Sit Down with Synthetic Biology

Welcome to iGEM UW

Who are we?

Introductions!

What is iGEM?

iGEM is an **international competition** for synthetic biology. Teams from around the world create, develop, and present projects to a panel of synthetic biology experts.

iGEM is an international synthetic biology competition

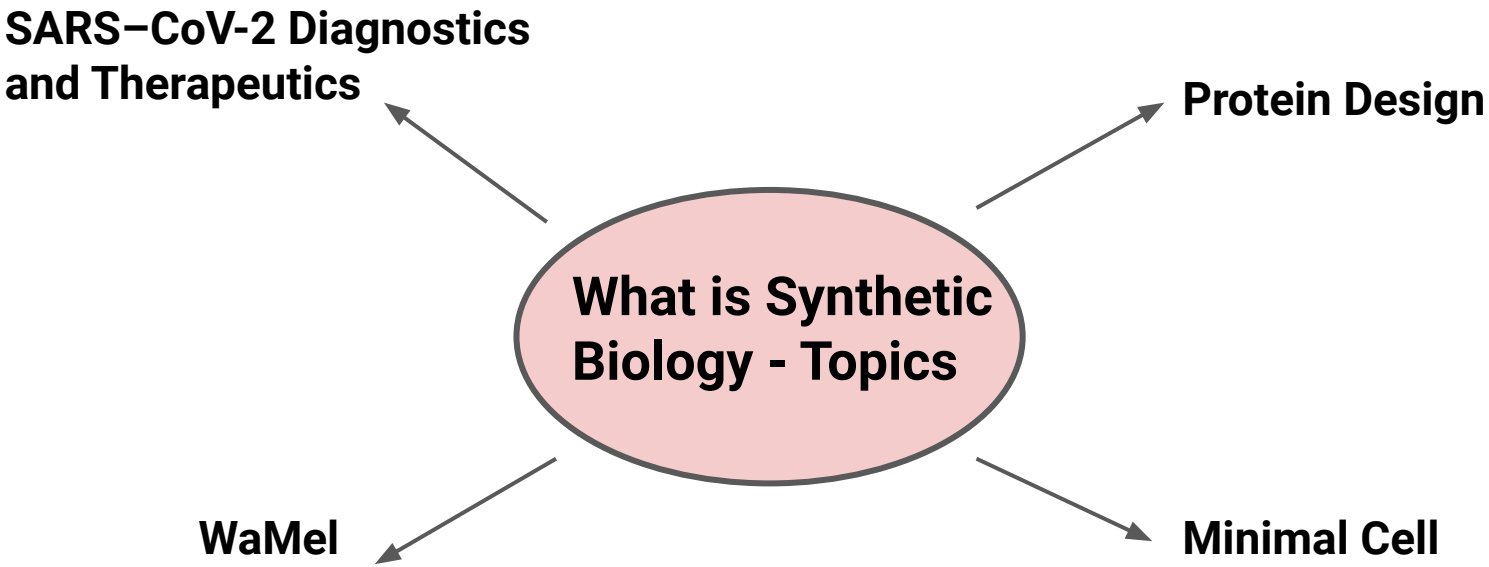
Teams work to use synthetic biology to solve problems

Competition is in the end of October each year.

We recruit new members in January!

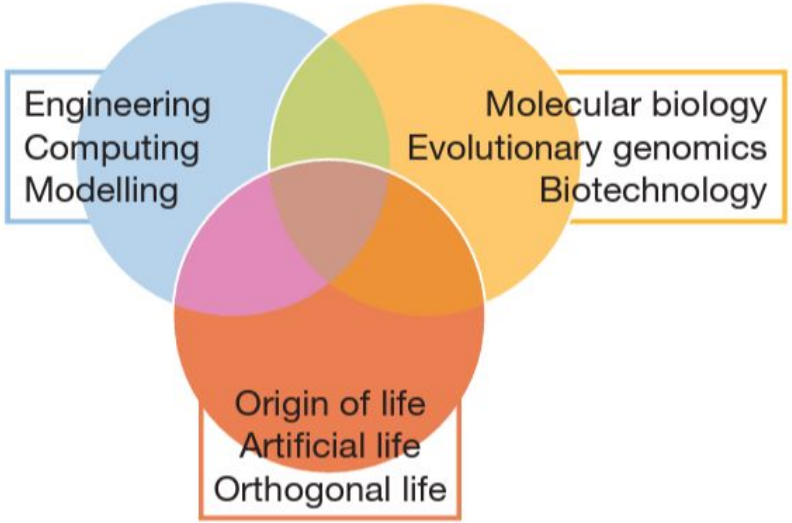
We are an interdisciplinary team

- Wetlab
  - Do Lab work. Most lab work occurs over the summer here at UW
- Drylab
  - Mathematical Modeling
  - Protein Modeling
- Human Practices
  - Communicate information about out topic
  - Talk with stakeholders and experts on the topic
- Fundraising
- Webdev
- Design

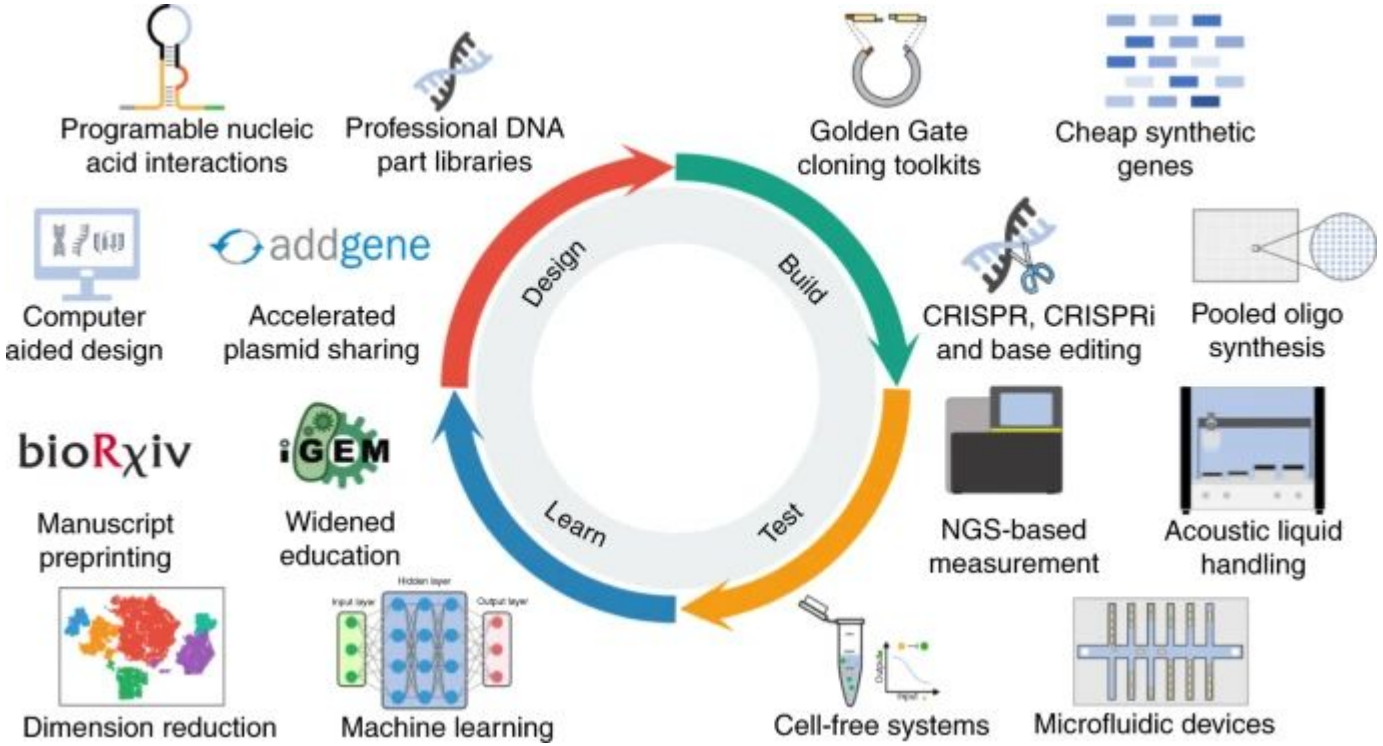

SARS–CoV-2 Diagnostics and Therapeutics
Protein Design
What is Synthetic Biology - Topics
WaMel
Minimal Cell

What is Synthetic Biology

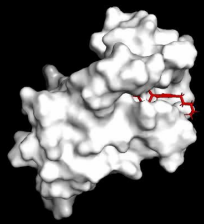
- Biological Tool Kit
- Design/redesign and construct new biological entities or give them new functions
- Concerns:
  - Gain in Function
  - Containment
  - Biosecurity

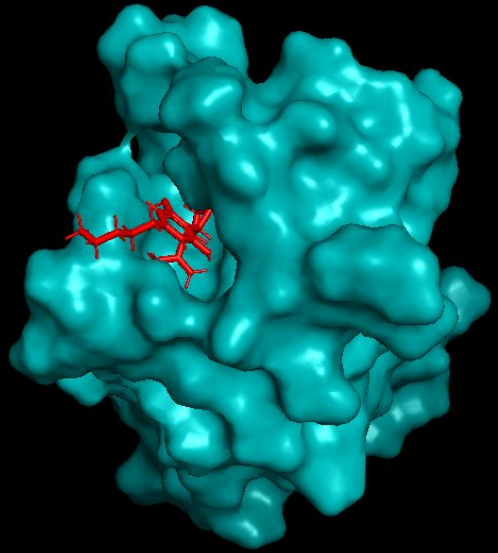


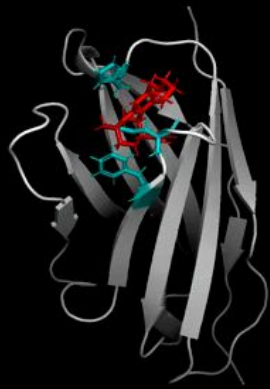
What is Synthetic Biology



Protein Design








INSTITUTE FOR
Protein Design
UNIVERSITY of WASHINGTON

Rosetta

- Utilizes command line interface
- Can be downloaded from Rosetta's website (https://www.rosettacommons.org/software)
- There are tutorials on the Rosetta website to help you get started!

Main areas of research:

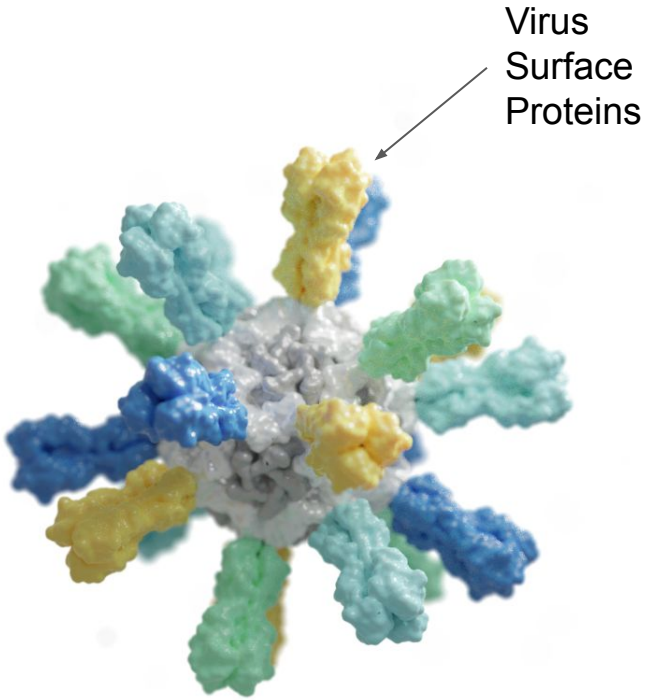
- Protein Structure Prediction and Determination
- Protein and Small molecule prediction
- New Protein Scaffolds
- Enzyme Design
- Self-assembling nanomaterials and vaccines

Recent Projects from the IPD

Nanoparticle vaccine development

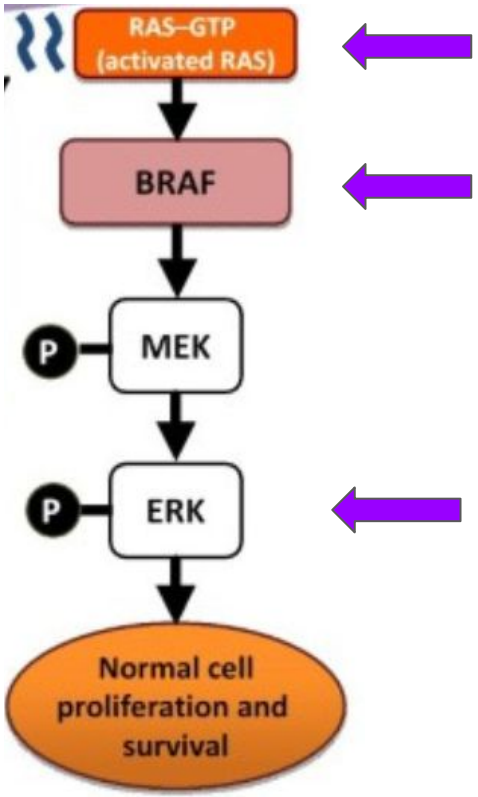
Developing vaccines against many illnesses including RSV (common respiratory virus) and COVID-19

Vaccine for covid is now approved for use in South Korea



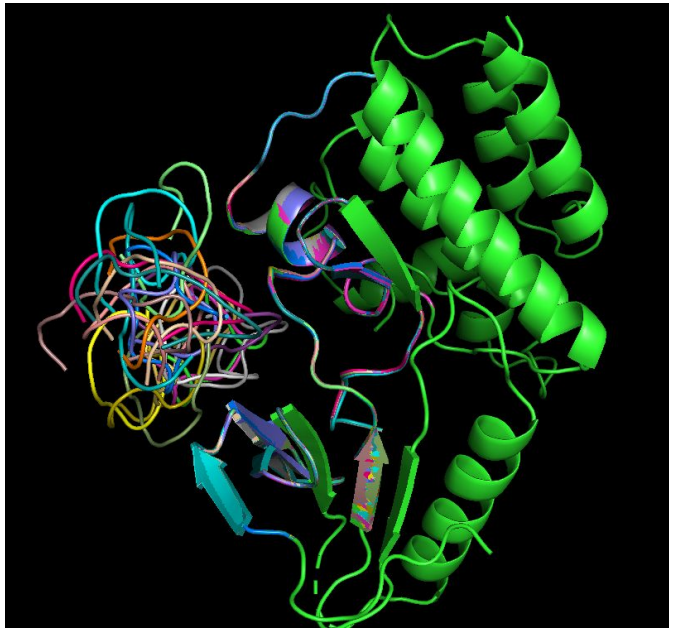

WASHINGTON iGEM

Development of Melanoma Therapeutic



We are using Rosetta to design peptides that bind to three proteins which are commonly mutated and cancer promoting in melanoma.

Development of Melanoma Therapeutic



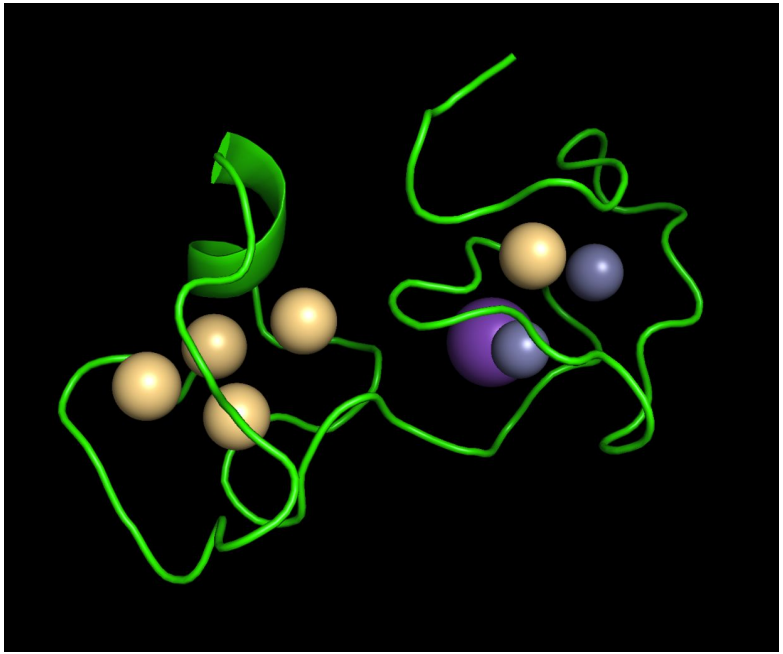
Work Flow:

- Docking 104 peptide backbone sets onto each protein
- Designing each peptide to better fit the binding site
- Altering the peptides so that they are cell-penetrating

Past Year's Project: Development of Arsenic Binding Proteins

Have soil bacteria express the protein, which will then bind arsenic. Extract arsenic pollution from soil

Altering naturally existing protein (Metallothionein) to bind specifically to arsenic

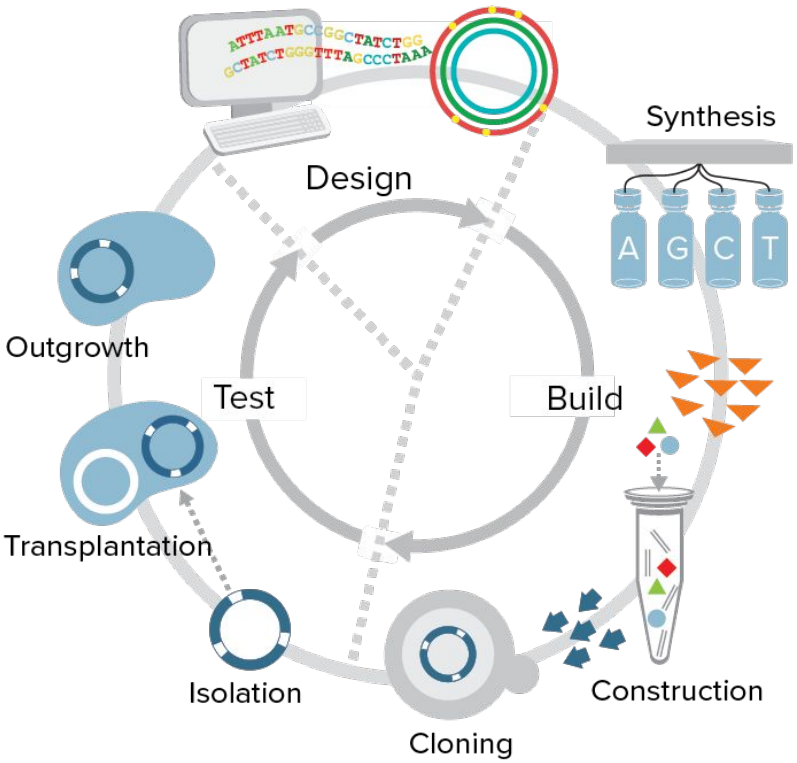


Topic 2: Minimal Cell

What is a minimal cell

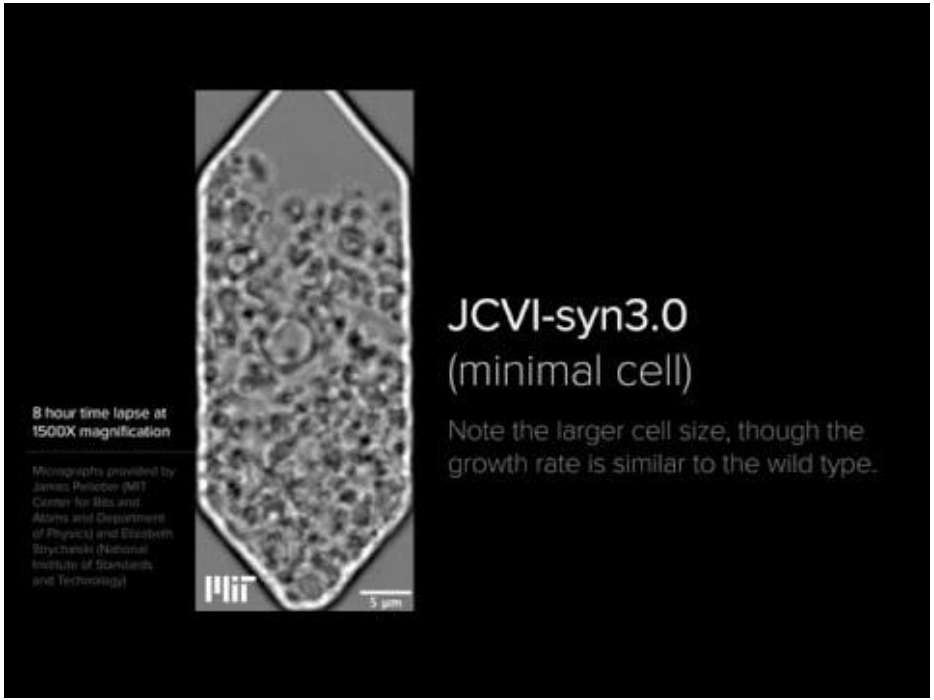
- Definition: a synthetic one-celled organism with genome that only encodes the minimal set of genes necessary for the cell to survive
- History:
  - 1995 – Mycoplasma genitalium genome sequence published
    - Posses the smallest genome of any living organism → 485 genes, of which ~100 are non-essential
  - 2010 – First synthetic cell *Mycoplasma mycoides* JCVI-syn1.0 was built
    - Mycoides chosen over genitalium → growth rate is faster
  - 2016 – JCVI-syn3.0 (first minimal cell) based off of 1.0 version but contains just 473 genes
    - Published in Science in 2016 – “Design and synthesis of a minimal bacterial genome”

DBT Cycle



1. Synthesise the minimal genome
    a. Yeast cells were employed to assemble the DNA in stages
2. Transplant it into a recipient cell
    a. Complete genome isolated from the yeast cell and transplanted into restriction deficient M. capricolum recipient
3. Proof of successful transplantation: recipient adopt the physical characteristics of its chromosomal donor

3.0 vs 1.0



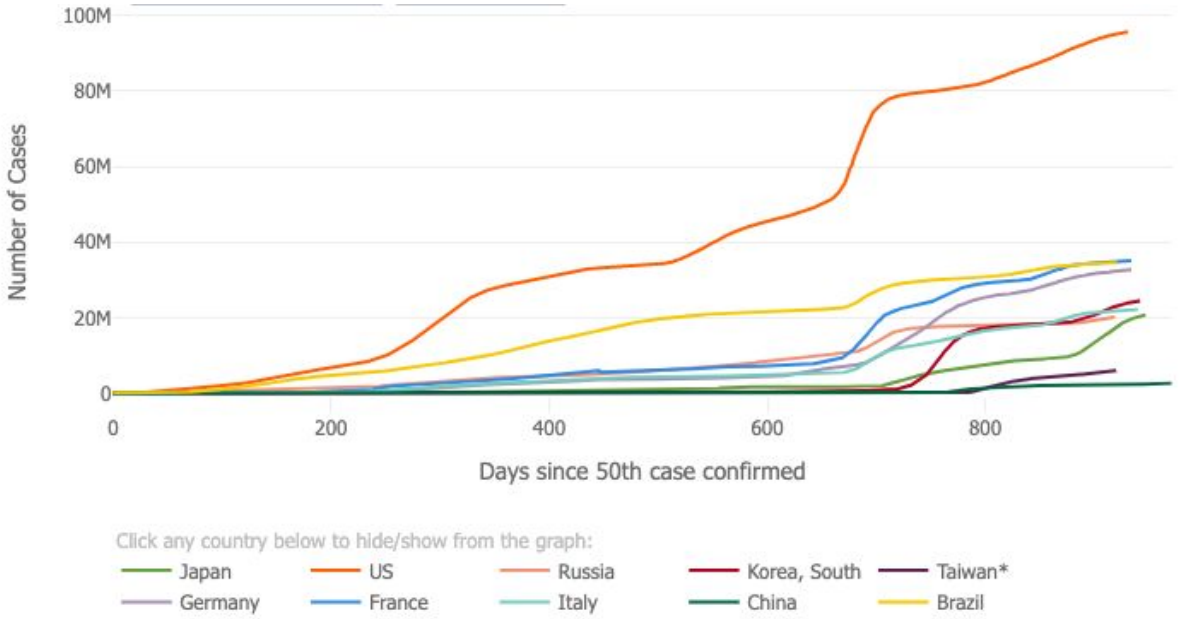
- Similar growth rates → successful transplantation
- Different morphology
  - Generated a 3.0A
  - 19 genes deleted from 1.0 → 3.0 retained in 3.0A responsible for difference in morphology

Moving Forward

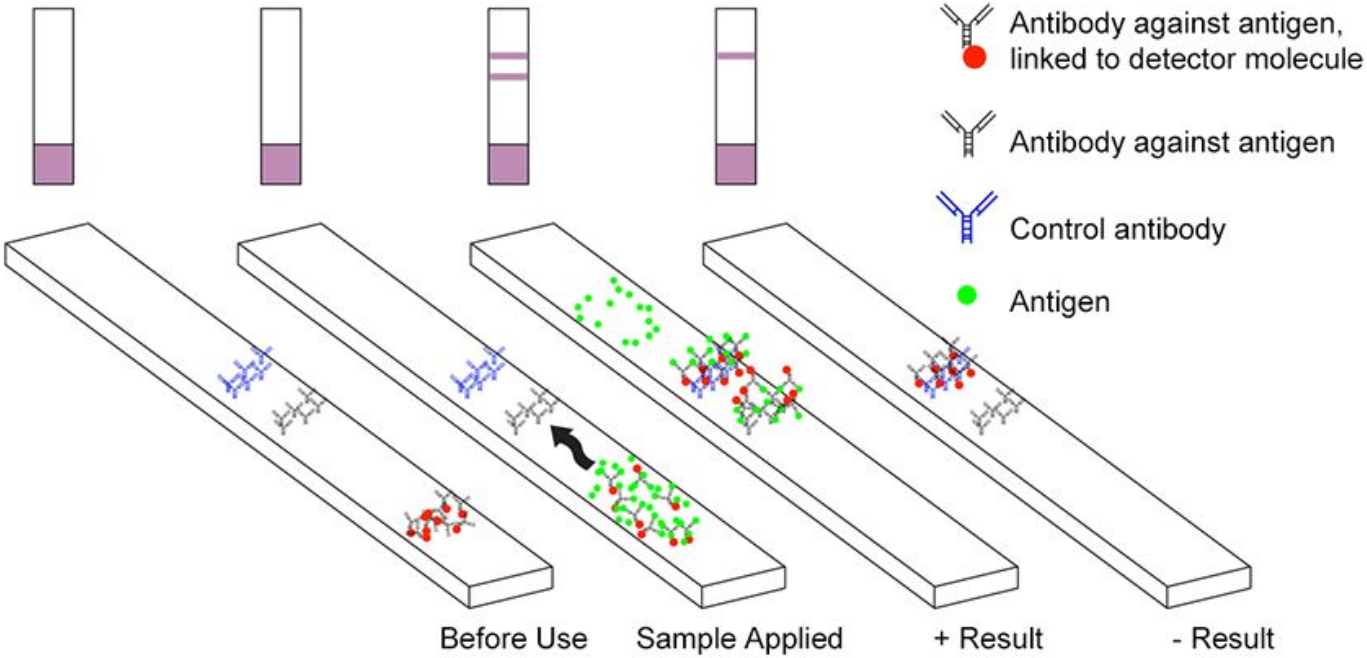
- Analyzing the function of every essential gene in a cell
- Clarify genes underlying core physiological processes
- Recreate life from scratch:
  - Design entirely new species for different purposes?
    - From biodegradation to biomedicine
  - Complete re-engineer a species
- Ethical Implications – Biosecurity concerns

Topic 3: SARS-CoV-2 Diagnostics and Therapeutics

The COVID-19 pandemic has infected over 612 million individuals worldwide, leading to over 6.5 million deaths.

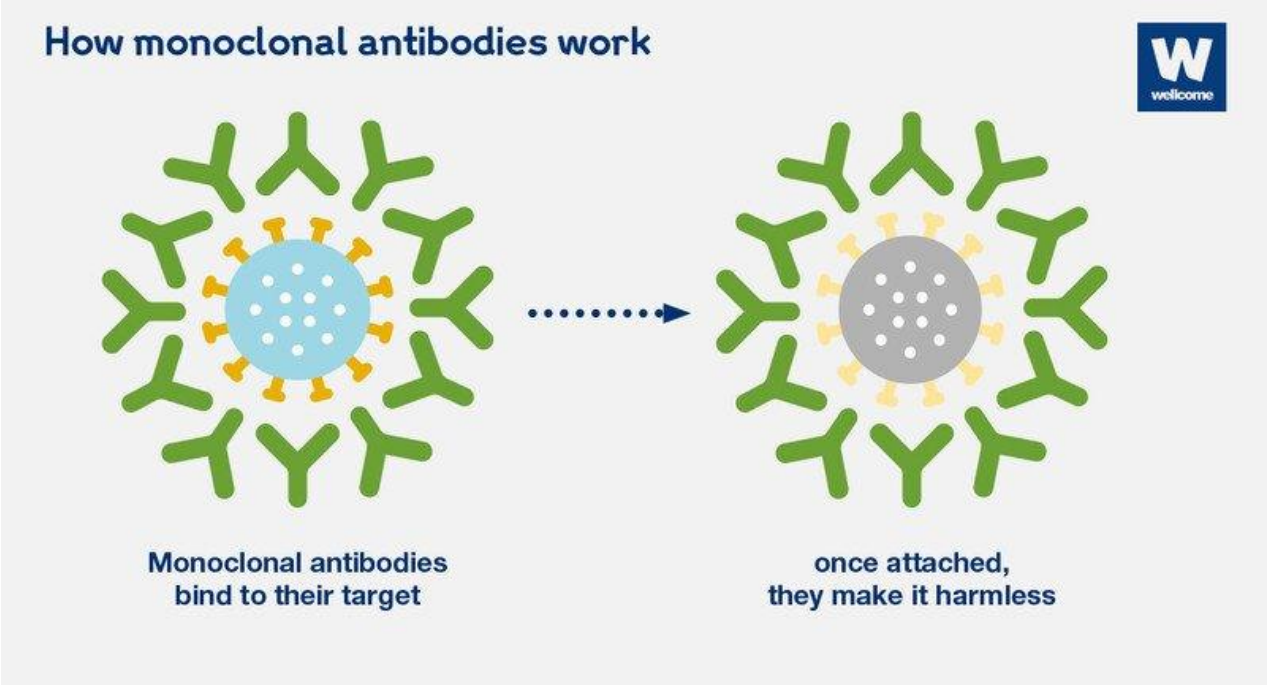


Traditional rapid antigen tests utilize antibody-based detection

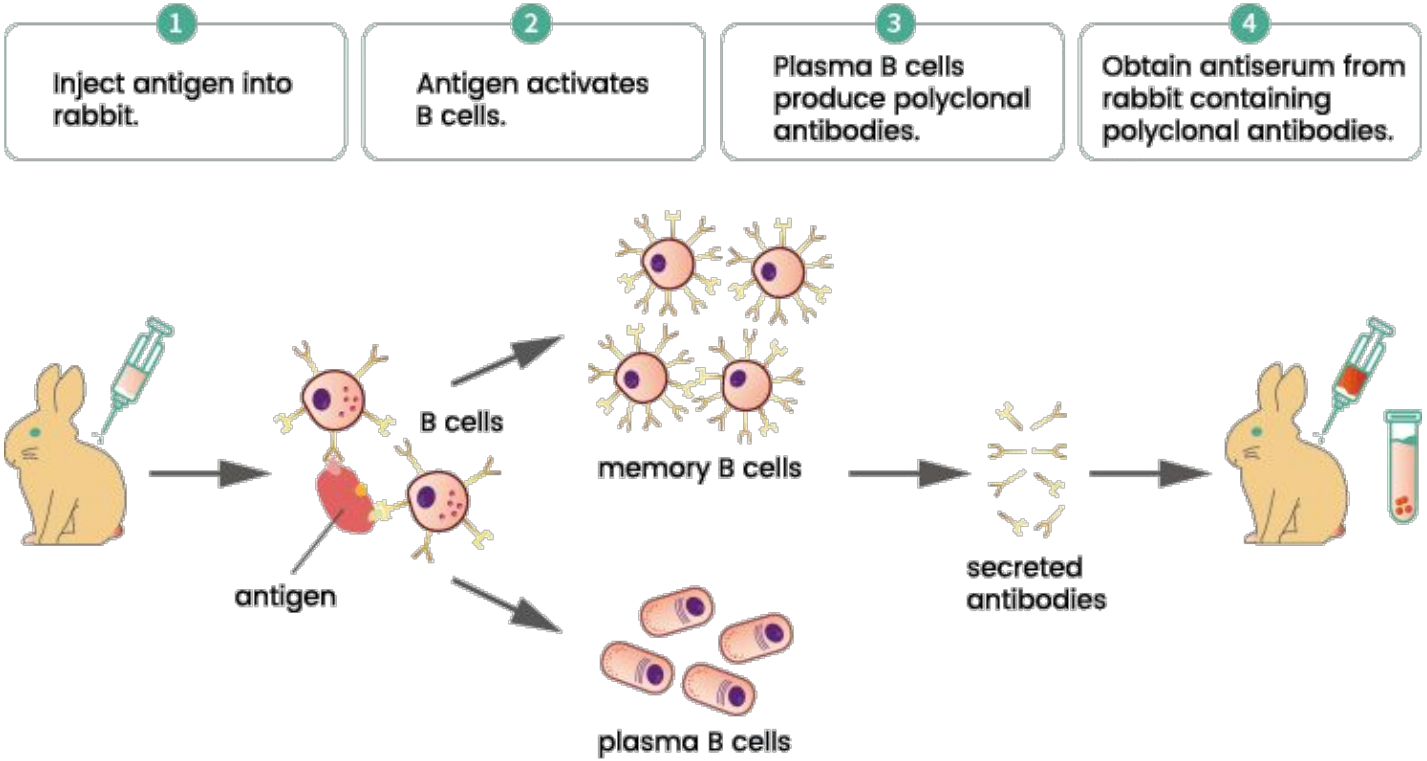


Antibodies are also used as a COVID-19 therapy

Monoclonal antibodies: a common COVID-19 therapy



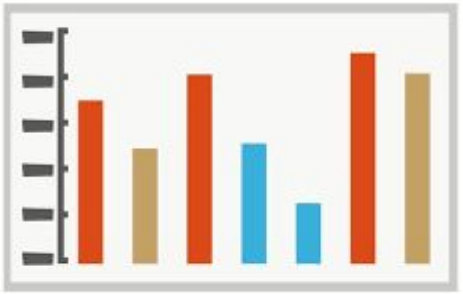
Antibodies are highly accurate, but also highly difficult to produce



Antibodies have several shortcomings

Cost: $400+

Time: 1-2 months



Consistency: Batch variation

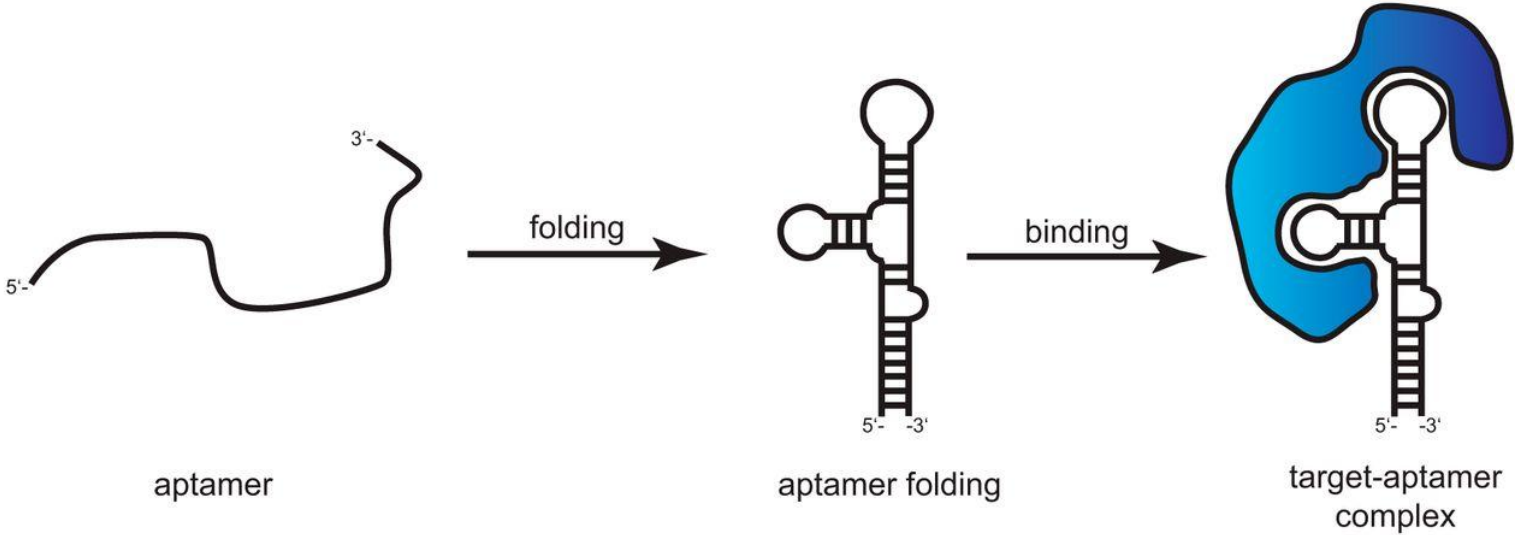
Addressing 3 primary issues in SARS-CoV-2 using synthetic biology

1. **Cost:** solution needs to be cost-effective and shelf-stable

2. **Time:** solution needs to be produced quickly and efficiently

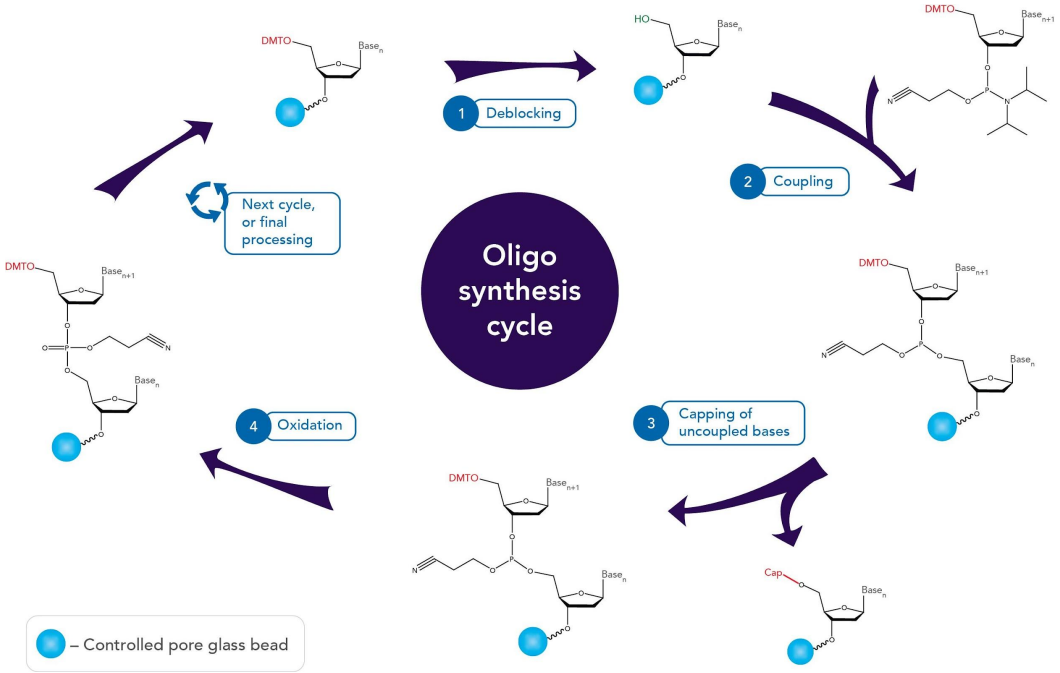
3. **Consistency:** solution needs to be invariable between batches

DNA aptamers are single-stranded oligonucleotides that can bind to biological targets with high affinity



Cost **Time** **Consistency**

The chemical nature of DNA aptamers makes them cheap **($1-5)** and shelf-stable



Cost | **Time** | **Consistency**

DNA aptamers are synthesized rapidly **(1-2 days)**

Cost | **Time** | **Consistency**

The chemical nature of DNA aptamers means that batches are identical

Batch 1

Batch 2

Batch 3

Cost → Time → **Consistency**

Rapid antigen tests using aptamers can be made for less than $1

Aptamer Sandwich Lateral Flow Assay (AptaFlow) for Antibody-Free SARS-CoV-2 Detection

Lucy F. Yang, Nataly Kacherovsky, Nuttada Panpradist, Ruixuan Wan, Joey Liang, Bo Zhang, Stephen J. Salipante, Barry R. Lutz, and Suzie H. Pun*

Cite This: *Anal. Chem.* 2022, 94, 7278−7285 Read Online

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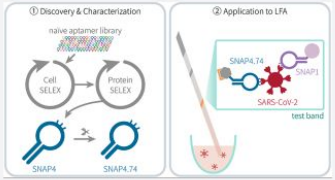
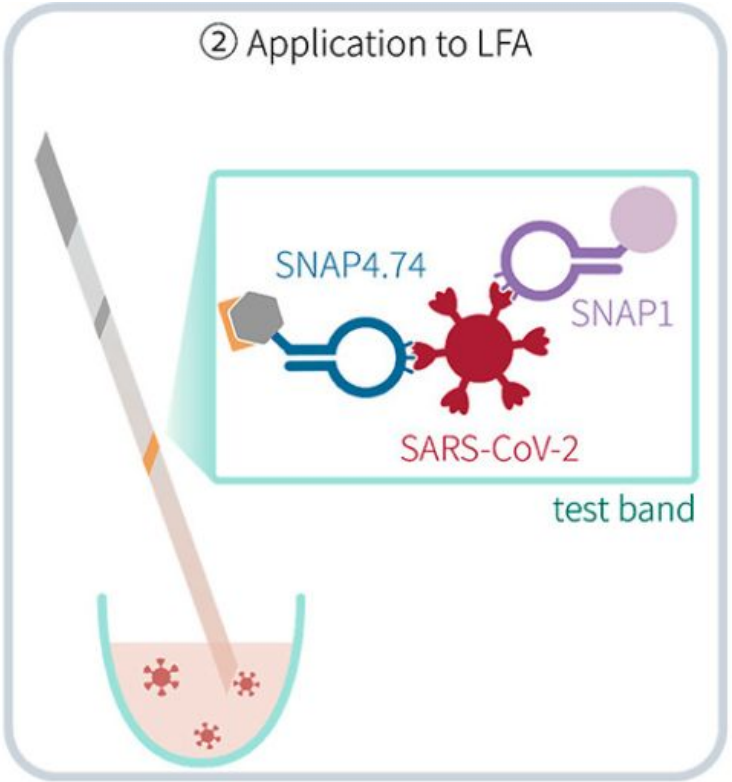
ABSTRACT: The COVID-19 pandemic is among the greatest health and socioeconomic crises in recent history. Although COVID-19 vaccines are being distributed, there remains a need for rapid testing to limit viral spread from infected individuals. We previously identified the SARS-CoV-2 spike protein N-terminal domain (NTD) binding DNA aptamer 1 (SNAP1) for detection of SARS-CoV-2 virus by aptamer−antibody sandwich enzyme-linked immunoassay (ELISA) and lateral flow assay (LFA). In this work, we identify a new aptamer that also binds at the NTD, named SARS-CoV-2 spike protein NTD-binding DNA aptamer 4 (SNAP4). SNAP4 binds with high affinity (<30 nM) for the SARS-CoV-2 spike protein, a 2-fold improvement over SNAP1. Furthermore, we utilized both SNAP1 and SNAP4 in an aptamer sandwich LFA (AptaFlow), which detected SARS-CoV-2 UV-inactivated virus at concentrations as low as 10^6 copies/mL. AptaFlow costs <$1 per test to produce, provides results in <1 h, and detects SARS-CoV-2 at concentrations that indicate higher viral loads and a high probability of contagious transmission. AptaFlow is a potential approach for a low-cost, convenient antigen test to aid the control of the COVID-19 pandemic.



The coronavirus disease 2019 (COVID-19) pandemic is one of the largest public health threats to date. Although COVID-19 vaccines have been authorized and administered to almost 4 billion people worldwide, vaccine dissemination remains a challenge,[1] and new variants of concern (VOCs) with increased transmissibility, severity, or vaccine escape capabilities continue to develop. Preventative measures, including frequent testing, are needed in addition to vaccines to control COVID-19.

Among COVID-19 diagnostics, rapid antigen tests for SARS-CoV-2 are important tools for curbing the pandemic.[2] Rapid antigen tests are inexpensive to produce and provide point-of-care results. Users can make better-informed risk decisions through more frequent testing, even if they are less

have been applied to a variety of fields, including therapeutics[7] and diagnostics.[8] A library selection method called systematic evolution of ligands by exponential enrichment (SELEX) is used to discover aptamer sequences.[9−11] A wide variety of selection targets can be used in SELEX, including whole cells[12] and proteins,[13] to yield highly specific and tight-binding aptamers. The spike (S) protein of SARS-CoV-2 is displayed on the viral surface and is therefore an attractive detection target for intact virus. The S protein is a homotrimer, and each monomer contains subunit 1 (S1) and subunit 2 (S2). Within S1, there are two distinct regions: the receptor-binding domain (RBD) and the N-terminal domain (NTD).[14,15] Several aptamers that bind SARS-CoV-2 S protein have been reported.[16−18] including the SARS-CoV-2 spike protein



Rapid antigen tests can be multiplexed to distinguish between different SARS-CoV-2 variants

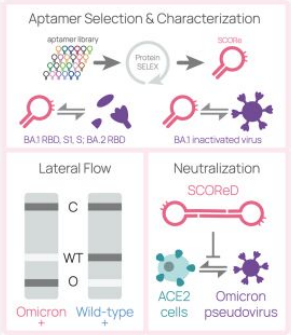
SCORe: SARS-CoV-2 Omicron Variant RBD-Binding DNA Aptamer for Multiplexed Rapid Detection and Pseudovirus Neutralization

Lucy F. Yang, Nataly Kacherovsky, Joey Liang, Stephen J. Salipante, and Suzie H. Pun*

Cite This: *Anal. Chem.* 2022, 94, 12683−12690 Read Online

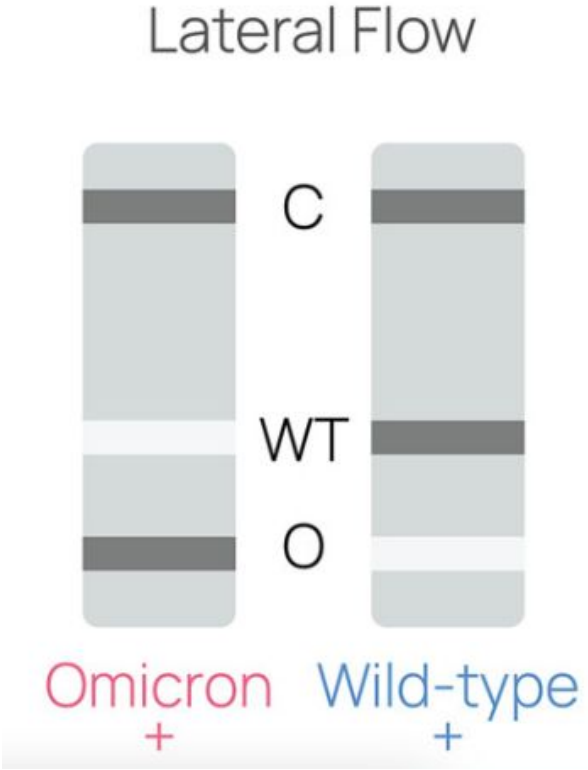
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: During the COVID-19 (coronavirus disease 2019) pandemic, several SARS-CoV-2 variants of concern emerged, including the Omicron variant, which has enhanced infectivity and immune invasion. Many antibodies and aptamers that bind the spike (S) of previous strains of SARS-CoV-2 either do not bind or bind with low affinity to Omicron S. In this study, we report a high-affinity SARS-CoV-2 Omicron RBD-binding aptamer (SCORe) that binds Omicron BA.1 and BA.2 RBD with nanomolar K_D. We employ aptamers SCORe.50 and SNAP4.74 in a multiplexed lateral flow assay (LFA) to distinguish between Omicron and wild-type S at concentrations as low as 100 pM. Finally, we show that SCORe.50 and its dimerized form SCOReD can neutralize Omicron S-pseudotyped virus infection of ACE2-overexpressing cells by >70%. SCORe therefore has potential applications in COVID-19 rapid diagnostics as well as in viral neutralization.

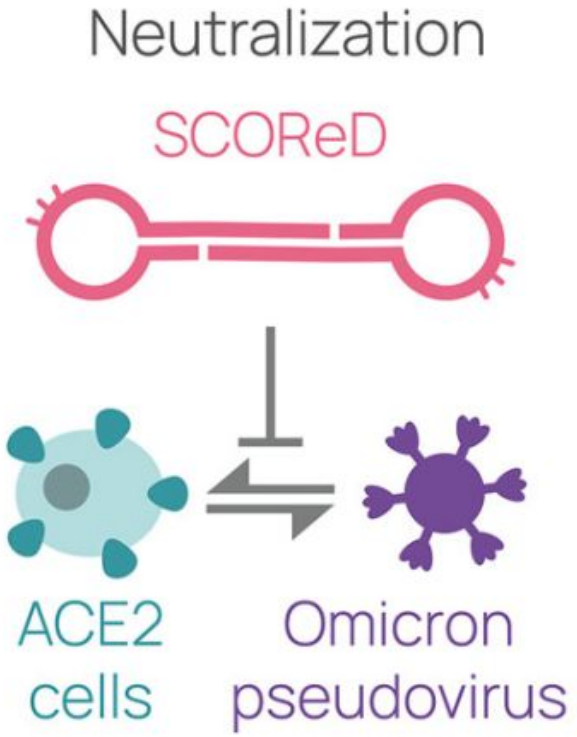


First identified to infect humans in 1967, the coronavirus subfamily has low pathogenic strains, such as HCoV-OC43, and severe disease-causing strains, such as SARS-CoV and SARS-CoV-2.[1] The evolution rate of coronavirus is impacted by its large RNA-based genome, low fidelity of viral polymerase, and recombination with other coronavirus variants within the host.[1] SARS-CoV-2, the pathogen responsible for the ongoing COVID-19 pandemic, has already evolved since its initial characterization in 2019. The World Health Organization has five SARS-CoV-2 variants as "variants of concern" (VOC) due to their increased global health significance: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), glycosylated protein that mediates virus binding to angiotensin-converting enzyme-2 (ACE2) on host cells. Each S monomer is comprised of the exposed subunit 1 (S1) connected to the membrane-anchored subunit 2 (S2) by a furin cleavage sequence.[5] S1 contains both the critical receptor-binding domain (RBD) that contacts the ACE2 receptor through the receptor-binding motif (RBM, residues 438−506) as well as the amino-terminal domain (NTD). S1 is the primary target for SARS-CoV-2-neutralizing antibodies, which have been reported to bind both the RBD and NTD domains of SARS-CoV-2 S protein.[6]

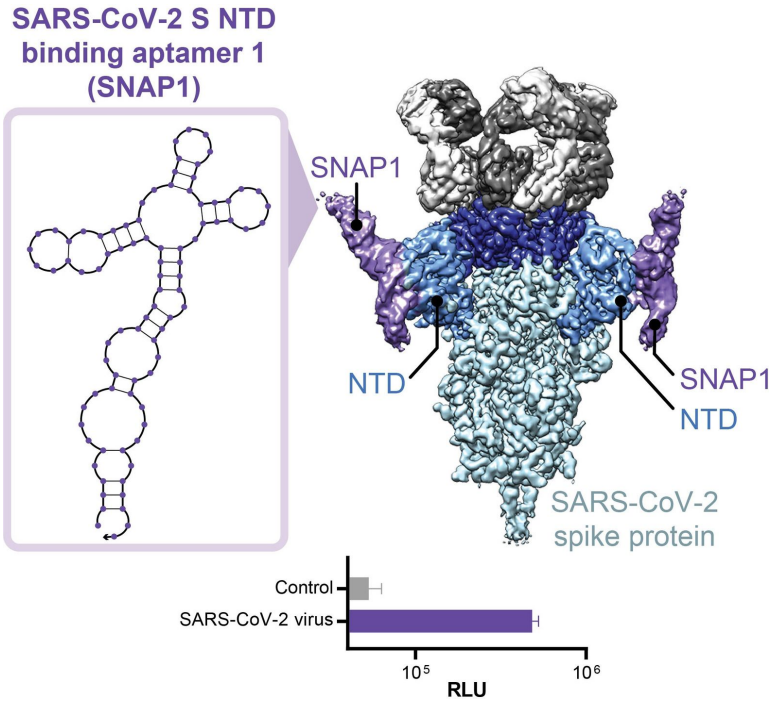
Molecular recognition agents that bind new variants are



Similar to monoclonal antibodies, DNA aptamers can also be applied as a therapeutic against SARS-CoV-2 infections



Aptamers accomplish this by binding to specific domains on the SARS-CoV-2 virus spike protein



Addressing 3 primary issues in SARS-CoV-2 using synthetic biology

1. **Cost:** solution needs to be cost-effective and shelf-stable
    a. Shelf-stable
    b. $1 - 5 rapid antigen tests

2. **Time:** solution needs to be produced quickly and efficiently
    a. 1-2 days for synthesis

3. **Consistency:** solution needs to be invariable between batches
    a. Batches are synthesized chemically and are identical

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Addressing 3 primary issues in diagnostics using synthetic biology

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Topic 4: Human Practices & WaMel

"We ask every team to think deeply and creatively about whether their project is responsible and good for the world." -iGEM website

-and also- will the idea actually work?

Interviewing Experts

Literature Review

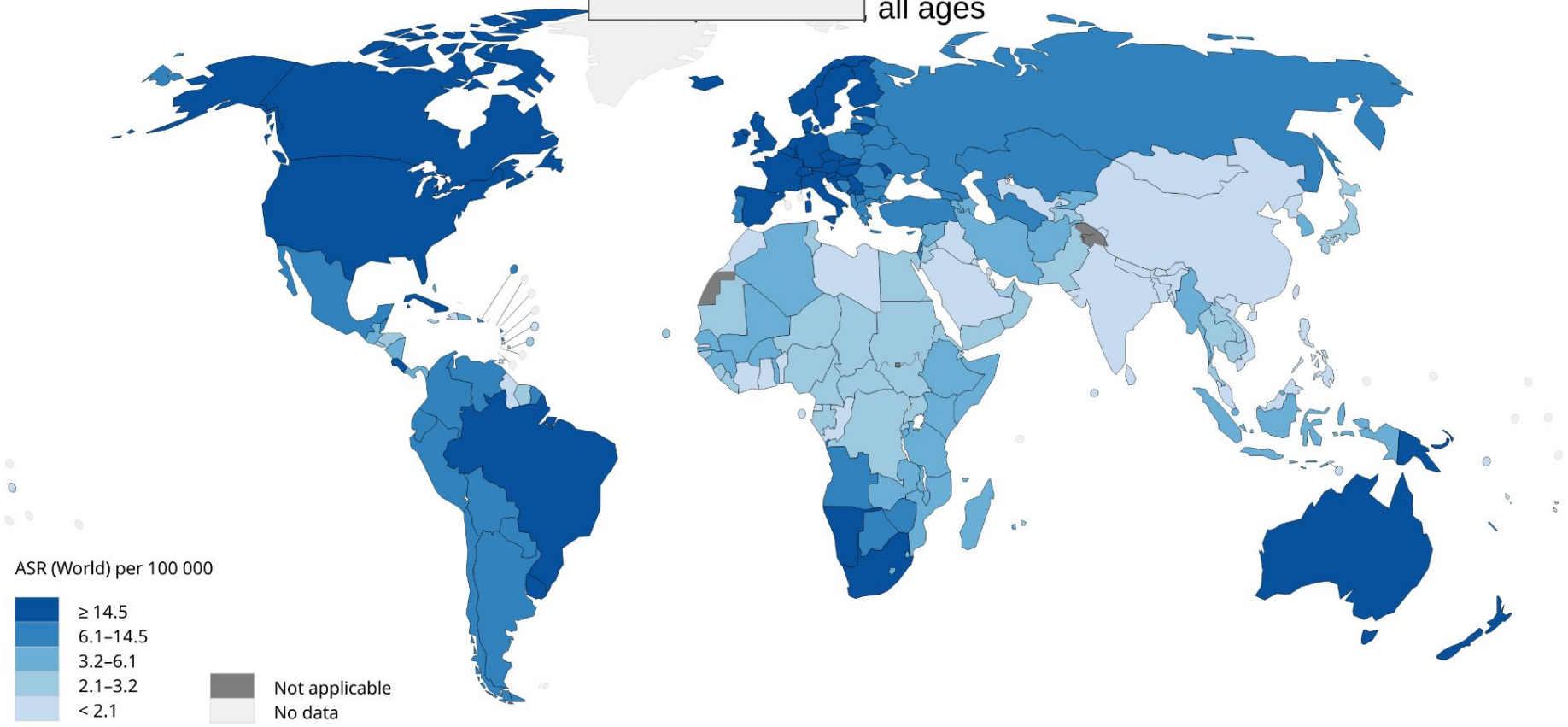
Trying to understand- Business, Policy, Bioethics

Topic 4: WaMel Basics (Owen)

What is the most common cancer in the world?

Hint: In the US ⅕ of people get it

Estimated age-standardized incidence rates (World) in 2020, all ages



Data source: GLOBOCAN 2020
Map production: IARC
(http://gco.iarc.fr/today)
World Health Organization

Topic 4: WaMel Basics (Owen)

Skin Cancer Diagnostic

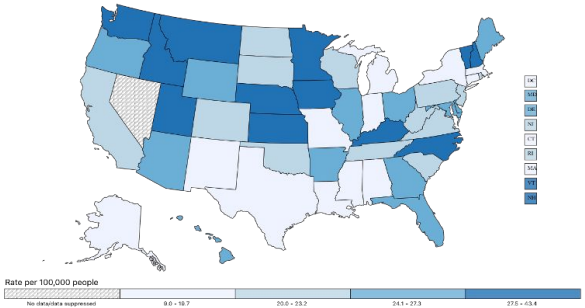
Early Diagnosis can save lives

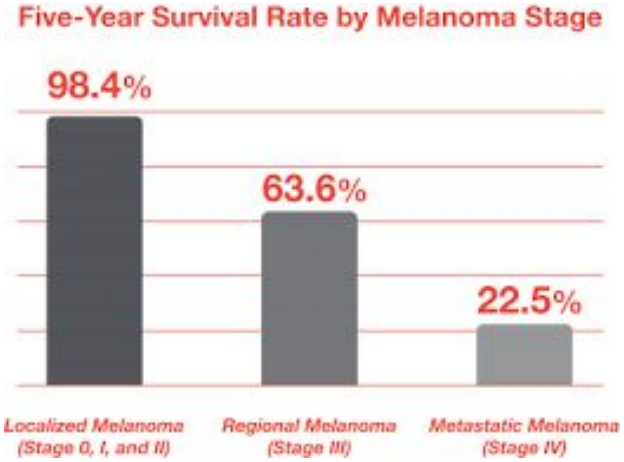
9,000 people in the US die

Melanoma- 6th most common in US

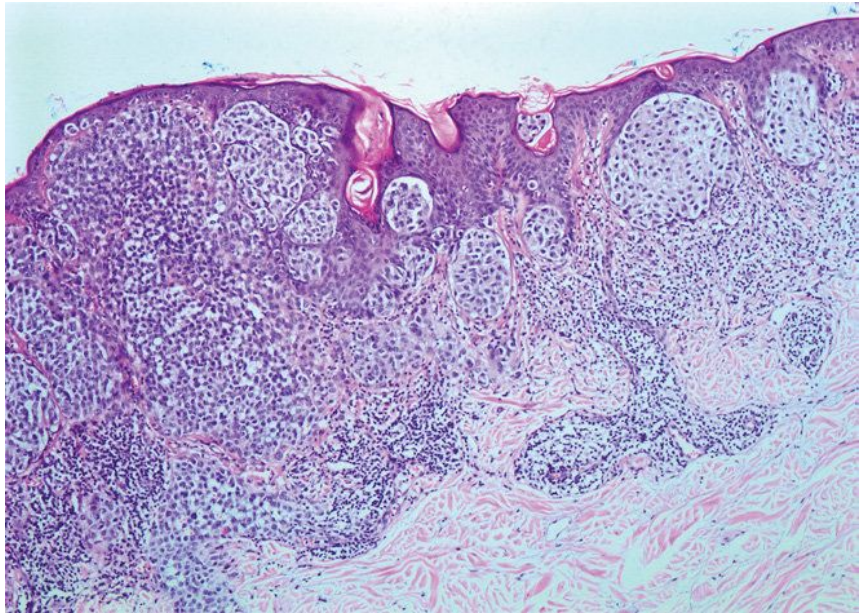
Cost of treatment
Inequalities of access based on geography, race, income

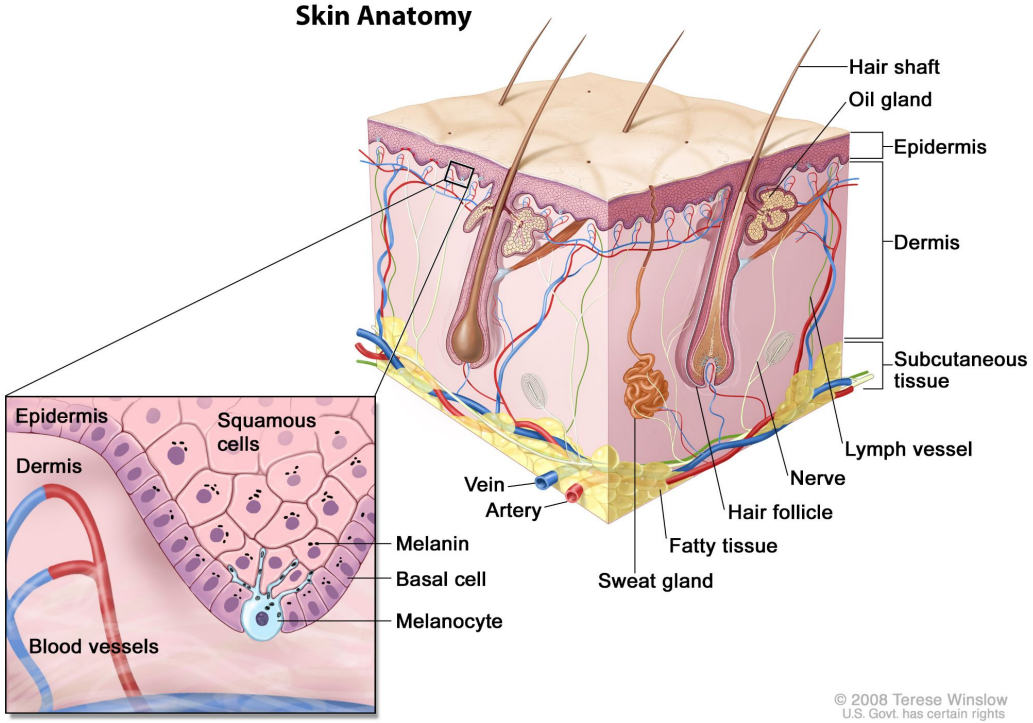
Rate of New Cancers in the United States, 2019
Melanomas of the Skin, All Ages, All Races and Ethnicities, Male and Female





Topic 4: What is melanoma and why do we need a special band aid?





BRAF and TERT mutations

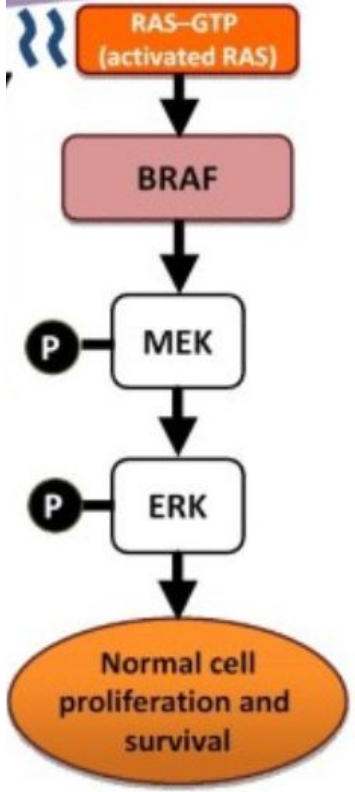
Braf V600 E- oncogene

TERT promoter mutations- telomerase reverse transcriptase

both: cause increased mitosis, thicker cancers

Prognostics

Correlated!



Subtypes of melanoma

~~1. Eye melanoma~~

~~2. Mucosal Melanoma~~

3. Skin Melanomas

   ~~a. Acral Lentiginous Melanoma~~

   b. Cutaneous melanoma (3 types)

      i. Superficial spreading,

      ii. nodular

      iii. lentigo

Where is the problem?

Where do you think the biggest disparity in melanoma outcomes is?

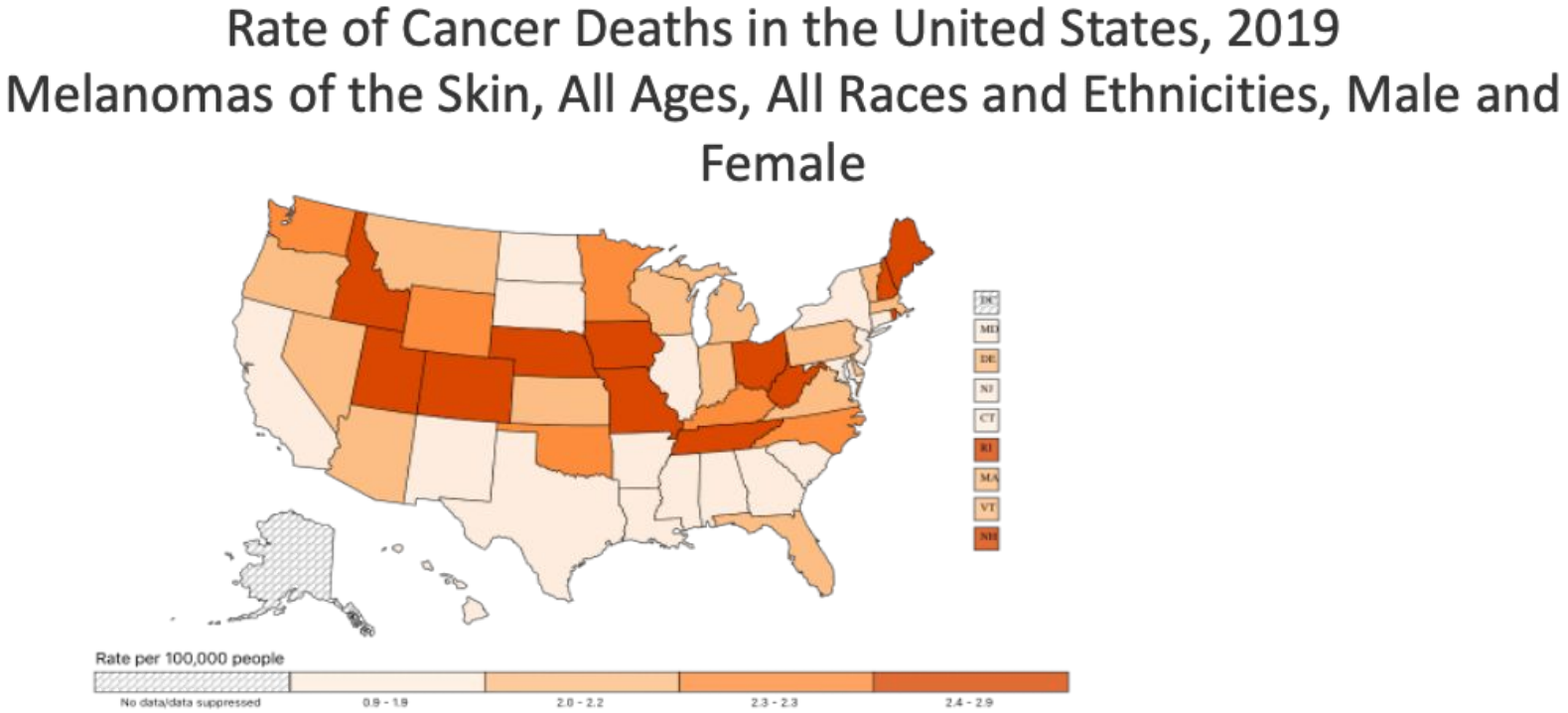
Racial Gap

Male vs. Female Gap

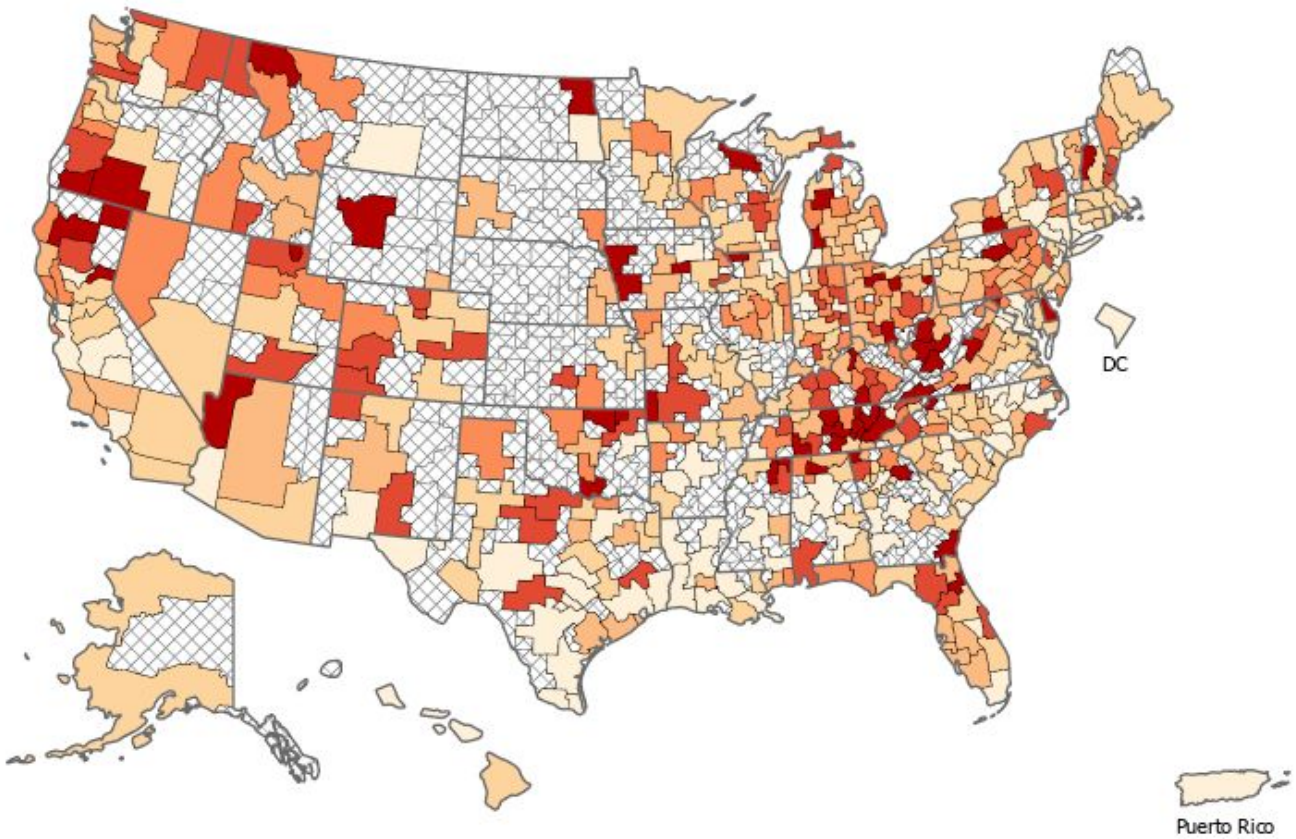
Poor vs. Rich Gap

Urban vs. Rural Gap

What does the urban gap look like?



Death Rates for United States by Health Service Area
Melanoma of the Skin, 2015 - 2019
All Races (includes Hispanic), Both Sexes, All Ages



Age-Adjusted
Annual Death Rate
(Deaths per 100,000)

Quantile Interval

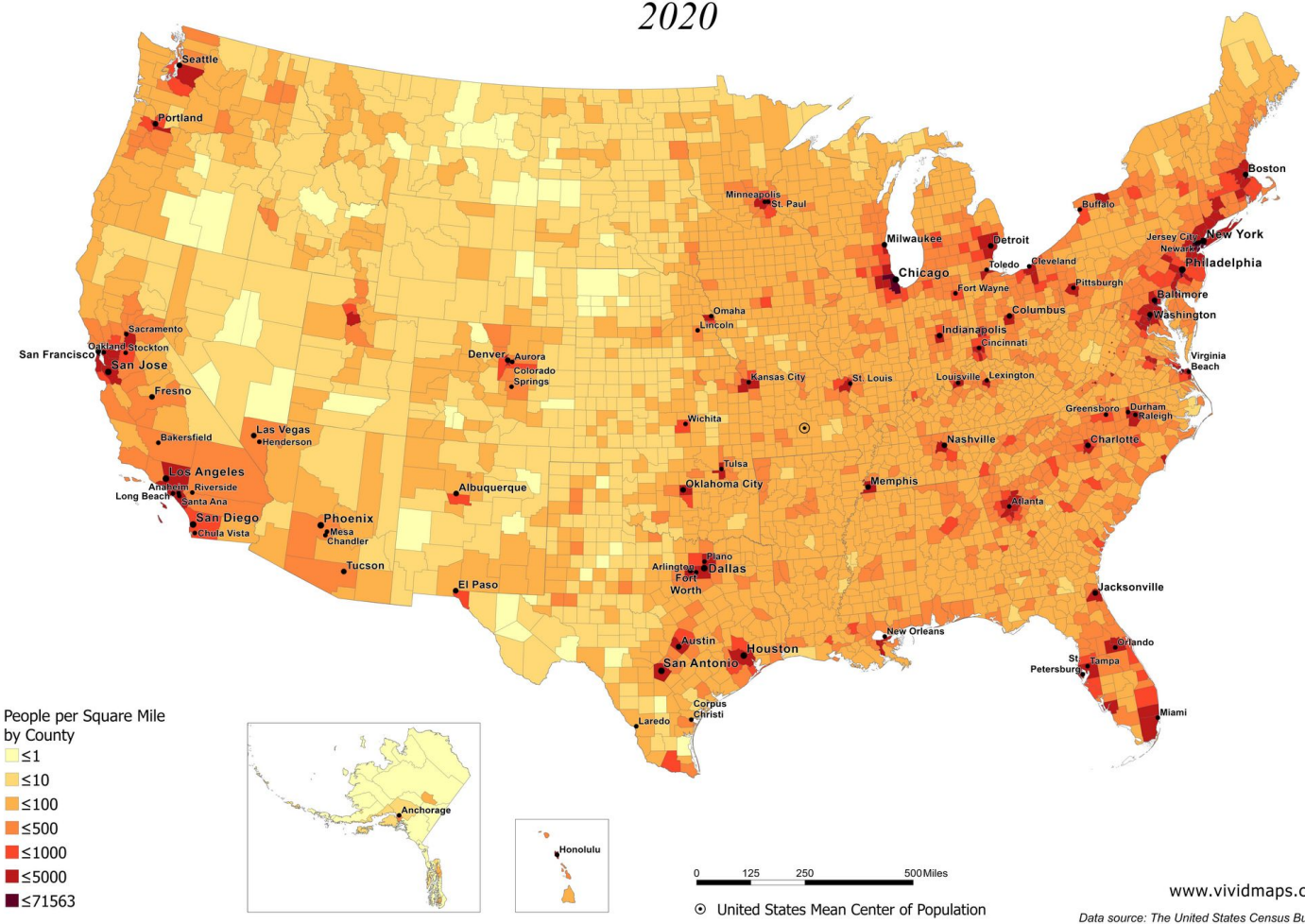
- 0.5 to 1.9
- > 1.9 to 2.3
- > 2.3 to 2.6
- > 2.6 to 2.8
- > 2.8 to 3.2
- > 3.2 to 5.3
- Suppressed *

United States
Rate (95% C.I.)
2.2 (2.1 - 2.2)

Healthy People 2020
Goal C-8
2.4

U.S. Population Density by County

2020



Acknowledgement: Dr. Sancy Leachman

Head of the OHSU Department of Dermatology and Director of the Knight Institute's Program for Melanoma

How does a test help

Tests can resolve issues for delayed diagnosis

-Inertia (patient passivity)

-Cost of appointments

-Time commitment of appointments

Acknowledgement
Professor Public Health Sciences Division at the Fred Hutch Cancer Center

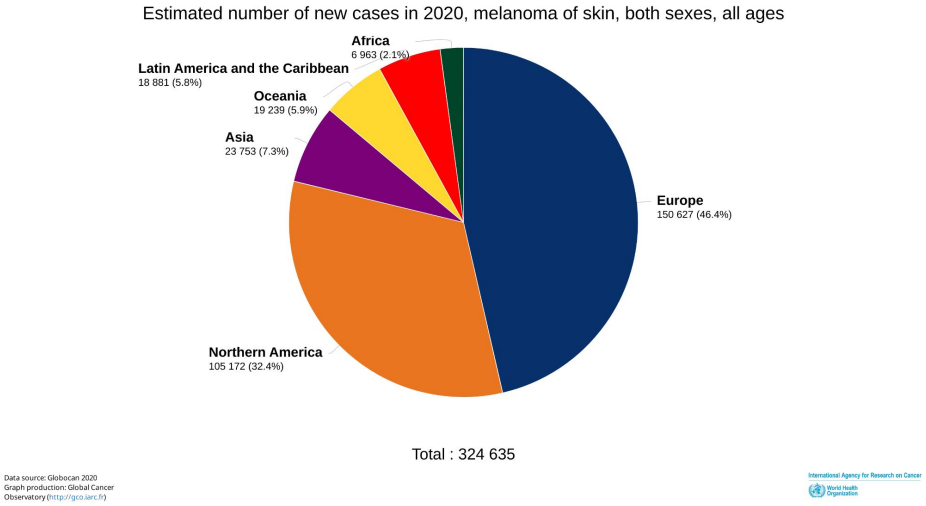
Why does this matter?

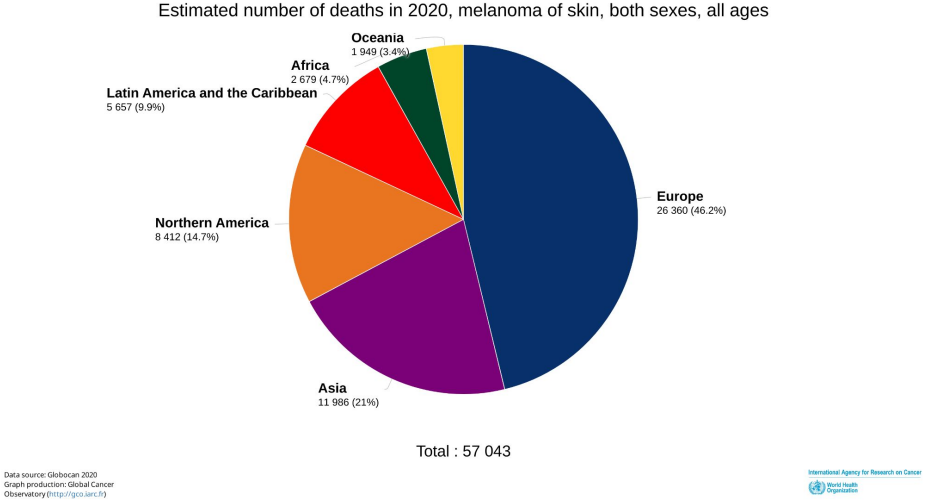
We can reduce melanoma mortality

99,780 melanomas diagnosed yearly with about 9,000 deaths

(.02)(99,780) = abt 2,000 deaths instead

People being diagnosed late is the first problem





Synthetic Biology at UW

- Research Database: https://www.washington.edu/undergradresearch/research-opportunities/find/
- Synthetic Biology Research Labs:
  - Institute for Protein Design
  - Bioengineering Faculty: https://bioe.uw.edu/faculty-staff/core-faculty/
- Email lists (department listservs)
  - Biology, microbiology, Chem-biochemugs mailing list
- Classes that discuss synthetic biology:
  - BIOEN 215 (Intro to BioE), BIOEN 423 (Intro to Synthetic Biology)
  - CSE 486 (Introduction to Synthetic Biology)
  - BIOEN 424/CSE 487 (Advanced Systems and Synthetic Biology)
  - BIOEN 425/CSE 488 (Laboratory Methods in Synthetic Biology)

How to get involved in synbio

Join our team! Washington iGEM

iGEM is an international synthetic biology competition

Teams work to use synthetic biology to solve problems

Competition is in the end of October each year.

We recruit new members in January!

We are an interdisciplinary team

- Wetlab
  - Do Lab work. Most lab work occurs over the summer here at UW
- Drylab
  - Mathematical Modeling
  - Protein Modeling
- Human Practices
  - Communicate information about out topic
  - Talk with stakeholders and experts on the topic
- Fundraising
- Webdev
- Design

Please sign up to join our email list

https://forms.gle/yXYtbeBT14V1MiD76

Contact us with any questions:

- uwigem@uw.edu

Follow our instagram!

- @washington_igem

http://students.washington.edu/uwigem/#/

In the next few days we will email you these slides!