## SUPPORTING INFORMATION

## Infrared Population Transfer Spectroscopy of Cryo-Cooled Ions: Quantitative Tests of the Effects of Collisional Cooling on the Room Temperature Conformer Populations

Christopher P. Harrilal, Andrew F. DeBlase, Joshua L. Fischer, John T. Lawler, Scott A. McLuckey, and Timothy S. Zwier Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084 U.S.A.

Figure S1. Background Subtraction from Conformation-Specific IR Spectra


Figure S1. Non-confomer specific gain spectra (red) taken off resonance at $35548.6 \mathrm{~cm}^{-1}$ overlaid on raw conformation-specific infrared spectra of conformer B at $35553.7 \mathrm{~cm}^{-1}$. The gain spectra were normailized to the unique NH stretch of conformer A in the on resonance signal at $3308 \mathrm{~cm}^{-1}$. The resulting subtraction in given in Figure S .3 (b).

Figure S2. Conformer specific IR-depletion on transitions labeled A and A*


Figure S2. Conformer specific IR depletion spectra taken on distinct UV transitions $35407.0 \mathrm{~cm}^{-1}$ and $35469.8 \mathrm{~cm}^{-1}$ in the hydride stretch (a) and amide I/II (b) regions. The UV transitions reported here are tentatively assigned to tyrosine rotamers (A and $\mathrm{A}^{*}$ ). Resulting IR spectra are identical, indicating conformations giving rise to the distinct UV transitions possess similar 3D structure.

Figure S3. Conformer Specific IR-depletion of family A and B along with global minimum structures


Figure S3. Conformer specific IR spectra in amide I/II and hydride stretch regions of family A (a) and B (b). Lowest energy representative structures for families A and B are shown in (c) and (d), respectively.


Global Minimum of Family $A$


Tyrosine ring rotation from global minimum structure

Figure S4. Comparison of the global minimum trans structure to the conformation differing only in the tyrosine hindered rotation, labelled with a short dashed red line in Figure 4. There are no further structural permutations to the structure pictured on the right.

Figure S5. Simulated cooling of nearly isoenergetic tyrosine rotamers


Figure S5. Equilibrium distribution as a function of internal energy between tyrosine OH rotamers of Conformer A with the proline puckered in the up position. Note the scale on the fractional populations, which are maintained near 0.500:0.500 throughout. Since the two conformations are nearly degenerate in free energy as a function of temperature there is no driving force to populate one over the other as internal energy is removed, thus it is possible to omit them from the kinetic cooling scheme.

Figure S6. UV trace of [YGGFL+H]+ under varied time delays between helium pulse and ion arrival to cold trap


Figure S6. UV trace of $[\mathrm{YGGFL}+\mathrm{H}]^{+}$under an increased delay between the helium pulse and ion arrival to the cold trap (a) compared to the delay under normal operations.

## Figure S7. Kinetic model used to simulate population distribution



Figure S7. A simplified reaction scheme that shows the relative isomerization rates for all species involved in the kinetics scheme used. A and B represent cis species while D, C and E represent trans species. More details are in the following text.

## Details of the cooling simulation:

Figure $\mathbf{S 7}$ shows the full reaction coordinate used to simulate the population distribution as a function of temperature and internal energy. For simplicity, letters are used to represent the various conformers which isomerize to one another. A $=$ Cis acid proline pucker up, $\mathrm{B}=$ Cis acid proline pucker down, $\mathrm{C}=$ Trans acid proline pucker down, $\mathrm{D}=$ Trans acid proline pucker up, $\mathrm{E}=$ Trans acid proline pucker up/tyrosine ring rotation. Prior to all simulations the equilibrium distribution was calculated for the 5 species as a function of temperature/internal energy. Each isomerization reaction considered can be grouped as a slow/fast reaction. The pucker isomerization, as well as the tyrosine ring rotation are both considered fast reactions as their isomerization rates are orders of magnitude faster than the cooling rate ( $10^{10} \mathrm{~s}^{-1} \mathrm{vs} .10^{3} \mathrm{~s}^{-1}$ ) and essentially remain so up until a few tens of wavenumbers above their respective barrier heights. On the other hand, the cis-trans isomerization reactions have initial isomerization rate constants at $\left\langle\mathrm{E}_{\mathrm{v}}\right\rangle=6700 \mathrm{~cm}^{-1}$ of $\mathrm{k}(\mathrm{E}) \sim 10^{6} \mathrm{~s}^{-1}$, and slow significantly prior to reaching their respective barrier heights. Thus, the reaction coordinate was broken down into a series of fast and slow steps as. To begin the simulation a set amount of temperature/internal energy was removed and the two slow steps were adjusted first. When only considering the two slow steps, they are independent of one another and are treated as two separate reactions of opposing equilibria ( $\mathrm{A} \leftrightarrow \mathrm{D}$ and $\mathrm{B} \leftrightarrow \mathrm{C}$ ). To simulate the population change within a given time period the following equation for opposing equilibria was used:

$$
\begin{equation*}
[A(t)]=A_{e}+x_{e} \exp \left[-\left(k_{1}+k_{-1}\right) t\right] \tag{S1}
\end{equation*}
$$

$$
\text { where } x_{e}=A_{0}-A_{e}
$$

$A_{e}$ is the equilibrium population for species $A$ at the given temperature/internal energy, $x_{e}$ is the difference between the starting concentration of $A,\left(A_{0}\right)$, and its final concentration $A_{e}, k_{1}$ and $k-1$ are the forward and backward rates at the specified temperature/internal energy, and $(t)$ is the time period between temperature/internal energy down steps. As temperature/internal energy is removed, the fully equilibrated population distribution among the 5 species changes. Using eqn. S1 it is possible to account for the shift in population between $A$ and $D$ as well as $B$ and $C$, under kinetic constraints, representing the cis-trans isomerization. The time allotted for these reactions are determined by the average time for the temperature of the ions to drop by $1 \mathrm{~K}(1 \mu \mathrm{~s})$ or to lose $13 \mathrm{~cm}^{-1}(1.9 \mu \mathrm{~s})$ of internal energy. After the distribution between A /D and B/C are adjusted the fast steps are considered. In considering only the fast steps the isomerization between $A$ and $B$ is independent of the isomerization between C, D, and E. Since the rates associated with the fast isomerization processes are much greater than the cooling rate, it is assumed that the species connected by these fast steps will maintain an equilibrated distribution throughout the cooling process. Thus, in this model, A/B maintain a near-equilibrium distribution throughout the cooling process while $A / D$ and $B / C$ do not. In order to make this adjustment the ratio of the new population between $A$ and $B$ (resulting populations after the slow step) is compared to the ratio of A to B from the fully equilibrated distribution. The population distribution between $A$ and $B$ is then adjusted such that new populations are in equilibrium. The same process is done for the trans species ( $\mathrm{C}, \mathrm{D}$ and E ). Once this is done another down step in temperature/internal energy is taken and the pro-
cess is repeated. In carrying out the simulation in this manner the change in the population distribution between the cis and trans species can be effectively model under kinetic constraints. A further constraint on the simulation is that the sum of the populations must equal one before the next down step is taken.

## Figure S8. Comparison of different cooling models employed to simulate population distributions vs. internal energy



Figure S8. A comparison of the two different cooling models employed to carry out the cooling simulations. Using the average cooling rate, it is assumed that the ion loses internal energy at a constant rate ( $\Delta \mathrm{E} / \Delta \mathrm{t}=6.7 \mathrm{~cm}^{-1} / \mu \mathrm{s}$ ) as a function of total internal energy (dashed line). Alternatively, vibrational cooling is often modeled as a constant fraction of total internal en$\operatorname{ergy}(\Delta \mathrm{E} / \mathrm{E}=$ constant). Figure 6 in the text demonstrates that ions entering the cold trap cool to a vibrational temperature of 10 K in 1 ms . Assuming that ions enter the trap at a vibrational temperature equivalent to room temperature, it is possible to fit a cooling rate of $\Delta \mathrm{E} / \mathrm{E}=-0.008$, leading to an exponential decay in internal energy with time (solid line). Simulations run with either energy loss function resulted in ending population distributions nearly identical to one another (Table S1). This insensitivity to cooling model is due to a slow change with temperature of the family populations. The proline pucker down structures shown in Figure 7 contain a majority of the total population ( $\sim 60 \%$ ) at room temperature. These structures remain relatively close in terms of free energy as a function of internal energy, thus the total cis to trans isomerization along this coordinate is rather small. While the proline pucker up structures have a large difference in energy between them, the higher member of this pair contains only $6 \%$ of the total population at room temperature. Thus, even though there is a larger driving force to isomerize from cis to trans along the pucker coordinate, there is very little population to be transferred. Consequently, the different rates at which energy is removed at early times has a small effect on the overall final distribution.

Table S1. Final simulated population distributions for each model

|  | Population Distribution |  |
| :--- | :---: | :---: |
| Cooling Model | Cis | Trans |
| Average Cooling Rate | $38 \%$ | $62 \%$ |
| Constant Fractional Loss | $36 \%$ | $64 \%$ |

Table S2. Distribution of low frequency modes in the 5 minima relevant for cooling simulations

|  | Minima |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :---: |
| Frequency <br> Range $\left(\mathrm{cm}^{-1}\right)$ | Cis Pucker up | Trans Pucker <br> up | Trans Pucker <br> down | Cis Pucker up | Trans Tyr. <br> Rotation |  |
| $0-100$ | 11 | 10 | 10 | 11 | 10 |  |
| $100-200$ | 10 | 11 | 11 | 10 | 11 |  |
| $200-300$ | 9 | 10 | 10 | 9 | 9 |  |
| $300-400$ | 10 | 8 | 8 | 7 | 8 |  |
| $400-500$ | 7 | 8 |  | 10 |  |  |

Table S3. Distribution of low frequency modes in the 5 transition states that connect the minima used for cooling simulations

|  | Transitions States |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :---: |
| Frequency <br> Range | Cis Pucker <br> Isomerization | Trans Pucker <br> Isomerization | Cis-Trans <br> Isomerization <br> Pucker up | Cis-Trans isom- <br> erization Pucker <br> Down | Trans Tyr. <br> Rotation |  |
| $0-100$ | 10 | 10 | 11 | 11 | 9 |  |
| $100-200$ | 11 | 11 | 9 | 9 | 11 |  |
| $200-300$ | 8 | 9 | 11 | 11 | 10 |  |
| $300-400$ | 10 | 8 | 9 | 9 | 8 |  |
| $400-500$ | 7 | 8 | 7 | 7 |  |  |

