Machine Learning Assisted Drug Discovery for SARS-CoV-2

Authors: Arun Sharma, Neeraj Chaturvedi, Dinesh Gupta*

(September 13th, 2023)

Presented by

Neeraj Chaturvedi
Senior Research Fellow
Translational Bioinformatics Group
ICGEB, New Delhi

Email: neeraj@icgeb.res.in; chaturvedineeraj.111@gmail.com

Reference: https://assets.researchsquare.com/files/rs-967196/v1/6e040cdf-a7d7-4af6-b201-00c95c013278.pdf?c=1635494778

Content

- About SARS-CoV-2
- Need for anti-SARS-CoV-2 drug discovery
- Current status of drug discovery for SARS-CoV-2
- Machine learning (ML) approaches used by our group for anti-SARS-CoV-2 drug discovery
- Performance of ML models with training and external validation datasets
- Deployment of the best ML models on ASCoVPred web-server and standalone
- Conclusion

About SARS-CoV-2

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA-enveloped virus that causes Coronavirus disease (COVID-19) in humans.

- Entire genome is 29881 bp in length (GenBank no. MN908947), encoding 9860 amino acids.

- Genes express structural and nonstructural proteins.

- The S, E, M, and N genes encode structural proteins.

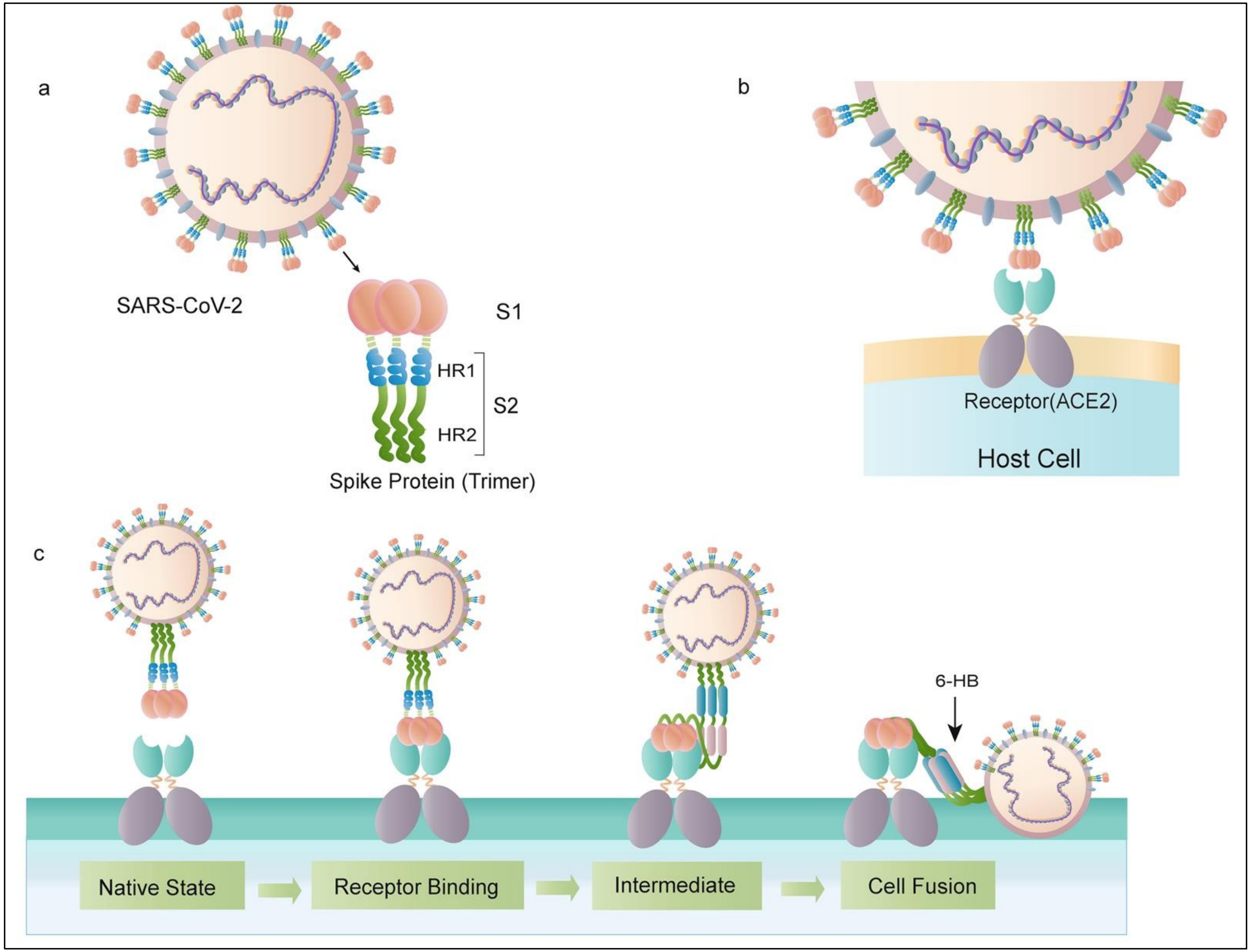
- Nonstructural proteins: 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase (RdRp).

- A large number of **glycosylated S proteins cover the surface of SARS-CoV-2** and **bind to the host cell receptor angiotensin-converting enzyme 2 (ACE2), mediating viral cell entry.**

- When the S protein binds to the receptor, TM protease serine 2 (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, promotes virus entry into the cell by activating the S protein.

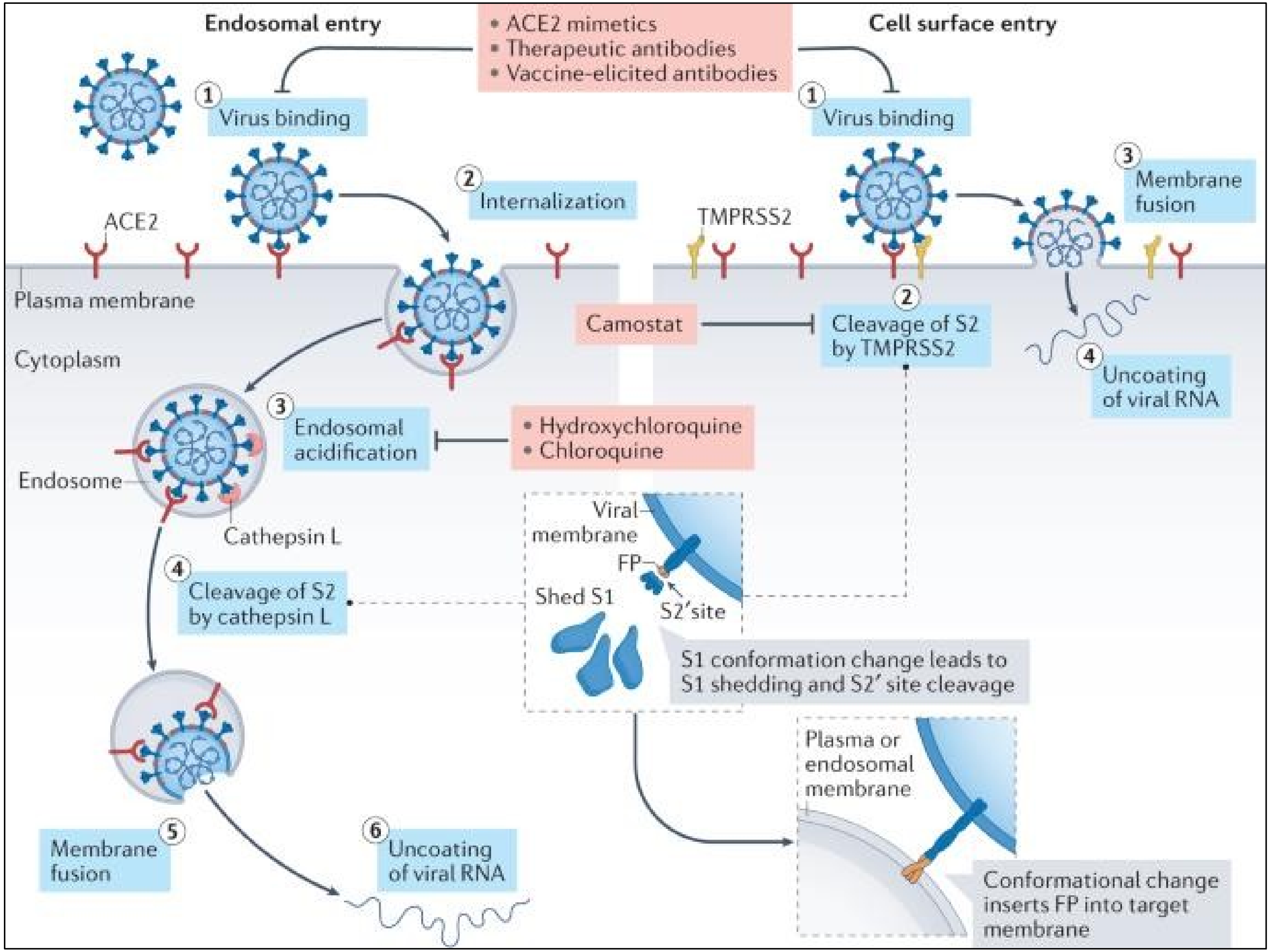
https://www.nature.com/articles/s41580-021-00418-x

Figure 1. a The schematic structure of the S protein. b The S protein binds to the receptor ACE2. c The binding and virus–cell fusion process mediated by the S protein.



https://www.nature.com/articles/s41401-020-0485-4

Figure 2. Modes of SARS-CoV-2 virus entry in the host cells. The S protein binds to the receptor, TM protease serine 2 (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, promotes virus entry into the cell by activating the S protein.



https://www.nature.com/articles/s41580-021-00418-x

Need for anti-SARS-CoV-2 drug discovery

- According to the World Health Organization (WHO) online dashboard, as of August 16, 2023, the SARS-CoV-2 has infected more than 769.80 million people, with nearly 7 million deaths globally.

- Treatment options, such as the development of antivirals, immunomodulators, neutralizing antibody therapies, cell therapy, etc., are **ongoing and yet to pass through different clinical trials**.

- However, ***in vitro* discovery of novel inhibitors** is **tedious, labor-intensive, time-consuming and costly exercise**.

- Although several vaccines have been developed to reduce the disease burden, effective antivirals are still required to treat infected and hospitalized patients.

- Computational predictions facilitate *in vitro* discovery by shortlisting the most effective chemical entities, saving time and cost.

https://covid19.who.int/ https://www.fda.gov/drugs/emergency-preparedness-drugs/coronavirus-covid-19-drugs

Current status of drug discovery for SARS-CoV-2

- **Veklury** (remdesivir), is the U.S. Food and Drug Administration (FDA) approved drug available for adult and certain pediatric COVID-19 patients.

- **Olumiant** (baricitinib) and **Actemra** (Tocilizumab) is approved for the treatment of COVID-19 in hospitalized adults requiring supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO).

- **S-protein** and **TMPRSS2** play a **vital role in SARS-CoV-2 entry into human target cells**.

- Computational approaches, including molecular docking and machine learning (ML)-based classification algorithm development, have been used to identify suitable anti-SARS-CoV-2 inhibitors.

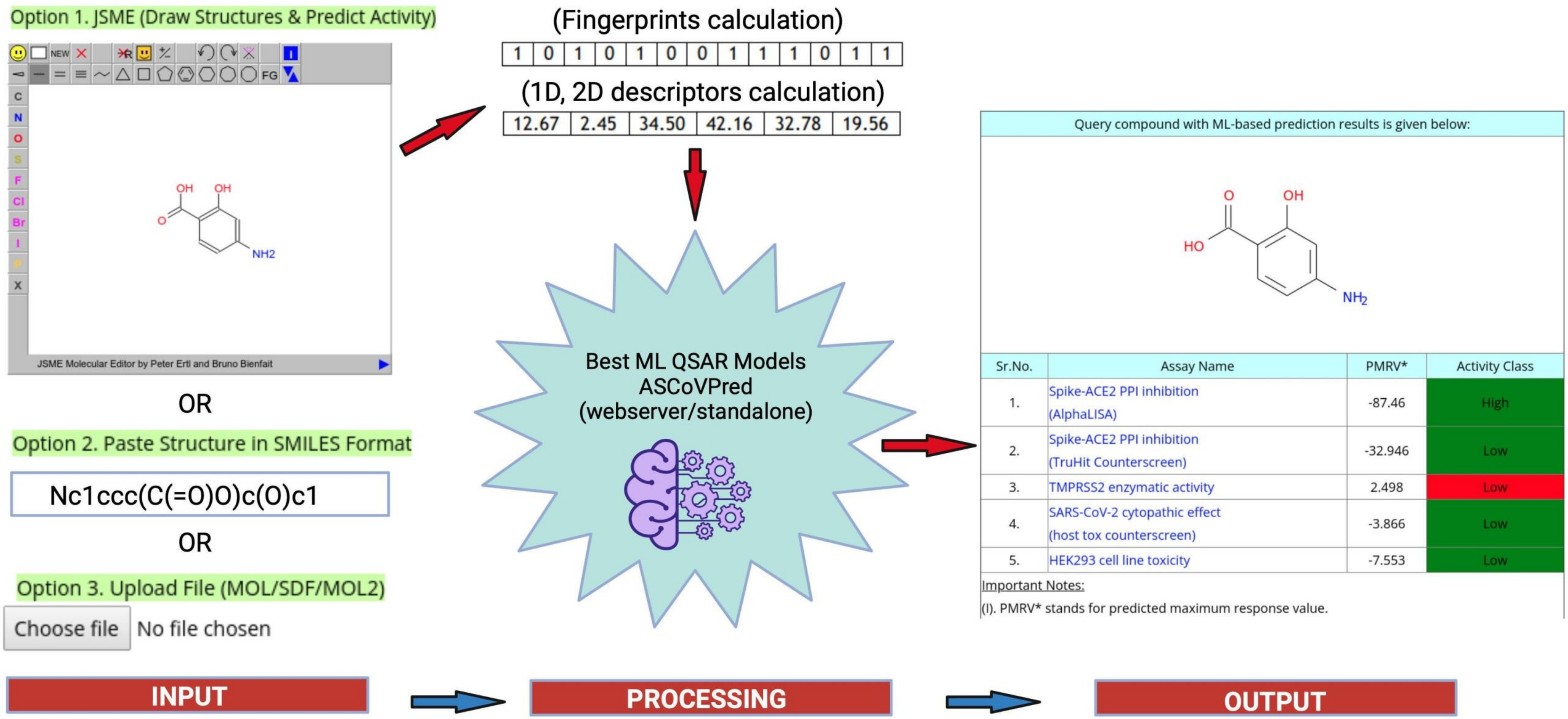
- Systematic attempts to develop **ML-based models through quantitative structure-activity relationship (QSAR) approaches are lacking**, which **motivated us to develop ML-based QSAR models that could rapidly screen large chemical libraries to identify anti-SARS-CoV-2 compounds**.

https://www.fda.gov/drugs/emergency-preparedness-drugs/coronavirus-covid-19-drugs

ML approaches used by our group for anti-SARS-CoV-2 drug discovery

(https://assets.researchsquare.com/files/rs-967196/v1/6e040cdf-a7d7-4af6-b201-00c95c013278.pdf?c=1635494778)

Figure 3. Anti-SARS-CoV-2 activity and human cell toxicity prediction of molecules through ASCoVPred webserver.



Option 1. JSME (Draw Structures & Predict Activity)

JSME Molecular Editor by Peter Ertl and Bruno Bienfait

OR

Option 2. Paste Structure in SMILES Format

Nc1ccc(C(=O)O)c(O)c1

OR

Option 3. Upload File (MOL/SDF/MOL2)

(Fingerprints calculation)

1	0	1	0	1	0	0	1	1	1	0	1	1

(1D, 2D descriptors calculation)

12.67	2.45	34.50	42.16	32.78	19.56

Best ML QSAR Models ASCoVPred (webserver/standalone)

Query compound with ML-based prediction results is given below:

Sr.No.	Assay Name	PMRV*	Activity Class
1.	Spike-ACE2 PPI inhibition (AlphaLISA)	-87.46	High
2.	Spike-ACE2 PPI inhibition (TruHit Counterscreen)	-32.946	Low
3.	TMPRSS2 enzymatic activity	2.498	Low
4.	SARS-CoV-2 cytopathic effect (host tox counterscreen)	-3.866	Low
5.	HEK293 cell line toxicity	-7.553	Low

Important Notes:

(I). PMRV* stands for predicted maximum response value.

INPUT → PROCESSING → OUTPUT

ASCoVPred webserver & standalone URL: https://apexbtic.icgeb.res.in/ascovpred/

- **Data source:** A total of **nine high-throughput screening (HTS) assays data were downloaded** from National Centre for Advancing Translational Sciences (NCATS) website and used for the machine learning (ML)-based models training and evaluation (apexbtic.icgeb.res.in/ascovpred/supple.php).

- The nine HTS assays used to test compounds' bio-activities by NCATS can be **broadly categorized into four different types:**

(i) Prevent viral entry into host cells.

(ii) Prevent viral replication into host cells.

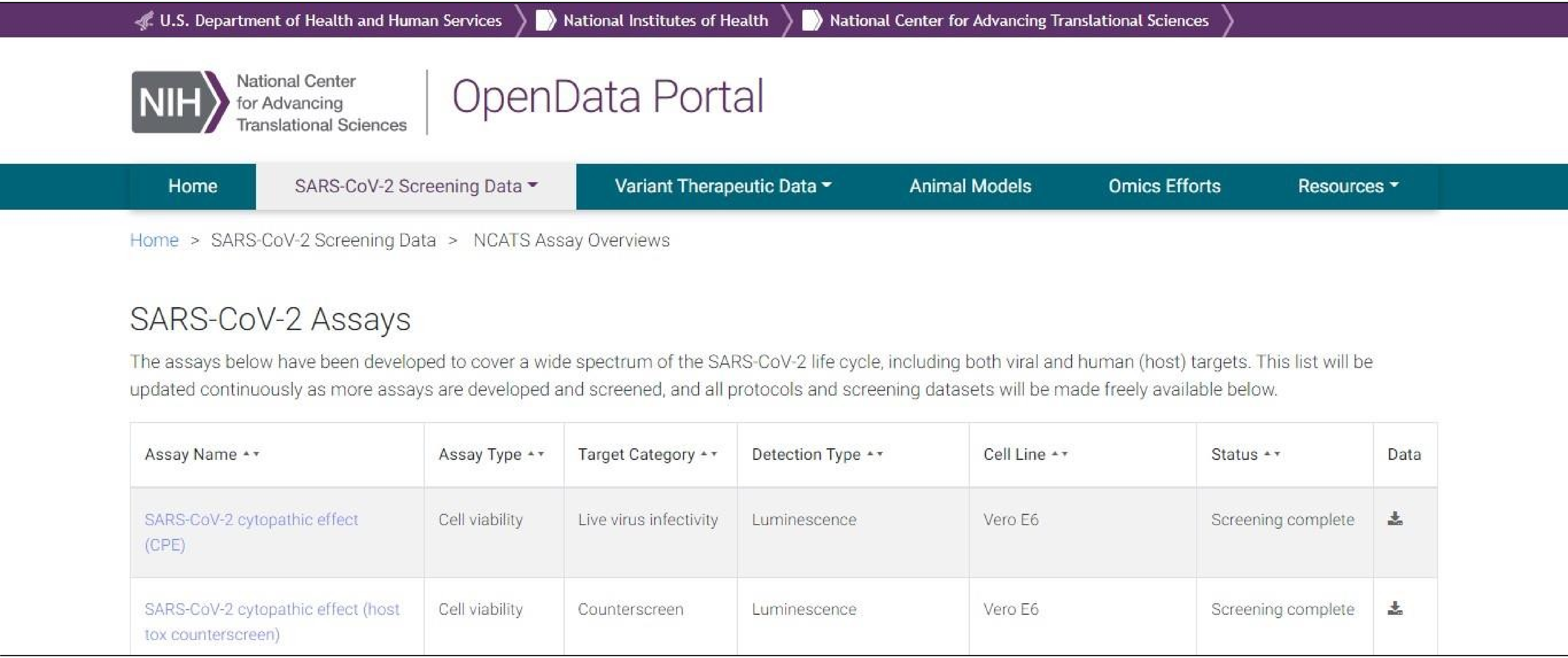
(iii) Reverse the cytopathic effect of host cells (caused by SARS-CoV-2 virion).

(iv) Show toxic effects against normal human/host cells.

- **Data pre-processing:** The parameters opted on PaDEL software (before actually starting the descriptors / FPs calculation) are "**Remove salt**", "**Detect aromaticity**", "**Standardize nitro groups**", "**Max. threads -1**", "**Max. waiting jobs -1**", "**Max. Running time per molecule: 12,00,000 milliseconds**", and "**Retain molecules order**".

- Only those molecules for which all the descriptor/fingerprint values are calculated have been used for ML-based models training, validation and further analysis.

A Screenshot from NCATS Open Data Portal


U.S. Department of Health and Human Services
National Institutes of Health
National Center for Advancing Translational Sciences
NIH National Center for Advancing Translational Sciences
OpenData Portal
Home
SARS-CoV-2 Screening Data
Variant Therapeutic Data
Animal Models
Omics Efforts
Resources
Home > SARS-CoV-2 Screening Data > NCATS Assay Overviews

SARS-CoV-2 Assays

The assays below have been developed to cover a wide spectrum of the SARS-CoV-2 life cycle, including both viral and human (host) targets. This list will be updated continuously as more assays are developed and screened, and all protocols and screening datasets will be made freely available below.

Assay Name	Assay Type	Target Category	Detection Type	Cell Line	Status	Data
SARS-CoV-2 cytopathic effect (CPE)	Cell viability	Live virus infectivity	Luminescence	Vero E6	Screening complete	⬇
SARS-CoV-2 cytopathic effect (host tox counterscreen)	Cell viability	Counterscreen	Luminescence	Vero E6	Screening complete	⬇

Table 1. A brief description of the nine assays used for training and evaluation of the prediction models.

Assay ID	Assay name	Assay description
1	**Spike-ACE2 protein-protein interaction (AlphaLISA)**	**Contains the therapeutic molecules that can potentially disrupt the interaction between SARS-CoV-2 Spike protein and the human host ACE2 receptor.**
2	**Spike-ACE2 protein-protein interaction (TruHit Counterscreen)**	**Helps identify false-positive compounds that interfere with the AlphaLISA readout in a non-specific manner.**
6	ACE2 enzymatic activity	Measures the ACE2 inhibitory potential of compounds to prevent the disruption of endogenous enzyme function.
8	**TMPRSS2 enzymatic activity**	**Specific to measure the TMPRSS2 inhibitory potential of compounds.**
9	3CL enzymatic activity	Measures the inhibitory potential of molecules against viral 3-chymotrypsin like protease (3CLpro), the main protease of SARS-CoV-2.
14	SARS-CoV-2 cytopathic effect (CPE)	Determines the ability of a compound to reverse the cytopathic effect caused by SARS-CoV-2 in Vero E6 host cells.
15	**SARS-CoV-2 cytopathic effect (host tox counterscreen)**	**To measure the toxicity of compounds against host (Vero E6) cells is used for the detection of such compounds.**
20	**HEK293 cell line toxicity**	**To measure the general toxicity of compounds against HEK293 cell line.**
21	Human fibroblast toxicity	To measure the general toxicity of compounds against Hh-Wt cell line.

https://opendata.ncats.nih.gov/covid19/assays

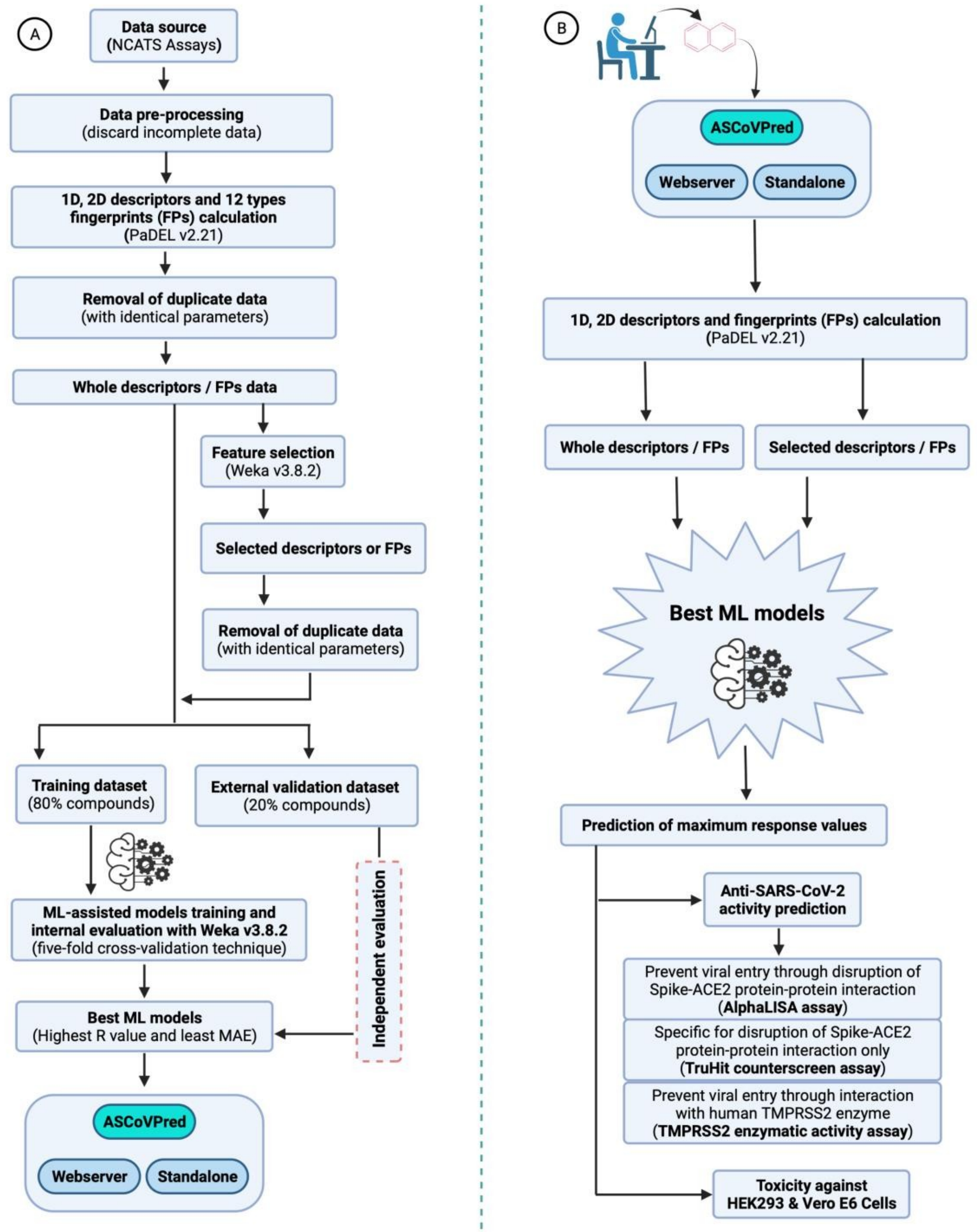
Figure 4. A systematic computational approach used for building ML-based QSAR prediction models and their usage by users.

(A)Flow diagram depicting the overall strategy used to train, evaluate and build the ML-based QSAR prediction models.

(B) The best prediction models can be used by users to predict the anti-SARS-CoV-2 activity and human cell toxicity of compounds.

Preparation of datasets for models training and validation:

- Data pre-processing and filtering are followed by redundancy removal to retrieve the dataset of unique molecules.
- Therefore, the molecules possessing identical descriptor or FPs values and maximum response values are included only once.
- The unique dataset of molecules was further split into a **training dataset (80% molecules)** and a **external validation dataset (20% molecules)**.


A
Data source
(NCATS Assays)
Data pre-processing
(discard incomplete data)
1D, 2D descriptors and 12 types fingerprints (FPs) calculation
(PaDEL v2.21)
Removal of duplicate data
(with identical parameters)
Whole descriptors / FPs data
Feature selection
(Weka v3.8.2)
Selected descriptors or FPs
Removal of duplicate data
(with identical parameters)
Training dataset
(80% compounds)
External validation dataset
(20% compounds)
ML-assisted models training and internal evaluation with Weka v3.8.2
(five-fold cross-validation technique)
Independent evaluation
Best ML models
(Highest R value and least MAE)
ASCoVPred
Webserver
Standalone
B
ASCoVPred
Webserver
Standalone
1D, 2D descriptors and fingerprints (FPs) calculation
(PaDEL v2.21)
Whole descriptors / FPs
Selected descriptors / FPs
Best ML models
Prediction of maximum response values
Anti-SARS-CoV-2 activity prediction
Prevent viral entry through disruption of Spike-ACE2 protein-protein interaction
(AlphaLISA assay)
Specific for disruption of Spike-ACE2 protein-protein interaction only
(TruHit counterscreen assay)
Prevent viral entry through interaction with human TMPRSS2 enzyme
(TMPRSS2 enzymatic activity assay)
Toxicity against HEK293 & Vero E6 Cells

- The training datasets are used for training and internal validation (through five-fold cross-validation technique) of the ML-based models, while external validation datasets are kept separate for the final or external validation of the developed models.

- **Descriptors or feature selection:** A feature selection technique in WEKA v3.8.2 is applied to determine the most relevant descriptors and fingerprints associated with the biological activity of the molecules.

- "**CfsSubsetEval**" (with default parameter values) as "**Attribute Evaluator**" with "**BestFirst**" as "**Search Method**" (with default parameter values) is used as feature selection techniques for the present study.

- **Tools used for model building:** An open-source data mining and ML tool, WEKA (v3.8.2), has been used in the present study to train and
validate the prediction models.

- **Cross-validation technique used:** Selection of the best models is made through the five-fold cross-validation technique.

Formulae used to evaluate the models' performance: The in-built functions available with WEKA (v3.8.2), such as Pearson Correlation Coefficient (R), mean absolute error (MAE) and root mean squared error (RMSE), have been used to evaluate the models' performance through five-fold cross-validation technique. In both internal and external validation, **the models with the highest R-value and lowest MAE and RMSE values are selected as the best prediction models.**

$$R = \frac{\left(\sum Xi\ Yi - \frac{\sum Xi\ \sum Yi}{N}\right)}{\sqrt{\left(\sum Xi^{2} - \frac{(\sum Xi)^{2}}{N}\right)\ \left(\sum Yi^{2} - \frac{(\sum Yi)^{2}}{N}\right)}} \qquad (1)$$

$$\mathrm{MAE} = \frac{\sum_{i=1}^{N} |Yi - Xi|}{N} \qquad (2)$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\ \sum_{i=1}^{N}\ (Yi - Xi)^{2}} \qquad (3)$$

For ith compound, Yi and Xi represent predicted and actual maximum response value, respectively. N is total number of compounds. **The value of R is used to measure the quality of model. The value of R varies from −1 to +1. The negative value of R shows the negative correlation with a particular property or feature.** Thus, **higher the value of R, better will be the quality of model in terms of the predicted maximum response value of the compounds.**

- We have developed machine learning (ML) models for the rapid discovery of molecules potentially inhibitory to SARS-CoV-2, with negligible or no human cell toxicity.

- The ML QSAR models were trained and optimized with features (descriptors and fingerprints) based on the activity assays of experimentally validated SARS-CoV-2 inhibitory compounds.

- The feature selection for selecting the best descriptors for ML training helped identify a set of decisive training descriptors and fingerprints that correlate positively or negatively with the anti-SARS-CoV-2 activity and toxicity of the compounds.

- The selected features were used to train thousands of different ML models. The best-optimized models are deployed as **ASCoVPred webserver and standalone software that provides easy and free access to the models.**

Performance of ML models with training and external validation datasets

Table 2. The number of molecules used for training and evaluation of the best prediction models.

Assay ID	Assay name	Number of unique molecules	Number of training dataset molecules	Number of external validation dataset molecules
1	**Spike-ACE2 protein-protein interaction (AlphaLISA)**	**3370**	**2696**	**674**
2	**Spike-ACE2 protein-protein interaction (TruHit Counterscreen)**	**3235**	**2588**	**647**
6	ACE2 enzymatic activity	3376	2701	675
8	**TMPRSS2 enzymatic activity**	**5144**	**4115**	**1029**
9	3CL enzymatic activity	11007	8806	2201
14	SARS-CoV-2 cytopathic effect (CPE)	9909	7927	1982
15	**SARS-CoV-2 cytopathic effect (host tox counterscreen)**	**9080**	**7264**	**1816**
20	**HEK293 cell line toxicity**	**9694**	**7755**	**1939**
21	Human fibroblast toxicity	4491	3593	898

Table 3. Results of the evaluation for best prediction models.

*SubstructureFingerprintCount; #Not deployed on ASCoVPred webserver

Performance evaluation of best models with training dataset compounds (five-fold cross-validation)									Performance evaluation of best models with external validation dataset compounds			
Assay ID	Assay name	Descriptors or fingerprints type	Number of input features	R	R^2	MAE	RMSE	WEKA technique used for training the model with training dataset of compounds	R	R^2	MAE	RMSE
1	**Spike-ACE2 protein-protein interaction (AlphaLISA)**	**SubFPC***	**307**	**0.67**	**0.44**	**14.96**	**21.22**	**weka.classifiers.meta.AdditiveRegression (RandomForest)**	**0.66**	**0.42**	**17.41**	**24.95**
2	**Spike-ACE2 protein-protein interaction (TruHit Counterscreen)**	**1D & 2D**	**50**	**0.72**	**0.51**	**13.51**	**18.83**	**weka.classifiers.meta.AdditiveRegression (RandomForest)**	**0.74**	**0.55**	**14.44**	**20.50**
6	ACE2 enzymatic activity	ExtendedFingerprinter#	28	0.32	0.10	14.31	37.09	weka.classifiers.trees.RandomForest	0.57	0.32	16.68	44.88
8	**TMPRSS2 enzymatic activity**	**SubFPC***	**307**	**0.52**	**0.24**	**11.12**	**36.47**	**weka.classifiers.trees.RandomForest**	**0.73**	**0.36**	**14.99**	**44.64**
9	3CL enzymatic activity	SubFPC*,#	307	0.40	0.16	5.90	11.31	weka.classifiers.meta.RandomCommittee (RandomForest)	0.45	0.19	5.11	9.14
14	SARS-CoV-2 cytopathic effect (CPE)	1D & 2D#	1444	0.50	0.25	7.43	14.59	weka.classifiers.meta.RandomSubSpace (RandomForest)	0.43	0.18	8.23	14.90
15	**SARS-CoV-2 cytopathic effect (host tox counterscreen)**	**1D & 2D**	**1444**	**0.66**	**0.43**	**13.12**	**21.13**	**weka.classifiers.meta.AdditiveRegression (RandomForest)**	**0.65**	**0.42**	**14.11**	**21.93**
20	**HEK293 cell line toxicity**	**SubFPC***	**307**	**0.66**	**0.44**	**26.68**	**34.16**	**weka.classifiers.meta.RandomCommittee (RandomForest)**	**0.68**	**0.46**	**26.41**	**33.87**
21	Human fibroblast toxicity	1D & 2D#	45	0.43	0.18	12.61	19.69	weka.classifiers.meta.RandomCommittee (RandomForest)	0.51	0.26	11.99	18.63

Table 4. The desired activity prediction profile for an ideal multi-target hit molecule.

Assay ID	Assay Name	Target Category	Predicted maximum response value (PMRV) threshold	Activity Class
1	Spike-ACE2 protein-protein interaction (AlphaLISA)	Viral entry	< -66	High
2	Spike-ACE2 protein-protein interaction (TruHit Counterscreen)	Counterscreen	> -33	Low
8	TMPRSS2 enzymatic activity	Viral entry	< -66	High
15	SARS-CoV-2 cytopathic effect(host tox counterscreen)	Counterscreen	> -33	Low
20	HEK293 cell line toxicity	Counterscreen	> -33	Low

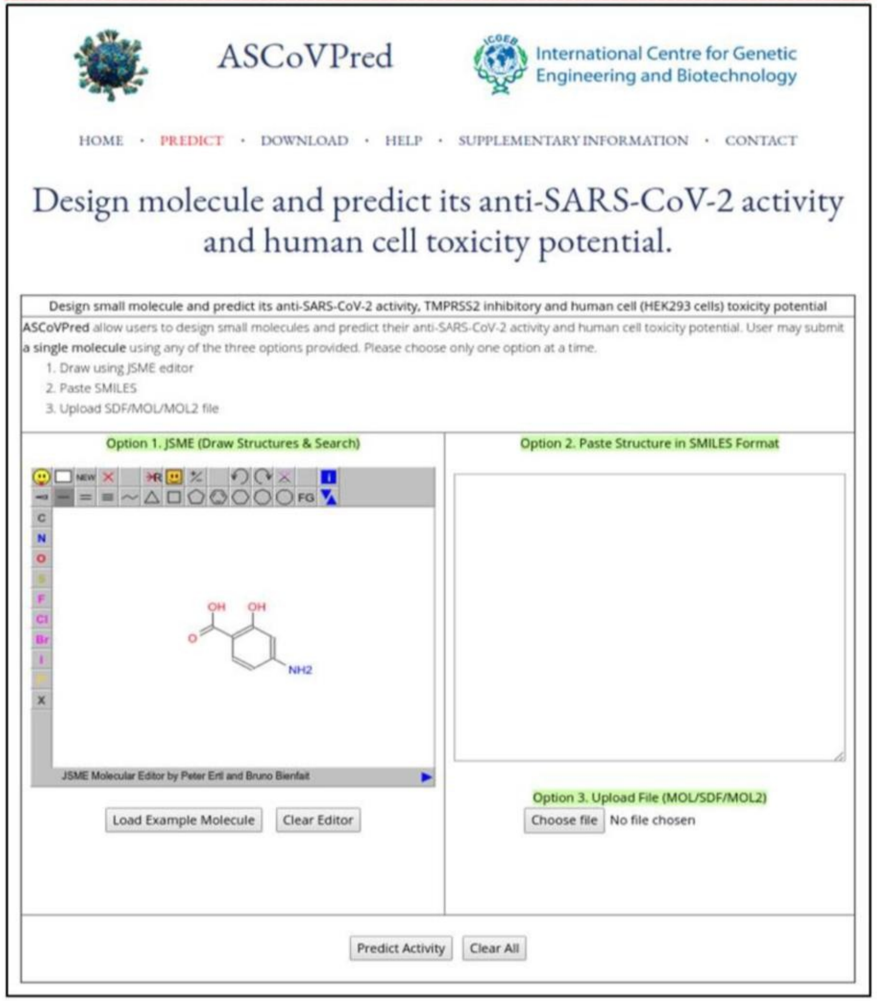
Deployment of the best ML models on ASCoVPred web-server and standalone

Figure 5. Screenshots of ASCoVPred webserver usage. Website link: https://apexbtic.icgeb.res.in/ascovpred/index.html

1

ASCoVPred webserver input options:

1. **Draw molecule structure**
2. **Paste Structure (in SMILES format only)**
3. **Upload structure file (in MOL/SDF/MOL2 format)**


ASCoVPred
International Centre for Genetic Engineering and Biotechnology
HOME • PREDICT • DOWNLOAD • HELP • SUPPLEMENTARY INFORMATION • CONTACT
OH
OH
O
NH2
JSME Molecular Editor by Peter Ertl and Bruno Bienfait
Load Example Molecule
Clear Editor
Choose file
No file chosen
Predict Activity
Clear All

Design molecule and predict its anti-SARS-CoV-2 activity and human cell toxicity potential.

Design small molecule and predict its anti-SARS-CoV-2 activity, TMPRSS2 inhibitory and human cell (HEK293 cells) toxicity potential

ASCoVPred allow users to design small molecules and predict their anti-SARS-CoV-2 activity and human cell toxicity potential. User may submit **a single molecule** using any of the three options provided. Please choose only one option at a time.

1. Draw using JSME editor
2. Paste SMILES
3. Upload SDF/MOL/MOL2 file

Option 1. JSME (Draw Structures & Search)

Option 2. Paste Structure in SMILES Format

Option 3. Upload File (MOL/SDF/MOL2)

2

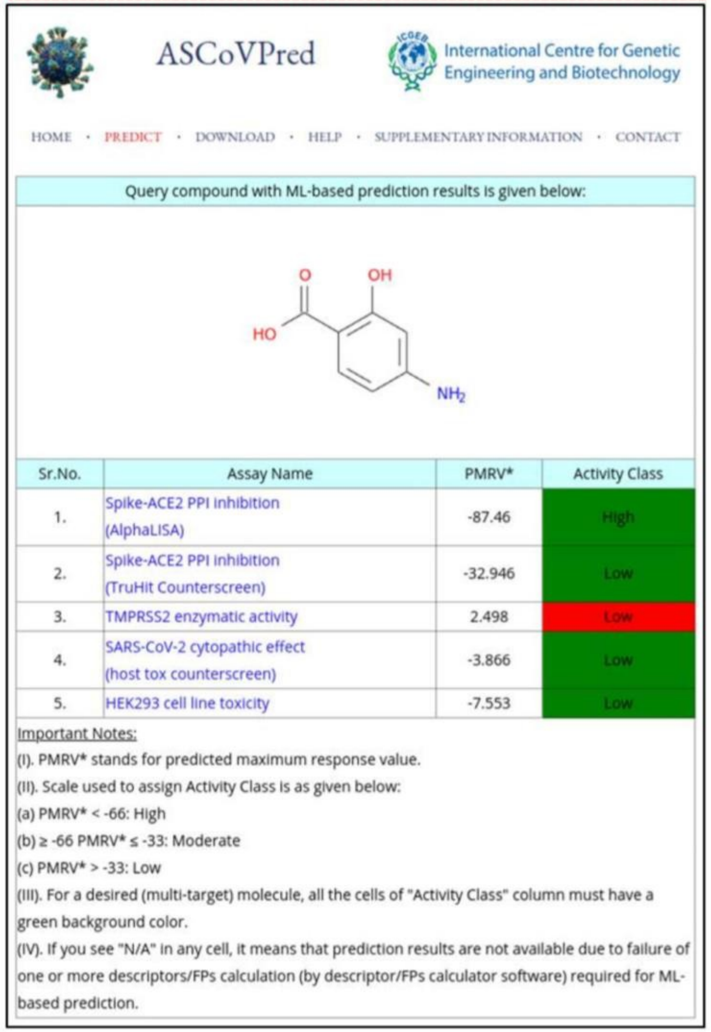
Processing of user submitted compound

Descriptors & FPs calculation, salt removal, prediction, image generation

3

ASCoVPred webserver output:

1. **Anti-SARS-CoV-2 activity prediction**
2. **Human cell toxicity prediction**


ASCoVPred
International Centre for Genetic Engineering and Biotechnology
HOME • PREDICT • DOWNLOAD • HELP • SUPPLEMENTARY INFORMATION • CONTACT
O
OH
HO
NH2

Query compound with ML-based prediction results is given below:

Sr.No.	Assay Name	PMRV*	Activity Class
1.	Spike-ACE2 PPI inhibition (AlphaLISA)	-87.46	High
2.	Spike-ACE2 PPI inhibition (TruHit Counterscreen)	-32.946	Low
3.	TMPRSS2 enzymatic activity	2.498	Low
4.	SARS-CoV-2 cytopathic effect (host tox counterscreen)	-3.866	Low
5.	HEK293 cell line toxicity	-7.553	Low

Important Notes:

(I). PMRV* stands for predicted maximum response value.

(II). Scale used to assign Activity Class is as given below:

(a) PMRV* < -66: High

(b) ≥ -66 PMRV* ≤ -33: Moderate

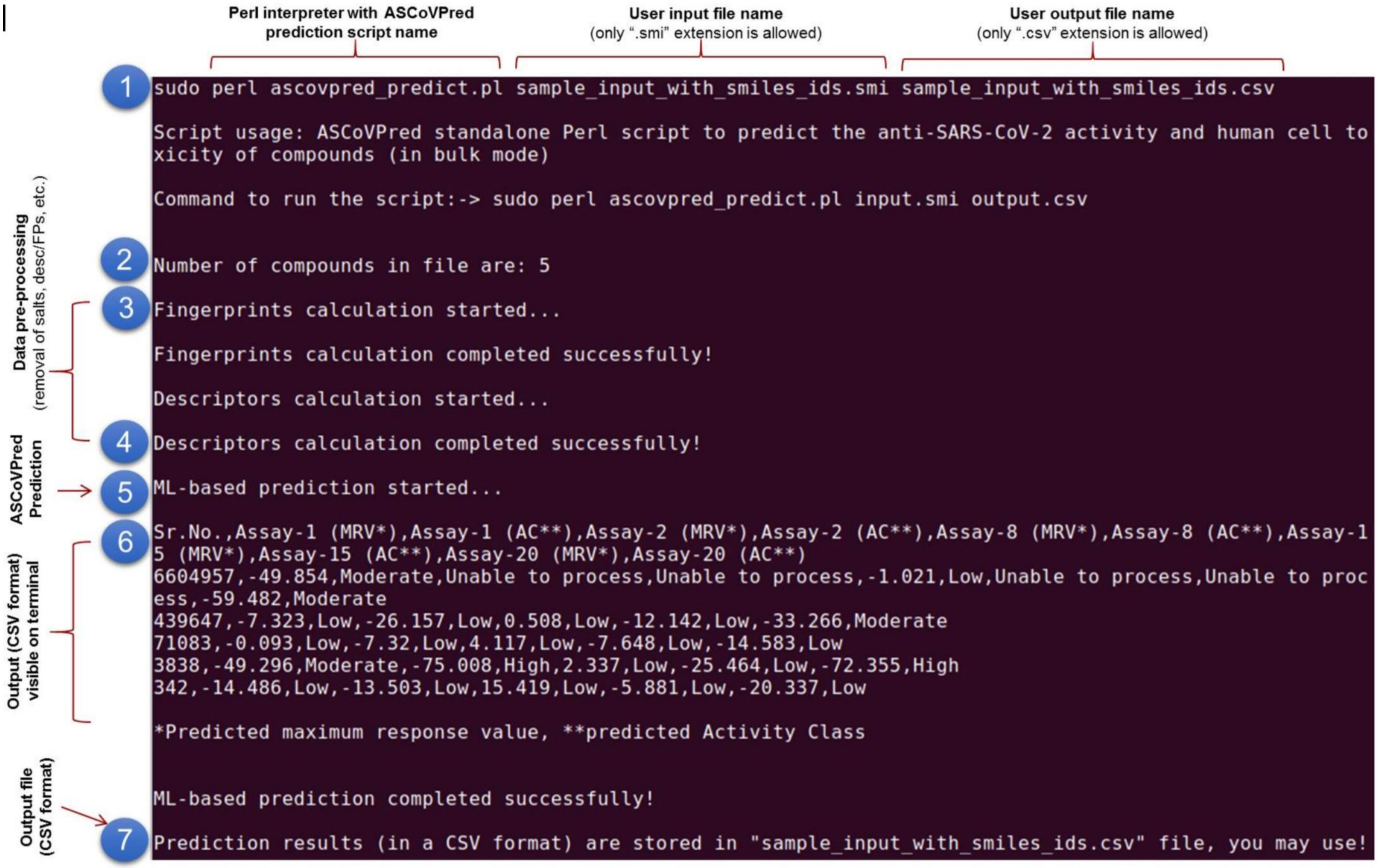
(c) PMRV* > -33: Low

(III). For a desired (multi-target) molecule, all the cells of "Activity Class" column must have a green background color.

(IV). If you see "N/A" in any cell, it means that prediction results are not available due to failure of one or more descriptors/FPs calculation (by descriptor/FPs calculator software) required for ML-based prediction.

INPUT **PROCESSING** **OUTPUT**

Figure 6. Screenshots of ASCoVPred standalone software usage. Website link: http://192.168.5.81/ascovpred/index.html


Perl interpreter with ASCoVPred prediction script name
User input file name (only ".smi" extension is allowed)
User output file name (only ".csv" extension is allowed)
1
2
3
4
5
6
7
Data pre-processing (removal of salts, desc/FPs, etc.)
ASCoVPred Prediction
Output (CSV format) visible on terminal
Output file (CSV format)

```
sudo perl ascovpred_predict.pl sample_input_with_smiles_ids.smi sample_input_with_smiles_ids.csv

Script usage: ASCoVPred standalone Perl script to predict the anti-SARS-CoV-2 activity and human cell to
xicity of compounds (in bulk mode)

Command to run the script:-> sudo perl ascovpred_predict.pl input.smi output.csv


Number of compounds in file are: 5

Fingerprints calculation started...

Fingerprints calculation completed successfully!

Descriptors calculation started...

Descriptors calculation completed successfully!

ML-based prediction started...

Sr.No.,Assay-1 (MRV*),Assay-1 (AC**),Assay-2 (MRV*),Assay-2 (AC**),Assay-8 (MRV*),Assay-8 (AC**),Assay-1
5 (MRV*),Assay-15 (AC**),Assay-20 (MRV*),Assay-20 (AC**)
6604957,-49.854,Moderate,Unable to process,Unable to process,-1.021,Low,Unable to process,Unable to proc
ess,-59.482,Moderate
439647,-7.323,Low,-26.157,Low,0.508,Low,-12.142,Low,-33.266,Moderate
71083,-0.093,Low,-7.32,Low,4.117,Low,-7.648,Low,-14.583,Low
3838,-49.296,Moderate,-75.008,High,2.337,Low,-25.464,Low,-72.355,High
342,-14.486,Low,-13.503,Low,15.419,Low,-5.881,Low,-20.337,Low

*Predicted maximum response value, **predicted Activity Class


ML-based prediction completed successfully!

Prediction results (in a CSV format) are stored in "sample_input_with_smiles_ids.csv" file, you may use!
```

Conclusion

- The designing of strategies for the rapid discovery of anti-SARS-CoV-2 compounds is an urgent need of the hour.
- Machine learning-based approaches in drug discovery and design are time-saving and cost-effective.
- The present study is based on computational designing of anti-SARS-CoV-2 compounds and estimates their toxicity against normal human cells.
- ASCoVPred webserver and standalone software are very useful in rapidly discovering inhibitors against SARS-CoV-2 and preventing viral entry into the human host cells.
- Also, the toxicity of molecules against normal human cells (HEK293 and Vero E6 cell-line) can be estimated with the help of toxicity prediction models deployed on the ASCoVPred platform.
- Functional groups associated with the anti-SARS-CoV-2 activity of the molecules may provide better insights while designing the better lead molecules.
- In the future, the development of more ML models (trained and evaluated with more NCATS assays data) could enhance the utility of the ASCoVPred platform. We will also continue to improve the performance of the deployed models and update those on the ASCoVPred platform.

Demonstration of the webserver

Website link: https://apexbtic.icgeb.res.in/ascovpred/index.html

Thank you