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Kinetics of formation of bile salt micelles from coarse-grained Langevin Dynamics simulations

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Simulations properly sample the configuration space of the system

As mentioned in the main text, investigating the mechanism(s) of micelle formation requires simulations where the number of fission and fusion events is sufficiently high to allow proper sampling of the configuration space of the system. In the main text we show that we achieve sufficient sampling by comparing the micelle size distribution obtained in our NVT simulations to that obtained using Grand Canonical Langevin Dynamics. Here we show further evidence indicating that our simulations achieve sufficient sampling: we demonstrate that individual molecules sample a wide range of micellar environments and that the reaction rates calculated from the micelle size distribution are identical to those directly measured in the simulation.

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Individual molecules sample a wide range of micellar environments

We assess whether molecules can sample a wide range of micellar environments by calculating the mean square displacement in aggregation number space, $\langle(\Delta n(t))^2\rangle$. This quantity is defined as

$$\left\langle(\Delta n(t))^2\right\rangle = \left\langle(n_i(t) - n_i(0))^2\right\rangle \qquad (1)$$

where $n_i(0)$ and $n_i(t)$ are the aggregation numbers at time 0 or t for the micelle to which molecule i belongs ($i = 1 \cdots N_{mol}$), and the average is over all molecules in the system and all time origins. In Figure 1 we show $\langle(\Delta n(t))^2\rangle$ for the lowest and highest concentration tested. For both concentrations, $\langle(\Delta n(t))^2\rangle$ saturates for times $\approx 10000\tau$. This saturation behavior is expected because we are performing simulations with a constant number of particles. As we demonstrate below, the mean square displacement of a particle diffusing freely in a 1-dimensional domain of length L should saturate to

$$\lim_{t\to\infty}\left\langle(\Delta x(t))^2\right\rangle = L^2/6 \qquad (2)$$

$$\lim_{t\to\infty}\left\langle(\Delta x(t))^2\right\rangle = 2\mathrm{Var}[x] \qquad (3)$$

where $\mathrm{Var}[x] = \langle(x - \langle x\rangle)^2\rangle$ is the variance of the position, x. Equation 2 does not strictly apply in our case because the probability distribution of micellar sizes is not uniform but, despite this limitation, we can use it together

with the saturation levels shown in Figure 1 to estimate an effective length, Δn_{max}, of the one-dimensional domain in n at both concentrations. We find that $\langle(\Delta n(t))^2\rangle$ indeed converges to a value that is close to 2Var[n], which provides further evidence that the positions, n, and displacements, Δn, are well-sampled; applying equation 2 we obtain $\Delta n_{max,1.8\mathrm{CMC}} = 12$ and $\Delta n_{max,18\mathrm{CMC}} = 25$. These values are almost identical to the maximum aggregate sizes that can be found with significant probability ($P \geq 0.001$) at each concentration. This result indicates that the simulations are long enough to allow each molecule to sample the full range of the most relevant micellar environments.

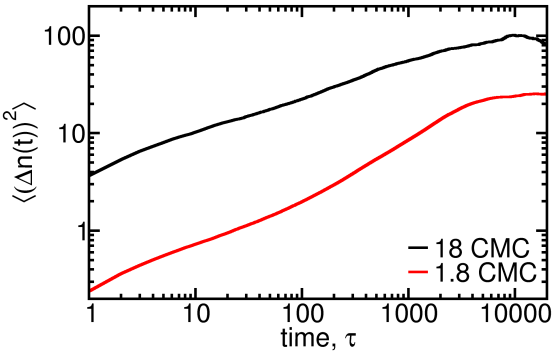

Figure 1: Mean square displacement in micellar size space, $\langle(\Delta n(t))^2\rangle$, at the lowest and highest concentrations investigated here.

The fission rate calculated from the micelle size distribution is identical to that directly measured in the simulation

The rate of fission of monomers or fragments of size m from micelles of size n can be calculated from the concentrations (C) and the fusion rate (k_+) as

$$k_- = \frac{C_n C_{n+m}}{C_m} k_+ \qquad (4)$$

In Figure 2 we compare the rates of fission of monomers, dimers or trimers from micelles calculated using Equation 4 against those directly measured from the simulations at 8.9 CMC. The rates coincide for all micellar sizes except those with very low probability of occurrence, indicating that the statistical uncertainty associated with k_- and k_+ is very low, and that our simulations may be used to gain insight into fusion and fission of oligomers to/from micelles.

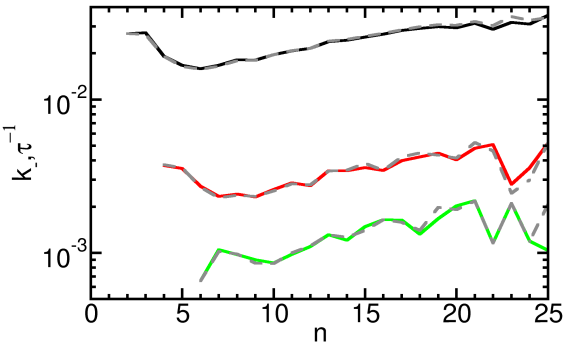

Figure 2: Rates of fission of monomers, dimers or trimers, at 8.9 CMC, directly measured from the simulation (black=monomers, red=dimers and green=trimers) or calculated from Equation 4 (grey lines). The calculated rates coincide with those directly measured from the simulation.

Dependence of rate constants on micelle size and total bile salt concentration

Rate constants depend only weakly on concentration

In models of micellar kinetics it is often assumed that the rate constants of fission and fusion are independent of total micelle concentration and, in the region of proper micelles, are independent of micelle size[1–3]. To investigate whether these assumptions hold for the case of bile salts, in Figure 3 we compare the rate constants of fusion and fission of monomers to/from micelles for different micelle sizes and bile salt concentrations. We find that monomer fission and fusion rate constants do not depend strongly on concentration. The fission rate constants at 18 CMC differ from those at 1.8 CMC

by a factor lower than 2, and the monomer fusion rates at the same concentrations may differ by a factor lower than 3. Qualitatively similar trends have also been found in simulations of model head-tail surfactants[4] performed at the CMC and at 3 CMC. Our results clearly show that assuming that rate constants are independent of concentration is a reasonable first approximation at least up a concentration of 18 CMC.

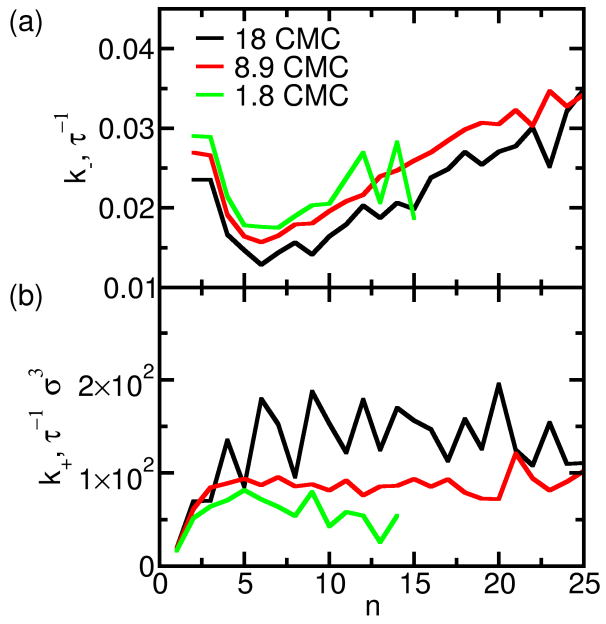

Figure 3: Rate constants for (a) fission and (b) fusion of monomers at different total bile salt concentrations. Each curve shows the fission or fusion rate constants as a function of the size n of the initial micelle.

Fusion rates are independent of micelle size

To investigate the dependence of the monomer fission and fusion rates on the micelle size, we examine the data shown in Figure 3 for 8.9 CMC only because it has the lowest statistical uncertainty. The trends seen here for pure bile micelles are qualitatively similar to those seen in simulations of model head-tail surfactants[4], again demonstrating that bile salts follow similar micellar kinetics to those observed for those surfactants. For all but the smallest aggregates (with $n < 3$), the fusion rates are independent of the size of the initial micelle. The rates of monomer fusion with other monomers or with dimers, however, are markedly lower than those observed for larger aggregates, for reasons that are not yet fully understood. The low rates of monomer fusion with other monomers or with dimers may arise because of the presence of a free energy barrier associated with the orientation of the incoming monomer relative to the monomer or the dimer with which it will merge: our prior results[5] show that bile salt molecules forming dimers and trimers have a marked preference for particular relative orientations; in contrast, neighboring bile molecules in larger aggregates have much lower preferences for particular relative orientations, consistent with their higher monomer fusion rates. The lower rates of fusion of monomers to other monomers or small oligomers may also have a contribution from differences in the radius and the diffusion coefficients between monomers, oligomers and micelles. These differences lead to smaller rates of barrierless fusion, as estimated using the Smoluchowsky model[6], for fusion of monomers to oligomers as compared to fusion of monomers to larger micelles.

Fission rates depend on micelle size

Contrary to what we observe for monomer fusion, the rates of monomer fission depend on micelle size in the entire range of micellar sizes observed in the simulations: Figure 3 demonstrates that the rates are large for dimers and trimers, decrease for aggregates of size 4, 5 and 6, and then again increase with increasing micellar size. This dependence is consistent with the micelle size distribution shown in the main text: a non-uniform micelle size distribution only arises when at least one of the rate constants is size-dependent.

For micelles with aggregation numbers $n > 6$, the monomer fission rate increases linearly with increasing n. Such a linear dependence is consistent with a free energy barrier, $\Delta G^{\ddagger}$, associ-

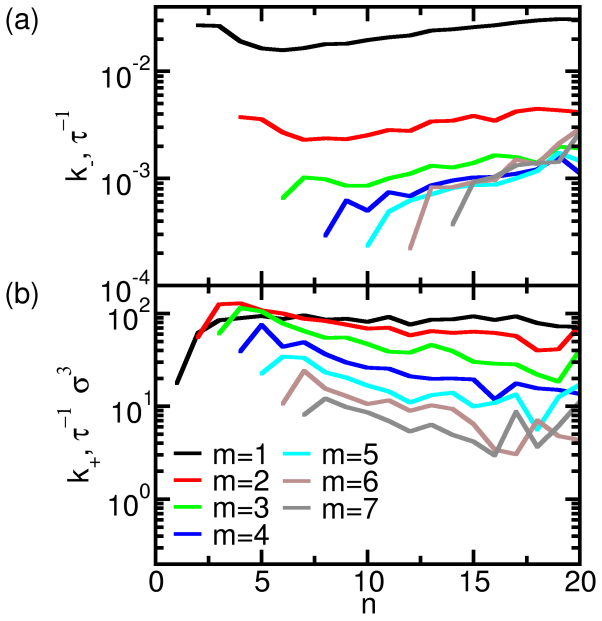

Figure 4: Rate constants for (a) fission and (b) fusion of bile salt fragments of different sizes ($m = 1, 2, \cdots, 7$, respectively) at 8.9 CMC, as a function of the size n of the initial bile micelle.

ated with monomer fission which is independent of micelle size. In these conditions, the theoretical expression for the rate of monomer removal from a micelle with aggregation number n is[3]

$$k_- = \frac{n}{\tau_d} \exp\left(-\beta \Delta G^{\ddagger}\right) \qquad (5)$$

In this expression, τ_d is the characteristic time for diffusional motion over a length scale associated with the free energy barrier for monomer fission. Equation 5 makes obvious that, if the free energy barrier to monomer removal is independent of micelle size, then the probability per unit time that monomers depart is simply proportional to the number of molecules composing the micelle. Equation 5 yields monomer fission rates less than one order of magnitude lower than those shown in Figure 3 (calculation not shown), i.e., it yields reasonable but rough estimates of monomer fission rates.

It would at first sight appear, then, that the models of micellar kinetics developed by Aniansson et al.[1–3], that rely on the assumption that the fission and fusion rates are independent of micelle size, are incorrect, because they would not recover the correct distribution of aggregation numbers. This conflict is only apparent, because in these models the shape of this distribution is given as input. Figure 3 makes clear that the monomer fission rates remain within the same order of magnitude within the entire region of proper micelles, so assuming that these rates are independent of micelle size when applying the models of Aniansson et al. to the interpretation of chemical relaxations of pure bile micelles is reasonable.

Rate constants of fission and fusion of fragments

In the main text we show that fission and fusion of fragments with $m \geq 2$ are still present to an appreciable extent in bile salt solutions, and suggest that it may be advisable to explicitly consider these events when interpreting experimental data at higher concentrations, where the contribution of these events to micellar kinetics is larger. In Figure 4 we show the dependence of fission and fusion rate constants on both the size of the micelle and the size of the fragment at a concentration of 8.9 CMC, the concentration for which the statistical uncertainty of the data is the lowest. In the region of proper micelles ($n > 6$), the rates of fission of dimers from micelles are one order of magnitude lower than the fission rates of monomers, and the rates of fission of trimers and other fragments are very similar to each other but lower than those of dimers by a factor of ≈ 5: for example, for $n = 15$, the fission rates for monomers, dimers and trimers are 0.026, 0.0038 and $0.0014\tau^{-1}$. Because the rates of fission of oligomers from micelles depend linearly on the size of the micelle, similarly to the rates of monomer fission, we can assume that equation 5 holds also for fission of fragments. Making the simplifying assumption that the timescale τ_d associated with fission of oligomers is similar to that for monomer fission, it follows that the free energy barrier to fission of oligomers should be $2-3k_BT$ higher than for monomers. Below we show free energy calculations that support this scenario.

The rates of oligomer fusion to proper micelles decrease visibly with increasing size n of the micelle (for $n > 3$), the decrease being more marked for larger sizes, m, of the incoming fragment. This dependence contrasts markedly with the independence of the rate of monomer fusion to micelles on the micelle size. For oligomers with $m < 5$ these rate constants are, at most, one order of magnitude lower than the rate constant of monomer fusion to micelles, which suggests that the barrier for oligomer fusion is, at most, $\approx 2k_{\rm B}T$ higher than that for monomer fusion. Our free energy calculations, presented below, suggest that the decrease in fusion rates with increasing size of micelle and incoming fragment may have two origins.

Comparing free energy profiles of monomers, dimers and trimers

Figure 5 shows the free energy profiles as a function of the distance r between the centers of mass of micelles and small fragments at 8.9 CMC total bile salt concentration. The fusion of monomers, dimers or trimers to proper micelles appears to be essentially barrierless: the free energy difference between $r = 10\sigma$ and the maximum in $A(r)$ (at $r = 5-7\sigma$, depending on m) is of order $1 - 2k_{\rm B}T$ in all cases and increases only slightly with increasing size of the incoming fragment. However, above we show that the rate constants of fusion of dimers or trimers to micelles are lower than the rates of monomer fusion. The decrease in the rate constant of fusion of small fragments to large micelles we observe is not yet fully understood. This decrease is qualitatively consistent with the predicted dependence of the rate constants of fusion of oligomers of increasing size to large micelles using the Smoluchowsky model[6], but it is also possible that a second reaction coordinate, related to the reorganization of micelle structure or to the relative orientation of micelles and oligomers, is necessary to explain the origin of the differences between the fusion rates of monomers and oligomers to micelles. Simulations using simplified models of surfactants with the typical head-tail configuration show that fusion of two micelles is an activated process, because of the large rearrangements that micelles must undergo in order to merge, and that the free energy barrier to fusion increases with increasing micelle size[7]. It seems possible that a similar molecular scale mechanism is behind the dependence of the rate constants of oligomer fusion on the size of the micelle and on the size of the oligomer. In any case, and similarly to what occurs for head-tail surfactants, reaction coordinates related to micellar structure or the relative orientation between bile micelles and oligomers are expected to play a small role only, because (i) the rates we measure are large compared to the fusion rate for barrierless attachment estimated from the Smoluchowsky model, and (ii) the rates of fusion of dimers and trimers to micelles are still of the same order of magnitude as the rates of fusion of monomers.

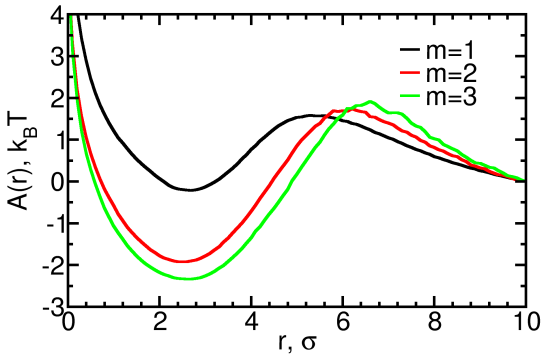

Figure 5: Free energy, $A(r)$, as a function of the distance r between micelles of size $n = 9 - 13$ and monomers, dimers or trimers ($m = 1, 2, 3$, respectively) at a total bile salt concentration of 8.9 CMC. Curves are shifted so that $A(r = 10) = 0$, for ease of viewing.

The magnitude of the free energy barrier associated with fission of oligomers from large micelles increases markedly with increasing size of the fissioned oligomer, and fission of dimers and trimers from micelles is clearly not barrierless: for example, fission of a trimer from a micelle is associated with a free energy barrier of $A(r = 6.5\sigma) - A(r = 2.5\sigma) = 4k_{\rm B}T$, whereas fission of a monomer is associated with a bar-

rier of $A(r = 5.5\sigma) - A(r = 2.5\sigma) \approx 2k_BT$. This difference in free energy barriers is quantitatively consistent with the rates of fission of trimers being ≈ 20 times smaller than those of fission of monomers, indicating that fission of oligomers can be well-described by the reaction coordinate r only.

Deriving equations 2 and 3

Consider a particle diffusing freely in a 1-dimensional domain of length L. The probability density of finding the particle at any position, x, at time t given that it was initially at position x' is denoted by $P(x, x', t)$. The mean square displacement, $\langle(\Delta x(t))^2\rangle$ of the particle is given by

$$\left\langle(\Delta x(t))^2\right\rangle = \frac{1}{L}\int_0^L \int_0^L P(x, x', t)(x-x')^2 dx dx' \tag{6}$$

and the variance, $\mathrm{Var}[x]$, of the position of the particle by

$$\mathrm{Var}[x] = \int_0^L P(x, \langle x\rangle, t)(x - \langle x\rangle)^2 dx \tag{7}$$

The average position of the particle is $\langle x\rangle = L/2$; $P(x, \langle x\rangle, t)$ is the probability of finding the particle in x given that it was initially at $L/2$. For long times[8],

$$\lim_{t\to\infty} P(x, x', t) = \lim_{t\to\infty} P(x, \langle x\rangle, t) = 1/L, \tag{8}$$

and

$$\left\langle(\Delta x(t))^2\right\rangle = L^2/6 = 2\mathrm{Var}[x] \tag{9}$$

We note that the equality $\langle(\Delta x(t))^2\rangle = 2\mathrm{Var}[x]$ was derived here for a uniform probability distribution, but is generally valid for 1-dimensional probability distributions.

In our case, $\langle(\Delta n(t))^2\rangle$ denotes a mean square displacement weighed by mass, so that

$$\left\langle(\Delta n(t))^2\right\rangle = 2\mathrm{Var}[n] = 2\left(\left\langle n^2\right\rangle - \langle n\rangle^2\right) \tag{10}$$

where

$$\langle n\rangle = \frac{1}{N_{mol}}\sum_{i=1}^{N_{mol}} n_i \tag{11}$$

and n_i is the aggregation number for the micelle to which molecule i belongs. Equation 10 is equivalent to

$$\left\langle(\Delta n(t))^2\right\rangle = 2\left(\frac{\langle n^3\rangle_{mic}}{\langle n\rangle_{mic}} - \left(\frac{\langle n^2\rangle_{mic}}{\langle n\rangle_{mic}}\right)^2\right) \tag{12}$$

where

$$\langle n\rangle_{mic} = \frac{\sum_{j=1}^{M} N_j j}{\sum_{j=1}^{M} N_j} \tag{13}$$

M is the maximum micellar aggregation number in the simulation, and N_j is the number of micelles of aggregation number j.

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