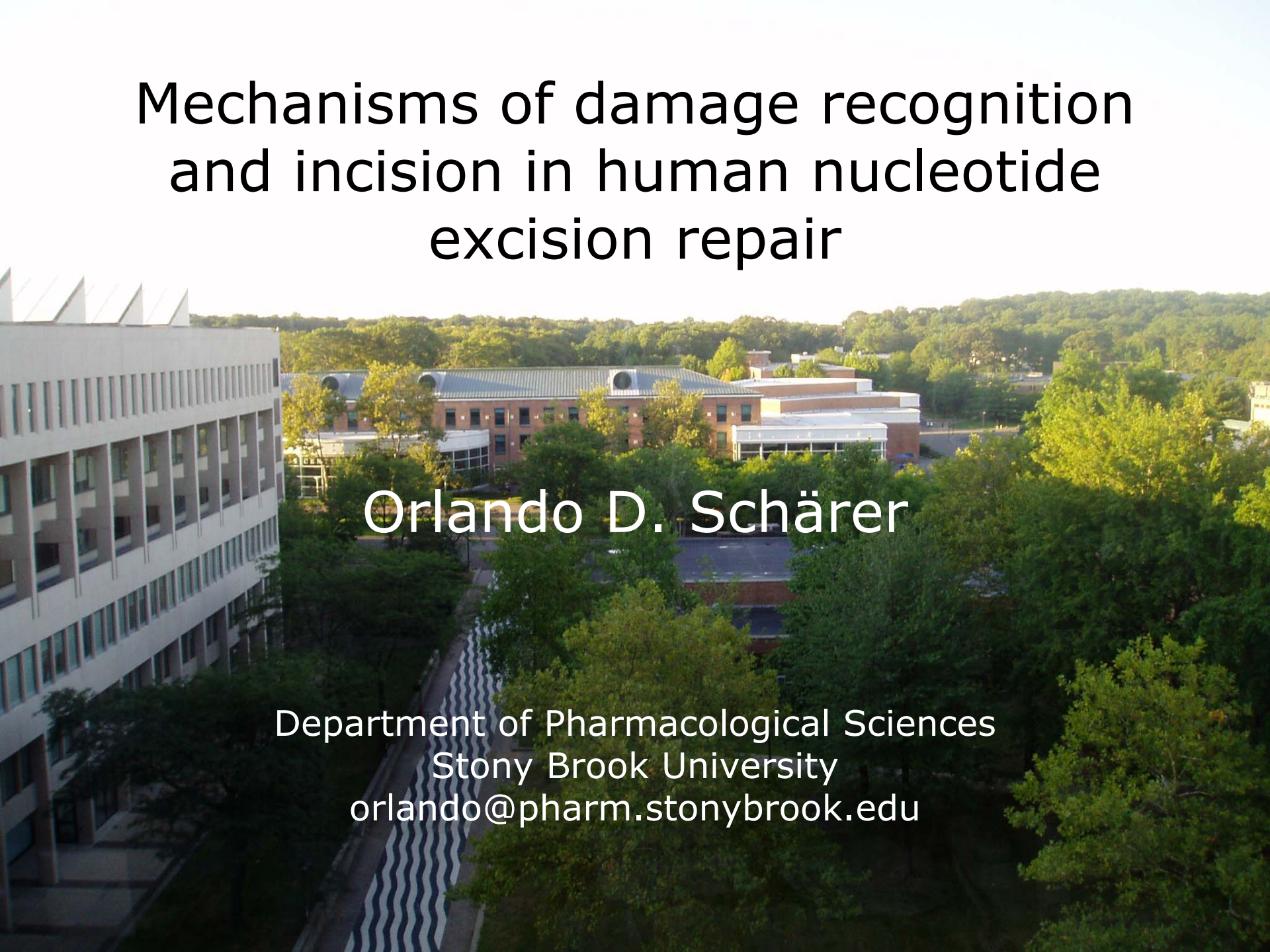
Mechanisms of damage recognition and incision in human nucleotide excision repair



Orlando D. Schärer

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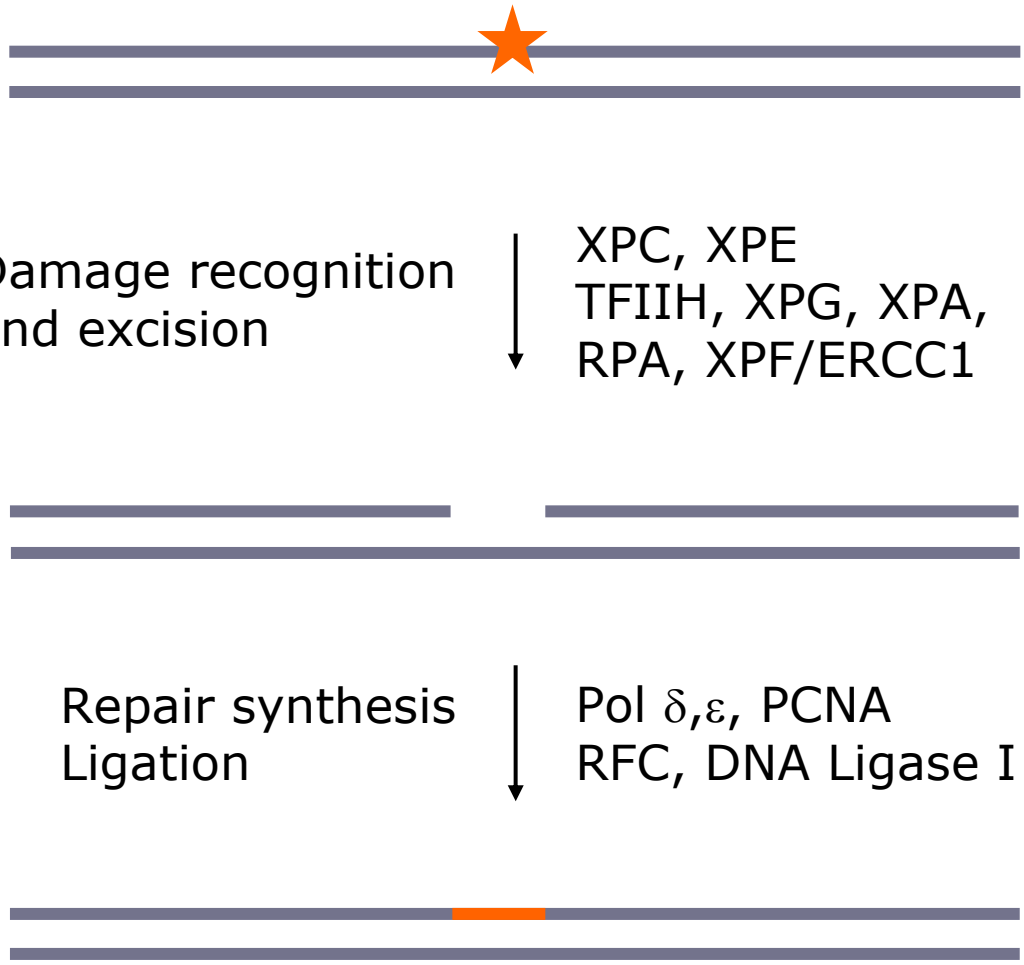
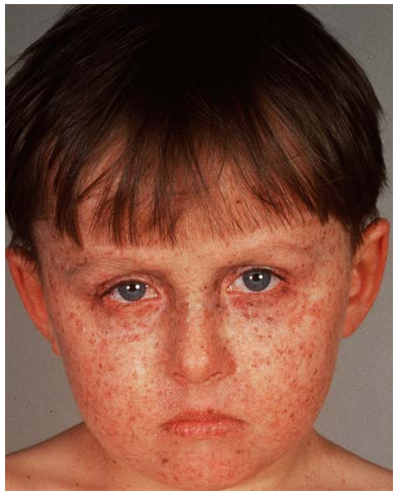
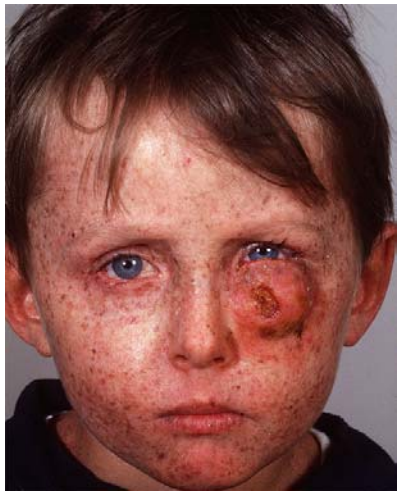
NUCLEOTIDE EXCISION REPAIR (NER)

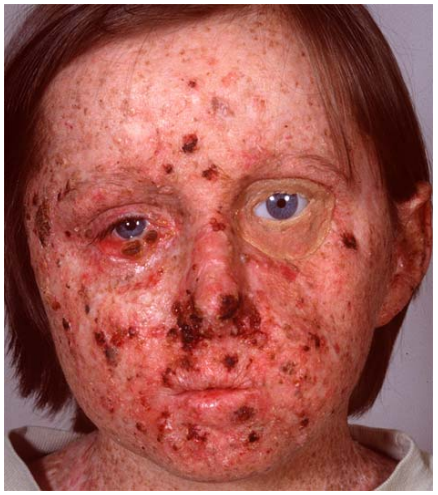


XERODERMA PIGMENTOSUM

- Inherited disorder with genetic and clinical heterogeneity
- Incidence $1:10^5$ - $1:10^6$
- Extreme sensitivity to UV radiation
- 2000-fold increased incidence of skin cancer
- Severe skin and eye abnormalities
- Neurological abnormalities (20% of patients)
- 30 year reduction in survival

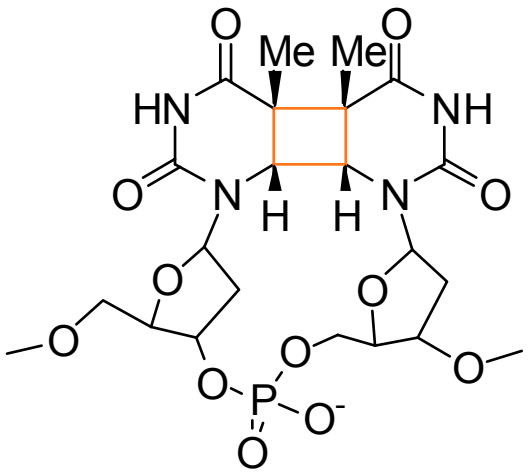


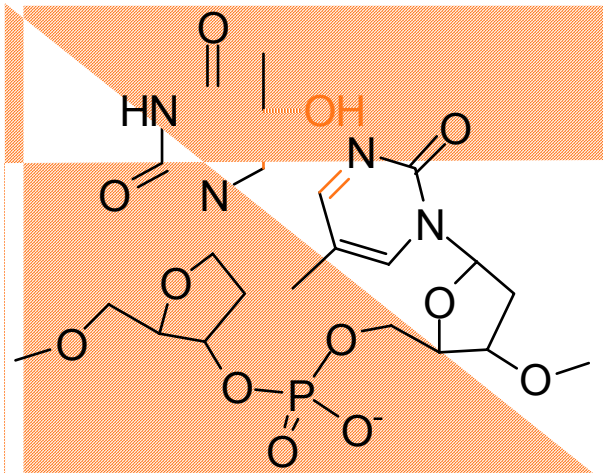


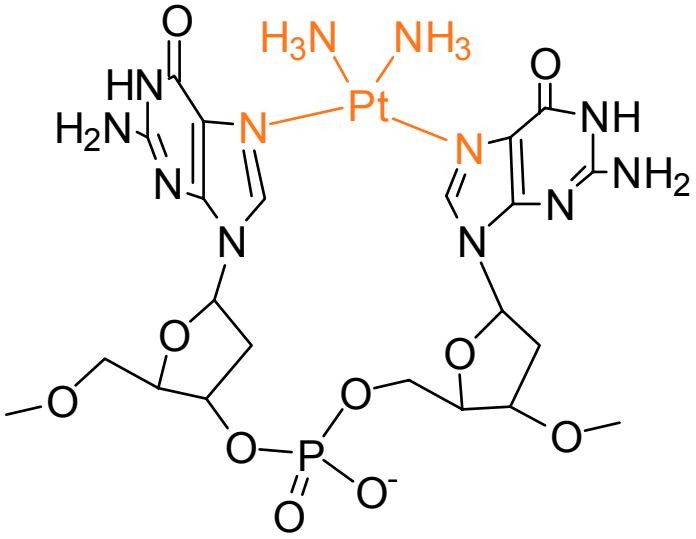


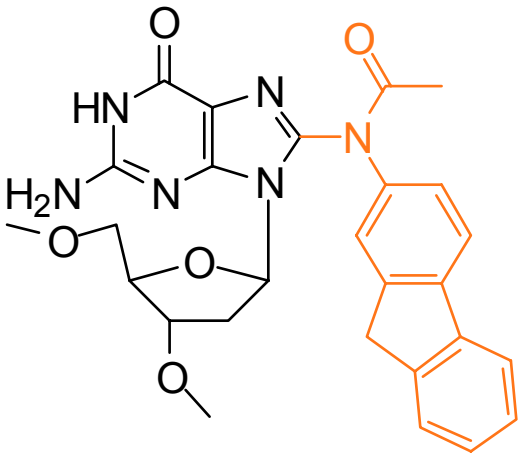
Photos courtesy of Prof. Reinhard Dummer, Dermatologische Klinik, Zürich

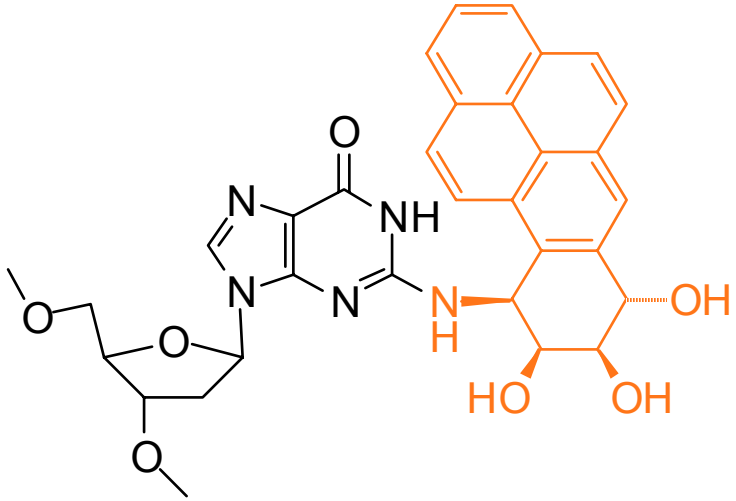
NER SUBSTRATES DISTORT THE DNA HELIX

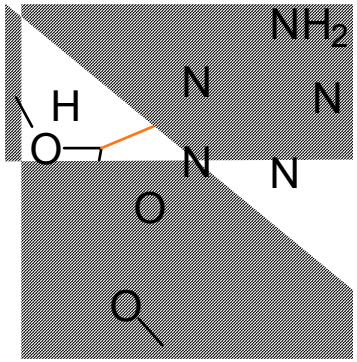




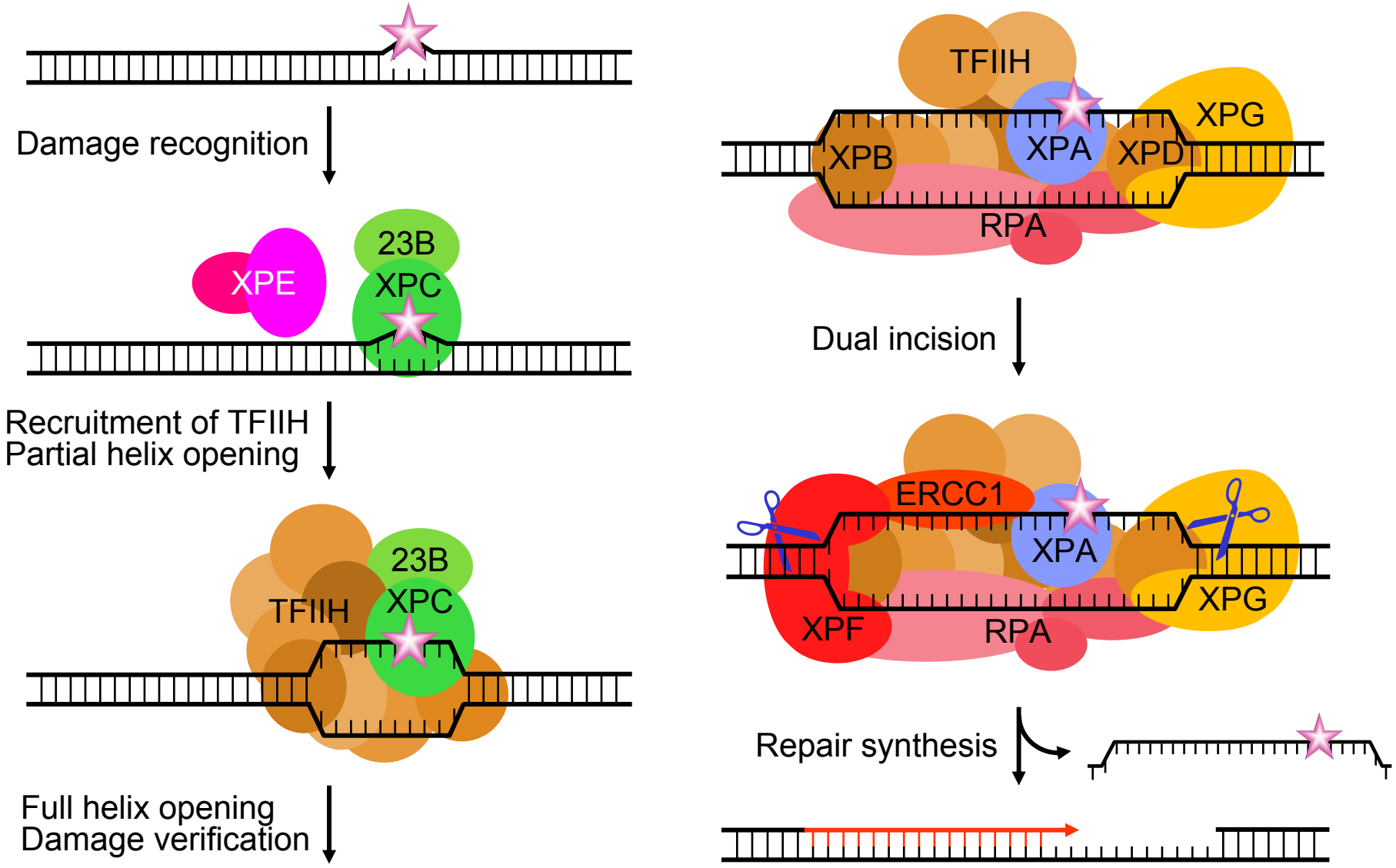




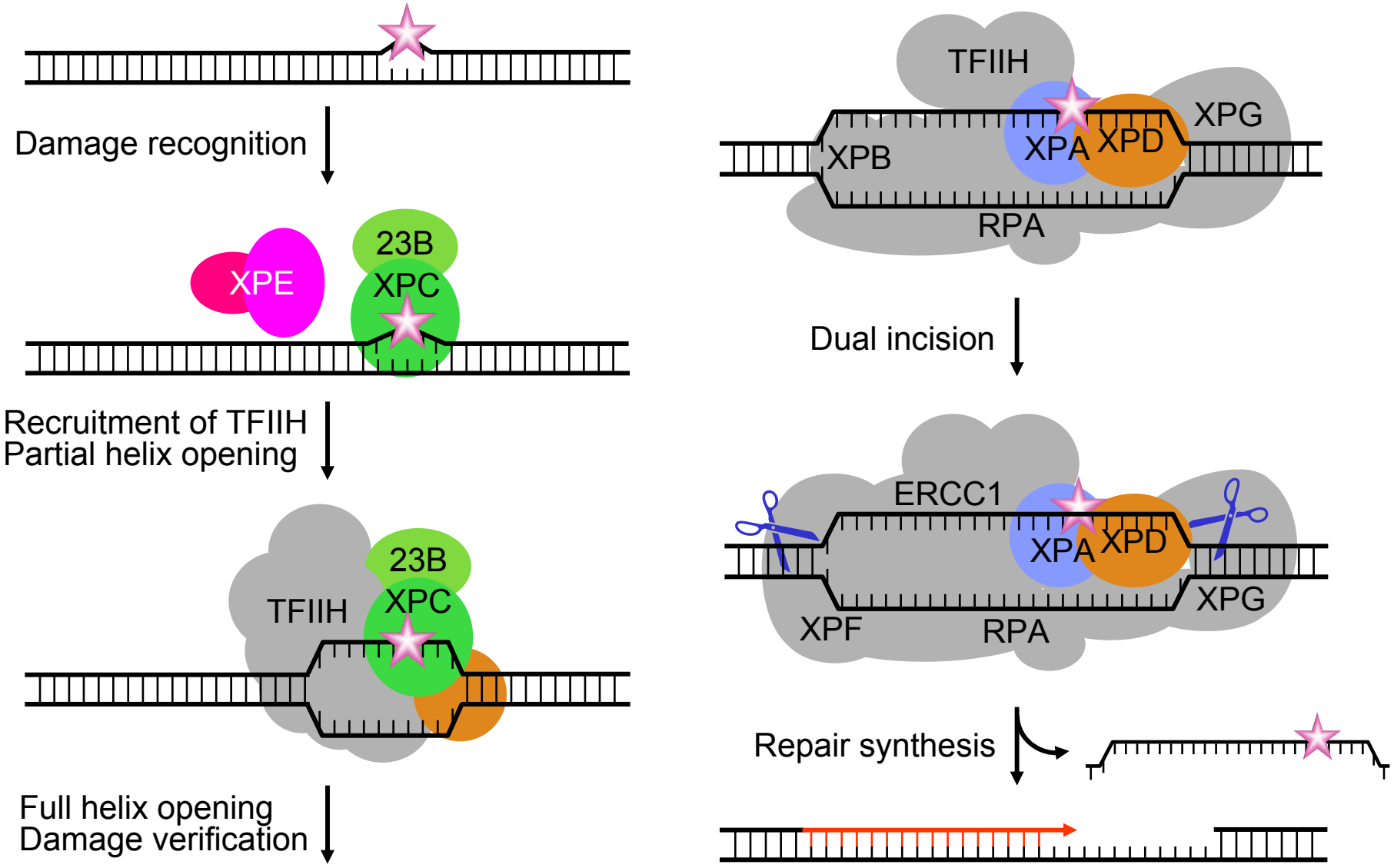




A MODEL FOR NUCLEOTIDE EXCISION REPAIR

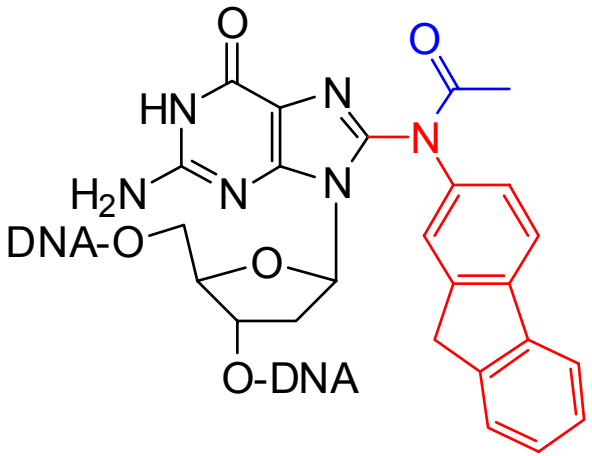


DAMAGE RECOGNITION IN NER

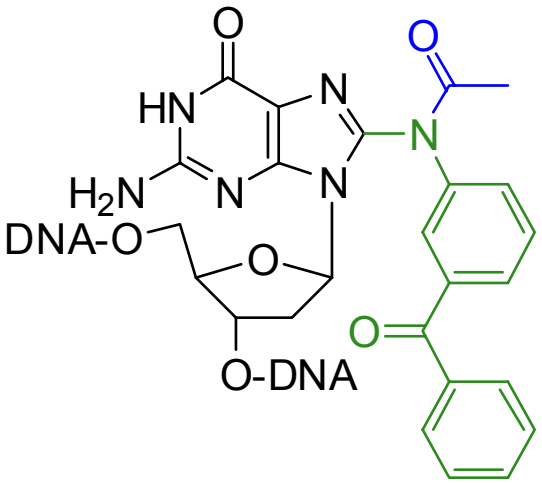


PHOTOACTIVE/FLUORESCENT NER SUBSTRATES

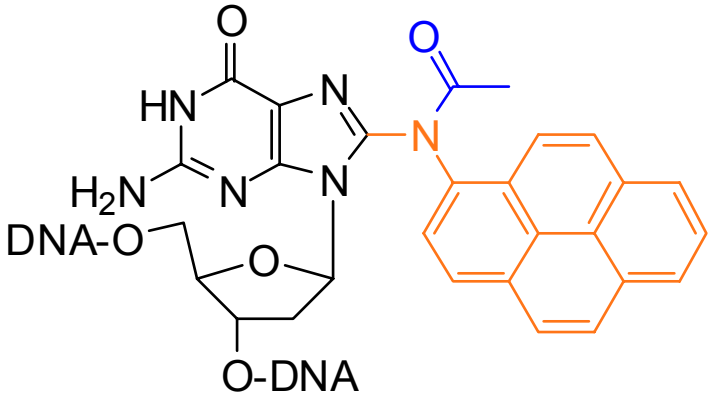
G(8)-AAF ADDUCT



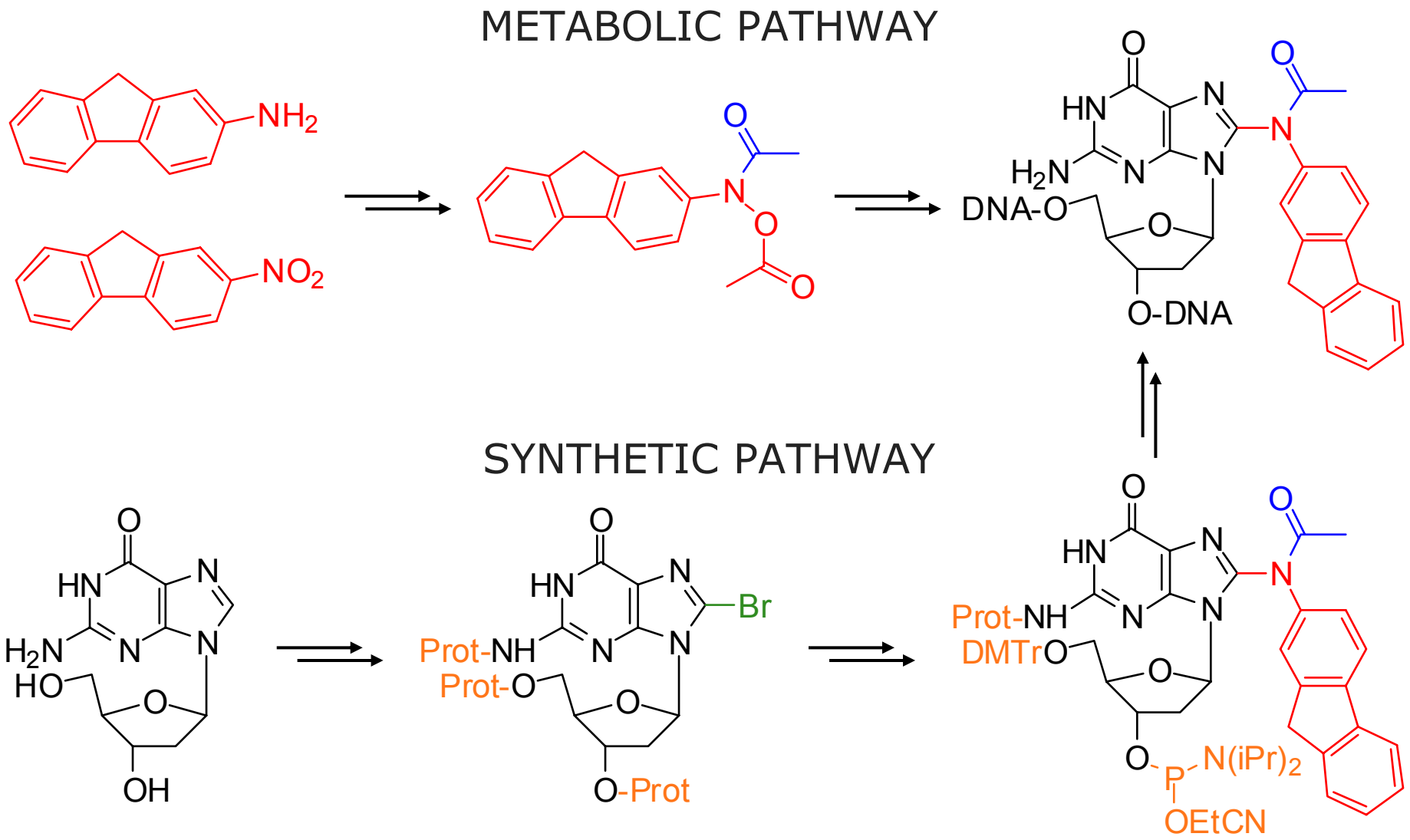
PHOTOREACTIVE DERIVATIVE



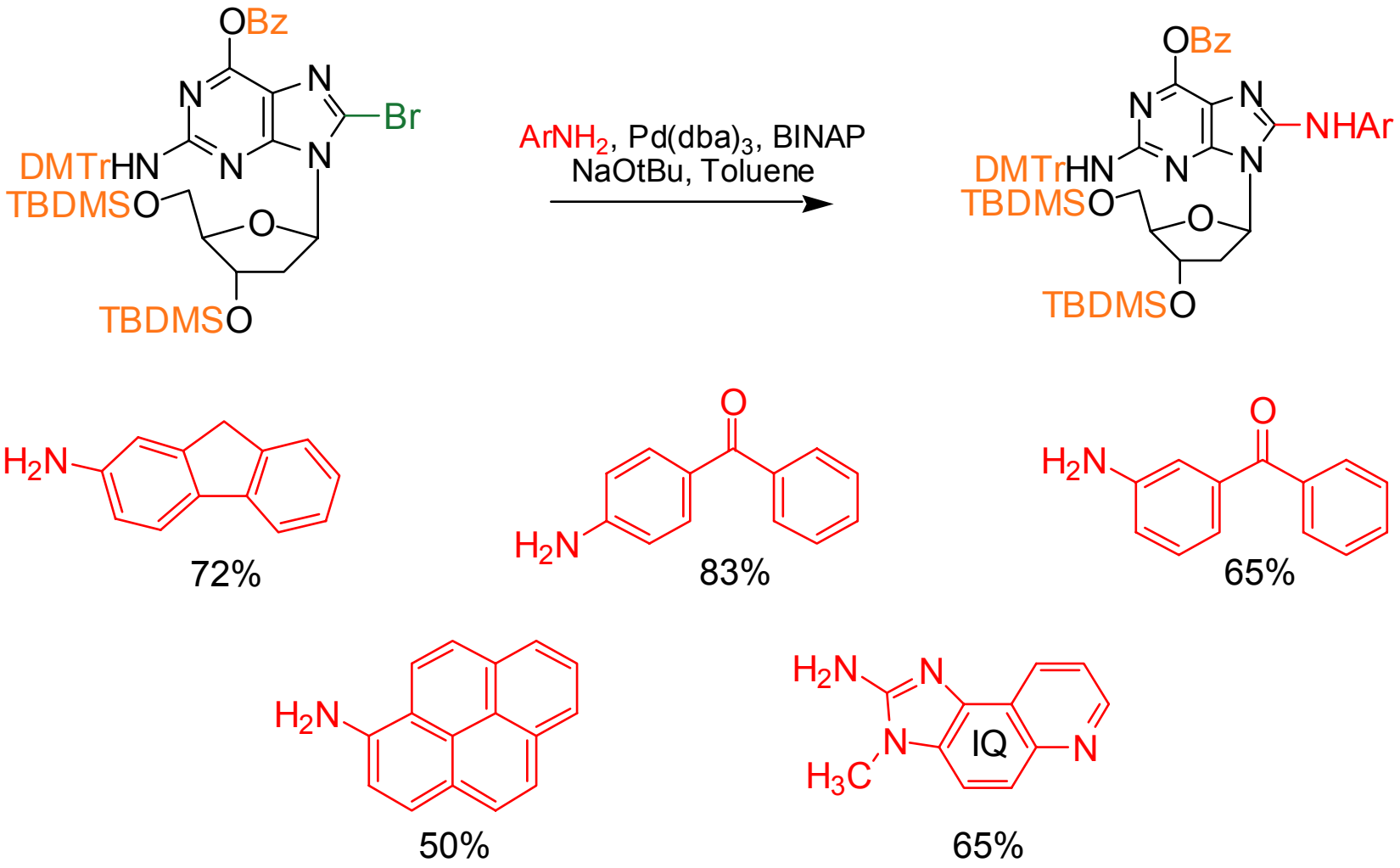
FLUORESCENT DERIVATIVE



SYNTHESES OF AAF ADDUCTS IN DNA

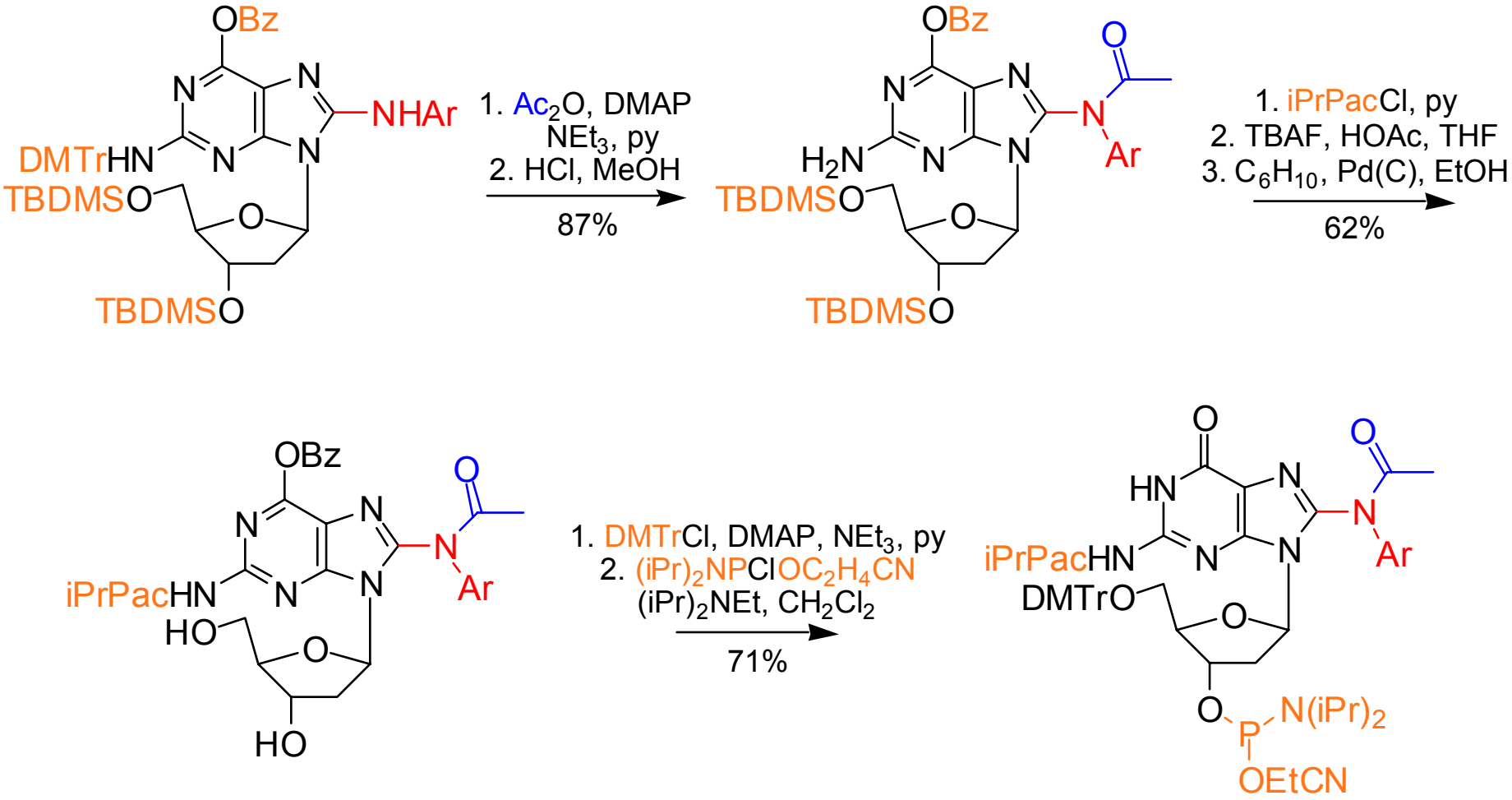


HARTWIG-BUCHWALD COUPLING OF 8-BrdG



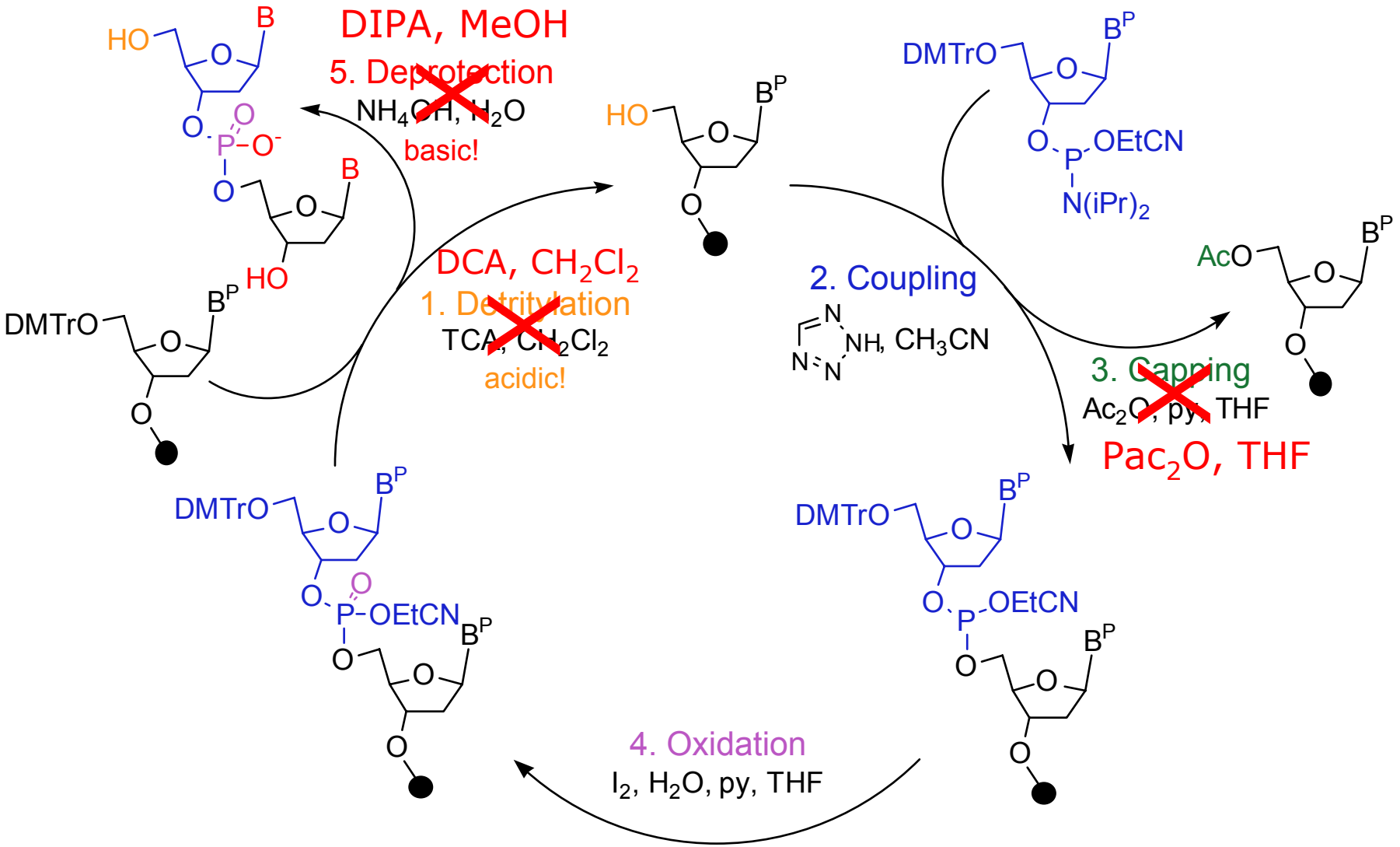
Gillet, L., Schärer, O.D. **Org. Lett.** *4*, 4205-4208 (2002)

ACYLATION AND CHANGE OF PROTECTION



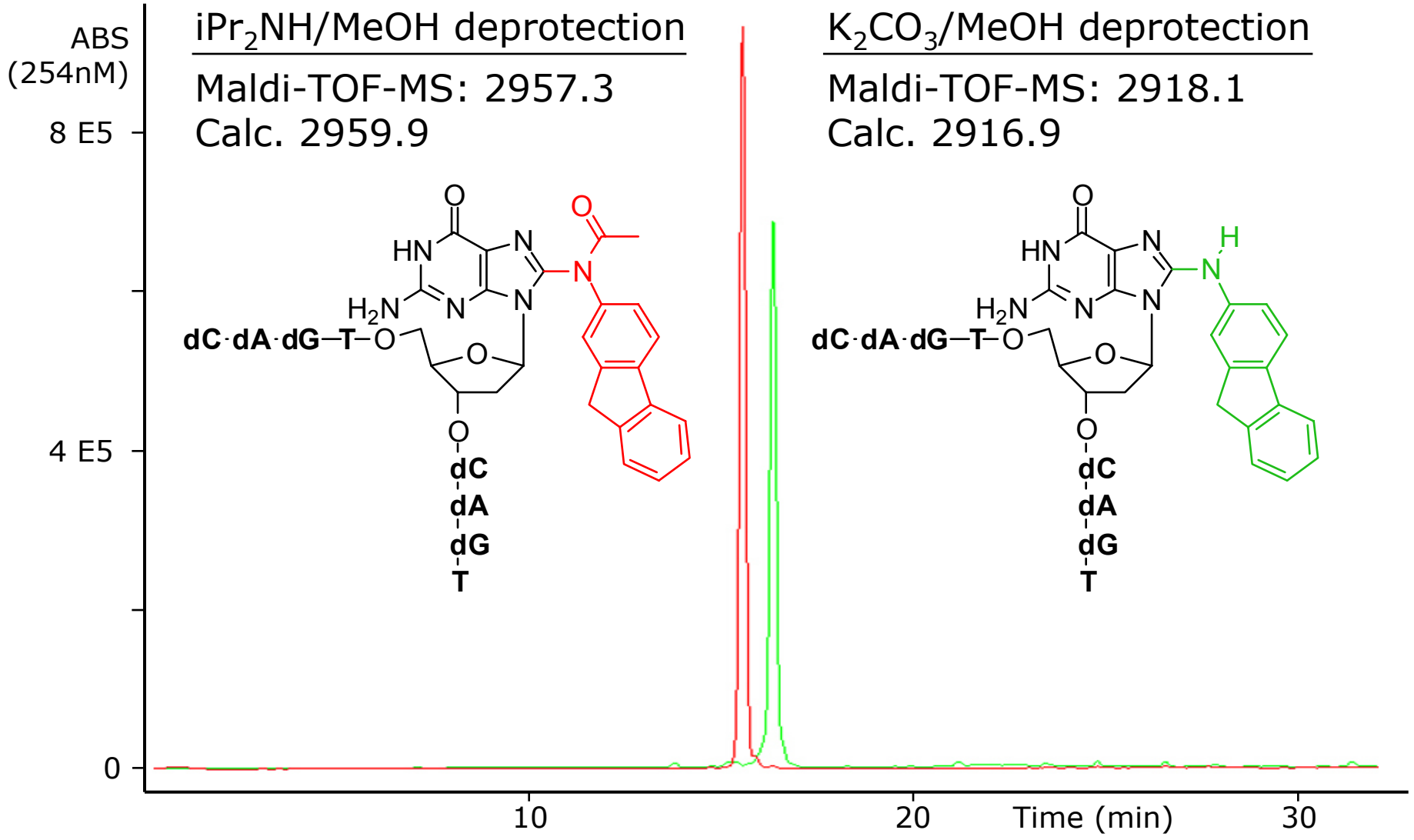
Gillet, L., Schärer, O.D. **Org. Lett.** *4*, 4205-4208 (2002)

MODIFIED DNA SYNTHESIS OF AAF OLIGOS



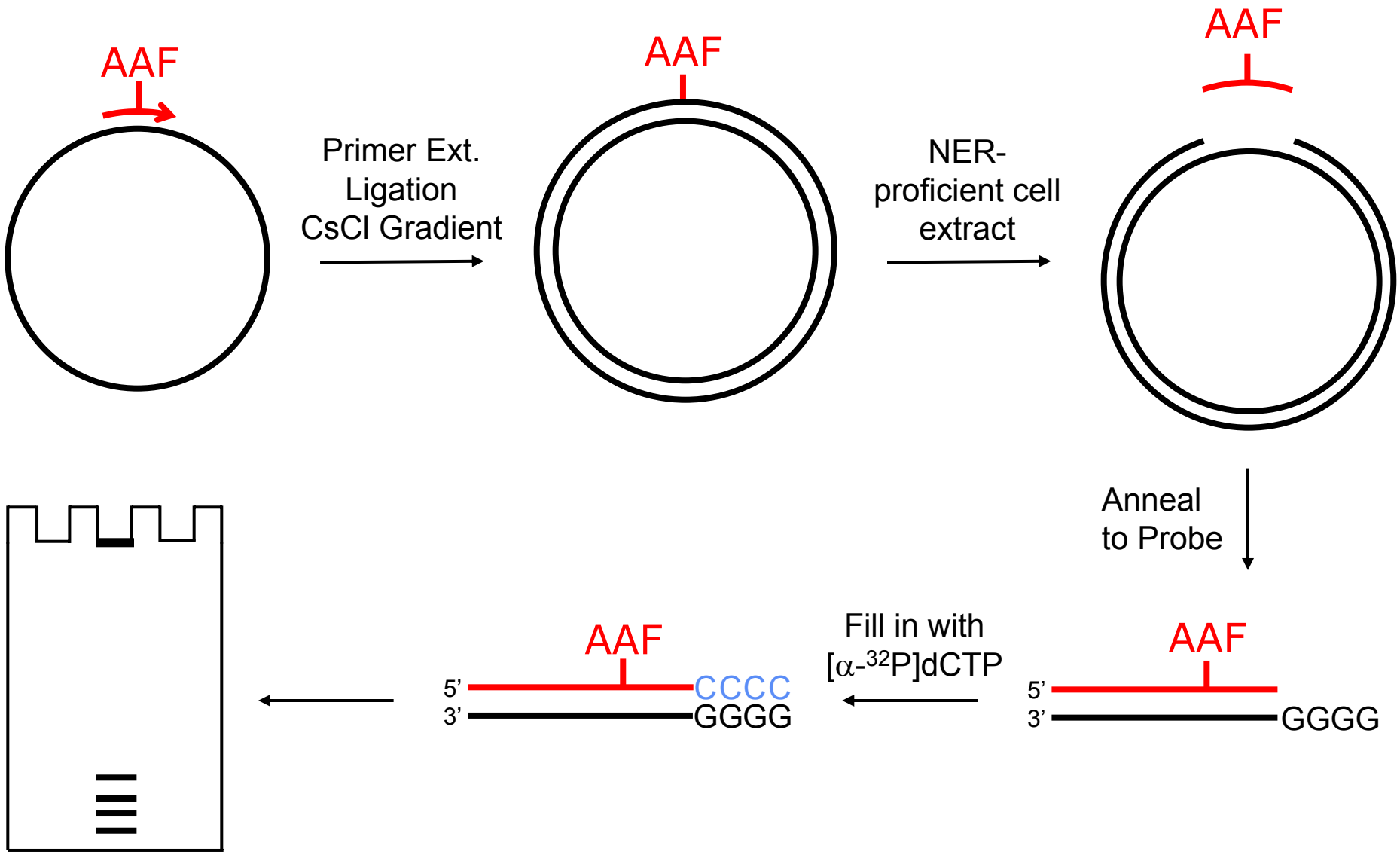
Jawad Alzeer, Ludovic Gillet

ANALYSIS OF dG-AAF AND dG-AF OLIGOS



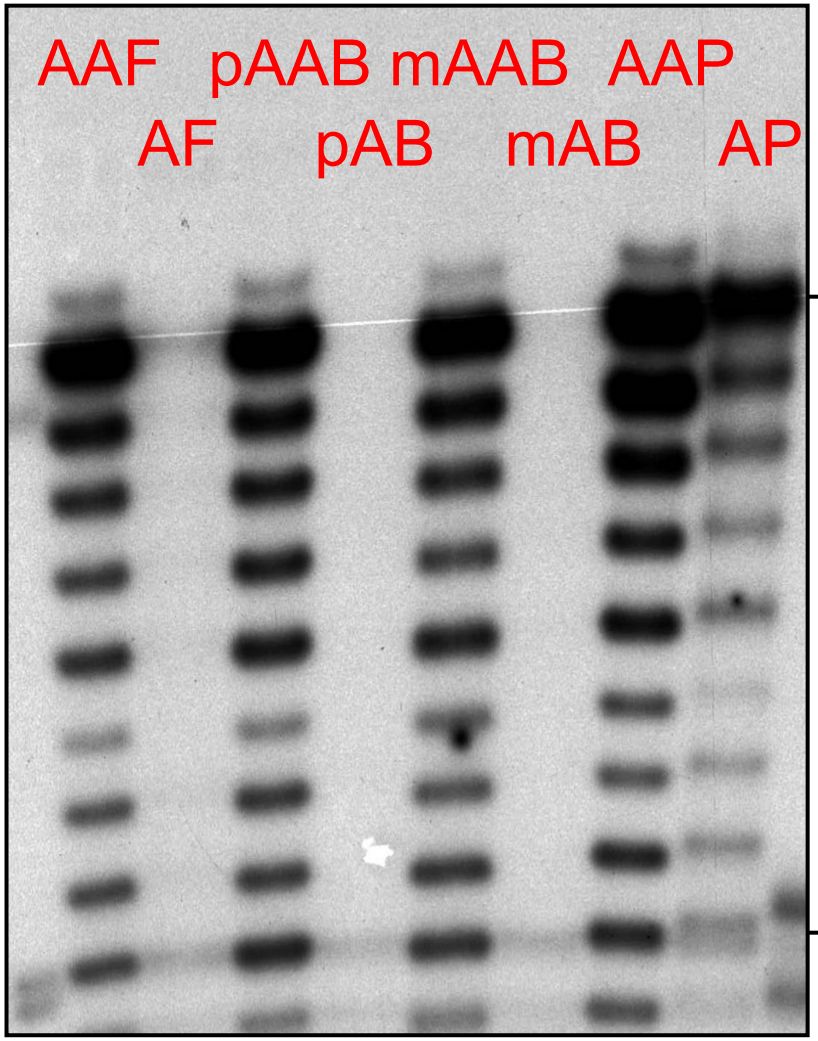
Gillet LCJ, Alzeer J, Schärer OD (2005) **NAR** *33*, 1961

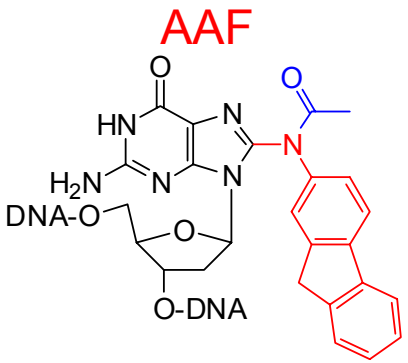
IN VITRO NER ASSAY

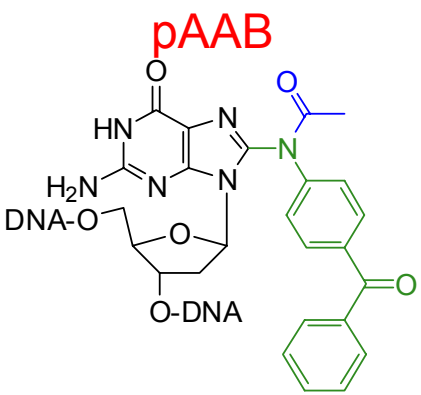


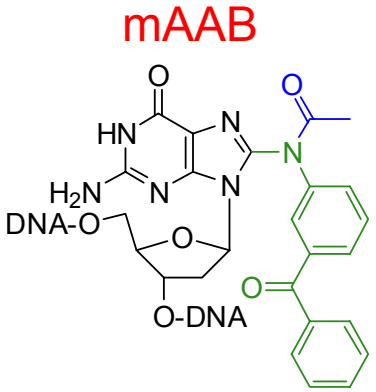
Jacqueline Enzlin, Ludovic Gillet

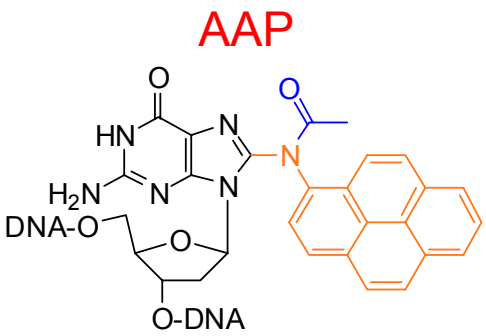
AAF/AF DERIVATES AS NER SUBSTRATES







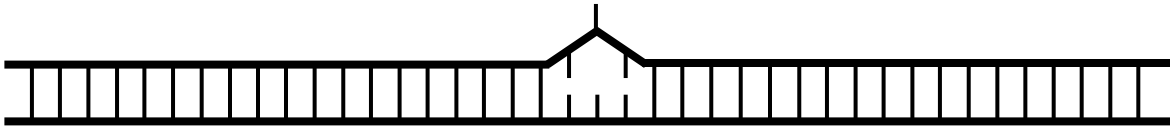




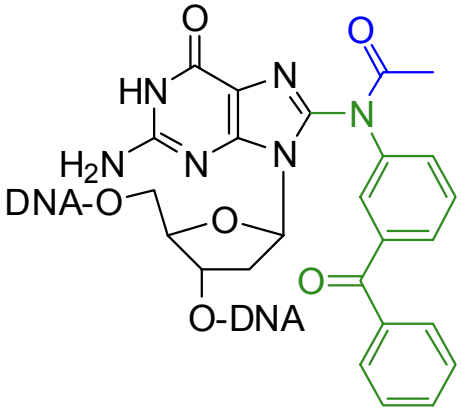
Ludovic Gillet

PHOTOACTIVE PROBES FOR NER

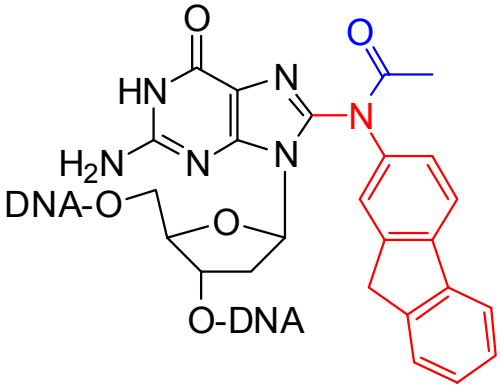
dG-AABP



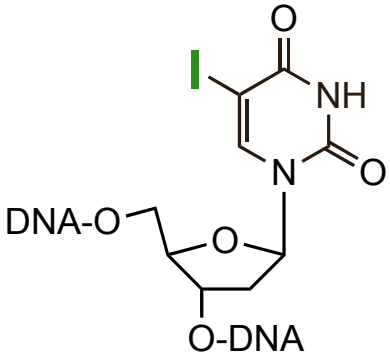
G(8)-AABP



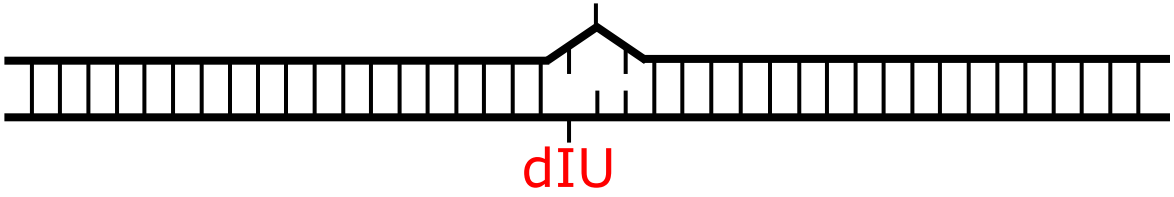
G(8)-AAF



5-dIU

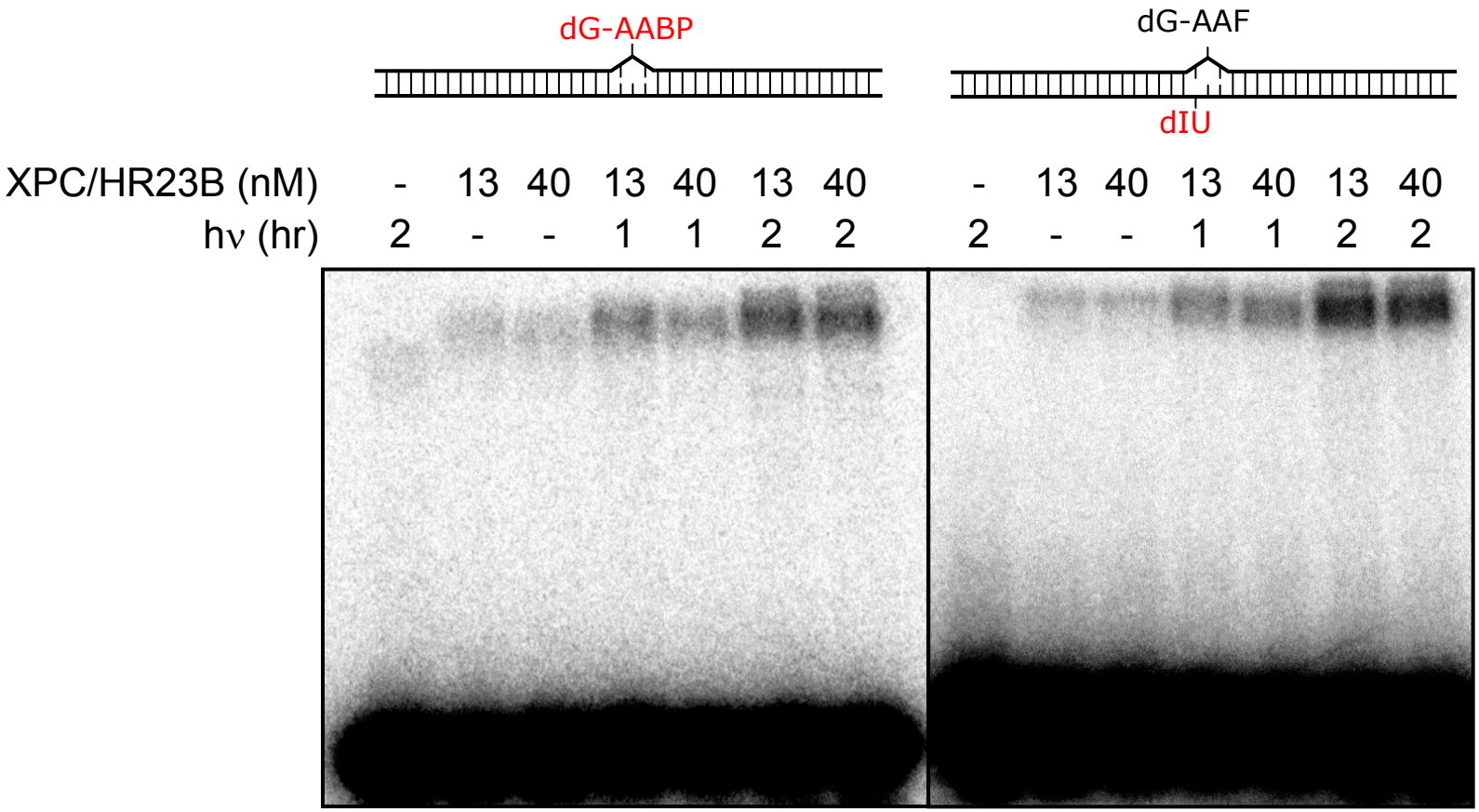


dG-AAF

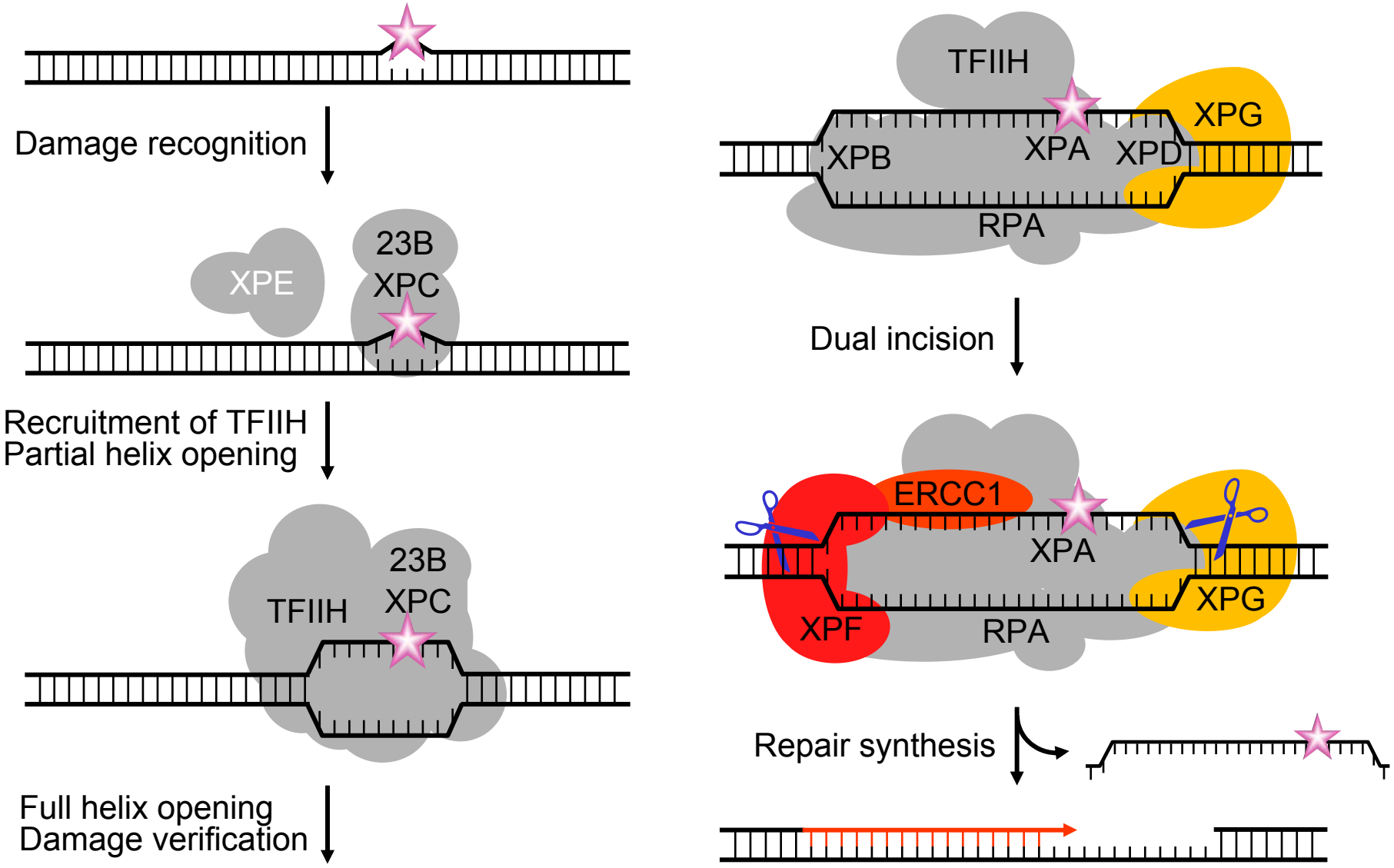


dIU

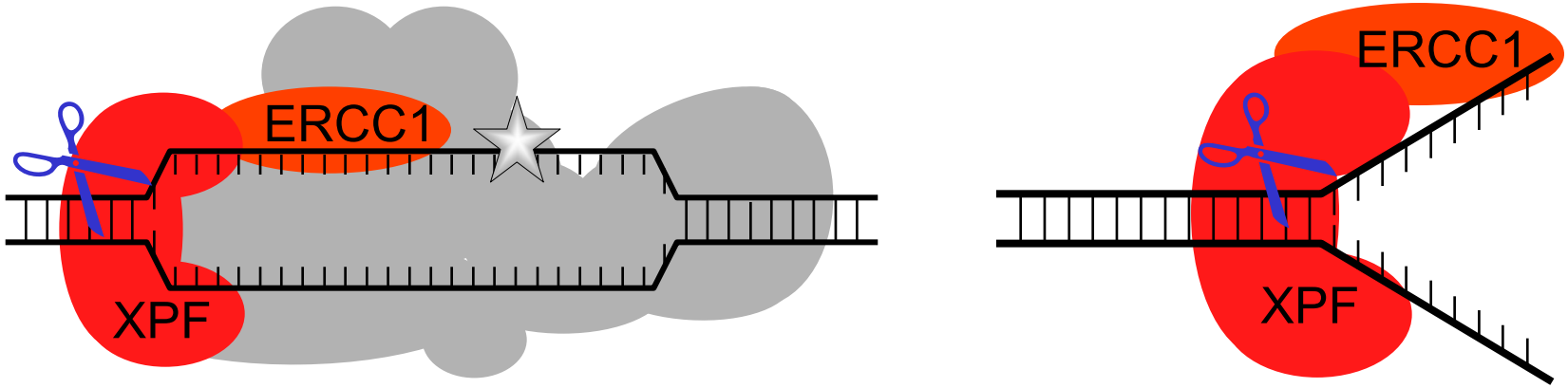
XPC/HR23B BINDS TO THE DAMAGED AND NON-DAMAGED STRANDS OF DNA

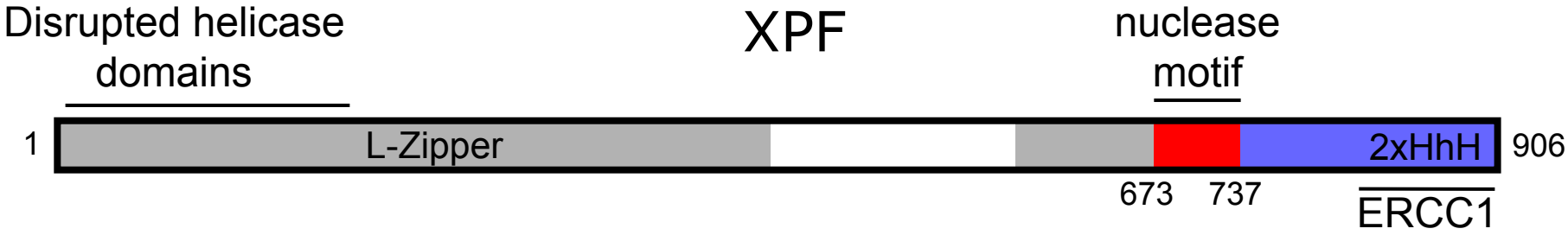


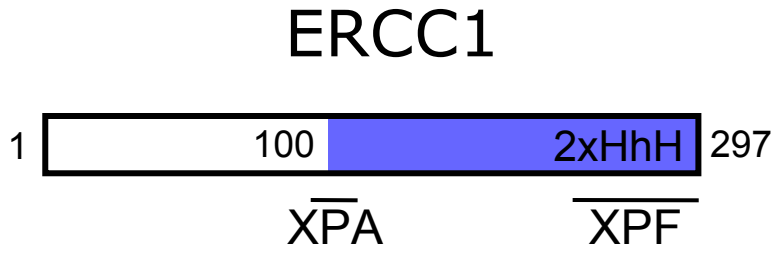
ENDONUCLEASES IN NER



THE XPF/ERCC1 HETERODIMER

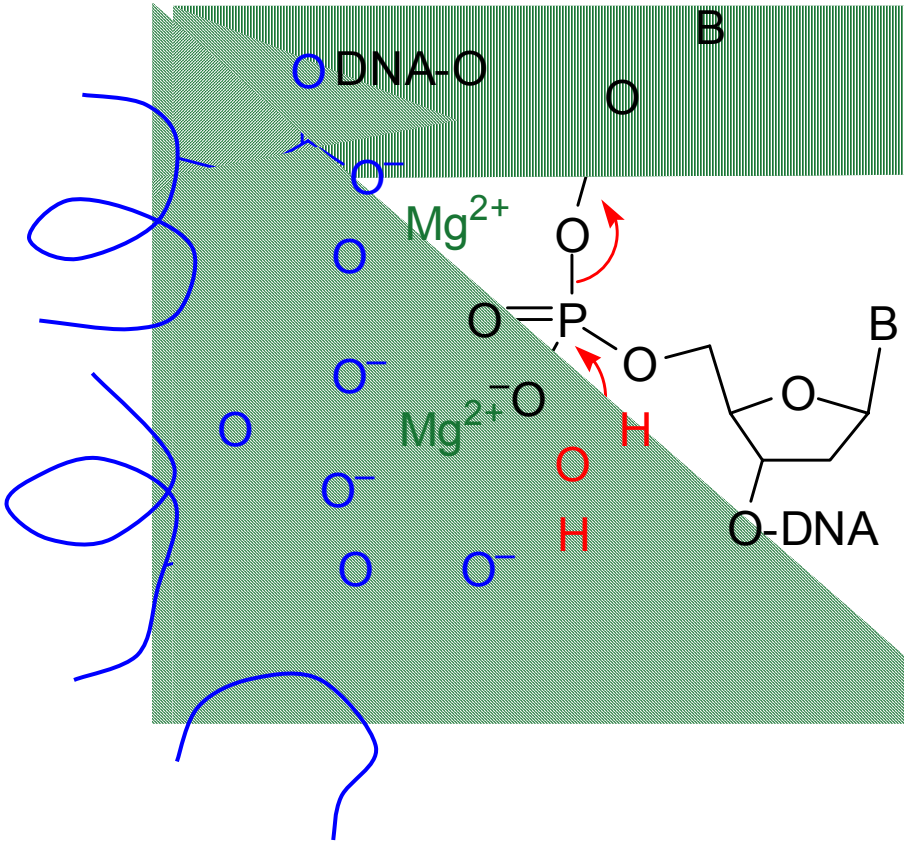




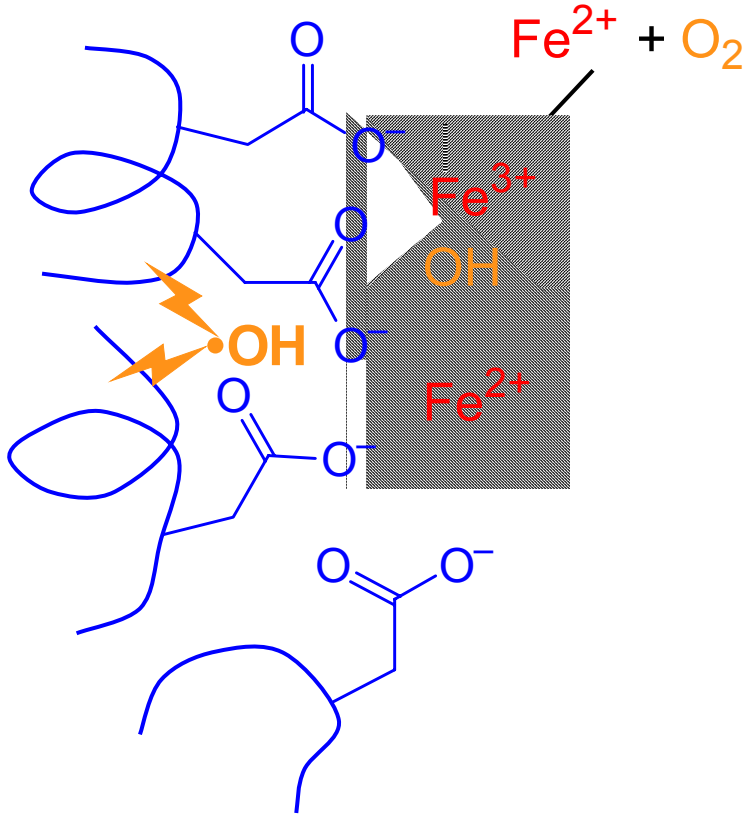


IDENTIFYING THE ACTIVE SITE OF XPF/ERCC1

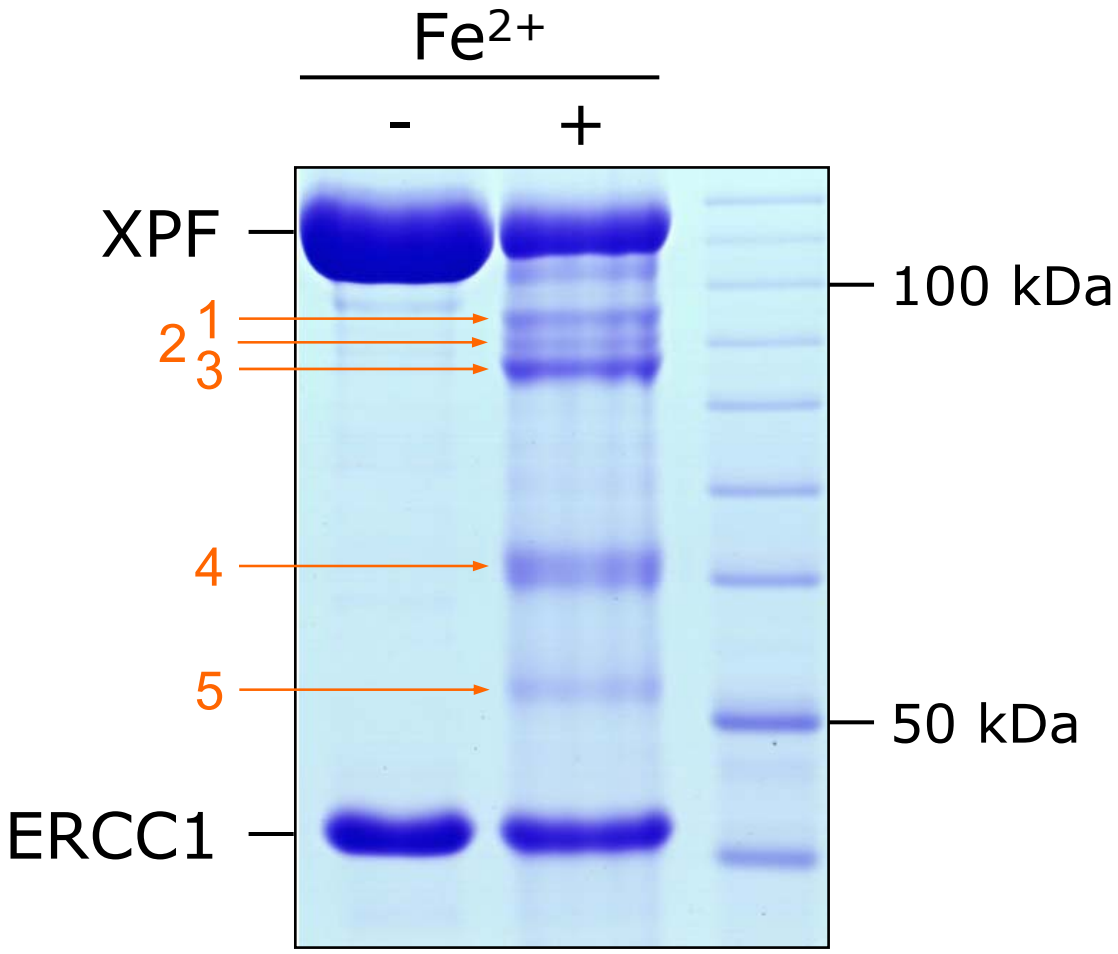
Mechanistic model for metal dependent endonucleases



Affinity cleavage reaction using Fe^{2+} and oxygen



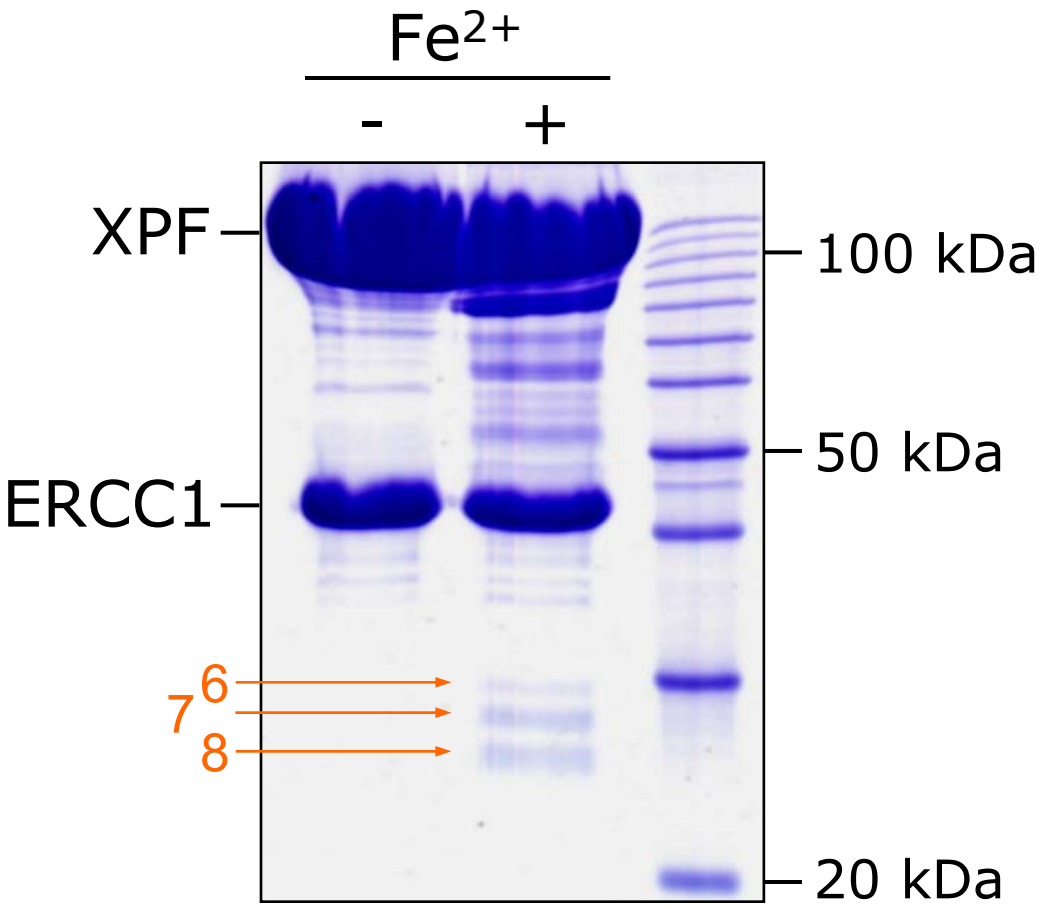
CLEAVAGE OF XPF USING FENTON CHEMISTRY



N-terminal sequencing: Bands 1-4 correspond to N-terminus of XPF
Band 5 could not be sequenced

Enzlin JH, Schärer OD **EMBO J.** *21*, 2045-2053(2002)

CLEAVAGE OF XPF USING FENTON CHEMISTRY



N-terminal sequencing:
Band 6: FRxELPxLIH ➲ 680-689 of XPF
Band 7: VGDYILRYTP ➲ 702-711 of XPF
Band 8: xISMSRYY ➲ 734-743 of XPF

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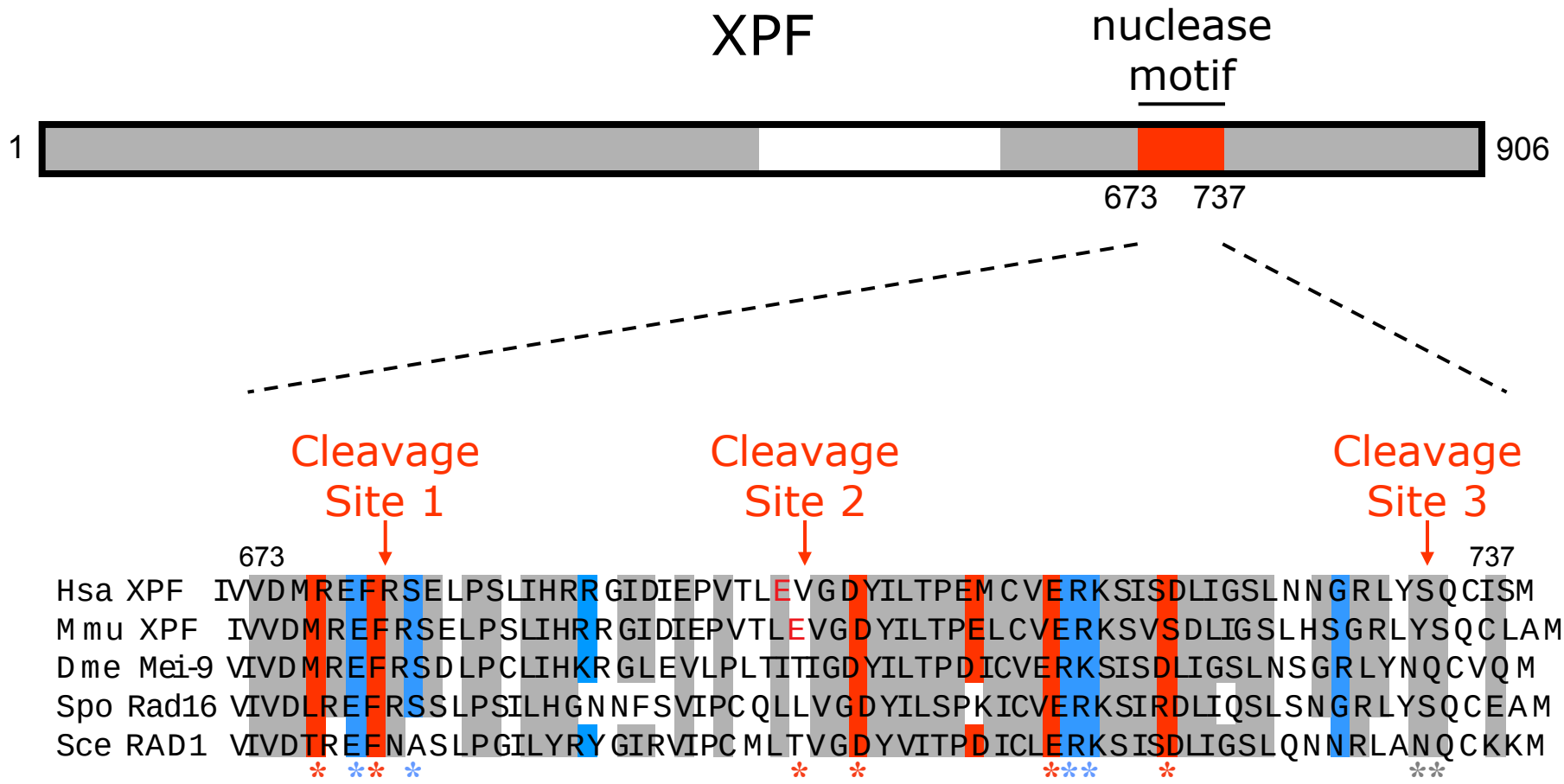
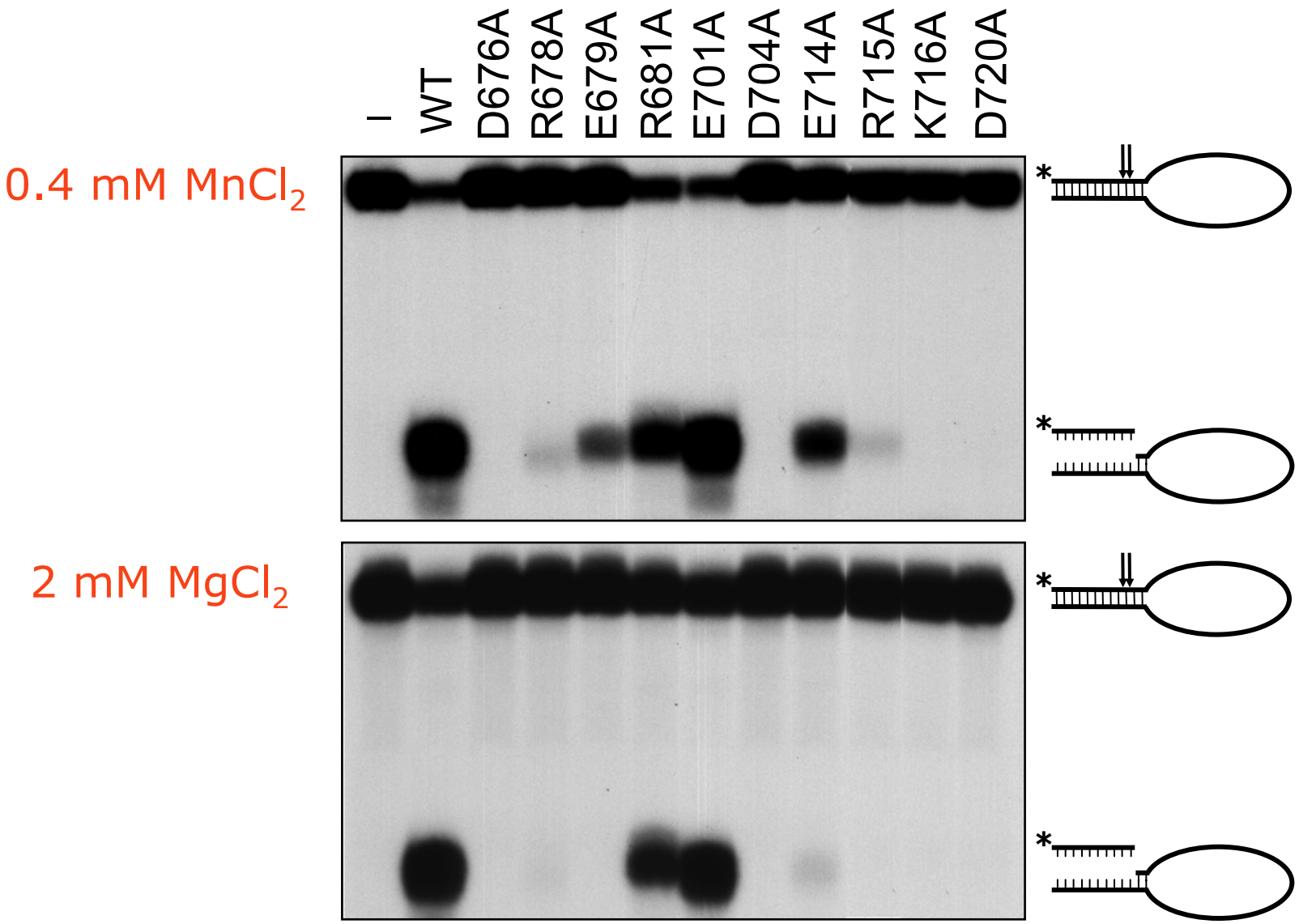
IRON-MEDIATED CLEAVAGE SITES IN XPF



ACTIVITY OF XPF ACTIVE SITE MUTANTS



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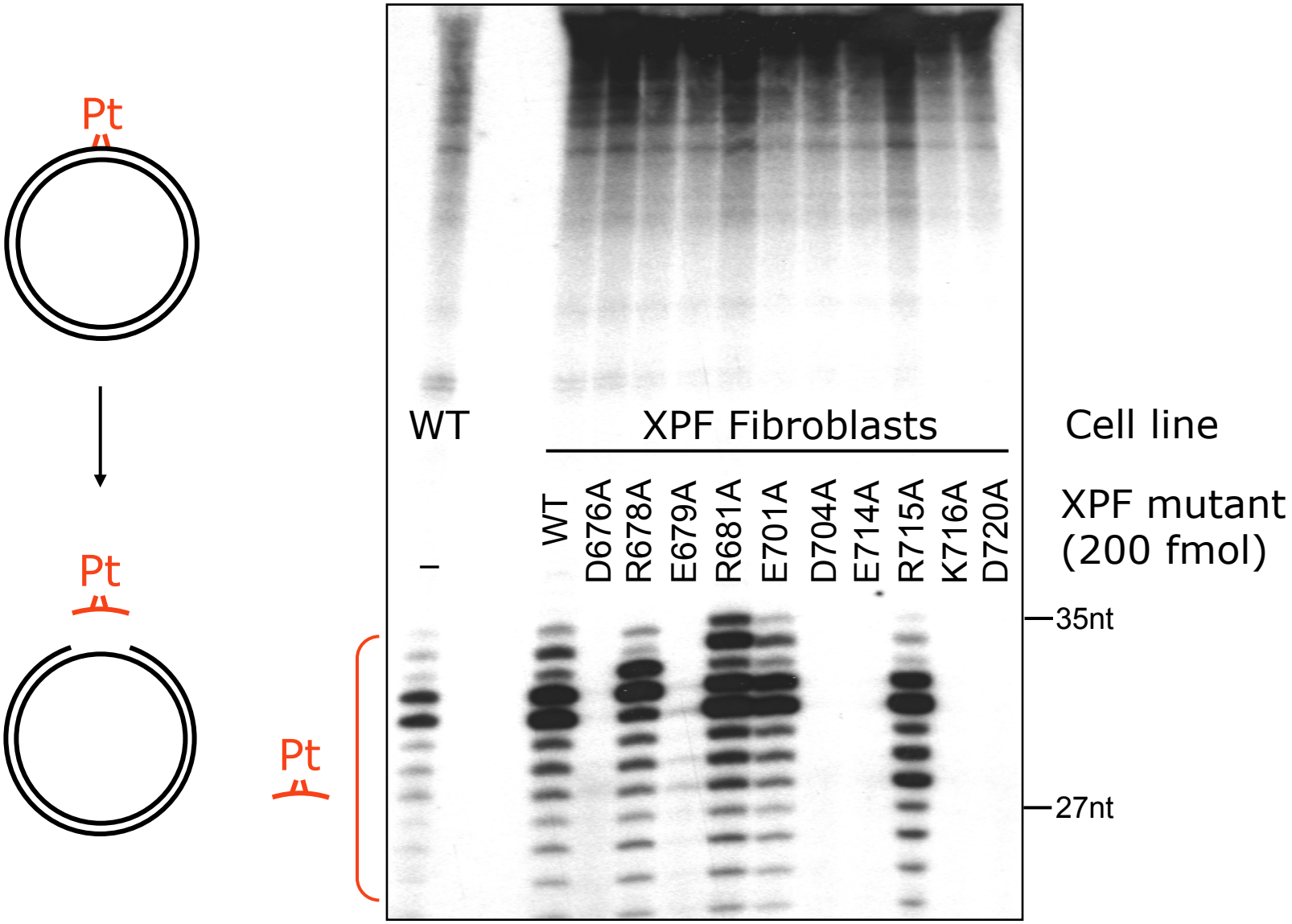
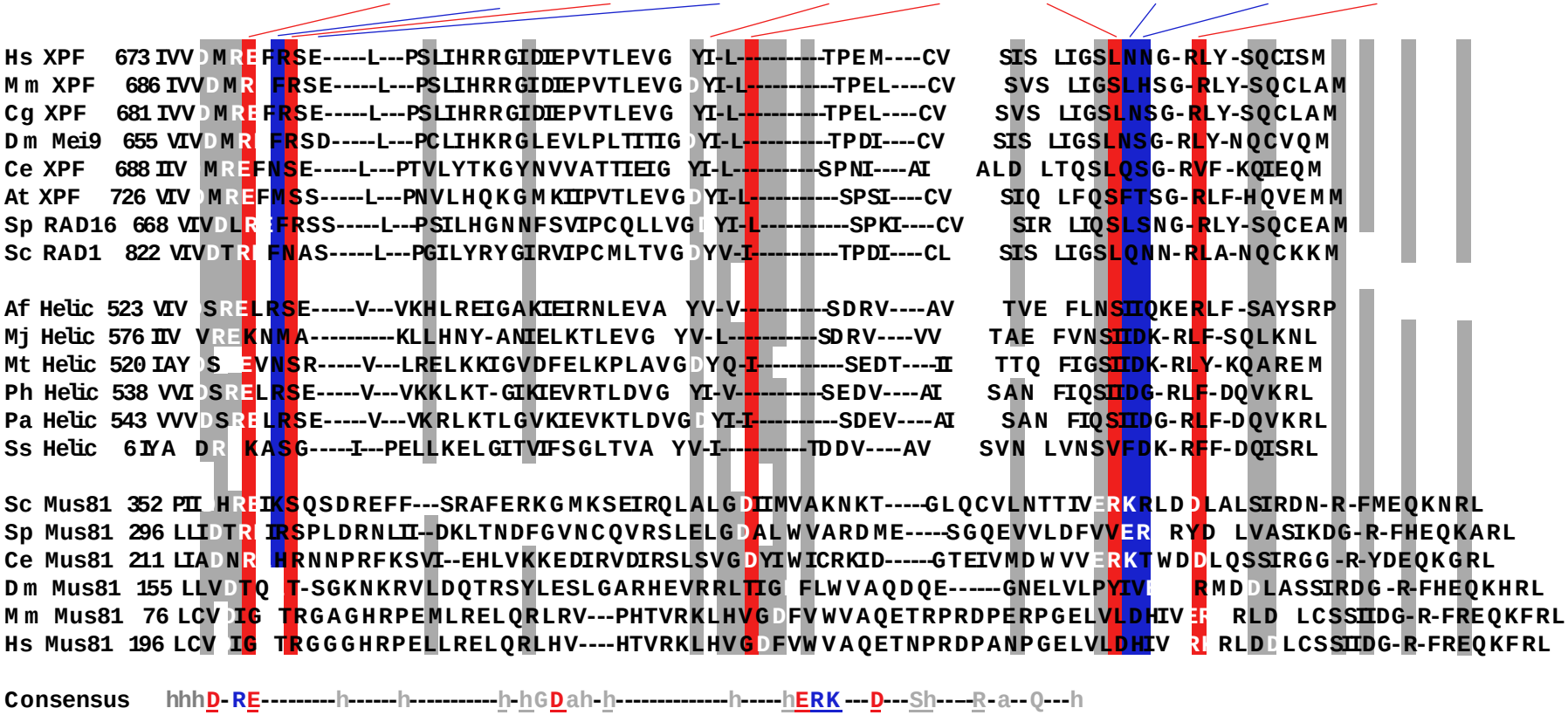
IN VITRO NER ASSAY WITH XPF MUTANTS



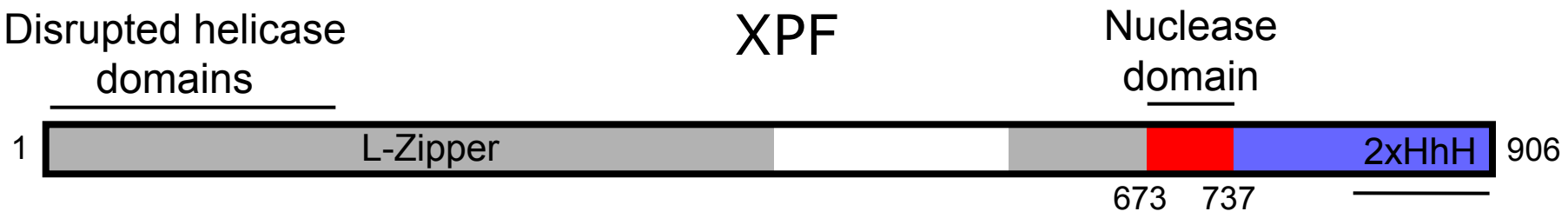
A CONSERVED NUCLEASE MOTIF IN XPF

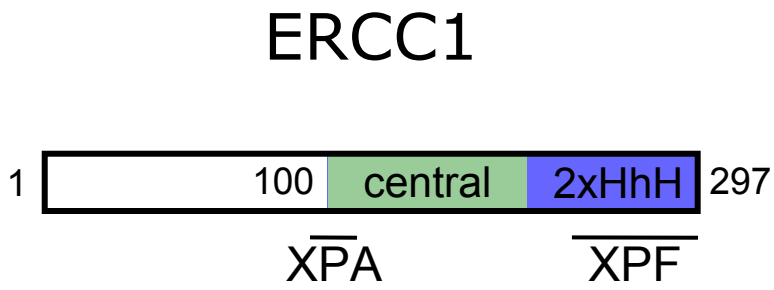
Residue	D676	R678	E679	R681	E701	D704	E714	R715	K716	D720
Nuclease activity	-	(+)	+	++	++	-	+	(+)	-	-
Cleavage by Fe^{2+}	-	+	-	+	+	-	-	+	+	+
DNA binding	+	+	+	+	+	+	+	+	+	+
In vitro NER activity	-	+	-	+	+	-	-	+	-	-

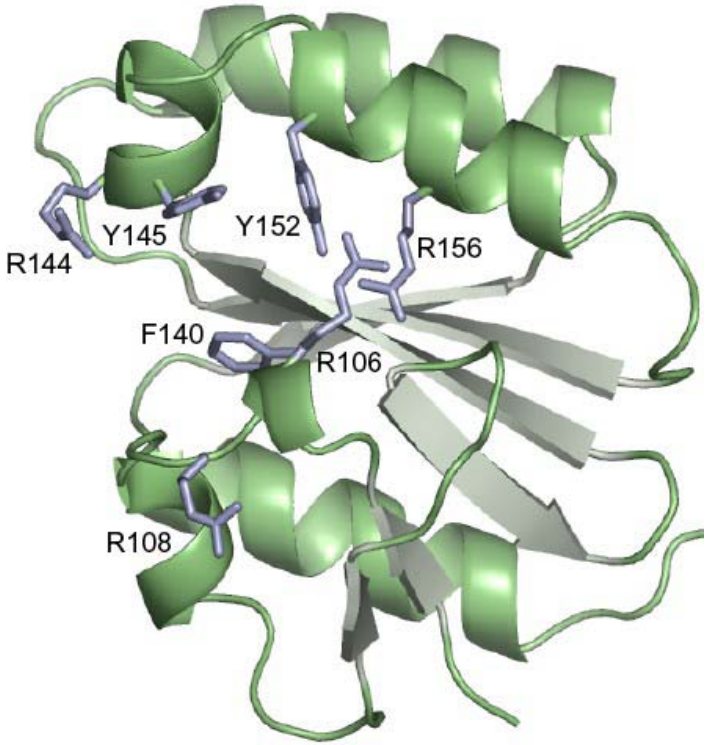


Enzlin JH, Schärer OD **EMBO J.** *21*, 2045-2053(2002)

DNA BINDING DOMAINS OF ERCC1/XPF

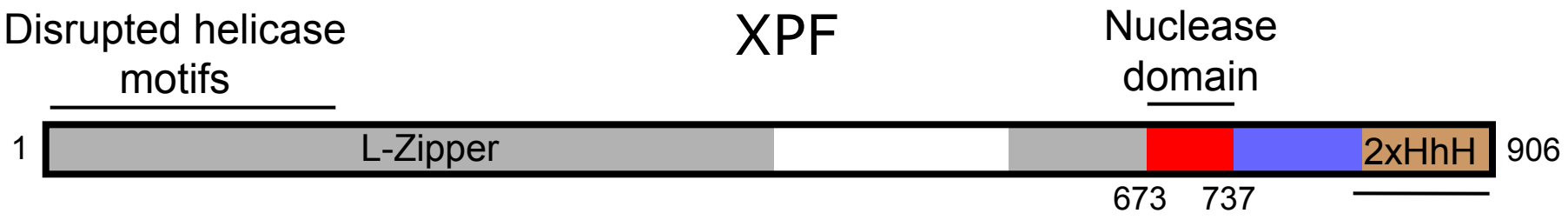


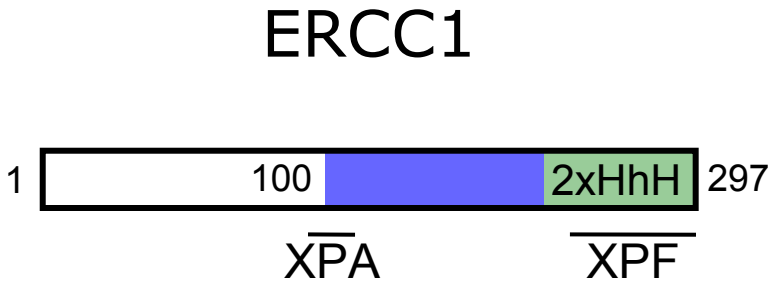


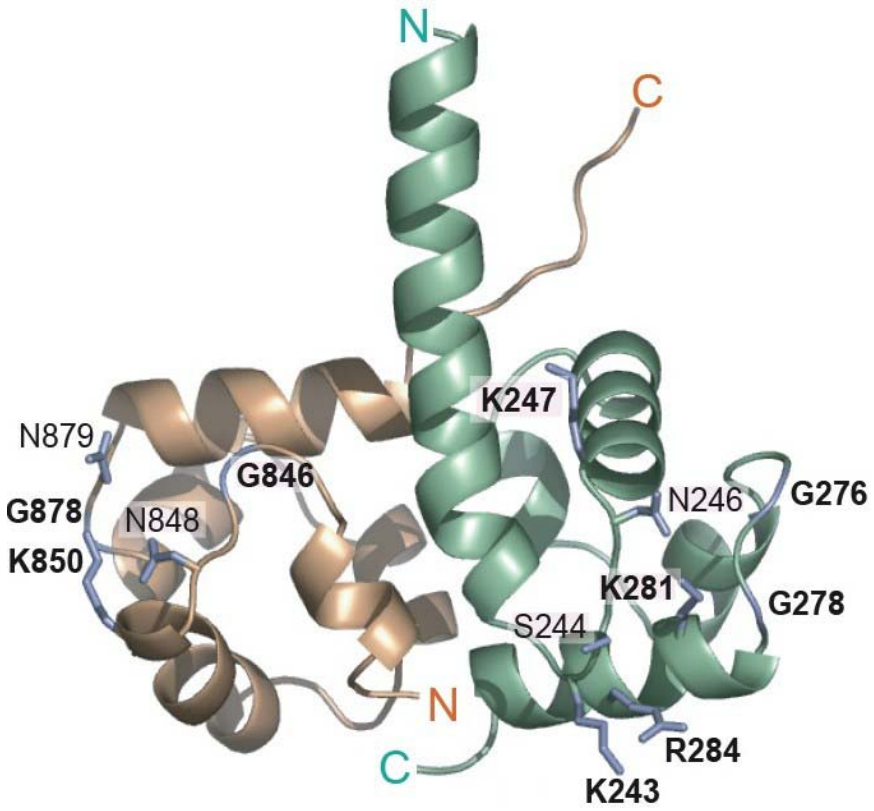


Tsodikov OV, Enzlin JH, Schärer OD, Ellenberger T **PNAS** *102*, 11236 (2005)

DNA BINDING DOMAINS OF ERCC1/XPF

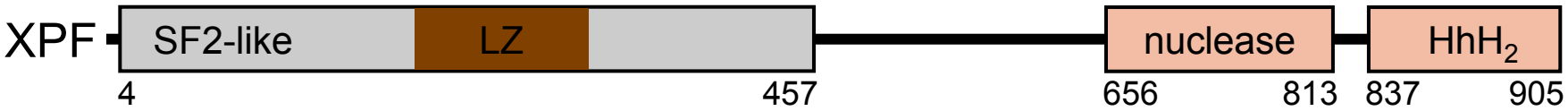


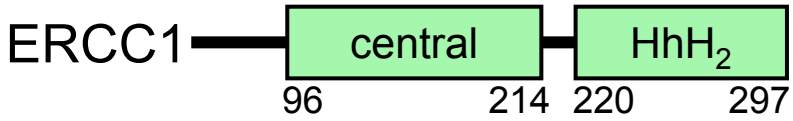


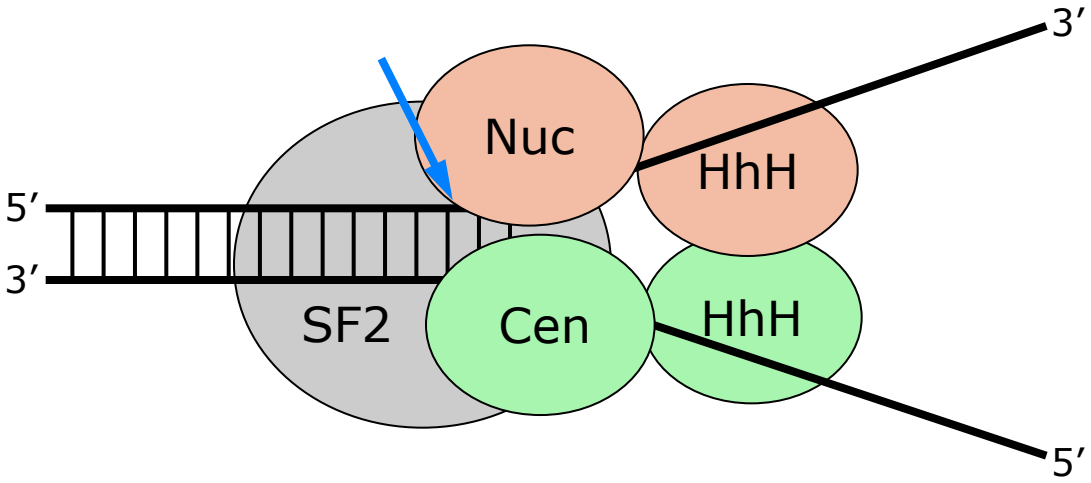


Tsodikov OV, Enzlin JH, Schärer OD, Ellenberger T **PNAS** *102*, 11236 (2005)

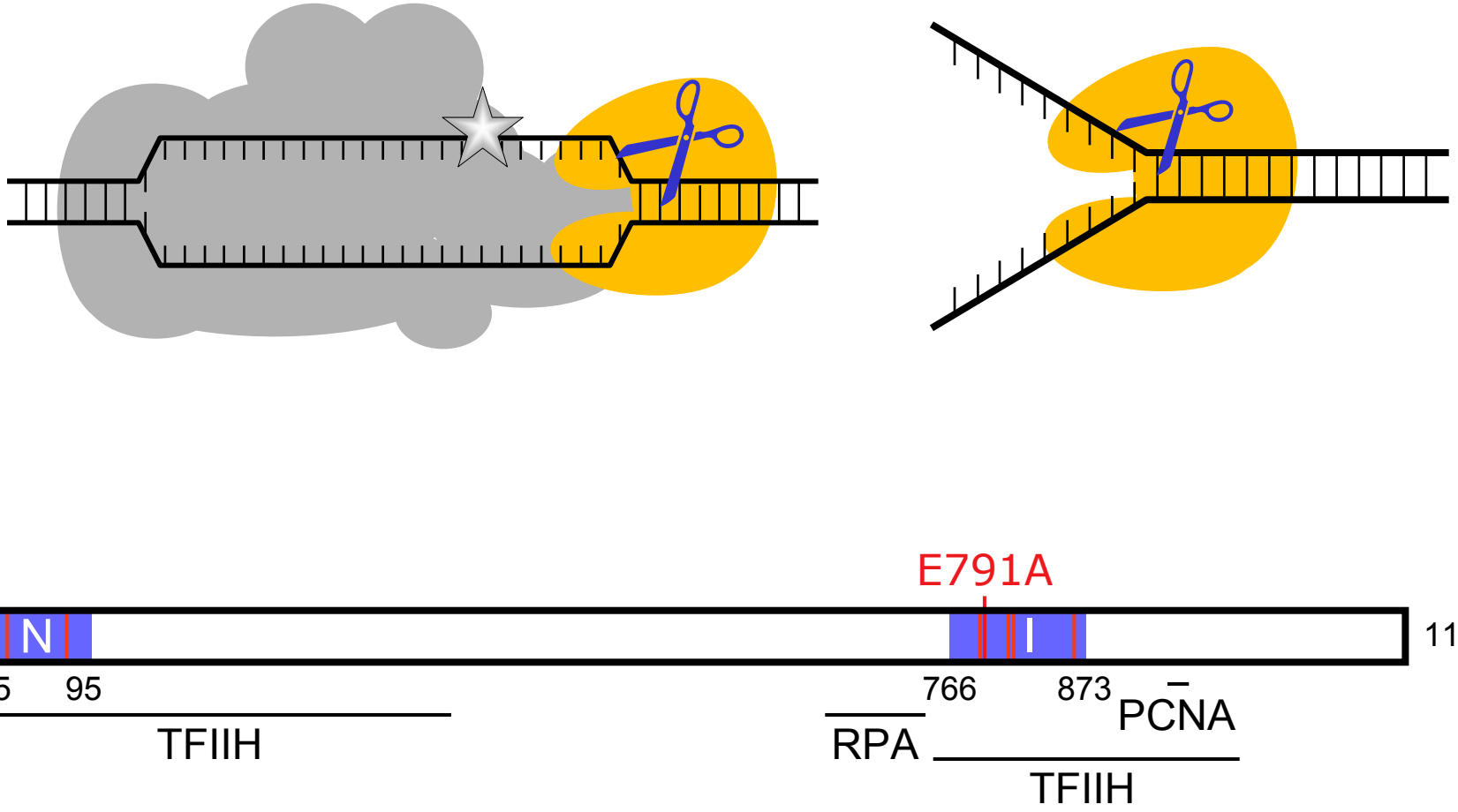
A MODEL FOR DNA BINDING BY ERCC1/XPF



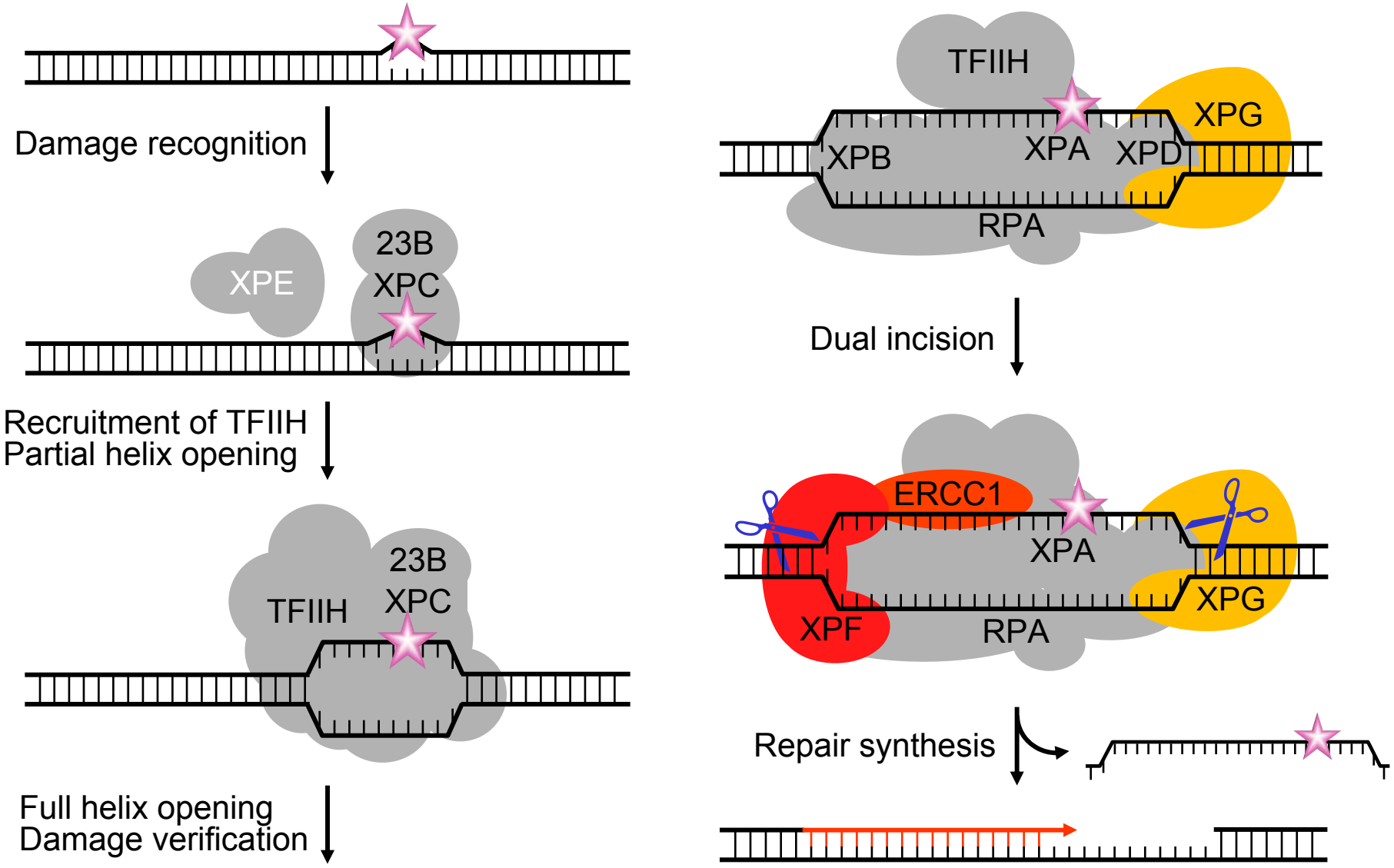




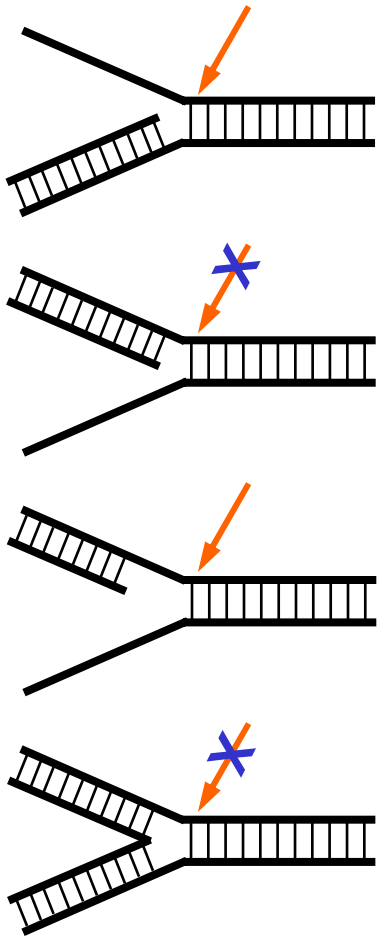
THE XPG ENDONUCLEASE



ENDONUCLEASES IN NER



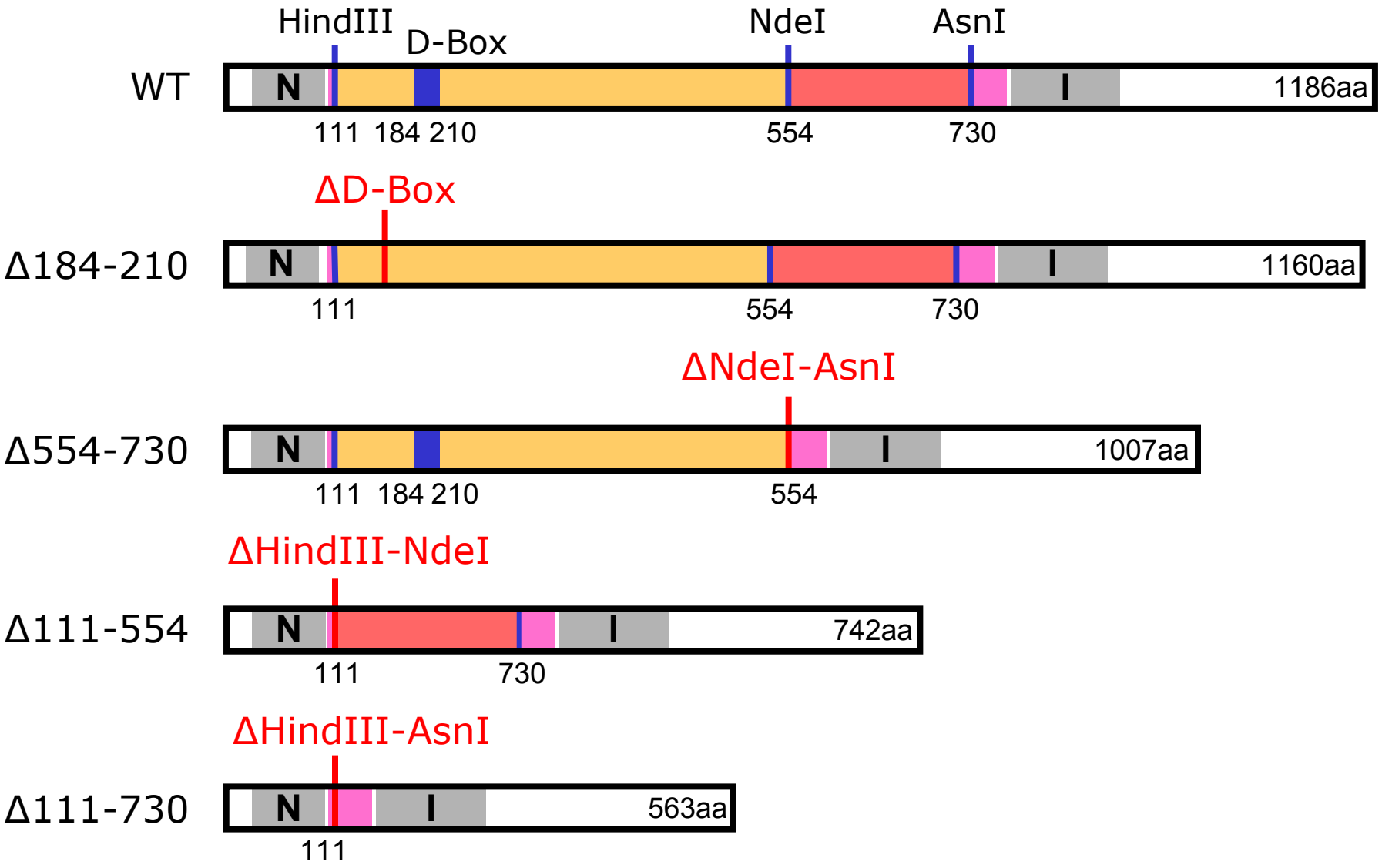
XPG SUBSTRATE BINDING AND CLEAVAGE



Cleavage	Binding
+	+
–	+
+	+
–	++

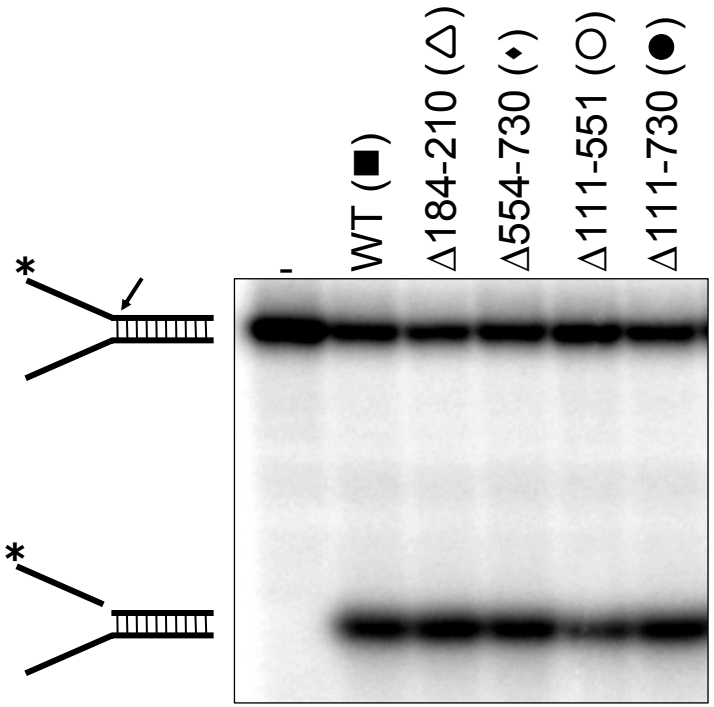
Hohl, M., Thorel, F., Clarkson, S.G., Schärer, O.D. (2003) **JBC** *278*, 19500.

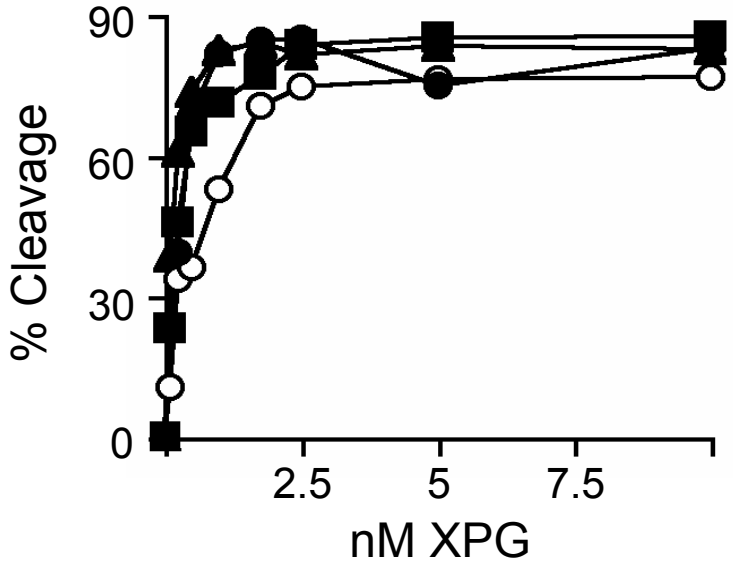
DELETIONS IN THE SPACER REGION OF XPG

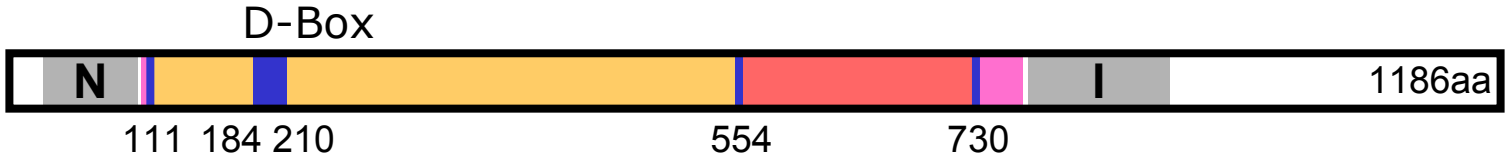


Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

ACTIVITY OF XPG MUTANTS ON Y SUBSTRATES

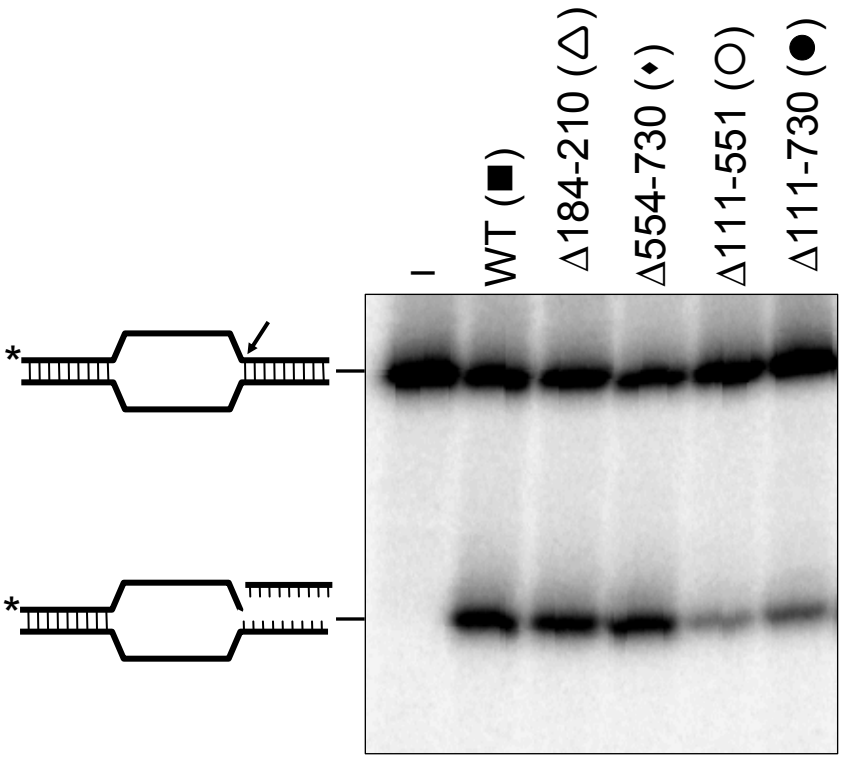


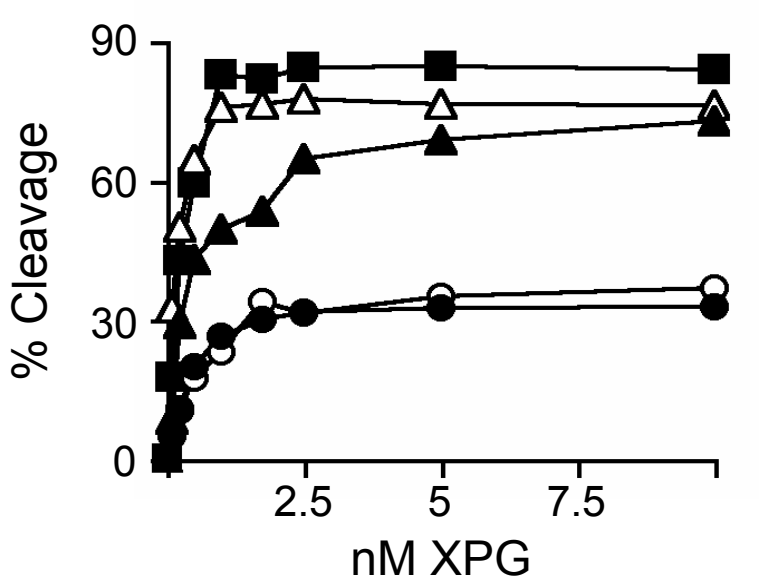


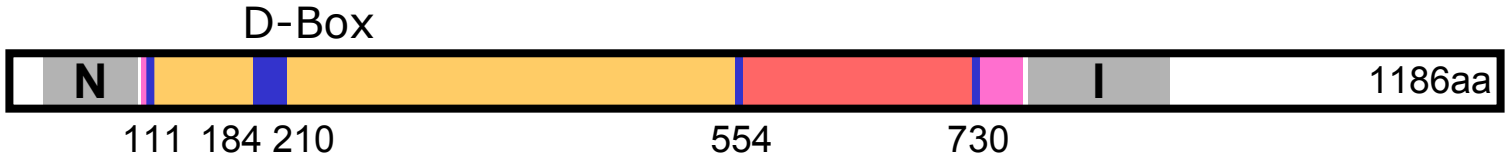


Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

ACTIVITY OF XPG MUTANTS ON BUBBLES

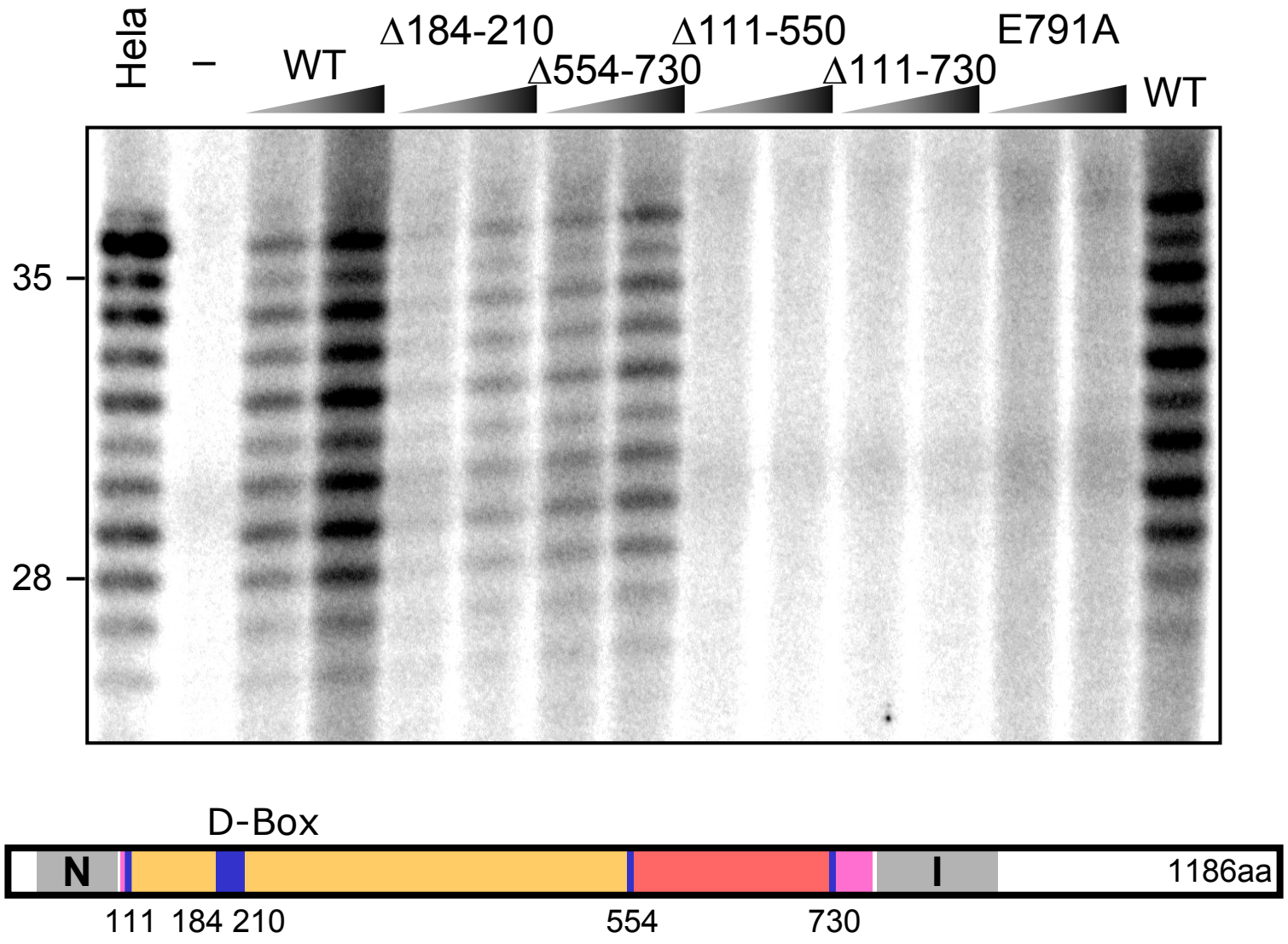






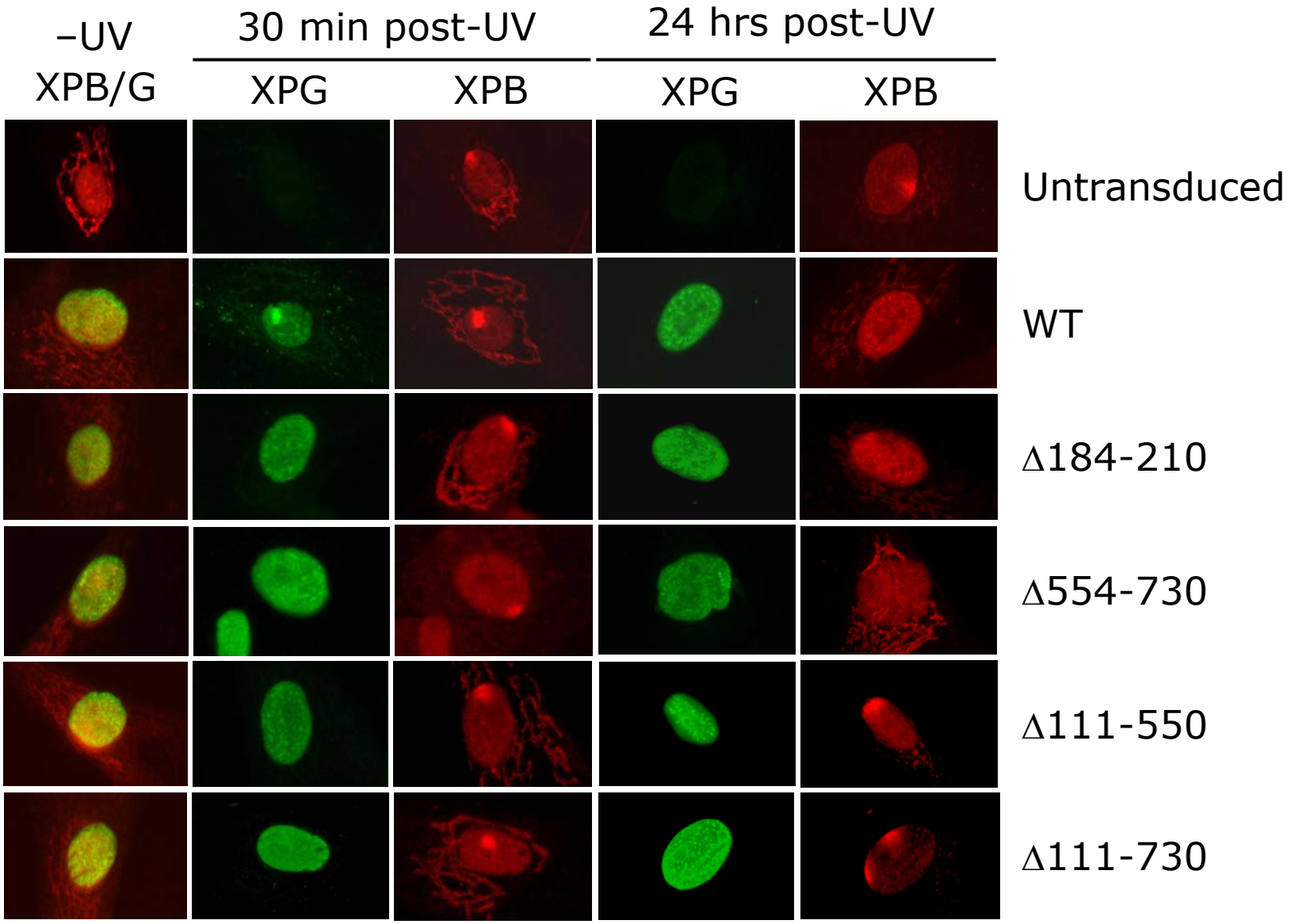
Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

IN VITRO NER ACTIVITY OF XPG MUTANTS



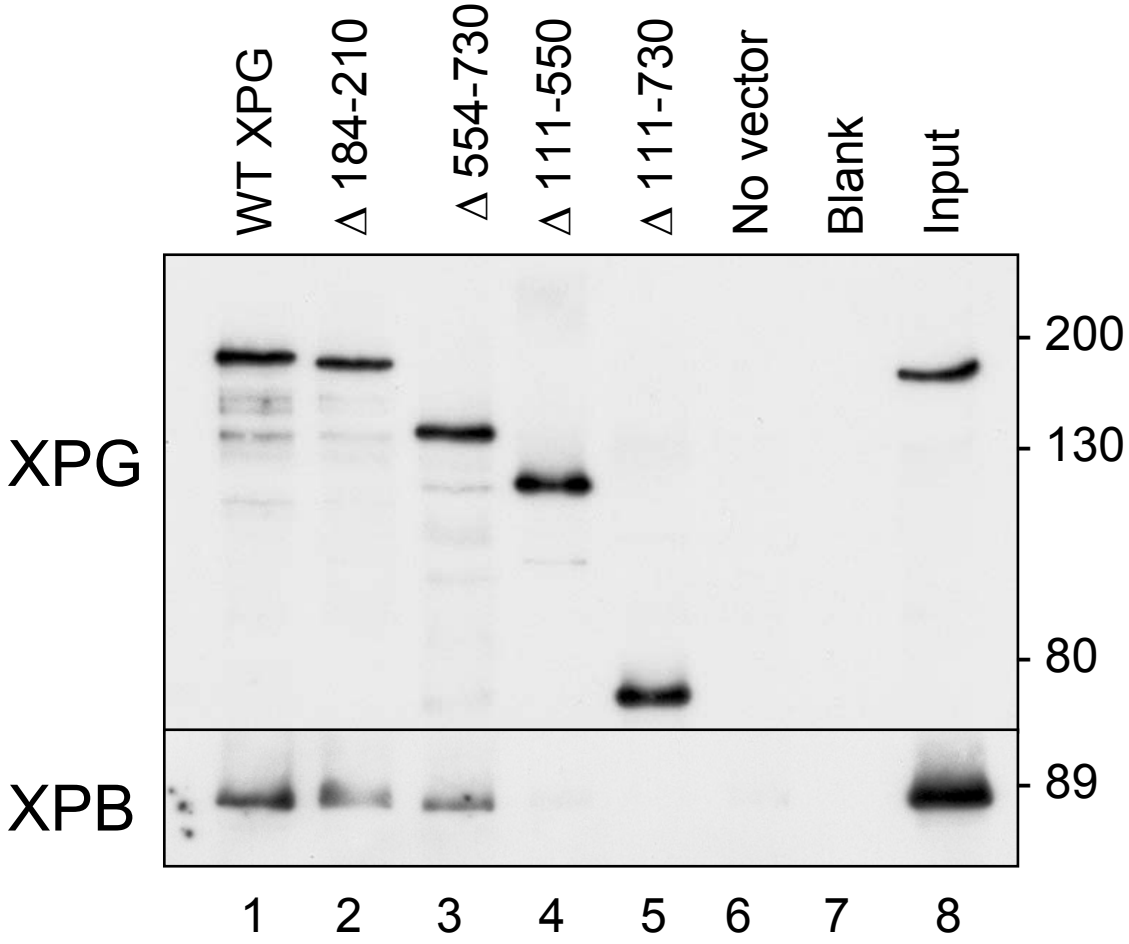
Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

MUTANT XPG IS NOT FOUND IN NER SITES



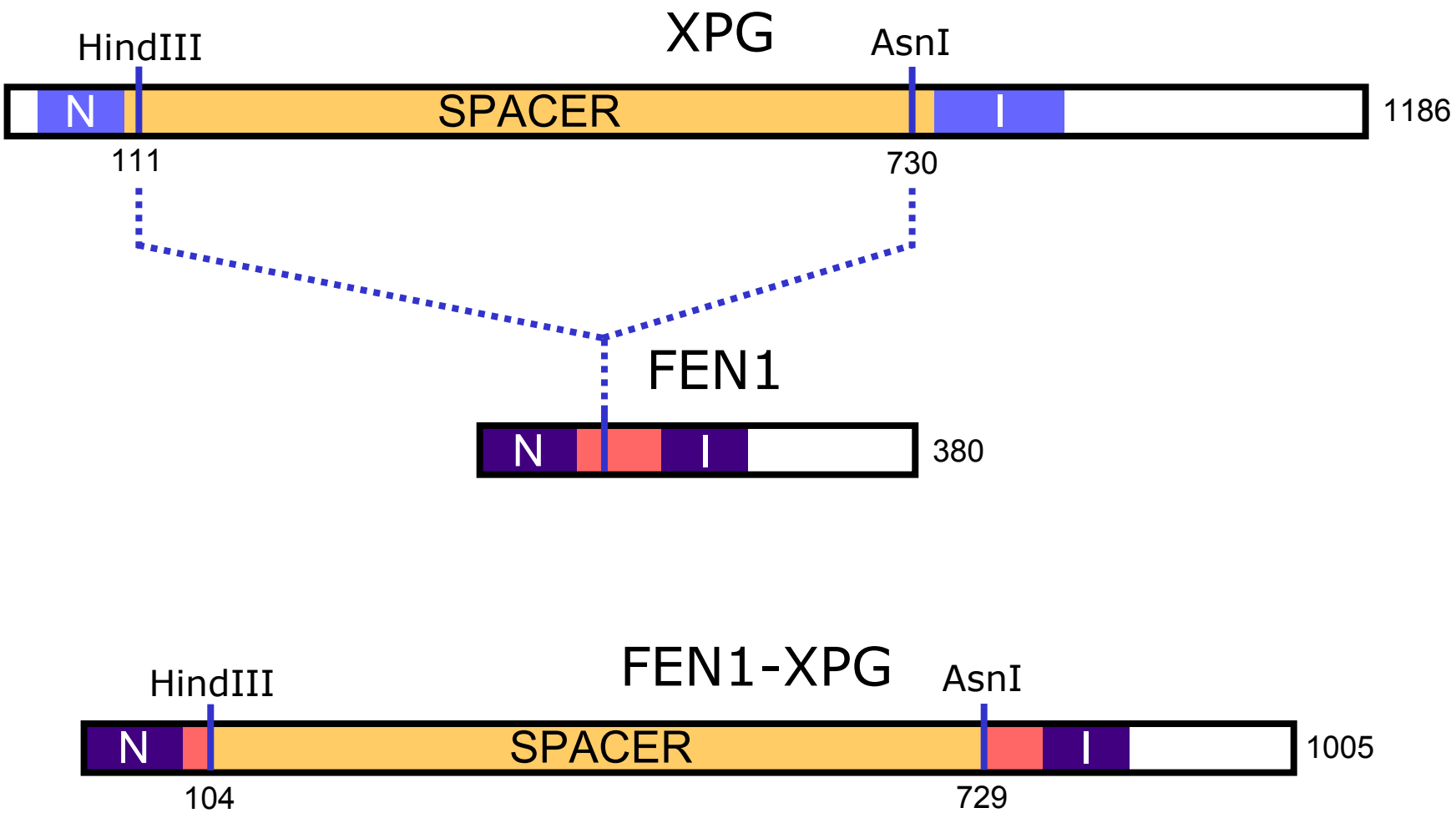
Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

MUTANT XPG HAS LOWER AFFINITY FOR TFIIH

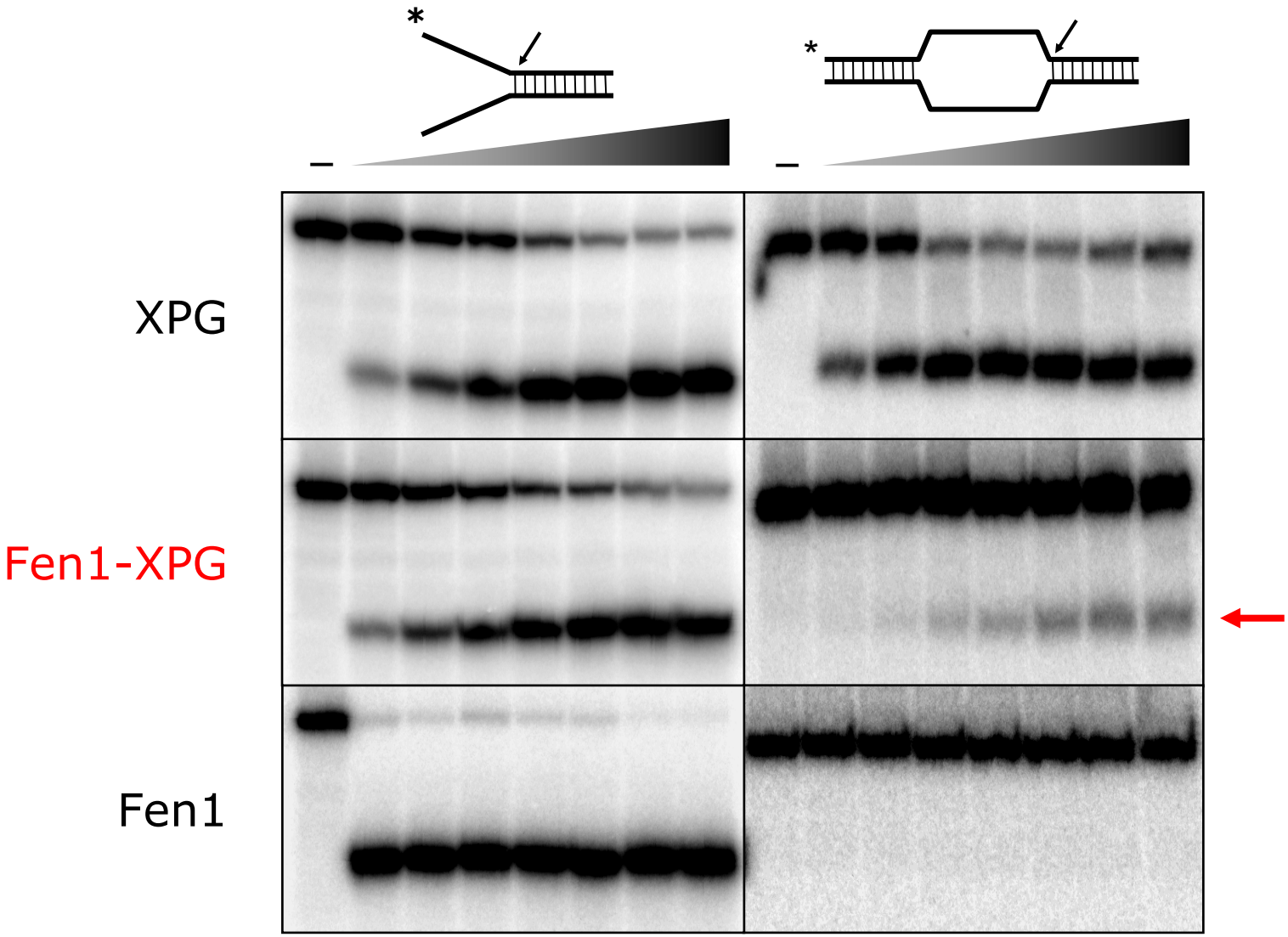


Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) **JBC** *280*, 7030

A FEN1-XPG HYBRID PROTEIN

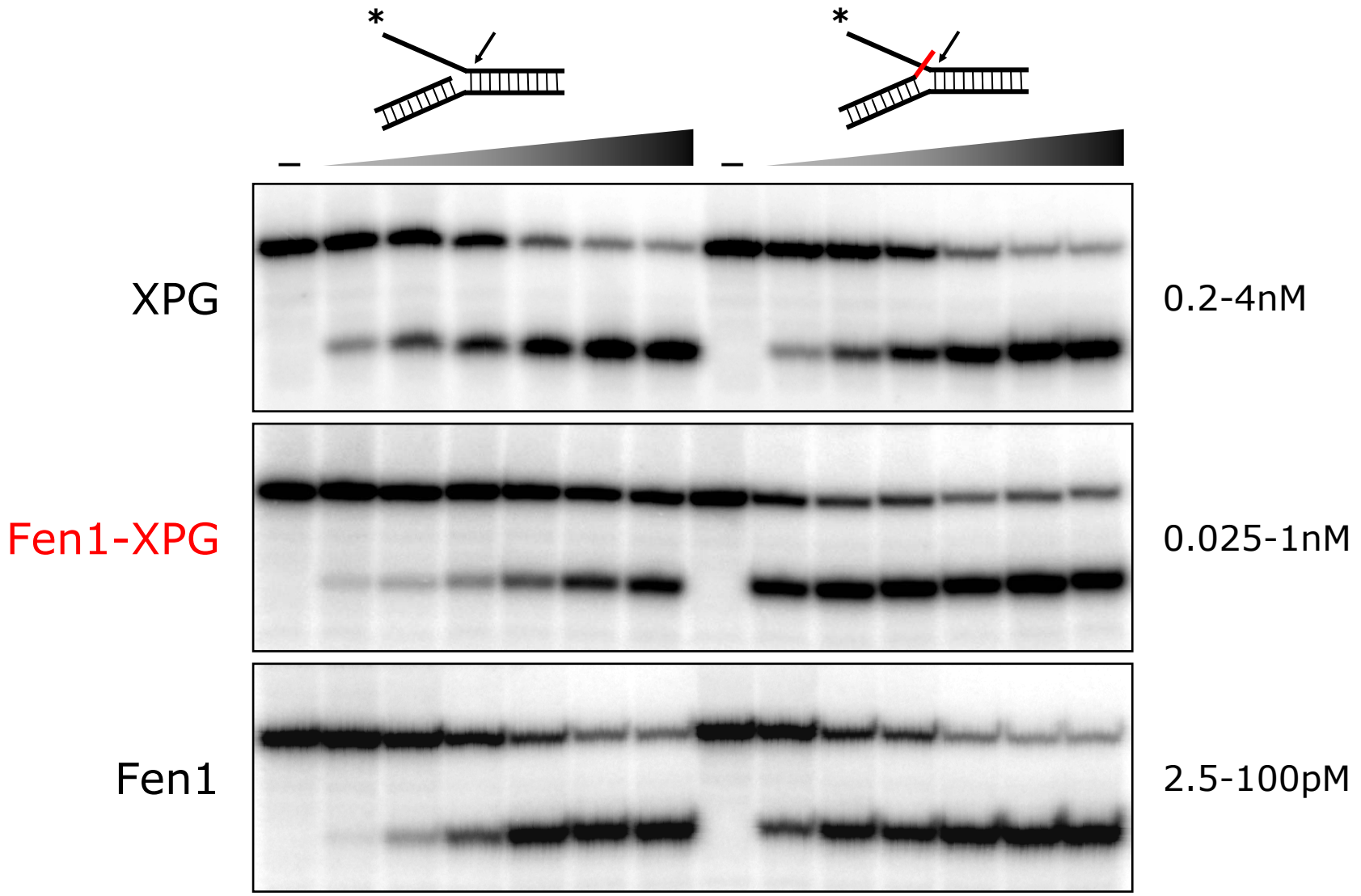


FEN-1/XPG CLEAVES BUBBLE SUBSTRATES



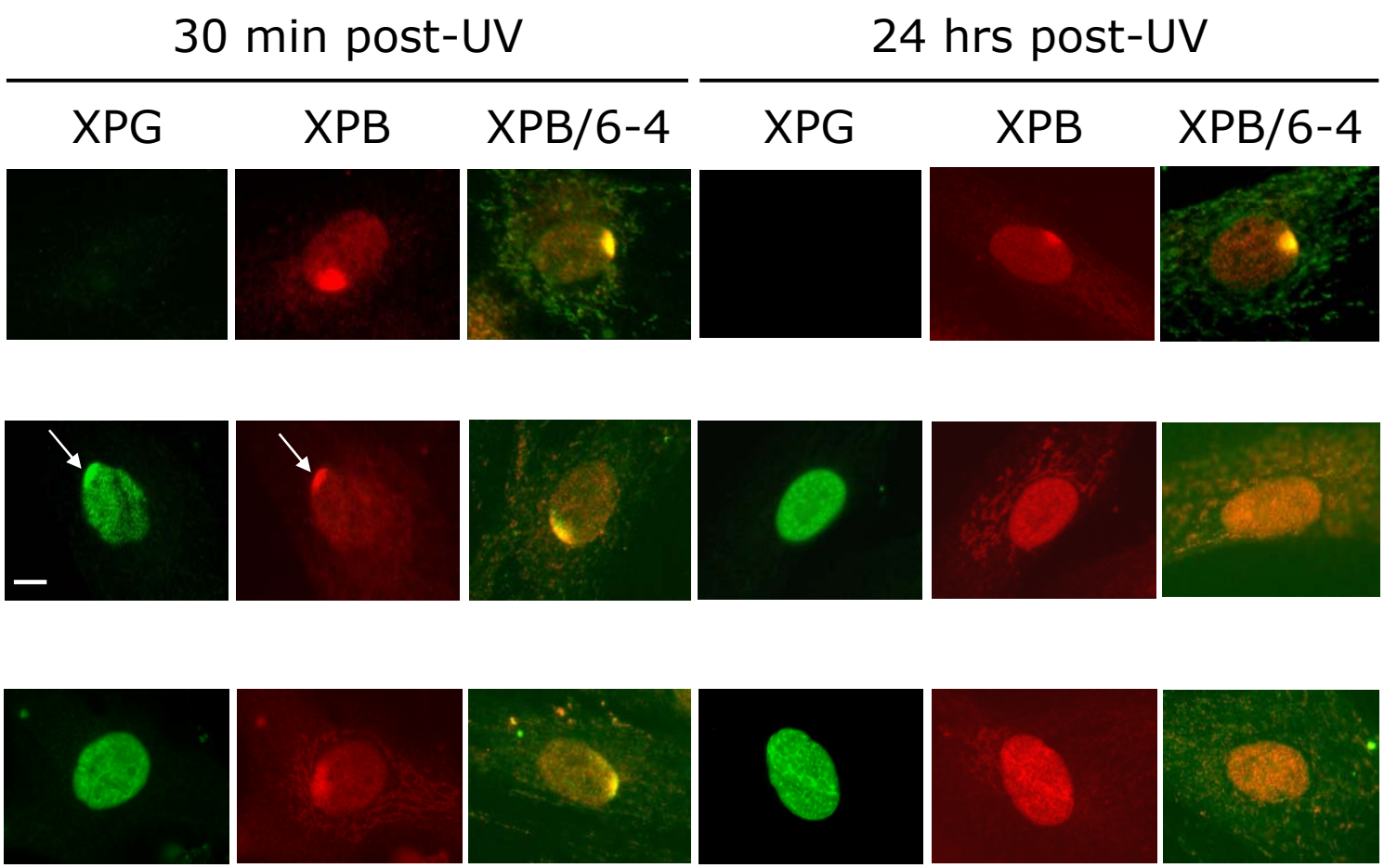
Marcel Hohl

FEN1-XPG PREFERS DOUBLE FLAPS



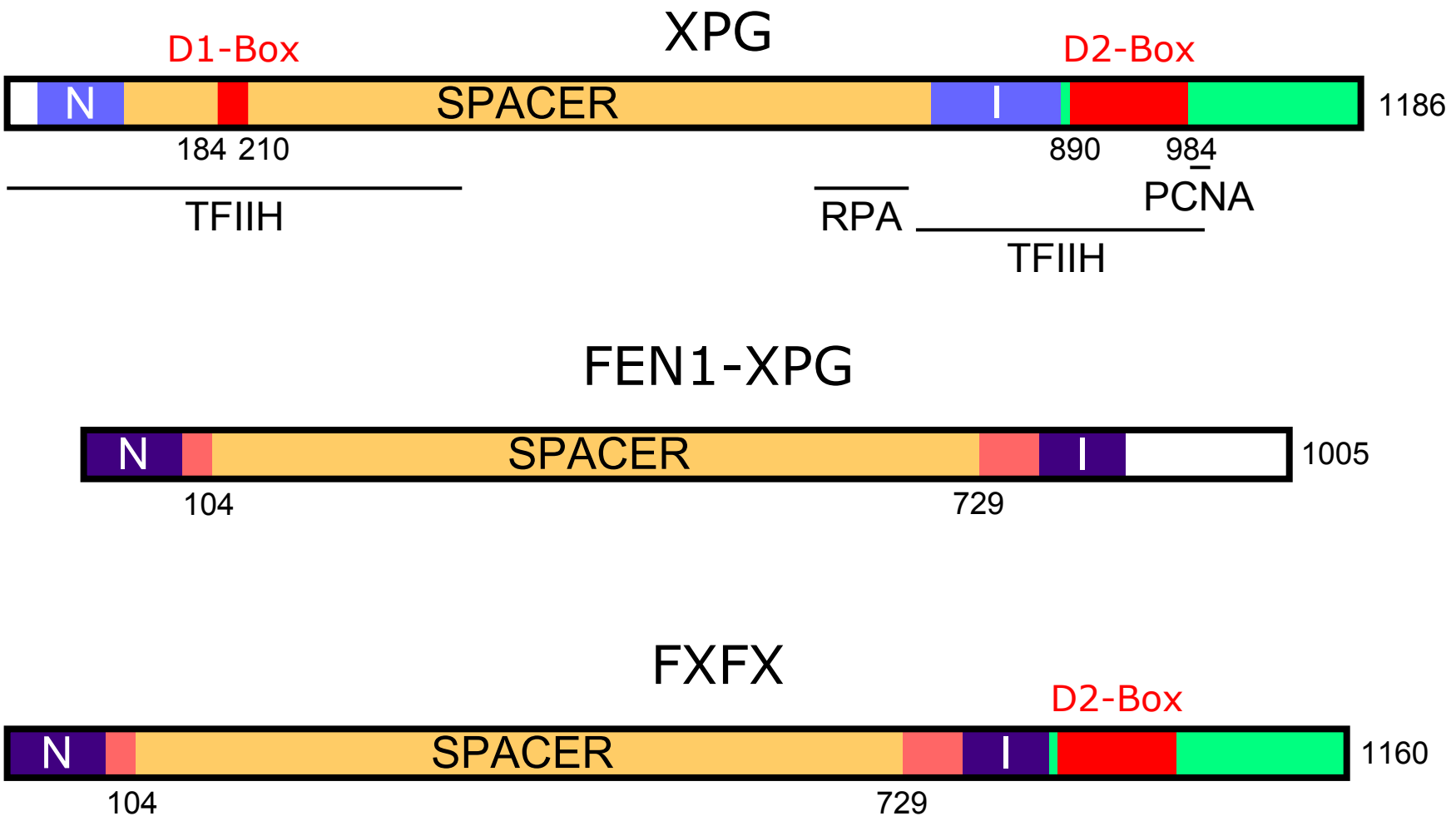
Marcel Hohl

FEN-1/XPG IS NOT FOUND IN NER SITES

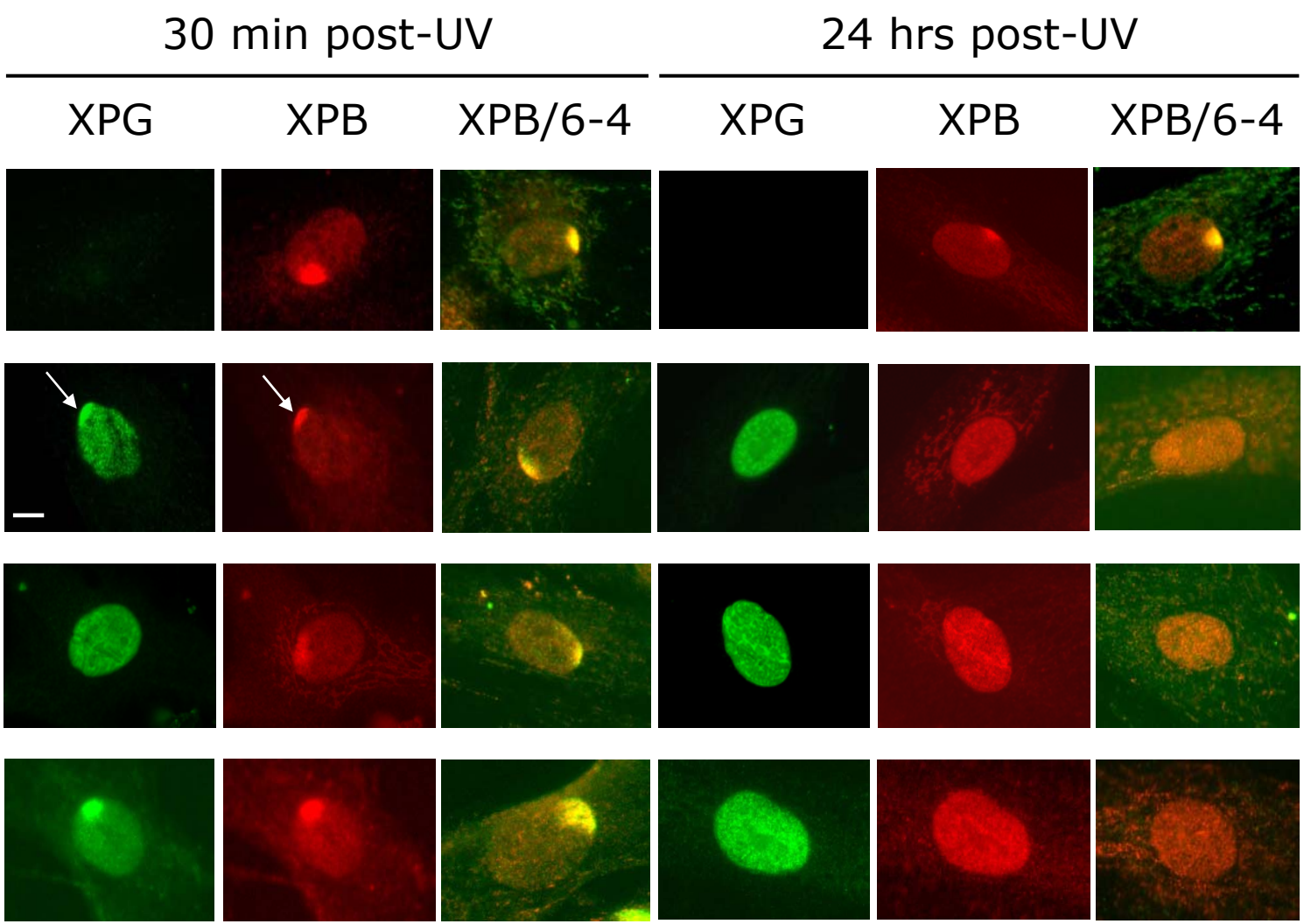


Isabelle Dunand-Sauthier

A ROLE FOR THE C-TERMINUS OF XPG IN NER?

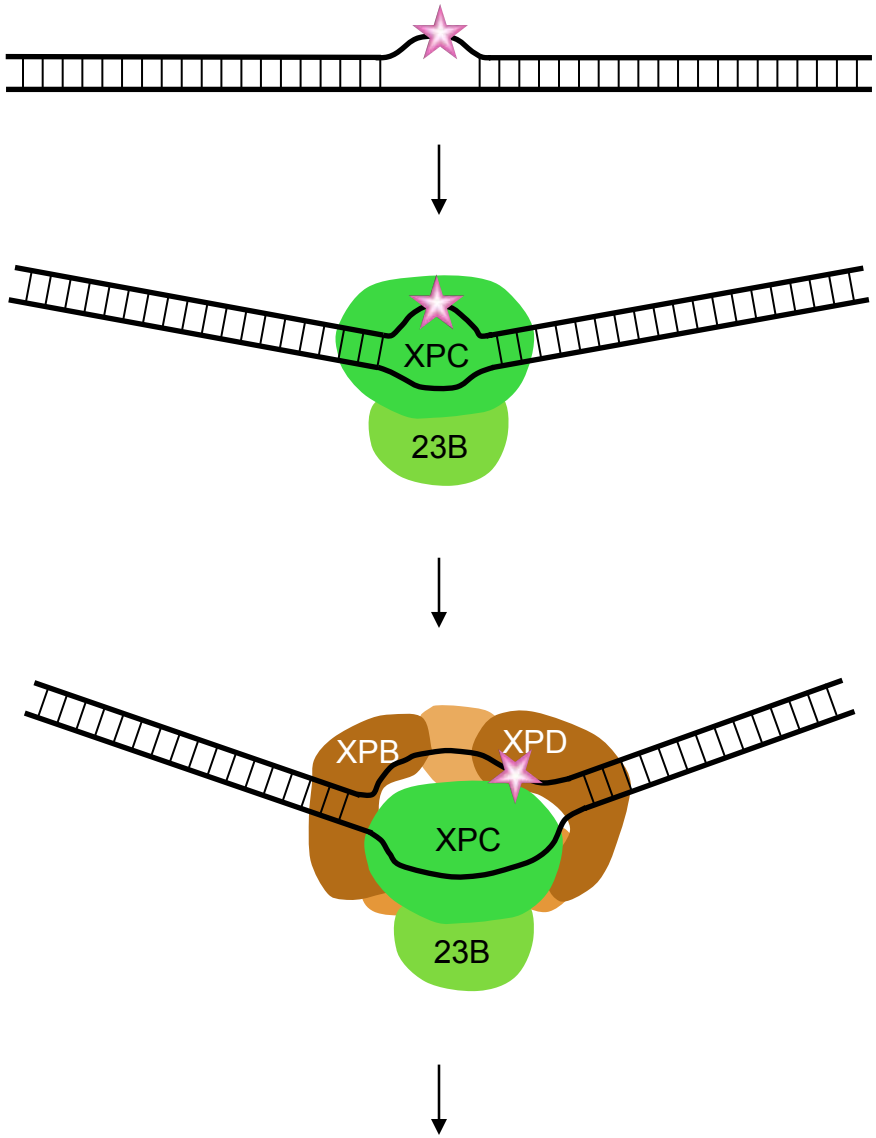


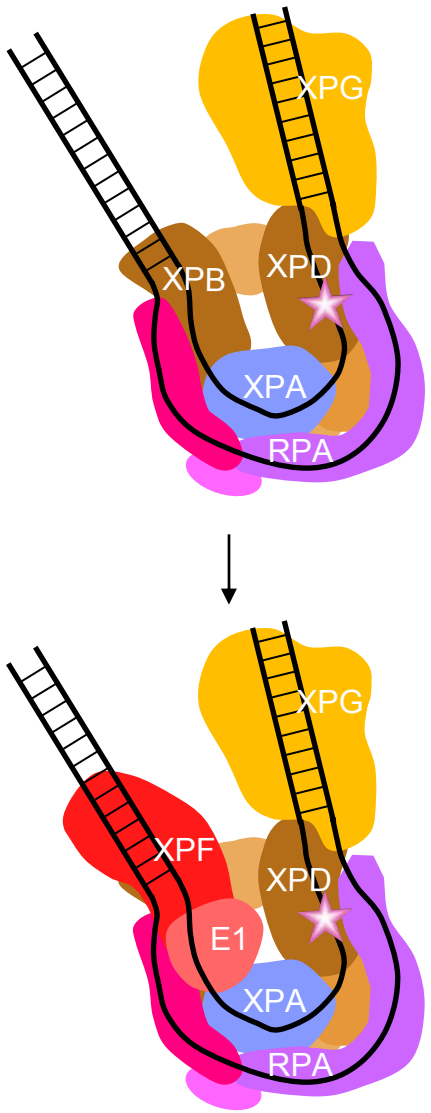
FXFX, BUT NOT FX IS FOUND IN NER SITES



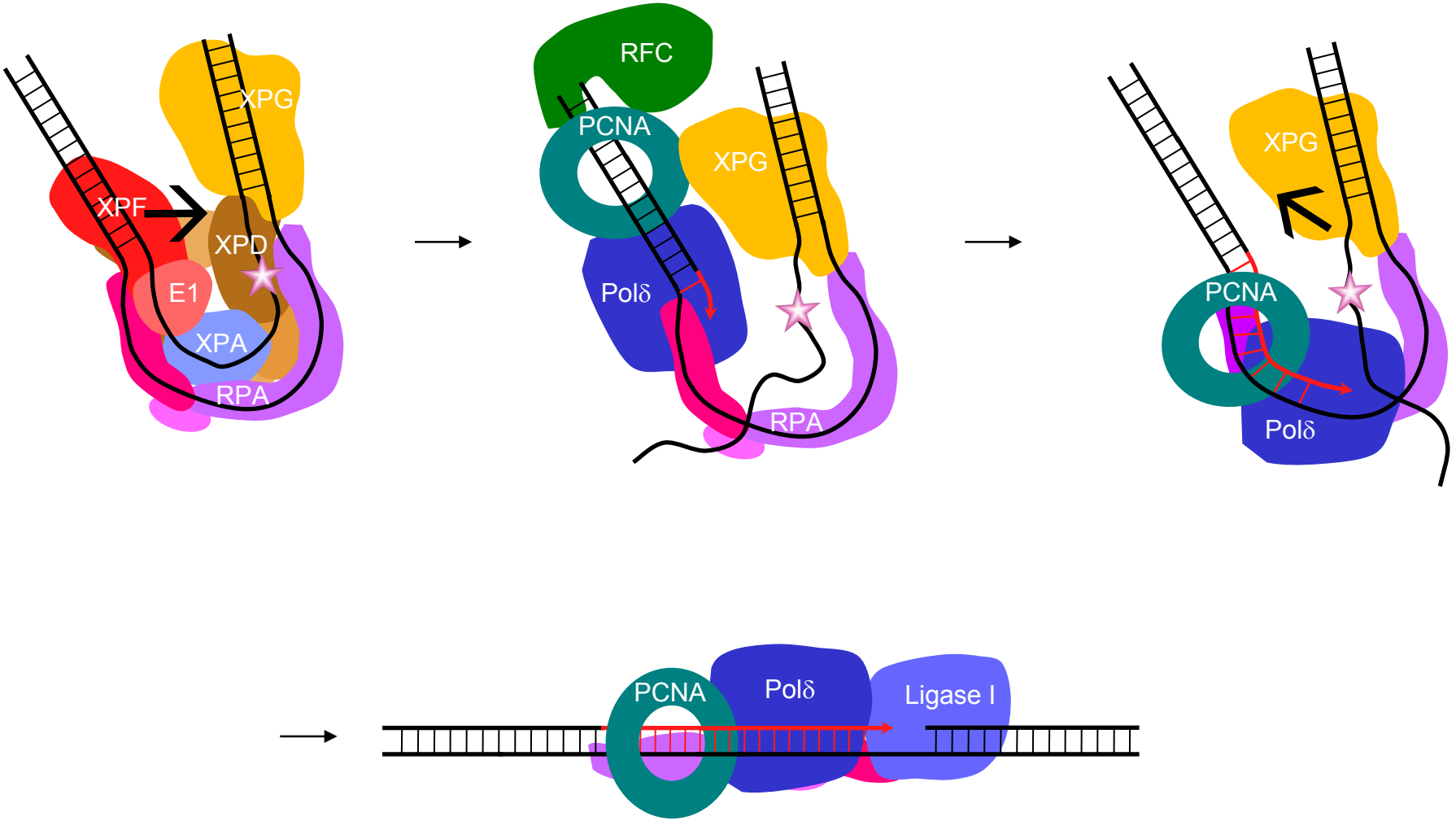
Isabelle Dunand-Sauthier

MODEL FOR NER COMPLEX FORMATION





FROM NER INCISION TO REPAIR SYNTEHSIS



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