SHORT COMMUNICATION

The use of Balasubramanian plots in the analysis of protein dynamics data

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A method for viewing the changes in protein mainchain dihedral angles is described. This facility is used in the analysis of protein molecular dynamics simulations. Each conformation resulting from the dynamics simulation has a Balasubramanian plot calculated. These 2D schematic plots are then viewed on the Winchester Graphics System, using the animation facility. This results in a 'movie' of the simulation being displayed.

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The molecular dynamics methodology has been described elsewhere[1,2] and is not repeated here. A molecular dynamics simulation of a protein results in a large number of sequential conformations. These conformations are normally modelled at regular time intervals, usually in the order of 0.001ps. A method of viewing these data using an interactive animation facility, has previously been described[3]. This facility allows the researcher to set up display lists for successive conformations on a mainframe computer. These frames can then be sent to a graphics stroke device at a rate that gives the viewer the impression of watching a film. At the same time, the viewpoint can be altered in realtime using I/O devices such as the tablet and pen and 3D joystick. The speed of animation is also under the control of the viewer, using programmed function keys. It is straightforward for the researcher to partition the data by using a relational database approach. This enables the researcher to look at fragments of the molecule of interest or to extract the mainchain so that the overall molecular motion can be observed.

Although this animation facility allows researchers to see the molecule vibrating, it does not give enough information about the nature of the motion. In particular it is very difficult to see which atoms move in a cooperative manner. In order to highlight these properties, a strategy of animating schematic plots has been adopted. The most successful of these schematic representations are the distance matrices and difference distance matrices[4]. Animation of these 3D representations allows researchers to recognize cooperative motion and other features. An interest in the nature of energy exchange through α helices presented the problem of analysing the changes in protein dihedral angles during a simulation. In order to view these changes, we began investigating animation of other types of schematic plots. This resulted in the addition of Balasubramanian plots to the animation facility.

BALASUBRAMANIAN PLOTS

Figure 1 shows part of a peptide mainchain and its dihedral angles. As the peptide bond is planar, these angles are important because they represent the only degrees of freedom of the protein mainchain. Rotation about the N–Cα bond corresponds to changing the ϕ angle and rotation about the Cα–C' bond corresponds to changing the ψ angle. The definition of the dihedral angles (whose conformation corresponds to 0° rotation)

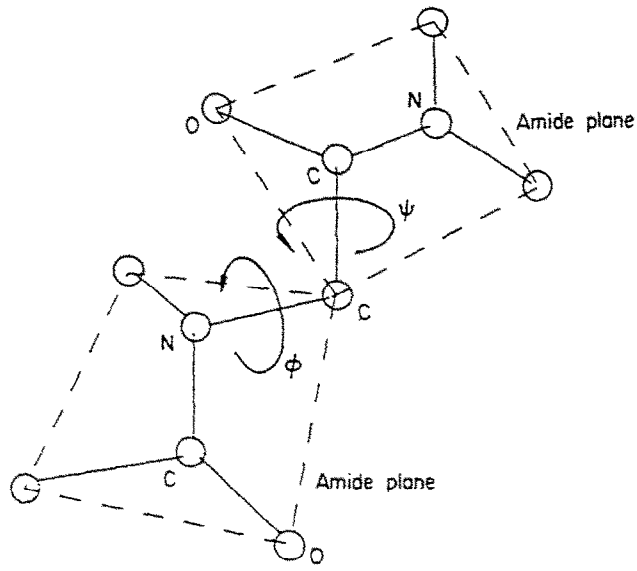

Figure 1. A fragment of the polypeptide chain showing the dihedral angles. These angles are the two degrees of freedom of the mainchain / amino acid residue

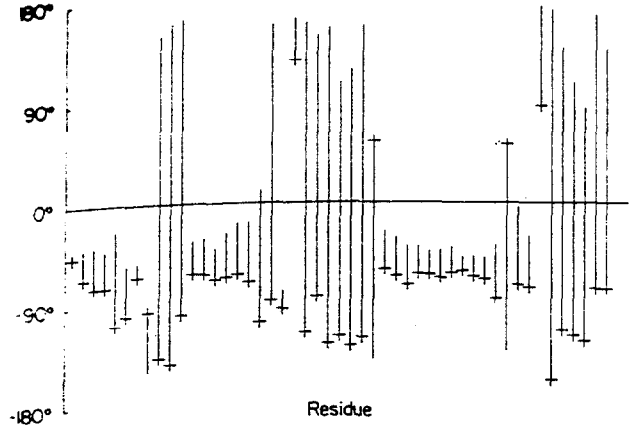

Figure 2. A Balasubramanian plot for the refined coordinates of pork insulin, taken from the structure of Isaacs and Agarwaal

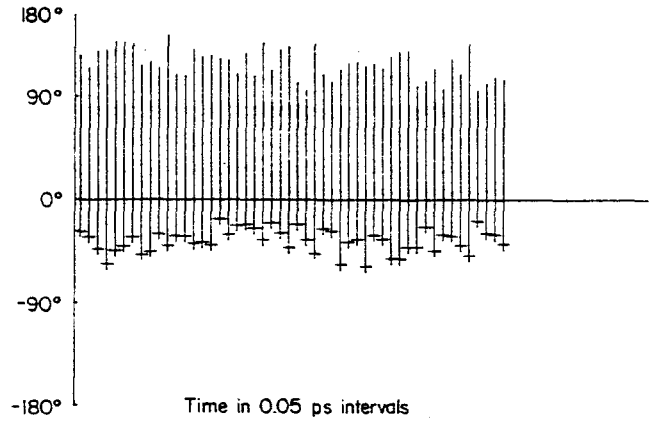

Figure 3. A derivative of the Balasubramanian plot used to show how a pair of dihedral angles has altered during a dynamics simulation. The example given here is taken from the Avian Pancreatic Polypeptide simulation and shows the variation in the dihedral angles of SER12. The interval between bars is 0.05ps

has been laid down by the IUPAC-IUB Commission on Biochemical Nomenclature[5].

A Balasubramanian Plot[6] provides a convenient way of examining the dihedral angles of a protein. One axis represents the amino acid residue number. In this paper, the residue axis is horizontal. The other axis represents the dihedral angle and ranges from $-180°$ to $+180°$. For each residue, the value of ϕ and ψ are marked on the plot and joined together by a vertical bar. A symbol is placed on one set of angle points, so that they can be identified. Figure 2 shows a Balasubramanian Plot for the refined pork insulin molecule[7], produced on a pen plotter. A + symbol has been placed on the ϕ end of each bar. The complete plot gives a 2D representation of the dihedral angles which allows the angles for a particular residue to be easily identified. This is not the case if the standard Ramachandran plot[8] is used, as the amino acid identifier has to be written on the plot. In large proteins this creates a confusing picture. The Balasubramanian plot also allows the researcher easily to identify regions of standard secondary structure, α helix and β strand. Regions of α helix appear as short bars just below the horizontal axis. Regions of extended conformation appear as long bars spanning the horizontal axis. Glycines can often be identified by the unusual vertical positions adopted by the bars.

ANIMATION

In order to use Balasubramanian plots to analyse the results from a molecular dynamics simulation, first a plot must be calculated for each conformation. These plots are then loaded into the mainframe memory and streamed to the graphics device in the manner described above[3,4]. This is a 2D representation and the 3D interactive capabilities of the system were found to be a problem at first. The distortion caused by viewing the plot at an angle was distracting to the user. This was overcome by adding a menu item that set the transform so that the screen corresponded with the graphics x, y plane, and then disabled the interactive devices.

When Balasubramanian plots are animated, the bars lengthen and shorten as the angles alter. With a little experience, the researcher is able to gain some insight into the motion of the mainchain from this representation.

A problem encountered with this representation is that caused by angles flipping between $-180°$ and $+180°$. Such flipping causes the bars to move considerably which makes them very conspicuous even though the change in the angle may be quite small. The answer is to keep a record of angles approaching $\pm 180°$ and to extend them beyond the limits of the plot rather than wrap around. This has not yet been implemented, however.

A static derivative of the Balasubramanian plot is useful for showing how the dihedral angles have altered during the simulation. Here, the horizontal axis becomes the modelled time. The bar for one pair of dihedral angles is then plotted against the time. A result is shown in Figure 3. This example has been taken from the dynamics simulation of Avian Pancreatic Polypeptide. The angles shown are from residue 12SER and the interval between each bar represents 0.05ps. Previously, publication of these data would have required two plots, one for each angle. Recently, Levitt has shown that two adjacent dihedral angles can alter in unison to allow large fluctuations of local conformation, without disturbing the surrounding structure[9]. A pair of these angles plotted on the static plot would appear as a bar of constant length, moving up and down. At the time of writing, this has not been tried.

APPLICATIONS

The animation facility has been developed as part of a joint project with Professor T L Blundell's group at Birkbeck College, London, UK. This group has been collaborating with a group in Groningen, the Netherlands, on a dynamics simulation of Avian Pancreatic Polypeptide. It is hoped that this new addition to the animation facility may provide more information about the nature of the atomic motion.

Animated Balasubramanian plots may also serve to

highlight a further feature of protein dynamics. It has been postulated that energy flows through proteins in the form of localised, mobile packets[10,11]. These energy packets are called solitons. Dynamics simulations are now being carried out with programs that include terms for soliton motion[12]. Strategically sampled distance matrices could indicate if such motion exists in a simulation. It is felt that the dihedral angles may give a stronger indication of soliton motion, however.

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Note added in proof: the problem of the lines flipping between −180° and +180° has been solved in the way described in the section on animation.