Genome Editing and Engineering

Course No: BT-637

LECTURE-5

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Introduction

- **FokI (Flavobacterium okeanokoites)**
- **578 aa; 64.5 kDa; Monomer**
- **Recognizes asymmetric DNA and Cleaves 9/13 bp away**
- **Foot printing “protection of rec. site”**
- **Cleavage site is active in presence of Mg^{+2}**
- **Single catalytic site = cleaves ds DNA??**

Structure of the multimodular endonuclease *Fok*I bound to DNA

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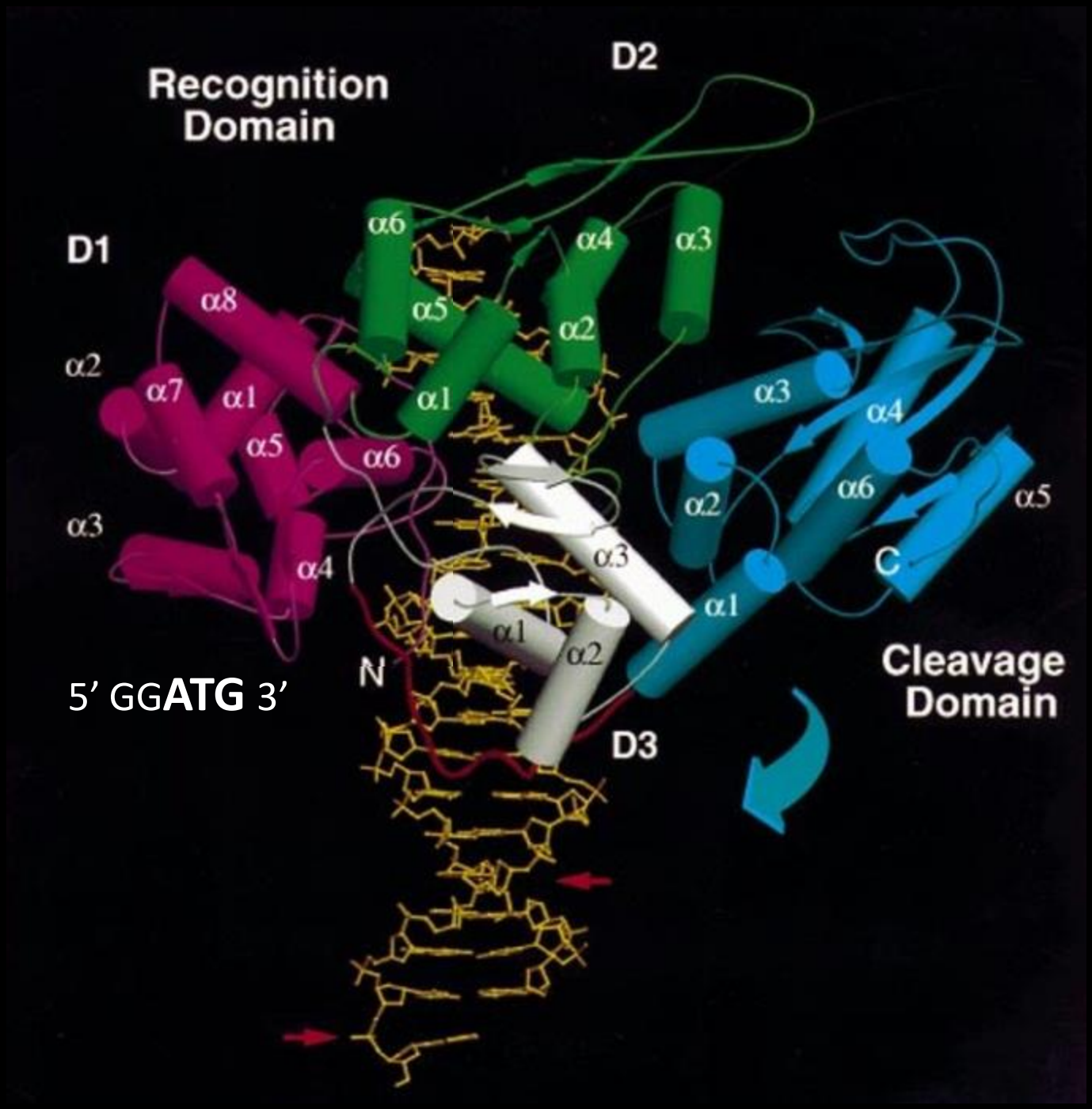
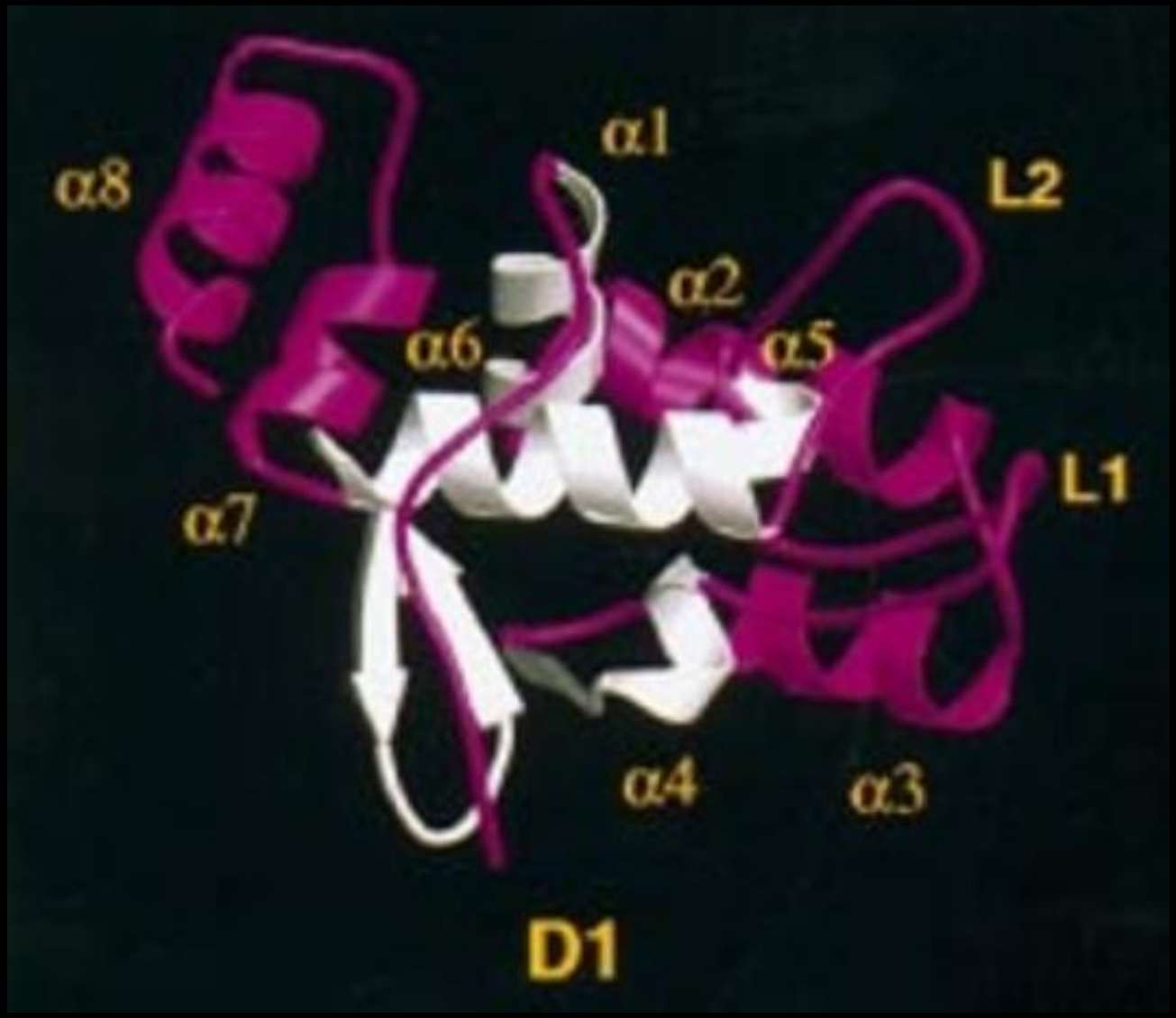
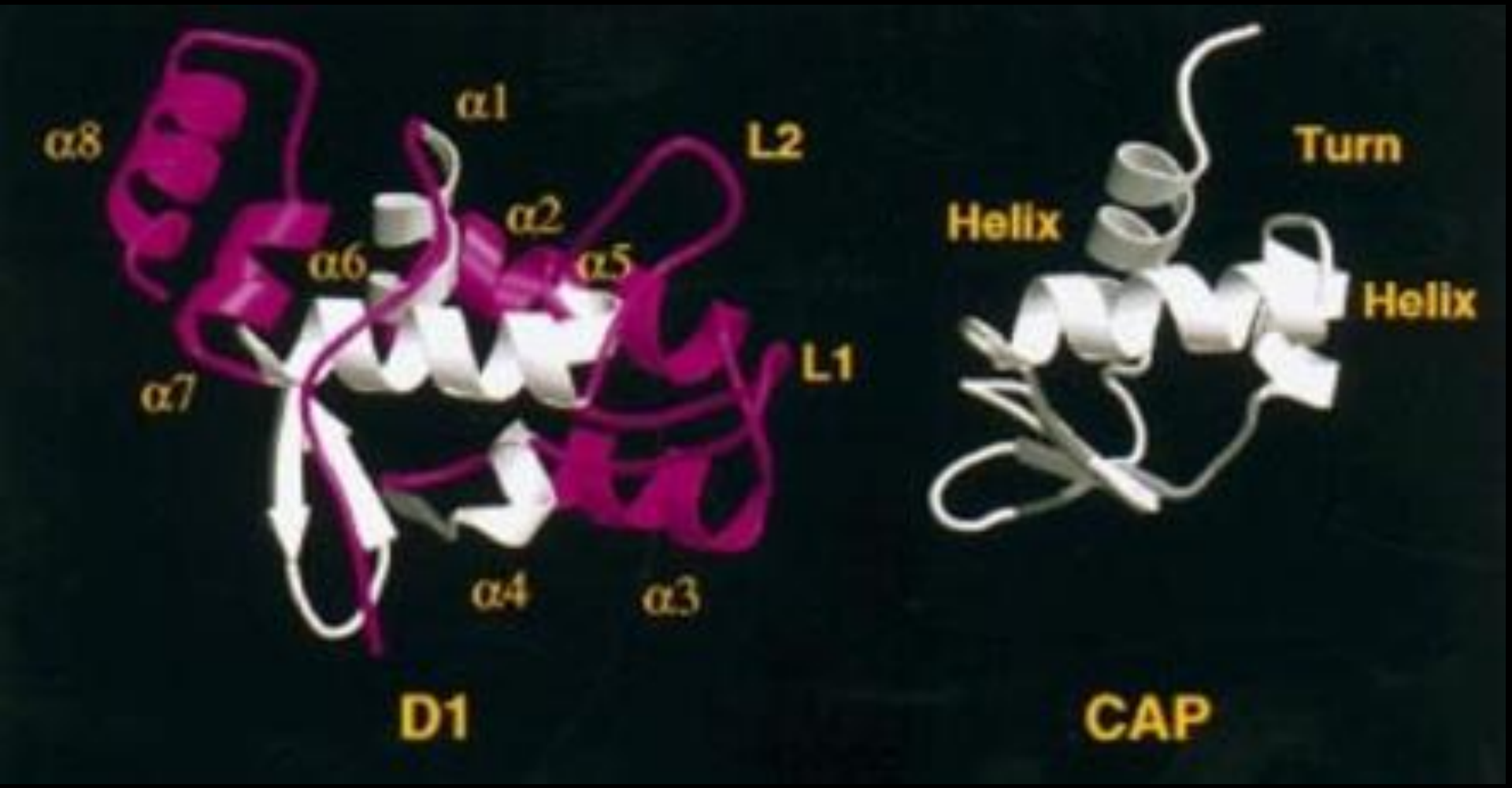
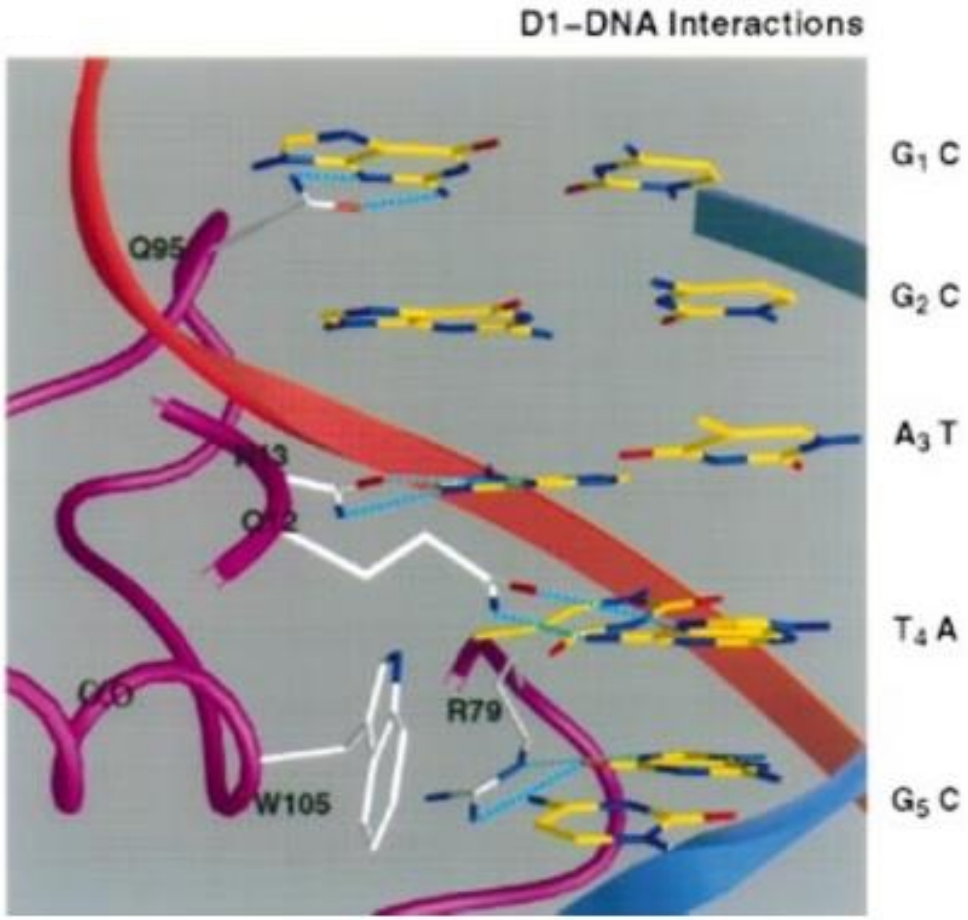
Macmillan


Recognition Domain
D2
D1
α6
α4
α3
α8
α5
α2
α2
α7
α1
α1
α3
α5
α6
α4
α2
α6
α5
α3
α4
C
α3
α1
α1
α2
N
Cleavage Domain
5’ GGATG 3’
D3

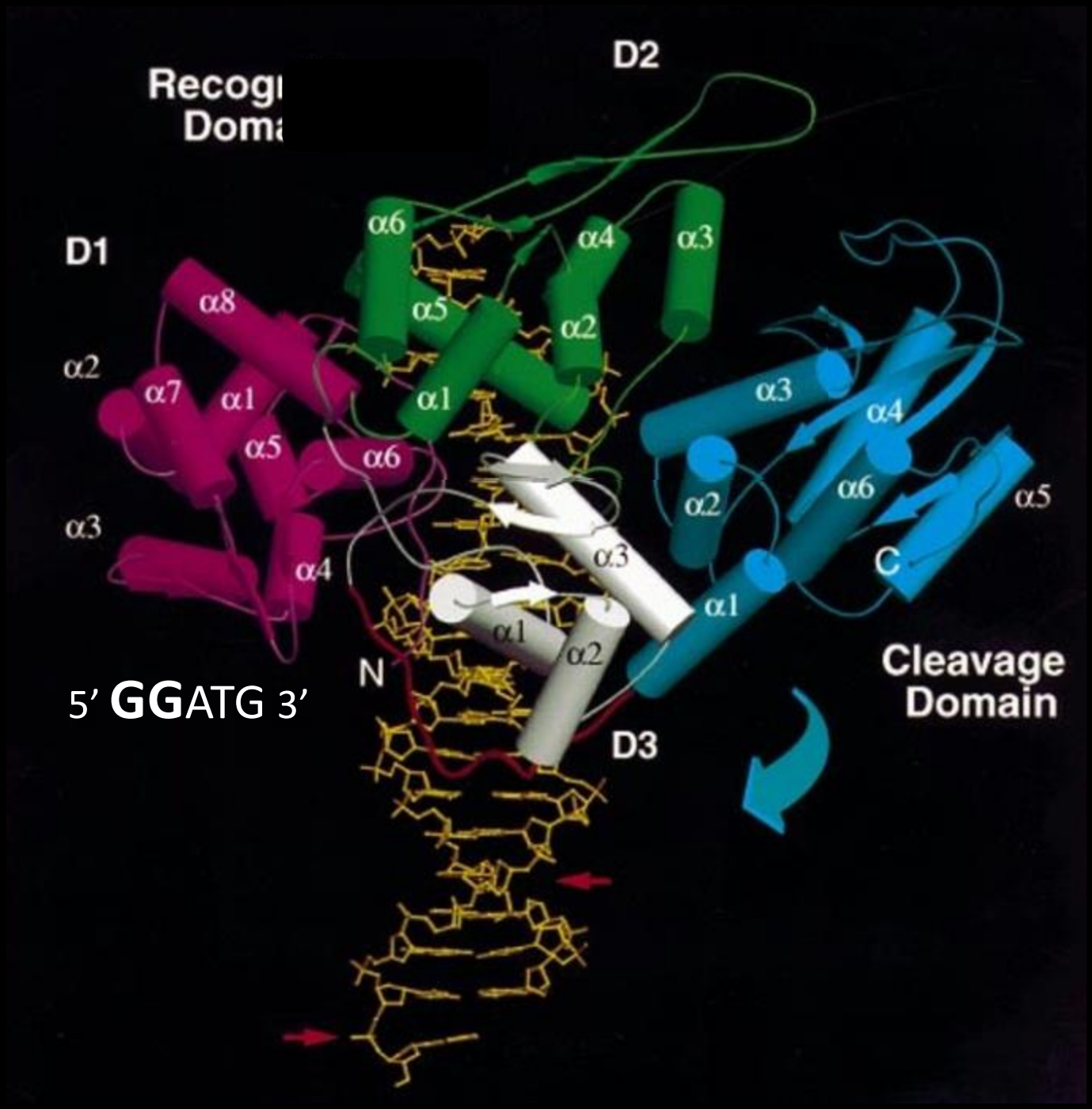

α1
α8
L2
α2
α6
α5
L1
α7
α4
α3
D1

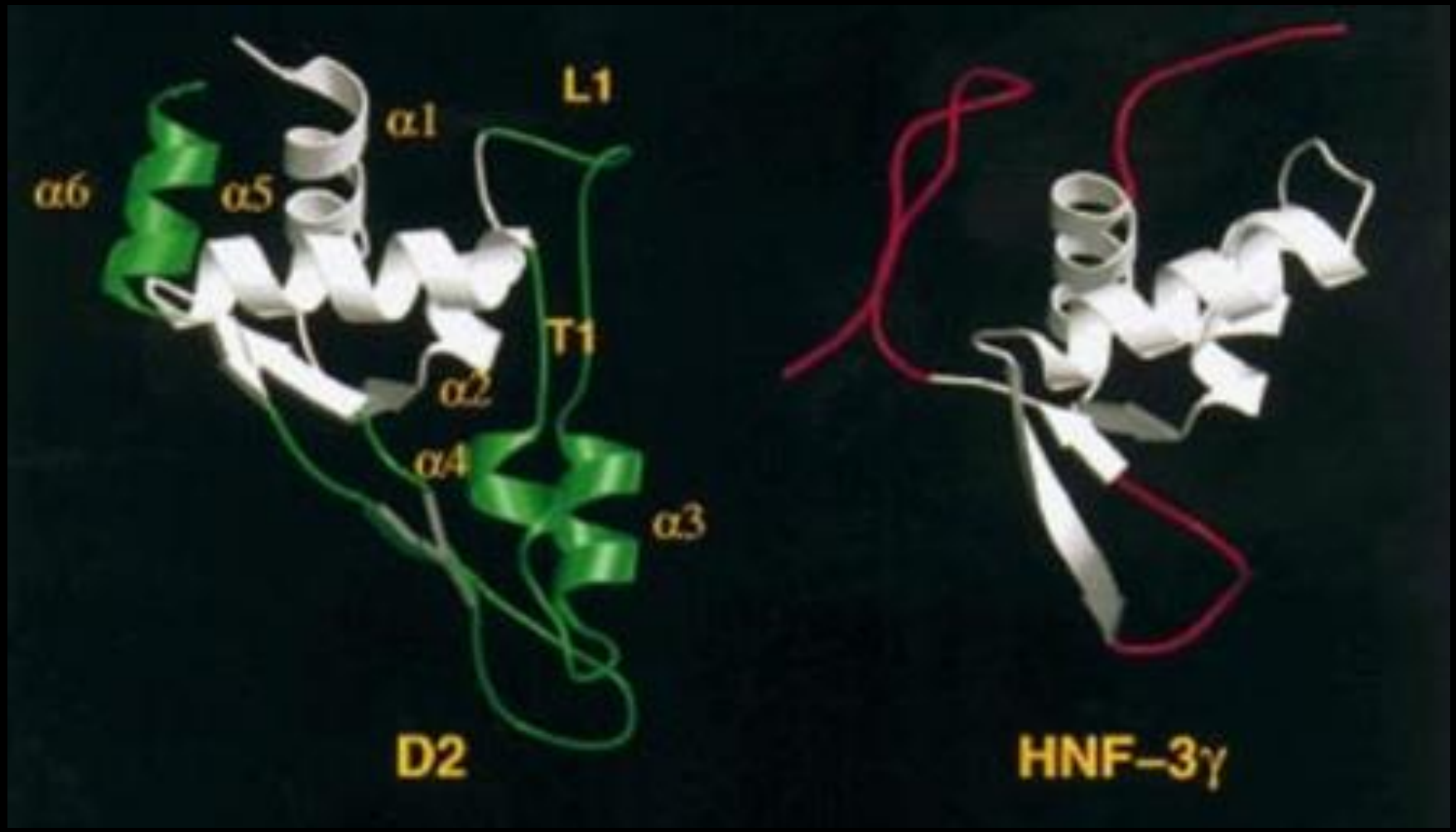

α8
α1
L2
α2
α6
α5
α7
L1
α4
α3
D1
Turn
Helix
Helix
CAP

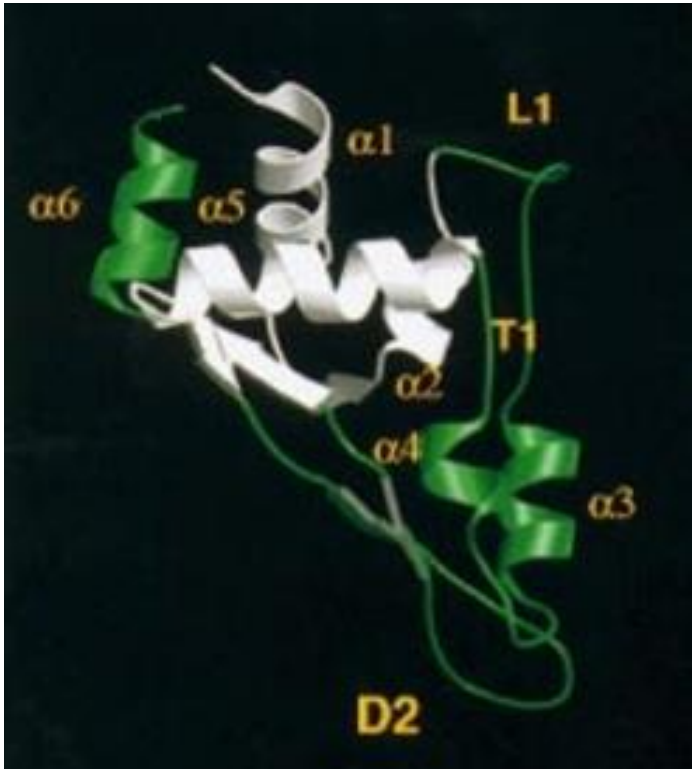


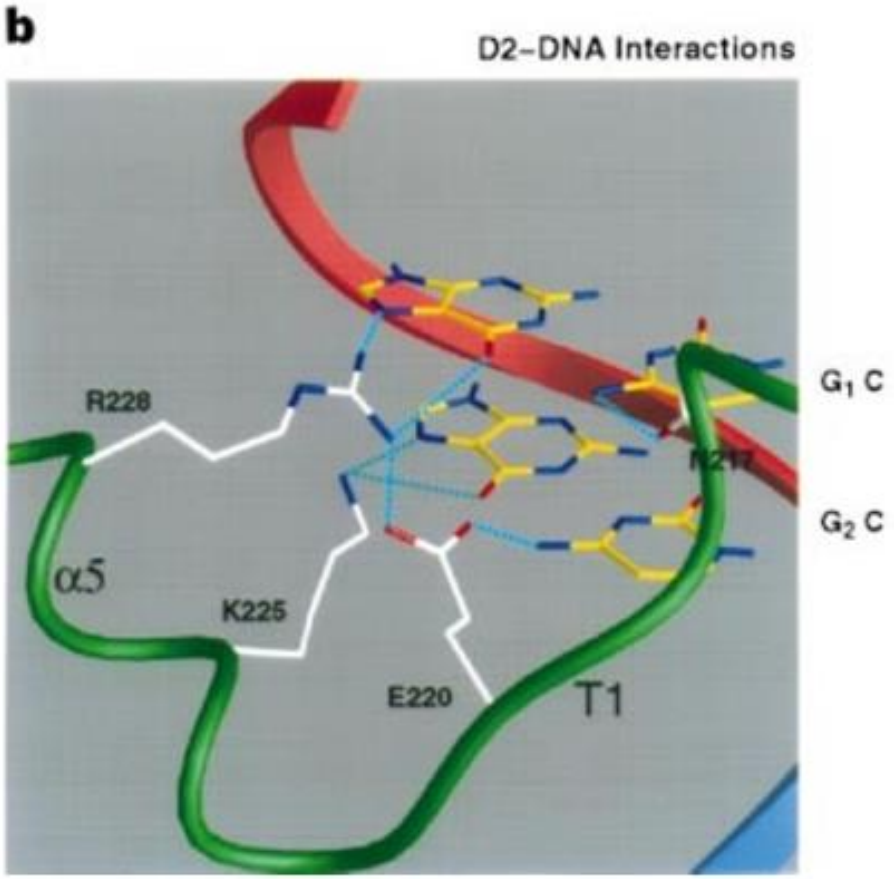
1. Loop 2 (L2) : Q95] minor groove
2. N-terminal arm : Q-12 ; N-13
3. Loop1 (L1) : R-79
4. Alpha 6 (α 6) : W105

major groove


Recogn
Doma
D2
D1
α6
α4
α3
α8
α5
α2
α2
α7
α1
α1
α3
α5
α6
α4
α2
α6
α5
α3
C
α4
α3
α1
α1
α2
N
Cleavage
Domain
5’ GGATG 3’
D3

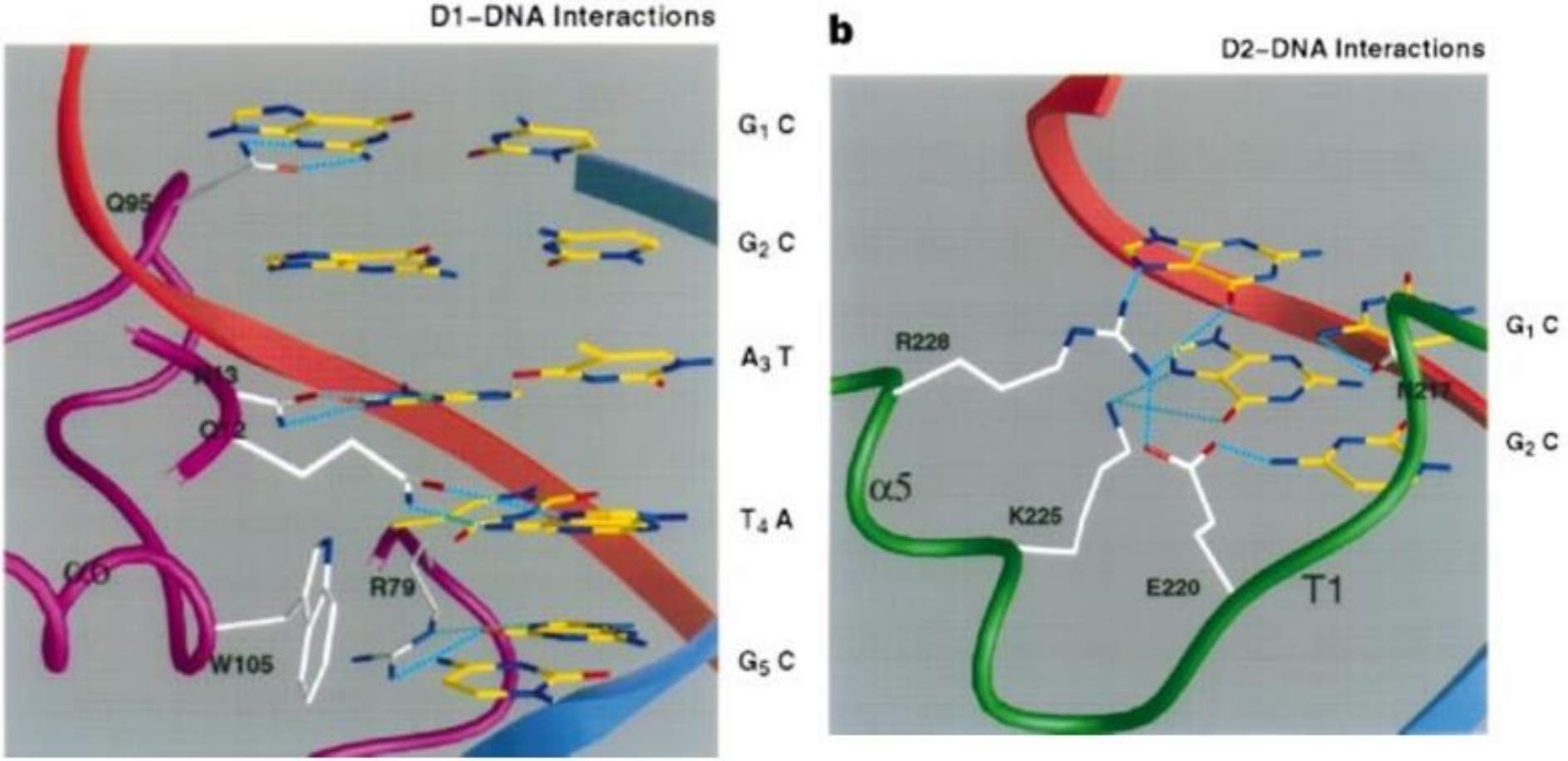

L1
α1
α6
α5
T1
α2
α4
α3
D2
HNF–3γ

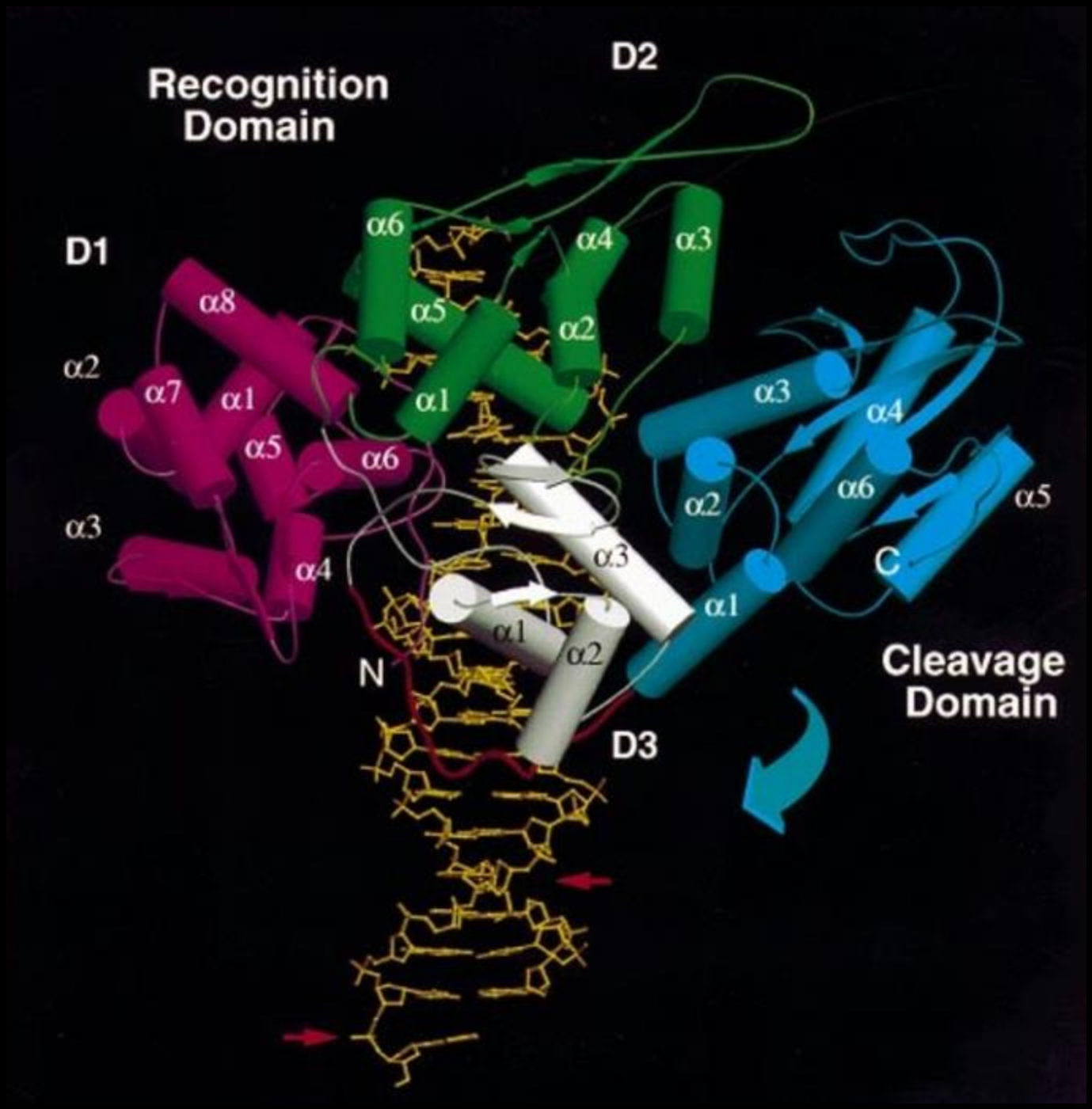


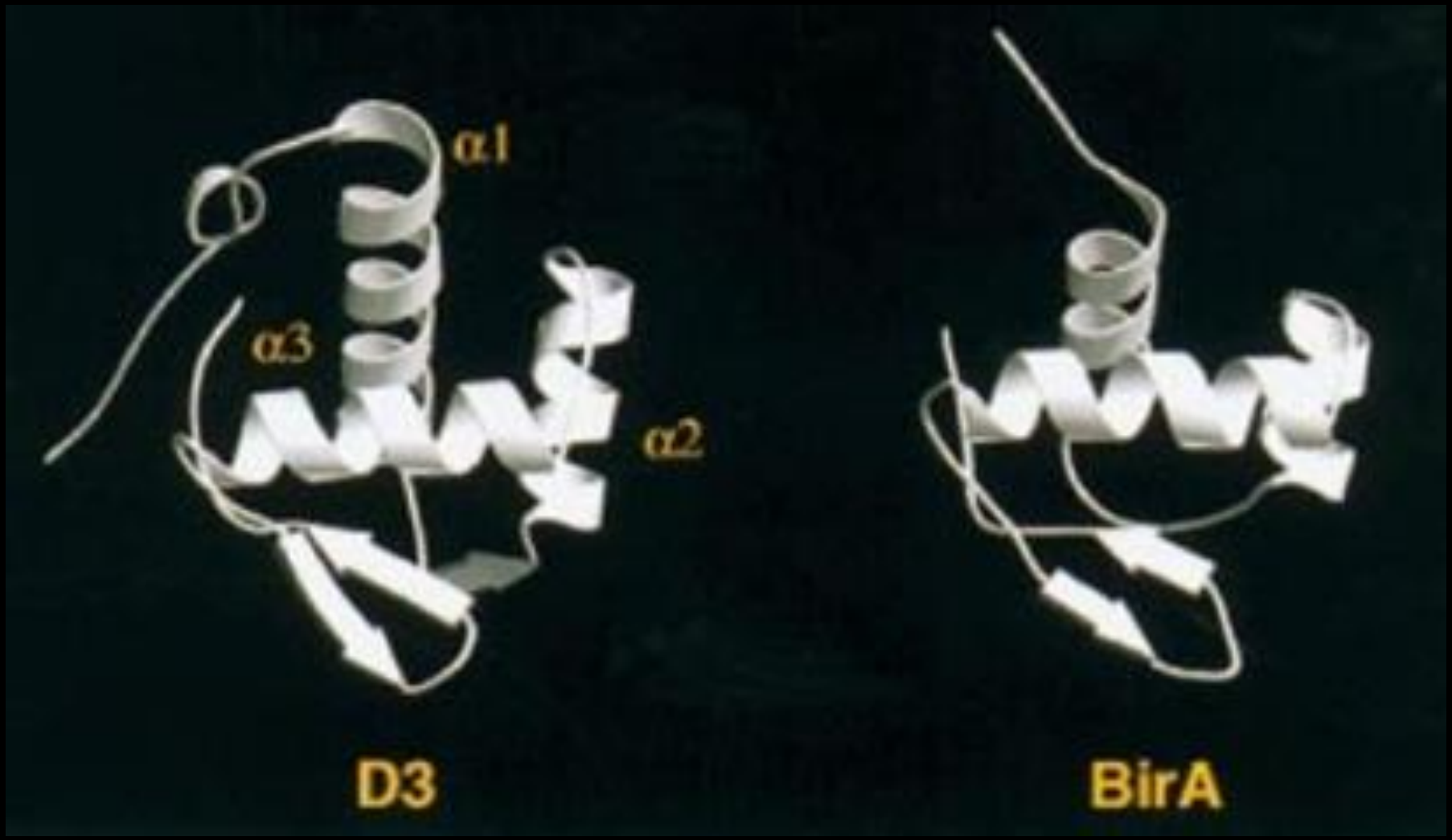


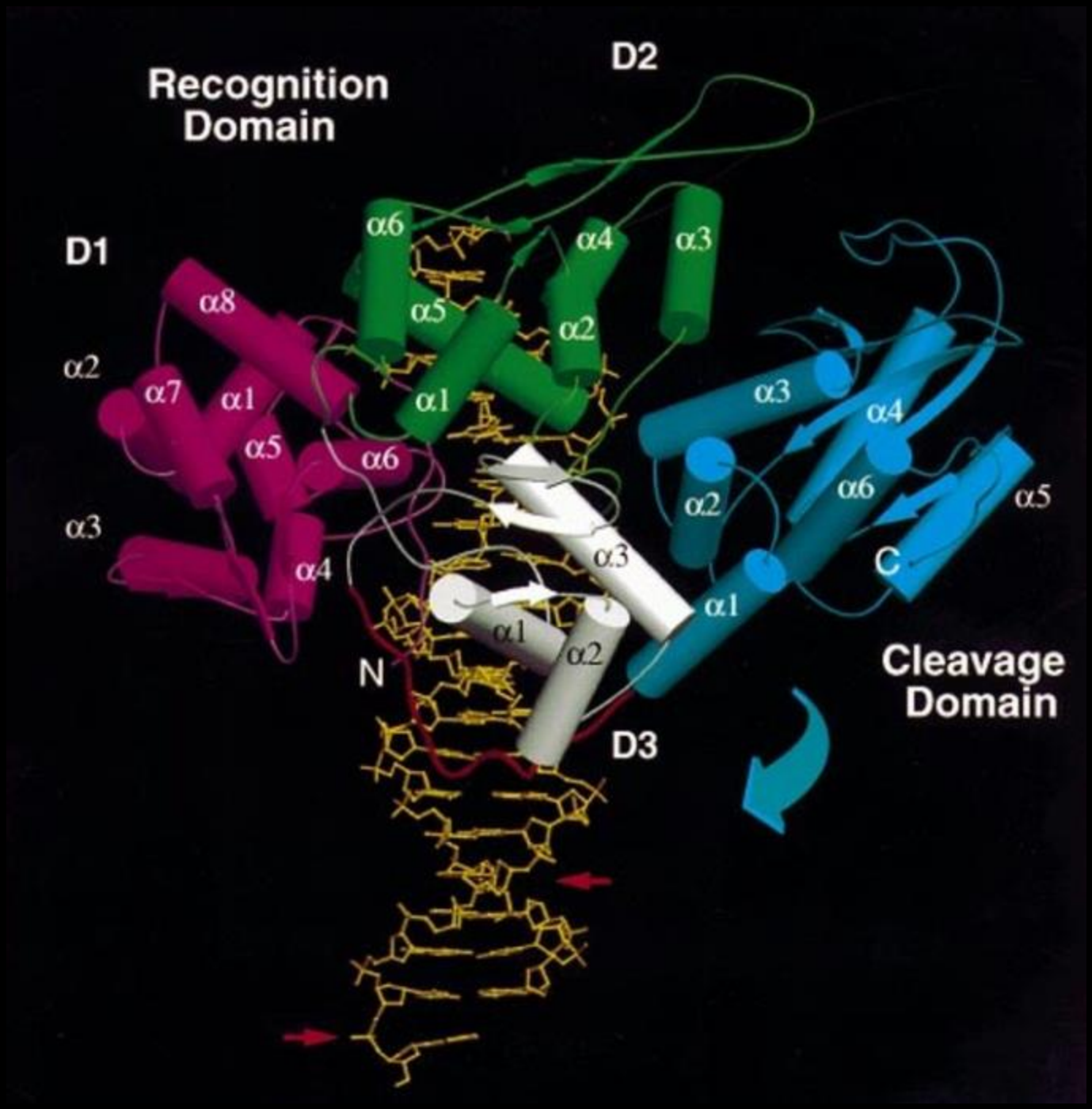
1. Recognition helix (α 5) : K225 ; R 228
2. T1 of "turn" : E220
3. Alpha 4 (α 4) "turn" : N217

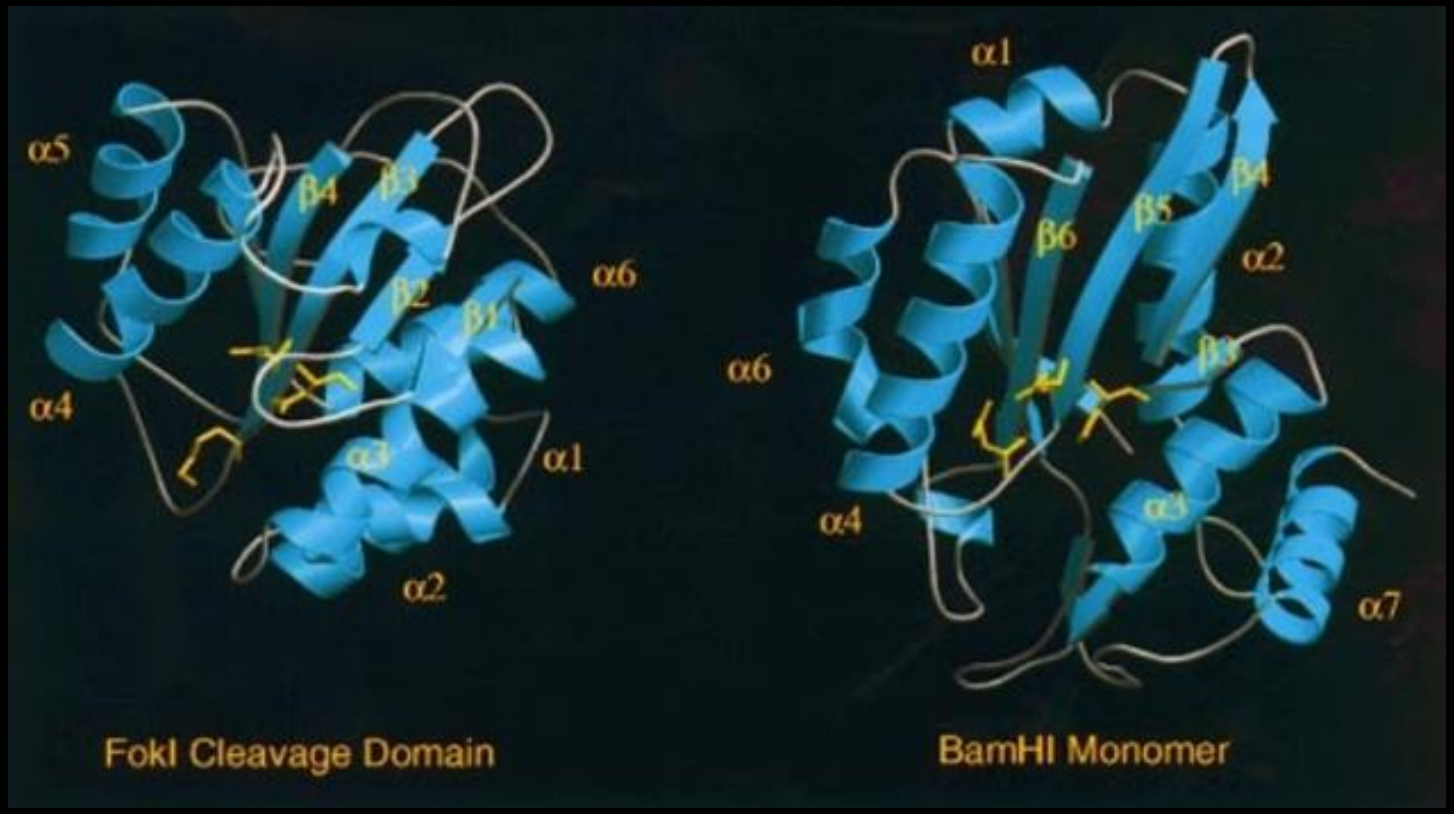
major groove


D1–DNA Interactions
Q95
G1 C
G2 C
A3 T
T4 A
G5 C
R79
W105
b
D2–DNA Interactions
R228
G1 C
G2 C
α5
K225
E220
T1

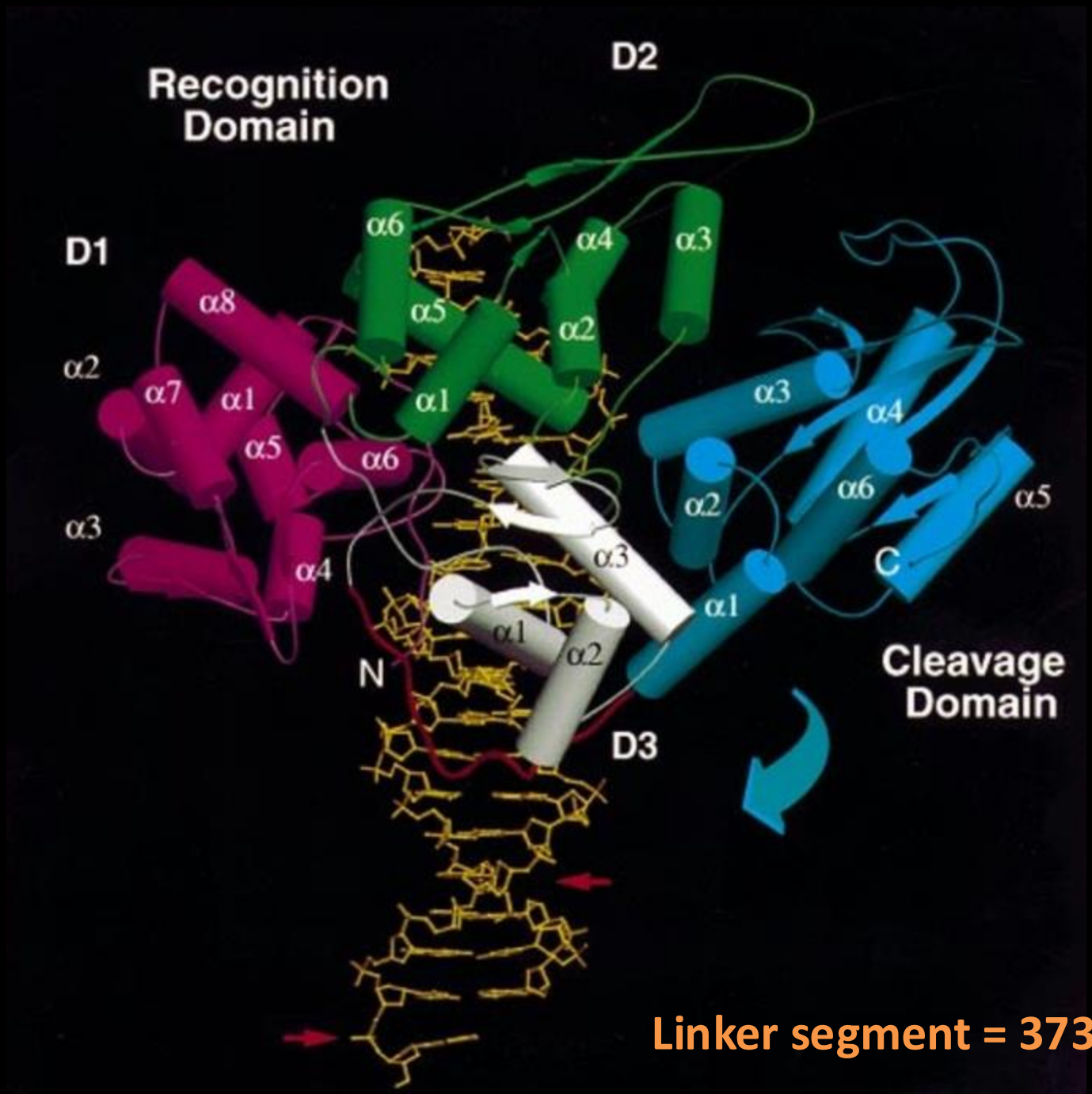

Recognition Domain
D2
D1
α6
α4
α3
α8
α5
α2
α2
α7
α1
α1
α3
α4
α5
α6
α2
α6
α5
α3
α4
α3
C
α1
α1
α2
N
Cleavage Domain
D3


α1
α3
α2
D3
BirA

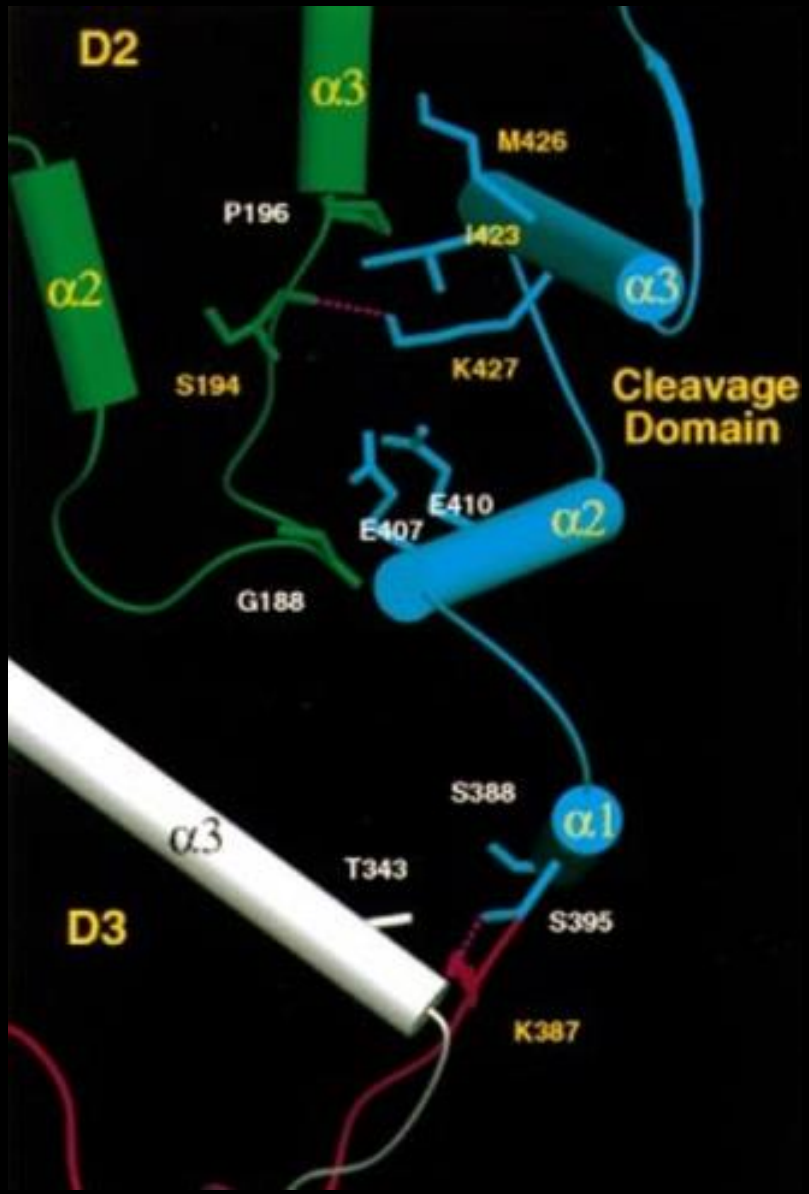

Recognition Domain
D2
D1
α6
α4
α3
α8
α5
α2
α2
α7
α1
α1
α3
α5
α6
α4
α2
α6
α5
α3
C
α4
α1
α1
α2
N
Cleavage Domain
D3



D450, D467 and K469



Linker segment = 373 -387 aa

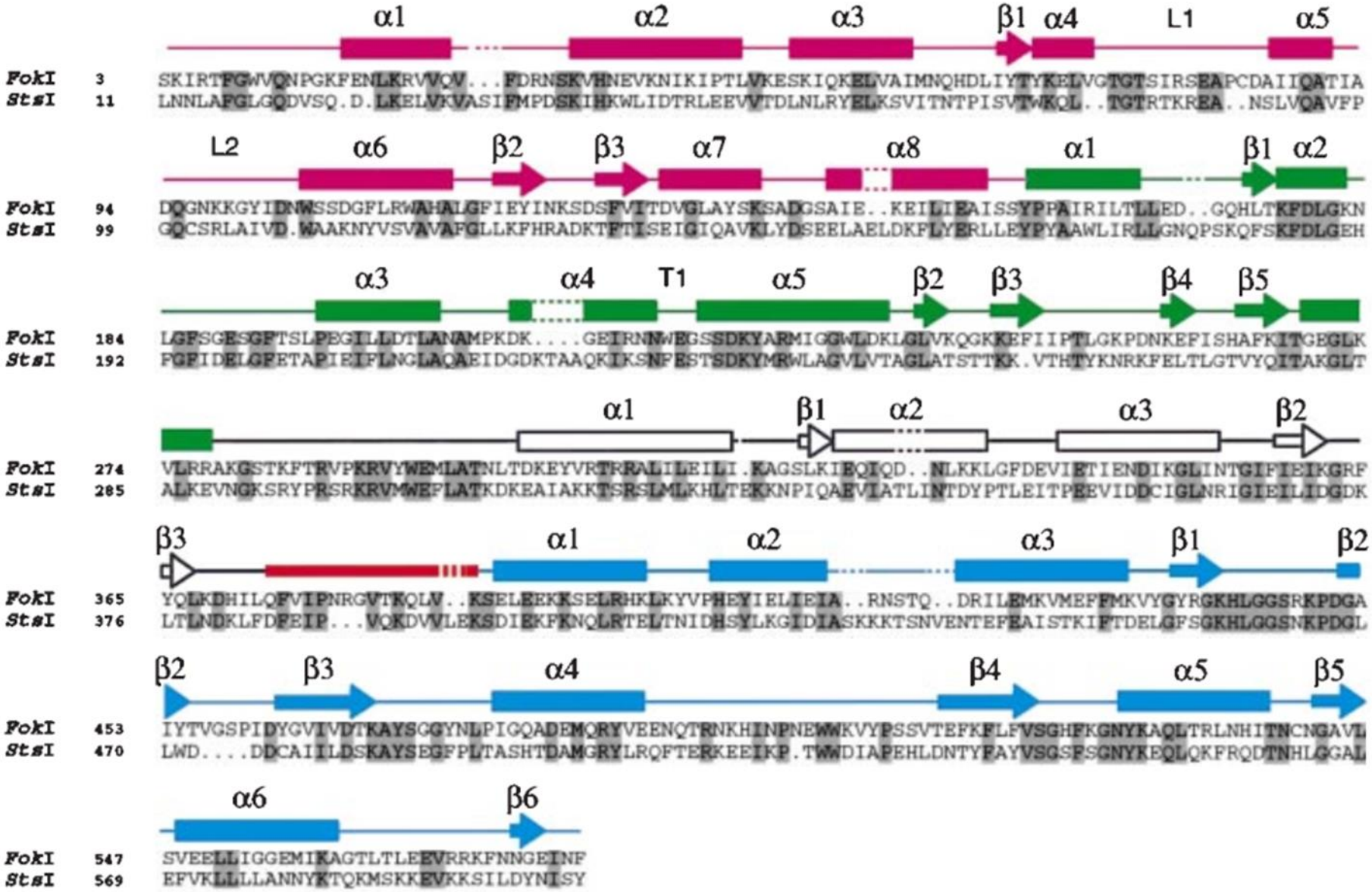


electrostatic interactions

subdomains D2 and D3

The linker segment &

Helices of the cleavage


α1 α2 α3 β1 α4 L1 α5
FokI 3 SKIRTFGWVQNPGKFENLKRVVQV...FDRNSKVHNEVKNIKIPTLVKESKIQKELVAIMNQHDLIYTYKELVGTGTSIRSEAPCDAIIQATIA
StsI 11 LNNLAFGLGQDVSQ.D.LKELVKVASIFMPDSKIHKWLIDTRLEEVVTDLNLRYELKSVITNTPISVTWKQL..TGTRTKREA..NSLVQAVFP
L2 α6 β2 β3 α7 α8 α1 β1 α2
FokI 94 DQGNKKGYIDNWSSDGFLRWAHALGFIEYINKSDSFVITDVGLAYSKSADGSAIE..KEILIEAISSYPPAIRILTLLED..GQHLTKFDLGKN
StsI 99 GQCSRLAIVD.WAAKNYVSVAVAFGLLKFHRADKTFTISEIGIQAVKLYDSEELAELDKFLYERLLEYPYAAWLIRLLGNQPSKQFSKFDLGEH
α3 α4 T1 α5 β2 β3 β4 β5
FokI 184 LGFSGESGFTSLPEGILLDTLANAMPKDK....GEIRNNWEGSSDKYARMIGGWLDKLGLVKQGKKEFIIPTLGKPDNKEFISHAFKITGEGLK
StsI 192 FGFIDELGFETAPIEIFLNGLAQAEIDGDKTAAQKIKSNFESTSDKYMRWLAGVLVTAGLATSTTKK.VTHTYKNRKFELTLGTVYQITAKGLT
α1 β1 α2 α3 β2
FokI 274 VLRRAKGSTKFTRVPKRVYWEMLATNLTDKEYVRTRRALILEILI.KAGSLKIEQIQD..NLKKLGFDEVIETIENDIKGLINTGIFIEIKGRF
StsI 285 ALKEVNGKSRYPRSRKRVMWEFLATKDKEAIAKKTSRSLMLKHLTEKKNPIQAEVIATLINTDYPTLEITPEEVIDDCIGLNRIGIEILIDGDK
β3 α1 α2 α3 β1 β2
FokI 365 YQLKDHILQFVIPNRGVTKQLV..KSELEEKKSELRHKLKYVPHEYIELIEIA..RNSTQ..DRILEMKVMEFFMKVYGYRGKHLGGSRKPDGA
StsI 376 LTLNDKLFDFEIP...VQKDVVLEKSDIEKFKNQLRTELTNIDHSYLKGIDIASKKKTSNVENTEFEAISTKIFTDELGFSGKHLGGSNKPDGL
β2 β3 α4 β4 α5 β5
FokI 453 IYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVL
StsI 470 LWD....DDCAIILDSKAYSEGFPLTASHTDAMGRYLRQFTERKEEIKP.TWWDIAPEHLDNTYFAYVSGSFSGNYKEQLQKFRQDTNHLGGAL
α6 β6
FokI 547 SVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF
StsI 569 EFVKLLLLLANNYKTQKMSKKEVKKSILDYNISY

Conclusions of Lecture-5

- Detailed structure of FokI
- Recognition Domain (RD) (D1,D2&D3)
- Linker (373-387aa)
- Cleavage domain (CD)
- CD is sequestered along the RD
- NO protection to the CD
- Only one catalytic site

Thank You!