

## Quantitative Determination of Noncovalent Protein-Ligand Interactions Automatic Chip-Based Nanoelectrospray

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### Abstract

The purpose of this experiment is to demonstrate automated nanoESI/MS analysis to determine micromolar and submicromolar dissociation constants as well as to measure the solution binding constants for the Ribonuclease A (RNase) complexes with cytidilic acid ligands.

### Benefits

- Allows extended acquisition time for better data quality
- Increases sample throughput
- Improves spray stability and reproducibility
- Automates nanoelectrospray with one time spray optimization
- No carryover

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## Introduction

### Automated Chip-Based Nanoelectrospray

Advion BioSciences, Inc. (Ithaca, NY) has developed a method and demonstrated the capabilities of the NanoMate System for quantitative determination of noncovalent interactions between proteins and ligands. The NanoMate 100 is a chip-based automated nanoelectrospray ionization system for mass spectrometry, and is readily integrated with the Waters Micromass Q-Tof micro.

Investigations of noncovalent protein-ligand interactions by nanoelectrospray ionization mass spectrometry (nanoESI/MS) are of great interest because of their relevance to molecular recognition and to combinatorial ligand library searching. This application note from Advion Biosciences introduces an experiment where automated nano ESI/MS analysis has been used to determine micromolar and submicromolar dissociation constants as well as to measure the solution binding constants for the Ribonuclease A (RNase) complexes with cytidilic acid ligands.



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*Waters Micromass Q-ToF micro Mass Spectrometer and the Advion NanoMate 100.*

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## Experimental

### Determination of Noncovalent Protein-Ligand Interactions

RNase complexed with cytidine 2'-monophosphate and cytidine triphosphate (see Figure 1), a well characterized model system, was used to demonstrate the method.

### Titration Experiments

RNase protein was maintained at 10  $\mu\text{M}$  and 4  $\mu\text{M}$ , respectively, in 10 mM ammonium acetate pH 6.8 for titration of 2'-CMP (1 to 20  $\mu\text{M}$ ) and CTP (1 to 8  $\mu\text{M}$ ), respectively. The solutions were then incubated at room temperature for 15 minutes prior to MS analysis.

$$K_d = \frac{[R] * [L]}{[RL]} = \frac{[R] * ([L_i] - [RL])}{[RL]}$$

$$[RL]/[R] = 1/K_d * ([L_i] - [RL])$$

## Competitive Binding Experiments

Equimolar solutions of 2'-CMP and CTP (4  $\mu$ M) were mixed with 4  $\mu$ M of RNase in 10 mM ammonium acetate pH 6.8. The solutions were then incubated at room temperature for 15 minutes prior to nanoelectrospray MS analysis.

$$K_{d_{RL1}} = [R] * ([R] + [RL_2]) / [RL_1]$$

$$K_{d_{RL2}} = [R] * ([R] + [RL_1]) / [RL_2]$$

## NanoESI/MS Analytical Conditions

Sample size:	3 $\mu$ L
Flow rate:	100 nL/minute
Spray voltage:	1.5 kV
Pressure:	0.3 psi
Acquisition time:	2 minutes
Instrumentation:	NanoMate100 with ESI Chip Micromass Q-ToF micro
Sample cone voltage:	30 V

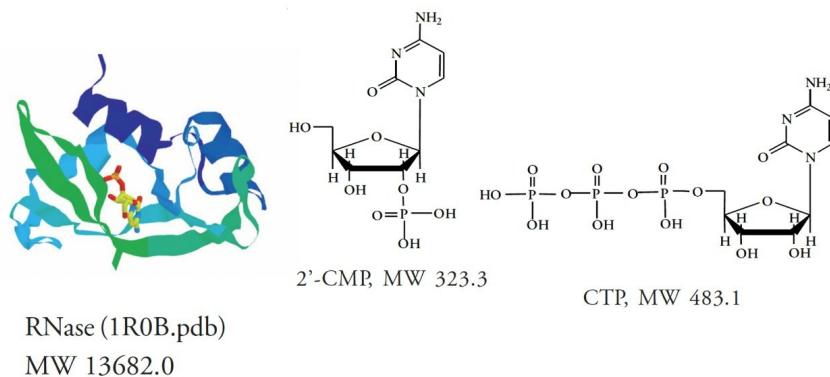
Source temp.:

45 °C

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## Results and Discussion

Each ligand was detected using the NanoMate100 System (Figures 2 and 3) and as a result titration and competitive binding experiments were performed (Figure 4). The results presented are in agreement with previously published results of circular dichroism (CD).<sup>1</sup>



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Figure 1. Protein and ligand structures.

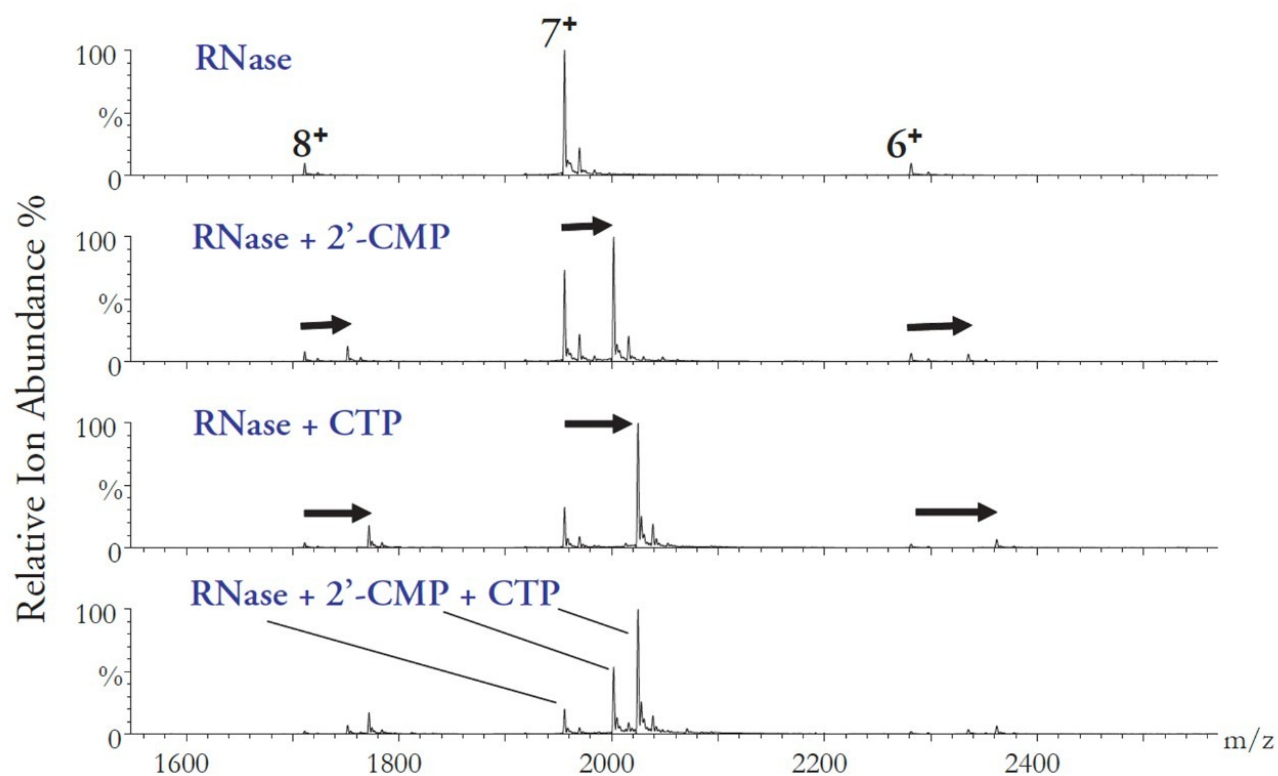


Figure 2. NanoESI mass spectra.

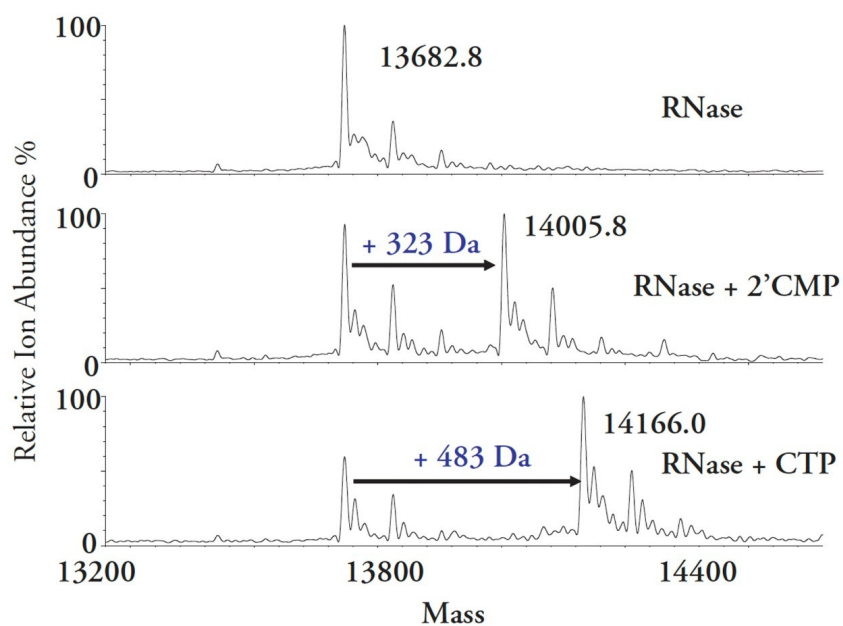


Figure 3. Deconvoluted mass spectra of the RNase-Ligand complexes.

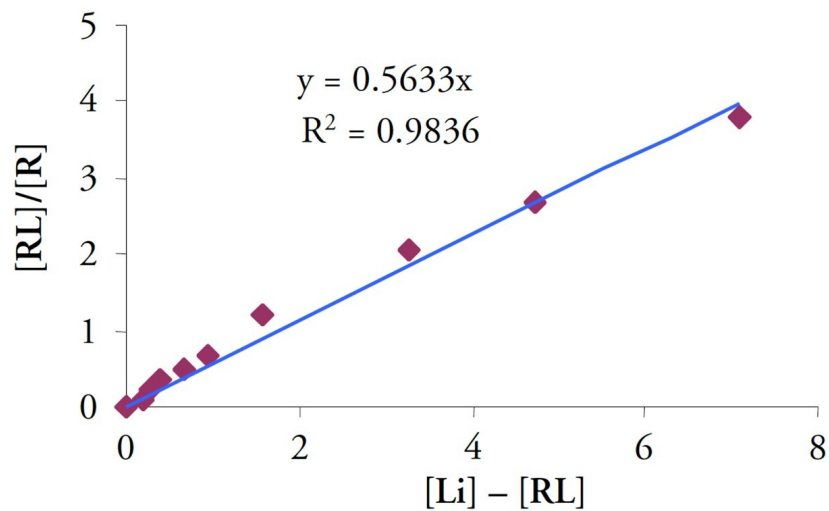


Figure 4. Titration assay for RNase (10mM) with 2'-CMP.

Ligand	K <sub>d</sub> (μM)		
	Titration experiment		Competitive binding experiment
	Ave. of individual points	Plot	
Cytidine 2'- monophosphate (2'-CMP)	1.71 ± 0.33	2.00 ± 0.43	2.3 ± 0.4
Cytidine triphosphate (CTP)	0.80 ± 0.2	0.74 ± 0.3	0.75 ± 0.4

Table 1. Summary of binding assay for RNase and cytidine nucleotide ligands using automated NanoESI/MS.

## Conclusion

The NanoMate100 System can be used to determine micromolar and submicromolar dissociation constants. In addition, an automated nanoESI/MS method can be used to measure solution binding constants for the RNase complexes with cytidilic acid ligands.

## References

1. Jones, C.L.; Fish, F.; Muccio, D.D. *Anal BioChem* 2002, 302, 184–190.
2. Application note based on Zhang, S.; Van Pelt, C.K.; Wilson, D.B. *Anal Chem* 2003, 75, 3010–3018.

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