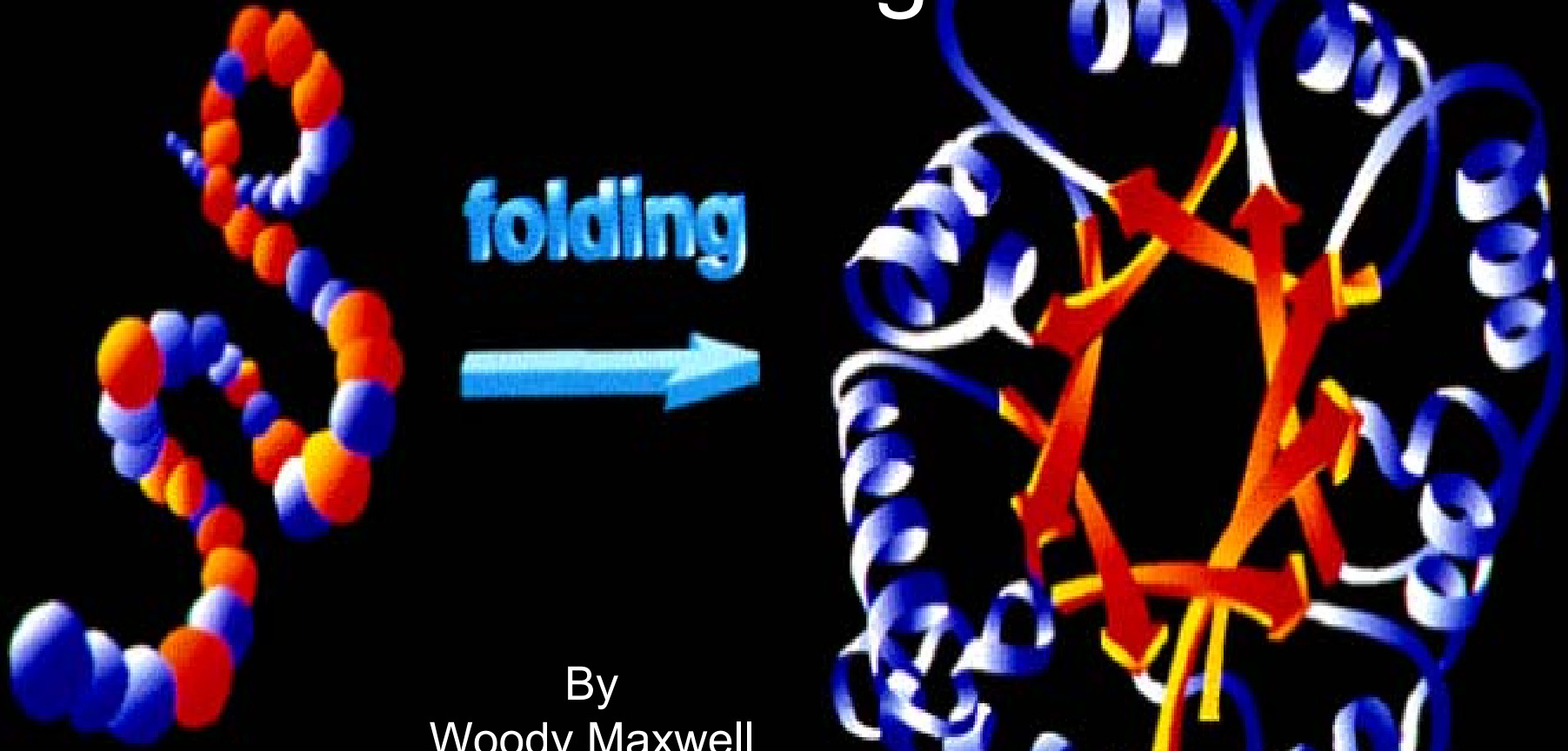
Probing the Mechanism of Protein Folding



By
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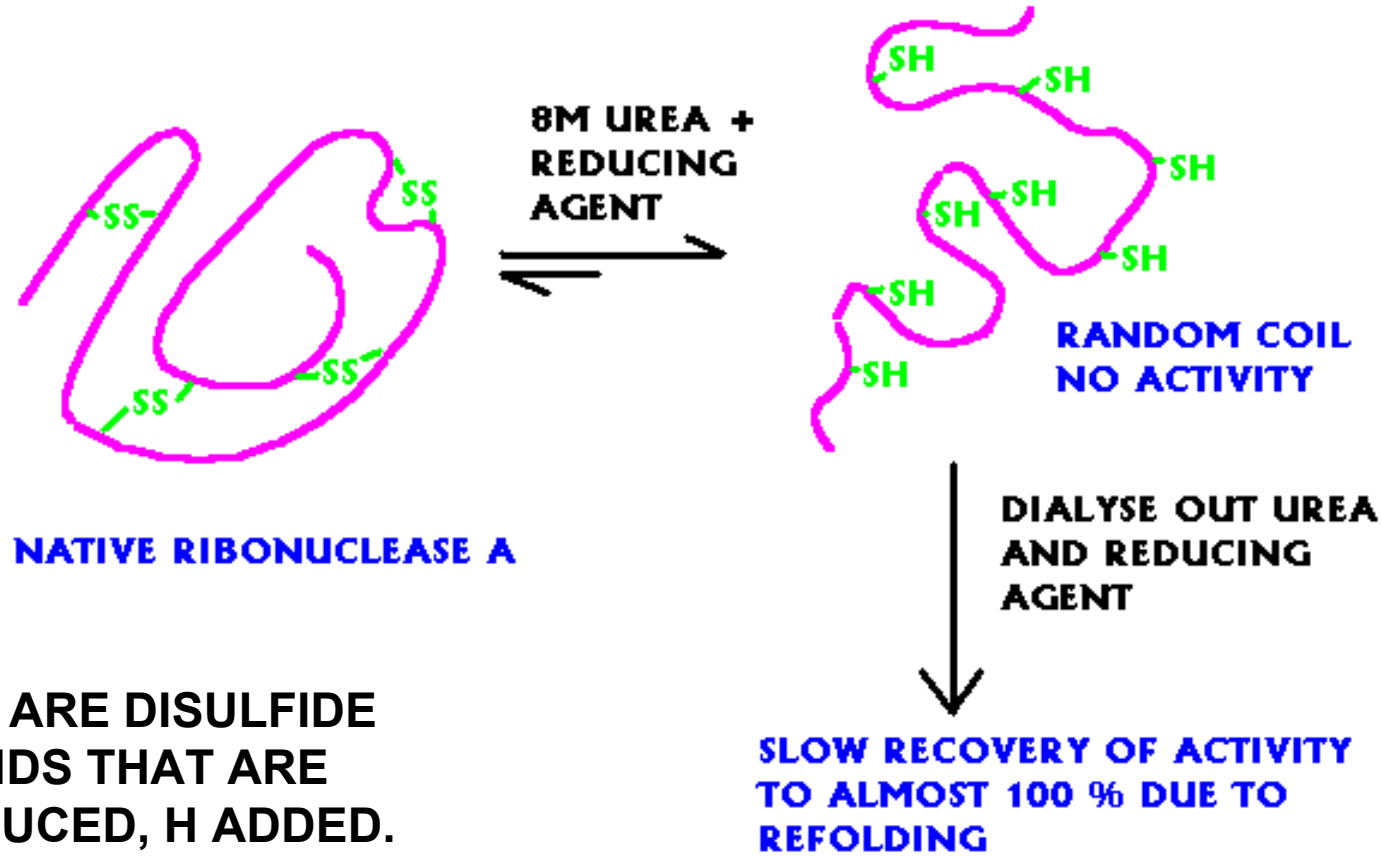
RET 1
Miguel De Los Rios, NIH grant

GOALS OF PROJECT

- UNDERLYING REASONS
- GENETIC RE-ENGINEERING OF PROTEINS AT SPECIFIC SITES
- CHARACTERIZE THE FOLDING AND UNFOLDING RATES

Anfinson's work on ribonuclease A

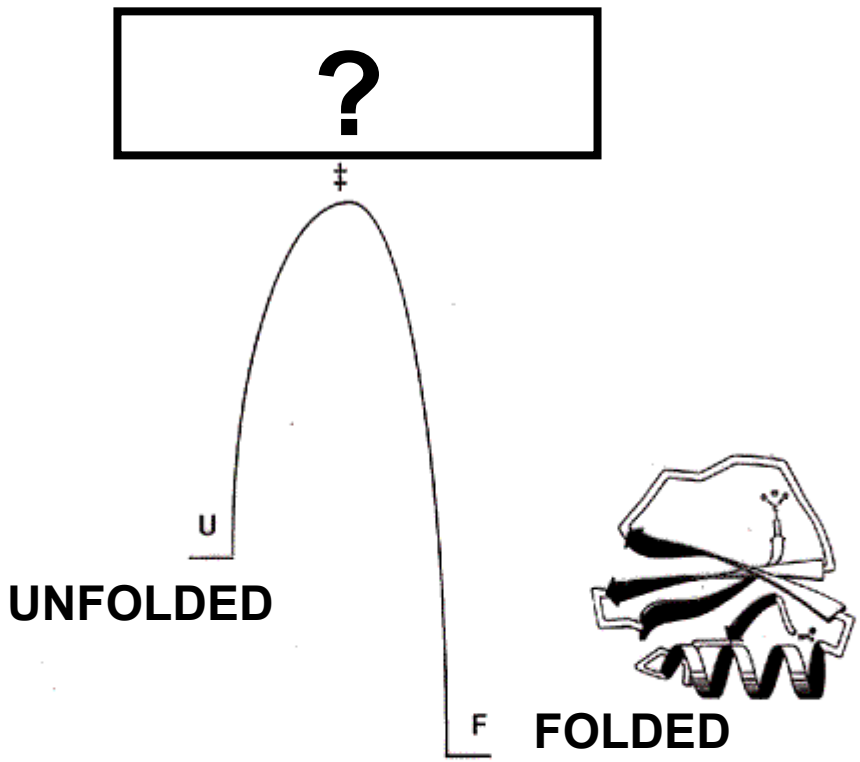


-SS- ARE DISULFIDE BONDS THAT ARE REDUCED, H ADDED.

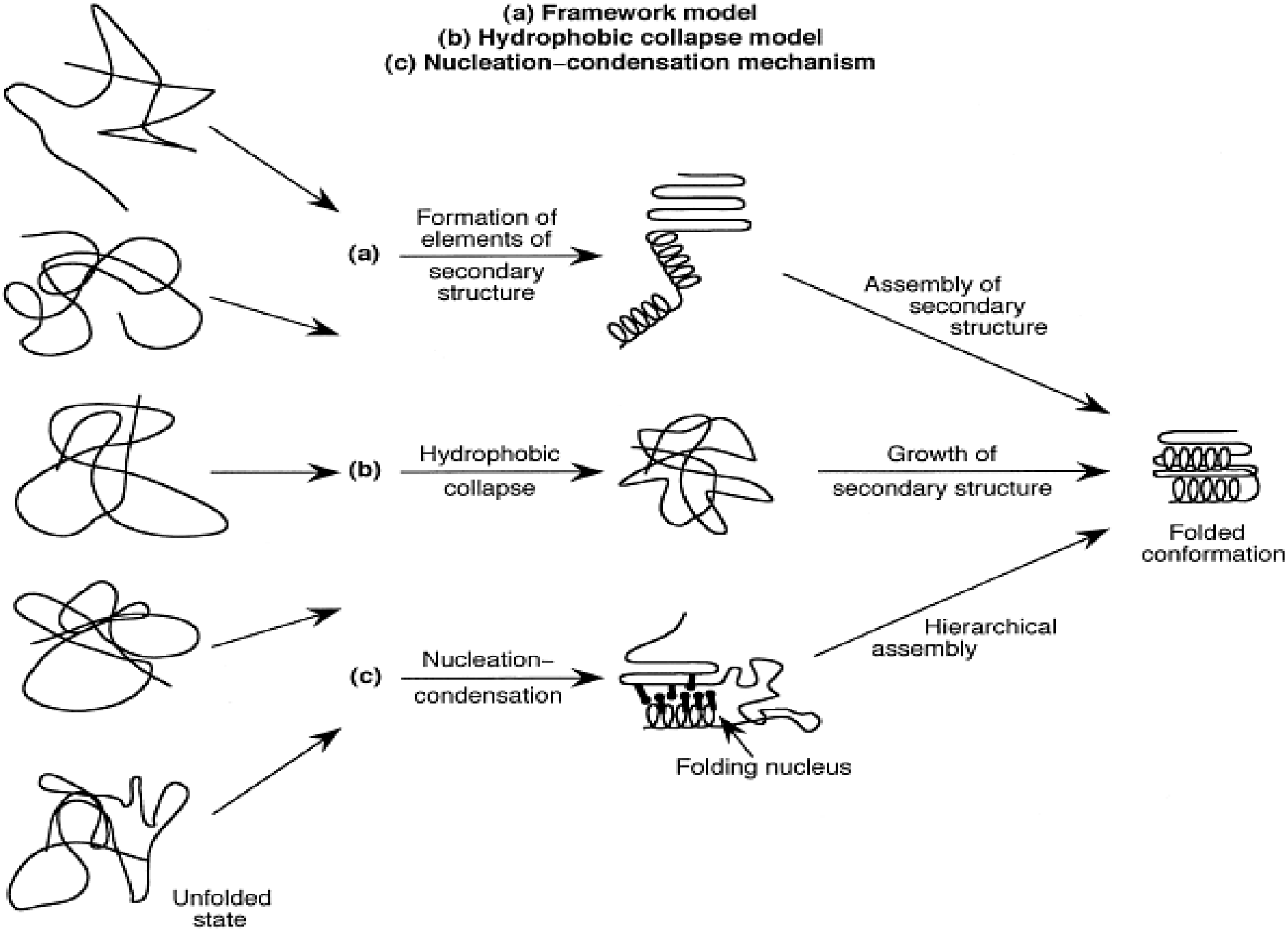
SIX ORDERS OF MAGNITUDE NEEDS TO BE EXPLAINED!

Protein	Rate	Reference
Cyt B_{562}	160,000	Wittung-Stafshede et al. 1999
Myoglobin	67,000	Wittung-Stafshede et al., 1998
PSBD	22,000	Spector & Raleigh, 1999
Cyt C	6,400	Winkler & Gray, pers com
λ-repressor	4,900	Ghaemmaghami et al., 1998
Ubiquitin	1,530	Khorasanizadeh et al., 1993
Im9	1,450	Ferguson et al., 1999
CspB	1,070	Perl et al., 1998
ADAh2	900	Villegas et al., 1998
Villin 14T	890	Choe et al., 1999
RP L9 (N-term)	735	Kuhlman et al., 1998
ACBP	700	B. Kragelund, pers com.
Protein G	500	Smith et al., 1996
U1A	320	Silow & Oliveberg, 1997
TI I27	160	Clarke et al., 1999
FynSH3	93	Plaxco et al., 1997
Protein L	61	Scalley et al., 1997
CI-2	48	Itzhaki et al., 1995
HPr	15	vanNuland et al., 1998
FKBP	3.8	Main et al., 1999
TnFNIII	2.9	Clarke et al., 1999
MerP	1.8	G. Aronsson, pers com.
Twitchin	1.5	Clarke et al., 1999
mAcP	0.2	vanNuland et al., 1998

The Folding of CI-2 is Two-State

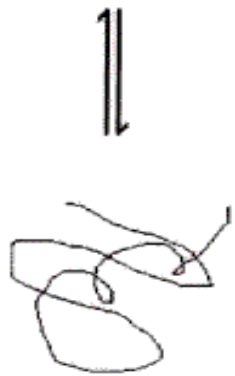


Jackson and Fersht, *Biochemistry*, **30**, 104280-10435 (1991)


Models for protein folding:
(a) Framework model
(b) Hydrophobic collapse model
(c) Nucleation–condensation mechanism
(a)
Formation of elements of secondary structure
Assembly of secondary structure
(b)
Hydrophobic collapse
Growth of secondary structure
Folded conformation
(c)
Nucleation–condensation
Hierarchical assembly
Folding nucleus
Unfolded state

Topomer Sampling Model

Non-native topomers



Native
Topomer

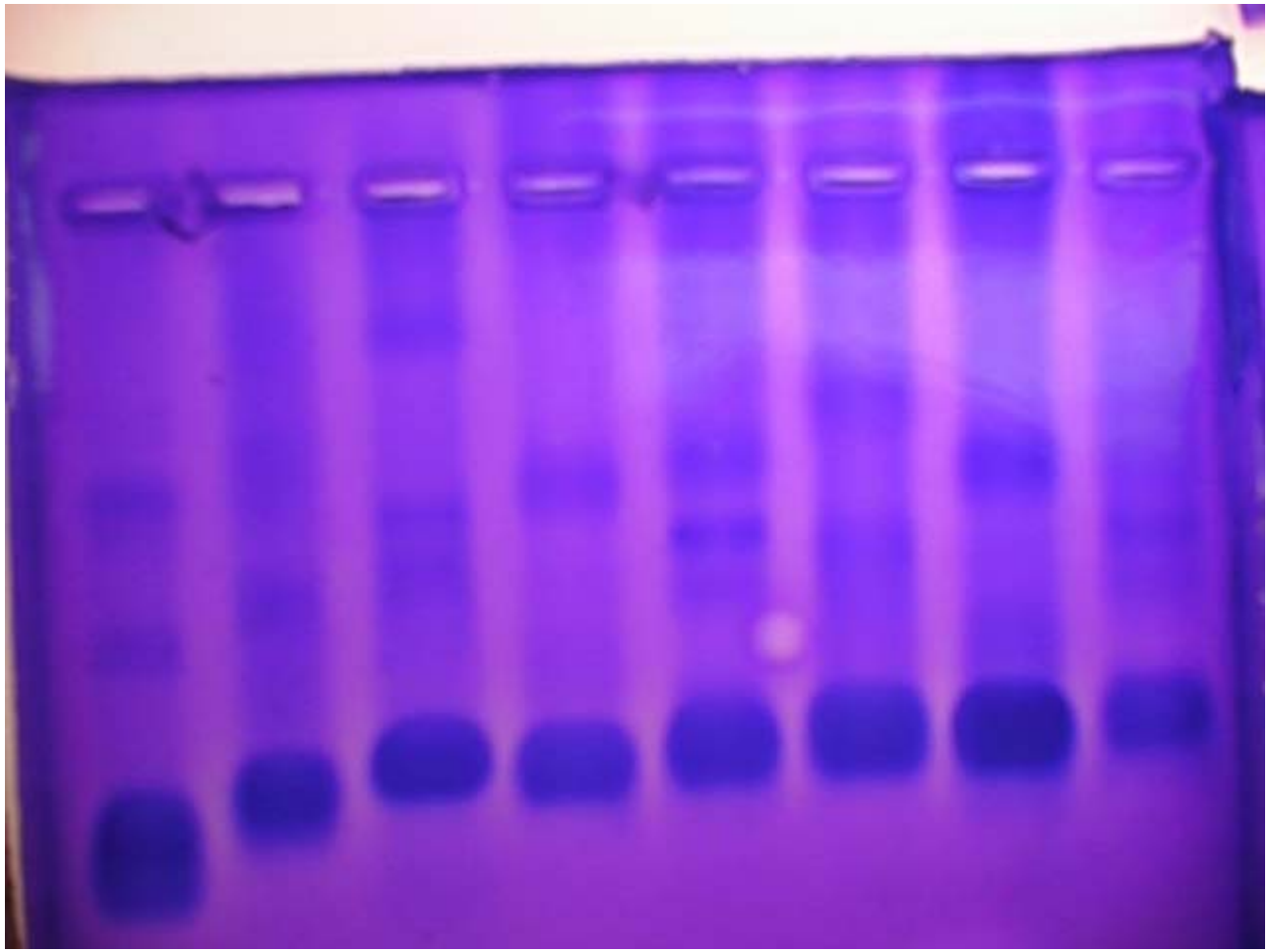
Folded

METHODS USED

- PCR
- PROTEIN AND DNA GEL ELECTROPHORESIS
- COLUMN CHROMOTOGRAPHY
- STOP-FLOW SPECTROPHOTOMETRY

PCR MACHINES

AGAROSE GEL ELECTROPHORESIS

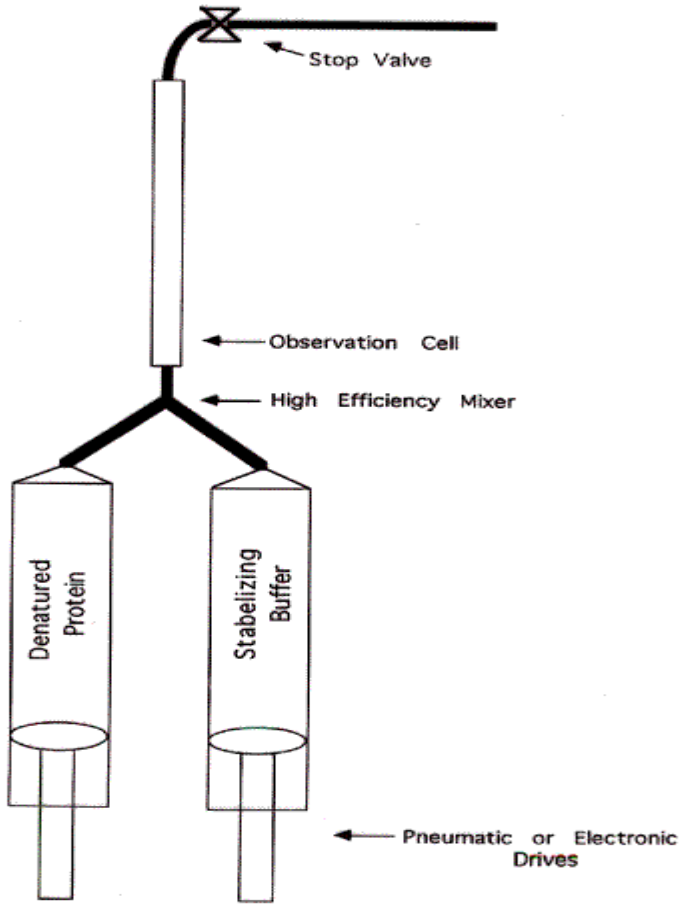


COLUMN CHROMATOGRAPHY

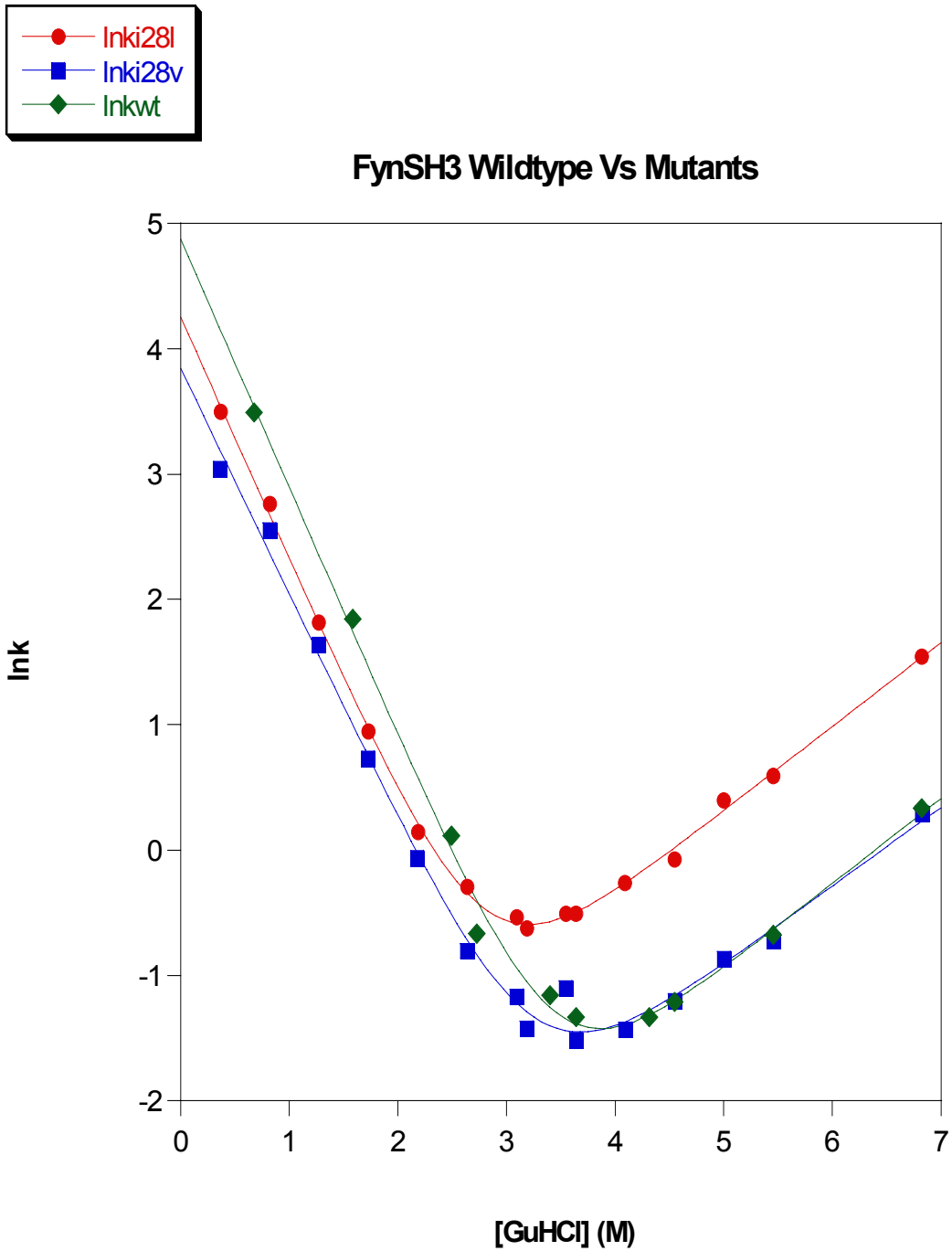
Nickel-containing compound that bonds strongly to the 6 His residues on our protein

STOP-FLOW SPECTROPHOTOMETRY

Biophysical Techniques



Plaxco and Dobson, *Curr. Op. Struct. Biol.*, 6, 630-636 (1996)



DATA FOR WILDTYPE VS. MUTANT FynSH3

	unfolding rate	folding rate	ΔG (kcal/mol)	ΔΔG (kcal/mol)
wild	-4.3434	4.8754	5.38	
I28V	-4.0639	3.8453	4.62	0.76
i28L	-3.0447	4.2515	4.26	1.12

CONCLUSIONS

- **PERSONAL**
  - **THE EXPERIENCE OF WORKING IN A TOP-RANKED UNIVERSITY LAB HAS BEEN VERY ENLIGHTENING.**
  - **THE PROCESS OF DISCOVERY IS INDIVIDUAL AS WELL AS COLLABORATIVE**
  - **THE EXPENSE AND TIME INVOLVED**
  - **PREVIOUS PROJECTS ARE TODAY'S TOOLS.**
- **SCIENTIFIC**
  - **ALTHOUGH THE FOLDING RATE VARIED, IT WAS STILL NOT SIGNIFICANT.**
  - **INDICATING THE REPLACEMENT OF INDIVIDUAL AMINO ACIDS HAS LESS EFFECT THAN THE GLOBAL SHAPE IN THE FOLDING RATE.**
  - **THE LITERATURE REPORTED THAT I28A HAD A HIGH Φ-VALUE, OUR DATA SHOWED THAT IT WAS MORE OF AN ANOMALY.**

THANK U'S

- Kevin Plaxco- the PI that allowed me to learn in his group.
- Miguel de los Rios- my actual boss who showed me some cool techniques and made my head hurt (again).
- The rest of the group for allowing me to take up space in their lab.
- RET (Martina) for enabling me to do research instead of summer school and meet some cool people.
- The chance to take this back to my classroom.