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# Scattering from laterally heterogeneous vesicles. III. Reconciling past and present work

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A recent series of papers have devised and successfully used a methodology for the detection and characterization of domains in laterally heterogeneous vesicles *via* small-angle neutron scattering. This methodology is in seeming contradiction to similar work devised by Knoll, Haas, Stuhrmann, Füldner, Vogel & Sackmann [*J. Appl. Cryst.* (1981), **14**, 191–202]. The present paper shows how these results may be reconciled.

## 1. Introduction

Cell membranes are an essential part of animal physiology, both as structural elements of tissues and organs, and as interfaces where chemical reactions and information and material transfer may take place. The ability of the cell membrane surface to reorganize itself into laterally heterogeneous, functional domains has stimulated the interest of both biologists and physicists.

While the idea of using small-angle scattering (SAS) to characterize membrane lateral heterogeneities is not new (Moody, 1975), there have been few such studies (detailed below). This is surprising given the large number of related studies using other techniques (*e.g.* Bagatolli, 2006, and references therein).

From a survey of literature we have found five groups that have been or are presently involved in studies of membrane domains using small-angle scattering. As mentioned, Moody (1975) recognized the potential for SAS to characterize membrane domains. However, to the best of our knowledge, Knoll and co-workers (Knoll, Haas *et al.*, 1981; Knoll, Ibel & Sackmann, 1981; Knoll, Schmidt & Ibel, 1985; Knoll, Schmidt, Ibel & Sackmann, 1985; Knoll *et al.*, 1991) were the first to use scattering methods to characterize laterally heterogeneous multilamellar vesicles. Small-angle neutron scattering (SANS) was used in order to take advantage of the high contrast offered by neutrons between the hydrogeneous and selectively deuterated lipids. Winter and co-workers also used SANS, but they examined heterogeneities in lipid multilayers rather than vesicles (Czeslik *et al.*, 1997; Winter *et al.*, 1999; Fahsel *et al.*, 2002; Nicolini *et al.*, 2004). More recently, we have further developed the general scattering theory and used SANS to examine domain formation in binary and ternary lipid mixtures (Pencer *et al.*, 2005, 2006, 2007; Anghel *et al.*, 2007). Two other groups have also recently performed similar studies (Hirai *et al.*, 2006; Masui *et al.*, 2006).

In comparing our work with that of Knoll, Haas *et al.* (1981), we have found seemingly contradictory predictions. Knoll, Haas *et al.* (1981) predicted that, under contrast-match conditions, the forward scattering from laterally heterogeneous vesicles can be used to calculate domain compositions, and have obtained data for homogeneous and heterogeneous systems that confirm their predictions. However, we have determined theoretically that the forward scattering from contrast-matched vesicles should be zero, regardless of whether they are heterogeneous or not (Anghel *et al.*, 2007). It is worth noting that, even with the potential contribution of forward scattered intensity, the method described by Pencer *et al.* (2006) can still be used to determine optimal conditions for domain studies.

In this paper, we discuss the assumptions made by Knoll, Haas *et al.* (1981), as well as by our group, and attempt to reconcile our seemingly disparate results. We find that the approach of Knoll, Haas *et al.* (1981) specifically applies to the case where heterogeneities are small and uncorrelated on a vesicle surface. Our approach, while more general, is shown to produce the same results as theirs when these same assumptions are made.

## 2. Predictions for low-angle scattering

As discussed by Moody (1975), the scattering from a heterogeneous vesicle can be calculated as

$$I(q) = \left\langle \left| \int_{\mathbf{r}} [\rho(\mathbf{r}) - \rho_s] \exp(-i\mathbf{q}\cdot\mathbf{r})\, d\mathbf{r} \right|^2 \right\rangle, \quad (1)$$

where $\mathbf{r}$ denotes a volume integral and $\langle \ldots \rangle$ refers to an orientational average. Orientational averaging leads to the Debye formula (Feigen & Sholer, 1975)

$$I(q) = \int_{\mathbf{r}'} \int_{\mathbf{r}} g(\mathbf{r}) g(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|}\, d\mathbf{r}\, d\mathbf{r}', \quad (2)$$

where the origin lies at the center of the vesicle, $g(\mathbf{r}) = \rho(\mathbf{r}) - \rho_s$ is the scattering length density (SLD) contrast, $\rho(\mathbf{r})$ is the (neutron or X-ray) SLD of the object, which may vary within the volume of the object, and $\rho_s$ is the mean SLD of the medium or solvent. If we assume a vesicle to be heterogeneous with $N$ discrete regions of constant SLD, $\rho_i$, we can expand the integral above into a sum of terms relating to self- and cross-correlation terms:



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$$I(q) = \sum_{i=1}^{N} \int_{\mathbf{r}'} \int_{\mathbf{r}} g_i(\mathbf{r}) g_i(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}' + \sum_{i=1}^{N} \sum_{j=1, i\neq j}^{N} \int_{\mathbf{r}'} \int_{\mathbf{r}} g_i(\mathbf{r}) g_j(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}' = \sum_{i=1}^{N} I_i(q) + \sum_{i=1}^{N} \sum_{j=1, i\neq j}^{N} \int_{\mathbf{r}'} \int_{\mathbf{r}} g_i(\mathbf{r}) g_j(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}'. \quad (3)$$

Note that, since the lipid headgroup SLD is typically different from that of the acyl chain region, the $N$ regions above will include lipid headgroup regions as well as lateral domains. In order to assess the forward scattered intensity, we assume that $|\mathbf{q}\cdot\mathbf{r}| \ll 1$. For a homogeneous particle, this expansion results in the familiar Guinier (1994) approximation:

$$I(q) = V^2(\rho - \rho_\mathrm{s})^2(1 - q^2R_\mathrm{g}^2/3), \quad (4)$$

where $V$ and $R_\mathrm{g}$ are the volume and radius of gyration of the particle, respectively. To first order, the expansion of the cross-correlation terms gives

$$g_i(\mathbf{r}) g_j(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}' \simeq g_i g_j. \quad (5)$$

Note that this approximation depends on the assumption that $q|\mathbf{r}-\mathbf{r}'| \ll 1$. We will discuss this in the following section. Nevertheless, for the time being, if we assume that $q|\mathbf{r}-\mathbf{r}'| \ll 1$ is true, the forward scattering can be calculated as

$$I(0) = \sum_{i=1}^{N} V_i^2(\rho_i - \rho_\mathrm{s})^2 + \sum_{i=1}^{N} \sum_{j=1, i\neq j}^{N} V_i V_j (\rho_i - \rho_\mathrm{s})(\rho_j - \rho_\mathrm{s}). \quad (6)$$

Under contrast-matching conditions (*i.e.* when the mean SLD, $\bar{\rho}$, equals the medium SLD, $\rho_\mathrm{s}$), the mean SLD is given by

$$\bar{\rho} = \sum_{i=1}^{N} V_i \rho_i / \sum_{i=1}^{N} V_i = \rho_\mathrm{s}. \quad (7)$$

Substitution of equation (7) into equation (6) shows that, under contrast-matching conditions, the forward scattering $I(0) = 0$. This directly contradicts the assumption made by Knoll, Haas *et al.* (1981), *i.e.*

$$I(0) = \sum_{i=1}^{N} P_i(\rho_i - \rho_\mathrm{s})^2, \quad (8)$$

where $P_i$ is the volume fraction of component $i$, since under contrast-matching conditions equation (8) does not result in $I(0) = 0$. However, as we will show below, under certain special conditions equation (8) is valid at intermediate angles.

## 3. Low- and mid-angle approximations

Let us return to equations (3) and (5) and make some additional assumptions. First, assume that $q$ is sufficiently small that all distances within any region $i$ satisfy $q|\mathbf{r}-\mathbf{r}'| \ll 1$. Secondly, assume that the distances between $i$ and $j$ are sufficiently large that in the cross-correlation terms, $q|\mathbf{r}-\mathbf{r}'| \gg 1$. If there is a $q_\mathrm{min}$ in the low- to mid-angle region that satisfies both these conditions, then

$$q|\mathbf{r}-\mathbf{r}'| \ll 1, \quad g_i(\mathbf{r}) g_i(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}' \simeq (\rho_i - \rho_\mathrm{s})^2$$
$$q|\mathbf{r}-\mathbf{r}'| \gg 1, \quad g_i(\mathbf{r}) g_j(\mathbf{r}') \frac{\sin q|\mathbf{r}-\mathbf{r}'|}{q|\mathbf{r}-\mathbf{r}'|} \, \mathrm{d}\mathbf{r} \, \mathrm{d}\mathbf{r}' \simeq 0, \quad (9)$$

and

$$I(q_\mathrm{min}) \simeq \sum_{i=1}^{N} V_i^2(\rho_i - \rho_\mathrm{s})^2. \quad (10)$$

Next, we assume that each region $i$ has the same volume $V_i$, that there are $M$ types of region and that there are $n_i$ domains of type $i$. If we now divide equation (10) by $V_i^2$ and the total number of regions $N$, we obtain

$$\frac{I(q_\mathrm{min})}{NV^2} \simeq \frac{1}{N} \sum_{i=1}^{M} n_i(\rho_i - \rho_\mathrm{s})^2 = \sum_{i=1}^{M} P_i(\rho_i - \rho_\mathrm{s})^2. \quad (11)$$

Thus, we recover the result of Knoll, Haas *et al.* (1981), which, while not strictly valid for evaluating $I(0)$, does apply in the intermediate $q$ range for small, uniform, uncorrelated domains.

## 4. Conclusions

In this short report we have shown that the seeming contradictions between our recent work (Pencer *et al.*, 2005, 2006; Anghel *et al.*, 2007) and that of Knoll, Haas *et al.* (1981) can be reconciled. In particular, we find that the general expression for scattering from laterally heterogeneous vesicles leads to that used by Knoll *et al.* when the following assumptions are made: (i) that the domains are small and (ii) that the domains are uncorrelated in space. Experiments are ongoing to test the method of Knoll, Haas *et al.* (1981) on heterogeneous ULV.

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