Genome Editing and Engineering

Course No: BT-637

LECTURE-6

Dr. Kusum K. Singh
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

Introduction

- FokI (Flavobacterium okeanokoites)
- Biochemical and crystal structure
- Single catalytic domain
- Rearrange in higher order structure
- Cleave both strands of DNA

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10564–10569, September 1998
Biophysics

Structure of *Fok*I has implications for DNA cleavage

DAVID A. WAH*, JURATE BITINAITE†, IRA SCHILDKRAUT†, AND ANEEL K. AGGARWAL*‡

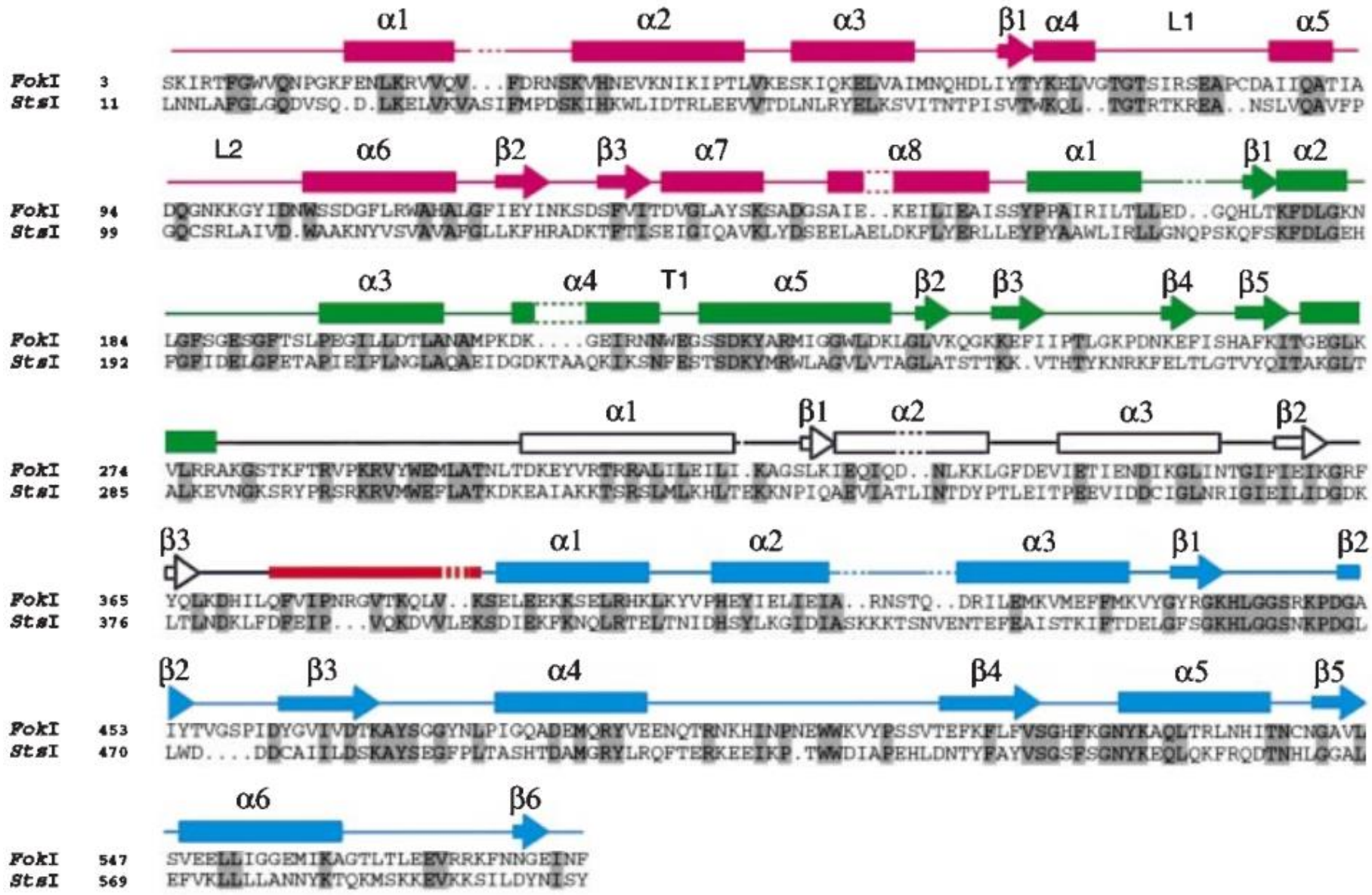
*Structural Biology Program, Department of Physiology and Biophysics, Box 1677, 1425 Madison Avenue, Mount Sinai School of Medicine, New York, NY 10029; and †New England Biolabs, 32 Tozer Road, Beverly, MA 01915

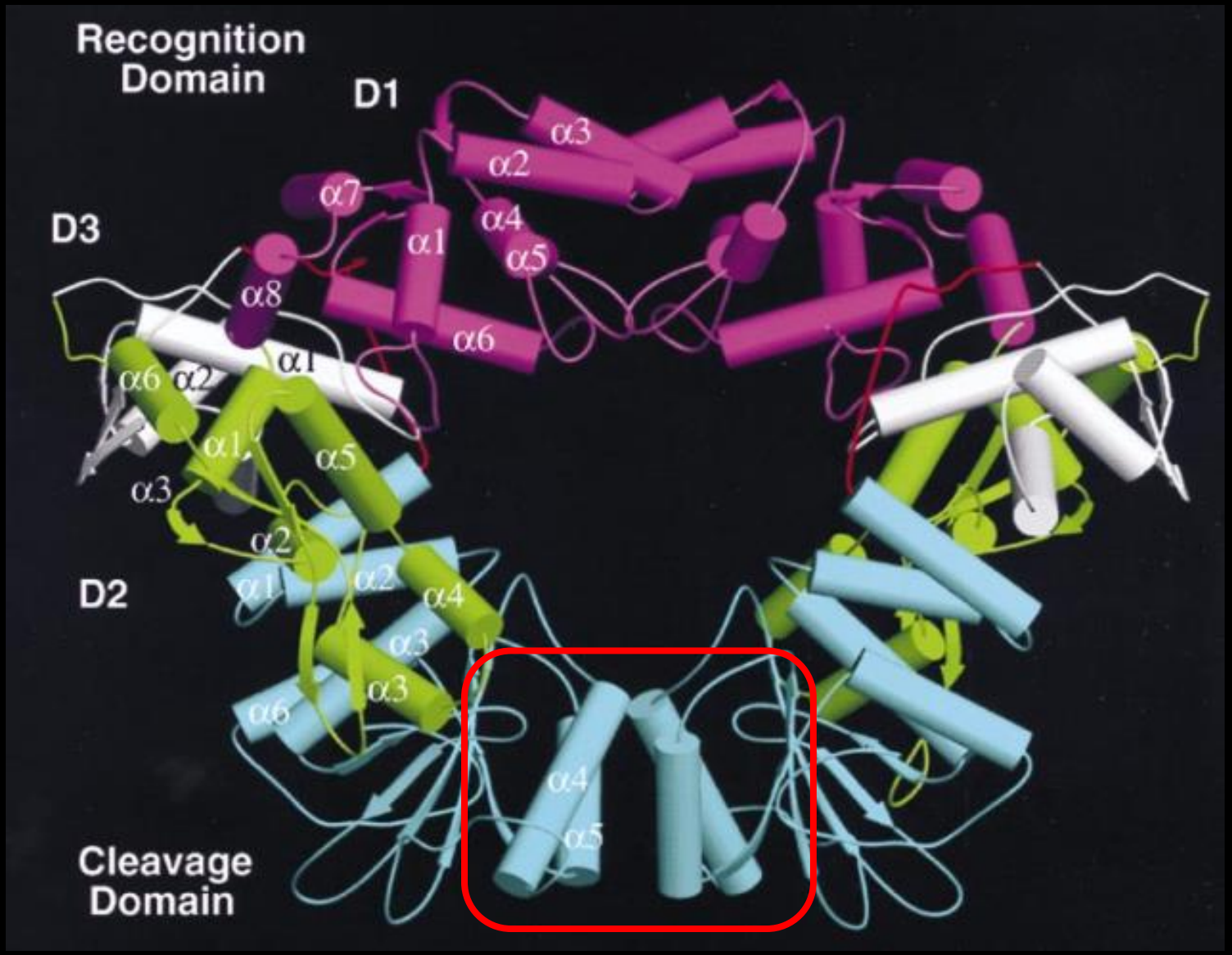
Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10570–10575, September 1998
Biochemistry

*Fok*I dimerization is required for DNA cleavage

JURATE BITINAITE*, DAVID A. WAH†, ANEEL K. AGGARWAL†, AND IRA SCHILDKRAUT*‡

*New England Biolabs, Inc., 32 Tozer Road, Beverly, MA 01915; and †Structural Biology Program, Department of Physiology and Biophysics, Box 1677, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, NY 10029


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..KEILIEAISSYPPAIRILTLLEED..GQHLTKFDLGKN
StsI 99 GQCSRLAIVD.WAAKNYVSVAVAFGLLKFHRADKTFTISEIGIQAVKLYDSEELAELDKFLYERLLEYPYAAWLIRLLGNQPSKQFSKFDLGEH
α3 α4 T1 α5 β2 β3 β4 β5
FokI 184 LGFSGESGFTSLPEGILLDTLANAMPKDK....GEIRNNWEGSSDKYARMIGGWLDKLGLVKQGKKEFIIPTLGKPDNKEFISHAFKITGEGLK
StsI 192 PGFIDELGFETAPIEIFLNGLAQAEIDGDKTAAQKIKSNFESTSDKYMRWLAGVLVTAGLATSTTKK.VTHTYKNRKFELTLGTVYQITAKGLT
α1 β1 α2 α3 β2
FokI 274 VLRRAKGSTKFTRVPKRVYWEMLATNLTDKEYVRTRRALILEILI.KAGSLKIEQIQD..NLKKLGFDEVIETIENDIKGLINTGIFIEIKGRF
StsI 285 ALKEVNGKSRYPRSRKRVMWEPLATKDKEAIAKKTSRSLMLKHLTEKKNPIQAEVIATLINTDYPTLEITPEEVIDDCIGLNRIGIEILIDGDK
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 EFVKLLLLANNYKTQKMSKKEVKKSILDYNISY


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


Dimer Interface
α5
R487
D483
α4
α4
D483
R487
α5

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10564–10569, September 1998
Biophysics

Structure of *Fok*I has implications for DNA cleavage

DAVID A. WAH*, JURATE BITINAITE†, IRA SCHILDKRAUT†, AND ANEEL K. AGGARWAL*‡

*Structural Biology Program, Department of Physiology and Biophysics, Box 1677, 1425 Madison Avenue, Mount Sinai School of Medicine, New York, NY 10029; and †New England Biolabs, 32 Tozer Road, Beverly, MA 01915

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10570–10575, September 1998
Biochemistry

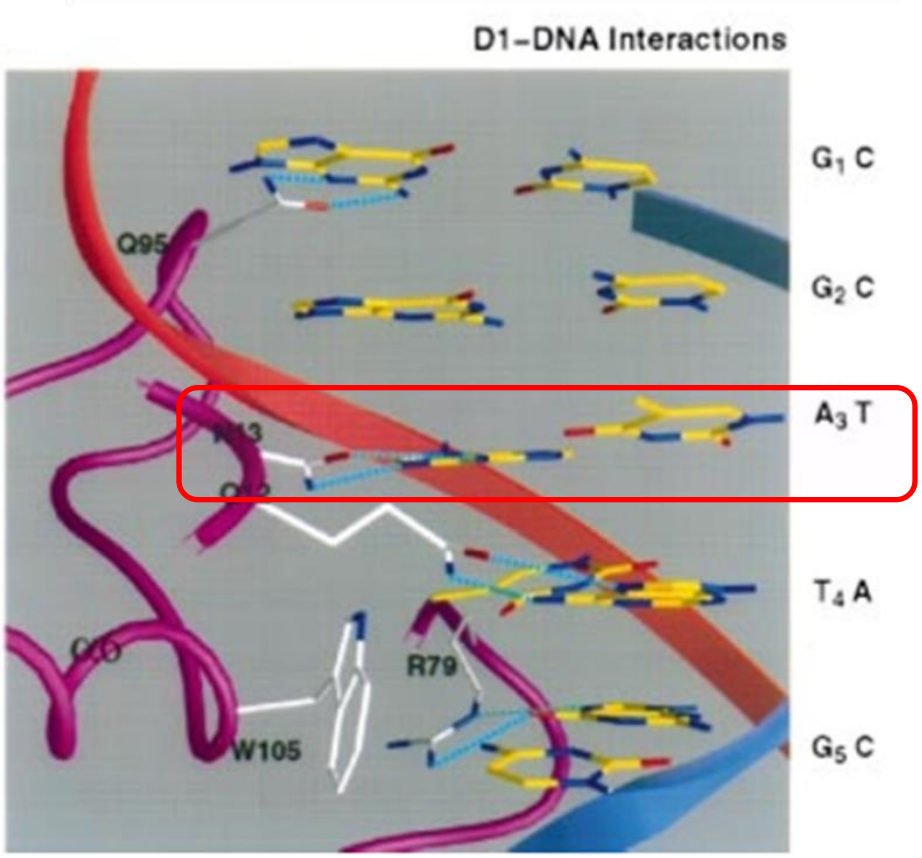
*Fok*I dimerization is required for DNA cleavage

JURATE BITINAITE*, DAVID A. WAH†, ANEEL K. AGGARWAL†, AND IRA SCHILDKRAUT*‡

*New England Biolabs, Inc., 32 Tozer Road, Beverly, MA 01915; and †Structural Biology Program, Department of Physiology and Biophysics, Box 1677, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, NY 10029

Construction of FokI mutants

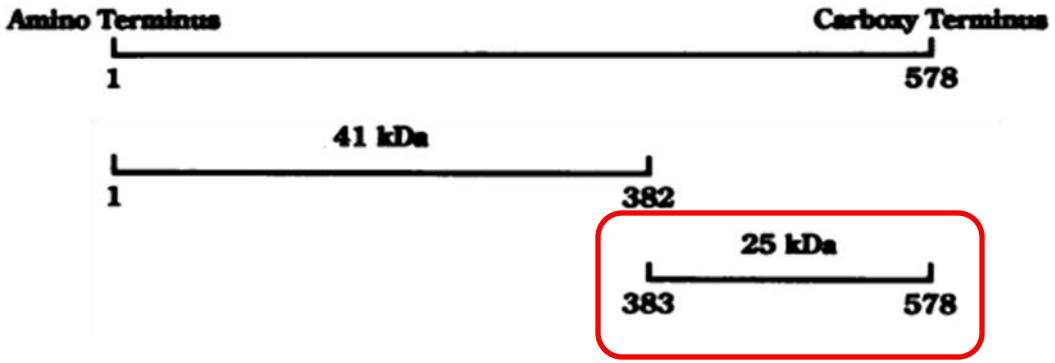
- **Fok N13Y**



Construction of FokI mutants

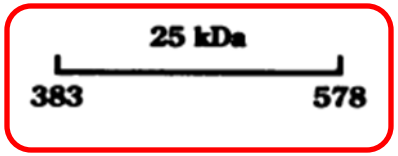
- Fok N13Y

- **Fok CD**
- **196 a.a.**
- **25 kDa**



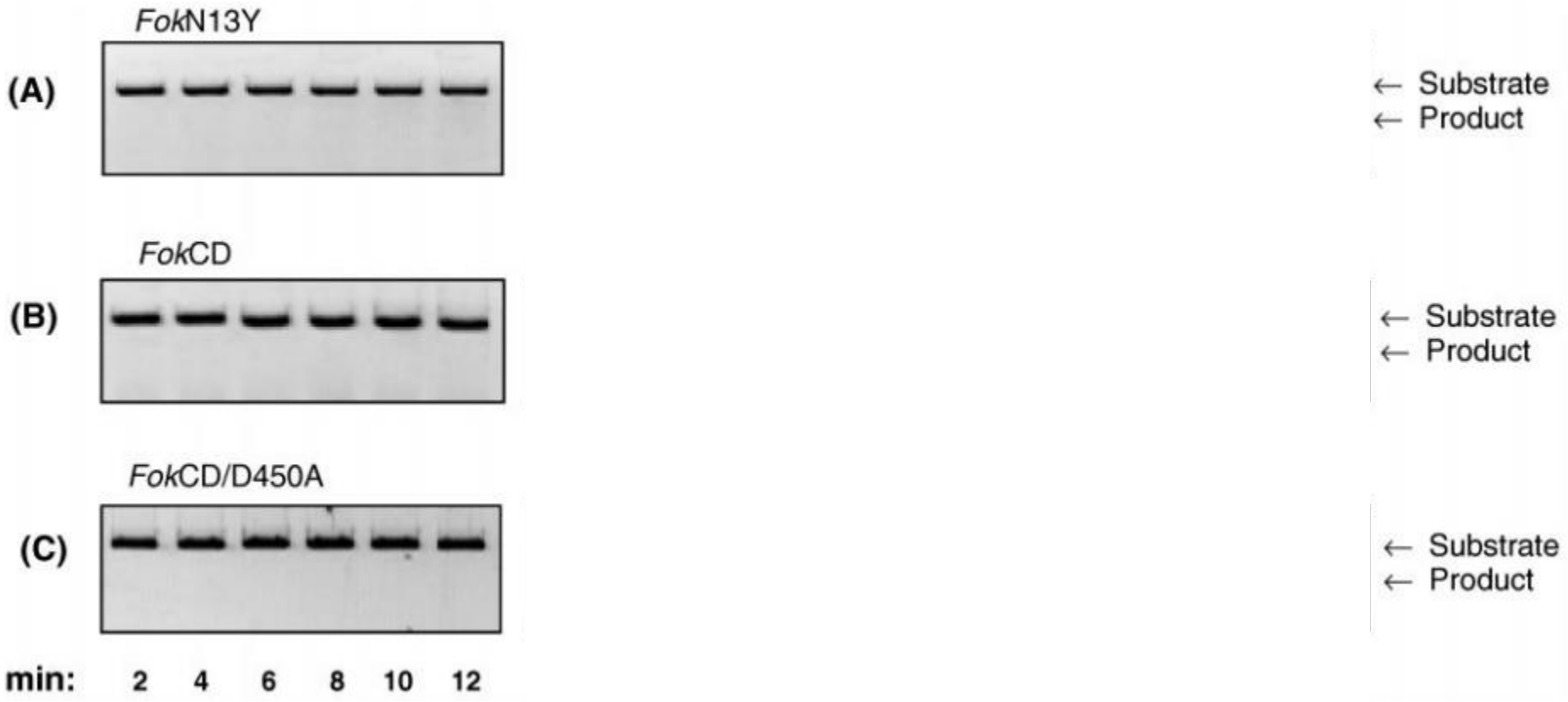
Construction of FokI mutants

- Fok N13Y
- Fok CD

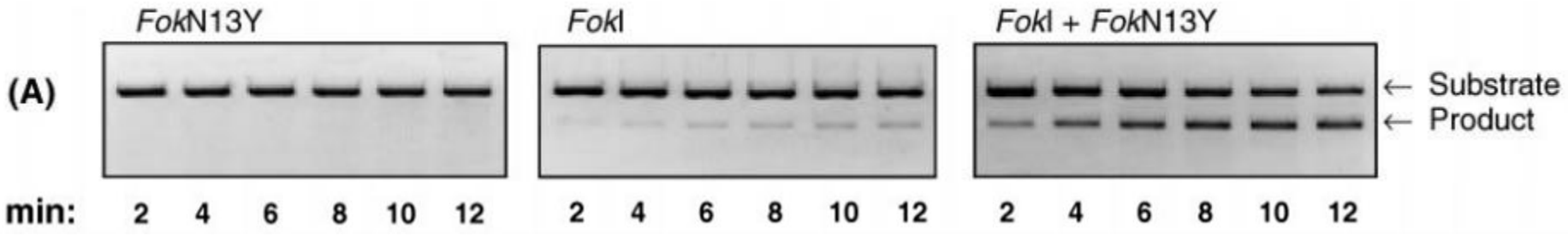


- **Fok CD/D450A** D450A;

Construction of FokI mutants

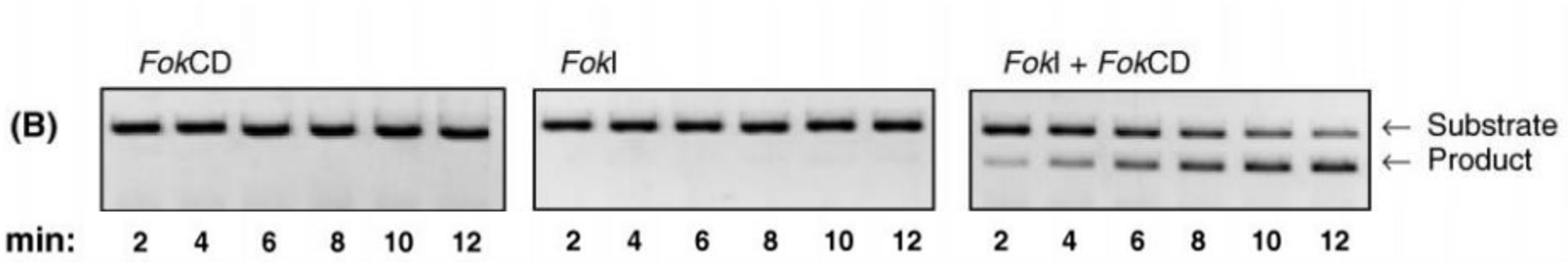


Construction of FokI mutants



Wt. FokI interacts with Fok N13Y

Construction of FokI mutants



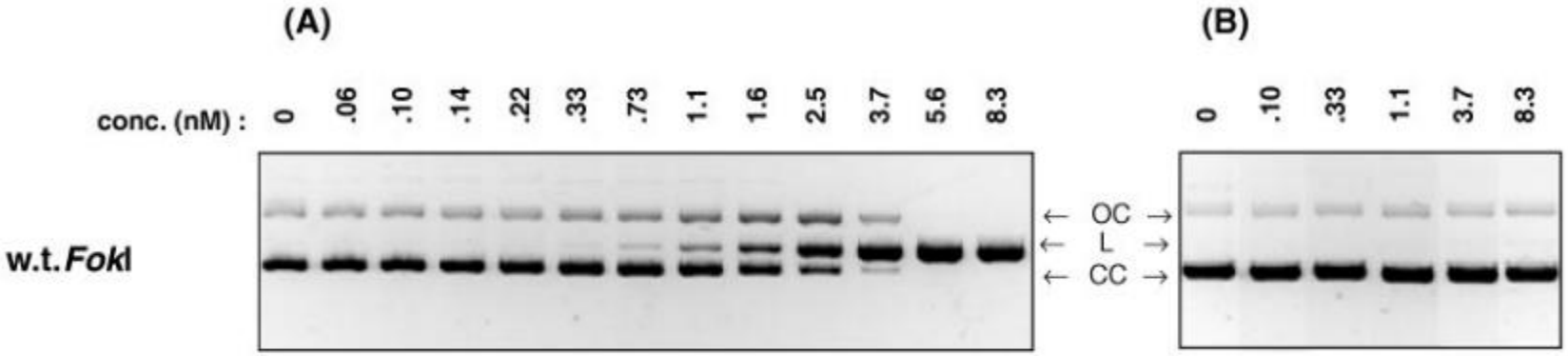
Wt. FokI interacts with Fok CD

Construction of FokI mutants

Wt. FokI does not interacts with Fok CD/D450A


Dimer Interface
α5
R487
D483
α4
α4
D483
R487
α5

Interface D483A; R487A mutants



Fok D483A, R487A = Reduced cleavage activity

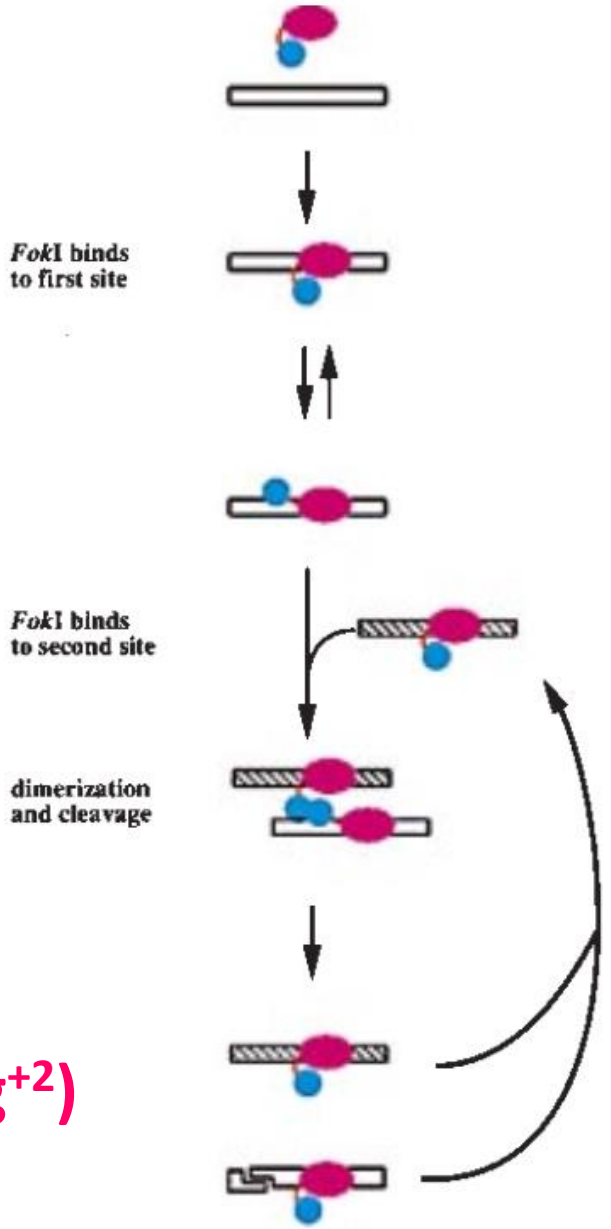
Dimer model

- FokI first binds to DNA as monomer
- The complex is inactive
- A second FokI monomer arrives:

i) when DNA is scanned until

ii) the monomer collides

- Correct orientation = dimerize
- Cleavage of ds DNA= Fok dimers (Mg^{+2})



Conclusions of Lecture-6

- Cleavage of both strand= after FOKI dimerization (Mg)
- Dimerization model offers (two control)

i) The release of CD depends on seq.-spe. binding

ii) Dimerization of CD = phosphodiester bond cleavage

Thank You!