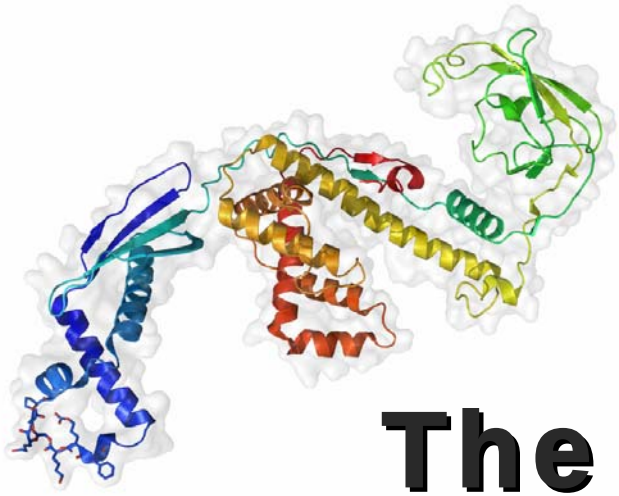
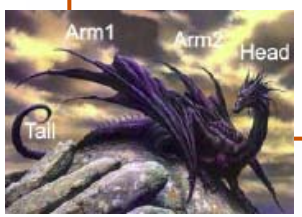


The Trigger Factor Chaperone

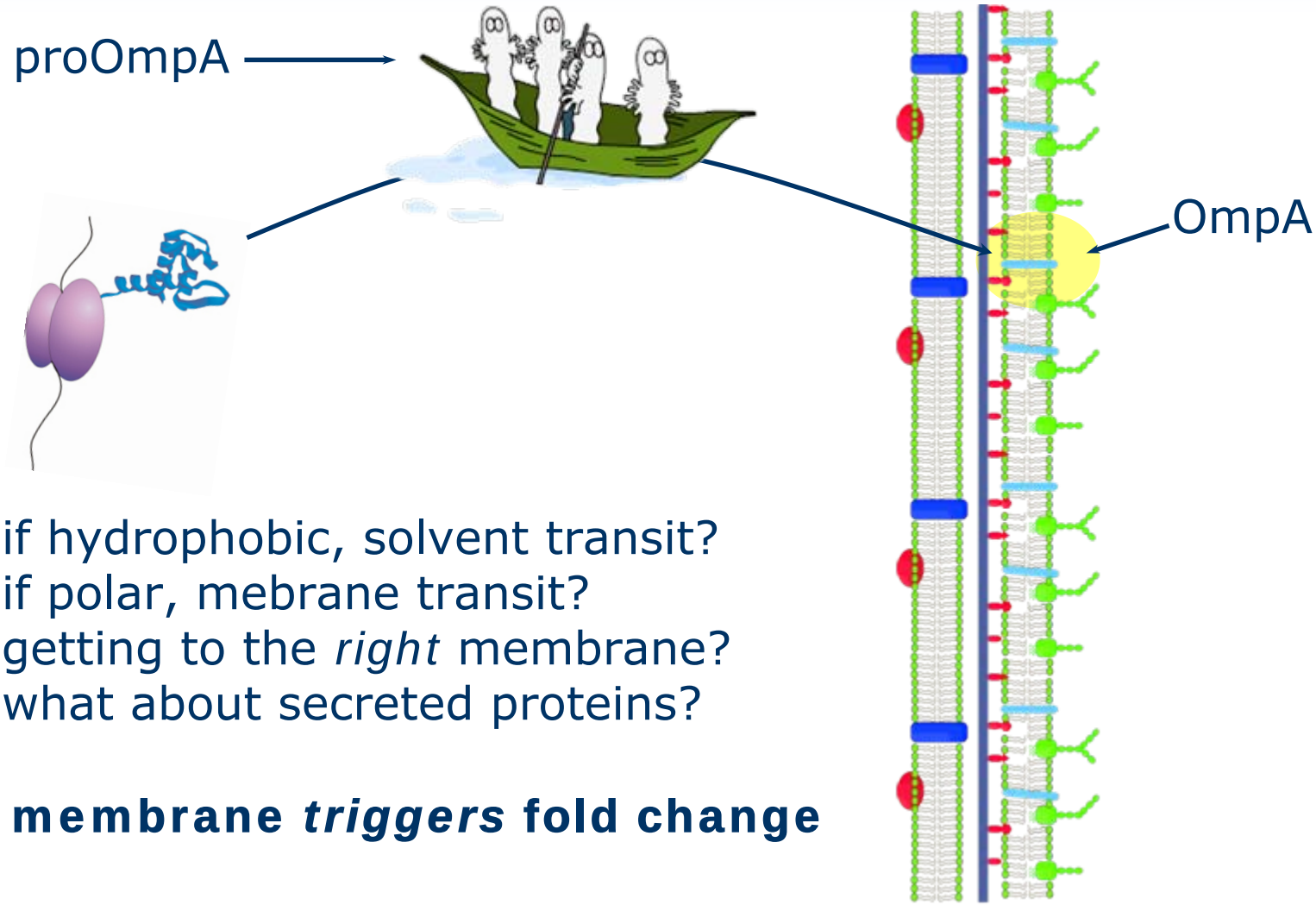
Mieszko Lis
Charles W. O'Donnell

Massachusetts Institute of Technology



December 1, 2006
MIT 7.88 Research Presentation

Crossing the membrane

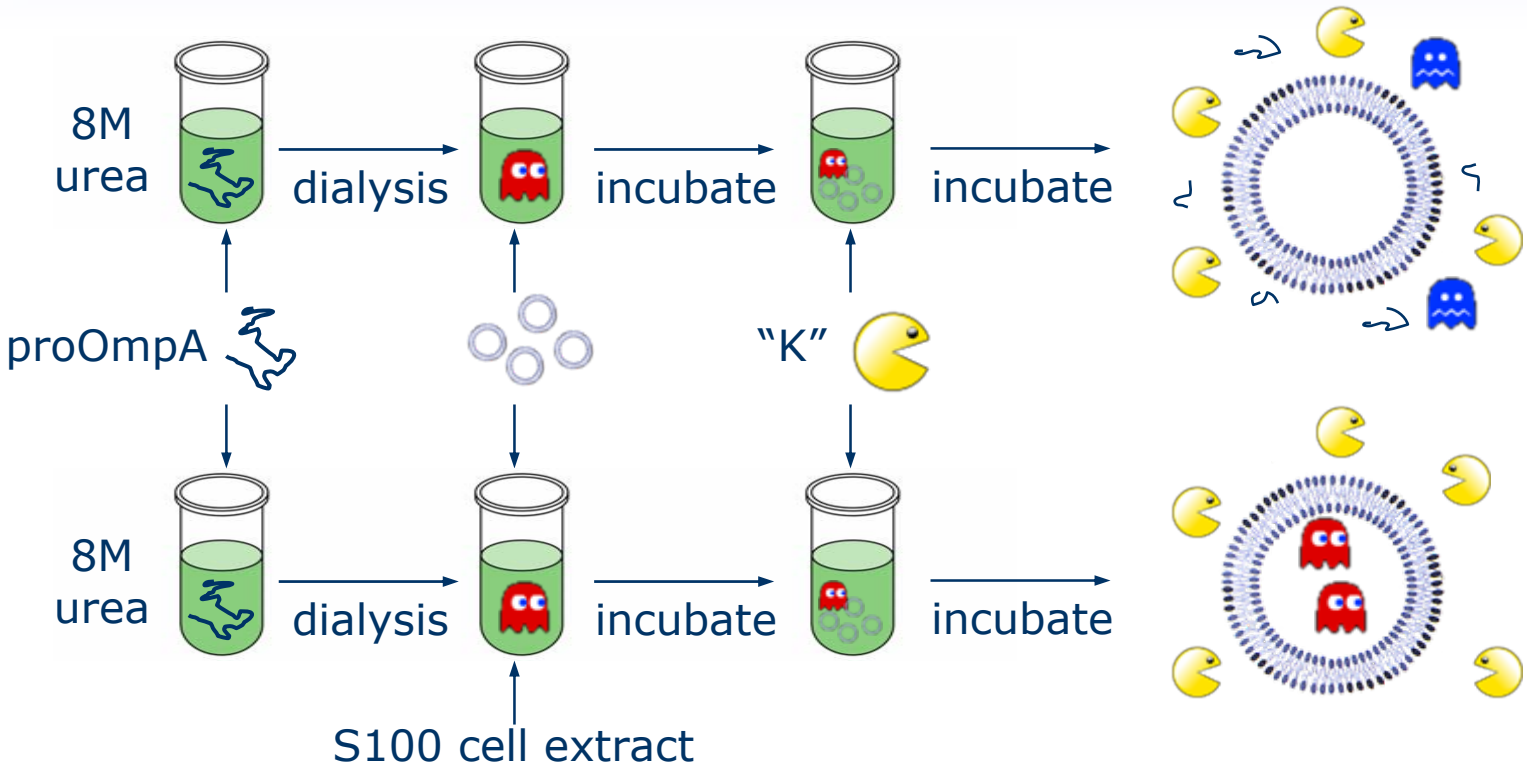


- if hydrophobic, solvent transit?
- if polar, mebrane transit?
- getting to the *right* membrane?
- what about secreted proteins?

Model: membrane *triggers* fold change

W. Wickner, Rev. Biochem. 48:23-45 (1979)

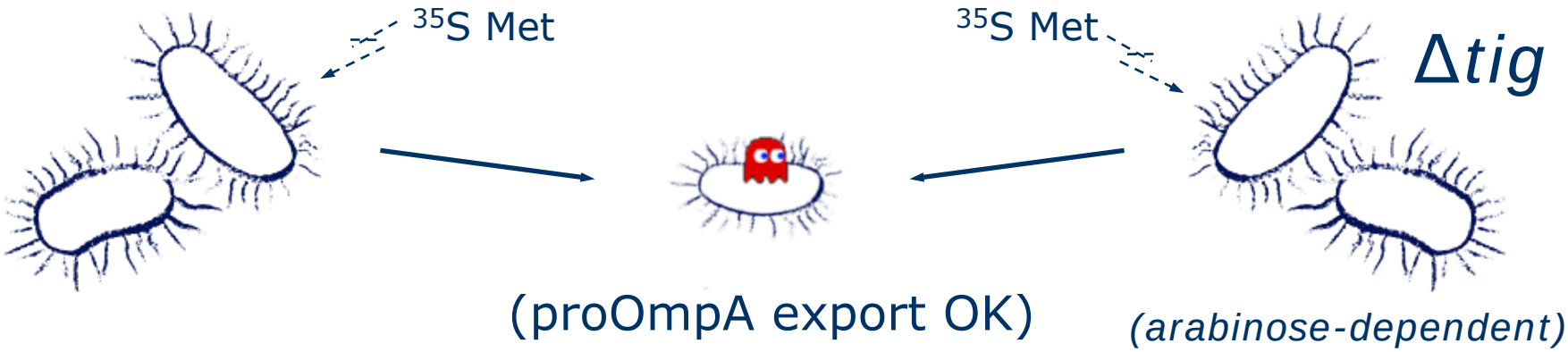
Trigger Factor



extract must contain some sort of **trigger factor** (later isolated from proOmpA and ribosomes)

Crooke, Wickner, PNAS 84:5216-5220 (1987), Lill, et alia, Cell 54:1013-1018 (1988)

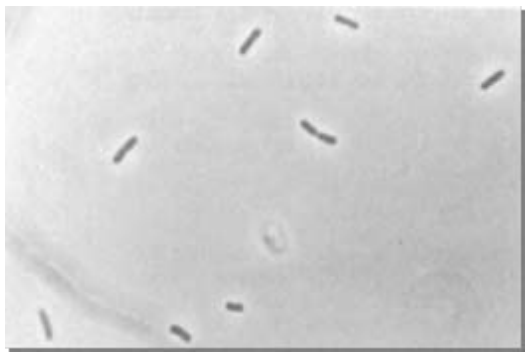
Close… but no trigger



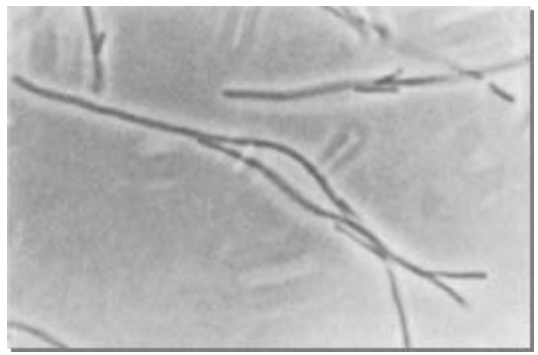
trigger factor *not* needed for translocation

TF < normal

TF = normal

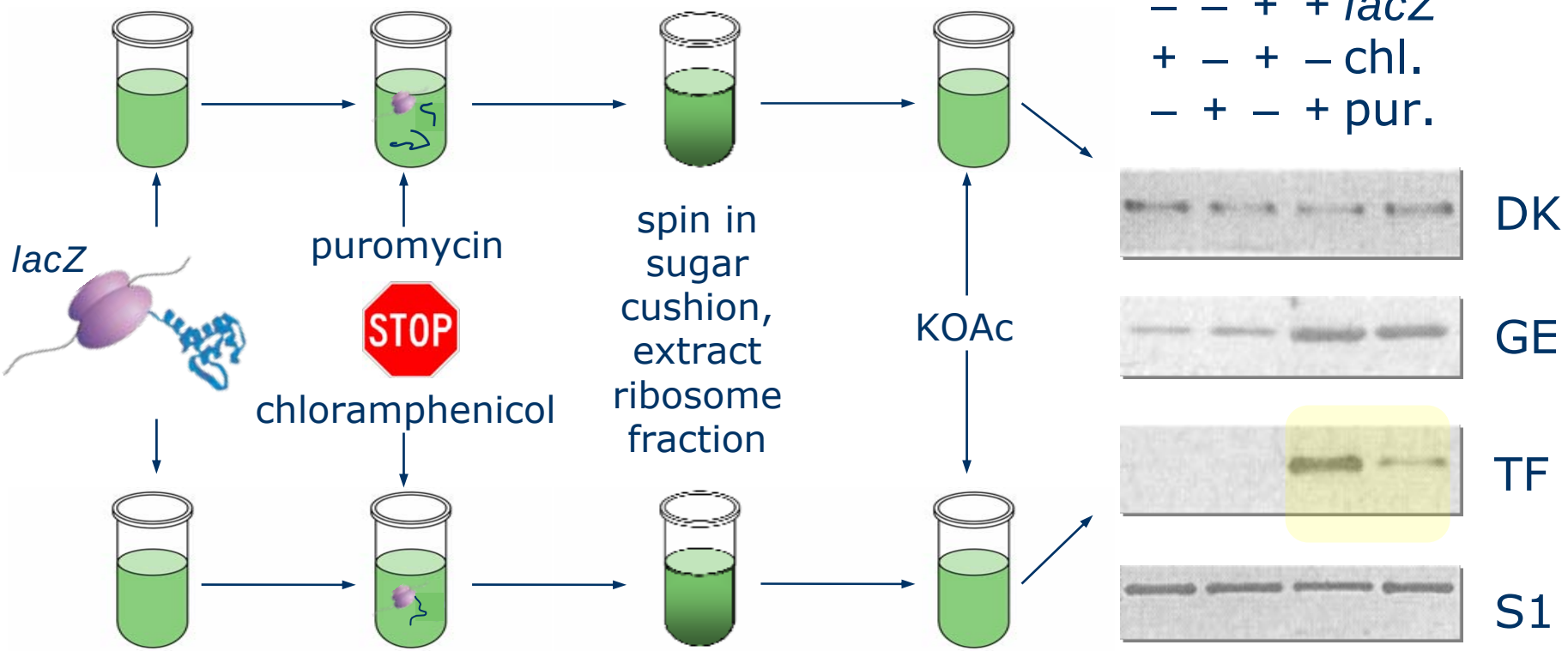


TF > normal



Guthrie and Wickner (UCLA), 1990

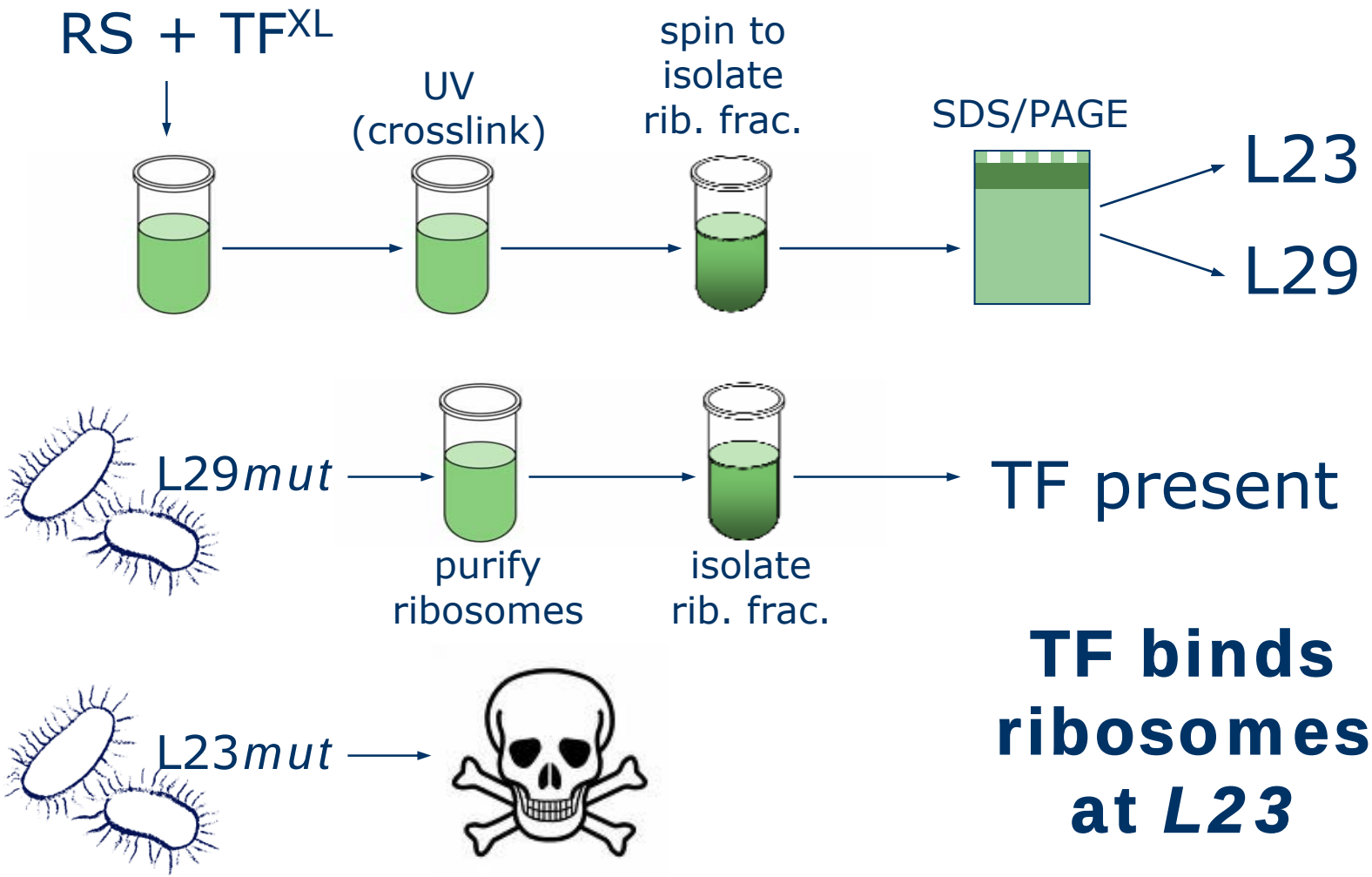
Binding to ribosomes



TF binds best to *translating* ribosomes (so… also to nascent chain?)

Hesterkamp, Bukau, et alia (Universität Heidelberg), 1996

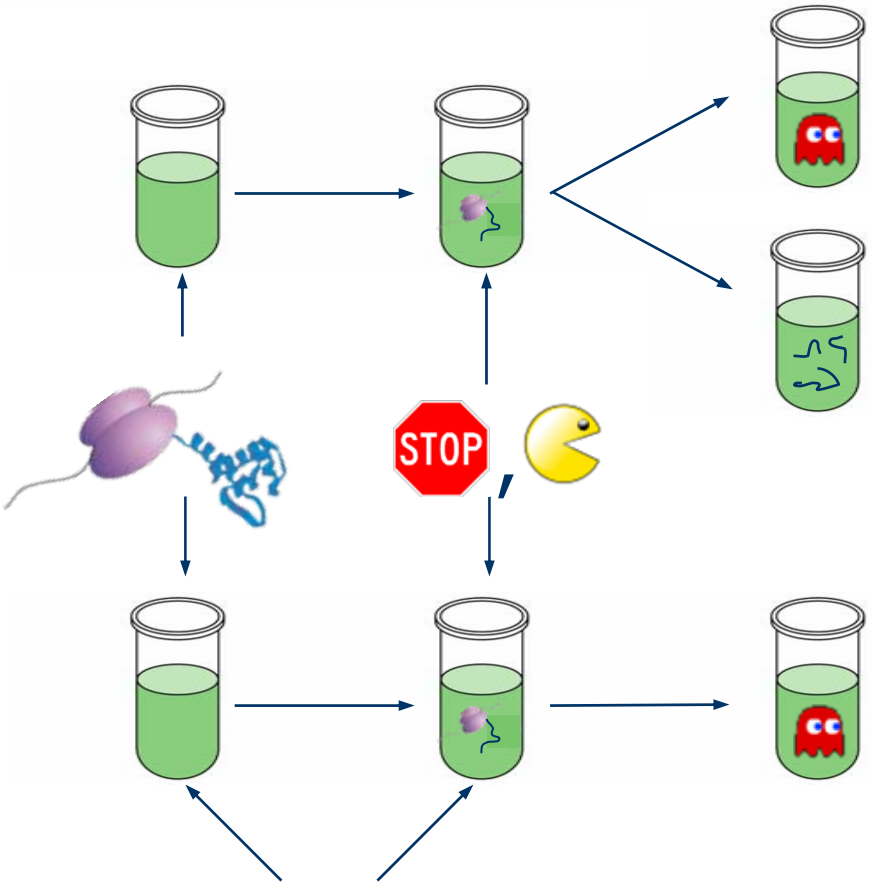
Where on the ribosome?



TF binds ribosomes at *L23*

Kramer, et alia, Science 295:1852-1858 (2002)

How to save a baby protein



e.g., SH3 of α-spectrin

e.g., SH3 *m10*, $ICDH_{177}$

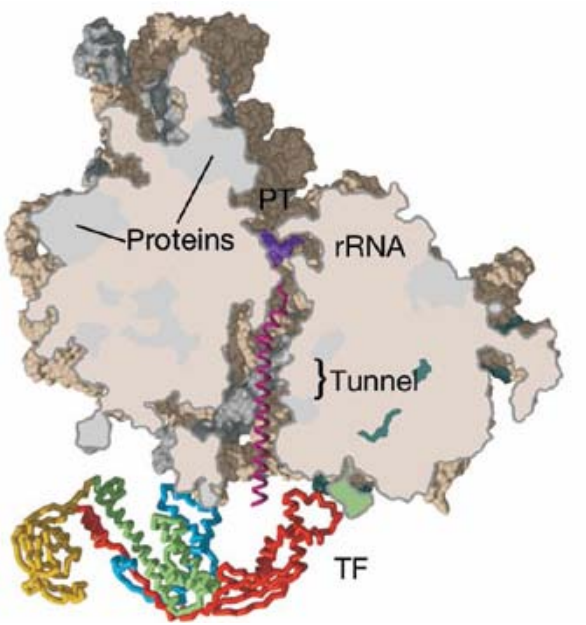
even big chains (e.g., 41 kDa)
only if TF bound to ribosome

TF protects nascent chains

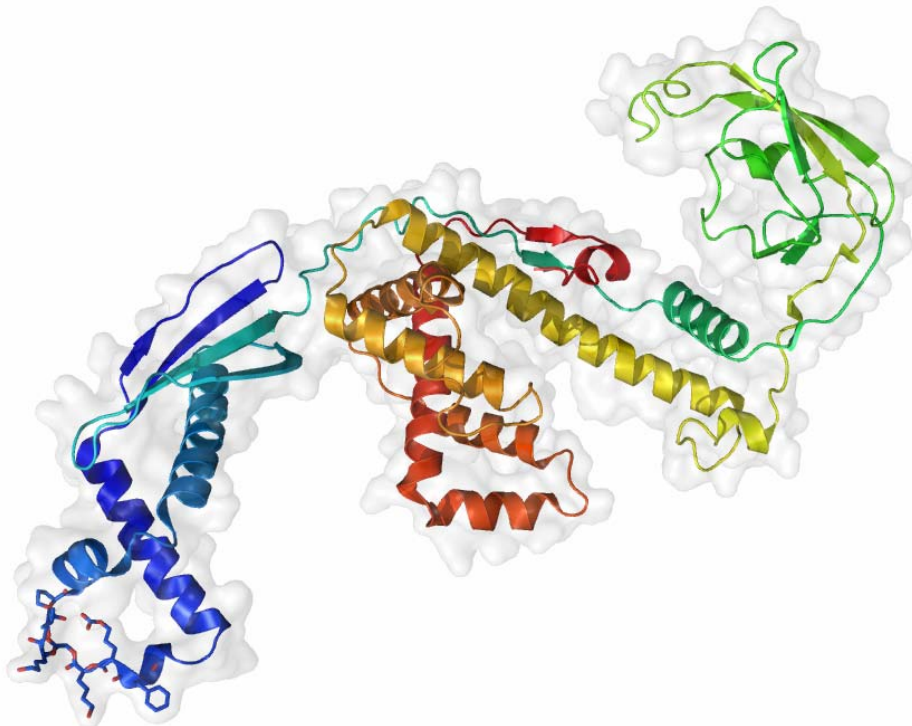
Crystal structure

Determined structure of *E. Coli TF* with *50S* ribosome unit (2004):

- N-Terminus *Tail*, PPIase *Head*, and C-Terminus *Arm* Domains
- *L23* Binding Motif
- Hydrophobic cradle formed over ribosome tunnel exit



High flexibility away from binding site



L. Ferbitz, et alia, Nature 431:590-596 (2004), L. Ferbitz PhD Dissertation, ETH Zurich (2005)

Domain homology

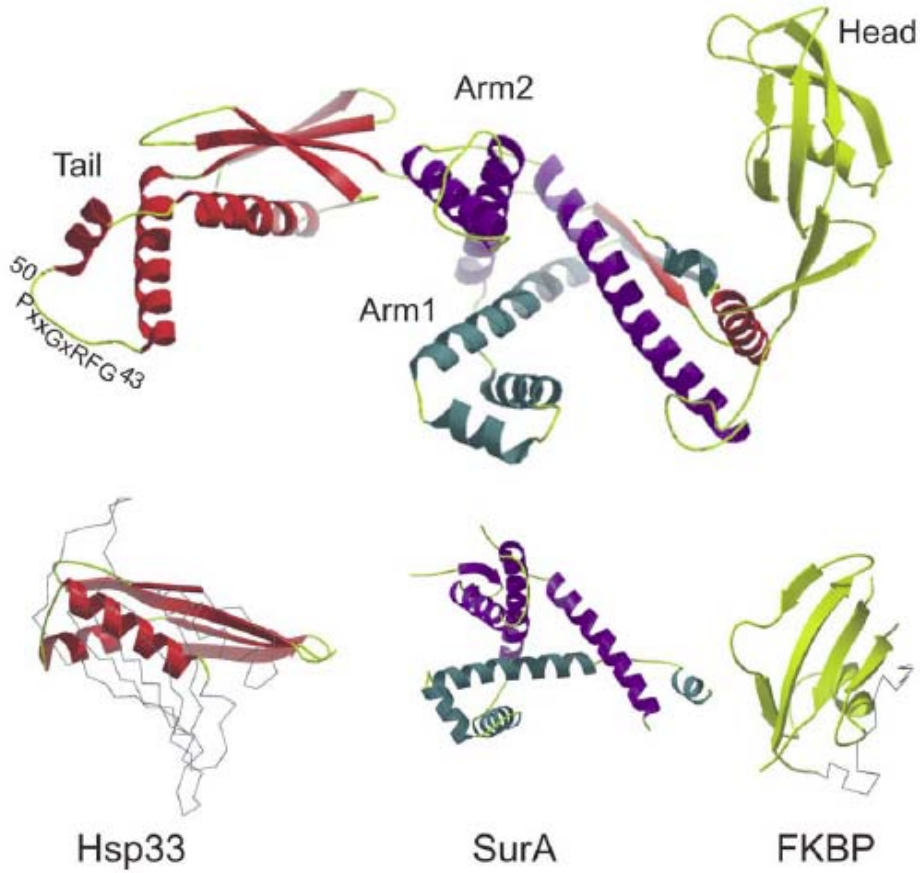
Domains show high structural resemblance to known proteins

Modular functional makeup:

FKBP – PPIase

SurA – IOM chaperone

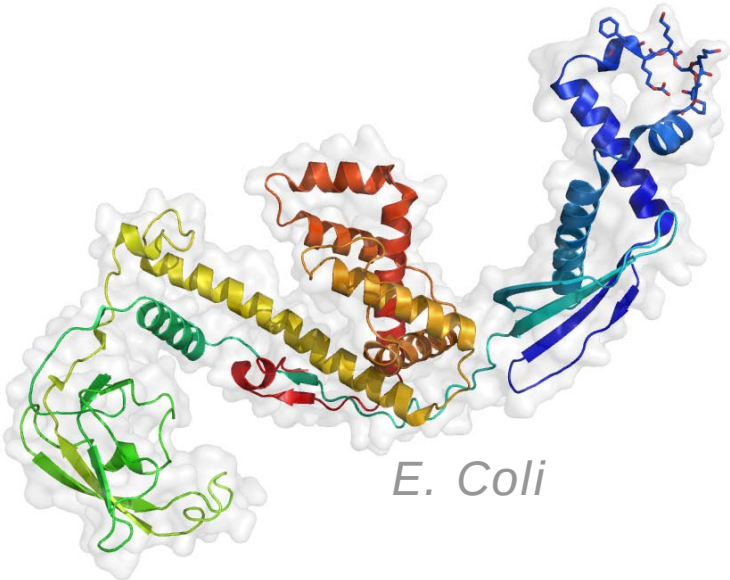
Hsp33 – Stress holdase

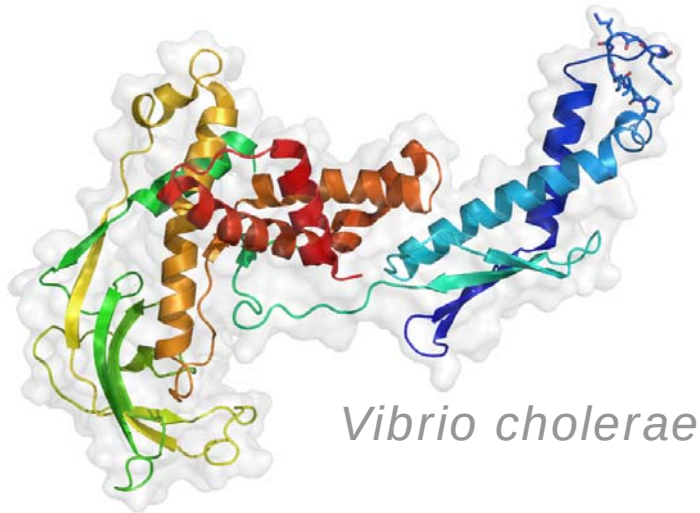


L. Ferbitz, et alia, Nature 431:590-596 (2004), L. Ferbitz PhD Dissertation, ETH Zurich (2005)

Almost scooped

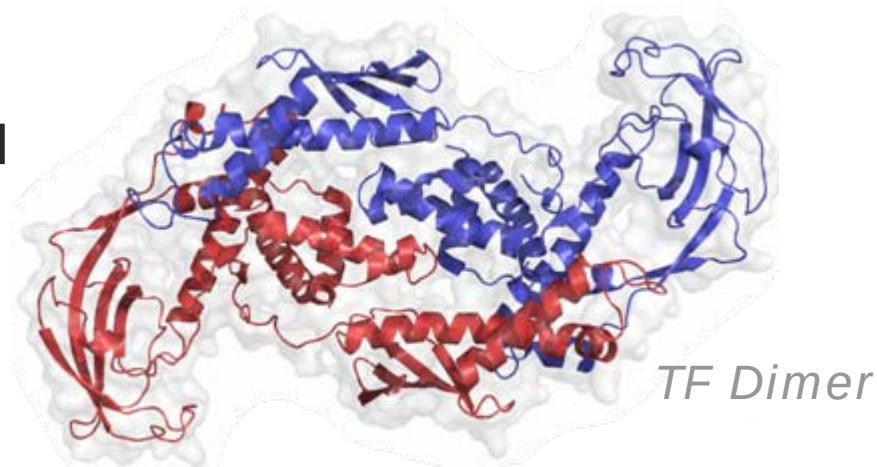
Vibrio cholerae TF published in same month! (70% homology)



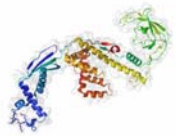


Dimer conformation compacted

N-Terminus domain buried
disallowing association,
PPIase binding site blocked



L. Ferbitz, et alia, Nature 431:590-596 (2004), A. V. Ludlam, et alia, PNAS 101(37):13436-13441 (2004)

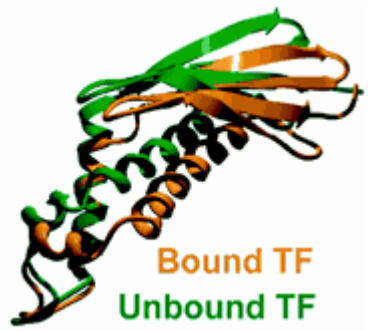


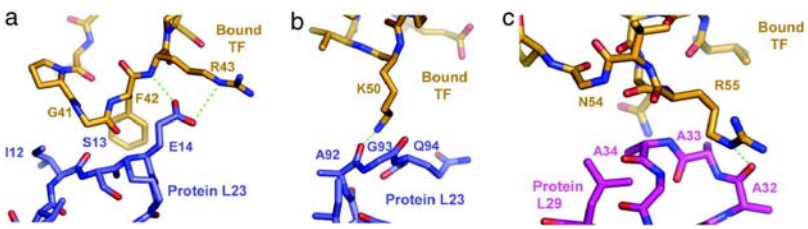
N-Terminus conformations

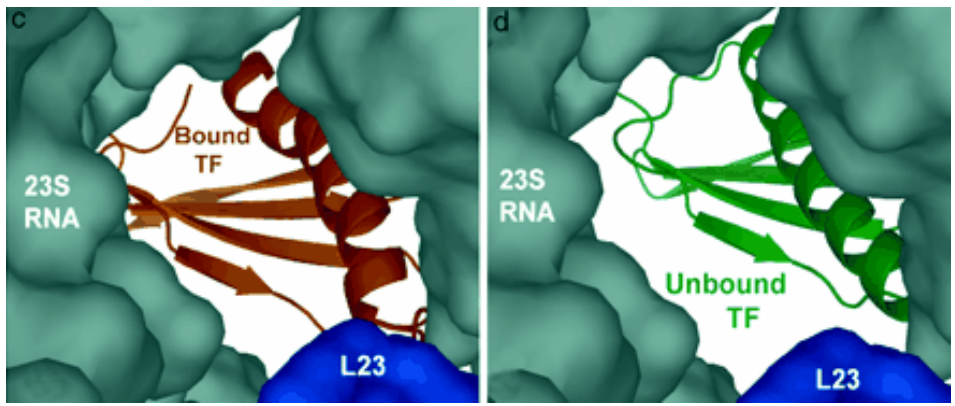
N-Terminus changes conformation when bound to ribosome

L23/L29 units bind loop regions, splays N-Terminus domain leaving hydrophobic cradle accessible

Very specific interactions along
N-Terminus domain loop-region
to L23/L29 binding site…
Motif highly conserved



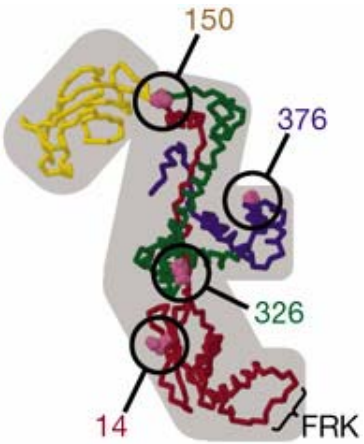




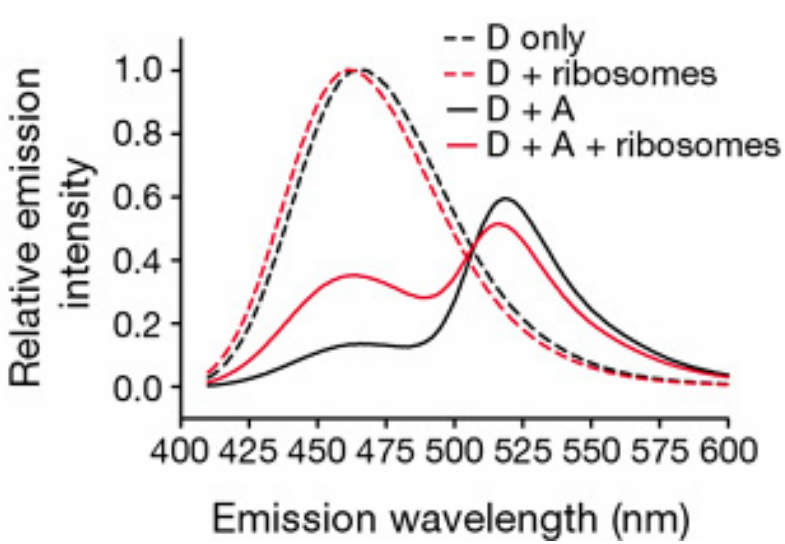
D. Baram, et alia, PNAS 102(34):12017-12022 (2005)

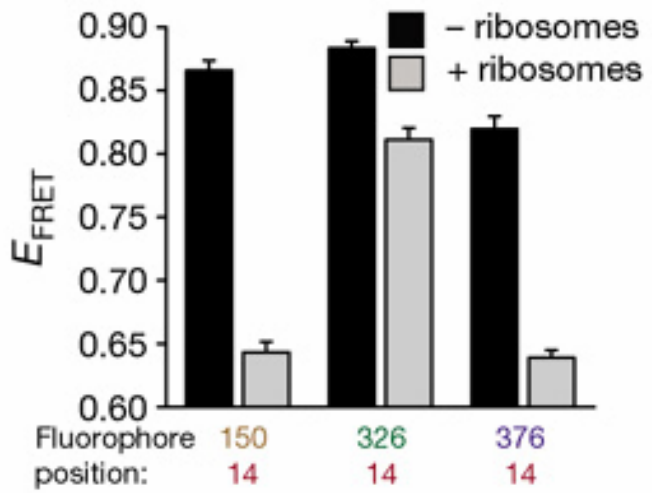
Observing conformation change

Real-time data shows structural rearrangement upon ribosome binding using FRET (*in vitro*)



Added fluorophores at 4 locations,
Compared unbound TF and concentration favoring 70% ribosome binding

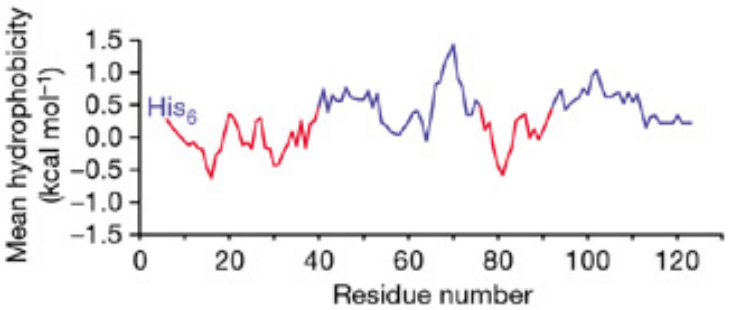




C. M. Kaiser, et alia, Nature 444:455-460 (2006)

Chain-length dependence

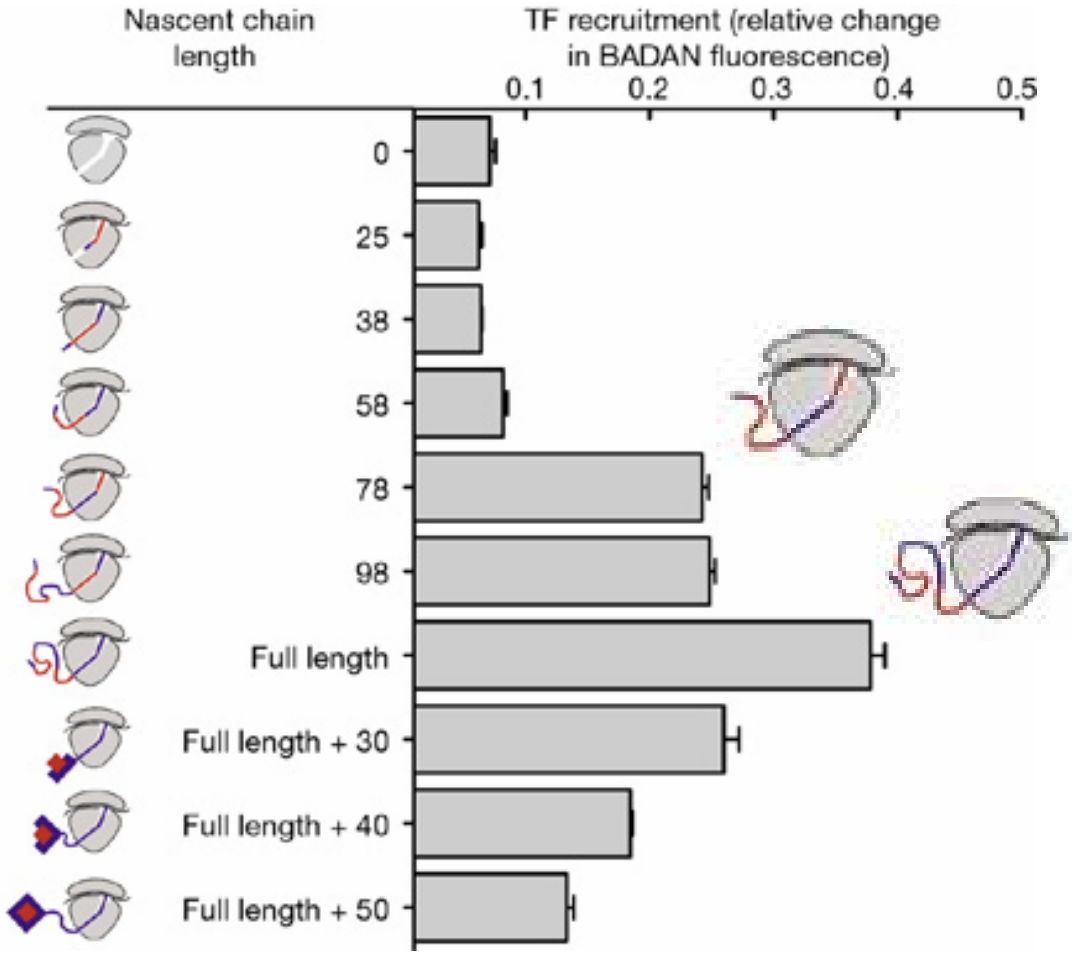
Multiple *TF*s can bind to separated hydrophobic regions of Titin domain I27



Arrest chain formation, BADAN-labeled PPIase

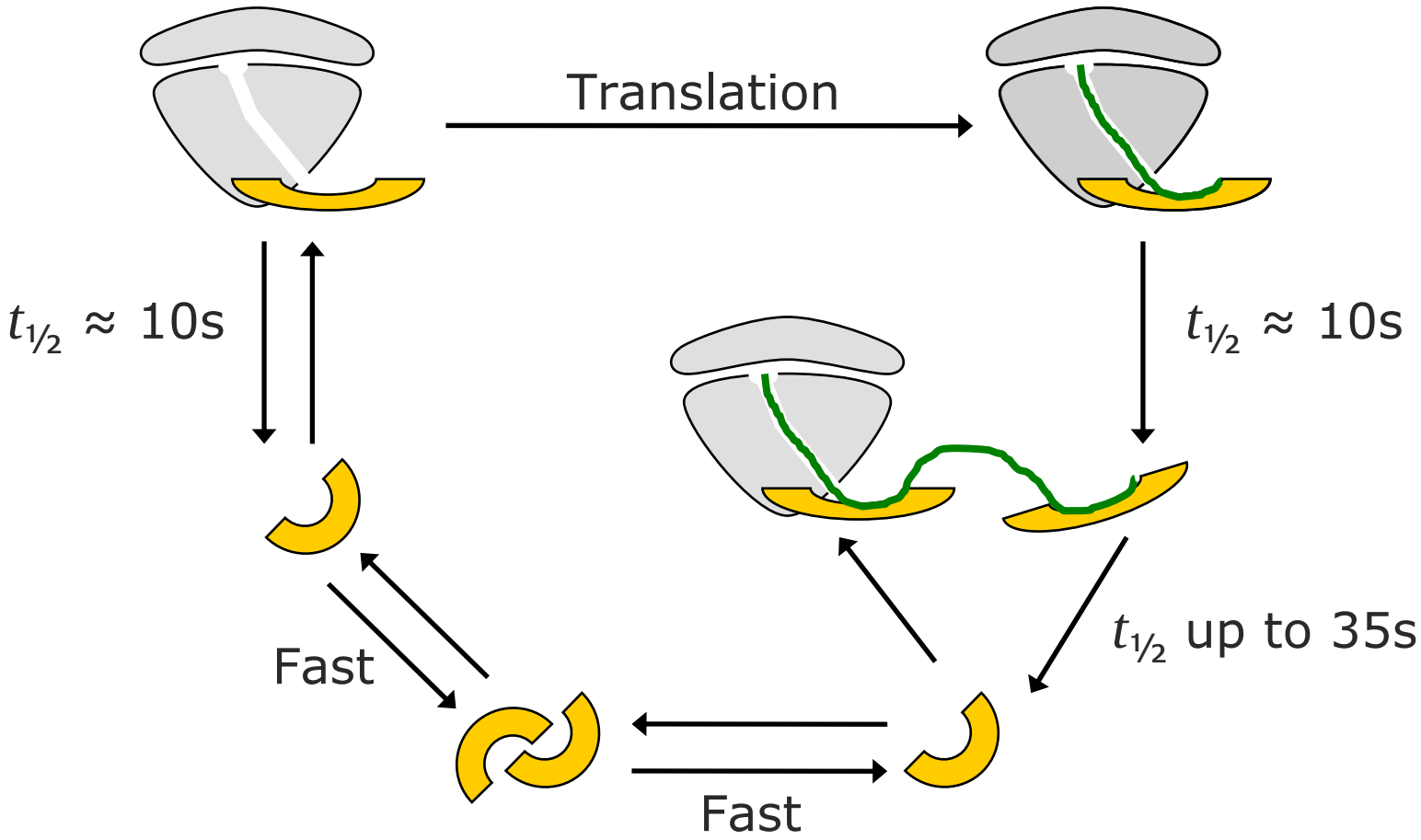
Little TF affinity until chain leaves tunnel

Two affinity jumps



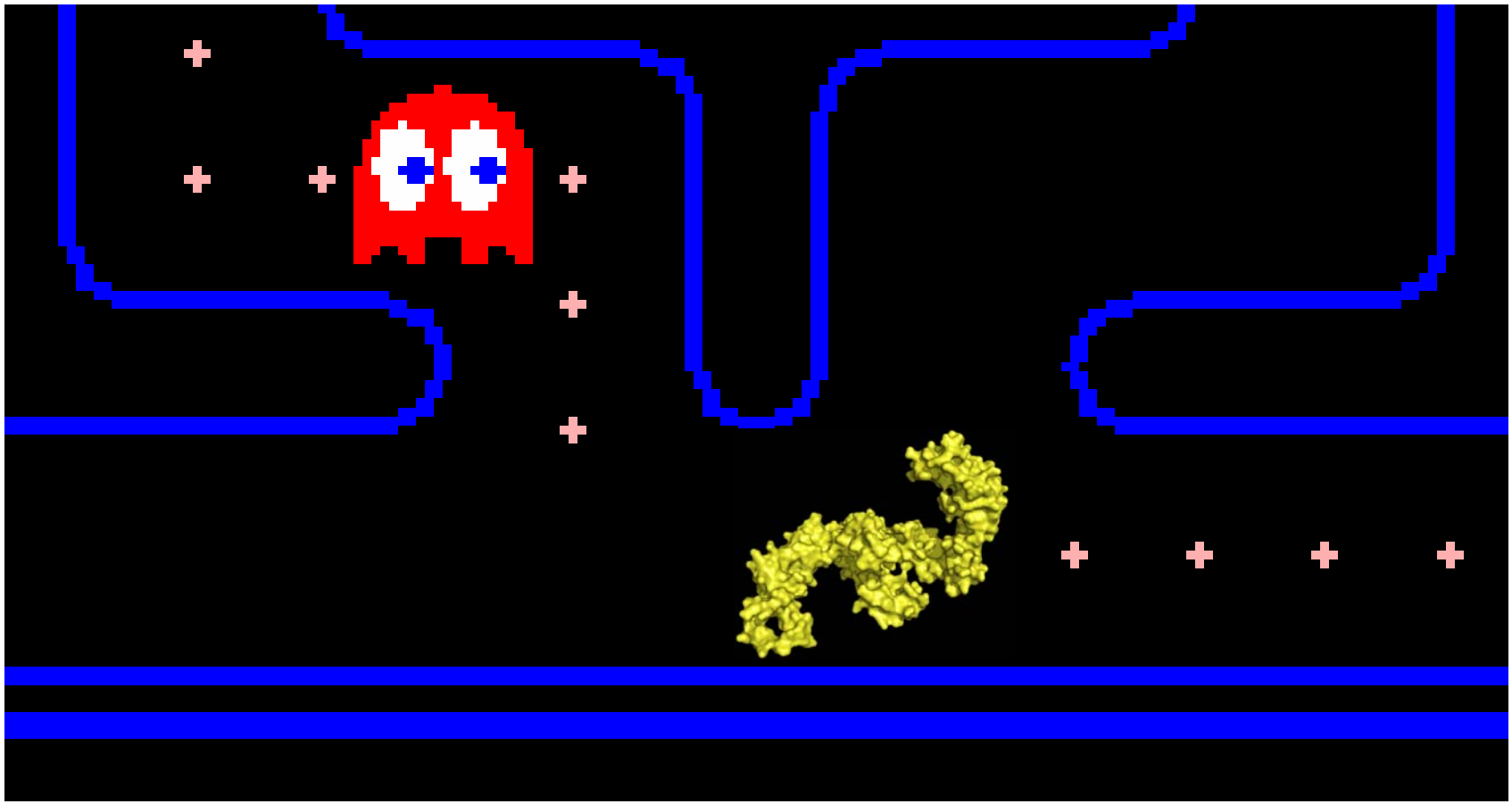
C. M. Kaiser, et alia, Nature 444:455-460 (2006)

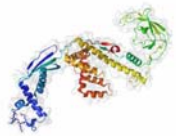
Current model of cycle



C. M. Kaiser, et alia, Nature 444:455-460 (2006)

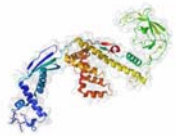
Game Over





Fame and glory await

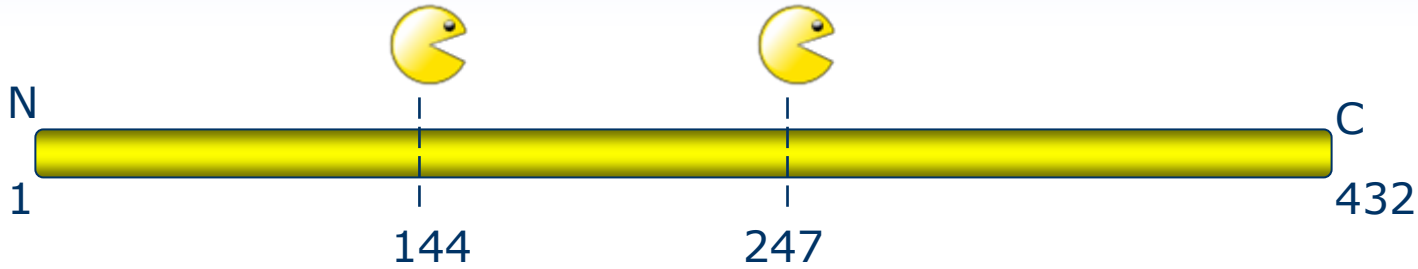
- Why is over-expressing trigger factor lethal?
- Why does TF bind to only certain nascent chains?
- Does L23 help signal attachment/detachment?
- Evolutionary purpose of attached PPIase?



Extra slides

Extra slides

Bits and pieces



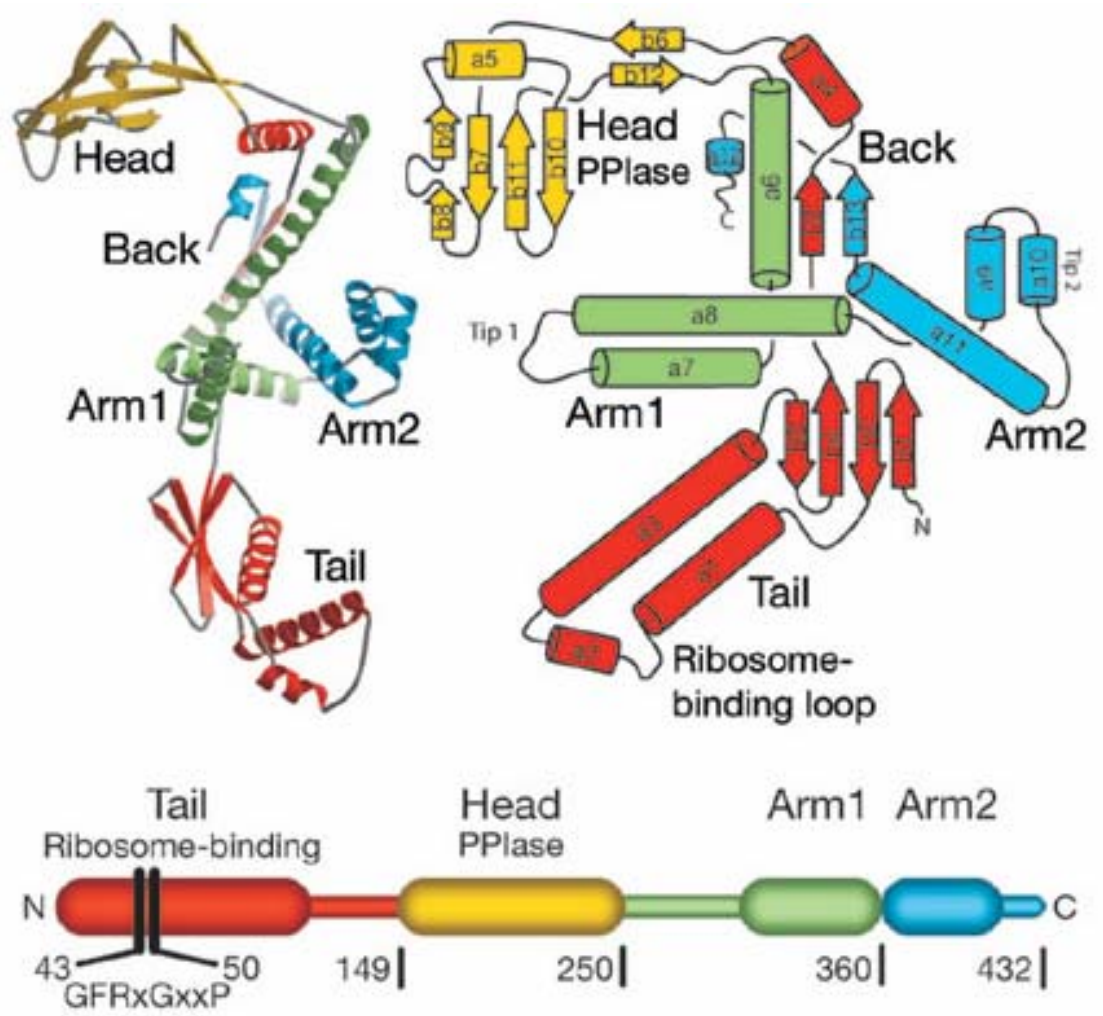
express domains separately

copurifies with ribosome

proline isomerase (~FKBP)

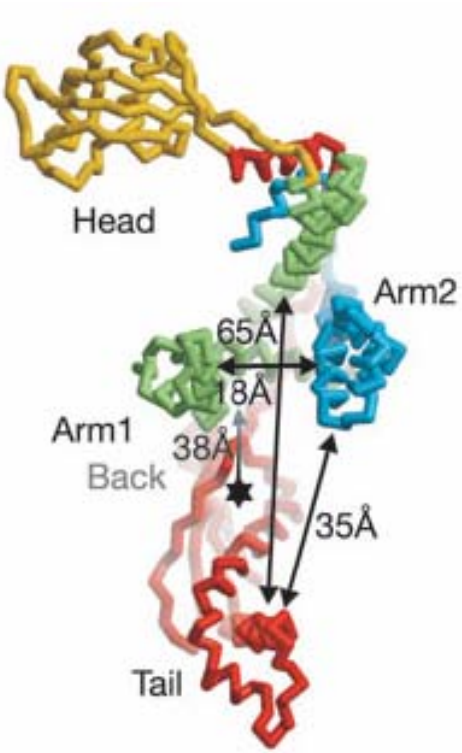
cross-links to nascent chains

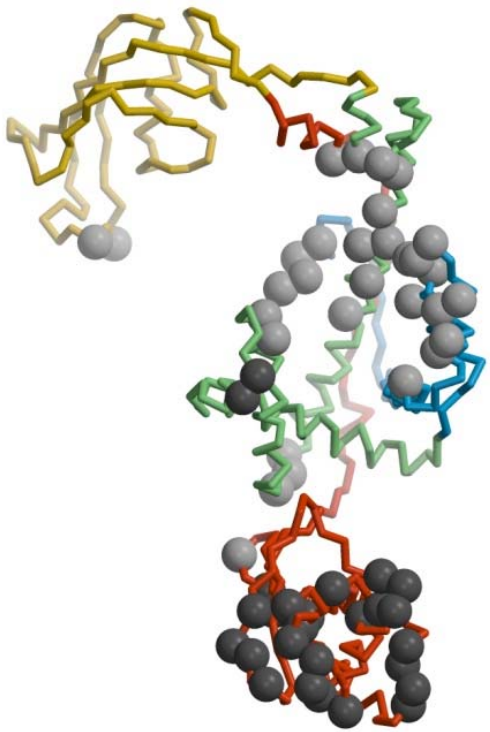
Secondary structure

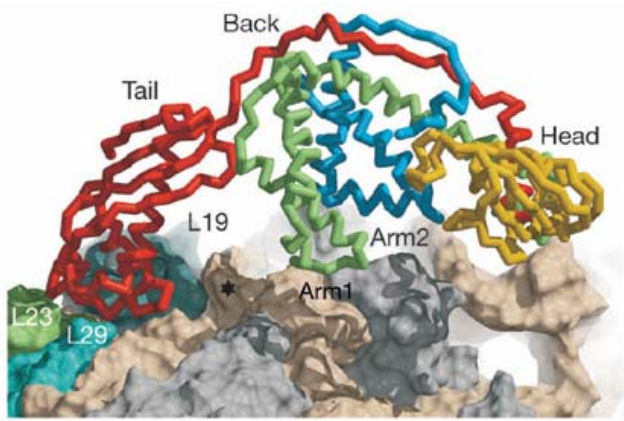


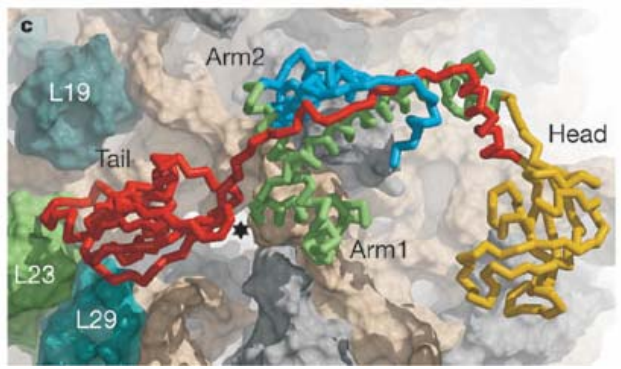
L. Ferbitz, et alia, Nature 431:590-596 (2004), L. Ferbitz PhD Dissertation, ETH Zurich (2005)

Hydrophobic cradle



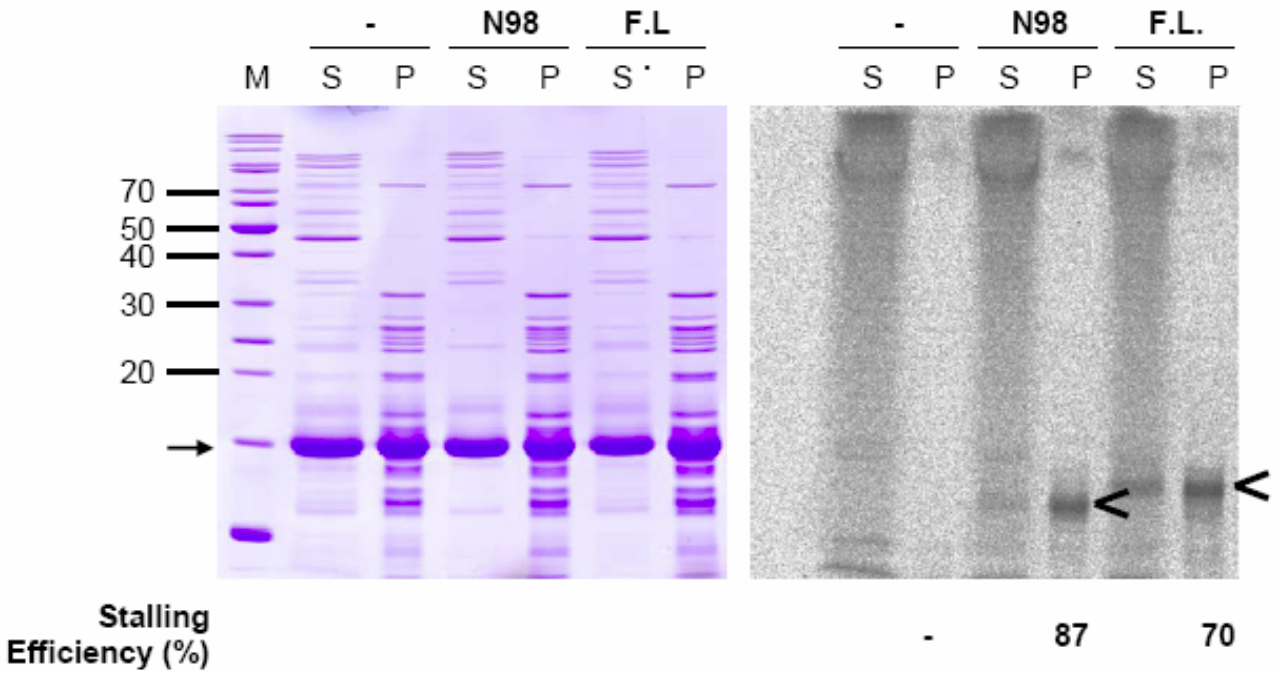






L. Ferbitz, et alia, Nature 431:590-596 (2004), L. Ferbitz PhD Dissertation, ETH Zurich (2005)

Efficiency of ribosome stall



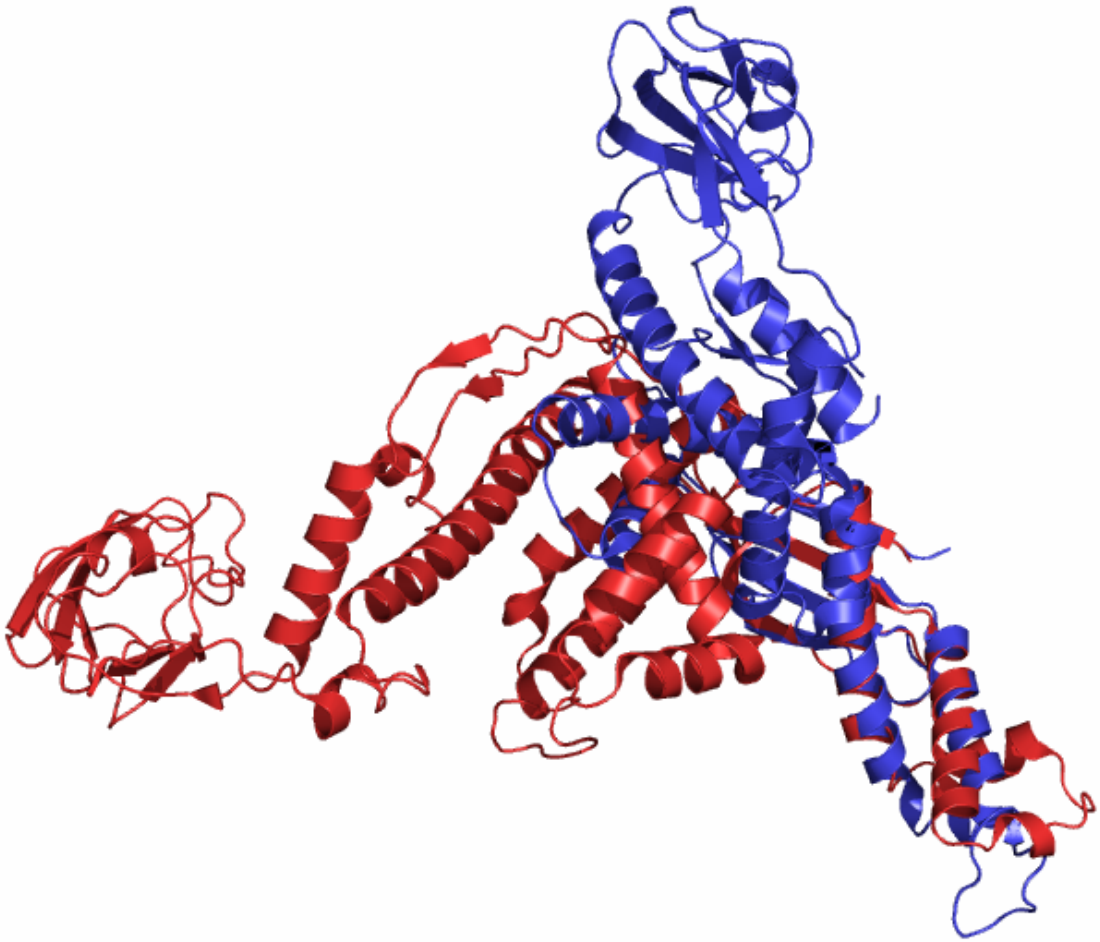
Left panel: Coomassie blue-stained gel of supernatant (S) and pellet (P) fractions after centrifugation (200,000 *g* for 70 min) of transcription/translation reactions containing no template DNA (-) or linear DNA of the titin I27 ORF ending at codon 98 (N98) or the fulllength ORF without a stop codon (F.L.). Reactions were radiolabeled with Promix (Amersham Biosciences) and treated with RNase A (position indicated by the arrow), which was added after 45 min of incubation at 30 ºC. The band pattern observed in the Coomassie blue-stained gel demonstrates efficient pelleting of ribosomes.

Right panel: autoradiogram of the gel shown in the left panel. Positions of the stalled nascent chains are indicated by the arrowheads. Efficiency of stalling (shown at the bottom of the panels) was determined by phosphorimager analysis.

C. M. Kaiser, et alia, Nature 444:455-460 (2006)

Alignment of structures

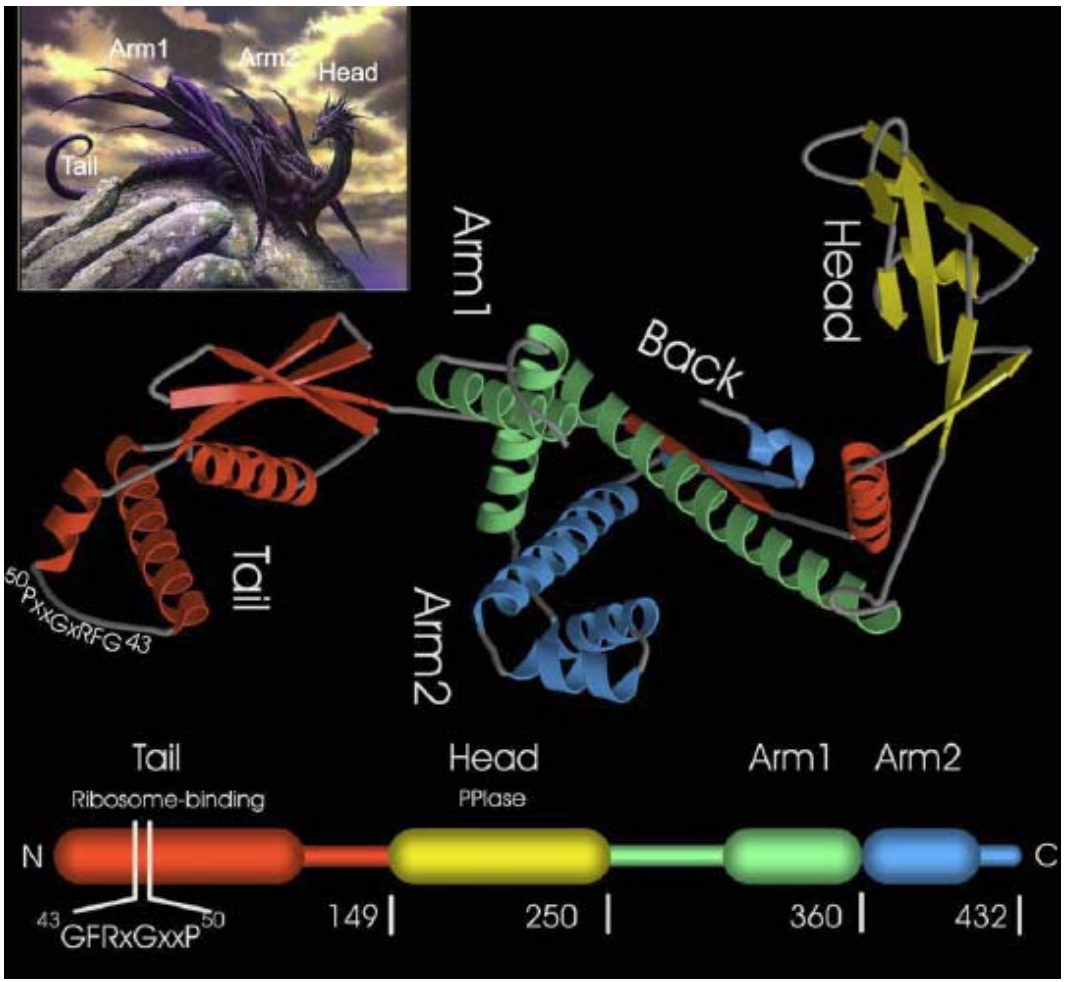
Alignment of N-Terminus of single molecule of dimerized *Vibrio cholerae TF* and monomer of *E. Coli TF*



L. Ferbitz, et alia, Nature 431:590-596 (2004), A. V. Ludlam, et alia, PNAS 101(37):13436-13441 (2004)

Why “The Dragon”

- Coined by Lars Ferbitz, his cartoon depiction:



L. Ferbitz, et alia, Nature 431:590-596 (2004), L. Ferbitz PhD Dissertation, ETH Zurich (2005)