



Eduardo Calçada

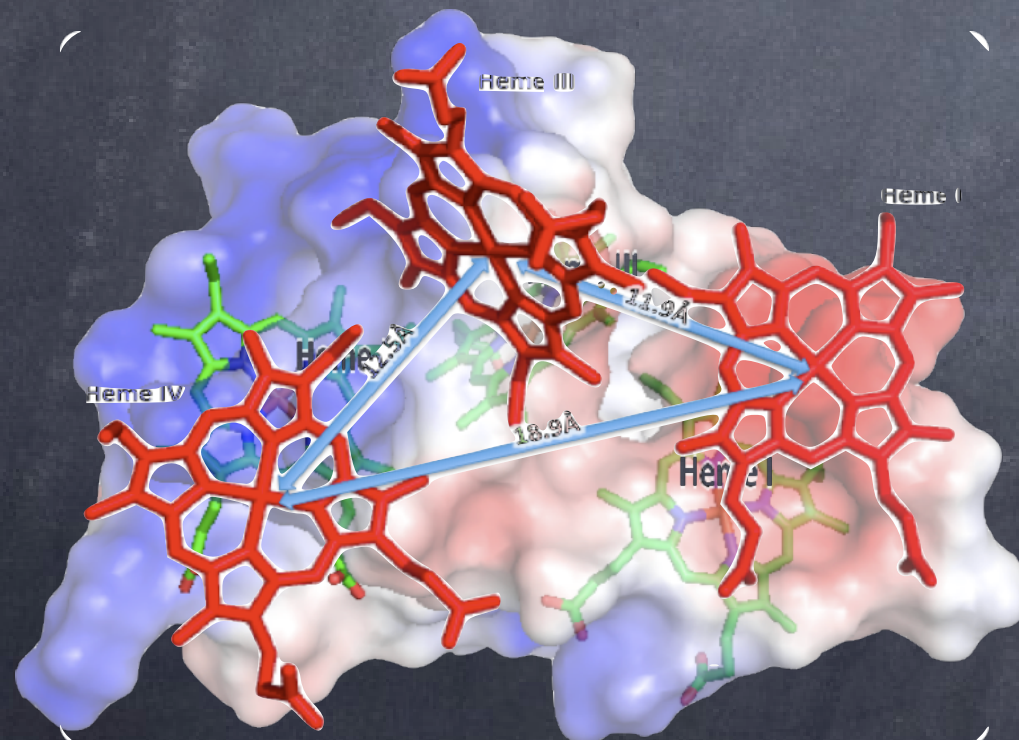
IDPbyNMR
CERM, Italy

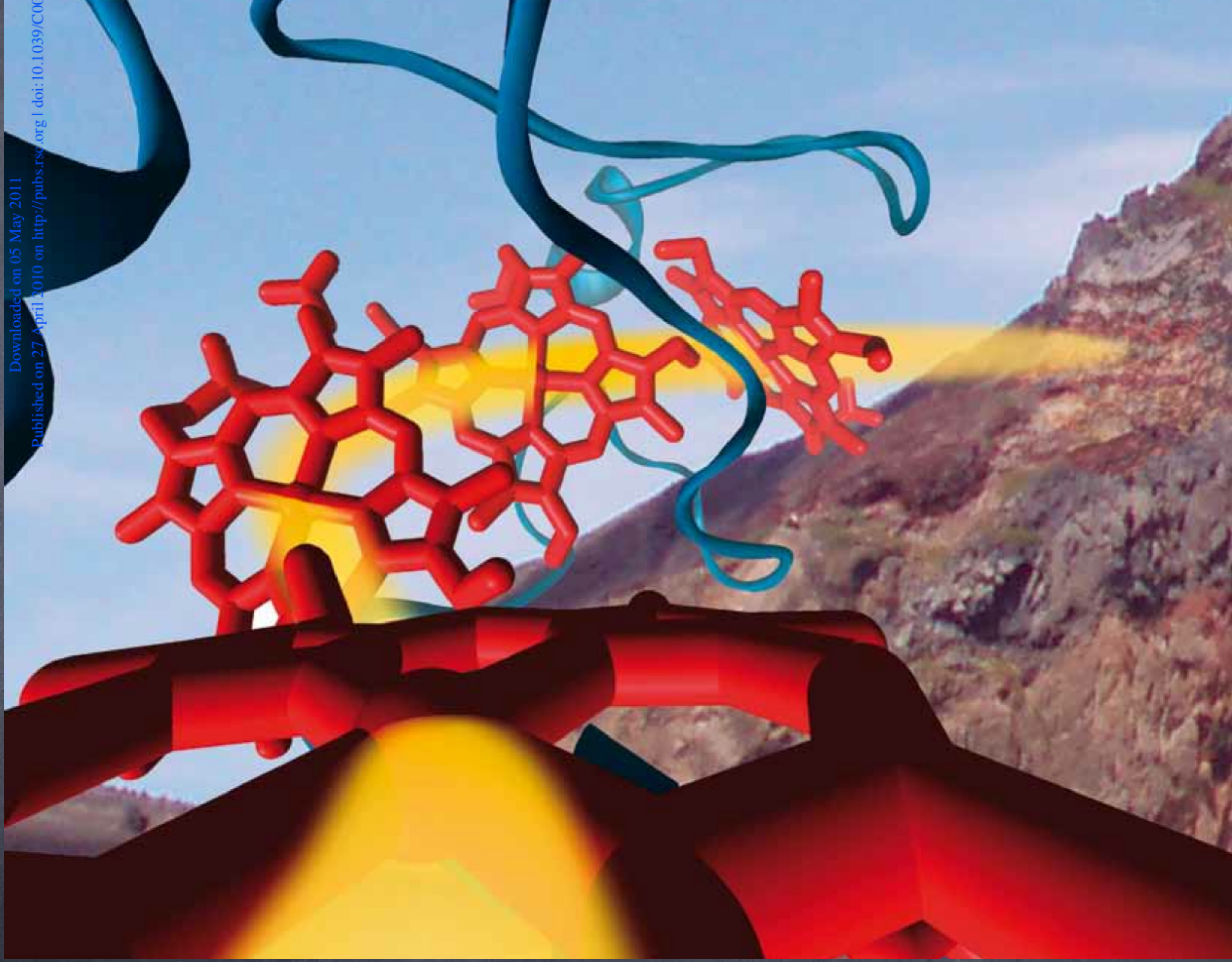


ITQB, Oeiras, Portugal

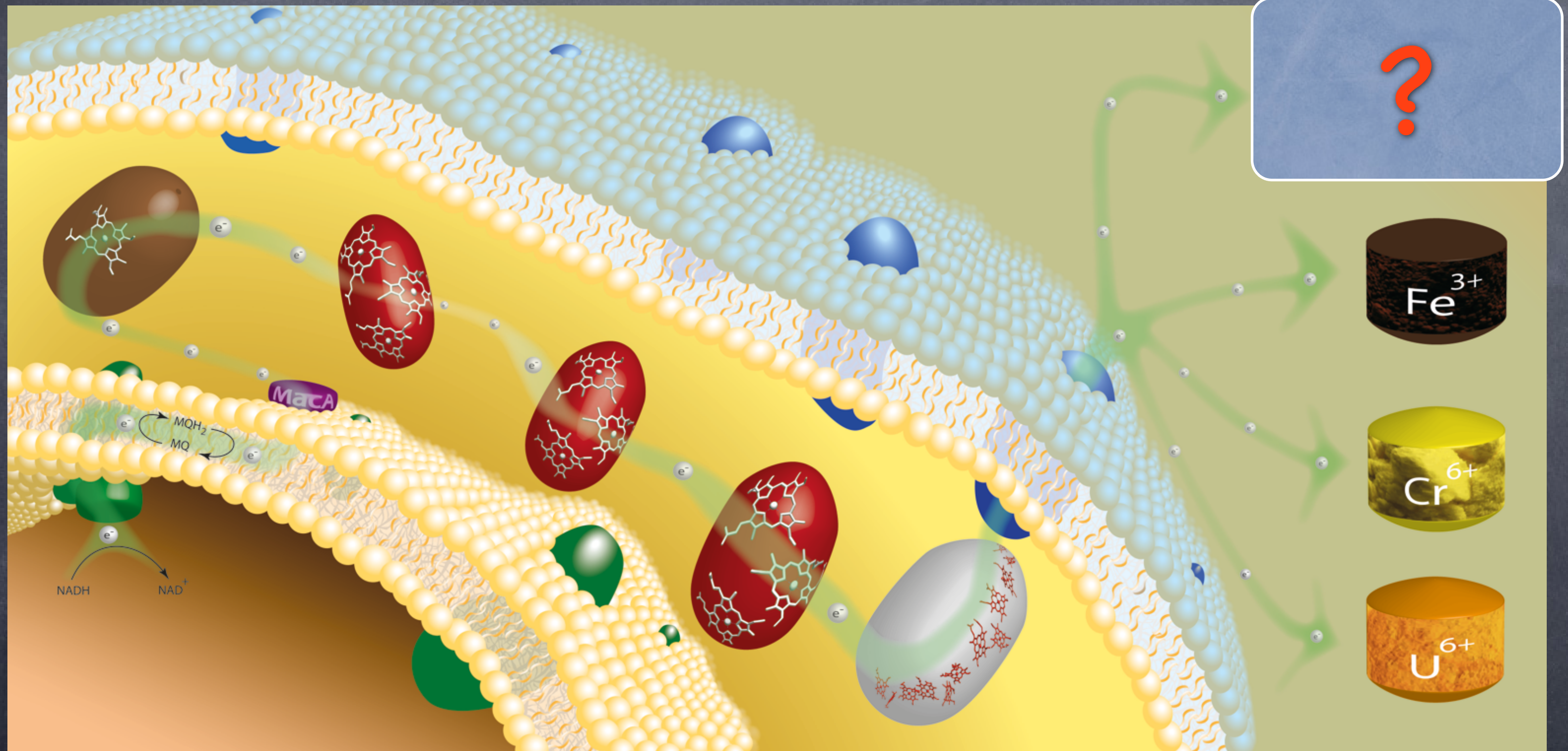
Dr. Ricardo O. Louro

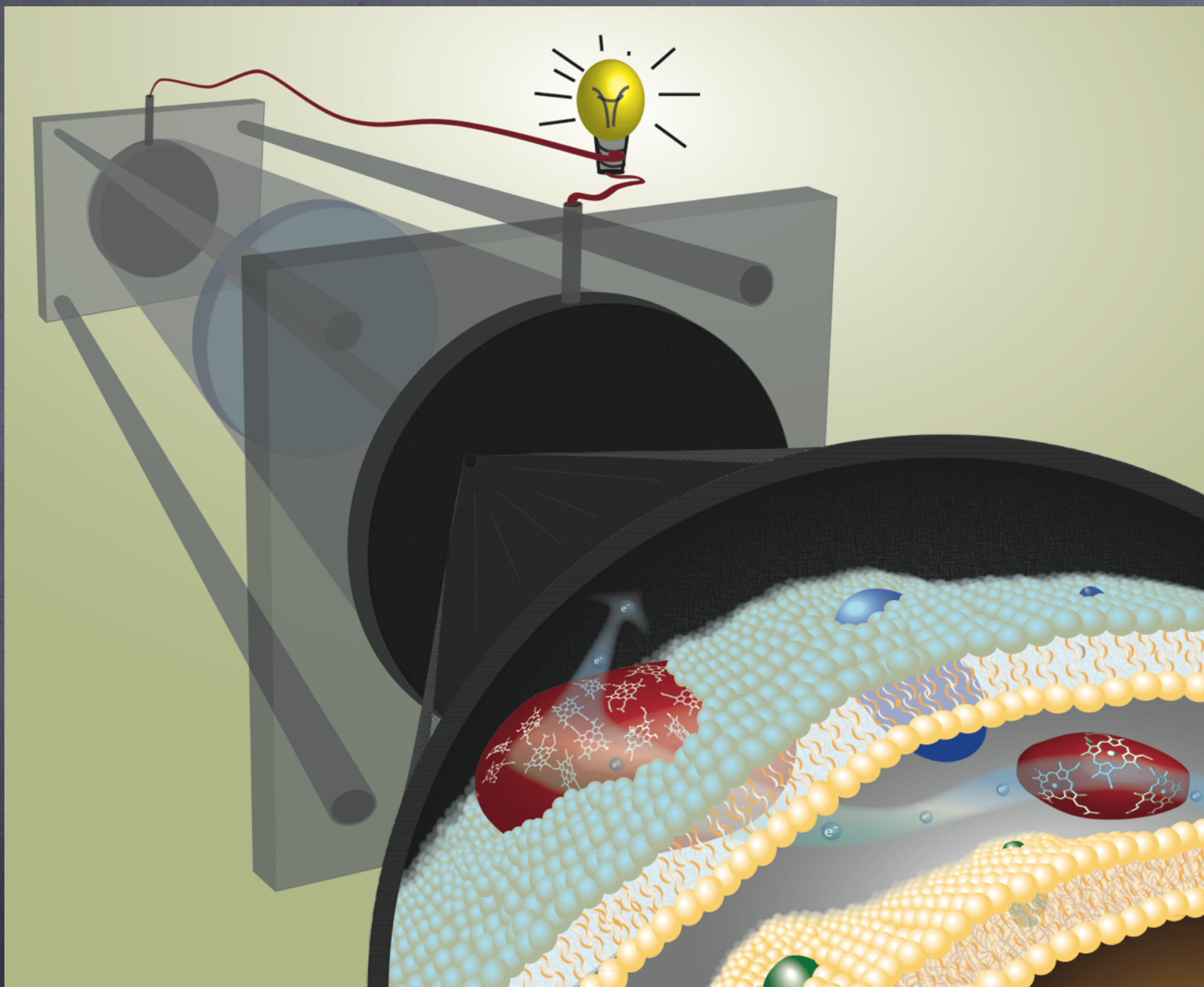
- cytochromes
- electron transfer mechanisms
- NMR and Stopped-flow





Respiratory chain







IDPs by NMR

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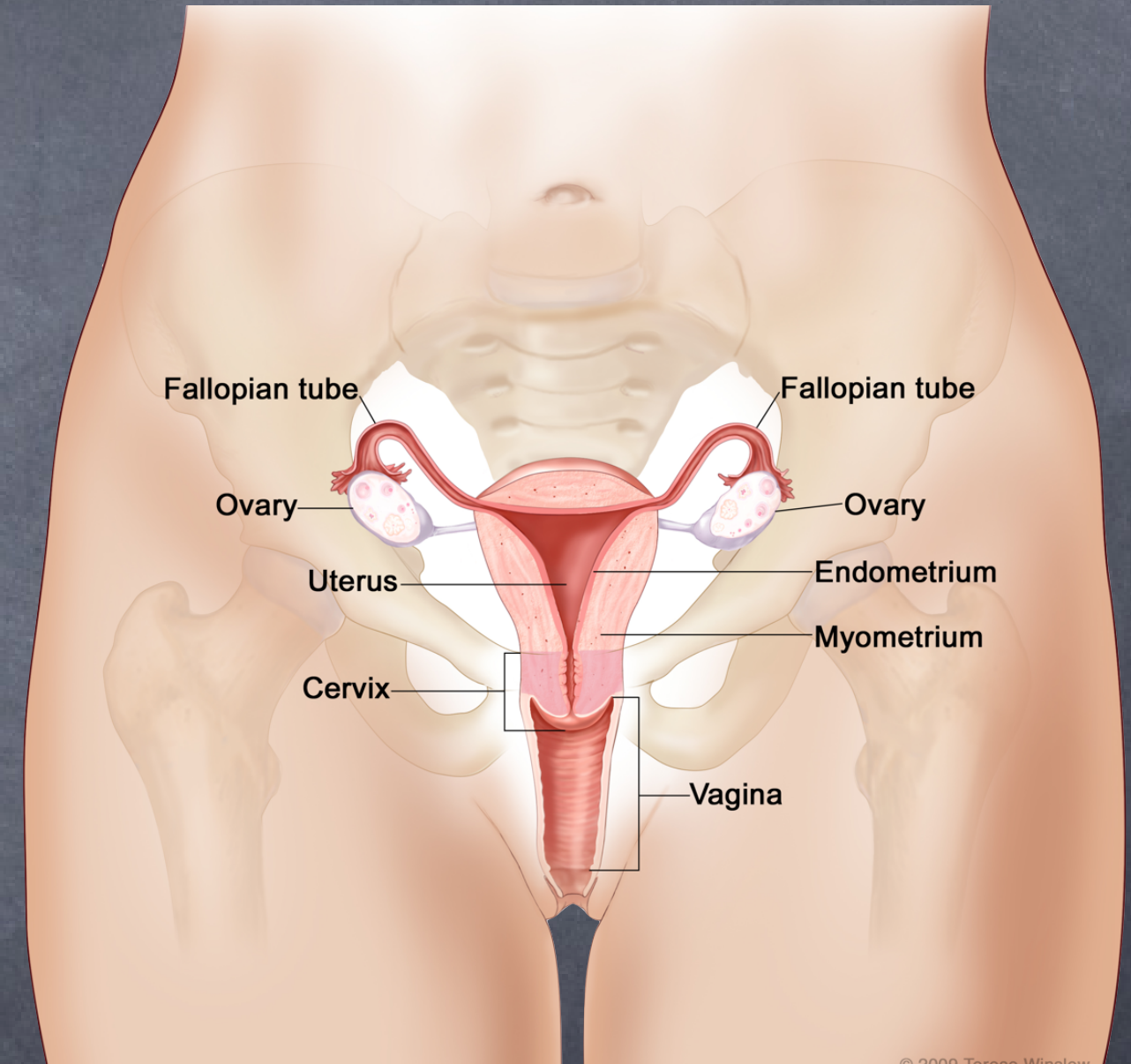


IDPs & Oncoproteins

- viral oncoproteins
- cancer development
 - Human papilloma virus
 - Cervical Cancer

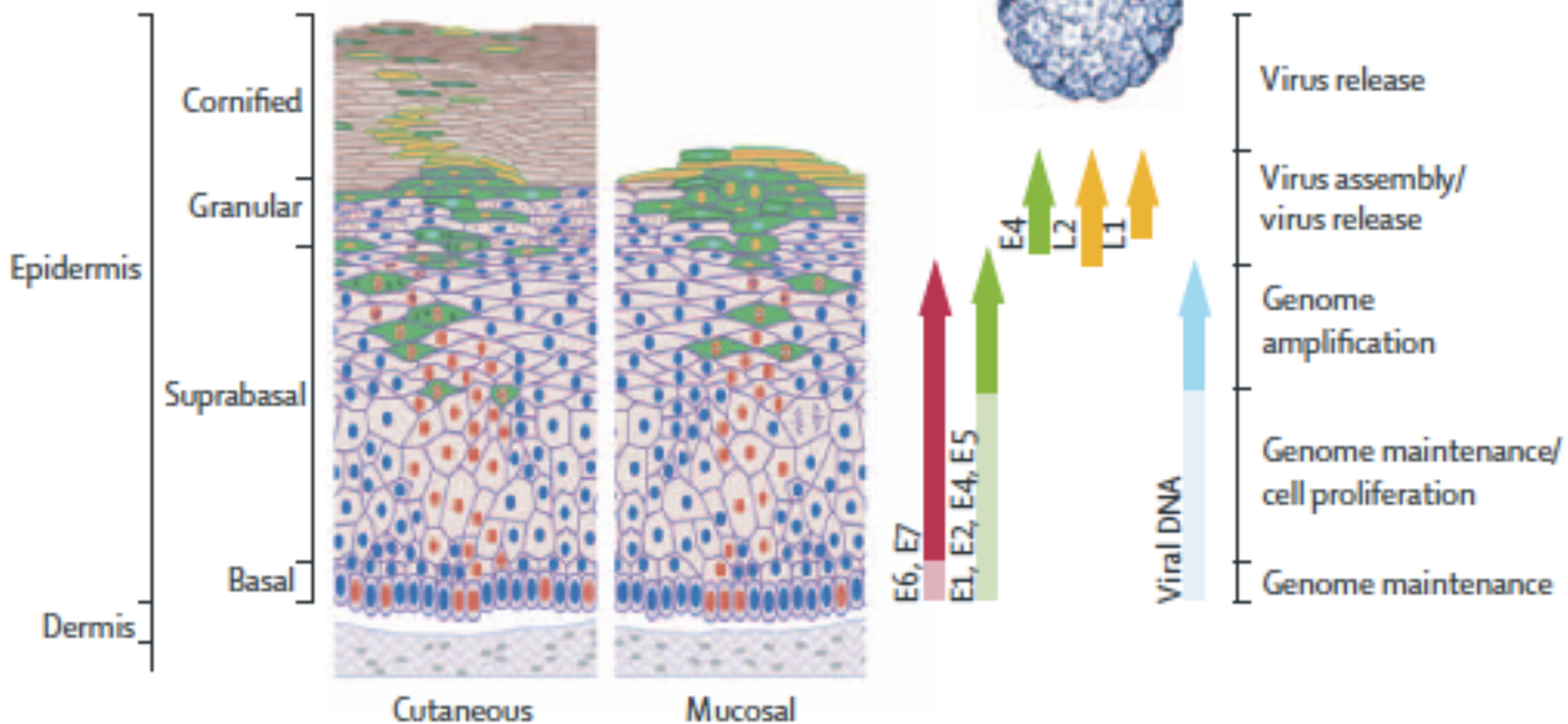
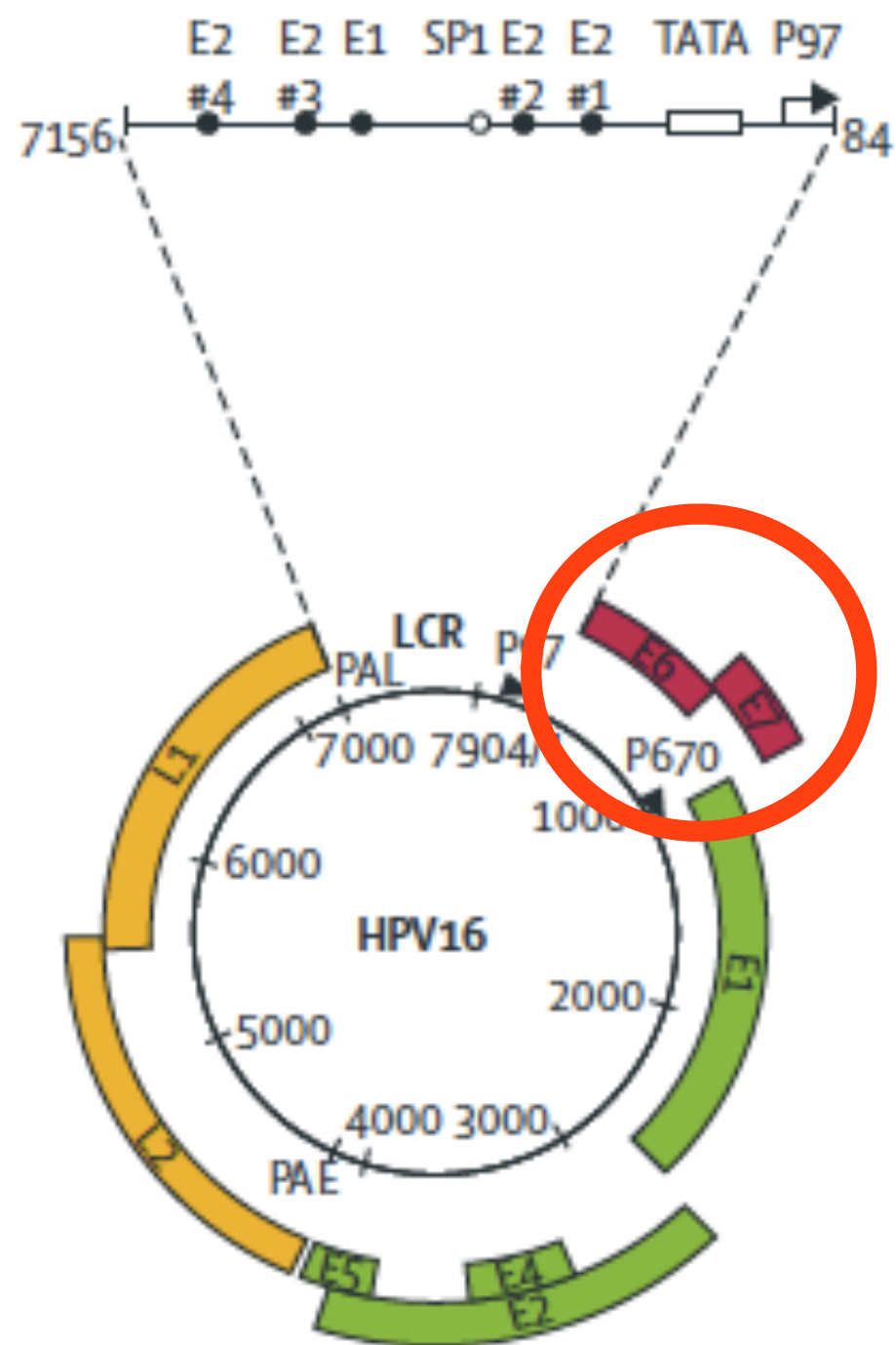
Human papilloma virus

- papilloma = small wart
- HPV attack in cervix
 - stratified squamous epithelium
- HPV family $\approx$ 200 viral types
- high-risk oncogenic (HPV16, HPV18, HPV45)





Human papilloma virus



Schiffman, M. et al., 2007. ScienceDirect - The Lancet : Human papillomavirus and cervical cancer. The Lancet.

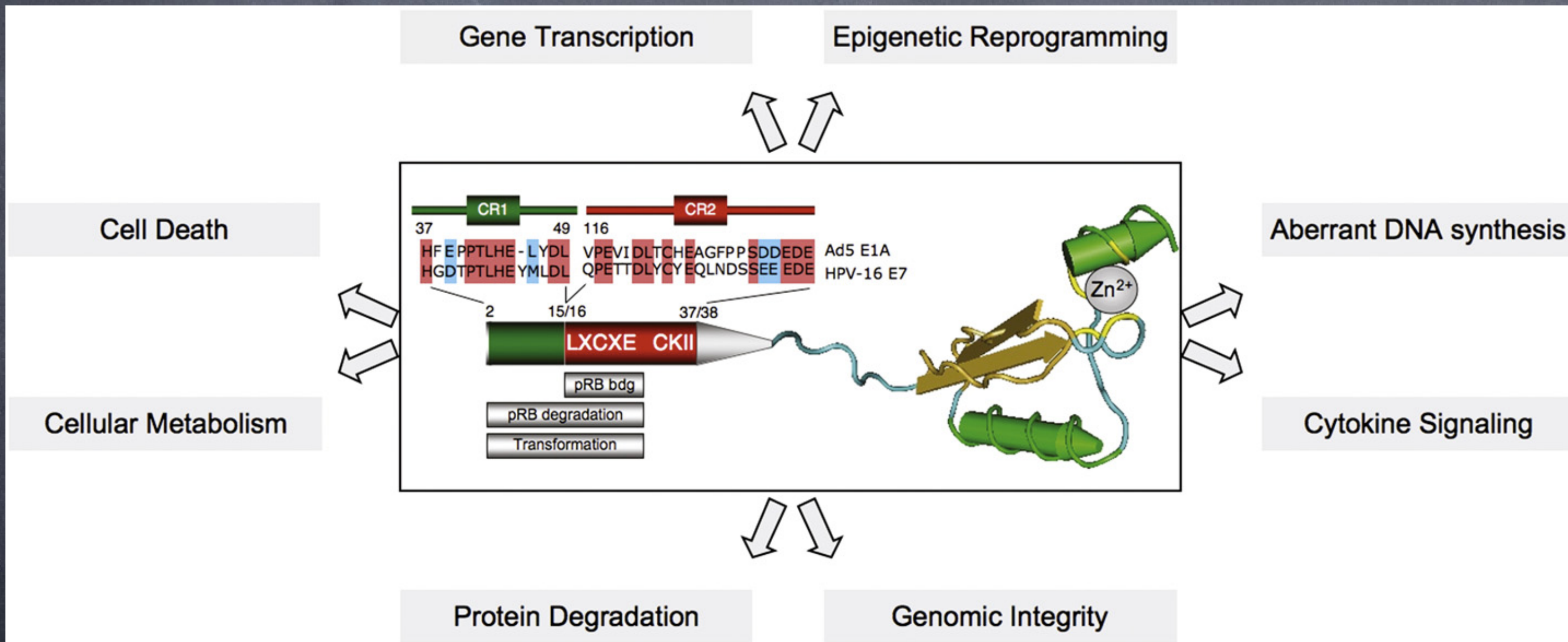


E7

Human Papilloma Virus

MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP

Cellular processes affected by E7





HPV16 E7 entire sequence



MHGDTPPTLHEYMLDL

QPETTDLYCYEQLNDSSEEEDE

IDGPAGQAEPDRAHYNIVTFCCKCDSTLRLC
VQSTHVDIRTLEDLLMGTLGIVCPICSQKP

MHGDTPPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP



E7 sequence



MHGDTPPTLHEYMLDL

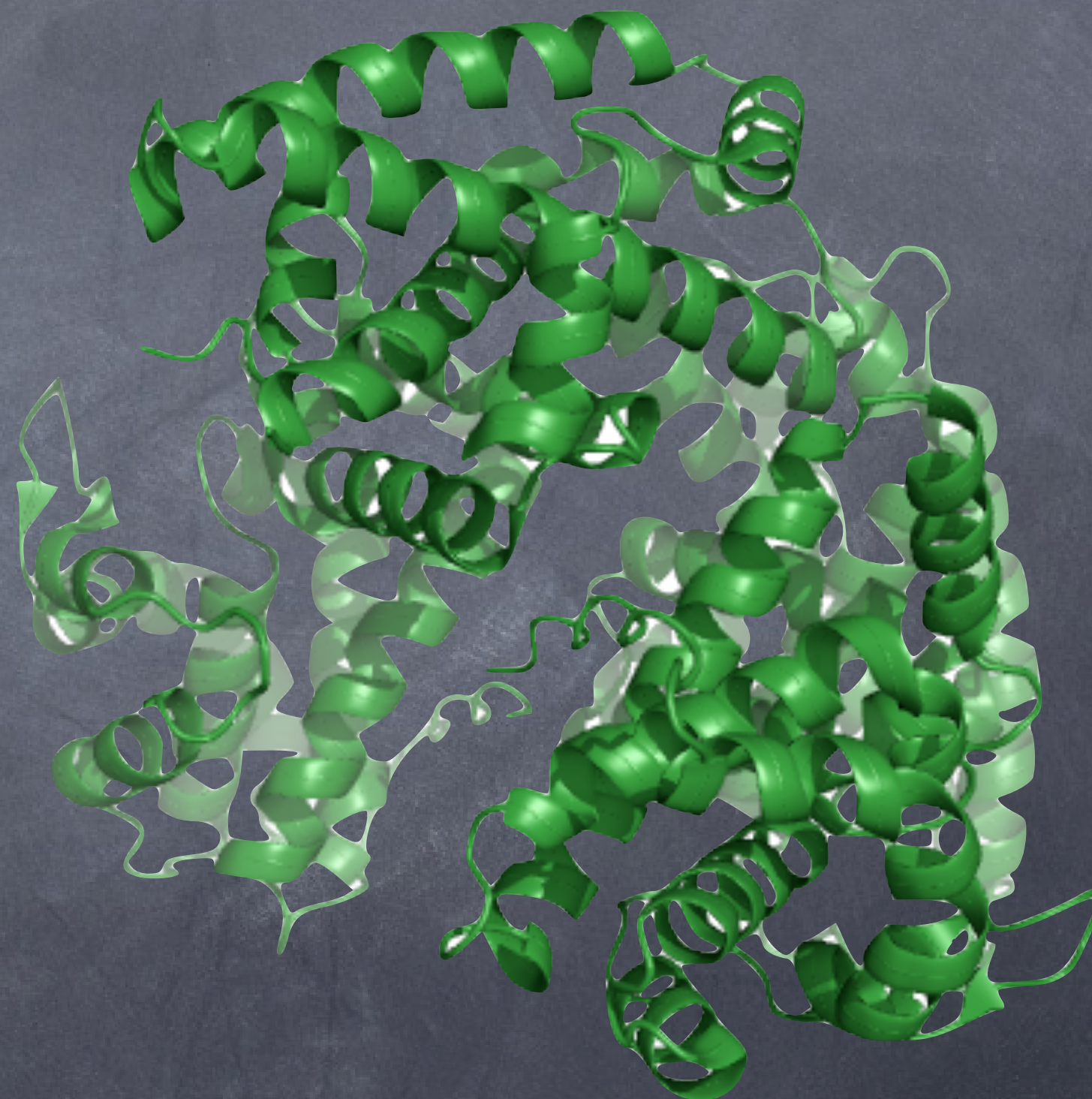
QPETTD**L****C**Y**E**QLNDSSEEDE

IDGPAGQAEPDRAHYNIVTF**C****C**K**C**DSTLRL**C**
VQSTHVDIRTLEDLLMGTLGIV**C**P**I****C**SQKP

MHGDTPPTLHEYMLDLQPETTDLYCYEQLNDSSEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP

LXCXE motif to pRB

- pRB is a tumor suppressor protein
- Retinoblastoma cancer
- it's unliganded structure was published early 2011
- pRB recognizes the LxCxE motif present in E7 protein
- E7 Disrupts the function of host retinoblastoma protein RB1/pRb





E7 sequence



MHGDTPPTLHEYMLDL

QPETTD**L****C**Y**E**QLNDSSEEDE

IDGPAGQAEPDRAHYNIVTF**C****C**K**C**DSTLRL**C**
VQSTHVDIRTLEDLLMGTLGIV**C**P**I****C**SQKP

MHGDTPPTLHEYMLDLQPETTDLYCYEQLNDSSEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP



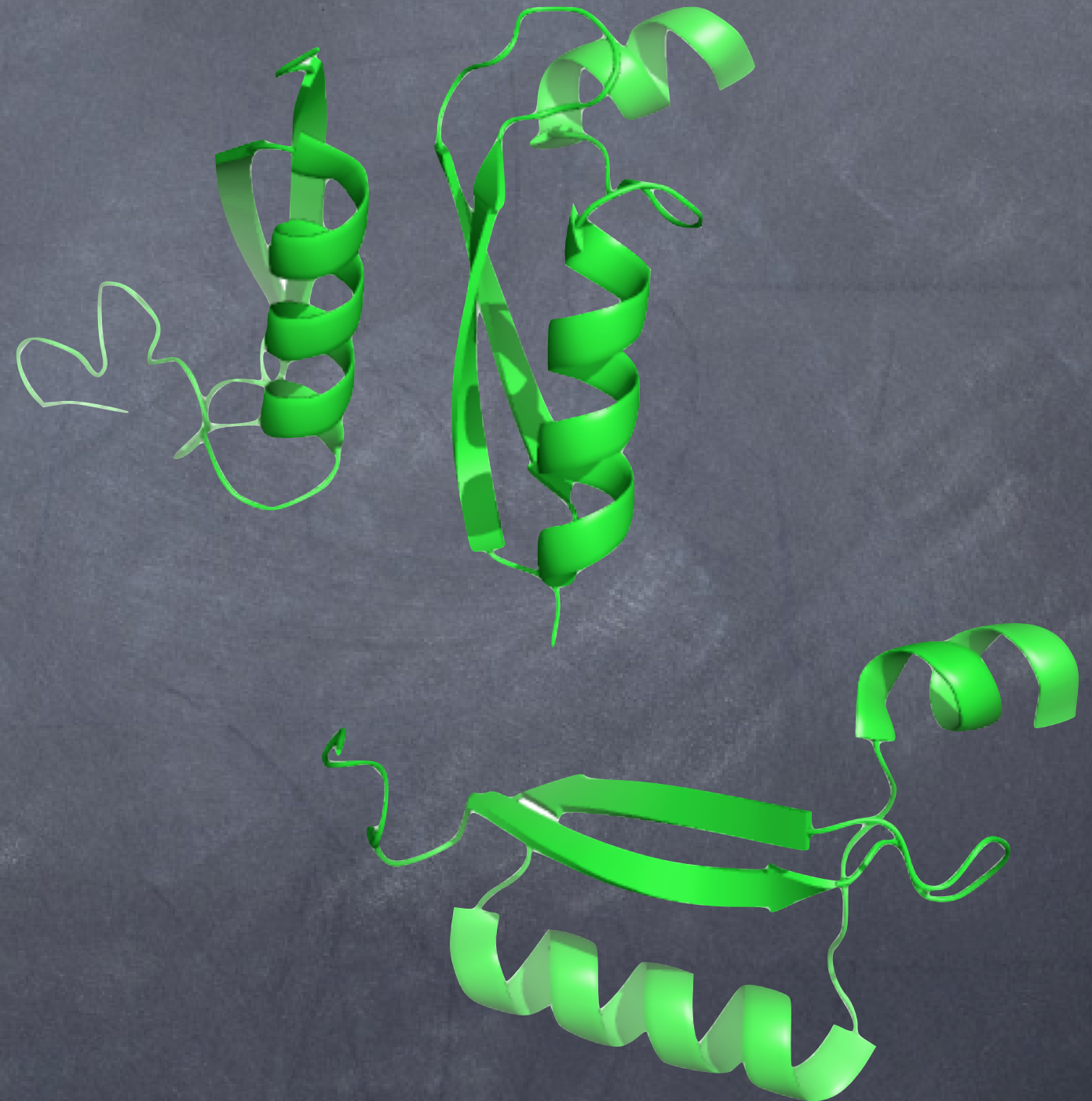
CXXC zinc-binding motifs

- CR3 contains two CXXC zinc-binding motifs
- separated by 29 residues
- ability to form big oligomers

Maria M García-Alai, Leonardo G Alonso, A. & de Prat-Gay, G., 2007. The N-Terminal Module of HPV16 E7 Is an Intrinsically Disordered Domain That Confers Conformational and Recognition Plasticity to the Oncoprotein.

Structures update (E7 CR3)

- 1993 E7 expression in *E. coli*
(Pahel G. et al 1993)
- 2005 Crystal, HPV1a E7 (44–93)
(Liu X. et al., 2005)
- 2006 NMR, HPV45 E7 (55–106)
(Ohlenschläger O. et al., 2006)
- 2006 considered an IDP
(Uversky V.N. et al., 2006)





HPV16 E7 expression

- E. coli BL21plys
- pET20 plasmid
- 1 purification step HisTag column
- E7 yield: 20mg/L

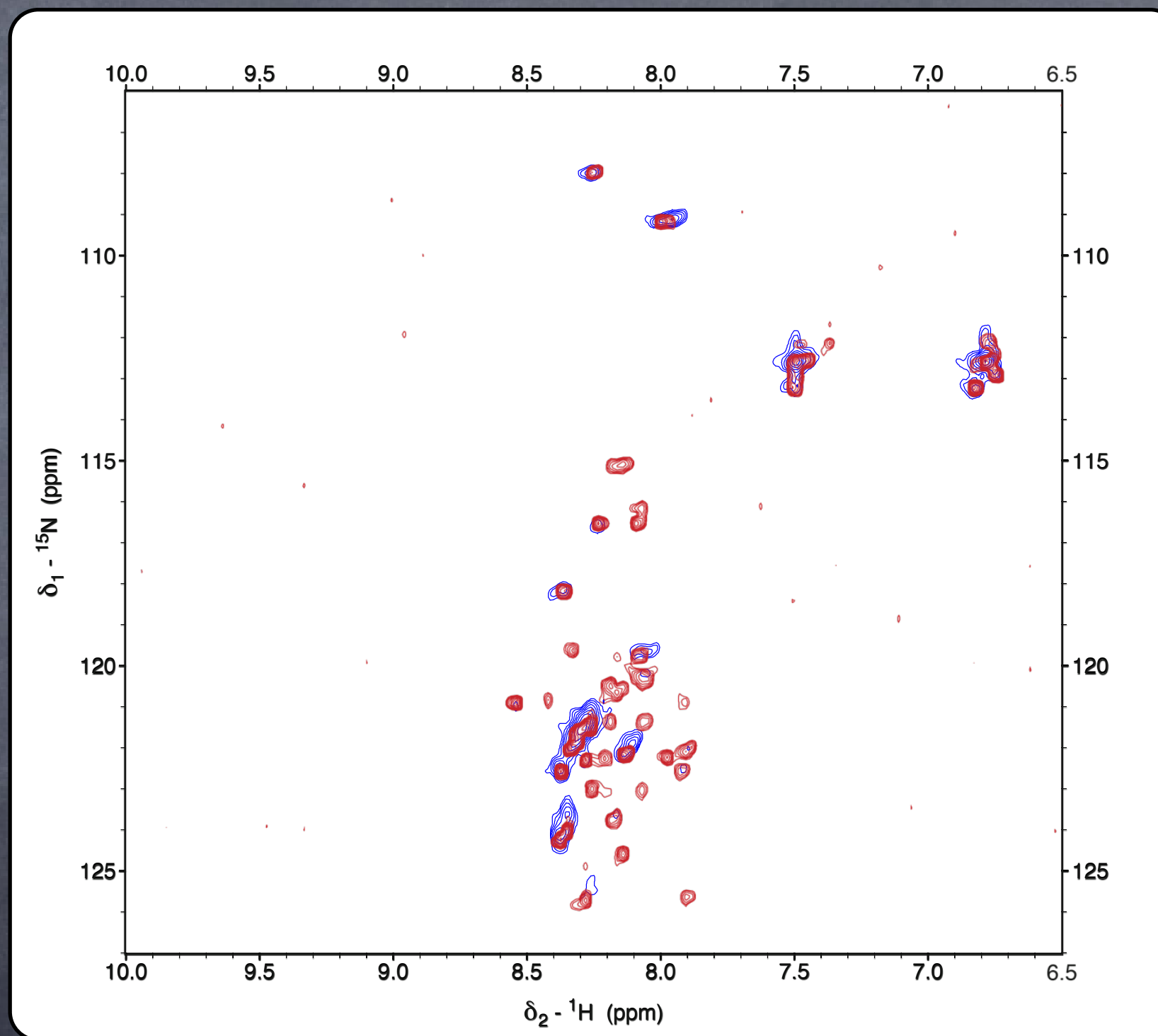


Optimization

- pH 5.5 – 8.5
- Temperature 278 – 308 K
- buffers
- anaerobic vs aerobic purification steps
- reductant agents
- denaturing conditions
- ^{15}N labeling



NMR – HSQC



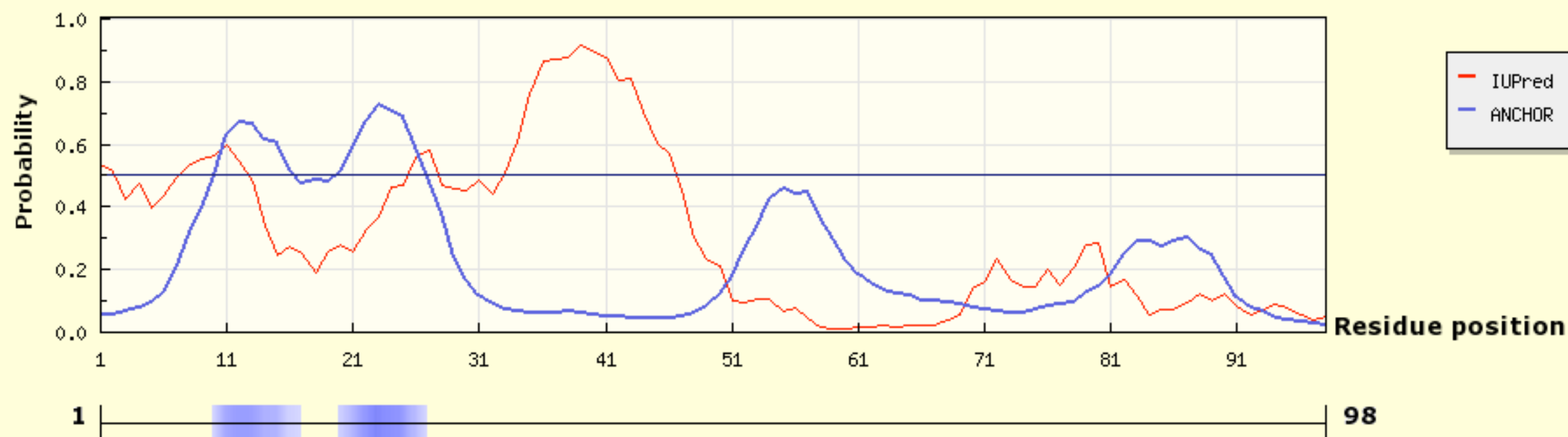
MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLRRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP



Future goals

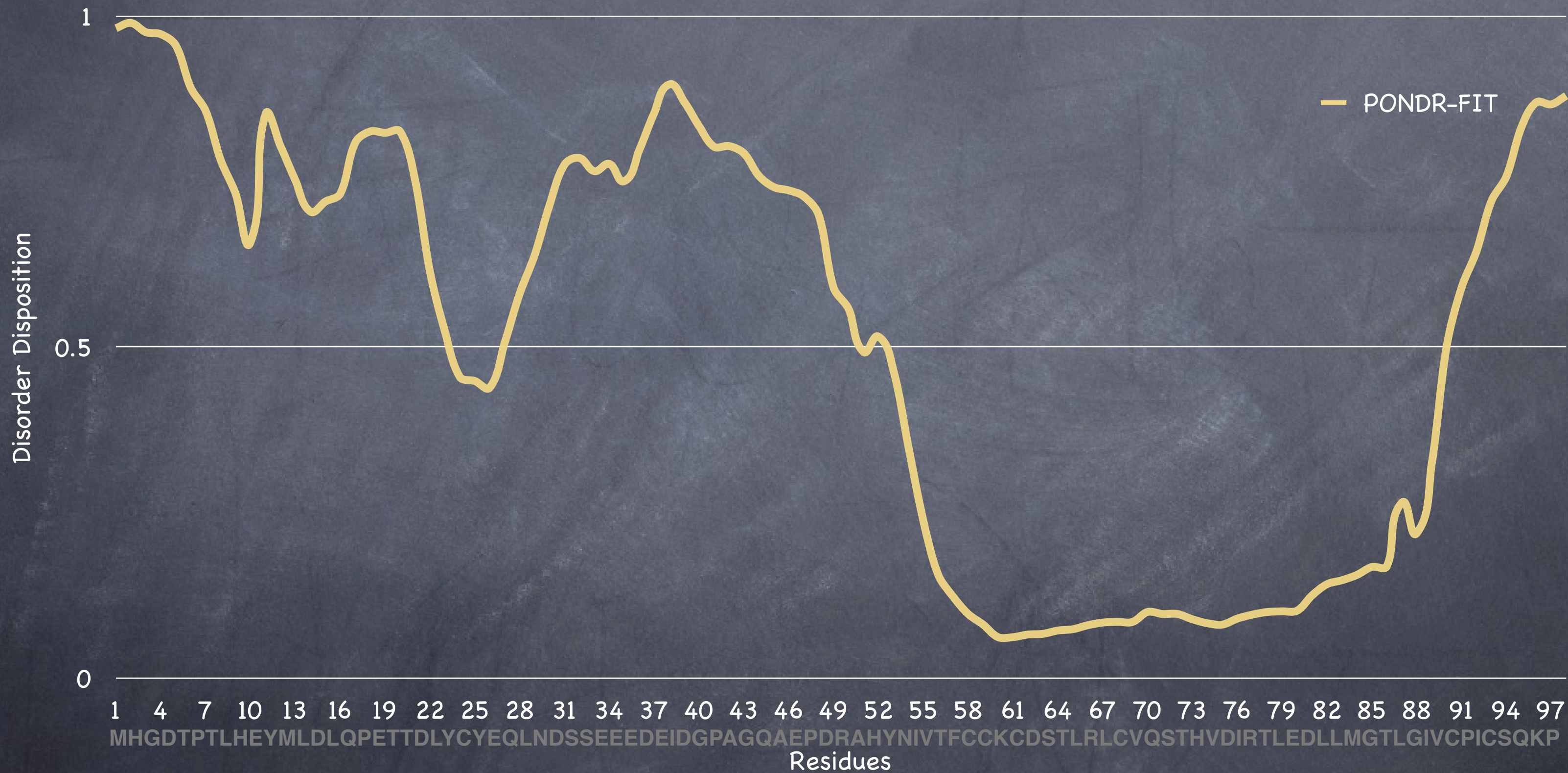
- ^{13}C labeling of E7 protein
- IDPs

Predictors: ANCHOR





Predictors: PONDR-FIT





Acknowledgments

- Professor Roberta Pierattelli
- Professor Isabella Felli
- IDPbyNMR

Thank you