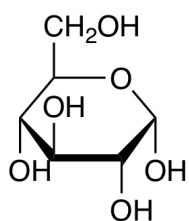


Corn starch  $\rightarrow$  cheap polymer  
of  $\alpha$ -D-Glucose  
 $\rightarrow$  Converted to  $\alpha$ -D-Glucose  
monomers using 2 enzymes:

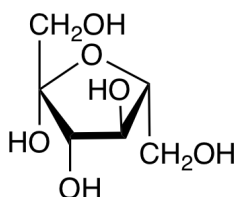
1)  $\alpha$ -Amylase  $\rightarrow$

2) Glucoamylase  $\rightarrow$

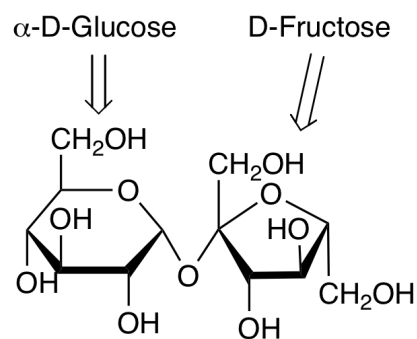
3) Some of the D-Glucose is converted  
to D-Fructose



$\alpha$ -D-Glucose



D-Fructose



Sucrose (Table Sugar)

Final high fructose corn  
syrup:

90 D-Fructose

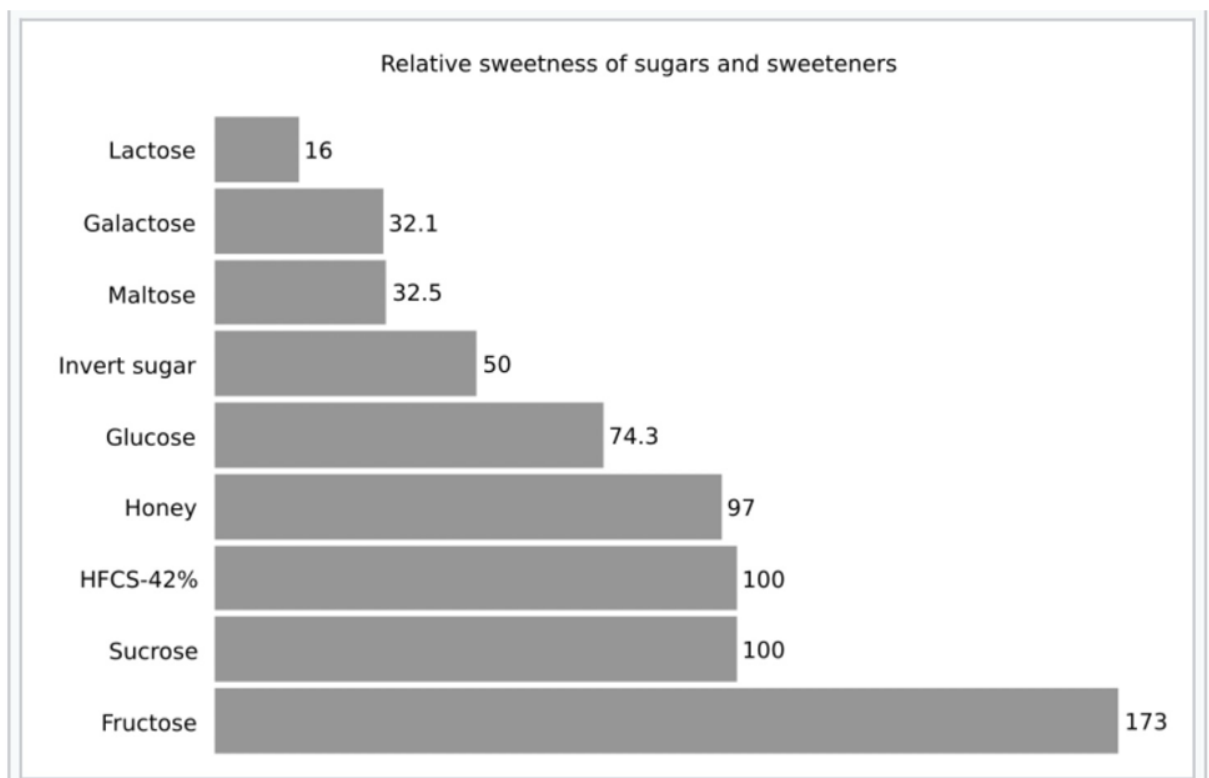
90 D-Glucose

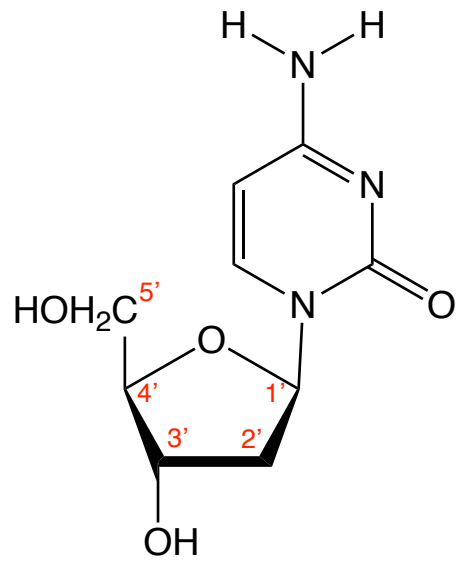
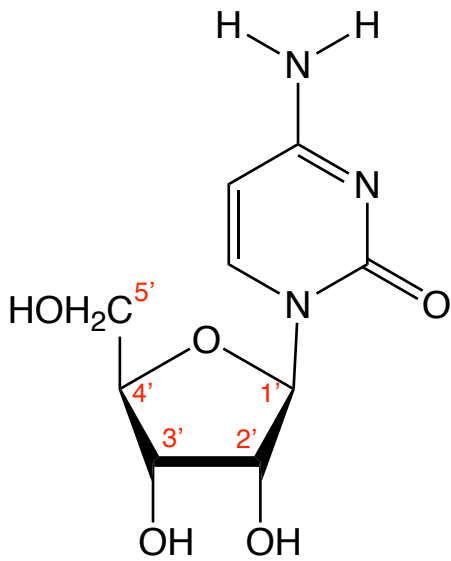
This is intended to mimic bee honey

90 D-Fructose

90 D-Glucose

90 Sucrose

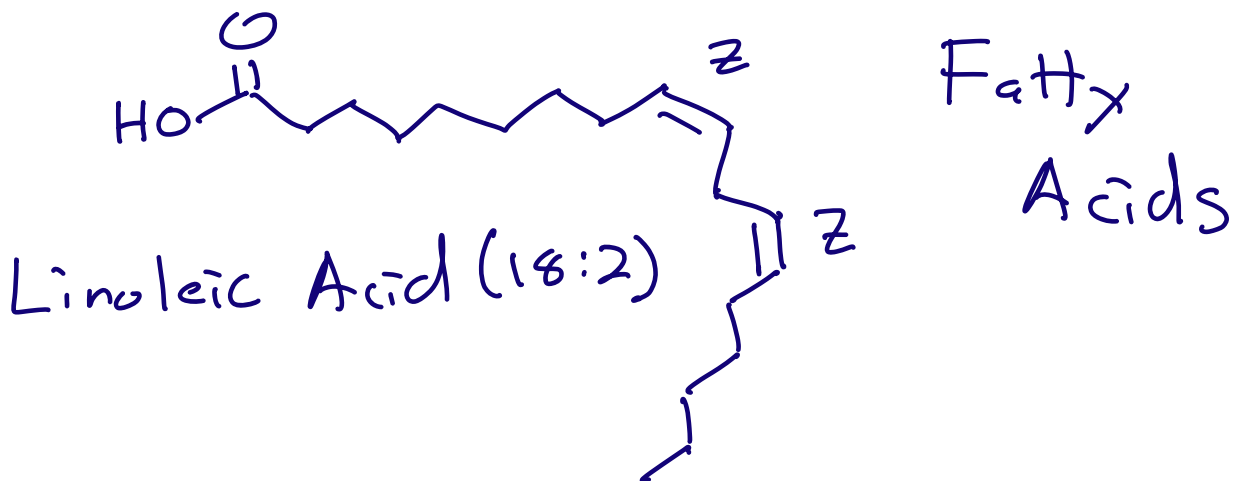
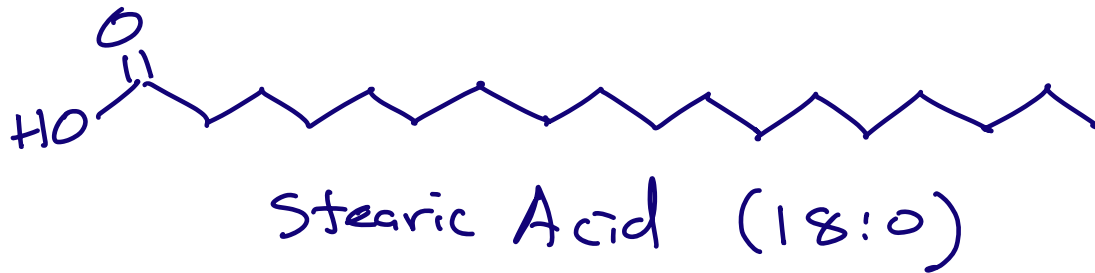
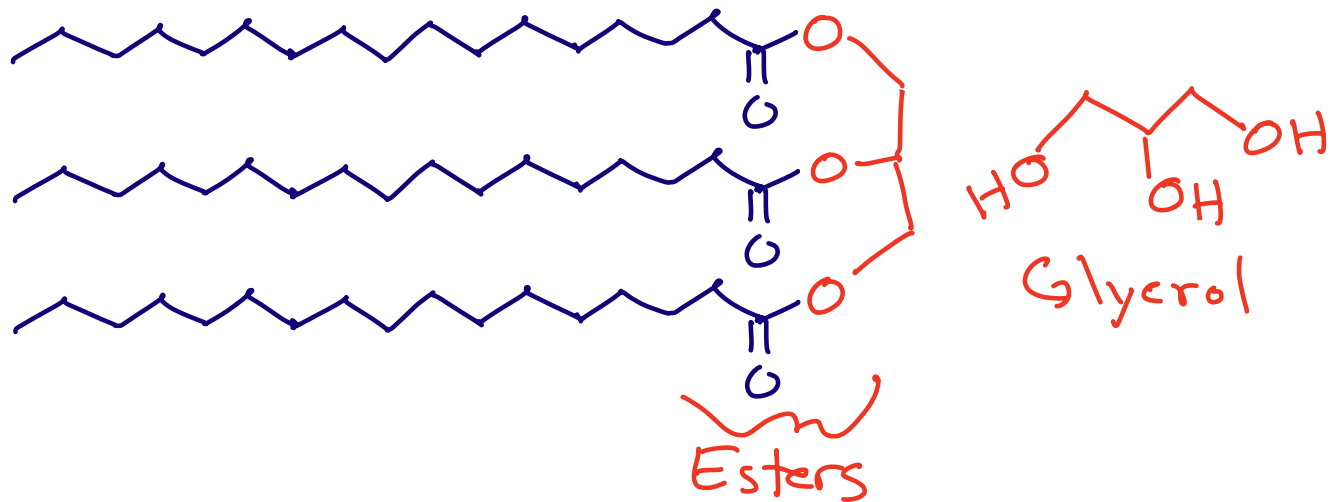




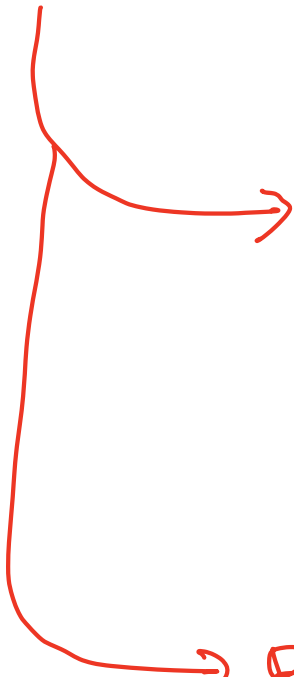
In RNA and DNA →

Lipids → Biological molecules that are not soluble in water

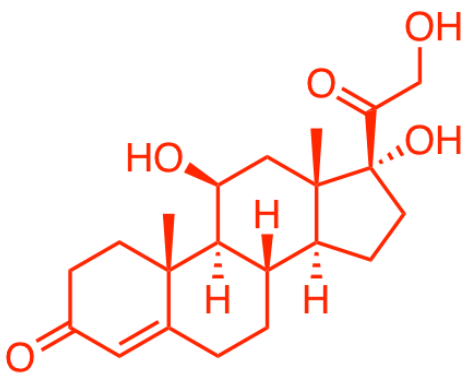
Triglycerides → Fats and oils



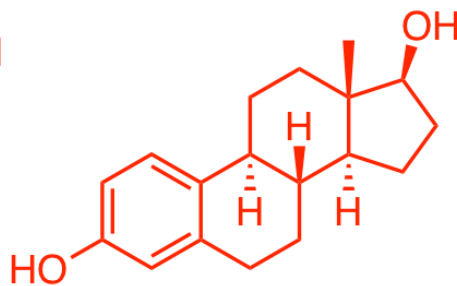
Steroids → Rigid multiring structures that are important signals



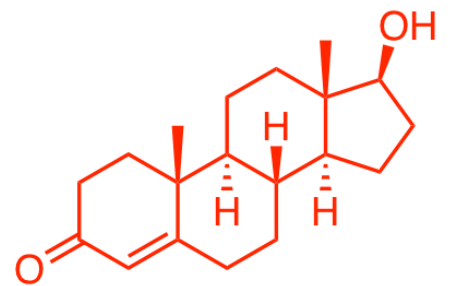
Rigid structure ensures high degree of for these critical functions



Cortisol



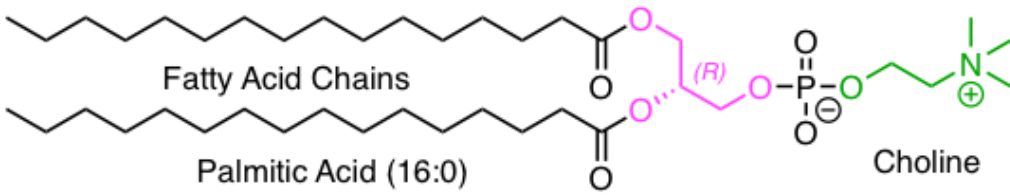
Estradiol



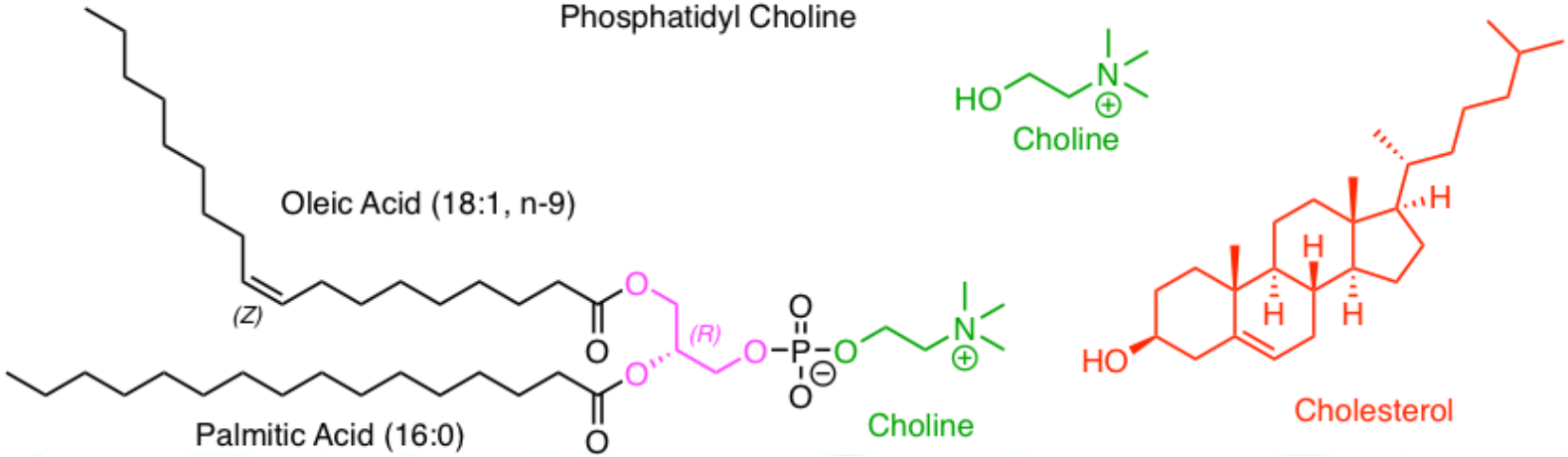
Testosterone

Phospholipids → Make up cellular membranes

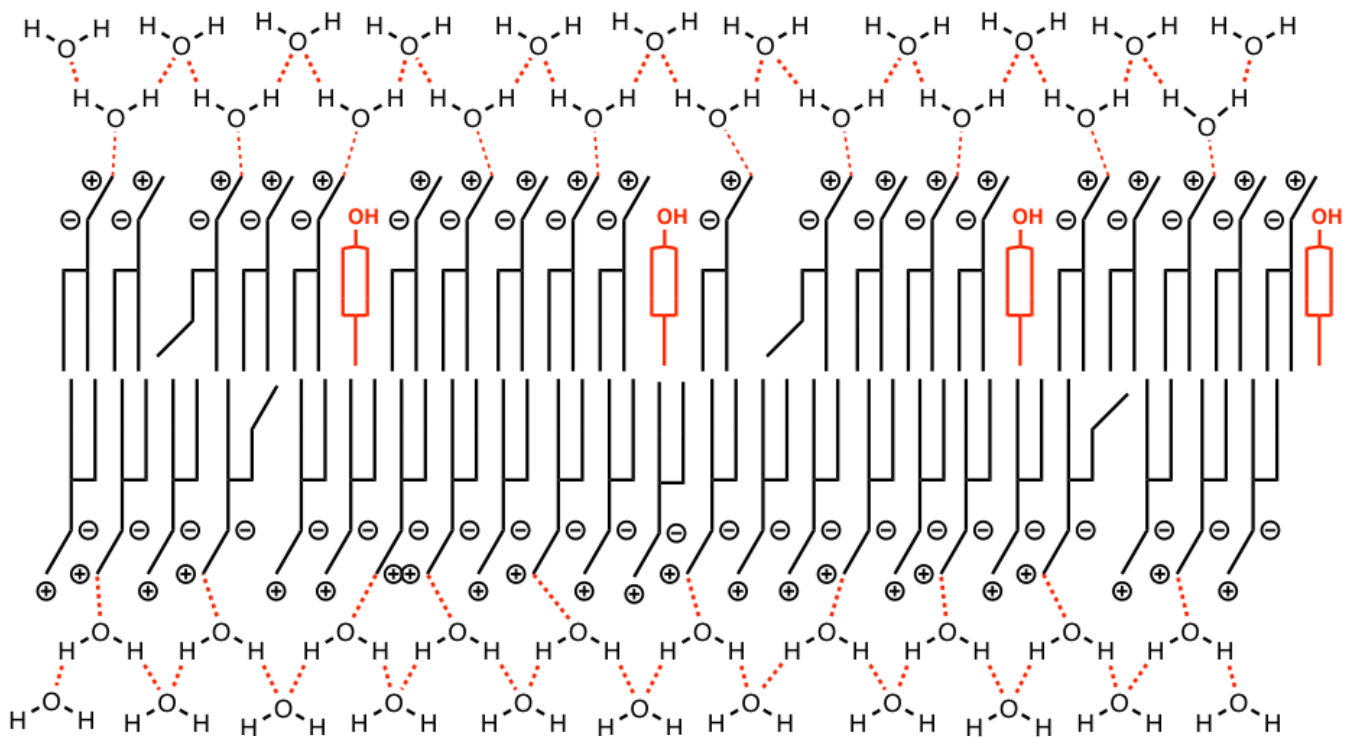
Palmitic Acid (16:0)



Phosphatidyl Choline



Phospholipid membrane bilayers → The  
and combine  
to make the membranes enough  
to operate effectively



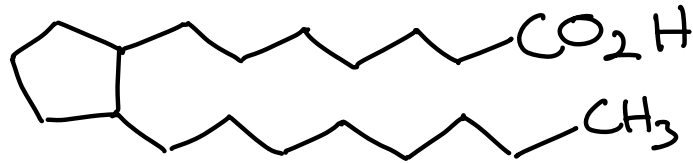
So what holds membranes together?

The fatty acid chains are not soluble in water → The in water prevent the from "dissolving" into the water → Only things that can interact with polar water molecules dissolve in water. → To break up hydrogen bonds a molecule must be charged and/or be capable of strong

"Like likes like" → Key rule of solvents

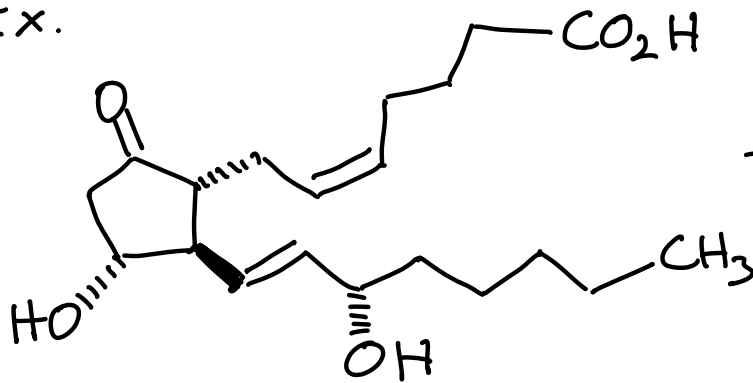
!!!  
→ The cannot interact with water so they must therefore associate providing the to hold membranes together

Prostaglandins → another important lipid



Prostaglandin general formula - 20 carbons

Ex.



Key mediator  
of inflammation  
and immunity

- swelling
- vasodilation
- suppresses  
T-Cell response

Speeds  
healing

PGE<sub>2</sub>

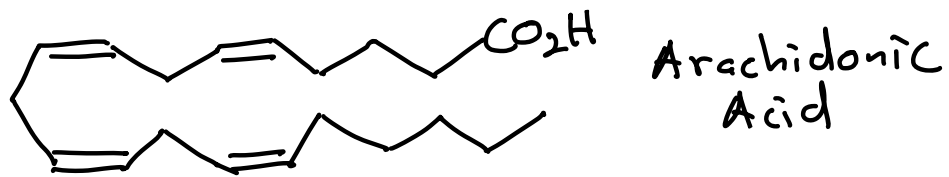
Induces uterine  
contractions

Enhances pain  
and causes  
fever

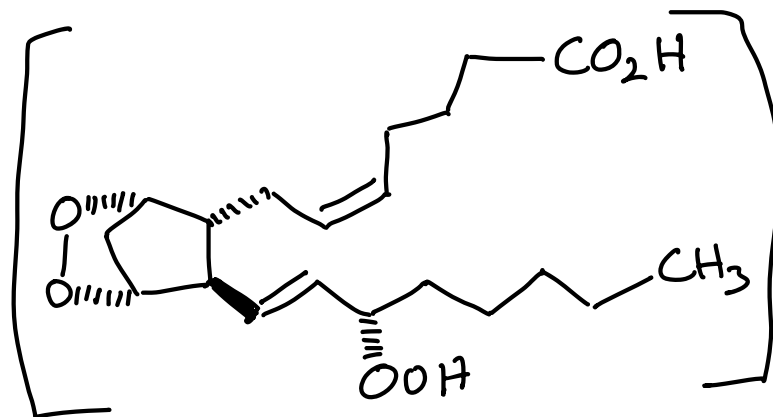
All of these are local effects

Prostaglandins are synthesized

# Important medication involving prostaglandins —



An enzyme called  
cyclooxygenase  
(COX)

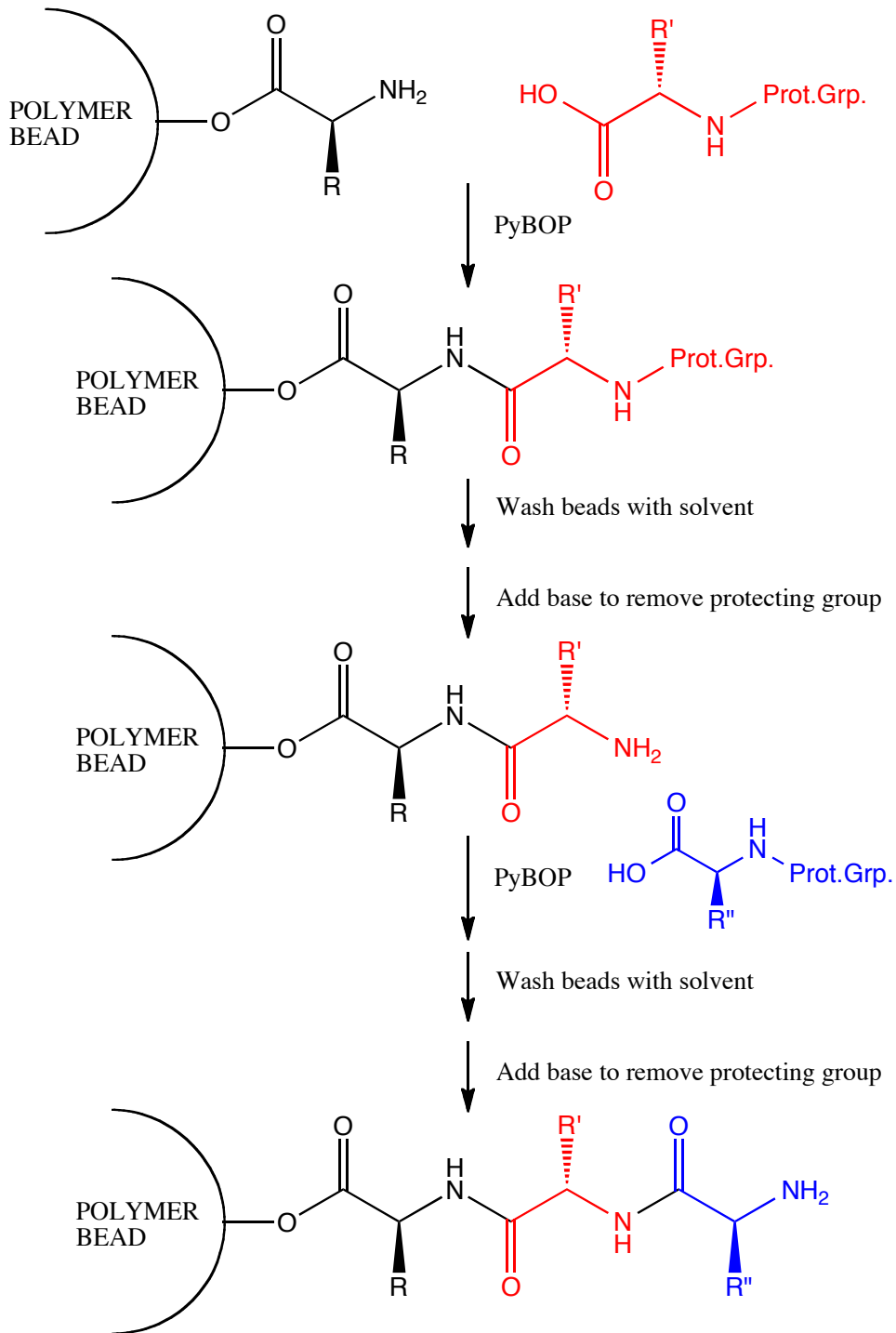


Unstable intermediate

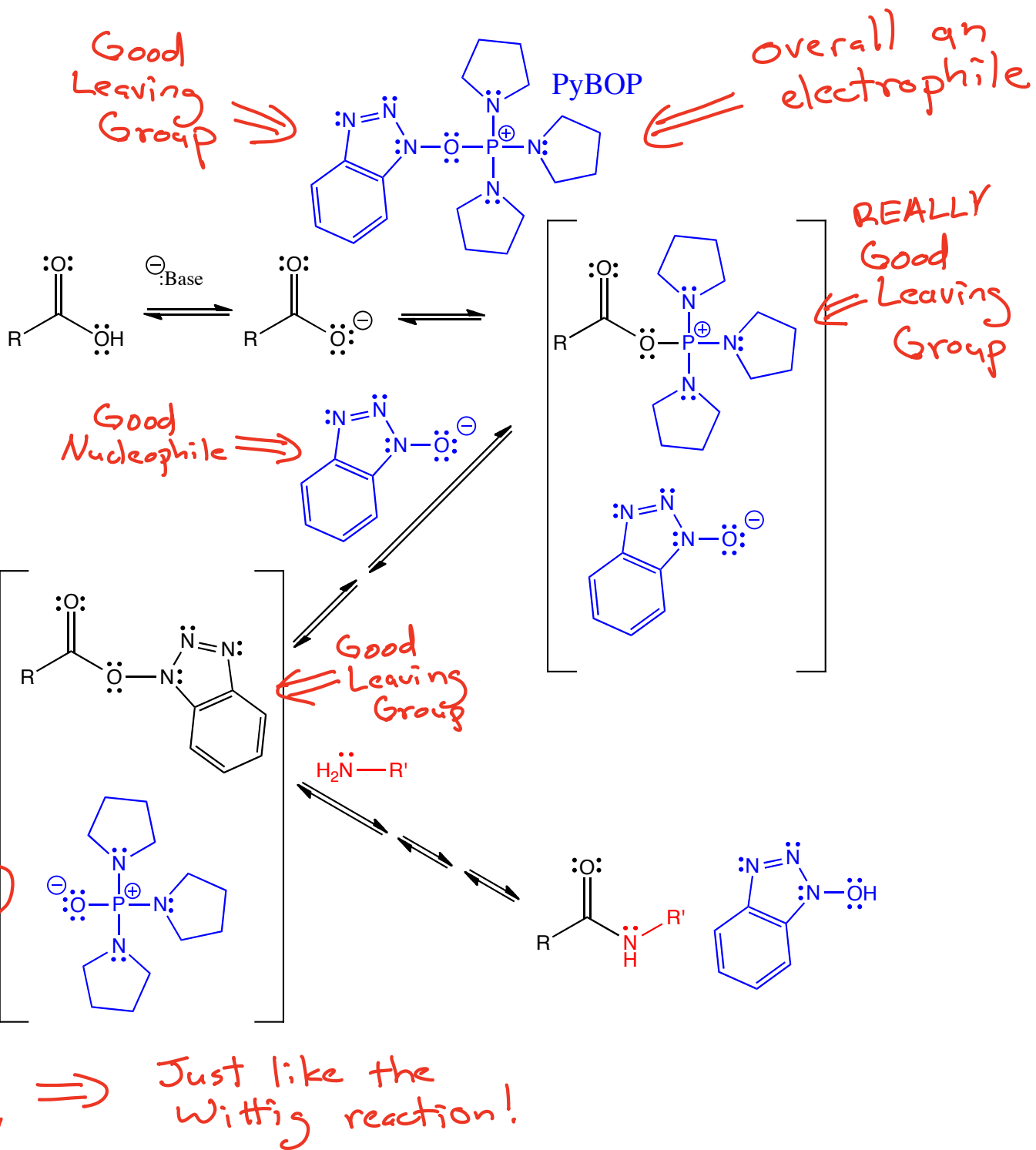
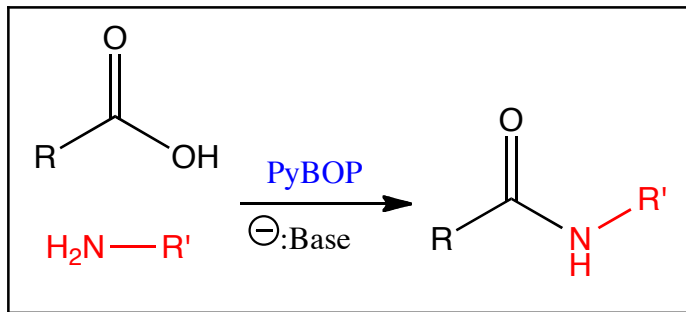
↓

Immediately converted to other prostaglandins that control various important functions involved with

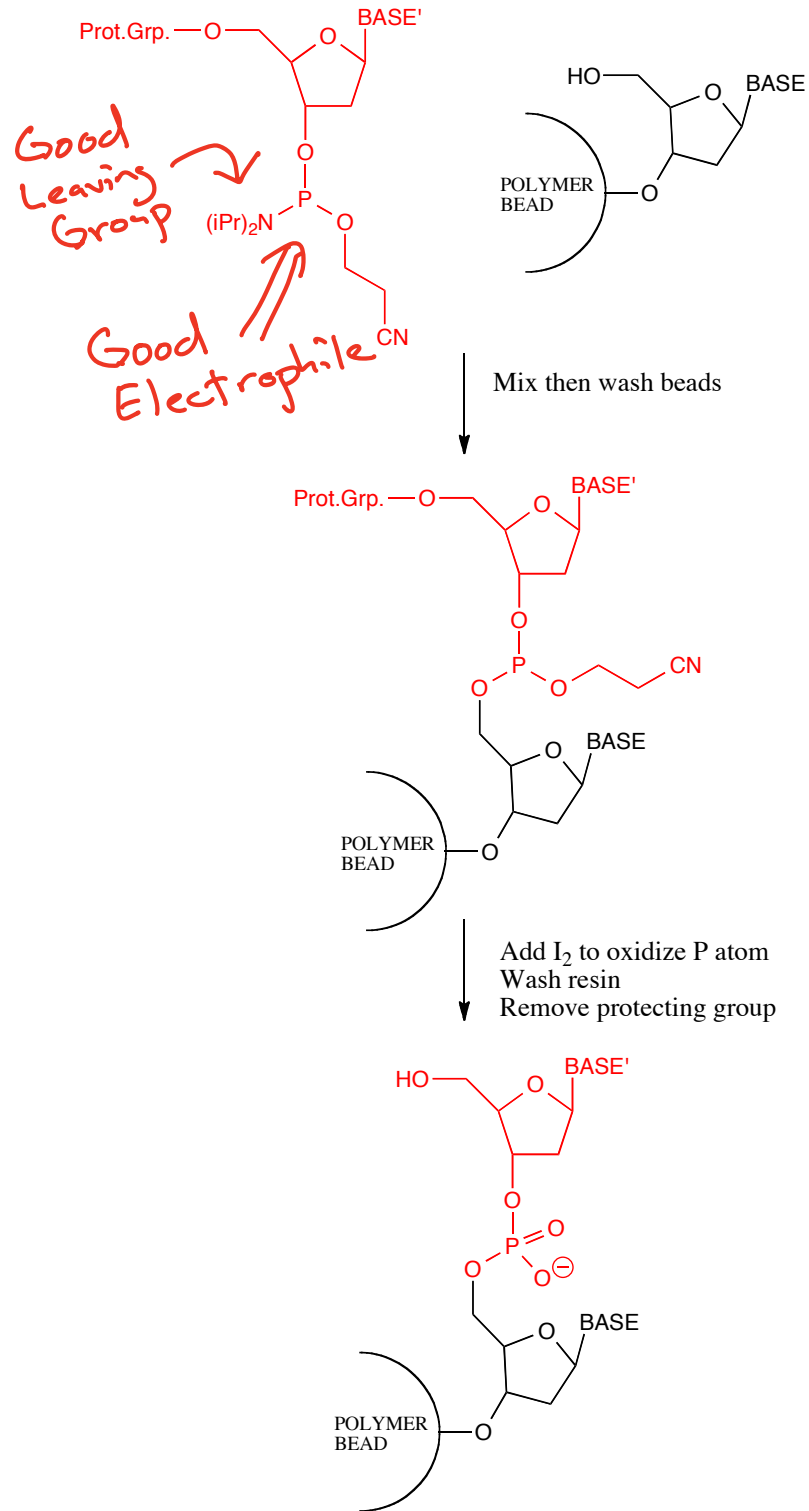
# Solid Phase Peptide Synthesis



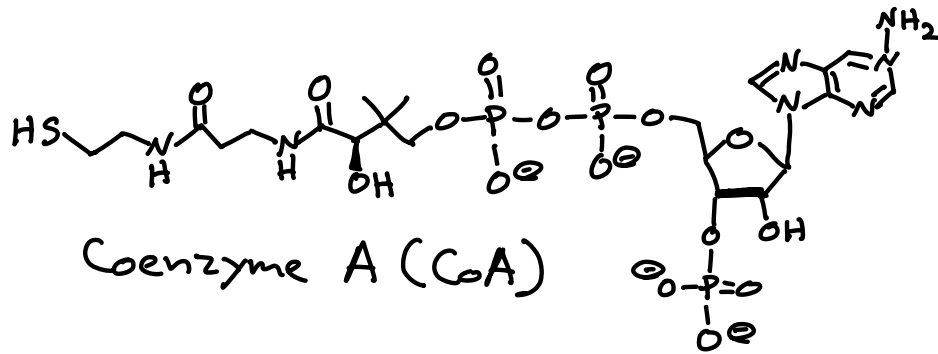
Repeat as necessary then remove from resin. Can add up to 100 amino acids this way.



# Solid Phase Synthesis of DNA

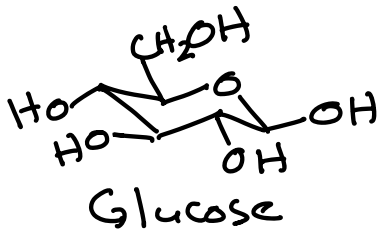


How does mother nature make C-C bonds? → ENOLATES!

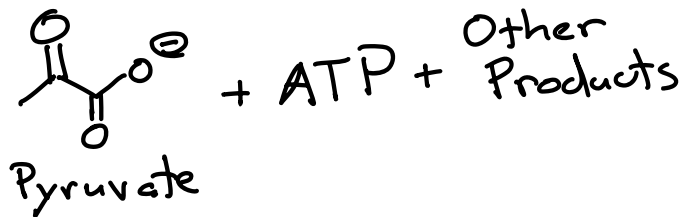


Thioesters → more reactive than esters →  $\ominus$ S-R is a better leaving group (more stable anion) than  $\ominus$ O-R.

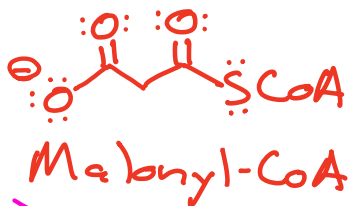
From food



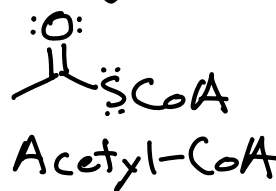
several enzyme reactions



enzymes



enzymes

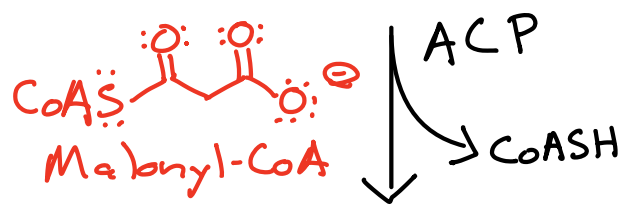
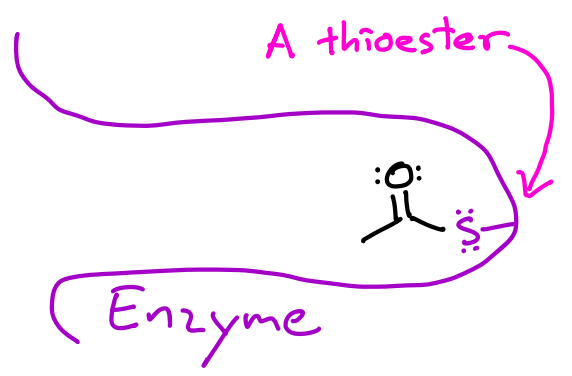


Both are thioesters

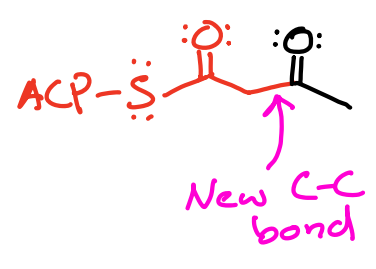
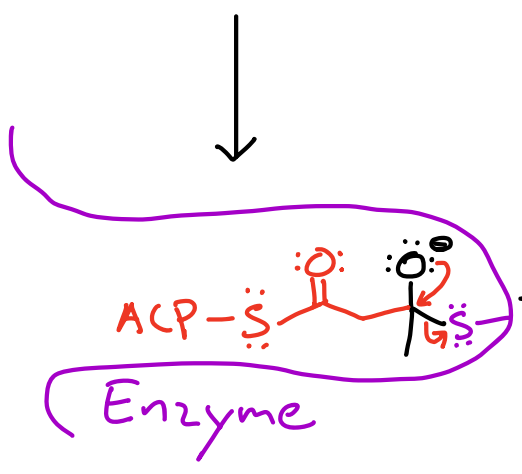
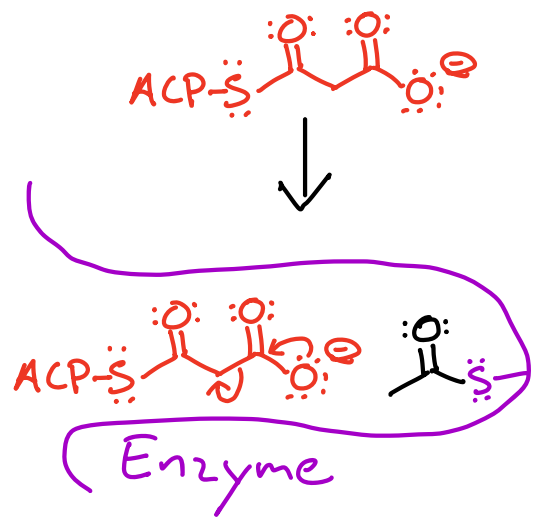
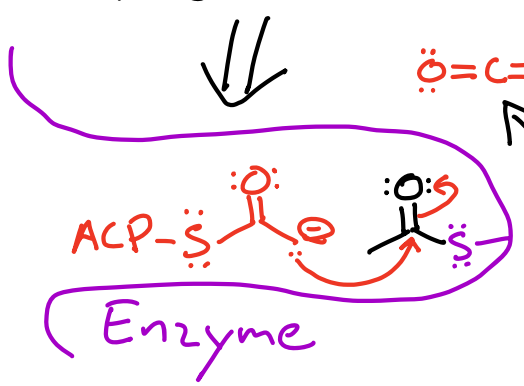
These are used to make C-C bonds in a reaction catalyzed by an enzyme that resembles a Claisen!

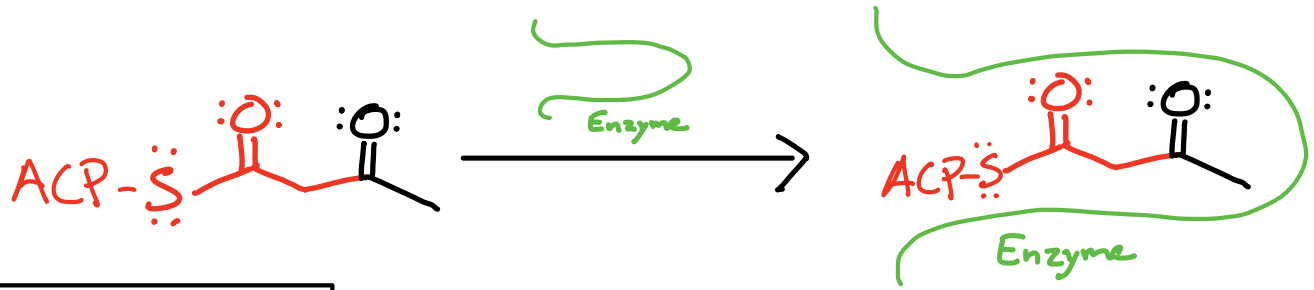


ACP = Acyl Carrier Protein



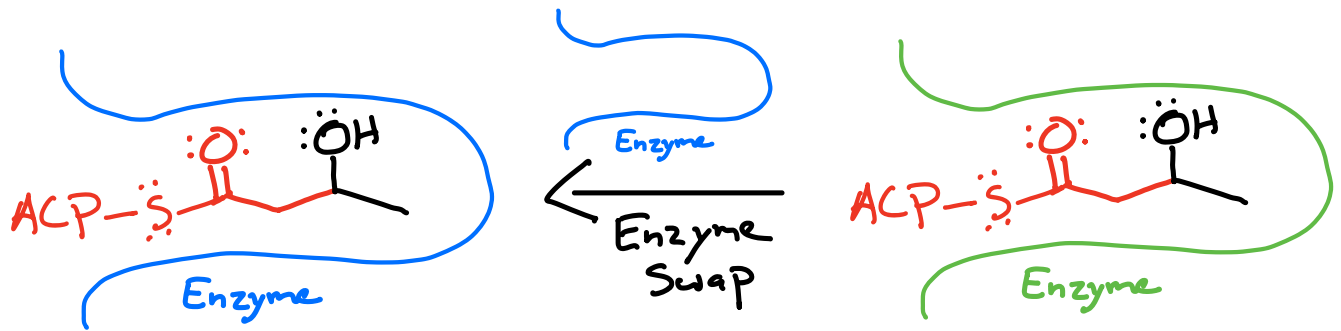
An enolate!



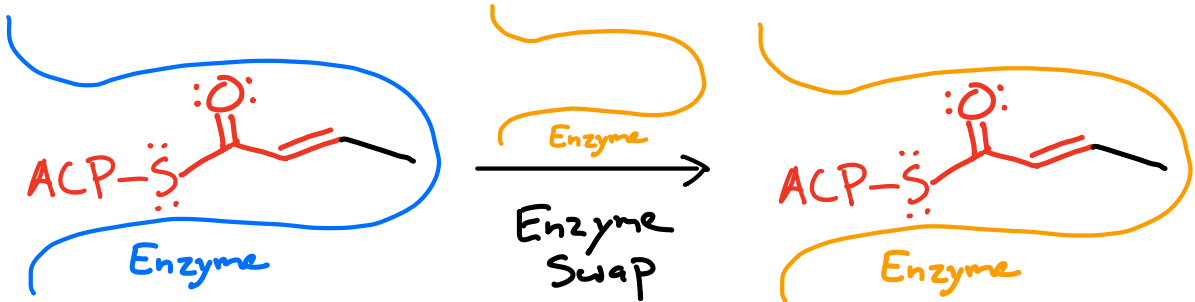


The substrate is handed off from one enzyme to another

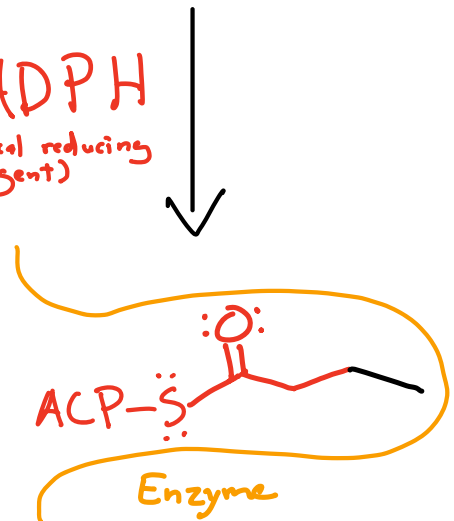
$\text{NADPH}$   
 (biological reducing agent)

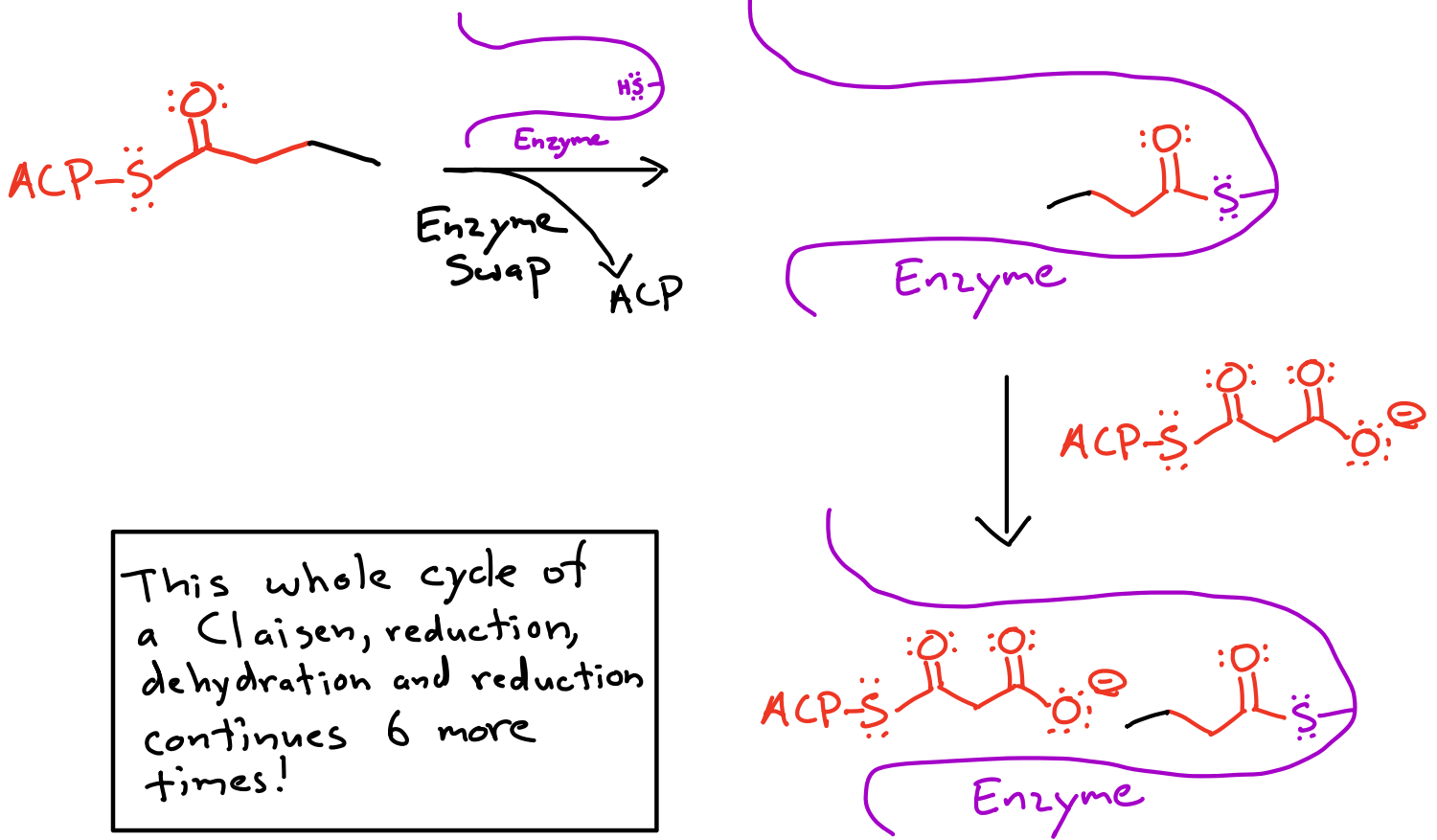


Dehydration

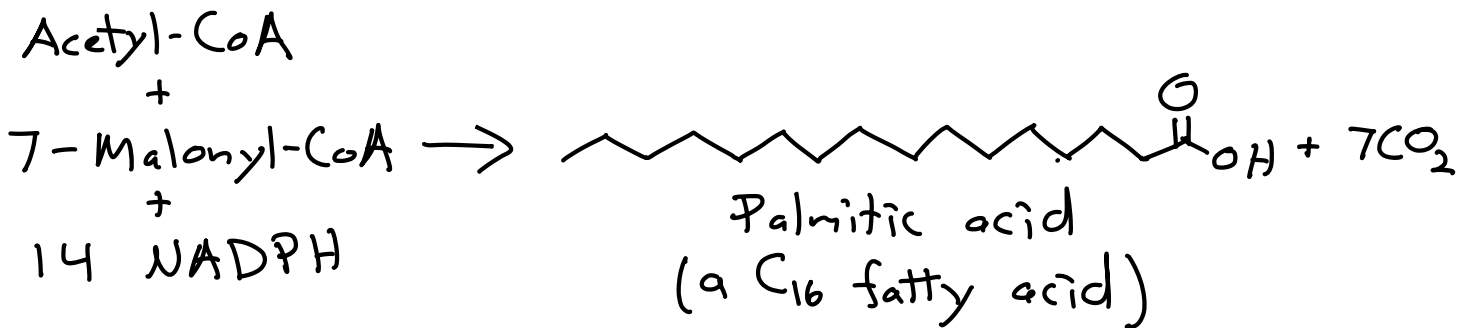


$\text{NADPH}$   
 (biological reducing agent)





## Overall Process:



Major Take Home Lesson

The chemistry of biological systems resembles the chemistry we have learned!

You are now well-prepared to crush biochemistry!



# How do energy drinks work?



Nutrition Facts	
1 Serving Per Container	
Serving Size 12 fl. oz (355mL)	
Amount Per Serving	10
Calories	%Daily Value*
Total Fat 0mg	0%
Sodium 5mg	0%
Total Carbohydrate 0mg	0%
Protein 0mg	
Calcium 50mg	4%
Vitamin C 60mg	70%
Riboflavin 1.7mg	130%
Niacin 20mg	130%
Vitamin B6 2mg	120%
Vitamin B12 6mcg	250%
Biotin 300mcg	1000%
Pantothenic Acid 10mg	200%
Chromium 50mcg	140%

Not a significant source of sat fat, trans fat, cholesterol, dietary fiber, total sugar, added sugars, vitamin D, iron, and potassium.

The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Caffeine Content: 200mg

**US BANG ENERGY BLUE RAZZ 16OZ. CAN**  
CONTAINS NO FRUIT JUICE

Nutrition Facts	
Serving size 1 can	
Amount per serving	
Calories 0	
	% Daily Value*
Total Fat 0g	0%
Sodium 40mg	2%
Total Carbohydrate 2g	1%
Total Sugars 0g	0%
Includes 0g Added Sugars	
<b>Protein 0g</b>	
Potassium 90mg 2%	Vitamin C 30%
Niacin 30% •	Vitamin B6 30%
Vitamin B12 60% •	Magnesium 2%

Not a significant source of saturated fat, trans fat, cholesterol, dietary fiber, vitamin D, calcium and iron.

\*The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

INGREDIENTS: CARBONATED WATER, CITRIC ACID, (pH CONTROL), NATURAL & ARTIFICIAL FLAVORS, CAFFEINE, SODIUM BENZOATE (PRESERVATIVE), POTASSIUM CITRATE, SUCRALOSE, ESSENTIAL AMINO ACIDS (L-LEUCINE, L-ISOLEUCINE, L-VALINE, L-THREONINE, POTASSIUM PHOSPHATE, ASCORBIC ACID (VIT. C), ACESULFAME POTASSIUM, POTASSIUM SORBATE (PRESERVATIVE), MAGNESIUM CHLORIDE, CALCIUM CHLORIDE, CALCIUM DOSODIUM EDTA, NIACINAMIDE (VIT. B3), COENZYMES B-12, PYRIDOXINE HYDROCHLORIDE (VIT. B6), METHYLCOBALAM (VIT. B12).

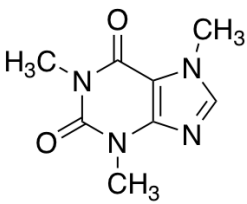
MANUFACTURED FOR BANG ENERGY LLC, 2380 ANSELMO DRIVE, CORONA, CA 92709 U.S.A.

©2023 BANG ENERGY LLC

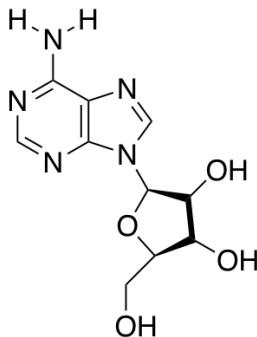
## CAFFEINE IN ENERGY DRINKS

INCLUDING CALORIES & SUGAR CONTENT

<b>3D</b> 200mg CAFFEINE 15 CALORIES 7g CARBS 0g SUGAR	<b>5 HOUR ENERGY SHOT</b> 200mg CAFFEINE 4 CALORIES 1g CARBS 0g SUGAR	<b>ALANI NU</b> 200mg CAFFEINE 10 CALORIES 6g CARBS 0g SUGAR	<b>BANG</b> 300mg CAFFEINE 0 CALORIES 0g CARBS 0g SUGAR
<b>BEYOND RAW LIT</b> 250mg CAFFEINE 20 CALORIES 4g CARBS 0g SUGAR	<b>C4 ENERGY</b> 200mg CAFFEINE 0 CALORIES 0g CARBS 0g SUGAR	<b>CELSIUS</b> 200mg CAFFEINE 10 CALORIES 2g CARBS 0g SUGAR	<b>GHOST</b> 200mg CAFFEINE 5 CALORIES 1g CARBS 0g SUGAR
<b>MTN DEW RISE</b> 180mg CAFFEINE 25 CALORIES 5g CARBS 3g SUGAR	<b>MONSTER</b> 160mg CAFFEINE 230 CALORIES 58g CARBS 54g SUGAR	<b>MONSTER ZERO SUGAR</b> 140mg CAFFEINE 10 CALORIES 3g CARBS 0g SUGAR	<b>NOCCO</b> 180mg CAFFEINE 12 CALORIES 0g CARBS 0g SUGAR
<b>NOS</b> 160mg CAFFEINE 200 CALORIES 54g CARBS 54g SUGAR	<b>REIGN</b> 300mg CAFFEINE 10 CALORIES 3g CARBS 0g SUGAR	<b>RED BULL</b> 80mg CAFFEINE 110 CALORIES 29g CARBS 27g SUGAR	<b>RED BULL SUGARFREE</b> 80mg CAFFEINE 10 CALORIES 2g CARBS 0g SUGAR
<b>ROCKSTAR</b> 160mg CAFFEINE 250 CALORIES 63g CARBS 63g SUGAR	<b>ROCKSTAR SUGAR-FREE</b> 160mg CAFFEINE 25 CALORIES 1g CARBS 0g SUGAR	<b>ZEVIA</b> 120mg CAFFEINE 0 CALORIES 0g CARBS 0g SUGAR	<b>ZOA</b> 160mg CAFFEINE 15 CALORIES 3g CARBS 0g SUGAR




Caffeine

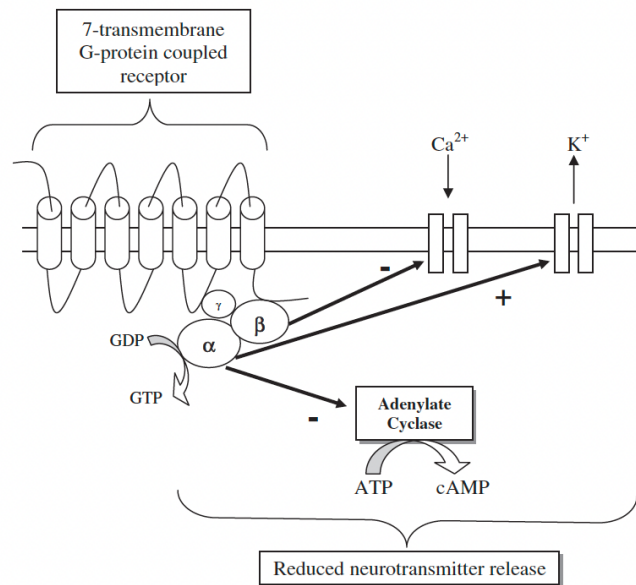
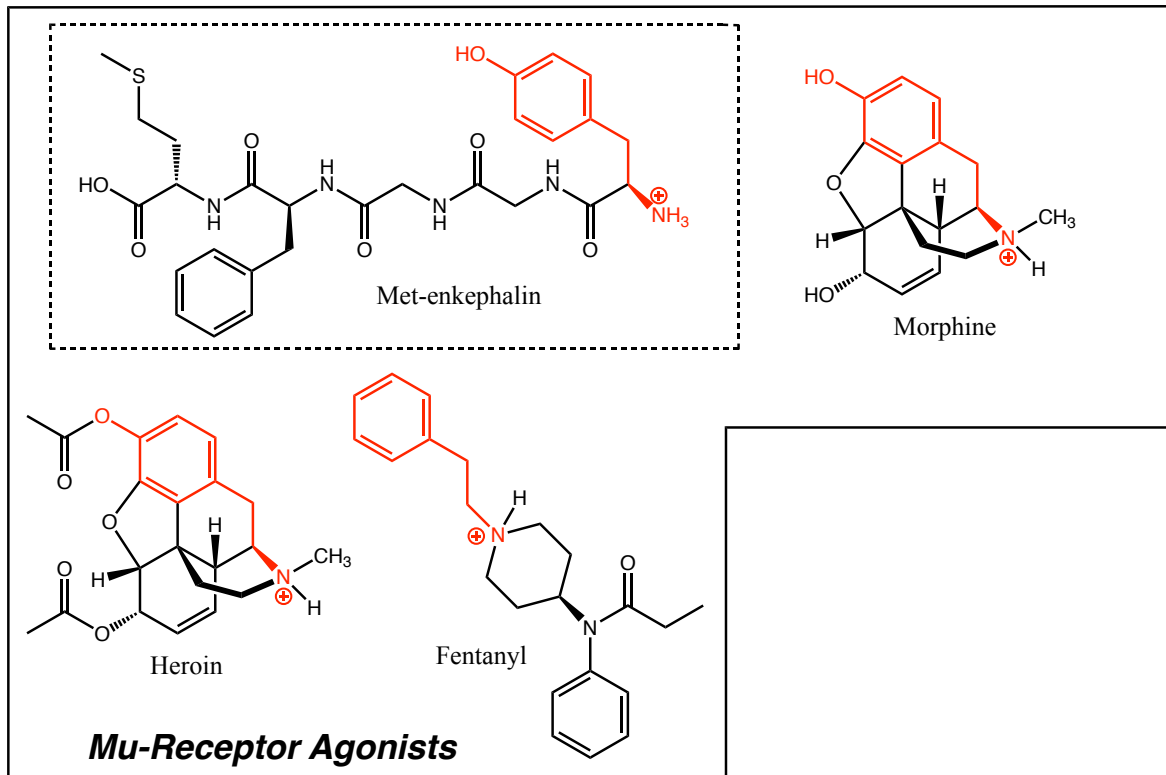


Adenosine

Coffee and tea drinks (coffee shops)	Serving size	Caffeine (mg)
Starbucks Coffee	20 oz. venti	390-490
Dutch Bros 9-1-1 Blended Freeze	large	440
Starbucks Coffee	16 oz. grande	315-390
Starbucks Iced Coffee	30 oz. trenta	320
Caribou Coffee	medium	305
Dunkin' Frozen Coffee	medium	295
Starbucks Coffee	12 oz. tall	235-290
Dunkin' Coffee	20 oz. large	270
Starbucks Caffè Americano	16 oz. grande	225
Dunkin' Coffee	14 oz. medium	210
Starbucks Cold Brew	16 oz. grande	205
Starbucks Flat White	16 oz. grande or 20 oz. venti	195

Antagonist  Caffeine → Turns off your  
 binds where binds because it  
 to signal low and  
 therefore .

Vitamins B<sub>6</sub> and B<sub>12</sub> → stimulate  
 production of in your body  
 so you have available



**Fig. 1** Seven transmembrane structure of opioid G-protein-coupled receptor. Receptor activation by opioid receptor ligands leads to initiation of intracellular transduction pathways that include stimulation of potassium efflux, inhibition of VSCCs and inhibition of adenylyl cyclase. In this diagram the G-protein is denoted  $\alpha$ ,  $\beta$ ,  $\gamma$  but the  $\alpha$ -subunit interacts with  $K^+/Ca^{2+}$  channel and adenylyl cyclase.

Fentanyl activates  $\mu$ -opioid receptors (Gi/o-coupled) on GABAergic interneurons in the VTA, decreasing their cAMP levels and suppressing GABA release.

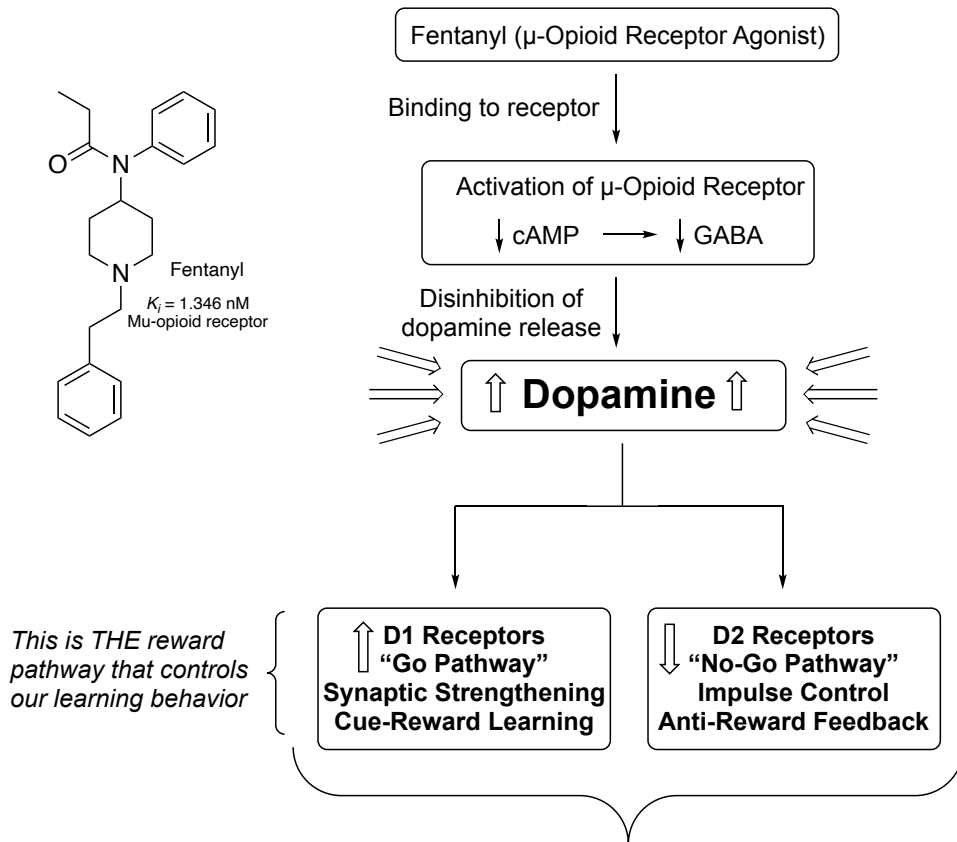
This disinhibits dopamine neurons and drives a surge of dopamine release into the nucleus accumbens.

Dopamine then acts on **D1 receptors** (Gs-coupled) to increase cAMP/PKA/CREB signaling, producing synaptic potentiation and strong reinforcement of fentanyl-associated cues.

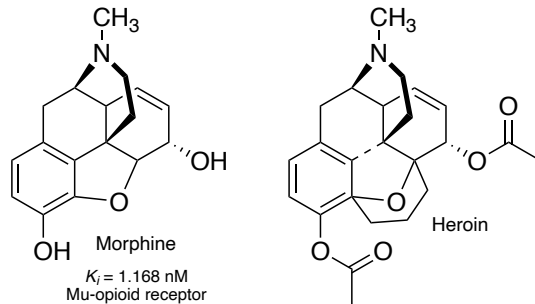
In parallel, chronic fentanyl use **downregulates D2 receptor** (Gi/o) signaling, which normally provides inhibitory control and reduces compulsive reward seeking.

The combined effect—**D1 sensitization plus D2 suppression**—shifts basal ganglia signaling toward reward acquisition, producing the neurochemical basis of fentanyl addiction and relapse vulnerability.

Fentanyl triggers addiction by disrupting the brain's normal reward and control systems. It activates opioid receptors in a part of the brain that normally keeps dopamine release in check, effectively **removing the brakes** and causing a strong surge of dopamine—the chemical that signals pleasure and reward. This dopamine then acts on two types of receptors. **D1 receptors** amplify the brain's "go" signals, strengthening the memory and motivation to use the drug again. **D2 receptors**, which normally help with self-control and resisting impulses, become less active with repeated fentanyl use. As D1 activity becomes stronger and D2 activity becomes weaker, the brain becomes increasingly wired to seek fentanyl while losing the ability to hold back, creating the powerful cycle of addiction.



**An individual is driven to pursue the drug and cannot anticipate negative consequences of drug use**



HEALTH

## Why fentanyl is deadlier than heroin, in a single photo

By Allison Bond Sept. 29, 2016

[Reprints](#)



Menu

# ONE PILL CAN KILL

## DEA Fentanyl Seizures in 2024

In 2023, DEA seized more than 79.5 million fentanyl-laced fake pills and nearly 12,000 pounds of fentanyl powder. The 2023 seizures are equivalent to more than 376.7 million lethal doses of fentanyl.

The 2024 fentanyl seizures represent over 93.7 million deadly doses. \*

17,900,000+

1,813+ lbs.

Millions of Fentanyl  
Pills Seized

Pounds of Fentanyl  
Powder Seized

\* 2 mg of fentanyl equates to a potentially deadly dose





PRESS RELEASE

# 13 Arrested in Connection with an LSD, Fentanyl and Methamphetamine Trafficking and Money Laundering Scheme Occurring in the West Campus Area of the University of Texas at Austin

Friday, December 4, 2020



For Immediate Release

U.S. Attorney's Office, Western District of Texas



[Click to download the full image](#)

Authentic Xanax® Front and Back



[Click to download the full image](#)

Counterfeit Xanax® Front and Back

# Jake Ehlinger's family releases statement saying Texas player died of accidental overdose



**Brian Davis**

Hookem

Published 4:56 p.m. CT Oct. 21, 2021 | Updated 3:54 p.m. CT Oct. 22, 2021



## Remembering Texas linebacker Jake Ehlinger

Jake Ehlinger, younger brother of former Longhorns quarterback Sam Ehlinger, was found dead on May 6, Austin police said. *Austin American-Statesman*

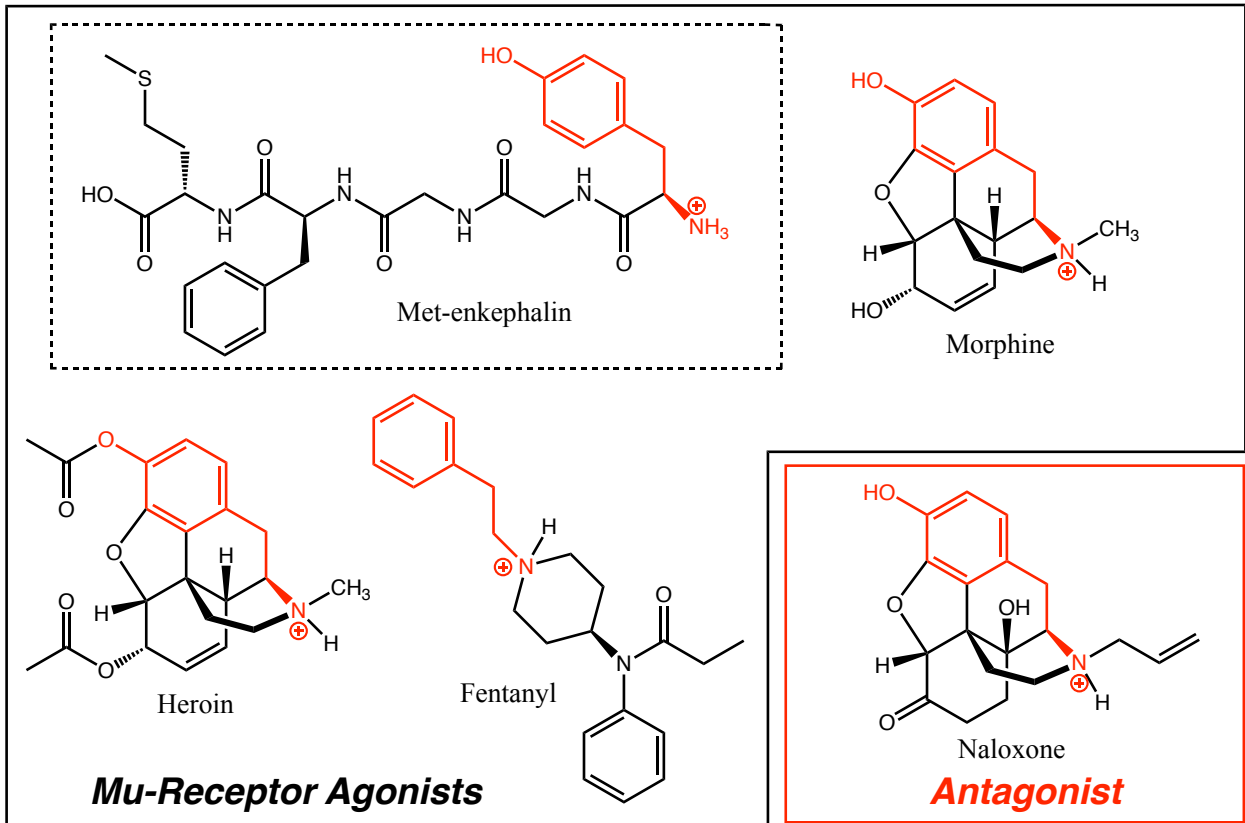


# SHIFT is ready for a different conversation.

For decades substance use and college campuses have been talked about as an inevitable rite of passage for college students, creating a norm that can far out shadow the dynamic pursuits of college students that revolve around academics and future opportunities.

SHIFT engages the community in dialogue that changes the culture of campus substance use from one of misuse to one of well-being.





## NARCAN<sup>®</sup> NASAL SPRAY – ANYONE CAN SAVE A LIFE

Be ready – at home or on the go. NARCAN<sup>®</sup> Nasal Spray is a treatment designed to rapidly reverse the effects of a life-threatening opioid emergency. Available as an over-the-counter medicine.



NARCAN<sup>®</sup>

Naloxone HCl Nasal Spray 4 mg

Emergency Treatment of Opioid Overdose

Original Prescription Strength

Easy to Use

Can Save a Life

Designed to Rapidly Reverse the Effects of a Life-Threatening Opioid Emergency

2 Single-Dose Nasal Spray Devices  
0.003 fl oz (0.1 mL) each

For use in nose only

## Should I carry naloxone?

Sometimes it can be difficult to imagine how one person can have an impact on the culture of substance use – but you can! By carrying naloxone and learning the right way to administer it to someone having an opioid overdose, you have the potential to save a life. Even if you don't personally know anyone using opioids, you may find yourself in a situation where having naloxone on hand could make a huge difference. By showing that you care and taking the time to learn, you're helping to raise awareness about how important it is for each of us to play a part in shifting the culture of substance use.

## How do I use naloxone?

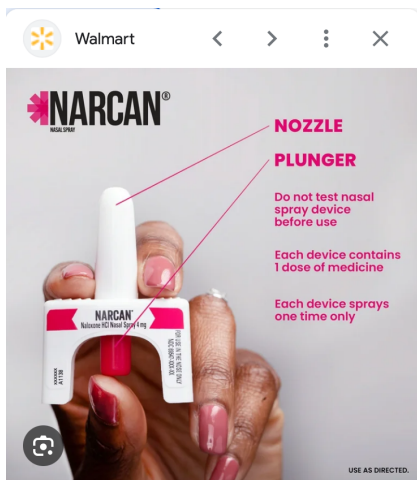
Okay, so now you know what naloxone is, and why it's so important – but how do you actually use it on someone experiencing an opioid overdose? Great question – luckily, Operation Naloxone at UT provides free trainings for students, staff, and faculty. Email [shift@utexas.edu](mailto:shift@utexas.edu) for more information. [Request an Operation Naloxone training.](#)

## How do you administer the nasal spray version of naloxone (Narcan)?

- Open the Narcan package, place the nozzle in the person's nostril and press the plunger.
- [View the CDC video](#) on how to administer Narcan.

## Where can I find naloxone?

- Naloxone is available for distribution to all students, faculty, and staff at the Perry-Casteneda Library security front desk, the Longhorn Wellness Center (SSB 1.106), and the Center for Students in Recovery (Belmont 222).
- Naloxone is available for emergency access at all residence hall front desks, Sid Richardson Library, the Life Sciences Library, the Perry-Casteneda Library, and through UTPD.
- Many pharmacies dispense naloxone without a prescription, but there may be a copay depending on the insurer. You can call your insurance provider in advance to learn more about the potential copay cost.
- In Texas, you can request free naloxone via mail at [MoreNarcanPlease.com](http://MoreNarcanPlease.com).
- [Map of free naloxone access sites in Austin.](#)



NARCAN Nasal Spray 4 mg, Emergency Treatment of...

[Visit >](#)

Images may be subject to copyright. [Learn More](#)

## Availability of Narcan on campus:

**EMERGENCY USE:** Originally placed in all residence halls; carried by UTPD; in the libraries across campus, Sid Richardson Hall, (Strategically covers all areas of our large campus)

**DISTRIBUTION:** Original site was the main PCL library (late night center of social/academic activity); Anyone can obtain a free box of Narcan Nasal Spray (2 doses) from the security desk no questions asked.

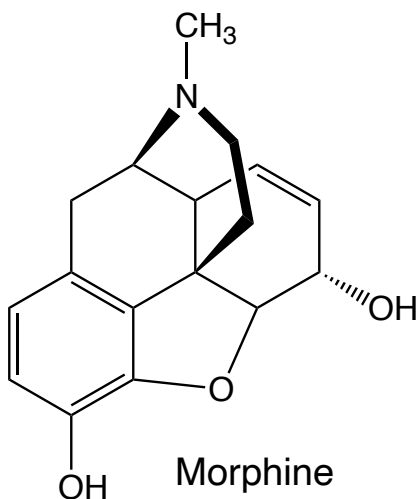
Hi Dr. Iverson,

I graduated with a BSA in Neuroscience in May 2023, and you were my organic chemistry professor during COVID! During your class, you shared about Operation Naloxone at UT Austin which was instrumental in my life when I was in need of Narcan a few years later for my loved one battling addiction...

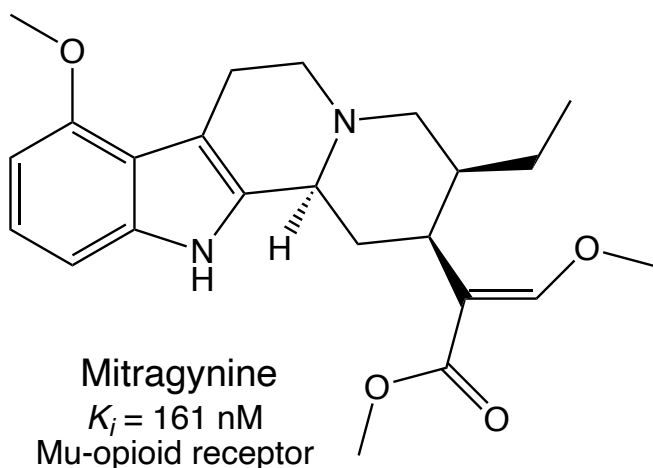
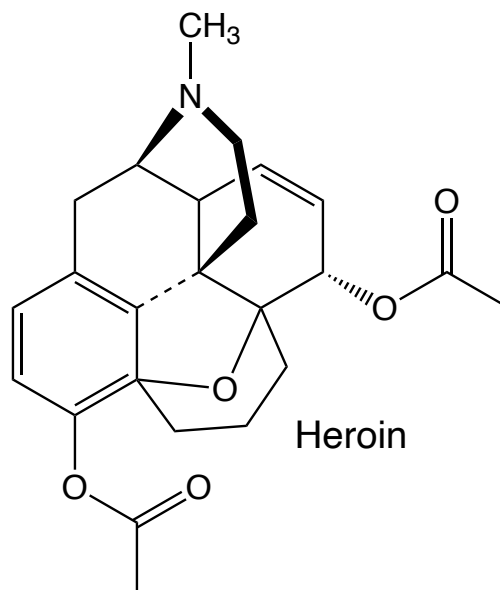
Day 1 was the day I found out he was using drugs recreationally since the age of 13. Year 6 was the year I accepted his substance abuse as a progressive illness. The darkness that lured him in changed my life forever when he disappeared one night and under the influence called our mom to let her know he was lost, the call disconnecting shortly after. I was afraid to know the substances he used, but one day with my heart ringing in my ears, I asked. This is how I knew I needed to request narcan from the free distribution services at my university that night. I tracked his last location on our shared family app and without knowing where or to what I was headed my mom and I drove to him. With shaking hands I read the narcan instructions and prepared for the worst. In the distance, I could see my brother's car and I could see his face and head resting back onto the driver's seat. The rest of the event's that night are oddly blurry yet so clearly ingrained in my memory. I remember having to put his car in park, trying to wake him up unsuccessfully, shouting out to my mom to call 911, using the Narcan, his eyes suddenly opening wide and him taking a deep breath in. Today, my brother is recovering after years of battling anxiety, depression, and substance abuse. His experiences from childhood to adulthood have been a driving force in my research endeavors and education in neuroscience.

I am grateful for the impact you have had on my academic journey and wanted to share this part of my story with you. Thank you for your inspiration and guidance!

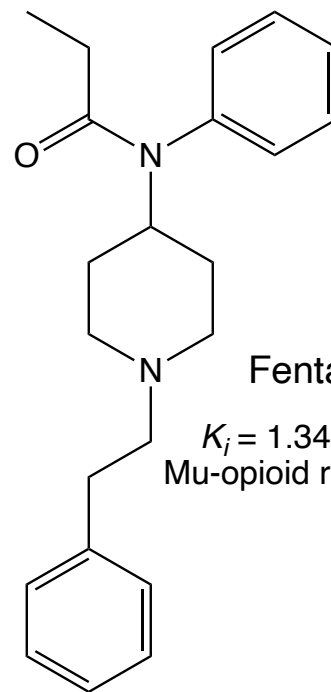
Best,



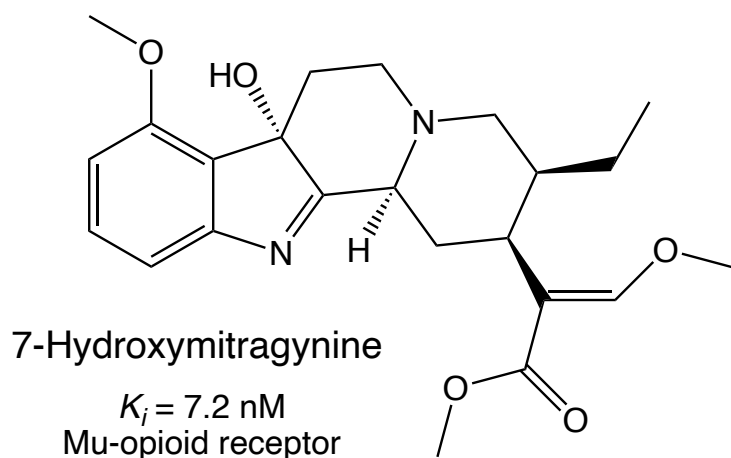
$K_i = 1.168$  nM  
Mu-opioid receptor



$K_i = 161$  nM  
Mu-opioid receptor



$K_i = 1.346$  nM  
Mu-opioid receptor



$K_i = 7.2$  nM  
Mu-opioid receptor

Regulatory Toxicology and  
Pharmacology  
Volume 59, Issue 3, April 2011,  
Pages 385-390

ACS Chem. Neurosci. 2023, 14,  
195-197



SHOP ONLINE ▼

STORE LOCATOR

LEARN ▼

PRESS



## THE HOTTEST SALE OF THE SUMMER

New subscription customers  
**GET \$80 OFF** the first two  
months (total) with code **HOT**

SHOP NOW



"The head is affected pleasantly; you feel friendly, not beer sentimental; you cannot hate with kava in you. Kava quiets the mind; the world gains no new color or rose tint; it fits in its place and in one easily understandable whole."

- Tom Harrison, author

### QUALITY CONTROLLED



Raw material, in-process, finished product, and product stability testing performed by trained quality control scientists.

### CERTIFIED LAB TESTED



Each batch's ingredients and final product pass multiple tests for active ingredient consistency and contaminant safety.

### MADE IN THE USA



Quality ingredients sourced from around the world, manufactured in an FDA-regulated facility in Tulsa, Oklahoma.

### What is Kava?

Kava, a member of the pepper family, is a traditional drink made from the roots of the kava plant. It has been enjoyed for centuries by the indigenous people of the South Pacific as a social and ceremonial beverage. Kava is known for its relaxing and calming effect on the mind and body.



# Kava-Forward Tonics and Supplements

Thoughtfully crafted to help you feel good.

SHOP NOW →

enjoy *feel free*® products when you want:



A CLEAN ENERGY BOOST WITHOUT THE JITTERS



A SOCIAL LIFT WITHOUT THE BOOZE (OR HANGOVER)



A FOCUS ENHANCEMENT WITHOUT THE CRASH

shop *feel free*® products



feel free tonic

SHOP NOW



CLASSIC tonic

SHOP NOW



CLASSIC capsules

SHOP NOW



NATURALLY CAFFEINATED

## feel free® tonic

Our newest feel-good wellness tonic is a kava drink made with kava, kola nut, lion's mane and rhodiola. These ingredients are known to enhance relaxation, cognitive function, and productivity.\* *feel free* is naturally caffeinated with 100mgs of caffeine per serving to boost and sustain your energy without the crash.

12-Pack Case (24 Servings) | 2oz (59ml) Bottles

<input checked="" type="radio"/>	<b>Subscribe &amp; Save</b>	<b>\$105</b>	<b>SAVE 29%</b>
<input checked="" type="checkbox"/>	Instant 29% savings ( <del>\$147</del> \$105)		
<input checked="" type="checkbox"/>	Delivery every 30 days		
<input checked="" type="checkbox"/>	Cancel / pause your monthly delivery anytime		
<input type="radio"/>	<b>One-Time Purchase</b>	<b>\$147</b>	

**Subscribe & Save | \$105**

- Ships within 5 business days
- Free shipping for every U.S. order
- Money back guarantee

GET \$40 OFF ✕

Free shipping on all feel free orders →



THE MINIMUM AGE REQUIRED TO PURCHASE AND CONSUME THIS PRODUCT MAY BE 18 OR 21 DEPENDING ON THE JURISDICTION. SHAKE WELL. REFRIGERATE AFTER OPENING.

Supplement Facts	Amount Per Serving	%DV
Serving Size: 1 oz (29.5 mL) Servings Per Container: 2		
Iron	0.4 mg	4%*
Kava root (extract)		
Total Kavalactones	260 mg	*
Kratom leaf (ground)		
Total Alkaloids	34 mg	*
Mitragynine	20 mg	*
7-hydroxymitragynine	<0.05 mg	*

\*Daily Value (DV) based on a 2000 calorie diet  
\*Daily Value (DV) not established

**Other ingredients:** water, pineapple juice, natural flavors, stevia leaf, citric acid.

**RECOMMENDED USE:** TAKE 1 OZ BY MOUTH. DO NOT EXCEED 2 OZ IN A 24-HOUR PERIOD.

**Note:** Anything that makes you feel good can become habit-forming including alcohol, caffeine, sugar, and kratom. Only use *feel free* CLASSIC in the recommended quantity and dosage. If consumed responsibly, *feel free* CLASSIC has not been shown to cause any serious physical or social harm. Caution: This product is not for use by or for sale to persons under the age of 18, or in some jurisdictions, 21. Consult a healthcare professional before taking any dietary supplement. Do not consume more than one serving at a time and no more than two servings per day. This product is not recommended for pregnant or nursing women or persons who are sensitive to the active ingredients like kava. Do not consume with excessive alcohol.

**These statements have not been evaluated by the FDA. This product is not intended to diagnose, treat, mitigate, cure, or prevent any disease or illness.**

BOTANIC TONICS, LLC  
13105 E. 61ST STREET, SUITE B • BROKEN ARROW, OK 74012

CAFFEINE FREE

## feel free® CLASSIC tonic

Our original feel-good wellness tonic is a kava drink made with kava and other ancient plants known to help with relaxation, productivity and focus.\* These plants hail from the South Pacific and Southeast Asia, where they've been used socially and in wellness for centuries.

12-Pack Case (24 Servings) | 2oz (59ml) Bottles

<input checked="" type="radio"/>	<b>Subscribe &amp; Save</b>	<b>\$105</b>	<b>SAVE 29%</b>
<input checked="" type="checkbox"/>	Instant 29% savings ( <del>\$147</del> \$105)		
<input checked="" type="checkbox"/>	Delivery every 30 days		
<input checked="" type="checkbox"/>	Cancel / pause your monthly delivery anytime		
<input type="radio"/>	<b>One-Time Purchase</b>	<b>\$147</b>	

**Subscribe & Save | \$105**

- Ships within 5 business days
- Free shipping for every U.S. order
- Money back guarantee

GET \$40 OFF ✕



## Federal authorities seize over \$3 million worth of kratom products

by Alexandria Tafoya, KTUL Staff | Tue, May 2nd 2023, 7:21 PM CDT



The Oklahoma state is seen at the Capitol in Oklahoma City on Feb. 3, 2020. (KTUL photo)




TULSA, Okla. (KTUL) — Northern District of Oklahoma reported on Tuesday that United States Marshals and investigators from the U.S. Food and Drug Administration seized over \$3 million worth of products containing Kratom.

According to the report, more than 250,000 units of dietary supplements and bulk dietary ingredients seized were or contained kratom, including more than 1,000 kilograms of bulk kratom.

The seized kratom products were manufactured by Botanic Tonics, LLC of Broken Arrow, and are marketed under the brand name "Feel Free Plant Based Herbal Supplement."

ADVERTISEMENT



**Parents love working for the School District of Palm Beach County!**

- Competitive salaries
- Comprehensive benefits packages
- Work-life balance
- Lots of available positions!

Join the family! [palmbeachschools.org/careers](https://palmbeachschools.org/careers)

NEWS > PALM BEACH COUNTY > REGION 5 PALM BEACH COUNTY > BOYNTON BEACH



# Judge awards \$11 million to family in death of Boynton Beach woman from kratom

Krystal Talavera, a mother of four who lived in Boynton Beach, died in 2021



TALK TO AN ANCHOR

**ONE ON ONE**

LET'S HEAR IT!

WASHINGTON STATE

## Dad found dead in living room after using kratom, lawsuit says. Jury awards family \$2.5M

BY JULIA MARNIN

UPDATED JULY 24, 2023 1:05 PM







**S+David's**  
**HEALTHCARE**

2ND  
**17** **11:34** **0**  
DOWN TO GO BALL ON

**DALL Technologies**

21	TOTAL PLAYS	14
246	TOTAL YARDS	26
8	NET DOWNS	2
10:31	TOP	7:55

*feel free*  
**CELEBRATION**



**THANK YOU FOR YOUR SERVICE**

**TCU** **TCU** **28**  
**TEXAS TECH** **35**

**MYERS-BONDS LEGACY CLUB**

**25**

**15 MOOREHEAD COMPLEX**

*feel free.*

[SHOP ALL](#) [STORE LOCATOR](#) [LEARN](#) ▼



Ships within 5 business days

Free shipping for every U.S. order

Money back guarantee



PLANT BASED



100% VEGAN



LAB TESTED



KOSHER CERTIFIED



ALCOHOL FREE



NUT FREE



GLUTEN FREE



**“It gives a very nice light feeling,  
like life is a little more effortless”**



**Official Tonic of  
FSU ATHLETICS**

**Official Tonic Sponsor of  
TEXAS  
ATHLETICS**

Hi Dr. Iverson,

I graduated with a BSA in Neuroscience in May 2023, and you were my organic chemistry professor during COVID! During your class, you shared about Operation Naloxone at UT Austin which turned out to be instrumental in my life when I was in need of Narcan a few years later for my loved one battling addiction. I am currently reflecting about this experience in my personal statement, and I thought I'd send you this paragraph from my rough draft:

Day 1 was the day I found out he was using drugs recreationally since the age of 13. Year 6 was the year I accepted his substance abuse as a progressive illness. The darkness that lured him in changed my life forever when he disappeared one night and under the influence called our mom to let her know he was lost, the call disconnecting shortly after. I was afraid to know the substances he used, but one day with my heart ringing in my ears, I asked. This is how I knew I needed to request Narcan from the free distribution services that you had told us about in OChem class. I tracked his last location on our shared family app and without knowing where or to what I was headed my mom and I drove to him. With shaking hands I read the Narcan instructions and prepared for the worst. In the distance, I could see my brother's car and I could see his face and head resting back onto the driver's seat. The rest of the events that night are oddly blurry yet so clearly ingrained in my memory. I remember having to put his car in park, trying to wake him up unsuccessfully, shouting out to my mom to call 911, using the Narcan, his eyes suddenly opening wide and him taking a deep breath in. Today, my brother is recovering after years of battling anxiety, depression, and substance abuse. His experiences from childhood to adulthood have been a driving force in my research endeavors and education in neuroscience.

I am grateful for the impact you have had on my academic journey and wanted to share this part of my story with you. Thank you for your inspiration and guidance!

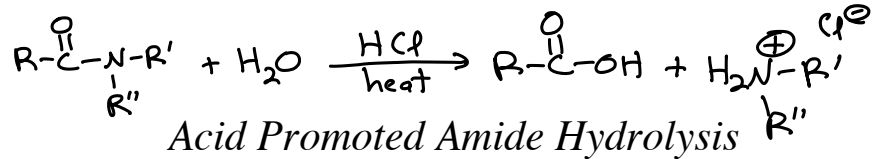
# Background on the HIV virus → AIDS

- 1) HIV is a retrovirus → single strand of RNA inside a protein coat → codes for 19 proteins
- 2) HIV infects cells of the immune system → HIV binds to specific receptors on these cells
- 3) Once inside the cell the RNA is reverse transcribed to DNA by a viral enzyme.  
"Reverse transcriptase"
- 4) The DNA is integrated into the host genome by another viral enzyme.  
"Integrase"  
→ The viral DNA remains inactive for some time within the DNA genome.

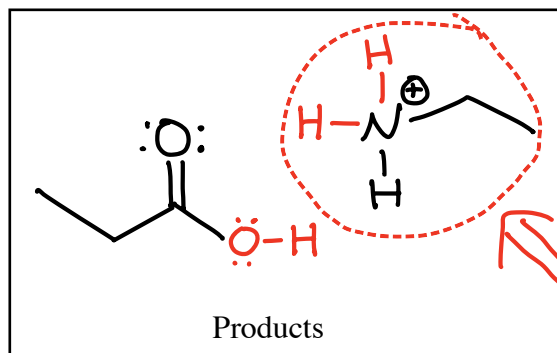
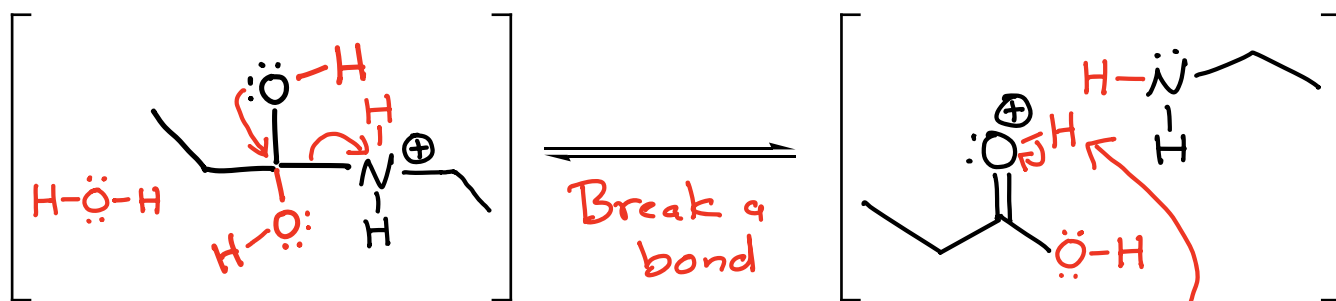
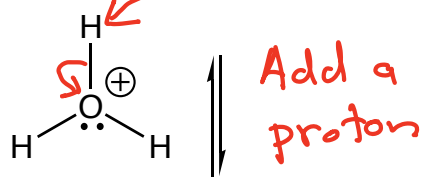
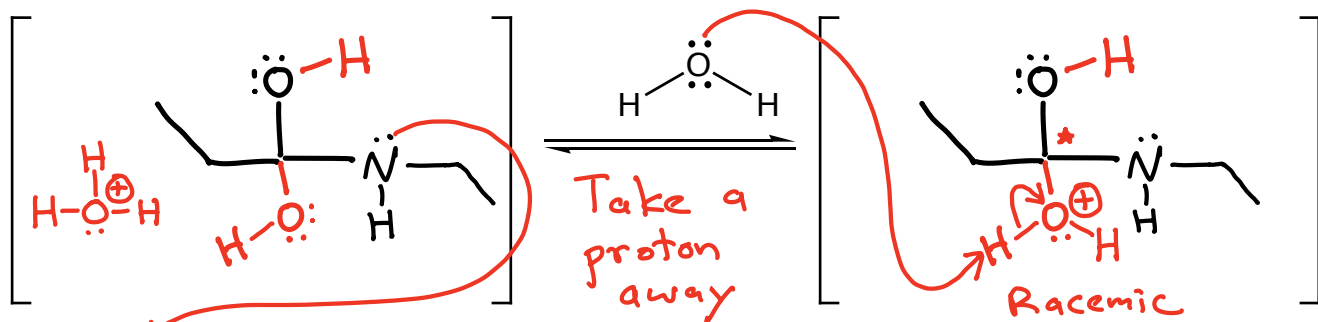
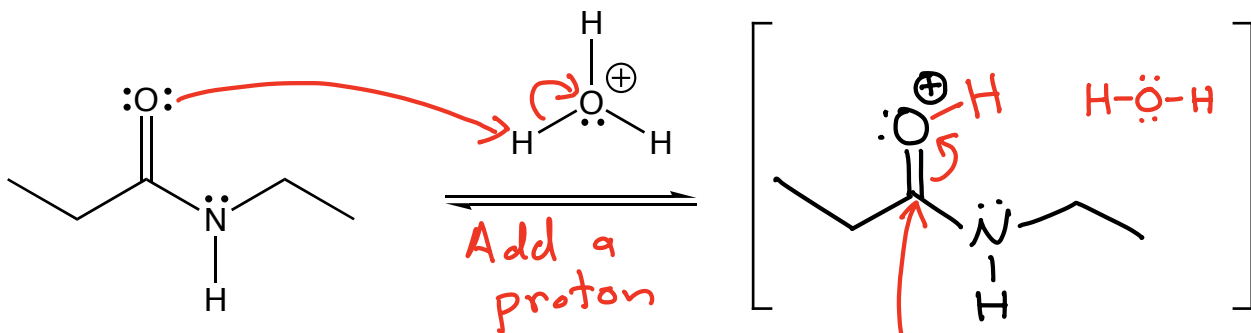
5) After an unknown signal, the viral DNA becomes active and produces a single long mRNA, that produces a very long HIV single protein. → called a polyprotein

6) The HIV polyprotein is cleaved into individual proteins by another viral enzyme called the AIDS protease → cleaves amide bonds at the correct sequences. → has two carboxylic acids (Asp) at the correct location to cleave the amide bond. → called an aspartyl protease because of the two key carboxylic acid groups on aspartic acid amino acids (Asp).

Enzymes  $\rightarrow$  catalyze reactions by having an active site that places acids, bases nucleophiles and sometimes metal atoms in exactly the correct 3-d space to make the reaction happen  $\rightarrow$  the active sites also lower the energy of reactions by stabilizing high energy intermediates

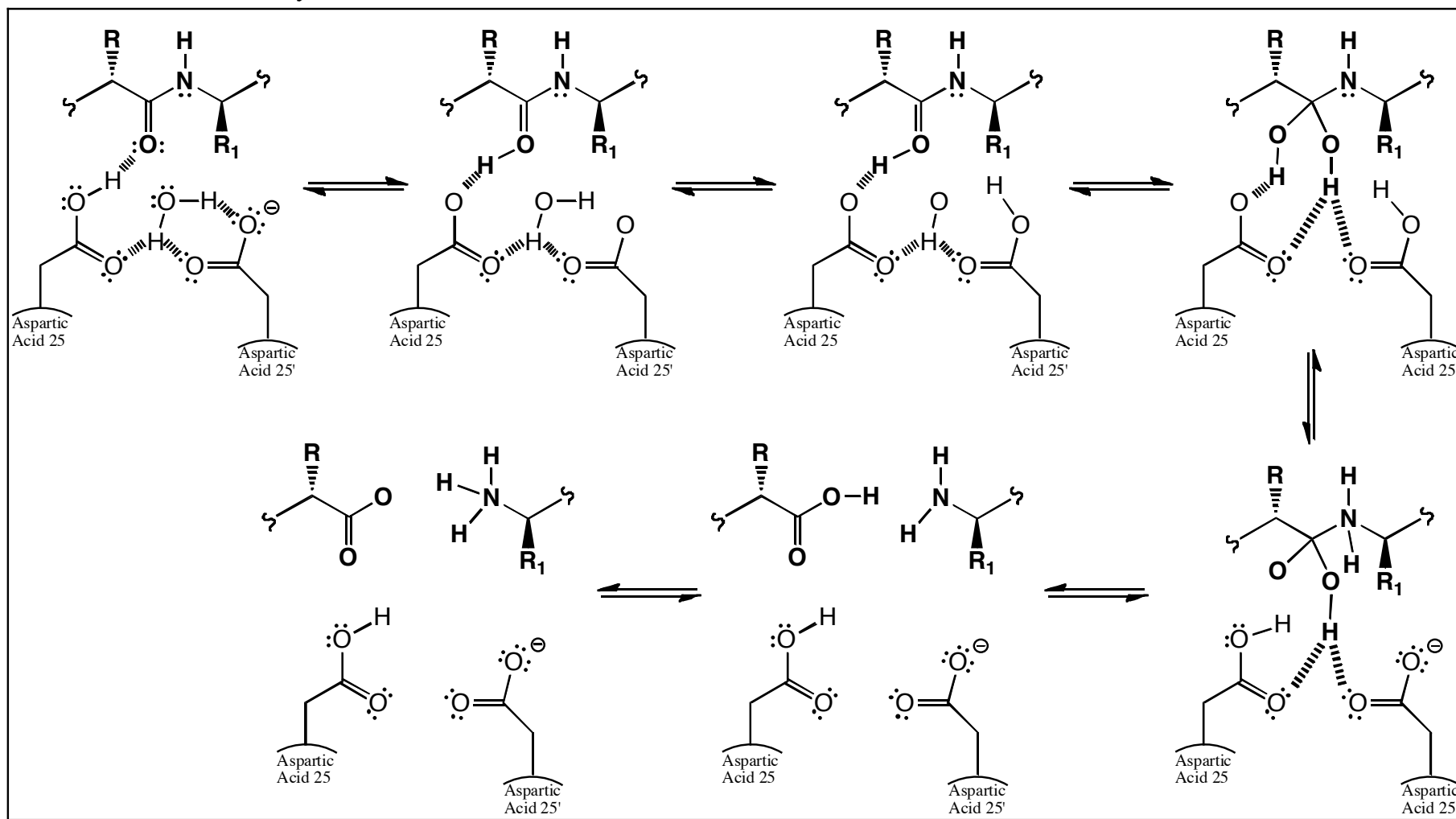
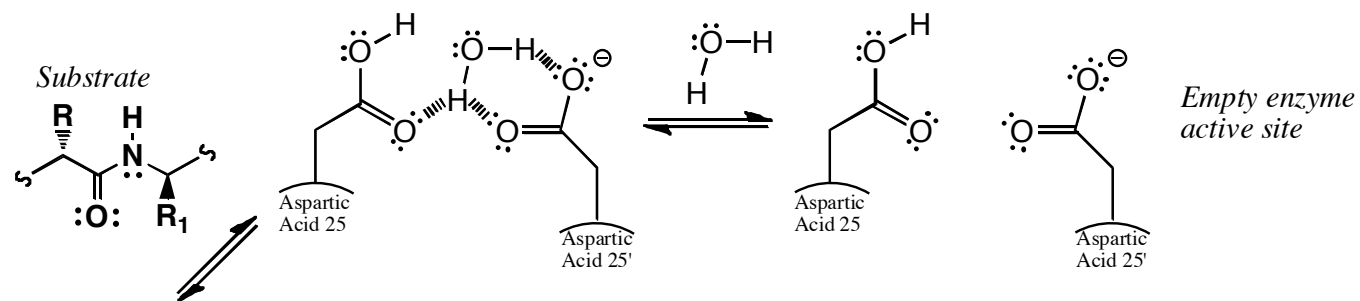


Acid Promoted Amide Hydrolysis



Take a proton away

This is NOT  $H_3O^+$ ,  
this reaction is  
NOT catalytic in  
acid



# HIV-1 protease: mechanism and drug discovery

Ashraf Brik and Chi-Huey Wong\*

Department of Chemistry and the Skaggs Institute for Chemical Biology,  
The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Received 22nd August 2002

First published as an Advance Article on the web 26th November 2002

## 1 Introduction

It has now been two decades since acquired immunodeficiency syndrome (AIDS) was first reported by the US Center for Diseases Control (CDC). A few years later, it was found that a retrovirus called human immunodeficiency virus (HIV) is the causative agent in AIDS.<sup>1</sup> In a short time, AIDS increased to epidemic proportions throughout the world, affecting more than 40 million people today and killing so far more than 22 million (UNAIDS, 2001).

Since the outbreak of the AIDS epidemic, tremendous efforts have been directed towards development of antiretroviral therapies that target HIV type 1 in particular (HIV-1). The identification of the HIV retrovirus and the accumulated knowledge about the role of the different elements in its life cycle led researchers around the world to develop inhibitors that target different steps in the life cycle of the virus. One of these targets is HIV-1 protease (HIV PR), an essential enzyme needed in the proper assembly and maturation of infectious virions. Understanding the chemical mechanism of this enzyme has been a basic requirement in the development of efficient inhibitors. In this review, we summarize studies conducted in the last two decades on the mechanism of HIV PR and the impact of their conclusions on the drug discovery processes.

## 2 The life cycle of HIV

HIV belongs to the class of viruses called retroviruses, which carry genetic information in the form of RNA. HIV infects T cells that carry the CD4 antigen on their surface. The infection of the virus requires fusion of the viral and cellular membranes, a process that is mediated by the viral envelope glycoprotein (gp120, gp41) and receptors (CD4 and coreceptors, such as CCR5 or CXCR4) on the target cell. As the virus enters a cell, its RNA is reverse-transcribed to DNA by a virally encoded enzyme, the reverse transcriptase (RT). The viral DNA enters the cell nucleus, where it is integrated into the genetic material of the cell by a second virally encoded enzyme, the integrase. Activation of the host cell results in the transcription of the viral DNA into messenger RNA, which is then translated into viral proteins. HIV protease, the third virally encoded enzyme, is required in this step to cleave a viral polyprotein precursor into individual mature proteins. The viral RNA and viral proteins assemble at the cell surface into new virions, which then bud from the cell and are released to infect another cell. The extensive cell damage from the destruction of the host's genetic system to the budding and release of virions leads to the death of the infected cells.

## 3 HIV protease

### 3.1 HIV protease: a logical target for AIDS therapy

Unless the HIV life cycle is interrupted by specific treatment, the virus infection spreads rapidly throughout the body, which results in the weakness and destruction of the body's immune system. From the analysis of the HIV life cycle, one could conclude that there are several steps that might be interfered with,

thus stopping the replication of the virus. For example, there are several commercially available drugs that inhibit the enzyme reverse transcriptase (RT). The first class of RT inhibitors is the nucleoside analogs such as AZT, ddI, ddC and d4T. These dideoxy compounds lack the 3'-hydroxy, causing DNA chain termination when they are incorporated into the growing DNA strand. The second class of inhibitors is the non-nucleoside inhibitors (NNIs); these inhibitors are known to bind in a pocket away from the polymerase active site, and are believed to cause a conformational change of the enzyme active site, and thus inhibit its action. Currently, there are three available non-nucleoside reverse transcriptase inhibitors (nevirapine, delavirdine, and efavirenz) for the treatment of AIDS.

Another critical step in the life cycle of HIV is the proteolytic cleavage of the polypeptide precursors into mature enzymes and structural proteins catalyzed by HIV PR. It has been shown that budded immature viral particles that contain catalytically inactive protease cannot undergo maturation to an infective form.<sup>2</sup> The necessity of this enzyme in the virus life cycle makes it a promising target for therapy of the HIV infection.<sup>3</sup>

### 3.2 Structure of HIV protease

Navia *et al.* from Merck laboratories were the first group to obtain a crystal structure of HIV PR;<sup>4</sup> a more accurate structure was reported subsequently by Kent and coworker.<sup>5</sup> HIV PR is a 99 amino acid aspartyl protease which functions as a homodimer with only one active site which is  $C_2$ -symmetric in the free form. More than 140 structures of the HIV-1 PR, its mutants and enzymes complexed with various inhibitors have been reported so far. A database dedicated to providing structural information about HIV PR has been created at the National Cancer Institute (<http://www-fbnc.ncifcrf.gov/HIVdb/>). The enzyme homodimer complexed with TL-3<sup>6</sup> is shown in Fig. 1 (PDB ID: 3TLH). Each monomer contains an extended  $\beta$ -sheet region (a glycine-rich loop) known as the flap, that constitutes in part the substrate-binding site and plays an important role in substrate binding, and one of the two essential aspartyl residues, Asp-25 and Asp-25' which lie on the bottom of the cavity. The substrate binds in its extended conformation, in which its interactions with the different amino

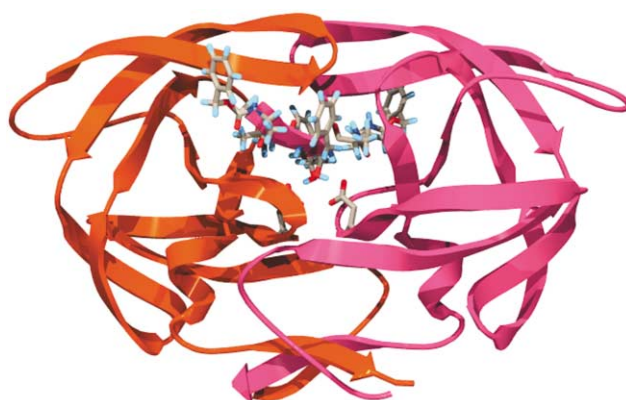


Fig. 1 Structure of HIV PR complexed with TL-3 (PDB: 3TLH).

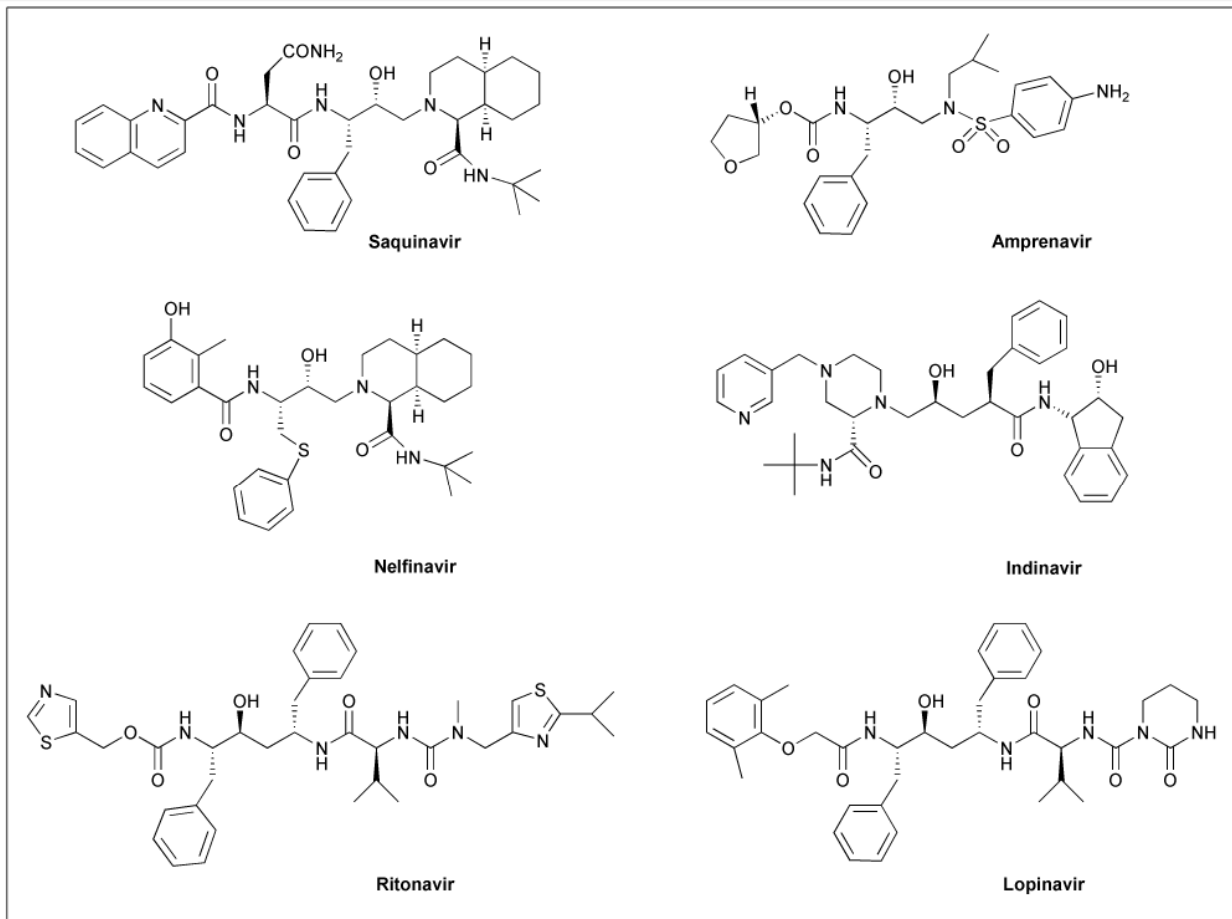
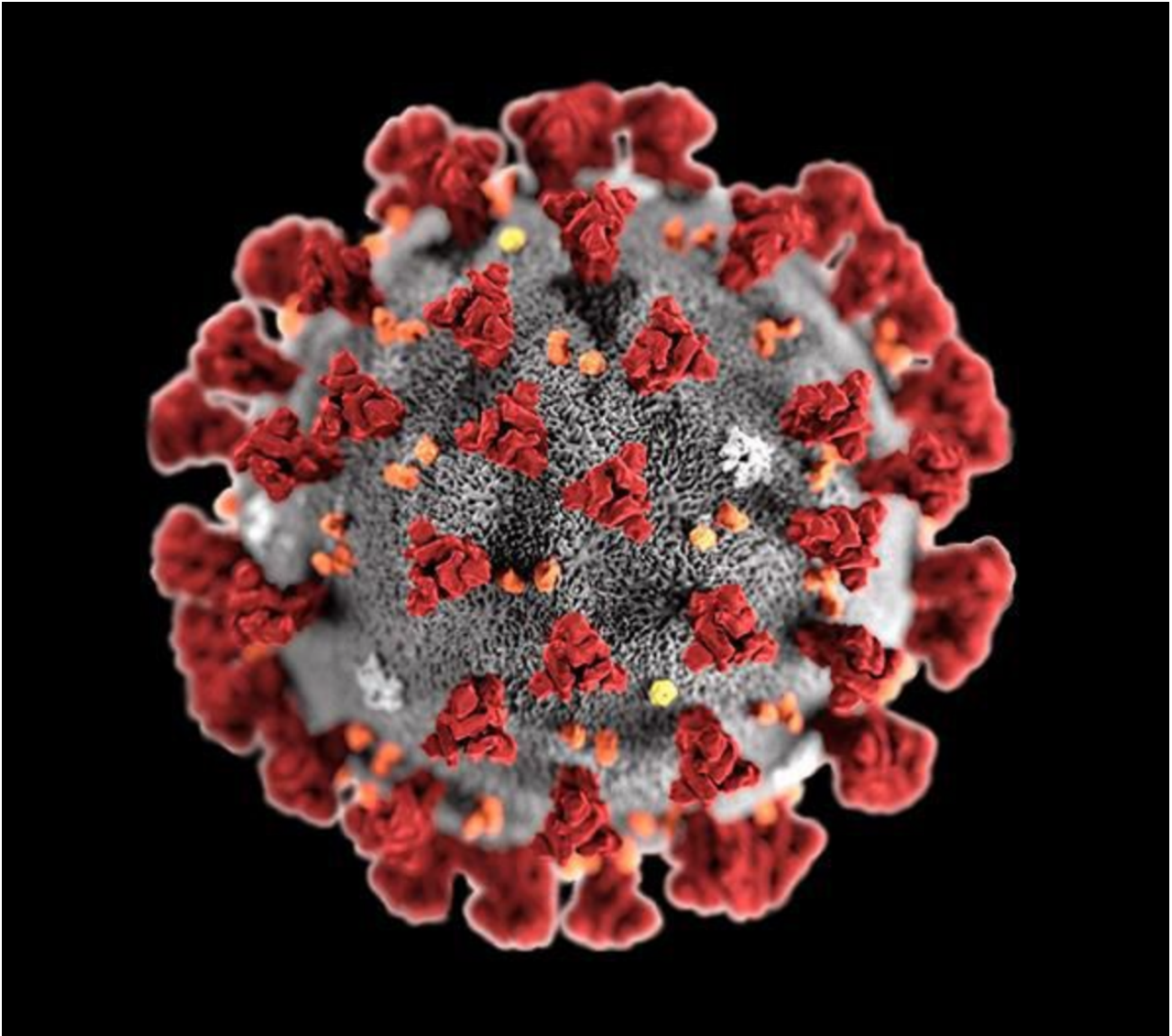


Fig. 10 FDA approved HIV-1 protease inhibitors.



# Structure-based design of prefusion-stabilized SARS-CoV-2 spikes

**Ching-Lin Hsieh<sup>1</sup>, Jory A. Goldsmith<sup>1</sup>, Jeffrey M. Schaub<sup>1</sup>, Andrea M. DiVenere<sup>2</sup>, Hung-Che Kuo<sup>1</sup>, Kamyab Javanmardi<sup>1</sup>, Kevin C. Le<sup>2</sup>, Daniel Wrapp<sup>1</sup>, Alison G. Lee<sup>1</sup>, Yutong Liu<sup>2</sup>, Chia-Wei Chou<sup>1</sup>, Patrick O. Byrne<sup>1</sup>, Christy K. Hjorth<sup>1</sup>, Nicole V. Johnson<sup>1</sup>, John Ludes-Meyers<sup>1</sup>, Annalee W. Nguyen<sup>2</sup>, Juyeon Park<sup>1</sup>, Nianshuang Wang<sup>1</sup>, Dzifa Amengor<sup>1</sup>, Jason J. Lavinder<sup>1,2</sup>, Gregory C. Ippolito<sup>1,3</sup>, Jennifer A. Maynard<sup>2\*</sup>, Ilya J. Finkelstein<sup>1,4\*</sup>, Jason S. McLellan<sup>1\*</sup>**

<sup>1</sup>Department of Molecular Biosciences, University of Texas, Austin, TX 78712, USA. <sup>2</sup>Department of Chemical Engineering, University of Texas, Austin, TX 78712, USA. <sup>3</sup>Department of Oncology, Dell Medical School, University of Texas, Austin, TX 78712, USA. <sup>4</sup>Center for Systems and Synthetic Biology, University of Texas, Austin, TX 78712, USA.

\*Corresponding author. Email: maynard@che.utexas.edu (J.A.M.); ilya@finkelsteinlab.org (I.J.F.); jmclellan@austin.utexas.edu (J.S.M.)

The COVID-19 pandemic has led to accelerated efforts to develop therapeutics and vaccines. A key target of these efforts is the spike (S) protein, which is metastable and difficult to produce recombinantly. Here, we characterized 100 structure-guided spike designs and identified 26 individual substitutions that increased protein yields and stability. Testing combinations of beneficial substitutions resulted in the identification of HexaPro, a variant with six beneficial proline substitutions exhibiting ~10-fold higher expression than its parental construct and the ability to withstand heat stress, storage at room temperature, and three freeze-thaw cycles. A 3.2 Å-resolution cryo-EM structure of HexaPro confirmed that it retains the prefusion spike conformation. High-yield production of a stabilized prefusion spike protein will accelerate the development of vaccines and serological diagnostics for SARS-CoV-2.

# Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability

Katalin Karikó<sup>1</sup>, Hiromi Muramatsu<sup>1</sup>, Frank A Welsh<sup>1</sup>, János Ludwig<sup>2</sup>, Hiroki Kato<sup>3</sup>, Shizuo Akira<sup>3</sup>, and Drew Weissman<sup>4</sup>

<sup>1</sup>Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA

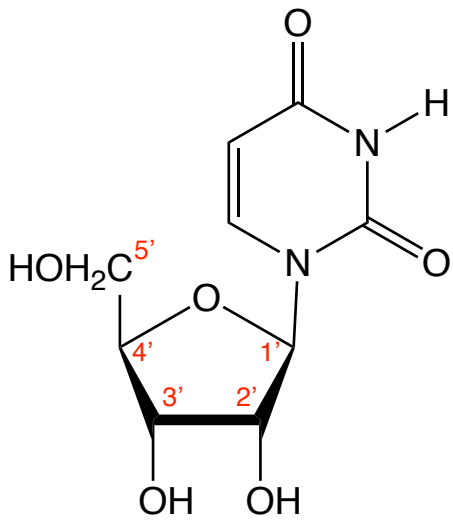
<sup>2</sup>Laboratory of RNA Molecular Biology, The Rockefeller University, New York, New York, USA

<sup>3</sup>Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

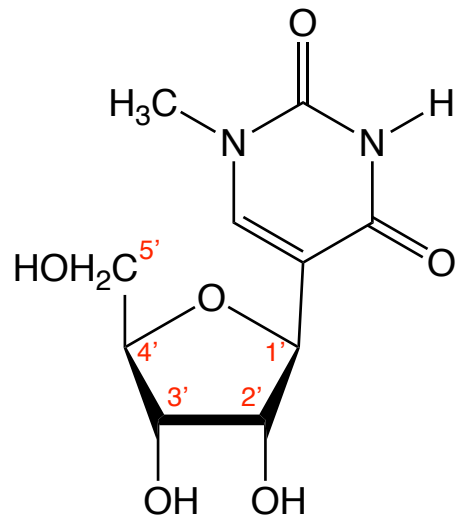
<sup>4</sup>Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

## Abstract

*In vitro*-transcribed mRNAs encoding physiologically important proteins have considerable potential for therapeutic applications. However, in its present form, mRNA is unfeasible for clinical use because of its labile and immunogenic nature. Here, we investigated whether incorporation of naturally modified nucleotides into transcripts would confer enhanced biological properties to mRNA. We found that mRNAs containing pseudouridines have a higher translational capacity than unmodified mRNAs when tested in mammalian cells and lysates or administered intravenously into mice at 0.015–0.15 mg/kg doses. The delivered mRNA and the encoded protein could be detected in the spleen at 1, 4, and 24 hours after the injection, where both products were at significantly higher levels when pseudouridine-containing mRNA was administered. Even at higher doses, only the unmodified mRNA was immunogenic, inducing high serum levels of interferon- $\alpha$  (IFN- $\alpha$ ). These findings indicate that nucleoside modification is an effective approach to enhance stability and translational capacity of mRNA while diminishing its immunogenicity *in vivo*. Improved properties conferred by pseudouridine make such mRNA a promising tool for both gene replacement and vaccination.



Uridine



*N1*-methylpseudouridine  
(m<sup>1</sup>Ψ)

# Nobel Prize in Physiology or Medicine 2023



© Nobel Prize Outreach. Photo:  
Clément Morin

**Katalin Karikó**

Prize share: 1/2



© Nobel Prize Outreach. Photo:  
Clément Morin

**Drew Weissman**

Prize share: 1/2

Amy Rhoden Smith



## Careers in Chemistry

Amy Rhoden Smith, PhD works at Precision BioSciences, a gene editing therapeutics company. As a program development leader, Dr. Rhoden Smith works with multiple teams to lead cell and gene editing therapy programs through preclinical and early clinical development. She loves working in biotechs and startups, where she can think about how to use cutting-edge science to bring meaningful therapies to patients. Dr. Rhoden Smith earned her BS in chemistry at the College of Charleston. She received her PhD in organic chemistry from the University of Texas at Austin.

Dr. Rhoden Smith has always been interested in applying her chemical knowledge to understand biological problems, so she started her career in biotechnology by joining Moderna Therapeutics. Her team used chemistry to create novel therapeutic mRNA conjugates for improved mRNA in vivo half-life and protein expression. She then moved to Intellia Therapeutics, where she led a team focused on generating Cas9 mRNA and synthetic guide RNAs for in vivo gene editing therapeutic applications. After working on projects that were discovery-based, she moved to Precision BioSciences, where she focuses on bringing products into the clinic.

Dr. Rhoden Smith says that organic chemistry gave her an understanding of how molecules interact with each other, which applies not only to small molecules, but also much larger ones like proteins, nucleic acids, and even cells. Just as importantly, it also taught her the value of problem-solving and gave her a passion for understanding complex problems, which she uses every day.

# A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Staci Sabnis,<sup>1</sup> E. Sathyajith Kumarasinghe,<sup>1</sup> Timothy Salerno,<sup>1</sup> Cosmin Mihai,<sup>1</sup> Tatiana Ketova,<sup>1</sup> Joseph J. Senn,<sup>1</sup> Andy Lynn,<sup>1</sup> Alex Bulychev,<sup>1</sup> Iain McFadyen,<sup>1</sup> Joyce Chan,<sup>1</sup> Örn Almarsson,<sup>1</sup> Matthew G. Stanton,<sup>1,2</sup> and Kerry E. Benenato<sup>1</sup>

<sup>1</sup>Moderna Therapeutics, 200 Technology Square, Cambridge, MA 02139, USA

**The success of mRNA-based therapies depends on the availability of a safe and efficient delivery vehicle. Lipid nanoparticles have been identified as a viable option. However, there are concerns whether an acceptable tolerability profile for chronic dosing can be achieved. The efficiency and tolerability of lipid nanoparticles has been attributed to the amino lipid. Therefore, we developed a new series of amino lipids that address this concern. Clear structure-activity relationships were developed that resulted in a new amino lipid that affords efficient mRNA delivery in rodent and primate models with optimal pharmacokinetics. A 1-month toxicology evaluation in rat and non-human primate demonstrated no adverse events with the new lipid nanoparticle system. Mechanistic studies demonstrate that the improved efficiency can be attributed to increased endosomal escape. This effort has resulted in the first example of the ability to safely repeat dose mRNA-containing lipid nanoparticles in non-human primate at therapeutically relevant levels.**

## INTRODUCTION

mRNA-based therapies have the potential to revolutionize the way we treat diseases. The surging interest in mRNA as a drug modality stems from the potential to deliver transmembrane and intracellular proteins, targets that standard biologics are unable to access due to their inability to cross the cell membrane.<sup>1</sup> One major challenge to making mRNA-based therapies a reality is the identification of an optimal delivery vehicle. Due to its large size, chemical instability, and potential immunogenicity, mRNA requires a delivery vehicle that can offer protection from endo- and exo-nucleases, as well as shield the cargo from immune sentinels. Lipid nanoparticles (LNPs) have been identified as a leading option in this regard.<sup>2</sup> Moderna Therapeutics has recently validated this approach by demonstrating safe and effective delivery of an mRNA-based vaccine formulated in LNPs.<sup>3</sup>

Key performance criteria for an LNP delivery system are to maximize cellular uptake and enable efficient release of mRNA from the endosome. At the same time, the LNP must provide a stable drug product and be able to be dosed safely at therapeutically relevant levels. LNPs are multi-component systems that typically consist of an amino lipid,

phospholipid, cholesterol, and a PEG-lipid.<sup>2</sup> Each component is required for aspects of efficient delivery of the nucleic acid cargo and stability of the particle. The key component thought to drive cellular uptake, endosomal escape, and tolerability is the amino lipid. Cholesterol and the PEG-lipid contribute to the stability of the drug product both *in vivo* and on the shelf, while the phospholipid provides additional fusogenicity to the LNP, thus helping to drive endosomal escape and rendering the nucleic acid bioavailable in the cytosol of cells.

Several amino lipid series have been developed for oligonucleotide delivery over the past couple of decades.<sup>4</sup> The literature highlights direct links between the structure of the amino lipid and the resultant delivery efficiency and tolerability of the LNP. The amino lipid MC3 (DLin-MC3-DMA) is the most clinically advanced oligonucleotide delivery system, as siRNA formulated in MC3-based LNPs has progressed to phase III for the treatment of transthyretin-mediated amyloidosis.<sup>5,6</sup> More recently, literature reports have demonstrated the effectiveness of MC3-based LNPs to deliver mRNA.<sup>7</sup> LNPs of this class are quickly opsonized by apolipoprotein E (ApoE) when delivered intravenously (*i.v.*), which enables cellular uptake into hepatocytes by the low-density lipoprotein receptor (LDLr).<sup>8</sup> Concerns remain that MC3's long tissue half-life could contribute to unfavorable side effects hindering its use for chronic therapies.<sup>9</sup> In addition, LNPs can induce activation of the immune system resulting in complement activation-related pseudoallergy (CARPA), an acute immunological response that can lead to anaphylactic-like shock.<sup>10</sup>

To unleash the potential of mRNA therapies for humans, we required a class of LNPs with increased delivery efficiency along with a metabolic and toxicity profile that would enable chronic dosing in humans.

---

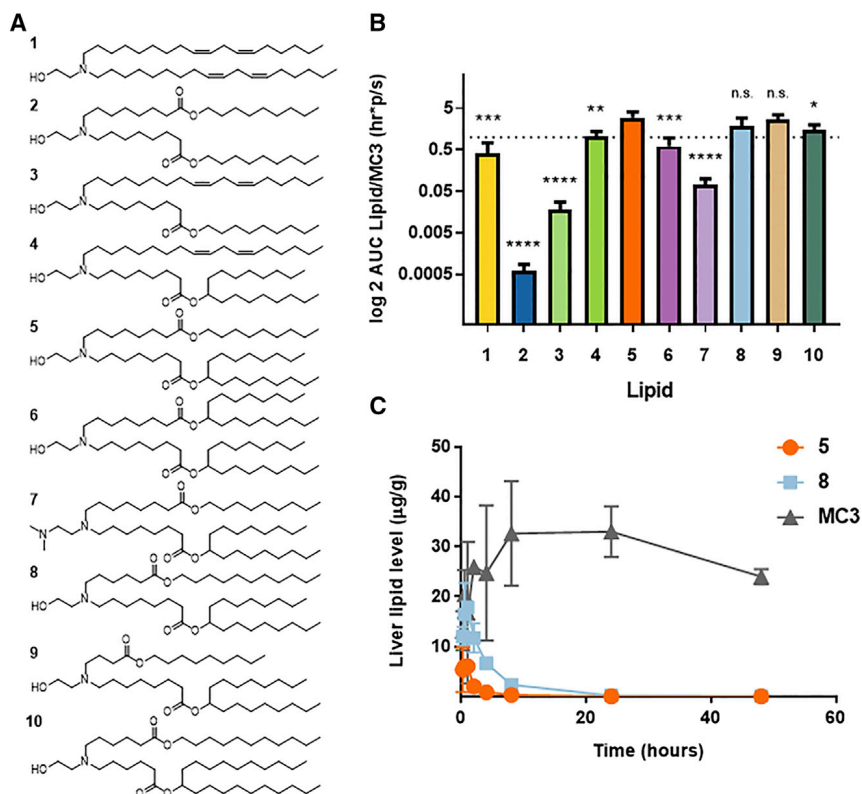
Received 20 November 2017; accepted 9 March 2018;  
<https://doi.org/10.1016/j.ymthe.2018.03.010>

<sup>2</sup>Present address: Generation Bio, 215 First Street, Suite 150, Cambridge, MA 02142, USA

**Correspondence:** Kerry E. Benenato, Moderna Therapeutics, 200 Technology Square, Cambridge, MA 02139, USA.

**E-mail:** [kerry.benenato@modernatx.com](mailto:kerry.benenato@modernatx.com)





**Figure 1. Optimization of Efficiency and Clearance of Amino Lipid**

(A) Structures of amino lipids. (B) Whole-body luciferase bioluminescence AUC of novel LNPs versus MC3 LNPs, measured in CD-1 mice ( $n = 6$  at 3 and 6 hr,  $n = 3$  at 24 hr), 0.5 mg/kg dose firefly luciferase (ffLuc) mRNA, i.v. bolus, error bars indicate SD of the ratio of novel lipid AUC versus MC3 AUC. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , n.s. = not statistically significant. (C) Parent amino lipid levels measured in liver tissue from Sprague-Dawley rats ( $n = 3$  per time point), 0.2 mg/kg dose hEPO mRNA, mean  $\pm$  SD,  $p < 0.05$  for lipids 5 and 8 AUC relative to MC3.

We took a rational medicinal chemistry approach to amino lipid optimization aiming to identify structural motifs that provide chemical stability, optimal tissue clearance, and mRNA delivery efficiency. Our initial rodent screens led to the identification of a lead lipid with good delivery efficiency and pharmacokinetics. The lead LNP was profiled further in non-human primate for efficiency of delivery after single and repeat dosing. Finally, the optimized LNPs were evaluated in 1-month repeat dose toxicity studies in rat and non-human primate.

## RESULTS

Initial screening of a broad chemical space identified ethanolamine as an amino lipid head group that could effectively drive mRNA encapsulation and provide LNPs with superior physicochemical properties. Combining the ethanolamine head group with di-linoleic lipid tails (lipid 1; Figure 1; Table 1) generated an LNP with high encapsulation of luciferase mRNA, small particle size, and low polydispersity index (PDI). The LNP with lipid 1 had a surface  $pK_a$  (apparent value for the particle) in the range that has been shown to be optimal for siRNA delivery.<sup>11,12</sup> To evaluate the efficiency of the new amino lipids, LNPs using the novel lipids were tested *in vivo* in mice using firefly luciferase mRNA as a reporter. An MC3 LNP was included as a control in each experiment, enabling us to compare LNPs from experiment to experiment. Measured luciferase activity also enabled us to determine protein bio-distribution. i.v. delivery of 0.5 mg/kg (mRNA dose level) of lipid 1-based LNPs to mice resulted in luciferase activity two-fold lower than an MC3 LNP control (Figure 1B).

Whole-body imaging clearly demonstrated that the majority of protein expression was localized in the liver (Figure S1). We found that the lipid had similar clearance to MC3 from liver tissue with 66% of the original dose remaining in liver tissue of mice 24 hr post-dose (Table 1).

To improve tissue clearance, we introduced ester linkages in the lipid tails (lipids 2 and 3; Figure 1A), which are reported to trigger metabolism by esterases *in vivo*.<sup>13</sup> This has been shown to be a viable strategy to improve lipid clearance in a MC3-based lipid structure.<sup>9</sup> First, we established that the lipids were chemically stable by measuring ethanol stability at room temperature and 37°C (Table S1). We observed less than 1% change in purity for all lipids tested. LNPs formed with lipid 2 were significantly larger with a surface  $pK_a > 7$  (Table 1). Removal of one ester (lipid 3) afforded LNPs with improved physicochemical characteristics and lower LNP surface  $pK_a$ . *In vivo*, neither lipid demonstrated efficient mRNA delivery (Figure 1B); however, we did observe rapid tissue clearance, with no lipid detected at 24 hr (Table 1).

Improvement in protein expression was observed when a secondary ester was introduced (lipid 4; Figures 1A and 1B). We observed equivalent expression to MC3 LNPs, but the clearance rate was slower than lipids 2 and 3 (67% lipid remaining; Table 1). Replacement of the linoleic tail with a primary ester-containing lipid tail (lipid 5; Figure 1A) provided increased expression (3-fold higher than MC3; Figure 1B) and optimal tissue clearance (no lipid detected at 24 hr; Table 1). To further increase expression an additional secondary ester was introduced (6), but this resulted in a lowering of the surface  $pK_a$  to 6.00 and lower luciferase activity. In addition, the lipid had a significantly slower tissue clearance with 68% remaining at 24 hr.

With an optimal lipid tail structure identified we re-visited the ethanolamine head group. Lipid 7 is one representative example (Figure 1A) where the alcohol functionality is replaced with a dimethylamine. This generated an LNP with comparable physicochemical properties, but complete loss of delivery efficiency (Figure 1B).

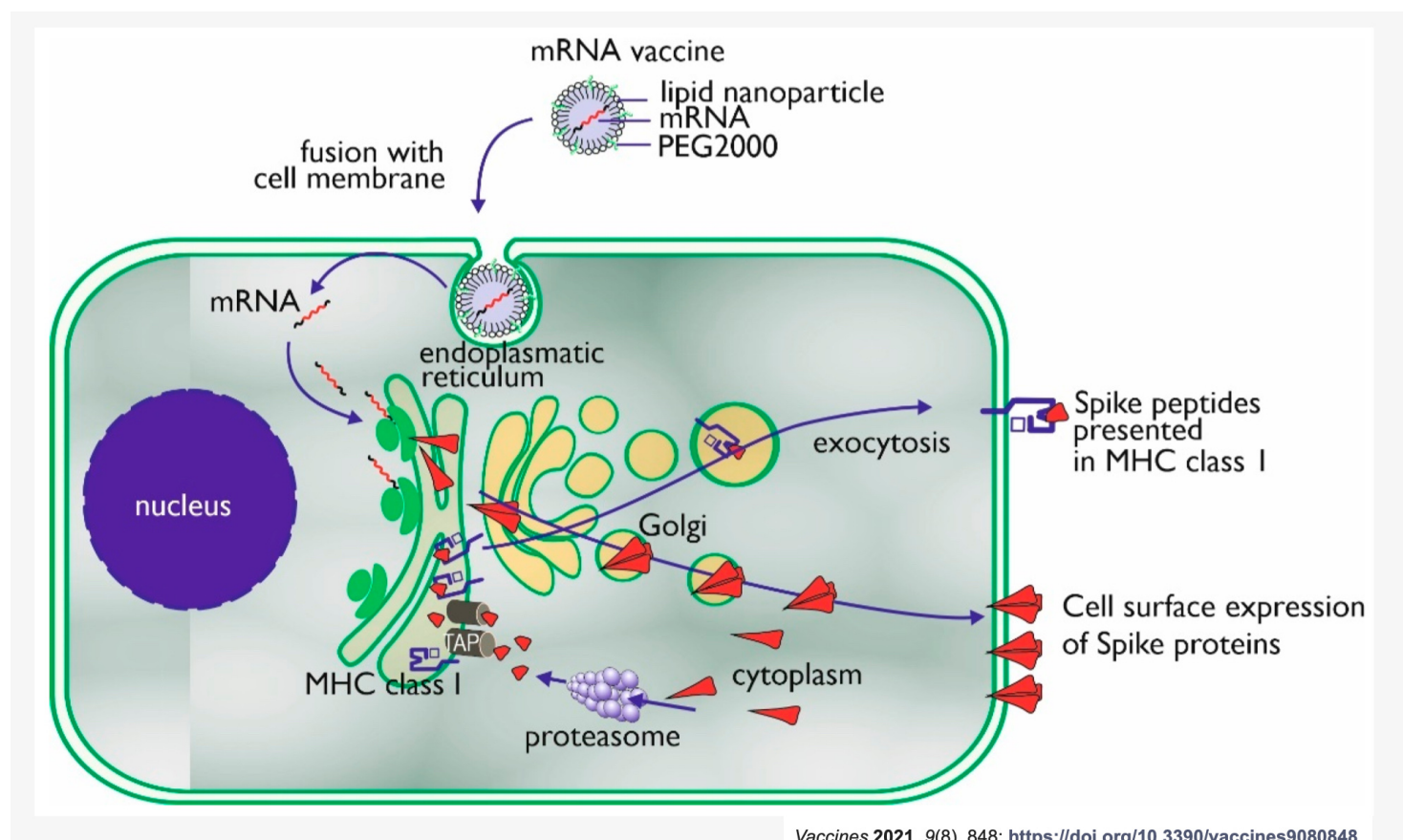
# Structure-based design of prefusion-stabilized SARS-CoV-2 spikes

Ching-Lin Hsieh<sup>1</sup>, Jory A. Goldsmith<sup>1</sup>, Jeffrey M. Schaub<sup>1</sup>, Andrea M. DiVenere<sup>2</sup>, Hung-Che Kuo<sup>1</sup>, Kamyab Javanmardi<sup>1</sup>, Kevin C. Le<sup>2</sup>, Daniel Wrapp<sup>1</sup>, Alison G. Lee<sup>1</sup>, Yutong Liu<sup>2</sup>, Chia-Wei Chou<sup>1</sup>, Patrick O. Byrne<sup>1</sup>, Christy K. Hjorth<sup>1</sup>, Nicole V. Johnson<sup>1</sup>, John Ludes-Meyers<sup>1</sup>, Annalee W. Nguyen<sup>2</sup>, Juyeon Park<sup>1</sup>, Nianshuang Wang<sup>1</sup>, Dzifa Amengor<sup>1</sup>, Jason J. Lavinder<sup>1,2</sup>, Gregory C. Ippolito<sup>1,3</sup>, Jennifer A. Maynard<sup>2\*</sup>, Ilya J. Finkelstein<sup>1,4\*</sup>, Jason S. McLellan<sup>1\*</sup>

<sup>1</sup>Department of Molecular Biosciences, University of Texas, Austin, TX 78712, USA. <sup>2</sup>Department of Chemical Engineering, University of Texas, Austin, TX 78712, USA. <sup>3</sup>Department of Oncology, Dell Medical School, University of Texas, Austin, TX 78712, USA. <sup>4</sup>Center for Systems and Synthetic Biology, University of Texas, Austin, TX 78712, USA.

\*Corresponding author. Email: maynard@che.utexas.edu (J.A.M.); ilya@finkelsteinlab.org (I.J.F.); jmclellan@austin.utexas.edu (J.S.M.)

The COVID-19 pandemic has led to accelerated efforts to develop therapeutics and vaccines. A key target of these efforts is the spike (S) protein, which is metastable and difficult to produce recombinantly. Here, we characterized 100 structure-guided spike designs and identified 26 individual substitutions that increased protein yields and stability. Testing combinations of beneficial substitutions resulted in the identification of HexaPro, a variant with six beneficial proline substitutions exhibiting ~10-fold higher expression than its parental construct and the ability to withstand heat stress, storage at room temperature, and three freeze-thaw cycles. A 3.2 Å-resolution cryo-EM structure of HexaPro confirmed that it retains the prefusion spike conformation. High-yield production of a stabilized prefusion spike protein will accelerate the development of vaccines and serological diagnostics for SARS-CoV-2.



## New UT cases

The University of Texas said two people in its community, an unnamed student and the Dean of Undergraduate Studies Brent Iverson, had tested positive for the virus. It is unclear if the pair are included in Travis County's count of confirmed cases.



## CORONAVIRUS

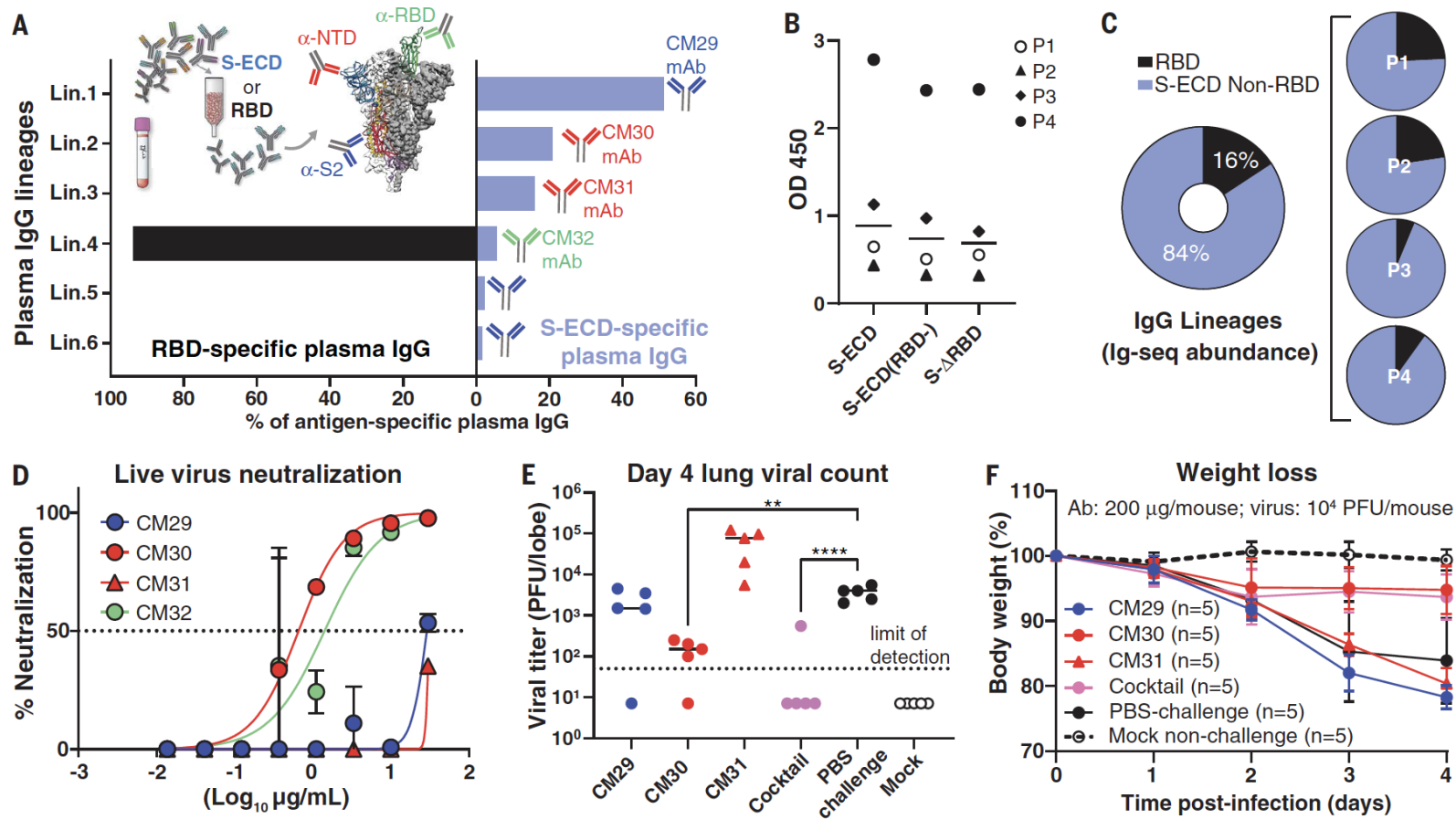
# Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes

William N. Voss<sup>1</sup>, Yixuan J. Hou<sup>2, #</sup>, Nicole V. Johnson<sup>1, #</sup>, George Delidakis<sup>3</sup>, Jin Eyun Kim<sup>4</sup>, Kamyab Javanmardi<sup>1</sup>, Andrew P. Horton<sup>1</sup>, Foteini Bartzoka<sup>1</sup>, Chelsea J. Paresi<sup>5</sup>, Yuri Tanno<sup>3</sup>, Chia-Wei Chou<sup>1</sup>, Shawn A. Abbasi<sup>6</sup>, Whitney Pickens<sup>1</sup>, Katia George<sup>1</sup>, Daniel R. Boutz<sup>1, 7</sup>, Dalton M. Towers<sup>3</sup>, Jonathan R. McDaniel<sup>8</sup>, Daniel Billick<sup>1</sup>, Jule Goike<sup>1</sup>, Lori Rowe<sup>9, 10</sup>, Dhvani Batra<sup>9</sup>, Jan Pohl<sup>9</sup>, Justin Lee<sup>9</sup>, Shivaprakash Gangappa<sup>11</sup>, Suryaprakash Sambhara<sup>11</sup>, Michelle Gadush<sup>12</sup>, Nianshuang Wang<sup>1</sup>, Maria D. Person<sup>12</sup>, Brent L. Iverson<sup>5</sup>, Jimmy D. Gollihar<sup>1, 7, 13</sup>, John M. Dye<sup>6</sup>, Andrew S. Herbert<sup>6</sup>, Ilya J. Finkelstein<sup>1</sup>, Ralph S. Baric<sup>2, 14</sup>, Jason S. McLellan<sup>1</sup>, George Georgiou<sup>1, 3, 4, 15</sup>, Jason J. Lavinder<sup>1, 3\*</sup>, Gregory C. Ippolito<sup>1, 13, 15\*</sup>

The molecular composition and binding epitopes of the immunoglobulin G (IgG) antibodies that circulate in blood plasma after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are unknown. Proteomic deconvolution of the IgG repertoire to the spike glycoprotein in convalescent subjects revealed that the response is directed predominantly (>80%) against epitopes residing outside the receptor binding domain (RBD). In one subject, just four IgG lineages accounted for 93.5% of the response, including an amino (N)-terminal domain (NTD)-directed antibody that was protective against lethal viral challenge. Genetic, structural, and functional characterization of a multidonor class of “public” antibodies revealed an NTD epitope that is recurrently mutated among emerging SARS-CoV-2 variants of concern. These data show that “public” NTD-directed and other non-RBD plasma antibodies are prevalent and have implications for SARS-CoV-2 protection and antibody escape.

## CORONAVIRUS

## Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes



# An Investigation of Nirmatrelvir (Paxlovid) Resistance in SARS-CoV-2 M<sup>pro</sup>

Rasha M. Yaghi, Dennis C. Wylie, Collin L. Andrews, Olivia H. Dickert, Anjana Ram, and Brent L. Iverson\*



Cite This: <https://doi.org/10.1021/acsbiochemau.4c00045>



Read Online

ACCESS |



Metrics & More



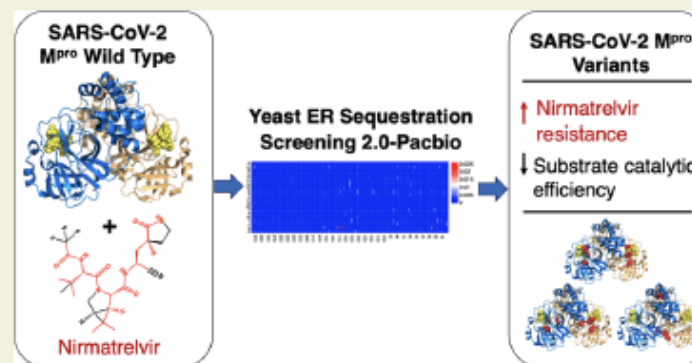
Article Recommendations



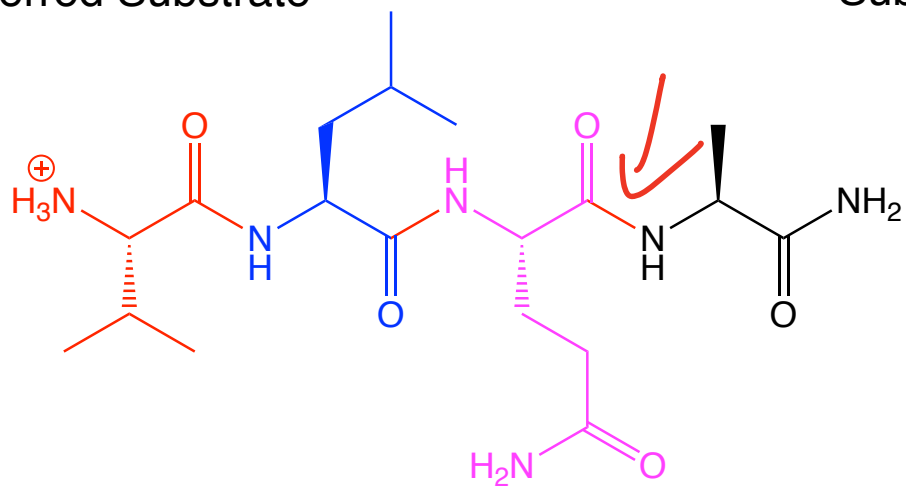
Supporting Information

**ABSTRACT:** The high throughput YESS 2.0 platform was used to screen a large library of SARS-CoV-2 M<sup>pro</sup> variants in the presence of nirmatrelvir. Of the 100 individual most prevalent mutations identified in the screen and reported here, the most common were E166V, L27V, N142S, A173V, and Y154N, along with their various combinations. *In vitro* analysis revealed that resistance to nirmatrelvir for these individual mutations, as well as all of the combinations we analyzed, was accompanied by decreased catalytic activity with the native substrate. Importantly, the mutations we identified have not appeared as significantly enriched in SARS-CoV-2 M<sup>pro</sup> sequences isolated from COVID-19 patients following the introduction of nirmatrelvir. We also analyzed three of the most common SARS-CoV-2 M<sup>pro</sup> mutations that have been seen in patients recently, and only a measured increase in nirmatrelvir resistance was seen when the more recently appearing A285V is added to both P132H and K90R. Taken together, our results predict that resistance to nirmatrelvir will be slower to develop than expected based on experience with other viral protease inhibitors, perhaps due in part to the close structural correspondence between nirmatrelvir and SARS-CoV-2 M<sup>pro</sup>'s preferred substrates.

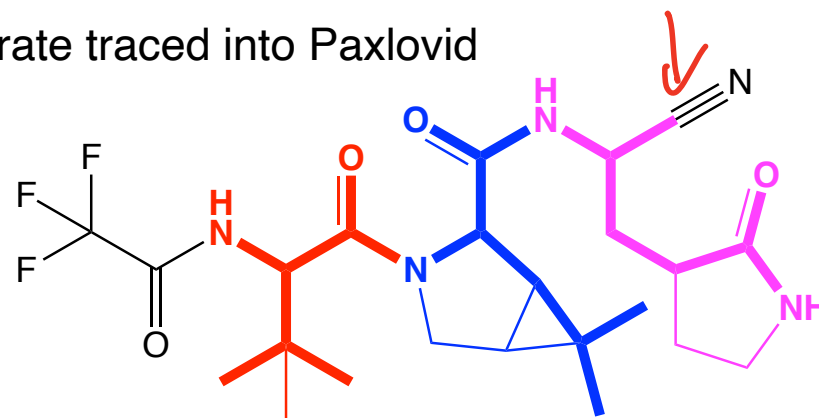
**KEYWORDS:** protease, YESS, COVID-19, Paxlovid, nirmatrelvir resistance



Preferred Substrate



Substrate traced into Paxlovid



Paxlovid

