Metal Coordination Groups in Proteins Some Comments on Geometry, Constitution and B-values

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Metals are found in a wide variety of proteins where they may have important functional (usually catalytic) or structural roles. In a large proportion of structures the coordination group around the metal atom is made up of donor groups (carboxylate, imidazole, etc) from several amino-acid side chains, usually, but not necessarily, within one polypeptide chain; water molecules or other small molecules incorporated in the crystal as inhibitors, substrate analogues, cofactors etc. may also participate in the coordination group. In iron proteins, haem groups and clusters like Fe_4S_4 are also common.

The aim of recent work here (Harding, 1999, 2000, 2001) has been to provide information on metal coordination geometry which could be of use to protein crystallographers determining structures - at the stage of interpreting an electron density map, or in the restrained refinement of structures where the data is of limited resolution, or in the validation of structures. The work included a systematic extraction of geometric data from the Protein Data Bank (PDB, Bernstein et al., 1977; Berman et al., 2000) for metalloproteins of six selected metals. This note describes some further work on these metal coordination groups, which although not geometrical, might also be of relevance in protein structure determination - a) a systematic description and listing of metal coordination groups in terms of constituent amino-acid donor groups and their relative positions in the amino-acid sequence of the polypeptide chain, and b) a brief comparison of reported B values for the metal atom and the donor atoms in some of these groups. Much of this information has been assembled in a website on metal coordination groups. This includes links to some other websites relevant to metal coordination chemistry in proteins; among these is metalloscripps, which contains extensive geometrical information and tools for manipulation. The present descriptions and listings take no account of biological function or other properties of the metal sites; Degtyarenko (2000) describes an approach in terms of 'bioinorganic motifs' which does take function into account, and gives information on the available databases relevant to the field.

1. Geometry around the metal atom

An analysis was recently made of the geometry of metal-ligand interactions in metalloproteins (Harding, 2001) based on protein structures reported in the PDB. It dealt with Ca, Mg, Mn, Fe, Cu and Zn, by far the commonest metals in the PDB. The analysis started with accurate structural information derived by diffraction methods (resolution < 0.9Å) for appropriate small molecule complexes of these metals. The information was extracted from the Cambridge Structural Database (CSD, Allen and Kennard, 1993), and used to prepare a set of 'target' values for distances between each metal and each type of donor atom. The agreement between these target values and the values actually observed in metalloprotein structures in the PDB was then assessed, a) for all structures determined with resolution < 1.6Å, and b) for a representative set of structures determined with resolution < 2.8Å (July 1999 release in both cases). With some very small adjustments the target distances were shown to be good and they can therefore be recommended for use in interpretation of electron density maps, in restrained refinement, or in validation of metalloprotein structures. Also available are fuller details of the metal coordination geometry, listed for each protein in a representative set (<2.8Å resolution, no two proteins with more than 30% sequence identity, Feb. 2001 release of PDB at present); an example of the information given is shown in Table 1. [Note too that the preferred geometry of metal in relation to donor groups such as carboxylate , imidazole, is described by Harding (1999), and that target distances and comments on the geometry of Na and K in protein structures are available on request from the author and will be published in due course.]

Table 1 Example of information provided on metal coordination geometry in one metalloprotein

1ctt resolution = 2.20; total no. atoms = 2286; no. metal atoms = 1

metal no.	coordn sphere	donor	dist (A)	dif from target	occ. product	B metal	B donor
1 ZN 296							
	1	ND1 HIS 102	2.02	0.02	1.0	22.4	26.3
	1	SG CYS 129	2.42	0.13	1.0	22.4	23.6
	1	SG CYS 132	2.11	-0.18	1.0	22.4	19.4
	1	O HOH 700	1.84	-0.25	1.0	22.4	13.7

cngroup is HCC with CN 4 Zn, sequence diffs 27 3

COORDINATION NUMBER: 4

nearest description of shape - tetrahedral
(r.m.s. devn from tet 7.7, r.m.s. devn from sqp 41.1 degrees)

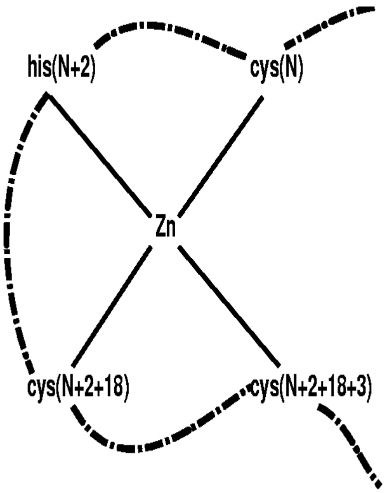
2. Constitution of metal coordination groups and relation to sequence

It may be useful to describe and categorise all these metal coordination groups in terms of the constituent donor groups and their relative positions in the amino-acid sequence of the polypeptide chain, and some attempts have been made to do this. Software which had already been developed for the analysis of the geometry of metal ligand interactions in metalloprotein structures needed only small further extensions to allow the present study.

The description of a coordination group, or *cngroup*, used here includes the nature of the amino-acid donor groups, their sequence and separation (number of residues apart) in the protein chain(s), together with the metal coordination number (which includes non-protein donors, water molecules and other ligands). This information can be summarised as in the two examples below, where it is assumed that N is a residue number, cysteine coordinates through the thiolate sulphur, and histidine through imidazole nitrogen.

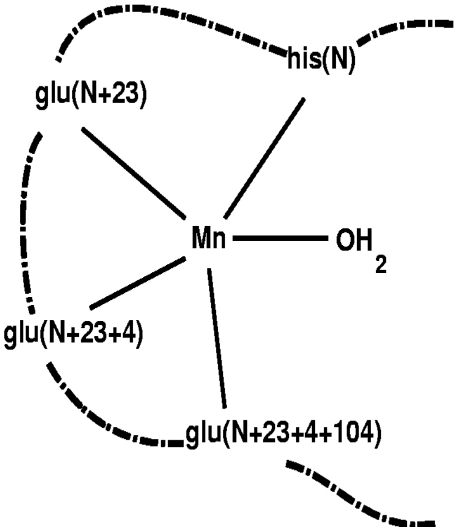
cngroup description

CHCC Zn 2 18 3, CN = 4 (in 1rmd)



cngroup description

HDDD Mn 23 4 104; CN=5 (in 1ria)



(Note that this takes no account of the nature of the other amino-acids in the protein chain, between those which contain donor groups, whereas sequence comparisons normally do.) Such *cngroup* sequences have been generated for Ca, Mg, Mn , Cu and Zn proteins.

Methods, procedures: The basis for generating the *cngroup* information is the program MP (Harding 2001, Acta Cryst D57,) which reads a PDB file, extracts coordinates and occupancy of each metal atom, and those of all atoms within 3.6 Å of the metal atom. Using target distances for each metal-donor atom combination it identifies atoms as donors if they lie within (target distance + tolerance) of the metal, and lists the amino-acids and residue numbers to which they belong, and the coordination number - as already illustrated in Table 1. It also evaluates sig, the r.m.s. deviation of the observed distances from the target values, and an alternative coordination number which would be found if an alternative, larger value of tolerance were used; these two, together with the resolution of the structure determination are useful in assessing the accuracy with which the *cngroup* has been identified. Metal coordination groups in which any atoms are disordered or have occupancy less than 0.7, are omitted.

Lists of PDB codes were obtained from the Jena Image Library search facility . (This gives a more complete listing than the PDB-3D Browser which searches in HET group names; some HET group names are odd, for example OC5 for Ca(OH2)52+, and in such cases no Ca atom is detected by the Browser.) From these lists protein and protein-nucleic acid complexes were selected, with structures determined by diffraction to a resolution ≤ 2.8 Å, and the program MP run, for all the structures available in the RCSB release of Feb2001. Additional smaller programs then gave the information on *cngroup* descriptions for the full lists or for selections from them. One such selection is a 'representative set' which excludes any structure which has more than 30% sequence identity with any other in the set; this used a culled PDB file 'cullpdb_pc30_res3.0_...'.

The list of *cngroup* descriptions is sorted in alphabetical order and gives sequence, metal, separation of coordinating residues in protein sequence, number of coordinating groups, pdbcode, 3 indicators of reliability, and the name of the metal and the first coordinated amino-acid in the chain. One letter amino-acid codes are used to specify the donor groups, and O indicates main chain carboxyl oxygen as donor. A carboxylate group (D or E) is always treated as one donor group, whether it is mono- or bidentate; at lower resolutions the distinction is not reliable, and particularly in Zn complexes, intermediate states are possible. The apaprent mono- or bi-dentate status is indicated at the end of the record. The presence of water molecules or donor atoms from non-protein molecules is indicated and an alternative output option includes these within the *cngroup* before sorting into sequence order .

Concerning cngroup definition: An atom is identified here as a donor when its distance from the metal atom is within (target distance + tolerance). The target distances have been carefully established using appropriate small molecule compounds in the CSD and checking against high resolution protein structures (Harding 1999, 2000, 2001). Errors in determination of atom positions, especially in low resolution structures might result in incorrect decisions on whether or not an atom is within the metal coordination group. For this reason structures determined at resolutions less good that 2.8 Å are not included at all. The tolerance was set at 0.75 Å after examining the distribution of (observed - target) distances. When the resolution is <1.8 Å there should be no 'wrong decisions' about whether an atom is within the metal coordination group; when the resolution is poorer, but still < 2.8 Å, some 'wrong decisions' will inevitably be made, but their number should be well under 5% of the whole. The three indicators given for each *cngroup* are provided to show more about the reliability of these decisions: i) the resolution, ii) the number of additional donor atoms which would have been found if the tolerance had been 0.95A, and iii) the r.m.s. deviation of observed from target distance in the *cngroup*.

A few metal atoms in the *cngroup* listings have coordination numbers lower than would normally be expected (i.e. <5 for Ca, <4 for Mg, Mn, Fe, Zn, <3 for Cu) . Usually this is the result of failure to identify a donor group such as a water molecule, in the electron density map, but in a few cases it could be the result of a shortcoming in the software, which does not (yet) detect when the metal atom is coordinated to a donor group in a neighbouring asymmetric unit of the crystal.

Lists generated and their possible uses: The lists now include all proteins containing any of the metals Ca, Mg, Mn, Cu, Zn, whose structures have been determined at resolution ≤ 2.8 Å. They include many repeat entries where the same metalloprotein molecule occurs more than once in the crystal asymmetric unit, as well as occurrences of the same *cngroup* sequence in very closely related proteins such as mutants. They are sorted so that identical *cngroups* in different proteins appear next to each other. From these lists summary lists are also provided, which group together all protein structures which have the same *cngroup* sequence . Lists are also provided for a representative set of proteins (no two having sequence identity > 30%), and the summary lists for these - Table 2 is an example showing the form of the summary list.

Table 2 Example of parts of summary lists for representative Ca and Zn proteins.

DDDN	Ca	88	2	1	.	.	.	.	6	1	1alv
DDDOD	Ca	2	2	2	5	.	.	.	6	1	2scp
DDDOE	Ca	2	2	2	5	.	.	.	6	1	1cdl
DDDOE	Ca	2	2	2	5	.	.	.	6	4	1acc 1sra 1vrk 2pvb
DDEOOD	Ca	2	7	34	3	10	.	.	6	1	1acc
DDND	Ca	2	5	1	.	.	.	.	6	1	2por
DDNNOE	Ca	2	2	.	2	5	.	.	6	1	2cdl
DDNOOE	Ca	2	2	2	2	3	.	.	6	1	1cdl
DDNOE	Ca	2	2	2	5	.	.	.	5	1	1cdl
DDNOE	Ca	2	2	2	5	.	.	.	6	2	1rec 1vrk
CCCC	Zn	3	7	6	.	.	.	.	5	1	1zme
CCCC	Zn	3	7	7	.	.	.	.	5	1	1hwt
CCCC	Zn	3	14	3	.	.	.	.	4	1	1dsz
CCCC	Zn	3	17	3	.	.	.	.	4	3	1dcq 1rmd 1zin
CCCC	Zn	3	22	3	.	.	.	.	4	1	1zbd
HCC	Zn	27	3	.	.	.	.	.	4	1	1ctt
HCCC	Zn	11	1	10	.	.	.	.	4	1	1btk
HCCC	Zn	13	10	3	.	.	.	.	4	1	1gpc
HCCC	Zn	30	3	16	.	.	.	.	4	1	1ptq

These lists may have a variety of uses. It would be quick and easy to check whether in a newly determined metalloprotein structure the constitution of the metal coordination group is the same as that in a known structure, or structures. Here we plan to make geometrical comparisons after selecting proteins which have the same sequence of coordinating amino-acids in the *cngroup*, or closely related sequences, even though the overall sequences of the proteins are very different; polypeptide chain conformations in the chelating loops and nearby regions of the structure will then be compared, either by examining sequences of torsion angles, or by graphical superposition. Several sets of *cngroup* descriptions are easily recognised as familiar motifs, e.g. Ca proteins with EF hands, some Zn fingers, etc., which suggests that these listings may have some use in classifying metalloprotein structures. A long term aim of this project is to establish a representative set (in constitution and geometry) of metal coordination groups in proteins; this would have considerable overlap with the list of metal coordination groups in a representative set of proteins, but not be identical to it. This aim bears some relationship, but is also complementary to work nearing completion at University College London (M.W.MacArthur - private communication). The project there, which is in a much more advanced stage than this one, involves building a library of structural motifs (of metal coordination sites) including 3 dimensional aspects. These motifs, which can be used as templates or probes for a systematic classification of sites, include the positions of donor atoms of constituent amino-acids relative to the metal, regardless of the positions in which the amino-acids occur in the polypeptide sequence.

Some statistics of the numbers of structures and sequences found are given in Table 3 . (A few of the results or sequences are trivial, e.g. the numerous examples of $Mg(OH_2)_n{}^{2+}$ cations with no protein donor groups. There are also examples where groups are separately reported although the difference is small - e.g. a difference in coordination number due to different numbers of water molecules located near the metal.)

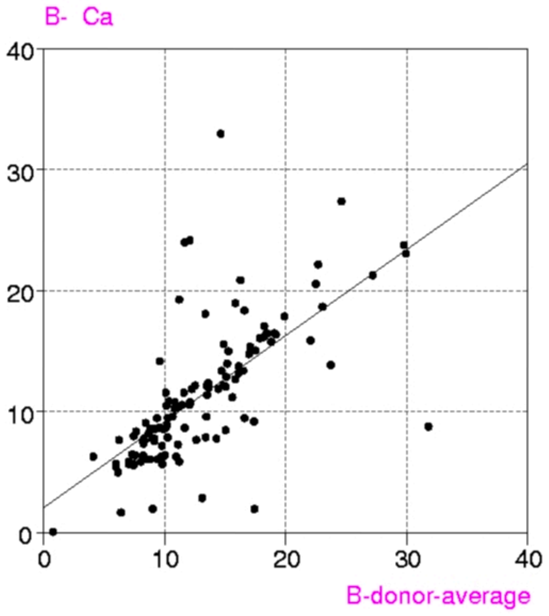
Table 3 Numbers of structures and cngroups

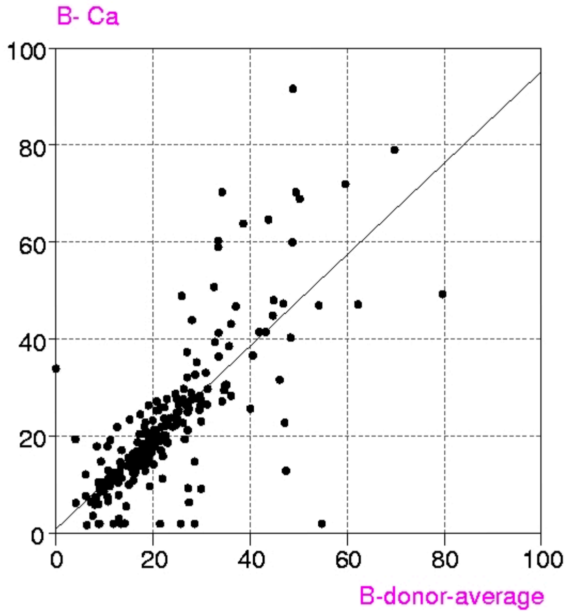
all metalloproteins		Ca	Mg	Mn	Cu	Zn
No. of structures		1081	546	207	183	782
No. of *cngroups* including repeats within asymmetric unit		2412	1113	571	434	1560
No. of *cngroups* occurring only once in different proteins		381	146	102	67	301
No. of *cngroups* occurring in different proteins	2 times	69	41	24	16	54
	3 times	49	23	10	4	18
	4 times	23	10	5	3	8
	5 times	87	30	11	10	65
representative set of metalloproteins		Ca	Mg	Mn	Cu	Zn
No. of structures		121	127	21	22	116
No. of *cngroups* including repeats within asymmetric unit		289	271	53	52	26
No. of *cngroups* occurring only once in different proteins		184	78	29	31	183
No. of *cngroups* occurring in different proteins	2 times	10	7	0	0	8
	3 times	1	5	1	2	3
	4 times	2	4	0	1	1
	>5 times	0	5	0	0	1

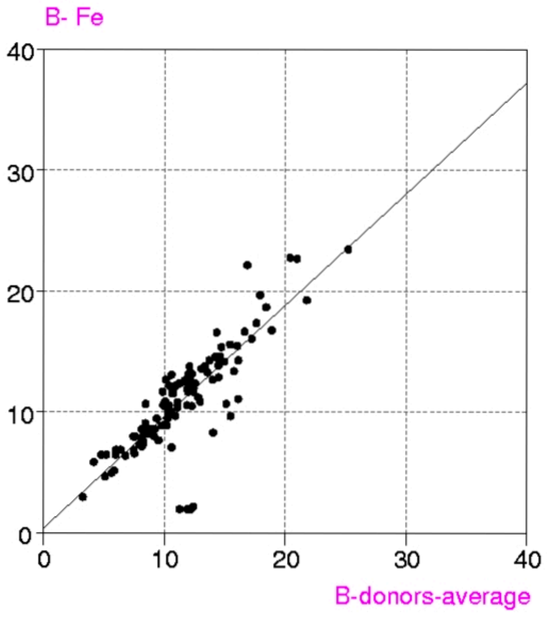
3. B-values in metal coordination groups

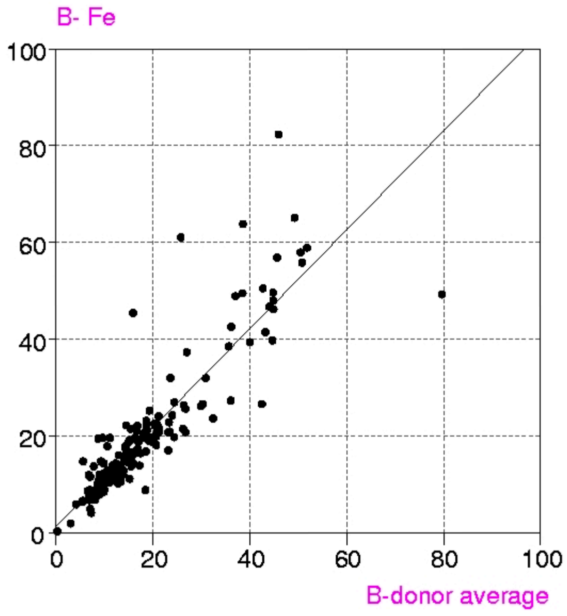
In addition to extracting geometrical information from PDB files the program MP extracted B values for metal atoms and donor atoms - see Table 1 for an example. Consideration of the value of B for the metal atom relative to the average B for the donor atoms might be helpful in identifying a metal atom, e.g an unexpectedly large B value for the metal relative to the surrounding donor atoms, could suggest that a metal of lower atomic number is actually present, or that the metal site is only partially occupied. Checking of the relative values might be useful in structure validation, but of course it would be essential to know what restraints had been applied to B values in refinement. A survey was made here of reported B values of donors relative to metal atoms, but without knowledge of the restraints applied.

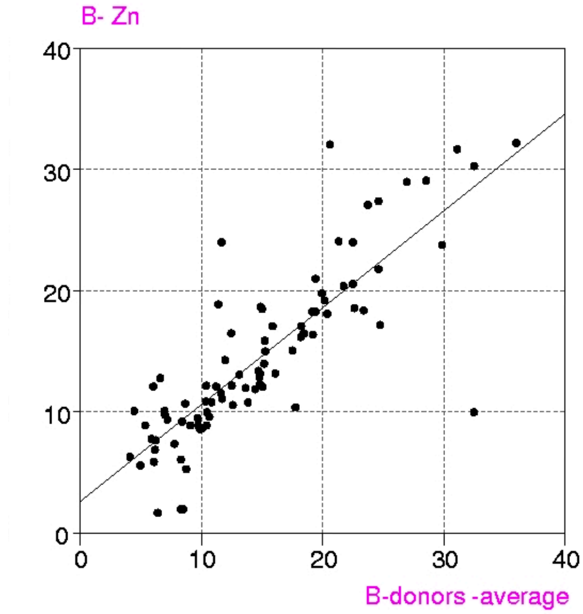
Procedures : Metalloproteins in the PDB up to July 1999 containing any of the metals Ca, Mg, Mn, Fe, Mn, Cu, Zn, have been examined in two groups, a) all structures with resolution $\leq$ 1.6 Å and b) 'representative macromolecules' with resolution up to 2.8 Å. Coordination groups where the metal atom occupancy or any donor atom occupancy is less than 1.0 were excluded. The results were displayed in a spreadsheet (VISTA of the CSD system), and included, for each metal coordination group, Bmetal, the mean, minimum and maximum values of Bdonor, as well as the coordination number, resolution, etc. Bdmean is the average value of B for all the donor atoms in the first coordination sphere around the metal atom, in this case those within (target distance + 0.5 A).

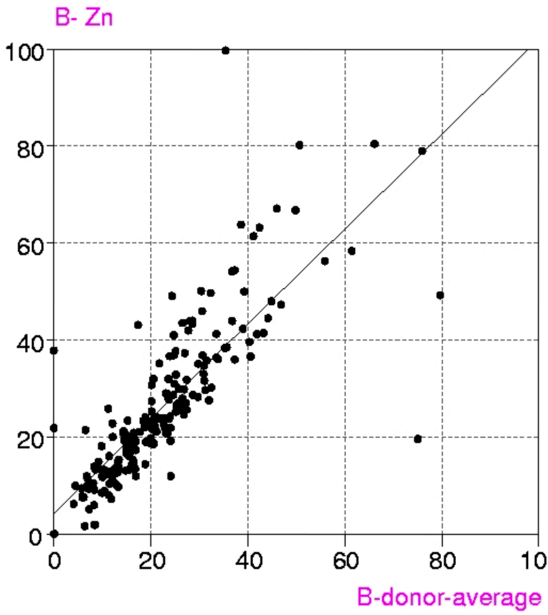

B- Ca
40
30
20
10
0
0
10
20
30
40
B-donor-average


B- Ca
100
80
60
40
20
0
0
20
40
60
80
100
B-donor-average


B- Fe
40
30
20
10
0
0
10
20
30
40
B-donors-average


B- Fe
100
80
60
40
20
0
0
20
40
60
80
100
B-donor average





Results, Comments: The figures show the distributions of the reported values of Bmetal and Bdmean for Ca, Fe and Zn; on the left are results for all metalloproteins with resolution $\leq$ 1.6 Å, on the right for the representative set of proteins with resolution $\leq$ 2.8 Å. Mg, Mn and Cu show similar trends but there are fewer observations. It is clear that for most Bdmean is similar to or slightly greater than Bmetal , as expected, and as generally found in 'small molecule' complexes. In the structure determinations at poorer resolution the scatter is greater, and the B values can be much larger. No correlation with coordination number was found. There are some very marked outliers in the distributions. Examination of some of the outliers showed that the refinements were done by several different frequently used programs. Bmetal=2.00 is curiously common among the outliers - perhaps a minimum value set by one of the programs , but it seems physically unreasonable in most cases. Outliers are much less common with Fe than with Ca or Zn (or Mg, Mn or Cu). This might be because, in the Fe structures, there is rarely an ambiguity about which metal is present, or an occupancy other than 1.00, whereas such uncertainties are more possible in Ca and Zn structures; alternatively it might be because Fe is most commonly bound within fairly rigid groups like haem or Fe_4S_4, while Ca or Zn atoms are sometimes more flexibly bound. These are very speculative suggestions. Further investigation of the outliers in these distributions has not been made here, but is desirable, and should start with a check on what restraints have been applied in the refinements. In all new structure determinations it is recommended that examination of Bmetal /Bdmean should be a useful step in validation.

4. Summary

The website metal coordination groups in proteins provides target values for metal-donor atom distances in metalloproteins for the six metals most commonly found in metalloproteins, together with fuller geometrical information on the geometry of coordination groups in a representative set of proteins. It also provides information on constitution of metal coordination groups in terms of coordinating amino-acids and their relative positions in the polypeptide chain sequence - for all metalloproteins containing Ca, Mg, Mn, Cu or Zn in the PDB (to 2.8 Å resolution, February 2001 release at present). The B values for metal and donor atoms, extracted from PDB files at the same time as the geometrical information, mostly follow expected patterns; these B values should be examined in structure validation, taking information on restraints into account. In future work the inclusion of some other metals will be considered, as well as extended geometrical comparisons of whole coordination groups of similar constitution.

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