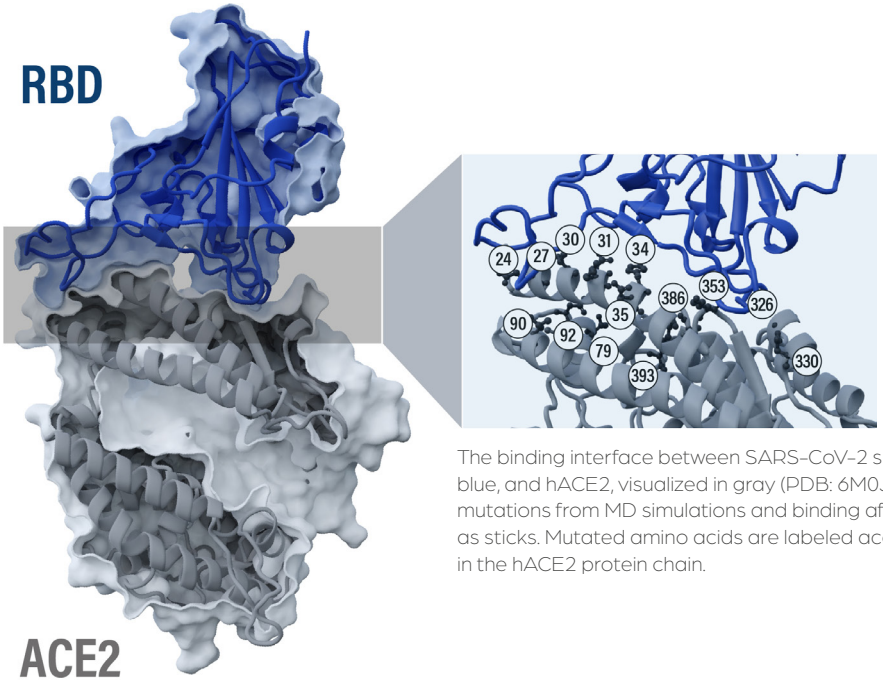


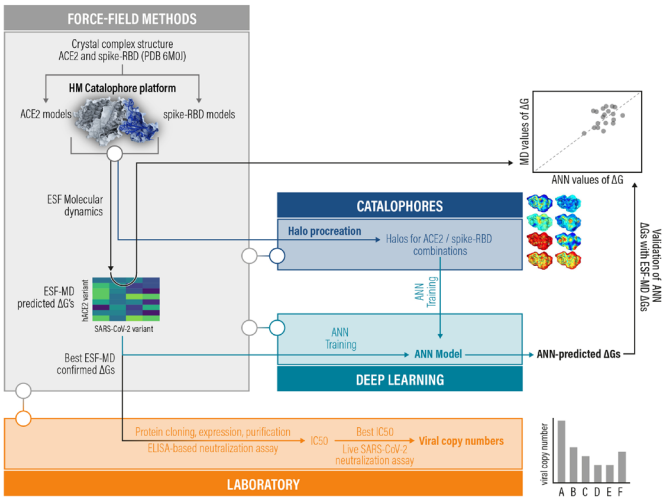
The binding interface between SARS-CoV-2 spike RBD, shown in dark blue, and hACE2, visualized in gray (PDB: 6M0J). Selection of hACE2 mutations from MD simulations and binding affinity assays are shown as sticks. Mutated amino acids are labeled according to their position in the hACE2 protein chain.

CASE STUDY | PROTEIN-LIGAND INTERACTIONS & DRUG DESIGN

Variant-specific **Therapeutic SARS-CoV-2 Decoys**

We identified hACE2-Fc K31W and multi-mutation variants as high-affinity candidates, which we validated *in vitro* with virus neutralization assays. We evaluated binding affinities of these ACE2 variants with the RBDs of Omicron BA.3, Omicron BA.4/BA.5, and Omicron BA.2.75 *in silico*. In addition, candidates produced in *Nicotiana benthamiana*, an expression organism for potential large-scale production, showed a 4.6-fold reduction in half-maximal inhibitory concentration (IC50) compared with the same variant produced in CHO cells and an almost six-fold IC50 reduction compared with wild-type hACE2-Fc. Our workflow could potentially be applied to other viral targets, such as the MERS entry receptor DPP4. However, our system is especially useful in assessing the efficacy of a given hACE2 decoy to a new VOC at an early stage, shortening timelines for hACE2-decoy adaptation and reducing the number of samples for *in vitro* selection.

Multi-level strategy to identify high-affinity human ACE2 (hACE2) variants for neutralization of diverse SARS-CoV-2 variants.



In brief, homology models (HM) of varying SARS-CoV-2 spike receptor binding domain (RBD) structures in complex with hACE2 variant structures served as input for molecular dynamics (MD) simulations analyzed via an empirical scoring function (ESF) closely related to the linear interaction energy (LIE) model.

Gibbs free energy predictions (ΔG) were combined with Catalophore Halos to train an artificial neural network (ANN). This enables the model to predict ΔG values for hACE2- and SARS-CoV-2 variants based on their Halos.

ANN ΔG predictions were validated with ESF ΔG values. The SARS-CoV-2 neutralizing potential of promising hACE2 variants was evaluated in ELISA-based competitive inhibition assays followed by live SARS-CoV-2-based neutralization assays.

Köchl, K., Schopper, T., Durmaz, V. et al. Optimizing variant-specific therapeutic SARS-CoV-2 decoys using deep-learning-guided molecular dynamics simulations. *Sci Rep* **13**, 774 (2023). https://doi.org/10.1038/s41598-023-27636-x