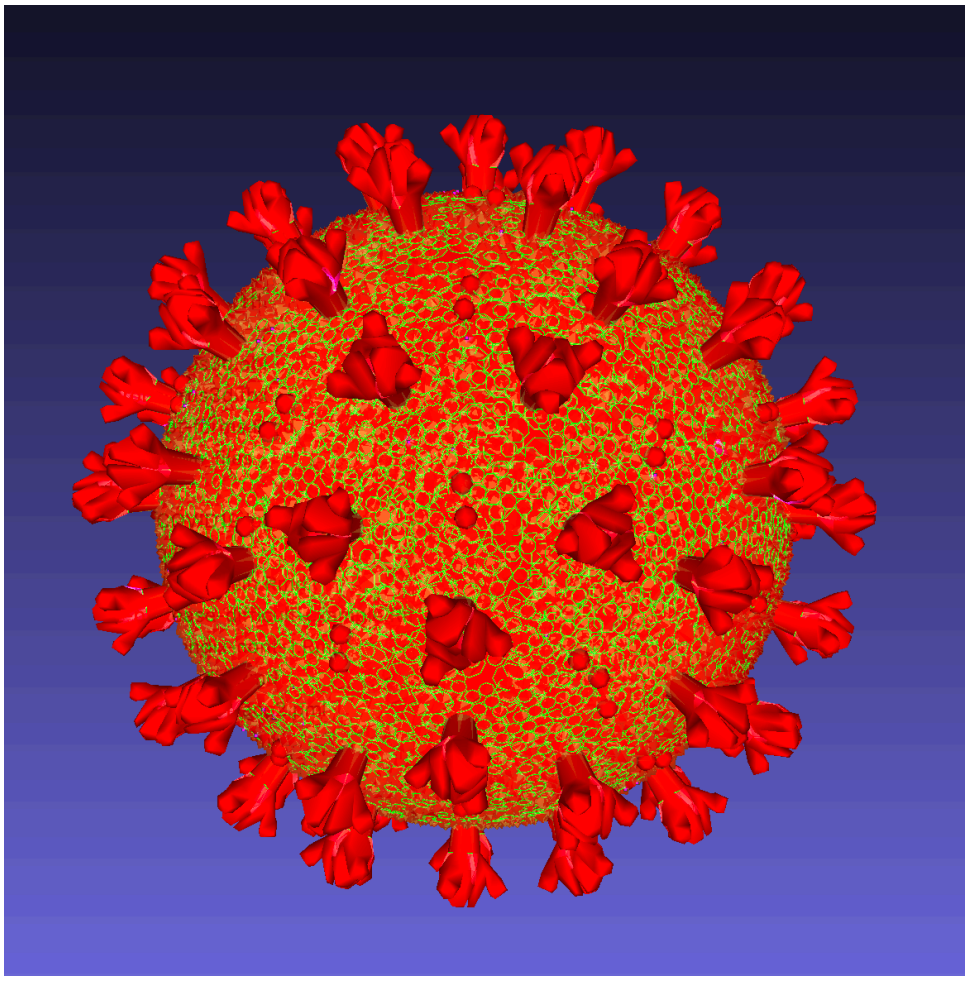
*Jean-Yves Sgro, Ph.D.*

# *PyMOL - Exploring the 3D PDB structures of SARS-CoV-2/COVID-19*



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Cover: created by JYS with MeshLab from 3D design file by Alejandro León (@alelepd). See text for more details

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# *Preface*

This booklet is meant for those that are already familiar with PyMOL [Schrödinger, LLC, 2020] as a molecular graphics software for macromolecular biological structures such as proteins and nucleic acids.

If you are novice you may benefit from finding the book "*Exploring Protein Structure: Principles and Practice*" [Skern, 2018]. If you are at the UW-Madison the information on my blog post[1] will guide you to download this book from the library.

You can also learn how to use PyMOL from my course material now availabe online [Sgro, Jean-Yves, 2017].

### Software information and conventions

I used the **knitr** package [Xie, 2015], the **bookdown** package [Xie, 2016] enhanced by the **bookdownplus** package to compile this book within the **RStudio** software [RStudio Team, 2015] in various formats (PDF, HTML etc.)

PyMOL figures were created *on the fly* by scripts run from within the **RStudio** framework every time the book was compiled. This was possible on a Macintosh by calling the internal PyMOL engine with `/Applications/PyMOL.app/Contents/MacOS/PyMOL` and providing a script.

[1] `http://tiny.cc/pybook`

On a Linux system the command would simply be `pymol` if the software is installed and within the `PATH` environment. On Windows I am not sure how this would or could work.

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**Acknowledgments**

"Summary" icon for special text boxes is from https://www.flaticon.com/free-icon/recorder_2450952

# *About the Author*

Jean-Yves Sgro, a senior scientist with years of experience in using and teaching computer programs, creates, organizes and teaches hands on workshops.

Jean-Yves has been at UW since 1986 after a Master in Physiology and a Ph.D. in Cellular and Molecular Biology from Joseph Fourier University[2], Grenoble, France, and researched at the European Molecular Biology Laboratory (EMBL[3]) where he already used large computers for sequence analysis.

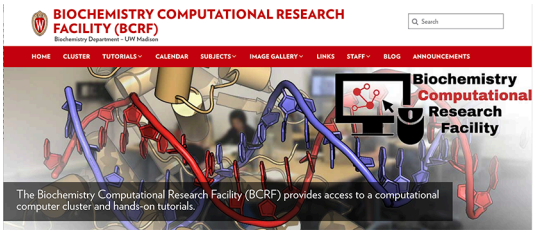
In Madison, at the Institute for Molecular Virology (IMV[4]) he continued developing computer expertise in addition to his wet-lab research – 3D molecular visualization (virusworld[5]), RNA-folding predictions, sequence and data analysis…

In 1996 he joined the UW Biotechnology Center[6] to better help Campus biologists analyze and visualize their data while continuing research at IMV until 2014 when this part-time position was transferred to the

[2] https://www.univ-grenoble-alpes.fr/english/
[3] https://www.embl.fr/
[4] http://virology.wisc.edu/
[5] http://www.virology.wisc.edu/virusworld
[6] https://www.biotech.wisc.edu/

Biochemistry Department[7] where he organizes and teaches hands-on tutorials on molecular graphics, data analysis as a support to the department personnel.

Tutorials are available on line from the The Biochemistry Computational Research Facility (BCRF[8].)




[7] https://biochem.wisc.edu/
[8] https://bcrf.biochem.wisc.edu/

# 1

# *Introduction*

We are in the midst of the COVID-19 pandemic, caused by the virus named SARS-CoV-2 [1]

This small booklet is an attempt to illustrate some of the SARS-CoV-2 know structures available as 3D atomic coordinates at the Protein Data Bank (PDB) with the PyMOL software.

**How to use this booklet:**

The original purpose was to provide a starting point to visualize known 3D strutures from the SARS-CoV-2 virus using the PyMOL molecular visualisation software.

All figures were created by script, and all complete scripts are availabe in the Appendix sections C, D, E,F,G.

Script lines can be copied one-by-one and pasted within the PyMOL command line.

Alternatively, the entire script can be copied within a *plain text* file and saved on the computer, then activated with a command such as: `@myscript.txt` from within the PyMOL command line area. (See section 3.3.1.)

[1] SARS: Severe Acute Respiratory Syndrome-related coronavirus

If your interest is to learn more PyMOL commands, I recommend to follow the scripts one line at a time:

Watch the result of each command (some results might not be visible immediately.)

Inquire about the command structure:

-. Use PyMOL `help` followed by command name for immediate info.
-. Find same info in `https://pymol.org/pymol-command-ref.html`
-. Search within `https://pymolwiki.org/`
-. Be curious with a search engine (*e.g.* Google)

I am creating this booklet with the principles of "Reproducible Research" in the sense that this very document can be recreated in its entirety, including the recreation of all PyMOL illustrations as they can be redrawn "on the fly" thanks to the use of the scripts that create them.

I am using *PyMOL 2.3.5 Incentive Product* but this version number would also be automatically adjusted should I update my PyMOL program and recompile the book.

I had started a different booklet and changed course when the pandemic started. I levied Chapter 4 from that prior (unfinished) project which explains its different style with some suggested exercises and more of the script written within the text. There were some "strange" difficulty with the ligand of the structure studied in this chapter which started the idea of these booklets.

The original format is **PDF** but other formats might be made available,

albeit there will be some incompleteness regarding cross-references, index, or even numbering of chapters etc.

# 2

# *SARS-Cov-2*

## 2.1 PDB Structures

Some SARS-Cov-2 viral proteins have been solved, mostly by X-ray crystallography, as of early April 2020:

- Main protease: 83 structures with various ligands (76 of which from the *PanDDA analysis group*.)
- Spike protein: 5 structures
- Papain-like SARS-CoV-2 structures: 1 structure
- Other SARS-CoV-2 structures (binding domains etc.): 17 structures

Tables with the PDB accession code and struture titles are available in Appendix A with information for the data source.

PDB structure 6LU7 is the first high-resolution crystal structure of SARS-CoV-2 main protease (released February 5, 2020) and is the object of study of chapter 4.

This booklet intention is to help with the rendering of the PDB files in PyMOL and provide all scripts to create PyMOL illustrations.

However, the complete virus particle has not been solved but various artistic "renderings" are available as discussed briefly in the next section.

## 2.2 SARS-CoV-2 - Artistic renderings

### 2.2.1 Painting

Scripps Prof. David Goodsell[1] [2], famous for his non-photorealistic illustrations of molecules, has created a beautiful rendering of the coronavirus entering the lung as a painting displayed on the Protein Data Bank web site[3].


**FIGURE 2.1:** SARS-CoV-2 painting and key, Pr. David Goodsell.

*This painting depicts a coronavirus just entering the lungs, surrounded by mucus secreted by respiratory cells, secreted antibodies, and several small immune systems proteins. The virus is enclosed by a membrane that includes the S (spike) protein, which will mediate attachment and entry into cells, M (membrane) protein, which is involved in organization of the nucleoprotein inside, and E (envelope) protein, which is a membrane channel involved in budding of the virus and may be incorporated into the virion during that process. The nu-*

[1] https://ccsb.scripps.edu/goodsell/
[2] Twitter: @dsgoodsell
[3] https://pdb101.rcsb.org/sci-art/goodsell-gallery/coronavirus

*cleoprotein inside includes many copies of the N (nucleocapsid) protein bound to the genomic RNA.*

## 2.3 3D models

Other artisitc renderings have been done by computer artists. The 3D file formats used are different than the format used for PDB files and cannot readily be opened with PyMOL. Many of them are for purchase on web sites.

Author Alejandro León[4] [5] created a free version that can be accessed already rendered within a web site[6] and that can be manipulated in 3D (See figure 2.2.)

A 12 seconds video of what this looks like is available on YouTube[7].

According to the author this 3d model of Coronavirus (SARS-CoV-2) based on the images from CDC/PHIL. The model is available to download for free (MIT License)[8].

In addition the 3D model file can be downloaded[9] as a compressed binary in the `.fbx` format that is typically used to allow transfer to and from various 3D illustration software.

A web site[10] can convert this file in other 3D formats[11]. Noteworthy is the fact that the most recent Photoshop version can open the `.obj` for-

[4] Twitter: `@alelepd`
[5] `https://alejandro.fun/`
[6] `https://spline.design/coronavirus3d/`
[7] `https://youtu.be/K0ORIaAuXb4`
[8] `https://opensource.org/licenses/MIT`
[9] `https://spline.design/coronavirus3d/_assets/coronavirus3d.fbx`
[10] `https://products.aspose.app/3d/conversion/`
[11] Available formats: FBX, OBJ, 3DS, DRC, AMF, RVM, DAE, GLTF, GLB, PDF, HTML, PLY, STL, U3D

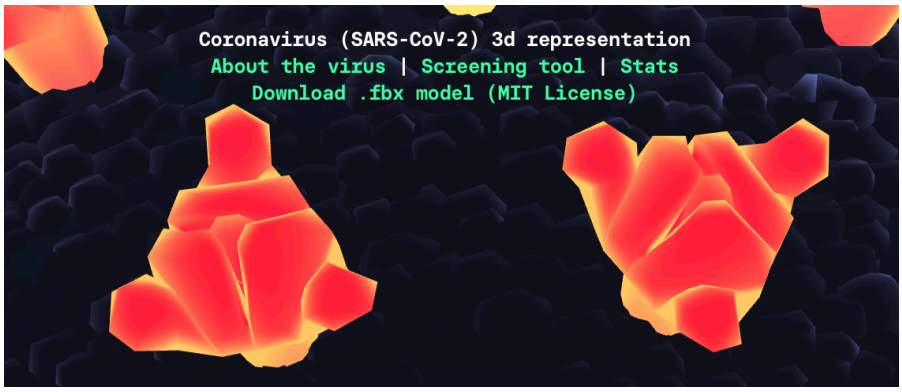



**FIGURE 2.2:** Details of coronavirus3d file shown on spline.design showing artisitic rendering of surface trimeric spike protein.

mat into a 3D layer where the various "layers" can be assigned a color or a texture (figure 2.3.)

Here is a stereo version created with this file with Photoshop:

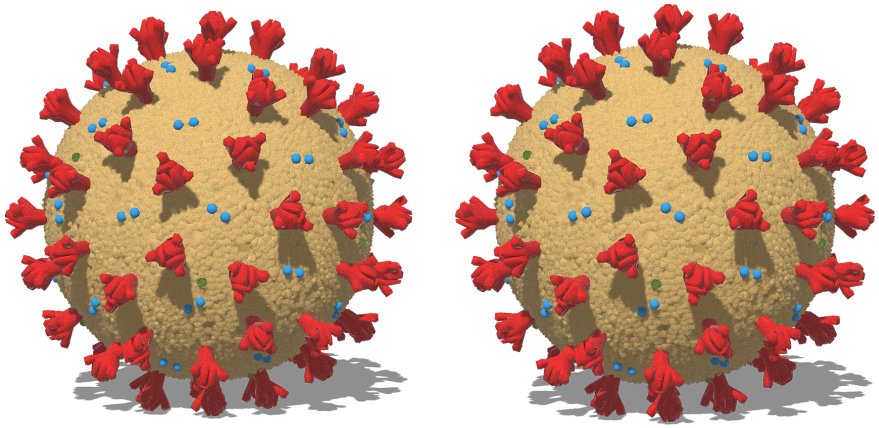


**FIGURE 2.3:** Stereo rendering of coronavirus3d file in Photoshop

The coronavirus3d (*e.g.* in `.obj` format) can also be rendered with the free 3D software MeshLab[12] available for Windows/Mac/Linux. Within this software all "layers" are given the same color, but vertices can be

[12] http://www.meshlab.net/

colored which can create “artistically nice” renderings as well (figure 2.4.)

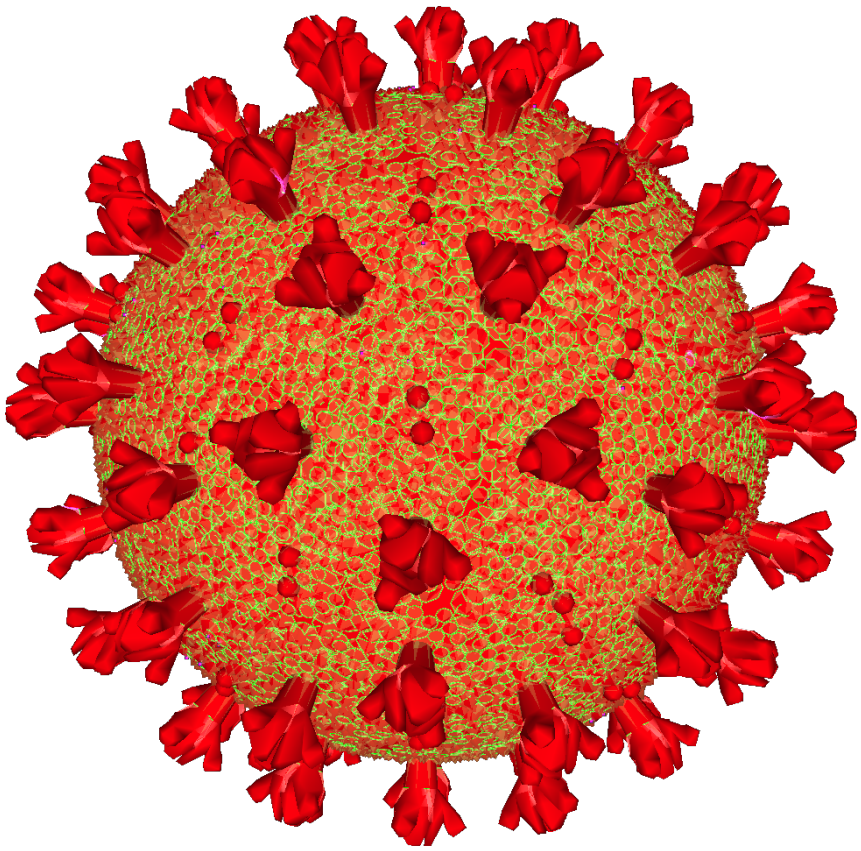


**FIGURE 2.4:** MeshLab rendering of coronavirus3d file.

# 3

# *PyMOL Scripts: Short Primer*

SUMMARY:
- Scripts are *plain text*
- PyMOL command ccripts are activated with `@`
- PyMOL python form scripts are activated with `run`

## 3.1 Scripts are "*plain text*"

A *plain text* file will:

- only contain printable characters that can be typed from the simplest of keyboards: letters, numbers and symbols such as !, @ and $.

- is not specific to any particular word processor format and is displayed with the default font within any word processor.

- contain a "line-break" or "end-of-line" code (specific to the operating system, which can be an inconvenience sometimes.)

Software that can easily create a *plain text* file:

- **Windows**: `Notepad` or one of many suggested replacements[1]

[1] https://alternativeto.net/software/notepad/?platform=windows

- **MacOS**: `TextEdit` or one of the many suggested replacements[2],[3]
- **Linux**: `nano`, `vim`, `emacs` or some other replacement[4]
- Cross-platform: `Atom`[5]

## 3.2 Scripts formats

There are different PyMOL scripts formats:

- `.pml`: Scripts containing lists of **PyMOL commands**
- `.py`:
  - Scripts comtaining lists of PyMOL command in **Python form** (See section 3.4.)
  - Scripts containing new functions, written in Python language.

*Note*: When activated through the command line, a file ending in `.txt` works as well.

## 3.3 Activating a command script

The activation of scripts may different depending on their nature if not activated by mouse menu.

With a mouse the following menu cascade can be used to activate any script form: **File > Run Script...** which then offers the choices:

- All Runnable (*.pml *.py *.pym)
- PyMOL Command Script (*.pml)

[2] https://alternativeto.net/software/textedit/?platform=mac
[3] "use menu: Format > Make plain text"
[4] https://alternativeto.net/software/nano/?platform=linux
[5] https://atom.io/

- PyMOL Command Script (*.txt)
- Python Script (*.py *.pym)
- Python Script (*.txt)
- All Files (*)

Note: in older versions of PyMOL a command file would need to end with `.pml` in order to be used with the mouse menu.

### 3.3.1 Command script

A PyMOL Command Script can be activated with the command line:

```
PyMOL>@myscript.pml
```

This assumes that the script file is located within the current directory.

### 3.3.2 Python script

A Python language script should use the PyMOL command `run` to be activated, even if `@` may work in most cases when the script is only a series of commands. PyMOL will provide a warning.

```
PyMOL>@myscript.py
Warning: use 'run' instead of '@' with Python files?
```

Therefore the command sould be given as:

```
PyMOL>run myscript.py
```

This is particilar true for files that provide new functions to PyMOL as we'll see later.

## 3.4 PyMOL command *vs* API

PyMOL is a molecular graphics software based on the Python language (hence its name.) This document is using *PyMOL 2.3.5 Incentive Product*

to create graphics automatically with scripts. (Scripts are located in the Appendix.)

### 3.4.1 PyMOL commands are API aliases

A PyMOL command has an equiallent in API[6] form (written in python) and there are also less documented API commands that do not exist in PyMOL command form.

This is best understood with a simple example, let's take the PyMOL command `color`. I can issue commands such as:

```
PyMOL>color blue
```

I can also specify the chain I'dlike to color if there are more than one:

```
PyMOL>color blue, chain A
```

The command `color`[7] is defined as:

```
color color [, selection ]
```

- The first word `color` is the command name
- The second `color` is the mandatory argument and should be a color name or number
- the selection is everything unless specified

But behind the scenes, PyMOL is running python language functions, and the command `color` is in fact defined with all its parameters[8] as:

```
cmd.color(string color, string selection, int quiet)
```

This can also be used directly within PyMOL, for example

[6] Application Programming Interface
[7] https://pymol.org/dokuwiki/doku.php?id=command:color
[8] https://pymol.org/dokuwiki/doku.php?id=api:cmd:color

```
PyMOL>cmd.color("blue")
```

Of course a chain name can also be specified:

```
PyMOL>cmd.color("blue", "chain A")
```

It is important that the arguments be placed within quotes or an error will occur. This is because within the definition the arguments are expeted to be *`string`* which means of *character* type (as opposed to a number for example.)

My opinionated conclusion is that the command form appears more concise to the eyes (less need for quotes or parenthesis) and therefore more "English-like" which in turn allows a better comprehension, or understanding of the tasks that PyMOL is asked to perform, such as changing a color.

## 3.5 Shell-like commands and PATH

One of the trickiest things about computer is all the assumptions behind ***where things are*** and what does the computer knows anbout *where things are*.

By default PyMOL will "look" within the home user directory. On modern desktop computers it is usually within the `/Users` directory. For example for me it would be `/Users/jsgro`.

If a script is not within the current directory, a *path* to the script can be given, either as absolute or relative path with the appropriate preceding `@` symbol or `run` command. For example `@../pyscripts/myscript.pml`. Most systems would also understand the ~ shortcut representing the home directory.

PyMOL also provides three commands identical to the shell commands[9].

- `cd`: Change the current working directory
- `ls`: List contents of the current working directory.
- `pwd`: Print current working directory.

In addition the command `system` executes a command in a subshell under Unix or Windows (not Mac.)

Therefore it is possible to change directory (`cd`,) check in which directory PyMOL is looking (`pwd`) and list current files in that directory (`ls`.)

The `cd` command works in the same way as in a Unix/Linux shell. BY default PyMOL will look within the home directory as detailed above. The standard shell commands apply, including

For example:

```
cd MyDirName

cd Desktop

cd ~/Desktop

cd ~/MyDirName2

cd ../
```

The `ls` command includes usage of wild card `*` and regular expressions in the search (*e.g.* `ls *[t].py`, `ls cla??29*.py`) and *tab-extension* is operational.

[9] https://pymol.org/pymol-command-ref.html

# 4

# *SARS-CoV-2 Protease Mpro*

SUMMARY:
Unexpected problems with a seemingly simple rendering of PDB coordinates of SARS-CoV-2 main proteinase Mpro.

This is an example of PyMOL Command Scripting to create figures that can automatically be generated from a script. The final script(s) can be found in Appendix C.

At this writing the Covid-19 pandemic caused by the 2019 novel coronavirus (SARS-CoV-2) is going on. The RNA genome is 82% identical to the SARS coronavirus (SARS-CoV) and has been named SARS-CoV-2 (Gorbalenya et al. [2020].) The disease caused by SARS-CoV-2 is called COVID-19.

The structure of the virus protease has been solved by X-ray diffraction (Jin et al. [2020]). The structure was deposited 2020-01-26 and released 2020-02-05 with PDB code 6LU7[1] with title *The crystal structure of COVID-19 main protease in complex with an inhibitor N3*. The biological assembly is a dimer.

The peptide-inhibitor is logdged within a groove on the protease surface. Its name is *PRD_002214*[2] in the *Biologically Interesting Molecule Reference Dictionary* (BIRD).

[1] http://dx.doi.org/10.2210/pdb6LU7/pdb
[2] https://www.rcsb.org/bird/PRD_002214

Within the PDB file it has sequence:

```
SEQRES   1 C    6  02J ALA VAL LEU PJE 010
```

When PyMOL (2.x) opens the file, showing the structure with the new default of cartoon, the protein is fine but zooming on the PRD_002214 it appears broken in 3 parts (see figure 4.1.)

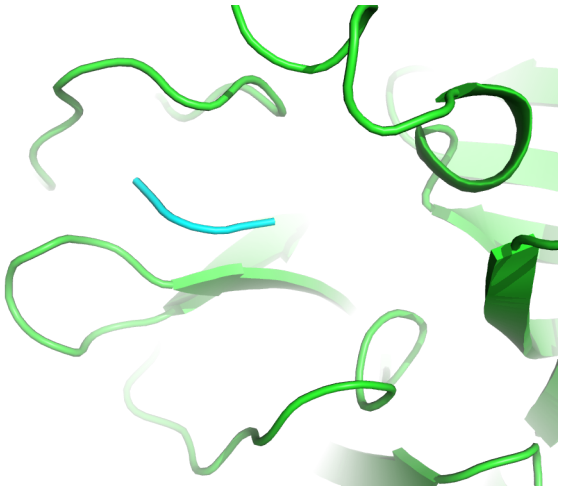


**FIGURE 4.1:** PRD_002214, cyan, appears broken into three parts.

The cause is the interpretation of the 3D coordinates for PRD_002214 in chain C. Closer inspection of the coordinates shows that its peptide-like nature causes its coordinates to be a mixture of `ATOM` and `HETATM` records (see Appendix B.)

Thanks to PyMOL command options we'll be able to modify the coordinates so that compound PRD_002214 may be represented as full in *stick* visual format.

## 4.1 Change record type

Even though chain C does not contain any termination TER records the result of the mixed atom records as ATOM and HETATM causes PyMOL to split the representation in 3 parts. One solution would be to change all records to HETATM which can be accomplished with PyMOL command `alter`[3].

The script below will accomplish this task:

```
fetch 6lu7, async=0
as cartoon

# Change ATOM records
alter (chain C),  type="HETATM"
show stick, chain C
hide cartoon, chain C
util.cbaw chain C

zoom 6lu7 and chain C
turn y, -50
turn z, -90
turn x, -30
```

This should now produce a complete stick representation of PRD_002214 compound.

Exercise:
In the script we used `alter (chain C),  type="HETATM"`.
You can also try `alter (seg B),  type="HETATM"`.

[3] https://pymol.org/pymol-command-ref.html#alter

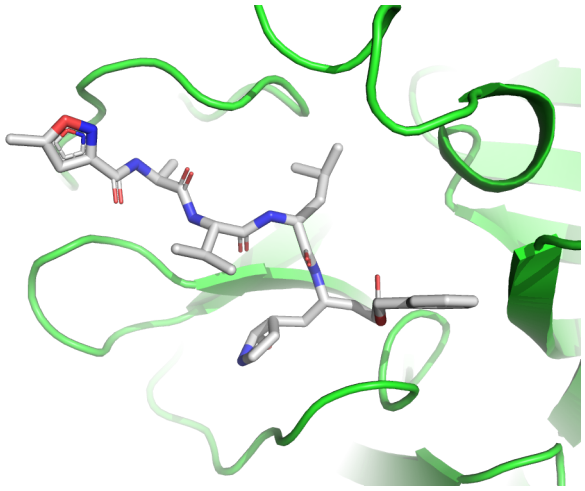


**FIGURE 4.2:** PRD_002214, sticks, CPK colors.

Does it work for both asymetric unit and biological assembly?
Why not?

Exercise:
Try to reproduce a similar image for the biological assembly (`type=pdb1`.)
Does the rotation translation provided above for the asymetric unit work as well?
Why or why not?

## 4.2 Surface representation: still an issue

If we now would like to use the standard surface representation that constructs a surface for the protein but not on the organic ligands, there will sill be a problem of representation, this time due to the fact

that the name of the residues that were originally presented as `ATOM` within the compound have natural amino acid names, and therefore, in spite of now being labeled `HETATM` will be included in the surface.

Residue names for the compound are indeed `02J ALA VAL LEU PJE 010` (see above) and the portion `ALA VAL LEU` will be selected for surface construction.

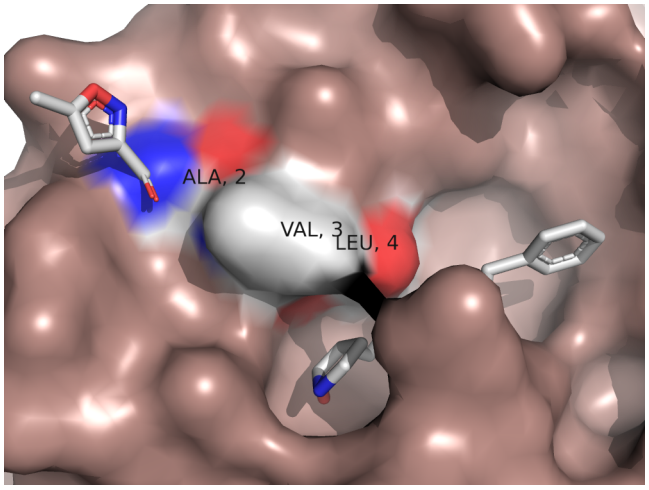



**FIGURE 4.3:** PRD_002214 middle portion ALA VAL LEU is included in surface.

One may think that a possible solution would simply to change the name of those amino acid to a generic `UNK` for unknown compound.

Exercise:
Test the hypothesis that changing the amino acid names within PRD_002214 to an "unknown" does (or does not) change the surface options.

Hint: `alter resn ala+val+leu and chain C, resn = "UNK"`

### 4.2.1 One solution: extract

One solution that works consists in extracting the ligand to a different PyMOL object (not a selection) so that the surface construction of the ramining structure will not include any of the ligand itself.

Assuming we already have the structure within PyMOL, this can be accomplished by the few commands below that:

1. Create a selection name for chain C as "C"
2. Extract the selection into a new object "PRD"
3. Show again surface for the rest of `6lu7`

```
select C, chain C
extract PRD, C
show surface, 6lu7
```

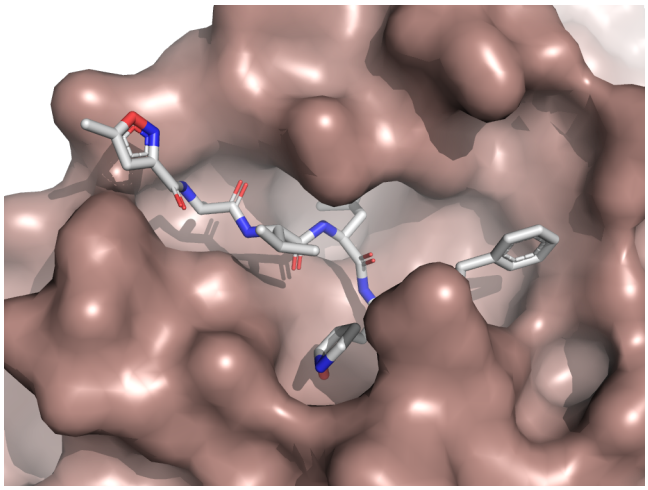


**FIGURE 4.4:** PRD_002214, extracted, in protein groove.

### 4.2.2 Final solution

At this point we could think that we are done. But we are not!

If we now try to create a surface for the new, independent "PRD" object we still obtain a partial surface of the molecule in the area of the (now) `UNK` residues.

For example with the command `show surface, PRD`.

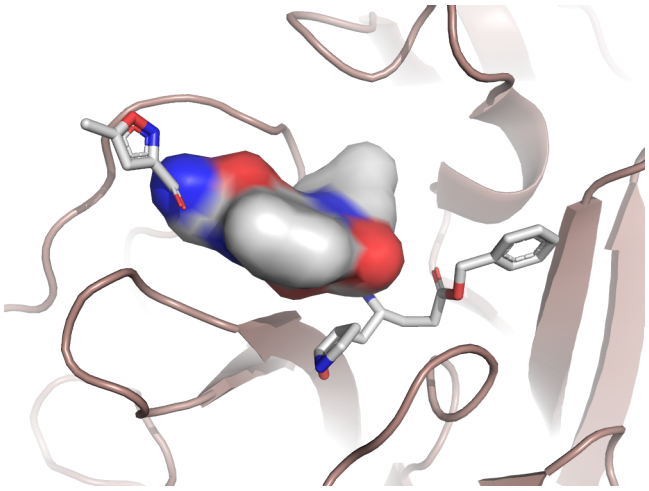


**FIGURE 4.5:** PRD_002214, partial surface.

The likely reason is that while we changed actual amino acid names from `ALA`, `VAL`, `LEU` to unknow `UNK`, PyMOL probably remembers the *properties* of these residues from *before they were changed*. Hence the surface creation.

To erase this residual memory of properties, one solution is the write the coordinates of `PRD` into a file, delete the object and re-read the coordinates within PyMOL.

Let's try if this will work with the few commands that follow.

```
save PRD.pdb, PRD
delete PRD
load PRD.pdb
hide cartoon, PRD
```

```
util.cbaw chain C
show surface, PRD
```

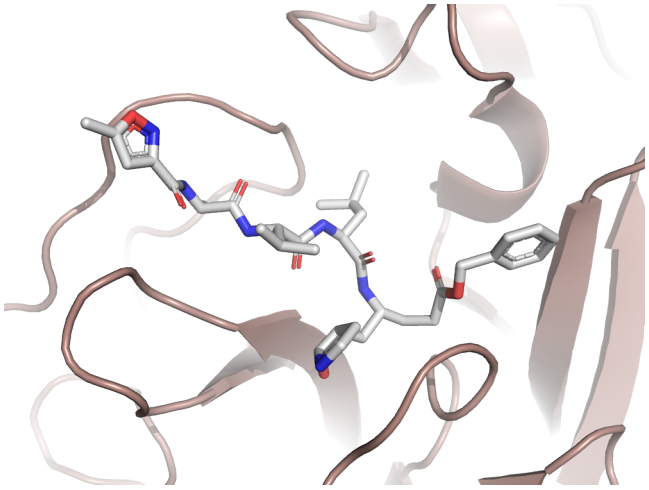


**FIGURE 4.6:** No surface created for PRD_002214 when reloaded.

Exercice:
Why is it necessary to have command `hide cartoon, PRD`?
What is the mouse menu equivallent to the `save` command?
Where is the mouse equivallent of the `delete` command?

Finally, the following settings may help when saving the coordinates into a file that will:

- Create `CONECT` records specifying which atoms are bonded
- Prevent the addition of `TER` records which could create gaps within graphics representations.

These were not necessary for the creation of the embedded illustration here but could be useful in other situations.

```
set pdb_use_ter_records, 0
set pdb_conect_all, on
# OR
set pdb_conect_nodup, 1  # no CONECT duplication is used
                         # to store bond order
```

See https://pymolwiki.org/ for more information on these commands

Exercice:
Where is the file PRD.pdb saved?
Could the file be saved in a different format?
Does it contain TER records?
Are there UNK residues present?

## 4.3 Biological assembly

The biologically active assembly is a dimer as shown in figure 4.7. See script in Appendix 4.

## 4.4 Comparing structures

Beyond scripting: if you are interested in the science of the protease the following papers would be of interest: Adem et al. [2020], Zhang et al. [2020] reporting *in silico* search for inhibitorts of the protease we have used in this chapter (Jin et al. [2020], PDB 6LU7.) This structure is very similar to that of the SARS coronavirus protease reported by Tan

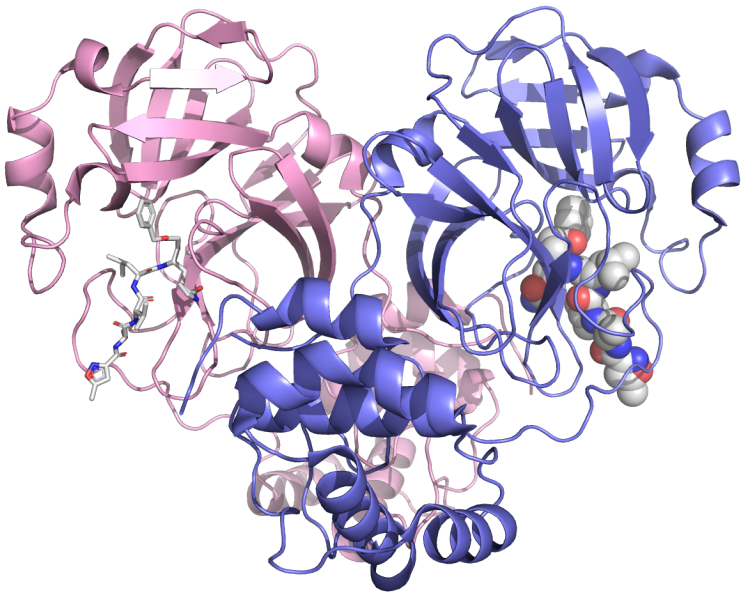


**FIGURE 4.7:** 6LU7 biological assembly. PRD_002214 shown as spheres or sticks in each monomer.

et al. [2005], (PDB 2BX4, no ligand,) and the new protease of SARS-CoV-2 has also been solved in *apo* form, without ligand (PDB: 6M03.)

Zhang et al. [2020] provide a good summary description of the protease function and cleavage specificity:

"*[...] this enzyme is essential for processing the polyproteins that are translated from the viral RNA (Hilgenfeld [2014]). The Mpro operates at no less than 11 cleavage sites on the large polyprotein 1ab (replicase 1ab, ~790 kDa); the recognition sequence at most sites is* `Leu-Gln↓(Ser,Ala,Gly)` *(↓ marks the cleavage site). Inhibiting the activity of this enzyme would block viral replication. Since no human proteases with a similar cleavage specificity are known, inhibitors are unlikely to be toxic.*"

These authors have also released the SARS-CoV-2 Mpro protease struc-

tures with and without bound ligands with the following PDB codes not cited within the paper:

- 6Y2E, no ligand
- 6Y2F and 6Y2G with complex ligand 06K

In the paper they state that *the r.m.s. deviation between the two free-enzyme structures is 0.53 Å for all Cα positions (comparison between SARS-CoV-2 Mpro structure and SARS-CoV Mpro, PDB entry 2BX4*.

How did they calculate this number?

My own search for calculating root-mean-square deviation (RMSD) *with PyMOL* pointed to many commands. However I believe that the following method was used as it provide the same answer. It is from the pymolwiki of PyMOL command `super` that aligns two selection with a sequence-independent structure-based dynamic programming alignment followed by a series of refinement cycles intended to improve the fit.[4]

The following script is taken from that page with only the PDB codes modified to those of the protease without ligand.

```
reinitialize

fetch 6Y2E, async=0
fetch 2BX4, async=0

# super 6Y2E, 2BX4

test=cmd.super("6Y2E","2BX4")
```

[4] https://pymolwiki.org/index.php/Super

```
python
writefile=open("rmsd_file_super.txt","a")
writefile.write(' '.join('%s' % x for x in test))
writefile.write('\n')
writefile.close()
python end
```

File `rmsd_file_super.txt` contains only one line:

```
0.5308237075805664 1928 5 1.130696415901184 2280 1361.6253662109375 303
```

The first value is indeed `0.53`.

One "trick" was to use the `cmd.super()` python form which allows the recoding of the data into a variable called `test`. The rest of the code is python for writing the content of the variable `test` within the `rmsd_file_super.txt` file.

Optional exercise:
Create a figure representing both structures superimposed and shown as `ribbon` or as `cartoon`.

Then create a new figure showing them side-by-side in the exact same orientation.

Hint: `grid_mode`

# 5

# *Spike Protein*

(Find all image scripts for this chapter in Appendix D.)

*The spike (S) protein is a surface trimer glycoprotein located on the envelope of the virions. It is cleaved into S1 and S2 subunits. The S1 subunit is responsible for host-receptor binding while the S2 subunit contains the membrane-fusion machinery*[1].

There are 5 published structures as shown in the following table:

**TABLE 5.1:** Spike protein PDB entries

| ID | Title |
|---|---|
| **6LZG** | Structure of novel coronavirus spike receptor-binding domain complexed with its receptor ACE2 |
| **6M0J** | Crystal structure of 2019-nCoV spike receptor-binding domain bound with ACE2 |
| **6VXX** | Structure of the SARS-CoV-2 spike glycoprotein $closedstate$ |
| **6VYB** | SARS-CoV-2 spike ectodomain structure $openstate$ |
| **6VSB** | Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up |

The first two 6M0J and 6LZG are both showing a spike receptor region (S1) bound to the Angiotensin-converting enzyme 2 (ACE2 ) receptor.

[1] https://www.rekombiotech.com/en/blog/detection-covid-19

Structures are almost indistinguishabel with an RMDS of 0.156 [2] are almost identical.

So choosing just one of them *e.g.* 6LZG is enough. We can illustrate the content of this PDB file showing how ACE2 and S1 fragment are bound. Both proteins have *N-Acetylglucosamine*[3] labeled NAG within the PDB file and bound to ASN residues (shown in red in figure 5.1.)

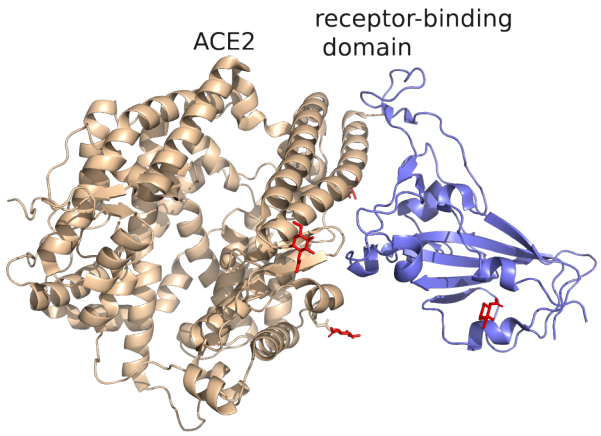



**FIGURE 5.1:** 6LZG: ACE2 and binding domain. NAG in red.

The other three structures are different configuration of the trimeric structure. Figures 5.2 and 5.3 show a side by side comparison between the closed structure (6VXX) and the trimeric structure of the spike glycoprotein with a single receptor-binding domain up 6VSB.

The figures are automatically colored by chain. In 5.1 structures are shown in the original orientation when the PDB is opened, looking into the trimeric structure as from the outside.

[2] see previous chapter 4.4 for RMSD calculation method
[3] https://en.wikipedia.org/wiki/N-Acetylglucosamine

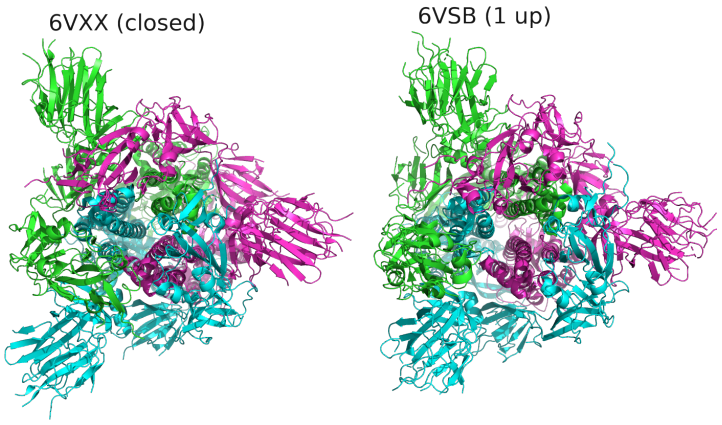



**FIGURE 5.2:** 6VXX (closed) and 6VSB (1 up) trimeric structure. Outside view.

To obtain figure 5.3 a simple rotation of 90 degrees around the $x$ axis is sufficient. It then becomes clearer to spot the "1 up" fragment (green) from structure 6VSB. This is the equivallent portion labeled "*receptor-binding domain*" of 6LZG displayed in figure 5.1.

*Note*: removed in these figures are the numerous glycosylation sites that would be shown by default as "floating" sticks.)

We'll use 6VSB trimeric structure of the spike glycoprotein with a single receptor-binding domain up. This domain corresponds to the spike receptor region (S1) of the 6M0J and 6LZG structures.

As an exercise we'll superimpose 6LZG over 6VSB to see how the receptor would look like and be positioned in relation to the trimeric structure. Color scheme is as in figure 5.1 for 6LZG and figures 5.2 and 5.3 for 6VSB.

The scripts can be found in Appendix D.

Within the script the structures are superimposed with the PyMOL

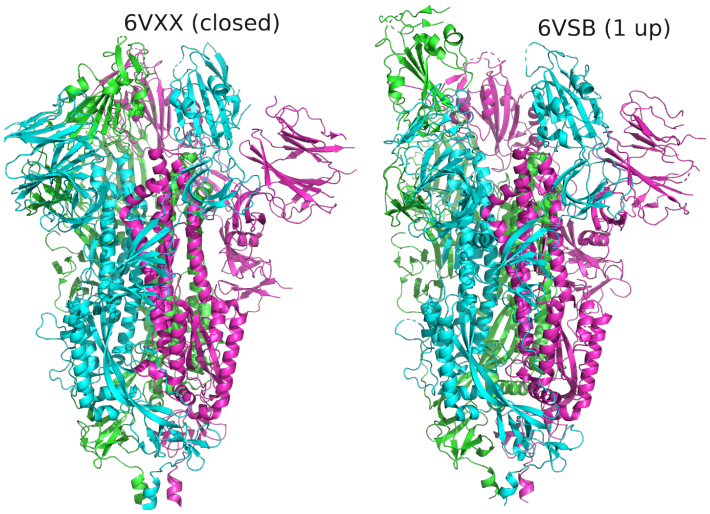



**FIGURE 5.3:** 6VXX (closed) and 6VSB (1 up) trimeric structure. Side view.

command `super` that is not relying on sequence. However, in this case the command `align` would work equally well.

To orient the molecules the script uses the PyMOL function `set_view` for which the data can be obtained when using the graphical interface by pressing the button "Get View" at the top right or on the command-line with the PyMOL command `get_view`. This method is an alternative to PyMOL commands `turn` and `move` used in other scripts, for simplicity.

After all this work we can now compare this "modeled" structure combination with a crystal structure of PDB file `6ACK` with title: "Trypsin-cleaved and low pH-treated SARS-CoV spike glycoprotein and ACE2 complex, ACE2-bound conformation 3".

This structures contains the trimeric spike glycoprotein trimer, one of which is attached to a molecule of *angiotensin-converting enzyme 2* (ACE2) in a similar way as our model above.

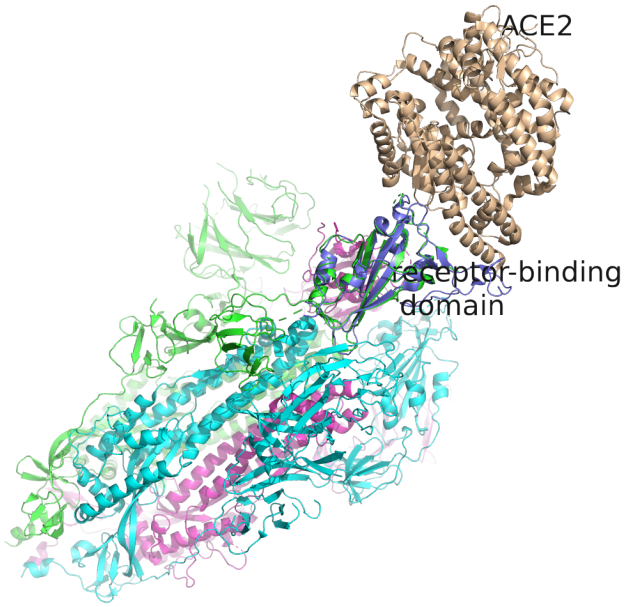



**FIGURE 5.4:** 6LZG (ACE2 and binding domain) superimposed onto 6VSB receptor-binding up domain.

We can select residues that are near ACE2 and show them as stick models and zoom for a more closer look (figure 5.6.)

Let's review some of the critical or new PyMOL commands from the scripts for this chapter (Appendix D.)

- `align` and `super` are two methods to superimpose structures. Unlike `align`, `super` does not rely on sequence information.
- `extract` is a useful command to separate a set of atoms that were previously selected. One of the effect is to allow a surface to be complete and independent of other molecules.
- `select byres chain C within 5 of ACE2` creates a selection based on distance with `within`
- `util.cbc` is a simple utility meaning *color by chain*
- `util.cbaw` is also a simple utility meaning *color by atom white*

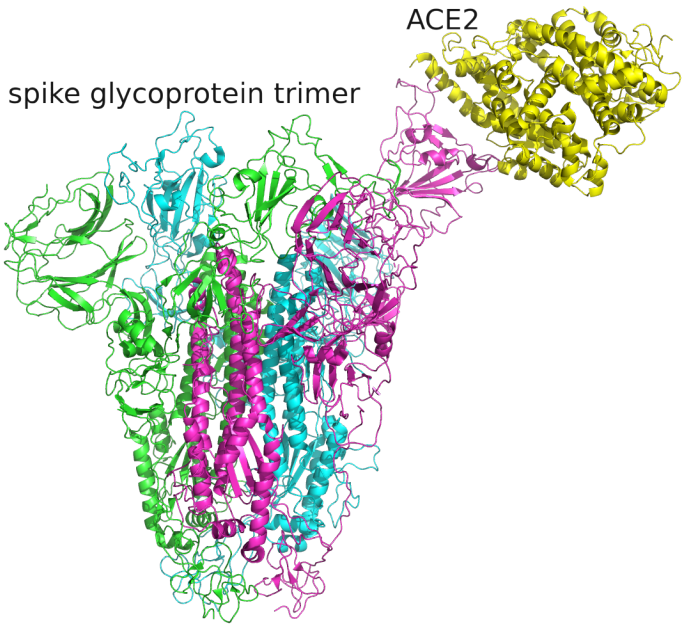



**FIGURE 5.5:** 6ACK: ACE2 bound to spike glycoprotein trimer.

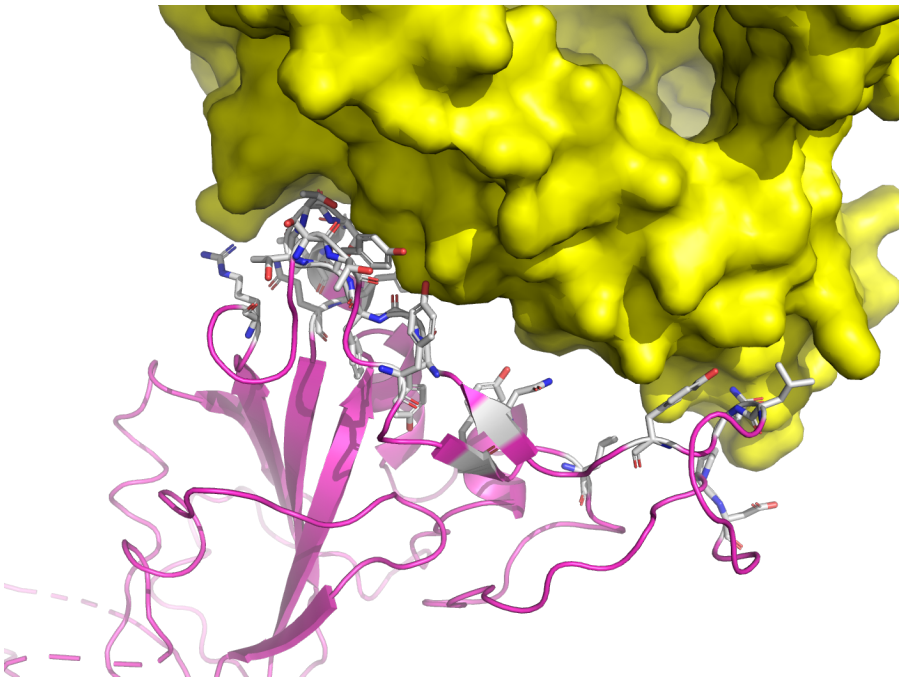


**FIGURE 5.6:** 6ACK: contact amino acids from spike glycoprotein trimer (chain C) with AC2 (surface.)

# 6

# *NSP3*

(Find all image scripts for this chapter in Appendix E.)

The multi-domain non-structural protein 3 (Nsp3) is the largest protein encoded by the coronavirus (CoV) genome, with an average molecular mass of about 200 kD. Nsp3 is a multifunctional protein comprising up to 16 different domains and regions and is an essential component of the replication/transcription complex. It comprises various domains, the organization of which differs between CoV genera, due to duplication or absence of some domains. However, eight domains of Nsp3 exist in all known CoVs. (Review: Lei et al. [2018].)

## 6.1 ADP ribose phosphatase of NSP3 from SARS CoV-2

There are 3 published structures for the SARS CoV-2 ribose phosphatase portion of NSP3 as shown in the following table:

**TABLE 6.1:** PDB entries for ADP ribose phosphatase portion of NSP3

| ID | Title |
|---|---|
| **6W02** | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in the complex with ADP ribose |
| **6VXS** | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 |

| ID | Title |
|---|---|
| **6W6Y** | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in complex with AMP |

Crystal Structure 6W02 with *ADP ribose phosphatase of NSP3 from SARS CoV-2 in the complex with ADP ribose* is very similar to that of MERS-Cov[1] (5HOL) with an RMSD of 0.63 [2] which is depicted in Figure 4 by Lei et al. [2018]. The two structures are easily superimposed (figure 6.1.)

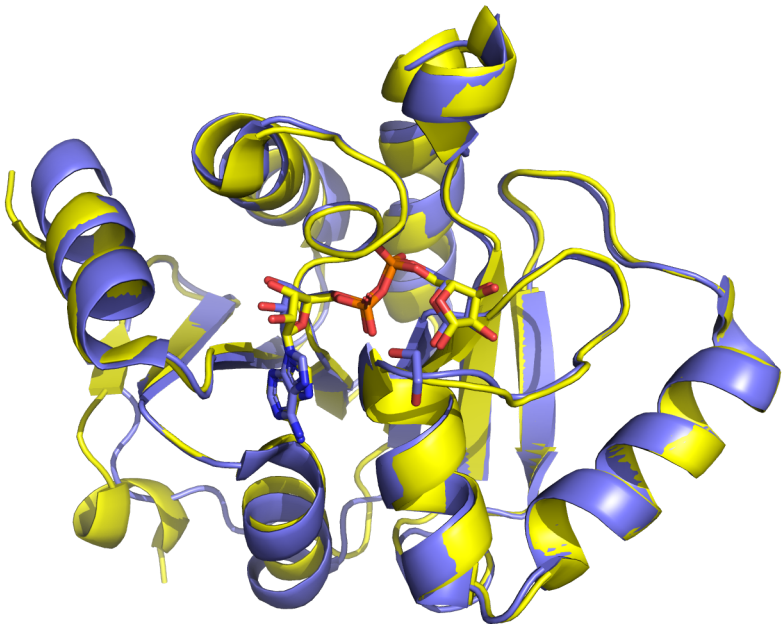


**FIGURE 6.1:** 6W02 aligned with 5HOL.

Figure 6.2 illustrates a closer view of ADP within structure 6W02. Also visible is ethylene glycol (alias 1,2-ethanediol) and labeled EDO within the PDB file. Amino acid ILE 131 has been shown only as sticks to facilitate view.

[1] Middle-East Respiratory Syndrome Coronavirus
[2] see 4.4 for RMSD calculation method

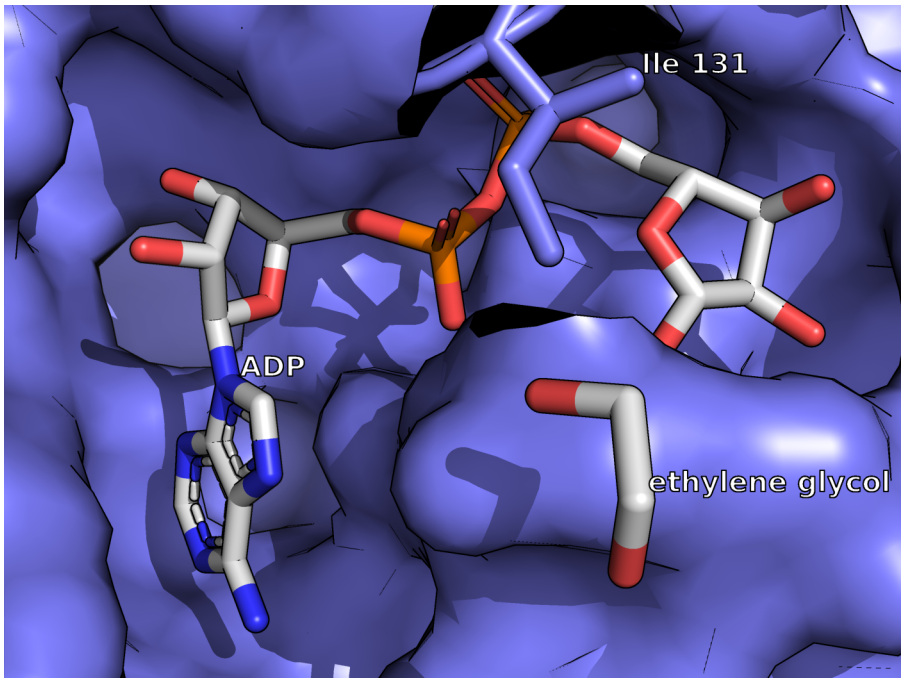



**FIGURE 6.2:** Close view of ADP within 6W02.

Here are a few highlights from the scripts for this chapter (Appendix E.)

- `util.cbaw`, `util.cbab` and `util.cbay` are utilities to *color by atom white*, *blue*, and *yellow* respectively.
- `hide nonbonded` is a way to hide the waters and other "floating" solvent molecules.
- `organic` usually serves to select ligands, but can also select specific organic solvents.

# 7

# *NSP15*

(Find all image scripts for this chapter in Appendix F.)

The title for structure `6W01` is *The 1.9 A Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2 in the Complex with a Citrate*.

This structure is a good illustration for the understanding of "asymetric unit" *vs* "biological assembly."

In simple terms, crystallographers solve the mathematical problem to obtain the smallest unit that can be reproduced almost *ad infinitum* to recreate the crystal. This "asymetric unit" is what gets deposited at the Protein Data Bank.

Fortunately these days, a set of symmetry information is built-in the PDB file so that it is possible to download the "biological assembly" which is the biologically active compound.

For this entry, the "asymetric unit" is composed of 2 molecules while the "biological assembly" is composed of a total of 6, consisting of 2 trimers.

Note that in other cases, the "biological assembly" may consist of a smaller number than the "asymetric unit."

Figure 7.1 illustrates the "asymetric unit" composed of 2 chains. Residues in the points of contact are shown as spheres. Within the PDB, information for the crystallographers would inform them that the "space group" if `P 63` and would also provide relevant information about symmetry operators.

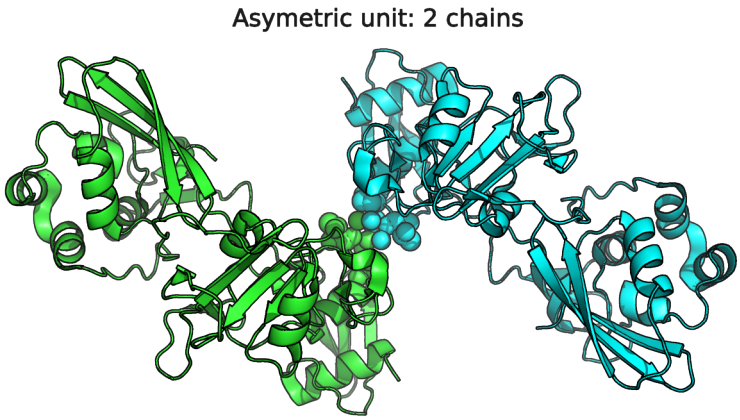



**FIGURE 7.1:** 6W01 asymetric unit.

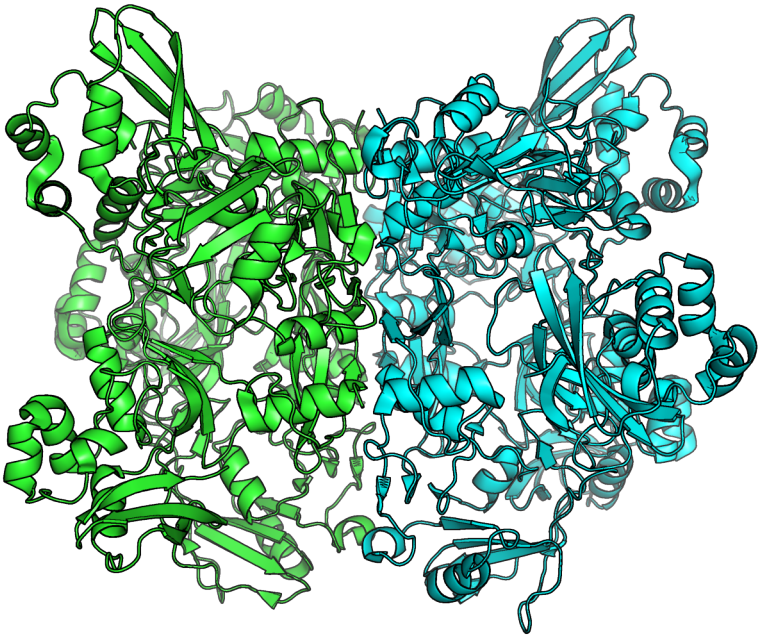


**FIGURE 7.2:** 6W01 biological assembly: 2 trimers.

Figure 7.2 is the first of two illustrations for the "biological assembly" that will appear as two trimers. All chains within a trimer have the same chain ID. One trimer is `A` and the other `B`. Therefore within one trimer it is not possible to uniquely address residues or atoms. This makes labeling more difficult and therefore there are no internal title within this illustration. (We'll fix that for the next illustration.) In addition, by default PyMOL will show only one of the chain for each trimer. Each trimer contains 3 "models" or "state", see command explanation below.

In this illusration alpha helices are shown as a helix reprentation. Compare with figure 7.3 using a different representation.

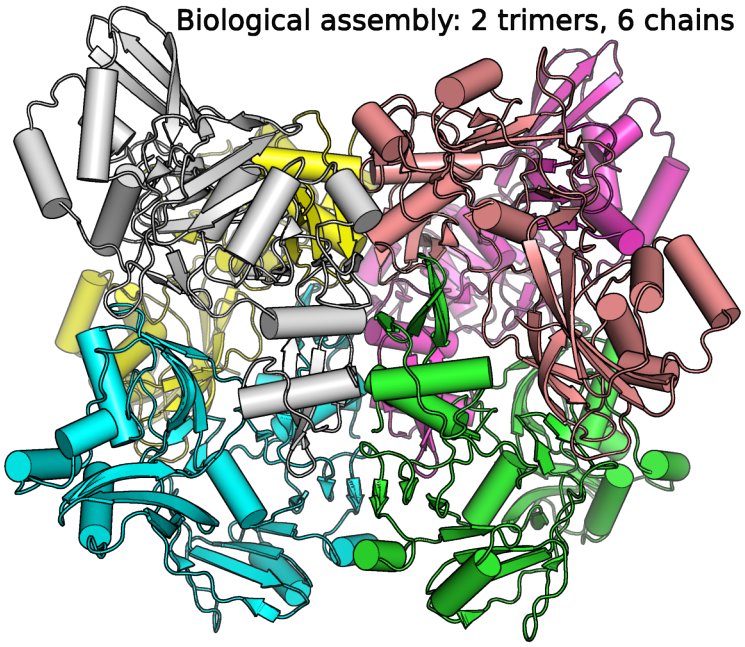



**FIGURE 7.3:** 6W01 biological assembly: all chains colored.

The script (Appendix F) contains a few critical PyMOL commands:

- `set all_states, on` will show all chains within each trimer

- `set cartoon_cylindrical_helices, on` to change the helix representaion mode
- `run flatten_obj.py` to acquire a new function to allow the renumbering of all chains. *Please note* that file `flatten_obj.py` has to be downloaded within the current directory (See pymolwiki [1] or download directly from Github[2] - short URL[3].

In addition the raytrace rendering was changed to provide a different look, with more "flat" coloring and a black line outline. This is thanks to the command `set ray_trace_mode, 1`. In addition cast shadows were removed with `set ray_shadow, off` as they would add confusion to this large structure.

[1] https://pymolwiki.org/index.php/Flatten_obj
[2] https://raw.githubusercontent.com/Pymol-Scripts/Pymol-script-repo/master/flatten_obj.py
[3] http://tiny.cc/flatten-obj

# 8

# *NSP12 polymerase*

(Find all image scripts for this chapter in Appendix G.)

The title for structure 6M71 is *2019-nCoV RNA-dependent RNA polymerase in complex with cofactors*.(Deposited: 2020-03-16 Released: 2020-04-01 )

It is very similar to structure 6NUR with title *SARS-Coronavirus NSP12 bound to NSP7 and NSP8 co-factors* (Deposited: 2019-02-01 Released: 2019-05-29) Kirchdoerfer and Ward [2019].

Figure 8.1 shows the structure as colored and oriented as in Kirchdoerfer and Ward [2019] figure 1b, middle figure with legend: *Structure of SARS-CoV nsp12 bound to nsp7 and nsp8 co-factors. b SARS-CoV nsp12 contains a large N-terminal extension composed of the NiRAN domain (dark red) and an interface domain (purple) adjacent to the polymerase domain (orange). nsp12 binds to a heterodimer of nsp7 (blue) and nsp8 (green) as well as to a second subunit of nsp8.*

With some extra PyMOLcommands the labels could be made to color-match the proteins.

Figure 8.2 compares structures 6M71 and 6NUR for which more of the nsp8 protein is visible (top right of the structure.)

Figure 1 legend continues as: *c–e Comparison of SARS-CoV nsp12 to the polymerase proteins of poliovirus24 (3OL6.pdb) [10.2210/pdb3OL6/pdb] and dengue virus55 (4V0R.pdb) [10.2210/pdb4V0R/pdb]. Viral RNA polymerases share an overall structural architecture with fingers (blue), palm (yellow), and*

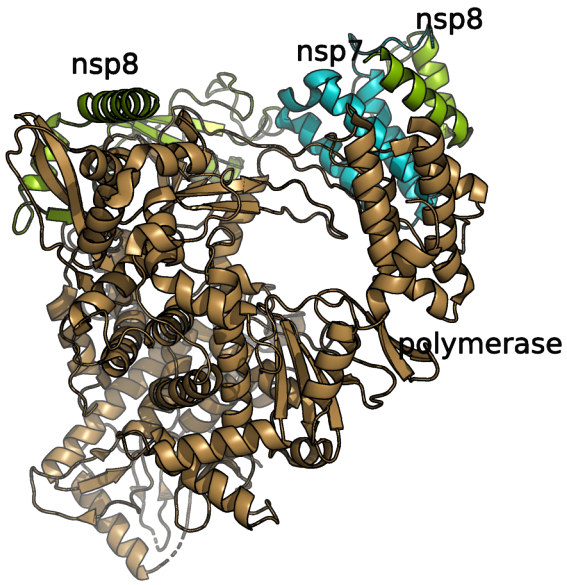



**FIGURE 8.1:** 6M71 RNA-dependent RNA polymerase nsp12 in complex with cofactors nsp7 and nsp8.

*thumb domains (red). Both SARS-CoV and dengue virus polymerase proteins contain N-terminal extensions colored green. The SARS-CoV nsp7 and nsp8 cofactors are colored in white and gray, respectively*

I leave the pleasure to the reader(s) to create the corresponding images for the SARS and the other viral polymerases.

The script (Appendix G) contains a few critical PyMOL commands:

- `hide everything, 6NUR`.Fetching the `6NUR1` structure early allows to color both structures at the same time. For the first image structure `6NUR` is hidden from view.
- `translate [40, 0, 0], 6M71` this command permits to separate the 2 structures for better comparison.

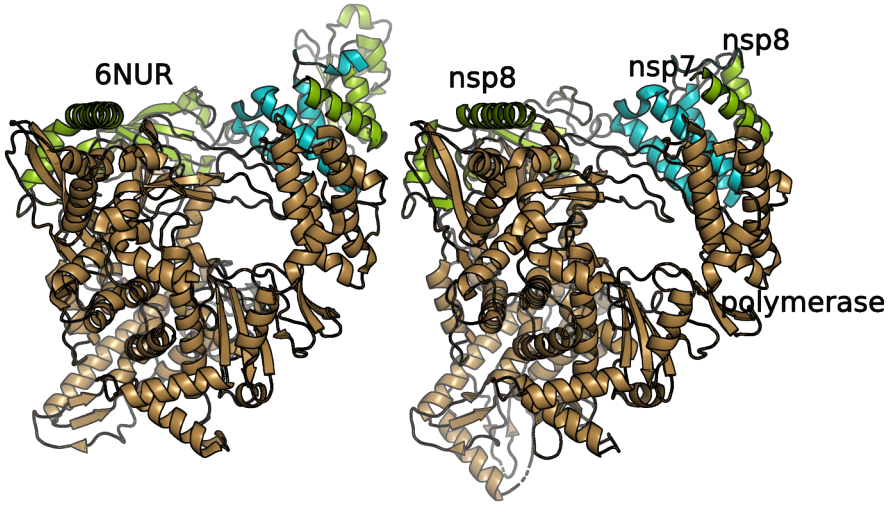



**FIGURE 8.2:** Comparison between Cov (6NUR) and Cov2 (6M71) RNA-dependent RNA polymerase nsp12 in complex with cofactors nsp7 and nsp8.

# 9

# *Epilogue*

I hope that this booklet will help you with your PyMOL skills. I probably left a few PyMOL commands unexplained that you can easily learn from a simple web search, as I often do.

I certainly learned or relearned many aspects of using PyMOL while working on this. Perhaps even more so as I wanted the image to be able to be reproduced *automatically* by script with the booklet creation method itself. As detailed in the Introduction (1) I have created this booklet using the "*markdown*" language augmented for *RStudio*, *bookdown* and *bookdownplus*. I have tried to use this method to add cross-references, appendices and a book index that I hope are useful and consistent. It sure was a learning curve for me, but it was an interesting endeavor even with some late night frustrations.

To know more about **bookdown**, see `https://bookdown.org`.

The next step will be to create a (smaller) booklet for printing and coloring structures with crayons. May this help those at home with little ones and ride the difficulties we face during the pandemic.

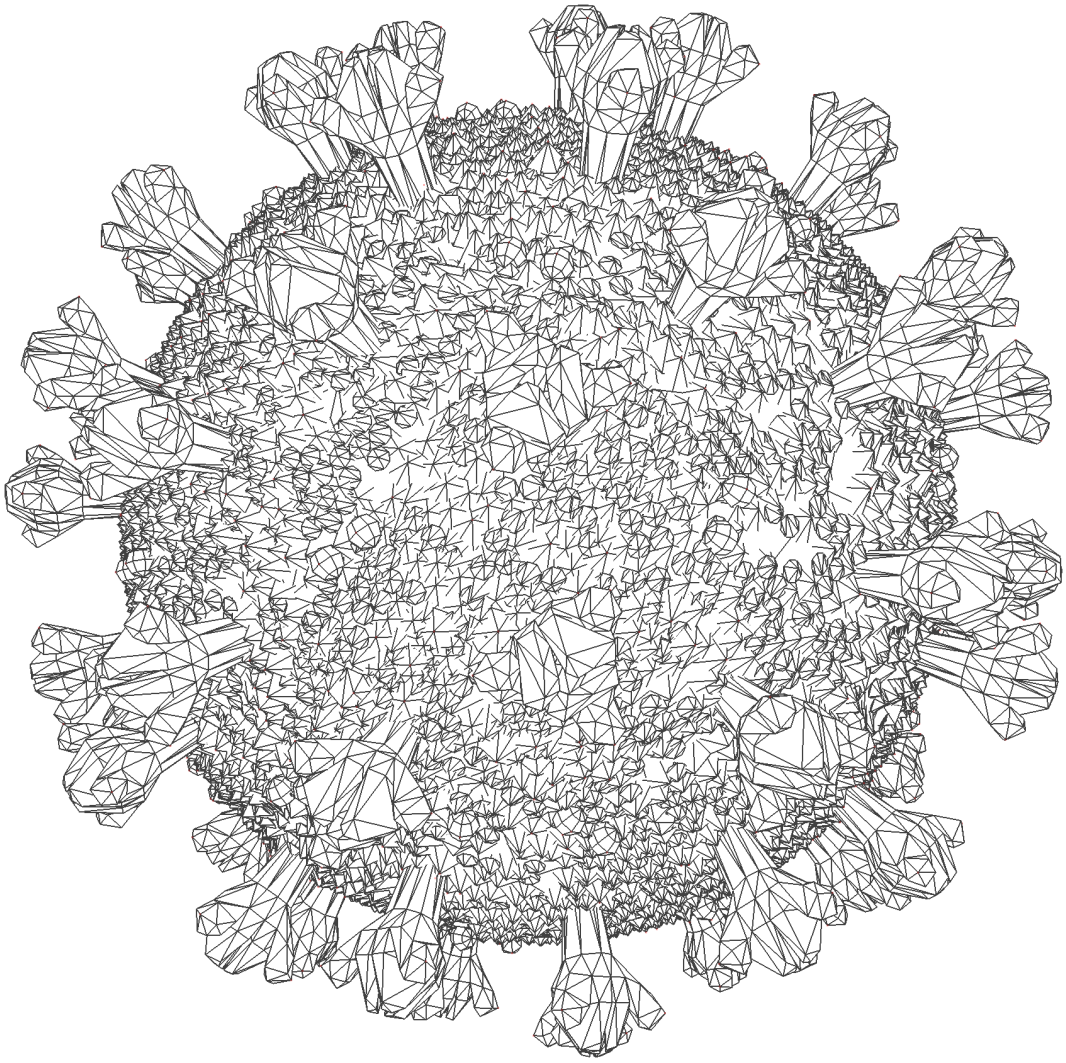


**FIGURE 9.1:** SARS-CoV-2 3D model for crayon coloring.

# A

## *SARS-CoV-2 PDB codes*

The information in these tables is derived from the Protein Data Bank web site SARS-CoV-2 article:

- Original link: "https://www.rcsb.org/news?year=2020&article=5e74d55d2d410731e9944f52&feature=true"
- Short URL: http://tiny.cc/PDBCovid19
- Short URL: https://bit.ly/2UXfwPQ
- Archived: 03April2020
  - Short URL: http://tiny.cc/PDBCovid19ARC
  - Short URL: https://bit.ly/2X8c0ob

### A.1 Main protease

*Note*: For clarity *PanDDA analysis group deposition – Crystal Structure of* has been removed for all PanDDA structures titles.

**TABLE A.1:** PDB IDs of SARS-CoV-2 main protease

| ID | Title |
|---|---|
| 5R8T | PanDDA analysis group deposition of ground-state model of SARS-CoV-2 main protease screened against DSI poised (Enamine), Fraglites and Peplites (Newcastle university), Mini Frags (Astex), York 3D (York university), electrophile cysteine covalent (Weizman institute) fragment libraries |
| 5RE4 | SARS-CoV-2 main protease in complex with Z1129283193 |
| 5RE5 | SARS-CoV-2 main protease in complex with Z33545544 |
| 5RE6 | SARS-CoV-2 main protease in complex with Z54571979 |
| 5RE7 | SARS-CoV-2 main protease in complex with Z30932204 |
| 5RE8 | SARS-CoV-2 main protease in complex with Z2737076969 |
| 5RE9 | SARS-CoV-2 main protease in complex with Z2856434836 |
| 5REA | SARS-CoV-2 main protease in complex with Z31432226 |
| 5REB | SARS-CoV-2 main protease in complex with Z2856434899 |
| 5REC | SARS-CoV-2 main protease in complex with Z1587220559 |
| 5RED | SARS-CoV-2 main protease in complex with Z2856434865 |
| 5REE | SARS-CoV-2 main protease in complex with Z2217052426 |
| 5REF | SARS-CoV-2 main protease in complex with Z24758179 |
| 5REG | SARS-CoV-2 main protease in complex with Z1545313172 |
| 5REH | SARS-CoV-2 main protease in complex with Z111507846 |
| 5REI | SARS-CoV-2 main protease in complex with Z2856434856 |
| 5REJ | SARS-CoV-2 main protease in complex with PCM-0102241 |
| 5REK | SARS-CoV-2 main protease in complex with PCM-0102327 |
| 5REL | SARS-CoV-2 main protease in complex with PCM-0102340 |
| 5REM | SARS-CoV-2 main protease in complex with PCM-0103016 |
| 5REN | SARS-CoV-2 main protease in complex with PCM-0102425 |
| 5REO | SARS-CoV-2 main protease in complex with PCM-0102578 |
| 5REP | SARS-CoV-2 main protease in complex with PCM-0102201 |
| 5RER | SARS-CoV-2 main protease in complex with PCM-0102615 |
| 5RES | SARS-CoV-2 main protease in complex with PCM-0102281 |

| ID | Title |
|---|---|
| 5RET | SARS-CoV-2 main protease in complex with PCM-0102269 |
| 5REU | SARS-CoV-2 main protease in complex with PCM-0102395 |
| 5REV | SARS-CoV-2 main protease in complex with PCM-0103072 |
| 5REW | SARS-CoV-2 main protease in complex with PCM-0102275 |
| 5REX | SARS-CoV-2 main protease in complex with PCM-0102287 |
| 5REY | SARS-CoV-2 main protease in complex with PCM-0102911 |
| 5REZ | SARS-CoV-2 main protease in complex with POB0129 |
| 5RF0 | SARS-CoV-2 main protease in complex with POB0073 |
| 5RF1 | SARS-CoV-2 main protease in complex with NCL-00023830 |
| 5RF2 | SARS-CoV-2 main protease in complex with Z1741969146 |
| 5RF3 | SARS-CoV-2 main protease in complex with Z1741970824 |
| 5RF4 | SARS-CoV-2 main protease in complex with Z1741982125 |
| 5RF5 | SARS-CoV-2 main protease in complex with Z3241250482 |
| 5RF6 | SARS-CoV-2 main protease in complex with Z1348371854 |
| 5RF7 | SARS-CoV-2 main protease in complex with Z316425948_minor |
| 5RF8 | SARS-CoV-2 main protease in complex with Z271004858 |
| 5RF9 | SARS-CoV-2 main protease in complex with Z217038356 |
| 5RFA | SARS-CoV-2 main protease in complex with Z2643472210 |
| 5RFB | SARS-CoV-2 main protease in complex with Z1271660837 |
| 5RFC | SARS-CoV-2 main protease in complex with Z979145504 |
| 5RFD | SARS-CoV-2 main protease in complex with Z126932614 |
| 5RFE | SARS-CoV-2 main protease in complex with Z509756472 |
| 5RFF | SARS-CoV-2 main protease in complex with PCM-0102704 |
| 5RFG | SARS-CoV-2 main protease in complex with PCM-0102372 |
| 5RFH | SARS-CoV-2 main protease in complex with PCM-0102277 |
| 5RFI | SARS-CoV-2 main protease in complex with PCM-0102353 |
| 5RFJ | SARS-CoV-2 main protease in complex with PCM-0103067 |
| 5RFK | SARS-CoV-2 main protease in complex with PCM-0102575 |
| 5RFL | SARS-CoV-2 main protease in complex with PCM-0102389 |
| 5RFM | SARS-CoV-2 main protease in complex with PCM-0102539 |

| ID | Title |
|---|---|
| 5RFN | SARS-CoV-2 main protease in complex with PCM-0102868 |
| 5RFO | SARS-CoV-2 main protease in complex with PCM-0102972 |
| 5RFP | SARS-CoV-2 main protease in complex with PCM-0102190 |
| 5RFQ | SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5RFR | SARS-CoV-2 main protease in complex with PCM-0102169 |
| 5RFS | SARS-CoV-2 main protease in complex with PCM-0102739 |
| 5RFT | SARS-CoV-2 main protease in complex with PCM-0102432 |
| 5RFU | SARS-CoV-2 main protease in complex with PCM-0102121 |
| 5RFV | SARS-CoV-2 main protease in complex with PCM-0102306 |
| 5RFW | SARS-CoV-2 main protease in complex with PCM-0102243 |
| 5RFX | SARS-CoV-2 main protease in complex with PCM-0102254 |
| 5RFY | SARS-CoV-2 main protease in complex with PCM-0102974 |
| 5RFZ | SARS-CoV-2 main protease in complex with PCM-0102274 |
| 5RG0 | SARS-CoV-2 main protease in complex with PCM-0102535 |
| 6W63 | Structure of COVID-19 main protease bound to potent broad-spectrum non-covalent inhibitor X77 |
| 6YB7 | SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19) |
| 5R7Y | COVID-19 main protease in complex with Z45617795 |
| 5R7Z | COVID-19 main protease in complex with Z1220452176 |
| 5R80 | COVID-19 main protease in complex with Z18197050 |
| 5R81 | COVID-19 main protease in complex with Z1367324110 |
| 5R82 | COVID-19 main protease in complex with Z219104216 |
| 5R83 | COVID-19 main protease in complex with Z44592329 |
| 5R84 | COVID-19 main protease in complex with Z31792168 |
| 6M03 | The crystal structure of COVID-19 main protease in apo form |
| 6Y84 | SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19) |
| 6Y2E | Crystal structure of the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease |

| ID | Title |
|---|---|
| 6Y2F | Crystal structure (monoclinic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b) |
| 6Y2G | Crystal structure (orthorhombic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b) |
| 6LU7 | The crystal structure of COVID-19 main protease in complex with an inhibitor N3 |

## A.2 Papain-like protease

**TABLE A.2:** PDB IDs of SARS-CoV-2 Papain-like protease

| ID | Title |
|---|---|
| 6W9C | The crystal structure of papain-like protease of SARS CoV-2 |

## A.3 Spike protein

**TABLE A.3:** PDB IDs of SARS-CoV-2 spike glycoprotein

| ID | Title |
|---|---|
| 6LZG | Structure of novel coronavirus spike receptor-binding domain complexed with its receptor ACE2 |
| 6M0J | Crystal structure of 2019-nCoV spike receptor-binding domain bound with ACE2 |
| 6VXX | Structure of the SARS-CoV-2 spike glycoprotein (closed state) |
| 6VYB | SARS-CoV-2 spike ectodomain structure (open state) |
| 6VSB | Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain up |

## A.4 Other SARS-CoV-2 structures

**TABLE A.4:** PDB IDs of other SARS-CoV-2

| ID | Title |
|---|---|
| 6M71 | 2019-nCoV RNA-dependent RNA polymerase in complex with cofactors |
| 6W41 | Crystal structure of SARS-CoV-2 receptor binding domain in complex with human antibody CR3022 |
| 6W61 | Crystal Structure of the methyltransferase-stimulatory factor complex of NSP16 and NSP10 from SARS CoV-2. |
| 6W6Y | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in complex with AMP |

| ID | Title |
|---|---|
| 6W75 | 1.95 Angstrom Resolution Crystal Structure of NSP10 - NSP16 Complex from SARS-CoV-2 |
| 6M3M | Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain |
| 6W4B | The crystal structure of Nsp9 RNA binding protein of SARS CoV-2 |
| 6W4H | 1.80 Angstrom Resolution Crystal Structure of NSP16 - NSP10 Complex from SARS-CoV-2 |
| 6M17 | The 2019-nCoV RBD/ACE2-B0AT1 complex |
| 6VYO | Crystal structure of RNA binding domain of nucleocapsid phosphoprotein from SARS coronavirus 2 |
| 6W01 | The 1.9 A Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2 in the Complex with a Citrate |
| 6W02 | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in the complex with ADP ribose |
| 6VW1 | Structure of 2019-nCoV chimeric receptor-binding domain complexed with its receptor human ACE2 |
| 6VWW | Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2. |
| 6VXS | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 |
| 6LVN | Structure of the 2019-nCoV HR2 Domain |
| 6LXT | Structure of post fusion core of 2019-nCoV S2 subunit |

# B

## *6LU7: PRD_002214 coordinates*

The 3D coordinates of compound PRD_002214 in PDB format, from structure 6LU7. The peptide-like n nature of compund PRD_002214 causes its coordinates to be a mixture of ATOM and HETATM records.

```
HETATM 2369  C4  02J C   1     -10.425   3.420  72.447  1.00 54.38
HETATM 2370  C5  02J C   1      -9.924   2.857  73.642  1.00 63.03
HETATM 2371  C6  02J C   1      -9.345   1.458  73.806  1.00 64.36
HETATM 2372  O1  02J C   1     -10.048   3.755  74.575  1.00 63.01
HETATM 2373  N2  02J C   1     -10.585   4.861  74.114  1.00 56.79
HETATM 2374  C3  02J C   1     -10.848   4.738  72.810  1.00 50.26
HETATM 2375  C41 02J C   1     -11.475   5.798  71.891  1.00 47.56
HETATM 2376  O42 02J C   1     -11.062   5.943  70.793  1.00 47.13
ATOM   2377  N   ALA C   2     -12.583   6.625  72.374  1.00 41.65
ATOM   2378  CA  ALA C   2     -13.166   7.625  71.495  1.00 42.30
ATOM   2379  C   ALA C   2     -12.115   8.750  71.241  1.00 41.96
ATOM   2380  O   ALA C   2     -11.466   9.159  72.125  1.00 41.95
ATOM   2381  CB  ALA C   2     -14.397   8.218  72.132  1.00 36.98
ATOM   2382  N   VAL C   3     -11.943   9.281  69.911  1.00 34.91
ATOM   2383  CA  VAL C   3     -10.955  10.336  69.666  1.00 36.29
ATOM   2384  C   VAL C   3     -11.663  11.606  69.273  1.00 40.78
ATOM   2385  O   VAL C   3     -12.381  11.617  68.328  1.00 36.69
ATOM   2386  CB  VAL C   3     -10.004   9.895  68.515  1.00 37.53
ATOM   2387  CG1 VAL C   3      -9.130  11.086  68.012  1.00 40.26
ATOM   2388  CG2 VAL C   3      -9.116   8.687  68.955  1.00 43.10
ATOM   2389  N   LEU C   4     -11.452  12.859  70.085  1.00 37.32
```

```
ATOM   2390  CA  LEU C   4      -12.124  14.096  69.716  1.00 39.95
ATOM   2391  C   LEU C   4      -11.582  14.561  68.334  1.00 38.38
ATOM   2392  O   LEU C   4      -10.411  14.570  68.131  1.00 44.55
ATOM   2393  CB  LEU C   4      -11.825  15.155  70.765  1.00 41.97
ATOM   2394  CG  LEU C   4      -13.001  15.397  71.764  1.00 45.86
ATOM   2395  CD1 LEU C   4      -13.834  14.100  72.019  1.00 43.70
ATOM   2396  CD2 LEU C   4      -12.487  15.982  73.082  1.00 43.98
HETATM 2397  C19 PJE C   5      -11.993  15.425  65.951  1.00 40.54
HETATM 2398  C20 PJE C   5      -12.499  16.817  65.647  1.00 36.98
HETATM 2399  C21 PJE C   5      -11.365  17.802  65.289  1.00 45.43
HETATM 2400  C22 PJE C   5      -10.167  17.764  66.278  1.00 52.38
HETATM 2401  C25 PJE C   5      -12.456  14.464  64.858  1.00 30.76
HETATM 2402  C26 PJE C   5      -11.827  13.003  65.048  1.00 34.19
HETATM 2403  C27 PJE C   5      -10.215  13.041  64.725  1.00 40.55
HETATM 2404  C28 PJE C   5       -9.925  12.061  63.836  1.00 39.60
HETATM 2405  N6  PJE C   5      -11.238  11.571  63.275  1.00 35.08
HETATM 2406  C29 PJE C   5      -12.383  12.148  64.256  1.00 34.70
HETATM 2407  O8  PJE C   5      -13.440  12.730  63.491  1.00 31.54
HETATM 2408  N5  PJE C   5      -12.524  14.987  67.260  1.00 34.60
HETATM 2409  O7  PJE C   5      -10.354  17.731  67.451  1.00 57.27
HETATM 2410  C   010 C   6       -7.830  17.582  66.759  1.00 63.14
HETATM 2411  O   010 C   6       -8.840  17.782  65.766  1.00 52.13
HETATM 2412  C1  010 C   6       -5.739  18.930  67.322  1.00 68.45
HETATM 2413  C2  010 C   6       -5.067  20.138  67.502  1.00 70.02
HETATM 2414  C3  010 C   6       -5.744  21.347  67.320  1.00 58.91
HETATM 2415  C4  010 C   6       -7.085  21.339  66.961  1.00 57.63
HETATM 2416  C5  010 C   6       -7.760  20.134  66.778  1.00 62.65
HETATM 2417  C6  010 C   6       -7.082  18.927  66.959  1.00 66.95
TER    2418      010 C   6
```

# C

## *Scripts for figures in Chapter 4*

### C.1 Stick and surface

```
# Script for "Protease Mpro"

# Load coodinates and change visual
fetch 6lu7, async=0
as cartoon
color darksalmon, chain A

# Alter atom type
alter (chain C),  type="HETATM"
show stick, chain C
hide cartoon, chain C
util.cbaw chain C

# Zoom and rotate
zoom 6lu7 and chain C
turn y, -50
turn z, -90
turn x, -60
turn y, 25
move z, 10

show surface
```

```
# label settings and labelling
set label_size, 20
set label_color, black
set label_position =[0.0, 1.5, 8]
label n. CG1 and resi 3 and chain C , "%s, %s" % (resn, resi)
label n. C and (i. 2 or i. 4) and chain C , "%s, %s" % (resn, resi)

bg_color white
png images/cov2-2-surf-01.png, 1000, dpi=300, ray=1

##############
# Next figure
hide surface, 6lu7
alter (chain C),  type="HETATM"
alter resn ALA+VAL+LEU and chain C, resn = "UNK"
# remove labels
label
select C, chain C
extract PRD, C
show surface, 6lu7
png images/cov2-2-surf-02.png, 1000, dpi=300, ray=1

##############
# Next figure
hide surface, 6lu7
show surface, PRD
png images/cov2-2-surf-03.png, 1000, dpi=300, ray=1

##############
# Next figure
save PRD.pdb, PRD
```

```
delete PRD
load PRD.pdb
hide cartoon, PRD
util.cbaw chain C
show surface, PRD
png images/cov2-2-surf-04.png, 1000, dpi=300, ray=1
```

## C.2 Biological assembly

```
reinitialize
fetch 6lu7, type=pdb1, async=0
set all_states, 1
split_states 6lu7
delete 6lu7
util.cbab 6lu7_0001
util.cbak 6lu7_0002
as cartoon
bg_color white
alter (chain C),  type="HETATM"
show sphere, chain C and 6lu7_0001
show sticks, chain C and 6lu7_0002
hide cartoon, chain C
util.cbaw chain C
orient
move z, 30
png images/cov2-dimer-1.png, 1400, dpi=300, ray=1
```

# D

## *Scripts for figures in Chapter 5*

### D.1 Display content of 6LZG

```
# spike ACE2 + S1 fragment
reinitialize
fetch 6LZG, async=0

color wheat, chain A
color slate, chain B

remove solvent
orient
move z, 50
move x, -7

# NAG = N-Acetylglucosamine
# Show bonded AA: ASN 53 and ASN 322
show stick, (resi 53 or resi 322) and chain A
color red, organic

bg_color white

# lablels:
set label_size, 20
set label_color, black
```

```
set label_position =[0, 3, 0]
label /6LZG/A/A/ASP`111/CA, "ACE2"
label /6LZG/B/B/ASN`481/CA, "receptor-binding \n domain"

png images/cov2-spike-ace2-1.png, 1200, dpi=300, ray=1
```

## D.2 Display content of trimeric structures 6VSB and 6VXX

```
# spike trimerics
reinitialize
# one open
fetch 6VSB, async=0
# closed
fetch 6VXX, async=0
util.cbc
as cartoon

align 6VSB, 6VXX

# Grid mode does not work in batch
# set grid_mode, 1

# Alternate option: translate
translate [50, 0, 0], 6VSB
translate [-70, 0, 0], 6VXX

bg_color white

# Labels
```

```
set label_size, 20
set label_color, black
set label_position =[2, 2, 8]
label /6VSB/A/A/ARG`246/CA, "6VSB (1 up)"
label /6VXX/A/A/HIS`245/CA, "6VXX (closed)"

png images/cov2-trimerics-1.png, 1400, dpi=300, ray=1
# cmd.png('images/cov2-trimerics-1.png', '30cm', '10cm', dpi=300, ray=1)

############
# Next figure
turn x, -90
label
label /6VSB/B/B/THR`500/CA, "6VSB (1 up)"
label /6VXX/A/A/LYS`444/CA, "6VXX (closed)"

png images/cov2-trimerics-2.png, 1400, dpi=300, ray=1
```

## D.3 Superimpose 6LZG and trimeric 6VSB

```
# spike trimer and ACE2 + S1 fragment
reinitialize
fetch 6VSB, async=0
fetch 6LZG, async=0
util.cbc

select S1, 6LZG and chain B
super S1, (6VSB and chain A)
as cartoon
# as ribbon
```

```
color wheat, 6LZG and chain A
color slate, S1
# show cartoon, 6LZG and chain A
# show cartoon, S1
# extract S1, S1

bg_color white
set_view (\
     0.569421589,   -0.808852911,    0.146665663,\
     0.195740119,   -0.039873410,   -0.979845762,\
     0.798400283,    0.586652577,    0.135621011,\
    -0.000902131,    0.000237614, -561.803344727,\
   216.354858398,  211.111328125,  255.279617310,\
   516.146301270,  607.512878418,  -20.000000000 )
select none

# lablels:
set label_size, 20
set label_color, black
set label_position =[0, 0, 8]
label /6LZG/A/A/ALA`614/CA, "ACE2"
label /6LZG/B/B/THR`478/CA, "receptor-binding \n domain"

png images/cov2-spike-trimer-1.png, 1400, dpi=300, ray=1
```

## D.4 6ACK spike glycoprotein trimer with bound ACE2

```
# spike glycoprotein trimer with ACE2 bound
reinitialize
fetch 6ACK, async=0
util.cbc
```

```
orient
turn z, 45
move z, 40

bg_color white

# lablels:
set label_size, 20
set label_color, black
set label_position =[0, 1, 8]
label /6ACK//D/GLU`87/CA, "ACE2"
label /6ACK//B/THR`433/CA, "spike glycoprotein trimer"

png images/cov2-spike-trimer-2-ace2-1.png, 1400, dpi=300, ray=1

##############
# Next figure

extract ACE2, chain D

select byres chain C within 5 of ACE2
show stick, sele
util.cbaw sele
show surface, ACE2

set_view (\
    -0.100384109,   -0.168694198,    0.980534792,\
    -0.242882282,    0.959854066,    0.140271023,\
    -0.964840829,   -0.224080622,   -0.137331456,\
     0.004348084,   -0.002141094, -107.068008423,\
   218.490600586,  243.002914429,  125.151054382,\
    52.256690979,  160.088317871,  -20.000000000 )
```

```
zoom sele
bg_color black
label

png images/cov2-spike-trimer-2-ace2-2.png, 1400, dpi=300, ray=1
```

# E

## *Scripts for figures in chapter 6*

### E.1 Nsp3 - ribose ADP

```
# spike glycoprotein trimer with ACE2 bound
reinitialize
fetch 6ACK, async=0
util.cbc
orient
turn z, 45
move z, 40

bg_color white

# lablels:
set label_size, 20
set label_color, black
set label_position =[0, 1, 8]
label /6ACK//D/GLU`87/CA, "ACE2"
label /6ACK//B/THR`433/CA, "spike glycoprotein trimer"

png images/cov2-spike-trimer-2-ace2-1.png, 1400, dpi=300, ray=1

#############
# Next figure
```

```
extract ACE2, chain D

select byres chain C within 5 of ACE2
show stick, sele
util.cbaw sele
show surface, ACE2

set_view (\
    -0.100384109,   -0.168694198,    0.980534792,\
    -0.242882282,    0.959854066,    0.140271023,\
    -0.964840829,   -0.224080622,   -0.137331456,\
     0.004348084,   -0.002141094, -107.068008423,\
   218.490600586,  243.002914429,  125.151054382,\
    52.256690979,  160.088317871,  -20.000000000 )

zoom sele
bg_color black
label

png images/cov2-spike-trimer-2-ace2-2.png, 1400, dpi=300, ray=1
```

# F

## *Scripts for figures in chapter 7*

### F.1 Nsp15 - asymetric unit and biological assembly

```
# NSP15 asymetric unit
fetch 6W01,async=0
as cartoon
util.cbc
orient
move z, 70
turn z, -5
# Select points of contact with 5 A
select byres chain A within 5 of chain B
show sphere, sele
select byres chain B within 5 of chain A
show sphere, sele
bg_color white
set label_size, 20
set label_color, black
set label_position =[0, 6, 8]
label /6W01/B/B/GLN`20/CA, "Asymetric unit: 2 chains"
set ray_trace_mode, 1
png images/cov2-nsp15-1.png, 1200, dpi=300, ray=1

###############
# Next image
```

```
# Biological assembly version 1
reinitialize
fetch 6W01, type=pdb1, async=0
set all_states, on
bg_color white
orient
move z, 30
turn y, 180
as cartoon
util.cbc
# set cartoon_cylindrical_helices, on
set label_size, 20
set label_color, black
set label_position =[2, 1, 8]
label /flat//F/SER`316/CA, "Biological assembly: 2 trimers"
set ray_trace_mode, 1
set ray_shadow, off
png images/cov2-nsp15-2.png, 1400, dpi=300, ray=1

###############
# Next image
# Biological assembly version 2
reinitialize
fetch 6W01, type=pdb1, async=0
# From: https://pymolwiki.org/index.php/Flatten_obj
run flatten_obj.py
flatten_obj flat, 6W01
bg_color white
orient
move z, 30
as cartoon
util.cbc
```

```
set cartoon_cylindrical_helices, on
set label_size, 20
set label_color, black
set label_position =[2, 1, 8]
label /flat//F/SER`316/CA, "Biological assembly: 2 trimers, 6 chains"
set ray_trace_mode, 1
set ray_shadow, off
png images/cov2-nsp15-3.png, 1400, dpi=300, ray=1
```

# G

## *Scripts for figures in chapter 8*

### G.1 SARS-CoV-2 nsp12 polymerase bound to nsp7 and nsp8 co-factors

```
# SARS-CoV-2 NSP12
fetch 6M71, async=0
fetch 6NUR, async=0
as cartoon
color sand
color  splitpea, chain B or chain D
color teal, chain C

align 6M71,6NUR
hide everything, 6NUR

set_view (\
    -0.086567082,   -0.070623048,   -0.993739665,\
    -0.930820167,    0.361255229,    0.055411216,\
     0.355083704,    0.929789126,   -0.097008832,\
     0.000127658,   -0.000919968, -318.655242920,\
   150.189804077,  138.051406860,  156.410263062,\
   264.730682373,  372.563507080,  -20.000000000 )

bg_color white
set label_size, 20
```

```
set label_color, black
set label_outline_color, white
set label_position =[0.5, 6, 16]
label /6M71/C/D/ARG`111/CA, "nsp8"
label /6M71/B/C/ARG`21/CA, "nsp7"
label /6M71/D/B/ARG`96/CA, "nsp8"
label /6M71/A/A/ASP`824/CA, "polymerase"

set ray_trace_mode, 1
png images/cov2-nsp12-1.png, 1400, dpi=300, ray=1

###############
# Next image - continue
# Side by side with SARS-CoV NSP12 6NUR
# reinitialize

show cartoon, 6NUR
translate [40, 0, 0], 6M71
translate [-40, 0, 0], 6NUR
move z, -75

label /6NUR/B/B/ASP`78/CA, "6NUR"

bg_color white
set ray_trace_mode, 1
set ray_shadow, off
png images/cov2-nsp12-2.png, 1400, dpi=300, ray=1
```

# *Bibliography*


Sevki Adem, Volkan Eyupoglu, Iqra Sarfraz, Azhar Rasul, and Muhammad Ali. Identification of potent covid-19 main protease (mpro) inhibitors from natural polyphenols: An in silico strategy unveils a hope against corona. *Preprints*, 2020. URL https://www.preprints.org/manuscript/202003.0333/v1.

A. E. Gorbalenya, S. C. Baker, R. S. Baric, R. J. de Groot, C. Drosten, A. A. Gulyaeva, B. L. Haagmans, C. Lauber, A. M. Leontovich, B. W. Neuman, D. Penzar, S. Perlman, L. L. M. Poon, D. V. Samborskiy, I. A. Sidorov, I. Sola, and J. Ziebuhr. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*, Mar 2020. [DOI:10.1038/s41564-020-0695-z[1]] [PubMed:32123347[2]].

R. Hilgenfeld. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *FEBS J.*, 281(18):4085–4096, Sep 2014. [DOI:10.1111/febs.12936[3]] [PubMed:25039866[4]].

Zhenming Jin, Xiaoyu Du, Yechun Xu, Yongqiang Deng, Meiqin Liu, Yao Zhao, Bing Zhang, Xiaofeng Li, Leike Zhang, Chao Peng, and et al. Structure of mpro from covid-19 virus and discovery of its inhibitors. *bioRxiv*, (2020.02.26.964882), Jan 2020. URL https://www.biorxiv.org/content/10.1101/2020.02.26.964882v2.


[1] https://dx.doi.org/10.1038/s41564-020-0695-z
[2] https://www.ncbi.nlm.nih.gov/pubmed/32123347
[3] https://dx.doi.org/10.1111/febs.12936
[4] https://www.ncbi.nlm.nih.gov/pubmed/25039866

R. N. Kirchdoerfer and A. B. Ward. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun*, 10(1): 2342, 05 2019. [PubMed Central:PMC6538669[5]] [DOI:10.1038/s41467-019-10280-3[6]] [PubMed:25451065[7]].

J. Lei, Y. Kusov, and R. Hilgenfeld. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. *Antiviral Res.*, 149:58–74, 01 2018. [DOI:10.1016/j.antiviral.2017.11.001[8]] [PubMed:29128390[9]].

RStudio Team. *RStudio: Integrated Development Environment for R*. RStudio, Inc., Boston, MA, 2015. URL http://www.rstudio.com/.

Schrödinger, LLC. The PyMOL molecular graphics system, version 2.0. 2020. URL https://pymol.org/.

Sgro, Jean-Yves. Molecular Graphics Essentials - PyMOL. 2017. URL https://bcrf.biochem.wisc.edu/2019/06/20/pymol-tutorial-books-released/.

Tim Skern. *Exploring protein structure: principles and practice*. Springer, 2018. ISBN 978-3-319-76857-1. URL doi:10.1007/978-3-319-76858-8.

J. Tan, K. H. Verschueren, K. Anand, J. Shen, M. Yang, Y. Xu, Z. Rao, J. Bigalke, B. Heisen, J. R. Mesters, K. Chen, X. Shen, H. Jiang, and R. Hilgenfeld. pH-dependent conformational flexibility of the SARS-CoV main proteinase (M(pro)) dimer: molecular dynamics simulations and multiple X-ray structure analyses. *J. Mol. Biol.*, 354(1):25–40, Nov 2005. URL doi.org/10.1016/j.jmb.2005.09.012.

[5] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6538669
[6] https://dx.doi.org/10.1038/s41467-019-10280-3
[7] https://www.ncbi.nlm.nih.gov/pubmed/25451065
[8] https://dx.doi.org/10.1016/j.antiviral.2017.11.001
[9] https://www.ncbi.nlm.nih.gov/pubmed/29128390

Yihui Xie. *Dynamic Documents with R and knitr*. Chapman and Hall/CRC, Boca Raton, Florida, 2nd edition, 2015. URL http://yihui.name/knitr/. ISBN 978-1498716963.

Yihui Xie. *bookdown: Authoring Books and Technical Documents with R Markdown*. Chapman and Hall/CRC, Boca Raton, Florida, 2016. URL https://github.com/rstudio/bookdown. ISBN 978-1138700109.

Linlin Zhang, Daizong Lin, Xinyuanyuan Sun, Ute Curth, Christian Drosten, Lucie Sauerhering, Stephan Becker, Katharina Rox, and Rolf Hilgenfeld. Crystal structure of sars-cov-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*, 2020. ISSN 0036-8075. doi: 10.1126/science.abb3405. URL https://science.sciencemag.org/content/early/2020/03/20/science.abb3405.

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