Physical Principles of Discrete Hierarchies Formation in Protein Macromolecules

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Abstract. A model for chiral periodicity with alternating chiral sense in hierarchies of protein and nucleic acid structures is proposed and substantiated. Regular alternation of the chirality sense is revealed in transitions from the lowest to higher levels of structural-functional organization in proteins where it is L-D-L-D. The stratification principle combines the ideas of biomacromolecules folding and molecular biological machines.

1. Chiral hierarchies of intra- and intermolecular structures of proteins and nucleic acids
Symmetries and symmetry breaking form a physical basis for the structure and functioning of living systems, their origin and evolution. We are talking about symmetry relations in the primary, secondary, etc. structural levels of nucleic acids and proteins. However, both systems must be constructed hierarchically to have executive and regulating subsystems. Chiral duality becomes a natural tool in combinatorics of structural correlations in these subsystems at all levels of structural organization. The purpose of the biological hierarchies is the ability to match the processes of different time and space scales.

New aspects of the theory of principles and mechanisms of sign-alternating chiral stratification in hierarchies of biological structures are proposed. It has previously been shown that the described discreteness principle combined ideas of biomacromolecules folding and molecular biological machines [1].

Earlier we detected the succession of the hierarchical levels with an alternating chirality sign in the structural hierarchy of DNA and proteins (Figure 1): from the "lower" asymmetric carbon atom to the helices, superhelices and supramolecular structures [1, 2]. The regular alternations of the chirality sign D-L-D-L for DNA and L-D-L-D for protein structures were observed during the transition to a higher level of the structural and functional organization [3]. There is a shift in chiral phase in proteins, because the protein primary structure is a sequence of L-amino acids residues, when the structure of the deoxyribose polymeric chain is taken to be the primary structure in DNA molecules.

Two basic primary polymer chains of DNA are composed of D-deoxyribose molecules. Nucleotides in the "left-handed" gosh-conformation together form "right-handed" DNA double helix (in the most common B-form). In bacteria, the circular "right-handed" helix is twisted into the "left-handed" superhelix. Negative supercoiling facilitates the melting of the double helix which is necessary for transcription and replication. In eukaryotes, the next level of organization is nucleosomal. The nucleosome is formed by a histone globule around which DNA wrapped in a superhelical manner. A histone octamer is the eight protein complex; it is a so-called "left-handed" superhelix. The right-handed DNA double helix wraps the histone octamer in the "left" way. It can be
assumed that such configuration creates the possibility for an easier way to open the helical structure of a lower scale. Structural hierarchy of proteins will be discussed more thoroughly.

![Periodic system of sign-alternating hierarchies from primary to quaternary chiral (helical) structures for DNA (left column) and proteins (right column).](image)

**Figure 1.** Periodic system of sign-alternating hierarchies from primary to quaternary chiral (helical) structures for DNA (left column) and proteins (right column). L denotes the left enantiomer or the helical configuration; D, the right enantiomer or the helical configuration.

The change of chirality sign provides for the evolutionary irreversibility of stratification (stratification is understood as the splitting of a hierarchical level of a system into two levels with different chirality signs, i.e. formation of chiral layers in a system). Indeed, to switch its chirality sign a chiral object, which does not possess a symmetry axis, must be disassembled and then reassembled from the same elements. The chirality of biological structures of different levels ensures that the process of L/D stratification is universal and hierarchical levels are deterministic and stable.

Our concept assumes that the same type of macromolecules tend to interact in the space of one type of chiral symmetry, L or D, and different types - in complementary pairs of different-valued chiral enantiomorphs.

One of the key properties of tRNA molecules is chiral selectivity for amino acids. Each amino acid has its own one or sometimes several different tRNAs. The addition of amino acids to tRNA is catalyzed by enzymes - aminoacyl-tRNA synthetases. Every such synthetase attaches a specific amino acid to the corresponding tRNA. The ATP molecule acts in this process as a source of energy. The process of aminoacylation of tRNA with aminoacyl-tRNA synthetase occurs in two stages: first, the enzyme activates the corresponding amino acid, and then a covalent bond occurs between the amino acid and tRNA. All amino acids (except glycine) are “left”, whereas tRNA is “right” at all structural levels. Thus, in the aminoacylation reaction, D- tRNA interacts with L-amino acids. Evolutionary such
chiral selectivity fixation could happen both from the excess of D-RNA and from the excess of L-amino acids in the RNA world.

A distributed resource of free energy appears due to the L-amino acids selection by tRNA from the racemate.

2. **Structural and functional hierarchy of proteins**

The protein primary structure is a sequence of L-amino acids residues. The polypeptide chain is laid in the helix or in the pleated sheet. Generally, α-helix is D-enantiomer, because the right-handed α-helix is more stable than the left-handed one [4]. The twist of β-sheet is right-handed. Interacting right-handed α-helices tend to form left superhelices. The quaternary structure of proteins is represented by the supramolecular structures, mainly formed with the left-handed superhelices interacting with the right-coiling structures. A phase shift is obvious because the protein hierarchy starts from L-amino acids, and the nucleotide hierarchy starts from D-carbohydrates deoxyriboses.

The "chiral stairs" in the intramolecular protein structure were thoroughly studied. To prove the dominance of the left-handed superhelices in all types of configurations, we analyzed all available protein structures from the "periodic table" [4, 5]. In almost all the cases where intermolecular overlap of α-helices was detected there was a tendency for the secondary left superhelix formation (Figure 2).

The structure analysis gives quantitative evaluations of the presence of lefthanded superhelical motif in each class selected from the table [4]. In the simplest case - for superhelix consisted of two α-helices, - left twisting tendency was observed almost in all structures in this class. Increasing a number of α-helices, number of structures with a similar trend is reduced, but in particular classes, which are presented by more complex superhelices, containing 5-9 α-helices, the percentage of structures with a tendency to left twisting increases sharply. In some cases it was difficult to classify the twisting due to accumulations of α-helices and β-sheets near the coiled coil. Sometimes it was difficult to observe the tendency because of too short fragments of α-helices in a superhelix. In addition in some cases (most often in superhelices based on 4 α-helices) there was almost a parallel layer of α-helices with a light left-handed twisting tendency, and we did not rank these structures as having left-handed motif. Today creating the program which can quantitative reveal the left-handed and right-handed motives in the local protein structures draws towards completion.

![Figure 2](image-url)

*Figure 2. Right-handed α-helices are twisted around each other forming a left-handed supercoil (coiled-coil structure): 1AA0 (based on 3 α-helices), 1QU7 (based on 4 α-helices) and 1MZ9 (based on 5 α-helices) in [6].*

Also we analyzed the functional class distribution of proteins containing certain types of superhelices from the database CC + [5]. Predominance of enzymes (25-45%) was observed in proteins with the most common types of superhelices - consisted of two, three and four α-helices. Furthermore, viral proteins (20%) and chaperones (9%) were frequently observed. This result essentially specifies the statement about "selected mechanical degrees of freedom" in the concept of "protein-enzyme-machine" [1, 2].
The evaluation of included in the α-helices and β-sheets in proteins amino acids percentage was made. For this purpose we used obtained from PDB [6] protein structure data for the samples from the table [4]. The calculations show that the average ratio of the quantity of making up α-helices amino acids to the total quantity of amino acids in protein is about 8% and about it is 2% for making up β-sheets amino acids to the total quantity of amino acids in protein.

The obtained results were used for quantitative thermodynamic analysis of hierarchical structure formation in typical protein molecules.

In our paper [3] there is a description of possible solution of Levinthal paradox: complementary sign-changing chiral sequences D–L–D–L and L–D–L–D for DNA and proteins form an Ariadne’s thread that directs macromolecular folding along the necessary trajectory toward Levinthal’s trap. It is known that a homochiral molecular substance (e.g., an amino acid or carbohydrate solution) undergoes racemization to equalize the concentration of enantiomers, raising the system’s entropy to its maximum and lowering its free energy. Meanwhile, a linear homochiral polymer is able to reduce the free energy not only through monomer racemization (“horizontal racemization”) but through “vertical racemization” as well (i.e., by creating higher level structures with the different chirality sign). In this case the "right-handed" and the "left-handed" structural units of different structural levels are the particles of the system. The system thus distributes its homochirality, lowering its free energy and leading to the development, among some (or all) macromolecules, of a more stable (longer lived, harder) shell, as opposed to the initial state.

3. Conclusions
A systemic regularity of molecular biology is considered: starting from the level of asymmetric carbon in the deoxyribose and amino acids there is a tendency toward alternating chirality sense of intramolecular structural levels of DNA and proteins, namely D-L-D-L for DNA and L-D-L-D for proteins. A special case of chirality is helicity.

In intermolecular interactions the sense of chirality of the highest intramolecular structural level directly involved in the interaction prevails in each of the participants. For molecules of the same nature (protein-protein, DNA-RNA, tRNA-mRNA, ribozymes) the interaction is realized mainly in the case of the same sense of chirality, either L-L, or D-D, and for molecules of different types (DNA-protein, tRNA-amino acids, enzyme-substrate) in the case of different senses, either D-L, or L-D.

Alternating sense of chiral hierarchy of conjugated levels of macromolecular structures in proteins and nucleic acids is of general biological importance: it determines discreteness of the levels, serves as an instrument of the folding, provides a structural basis for “preferred collective” (or “macroscopic mechanical”) degrees of freedom in constructions of macromolecular machines, as well as one of the mechanisms of block/saltatory development of the evolutionary process.

A new principal concept is put forward: homochirality of primary structures of DNA and proteins determines the amount of entropic component of the free energy which is used in the processes of folding and molecular rearrangements.

The resulting hierarchy of conjugated sign-alternating chiral structures kinetically stabilizes them by inhibiting spontaneous racemization; forms a conjugated system with selected degrees of freedom, which makes the work of biological machines possible; in living systems macromolecular and autowave chiral structures are conjugated as a consequence of their channeling through the structures of chiral biological machines; defines the vector of the general development of a system in the direction of an upper, “open-ended” hierarchical level [10].

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