Appendix

Materials and Methods
**A.1 Reagents and sample preparation:**

*E. coli* 0111: B4 LPS and LPS from *P. aeruginosa* serotype 10 were purchased from Sigma-Aldrich Co. (St. Louis, Mo.). Bovine Lactoferrampin (WR17, W268-R284), Indolicidin (IR13), Esc(1-21) and Esc(1-21)-lc were purchased from GL Biochem (Shanghai, China). All other peptides were synthesized in Solid Phase Peptide synthesizer (Aapptec Endeavor 90) using Fmoc protected amino acids and Rink Amide MBHA resin (substitution 0.69 mmol/g; Novabiochem, San Diego, California) by following a solid phase peptide synthesis protocol described elsewhere.\(^1\) \(^2\) Synthesized peptides were further purified by reverse phase HPLC system (SHIMADZU, Japan) using Phenomenix C\(_{18}\) column (dimension 250 × 10 mm, pore size 100 Å, 5-μm particle size) by linear gradient elution technique using dual solvent system (Water and Acetonitrile) containing 0.1 % TFA. Molecular weight and purity of the peptides were confirmed using MALDI-TOF and NMR. DNA duplexes, GG28 [d(5'-GCGCATGCTACGCG-3')\(_2\)] and GC28 [d(5'-GCGCATATTATGC-3')\(_2\)] were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India. HPLC-pure DNA sample was dissolved in aqueous phosphate buffer (20 mM sodium phosphate, 1 mM EDTA, and 50 mM NaCl) of pH 7.2 and annealed at 80 °C for 5 minutes in a water-bath, cooled down slowly to room temperature overnight. Reagents like 4, 4-dimethyl-4-silapentane-1-sulfonic acid (DSS) and deuterium oxide (D\(_2\)O) were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). Thioflavin T dye was purchased from Sigma-Aldrich (St. Louis, MO).

Cell culture dimethyl sulfoxide (DMSO), 4, 6-diamidino-2-phenylindole (DAPI), β-casein, Dulbecco Modified Eagle Medium (DMEM), Kanamycin sulfate, Trypsin-EDTA solution, Sodium Chloride, Potassium Chloride and fetal bovine serum were purchased from Sigma-Aldrich. 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES) was purchased from Himedia. Penicillin-Streptomycin and Neutravidin were purchased from Invitrogen. Sodium bicarbonate was purchased from Merck. Rhodamine-avidin dye was purchased from Vector Laboratories. Cover glass bottom dishes were purchased from SPL Life Sciences, Korea. All the materials were used without further purification. A549 (Adenocarcinomic human alveolar basal epithelial cells) cell line was purchased from NCCS, Pune (India) and cultured in our lab. All other chemicals and reagents were obtained from Sigma (St. Louis, MO) unless specified. Throughout the experiment, HPLC grade water was used for sample preparation. hIAPP was purchased from Genscript (Piscataway, NJ).

hIAPP was prepared by dissolving the peptide in hexafluoroisopropanol (Sigma-Aldrich) followed by lyophilization. Peptides stock was dissolved in 100 μM HCl (pH 4), sonicated for 1 min and kept on ice before use. Unlabeled samples of Aβ\(_{1-40}\) were purchased either from Genscript Inc. (Piscataway, NJ, USA) or GL Biochem (Shanghai, China) with >95 % purity. Uniformly \(^{15}\)N-labeled Aβ\(_{1-40}\) was purchased from rPeptide (Bogart, GA). The uniformly \(^{15}\)N/\(^{13}\)C-labeled Aβ\(_{1-40}\) was biologically produced as described elsewhere.\(^1\) To remove preformed aggregates, the purified peptide was dissolved in 1% ammonium hydroxide (v/v) at a concentration of 1 mg/ml followed by removal of the solvent by lyophilization for 24 hours in aliquots of 0.1 mg. The aliquoted peptide was then stored at -20 °C and used only once. Preparation of a primarily monomeric Aβ\(_{1-40}\) sample was performed as described previously. Briefly, 0.1 mg of the lyophilized peptide was first dissolved in 3 μl of DMSO and sonicated until the peptide was solubilized. The peptide solution was then dissolved in H\(_2\)O (or 10% D\(_2\)O for NMR measurements), 20 mM phosphate buffer, pH 7.4, 50 mM NaCl and diluted to a final peptide concentration of 80 μM. The peptide concentration was confirmed by UV absorbance at 280 nm (\(ε_{280} = 1490 \text{ M}^{-1} \text{ cm}^{-1}\)). For seeded NMR experiments, a fibrillar Aβ\(_{1-40}\) sample was prepared as...
described previously. The peptide was dissolved directly into a solution of 50 mM NaCl, 20 mM phosphate buffer with pH 7.4, and 0.01% NaN₃. The peptide concentration was adjusted to 150 μM (confirmed by UV absorbance). The sample was then kept at 37 ºC with agitation to produce samples to be used as preformed fiber seeds.

A.2 Biological Experiments

A.2.1 Antimicrobial activity assays

Bacterial Cells used for this assay, e.g. Bacillus subtilis, Xanthomonas campestris and Pseudomonas aeruginosa ATCC 27853, were cultured in Muller-Hinton (MH) broth at overnight. Overnight cultures were reinoculated and incubated for three hours at 37 °C to obtain log phase culture. Cells were centrifuged and washed twice with the assay buffer (10 mM sodium phosphate buffer, pH 7.4) and diluted to 10⁵ cells. 50 μl of the above cell suspensions were incubated for 5 hours at 37 °C, in a sterile 96-well microtiter plate, with an equal volume of peptides at various concentrations, ranging from 1 to 100μM, diluted from a stock solution of 1 mM (prepared in assay buffer). 200μL MH broth was added to each well and incubated overnight. Absorbance was measured at A₆₀₀. The minimum inhibitory concentration was expressed as the lowest concentration of the peptide where there was no growth of the bacteria.

A.2.2 ELISA

Enzyme-linked immunosorbent assays were performed to estimate the secreted TNF-α and IL-6 in LPS-treated cells in the presence of KR7 and after a 1.5hrs (TNFα) and 4hrs (IL6) incubation. Levels of these cytokines in the culture supernatant of untreated and LPS-treated cells were taken as positive and negative control. Cytokines and chemokines in culture supernatants of the experimental cells and mouse serum were measured by ELISA using ELISA kit (eBioscience) as per the manufacturer’s instructions.

A.2.3 Calcein leakage assay

This assay was performed according to the protocol published elsewhere.[³] In brief, POPC and POPG were mixed in a molar ratio of 3:1 in 2:1 chloroform/methanol solution to a mass of 20 mg. After that, it was lyophilized overnight and hydrated with calcein solution containing 70 mM calcein and 10 mM Tris-HCl, pH 7.0 and incubated at 40 ºC in water bath for an hour. Next, vortexing the lipid suspension created multilamellar vesicles which were sonicated for three minutes. The vesicle was centrifuged at 14,000 rpm for 10 minutes and extruded through two stacked 50 nm polycarbonate filters for 25 times. Free calcein was removed passing through hydrated Centri Sep spin columns. An iridescent light orange suspension was collected followed by centrifugation at 750 g for 2 min. The final concentration was of 50 μM in 600 μl of buffer containing 10 mM Tris HCl and 100 mM NaCl, pH 7.4. Fluorescence was measured in a Hitachi F-7000 FL spectrometer in a quartz cuvette having path length of 0.1 cm and bandwidth of 2.5 nm at 25 ºC. The excitation and emission maxima were 490 nm and 520 nm, respectively. After stabilization of calcein fluorescence, peptides were added in increasing concentration ranging from 5 μM to 35 μM and fluorescence intensity increase due to calcein leakage was measured after 5 minutes of incubation. 5 μl of 10 % (v/v) Triton X 100 was used as a positive control to yield the maximum fluorescence intensity or in other words maximum leakage (F_T). Percentage of leakage or SUV permeabilization (P) was measured using the formula as follows:

\[ P = \frac{(F-F_0)}{(F_T-F_0)} \times 100 \]
Where \( F_0 \) is the initial basal fluorescence intensity, \( F \) is the final fluorescence intensity, 5 minutes after addition of peptide and \( F_T \) is the maximal fluorescence intensity after addition of Triton-X 100.

### A.2.4 NPN dye uptake assay

Midlog phase cells of *E. coli* BL21(DE3) (OD\(_{600}\) of 0.5), obtained from an overnight culture of stationary phase cells in LB, were used in this assay. The cells were pelleted by centrifuging at 8000 rpm for 10 min at 25 °C and washed with 10 mM phosphate buffer of pH 7.4 twice and then re-suspended in the same buffer. A hydrophobic dye 1-N-phenylnaphthylamine (NPN) was added to the re-suspended cell with a concentration of 10 μM (prepared in acetone) and was allowed to stabilize for 30 minutes. Peptides were added to cells in increasing concentrations, between 5 μM and 30 μM and the increase in NPN fluorescence, due to permeabilization of the outer membrane was measured on a Hitachi F-7000FL spectrophotometer. NPN fluorescence was measured at an excitation wavelength of 350 nm and a bandwidth of 5 nm and scanning its emission maximum at 410 nm. The baseline fluorescence of the free NPN was subtracted from the total fluorescence for each peptide, and percentage increase of NPN fluorescence was calculated in each case, considering Polymyxin B (10 μl of 0.64 μg/ml) to show 100 % increase in NPN fluorescence.\(^3,4\)

### A.2.5 Neutralization of endotoxin

Neutralization of endotoxin by the designed peptides was estimated using a limulus amoebocyte lysate (LAL) with a Lonza PYROGENT\textsuperscript{TM} Plus kit. The protocol was followed to perform this experiment as per the guidance provided in the kit. Briefly, peptide stocks were prepared in pyrogen-free water given in the kit. 1, 5, 10, 15, and 25 μM peptides were then incubated with endotoxin, in a final volume of 100 μl, at three different concentrations or endotoxin units (EU) of LPS of *E. coli* O55:B5, that were 0.125, 0.25, 0.5, and 1EU/ml (1 EU =0.13 ng of LPS) at 37 °C for 30 min to allow peptide binding to LPS.\(^5\) This was next added to an equal volume of lysate and the mixture was further incubated for one hour. Formation of a clot (firm gel) that remains intact momentarily upon inversion of tube characterizes a positive reaction. This suggests the presence of endotoxin and consequently the absence of any neutralization. The experiments were repeated thrice, and the average values have been reported.

### A.3 Optical Spectroscopy

#### A.3.1 Fluorescence Spectroscopy

All the fluorescence experiments were performed using Hitachi F-7000 FL spectrometer with a 0.1 cm path length quartz cuvette at 25 °C. Excitation and emission slit were set to 5 nm. All the peptides and LPS were dissolved in 10 mM Phosphate buffer at pH 6.0/7.2. The molecular mass of 10 KDa was considered for LPS.\(^6\) The intrinsic fluorescence of Tryptophan was used to determine the binding interaction of the peptide with LPS using excitation wavelength of 280 nm and emission in a range of 300-400 nm. Increasing concentration of LPS (ranging from 0 to 20 μM) was titrated against 5 μM peptides. Binding constant (equilibrium dissociation constant, \( K_D \)) of the peptides with LPS were measured by plotting difference in emission maxima as a function of LPS concentration using standard single-site binding curve fitted to following equation:

\[
f = B_{\text{max}}L \cdot (K_D + L)^{-1}
\]
where, \( f \) = fractional saturation of the peptide with respect to LPS expressed in terms of difference in wavelength, \( \Delta \lambda_{\text{max}} = \lambda_{\text{max}} - \lambda(0)_{\text{max}} \), \( \lambda_{\text{max}} \) = emission maxima of the peptide on successive addition of LPS in nm, \( \lambda(0)_{\text{max}} \) = emission maxima of the peptide without addition of LPS in nm, \( L = \) ligand (LPS) concentration (\( \mu \)M), \( K_D = \) Equilibrium Dissociation constant (\( \mu \)M).

Quenching experiments were performed with free peptides and LPS bound peptides on adding increasing concentration of acrylamide (ranging from 0-0.5 M). Stern-Volmer’s constant (\( K_{sv} \)) was calculated using the equation:

\[
\frac{F_0}{F} = 1 + K_{sv} [Q]
\]

where \( F_0 \) = fluorescence intensity in the absence of quencher, \( F \) = fluorescence intensity in the presence of quencher at each titration, \( [Q] \) = concentration of quencher in molarity.

Red Edge Excitation Shift (REES) experiment was performed for the free peptide and LPS bound peptide to understand solvent accessibility. The excitation wavelength was varied from 280 to 310 nm and emission profile was monitored in the range of 320-400 nm in each case keeping other parameters constant. For each excitation wavelength, the emission maxima were plotted for free peptide and LPS bound peptide.

Steady-state anisotropy was recorded with a Hitachi model F-7000 FL spectro-meter equipped with a polarization accessory. Anisotropy values were calculated based upon the intrinsic Trp fluorescence property. The fluorescence anisotropy (\( r \)) values were obtained using the following equation:

\[
r = \frac{I_{VV} - G*I_{VH}}{(I_{VV} + 2*G*I_{VH})}
\]

where \( I_{VV} \) and \( I_{VH} \) are the vertically and horizontally polarized components of the probe with excitation by vertically polarized light at 280 nm. \( G \) is the sensitivity factor of the instrument. The slits for excitation and emission were set to 5 nm. Each peptide of 5 \( \mu \)M was titrated with increasing concentrations of LPS up to 30 \( \mu \)M.

To monitor GG28 and IR13 binding interaction, 10 \( \mu \)M N-ter FITC-Labelled IR13 was titrated with an increasing concentration of GG28 up to 25 \( \mu \)M. All the experiments were performed in 10 \( \mu \)M in 20 mM sodium phosphate buffer (pH 7.2), containing 1 mM EDTA and 50 mM NaCl. FITC (5(6)-fluorescein isothiocyanate), the labeling group was excited at 485 nm and emission profile was monitored at 500-600 nm range.\(^7\) The % enhancement of fluorescence emission intensity of FITC-IR13 was calculated using equation (1) and plotted against GG28 (concentration in \( \mu \)M) to calculate the equilibrium dissociation constant (\( K_D \)).

\[
\% \text{ Enhancement of fluorescence intensity} = \frac{(F-F_0)}{F_0} \times 100\%
\]

Where, \( F \) = emission intensity maxima of the FITC labeled IR13 for each successive addition of GG28, \( F_0 \) = emission intensity maxima of the FITC labeled IR13 without the addition of GG28. To obtain the \( K_D \) value, two independent single site binding equations were used to fit each of the two phases in the resulted biphasic curve.

To determine whether IR13 binds to the minor groove of GG28, a minor groove binding fluorescent stain 4’, 6-diamidino-2-phenylindole (DAPI), was used which binds selectively A-T rich minor groove of Duplex DNA.\(^8\) The excitation wavelength was 372 nm and emission profile was monitored at 400-520 nm.
Intrinsic tyrosine fluorescence property was used to monitor the self-assembly property for tyrosine containing peptides TK9, TY5, YR5 using an excitation wavelength of 274 nm and emission in a range of 290-370 nm. The excitation and emission slit both were 5 nm. The peptide concentrations were 25 µM throughout the experiment. Thioflavin-T experiments were performed on a BioTek multiplate reader using an excitation wavelength of 440 nm and an emission wavelength of 485 nm. Samples were prepared by adding hIAPP (10 µM) to a buffer solution (10 mM phosphate, 150 mM NaCl, pH 7.4) contain ThT (20 µM) and varying concentrations of TK9 monomer. TK9 aggregates for the ThT assay were prepared by incubation at 37 °C for 7 days.

A.3.2 Measurement of Depth of Insertion of the Peptides into LPS by spin-labeled lipids

LPS bilayer vesicles (LUVs) were prepared and characterized as described elsewhere. Approximately 5 mg of E.coli 0111: B4 LPS was dissolved in 2:1 (v/v) chloroform/methanol solution and it was dried under a nitrogen atmosphere. Trace of the organic solvent was evaporated to dryness under vacuum pump. After that the lipid film was hydrated with 10 mM phosphate buffer (pH 6.0) at 60 °C in a water bath and vortexed repeatedly for 30 minutes. Next, the lipid suspension was immersed in liquid nitrogen followed by water in a water bath at 60 °C for 10 cycles and extruded through a 0.1 µM membrane with the extruder (Avanti Polar Lipids, Alabaster, AL) for 10 times. 5-DSA (shallow quencher) and 16-DSA (deep quencher) stock solutions were prepared in methanol solution and subsequently added to the solution containing 5 µM WR17 and WG12 with 40 µM LPS vesicles. The location of Tryptophan in the LPS bilayer (Zcf) was determined from the following equation using Parallax analysis method.

\[
Z_{cf} = L_{c1} + \left( \frac{-1}{1 + \pi C \ln(F_1/F_2) - L_{21}} \right)^2 / 2L_{21}
\]

Where \(L_{c1}\) is the difference in depth between the shallow quencher and the bilayer center, \(F_1\) and \(F_2\) are the relative Trp fluorescence intensities in the presence of the shallow and deeper quencher, respectively, \(L_{21}\) is the difference in depth between the shallow and deeper quencher, \(C\) is the mole fraction of quencher per unit area assuming the surface area of the LPS bilayer to be 70 Å.

A.3.3 UV-visible Spectroscopy

All absorbance spectra were measured on a Hitachi U-2910 double beam double monochromator spectrophotometer at 25 °C in 1 cm path length quartz cuvettes. To understand whether IR13 binds to the major groove of GG28, we performed UV-visible spectroscopy by using a major groove binding dye methyl green. Briefly, methyl green was mixed with GG28 in 1:1 molar ratio and its absorption spectrum were scanned in the range of 500 to 700 nm. The change in the absorption at the \(\lambda_{max}\) of the dye (632 nm) was monitored at each successive addition of peptides (IR13 and IR13AA) and plotted against peptide concentration. All the experiments were performed in 20 mM sodium phosphate buffer (pH 7.2), containing 1 mM EDTA and 50 mM NaCl.

A.3.4 Circular dichroism spectroscopy

Peptide secondary structure was determined using Jasco J-815 spectrometer. Solutions were prepared using 10 mM phosphate buffer at pH 6.0. CD spectra were recorded at 25 °C by titrating the increasing concentration of LPS (25-50 µM) against peptides (25 µM). Spectra were obtained with an accumulation of three scans, at a speed of 100 nm.min\(^{-1}\) over a range of 190-260 nm, at 1 nm data interval. Quartz cuvette of path length 0.2 cm and 0.5 cm were used. The buffer subtracted spectral data obtained in milli-degrees were converted to molar ellipticity (\(\theta\)) (deg.cm\(^{-2}\).dmol\(^{-1}\)), using following equation:

\[
\text{Molar ellipticity (}\theta\text{)} = m_0M/10 \times L \times C
\]
where \( m_0 \) is milli-degrees, \( M \) is molecular weight (g.mol\(^{-1}\)), \( L \) is path length of cuvette (cm) and \( C \) is concentration (M).

The initial concentration of DNA was 10 \( \mu \)M in 20 mM sodium phosphate buffer (pH 7.2), containing 1 mM EDTA and 50 mM NaCl. This is used for subsequent titration with varying concentrations of IR13 ranging from 5 to 20 \( \mu \)M. The samples were scanned over the range of 210 to 320 nm at a scanning speed of 100 nm.min\(^{-1}\). Data points were taken at an interval of 1 nm, averaged over 4 repetitive scans using 2 mm path length quartz cuvette. The data obtained in millidegree were converted to molar ellipticity (ME) (deg.cm\(^2\).dmol\(^{-1}\)) for analysis.

CD Melting experiments were performed for GG28, GG28-IR13 and GG28-IR13AA/FF/HH complex at a 1:1 molar ratio using the same protocol stated above. The sample was heated from 5 to 95 °C with a heating rate of 2.5 °C. Min-1. Before data acquisition, the sample cell was equilibrated for 5 minutes at each temperature. The cuvette-holding chamber was flushed with a constant stream of dry nitrogen gas to avoid condensation of water vapor exteriorly over cuvette at low temperature. The fraction of GG28 in the duplex state was calculated using equation (2) and plotted against temperature.\(^{[12]}\) Melting Temperature (\( T_m \)) was calculated by fitting to a sigmoidal curve assuming two state models.

\[
\alpha = \frac{\theta_t - \theta_s}{\theta_d - \theta_s}
\]

Where, \( \theta_t \) = observed CD value in milli-degree at Temperature \( T \), \( \theta_d \) and \( \theta_s \) are the observed CD values in millidegree when DNA is in fully double stranded and single stranded conformation, respectively, i.e., at the starting and end temperature of the melting experiment. The following equation was used to fit the resulted duplex fraction (\( \alpha \)) against different temperatures (\( T \)), to obtain the melting temperature (\( T_m \)).

\[
\alpha = \frac{a}{1 + \exp\left(-\frac{(T - T_m)}{b}\right)}
\]

where \( a \), \( b \) are adjustable fitting parameters.

The thermodynamic parameters, i.e., \( \Delta H \), \( \Delta G \), \( \Delta S \) for the free DNA and DNA-peptide complex were calculated using van't Hoff equation by the literature.\(^{[13, 14]}\)

\[
d\ln K/dT = \frac{\Delta H}{RT^2}
\]

Where \( K \) = equilibrium constant at temp \( T \) in Kelvin, \( \Delta H \) = enthalpy change for the process, \( R \) = Universal gas constant.

**A.3.5 Dynamic Light Scattering (DLS)**

DLS experiments were performed using Malvern Zetasizer Nano S (Malvern Instruments, UK) furnished with a 4-mW He-Ne laser (\( \lambda \approx 633 \) nm) and 173° backscattering angle. 1 \( \mu \)M LPS and LPS and peptide at a molar ratio of 1:1 were prepared in 10 mM sodium phosphate (pH 6.0). All samples were filtered using 0.45-\( \mu \)M filter papers (Whatman Inc) and degassed before use and measured at 298 K using low volume disposable sizing cuvette. The viscosity (0.8924) and refractive index (1.330) of 10 mM sodium phosphate buffer were used for data analysis.

**A.4 Isothermal Titration Calorimetry (ITC)**

ITC was carried out to determine thermodynamics of binding of peptides with LPS using VP-ITC microcalorimeter (MicroCal Inc., Northampton, MA). All peptides and LPS were dissolved in 10
mM phosphate buffer at pH 6.0 and degassed. A sample cell is containing (volume ~1.5 ml) 10 µM LPS was titrated against peptides from a stock solution of 250 µM at 298 K. A total of 35 injections were carried out at an interval of 4 minutes with 5 µL of peptides aliquots per injection. The raw data was plotted using Micro Cal Origin 5.0 software supplied with the instrument. A single site binding model was used to analysis the association constant (K_A), change in the heat of enthalpy of reaction (∆H), free energy of binding (∆G) and entropy (∆S) were evaluated using the equations ∆G = -RT ln K_A and ∆G=∆H-T∆S, respectively.

A.5 Nuclear Magnetic Resonance (NMR) experiments

NMR experiments were performed on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm cryogenic probe or on a Bruker Avance III 500 MHz NMR spectrometer equipped with a 5 mm SMART probe or on a Bruker Avance 800 MHz spectrometer equipped with a 5 mm cryogenic probe. NMR experiments were performed at 10 °C or 25 °C. Data acquisition and data processing were carried out using Topspin™ v3.1 software (Bruker Biospin, Switzerland). Two-dimensional total correlation spectroscopy (TOCSY) and nuclear overhauser effect spectroscopy (NOESY) spectra of free peptides and peptide in LPS were acquired in an aqueous solution containing 10% D2O at pH 4.5 with peptide concentration 1 mM. TOCSY mixing time was 80 ms whereas four different NOESY mixing times, 80, 100, 150 and 200 ms were used for experiments. DSS (2, 2-dimethyl-2-silapentane 5-sulfonate sodium salt) was used as an internal standard (0.0 ppm). Two-dimensional trNOESY experiments were performed using 1 mM peptides by titrating various concentration of LPS ranging from 5 to 25 µM. A series of one-dimensional proton-decoupled 31P NMR spectra using the Bruker pulse program “zgpg30” were recorded on a Bruker AVANCE III 500 MHz NMR spectrometer to monitor LPS-peptide interactions at 298 K. The 31P NMR spectra of LPS of 0.5 mM concentration were acquired with 3,072 scans. The sample was prepared by dissolving 5 mg LPS in 1 ml Milli-Q water only, and pH was adjusted to 4.5. The LPS solution was then titrated with increasing concentrations of peptides.

The NOESY experiments for free DNA and DNA-peptide complex at 1:1 molar ratio were performed with four different mixing times, ca. 80, 100, 120 and 150 ms with 20 ppm spectral width. DQF-COSY spectra were acquired with a spectral width of 20 ppm in both the t2 and t1 dimensions, with 2048 (ω2) and 128 (ω1) data points and a saturation delay of 2.0 s. The inverse 1H-31P-correlation spectra were measured with the delay, τ, in the INEPT step adjusted to give JHP of 5 to 20 Hz, 256 scans, and 256 experiments. A series of one-dimensional temperature dependent 1H proton NMR spectra for both the free DNA duplex as well as the IR13 bound DNA at a molar ratio of DNA: IR13= 1: 1, were recorded at 500 MHz NMR spectrometer to measure the relative thermal stability of IR13 bound DNA in comparison to the iso-sequential free DNA. The 1D spectra were acquired using excitation sculpting scheme for water suppression. The acquisition parameters were, 20 ppm for spectral width and 128 transients, a relaxation delay of 2 s, and an acquisition delay of 1.5 s.

One dimensional relaxation experiments for protons namely, T1, T2, and T1ρ were performed on a 500 MHz Bruker Avance III spectrometer. The T1 experiments were performed with the inversion recovery delays of 50ms, 150 ms, 300 ms, and 450 ms, 600 ms, 800 ms, 1s, 1.2s, 1.4s, 1.6s, 1.8s, 2s, 3s, 5s, and 7s. The measurement for T2 using CPMG refocusing experiments was performed with increasing delays of 1ms, 3ms, 7ms, 12 ms, 15 ms, 18ms, 25ms and 50 ms. On-resonance T1ρ experiments were performed at the spin-lock field strengths of 3 KHz to 6KHz. R_ex was estimated
from the $R_{\text{ex}}$ value calculated at 3 KHz and 6 KHz using the formulae, $R_{\text{ex}} = R_{1p} (3 \text{ KHz}) - R_{1p} (6 \text{ KHz})$ and $R_{1p} = R_0 + R_{\text{ex}}$, where, $R_0$ is the T2 relaxation rate devoid of any contributions from the chemical exchange ($K_{\text{ex}}$). Here we made a valid assumption that the contribution of $R_{\text{ex}}$ is negligible at higher spinlock field (6 KHz) compared to a lower spin lock field (3 KHz).

Diffusion NMR spectra were acquired on a Bruker 500 MHz spectrometer equipped with a 5-mm smart probe head at 288 K. All diffusion measurements were recorded using a standard 1D stimulated echo pulse program with a bipolar gradient pulse for diffusion (“stebpgp1s191d”). In the subsequent measurements, the gradient strength was varied in 16 increments from 2% to 95% with 128 scans and 16 dummy scans for each experiment, using a relaxation delay of 2.0 s and 12 ppm spectral width. The diffusion time ($\Delta$) was 200 ms, and the duration of the gradient pulse, ($\delta$) was 1.5 ms. An exponential window function with 3 Hz line broadening was applied before Fourier transformation, followed by polynomial baseline correction using the Topspin software package (Bruker). To calculate diffusion coefficient (D) for free and LPS bound peptide peak intensities were collected at each gradient strength and fitted to the following exponential equation:

$$I = I_0 \exp \left[ -D \gamma^2 g^2 \delta^2 (\Delta - \delta/3) \right] = I_0 \exp(-K^2D) \quad (K \text{ is a constant factor})$$

Where $I_0$ and $I$ are the signal intensity in the absence and presence of a gradient pulse, $\delta$ is the duration of the gradient pulse, $\gamma$ is the gyromagnetic ratio of the nucleus, and $\Delta$ is diffusion time [18].

Saturation transfer difference (STD) NMR experiments for a sample containing TK9 (0.5 mM) and hIAPP monomers (10 $\mu$M) were carried at 25 ºC using a Bruker 600 MHz spectrometer equipped with a cryoprobe. The on- and off- resonance frequency was -1 and 40 ppm, respectively for the duration of 2 s with 128 scans. The 49 ms of the consecutive Gaussian pulse with an interval of 1 ms at 30/50 dB was used for this experiment.

Before adding a fibril seed to the NMR tube containing monomeric Aβ1-40, a 2D band-Selective Optimized-Flip-Angle Short-Transient (SOFAST) heteronuclear multiple quantum coherence (HMQC) spectra was acquired to compare differences immediately before and after titration with fibrillar Aβ. Resonance assignments of the monomer 2D $^1$H/$^{15}$N HMQC spectra were taken from the literature while the new peaks were assigned as described below. To monitor Aβ1-40 aggregation with atomic resolution, real-time 2D NMR experiments were performed by consecutively acquiring $^1$H/$^{15}$N SOFAST-HMQC spectra. Each $^1$H/$^{15}$N SOFAST-HMQC spectrum was obtained from 64 t1 experiments, 48 scans, 8 dummy scans, and a 0.1 s recycle delay. The spectral widths were 12 and 26 ppm, and the offsets were 4.7 and 118 ppm, for the proton and nitrogen dimensions, respectively. For seeded real-time 2D NMR experiments, Aβ1-40 fibril seeds were added to monomer solutions at concentrations of 5% up to 30% of the total peptide concentration. At 1:10 molar ratios of fiber to the monomer or lower and at a temperature of 25 ºC or lower, real-time 2D NMR experiments could be used to track the time-course intensity depletion of monomer peaks in a residue-specific manner. To achieve sequence-specific backbone resonance assignments, several triple resonance experiments HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH were performed in an 800 MHz Bruker spectrometer using uniformly $^{15}$N/$^{13}$C-labeled Aβ1-40 at a concentration of 80 $\mu$M in the presence of 10 mol% preformed fiber seeds in 50 mM NaCl, 20 mM phosphate buffer with pH 7.4. The HSQC peaks were identified for all the residues except V18, F19 and F20. The RDC experiments were acquired using the standard Bruker pulse program $hsqcfgpiaphsiwg$, with sensitivity enhancement and TPPI based quadrature.
detection along t1 dimension. The experiment was acquired as in-phase and antiphase spectra in an interleaved manner. The final high-field and the low-field spectra were obtained by the addition and subtraction of the interleaved in-phase and anti-phase spectra, respectively. Other experimental parameters include 128 t1 increments, 96 scans and 1s recycle delay. Two experiments were performed on the Aβ1-40 monomer sample (80 µM in 20 mM PO4 buffer pH 7.4, containing 50 mM NaCl) at 4 °C: one in the presence and another in the absence of a 10 mol% pre-seeded fibril. The data was processed using NMRPipe (6) and analyzed using SPARKY. The RDC values were obtained by finding the difference in N-H couplings measured from the fibril free and fibril seeded spectra.

A.6 Calculation of NMR-derived structures

CYANA program v2.1 calculated all the NMR structures of peptides.[19] NOE intensities were qualitatively characterized as strong, medium, and weak based on their respective cross-peak intensities from trNOESY spectra obtained at a mixing time of 150 ms in the presence of LPS. This was further translated to interproton upper-bound distances of 3.0, 4.0 and 5.0 Å respectively. The lower bound distance was kept constant at 2.0 Å. The backbone dihedral angle (phi) was varied from -30° to -120° to restrict the conformational space for all residues. No hydrogen bonding constraints were used for structure calculation. Several round of structure refinements were performed and based upon the NOE violations; the distance restraints were adjusted accordingly. The twenty lowest energy structures were selected to generate ensembles of structures of peptides bund to LPS. The structures were analyzed using pymol. The quality of the structures was evaluated using PROCHECK and Protein Structure Validation suite.[20, 21]

Model of DNA duplex GG28 was built using NAB from David Case Group (http://structure.usc.edu/make-na/). Leap program was used from AMBER 11[22] to read in a force field (ff99SB)[23] topology and coordinate information to generate requisite files. A short minimization was performed with a GG28 model to obtain a starting point for structure calculations with experimental restraints using sander program. By NOE cross peak intensities from 1H-1H 2D NOESY spectrum, NOE intensities were qualitatively categorized as strong, medium and weak and translated to the upper distance limit to 3.0, 4.0 and 5.0 Å, respectively. The lower limits were varied from 1.8 to 3.5 Å. In addition to 355 interproton NMR restraints, 62 Watson-Crick, 28 sugar pucker and 164 backbone torsion angle restraints were used for the structure calculation (Table S2 and S3). The restrained files were processed in AMBER using nmropt = 1 option and then minimization of the model is performed with lower and upper bound force constants of 10 kcal. mol¹. A simulated annealing procedure was applied in a vacuum with Generalized Born model as a final step in structure calculation. The model was heated up to 400 K for 10 ps and then it was slowly cooled to 300 K in next 10 ps and then cooled to 0 K for next 5 ps. The cycle was repeated for five times. The NMR ensembles were obtained with lowest rmsd values. Structures were visualized using Chimera, PyMOL and Maestro software and the solution structures were validated using X3 DNA software suite (http://x3dna.org/).[24]

A.7 Docking calculation of peptides with LPS and DNA

Docking calculation of all peptides with LPS was carried out using AutoDock program.[25] Coordinates of LPS were obtained from pdb accession code 1QFG[26] and for peptides, the trNOE derived structures were used. Peptides were used as a ligand in each docking calculations, with a
rigid backbone and flexible side chains. A grid spacing of 0.37 Å was used centering the H2 atom of glucosamine II residue of LPS for grid preparation using AutoGrid. All the energy-storing grids have the same size (grid points 70 × 116 × 80) with external grid energy fixed to 1000. Lamarckian genetic algorithm (LGA) was employed as search engine (50 runs) and a LUDI type scoring function. Some individuals in each population was set to 150. Iteration steps employed for energy evaluation was fixed to 2500,000, and a maximum number of generations was fixed to 27,000. The rate of gene mutation and crossover was set to 0.02 and 0.8, respectively. Gasteiger-Marsili charges were used for docking calculations. A charge of +1 value was added to each phosphorus atom to neutralize the system according to De-tering and Varani. The generated binding conformations were grouped into clusters based on root-mean-squared tolerance of 1 Å for analysis.

The three-dimensional structure of IR13 (PDB accession code: 1G8C) was docked to the solution structure of GG28 using Hex. The docking model correlated well with the NMR chemical shift perturbation of the DNA-peptide complex with respect to the free DNA. Furthermore, the final complex structure was refined by energy minimization protocol.

### A.8 Molecular Dynamic Simulation:

The NMR-derived GG28 structure was used for a 50 ns MD simulation in Desmond (Desmond Molecular Dynamics System, version 3.1, D.E. Shaw Research, New York, NY, 2012. Maestro-Desmond Interoperability Tools, version 3.1, Schrödinger, New York, NY, 2012) using AMBER ff99SB force field. To study the structural changes of DNA, the interacting refined model of the GG28-IR13 complex was also processed for MD simulation for the same period. Both the system was solvated in orthorhombic water-box using TIP3 water models using a cut-off radius of 12 Å. For neutralization of the system, appropriate Na+ counterions were added in both the solvated models. The initial convergence of the system to its near minima for adjustment of solute and solvent was performed with a minimization threshold of 1.0 kcal.mol⁻¹ Å⁻¹. The initial relaxation and equilibration of the system before the production run was done using Berendsen thermostat and barostat in NPT ensemble and finally the temperature of the system was raised up to 300 K to provide iso-thermal-isobaric ensemble was adopted as the microcanonical ensemble for MD simulation of both the models under study. The ensemble was processed using thermostat method and barostat method. Smooth Particle Mesh Ewald (PME) was applied for the calculation of non-bonded interactions of the dynamics system. SHAKE algorithm was implemented to freeze the vibrations of bonds involving hydrogen with an integration time step of 2 ps. The trajectories were saved at an interval of 5 ps for the analysis. All figures were prepared using Pymol and Chimera visualizing software.

### A.9 Microscopy Experiments

#### A.9.1 Study of DNA-peptide interaction on biotin micropattern:

Biotin micropatterned surfaces were generated using square micropatterned photo mask and UV light. A flow chamber was made using that micropatterned glass surface and poly-L-lysine (PLL)-PEG passivated counter glass and equilibrated for 7 minutes with a β-casein solution (1 mg/mL) in HEPES buffer keeping it in cold metal block. To study the binding of 561-labelled DNA, GG28 (F), on the biotin micropattern, we incubated the flow chamber with neutravidin (0.3 µM) solution in HEPES buffer for 7 minutes, excess neutravidin was washed out by HEPES buffer and after that 62 µM solution of the DNA was flowed into the flow chamber and incubated for 10
Excess DNA was removed by washing with the HEPES buffer. Finally, 20 µL solutions of those peptides (FITC-IR13, FITC-IR13AA) in HEPES buffer were loaded into the flow chamber (all the peptide concentrations were 100 µM) and incubated for 10 min. The excess peptide solution was washed out by HEPES buffer. After the termination of the experiment, Flow chambers were observed by Nikon Eclipse Ti-U inverted fluorescence microscope in 561 and 488 nm channels.

A.9.2 Cellular uptake of peptides
A549 cells were cultured in Dulbecco Modified Eagle Medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum at 37°C and 5% CO₂ atmosphere. For cellular uptake experiment, 5000 cells were seeded onto a cover glass bottom dish, one day before incubation with the FITC-IR13 and FITC-IR13AA peptides. All these peptides are commercially purchased and used without further purification. All those peptides were dissolved in autoclaved water and from that peptide solution of 100 µM concentration were prepared in serum-free DMEM medium. 200 µL of a solution of each peptide was treated with the cells after washing with the 1X phosphate-buffered saline (PBS) and incubated for 4 hours at 37°C under 5% CO₂ atmosphere. The nucleus was stained with 3 µM solution of 4, 6-diamidino-2-phenylindole (DAPI) in DMEM medium having <0.1%. DMSO concentration (Stock solution was prepared in cell culture DMSO) was loaded into the cell for 1 hour. Cellular internalization of those peptides was imaged by Nikon Eclipse Ti-U inverted fluorescence microscope in 405 & 488 nm channels along with the bright field.

A.9.3 Scanning electron microscopy (SEM)
The incubated peptide solutions were deposited on a glass slide (1 cm²) and dried oven night in air. The slide was then coated with gold for 120 s at 10 kV voltage and 10 mA current. The samples were viewed on a ZEISS EVO-MA 10 scanning electron microscope equipped with a tungsten filament gun operating at 10 kV.

A.9.4 Transmission electron microscopy (TEM)
The 500 µM peptide stock solutions were incubated at room temperature for up to 15 days, and 10 µL aliquots of the solution were placed on300 mesh Formvar/carbon coated copper TEM grids (Ted Pella, Redding, CA 96049, USA). It was allowed to adsorb on TEM grid for about 3-4 minutes, and excess volume was removed with filter paper. The grid was negatively stained with 5 % (v/v) freshly prepared uranyl acetate in water. After 5-minute excess dye was removed and the grids were viewed on a JEOL JEM 2100 HR-TEM microscope operating at 80 kV. Digital images were acquired using Gatan Digital Micrograph 2.3.0 Image Capture Software.