Abstract

The acoustic ratio ($\Delta D/\Delta F$) of the dissipation ($\Delta D$) and frequency ($\Delta F$) change measured with a Quartz Crystal Microbalance (QCM-D) has been proposed as a fixed value related to the intrinsic viscosity of the molecules attached to the surface and therefore with their three dimensional structure. The group of Prof. Dr. Electra Gizeli in Heraklion (Greece) has been pioneer in this way to analyze and interpret the QCM-D data being successful in discriminating the length and shape of DNA molecules\textsuperscript{[1]}. This new theoretical framework opens the door to use the QCM-D for structural analysis of biomolecules.

During my stay in Heraklion, and in the context described above, we used QCM-D applied to study structural conformational changes of the bacterial protein ZipA. ZipA is a 36.4 KDa $E. coli$ membrane protein with an unstructured domain linking the N-terminal transmembrane helix with its C-terminal globular domain that has been postulated as a flexible tether of the cytoskeleton protein FtsZ\textsuperscript{[2]} involved in bacterial cell division. We have used two soluble protein constructs of ZipA in which the transmembrane domain was substituted by a histidine tag for oriented attachment to a membrane containing nickel chelating lipids. The constructs s1-ZipA and s2-ZipA differ, respectively, in the presence or absence of the unstructured domain (see figure 1A). We were successful in following in real time the expansion and collapse of the unstructured domain of ZipA induced by changes in the ionic strength of the buffer solution (figure 1B).
**Experiments and Results**

The experiments to study structural conformational changes of the soluble forms of ZipA comprise three stages:

1. **The formation of a Supported Lipid Bilayer (SLB)** on the surface of the crystal sensor of the QCM-D.

2. **Anchoring and orientation of s1-ZipA (or s2-ZipA) on the bilayer containing nickel chelating lipids (DGS-NTA).** We verified the specificity of the binding and orientation of the soluble constructs of ZipA to the lipid bilayer running two independent controls. First, a negative control observing the absence of protein adsorption when exposing s1-ZipA (or s2-ZipA) to SLBs lacking nickel chelating lipids. Second, a positive control where we observed a total elution of s1-ZipA and s2-ZipA anchored to the surface upon addition of imidazole.

3. **Inducing the expansion and collapse of the unstructured domain of ZipA reducing or increasing the salt concentration of the buffer respectively.** After s1-ZipA and s2-ZipA were anchored on the bilayer we have registered the dissipation (ΔD) and frequency (ΔF) changes upon exchanges of running buffer with different ionic strength. Notice that to extract the actual change in the acoustic ratio (ΔD/ΔF) of s1-ZipA and s2-ZipA at each ionic strength is necessary to subtract ΔD and ΔF on the bare bilayer induced by each buffer exchange.

The acoustic characterization of s1-ZipA and s2-ZipA anchored and oriented on supported lipid bilayers has led to the following results:

1. The acoustic ratios measured for s1-ZipA and s2-ZipA are clearly different revealing the presence and the absence of the unstructured domain of ZipA respectively.

2. We observed an increase in the acoustic ratio of s1-ZipA while the ionic strength of the buffer is reduced. In contrast, the ionic strength of the buffer does not affect the acoustic ratio of s2-ZipA.

In terms of the intrinsic viscosity theory a larger value of the acoustic ratio of a molecule anchored to the surface indicates that its shape protrudes further away from the surface. Both the increase of the acoustic ratio of s1-ZipA at low ionic strength and the lack of dependency of the acoustic ratio of s2-ZipA with the salt concentration of the buffer indicate that the unstructured domain of ZipA is flexible enough to be expanded and collapsed depending on the environment.

**Conclusions and Perspectives**

The experimental results obtained during this STMS extend the application of the QCM to structural studies of proteins. Specifically, we have applied this technique to follow conformational changes of an unstructured protein domain. This is of special interest due to
the impossibility of study these nonstructured protein domains with other techniques as X-ray diffraction.

We have discussed the results and outlined a preliminary draft of a manuscript for a future submission of this work. We have prepared a set of potential figures for the manuscript and we agreed in the needed of complementary measurements with an independent technique to confirm the expansion of the nonstructured domain of ZipA at low ionic strength.

References