Datum: July 3rd, 2013
Tijd: 2-5 pm
Zaal: C1
Docent: Dr. Sylvestre Bonnet

Voorzie het 1e blad van naam, adres, email, jaar van aankomst en nummer collegekaart. Schrijf op de andere losse bladen alleen de naam. Bij het tentamen is het gebruik van de syllabus of mobiele telefoon niet toegestaan. Voor elke vraag is de waardering aangegeven.

On page 1, write your name, address, e-mail, year of enrolment and the number of the college card. At the following pages not your name. 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.

Part A: Bioinorganic chemistry of Titanium (7 points)

The periodic table below in Figure 1 gives, for each element, the atomic number Z and the electronegativity E. Figure 2 gives the ionic radii of a selection of ions.

This exam is dealing with titanium, an element with Z=22. The two most common oxidation states of Ti are Ti(III) and Ti(IV). The potential at low pH, formulated for the reduction of the titanyl $(Ti(IV)=O)^{2+}$ species, is as follows:

$$TiO^{2+} + 2H^{+} + e^{-} \rightarrow Ti^{3+} + H_2O \qquad E_0=0.09V \text{ vs. NHE}$$

In an aerobic atmosphere, particularly near neutral pH, Ti(IV) is favored, and most of the Ti in the environment is oxidized. In reducing environments, including the ones that may have occurred during the evolution of the Earth, Ti(III) can be favored. Titanium(III) citrate notably finds an important modern use as a biocompatible reductant, for example, as an agent to maintain anaerobic cell culture. Near neutral pH, the neutral hydroxide species of Ti^{4+} dominate $(Ti(OH)_4$ or $TiO(OH)_2)$, each probably further forming polynuclear clusters.

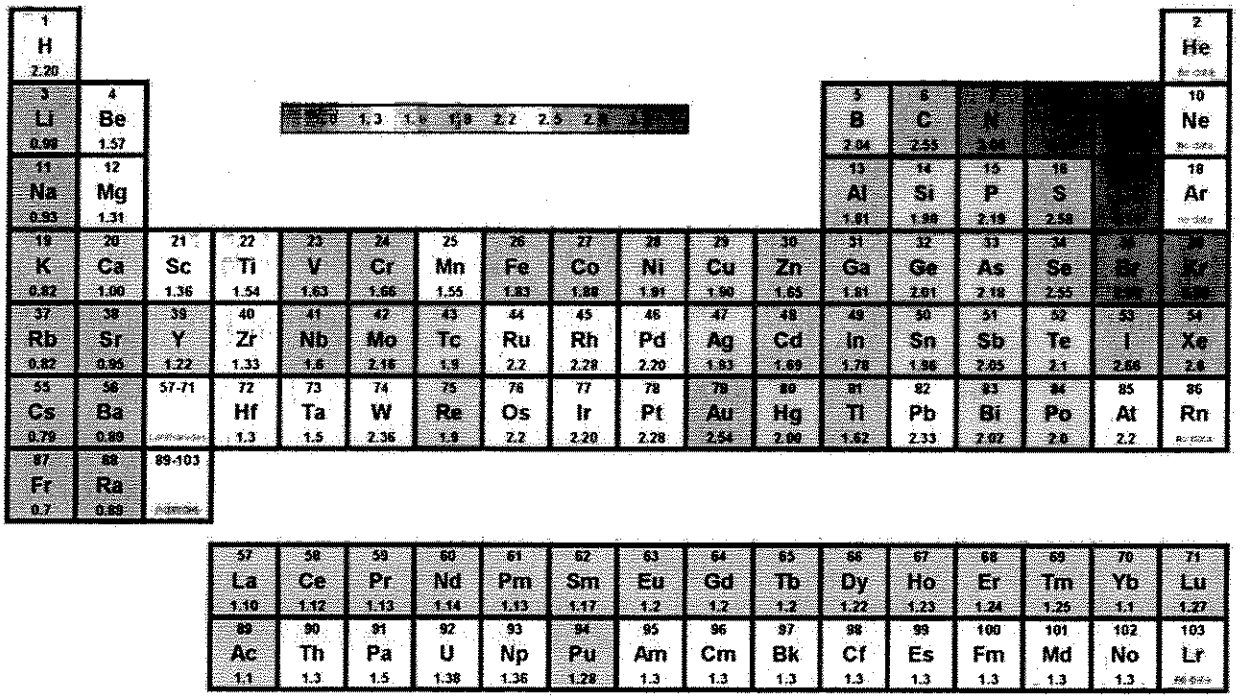


Figure 1. Electronegativities of the elements.

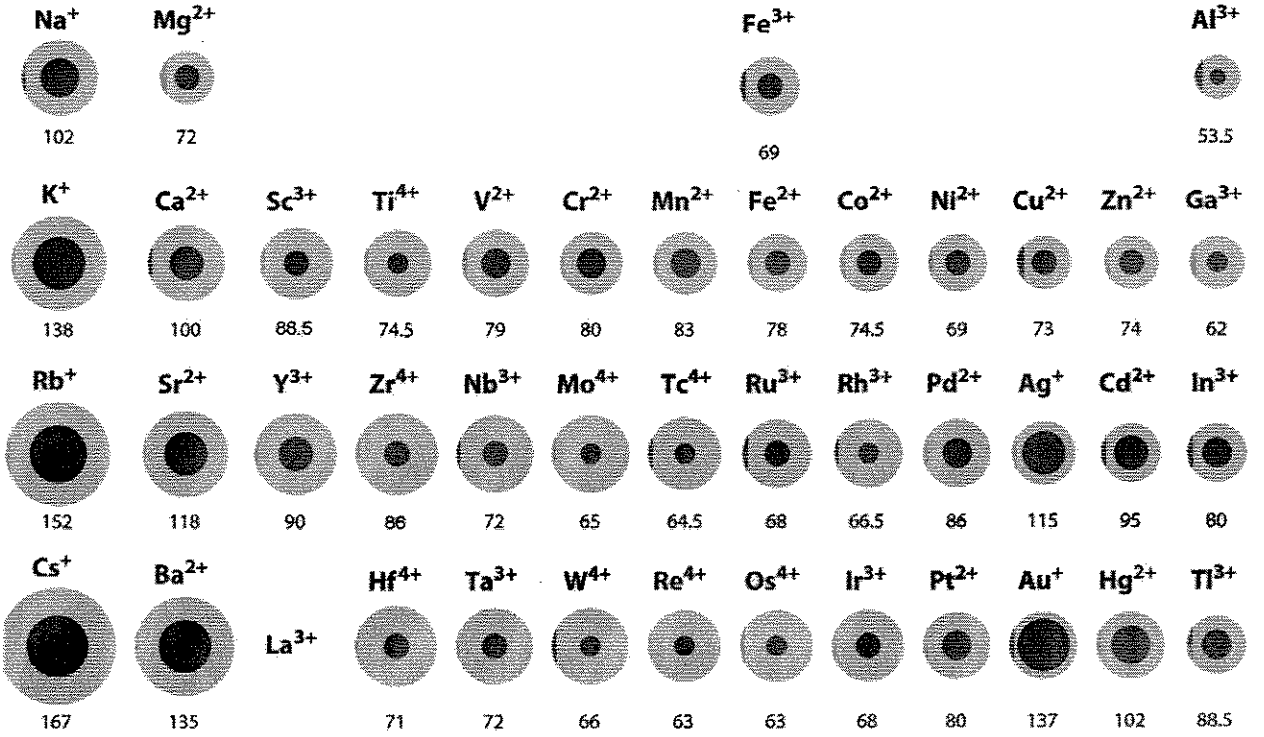


Figure 2. Selection of ionic radii (in pm).

Answer the following questions:

1. Is titanium an alkaline metal, an alkaline earth metal, a transition metal, a semi-metal, a lanthanide, an actinide, or a non-metal? Justify. (0.5 point)
2. Compare the hardness/softness of Ti^{4+}, in the HSAB theory, to that of Sc^{3+}, Zr^{4+}, and Ti^{3+}. (0.5 point)
3. Is Ti^{4+} a hard or a soft ion? Will it bind better to nitrogen-, oxygen-, or sulfur-based ligands? Charged or uncharged ligands? Justify. (0.5 point)

Titanium is the ninth most abundant element in the Earth's crust; soluble Ti concentrations are typically of 4 pM in the surface ocean, and titanium concentrations in rivers and near coastal waters vary between 1 pM and >100 nM. Titanium has never been demonstrated to be essential for any organism, nor to occur natively in any metalloenzyme. However, the essentiality of Ti for organism(s) would be quite easy to miss as its analytical detection in biological medium has been challenging (it is not magnetic, its complexes are often not colored and not fluorescent, etc.). However, recent work using modern mass spectrometry has begun to include Ti among the elements analyzed for in the characterization of metalloproteomes, and high Ti levels in some cells were detected. For many of the same reasons, demonstrating that Ti is not required is quite difficult and has never been done. Between 10 and 20 mg are found in the body of the Standard Man, making Ti the 14th most abundant element there. In adults it is concentrated in the lung, liver, spleen, and kidney. Its concentration has been reported as 116.7 ppb (~2 μM) in human blood serum and 250 ppb (~5 μM) in milk. Contradicting these results, lower Ti concentrations (mid-nM concentrations in serum) are reported as normal baseline levels in negative controls in Ti toxicity studies.

4. Give three metal ions, different from Ti^{4+} or Ti^{3+}, that are also found in humans around the globe, but that do represent a danger for human health. Which typical illnesses have they been shown to provoke upon acute poisoning? (0.5 point)

5. What is the "chemical burden" as discovered by the CDC – National Biomonitoring project in the USA? Give at least two *scientific* questions this project has raised. (0.5 point)

Ti toxicity studies were actually performed when Ti-based anticancer compounds started to be considered. Because of its propensity for hydrolysis, well-characterized compounds of Ti(IV) that are prepared and/or stable in aqueous solution are somewhat rare. The two complexes shown in Figure 3 have been prepared and clinically tested as anticancer compounds on humans. They are somewhat stable, but do hydrolyze in aqueous solution (see below).

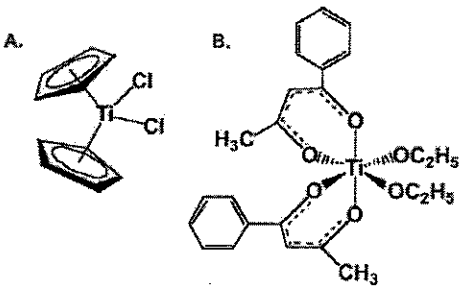
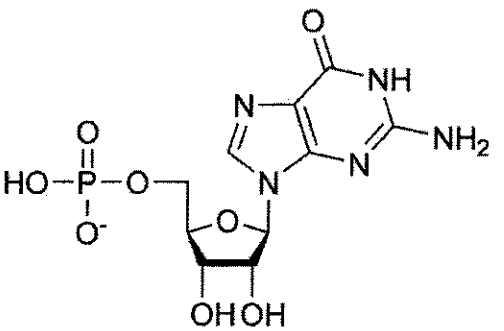


Figure 3. Two Ti anticancer drugs used in human clinical trials. (A) Titanocene dichloride, Cp_2TiCl_2. (B) Bis(β-diketonato) complex, also known as "budotitane". Other isomers are possible.

6. What does the fact that both compounds show similar activity and toxicity patterns, suggest to you? (0.25 point)
7. I was proposed that the ligands are lost and that Ti(IV) is carried into the tumor cell by a serum ion transport protein. Which serum protein do you know that usually performs metal ion transport and storage? For which naturally occurring metal ion does this protein notably function? In which ionization state is the metal usually stored? (0.5 point)
8. Figure caption of Figure 3 mentions that "other isomers are possible". Can you draw three isomers of budotitane that are different from the one shown in Figure 3? (0.25 point)
9. Do you expect similar or different biological activity for the three isomers in front of you? Justify. (0.25 point)

DNA has long been invoked as the probable target for Ti anticancer drugs, partly because of the similarity to cisplatin of both Cp_2TiCl_2 and budotitane, with their labile ligands in cis-configuration. This notion was supported by early work, which found that Ti localizes to the chromatin and inhibits DNA synthesis. An X-ray fluorescence intracellular mapping study of Cp_2TiCl_2-treated hamster lung cells revealed Ti distributed throughout the cell, but somewhat concentrated in the nucleus.

10. Below is a picture of a guanosine monophosphate, a small molecule modeling DNA. On which atoms do you expect Ti(IV) to bind to? Based on this observation, do you expect titanocene to show the same biological mechanism of action as cisplatin? (0.5 point)



Both compounds shown in Figure 3 hydrolyze quickly into aggregates and ultimately into TiO_2. At pH 5 the two chloride ligands of titanocene dichloride are lost with a $t_{1/2}$ of a few minutes, while hydrolysis of the Cp^- ligands is a bit slower ($t_{1/2}$ ca. 54 h). A new generation of Ti compounds, such as the phenolato complexes shown in Figure 4, was considered. These compounds would hydrolyze less quickly in a biological environment than budotitane or titanocene dichloride.

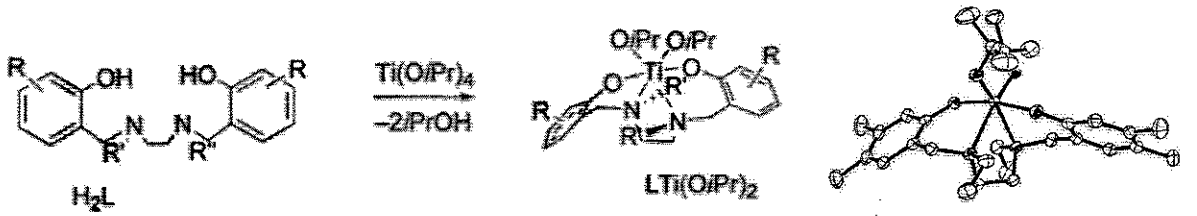


Figure 4. Synthesis of new generation anticancer titanium compounds [LTi(OiPr)$_2$]. Right: X-ray structure of one example with R=Me$_2$ and R'=Me.

11. Which primary design strategy did the scientists use, considering that they wanted to avoid quick hydrolysis? Why would this family of compounds hydrolyze less quickly than for example titanocene dichloride? (0.5 point)
12. Compounds of the type [LTi(OiPr)$_2$] are indeed very slow to hydrolyze. In addition, the rate of hydrolysis is very dependent on the steric hindrance of the substituents, with half-reaction times for the hydrolysis of the isopropylate ligand ranging from 5 min to 5 h. Why is this property an advantage in terms of medicinal chemistry? (0.25 point)

The cytotoxicity of a whole range of the phenolato complexes shown in Figure 4 with different substituents has been measured on two types of cancer-cell lines, colon HT- 29 and ovarian OVCAR-1 cells. In general, similar activity patterns were observed for both cell types. In some cases, measurements were conducted in the presence of a supplement, apo-transferrin (Tr). In addition, variations of incubation times allowed estimating the general stability and the time scale of activity and cell penetration.

	Reagent	Tr	HT-29	OVCAR-1
1	Cp_2TiCl_2	–	710 ± 120	780 ± 90
2	$(bzac)_2Ti(OiPr)_2$	–	53 ± 1	53 ± 1
3	cisplatin	–	33 ± 3	17 ± 4
4	$L^1Ti(OiPr)_2$	–	12 ± 1	14 ± 1
5	$L^2Ti(OiPr)_2$	–	12 ± 1	12 ± 1
6	$L^3Ti(OiPr)_2$	–	inactive	inactive
7	$L^4Ti(OiPr)_2$	–	–[a]	–[a]
8	$L^5Ti(OiPr)_2$	–	inactive	inactive
9	$L^1Ti(cat)$	–	20 ± 2	40 ± 4
10	Cp_2TiCl_2	+	460 ± 40	520 ± 30
11	$(bzac)_2Ti(OiPr)_2$	+	56.9 ± 0.6	65.0 ± 0.6
12	$L^1Ti(OiPr)_2$	+	16 ± 3	15 ± 3
13	$L^2Ti(OiPr)_2$	+	20 ± 3	39 ± 4
14	$L^3Ti(OiPr)_2$	+	inactive	inactive

[a] Cell-growth inhibition does not exceed 50%.

Figure 5. IC50 (μM) values for selected diamine bis(phenolato) bis-(isopropoxo) complexes on HT-29 and OVCAR-1 cancer cells, and comparison to known compounds with or without a supplement of apo-transferrin (Tr).

13. What is apo-transferrin? Does it have a significant effect on the cytotoxicity of the titanium compounds? Which conclusion can you draw on the uptake mechanism of these compounds by HT-29 and OVCAR-1 cancer cells? (0.5 point)
14. Are the bis(phenolato) bis-(isopropoxo) complexes more or less cytotoxic than cisplatin? What do this result tell you about the pharmacological interest of these compounds? (0.5 point)

15. It was observed that hydrolytically labile compounds such as titanocene dichloride quickly form stable O-bridged aggregates in contact with water. Upon the much slower hydrolysis of the isopropoxo groups, bis(phenolato) bis-(isopropoxo) complexes such as $[L^1Ti(OiPr)_2]$ also form multinuclear clusters such as the one shown on the right of Figure 6. In general it seems that only complexes that slowly yield a stable polynuclear hydrolysis product upon water addition (in test tubes) are significantly cytotoxic (in cells). The correlation between polynuclear complex formation and cytotoxic activity raises the possibility of the cluster itself being the active species. In order to check this hypothesis the cytotoxicity of the isolated trinuclear complex $[L^1{}_3Ti_3O_3]$ was measured by growing cancer cells in presence of decreasing concentrations of the trinuclear complex (see data in Figure 7). Is the trimer cytotoxic towards OVCAR-1 cell? Can you emit an hypothesis on the reason why $[L^1Ti(OiPr)_2]$ and $[L^1{}_3Ti_3O_3]$ have a different behaviour? (0.5 point)

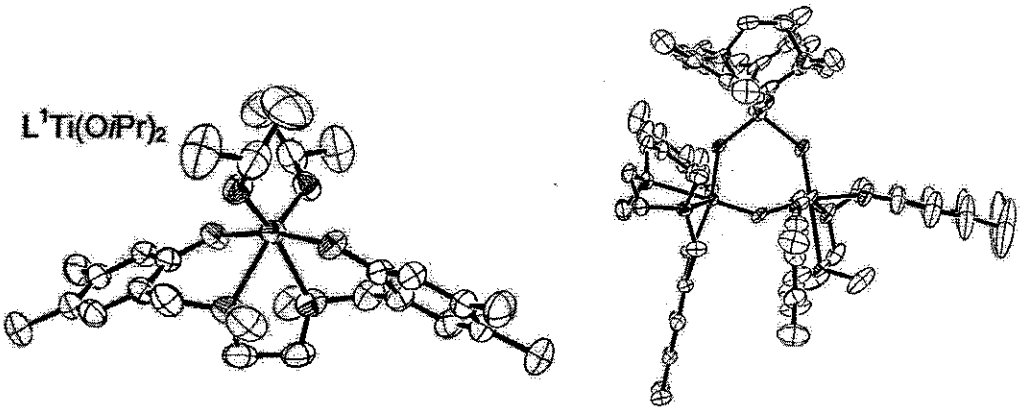


Figure 6. X-ray structure of $[L^1Ti(OiPr)_2]$ and of its hydrolyzed trinuclear product $[L^1{}_3Ti_3O_3]$.

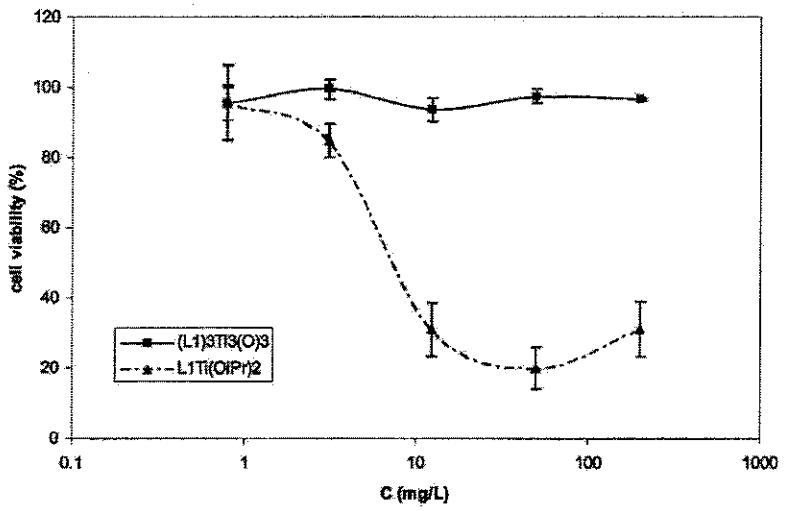


Figure 7. Dependence of OVCAR-1 cell viability after 3 days incubation period on administered concentration of $[L^1Ti(OiPr)_2]$ and $[L^1{}_3Ti_3O_3]$ presented in a logarithmic scale.

16. The ^{45}Ti isotope of titanium has a half-life of 3.1 h and decays mostly by positron emission with an energy $E^{\beta+}{}_{max}$ = 1.04 MeV. The isotope can be prepared in good yield in biomedical cyclotrons by the bombardment of scandium with protons. Would you use this nucleide for imaging or for therapy? Justify. (0.5 point)

End of the exam.