Electronic Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Human Cathelicidin Peptide LL37 Binds Telomeric G-Quadruplex

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1. MATERIALS AND METHODS

Commercially synthesized LL37 and LL37AA were purchased from G.L. Biochem (Shanghai, China) and purified using reverse phase HPLC system (SHIMADZU, Japan) using Phenomenix C_{18} column (Dimension 250×10.0 mm, pore size 100Å, 5 µm particle size) by linear gradient elution using dual solvent system (Water and Acetonitrile) containing 0.1 % TFA. Characterization of peptides was done by NMR spectroscopy and MALDI-TOF. All other chemicals used are of reagent grade from Acros organics and Sigma.

1.1 Circular Dichroism (CD) Spectroscopy

The secondary structure of G-Quadruplex DNAs and LL37 were measured using Jasco 815 spectrometer. The solutions were prepared using 10 mM phosphate buffer containing 100 mM KCl and 10 mM EDTA at pH 7.0. CD spectrums were recorded at room temperature by titrating LL37 with DNA in one phase and DNA with LL37 in another phase. Spectra were obtained with accumulation of three scans, at a speed of 100nm/min, scanned over a range of 210-320 nm and 205-260 nm for DNA and peptide, respectively with data interval of 1 nm. Cuvette of path length 0.1 cm and 0.5 cm were used. The spectra obtained were baseline corrected. The data obtained in millidegrees (θ) were converted to molar ellipticity ($deg.cm^2.dmol^{-1}$) by using the equation: Molar ellipticity = $m_oM/10\times L\times C$, where m_o is millidegrees, M is molecular weight (g/mol), L is path length of cuvette (cm) and C is concentration (g/L). 10 µM of DNA was titrated with increasing concentration (1-10 µM) of LL37. The 10 µM LL37 was titrated with increasing concentration (1-3µM) of DNA. CD melting studies were performed with DNAs and DNA:Peptide 1:1 ratio from 10-90 ^{0}C temperature range separately. Temperature interval and Ramp rate were 5 ^{0}C and 2.5 ^{0}C/min, respectively.

1.2 Fluorescence Spectroscopy

All fluorescence experiments were carried out using Hitachi spectrophotometer (F-700 FL spectrophotometer). Fluorescence spectra were measured using 0.1cm cuvette. All DNAs and peptide were dissolved in 100 mM KCl, 10 mM EDTA at pH 7.0 for fluorescence measurement. Excitation and emission slit were 5 nm. Response was 0.5s. The binding interaction of DNA and peptide were measured from 300-500nm with excitation wavelength of 260nm. Initial concentration of four DNAs was 30 µM. DNAs were titrated with increasing concentration (5-60µM) of LL37. Quenching of G-Quadruplexes were measured with free DNAs and DNA bound with LL37 by titrating increasing concentration (0.05-0.5 M) of acrylamide.

1.3 UV Spectroscopy

All UV absorbance spectra of four G-Quadruplex DNAs and LL37 were measured by Simadzu UV spectrophotometer (UV 1700 series). G-Quadruplex DNAs and LL37 were dissolved in 10 mM phosphate buffer containing 100 mM KCl, 10 mM EDTA at pH 7.0. The 2 µM of G-Quadruplex DNAs were titrated with LL37 (1-4 µM), scanned over range 230-350 nm.

1.4 Transmission Electron Microscopy (TEM)

100nM of Peptide LL37, 100nM DNA P4 and 1:2 mixture (volume/volume) of 100 nM DNA - P4 and 100 nM Peptide LL37 solutions were prepared in buffer containing 10 mM phosphate, 100 mM KCl, 10 mM EDTA at pH 7.0 and incubated for 10 days at 37°C. The 10 µL aliquot of each samples were placed on a 400 mesh carbon coated copper grids. After 1 minute, excess fluid was removed and the grids were stained with 2% uranyl acetate in water. Excess staining solution was removed from the grids after 30 second. Samples were viewed using a TECNAI G2 SPIRIT BIOTWIN CZECH REPUBLIC 120 KV electron microscope operating at 80 kV.

1.5 Molecular Dynamics (MD) simulations

MD simulation for 30 ns timescale was carried out in Desmond[1] using Amber ff99SB force field.[2] Explicit solvation of complex was done in an orthorhombic system using pre-equilibrated TIP3P water model[3] (7140 molecules) with requisite Na^+ counter ions for neutralization. Systematic adaptation of the model to the solvated condition was accomplished through an initial minimization with convergence threshold of 1.0 $kcal.mol^{-1}$. The model system was relaxed prior to NPT ensemble simulation run with NVT ensemble – Berendsen thermostat with restraints on heavy atoms (temp 10 K, 12 ps), NPT ensemble – Berendsen thermostat and barostat (temp 10 K, pressure 1 atm, 12 ps), NPT ensemble – Berendsen thermostat (temp 300 K, pressure 1 atm, 24 ps) with and without restraints on heavy atoms.[4] SHAKE[5] algorithm was employed for constraining the bond length involving hydrogen atoms with reference to their equilibrium positions. Newton's equation of motion for every atom was integrated with a time step of 2 fs. A cut-off of 10 Å was used for accounting the long range non-bonded interactions. [6]The trajectory was recorded at an interval of 5 ps in simulation time course.

2. Figure Legends

Figure S1. UV spectra of G-Quadruplex DNAs (2 µM) vs LL37 with concentration ranging from 1 µM to 4 µM. **A-D**: Individual spectra of P1, P2, P3 and P4, respectively. All the experiments were performed in 10 mM phosphate, 100 mM KCl and 10 mM EDTA (pH 7.0).

Figure S2. Fluorescence spectra of G-Quadruplex (30 µM) with LL37 (varying concentration of 5 µM to 60 µM). **A-C:** Obtained blue shift is in range of 16, 16 and 12 nm for P1, P2 and P3, respectively. **D-F:** A plot of Fo/F (Fluorescence quantum yield) vs acrylamide concentration shows that at the free statethe G-quartet face is more exposed compared to that of the bound state of LL37. All the experiments were performed in 10 mM phosphate, 100 mM KCl and 10 mM EDTA (pH 7.0).

Figure S3. A-C: CD spectra of G-Quadruplex (10 µM) titrated with LL37 concentration ranging from 1 µM to 10 µM for P1, P2 and P3, respectively. **D-F:** CD spectra of LL37 (10 µM) titrated with G-Quadruplex concentration ranging from 1 µM to 3 µM for P1, P2 and P3, respectively. **G, I and K:** CD Melting spectra of G-Quadruplex (10 µM) with varying temperature ranging from 10 to 90 ^{0}C for P1, P2 and P3, respectively. **H, J and L** showed CD Melting spectra of G-Quadruplex in presence of LL37 with 1:1 ratio with varying temperature ranging from 10 to 90 ^{0}C for P1, P2 and P3, respectively. All the experiments were performed in 10 mM phosphate, 100 mM KCl and 10 mM EDTA (pH 7.0).

Figure S4. Fluorescence Resonance Energy Transfer (FRET) of G-Quaduplexes (A) P1, (B) P2, (C) P3 and (D) P4 in presence of N-terminal densylated-LL37. The increase in emission intensity at 490 nm with the increase of denylated-LL37 clearly indicates the proximity of LL37 towards the G-face of the G-quartet. As the emission intensity at 337 nm increases with increase in densylated-LL37, the Fröster distance calculation is not possible. This anomolous increase in fluorescence intensity at 337 nm is due to exposure of adenine residues in solution side by side. It is noteworthy to mention that the G-quartet and adenine residues fluoresce at ~ 330-340 nm range, it is impossible to disect individual's contributions in the fluorescence emission spectra. All the experiments were performed in 10 mM phosphate buffer containing 100 mM KCl and 10 mM EDTA (pH 7.0).

Movie S1. H-Bonding of **Arg7** and **Arg23** with phosphate backbone of G-Quadruplex P4 as a key feature in LL37 - G-Quadruplex interaction. **Arg7** drives out to interact with the phosphate backbone in the initial phase of simulation. Both **Arg7** and **Arg23** are attached to the phosphate backbone throughout the 30 ns simulation.

Movie S2. Conformational change of α-helical LL37 to unstructured secondary structure thereby adopting circular necklace like structure goverened by the interaction with G-Quadruplex P4. The necklace like circular structure is stabilized and exist for 15-30 ns in the simulation time course.

Movie S3. Salt-bridge formation between **Leu1** and **Glu36** after 15 ns simulation time and stabilization of the salt-bridge till the end of the simulation (15-30ns).

3. Figures

Figure S1

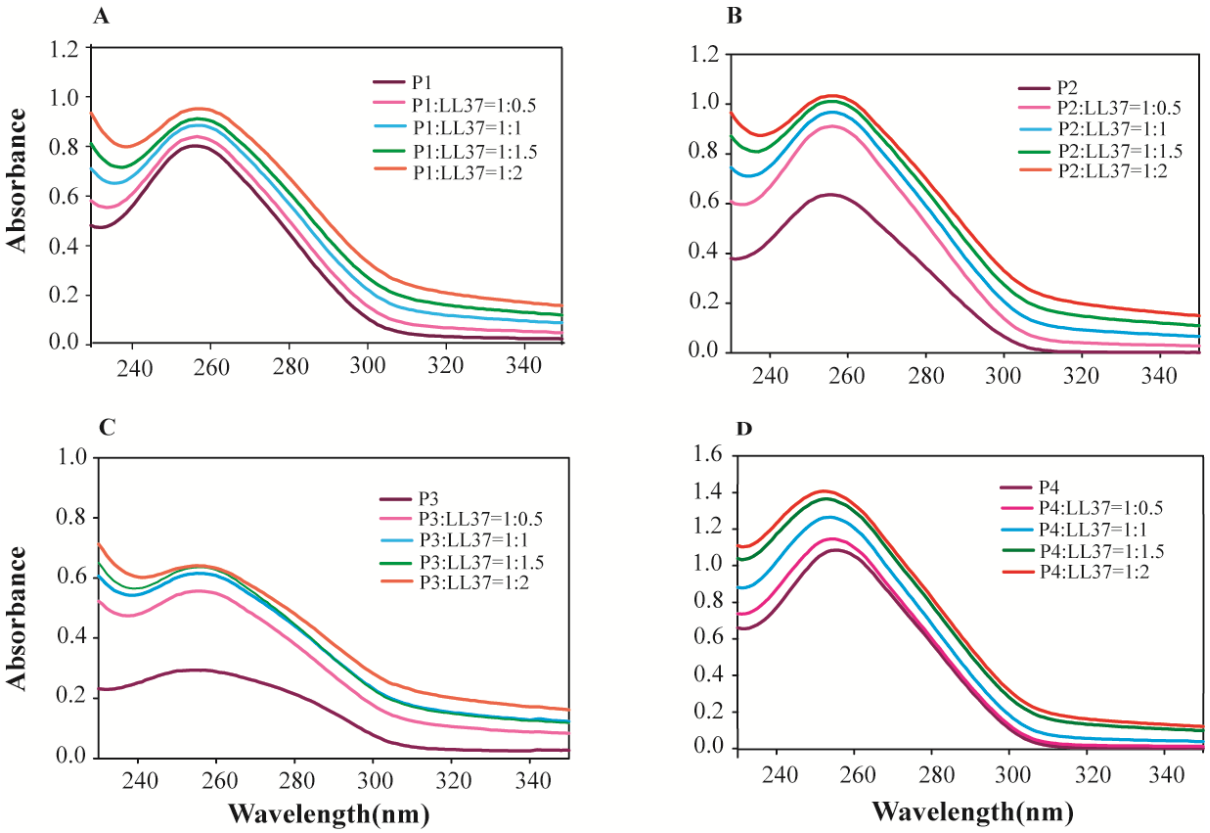


Figure S2

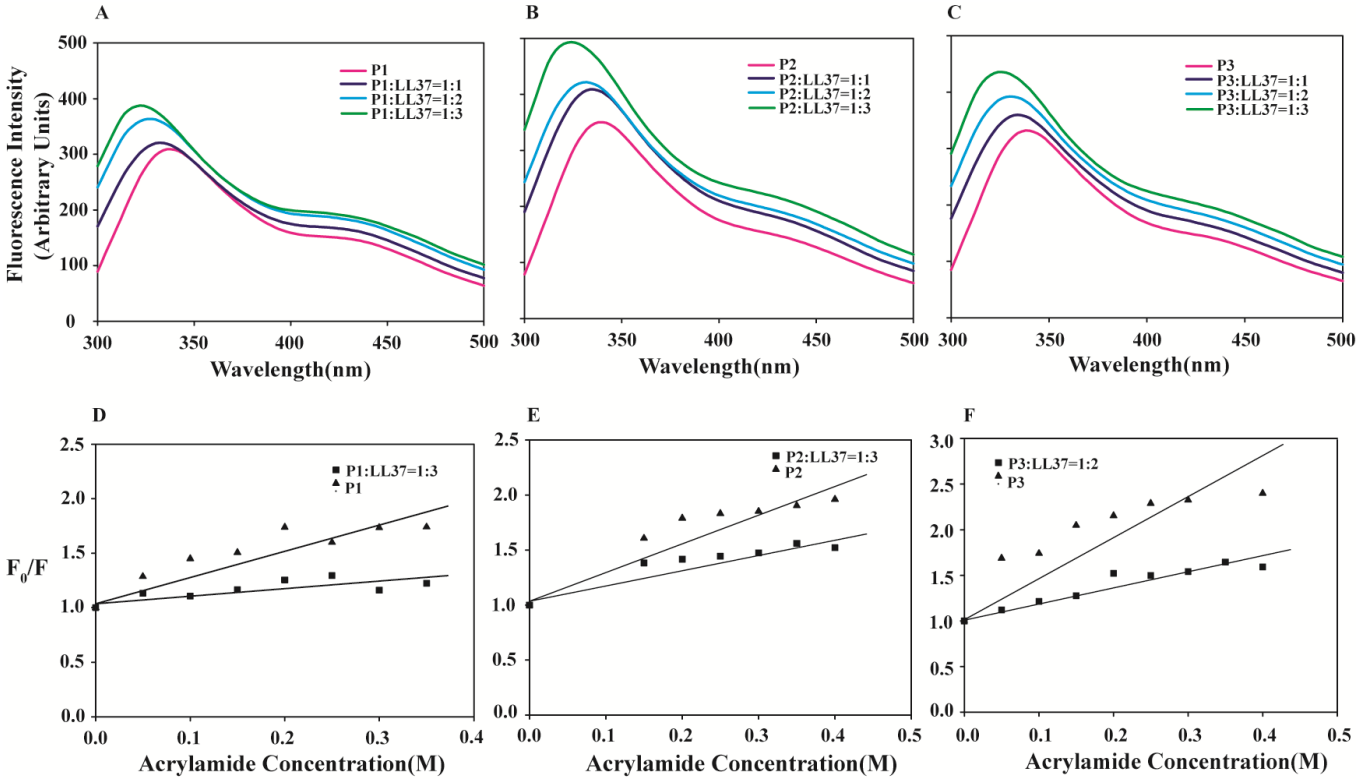


Figure S3

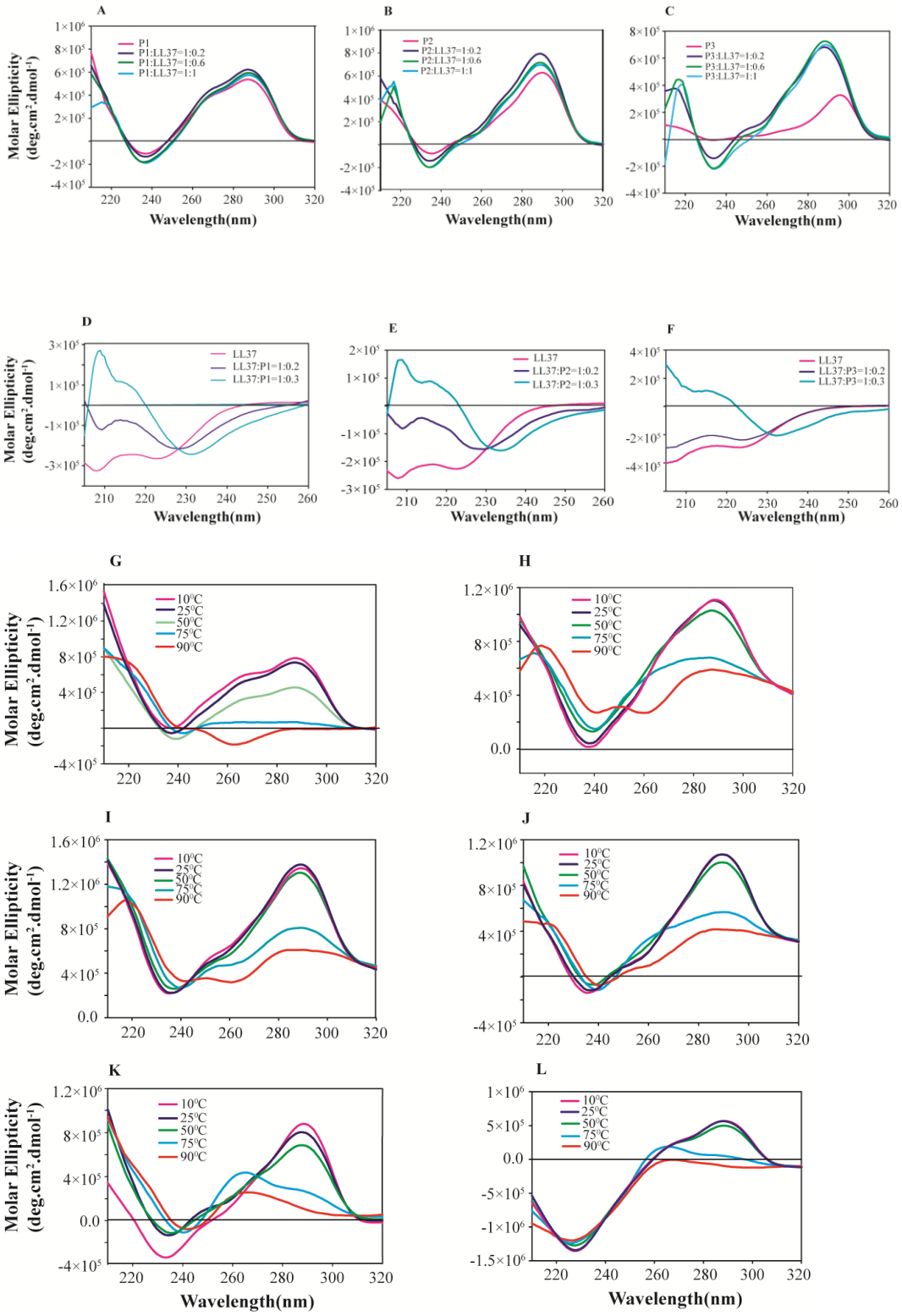


Figure S4

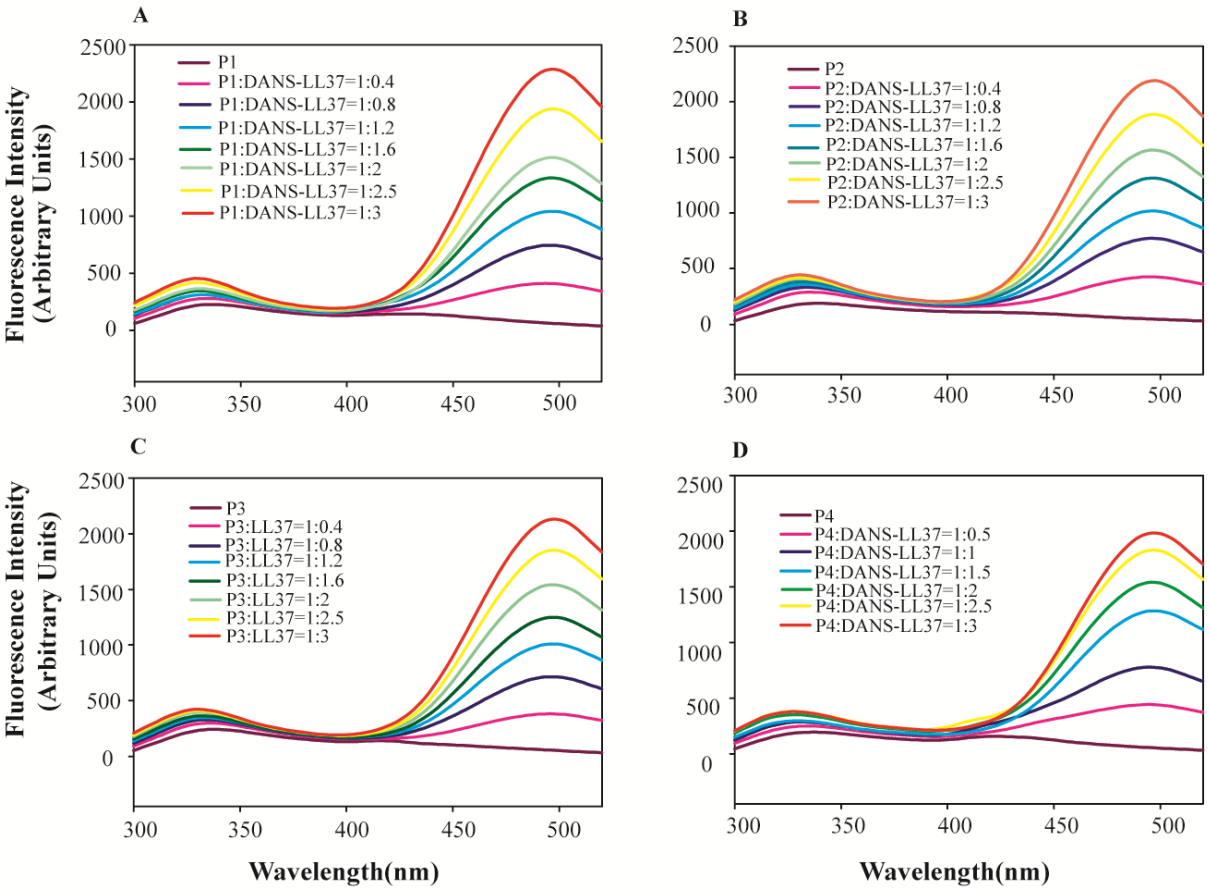
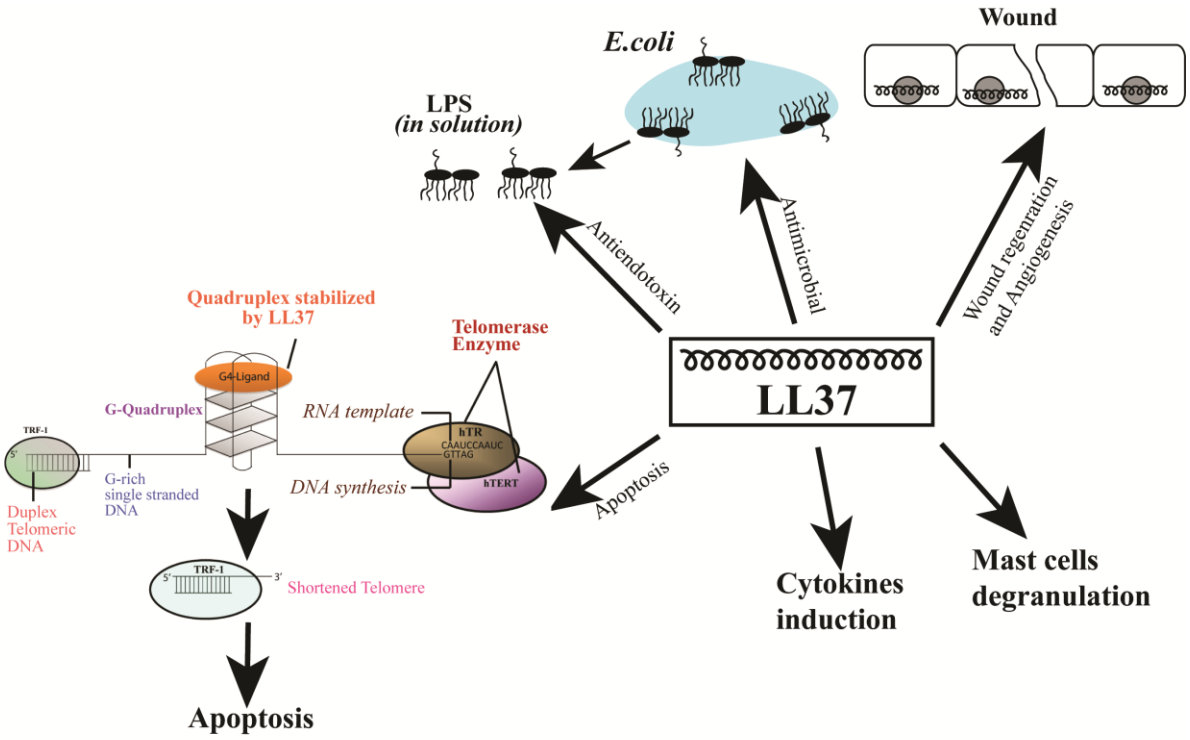


Table S1. Fluoremetric paramaters of LL37 – G-Quadruplex binding.

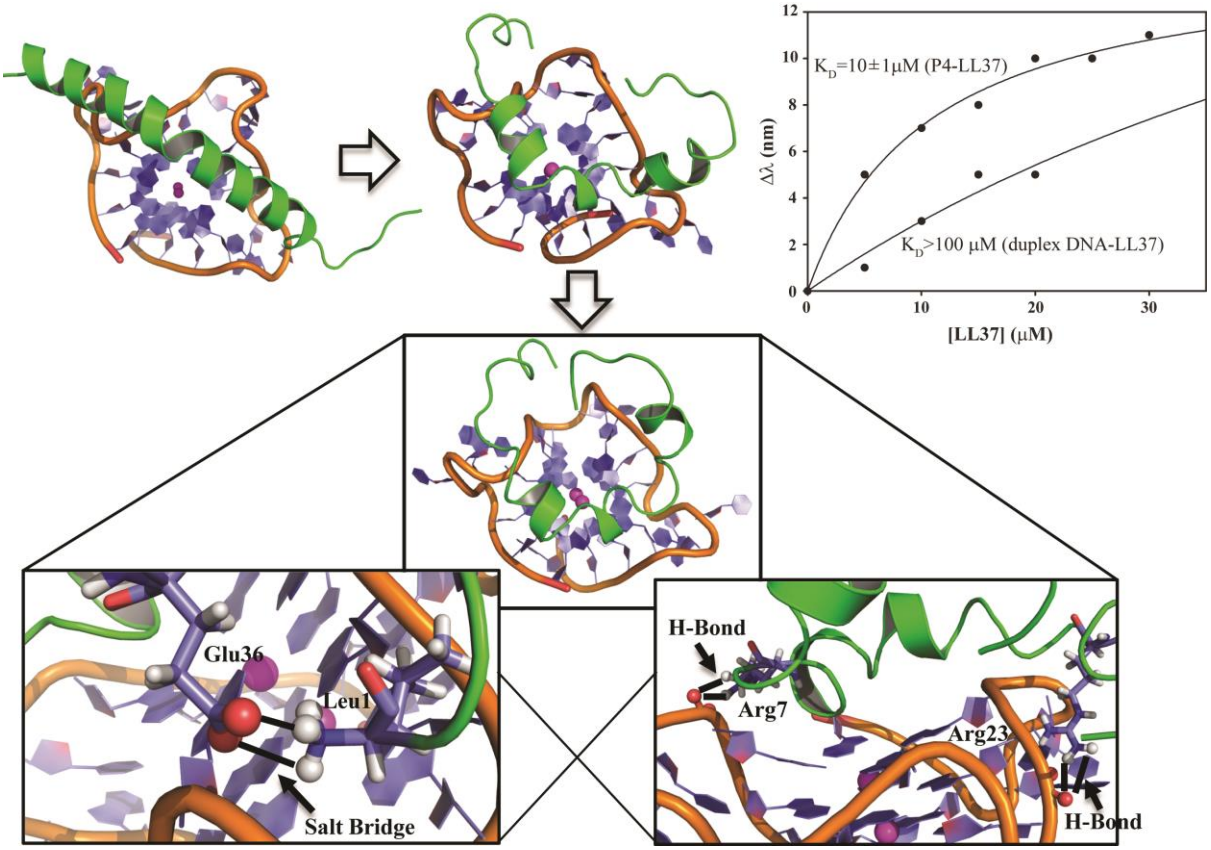
	LL37				LL37AA
Sequences	**BS[a]**	**K_{sv1}[b]**	**K_{sv2}[c]**	**K_D (μM)[d]**	**K_D (μM)[e]**
Duplex DNA $(GCGCATGCTACGCG)_2$	-	-	-	>100	-
P1[$TAGGG(TTAGGG)_3TT$]	16	2.6	0.8	37.1±9.7	70.7±20
P2[$GGG(TTAGGG)_3T$]	16	2.6	1.5	42.9±14	67.0±18
P3[$GGG(TTAGGG)_3TT$]	14	4.2	1.8	24.4±7.6	79.6±38
P4[$TAGGG(TTAGGG)_3$]	14	7.0	1.8	10.5±1.7	38.0±18

[a]BS=Blue Shift(nm); [b]Stern-Volmer co-efficients of free G-Quadruplex DNAs; [c]Stern-Volmer co-efficients of G-Quadruplex DNAs bound with LL37; [d]Binding constant of G-Quadruplex DNAs with LL37 (μM) ; [e]Binding constant of G-Quadruplex DNAs with LL37AA (μM).

Scheme S1. Biological action of LL37 and proposed mechanism of its apoptotic function.



TOC Graphic



4. References

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