

Supplementary Information

July 30, 2025

Supplementary Tables

Supplementary Table 1: Single-point mutation effect on Gibbs Free Energy predictions of multiple softwares

Mutation	mCSM	SDM	DUET	DynaMut2	DDGun	MAESTRO	SAAFEC
C10R	0.048000	0.000000	0.337000	1.090000	0.100000	0.095675	-0.980000
L12P	-1.623000	-4.310000	-2.304000	-1.510000	-2.900000	3.504819	-1.690000
M13I	-1.911000	0.970000	-1.285000	-1.210000	-0.100000	0.480930	-0.610000
D18G	-0.906000	-0.900000	-0.968000	-0.480000	-1.800000	2.058429	-0.730000
D18N	-0.815000	0.440000	-0.504000	-1.040000	-0.400000	1.346156	-0.500000
D18E	-0.579000	-0.290000	-0.338000	0.020000	-0.800000	1.176819	-0.550000
A19D	-0.522000	-0.430000	-0.223000	-0.170000	-1.300000	1.805418	-0.850000
V20I	-0.393000	0.030000	-0.067000	-0.110000	-0.200000	-0.160910	-0.900000
R21Q	-0.282000	-0.380000	-0.171000	-0.270000	-0.400000	-0.036758	-0.200000
S23N	-0.411000	0.690000	0.011000	0.060000	-0.100000	1.436890	-0.570000
P24S	-1.185000	0.530000	-0.747000	-1.320000	-2.900000	0.872833	-1.220000
A25S	-1.950000	-1.860000	-2.034000	-1.560000	-1.100000	2.452239	-1.130000
A25T	-1.694000	-1.580000	-1.711000	-1.350000	-1.100000	2.044710	-1.100000
V28S	-2.668000	-3.200000	-2.970000	-3.430000	-3.800000	3.216426	-2.310000
V28M	-0.918000	-1.590000	-1.181000	-1.370000	-1.000000	1.022958	-1.330000
V30G	-2.920000	-3.200000	-3.472000	-4.180000	-5.400000	4.724899	-2.640000
V30A	-2.428000	-2.660000	-2.887000	-2.580000	-3.400000	3.477366	-1.670000
V30M	-0.949000	-2.390000	-1.400000	-1.010000	-1.800000	1.659947	-1.240000
V30L	-1.071000	-1.500000	-1.166000	-1.320000	-1.500000	1.067225	-0.950000
V32G	-2.267000	-3.200000	-2.811000	-3.290000	-3.500000	4.482067	-2.670000
V32A	-1.671000	-2.660000	-2.082000	-2.330000	-2.100000	2.085456	-1.620000
F33C	-1.490000	0.270000	-1.259000	-2.060000	-2.300000	1.275398	-1.470000
F33L	-1.847000	0.110000	-1.675000	-2.210000	-0.800000	1.191928	-1.460000
F33V	-2.270000	1.050000	-1.911000	-3.270000	-0.900000	1.433640	-1.360000
F33I	-1.847000	0.590000	-1.584000	-2.430000	-0.800000	1.298581	-1.450000
R34G	-2.396000	0.190000	-2.079000	-3.130000	-1.900000	1.555289	-1.010000

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Mutation	mCSM	SDM	DUET	DynaMut2	DDGun	MAESTRO	SAAFEC
R34T	-1.829000	-0.480000	-1.719000	-2.080000	-0.900000	0.926791	-0.450000
K35T	-1.401000	0.340000	-1.094000	-1.330000	0.800000	0.939181	-0.250000
K35N	-1.061000	-0.390000	-0.942000	-1.020000	0.200000	1.076140	0.010000
A36D	-1.381000	-1.790000	-1.436000	-0.550000	0.200000	1.742734	-0.120000
A36P	-0.695000	-1.750000	-0.814000	-0.150000	-0.100000	1.086776	-0.200000
D38V	0.188000	-0.120000	0.390000	-0.120000	-0.100000	0.641339	0.080000
D38A	0.037000	-0.480000	0.150000	-0.070000	-0.400000	1.333339	0.010000
D39V	0.040000	1.180000	0.523000	-0.220000	0.000000	0.486930	0.050000
W41L	-2.187000	-0.130000	-1.754000	-1.430000	-2.200000	1.562413	-1.600000
E42G	-1.208000	0.160000	-1.014000	-2.030000	-0.800000	0.284824	-0.530000
E42D	-0.993000	-0.960000	-0.949000	-0.900000	-0.400000	0.331035	-0.160000
F44S	-2.761000	-2.800000	-2.899000	-3.530000	-2.300000	3.008940	-2.820000
F44L	-1.120000	-2.310000	-1.323000	-2.270000	1.000000	1.170348	-1.700000
F44Y	-0.611000	-0.160000	-0.366000	-0.010000	-0.300000	0.730337	-1.530000
A45S	-1.056000	-1.530000	-1.025000	-0.350000	-0.600000	1.588476	-0.310000
A45D	-0.628000	-1.010000	-0.460000	-0.240000	-1.700000	2.452025	-0.400000
A45T	-0.861000	-0.290000	-0.575000	-0.250000	-0.300000	0.943673	-0.330000
A45G	-0.859000	0.530000	-0.441000	-1.030000	-1.200000	1.678345	-0.400000
G47E	-1.077000	-0.380000	-0.795000	-0.480000	-0.600000	1.876033	-0.810000
G47R	-0.785000	-0.510000	-0.582000	-1.030000	0.100000	0.517850	-0.590000
G47A	-0.568000	-0.240000	-0.314000	-0.110000	0.300000	-0.074155	-0.570000
G47V	-0.411000	0.940000	0.112000	-1.150000	0.700000	-0.069220	-0.910000
T49S	-1.373000	-0.980000	-1.370000	-1.110000	-1.700000	1.359749	-1.090000
T49P	-0.470000	-0.600000	-0.424000	-0.210000	-2.600000	1.369659	-1.070000
T49A	-0.862000	0.830000	-0.444000	-0.450000	-2.400000	1.389565	-1.250000
T49I	-0.314000	0.410000	0.013000	-0.400000	-1.300000	0.438767	-1.310000
S50I	-0.191000	1.250000	0.341000	-0.470000	-0.500000	-0.192585	-0.770000
S50R	-0.368000	0.330000	-0.043000	0.160000	0.400000	-0.347085	-0.590000
E51G	-0.662000	-0.160000	-0.510000	0.090000	-0.600000	0.121036	-0.290000
S52P	-0.326000	-0.340000	-0.199000	-0.110000	-0.600000	0.692510	-0.180000
G53E	-2.923000	-2.740000	-3.220000	-1.090000	-2.800000	2.744853	-1.700000
G53R	-1.014000	-3.520000	-1.195000	-1.260000	-1.900000	2.180179	-1.560000
G53A	-0.734000	-1.540000	-0.806000	-0.340000	-2.200000	0.615396	-1.140000
E54D	-1.089000	-0.850000	-1.026000	-1.160000	-0.500000	1.243156	-0.680000
E54K	-0.395000	-0.150000	-0.105000	-0.030000	0.900000	1.948915	-0.800000
E54Q	-0.552000	-0.720000	-0.423000	-0.480000	0.700000	0.866636	-0.570000
E54G	-0.665000	0.560000	-0.332000	-0.010000	-1.100000	0.916399	-1.040000
E54L	0.452000	0.630000	0.814000	1.350000	0.400000	-0.406742	-0.710000
L55P	-1.386000	-2.190000	-1.672000	-1.090000	-1.900000	2.680218	-1.990000
L55Q	-1.466000	-1.040000	-1.363000	-2.150000	-1.800000	2.513701	-1.970000
L55R	-0.853000	-1.460000	-0.778000	-0.590000	-1.200000	2.331249	-1.890000
H56R	-0.060000	-0.610000	0.094000	0.440000	0.100000	0.140162	-0.560000
G57R	-0.443000	-2.390000	-0.509000	-0.270000	1.000000	0.355795	-0.600000

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Mutation	mCSM	SDM	DUET	DynaMut2	DDGun	MAESTRO	SAAFEC
L58R	-0.560000	-2.460000	-0.630000	1.070000	-2.200000	2.739356	-1.190000
L58H	-0.839000	-0.720000	-0.847000	-0.580000	-2.500000	2.273616	-1.260000
T59K	-0.346000	-1.490000	-0.338000	-0.080000	-1.100000	2.223868	-1.070000
T59R	-0.541000	-1.350000	-0.506000	-0.170000	0.000000	1.994997	-0.930000
T60A	-0.740000	-0.250000	-0.615000	0.120000	-0.200000	0.890968	-0.140000
E61G	-0.827000	-0.160000	-0.688000	-0.540000	0.200000	0.053070	-0.270000
E61K	-0.016000	-0.270000	0.264000	0.200000	0.000000	-0.172136	-0.220000
E62K	0.413000	-0.270000	0.695000	1.310000	-0.100000	-0.441654	-0.380000
F64S	-2.944000	-3.120000	-3.093000	-3.460000	-2.300000	3.257644	-2.600000
F64I	-0.572000	-0.070000	-0.398000	1.260000	-0.200000	1.765091	-1.920000
F64L	-0.572000	-2.240000	-0.743000	0.520000	0.300000	1.263882	-1.950000
G67R	-1.003000	-0.250000	-0.756000	-1.340000	-1.500000	2.123210	-1.350000
G67E	-1.671000	-0.650000	-1.490000	-1.300000	-2.300000	1.633188	-1.270000
I68L	-0.383000	-0.560000	-0.193000	-0.210000	-0.400000	0.450980	-0.750000
Y69H	-2.277000	-0.900000	-2.258000	-3.060000	-2.700000	3.411389	-1.780000
Y69I	-1.148000	0.570000	-0.828000	-1.700000	-3.100000	2.176070	-1.480000
K70N	-1.977000	-0.630000	-1.957000	-2.360000	-0.300000	0.780808	-0.470000
V71A	-2.708000	-2.660000	-3.176000	-3.440000	-2.300000	2.510127	-1.680000
E72G	-2.431000	1.080000	-2.031000	-2.420000	-0.700000	1.048607	-0.470000
I73V	-2.018000	-2.380000	-2.399000	-1.11	-0.500000	1.544197	-1.320000
D74H	-1.138000	0.380000	-0.881000	-1.540000	1.000000	0.926688	-0.690000
S77Y	-0.631000	0.710000	-0.339000	-1.360000	0.300000	-0.074396	-0.860000
S77F	-0.918000	0.760000	-0.605000	-0.280000	0.300000	-0.470808	-0.730000
Y78F	-0.723000	-0.510000	-0.542000	-0.440000	-1.500000	0.914390	-1.340000
A81T	-0.663000	-1.970000	-0.696000	0.290000	0.000000	0.360463	-0.250000
A81V	-0.219000	-1.030000	-0.130000	-1.120000	0.000000	0.334558	-0.160000
G83R	-0.388000	-2.390000	-0.457000	-0.290000	-0.100000	0.071862	-0.990000
I84S	-1.771000	-2.260000	-1.918000	-1.320000	-0.800000	1.452406	-0.800000
I84N	-1.025000	-1.430000	-0.978000	-1.360000	-0.900000	1.104595	-0.480000
I84T	-1.526000	-1.460000	-1.510000	-1.230000	-0.400000	1.403141	-0.600000
H88R	-0.823000	-0.210000	-0.623000	-0.460000	-0.700000	1.272331	-0.770000
E89Q	-0.269000	-0.900000	-0.162000	-0.040000	0.400000	-0.037222	-0.790000
E89K	-0.005000	-0.400000	0.250000	0.220000	-0.200000	0.046183	-1.070000
H90N	-0.107000	-0.980000	-0.158000	0.390000	-1.000000	0.819017	-1.380000
H90D	-0.259000	-0.820000	-0.227000	0.400000	-1.500000	1.256770	-1.120000
A91S	-1.801000	-2.420000	-1.977000	-2.010000	-0.900000	1.682146	-1.300000
E92K	-1.138000	-0.580000	-0.990000	-1.170000	-0.500000	0.200088	-0.820000
V93M	-0.439000	-2.060000	-0.967000	-1.070000	-1.300000	0.892321	-1.190000
V94A	-1.122000	-1.410000	-1.200000	-1.410000	-1.300000	1.277868	-1.470000
A97S	-1.055000	-2.420000	-1.192000	-0.330000	-0.400000	1.529493	-1.130000
A97G	-0.832000	-0.430000	-0.643000	-1.170000	-1.400000	1.744698	-1.120000
G101S	-0.678000	-3.480000	-0.988000	-0.240000	0.600000	0.990593	-0.560000
P102R	-0.110000	0.070000	0.124000	0.160000	0.600000	0.278971	-0.040000

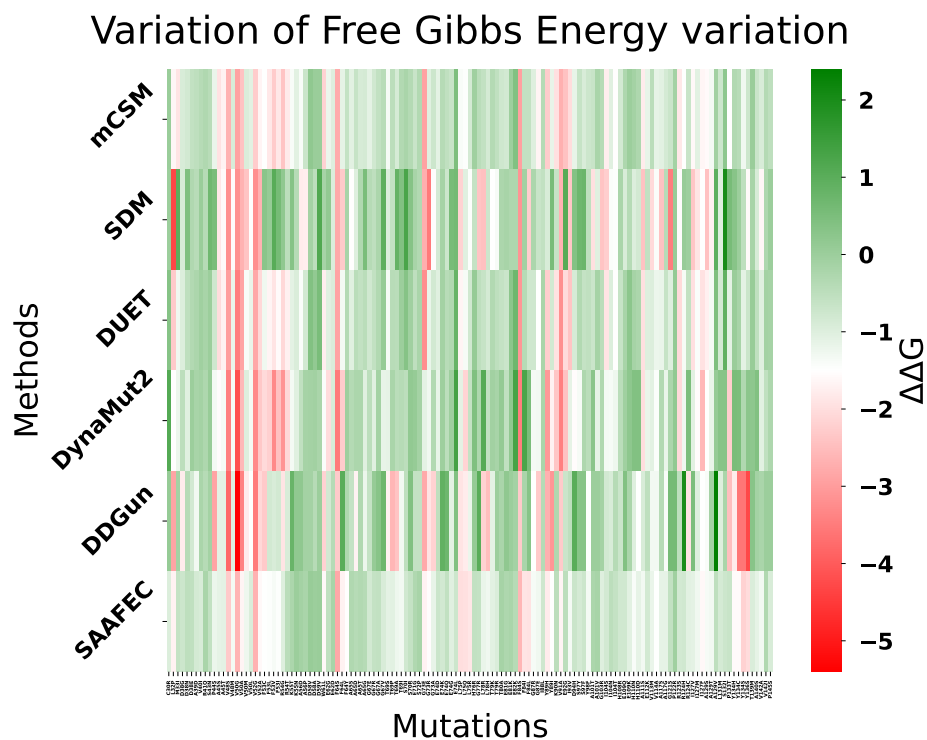
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Mutation	mCSM	SDM	DUET	DynaMut2	DDGun	MAESTRO	SAAFEC
R103S	-1.851000	-1.560000	-2.057000	-2.050000	-0.500000	1.293087	-0.810000
R104H	-1.485000	0.070000	-1.372000	-1.260000	2.000000	0.126805	-0.790000
R104C	-0.669000	-0.030000	-0.567000	0.510000	-1.900000	0.037933	-1.060000
I107V	-1.620000	-2.380000	-1.968000	-1.050000	0.500000	1.472897	-1.100000
I107M	-1.071000	-1.910000	-1.424000	-1.560000	-1.000000	1.844882	-1.270000
I107F	-1.635000	-1.490000	-1.942000	-2.590000	-1.500000	1.954769	-1.600000
A109S	-1.575000	-2.420000	-1.739000	-1.470000	-1.400000	2.446090	-1.350000
A109T	-1.341000	-1.720000	-1.364000	-1.170000	-0.100000	1.447455	-1.290000
A109V	-0.092000	0.950000	0.434000	0.100000	2.400000	0.271922	-0.510000
L111M	-0.737000	-0.990000	-0.837000	0.300000	-1.000000	1.952754	-0.830000
S112I	0.236000	2.020000	0.896000	0.310000	-0.200000	-0.011485	-0.730000
P113T	-1.518000	0.550000	-1.083000	-2.250000	-2.500000	0.927692	-0.670000
Y114H	-0.330000	0.300000	0.040000	0.520000	-1.800000	-0.125850	-1.570000
Y114C	-0.409000	0.020000	-0.165000	0.500000	-3.600000	-0.017269	-1.570000
Y114S	-1.264000	-0.500000	-1.074000	-0.300000	-3.600000	-0.295191	-2.200000
Y116S	-0.728000	-1.590000	-0.688000	0.250000	-4.300000	2.405803	-2.030000
T119M	0.031000	-0.160000	0.065000	0.230000	0.300000	-0.270595	-0.950000
A120S	-0.700000	-0.910000	-0.528000	0.390000	-0.100000	0.715069	-1.120000
V122A	-0.904000	-1.650000	-1.018000	-1.070000	-0.200000	1.091749	-1.300000
V122I	-0.367000	-0.280000	-0.111000	-0.540000	0.000000	0.150486	-0.290000
P125S	-0.496000	0.000000	-0.145000	-0.270000	0.000000	0.187510	-0.920000

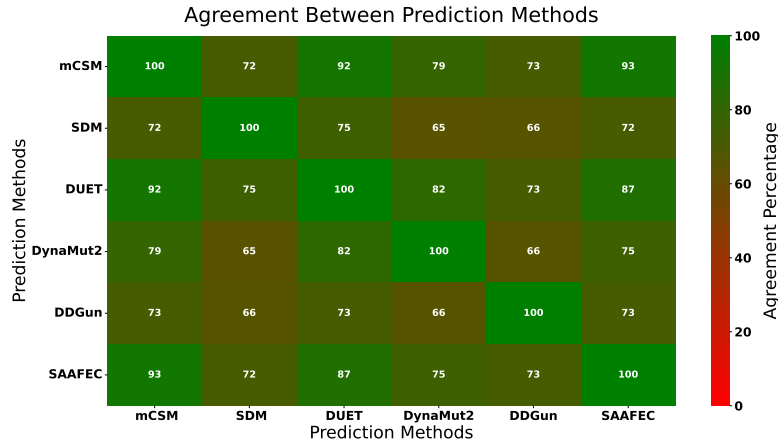
Parameter	Protein	Protein-Ligand complex
Simulation Time	100 ns	100 ns
Integration timestep	0.02 fs	0.02 fs
Water model	tip3p	tip3p
Simulation box shape	cubic	cubic
Simulation box dimension	11-12*	11-12*
Position restrain NVT and NPT	DPOSRES	DPOSRES
LINCS constrains	h-bonds	h-bonds
Electrostatic force calculation	PME	PME
Force field	CHARMM36	CHARMM36
Temperature	300	300

Supplementary Table 2: Molecular dynamics parameters used. (*Box dimension varies between different TTR variants, created with a distance between the solute and the box of 2 nm.)

Supplementary Figures

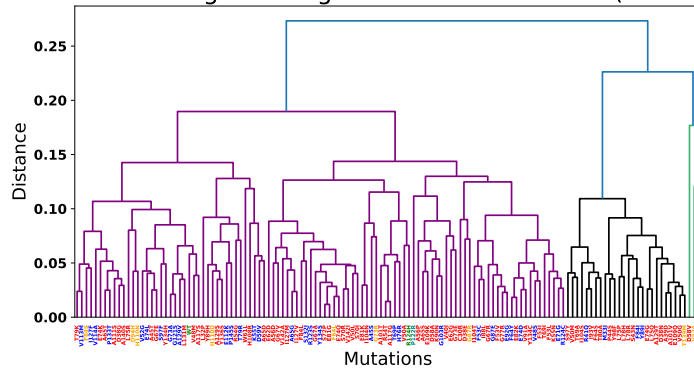


Supplementary Figure 1: Heatmap showing the predicted Gibbs free energy changes ($\Delta\Delta G$) for each TTR mutation across different computational tools. Red and green colours indicate destabilizing and stabilizing mutations, respectively. The use of multiple predictors highlights variability in estimated mutation effects.

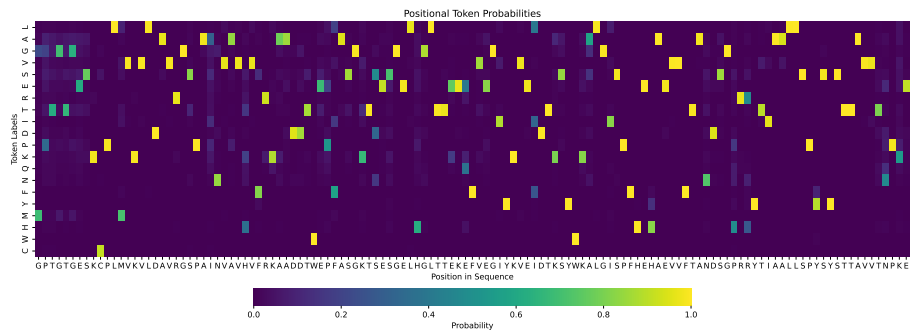


Supplementary Figure 2: Agreement heatmap for stabilizing versus destabilizing classification, highlighting tool concordance in pathogenicity prediction. Agreement values are reported using a green-red colormap, with green cells reporting methods with high agreement while red depicting methods with low agreement in $\Delta\Delta G$ prediction.

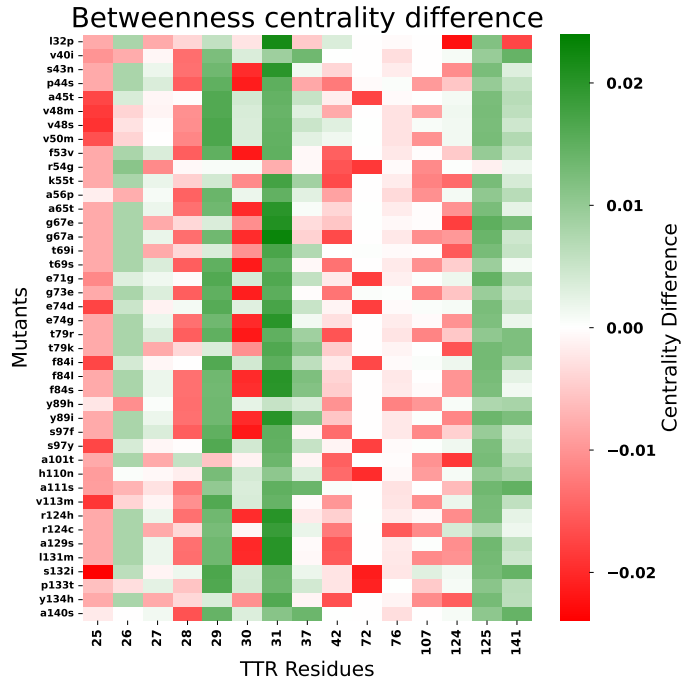
Hierarchical Clustering Dendrogram for TTR monomer (Average Linkage)



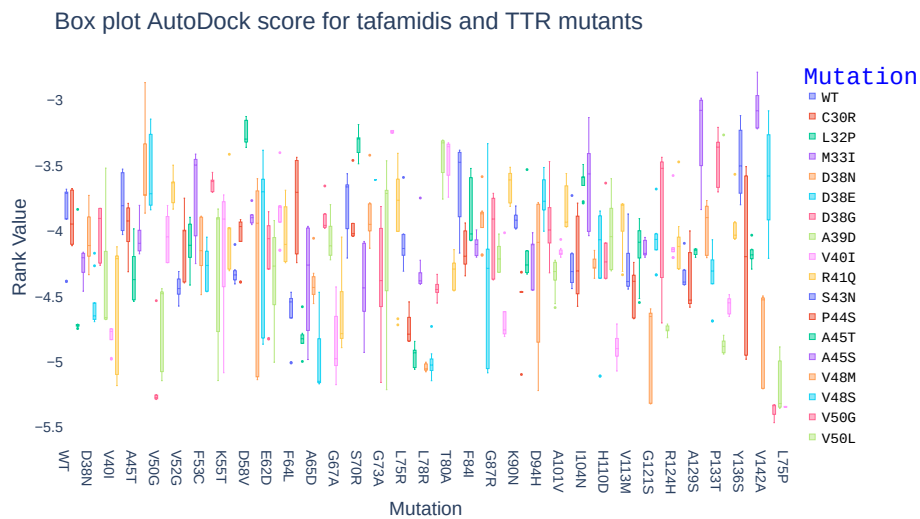
Supplementary Figure 3: Hierarchical clustering analysis of TTR variants based on TM-score similarity. Clinical categories are color-coded: red (Pathogenic), orange (Likely pathogenic), green (Benign), teal (Likely benign), and blue (Unknown). Three clusters are reported in the dendrogram, colored in purple, black and sea-green.



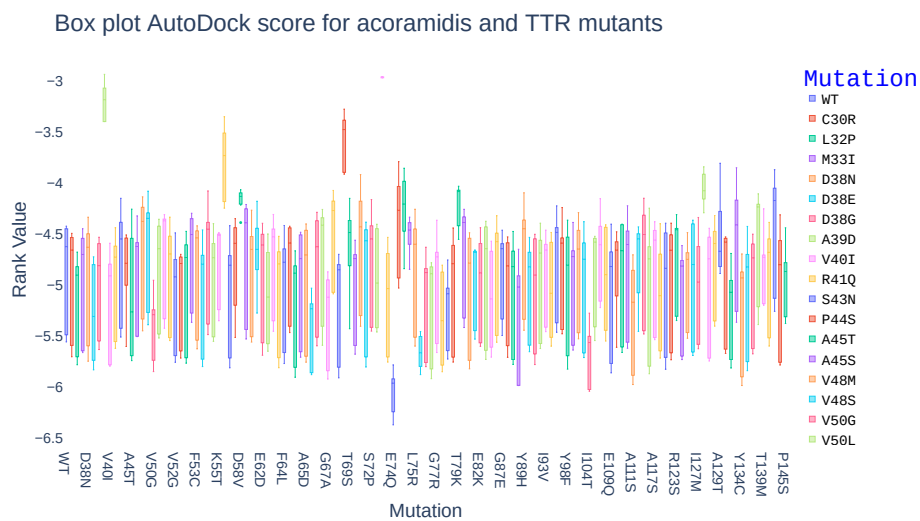
Supplementary Figure 4: **Residue-level mutation probabilities predicted by ESM2 for the wild-type TTR monomer.** The heatmap depicts the estimated mutability at each residue position, with higher values (depicted in green-yellow) indicating regions of increased evolutionary flexibility. Structural or functional hotspots typically exhibit lower mutability.



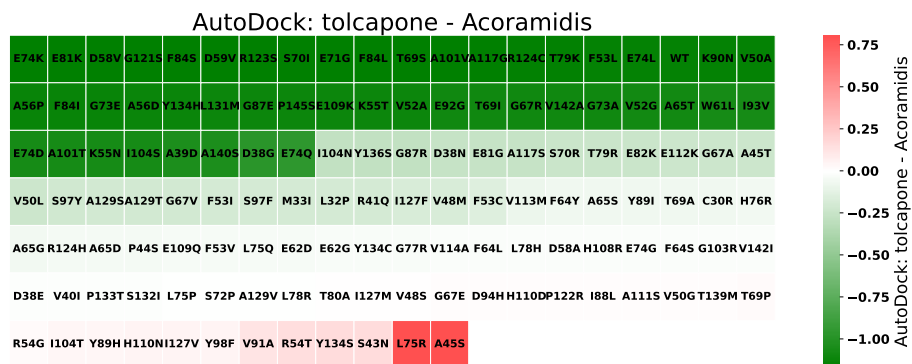
Supplementary Figure 5: Residue-level differences in betweenness centrality between wild-type and mutant TTR tetramers. Only one representative chain is shown; figure includes only mutations with at least five altered residues.



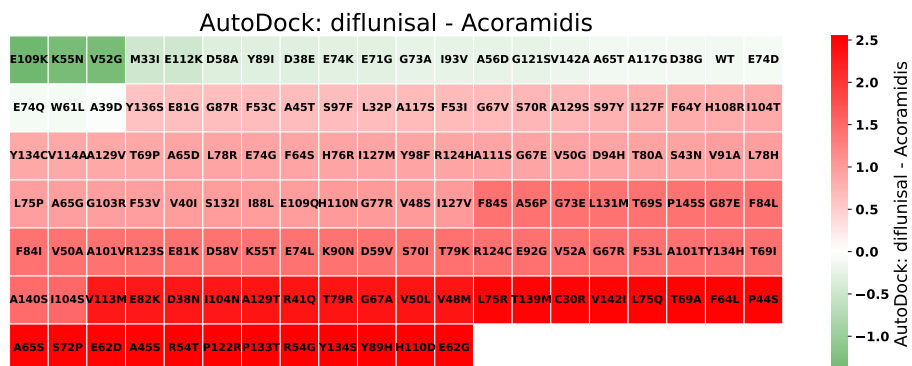
Supplementary Figure 6: Distribution of AutoDock Vina binding affinities across TTR tetramer mutants for tafamidis.



Supplementary Figure 7: Distribution of AutoDock Vina binding affinities across TTR tetramer mutants for acoramidis.

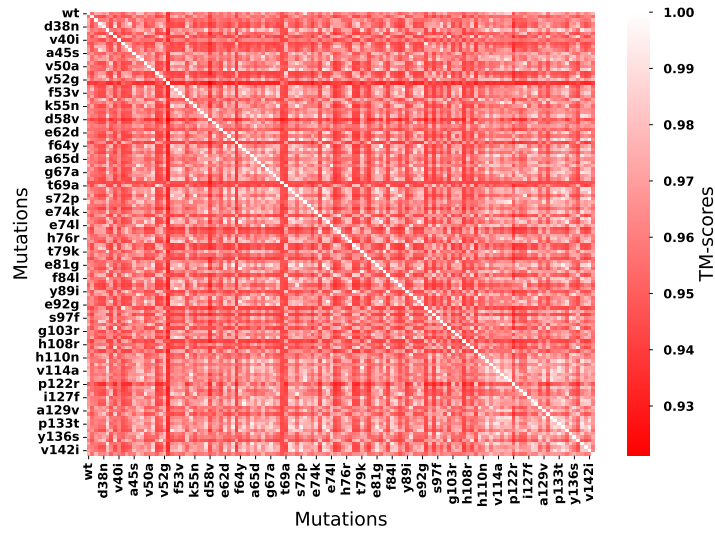


Supplementary Figure 8: Evaluation of tolcapone ligand targeting TTR mutants. White cells depicts mutations that report approximately no changes in binding affinity, while red and green ones depict mutations with decreased or increased binding affinity againsta acoramidis ligand.



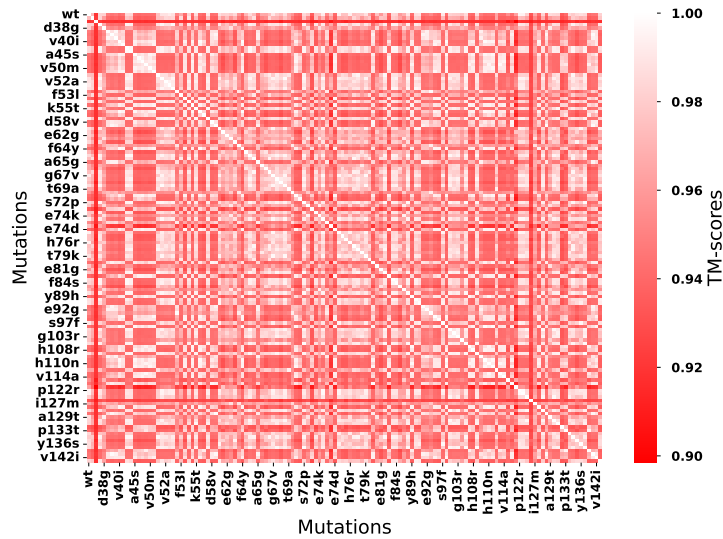
Supplementary Figure 9: Evaluation of diflunisal ligand targeting TTR mutants. White cells depicts mutations that report approximately no changes in binding affinity, while red and green ones depict mutations with decreased or increased binding affinity againsta acoramidis ligand.

TM-scores between TTR mutants in monomer conformation



Supplementary Figure 10: Heatmap with TM-Score calculated for the monomer.

TM-scores between TTR mutants in tetramer conformation



Supplementary Figure 11: Heatmap with TM-Score calculated for the tetramer.

TTR Mutations in Italian Regions



Supplementary Figure 12: Geographical distribution of most common TTR mutations in Italian regions. Data obtained from [1]

References

- [1] Massimo Russo, Laura Obici, Ilaria Bartolomei, Francesco Cappelli, Marco Luigetti, Silvia Fenu, Tiziana Cavallaro, Maria Grazia Chiappini, Chiara Gemelli, Luca Guglielmo Pradotto, Fiore Manganelli, Luca Leonardi, Filomena My, Simone Sampaolo, Chiara Briani, Luca Gentile, Claudia Stancanelli, Eleonora Di Buduo, Paolo Pacciolla, Fabrizio Salvi, Silvia Casagrande, Giulia Bisogni, Daniela Calabrese, Fiammetta Vanoli, Giuseppe Di Iorio, Giovanni Antonini, Lucio Santoro, Alessandro Mauro, Marina Grandis, Marco Di Girolamo, Gian Maria Fabrizi, Davide Pareyson, Mario Sabatelli, Federico Perfetto, Claudio Rapezzi, Giampaolo Merlini, Anna Mazzeo, and Giuseppe Vita and. Attrv amyloidosis italian registry: clinical and epidemiological data. *Amyloid*, 27(4):259–265, 2020. PMID: 32696671.