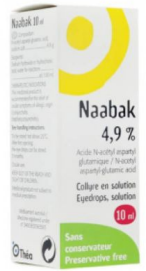
Computational Characterization of N-acetylaspartylglutamate Synthetase: From the Protein Primary Sequence to Plausible Catalytic Mechanism

Kniga A.E[1,2], Polyakov I.V[1,2], Nemukhin A.V.[1,2]

[1]Lomonosov Moscow State University
[2]Emanuel Institute of Biochemical Physics RAS

XXVIII Symposium on *Bioinformatics* and Computer-Aided Drug Discovery
May 25, 2022

N-acetyl-aspartyl-glutamate - the most abundant peptide neurotransmitter



Targets:

1. mGluR3 +, EC50= 11-100 uM
2. NMDA -/+, EC50 = 666 uM

mGluR3 --| AC --| L-Ca^{2+}

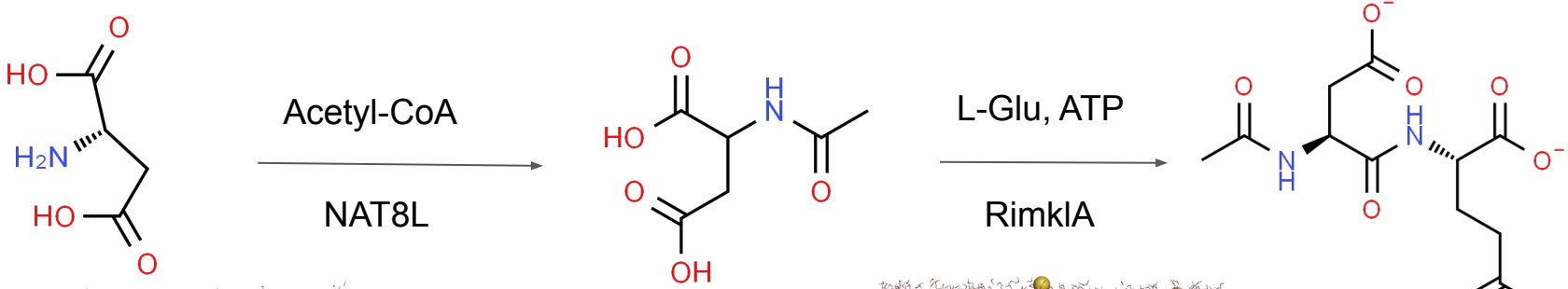
A number of studies stipulate its implication in:

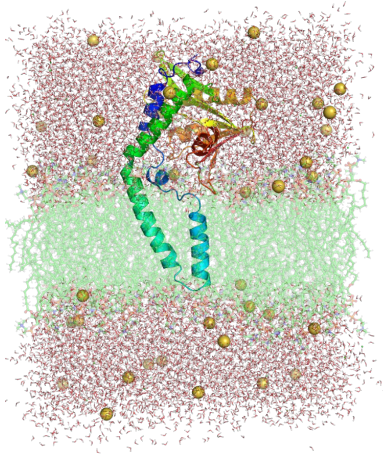
1. Schizophrenia
2. Ischaemia
3. Pain
4. Epilepsy
5. Traumatic Brain Injury

INN: spaglumic acid
Antiallergic - mast cell stabilizer

Morland, C.; Nordengen, K. N-Acetyl-Aspartyl-Glutamate in Brain Health and Disease. Int. J. Mol. Sci. 2022, 23, 1268. https://doi.org/10.3390/ijms23031268

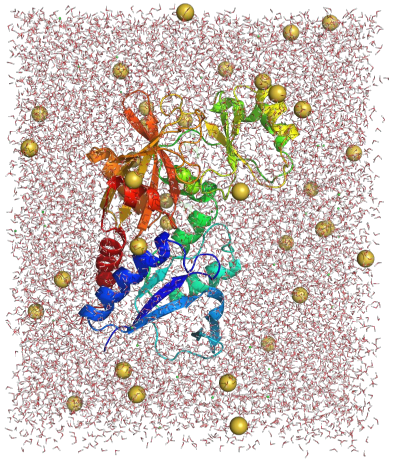
Metabolic pathway for NAAG biosynthesis in brain





in vitro:
K_M(Asp) = 0.16 ± 0.05 mM
k_{cat} = 0.071 ± 0.006 U/mg

Wang Q., Zhao M., Parungao G.G., Viola R.E.: Purification and characterization of aspartate N-acetyltransferase: A critical enzyme in brain metabolism. Protein Expr Purif. 2016 Mar;119:11-8. doi: 10.1016/j.pep.2015.11.001.

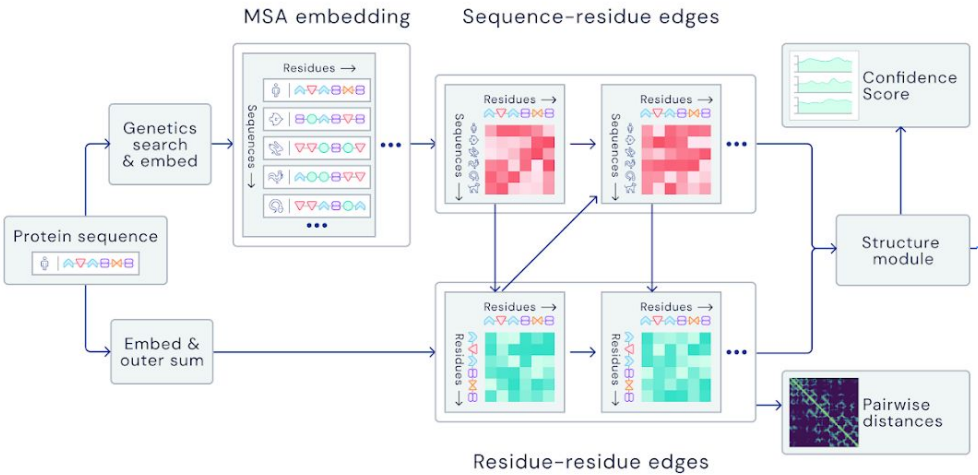


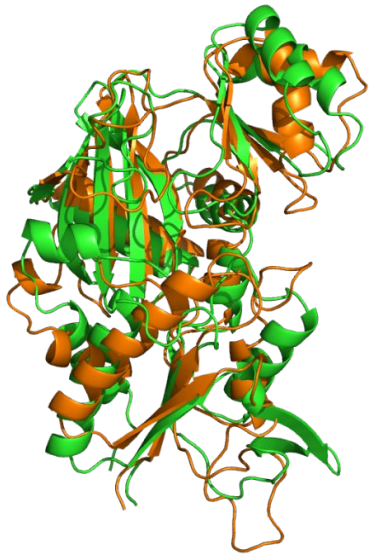
in vitro:
K_M(NAA) = 1.48 mM
K_M(Glu) = 0.88 mM
K_M(mgATP) = 0.065 mM
k_{cat} = 2.6 s^{-1}

Collard F., et al.: Molecular identification of N-acetylaspartylglutamate synthase and beta-citrylglutamate synthase. J Biol Chem. 2010 Sep 24;285(39):29826-33. doi: 10.1074/jbc.M110.152629.

Polyakov IV, Kniga AE, Grigorenko BL, Nemukhin AV. Structure of the Brain *N*-Acetylaspartate Biosynthetic Enzyme NAT8L Revealed by Computer Modeling. ACS Chem Neurosci. 2020 Aug 5;11(15):2296-2302. doi: 10.1021/acschemneuro.0c00250.

Coevolutionary-based protein structure prediction with AlphaFold2





3.5 Å RMSD

AlphaFold2* have been widely recognized as a new state-of-the art protein prediction method after its success in 14th Critical Assessment of protein Structure Prediction (CASP14)

Comparison of the homology model (orange) of RIMKLA structure to AlphaFold2 (green).

*Jumper, J., et al.: Highly accurate protein structure prediction with alphafold. Nature 596(7873), 583–589 (Aug 2021). https://doi.org/10.1038/s41586-021-03819-2

Classical MD of RIMKLA

MD SETUP:

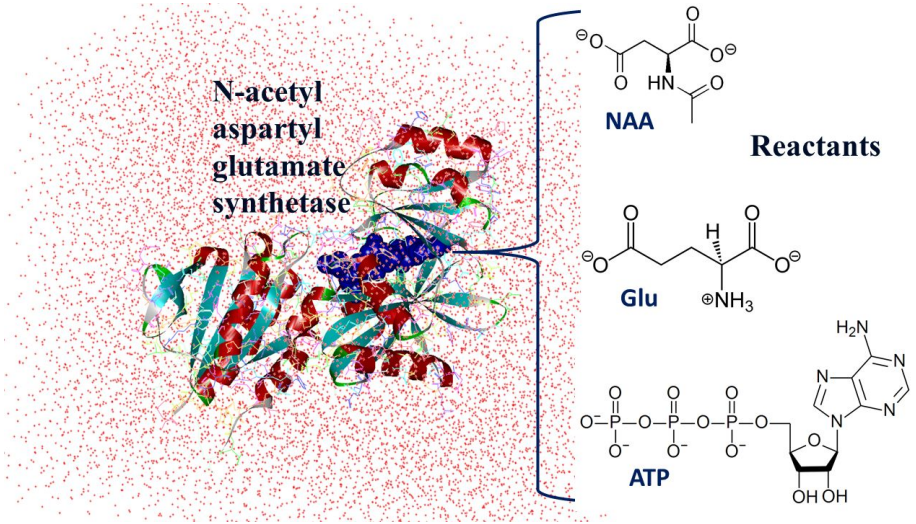
Software: GROMACS, NAMD

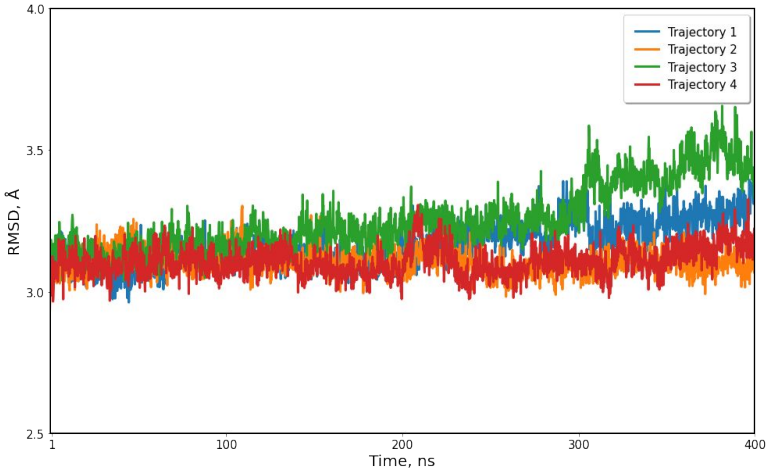
Number of atoms: ~ 40 000

Force field: CHARMM27

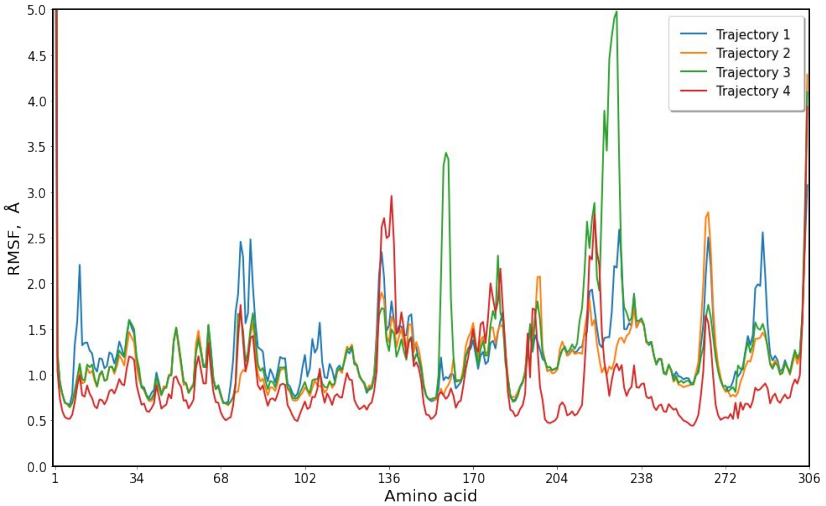
Periodic boundary conditions & Ewald summation scheme

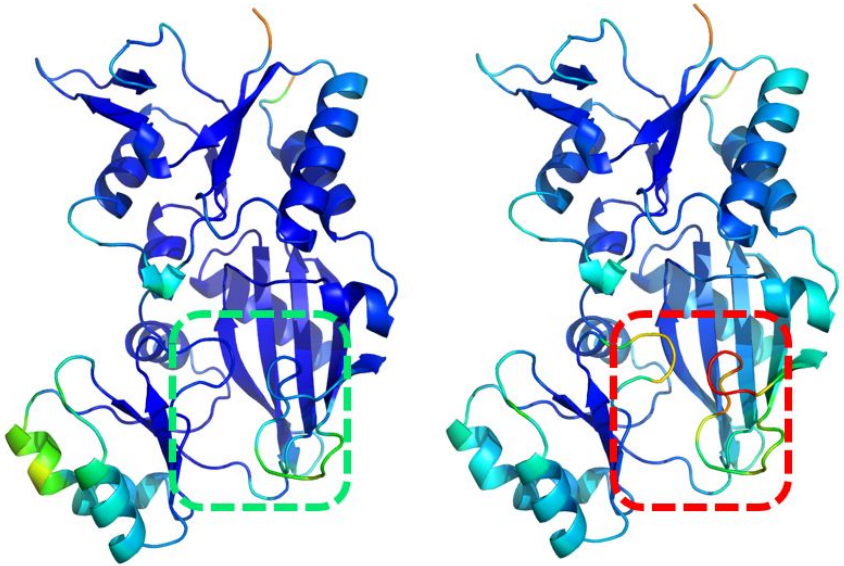
T = 300 K, p = 1 atm





MD results in refinement of active site



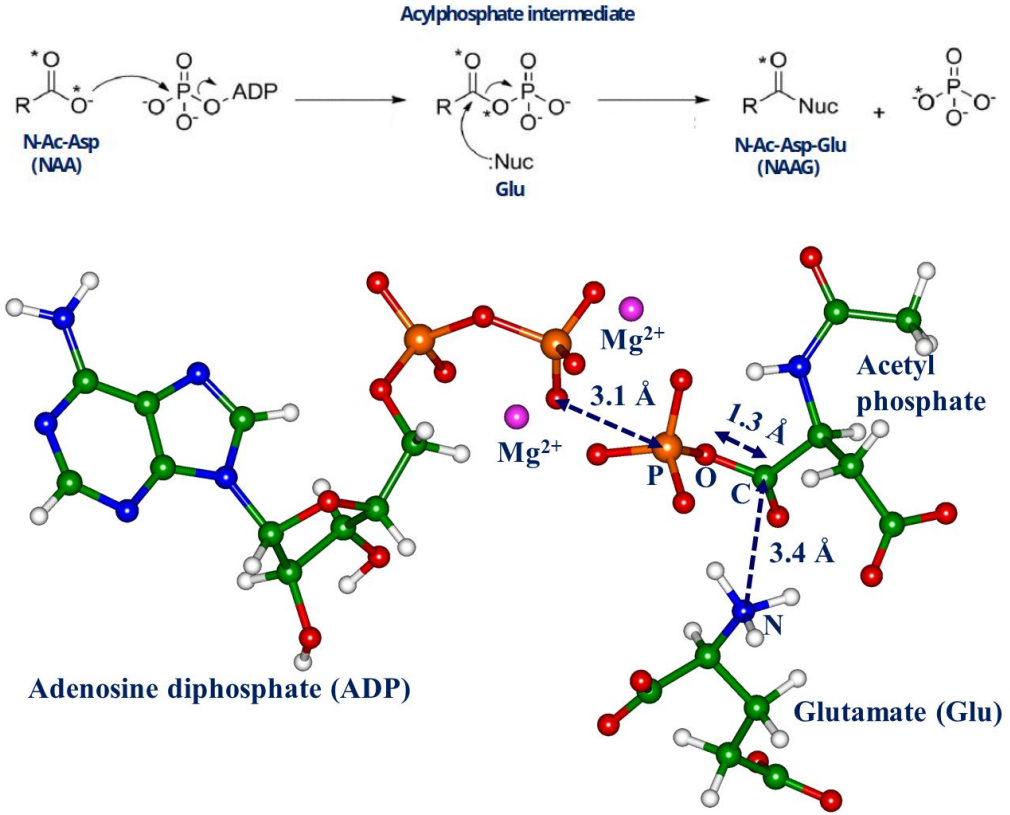


But only with multiple trajectories, we can expect to see actual active site's side chain packing improvement. Trajectories may also terminate in the actual collapse of the active site and the exit of ligands, despite the retention of the domain structure as a whole!

Active site - Mechanism - Intermediate

Proposed reaction mechanism:

1. Transfer of phosphate to N-Ac-Asp leading to acyl phosphate intermediate.
2. Nucleophilic attack N-C leading to NAAGS.



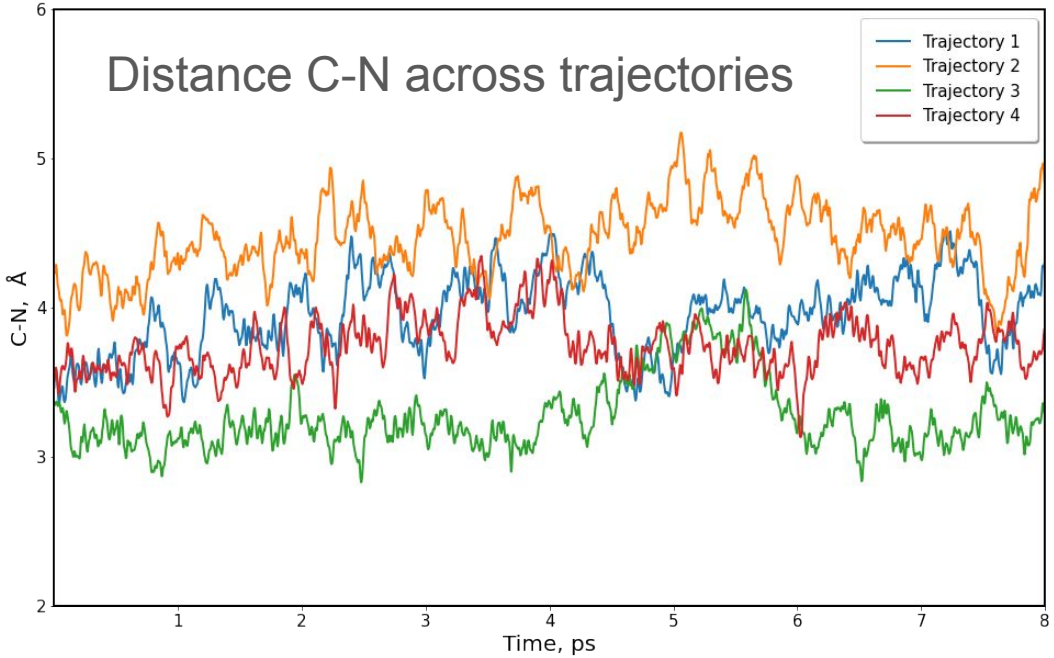
Another example of substrate-assisted catalysis !?

Reactionary configurations - QM/MM MD of RIMKLA-Intermediate complex

NAMD-TeraChem interface*

The QM part contained 135 atoms described by the density function theory with the PBE0 hybrid functional, D3 dispersion corrections and 6-31G** basis set with 1395 basis functions in total. The QM system included NAA-PO_3, ADP (cut on the C4'-C5' bond), two magnesium atoms; Arg160, Arg201, Arg215, Glu273, Asp260, Asn275 side chains and water molecules.

Distance C-N across trajectories vary from 3A to 5A, but consistently low (3A) distance signals that under the condition of attacking amino group deprotonation, the reaction is plausible.



*Khrenova, M.G., Polyakov, I.V., Nemukhin, A.V.: Molecular dynamics of enzyme-substrate complexes in guanosine-binding proteins. Khimicheskaya Fizika 41(6), 66–72 (2022). https://doi.org/10.31857/S0207401X22060061

Conclusion

1. Starting from primary sequence, we obtained a model of RIMKLA in the holo-form and studied its dynamical properties using classical MD.
2. Notably, we were able to reconstruct the structure of the active site including reaction intermediate, coordinated with two Mg2+ ions, ADP and glutamate, that turned out to be stable in some of the trajectories we obtained, including those featuring QM/MM potential.
3. Obtained model suggests that catalysis is likely to proceed through substrate-assisted mechanism with acetyl phosphate or glutamate carboxyl groups as proton acceptors in the second step of the reaction.

Thank you for your attention!