Structural Studies on papillomavirus oncoproteins

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Papillomavirus and cancer

mammals, birds & reptiles

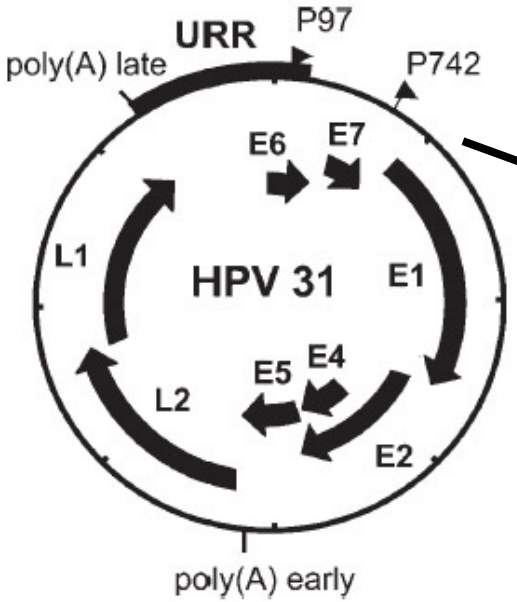
orogenital and cutaneous epithelia

> 200 PV strains, > 100 HPVs

80% → low risk: **warts and lesions**

20% → high risk: skin cancers, **cervical cancer**

Very small genome (8kb)

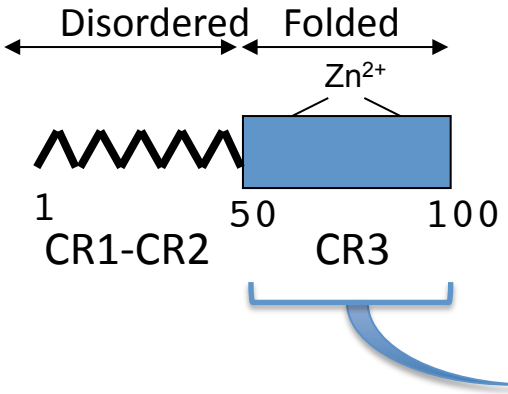


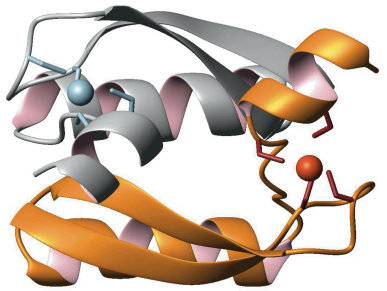
E6 & E7: the oncoproteins of HPV
-> Cell proliferation priming

- E6 and E7 bind / degrade MANY cellular proteins (>100)
- E6 and E7 hijack cellular ubiquitin ligases (E6AP, cullin 2...)
- E7 alters cell cycle checkpoints (Rb proteins, cyclins…)
- E6 alters apoptosis control (p53, Bak…)
- E6 alters cell adhesion pathways (PDZ-containing proteins)

HPV E6 and E7 oncoproteins: Structural information remains scarce

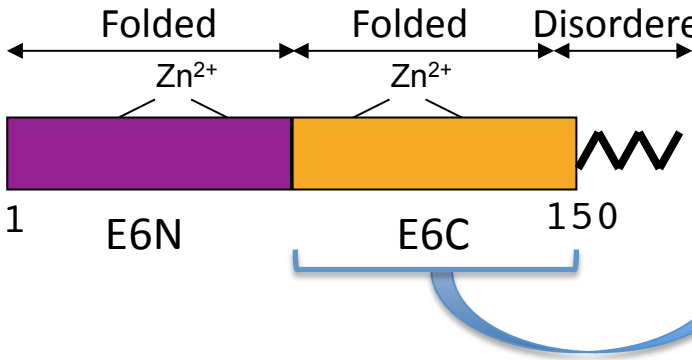
E7 (mammalian PVs) :

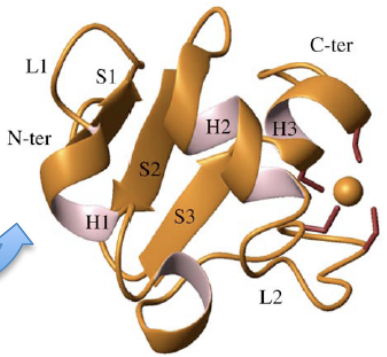




HPV1 E7 CR3 (Liu et al., JBC 2006)
HPV 45 E7 CR3 (Ohlenschlaeger et al. 2006)

E6 (mammalian PVs) :





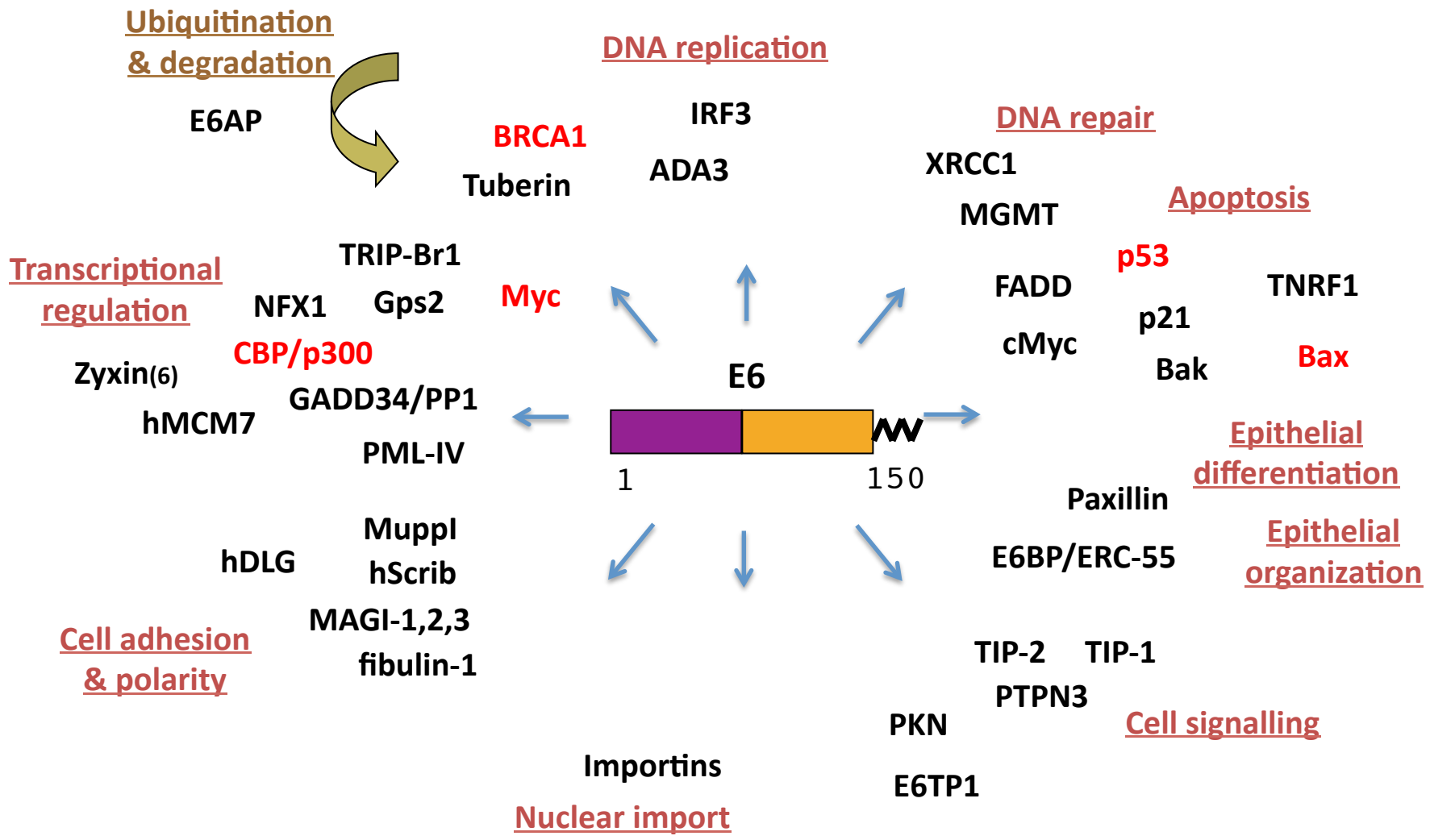
HPV 16 E6C (Nominé et al. 2006)

-Structure of a full-length E6 ? Still unknown (expected since ≈1985).

-Origin of E6C and E7 CR3 folds ? These folds are not observed in any other living organism .

-Structures of target-bound E6 and E7 ? Needed for design of small molecule inhibitors of HPV

HPV 16 E6: > 50 cellular targets

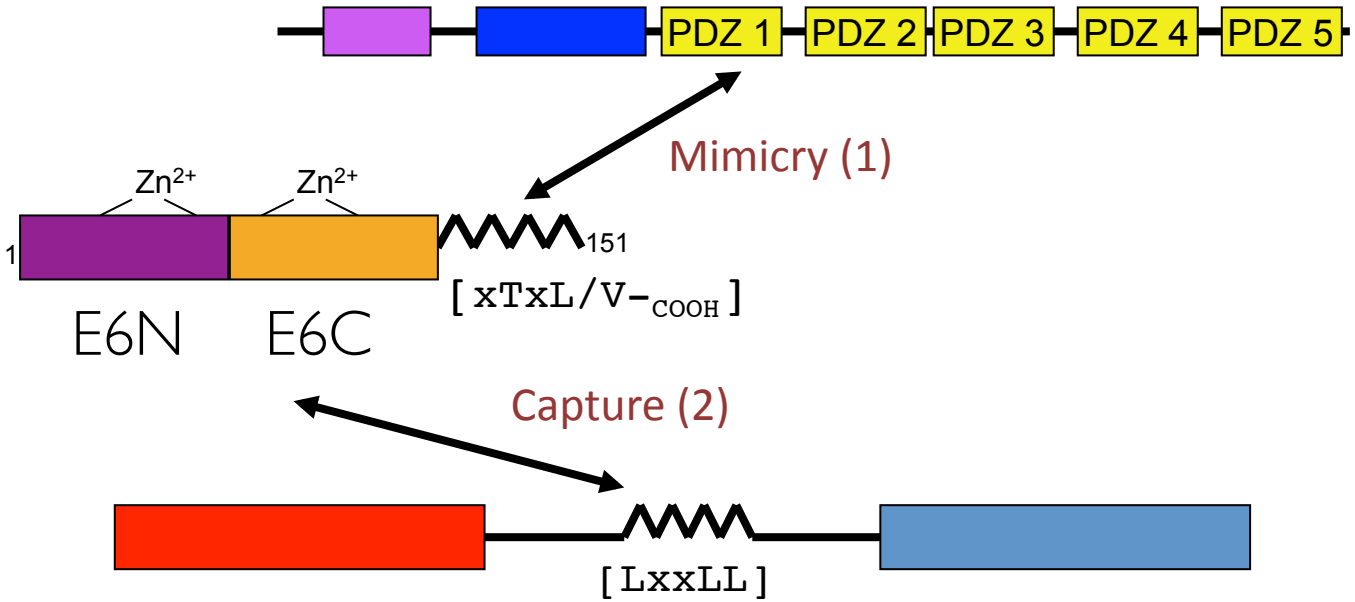


How does such a small protein as E6 interact with so many targets ?

→ Molecular and structural basis for E6 multifunctionality ?

A molecular explanation for E6 multifunctionality: Viral hijacking of domain-motif interactions

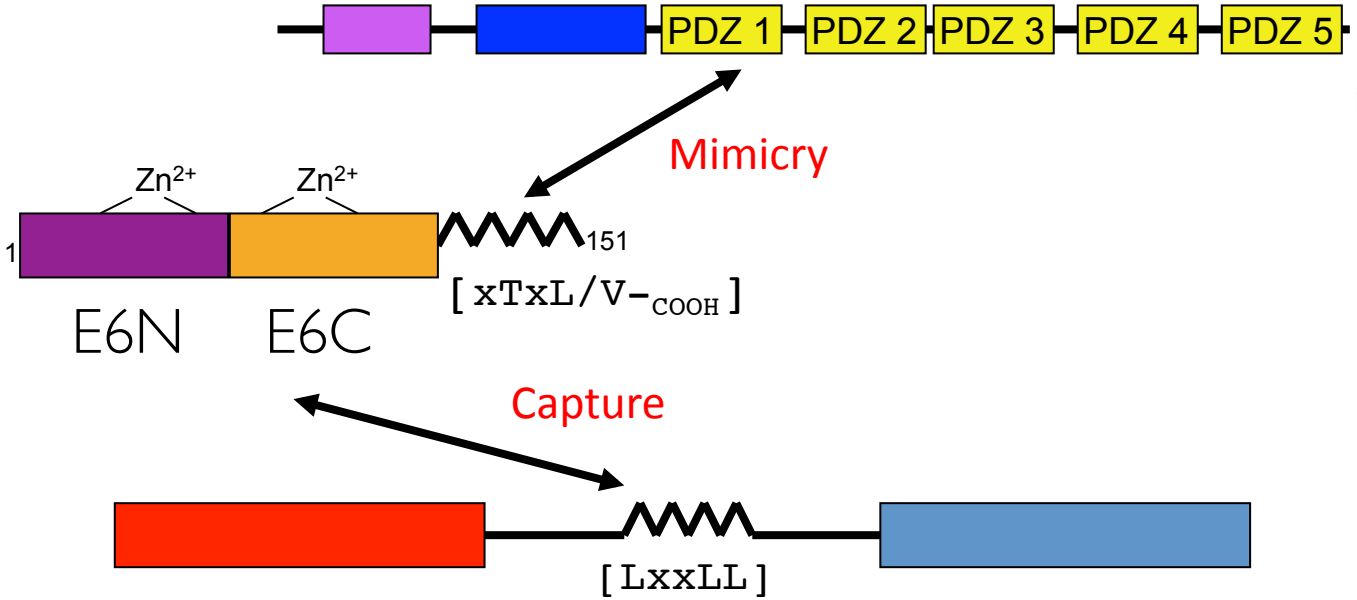
(1) C-term of E6 *mimics* a PDZ-binding motif to « hook » cellular PDZ domains
PDZs are involved in cell communication, adhesion, signalling



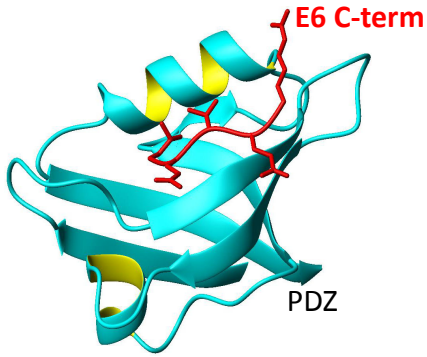
(2) Folded part of E6 *captures* cellular acidic LxxLL motifs
LxxLL frequent in proteins controlling cell proliferation, adhesion, & apoptosis
(p53, Ub-ligase E6AP, focal adhesion protein Paxillin…)

-Motif hijacking concepts are original IP of Toby Gibson (EMBL, Heidelberg) !
-for a review, see « How viruses hijack cell regulation », Davey et al. TIBS 2010

Current structural knowledge on E6 hijacking complexes ?



Several E6-PDZ structures (*)

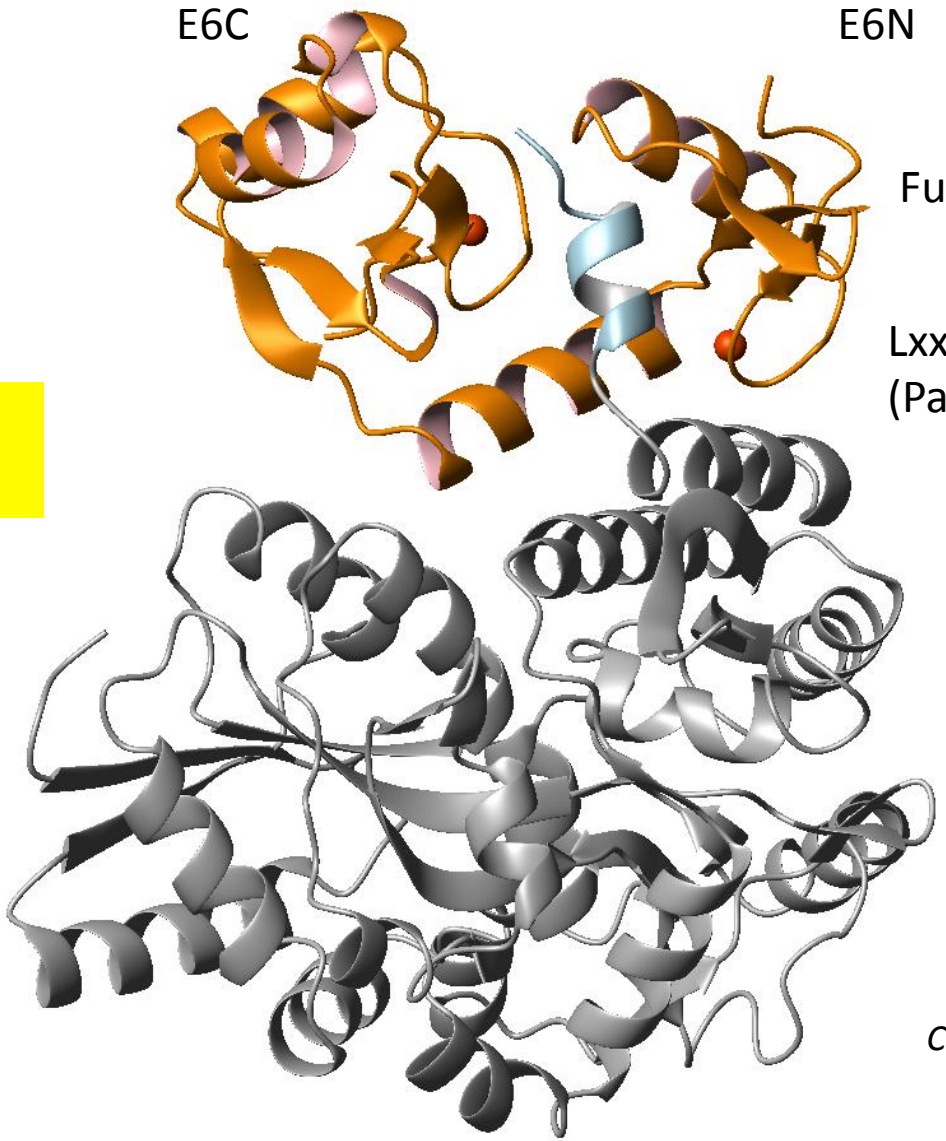


No E6-LxxLL structure

(*) Zhang et al, 2007: E6- MAGI1 PDZ1, E6-hDLG PDZ2, E6-hDLG PDZ3 (X-ray)
(*) Liu et al., 2007: E6-hDLG PDZ2 (NMR)
(*) Charbonnier et al., 2011: E6- MAGI1 PDZ1 (NMR)

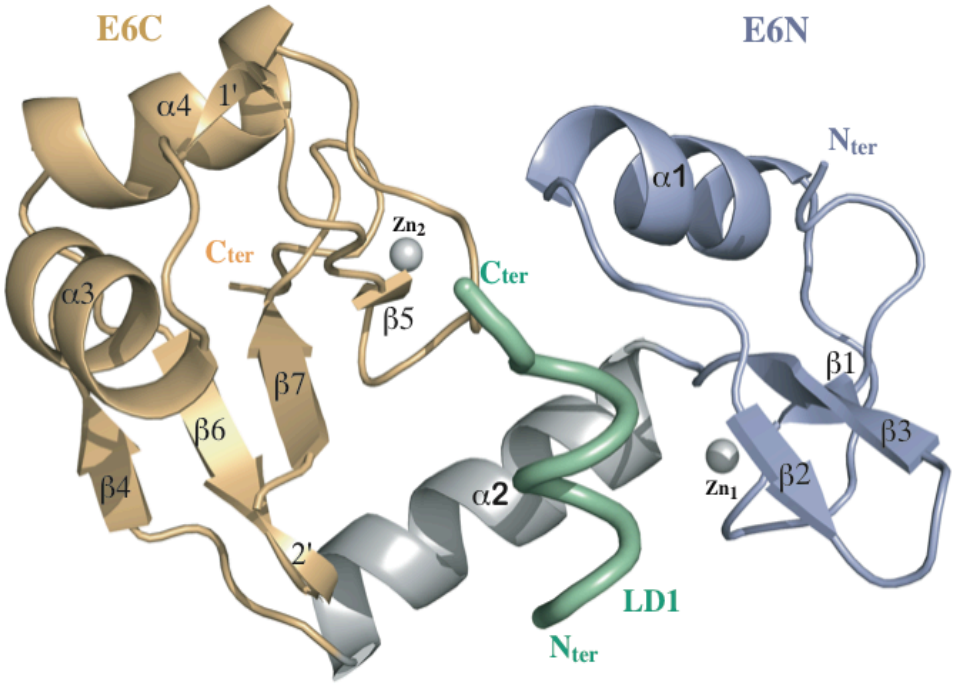
Recombinant E6 proteins misfold and/or aggregate:
Successfull crystallisation of a triple MBP-LxxLL-E6 fusion

MBP-LxxLL-BPV E6 fusion



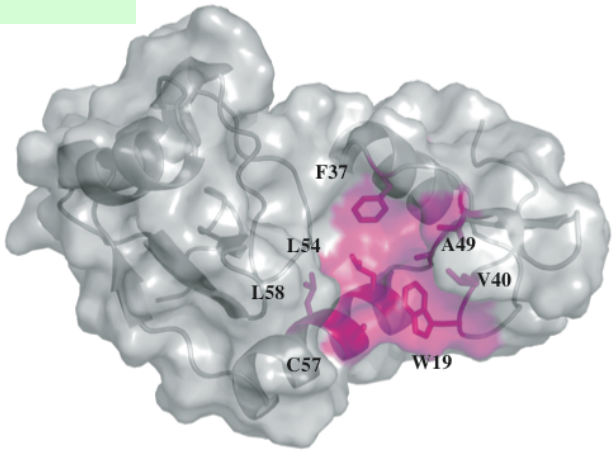
Charbonnier et al., unpublished

X-ray Structure of full-length BPV E6 bound to paxillin LxxLL motif

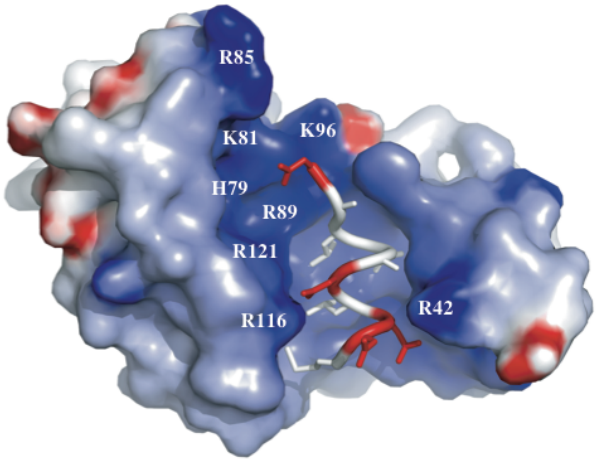


The **first structure of full-length E6**:
Two zinc domains separated by a linker helix

The prototype of **E6-LxxLL motif recognition**:
-> Helical LxxLL motif grabbed by the two domains
-> A plausibly **druggable** viral pocket !



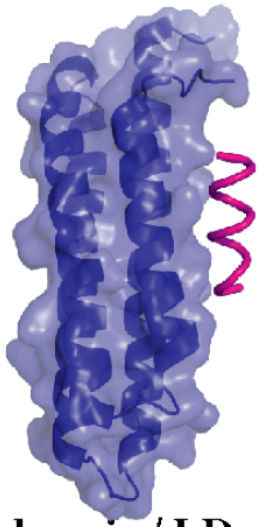
hydrophobic pocket
docks the three conserved Leucines



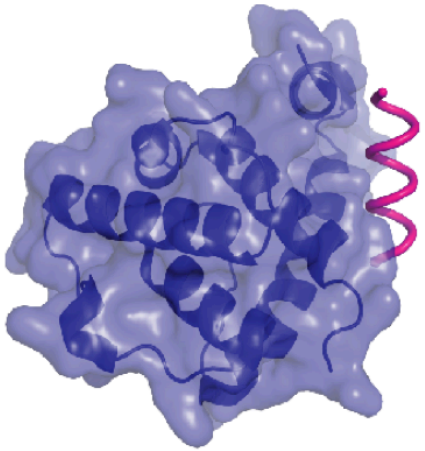
positively charged (blue) surface
explain the preference for acidic LxxLL motifs

Comparing E6-LxxLL complex to cellular domain-LxxLL complexes

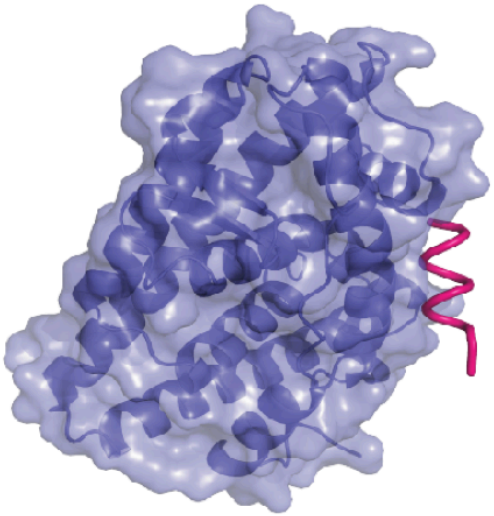
-Cellular domains bind LxxLL through weak surface interactions
Kd 10^{-5} M (transient, for signalling)



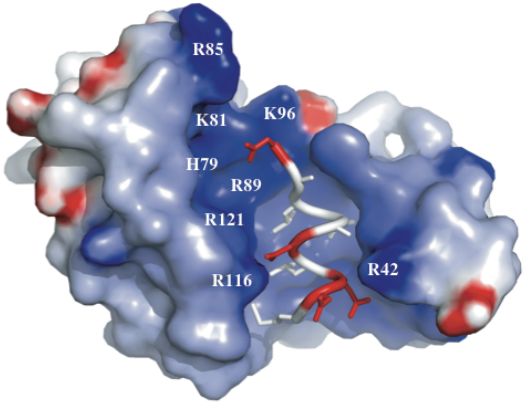
FAT domain / LD complex



CH domain / LD complex



LBD / LxxLL complex



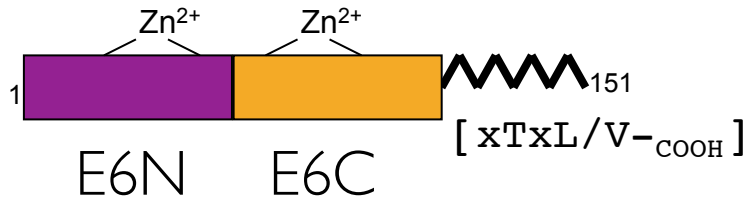
E6 / LxxLL complex

-Viral E6 grasps LxxLL motif inside a rather deep pocket
Kd 10^{-7} M (tight)

→ E6 may out-compete efficiently the cellular LxxLL binders

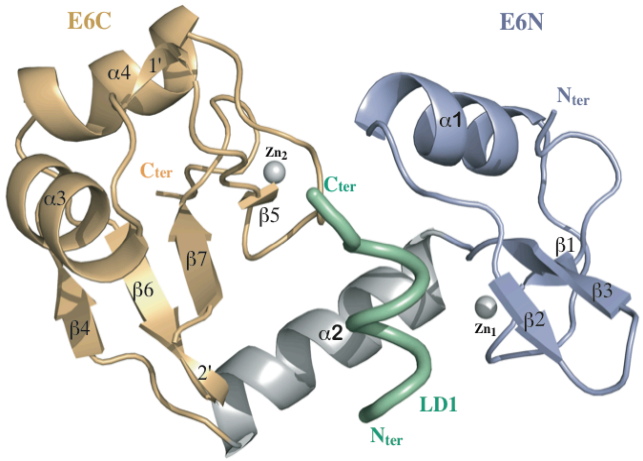
E6-LxxLL

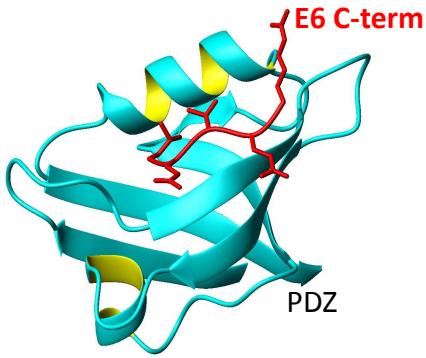
Can we predict the interactome of E6 oncoprotein
using structure and binding selectivity knowledge
on E6-motif and E6-domain complexes ?



Capture

Mimicry





Prediction of E6 targets
containing LxxLL motifs ?

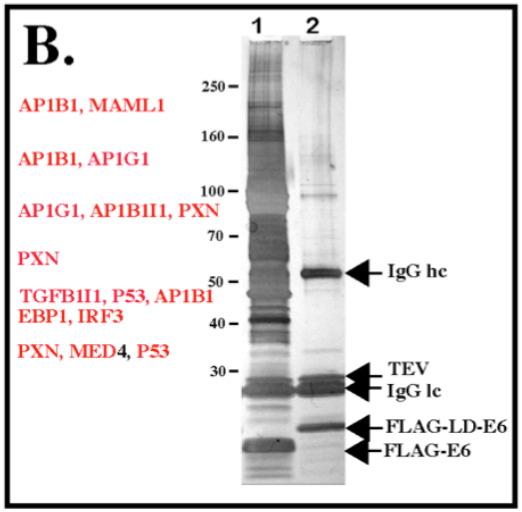
Prediction of E6 targets
containing PDZ domains ?

Bioinformatic search of E6-binding LxxLL proteins is surprisingly accurate !

I/ Fine analysis of E6-LxxLL preferences:
-Structural analysis of key contacts
-Systematic mutagenesis
-Phage Display selections

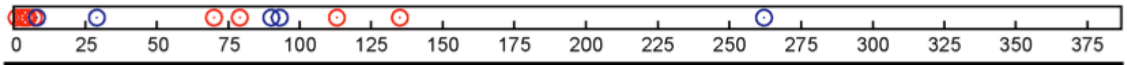
II/ Bioinformatic search on full proteome:
-Regular expression search
-PSSM-based ranking
-search only presumably unfolded regions

III/ Experimental search on cell extracts:
-pull down with double-tagged E6
-mass spec analysis of binders



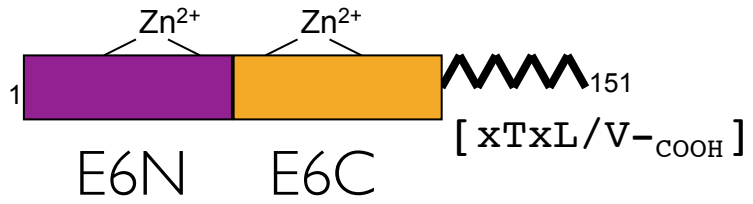
Luck et al., unpublished

A. Distribution of hits from proteomic screen in list of predicted binders



n° prediction (out of 387)	target protein	motif-containing sequence	localization in sequence	net charge	LD-motif	regulation of transcription	Epithelium	cytoskeleton
1	paxillin (PXN_1)	MDDLDALLAD LESTT	1-11	-4	yes		x	x
2	ninein	QKRLS WDKLDHLMNE EQQLL	1838-1848	-1	no			x
3	TGFβ1 induced transcript 1 (TGFB1I1_1)	MEDLDALLSD LETTT	1-11	-4	yes	x		x
4	mediator complex subunit 13 (MED13)	DLAVS YTDLDNLFNS DEDEL	778-788	-2	no	x	x	
5	tumor protein p53 (P53)	LSQET FSDLWKLLPE NNVLS	19-29	-1	no	x		
7	mastermind-like 1 (MAML1)	TSEEW MSDLDDLLGS Q	1006-1016	-3	no	x		
8	TGFβ1 induced transcript 1 (TGFB1I1_2)	VLGTG LCELDRLLQE LNATQ	73-83	-2	yes	x		x
9	amyloid beta (A4) precursor protein-binding. family B. member 1 interacting protein	LNALE DQDLDALMAD LVADI	61-71	-4	yes			x
14	meningioma 1	KSAMS TIDLDSLMAE HSAAW	1213-1223	-3	no		x	
19	Decidual protein induced by progesterone	PMADT VDPLDWLFGE SQEKQ	102-112	-3	no			
20	golgi-associated gamma adaptin ear containing ARF binding protein 3	SALHH LDALDQLLEE AKVTS	516-526	-4	no			
29	paxillin (PXN_2)	SLGSN LSELDRLLLE LNAVQ	142-152	-2	yes		x	x
43	kinesin light chain 2	KGDVP KDTLDDLFPN EDEQS	161-171	-2	no		x	x
44	spindlin family. member 2B	ITQWK GTVLDQLLDD YKEGD	70-80	-3	no			
58	consortin. connexin sorting protein	CGNNQ ISDLGILLPE VCMAP	516-526	-2	no			
64	protocadherin 10	HSTLE RKELDGLLTN TRAPY	1015-1025	0	no			
70	adaptor-related protein complex 1. beta 1 subunit (AP1B1)	AVDLL GGGLDSLMGD EPEGI	660-670	-2	no			
79	interferon regulatory factor 3 (IRF3)	TSDTQ EDILDELLGN MVLAP	137-147	-4	no	x		
90	paxillin (PXN_4)	SASSA TRELDELMAS LSDFK	263-273	-2	yes		x	x
93	TGFβ1 induced transcript 1 (TGFB1I1_3)	SATSA TLELDRLMAS LSDFR	136-146	-1	yes	x		x
95	clathrin interactor 1	PAASN SSDLFDLMGS SQATM	402-412	-2	no		x	
97	eukaryotic translation initiation factor 4E nuclear import factor 1	QKA KVDLKPLLSS LSANK	4-14	1	no			
105	centrobin. centrosomal BRCA2 interacting protein	QQVAE DYELRLLLLD PPAPG	520-530	-2	no			x
113	adaptor-related protein complex 1. gamma 1 subunit (AP1G1_1)	KPSSA GGELLDLLGD INLTG	651-661	-3	no			
135	proliferation-associated 2G4 (EBP1)	EMEVQ DAELKALLQS SASRK	349-359	-1	no	x	x	
262	adaptor-related protein complex 1. gamma 1 subunit (AP1G1_2)	QPTSQ ANDLLDLLGG NDITP	626-636	-2	no			

Prediction of cellular targets of E6 protein based on E6-LxxLL or E6-PDZ binding selectivity information ?



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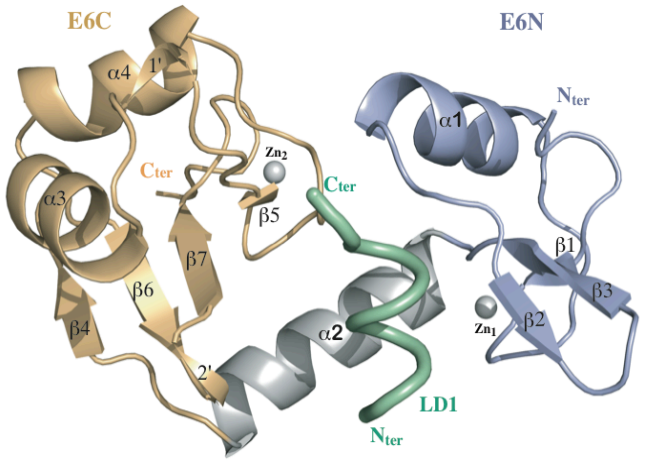
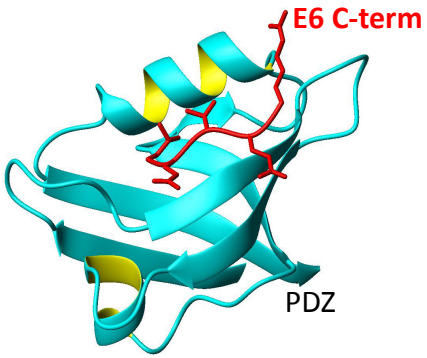




Prediction of E6 targets containing LxxLL motifs ?

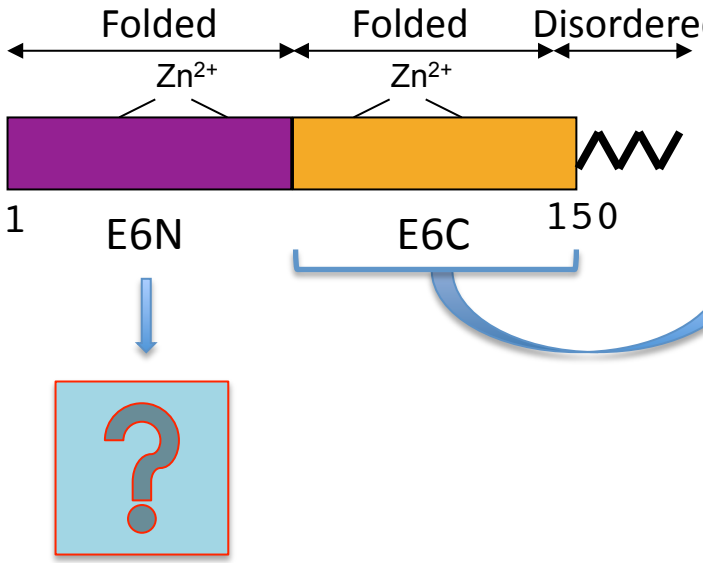
→ Quite accurate !

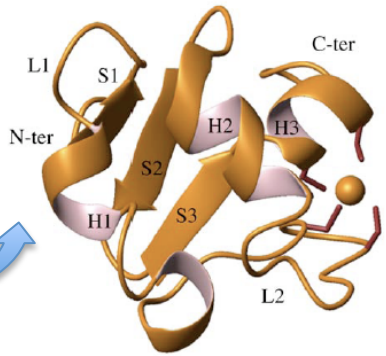
Prediction of E6 targets containing PDZ domains ?

→ In progress.

HPV16 E6 oncoprotein (*):
Structural information on full-length protein ?

E6 (HPV16) :





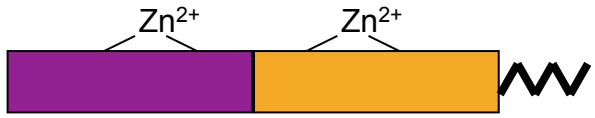
HPV 16 E6C (Nominé et al. 2006)

(*) HPV16 is the highest risk type, responsible for 60-70% of cervical cancers

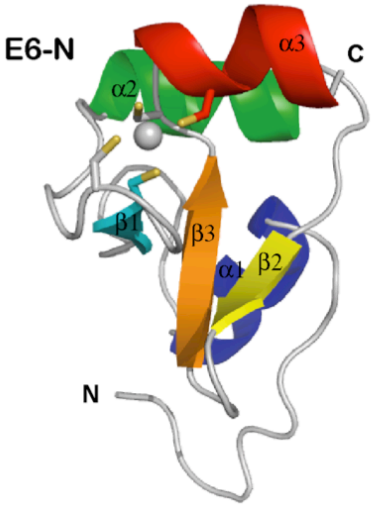
HPV16 E6 structure

Surface mutagenesis to prevent aggregation:
-> NMR structures of HPV 16 E6N and E6C domains

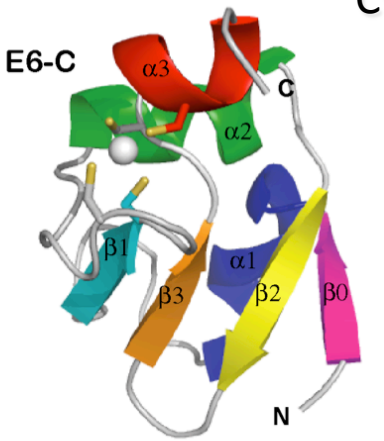
F47R mutation prevents E6N dimerization



E6-N F47R



E6-C 4C/4S



4C4S mutation prevents Cys-driven aggregation

Nominé et al. (Mol Cell 2006)

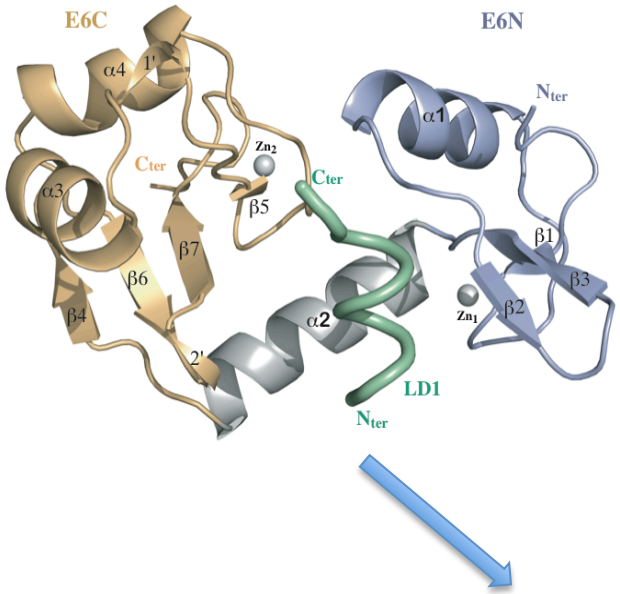
-> A first structural view of full-length unbound HPV16 E6 protein

Katia Zanier et al., (submitted)

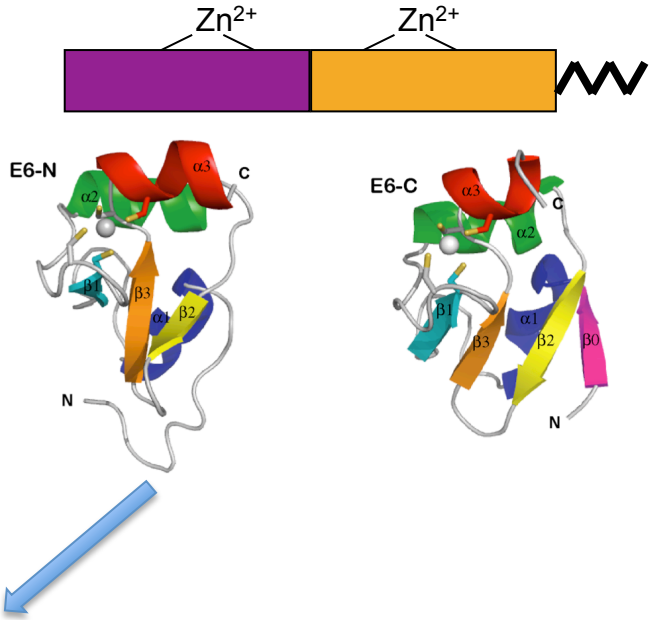
HPV16 E6 structure

Towards full-length structure of HPV 16 E6 bound to E6AP LxxLL motif (the cervical cancer-causing complex)

BPV1 E6 + LxxLL of paxillin



HPV16 E6N and E6C (free)



HPV16 E6 + LxxLL of E6AP

Structure of HPV16 E6 bound to the LxxLL motif of ubiquitine ligase E6AP:
The complex required for degradation of tumour p53 by oncogenic HPVs
A druggable viral pocket to design therapeutic molecules against cervical cancer

Conclusion

Novel structural data on PV E6 oncoprotein
Increase our understanding and open new perspectives

Main findings:

-Two viral strategies for hijacking of cellular networks:
  -mimicking cellular motifs to capture domains (e.g. E6-PDZ interactions) (frequent)
  -capturing cellular motifs (e.g. E6-LxxLL interactions) (rare)

-E6 tightly grasps acidic LxxLL motifs through a hydrophobic pocket
  flanked with positive charges: a druggable viral pocket

-Fine structural & molecular analysis of E6-LxxLL recognition specificities
  provided remarkably accurate predictions of cellular targets of E6

Perspectives:

-Solve the structure of HPV16 E6 – E6AP LxxLL complex (on the way)

-Predict and validate LxxLL-containing cellular proteins targeted by HPV E6 proteins
  from HPV strains (i.e. high-risk, low-risk, mucosal, cutaneous…)

-Design drugs or recombinant inhibitors of the E6-E6AP interaction responsible for p53
  destruction in HPV positive tumor cells

Thanks !