Supplementary material

Familial L723P mutation can shift the distribution between the alternative APP transmembrane domain cleavage cascades by local unfolding of the ε-cleavage site suggesting a straightforward mechanism of Alzheimer's disease pathogenesis

Eduard V. Bocharov[1,2*], Kirill D. Nadezhdin[1,2], Anatoly S. Urban[1,2], Pavel E. Volynsky[1], Konstantin V. Pavlov[3], Roman G. Efremov[1,2,4], Alexander S. Arseniev[1,2], Olga V. Bocharova[1,2]

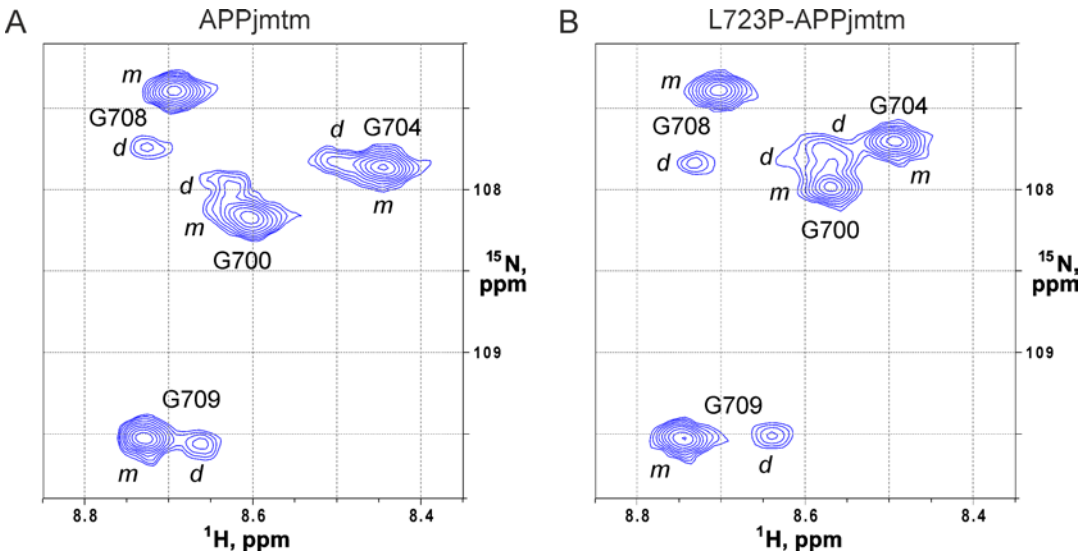
[1] *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation;*

[2] *Moscow Institute of Physics and Technology, Dolgoprudnyi, Russian Federation;*

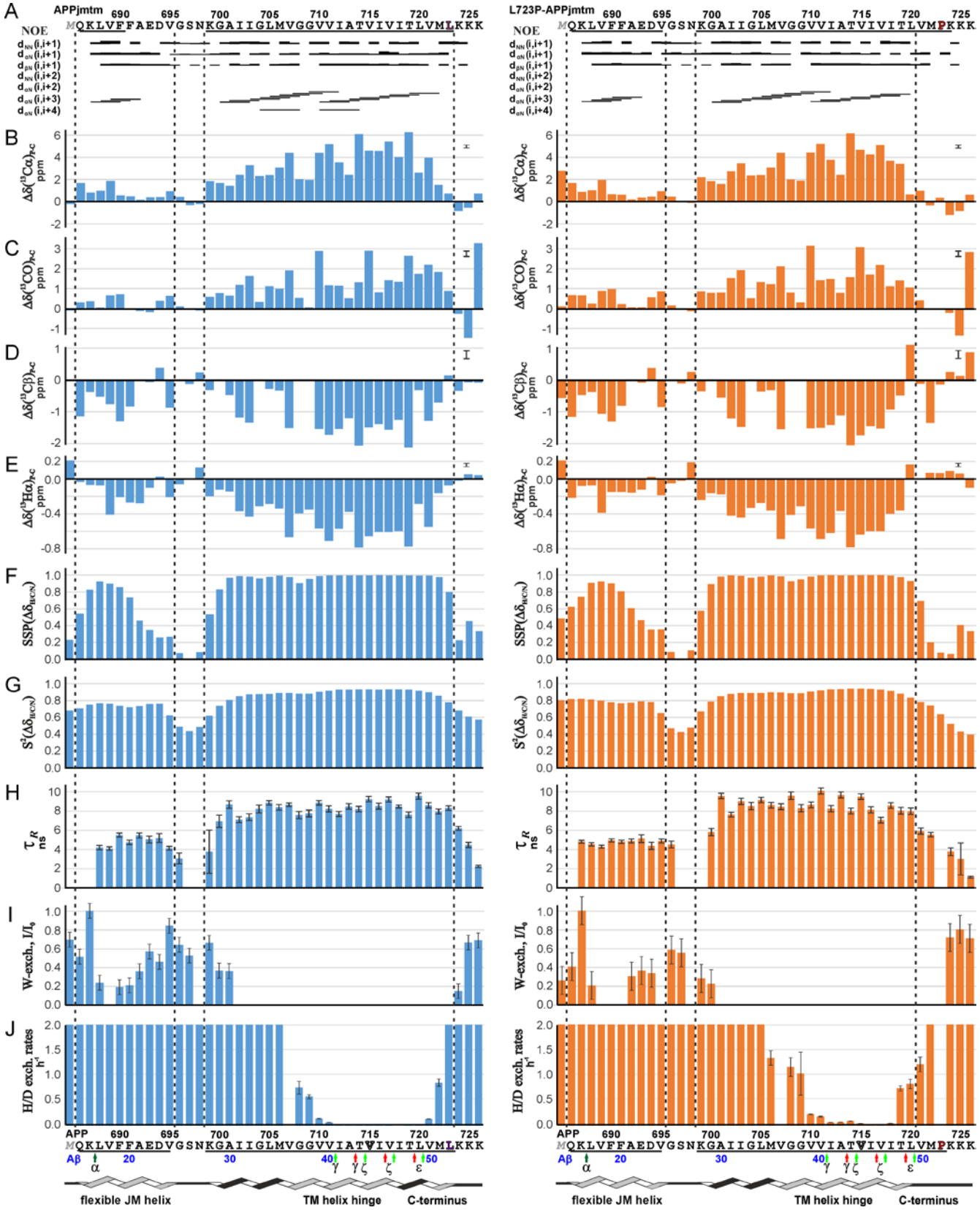
[3] *Federal clinical center of physical-chemical medicine of FMBA, Moscow, Russian Federation;*

[4] *National Research University Higher School of Economics, Moscow, Russian Federation.*

[*] **Correspondence:** bon@nmr.ru



Supplementary Figure S1. Comparison of the ^{1}H-^{15}N signal doubling of TM glycine amide groups upon dimerization of the wild-type APPjmtm peptide and its L723P mutant in the micellar environment. Fragments of the heteronuclear ^{1}H/^{15}N-HSQC NMR spectra acquired for fresh samples of APPjmtm (**A**) and L723P-APPjmtm (**B**) embedded into the DPC micelles at peptide/detergent ratio (P/D) of 1/110, 318 K and pH 6.9. The ^{1}H-^{15}N backbone resonance assignments of the amide groups of the glycine residues situated in the APP TM domain are shown. In both cases the APP TM domain undergoes a slow monomer-dimer transition, as proved by the appearance of P/D-dependent signal doubling in the NMR spectra of APPtmtm (ref. 13). The cross-peaks corresponding to the monomeric and dimeric states are marked by '*m*' and '*d*', respectively. The pattern of chemical shift changes of the mutant upon dimerization is similar to that of the wild type APPjmtm.


A
APPjmtm
L723P-APPjmtm
NOE
B
C
D
E
F
G
H
I
J
APP
Aβ
flexible JM helix
TM helix hinge
C-terminus

Supplementary Figure S2. Summary of the structural-dynamic NMR data for the wild-type APPjmtm peptide (in left) and its L723P mutant (in right) in the micellar environment. (**A**), Sequential and medium range ^{1}H-^{1}H NOE connectivities observed in 3D ^{15}N-edited NOESY-HSQC spectra (80 ms mixing time) are shown by horizontal lines. The absence of the $d_{\alpha N}(i, i+3)$ connectivity between residues T719 and M722 of L723P-APPjmtm indicates unfolding of the last turn of APP TM helix. **(B-E**), Secondary ^{13}Cα, ^{13}CO, ^{13}Cβ and ^{1}Hα chemical shifts are given as the difference between the actual chemical shift and the typical random-coil chemical shift for the given residue. Substantially positive $\Delta\delta(^{13}C\alpha)_{P\text{-}C}$ and $\Delta\delta(^{13}CO)_{P\text{-}C}$ values, as well as negative $\Delta\delta(^{13}C\beta)_{P\text{-}C}$ and $\Delta\delta(^{1}H\alpha)_{P\text{-}C}$ values are indicative of the helical structure of both peptides. Uncertainties are shown by bars in the upper right corners. (**F)** and **(G**), The secondary structure probabilities (SSP) and local order parameters S^2 derived from ^{1}H, ^{13}C and ^{15}N chemical shifts using the TALOS-N software, respectively, illustrate the helical structure distribution and its stability (on the ps-ns and possibly higher timescales) along the sequence of the peptides. (**H**) The ^{15}N-relaxation data of the amide groups off the peptides are presented as local rotation correlation times (τ_R) estimated from ^{15}N CSA/dipolar cross-correlated transverse relaxation. The uncertainties are shown by bars. Smaller relaxation times correspond to higher backbone flexibility. (**I**) Accessibility of the peptide residues to water assessed using CLEANEX experiment, which reveals the backbone amide protons undergoing rapid exchange with water. The uncertainties are shown by bars. (**J**) H/D exchange rates of the backbone amide protons of the peptides are plotted, revealing slow processes of exchange with water. Fast exchange rates above 2 h^{-1} for N- and C-terminal residues could not be properly detected due to finite reconstruction period of lyophilized samples of peptides embedded into DPC micelles. The uncertainties are shown by bars.