Hot Science Cool Talks

UT Environmental Science Institute

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Diagnosing Ourselves: Take Two Assays and Don't Call Me in the Morning

Dr. Andrew Ellington

April 4, 2013

Take two assays and don't call me in the morning

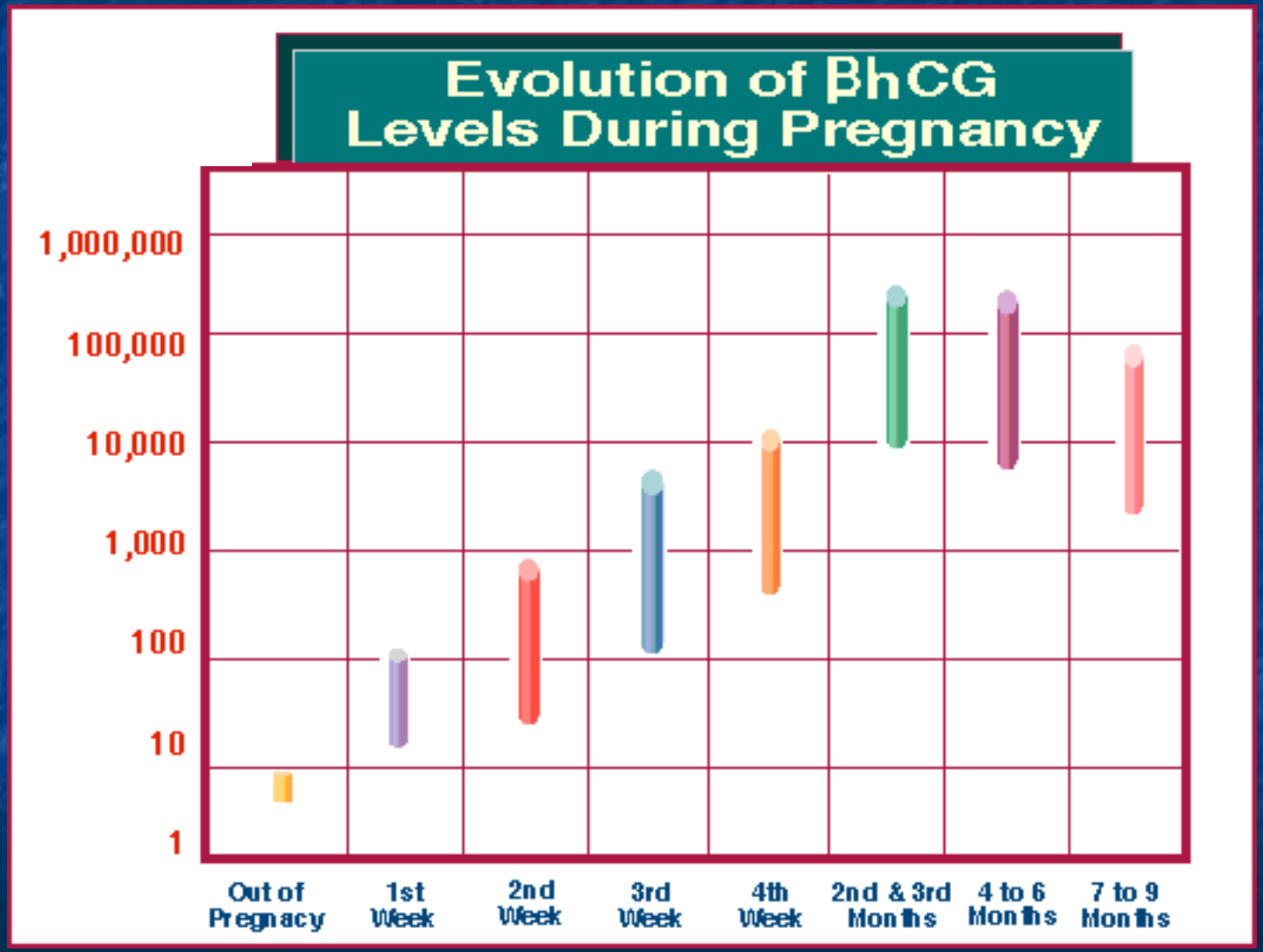
Andrew D. Ellington
Fraser Professor of Biochemistry
Center for Systems and Synthetic Biology
University of Texas at Austin

Our story starts in a somewhat odd place: how does a woman know if she's pregnant?

While "the rabbit died" became a common way to announce a pregnancy after being popularized by Lucille Ball on a 1952 episode of "I Love Lucy", injecting a rabbit with a pregnant woman's urine will not kill the rabbit. Researchers did inject female rabbits and other animals with pregnant women's urine throughout the 20th century in an effort to discern pregnancy. They theorized that a chemical in a pregnant women's urine, known as human chorionic gonadotropin (hCG), would stimulate the rabbit's ovaries. Unfortunately for the rabbit, the quickest way to see whether or not her ovaries were affected by the urine was to kill and dissect her.

Read more: Homemade Pregnancy Tests | eHow.com http://www.ehow.com/about_5390948_homemade-pregnancy-tests.html#ixzz1tQjG8weS

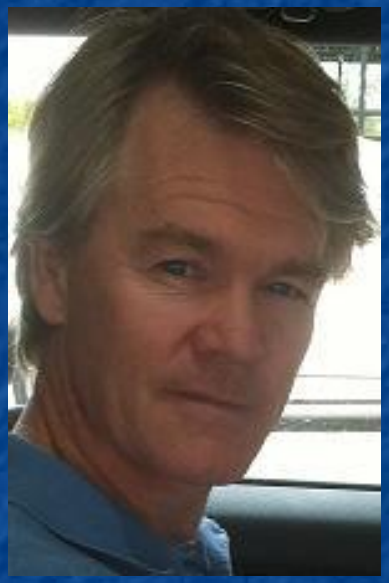
Of course, what the rabbits were really measuring was human chorionic gondaotropin, a hormone induced early in pregnancy.



And if rabbits could measure it, surely we could, too?
If only someone could figure out … how?

Monoclonal antibody technology

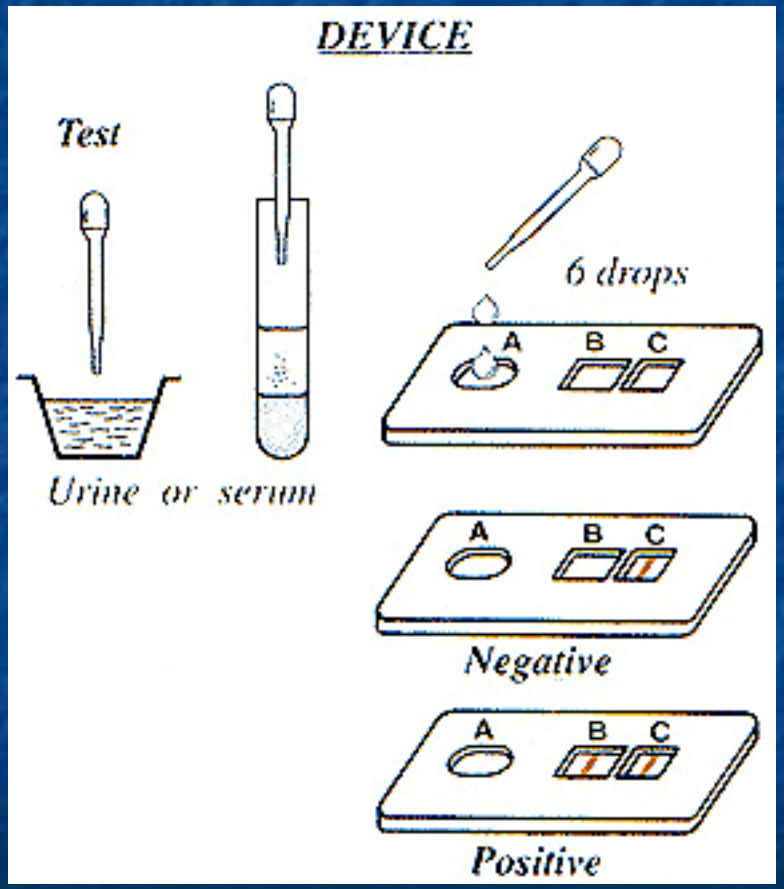
Materials science

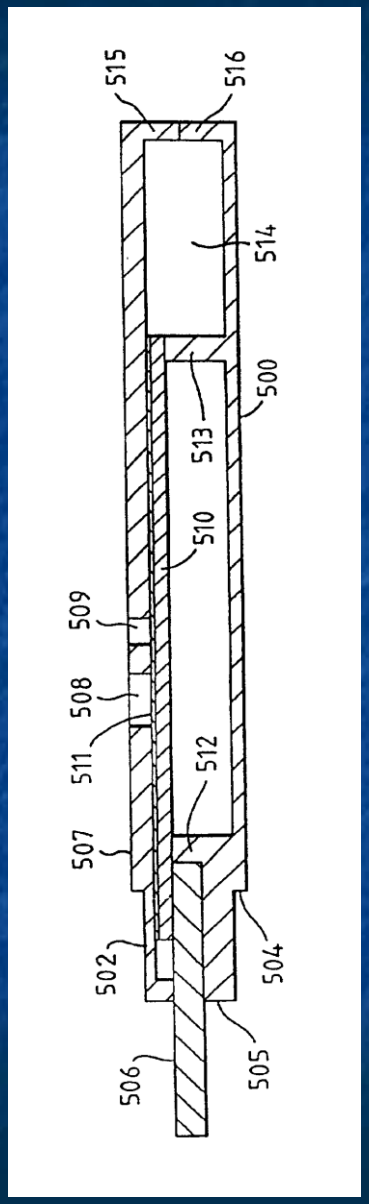


This is where Dr. Ian Richards, amongst others, comes in. Ian in the end made a very complex task … very simple.

Social forces

What Ian did was to take a laboratory assay for hCG that was cumbersome, and make a complex device that was simple in design and use.

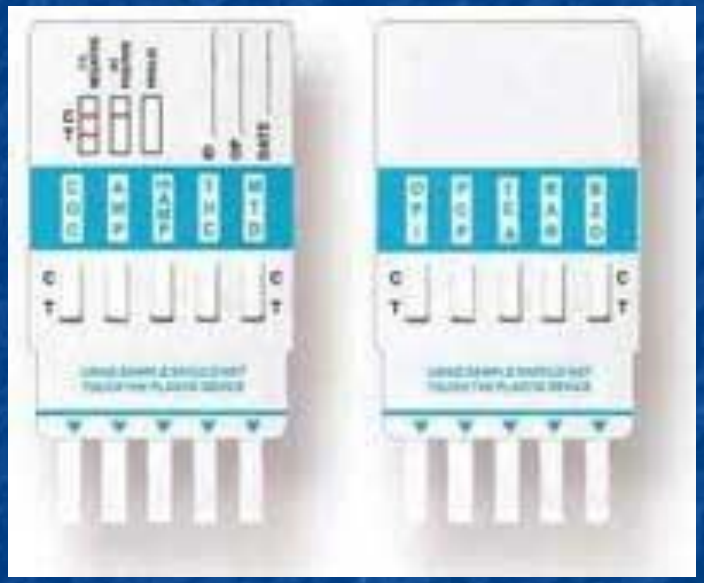




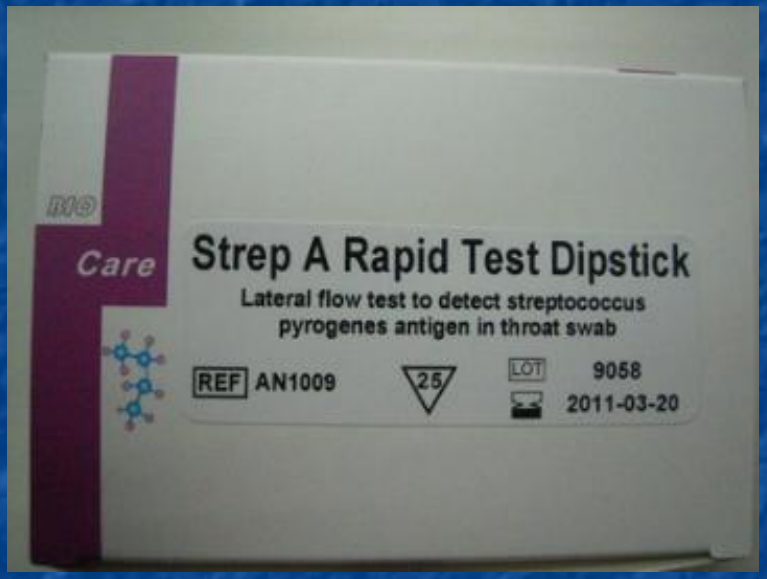
And this is the device, updated, that is still used today.

And now, we are finally to the point of the talk:

If we can measure hCG easily, why can't we, and why don't we, measure lots of other things in a similar manner?



Drugs of abuse

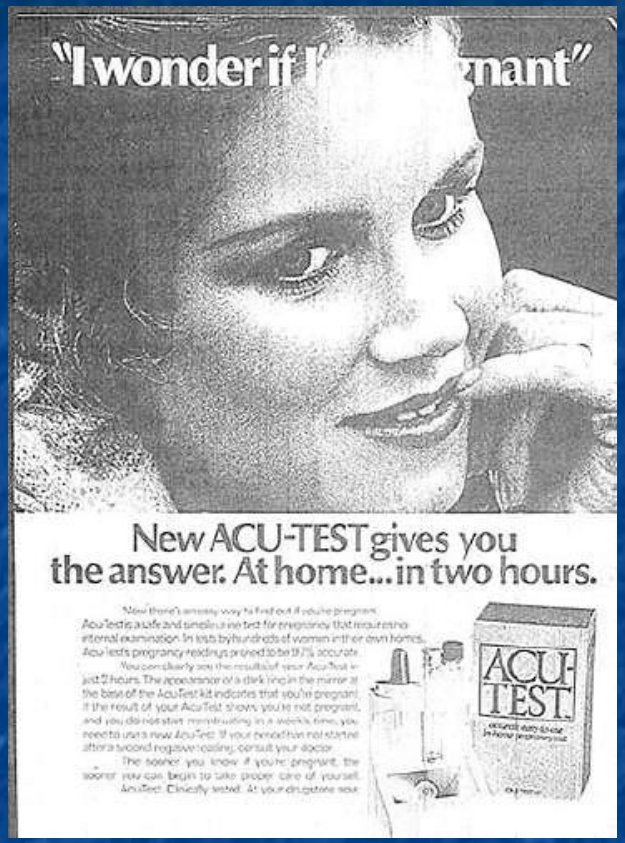


Bacteria of nastiness

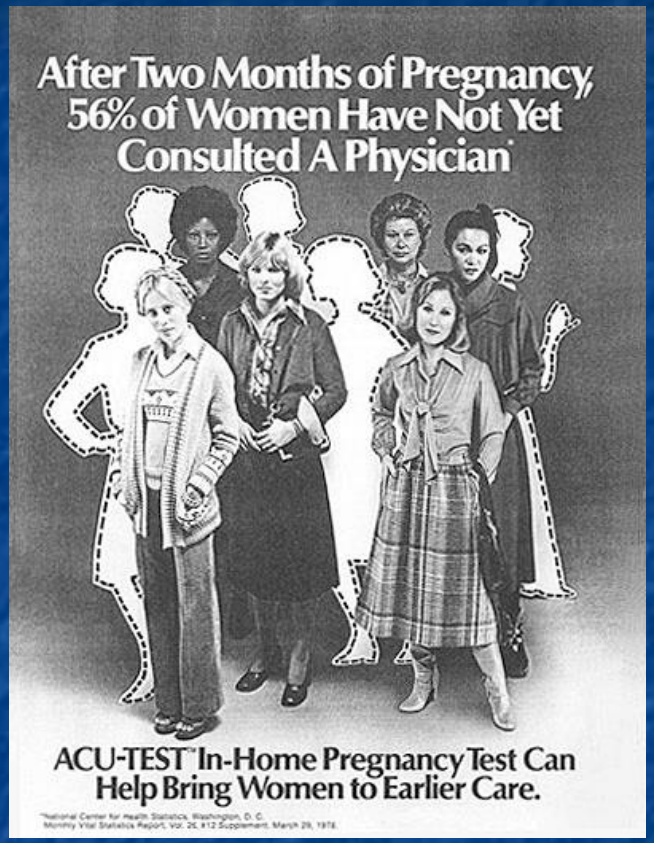
Well, we do, sort of. It's possible, but it hasn't really caught on.

What Ian reminded me of, was that there was a social revolution going on in parallel with the scientific one.

We go from women not being trusted to make decisions that a doctor 'should' make, to ….



Circa 1976

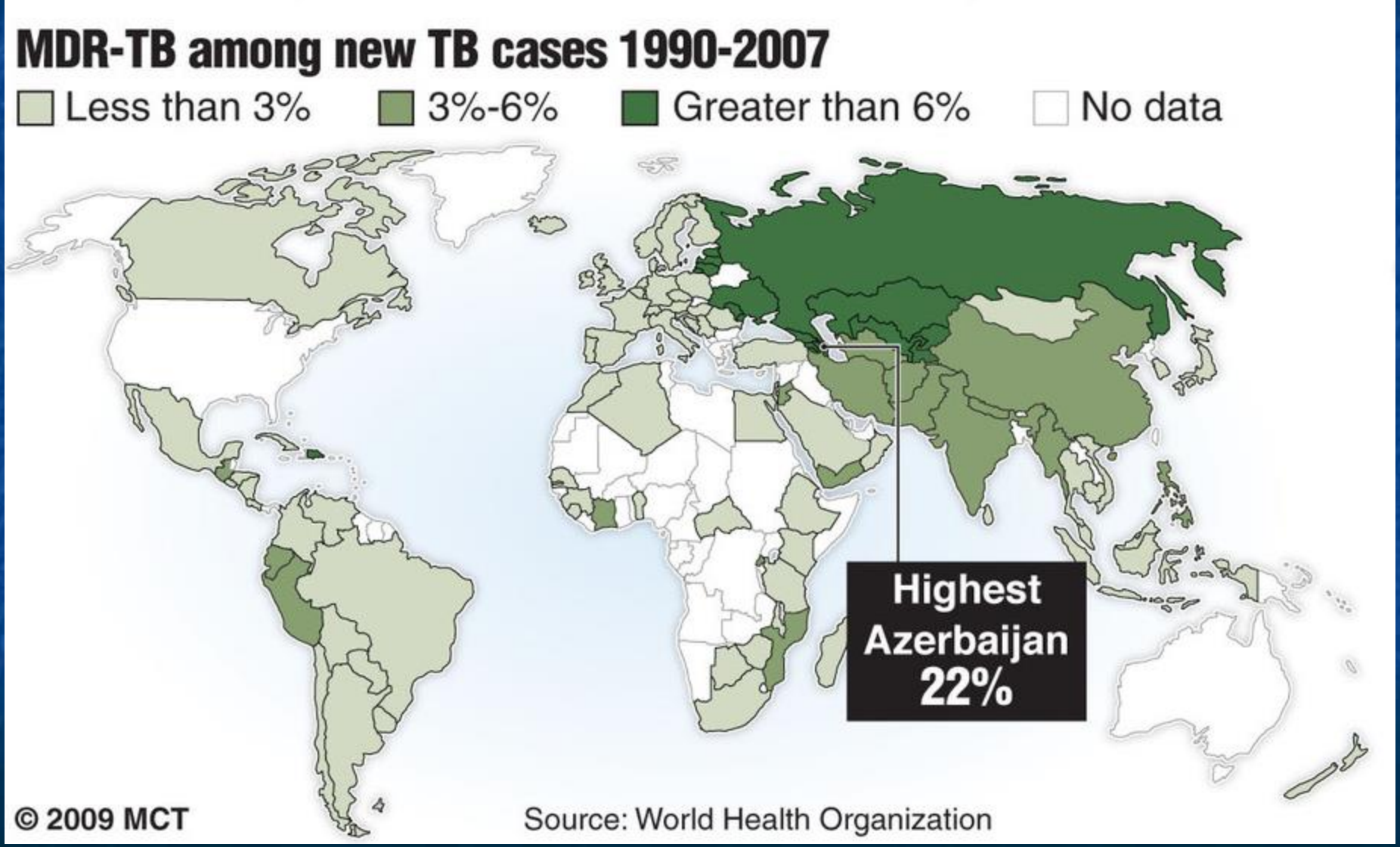


Circa 1979

And here's where my research comes in …..

Multidrug-resistant tuberculosis

There are nearly half a million new MDR-TB cases a year worldwide.

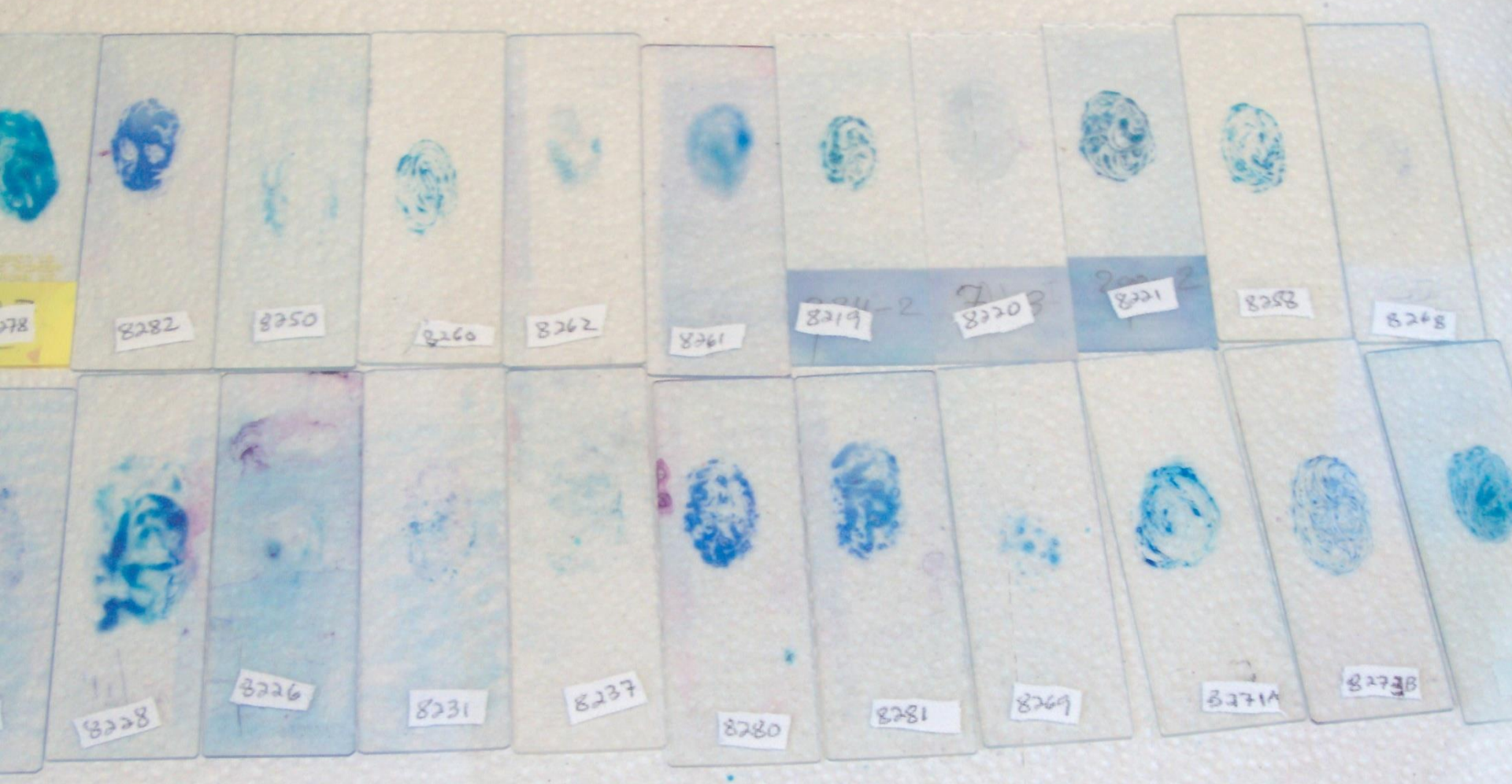


A different woman, a different social revolution: monitoring drug resistant tuberculosis in Afghanistan

-Collect slide material
-Boil, centrifuge, collect supernatant
---*Phenol chloroform extraction*
-Nested PCR of *rpoB*
-Sequencing
-Analysis

- Gain a picture of the extent of rifampin resistance in primary tuberculosis isolates.
- Identify relative frequency of resistance-conferring mutations to set up interpretation of new resistance tests.
- Determine geographical distribution of resistance.
- Evaluate the feasibility of molecular mutation detection for the Afghanistan National Tuberculosis Program, utilizing the existing infrastructure as an alternative to phenotypic susceptibility testing.

Sputum from Afghanistan

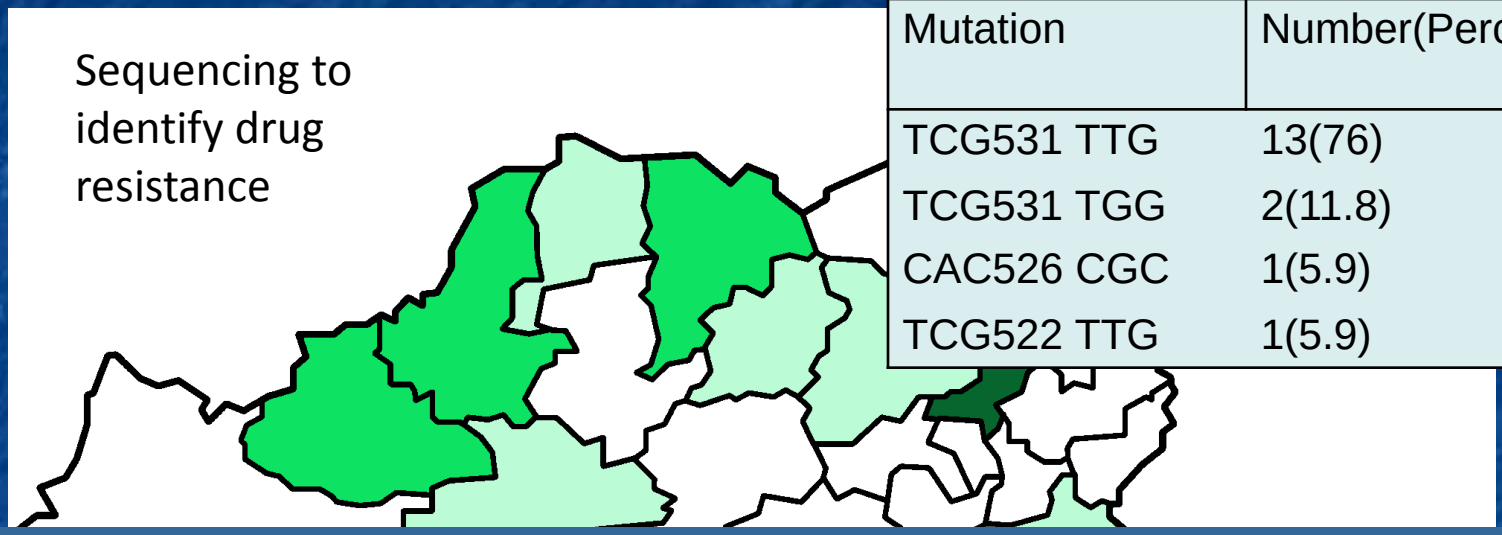


Clean, number, and select slides

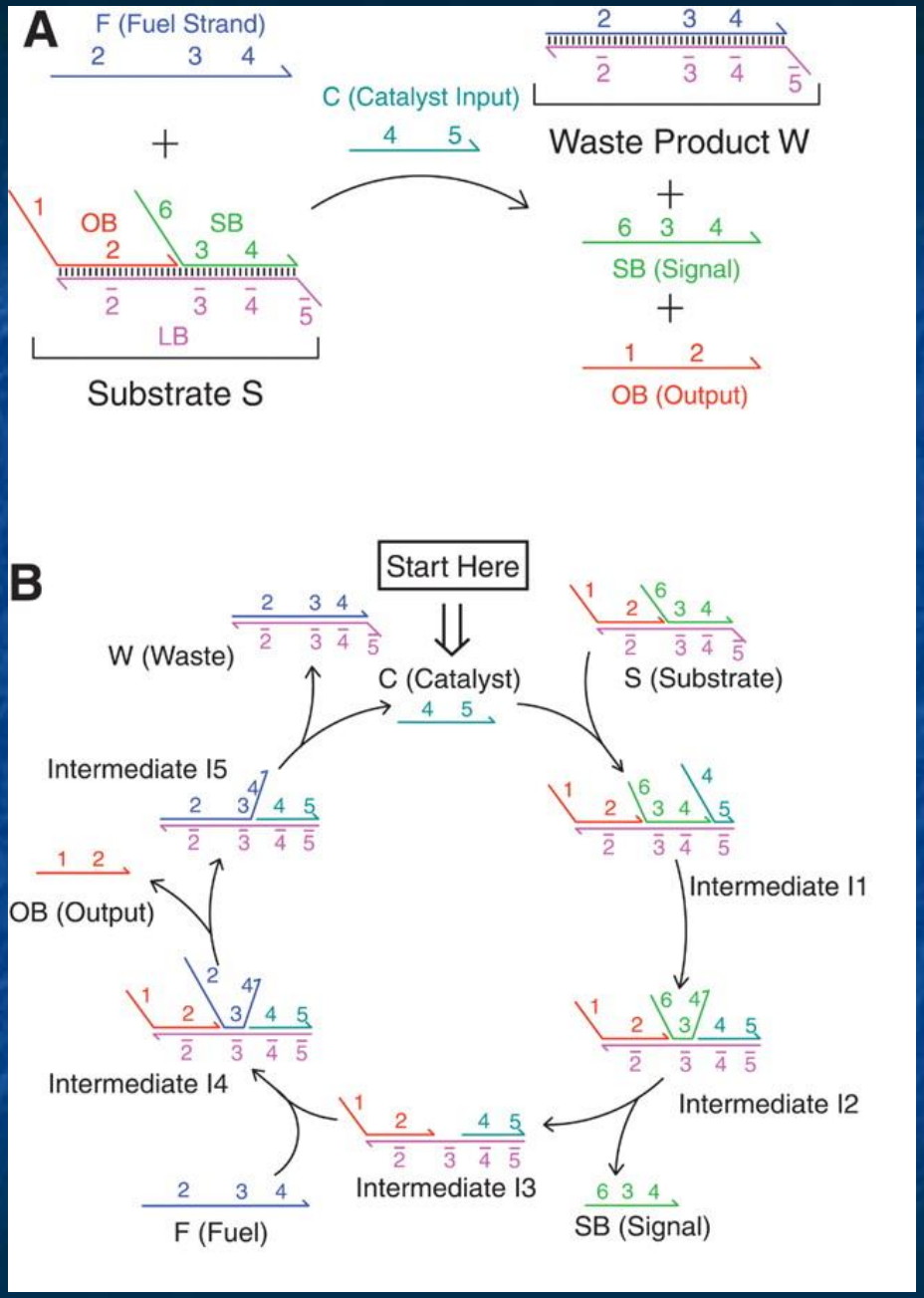
Afghan Cases	Slides Received	Slides Chosen	Samples Amplified	Resistant Sequences
~5000	1120	511	150	17

Sequencing to identify drug resistance

Mutation	Number(Percent)
TCG531 TTG	13(76)
TCG531 TGG	2(11.8)
CAC526 CGC	1(5.9)
TCG522 TTG	1(5.9)



So, we've gone from pregnancy tests of convenience, to life-or-death decisions where medical care is sparse … but the story gets weirder (this is Austin, after all):



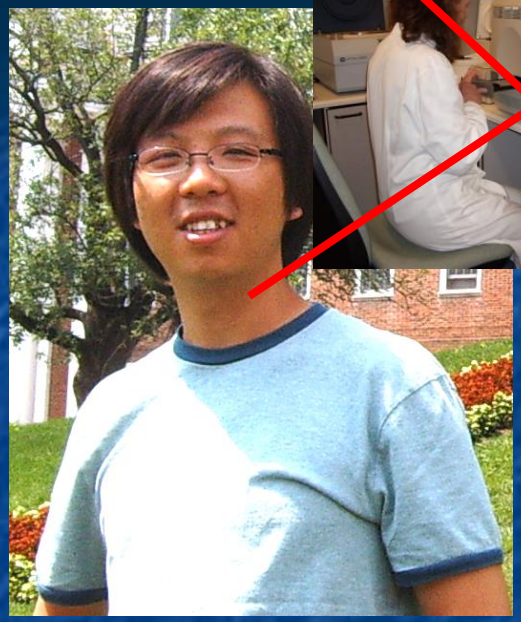
Zhang et al. (2007) Science 318:1121

How to make computers with carbon, rather than silicon

Erik Winfree

Niles Pierce

Peng Yin

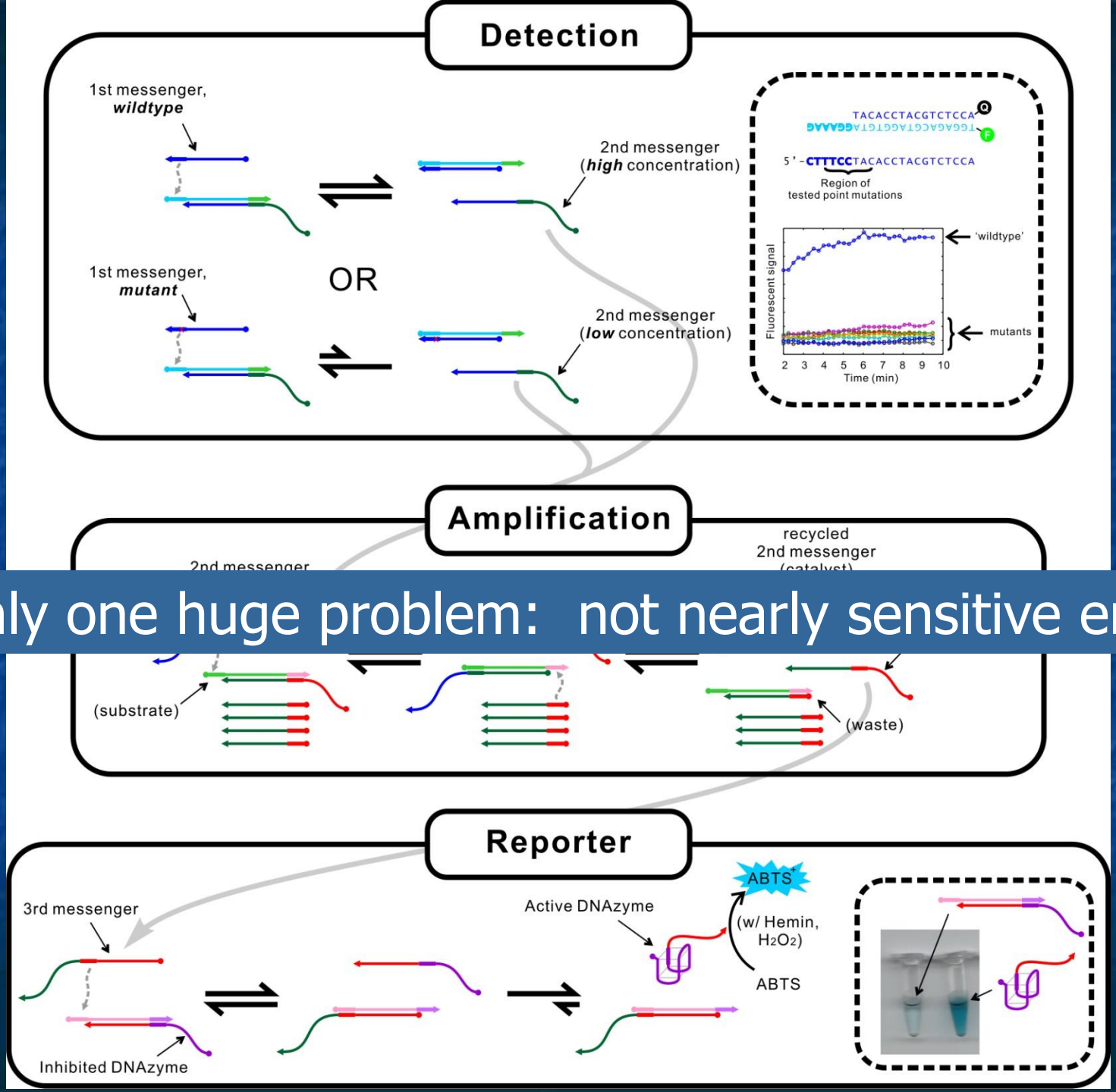


Xi Chen, brilliant and driven graduate student

+

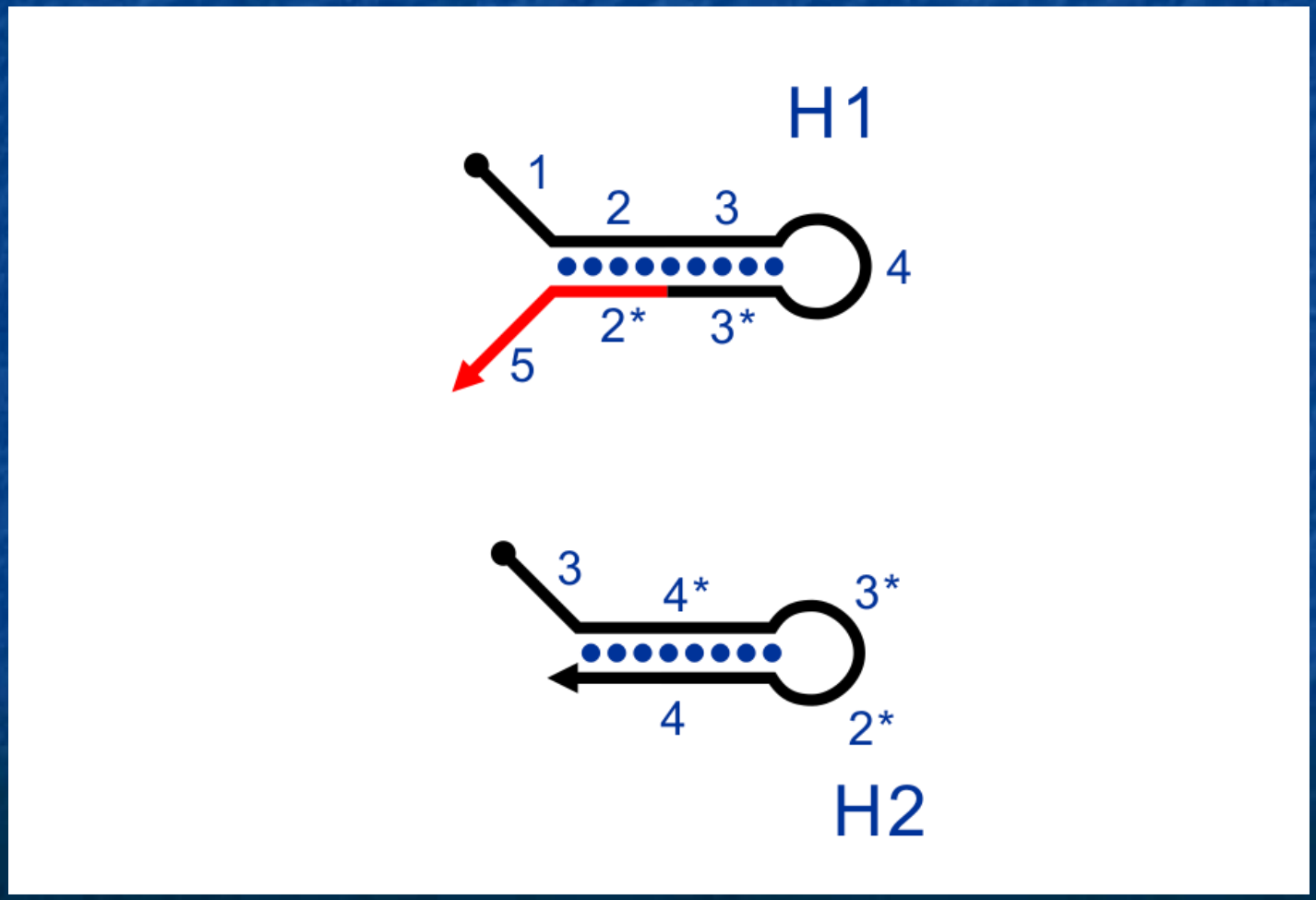
ce Eckhoff, brilliant
compassionate
undergra

(And this, my friends, is why you go to th
Texas at Austin, rather than the University
at Online)

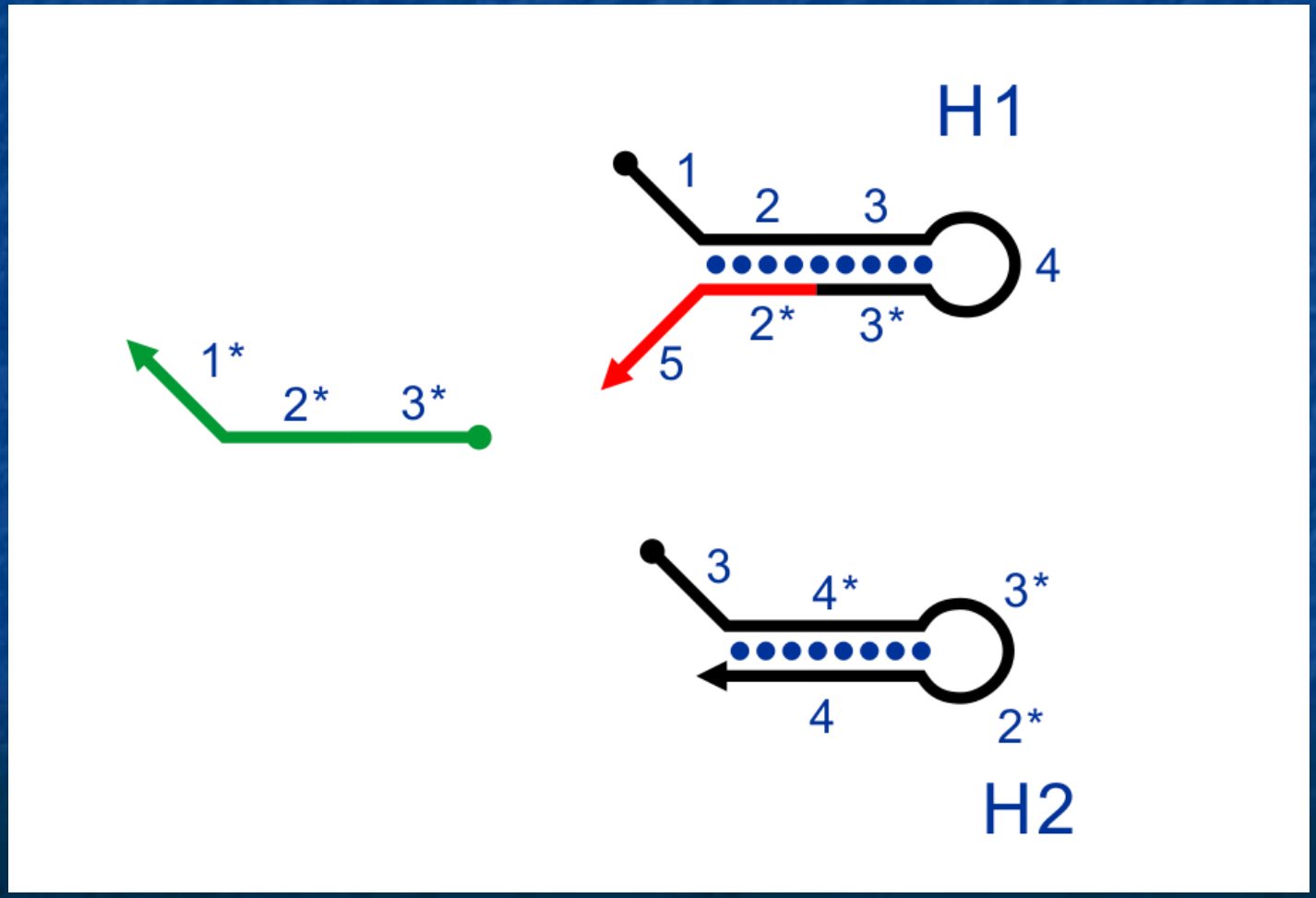

Detection
1st messenger,
wildtype
2nd messenger
(high concentration)
OR
1st messenger,
mutant
2nd messenger
(low concentration)
TACACCTACGTCTCCA
5'-CTTTCCTACACCTACGTCTCCA
Region of
tested point mutations
'wildtype'
mutants
Fluorescent signal
Time (min)
Amplification
recycled
2nd messenger
(substrate)
(waste)
Only one huge problem: not nearly sensitive enough
Reporter
3rd messenger
Active DNAzyme
ABTS
(w/ Hemin,
H2O2)
ABTS
Inhibited DNAzyme

A brief scientific digression, so you know what I'm talking about:

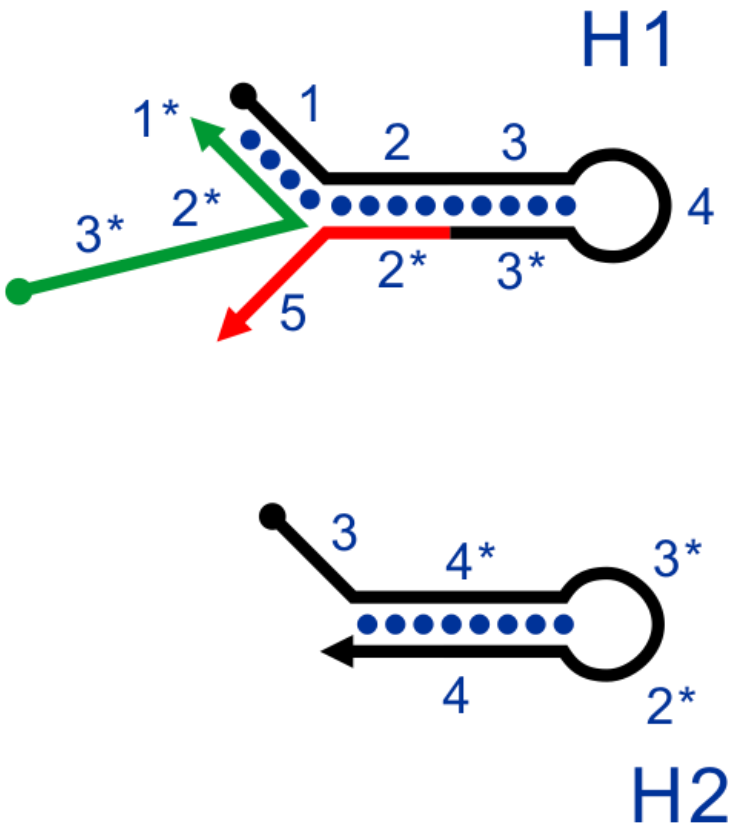
Catalyzed hairpin assembly



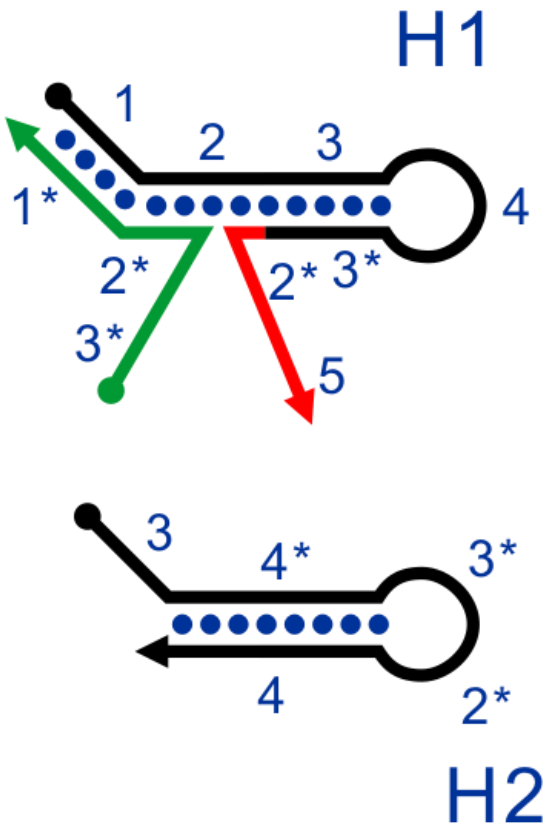
Catalyzed hairpin assembly



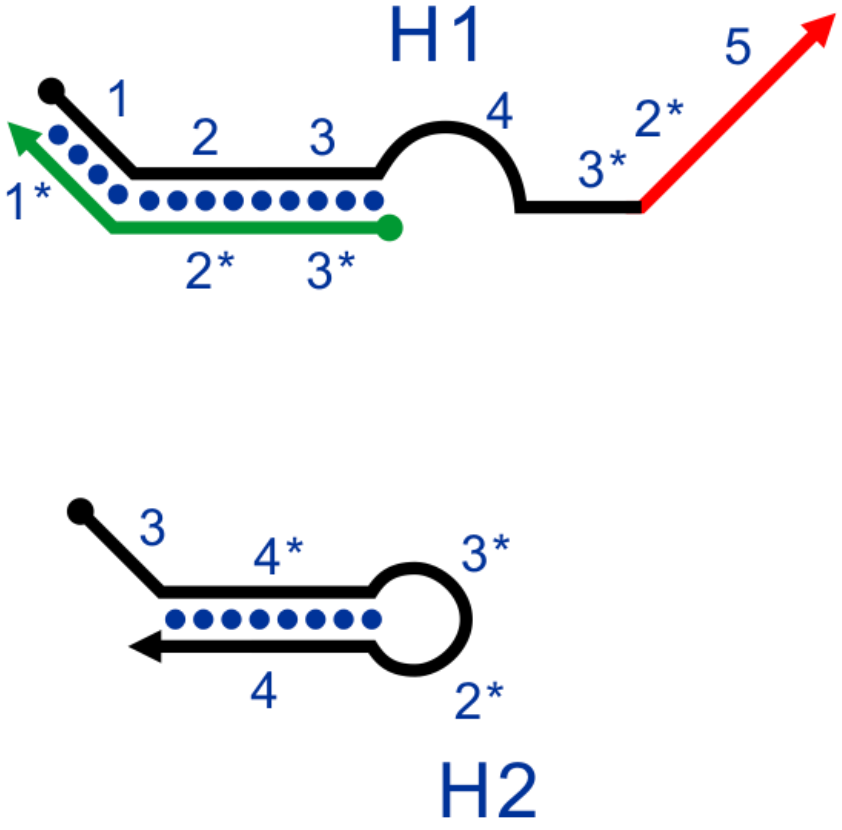
Catalyzed hairpin assembly



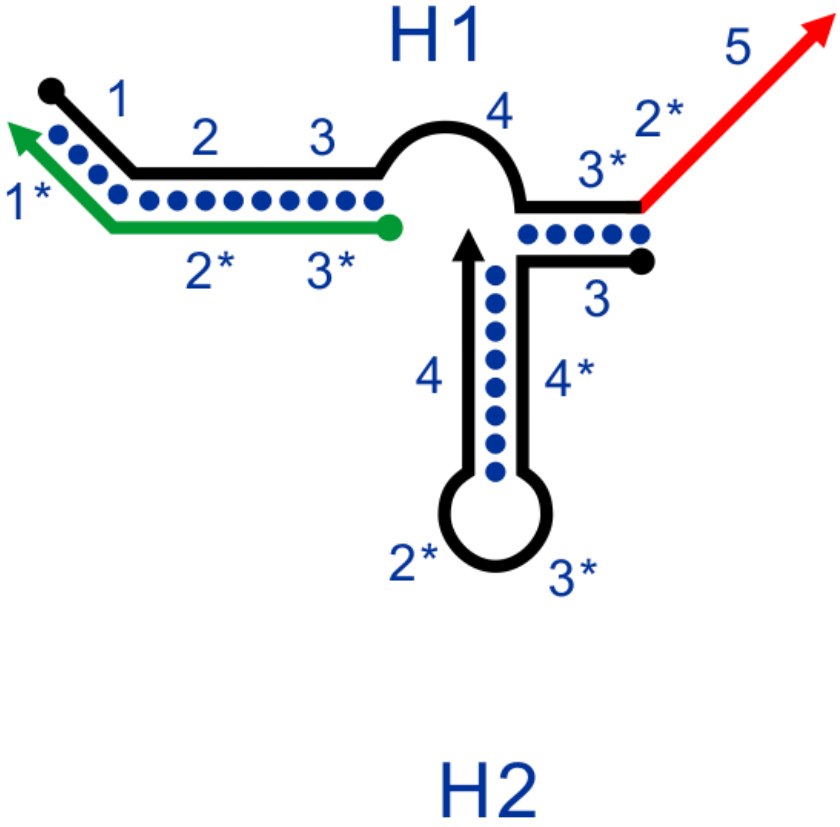
Catalyzed hairpin assembly



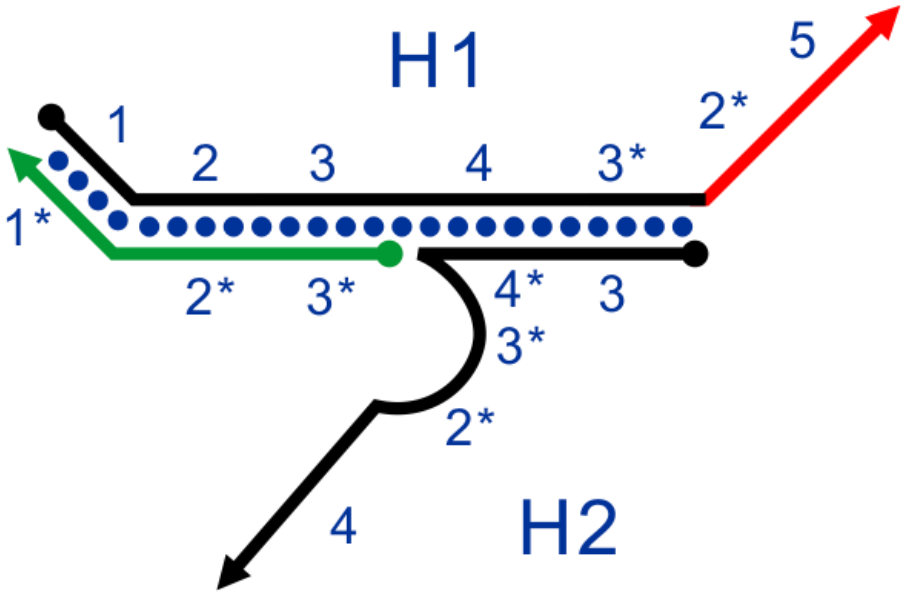
Catalyzed hairpin assembly



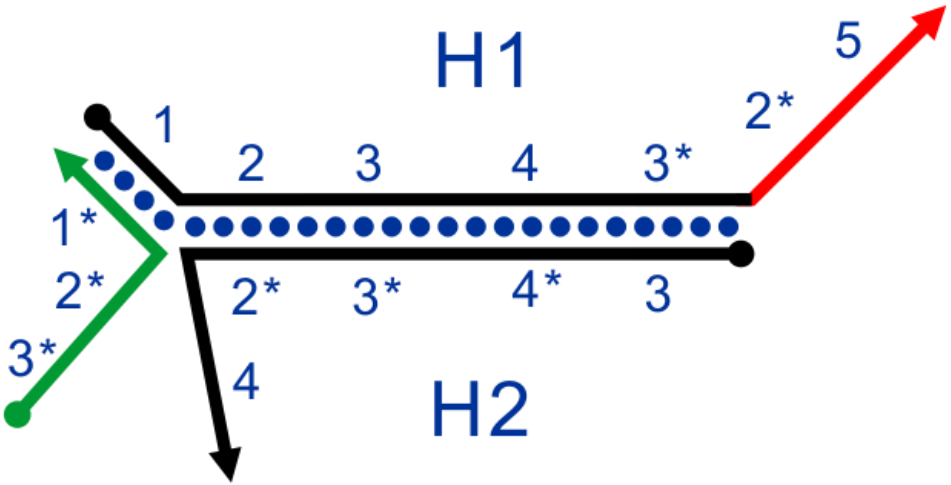
Catalyzed hairpin assembly



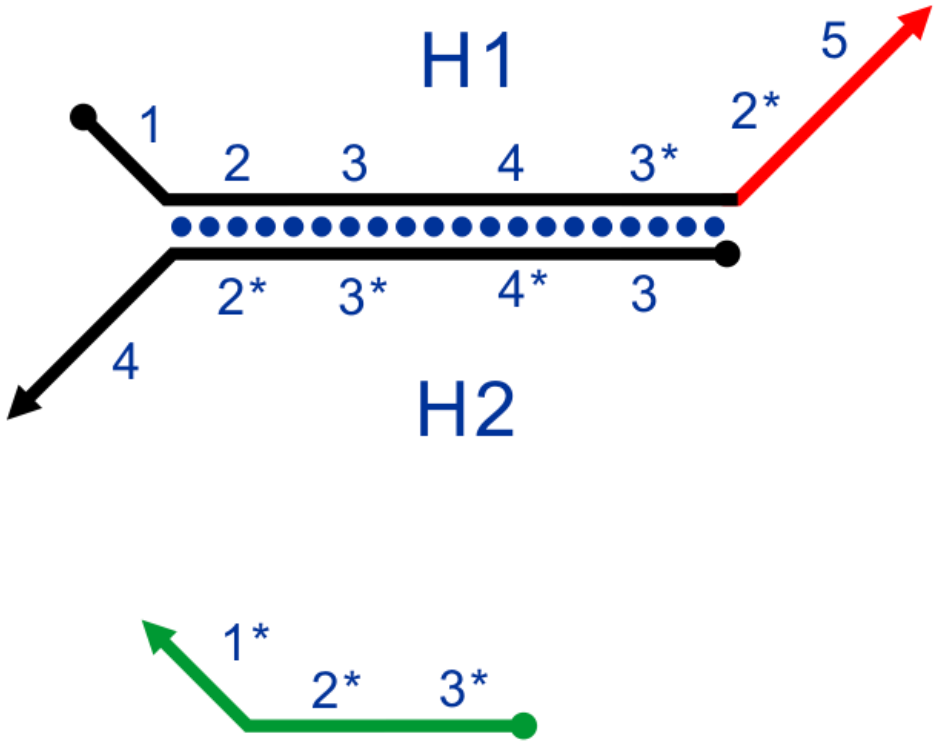
Catalyzed hairpin assembly



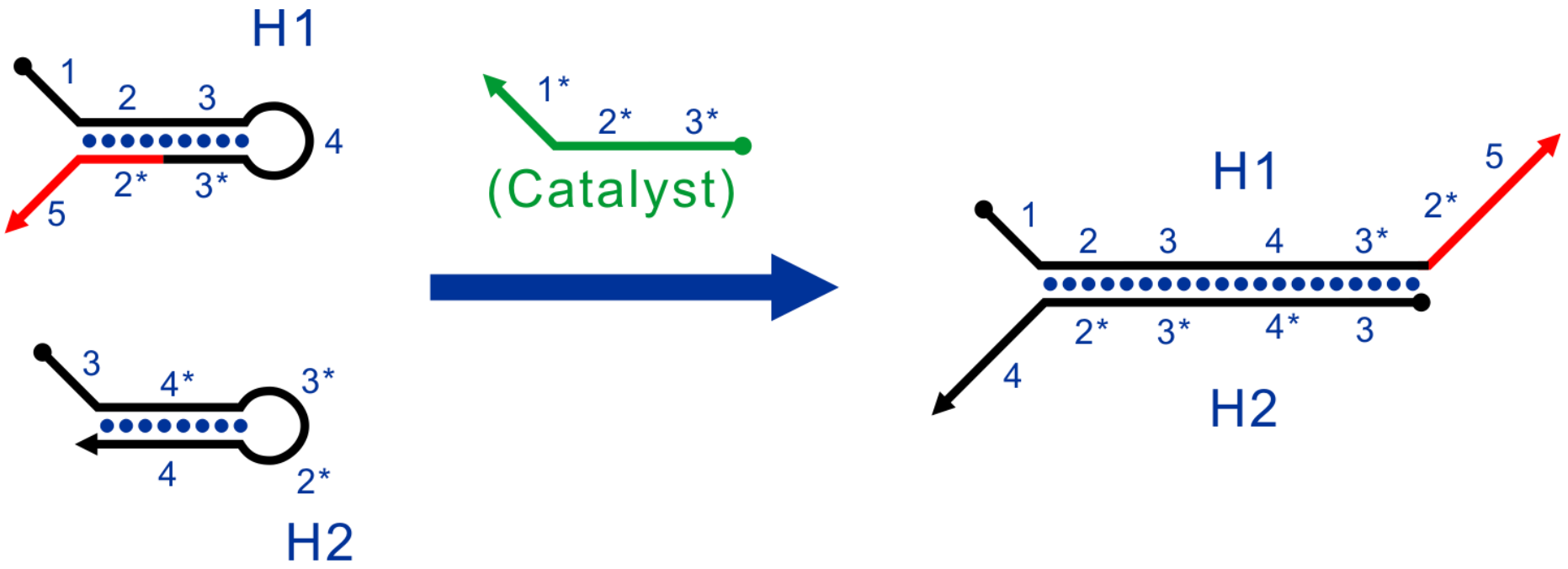
Catalyzed hairpin assembly



Catalyzed hairpin assembly

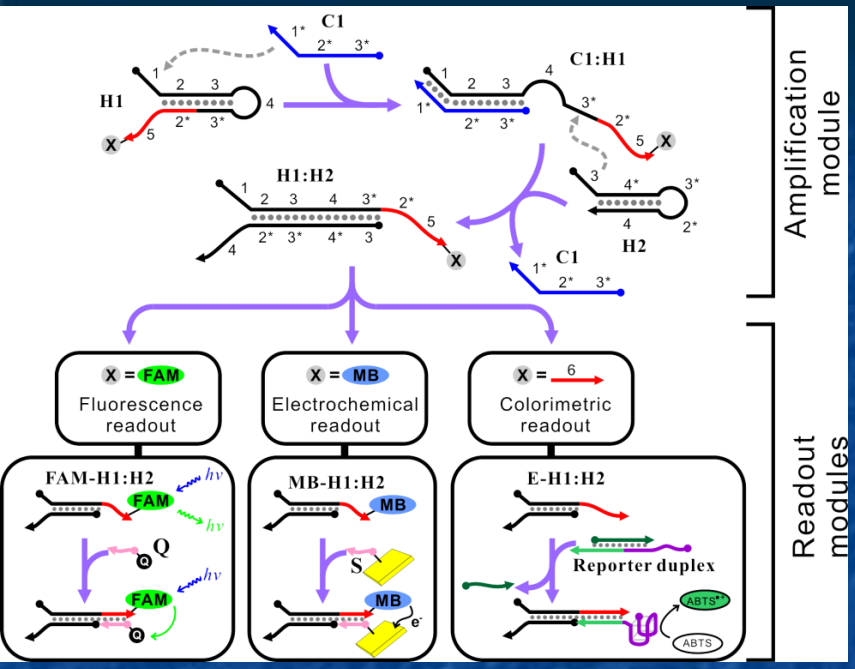


Catalyzed hairpin assembly

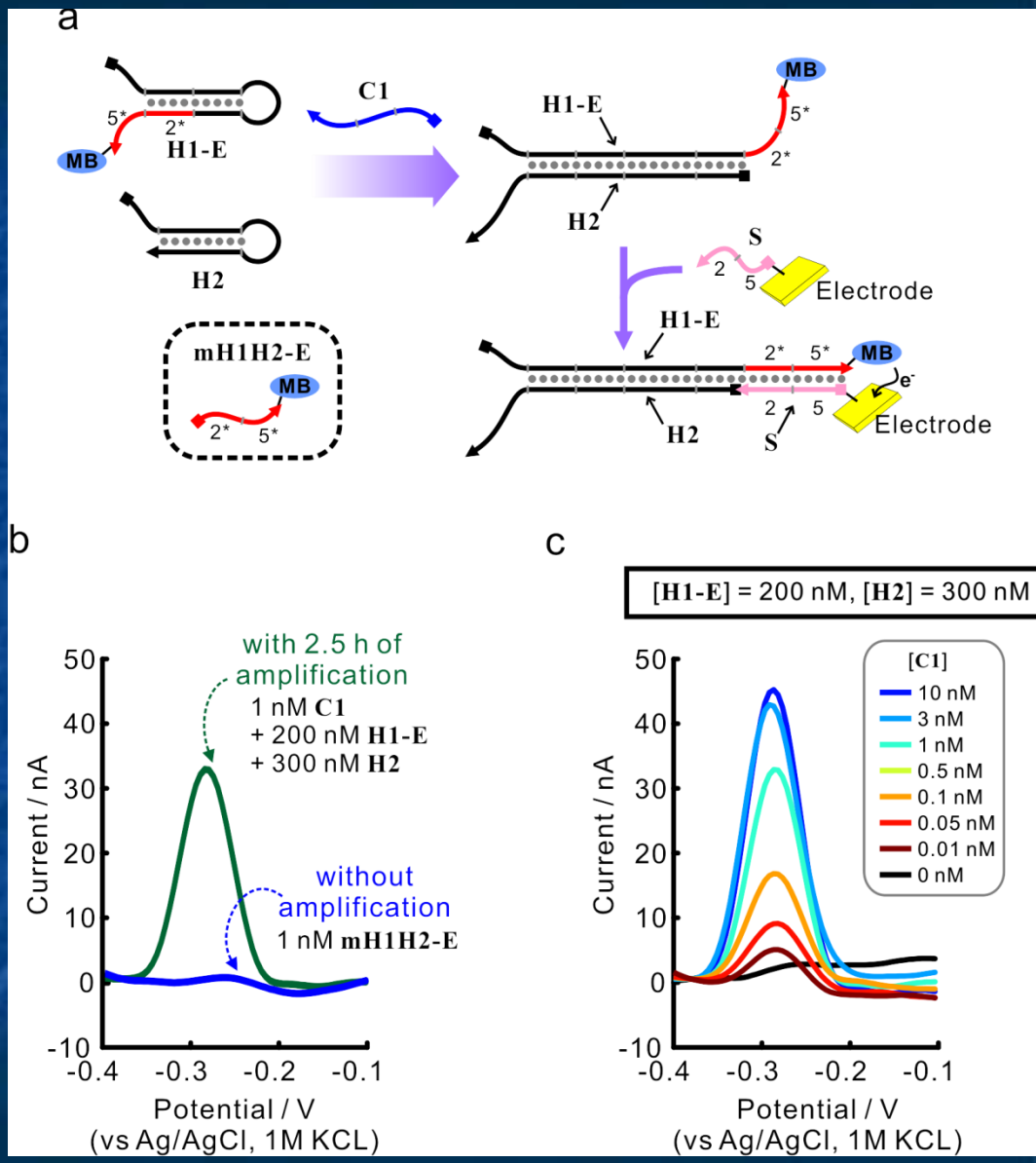


Overall reaction

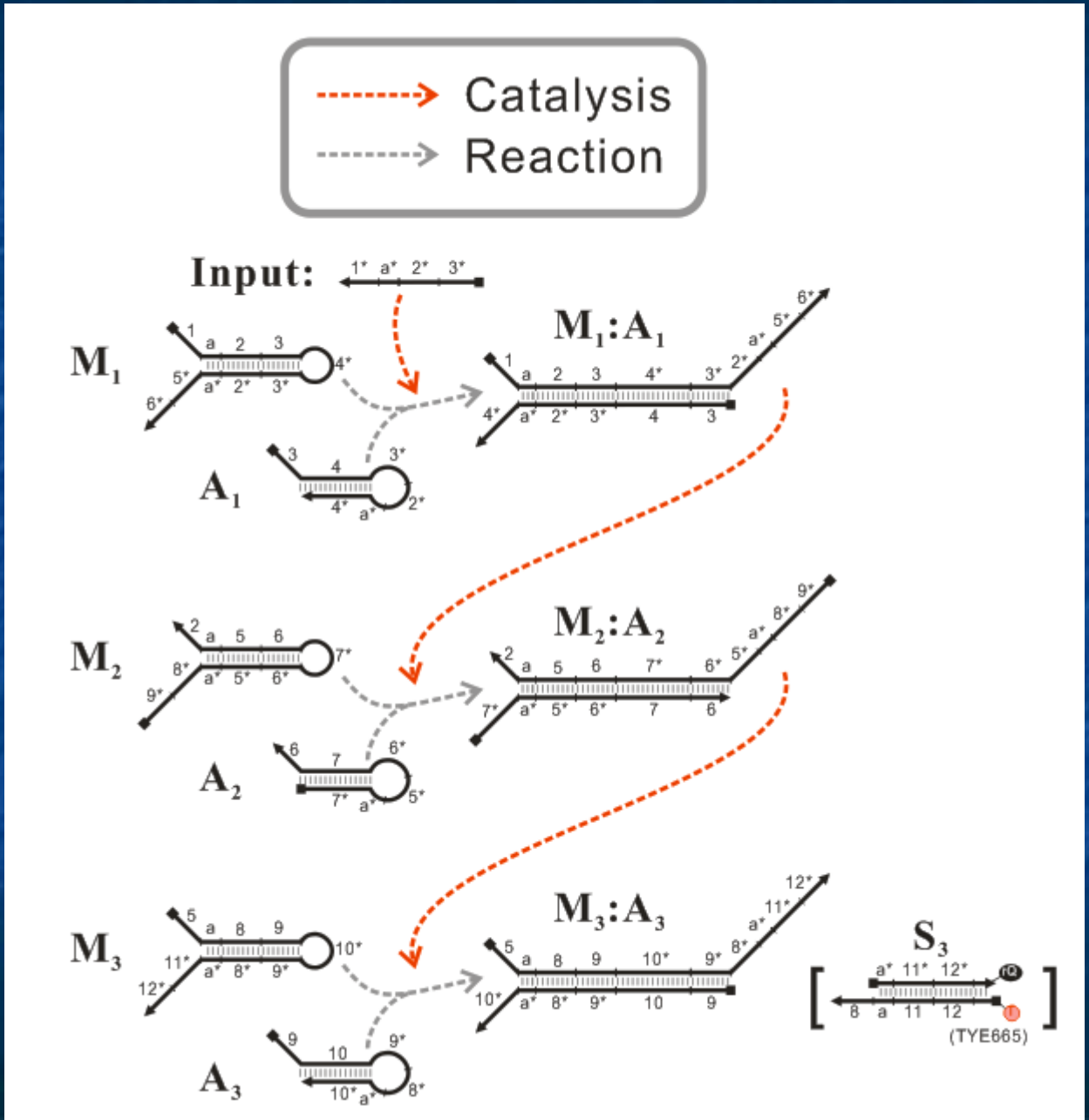
Adaptation of catalytic hairpin assembly to detection



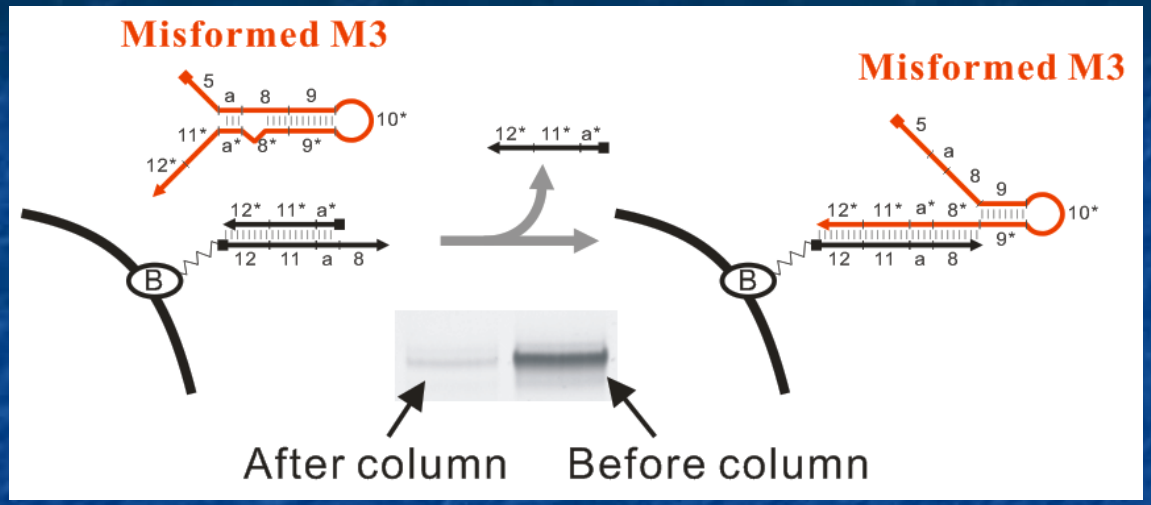
- Multiple different ways to look at the same reaction.
- Modularity of adaptation to platforms.
- 100-fold amplification (still not good enough!)
- A little slow (1/min turnover)



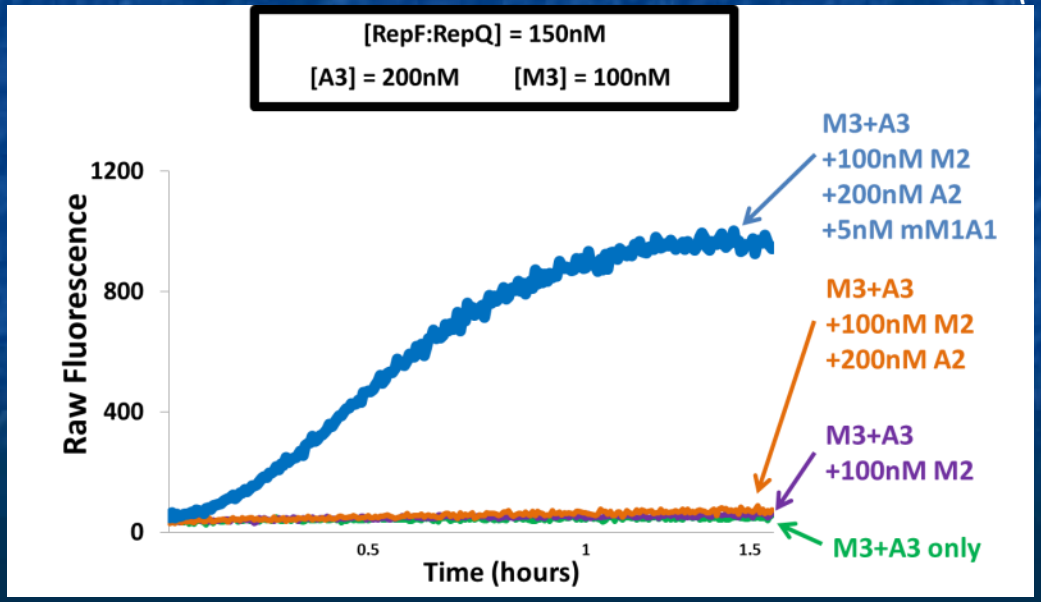
If one reaction isn't good enough, maybe we can stack them, just like electronic circuits?



Problem: background leakage from misformed hairpins → Get rid of the mis-formed material!

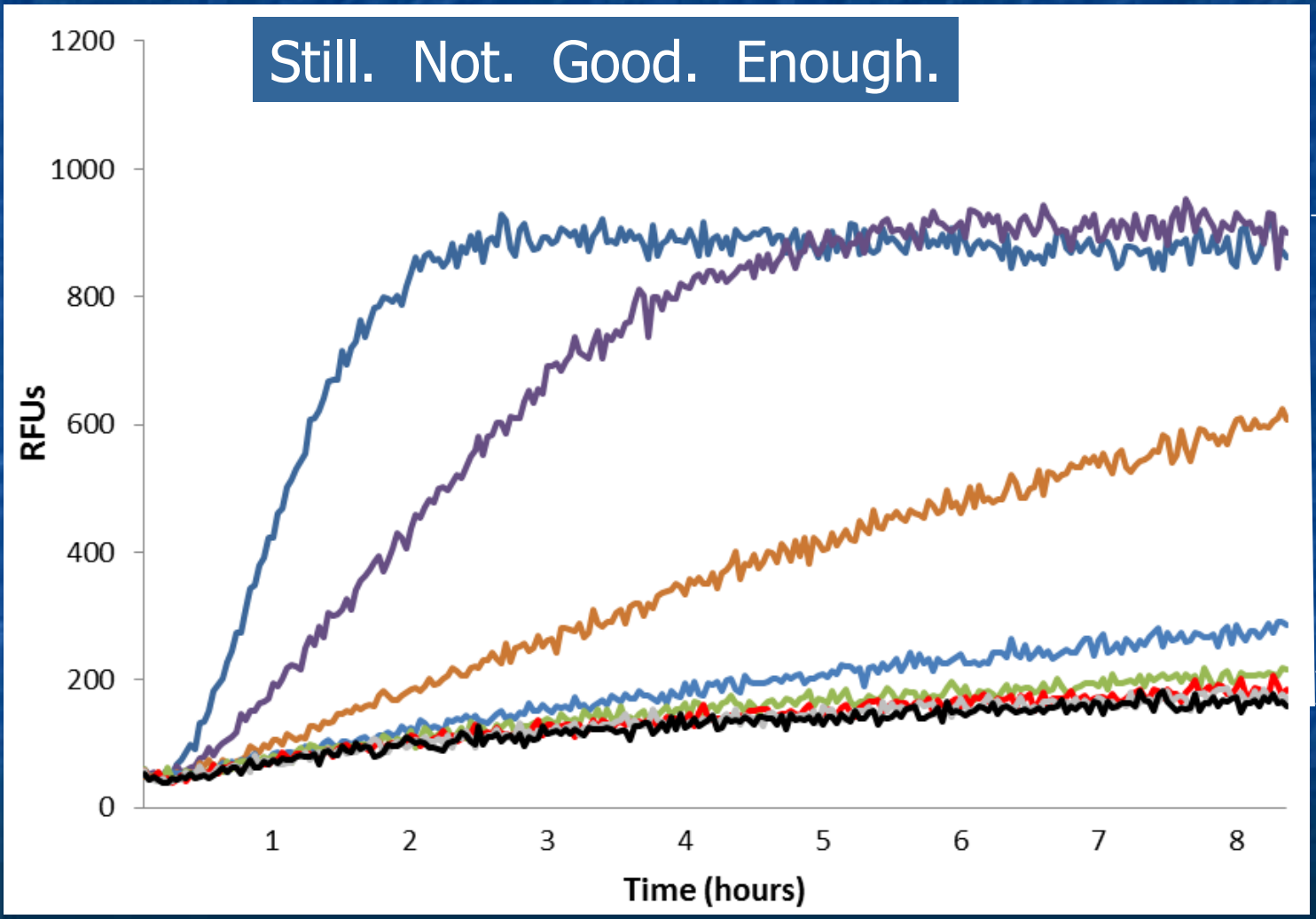


(Credit: Jeremy McLain)



(Credit: Neima Briggs)

Results – 1,000x amplification achieved with extensively purified DNA



(Credit: Neima Briggs)

Eliminate impurities with arge-scale (>1 nmole), enzymatic oligonucleotide synthesis

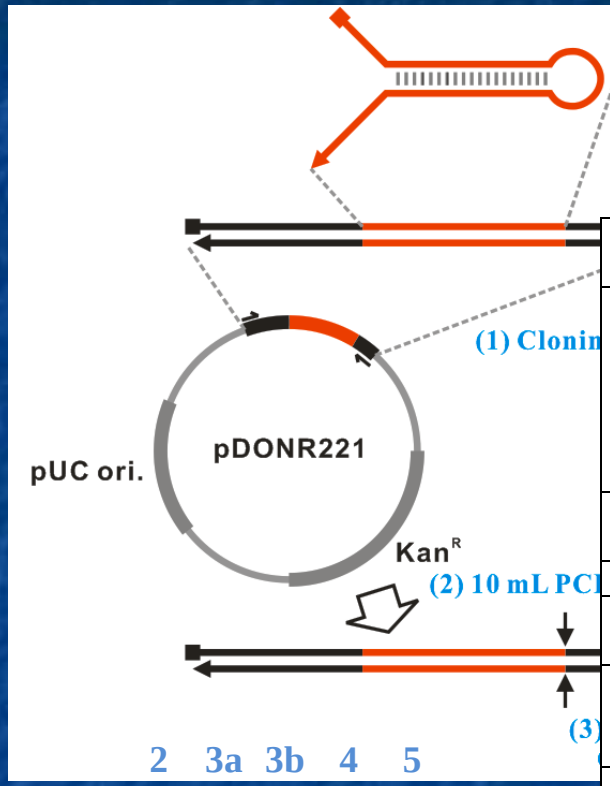
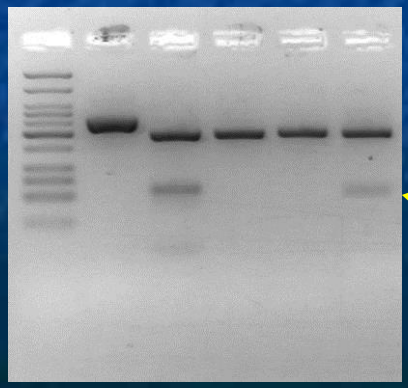


Table 1. Estimated cost for each batch of DNA hairpin

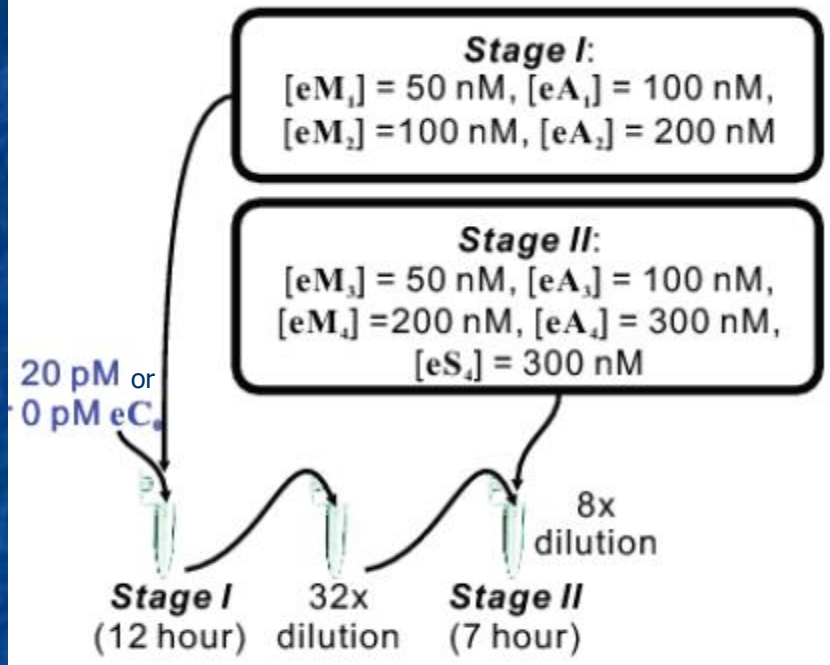
Step	Major costly materials	Amount	Cost	Source
PCR	DNA polymerase (Pfu-Sso7d or Vent)	0.003 mg	$0.03	home-made[1]
	dNTPs	1 mg each nucleotide	$2	Chem-Implex Intl.
Restriction digestion	EcoRV-HF or PvuII-HF	0.001 mg	$0.01	home-made[1]
Nicking	Nt.BstNBI	0.001 mg	$0.01	home-made[1]
Strand displacement	Vent	0.01 mg	$0.1	home-made[1]
Other cost	Buffers, tubes, depreciation of equipments, etc	N/A	~$2	N/A
Total			~$4	

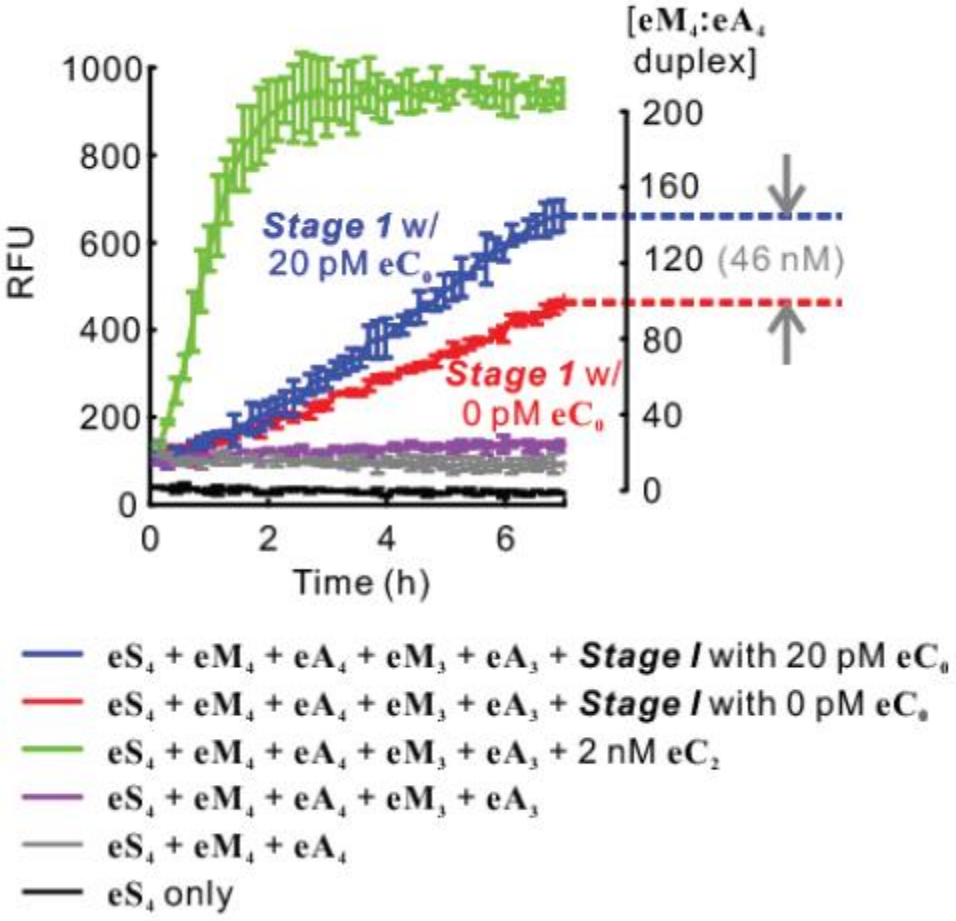
[1] We assume 1 L E.coli culture can yield at least 1 mg of protein of interest. Major costly materials in protein purification include LB broth (~$2 for 20 g), Ni-NTA beads (~$2.5 for 1 mL, considering re-use for at least 4 times), and other chemicals including antibiotics, IPTG, BME, imidazole (~$5 total). These add up to $10. Therefore we use a standard rate of $10 per mg protein of interest when estimating cost.



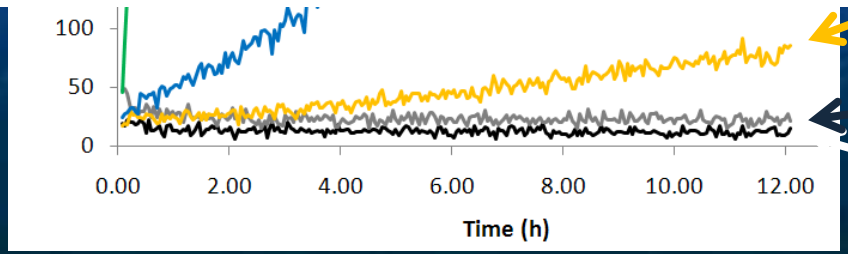
Cost / reaction = 5 cents!

Unprecedented purity = unprecedented amplification





Update: two-stage, 10^6-fold amplification!



Leakage is much more manageable.

Reporter + 2nd layer hairpins

Reporter only

Brief intermission:

- Great idea, non-enzymatic amplifiers that recognize sequence.
  Except no amplification.
- So, make a different mousetrap, 100-fold amplification.
- And stack the mousetraps (painful analogy?), 1,000-fold amplification.
- And optimize preparation, 10,000-fold amplification.
- And stage the background, 1,000,000-fold amplification.
- You know what comes next.

STILL NOT GOOD ENOUGH

- But only because it's too slow, sigh. Back to the drawing board.

When life gives you lemons … the heck with lemonade, cheat

- **Use enzymes, but in a convenient format, LAMP**
  Amplification with a DNA polymerase with strand displacement activity at a constant temperature (about 65° C).

- **High amplification efficiency**
  10^9-10^{10}-fold in 15-60 minutes

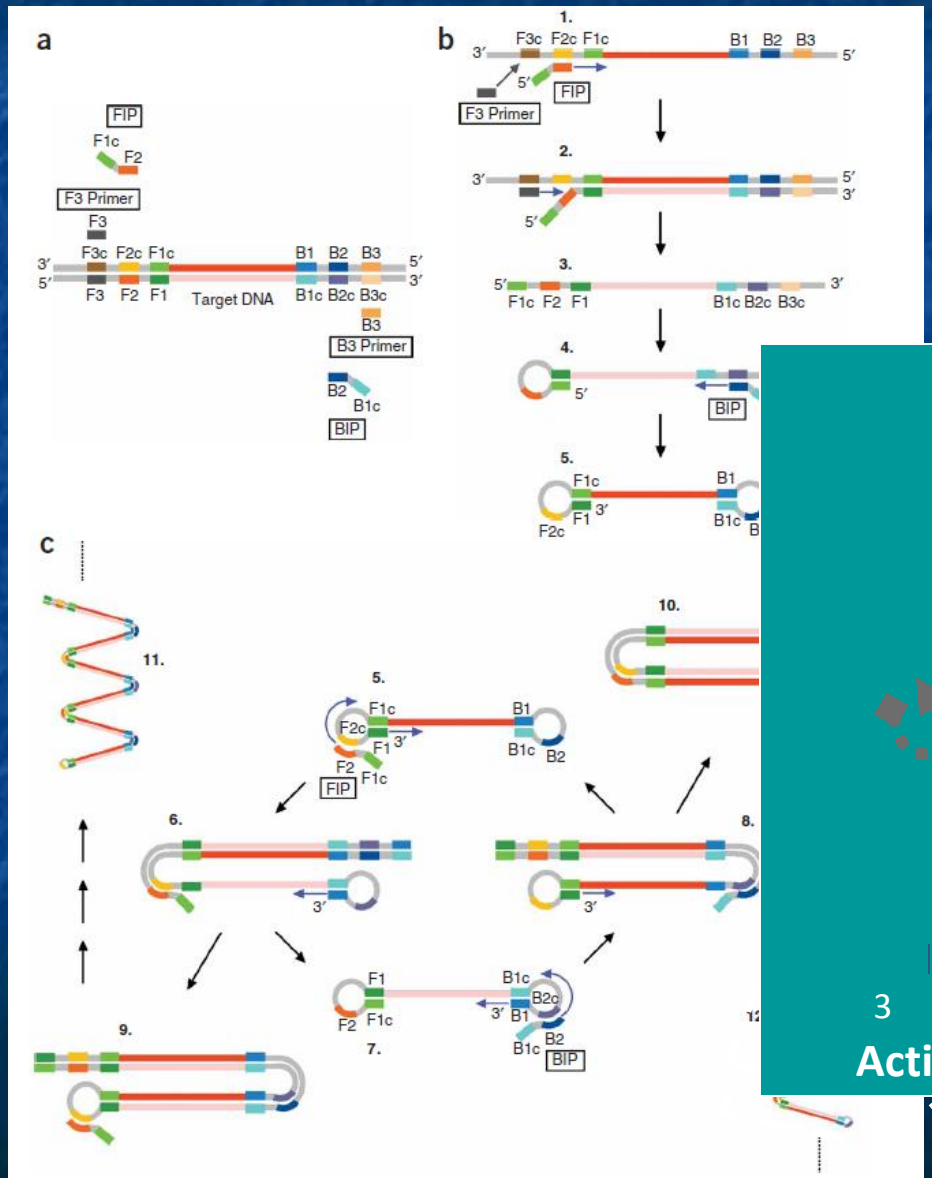
- **High specificity**
  Single mismatch discrimination ability

Bingling Li

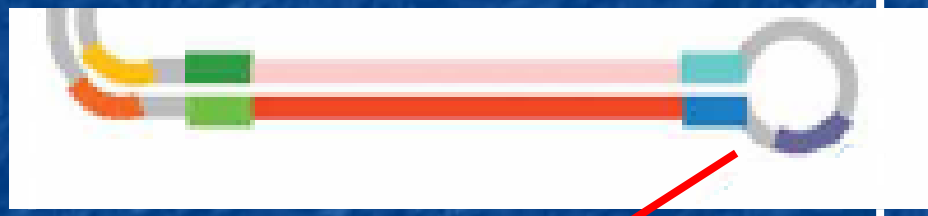
Asuragen®

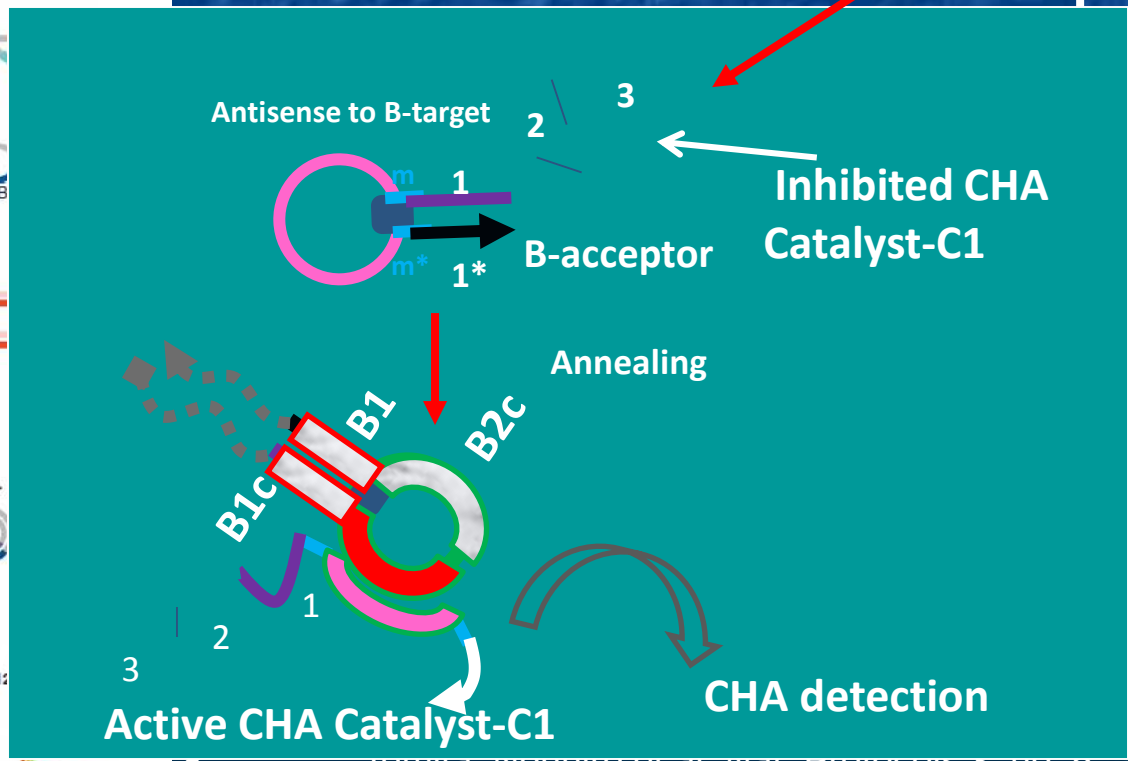
Sanchita Bhadra and Asuragen's ERI Team

Only one problem: it's too good; high background, many false positives



Specific, single-stranded, loop



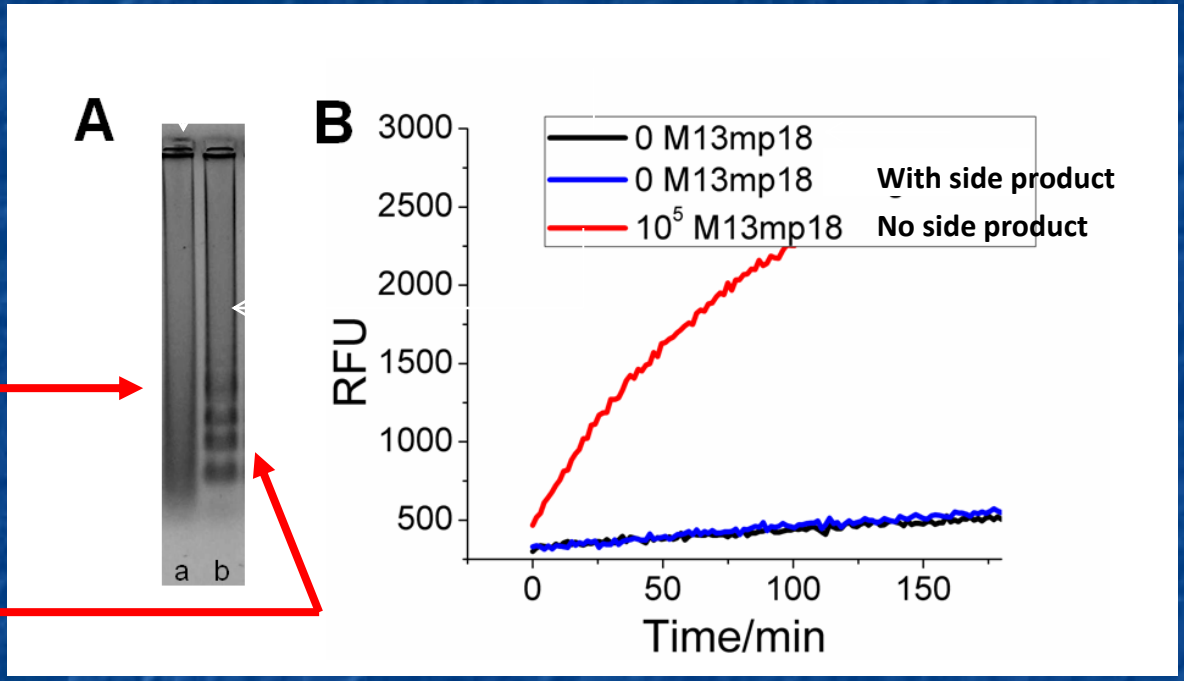


Tomita, Nohiro et al. *Nat. Protocols* 3, no. 5 (April 2008): 877-882.

Telling signal from noise

This is muck

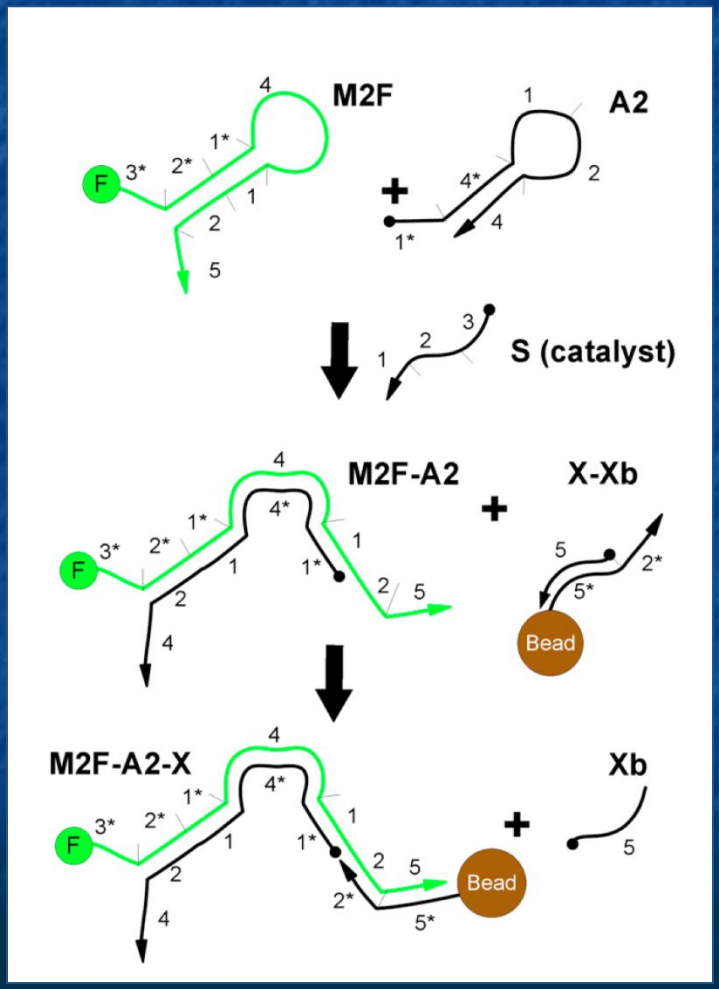
This is not

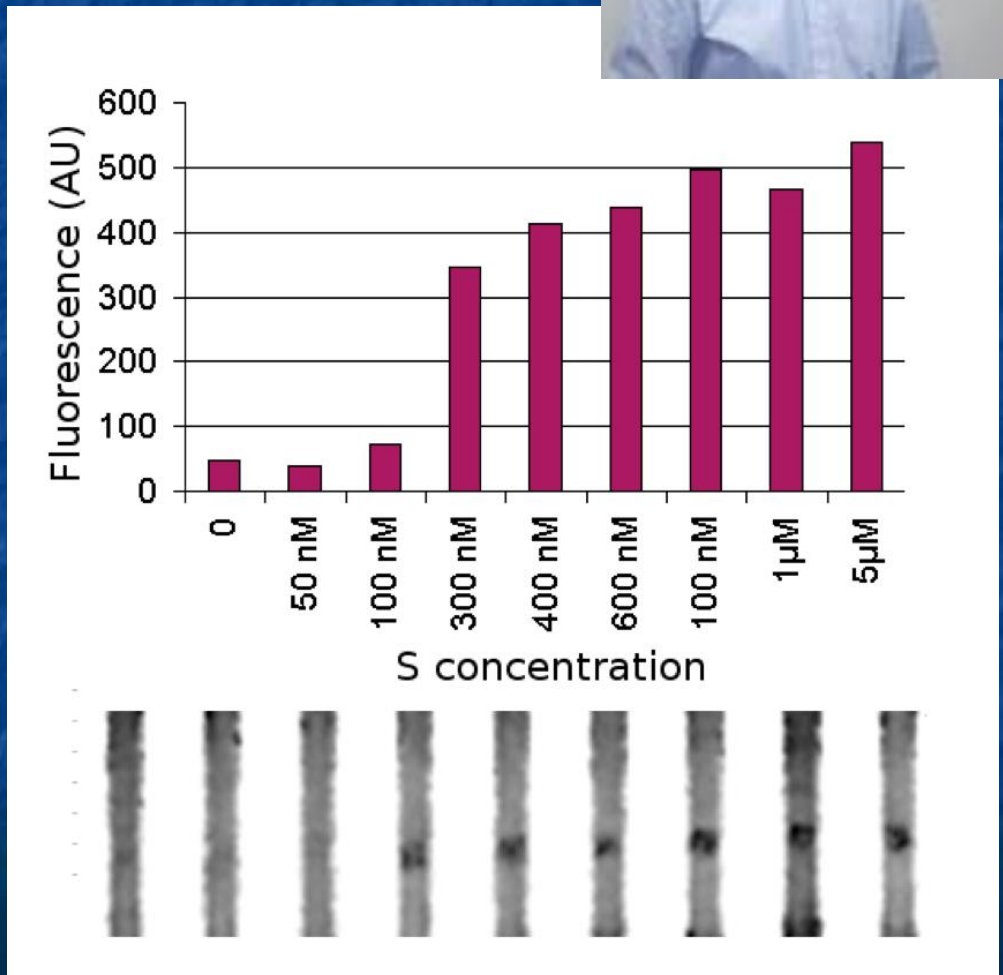


Detection of true versus spurious LAMP amplicons. (A) 2% agrose gel electrophoretic analysis of a LAMP reaction without betaine after 90 min. The reactions in lanes **a** and **b** were seeded with 0 and 10^5 copies of M13Mp18, respectively. (B) CHA kinetic curves of LAMP products.

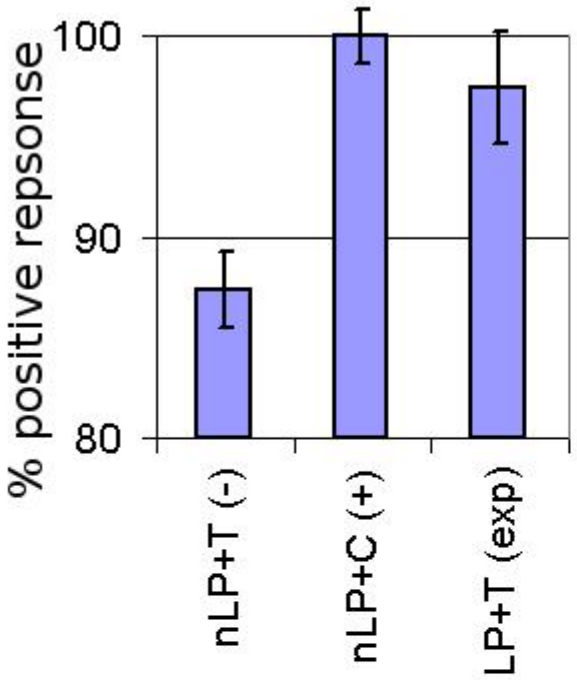
The return of paper!

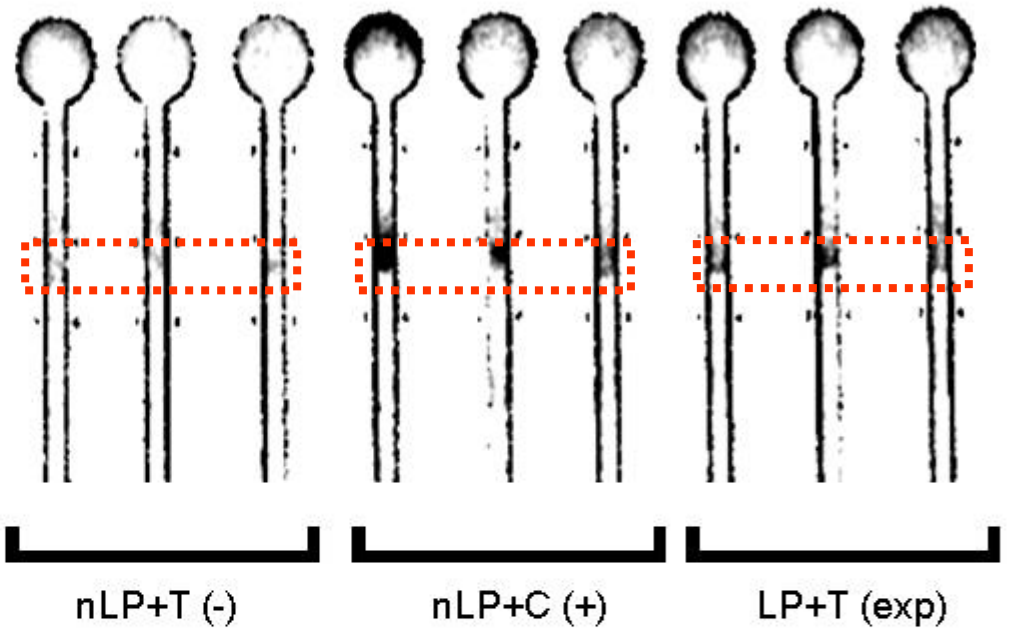
Peter Allen



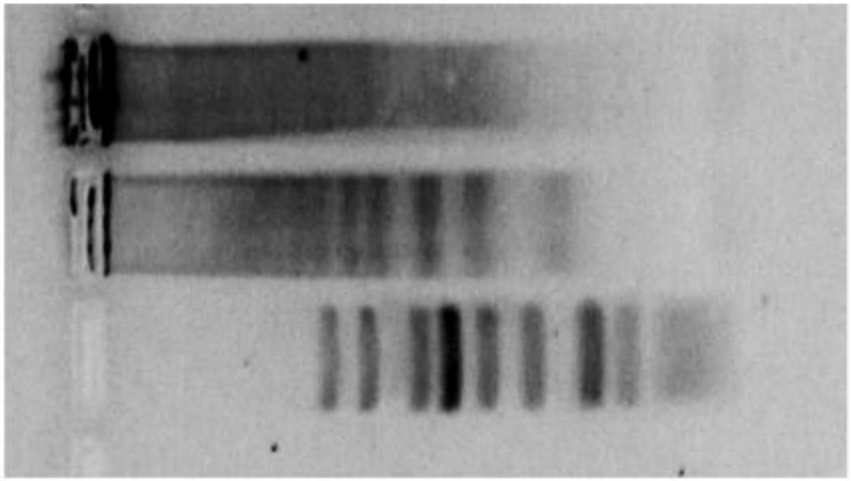


And finally … good enough. That'll do, enzyme. That'll do.





@ 1200 molecules

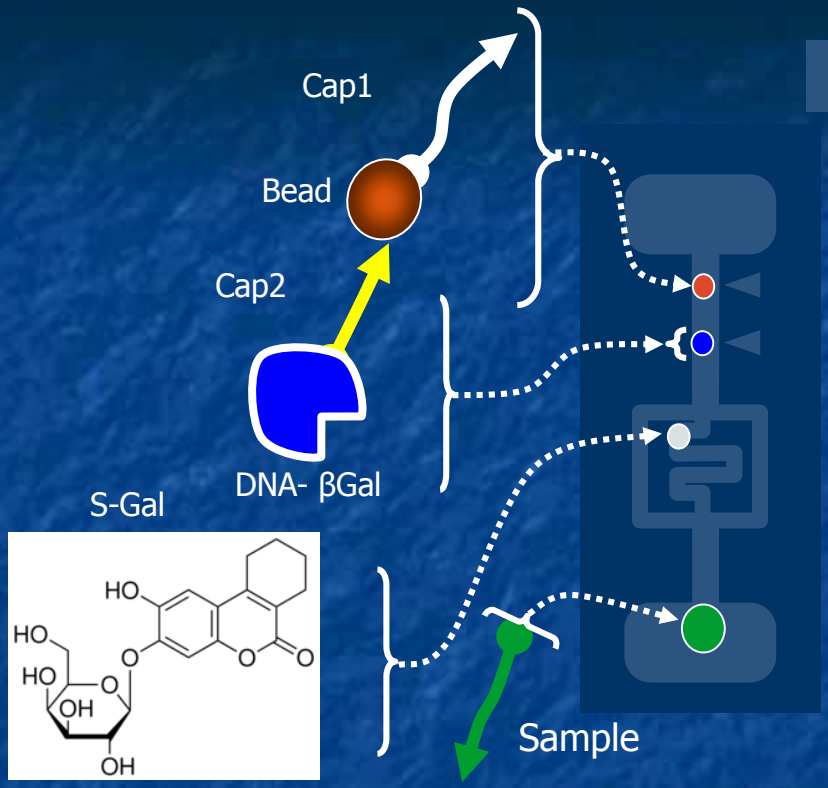


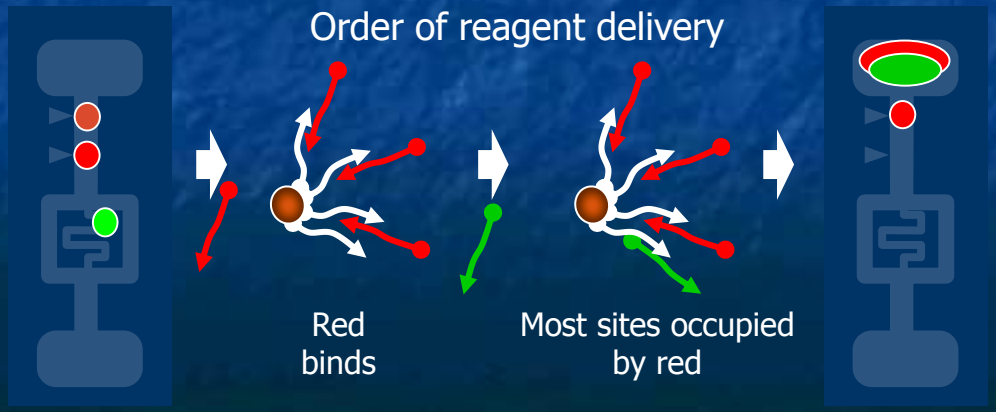
no-template LAMP (nLP)

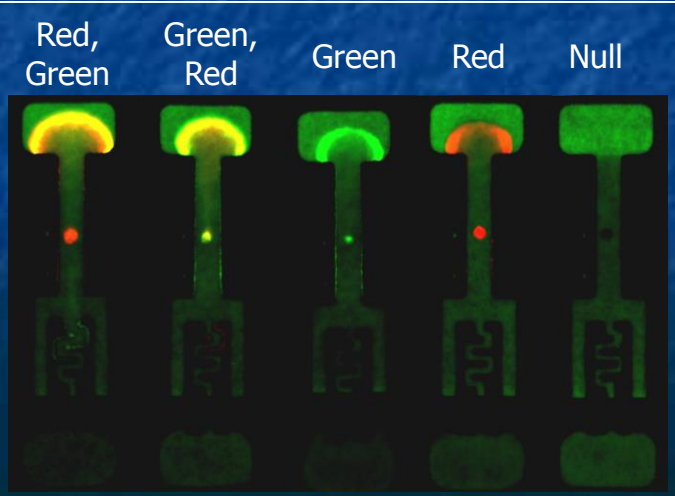
LAMP product (LP) @ 1200 molecules

Ladder

Getting reagents to the enzymes … flow sets the staging







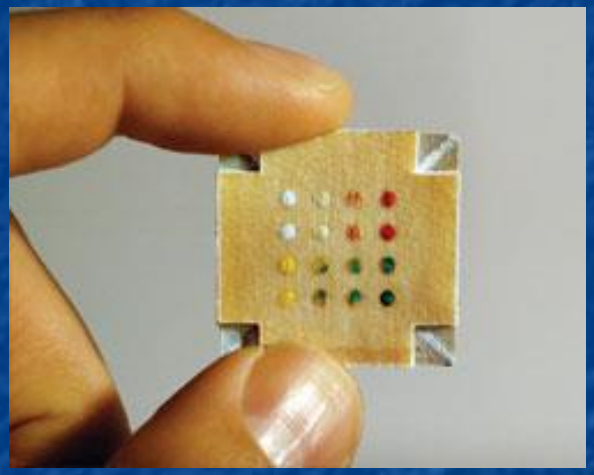
Except … we have to detect multiple mutations in parallel

Overlap PCR with mutated primers will be used to generate the following mutated alleles:

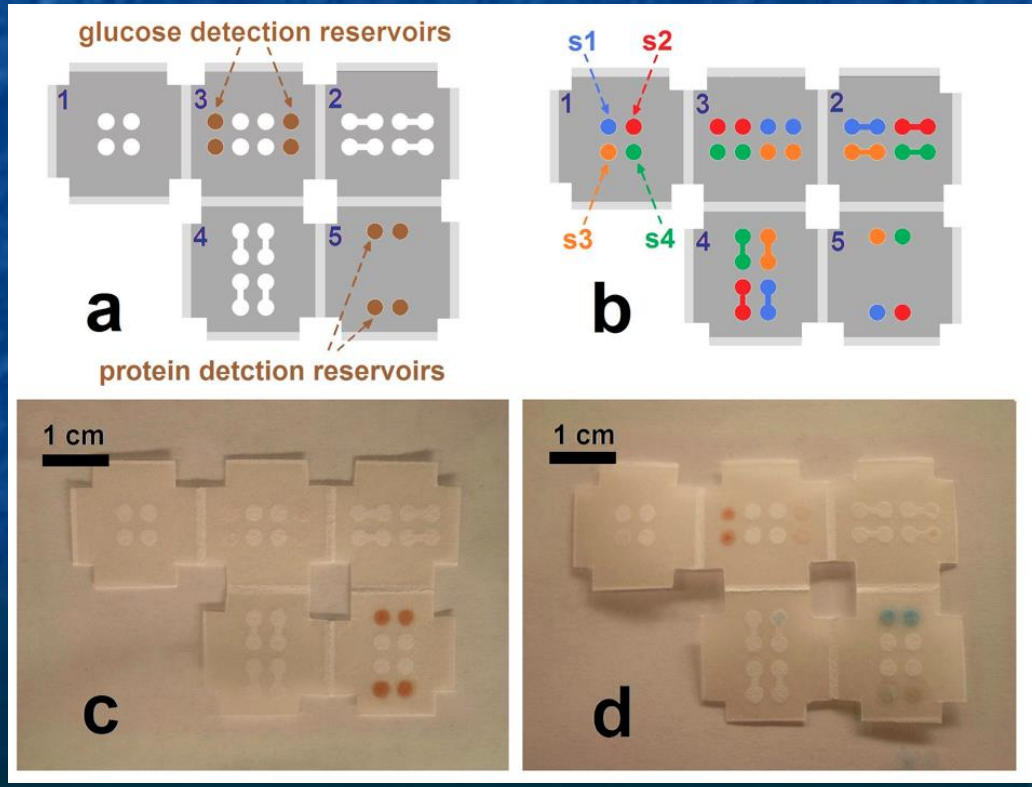
Drug	Gene	Mutated codon (mutant base shown in red)
Rifampicin	*rpo*B (RNA polymerase beta subunit)	S531L (TCG→T**T**G) H526Y (CAC→**T**AC) Q513L (CAA→C**T**A)
Isoniazid	*kat*G (catalase-peroxidase)	S315T (AGC→A**C**C)
Streptomycin	*rps*L (ribosomal protein S12)	K43R (AAG→A**G**G)
Ethambutol	*emb*B (indolylacetylinositol arabinosyltransferase)	M306V (ATG→**G**TG)
Fluoroquinolones	*gyr*A (DNA gyrase subunit A)	D94G (GAC→G**G**C)

- Isoniazid, rifampicin, streptomycin and ethambutol are essential first-line antituberculosis drugs.
- Ethambutol and streptomycin are also included in the group 1 and 2 drugs for treatment of MDR-TB while fluoroquinolones are part of the reserve second-line drugs.

But Dick Crooks at Texas … had a better idea.

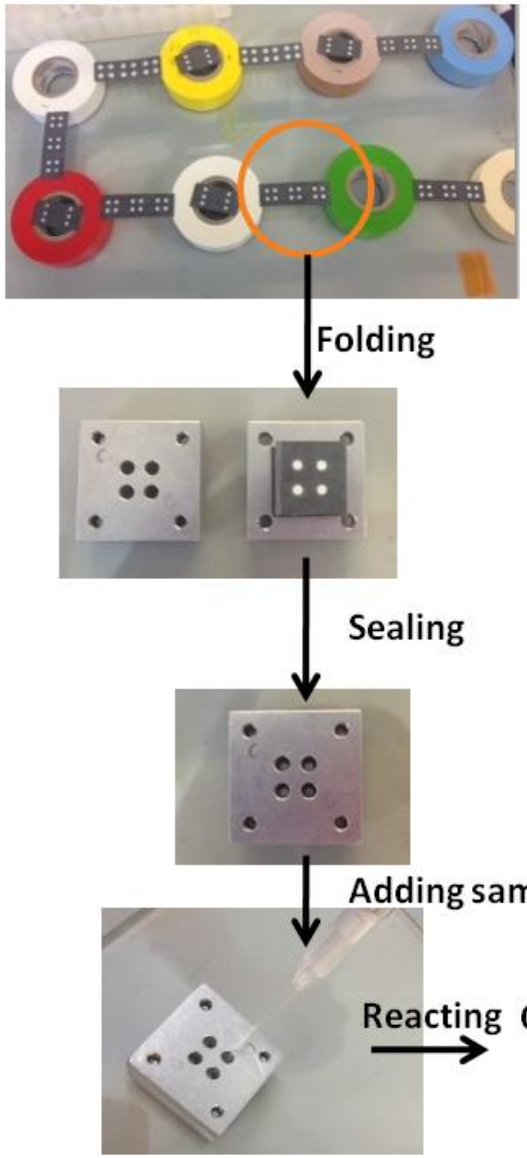


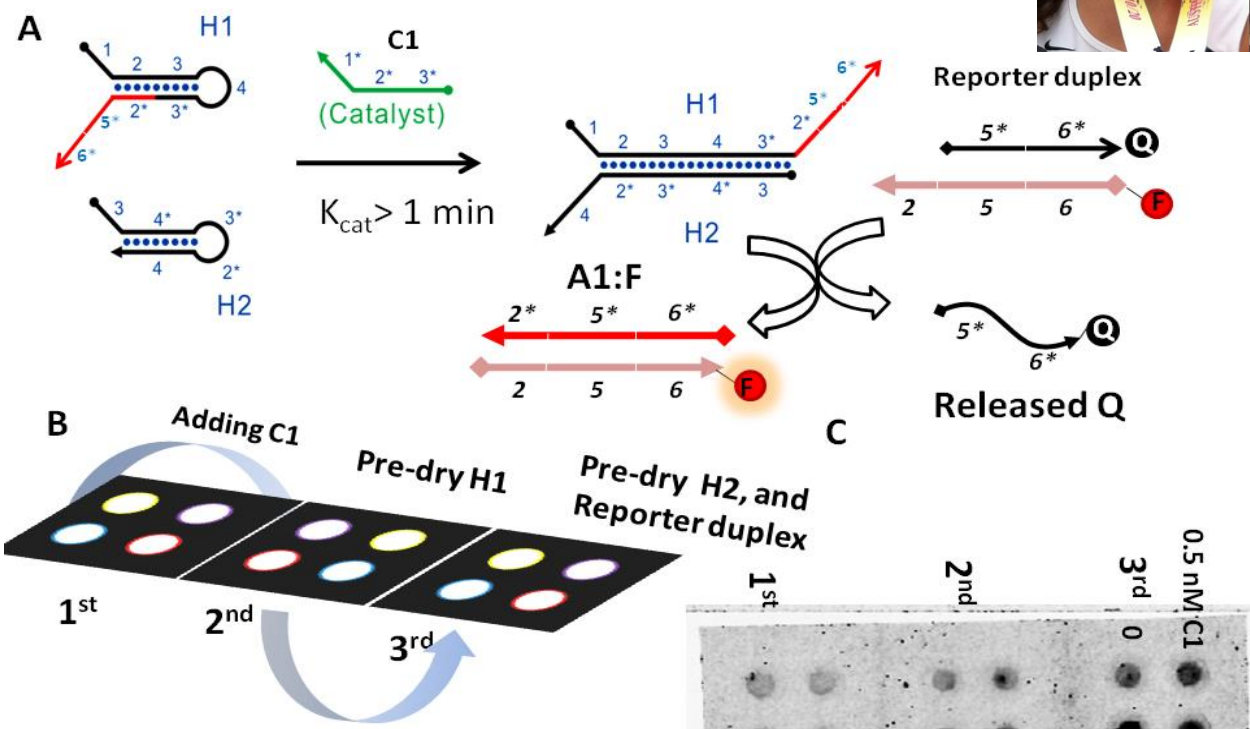
George Whitesides at Harvard is a very clever fellow who can make paper do many things.



Our assays on their paper

Karen Scida





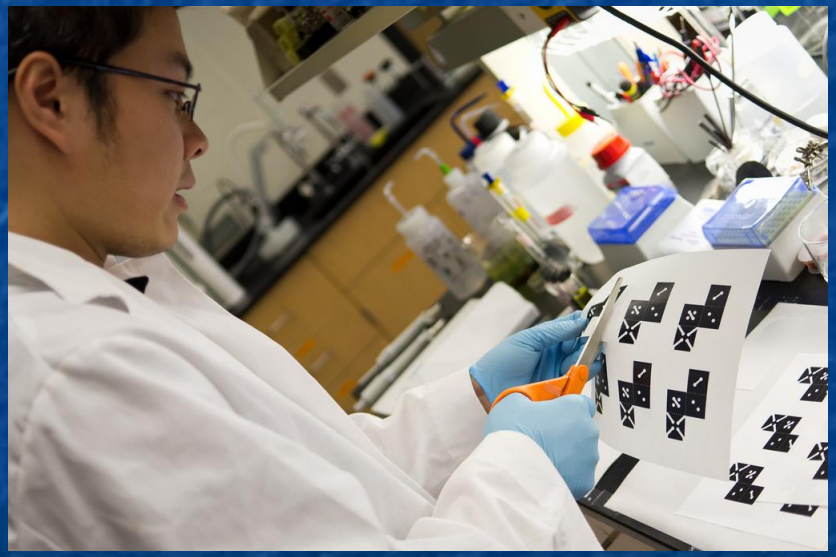
o-PAD 2

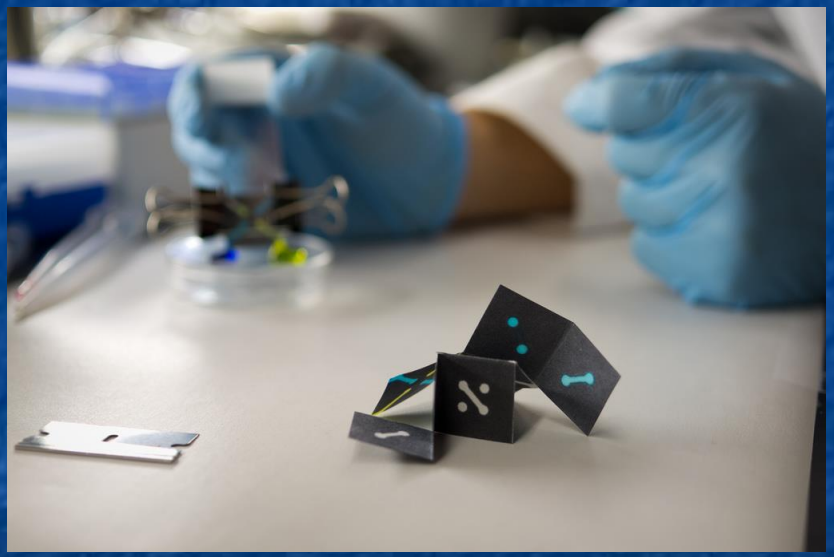
Improvements

Photolithography is eliminated in favor of wax printing using a $700 office printer

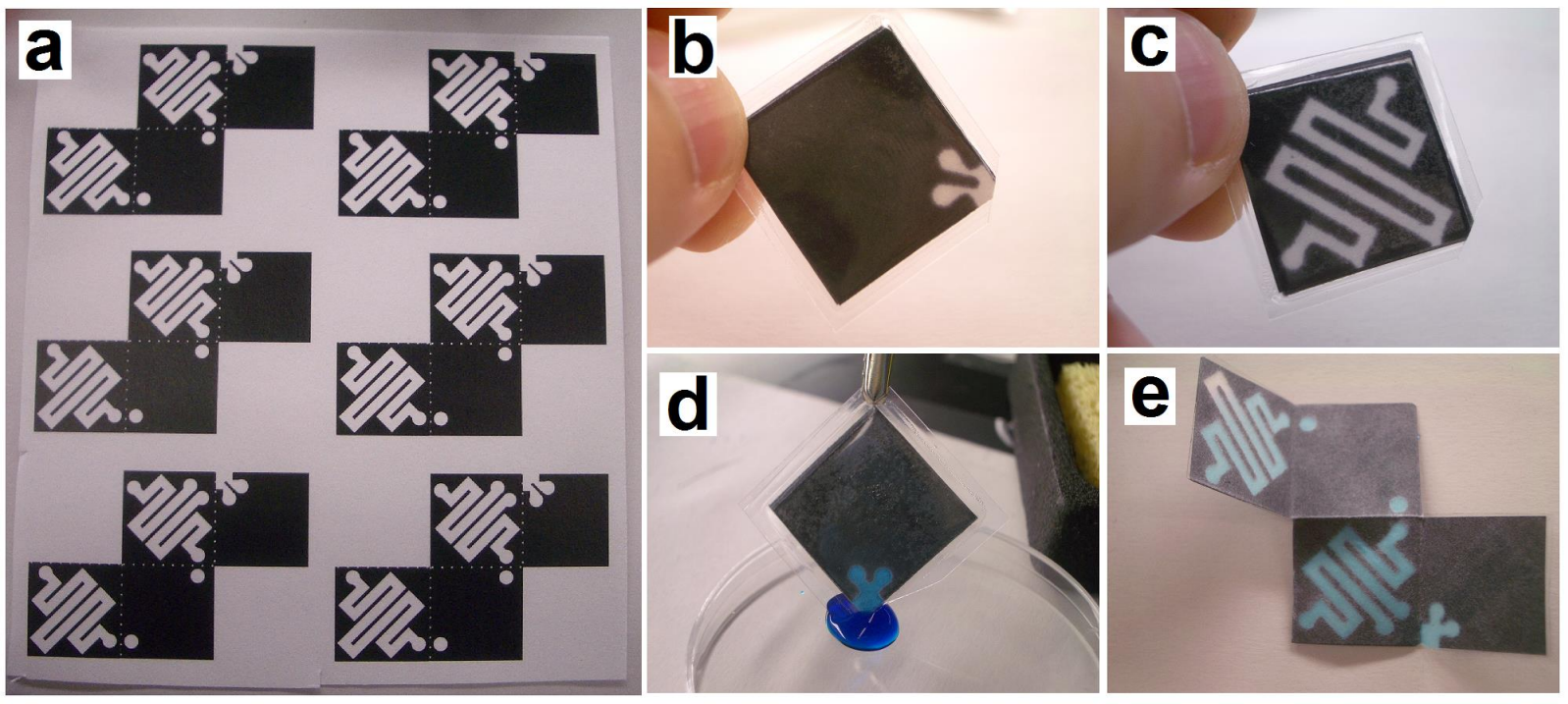
Device is laminated to prevent evaporation during assays and to prevent contamination

A voltmeter is used for read-out, which is both sensitive and quantitative





o-PAD 2 Fabrication and Fluidics



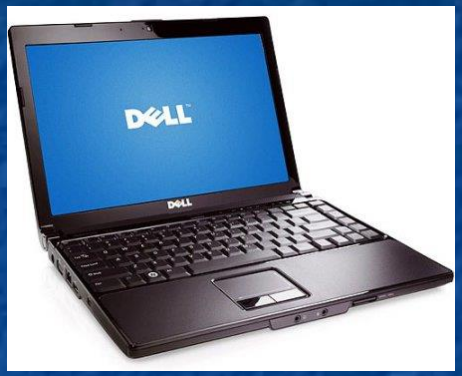
(a) A sheet of paper printed using an office printer (6 devices per sheet of paper)
(b) The folded and laminated o-PAD
(c) A corner is snipped off to admit the analyte solution
(d) The analyte is introduced into the fluidics
(e) The unfolded device showing the results of 3-D fluidic penetration

If we can deliver paper, we can deliver health care.

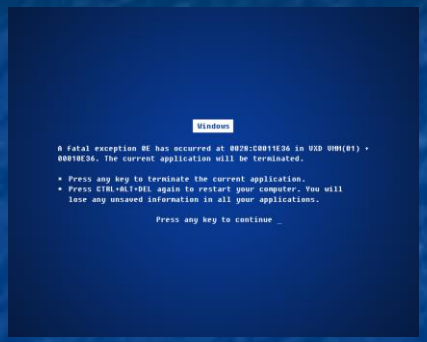
India has some 545 million cell phones, enough to serve about 45 per cent of the population, but only about 366 million people or 31 per cent of the population had access to improved sanitation in 2008.

But it's bigger than that, and impacts us here at home, too.

Complex system diagnostics: then



Crud, it doesn't work



And now

Complex system diagnostics: then

And now

What if … we could just logon to get our health status?

The Traitwise Technology

[Full disclosure: I own equity that is worth almost $10.52]

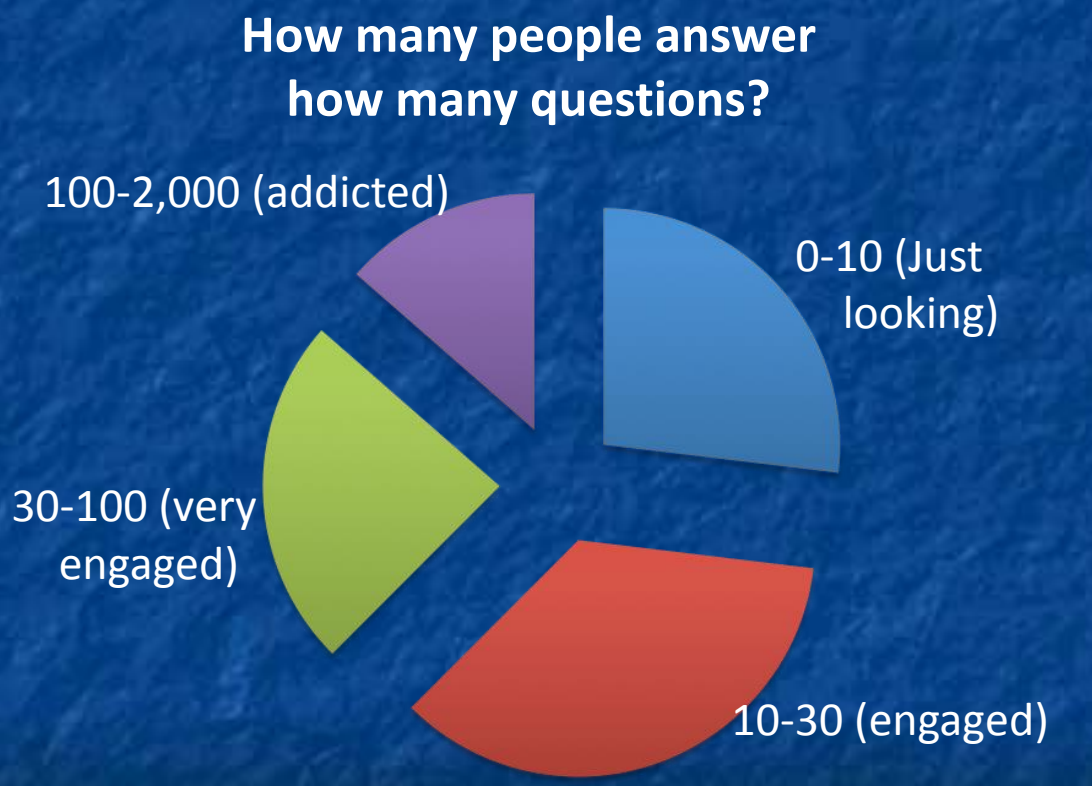
- We crowd-source information by having users answer personalized questions & ask questions of their own
- By using gaming paradigms we optimize every panel to engage the user.
- New questions emerge at the top of the page to create user anticipation and eliminate page navigation
- Traitwise becomes a dialog to create a personalized experiences

Fun survey interface designed by game developers

Zack Simpson

The Traitwise Technology (continued)

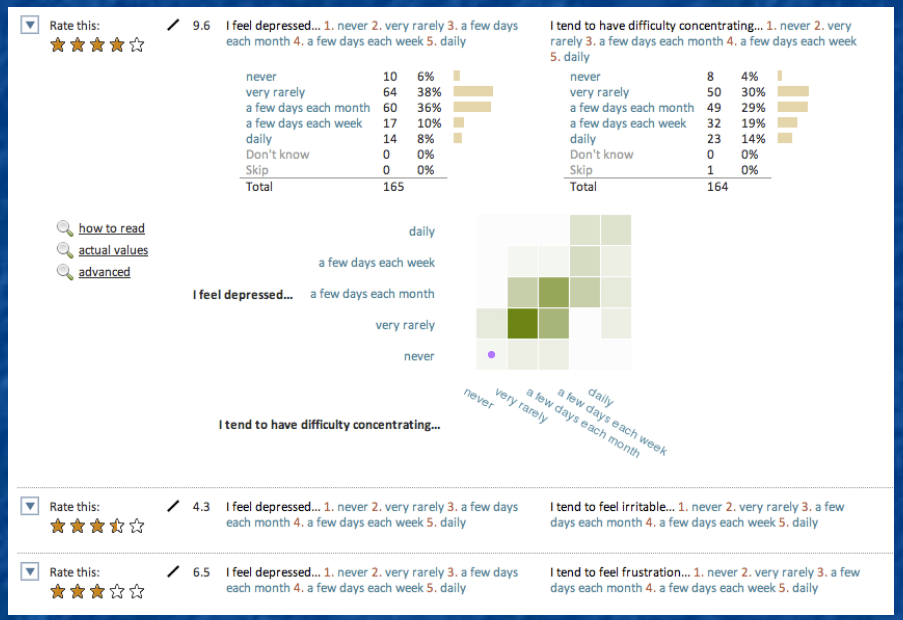
- Traitwise provides instant feedback and survey results
- The average number of questions answered using Traitwise is over 50
- Nearly 20% of participants answer over 100 questions with a tail of distribution going out to over 2,000.



Fun and addictive!

Research Using Patient Reported Outcomes

- Patient reported outcomes are being used to reduce health care costs by comparative effectiveness research
  - By a combination of screening and patient reported outcomes, researchers are identifying the treatment effectiveness
  - Patent reported research also “reveals acquired behaviors and individual responses to health programs” necessary for improved health (1)



Example of depression correlations

(1) Jethwani, K. et al. // www.medscape.com // Jan. 2011

Used in Commercial, Research, and Clinical Settings

What is the Freshman Research Initiative?

- *An innovative, ground breaking, faculty initiated program*
- ***Developed to***
  - Tap the resources of the research university – ideas, expertise, mentorship, facilities, etc.
  - To benefit of the education of our undergraduate students.

- ***Provides a research experience and all its benefits***
  - Excitement and engagement, retention, relationships, etc.
  - To students early in their careers
- **Operates *at scale*** – hundreds of students each year.
  - Over 7 years > 3,000 new UT students have participated
  - Again in 2012, ~ 700 students will start FRI, ~33% of the incoming Natural Sciences class

FRI
THE UNIVERSITY OF TEXAS AT AUSTIN

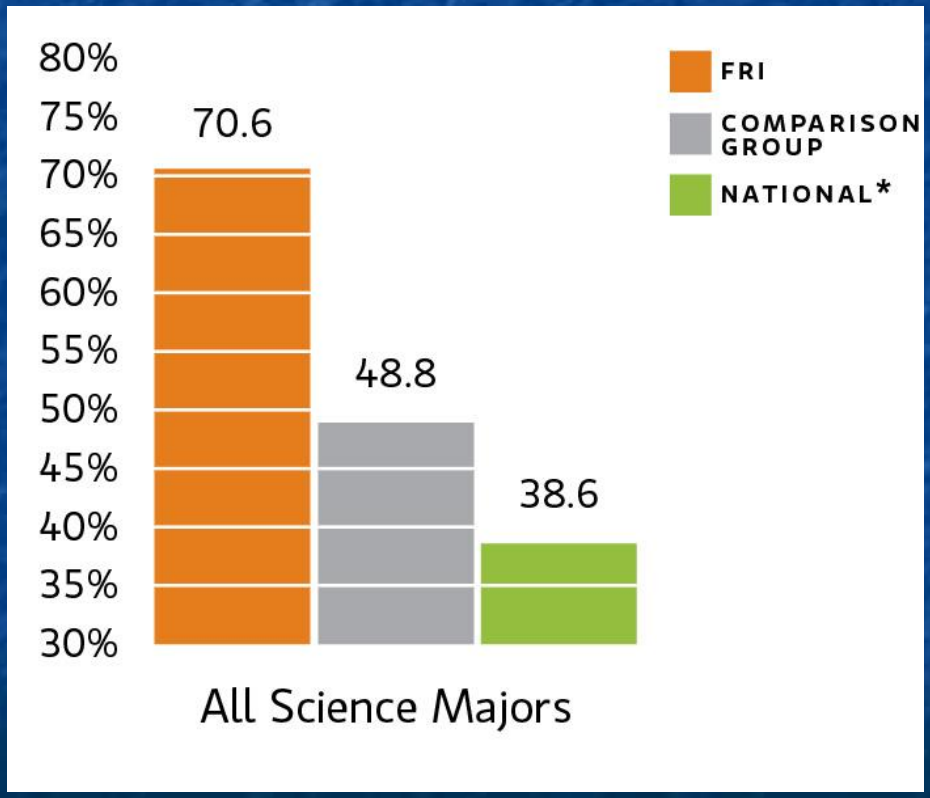
FRI Student Timeline

FRI
THE UNIVERSITY OF TEXAS AT AUSTIN

It works!

- **Improves retention and Increases overall STEM graduation rates**
  - 35% more students graduate with a science or math degree if they participated in FRI.

4 Year Retention Percentages (students graduated or on track to graduate)



* 38.6% = National STEM 6-year graduation rate

FRI student-authored publications

	Number of papers	*Papers on FRI stream research*	*Number of student authors*	*Number of Risk student authors*
In preparation	21	16	35	10
Submitted	8	8	13	4
Published or in press	115	96	84	20
Total	**143**	**120**	**132**	**34**

Undergraduate student authorship is not tracked at the university. A survey of 15 faculty recognized for their undergraduate research track-records, gives a generous estimate of 2% of Chem/Biochem majors become coauthors each year.

11% of the FRI06 cohort and 9% of the FRI07 cohorts are published authors.

FRI
THE UNIVERSITY OF TEXAS AT AUSTIN

Re-gifting the Gates Computer Science Building ….

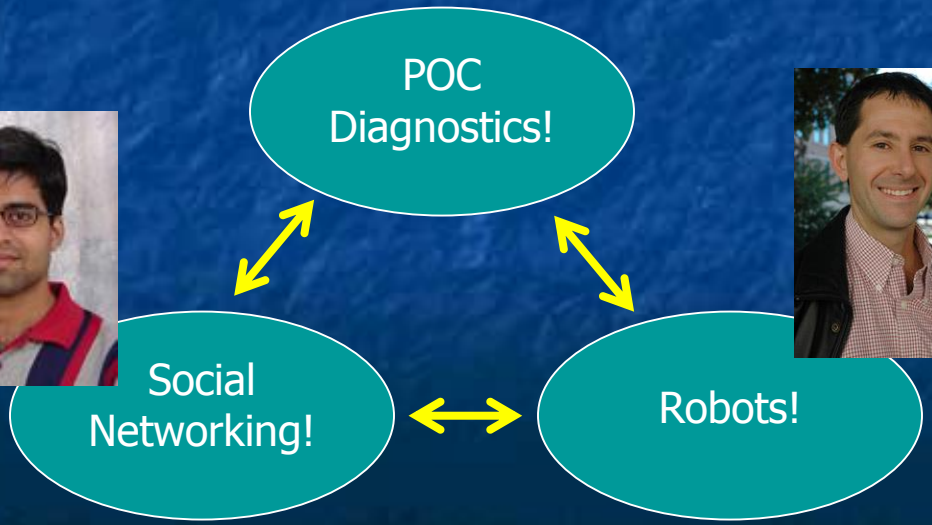
D.I.Y. Disease Diagnostics Stream

FRESHMAN RESEARCH INITIATIVE
THE UNIVERSITY OF TEXAS AT AUSTIN

FRESHMAN RESEARCH INITIATIVE
THE UNIVERSITY OF TEXAS AT AUSTIN

An extremely generous gift of Bob and Cathy O'Rear

Pradeep Ravikumar, Computer Science

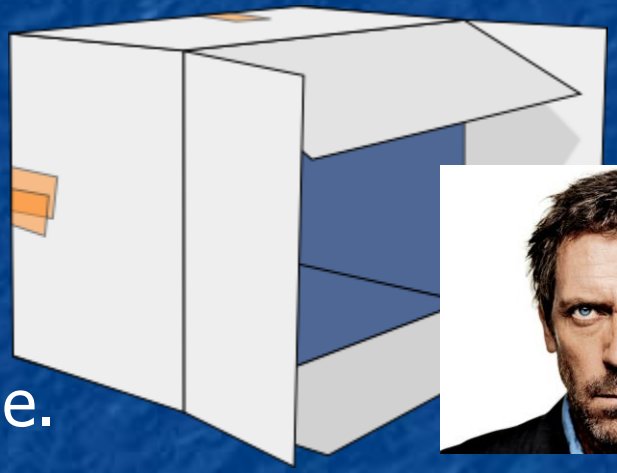


Peter Stone, Computer Science

The future of health care?

So, what does the future look like?

- Complex diagnostics, made cheap and uploadable (is that a word?)



- Complex analyses, easy and online.
- People taking control of their own healthcare.

The folks responsible for the rainbows and unicorns:

Grace Eckhoff, Marshall Scholar

Xi Chen, Harvard Fellow

Bingling Li
Sanchita Bhadra
Peter Allen
Zack Simpson

Matt Winkler and Asuragen ERI
Jeff Taylor, John Jacob, Oscar Ayala

Dick Crooks, Karen Scida

Peter Stone, Pradeep Ravikumar

NIH
DARPA
Gates Foundation

The great state of Texas
(I am a state employee, something I seldom forget)

The Freshman Research Initiative!
Gwen Stovall, Research Educator;
Sarah Simmons, The Awesome!

Dr. Andrew Ellington

Dr. Ellington's research focuses on using evolutionary techniques to engineer biopolymers and cells. Researchers in his lab select binding species (aptamers) and ribozymes from random sequence populations. They then attempt to apply the selected species to solve real-world problems. For example, his lab members are exploring how aptamers can be used to block viral replication. His team has also developed methods for evolving proteins with novel functions, and they are attempting to use the evolved proteins in medical or biotechnological applications.