NMR characterization of the intrinsically disordered N-terminal region of the E7 protein from Human Papillomavirus

Ivano Bertini[1,2], Eduardo Calçada[1], Isabella C. Felli[1,2], Tomáš Hošek[1], Roberta Pierattelli[1,2]

[1]Magnetic Resonance Center (CERM), University of Florence, Via L. Sacconi 6, Sesto Fiorentino 50019, Florence, Italy (hosek@cerm.unifi.it)
[2]Department of Chemistry, University of Florence, Via Delle Lastruccia, Sesto Fiorentino 50019, Florence, Italy

Many functional proteins are devoid of stable secondary and tertiary structure, and recent evidence shows that these proteins gain functional advantages by remaining natively unstructured. Intrinsically disordered proteins (IDPs) could be either completely or partially unstructured, and were shown to play important biochemical functions including molecular recognition, signaling, and regulation with implication in several human diseases like Alzheimer, Parkinson, cancer and many viral infections.

The protein E7 from HPV-16 has a key role in the development of cervical cancer, the second most common cancer in women worldwide, and it is known to be partially unstructured. It is a 98 residues protein composed by three conserved regions, CR1 and CR2 in the N-terminal half and CR3 in the C-terminal half. The N-terminal part has been found to interact with a high variety of targets, like the Retinoblastoma tumor suppressor protein[1], contains phosphorylation site[2], and it is of crucial importance to achieve its structural and dynamical characterization, despite the absence of a stable structure. Nuclear magnetic resonance spectroscopy has been proven to be a useful tool to obtain atomic resolution information on IDPs.

A combination of ^{1}H detected and ^{13}C detected NMR experiments were utilized to achieve the structural and dynamics characterization of the N-terminal disordered part of the HPV-16 E7.

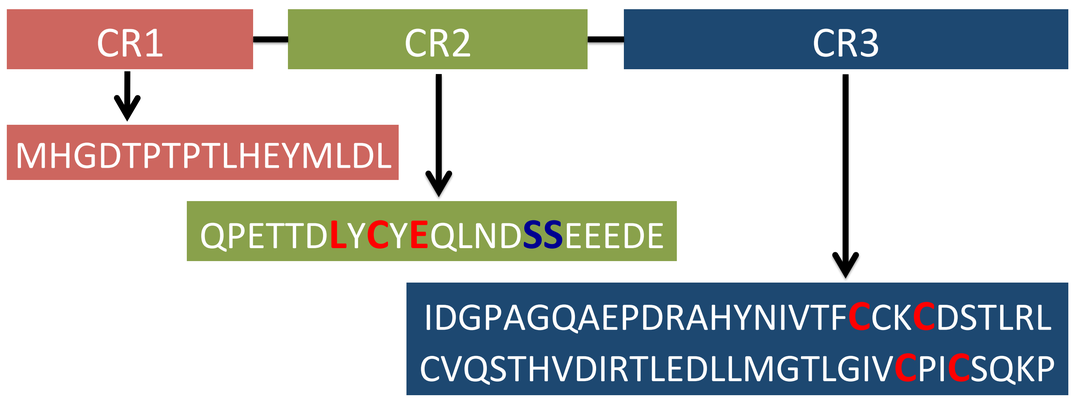

Figure 1: The sequence of oncoprotein E7 from HPV 16. Short linear motifs are highlighted in red: Retimoblastoma tumor suppressor binding motif (LxCxE), Zinc binding motifs (CxxC). Phosphorilation site (SS) is highlighted in blue.

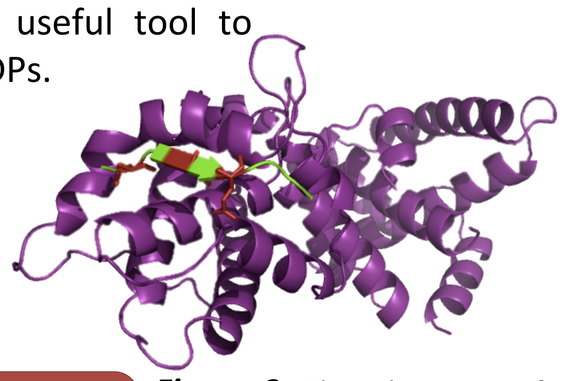

Figure 2: Short linear motif LxCxE (green-red) forms extended β-strand conformation upon binding to Retinoblastoma tumor suppressor protein (purple).[1]

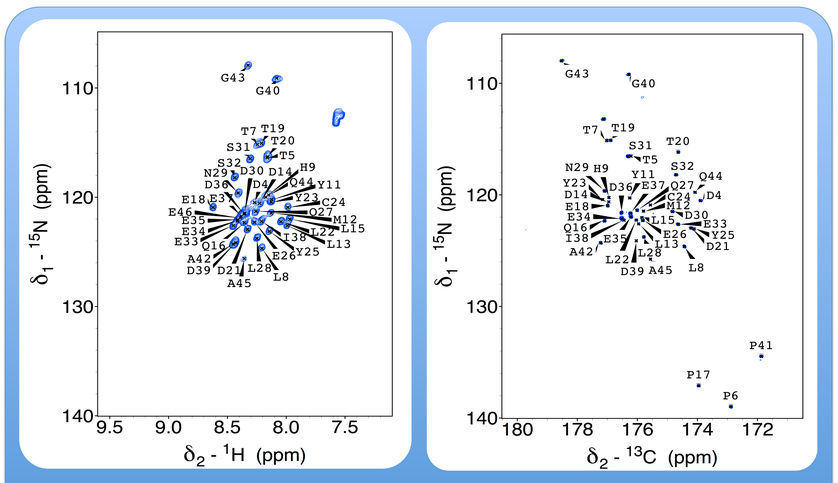

Figure 3: The assigned ^{1}H-^{15}N SFHMQC and ^{13}C-^{15}N CON-IPAP spectra of HPV-16 E7 oncoprotein.

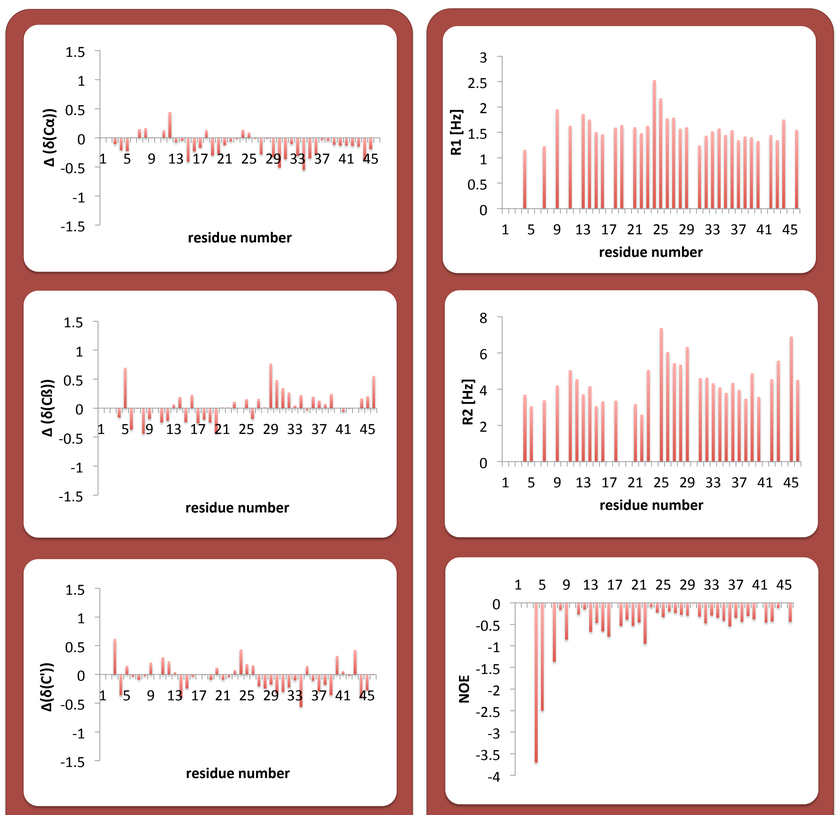

Figure 4: Chemical shift index of C^{α}, C^{β} and $C^{/}$ carbon atoms.

Figure 5: ^{15}N R_1 and R_2 relaxation rates and ^{1}H-^{15}N NOEs.

The chemical shift index analysis suggests slight tendency of the region around LxCxE short linear motif (residue number 22 to 26) to adopt β-strand conformation. Also ^{15}N relaxation experiments show smaller flexibility of this region comparing to the rest of N-terminal half of E7.

This results are in accordance with the obsevation that LxCxE SLiM forms extended β-strand conformation upon binding to Retinoblastoma protein.[1]

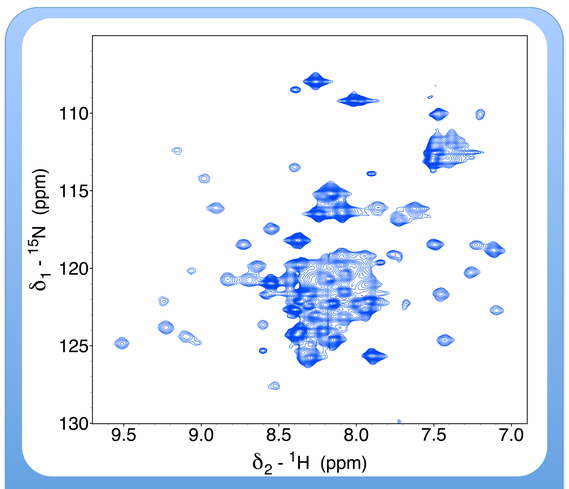

Figure 6: The signals from structured CR3 region appeared in the ^{1}H-^{15}N SFHMQC spectra as the result of improved expression protocol.

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References:

[1] Lee, J. O., Russo, A. A., & Pavletich, N. P. (1998). Structure of the retinoblastoma tumour-suppressor pocket domain bound to a peptide from HPV E7. Nature, 391(6670), 859–865.
[2] Chien, W. M., Parker, J. N., Schmidt-Grimminger, D. C., Broker, T. R., and Chow, L. T. (2000) Casein kinase II phosphorylation of the human papillomavirus-18 E7 protein is critical for promoting S-phase entry, Cell Growth Differ. 11, 425-435.