

Gas Phase Dopant-Induced conformational changes monitored with Transversal Modulation Ion Mobility Spectrometry.

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1. Comparison of the measurement-based and the theoretical estimations of the ratio of the collision cross section (CCS) of cytochrome in hexane over the CCS in N₂

We found that the observed CCS shifts of cytochrome c due to the addition of hexane in the gas phase were remarkably high. This suggested that, although hexane is non-polar, it was clustering with the ion due to an inelastic collision. Note in this respect that, at the concentrations of our experiments, the saturation of hexane was below 10%, and hence the formation of hexane-hexane clusters was very unlikely.

In order to distinguish whether hexane and the protein experience an inelastic or an elastic collision, we have estimated the CCS of the protein-hexane interaction under the hypothesis that all collisions are elastic and therefore no clusters are formed, both theoretically, and using our experimental data. Comparing the empirically estimated value with the theoretical value we can prove or discard our initial hypothesis.

1.1. Empirical estimation of the ratio of the CCS of the pair protein-hexane over the CCS of the pair protein-N₂:

On the one hand, using the definition of the collision cross section¹, our measurements provide a direct estimation of the ratio of the collision cross section of cytochrome c in hexane (Ω_{g2}) over the collision cross section in N₂ (Ω_{g1}). According to the Blanc's empirical equation, the mobility in a mixture of nitrogen and hexane (K_{12}) is given by²:

$$\frac{1}{K_{12}} = (1 - \alpha) \frac{1}{K_1} + \alpha \frac{1}{K_2} \quad (s1)$$

where α is the volumetric concentration of hexane, K_1 is the mobility in N₂, and K_2 the mobility that the protein would be in pure hexane. On the other hand, our measurements show empirically that the ratio K_1/K_{12} follows:

$$\frac{K_1}{K_{12}} = 1 + G\alpha \quad (s2)$$

where G is a dimensionless parameter which is determined experimentally (see figure s1).

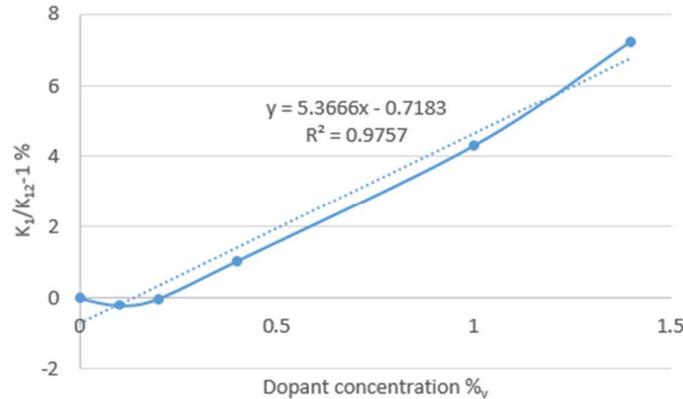


Figure s1: Ratio of the measured mobility of cytochrome c in the mixture of nitrogen and hexane over the mobility of cytochrome c in pure N₂ as a function of the volumetric concentration of hexane in the gas mixture.

Rearranging equations s1 and s2 yields:

$$\frac{K_1}{K_2} = 1 + G \quad (s3)$$

Figure s1 show that G is 5.5 ± 0.7 . Accordingly, the mobility ratio is $K_1/K_2 = 6.5 \pm 0.7$.

In order to obtain the CCS ratio, we use the Mason-Schamp equation. Based on the definition of the collision cross section, the ratio of the mobility of an ion in a gas g_1 over the mobility in second gas g_2 is¹:

$$\frac{K_1}{K_2} = \sqrt{\frac{m_{g2} m_i + m_{g1} \Omega_{g2}}{m_{g1} m_i + m_{g2} \Omega_{g1}}} \quad (s4)$$

Where m_i , m_{g1} and m_{g2} are the molecular weights of the analyte ion and the gases g_1 (N₂) and g_2 (hexane), and Ω_{g1} , Ω_{g2} are the collision cross sections of the ion in the two gases. Finally, Introducing the molecular masses in eq. s4, the ratio of the collision cross section of cytochrome in hexane over N₂ is $\Omega_{g2}/\Omega_{g1} = 3.8 \pm 0.4$.

1.2. Theoretical estimation of the ratio of the CCS of the protein-hexane pair over the CCS of the protein-N₂ pair:

The theoretical ratio of the collision cross sections of cytochrome c in hexane and in N₂ can be estimated under the assumption that the gas molecules and the protein collide elastically, using a projection area approximation corrected by an ion-induced dipole enhancement factor³. In first approximation, considering that native cytochrome c is compact, yet much larger in size than N₂ and hexane, the projection approximation method for a spherical ion yields:

$$\frac{\Omega_{g2}}{\Omega_{g1}} = \frac{(d_i+d_{g2})^2 L(\psi_{g2})}{(d_i+d_{g1})^2 L(\psi_{g1})} \quad (s5)$$

Where d_i , d_{g1} and d_{g2} are the effective collision diameters of the ion and the two gases. The ion-induced dipole correction factors for the two gasses $L(\psi_{g1})$ and $L(\psi_{g2})$ are given by:

$$L = \begin{cases} 1 + \psi \left(\frac{1}{3.1} + \frac{1}{\xi} \left(\frac{1}{16} + \frac{4}{33} \psi \right) \right) & \text{for } \psi \leq 1 \\ 1 + \psi \left(\frac{1}{4} - \frac{2.3}{1000} \psi + \frac{1}{\xi} \left(\frac{9}{56} - \frac{6.8}{1000} \psi \right) \right) & \text{for } \psi > 1 \end{cases} \quad (s6)$$

Where $\xi = 1.36$ is the momentum scattering factor, which derives from the measurements of Millikan, and ψ_g is the polarization to thermal energy ratio defined as:

$$\psi_g = \frac{\alpha_{pol} z^2 e^2}{\pi \epsilon_0 k_B T (d_i + d_g)^3} \quad (s7)$$

Where α_{pol} , z , e , ϵ_0 , k_B and T are the polarizability of the gas, the charge state of the ion ($z=7$ in the data of figure 3), the unitary charge, the vacuum permittivity, the Boltzmann constant, and the temperature of the gas (300 K). Introducing the polarizabilities for N₂ and hexane (1.71×10^{-30} m³ and 11.7×10^{-30} m³ respectively) in equation s7, considering that d_g for hexane is smaller than the molecular length (0.6 ± 0.1 nm), using the measured value of Ω_{g1} ($\sim 13 \pm 1$ nm² in N₂), taking $d_i = 2\sqrt{\Omega_{g1}/\pi} = 4.1 \pm 0.15$ nm and $d_g = 0.1 \pm 0.1$ nm for N₂, we get $\psi_g = 0.038$ for N₂ and $\psi_g = 0.4$ for hexane. Accordingly, the ion-induced dipole correction factors for N₂ and hexane are 1.01 and 1.17, respectively.

Finally, introducing these factors in equation s5, we can conclude that the theoretically estimated ratio Ω_{g2}/Ω_{g1} for cytochrome, hexane and N₂ is smaller than 1.45 ± 0.15 . ($\Omega_{g2}/\Omega_{g1} < 1.45 \pm 0.15$) Note that, by using the length of hexane to define its effective collision diameter we are providing a conservative calculation that overestimates Ω_{g2}/Ω_{g1} .

1.3. Comparison of the empirical and theoretical estimation of the CCS ratio of the CCS of the protein-hexane pair over the CCS of the protein-N₂ pair:

We estimated the CCS ratio empirically and theoretically under the hypothesis that hexane and the protein collide but do not aggregate.

We used a projection area approximation corrected by an ion-induced dipole enhancement factor¹. Different methods have been developed to estimate the integral collision cross section for different ions and gases with much greater precision and at moderate computational costs³⁻⁵. However, for the purpose of the present discussion, this approximation was sufficient to evaluate whether the observed drift could be explained under the assumption that ions and molecules collide elastically and therefore do not aggregate, or if other models are required to explain the observed mobility shift.

According to our models, the theoretically predicted ratio of the collision cross section of cytochrome c in hexane over N₂ is $\Omega_{g2}/\Omega_{g1} = 1.45 \pm 0.15$, while a much larger ratio was obtained from experimental measurements, $\Omega_{g2}/\Omega_{g1} = 3.8 \pm 0.4$.

This strong discrepancy shows that the initial hypothesis is wrong, and hence that the collision of hexane and the protein is not always elastic but rather inelastic. Due to this, hexane molecules stick to the protein and substantially change its CCS by forming a cluster.

2. Literature Values:

Table s1: Summary of all referenced literature values. Values measured in He were corrected by using the interpolation of the linear relationship resulting from plotting helium and nitrogen values reported by Bush et al.⁶

Charge state	Measured CCS (nm ²)						CCS (nm ²) corrected to N ₂		
	Shelimov et al. ⁷	Valentine et al. ⁸	Faull et al. ⁹	Bush et al. ⁶	Smith et al. ¹⁰	Sivalingam et al. ¹¹	Shelimov et al. ⁷	Valentine et al. ⁸	Faull et al. ⁹
	Measured in He. Multiple values corresponding to one charge state indicate multiple resolvable peak maxima. This suggests the coexistence of multiple conformations.	Measured in He. Multiple values corresponding to one charge state indicate multiple resolvable peak maxima. This suggests the coexistence of multiple conformations.	Measured in He.	Measured in N ₂ .	Measured in N ₂ . Multiple values corresponding to one charge state indicate multiple resolvable peak maxima. This suggests the coexistence of multiple conformations	Measured in N ₂ .			
+7	12.47 17.85 16.02 20.07		15.46	15.9	15.5	18.5	10.30 19.48 16.36 23.26		15.40
+8	12.5 17.02 18.45 20.61	12.6 21.29	18.65		17	19	10.36 18.06 20.50 24.18	10.53 25.34	20.84
+9	22.15 19.64	13.52 21.93	20.66		18 20	20	26.80 22.53	12.10 26.43	24.26
+10	22.26	15.22 22.57	22.28		20.5	20.5	26.99	14.99 27.52	27.02
+11	23.03	23	22.55		21	21	28.30	28.25	27.48
+12	23.35	23.31	24.07		21	21.5	28.85	28.78	30.07
+13	23.91	24.06		30.8	21.5	23.5	29.80	30.06	
+14	24.73	24.86		32	21.75	24.5	31.20	31.42	
+15	25.79	25.73		33.3	22.5	25.5	33.01	32.90	

3. Mobility Spectra:

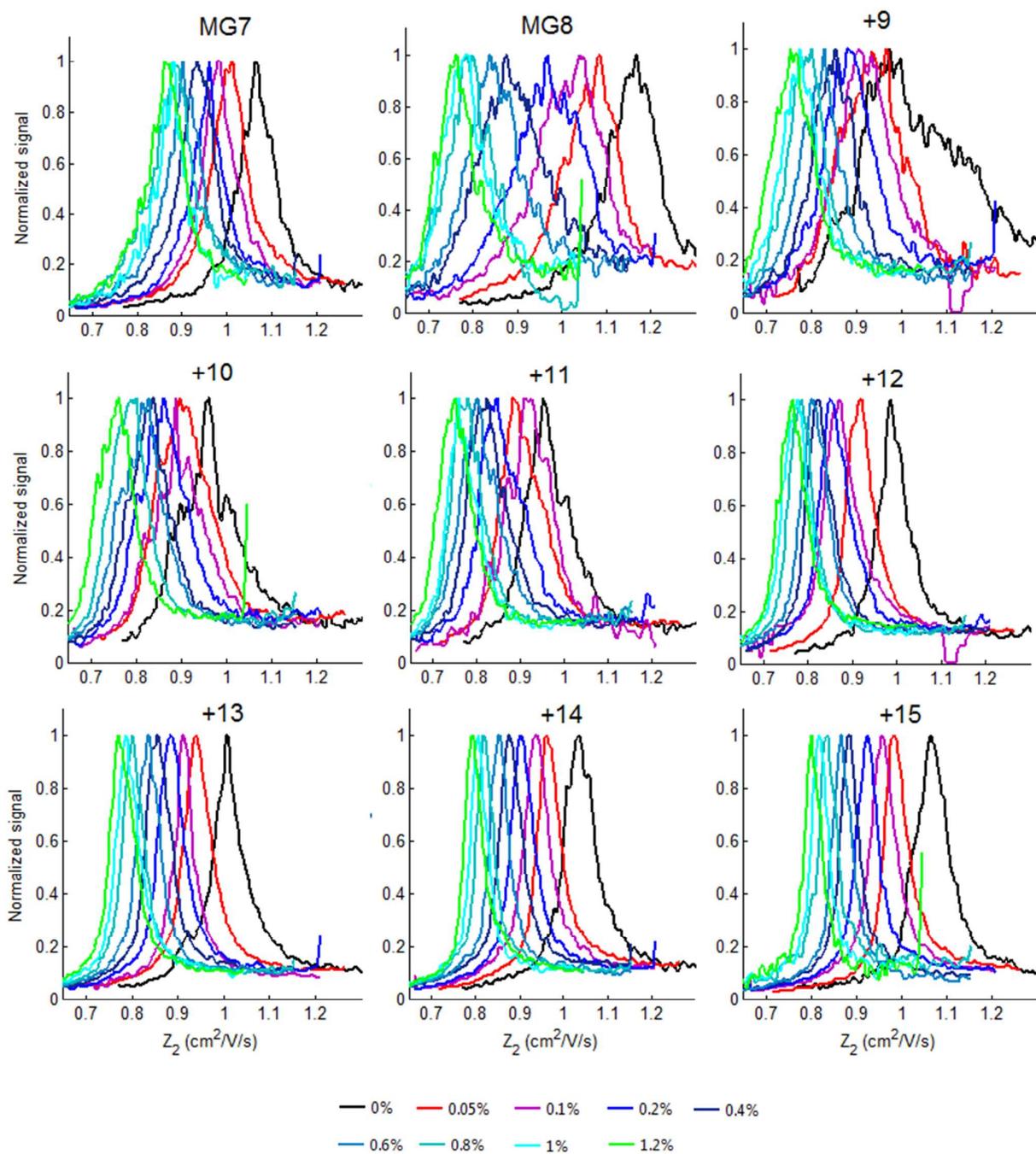


Figure s2: Mobility spectra of the different charge states of denatured cytochrome c for increasing isopropanol concentrations

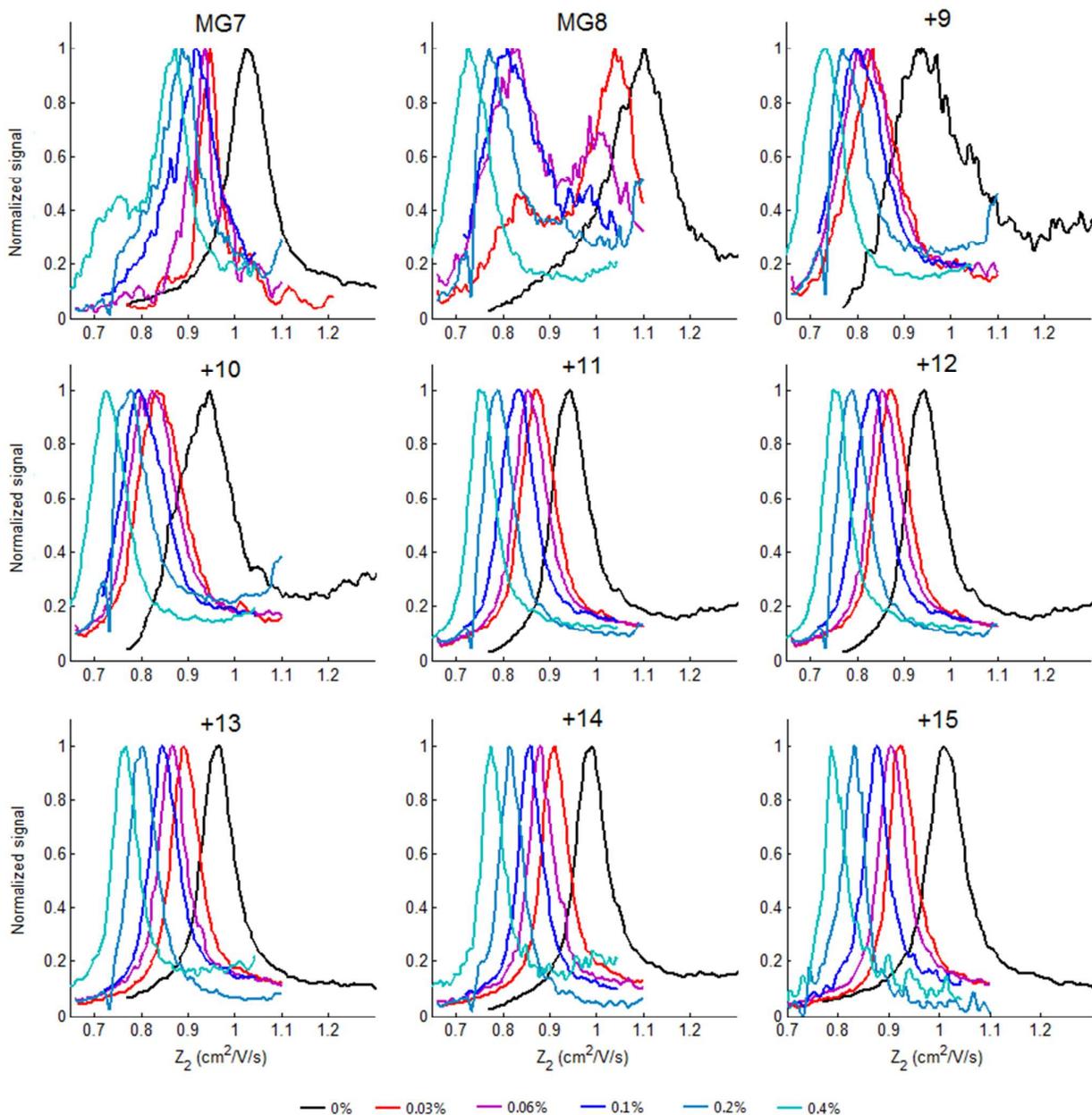


Figure s3: Mobility spectra of the different charge states of denatured cytochrome c for increasing tert-pentanol concentrations

4. References

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