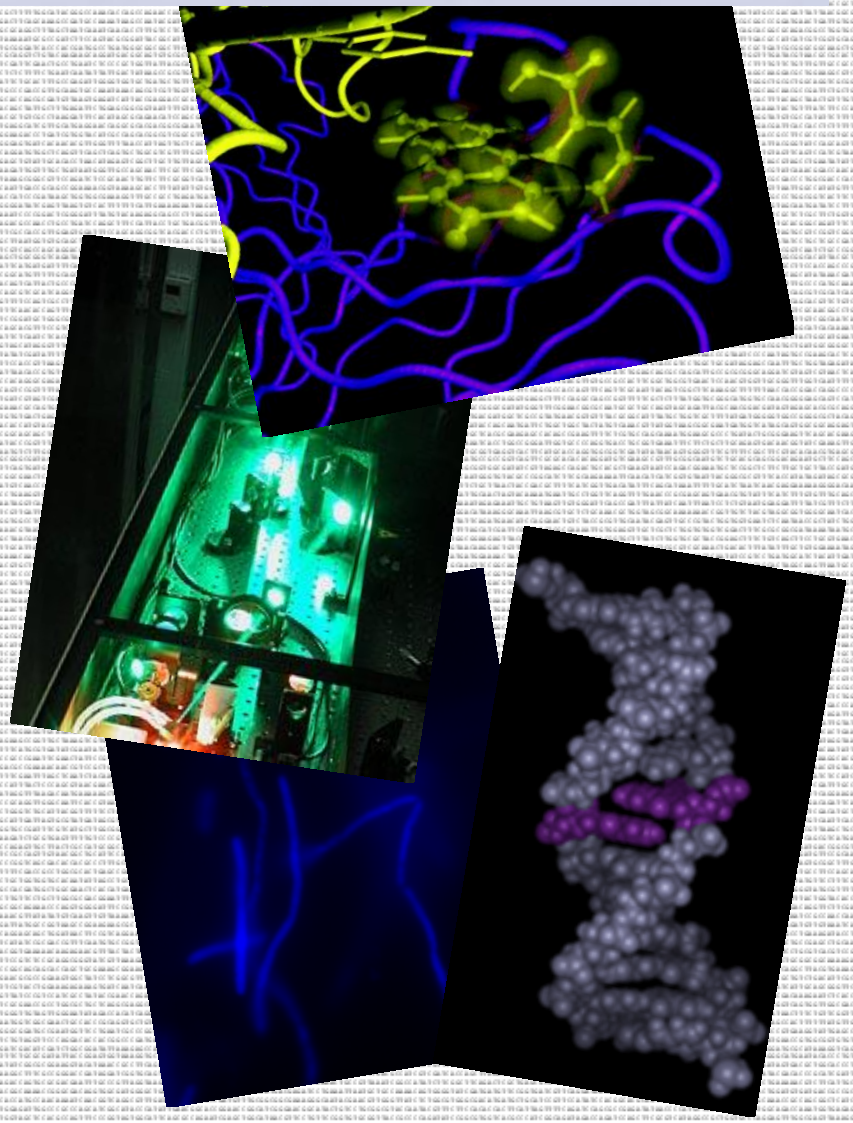
Artificial DNA in Living Cells

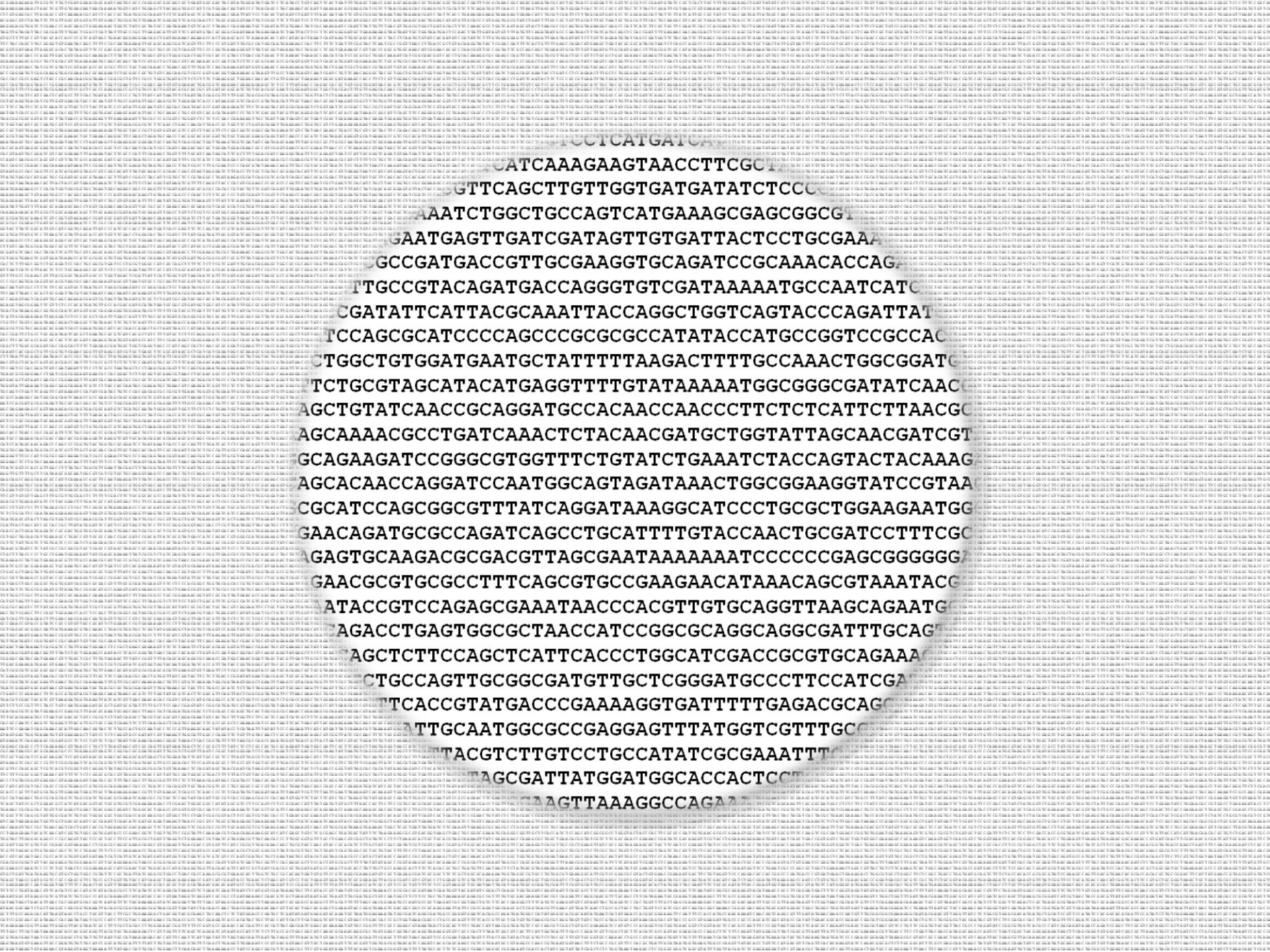
Floyd E. Romesberg, Ph.D.
National Academy of Sciences Forum on Synthetic Biology
19 September 2014
Washington, DC

Chemical Biology and Biophysics

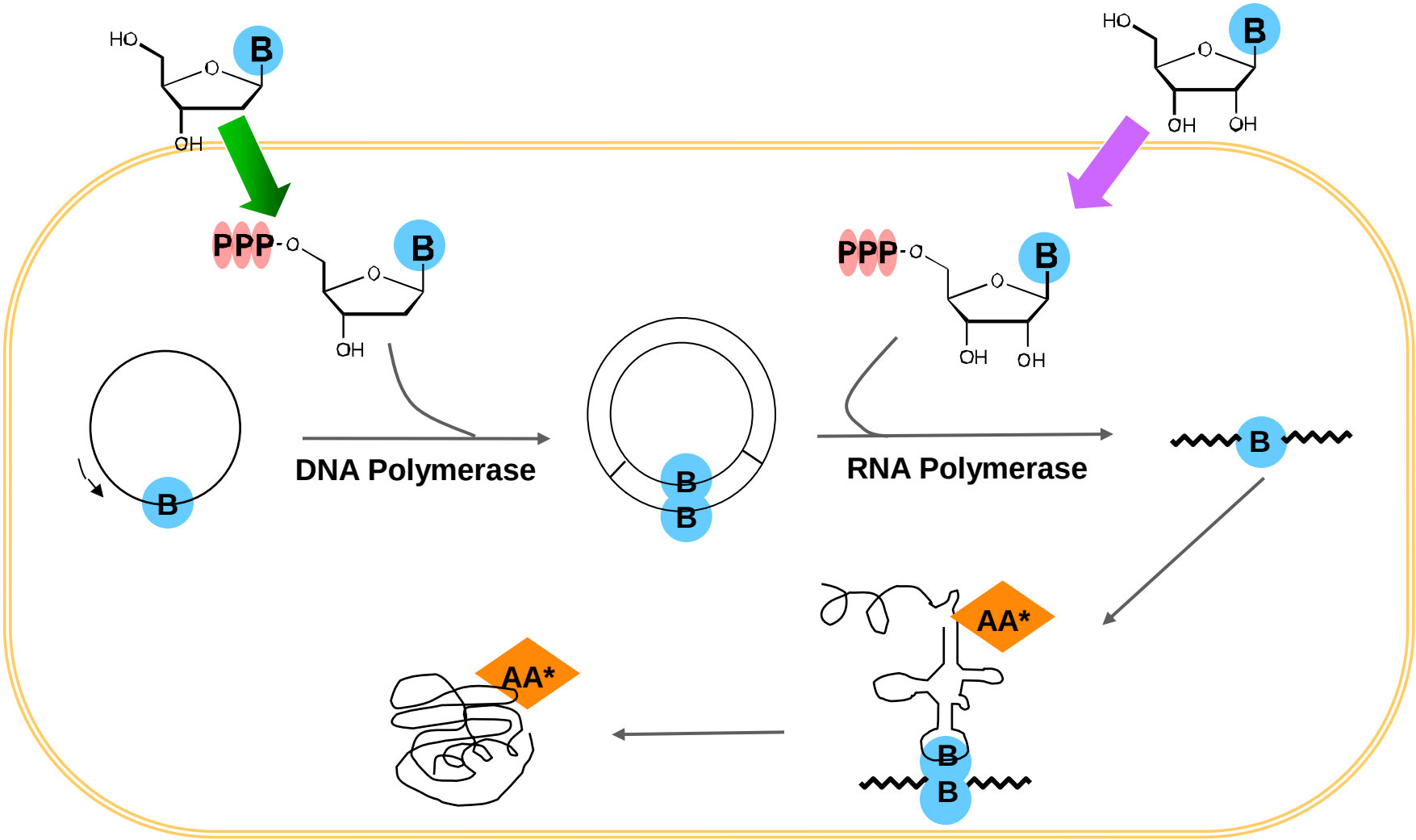
ROMESBERG LAB

- **Efforts to expand the genetic alphabet by unnatural base pair design and polymerase evolution**
- **Novel evolution-inspired approaches to antibiotic discovery**
- **Biophysical characterization of how protein dynamics are evolved for biological function**



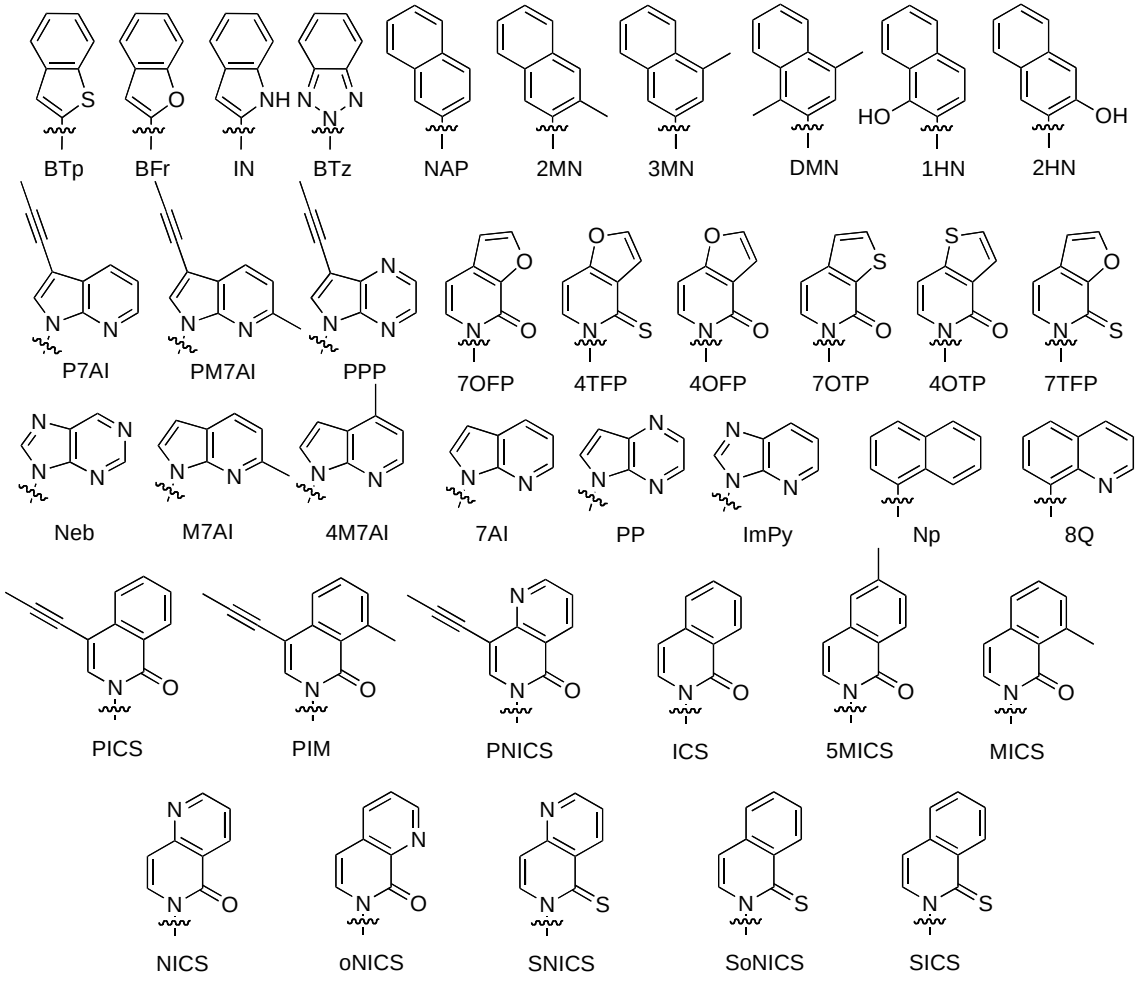


Expansion of the Genetic Alphabet/Code



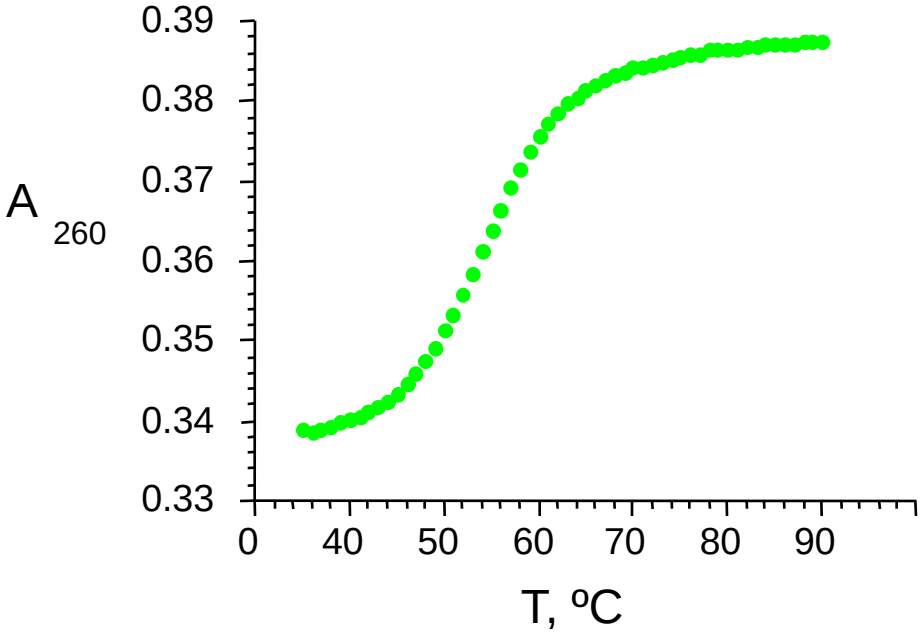
Seminal work of Steve Benner (FAME) Peter Schultz (TSRI)

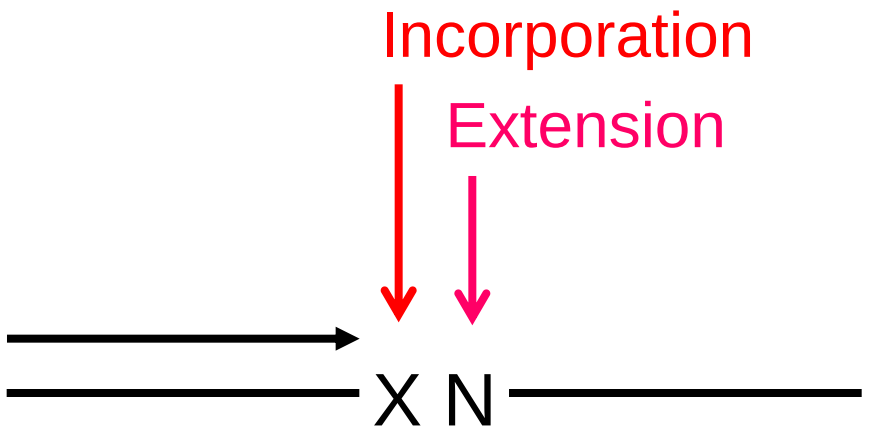
Predominantly Hydrophobic Nucleotides
(sugar & phosphate not shown)



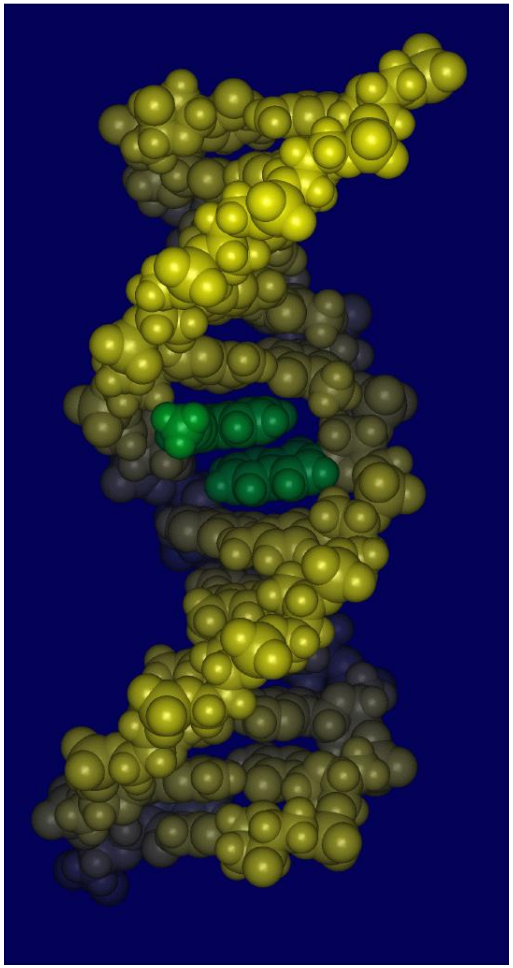
Assays: Thermostability and Kinetics

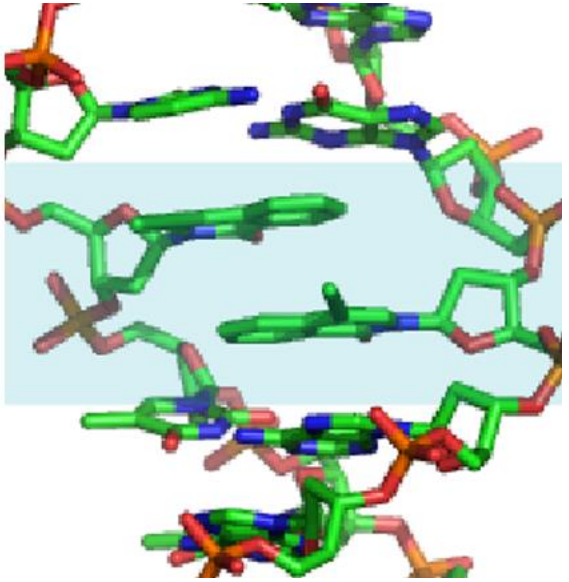
5’-GCGTACXCATGCG-3’
3’-CGCATGYGTACGC-5’

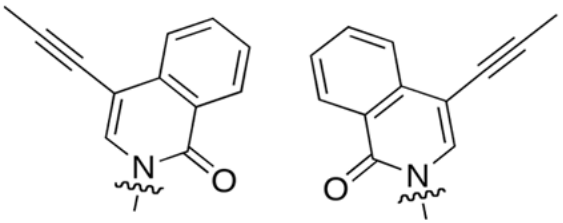




Structure of a First Generation Hydrophobic UBP



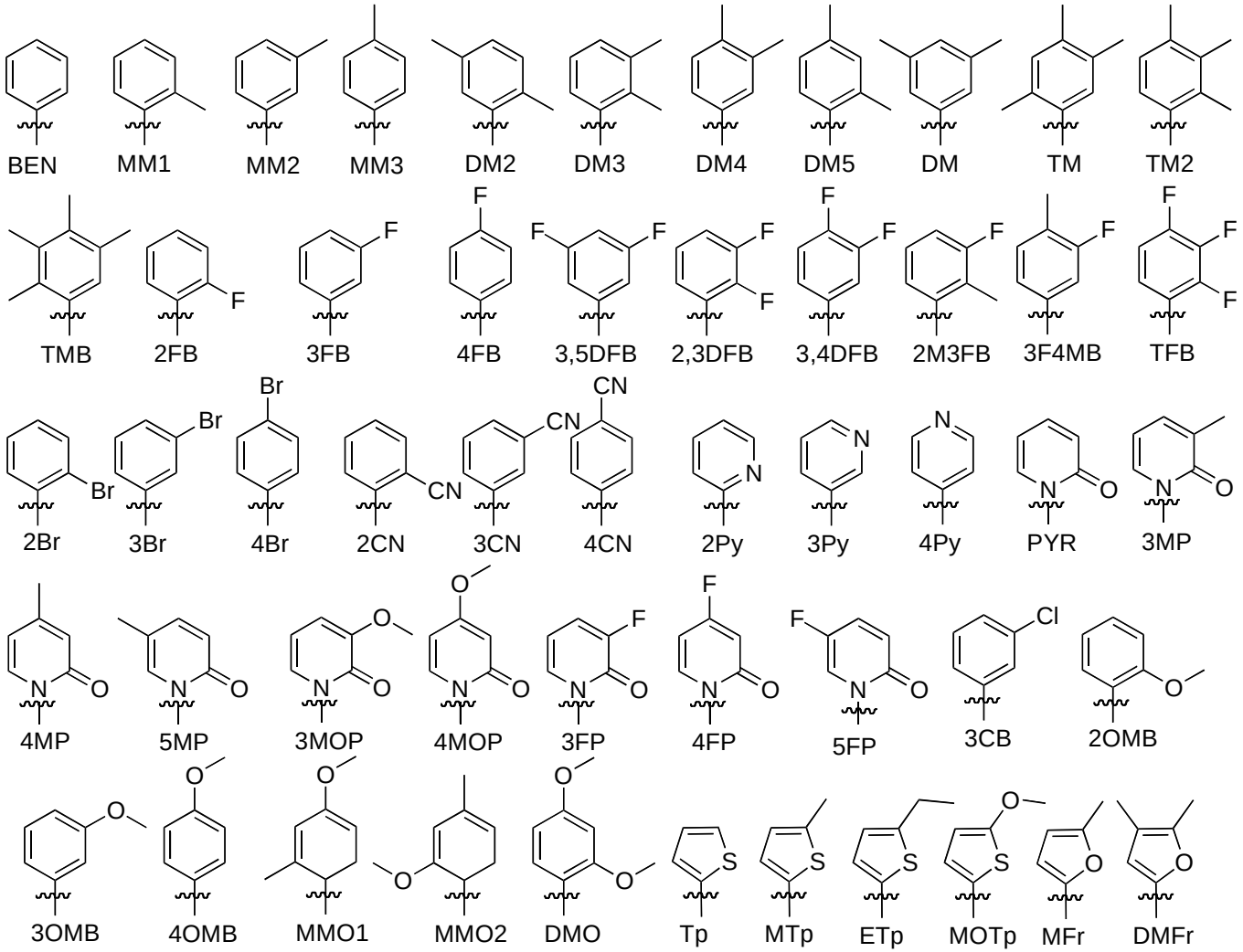




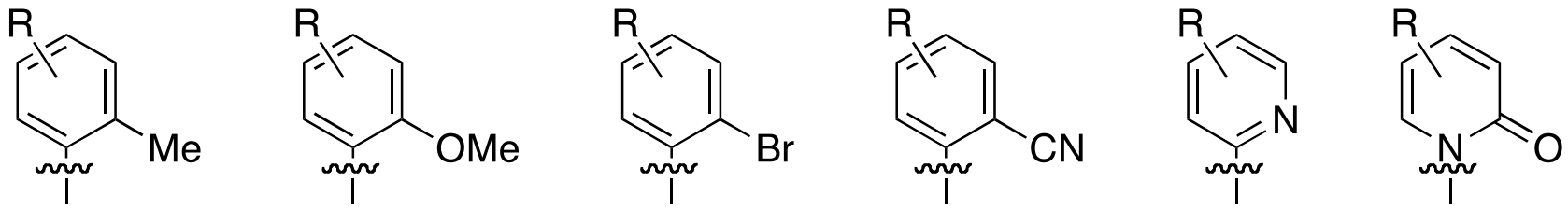
dPICS:dPICS

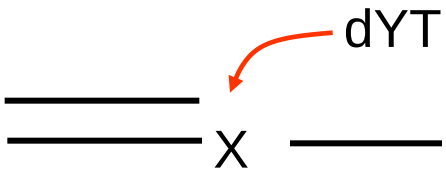
With D.Wemmer (UC Berkeley) and P. Schultz (TSRI)

Second Generation Hydrophobic Nucleotides

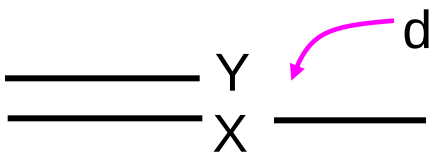


SAR Issue





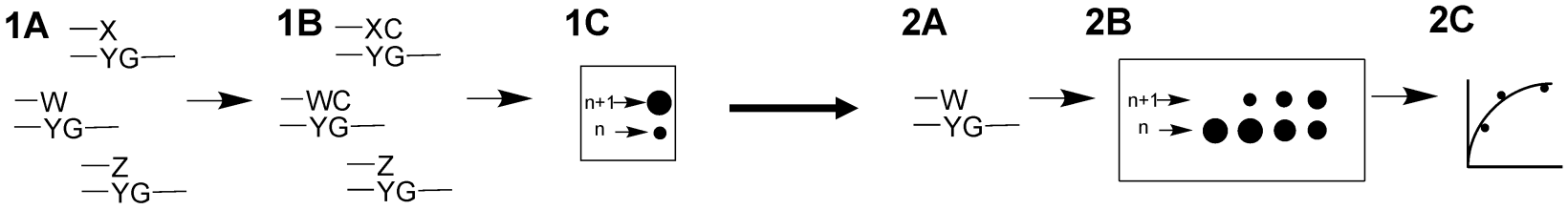
Efficient dYTP Insertion: dYTP–minor groove hydrophobic group
template dX–minor groove hydrophobic group



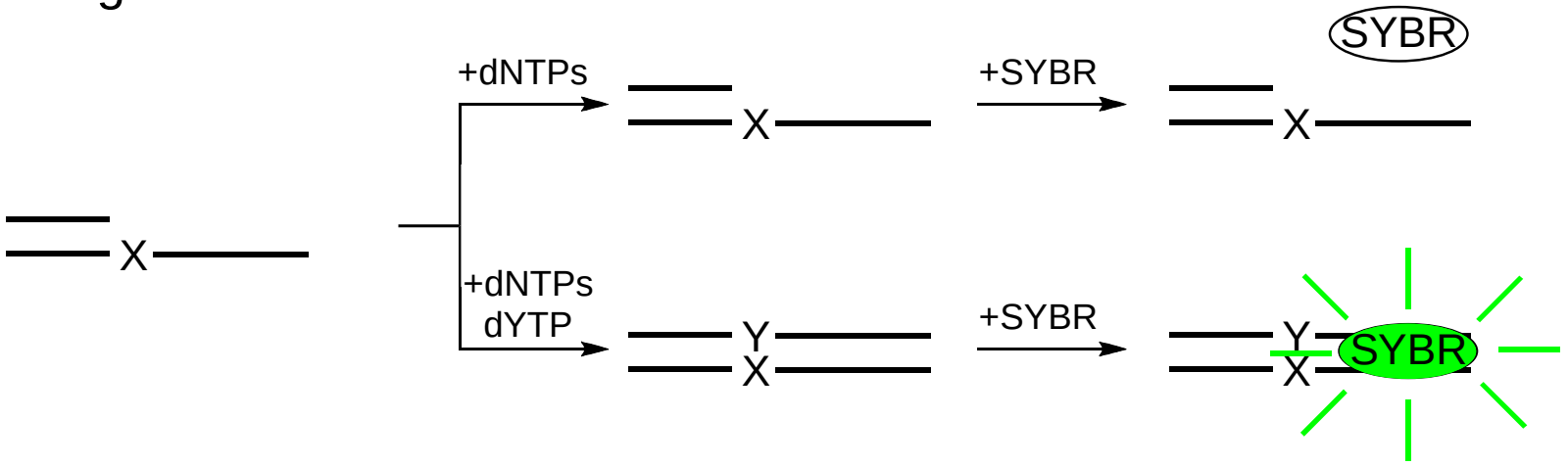
Efficient extension: Primer terminus dY–minor groove hydrogen-bond acceptor
Template dX–minor groove hydrophobic group

Two Screens of 3600 Candidate UBPs

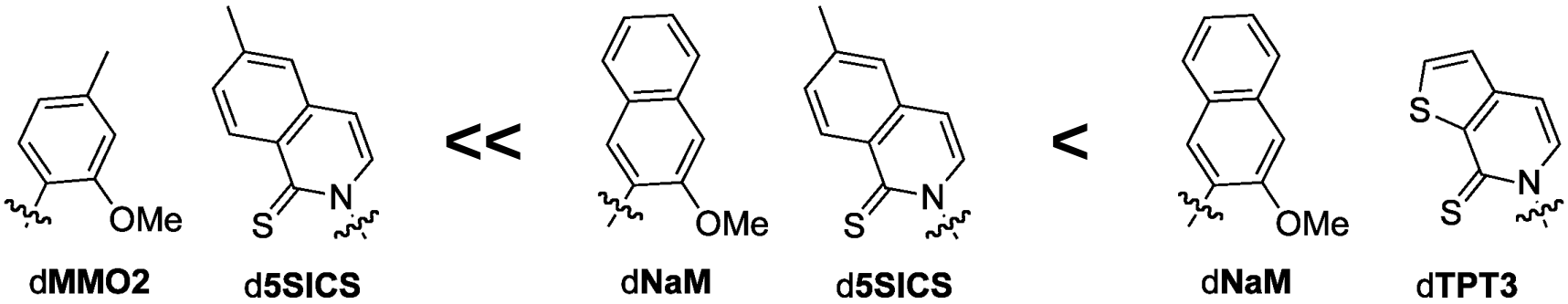
Extension Screen



Full Length Screen

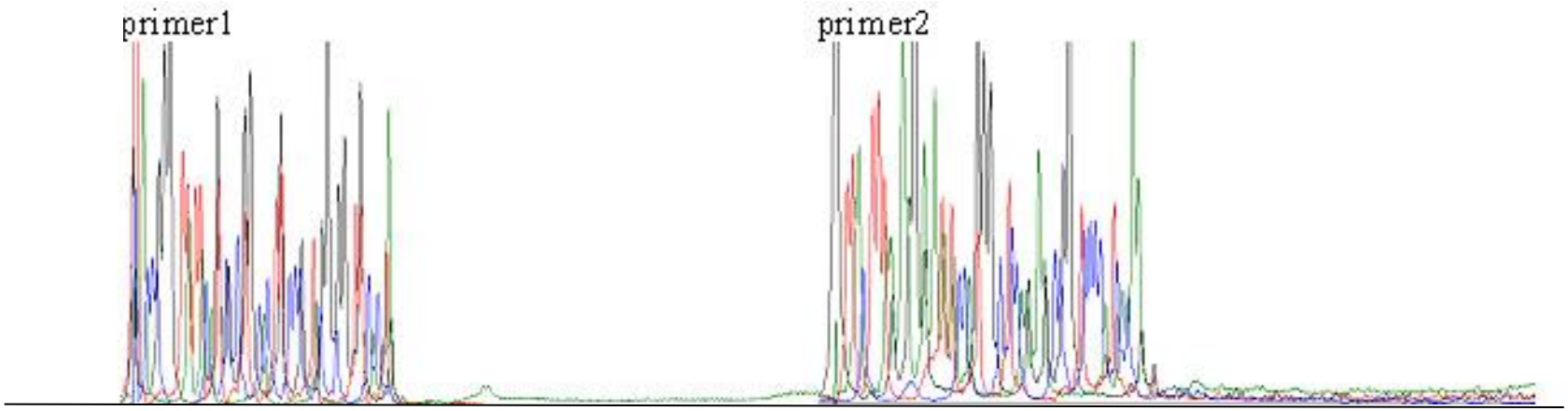


A Family of UBPs that are Efficiently Replicated and Transcribed *in Vitro*

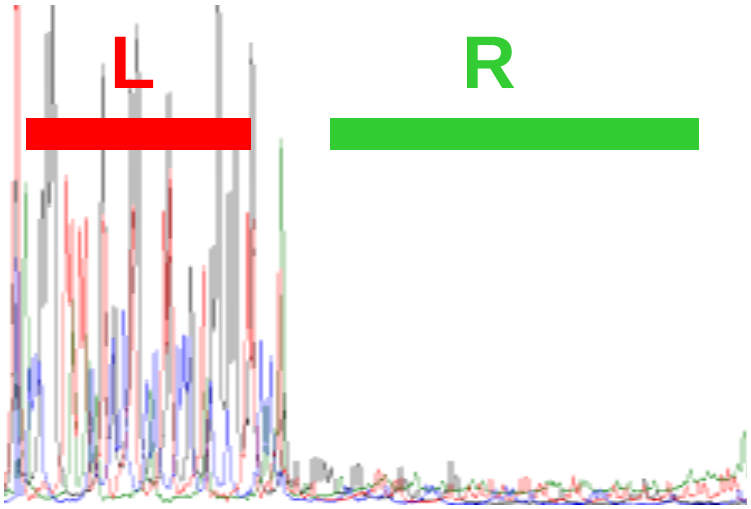


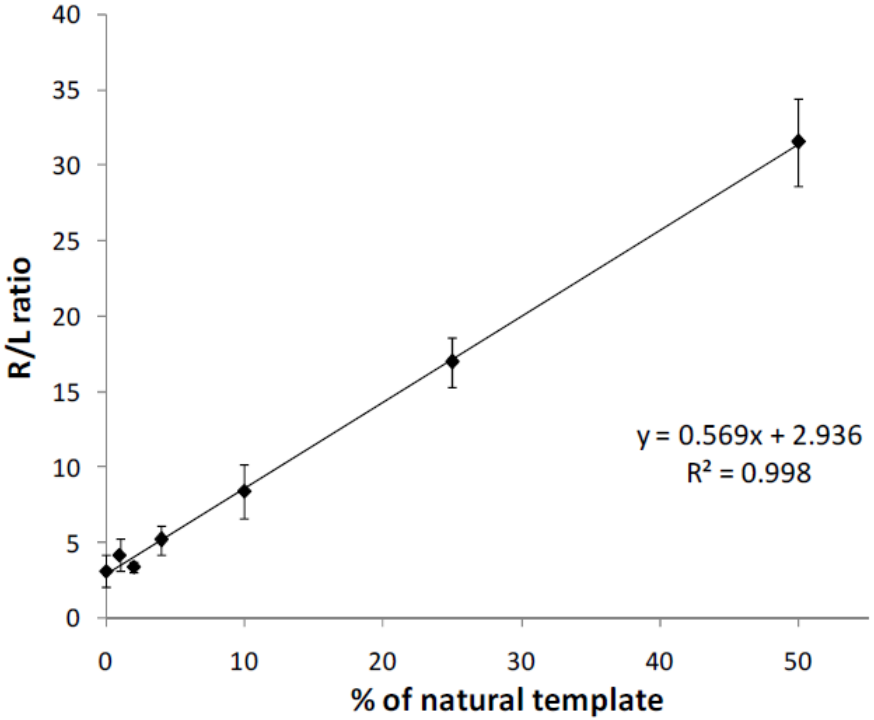
dMMO2 **d5SICS** **dNaM** **d5SICS** **dNaM** **dTPT3**

Sequencing Analysis of Replication Fidelity



Calibration Curve for d**5SICS**-d**NaM** Replication

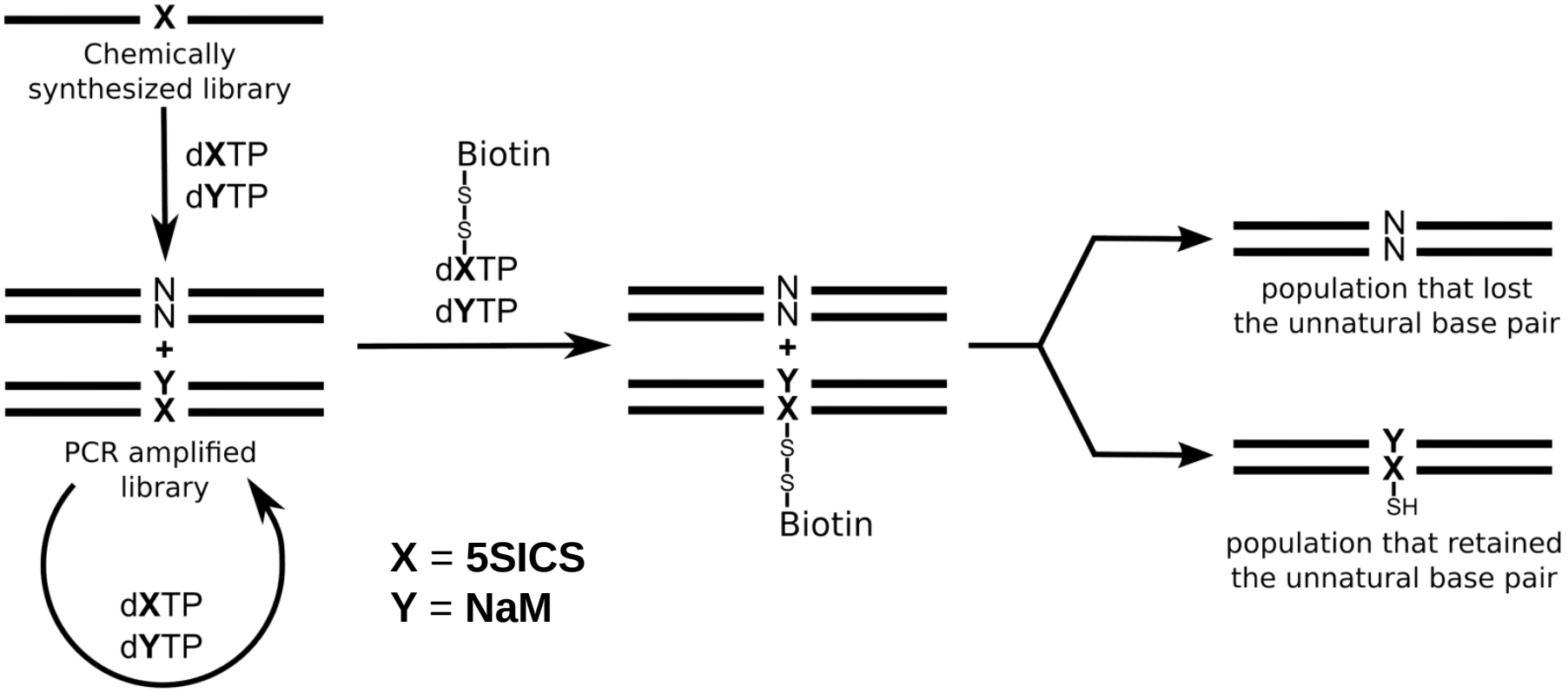




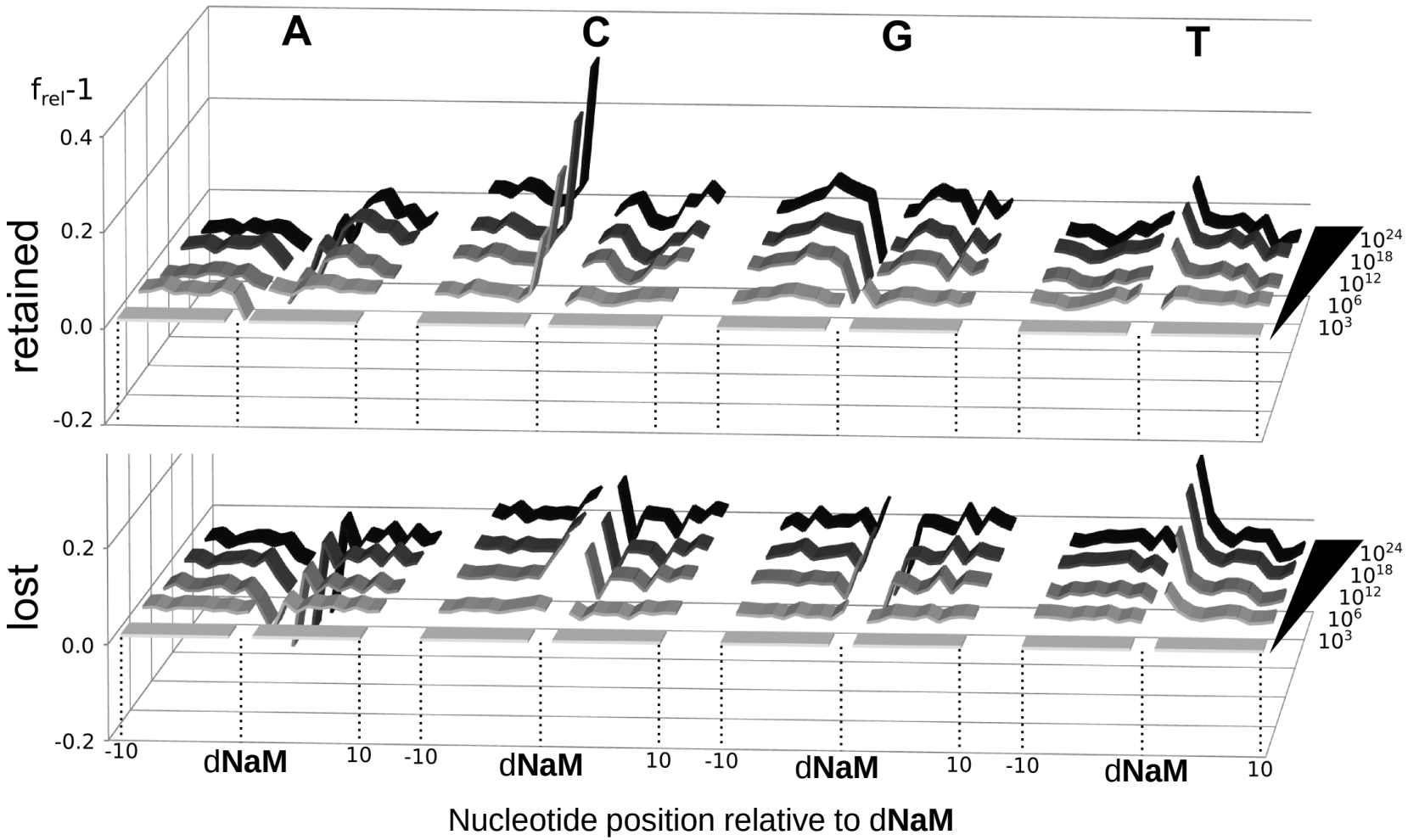
One*Taq* PCR with d**5SICS**-d**NaM**

DNA	Amplification	Efficiency (%)	Fidelity (%)
ACT β GTG	2.5×10^{12}	97	99.945 ± 0.016
GTC β GGT	1.5×10^{12}	95	99.874 ± 0.035
AGC β CGT	3.5×10^{12}	96	99.930 ± 0.003
CCG β GAA	8.1×10^{12}	>99	99.664 ± 0.037
GTA β TGT	3.1×10^{12}	95	99.987 ± 0.021
AGA β AGT	8.5×10^{12}	>99	>99.98
CCT β AAA	8.4×10^{12}	>99	99.866 ± 0.014
GGT β TCC	2.6×10^{12}	94	99.958 ± 0.012
ACT β β GTG	3.8×10^{12}	96	≈ 99.5
ACT β A β GTG	2.7×10^{12}	94	≈ 99.5
ACT β GTGACT β GTG	2.0×10^{12}	93	99.47
NNN β NNN	4.8×10^{12}	97	99.925 ± 0.008

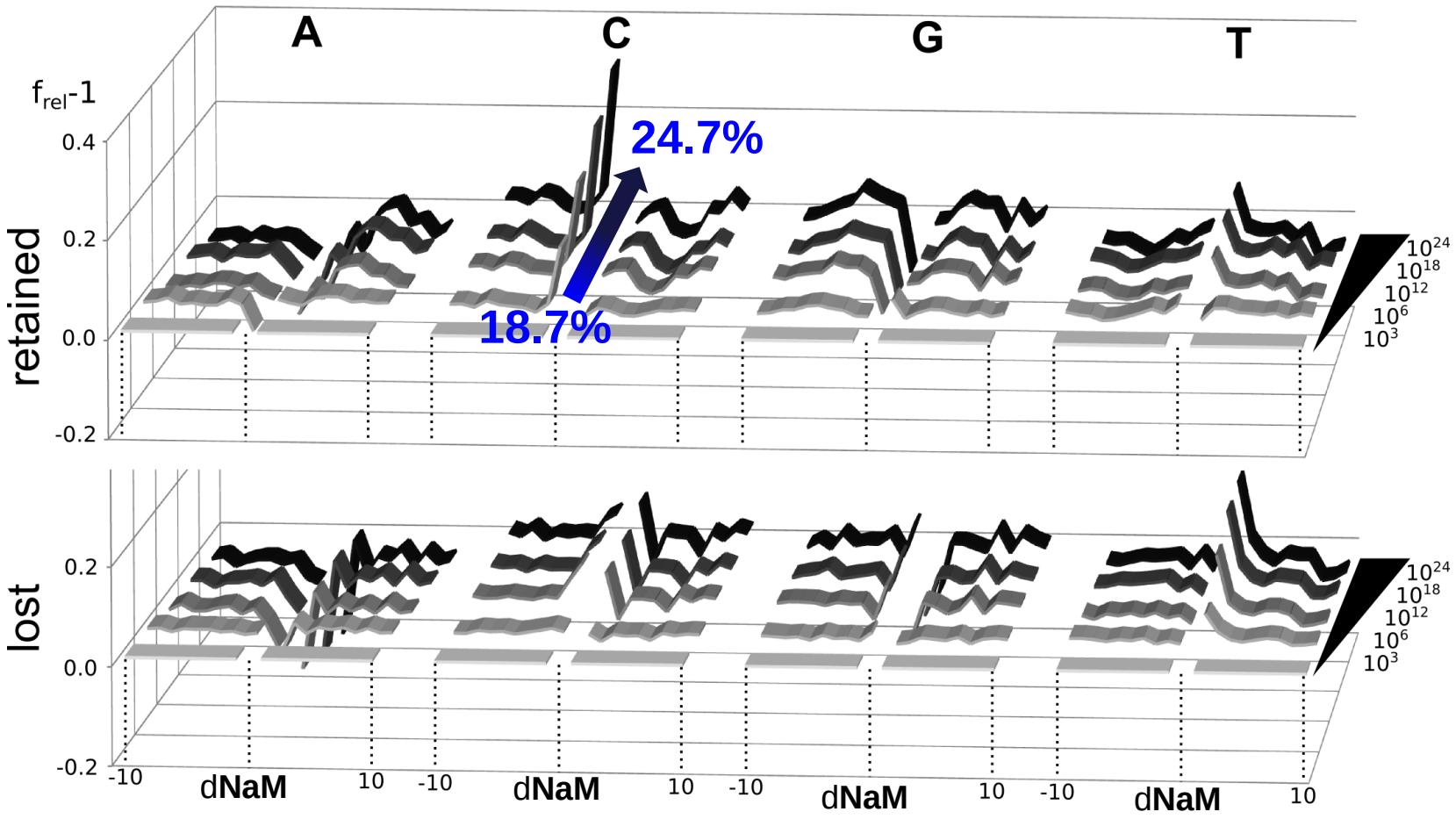
PCR Selection



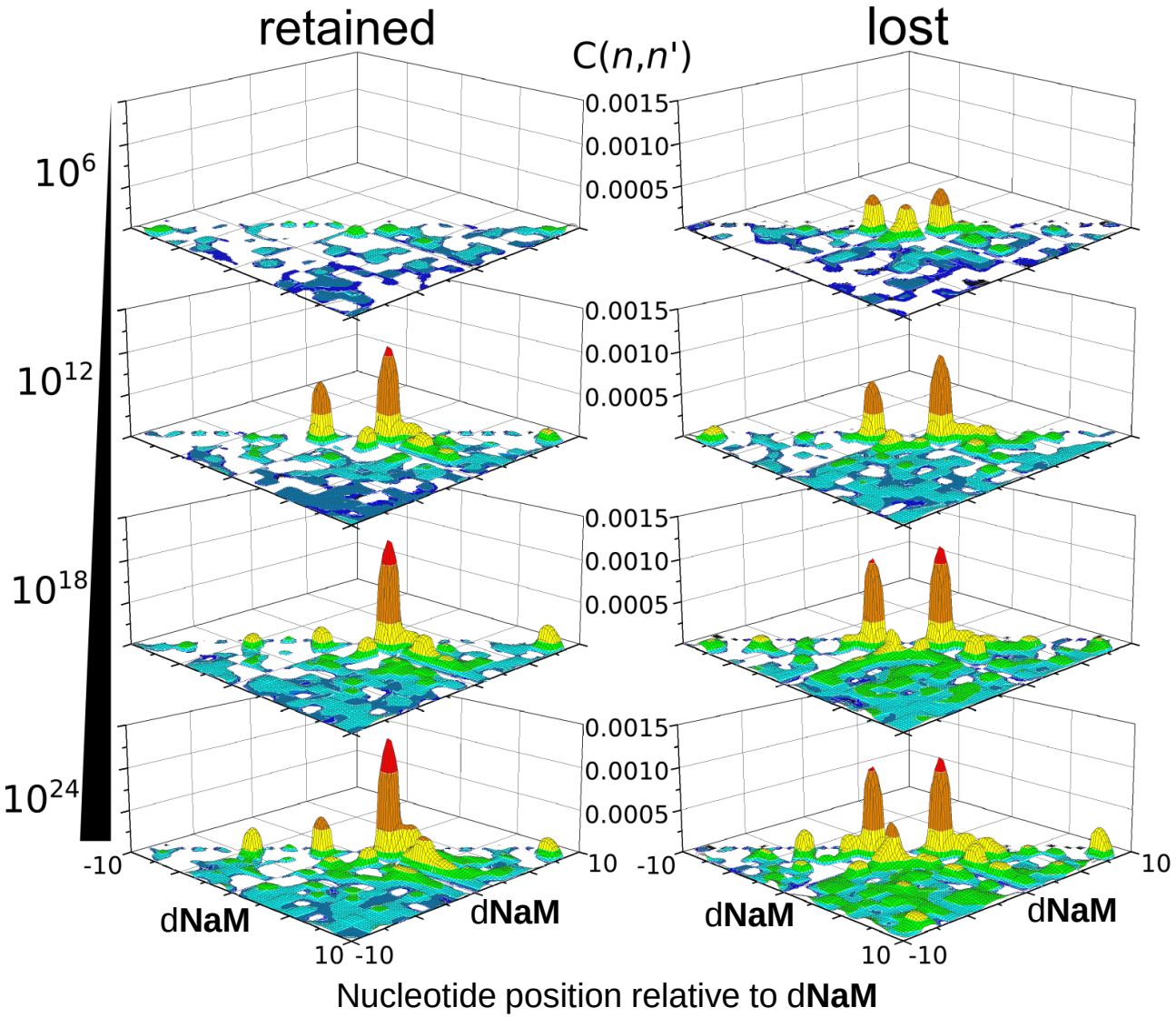
Single Nucleotide Frequency



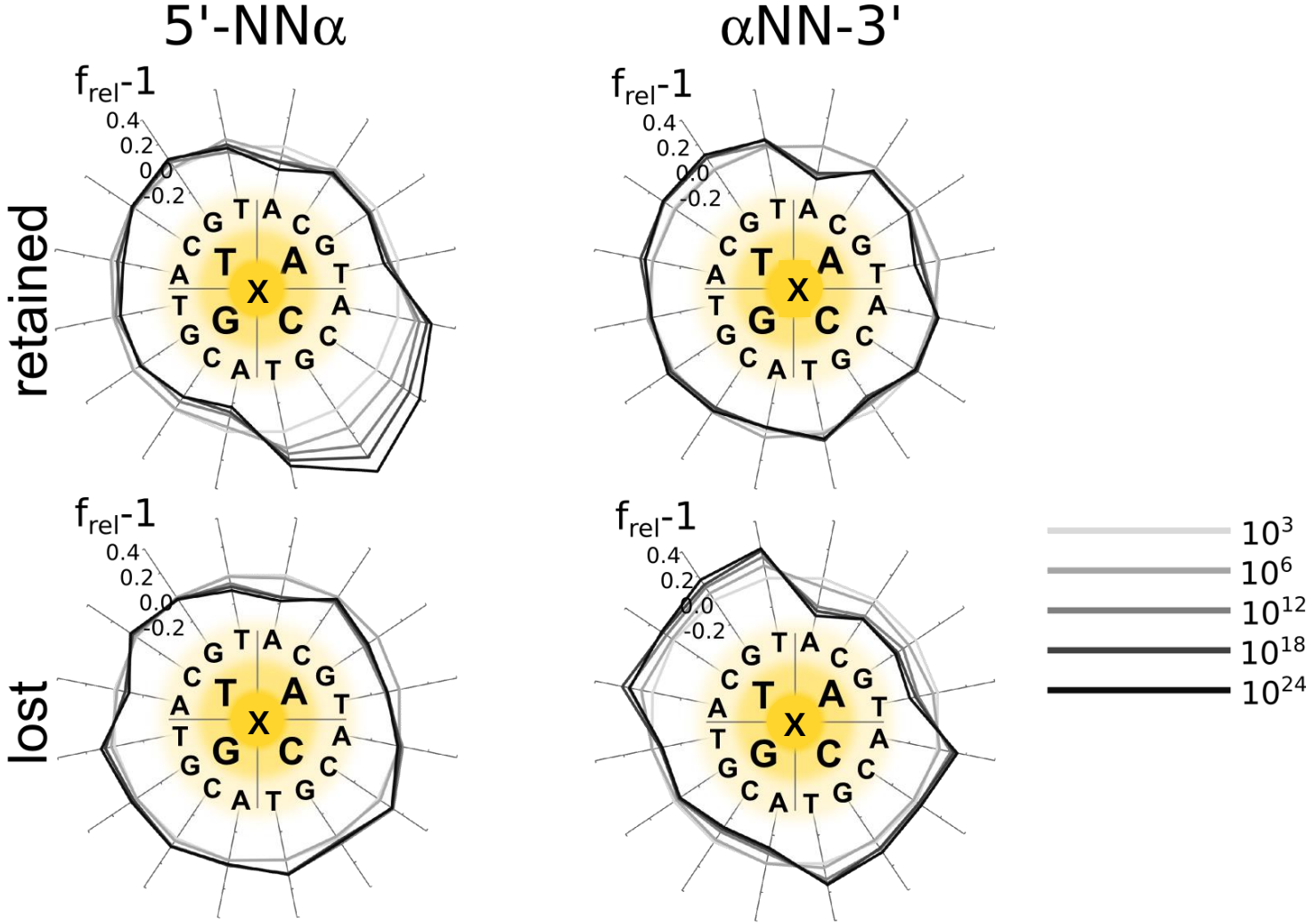
Single Nucleotide Frequency



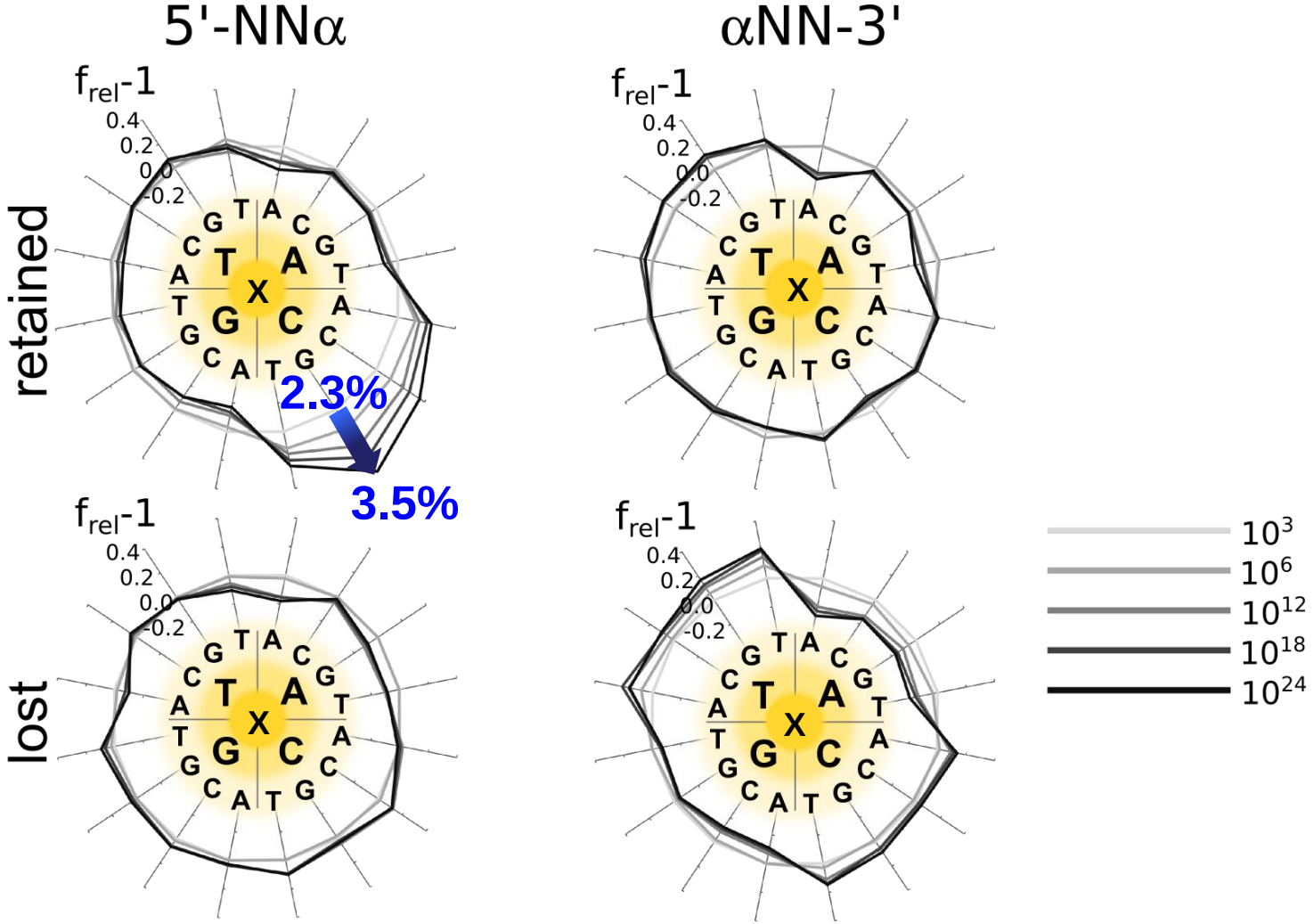
Correlation Analysis



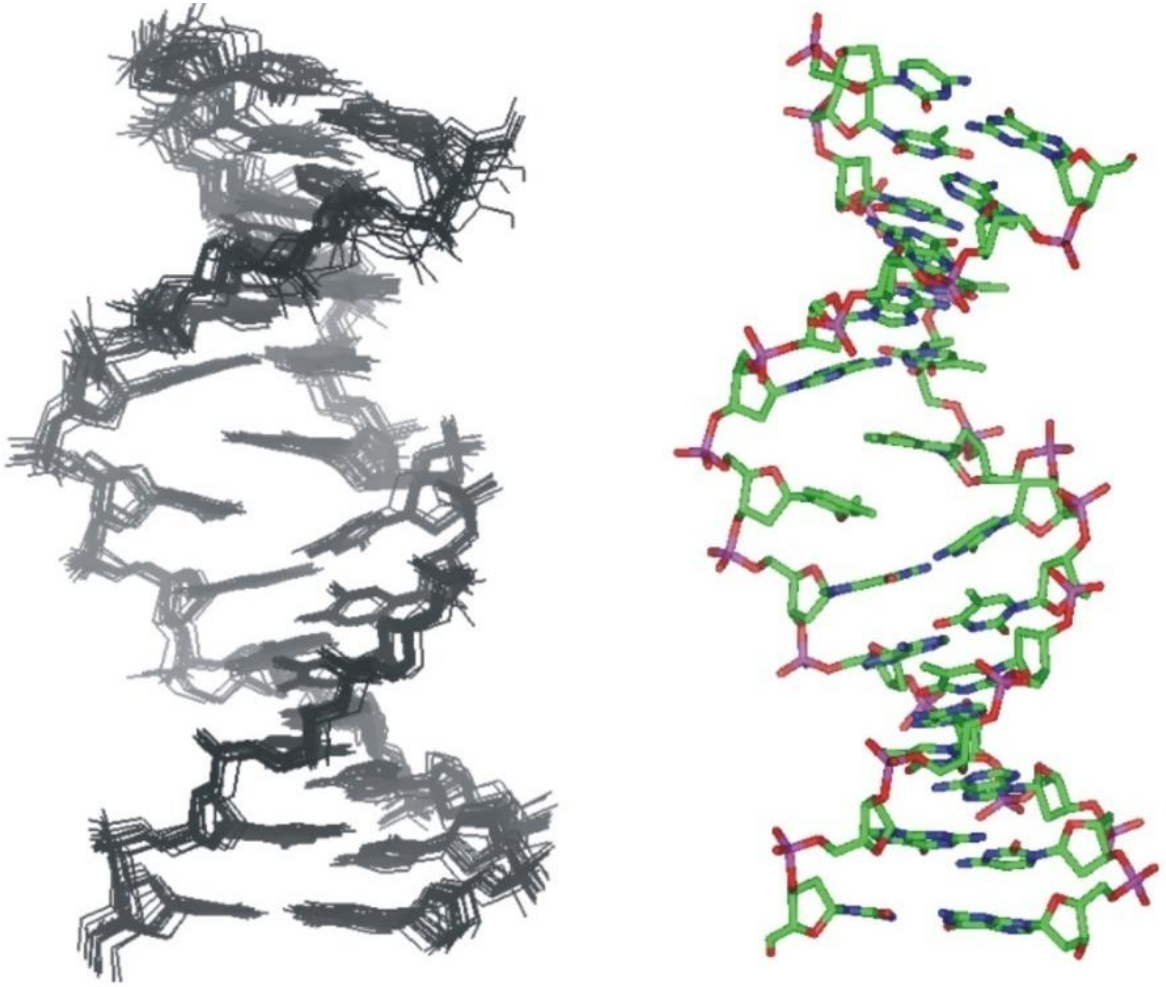
Dinucleotide Frequency



Dinucleotide Frequency

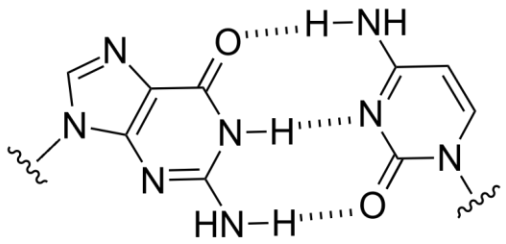


Structural Studies of d**5SICS**-d**NaM**

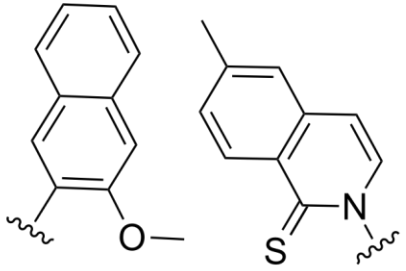


With Tammy Dwyer (U. San Diego)

Structural Studies of d**5SICS**-d**NaM**: Two Questions

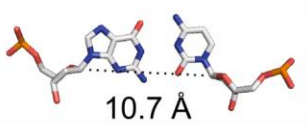


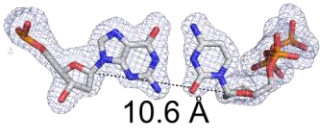
dG dC

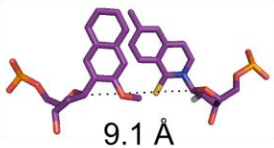


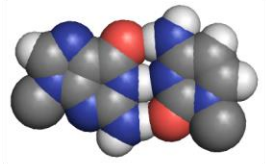
d**NaM** d**5SICS**

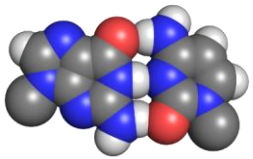
duplex polymerase duplex

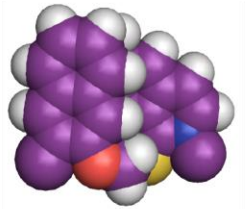


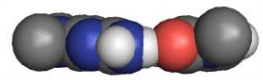


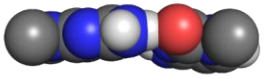


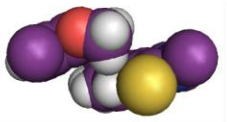




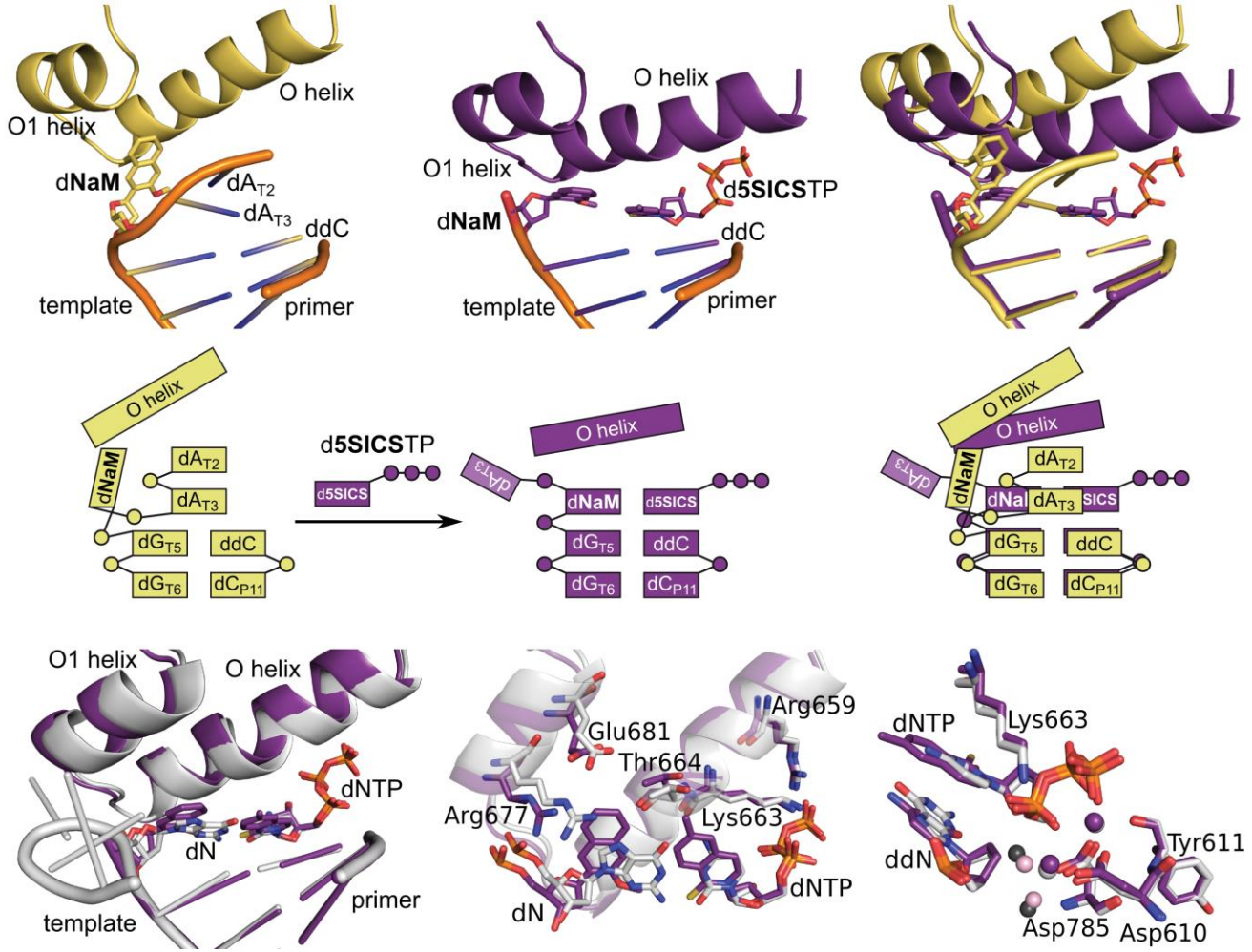






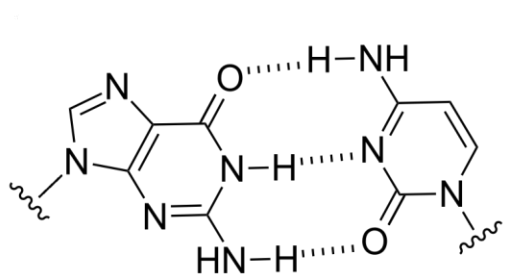


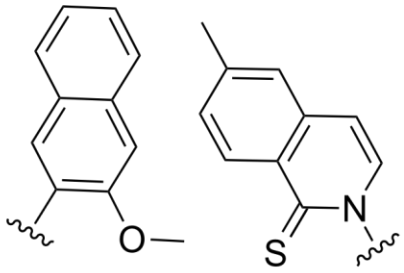
Structural Studies of d**5SICS**-d**NaM**



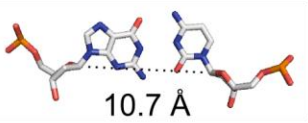
With K. Betz & A. Marx

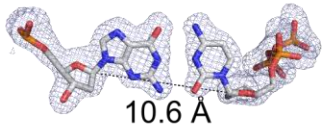
Structural Studies of d**5SICS**-d**NaM**

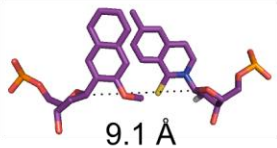


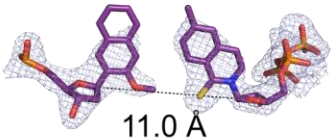


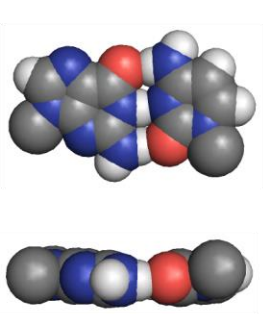
duplex | polymerase | duplex | polymerase

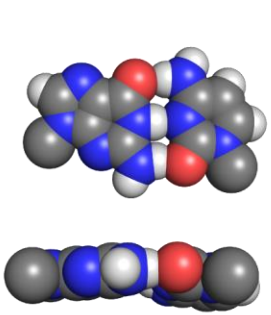


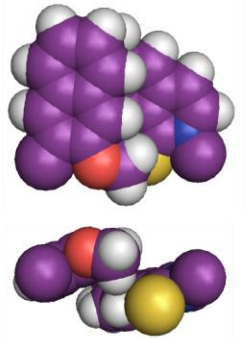


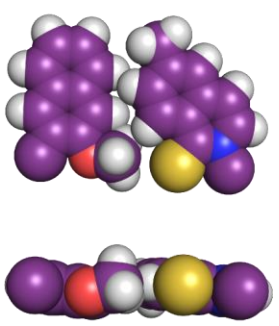




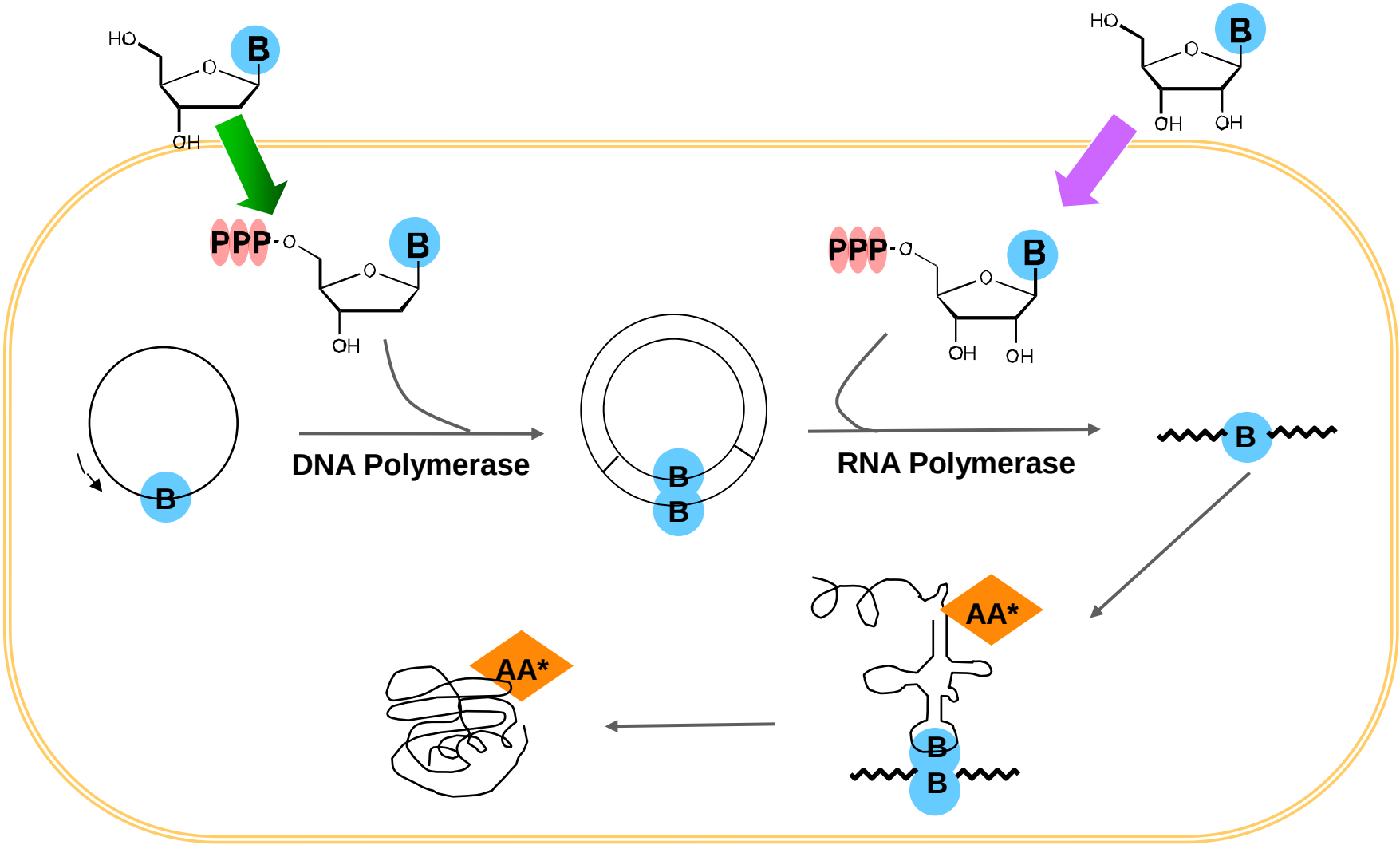




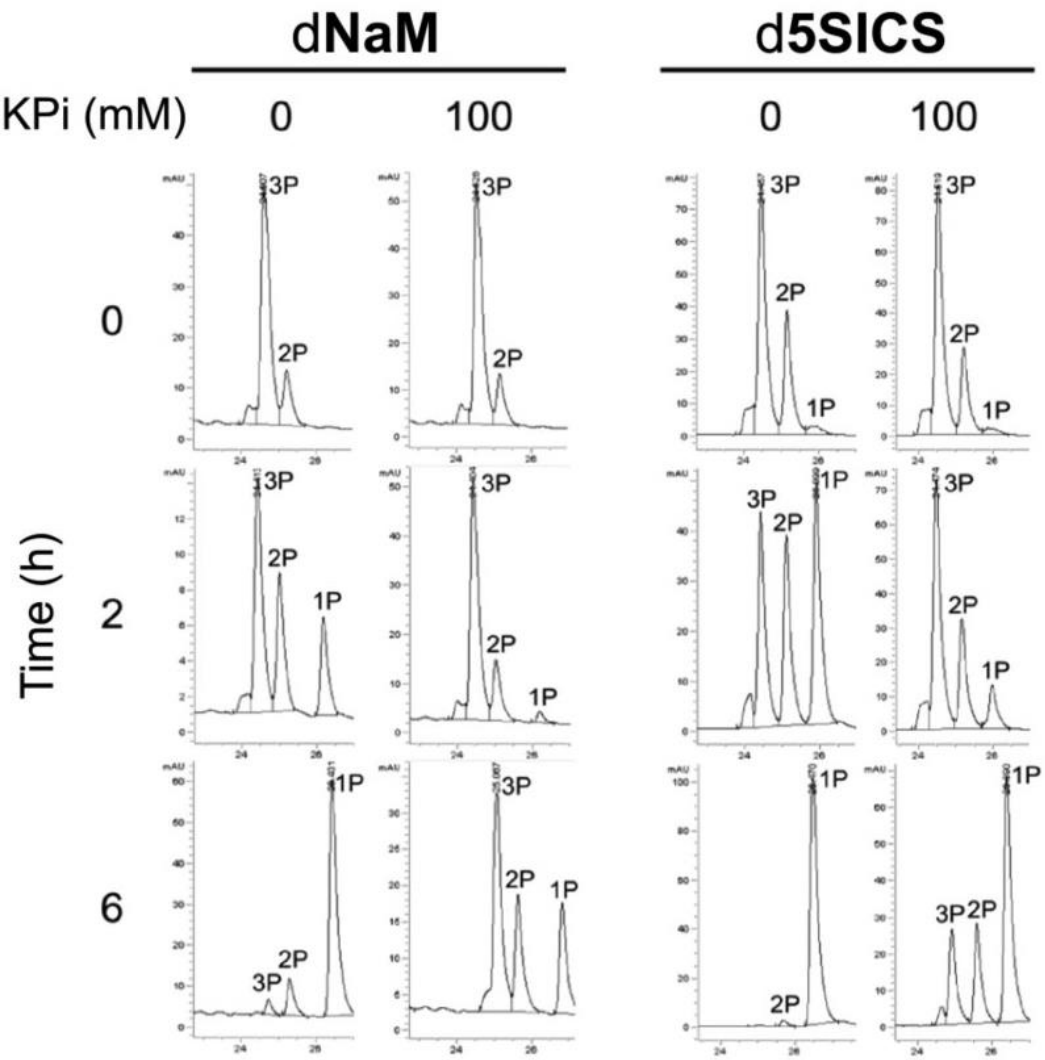




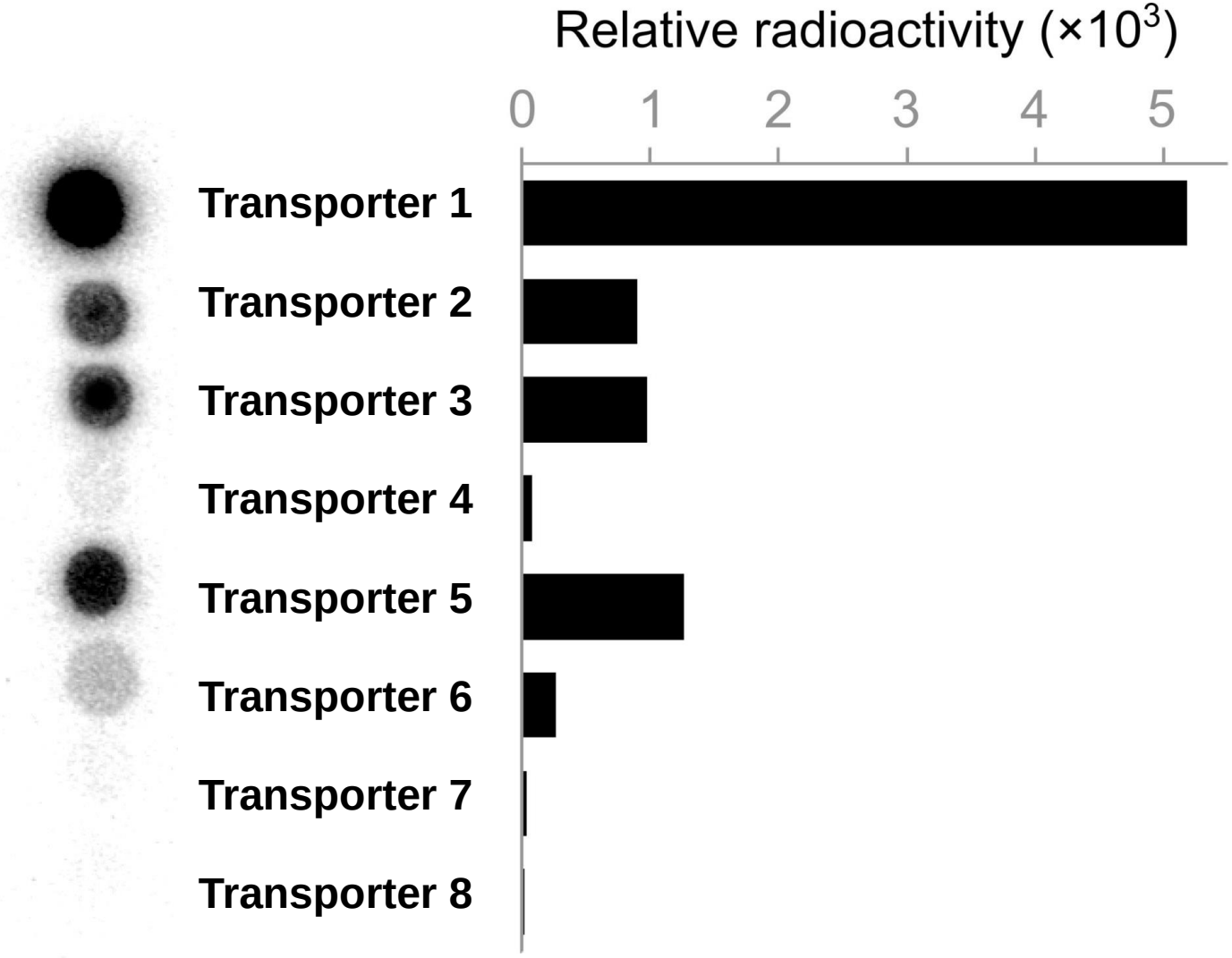
Expansion of the Genetic Alphabet/Code



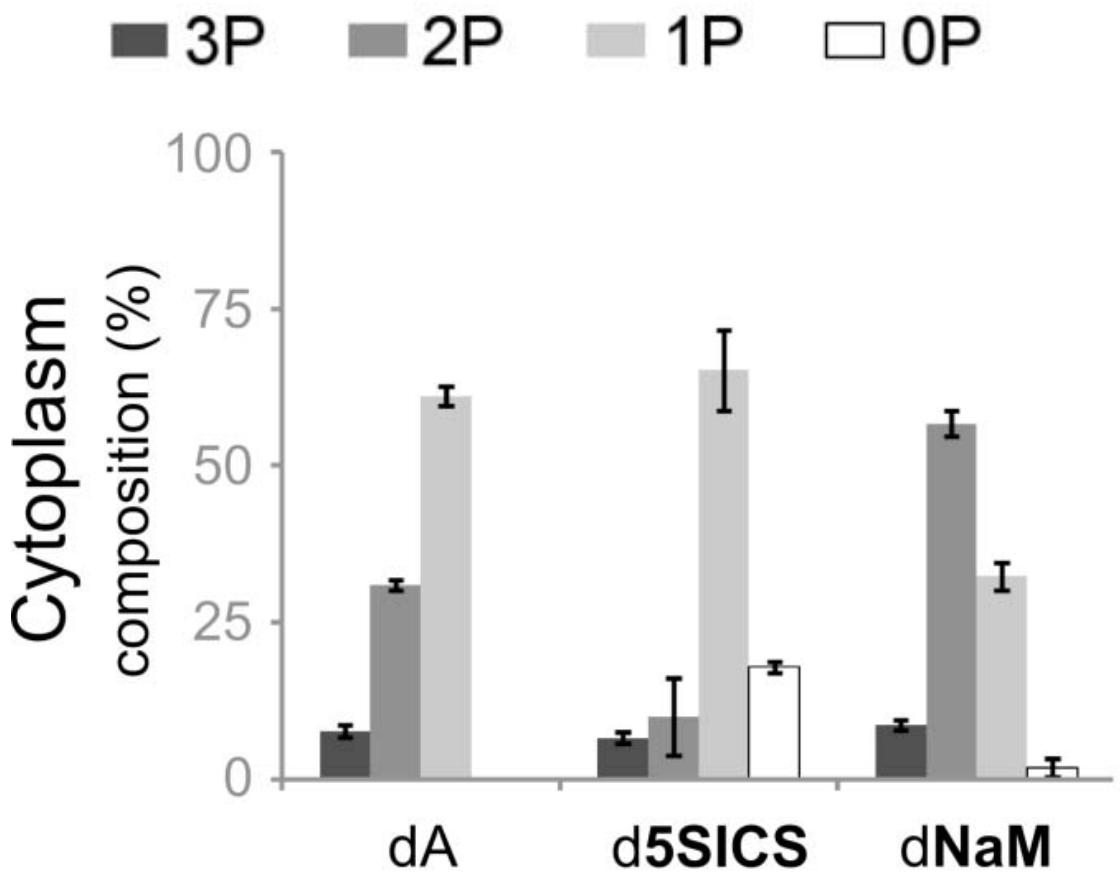
Extracellular Degradation of d**NaM** and d**5SICS**



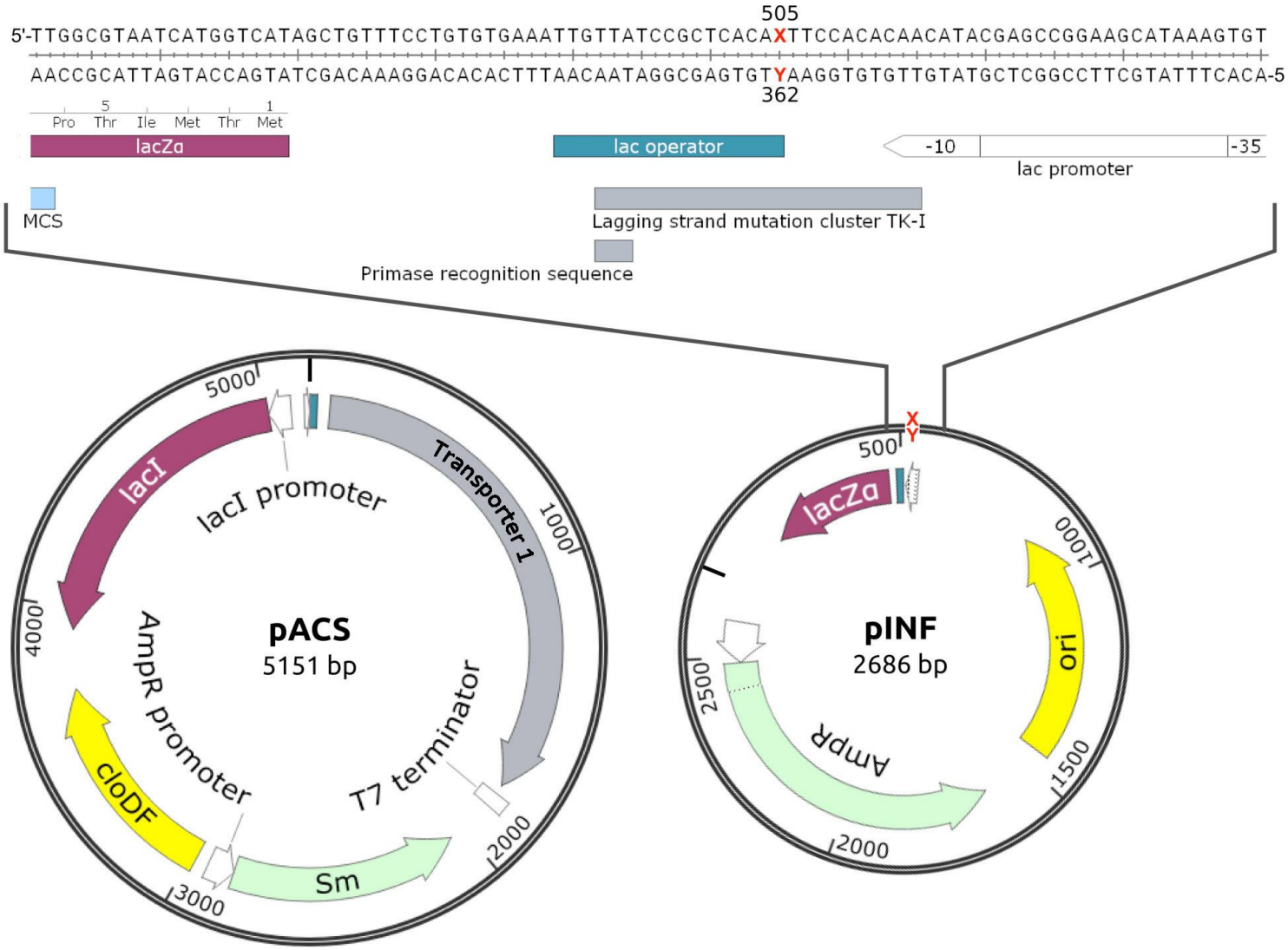
Activity of *Phaeodactylum tricornutum* NTT (*Pt*NTT2) in *E. coli* (dATP uptake)



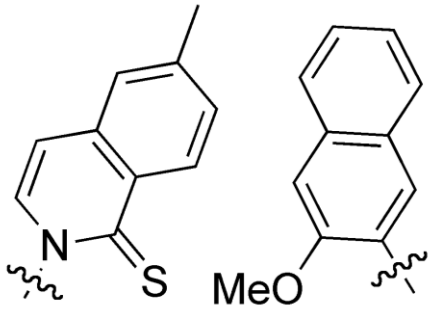
*Pt*NTT2-Mediated Uptake in *E. coli*



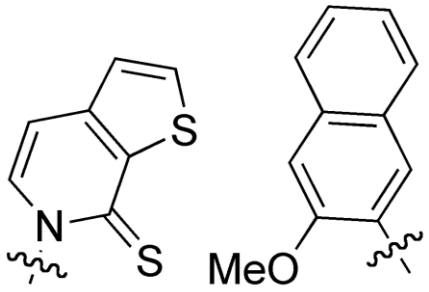
pACS and pINF (X=**NaM**, Y=**TPT3**)



d5SICS-dNaM and dTPT3-dNaM



d5SICS-dNaM



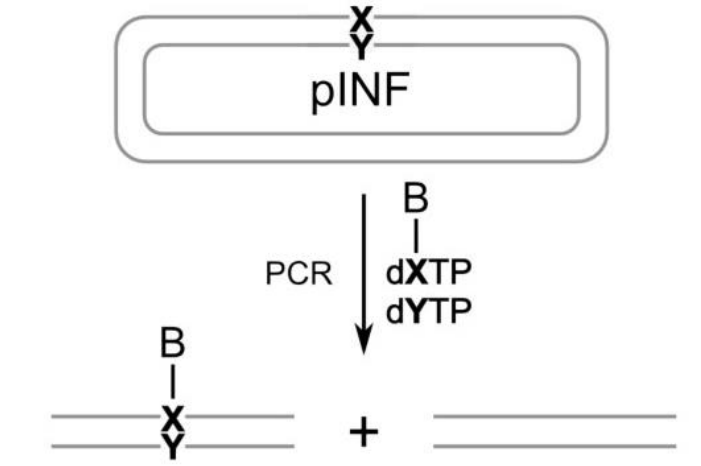
dTPT3–dNaM

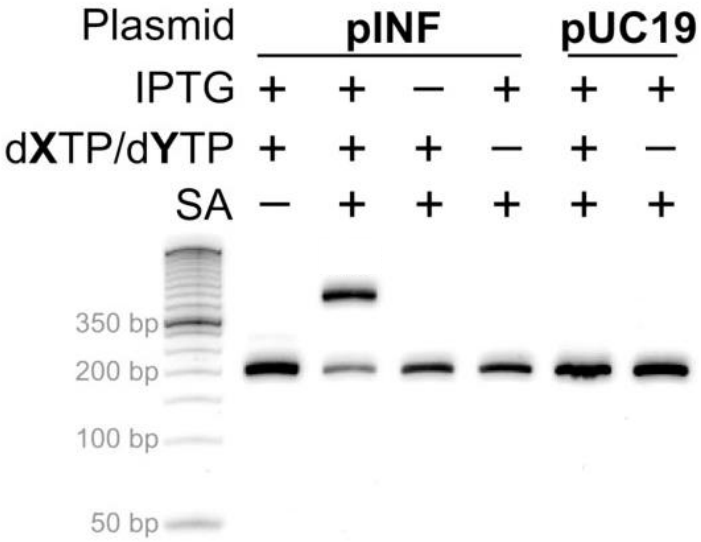
The Experiment

Transform *E. coli* with pACS, induce *Pt*NTT2, add dXTPs, transform with pINF, grow, recover pINF and characterize

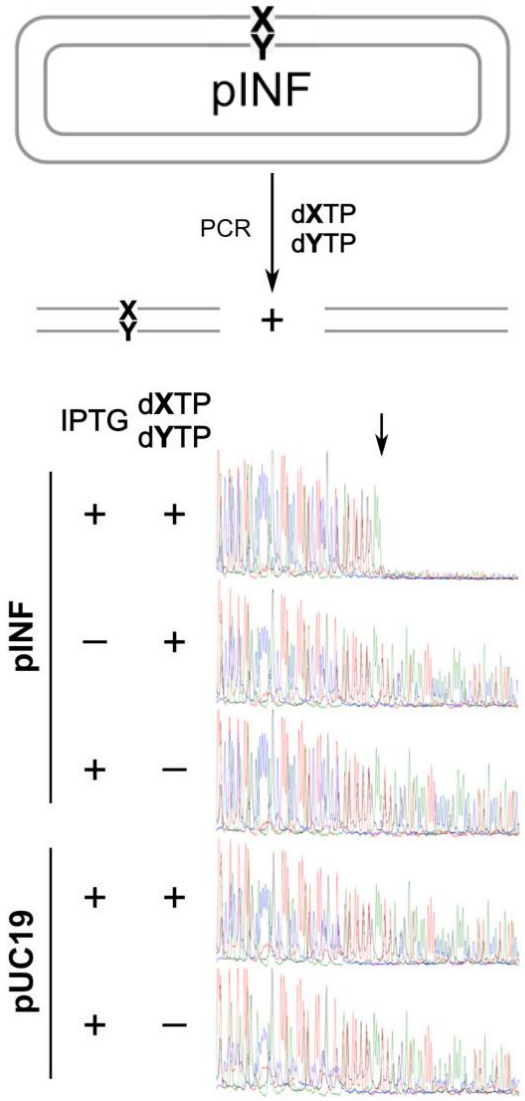
Controls: **transform with pUC19 instead of pINF**
do not induce *Pt*NTT2
do not add dXTPs

Analysis of pINF After Growth in *E. coli* (15 h, 22 Doublings, 10^7-Fold Amplification)

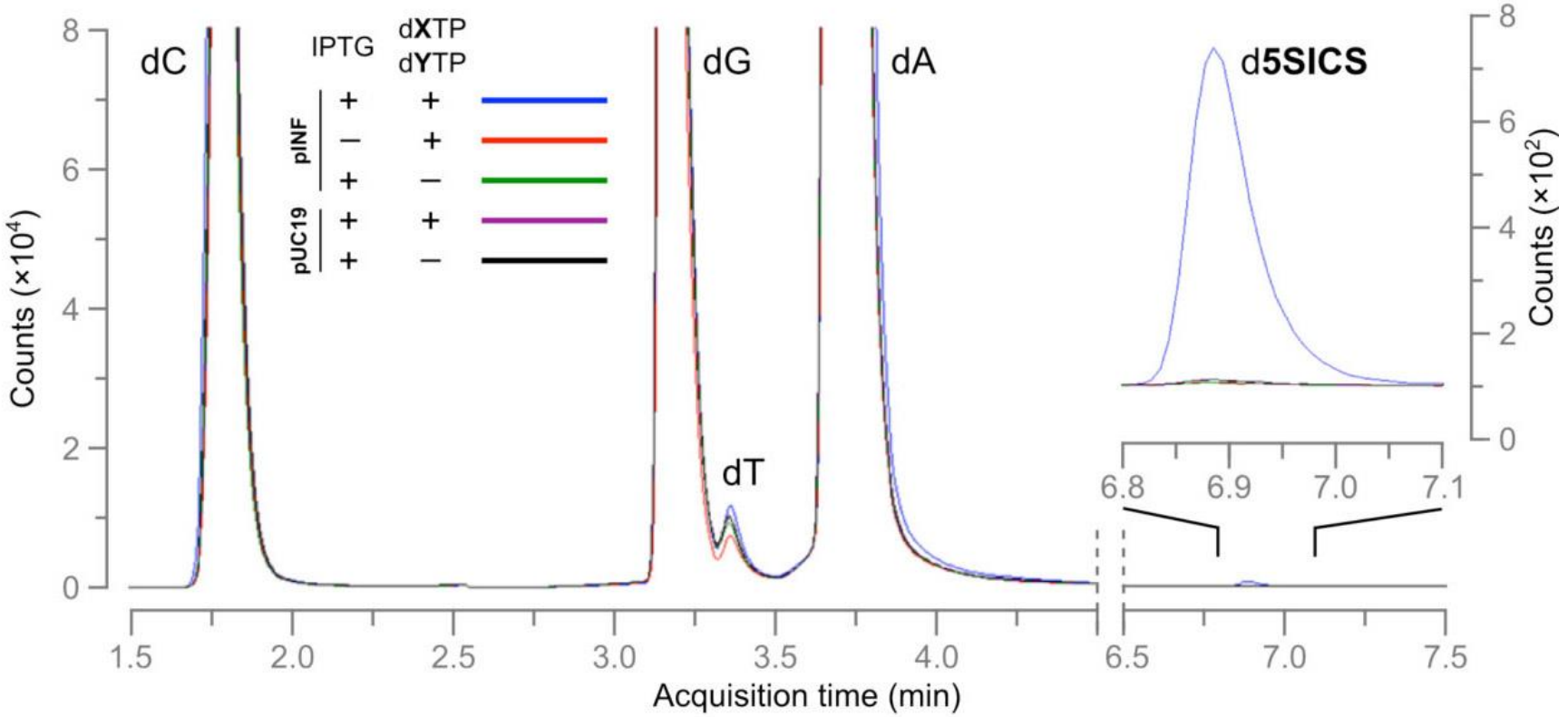




Sequencing Analysis of pINF After Growth in *E. coli* (15 h, 22 Doublings, 10^7-Fold Amplification)

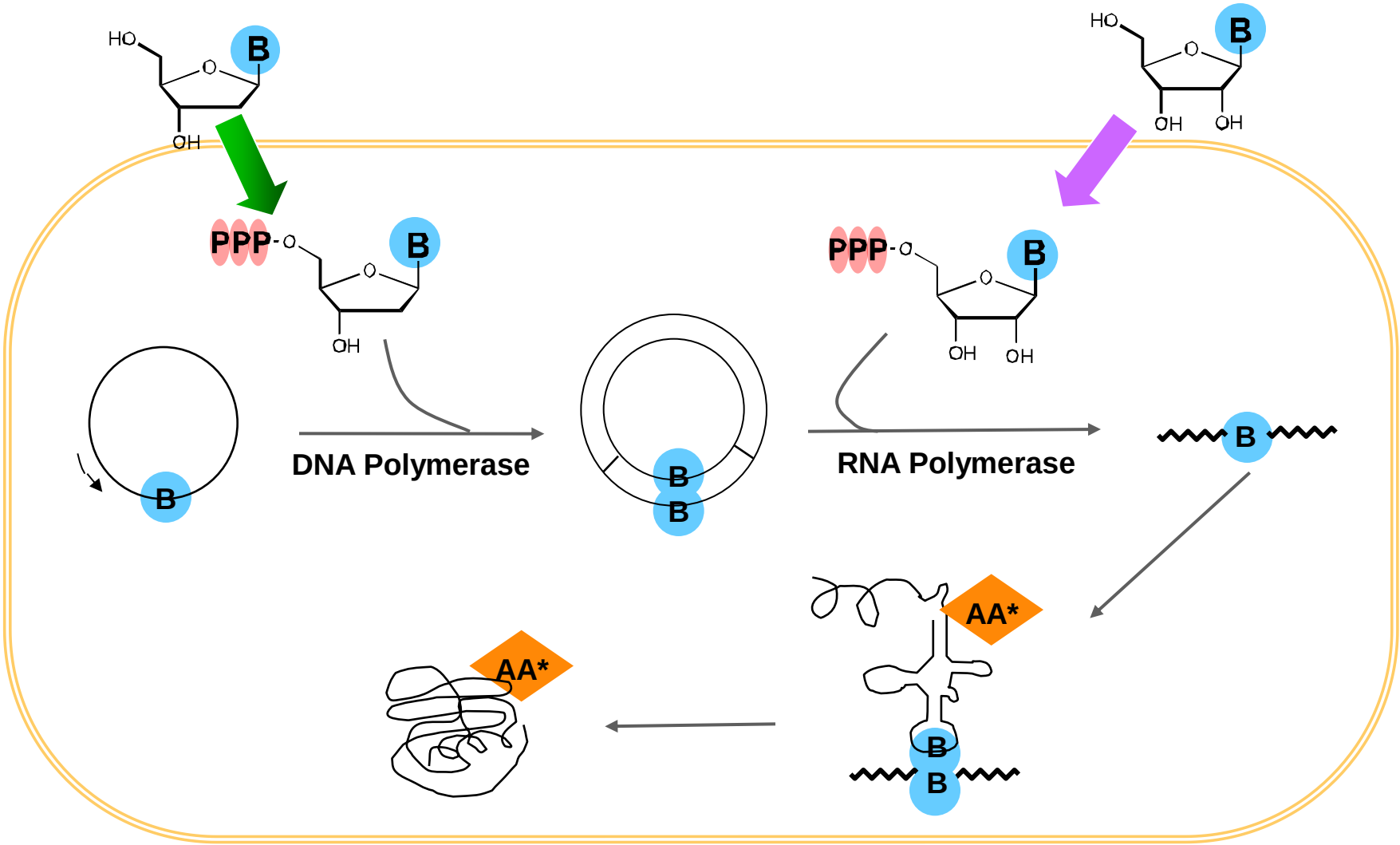


Global Nucleoside Content in pINF and pUC19 via LC-MS/MS

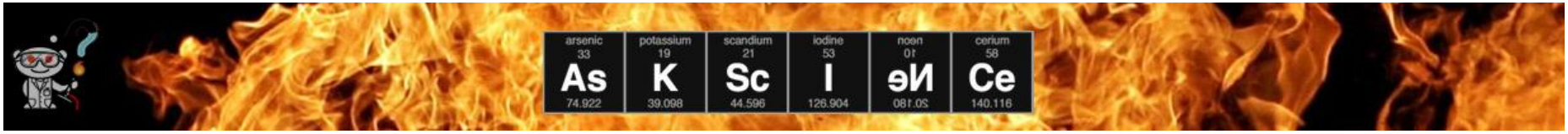


With Ivan Correa, Nan Dai, and Jeremy Foster (NEB)

Expansion of the Genetic Alphabet/Code



Reddit AMA



Chemistry We've added new, artificial letters to the DNA alphabet. Ask Us Anything about our work!

- *773 comments; score of 3056 (93% upvoted)*
- *Much of the dialogue was technical, unlike what was generated by the NYT and NPR stories*

[–] **state_your_case** 2 points 3 months ago

I'm wondering about how you plan to deal with the blowback from the less scientifically minded? I ask because there seems to be a consistent pattern of discovery and then defense whenever a new technology is discovered (like cloning for instance) as those who fear what they don't understand challenge the science that underpins the new technology in question.

Along similar lines, do you anticipate any negatives from your discovery, and what sort of steps are you taking to uncover any potential downside to your work?

[–] **_Illuvatar_** 1 point 3 months ago

First of all, congratulations! It is really exciting to see this kind of breakthrough and I'm sure there will be plenty of awesome discoveries that this leads to! Do you foresee any possible negative uses for this technology? Is it possible that down the line this could be used to create something dangerous?

Jim Thomas Comments on Legal/Regulatory Implications

BUSINESS DAY

184 COMMENTS

Scientists Add Letters to DNA's Alphabet, Raising Hope and Fear

By ANDREW POLLACK MAY 7, 2014

"The arrival of this unprecedented 'alien' life form could in time have far-reaching ethical, legal and regulatory implications," Jim Thomas of the ETC Group, a Canadian advocacy organization, said in an email. "While synthetic biologists invent new ways to monkey with the fundamentals of life, governments haven't even been able to cobble together the basics of oversight, assessment or regulation for this surging field."

Comments in Response to Pollack's *NYT* Article

BUSINESS DAY

184 COMMENTS

Scientists Add Letters to DNA's Alphabet, Raising Hope and Fear

By ANDREW POLLACK MAY 7, 2014

Garrett Clay San Carlos, CA · 8 May 2014

The Titanic is unsinkable.

We will figure out a way to safely store nuclear waste in this decade, the 1960s.

Three Mile Island was an anomaly, nuclear power is safe.

Three Mile Island and Chernobyl were anomalies, we will soon figure out a way to safely store nuclear waste.

Three Mile Island, Chernobyl, and Fukashima were all anomalies, we will figure out a way to safely store nuclear waste.

Nuclear power is safe using new technologies being developed now, we just need government loan guarantees and government insurance.

GMO crops will reduce pesticide use.

Shall I go on?

12 Recommend ·

Sharkie Boston · 7 May 2014

Alien DNA. Some corporate scientist is going to make an alien DNA life form. Don't we have special forces to handle this? What's the consequence of cells making their own mutant nucleotides, allowing cells to survive on their own - something that doesn't die. What do you think Monsanto's going to do with this. I hope whatever freak they make gets them first.

15 Recommend ·

Comments in Response to Pollack's *NYT* Article

BUSINESS DAY

184 COMMENTS

Scientists Add Letters to DNA's Alphabet, Raising Hope and Fear

By ANDREW POLLACK MAY 7, 2014

Erik Dorthe San Diego • 7 May 2014

All these comments saying things like "mad science again!" seem to leave out one vital piece of information: What past horror of science are you referencing? Sure, there are risks. There are risks to many scientific endeavors, and many non-scientific endeavors. Don't panic just because someone created something you cannot understand.

Matthew Duluth, MN • 7 May 2014

Scientists are the authorities society should look to when it wants to know what the biological implications of research might be, but should the scientific community really have a monopoly on the discussion of scientific ethics?

Both scientists and informed laypeople can be trusted to offer valid ethical opinions on research. It usually doesn't take an advanced degree to understand enough to make a sound moral judgement, and no one save the completely ignorant should be "[left out] of serious discussions."

Comments in Response NPR Health Blog

Chemists Expand Nature's Genetic Alphabet

by **NELL GREENFIELDBOYCE**

May 07, 2014 2:07 PM ET

KevinS78 • 4 months ago

How about we NOT mess with the basic structure of life forms? What do we have to gain? More drugs? GREAT. You're gonna need them for the increase in cancer and other genetic malfunctions that will proliferate as a result of genetic tampering.

Tom Bombadil • 4 months ago

If messing with genetics is safe, why has the EU banned GMO? Why our government has not banned them is obvious.

"you are what you eat" now has different connotations. Monsanto's misplaced genes change the genetic make up of the eater.and we all have to eat. When you digest food, the proteins are not broken down all the way to individual amino acids. Rather, short chains of the amines are incorporated into the proteins of he who eats. Monsanto's misplaced genes incorporated into cells undergoing meiosis has dire consequences.for the fetus..Their anomalous genes consumed by adults can also have undesirable consequences such as cancer.

Dave R ↗ Tom Bombadil • 4 months ago

Whether we add genes by taping seed halves together or using bacterial enzymes makes no difference to the final product. In fact, genetic modification gives us much better control over what we do and don't want to add, making the process much safer.

Objections to Comparison with Nature

Imagine writing a story with a language with only four letters. A fifth and sixth letter would let you write more Interesting stories.

Objections to Comparison with Nature

Imagine writing a story with a language with only four letters. A fifth and sixth letter would let you write more Interesting stories.