Supporting Information

Antifungal activity of designed α -helical antimicrobial peptides

Ruicheng Xu^{1,4}, Jing Tang^{1,4}, Roja Hadianamrei², Suyu Liu², Songwei Lv¹, Rongrong You¹, Fang

Pan¹, Peng Zhang³, Nan Wang¹, Zhiqiang Cai¹, Xiubo Zhao^{1,2, *}

¹School of Pharmacy, Changzhou University, Changzhou 213164, China

²Department of Chemical and Biological Engineering, University of Sheffield, Sheffield S1 3JD, UK

³School of Materials Science and Engineering, Changzhou University, Changzhou 213164, China

⁴The Authors contributed equally to this work.

*Author for correspondence: X Zhao, E-mail: xiubo.zhao@cczu.edu.cn



Figure S1. HPLC chromatograms of At1-At12 peptides. HPLC conditions: The peptide concentration was fixed at 1 mg/mL. Analytical column type: SHIMADZU Inertsil ODS-SP (4.6 x 250 mm x 5 μ m). Eluent A (0.1% trifluoroacetic in water) and eluent B (0.1% trifluoroacetic in acetonitrile). The flow rate was 1 mL/min and the UV detector was set at 214 nm.



Figure S2. α-helical content of the designed antifungal peptides in DPPC and DPPG SUVs mimicking the membrane of normal mammalian cells and fungi respectively.



Figure S3. MIC Assay of AMPs to (**A-B**) *Candida albicans* (ATCC 10231), (**C-D**) *Candida albicans* (clinical isolate), (**E-F**) *Candida tropicalis* (clinical isolate), (**G-H**) *Candida lusitaniae* (clinical isolate) after 24 h incubation with peptides.



Figure S4. Determining the minimum fungicidal concentration of the selected AMPs (At3, At5, and At10) against *Candida albicans* (ATCC 10231).



Figure S5. Antifungal dynamics of At3, At5 and At10 to *Candida albicans* (10231). Peptide concentration was fixed at $3.2 \mu M$.



Figure S6. Live/Dead assay of the antifungal peptides. CLSM images of (**A**) *Candida albicans* (clinical isolate), (**B**) *Candida tropicalis* (clinical isolate), (**C**) *Candida lusitaniae* (clinical isolate) treated with At5. The PBS-treated groups were used as the negative controls; scale bar = 10 μm.



Figure S7. Live (green) /Dead (red) assay of the antifungal peptides. Confocal laser scanning microscopy (CLSM) images of *Candida albicans* (ATCC 10231) treated with At3 for 1 h, at different peptide concentrations (1/4, 1/2, and 1 x MIC). The green fluorescence indicates live/healthy fungi, and the red fluorescence indicates dead/damaged fungi. The PBS-treated fungi were used as the negative controls; scale bar = 10 μ m (magnification, × 100).



Figure S8. Live (green) /Dead (red) assay of the antifungal peptides. Confocal laser scanning microscopy (CLSM) images of *Candida albicans* (ATCC 10231) treated with At10 for 1 h, at different peptide concentrations (1/4, 1/2, and 1 x MIC). The green fluorescence indicates live/healthy fungi, and the red fluorescence indicates dead/damaged fungi. The PBS-treated fungi were used as the negative controls; scale bar = 10 μ m (magnification, × 100).



Figure S9. Hemolytic activities of At3, At5, and At10. Human red blood cells (hRBCs) were incubated in PBS with different concentrations of peptides for 1 h at 37 °C, followed by the monitoring of hemoglobin release at 540 nm.



Figure S10. Fluorescent images of the FITC-labelled peptide (At3, At5 and At10) distributions in coculture systems containing Hff1 cells (model mammalian host) and (**A**) *Candida albicans* (clinical isolate), (**B**) *Candida tropicalis* (clinical isolate), and (**C**) *Candida lusitaniae* (clinical isolate). Adherent cells are Hff1, and round cells are Candida. Bar = 25 µm.



Figure S11. Antifungal resistance (AFR) test in *Candida albicans* (ATCC 10231) treated with At3 (**A**), At5 (**B**), At10 (**C**) and fluconazole (**D**) after multiple treatments cycles (n = 1-25) with the peptides at the concentration of $1 \times MIC$.



Figure S12. Development of antimicrobial resistance (AMR). AMR of *Candida albicans* (clinical isolate) against At3 (**A**), At5 (**B**), At10 (**C**), and (**D**) fluconazole after multiple treatments (n = 1-25) with the peptide concentrations at 1 × MIC. AMR of *Candida tropicalis* (clinical isolate) against At5 (**E**) and (**F**) fluconazole after multiple treatments (n = 1-25) with the peptide concentrations at 1 × MIC. AMR of *Candida tropicalis* (clinical isolate) against At5 (**E**) and (**F**) fluconazole after multiple treatments (n = 1-25) with the peptide concentrations at 1 × MIC. AMR of *Candida lusitaniae* (clinical isolate) against At5 (**G**) and (**H**) fluconazole after multiple treatments (n = 1-25) with the peptide concentrations at 1 × MIC.



Figure S13. SEM images showing extensive damage to *Candida albicans* (clinical isolate), *Candida tropicalis* (clinical isolate), and *Candida lusitaniae* (clinical isolate) membranes after 2 h treatment with At5 at $1/4 \times$, $1/2 \times$, and $1 \times$ MIC in contrast to the intact membrane surfaces of respective controls incubated with SDA medium.