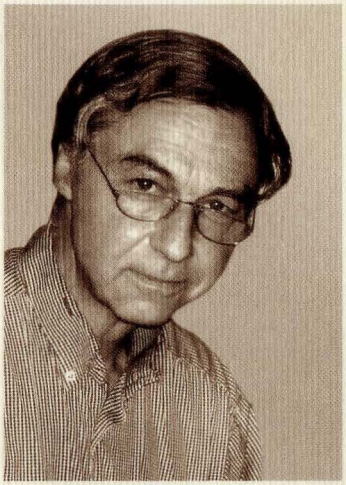
Doctor Honoris Causa

ROBERT HUBER



Facultat de Ciències

Sant Albert 2000

Universitat Autònoma de Barcelona

Doctor Honoris Causa

ROBERT HUBER

Discurs llegit a la
cerimònia d'investidura
celebrada a la sala d'actes
de la Facultat de Ciències
el dia 15 de novembre
de l'any 2000

Bellaterra, 2000

Universitat Autònoma de Barcelona

PRESENTACIÓ DE ROBERT HUBER PER F. XAVIER AVILÉS

Excel·lentíssim i Magnífic Senyor Rector,
Il·lustríssim Degà,
Il·lustríssimes Senyores, Il·lustríssims Senyors,
Senyores i Senyors,
Benvolguts companys,

Som aquí per honorar el professor Robert Huber, i és preceptiu que justifiquem la proposta que des de l'Institut de Biologia Fonamental, el Departament de Bioquímica i de Biologia Molecular i la Facultat de Ciències vam fer en aquest sentit a la Universitat Autònoma de Barcelona. Aquesta és, però, una tasca, per a mi, al mateix temps fàcil i difícil. Fàcil, perquè en la comunitat científica de les ciències biològiques són molt reconegudes les sòlides i nombroses aportacions que ell hi ha fet, i se'n poden posar molt exemples. Difícil, perquè el considero un dels meus mestres, un dels grans científics actuals en el nostre camp, amb el qual m'uneix des de fa temps una relació amistosa i, per tant, si no encerto en la selecció dels seus mèrits que vull exposar, en els adjectius escollits, en la visió que us voldria trametre, seré jo el que no tindré justificació.

Permeteu-me que comenci en anglès, la llengua franca actual en ciència, per poder-li donar una benvinguda entenedora per a ell, encara que em consta que acceptarà plenament que a continuació passem al català, atès que és un home molt amant de la seva cultura regional. Curiosament, ell, en la seva xerrada, establirà relacions entre llengua i codificació gènica, quelcom que també es podria utilitzar per indicar la profunditat i importància que té per a nosaltres la nostra llengua.

M'agradaria remarcar alguns detalls de la seva trajectòria humana i científica. Ell va néixer a Munic, on s'ha format i on ha desenvolupat pràcticament tota la seva tasca professional. Es pot, per tant, entendre fàcilment que sigui un enamorat de Baviera, un país entranyable i molt cuidat pels seus habitants. Va fer els seus estudis universitaris a la Universitat Tècnica de Munic,

d'on avui és professor. Allí es va trobar amb tota una generació excel·lent de professors, entre els quals podríem citar Karlson, Braunitzer i Hoppe, amb qui va caracteritzar l'ecdisona, una hormona de metamorfosi d'insectes, treball que va seguir posteriorment amb la seva anàlisi cristal·logràfica, tema de la seva tesi doctoral presentada el 1963. El 1968, va ser habilitat com a docent a la mateixa Universitat, de la qual va ser nomenat professor el 1976. L'any 1972, va accedir al càrrec de director del Departament d'Investigació Estructural del Max Planck Institut de Bioquímica de Munic, un dels centres de ciències biològiques fonamentals de més prestigi al món, càrrec que ha ocupat des de llavors, junt amb el de director del centre, diverses vegades.

És interessant constatar que les seves aportacions científiques, centrades essencialment en el món de la biologia estructural, s'han basat en una gran varietat de sistemes biològics, des dels més senzills fins als més complicats, i han contribuït de manera significativa a la visió microscòpica molecular i tridimensional que tenim de les estructures dels éssers vius. Cal tenir en compte que una de les característiques més sobresortints de la biologia molecular és la importància que hi té la tridimensionalitat. Per això es consideren fites essencials la resolució de l'estructura tridimensional de l'ADN i la de la mioglobina, el 1953 i el 1957, ambdues per cristal·lografia de raigs X. Doncs bé, el laboratori de Robert Huber és un dels que ha resolt fins ara més estructures tridimensionals de proteïnes, i ens n'ha facilitat la visió esmentada. Això ho ha fet per mitjà de les més de vuit-centes publicacions científiques del seu grup. Aquesta és una tasca de gran importància en l'anàlisi comparativa que s'intenta fer avui dia per desxifrar la codificació gènica a nivell seqüencial i conformacional, i que ell citarà en la seva intervenció com la pedra de Rosetta de la genòmica i de la proteòmica modernes. Quin paral·lelisme tan bonic!

Entre els seus treballs postdoctorals immediats es pot citar la determinació de l'estructura tridimensional de l'eritrocruorina, una proteïna d'insecte que va resultar ser similar a la de globines de mamífers, resoltes pocs anys abans per Kendrew i Perutz. També, la seva investigació sobre la conformació de l'inhibidor de tripsina pancreàtic bàsic (BPTI), un model en química i biofísica de proteïnes, i dels seus complexos amb la proteasa digestiva tripsina. D'aquí va néixer el seu interès per l'estudi de les interaccions proteïna-proteïna i proteïna-lligand, essencials en molts processos biològics. Tot això el va portar, posteriorment, a especialitzar-se en el món del control de l'activitat enzimàtica, a partir de precursors processables o mitjançant inhibidors naturals o artificials, particularment en el camp dels enzims hidrolítics (p.e. proteases). Sens dubte, el seu laboratori ha estat i és un dels referents en aquests camps, en el qual vam començar a col·laborar. Des de llavors, ha estès la seva recerca a molts sistemes biològics fonamentals que involucren aquests enzims: coagulació de la sang, fibrinòlisi, inflamació, sistema immunitari i de defensa, proliferació viral, càncer, etc., i molts d'altres que impliquen degradació de proteïnes.

En paral·lel o posteriorment, ha estès la seva recerca cap a una gran varietat de biomolècules importants, com ara immunoglobulines, enzims de passos o rutes metabòliques essencials (com la que porta de GTP a flavines i folats, o la síntesi de lisina i metionina), i enzims involucrats en processos xenobiòtics, molts d'ells de gran interès biotecnològic i biomèdic.

Una fita important en la seva trajectòria va ser la resolució, al començament dels anys vuitanta, de l'estructura tridimensional del centre de reacció fotosintètic del bacteri purpuri *Rhodopseudomonas viridis,* un complex de quatre proteïnes, incloent-hi els grups prostètics, per la qual li van concedir el Premi Nobel de Química l'any 1988, juntament amb Deisenhofer i Michel. Aquest treball ja és un clàssic en bioquímica, perquè no solament va constituir la primera estructura detallada de la part central de la maquinària fotosintètica, essencial per a la vida, sinó també d'una proteïna de membrana transductora d'energia i transferidora d'electrons. Hauríem de recordar que, avui dia, la resolució de les estructures tridimensionals de proteïnes de membrana és una de les fites encara no assolides de manera sistemàtica per la biologia estructural, malgrat la seva importància quantitativa (entre el 20 i el 30 % dels gens sembla que codifiquen per aquestes proteïnes) i biomèdica (són un dels principals llocs d'unió dels fàrmacs). No hi ha dubte, per tant, que Robert Huber va ser un innovador en aquest camp. De tota manera, els que ens hi movem creiem que, amb aquell premi, al mateix temps es reconeixia la seva amplíssima tasca en molts altres temes.

També han estat especialment significatives les seves aportacions sobre metal·loproteïnes, els complexos entre proteïnes i metalls que actuen de cofactors (zinc, ferro, coure, vanadi, molibdè, etc.) essencials en molts mecanismes de catàlisi enzimàtica i de transferència electrònica, de paper crucial en processos fisiològics i fisiopatològics.

Cap als anys setanta, el seu grup va descriure que algunes de les proteïnes analitzades presentaven flexibilitat a gran escala. Aquesta propietat, que posteriorment ells mateixos i altres grups van estudiar profundament per gran varietat d'aproximacions (crioespectroscòpia, NMR, modificacions químiques, dinàmica molecular...), ha resultat essencial per comprendre les propietats de les proteïnes. Avui dia, s'accepta que flexibilitat conformacional i desordre proteic són propietats rellevants, i que la seva modificació o interconversió, a escala global o regional, té un paper significatiu en la regulació de les propietats de moltes proteïnes i dels sistemes biològics dels quals formen part.

No cal dir que, després d'una trajectòria tan productiva i extensa en cristal·lografia de raigs X, el grup de Robert Huber havia de tenir un rol important en el desenvolupament d'aquesta tecnologia i d'altres associades: mètodes i programes per avaluar la intensitat de les dades, la correcció de l'absorció, recerca i ús de models, tractament de geometries internes, traçat i afinament de models atòmics sobre densitat electrònica, etc. També la seva

extensió cap a un millor ús de la moderna tecnologia basada en sincrotons, com el marcatge específic de proteïnes amb seleni, un bon dispersor anòmal. Aquest és, precisament, un exemple de la seva polivalència metodològica i estratègica, ja que va requerir incorporar subgrups treballant en mutagènesi i enginyeria de proteïnes. El mateix van fer, a la pràctica, en el camp de la biocomputació i de la ressonància magnètica nuclear, amb subgrups potentíssims integrats al seu departament. A un altre nivell, aquest tarannà ha quedat reflectit en l'empresa biotecnològica «Proteros», que va muntar fa uns anys als afores del seu institut, amb la col·laboració del *Land* de Baviera.

Tornant a l'home, a més de la seva finor intel·lectual, que tothom li reconeix, crec que cal remarcar la seva capacitat organitzativa i perseverança, aquesta molt lligada a un entusiasme sense límits pel seu treball. És sorprenent i, sens dubte, estimulant per als seus col·laboradors joves veure com s'asseu al seu costat per ajudar-los a muntar un cristall o resoldre sobre la pantalla del computador una estructura difícil, especialment en caps de setmana. És revelador constatar que sempre ha col·laborat d'una manera clara en l'elaboració de les més de vuit-centes publicacions del seu grup. Això ho ha fet compatible amb la participació freqüent en actes festius interns del grup, i en la multitud de reunions científiques pròpies i alienes en què un grup tan gran com el seu es veu involucrat. Costa d'entendre d'on treu temps per a tot!

Per acabar, voldria indicar breument la relació de Robert amb la UAB i amb altres institucions de països europeus meridionals, que ens és coneguda. Encara que nosaltres havíem seguit estretament des de feia temps la seva trajectòria i els seus assoliments, el primer contacte seriós amb ell el vam tenir l'any 1987, a Marsella, durant la jornada d'homenatge a un altre gran expert en proteases, el professor Pierre Desnuelle, a la qual vam ser convidats conjuntament. Des de llavors, hem mantingut una relació científica i humana productiva, amistosa, molt agradable i de la qual em sento honorat. D'aquesta relació ha nascut una sèrie de projectes (de l'EC, AI, i altres), que s'han portat a terme i que han permès que tot un conjunt d'investigadors catalans, molts de la UAB o que hi estan relacionats, s'hagin involucrat en treballs conjunts o s'hagin format en biologia estructural i cristal·lografia de proteïnes al seu laboratori: Miquel Coll, F. Xavier Gomis-Ruth, Alícia Guasch, Mariola Gómez, David Reverter, Eva Estébanez, Marta Garrido, Mireia Comellas i jo mateix, entre d'altres. Sense el seu ajut, les nostres aportacions al camp de les proteases haurien quedat molt limitades. A més, altres investigadors espanyols, portuguesos o procedents d'altres indrets de l'àrea mediterrània, dels quals em permeto assumir avui una representació, s'han format també al seu laboratori: Antonio Romero, Javier Medrano, Carlos Fernández-Catalán, Maria Joao Romao, Pedro Pereira, Sandra Ribeiro, Martino Bolognesi, Doriano Lamba, etc. Cal esmentar, també, que Robert Huber ha acceptat actuar com a conferenciant en diversos cursos, tallers o congressos que hem organitzat o en els quals hem participat, malgrat la seva evident manca de temps. Per tot això,

per la seva hospitalitat, i per l'amabilitat amb què sempre ens ha tractat, volem dir-li: moltes gràcies Robert! Benvingut a la nostra comunitat universitària.

Sobre la base del que he exposat, Excel·lentíssim i Magnífic Senyor Rector, sol·licito que el professor Robert Huber sigui investit en el dia d'avui amb el grau de doctor *honoris causa* per la Universitat Autònoma de Barcelona, segons proposta formulada al seu dia per la Facultat de Ciències, el Departament de Bioquímica i de Biologia Molecular, i l'Institut de Biologia Fonamental.

Moltes gràcies.

DISCURS
DE
ROBERT HUBER

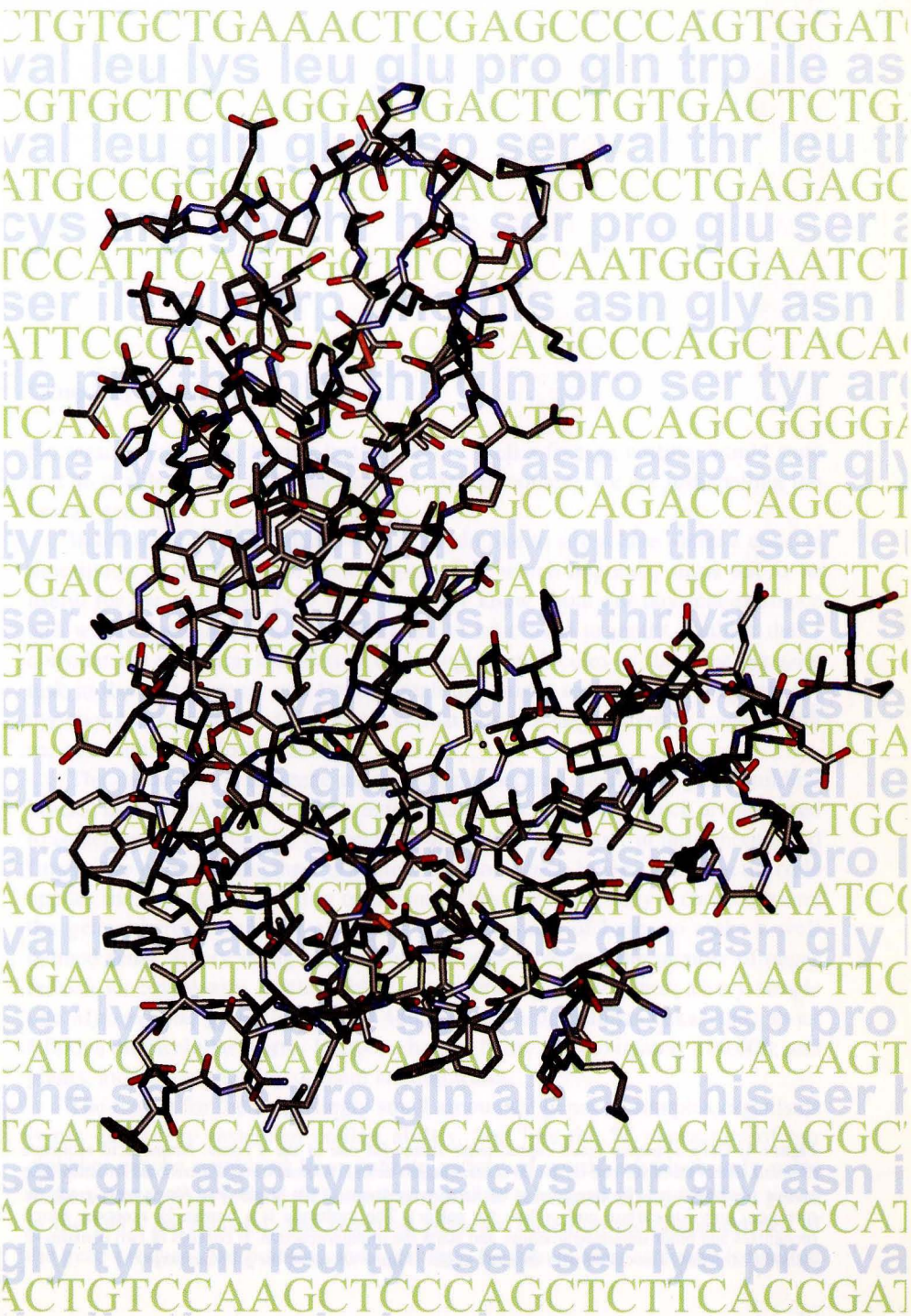


Figure 1: Gene sequence (each capital letter stands for one of the 4 bases. Amino acid sequence (3 small letters stand for one of the 20 natural amino acids). Atomic three dimensional structure (the atomic positions are to be imagined at the cross over points of the bonds. Red for oxygen, green for nitrogen, grey for carbon and yellow for sulfur). The molecule is a human Fc receptor which recognizes and binds immunoglobulines and starts the immunoreaction. It consists of two domains of a similar structure, associated with an acute angle. *(Figure shown in the previous page)*

Genes: an endless puzzle ?

No reasonable person having just learned to spell a foreign writing, would state that he is able to read and understand it. At the moment, we are just spelling the human genome.

In the sense of this realistic view, I would like to stress here –after a concise description of the basics of gene engineering, which hopefully turns out to be useful for the audience– how little we really know about our physical existence and which gaps in our knowledge have to be closed urgently. I admit that I consider the structural elucidation of biological molecules as the most pressing task in the post-genome era. Even though this might sound like propaganda for my own special research field, I believe that it is to a large extent in consent with the community of biosciences.

The metaphor of the gene as a writing describes clearly the gene building blocks, the coding DNA bases as letters, the genes as words, which are arranged to sentences of functionally cooperating units, the "operons", in the genome, and finally the entire work, the "genome", the book of a being. The genetic code applies universally to all living organism. It has, however, only 4 characters and the genes therefore consist of a very long sequence of bases to make a well defined sense. Every being has its own genome-book, and the number of pages reflects its complexity. Bacteria have approximately 1.000 genes; a very simple one *Mycoplasma genitalium*, only 480; yeast, an unicellular eukaryont, about 6.000; a multicellular worm, 19.000; a human being, approximately 100.000; and plants, more, though many of them are duplicates.

The reason for the surprisingly small amount of genes in complex organisms is not caused by restricted coding ability. Just like the 26 characters of our alphabet can be combined to an infinite variety of words, of which only some make sense, only a few of the countless possible arrangements of bases in genes in the DNA are realized. The selection rules are based on the characteristics of the gene products, the proteins, their stability, the rate of their structure formation

from an unfolded chain of amino acids (as will be explained later), and their history and development from simple antecedents in the course of evolution.

A German Dictionary, the Duden, contains approximately 120.000 "catchwords" (coincidentally just as many as the human being has genes), a few thousand more than the previous edition. Most of the new words are compositions of several different words, like "Maschendrahtzaun", or have been adapted from foreign languages.

Composed words have their analogues in the building plan of many genes which are also pieced together by different parts. These parts exist in different combinations in the genome and appear as autonomously folded domains, threaded like pearls on the string of a polypeptide chain in the proteins.

Our metaphor is still applicable, because the adaptation of words from foreign languages reminds of the horizontal gene transfer among the species, which occurs primarily in bacteria. Gene engineering takes advantage of this natural event. Sequencing of the genomes and the production of transgenic organisms, bacteria, animals and plants, are based on the technical development of this natural process.

Approximately two dozens of complete genomes and archaebacteria, bacteria and uni- and multi-cellular eukaryonts have been sequenced up to now, first of all the bacteria *Haemophilus influenzae* in 1995. The sequencing of the human genome is another milestone in biological research, although the methodical bases of gene sequencing had been discovered and developed earlier. There is good reason for the opinion that the analysis of the genome of pathogene microorganisms is more important than of the human genome because this helps to advance drug design more directly.

The difference between our genome and that of the worm is amazingly small, not only in size but also with respect to the sequence of most of the genes. Bacteria, yeast, worm, plants, fly, mouse and the human being have many important physiologic functions and the necessary genes in common. The analysis of the hereditary material of man, of the "crown of creation", does therefore not give reason for arrogance.

The similarity of related genes in different organisms is in fact an important aid to learn about the function of the gene products, of the proteins, because they can be studied more easily in lower organisms, with less technical problems than in mammalia and without ethical problems as in research on humans. Similar proteins have similar functions in bacteria and humans. The comparative genome analysis reminds of the deciphering of the Egyptian writings on the Rosetta stone, which also contained the legible Greek text.

The meaning of a word describing an object depends on experience and perception: reading the word "house" evokes a building in our imagination. A book is the same to all readers, although its interpretation may vary from person to person.

The interpretation of the genome in living organisms, however, is very complicated: the genomes are translated three times before they appear as gene products, as proteins: the DNA is transcribed into messenger RNA which serves as a matrix for the translation into proteins. While the transcription from DNA into messenger RNA happens base by base, a "triplet-code" is being used for the translation into proteins, whereby three bases encode one of the 20 different amino acids. Under the direction of the messenger RNA the amino acids are connected at the ribosome, a big intracellular synthesis machine, to linear chains by peptide bonds. Proteins emerge as flexible chain molecules. In a third step, the proteins fold from a flexible chain to well arranged three dimensional structures. The triple translation of the genome is not a linear process. In higher organisms there are branch points at each translation: in the course of the transcription of the DNA into RNA different segments may be cut out so that different messenger-RNAs are generated, which in the translation step result in a variety of proteins. Proteins are chemically modified during and after the folding process, and their properties altered.

In certain organs the genes, too, undergo somatic changes, for instance in immune cells (lymphocytes) in such a way that in different cells, by any combination of gene fragments, a huge repertoire of genes for antibodies is produced which exceeds even the number of germ cell genes. Different antibodies possess different binding properties for the recognition and defense of antigens, which stimulate "their" respective lymphocytes (Clonal selection). The immune system is thus armed against all sorts of foreign macromolecular materials.

Genes define the physical existence and the possibilities of an organism. The missing of one single gene or the mis-function of one single gene-product can be life threatening as we know from the studies of human hereditary deseases. The expression of the genes, however, is correlated with the surroundings. Our cognitive existence, life and spirit, are mainly imprinted by the socio-cultural surroundings. We are no slaves of our genes, of which we are indeed dependent, but which do not command us. The genome analysis therefore does not offer a description of the functioning of an organism, not even a building plan; it gives us rather an incoherent list of parts –incoherent because we know very little about the interplay between the genes and the gene products. It is even more fatal that we know almost nothing about the function of many of the parts. To find the answer, we have to examine the gene-products: the proteins.

It is therefore timely to have a closer look at them. I have already mentioned that proteins have well defined three dimensional structures and multiple functions as building elements, catalysis, ligands regulators, inhibitors in living organisms. Once their structure is destroyed, they loose their functional ability. No chick slips from a boiled egg.

Protein molecules are very small. A supermicroscope using x-rays is needed to make them visible. Many thousands of atoms of the amino acid building

blocks arrange themselves to a rigid molecule in which each one of them knows its well defined place. Let us remember that proteins are synthesized from flexible chains of amino acids. How do they find their three dimensional shape in a short time? The folding of an amino acid chain can be described approximately by two dihedral angles of each of their building blocks, and has thus 200 degrees of freedom if the length of the chain is 100 amino acids. Even if each dihedral angle had only 4 allowed values, we could count 4^{100} different configurations. From such a large number of possibilities, it would be impossible to find the correct one by trying, even if the rate of change of configurations were very rapid. However, in each cell many thousands of protein molecules are synthesized every second, which fold correctly according to rules and mechanisms which are largely unknown and which need to be studied and analysed urgently. As long, however, as we cannot compute protein structures from their amino acid sequence, we have to define them experimentally through the troublesome x-ray crystallographic analysis.

Development of methods, technique and instrumentation have facilitated and speeded up protein crystallography during the last years. By molecular biology and gene engineering we obtain sufficient material for experiments with rare proteins. We have sources for x-ray light with the synchrotrons, which had been built by physicists to investigate elementary particles and which emit strong focussed and tunable x-rays, a burden and trash for the physicists, an irreplaceable tool for the structure researchers. During the last years, synchrotrons as dedicated x-ray sources have therefore been built for structure research. In addition to that, synchrotrons are versatile sources also of light of longer wavelength which is indispensable for spectroscopy, microscopy, lithography in physics, chemistry, material research and nanotechnology.

The trouble of structure analysis pays because only the atomic structure reveals the function. Figure 2, for instance, shows the surface of the central enzyme of blood coagulation, of thrombin. Thrombin cleaves a big protein molecule in the blood, fibrinogen, activated by signals which are released by injured blood vessels. The cleavage product, fibrin, together with other blood substances, forms a network, the thrombus, which closes the wound. The structure of thrombin not only shows how fibrinogen substrate is bound, but also allows to plan and to test inhibitors. Thrombin is a most important molecule for the development of drugs against malfunction of blood coagulation, like in heart infarction or stroke. The small organic molecule, which is drawn as blue lines model on the yellow surface, is such an inhibitor, synthesized as designed. Let us have one more look at thrombin, which can initiate blood coagulation, but also terminate. This happens when thrombin binds to another protein molecule, thrombomodulin. In this complex the binding of fibrinogen is suppressed, but that of another molecule, protein C, is enhanced. Protein C is cleaved and thus

activated in the ternary complex. Activated protein C destroys other coagulation factors and thus stops coagulation.

We know all this from x-ray crystallographic analyses in atomic detail. Only a small part of the three dimensional structures of human proteins have been analysed so far. We have to examine all of them in order to understand the human genome, but will concentrate on those with known close connections to diseases, because these studies will contribute to a basic understanding and therapy.

Every kind of being has its own genome. Several million species are populating our planet. Not each one of their genomes has to be analysed, because reliable conclusions can be drawn from related species. Obviously, there is quite a bit of fascinating research work left for the coming generations, especially with resepect to the analysis of the genome of pathogenic microbes or of plants, where useful applications are obvious.

Let us finally come back to the motto of this talk: the sense of the genome. The realization of the information in a living organism, which is layed down in the sequence of the bases of genomic DNA, takes place in many steps: copying of the DNA of the germ cell during cell division, transcription of the DNA into messenger RNA, translation of the RNA into protein, folding of the peptide chain into a three dimensional structure, formation of a functioning protein, development of protein-protein and protein-nucleic-acid complexes, interaction of molecule complexes in cells, cell-cell interplay in organisms and organs, and, last but not least, interaction of organs in higher living creatures.

Each one of these translation steps includes ramifications, possible choices and control, which for instance determine when, where and in which quantities proteins are synthesized and with which molecules they interact, which in return modulate their function. Embryogenesis, the wonderful development of a fertilized egg, equipped with the complete complement of genes, to a complicated organism, is based on such complex, in space and time exactly controlled regulatory cascade.

One such molecular step of the coagulation cascade I have described with thrombin and its function. A much more complex structure we find with the proteasome which consists of 28 protein subunits (Figure 3). Each subunit knows its precise position in the molecule. They are arranged in four rings and form a hollow cylinder. The active sites of the proteasome, which functions to cut protein waste in cells, are located on the inner wall of the middle rings and have small synthetic inhibitor molecules bound. Their structures are moduled with white connected spheres. Only 6 of the 28 subunits are enzymatically active, but all are required to build the macromolecular complex like the construction elements of a house supporting the functional components. The proteasome is in a latent state in cells and becomes activated when needed in the cell cycle. With thrombin and the proteasome the molecular basis of their function and regulation

is understood because their spatial structures are known. However, most of the very many regulatory mechanisms which operate in the course of the translation of the genome are yet unknown.

In general, the correlation between genotype and phenotype becomes less sharply determined with continuing translation and increasing complexity and is more influenced by the environment and its change in time, by chance and also by our personal will. Human freedom is possible.

Also the possibilities of scientists to study the molecular basis of life have grown enormously and let us expect that we will precisely know within one generation the first 6 steps of the translation of the genome of a few bacterial species as defined above.

Methods and instruments are in principle available also for the next steps and applicable for higher organisms. We must stifle the enthusiasm of young people for the biosciences, for adventure and discoveries awaiting them, and for research, which like no other is needed to provide food and health for mankind in this century.

We must also encourage international collaborations to combine the strength and expertise of scientists from different laboratories. Collaborations have scientific and human social value. I have the privilege of a long fruitful time of joint research with the Universitat Autònoma de Barcelona. It led to a deep respect for the achievements in research of professor Francesc Xavier Avilés, and to friendship. I am happy and feel deeply honoured about my formal ties with the Universitat Autònoma de Barcelona.

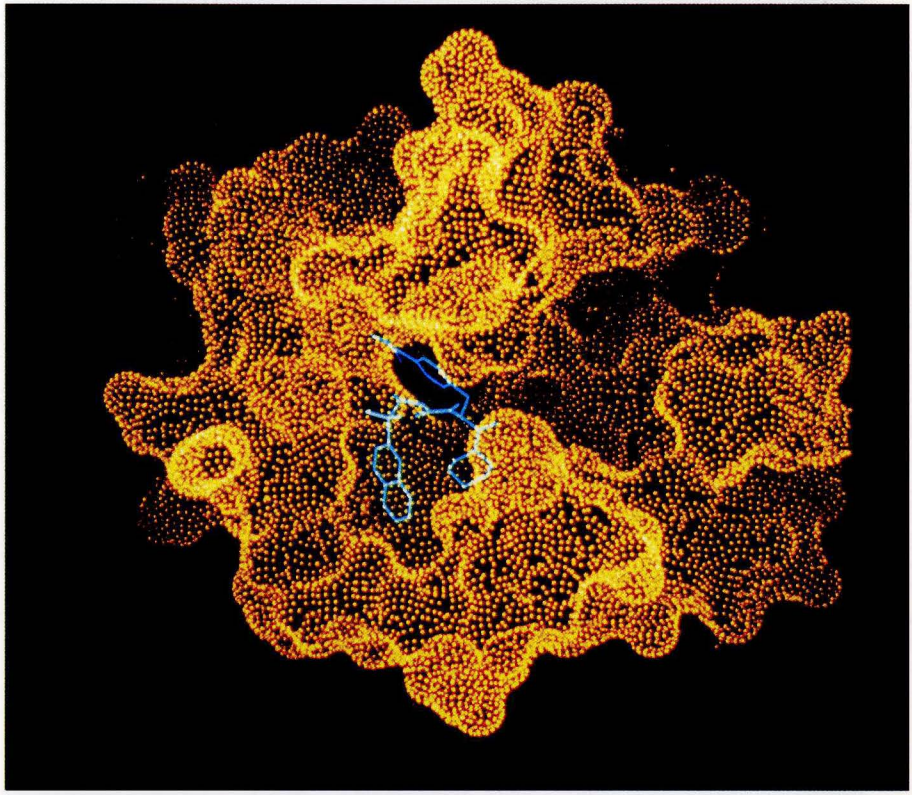


Figure 2: Surface representation of human thrombin. The active site cleft which devides the molecule in a northern and a southern part is the substrate binding region which binds fibrinogen. The blue skeleton model is a small synthetic inhibitor which prevents binding and cleavage of fibrinogen.

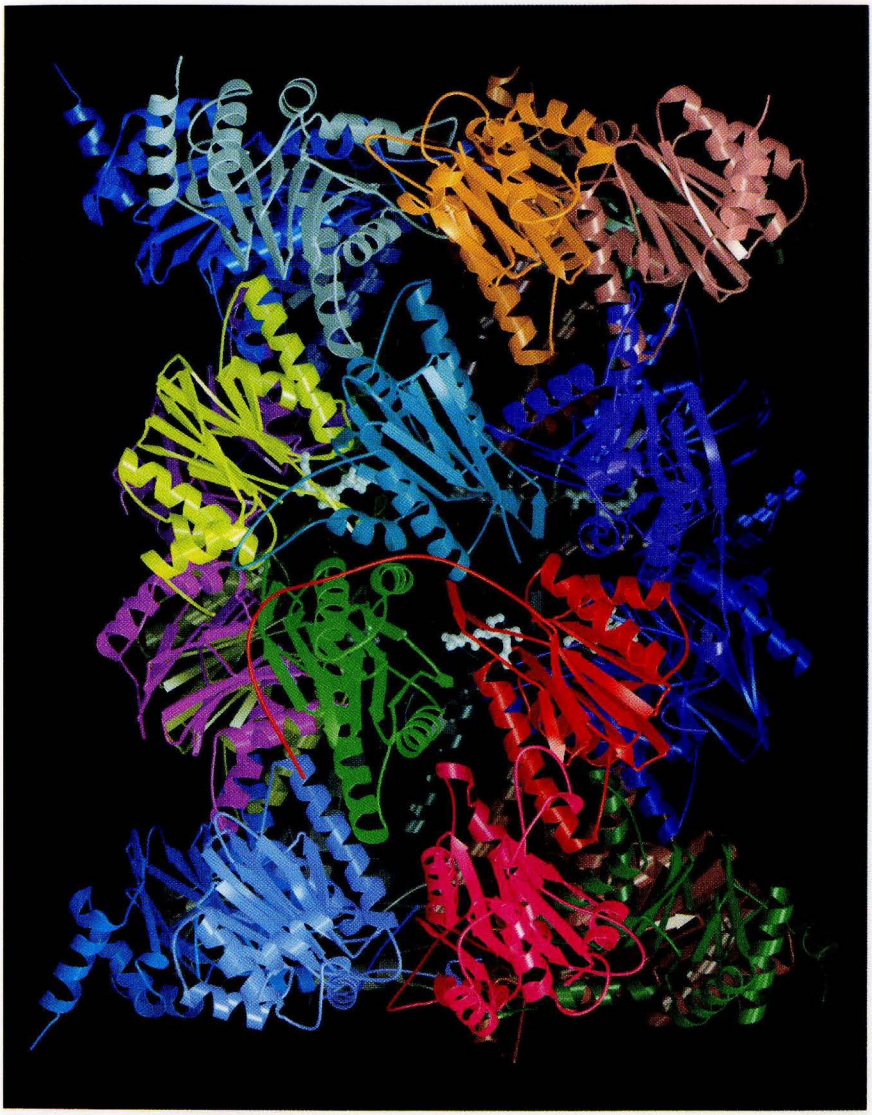


Figure 3: A ribbon picture of the 28 subunits of the proteasome molecule with different colours. The molecule is a hollow cylinder. The active sites are located on the inner wall and are marked with inhibitor molecules which appear as white objects.

CURRICULUM VITAE
DE
ROBERT HUBER

Robert Huber
Born: February 20, 1937 in Munich
Grammar school and Humanistisches Gymnasium in Munich

Professional education and positions
- Diploma in chemistry, Technische Universität München (1960)
- Dr. rer. nat., Technische Universität München (1963)
- Habilitation, Technische Universität München (1968)
- Scientific Member of the Max-Planck-Gesellschaft and Director at the Max-Planck-Institut für Biochemie (1972-2000)
- Apl. Professor, Technische Universität München (1976)
- Editor: *Journal of Molecular Biology*

Societies
- Member of the Deutsche Chemische Gesellschaft
- Member of the Gesellschaft für Biologische Chemie
- Honorary Member of the American Society of Biological Chemists
- Honorary Member of the Swedish Society for Biophysics
- Member of EMBO
- Honorary Member of the Japanese Biochemical Society
- Member of the EMBO Council
- Member of the Bayerische Akademie der Wissenschaften
- Member of the Deutsche Akademie der Naturforscher, Leopoldina
- Corresponding Member of the Croatian Academy of Sciences and Arts
- Member of the Accademia Nazionale dei Lincei, Rome
- Member of the Ordre Pour le Mérite für Wissenschaften und Künste
- Associate Fellow, Third World Academy of Sciences, Trieste
- Foreign Associate, National Academy of Sciences, USA
- Fellow, American Academy of Microbiology
- Fellow of the Royal Society, London

Honors
- E. K.-Frey Medal, Gesellschaft für Chirurgie (1972)
- Otto-Warburg Medal, Gesellschaft für Biologische Chemie (1977)
- Emil von Behring Medal, Universität Marburg (1982)
- Keilin Medal, Biochemical Society, London (1987)
- Richard-Kuhn Medal, Gesellschaft Deutscher Chemiker (1987)
- Doctor *honoris causa*, Université Catholique de Louvain (1987)

– Nobel Prize in Chemistry (1988)
– E. K. Frey-E. Werle Gedächtnismedaille (1989)
– Doctor *honoris causa*, University of Ljubljana, Slovenia (1989)
– Kone Award, Association of Clinical Biochemists, United Kingdom (1990)
– Doctor *honoris causa* for Medicine and Surgery, Università Tor Vergata, Rome, Italy (1991).
– Sir Hans Krebs Medal, Federation of European Biochemical Societies (1992)
– Bayerischer Maximiliansorden für Wissenschaft und Kunst (1993)
– The Linus Pauling Medal (1993/94)
– Miami Winter Symposia, The Distinguished Service Award (1995)
– Max Tishler Prize, Harvard University, USA (1997)
– Max-Bergmann-Medaille des Max-Bergmann-Kreises zur Förderung der peptidchemischen Forschung, (1997)
– Das Grosse Verdienstkreuz mit Stern und Schulterband der Bundesrepublik Deutschland (1997)
– Doctor *honoris causa*, Universidade Nova de Lisboa, Portugal (2000)

Professional interests

Structure and function of biological macromolecules

– Structural basis of protein-protein and protein-ligand interaction; flexibility and its functional importance. Systems: protease inhibitors, proteases, antibodies, and enzyme complexes, proteins of photo-synthesis, proteins of energy and electrontransport.

Experimental and theoretical methods for the X-ray crystallography of proteins

– Patterson methods, structural refinement, diffraction intensity measurements.

Publications

– More than 823 articles in referenced scientific journals (in 10-2000).
– Numerous reviews and book chapters.

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Robert Huber. A personal account

I was born February 20, 1937 in Munich as the first child of Sebastian and Helene Huber. My father was cashier at a bank and my mother kept the house and brought up the children, me and my younger sister, a difficult task during the war, a continuous struggle for some milk and bread and search for airraid shelters. There was no Grammar school in 1945 and 1946 and I entered the Humanistische Karls-Gymnasium in Munich 1947 with intense teaching of Latin and Greek, some natural science and a few optional monthly hours of chemistry. I learned easily and had time to follow my inclination for sports (light athletics and skiing) and chemistry, which I taught myself by reading all textbooks I could get.

I left the Gymnasium with the Abitur in 1956 and began to study chemistry at the Technische Hochschule (later Technische Universität) in Munich, where I also made the Diploma in Chemistry in 1960. A stipend of the Bayerisches Ministerium für Erziehung und Kultur and later of the Studienstiftung des Deutschen Volkes helped to relieve financial problems of my family and allowed me to study without delay. The most impressive teachers I remember were W. Hieber and the logical flow and impressive diction of his lectures in inorganic chemistry, E. O. Fischer, the young star in metalloorganic chemistry, F. Weygand and his deep knowledge of organic chemistry; and G. Joos and G. Scheibe, the physicist and physiochemist, respectively. I joined the crystallographer W. Hoppe's laboratory for my diploma work on crystallographic studies of the insect metamorphosis hormone ecdysone. Part of these studies were made in Karlson's laboratory at the Physiologisch-Chemisches Institut der Universität München, where I found by a simple crystallograpic experiment the molecular weight and probable steroid nature of ecdysone which I and Hoppe later elucidated in atomic detail after my thesis work which was on the crystal structure of a diazo compound (1963). This discovery convinced me of the power of crystallography and led me to continue in this field.

After a number of structure determinations of organic compounds and methodical development of Patterson search techniques, I began in 1967 with Hoppe's and Braunitzer's support crystallographic work on the insect protein erythrocruorin. The elucidation of this structure and its resemblance to the mammalian globins as determined by Perutz and Kendrew in their classical studies suggested for the first time a universal globin fold. 1971 the University of Basel offered me a chair of structural biology at the Biozentrum and the Max-Planck-Gesellschaft the position of a director at the Max-Planck-Institut für Biochemie which I accepted. I remained associated with the Technische Universität München, where I became Professor in 1976.

I had begun in 1970 work on the basic pancreatic trypsin inhibitor which has later become the model compound for the development of protein NMR,

molecular dynamics, and experimental folding studies in other laboratories. Work in the field of proteolytic enzymes and their natural inhibitors has been continued in my steadily growing laboratory and extended to many different inhibitor classes, proteases, their proenzymes, and complexes between them and other hydrolytic enzymes, of which the a-amylases and their natural inhibitor complexes deserve particular attention. The potential of these systems for drug and protein design has spurred our interest until today with a focus on proteins of the coagulation and fibrinolysis cascade. Studies of these systems included natural inhibitors of thrombin and factor Xa which were often found to bind in a non-canonical manner to their target enzymes. Our recent structural characterization of the proteasome and the HslV protease highlights the aspect of active site shielding and compartimentalization as a regulatory means of these intracellular protease complexes.

Early in the seventies I began to work on immunoglobulins and their fragments which culminated in the elucidation of several fragments, an intact antibody and its Fc fragment, the first glycoprotein to be analysed in atomic detail. Work was extended to proteins interacting with immunoglobulins, most recently the Fc receptor and to complement proteins. We also studied a variety of enzymes leading to the elucidation of the structure and the chemical nature of the selenium moiety in glutathion peroxidase. We determined the structures of citrate synthase in different states of ligation and of a very large multienzyme complex, heavy riboflavin synthase.This led recently to a programme aiming at the elucidation of many or all of the proteins in the metabolic pathway from GTP (guanosintriphosphate) to flavins, tetrahydropterins, and folates. We have made considerable progress and analysed 6 enzyme structures including many variants. Similarly we are interested in enzymes involved in the biosynthesis of the amino acids lysine and methionine and in the metabolism of xenobiotics and herbicide. Here the aspect of design and development of efficient herbicides, often in collaboration with industry, provides a strong impetus for the work.

Early in the 80ths we began with studies of proteins involved in excitation energy and electron transfer to understand the physical basis of these processes, light harvesting proteins, the reaction centre, the first membrane protein yielding high resolution crystals and ascorbate oxidase, a multicopper enzyme.

The interplay between proteins and their metal cofactors spurred our interest leading recently to structural studies of many more metalloenzymes, containing copper, iron, vanadium, molybdenum, zinc. In our recent studies of the families of zinc metalloproteases, both aspects of metalcoordination chemistry and medical interest are realized because these proteins and their natural inhibitory counterplayers play crucial roles in physiological and pathphysiological processes.

We had discovered in early 1970 that some of the proteins analysed showed large-scale flexibility which was functionally significant. The trypsinogen serine

protease system was investigated in great detail by low temperature crystallography, x-ray spectroscopy, chemical modification and molecular dynamics calculations, but it required some years before the scientific community in general accepted that flexibility and disorder are very relevant molecular properties also in other systems. It recently became clear that reversible order-disorder transitions are a physiologcal regulatory mechanism the serine protease plasminogen activator.

The development of methods of protein crystallography has been in the focus of my laboratory's work from the beginning and led to the development of refinement in protein crystallography and recently to the definition of standard group geometries and forces on the basis of experimental structures, to the development of Patterson search methods, to methods and suites of computer programmes for intensity data evaluation and absorption correction for protein crystallographic computing, for computer graphics and electron density interpretation and refinement (FRODO, Jones; MAIN, Turk), and for area detector data collection (MADNES). These methods and programmes are in use in many laboratories in the world today. Many of these structural studies were collaborative undertakings with other laboratories, often in industry, many of them from foreign countries. With the development of more efficient and faster crystallographic methods much of the workload has been shifted towards molecular biology and protein chemistry also carried out in my laboratory. This is not the place to name all of the numerous students and collaborators who joined my laboratory over the years, but without their contributions little would have been achieved. I just point out a few who were responsible for major achievements of the laboratory: Bode, Steigemann, Epp, Deisenhofer, Messerschmidt, Groll, Clausen, Steinbacher, some of whom are still with me.

I married Christa Essig in 1960. We have four children. The eldest daughter (1961) and the two sons (1963, 1966) have studied economics and are in various businesses. The youngest daughter (1976) studies English history in England.

SELECTED ORIGINAL ARTICLES (10 out of 823, October 2000)

- SONDERMANN, P.; HUBER, R.; OOSTHUIZEN, V.; JACOB, U. (2000). [822] «The 3.2-Å crystal structure of the human IgG1 Fc fragment-FcgRIII complex». *Nature* 406, 267-273.

- SOULIMANE, T.; BUSE, G.; BOURENKOV, G. P.; BARTUNIK, H. D.; HUBER, R.; THAN, M. E. (2000). [819] «Structure and mechanism of the aberrant ba3-cytochrome c oxidase from *Thermus thermophilus*». *EMBO J.* 19, 1766-1776.

- FUENTES-PRIOR, P.; IWANAGA, Y.; HUBER, R.; PAGILA, R.; RUMENNIK, G.; SETO, M.; MORSER, J.; LIGHT, D. R.; BODE, W. (2000). [811] «Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex». *Nature* 404, 518-525.

- BOCHTLER, M.; HARTMANN, C.; SONG, H. K.; BOURENKOV, G.; BARTUNIK, H.; HUBER, R. (2000). [791] «The structure of HslU and the ATP-dependent protease HslU-HslV». *Nature* 403, 800-805.

- DITZEL, L.; LÖWE, J.; STOCK, D.; STETTER, K. O.; HUBER, H.; HUBER, R.; STEINBACHER, S. (1998). [693] «Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT». *Cell* 93, 125-138.

- GROLL, M.; DITZEL, L.; LÖWE, J.; STOCK, D.; BOCHTLER, M.; BARTUNIK, H. D.; HUBER, R. (1997). [596] «Structure of 20S proteasome from yeast at 2.4 Å resolution». *Nature* 386, 463-471.

- ROMÃO, M. J.; ARCHER, M.; MOURA, I.; MOURA, J. J. G.; LEGALL, J.; ENGH, R. A.; SCHNEIDER, M.; HOF, P.; HUBER, R. (1995). [534] «Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from D. gigas». *Science* 270, 1170-1176.

- LÖWE, J.; STOCK, D.; JAP, B.; ZWICKL, P.; BAUMEISTER, W.; HUBER, R. (1995). [496] «Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 Å resolution». *Science* 268, 533-539.

- DEISENHOFER, J.; EPP, O.; MIKI, K.; HUBER, R.; MICHEL, H. (1985). [204] «Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution». *Nature* 318, 618-624.

- HUBER, R.; DEISENHOFER, J.; COLMAN, P. M.; MATSUSHIMA, M.; PALM, W. (1976). [87] «Crystallographic structure studies of an IgG molecule and an Fc fragment». *Nature* 264, 415-420.

ORIGINAL ARTICLES IN 2000 (out of 823, October 2000)

- MINKS, C.; HUBER, R.; MORODER, L.; BUDISA, N. (2000). [823] «Noninvasive tracing of recombinant proteins with "Fluorophenylalanine-Fingers"». *Analytical Biochemistry* 284, 29-34.

- SONDERMANN, P.; HUBER, R.; OOSTHUIZEN, V.; JACOB, U. (2000). [822] «The 3.2-Å crystal structure of the human IgG1 Fc fragment-FcgRIII complex». *Nature* 406, 267-273.

- GARRIDO FRANCO, M.; HUBER, R.; SCHMIDT, F. S.; LABER, B.; CLAUSEN, T. (2000). [821] «Crystallization and preliminary X-ray crystallographic analysis of PdxJ, the pyridoxine 5'-phosphate synthesizing enzyme». *Acta Cryst.* D56, 1045-1048.

- KRUPKA, H. I.; HUBER, R.; HOLT, S. C.; CLAUSEN, T. (2000). [820] «Crystal structure of cystalysin from *Treponema denticola*: a pyridoxal 5'-phosphate-dependent protein acting as a haemolytic enzyme». *EMBO J.* 19, 3168-3178.

- SOULIMANE, T.; BUSE, G.; BOURENKOV, G. P.; BARTUNIK, H. D.; HUBER, R.; THAN, M. E. (2000). [819] «Structure and mechanism of the aberrant ba3-cytochrome c oxidase from *Thermus thermophilus*». *EMBO J.* 19, 1766-1776.

- CLAUSEN, T.; KAISER, J. T.; STEEGBORN, T.; HUBER, R.; KESSLER, D. (2000). [818] «Crystal structure of the cystine C-S lyase from *Synechocystis*: Stabilization of cysteine persulfide for FeS cluster biosynthesis». *Proc. Natl. Acad. Sci. USA* 97, 3856-3861.

- THEODORATU, E.; PASCHOS, A.; MAGALON, A.; FRITSCHE, E.; HUBER, R.; BÖCK, A. (2000). [817] «Nickel serves as a substrate recognition motif for the endopeptidase involved in hydrogenase maturation». *Eur. J. Biochem.* 267, 1995-1999.

- SPERL, S.; JACOB, U.; DE PRADA, N. A.; STÜRZEBECHER, J.; WILHELM, O. G.; BODE, W.; MAGDOLEN, V.; HUBER, R.; MORODER, L. (2000). [816] «(4-Aminomethyl)phenylguanidine derivatives as nonpeptidic highly selective inhibitors of human urokinase». *Proc. Natl. Acad. Sci. USA* 97, 5113-5118.

- HUBER, R. (2000). [815] «Warum sind die Blätter an den Bäumen grün». *SZ Magazin* 20/2000, 20-21.

- WORBS, M.; WAHL, M. C. (2000). [814] «Expression, purification, crystallization and preliminary X-ray diffraction studies of bacterial and archaeal L4 ribosomal proteins». *Acta Cryst.* D56, 645-647.

- RESTER, U.; MOSER, M.; HUBER, R.; BODE, W. (2000). [813] «L-Isoaspartate 115 of porcine b-trypsin promotes crystallization of its complex with bdellastasin». *Acta Cryst.* D56, 581-588.

- REVERTER, D.; FERNÁNDEZ-CATALÁN, C.; BAUMGARTNER, R.; PFÄNDER, R.; HUBER, R.; BODE, W.; VENDRELL, J.; HOLAK, T. A.; AVILÉS, F. X. (2000). [812] «Structure of a novel leech carboxypeptidase inhibitor determined free in solution and in complex with human carboxypeptidase A2». *Nature Struct. Biol.* 7, 322-328.

- FUENTES-PRIOR, P.; IWANAGA, Y.; HUBER, R.; PAGILA, R.; RUMENNIK, G.; SETO, M.; MORSER, J.; LIGHT, D. R.; BODE, W. (2000). [811] «Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex». *Nature* 404, 518-525.

- LOIDL, G.; MUSIOL, H. J.; BUDISA, N.; HUBER, R.; POIROT, S.; FOURMY, D.; MORODER, L. (2000). [810] «Synthesis of b-(1-azulenyl)-L-alanine as a potential blue-colored fluorescent tryptophan analog and its use in peptide synthesis». *J. Peptide Sci.* 6, 139-144.

- HOFMANN, A.; RAQUÉNÈS-NICOL, C.; FAVIER-PERRON, B.; MESONERO, J.; HUBER, R.; RUSSO-MARIE, F.; LEWIT-BENTLEY, A. (2000). [808] «The annexin A3-membrane interaction is modulated by an N-terminal tryptophan». *Biochemistry* 39, 7712-7721.

- DAMS, T.; AUERBACH, G.; BADER, G.; JACOB, U.; PLOOM, T.; HUBER, R.; JAENICKE, R. (2000). [807] «The crystal structure of dihydrofolate reductase from thermotoga maritima: molecular features of thermostability». *J. Mol. Biol.* 297, 659-672.

- BODE, W.; HUBER, R. (2000). [805] «Structural basis of the endoproteinase-protein inhibitor interaction». *Biochimica et Biophysica Acta* 1477, 241-252.

- WAHL, M. C.; HUBER, R.; MARINKOVIÇ, S.; WEYHER-STINGL, E.; EHLERT, S. (2000). [803] «Structural investigations of the highly flexible recombinant ribosomal protein L12 from *Thermotoga maritima*». *Biol. Chem.* 381, 221-229.

– Rebelo, J.; Macieira, S.; Dias, J. M.; Huber, R.; Ascenso, C. S.; Rusnak, F.; Moura, J. J. G.; Moura, I.; Romão, M. (2000). [802] «Gene sequence and crystal structure of the aldehyde oxidoreductase from *Desulfovibrio desulfuricans* ATCC 27774». *J. Mol. Biol.* 297, 135-146.

– Huber, R. (2000). [801] «Ufos im Körper. Kann die Chemie jede Krankheit heilen?» *Kultur und Technik* 2/2000, 16-19.

– Worbs, M.; Huber, R.; Wahl, M. C. (2000). [799] «Crystal structure of ribosomal protein L4 shows RNA-binding sites for ribosome incorporation and feedback control of the S10 operon». *EMBO J.* 19, 807-818.

– Kaiser, J. T.; Clausen, T.; Bourenkov, G. P.; Bartunik, H.; Steinbacher, S.; Huber, R. (2000). [798] «Crystal structure of a NifS-like protein from *Thermotoga maritima*: implications for iron sulphur cluster assembly». *J. Mol. Biol.* 297, 451-464.

– Schuler, B.; Fürst, F.; Osterroth, F.; Steinbacher, S.; Huber, R.; Seckler, R. (2000). [797] «Plasticity and steric strain in a parallel b-helix: rational mutations in the P22 tailspike protein». *Proteins: Structure, Function, and Genetics* 39, 89-101.

– Clausen, T.; Schlegel, A.; Peist, R.; Schneider, E.; Steegborn, C.; Chang, Y.; Haase, A.; Bourenkov, G. P.; Bartunik, H.; Boos, W. (2000). [796] «X-ray structure of MalY from *Escherichia coli*: a pyridoxal 5' - phosphate-dependent enzyme acting as a modulator in *mal* gene expression». *EMBO J.* 19, 831-842.

– Hofmann, A.; Proust, J.; Dorowski, A.; Schantz, R.; Huber, R. (2000). [795] «Annexin 24 from *Capsicum annuum*». *J. Biol. Chem.* 275, 8072-8082.

– Groll, M.; Kim, K. B.; Kairies, N.; Huber, R.; Crews, C. M. (2000). [793] «Crystal structure of epoxomicin: 20S proteasome reveals a molecular basis for selectivity of a', b'-epoxyketone proteasome inhibitors». *J. Am. Chem. Soc.* 122, 1237-1238.

– Gremer, L.; Kellner, S.; Dobbek, H.; Huber, R.; Meyer, O. (2000). [792] «Binding of flavin adenine dinucleotide to molybdenum-containing carbon monoxide dehydrogenase from *Oligotropha carboxidovorans*». *J. Biol. Chem.* 275, 1864-1872.

– Bochtler, M.; Hartmann, C.; Song, H. K.; Bourenkov, G.; Bartunik, H.; Huber, R. (2000). [791] «The structure of HslU and the ATP-dependent protease HslU-HslV». *Nature* 403, 800-805.

– Loidl, G.; Musiol, H. J.; Groll, M.; Huber, R.; Moroder, L. (2000). [790] «Synthesis of bivalent inhibitors of eucaryotic proteasomes». *J. Peptide Sci.* 6, 36-46.

– Strobl, S.; Fernández-Catalán, C.; Braun, M.; Huber, R.; Masumoto, H.; Nakagawa, K.; Irie, A.; Sorimachi, H.; Bourenkov, G.; Bartunik, H.; Suzuki, K.; Bode, W. (2000). [789] «The crystal structure of calcium-free human m-calpain suggests an electrostatic switc mechanism for activation by calcium». *Proc. Natl. Acad. Sci. USA* 97, 588-592.

– Wahl, M. C.; Bourenkov, G. P.; Bartunik, H.; Huber, R. (2000). [788] «Flexibility, conformational diversity and two dimerization modes in complexes of ribosomal protein L12». *EMBO J.* 19, 174-186.

– Skirgaila, R.; Grazulis, S.; Bozic, D.; Huber, R.; Siksnys, V. (2000). [787] «Structure-based redesign of the catalytic/metal binding site of Cfr10I restriction endonuclease reveals importance of spatial rather than sequence conservation of active centre residues». *J. Mol. Biol.* 279, 473-481.