NATURAL ENZYMES WERE NOT DESIGNED
THEY AROSE BY EVOLUTION

ENZYMATIC BEHAVIORS REFLECT 3 PROCESSES:

1. NATURAL SELCTION. The behavior assists the host organism struggling to survive and reproduce

2. NEUTRAL DRIFT. Structural and behavioral variation with no impact on survival accumulates during divergent evolution

3. CONSERVATION. A behavior may serve no direct function but nevertheless be conserved because there is no easy way for it to drift. Such traits are vestiges of ancestral enzymes synthesized by organisms that lived in earlier times.

HOW WE INTERPRET A PARTICULAR ENZYMATIC BEHAVIOR DEPENDS ON THE PROCESS THAT PRODUCED IT

AN EVOLUTIONARY STATUS CAN NOW BE ASSIGNED TO MOST ENZYMATIC TRAITS, AND A UNIFIED PICTURE OF BIOCHEMISTRY IN TERMS OF EVOLUTION IS AVAILABLE FOR EXPERIMENTAL INVESTIGATION

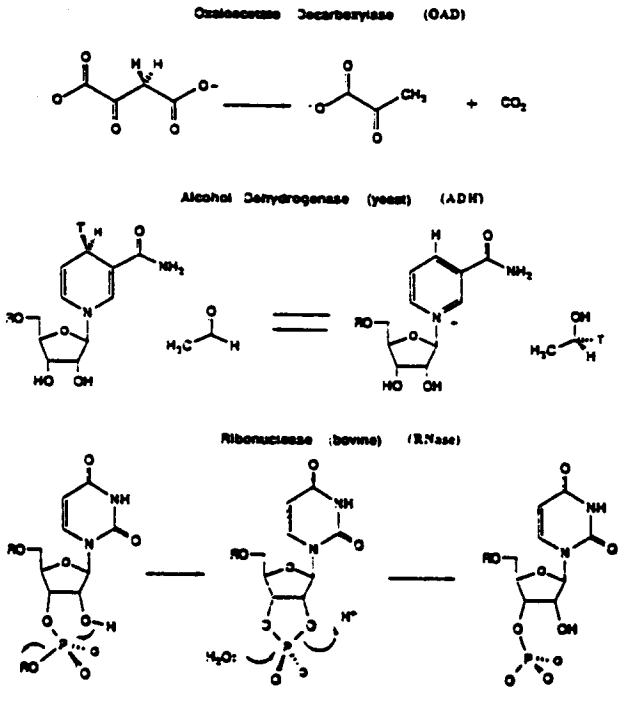
Benner, Redesigning the Molecules of Life, (1988) Heidelberg, Springer-Verlag, 115-175

Benner, Ellington, CRC Crit. Rev. Biochem. (1988) in press

Benner, Glasfeld, Piccirilli, Top. Stereochem. (1988) 19, in press

Benner, Chem. Rev. (1988) submitted.

WE WILL DISCUSS HOW ONE MIGHT USE THIS PICTURE TO DIRECT EXPERIMENTS WITH BIOLOGICAL MACROMOLECULES



THE DESIGN OF POLYPEPTIDES THAT FOLD IN SOLUTION AND CATALYZE REACTIONS

PREDICTION OF TERTIARY STRUCTURE OF NATURAL PROTEINS FROM SEQUENCE DATA

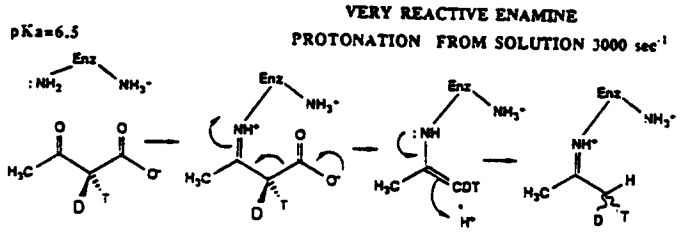
MANIPULATION OF THE STRUCTURE OF NATURAL PROTEINS TO ALTER THEIR BEHAVIORS

UNDERSTANDING THE STRUCTURAL BASIS FOR THE BIOLOGICAL ACTIVITY OF TUMOR GROWTH PROMOTERS AND INHIBITORS

RECONSTRUCTION OF EVENTS OCCURRING EARLY IN THE EVOLUTION OF LIFE

SELECTED AND NEUTRAL BEHAVIORS IN BETA-DECARBOXYLASES

ACETOACETATE DECARBOXYLASE PRODUCES RACEMIC PRODUCT

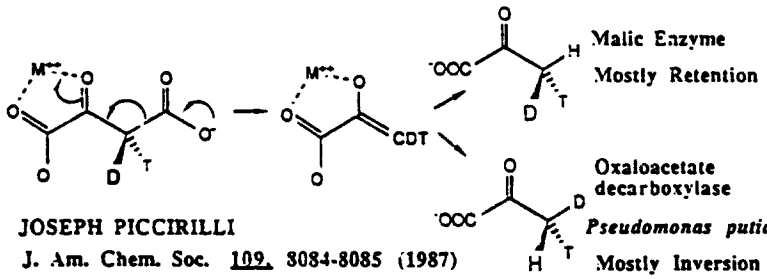


DAVID ROZZELL

J. Am. Chem. Soc. 106, 4937-4941 (1984)

Ca. 50% retention
50% inversion

2 OXALOACETATE DECARBOXYLASES USING METALS AS ELECTROPHILES HAVE OPPOSITE STEREOSPECIFICITIES



Malic Enzyme
Mostly Retention

Oxaloacetate decarboxylase
Pseudomonas putida
Mostly Inversion

JOSEPH PICCIRILLI

J. Am. Chem. Soc. 109, 8084-8085 (1987)

CONCLUSIONS:

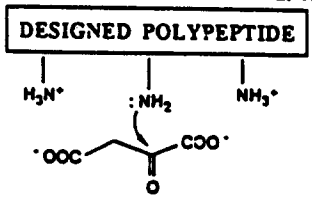
STEREOSPECIFICITY IN BETA-DECARBOXYLASES IS NOT A SELECTED TRAIT

THE PROTONATION STEP IS NOT VERY IMPORTANT IN DESIGNING A CATALYST

HOWEVER, AN AMINE THAT IS UNPROTONATED AT LOW pH IS IMPORTANT IF ONE WANTS TO USE A SCHIFF'S BASE AS AN ELECTRON SINK.

DESIGN OF A SMALL PROTEIN THAT FOLDS IN SOLUTION & CATALYZES THE DECARBOXYLATION OF OXALOACETATE

1. IN THE FOLDED FORM, SEVERAL LYSINES MUST COME TOGETHER
2. THE pKa OF ONE OF THE LYSINES MUST BE LOW, PERMITTING IT TO BE UNPROTONATED AT NEUTRAL pH, ALLOWING IT TO FORM A SCHIFF'S BASE WITH OXALOACETATE
3. THE REMAINING CHARGED LYSINES BIND THE SUBSTRATE
4. NO PROVISION NEED BE MADE FOR CATALYZING THE PROTONATION OF THE INTERMEDIATE ENAMINE

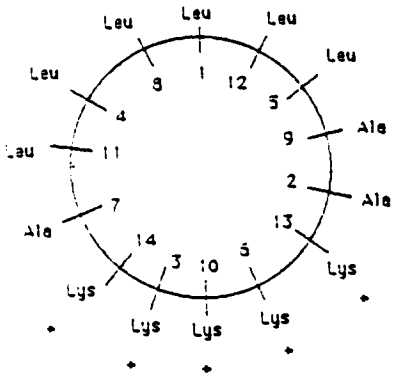


UNDERSTANDING IS THE GOAL

DESIGNED ARTIFICIAL OXALOACETATE DECARBOXYLASE

5 10

H_2N-Lys-Lys-Leu-Leu-Lys-Ala-Leu-Ala-Lys-Leu-Leu-Lys-Ala-Leu-COOH

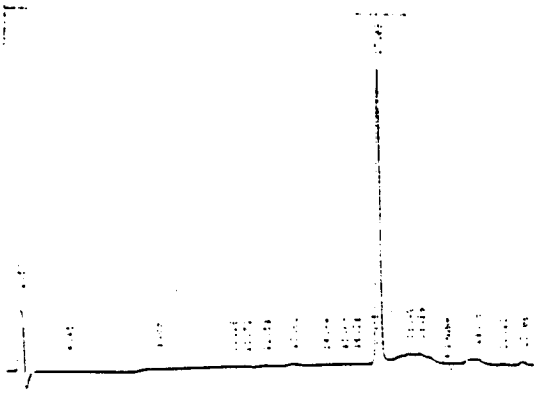


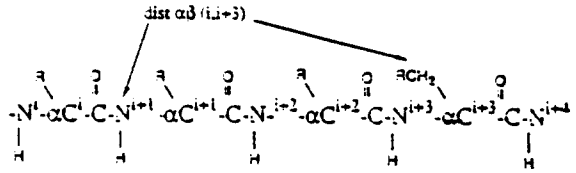
Should form an amphiphilic helix, bringing all of the lysine residues together:

(a) In the presence of an organic-aqueous interface
(b) In the presence of small amounts of "structure-forming solvents" (e.g. trifluoroethanol).

Rudolf Allemann
Kai Johnsson

HPLC-Diagram des gereinigten Peptides:





pKa values: 7.25 9.5 10.6

Circular dichroism suggests that helix content is higher at high pH, and increases upon addition of trifluroethanol

But circular dichroism provides only a crude estimate of conformation. It is no more a "structure proof" for peptides than it is for other organic molecules

COSY IDENTIFIES SPINS THAT ARE COUPLED

Can "walk" down the chain of amino acids, facilitating assignment of resonances secondary structure

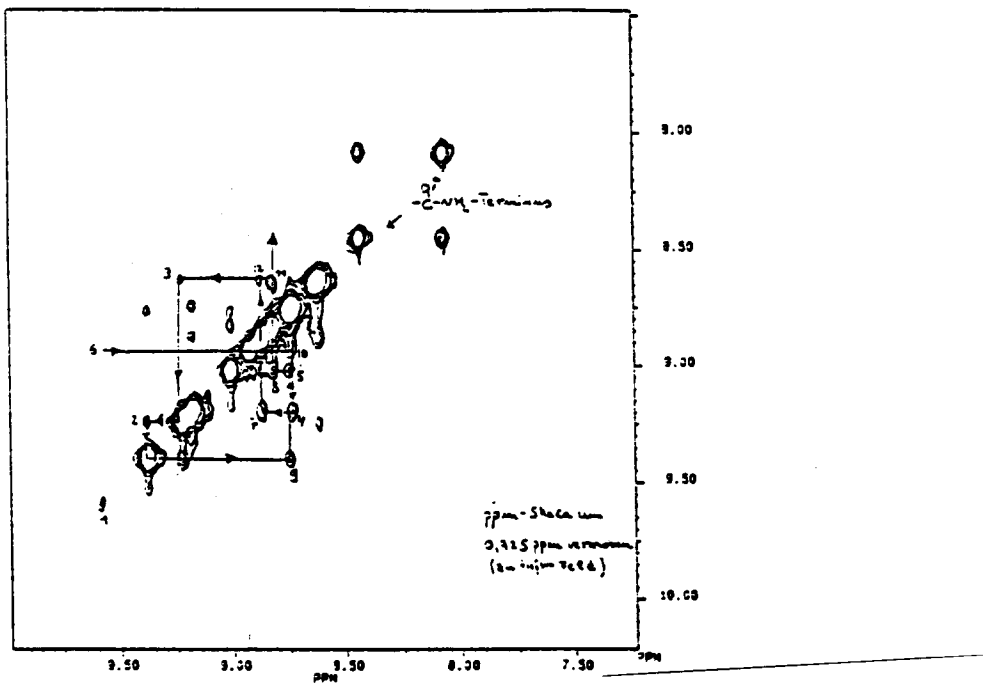
α-Helix (ϕ= -57°) $J_{NH\alpha}$ = 3.9 Hz

β-Sheet (Antiparallel) (ϕ = -139°) $J_{NH\alpha}$= 8.9 Hz

β-Sheet (parallel) (ϕ= -119°) $J_{NH\alpha}$= 9.7 Hz

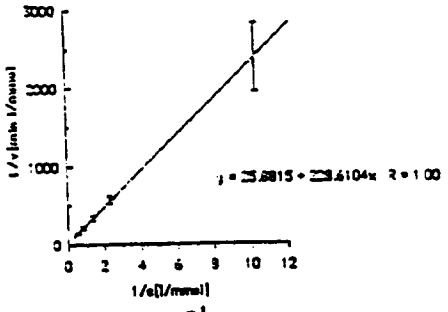
NOESY IDENTIFIES SPINS THAT ARE CLOSE IN SPACE

Allowing assignment of overall folded form



-11-8-5-9-2-3-12-7-4-10-6-

The peptide has catalytic activity as an oxaloacetate decarboxylase, showing Michaelis Menten kinetics



k_{cat} = 0.4 min^{-1}
K_M = 8.8 mM

The catalytic activity increases with increasing concentration of TFE, as does the percentage helical content, suggesting that the helical form of the polypeptide is catalytically active

The catalytic activity of lysine as an oxaloacetate decarboxylase goes <u>down</u> with increasing TFE concentrations, suggesting that the result above is not simply a solvent effect

	k_{cat} (sec^{-1})	K_M (mM)	log relative rate
Oxaloacetate decarboxylase (natural)	1000	3.0	9
Oxaloacetate decarboxylase (designed)	.01	8.8	4
Spontaneous decarboxylation	.000001	---	0

CONCLUSIONS

1. The designed oxaloacetate decarboxylase has a helical structure in solution and a single lysine with a low pKa
2. It catalyzes the decarboxylation of oxaloacetate, probably from the helical conformer, with Michaelis-Menten kinetics
3. The Km is similar to that of the natural enzyme; the designed peptide accelerates the rate of decarboxylation of oxaloacetate by 3 orders of magnitude at pH 7, compared to an increase of 8 orders of magnitude effected by the enzyme

EVOLUTION AND THE STABILITY OF FOLDED PEPTIDES

Predicting tertiary structure from sequence

If all of the "rules" for folding a protein are followed, the tertiary structure will be very stable

Instability is a selected trait in proteins, as it permits the turnover of proteins when no longer needed.

Therefore, natural proteins violate folding rules to have the desired level of instability

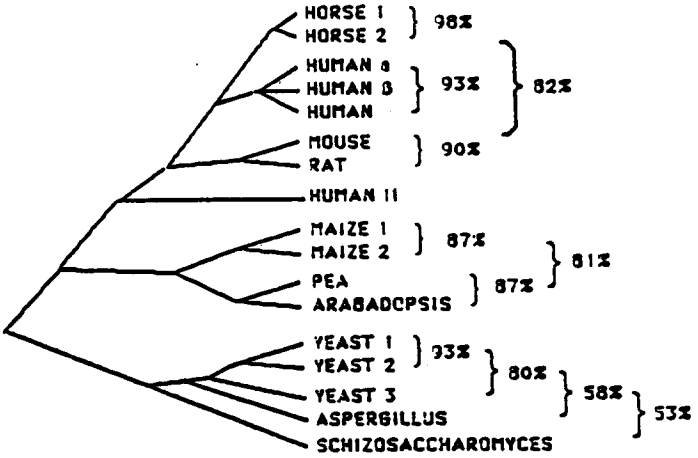
It is relatively easy to increase the stability of most proteins by point mutation (enzyme engineering)

Even if we can learn perfectly the "rules" governing protein folding, a sequence of a protein will deceive the chemist trying to apply them to predict tertiary structure

Patterns of sequence divergence within a set of homologous proteins can provide information about tertiary structure.

Benner, <u>Adv.Enzyme Reg.</u> (G. Weber, ed.) in press

ALIGNMENT OF ALCOHOL DEHYDROGENASES BASED ON COMPARISON OF SEQUENCES (OVERALL 21%)



ALGORITHM FOR PREDICTING SURFACE RESIDUES

1. Pick subgroups of proteins that are highly similar in sequence (>85% sequence identity)

2. Make a list of positions in the alignment where variation that includes polar substitution is observed in two or more of the subgroups.

3. These are assigned to be surface residues.

RATIONALE

1. Neutral variation (that having no impact on a selectable behavior) must occur on the surface of proteins with high sequence identity.

2. Adaptive variation can occur virtually anywhere in the structure; it is intended to alter behavior.

3. It is unlikely that the same adaptive variation will occur in more than one subgroup. Requirement 2 "filters" variation that might be adaptive (at the expense of missing some variation that might be neutral

RESULTS IN YEAST ALCOHOL DEHYDROGENASE

34 residues (10% of the alignment) are assigned "surface residues" by this algorithm. The algorithm is 100% successful; all residues assigned to the surface are found on the surface.

Analogous algorithms assign active site residues, residues in hydrophobic cores, and the interruption of secondary structure. With structures such as beta barrels, predictions of tertiary structure to medium resolution can be made from sequence data alone.

10-15 homologous sequences are needed with homologies from 90% to 25%.

TOGETHER WITH "CLASSICAL" APPROACHES FOR ANALYZING PROTEIN STRUCTURE, THESE APPROACHES PERMIT THE ASSIGNMENT OF TERTIARY STRUCTURE FROM AN ALIGNMENT OF SEQUENCES

SEQUENCES THAT CAN FORM AMPHIPHILIC HELICES SOMETIMES ARE HELICAL IN PROTEINS. BUT THE PREDICTIVE SUCCESS IS NOT EXCELLENT

AN AMPHIPHILIC HELIX HAS A REASON TO FORM IF IT IS ON THE SURFACE OF A PROTEIN

14 AMPHIPHILIC "HELICES" DETECTED BY THE ALGORITHM IN ADH, 6 ARE FALSE

Mammalian Adh's are dimers, with broad and variable substrate specificity; variability is probably adaptive

Yeast Adh's are tetramers, with narrow and invariant substrate specificity

We have a crystal structure for Horse Adh, but not for Yeast Adh.

Patterns in the evolutionary divergence of sequence in the two groups help us extrapolate the structure for Yeast Adh from the structure for Horse Adh.

1. Select a subgroup of dimeric enzymes with sequence identities >85%, and a subgroup of tetrameric enzymes with overall sequence divergence of 50%. Thus, the dimers that we compare have diverged less than the tetramers that we compare.

2. Identify positions in the alignment where the amino acid in the dimers has diverged, but where the sequence in the tetramers is conserved.

Residues identified by this algorithm are those functionally constrained from drifting in the yeast enzymes and not in the horse enzyme:

(a) Residues in the active site of the mammalian enzyme that bind the side chain of the substrate, and very to alter the substrate specificity
(b) Residues making quaternary contacts in the tetramer that are not made in the dimer

Strong Signals	Moderate Signals	Weak Signals	Residues in question	Comments	Prediction
	Pro/Pro/KE 30				
Pro 31			32-43	Surf 33,34 Conserved E 35	BETA
		Gly/Gly/SA 44	45-55	Amphiphil 45-53 AS 45,47,48,50	HELIX
	Deletion 56-57				
	Pro/STVA/CL 59 Pro(A)/VL/K 60		56-62	Surf 56 AS 56,57	
Pro 62			61-64	AS 63	BETA
		Gly/ALVF 65			
Gly 66			67-70	AS 67,69	BETA
Gly 71			72-76	Core 73	BETA
Gly 77					
		Gly/Gly/GNL 79	77-86	Surf 82,84	BETA ?11 80
	Deletion 82-83 Pro/Pro/TV 85			Core 80	LOOP?
Gly 86			87-90	Cons D 90	BETA
	Pro/Pro/TV 91		92		
	Pro/Gly/Asn 95 Deletion 95-96		97-110	Surf 99,101 Cons C very polar	LOOP
	Deletion 111-112 Pro/GA/AE 112		113-118	Surf 115,117, 118	LOOP
Deletion 119-119			140-141		TURN
		Gly(S)/Gly 142			
	Ser/Ser/Gly 144		145-146	Core 146	TURN BETA
	Ser/Ser/G 147G		148-160	Surf 156 Core 160 Not amphi Too long for single Beta	UNCERTAIN ? BETA
	Pro(Q)/Asp/Asn 161				
		P/DGE/DGA 162	161-164	Polar	TURN
	Pro/P(N)/P(D) 165				
			166-185	Amphi 169-188 Surf 185 Core 166 P/L/VI 171	HELIX perhaps irregular
	Deletion 186				
			187-188		UNCERTAIN
	Pro/VAL 189				
		PG/PK/PA 191	189-192	Surf 190,191	TURN
Gly 192					
		S/SG/GHDE 193	192-193		TURN
			194-198	Core 196	BETA
Gly 199					
	Deletion 200-201				
Gly 201					
Gly 204					
		Gly/G/A 202	199-204		LOOP
		G/G/Y 210	205-214	Amphi, no surf AS 207	POSSIBLE HELIX (distorted?)
Gly/A 215			215-217		TURN
	Deletion 217				
		GA/GA/G 221	218-221		BETA (CF)
			222-235	Amphi 226-235 Surf 227,231,233	HELIX
Gly 236					
			237-242		BETA
	Pro/Pro/Phe 243				
			244-248	Surf 247	TURN
	Pro/Pro/VIN 249				
			250-255	Core 253	BETA
	Deletion 256				
			257-259	AS 258 Following segment is certainly a loop, making unlikely that 258 is truly at the active site	UNCERTAIN
Gly 260					
Gly 261					
	Deletion 261-262		260-262		LOOP
			263-269	Splits	BETA
		G/G/VT 270			
			271-283	Surf 277 Amphi 267-83	HELIX
	Deletion 284				
		PSA/G 285	284-287	AS 283,284 Deletion makes assignment to active site likely to be incorrect	TURN
Gly 287					
			288-292	Core 290,292	BETA
Gly 291					
			293-296		TURN
Pro(A) 295					
	Pro(A)/ESA 296				
			297-304	AS 298 Surf 297,300,303 3 turns in a row is unlikely middle is assigned Beta	TURN
	Pro/Pro/Phe 305				
			306-310	Surf 307,310	TURN
	Deletion 311-313				
		G/G/IL 316	314-319	AS 318	BETA

Gly 120			
Gly/Asn/Ser 121 Ser/Pro/Gly 124		320-324	TURN
		325-328 Surf 327	UNCERTAIN could be part of helix that follows
Pro/Pro/GTTS 329			
		329-338 Amphi 325-39 Surf 327 AS 326	HELIX
Deletion 339-340 Gly/PNSA/E 341		339-341 AS 341	LOOP
		342-350 Core 342 AS 344 Pro in 344,45,46	LOOP till 346 BETA till 350
Pro/Pro/PG 351			
	Pro/N/N 356	351-356 357-364 Amphi Surf 363	UNCERTAIN Helix
Gly(N) 365			
Deletion 366-367		365-367	LOOP

CONCLUSIONS

1. The patterns of sequence divergence in proteins provides a method for reliably predicting the tertiary structure of proteins with unknown structure, given an alignment of 10-15 homologous proteins with a range of sequence divergence.

2. Given the desire of evolution to produce unstable proteins, it is unlikely that predictions can be made with much less information.

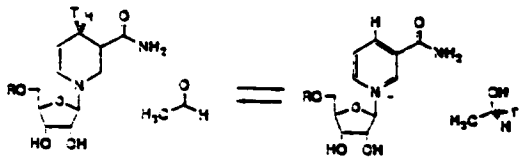
3. Patterns of sequence divergence also help extrapolate a known structure to the structure of a distant homolog.

4. In tetrameric yeast Adh, the position of the quaternary contacts is significantly different from the position of the dimer contacts in horse Adh.

THE DESIRE TO EXTRAPOLATE A STRUCTURE FOR YEAST ADH FROM THE STRUCTURE OF THE DISTANTLY RELATED (30%) HORSE ADH IS MORE THAN ACADEMIC

WE WISH TO ENGINEER THE BEHAVIOR OF YEAST ADH BY CHANGING AMINO ACIDS IN THE PROTEIN. THIS REQUIRES THAT WE HAVE A WORKING MODEL FOR THE STRUCTURE

ONE MEASURE OF THE QUALITY OF OUR STRUCTURAL EXTRAPOLATION IS THE SUCCESS OF ENGINEERING EFFORTS THAT ARE GUIDED BY THE MODEL DERIVED FROM IT.



STEREOSPECIFICTY WITH RESPECT TO COFACTOR IS A SELECTED TRAIT IN MANY ALCOHOL DEHYDROGENASES

1. IN SOME DEHYDROGENASES (e.g. MALATE DEHYDROGENASE), STEREOSPECIFICITY IS HIGHLY CONSERVED

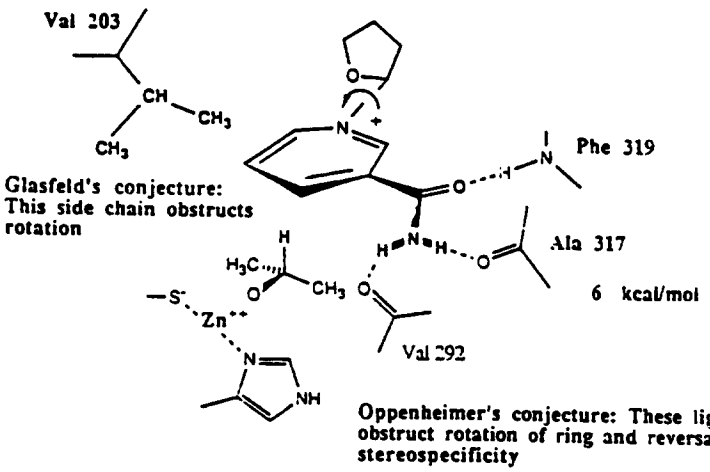
2. IN OTHER DEHYDROGENASES (e.g. ENOYL CoA REDUCTASE, HMG-CoA REDUCTASE, ALCOHOL DEHYDROGENASE), STEREOSPECIFICITY APPARENTLY DIVERGES.

3. THE PATTERN OF DIVERGENCE AND CONSERVATION IS CONSISTENT WITH A FUNCTIONAL MODEL THAT ASSUMES THAT THE REDOX POTENTIAL OF THE NATURAL SUBSTRATE IS THE STEREOCHEMICAL DETERMINANT.

HOWEVER, MOST BIO-ORGANIC CHEMISTS BELIEVE THAT STEREOSPECIFICITY IS A NON-FUNCTIONAL TRAIT REFLECTING ANCIENT HISTORICAL ACCIDENT.

IN THIS VIEW, STEREOSPECIFICITY IS CONSERVED BECAUSE IT IS TIGHTLY COUPLED TO OTHER STRUCTURAL FEATURES THAT SERVE IMPORTANT FUNCTIONS

Oppenheimer, et. al. Mechanisms of Enzymatic Reactions:Stereochemistry P.A. Frey, ed. Elsevier, 15-28 (1986)



Glasfeld's conjecture: This side chain obstructs rotation

Oppenheimer's conjecture: These ligands obstruct rotation of ring and reversal of stereospecificity

IF WE CAN REDUCE STEREOSPECIFICITY BY REDUCING THE SIZE OF RESIDUE AT POSITION 182 IN YEAST Adh (an Ile corresponding to Val 203 in the horse enzyme), THEN WE:

(a) Confirm our extrapolation of structure

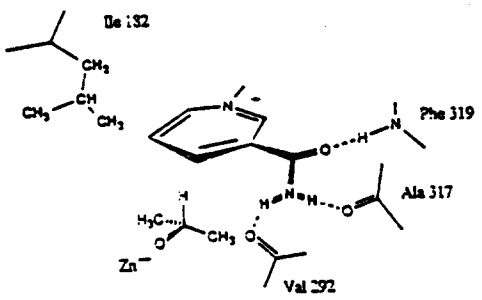
(b) Show that it is incorrect to argue that the side chains of critical amino acids coordinating the active site zinc obstruct the syn conformer of NADH

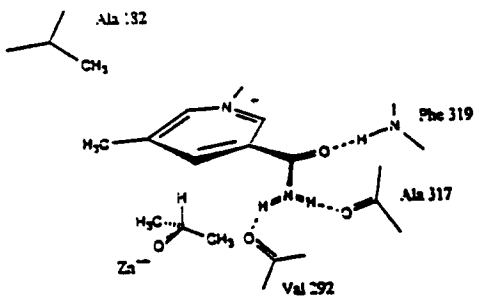
OF COURSE, STEREOSPECIFICITY WILL NOT BE LOST COMPLETELY; THE GROUPS FORMING HYDROGEN BONDS TO THE CARBOXAMIDE WILL REMAIN

Fersht and his coworkers have estimated that each hydrogen bond of this type is worth ca. 2 kcal/mol

Enzyme	$K_m NAD^+$	$K_m NADH$	$k_{cat}ox.$*	$k_{cat}red.$**
L182A	1.49±.14mM	720±120 µM	169±16 units	570±130 units
L182V	223±11 µM	297±63 µM	502±25 units	1500±320 units
WT	191±12 µM	130±12 µM	411±25 units	845±70 units

Table 3.3: Kinetic data for YEDH mutants and wild type enzyme. Values were calculated by plotting initial rate data on Lineweaver-Burk plots. Error was calculated from the standard deviations of slope and intercept. *k_{cat}ox. refers to rate of ethanol oxidation at saturating cofactor concentrations when [ethanol] = 330 mM. **k_{cat}red. refers to the rate of acetaldehyde reduction at saturating cofactor concentrations when [acetaldehyde] = 10 mM.





5-METHYLNICOTINAMIDE ADENINE DINUCLEOTIDE IS NOT A SUBSTRATE FOR NORMAL YEAST ALCOHOL DEHYDROGENASE

IT IS A SUBSTRATE FOR THE MUTANT

ELMAR WEINHOLD

CONCLUSIONS

1. Reducing the side chain at position 182 creates space that can accomodate either the syn conformer of NADH (reversing stereospecificity), or a methyl group of 5-methyl-NAD

2. The stereospecificity of the mutant is almost exactly that expected from hydrogen bonding alone.

3. The ligands to Zn do not prevent a reversal in stereospecificity; when stereospecificity is conserved, it cannot be attributed to a functional constraint on drift involving these ligands.

4. As alternative functional constraints are removed one by one, it becomes more likely that the conservation of dehydrogenase stereospecificity observed is due to direct selection for a functional role played by stereospecificity itself.

5. The structural model for yeast Adh appears to be a good working model.

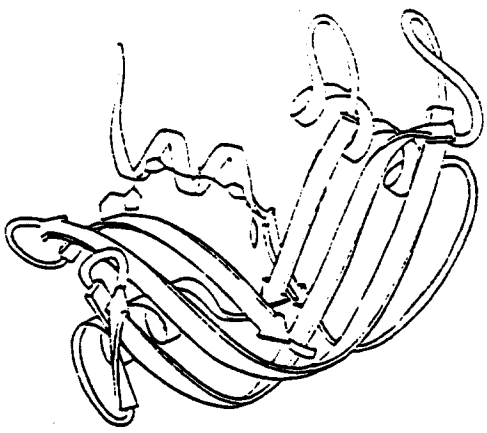
BUT WHAT ABOUT CANCER?

IN PROTEINS WHERE STRUCTURE IS FULLY KNOWN,
EVOLUTIONARY INFORMATION CAN BE VERY VALUABLE
IN DIRECTING MUTAGENESIS EXPERIMENTS

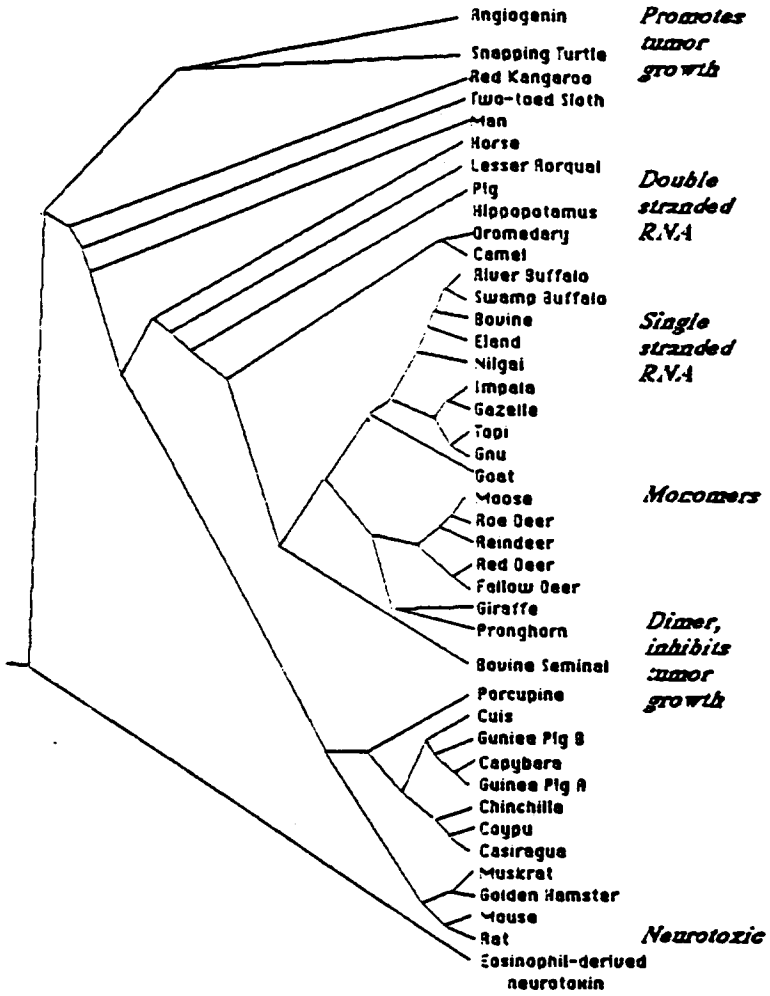
OFTEN, VERY COMPLEX CHEMICAL AND BIOLOGICAL
BEHAVIORS CAN BE INVESTIGATED

RIBONUCLEASE (RNase) IS AN EXCELLENT MODEL

9 CRYSTAL STRUCTURES
OVER 60 SEQUENCES ARE AVAILABLE



Ribonuclease



RNase POTENTIATES THE CYTOSTATIC EFFECT OF ACTINOMYCIN D
Sartorelli Nature (1964) 203 377-78

BOVINE SEMINAL RNase HAS STRONG ANTI-TUMOR ACTIVITY IN VIVO AND IN VITRO
DIMER ACTING ON DOUBLE STRAND NUCLEIC ACIDS
Matousek Experientia (1973) 29 858-859
Vescia et al. Cancer Res. (1980) 40 3740-44

ANGIOGENIN, SECRETED BY TUMORS TO ATTRACT BLOOD VESSELS, IS AN RNase HOMOLOG
Strydom et al. Biochem. (1985) 24 5486-94

EOSINOPHILE DERIVED NEUROTOXIN: AN RNase HOMOLOG
Gleich et al. Proc.Nat.Acad.Sci (1986) 83 3146-50

EXTRACELLULAR RNases AND RNase INHIBITORS WITH BIOLOGICAL ACTIVITY IMPLIES THE EXISTENCE OF EXTRACELLULAR RNA
Benner FEBS Lett. (1988) 233 225-28

National Institutes of Health (1984)
"If RNase had one tenth the potential as implied by Dr. Benner, dozens of scientists would already be busily investigating. This hasn't happened!"

THE BIOLOGICAL ACTIVITY OF TUMOR ANGIOGENESIS FACTOR IS DESTROYED BY RNase
Folkman et al. J. Exp. Med. (1971) 133 275-88

MACROMOLECULAR RNA IS TRANSFERRED BETWEEN CELLS
Kolodny Cell Comm. (1974) Wiley, 97-111

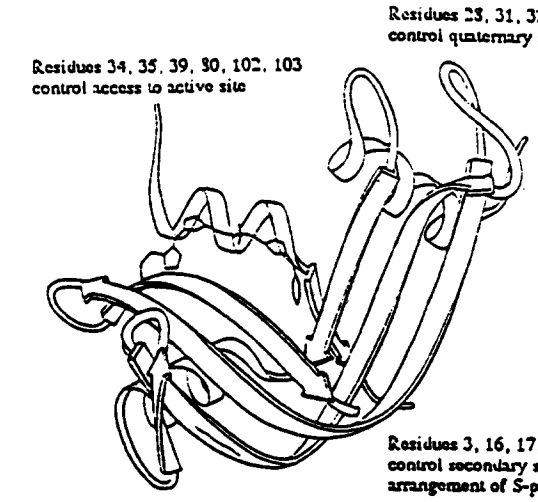
ANGIOTROPIN IS A RIBONUCLEOPROTEIN CONTAINING AN RNA MOLECULE 43 BASES IN LENGTH
Wissler et al. Prot.Biol.Fluids (1986) 34 525-36

DIMERIC RNases HAVE MORE ANTITUMOR ACTIVITY THAN MONOMERIC RNases.

DIMERIC RNases HYDROLYZE DOUBLE STRANDED RNA BETTER THAN MONOMERIC RNases

THIS PROVIDES A SOLID RATIONALE FOR THE ANTITUMOR ACTIVITY OF DIMERIC RNases

EVOLUTION HAS ENGINEERED DIMERIC RNases FROM MONOMERIC RNases BY CHANGING 23 AMINO ACIDS

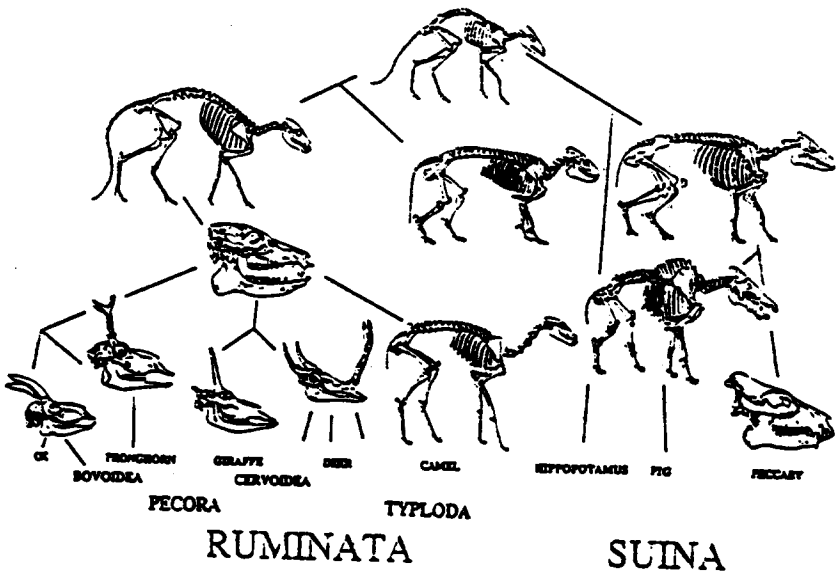


MUTANT 1
Ser16-Gly Thr17-Asp Ala19-Pro Ala20-Ser
the "hinge" region incorporates residues in dimer

MUTANT 2
Gln28-Leu
Hydrophobic contact as in dimer

PROTEIN IS A MONOMER
THUS, THE DISULFIDE RESIDUES SEEM TO BE NECESSARY FOR DIMER FORMATION

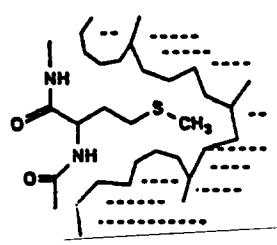
Jerry McGeehan
Marianthi Kalamaraki
Germaine Seewer



PARSIMONY

37	38	39	40	41	42	43	
Arg	Asn	Met	Thr	Lys	Asp	Arg	Protobovoid *
Arg	Asp	Met	Thr	Lys	Asp	Arg	Eland
Arg	Asn	Leu	Thr	Lys	Asp	Arg	Ox
Arg	Asn	Met	Thr	Ser	Asp	Arg	River Buffalo
Arg	Ser	Met	Thr	Ser	Asp	Arg	Swamp Buffalo

M39L
D38N
K41S
N38S

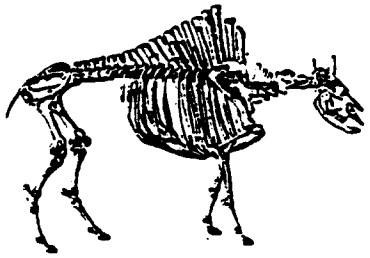


PARSIMONY MAKES AN UNUSUAL PREDICTION

PROTOBOVOID RNase HAS Met IN THIS POSITION WITHOUT A COMPENSATING CHANGE IN ANOTHER POSITION NEARBY

	K_m (μM)	k_{cat} (sec^{-1})
Modern RNase	164 ±11	1925 ±90
Ala 19 -> Ser	195 ±30	2160 ±180
Leu 35 -> Met	164 ±36	2270 ±390
Ala 19 -> Ser Leu 35 -> Met (Protobovoid)	177 ±33	2200 ±570

PARSIMONY HAS PROVIDED FULLY ACTIVE PROTEINS; THE BEHAVIOR OF THESE ANCIENT RNases SUGGESTS A PATTERN OF NEUTRAL DRIFT



THE HOMOLOGY OF RNases, ANGIOGENINS, NEUROTOXINS, AND TUMOR-GROWTH INHIBITORS SUGGESTS THAT EVOLUTION HAS USED THE RNase STRUCTURE REPEATEDLY TO SOLVE PROBELMS PRESENTED BY A CHANGING ENVIRONMENT

DIGESTIVE RNase AROSE TO ADDRESS PROBLEMS PRESENTED BY A RUMINANT DIGESTION

BUT RNase CAN NOW BE TRACED TO THE ORIGIN OF MULTICELLULAR LIFE, 700 MILLION YEARS AGO

RECONSTRUCTION OF ANCIENT LIFE FORMS BY APPLICATION OF THE "RULE OF PARSIMONY" CAN TAKE US STILL FARTHER BACK IN TIME

HERE AGAIN, WE COMPARE BIOCHEMICAL TRAITS IN MODERN ORGANISMS, IDENTIFY THOSE THAT ARE THE PRODUCTS OF ADAPTIVE EVOLUTION AND THOSE THAT ARE THE PRODUCTS OF NEUTRAL DRIFT.

WHAT REMAINS ARE TRAITS THAT ARE VESTIGES OF EARLIER FORMS OF LIFE

THE EVOLUTION OF LIFE ON EARTH MOST PROBABLY OCCURRED IN 3 EPISODES

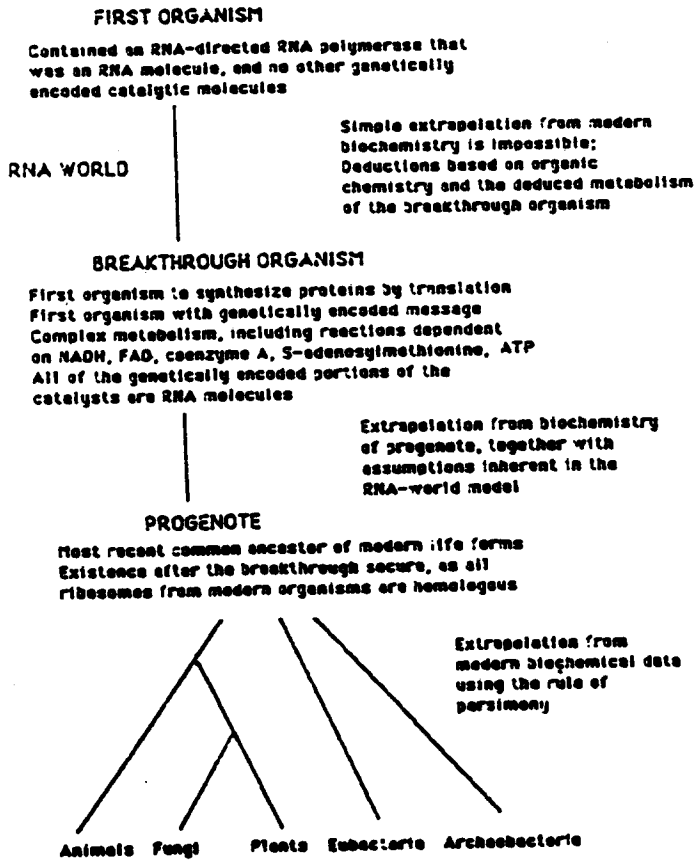
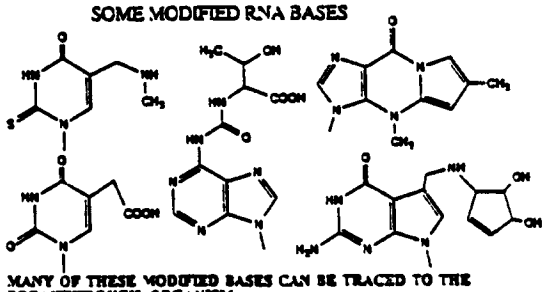


Figure 5: An outline for the origin of modern life.

SOME MODIFIED RNA BASES



MANY OF THESE MODIFIED BASES CAN BE TRACED TO THE BREAKTHROUGH ORGANISM

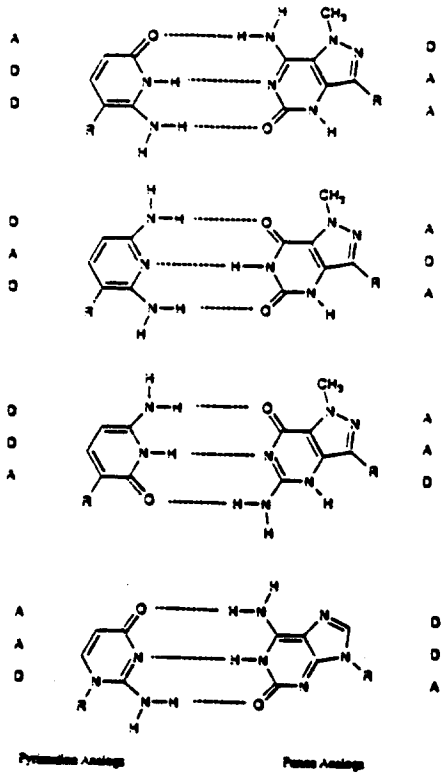
THEY APPEAR TO BE LATE PRODUCTS OF THE RNA WORLD

IT SEEMS ALMOST AS IF THE RIBOORGANISM WAS TRYING TO OBTAIN A RANGE OF FUNCTIONAL GROUPS CHARACTERISTIC OF PROTEINS, BUT ON AN RNA BACKBONE.

HOWEVER, THE BASES BEARING FUNCTIONAL GROUPS HAVE HYDROGEN BONDING SCHEMES JUST LIKE NORMAL BASES.

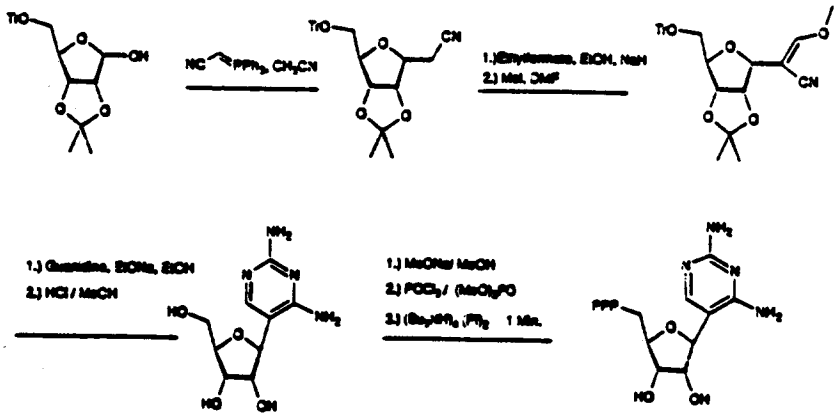
THIS MEANS THAT THEY CANNOT BE INCORPORATED INTO A REPLICATING GENE; THEY MUST BE MADE POST-TRANSCRIPTIONALLY, WHICH IS EXPENSIVE

Alternative Base Pairing Schemes



Pyrimidine Analogs Purine Analogs

Synthesis of KTP

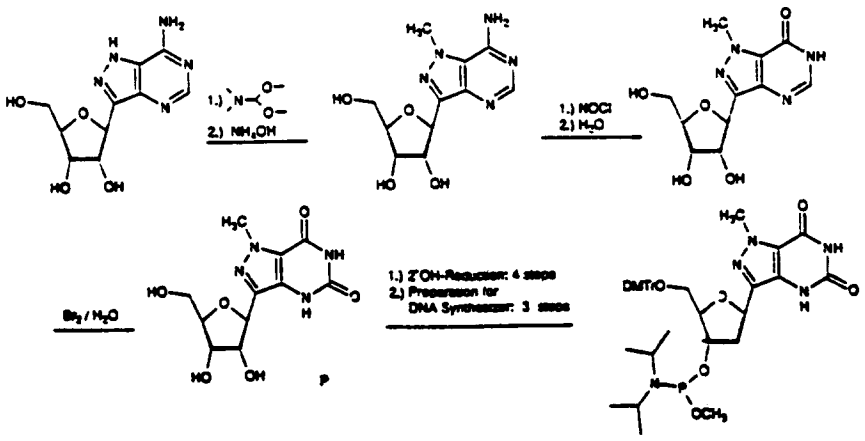


Moffatt et al. JACS 92, 4992 (1970)

Wittenburg et al. JOC 42, 711 (1977)

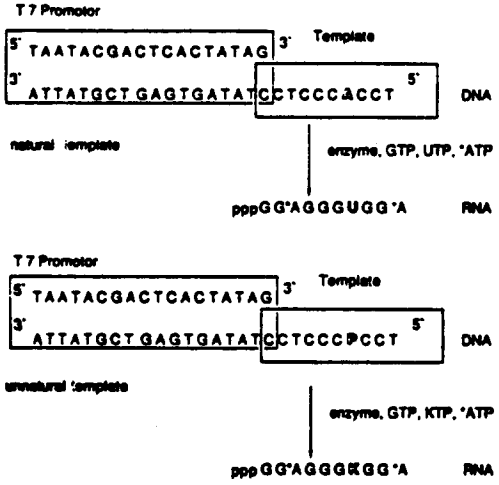
Ludwig et al. Acta. Biochim. Biophys. Acad.Sci. Hung. 8 , 131 (1981)

Synthesis of deoxy - Methyloxoformycin B dP

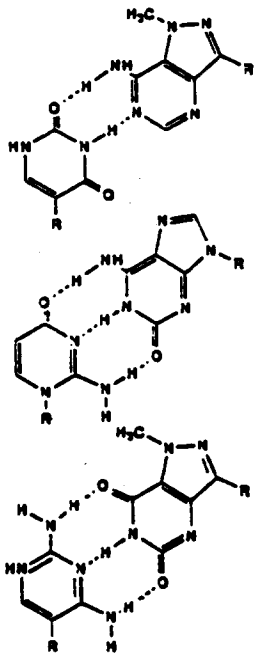


JACS 97, 5896 (1975)
Nucleosides, Nucleotides 3, 223 (1984)
J. Het. Chem. 21, 1885 (1984)

Transcription with T 7 RNA Polymerase



ENZYMATIC INCORPORATION OF UNNATURAL BASES



ACCEPTED BY RNA POLYMERASE: DEMONSTRATES THAT ENZYME WILL ACCEPT 2 C-GLYCOSIDES, AND THE FORMYCIN RING SKELETON WITH A 7 METHYL GROUP

INCORPORATED BY DNA POLYMERASE AND RNA POLYMERASE: DEMONSTRATES THAT THESE ENZYMES CAN ACCEPT ALTERED HYDROGEN BONDING SCHEMES

SYNTHESIZED; NOT YET INCORPORATED

THE DESIGN OF POLYPEPTIDES THAT FOLD IN SOLUTION AND CATALYZE REACTIONS

PREDICTION OF TERTIARY STRUCTURE OF NATURAL PROTEINS FROM SEQUENCE DATA

MANIPULATION OF THE STRUCTURE OF NATURAL PROTEINS TO ALTER THEIR BEHAVIORS

UNDERSTANDING THE STRUCTURAL BASIS FOR THE BIOLOGICAL ACTIVITY OF TUMOR GROWTH PROMOTERS AND INHIBITORS

RECONSTRUCTION OF EVENTS OCCURRING EARLY IN THE EVOLUTION OF LIFE

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PROTEIN FOLDING
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ALCOHOL DEHYDROGENASE
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