Supplementary Material. Manuscript Title: Reversible and Irreversible Aggregation of Magnetic Liposomes

CHARACTERIZATION OF THE FORM FACTOR BY A SLS MEASUREMENT

We performed a characterization of the liposome form factor, $P(q)$, experimentally based on SLS measurements and analytically described by a multimodal approach which takes into account different particle size populations. With this approach we avoid those systematic errors involved in the Laplace transform of the field autocorrelation function, $g_E(q;\tau)$, obtained by a DLS measurement which, in principle, could contain a wide spectrum of relaxation modes (H. Schnablegger and O. Glatter, Appl. Opt. **30**, 4889 (1991), Ref. [80] in the main text).

As mentioned in the main text (section III.A), we describe the experimental form factor, $P_{exp}(q)$, by assuming a trimodal distribution whose first five moments are distributed according to a Schulz distribution. Indeed we considered a Schulz distribution due to its efficiency to describe the size distribution of polydisperse systems of mesosized particles coming from different synthesis protocols (B. D'Aguanno and R. Klein, J. Chem. Soc. Faraday Trans., **87**(3), 379 (1991) and B. D'Aguanno and R. Klein, Phys. Rev. A, **46**, 7652 (1992), Refs. [78, 79] in the main text).

Our first step to reach our characterization of $P_{exp}(q)$ involves the transformation of a generic continuous Schulz distribution with a given mean and standard deviation (polydispersity) into an optimal trimodal distribution. The continuous Schulz distribution for a variable size diameter, σ, with mean value $\bar{\sigma}$ and variance $\langle\sigma^2\rangle$ is given by:

$$f_{Schulz}(\sigma) = \left[\frac{t+1}{\bar{\sigma}}\right] \frac{\sigma^t}{\Gamma(t+1)} exp\left[-\frac{t+1}{\bar{\sigma}}\sigma\right] \tag{1}$$

Where $\Gamma(x)$ is Euler's Gamma function and t a measure of the distribution width defined in terms of $\bar{\sigma}$ and $\langle\sigma^2\rangle$:

$$t = \frac{2\bar{\sigma}^2 - \langle\sigma^2\rangle}{\langle\sigma^2\rangle - \bar{\sigma}^2} \tag{2}$$

To obtain the optimal trimodal distribution which better approaches a Schulz distribution, we impose the equality of the first five moments of both distributions (Schulz and trimodal) and solve the corresponding five non-linear equations by a Newton-Raphson method. In Table I we show our results for five different polydispersities. Thus, a given polydispersity has three associated modal diameters, σ_i ($i \in \{1,2,3\}$), and three corresponding relative frequencies, c_i where $\sum_{i=1}^{3} c_i = 1$.

TABLE I: **Parameters corresponding to a trimodal distribution whose first five moments are distributed according to a Schulz distribution normalized by $\bar{\sigma}$. We have highlighted those values associated to a polydispersity of 0.2 which corresponds to the value obtained for the magnetic liposomes presented in the main text.**

Polydispersity ($\sqrt{\langle\sigma^2\rangle}/\bar{\sigma}$)	c_1	c_2	c_3	$\sigma_1/\bar{\sigma}$	$\sigma_2/\bar{\sigma}$	$\sigma_3/\bar{\sigma}$
0.10	0.617	0.358	0.025	1.059	0.877	1.311
0.15	0.716	0.255	0.029	0.933	1.236	0.582
0.20	**0.635**	**0.271**	**0.094**	**1.013**	**0.730**	**1.447**
0.25	0.076	0.611	0.313	1.607	1.085	0.689
0.30	0.065	0.345	0.590	1.773	0.648	0.583

The second step consists in finding those σ_i and c_i values (Table I) which better reproduce the experimental form factor, $P_{exp}(q)$, that is, those σ_i and c_i values for which the deviation, δ, is minimum over all the explored q values (scattering angles), N_q:

$$\delta = \sum_{j=1}^{N_q} \left[P_{exp}(q_j) - \left(\sum_{i=1}^{3} c_i P_i(q_j) \right) \right]^2 \tag{3}$$

As mentioned in the main text, each $P_i(q)$ appearing in Eq. (3) adopts the form of a solid sphere in the context of the Rayleigh-Gans-Debye (RGD) theory (J. Dhont, *An Introduction to Dynamics of Colloids*, Amsterdam: Elsevier (1996), Ref. [74] in the main text):

$$P_i(q) = \left[\frac{3\,(sin(qR_i) - qR_i cos(qR_i))}{(qR_i)^3} \right]^2 \tag{4}$$

Where $R_i = \sigma_i/2$ is the radius of species $i \in \{1,2,3\}$. Therefore we performed a multimodal (in particular trimodal) description of our form factor $P_{exp}(q)$ (Fig.2a) in the main text) as:

$$P_{exp}(q) \cong \sum_{i=1}^{3} c_i P_i(q) \tag{5}$$

The optimal values we obtained for our approximation are those corresponding to a polydispersity of 0.2 (highlighted in Table I). This corresponds to the σ_i and c_i values represented in Fig. 1.

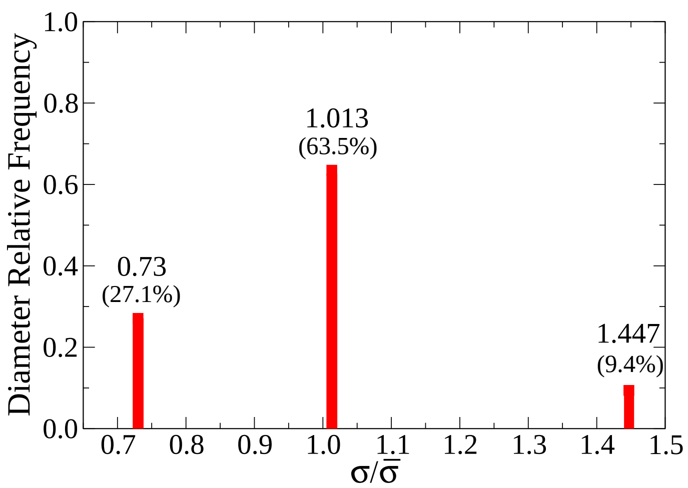

Fig.1. Diameter relative frequencies from a trimodal distribution for the magnetic liposomes presented in the main text as obtained from the fitting of $P_{exp}(q)$ according to Equations (3) and (4) and Table I. The different populations would correspond to $\sigma_1 = 182.3$ nm, $\sigma_2 = 131.4$ nm, and $\sigma_3 = 260.5$ nm, where $\bar{\sigma} \cong 180$ nm.

MAGNETIC NANOPARTICLE IN A MAGNETIC FIELD: MAGNETIC DRIFT VERSUS DIFFUSION

In this section we show that the effect on the magnetic liposome motion due to a hypothetical velocity induced by the magnetic field is negligible compared to the effect of the purely diffusive (Brownian) motion. To prove this statement we will show that:

- The hypothetical velocity induced by the magnetic field would reach a stationary modulus and have a direction which would be parallel to the magnetic field.

- The modulus of the hypothetical velocity due to the presence of the magnetic field would induce displacements within the correlation time of the field autocorrelation function, $g_E(q;\tau)$, which are negligible compared to those displacements induced by diffusion.

Let us consider the first point.

The force, $\vec{F}_{mag}$, acting on a magnetic particle immersed in a magnetic field, $\vec{B}$, is (S.S. Shevkoplyas, A.C. Siegel, R.M. Westervelt, M.G. Prentiss, and G.M. Whitesides, Lab Chip, **7**, 1294 (2007), Ref. [77] in the main text):

$$\vec{F}_{mag} = \frac{V\Delta\chi}{\mu_0}\left(\vec{B}\cdot\vec{\nabla}\right)\vec{B} \tag{6}$$

Where V is the particle volume, $\Delta\chi$ the difference in magnetic susceptibilities between the particle and the surrounding medium, and μ_0 the permeability of vacuum.

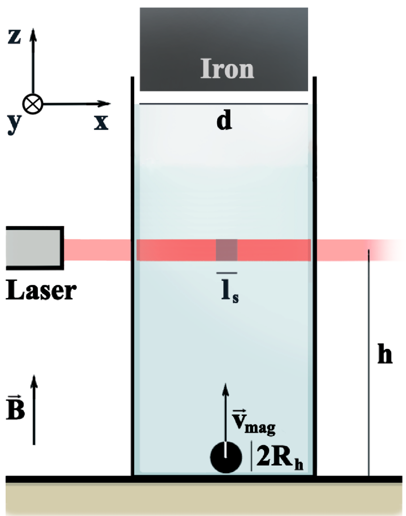

Fig.2. Sketch of a particle moving vertically from the bottom of the scattering cell at a constant velocity, $\vec{v}_{mag}$, which results from the balance between magnetic and fluid friction forces. Both the particle size (given by R_h) and the scattering volume (defined by the linear length $l_s \lesssim 0.5$ mm) have been magnified in the figure. The diameter of the cylindrical iron bar is $d = 22 \pm 1$ mm whereas the distance between the bottom of the cell and the laser beam is $h \cong 5$ mm (note that the figure is merely illustrative and the real ratio $d/h \cong 4$ is not as the aspect ratio shown in the figure).

As show in Fig. 2, in our experiments the homogeneous magnetic field line confinement and the fact that the linear size, l_s, defining the scattering volume is much smaller than the length scale defining the iron bar, d, result in a magnetic field with only z-component, $\vec{B} = B_z\hat{k}$. Thus Eq. (6) leads us to a magnetic force which only has z-component, $\vec{F}_{mag} = F_z\hat{k}$:

$$\vec{F}_{mag} = \frac{V\Delta\chi}{\mu_0} B_z \frac{\partial B_z}{\partial z}\hat{k} = F_z\hat{k} \tag{7}$$

From Eq.(7) we see that the magnetic force would come from the variation of the magnetic field along the vertical axes ($\partial B_z/\partial z$). In principle, we can expect a small vertical magnetic field gradient due again to the vertically homogeneous magnetic line confinement inside the tiny scattering volume. In the second point of this dissertation we will show that, if this vertical gradient is present, it would induce extremely slow velocities.

Since the magnetic particle is immersed in a fluid, the vertical magnetic force (Eq. (7)) will be balanced by a fluid friction force proportional to the particle velocity, $\vec{F}_{fric} \sim \vec{v}$, which has the same direction of $\vec{F}_{mag}$ but opposite sense. As a consequence, the magnetic particle will immediately reach an equilibrium for the z-component which results in a constant velocity, $\vec{v}_{mag} = |\vec{v}_{mag}|\hat{k}$ (see for instance J. Dhont, *An Introduction to Dynamics of Colloids*, Amsterdam: Elsevier (1996), Ref. [74] in the main text):

$$\vec{F}_{mag} + \vec{F}_{fric} = (F_z - \gamma|\vec{v}_{mag}|)\,\hat{k} = 0 \tag{8}$$

Where γ is the Stokes coefficient. Therefore Eq. (8) shows the first result we wanted to prove.

At this point we should note that for small velocities, $\vec{v}_{mag}$, *i.e.* velocities inducing displacements similar to (or smaller than) those induced by diffusion, we would not detect the effect of $\vec{v}_{mag}$ in the field correlation function obtained by our DLS measurements. Indeed for such a velocity the field autocorrelation function would read (B. Berne and R. Pecora, *Dynamic Light Scattering With Applications to Chemistry, Biology, and Physics*, Dover Publications, Mineola, New York (2000), Ref. [72] in the main text):

$$g_E(q;\tau) = exp(i\vec{q}\cdot\vec{v}_{mag}\tau)exp(-Dq^2\tau) \tag{9}$$

Since the scattering vector $\vec{q}$ is included in the scattering plane (XY-plane in Fig. 2), we have $\vec{q}\cdot\vec{v}_{mag} = 0$. Therefore, in this case Eq. (9) would just contain the diffusive motion and would result in:

$$g_E(q;\tau) = exp(-Dq^2\tau) \quad ; \quad \vec{q}\cdot\vec{v}_{mag} = 0 \tag{10}$$

Once this result (Eq. (10)) has been reached we just have to prove that the displacements induced by $\vec{v}_{mag}$ are similar to (or smaller than) those induced by diffusion within the time needed to decorrelate the field autocorrelation function by pure Brownian motion (this is indeed the second point of this dissertation). We will prove that the displacements induced by $\vec{v}_{mag}$ are indeed negligible compared to those induced by the particle diffusion. This is an important result since the effect of $\vec{v}_{mag}$ when interpreting DLS data would be negligible not only in the experiments we performed in the present work (where $\vec{q}\cdot\vec{v}_{mag} = 0$) but even in those experiments where $\vec{v}_{mag}$ has a non-zero projection into the scattering plane ($\vec{q}\cdot\vec{v}_{mag} \neq 0$). To prove this (final) statement we will consider a crude experimental fact: in all the light scattering experiments we performed the time average scattered light intensity, $\langle I(q;t)\rangle$, reaches a final stationary value which never decays within the duration of our DLS experiments (see sections II.D.2, III.B, and III.C in the main text).

The typical duration of our experiments for an aggregation kinetics experiment is of the order of $t_{exp} \sim 1$ hour (see section III.C in the main text). If $|\vec{v}_{mag}| > h/t_{exp}$ all the particles coming from the bottom of the scattering cell (see Fig. 2) would overpass the laser beam and the scattered intensity would immediately drop (there will be no particles in the scattering volume). However, as mentioned before, $\langle I(q;t)\rangle$ remained constant during our experiments. This experimental fact introduces an upper bound for the velocity induced by the magnetic field given by:

$$|\vec{v}_{mag}| \leq \frac{h}{t_{exp}} \cong 1 \ \mu m/s \tag{11}$$

Let us now consider the time needed to decorrelate the field autocorrelation function, $g_E(q;\tau)$, by pure Brownian diffusion. This time is $\tau_B = (Dq^2)^{-1}$ (see Eq. (10)), where D is the particle diffusion coefficient and $q = (4\pi n/\lambda)\sin(\theta_f/2)$, being $\theta_f = \pi/2$ the scattering angle, $\lambda = 633$ nm the laser wavelength, and $n = 1.33$ the water refraction index (section II.D.2 in the main text). The particle displacement, Δl_B, during this time due to pure Brownian diffusion will be given by Einstein's equation ($\langle \Delta\vec{r}(\tau_B)^2\rangle = 6D\tau_B$):

$$\Delta l_B = \sqrt{\langle \Delta\vec{r}(\tau_B)^2\rangle} = \sqrt{6D\tau_B} = \sqrt{6D\frac{1}{Dq^2}} = \frac{\sqrt{6}}{q} \tag{12}$$

The resulting Brownian displacement is $\Delta l_B \cong 130$ nm. Let us now compare this displacement with the displacement, Δl_{mag}, that we could in principle obtain due to the magnetic drift according to Eq. (11) for the same time τ_B:

$$\Delta l_{mag} = |\vec{v}_{mag}|\tau_B = |\vec{v}_{mag}|\frac{1}{Dq^2} \tag{13}$$

To evaluate Eq. (13) we use Stokes-Einstein relation $D = k_B T/6\pi\eta R_h$, where k_B is Boltzmann's constant, $T = 298°$K the absolute temperature, $\eta = 9.1 \cdot 10^{-4}$ Pa·s the water shear viscosity at 298°K, and R_h the particle hydrodynamic radius. In particular, we evaluate Eq. (13) for the typical single liposome radius, $R_h \cong 100$ nm, and for the radius of the biggest aggregates we measured in our aggregation kinetics experiments, $R_h \cong 500$ nm (see $B = 38.8$ mT, Fig. 3, section III.B, in the main text). According to Eq. (11), $|\vec{v}_{mag}| \leq 1$ μm/s, we obtain: $\Delta l_{mag} \leq 1.2$ nm for $R_h = 100$ nm and $\Delta l_{mag} \leq 6$ nm for $R_h = 500$ nm. In both cases the maximum displacement we can expect due to the magnetic drift is about two orders of magnitude smaller than that corresponding to the Brownian displacement for the same time, $\Delta l_B \cong 130$ nm. In conclusion we can neglect the effect of the magnetic drift in our DLS experiments by two independent arguments, the first one based on the direction of $\vec{v}_{mag}$ (Eq. (10)) and the second one based on the small modulus of $\vec{v}_{mag}$, which would lead to really small displacements compared with the diffusive ones.