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The disposition of the LZCC protein residues in wenxiang diagram provides new insights into the protein–protein interaction mechanism

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ABSTRACT

Wenxiang diagram is a new two-dimensional representation that characterizes the disposition of hydrophobic and hydrophilic residues in α-helices. In this research, the hydrophobic and hydrophilic residues of two leucine zipper coiled-coil (LZCC) structural proteins, cGKI 1–59 and MBSCT35 are dispositioned on the wenxiang diagrams according to heptad repeat pattern (abcdefg n ), respectively. Their wenxiang diagrams clearly demonstrate that the residues with same repeat letters are laid on same side of the spiral diagrams, where most hydrophobic residues are positioned at a and d, and most hydrophilic residues are localized on b, c, e and g polar position regions. The wenxiang diagrams of a dimeric LZCC can be represented by the combination of two monomeric wenxiang diagrams, and the wenxiang diagrams of the two LZCC (tetramer) complex structures can also be assembled by using two pairs of their wenxiang diagrams. Furthermore, by comparing the wenxiang diagrams of cGKI 1–59 and MBSCT35, the interaction between cGKI 1–59 and MBSCT35 is suggested to be weaker. By analyzing the wenxiang diagram of the cGKI 1–59 • MBSCT42 complex structure, most affected residues of cGKI 1–59 by the interaction with MBSCT42 are proposed at positions d, e, and g of the LZCC structure. These findings are consistent with our previous NMR results. Incorporating NMR spectroscopy, the wenxiang diagrams of LZCC structures may provide novel insights into the interaction mechanisms between dimeric, trimeric, tetrameric coiled-coil structures.

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1. Introduction

Coiled coil (CC) structural proteins usually contain a repeated pattern of nonpolar and charged amino-acid residues, referred to as a heptad repeat, which is denoted (abcdefg)n. Where the positions for a and d are predominantly hydrophobic, often being occupied by leucine, isoleucine, or valine, and the positions at b, c, e and g are typically charged or polar (Chambers et al., 1990; Crick, 1952; Hartmann et al., 2009; Lupas and Gruber, 2005; Surks et al., 1999). It has been known that many CC type proteins are involved in important biological functions such as the regulation of gene expression. Representative examples include the oncogenes c-fos and jun, and the muscle protein tropomyosin (Hartshorne, 1997; Hartshorne and Hirano, 1999; O’Shea et al., 1989; Surks and Mendelsohn, 2003).

Usually, a leucine zipper (LZ) structure is made up of two α-helical segments of a protein; it has leucines and/or isoleucines facing each other along the length of the helices, allowing them to dimerize and form a symmetric interface that can bind to the DNA on both sides of the double helix (Lupas and Gruber, 2005) or other proteins. As a three-dimensional structural motif, LZ structures are widely found in both eukaryotic and prokaryotic regulatory proteins (Baker et al., 2005; Rybalkin et al., 2002; Schlossmann et al., 2000; Surks et al., 1999). The protein’s domain possessing LZ motif usually is a CC structure. Thus this kind of protein is also referred as leucine zipper coiled-coil (LZCC) structural proteins. Evidences that the proteins of Leucine Zipper sequences belong to coiled-coil structures have been reported by many workers (O’Shea et al., 1989; Surks et al., 1999; Surks and Mendelsohn, 2003).

Previous studies have shown that both cGMP-dependent protein kinase I, cGKI 1–59 and the C-terminal 180 amino acids (residues 929–970) of the myosin binding subunit (MBS) of the myosin light-chain phosphatase (PP1M), MBSCT180 contain LZCC domains. cGKI 1–59 functions in the nitric oxide (NO) mediated relaxation of vascular smooth muscle (Lincoln, 1994). The cyclic GMP-mediated vascular smooth muscle cell relaxation is characterized by both a reduction of intracellular calcium concentration and by activation of PP1M, and thus results in sensitivity reduction of the contractile apparatus to intracellular calcium (Lincoln, 1994; Morgan and Morgan, 1984; Surks et al., 1999). The state of contraction or relaxation of vascular smooth muscle cells is closely coupled to phosphorylation and dephosphorylation of the regulatory myosin light chain, which is in part regulated by the binding of cGKI1 to MBS of the PP1M. The disruption of the cGKI-MBS interaction impairs cGMP-mediated dephosphorylation of myosin light chain, the critical determinant of smooth muscle cell contractile state.
(Nakamura et al., 2007). More recently, the specific interaction between cGKI(1–59) and MBS(592–970), the C-terminal 42 amino acids of MBS protein has been further confirmed by NMR, biochemical and other biophysical methods such as glutathione S-transferase pulldown experiments, chemical cross-linking, size exclusion chromatography, circular dichroism, and isothermal titration calorimetry (Sharma et al., 2008; Zhou, 2011). These results further supported that the interaction between the cGKI and MBS proteins actually is that between the LZCC of cGKI(1–59) and the LZCC of MBS(592–970).

In view of this, the present study was initiated in an attempt to utilize wenxiang diagram to characterize the LZCC domains of both cGKI(1–59) and the LZCC of MBS(592–970). It has been known that many α-helices in proteins are amphiphilic, i.e., formed by the hydrophobic and hydrophilic amino acids according to a special order along the helix chain (Mercier et al., 1998; Schnell et al., 2005; Zhou, 2011). Besides, it has also been known that different types of proteins have different amphiphilic features, corresponding to different hydrophobic and hydrophilic order patterns (Chou, 2005; Kurochkina, 2010). As summarized in an article in Wikipedia (http://en.wikipedia.org/wiki/Alpha_helix, 2010), the wenxiang diagram (Chou et al., 1997) has the following advantages: (1) able to show the relative locations of the amino acids in an alpha-helix regardless how long it is; (2) able to indicate the direction of an alpha-helix; and (3) having the capacity to provide more information about each of the constituent amino acid residues in an α-helix. With these features, the wenxiang diagram can provide an easily visualizable picture in a 2D space for clearly characterizing the disposition of amphiphilic helices in proteins.

Using graphical approaches to study biological problems can provide an intuitive picture or useful insights for helping analyzing complicated mechanisms in these systems, as demonstrated by many previous studies on a series of important biological topics, such as enzyme-catalyzed reactions (Andraos, 2008; Chou, 1980, 1981, 1989; Chou and Forsen, 1980; Cornish-Bowden, 1979; King and Altman, 1956; Myers and Palmer, 1985; Zhou and Deng, 1984), protein folding kinetics and folding rates (Chou, 1990; Chou and Shen, 2009), inhibition of HIV-1 reverse transcriptase (Althaus et al., 1993a, 1993b, 1993c), inhibition kinetics of processive nucleic acid polymerases and nucleases (Chou et al., 1994), drug metabolism systems (Chou, 2010), analysis of DNA sequence (Xie and Mo, 2011; Yu et al., 2009), and protein sequence evolution (Wu et al., 2010). Moreover, graphical methods have been utilized to deal with complicated network systems (Gonzalez-Diaz et al., 2009; Munteanu et al., 2009) and identify the hub proteins from complicated network systems (Shen et al., 2010). Recently, the “cellular automaton image” (Wolfram 1984, 2002) has also been applied to study hepatitis B viral infections (Xiao et al., 2006), HBV virus gene missense mutation (Xiao et al., 2005a), and visual analysis of SARS-CoV (Wang et al., 2005), as well as representing complicated biological sequences (Xiao et al., 2005b) and helping to identify various protein attributes (Xiao et al., 2009, 2010). The present study was initiated in an attempt to use the elegant wenxiang diagram (Chou et al., 1997) to investigate protein–protein interactions in hope to gain useful insights for understanding some of their subtle action mechanisms. Because LZ and CC proteins have amphiphilic α-helical feature, it may be rewarding to use wenxiang diagram to investigate the disposition of their hydrophobic and hydrophilic residues. Our results will indicate that the heptad repeat and the dispositions of some key residues of cGKI(1–59) in the wenxiang diagrams are basically consistent with our previous NMR experimental results (Sharma et al., 2008).

2. Materials and methods

2.1. The sequences of cGKI(1–59) and MBS(592–970) proteins

The sequence of cGKI(1–59) is

T1SELEDFAK10ILMLKEERK16ELEKRLSKE19EIEIQLKRRK20HKMQSLVTVP56STHGIPRT59.

The sequence of MBS(592–970) protein is

S529TD531FKKLY535EQILA541ENEKLKAQLHDTNMELTD
KLMQLEKATQR570.

The coiled-coil predictions of the heptad repeat region were made using the programs COILS (Lupas et al., 1991; Berger et al., 1995).

2.2. Ensemble principle of wenxiang diagram of a coiled-coil dimer

It has been clearly known that all amphiphilic helical proteins, with most hydrophobic residues being distinctly distributed in one-half of each wenxiang diagram, and most hydrophilic residues being distributed in the other half (Hartshorne, 1997; Sharma et al., 2008). Thus, the wenxiang diagrams of any LZCC structures should have similar characteristics. The hydrophobicity-weighed (w+ (i)) and hydrophilicity-weighed (w− (i)) contributions of each residue, and hydrophobic and hydrophilic centroids of each α-helix can be determined by the wenxiang diagram coordinate system (Hartshorne, 1997). Furthermore, the inclination angle Ω, which is defined as the angle formed by the axis of the helix and its projected line on the plane S (Fig. 1) can be calculated using the following equation:

\[ \Omega = \sin^{-1} \left[ \frac{1}{2} \left( C_3^2 + C_2^2 + C_1^2 \right)^{-1/2} \right] \]  

where \( C_1, C_2, C_3 \) are directly related to the hydrophobic atom coordinate system (\( x_1^+, y_1^+, z_1^+ \)), hydrophilic atom coordinate system (\( x_2^-, y_2^-, z_2^- \)), hydrophobicity-weighed \( w^+ (i) \), and hydrophilicity-weighed \( w^- (i) \) (Hartshorne, 1997).

According to the ensemble principle of wenxiang diagram, an α-helix can be visualized as a two-dimensional diagram generated by

Schematic drawing showing the inclination angle Ω and the intercept Tz of the interfacial plane S. Q is the intersection point of the helix axis z with the interfacial plane S. As shown in the figure, Ω is the angle between the helix axis Z and its projection on the plane S, whereas Tz is the distance from the projection of the first C1 on the helix axis to Q (Hartshorne, 1997).
Fig. 2. Conical projection of a hollow cylinder \( \Phi \) onto a plane perpendicular to the cylinder. The dot-dash lines (---) represent the radiating lines from the apex \( \psi \). The distance \( \psi \) from the projection plane is arbitrary and is chosen to ensure that the resulting projection graph clearly portrays the relevant information. The image of the hollow cylinder on the projection plane is the ring \( \psi \).

its conical projection (Fig. 2), where the projected image of a hollow cylinder \( \Phi \) onto a plane perpendicular to it by conical projection will become a ring \( \psi \). The outer and inner circles of the ring \( \psi \) correspond to the top and bottom circles of the hollow cylinder, respectively. Thus, the projected image of a helix could be viewed as a planar spiral with a continuous varying radius. If a helical COOH-terminal is close to its projected plane, and NH2-terminal is far away the plane, the COOH-terminal of the helix lies near the center of the projected plane, and NH2-terminal lies at the outer rim of the diagram. The disposition of a coiled-coil dimmer could be expressed by a pair of two-dimensional diagrams, i.e., two same monomer wenxiang diagrams.

3. Results and discussion

3.1. Distribution characteristics of the residues of cGKlx1–59 and MBS\(_{\text{CT55}}\) in the wenxiang diagrams

In previous studies, we have used programs COILS (Lupas, et al., 1991) to predict the coiled-coil region in cGKlx\(_{1–59}\) domain, which is from the residue L12 through residue H53. This result has been confirmed by the analysis of our NMR relaxation data and RDC data (Schnell et al., 2005). This domain contains a repeating pattern of amino acids \( a, b, c, d, e, f, \) and \( g \) (Fig. 3A). Thus, the monomer structure of cGKlx\(_{1–59}\) could be characterized by a wenxiang diagram. Where each residue of the denoted repeating amino acids (abcdefg\(_1\)) in Fig. 3B is represented by a circle and repeated letters \( a, b, c, d, e, f, \) and \( g \), respectively. It is clearly shown with the wenxiang diagram that the residues with same letters are laid on same side of the spiral diagram, and most hydrophobic residues are positioned on \( a/d \) region except Lysine 15 and 29 (Fig. 3B).

Because a LZ domain must be a typical amphiphilic helix, i.e., part of the helix flank is dominated by hydrophobic residues and the other part by hydrophilic residues, their inclination angles are all very close to \( 0^\circ \), which is fully consistent with the requirement of minimum free energy in a amphiphilic helical system (Hartsorne, 1997).

It has been suggested that a critical region responsible for interacting with cGKlx\(_{1–59}\) is another LZ coiled-coil domain, which is located within the C-terminal 100 amino acids (residues 930–1030) of the MBS (Baker et al., 2005; Lincoln, 1994). This region has been further confirmed to be MBS\(_{\text{CT42}}\), the C-terminal 42 amino acids (residues 929–970) of the MBS protein by our NMR data (Sharma et al., 2008). Actually, according to our pair coil analysis, the LZCC domain with the highest probability (1.0) should be the C-terminal 35 amino acids of the MBS (MBS\(_{\text{CT55}}\), which contains a repeating pattern of amino acids \( a, b, c, d, e, f, \) and \( g \) from residues 930 to 970 (Fig. 4A). Because all residues of MBS\(_{\text{CT55}}\) are included in MBS\(_{\text{CT42}}\) domain, the interaction between it and cGKlx\(_{1–59}\) has been confirmed by our biochemical and NMR data (Lincoln, 1994). According to this information, we obtained a wenxiang diagram of the monomer of MBS\(_{\text{CT55}}\), where all residues with same letter are also distributed on the same side of the spiral wenxiang diagram (Fig. 4B). It can be seen from both Figs. 3 and 4B and that most nonpolar residues are localized on the hydrophobic \( a/d \) regions.

According to the calculated results for a number of proteins, the inclination angles for most helices therein are small (\( \Omega \approx 25^\circ \)).
This suggests that driven by the free energy an amphiphilic helix will tend to seek its own arrangement in a protein such that approximately half of its face is buried in the protein whereas the other half is exposed to the aqueous environment that surrounds the protein (Hartshorne, 1997). Thus, a coiled-coil dimer structure can be further expressed by a combination of two identical two-dimensional wenxiang diagrams. The ensemble principle of such wenxiang diagram should observe the following rule: a/d region of one monomer wenxiang diagram must be faced to the a/d region of another identical monomer wenxiang diagram. Because most hydrophobic residues are localized at the a/d positions, the approach to each other between the two monomer’s a/d regions should be helpful for the formation of a stable coiled-coil dimer structure due to the hydrophobic interactions of the nonpolar residues between the two a/d regions. According to such an ensemble rule, the wenxiang diagrams of the cGKI lysine (K15 and K29) in position a/d are shown in red. (Fig. 6) based on the previous NMR experimental data (Surks and Mendelsohn, 2003).

As shown in Fig. 5, the hydrophobic side chains of leucine and isoleucine in positions a and d, and two side chains of lysine (K15 and K29) in position d should be clearly displayed on the nearest neighbor region between the two wenxiang diagrams. This observation has been verified by our 3D structure of cGKI (Fig. 6) based on the previous NMR experimental data (Surks and Mendelsohn, 2003).

Fig. 6. 3D structure of LZCC domains of cGKI1–59. (A) Bundle of 20 refined parallel coiled-coil dimer structures with lowest RDC energies superimposed on the backbone heavy atoms (blue). The hydrophobic side chains of leucine and isoleucine in positions a and d are shown in green, and the two side chains of lysine which are in position d are shown in red. (B) End-on view from the N-terminus of a representative refined dimer. The backbone structure is shown as a ribbon diagram, and side chains are colored as in (A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
diagrams of MBSCT35 LZCC structure. In Fig. 5A, although the seven apolar residues (M13, L14, Y46, P48, V49, G55, and P56) of cGKI<sup>1-59</sup> localize the outside of the a/d position region, most of them are still close to the a/d region except residues M13 and L14. However, for the wenxiang diagrams of MBSCT35, an interesting feature is that most apolar residues of outside the hydrophobic a/d position region such as 941 A, 948 A, 955 M and 962 L are located at position f (where usually are occupied by the hydrophilic residues) and are little far away from the hydrophobic a/d position region (Fig. 5B). These differences between Figs. 5A and B suggest that the either hydrophobic or hydrophilic interactions between cGKI<sup>1-59</sup> and MBSCT35 may be weaker due to the lack of the contributions of the more apolar residues near the a/d position region of MBSCT35, or lack of the contributions of polar residues from g, b, c, and e positions of cGKI<sup>1-59</sup>. This may explain why the molar ratio of the interaction between MBS<sub>CT35</sub> and cGKI<sup>1-59</sup> has to be 2:1 (Lincoln, 1994).

3.2. Analysis of the interaction between cGKI<sup>1-59</sup> and MBS<sub>CT35</sub> incorporating wenxiang diagrams and NMR data

In order to determine whether an interaction exists between cGKI<sup>1-59</sup> and MBS<sub>CT42</sub>, Figs. 5A and B were combined to form the wenxiang diagrams of a hexamer complex structure according to the molar ratio of cGKI<sup>1-59</sup> and MBS<sub>CT35</sub> (1:2) (Fig. 7).

The ensemble of these combined wenxiang diagrams should be reasonable. Because most hydrophobic residues (position a/d regions, or nearby a/d regions) are buried inside the bundle and most hydrophilic residues are exposed to the aqueous environment that surrounds the cGKI<sup>1-59</sup>-MBS<sub>CT42</sub> hexamer complex. As shown in Fig. 7, the a-e position region of cGKI<sup>1-59</sup> is close to the d-g position region of MBS<sub>CT35</sub>, and the d-g position regions of cGKI<sup>1-59</sup> is close to the a-e position regions of MBS<sub>CT35</sub>. The all residues (E16, E23, E30, K37, Q44 and S51) at position e of cGKI<sup>1-59</sup> are all polar residues. In the other hand, all residues (942E, 949Q, 956E, 963Q and

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**Fig. 7.** The wenxiang diagrams of the hexamer complex structure of cGKI<sup>1-59</sup> (middle pair diagrams) and two MBS<sub>CT35</sub> (the topper and lower pair diagrams). Where, the a-e region of one molecule is close to the d-g region of another molecule.
970R) at position g of MBSCT35 are also polar residues. According to the previous studies (Adamian and Liang, 2002), the salt bridge residue pairs of E–R may be formed between E16, E23, or E30 of cGK1z1–59 and 970R of MBSCT35, and the salt bridge residue pairs of R–E may be formed between R37 of cGK1z1–59 and 942E or 956E of MBSCT35. These salt bridge pairs have high propensity for the interhelical-polar-polar atomic contacts. In addition, other ionizable-polar residue pairs such as Q–R or R–Q (Q44–970R, or R37–949Q, or R37–963Q), and S–R (S51–970R) between cGK1z1–59 and MBSCT35 may also have high propensity to form the interhelical-polar-polar interactions (Adamian, and Liang, 2002). Similarly, the all residues (R18, R25, E32, K39, and H53) at position g of cGK1z1–59 are all polar residues, and most residues (947K, 954N, 961K, and 968T) at position e of MBSCT35 are also polar residues. The ionizable-polar residue pairs such as R–N (R18–954N), or K–N (K39–954N), or H–T (H53–968T) between cGK1z1–59 and MBSCT35 may also be formed to contribute the interhelical-polar-polar interactions.

Such ensemble of the wenxiang diagrams suggests that most affected residues of cGK1z1–59 and MBSCT35 might be at positions d, a, e and g of these two LZCC structures. This prediction has been supported by the previous NMR data (Lincoln, 1994; Sharma et al., 2008).

NMR spectroscopy is the preferred method for characterizing the structural details of protein–protein and protein-ligand interaction interfaces following complex formation in an aqueous environment (Chou et al., 1997). Our 15N-1H-HSQC titration data in which interfaces following complex formation in an aqueous environment structural details of protein–protein and protein-ligand interaction et al., 2008).

Based on similar data obtained in studies of F-actin interacting with skeletal myosin light chain 1 and ubiquitin hydroxase interacting with its native substrate, it is likely that these residues are within the interaction interface between these proteins although additional experiments evaluating the on/off rate of the interaction may be necessary (Naik et al., 2001; Sakamoto et al., 2005; Hutchings et al., 2003). As has been previously identified for the GCN4 LZ, upon tetramer formation (pairing of two dimers) there is an increase in packing density within the intermolecular interface, which results in additional perturbation of residues within the a, d, e, b and g positions that is unlike the typical packing of the a and d side chains of isolated helices (Harbury et al., 1994). Interestingly several of the residues of cGK1z1–59 that are conformationally perturbed following the addition of MBS are within a, d, e, g and b positions of the repeating heptads layer (Fig. 7). In addition, the previous studies about the GCN4 tetramer LZ, an asialoglycoprotein receptor heterotetramer, and an extensive study of Crick’s knobs-into-holes packing for 3-, 4- and 5-stranded structures have provided additional geometric, conformational packing information for generating this model (Harbury et al., 1993; 1994;1995; Walshaw and Woolfson, 2003). Moreover, in the crystal structure of the GCN4 trimer and tetramer the authors observe significant packing and numerous interactions between residues in the a/d hydrophobic interface and the hydrophilic residues at positions e and g, which and our cGK1z1–59. MBSCT42 complex wenxiang diagrams may be consistent and helpful for a better understanding the packing mechanism that present between the cGK1z1–59 LZCC domain and MBSCT42 LZCC motif.

4. Conclusion

Although many leucine zipper domains of proteins have been characterized by a heptad repeat of leucine residues in a helical wheel (Jones et al., 1992), the information thus provided about the α-helices and their lengths are very limited. If the helical wheel diagrams of a LZCC structure are used to represent two α-helices longer than 20 residues, they must crowd into a very limited space or overlap with each another, and will be difficult to be distinguished from each other. It is particularly difficult to represent a LZCC-LZCC tetramer complex using four helical wheel diagrams. In contrast, the wenxiang diagrams can be used to represent two α-helices regardless how long they are. Furthermore, wenxiang diagrams of LZCC structure can provide much more information about the physico-chemical features of the constituent amino acids as well as their distribution even for four-alpha-helix bundle with heptad repeat residues. Furthermore, our results also suggest that the wenxiang diagrams of a LZCC structure can be used to identify the key residues that play the most important role for its interaction with another LZCC protein. It is demonstrated through this study that wenxiang diagram holds a great potential in providing more information about each of the constituent amino acid residues in a heptad’s repeat. Incorporated with NMR or other biological/biophysical experimental results, wenxiang diagram may provide useful insights into the 3D structural model of LZCC protein and the interaction mechanism between LZCC and LZCC proteins.

References

Adamian, L., Liang, J., 2002. Interhelical hydrogen bonds and spatial motifs in membrane proteins: polar clamps and serine zippers. Protein: Struct. Funct. Genet. 47, 209–218.
Althaus, I.W., Chou, J.J., Gonzales, A.J., Diebel, M.R., Chou, K.C., Keedy, F.J., Romero, D.L., Aristoff, P.A., Tarpley, W.G., Reusser, F., 1993a. Kinetic studies with the nonnucleoside HIV-1 reverse transcriptase inhibitor U-88204E. Biochemistry 32, 6548–6554.
Althaus, I.W., Chou, J.J., Gonzales, A.J., Diebel, M.R., Chou, K.C., Keedy, F.J., Romero, D.L., Aristoff, P.A., Tarpley, W.G., Reusser, F., 1993b. Steady-state kinetic studies with the non-nucleoside HIV-1 reverse transcriptase inhibitor U-87201E. J. Biol. Chem. 268, 6119–6124.
Althaus, I.W., Gonzales, A.J., Chou, J.J., Diebel, M.R., Chou, K.C., Keedy, F.J., Romero, D.L., Aristoff, P.A., Tarpley, W.G., Reusser, F., 1993c. The quinoline U-78036
is a potent inhibitor of HIV-1 reverse transcriptase. J. Biol. Chem. 268, 14875–14880.

Andraos, J., 2008. Kinetic plasticity and the determination of product ratios for kinetic schemes leading to multiple products without rate laws: new methods based on directed graphs. Can. J. Chem. 86, 342–357.

Baker, P.R.S., Lin, Y., Schopfer, F.J., Woodcock, S.R., Groeger, A.L., Bathyanthy, C., Sweeney, S., Long, M.H., Iles, K.I., Baker, M.L.S., Branchaud, B.P., Chen, Y.E., Freeman, B.A., 2005. Fatty acid transduction of nitric oxide signaling: multiple nitrate unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous PPAR ligands. J. Biol. Chem. 280 (51), 42475–42466.

Berger, B., Wilson, D.B., Wolf, E., Tonchev, T., Milla, M., Kim, P.S., 1995. Predicting coiled coils by use of pairwise residue correlations. Proc. Natl. Acad. Sci. USA 92, 8259–8261.

Chou, K.C., 1980. A new schematic method in enzyme kinetics. Eur. J. Biochem. 113, 195–198.

Chou, K.C., Forsen, S., 1980. Graphical rules for enzyme-catalyzed rate laws. Biochem. J. 187, 829–835.

Chou, K.C., 1981. Two new schematic rules for rate laws of enzyme-catalyzed reactions. J. Theor. Biol. 89, 581–592.

Chou, K.C., 1989. Graphical rules in steady and non-steady enzyme kinetics. J. Biol. Chem. 264, 12074–12079.

Chou, K.C., 1990. Review: applications of graph theory to enzyme kinetics and protein folding kinetics. steady and non-steady state systems. Biophys. Chem. 35, 1–24.

Chou, K.C., Kezdy, F.J., Reusser, F., 1994. Review: Steady-state inhibition kinetics of processive nucleic acid polymerases and nucleases. Anal. Biochem. 221, 217–230.

Chou, K.C., Zhang, C.T., Maggiora, G.M., 1997. Disposition of amphiphilic helices in heteropolar environments. Proteins: Struct. Funct. Genet. 28, 99–108.

Chou, K.C., 2005. Using amphiphilic pseudo amino acid composition to predict enzyme subfamily classes. Bioinformatics 21, 10–19. doi:10.1093/bioinformatics/bth065.

Chou, K.C., Shen, H.B., 2009. FoldRate: a web-server for predicting protein folding rates from primary sequence 3, 31–500pen Bioinf. J. 3, 31–50 (openly accessible at http://www.bentham.org/open/tobioij/).

Chou, K.C., 2010. Graphic rule for drug metabolism systems. Curr. Drug Metab. 11, 369–378.

Chambers, P., Pringle, C.R., Easton, A.J., 1990. Heptad repeat sequences are located adjacent to hydrophobic regions in several types of virus fusion glycoproteins. J. Genet. Virol. 71, 3075–3080 (Printed in Great Britain).

Crick, F.H., 1952. Is alpha-keratin a coiled coil? Nature 170 (4334), 882–883.

Cornish-Bowden, A., 1979. Fundamentals of Enzyme Kinetics. Butterworths, London (Chapter 4).

Gonzalez-Diaz, H., Perez-Montoto, L.G., Duardo-Sanchez, A., Paniagua, E., Vazquez-Prieto, S., Vilas, R., Da-Ayuela, M.A., Bola-Fernandez, F., Munteanu, C.R., Dorado, J., Costas, J., Ubeira, F.M., 2009. Generalized lattice graphs for 2D-visualization of biological information. J. Theor. Biol. 261, 136–147.

Harbury, P.B., Zhang, T., Kim, P.S., Albert, T., 1993. A switch between two-, three-, and assemblies. J. Theor. Biol. 264, 585–592.

Hartshorne, D.J., Hirano, K., 1999. Review interactions of protein phosphatase type 1, with a focus on myosin phosphatase. Mol. Cell. Biochem. 190, 79–84.

Harbury, P.B., Zhang, T., Kim, P.S., Albert, T., 1993. A switch between two-, three-, and assemblies. J. Theor. Biol. 264, 585–592.

Hartshorne, D.J., 1997. Interactions of the subunits of smooth muscle myosin phosphatase. J. Biol. Chem. 272, 3683.

Hartshorne, D.J., 1997. Interactions of the subunits of smooth muscle myosin phosphatase. J. Biol. Chem. 272, 3683.

Harbury, P.B., Zhang, T., Kim, P.S., Albert, T., 1993. A switch between two-, three-, and assemblies. J. Theor. Biol. 264, 585–592.

Hartshorne, D.J., Hirano, K., 1999. Review interactions of protein phosphatase type 1, with a focus on myosin phosphatase. Mol. Cell. Biochem. 190, 79–84.

Hartmann, M.D., Ridderbusch, O., Zeth, K., Albrecht, R., Testa, O., Woolfson, D.N., Sauer, G., Dunin-Horkawicz, S., Lupas, A.N., Alvarez, B.H., 2009. A switch between two-, three-, and complex coiled-coil assemblies. J. Struct. Biol. 144, 349–361.

Hutchings, N.J., Clarkerson, N., Chalkley, R., Barclay, A.N., Brown, M.H., 2003. Linking the T cell surface protein CD2 to the actin-capping protein CAPZ via CMS and biopolymers. J. Theor. Biol. 264, 113–115.

Munteanu, C.R., Magalhaes, A.L., Uriarte, E., Gonzalez-Diaz, H., 2009. The structural determinations of the leucine zipper coiled-coil protein and its potential use in sensor applications. Biosens. Bioelectron. 16 (9-12), 1051–1057.

Naik, R.R., Kirkpatrick, S.M., Stone, M.O., 2001. The thermostability of an alpha-helical coiled-coil protein and its interaction with the myosin binding subunit of the myosin light chain phosphatase. Circul. Res. 101, 712.

O’Shea, E.K., Rutkowski, R., Kim, P.S., 1989. Evidence that the leucine zipper is a coiled coil. Science 243, 538–542.