Chapter 15. Ouellette, 2/e. Chemistry X402.

Ch 15. Amino acids, peptides, and proteins.

<u>**Skip**</u>: Sect 6.

<u>**Essays**</u>: Not required. Both involve nutrition. One was mentioned in the Ch 13 handout, re cholesterol.

⇒ A **practice quiz** for Ch 15 (Amino acids and proteins) is at the web site. Much of it focuses on relating amino acid structures to those of other chemicals.

<u>Notes</u>

General. Much of this chapter builds on Ch 12 & 14. Amino acids contain amino groups (Ch 14) and carboxylic acid groups (Ch 12). In proteins, the amino acids are linked together by amide bonds (Ch 14). The big new -- and very biochemical -- issue in this Ch is protein structure.

Sect 2. p 421, Table 15.1. Ouellette's classification of the amino acids is quite reasonable. However, you should be aware that some parts of the scheme are somewhat arbitrary. In fact, about 1/4 of the amino acids are classified differently by different authors. For example, one could argue that tyrosine (a phenol) has an acidic side chain. Yes, but at physiological pH it is rarely ionized, so most authors do not classify it as an acidic amino acid.

Try to see <u>why</u> each amino acid is classified the way it is, using your own understanding of the side chain. Ask about those you would consider classifying otherwise.

⇒ Table 15.1 will be provided on the test (if needed). This table should make sense to you, but you are not responsible for knowing the specific amino acids. (You should know the general nature -- structure and properties -- of amino acids.)

Sect 3. Skip the subsection on pK_a values.

Sect 4. "Advanced." The key idea is that at a certain pH a molecule with both + and - charged groups will be neutral. This pH is known as the pI (I = isoionic or isoelectric point). This has an effect on protein behavior (proteins are usually least soluble at their pI), and is useful in certain kinds of protein analysis. But we don't need it here. Ok to skip this section completely, if you want.

Sect 7. "Advanced"; ok to skip, <u>except</u>… There are two sub-sections on p 433 that introduce enzymatic methods for analysis of a protein. These two sub-sections are important in that they introduce the idea of enzyme specificity: different proteases (enzymes that degrade protein) do so in different ways, because they recognize different features of the protein. These two sub-sections are required reading for the Metabolism section, but they are not important now.

Sect 8. Very important.

As Ouellette notes, the division of levels of protein structure is somewhat arbitrary. For example, Ouellette classifies disulfide bonds as part of primary structure. Classifying them as part of tertiary structure is common (side chain interactions). It isn't important exactly how they are classified; what is important is understanding what disulfide bonds are, and how they play a role in protein structure. The general idea of levels of protein structure is clear enough, but exactly which features should be classified as what level is subject to opinion. It is much more important to understand the general picture of how proteins fold than to get hung up on terms, especially when those terms are not used consistently.

Ouellette says that the chains in a β-sheet structure are anti-parallel (p 439). That is perhaps most common, but is not always so. Parallel chains can form β-sheets.

The use of the word "chain" (above, and in the book) carries an ambiguity. The two (or more) "chains" in a β-sheet may or may not be part of the same "molecule" (primary sequence). That is, strictly speaking, β-sheets may be intra-molecular or inter-molecular. If the latter, they would properly be thought of as being part of the quaternary structure. I will try to illustrate this in class.

Errata and notes

p 419. Last line says there are peptide hormones as short as 9 amino acids. In fact, peptide hormones as short as 4 amino acids are known -- and are shown in Table 15.4, p 429. ☺

p 444, #9-10.

- In "estimating" the isoelectric point, it is sufficient here to answer "near neutral", or acidic, etc.
- Since the question asked for the isoelectric point, it would be better if the answer said pH_i (often called pI), rather than just pH.
- For 10c, emphasize that histidine is a very weak base. Note the near-neutral pH_i. Its basicity is physiologically relevant.

p 444, #13-14. Given answers are weak. It would be better to say that there is an excess of basic amino acids (in #13), i.e., more basics than acidics.

Suggested problems

Models. Good exercise, all related. Even doing just the first one is useful.
Exercises. 1-8 are good. 9-14 are "advanced", but if you could do some of them it would be good; emphasize the odds, so you can check yourself. Even doing #9 qualitatively, just ranking them, is good. 15-22 are good; #20 raises an interesting point. (Note that Ouellette has introduced glutathione before; check the index.) 23-34 are "advanced"; maybe try #25 & 27. 35-40 are advanced; maybe try #35. 41-46 are important.

Overview of skipped section

Sect 6. How to make peptides in the lab.

Further reading

Book

P Forbes, The Gecko's Foot - Bio-inspiration: Engineering new materials from nature. 2005. The lotus leaf is easily rinsed clean; the gecko can climb a glass wall. Why? And, can we make use of the principles that Nature has used to achieve these remarkable accomplishments? Those are just two of the topics in this delightful book -- one of which is reflected in its title. The theme is bio-inspiration (sometimes called biomimetics), in which we look to Nature for an idea about how to do something. The hook-and-loop fastener, popularly known by the tradename Velcro, is an example of old, but the field has now taken on an identity that reflects a more focused effort to discover and exploit what Nature has already learned. Spider silk is among the other topics of the book; more about this below. For more about the book, see my web page of book suggestions.

Articles. (See Old Articles page at web site for more.)

H E Huxley, Obituary: Max Perutz (1914-2002). Nature 415:851, 2/21/02. I don't normally include obituaries in the reading lists, but this must be an exception. Max Perutz, "One of the principle founders of molecular biology", died in 2002. The simple story is that he determined the structure of the protein hemoglobin (pp 437-8), for which he received the Nobel prize. As a pioneer and advocate, he was an influential figure for decades; Perutz's work was part of that magic era of the 1950's and 60's when it seems that the core of our modern understanding of the molecular basis of life was established. His work was part of the magnet that attracted two generations of young students, including me, to this developing field. Nature gave Hugh Huxley two full pages to retell the hemoglobin story as well as the story of Perutz's influence; worth reading. (And for more… another two page obituary, by Aaron Klug, Science 295:2382, 3/29/02.)

J Marx, Neuroscience: Minor variation in growth-factor gene impairs human memory. Science 299:639, 1/31/03. News. A small part of a very big story. People with a single amino acid change in a particular brain protein don't do as well on a long term memory test as those with the normal version of the protein. Other work had implicated this protein in the process of "long term potentiation" -- converting a memory to "long term". The mutant protein seems to be altered in its ability to get to the right place. The effect of this variant protein, which is common in the population, is quite small. But it opens the issue of genetic factors that affect brain function. It also raises the question of whether people with this variant might be more susceptible to neurodegenerative diseases, such as Alzheimer's.

F Schotte et al, Watching a protein as it functions with 150-ps time-resolved X-ray crystallography. Science 300:1944, 6/20/03. High intensity X-ray beams, and other technical developments, actually allow some observation of "enzyme action". In this case, they watch the light-stimulated dissociation of CO from myoglobin. This work was done at 10°C; early work on X-ray observation of protein action was done at very low T, to slow it down. Also see Huxley (2002), above, for more on X-ray crystallography.

H Jakubowski, Molecular basis of homocysteine toxicity in humans. Cellular and Molecular Life Sciences 61:470-487, 2/04. Review. The "non-standard" amino acid homocysteine is actually a normal metabolite. High levels correlate with heart disease, but the causal link is uncertain. This article reviews the biology of homocysteine. [The vitamin folic acid may serve to reduce homocysteine levels; that is not discussed here.] So what is homocysteine, and why is it a normal metabolite? See the practice quiz for this Ch.

A Ault, The monosodium glutamate story: The commercial production of MSG and other amino acids. J Chem Educ 81:347, 3/04. As the extended title suggests, this article is useful as a broad overview of amino acid production, both by chemical and biological processes.

A Korkegian et al, Computational thermostabilization of an enzyme. Science 308:857, 5/6/05. Although calculating the tertiary structure of a protein based on its primary sequence is still an elusive goal, one can calculate the effect of small changes of primary structure on tertiary structure. Here they use a computer model to predict that certain amino acid changes will result in increased thermostability of a protein; they also used considerable judgment about where in the protein to focus their efforts. They tested the predicted changes: a high percentage of them did indeed result in increased thermostability.

A Damasio, Human behaviour: Brain trust. Nature 435:571, 6/2/05. News. Oxytocin is a small peptide hormone. Ouellette mentions it on p 429, noting its role in inducing uterine contractions. But oxytocin has also been implicated in a wide range of social behaviors. In the work discussed here, evidence is presented suggesting a role for this hormone in developing trust in a stranger. An interesting -- and quite readable -- story about human behavior, and one hormone that affects it.

R M Fairhurst et al, Abnormal display of PfEMP-1 on erythrocytes carrying haemoglobin C may protect against malaria. Nature 435:1117, 6/23/05. The malaria parasite grows in red blood cells (during one phase of its complex life cycle). Interestingly, people with certain forms of hemoglobin (Hb) have reduced susceptibility to malaria. Unfortunately, the best known Hb variants that provide protection from malaria are themselves harmful, especially when homozygous; these include sickle cell hemoglobin (Ouellette p 438), and some thalassemias. An interesting case is HbC, found in some West African populations. HbC is quite protective, at least when homozygous, and is apparently without harm to the person. Here they show that HbC erythrocytes have altered surface properties, which might account for the resistance. Much remains to be figured out.

P M Conn & J A Janovick, A new understanding of protein mutation unfolds. Amer Sci 93:314, 7/05. Some mutations that cause disease prevent the protein from folding properly. If, somehow, one can get it to fold, it will function ok. The idea, then, is to find "drugs" that will help guide the protein to correct folding.

R Daw, Materials science: At a stretch. Nature 437:961, 10/13/05. News. Report of work on designing a rubbery polymer based on part of a natural elastic protein.

O Schueler-Furman et al, Progress in modeling of protein structures and interactions. Science 310:638, 10/28/05. Review.

A Buckling & M Brockhurst, Microbiology: RAMP resistance. Nature 438:170, 11/10/05. News. RAMP = ribosomally encoded antimicrobial peptides. A wide range of organisms -- animals, plants, microbes -- make small proteins (peptides) that are broadly toxic to bacteria, by interacting with their membranes. These peptides are part of our natural defenses. (This has nothing to do with the adaptive immune system that we usually think about.) Now, there is evidence that bacteria can develop resistance to this possible new class of antibiotics. Of special concern would be if resistance to one RAMP also provided resistance against our natural peptide antibiotics.

J Winkler, Misfolded proteins and Parkinson's disease. Engineering & Science Vol LXVIII #3, 2005, p 14. Free online: http://pr.caltech.edu/periodicals/EandS/articles/LXVIII_3/Winkler.pdf. This article is based on a public lecture, and much of it should be at a level appropriate for X402 students. It generally discusses proteins and how they fold, and focuses on the specific case of a protein whose misfolding is implicated in Parkinson's disease.

F Garczarek & K Gerwert, Functional waters in intraprotein proton transfer monitored by FTIR difference spectroscopy. Nature 439:109, 1/5/06. The solvent is an important part of protein structure -- and function.

C Tokarski et al, Identification of proteins in renaissance paintings by proteomics. Analytical Chem 78:1494-1502, 3/1/06. A particular question of interest is whether egg was used as a binder material.

V J Vinson, Proteins at work. Science 312:211, 4/14/06. Introduction to a feature section, Tools for Biochemistry, with three reviews on protein methodology. The articles are: Mass Spectrometry and Protein Analysis; The Fluorescent Toolbox for Assessing Protein Location and Function; New Tools Provide New Insights in NMR Studies of Protein Dynamics. Also see Schotte et al (2003) and Computer Resources, below.

J R Barone & W F Schmidt, Products of chemistry: Nonfood applications of proteinaceous renewable materials. J Chem Educ 83:1003, 7/06. Glue. Clothing. Cosmetics. Read the article for details, and more.

H Lee et al, Single-molecule mechanics of mussel adhesion. PNAS 103:12999, 8/29/06. From their abstract: "The glue proteins secreted by marine mussels bind strongly to virtually all inorganic and organic surfaces in aqueous environments in which most adhesives function poorly. Studies of these functionally unique proteins have revealed the presence of the unusual amino acid 3,4-dihydroxy-L-phenylalanine (dopa), which is formed by posttranslational modification of tyrosine. However, the detailed binding mechanisms of dopa remain unknown, and the chemical basis for mussels' ability to adhere to both inorganic and organic surfaces has never been fully explained."

R J Abergel et al, Anthrax pathogen evades the mammalian immune system through stealth siderophore production. PNAS 103:18499, 12/5/06. Iron(III) ions are essential to most aerobic organisms -- and quite insoluble. So organisms have tricks to acquire -- and hoard -- iron. In humans this includes the iron-carrying protein, transferrin. Microbes make various small compounds, called siderophores, to extract iron from the environment. Researchers, including a group from UC Berkeley, have explored the tricks that anthrax bacteria use to get the iron they need for growth. They found that these bacteria make two siderophores to steal iron from their host. One of these is attacked by the human immune system; however, the other -- the more novel one -- evades it, and actually succeeds in supplying iron to the bacteria. They suggest that this novel siderophore might be a good target for anti-anthrax drugs, or simply a marker for detection of this pathogen. This paper is listed for my web page on Biotechnology in the News (BITN), Miscellaneous, under Anthrax; a story from the UC Berkeley student newspaper is also noted there.

A G Smith et al, Greenbottle (Lucilia sericata) larval secretions delivered from a prototype hydrogel wound dressing accelerate the closure of model wounds. Biotechnol Prog 22:1690-1696, 12/06. Maggots may be used to help keep wounds clean. They work by protease action. Here, they explore a way to deliver the proteases as an extract, without use of live maggots.

W Liu et al, Genetic incorporation of unnatural amino acids into proteins in mammalian cells. Nature Methods 4(3):239, 3/07. They show how to manipulate the genetic code and the translation apparatus to put novel amino acids into proteins. This had previously been done with microbes; now they do it with mammalian cells. Compare this with Ragsdale (2011)… Both involve understanding and manipulating the genetic code. The 2011 paper describes a natural process, whereas this paper describes an artificial process, engineered by scientists.

G Grotenbreg & H Ploegh, Chemical biology: Dressed-up proteins. Nature 446:993, 4/26/07. News. “Proteins aren’t just defined by their constituent amino acids - structural modifications can yield complex mixtures of protein forms. An approach that controls the addition of such modifications may help to define their role.” (There is more about glycoproteins in the Ch 11 handout. Included is a Nature Insight feature section on Glycochemistry & glycobiology, from this same issue.)

J Kopecek, Hydrogel biomaterials: A smart future? Biomaterials 28:5185-92, 12/07. Review. Hydrogels are polymer networks that swell in water. One application is soft contact lenses. Polypeptides are used in some hydrogels. An advantage of using polypeptides is the ability to make chains of well defined composition and length using the biological process of protein synthesis. This article is an overview of the nature of hydrogels.

A Cossins & M Berenbrink, Physiology: Myoglobin’s new clothes. Nature 454:416, 7/24/08. News. Myoglobin is the major “red” protein of muscle. Like the hemoglobin in blood, it carries oxygen; that was long thought to be its “purpose”. However, mice lacking myoglobin exercise fine; this result is not consistent with the proposed major role of myoglobin. New work shows that myoglobin is the enzyme that reduces nitrite ion (NO_2^-) to nitric oxide (NO) -- but only when it is starved of oxygen. The NO is known to limit tissue damage under oxygen deprivation. Thus this work suggests that one key role of myoglobin is making NO.

T-W Mu et al, Chemical and biological approaches synergize to ameliorate protein-folding diseases. Cell 134, 769-781, 9/5/08. Defects in protein folding are involved in numerous diseases. This article discusses some possible therapeutic approaches to such diseases.

K Hollemeyer et al, Species identification of Oetzi's clothing with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on peptide pattern similarities of hair digests. Rapid Commun Mass Spectrom 22:2751-67, 9/08. An example of the use of mass spec to analyze proteins. In this case, the sample is the clothing of a 5000 year old human.

B C Berks, Biochemistry: Cells enforce an ion curtain. Nature 455:1043, 10/23/08. News. How does a metalloprotein get the correct metal ion? Cu ions bind more tightly than Mn ions, so how can we make a Mn-protein? One answer is to insert the metal ion for a Mn-protein in a cellular location where there is no Cu available.

M E Linder, Neuroscience: Greasy proteins of the neuron. Nature 456:887, 12/18/08. News, about a lipoprotein. "An analysis of neuronal proteins reveals that many are regulated through covalent attachment of the lipid palmitate. This reversible modification seems to affect the form and function of synaptic junctions."

D S Burz & A Shekhtman, Structural biology: Inside the living cell. Nature 458:37, 3/5/09. News. The classic method for determining protein structure is X-ray crystallography. NMR is being used more and more; a key advantage is that it is used on proteins in solution. Now, NMR is being used to study proteins inside the cell.

R L Koder et al, Design and engineering of an O_2 transport protein. Nature 458:305, 3/19/09. "The authors used de novo protein design to generate a simple unnatural oxygen transport protein, akin to human neuroglobin; the design strategy involved the assembly of a short helix-forming sequence into a four-helix bundle that contained histidine residues at key positions. The O_2 on-rate, off-rate and dissociation constants are similar to those of human neuroglobin and other naturally occurring globins." This is something of an academic exercise: they design a protein from scratch, rather than building on anything known in Nature. Thus they can freely explore novel approaches to protein structure and function.

J R Tolman, Structural biology: Protein dynamics from disorder. Nature 459:1063, 6/25/09. News. "The functions of proteins are often crucially dependent on how they move, but measuring the absolute magnitudes of protein motions hasn't been possible. A spectroscopic method looks set to change all that."

S. K. Maji et al, Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. Science 325:328, 7/17/09. Amyloid is a form of aggregated protein that we most commonly associate with disease, such as Alzheimer's disease. Here they show that cells may store hormones in an amyloid form as a reserve. They note other examples of amyloids that are "normal" or "good".

A E X Brown et al, Multiscale mechanics of fibrin polymer: gel stretching with protein unfolding and loss of water. Science 325:741, 8/7/09. Blood clots are composed largely of the protein fibrin. This paper explores the properties of fibrin, and how they relate to clots.

S R Blanke, Expanding functionality within the looking-glass universe. Science 325:1505, 9/18/09. News. Although L-amino acids are universally used for ordinary protein synthesis, there are specific biological roles for some D-amino acids. It has long been known that D-Ala and D-Glu are part of the bacterial cell wall. Here is a story about new work showing a regulatory role for some D-amino acids. They even mention some roles for D-amino acids in higher organisms.

F G Omenetto & D L Kaplan, New opportunities for an ancient material. Science 329:528, 7/30/10. Review. Silk protein is a classic example of a fibrous protein rich in β-sheets (p 439). It is a particularly interesting protein because of its strength. Silk strength depends both on the source and on the spinning technique. Silkworm silk and spider silk both get much attention. This is an overview of the field of learning about diverse silks.

S W Ragsdale, Biochemistry: How two amino acids become one. Nature 471:583, 3/31/11. News. We say that there are 20 standard amino acids. That statement may imply that there are more -- some that are non-standard. Molecular biology has worked out how the standard 20 amino acids are made, specified and incorporated; Ch 16 introduces much of this. This news story discusses how one of the non-standard amino acids, pyrrolysine, is made. Pyrrolysine, recently discovered, is an amide of lysine; the new work shows that it is made from two molecules of lysine. Also see Liu et al (2007).

Computer resources (See web page for details and links.)

The 2002 Nobel Prize in Chemistry was awarded to three scientists for the development of methods for use of mass spectrometry (MS) and nuclear magnetic resonance (NMR) with proteins. Also see related articles in JCE: 1) M S Vestling, Using mass spectrometry for proteins. 2) S Cavagnero, Using NMR to determine protein structure in solution. J Chem Educ 80:122 & 125, 2/03.

The 2004 Nobel Prize in Chemistry was awarded to three scientists for their key roles in understanding how proteins are degraded. Specifically, they discovered the role of ubiquitin, a special protein that is attached to proteins to tag them for degradation. The bigger story is the increasing recognition of the importance of protein degradation. Many proteins are made defective, and must be degraded rapidly. Some proteins are supposed to act for only a brief time, and must be promptly degraded. And some mutations lead to proteins that are unstable, and get marked for rapid degradation.

The RasMol computer program, which was introduced in the Ch 3 handout, is particularly useful for viewing protein structures. This would be a good time to go to the RasMol tutorial at my web site, and work through the sections on proteins.

Once again, a reminder that my web page on Primary (etc.) summarizes all the various meanings of these terms that we have encountered, in various contexts.

For more about the properties of peptide bonds, which are amide linkages, see my page on Amides. The page discusses the non-basicity and the planarity of the amide linkage, and presents the resonance structures that account for these properties. This page was originally mentioned along with Ch 14, on amides. Ouellette's coverage of this is fine.

A good Internet site to view the amino acids (with accompanying text in German and English).

The Univ Akron site, listed as a general resource, includes animations of the processes of DNA replication, RNA synthesis and protein synthesis. They all simplify the processes, but are still helpful. The one on protein synthesis is probably the best.