

# Heterogeneous Nucleation in Droplet-Based Nucleation Measurements

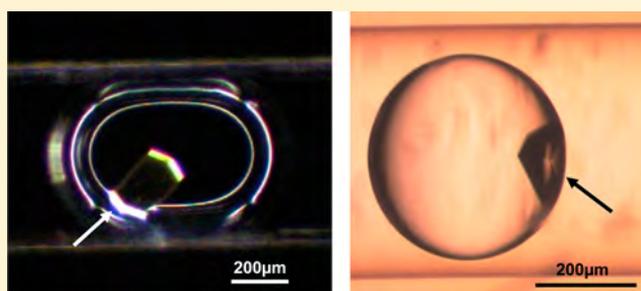
Published as part of a *Crystal Growth and Design* virtual special issue of selected papers presented at the 10th International Workshop on the Crystal Growth of Organic Materials (CGOM10).

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## S Supporting Information

**ABSTRACT:** Droplet-based nucleation experiments reveal discrepancies in kinetic and thermodynamic factors. Here we examine how the chemical nature of the water–oil interface, and not the type of the device, used in different set-ups by three different groups impacts nucleation rate and explain discrepancies among lysozyme interfacial energies and pre-exponential factors encountered in the literature.



Microfluidics is a powerful tool for studying nucleation<sup>1–4</sup> allowing a large number of experiments to be realized under identical conditions. This permits, for instance, the accurate determination of nucleation frequencies.<sup>5</sup> In this paper, using a droplet-based method, we demonstrate the influence of heterogeneities in supersaturated solutions on nucleation rate measurements of lysozyme. We discuss, in the light of our recent experiments<sup>5,6</sup> and data from Fraden and Vekilov groups,<sup>7,8</sup> the influence of heterogeneous nucleation in the determination of thermodynamic and kinetic factors. Lastly, we comment on the discrepancy between different results in the literature, and we demonstrate how the chemical nature of the water–oil interface induces heterogeneous nucleation.

## 1. THEORETICAL BACKGROUND

**Classical Nucleation Theory (CNT) for Homogeneous Nucleation (HON).**<sup>9–11</sup> In the CNT, the nucleation rate  $J$  (number of crystals·s<sup>-1</sup>·m<sup>-3</sup>) can be expressed as<sup>12</sup>

$$J = K_0 \exp\left(\frac{\Delta G_{\text{HON}}^*}{kT}\right) \quad (1)$$

with  $K_0$  the kinetic factor (m<sup>-3</sup>·s<sup>-1</sup>),  $\Delta G_{\text{HON}}^*$  the activation free energy for homogeneous nucleation ( $J$ ),  $k$  the Boltzmann constant (J·K<sup>-1</sup>), and  $T$  the temperature (K). The CNT assumes a spherical form for the critical nucleus; this point was mentioned by Fletcher<sup>13</sup> and is in good agreement with our observations of crystals just after nucleation.<sup>14</sup> In this ideal case an isotropic interfacial energy,  $\gamma$  (J·m<sup>-2</sup>), of the critical nucleus is used, and eq 1 becomes

$$J = K_0 \exp\left(-\frac{16\pi}{3} \frac{\Omega^2 \gamma^3}{(kT)^3 \ln^2 \beta}\right) \quad (2)$$

with  $\Omega$  the volume of one molecule in the critical nucleus (m<sup>3</sup>) and  $\beta$  the supersaturation defined as the ratio of the concentration in solution versus the solubility.

**Model for Heterogeneous Nucleation (HEN) under CNT.** The nucleating solution itself has heterogeneities: foreign molecules or particles, bubbles, crystallizer walls, liquid–liquid or liquid–air interfaces. The surface of these foreign substances can be a place on which nucleation can preferentially occur, thus acting as a nucleation catalyst.<sup>15</sup> This is known as heterogeneous nucleation (HEN) in the literature,<sup>11,12,16,17</sup> the main idea being that the foreign substance decreases the activation free energy required to form the critical cluster,  $\Delta G_{\text{HEN}}^*$ . This decrease is determined by the ratio between the volumes of the cluster onto the foreign substance and the corresponding homogeneously formed spherical cluster. Introducing a factor  $f(\theta)$  with  $\theta$  the contact angle of the cluster onto the foreign substance,  $J$  becomes

$$J = K_0 \exp\left(-\frac{\Delta G_{\text{HEN}}^*}{kT}\right) \quad (3)$$

with

$$\Delta G_{\text{HEN}}^* = f(\theta) \times \Delta G_{\text{HON}}^* \quad (4)$$

**Received:** January 28, 2013

**Revised:** March 27, 2013

**Published:** April 1, 2013

Assuming a constant shape for the cluster (here spherical) and depending on the affinity of the cluster to the foreign substance, as characterized by a contact angle, defined from 0 to  $\pi$ , we deduce

$$0 < f(\theta) < 1 \quad (5)$$

The factor  $f(\theta)$  was generalized to different shapes for clusters and foreign substances (for details see Fletcher,<sup>13</sup> Kashchiev,<sup>11</sup> and Liu<sup>16</sup>). To summarize,  $f(\theta)$  represents the thermodynamic part of the catalytic effect of the foreign substance on nucleation. In practice, an effective interfacial energy  $\gamma_{\text{ef}}$  was introduced depending on  $f(\theta)$ <sup>11</sup>

$$\gamma_{\text{ef}} = f(\theta)^{1/3} \gamma \quad (6)$$

Note that the lower  $f(\theta)$  or  $\gamma_{\text{ef}}$  the greater the affinity of the cluster to the foreign substance. The effect of heterogeneity on  $K_0$ , the kinetic factor, will be discussed in the Results and Discussion section. Finally, we can write a general equation for (primary) nucleation

$$J = K_0 \exp\left(-\frac{16\pi}{3} \frac{\Omega^2 \gamma_{\text{ef}}^3}{(kT)^3 \ln^2 \beta}\right) \quad (7)$$

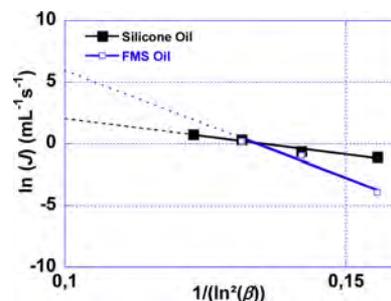
Thus, plotting logarithm of  $J$  as a function of the inverse of the square logarithm of supersaturation gives the effective interfacial energy and the kinetic factor.

## 2. EXPERIMENTS

In previous reports,<sup>5,18</sup> we presented different microfluidic set-ups for studying nucleation using the double pulse technique. This technique allows a direct determination of the steady-state rate of primary nucleation, decoupling crystal nucleation from the growth process and not by an estimation of the induction time. In this paper, we reanalyze our experimental data on lysozyme using the concept of  $\gamma_{\text{ef}}$ . Microfluidic devices and experimental procedures have already been fully described.<sup>5,18</sup> Lysozyme from Sigma (15–50 mg/mL) was crystallized at pH = 4.5 (acetate 80 mM buffer) in 0.7 M NaCl solution. The experiments were performed with the same solutions in both a PDMS device with silicone oil (Sigma oil AP 100) and a PEEK/Teflon device with fluorinated oil (poly-3,3,3-trifluoropropylmethylsiloxane, Hampton Research HR2-595). The microfluidic device allowed us to generate and store droplets of a volume of 250 nL and a volume polydispersity of a few percent.<sup>19,20</sup> During generation and storage, the devices and the solution were thermostatted. Droplets were observed under a stereomicroscope equipped with a CCD camera. In the PDMS device droplets were stored for 24 h maximum; for longer storage times evaporation is no longer negligible.<sup>6,21</sup> In contrast, in the PEEK/Teflon device droplets can be stored for months without significant evaporation.<sup>5</sup> Supersaturation was achieved by mixing a solution of protein with a salt solution, and droplets were generated using flow-focusing and cross-flowing geometries for PDMS and PEEK/Teflon respectively. Since temperature was controlled throughout, the double pulse technique was used to measure the nucleation rate of lysozyme at 20 °C.<sup>6,8,22,23</sup> The double pulse technique needed the determination of the metastable zone limit (details of the procedure is provided in the Supporting Information).

## 3. RESULTS AND DISCUSSION

Figure 1 summarizes nucleation frequencies we had previously measured with different microfluidic devices using different



**Figure 1.**  $\ln(J)$  as a function of  $1/(\ln \beta)^2$  from Ildefonso et al.,<sup>5,6</sup> error bars have the size of the markers.

oils.<sup>5,6</sup> The values of  $K_0$  and  $\gamma_{\text{ef}}$  are computed, and the results are summarized in Table 1. The errors on  $K_0$  and  $\gamma_{\text{ef}}$  are determined graphically. Note that each nucleation frequency requires from 400 to 800 independent experiments.

**Table 1.** Estimated CNT Parameters  $K_0$  and  $\gamma_{\text{ef}}$  from eq 7<sup>a</sup>

chemical nature device – oil	$\ln(K_0)$ ( $\text{mL}^{-1}\cdot\text{s}^{-1}$ )	$K_0$ ( $\text{mL}^{-1}\cdot\text{s}^{-1}$ )	$K_0$ ( $\text{m}^{-3}\cdot\text{s}^{-1}$ )	$\gamma_{\text{ef}}$ ( $\text{mJ}\cdot\text{m}^{-2}$ )
PDMS – silicone	7.9 (3)	$2.7 \times 10^3$	$2.7 \times 10^9$	0.62 (0.13)
PEEK/Teflon – fluorinated	21.0 (4)	$1.32 \times 10^{10}$	$1.32 \times 10^{16}$	0.88 (0.05)

<sup>a</sup>The deviation in absolute is given in parentheses.

**Effective Interfacial Energies  $\gamma_{\text{ef}}$ .** We observe that  $\gamma_{\text{ef}}$  (PEEK/Teflon – fluorinated oil) >  $\gamma_{\text{ef}}$  (PDMS – silicone oil) pointing to a better catalytic effect on nucleation using the PDMS device with silicone oil than using the PEEK/Teflon device with fluorinated oil. Moreover, the ratio of the slopes of Figure 1, a quantity that can be determined via the experiment without any assumptions, is equal to the ratio of activation free energy in the different experimental conditions. Finally, we have the following relation between the activation free energies, confirming that nucleation is easier in the PDMS device with silicone oil than in the PEEK/Teflon device with fluorinated oil.

$$\begin{aligned} \Delta G_{\text{HEN}}^* (\text{PEEK/Teflon – fluorinated oil}) \\ = 3 \times \Delta G_{\text{HEN}}^* (\text{PDMS – silicone oil}) \end{aligned} \quad (8)$$

The modification of device and oil in order to generate and store droplets significantly changes the kinetics of heterogeneous nucleation. However, at this stage of the discussion, we cannot differentiate the effect of the type of device from that of the type of oil on heterogeneous nucleation. Note that solution purities are the same. In order to discriminate the effect of the type of device from that of the type of oil, we compare our data with experiments carried out by other groups.

Recently, two groups measured  $\gamma_{\text{ef}}$  and  $K_0$  values for lysozyme in identical crystallization conditions (0.7 M NaCl, pH = 4.5) with different set-ups.<sup>7,8,24</sup> The Fraden group used a PDMS microfluidic device with fluorinated oil (FC43, Acros) in a supersaturation range of 24–55, and the Vekilov group used droplets suspended in silicone oil in Teflon wells in a supersaturation range of 5–10, both using direct determination of primary nucleation rate (decoupling crystal nucleation from

growth process) and not by an estimation of the induction time. It is difficult to extract the  $J$ -data from the paper of Fraden. However the plot of the  $J$ -data of Vekilov and Ildefonso (both with silicone oil) on the same curve shows a good agreement (Supporting Information). Note that our data are in a supersaturation range of 13–17. Hence  $\gamma_{\text{ef}}$  and  $K_0$  values determined from nucleation experiments of lysozyme in a supersaturation range of 5–55 are summarized in Table 2 and clearly show discrepancies between different groups.

**Table 2. Parameters  $K_0$  and  $\gamma_{\text{ef}}$  Determined for Different Chemical Natures of Devices and Oils, by Different Groups<sup>a</sup>**

chemical nature	Ildefonso et al. <sup>18</sup>	Vekilov group <sup>8,24</sup>	Ildefonso et al. <sup>5</sup>	Fraden group <sup>7</sup>
device	PDMS	Teflon wells	PEEK/Teflon	PDMS
oil	silicone	silicone	fluorinated	fluorinated
$\beta$ -range	13–17	5–10	13–17	24–55
$\gamma_{\text{ef}}$ (mJ·m <sup>-2</sup> )	0.62 (0.13)	0.56	0.88 (0.05)	0.91
$K_0$ (m <sup>-3</sup> ·s <sup>-1</sup> )	$2.7 \times 10^9$	$3.6 \times 10^8$	$1.32 \times 10^{16}$	$1.2 \times 10^{13}$
$\ln(K_0)$ (mL <sup>-1</sup> ·s <sup>-1</sup> )	7.9 (3)	8.2	21.0 (4)	16.3

<sup>a</sup>The deviation in absolute is given in parentheses, errors are not available in<sup>7,8,24</sup>

**Effective Interfacial Energies  $\gamma_{\text{ef}}$ : Comparison to Literature (Table 2).** The discrepancy between the different results in the literature can be reanalyzed in the light of effective interfacial energies, i.e., the catalytic effect on nucleation of foreign substances: oil or type of device. According to Table 2, we observe that data from different groups with the same oil gave the same effective interfacial energies. Moreover, we observe that  $\gamma_{\text{ef}}$  (fluorinated oil) >  $\gamma_{\text{ef}}$  (silicone oil) pointing to a better catalytic effect on nucleation using silicone oil than using fluorinated oil, thus indicating that the interface between oil and protein solution represents the main heterogeneity for lysozyme nucleation, that is to say, the “foreign substance” for HEN and not the type of device. While this is an experimental confirmation of the efficiency of oil for avoiding contact between crystallizing solution and device walls by creating a “containerless” environment as pointed out by Chayen,<sup>25</sup> results clearly show the importance of the type of oil used. It also illustrates the difficulty of comparing data from different experiments by different groups on the same molecules using equivalent experimental procedures. For instance, Selimovic et al.<sup>7</sup> identified heterogeneous nucleation — the way supersaturation is achieved — the expression used for the nucleation rate as potential sources of discrepancy between their data and the data of Galkin and Vekilov.<sup>8</sup>

#### Kinetic Pre-Exponential Factor.

(1)  $K_0$  values presented in Table 1 for both experiments ( $\sim 10^9$  and  $\sim 10^{16}$  m<sup>-3</sup>·s<sup>-1</sup>) are orders of magnitude lower than the  $10^{20}$  m<sup>-3</sup>·s<sup>-1</sup> for HEN proposed by Kashchiev<sup>17</sup> for small molecules. For HON, values of the order of  $10^{32}$  m<sup>-3</sup>·s<sup>-1</sup> are expected.<sup>17</sup> Thus, it confirms that the lysozyme nucleation experiments summarized in Table 2 are heterogeneous, even when experiments are conducted at high supersaturation: up to  $\beta = 55$  for the group of Fraden. For HEN, the kinetic pre-exponential factor is proportional to the monomer attachment frequency and the number of nucleation active centers in the system.<sup>17,28</sup> The monomer attachment frequency is linearly dependent on the diffusion coefficient ( $D$ ); for instance,  $D$  of lysozyme is 1 order of magnitude smaller than that of

chloride.<sup>26</sup> In the literature, only 2–3 orders of magnitude lower growth rates are observed for proteins than for inorganic crystals,<sup>27</sup> far less than the difference measured here for lysozyme nucleation rates. This discrepancy may also be due to a lower number of nucleation active centers in the system, a value difficult to estimate. Note that in the case of the droplet method an initial volume of 50  $\mu\text{L}$  is divided in 200 small volumes of 250 nL; hence, the number of nucleation active centers is also divided by 200, as pointed out by Kashchiev.<sup>28</sup> Moreover, in a recent paper Kadam et al.<sup>29</sup> found even lower  $K_0$  values for the paracetamol–water system, on the order of  $10^3$  m<sup>-3</sup>·s<sup>-1</sup>.

(2)  $K_0$  values presented in Table 1 differ by many orders of magnitude. This difference is principally due to the different type of oil used which impacts nucleation rates. This point is developed in (3). However, the simplicity of the model<sup>12</sup> can be also incriminated:  $K_0$  is assumed to be independent of  $\beta$ , an assumption also accepted by Kadam et al.,<sup>29</sup> and  $\ln(K_0)$  is extrapolated to infinite supersaturation, outside the experimentally accessible range, a domain where CNT fails to describe nucleation, for instance, when  $\beta \rightarrow \infty$  CNT predicts  $J \rightarrow K_0$ .

(3) Lastly, we observe a discrepancy among  $K_0$  values from the literature concerning lysozyme nucleation in identical chemical conditions (Table 2). Note that it is more appropriate to compare  $\ln(K_0)$  values, because the kinetic pre-exponential factor is extracted from a plot of logarithm of  $J$  as a function of the inverse of the square logarithm of supersaturation. Thus, we observe that data from different groups with the same oil gave qualitatively the same  $\ln(K_0)$  values. Moreover, we observe that  $\ln(K_0)$  (fluorinated oil) >  $\ln(K_0)$  (silicone oil). According to Kashchiev,<sup>17</sup> the smaller values of  $\ln(K_0)$  in silicone oil indicate a better catalytic effect on nucleation using silicone oil than using fluorinated oil.

## 4. CONCLUSION

We present a discussion on the influence of heterogeneous nucleation in the determination of thermodynamic and kinetic factors in droplet-based nucleation experiments. Our comments on lysozyme nucleation rate measured by three different groups are intended to highlight how heterogeneities in supersaturated solutions, here, the chemical nature of the water–oil interface, influence nucleation rate. We show that the discrepancies among lysozyme interfacial energies and kinetic pre-exponential factors encountered in the literature arise from the type of oil used and that most of the data presented in the literature concern heterogeneous nucleation of lysozyme.

### ■ ASSOCIATED CONTENT

#### Supporting Information

Figure S1: Tetragonal lysozyme solubility versus temperature and MZL; Figure S2: primary nucleation rate vs supersaturation, at 20 °C. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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#### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank M. Sweetko for English revision and N. Ferté for protein characterization and fruitful discussions.

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