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ORIGINAL PAPER

Teaching Molecular Biology using computational tools and tacking into account the learning styles of students

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Abstract

A challenge in educating undergraduate and graduate students is to introduce them to the Internet, especially to visualization and molecular modeling software, and to teach them how to use these programs in their future research. This paper illustrates a few ways to use computer science for teaching and learning the modeling of biological molecules. The results demonstrate that effective use of molecular visualization can improve students understanding of protein structure and functions, independently of the modalities they use to acquire information. Using the molecular graphic programs, students obtain hands-on experiences to explore the structural aspects of macromolecular systems. All the applications are made for calcium binding proteins. The use of the Internet tools and of the molecular visualization software in biochemistry and molecular biophysics classroom, with their advantages and disadvantages, is discussed.

Keywords: learning styles, active project-based learning, Internet, molecular biology education.

Introduction

Over the past decade an intense use of the Internet and educational software in high schools, colleges and universities has been noted. These methods are constantly developing, involving a considerable effort both for developers and users (or more experienced novices) [1]. For these reasons it is a real challenge to use visualization software and molecular modeling in student education [2]. There are several ways of using computers, we will refer to the online tools to teach and learn modeling and simulation, physicochemical parameters calculation and prediction of structures and functions of the biological molecules [3, 4].

The first items on biological molecules structure are taught in the course of Biochemistry and some of the students are familiar with the computational tool for visualization and investigation of protein structure. Since these programs include many tools, they can be used later in the master cycle for studying molecular mechanics and molecular dynamics of biological macromolecules and/or of their complexes. All these online programs, used properly and responsible, can lead to great results for students, improving the understanding level of the student related to biological molecules structures and functions [5]. It also offers a powerful tool which leads to addressing the problems by using the scientific research method.

Knowing the different learning styles, the question to be answered within this study is how the educational process can be improved, in particular with reference to the course of "Molecular basis of therapeutics action" uniting in an efficient way the learning style, the active methods and the Internet-based programs free available online. During the applicative activities for this course, the active learning based on projects is used. The students are

encouraged to obtain general information about a class of biological molecules and to design a project in which they need to visualize, to model and to study the properties of these molecules. After they establish the goals of this project, the methods and activities, every activity is organized in such a way that they work in groups to achieve the goals of the project. After each didactic sequence, there are discussions concerning the results of the research and the biological meaning of these results. At the end of the semester the results are organized as a report for their project. In this way, we are interested to obtain maximum results, leading to a profound learning for students and last, but not least, they may do their applicative activities with pleasure.

Material and Method

LEARNING STYLES

Learning styles refers of how students learn. There are a variety of models that characterize the modality of human information acquisition. We can mention some prominent schools that have studied and classified the learning styles [6, 7]: Dunn & Dunn's environmental preferences, VARK classification, Gardner's multiple intelligences, Kolb's experiential learning cycle, Honey & Mumford's learning types and Gregorc's mind style. It is generally agreed that an understanding of the dominant learning preference will be useful in designing effective instructional strategies to facilitate learning and to capitalize on the individual's potential.

***Dunn & Dunn's environmental preferences*:** the three basic perceptual learning styles are visual**,** verbal and kinesthetic/tactile**.** Visual learners learn using visual displays like notes, pictures, diagrams, write down key points and follow written instructions. Auditory (verbal) learners learn from verbal lectures, discussions, they participate with pleasure in class debates and are good in speeches and presentations. Kinesthetic/tactile learners learn through doing and touching and, generally, they use hands-on approaches to explore the physical world around them.

VARK learning styles: there are four basic learning styles: visual/auditory, read/write, kinesthetic/tactile and a combination of them, the multimodal style. Read/write learners take the information from manuals, textbooks, notes and write definitions, list, and exam answers. Multimodal learners (60%) can adopt the mode being used or requested.

Gardner's Multiple Intelligences considers seven intelligences: linguistic/ verbal, spatial/visual, bodily/kinesthetic, logical/mathematical, musical, interpersonal, intrapersonal and it represent a more holistic approach to learning in the real world.

Honey & Mumford's Learning Model sorts people in four learner types: activists, reflectors, theorists and pragmatists.

In ***Gregorc's Mind Styles*****,** people are sort along two continua: abstract—concrete and sequential—random.

If we refer to the VARK classification [6], specialized studies show that 60% of students take a multimodal learning style, 20% adopt mostly visual style, other styles being adopted by less than 10% (we refer to young people between 18 and 30 years) [8].

Knowing the different learning styles, the question to be answered is how the educational process can be improved, in particular with reference to course "Molecular basis of therapeutics action". We consider this topic because, according to its analytical program, it addresses to the various ways of gaining information by students. The study in cooperation by groups of students is used and students are involved in an active learning [9, 10]. Perhaps the best used method would be project based learning, which involves the collaboration of students in carrying out and presenting a project [11]. Based on course schedule mentioned

above, students are encouraged to seek online structural data banks and computational tools for modeling and visualization of biological molecules, to use them to achieve their project. Using the Internet and the online software for visualization and investigation of structural and dynamic features of biological molecules in the elaboration of a draft may be one of the most effective methods of active learning and may lead students to a professional approach in a manner and a rhythm of their own, of the studied topic. It should be noted that in the course mentioned above, the issues are, in their great majority, presented in the form of slides which involve a maximum impact on students who learn visual. Oral lectures and classical courses are used, as well as written material.

Active project-based learning using on-line molecular graphic programs

Knowing the biological molecules structures leads to important information related to their functions and mechanisms of action [12]. The protein structure can be determined by crystallographic and NMR methods and the obtained models are stored in structural data banks [13, 14]. Once the structure of protein is obtained, different software to determine the molecular properties can be used: energetic or electrostatic calculations, determination of geometric parameters (molecular surface, radius of gyration, volume), determination of cavities and functional sites, docking points of various drugs respectively. Thus it is important to refine different regions in search of some more flexible or more rigid regions. There is a multitude of online programs that can perform the studies mentioned above. To find them we can use either the usual search engines (Google for example) or we can use articles based on this issue. In the latter case items become scientific learning material [15]. Without whishing to enumerate all existing online programs, we can still remember some of the programs discovered and used by students in biomolecular sciences:

- ***Data banks***: RCSB (PDB) [13], SWISS PROT[14], Cambridge Structure Database[16];
- ***Visualization and modeling tools***: PyMol[17], RasMol[18], Swiss-PdbViewer[19], VMD[20], Kinemage[21], JMol [22], MolMol[23], First Glance[24], PPG [25];
- ***Primary structure analysis*****:** similarity search (BLAST [26]), pair wise sequence alignment (PyMol [17], JAligner [27]), multiple sequence alignment (CLUSTALW [28], T-Coffee [29], FSA [30], KALIGN [31]), pattern search (ScanProsite [32], MotifScan [33]), computation of physicochemical parameters (ProtParam, ProtScale [34]);
- ***Secondary and tertiary structure prediction tools:*** SOSUI [35], Geno3D [36];
- ***Electrostatic interactions:*** PCE (Protein Continuum Electrostatic) [37], Protein Dipole Moments Server [38];
- ***Functional sites, druggable pockets, protein docking:*** Qgrid [39], SCREEN [40], SitesBase [41], JCB Protein-Protein Interaction Website [42].

We illustrate the use of these programs for studying calcium binding proteins (CABPs) structural and dynamical properties. They are proteins that bind calcium ions to perform catalytic activity or to stabilize their conformations. Usually these proteins contain a so called “EF hand” region (helix-loop-helix region). The EF-hand CaBPs were grouped into two categories: calcium buffers (that bind calcium to transport or regulate the concentration) and calcium sensors (which bind calcium to decode its signal). Between these EF-hand categories, there are structural and functional differences. Calcium sensors usually present an extended tertiary structure and are conformational sensitive to calcium binding and, in opposite, calcium buffers present a compact structure and are much less conformational sensitive to calcium binding [43].

Results and Discussions

After the students browse the scientific literature concerning calcium binding proteins structures and dynamics and they establish the goals of their project, they are encouraged to find web pages of visualization and molecular modeling tools. All the applications are made for some of CaBPs, usually for calmodulin (CaM), which is a calcium sensor. CaM mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, intracellular movement, short-term and long-term memory, nerve growth and the immune response. Many of the proteins that CaM binds are unable to bind calcium themselves, and they use CaM as a calcium sensor and signal transducer. Calmodulin is a small, acidic protein approximately 148 amino acids long [43].

The students find available structures in structural data banks (such as PDB), both *apo* and *calcium loaded* structures of CaBPs, some examples of codes entry being 1CMF, 1OSA, 2SCP, 1CLL, 3CNL. There are also some structures of mutants, examples of CaM mutants code entries being 1AHR, 1CKK. The students investigate a lot of CaBPs and make several visualizations of them.

For the human CaM, using free accessible data bases and computational tools, they find the following information:

PDB entry code 1CLL (*isoform 1*, *Homo sapiens,* full length protein, pH 5 Crystal structure, 1.7 angstrom resolution, R-value = 21.6, 4 Ca ions bound).

SWISSPROT code P62158.

Using similarity search tool BLAST [26] the students identify the sequences having a high similarity with that of human CaM. A part of the BLAST output file for this case is shown in the figure 1.

```
sp P62155CALM_XENLA Calmodulin (CaM)[calm1] [Xenopus laevis (A...  296 2e-79
sp P62151CALM_TORCA Calmodulin (CaM)[Torpedo californica (Paci...  296 2e-79
sp Q6YNX6CALM_SHEEP Calmodulin (CaM)[CALM2] [Ovis aries (Sheep)]   296 2e-79
sp P62161CALM_RAT   Calmodulin (CaM)[Calm1] [Rattus norvegicus (...296 2e-79
sp P62160CALM_RABIT Calmodulin (CaM)[CALM] [Oryctolagus cunicu...  296 2e-79
sp Q5RAD2CALM_PONAB Calmodulin (CaM)[CALM] [Pongo abelii (Suma...  296 2e-79
sp Q71UH6CALM_PERFV Calmodulin (CaM)[calm] [Perca flavescens (...  296 2e-79
sp P62156CALM_ONCSP Calmodulin (CaM)[calm] [Oncorhynchus sp. (...  296 2e-79
sp P62204CALM_MOUSE Calmodulin (CaM)[Calm1] [Mus musculus (Mou...  296 2e-79
sp P62158CALM_HUMAN Calmodulin (CaM)[CALM1] [Homo sapiens (Hum...  296 2e-79
```

Fig. 1. A fragment of BLAST OUTPUT for human CaM

All the proteins presented in the figure 1 are CaMs belonging to different organisms, as it is specified in the first six columns. We notice high alignment scores (column 7) and the probability the sequence similarity to be random is very small (column 8).

Using multiple sequence alignment tool CLUSTALW [28] the students may illustrate the conserved positions of amino acids. A fragment of the sequence alignment for a few CaBPs is shown in the figure 2.

```
1OSA_A|PDBID|CHAIN|SEQ-EAFKVFDRDGNGLISAAELRHVMTNLGEKLTDDEVDEMIREADIDGDGH 135
1CLM_A|PDBID|CHAIN|SEQ-EAFKVFDRDGNGLISAAELRHVMTNLGEKLTDDEVDEMIREADIDGDGH 135
1CLL_A|PDBID|CHAIN|SEQ-EAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQ 135
1CMF_A|PDBID|CHAIN|SEQ-EAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQ 60
3CLN_A|PDBID|CHAIN|SEQ-EAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREANIDGDGQ 135
1AHR_A|PDBID|CHAIN|SEQ-EAFRVFDKDGNGFISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQ 133
2SCP_A|PDBID|CHAIN|SEQPLFFRAVDTNEDNNISRDEYGIFFGMLG--LDKTMAPASFDAIDTNNDGL 144
                        *:..* : :. **  *   .:  **  * .  .   :   : :.**
```

Fig. 2. A fragment of the sequence alignment made for a few CaBPs. The stars corresponds to the totally conserved amino acids, the double points show the replacement of an amino acids by another with a similar physical property and the point shows a substitution of an amino acid by another which has not a totally opposite physical property.

Using the entire alignment profile for these CaBPs, it may be noticed that the calcium binding regions are very well conserved (data not shown). The identification of these regions may be used in other applications, such as domains identification and/or prediction of the secondary and tertiary structures.

Using ProtParam [34] tool, the students identify the physicochemical parameters of CaBPs. For the human CaM some of these parameters are: number of amino acids 149, molecular weight 16837.5 Da, theoretical pI 4.09, Instability index 28.21 (and this classifies the protein as stable), grand average of hydropathicity -0.654. This information is useful in combination with other applications.

Using ProtScale [34] tool, the students may analyze how a physical property varies along the protein chains of CaBPs. In the figure 3 we illustrate the hydrophobicity variation along the human centrin 2 chain using Kyte and Doolittle scale. Within this picture we may notice the predicted hydrophobic (more than -1 on the values of y axis) and hydrophilic regions of the protein. It is an important tool because the hydrophobic interactions are strongly involved in the protein folding and stabilization. In the figure 3 we notice predicted hydrophobic clusters in the 37-61, 82-97, 105-115 and 120-135 regions of the chain. This result is in good agreement with known structural data showing that these regions are located in the interior of the N-terminal and C-terminal domains of the protein (PDB code 2GGM).

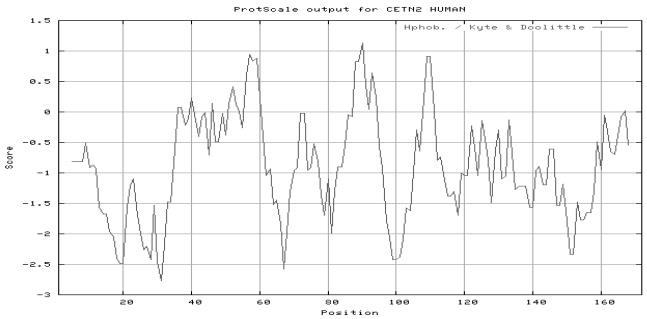
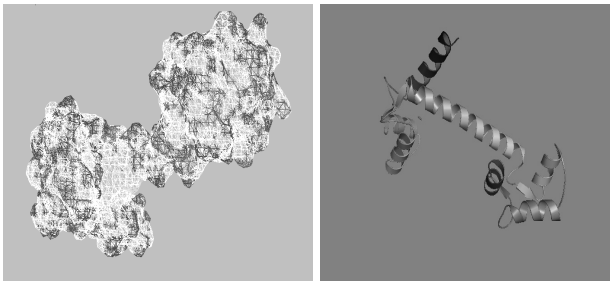


Fig. 3. Hydropaticity variation along the chain of human centrin 2. The Kyte and Doolittle scale for hydrophaticity has been used.

Using visualization tools, the student may obtain different views for a protein. They may visualize the protein surface (figure 4.a), the protein backbone, the protein tertiary structure illustrating also its content in secondary structural elements (figure 4.b), and the electrostatic potential distribution and so on.



(a) **(b)**

Fig. 4. - (a) Computed molecular surface obtained with SPdb-Viewer [19] for CALM_HUMAN created using Geno3D (1CLL);
(b) Tertiary structure of human CALM_HUMAN obtained with JMol [22]

These tools also allow some molecular mechanics and dynamics studies, such as minimization, calculation of the electrostatic potential, calculation of the molecular surface, superposition and fitting of structures, etc.

The results obtained by students concerning CaBPs structural features are correlated with their structural classification and biological function and they are presented as a report of the scientific project. The students were motivated to perform the activities and to achieve the goals and they were delighted to obtain results in good correlations with other data presented in specific literature. Their opinion after finishing this course was that project based learning was the most interesting way to do applicative activities in their studying period.

In the working groups we have observed that each student has exploited its own style of learning, but also adopted information gathered by colleagues independently of the way in which they were originally processed. The visual learners had the greatest benefits, but also kinesthetic/tactile learners for them being a real pleasure to look for programs and to learn to use them. Read/write learners were able to acquire information in the style of their own, reading textbooks, online articles, etc. Perhaps these tools have been found the less attractive by the verbal learners, but they have benefited fully from discussions with colleagues and had a major contribution to the oral presentation of the project. From the perspective of other learning modes, we can say that each of them has been found more ore less in these activities.

Although in this case learning was to a great extent influenced by the own style, we cannot say that keeping permanently account of the learning style of every student is a decisive fact for the success in the process of teaching. As it was shown in other studies [1-3, 5, 7-8, 10], the use of active, content appropriate, varied methods of teaching/learning is recommended.

Conclusions

There is a growing trend toward the use of computational procedures to model laboratory results before actual physical experimentation. The methods presented here may be used to predict 3D structures, potential protein-protein interaction sites, druggable pockets, and other molecular functions.

The use of the Internet and online programs presents certain advantages and disadvantages. Several advantages are:

- they are very actual for researches in the field and allow to obtain detailed information for every structural level of biological molecule;
- the resources are available anytime and anywhere;
- they allow a student-centered learning environment;
- they allow the use of learning strategies that are aligned with a student modality preferences;
- information being accessed using student learning preferences strategies can be understood better and can be motivating.

Some disadvantages are:

- the molecular modeling does not stand for the experiment itself;
- using the Internet and the educational software is beneficial for the students who have a visual and kinesthetic learning style, and is not so effective for those who have an auditory learning style;
- the heterogeneity of students group, their levels in biomolecular sciences were very different, their computer skills were totally unsatisfactory (with only a few exceptions) and the students competences in English were weak.

Even if computational methods are useful both for learning and research, we must underline that software cannot replace a good lecturer and a simulated experiment cannot replace a *real* laboratory experience. They represent additional, powerful pedagogic vehicles that complement others. Once effective software has been used in teaching and learning, it becomes an indispensable tool. They can be used to engage the student intellect, to motivate learning, to allow students to learn in their own style, to make learning interesting and fun.

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