Conformational profile assessment of Human cathelicidin (LL-37) as suppressor of neutrophil apoptosis via the activation of FPRL1 using Molecular dynamics Simulations

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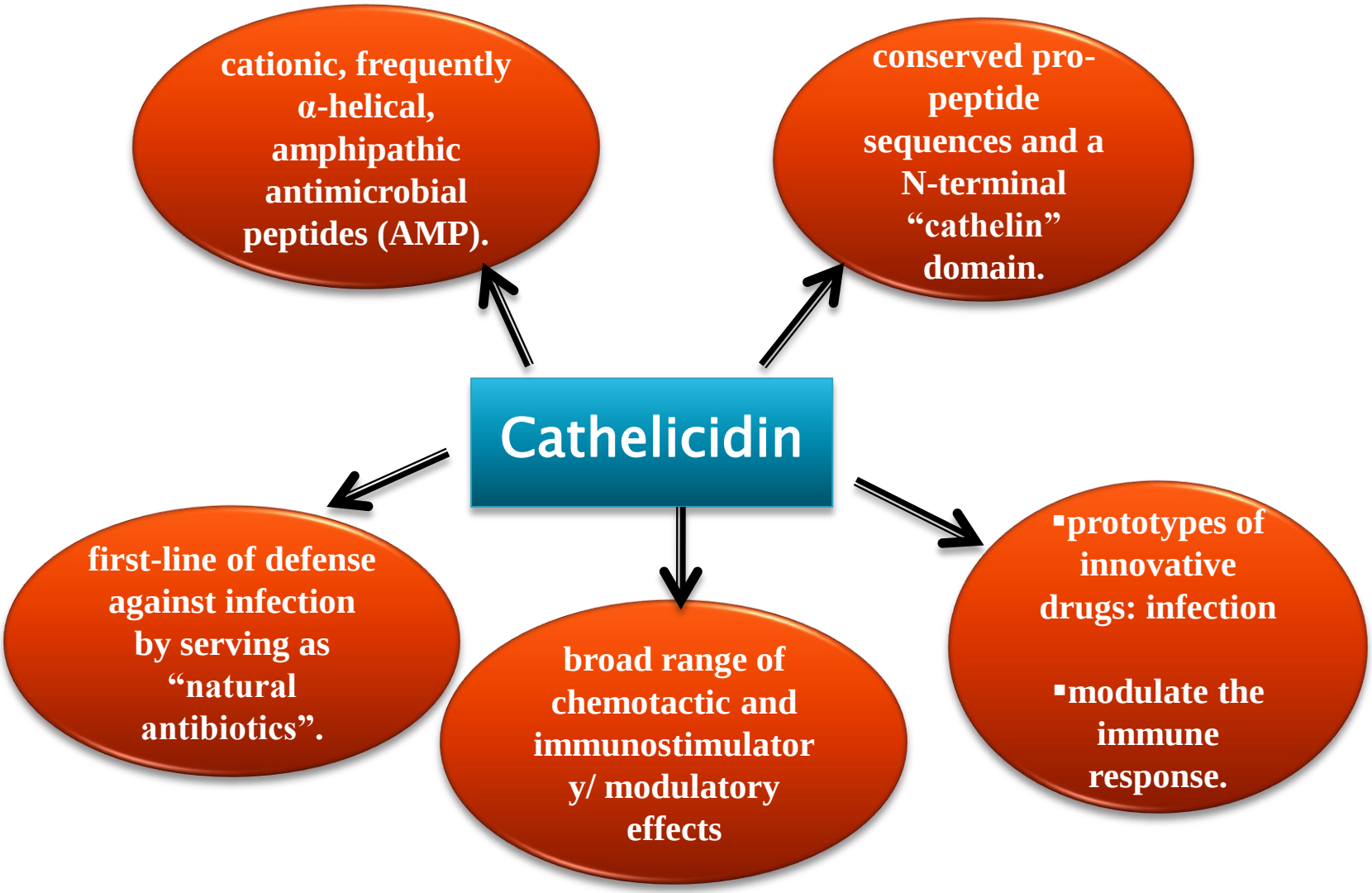
AIIMS

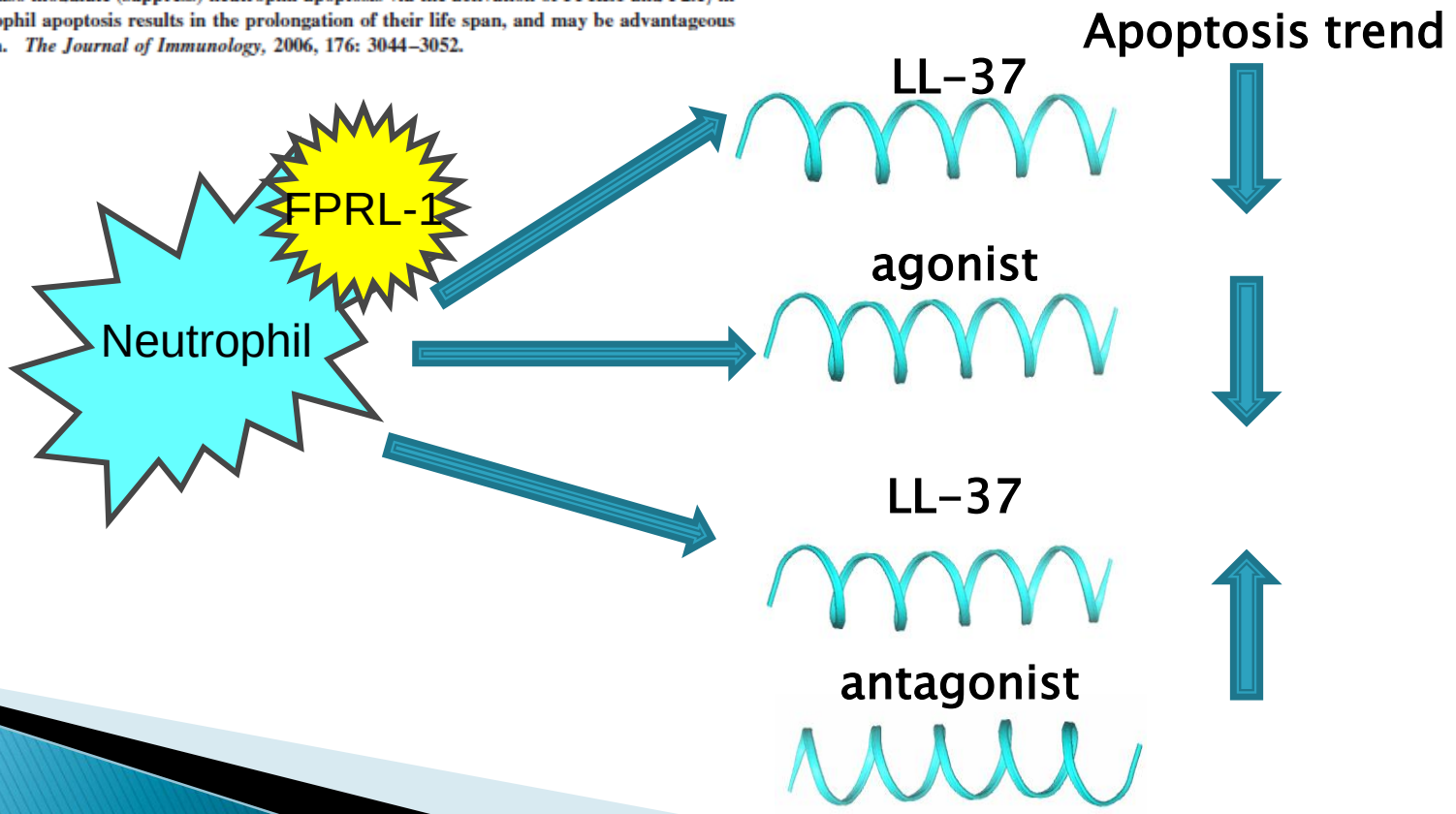

cationic, frequently α-helical, amphipathic antimicrobial peptides (AMP).
conserved pro-peptide sequences and a N-terminal "cathelin" domain.
Cathelicidin
first-line of defense against infection by serving as "natural antibiotics".
broad range of chemotactic and immunostimulatory/ modulatory effects
▪prototypes of innovative drugs: infection
▪modulate the immune response.

An Antimicrobial Cathelicidin Peptide, Human CAP18/LL-37, Suppresses Neutrophil Apoptosis via the Activation of Formyl-Peptide Receptor-Like 1 and $P2X_7$[1]

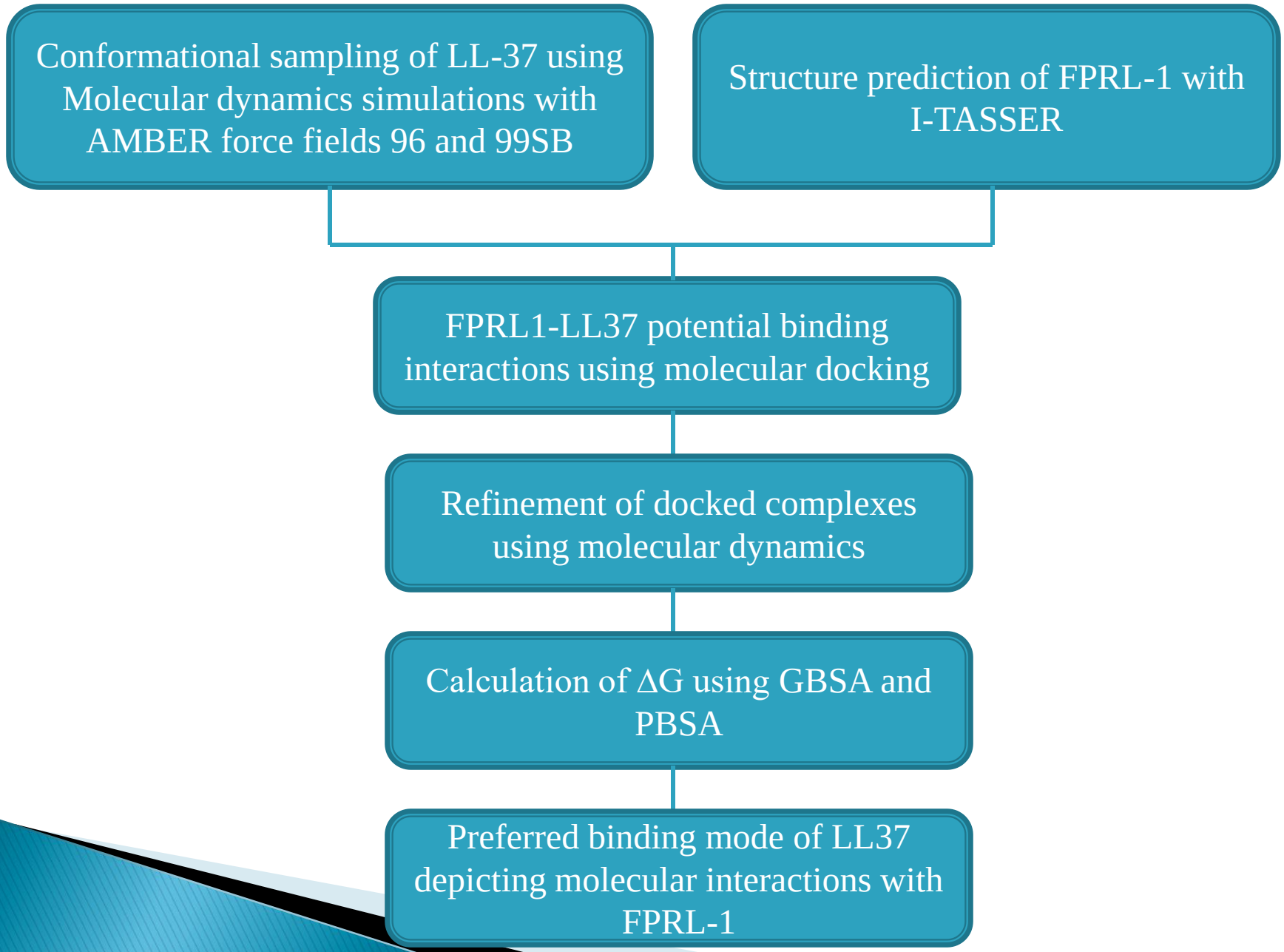
J Immunol 2006; 176:3044-3052;

Isao Nagaoka,[2]* Hiroshi Tamura,† and Michimasa Hirata‡

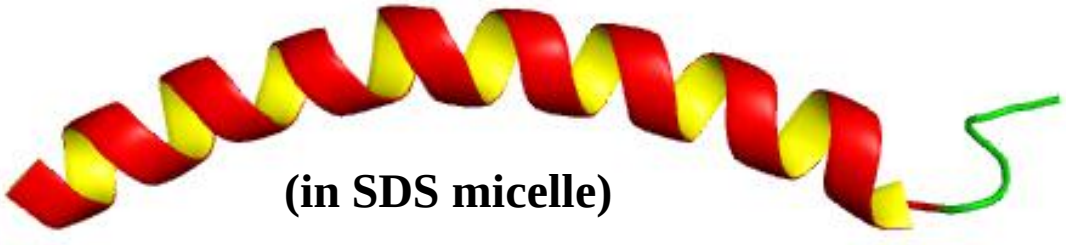
Peptide antibiotics possess the potent antimicrobial activities against invading microorganisms and contribute to the innate host defense. An antibacterial cathelicidin, human cationic antibacterial protein of 18 kDa/LL-37, not only exhibits potent bactericidal activities against Gram-negative and Gram-positive bacteria, but also functions as a chemoattractant for immune cells, including neutrophils. During bacterial infections, the life span of neutrophils is regulated by various pathogen- and host-derived substances. In this study, to further evaluate the role of LL-37 in innate immunity, we investigated the action of LL-37 on neutrophil apoptosis. Neutrophil apoptosis was assessed using human blood neutrophils based on the morphological changes. Of note, LL-37 dose dependently (0.01–5 μg/ml) suppressed neutrophil apoptosis, accompanied with the phosphorylation of ERK-1/2, expression of Bcl-x_L (an antiapoptotic protein), and inhibition of caspase 3 activity. Interestingly, LL-37-induced suppression of neutrophil apoptosis was attenuated by the antagonists for formyl-peptide receptor-like 1 (FPRL1) and $P2X_7$ nucleotide receptor. Of importance, the agonists for FPRL1 and $P2X_7$ apparently suppressed neutrophil apoptosis. Collectively, these observations indicate that LL-37 cannot only kill bacteria, but also modulate (suppress) neutrophil apoptosis via the activation of FPRL1 and $P2X_7$ in bacterial infections. Suppression of neutrophil apoptosis results in the prolongation of their life span, and may be advantageous for host defense against bacterial invasion. *The Journal of Immunology,* **2006, 176: 3044–3052.**



Modeling Approach Workflow

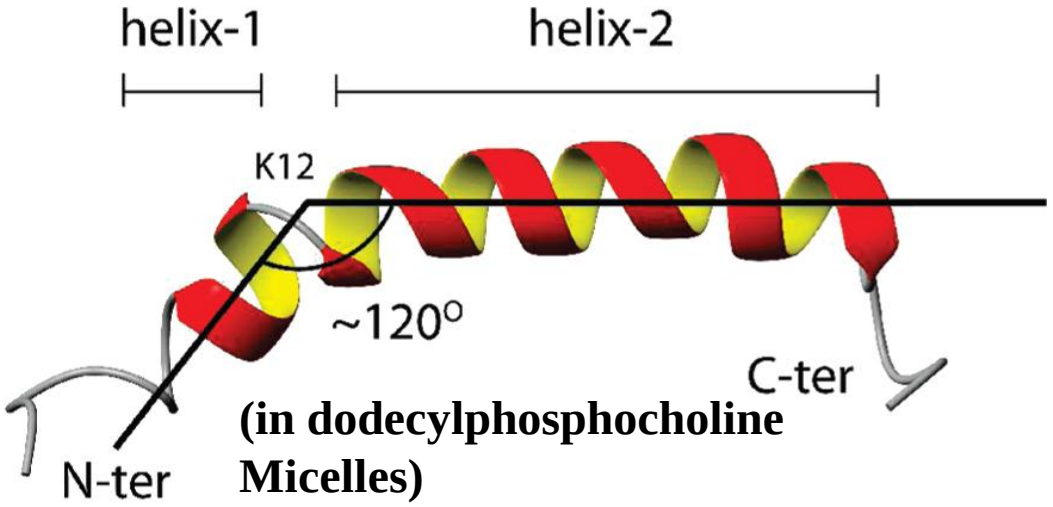


Available NMR structures of LL37



(in SDS micelle)

Wang G., JBC 2008, 283, 32637

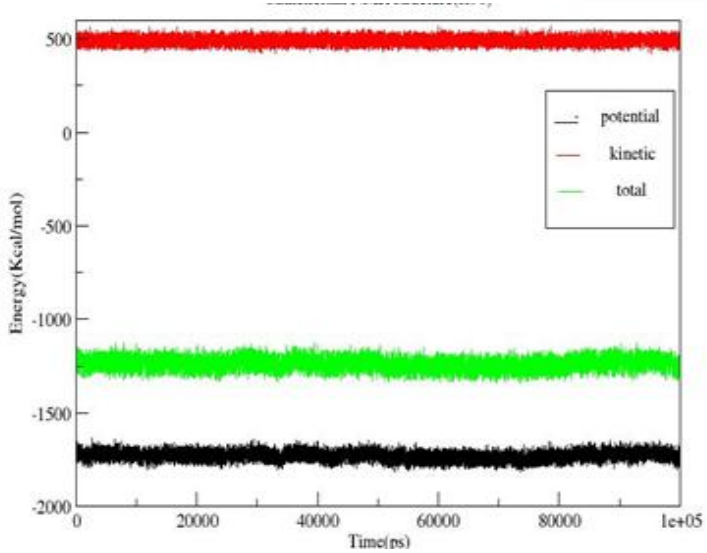


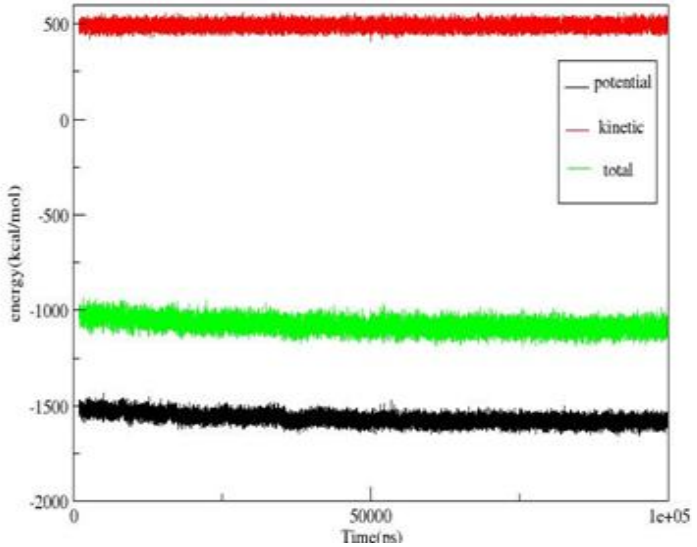
(in dodecylphosphocholine Micelles)

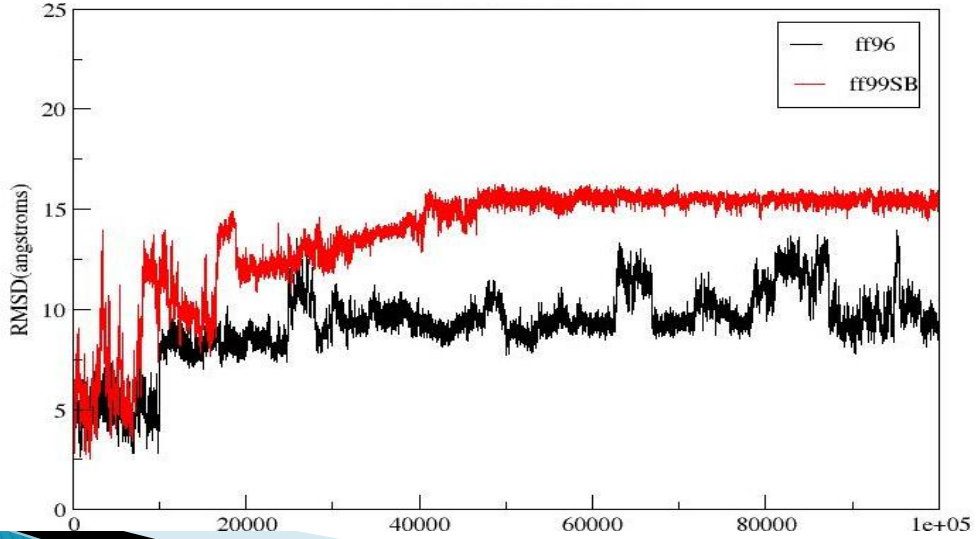
Porcelli F., Biochem. 2008, 47, 5565

Parameters of MD trjectory

ff96 | **PDB: 2K6O** | **ff99SB**

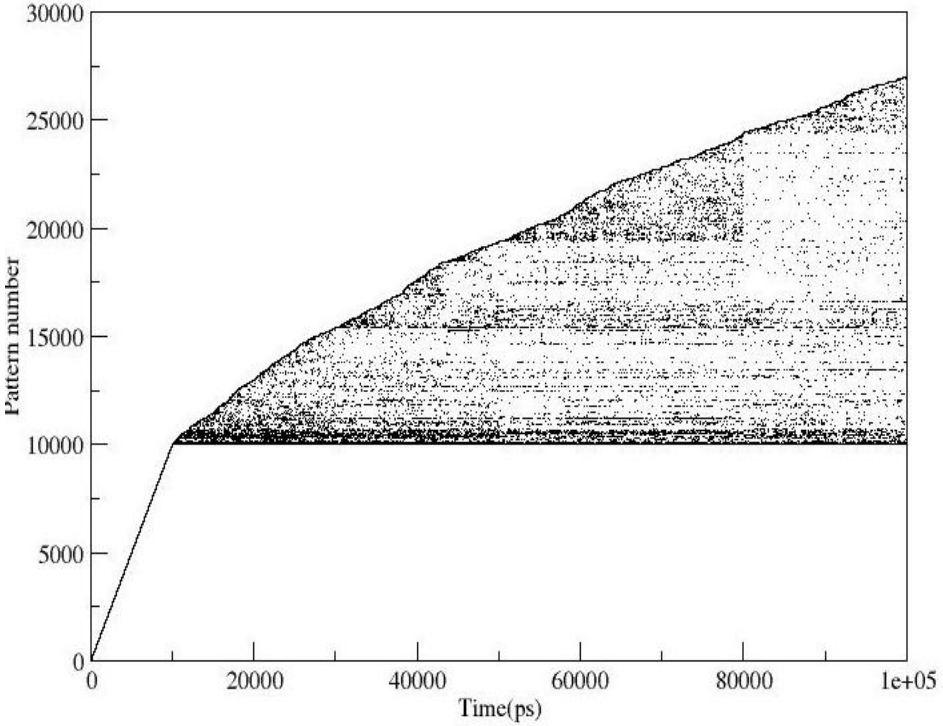




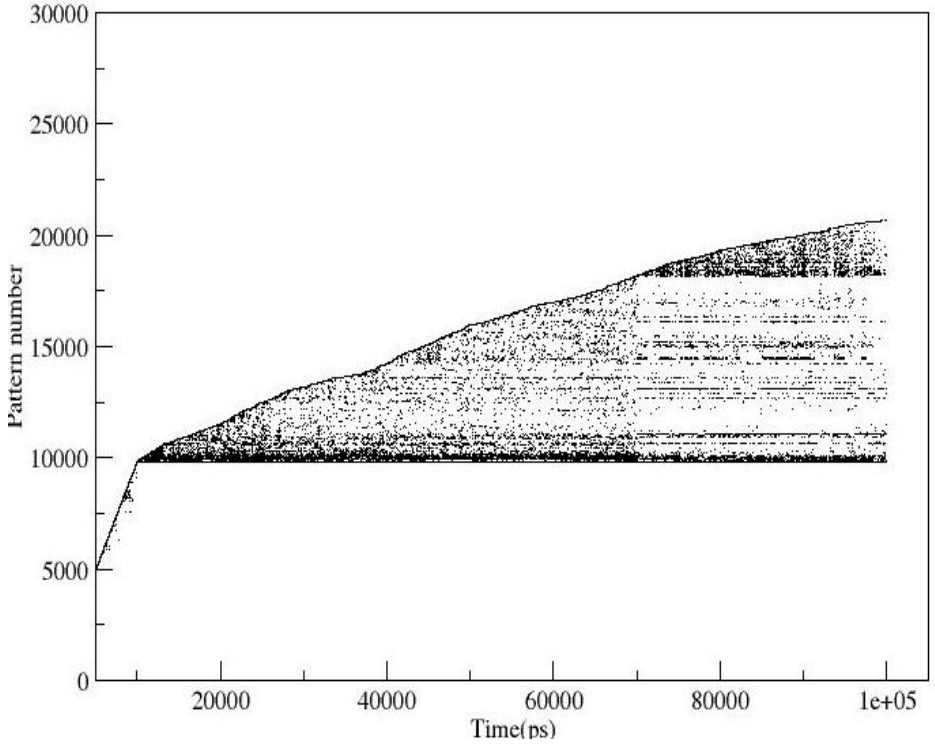


PDB: 2K6O

ff96



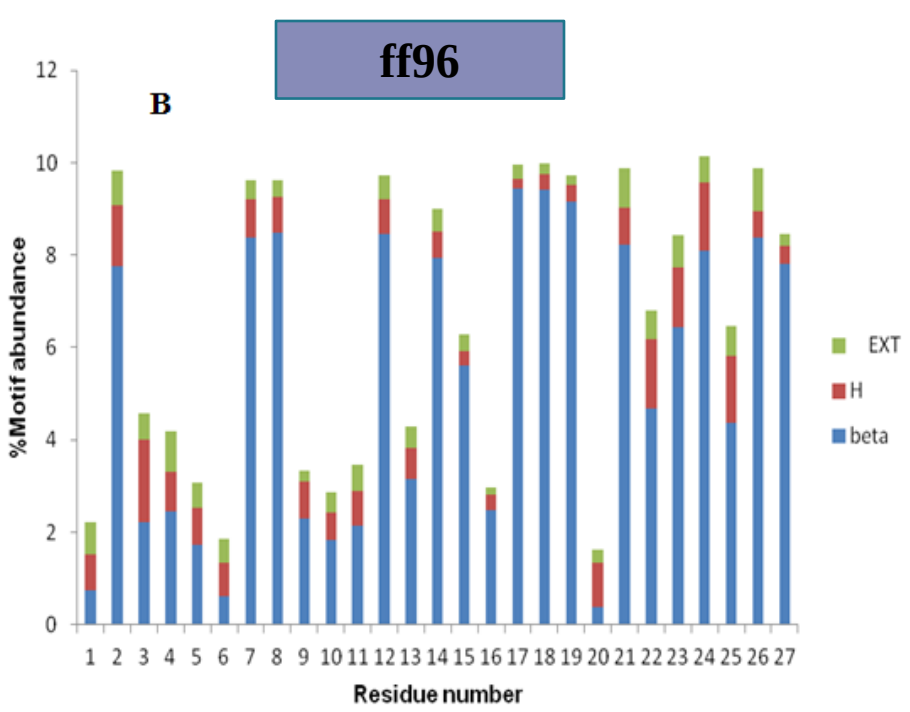
ff99SB

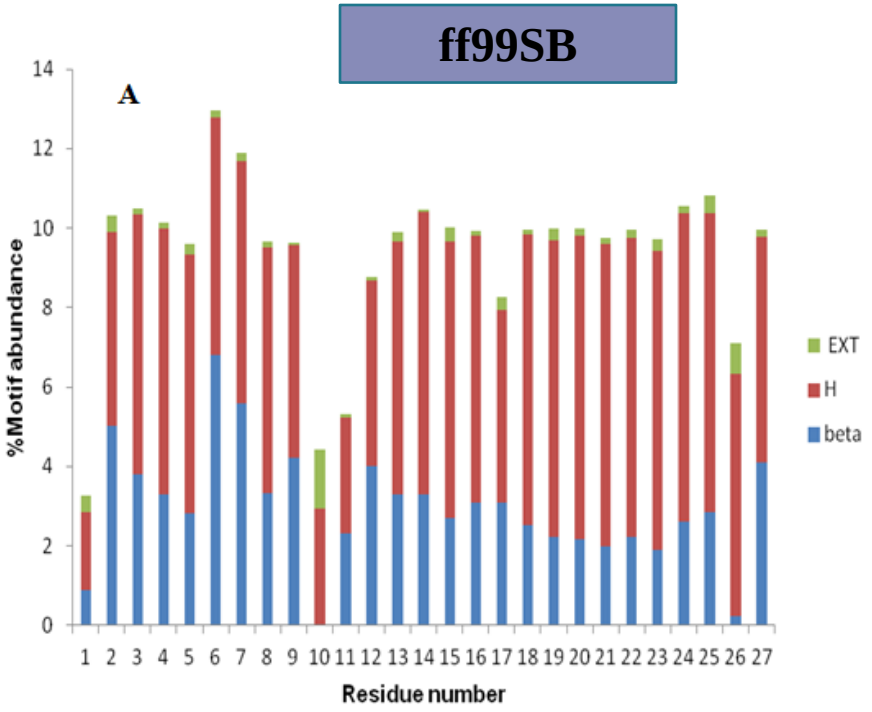


Dark areas are the number of conformations trapped in the configurational space

- **Percent efficiency for generating new patterns in case of MD ff96 was 26% for ff96 and 20%for ff99SB.**
- **New patterns have developed earlier (around 5ns) in case of ff99SB as compared to ff96 (after 10ns).**

Secondary structural profile of each residue during total simulation time

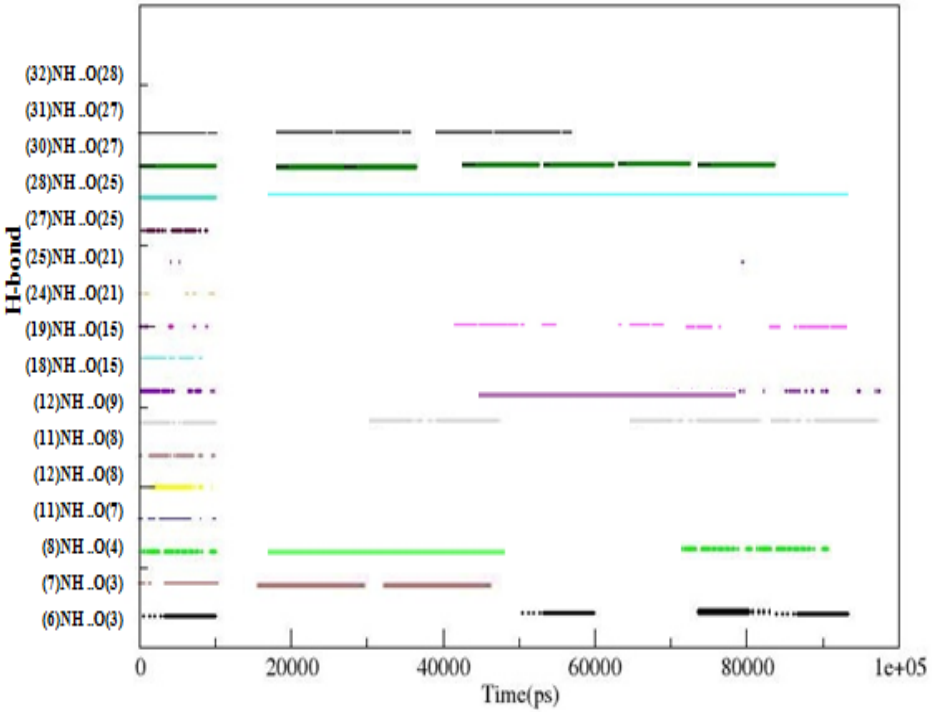




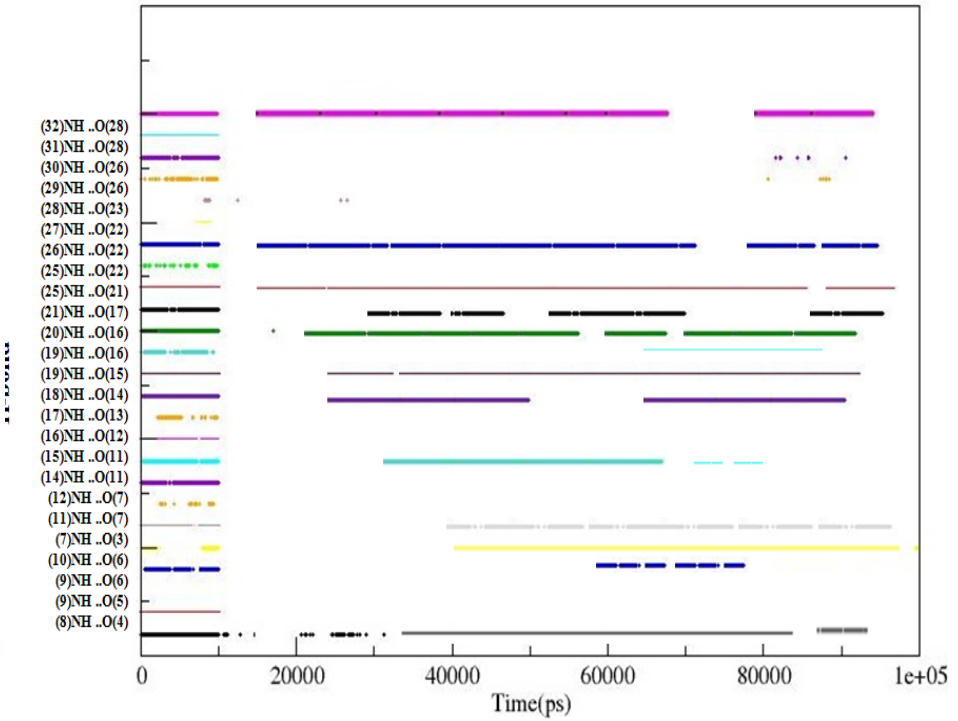
- beta turns were again classified into different types using CASICO program
- type 1 beta turns were found to be maximum, three residue window is considered for a turn in CLASICO
- Consecutive beta turns resemble 3-10 helix

Secondary structure conformations of LL37 based on H-bonds during MD simulation

ff96

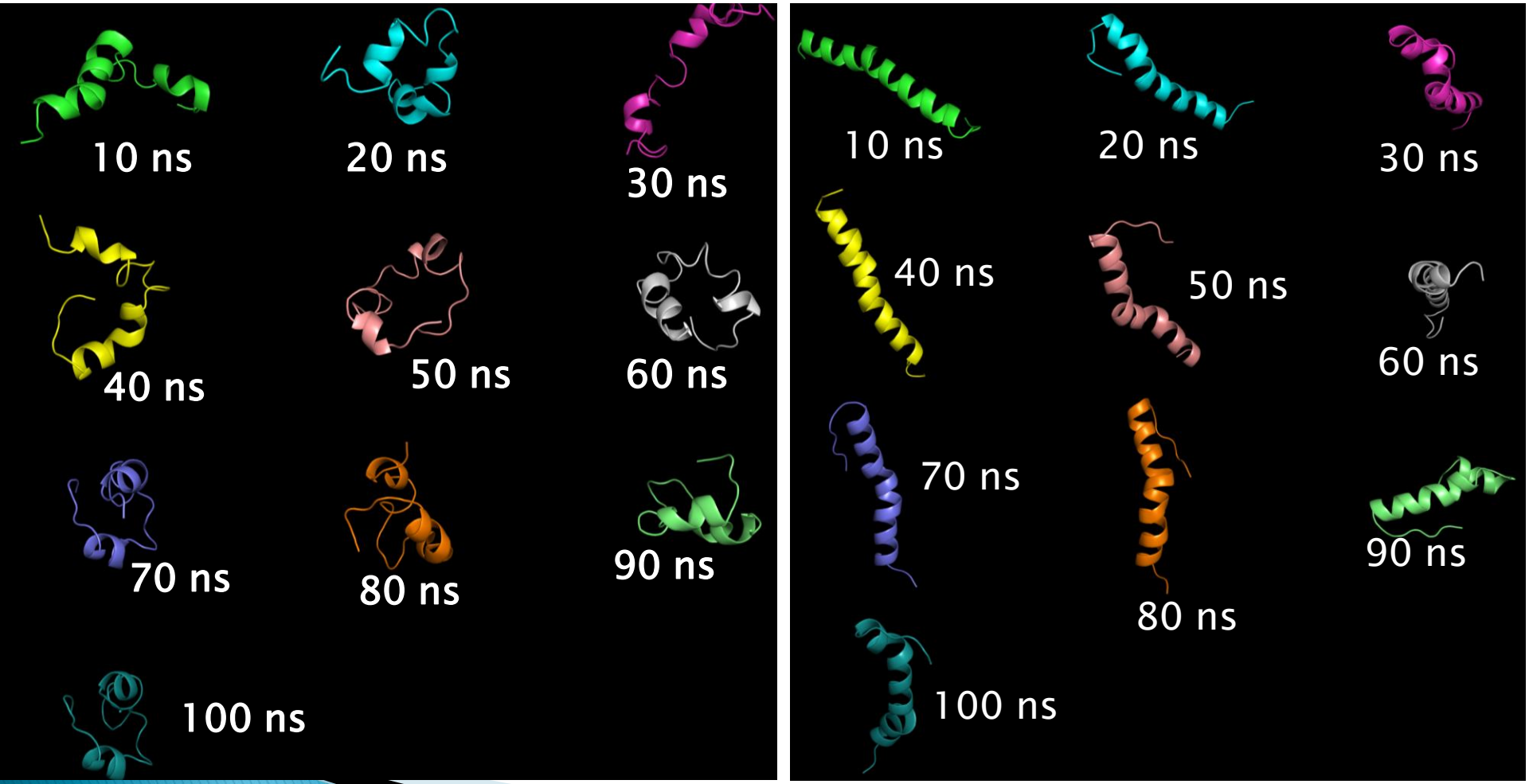


ff99SB



Conformations obtained during MD trajectory

PDB: 2K6O

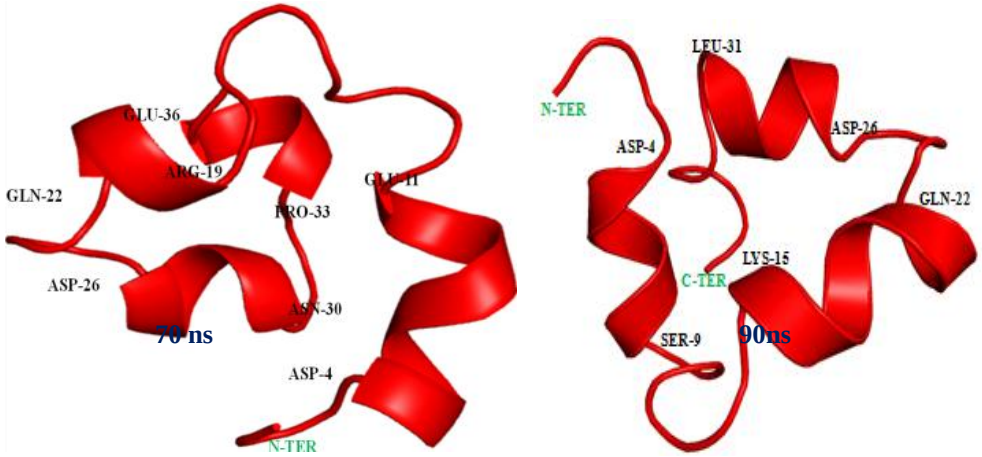


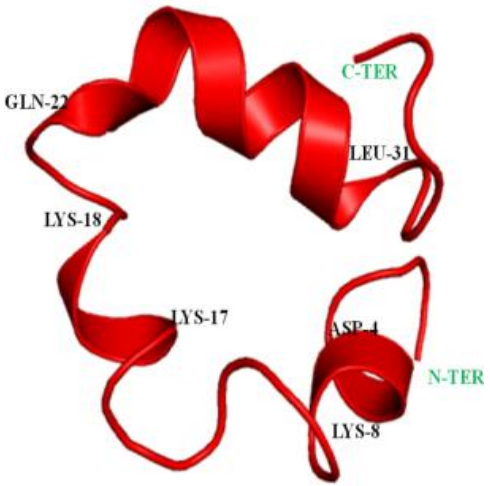
Major structural motifs found during MD simulations

ff96

π helix towards C-terminal from residue ASP^{26} - ASN^{30}

3$_{10}$ helix from ASP^{4} –SER^{9} .



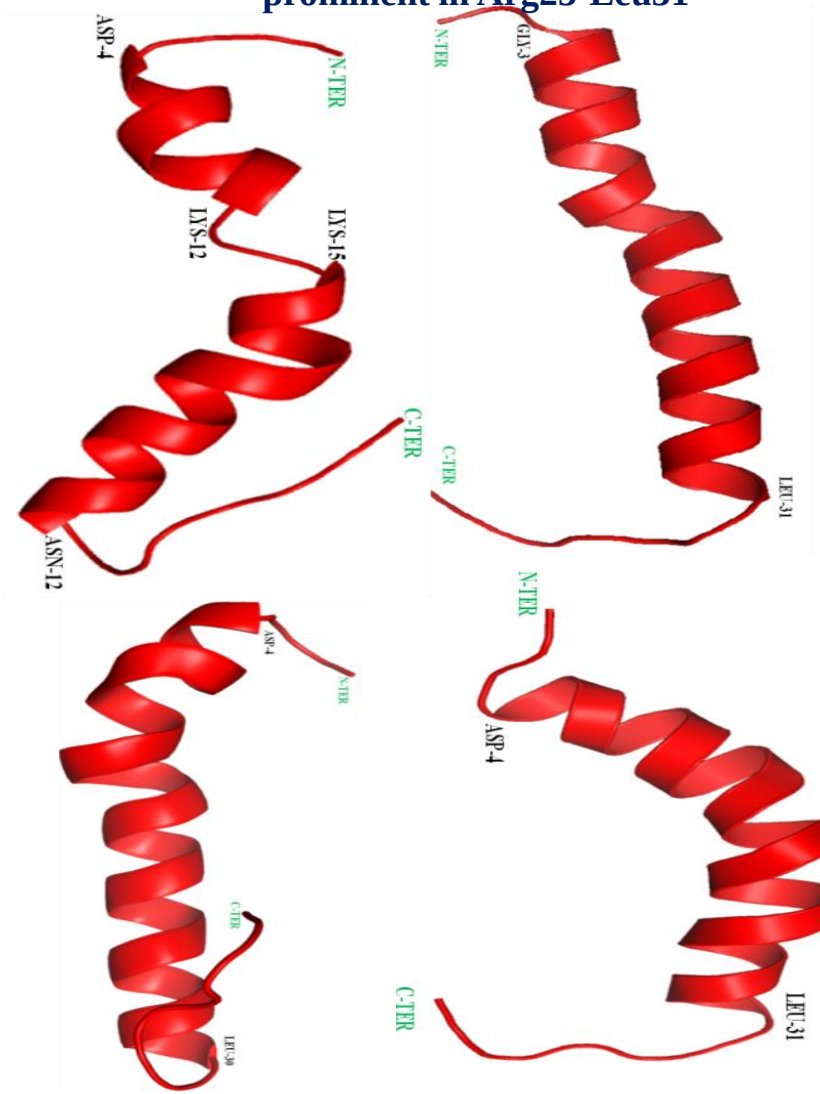


100ns

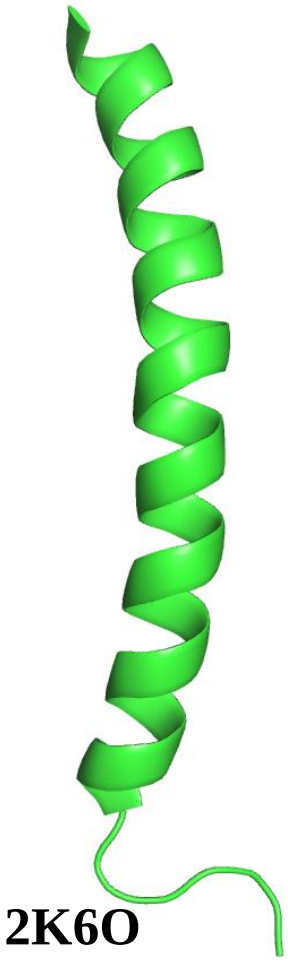
α-helix from ASP^{4}-LYS^{8} towards N-terminal

ff99

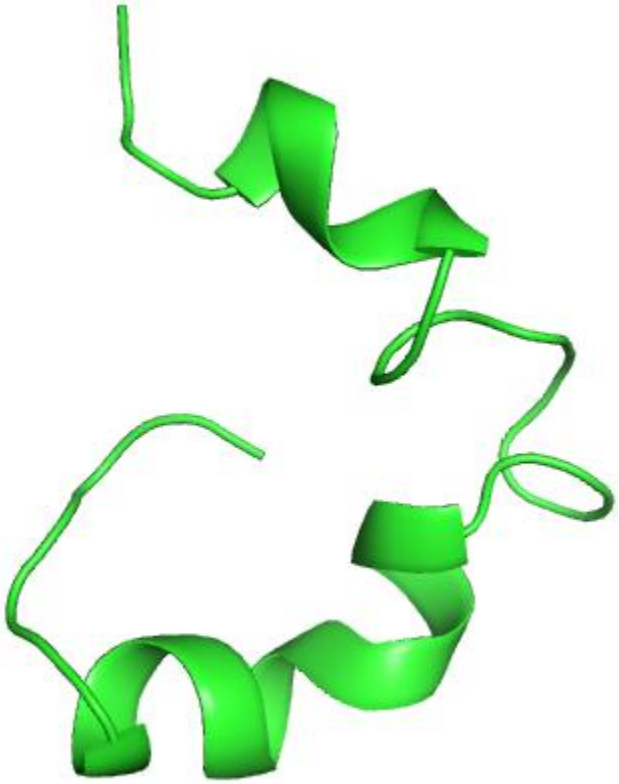
α- helical region is more prominent in Arg23-Leu31



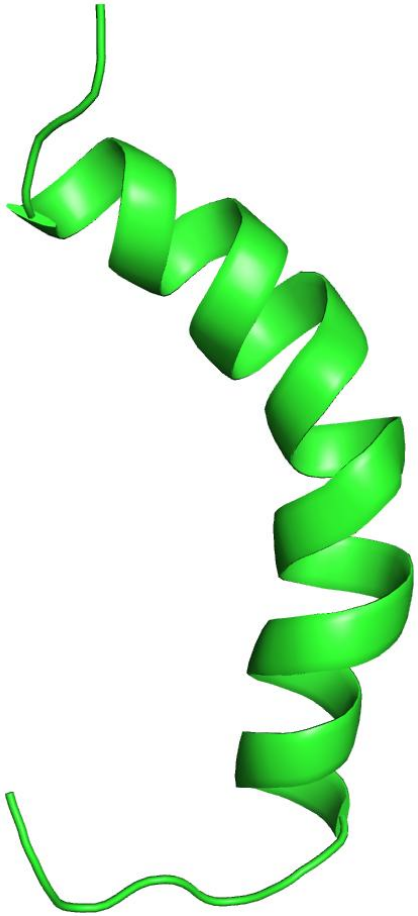
Final conformations of LL-37 used for docking study with FPRL1



2K6O



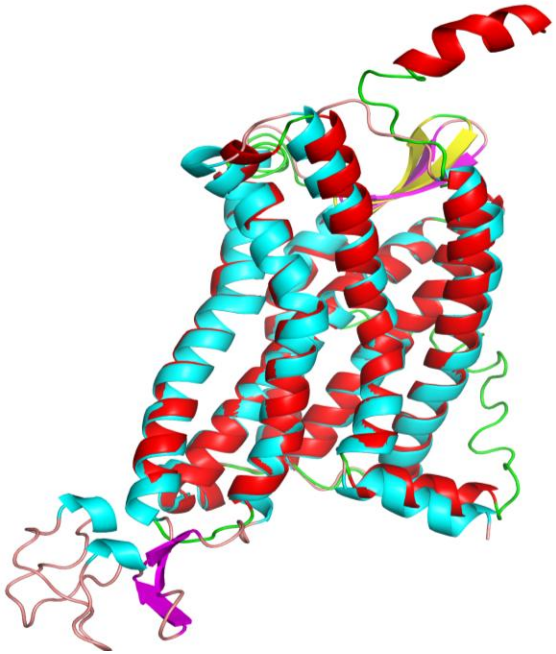
ff96

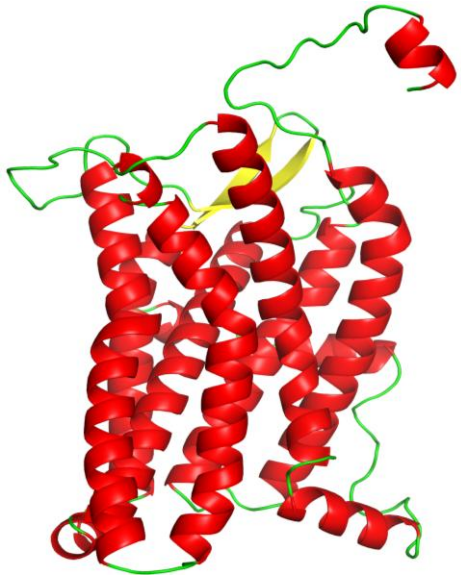


ff99

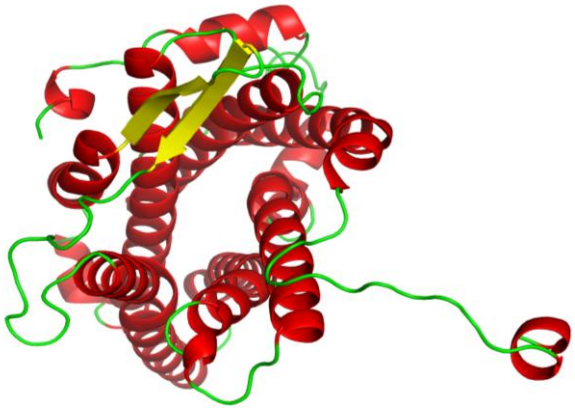
3D Model of Formyl Peptide Receptor like -1

RANK	PDB Hit	TM-SCORE	RMSD	IDEN	COV.
1	4mbsa	0.821	0.83	0.264	0.832
2	4yay	0.755	1.06	0.268	0.858
3	3oduA	0.727	2.24	0.279	0.792
4	4ea3B	0.717	2.97	0.277	0.806
5	2ksaA	0.708	3.53	0.207	0.823
6	4ib4A	0.695	3.02	0.179	0.798
7	1gzmB	0.694	3.47	0.179	0.812
8	4djhA	0.690	2.99	0.284	0.786
9	1kpnA	0.687	3.61	0.150	0.809
10	1kadA	0.681	4.06	0.183	0.832



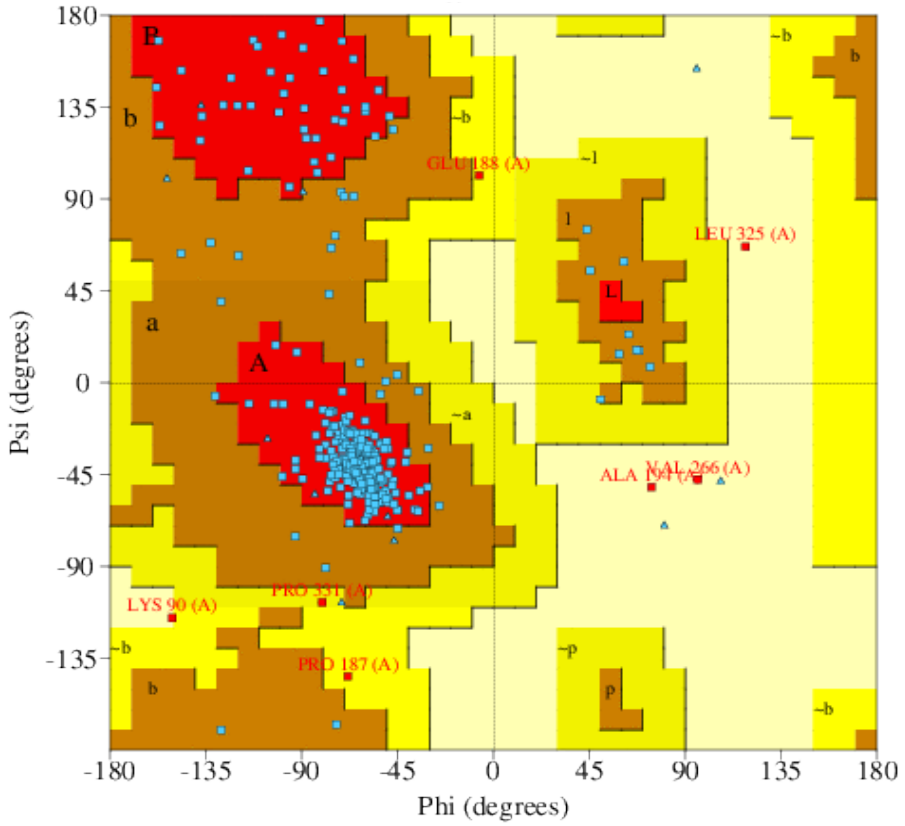


Side View



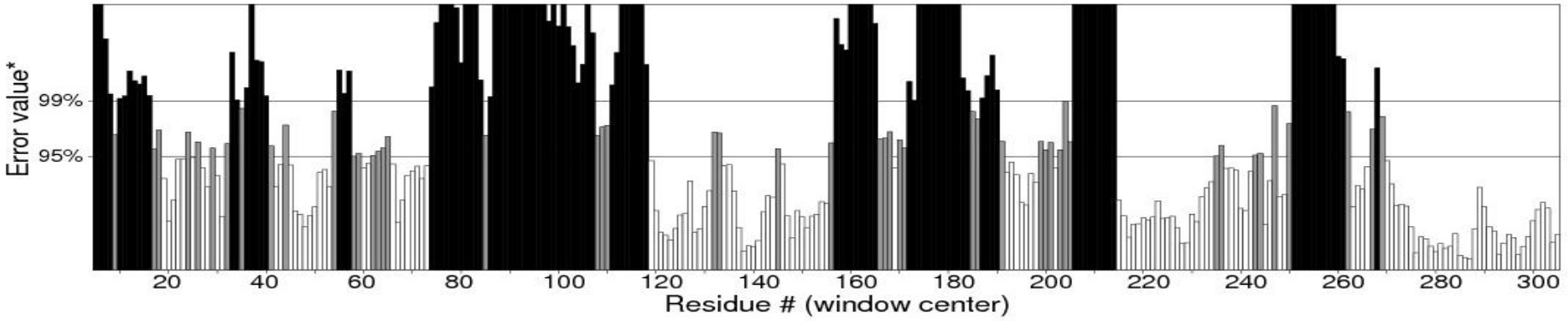
Top View

PROCHECK



Ramachandran's map	% of residues
Most favoured regions	89.7%
Additional allowed regions	8.7%
Generously allowed regions	0.3%
Disallowed regions	1.3%

ERRAT2

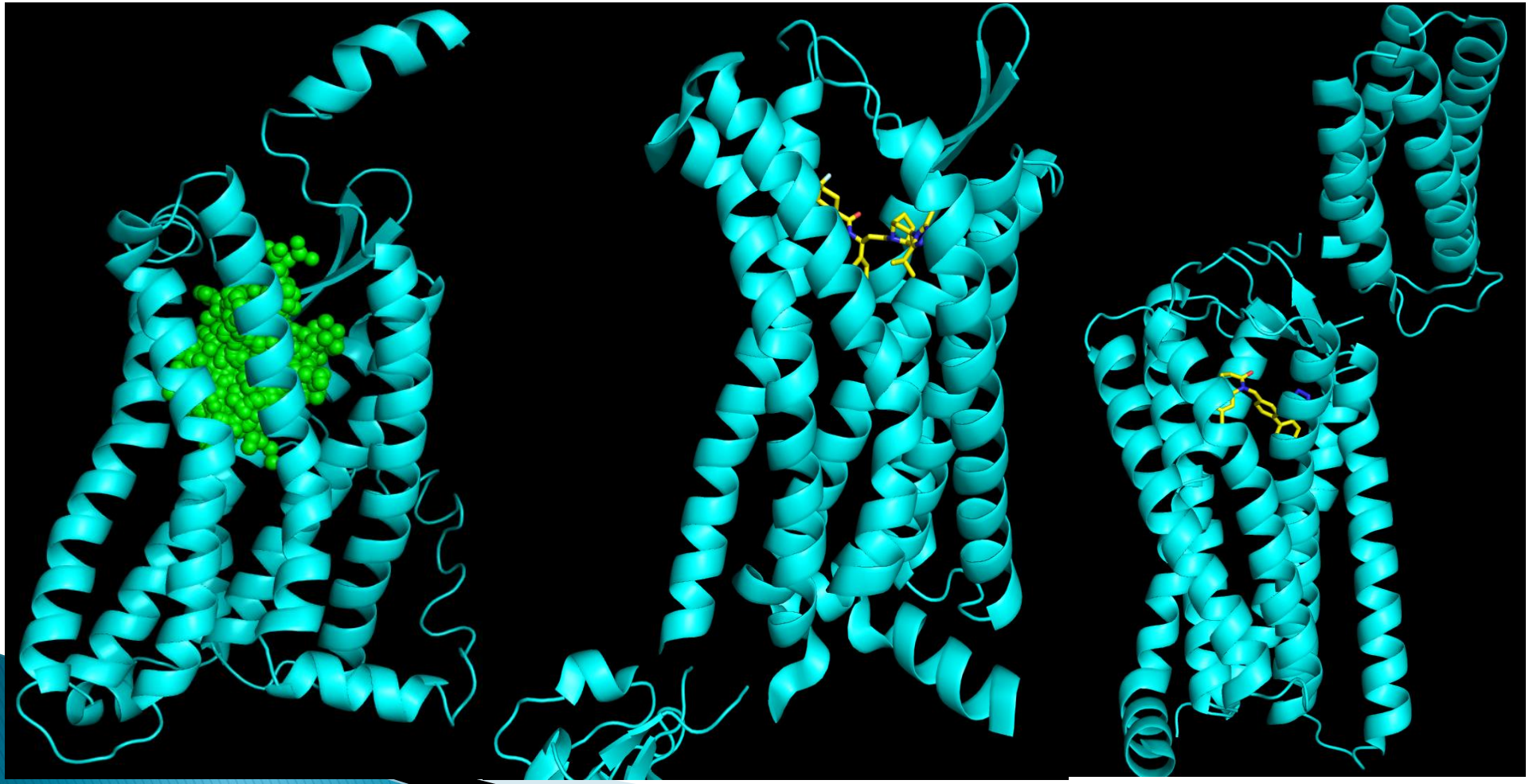


Prediction of binding site in FPRL-1 for docking of LL-37

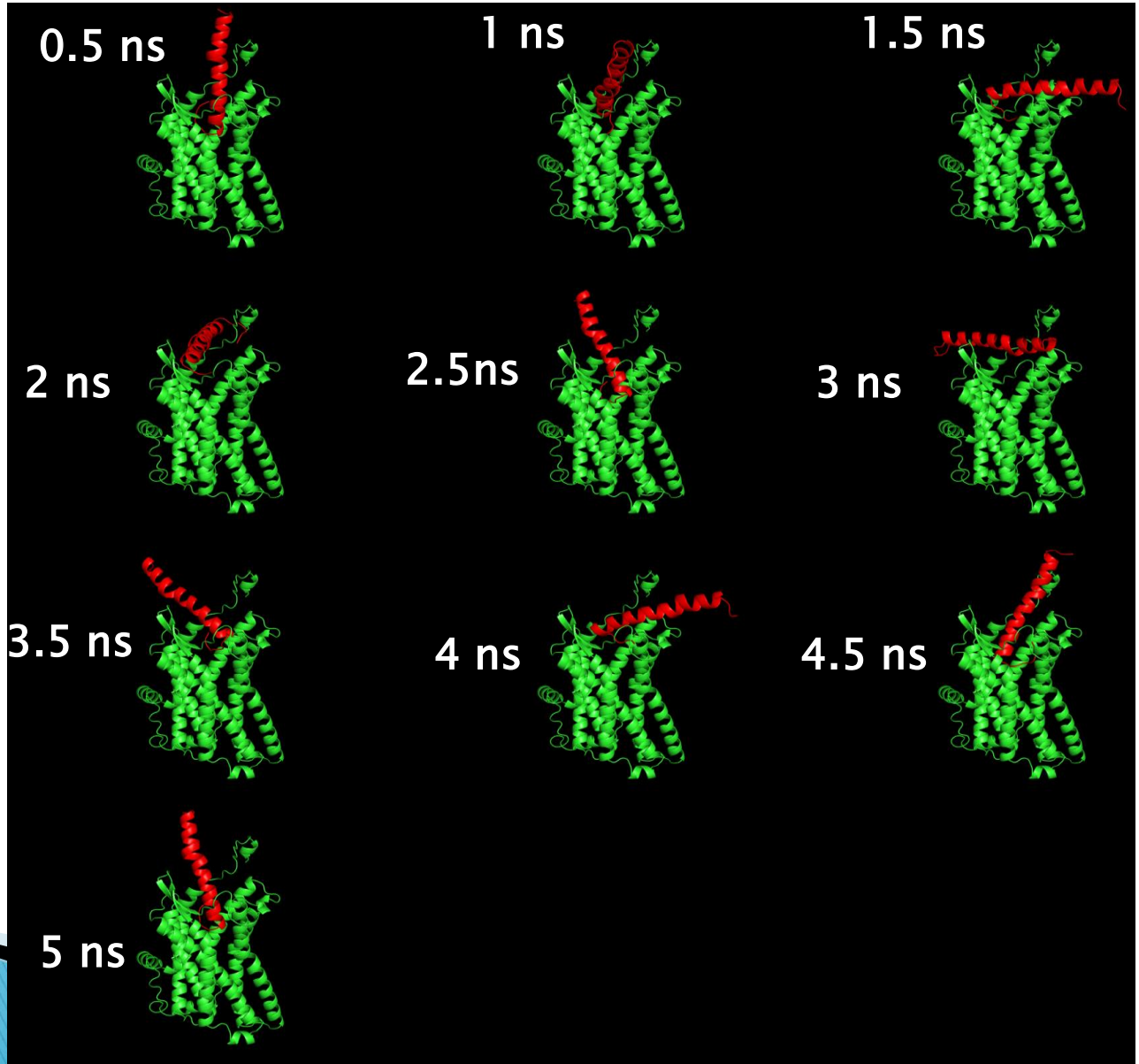
TM-SITE and S-SITE programs

PDB: 4MBS; CCR5 chemokine receptor with marketed HIV drug maraviroc

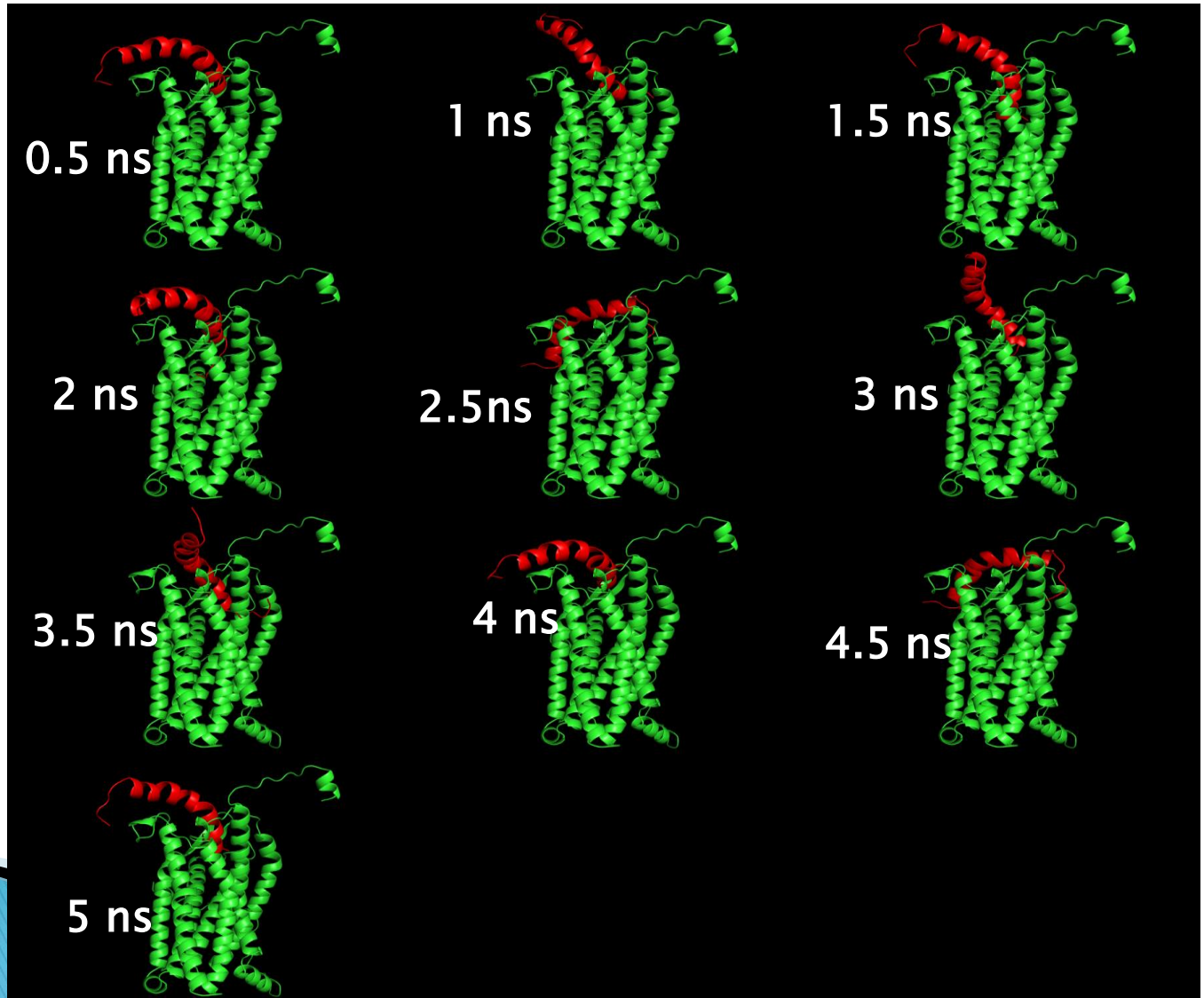
PDB: 4YAY; Human Angiotensin receptor with its selective antagonist ZD7155



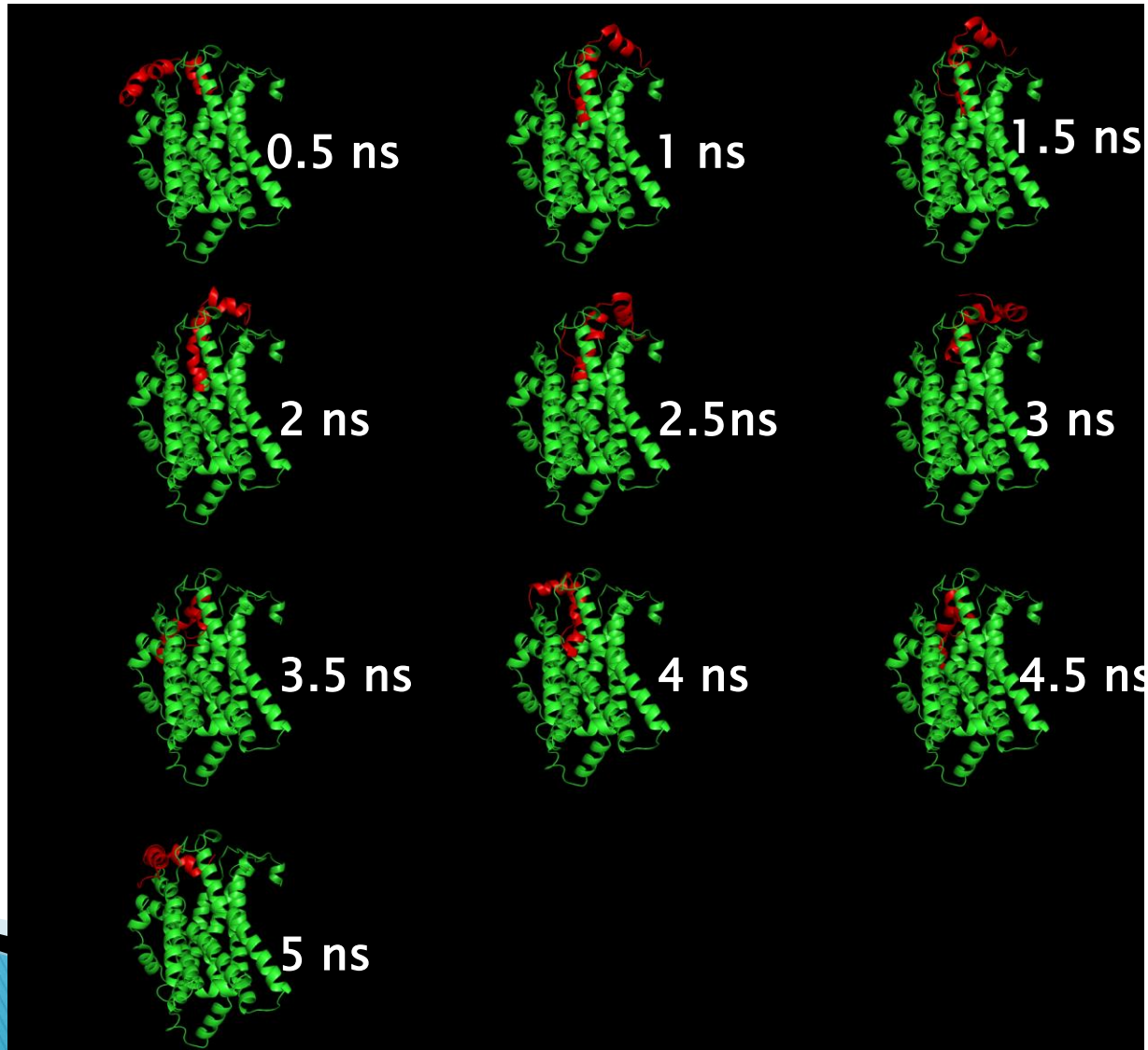
Snapshots taken during MD simulation for FPRL1 docked with 2K6O



Snapshots taken during MD simulation for FPRL1 docked with helix-bend-helix conformation sampled with ff99SB



Snapshots taken during MD simulation for FPRL1 docked with kink at K12 conformation sampled with ff99SB



MMPBSA binding energy calculations

Complex ---> method	FPRL1-LL37 (ff96)	FPRL1-LL37(ff99SB)	FPRL1-2K60
GENERALIZED BORN (at 1ns)	-104.4617(Kcal/mol)	-120.8217(Kcal/mol)	-76.8715 (Kcal/mol)
(at 5ns)	-108.2894(Kcal/mol)	-124.3812(Kcal/mol)	-81.7816(Kcal/mol)
POISSON BOLTZMANN (at 1ns)	-204.0037(Kcal/mol)	-220.7338(Kcal/mol)	-159.2902(Kcal/mol)
(at 5ns)	-214.0543(Kcal/mol)	-237.6425(Kcal/mol)	-165.4324(Kcal/mol)

FPRL1-LL37 (ff96)

FPRL1-LL37(ff99SB)

2K6O

Conclusion

- The results demonstrate that the LL-37 has a tendency to attain folded and unfolded conformations, probably due to the low energy barrier between the states accounting for the high level of flexibility of the peptide.
- Analysis of the MD trajectories of LL-37 depicts the propensity of this peptide to attain α-helices and β-turns under the influence of force fields AMBER ff96 and AMBER ff99SB.
- With AMBER ff96, conformation of LL37 changed from linear helix to two small helices with kink as proposed by NMR structure (Porcelli, 2008)
- With AMBER ff99SB force field the peptide attains a stable helical structure with the N and C-terminals being extended and flexible.
- Docked conformations of peptide by MD using ff99SB with FPRL1 was having maximum interactions and free binding energy as compared to NMR structure. This could further give new insight into the neutrophil apoptosis role of LL-37
- Additionaly, the functional diversity of cathelicidin makes it a relevant and lucrative target to investigate.