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, or SDF) 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.](image)

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).
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 a 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 2.3.5) and to pick atom pairs for calculating distances (see paragraph 2.3.4).
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).

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. S2A).

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 gray scale coloring, set background color, set interior color (for spheres and sticks representation), vii) apply outlines, change outlines color, apply shadowing effect and modify its parameters (see also paragraph S2.6) (Fig. S5C).

2.3.4. 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. S5D).

2.3.5. The **Membrane** subpanel allows to place a membrane representation in the **NGL molecular viewer window**. The placed membrane is shaped like a circular bilayer made of small spheres. The membrane is originally restrained in the **NGL molecular viewer window** allowing it to be placed correctly by moving the protein structure 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.

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![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 Distances subpanel. (E) The Membrane subpanel. For details on the function of each subpanel, see paragraph 2.3.](image-url)
The Protein Imager

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.](image)

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).

![Fig. S7. Selection representation modes.](image)

- (A) Sphere; (B) Surface; (C) Tube; (D) Stick; (E) Cartoon. (F) Label options.

![Fig. S8. Coloring methods.](image)

- (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. 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.

#### 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).

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

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

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.
2.5.4 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. 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”.

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 (“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).

The 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. S1).

2.6.1. Request the rendering as 3D Mesh

It is also possible to require the current project in VRML2 format from the Main menu (“Request rendering” -> “Get Mesh” – orange button). This can be useful to export representations geometries and colors that can be used on other 3D graphics tools and also for 3D Printing.

Fig. S11. 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).

References
