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Supporting Material

Probing structural transitions in the intrinsically disordered C-terminal domain of the measles virus nucleoprotein by vibrational spectroscopy of cyanylated cysteines

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Supporting Information

1. Far-UV circular dichroism

A crucial point for further analysis was to assess whether cysteine cyanylation could affect the overall secondary structure content of N_{TAIL}. Notably, previous studies revealed that neither cysteine substitution nor introduction of a nitroxide radical affected the overall secondary structure content and folding propensities of N_{TAIL} (Belle et al. 2008, ref. 13) thus suggesting that no notable effects were to be expected upon cysteine cyanylation.

Methods: far-UV circular dichroism. All CD spectra were recorded between 190 and 260 nm using 1-mm pathlength quartz cells in 10 mM sodium phosphate buffer pH 7 at 25°C. The spectra of the *wt* protein and all cyanylated variants were collected using an AVIV model 410 spectropolarimeter, equipped with a Peltier themoregulation system which held the cell temperature constant at 25ºC. All spectra were measured with a scanning speed of 20 nm/min and a data pitch of 1.0 nm, and were not smoothed. Mean ellipticity values per residue (MRE) ([Θ]) were calculated as $[\Theta] = 3300\ m\ \Delta A/(l\ c\ n)$, where l (path length) = 0.1 cm, n (number of residues) = 132, m (molecular mass) = 14,632 Da for *wt* N_{TAIL}, 14,673 Da for S407C* and S491C*, 14,647 Da for L496C*, and 14,657 Da for V517C* and c (protein concentration) = 0.1 mg/mL.

Discussion. The far-UV CD spectra of the cyanylated N_{TAIL} variants at neutral pH were found to superimpose well onto that of *wt* N_{TAIL}, being all typical of unstructured proteins, as seen by their large negative ellipticity at 200 nm and moderate ellipticity at 190 nm (see Figure S1). These data indicate that the replacement of the native side chain by a cyanylated cysteine induces little, if any, structural perturbations. No significant

variations were observed between the spectra of any of the cyanylated samples and the *wt* protein.

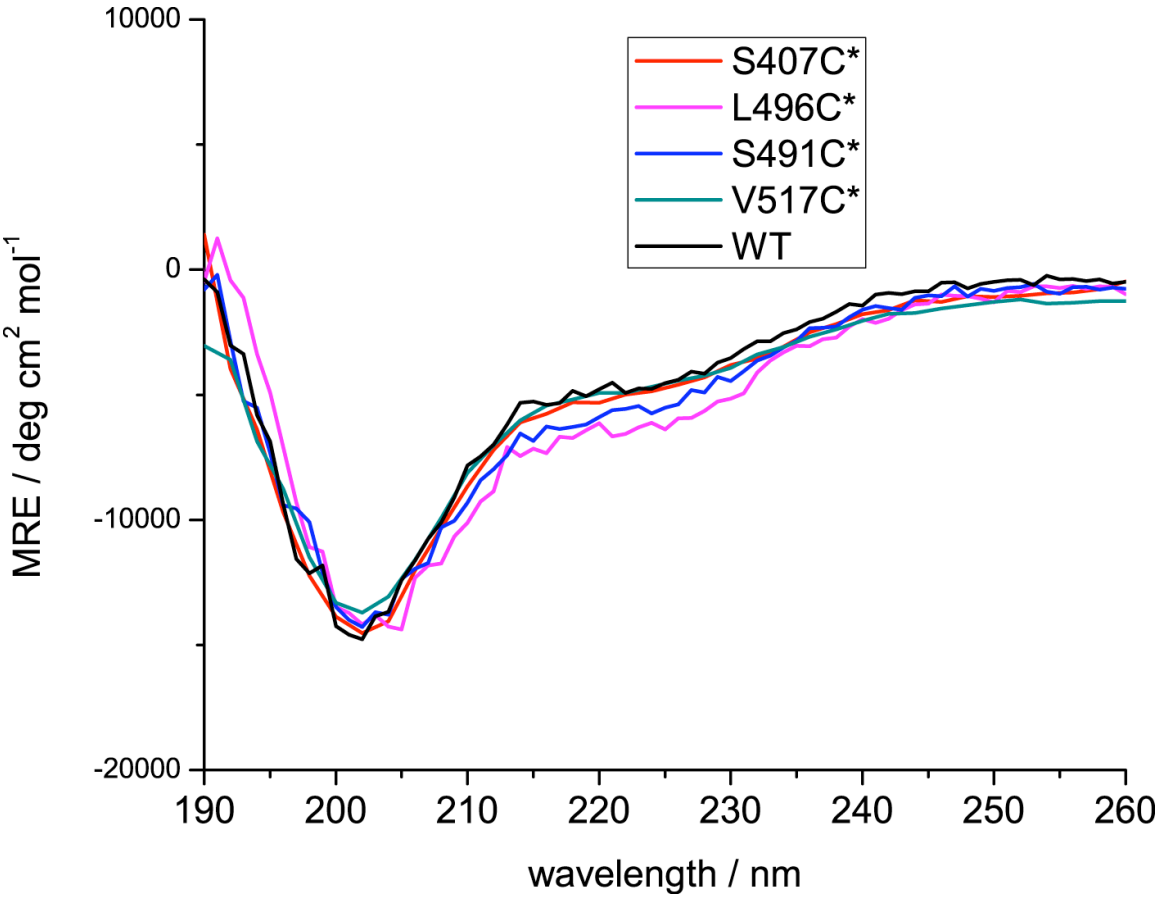

Figure S1. Far-UV CD spectra of native and cysteine cyanylated N_{TAIL} variants.

2. Infrared spectroscopy of MeSCN in THF/water mixtures

The infrared spectra of methyl thiocyanate (MeSCN) in mixed solvents varying from 0% to 100% THF in water are presented in Figure 3. Figure S2 shows the central frequency and linewidth of each of these CN stretching peaks; the gray filled contour area represents the 50% intensity level of each band, and thus the height of the gray areas are a representation of the bands' changing widths.

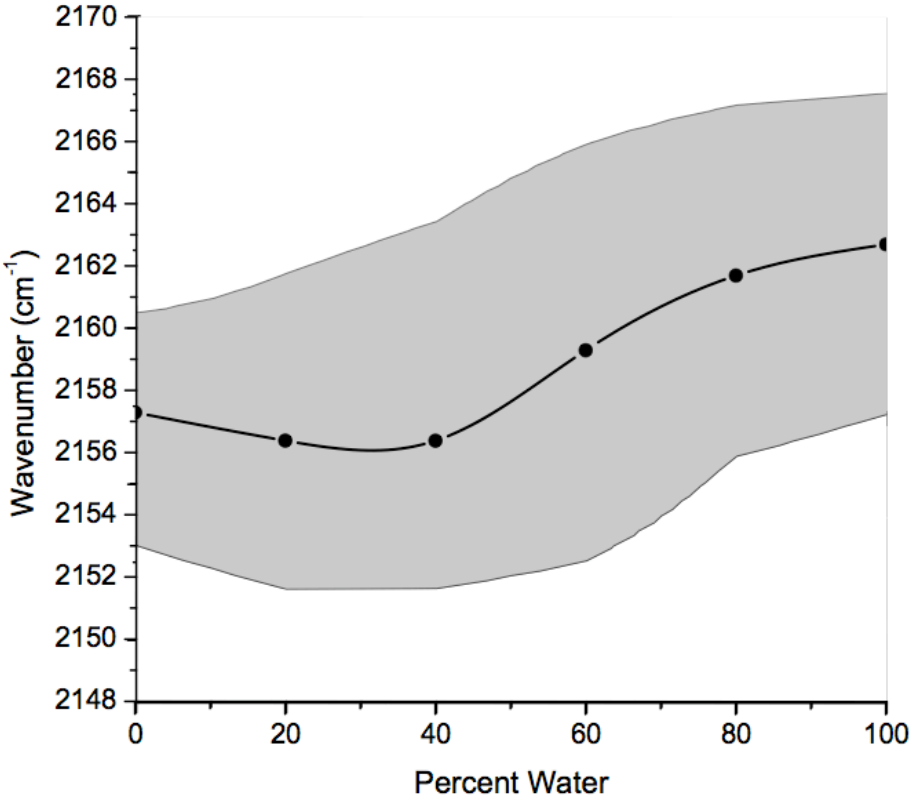

Figure S2. Maximum frequency and linewidth (full width at half maximum, displayed at 50% contour level and filled in gray) for the CN stretching bands in Figure 3 as a function of solvent composition.

For mixed solvents containing a high percentage of water, the central frequency decreases with increasing THF, as expected. However, throughout the varying solvent mixtures, both the frequency and linewidth change in non-monotonic ways. The frequency reaches a minimum at 40% THF and then slightly increases as the amount of

water approaches zero. The linewidth broadens from its value in 100% water as THF is added to the solvent mixture, stays broad for most mixed solvents, and then narrows substantially for 100% THF. Previous analyses of model compounds with carbon-bound nitrile vibrations in THF/water mixtures (SI-1, SI-2) have interpreted similar broadening in mixed solvents as evidence of multiple spectral subpopulations, invoking three subpopulations and fitting the single nitrile stretching band to linear combinations of these subpopulations as the solvent mix is changed.

The current situation is somewhat different from those previous studies for two reasons: 1) the shift in frequency due to the solvent is smaller compared to the linewidth, and 2) additional low-frequency components are observed in solvent mixtures below the bandshape observed in pure THF. Compared to the 100% THF spectrum, the 60% and 80% THF spectra include both higher and lower frequency components. Without extensive line-fitting of the current data to multiple underlying peaks (which would be somewhat self-serving in the current case due to the lack of more clear underlying subpopulations), it is likely that the behavior observed here is due to the presence of multiple subpopulations which come from different mixed solvent environments around the vibrational chromophore. These environments could be different in their hydrogen bonding character, their dielectric constant, their mean dipolarity, and the time scale for reorientation of solvent dipoles. There is no particular reason that any of these environmental factors should particularly resemble the environment around the side chain of an artificial amino acid in a protein, other than the presence or absence of water.

For the sake of interpreting the data for the V517C* mutant in the absence and presence of XD, the 0% THF and 20% THF spectra provide a reasonable empirical comparison since they have similar linewidths, a similar frequency shift, and no notable asymmetry. The spectra of MeSCN in mixed solvents are clearly more complicated than just reflecting the extent of water exclusion and indicate, most likely, that MeSCN in THF and THF/water mixtures is not necessarily a useful general model for interpretation of the extent of water exposure of this vibrational label in all scenarios.

Additional References

SI-1. Getahun, Z., C. Y. Huang, T. Wang, B. De Leon, W. F. DeGrado, and F. Gai. 2003. Using nitrile-derivatized amino acids as infrared probes of local environment. J. Am. Chem. Soc. 125:405-411.

SI-2. Watson, M. D., X. S. Gai, A. T. Gillies, S. H. Brewer, and E. E. Fenlon. 2008. A Vibrational Probe for Local Nucleic Acid Environments: 5-Cyano-2 '-deoxyuridine. J. Phys. Chem. B 112:13188-13192.