Development of models pieces to represent amino acid and to building protein structures: evaluation by graduation students and teachers from public schools of São Paulo State.

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ABSTRACT

It was developed a set of plastic pieces that allow the assembly and representation of the most common amino acids, as well as the construction of secondary protein structures. During and after development the material was submitted to several stages of evaluation by teachers and students from graduate, undergraduate and high schools levels. The first step was the development of models in the computing environment, followed by prototyping of parts, discussion with the scientific community. Molds for thermoplastic injection were designed, detailed and constructed under our supervision. Parts representing the structures of amino acids and bonds were produced in large scale and it was started the final process of evaluation. The pieces had good geometric relationships with the structural formulas of amino acids obtained from databases and textbooks. After to assemble amino acids units it is possible to build a polypeptide chain and, through hydrogen bonding, is possible to obtain the main secondary structures (α-helix and β-structures). The results of all evaluations were extremely positive and it is important to highlight the amount and content of approving comments for the potential of use of the material. The material may even assist to solve conceptual gaps that exist in teacher instruction and that were observed during the evaluation activities. This set of pieces was submitted to the analysis of Brazilian Education Ministerial (MEC) and certified by this organization.

Keywords: Amino Acids Models, Protein Models, Biochemistry Education, Plastic Models.

INTRODUCTION

The comprehension of structure and function of proteins has a tight relationship with the development of structural biology. Concerning to the learning process and future knowledge utilization, it is essential the possibility of effective three-dimensional structure visualization since the beginning of scholar education. However, students usually have difficulty to visualize the biomolecule structures when only the schematic drawings of didactic books or computer figures are used. Even more elaborated figures made with computational resources do not permit all the necessary structures understanding. The representation of three-dimensional structures of some biomolecules with handling models, built with representative units, have supplied the students and teachers (of all educational levels) a successfully experience to better visualize the structures and correlate them to the real molecules [1]. The present work will show the development of models which began with a detailed study of the structures of the 20 main amino acids [2], their assembly to build amino acid, protein primary and secondary structures, and the evaluation in the teaching/learning process by graduation students from biomolecular physics area and natural science teachers from public schools of São Paulo state. The following criteria were taken into account to the development of the models: a) pieces should provide easy handling; b) the geometric representation of the chemical structure of the Cα, amine, carboxyl, hydrogen, and side chains of amino acids should be designed in order to facilitate understanding of them; c) the scale of the models should be approximately 1nm = 1 cm; d) the primary structure obtained with the representative units of amino acids should represent the peptide bonds with their characteristic rigidity and angles Φ and Ψ; e) after obtain the peptide sequence, the model should allow the assembly of secondary structures like helices, beta-structures and turns; f) the plastic models should demonstrate the interaction among the functional groups, such as polarity, electro negativity, mass, that should by represented by colors, shapes, appropriate sizes,

facilitating the assembly and interpretation of the 3-D structure. After industrial pieces production there were assembled as a kit denoted “Building amino acid molecules and secondary proteins structures” and evaluation process in two steps, by graduate students and high school teachers using specific assessment questionnaires [3].

METHODS

The three-dimensional structures of amino acids in the pattern of balls and sticks, were obtained from the Protein Data Bank (PDB) [4] and the visualization and measurement made with RasMol program [5]. To create the representative side chains units we sought a view that could be adapted to a flat two-dimensional representation. Computer Aided Design (CAD) software used was Blender software, version 2.39, which is distributed free by Blender Foundation [6]. Using computational modeling it was possible to view parts of the models in sizes, colors and forms of assembly interactively. Models were designed for Cα, amine group, carboxyl group, with a stick that represents the peptide bound, hydrogen, side chains, hydrogen and disulfide bounds. All the pieces are produced in different colors. According to Salmoria et al [7] and Karania and Kazmer [8], during the development of new products the manufacture of thermoplastic mold is one the most critical stage due to high costs and long time required to manufacturing. Considering this aspect, a prototyping step was made using fused deposition modeling (FDM) and water cutting process. After prototypes evaluation, the models were adjusted and four thermoplastic injection molds were developed. The pieces that represent the amino acid lateral chains, hydrogen, hydrogen and disulfide bounds, were manufactured using the CAM (Computer Aided Manufacturing) software Power Mill 8.0 in a "high speed" milling machine (HERMLE - I model C800V and command CNC SIEMENS 840), in the Optimization of Production Laboratory (OPL), of campus USP São Carlos. The other molds were constructed in specialized company under our technical support. The plastic pieces were made of polypropylene thermoplastic due to the flexibility of this material. Finally, the pieces were organized as a kit to be used by teachers and students of all teaching levels.

The evaluation data presented in this paper are related to the courses carried out with: a) 26 graduated students (course denoted “Biomolecules: structure and function”) from PhD course of Physics, sub area Biomolecular Physics; b) 256 teachers from elementary and secondary public schools of the fields of the Natural Sciences. This latter course, called “Structural Molecular Biology and its relations with Biotechnology”, was offered to teachers that are coordinators of pedagogical workshops (TCPW) related to the mentioned fields, and work along with the 91 Departments of Education of the Agency of Education of the State of São Paulo, being responsible for advising and training elementary and secondary teachers registered at these departments. The courses included lectures and/or video conferences on themes related to structural biology and biotechnology, presentation, utilization, and evaluation of instructional resources intended to be used on teaching of such themes, as developed by our group [9, 10, 11, 12]. These evaluations were carried out through a written questionnaire containing questions (open and multiple-choice) about specific contents presented in the course, as well as about how teachers can use the instructional material proposed in the course. In both courses, the stage of formulation of the evaluation questionnaires and the treatment of the answers obtained were based on Gil [3] and Bogdan and Biklen [13].

RESULTS AND DISCUSSION

Representative pieces

The development of all models was made in a 3D CAD environment. The start point was the definition of a suitable design for the Cα, model that is fundamental to make the connections and to simulate correctly the bond angles among the atoms. Several configurations were tested for this model and for each one it was checked its capacity to simulate the bonds while keeping the model set viable under the mold design point of view point. The final model pieces are showed in the figure 1. The configuration used for Cα was able to simplify the side chains and also the models of amine and carboxyl presented in the figure 1. These last models have an identifier according to the configuration adopted during the peptide bond. Should be noted the square connector of the carboxyl that fits at a right position of the amine unit. This square connection permits to simulate the double bond character that avoids the free rotation

of the peptide bond. For other hand, the hole near the identifier is used to make the hydrogen bonds.

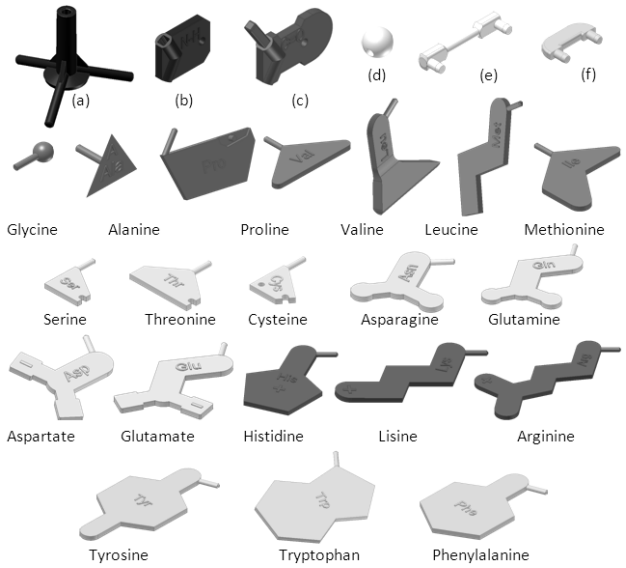

Figure 1 – Developed models: Cα ((a), amine group (b), carboxyl group (c), hydrogen (d), hydrogen bound (e), disulfide bound (f) and side chains.

The figure 2 shows the logic used to design the side chain models, that is, figures of low complexity which present similarity with the ball and stick models. In the same figure, it is also observed the artifice to identify the side chains of positive and negatively charged groups.

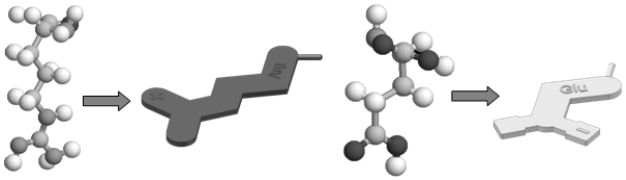

Figure 2 – Logic used to design the side chain models and polarity identifier signal.

Building the amino acids units

Using the developed units it is possible to assembly all the amino acids, for example, the leucine and proline, which uses a special unit to represent the pyrrolidine ring of proline (figure 3). Except by the peptide bond, all the bonds permit to rotate the units. Thus, it is possible to simulate the steric hindrance of the chains.

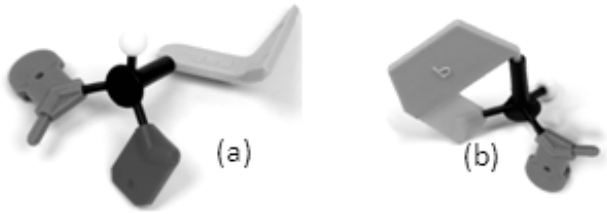

Figure 3 – Examples of amino acids assembling: Leucine (a) and Proline (b)

The amino acids residues can be linked to each other by peptide bond producing the primary structure of protein. The disulfide bond model is used to join two cystein side chains (figure 4).

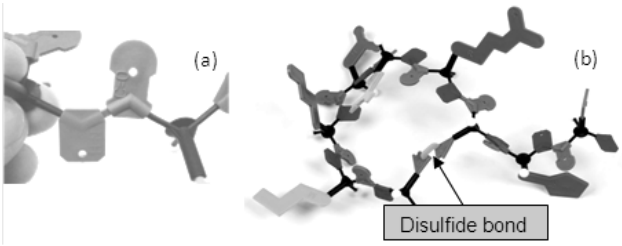

Figure 4 – Peptide bond (a) and primary structure with a disulfide bond (b)

Considering the plan of Cα, were lies the lateral chain bond and hydrogen, there are two possible positions of amino group and carboxyl group. However, in nature, the most common form is the L-amino acid, represented in figure 5. Thus, with the lateral chain pointed up, the following sequence in clockwise direction (H–CO– NH) must be followed to correctly assembly the secondary structures.

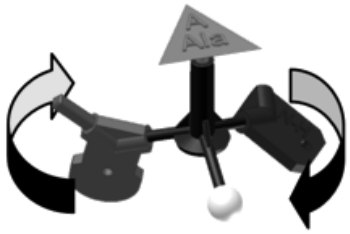

Figure 5 – Correct assembly of L-amino acid.

Building 3-D secondary protein structures

In order to assemble an α-helix it is necessary to consider the information indicated in the figure 6.

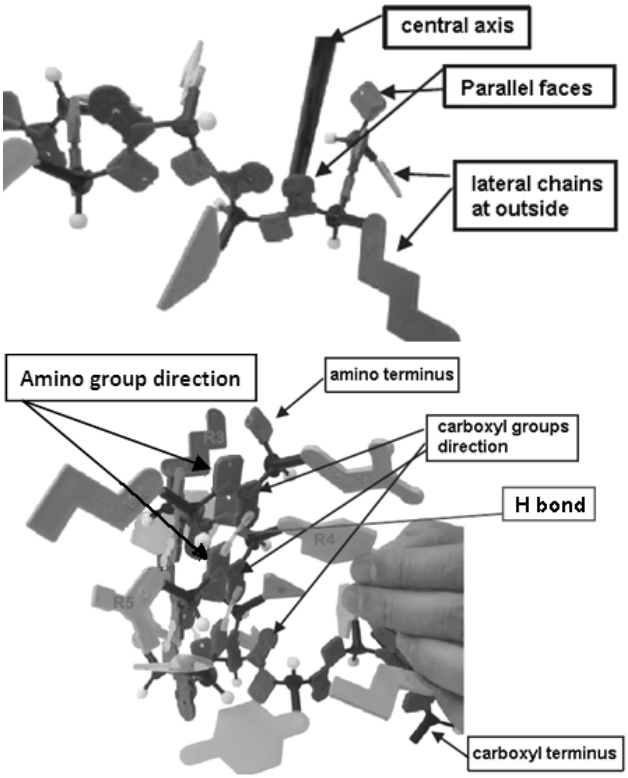

Figure 6 – Steps to assembly an α-helix.

The assembly is made using a primary structure and following the steps:

1. Consider a imaginary central axis to start the assembling;
2. Rotate the amino acid residues until the amino and carboxyl groups be parallel to the axis;
3. The side chain must be at the outside of the helix;
4. Amino groups are oriented to amino terminus of the helix;
5. Carboxyl groups are oriented to carboxyl terminus of the helix;
6. Fix hydrogen bond between the amine of the residue 1 (R1) and the carboxyl of the residue 5 (R5);
7. At the sequence, one hydrogen bond is added at each pairing carboxyl-amine;
8. The result will be any helix with a triangular shape and peptide bonds at the faces of the triangle, figure 7.

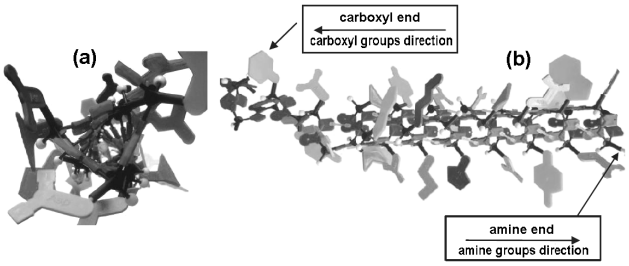

Figure 7 – α-helix: top view (a); side view (b).

The assembling of β structures always begins with the alignment of carboxyl and amino groups at 180° from each other, a zig-zag configuration named β-strand, figure 8a. Making a β-turn and fixing the units which represent the hydrogen bond it is obtained a β-sheet with two strands, an structure termed a ladder, figure 8b. Adding new β-turns at the same primary structure and using the hydrogen bonds models it results in an anti-parallel β-sheet, figure 7c.

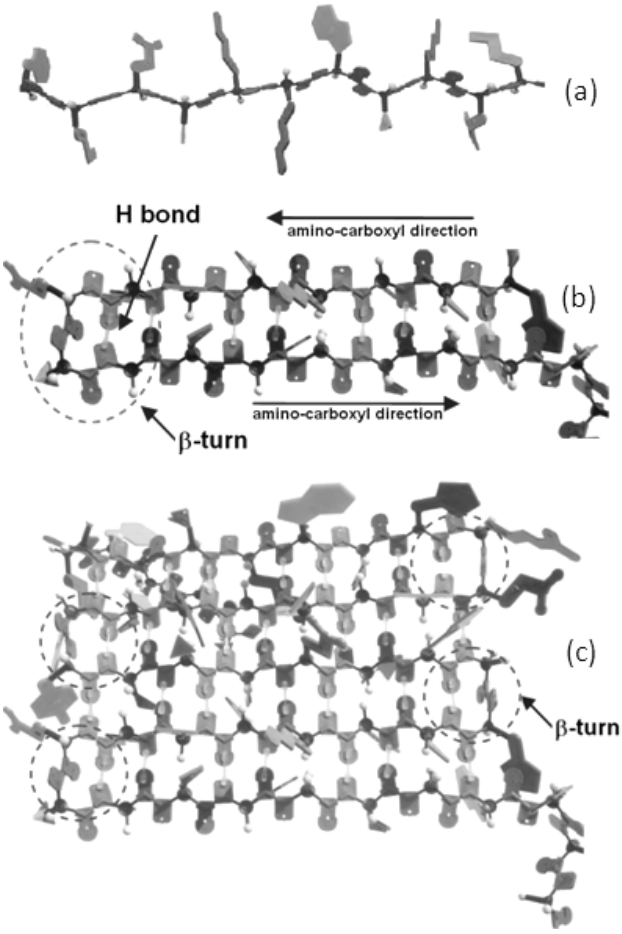

Figure 8 – β-strand (a); ladder (b); β-sheet (c)

Evaluation

The table 1 shows the opinion of 26 graduation students of Biomolecular Physics course from Physiscs Institute of São Carlos, University of São

Paulo (IFSC-USP). Some of the students had knowledge in biochemistry and a few already taught the subject at high school and undergraduates courses.

Table 1: Opinion of graduate students at Biomolecular Physics course from IFSC-USP about the kit "Building Amino Acids and Proteins" as teaching tool.

Evaluated Aspects	Totally agree	Partially agree	Neutral	Partially disagree
Is it suitable for a reduced number of teaching hours?	57	39	4	0
Can it make learning more attractive?	91	9	0	0
Can it encourage reflexive reasoning?	74	26	0	0
Can it arouse curiosity?	78	22	0	0
Can it facilitate learning of the fundamental concepts of amino acids?	61	30	9	0
Can it make the classes more dynamic?	83	13	4	0
This first contact with the models intensified the knowledge about the three-dimensional structure of amino acid or secondary structure of proteins.	48	22	26	4

Data are expressed as percentage of all questionnaires.

Bossolan at al. [13] showed the perception teachers from Natural Science fields have on the use of instructional strategies that make use of models to represent biomolecules. The data showed that the teachers approved the use of 3-D biomolecular and pointed out some advantages and obstacles to the use of such materials.

The questions below are examples of those used during the material evaluation with 256 teachers from São Paulo State public schools. The questions explore the reaction of the teachers concerning to the knowledge improvement of biomolecules structures after work with the amino acids models.

Questions:
1) Have the visualization of a β-sheet segment, assembled with the models, improved your understanding of this secondary structure?

2) Have the visualization of a α-helix segment, assembled with the models, improved your understanding of this secondary structure?

3) Have the utilization of the kit models improved your understanding of the RNA translation in the protein synthesis process?

The figure 9 shows the answers for the three questions.

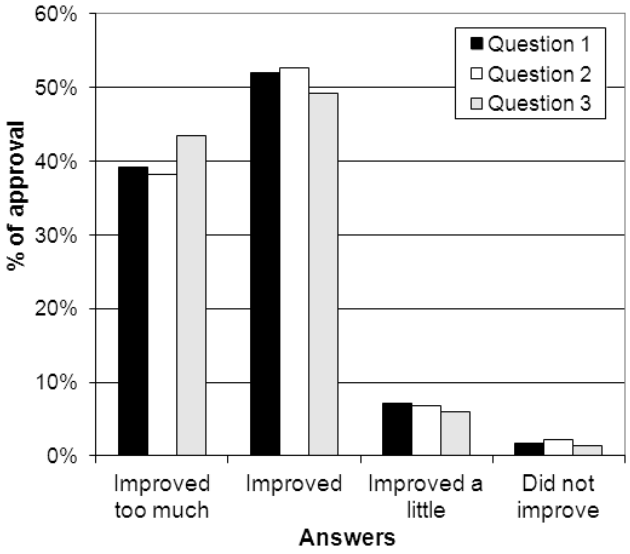

Figure 9 – Answers of the teachers about the knowledge improvement after models utilization.

Both evaluation results, with graduation students and high school teachers, were very grateful and showed that this kit can help teachers make learning of this topic more attractive, awake students' curiosity and promote a dynamic in the classes.

CONCLUSIONS

The main challenge of this work was the integration of scientific knowledge, different technical areas (computer designer, mold design/construction, plastic injections, etc.) and science education. Besides these aspects, development of molds is usually made by companies with focus in market share and profits. The development of this educational tool has been conducted by our group as a consequence of a hard work in teaching/learning area at the Centre for Structural Molecular Biotechnology (CBME) research group. In this sense, this work represented a contribution to teaching process in structural biology and biotechnology area, and provided a useful tool to learning process. This kit represents a new teaching tool because there are not similar patents available in this area which contain the aspects developed on it. This set of pieces, arranged in the form of the kit "Building Structures of Amino Acids and Proteins", was submitted to the analysis of Brazilian Education Ministerial (MEC) and certified by this organization,

starting to integrate the Guide of Educational Technology 2009.

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