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Geometric characteristics of hydrogen bonds involving sulfur atoms in proteins

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ABSTRACT

Sulfur atoms have been known to participate in hydrogen bonds (H-bonds) and these sulfur-containing H-bonds (SCHBs) are suggested to play important roles in certain biological processes. This study aims to comprehensively characterize all the SCHBs in 500 high-resolution protein structures (≤1.8 Å). We categorized SCHBs into six types according to donor/acceptor behaviors and used explicit hydrogen approach to distinguish SCHBs from those of nonhydrogen bonding interactions. It is revealed that sulfur atom is a very poor H-bond acceptor, but a moderately good H-bond donor. In α-helix, considerable SCHBs were found between the sulphydryl group of cysteine residue *i* and the carbonyl oxygen of residue *i*-4, and these SCHBs exert effects in stabilizing helices. Although for other SCHBs, they possess no specific secondary structural preference, their geometric characteristics in proteins and in free small compounds are significantly distinct, indicating the protein SCHBs are geometrically distorted. Interestingly, sulfur atom in the disulfide bond tends to form bifurcated H-bond whereas in cysteine-cysteine pairs prefer to form dual H-bond. These special H-bonds remarkably boost the interaction between H-bond donor and acceptor. By oxidation/reduction manner, the mutual transformation between the dual H-bonds and disulfide bonds for cysteine-cysteine pairs can accurately adjust the structural stability and biological function of proteins in different environments. Furthermore, few loose H-bonds were observed to form between the sulphydryl groups and aromatic rings, and in these cases the donor H is almost over against the rim rather than the center of the aromatic ring.

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Key words: sulfur-containing hydrogen bond; hydrogen bond geometry; weak interaction; sulfur atom; protein structure.

INTRODUCTION

Hydrogen bonds (H-bonds) play critical roles in structure and function of proteins, including features such as overall folding, local architecture, protein-ligand recognition, enzymatic activity, protein hydration, and macromolecular dynamics.[1,2] For a long time, research has concentrated on H-bonds X-H...A in which X and A both are very electronegative atoms (mainly N and O). However, H-bonding is a very broad phenomenon that is not restricted to N and O, but may involve less electronegative atoms. In structure biology, some of the noncanonical H-bonds have recently shown to be of greater importance,[3] in particular the variants C—H...O,[4] X-H...π,[5] and C—H...π,[6] etc. They have been systematically inspected on geometry and energetics properties using quantum chemistry, molecular mechanics, and statistics.[7–12]

Sulfur-containing hydrogen bond (SCHB) is an elusive weak H-bond.[13] In early study on a simple dimer H_2S, S—H...S was inferred to be quite weak with a bond strength of about 1 kcal/mol.[14,15] Platts *et al.*[16] have used *ab initio* calculation to analyze the directionality of H-bonds to sulfur and oxygen, indicating that H-bond formation to oxygen is driven by charge–charge interactions, whereas with sulfur the stabilization arises principally from the interaction of the charge on the acidic hydrogen with the dipole and quadrupoles of sulfur. Similar approach was used to study the dimethylsulfide-methanol complex, and the results showed that at the coupled cluster level the binding energy of O—H...S is −5.46 kcal/mol, only slightly less than the H-bond energy of −5.97 kcal/mol for the corresponding oxygen analog.[17] All these works indicate that SCHBs are not so weak as early believed. In addition, Allen[18] and Steiner[19] *et al.* have comprehensively examined the crystal structures of organic compounds in the Cambridge Structural Database and derived much valuable geometrical information for SCHBs. Furthermore, numerous experimental works also suggested that SCHBs play important roles in intermolecular interactions[20,21] and physicochemical processes such as electron transfer activity of metal-sulfur complexes.[22]

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Although SCHBs in small organic compounds were widely investigated, few works were concerned on biomolecular SCHBs. In many cases, SCHBs essentially participate in various biological processes (e.g., protein stabilization, macromolecular recognition, and enzyme activity). As an instance, sulfur atoms in the active site of the sulphydryl proteases such as papain[23] and cysteine proteases[24] directly participate in catalytic reaction, with H-bond formation with substrates to thus significantly enhance binding strength and to accurately locate the ligand. In redox proteins such as ferredoxin and rubredoxin, intensive SCHB networks are formed to stabilize the active clusters.[25,26] On the other side, the sulfur atom can also be an electrophile/nucleophile to interact with amide, carbonyl group, and aromatic ring that do not need any participation of proton (nonhydrogen bond interactions). Pal and Chakrabarti[27–29] have made a series of investigations on these electrophile–nucleophile interactions and suggested that the intraresidue S. . .C=O interaction constrains the protein main-chain and may have a role in lowering the pK_a of cysteine in enzyme active sites. Reid *et al.*[30] found the sulfur-aromatic interactions occur most frequently in the interior of proteins and may contribute to protein stability.

Early studies on protein SCHBs are quite deficient, even some conclusions from different works are conflictive to each other. For example, although the sulphydryl group was assumed to be an acceptor in one study,[31] another[27] found it to be less of an acceptor. Ippolito *et al.*[32] indicated that a slight angular preference at 60°of χ_2 for cysteine S—H. . .X pairs, but in Pal's report[27] the distribution peak of χ_2 is near 90°. As our knowledge, the work made by Gregoret *et al.*[33] was the only publication that systematically examined the protein SCHBs. However limited to the poor quantity and quality of protein crystal structures available at the time, they could not give a comprehensive characterization for protein SCHBs. Following Gregoret, although several groups have also made some analyses on protein SCHBs,[34–36] their works were too curt to provide enough information on the detailed geometric characteristics of protein SCHBs.

In this study, statistical analysis for protein SCHBs was performed over 500 high-resolution protein crystal structures, attempting to make a comprehensive insight into geometric characteristics of different protein SCHBs. In contrast to previous works made by Gregoret *et al.*,[33] this study has been significantly improved in following aspects: (i) based on 500 high-resolution protein structures collected from Top500 database, our dataset is greatly enhanced in both the quantity and quality in comparison with that of Gregoret *et al.*, thus significantly improving the statistical accuracy and reliability; (ii) SCHBs were elaborately categorized according to donor/acceptor behaviors, and different geometric criteria were defined for corresponding SCHBs; (iii) all the SCHBs were analyzed using explicit hydrogen approach, thus SCHBs can be clearly distinguished from the nonhydrogen bond interactions; (iv) besides conventional SCHBs, we also inspected some special SCHBs such as S—H. . .S and S—H. . .π; (v) detailed geometrical parameters for different SCHBs were presented, and these information would be of interest to experimentalists and theoreticians.

METHODS

Top500 database

Different to other kinds of nonbonding interactions, H-bond possesses directionality and can be saturated. Considering protein's quality is more essential than the quantity, the top500 database[37] developed by Richardson's group was used for data analysis in the present work (see Supp. Info.). Top500 database consists of 500 elaborately selected low-homology protein structures, and these proteins satisfy the following criteria.

i. Resolution 1.8 Å or better.

ii. Clash score (for atoms B-factor $<$ 40) $<$ 22/1000 atoms (clash score is defined by Word *et al.*[38]).

iii. Fewer than 10/1000 atoms whose main-chain bond angles (incline to C_β) are $>$5 standard deviations from Engh & Huber geometry.[39]

iv. No unusual amino acids with main-chain substitutions.

v. No free-atom refinements.

vi. Wild type preferred to mutant if otherwise approximately equivalent, and if proteins related but not same, took best combination of resolution and clash score.

Because the sulfur atom can be an electrophile/nucleophile to interact with polar atom and group,[27–29] accurate coordinates of hydrogen atoms in proteins are essential for distinguishing SCHBs from those of non-H-bonding interactions. All protein hydrogen atoms in the Top500 database were added and optimized by REDUCE.[40] In this way, hydrogen atoms involved in SCHBs are explicitly located, and H-bonding interactions are thus identified more accurately.[41,42]

REDUCE assessment

Because the accuracy of hydrogen positions is directly related to the reliability of identified SCHBs, REDUCE-predicted hydrogen positions are systemically assessed using neutron diffraction-determined protein structures. Here protein hydrogens are classified into three categories (see Fig. 1): (i) constrained H, their spatial positions are completely determined by heavy atoms and chemically geometric constraints, (ii) rotatable H, such as —OH, —SH, —NH_3^+ that are positionally uncertain hydrogens because of single bond rotating, and (iii) flipable H, referring to those hydrogens in amide groups of asparagines/glutamines and in side-chains of histidines, due to

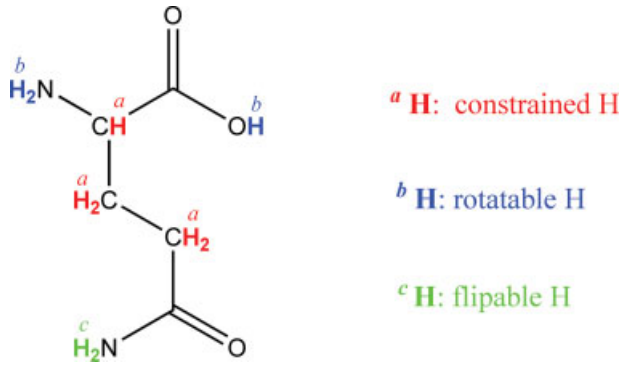

Figure 1

An instance for constrained H, rotatable H, and flipable H. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the amide groups and aromatic rings can perform 180° flip.

PDB entries and the root mean square error (RMSE) between the RUDUCE-predicted and experimentally measured hydrogens for 19 neutron diffraction-determined proteins are listed in Table I. It is revealed the REDUCE has a very favorable prediction accuracy for constrained H and flipable H, with the RMSE of about 0.15 Å, which is quite close to the experimental error level; although for the rotatable H where single bonds can be freely rotated, the prediction error is larger than that of constrained H and flipable H, with an average value of 0.42 Å. In addition, the REDUCE-prediction error is significantly correlated with the resolution of protein structures (correlation coefficient $r = 0.827$), indicating the accuracy of REDUCE-predicted hydrogens depends on the quality of protein structures. The average error for all predicted hydrogens in the 19 proteins is 0.22 Å, it is satisfactory and eligible for our study due to the 0.2 Å error was considered as the best prediction in previous study of Forrest *et al.*[43]

Classification

In present study, SCHBs were categorized into two types, one is conventional SCHB and another is nonconventional SCHB. In the conventional SCHB, sulfur atoms H-bond with nitrogen or oxygen atoms. Conventional SCHB includes three cases: (i) methionine S served as H-bond acceptor, (ii) cysteine S served as H-bond donor, and (iii) cysteine S served as H-bond acceptor. Nonconventional SCHBs are those special H-bonds containing sulfur atoms, also classified into three cases: (i) half-cystine (in disulfide bond) S served as H-bond acceptor, (ii) S served as H-bond donor and acceptor, and (iii) cysteine S served as H-bond donor interacts with π-electron acceptor.

For convenience, above-mentioned SCHBs are abbreviated as follows:

i. methionine S served as H-bond acceptor: Met←X.
ii. cysteine S served as H-bond donor: Cys→X.
iii. cysteine S served as H-bond acceptor: Cys←X.
iv. half-cystine S served as H-bond acceptor: Hcys←X.
v. S served as H-bond donor and acceptor: Cys→S.
vi. cysteine S served as H-bond donor interacts with π-electron acceptor: Cys→π.

Where X denotes N or O, arrow is pointed from H-bond donor to acceptor.

Geometry and definition of SCHBs

SCHBs are longer than those formed between nitrogen or oxygen atoms because of sulfur's larger size and more diffuse electron cloud. In previous works, the adopted geometric criteria for SCHBs are quite rough that cannot exactly define and identify SCHBs.[33–36] In the present work, a series of fine criteria was set for different kinds of SCHBs. In Figure 2, geometric parameters d, D, θ, ψ, φ, and χ for six kinds of SCHBs are defined. In which sulfur, hydrogen, donor, acceptor, and acceptor antecedent (heavy atom) are denoted by S, H, D, A, and AA, respectively. (i) When the S is served as H-bond acceptor and N or O as H-bond donor [Fig. 2(a,c,d)], the geometric criteria is defined as $d < 3.2$ Å, $D < 4.1$ Å, and $\theta > 90°$. (ii) When the N or O is served as H-bond acceptor and S as H-bond donor [Fig. 2(b)], the $d < 3.0$ Å,

Table I

Comparing RUDUCE-Predicted H Positions with the Neutron Diffraction-Measured H Positions for 19 Protein Structures

		RMSE between the predicted and measured H positions (Å)			
PDB entry	Resolution (Å)	Constrained H	Rotatable H	Flipable H	All
1C57	2.40	0.241	0.586	0.143	0.323
1CQ2	2.00	0.187	0.472	0.139	0.221
1GKT	2.10	0.179	0.458	0.119	0.238
1IO5	2.00	0.134	0.345	0.187	0.156
1IU6	1.60	0.112	0.257	0.122	0.128
1L2K	1.50	0.117	0.348	0.174	0.136
1LZN	1.70	0.154	0.377	0.161	0.189
1NTP	1.80	0.148	0.418	0.108	0.167
1VCX	1.50	0.121	0.381	0.072	0.145
1WQ2	2.40	0.204	0.459	0.179	0.267
1XQN	2.50	0.215	0.489	0.137	0.241
2DXM	2.10	0.234	0.478	0.141	0.287
2EFA	2.70	0.224	0.394	0.113	0.281
2GVE	2.20	0.201	0.447	0.184	0.279
2INQ	2.20	0.198	0.379	0.175	0.213
2MB5	1.80	0.157	0.336	0.124	0.189
2VS2	2.00	0.169	0.412	0.117	0.196
2YZ4	2.20	0.224	0.518	0.159	0.264
3CWH	2.20	0.258	0.484	0.125	0.297
Average	2.04	0.183	0.423	0.141	0.222

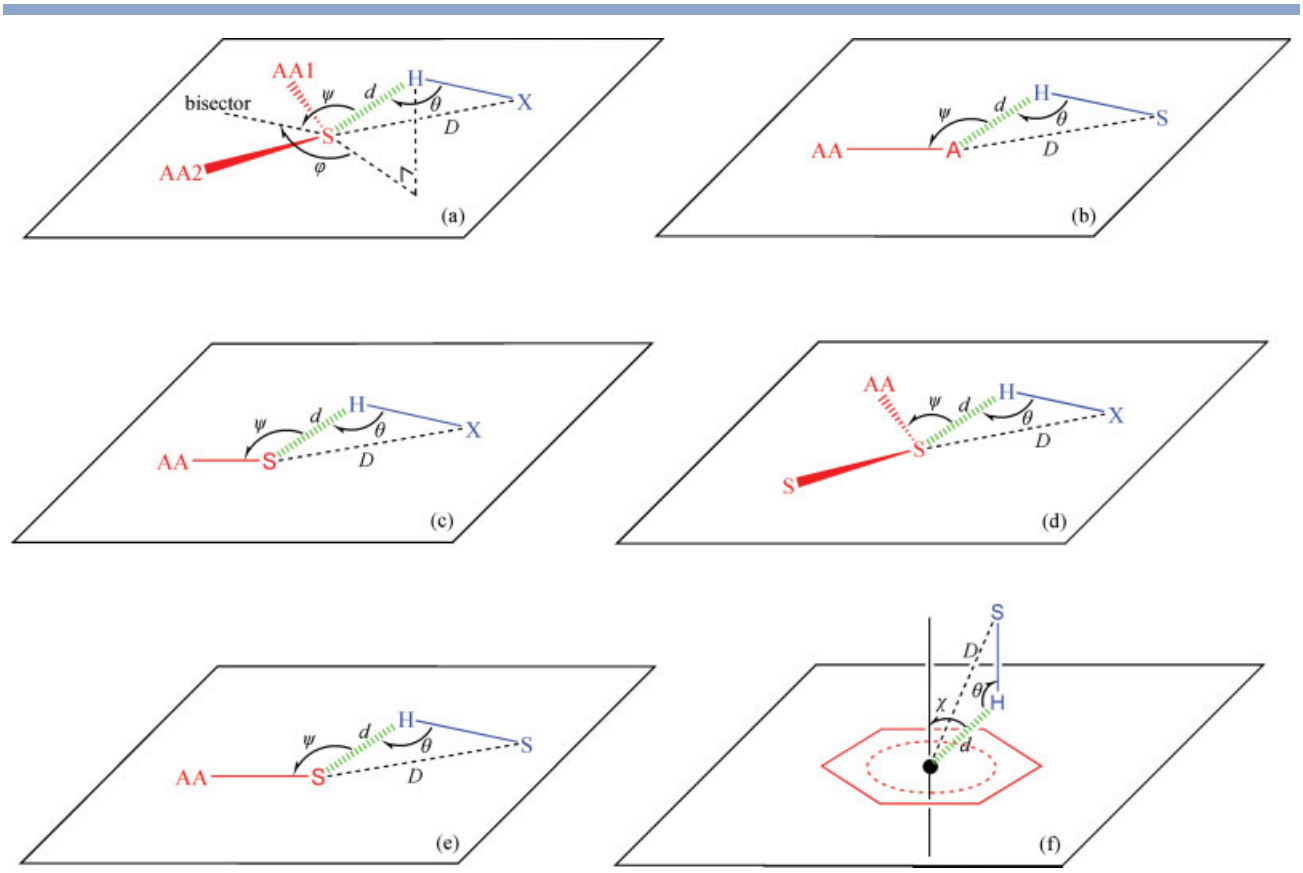

Figure 2

H-bond geometry (X = O or N). (**a**) Methionine sulfur atom served as H-bond acceptor. (**b**) Cysteine sulfur atom served as H-bond donor. (**c**) Cysteine sulfur atom served as H-bond acceptor. (**d**) Disulfide bond sulfur atom served as H-bond acceptor. (**e**) Cysteine/methionine sulfur atom served as H-bond donor/acceptor. (**f**) Cysteine sulfur atom/aromatic ring served as H-bond donor/acceptor.

$D < 4.3$ Å, and $\theta > 90^\circ$ (because S—H is longer than O—H and N—H, the d is shorter and D is longer in contrast with the case (i)). (iii) When the H-bond donor and acceptor are both S [Fig. 2(e)], the $d < 3.3$ Å, $D < 4.5$ Å, $\theta > 90^\circ$. Obviously, the d and D are longer than the former two cases. (iv) When H-bond donor S interacts with π-electron acceptor [Fig. 2(f)], it is fundamental that donor heavy atom is positioned "over" the π-face of the acceptor. Steiner and Koellner[44] have systematically examined geometric characteristics of X-H...π (X = O, N, S, or acidic C). In the light of their works, the criteria for S—H...π is set as $d < 3.3$ Å, $D < 4.5$ Å, and $\theta > 120^\circ$ in consideration of the particular properties of sulfur atom and large geometric flexibility of π-electron H-bonds.[3] Tryptophan, phenylalanine, tyrosine, and histidine can all be served as π-electron acceptor, and in case of tryptophan, the five-membered and six-membered ring systems are considered separately. In addition, previous studies demonstrated that the angle ψ of SCHBs in small organic complexes is close to 90° and presents a L-shape,[45–47] this is distinct from that of canonical H-bonds, so in this study the angle is set as $\psi > 80^\circ$.

Geometric parameters of SCHBs were calculated using in-home program HB_catcher written in C++, and this program was designed to perform batch processing of PDB files.

RESULTS AND DISCUSSION

Methionine S served as H-bond acceptor (Met←X)

In our data set, there are 2143 methionines. Using the criteria specified in method section, 232 Met←Xs have been identified. Each protein includes 0.464 (232/500) Met←Xs and each methionine can form 0.108 (239/2143) Met←Xs on average. It is revealed that SCHB is not particularly prevalent among methionine residues. Such a phenomenon can be ascribed to two aspects: methionine is hydrophobic and thus surrounded mostly by carbon atoms (rather than nitrogen and oxygen atoms), and the methionine Sis a poor H-bond acceptor.

Then to investigate donor influence on the Met←Xs, donor is categorized into five types: (i) backbone N;

Table II
Number, Geometric Mean, and Standard Deviation of Met←Xs with Different Donor Types

Donor type	Number	*d*	*D*	θ	ψ	φ
Backbone N	105	2.74 (0.27[a])	3.54 (0.25)	142.6 (21.0)	117.6 (20.6)	140.0 (30.1)
Amide N	48	2.81 (0.26)	3.59 (0.19)	140.3 (20.9)	124.2 (23.5)	141.8 (26.9)
Charged N	42	2.86 (0.23)	3.54 (0.21)	130.1 (22.4)	115.5 (21.4)	134.9 (31.1)
Aromatic N	13	2.71 (0.27)	3.51 (0.24)	139.0 (16.1)	117.7 (22.7)	133.0 (35.9)
Hydroxyl O	24	2.42 (0.25)	3.32 (0.25)	156.8 (19.4)	122.0 (19.6)	138.7 (27.8)
All	232	2.74 (0.29)	3.52 (0.24)	141.1 (21.9)	119.0 (21.6)	137.8 (29.8)

[a]Data in bracket are standard deviation.

(ii) amide N in the side chain of Asn and Gln (amide N); (iii) charged N in the side chain of Arg and Lys (charged N); (iv) aromatic N in the side chain of Trp and His (aromatic N); (v) hydroxyl O in the side chains of Ser, Thr, and Tyr (hydroxyl O). The number, geometric mean and standard deviation of different donors are listed in Table II. The most frequent donor atom for Met←Xs is backbone N that accounts for nearly half of the Met←Xs, whereas the occurrences of other four donors are all below 50. In proteins, the abundance of backbone N is remarkably more than the other four donors, thus leading to a greater probability of participating in Met←Xs. To compare H-bond parameters in Table II, the Met←Xs involving oxygen atom have a shorter bond length and a larger bond angle than that involving nitrogen atom. In contrast with nitrogen, the oxygen possesses smaller van der Waals radii and stronger electronegativity. There is no significant geometric difference between different Met←Xs, and the average *d*, *D*, θ, ψ, and φ are 2.74 Å, 3.52 Å, 141.1°, 119.0°, and 137.8°, respectively.

We used principal component analysis (PCA) to process the geometric parameters of all Met←Xs, and the extracted top three principal components (PCs) accumulatively account for 94.3% variance of original data matrix (including five geometric parameters of the Met←Xs). Then all the Met←Xs were plotted in the three PC spaces and different kinds of Met←Xs were denoted using different symbols. As shown in Figure 3, the Met←Xs with different donors have been distinguished poorly in the PC spaces, this evidence further confirms that the geometric characteristics of Met←Xs are not related significantly with its donor types.

In vacuum, the H-bond geometry of dimethylsulfide(acceptor)-methanol(donor) was optimized in RI-MP2 level by Wennmohs *et al.*,[17] and the resulted optimal *d*, θ, and ψ were 2.34 Å, 151,° and 81°, respectively. Comparing these parameters with the geometric mean of protein Met←Xs (X = hydroxyl O) (*d*, θ, and ψ are 2.42 Å, 156.8,° and 122.0°, respectively, see Table II), the both are not considerably different in *d* and θ, whereas the ψ in proteins is remarkably larger than that in theoretically optimized small molecules. According to Legon and Mille,[47] the axis of the donor XH (X = O or N) in free-state coincides with the supposed axis of a nonbonding electron pair as conventionally envisaged. Because the nonbonding electron pairs of sulfur atom are nearly perpendicular to the AA1-S-AA2 plane, the ψ of small molecular complexes with little conformational constraints would approach 90° and shows L-shape (just as the case reported by Wennmohs *et al.*[17]) [Fig. 4(a)]. However, due to proteins are closely packed in physiological state, conformational space of side chains is greatly restricted, so the ψ is remarkably deviated from a standard geometry [Fig. 4(b)]. According to our statistical analysis, the ψ of Met←Xs (X = hydroxyl O) in proteins is closer to that of dimethyloxygen(acceptor)-hydroxyl(donor) [Fig. 4(c)] than of dimethylsulfide(acceptor)-hydroxyl(donor) [Fig. 4(a)]. Such a conclusion is also suitable for other protein Met←Xs.

Cysteine S served as H-bond donor (Cys→X)

Our dataset comprises 753 nondisulfide bonding cysteines and 465 Cys→Xs were identified. Each protein and

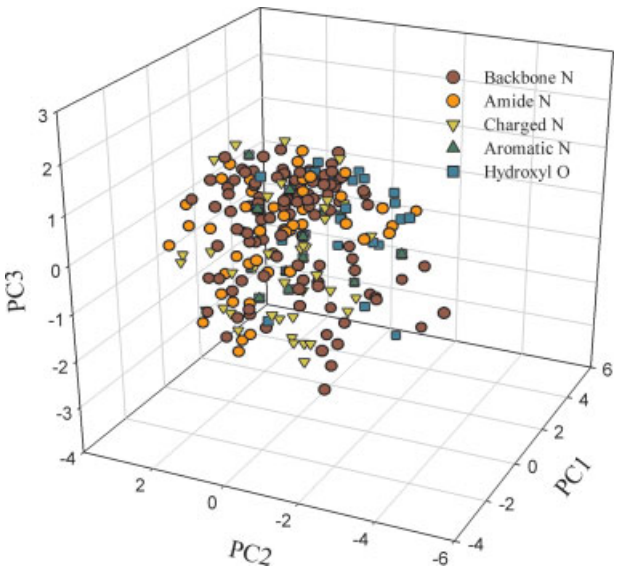

Figure 3
Sore scatters of 232 Met ← Xs in the top three principal component spaces. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

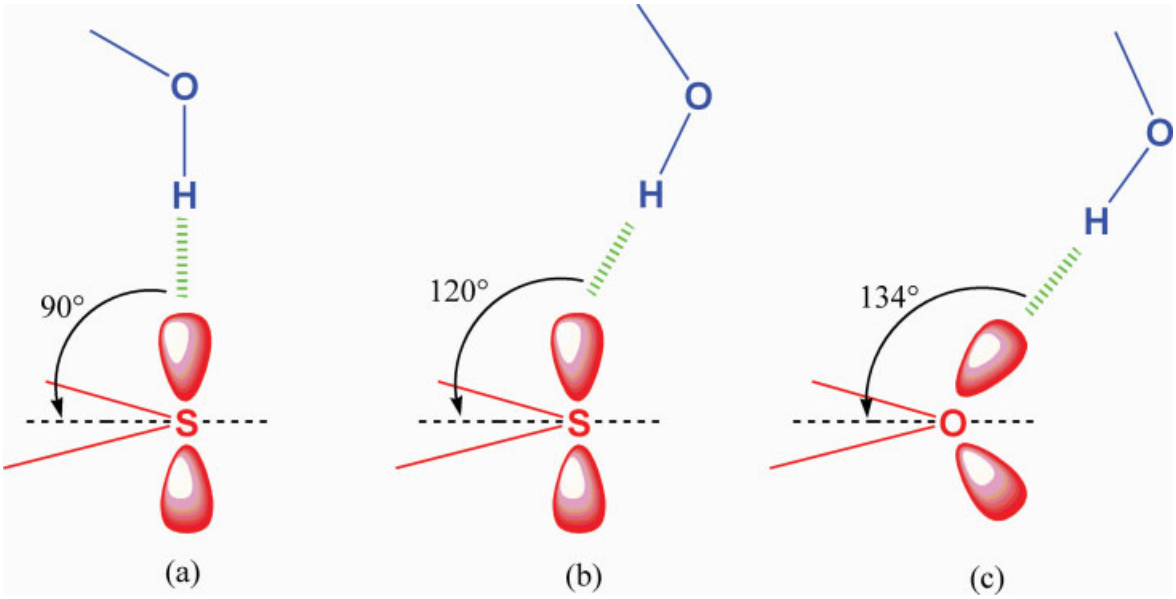

Figure 4

(**a**) ψ of the dimethylsulfide-hydroxyl H-bond in free-state.[47] (**b**) ψ of methioninehydroxyl H-bond in proteins (in present study). (**c**) ψ of dimethyloxygen-hydroxyl H-bond in free-state.[47] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

each cysteine on average possess 0.930 (465/500) and 0.618 (465/753) Cys→Xs, respectively. The sulphydryl group of cysteine is a moderately good H-bond donor.

Acceptor of Cys→Xs is categorized into eight types: (i) backbone N; (ii) amide N in the side chains of Asn and Gln (amide N); (iii) charged N in the side chains of Lys and Arg (charged N); (iv) aromatic N in the side chains of Trp and His (aromatic N); (v) carbonyl O in the backbone (backbone O); (vi) amide O in the side chains of Asn and Gln (amide O); (vii) carboxyl O in the side chains of Glu and Asp (carboxyl O); (viii) hydroxyl O in the side chains of Ser, Thr, and Tyr (hydroxyl O). Table III lists the statistical results of different kinds of Cys→Xs. When compared with the acceptor N, the Cys→Xs with acceptor O have shorter bond length but larger bond angle. The sulphydryl group of cysteine is prone to form H-bond with the backbone O and backbone N, accounting for 85.4% of the total Cys→Xs (397/465). As the acceptors for Cys→Xs, the backbone N is nearly one-thirds of backbone O (83/314). It is revealed that backbone N served as the acceptor for Cys→Xs is prevalent owing to the high abundance of backbone N in proteins, which is similar to the case of backbone N served as the Met←Xs donor. For backbone O, the preference is not only due to its abundance, and more importantly, it is H-bonded with the sulphydryl group of cysteine to thus stabilize the α-helix (see Fig. 5), which is similar to the cases of serine and threonine.[49] This phenomenon was also observed in previous work.[33]

Table III
Number, Geometric Mean, and Standard Deviation of Cys→Xs with Different Acceptor Types

Acceptor type	Number	*d*	*D*	θ	ψ
Backbone N	83	2.84 (0.12[a])	3.50 (0.31)	111.8 (21.6)	109.0 (20.1)
Amide N	6	2.83 (0.11)	3.65 (0.22)	121.7 (15.3)	117.1 (24.4)
Charged N	6	2.79 (0.18)	3.75 (0.23)	131.8 (18.5)	111.3 (24.8)
Aromatic N	17	2.58 (0.22)	3.63 (0.25)	139.7 (18.7)	113.1 (21.9)
Backbone O	314	2.41 (0.26)	3.48 (0.21)	142.5 (20.1)	120.1 (18.2)
Amide O	11	2.45 (0.36)	3.51 (0.23)	144.2 (22.9)	119.1 (21.7)
Carboxyl O	8	2.44 (0.25)	3.50 (0.22)	139.8 (16.6)	111.3 (20.2)
Hydroxyl O	20	2.45 (0.30)	3.54 (0.18)	146.4 (23.3)	117.9 (27.2)
All	465	2.51 (0.30)	3.50 (0.24)	136.5 (23.5)	117.4 (19.8)

[a]Data in bracket are standard deviation.

In the 314 Cys→backbone Os, 115 were found between sulphydryl group of cysteine residue *i* and the carbonyl oxygen of residue *i*-4, and the distance four is approximate to the standard thread pitch of 3.6 for α-helix; whereas in all of 83 Cys→backbone Ns, none was formed between the residues *i* and *i*-4. Figure 6 shows the distribution of bond length *d* and angle θ for Cys→backbone Os, and the distribution center and variance fitted using Gaussian function are 2.28 Å, 0.016 Å^2, and 138.5°, 589.3°^{2}, respectively (the center and variance indicate the peak position and preference, respectively). It is evident that the distribution of bond length obeys Gaussian distribution well (adjusting correlation coefficient Adj. r^2 = 0.817). As shown in Figure 6(a), bond length *d* achieves its peak at 2.28 Å with a small variance

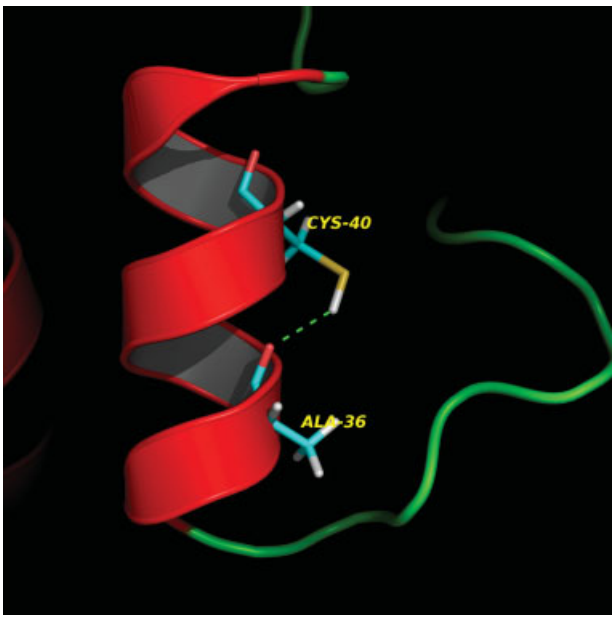

Figure 5

A Cys → X formed between the backbone O of Ala36 and the sulfhydryl group in side chains of Cys40 (PDB entry: 1ay7[48]). This hydrogen bond helps to stabilize the α-helix, its *d*, *D*, θ and ψ of 2.34 Å, 3.43 Å, 139.0°, and 137.7°, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of 0.016 Å^2, indicating that the *d* has an obvious preference around the peak and less appears in other regions. In Figure 6(b), the peak of bond angle distribution fitted by Gaussian function is located at 138.5°, with the variance of 589.3°^{2}, showing an obscure distribution preference. In addition, most samples are presented in the range >120°, and suggested by adjusting correlation coefficient (Adj. r^2 = 0.622), the θ does not obeys the Gaussian distribution well.

Cysteine S served as H-bond acceptor (Cys←X)

151 Cys←Xs have been identified in our dataset. Each protein includes 0.302 (151/500) Cys←Xs and each cysteine can form 0.201 (151/753) Cys←Xs on average. It is indicated that the Cys←X is more frequent than Met←X in proteins. However, if we attached no constraints on the ψ, a large number of small-angle Cys-X interactions which is distinct to the normal Cys←Xs would be found in proteins. These small-angle interactions are found to be nearly all formed between the side chain Sof cysteine *i* and the backbone N of this residue *i* or the next residue *i*+1. Pal and Chakrabarti[27] demonstrated that cysteine S can form non-H-bonding interaction with carbonyl group. In present study, we found nearly three-fourths of the sulfur atoms in small-angle Cys-X interactions participate the intraresidue S...C=O. As shown in Figure 7(a), an intraresidue small-angle interactions and an intraresidue S...C=O are simultaneously formed in chain A Cys308 of regulator of chromosome condensation (RCC1). Cys308 S served as acceptor forms an 2.929 Å adjacent Cys←X with the backbone N—H. Indicated by Newman projection diagram [Fig. 7(b)], the side chain of Cys308 is in the *g*-state (χ_1 = 60°), thus leading

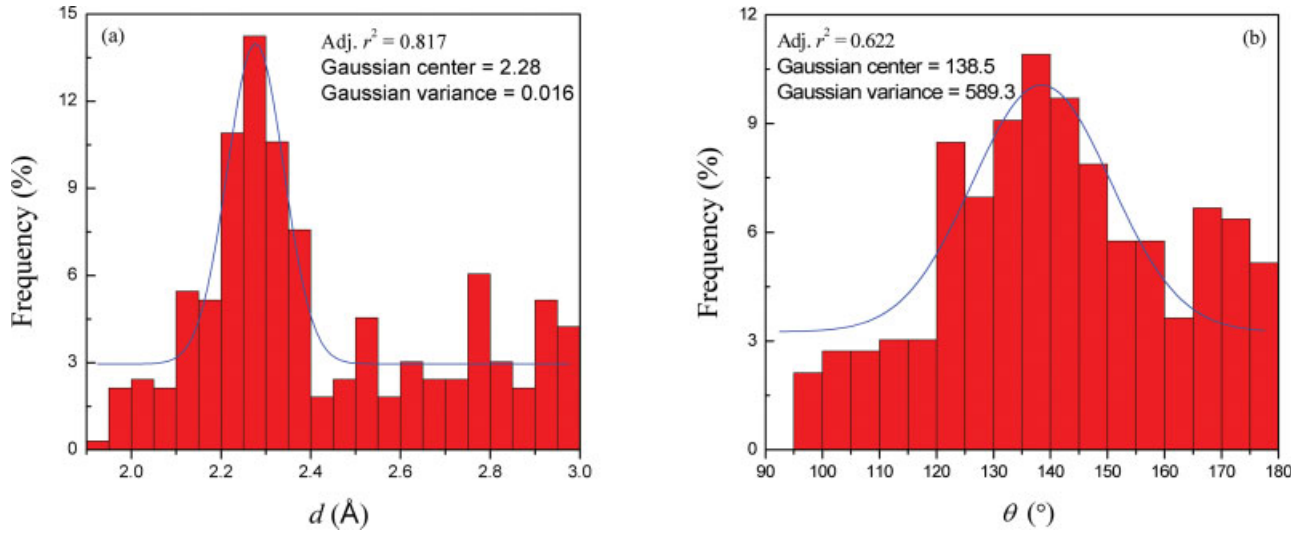

Figure 6

Distributions of bond length d (**a**) and angle θ (**b**) for the 314 Cys → Xs (X = backbone O). The distributions are fitted using the standard Gaussian function, yielding the distributional center and variance for *d* and θ. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

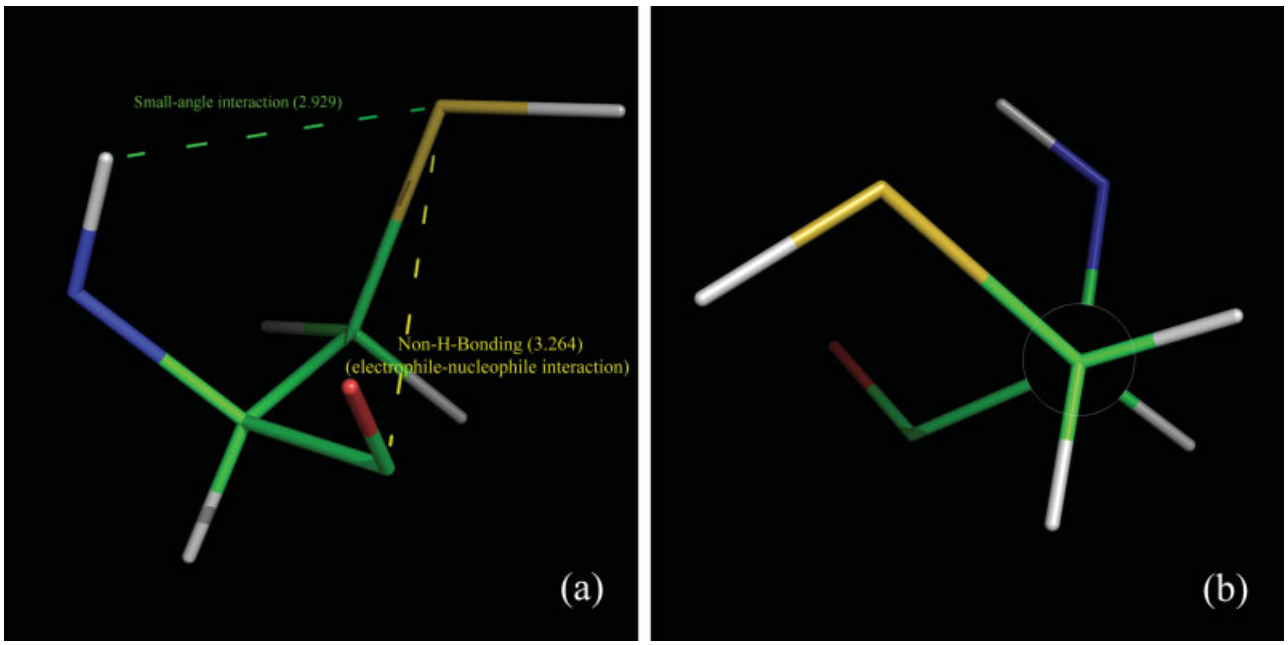

Figure 7

(**a**) An intra-residue small-angle Cys-X interaction and an intra-residue non-hydrogen bonding interaction (electrophile-nucleophile interaction) simultaneously formed in chain A Cys308 of protein RCC1 (PDB entry: 1a12[50]); (**b**) Newman projection diagram for Cys308, the side chain is in the g-state ($\chi_1 \approx 60^\circ$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sulphydryl S approaches the carbonyl group to form intraresidue S. . .C=O.

Compared to that of the small-angle interactions, the average angle θ and ψ of normal Cys←Xs are much larger, while the average bond length *d* and *D*, the *d* of small-angle interactions is much longer but *D* is shorter. It can be considered as the conformation of small-angle interactions is distorted remarkably. Because the small-angle interactions cannot be confirmed as the valid H-bonding, they are not considered as SCHBs in present study. Table IV lists a series of statistical results for normal Cys←Xs. Each cysteine can form about 0.2 (151/753) Cys←Xs, which is similar to the case of Met←Xs. Because of the high abundance in proteins, the backbone N is usually served as the donor for Cys←Xs. Besides, we found the Cys←Xs are presented in different second structures such as α-helix, β-sheet, and coil, with the average bond length *d* and angle θ of 2.79 Å and 142.1°, respectively. When served as H-bond acceptor, sulfur atoms from cysteine and methionine possess similar behavior, and the both are poor H-bond acceptor.

Table IV
Number, Geometric Mean, and Standard Deviation of Cys←Xs with Different Donor Types

Donor type	Number	*d*	*D*	θ	ψ
Backbone N	95	2.79 (0.23[a])	3.58 (0.19)	143.5 (24.6)	104.6 (17.7)
Amide N	12	2.87 (0.25)	3.61 (0.23)	136.8 (25.0)	120.6 (28.7)
Charged N	18	2.78 (0.33)	3.44 (0.23)	128.2 (22.2)	119.6 (23.2)
Aromatic N	8	3.05 (0.12)	3.50 (0.18)	109.4 (15.4)	124.9 (31.3)
Hydroxyl O	18	2.76 (0.35)	3.45 (0.24)	133.1 (27.8)	120.1 (22.6)
All	151	2.80 (0.26)	3.54 (0.21)	138.1 (25.6)	110.6 (22.0)

[a]Data in bracket are standard deviation.

Half-cystine (disulfide bond) S served as H-bond acceptor (Hcys←X)

Allosteric disulfide bond regulates protein function when they break and/or form,[51,52] and it can be also served as H-bond acceptor to interact with the surrounding.[33] In our dataset, 1048 half-cystines were found to participate in disulfide bonding (i.e., 524 disulfide bonds) and 309 Hcys←Xs were identified. Each protein comprises 0.618 (309/500) Hcys←Xs and each half-cystine can form 0.295 (609/1048) Hcys←Xs on average, slightly more than the case of nonadjacent Cys←Xs and Met←Xs. In contrast with cysteines, the number of half-cystines is much more, but the both are nearly equivalent in H-bonding ability. Furthermore, there are also many small-angle Hcys-X interactions, this is similar to that of Cys←Xs.

Table V lists statistical results of Hcys←Xs with different kinds of donors. The short distances ($D < 3.5$ Å) between the oxygen atoms of hydroxyl groups and the sulfur atoms of half-cystines were observed by Gregoret *et al.*, and short distances to nitrogen atoms are rarer.[33] But in our observation, short contacts between the oxy-

Table V
Number, Geometric Mean, and Standard Deviation of Hcys←Xs with Different Donor Types

Donor type	Number	*d*	*D*	θ	ψ
Backbone N	255	2.68 (0.25[a])	3.49 (0.20)	144.1 (22.3)	109.0 (16.1)
Amide N	13	2.80 (0.18)	3.48 (0.22)	129.9 (22.2)	125.6 (25.8)
Charged N	12	2.87 (0.26)	3.41 (0.12)	118.3 (22.6)	113.4 (18.5)
Aromatic N	4	2.21 (0.88)	3.09 (0.84)	149.6 (20.7)	107.5 (17.9)
Hydroxyl O	25	2.58 (0.33)	3.39 (0.23)	146.0 (24.5)	114.0 (32.4)
All	309	2.68 (0.28)	3.47 (0.22)	142.7 (23.1)	110.2 (18.7)

[a]Data in bracket are standard deviation.

gen and sulfur atoms are also prevalent. In investigation of geometric parameters for Hcys←Xs, Cys←Xs, and Met←Xs, their average angle θ and ψ are very approximate, but the average bond lengths *d* and *D* of Hcys←Xs are slightly shorter. By molecular graphics exhibition, we found the two sulfur atoms in the disulfide bond are very close, thus facile to form bifurcated Hcys←Xs, that is, one H-bond donor is simultaneously interacting with the two sulfur atoms of a disulfide bond, hence shortening the donor-acceptor distance. In proteins, the bifurcated Hcys←X is frequently observed. Figure 8 shows an example of bifurcated Hcys←X that links two close α-helixes together. For the bifurcated Hcys←X, its number is larger and strength much stronger than other Hcys←Xs, so we believe it play an important role in stabilizing tertiary structure of proteins.

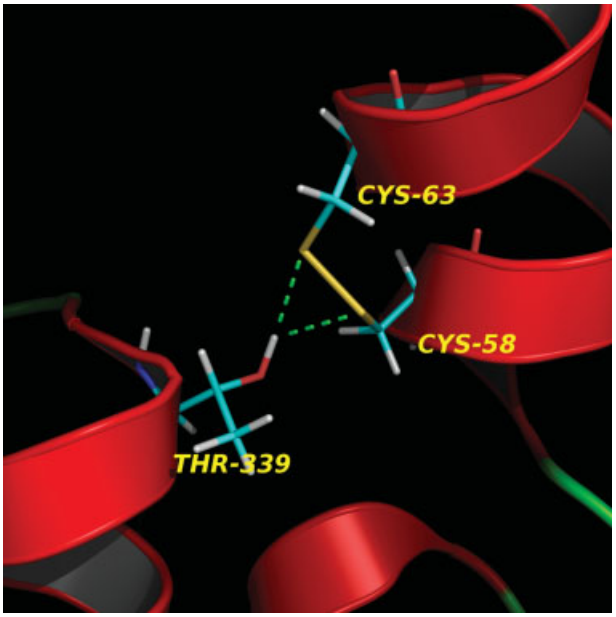

Figure 8
A bifurcated Hcys ← X formed between the hydroxyl group in Thr339 and the two sulfur atoms in disulfide bond Cys58-Cys63. The bifurcated Hcys ← X links two α-helixes together (PDB entry: 3grs[53]), and the *d*, *D*, θ, and ψ of the two H-bonds in the bifurcated Hcys ← X are 2.66 Å, 3.34 Å, 126.1°, 68.2° and 2.51 Å, 3.48 Å, 163.1°, 159.5°, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

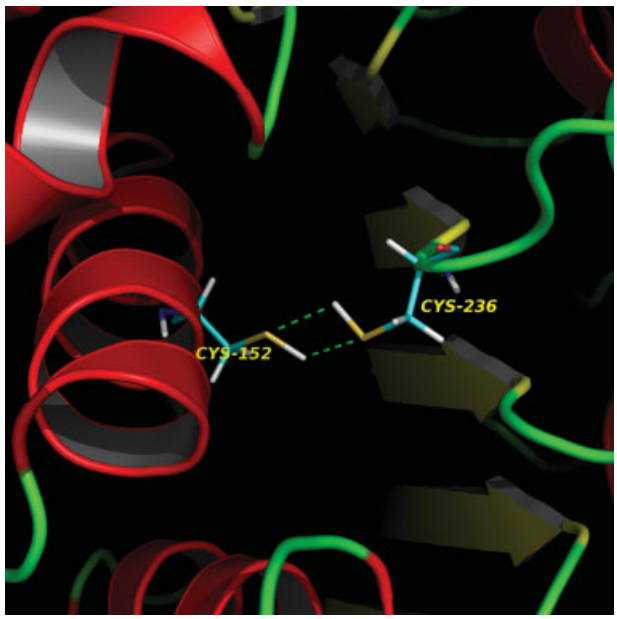

Figure 9
A dual Cys → S formed between the reduced Cys152-Cys236 pair. This dual Cys → S links a α-helix with a β-strand end together (PDB entry: 1a4i[54]), and the *d*, *D*, θ, and ψ of the two H-bonds in the dual Cys → S are 2.64 Å, 3.35 Å, 111.7°, 174.3° and 2.65 Å, 3.35 Å, 111.5°, 166.7°, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

S served as H-bond donor and acceptor (Cys→S)

Only sulphydryl group of cysteine can be served as the donor for Cys→Ss. Because sulfur atoms are relatively scarce in proteins, H-bonds formed between two sulfur atoms are quite rare. In fact, only 22 Cys→Ss were identified in our dataset and the number of methionines, cysteines, and half-cystines (whose S served as acceptor in Cys→Ss) is 6, 12, and 4, respectively. In the three residues, cysteine is least observed in proteins, whereas it forms the largest number of Cys→Ss (when served as acceptor). Analyses of crystal structures we found that: (i) nondisulfide-bonding cysteine–cysteine pairs (in reduced state) are usually connected by Cys→Ss, and (ii) due to the cysteine simultaneously play double roles of H-bond donor and acceptor, cysteine–cysteine pairs are mostly behaved as dual Cys→Ss. Figure 9 shows a dual Cys→S formed between a cysteine-cysteine pair, and obviously, the two cysteines can be further oxidized to a disulfide bond. It may be inferred that under a mild condition, protein tertiary structures are fixed by the dual Cys→Ss formed between the cysteine–cysteine pairs, whereas when the condition is changed, for example, the

Table VI
Number, Geometric Mean, and Standard Deviation of Cys→Ss with Different Acceptor Residue Types

Acceptor residue type	Number	*d*	*D*	θ	ψ
Methionine	6	2.49 (0.57[a])	3.68 (0.47)	156.4 (19.8)	110.9 (28.5)
Cystine	12	2.62 (0.46)	3.62 (0.32)	140.5 (28.7)	131.0 (35.5)
Half-cystine	4	2.29 (0.40)	3.39 (0.18)	141.3 (19.4)	114.1 (44.8)
All	22	2.55 (0.47)	3.61 (0.34)	144.8 (25.7)	123.9 (34.0)

[a]Data in bracket are standard deviation.

proteins are transported into extracelluar oxidizing environment from intracellular reducing environment, a covalent disulfide bond would be formed to reinforce the strength of cysteine–cysteine pair.

The statistical results of different kinds of Cys→Ss are summarized in Table VI, it is revealed that the bond length of S—H...S in proteins is shorter than that in small compounds (e.g., in thiosalicylic acid, the *d* and *D* of S—H...S are 2.72 Å and 3.99 Å, respectively[19]). In contrast with cysteine and half-cystine, ψ of methionine is slightly smaller, indicating the donor sulphydryl group is preferential to approach methionine S in the direction perpendicular to the AA1-S-AA2 plane. Surprisingly, the average *d* of Cys→Ss is remarkably shorter than above mentioned other SCHBs, whereas its standard deviation is larger. It may be considered as the dual Cys→Ss formed between the cysteine–cysteine pairs lead to the bond length *d* shortened sharply, which is similar to that of bifurcated Hcys←Xs, but the bond length of other nondual Cys→Ss is much longer. Therefore, the average *d* of Cys→Ss is generally shorter, but yielding bigger variance. Because of the Cys→Ss are rarely found in proteins, we think such a kind of SCHB is not important for the structure and function of proteins. However in some special cases, the protein structure and function can be smartly controlled and regulated using the peculiarity of Cys→Ss (e.g., cysteine–cysteine pair is prone to form dual H-bond and also to form disulfide bond).

Cysteine S served as H-bond donor interacting with π-electron (Cys→π)

Our dataset consists of 2564 histidines, 4423 phenylalanines, 3967 tyrosines, and 1640 tryptophans. Using the criteria defined in method section, 51 Cys→πs have been identified from the dataset. The statistical parameters of these SCHBs are listed in Table VII. The probability of forming Cys→πs in proteins is quite low, and in the four kinds of aromatic residues, the most efficient π-electron acceptor is the side chain of tryptophan (for each tryptophan, about 0.006 Cys→πs are formed on average), which is consistent with the situation that the donor is N—H or O—H.[44] Comparing geometric parameters of Cys→πs with the other five kinds of SCHBs, Cys→πs possess longer bond length *d* and larger angle θ. Being very poor H-bond acceptor, π-electron accepting polar H is not as good as the electron pair of polar atoms,[47] thus forming weak Cys→πs. Figure 10 shows a typical Cys→π, and this H-bond links a helix with a loop together.

Different kinds of Cys→πs were found to be geometrically distinct. The *d*, *D*, θ, and ψ distributions of 51 Cys→πs are separately shown in Figure 11, indicating that all parameters do not obey well the Gaussian distribution. For bond length *d*, two peaks are located around 2.7 Å and 3.0 Å, with a 2.87 Å mean. Bond length *D* is widely distributed, achieving the peak around 3.9 Å. Bond angle θ is mainly concentrated around 140°, with rare appearance in the range >160°. ψ, an important parameter for Cys→π, indicates deviations of sulphydryl H of the cysteine from π-electron center. The distribution

Table VII
Number, Geometric Mean, and Standard Deviation of Cys→πs with Different Acceptor Residue Types

Acceptor residue type	Number	*d*	*D*	θ	χ
Histidine	9	2.83 (0.19[a])	3.98 (0.24)	147.3 (10.0)	50.3 (7.9)
Phenylalanine	16	2.80 (0.21)	3.84 (0.22)	137.1 (11.1)	43.2 (8.0)
Tyrosine	16	2.93 (0.22)	4.02 (0.22)	144.0 (14.2)	42.7 (7.6)
Tryptophan	10	2.91 (0.22)	4.00 (0.24)	135.7 (7.6)	46.2 (7.0)
All	51	2.87 (0.22)	3.95 (0.24)	140.8 (12.3)	44.9 (8.1)

[a]Data in bracket are standard deviation.

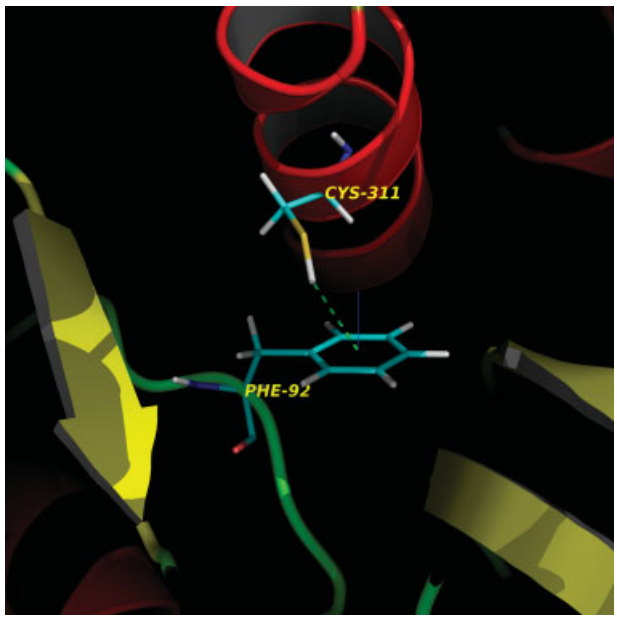

Figure 10
A Cys → π formed between the aromatic ring of Phe92 and the sulphydryl group in side chain of Cys311. This H-bond links an α-helix with a loop (PDB entry: 1gso[55]), its *d*, *D*, θ, and ψ are 2.96 Å, 4.13 Å, 150.3°, and 52.0°, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

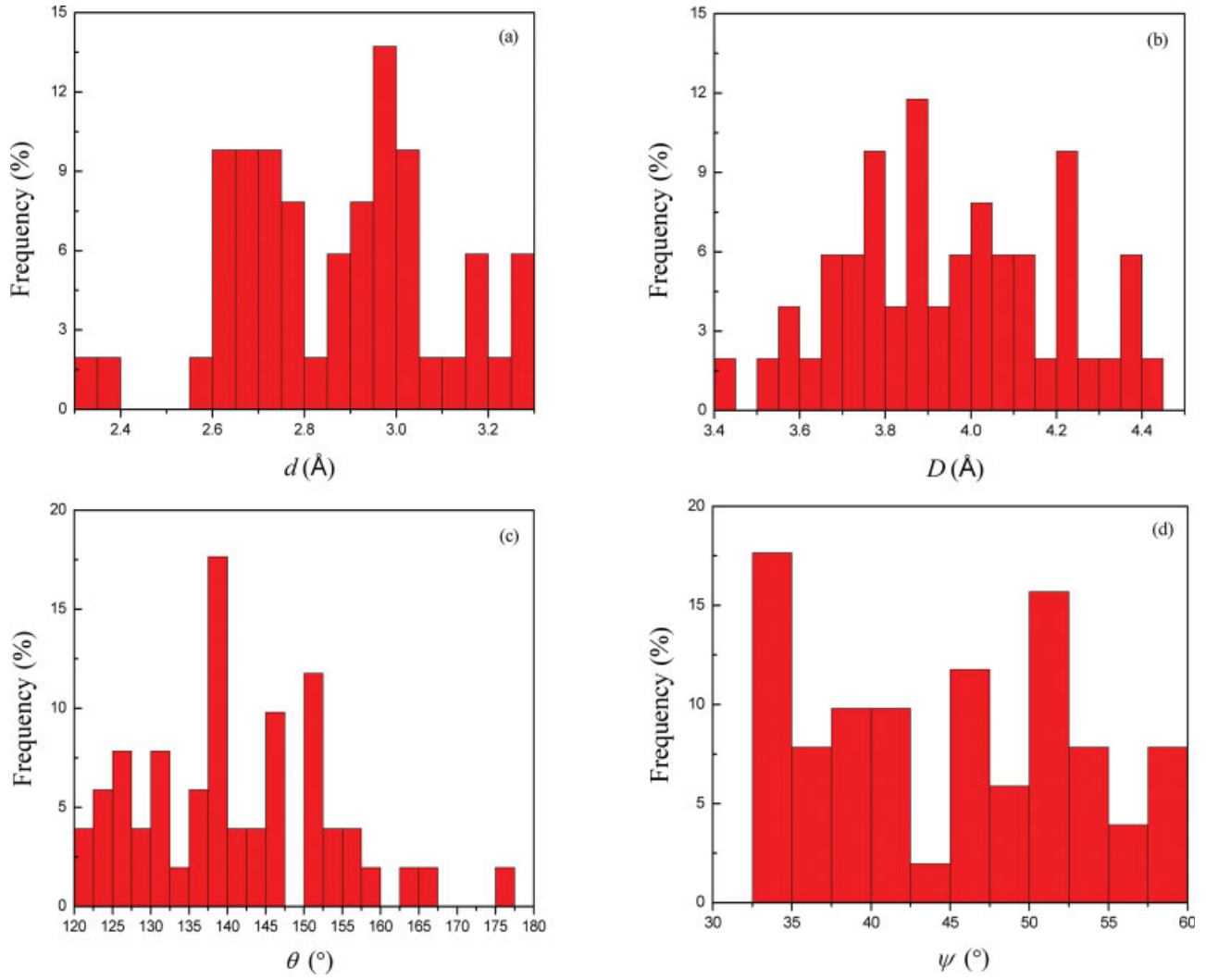

Figure 11

Distributions of (**a**) bond length d, (**b**) bond length D, (**c**) bond angle θ and (**d**) bond angle ψ for 51 Cys → πs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

scope of ψ is very narrow, and all the ψ are concentrated in the narrow interval of 30–60°, which implies the sulphydryl H is possibly over against the rim of the aromatic ring. Similar to the Cys→S, Cys→π has weak strength and small number, thus playing limited roles in protein functions.

CONCLUSIONS

Both the number and strength of protein SCHBs are significantly lower than canonical H-bonds. However due to the particular property of sulfur atom, SCHBs play important or even crucial roles in stabilizing of protein structure and in regulation of protein functions. For a long time, protein SCHBs has not received much attention and related researches are still in lack. In present study, we used statistical approaches to thoroughly investigate the detailed geometric characteristics of protein SCHBs and attempted to make a comprehensive insight into this kind of weak H-bond.

We observed sulfur atom of methionine and cysteine is poor H-bond acceptor, but the sulphydryl group of cysteine is a moderately good H-bond donor. This is consistent with previous reports.[27,33] By statistical analysis, it is revealed that sulfur atom is more capable of donating than accepting hydrogen bonds and the ratio of sulfur severed as donor to acceptor is about 5:1. In α-helixes,

considerable SCHBs were found between sulphydryl group of cysteine residue *i* and the carbonyl oxygen of residue *i*-4, which was also observed in Gregoret's work,[33] indicating Cys→Xs play an important role in stabilizing of helix, which is similar to the serine and threonine.[49] This may be the reason that the cysteine preferentially adopts a helical conformation.[51]

In this study, five conclusions can be proposed.

i. In our dataset, total of 1175 SCHBs (except Cys→πs) were identified. The average *d*, *D*, θ, and ψ (as well as standard deviation) are 2.66 Å (0.32 Å), 3.53 Å (0.25 Å), 140.6° (24.0°), and 116.2° (23.2°), respectively. In contrast with canonical H-bonds, SCHBs has longer bond length *d* and *D* but generally smaller angle θ and ψ.
ii. When sulfur atoms are served as H-bond acceptor in proteins, the H-bond donor X-H (X = N or O) usually forms a dihedral angle ψ of about 120° with the AA1-S-AA2 plane. This is distinct to that of sulfur atoms served as H-bond acceptor in free small molecules ($\psi \approx 90°$), but more likes the case that the oxygen atom is served as H-bond acceptor in free small molecules ($\psi \approx 134°$). However, there are two exceptions: when the methionine S is served as H-bond acceptor, the angle ψ of Cys→Ss is close 90°, and small-angle interactions are distorted severely, thus leading to its ψ around 60°.
iii. When the cysteine and half-cystine S are served as H-bond acceptor, they are prone to form small-angle interactions with the backbone N of their own or of the next residue. Such an interaction has a small ψ value and shows distorted conformation. Small-angle interactions are very weak and cannot be demonstrated to form valid H-bonding.
iv. Disulfide bonds served as acceptor are prone to form bifurcated H-bonds, but the cysteine-cysteine pair trend to form dual Cys→Ss. In these ways, SCHB strength is significantly enhanced, and the local stability of proteins is thus improved. If possible, cysteine–cysteine pairs can be further oxidized to the covalent disulfide bonds that would greatly reinforce the linkage strength.
v. Sulphydryl group of cysteines can loosely H-bond with π-electron acceptor, which has a longer bond length than the other SCHBs. The distribution of angle ψ of such π-electronic H-bonds was concentrated in the narrow interval of 30–60°, leading to the sulphydryl H oriented against the rim but not the center of the aromatic ring.

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