



Department of Chemistry  
NMR Facilities  
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## NMR NEWS

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\* To check on the [instrumental status](#) and [reservation system](#), and find tutorials, links and more, visit our website [www.chem.utk.edu/nmr](http://www.chem.utk.edu/nmr)

**\* Varian Mercury 300 MHz upgrade:  $^2\text{H}$  gradient shims.**

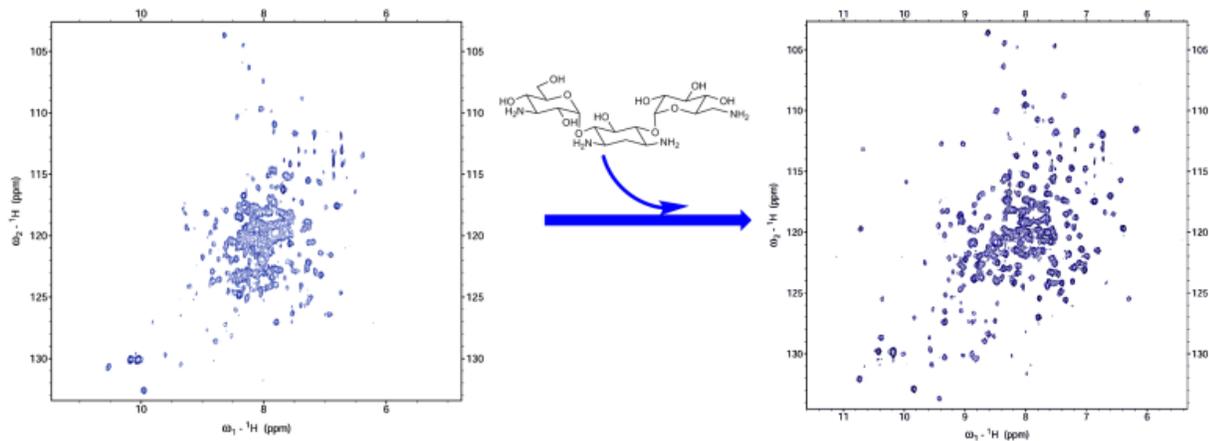
Besides a new computer and software, the Varian 300 spectrometer has now “Deuterium Gradient Shims”. This new feature allows automatic gradient shims on deuterated solvents with the click on a button. In this way, you get the best shims possible in no time.

**\* Comparing proton and  $^{13}\text{C}$  sensitivity (signal to noise) on the different liquid state spectrometers.**

	AC250	V300	B400 (QNP)	V600 (cold probe)
$^1\text{H}$	70:1	105:1	131:1	4600:1
$^{13}\text{C}$	50:1	75:1	259:1	524:1

**\* Dr. E. Serpersu research work. Dept. of Biochemistry & Cellular and Molecular Biology. JACS 131,8587-8594 (2009).**

Our laboratory studies the physico-chemical properties of enzyme–antibiotic complexes using several different enzymes that modify aminoglycoside antibiotics and render them useless against infectious bacteria. In our recent work, hydrogen deuterium exchange detected by NMR spectroscopy is used to determine the dynamic properties of the aminoglycoside phosphotransferase 3'-IIIa (APH), a protein of intense interest due to its involvement in conferring antibiotic resistance to both gram negative and gram positive microorganisms. This work represents the first characterization of dynamic properties of an aminoglycoside modifying enzyme. Herein we describe *in vitro* dynamics of apo, binary and ternary complexes of APH with kanamycin A, neomycin B, and metal–nucleotide. Regions of APH in different complexes that are superimposable in crystal structures show remarkably different dynamic behavior.  $^{15}\text{N}$ - $^1\text{H}$  HSQC spectra shown below illustrates that the apo-enzyme is highly dynamic (left panel) as it is apparent from the low dispersion and high overlap of the backbone amides. Addition of any aminoglycoside alters the spectrum dramatically (right panel) and shows that the enzyme adopts a well-defined conformation in solution (Spectra shown in the right hand panel is also displayed at the NMR page of the Complex Carbohydrate research center at The Univ. Georgia as a example how well 900 MHz NMR spectrometer can resolve resonances of a 31 kDa protein).



Furthermore a *complete exchange* of backbone amides is observed within the first 15 hours of exposure to  $\text{D}_2\text{O}$  in the apo form of this 31 kDa protein. Binding of aminoglycosides to the enzyme induces significant protection against exchange and  $\sim 35\%$  of the amides remain unexchanged up to 90 hours after exposure to  $\text{D}_2\text{O}$ . Our data also indicate that neomycin creates greater solvent protection and overall enhanced structural stability to APH than kanamycin. Surprisingly, nucleotide binding to the enzyme–aminoglycoside complex *increases* solvent accessibility of a number of amides and is responsible for destabilization of a nearby  $\beta$ -sheet thus providing a rational explanation for previously observed global thermodynamic parameters. Our data also provide a molecular basis for broad substrate selectivity of APH.

Our current work is concentrated characterization of another aminoglycoside-modifying enzyme, the aminoglycoside acetyltransferase(3)-IIIb in a similar manner described above. Our ultimate goal is to use representative enzymes from each of the three catalytically-distinct group of aminoglycoside-modifying enzymes to determine shared physicochemical

properties of enzyme–antibiotic complexes to understand the general molecular principles that allow these enzyme to recognize the same molecules despite differences in their primary structures.