The Equilibrium Distribution at Room Temperature

As we stated in Analysis of the Experimental Data, the validity of our approach strongly depends on the assumption that the gallery of snapshots describes a system at thermodynamical equilibrium. This assumption should be carefully checked in the case of a system that undergoes a temperature quench between room temperature ($T_{\rm amb} \simeq 20^{\rm o}$C) and the temperature of dynamical arrest (estimated as the temperature of divergence of water viscosity $T_\infty \simeq -45^{\rm o}$C).

We can identify two limiting cases for this process. In the case of an almost instantaneous temperature quench the probability density of states of the system would not have the time to significantly relax away from the equilibrium distribution at room temperature. The latter one would then be the distribution of states observed in the experiment. In the opposite case of an almost adiabatic temperature quench, the system would have time to gradually thermalize and it will approach the equilibrium distribution at T_∞ through a series of consecutive quasi-equilibrium states. At realistic values of the quenching rate, the situation will be a compromise between these two extremes, so that the observed density of states would correspond as a first approximation to thermodynamic equilibrium at a temperature intermediate between $T_{\rm amb}$ and T_∞.

To gain a quantitative insight into this problem, let us focus on the equilibrium distribution at room temperature in the two limiting cases $T_0 = T_{\rm amb}$ and $T_0 = T_\infty$. By introducing in Eq. **2** the Eqs. **11** and **12** for the effective potential, one obtains the following relation:

$$\rho(\phi_1, \phi_2, \theta, T_{\rm amb}) = \frac{1}{Z(T_{\rm amb})} \left[\sin\phi_1 \sin\phi_2\right]^{1-T_0/T_{\rm amb}} \left[1 + \left(\frac{\theta - \theta_0}{\sigma_\theta}\right)^2\right]^{-T_0/T_{\rm amb}} \tag{1}$$

where $\phi_{\rm min} < \phi_1, \phi_2 < \phi_{\rm max}$ and $\theta \in [0, 360]$. The normalized profiles $\rho_1(\phi, T_{\rm amb})$ and $\rho_2(\theta, T_{\rm amb})$, and their corresponding cumulative distributions, are computed from Eq. **1** and plotted in Figures 1 *a* and *b*, respectively. The two distributions differ only slightly. More precisely, the difference between them is smaller than the average fluctuation displayed by the experimental data (i.e. statistical error). As a consequence, we can claim that the measured distribution is consistent with the stationarity hypothesis.

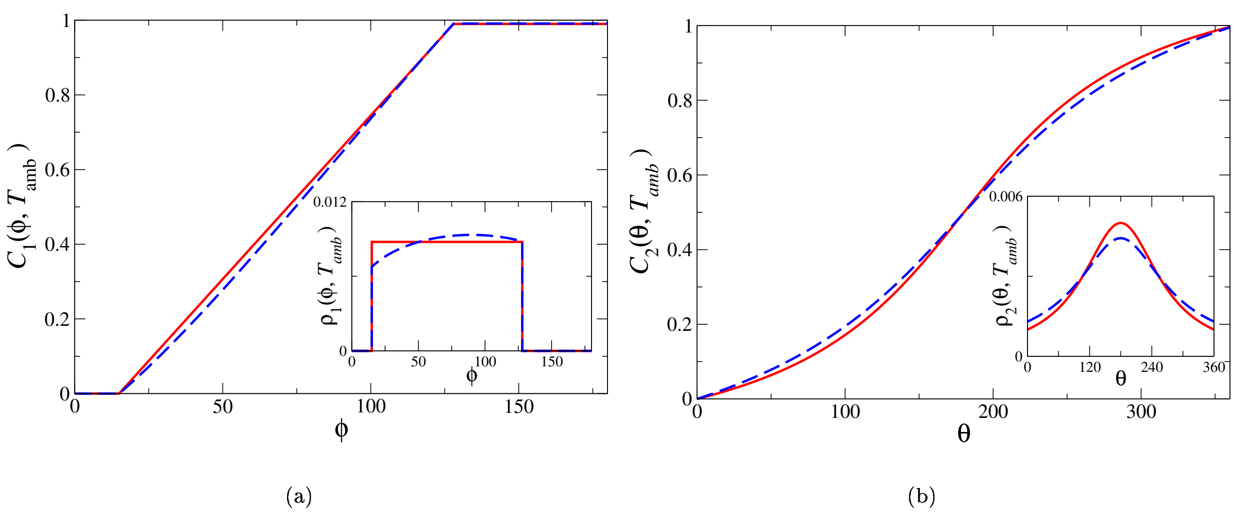

Figure 1: *(a)* Cumulative distributions of the ϕ angles at room temperature (main plot) and associated normalized histograms (inset). Both profiles are computed from Eq. **1**. The solid line refers to $T_0 = T_{\rm amb}$ whereas the dashed line is found by setting $T_0 = T_\infty$. *(b)* Cumulative distributions of the θ angles at room temperature (main plot) and associated normalized histograms (*Inset*). The curves are derived from Eq. **1**. The solid line refers to $T_0 = T_{\rm amb}$ while the dashed line is found by setting $T_0 = T_\infty$.

It should be noted that this conclusion does not help determining the best value for T_0 to use in Eq **12**. However, we can provide an estimate of T_0 by studying the ratio of the correlation time of the molecule τ_γ and the experimental quenching time Δt_q. A good estimate of τ_γ is given by the time constant of the exponential decay of the position autocorrelation function of an harmonically bound Brownian particle in the over–damped limit,

$$\tau_\gamma = \frac{\gamma}{M\omega^2} \quad , \tag{2}$$

where γ is the friction coefficient of a single domain of the IgG molecule, M its mass and ω is the frequency of the harmonic oscillations. We can approximate the friction coefficient γ of a single Fab domain with that of a rigid sphere of radius $L/2$, $\gamma = 6\pi\eta_W(L/2)$. Therefore, recalling Eq. **17**, we obtain a ratio $\tau_\gamma/\Delta t_q$ of the order 10^{-4} at $T = T_{\rm amb}$ for the slowest degree of freedom (oscillations around $\phi_{\rm min}$). This means that the quenching process is significantly adiabatic. Therefore, it appears reasonable to assume as a first approximation $T_0 = T_\infty$.

Finally, as a side remark, let us shortly discuss the issue of determining the two limiting angles $\phi_{\rm min}$ and $\phi_{\rm max}$ in the distribution $\rho_1(\phi)$. By analyzing the experimental data, it is indeed reasonable to guess the existence of a potential barrier (or, alternatively, of a steep repulsive potential) around the configuration $\phi_{\rm min}$ and $\phi_{\rm max}$. Unfortunately, the statistic is at present too poor to allow for a satisfactory interpretation of the distribution $\rho_1(\phi)$ in those regions. Therefore, we chose to approximate the potential with two infinite potential barriers located at $\phi_{\rm min}$ and $\phi_{\rm max}$. This amounts to labeling as unaccessible regions that most probably are just rarely visited.