Human Papilloma Virus intrinsically disorder protein E7 characterization by NMR spectroscopy

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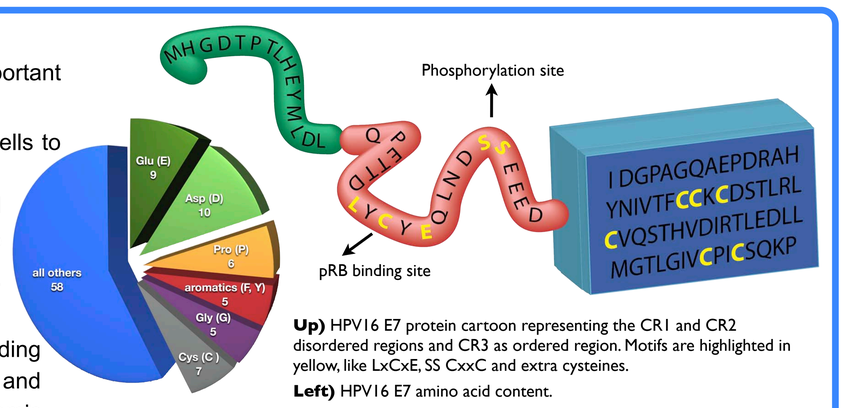
Introduction

Intrinsically disordered proteins (IDPs) are known to gain functional advantages by remaining natively unstructured, playing important biochemical functions including molecular recognition, signaling, and regulation with implication in several human diseases.

Human Papilloma Virus (HPV) genome encodes only 8 proteins were E6 and E7 proteins play key role on infection, forcing quiescent cells to enter the cell division cycle initiating the progression to cervix cancer. HPV type 16 (HPV16) is know to be the most carcinogenic viral type. The oncoprotein E7 from HPV16 contains 98 residues (12 kDa) composed by three conserved regions, CR1 and CR2 in the N-terminal (first 37 residues) and CR3 in the C-terminal (last 61 residues). CR1 and CR2 are predicted as disordered regions [1]. CR2 contains the motif LxCxE know to interact and inactivate the pRB (Retinoblastoma tumor suppressor protein) an important regulator of cell growth [2]. CR2 contains also a SS phosphorylation site believed to make E7 oncoprotein engage cell cycle mechanisms [3].

CR3 region is predicted to be structured due to the presence of two CxxC motifs separated by 29 residues known to form a zinc-binding domain. Only two analogous structures of just the CR3 domain of E7 protein are available on PDB. The X-ray structure of HPV1a E7 CR3 and solution structure HPV45 E7 CR3. Nuclear magnetic resonance (NMR) spectroscopy has been proven to be a useful tool to obtain atomic resolution information on IDPs [4].



Up) HPV16 E7 protein cartoon representing the CR1 and CR2 disordered regions and CR3 as ordered region. Motifs are highlighted in yellow, like LxCxE, SS CxxC and extra cysteines.
Left) HPV16 E7 amino acid content.
Bottom) Alignment of E7 proteins from HPV1a, 45 and 16.

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E7 HPV-45   MHGPRATLQEIVLHLEPQNELDPVDLLCYEQLSESEEENDEADGVSHAQLPARRAEPQR--HKILCVCCKCDGRIELTVESSADDLRTLQQLFLSTLSFVCPWCATNQ- 106
E7 HPV-16   MHGDTPTLHEYMLDLQP----ETTDLYCYEQLNDSSEEEDEIDG------PAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP- 98
E7 HPV-1a   MVGEMPALKDLVLQLEP--SVLDLDLYCYEEVPPDDIEEELVS----------PQQP-YAVVASCAYCEKLVRLTVLADHSAIRQLEELLLRSLNIVCPLCTLQRQ 93
            *.*. ..*.*.:* *:*   .. .**.***::.....*.:...      .. ...*.:  *  *     * * :.*.* :.. .:*.*:.*.: :*..***.*  ..
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45% homology 38%

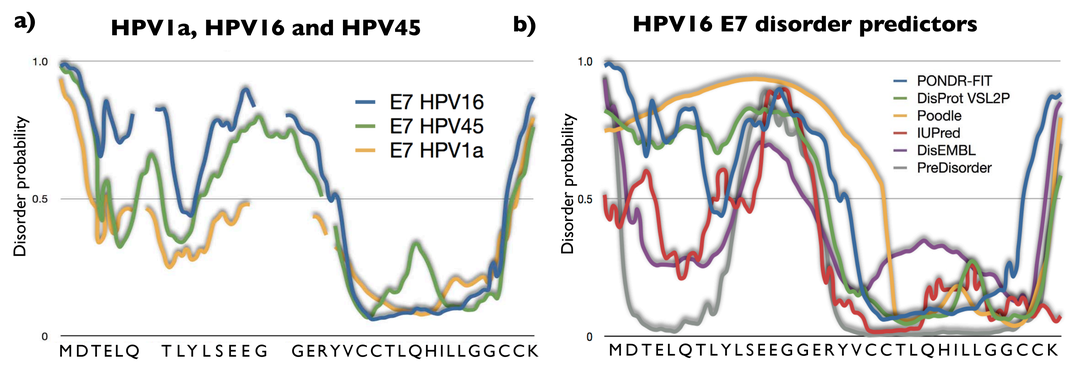
Objectives

We will present the expression, purification and characterization of the full-length HPV-16 E7 protein. Double-labeled (^{15}N, ^{13}C) samples suitable for multinuclear NMR characterization and the results from complementary spectroscopic techniques will be shown, providing a framework to understand the functional role of this protein.

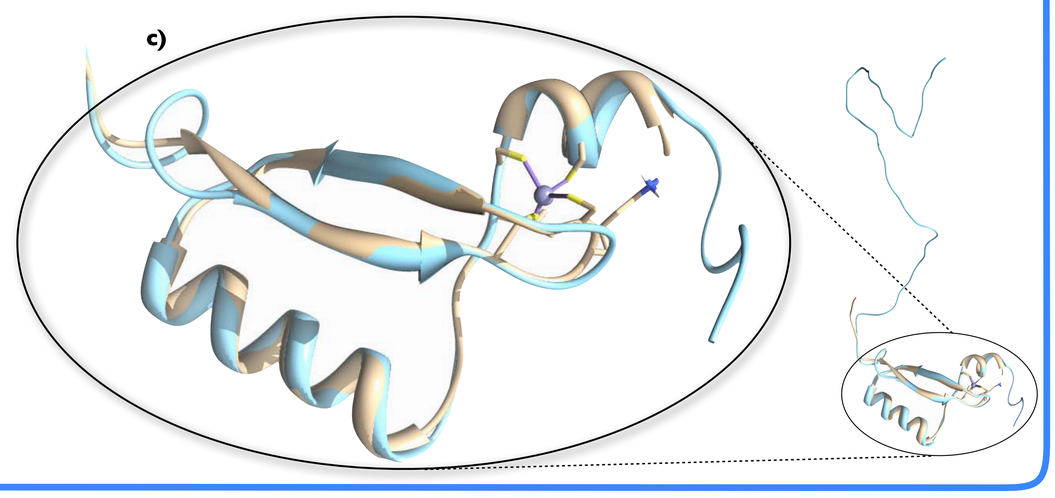
Materials and Methods

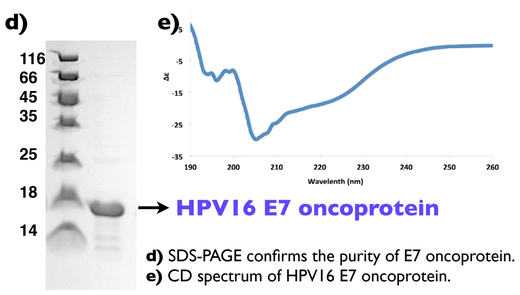
Bioinformatic analysis of E7 HPV16 was done to predict its order and disorder regions. The complete sequence of HPV16 E7 protein was modulated with MODELLER 9.10 using HPV45 E7 CR3 protein as template. Expression system was *E.coli* BL21/pLys transformed with pET20(b)+ containing the full length E7 HPV16 protein used for double labeling with ^{13}C and ^{15}N. Purification was done under anaerobic conditions and confirmed by SDS-PAGE. Structural and dynamics characterization was achieved by a combination of ^{1}H detected and ^{13}C detected NMR experiments. Complementary biophysical techniques were used like CD, MALDI, ESI, DLS and SAXS spectroscopies. Molecular weight, shape, radius of gyration were measured and α-helix and β-sheet were estimated.

Results and Discussion



a) PONDR-FIT disorder predictor of E7 proteins from HPV1a, HPV16 and HPV45.
b) E7 HPV16 protein analyzed by different disorder predictors PONDR-FIT, Disprot VSL2P, Poodle, IUPred, DisEMBL and PreDisorder.
c) Modeling of HPV16 E7 (blue) protein based on HPV45 E7 CR3 region (brown); CR3 region is zoomed for better comparison.





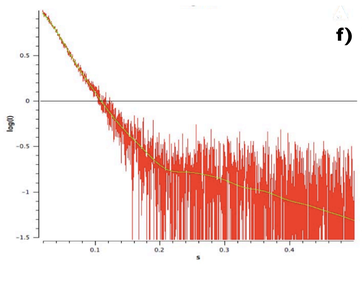
d) SDS-PAGE confirms the purity of E7 oncoprotein.
e) CD spectrum of HPV16 E7 oncoprotein.

HPV16 E7 secondary structure contents

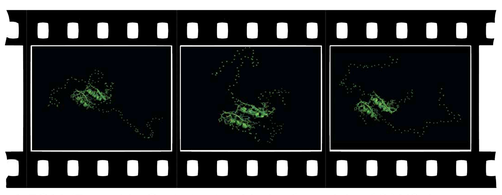
E7 protein	α-helix	β-sheet	loop
E7 HPV16 modeled with E7 HPV1a CR3	13.21%	14.15%	72.64%
E7 HPV16 modeled with E7 HPV45 CR3	21.7%	14.15%	64.15%
E7 HPV16 CD data	51.03%	8.95%	40.02%

HPV16 E7 protein molecular weight

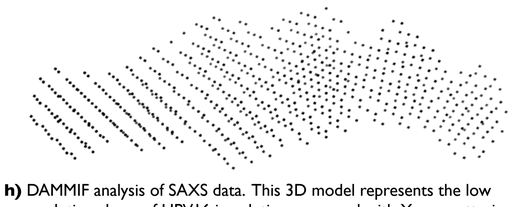
Theoretic - ExPASy	12152 Da
ESI	12149 Da
MALDI	12219 Da
DLS	24 ± 6 kDa
SAXS	30 ± 4 kDa



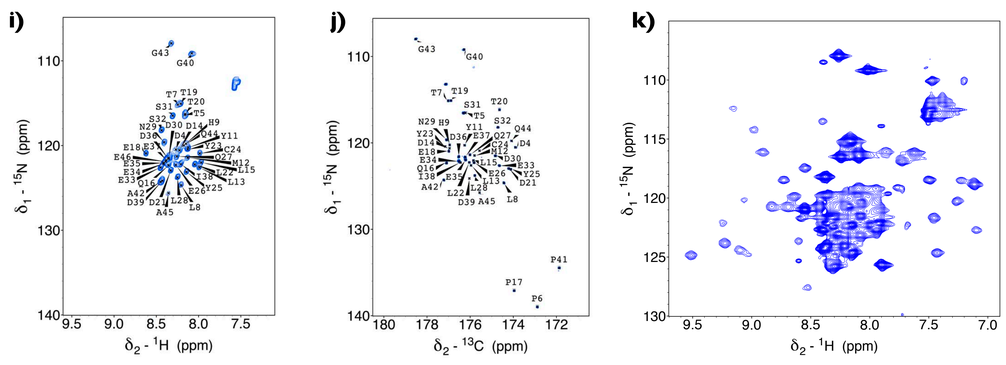
f) X-ray scattering; (red) experimental data with error bars; (green) computed scattering from models with a typical fit by the selected ensemble of structures; The logarithm of the scattering intensity is plotted against the momentum transfer.



g) EOM (Ensemble Optimization Method) models of SAXS data using the homologous HPV1a structured part.



h) DAMMIF analysis of SAXS data. This 3D model represents the low resolution shape of HPV16 in solution measured with X-ray scattering.



i) Assigned ^{1}H-^{15}N SFHMQC and **j)** ^{13}C-^{15}N CON-IPAP spectra of HPV16 E7 oncoprotein.
k) SFHMQC spectrum of HPV16 E7 oncoprotein showing both order and disorder regions. The signals from structured CR3 region appeared in the ^{1}H-^{15}N SFHMQC spectra as the result of improved expression protocol.

Conclusions and Perspectives

- The HPV16 E7 oncoprotein complete construct has been analyzed for the first time.
- CD and SAXS showed that the E7 oncoprotein contains folded and unfolded regions. SAXS showed a shape similar to a dimer, with high tendency to form aggregates when increasing concentrations and temperatures. SAXS and DLS also showed high tendency for aggregates formation.
- The chemical shift index analysis suggests slight tendency of the region around LxCxE short linear motif to adopt β-strand conformation, in accordance with the observation that LxCxE SLiM forms extended β-strand conformation upon binding to Retinoblastoma protein [2].
- The E7 oncoprotein is believed to follow disordered to ordered transitions. Interaction with its physiologic partner pRB will enable us to understand how this interaction occurs.
- The phosphorylation site present in HPV16 E7 oncoprotein residues S31 and S32 can be phosphorylated by KII [3] and the dynamics and structures properties of this transition can be followed by NMR [4].

References

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