Supporting information to manuscript:

Nonspecific Protein Adsorption at the Single Molecule Level Studied by Atomic Force Microscopy

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Experimental Section.

Ferritin (25 mg/ml in 150 mM NaCl, 0.1% NaN_3, chromatographically purified, purity:> 90%) from equine spleen was purchased form Merck Cellbiochem (order no.: 341476) and used as received.

Sample preparation. For atomic force measurements ultraflat gold surfaces were prepared by template stripping according to the procedure of Hegner and Wagner et al.[1]. A 60 µl droplet of buffer (50 mM HEPES, 10 mM NaCl, 1 mM EDTA pH 7,5) was placed on a freshly prepared gold surface and mounted to the AFM liquid cell. Ferritin was added to the buffer droplet using a solution of 25 µg/ml in the same buffer with a 25 µl Hamilton microliter syringe.

Atomic Force Microscopy. For AFM measurements a Nanoscope IV multimode instrument (Veeco/digital instruments, Santa Barbara, California) equipped with a 12 µm scanner (E scanner) was used. Tapping in liquid was performed in the buffer droplet in a tapping-mode liquid cell. Narrow-legged cantilevers (OMCL-TR400PSA, Olympus Ltd., Tokyo, Japan) with oxide sharpened Si_3N_4 tips were used. These 100 µm long V-shaped cantilevers have nominal spring constants of 80 pN/nm. Cantilevers were driven at resonance frequencies of 8.4 ± 0.5 kHz with piezo drive amplitudes of 50-100 mV resulting in cantilever amplitudes of ca. 0.5 V. Scanning was performed at a speed of 1.606 Hz. (ca. 320 s per image).

Image processing (2^{nd} order flattening) and data analysis (particle analysis) was performed with the Nanoscope software version 5.12r5. ImageJ (1.37v) was used to count the number of

particles and extract the x-y positions of particle centres in each image. The real surface coverages were calculated by multiplying the number of particles with the projected area of a ferritin molecule of 13x13 nm² and related to the total surface area of 3x3 µm².

The radial distribution function (RDF) was calculated by counting the number of particles in circular shells around each particle. The RDF was normalized by dividing the number of found particles in each shell by the number expected in that shell in case of a uniform distribution. The x-axis gives r/a (distance between particles divided by particle radius (taken to be 6.5 nm). Y axis gives the RDF function.

The 2-dimensional radial distribution function, g(r), is given by:

$$g(r) = \frac{N(r - \frac{1}{2}w, r + \frac{1}{2}w) \cdot A_{total}}{2\pi \cdot r \cdot w \cdot N_{total}}$$

Where N(r-½w, r+½w) is the number of particles found in a circular strip with width w and central radius r, and A_{total} and N_{total} are the total number of particles in the image and the total surface of the image respectively.

(1) (a) M. Hegner, P. Wagner, and G. Semenza, Surf. Sci., 1993, 291, 39;

(b) P. Wagner, M. Hegner, H.-J. Güntherodt and G. Semenza, Langmuir, 1995, 11, 3867.

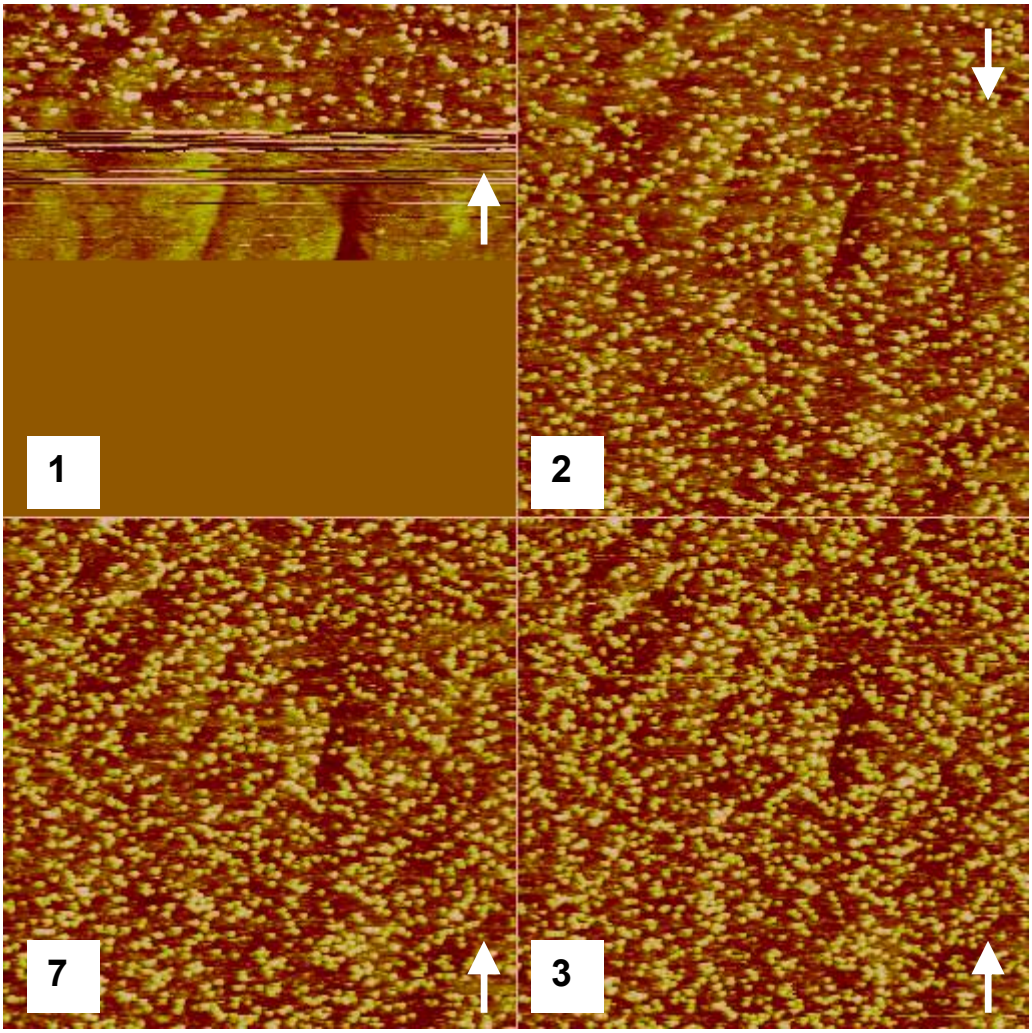

Figure 1 : images no 1, 2, 3 and 7 of a consecutive series of 8 AFM topographic images obtained upon addition of ferritin solution (final concentration 3,6 µg/ml). Scan rate: 1.606 Hz, frame size: 3 x 3µm², z = 15 nm; white arrows indicate scan directions. Distinct horizontal lines in 1 are disturbances of the topographic signal during the interval of protein addition, mixing and stabilization of the measurement signal.

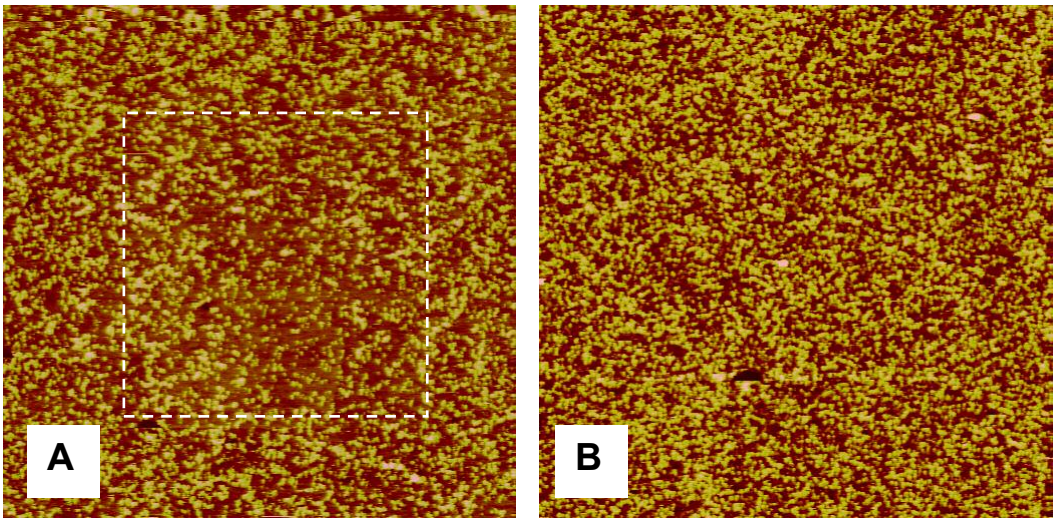

Figure 2: topographic images obtained after the initial protein adsorption/measurement experiment, A: zoom out after the initial ferritin adsorption experiment, observing a larger 5x5 µm² area, B: 5x5 µm² image at a different location, ~ca. 1 mm away from the original area. z-scales: 10 nm; dashed square indicates scanning area during adsorption experiment.

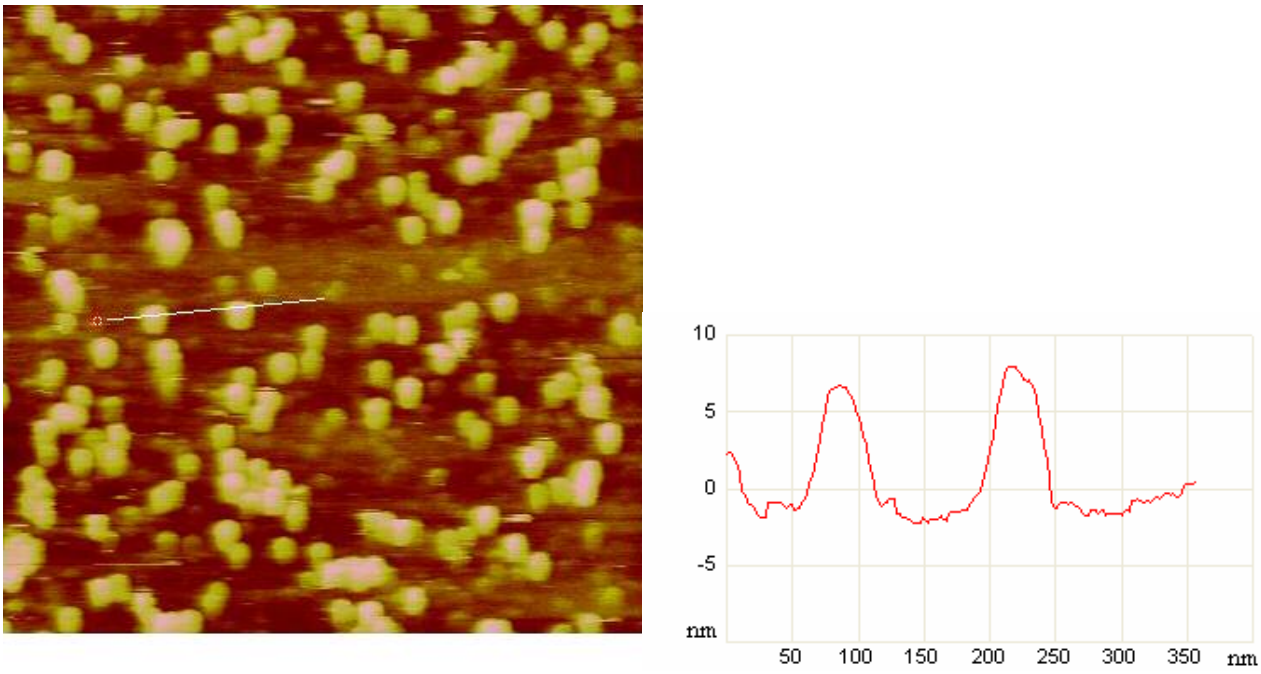

Figure 3: AFM topographic image (1x1 µm², z-scale= 10nm) and corresponding cross-section indicating significant broadening of ferritin molecules to an apparent diameter of ca. 50 nm.

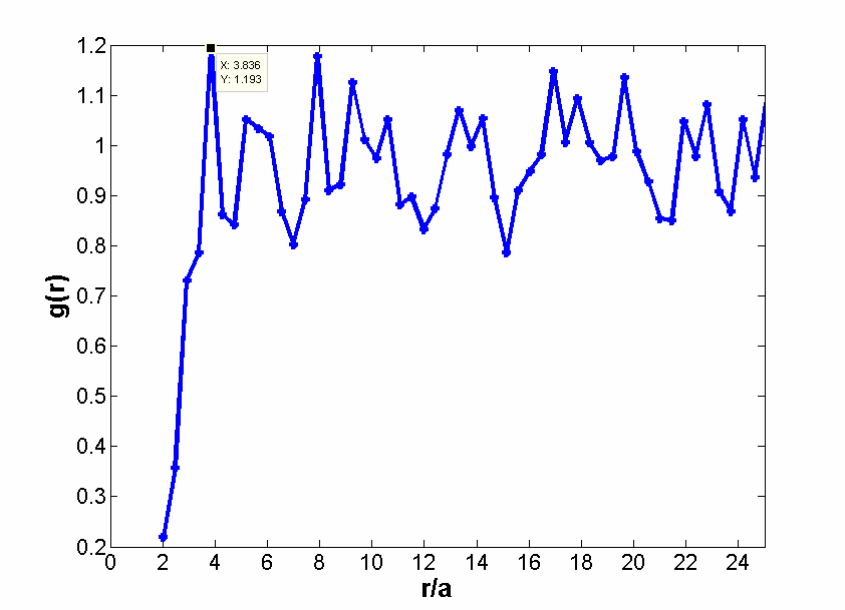

Figure 4: Radial distribution function (RDF) as obtained for an AFM topographic image at saturation coverage at a ferritin solution concentration of 3,6 µg/ml; based on the counted large particles

Description to Movie Files

The adsorption process can be studied in situ at single molecule resolution.

Movie 1 shows the adsorption at low ferritin concentration (0.36 µg/ml) After obtaining stable imaging conditions in the absence of ferritin, a small volume of protein stock solution was administered to the buffer droplet using a Hamilton microliter syringe. After circa 1 min the first protrusion was detected in the upper right, which we interpret as a single adsorbed protein molecule. The number of protrusions continuously increased with scanning time. In total a number of 11 topograhic images were collected at a protein concentration of 0.36 µg/ml until no significant increase in adsorption could be observed. Then protein concentration was increased to 3.6 µg/ml.

Movie 2 shows the adsorption at high ferritin concentration (3.6 µg/ml). After obtaining stable imaging conditions on bare gold ferritin was administered to the liquid droplet yielding a solution concentration of 3.6 µg/ml.

Movie 3 shows a switching between the topographic images no 9 and no 10 of the experiment shown in Figure 1 and Movie 1 respectively, with indications of different rare events observed; circles. white: dissociation, black: lateral movement, green: desorption.

Movie 4 shows an individual moving molecule, to be observed in-between two consecutive scans, taken from the experiment shown in Figure 1 and Movie 1; indicated with a black circle.