Supplementary materials

Structural evolution of primate glutamate dehydrogenase 2 as revealed by *in silico* predictions and experimentally determined structures

Ionela Litso[1], Andreas Plaitakis[1], Vasiliki E. Fadouloglou[2], Mary Providaki[3], Michael Kokkinidis[3,4] and Ioannis Zaganas [1,*]

[1] Neurology/Neurogenetics Laboratory, School of Medicine, University of Crete, Voutes, Heraklion, 71003 Crete, Greece; medp2012038@med.uoc.gr ; andreasplaitakis@gmail.com

[2] Department of Molecular Biology and Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece; fadoulog@mbg.duth.gr

[3] Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology-Hellas, Heraklion, Greece; providak@imbb.forth.gr; kokkinid@imbb.forth.gr

[4] Department of Biology, University of Crete, Vasilika Vouton, GR 71409, PO Box 2208, Heraklion, Crete, Greece; kokkinid@imbb.forth.gr

* Correspondence: zaganas@uoc.gr

		Dynamut	iMutant	MUpro	iSTABLE	PremPS	MaestroWeb	SDM
	Mutations	ΔΔG (kcal/mol)	ΔΔG (kcal/mol)	Conf. score	Conf. Score	ΔΔG (kcal/mol)	ΔΔGpred (kcal/mol)	ΔΔGpred (kcal/mol)
Human Node A-B	***A3V***	**1.13 (S)**	-0.31(D)	**0.18(S)**	0.63(D)	**-0.17(S)**	**-0.08(S)**	**1.16(S)**
Human Node A-B	***E34K***	0.54 (S)	**-0.81(D)**	**-1.00(D)**	**0.87(D)**	**0.22(D)**	**0.27(D)**	**-0.86(D)**
Human Node A-B	***D142E***	**0.27 (S)**	-0.69(D)	**1.00(S)**	**0.69(S)**	**-0.17(S)**	**-0.44(S)**	**1.25(S)**
Human Node A-B	***S174N***	**0.15 (S)**	**-0.30(S)**	**0.12 (S)**	**0.76(S)**	0.16(D)	**-0.12(S)**	**0.29(S)**
Human Node A-B	***R443S***	**-0.27 (D)**	**-1.11(D)**	**-1.00 (D)**	**0.75(D)**	**0.29(D)**	-0.21(S)	**-0.92(D)**
Human Node A-B	***G456A***	**0.35 (S)**	-0.90(D)	**0.54 (S)**	**0.61(S)**	1.26(D)	**-0.60(S)**	**1.57(S)**
Human Node A-B	***N498S***	**-0.51 (D)**	0.12(S)	**-0.66(D)**	**0.59(D)**	**0.77(D)**	-0.41(S)	**-0.07(D)**
Human Node B-C	***V3L***	0.75(S)	**-0.68(D)**	**-0.28(D)**	**0.87(D)**	-0.22(S)	**0.12(D)**	**-0.15(D)**
Human Node B-C	***R39Q***	0.35(S)	**-0.93(D)**	0.02(S)	**0.53(D)**	**0.34(D)**	-0.13(S)	**-0.06(D)**
Human Node B-C	***K299R***	**-0.51(D)**	**-0.07(D)**	**-0.18(D)**	**0.60(D)**	**0.47(D)**	-0.22(S)	0.01(S)
Human Node B-C	***S331T***	0.12(S)	0.10(S)	**-0.55(D)**	**0.53(D)**	**0.24(D)**	**0.79(D)**	0.23(S)
Human Node B-C	***M370L***	**0.13(S)**	-1.52(D)	-1.00(D)	0.82(D)	**-0.72(S)**	**-0.01(S)**	**0.29(S)**
Human Node B-C	***R470H***	0.08(S)	**-1.34(D)**	**-0.53(D)**	**0.85(D)**	**0.56(D)**	-0.40(S)	**-0.15(D)**
Human Node C-D	***I166V***	**-0.27(D)**	**-0.64(D)**	**-1.00(D)**	**0.81(D)**	**0.26(D)**	-0.08(S)	0.04(S)
Human Node C-D	***G247R***	0.57(S)	-0.22(S)	**-0.40 (D)**	0.56(S)	**0.25(D)**	**0.14(D)**	**-2.36(D)**
Human Node C-D	***A321V***	-0.06(D)	**-0.02(S)**	**0.02(S)**	**0.69(S)**	**-0.07(S)**	**-0.19(S)**	-0.47(D)
Human Node D-E	***R299K***	**-0.83(D)**	**-0.89(D)**	**-1.00 (D)**	**0.67(D)**	-0.61(S)	-0.08(S)	**-0.72(D)**
Human Node D-E	***M415L***	**-0.02(D)**	**-0.95(D)**	**-0.60 (D)**	**0.70(D)**	**0.48(D)**	**0.14(D)**	0.54(S)
Chimpanzee Node E	***I305L***	**-0.49(D)**	**-0.50 (D)**	**-0.04 (D)**	**0.61(D)**	-0.183(S)	**0.09(D)**	0.09(S)
Chimpanzee Node E	***V321I***	0.15(S)	**-0.25(D)**	**-1.00 (D)**	**0.78(D)**	**0.099(D)**	-0.05(S)	**-0.39(D)**
Gorilla Node D	***S66C***	**-0.03(D)**	**-1.06(D)**	**-0.72 (D)**	**0.62(D)**	**0.44(D)**	-0.37(S)	0.7(S)
Gorilla Node D	***K362R***	-0.12 (D)	-0.36(D)	**0.39 (S)**	**0.58(S)**	0.21(D)	**-0.35(S)**	**0.26(S)**
Gorilla Node D	***L365Q***	**-0.50(D)**	**-2.38(D)**	**-1.00(D)**	**0.85(D)**	**1.64(D)**	-0.52(S)	0.17(S)
Gorilla Node D	***E439D***	**-0.53(D)**	-0.28(S)	**-0.98(D)**	0.58(S)	**0.06(D)**	-0.08(S)	**-1.48(D)**
Orangutan Node C	***I239N***	**-0.38(D)**	**-1.71(D)**	**-1.00(D)**	**0.81(D)**	**0.67(D)**	-0.77(S)	**-0.86(D)**
Orangutan Node C	***L240V***	**-1.53(D)**	**-1.51(D)**	**-0.46(D)**	**0.88(D)**	-0.28(S)	-0.27(S)	**-3.06(D)**
Orangutan Node C	***I275V***	**-1.02(D)**	**-0.93(D)**	**-1.00(D)**	**0.83(D)**	**0.51(D)**	**0.06(D)**	**-1.91(D)**
Orangutan Node C	***L375V***	**-1.57(D)**	**-1.75(D)**	**-1.00(D)**	**0.80(D)**	**1.14(D)**	-0.35(S)	**-3.07(D)**
Orangutan Node C	***Q441R***	**-0.50(D)**	0.19(S)	**-0.76(D)**	0.57(S)	**0.3(D)**	**0.01(D)**	**-0.23(D)**
Gibbon Node B	***E8K***	0.35(S)	**-1.00(D)**	**-1.00(D)**	**0.76(D)**	-0.19(S)	**0.35(D)**	**-0.16(D)**
Gibbon Node B	***T101A***	**-0.54(D)**	**-1.16(D)**	**-0.77(D)**	**0.71(D)**	**0.36(D)**	**0.16(D)**	0.12(S)
Gibbon Node B	***L377V***	**-1.15(D)**	**-1.70(D)**	**-1.00(D)**	**0.81(D)**	**0.79(D)**	-0.27(S)	**-2.90(D)**

Table S1. Quantitative analysis of the predicted effect of amino acid substitutions during evolution. Effect: (D)Destabilizing, (S) Stabilizing. The majority consensus among methods is highlighted in bold (energy trend estimated by three or more methods).

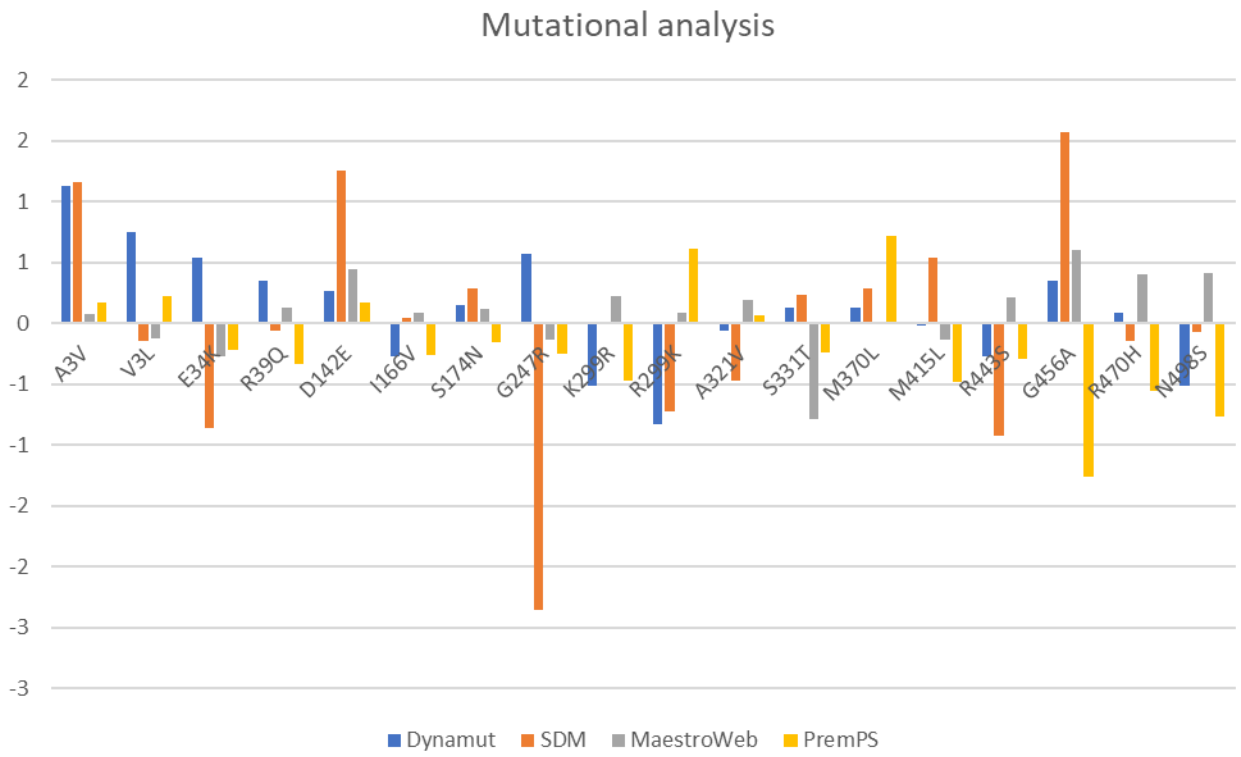

Figure S1. Effects of amino acid substitutions occurring during hGDH2 evolution on enzyme stability analyzed by four different webservers (Dynamut, SDM, Maestroweb, PremPS). Results from MastroWeb and PremPS have been multiples by -1 so that all values indicating stabilization are above zero and all values indicating destabilization are below zero.