Experimental Report

Proposal: **9-13-419** **Council:** 10/2011

Title: Ion-Ion pairing in solutions of guanidinium salts

This proposal is a new proposal

Researh Area: Biology

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Samples: N3 C H6 (Cl and CO3 salts), D2O Solutions, (15) N3 C H6 (Cl and CO3 salts), D2O Solutions

Instrument	Req. Days	All. Days	From	To
D4	4	5	18/11/2012	23/11/2012

Abstract:

Structure factors will be measured for aqueous 6M GdmCl D2O solutions labeled with 15N, natN and mixN atomic concentrations of nitrogen. An identical experiment will be conducted for Gdm2CO3. From these measurements the functions SNN(Q) and gNN(r) will be calculated and the nature of the guanidinium-guanidinium interaction examined as a function of counter-ion. Despite the small nature of these differences, with suitable care and appropriate experimental methodologies we have successfully measured comparable differences previously. There is an increasing need for structural data on the nature of this interaction in the field of protein science due to the important role of the guanidinium moiety in the side chain of the amino acid arginine, and the role it plays in protein folding/ protein aggregation. It is also of significance to the mechanism by which guanidinium (one of the most commonly used denaturant) destabilizes proteins.

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The guanidinium motif is ubiquitous in biochemistry. It occurs as the side chain of the amino acid arginine, as a neutral motif in the base pair guanine, and is isoelectronic with the denaturant and metabolically important compound urea. The guanidinium cation is also a powerful denaturant and an interesting ion in its own right. In molecular dynamic simulations it shows a wide variety of interactions with both with itself and with other anions. The goal of this study was to gain some insight into these ion-ion interactions by neutron scattering.

In order to obtain the highest chance of success on this technically challenging experiment, it was necessary to use almost the highest concentration of guanidinium possible. 6 molal solutions of GdmCl and $^{15}N_3$-GdmCl were prepared in D_2O. From these solutions raw total neutron scattering patterns were measured. An exactly 50:50 mix was then taken of these two solutions and the raw total neutron scattering pattern measured. The remaining GdmCl and $^{15}N_3$-GdmCl solutions were then sent through an ion exchange resin and titrated up to form solutions of 3 molal (6molal of Gdm^+) Gdm_2SO_4 and $^{15}N_6$-Gdm_2SO_4 in D_2O. Raw total neutron scattering patterns were then collected on these two solutions and again on an exact 50:50 mixture of these two solutions. The acquired data was subject to multiple scattering and absorbtion corrections and were normalized versus a standard vanadium rod in order to obtain corrected total neutron scattering patterns F(Q). From the first order difference using neutron scattering with isotopic substitution (NDIS) the functions $\Delta S_{NX}(Q)$ were calculated for both sets of solutions (where X is any atom in the system). The constitution of the two first order difference functions $\Delta S_{NX}(Q)$ are as follows for the GdmCl and Gdm_2SO_4 solutions respectively.

$$^{GdmCl}\Delta S_{NX}(Q) = 0.77\ S_{NC}(Q) + 6.3\ S_{NO}(Q) + 19.1\ S_{ND}(Q) + 1.1\ S_{NCl}(Q) + 2.76\ S_{NN}(Q)$$

$$^{Gdm_2SO_4}\Delta S_{NX}(Q) = 0.72\ S_{NC}(Q) + 7.1\ S_{NO}(Q) + 17.7\ S_{ND}(Q) + 0.15\ S_{NS}(Q) + 2.56\ S_{NN}(Q)$$

The measured functions ${}^{GdmCl}\Delta S_{NX}(Q)$ and ${}^{Gdm_2SO_4}\Delta S_{NX}(Q)$ are shown in Figure 1.

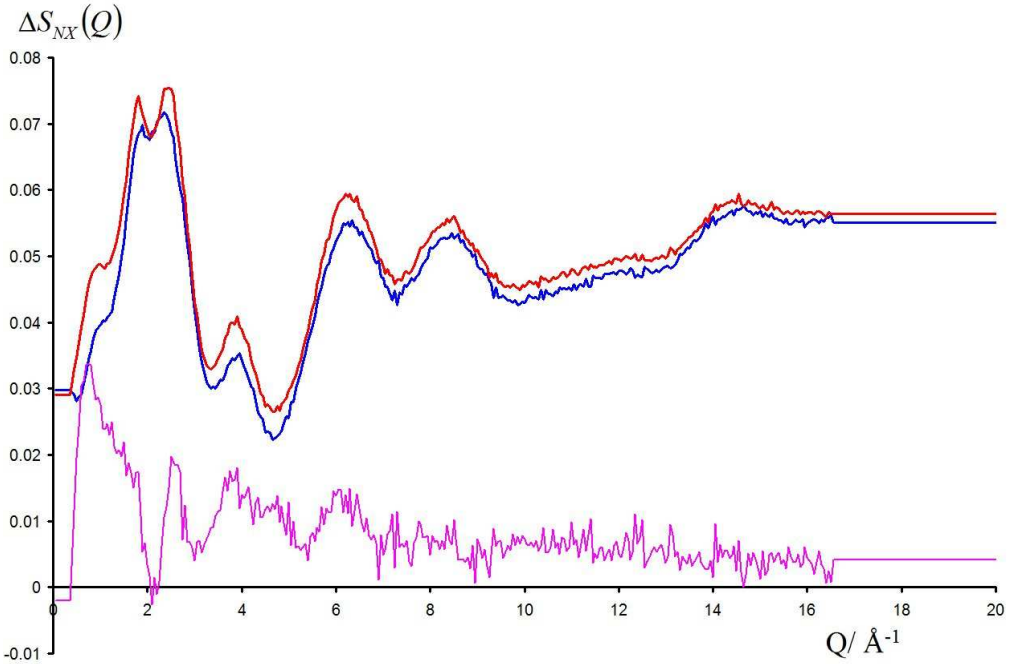

Figure 1, the functions ${}^{GdmCl}\Delta S_{NX}(Q)$ (red) and ${}^{Gdm_2SO_4}\Delta S_{NX}(Q)$ (blue). Shown in purple (scaled by a factor of 3) is the difference between these two functions, highlighting the difference in the ion-ion ordering in these solutions.

As the NDIS experiment on the Gdm^+ ion yields the structure around the Gdm^+ ion (both ions and water), this means that any difference in these functions must be due to different ion-ion and ion-water ordering. This difference is also shown in Figure 1, and is a demonstration that using stable, high counting instruments such as D4C combined with well prepared samples that it is possible to gain insight into the ion-ion ordering in aqueous solution. This was the primary goal of this proposal.

Currently we are running molecular dynamics studies on systems mirroring those in these experiments in order to better interpret the experimental results. These results are already well on their way to being written up as a paper.