Giorgio Carta and Alois Jungbauer

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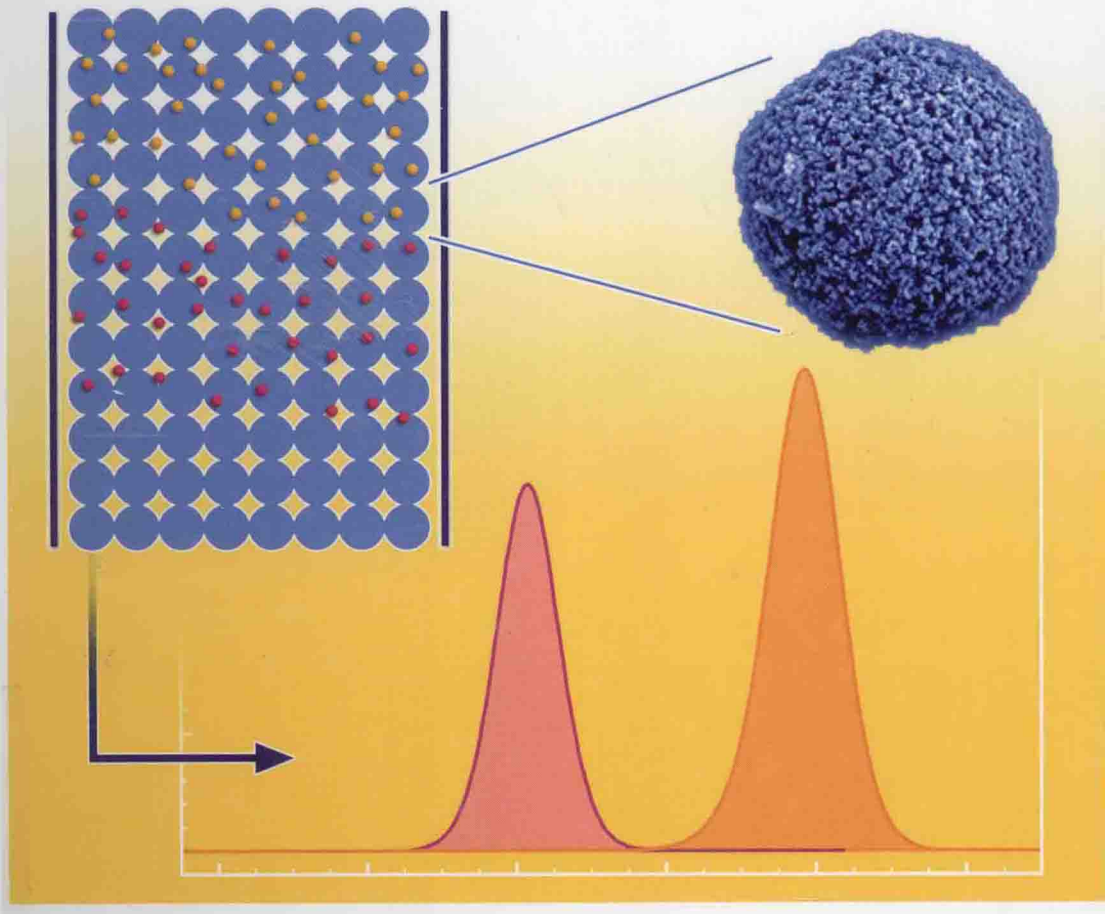
Protein Chromatography

Process Development and Scale-Up



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Preface

Chromatography has become an essential unit operation in the production of biopharmaceuticals. This method facilitates the processing of the complex mixtures encountered in this industry using readily available stationary phases and equipment suitable for large-scale sanitary operation. Moreover, its practice as a process purification tool is recognized by regulatory agencies so that chromatography is an integral part of essentially all licensed biopharmaceutical processes. An in-depth understanding of the process is desirable and is increasingly being sought by regulatory agencies. As a result, chemists, engineers, and life scientists working in this field need to become familiar with the theory and practice of process chromatography.

While, in general, the theory of chromatography is well established for small molecule separations, the design and scale-up of chromatography units for biopharmaceutical purification remain largely empirical. Thus, optimum designs often remain elusive. On one hand, engineers, while possessing a strong foundation in transport phenomena and unit operations, often have a limited understanding of biomolecular properties. On the other, biochemists and biologists often have a limited understanding of the key scale-up relationships and models needed for optimum design. In an effort to address this dichotomy, in 2000 we started a new short course at BOKU in Vienna, Austria, with the principal aim of merging the theory and practice of biochromatography to achieve optimum design and scale-up of process units. Our goal was to help educate engineers who understand the biophysical properties of proteins and other bio-macromolecules and can implement this understanding in the bioprocess setting; and life scientists who understand transport phenomena and engineering models and who can apply these tools to the design of process units. Since 2000, the course, which has been open to both industrial and academic participants, has been held annually both in Vienna and at the University of Virginia, in Charlottesville, Virginia, USA. The course has both theoretical and practical, hands-on components. The participants learn the fundamentals of protein production, their structural and biophysical properties, and the varied nature of their contaminants. In the lectures, they learn the theory of chromatography, the properties of stationary phases, how to describe the equilibrium and kinetic factors that govern process performance, and how to achieve a proper balance of separation efficiency and productivity. In the labora-

Protein Chromatography: Process Development and Scale-Up. Giorgio Carta and Alois Jungbauer

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tory, they learn to pack columns which are useful as scale-down models, plan experiments to identify critical parameters, and use advanced chromatography workstations to measure the critical physiochemical properties needed to model retention and band broadening in different types of chromatographic operations. Ultimately, the participants complete a design exercise, in which they are asked to design an optimized column on the basis of the laboratory measurements and theories learned during the course.

This book is based on the same framework. After teaching the course for more than ten times and after discussions with several hundred participants with very broad ranges of educational backgrounds and job functions, we now have a better understanding of the main difficulties that are encountered in understanding protein chromatography from both theoretical and practical viewpoints. Therefore, following the spirit of the course, we begin with a chapter on the biochemical and biophysical properties of proteins and their contaminants. We focus on the properties that are relevant for chromatography such as size, surface charge and hydrophobicity, solution viscosity, and diffusivity and on how to preserve biological activity. In Chapter 2, we provide a succinct, general introduction to chromatography identifying the key factors that are important for design and scale-up. This allows the reader who is not familiar with chromatography to put the various issues discussed in Chapters 3 to 10 into proper context. Chapter 3 addresses the chemistry and structure of many different stationary phases while Chapter 4 discusses laboratory and process columns and equipment. Both of these chapters are limited in scope to familiarizing the reader with examples of commercially available materials and equipment. No attempt has been made to provide comprehensive coverage, in large part because the field is rapidly expanding and new media and equipment are constantly being introduced. The mechanical design of equipment has also been omitted, since separation scientists and engineers in the biopharmaceutical production setting are rarely required to undertake this task. Chapters 5 to 9 are structured to acquaint the reader with theory and models to design and scale-up chromatography units. Emphasis has been placed on phenomenological models whose parameters can be determined using suitable experimental studies. Many specific numerical examples are provided to illustrate the application of these models to the analysis of laboratory data and to the prediction of column performance. A great deal of emphasis has been placed on describing transport in the stationary phase, since adsorption kinetics is often limiting in industrial applications of biochromatography. Thus, Chapter 6 provides a detailed coverage of mass transfer effects and their relationship to the structure of the stationary phase. Chapter 7 explores the dynamic behaviour of chromatography columns to establish a link between equilibrium properties, which are described in Chapter 5, and column behaviour. Chapter 8 discusses how equilibrium and rate factors combine to determine column performance and how to model band broadening for practical conditions. Chapter 9 focuses on gradient elution chromatography. We chose to devote a separate chapter to this mode of operation, since, in our experience, it is frequently less well understood despite its major importance in the practice of biochromatography. Finally, Chapter 10 is designed

in hopes of bringing everything together and providing guidance for the optimum design of process units. Although most of the emphasis is on conventional, batch chromatography processes, we conclude with an overview of continuous or semi-continuous multicolumn systems that are attracting increasing interest for biopharmaceutical applications. It should be noted that the main intent of this book is not to address *de novo* process development – rather, the main focus is on the optimal design and scale-up of columns for a process whose steps have already been defined. Nevertheless, understanding these concepts will also aid the scientist who is involved in early process development to identify process steps that are scalable and can be efficiently translated from the laboratory to the manufacturing suite.

We are convinced that proper application of theory combined with adequate experiments is instrumental to the successful application of biochromatography on a large scale. We would be happy, of course, if the book encouraged some of the readers to attend our course and learn about the practical, laboratory aspects that accompany the theory. The book also provides extensive references to original literature, textbooks, and books on chromatography, for those seeking greater detail. We have endeavored to make the notation consistent throughout the book and to check the correctness of the mathematical equations. Notwithstanding these efforts, we strongly suspect that there may still be some inconsistencies. We would be very grateful to readers who inform us of any such issues so that they can be remedied.

Finally, we would like to thank our students who, over the years, have helped us to develop and teach the laboratory and discussion sessions used in our short courses, which could not have been held without their input and enthusiastic support. We would particularly like to thank our students Timothy Pabst, Emily Schirmer, Jamie Harrington, Melani Stone, Jeremy Siebenmann-Lucas, Theresa Bankston, Yinying Tao, Robert Deitcher, and Ernie Perez-Almodovar at the University of Virginia and Tina Paril, Kerstin Buhlert, Rene Überbacher, Anne Tscheliesnig, Alfred Zoechling, and Christine Machold at BOKU and our colleague Rainer Hahn for their support. We also thank all the participants who have attended our courses and who have provided very valuable feedback and have shared with us much of their practical experience.

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Nomenclature

a	coefficient in dimensionless van Deemter equation (2.4, 2.5, 8.50) or isotherm parameter
A	coefficient in van Deemter equation (8.49), m
$A_{external}$	surface area outside particles per unit column volume (3.11), m^2/m^3
A_i	combined equilibrium parameter for retention in IEC (9.24), RPC (9.36) and HIC (9.42), variable units
$A_{internal}$	surface area inside particles per unit column volume (3.10), m^2/m^3
A_s	asymmetry factor (8.12)
b	coefficient in dimensionless van Deemter equation (2.4, 2.5, 8.50) or isotherm parameter
B	coefficient in van Deemter equation (8.49), m^2/s
B_0	hydraulic permeability (= $\eta Lu/\Delta P$), m^2
c	protein concentration in pore liquid, kg/m^3, or coefficient in dimensionless van Deemter equation (2.4, 2.5, 8.50).
$\overline{c}$	average concentration in pore liquid, kg/m^3
C	protein concentration in mobile phase, kg/m^3, or coefficient in van Deemter equation (8.49), s
C_f	peak compression factor in linear gradient elution (9.15, 9.28, 9.40)
C_F	concentration in feed, kg/m^3
C_M	mobile phase modifier concentration in IEC and HIC, M
C_0	initial concentration, kg/m^3
C_s	protein concentration in mobile phase at particle surface, kg/m^3
C^*	mobile phase protein concentration in equilibrium with stationary phase, kg/m^3
CV	number of column volumes of mobile phase passed through column
d_c	column diameter, m
d_p	particle diameter, m
d_{pore}	pore diameter, m
D_0	molecular diffusivity in mobile phase, m^2/s
D_L	axial dispersion coefficient (see Equations 7.1 and 8.46), m^2/s
D_e	effective pore diffusivity (6.9), m^2/s
$D_{e,b}$	effective diffusivity in mobile phase (8.46), m^2/s
D_s	effective adsorbed-phase or surface diffusivity (see 6.14), m^2/s

Protein Chromatography: Process Development and Scale-Up. Giorgio Carta and Alois Jungbauer

ISBN: 978-3-527-31819-3

$\tilde{D}_e$	convection-enhanced effective intraparticle diffusivity (6.20), m^2/s
DBC	dynamic binding capacity or amount of protein held in column at a specified percentage of breakthrough (see 2.15, 8.59, 10.5), kg/m^3
E_D	eddy diffusivity (8.47), m^2/s
EBC	equilibrium binding capacity or amount of protein held in column at equilibrium with feed, kg/m^3
F	fractional approach to equilibrium (= $\langle\hat{q}\rangle/\hat{q}^*$)
F_p	ratio of intraparticle and column superficial velocities (6.15 and 6.18)
h	reduced HETP (= H/d_p, see Equations 2.6 and 8.50)
H	height equivalent to a theoretical plate (HETP, see Equations 2.3 and 8.4), m
$H(t)$	unit step function
I	ionic strength (1.16), mol/m^3
J	mass transfer flux, $kg/m^2 \cdot s$
$J(n, n\tau_1)$	J-function (item B in Table 8.1 or Equations 8.38 and 8.39)
k	rate coefficient for LDF model with adsorbed phase concentration driving force (8.30), s^{-1}
k_a	second order adsorption rate constant (Equations 6.22 and 8.62), $m^3/kg \cdot s$
k_b	Boltzmann constant (= 1.38×10^{-23} joule/K)
k_c	rate coefficient for LDF model with mobile phase concentration driving force (8.30a), s^{-1}
k'	retention factor (= $\phi\hat{q}_F/C_F$ or = ϕm for the linear isotherm case Equation 2.10)
$\bar{k}'$	average retention factor (2.13)
k_f	film or boundary layer mass transfer coefficient (6.1 and 6.3–6.5), m/s
K	adsorption equilibrium constant (e.g. Equation 5.7), m^3/kg
K_D	distribution coefficient (1.11 and 3.15)
K_e	equilibrium constant for ion exchange (5.15)
ℓ	length of packed column in SMB separator (10.48), m
L	length of packed chromatographic column or zone length in SMB separator, m
L_0	uncompressed column length (see Equation 10.18), m
L_{cri}	critical, compressed column length (see Equations 10.18 and 10.19), m
m	linear isotherm slope or Henry constant (e.g. Equation 5.5) (= $\hat{q}^*/C$)
M	mobile phase modifier or amount injected, kg
M^j	flow rate ratio in zone j of SMB separator (10.45)
M_r	molecular mass
n	number of transfer units (Table 8.2)
N	number of plates (2.3 and 8.5)
p	switch time for SMB separator (10.48), s
P	productivity (10.1), $kg/m^3 \cdot s$
ΔP	column pressure drop, Pa
q	adsorbed protein concentration, kg/m^3

q_F	adsorbed protein concentration in equilibrium with feed, kg/m^3
q_m	maximum protein adsorption capacity (e.g. Equation 5.4), kg/m^3
q_{max}	maximum protein adsorption capacity (in SD or SMA models (5.21 and 5.23)), kg/m^3
q_0	concentration of charged ligands in the stationary phase (e.g. see Equation 5.17), mol/m^3
$\overline{q}$	adsorbed protein concentration averaged over particle volume (see Equation 6.29), kg/m^3
$\hat{q}$	total protein concentration in stationary phase including amounts adsorbed and held in the pores (6.24), kg/m^3
$\langle\hat{q}\rangle$	total concentration in stationary phase averaged over particle volume (6.29), kg/m^3
$\hat{q}^*$, q^*	adsorbed concentrations in equilibrium with mobile phase, kg/m^3
Q	volumetric flow rate, m^3/s
r	particle radial coordinate, m
r_h	hydrodynamic radius (e.g. see Equation 1.8), m
r_m	molecule radius, m
r_p	particle radius, m
r_{pore}	pore radius, m
$\overline{r}_p$	volume-average particle radius (3.16), m
R	separation factor isotherm parameter (5.7); $R = 1$ for a linear isotherm, $R = 0$ for a rectangular isotherm
R_s	chromatographic resolution (2.9 and 10.22 or 10.30)
S	sensitivity coefficient for retention in RPC and HIC (3.6 and 3.8 or 9.37 and 9.42), or column cross sectional area, m^2
t_b	breakthrough time (see Figure 8.13), s
t_C	total cycle time (see Figure 10.3), s
t_F	duration of feed injection, s
t_G	parameter in EMG function (8.14), s, or duration of gradient, s
t_{max}	time elapsed from injection at peak maximum, s
t_R	retention time (see Equation 7.22), s
t_s	time required for separation (2.14), s
t_{sh}	time at which shock emerges from column, s
T	temperature, K
u	superficial mobile phase velocity (4.1), m/s
u_s	adsorbent superficial velocity in SMB separator (see Figure 10.8), m/s
u^j	superficial mobile phase velocity in zone j of TMB-equivalent to SMB separator (see Figure 10.8), m/s
u^j_{SMB}	superficial mobile phase velocity in zone j of actual SMB separator (10.49), m/s
v	interstitial velocity of mobile phase (= u/ε, Equation 4.2), m/s
v'	reduced velocity (= vd_p/D_0, Equations 2.7 and 8.51)
v_c	chromatographic velocity for simple waves (7.28), m/s
v_{sh}	shock velocity (7.30), m/s
V	liquid phase volume, m^3

V_b mobile phase volume passed through column at breakthrough, m^3
V_c column volume, m^3
V_F feed volume loaded to column, m^3
V_0 column extraparticle void volume ($= \varepsilon V_c$), m^3
V_p volume of adsorbent particles, m^3
V_R retention volume, m^3
w solubility in solution, kg/m^3
w_0 solubility in pure water, kg/m^3
W baseline width of pulse response peak (Figure 8.1), s or m^3
X dimensionless protein concentration in mobile phase (7.12)
Y dimensionless protein concentration in stationary phase (7.12)
z protein effective charge (5.17) or column axial coordinate, m

Greek Symbols

α selectivity ($= k'_B/k'_A$)
β gradient slope (9.6) mM/s or mM/m^3, or safety margin for SMB
$\overline{\varepsilon}$ separator (10.46)
δ stagnant film or boundary layer thickness (6.2), m
δ_i^j SMB separator parameter (10.63)
$\delta(t)$ delta function
Δ peak width at half-peak height (Figure 8.1), s or m^3
ε extraparticle void fraction (4.3)
ε_p intraparticle void fraction (see Figure 2.7)
ε_0 extraparticle void fraction of uncompressed bed (see Example 10.2)
ε_t total column void fraction (2.1)
$\overline{\varepsilon}$ power input per unit mass in an agitated tank (see Equations 6.6 and 6.7), m^2/s^3
ϕ ratio of stationary and mobile phase volumes in column ($= (1 - \varepsilon)/\varepsilon$)
γ normalized gradient slope ($= \beta L/v = \beta V_0/Q$, see Equation 9.11), mM or
$\dot{\gamma}$ shear rate, s^{-1}
η mobile phase viscosity, Pa·s
η_E elution recovery yield, (see Equation 10.2)
$[\eta]$ intrinsic viscosity (1.24), ml/g
φ volume fraction of organic modifier in RPC
λ_D Debye length (3.9), m
λ_{cri} critical bed compression factor ($= (L_0 - L_{cri})/L_0$, see Example 10.2)
λ_m ratio of protein and pore radii ($= r_m/r_{pore}$)
μ_0 zeroth moment of pulse response peak (8.1), kg·s/m^3 or kg
μ_1 first moment of pulse response peak (8.2), s or m^3
ρ density of mobile phase, kg/m^3

σ	steric hindrance parameter in SMA model (5.22) or standard deviation of pulse response peak (8.3), s or m^3
σ_G	parameter in EMG function (8.14)
τ	dimensionless time (= $\varepsilon vt/L$, see Equation 7.13) or shear stress (1.22)
τ_a	time constant for affinity binding, s
τ_G	parameter in EMG function (8.14), s
τ_p	tortuosity factor for intraparticle diffusion (see 6.9)
τ_1	dimensionless time (= $(vt/L - 1)C_F/\phi q_F$ at column exit, see Equation 7.17)
ψ_p	hindrance parameter for pore diffusion (6.10 and 6.11)
ζ	dimensionless column length (7.13)

Dimensionless Transport Parameters

Bi	Biot number (= $r_p k_f/D_e$)
Pe_L	Peclet number based on column length (= vL/D_L)
Pe_p	intraparticle Peclet number (see Equation 6.21)
Re	Reynolds number (= $\rho u d_p/\eta$)
Sc	Schmidt number (= $\eta/\rho D_0$)
Sh	Sherwood number (= $k_f d_p/D_0$)
St	Stanton number (= $(1 - \varepsilon)kL/u_s$)
n_{film}	number of transfer units for film mass transfer (= $3\phi k_f L/vr_p$, see Table 8.2)
n_{pore}	number of transfer units for pore diffusion (= $15\phi D_e L/vr_p^2$, see Table 8.2)
n_{solid}	number of transfer units for solid diffusion (= $15\phi D_s q_F L/vr_p^2 C_F$, see Table 8.2)

Contents

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1
Downstream Processing of Biotechnology Products

1.1
Introduction

Biological products are important for many applications including biotransformations, diagnostics, research and development, and in the food, pharmaceutical, and cosmetics industries. For certain applications, biological products can be used as crude extracts with little or no purification. However, biopharmaceuticals typically require exceptional purity, making downstream processing a critical component of the overall process. From the regulatory viewpoint, the production process itself defines the biopharmaceutical product rendering proper definition of effective and efficient downstream processing steps crucial early in process development. Currently, proteins are the most important biopharmaceuticals. The history of their development as industrial products goes back more than half a century. Blood plasma fractionation was the first full-scale biopharmaceutical industry with a current annual production in the 100-ton scale [1, 2]. Precipitation with organic solvents has been and continues to be the principal purification tool in plasma fractionation, although, recently, chromatographic separation processes have also been integrated into this industry. Anti-venom antibodies and other anti-toxins extracted from animal sources are additional examples of early biopharmaceuticals, also purified by a combination of precipitation, filtration and chromatography. In contrast, current biopharmaceuticals are almost exclusively produced by recombinant DNA technology. Chromatography and membrane filtration serve as the main tools for purification for these products.

Figure 1.1 shows the 2006 market share of various biopharmaceuticals. Approximately one-third are antibodies or antibody fragments [3], nearly 20% are erythropoietins, and 14% are insulins. The rest are enzymes, growth factors and cytokines [3]. Although many non-proteinaceous biomolecules such as plasmids, viruses or complex polysaccharides are currently being developed, it is likely that proteins will continue to dominate as biopharmaceuticals. Proteins are well tolerated, can be highly potent, and often posses a long half-life after administration, making them effective therapeutics. Some of these properties also make proteins potentially useful in cosmetics, although applications in this field are complicated in part by the US and European legal frameworks that do not allow the use of phar-

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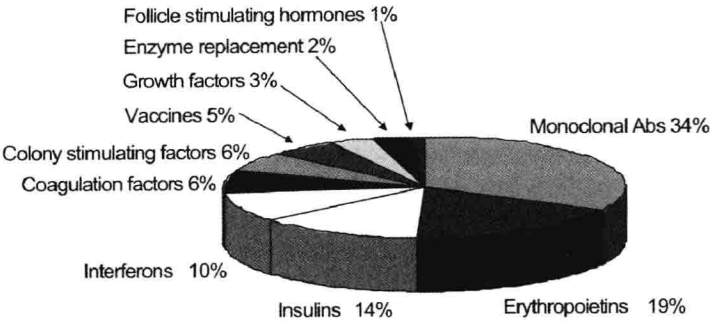

Figure 1.1 Biopharmaceuticals market share in 2006. Approximately 160 protein therapeutics have gained approval in the USA and EU. Data from La Merie Business Intelligence (www.lamerie.com).

macologically active compounds in cosmetics. Currently only a few proteins are used in this area. The most prominent one is the botulinum toxin, Botox®, used for skin care [4]. This and similar compounds are exclusively administered by physicians and thus are not considered to be cosmetics.

1.2 Bioproducts and their Contaminants

This chapter gives an overview of the chemical and biophysical properties of proteins and their main contaminants such as DNA and endotoxins. The description is not comprehensive; only properties relevant to chromatographic purification will be considered. A detailed description of the chemistry of proteins and DNA is outside the scope of this book and can be found in a number of excellent biochemistry or molecular biology texts [5, 6].

1.2.1 Biomolecules: Chemistry and Structure

1.2.1.1 Proteins

Proteins constitute a large class of amphoteric biopolymers with molecular masses ranging from 5 to 20 000 kDa, which are based on amino acids as building blocks. There are enormous variations in structure and properties within this class. Insulin, for example, a peptide with molecular mass of 5808 Da, has a relatively simple and well-defined structure. On the other hand, human van Willebrand factor, a large multimeric glycoprotein with a molecular mass of 20 000 kDa, has an extremely complex structure consisting of up to 80 subunits, each of which is 250 kDa in mass. Most proteins have a molecular mass well within these two extremes, typically between 15 and 200 kDa. Proteins are generally rather compact