The Transfer of Iron between Ceruloplasmin and Transferrins

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Abstract

Background: It is over 60 years since the discovery and isolation of the serum ferroxidase ceruloplasmin. In that time much basic information about the protein has been elucidated including its catalytic and kinetic properties as an enzyme, expression, sequence and structure. The importance of its biological role is indicated in genetic diseases such as aceruloplasminemia where its function is lost through mutation. Despite this wealth of data, fundamental questions about its action remain unanswered and in this article we address the question of how ferric iron produced by the ferroxidase activity of ceruloplasmin could be taken up by transferrin or lactoferrin.

Methods: Overlapping peptide libraries for human ceruloplasmin have been probed with a number of different lactoferrins to identify putative lactoferrin-binding regions on human ceruloplasmin. Docking software, 3D-Garden, has been used to model the binding of human lactoferrin to human ceruloplasmin.

Results: Upon probing the human ceruloplasmin library with human lactoferrin, three predominantly acidic lactoferrin-binding peptides, located in domains 2, 5 and 6 of human ceruloplasmin, were identified. The docking software identified a complex such that the N-lobe of human *apo*-lactoferrin interacts with the catalytic ferroxidase centre on human ceruloplasmin.

General Significance: In vitro binding studies and molecular modelling indicate that lactoferrin can bind to ceruloplasmin such that a direct transfer of ferric iron between the two proteins is possible. A direct transfer of ferric iron from ceruloplasmin to lactoferrin would prevent both the formation of potentially toxic hydroxyl radicals and the utilization of iron by pathogenic bacteria.

Keywords: Ceruloplasmin; Ferroxidase; Transferrin; Lactoferrin

Abbreviations: Cp, ceruloplasmin; Lf, lactoferrin

1. Introduction

Part of systemic iron homeostasis involves mobilisation of iron from tissue stores for transport to other regions of the body. Iron exported from tissues such as liver, or from macrophages, leaves the tissue or cells as ferrous iron and is converted to ferric iron by the plasma ferroxidase ceruloplasmin, after which the ferric iron is taken up by the iron transport protein transferrin. Upon activation, macrophages release ferrous iron which is then oxidised by ceruloplasmin and taken up by lactoferrin. In this report we focus on the molecular interactions between the serum ferroxidase ceruloplasmin and transferrin and lactoferrin. We start by briefly reviewing ceruloplasmin and its interaction with transferrin and lactoferrin and then present some data from *in vitro* and modelling studies.

1.1 Ceruloplasmin

In 1948, as part of an investigation of the transport of copper in blood, Holmberg and Laurell described the first purification of a blue protein from serum that contained most of the serum copper [1]. They named the protein ceruloplasmin and an investigation of the redox chemistry soon revealed a copper–dependent oxidase enzyme activity, with p-phenylene-diamine the best of a number of substrates tried [2]. The now familiar characteristics of ceruloplasmin oxidase activity, namely inhibition by azide, pH optimum between 5.0 and 6.0 and a requirement for oxygen, were also established [2]. Just prior to the description of ceruloplasmin Holmberg and Laurell had described a distinct iron-binding protein in serum that they named transferrin [3], but it was not until 1960 that the first report of iron, in the form of Fe^{2+}, affecting ceruloplasmin activity was published [4]. Ferrous iron had a marked stimulatory effect on ceruloplasmin oxidase activity and at the time it was known that serum possessed strong iron-oxidising activity, but it was not linked to ceruloplasmin. It was also speculated that when iron was loaded onto transferrin it was initially made available as ferrous iron and that oxidation for loading onto transferrin was necessary and was carried out by oxidases of unknown identity [4]. A definitive study from Frieden's lab confirmed that the marked ferroxidase activity of serum was due to ceruloplasmin [5] and their publication in 1966 contained the first description of the role of ceruloplasmin in iron metabolism in which it speculated that ferrous iron exported from mucosal cells is oxidised to ferric iron for loading onto transferrin and transport to the bone marrow for red blood cell synthesis [5]. The model was tested, and confirmed by *in vivo* studies in which ceruloplasmin injected into copper-deficient, and therefore anemic, pigs caused an immediate and sustained increase in serum iron [6]. Moreover when liver was perfused with ceruloplasmin there was an immediate increase in iron loading onto transferrin in the perfusate [7]. A further study in copper-deficient pigs confirmed and extended these findings and estimated that the high ferroxidase activity of ceruloplasmin meant that levels of ceruloplasmin needed to be reduced to 1% or less than normal in order to have an inhibitory effect on iron mobilisation from tissues [8]. On the basis of measurements of plasma iron turnover it was speculated that the high levels of ceruloplasmin found in normal individuals could accommodate the high rates of iron turnover needed in pathological conditions of haemolysis or haemorrhage [8].

The availability of purified serum ceruloplasmin allowed direct sequencing of the protein to commence with the first fragment in 1979 [9] and the full sequence in 1984 [10]. As details of the sequence emerged it became apparent that the protein has three homologous domains, is capable of binding six to seven copper atoms and is glycosylated in three or four positions [9, 10]. The full sequence of the cDNA followed in 1986 [11] and homology with blood clotting Factor VIII was noted [11]. Cp belongs to the blue multi-copper oxidase protein family which includes laccase, ascorbate oxidase, nitrate reductase, bilirubin oxidase, yeast FET3, factor V and factor VIII.

The X-ray structure of ceruloplasmin isolated from human serum was achieved 10 years later and revealed a triangular array of six plastocyanin-type domains [12]. Six copper atoms are in the structure, three forming a trinuclear cluster at the interface between domains 1 and 2 and the three mononuclear copper atoms are in domains 2, 4 and 6. The trinuclear group of copper atoms and the type 1 copper atom in domain 6 form a cluster similar to that found in ascorbate oxidase [12]. In a further, more refined structure a dioxygen species was modelled binding to the trinuclear centre [13] and soaking experiments with $FeSO_4$ and $FeCl_3$ provided further evidence of iron-binding sites in domains 4 and 6, close to the mononuclear coppers. The binding sites can also be occupied by other divalent transition metals ions including Cu^{2+} and Co^{2+}. The type 1 mono-nuclear coppers of domains 2, 4 and 6 are close enough to the tri-nuclear copper to allow transfer of electrons, providing a clear means by which the ferroxidase activity of ceruloplasmin can operate, although there is some uncertainty as to whether the type 1 Cu in domain 2 is active [13].

Other features revealed by the refined model include the presence of Na^+ and Ca^{2+} ions in the protein structure. A Na^+ ion is located in each of three protuberances above the main body of the protein, and plays a structural role. The protuberances are suggested to limit access of larger molecules to the type 1 oxidation sites in domains 4 and 6. The Ca^{2+} ion also appears to have a structural role, being part of domain 1, but it is close enough to the surface to have a role in the binding of ceruloplasmin to erythrocytes.

Studies in which organic substrates were soaked onto crystals have identified two major binding sites for aromatic amines. Diamines bind near the bottom of domain 4 and biogenic amines such as adrenaline, noradrenaline, serotonin and dopa bind to domain 6 close to the site of cation oxidation [13, 14].

1.2 Interaction of Ceruloplasmin with Transferrin and Lactoferrin

Kinetic studies of ceruloplasmin with ferrous iron as substrate and using *apo*-transferrin as acceptor had indicated rapid oxidation kinetics [15] but attempts to study an interaction with transferrin have not been published until relatively recently. Although ferroxidase activity had long been measured by monitoring formation of *holo*-transferrin by its absorbance at 460 nm, the question of whether transferrin was capable of binding ceruloplasmin to receive the ferric iron directly, or whether ferric iron was released to the medium and taken up by transferrin in a stochastic interaction has not been extensively addressed.

While most work on transfer of ferric iron from ceruloplasmin to transporter protein has involved transferrin as recipient, in an investigation of lactoferrin as a gene transfer reagent Zakharova et al. found that Cp bound strongly to lactoferrin [16] with K_d 1.8 μM and a stoichiometry Lf:Cp of 1:1 or 2:1. Addition of exogenous lactoferrin could titrate the ceruloplasmin from whole plasma [15], as well as form complexes with ceruloplasmin *in vitro* [16, 18-20], suggesting that the complexes could exist in the blood *in vivo*. Lactoferrin is known to be secreted by activated inflammatory cells [17] and the interaction with ceruloplasmin would allow the ferrous iron released during inflammatory reactions, which is potentially harmful, to be rapidly oxidised to ferric iron and taken up by lactoferrin [16]. Complexes of ceruloplasmin with lactoferrin could also be found in breast milk [18]. The complex can be dissociated by 0.3 M NaCl, low pH and other conditions suggestive of an ionic interaction possibly reflected by the large difference in pIs, 8.6 for lactoferrin and 4.5 for ceruloplasmin [19]. Accordingly dissociation of the complex was attempted using cationic peptides taken from the sequence of lactoferrin and anionic peptides taken from the sequence of ceruloplasmin, and was achieved using the peptide R-R-R-R corresponding to residues 2 to 5 of lactoferrin.

A 1:1 *holo*-Lf:Cp complex was studied by small angle X-ray scattering (SAXS) and two models emerged, with either the N-lobe or C-lobe of lactoferrin binding the C-terminal region of ceruloplasmin [20]. The model with the N-lobe binding of Lf to Cp was favoured, based on the ability of the lactoferrin arginine tetrapeptide to dissociate the complex, as mentioned above, and other binding data [discussed in 20]. The study confirmed earlier data from CD measurements of the complex that neither Cp nor Lf underwent conformational changes on formation of the complex [16].

While ceruloplasmin can form stable complexes with lactoferrin that can be detected by standard biochemical techniques such as non-denaturing electrophoresis or gel filtration, an attempt to detect transferrin-ceruloplasmin complexes by non-denaturing electrophoresis failed [21], suggesting that transferrin binding to ceruloplasmin, if it occurs, is too weak to detect by these methods. A more sensitive technique for probing ceruloplasmin-transferrin interactions, surface plasmon resonance, has been attempted using ceruloplasmin as the immobilised ligand, but no interactions could be detected [21]. More recently a study exploiting changes in the intrinsic fluorescence of lactoferrin and transferrin successfully demonstrated formation of complexes of either protein with ceruloplasmin at equilibrium [22]. The data confirmed the initial report that two molecules of *holo*-lactoferrin could bind to one molecule of ceruloplasmin [16] and binding constants of 1.5 μM and 11 μM were reported. The sensitivity of the fluorescence measurement allowed a wide range of transferrin concentrations to be used and 1:1 complexes of ceruloplasmin with *apo-* and *holo*-transferrin were detected with K_Ds of 19 μM and 12 μM, respectively [22]. The value for *holo*-transferrin is much higher than the binding constant for transferrin receptor and the interaction with ceruloplasmin will not compete with binding to the transferrin receptor. Based on concentrations of ceruloplasmin and transferrin of 2.5 μM and 25 μM, respectively, in the blood circulation it was estimated that 70% of ceruloplasmin could be complexed to transferrin in the blood [22]. However no attempts have been reported yet to detect such complexes in whole blood or serum.

These studies suggest the possibility of complex formation between ceruloplasmin and transferrin or lactoferrin facilitating direct transfer of ferric iron within the complex. In this model, once ferric iron moves from the “holding” site of ceruloplasmin [13] and binds to the cleft between domains of the N-lobe or C-lobe of transferrin or lactoferrin, a conformational change would be induced that would promote closure of the domains of the binding lobe of transferrin or lactoferrin and dissociation from ceruloplasmin.

We have used peptide libraries and docking modelling to model the interaction of ceruloplasmin with lactoferrin. The binding sites identified by the two approaches overlap and the data suggest a model in which the two domains of the N-lobe of lactoferrin bind to either side of a domains 4 and 6 of ceruloplasmin. Potential binding sites are discussed in relation to known structural data.

2. Materials and methods

2.1. SPOTs peptide library construction

An overlapping peptide library representing the human ceruloplasmin primary sequence was generated by the SPOTSCAN method (Sigma-Genosys). The library consisted of a series of 13-amino-acid linear overlapping peptides, with a 6-amino-acid overlap, immobilised onto a cellulose membrane by N-terminal acetylation.

2.2. SPOTs peptide library mapping

Before use, the SPOTs membrane (Sigma-Genosys) was allowed to warm to room temperature and rinsed in methanol for 5 minutes. The membrane was sealed in a polypropylene box and rocked for 10 minutes in 30 ml of 50 mM TRIS, 137 mM NaCl, 2.7 mM KCl, pH 8 (TBS). This washing procedure was repeated twice. Blocking of the membrane was carried out at room temperature for two hours in 30 ml of 1 x SPOTs blocking buffer (Sigma-Genosys), before being washed for 10 minutes in 30 ml of 50 mM TRIS, 137 mM NaCl, 2.7 mM KCl, 0.05% Tween 20, pH 8 (T-TBS). The membrane was then left to incubate for 4 hours on a rocking platform in 30 ml of SPOTs blocking buffer containing human lactoferrin isolated from milk and conjugated with horseradish peroxidase at a final concentration of 1 μg/ml. HRP-labelled human Lf was prepared according to the manufacturer's instructions using the SureFIRE^{TM} HRP conjugation kit (KPL, USA). Unbound conjugated protein was removed by washing the membrane three times for 10 minutes in 30 ml of T-TBS. Finally, the membrane was developed using the substrate of peroxidase prepared as follows: 20 mg of 4-chloro-1-naphthol were dissolved in 1 ml methanol and 100 μl of this mixture were added to 10 ml of PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) together with 5 μl of H_2O_2. The membrane was regenerated using the procedure recommended by the manufacturer. The peptide library was then probed a further five times with HRP-labelled proteins in the following order: HRP-labelled human Lf, HRP-labelled human transferrin, HRP-labelled bovine Lf, HRP-labelled camel Lf and HRP-labelled human Lf. The membrane was finally probed with horseradish peroxidase.

2.3 Protein reagents

Human lactoferrin was isolated from outdated human milk using heparin-Sepharose following the procedure of Blackberg and Hernell [23]. Bovine lactoferrin was a gift from Dr. Rex Humphrey of the Tatua Dairy Cooperative, New Zealand. Human transferrin was purified from plasma by immunoaffinity chromatography [24]. Camel lactoferrin was isolated from camel milk by ion-exchange chromatography on SP-Sepharose [25, 26]. Lactoferrins prepared by these methods are predominantly in the *apo* form [26].

2.4 Modelling Cp-Lf Interactions

Docking software 3D-Garden was used to model binding of lactoferrin to ceruloplasmin [27]. Co-ordinates of protein structures were obtained from the Worldwide Protein Data Bank for human ceruloplasmin (PDB ID: 1KCW) [12], human *apo*-lactoferrin (PDB ID: 1CB6) [29] and human *holo*-lactoferrin (PDB ID: 1B0L) [30] were used. Models were analysed for proximity of the iron binding site on lactoferrin and the active site of ceruloplasmin and the model with the sites closest was selected and analysed using Jmol, an open-source Java viewer for chemical structures in 3D [http://www.jmol.org/].

3. Results

When the human ceruloplasmin peptide library was probed with HRP-labelled human lactoferrin the protein was observed to bind to three peptides (Figure 1) corresponding to residues 204-216, 715-727 and 904-916, with amino acid sequences VVDENFSWYLEDN, YYIAAVEVEWDYS and FDENESWYLDDNI, respectively. The same three predominantly acidic peptides were also visualised when the library was probed a second time with HRP-labelled human Lf and then with HRP-labelled bovine lactoferrin, however, no binding was observed with HRP-labelled human transferrin or HRP-labelled camel lactoferrin (data not shown). In order to check the integrity of the peptide library after it had been probed with HRP-camel lactoferrin it was probed a third time with HRP-labelled human Lf which once again was observed to bind to the same three acidic peptides identified earlier. When the library was subsequently probed with horseradish peroxidase, no binding of the enzyme to themembrane was observed confirming that the observed binding of HRP-human Lf and HRP-labelled bovine Lf to the same three peptides was due to the interaction of the lactoferrins, and not the enzyme label, HRP with the peptide library. When the three peptides were mapped onto the solved crystal structure of ceruloplasmin [12] they were found to be located in domains 2, 5 and 6 respectively (Figure 2).

The most favourable complex of ceruloplasmin and *apo*-lactoferrin predicted by 3D-Garden with the constraint of minimal distance between the active site of ceruloplasmin and an iron-binding site on lactoferrin is shown in Figure 3. The N-lobe of lactoferrin has an open conformation and is bound to domains 5 and 6 of ceruloplasmin and contact points include residues within two of the sequences identified from the ceruloplasmin peptide library to which lactoferrin could bind, namely YYIAAVEVEWDYS corresponding to residues 715-727 and FDENESWYLDDNI corresponding to residues 904-916. The two domains of the N-lobe of lactoferrin bind to either side of one of the protuberances on the upper surface of ceruloplasmin [12, 13] composed of regions of domains 5 and 6, and the iron-binding site of lactoferrin is at the end of the cleft formed by the N-lobe domains (Figure 3). Key residues involved in contact include i) asp913 in contact with ser814, ser185 and gln186 of the N2 domain on the N-lobe of lactoferrin; ii) glu723 in contact with ser12 and pro14 of the N1 domain of lactoferrin; iii) glu721 in contact with asp40, ser42 and pro42 of domain N1 of lactoferrin. The N-terminal arginines of lactoferrin, residues 2 to 4 on the structure, are clearly on the outside of one of the N-lobe domains and are not involved in the interactions between lactoferrin and ceruloplasmin in this model.

The complex suggests a model for transfer of ferric iron from the active site of ceruloplasmin to the binding site on lactoferrin in which the Fe3+ ion generated at the active site moves to a putative holding site on glu935 [13] and thereafter is constrained to move into the binding site of the N-lobe of lactoferrin by the presence of a series of three acidic amino acids that form a path into the lactoferrin binding site (Fig. 4). The distances between the carboxylate oxygens on the holding site at glu935 and the nearest residues on the iron binding site of lactoferrin, asp60 and tyr192, are 34 and 43 Å, respectively. The shortest distance between the carboxylate oxygens on the last residue on the path, asp914, and the oxygens on asp60 and tyr192 are 11.4 and 14.5 Å, respectively, suggesting that ferric ion can readily position itself for the final binding step from asp914 on ceruloplasmin.

4. Discussion

The formation of the lactoferrin-ceruloplasmin complex both *in vitro* and *in vivo*, with a dissociation constant of K_d = 1.8 μM, has been shown to increase the ferroxidase activity of ceruloplasmin [19]. Such an interaction has been proposed to have a marked electrostatic character, with the suggested participation of the positively charged arginine cluster at the N-terminus of human lactoferrin, and a negatively charged stretch in the C-terminus of ceruloplasmin. This model is supported by the fact that the complex can be dissociated at low pH or by addition of polyanions such as DNA, heparin or lipopolysaccharide [31]. The molar ratio of the Cp:Lf complex is 1:2 or 1:1 *in vitro*, while only the 1:1 species was found *in vivo* [19, 22, 31]. Structural analysis of a 1:1 complex by SAXS indicated that neither protein undergoes major conformational changes on complex formation [20]. Sokolov et al. [32] have found that some peptides of ceruloplasmin resulting from its partial hydrolysis, with molecular masses of about 5 and 7 kDa, could interact with lactoferrin and myeloperoxidase. These peptides were found to be located in domains 1 and 6 of the ceruloplasmin tertiary structure and close to the tri-nuclear copper catalytic centre of the protein, located between the domains 1 and 6, and the mononuclear copper in domain 6 where ferrous iron is oxidised to ferric iron. The peptides that we have identified in the ceruloplasmin peptide library differ from the partial peptides of ceruloplasmin, with only our third peptide, corresponding to the amino acid stretch (904-916), being very close to those reported by Sokolov et al [32] and therefore to the catalytic centre. Such a location close to the catalytic centre is in agreement with a physiological role for the Lf:Cp complex. In such an arrangement, ceruloplasmin could make oxidised iron available directly to lactoferrin during the inflammatory process therefore not only preventing the formation of potentially toxic hydroxyl radicals but also preventing the utilization of such iron by pathogenic bacteria which may be present at the foci of inflammation.

We attempted to model the docking of either *apo*- or *holo*- lactoferrin to ceruloplasmin using 3D-Garden [27]. Out of 25 models produced with *apo*-lactoferrin the fifth most stable had a configuration that was most compatible with modelling transfer of ferric ion between a catalytic site on ceruloplasmin and an iron-binding site on lactoferrin (Figure 3). The structure of *apo*-lactoferrin used for modelling had the two domains of the N-lobe open, but the corresponding domains of the C-lobe were closed [30]. The open configuration of the N-lobe domains was essential in order to allow binding of the lactoferrin to the protuberances of ceruloplasmin [12, 13] allowing juxtaposition of the cleft of lactoferrin, and the iron binding site in it, over the protuberances, ready to receive ferric ions released from the “holding” site in domain 4 of ceruloplasmin [13]. It would be of interest to repeat the modelling with a fully open structure of *apo*-lactoferrin, currently unavailable in the Protein Databank, to see whether the C-lobe domains could bind in a similar fashion. The model predicted by 3D-Garden is consistent with evidence from the peptide library reported in this study in which peptides corresponding to residues 715-727 and 904-916 are close together in the tertiary structure of Cp (Figure 2). The third Lf-binding peptide identified from the library, corresponding to residues 204–216 of ceruloplasmin, is further removed from the other two, being located in domain 2, and may reflect a weaker, but detectable, interaction. In models of holo-Lf bound to Cp there were none in which Lf was in contact with peptides 715–727 or 904–916, suggesting that the opening of the domains in either lobe of Lf is critical for binding that facilitates transfer of iron from Cp to Lf. A model for transfer of the ferric ion from the holding glu935 to the binding site in the N-lobe of lactoferrin is shown in Figure 4. The ferric ion must travel a distance of around 35 Å to reach the nearest residues of the binding site in lactoferrin, asp60 and tyr192. We speculate that a series of acidic amino acids, including glu932, glu753 and asp921 of ceruloplasmin and asp297 of lactoferrin, facilitates passage of Fe3+ to the lactoferrin binding site. These residues were selected because they form a string of negative charges that is not interrupted by basic residues present on the surface of ceruloplasmin. These studies provide a focus for future work in determining the mechanism of iron transfer from ceruloplasmin to ferric binding proteins.

4.1. Conclusions and perspectives

A growing body of biochemical and biophysical evidence supports the idea that ceruloplasmin, and by inference ferroxidase homologues hephaestin and zyklopen, can bind and interact directlywith transferrin or lactoferrin. Evidence that provides molecular details of the interaction has been largely limited to inhibition data with peptides targeted at specific regions of lactoferrin [e.g. 17]. A model of the structure of a ceruloplasmin–lactoferrin complex based on small-angle X-ray scattering has been published [20], but this was of a complex of ceruloplasmin with holo-lactoferrin in which both the N-lobes and C-lobes are closed. The structure of apo-lactoferrin from the Protein Data Bank (PDB file 1CB6) has the N-lobe, but not the C-lobe, in the open conformation characteristic of apo-transferrins. We have combined biochemical and modelling approaches to probe the interaction between ceruloplasmin and apo-lactoferrin as part of an effort to understand in molecular detail how ferroxidases can transfer ferric iron to lactoferrin and transferrin. The two approaches provided some converging evidence implicating particular amino acids to have a role inmediating contacts between ceruloplasmin and the open N-lobe of apo-lactoferrin, such as glu721, glu723 and asp913. However, some other residues in the model were not identified by screening the peptide library and this may reflect weaker binding of apo-lactoferrin to other peptides in the library. It may be therefore be useful to repeat the screeningwith a more sensitive detectionmethod such as chemiluminescence.While the use of modelling software will not produce models that are 100% accurate [27] it would be of interest to repeat these approaches with apo-transferrin and to model interactions between transferrins and the extracellular domains of hephaestin and zyklopen. In particular it would be of interest to establish whether or not there is commonality of key residues amongst these highly homologous ferroxidases, whose role could be assessed by site-directed mutagenesis. We are still at the early stages of a molecular understanding of this critical process of ferric iron transfer, and the use ofmodelling to complement in vitro studies may help to provide further insights and focus for future work.

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Fig. 1. Human ceruloplasmin peptide library. The binding of human lactoferrin labelled with HRP was detected using the substrate peroxidase, 4-chloro-1-naphthol. The three positive peptides are circled in yellow, red and green and correspond to residues 204-216 (VVDENFSWYLEDN), residues 715-727 (YYIAAVEVEWDYS) and residues 904-916 (FDENESWYLDDNI), respectively.

Fig. 2. Mapping of the peptides on human ceruloplasmin involved in its interaction with human lactoferrin onto the 3-D structure of human ceruloplasmin. The peptide corresponding to residues 204-216 (VVDENFSWYLEDN), residing in domain 2, is shown in yellow, the peptide corresponding to residues 715-727 (YYIAAVEVEWDYS), residing in domain 5, is shown in red and the peptide corresponding to residues 904-916 (FDENESWYLDDNI), residing in domain 6, is shown in green.

Fig. 3. Model of *apo*-Lf:Cp complex produced by 3D-Garden. Ceruloplasmin is the lower protein and the three peptides identified in the peptide library are shown. The peptide corresponding to residues 715-727 (YYIAAVEVEWDYS) in domain 5 is coloured red and the peptide corresponding to residues 904-916 in domain 6 is shown in green. The N-terminus of lactoferrin, the upper protein makes contact with the peptides from domains 5 and 6 but not from domain 2, coloured in yellow. The spacefilled residues in white on hLF are three arginines, residues 2-4, at the N-terminus. Copper atoms are shown as orange spheres.

Fig. 4. Model of transfer of ferric ion from ceruloplasmin to the N-lobe of lactoferrin. The upper polypeptide is the N-lobe of lactoferrin with the N1 domain in blue and the N2 domain in cyan. The Fe3+ binding site residues are represented in wireframe. The lower polypeptide shows domains 4, 5 and 6 of ceruloplasmin with library peptides 715–727 and 904–916 highlighted in green and red, respectively. Key acidic amino acids putatively involved in the transfer of Fe3+ from the holding glu935 to the Fe3+ binding site of lactoferrin are labelled. The copper atoms close to glu935 are shown as orange spheres.

Figure 1

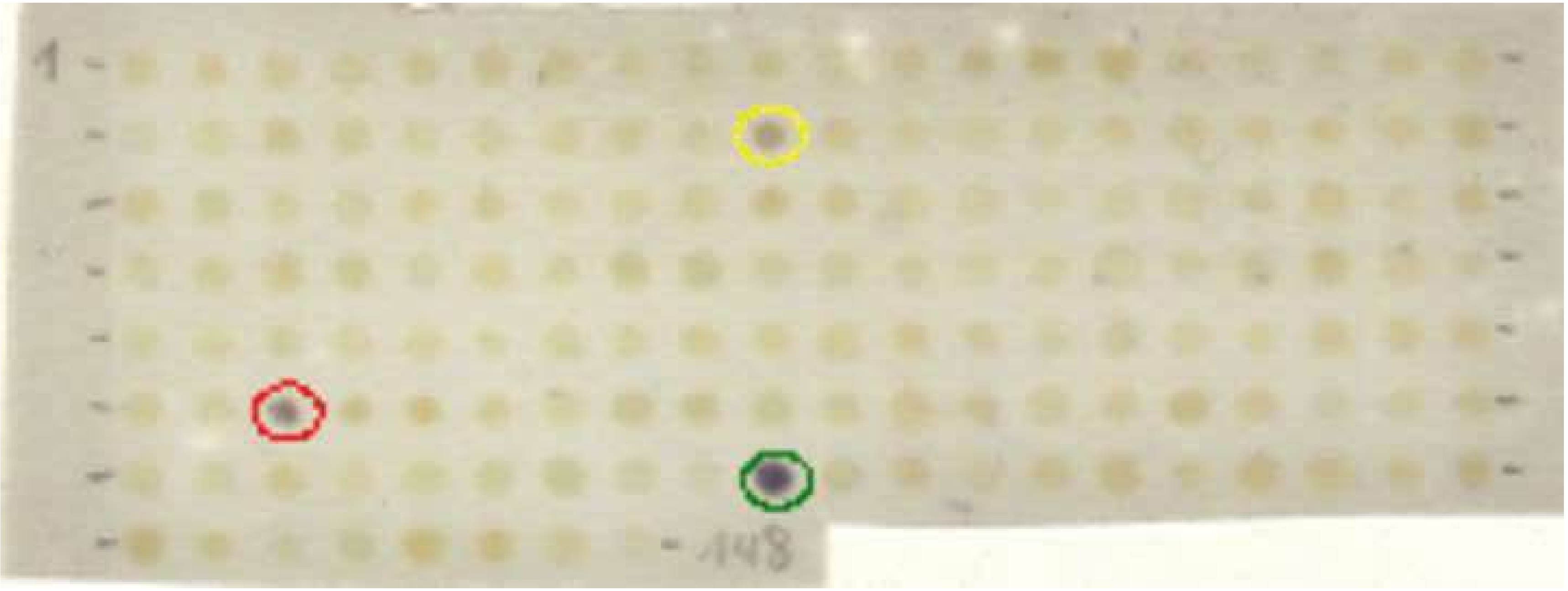

Figure 2

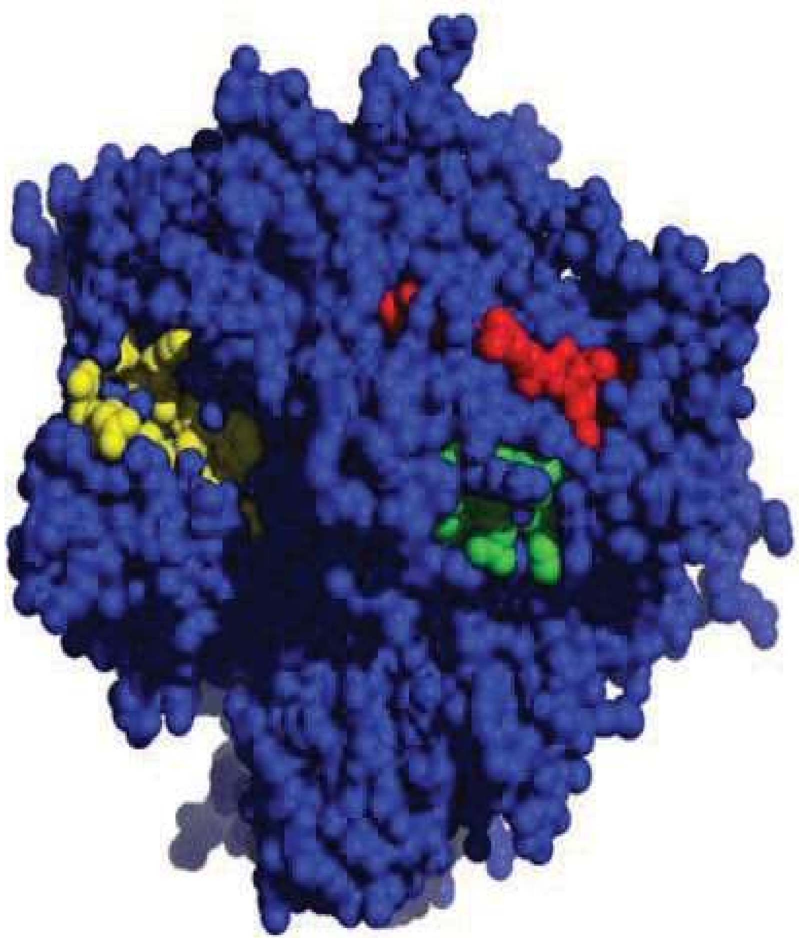

Figure 3

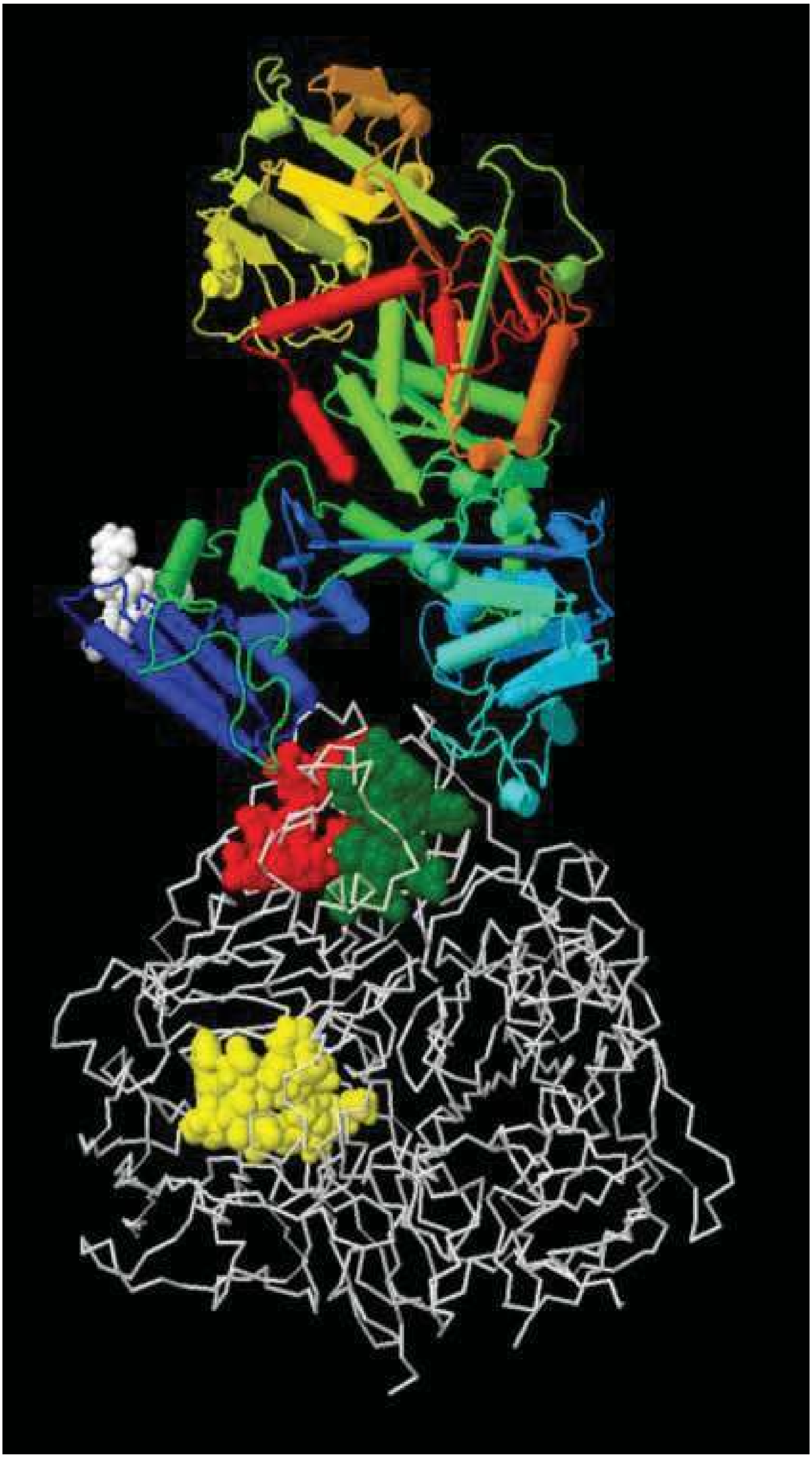

Figure 4