Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/3/4/e1602794/DC1

Supplementary Materials for

Structural and biochemical differences between the Notch and the amyloid precursor protein transmembrane domains

Catherine L. Deatherage, Zhenwei Lu, Brett M. Kroncke, Sirui Ma, Jarrod A. Smith, Markus W. Voehler, Robert L. McFeeters, Charles R. Sanders

Published 12 April 2017, *Sci. Adv.* **3**, e1602794 (2017)
DOI: 10.1126/sciadv.1602794

This PDF file includes:

Supplementary Methods

Structure Refinement with Restrained-Molecular Dynamics

Ten of the top 1%-scoring Xplor-NIH-generated Notch-TMD structures were selected for further structural refinement in an explicit membrane bilayer using restrained molecular dynamics (rMD). These 10 starting structures were chosen based on consistency with previous experimental membrane topology measurements (*40*). For example, structures were not chosen if the conformation of the N-terminal JM domain (known to be located in the aqueous phase) would place it in the membrane. The ten selected structures were solvated in an explicit DMPC bilayer using the CHARMM-GUI server (*94, 95*). Using GPU-accelerated AMBER14 (*96*), we conducted restrained minimization of each representative structure for 30000 steps using steepest descent followed by 30000 steps using conjugate gradient with protein atoms restrained to initial positions. Following restrained minimization, structures were minimized without restraints, with 1500 steps each of a steep descent gradient followed by a conjugate gradient. With the Notch-TMD still restrained to initial Xplor-NIH coordinates, heating of lipid and water to 10 K over 500 steps was performed using constant volume boundary conditions and Langevin dynamics with a rapid collision frequency of 10000 ps^{-1} to ensure that forces/velocities remained stable. The system was then heated to 100 K over 2500 steps. The time step was increased from 1 fs to 2 fs for system heating to 318 K over 100 ps with constant pressure dynamics and anisotropic pressure scaling while the protein and lipid bilayer were restrained. The system was equilibrated with backbone atoms constrained to starting position while allowing side-chains to move for 5 ns with NMR restraints applied with a weight of 100%. After equilibration, all atoms were released with NMR restraints continuing to be applied with a weight of 100% at 318K for 60 ns, using constant pressure periodic boundary conditions and anisotropic pressure scaling. A representative structure for each of the ten production trajectories was selected by determining which frame contains the Notch-TMD structure closest to the mean structure for that trajectory. Determining these average structures as well as

calculating water penetrance and side-chain/lipid interactions employed CPPTRAJ for processing of atomic positions across time (*93*).

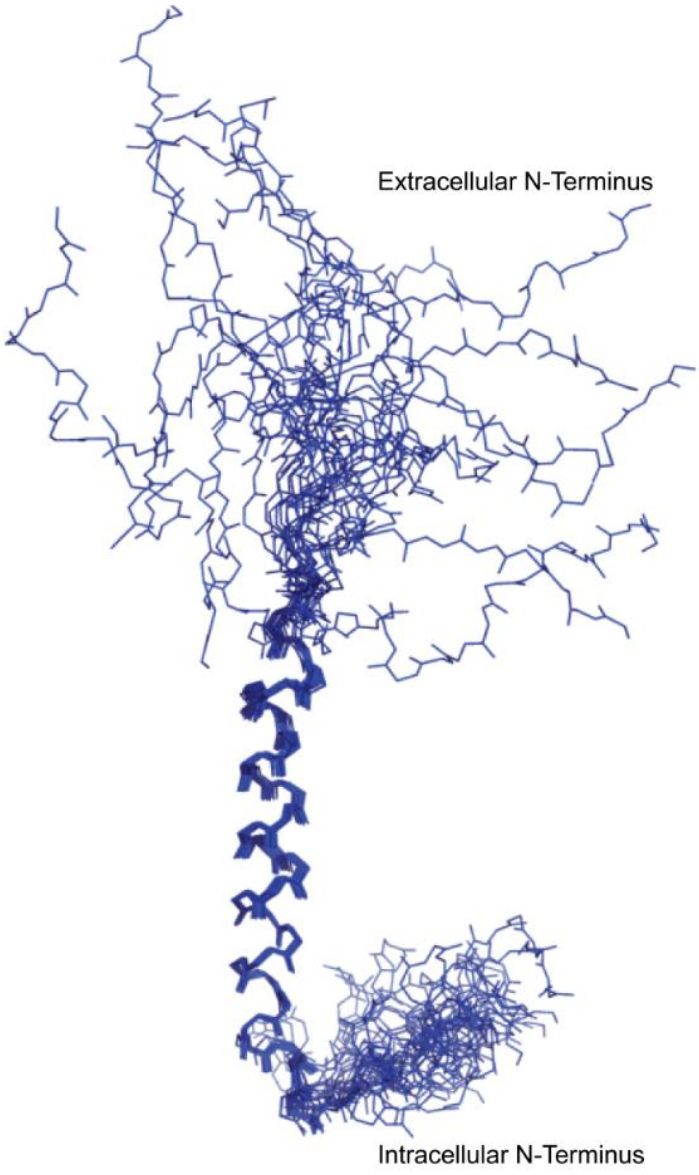

fig. S1. Top 20 NMR-determined XPLOR-NIH structures of the Notch-TMD.

table S1. Statistics for Notch-TMD structure determination and structure quality for the 10 final XPLOR-NIH NMR structures and for the 10 representative restrained MD structures.

NMR Distance and dihedral restraints

Distance Restraints	
Total NOE distances	337
Intra-residue NOEs	85
Inter-residue NOEs	244
Ambiguous NOEs	8
PRE distances	81
Total dihedral angle restraints	70

Structure Statistics*

	XPLOR-NIH	**rMD**
Violations		
Distance restraints (Å)	0.067±0.001	0.121±0.11
Dihedral angle restraints (°)	1.129±0.064	9.067±1.131
Average backbone pairwise r.m.s.d. (Å)**	0.425	1.165
Ramachandran plot (%)***		
Most favored regions	90%	90.9%
Additionally allowed regions	10%	9.1%
Generous allowed regions	0%	0%
Disallowed regions	0%	0%

*10 XPLOR-NIH NMR structures were used to start the explicit membrane restrained-MD simulations.

** RMSD calculated for TM segment only.

***Ramachandran plot percentage is from Procheck.