446.
[18] W. Bakker, *Versl. Landbouwkd. Onderz.* **1974**, *829*, 152–161.
[19] Stephen D. Fuerstenau and W. Henry Benner reported at the 44th ASMS Conference on Mass Spectrometry and Allied Topics, **1996**.
[20] J. A. Loo *J. Mass. Spectrom.* **1995**, *30*, 180–183.
[21] E. J. Nettleton, M. Sunde, Z. Lai, J. W. Kelly, C. M. Dobson, C. V. Robinson *J. Mol. Biol.* **1998**, *281*, 553–564.
[22] A. A. Rostom, C. V. Robinson, *J. Am. Chem. Soc.* **1999**, *121*, 4718–4719.
[23] H. H. Maurer, *J. Chromatogr. B* **1998**, *713*, 3–25.
[24] J. J. Thomas, R. Bakhtiar, G. Siuzdak, *Acc. Chem. Res.* **2000**, *33*, 179–187.
[25] C. Fauquet, J. C. Thouvenel, *Plant Dis. Rep.* **1977**, 61, 443–446.

Engineering Catalysts for Enantioselective Addition of Diethylzinc to Aldehydes with Racemic Amino Alcohols: Nonlinear Effects in Asymmetric Deactivation of Racemic Catalysts**

Jiang Long and Kuiling Ding*

"Asymmetric amplification" or the "positive nonlinear effect" ((+)-NLE) is a very attractive phenomenon in catalytic asymmetric processes, since it gives enantioselectivities which are improved with respect to the expectations based on the *ee* value of the auxiliary.[1–7] Therefore, because of asymmetric amplification, high-enantiopurity chiral ligands need not necessarily be applied to achieve high enantiopurities of product. Nevertheless one has to make a partial resolution of the chiral ligands from their racemic forms. Interestingly, asymmetric catalysis with racemic catalysts has been successfully achieved by "chiral poisoning"[8] or "asymmetric activation"[9] strategy.

After the first report on the nonlinear effect in asymmetric catalysis by Kagan and co-workers,[2] the early experiments with strong asymmetric amplification were demonstrated by Oguni et al. in 1988 and by Noyori et al. in 1989 for enantioselective addition of diethylzinc to aldehydes.[3] In this type of alkylation, the asymmetric amplification is well recognized to be a consequence of an in situ increase in the *ee* value of the active catalyst, since racemic ligand is trapped in the more stable, unreactive *meso* species.[7] The recent treatment by Noyori and co-workers [7b] of the systems involving the dynamic monomer–dimer equilibria where the monomers are active species is complementary to the ML_n models of Girard and Kagan.[1b] In principle, if racemic ligands are used alone, the reaction will definitely give racemic product. The addition of an alternative nonracemic additive (which should be cheap and easily obtainable) to the racemic catalyst system may enantioselectively generate a new species of dinuclear zinc complex with one enantiomer of racemic ligand through "non-self-recognition"[7a] to release the opposite enantiomer of catalyst for asymmetric catalysis. To exemplify this strategy, we choose Oguni et al.'s racemic amino alcohols to carry out asymmetric catalysis by adding nonracemic additives. In this contribution, we report our results on the first example of highly enantioselective addition of diethylzinc to aldehydes with the catalysis of racemic amino alcohols in the presence of chiral additives.

[*] Prof. Dr. K. Ding, Dr. J. Long
Laboratory of Organometallic Chemistry
Shanghai Institute of Organic Chemistry
Chinese Academy of Sciences
354 Fenglin Road, Shanghai 200032 (China)
Fax: (+86) 21-6416-6128
E-mail: kding@pub.sioc.ac.cn

[**] Financial support from the National Natural Science Foundation of China, the Chinese Academy of Sciences, the Major Basic Research Development Program of China (Grant no. G2000077506), and the Science and Technology Commission of Shanghai Municipality is gratefully acknowledged.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

  0044-8249/01/11303-0562 $ 17.50+.50/0