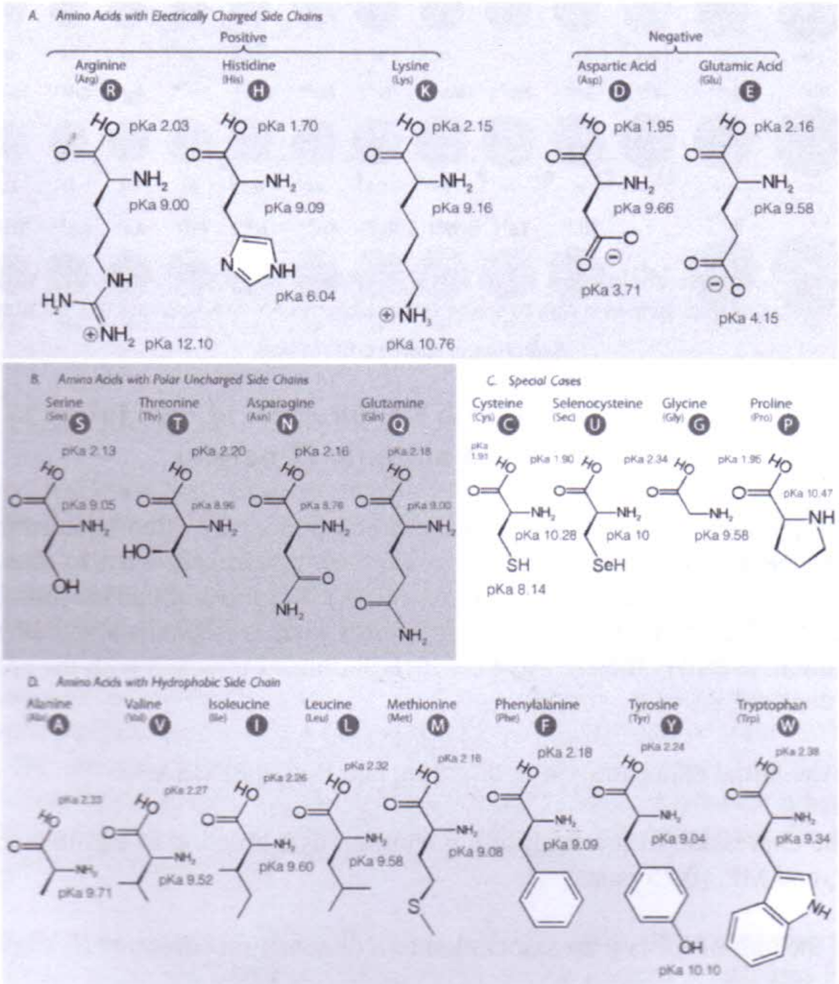
"Metals and life" re-take exam
Datum: February 2^{nd}, 2016
Tijd: 2-5 pm
Zaal: L144
Docent: Dr. Sylvestre Bonnet

Voorzie elke blad van naam en nummer collegekaart.
Bij het tentamen is het gebruik van de syllabus of mobiele telefoon niet toegestaan. Voor elke vraag is de waardering aangegeven.

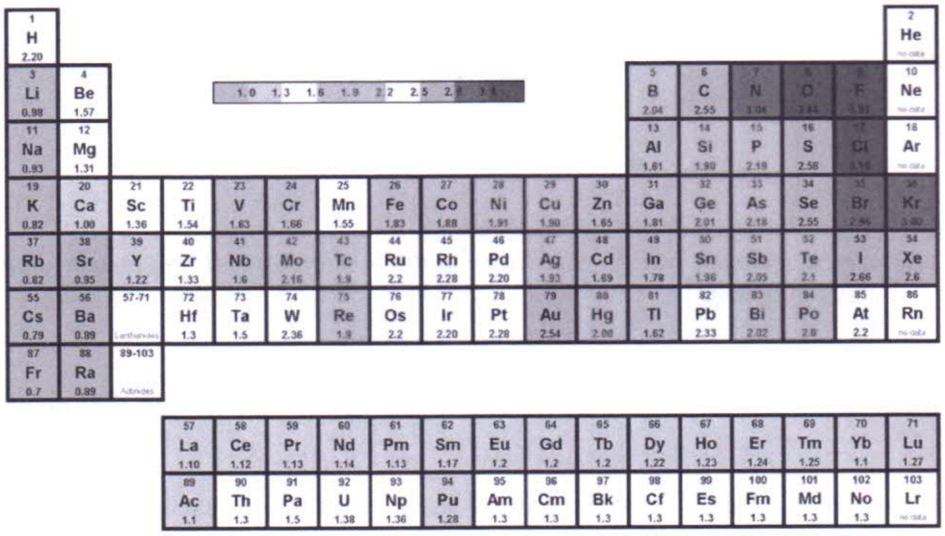
On each page write your name and the number of the college card.
It is not allowed to use the syllabus or a cell phone during the examination. For each question the rating is given.

When a justification is asked it counts at least as many points as the answer itself. The number of points per question is indicative and may be re-evaluated.

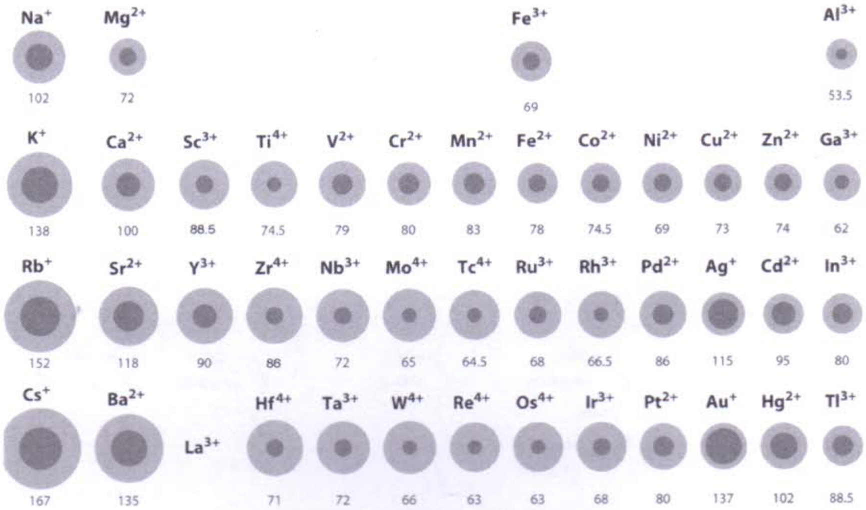
Important advice: *do not lose time on questions you can't do. First answer the questions you can answer, and then spend time on the more difficult ones.*
Also, write your answer clearly enough so that I can read it. If I cannot read you, you won't get the points.



Essential aminoacids with pKas and side chains.



Electronegativities of the elements.



Selection of ionic radii (in pm).

Part A. Binding of cisplatin to serum albumin (2 points)

Drugs such as cisplatin, a clinically used anticancer compound, are often injected in patient's blood where they often bind to serum protein. In this exercise researchers want to measure the binding constant of cisplatin and human serum albumin (HSA), the most abundant protein in the plasma with an average blood content of 40–45 $g.L^{-1}$ in healthy humans. HSA is a 585 aminoacids, 66 kDa single-chain protein. It is hypothesized that the metallodrug M interacts with the protein via a single equilibrium of constant K:

$$M + P \rightleftarrows PM$$

Let us note M_0 the initial concentration in cisplatin, and P_0 that of HSA.

1) Write the expression of the equilibrium constant as a function of equilibrium concentrations in M, P, and MP. (0.25 point)

2) What is the unit of K? Is it an association or a dissociation constant? (0.25 point)

3) To study this equilibrium the researchers use absorption spectroscopy. At 270 nm unbound cisplatin hardly absorbs compared to the HSA protein and its cisplatin adduct. The

researchers measured the absorbance of several solutions containing M and P, and vary M_0 at fixed P_0. Express A_0, the initial absorbance at t=0, and A, the absorbance of the solution at the equilibrium, as a function of M_0, of P_0, of the advancement of the reaction x (in $mol.L^{-1}$), the optical pathlength l of the spectrometer, and of the extinction coefficients ε_P and ε_M. (0.25 point)

4) The double reciprocal plot of $1/(A-A_0)$ vs. $1/M_0$ is shown in Figure 1. By assuming that the cisplatin binding fraction is small, demonstrate that the plot of $1/(A-A_0)$ vs. $1/M_0$ should be linear, and express the slope and intercept of the curve in Figure 1 as a function of the binding constant K, the optical pathlength l of the spectrometer, the initial concentration in protein P_0, and the difference $\Delta\varepsilon$ between the extinction coefficient of the metallated protein and that of the metal-free protein at 270 nm. (0.5 point)

5) Calculate the approximate numerical value of K (*be careful with units*). (0.5 point)

6) Which fraction of cisplatin is bound at a cisplatin concentration of 0.1 mM? Is your hypothesis confirmed in such conditions? (0.25 point)

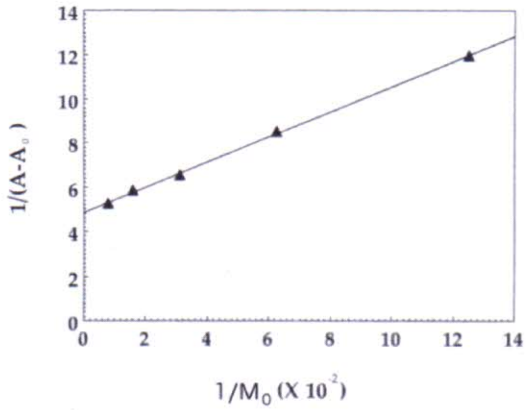


Figure 1. The plot of $1/(A-A_0)$ vs. $1/M_0$ ($\times 10^{-2}$ $mol^{-1}.L$) for HSA and its cisplatin complexes where A_0 is the initial absorbance of protein (at 270 nm) and A is the recorded absorbance at different drug concentrations M_0.

Part B. Role of metal ions in Alzheimer's disease (4.25 points)

Metal ions play an important role in Alzheimer's disease (AD). A common hallmark of neurodegenerative diseases and neurological disorders is lipid oxidation. Abnormally high levels of oxidized lipids have been found in the brains of patients with Parkinson's disease, Alzheimer's disease, and autism, compared with healthy subjects. In a recent paper, Cremer et al studied the interaction between free copper ions and biological membranes, and more precisely those containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanol-amine (POPE, see formula Figure 2). Phosphatidylethanolamine lipids (PE) are the second most abundant lipids in mammalian cells (25% of membrane lipids), and they are present at elevated levels in the brain (45% of membrane lipids). Unlike the phosphatidylserines (e.g. POPS shown in Figure 2) PE lipids have not historically been considered among the metal-binding lipids, as PE do not bear a negative charge at physiological pH. In this whole part, POPC is taken as non-coordinating basis for the lipid bilayer formulation (see POPC formula in Figure 2).

To study the interaction between free Cu^{2+} ions and PE lipids, the researchers first prepared supported lipid bilayers (SLBs) inside a microchannel device (Figure 3 left, and Figure 3A). The lipid bilayer was deposited at the surface of the microchannel following a protocol that leaves the bilayer intact. By including in the formulation of the lipid bilayer a few percent of the red