we are excited to have dr barry okeefe

dr okeefe received a bachelor of

science degree in botany from michigan

state university and a phd in

pharmacognosy from the university of

illinois at chicago

at the nci dr okeefe currently leads

the protein chemistry and molecular

biology section and is deputy chief of

the molecular targets laboratory

he is also chief of the natural products

branch which is responsible for the

collection extraction prefractionation

and discovery of bioactive natural

products

im confident youre going to enjoy

todays lecture

hello my name is barry okeefe im the

chief of the natural products branch

here at the national cancer institute

and what ive come to talk to you about

today are is natural products research a

lot of you might be familiar with

natural products a majority of the

population of the world relies on plants

and microbes and a variety of other

natural products for their health needs

the nci in fact has had collections of

natural products taking place for over

0 years and has amassed an amazing

repository of biodiversity which the nci

uses

to test for compounds that might be

useful in cancer treatment what id like

to talk to you about today is the

aspects of the nci involved with

screening natural product extracts to

identify modulators of targets and

pathways in cancer

so with that ill move along to my

presentation

so to start out the outline for todays

presentation well discuss why we even

screen natural products in the first

place and then well discuss some of the

challenges associated with screening

natural products

and there are a variety of those and

they entail the necessity of making a

variety of screening decisions on how

you intend to use your natural products

in your development of screens to be
able to identify new molecules ill then
discuss a couple examples of cells free
screens that weve performed in our
laboratory and then well conclude with
some new avenues for improving the
efficiency and increasing the output of
natural products research
so why investigate natural products well
first what youre seeing here is a graph
that shows the yeartoyear new drugs
that are approved by the fda and this
shows in the green bars the percent of
those new drugs that are derived from
natural products
so as you can see theres a significant
proportion of all drugs that are natural
product derived in this case about 0
percent of all small molecule drugs
approved by the fda
over time have been natural product
chemotypes and for anticancer in
particular greater than 0 percent of
all anticancer drugs are derived from
natural products so this is a very
fruitful area of research for the

discovery of new natural products

so just to give you a more pictorial

example here im showing a variety of

the unique chemical structures that are

found in natural organisms that have

been approved drugs these are just those

that were approved from 00 to 0 and

what you see here are different

compounds that have been approved for

either cancer or in one case we have one

here that was approved as an antibiotic

and you can see theres a lot of

structural complexity and a lot of

difference to the structures here and

thats based on the fact that some are

from plants some are from microbes some

are marine organisms

what is most fascinating to me however

being involved in natural product

discovery is that even though weve had

this level of success with natural

product research less than one percent

of the total biome of potential

organisms for natural product research

have been examined so theres still a

lot of work to do

so

if natural products are so successful

and theyre responsible for so much of

the drug pharmacopoeia that we use on a

daily basis

one of the things thats most vexing to

us natural products researchers is the

fact that

recently over the last decade or so high

throughput screening has been

development in drug discovery so these

are large libraries that are screened in

a very high throughput to identify

molecules that could be useful drugs and

what i show on this slide is a graph

that was put together by my colleagues

and what you see here in the white bars

are the number of papers in pubmed that

show an increasing amount of high

throughput screening or hts so these are

all the citations for high throughput

screening on a yeartoyear basis

what you see in red

barely visible down at the bottom are

those screens that actually involve

natural product extracts

so even though natural products have
been responsible for such a high
percentage of the drugs the amount of
modern drug discovery screening that
actually uses natural products is only a
very tiny percentage shown by those
little red bars there at the bottom of
this graph
so the question is how do we get more
people screening natural products and
get them involved in this area of
research
well one way is the nci has a natural
products branch which is responsible for
collecting source organisms from around
the globe and growing microbial source
organisms we also then process and
extract these organisms to produce
aqueous and organic extracts that are
then provided to researchers worldwide
both in vials and as screening plates
we also involved the isolation and
identification of active compounds from
these extracts and also we can work with
people to reisolate bulk quantities of
the compounds that are isolated so that

there are more compounds available for
animal studies and preclinical
development were also responsible for
large data fields of where these
organisms were collected the gps
coordinates what the taxonomy is we also
have agreements with the countries that
allow us to collect these organisms
there are letters of collection that are
in fact almost a treaty between the
united states and the host countries
that ensure that the host countries
rights are respected so should anything
come that is commercially developed out
of one of these organisms there are
responsibilities for those developing
that and licensing that that they have
equitable benefit sharing with the host
country and we think thats very
important
so where are collections located here
you see a world map and you can see
different hexagons and blue green
red and also black and these show the
locations of the collections of the
natural products branch around the globe

you can see the majority of them are

equatorial this is because what the

natural products branch looks for is

unique organisms that are in very rich

environments which requires them to

compete with each other because we feel

that these organisms then are more

likely to have to develop chemical

defenses and unusual chemistry that

allows them to compete with such an over

abundance of biota and so those are the

collections we currently have

what you see here is pictures of our

extraction laboratory

bags of these extracts whether theyre

plant or or marine organisms come to the

nci in frederick and they are ground and

extracted in the purple columns that you

see on the upper left

they are then dried down into these

bottles that you see in the center

using rotary evaporators that you see on

the lower lefthand side of this

slide we then have

lots of bins of viled extracts that you

see there in the upper right hand corner

and then we take these vials and create

plates for screening 9 well plates

well plates that we then ship to

researchers around the world to do

research on cancer and we provide them

for free the nci provides these for free

to researchers all they have to do is

pay for shipping

so what exactly is in the repository

well heres a quick slide that shows you

we have more than 00 000 different

extracts in the ncaa repository at this

time actually its closer to 0 000 at

this time and you can see here the

breakdown of them about 0 000 plant

extracts about 0 000 marine extracts

and about 0 000 microbial extracts

these we have both aqueous and organic

extracts for each source organism and as

i said these are all plated out at this

point and can be made available to

researchers worldwide

so what are the challenges we now know

that the nci has produced a vast

repository of extracts that should make

screw available to researchers and

should make screening extracts

worthwhile endeavor and reduce a lot of

the costs that might be associated with

researchers doing this work

so what are the challenges well for one

natural product extracts are difficult

to screen in their crude form because

they have cytotoxic compounds they have

common nuisance compounds that

nonspecifically bind to proteins they

also have

fluorescent compounds and colored

compounds and so this makes working with

some of the modern high throughput

screening systems challenging and so

researchers have to get some familiarity

with how to work with screens with

natural products

also each extract contains hundreds of

compounds and so once you get an extract

thats active you then have to go

through the process of identifying the

single active agent or family of agents

that is in that extract and thats a

challenge for some researchers

and that type of purification and

structural characterization of the
active components thats a unique skill
set and the time frames and the
requirements for that havent always
meshed with modern high throughput
screening time frames and i think thats
part of the reason why weve seen not a
lot of natural product extracts being
used in the high throughput screens
theres a requirement for a long term
lower throughput bioassay guided
fractionation support and theres also a
continual accrual of additional lead
molecules that might not work in the
time frames and such rapid screening
paradigms that we use today
but i know theres a lot of words on
this slide so the easiest way to think
about that is a picture is worth a
thousand words why is it difficult to
screen natural products
because they look like this this is a
plate of natural product extracts that
are meant for screening and you can see
here theyre colored theyre sticky
theyre viscous theyre fluorescent and

so having compounds and extracts like

this put into screens is a challenge for

many researchers

so what sort of decisions do you need to

make when youre going to screen natural

products well what kind of density are

you doing 9 well plates well plates

ma the most researchers now use well

plates for their screenings but also

recently we have completed a screen with

natural product extracts in a

higher density format

also theres the assay endpoint theres

common interfering compounds you might

imagine that a plant extract is going to

have chlorophyll in it chlorophyll is

green if you have a green labeled

compound that youre trying to find in

your reporter assay theres going to be

complications with the green compounds

that are present in those extracts

also extract concentration

theres several tradeoffs here

you can screen at lower and lower

concentrations of the extract but then

youre more and more likely to find

common compounds that are only a high

percent of the total mass of an extract

so theres assay optimization that you

can do to reduce these false positives

you ask also have to define your hit is

your hit the same if youre screening

marine extracts as it is if youre

screening plant extracts as it is if

youre screening microbial extracts

sometimes it can be significant

differences in the type of molecules

that are present in extracts from

different sources and so you might have

to adjust your paradigms for screening

based upon those source organisms

theres also hit prioritization which is

a requirement for this its high

throughput orthogonal and it helps weed

through some of these nonspecific hits

so

two things i want to talk about in more

detail are assay optimization and hit

prioritization to give you an example of

why this is important

but the bottom line is that taking the

time to address these decisions early on

will result in a screening program that

can effectively assay natural products

and work better with the chemists and

the downstream

users of your data

so

assay optimization what can you do to

help

for example you need to optimize assay

kinetics in a cell free assay system and

you have to optimize extract

concentration that means testing at a

variety of concentrations putting in a

variety of enzymes also as i mentioned

green

labeled compounds can be challenging to

use with natural products so you could

use a fluorophore or label that has a

longer wavelength shifted into the red

region this should help to remove some

of the interference that you get from

natural products

one important thing is just a simple

wash step if you can use a step where

your label is bound to the plate and

then youre able to wash away the

unbound extract thats not interacting

with your target that allows you to wash

away many of these interfering compounds

and finally addition of something like

an excipient protein like bovine serum

albumin or gelatin to your assay buffer

can reduce the effect of nonspecific

binding of certain compounds to proteins

because now the protein of interest is

only a small percentage of the total

protein in the well and so that reduces

the effect of those compounds on that

protein of interest

and just to give you a visual example

here is an example of what an assay

looked like before modification and you

can see that we have controls on the

left hand side of substrate its not

inhibited and then you see the presence

of the enzyme there tdp reduces the

signal now down and that window between

those two points the one on the left and

the one in the middle those two lines

thats your effective window of what

might be a hit

what you can see on the right hand side

is a screen of actual compounds in this

assay

so what how many hits are in this assay

and you can see anything above that

dashed line in the middle would be

considered a positive result something

that is inhibiting that enzyme in this

case tdp

thats an assay as it might come into

the laboratory i work in and its not

modified this assay in our hands would

be considered to have too high a hit

rate theres too many molecules that are

becoming active here and so wed have a

hard time weeding through them so what

we do is modify that assay using some of

the techniques shown above

and this is what we get and what you see

here now you have your controls this

time shown in red and blue in the same

positions they were theyre tighter

theres a larger window between the two

and now if we take a look at the same

exact compound screened in this assay

now you see only a few compounds that

have risen above that dashed line that

indicates that the assay is now

significantly more rigorous its more

challenging for something to inhibit

that assay and thats an assay that now

might be more amenable to screening

something as challenging as natural

products

so

what other prioritization techniques can

we use theres a lot of things you can

use here and you have to really think

down the line youre still going to get

a significant number of hits if you

screen all 00 000 extracts you might

still end up with a thousand hits even

after optimizing your assay so you have

to use additional assays biological

assays to determine cellular activity

gene transcript analysis pathway

analysis or biochemical assays that look

at direct modulation of your target do

can you get direct binding constants can

you look for selectivity amongst related

proteins with similar structures and can

you look at things such as reversibility

of the inhibition and allosterism or

noncompetitive inhibition

also one could look at taxonomic and

geographic if you have a lot of hits

from the same genus of plants they might

be one project instead of several

chemical metabolomics can also help this

gives you an idea of the chemistry

present in different extracts and so

some can be grouped and related by their

chemistry rather than their taxonomy

and finally theres just practical

considerations how much extract is there

is it able to work in your separation

techniques is it a very challenging

extract or easy so all of these

ideas can be put to use in prioritizing

your projects

here im going to show you just an

example of something weve worked up on

a project that we did with the sanford

burnham research institute its a list

of a variety of different criteria that

we would like an active extract to have

and you can see theres some activity

that fractionates well there are things

that are analytical as far as chemistry

and theres also taxonomic

considerations and every extract that

came out as a hit got a points

associated with being able to pass

certain of these criteria were then

able to grade the extracts and come up

with a triage triangle like you see here

so there were over 000 initial extracts

that hit but you can see after we did

confirmation and validated them through

certain techniques and then we ended up

with only a hundred that were

fractionation candidates by the time we

got down to structural elucidation

candidates we only had extracts which

is a number thats easily doable by most

chemistry labs and this then yielded a

couple of interesting molecules that

might be useful for drug development and

thats the type of triage that you have

to do to be able to weed through all of

the natural products

so im just going to go over a couple

details on a couple different assays

that weve done recently to give you

examples of the type of screens done in

the nci and in this case were going to

talk about malt which is an enzyme and

also tdp a different enzyme that weve

done in our lab and theyre different

types of formats

these are just a list that you see here

a variety of assays that have been done

in the molecular targets laboratory in

which i work

so the first project is a malt one and

this is involved with diffuse large

b cell lymphomas

so these are forty percent of

nonnonhodgkins lymphoma and there are

about diagnoses per year

unfortunately this only has a 0 cure

rate and approximately 0 000 deaths per

year

so

there was a genetic analysis done by lou

stouts laboratory in the ccr that was

able to find different genotypes of

lymphomas to separate them based on the

actual

genetic and proteinaceous differences

between these types of lymphomas to get

a better idea how they could be targeted

what he found was that an activated b

cell like diffuse large b cell lymphomas

were the most pernicious form they had

the least survival rates they were the

most challenging to treat and so he came

to us looking for ways that you might be

we might be able to find molecules that

target

these particular types of lymphoma

and so one of the aspects that he found

was that nf kappa b was found to be

constitutively active in these activated

bcell lymphomas

so

this shows some of the key pathways and

i know its a very complex diagram here

so im going to home in on just some

areas right in the center here and what

you see there are a variety of molecules

in this case proteins card malt

bcl0 and you can see a little box

there called malt inhibitors

and what were looking for are things if

you look below these things all turn on

nf kappa b pathway and what we want to

do is turn that off in these lymphomas

and so what we did is look for which

molecules we might be able to inhibit

and it turned out that malt is an

enzyme a protease and that was what we

targeted

so we set up an assay for inhibitors of

malt which is a translocation protein

and its active in these diffuse beasts

large bcell lymphomas and its a

hallmark of them so that the malt one is

also an interesting protein in itself

its a pear capsaice which is unusual in

the human genome in that it is an

arginine specific protease and there

arent many of those so that made it

potentially interesting also we uh dr

stouts lab used si rna and peptide

inhibitors of this protein and showed

they were selectively toxic to malt one

dependent diffuse large cell b lymphomas

so

this is the protein you see here is the

pear capsules thats is malt and what

we set up was an assay to identify small

molecules so this is a substrate for

bolt it has four amino acids and it

has a label on the right hand side here

malt one will cleave

that amino bond that you see there that

amide bond and it will free

that fluorescent label so what we did is

we set up an assay that could cleave

that label off of it and that would show

us the activity of molten one if we

added extracts to that they would

inhibit malt and they would increase

the fluorescence of that substrate

because malt one had been inhibited

so

we first thing you have to do for an

assay of this type is you have to

optimize the kinetics and the

biochemistry of the enzyme so we looked

at a variety of different forms of the

enzyme we looked at the time scale of

the reaction and the velocity of that

reaction and you can see here the gst

mult one had about a hundred micromolar

km so that seemed a good level of enzyme

kinetics and turnover rate for us to use

for the assay you can also see that we
selected a time point of 0 minutes
which was right in the middle of that
velocity curve so right theres still
linear velocity out to 0 minutes so if
we ran the assay for 0 minutes there
would be a constant rate of enzyme
activity and thats important to give
you optimal use of an assay
and what you see on the lower right hand
side is an assessment of assay
repeatability the common
term that is used is a z factor and a z
factor should be above 0 to be useful
for screening and it tests a whole
plates multiple plates on multiple days
at multiple times so each plate has
wells in it and each well as an
individual assay and you repeat that
multiple times and show that it has
significant repeatability you get the
same results day in day out no plate
differences and you can see here that
our averages were well above 0 in all
cases indicating that this assay was
useful for screening and robust enough

to do natural product research

here like the triangle i showed you

before gives you an idea of what our

throughput was we screened 0 000

extract samples we found 9 that were

originally appeared to be active again

we can confirm those in quadruplicate we

did secondary assays for selectivity and

reversibility and then we had selective

cytotoxicity in a cellbased assay so

our primary assay was biochemical

looking at just inhibition of the enzyme

that secondary assay was in whole cells

looking for selective killing of malt

cells we ended up with projects that

were worthwhile for chemistry this just

gives an example of a few of the ways we

looked at selectivity or specificity we

tested against two other enzymes arch c

which is another arginine specific

protease and also caspase9 which is

similar in structure to malt and we

took a look to see if any of these

molecules were selective and what you

see here are the inhibition constants in

micromolar for the different

molecules we found and what ill show

you here is how we did extracts with

extracts in the cellbased assay we were

actually looking for changes in an

nfkappab specific reporter so we know

we have a molecule that inhibits the

enzyme of interest it shows selectivity

and now we want to see in cells does it

selectively down regulate nf kappa b

which is really the end point we want to

be able to attack these lymphoma cells

and what you can see here in the graph

that we want to see the positive control

in this case a brute nib you can see

that the left hand side of those graphs

move in an upward direction going from

blue to green to purple and on the right

hand side you can see they go down in

the opposite direction that is the

positive control thats the signal were

looking for in extracts

we tested extracts and showed

activity like that where that first one

was going up and the second one was flat

or going down and this is an example of

what one of those positive extracts

would look like

we then took a look at this particular

extract which was a marine extract and

we saw that it had activity again making

the luciferase go up and leaving the nf

kappa b flat we saw that it inhibited

the enzyme and it turned out that we

were able to isolate these molecules

that you see here the calibulones

they were

isoquinoline quinones and they were

isolated originally from a bryozoan a

marine organism they showed activity

against malt at a micromolar

concentration and that was not affected

by glutathione they showed selectivity a

little better selectivity for caspase9

but then we took a look at this molecule

which is a synthetic derivative of the

calibulones and you can see here in

cellbased assays what were looking for

is something at the top that is not

affecting growth and in the bottom graph

that is excuse me the opposite way and

the top is affecting growth but in the

bottom is not and so what you can see

here the top

graph shows activated b cell lymphomas

the bottom graph shows germinal b cell

lymphomas so thats the difference the

top has nf kappa b active and thats

what we want to inhibit the bottom graph

doesnt so we want to see activity up

top and not at the bottom and what you

can see here is that compound i showed

on the previous screen selectively kills

the activated bcell lymphoma cells but

does not touch at 0 micromolar the

other cells and thats the type of

selectivity we want and so that molecule

is moving forward for further study and

potential modifications to increase its

potency now id like to move on to a

different type of assay this is actually

looking at an assay that attempts to

restore sensitivity to topoisomerase

inhibitors and this was a collaboration

we did with eve palmier in the

developmental therapeutics branch

so what is topoisomerase topoisomerase

is an enzyme involved in dna replication

and repair its an important enzyme your

cells cant grow without it however

cells that are rapidly turning over like

cancer cells are more sensitive than

topoisomerase inhibitors than others and

in fact there are known natural products

such as camptothecan which are

inhibitors of topoisomerase theyve been

used in the clinic for years theyre

very effective and what you see here in

the bottom lower right hand side of this

is that you see topoisomerase comes it

makes a cut in dna and then it allows it

to unwind and then repairs it what

happens is when you add camp to thicken

into this camptothecan binds and

prevents the topoisomerase from being

released

and so you build up these perturbations

to the dna and the cell cannot tolerate

that so after a period of time the cell

dies

however there are mechanisms for

recovery from these type of injuries

over time in evolution cells had to

adapt to be able to handle this type of

perturbation and so what you see in the

next is theres an enzyme called

tyrosine dna phosphodiesterase or

tdp

now what tdp do does is hydrolyze that

covalent bond to topoisomerase and so

when camptothecan blocks top isomerase

and freezes it on the dna tdp can come

and cut that off and that allows cells

to become more resistant to

topoisomerase inhibitors like the

natural product camp to thicken

so what we wanted to do was develop an

assay to look for inhibitors of that

enzyme to inhibit tdp then maybe we

restore sensitivity to topoisomerase

inhibitors and so as you can see here we

replace topoisomerase one this time with

a different fluorophore a redshifted

fluoresce force so we could see the

activity of this enzyme

and as i showed you previously we were

able to optimize the enzyme kinetics and

all the parameters of that assay to have

a very robust assay and this just shows

some of the

statistics for that assay we screen

synthetic compounds partially purified

natural products natural product

extracts and you can see overall we

screened over 00 000 different samples

and had a total number of hits of about

only so this was a very rigorous

assay

what you can see here is a secondary

assay that was done even though we had

only a low number of confirmed hits they

had to be confirmed in this assay

instead of looking for changes in color

or fluorescence were looking for

changes in a gel shift assay for the

presence or absence of a band that shows

that tdp was able to cut and what you

can see here for example in this marine

extract

c0 you can see that the bands are

missing at the higher concentration

shown by the fat side of the triangle

above the gel bands what you can see

there is that in the first three highest

doses theres no lower band indicating

that tdp is unable to cut that piece

of dna meaning that it only goes at the

higher molecular weight and theres no

lower molecular weight band and you can

see as we go to lower concentrations

that yes we are able to see that lower

band because we get below an effective

therapeutic concentration

so what are the compounds that were

able to isolate here we found additional

marine compounds

this time from a sponge called

zestospongia there was a family of

compounds the helenoquinols they were

isolated from this they were confirmed

in the gel shift assay they also were

tested to increase the presence in cells

of topoisomerase adducts which they did

and they did not inhibit a selective

they selectively inhibited tdp but not

a similar enzyme called tdp

and so these look like potentially

interesting molecules but how do they

work

well one of the things you can do is

look at the biochemistry of how these

work what you see on the upper lefthand

side is a graph that shows

changes in the velocity of enzyme

activity after the enzyme has been

treated with drugs but the drugs have

been washed away so you pretreat the

enzyme with drugs you then dilute the

drugs out to well below any effective

concentrations and then you look at

enzyme activity and see if the enzyme

can still be active and what you see in

the circled areas and in those lines

that are down near the bottom of the

graph is that there those are indicative

of irreversible inhibition so that once

we treat the protein with the drug even

though we wash the drug away its still

stuck to that protein still stuck to

tdp and still able to inhibit its

activity and that was the case with

these drugs so dilution of helena quinol

sulfate away from tdp did not reduce

its inhibition activity indicating that

it was likely covalently bound to the

protein a suicide inhibitor if you will

the other aspect we looked at was what

type of inhibition

tdp normally uses a certain substrate

its going to bind to dna but it also

uses atp

now one of the things you look for in

enzyme inhibition

if you are competing with the substrate

for binding to the same site the dna

does that would be considered

competitive inhibition what were

looking at are things that are going to

be noncompetitive so they dont bind to

the same site as the dna you dont

necessarily want a dna memetic you want

something that acts at a different point

of the enzyme what you can see here from

the lower graph on the left hand side is

classic michaelismenten kinetics

indicating noncompetitive inhibition

for helena quinoal sulfate indicating

that its binding somewhere other than

the substrate binding site

and thats important because that means

we might be able to identify new

biochemistry and new biology about this

protein and find some new site that

drugs could be able to inhibit

so

helenoquinol itself is an interesting

molecule it could react to covalently

bind two different ways one binding to

cysteine residues and one binding to

lysine residues so which way were we

seeing this react that was one of the

things we wanted to look at well it

turns out in fact that helinoquinone

sulfate

which only has

area two here that you see the lysine

press preference possible because area

one is no longer a quinone it is

sulfated at that position and so is

unable to react with cysteines so we

knew that this molecule and the

mechanism by which it was inhibiting

tdp had to go through an addict onto

lysine rather than cysteine

so if we take a look theres already

been a drug in clinical trials phase two

clinical trials called wertmannon or an

analog of workman which you see on the

upper left hand side here and where

mannin has a very similar structure if

you look to helena quinol sulfate

especially in the bottom part of it that

reacts with lysine and so we wanted to

take a look to see if what manned would

work in a similar mechanism as hellenic

quinoa sulfate against tdp

what we found is that what mannin was

inactive against tdp

which indicated that the other parts of

this molecule outside of that red circle

must be important for the selectivity of

helinequinol inhibition against tdp

so what is the structural basis for the

inhibition what part of tdp has a

pocket that will fit hellenic when all

but wont fit wart manning and how does

that work

to get that answer

we were able to do structural studies

and what you see here is a

threedimensional structure of tdp

we were able to get crystals a colleague

of mine

tanush mulaway who worked in our

laboratory was able to get cocrystals

of tdp with helena quinol sulfate

now

tdp one itself has different lysines

so there are different possibilities

that hellenic quinoa sulfate could bind

what we found however was that this

region that you see here highlighted

is an unstructured loop

that was found in the back of tdp one

away from the substrate binding site and

when we took a look at where tdp one was

bound

we found that it selectively bound into

that site with those three lysines and

if we look in more detail in the

preliminary xray crystal data there was

a particular lysine lysine number

that helenoquinol sulfate bound to when

we use mass spectrometry to identify if

there were any other lysines that were

modified by tdp we did not find any

other lysines only a single lysine in

tdp was modified by hellenic one all

sulfite indicating that this region was

sufficient to inhibit the enzyme even

though its 0 degrees away from the

binding site for substrates so that

gives us important information of

additional molecules that might be able

to be designed to bind into this site to

be more druglike and inhibit tdp

you can see here some of the points of

that interaction

so

ive shown you some examples of how

to screen natural products and the ways

we do that and some of the challenges so

how can we make this better you know a

significant cost in time and resources

is the effort to do the natural products

isolation and structural elucidation

so here youre going from a crude

extract through fractionation in a

column like you see here

to the characterization of an active

molecule you can see here an example of

some of the chemists that might be

working in your laboratory doing this so

how do we get natural products to be a

little more efficient for us

one of the ways is to prefractionate

the extracts themselves so if we

partially purify the extracts what

effect will that have what we see is

that in cellbased assays we see an

increased hit rate for those enzymes

indicating that we might have

concentrated minor components

and increased the percent of the active

compounds in those fractions

in cellfree assays like the ones i just

showed you for example tdp we see

lower initial hit rates meaning that we

have purified away some of those

nuisance compounds that gave us

nonspecific activity and so we get a

higher percentage of hits that confirm

because those

extracts that show activity or fractions

that show activity now have a higher

rate of confirmation because theyre

freer from those nuisance compounds

so prefractionation removes some of the

nonspecific false positives and also

increases that reconfirmation rate

so

what the nci has decided to do is

theyre undertaking a large automated

prefractionation of natural product

extracts thats going on right now

and what there are plans to do is create

a one million fraction library of

semipure natural products for screening

we hope that this will help sequester

nuisance compounds and concentrate these

low percentage active metabolites and

this will provide a value added as wed

like to call it screening library unlike

anything currently available and like

the crude extracts themselves this will

be made available to researchers

worldwide for free

and then the use of this

prefractionated library should increase

the efficiency of both highthroughput

screening and subsequent chemistry

efforts

just to show you an example were also

in undertaking rapid secondary analysis

so in this case we have

prefractionation of a crude extract and

you see the extract up at the top and it

shows activity the farther those bars

progress to the right of those graphs

the more active the component in that

fraction you can see that that activity

was concentrated in just fractions four

or five here fractions one two three six

and seven the graph bars are much

shorter indicating theres not much

activity there whereas fractions four

and five the bars on those graphs are

longer indicating more potent activity

in those fractions

so what we now undertake is a rapid

secondary analysis and weve built high

throughput mechanisms for this so that

we can create a thousand subfractions

per day we can basically take that

initial fraction that showed activity

and further purified into

subfractions these fractions can be

done in an automated manner where we can

do fractions and hours on one

instrument

and what we can then see are

standardized chromatography runs like

you see below separating these compounds

based upon their lipophilicity or c log

p

so what will this allow us well rapid

prioritization

of hit list so now if you get a hundred

to two hundred things weve set up a

system whereby you can screen

00 of these through the second level

purification and get those plates back

and have that complete within two weeks

this should improve the speed and

efficiency of hit confirmation by

screening laboratories and it will

assist both intramural and extramural

research researchers in the

identification of new active natural

products

and also an important point here

although its the last one listed here

is this will minimize the waste of the

extracts the natural products repository

is a national treasure its

un

usual anywhere in the world it is

something that is not being recreated

some of these extracts are the only ones

we have of those so finding the chemical

diversity in them is important by using

this methodology we use much less of the

extracts which preserves that repository

for future generations and future

researchers and so thats very important

as well

so one of the ways we can look at data

is important too so now with

bioinformatics we can look at large

swaths of data and with the new

prefractionated library being large and

0 000 extracts to start theres a lot

of data to look at and what you see here

is something we call a selforganizing

map that members of our laboratory

created to evaluate the biological

activity of the extracts in aggregate so

we look at all the extracts at once in

this case were looking at their

activity in the nci 0 cell line panel

so that is a panel of 0 different human

tumor cell lines so each of the extracts

was tested in five different doses

against all 0 cell lines so that gives

you 00 data points for each extract and

what the selforganizing map does is

look at the pattern of activity

basically its like a

pattern of dots of the activity against

those 0 cell lines and they compare

that to the pattern of dots from every

other cell line and they keep comparing

them and comparing them and its self

learning so it what it does is it keeps

looking and looking for matches and

closer and closer and when it finds

things that are close enough they go in

individual nodes which are the circles

you see on the graph here each one of

those circles has approximately you know

0 or so extracts in it indicating that

their activity in the 0 cell panel the

pattern of that was close enough that

they might be related and so what we do

is we think this puts those extracts

into biological space which is can

basically our way in this assay of

looking at their activity against cancer

cells and what we do is we parse this

selforganizing map by increased lethal

response or by increased potency or

lower initial concentration of response

now when you look at it in aggregate you

get a huge map like this this is looking

at every extract thats ever been tested

in the nci0 panel and every pure

compound thats ever been tested in the

nci0 panel and what you see here are

individual nodes now that contain about

compounds those nodes that are

lighter in color than the other nodes

are nodes that only contain extracts

there are no pure compounds in those

nodes indicating that maybe that region

of biological space might be a good area

to look for new chemistry

and what you can see here are numbers on

a few of these and those numbers are

particular extracts that might be of

interest

this is an example of something we

worked on so we looked at pure

compounds through this and were able in

a short period of time to identify

pure compounds two new natural products

and do that in a period of only six

months

one of the aspects in looking at natural

products are these going to be drugs and

so what we did is we wanted to look at

the chemical space coverage of these and

this basically takes a look at different

parameters that are involved in

chemistry the weight of something its

lipophilicity its aromaticity perhaps

its flexibility by sp carbons what you

see here is all those molecules that

we isolated on a threedimensional graph

of lipophilicity molecular weight and

aromaticity and you can see theyre

nicely spread amongst those parameters

here weve exchanged the x axis for

flexibility this time while retaining

aromaticity and lipophilicity and what

you can see is again we have a nice

spread of compounds with a nice amount

of sp carbons indicative of what is

typical for natural products

now importantly what we wanted to do is

compare what these look like as drugs

and so there were methodologies that are

used to compare compounds to each other

in this case we wanted to compare them

to all approved natural product drugs

and what you see here we took natural

products approved from 9 to 00 and

compared them by the same parameters we

just compared those molecules that we isolated using a statistical program chem gps and what you can see in this next graph is you see in three dimensions again natural product drugs aromaticity molecular weight and lipophilicity the natural product drugs are shown in blue the natural products that we isolated with our test system for this new high throughput methodology are shown in orange what you can see is theres a significant overlap in the chemical space in this case instead of the biological space we looked for with the psalm now were looking at chemical space and what you can see is a significant overlap of known drugs with the type of compounds were finding through these methodologies the only area thats missing is you can see this area down here and that is an area that is a much more polar compounds those dont seem to be present but that is likely because our test set was only

organic extracts not aqueous extracts
and aqueous extracts are going to be
more likely to contain the type of polar
compounds that you see down in that
lower region so as we get more data in
the future on both aqueous and organic
extracts we are hopeful that we will
have continual overlap with what is
required from natural product drugs so
that we know were identifying the type
of molecules that could lead to
clinically useful drugs
so with that i would just like to end
and basically let you know that this
process of prefractionation
with selforganizing map analysis
metabolomics rapid isolation and
structural illusionation will come up
with pure natural products that are
useful and i hope some takehome
messages are that you will see that
natural products continue to be an
important source of chemotypes resulting
in approved drugs that screening new
crude natural product extracts requires
assay modifications and hopefully youve

learned a few of those today and that
the high hit rates with extracts require
you to use different prioritization
techniques to be able to weed through
those extracts and find those that could
be most useful
also prefractionation can make a
significant difference
and new automation technologies in the
throughput and efficiency of natural
product efforts and finally these new
bioinformatic interfaces will allow sort
of an omic level of prioritization and
evaluation of natural product research
and hopefully improve natural product
discovery
and with that i just want to acknowledge
several people in different laboratories
who contributed to the data i showed you
today
so with that i would like to thank you
very much for your attention i hope that
ive been able to show you some new
aspects of natural product research and
hopefully some of what youve seen today
will encourage some of you to get

involved with natural product research

because theres a lot more things to

find out there and hopefully well find

more molecules that will be helpful to

the cancer patients we try and treat

here at the nci

you