MolecularEcologyStudies with Nucleic Acids

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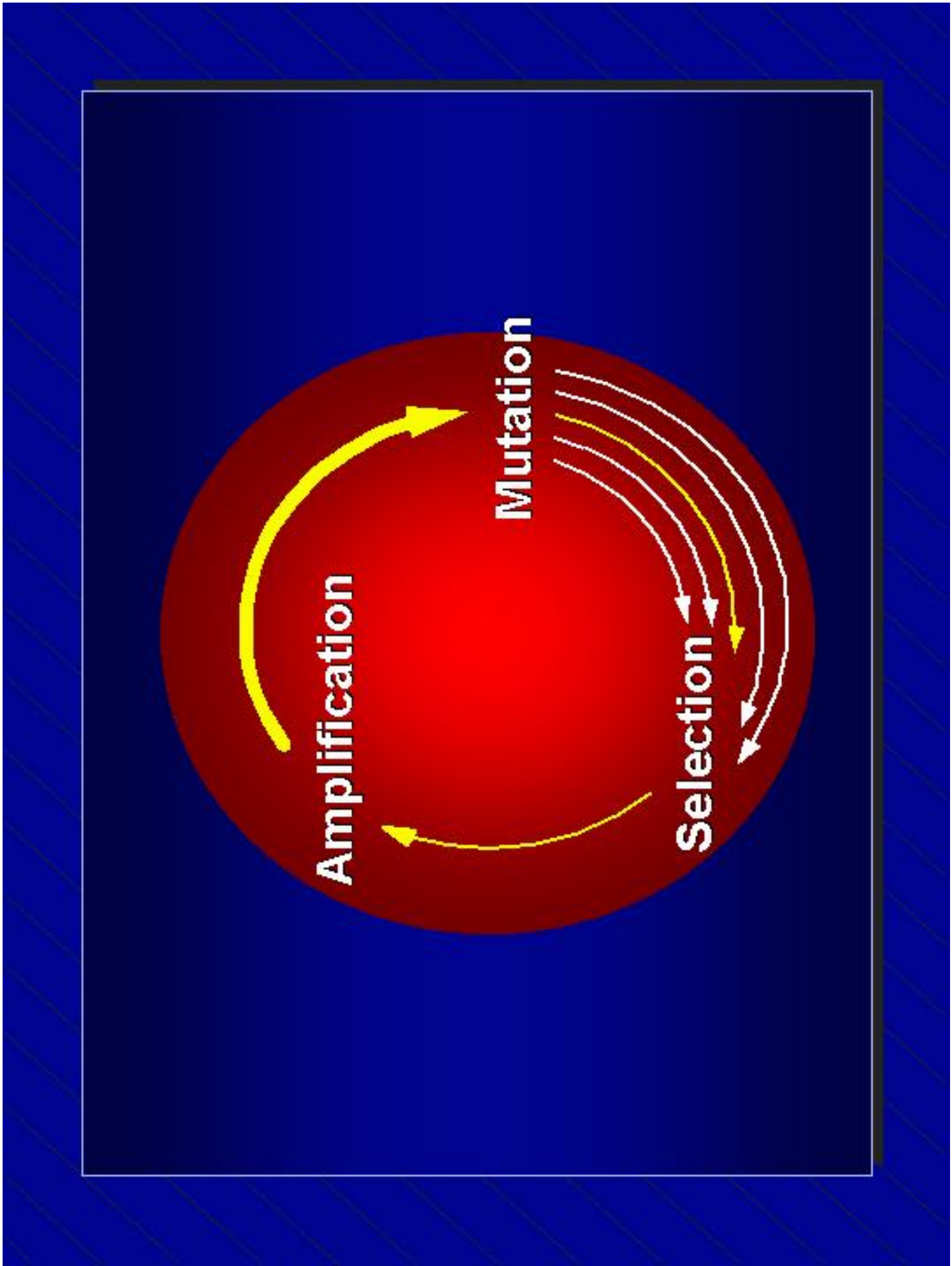
[02] Dr. P. Ordoukhanian, Scripps (ITP 4/25/01) Molecular Ecology Studies with Nucleic Acids

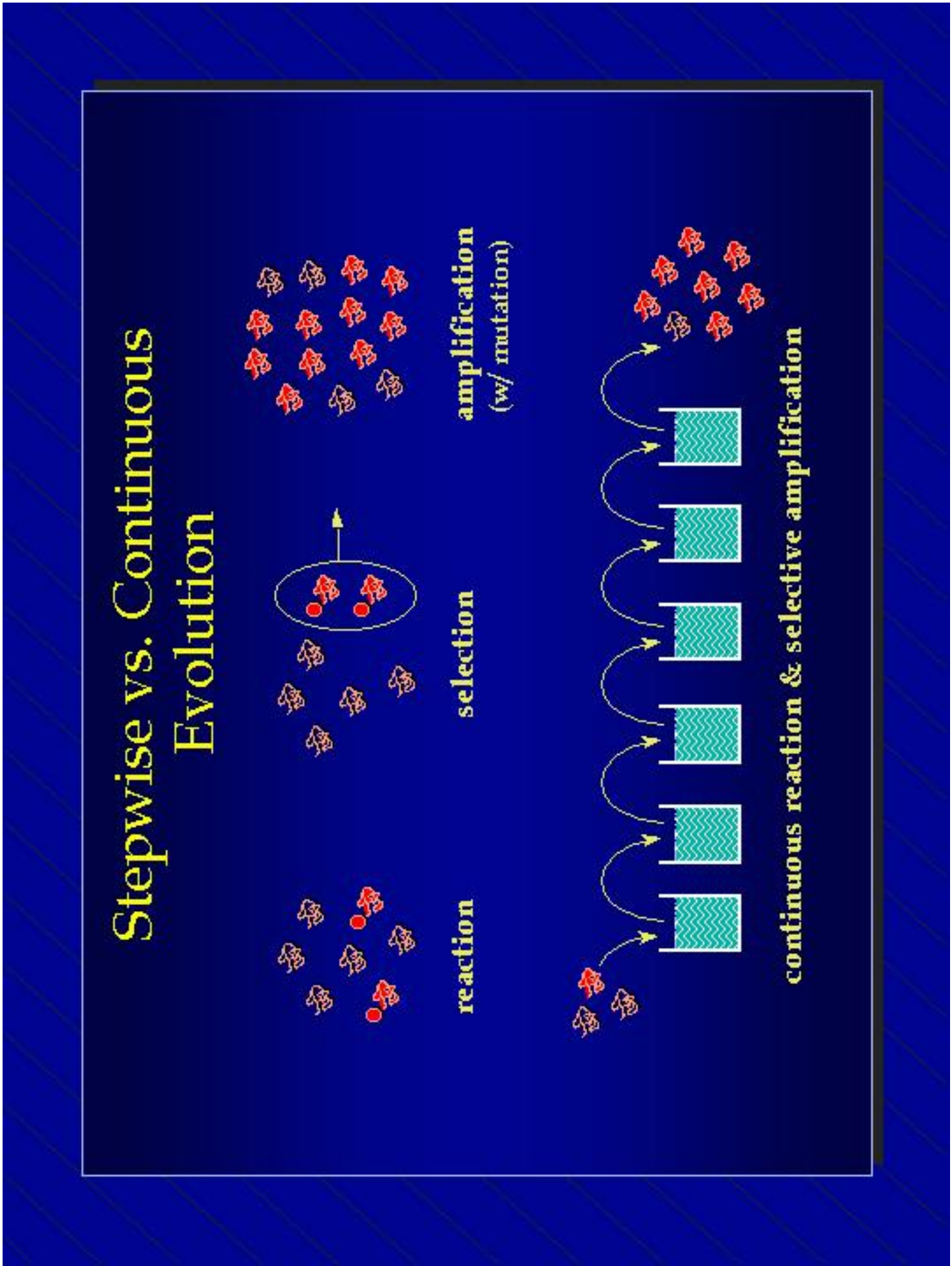


- **A molecular description of the evolution of resistance**

  Ordoukhanian, P. and Joyce, G.F. *Chem. Biol.*, **6**, 881-889 (1999)

- **Non-unity molecular heritability demonstrated by continuous evolution *in vitro***

  Schmitt, T. and Lehman, N. *Chem. Biol.*, **6**, 857-869 (1999)


Stepwise vs. Continuous Evolution
reaction
selection
amplification
(w/ mutation)
continuous reaction & selective amplification

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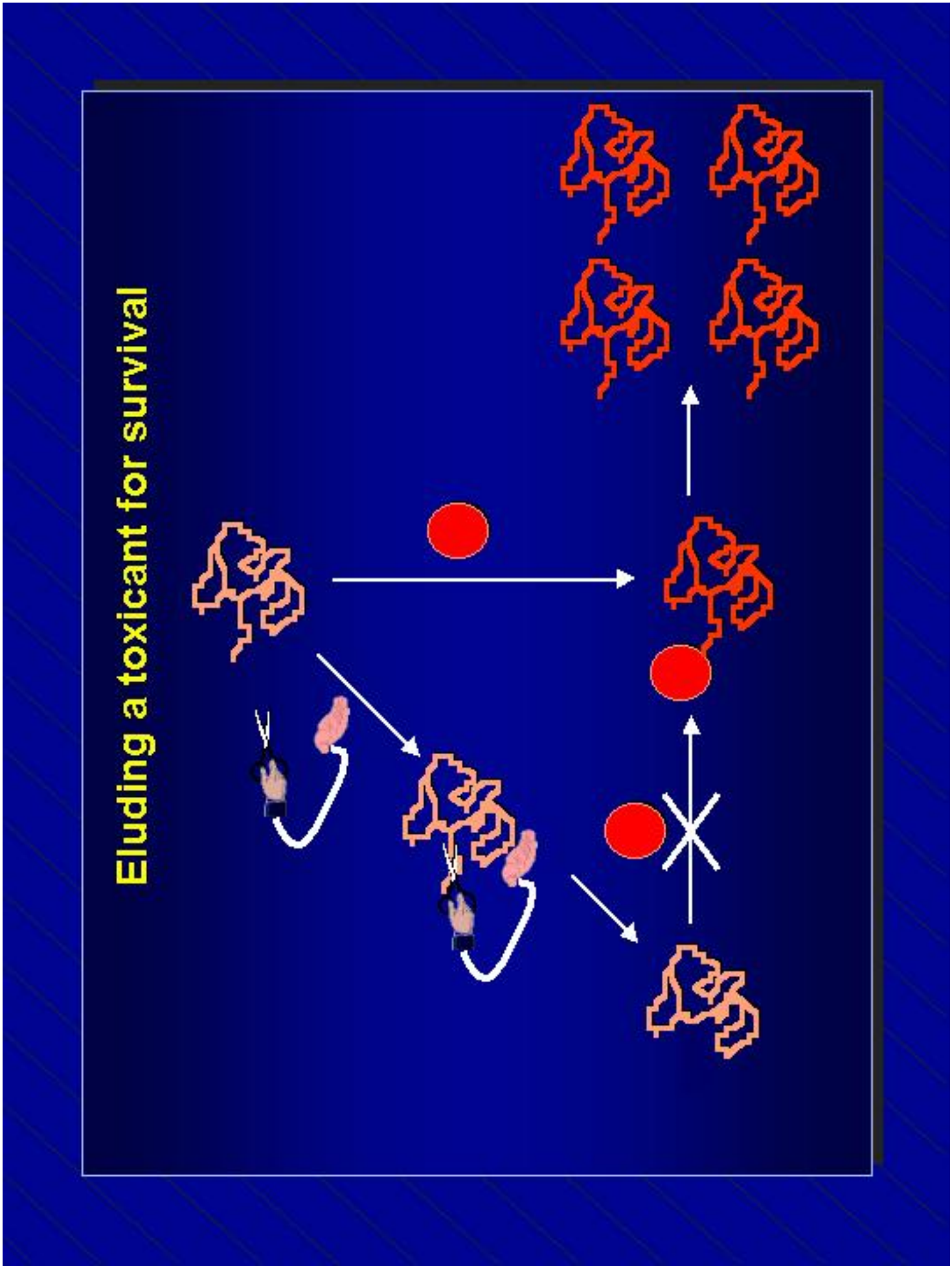
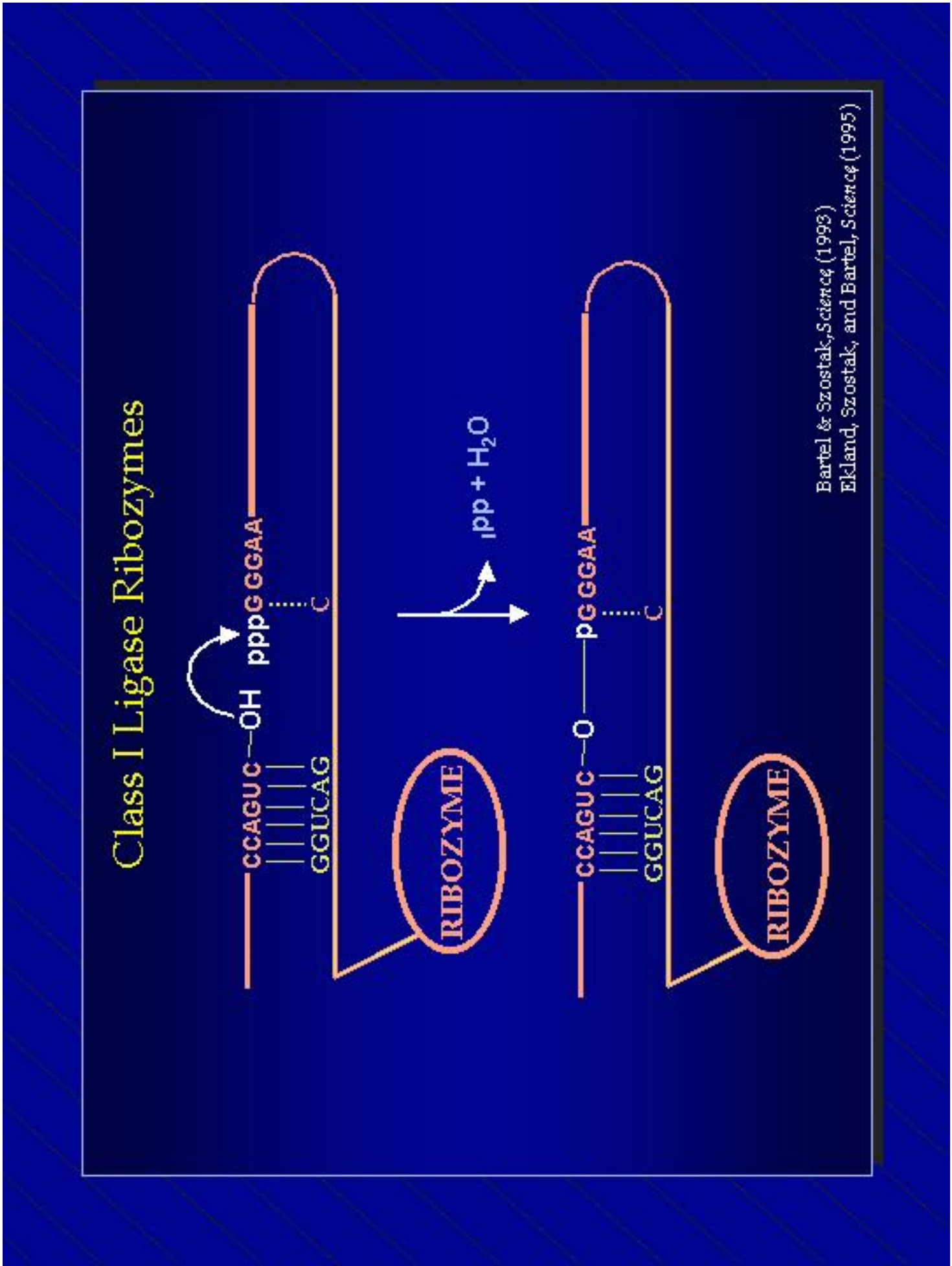
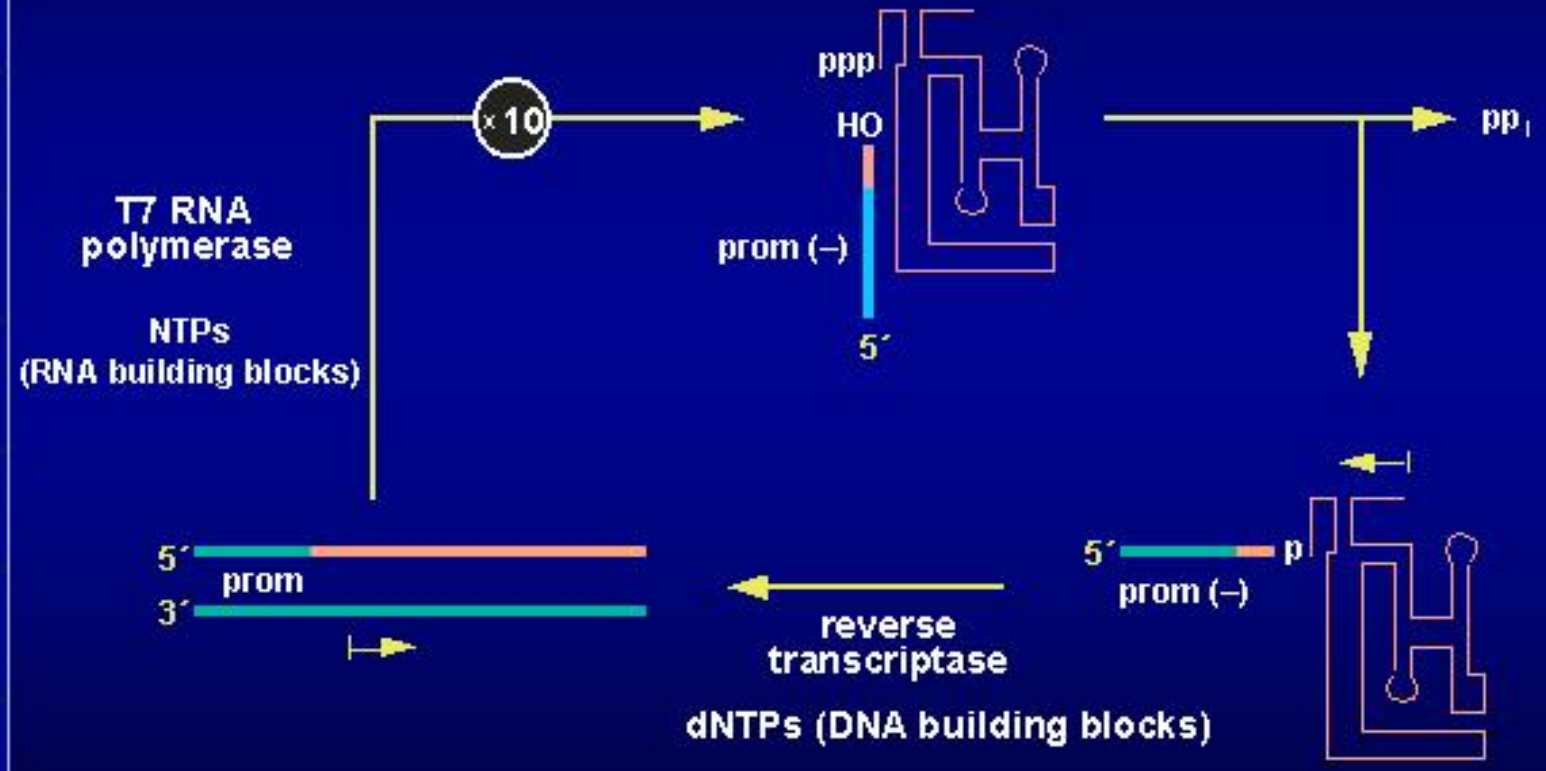



Class I Ligase Ribozymes
CCAGUC—OH pppGGGAA
GGUCAG
RIBOZYME
ppp + H2O
CCAGUC—O—pGGGAA
GGUCAG
RIBOZYME
Bartel & Szostak, Science (1993)
Ekland, Szostak, and Bartel, Science (1995)

Coupled Catalysis / Amplification



M. Wright and G.F. Joyce, *Science*, **276**, 614-7 (1997)

10-23 DNA enzyme
3'
5'
YR
5'
3'
R
G
G
C
T
A
G
C
T
A
C
A
A
G
C
Y = U or C
R = A or G
S. Santoro and G.F. Joyce, PNAS, 94, 4262-6 (1997)

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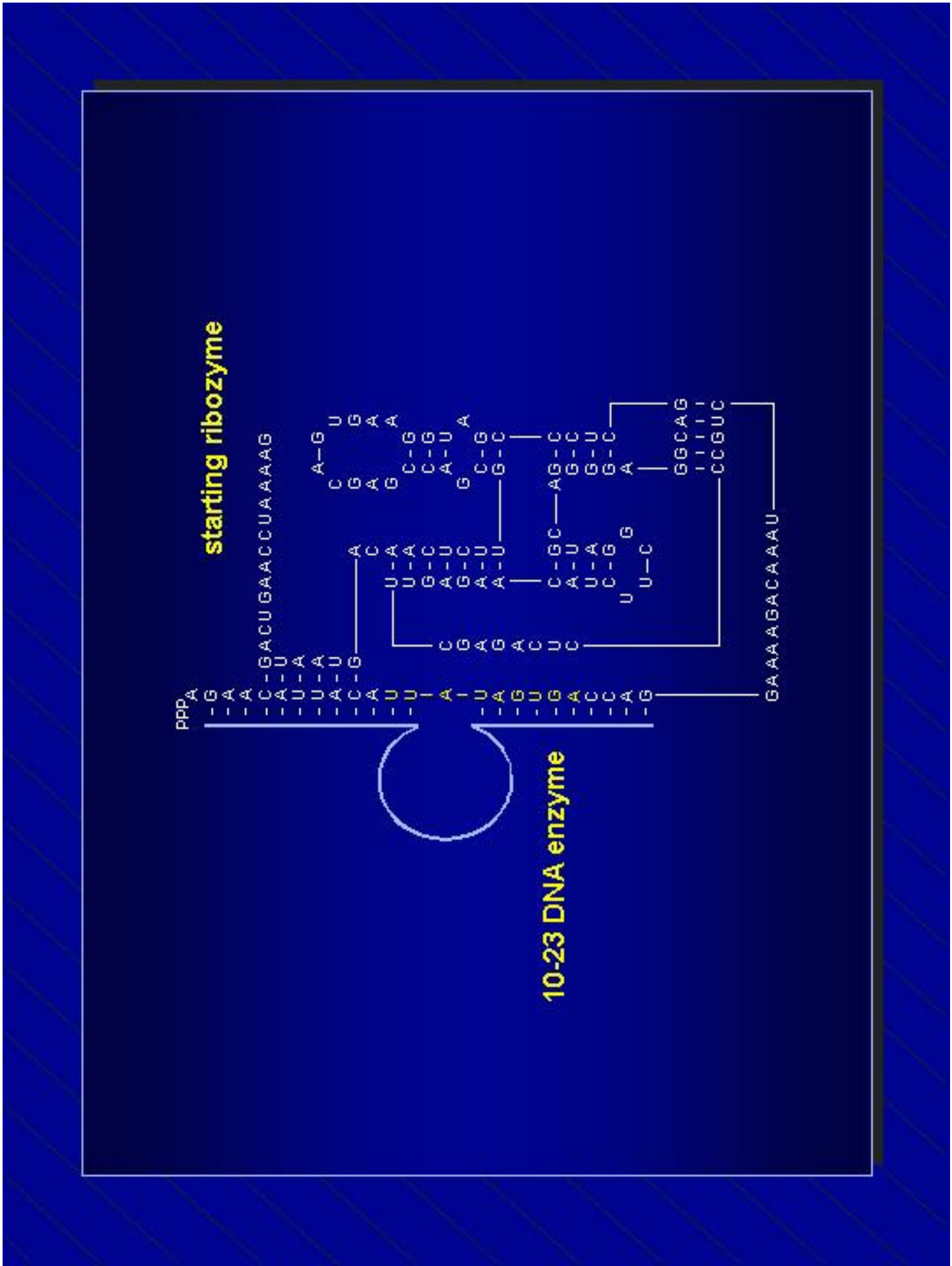

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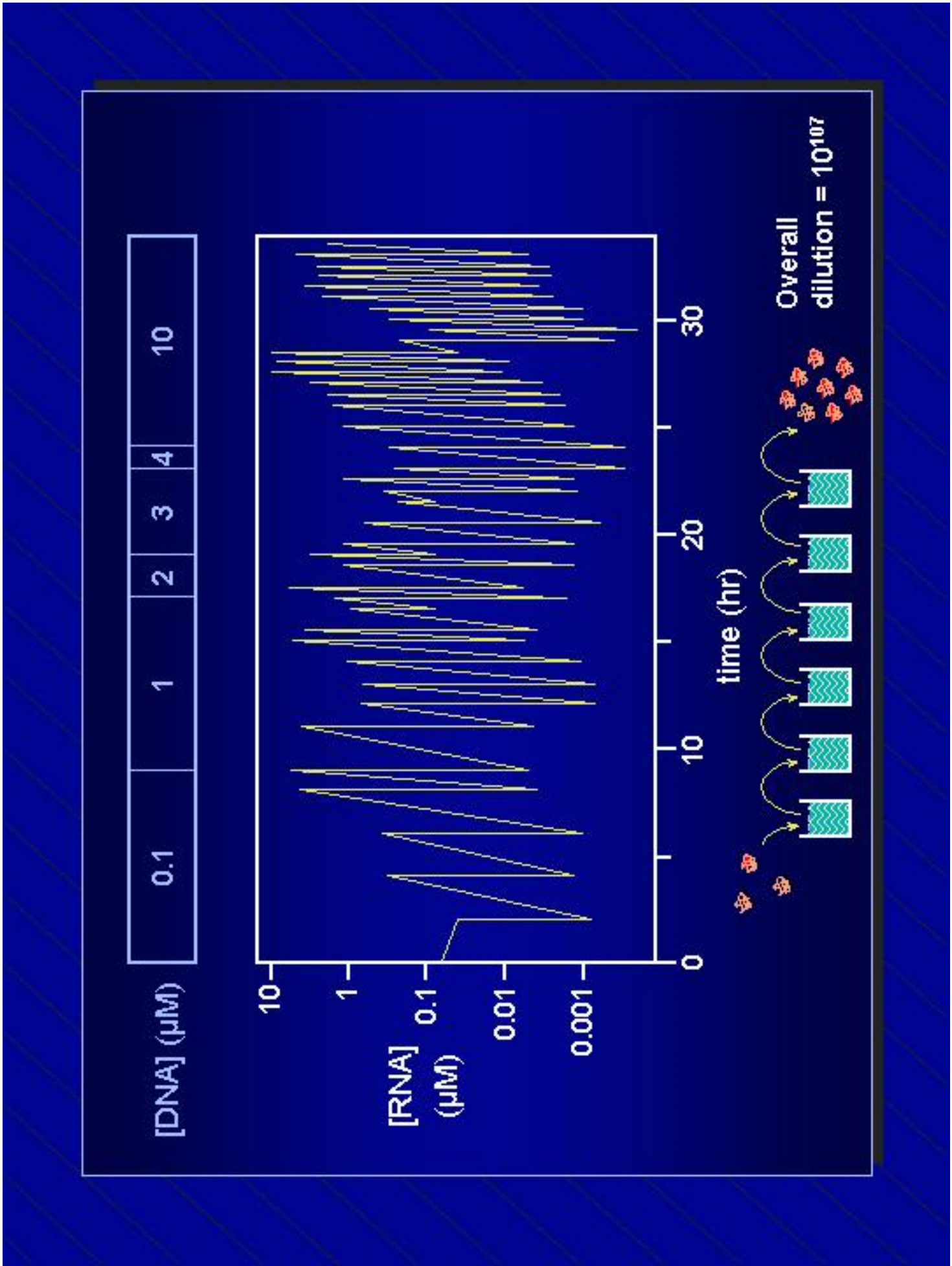

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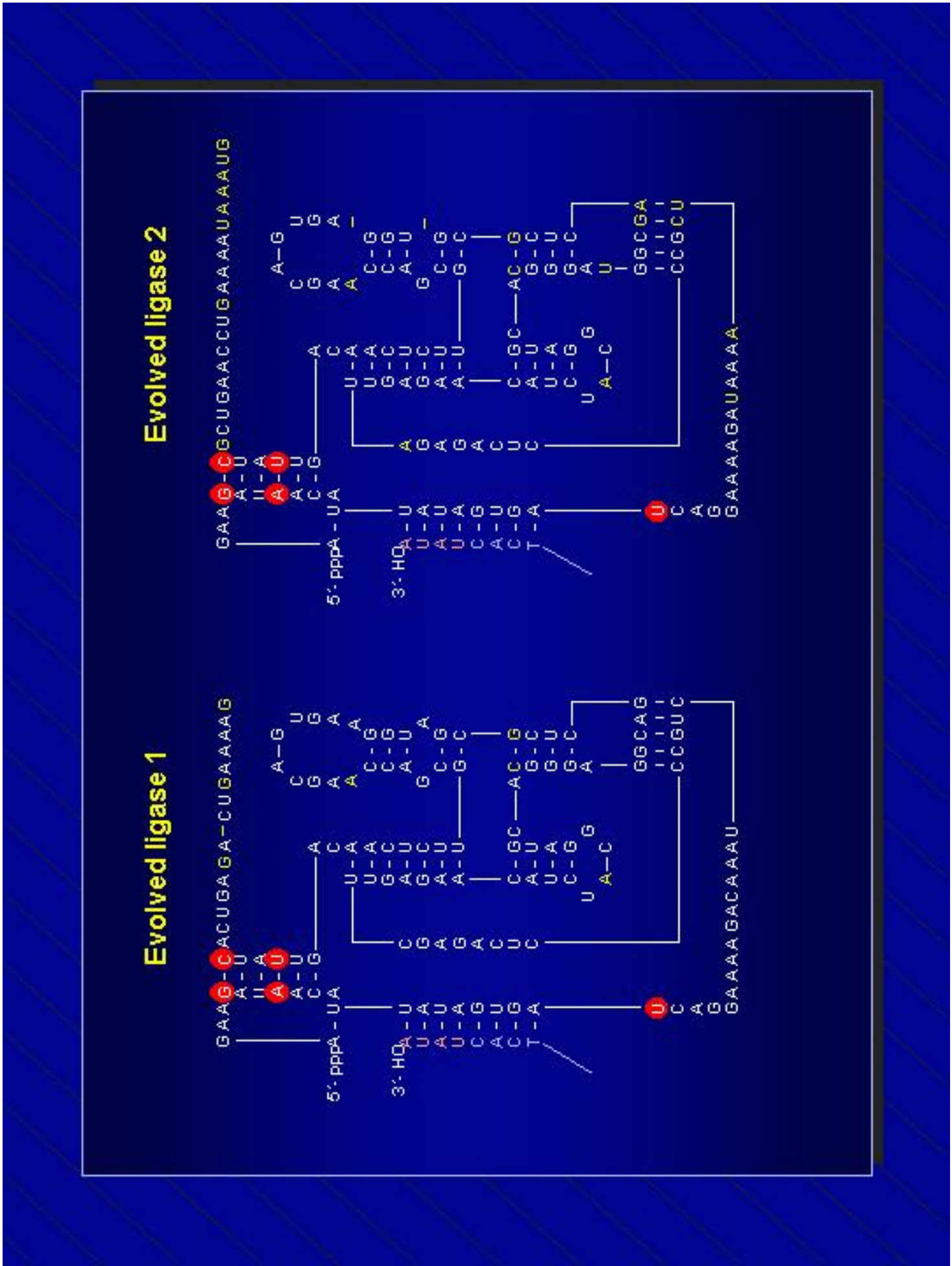

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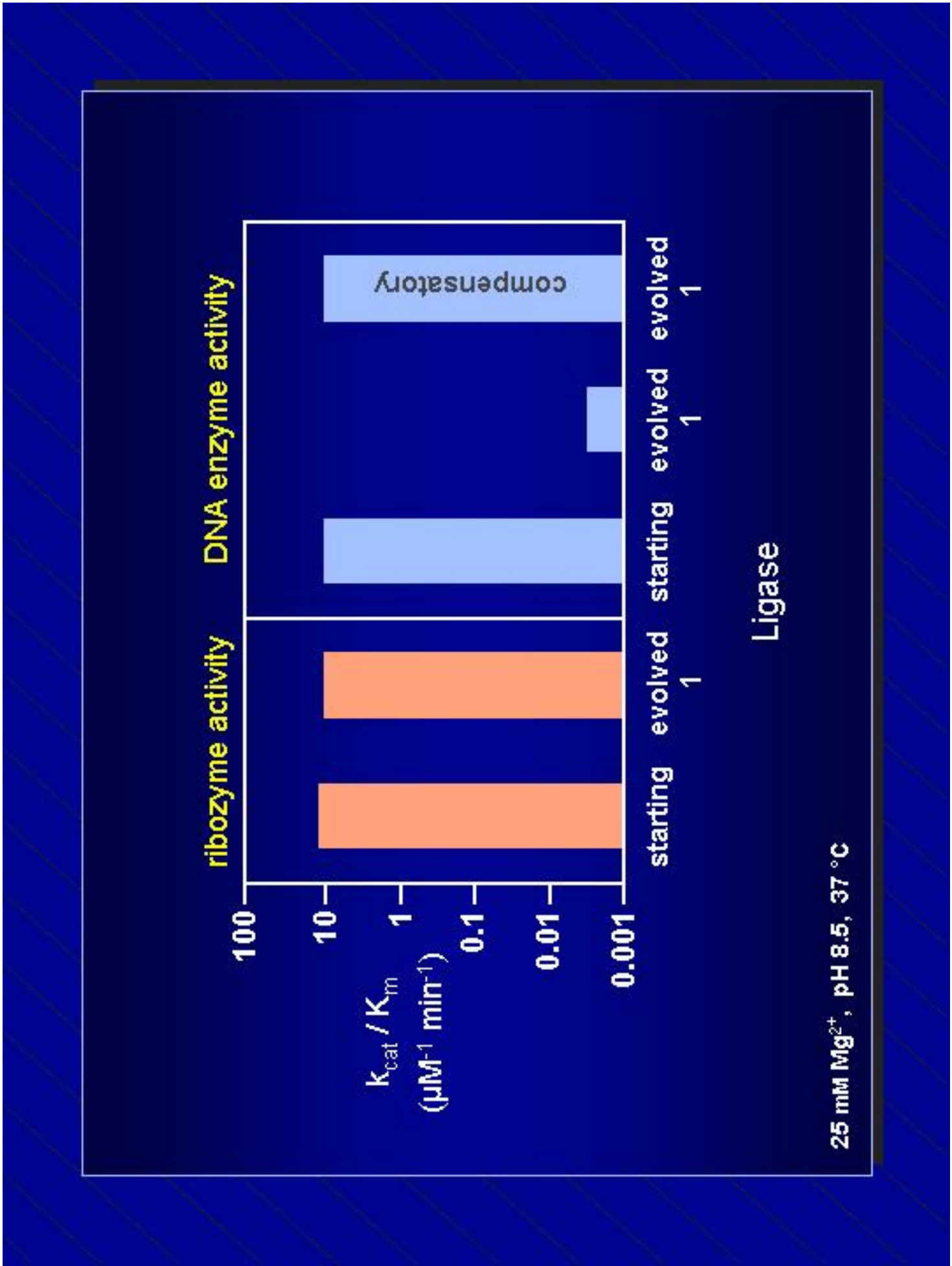
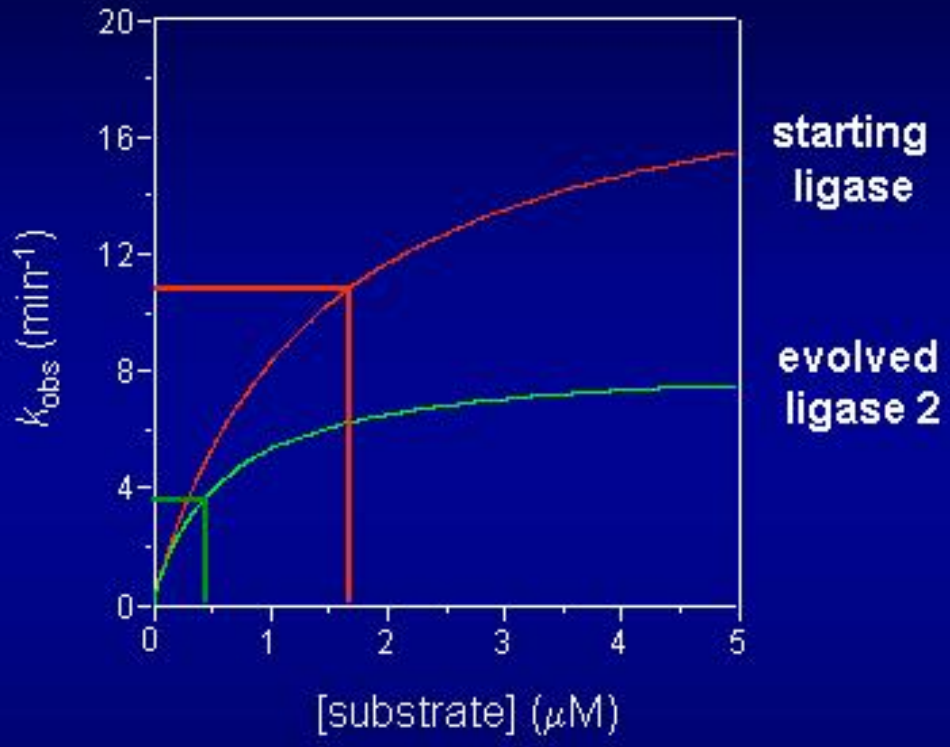


Comparison of K_m's for Starting and Evolved Ligase 2



$[substrate]_o$ = 2.5 µM

starting 60% saturated

evolved 2 80% saturated

25 mM Mg^{2+}, pH 8.5, 37 °C

K_m = concentration of substrate where the rate is half maximal.

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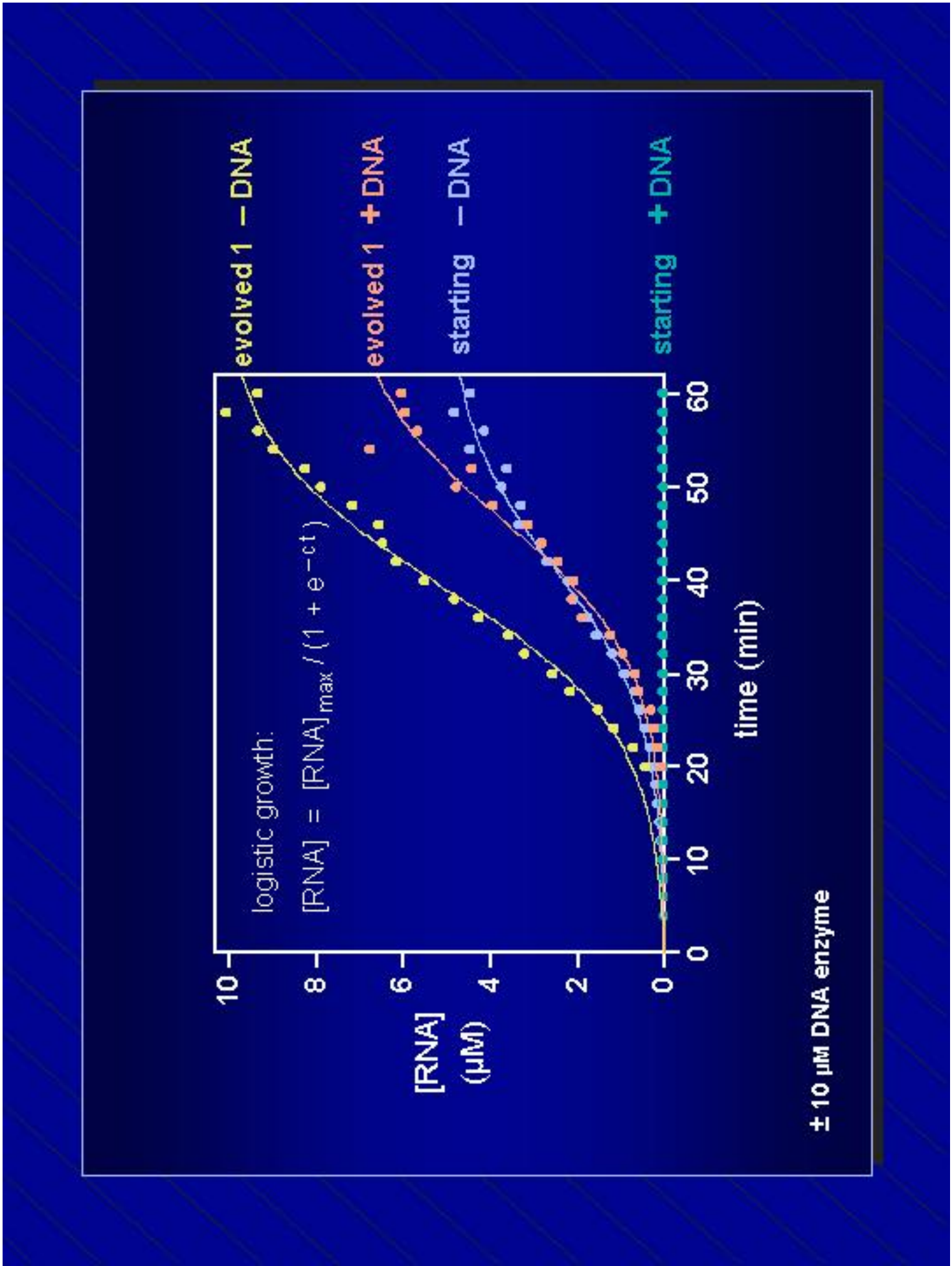

Predicting the Growth Rate in the Presence of the DNA enzyme

- The growth rate is dependent upon the catalytic efficiency of the ligase and the DNA enzyme.
- Comparing the specificity of ligase with its substrate vs. the DNA enzyme for cleaving the ligase .

$$\frac{\nu_{\text{ligase}}}{\nu_{\text{DNA enzyme}}} = \frac{(k_{cat} / K_m)_{\text{ligase}} \bullet [\text{substrate}]}{(k_{cat} / K_m)_{\text{DNA enzyme}} \bullet [\text{DNA enzyme}]}$$

$$= \frac{(20\ \text{min}^{-1} / 1.7 \times 10^{-6}\ \text{M}^{-1}\ \text{min}^{-1})(2.5\ \mu\text{M})}{(0.4\ \text{min}^{-1} / 4.8 \times 10^{-8}\ \text{M}^{-1}\ \text{min}^{-1})(10\ \mu\text{M})}$$

$$= \underline{0.3}$$

- Growth rate in the absence of DNA enzyme = 0.13 min^{-1}
- **Predicted** growth rate with DNA enzyme = (0.3)(0.13) = **0.04 min^{-1}**
- **Observed** growth rate with DNA enzyme = **0.06 min^{-1}**

Summary

Two mechanisms of ligase resistance

- Alteration of toxicant binding site, without disruption of the substrate binding site or catalytic function, resulted in a 2,000-fold lower susceptibility to the toxicant.

- Greater initial substrate saturation of the evolved 2 ligase protects the ribozyme by blocking the toxicant binding site.

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Heritability of a Trait

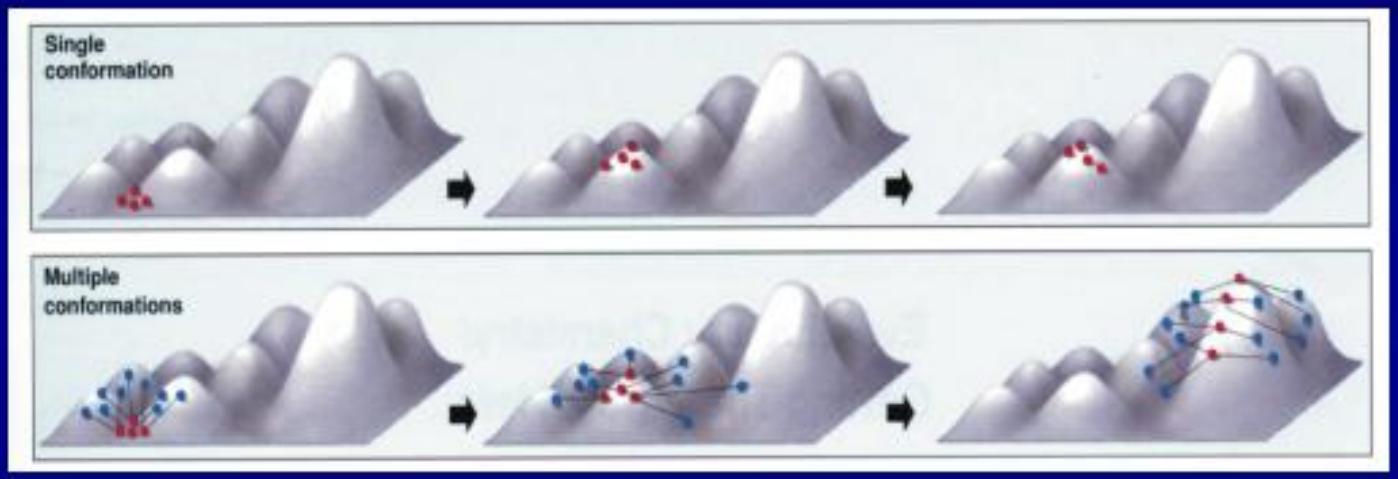
$$0 \geq H^2 \geq 1$$

It is a measure of the response of a population's phenotypic variation of a trait after an evolutionary period, which is dependent upon the genotypic variation that existed before the period.

$H^2 = 1$, the trait is more easily affected by natural selection during the evolution process.

Non-unity heritabilty, the trait responds slower to natural selection in the evolution process.

Multiple Conformations vs. a Single Conformation on a Fitness Landscape



Phenotype = Fitness = Σ (fraction of conformer)(conformer reactivity)

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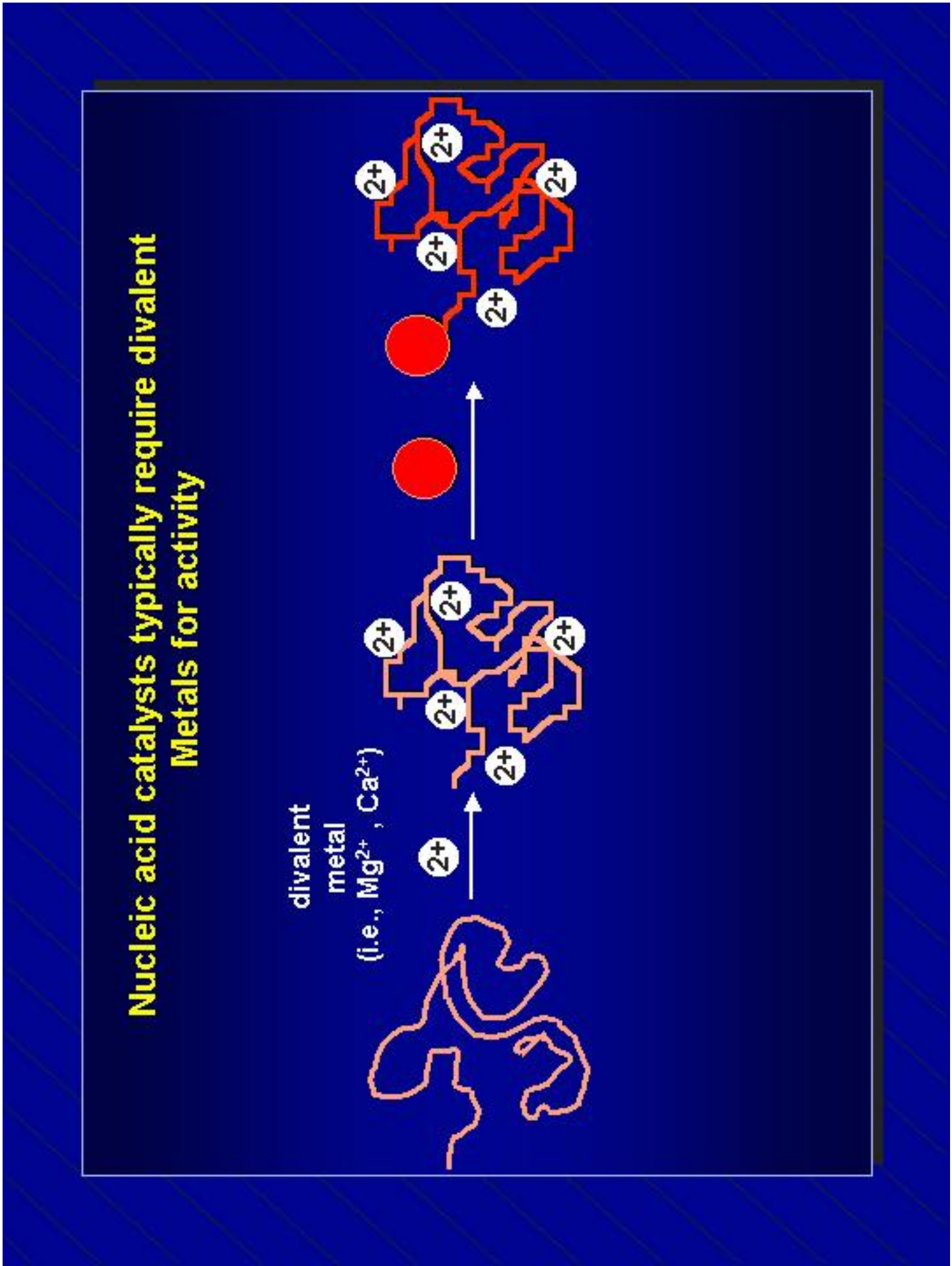

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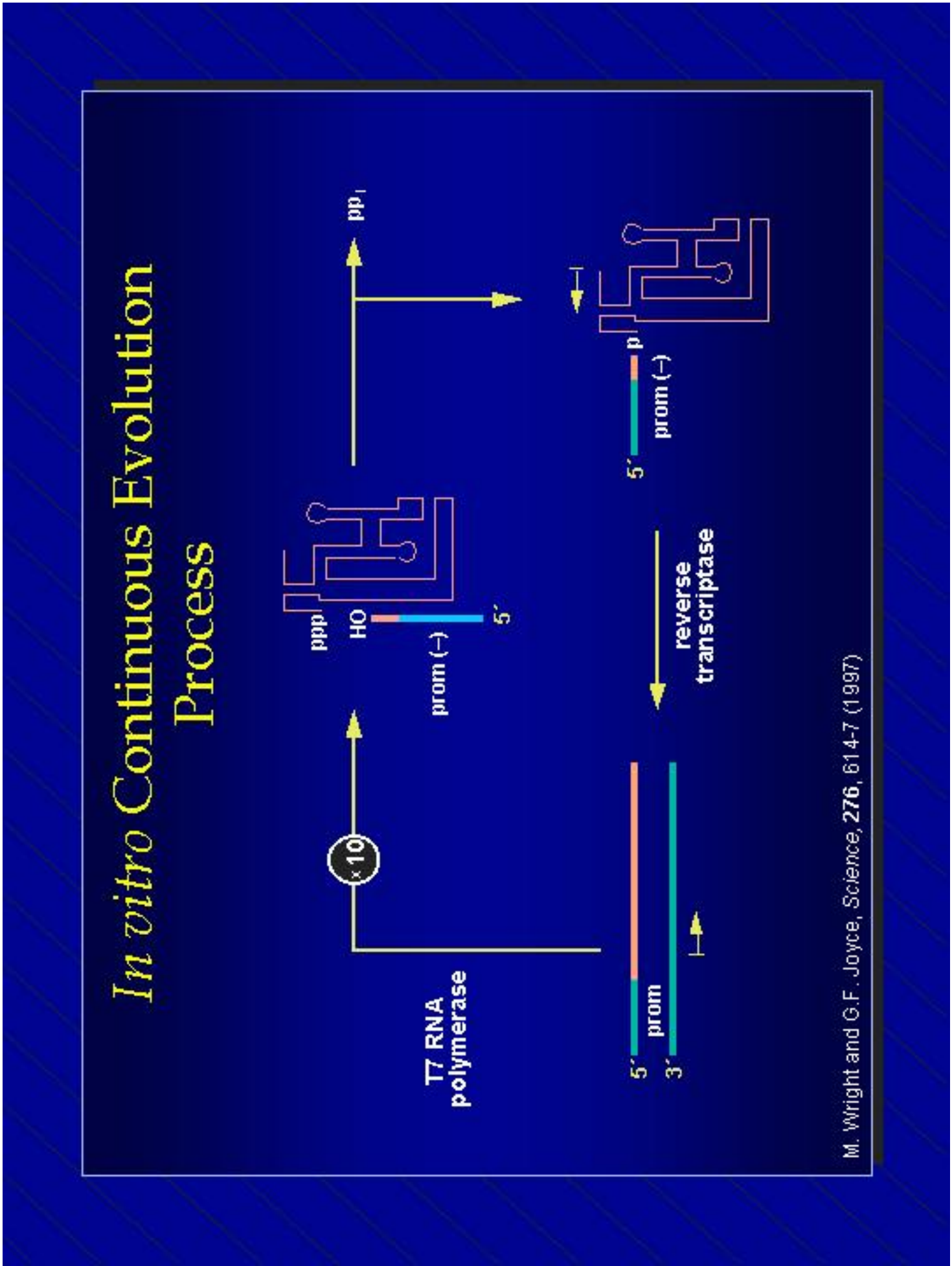

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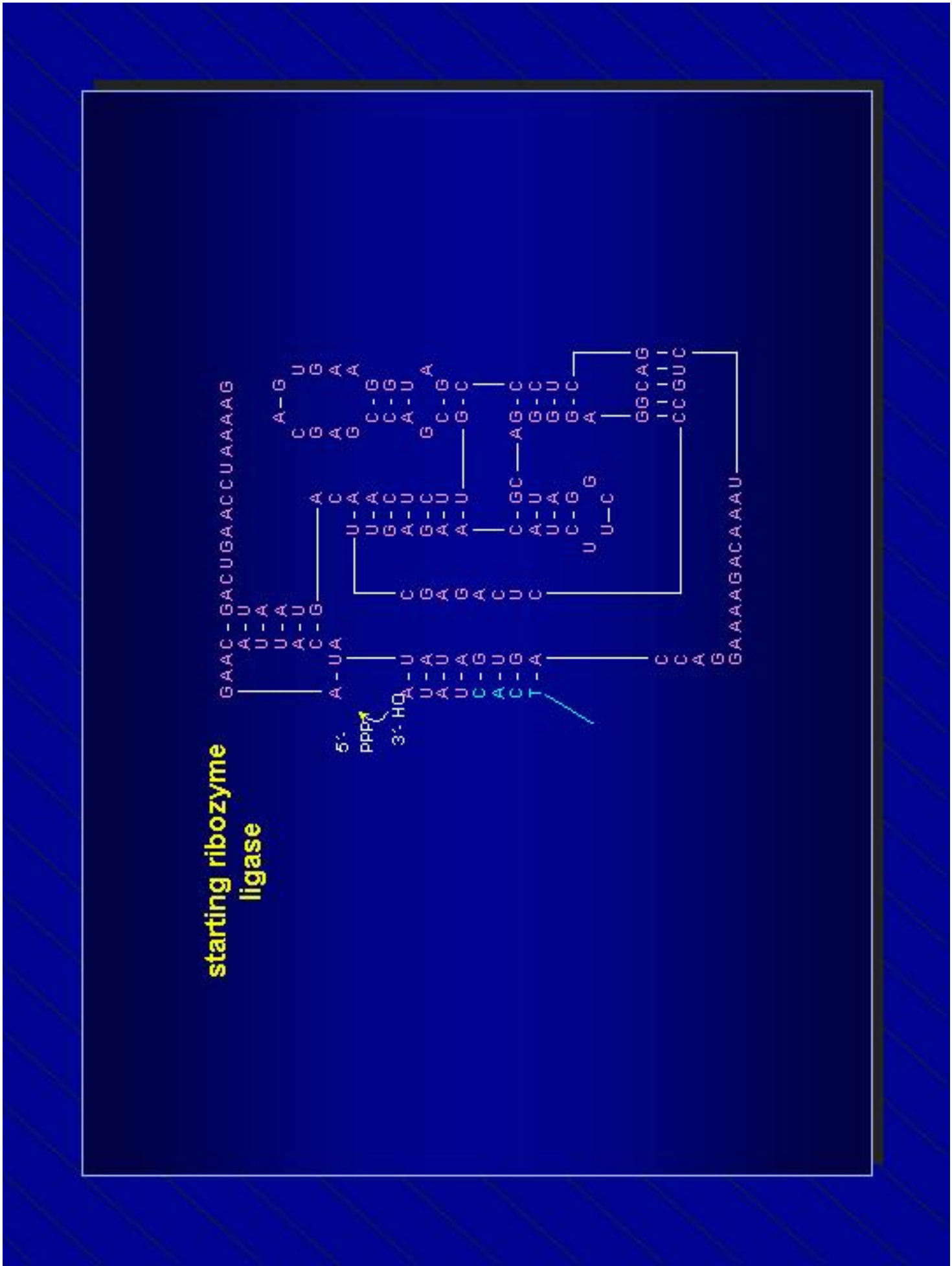

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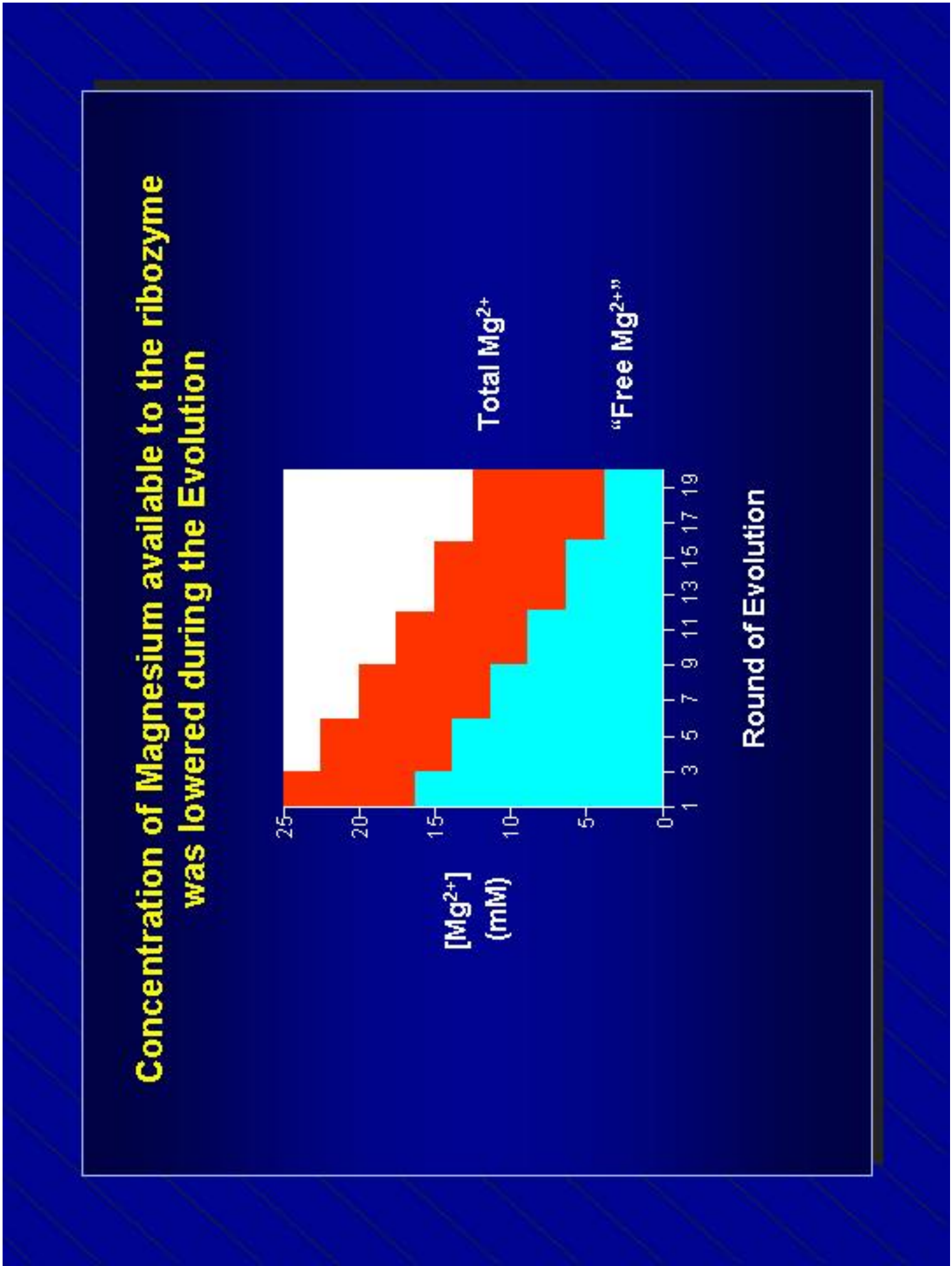

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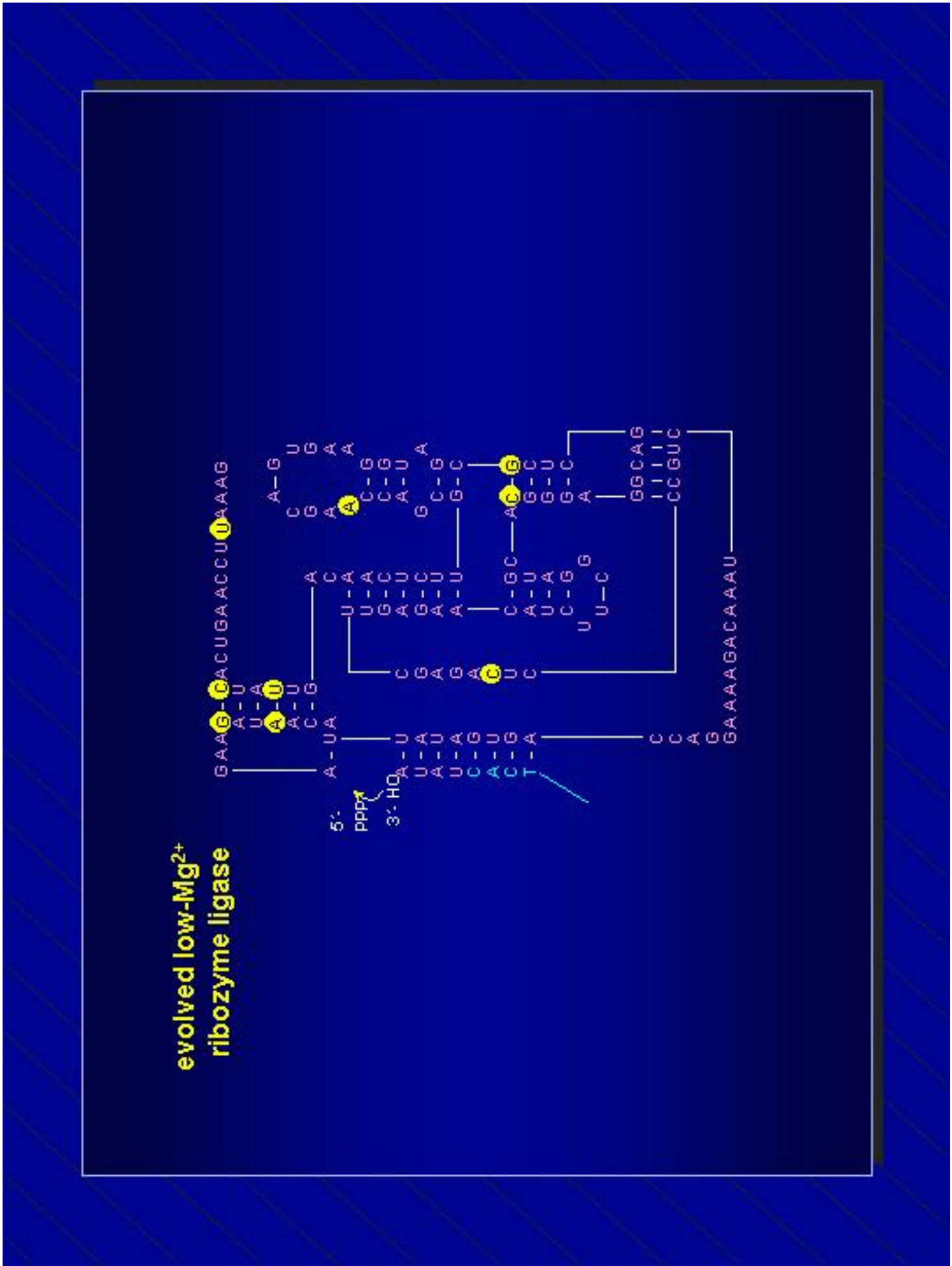
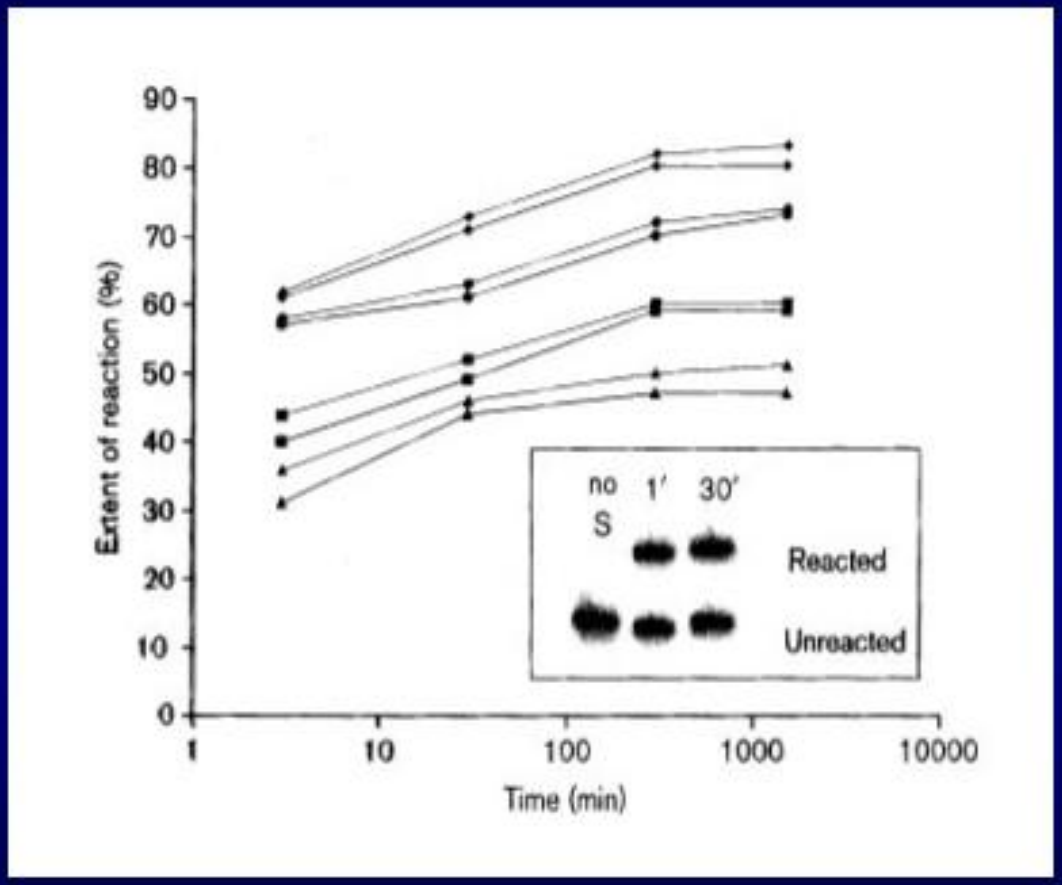
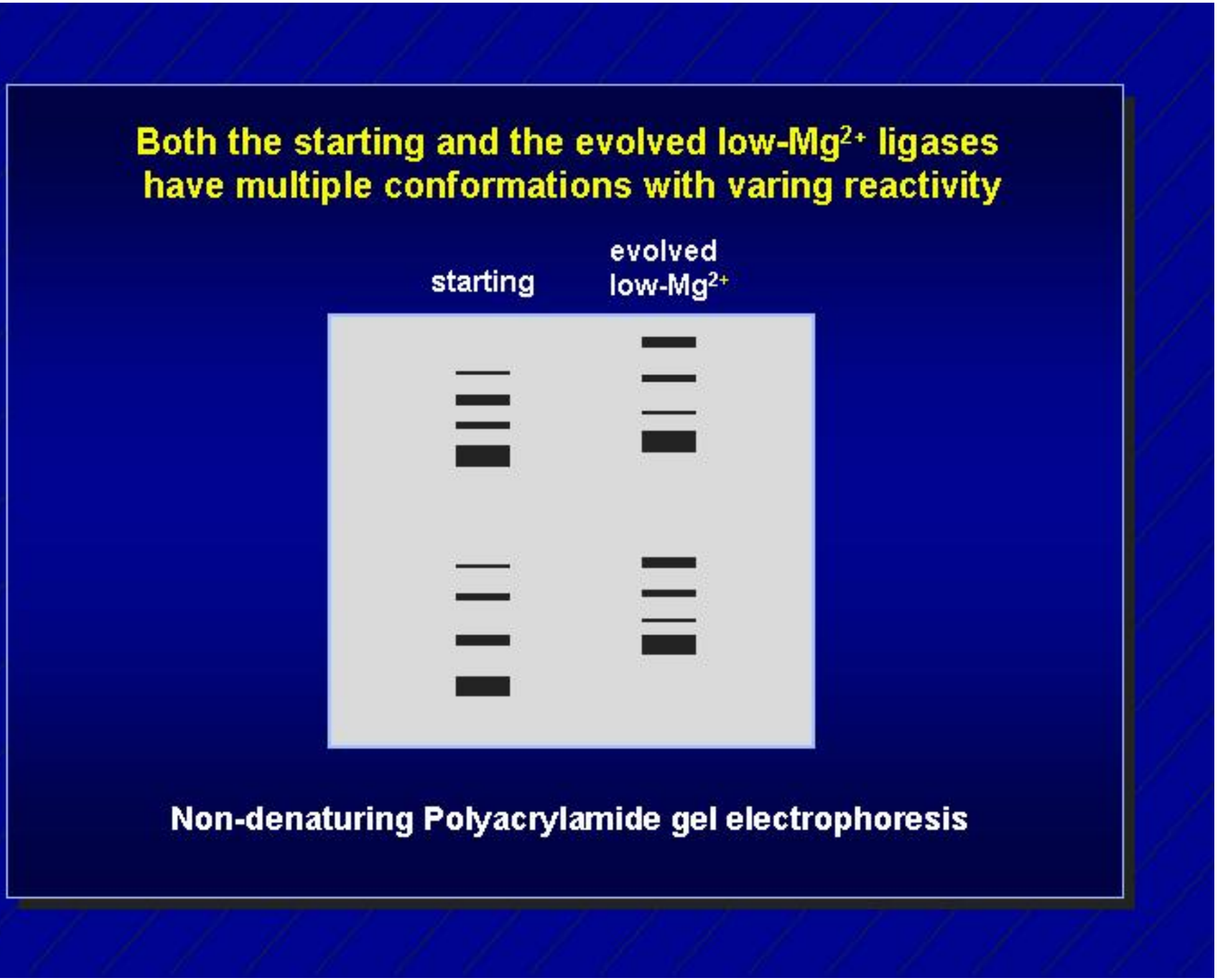


Maximum Extent of the Ligases



Ligase	[Mg2+]
evolved low-Mg^{2+}	25 mM
evolved low-Mg^{2+}	10 mM
starting	25 mM
starting	10 mM



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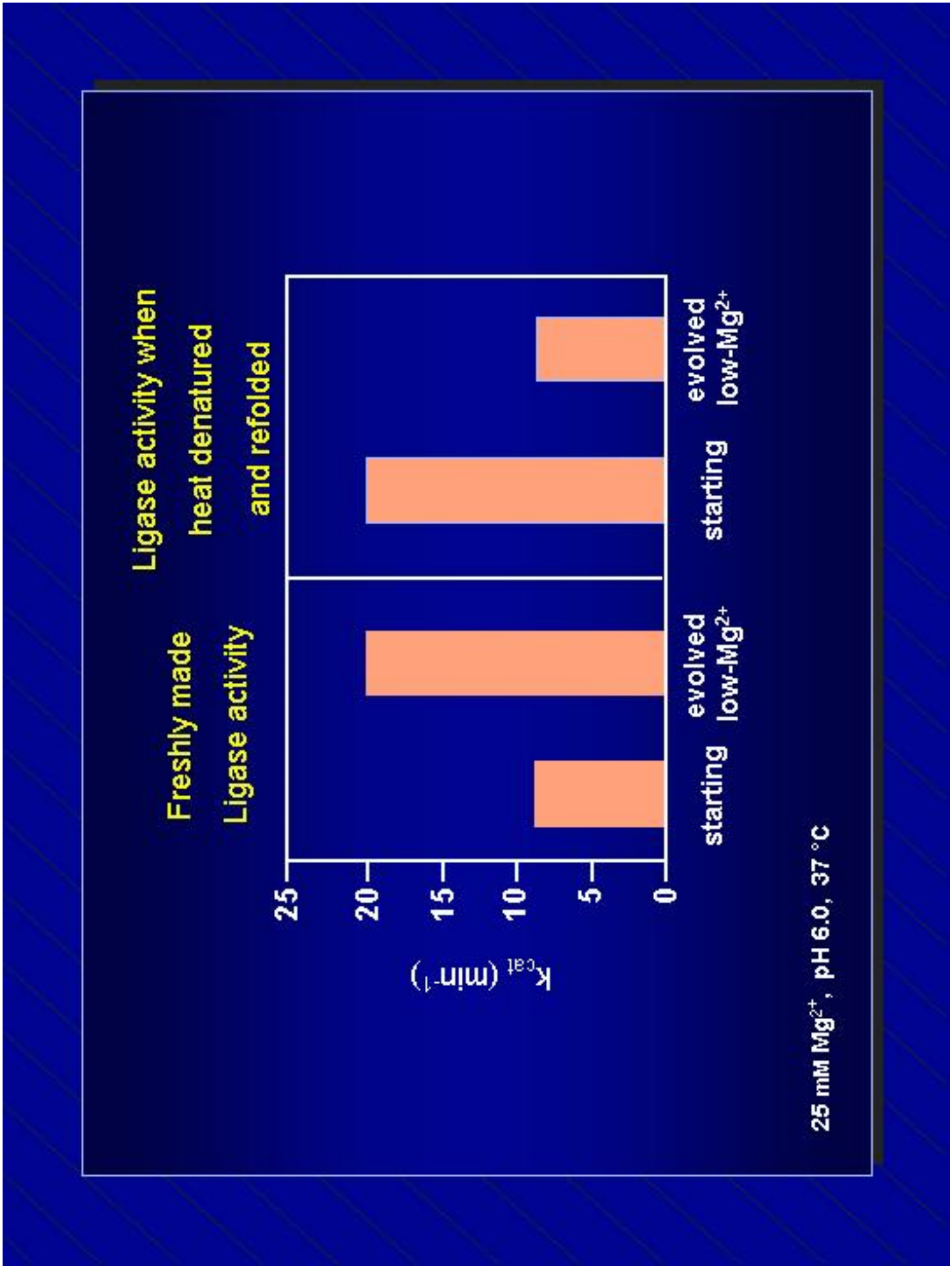

Activity of the Dominant Conformer when Transcribed in the Presence and Absence of Substrate

	substrate (+)	substrate (—)
starting ligase	inactive	active
Evolved low-Mg $^{2+}$	active	active

Calculating the Heritability of the Trait

$$H^2 = R / S$$

Mean phenotype, $z = \Sigma$ (amount of conformer)(conformer reactivity)

Starting ligase, $z_{before} = 2.1\%$
Evolved low-Mg^{2+} ligase, $z_{after} = 5.9\%$

Where **R** is the **response to the evolution.**

R = (mean phenotype **after** the evolution) — (mean phenotype **before** the evolution)

= 5.9% — 2.1% **= 3.8%**

And **S** is the **maximum possible response to the evolution.**

S = total max. response — starting response

= 100% — 2.1% **= 97.9%**

So, $H^2 = 0.038 / 0.979 = 0.04$

Summary

- $H^2 = 0.04$, is close to zero.

- The overall response to the evolution experiment was small, and so, the heritability of the trait of conformational reactivity responds slowly to evolutionary pressures.

- However, there was a measurable response:
  1) The starting ligase and the evolved low-Mg^{2+} were both conformationally heterogenous and the various conformers had differing reactivity.
  2) The evolved low-Mg^{2+} ligase was able to adopt more active conformations as shown by the maximum extent experiment.
  3) The evolved low-Mg^{2+} was able to fold better "on the fly" during transcription.
     a) Kinetics: the evolved low-Mg^{2+} ligase was faster when transcribed and slower when denatured and refolded. The reverse was true for the starting ligase.
     b) The substrate was able to inhibit the folding of the starting ligase if present during transcription, but not the evolved low-Mg^{2+} ligase.

Conclusions

- The use of isothermal amplification methods can be used to study a variety of ecological problems on reasonable time scales.
- Since these methods make use of nucleic acids, whose sequence can be obtained and activity quantitated, it allows for a molecular level of description of the changes that occured during the course of the evolution experiment.
- As a result, mechanisms can be proposed to account for these changes.

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