# Synthetic biology challenges long-held hypotheses in translation, codon bias and transcription

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Presented by Gonzo and Holly

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All native aminoacyl-tRNAs are *not* equally efficient in translation at equivalent concentrations.

# Translation Simplified

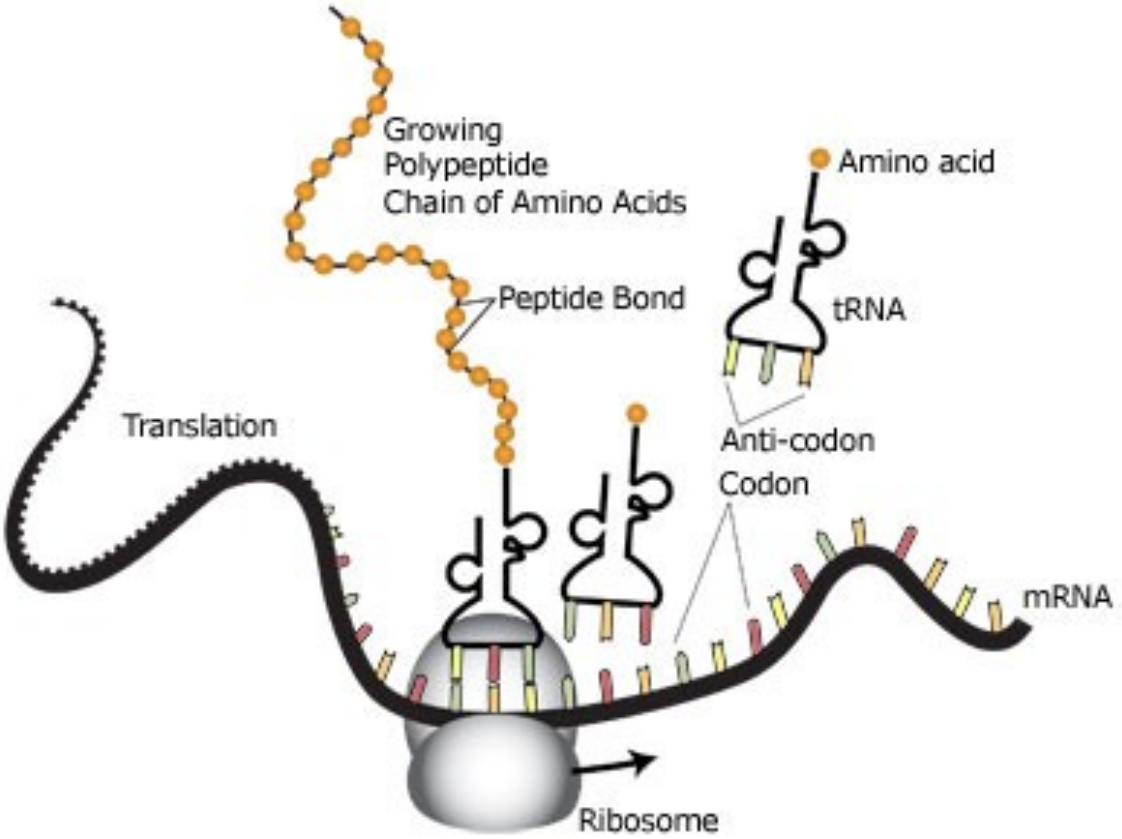
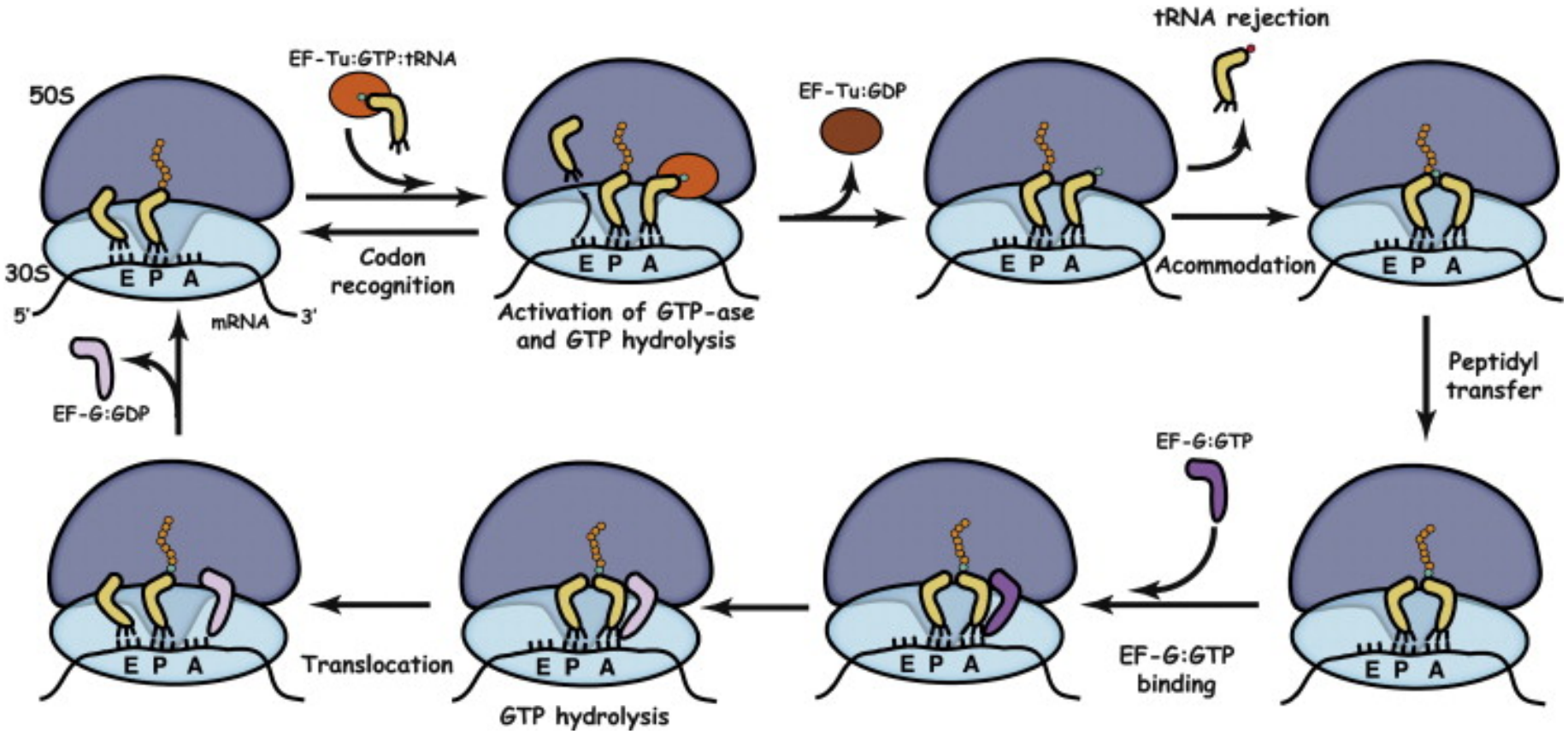



Image adapted from: National Human Genome Research Institute.



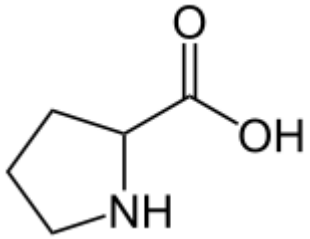

http://ars.els-cdn.com/content/image/1-s2.0-S1874939909000777-gr1.jpg

# tRNA Incorporation

- Uniform-decoding rate hypothesis
  - Past experiments show uniform levels of binding affinity for all AA-tRNAs
- How does this occur when AAs are different?
  1. tRNA portion allows for mutations to tweak affinities
  2. Rate-limiting conformational change by ribosome

# N-alkyl-AA Incorporation

- Hypothesis
  - N-methyl-AAs will incorporate as well as canonical AAs
- Outcome
  - Cut peptide product yield in half
  - Steric hindrance




Proline

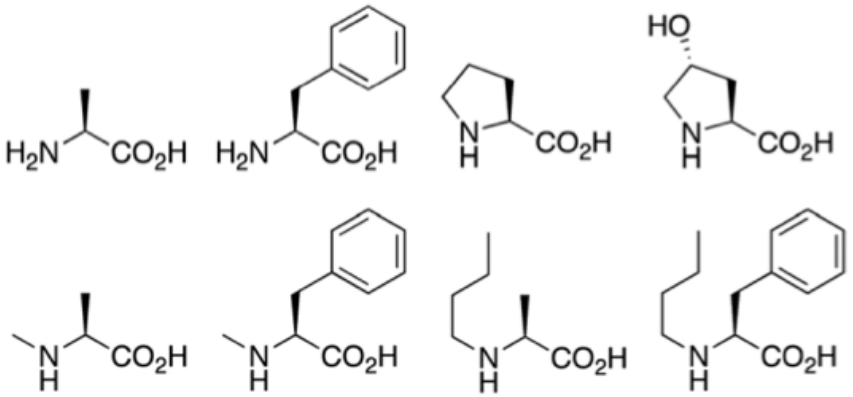
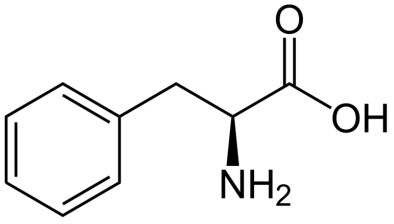
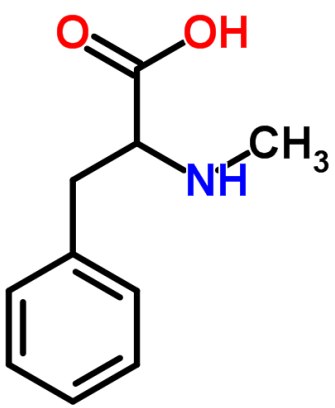



**Figure 1.** Structures of AAs and N-alkyl-AAs compared for translation incorporation and translocationfrom [35].

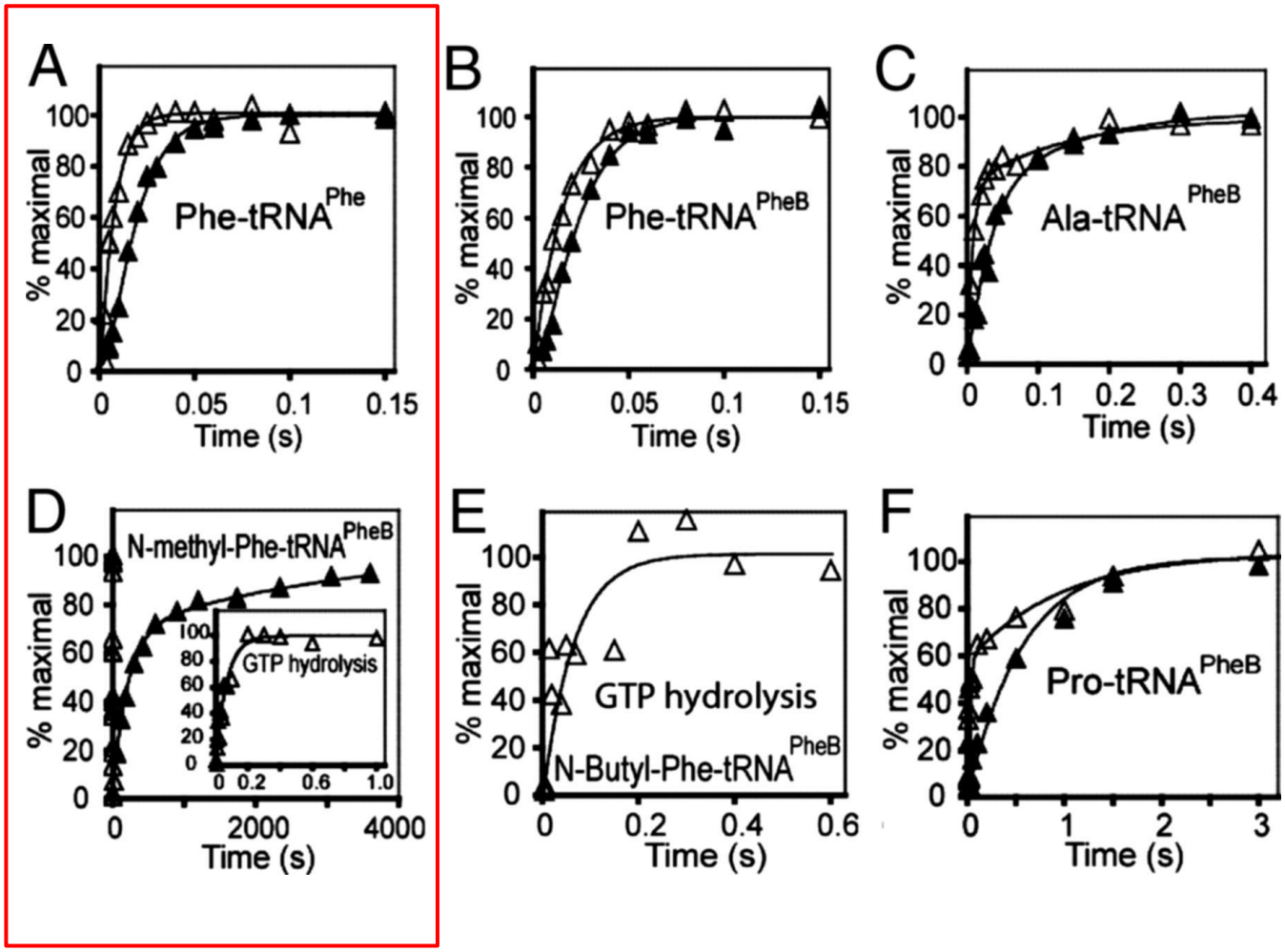
# Modified Phe Incorporation




Phenylalanine




N-Me-Phenylalanine



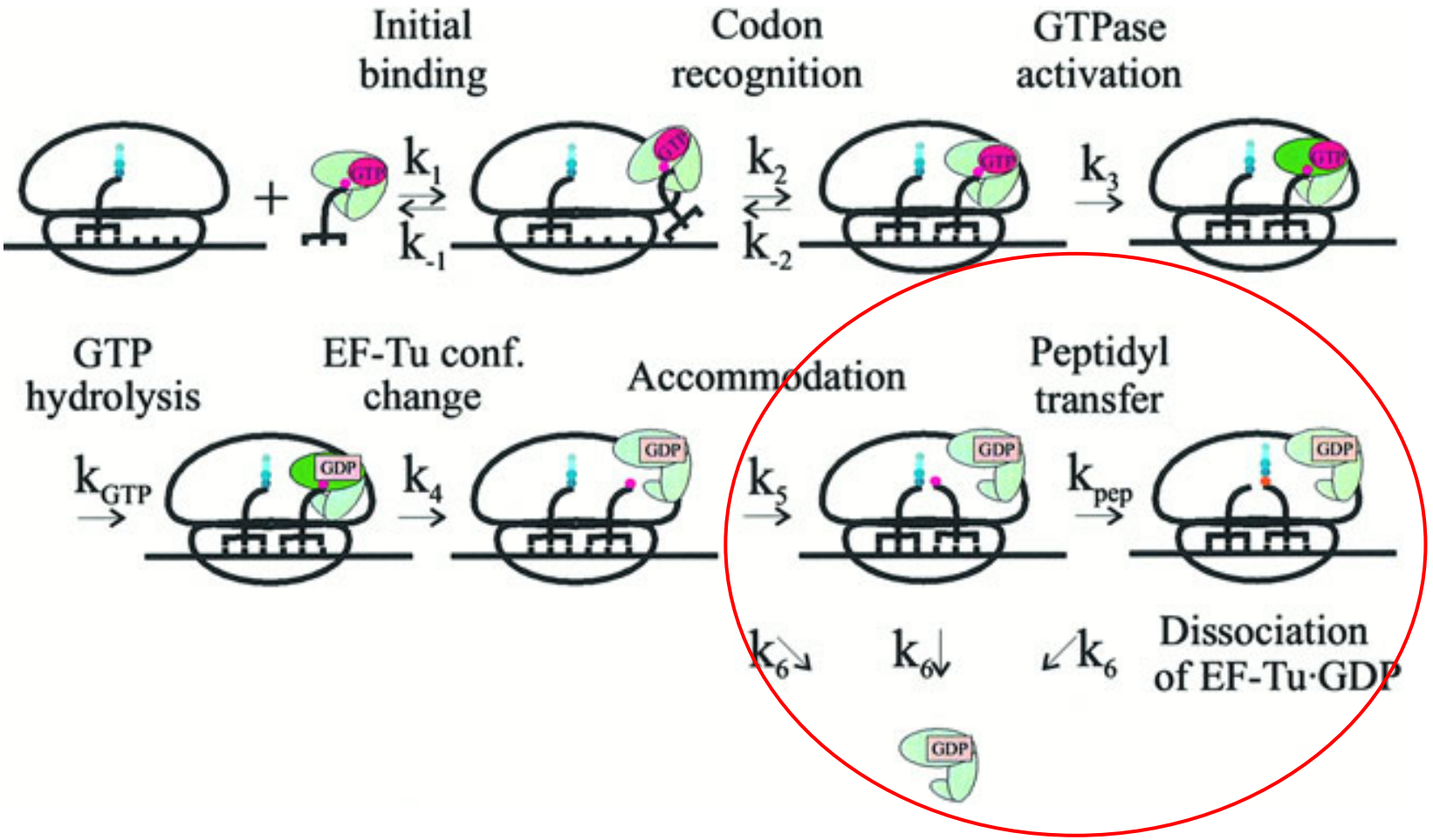

Pavlov M Y et al. PNAS 2009;106:50-54

# Conclusions

- Uniform decoding rate hypothesis disproved
  - Proline vs. Phenylalanine comparison
- Proline is incorporated naturally at "stalling sites"
- Steric hindrance matters in design of AAs

Accommodation is *not* always rate limiting in translation

# Background


Initial binding
Codon recognition
GTPase activation
GTP hydrolysis
EF-Tu conf. change
Accommodation
Peptidyl transfer
Dissociation of EF-Tu·GDP
GTP
GDP

# So which one is rate-limiting?

- Right now impossible to measure the three steps between GTP Hydrolysis and peptide bond formation
  - Release of AA-tRNA from EF-Tu-GDP
  - Accommodation into A/A Site
  - Nucleophilic attack of the ester

# Experiment in Question?

- Monitored accommodation using Phe charged on fluorescently-labeled tRNA
- Concluded that accommodation is the rate-limiting step in dipeptide formation and is more than 12-times slower than peptide bond formation
- Large fluorescent label may have affected the rate of accommodation.
- Peptide bond formation was also slower than physiological rates.

# Chemical Reactivity Hypothesis>Accommodation?

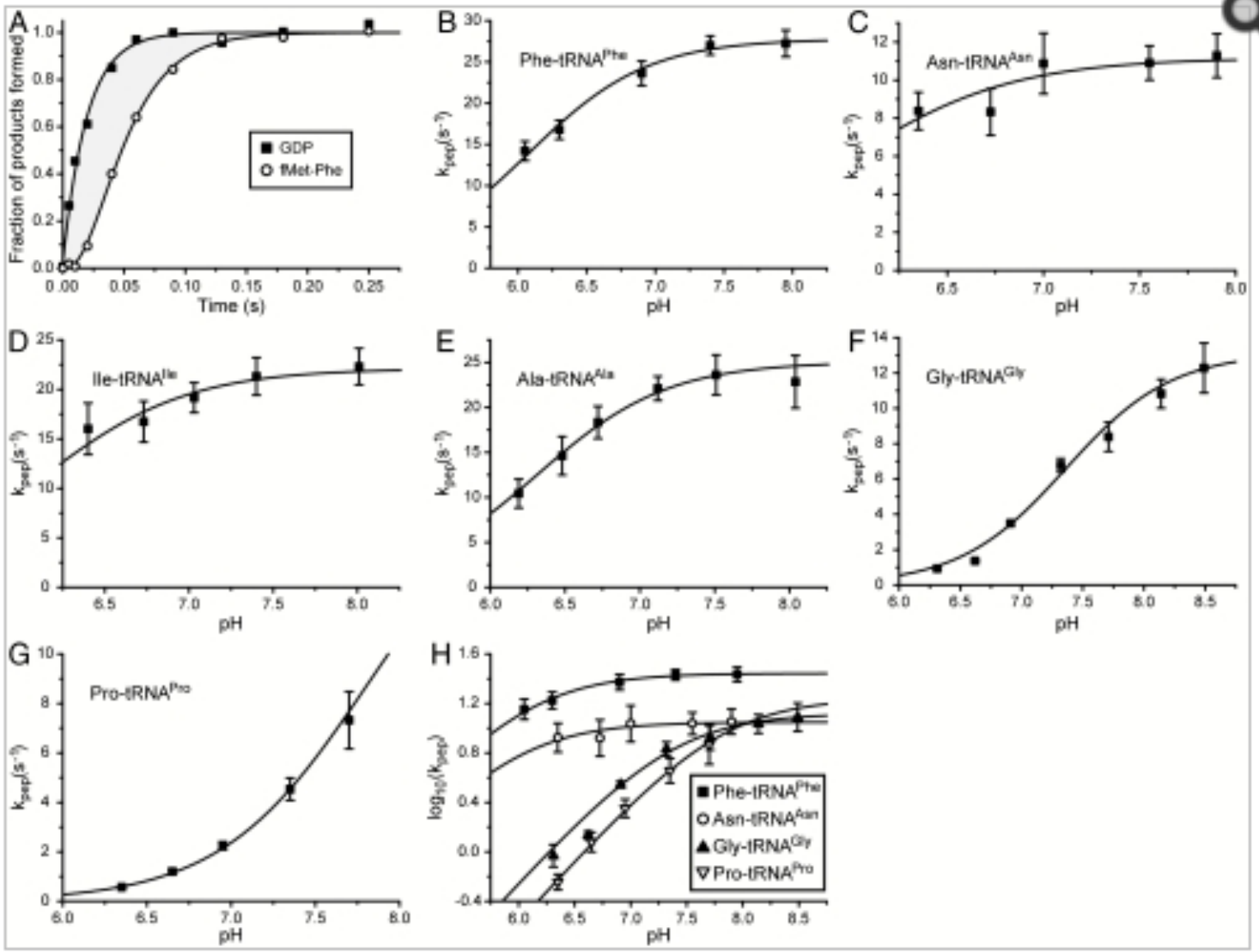
- Chemical model system lacking ribosomes, the fMet electrophile was activated by N-hydroxysuccinimide ester formation
- Striking similarities with rates of same AA on the ribosome
- Steric effect >> pKa and inductive effects in model

# Other Evidence?

- Measured activation enthalpy and entropy parameters of dipeptide formation from AA-tRNA are similar to peptide formation from puropycin.
- Rate of GTP hydrolysis, measure of the rate of delivery of AA-tRNA to ribosome by EG-Tu, is roughly the same for N-alkl-AA-tRNA and AA-tRNA
  - Rate-limiting step must follow GTP hydrolysis

# Recent Evidence

- Dipeptide formation exhibits sensitivity to pH dictated by the pKA of the AA of the AA-tRNA in the A site of ribosome.
  - Extends hypothesis of rate-limiting peptide bond formation beyond n-alkyl-AA to AA tested.


A
Fraction of products formed
Time (s)
GDP
fMet-Phe
B
Phe-tRNA^Phe
pH
C
Asn-tRNA^Asn
pH
D
Ile-tRNA^Ile
pH
E
Ala-tRNA^Ala
pH
F
Gly-tRNA^Gly
pH
G
Pro-tRNA^Pro
pH
H
Phe-tRNA^Phe
Asn-tRNA^Asn
Gly-tRNA^Gly
Pro-tRNA^Pro
pH

# Relevance to Synthetic Biology

- What value does the paper bring?
  - accurate modeling
  - gates, biological clock
  - modifications to amino acids
- Can't abstract away the encoding machinery
- "Presets" for different cell types and systems may vary

# Discussion Questions

- Can this level of detail realistically lead to something useful?
- Is this good science writing? How would you advise the author to improve his work?