

Genome Editing and Engineering

Course No: BT-637

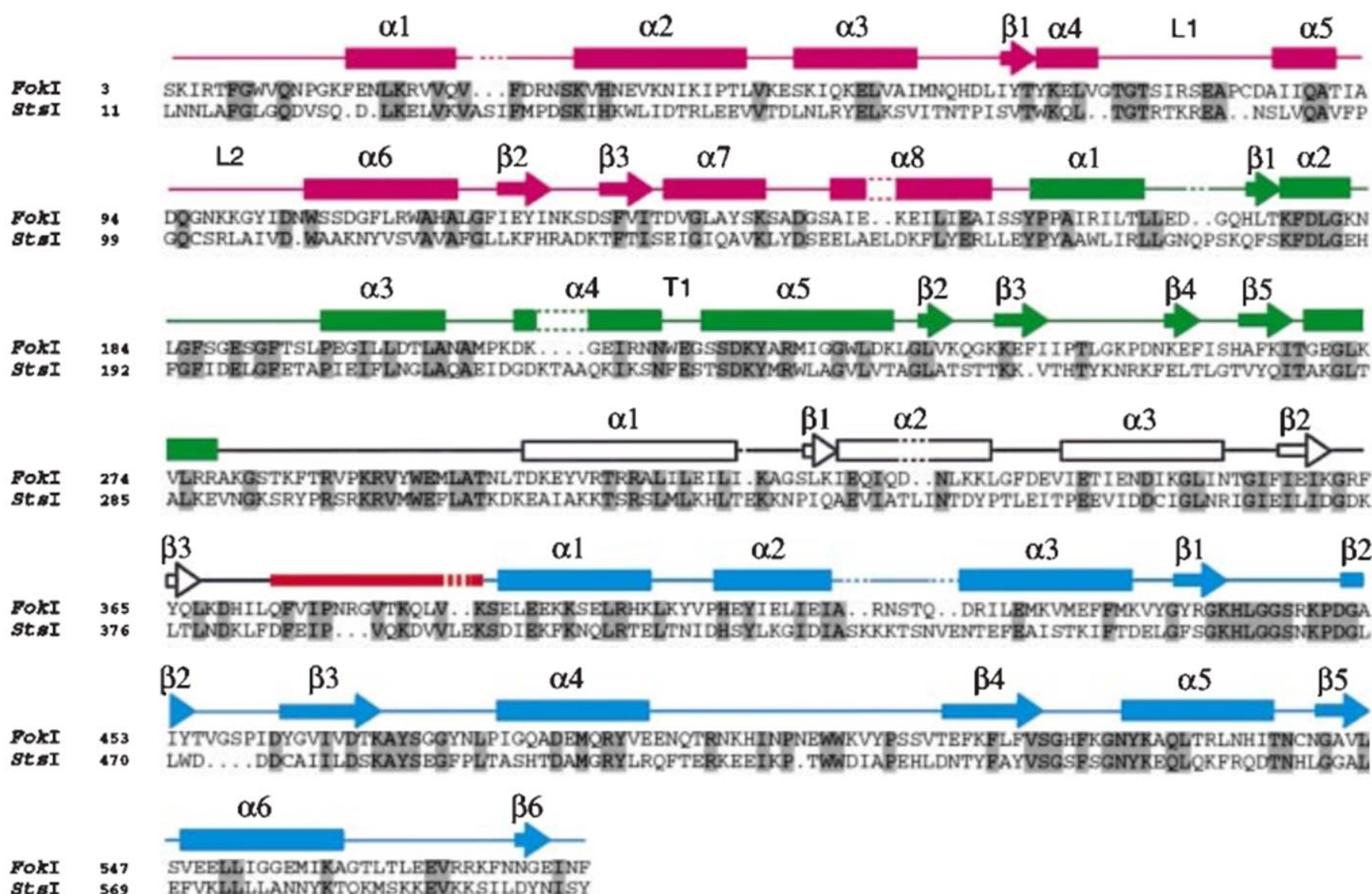


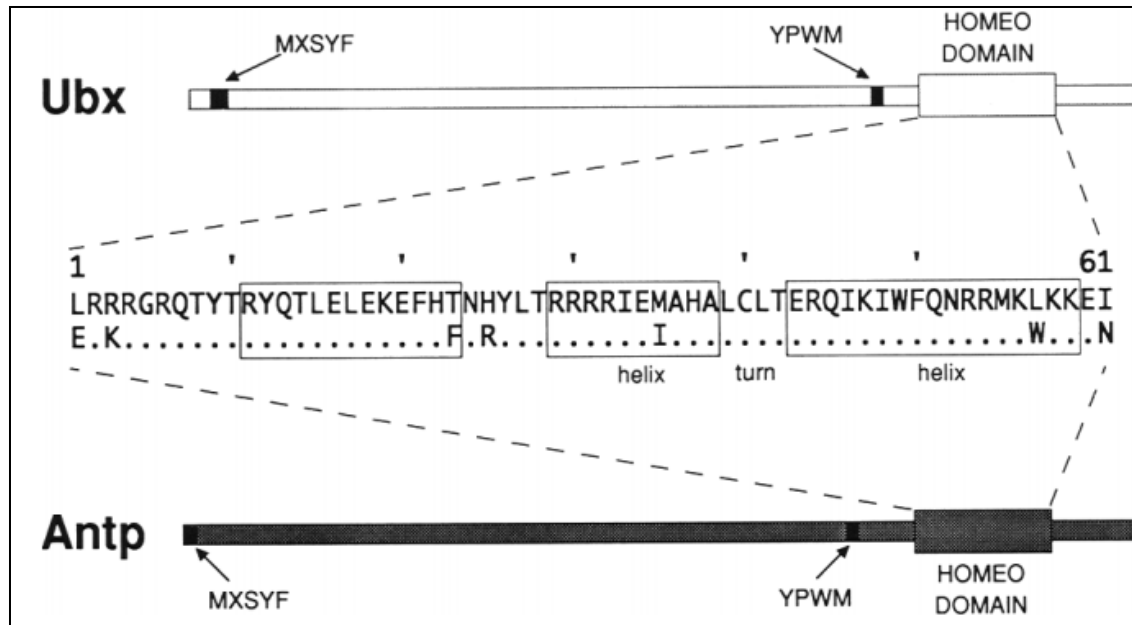
LECTURE-11

Dr. Kusum K. Singh

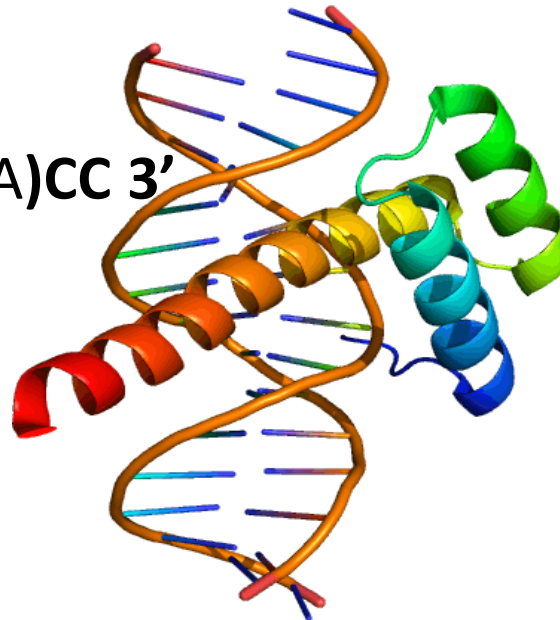
Department of Biosciences and Bioengineering

Indian Institute of Technology Guwahati





5' TTAAT(G/T)(G/A)CC 3'



1 8 13 17 23 26 30

T G E K * P (Y) V (C) . . D G (C) D K R (F) T K K . . (L) K * R (H) . . * . (H)

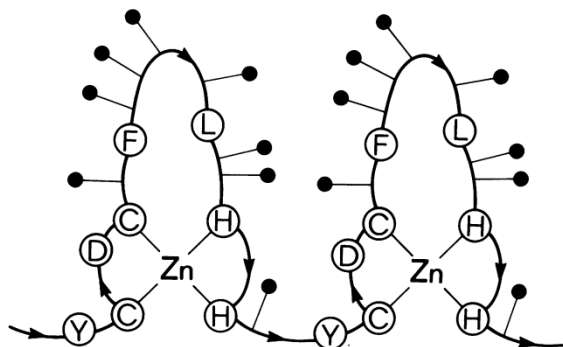
1 (M G E K A L P V V Y K R)

12

1 (Y) I (C) S F A D (C) G A A (Y) N K N W K (L) Q * A (H) L C * K (H) 37
 2 T G E K * P (F) P (C) K E E G (C) E K G (F) T S L H H (L) T * R (H) S L * T (H) 67
 3 T G E K * N (F) T (C) D S D G (C) D L R (F) T T K A N M K * K (H) F N R F (H) 98
 4 N I K I C V (Y) V (C) H F E N (C) G K A (F) K K H N Q (L) K * V (H) Q F * S (H) 129
 5 T Q Q L * P (Y) E (C) P H E G (C) D K R (F) S L P S R (L) K * R (H) E K * V (H) 159
 6 A G - - * - (Y) P (C) K K D D S (C) S (F) V G K T W T (L) Y L K (H) V A E C (H) 188
 7 Q D - - * L A V (C) - - D V (C) N R K (F) R H K D Y (L) R * D (H) Q K * T (H) 214
 8 E K E R T V (Y) L (C) P R D G (C) D R S (Y) T T A F N (L) R * S (H) I Q S F (H) 246
 9 E E Q R * P (F) V (C) E H A G (C) G K C (F) A M K K S (L) E * R (H) S V * V (H) 276

277

(D P E K R K L * K E K C P R P K R S L A S R L T G Y I P P K S K E K (N) A 311
 S V S G T E K T D S L V K N K P S G T E T (N) G S L V L D K L T I Q) 344



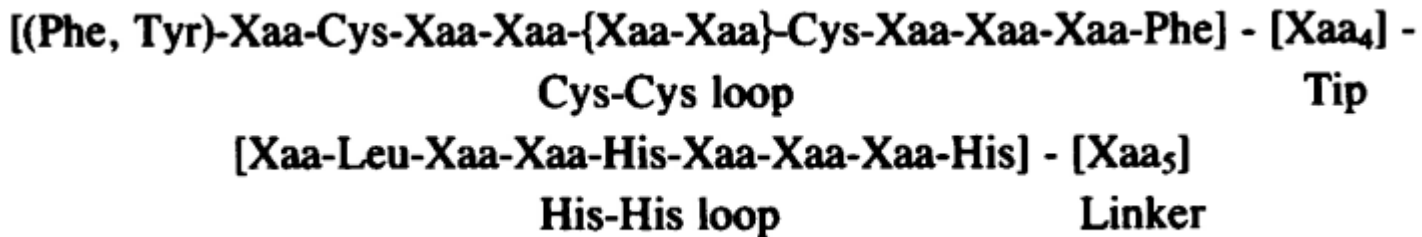
Introduction

- TF was isolated, purified and characterized from *Xenopus laevis*.
- This factor showed characteristic arrangements of Cys & His.
- These C₂H₂ = fundamental = folding of the finger = coordinating Zn ion.
- Apart from Cys & His, other conserved = Tyr/Phe 6; Phe 17 and Leu 23 = hydrophobic structural core.
- Seven conserved amino acid in each unit provide the framework of tertiary folding.
- The variable residues determine the specificity of each domain.

Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins

JEREMY M. BERG

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 99–102, January 1988
Biophysics



Rubredoxin

4

1 Tyr-Thr-Cys-Thr-Val-Cys-Gly-Tyr-Ile-Tyr

37

2 Trp-Val-Cys-Pro-Leu-Cys-Gly-Val-Gly-Lys

Aspartate transcarbamoylase

136

3 Leu-Lys-Cys-Lys-Tyr-Cys-Glu-Lys-Glu-Phe

107

4 Leu-Val-Cys-Pro-Asn-Ser-Asn-Cys-Ile-Ser-His-Ala

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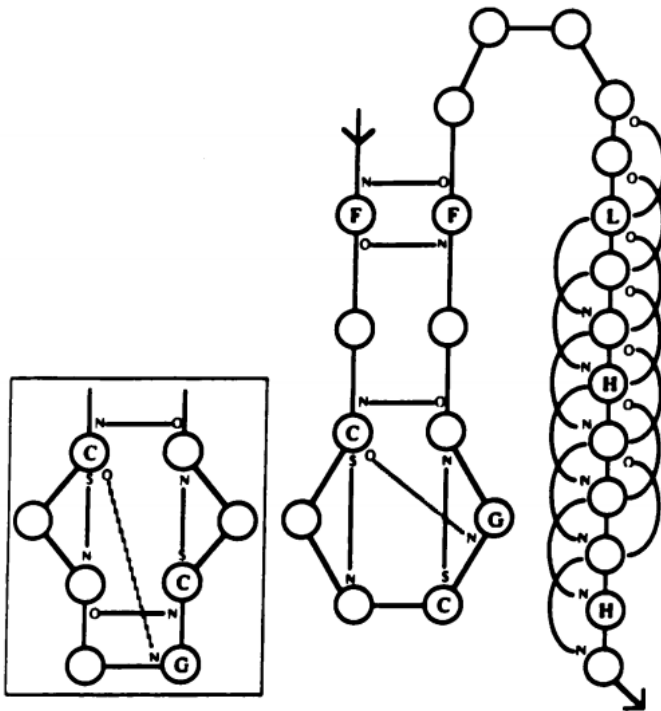
Comparisons with metalloproteins with known structures have allowed the development of a detailed three-dimensional model for these domains consisting of an antiparallel β -sheet followed by an α -helix.

Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins

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Proc. Natl. Acad. Sci. USA
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[(Phe, Tyr)-Xaa-Cys-Xaa-Xaa-{Xaa-Xaa}-Cys-Xaa-Xaa-Xaa-Phe] -
Cys-Cys loop



(Left) Hydrogen-bonding pattern for the predicted zinc-binding domain structure. The main figure shows the hydrogen bonding for a Cys-Xaa-Xaa-Cys loop; the insert corresponds to a Cys-Xaa-Xaa-Xaa-Cys loop. Glycine (G) residues, labeled in outline, occur frequently

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[(Phe, Tyr)-Xaa-Cys-Xaa-Xaa-{Xaa-Xaa}-Cys-Xaa-Xaa-Xaa-Phe] - [Xaa₄] -

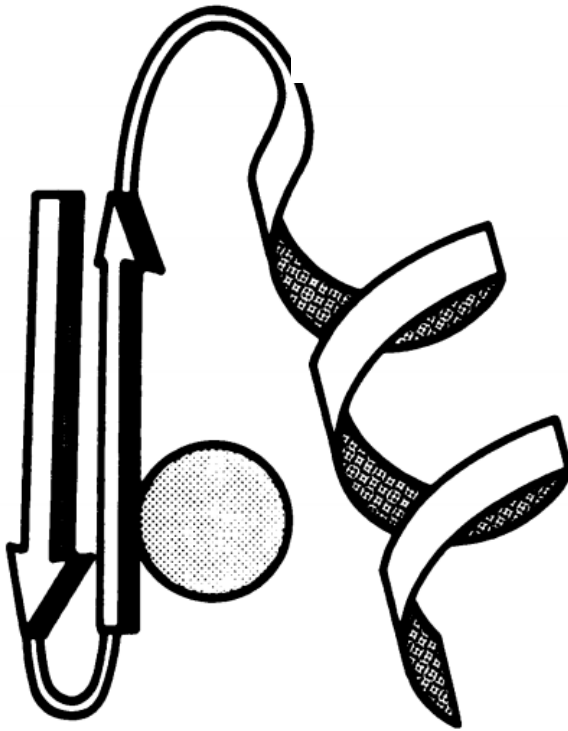
Cys-Cys loop

Tip

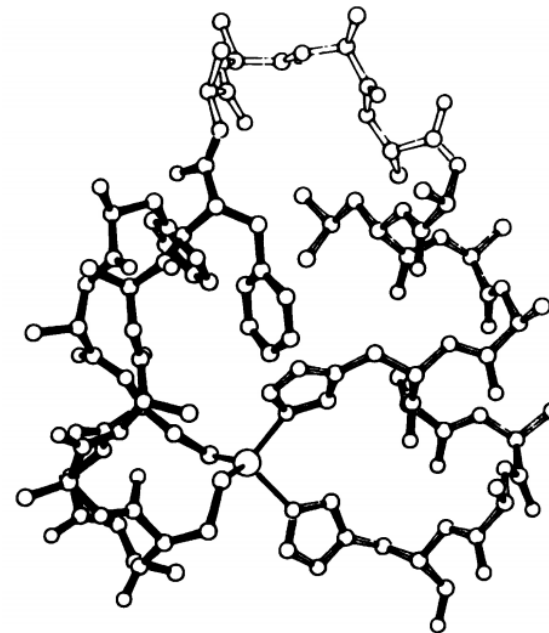
[Xaa-Leu-Xaa-Xaa-His-Xaa-Xaa-Xaa-His] - [Xaa₅]

His-His loop

Linker



Schematic drawing of the proposed zinc finger structure.



An ORTEP drawing of the proposed structure showing the side chains of the conserved metal-binding and hydrophobic regions. The Cys-Cys loop (dark), His-His loop (medium), and Tip (light) regions are indicated.

Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins

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[(Phe, Tyr)-Xaa-Cys-Xaa-Xaa-{Xaa-Xaa}-Cys-Xaa-Xaa-Xaa-Phe] - [Xaa₄] -

Cys-Cys loop

Tip

[Xaa-Leu-Xaa-Xaa-His-Xaa-Xaa-Xaa-His] - [Xaa₅]

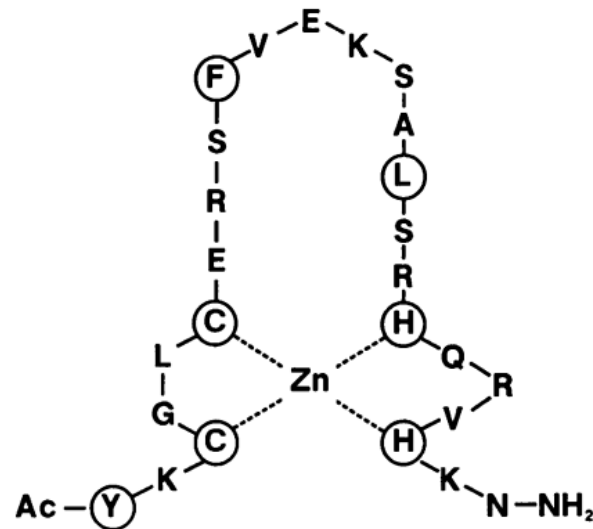
His-His loop

Linker

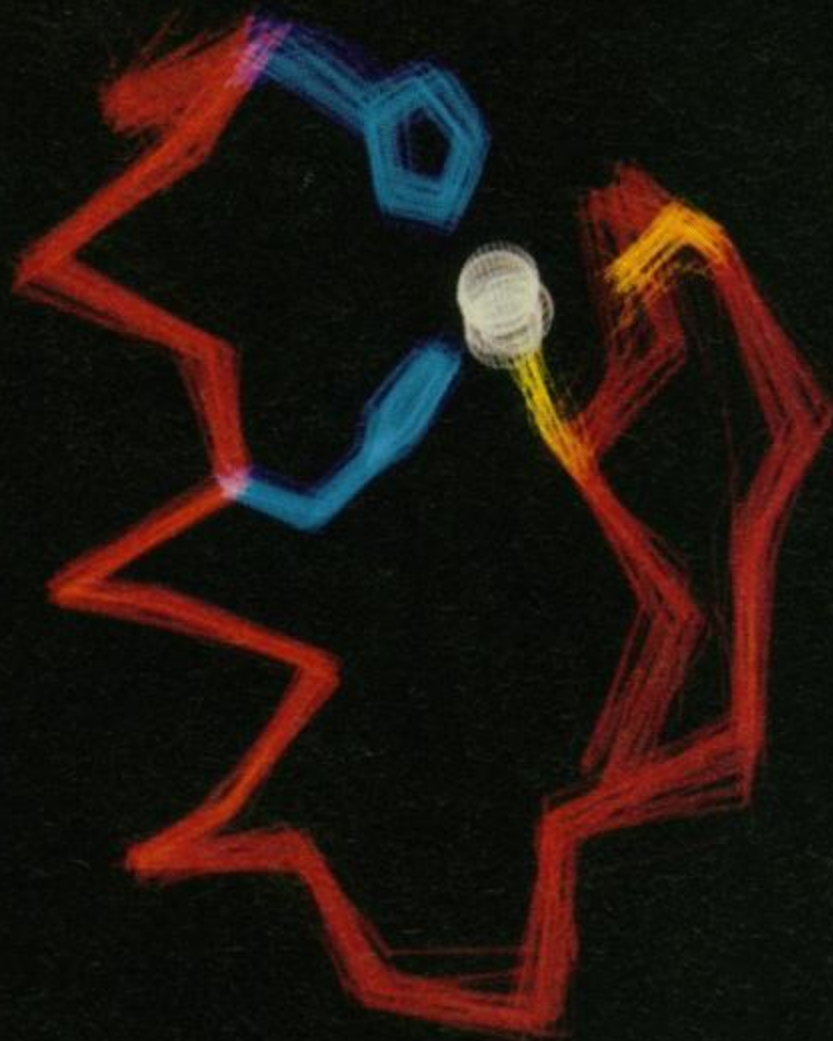


Three-Dimensional Solution Structure of a Single Zinc Finger DNA-Binding Domain

MIN S. LEE, GARRY P. GIPPERT, KIZHAKE V. SOMAN, DAVID A. CASE, PETER E. WRIGHT*



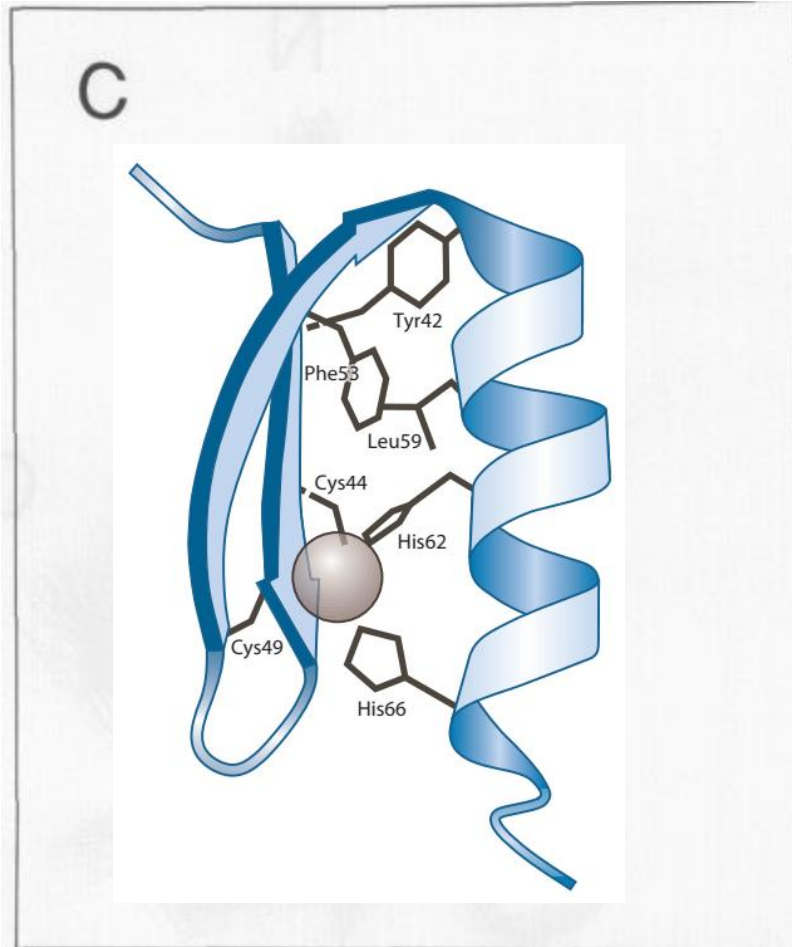
Xenopus protein *Xfin* (13)



Solution Structures of Two Zinc-finger Domains from SWI5 Obtained Using Two-dimensional ^1H Nuclear Magnetic Resonance Spectroscopy

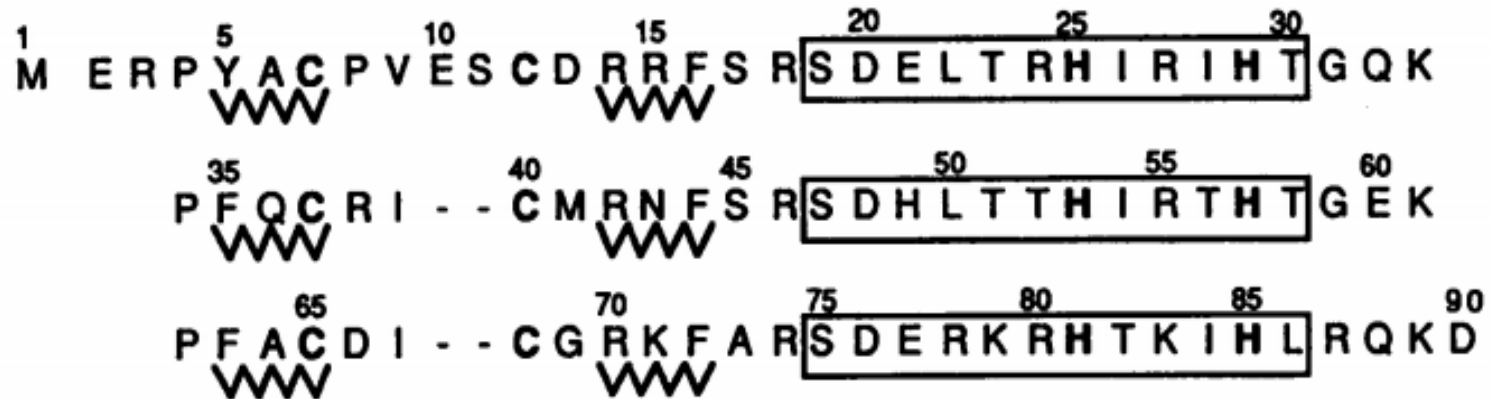
A Zinc-finger Structure with a Third Strand of β -Sheet

David Neuhaus, Yukinobu Nakaseko[†], John W. R. Schwabe and Aaron Klug



Zinc Finger–DNA Recognition: Crystal Structure of a Zif268-DNA Complex at 2.1 Å

NIKOLA P. PAVLETICH AND CARL O. PABO



antiparallel β - sheet

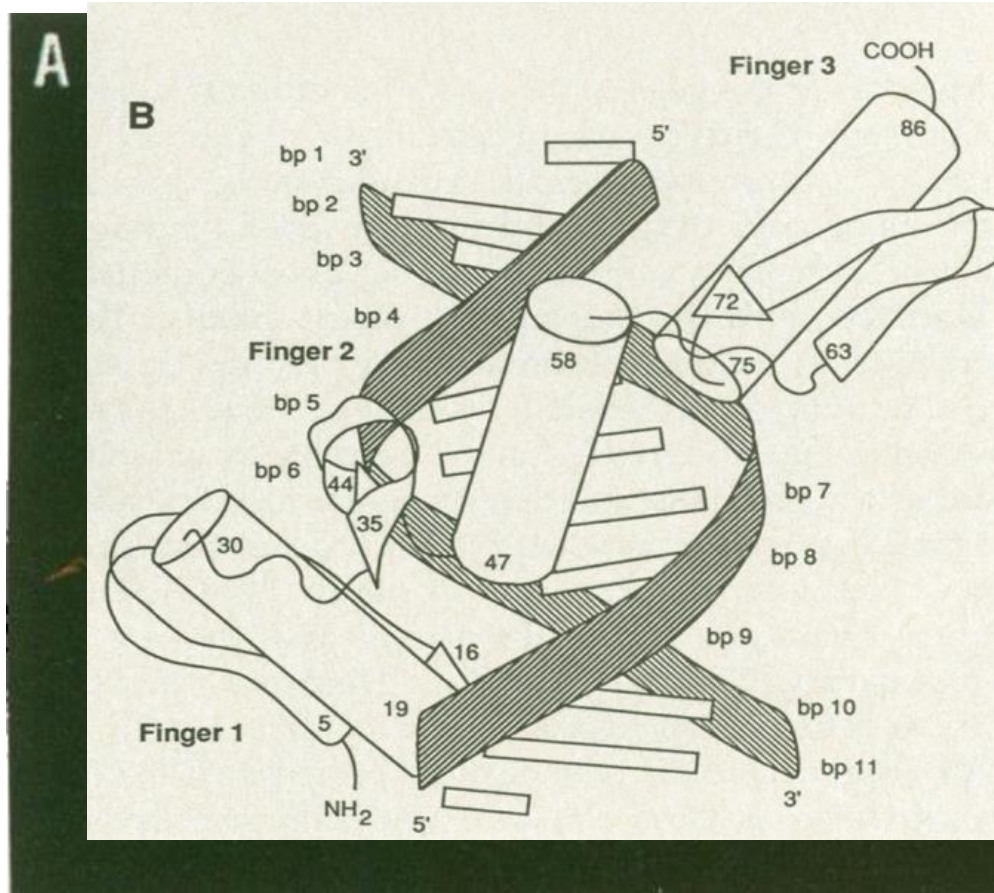
α - helix

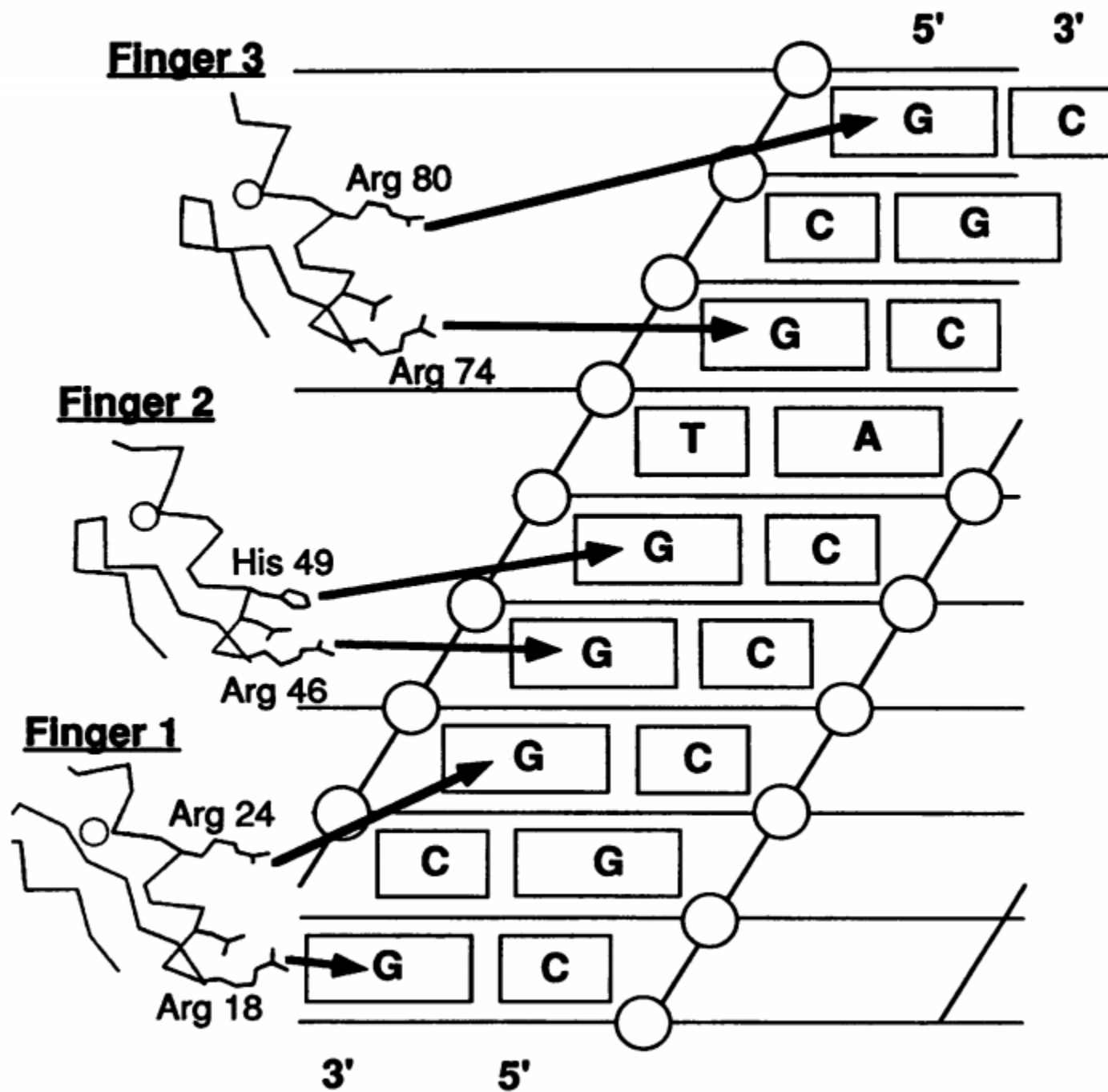
B

1	2	3	4	5	6	7	8	9	10	11
A	G	G	T	G	G	G	C	G	T	
	C	G	C	A	C	C	G	G	C	A

Zinc Finger–DNA Recognition: Crystal Structure of a Zif268-DNA Complex at 2.1 Å

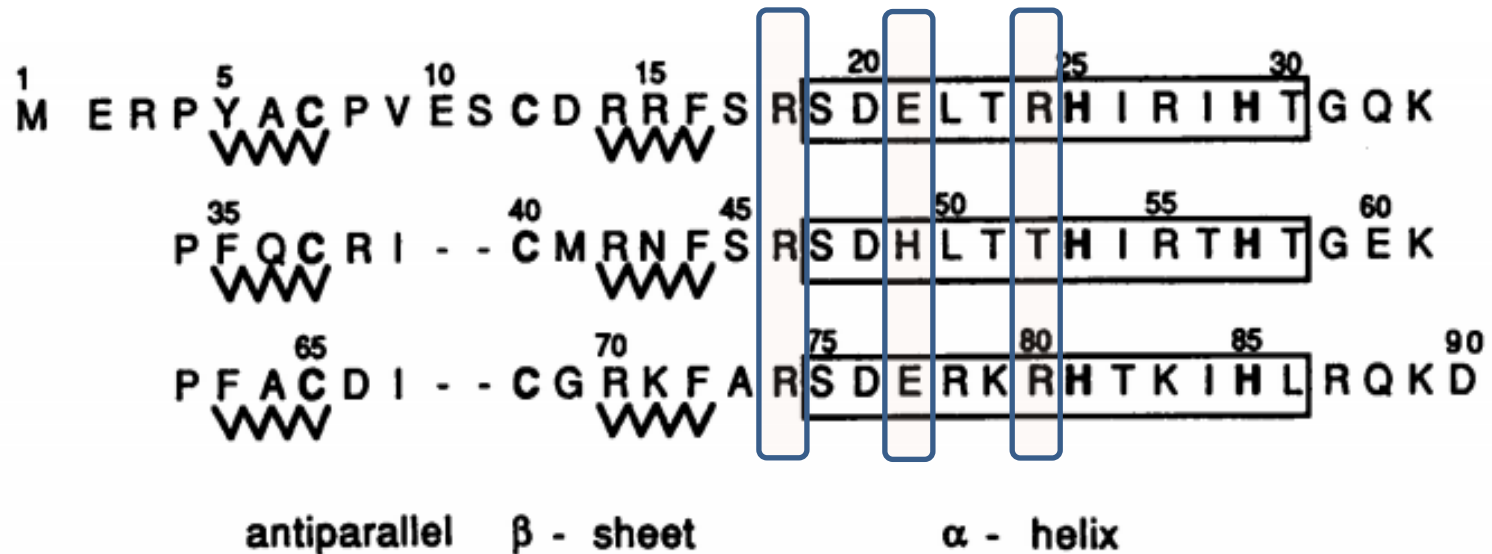
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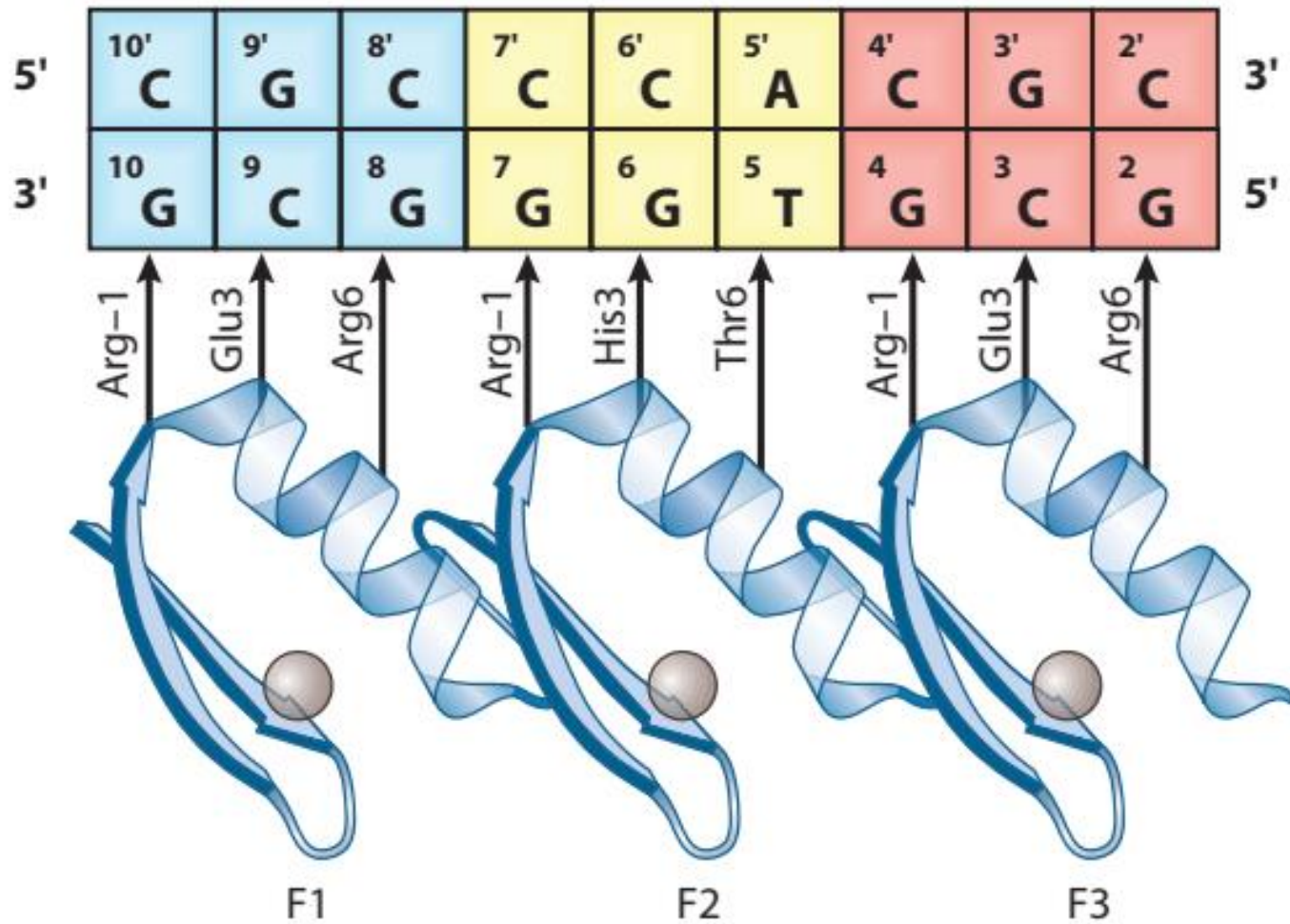
Zinc Finger–DNA Recognition: Crystal Structure of a Zif268-DNA Complex at 2.1 Å

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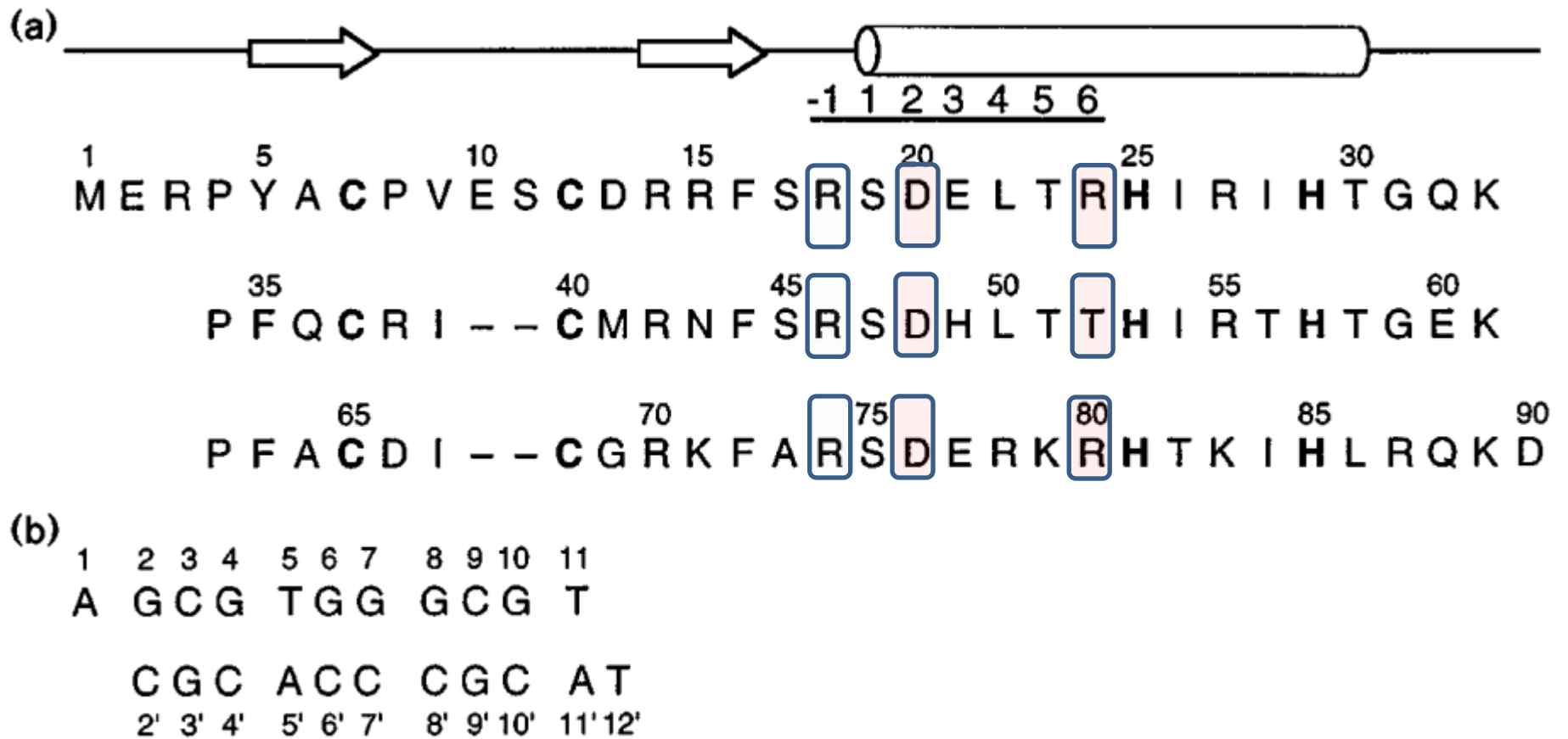
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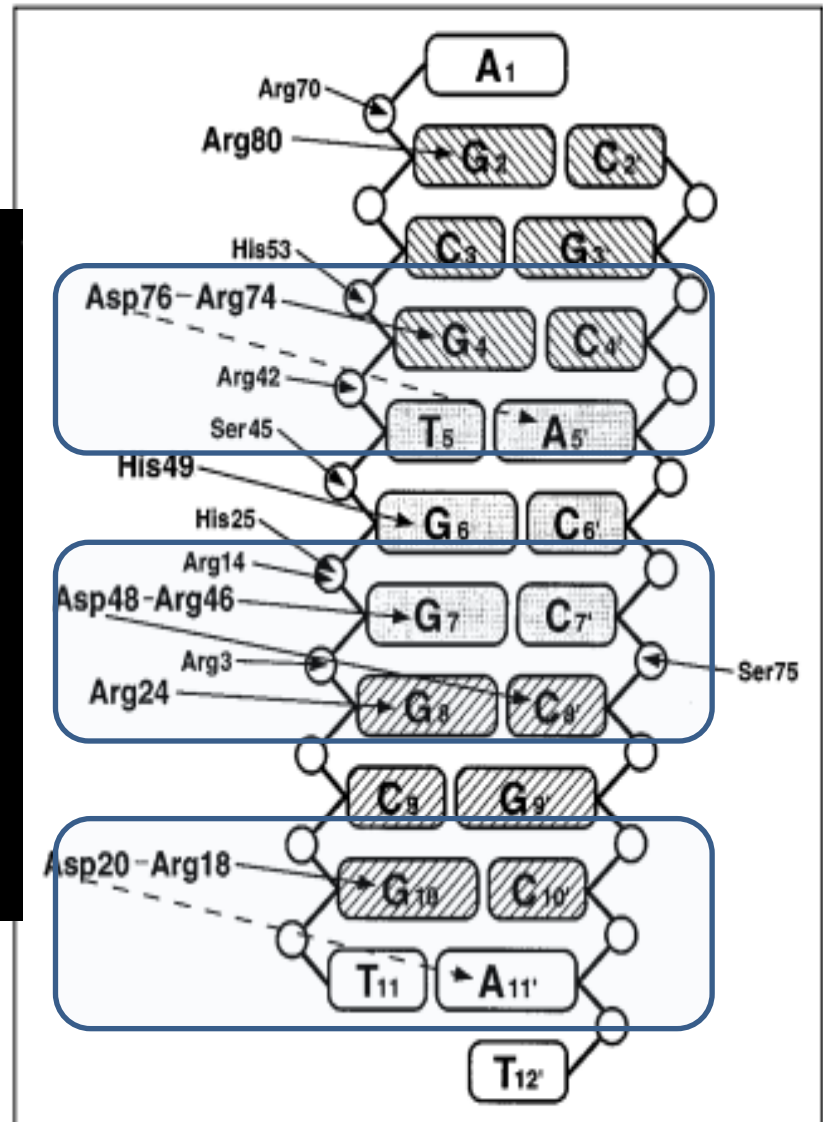
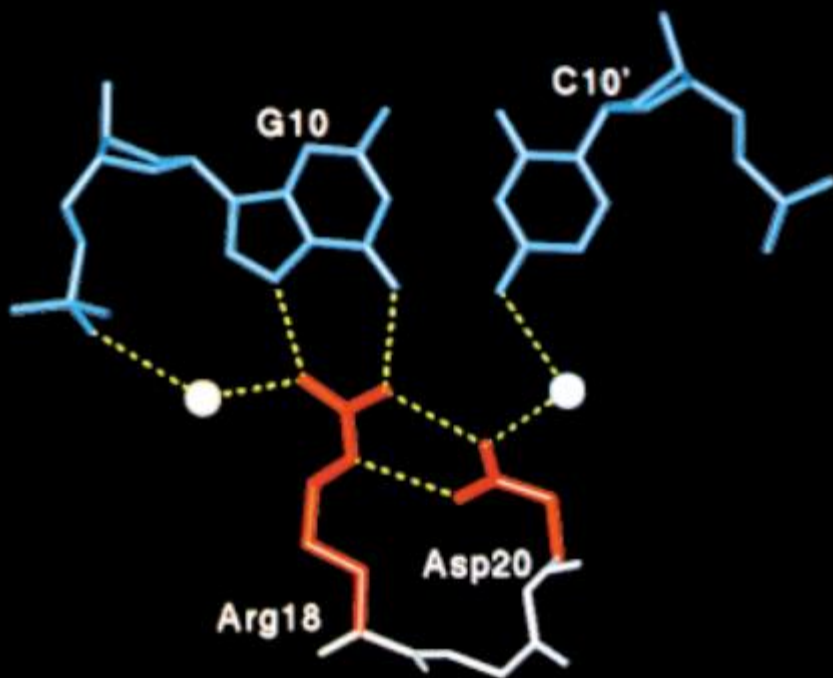
1	2	3	4	5	6	7	8	9	10	11
A	G	G	T	G	G	G	C	G	T	
	C	G	C	A	C	C	G	C	C	A



Zif268 protein–DNA complex refined at 1.6 Å: a model system for understanding zinc finger–DNA interactions

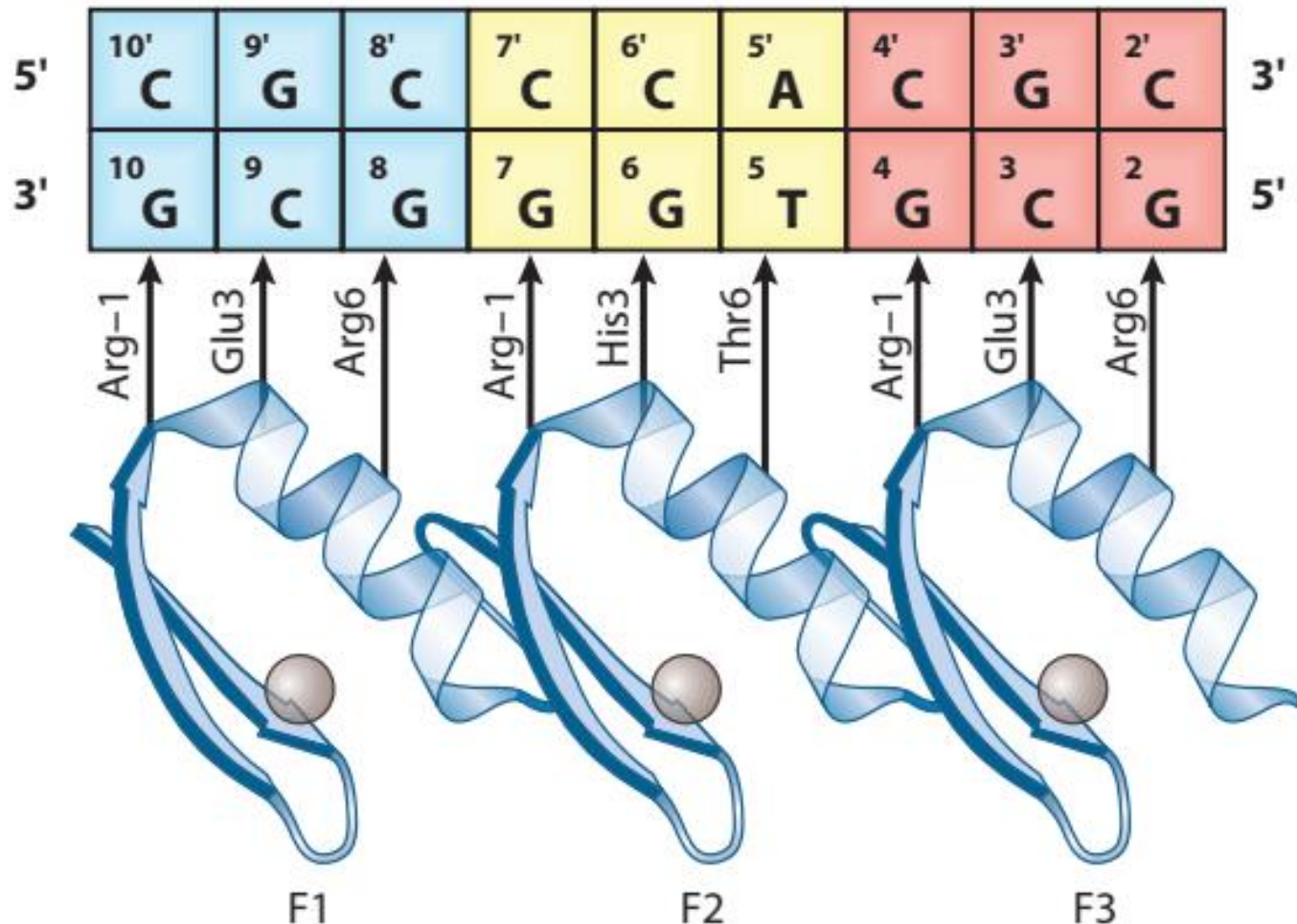
Monicia Elrod-Erickson, Mark A Rould, Lena Nekludova and Carl O Pabo*





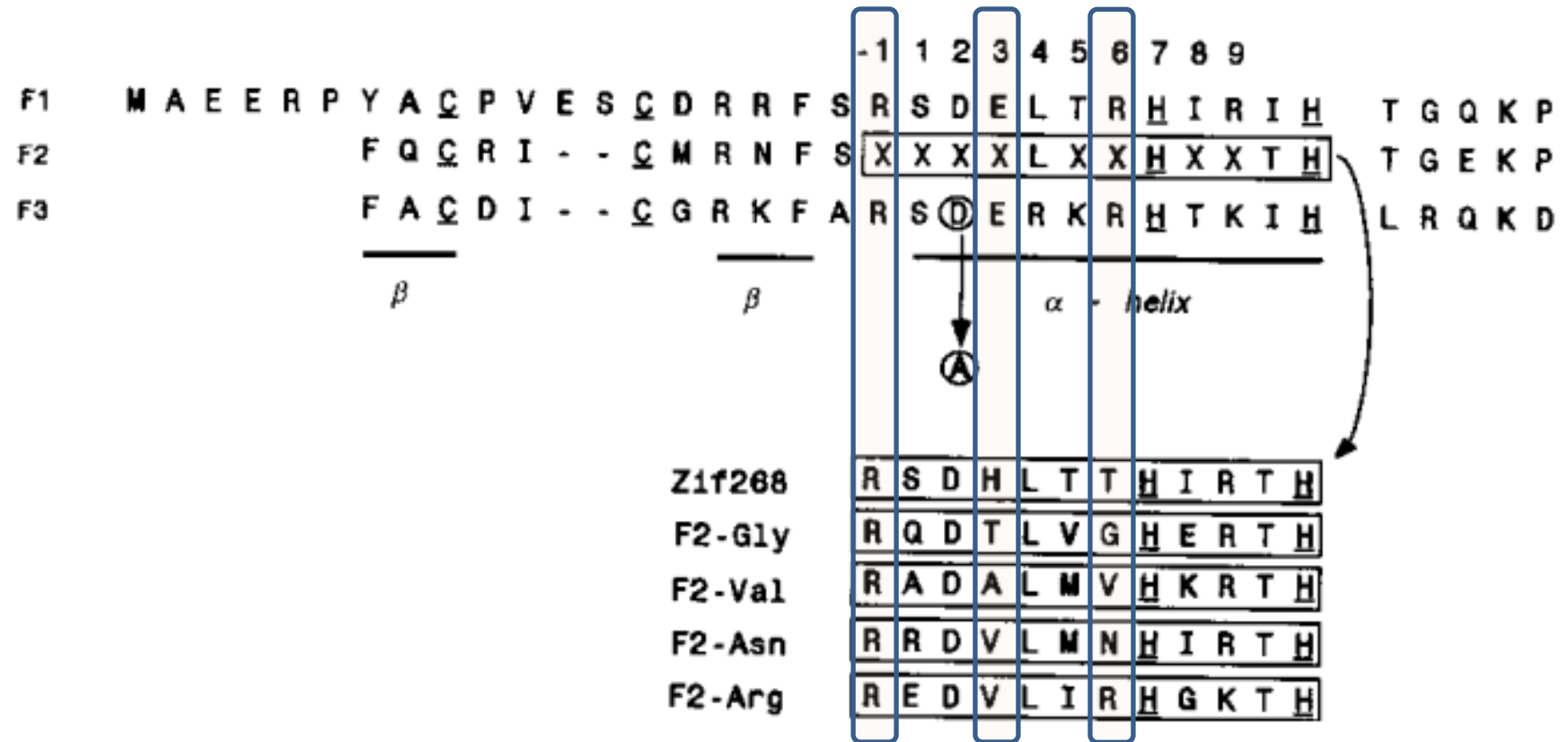
Synergy between adjacent zinc fingers in sequence-specific DNA recognition

MARK ISALAN, YEN CHOO*, AND AARON KLUG



Synergy between adjacent zinc fingers in sequence-specific DNA recognition

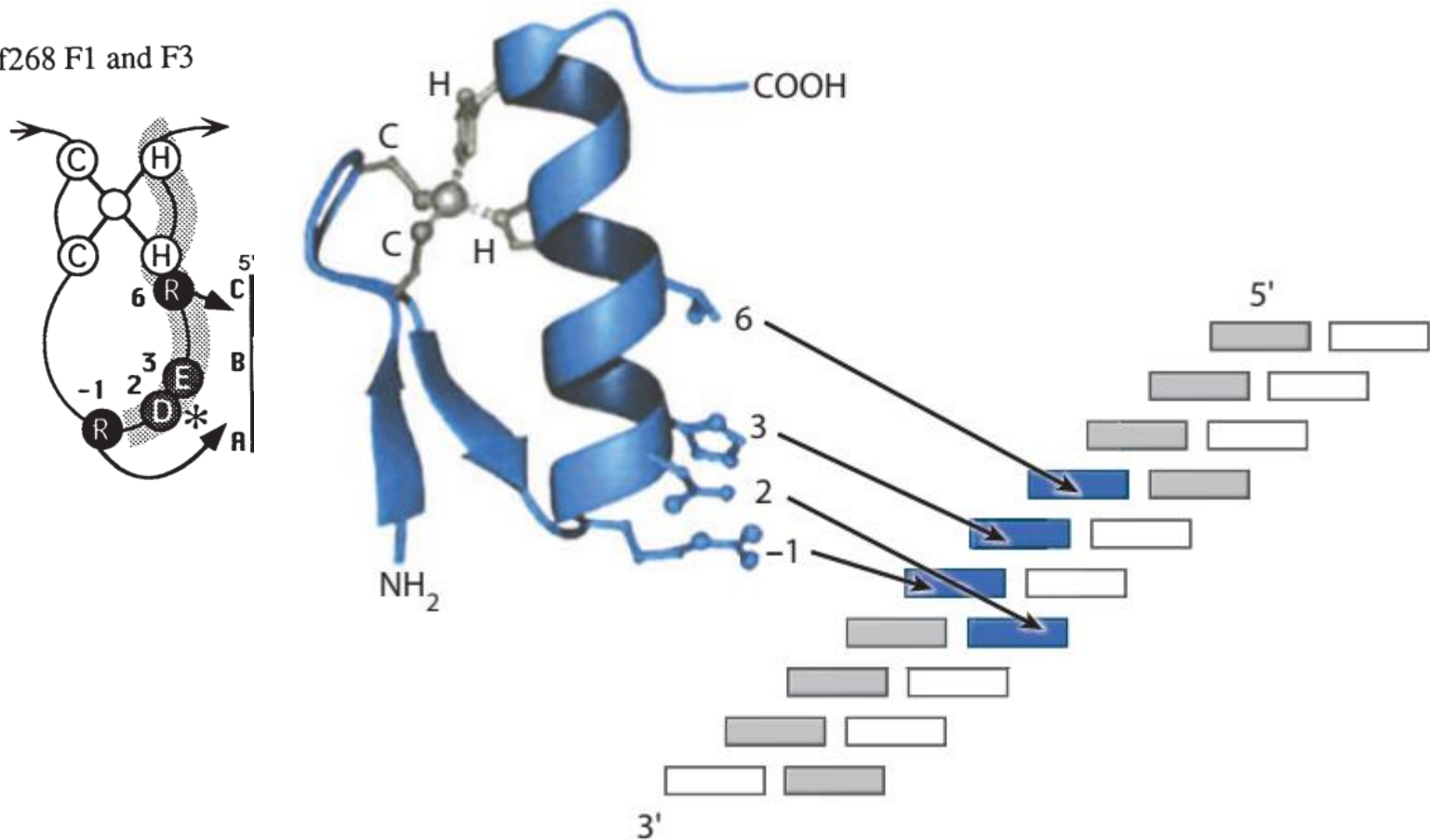
MARK ISALAN, YEN CHOO*, AND AARON KLUG



The crystal structure of a two zinc-finger peptide reveals an extension to the rules for zinc-finger/DNA recognition

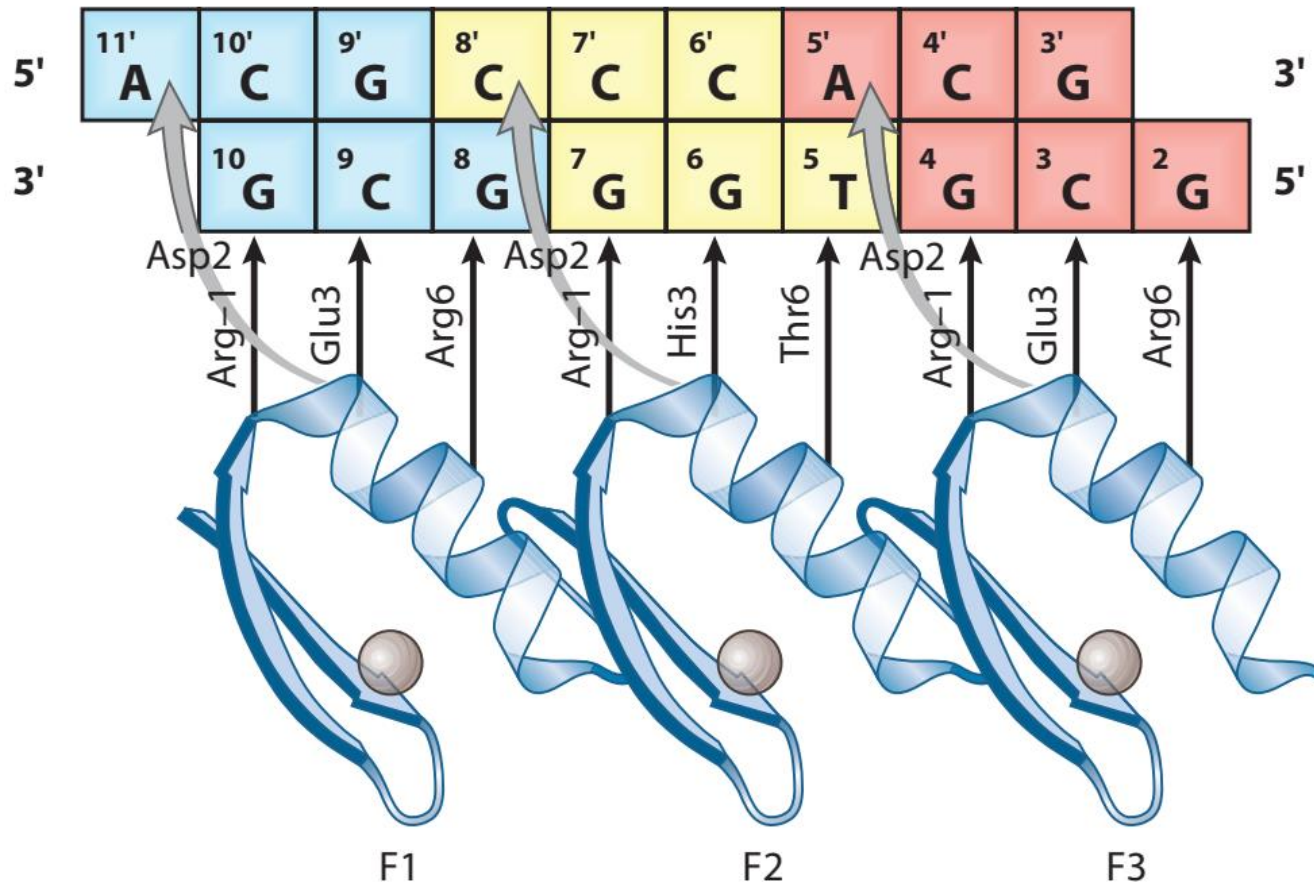
Louise Fairall, John W. R. Schwabe,
Lynda Chapman, John T. Finch
& Daniela Rhodes

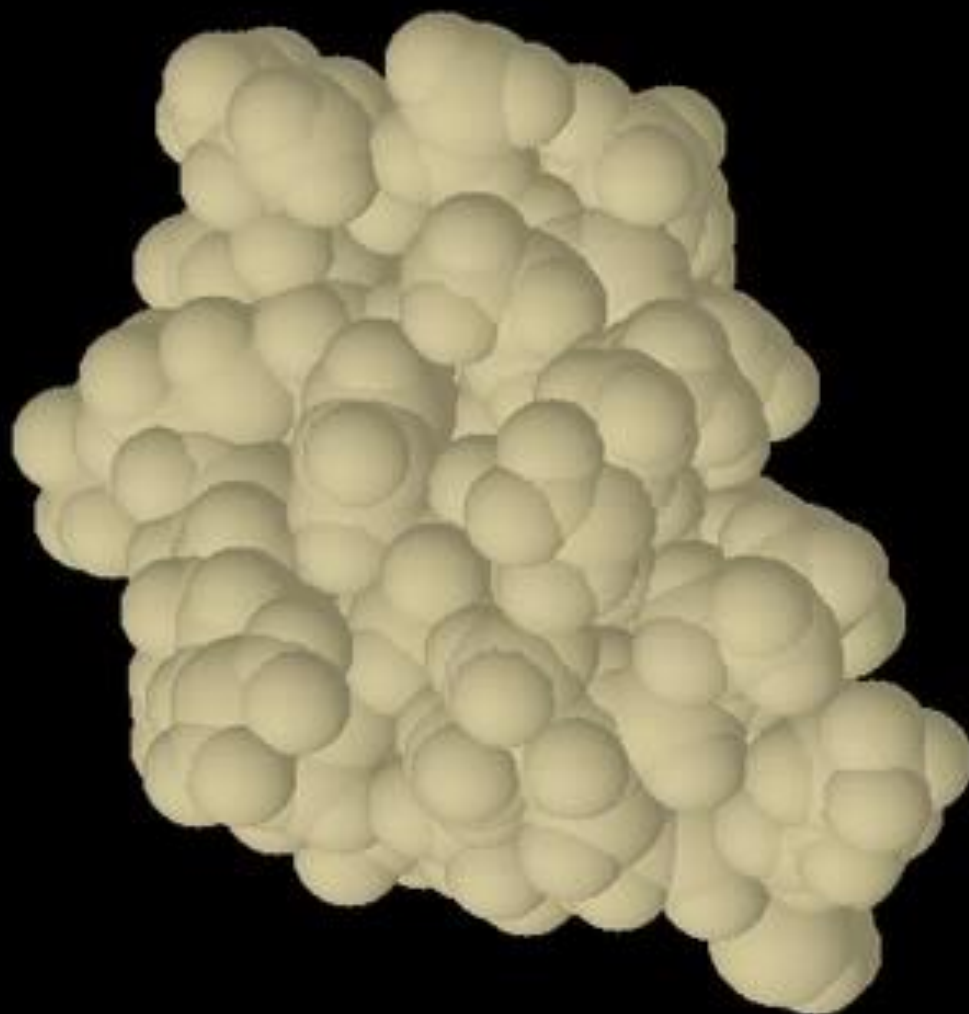
Zif268 F1 and F3



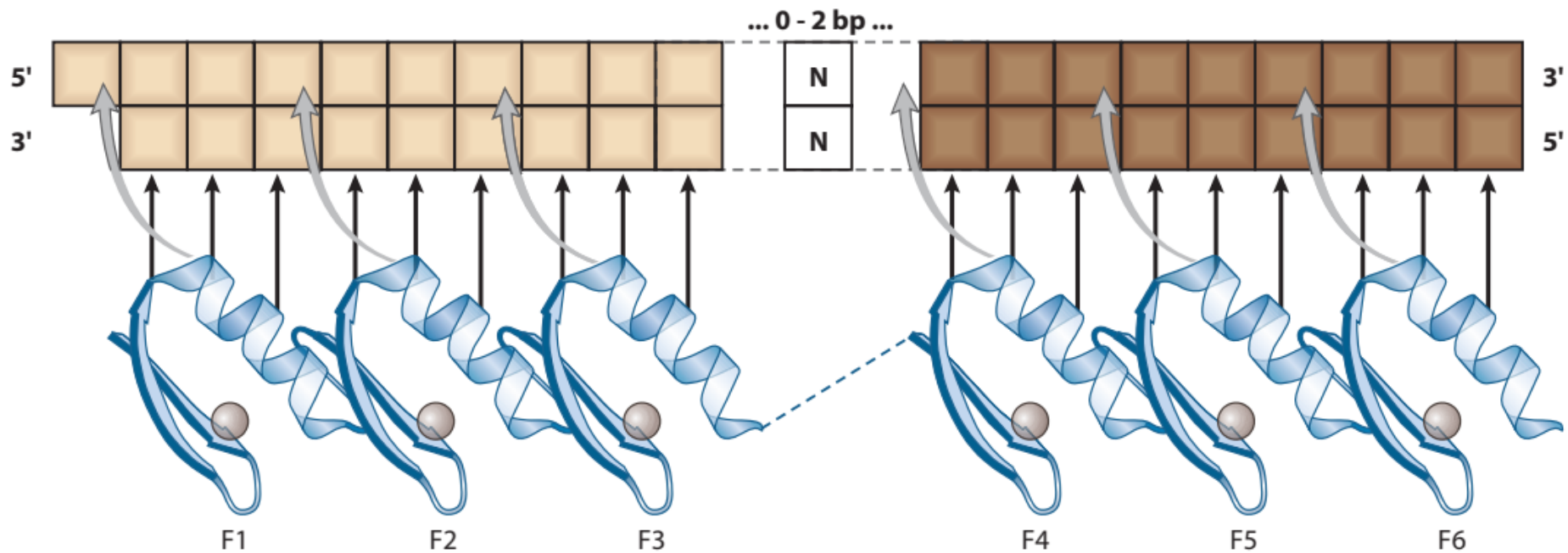
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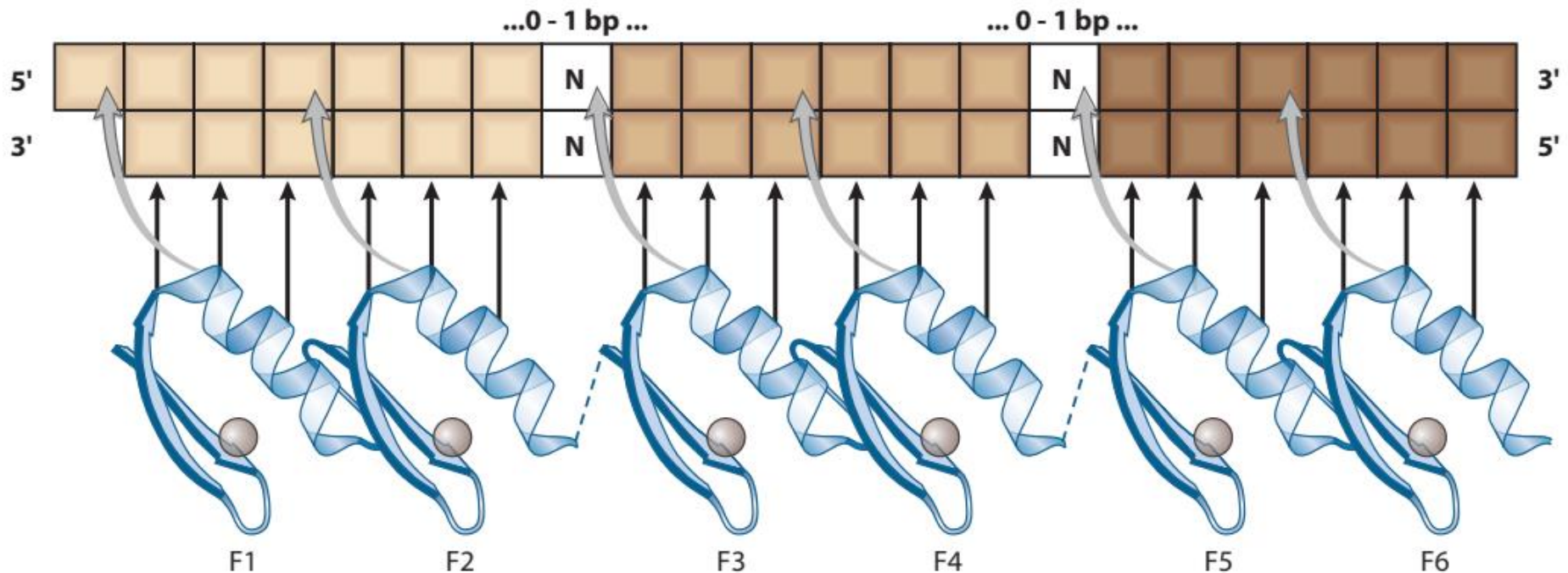


Periodicity of packed finger \neq DNA periodicity



Two modes of generating six-zinc finger proteins for specific recognition of 18-bp sequences (35, 36). (a) Two three-finger peptides fused together using an extended canonical linker ($2 \times 3F$ scheme). (b) Three two-finger peptides linked using canonical linkers

Periodicity of packed finger \neq DNA periodicity



fused together using an extended canonical linker ($2 \times 3F$ scheme). (b) Three two-finger peptides linked using canonical linkers extended by an insertion of either a glycine residue or a glycine-serine-glycine sequence in the canonical linkers between fingers 2 and 3 and fingers 4 and 5, respectively.

Conclusions of Lecture-11

- Today we saw the Structure of Zinc Finger proteins.
- Zinc Fingers have beta sheet and alpha helix.
- The alpha helix contains 7 conserved amino acids.
- The amino acids in the helix at (-1, 2, 3 and 6) form contacts with bases in overlapping subsites.
- So 1 Finger = 3 base, $\therefore 18\text{bp} = 3 \times 2\text{Finger}$ via artificial linker (G-S-G) residues.

Questions??