Overview of “The Protein Imager” interface

“The Protein Imager” (https://3dproteinimaging.com/protein-imager) is the interface of an online molecular viewer for visualizing macromolecular structures directly in a web browser, without any plugins or installation of software on a local computer. This tool is also connected with a server-side system able to produce high-resolution, publication-quality molecular illustrations.

The interface of “The Protein Imager” has been designed to be user friendly but, at the same time, to be powerful and flexible, allowing users to produce very complex molecular representations.

The aim of this document is to introduce some of the major features of "The Protein Imager" interface.

1. Loading files & projects

“**The Protein Imager**” can load structural data from local files (accepted file formats: PDB, PDBQT, CIF, MMTF, GRO, PQR, SDF, MOL or MOL2) or directly fetch structures from the Protein Data Bank (Berman et al., 2000) allowing to choose between different initial representation and viewing style presets (Fig. S1A). The interface allows also to save or load project files on a local computer or on the server (Fig. S1B). Different tutorial projects available on the website aim to help the user explore the graphic user interface of “The Protein Imager” (Fig. S1C).

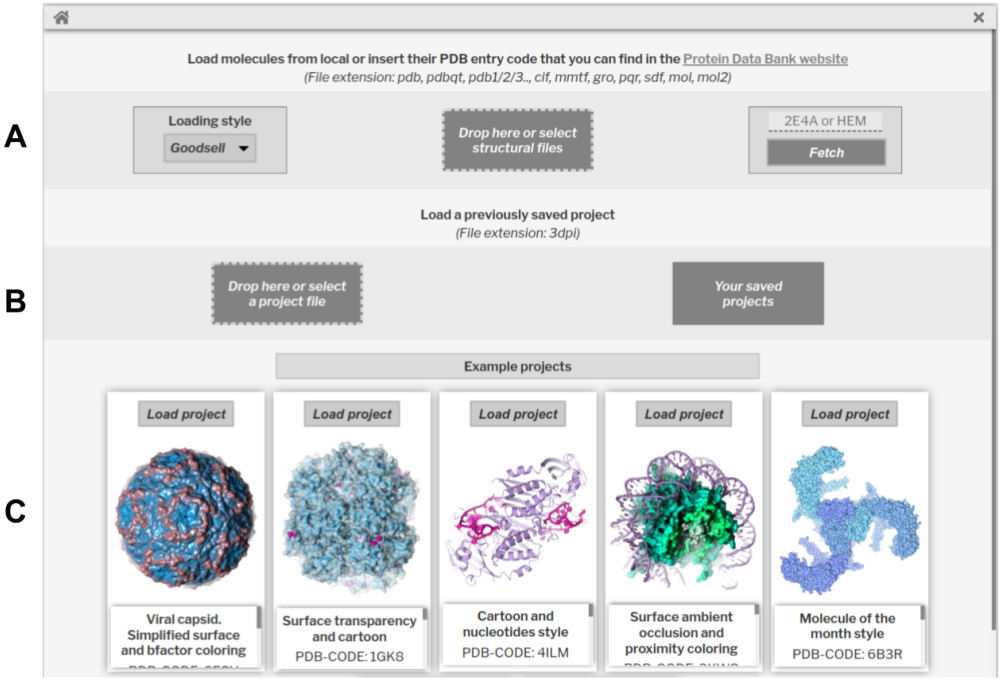

Fig. S1. The files & projects loading window. *The main interface for loading molecular structures, user’s projects, or sample projects.*

2. The graphical interface

“The Protein Imager” is composed of the **Top Bar** containing the **Main Menu** (Fig. S2A), the interactive **NGL molecular viewer window** (Fig. S2B), which includes a **Bottom panel** (composed of the **Log history**, the **Sequence viewer**, the **Viewing options**, the **Distances** and the **Membrane** subpanels) (Fig. S2C), the **Right panel** (composed of the **Structures**, **Current structure** and **Selections** subpanels) (Fig. S2D), and the **Left panel** (composed of the **Visual selection**, **Structure hierarchy,** and **Advanced selections** subpanels) (Fig. S2E).

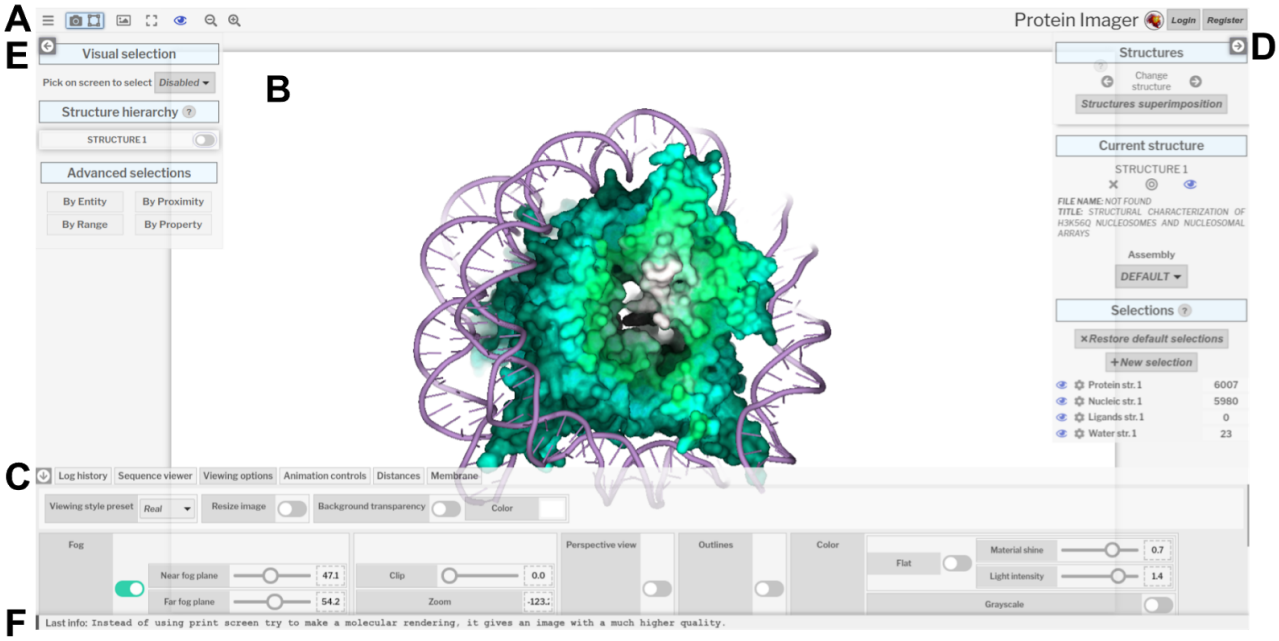

Fig. S2. The Protein Imager Interface. *(A) The menu. (B) The interactive NGL molecular viewer window with (C) the Bottom panel, (D) the Right panel, and (E) the Left panel.*

2.1. The Top Bar and the Main Menu

The Top Bar (Fig. S2A) contains the Main menu, which is accessible by pressing the hamburger icon. Using the **Main Menu**, the user can:

- Open the window to load new structures and projects (Fig. S1).
- Open the "Image rendering options" panel to set the desired molecular rendering options and styles (Fig. S3).
- Request server-side molecular rendering of the current representation (also accessible by a specific icon on the Top Bar).
- Download the requested high-quality renderings (also accessible by a specific icon on the Top Bar) (Fig. S4).
- Manage the user's projects.
- Save projects on the server.
- Download projects on a local computer.
- Reinitialize the current project.
- Open the tutorial page.
- Return to the home page.
- Send feedback.

Specific icons on the **Top Bar** allow the user to toggle the Full Screen Mode, to show/hide the panels, and to increase/decrease the font size.

2.2. The interactive NGL molecular viewer window

The interactive **NGL molecular viewer window** is an interactive **canvas** showing the currently loaded structures by the desired representations (Fig. S2B). The user can directly interact with **NGL molecular viewer window** to highlight the representations on the **Right Panel** and show atomic information in the **Bottom Panel** (by pressing the left mouse button), to center the view on a desired atom (by pressing the middle mouse button) or to rotate, translate and zoom the currently loaded structures. The **NGL molecular viewer window** is also used to modify selections (see paragraph 2.5.1), to position the membrane representation (see paragraph 2.3.6) and to pick atom pairs for calculating distances (see paragraph 2.3.5).

2.3. The bottom panel of the NGL molecular viewer window

The **Bottom Panel** contains five subpanels (Fig. S2C) and a text line presenting the latest information based on the user actions (Fig. S2F).

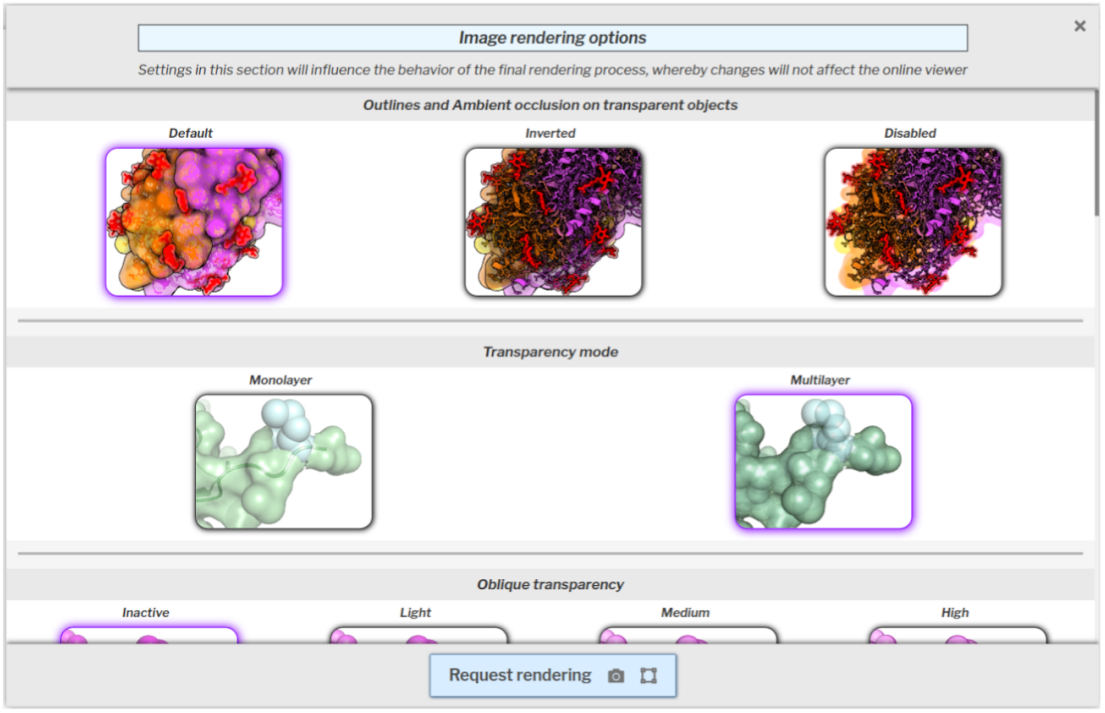

Fig. S3. Rendering Options Panel. *Preferred settings for the server-side molecular rendering procedure can be chosen on this panel. Available settings allow to: toggle transparent background, alter transparent objects' appearance and interaction, alter the mesh thickness, manage lighting effects and shadows, etc.*

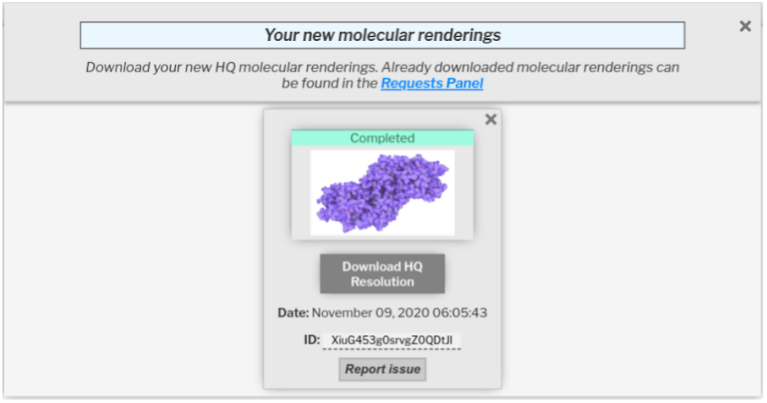

Fig. S4. Molecular renderings Window. *This panel shows the high-quality rendered projects that can be downloaded. The panel is automatically updated any time a rendering request is completed.*

2.3.1. The **Log history** subpanel shows (in chronological order) all notifications displayed to the user during the current session in the text line (Fig. S5A).

2.3.2. The **Sequence viewer** subpanel (enabled by default) shows the sequence of a specific protein or nucleic acid chain of the loaded structure(s) and highlights (in cyan) the active selection (Fig. S6E). Residues or nucleotides directly selected on the sequence (by a click-and-drag action) are added to the active selection (or removed, if they are already part of the selection itself) (Fig. S5B). Pressing the middle mouse button on a specific residue centers the view on that residue.

2.3.3. The **Viewing options** subpanel can be used to modify parameters that globally affect the view. In detail, the user can i) choose between different viewing styles presets ("Real", "Goodsell" or "Outlines only"), ii) change the canvas width/height ratio, iii) modify the position of the near and far fog planes, iv) change the front clipping plane and the zoom level to slice the macromolecule, v) switch between orthographic or perspective view, vi) apply flat coloring, modify the material sheen, modify light intensity, apply grayscale coloring, set background color and transparency, set interior color (for spheres and sticks representation), vii) apply outlines, switch to outlines-only style, change outlines color, sensitivity and thickness, apply shadowing effect and modify its parameters (see also paragraph S2.6) (Fig. S5C).

2.3.4. The **Animation controls** subpanel can be used to apply basic animations (rock and spin) to the whole scene (Fig. S5D).

2.3.5. The **Distances** subpanel is used to measure the distance between two atoms directly by selecting them in the **NGL molecular viewer window**. The size and color of labels and connectors can also be customized (Fig. S5E).

2.3.6. The **Membrane** subpanel allows placement of a membrane representation in the **NGL molecular viewer window** (Fig. S5F). The placed membrane is shaped like a circular bilayer made of small spheres (Fig. S12). The membrane is originally restrained in the **NGL molecular viewer window** allowing it to be placed correctly by moving the protein structure with respect to the membrane. The membrane can be easily unrestrained using a switch that appears on the **NGL molecular viewer window**. Different options are available in the **Membrane** subpanel to alter the membrane radius, the bilayer spacing, the grid spacing, the spheres size and the color of each layer.

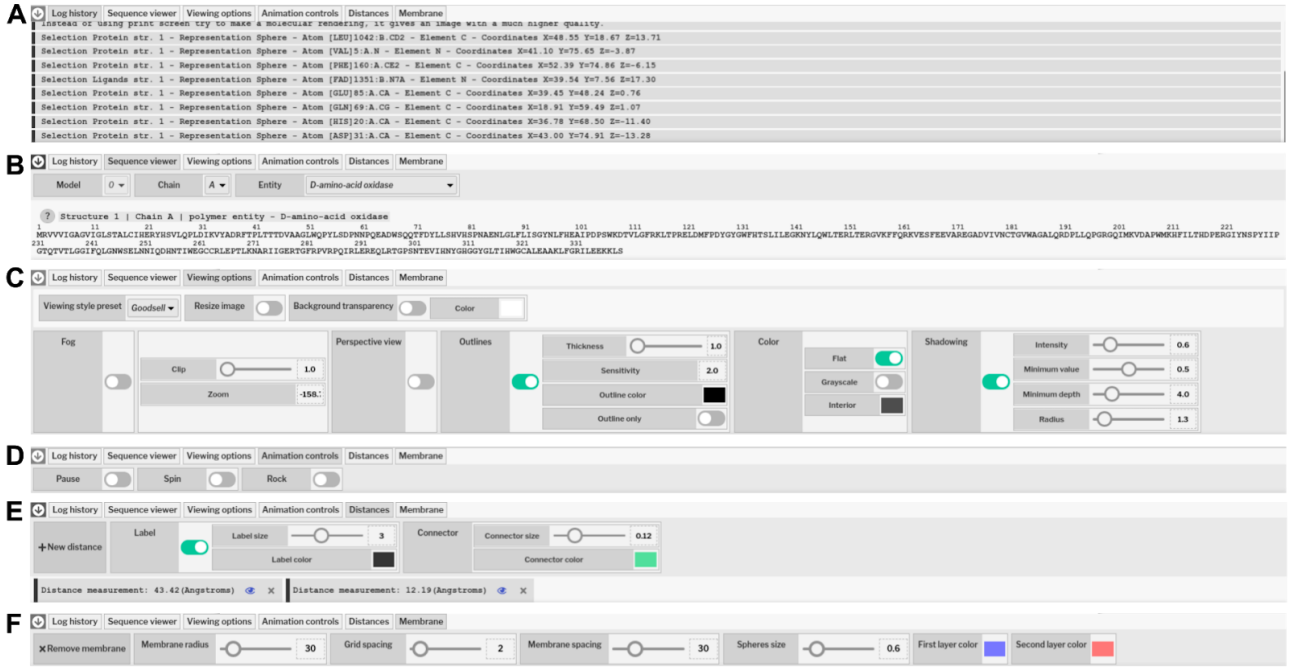

Fig. S5. The bottom panel of the NGL molecular viewer window. *(A) The* ***Log history*** *subpanel. (B) The* ***Sequence viewer*** *subpanel. (C) The global* ***Viewing options*** *subpanel. (D) The* ***Animation controls*** *subpanel. (E) The* ***Distances*** *subpanel. (F) The* ***Membrane*** *subpanel. For details on the function of each subpanel, see paragraph 2.3.*

2.4. The Right Panel

2.4.1. At the **Structures** subpanel the user can switch between molecular structures that are loaded on the interface (Fig. S6A) and perform structural superimpositions between different structures (Fig. S6AB).

2.4.2. Using the **Current structure** subpanel the current structure can be centered, hidden, or deleted and the desired biological unit (BU) of the crystallographic structure assembly selected (Fig. S6C)**.**

2.4.3. Using the **Selections** subpanel new selections can be created, or default selections restored. A list of the current selections is shown. Selections can be visualized or hidden by clicking on the eye icon next to the selection name (Fig.

S6D).

Left clicking on the selection name (or on the adjacent "gear" icon) activates the selection and reveals the visualization options (cyan background, Fig. S6E). Self-explanatory icons allow to center, copy, or remove elements of the corresponding selection. The available representation modes are listed in Tab. S1. The structural entities belonging to the active selection are also shown (in cyan) in the **Structure hierarchy** subpanel on the **Left panel** (see paragraph 2.5.2 and Fig. S9B) and on the **Sequence viewer** subpanel on **the bottom panel** of the **NGL molecular viewer window** (see paragraph 2.3.2, Fig. S5B).

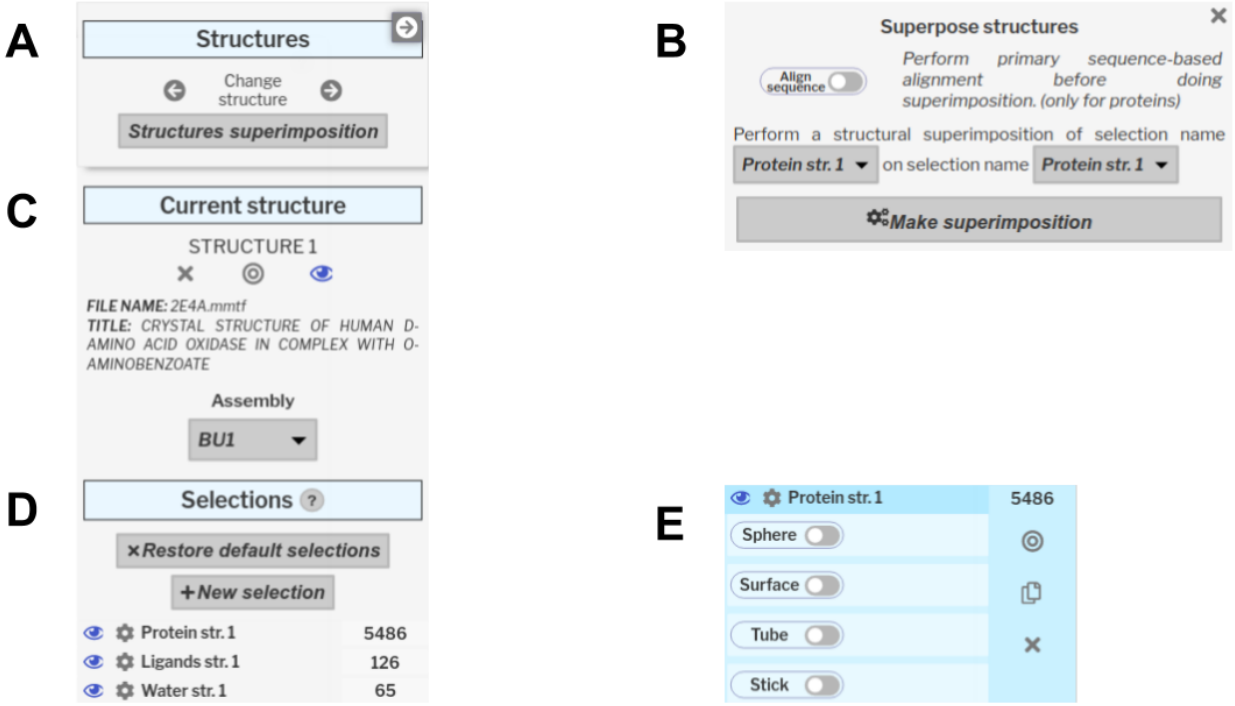

Fig. S6. The Right Panel. *(A) The* ***Structures*** *subpanel and (B) the corresponding* ***Superpose structures*** *window. (C) The* ***Current structure*** *subpanel. (D) The* ***Selections*** *subpanel. (E) The options for the active selection (specific for each selection) that are toggled by left clicking on the selection name or gear icon.*

Different settings are available for each representation method (Fig. S7 and Table S1).
The available coloring methods are (Fig. S8 and Table S1):

- **Goodsell-like.** A specific atomic-type coloring scheme with different shades between two colors is applied (Goodsell D. S., 2009) (Fig. S8A).
- **Uniform**. A uniform color is applied to all the structural entities of the selection (Fig. S8B).
- **By element.** Colors are applied based on the atomic element type. Each element color can be modified (Fig. S8C).
- **By moiety**. A uniform color is applied on different moieties of the selection based on properties such as the chain name, the residue (or atom) range, number or name, or the type of secondary structure. Multiple coloring methods can be applied to a subset of entities of the same selection (Fig. S8D).
- **Proximity coloring** (only available for sphere and surface representations). Atoms are colored using a color gradient based on the distance from a specific selection or point (Fig. S8E).
- **B-factor.** A custom color gradient is applied to the representation based on the data stored in the temperature factor column of the PDB file (when available) (Fig. S8F).

Table S1. Available representation and coloring methods and corresponding settings.

Representation method	Description	Parameters	Available coloring methods
Sphere	Atoms are represented as a sphere with a size corresponding to their Van der Waals radius.	*Transparency* *Scale*	*Goodsell-like* *By element* *Uniform color* *By B-factor* *By moiety* *By proximity*
Stick	Atoms are shown along with their connections.	*Transparency* *Scale* *Valence* *Ball-stick ratio* *Small hydrogens*	*Goodsell-like,* *By element,* *Uniform color* *By B-factor* *By moiety*
Surface	Atoms are shown as a continuous surface computed according to their atomic Van der Waals radius.	*Transparency* *Mesh* *Filter*[1] *Simplify*	*Goodsell-like,* *By element* *Uniform color* *By B-factor* *By moiety* *By proximity*
Cartoon[2]	The secondary structure of the molecule is shown (coils, α-helices, and β-sheets).	*Transparency* *Smooth sheets* *Show nucleic* *Nucleic color* *Nucleic Size*	*Uniform color* *By B-factor* *By moiety*
Tube[2]	The backbone of the molecule is shown as a tube.	*Transparency* *Cylindrical helices* *Radius type* *Show nucleic* *Nucleic color* *Nucleic size*	*Uniform color* *By B-factor* *By moiety*
Label	Labels correspond to atoms or residues of the selection are shown.	*Method* *Alter residue index* *Depth* *X and Y offset* *Size* *Bold* *Italic* *Outline*	*By element* *Uniform color* *By moiety*

[1] *Using the filter setting only the portion of the surface within a certain distance from another selection is shown.*

[2] *Available only for proteins and nucleic acids.*

2.5. The Left Panel

2.5.1 With the **Visual selection** subpanel structural entities can be added or removed from the active selection (Fig. S6E) on the **NGL molecular viewer window** by directly selecting the desired entity type (atom, residue, chain or model) in the "Pick on screen to select" options dropdown menu (Fig. S9A). Selected entities are shown in a dark color. Clicking on an entity from the active selection removes that entity from the selection. Clicking on a structural entity of an inactive selection will add that specific entity to the active selection.

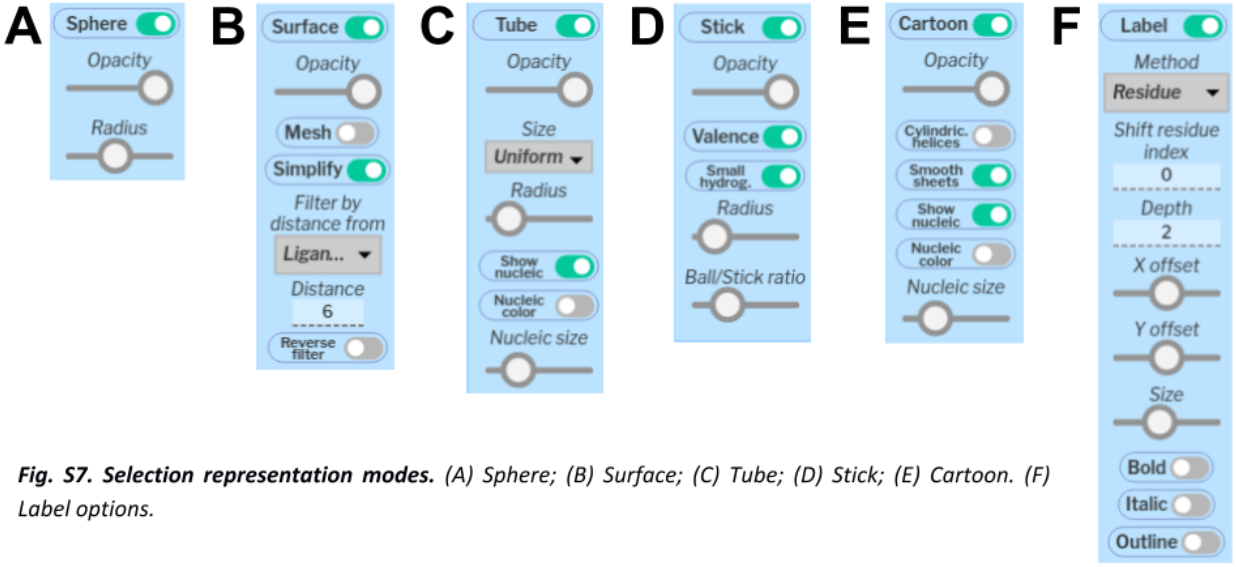

Fig. S7. Selection representation modes. *(A) Sphere; (B) Surface; (C) Tube; (D) Stick; (E) Cartoon. (F) Label options.*

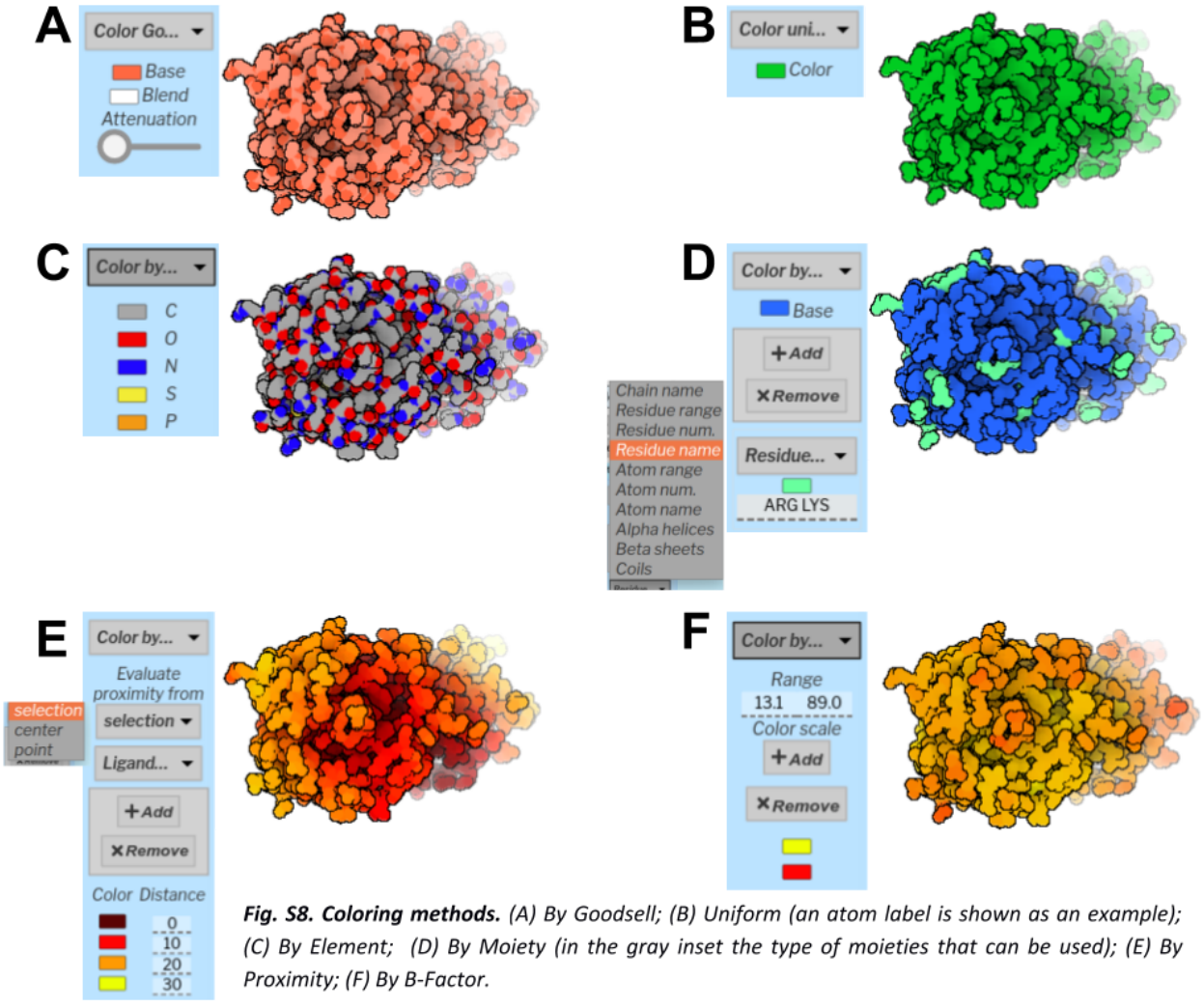

Fig. S8. Coloring methods. *(A) By Goodsell; (B) Uniform (an atom label is shown as an example); (C) By Element; (D) By Moiety (in the gray inset the type of moieties that can be used); (E) By Proximity; (F) By B-Factor.*

2.5.2. The Structure hierarchy subpanel can be used to add or remove individual structural entities (models, chains, residues, or atoms) from the active selection. Multiple structural entities can be selected by pressing the left mouse button and dragging over the entities.

2.5.3. The "Advanced selections" subpanel is a very powerful tool to create (or modify) complex selections based on different criteria ("By Entity", "By Proximity", "By Range", or "By Property"). The unique mode of action of the "Advanced selections" subpanel doesn't require a target selection to be active (Fig. S9C).

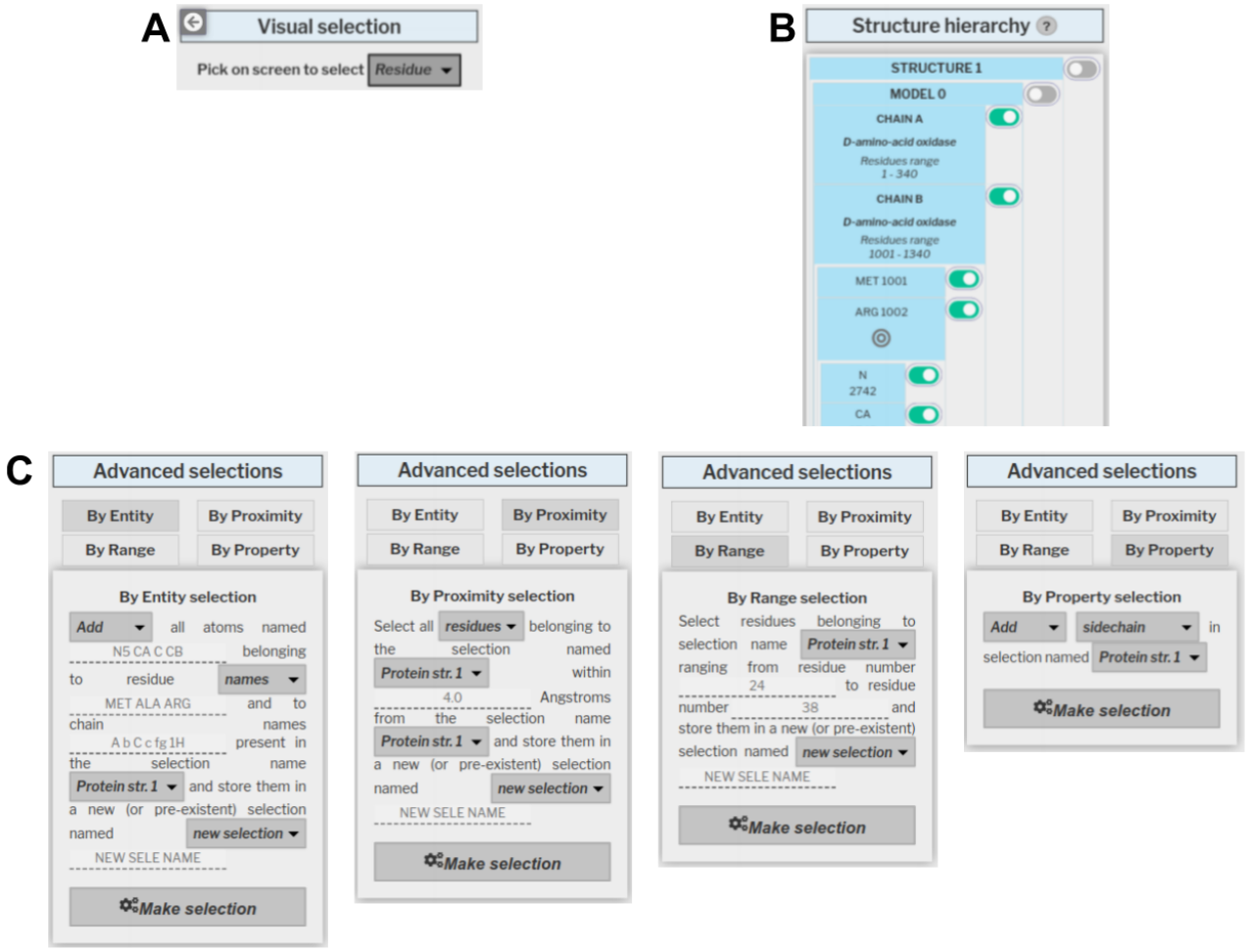

Fig. S9. The Left Panel. *(A) Via* ***Visual selection*** *the active selections can be colored in cyan on the* ***NGL molecular viewer window*** *and the entities (atom, residue, chain, or model) belonging to the active selection can be modified by picking them. (B) Via the* ***Structure Hierarchy*** *subpanel entities belonging to the currently active selection can be inspected and modified (Fig. S6E). (C) With the* ***Advanced selections*** *subpanel new selections can be modified or created using different selection methods: "By Entity", "By Proximity", "By Range", and "By Property".*

2.5.4 Working with NMR structures.

PDB files containing structures solved by NMR usually contain multi-state structures. In "The Protein Imager" each NMR state is stored in a different "MODEL" entity (Fig. S10). Each NMR state can be individually/collectively selected through the **Structure hierarchy** subpanel in the **Left panel** (Fig. S9B).

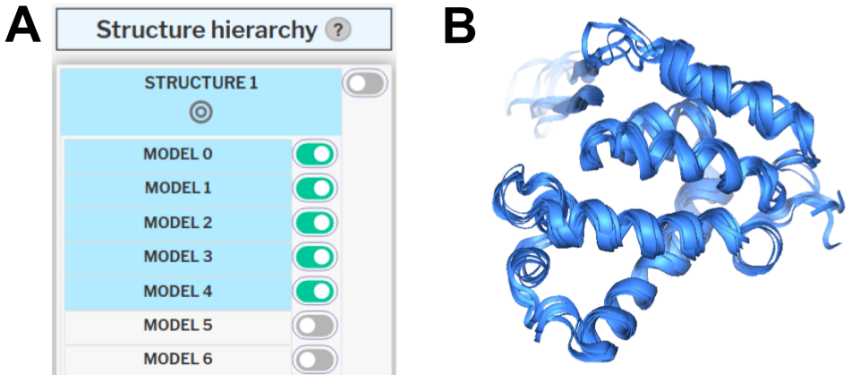

Fig. S10. Multi-state NMR structures. *The* ***Structure hierarchy*** *subpanel (A) can be used to select multi-state structures (B).*

2.6. Render the final illustration

High-quality molecular renderings are produced by launching the server-side rendering process through the **Main menu** (Fig. S11) ("**Request rendering**" -> "**Get Image**" – green button). Rendering parameters are set in the "**Image rendering options**" ("**Request rendering**" -> "**Options**" – light blue button Fig. S3). The canvas can be resized to the desired proportions before starting the rendering through the **Viewing options** subpanel (Fig. S5C).

2.6.1. Request the rendering as 3D Mesh

Is also possible to require the current project in VRML2 format from the **Main menu** ("**Request rendering**" -> "**Get Mesh**" – orange button Fig. S11). This can be useful to export representations geometries and colors that can be used on other 3D graphics tools for illustration or 3D printing purposes.

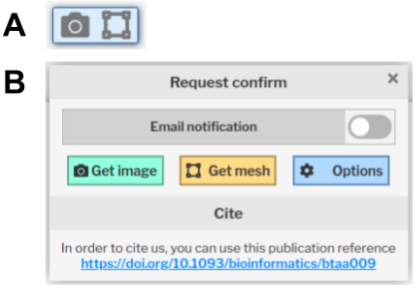

Fig. S11. Rendering & Mesh request confirmation box. *(A) Button in the Main menu to trigger request confirmation box. (B) The request confirmation box. The green button "Get Image" starts the illustration rendering request. Instead, orange button "Get mesh" starts the mesh export request. Blue button "Options" shows the* ***Rendering options panel*** *(Fig. S3).*

User is notified when the rendering procedure is complete by a pop-up message on "The Protein Imager" interface and, if the corresponding option has been selected, also by email. The rendered high-quality rendering can be downloaded through the "**Your new molecular renderings**" window (Fig. S4), which can be accessed from the "**Your requests**" item of the **Main menu** or from the icon in the **Top Bar** (see paragraph S2.1). Examples of high-quality representations are shown in Fig. S12).

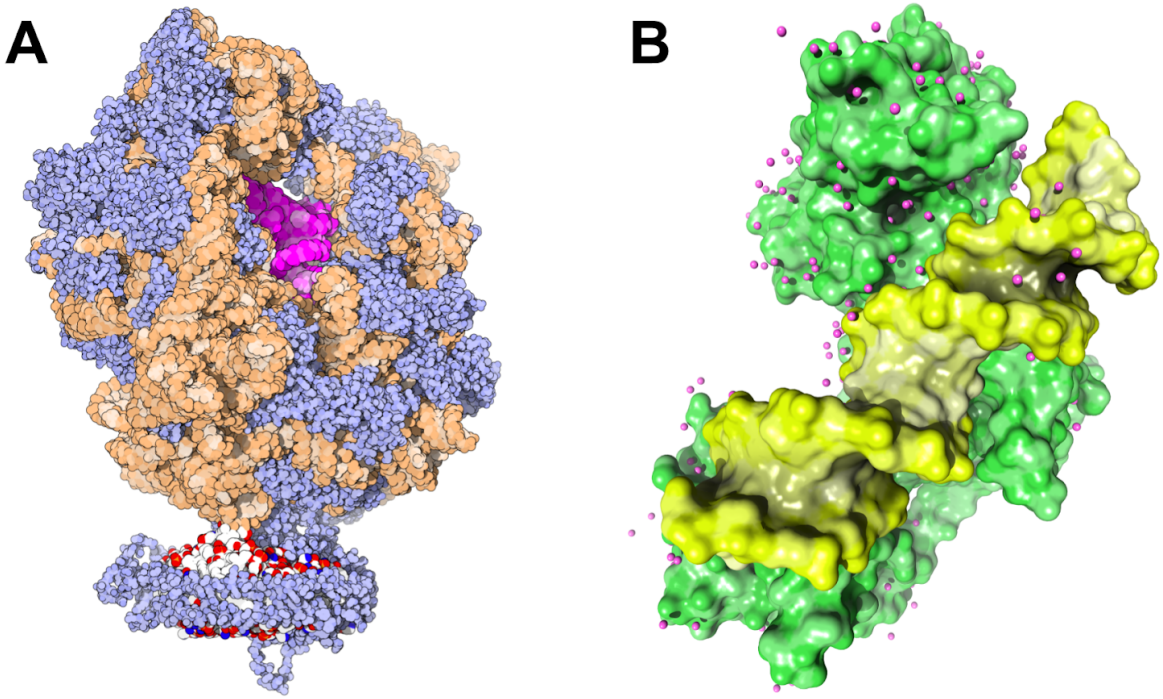

Fig. S12. High-quality rendered illustrations of macromolecules. (A) Structure of the ribosome-SecYE complex in the membrane environment (PDB code: 4V6M). (B) Tumor suppressor p53 complexed with DNA (PDB code: 1TUP).

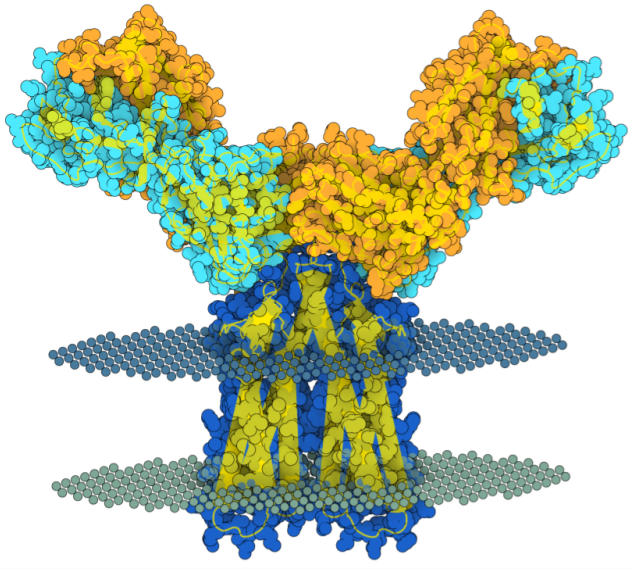

Fig. S13. Example of membrane protein with added membrane. *Structure of full-length CD20 in complex with Rituximab Fab (PDB code: 6Y90).*

References

Goodsell,D.S. (2009) The machinery of life. *New York: Springer-Verlag*.

Berman,H.M. *et al.,* (2000) The Protein Data Bank Nucleic Acids Research, **28**: 235-242. *URL: www. rcsb. org Citation*