scopic single-crystal planes where waves triggered from the boundaries will die out within short (~1 μm) distances.

It has to be emphasized that the phenomena reported here are fundamentally different from the concepts of 'spillover' or 'remote control' as frequently discussed in heterogeneous catalysis, where reacting species are supplied to the active centres by diffusion from other (more inactive) parts of the surface. With the present system, chemisorbed oxygen atoms would, at 300 K, not be mobile enough to account for the observed changes of the surface concentrations. By contrast it is the local coupling between diffusion and reaction that gives rise to rapid propagation of reaction fronts. The propagation speed of such 'chemical waves' is, to a first approximation, determined by the product of a diffusion coefficent with an effective rate constant[15,21,22].

The present findings are of essential relevance to understanding heterogeneous catalysis. They indicate that the catalytic properties of a small particle exhibiting various crystal planes of restricted dimensions (typically < 10 nm) cannot simply be regarded as a superpostion of contributions from its individual parts. □

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Counting polymers moving through a single ion channel

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THE change in conductance of a small electrolyte-filled capillary owing to the passage of sub-micrometre-sized particles has long been used for particle counting and sizing. A commercial device for such measurements, the Coulter counter, is able to detect particles of sizes down to several tenths of a micrometre[1–3]. Nuclepore technology (in which pores are etched particle tracks) has extended the lower limit of size detection to 60-nm particles by using a capillary of diameter 0.45 μm (ref. 4). Here we show that natural channel-forming peptides incorporated into a bilayer lipid membrane can be used to detect the passage of single molecules with gyration radii as small as 5–15 Å. From our experiments with alamethicin pores we infer both the average number and the diffusion coefficients of poly(ethylene glycol) molecules in the pore. Our approach provides a means of observing the statistics and mechanics of flexible polymers moving within the confines of precisely defined single-molecule structures.

The underlying idea of our experiment is very similar to the resistive pulse principle that has been used in Coulter counters since 1953. If a nonconducting particle suspended in a conducting medium moves into a small capillary, it decreases the conductance of the capillary relative to that of the capillary filled with the conducting medium alone. The magnitude of this decrease in conductance is related to particle size (ref. 3).

For a molecular pore of ~50 Å length and ~10 Å radius (similar to alamethicin pore dimensions[5,6]), the brownian motion of particles dominates over directional flow at all reasonable hydrostatic pressure differences across the membrane. Estimates for flow transient time and diffusion relaxation time (see below) show that for 5-Å-radius particles in water these times become equal only at hydrostatic pressure differences of $\sim 10^8$ Pa, equivalent to a 10-km-high water column.

To study diffusion-driven exchange of poly(ethylene glycol)s (PEGs) of different relative molecular masses (M_r) in a molecular pore, we analysed polymer-induced conductance fluctuations of a single alamethicin channel[7] in the experiment schematically shown in Fig. 1. Addition of polymers to the membrane-bathing solution changes the conductance of the pore-containing membrane: the amplitudes of conductance states decrease and the current noise of the open channel increases. Figure 2 illustrates these changes for three states of the alamethicin channel, comparing spontaneous current "bursts" in polymer-free solution with those recorded in solutions of 15 wt% PEG with M_r 600 (PEG 600).

Figure 3 shows that the magnitude of polymer-generated excess noise is different for different conductance levels and

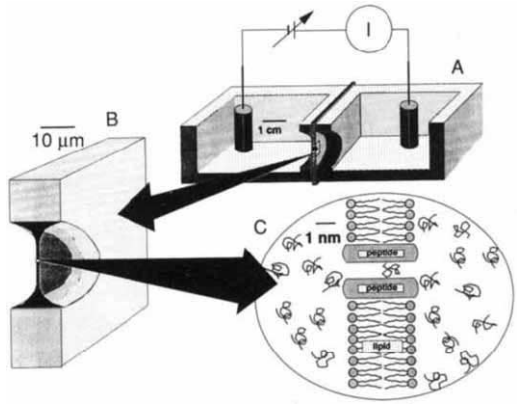

FIG. 1 Schematic representation of the cell (A), Teflon partition with a lipid bilayer (B), and a single ion pore exchanging polymers with bulk solution (C). Bilayer lipid membranes were prepared as described by Montal and Mueller[18]. The membrane-forming solution was L-α-diphytanoyl lecithin in *n*-pentane. Hexadecane in *n*-pentane (1:10) was used for aperture pre-treatment. Natural alamethicin, purified as described elsewhere[19], was added as an ethanolic solution (after membrane formation) to the bathing solution from one side of the membrane only. Single-channel spontaneous insertions were detected as current 'bursts' shown in Fig. 2. PEGs of different M_r were added at 15% w/w concentration to aqueous 1 M NaCl solutions buffered at pH 6.2 by MES. All measurements were made at room temperature, at a transmembrane voltage of +150 mV on the side of peptide addition.

depends on polymer M_r. Noise is not monotonic in polymer size. Rather, there is a clear maximum for M_r 600–1,000. Small polymers (M_r 200–400) produce relatively less noise, presumably because their effect is an average over a large number of small events. Large polymers ($M_r > 1{,}000$) are geometrically restricted from even entering the channel.

The average number of polymer molecules in the channel, $\langle N(w)\rangle$, can be determined from the degree of channel conductance reduction on addition of polymer[5,8]. If the entry of a single polymer of M_r w lowers single-channel conductance by an amount $h(w)$, and if one is working at a level of occupancy $\langle N(w)\rangle$ such that $h(w)\langle N(w)\rangle$ is much smaller than the mean conductance of polymer-free channel, one can write

$$h(w)\langle N(w)\rangle = h_{ch}(\infty) - h_{ch}(w)$$

Here $h_{ch}(w)$ is the mean conductance of the channel in the presence of polymers of M_r w, and $h_{ch}(\infty)$ its conductance in the presence of totally excluded, large polymers. These average conductances are measured directly (see Fig. 2).

We have verified previously[5] that the relative reduction of alamethicin channel conductance in the presence of small, easily penetrating PEG 200 is close to the relative reduction in the conductivity of bulk electrolyte solutions on polymer addition. This was also shown for another mesoscopic channel formed by staphylococcal α-toxin[9]. Taking into account the large size of these channels, this means that in the case of small polymers their incremental (negative) contribution to channel conductance is close to the contribution that one would expect for the conductance of an electrolyte layer whose thickness is equal to the channel length. This equivalence permits a straightforward derivation of $h(w)$ from the polymer-induced reduction in solution conductivity. As polymer size is increased, the channel starts to exclude polymers; the proportional effect of polymer addition on channel conductance decreases[5]. As long as the size of a polymer coil does not significantly exceed the size of the pore, and thus its shape and monomer density are not heavily distorted by the channel, $h(w)$ obtained in this way may serve as a good approximation.

An alternative way to determine $\langle N(w)\rangle$, and thus $h(w)$, is based on the observation that the reduction in solution conductivity is proportional only to the wt% concentration of polymer, that is, to monomer density, and does not depend on polymer M_r (ref. 5). Supposing that the relative reduction in the pore conductance is also proportional to the density of monomers inside the pore, we calculate pore-bulk polymer partitioning as a function of polymer M_r to obtain $\langle N(w)\rangle$. Both methods give similar results; we use this second method as it employs less restrictive assumptions. It is also reassuring that polymer partitioning into the pore can be verified independently by measuring size-modulated osmotic response[8].

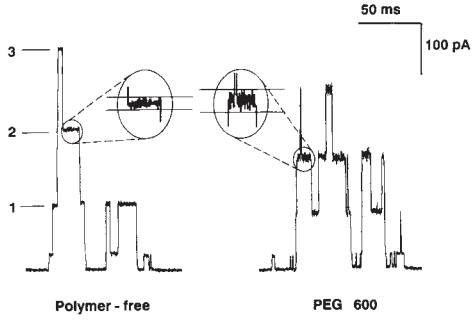

FIG. 2 Ion current of a single alamethicin channel as changed by polymer addition. (Left, polymer-free; right, after addition of PEG 600). PEG 600 decreases the amplitude of different conductance levels (numbers 1, 2 and 3 at left) and induces additional current noise. Current records filtered by an 8-pole Bessel filter at 3 kHz are shown enlarged (2×) in insets for comparison. Different conductance levels of the channel correspond to different numbers of closely packed molecular pores (Fig. 1) of nearly uniform dimensions[5,20]. The time/current scale in the right upper corner is common to both records.

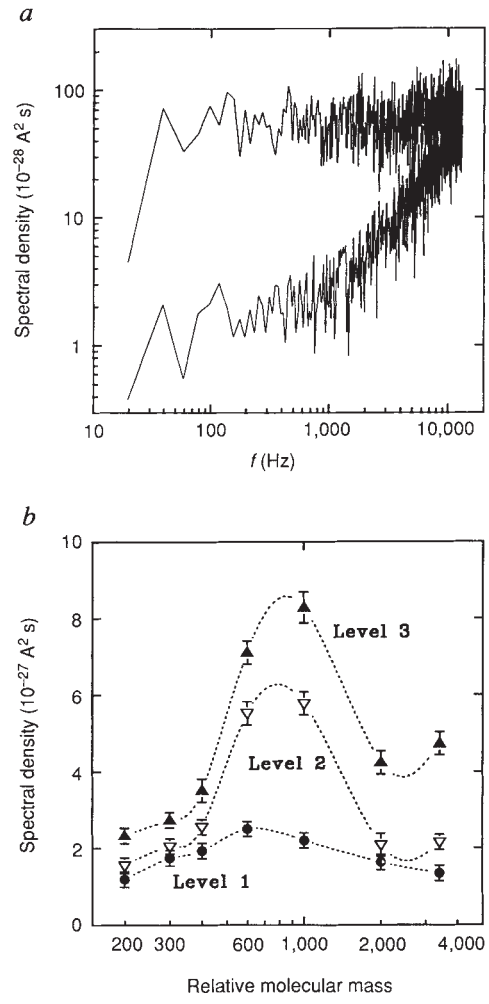

FIG. 3 Measurement of polymer-induced current fluctuations. *a*, Spectrum of open-channel noise in the presence of PEG 600 at level 2 (upper trace) is shown in comparison with the spectrum of the background noise obtained from the parts of the current recordings when the channel was closed (lower trace). *b*, Amplitude of the 'white' spectral part of the open-channel noise changes with polymer relative molecular mass, M_r. The spectrum was averaged over a 200–2,000 Hz frequency range with the background subtracted.

METHODS. In spectral measurements an 8-pole Butterworth filter was employed, the corner frequency being chosen to be three-eighths of the sampling frequency (40 kHz). To separate open-channel noise from a strong low-frequency contribution of the channel switching between different conductance states, we first recorded the segments of the current corresponding to a particular conductance state into the computer memory; then, after cutting 0.25 ms off both ends of each segment to eliminate transient currents and after zeroing the mean value of each segment, we connected them into a 2,048-point vector to be used in Fourier transformation. This signal processing procedure is justified by the fact that characteristic times of current fluctuations within a particular state are orders of magnitude smaller than inter-state switching times. Nevertheless, to check for possible spectrum distortions due to a limited lifetime of the channel at a particular state, we performed a specially designed test. A signal from a calibrated noise generator was admixed to the channel current recording and processed as described above. The spectra so obtained were then compared to those measured directly from the generator output. It turned out that the damping of the admixed noise spectra is significant only at frequencies lower than 50 Hz.

The mean value of square conductance fluctuations, $h(w)^2\langle N(w)\rangle$, can also be calculated theoretically as a product of the low-frequency limit of conductance fluctuation spectral density $S_h(0)$ times the spectral width created by diffusion relaxation times. General considerations show that the bandwidth of these fluctuations should be proportional to the diffusion coefficient D of polymer inside the pore. To calculate the bandwidth for the case of a long pore we used a one-dimensional approximation. For the probability $P(x_0, x, \tau)$ to find a particle in position x at time τ if it had been in position x_0 at $\tau=0$, we have

$$D(\partial^2 P(x_0, x, \tau)/\partial x^2) = \partial P(x_0, x, \tau)/\partial \tau$$

For a narrow channel, the probability that a particle that has left the channel will return diminishes rapidly as the particle moves from the mouth of the channel. Placing absorbing boundaries at both ends of the capillary, that is at $x=0$ and $x=L$, we solve the problem using methods described elsewhere[10].

Formally, for the spectral density, $S_h(0)$, of conductance fluctuations at frequency $f=0$, we obtain

$$S_h(0) = S_h(f)|_{f=0} = \langle(\delta h)^2\rangle L^2/3D$$

where $\langle(\delta h)^2\rangle$ is the time-averaged square fluctuation. The diffusion-generated bandwidth of pore conductance fluctuations is $3D/L^2$. The corresponding time constant that describes averaged relaxation of polymer number fluctuation inside the pore is equal to $L^2/6\pi D$. This value is remarkably close to the intuitive result $L^2/12D$ reported by Feher and Weissman[11].

Experimentally, we obtain $S_h(0) = S_I(0)/V^2$ from the measured low-frequency 'white' part of the current noise spectrum $S_I(0)$ and transmembrane voltage V. Equating time-averaged and ensemble-averaged square fluctuations, $\langle(\delta h)^2\rangle$ and $h(w)^2\langle N(w)\rangle$, for the polymer diffusion coefficient inside the pore we have

$$D(w) = h(w)^2 L^2 \langle N(w)\rangle V^2/3S_I(0)$$

The diffusion coefficients calculated for polymers of different M_r are shown in Fig. 4, together with their values in bulk aqueous solution. We assume channel length $L=40$ Å and radius $R=6.5$ Å, based on results of measurements[5] and on molecular model considerations[6].

The smallest polymer used in our experiments, PEG 200, has a gyration radius of 4.0 Å (ref. 12), already quite close to the pore radius. Formal application of restricted-diffusion theory[13] in this case gives the right order of magnitude for the reduction in diffusion rate (Fig. 4) even though its application is highly questionable here. Restricted-diffusion theory is developed for hard spheres, whereas PEG polymers in aqueous solutions exist as well-characterized random coils[14]. The observed order-of-magnitude-slower polymer diffusion in the pore can have many causes: size restriction, friction with the walls, higher viscosity of water in the pore, and so on. The absence of any significant dependence of the diffusion coefficient on the polymer M_r disagrees with the expected results of restricted-diffusion considerations for hard spheres, and might reflect the confinement of flexible coil in a pore whose dimensions are comparable to the size of the polymer. Although the viscosity of water inside the channel may be different from the bulk, the observed decrease in diffusion coefficient does not provide a quantitative measure of this.

It is important to note here that the diffusion coefficient obtained in our experiments describes transport of polymers only once they are inside the pore. To describe the overall diffusion of polymers across the whole membrane, one should take into account partitioning of polymers between the pore and bulk solution. Partitioning of polymers in the pore quickly decreases with increasing polymer M_r (Fig. 4); so does diffusion across the membrane.

Fluctuation analysis of currents through ion channels, performed to obtain dwell times of charged permeant species, has been successfully used previously. Classic examples include studies on Ca^{2+} block of monovalent currents through calcium channels[15] and on Na^+ block of proton currents through the gramicidin A channels[16]. Now that strong evidence for the existence of protein-conducting channels is starting to emerge[17], the importance of studies on neutral-permeant transport is obvious. Using the approach outlined here, it should be possible to conduct such studies at a single-channel level. □

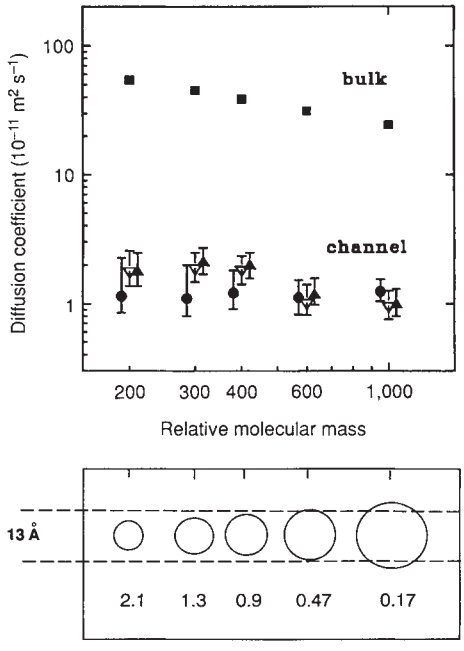

FIG. 4 Top, Diffusion coefficients of polymers inside the channel calculated from polymer-induced current noise (symbols for different levels correspond to those in Fig. 3*b*). An order of magnitude decrease in comparison with their values in bulk water is already expected from restricted-diffusion theory[13] for the smallest polymer, PEG 200. Bottom, PEG gyration diameters are compared with the size of the alamethicin channel opening (13 Å). Numbers below the gyration diameters correspond to the average number of PEG molecules in the channel (level 1).

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