Dynamics of unfolded protein transport through an aerolysin pore

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Supporting Information available

Materials and Methods

Bilayers. Membrane lipid bilayers were made by using the previously described method [1,2].

Protein production and purification. Aerolysin was produced in *Escherichia coli* as proaerolysin as described before [3,4] and it was activated by digestion with trypsin 10 min at room temperature prior to introduction in *cis* compartment of the chamber to eliminate propeptide sequence allowing monomers to polymerise [5]. The recombinant maltose binding protein of *Escherichia coli* (MalE) [6]was used as a model substrate. This monomeric protein with 370 residues (Mr = 40707) is negatively charged (with a net charge Z=−8e) at physiological pH. The wild-type (MalEwt) and variant (MalE219) proteins were purified as described [7]. The tandem protein, MalEwt-MalEwt, was constructed by subcloning a PCR-amplified DNA fragment of the corresponding mature sequence of MalE with primers containing a HindIII adaptor as described [8]. In experiments, proteins are entering by the stem side first (figures 2, 3, 5, 6) or by stem or vestibule side (figure 4).

Data acquisition. The ionic current through one aerolysin channel was measured with an Axopatch 200B amplifier. Data were filtered at 10 kHz and acquired at 4 μs intervals (250 kHz) with the DigiData 1322A digitizer coupled with Clampex software (Axon Instruments, Union City, USA). The measurements of the transients were based on the statistical analysis of the current traces. Data were systematically checked for reproducibility with several pores. The main difficulty of the data analysis is to separate the pulses of electric current from the noise. We define a first threshold th1 as the average current of the open pore $<I_0>$ minus the standard deviation σ of its distribution, th1 = $<I_0>$ - 2σ. We define a second threshold th2 as the end of this distribution. The average frequency of blockades is deduced from the distribution of the time intervals Ti between two successive blockades. The average duration of

blockades is deduced from the distribution of blockade duration Tt. The two blockade time distributions of independent events are adjusted with 2 separate exponential functions, $y = A1 \exp(-t/\tau 1)$ and $y = A2 \exp(-t/\tau 2)$ and with a double exponential function, $y = A1 \exp(-t/\tau 1)$ and $y = A2 \exp(-t/\tau 2)$. All statistical analyzes were performed with the software Igor Pro (WaveMetrics Inc.).

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Tables

Table S1. Fitting parameters of the figure 5a and 5b. *The histogram of the blockade distribution is fitted by single exponential for each distribution; ¤ the histogram of the blockade distribution is fitted by double exponential.

V = 130 mV	$*t_{short}$	$*t_{long}$	$¤t_{short}$	$¤t_{long}$	$<t_{short}>$	$<t_{long}>$
[MalEwt] = 0.35 µM	*42 ±2 µs*	*318 ±28 µs*	*37 ±3 µs*	*324 ±29 µs*	*40 ±3 µs*	*321 ±2 µs*
[MalE219] = 0.35 µM	*33 ±5 µs*	*286 ±30 µs*	*24 ±1 µs*	*309 ±35 µs*	*29±4 µs*	*298±12 µs*

Table S2. Fitting parameters of the figure 6a, 6b, 6d, 6e. The histogram of the time inter events is fitted by single exponential;*the histogram of the blockade distribution is fitted by single exponential for each distribution; ¤ the histogram of the blockade distribution is fitted by double exponential.

V = 70 mV	*frequency*	$*t_{short}$	$*t_{long}$	$¤t_{short}$	$¤t_{long}$	$<t_{short}>$	$<t_{long}>$
[MalEwt] = 2 µM	*9.6 ±0.6Hz*	*93 ±15 µs*	*613 ±38 µs*	*61 ±1.5 µs*	*500 ±23 µs*	*77 ±16 µs*	*557 ±57 µs*
[(MalEwt)2] = 2 µM	*7.8 ±0.2Hz*	*62 ±6 µs*	*1000 ±62 µs*	*71 ±8 µs*	*833 ±62 µs*	*67±5 µs*	*917±84 µs*

Figure

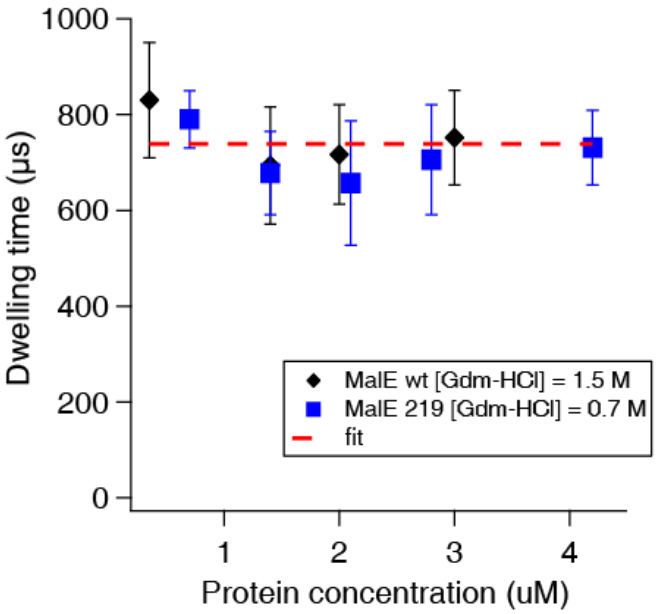

Figure S1. Duration of the dwelling time (translocation time or long time) of unfolded proteins as a function of protein concentration. The applied voltage is 70 mV. Experiments are made at 1 M KCl, 5 mM HEPES pH 7.4, the final guanidium concentrations are respectively: 1.5 M for MalEwt and 0.7 M for MalE219. The mean dwelling time is <t> = 732 ± 31 µs (dotted line).