A Numerical Study on the Effect of the Particle Surface Coverage on the Quartz Crystal Microbalance Response

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- 14
- 15 <u>Abstract</u>

16 The quartz crystal microbalance (QCM) is a surface-sensitive measurement technique to characterize 17 adsorption processes at solid-fluid interfaces. While QCM measurements are routinely applied to study homogenous thin films, characterizing heterogeneous films of adsorbed particles remains 18 19 challenging because QCM is sensitive to not only the mass of adsorbed particles but also that of 20 hydrodynamically-coupled fluid. To extract information about adsorbed particles, it is necessary to 21 model these complex hydrodynamic effects, however, current QCM models are restricted to the limit 22 of either a very low surface coverage or to the extrapolated limit of saturation coverage. Herein, we 23 investigated QCM measurement responses in the intermediate surface coverage regime, by 24 conducting lattice Boltzmann simulations of monodisperse, spherical particles that are attached to an 25 oscillating surface. From the simulations, we relate the overtone-dependent QCM frequency and bandwidth shifts to particle size, interparticle distance, and the relevant hydrodynamic length scale. 26 27 The corresponding results are in qualitative agreement with experimental OCM data for sub-100 nm, 28 gel-phase liposomes. Furthermore, the data provide a theoretical basis for extracting particle sizes 29 from QCM data in the high surface coverage limit.

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32 Introduction

The quartz crystal microbalance (QCM) is a widely used, surface-sensitive measurement technique to characterize adsorption events at solid-fluid interfaces.^{1,2} QCM measurements involve tracking the resonance behavior of shear-thickness oscillations of a piezoelectric quartz crystal at its fundamental frequency and odd overtones thereof.^{3,4} The presence of an adsorbate on the QCM sensor surface influences this resonance behavior, and corresponding shifts can be monitored in order to obtain information about physical properties of the adsorbate.^{5,6}

39 The QCM detects not only the adlayer but also the fluid that is coupled to the oscillating adlayer.^{7,8} As a result, translating QCM data to adlayer properties requires so-called 'QCM models', 40 41 which are based on hydrodynamic analysis. Analytical OCM models have been developed to extract the mass and viscoelastic properties of homogenous, thin-film adsorbates.⁹⁻¹² On the other hand, 42 43 layers of adsorbed particles are governed by heterogeneous hydrodynamic coupling between the adlayer and surrounding fluid.^{3,12-20} These complex hydrodynamic effects have hindered the 44 development of corresponding OCM models that relate OCM measurement responses, via the 45 46 hydrodynamic force, to the properties of adsorbed particles, *e.g.*, surface coverage, size, and shape. As a result, current QCM models are restricted to the limit of either a very low surface coverage^{21,22} 47 or to the extrapolated limit of saturation coverage.^{23,24} Specifically, this latter model extrapolates 48 49 QCM data to a hypothetical scenario, where the adsorbed particles behave as a rigid, densely-packed layer. The thickness of the corresponding adlayer is determined by using a simple analytical model, 50 which is valid for homogenous films.⁹ In some cases, the resulting adlayer thickness agreed well with 51 the known size of adsorbed particles.²³⁻²⁷ However, a theoretical basis for this 'extrapolation method' 52 53 remains to be established.

54 Constructing such theory requires analyzing the hydrodynamic force acting on a collection of 55 particles attached to an oscillating surface. Previously, we combined numerical simulation with 56 dimensional analysis to study the corresponding problem for a single spherical²¹ or ellipsoidal²² 57 particle. From those analyses, we derived QCM models for extracting the size and shape of adsorbed 58 particles at low QCM surface coverages.^{21,22} Herein, we extend this methodology and investigate the hydrodynamics of adsorbed particles, not only for a low surface coverage but also for higher surface 59 coverages where hydrodynamic interactions between adsorbed particles are important. By 60 61 systematically varying the independent, dimensionless parameters in the simulation, we provide a comprehensive description of the hydrodynamic interaction between monodisperse, spherical 62 particles adsorbed on the QCM sensor surface, as a function of particle size and surface coverage. 63 Guided by the simulation outcome, we also provide a theoretical basis for the aforementioned 64 extrapolation method to determine the size of adsorbed particles from QCM data at high surface 65 66 coverages.

67 **Theoretical Considerations**

We consider the QCM measurement response that occurs due to the adsorption of N solid, spherical 68 69 particles of radius a. The surface area of the quartz crystal is A_0 and the distance between the adsorbed particles is $L = (A_0/N)^{1/2}$, which corresponds to a surface coverage of $\phi = \pi (a/L)^2$. We assume that the 70 71 particles are rigidly adhered to the substrate and remain spherical in the adsorbed state. $\Delta \hat{F}$ denotes 72 the complex-valued amplitude of the force oscillation, which is exerted on the quartz crystal due to the adsorption of one particle, and the hat \wedge signifies a complex number. The force amplitude, $\Delta \hat{F}$, 73 74 has an inertial (real) component, which is in phase with the quartz acceleration and responsible for the frequency shift, Δf , and a dissipative (imaginary) component, which is in phase with the quartz 75 velocity and responsible for the bandwidth shift, $\Delta \Gamma = f \Delta D / \pi$. The bandwidth shift is equivalent to 76 77 the dissipation shift, ΔD , where $f = f_0 n = \omega/2\pi$ is the (overtone) frequency, n is the overtone number, f_0 is the fundamental frequency, and ω is the (overtone) angular frequency of the quartz. In this work, 78 79 the QCM data is presented in terms of the frequency and bandwidth shifts (Δf , $\Delta \Gamma$). Since $\Delta \Gamma$ is dimensionally equivalent to Δf , this representation offers a seamless comparison between the two 80 81 quantities. It also allows us to compare our theoretical approach with the extrapolation method, which was described in the Introduction and is henceforth referred to as the $\Delta\Gamma/\Delta f$ extrapolation method. 82 Specifically, the $\Delta\Gamma/\Delta f$ extrapolation method involves plotting the $-\Delta\Gamma/\Delta f$ ratio versus $-\Delta f/n$ at 83

84 multiple overtones in order to determine the adlayer height.^{23,24}

Based on the above definitions, the QCM frequency shift, Δf , and bandwidth shift, $\Delta \Gamma$, can be expressed as follows:^{3,4}

$$87 \quad -\Delta f + i\Delta\Gamma = \frac{fN\Delta\hat{F}}{\omega Um_Q},\tag{1}$$

88 where $m_Q = A_Q l_Q \varrho_Q$ is the mass, l_Q is the thickness, ϱ_Q is the mass density of the quartz crystal, and 89 $i = \sqrt{-1}$ is the imaginary unit. The force per adsorbed particle $\Delta \hat{F} = \Delta \hat{F}_H + m_P \omega U$ is the sum of the 90 hydrodynamic force, $\Delta \hat{F}_H$, and the inertia, $m_P \omega U$, of one adsorbed particle, where *U* is the (real-91 valued) quartz velocity amplitude and $m_P = \varrho_F (4\pi/3)a^3$ is the mass of one particle, and it is assumed 92 that the particle mass density equals the fluid mass density, ϱ_F . The hydrodynamic force per particle, 93 $\Delta \hat{F}_H$, is the force acting on the combined system of *N* particles and the substrate minus the force acting 94 on the substrate without the particles, divided by *N*.

We use dimensional analysis to derive the general form of the governing relation between $\Delta \hat{F}_{\mathrm{H}}$ 95 and the operating conditions. The number of variables in the problem is seven, *i.e.*, $\Delta \hat{F}_{\rm H}$, U, ω , a, ϕ , 96 97 $\rho_{\rm F}$, and the fluid kinematic viscosity, $v_{\rm F}$. According to the Buckingham-Pi theorem,²⁸ one can combine seven variables (with three units being length, time, and mass) into 7 - 3 = 4 independent 98 dimensionless parameters such that $\widehat{\Pi}_i = \Delta \widehat{F}_H^{\alpha} U^{\beta} \omega^{\gamma} a^{\delta} \phi^{\varepsilon} v_F^{\zeta} \rho_F^{\eta}$, where i = 1, 2, 3 or 4 and $\alpha, \beta, \gamma, \delta$, 99 ε , ζ and η are determined by requiring that $\widehat{\Pi}_i$ be dimensionless. This procedure results in the 100 dimensionless force $\widehat{\Pi}_1 = \Delta \widehat{F}_H / (\rho_F a^3 \omega U)$, the dimensionless size $\Pi_2 = a \omega^{1/2} v_F^{-1/2}$, the surface 101 102 coverage $\Pi_3 = \phi$, and the dimensionless velocity $\Pi_4 = Ua/v_F$. The parameter Π_4 is known as the Reynolds number, Re, which is estimated to be Re = 10^{-2} based on $U = 10^{-1}$ m s⁻¹,²⁹ $a = 10^{-7}$ m, and 103 $v_{\rm F} = 10^{-6} \text{ m}^2 \text{s}^{-1}$. Since Re is small compared to unity, the problem is independent of Re, and the 104 dimensionless force $\widehat{\Pi}_1$ is therefore a dimensionless, complex-valued function of only two 105 dimensionless variables: Π_2 and Π_3 , *i.e.* $\widehat{\Pi}_1 = \widehat{\Pi}_1(\Pi_2, \Pi_3)$. Expressing the dimensionless size as Π_2 106 107 = a/δ where the penetration depth is given by $\delta = (2v_F/\omega)^{1/2}$, the governing relation is written as:

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$$\Delta \hat{F}_{\rm H} = \rho_F a^3 \omega U \widehat{\Pi}_1 \left(\frac{\delta}{a}, \phi\right). \tag{2}$$

109 As described below, $\widehat{\Pi}_1(\delta/a, \phi)$ is determined by using numerical simulation.

110 Materials and Methods

111 Numerical Simulation. A lattice Boltzmann (LB) method was employed to compute the 112 hydrodynamics of a single spherical particle, with radius *a*, which is attached to an oscillating surface. The specific details of the LB method are provided in the Supporting Information. The geometry and 113 114 coordinate system of the simulation setup are presented in Fig. 1a. The surface has its normal in the 115 z direction and oscillates in the x direction. The y direction is normal to the x and z directions. The domain sizes are L in the horizontal (x and y) directions and L_z in the vertical (z) direction. In the 116 117 horizontal directions, periodic boundary conditions are employed. This means that the system 118 represents a periodic array of spheres, with an interparticle separation distance of L. Thus, by varying the horizontal domain size L, we can modulate the particle surface fraction, $\phi = \pi (a/L)^2$. 119

The numerical method to solve the hydrodynamics problem is based on a three-dimensional staircase approximation of the spherical particle shape, as shown in **Fig. 1b**. The fluid kinematic viscosity was fixed at $v_F = \Delta x^2/(6\Delta t)$, where Δx and Δt are the lattice spacing and computational time step, respectively. The simulations were conducted using a range of values for the horizontal and vertical domain sizes, *L* and *L*_z, particle radius *a*, and oscillation frequency *f*, which resulted in a surface coverage between $\phi \approx 0.01$ and 0.8 and a scaled, viscous penetration depth that varied between $\delta/a \approx 0.6$ and 4.6. All simulation parameters are listed in **Supporting Table S1**.



Figure 1. (a) Computational domain of the lattice Boltzmann (LB) simulation. (b) Staircase approximation of a spherical particle in the LB simulation, where the particle radius spans ten lattice spacings.

132 Liposome Preparation. Liposomes (lipid vesicles), which are in the gel phase at room temperature, were prepared by the extrusion method.³⁰ A dried film of 1,2-dipalmitoyl-sn-glycero-3-133 phosphocholine (DPPC, Avanti Polar Lipids, Alabaster, AL, USA) lipids was hydrated in an aqueous 134 135 buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl) at a lipid concentration of 5 mg/mL, using Milli-Q-treated water with a minimum resistivity of 18.2 MQ \cdot cm (Millipore, Billerica, MA, USA). 136 137 After vortexing the sample, the liposomes were extruded through a track-etched polycarbonate 138 membrane with a 50-nm diameter nominal pore size. As the fluid-to-gel phase transition temperature 139 of the DPPC lipid is around 41 °C, the liposome suspension was heated to ~60 °C during the extrusion process, so that there was efficient sizing of the DPPC liposomes in the fluid-phase state.³¹ After the 140 141 extrusion process was completed, the DPPC liposome suspension was cooled down to room temperature, and the liposomes returned to the gel-phase state. The mean and standard deviation of 142 143 the liposome radius was measured by dynamic light scattering (DLS, Brookhaven Instruments, Holtsville, NY, USA), and was determined to be $a = 38 \pm 7$ nm. The corresponding size distribution 144 145 is given in Supporting Figure S1. Immediately before QCM experiments, the liposomes were diluted to a 0.05 mg/mL lipid concentration in 10 mM Tris buffer [pH 7.5] with 250 mM NaCl. The selected 146 147 ionic strength promotes a strong adhesion force by shielding electrostatic repulsion between the TiO₂coated substrate and DPPC liposomes,³² while it should be noted that the adsorbed, gel-phase DPPC 148 149 liposomes remain spherical and do not deform appreciably, under these conditions.³³

150 **QCM Experiments.** Liposome adsorption was measured by using the Q-Sense E4 QCM instrument 151 (Biolin Scientific AB, Stockholm, Sweden). We used quartz crystal sensor chips, which had a sputter-152 coated, 50-nm thick layer of TiO₂ (model no. QSX 310, Biolin Scientific AB) and a fundamental frequency of $f_0 = 5$ MHz, thickness of $l_0 = 0.3$ mm, and mass density of $\rho_0 = 2.65$ g/mL. A peristaltic 153 pump (Reglo Digital, Ismatec, Glattbrugg, Switzerland) was used to inject liquid sample into the 154 155 measurement chamber at a flow rate of 50 μ L/min. The temperature in the measurement chamber was maintained at 25.0 \pm 0.5 °C. The experimental data were collected at the 3rd to 11th odd overtones 156 157 using the QSoft software program (Biolin Scientific AB). Baseline signals in aqueous buffer solution (without liposomes) were recorded for 7 min prior to liposome injection under continuous flowconditions.

160 **<u>Results</u>**

161 Flow Field Visualizations

Fig. 2 shows the simulated *x*-component of the fluid velocity in the (x, z)-plane for four different values of ϕ at a fixed value of $\delta/a \approx 0.6$. These visualizations illustrate the physical mechanism that is responsible for the overtone- and time-dependent frequency and bandwidth shifts, which are typically observed during particle adsorption. Below, we analyze these dependencies in detail, through both simulation and experiment.



- Figure 2. Simulated *x*-component of the fluid velocity field around spherical particles attached to an oscillating surface at different surface coverages: (a) $\phi \approx 0.01$, (b) $\phi \approx 0.05$, (c) $\phi \approx 0.2$, and (d) $\phi \approx$ 0.8. The snapshots are taken at the instant in the oscillation, when the velocity at the surface equals zero. For all cases, the viscous penetration depth scaled to the particle radius is $\delta/a \approx 0.6$.
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174 FIG 3. (a) Simulated real part of the QCM force $\Delta \Re(\hat{F})$ (corresponding to frequency shift) per 175 particle versus scaled viscous, penetration depth δa for various surface coverages ϕ . (b) Simulated 176 imaginary part of QCM force $\Delta \Im(\hat{F})$ (corresponding to dissipation shift) per particle versus scaled 177 penetration depth δ/a for various surface coverages ϕ . The simulated force $\Delta \hat{F}$ is scaled with $a^3 \rho_F \omega U$, where a is the particle radius. $\rho_{\rm F}$ is the fluid mass density. ω is the OCM (overtone) angular frequency. 178 179 and U is the QCM (overtone) velocity amplitude. (c) Measured, negative QCM frequency shift per overtone $-\Delta f/n$ versus scaled penetration depth δ/a for various time points t. (d) Measured QCM 180 bandwidth shift per overtone $\Delta\Gamma/n$ per particle *versus* scaled penetration depth δ/a for various time 181 182 points t. In order to compare the experimental data in (c, d) to the simulation data in (a, b), we divided $-\Delta f/n$ and $\Delta \Gamma/n$ by the adsorption time t, which is, prior to saturation, assumed to be proportional to 183 184 the number of adsorbed particles.

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186 QCM Response as a Function of Overtone Number

In Figs. 3a and 3b, we investigate the simulated QCM force per particle, $\Delta \hat{F}$, as a function of the scaled penetration depth, δ/a , for various fixed values of ϕ . As mentioned above, $\Delta \hat{F}$ is a complexvalued force amplitude, and the real and imaginary parts correspond to the QCM frequency and bandwidth shifts, respectively [*cf.* Eq. (1)]. Fig. 3a shows the real part $\Delta \Re(\hat{F})$, which is the sum of the inertial force of the particle and that of the coupled fluid. The results indicate that, for large $\phi \approx$ 0.8, $\Delta \Re(\hat{F})$ is independent of the penetration depth. In this regime, $\Delta \Re(\hat{F})$ consists solely of particle

193 inertia, while there is no contribution from the coupled fluid, *i.e.*, the coupled fluid resembles that of a flat surface, which can be seen from the visualization as presented in Fig. 2d. On the other hand, at 194 low $\phi \approx 0.01$, $\Delta \Re(\hat{F})$ contains the particle inertia (constant component as a function of δ/a) as well 195 as the inertia of the coupled fluid, which is reflected by an increase of $\Delta \Re(\hat{F})$ as a function of δa . 196 197 However, it is observed that $\Delta \Re(\hat{F})$ does not continually increase as δ/a becomes larger. At large δ/a , $\Delta \Re(\hat{F})$ levels off, which reflects the hydrodynamic interactions between neighboring particles, *i.e.*, 198 199 the overlap of the coupled fluid of neighboring particles. With increasing ϕ , the distance between 200 neighboring particles decreases, and the hydrodynamic coupling is therefore observed to set in at 201 smaller δ/a .

Fig. 3b shows the imaginary part of the force per particle, $\Delta \mathfrak{I}(\hat{F})$, which measures the frictional force of the coupled fluid that is exerted at the solid-fluid interface. For $\phi \approx 0.8$, the particles form a densely packed layer (*cf.* Fig. 2d) and the friction resembles that occurring on a flat surface, *i.e.*, $\Delta \mathfrak{I}(\hat{F}) = 0$. On the other hand, for smaller ϕ , $\Delta \mathfrak{I}(\hat{F})$ shows non-monotonic behavior as a function of δ / a , *i.e.*, as a function of δ / a , $\Delta \mathfrak{I}(\hat{F})$ first increases and then decreases towards zero, due to the overlap of the coupled fluid of the neighboring particles.

Figs. 3c and 3d show the experimentally measured force per particle as a function of δ/a , which 208 209 corresponds to various overtones n, and for various ϕ , which corresponds to various time points t. The relative penetration depth, δ/a , is related to the overtone, *n*, by $\delta/a = (v_F/\pi f_0 n)^{1/2}$. The real and 210 imaginary parts of the force are represented by the (negative) frequency shift per overtone number, -211 212 $\Delta f/n$, and by the bandwidth shift per overtone number, $\Delta \Gamma/n$. These quantities correspond to the force 213 induced by all adsorbed particles. In order to obtain quantities that are proportional to the force per 214 adsorbed particle, we need to divide $-\Delta f/n$ and $\Delta \Gamma/n$ by the (areal) number (density) of adsorbed particles. Since the number density of adsorbed liposomes is not directly available, we take into 215 account that the liposome adsorption process is diffusion-limited,³⁴ which implies that the number 216 217 density is proportional to the adsorption time t. For this reason, we divide the frequency and bandwidth shifts (per overtone number) in Figs. 3c and 3d by *t*. Under diffusion-limited adsorption conditions, the resulting quantities, $-\Delta f/nt$ and $\Delta \Gamma/nt$, are equal to the force per particle, up to an unknown proportionality constant. We do not further consider this constant for the present purpose, which is focused on comparing the simulated and measured QCM force dependencies on δ/a and ϕ . The experimental data in Figs. 3c and 3d compare well with the simulated data in Figs. 3a and 3b, in terms of the aforementioned dependencies on δ/a and ϕ .

224 QCM Response as a Function of Time

We now turn our attention to the time dependence of the frequency and bandwidth shifts due to the entire ensemble of adsorbed particles. For this purpose, we multiply the real and imaginary parts of the simulated force per particle (*cf.* Figs. 3a and 3b) by the surface coverage, ϕ . In Figs. 4a and 4b, we plot the resulting $\phi \Delta \Re(\hat{F})$ and $\phi \Delta \Im(\hat{F})$ as functions of ϕ , for various δ/a . Experimentally, this representation corresponds (up to an unknown proportionality constant) to the frequency and bandwidth shifts as functions of time for the various overtones, which are shown for comparison in Figs. 4c and 4d.

The simulated inertial force on the QCM response increases monotonically with ϕ and with δ/a (Fig. 4a), and these trends agree well with the experimental data (Fig. 4c). At saturation coverage, the different overtones collapse, *i.e.*, the saturated layer of regularly distributed particles behaves as a uniform film.⁹ In the experiment, one nuance is that the different overtones do not collapse at saturation, which reflects that the saturated layer of adsorbed liposomes does not fully behave as a uniform film. This might reflect, the irregular, spatial particle arrangement, with gaps between the particles, as opposed to the regular, and closed packed particle arrangement in the simulation.

The simulated frictional force on the QCM response is a non-monotonic function of ϕ , which increases at low ϕ and decreases towards zero at high ϕ (Fig. 4b). These trends are also observed in the experimental data, albeit the decrease at higher ϕ does not continue to zero but instead saturates at a finite value (Fig. 4d). This again indicates, that the saturated layer is not closed packed.





FIG 4. (a) Simulated real part of QCM force per πa^2 surface area $\phi \Delta \Re(\hat{F})$ (corresponding to frequency shift), as a function of surface coverage ϕ , for various scaled penetration depths δ/a . (b) Simulated imaginary part of QCM force per πa^2 surface area $\phi \Delta \Im(\hat{F})$ (corresponding to dissipation shift), as a function of surface coverage ϕ , for various scaled penetration depths δ/a . (c) Measured QCM frequency shift per overtone $-\Delta f/n$ as a function of time for various overtones *n*, corresponding to various scaled penetration depths δ/a . (d) Measured QCM bandwidth shift per overtone $\Delta\Gamma/n$ as a function of time for various overtones *n*, corresponding to various scaled penetration depths δ/a .

It is noted that the transient maximum in the bandwidth shift (*cf.* Figs. 4b and 4d) has been observed previously for globular proteins and virus particles, in which cases it was attributed to the softness of

the linkers between the particles and substrate.^{3,27} Interestingly, the present results support that similar

255 transient maxima occur in purely rigid systems, originating from the hydrodynamic interactions

256 between particles and hence providing a physical mechanism to explain past experimental

- 257 observations with adsorbed liposomes as well.^{24,33}
- 258 Onset of Hydrodynamic Coupling

259 We continue by studying the relation between the hydrodynamic force per particle and the average

260 distance between the particles, $L = a(\pi/\phi)^{1/2}$, or equivalently the surface coverage ϕ . To this end,





FIG 5. (a) Simulated real part of the QCM force per particle $\Delta \Re(\hat{F})$ as a function of the surface coverage ϕ for various values of the scaled penetration depth δ/a . (b) Simulated imaginary part of the QCM force per particle $\Delta \Im(\hat{F})$ as a function of the surface coverage ϕ for various values of the scaled penetration depth δ/a . (c) Simulated real part of the QCM force per particle $\Delta \Re(\hat{F})$ as a function of the scaled interaction length $(a^2 \delta)^{1/3}/L$ for various values of the scaled penetration depth δ/a . (d) Simulated imaginary part of the QCM force per particle $\Delta \Im(\hat{F})$ as a function of the scaled interaction length $(a^2 \delta)^{1/3}/L$ for various values of the scaled penetration of the scaled interaction length $(a^2 \delta)^{1/3}/L$ for various values of the scaled penetration depth δ/a .

for various fixed values of δa , the simulated inertial force $\Delta \Re(\hat{F})$ and frictional force $\Delta \Im(\hat{F})$ per 270 271 particle are presented as functions of ϕ in **Figs. 5a** and **5b**, respectively. For small ϕ (or large L), there 272 is no hydrodynamic coupling between the particles, and hence the forces are independent of ϕ . With 273 increasing ϕ , the separation between adsorbed particles decreases and hydrodynamic coupling sets in above a critical ϕ^* , as reflected by decreases in $\Delta \Re(\hat{F})$ and $\Delta \Im(\hat{F})$ as functions of ϕ . Since the volume 274 of the coupled fluid per particle increases with increasing δ (cf. Fig. 3a), the critical ϕ^* , which marks 275 276 the onset of hydrodynamic coupling, decreases with increasing δa (cf. Figs. 5a and 5b). As the volume of the coupled fluid scales as $a^2 \delta$ (cf. Fig. 3a), we define the interaction length scale as $\ell \sim$ 277 $(a^2 \delta)^{1/3}$, and equating this length scale with the interparticle distance, $L \sim a \phi^{1/2}$, provides an onset 278

condition for hydrodynamic coupling, $\phi^* \sim (a/\delta)^{2/3}$. To test this scaling hypothesis, plots of $\Delta \Re(\hat{F})$ and $\Delta \Im(\hat{F})$ are presented in **Figs 5c** and **5d**, respectively, as functions of $\ell/L \sim (a^2 \delta/L^3)^{1/3}$ for various δ/a . Hydrodynamic coupling is evident from the decreases in $\Delta \Re(\hat{F})$ and $\Delta \Im(\hat{F})$ that occur beyond $(a^2 \delta/L^3)^{1/3} \approx 0.25$, and this value is independent of δ/a , which validates the proposed scaling, $\ell \sim$ $(a^2 \delta)^{1/3}$.

284 Theoretical Basis for the $\Delta\Gamma/\Delta f$ Extrapolation Method

It is noted that the present simulation is based on the assumption of a regular spatial distribution of particles, and the corresponding hydrodynamic interactions are weaker than in the more realistic case of a random distribution, see, *e.g.*, Refs. [16,20]. However, the qualitative behavior of the hydrodynamic interaction does not depend on the details of the spatial distribution, as demonstrated by the agreement between simulation and experiment in Figs. 3 and 4.

This agreement supports the significance of the simulations for QCM data analysis. Consequently, we now analyze the asymptotic behavior of the simulations when ϕ approaches the saturation density, ϕ_s , and provide a theoretical basis for the $\Delta\Gamma/\Delta f$ extrapolation method to extract particle sizes from QCM data at high surface coverage.²³ We start by recalling that the frequency and bandwidth shifts are proportional to the real and imaginary components, respectively, of the force density, $\phi\Delta\hat{F}$, which is the force per πa^2 surface area of the QCM response. Using $N = A_Q \phi/\pi a^2$, Eq. (1) can be rewritten as:

$$297 \quad -\Delta f + i\Delta\Gamma = \frac{f\phi\Delta\hat{F}}{\pi a^2 l_Q \rho_Q U\omega}.$$
(3)

In **Fig. 6a**, it seen that for $\phi \to \phi_{S}$, the real part of $\phi \Delta \Re(\hat{F})$ approaches a constant that is independent of δ/a , *i.e.*, $\phi \Delta \Re(\hat{F}) \to \chi_R \phi \rho_F \omega U a^3$. This situation resembles the rigid oscillation of a homogeneous film (*cf.* Fig. 2d). **Fig. 6b** shows that the imaginary part of the force density $\phi \Delta \Im(\hat{F}) \to 0$, when $\phi \to \phi_{S}$. If we choose $\phi_{S} \approx 0.7$, then the relation between $\phi \Delta \Im(\hat{F})$ and the void fraction $\phi^2 = \phi_{S} - \phi$ is linear for small ϕ^2 , *i.e.*, $\phi \Delta \Im(\hat{F}) \to \chi_I \rho_F \omega U a^3 \phi'$, which is shown by the straight lines in **Fig. 6b**. Here the proportionality constant χ_I is seen to depend on δ/a . It is noted that the saturation surface coverage,



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FIG 6. (a) Simulated real part of QCM force per πa^2 surface area $\phi \Delta \Re(\hat{F})$ (corresponding to 306 frequency shift), as a function of the void fraction $\phi' = \phi_{\rm S} - \phi$, for various scaled penetration depths 307 δ/a . (b) Simulated imaginary part of QCM force per πa^2 surface area $\phi \Delta \Im(\hat{F})$ (corresponding to 308 dissipation shift), as a function of the void fraction $\phi' = \phi_s - \phi$, for various scaled penetration depths 309 δ/a . The data are fitted by linear functions that intercept the origin when choosing $\phi_{\rm S} \approx 0.7$. (c) 310 311 Simulated ratio of imaginary and real parts of the QCM force as a function of the real part of the force density. In agreement with Eq. (7), the data for different δ/a follow linear trends that intercept the 312 313 horizontal at the same point. (d) Experimental ratio of bandwidth shift $\Delta\Gamma$ and negative frequency shift $-\Delta f$ as a function of negative frequency shift per overtone $-\Delta f/n$ for various overtones 314 (corresponding to various δ/a). The data for the different overtones follow linear trajectories, which 315 316 intercept the horizontal axis at a frequency shift that, according to Eqs. (8) and (9), corresponds to a saturation surface coverage of $\phi_{\rm S} \approx 0.6$. 317 318

and rigid film, is slightly smaller than the value at close packing of the current system, $\phi_{\rm S} \approx 0.8$.

320 Furthermore, it is expected that the value for ϕ_s varies under different experimental conditions,

- 321 including the size distribution and spatial distribution of adsorbed particles. Inserting the above-
- 322 mentioned scaling relations for the real and imaginary parts of $\phi \Delta \hat{F}$ into Eq. (3), we find:

323
$$-\frac{\Delta f}{n} \approx \chi_R \frac{f_0 \rho_F a}{\pi \rho_Q l_Q} (\phi_S - \phi'), \tag{4}$$

324 and

$$325 \qquad \frac{\Delta\Gamma}{n} \approx \chi_I \frac{f_0 \rho_F a}{\pi \rho_Q l_Q} \phi'. \tag{5}$$

326 Dividing Eq. (5) by Eq. (4) and Taylor expanding the result up to the first order in ϕ ':

$$327 \qquad -\frac{\Delta\Gamma}{\Delta f} \approx \frac{\chi_I \phi'}{\chi_R \phi_S},\tag{6}$$

328 and combining Eqs. (4) and (6) yields:

$$329 \qquad -\frac{\Delta\Gamma}{\Delta f} \approx \frac{\chi_I}{\chi_R} \left(1 + \frac{\Delta f \pi \rho_Q l_Q}{n f_0 \chi_R \phi_S \rho_F a} \right),\tag{7}$$

330 *i.e.*, on $(-\Delta f/n, -\Delta \Gamma/\Delta f)$ coordinates, the QCM data appear as a straight line that intercepts the 331 horizontal at:

$$332 \qquad -\frac{\Delta f}{n} = \frac{f_0 \rho_F h}{\rho_Q l_Q},\tag{8}$$

and corresponds to the frequency shift for a uniform layer with a thickness of:

$$334 h = \frac{a\chi_R\phi_S}{\pi}. (9)$$

335 Importantly, this result provides a theoretical basis for the $-\Delta\Gamma/\Delta f$ extrapolation method in order to 336 determine the particle size from QCM data at high surface coverages.^{23,24}

Fig. 6c shows numerical data on $\left[\frac{\phi \Delta \Re(\hat{F})}{\rho_F a^3 \omega U}, \frac{\Delta \Im(\hat{F})}{\Delta \Re(\hat{F})}\right]$ coordinates, which are equivalent (up to some 337 proportionality constants) to the $(-\Delta f/n, -\Delta \Gamma/\Delta f)$ coordinates. On these equivalent coordinates, the 338 339 numerical data for the different overtones (different δ/a values) are observed to follow linear 340 trajectories, which agree well with Eq. (7) and intercept the horizontal at the same point. Interestingly, 341 while the linear relation [Eq. (7)] was derived by assuming $\phi^2 \ll 1$, the numerical data in Fig. 6c follow a linear relationship for nearly the entire domain, especially for relatively large $\delta/a \ge 2$. The 342 343 same is observed in the experimental data in Fig. 6d, where according to Eq. (7), the intercept at - $\Delta f/n \approx 300$ Hz corresponds to a saturation density of $\phi_{\rm S} \approx 0.6$ for the case we have used of a = 38 nm 344 and $\chi_{\rm R} = 7.1$. 345

346 Effect of Particle Polydispersity

347 Let us finally comment on the effect of particle polydispersity. Olsson *et al.* used the $\Delta\Gamma/\Delta f$ 348 extrapolation method to measures the effective film height [*h* in Eq. (8)] of a bimodal mixture of spheres, with radii of $a_1 = 13$ nm and $a_2 = 55$ nm, using various sphere number ratios N_1/N_2 . ²⁵ Their measured, effective particle radius $a_{eff} = h/2$ is well captured by:

351
$$a_{\text{eff}} = \frac{N_1 a_1^3 + N_2 a_2^3}{N_1 a_1^2 + N_2 a_2^2},$$
 (10)

which corresponds to the frequency shift, that one would get in vacuum, *i.e.* proportional to particle volume (numerator) per area (denominator). We generalize Eq. (10) to the case of a continuous particle size distribution N(a):

355
$$a_{\text{eff}} = \frac{\int N(a)a^3 da}{\int N(a)a^2 da},$$
 (11)

and estimate the effect of polydispersity on the effective particle radius, obtained by the $\Delta\Gamma/\Delta f$ extrapolation method, applied to our QCM-D data. The particle radius in our experiment has a mean value of $\bar{a} = 58$ nm and a standard deviation of a' = 7 nm. The particle size distribution (measured by DLS) is log-normal (Supporting Fig. S1). However, when $(a'/\bar{a})^2 \ll 1$, the log-normal is well approximated by the Gaussian:

361
$$N(a) \sim \exp\left[-\frac{(a-\bar{a})^2}{2a'^2}\right].$$
 (12)

Using that $(a'/\bar{a})^2 \approx 0.04 \ll 1$, Eqs. (11) and (12) give an effective particle radius of $a_{\text{eff}} \approx \bar{a}[1 + 2(a'/\bar{a})^2]$, which is estimated to be 8% larger, than the mean value \bar{a} .

364 It is finally noted, that the particle flux *J* from the bulk solution to the QCM-D surface is 365 limited by diffusion,³⁴ and according to boundary layer theory:³⁵ $J \sim a^{-2/3}$. Therefore, the size 366 distribution on the surface $N_s(a)$ differs from that in the bulk N(a):

367
$$N_s(a) \sim N(a) a^{-2/3}$$
. (13)

368 Combining Eqs. (12) and (13), and dropping the (irrelevant) constant terms in the exponent gives:

369
$$N_s(a) \sim \exp\left[-\frac{\left(a - \bar{a}\left[1 - \frac{2}{3}\left(\frac{a'}{\bar{a}}\right)^2\right]\right)^2}{2{a'}^2}\right].$$
 (14)

This means that the surface size distribution has a mean value of: $\bar{a}_s \approx \bar{a}(1 - \frac{2}{3}(a'/\bar{a})^2)$, which is 371 3% smaller than in bulk. Since this effect is small and counteracting the first (~10%) effect, we

- 372 conclude, that in the present QCM-D experiment, effects of polydispersity have a minor effect on the
- 373 effective particle height obtained from the $\Delta\Gamma/\Delta f$ extrapolation method.

374

375 <u>Conclusion</u>

376 We have conducted numerical simulations of the hydrodynamics of spherical particles that are 377 attached to an oscillating surface across a range of surface coverage values, ϕ , and scaled penetration 378 depths, δ/a . The simulations compare well to experimental QCM data of adsorbed gel-phase liposomes (38-nm mean radius), offering a quantitative framework to understand how hydrodynamic 379 380 coupling between adsorbed particles influences QCM measurement responses. In particular, the numerical data show that hydrodynamic coupling sets in beyond a critical surface coverage, $\phi^* \sim$ 381 $(a/\delta)^{2/3}$. The effect of the coupling is a decrease in the contribution per particle to both the frequency 382 383 and bandwidth shifts. Guided by the simulation outcome, and by expanding the frequency and bandwidth shifts in the void fraction (gaps between particles), we derived a theoretical basis for the 384 385 $\Delta\Gamma/\Delta f$ extrapolation method, further validating its utility for extracting particle size from QCM data 386 collected at high surface coverages.

387 Associated Content

- 388 Supporting Information
- 389 Supporting Information contains additional details about the lattice Boltzmann simulations, including
- 390 simulation parameters.
- 391 <u>Notes</u>
- 392 The authors declare no competing financial interest.

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