THE EUROPLANET TNA ACTIVITY REPORT

TITLE PROJECT: Investigation of the adsorption, polymerisation and stability under various conditions of amino acids and peptides on gold, TiO_2 and SiO_2

PROJECT LEADER: Prof. Claire Marie Pradier
COLLABORATOR: Prof. Jean-François Lambert
HOST: Dr. Eva Mateo-Martí
DATES OF VISIT: 19th July2010 – 23th July2010
EuroPlanet PROPOSAL: TNA2

Purpose of the visit

Objectives of the experiment at the planetary simulation chamber

The objective of the proposal was towfold, for this allocation period:

- investigating the stability (chemical form, dissociation?) of some biomolecules, Gly-Pro and IGF under various environments, vacuum, oxygen and UV Irradiation

Various surfaces of interest had been mentioned, Au(111), SiO_2 powder and a TiO_2 surface

- characterising the polymerisation of the amino acids constituting these peptides, lysine, proline and glutamic acid, under UV irradiation;

Answers to these questions was expected by analysing the surfaces by IR in the reflection mode; the various vibrational bands, in particular the peptide bands in the 1500-1700 cm^{-1} region; the use of the Planetary Simulation chamber is a prerequisite to conduct these experiments.

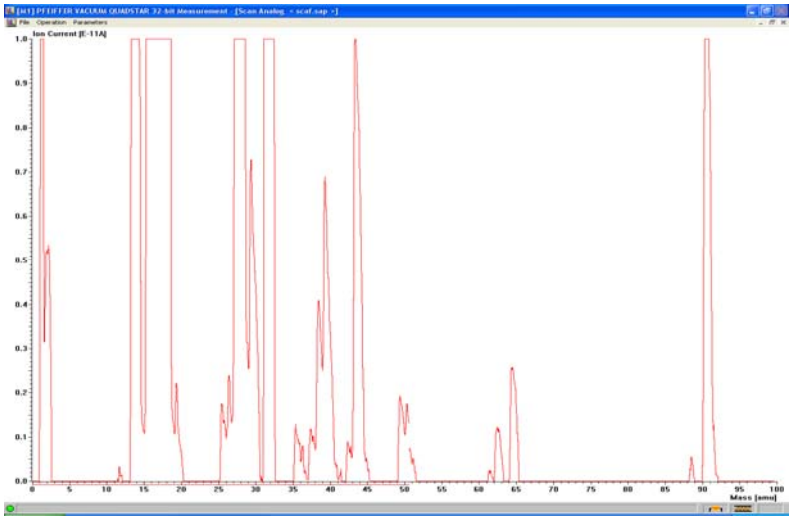
Description of the work carried out during the visit

Experiments at the Simulation chamber, INTA, 19-24 July 2010

The first series of experiments consisted in characterising the adsorption of one dipeptide, Gly-Pro, on gold and its stability/reactivity under UV-irradiation. Two main techniques were used, all along the experiments, FT-IRRAS and Mass spectrometry
.The chamber was ready to be used with a gold wafer and IGF powder in place and pumped down to P = $9x10^{-8}$ mbars + Mass spectrometer and IRRAS spectrometer working fine.

The following experiments have been performed:

Exp. n° 1: Adsorption of Gly-Pro on a gold wafer
When T powder =50°C, some IR weak band at ca 1590 cm^{-1}
Result: almost no IR signal
The mass spectrum showx peaks at m= 18, 28 atributed to H_2O or N_2 respectively; another peak at 40 mau could not be attributed (?)



Mass spectra during dosing Gly-Pro

Monday evening: IGF put into the doser, new gold wafer, rinsed in ethanol, in the vacuum chamber

Exp. n° 2: Adsorption of IGF on a gold wafer (Tuesday)

Same experiment as with Gly-Pro

T up to 200°C, T sample = RT

Almost no IR signal, but appearance of intense peaks in the mass spectra (m = 50, 51, 91) which hardly correspond to the the peptide fragments. Could it be due to some degassing?

Exp. n° 3: Analysis after adsorption of IGF (from liquid solution, 0.01M) on a gold wafer

Almost no IR signal

More IGF was added in the solution and the sample was left overnight

Exp. n°4: test the IR signal on a SiO_2 pellet (broken pellet), sample JFL n°2

No IR reflection

Exp. n°5: Test IR signal on a silica wafer

No IR reflection

Tuesday evening: sample holder with no sample and IGF in the doser

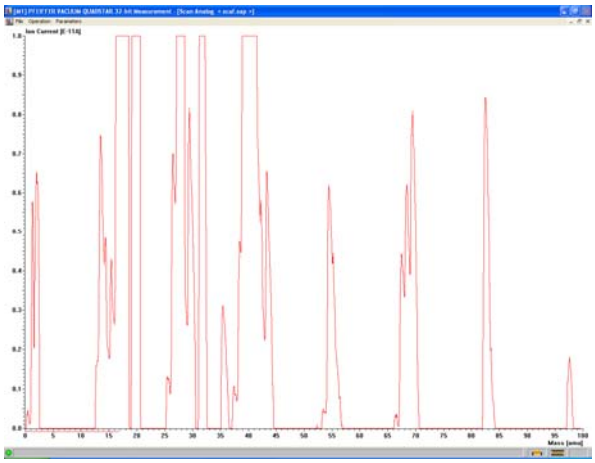
Exp. n° 6 : evaporation of IGF with the sample holder (gold) at low T

T sample holder = 250K to 70K,

Appearing masses in the MS: 55, 68, 83, 91, which are not obvious fragments of IGF.

Weak IR signals at ca 1650-1730

Mass spectra during dosing IGF



Then: UV irradiation, with the sample holder at 66 K, up to 85 min
Some very interesting changes in the IR spectra have been observed : a decrease of the band at 1730 cm^{-1} and ca 1680 cm^{-1}, which suggests that UV irradiation breaks the bond between the molecule and the carboxyl groups. It indeed destroys the peptide molecule.

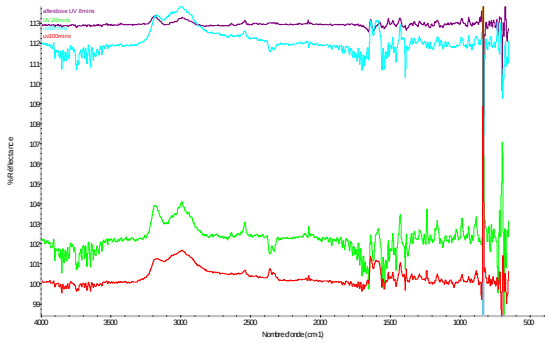


Figure 1: IRRAS spectra of the gold surface (sample holder) upon exposure to IGF tripeptide)

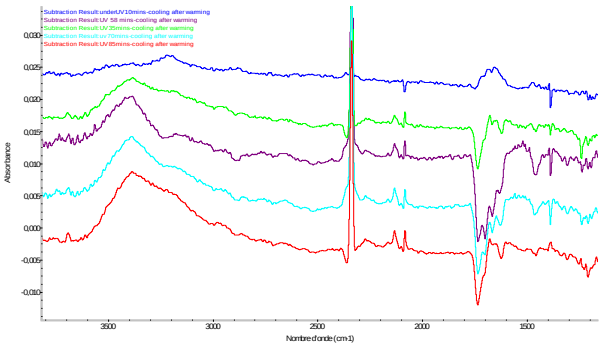


Figure 2: The same surface upon UV-irradiation after IGF exposure (subtraction spectra against the spectrum at t=0 irradiation)

Note that UV stopped, dosing again IGF and warming up the sample: no IR change, were observed.

Exp. n°7: Analysis after adsorption of IGF (from liquid solution, 0.02M, more than 24h) on a gold wafer

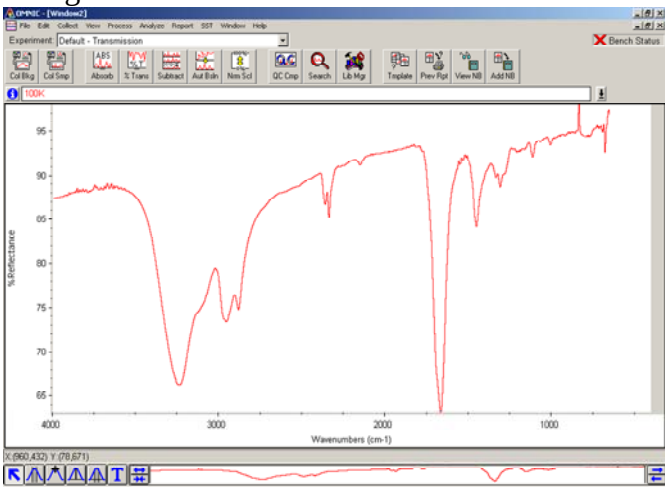
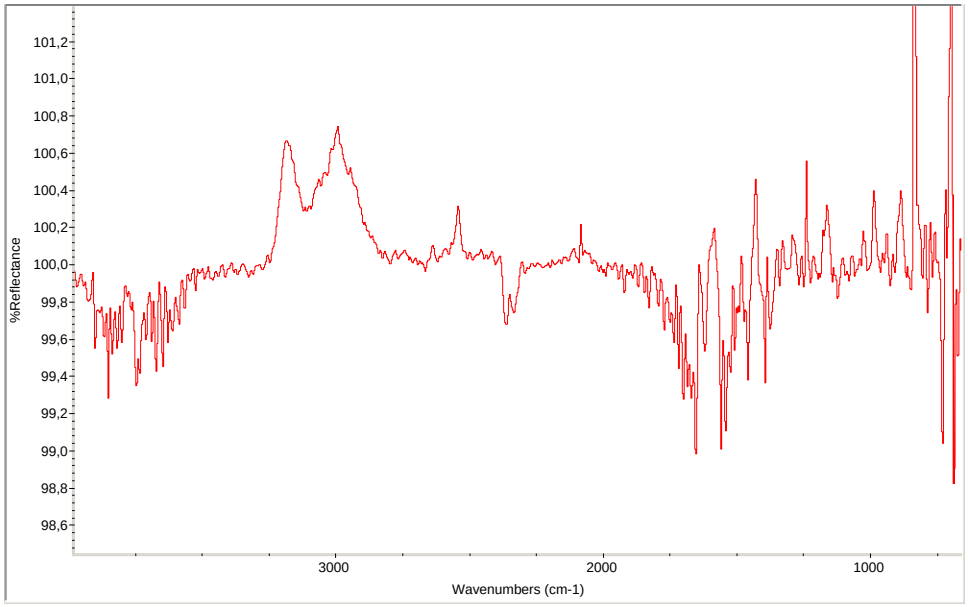


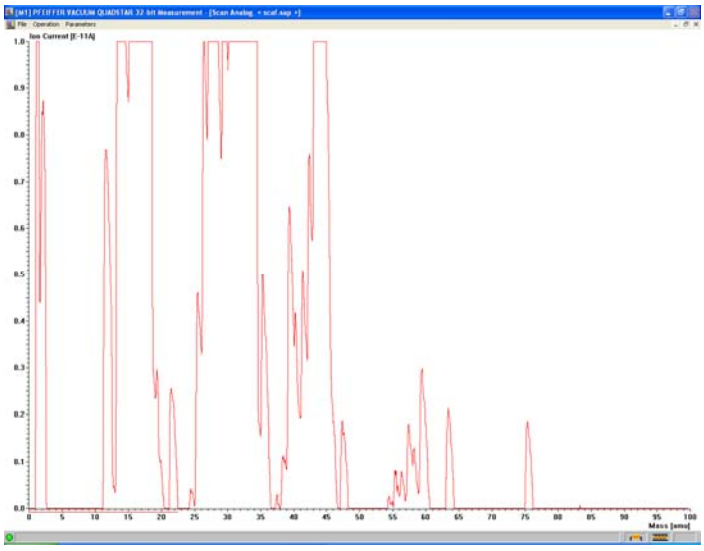
Figure 3: IRRAS spectrum of a gold sample after adsorption of the IGF tripeptide in a liquid solution.

Exp. n° 8: test the IR signal on SiO_2 + Gly pellet (JFL n°1)
It was deposited on a gold wafer and the bcknd was the gold wafer surface: no IR good signal except a huge absorption at 1000-1200 cm^{-1} due to Si-O in SiO2.

Exp n° 9: Ads of Cysteine on a gold wafer

T sample holder = 25ºC,
Appearing masses in the MS: 44, 58,64, 76
UV during 100 mins (figura shows IR after 20 mins UV). Bands appearing at ca 300 cm^{-1} suggest a loss of hydrocarbon residues on the surface. Other bands are not distinct due to the signals from water.

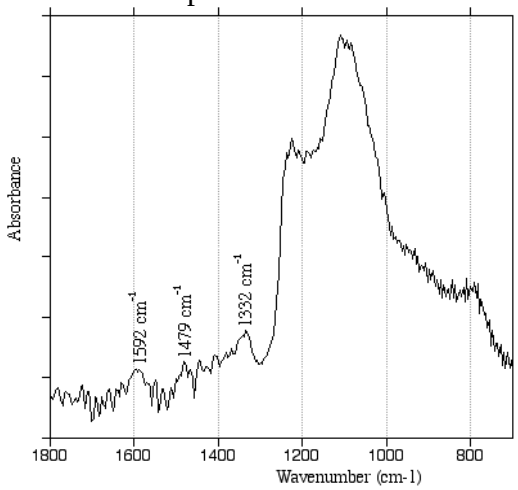




Mass spectra during cys dosing

Glycine on Aerosil silica (powder), 0.8 mmol/g

Glycine was deposited on high-surface area fumed silica (Aerosil) from aqueous solution, following which the samples were pressed into a self-supporting pellet; this pellet was fixed on a copper sample holder with graphite paste, and the IRRAS spectrum was recorded , first under air, then after UV irradiation under air, UV irradiation under vacuum, and further heating to 50°C under vacuum. The last spectrum is shown as an example.



In spite of the unfavorable optical conditions (the beam has to cross a rather thick pellet twice), exploitable spectra can be obtained. They are dominated by the absorption bands of the silica support between 800 and 1250 cm^{-1}; several discernable bands can be assigned to the adsorbed amino acid at 1332 cm^{-1} ($\rho_w CH_2$), 1479 cm^{-1} ($\delta_{sym} NH_3^+$), and 1592 cm^{-1} (mainly $\delta_{asym} COO^-$). These positions are fully compatible with previously published data for the glycine zwitterion in this system.

Additional experiments were conducted on a dipeptide (Gly-Pro) and a tripeptide (Gly-Pro-Glu; these are the same peptides as used for the experiments on gold surfaces above). The amide band was clearly observable, opening the possibility of a discrimination between monomeric (unpolymerized) and polymerized amino acids.

However, no clear differences were observed after the various pretreatments (UHV, UV irradiation, moderate heating). It is obvious that the data collection conditions have to be optimized before fine points of prebiotic molecules reactivity can be addressed on divided oxide surfaces.

Description of the main results obtained

These series of experiments were the first on this machine performed on planar samples usinf FT-IRRAS spectroscopy in the presence of a gas phase. Moreover, adsorption of peptides on metal or oxide surfaces is a rather innovative way of testing their stability under irradiations; protocols had to be optimised

This implied a number of new setups and methods for example to record satisfying reference spectra and sample spectra, to avoid contamination from valve degassing, or to irradiate at the optimal temperature in order to “keep” molecules on the surface even after peptide exposure.

The first three days, we got satisfying spectra of peptides adsorbed on gold… but no success when monitoring the adsorption in situ. The reason for that is likely the two low signal-over-noise ratio, due partly to the necessity to perform a reference spectrum before staring the experiments.

The most interesting and promising result was obtained, upon exposure to UV irradiation of a layer of IGF, with a net disappearance of the carboxyl group signals which shows a damage of the molecule.

Regarding the experiments on powder samples, our results constitute a proof of concept in that several exploitable bands of adsorbed biological molecules were obtained. However, the method has to be optimized before providing useful results on their reactivity (longer accumulation times, optimisation of the pellet preparation procedure).

Future collaboration with host institution (if applicable)

After these first series of experiments, though many experiments did not lead to exploitable data, we had some promising slight results which encourage us to continue ouir collaboration with Dr Eva Mateo at the Center of Astrobiology of Madrid.

Knowing the functioning of the machine better, we now would like to investigate the following questions: does a protein denaturate upon UV-irradiation? Can it be detected by IRRAS and, in complement does the protein loose its biorecognition properties?

We would like to address these three questions, and we think that valuable results could be obtained thanks to complementary measurements in France and at the EUROPLANET center.