

Supplementary Materials for

Human β -defensin 2 kills *Candida albicans* through phosphatidylinositol 4,5-bisphosphate-mediated membrane permeabilization

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Fig. S2. The three-dimensional structure of HBD-2 is important for its antifungal activity and liposome permeabilization.

Table S1. SAXS data collection and scattering-derived parameters.

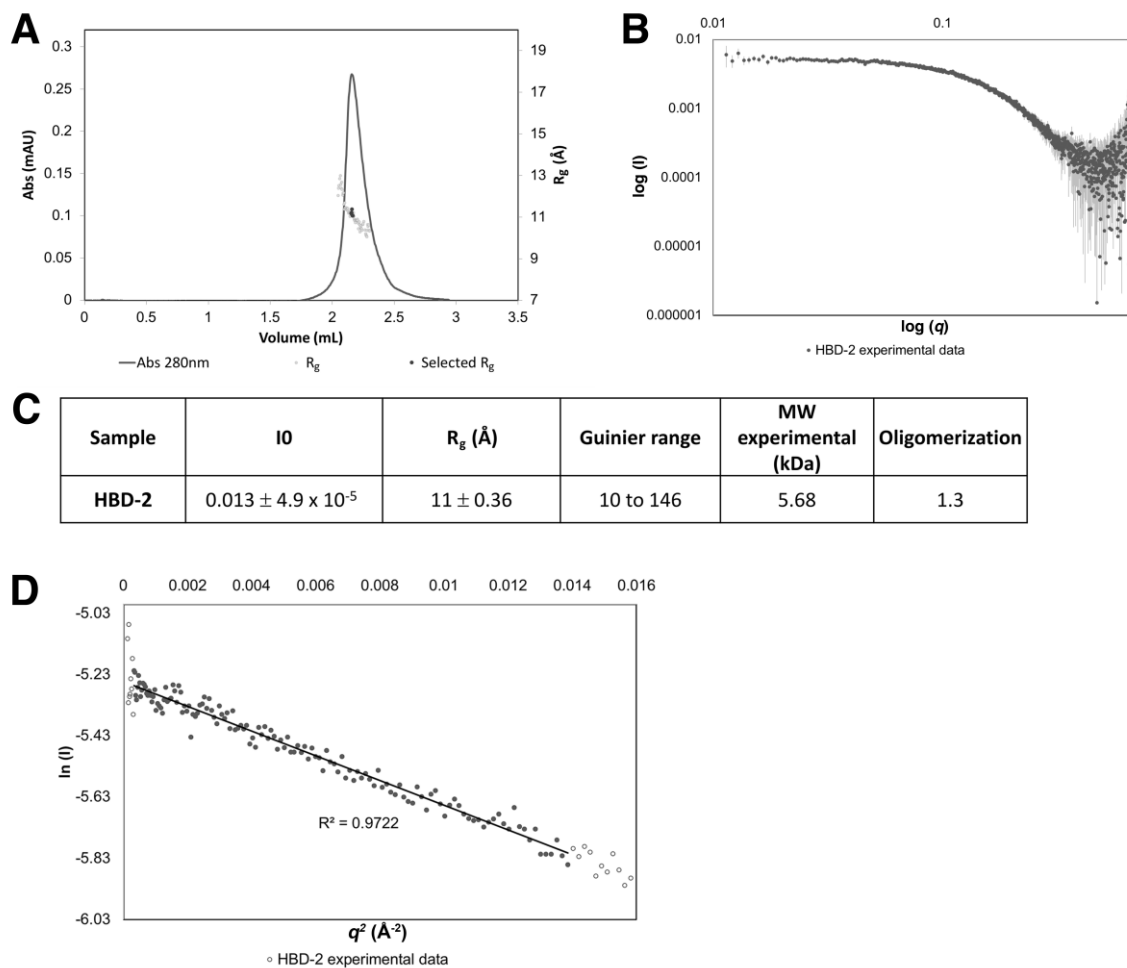


Fig. S1. SEC-SAXS analysis of HBD-2. (A) In-line size exclusion SAXS chromatogram of HBD-2. The dark dots represent the frames used for averaging the scattering profiles based on R_g (dots). (B) Log-log representation of HBD-2 scattering profile extracted from fractionated peaks obtained from in-line SEC-SAXS. (C) Oligomerization analyses of HBD-2 based on Guinier range. (D) Guinier plot of HBD-2 with Guinier range highlighted with dots.

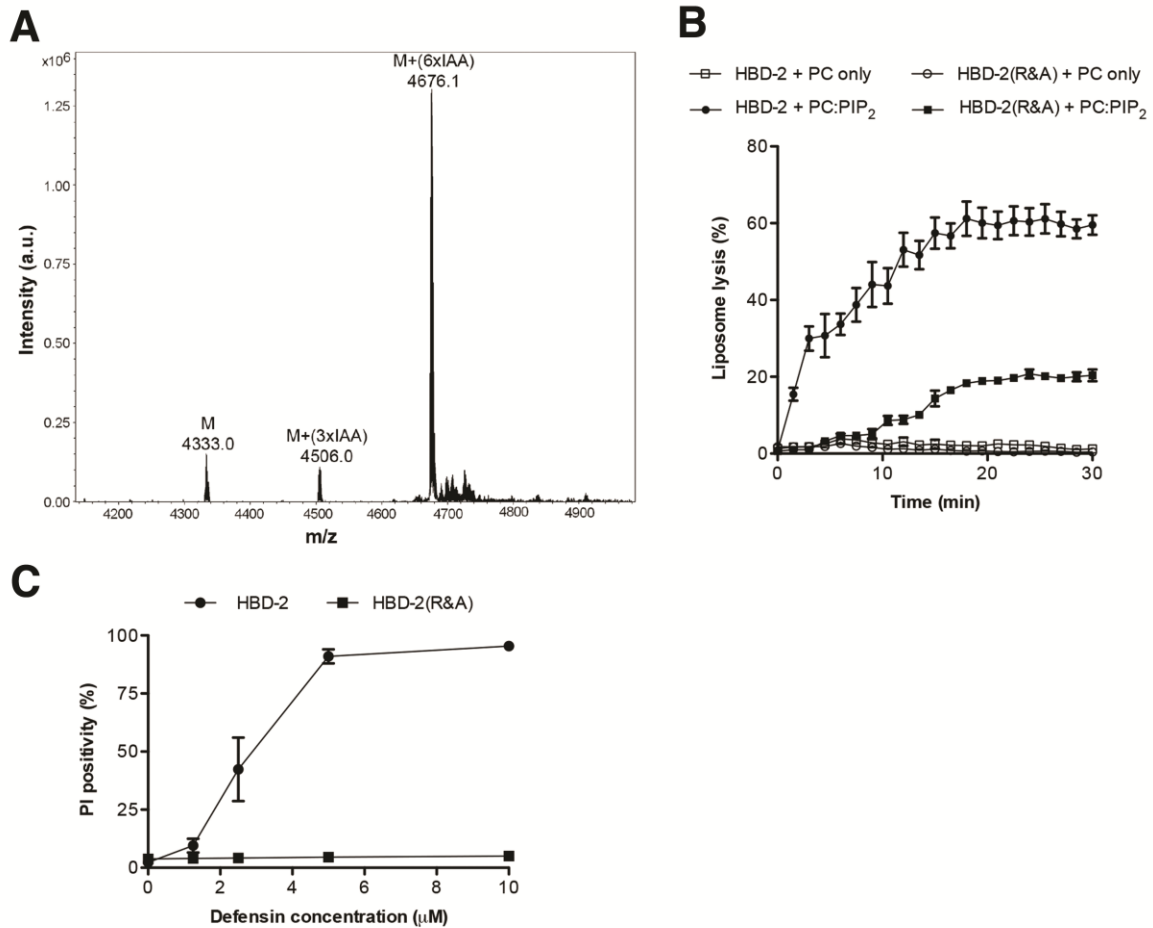


Fig. S2. The three-dimensional structure of HBD-2 is important for its antifungal activity and liposome permeabilization. (A) ESI-Q-TOF mass spectrum of reduced and alkylated HBD-2 (HBD-2(R&A)) sample with masses (M) and number of conjugated iodoacetamide (IAA) explicitly indicated. (B) Fungal cell permeabilization by HBD-2(R&A) in comparison to HBD-2. *C. albicans* was treated with defensins at indicated concentrations for 30 min, followed by PI staining prior to flow cytometry analysis. (C) Liposome lysis with 50 μ M HBD-2 or HBD-2(R&A) using calcein-encapsulated PC only or PC:PIP₂ liposomes. Liposome lysis was normalized against triton X-100 treatment. Data in (B) and (C) represent mean \pm SEM of three independent experiments.

Table S1. SAXS data collection and scattering-derived parameters.

HBD-2	
Data collection parameters	
Instrument	SAXS/WAXS beamline, Australian Synchrotron
Beam geometry (μm)	250 x 450
Fractional sample flow rate	0.5
Wavelength (keV)	12
Flux (ph.s^{-1})	6×10^{12}
q range (\AA^{-1})	0.011-0.65
Exposure time (s)	1 (detector integration)
Temperature (K)	299.15
Structural parameters †	
$I(0)$ (from Guinier) (cm^{-1})	$0.013 \pm 4.85 \times 10^{-5}$
R_g (from Guinier) (\AA)	11.00 ± 0.36
Molecular-mass determination †	
Partial specific volume ($\text{cm}^3 \text{g}^{-1}$)	0.739
Contrast ($\Delta\rho \times 10^{10} \text{cm}^{-2}$) §	2.897
Molecular mass M_r [from $I(0)$] (kDa) §	5.68
Calculated monomeric M_r from sequence (kDa)	4.33
Software employed	
Primary data reduction	SAXS/WAXS beamline software
Data processing	<i>PRIMUS</i>
Theoretical scattering calculations	<i>CRYSOL</i>
Three-dimensional graphics representation	<i>PyMOL</i>
Graphics representation	<i>EXCEL</i>

† Reported for peaks (Fig. S1) § Determined with MULCh (Whitten *et al.*, 2008)