

January 2000

Clusterin is a secreted mammalian chaperone

M. R. Wilson

University of Wollongong, mrw@uow.edu.au

S. B. Easterbrook-Smith

University of Sydney

Follow this and additional works at: <https://ro.uow.edu.au/scipapers>



Part of the [Life Sciences Commons](#), [Physical Sciences and Mathematics Commons](#), and the [Social and Behavioral Sciences Commons](#)

Recommended Citation

Wilson, M. R. and Easterbrook-Smith, S. B.: Clusterin is a secreted mammalian chaperone 2000.
<https://ro.uow.edu.au/scipapers/16>

Clusterin is a secreted mammalian chaperone

Abstract

[Extract] By any criteria, clusterin is an interesting protein. It was first described in 1983 as a secreted glycoprotein in ram rete testis fluid that enhanced aggregation (“clustering”) of a variety of cells in vitro 1. Many homologues in other species were subsequently discovered. Typically, each “discovery” of clusterin in a different species or by a different research group led to it being assigned another name. By the early 1990s clusterin was known under many aliases 2, some of which persist in the literature. However, the inaugural international workshop on clusterin (Cambridge, 1992) agreed to the name clusterin, in deference to the original reports of the protein’s properties.

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

This article was originally published as Wilson, MR and Easterbrook-Smith, SB, Clusterin is a secreted mammalian chaperone, Trends in Biochemical Sciences, 25, 2000, 95-98. Copyright Elsevier. Original journal available [here](#).

Clusterin is a Secreted Mammalian Chaperone

Mark R Wilson^{1,2} and Simon B Easterbrook-Smith³

¹ Department of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW, Australia. 2522. Email: mrw@uow.edu.au

² To whom correspondence should be addressed

³ Department of Biochemistry, University of Sydney, Sydney, NSW, Australia. 2006. Email: sbe@biochem.usyd.edu.au

Introduction

By any criteria, clusterin is an interesting protein. It was first described in 1983 as a secreted glycoprotein in ram rete testis fluid that enhanced aggregation (“clustering”) of a variety of cells *in vitro* ¹. Many homologues in other species were subsequently discovered. Typically, each “discovery” of clusterin in a different species or by a different research group led to it being assigned another name. By the early 1990s clusterin was known under many aliases ², some of which persist in the literature. However, the inaugural international workshop on clusterin (Cambridge, 1992) agreed to the name clusterin, in deference to the original reports of the protein’s properties.

Clusterin structure and distribution

Clusterin is a 75-80 kDa disulfide-linked heterodimeric protein with 30% of its mass being N-linked carbohydrate. It is encoded by a single gene and the translated product is internally cleaved to produce its α and β subunits prior to secretion from the cell. Sequence analyses predict that clusterin contains a disulfide-linked core region flanked by sections of the α and β subunits containing three amphipathic α -helices and two coiled-coil α -helices (Fig. 1).

**** Fig 1 here, containing refs 3, 4 ****

The breadth of its biological distribution is striking. In animal tissues clusterin mRNA is near ubiquitous, being found in locales as diverse as the rat prostate gland and quail neuroretinal cells. Across species clusterin maintains a high level of sequence homology, comparisons between mammals being in the range 70-80% ². There is extensive evidence of a correlation between clusterin expression and diseases (eg Alzheimer’s disease, gliomas) or pathological stress (eg pressure or ischemic kidney insult) ².

Previous ideas about clusterin’s function

The function of clusterin has been an elusive goal. It has been ascribed many functions, including:

Controlling cell-cell and cell-substratum interactions Although clusterin was named after its ability to mediate clustering of cells ¹ and exogenous clusterin promotes formation of “nodules” in cultured porcine cells ⁵, the relevance of these observations to *in vivo* physiology remains to be established.

Regulating apoptosis This idea was popular following the discovery that clusterin mRNA was upregulated in apoptotic rat prostate ⁶. However, subsequent studies showed that clusterin expression was not increased in other models of apoptosis.

Acting as a “membrane policeman” protecting cells at fluid-tissue interfaces from stresses ⁷. Since it is known that heat shock proteins protect cells from a variety of stresses, this idea is consistent with recent data that suggests clusterin is a novel type of heat shock protein (see below).

Transporting lipids A fraction of clusterin in human plasma is associated with high density lipoprotein (HDL) particles. Clusterin is secreted from HepG2 cells associated with lipids ⁸ and exogenous clusterin can promote the *in vitro* efflux of cholesterol from lipid-loaded mouse macrophages ⁹. It remains to be established whether clusterin has a lipid transport role *in vivo*.

Regulating complement Several reports show that *in vitro* clusterin can influence complement-mediated cytolysis effected with purified complement components ². Hence, the notion that clusterin is a complement regulator was popular. However, our recent work suggests that, under physiological conditions, clusterin is unlikely to be an important complement regulator ¹⁰.

Thus, until recently clusterin’s biological function(s) was clouded in uncertainty and confounded by a proliferation of putative functions. Part of the reason for this and, we suggest, part of the answer, lies in the diversity of clusterin-binding ligands.

****Table 1 here, contains refs 11-23****

Clusterin binds to many different biological ligands

Clusterin binds to a wide array of biological ligands (Table 1). In many cases, the discoverers of a new ligand also proposed a new function for clusterin, associated with the ligand. For example, when we reported that clusterin binds to IgG we suggested that clusterin's function might involve association with IgG¹⁸. Arguably, the sheer number of clusterin-binding ligands makes it unlikely that each relates to a separate function. A reasonable hypothesis is that many of these interactions result from a single underlying property of clusterin, related to a primary function. The first tangible clue for this function came from a study of the clusterin promoter.

Is clusterin a functional heat shock protein?

Mammalian HSPs are a diverse group of intracellular proteins that protect cells against heat and other stresses. Individual HSPs differ in their expression and mode of action but all are chaperones, in that they share the characteristic of binding to exposed hydrophobic regions of proteins that are either incompletely folded or are partly unfolded as the result of stress. By this action, chaperones can stabilize conformations of proteins that are otherwise unstable. This is believed to be the primary function of the small HSPs (sHSPs;²⁴). *In vivo*, different HSPs are thought to cooperate in stabilizing partially unfolded proteins (eg, the action of the sHSPs) and then refolding them (eg, via the action of HSP70). These combined actions might protect cells from stress by preserving the integrity of critical proteins²⁵.

In 1997 it was reported that a 14-bp element in the clusterin promoter, the sequence of which is strictly conserved in vertebrates, is specifically recognized by transcription factor HSF1 and can mediate heat-shock-induced transcription in transient expression assays. This suggests that clusterin is a heat shock protein (HSP)²⁶. A recent review made the same suggestion and proposed a model in which clusterin acts as a “biological detergent”, binding to hydrophobic complexes and denatured proteins to aid their uptake or clearance²⁷. However, this simple model did not incorporate either (i) the concept that, like all known mammalian HSPs, clusterin may be a chaperone, or (ii) recently published data showing that clusterin can potentially inhibit stress-induced protein precipitation¹⁴. It is clear that, like HSPs, clusterin expression is induced by heat shock²⁷. However, if clusterin actually *functions* as a HSP, it

should stabilize stressed proteins and, acting alone or in concert with other HSPs, protect cells from stress.

The first of these criteria was satisfied by a recent report that clusterin forms high molecular weight “solubilized” complexes with heat- or reduction-stressed proteins, inhibiting their precipitation¹⁴. On a molar basis, clusterin is substantially more potent than sHSPs at inhibiting this protein precipitation^{14, 28}. Clusterin did not protect the two enzymes tested, catalase and glutathione-S-transferase, from heat-induced loss of function¹⁴, nor did it promote recovery of enzyme activity following heat stress (D. Humphreys, unpub). Thus, clusterin can stabilize stressed proteins but cannot catalyse protein refolding, properties shared with sHSPs.

In relation to the second criterion for a functional HSP, it appears likely that clusterin can protect cells from many environmental stresses. LNCaP cells transfected with anti-sense clusterin cDNA are sensitized to TNF α -mediated death²⁹. Furthermore, overexpression of clusterin protects LNCaP²⁹ and L929³⁰ cells from TNF α . Although studies involving overexpression of proteins must be interpreted with caution, taken together, these results suggest that clusterin protects cells from the cytotoxicity of TNF α . A correlation between clusterin expression and cell survival was reported for a variety of models of apoptosis, ranging from UV irradiation of U937 cells to dexamethasone treatment of CEM-C7 cells³¹. Recently, it was shown that A431 cells transfected with anti-sense clusterin cDNA are more susceptible to heat shock or oxidative stress than controls³². Finally, it has been shown that exogenous clusterin can protect LLC-PK₁ cells from oxidative stress³³ and protects a variety of human cell lines from heat stress (S. Poon, unpub). Although further confirmatory evidence will be valuable, collectively, these results strongly suggest that clusterin is a cytoprotective molecule.

Taken together, this evidence strongly supports the hypothesis that clusterin is a functional HSP. In this context, it is interesting to note that there are limited sequence similarities between residues 230-422 of clusterin and the α B-crystallins (a type of HSP; Fig. 2). The overall sequence similarity is low at 17% (33/193 residues identical or highly conserved), however, it is noteworthy that there is 25% similarity between residues 286-343 of clusterin and residues 57-107 of α B-crystallin. This region of

α B-crystallin forms part of its chaperone active site^{35, 36}. The equivalent region of clusterin contains a discrete motif; the coiled-coil α -helix in the β chain (Fig. 1).

**** Fig 2 here, contains refs 34-36 ****

To summarize: (i) at least one common element (HSF1) can control expression of both clusterin and HSPs, (ii) like HSPs, clusterin can inhibit stress-induced protein precipitation and appears likely to protect cells from stresses, and (iii) clusterin shares some sequence similarity with HSPs. Thus, at the levels of gene control and protein function, clusterin shares many similarities with HSPs, an established group of chaperones. Amino acid sequence analyses indicate that there may be a distant evolutionary relationship between clusterin and HSPs (represented by α B-crystallin) but also indicate that clusterin is a unique protein with no closely related family members yet identified.

Site of Action

Clusterin is constitutively secreted by mammalian cells, although stress might induce intracellular forms of the protein. A truncated form, possibly resulting from differential translation, has been reported in TGF β -treated cells³⁷; corroboration of this or comparable observations by independent laboratories would be of interest. Furthermore, a recent study has clearly demonstrated that in cell lysates (i) clusterin associates with a DNA-binding protein known as Ku70 and (ii) clusterin inhibits binding of Ku70 to a short, ³²P-labelled double-stranded oligonucleotide³⁸. Purified clusterin and Ku70 were also clearly shown to bind to each other³⁸. These findings are interesting and raise the possibility that clusterin may interact with Ku70 within cells. However, all these results could also be explained with the hypothesis that, when liberated by detergent-mediated cell lysis from its normal intracellular compartments (eg the lumen of the endoplasmic reticulum), clusterin binds to regions of exposed hydrophobicity on Ku70, thereby inhibiting its interactions with DNA. Lastly, it was recently reported that clusterin is normally intracellular in chickens³⁹.

For mammalian cells, although an intracellular action of clusterin during stress remains possible, more evidence is required to establish this. Thus, as clusterin is in most physiological settings a secreted

protein, and in light of direct demonstrations of an extracellular cytoprotective action, we suggest that clusterin acts *in vivo* as an extracellular chaperone. Known mammalian HSPs exert their chaperone effects intracellularly to protect cells. Secreted HSPs have been identified in yeast and plants - following heat shock, soybean seeds secrete a 168 kDa glycoprotein (basic 7S globulin)⁴⁰ and the yeast *Hansenula polymorpha* secretes a 198 kDa glycoprotein⁴¹. However, in neither case are the functions of these HSPs known. There are no previous reports of mammalian proteins being secreted in response to heat or acting as extracellular chaperones. Thus, clusterin may be the first identified secreted mammalian chaperone.

In the extracellular milieu, in addition to its apparent direct effects on cell viability, clusterin might bind to damaged or toxic molecules, or both, and target them for removal via megalin. Megalin, an endocytic receptor expressed by a variety of cells, can internalize clusterin which is then degraded^{15, 16}.

The challenges ahead

The realization that clusterin is a novel HSP with chaperone activity is an exciting breakthrough. However, many areas of poor understanding remain. We know little about how clusterin exerts its potent chaperone action to stabilize stressed proteins. Another area of interest is clusterin's site of action; can clusterin act both intracellularly and extracellularly to protect cells from stresses? Further, there is the question of the targets that clusterin acts on during stress. Does clusterin protect membrane proteins or exert a direct effect on lipids to stabilize cell membranes? Lastly, does extracellular clusterin act as a hydrophobic "sink", sequestering toxic or damaged molecules, directing them away from sensitive cellular sites to a safe disposal route? Obtaining the answers to these questions will keep many of us busy for years to come.

References

- 1 Blaschuk, O., Burdzy, K. and Fritz, I. B. (1983) Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. *J Biol Chem* 258, 7714-7720
- 2 Jenne, D. E. and Tschopp, J. (1992) Clusterin: The intriguing guises of a widely expressed glycoprotein. *Trends Biochem Sci* 17, 154-159
- 3 de Silva, H. V. *et al.* (1990) Apolipoprotein J: structure and tissue distribution. *Biochemistry* 29, 5380-5389
- 4 Lupas, A. (1991) Predicting coiled-coils from protein sequences. *Science* 252, 1162-1164
- 5 Thomas-Salgar, S. and Millis, A. J. T. (1994) Clusterin expression in differentiating smooth muscle cells. *J Biol Chem* 269, 17879-17885
- 6 Montpetit, M. L., Lawless, K. R. and Tenniswood, M. P. R. (1986) Androgen-repressed messages in the rat ventral prostate. *Prostate* 8, 25-36
- 7 Jordan-Starck, T. C., Witte, D. P., Aronow, B. J. and Harmony, J. A. K. (1992) Apolipoprotein J: a membrane policeman? *Curr Opin Lipidol* 3, 75-85
- 8 Burkey, B. F., Stuart, W. D. and Harmony, J. A. K. (1992) Hepatic apolipoprotein J is secreted as a lipoprotein. *J Lipid Res* 33, 1517-1526
- 9 Gelissen, I. C. *et al.* (1998) Apolipoprotein J (clusterin) induces cholesterol export from macrophage-foam cells: a potential anti-atherogenic function. *Biochem J* 331, 231-237
- 10 Hochgrebe, T. T., Humphreys, D., Wilson, M. R. and Easterbrook-Smith, S. B. (1999) A reexamination of the role of clusterin as a complement regulator. *Exp Cell Res* 249, 13-21
- 11 Jenne, D. E. *et al.* (1991) Clusterin (complement lysis inhibitor) forms a high density lipoprotein complex with apolipoprotein A-I in human plasma. *J Biol Chem* 266, 11030-11036
- 12 Ghiso, J. *et al.* (1993) The cerebrospinal-fluid soluble form of Alzheimers amyloid-beta is complexed to SP-40,40 (apolipoprotein-J), an inhibitor of the complement membrane-attack complex. *Biochem J* 293, 27-30

- 13 Tschopp, J., Chonn, A., Hertig, S. and French, L. E. (1993) Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 β , and the β domain of C9. *J Immunol* 151, 2159-2165
- 14 Humphreys, D., Carver, J., Easterbrook-Smith, S. B. and Wilson, M. R. (1999) Clusterin has chaperone-like activity similar to that of small heat-shock proteins. *J Biol Chem* 274: 6875-6881
- 15 Kounnas, M. Z. *et al.* (1995) Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin. *J Biol Chem* 270, 13070-13075
- 16 Hammad, S. M. *et al.* (1997) Interaction of apolipoprotein J-amyloid beta-peptide complex with low density lipoprotein receptor-related protein-2 megalin - a mechanism to prevent pathological accumulation of amyloid beta-peptide. *J Biol Chem* 272, 18644-18649
- 17 Pankhurst, G. J., Bennett, C. A. and Easterbrook-Smith, S. B. (1998) Characterization of the heparin-binding properties of human clusterin. *Biochemistry* 37, 4823-4830
- 18 Wilson, M. R. and Easterbrook-Smith, S. B. (1992) Clusterin binds by a multivalent mechanism to the Fc and Fab regions of IgG. *Biochim Biophys Acta* 1159, 319-326
- 19 Kelso, G. J. *et al.* (1994) Apolipoprotein-J is associated with paraoxonase in human plasma. *Biochemistry* 33, 832-839
- 20 McHattie, S. and Edington, N. (1999) Clusterin prevents aggregation of neuropeptide 106-126 in vitro. *Biochem Biophys Res Commun* 259, 336-340
- 21 Akesson, P., Sjöholm, A. G. and Björck, L. (1996) Protein SIC, a novel extracellular protein of *Streptococcus pyogenes* interfering with complement function. *J Biol Chem* 271, 1081-1088
- 22 Partridge, S. R., Baker, M. S., Walker, M. J. and Wilson, M. R. (1996) Clusterin, a putative complement regulator, binds to the cell surface of *Staphylococcus aureus* isolates. *Infect Immun* 64, 4324-4329
- 23 Reddy, K. B., Karode, M. C., Harmony, J. A. K. and Howe, P. H. (1996) Interaction of transforming growth factor beta receptors with apolipoprotein J/clusterin. *Biochemistry* 35, 309-314
- 24 Ehrnsperger, M., Buchner, J. and Gaestel, M. (1997) in *Molecular chaperones in the life cycle of proteins. Structure, function and mode of action.*, pp 533- 575, Fink, A.L. and Goto, Y. (1997) eds, Marcel Dekker, New York.

- 25 Veigner, L., Diamant, S., Buchner, J. and Goloubinoff, P. (1998) The small heat-shock protein IbpB from *Escherichia coli* stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. *J Biol Chem* 273, 11032-11037
- 26 Michel, D., Chatelain, G., North, S. and Brun, G. (1997) Stress-induced transcription of the clusterin/ApoJ gene. *Biochem J* 328, 45-50
- 27 Bailey, R. and Griswold, M. D. (1999) Clusterin in the male reproductive system: localization and possible function. *Mol Cell Endocrin* 151, 17-23
- 28 Farahbakhsh, Z. T. *et al.* (1995) Interaction of alpha-crystallin with spin-labelled peptides. *Biochemistry* 43, 509-516
- 29 Sensibar, J. A. *et al.* (1995) Prevention of cell death induced by tumor necrosis factor alpha in LNCaP cells by overexpression of sulfated glycoprotein-2 (clusterin). *Cancer Res* 55, 2431-2437
- 30 Humphreys, D. *et al.* (1997) Effects of clusterin overexpression on TNF α - and TGF β -mediated death of L929 cells. *Biochemistry* 36, 15233-15243
- 31 French, L. E. *et al.* (1994) Human clusterin gene expression is confined to surviving cells during in vitro programmed cell death. *J Clin Invest* 93, 877-884
- 32 Viard, I. *et al.* (1999) Clusterin gene expression mediates resistance to apoptotic cell death induced by heat shock and oxidative stress. *J Invest Dermatol* 112, 290-296
- 33 Schwochau, G. B., Nath, K. A. and Rosenberg, M. E. (1998) Clusterin protects against oxidative stress in vitro through aggregative and nonaggregative properties. *Kidney Int* 53, 1647-1653
- 34 Corpet, F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucl Acids Res* 16, 10881-10890
- 35 Sharma, K. K., Kaur, H. and Kester, K. (1997) Functional elements in molecular chaperone α -crystallin: identification of binding sites in α B-crystallin. *Biochem Biophys Res Commun* 239, 217-222
- 36 Sharma, K. K., Kumar, G. S., Murphy, A. S. and Kester, K. (1998) Identification of 1,1'-bi(4-anilino)naphthalene -5,5'-disulfonic acid binding sequences in α -crystallin. *J Biol Chem* 273, 15474-15478
- 37 Reddy, K. B. *et al.* (1996) Transforming growth factor β (TGF β)-induced nuclear localization of apolipoprotein J/clusterin in epithelial cells. *Biochemistry* 35, 6157-6163
- 38 Yang, C. R. *et al.* (1999) Isolation of Ku70-binding proteins (KUBs). *Nucleic Acids Res* 27, 2165-2174

- 39 Mahon, M. G. *et al.* (1999) Multiple involvement of clusterin in chicken ovarian follicle development - binding to two oocyte-specific members of the low density lipoprotein receptor gene family. *J Biol Chem* 274, 4036-4044
- 40 Kagawa, H., Hirano, H., Tomotake, M. and Kikuchi, F. (1993) A seed protein induced by heat treatment in soybean. *Food Chem* 48, 159-163
- 41 Tsiomenko, A. B., Plekhanov, P. G., Tuymetova, G. P. and Kononova, S. V. (1997) Secretory heat-shock protein of the thermotolerant yeast *Hansenula polymorpha*. Identification and comparative characteristics. *Biochemistry (Moscow)* 62, 123-128

Table 1 Reported clusterin binding ligands

Ligand	Reference
apoA-I	11
β -amyloid peptide	12
complement components	13
glutathione-S-transferase	14
gp330/megalin	15, 16
heparin	17
immunoglobulins	18
lipids	8
paraoxonase	19
prion peptide	20
SIC (Streptococcal inhibitor of complement)	21
<i>Staphylococcus aureus</i> cell surface	22
TGF- β receptor	23

Legends to Figures

Figure 1: Cartoon of the predicted structure of clusterin

The cartoon shows mature human clusterin after cleavage of its 22-mer signal peptide and internal cleavage at its arg227-ser228 bond, generating the α chain (residues 23-227) and β chain (residues 228-449). The three predicted amphipathic α -helices (yellow ovals, residues 173-184, 234-250 and 424-441) were identified by helical wheel analysis³. The two predicted coiled-coil α -helices (solid rectangles, residues 40-99 and 318-350) were identified using the COILS algorithm⁴. The central disulfide-rich region (green hatched ovals) spans residues 102-129 in the α chain and residues 285-313 in the β chain.

Figure 2: Sequence alignment of clusterin and α B-crystallin

The alignment shown is between the C-terminal halves of matched species homologs of clusterin and α B-crystallin. The sequences were from the SwissProt database and the alignment was carried out using the Multalin algorithm³⁴. The residues in red are identical or highly conserved between clusterin and α B-crystallin and the residues in blue are identical between the species homologs of α B-crystallin. The boxed regions of α B-crystallin show regions identified as being part of its chaperone active site from chemical crosslinking to alcohol dehydrogenase (ADH, orange box) and from photochemical crosslinking to bisANS (green box)^{35, 36}. The overall sequence similarity between α B-crystallin and residues 230-422 of clusterin is 17% (33 residues identical or highly conserved/193 residues).

Figure 1

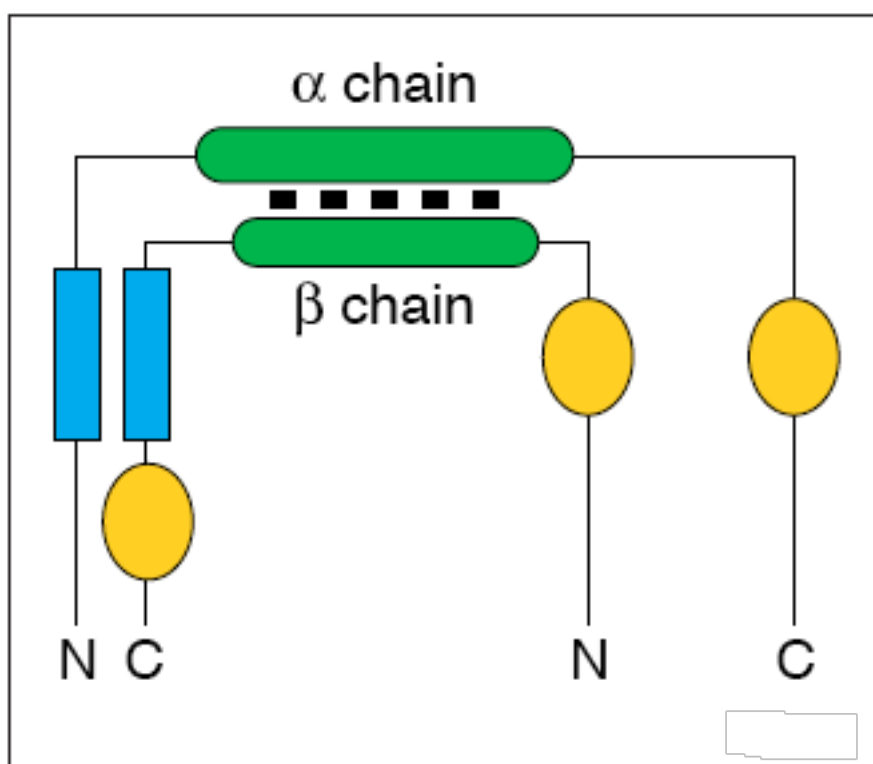


Figure 2

