

Electronic supplementary information (ESI) for

Synchronization of opening and closing of two gramicidin A channels pulled together by a linker: possible relevance to channel clustering

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Bilayer lipid membranes (BLMs) were formed from a 2% solution of diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids, Alabaster, AL) in *n*-decane (Merck, Darmstadt, Germany) by the brush technique on a hole in a Teflon partition separating two compartments of a cell containing aqueous buffer solutions. The cell with the 0.15-mm diameter hole was used in single-channel experiments. The biotinylated analogs of gramicidin A (generous gifts of F. Separovic, University of Melbourne, Australia) with a biotin group attached to the C-terminus of gramicidin A through a linker arm comprising five (gA5XB) aminocaproyl groups were added from stock solutions in ethanol to the bathing solutions at both sides of the BLM and routinely incubated for 15 min with constant stirring. Streptavidin was from Fluka (Buchs, Switzerland). In all the experiments the solution was 1 M KCl, 10 mM Tris, 10 mM MES, 10 mM β -alanine, pH=7.0. All the experiments were carried out at room temperature (22-24 °C).

The electric currents (*I*) were recorded under voltage-clamp conditions. Voltages were applied to BLMs with Ag-AgCl electrodes placed directly into the cell. The currents, measured by means of a patch-clamp amplifier (OES-2, OPUS, Moscow) in single-channel experiments, were digitized by using a LabPC 1200 (National Instruments, Austin, TX) and analyzed using a personal computer with the help of WinWCP Strathclyde Electrophysiology Software designed by J.Dempster (University of Strathclyde, UK). Single-channel currents were low-pass filtered with a cutoff frequency of 100 Hz and sampled at 1 kHz.

Figures S1-S3. Single-channel traces of gA5XB after incubation with 1 μ g/ml streptavidin added to both sides of a BLM. The BLM voltage was 50 mV. The solution was 1 M KCl, 10 mM Tris, 10 mM MES, 10 mM β -alanine, pH=7.0. Planar bilayers were from DPhPC. The low-pass digital filter with a cutoff frequency of 53 Hz was used in these recordings.





