

Design for Biosecurity
Prof. Mainak Das
Department of Design
Indian Institute of Technology, Kanpur

Lecture 11

Unraveling the Ingenious Bacillus Anthracis Attack and Case Study for Anthrax Sensor

Welcome back! We've now completed two weeks of this course, and today marks the start of our third week with the 11th lecture. As we progress, I want to mention a slight shift in our approach. We will delve into the background, history, geography, epidemiology, and sensor technology related to various diseases. Along the way, I will also share some of my notes, inspired by global events that pushed me to uncover some hidden stories, stories that were once shrouded in secrecy but have since become public knowledge. These events have had a profound impact on the human psyche, often leaving deep emotional scars.

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WHY ANTHRAX IS A POTENT BIOTERRORISM AGENT?

Handwritten notes on the right side of the slide:

- nasab
- ovula
- certancup

- This is due to the properties of this bacterium, the ease with which it can be produced in large quantities, and its many possible applications.

These properties mean that laboratory conditions are not necessary for producing both vegetative forms and spores. Moreover, the spores of this microorganism in particular can be secretly transported around the world. Although, at present, the use of Bacillus anthracis as a biological weapon on a large scale may be considered highly unlikely, the use of this bacterium in a bioterrorist attack is not only possible, but has already taken place. In addition, these attacks have been effective, as they have led to the deaths not only of those targeted by these unlawful acts but also of bystanders.

<https://link.springer.com/article/10.1007/s11259-023-10238-3#Sec110>

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Anthrax is one such story. As I mentioned in the last class, everything began in 2001, marking a significant chapter in the history of bioterrorism. In our previous lecture, we explored the possibility of an anthrax pandemic or epidemic, examining different global scenarios, from conservative estimates to highly liberal predictions of how anthrax could devastate human populations. Today, we will dive deeper into why anthrax is considered a potent bioterrorist agent. Over the past 25 years, anthrax has emerged as the most well-known bioterrorist weapon, and its notoriety is well-documented.

While other bioterrorist incidents have occurred, we often lack concrete data or evidence, with some information being suppressed. However, the anthrax case is relatively well-documented, though much of it remains within the confines of classified information. We may never fully understand the extent of what occurred, but we do have some insights.

Let's now move on to the critical question: Why is anthrax such a potent bioterrorist agent? What makes it so attractive for use in bioterrorism? The answer lies in the bacterium's unique properties. First, it can be produced in large quantities with relative ease, making it a feasible option for mass destruction. Additionally, anthrax can enter the human body through multiple routes: nasal, oral, and cutaneous (skin) pathways. This versatility in entry points, through inhalation, ingestion, or skin contact, adds to its lethality.

When considering the use of microbes as a weapon, a few key factors come into play. The ease of production is paramount, as mass destruction requires a readily available agent. Anthrax fits this criterion perfectly, as it can be aerosolized to enter the nasal cavity, contaminate food to be ingested, or be transmitted through cuts and wounds on the skin. These properties make it possible to produce both vegetative forms and spores without needing a highly specialized laboratory environment. This means that large quantities of anthrax can be manufactured and transported secretly around the world.

However, despite these properties, the large-scale use of *Bacillus anthracis* as a biological weapon is currently considered unlikely. Nonetheless, the potential for its use in bioterrorist attacks remains real, and as we know from the events in the United States in 2001, it has already been used with devastating effects.

These attacks have been devastating, resulting in the deaths not only of the intended targets but also of innocent bystanders who happened to be nearby. While some may be skeptical and claim that such events could never happen again, history serves as a stark reminder that they have already occurred. We cannot afford to be negligent and allow these threats to proliferate without taking proper precautions.

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2001

The Washington Post
31 Exposed to Anthrax on Capitol Hill;
House Shuts Down; Senate Offices Close

U.S. Senate
Investigation: One Man's Letters
to the "9/11" and Anthrax Cases

SENATOR DASCHLE
509 HART SENATE OFFICE
BUILDING
WASHINGTON, DC 20540-0507

4TH GRADE
GREENDALE SCHOOL
FRANKLIN PARK NJ 07852

Anthrax attacks in September 2001, known as "Amerithrax," are an example in which workers have potential exposure to anthrax on the job. In this event, BA-laced letters, including the one pictured above, mailed to news media offices and the U.S. Congress resulted in a total of 22 people contracting anthrax through inhalation or skin exposure. These cases, including five that were fatal, included people at targeted locations, U.S. Postal Service workers at sorting facilities, and individuals handling cross-contaminated mail at the destination facilities.

Bruce E. Ivins, a biochemist the F.B.I. says was responsible for five anthrax deaths. Dr. Ivins, a microbiologist who had worked with anthrax for decades as part of the vaccine program at the Army's biodefense laboratory at Fort Detrick, Md., took a fatal overdose of Tylenol in July 2008 at the age of 62, after months of intense scrutiny by the F.B.I., which had placed a GPS device on his car, examined his trash and questioned his wife and two children.

Dropped into a mailbox in downtown Princeton, N.J., the anthrax letters were addressed to news organizations and two United States senators and contained notes with radical Islamist rhetoric that appeared to link them to the Sept. 11 attacks, which occurred a week before the first of the two mailings. In the jittery wake of 9/11, they set off a nationwide panic over random discoveries of white powder that people feared might be more anthrax. The real anthrax — a few teaspoons of very fine powder — infected at least 22 people, including several postal workers, and killed 5.

VSPS

<https://www.osha.gov/anthrax/background> <https://www.nytimes.com/2010/02/20/us/20anthrax.html> Credit...U.S. Army

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Now, let me move on to the next topic, which is my personal journey in understanding anthrax from a very different perspective, starting with the year 2001. The year 2001 was marked by tragedy. It was the year of the 9/11 attacks, when the Twin Towers were destroyed and the Pentagon was severely damaged. But from the standpoint of bioterrorism, this year is also infamously remembered for the anthrax attacks in September, often referred to as "Amthrax."

These attacks involved letters laced with Bacillus anthracis being sent to news media offices and the U.S. Congress. As a result, 22 people contracted anthrax through inhalation or skin exposure, five of whom tragically lost their lives. The victims included not only the

people at the targeted locations but also USPS workers at sorting facilities and individuals who handled cross-contaminated mail at the destination facilities. Orchestrating an attack of this magnitude required an in-depth understanding of anthrax biology, which highlights the expertise needed to execute such an operation.

According to the FBI's investigation, the person considered to be the mastermind behind these attacks was Bruce Ivins. Bruce Ivins's story is a particularly intriguing one. He was, until his death, one of the world's leading experts on anthrax. He wasn't just a random figure; he was well-known within the U.S. Army and other intelligence agencies, who often consulted him on the various aspects of anthrax and its potential uses. Despite his expertise, Ivins was known to be somewhat eccentric and had several personal issues, but he was also described as a remarkable gentleman, at least, that's what the documentation suggests.

Interestingly, there is a movie on Netflix about this anthrax issue, which I highly recommend you watch. Many of you were not even born at the time these events occurred, so watching the movie could give you a better understanding of the situation and the impact it had.

Returning to the events of 2001, it was an extremely challenging time. The United States was still reeling from the 9/11 disaster when the anthrax attacks occurred, exacerbating an already tense and volatile situation. These attacks had the potential to inflame religious hatred, making an already unusual time even more precarious.

Bruce Ivins, a biochemist, was the individual the FBI believed to be responsible for the five anthrax-related deaths. Dr. Ivins had worked with anthrax for decades as part of the vaccine program at the Army's Biodefense Laboratory at Fort Detrick, Maryland. In July 2008, at the age of 62, Ivins took a fatal overdose of Tylenol after months of intense scrutiny by the FBI. The FBI had placed a GPS device in his car, examined his trash, and questioned his wife and two children. Their suspicions grew over time as they monitored his activities. For example, they noted his peculiar habit of traveling long distances at odd hours and mailing items far from his home, which further fueled their concerns.

Imagine this scenario: it's post-2001, a time of heightened tension and fear. During this period, certain unusual behaviors began to emerge, and among these was Bruce Ivins, who had a history of issues with some of his female colleagues, possibly due to his temper. We may never fully understand the extent of his personal struggles, but what we do know is that he was one of the finest experts in his field.

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A Coded Message?

The F.B.I. says Bruce E. Ivins embedded a complex coded message in the notes that he mailed with anthrax in 2001. The coded message, based on DNA biochemistry, alluded to two female colleagues with whom he was obsessed, it says.

Investigators separated the boldface letters, TTT AAT TAT, underlined here. The letters "T" and "A," they say, represent Thymine and Adenine, two of the four nucleotides present in DNA sequences.

Each sequence of three nucleotides codes for an amino acid.

Each amino acid also has a single-letter designator.

"PAT" was the nickname of a former colleague of Dr. Ivins, when the report describes as "one of his only friends" and the "object of excessive affection and attention by Dr. Ivins."

"FNV" was "a verbal assault on New York" and also associated with another former colleague. In an e-mail message to the colleague, Dr. Ivins accused New Yorkers of "overplaying" the 9/11 attacks.

Letter sent to Tom Brokaw, the former NBC news anchor.

AMINO ACIDS

TTT - Phenylalanine
 AAT - Asparagine
 TAT - Tyrosine

Amino acid code

A - Alanine	G - Glycine	M - Methionine	S - Serine
C - Cysteine	H - Histidine	N - Asparagine	T - Threonine
D - Aspartic acid	I - Isoleucine	P - Proline	V - Valine
E - Glutamic acid	K - Lysine	Q - Glutamine	W - Tryptophan
F - Phenylalanine	L - Leucine	R - Arginine	Y - Tyrosine

<https://archive.nytimes.com/www.nytimes.com/imagepages/2010/02/20/us/20anthraxr.html?ref=us>
<http://web.archive.org/web/20201120064637/http://www.yourgenome.org/facts/what-does-dna-do>

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Now, consider this: anthrax-laced letters were being dropped into a mailbox in downtown Princeton, New Jersey. These letters were addressed to major news organizations and two United States senators, and they contained notes with radical Islamist rhetoric. This was clearly an attempt to link the anthrax attacks to the 9/11 terrorist attacks, which had occurred just a week before the first of these mailings. In the jittery aftermath of 9/11, these letters sparked a nationwide panic, with people fearing that any random discovery of white powder might be anthrax. The reality was that just a few teaspoons of this fine anthrax powder infected at least 22 people, including several postal workers, and tragically claimed five lives.

Think about the situation: the country was already under immense stress from the 9/11 attacks, and then, right on its heels, came a bioterrorist attack. The letters themselves indicated a particular religious sentiment, further misleading the system and sowing seeds of confusion. This was one of the first major homegrown terrorist attacks on U.S. soil, orchestrated by a U.S. citizen. It was a profoundly misleading and dangerous act that could have had catastrophic consequences, potentially sparking religious wars at a time when there was already significant apprehension about immigrants and people from outside the United States. Adding this bioterrorist threat into the already volatile mix made for an extraordinarily difficult and dangerous time.

Let's delve into some of the coded messages from the New York Times that accompanied these letters. The messages were both cryptic and disturbing: "Take penicillin now, death to America, death to Israel. Allah is great." At first glance, these lines might seem like nothing more than radical rhetoric, but let's take a closer look, especially at the repetition of the letter "T."

Now, think back to your studies on the genetic code, Hargobind Khurana, who won the Nobel Prize for his work on this, established the significance of genetic coding. Consider the sequence "T T T," which corresponds to the amino acid phenylalanine, represented by the letter "F." This kind of creativity shows just how much thought and effort went into crafting these messages.

Now, let's look at another sequence: "A A T," which corresponds to asparagine, represented by the letter "N." Finally, we have "T A T," which corresponds to tyrosine, represented by the letter "Y." When you put these letters together, F, N, and Y, they spell out a coded assault on New York, possibly linked to a former colleague of Ivins. In an email, Dr. Ivins had accused New Yorkers of playing a role in the 9/11 attacks, further deepening the sinister nature of this entire episode.

This level of coded communication, the psychological manipulation involved, and the potential to cause widespread panic and confusion highlight the incredible and terrifying creativity that went into these attacks.

Consider the case of Pat, a nickname for a former colleague of Dr. Bruce Ivins. According to the FBI report, Pat was one of Ivins' only friends and the object of his excessive affection and attention. This detail offers a glimpse into the complex and creative mind of Ivins, a mind that misled investigators and caused widespread chaos. It took the FBI an extended period to decipher the intricate web he wove.

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F.B.I., LAYING OUT EVIDENCE, CLOSES ANTHRAX CASE

By Scott Shane, Feb. 19, 2010

WASHINGTON — More than eight years after anthrax-laced letters killed five people and terrorized the country, the F.B.I. on Friday closed its investigation, adding eerie new details to its case that the 2001 attacks were carried out by Bruce E. Ivins, an Army biodefense expert who killed himself in 2008.

- A 92-page report, which concludes what by many measures is the largest investigation in F.B.I. history, laid out the evidence against Dr. Ivins, including his equivocal answers when asked by a friend in a recorded conversation about whether he was the anthrax mailer.
- The report disclosed for the first time the F.B.I.'s theory that Dr. Ivins embedded in the notes mailed with the anthrax a complex coded message based on DNA biochemistry, alluding to two female former colleagues with whom he was obsessed.

2001 — 2010 → 26th Feb

2001-2010

<https://www.osha.gov/anthrax/background> <https://www.nytimes.com/2010/02/20/us/20anthrax.html>

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Now, let's move on to the next significant point: the closure of the anthrax case by the FBI. The date is crucial, February 19, 2010. To give you some personal context, I remember this date vividly. It was just a week before, on February 26, 2010, that I left the United States after spending ten long years there. This case had become quite close to my heart, especially because it transformed what was once just another topic in a microbiology textbook into a profound real-life event. This case turned a theoretical understanding into something far more tangible and imaginative for me.

More than eight years after the anthrax letters claimed five lives and terrorized the nation, the FBI finally closed its investigation. On that Friday, they released new, eerie details in

what was considered the largest investigation in FBI history. The FBI concluded that the 2001 anthrax attacks were orchestrated by Dr. Bruce Ivins, an Army biodefense expert who took his own life in 2008 using an overdose of Tylenol. For those unfamiliar with Tylenol, it's a common over-the-counter medication similar to ibuprofen, typically used to reduce fever.

The 92-page report laid out the extensive evidence against Dr. Ivins, including a recorded conversation in which he gave an equivocal answer when asked by a friend if he was the anthrax mailer. For the first time, the report disclosed the FBI's theory that Dr. Ivins embedded a complex coded message in the notes sent with the anthrax, based on DNA biochemistry. This code alluded to a female colleague with whom he was obsessed. The gravity of the situation is underscored by this chart from February 19, 2010, which I am sharing from an exact New York Times paper cutting.

The anthrax case was finally put to rest, but it raises a chilling thought: if such an attack was feasible 24 or 25 years ago, consider the potential for destruction today. With the advanced molecular biology tools, biochemistry techniques, physical chemistry innovations, synthetic biology, synthetic chemistry, and sophisticated engineering methods available now, the scope for bioterrorism is vast. Imagine the damage that could be inflicted on a population, plunging them into fear and chaos.

This leads to the inevitable conclusion that biosecurity is poised to become one of the most critical and specialized fields in the coming years and decades. Unlike nuclear warfare, where satellite imagery can detect the transfer of nuclear stockpiles, biowarfare is insidious. A pathogen can be transported across borders in a tiny ampoule, hidden in something as innocuous as toothpaste, tooth powder, or cosmetics. It can slip through any border undetected, only to be activated later.

This isn't a war fought with heavy artillery or nuclear weapons; it's a war on the human body, on the very ecosystem we inhabit. It's a war on biodiversity, which, as I've mentioned before, can be utterly destroyed. The need for investment in biosecurity cannot be overstated.

This type of warfare is exceedingly difficult to confront, especially because it is a war for which we are unprepared. It underscores the critical need to deeply understand biology, to study it rigorously, and to invest in grasping the molecular intricacies of biology, chemistry, physics, engineering, and epidemiology. We must be thorough in our informatics to anticipate and counter such threats effectively.

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CASE STUDY FOR BACILLUS ANTHRACIS (ANTHRAX) SENSOR

Bacillus anthracis, present in two forms of vegetative cells and spores, is a pathogen that infects humans through contact with infected animals or contaminated animal products and is also maliciously used in terrorist acts. Therefore, a rapid and sensitive test for *B. anthracis* is necessary but challenging. The challenge comes from the following aspects:

- an accurate distinction of *B. anthracis* from other *Bacillus* species due to their high genomic similarity and the horizontal gene transfer between *Bacillus* members;
- direct detection of the *B. anthracis* spores without damaging them for component extraction to avoid the risk of spore atomization;
- and the rapid detections of *B. anthracis* in complex samples, such as soil and suspicious powders, without sample pretreatments and expensive large-scale equipment.

Acc. Chem. Res. 2021, 54, 24, 4451–4461

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Now, let's examine the case study for Bacillus anthracis sensors. Bacillus anthracis exists in two forms: vegetative cells and spores. This pathogen infects humans through contact with infected animals or contaminated animal products and has been maliciously used as a weapon of terrorism. Therefore, developing a rapid and sensitive test for Bacillus anthracis is essential but presents several significant challenges.

Here are the challenges:

1. Accurate Distinction: Differentiating Bacillus anthracis from other closely related Bacillus species is difficult due to their high genomic similarity and the occurrence of

horizontal gene transfer between *Bacillus anthracis* and other *Bacillus* members. This makes it challenging to identify the pathogen accurately.

2. Direct Detection of Spores: Detecting *Bacillus anthracis* spores directly, without damaging them for component extraction, is crucial to avoid the risk of spore aerosolization, which could spread the pathogen. Therefore, understanding and developing methods that allow for non-destructive detection is vital.

3. Detection in Complex Samples: Rapid detection of *Bacillus anthracis* in complex samples, such as soil and suspicious powders, is another significant challenge. This process must be done without extensive sample pretreatment or the need for expensive, large-scale equipment. Not every country has access to sophisticated instrumentation for every pathogen, which highlights the need for more accessible and efficient detection methods.

In other words, we may need to develop comprehensive libraries of antidotes and establish task forces that remain vigilant at all times. These task forces should continuously update the government about potential epidemic or pandemic threats or possible bioterrorist attacks. It is far better to take preventive measures than to be caught unprepared and descend into chaos.

These signals and instances provoke us to think judiciously, emphasizing where we must allocate our resources. A significant focus should be on encouraging young minds to engage in this field, as it represents one of the most challenging zones humanity is entering, the world of microbes and their potential to be used as terrorist agents.

This will be a long and complex journey, and in the next class, we will discuss the various types of sensors that have been developed so far for detecting *Bacillus anthracis*. Thank you.