

eMovie: a storyboard-based tool for making molecular movies

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The 3D structures of macromolecules are difficult to grasp and also to communicate. By their nature, movies or animations are particularly useful for highlighting key features by offering a ‘guided tour’ of structures and conformation changes. However, high-quality movies are rarely seen because they are currently difficult and time consuming to make. By adopting the traditional movie ‘storyboard’ concept, which gives guidance and direction to filming, eMovie makes the creation of lengthy molecular animations much easier. This tool is a plug-in for the open-source molecular graphics program PyMOL, and enables experts and novices alike to produce informative and high-quality molecular animations.

Introduction

Soon after the first 3D structures of biological macromolecules were determined, it became clear that visualization and comparison of these structures is crucial to understand their structure and function. The field of molecular graphics was pioneered by Cyrus Levinthal in the 1960s [1], the ‘3D effect’ being achieved by rotating the macromolecular structure constantly on the screen, like a ‘real-time’ movie. The largest drawback was that the computer system – a specialized and extremely large Digital Equipment Computer dedicated to this task, nicknamed the ‘Kluge’ – cost well over US\$300 000. However, during the past 40 years, both hardware and software have progressed enormously (<http://www.umass.edu/microbio/rasmol/history.htm#tams>), making it possible to accomplish much more on a standard desktop computer than was ever possible using the Kluge. A few notable examples of molecular visualization programs that have taken advantage of the improved technology include Kinemage [2], the RasMol-based [3] 3DBrowser [4,5] and ProteinExplorer (http://www.umass.edu/microbio/chime/pe_beta/pe/protexpl/frntdoor.htm), and the jMol-based (<http://www.jmol.org>) FirstGlance (<http://molvis.sdsc.edu/fgj/index.htm>).

Recently, there has been a trend of animating 3D structures in short movies, as exhibited by the creation of PDB2MultiGIF [6], MovieMaker [7], ‘Morph Server’ [8],

CCP4mg [9], iSee [10], the animation features of PyMOL (<http://pymol.sourceforge.net>), and VMD [11]. Molecular movies, as shown dramatically by Jones, Kleywegt and Olson [12,13] (http://alpha2.bmc.uu.se/usf/mol_morph.html), lend a degree of three-dimensionality – which is impossible in a 2D picture – to the displayed molecules through movements of the viewing angle. Movies are also much more demonstrative than pictures, especially in illustrating transitions between multiple states of a molecule. In addition, they can be used to highlight structural features, model chemical reactions, compare structures and show docking events. Such movies can function as ‘guided tours’ of a molecule, engaging the audience through motion and dynamic change, and appealing to scientists and non-scientists alike.

Some of the current tools enable the creation of simple molecular animations, such as rotating a PDB structure (e.g. PDB2MultiGIF and MovieMaker). CCP4mg and MorphServer are used to focus on transitions between conformations, and iSee provides a library of prepared molecular structures that can be browsed interactively. VMD can be used to create animated molecules resulting from a molecular dynamics trajectory. These tools are fairly intuitive and easy to use, but their functionality is limited.

Here, we present ‘eMovie’ – a tool for making the process of molecular movie creation more fluid and natural. It is more similar to traditional movie-editing programs and can be used to create extended, complex animations. eMovie introduces a storyboard to the world of molecular animation in addition to modular, insertable actions. Thus, the user can focus on the scientific story rather than the technicalities of animation.

eMovie has been created as a plug-in for the molecular display program PyMOL. PyMOL combines superior picture quality with a highly advanced scripting interface, including versatile animation commands that can create long, complex movies; however, accessing the animation commands through the command line is difficult and error-prone. A new user is faced with a steep learning curve given the complicated animation commands and the necessity for writing external scripts. eMovie resolves these issues by providing a simple graphical interface through which the user can interact with the powerful movie-making capabilities of PyMOL without needing familiarity with commands, syntax or external scripts.

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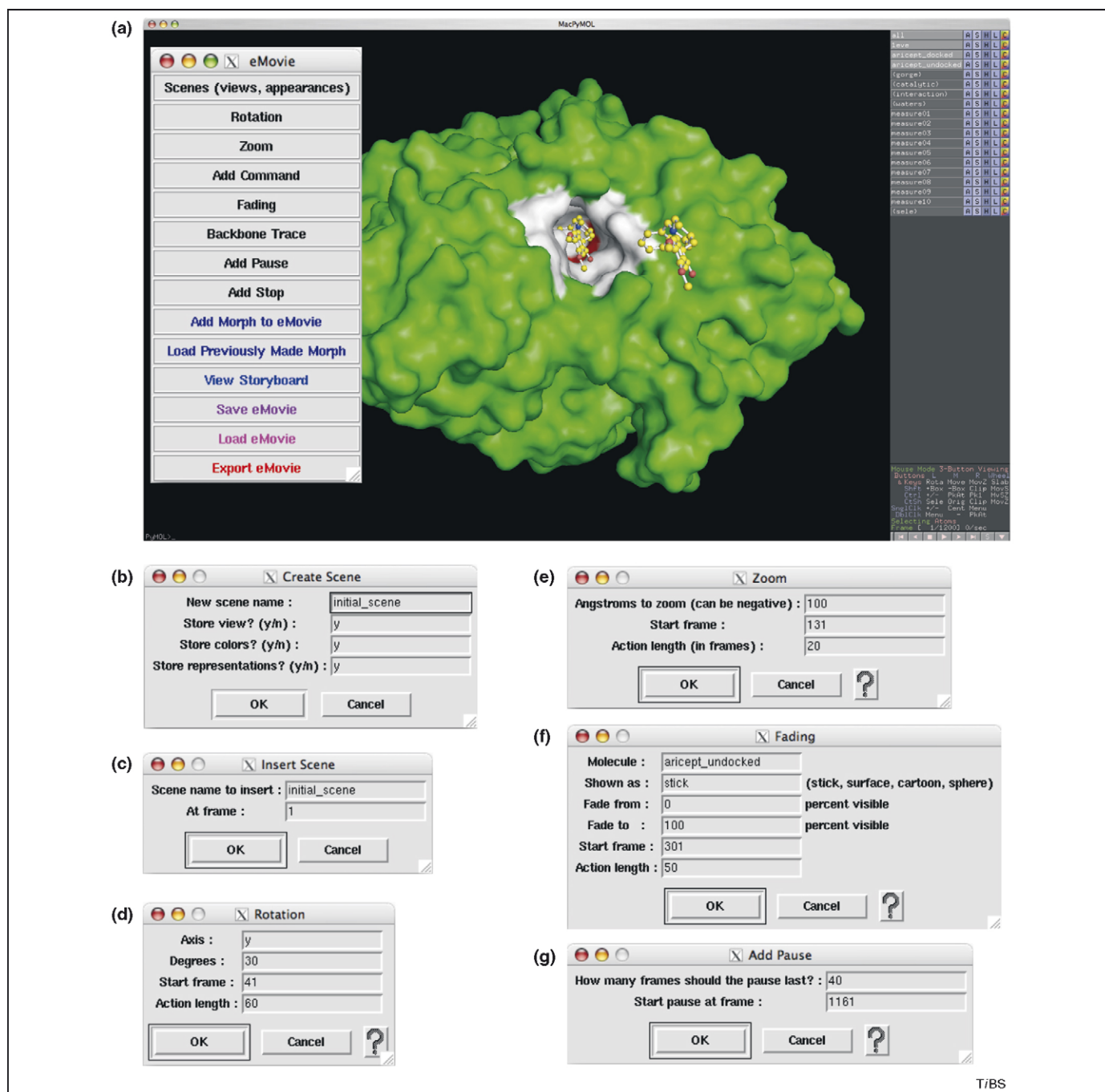


Figure 1. Snapshot of eMovie in action. Docking of Aricept[®], a key Alzheimer's drug, to AChE. (a) The eMovie menu bar (see Box 1 for descriptions of functions) with the overall, all-inclusive PyMOL session. (b) Creating a new scene that saves the current view, colors and representations as a scene entitled 'initial_scene'. (c) Inserting the created scene at frame 1. (d) Inserting a rotation about the y-axis of 30 degrees starting at frame 41 and lasting 60 frames. (e) Insertion of a 100-Å zoom at frame 131 lasting 20 frames. (f) Fading in of the stick representation of the aricept_docked object from 0% visible to 100% visible starting at frame 301 and lasting 50 frames. (g) Inserting a 40-frame pause at the end of the movie.

eMovie in action

eMovie runs on Macintosh OS X (the X11 version, i.e. PyMOLX11Hybrid)*, UNIX/LINUX and Windows. The user is presented with several buttons for interactive movie production, and instructive help buttons assist

* Switching from the Macintosh version of PyMOL (MacPyMOL) to the X11 version of PyMOL (PyMOLX11Hybrid) is simply accomplished by renaming the filename of MacPyMOL as PyMOLX11Hybrid (switching the name back reverts from PyMOLX11Hybrid to MacPyMOL). The authors find it useful to keep a copy of both versions on the Mac.

first-time users (Figure 1a and Box 1). eMovie expects the user to be familiar with PyMOL and its graphical user interface (GUI), which is mostly self-explanatory. In PyMOL, each movie is treated as an ordered sequence of frames (pictures), to which particular actions are assigned. eMovie enables the user to enter actions in a modular way, generally by defining the starting point, the duration in frames, and the type of event that is required. Each action appears on an editable storyboard.

Box 1. Main functions of eMovie

The buttons of the eMovie menu bar are listed here with a brief explanation of their functions.

Scenes (views, appearances): manage PyMOL scenes, including creating new scenes, recalling existing scenes, deleting scenes and inserting scenes into the movie.

Rotation: insert camera-angle rotations.

Zoom: insert either a zooming in or out action.

Add Command: insert a PyMOL command to be executed during a specified frame.

Fading: fade a molecule or selection in or out.

Backbone Trace: insert a C α backbone trace of a protein, which adds one residue at a time to the trace. The trace takes a rainbow color, ranging from blue (N terminal) to red (C terminal).

Add Pause: insert a pause into the movie, which is often most useful at the end of movies.

Add Stop: insert a stop command at a specific point in the movie that automatically stops the movie when reached. The movie can then be restarted using the PyMOL GUI. This is particularly useful for live presentations.

Make Morph: create a morph (an interpolated transition between two states) between two molecules. (This button is hidden if the version of PyMOL used does not have the RigiMOL module.)

Add Morph to eMovie: after a morph has been created using 'Make Morph', it can be inserted at any frame and played forwards, backwards or in a loop (forwards and then backwards).

Load Previously Made Morph: load a morph from a file into the movie. The morph can then be inserted into the movie using 'Add Morph to eMovie'.

View Storyboard: view the current list of actions (the storyboard) comprising the movie. Actions on the storyboard can be selected and deleted at any time.

Save eMovie: save the current movie.

Load eMovie: load a previously saved movie.

Export eMovie: export the movie as a sequence of numbered images (.png) with a specified title.

Creating a Movie

Aricept[®] is a drug that is currently widely used for the symptomatic treatment of Alzheimer's disease. Based on the 'cholinergic hypothesis' [14], Aricept[®] is believed to exert its action by inhibiting the synaptic enzyme acetylcholinesterase (AChE), thus partially alleviating the deficiency in levels of the neural transmitter acetylcholine in the brains of Alzheimer's patients. The 3D structure of the AChE–Aricept[®] complex was determined by X-ray crystallography [15]. It shows that, although Aricept[®] binds tightly to AChE [16] along the entire length of the active-site gorge of AChE, there are no direct hydrogen bonds or salt bridges between the drug and the enzyme. This surprising observation is best illustrated by showing the details of the complex as a simple movie.

To produce a molecular animation using eMovie, the user is advised to go through eight steps for its creation (as follows). The complete movie, the making of which is described here, plus a demonstration of eMovie in use is available as [Supplementary Material](#).

(i) Define what you want to show (a still image might be more appropriate)

This initial step, although not directly connected to the technicalities of movie making, is the most difficult task. A movie has the potential to give a 3D feeling to the molecules and their motions, but bringing a molecular story to life in an appealing way requires thought. In the

example described here, the aim is to show the binding pocket of Aricept[®] from different angles, with the protein and ligand parts clearly distinguishable. It is also important that the proximity of the catalytic site is clear.

(ii) Sketch a rough, conceptual storyboard

The following is the plan for the AChE–Aricept[®] movie (see [Supplementary Material](#)). The movie begins by viewing down the deep gorge of AChE, which is presented in a surface representation, while rocking the molecule back and forth to provide perspective. The view then zooms into the gorge and, again, rocks back and forth. The Aricept[®] molecule (in ball-and-stick representation) fades in, and then zooms out to give the audience a better view. The view is rotated 360° around the Aricept[®] molecule to display its structure. It then zooms again into the gorge of AChE, and the Aricept[®] molecule fades into its docked position in the gorge. Now, the surface representation of AChE fades out to reveal its underlying simple chain representation, highlighting in stick representation the residues that interact with Aricept[®]. The view is then changed so that the gorge is vertical on the screen and is rotated 360° to display the interaction of Aricept[®] with AChE through four water molecule intermediaries. Finally, the initial view of looking down the gorge is re-established, and the surface representation of AChE is faded back in. The catalytic triad, the binding gorge and the residues that interact with Aricept[®] are shown in different colors.

(iii) List the conceptual storyboard as a series of eMovie actions

This step reveals how the conceptual storyboard can be translated into eMovie actions. The planned movie can be created in eMovie using scenes (to help change colors, representations and views), rotations, zooms and a pause.

(iv) Create morphs

The movie used here as an example does not require any morphs; however, it is worth noting that a subscription version of PyMOL is required for the creation of morphs. This is the only function that eMovie lacks without a subscription version of PyMOL containing the RigiMOL morphing module. To create a morph, the user would load into PyMOL the two structures to be morphed. Usage of the 'Make Morph' button leads to the RigiMOL module's creation of the transition between the structures as a sequence of conformations, or morph. A morph previously created using eMovie can be loaded into the current movie by using the 'Load Previously Made Morph' button.

(v) Create one, all-inclusive PyMOL session containing each object and selection to be used in the movie

All necessary molecules and selections necessary should be prepared in PyMOL-proper before adding scenes and actions using eMovie. It must be considered which parts of the scenery need to be defined as individual objects (in some situations, even duplicate objects of the same molecule can be useful).

Now it is time to start PyMOL and load the PDB file, or files, that will be used to make the movie. In this example, we load 1eve.pdb, which contains the crystal structure of AChE with Aricept[®] bound to it. Using the GUI of PyMOL, the bound Aricept[®] molecule is duplicated and dragged out of the AChE gorge into empty space. Thus, there is a bound Aricept[®] object (aricept_docked), and an unbound Aricept[®] object (aricept_undocked). Again using GUI of PyMOL, four selections are made within the AChE object: the catalytic triad, the gorge, the residues that interact with Aricept[®] and the water molecules that interact with Aricept[®]. The GUI is also used to create broken lines between interacting atoms (these are actually measurement objects) (Figures 1a and 2a).

(vi) Store as scenes each distinct combination of representations, colors and views to be presented in the movie

Each distinct combination of representations, colors and views that are planned for the movie are carefully designed, including the initial scene, the docking scenes, the interaction scenes and the final scenes. Each time a distinct combination of representations, colors and views is decided upon for a particular scene, it is saved using the 'Scenes (views, appearances)' button (Figure 1b).

(vii) Insert the action sequence into eMovie. First insert the morphs, and then the rest of the actions

If the planned movie contains a morph, the morph would now be inserted into the movie using the 'Add Morph to

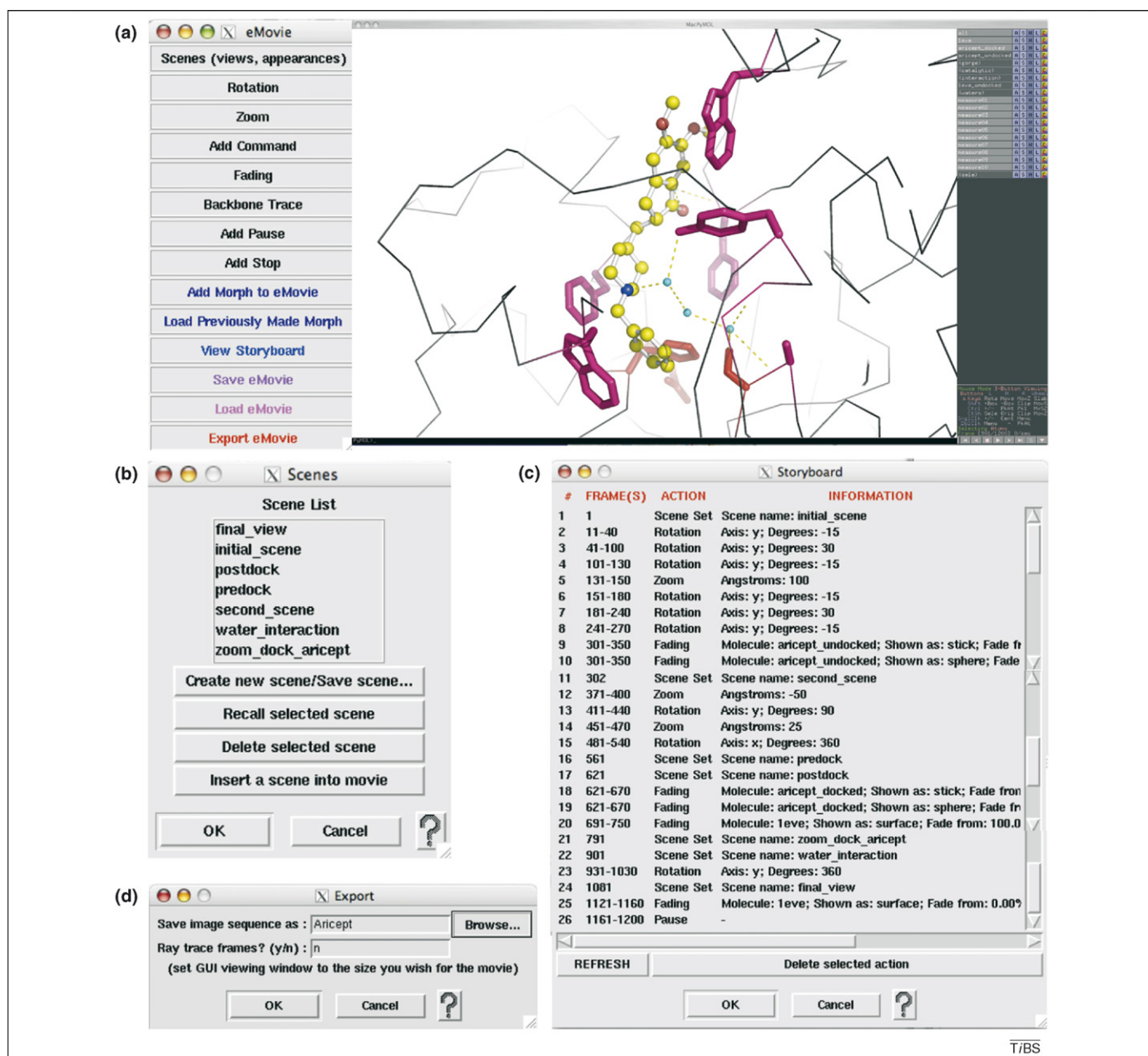


Figure 2. Snapshot of a finished movie. (a) The scene entitled water_interaction with Aricept[®] (yellow ball-and-stick) docked in the AChE gorge (pink and red sticks) through water intermediaries (cyan spheres). (b) The list of scenes used to make the movie, a window opened by clicking on 'Scenes (views, appearances)'. (c) The completed storyboard window, which is opened by clicking 'View Storyboard', showing the 26 steps that comprise the movie. (d) How the movie is exported, using 'Export eMovie', as an image sequence for use in conversion to common movie file format (e.g. .mov, .gif or .mpeg).

eMovie' button, specifying whether the morph should be played forwards, backwards or in a loop (forwards and then backwards). The modular actions are inserted into the movie by first setting the initial scene (Figure 1c). Next, to rock the molecule back and forth, the 'Rotation' button is used to add a rotation at frame 11 of -15° on the y -axis taking 30 frames, followed by a rotation at frame 41 (30 frames later) of 30° on the y -axis taking 60 frames, and a rotation at frame 101 (60 frames later) of -15° on the y -axis taking 30 frames (Figure 1d). Next, to zoom into the gorge and again rock back and forth, the 'Zoom' button is used to add a zoom of 100 \AA at frame 131 taking 20 frames for completion (Figure 1e), and rotations are inserted (as before) to rock the molecule back and forth. At this point, the conceptual planning dictates a fading in of the Aricept[®] molecule. This is accomplished by using the 'Fading' button to insert a fading in of the aricept_undocked object in both the stick and the sphere representation (because it is shown as ball-and-stick) from 0% visible to 100% visible, starting at frame 301 and taking 50 frames (Figure 1f). To display the newly shown Aricept[®] molecule, a zoom of -50 \AA is inserted followed by a rotation along the y -axis of 90° , a zoom of 25 \AA and a rotation along the x -axis of 360° . The pre-docking scene that occurs before Aricept[®] fades into the AChE gorge is first inserted, and then the 'Fading' button is used to enable the aricept_docked object to fade into view. Next, the 'Fading' button is used again to fade out the surface representation of AChE, followed by insertion of a scene showing Aricept[®] vertically positioned in the gorge. A similar scene is inserted a few frames later to highlight the interactions of Aricept[®] with the gorge and the four water molecules. After inserting a 360° rotation about the y -axis to fully display the scene, it is time for the final scene, which has the same viewpoint as the initial scene, and the surface representation of AChE is faded back in. The movie is finished by adding a pause of 40 frames (Figure 1g).

(viii) Review the movie and make adjustments

At this point, the movie is complete. Each scene can be listed using the 'Scenes' button, and each individual action can be viewed on the eMovie storyboard by clicking 'View Storyboard' (Figure 2b,c). Adjustments can be made by deleting individual actions using the 'Delete selected action' button on the storyboard window and reinserting the desired actions. The movie should be saved periodically throughout the creation process and upon completion using 'Save eMovie'.

When the animation is finished, there are two options for playing it in a program other than PyMOL. One option is to use the 'Export eMovie' button (Figure 2d) to export the movie as an image sequence and to subsequently merge the sequence to a common movie format (e.g. .mov, .gif or .mpeg) using programs such as Mencoder (UNIX and Windows), Graphic Converter (Macintosh OS X), Movie Maker (Windows), VideoMach (Windows), Adobe Premiere (Windows), or Berkeley encode_mpeg (UNIX or LINUX). A second option (for Macintosh users) is to save the finished eMovie using the PyMOL 'File/Save Session' feature; this saved session .pse file can then be opened in

MacPyMOL and exported as File/Save_movie/QuickTime. Other programs can be used to apply titles, voice-over, music, labels and end credits.

Concluding remarks

As a tool that facilitates the making of molecular movies, eMovie is a key stop-gap in the niche of intuitive molecular animation. Ideally, the next-generation molecular movie-making software would either be a stand-alone program or an intuitive, powerful GUI built directly into an existing molecular visualization program. This new program would function similarly to modern movie-editing software, incorporating a visual storyboard or timeline that a user can both scroll through and edit through 'drag and drop'. In addition to the capabilities of eMovie, this (hypothetical) new program would combine the following features: (i) facilitation of complex operations such as alterations of parts of the molecules and chemical reactions; (ii) support of automatic docking of multiple objects while avoiding collisions; (iii) freely placeable, visually appealing labels; (iv) inclusion of molecular dynamics trajectories; (v) the option to include, standard, insertable schematic non-molecule objects symbolizing lipid membranes or proteins interacting with unsolved structures; (vi) a zoom out function to display processes on a cellular level; (vii) simple addition of audio commentary; and (viii) seamless interaction with other molecular graphics programs such as CCP4MG and iSee.

The availability of a storyboard-based, molecular movie-making tool such as eMovie has, without doubt, the potential to affect the way science is communicated, as evidenced by the quick adoption of and positive response to the predecessor of eMovie, the movie.py PyMOL plug-in (<http://www.rubor.de/bioinf>). By making the creation of molecular movies more fluid through an editable storyboard, eMovie enables more scientists to take advantage of this media. An unfamiliar user can prepare an illustrative molecular movie for a presentation or a website within hours or even minutes.

eMovie can be downloaded from <http://www.weizmann.ac.il/ISPC/eMovie.html>, and PyMOL can be downloaded from <http://pymol.sourceforge.net>.

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Supplementary data

The movies described here and a brief movie demonstrating eMovie in use can be found online at [doi:10.1016/j.tibs.2007.03.008](https://doi.org/10.1016/j.tibs.2007.03.008).

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Letters

Life on the edge: a link between gene expression levels and aggregation rates of human proteins

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We have found that expression levels of human genes *in vivo* are remarkably anti-correlated with the aggregation rates of the corresponding proteins measured *in vitro* by experiment. This result indicates that human proteins have evolved to resist aggregation and to function efficiently, but with almost no margin of safety to respond to genetic and environmental factors that decrease their solubility or increase their concentration *in vivo*. We speculate that this result provides a compelling reason for the existence of disorders that are associated with protein aggregation, such as Alzheimer's and Parkinson's diseases [1].

Evidence for this conclusion is presented in Figure 1, where the experimental *in vitro* aggregation rates of a set of peptides and proteins are plotted against the *in vivo* expression levels of their respective genes, as assessed using DNA microarray technology [2]. The plot includes all the experimental data we could extract from the literature (Table 1), and the correlation coefficient between expression levels and aggregation rates is an astonishing 0.97.

The existence of such a strong degree of anti-correlation for this set of peptides and proteins indicates that the aggregation propensities of the proteins needed by the cell are precisely tuned by mutation and evolutionary selection to levels that enable them to be functional at the concen-

trations required for optimally efficient performance. It also indicates that protein molecules have co-evolved with their cellular environments to be sufficiently soluble for their biological roles, but not more so. Hence, aggregation can result from even minor changes in the chemistry (e.g. as a result of oxidative stress) and in the regulation (e.g. as a result of changes associated with ageing) of otherwise harmless proteins. Indeed, when proteins are expressed at higher levels than those found naturally, it is unlikely that their aggregation can be avoided, except for relatively short lengths of time.

Table 1. Conditions used to monitor the aggregation rates for the peptide and proteins considered in this work

Name	EL ^a	Rate	pH	IS	Conc.	Refs
Acylphosphatase	0.7	–3.0	5.5	43.0	0.04	[7]
Prion106–126	0.5	–3.5	5.0	1.2	0.3	[8]
Calcitonin	0.9	–3.5	7.4	25.0	1.5	[9]
Tau protein	0.8	–3.6	7.6	50.0	0.004	[10]
Acetylcholinesterase _{586–599}	1.4	–3.7	7.0	7.7	0.2	[11]
Aβ ₄₀ peptide	1.6	–4.6	7.4	81.0	0.03	[12]
Aβ ₄₂ peptide	1.4	–4.3	7.4	81.0	0.01	[12]
Amylin	1.8	–4.5	5.0	0.06	0.001	[13]
Transthyretin	2.1	–5.2	4.4	130.0	0.01	[14]
α-synuclein	2.6	–5.9	7.4	2.0	0.1	[15]
Glucagon	2.8	–6.0	7.0	10.0	0.8	[16]
Pmel17	2.7	–0.1	7.4	100.0	0.01	[4]

^aEL: normalized expression levels [2,17]. Rate: logarithm in base 10 of the aggregation rates measured in seconds. The ionic strength (IS) and the protein concentration (Conc.) are in mM units. See Ref. [1] for further information about the proteins and their links (or absence thereof) with disease.

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