

In Silico Mutagenesis of Protein–Protein Interfaces: A DMS-Validated, Three-Tier NOVA/SCWRL Web Pipeline Applied to the SARS-CoV-2 Spike–ACE2 Interface

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Protein–protein interfaces govern molecular recognition and viral entry, and single interface mutations can decisively alter binding. Experimental deep mutational scanning (DMS) maps such effects exhaustively but is slow and system-specific, motivating fast computational approaches. We present an interactive, experimentally validated pipeline for *in silico* mutagenesis of any protein–protein interface, demonstrated on the SARS-CoV-2 Spike receptor-binding domain (RBD)–ACE2 interface. Interface residues are detected with 4 Å and every interface position is comprehensively mutated. Fast protocol scores each mutation: Tier 1 (always, ~5 s/mutation) applies SCWRL rotamer repacking and per-residue NOVA non-bonded energy; NOVA (protein Optimization in VAcuo, 10.5 Å) scales charged-residue charges to emulate solvent screening. Later on, MD simulated annealing and LightDock re-docking for flagged edge cases can be applied. The pipeline is delivered as a web application returning NOVA heatmaps, per-mutation triage and biophysical descriptors. Validated against two independent DMS datasets (Starr et al.), the method reproduces the direction of mutation effects with ~82% sign agreement. Crucially, this directional accuracy is robust: across 19 independent Spike–ACE2 interface scans spanning wild-type and Alpha/Beta/Gamma/Omicron/XBB structures, the median sign agreement is 82% (17/19 scans ≥80%). On the same Spike–ACE2 DMS benchmark, the only prior systematic comparison found that no structure-based predictor achieved meaningful correlation (FoldX best, R = -0.51, 64% binary classification; Ozden et al., 2024), so the directional agreement reported here is a substantial improvement.

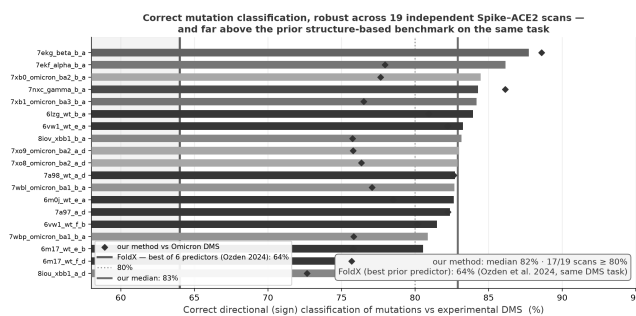


Figure 1. Directional (sign) classification agreement with experimental DMS across 19 independent SARS-CoV-2 Spike–ACE2 interface scans (wild-type and variants). Bars: vs ancestral Bloom DMS; diamonds: vs Omicron DMS. Median 82%; 17/19 scans ≥ 80% (dashed line).

References. [1] Starr T.N. et al. Cell 182, 1295–1310 (2020). [2] Starr T.N. et al. Science 377, 420–424 (2022). [3] Krieger E. et al. Proteins 57, 678–683 (2004). [4] Krivov G.G. et al. Proteins 77, 778–795 (2009). [5] Jiménez-García B. et al. Bioinformatics 34, 129–131 (2018). [6] Ozden B. et al. Proteins 92, 529–539 (2024). *Funding:* ARIS Slovenia grant J7-50043.