

Electronic Supplementary Information

Repurposing an antimicrobial peptide for the development of a dual ion channel/molecular receptor-like platform for metal ions detection

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Materials and methods

Design of alamethicin (Alm) – peptide nucleic acids (PNA) chimeras. As a research strategy, we employed herein as a novel ion channel-based receptor for selected metal ions, the nanopore-forming alamethicin peptide (Alm), due to a number of intrinsic qualities, including: (i) it is relatively cheap to synthesize; (ii) it can be tailored for the specific detection of analytes via derivatization with peptide nucleic acids (PNA) moieties, attached covalently to the C-terminus of Alm; (iii) the synthetic Alm-PNA chimeras preserve the spontaneous self-incorporation into planar bilayers of the native Alm and display the well-defined, discrete ion conductance values suggestive of reversible oligomers formation.

The synthetic (Alm-PNA) chimeras used herein were based on the native Rf30 form of the Alm peptide, in which Aib residues were replaced with Leu residues, and was engineered at the C-terminus with either T- or G-rich PNA segments via a flexible linker. The N-terminus of the modified Alm peptide, whose movement across the membrane is critical for attaining the voltage-dependent activity of the peptide, was left unaltered (Table S1).

Table S1 The structures of the synthetic (Alm-PNA) chimeras used herein. In red and underlined we represented the Leu-substituted Aib amino acids from the native Rf30 alamethicin, and the linker connecting either T₅ or G₅ PNA segments to the peptide's C-terminus is shown in purple.

Name	Primary sequence	Mw (g/mol)
Alamethicin (Alm)	Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib- Pro-Val-Aib-Aib-Glu-Gln-Pho	1964.34
Alm_T	Ac- <u>Leu</u> -Pro- <u>Leu</u> -Ala- <u>Leu</u> -Ala-Gln- <u>Leu</u> -Val- <u>Leu</u> -Gly-Leu- <u>Leu</u> - Pro-Val- <u>Leu</u> - <u>Leu</u> -Glu-Gln- <u>Gly-Gly-Ser-Gly-(T-T-T-T-T)</u>	3645.0
Alm_G	Ac- <u>Leu</u> -Pro- <u>Leu</u> -Ala- <u>Leu</u> -Ala-Gln- <u>Leu</u> -Val- <u>Leu</u> -Gly-Leu- <u>Leu</u> - Pro-Val- <u>Leu</u> - <u>Leu</u> -Glu-Gln- <u>Gly-Gly-Ser-Gly-(G-G-G-G-G)</u>	3770.2

To maintain a space between the Alm and PNA segments and allow the ions to flow through the Alm-PNA oligomers, the linkers used in the construction of the chimeras were based on the Gly-Gly-Ser peptide motif.^{1,2}

Reagents. The Alm-PNA peptide chimeras were purchased from Panagene Inc., Daejeon, Republic of Korea, and short ssDNA fragments TTTTT (T₅) and GGGGG (G₅) were purchased from Sigma-Aldrich, Darmstadt, Germany. Other reagents, including potassium chloride (KCl), sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), Tris(hydroxymethyl)aminomethane (Tris), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ultrapure DNAase and RNAase-free water, n-pentane, hexadecane and metal salts

(CuCl₂, ZnCl₂, HgCl₂ and AlCl₃) were purchased from Sigma-Aldrich, Darmstadt, Germany. The 1,2-diphytanoyl-sn-glycerophosphocholine (DPhPC) lipids were procured from Avanti Polar Lipids (Alabaster, AL, USA).

Sample preparation. The dried form of Alm-PNA peptide chimeras was dissolved in ethanol and when needed, a small amount of dimethyl sulfoxide (DMSO) was added for better solvation. The ssDNAs samples were dissolved in ultra-pure water containing 1 M NaCl, buffered with TE (10 mM Tris, 1 mM EDTA) at pH = 8.2, heated up to 95 °C using an IKA Digital Block Heater (Cole-Parmer, USA) and slowly cooled down to room temperature, to assure rehydration. All solvated samples were vigorously stirred using a Stuart BioCote vortex mixer (Sigma–Aldrich, Germany) at 1,400 rpm, for 3 min, in continuous mode and then were divided in aliquots and kept at – 20 °C.

Electrophysiology Experiments. As reported previously,³ the bilayer lipid membranes-BLMs were formed using DPhPC lipids dissolved in HPLC-grade n-pentane (10 mg mL⁻¹), onto an aperture of ~100 μm diameter generated in a 25 μm thick Teflon septum, clamped between two Teflon chambers each of 1 mL volume. Throughout experiments, both the cis (grounded) and trans chambers of the bilayer setup contained 3 M KC buffered with 10 mM HEPES (for the experiments with Hg²⁺, Cu²⁺ or Zn²⁺) or 50 mM HEPES (for the experiments with Al³⁺) at pH = 7. Before membrane formation, the septum was pretreated with 10% (v/v) hexadecane in highly purified n-pentane and the formation of a bilayer was monitored by observing the increase in capacitance to a value of approximately 60–100 pF. After testing the stability of the formed lipid bilayer by applying positive and negative transmembrane voltages, Alm-PNA monomers were added from stock solution to the cis chamber connected to the ground. Metals were added from stock solution made in ultrapure water on the same side as the peptides (cis chamber). All measurements were performed in a Faraday cage (Warner Instruments, USA) and with the bilayer cell placed on a

vibration-free platform BenchMate 2210 (Warner Instruments, USA), at a room temperature of ~25 °C. The ionic currents flow through the oligomers generated with the chimeric peptides described herein were recorded in the voltage-clamp mode via two Ag/AgCl electrodes connected to a Multiclamp 700B amplifier (Molecular Devices, USA), with the help of a virtual instrument developed within the LabVIEW 8.20 platform (National Instruments, USA). The electrical signals were digitized at a sampling frequency of 50 kHz with a NI PCI 6221 16-bit acquisition board (National Instruments, USA) and low-pass filtered at 12 kHz. Alternatively, current recordings were undertaken by employing the ePatch amplifier (ELEMENTS SRL, Italy). All numerical analysis, including post-recording low-pass filtering ($f_c = 5000$ Hz) and spectral analysis, were done using a custom designed program in LabVIEW 2020 (National Instruments, USA). The graphic representations of the recorded data were done using Origin 6 (OriginLab, Northampton, MA, USA). The I- Δ V diagrams were drawn automatically with a custom designed program written in LabVIEW 2020 (National Instruments, USA), which averaged electrical current fluctuations data comprising 35.000 points, recorded at various holding potentials. The average power (pA^2) contained in the ionic current fluctuations was derived via Fourier analysis, whereby the corresponding power-spectrum density graphs resulting from analysis undertaken on cvasi-stationary recordings were calculated with a Hanning window, and integrated over the 1–5000 Hz frequency domain; for simplicity, the resulting values were rounded to the nearest integer.

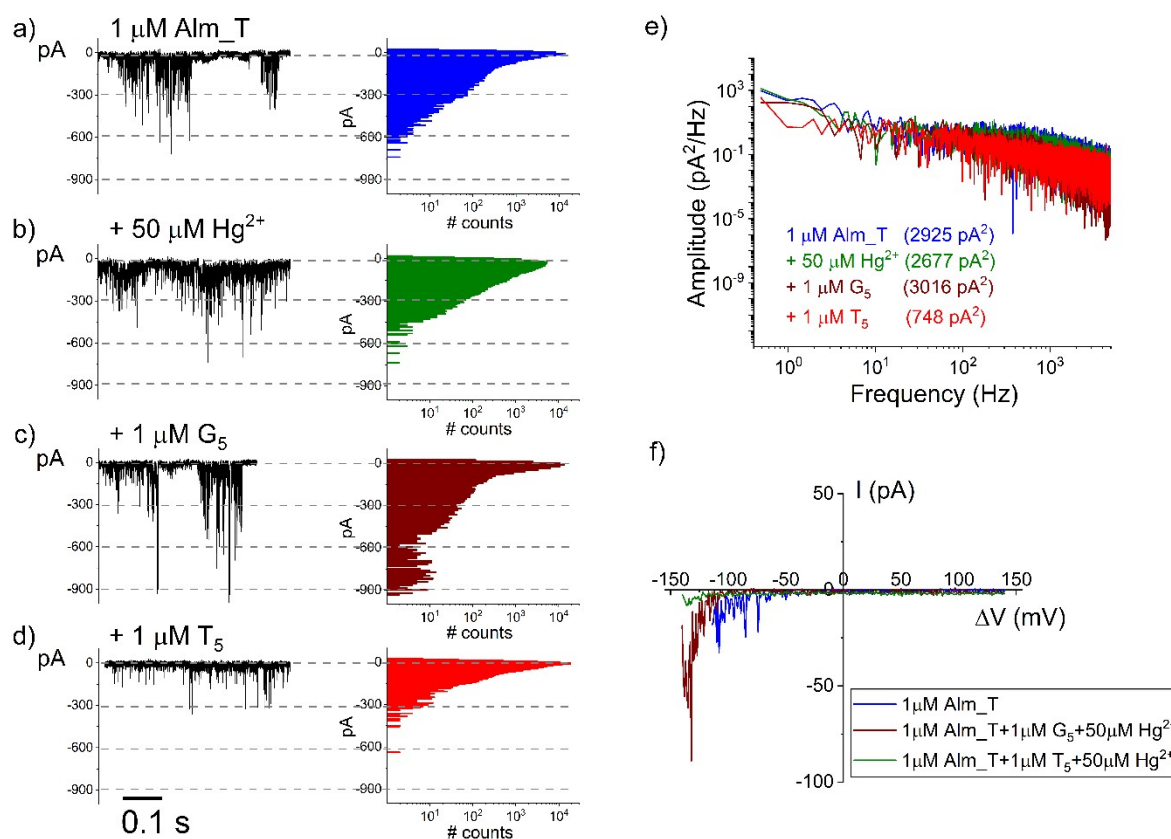


Fig. S1 Hg^{2+} ions detection with the Alm_T oligomers requires the presence of T_5 ssDNA in the bathing electrolyte. Selected traces recorded at $\Delta V = -140mV$ displaying the bare activity of Alm_T oligomers generated upon cis-side addition of 1 μM from the chimeric peptide in the absence (a) and the presence of successively cis added Hg^{2+} [50 μM] (b), G_5 ssDNA [1 μM] (c) and respectively T_5 ssDNA [1 μM] (d). On the corresponding right-hand-side panes are displayed the all-point histograms of ionic current fluctuations recorded under the specified conditions. (e) The power-spectrum density traces of the ionic current fluctuations recorded on the experimental conditions specified in panels a-d. Numerical data in the legends indicate the average power contained in the corresponding current fluctuations, calculated as the graph's integrals. (f) I- ΔV diagrams demonstrating the conjugated, inhibitory effect of the T_5 ssDNA and Hg^{2+} present simultaneously on the cis-side electrolyte, on the ionic transport mediated by Alm_T oligomers.

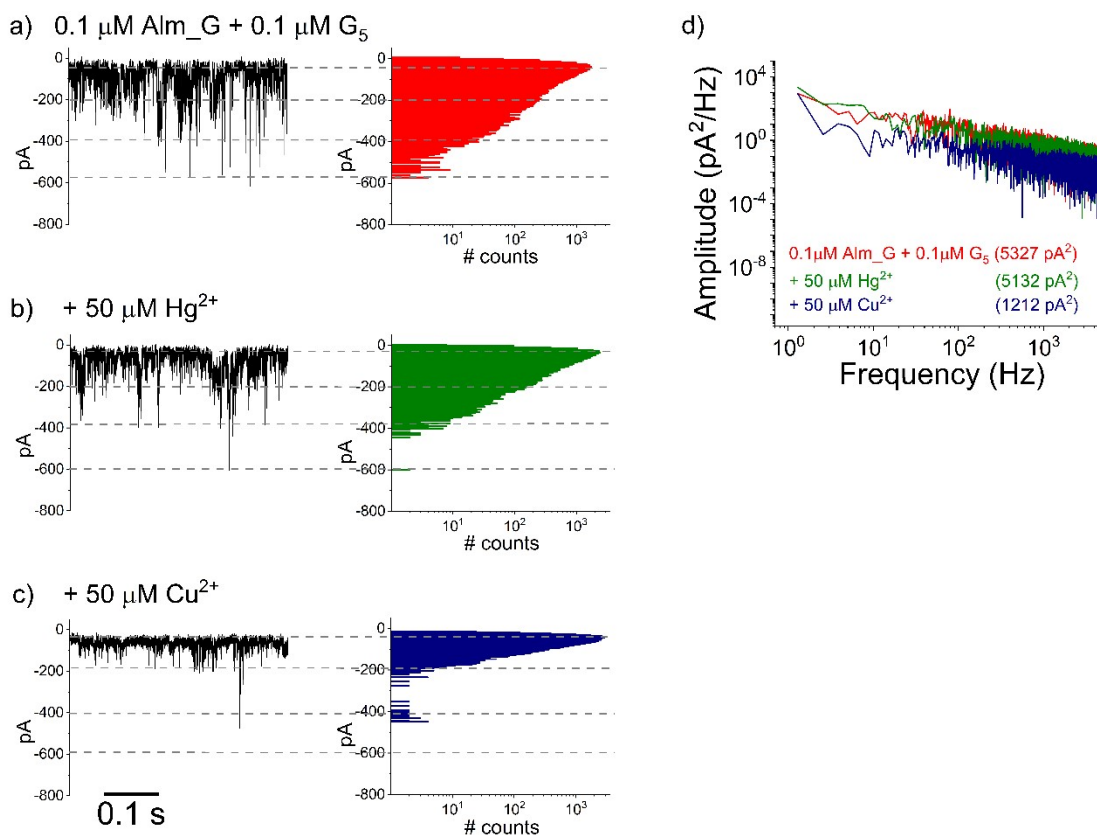


Fig. S2 The (Alm_G; G₅ ssDNAs) system probes selectively Cu²⁺ over Hg²⁺ metal ions. Selected recordings displaying the ionic current fluctuations activity and all-point histograms of active Alm_G oligomers embedded in a planar lipid membrane, clamped at a potential difference of $\Delta V = -100$ mV, when added together with the T₅ ssDNA (1 μM) at a 1:1 molar ratio (a) and in the presence of successively added Hg²⁺ [50 μM] (b) and respectively Cu²⁺ [50 μM] (c). As a quantitative report of the effects pertaining to the recordings presented above, in panel d we illustrate the power-spectrum analysis of such ionic current fluctuations (panels a-c) together with the average power contained calculated as the graph's integrals, displayed in the panel's legend.

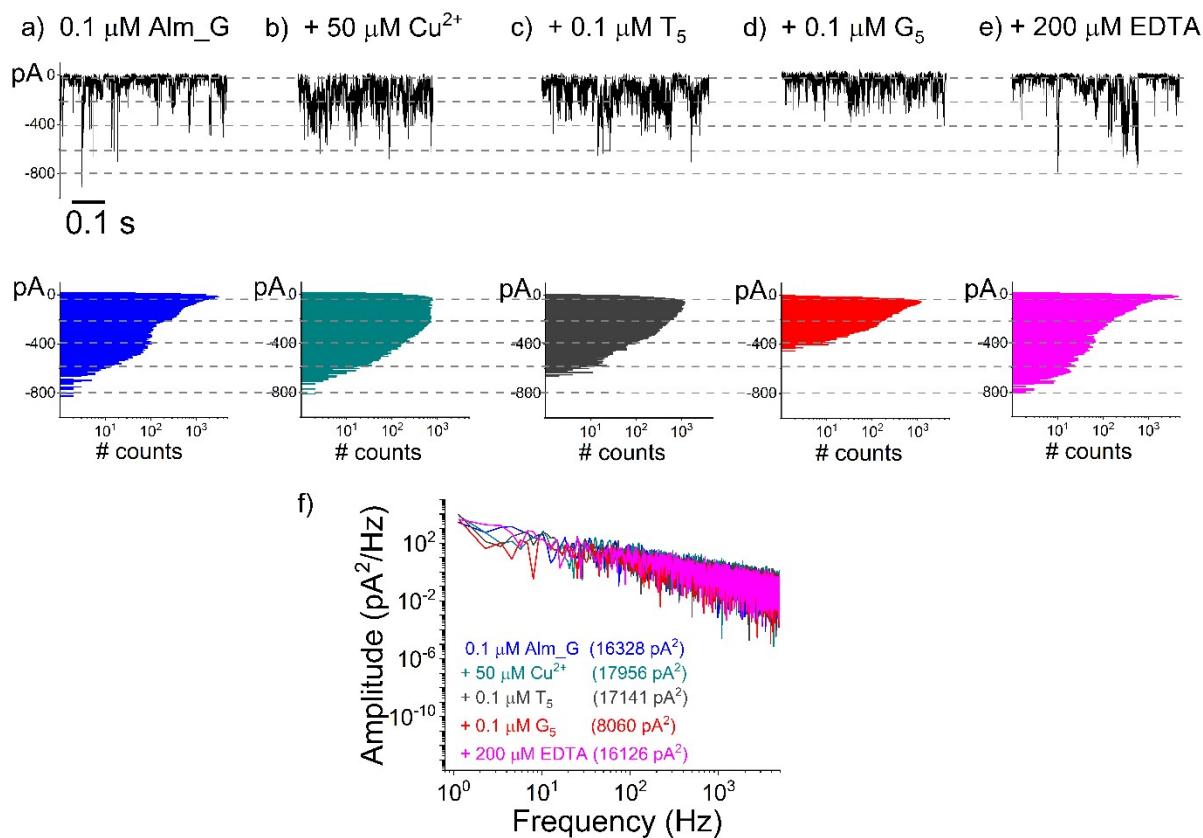


Fig. S3 The Alm_G chimeric construct probes Cu^{2+} metal ions detection in a heterogeneous electrolyte. The ionic current signature of functional Alm_G oligomers in planar lipid membranes held at $\Delta V = -100$ mV, bathed in an electrolyte containing 3 M KCl alone (a), and in the presence of successive cis-side additions at the indicated micro-molar concentrations of Cu^{2+} (b), T_5 ssDNAs (c), G_5 ssDNAs (d) and EDTA (e). In the panels beneath each trace we display the all-point histograms of the corresponding current fluctuations. (f) The power-spectrum analysis of the traces shown in panels a-e. In the legend, we display the extracted average power values contained in such current fluctuations, calculated as the graph's integrals.

References

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