Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Figures and Discussion

The Impact of Physiological Crowding on the Diffusivity of Membrane Bound Proteins

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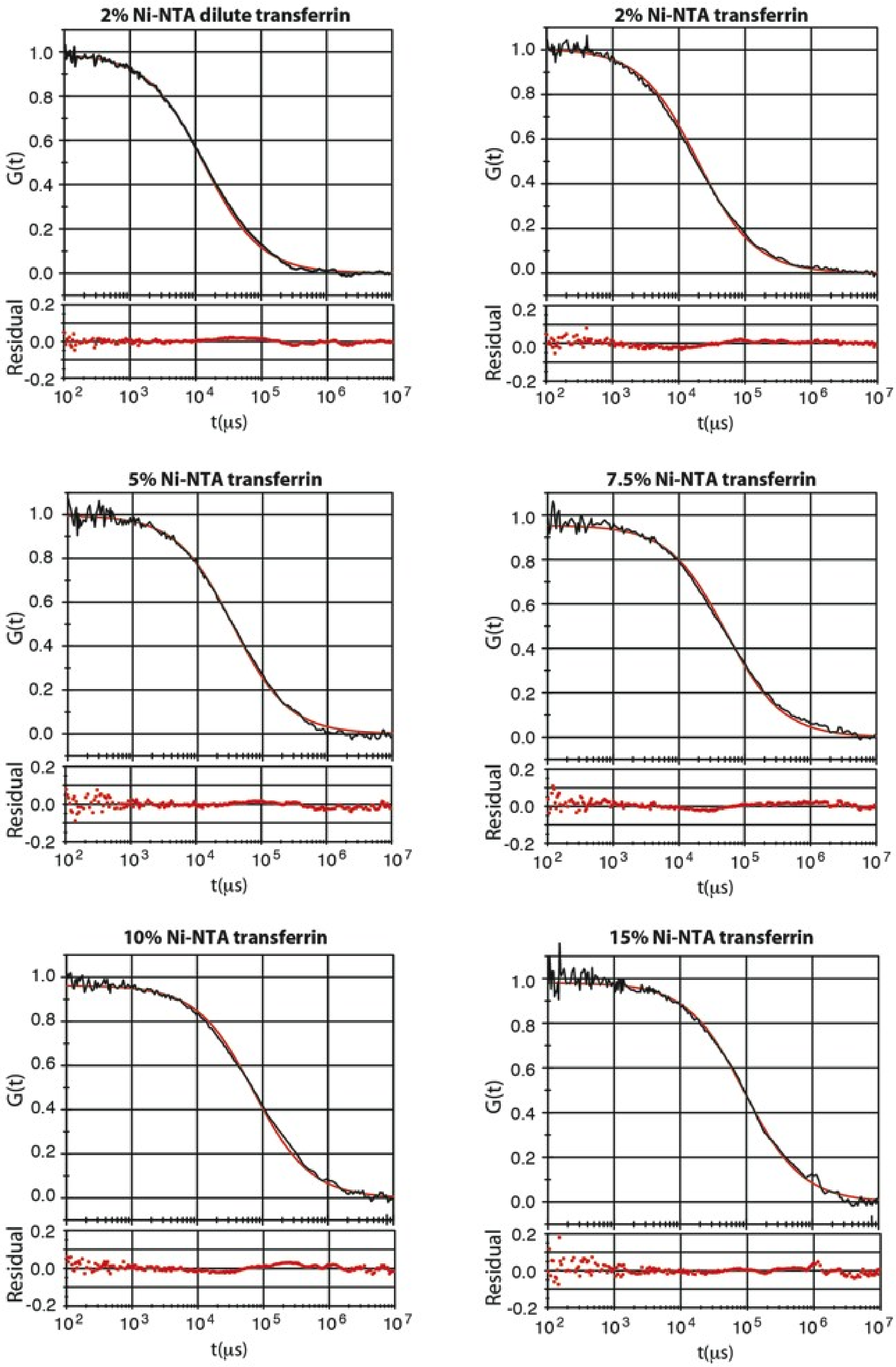
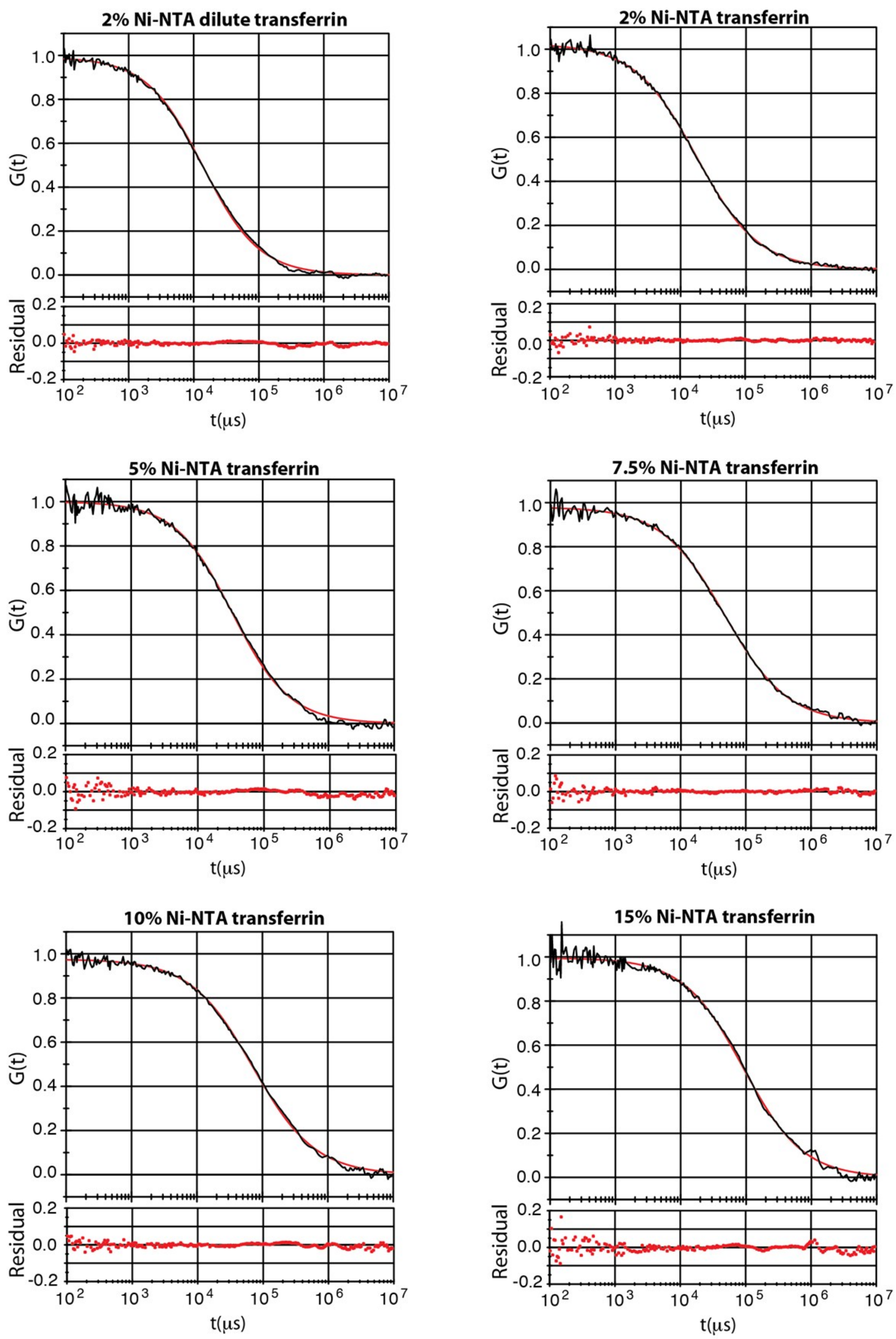


Supplementary Figure S1. Example fitting of the standard 2D autocorrelation function (equation S1, red) to experimental autocorrelation curves, black. Membranes contained increasing concentrations of Ni-NTA-DOGS lipids and were crowded by membrane-bound transferrin proteins.



Supplementary Figure S2. Example fitting of the anomalous 2D autocorrelation function (equation S2, red) to experimental autocorrelation curves, black. Membranes contained increasing concentrations of Ni-NTA-DOGS lipids and were crowded by membrane-bound transferrin proteins.

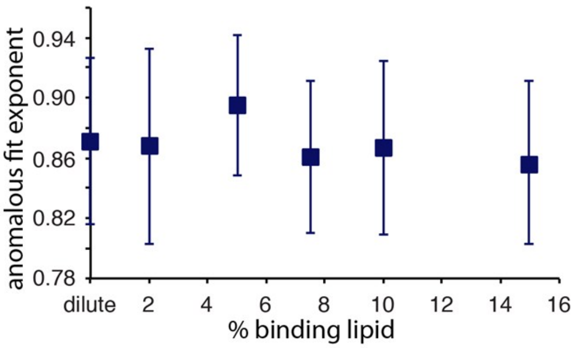
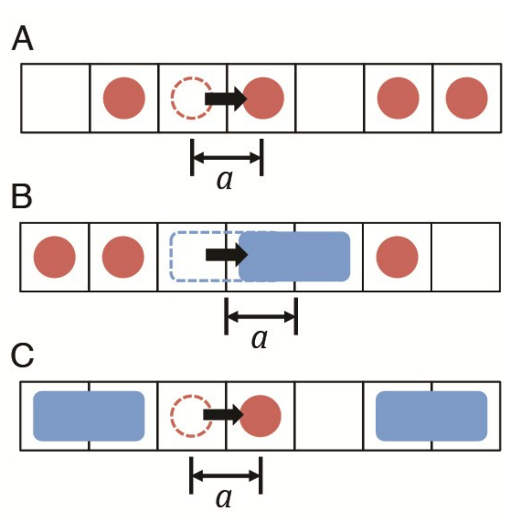


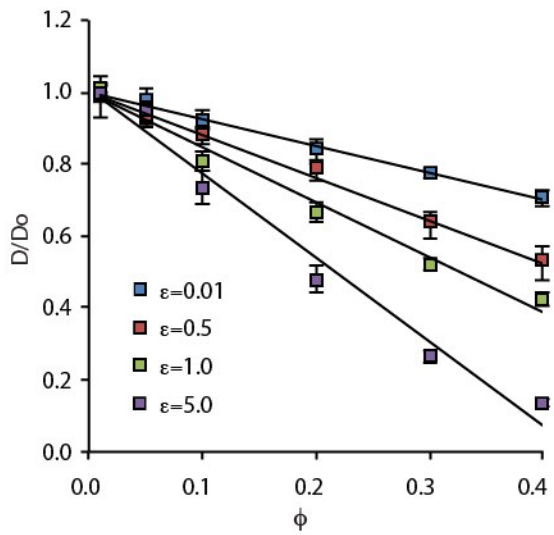
Figure S3. The anomalous diffusion exponent, γ, as a function of the concentration of protein binding sites. These data come from fits to FCS curves for membrane-bound, fdiffusing transferrin proteins.

Steric exclusion model of the influence of protein crowding on membrane protein diffusion.

A simple Boltzmann lattice model predicts that the diffusivity of non-interacting spherical particles over length scales much longer than the molecular size will decrease linearly with increasing molecular coverage [1]. Specifically, for a one dimensional lattice, which can be generalized to two or three dimensions, a molecule at a given position within the lattice can either move to the left or right by a step size, a, or stay in its present position at each time step. Steps to the left and right are equally probable. Under dilute conditions, these probabilities are each ½, and the probability of staying in place is zero for sufficiently long time steps. However, if the space to either the left or right of the particle is occupied, the particle cannot move in these directions and the probability of staying in place increases accordingly. For a given value of coverage, ϕ, the probability of moving to the left and right are each $\frac{1}{2}(1-\alpha\phi)$, and the probability of staying in place is ϕ, where α is a constant, approximately equal to 2 for 2D diffusion[2]. Accordingly, the root mean squared displacement of each particle is simply the displacement associated with each possible outcome of the time step multiplied by the probability of that outcome. Since the displacement associated with staying in place is zero, the root mean squared displacement is simply $\langle x^2 \rangle = a^2(1 - \alpha\phi)$. Under dilute conditions ϕ approaches zero such that the ratio of the root mean squared displacements under crowded and dilute conditions is $\frac{\langle x^2 \rangle}{\langle x^2 \rangle_0} = \frac{a^2(1 - \alpha\phi)}{a^2} = \frac{D}{D_0}$, which is equal to the ratio of crowded and dilute diffusivities. The step size cancels in this expression yielding the following expression for the crowded diffusivity as a function of the dilute diffusivity and ϕ, $D = D_0(1 - \alpha\phi)$. Notably, the probability that a tracer protein will make a step at each time interval is independent of the size of both the tracer and surrounding crowders, depending only on the overall coverage of the membrane surface. This can be explained as seen in Supplementary Fig. S2. When the lattice step size is governed by the smallest particle, size a, both the large and small particles can diffuse over the same step size a. The probability of moving into a left or right adjacent lattice position remains p=0.5(1 – ϕ), for constant ϕ, regardless of crowder and tracer size, provided all particles have the same diffusive rate under dilute conditions.



Supplementary Figure S3. Cartoon representation of diffusion along a 1-D lattice, adapted from [1]. (A) Homogeneous diffusion of equally-sized tracer and crowder particles, with a diffusion step size of *a*, the lattice size of the particle. (B) Diffusion of a large tracer particle among small crowders. Note that the step size is still *a*, the lattice size of the smaller crowder particle. (C) Diffusion of a small tracer particle among large crowders.



Supplementary Figure S4. Diffusivity calculated from MD simulations as a function of molecular coverage ϕ for a range of ε (Lennard-Jones potential minimum energy well depth) values, in units of kcal per mole. Lines represent linear best fits.

SUPPLEMENTARY REFERENCES

1. Phillips, R., Kondev, J. & Theriot, J. *Physical biology of the cell.* 487-498 (Garland Science, 2009).
2. Ackerson, B. J. & Fleishman, L. Correlations for Dilute Hard-Core Suspensions. *Journal of Chemical Physics* **76**, 2675-2679, (1982).