

Science AMA series: We're Thomas Bartlett and Benjamin Bratton from Princeton University. The bacterium that causes cholera is curved. People have known this for 160 years, but never known how or why it's curved. We figured it out... Ask us anything!

Gitai_{Lab}¹*andr/ScienceAMAs*¹

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Abstract

The first observations made about most bacteria include a description of their cell shape. Only recently have we started to figure out how all of these different shapes arise, and to understand their purpose. *Vibrio cholerae*, the bacterium that causes the deadly epidemic disease cholera, is curved. We've known this since its discovery 160+ years ago, but never figured out how or why. Aside from the basic research angle (how does something so small self-organize into a complicated shape?) this question has serious human health implications – the world is currently gripped by a global cholera pandemic, infecting millions and killing over 100,000 annually. We are Thomas Bartlett (graduate student/PhD candidate, bacterial cell biologist) and Benjamin Bratton (postdoc, biophysicist and quantitative biologist), and we discovered the gene (and protein) necessary for *V. cholerae* curvature, *CrvA* (for curvature regulator in *vibrio A*). We found that *CrvA* curves the cell by causing one side of the cell to grow faster than the other, and developed some new tools/took some cool pictures along the way. We also found that curvature helps *V. cholerae* to swim in gels, as well as to colonize and pathogenize the host gut. Our paper just came out on Thursday, January 12th, in the journal *Cell*. We will be back at 3 pm ET to answer your questions, Ask us anything! Here is a write-up of our research! - A great write-up without all of the technical detail; also not behind a paywall! Here is our paper! Find Benjamin Bratton | Twitter | Google Scholar Find Thomas Bartlett | Twitter | Google Scholar EDIT 1: Aaaaaaaaaaaaaand we're live! Thanks for all of the attention and great questions! We'll do our best to answer them all. EDIT 2: Okay, we are going to call it (for now, anyway)! Thanks for all of the great questions (and answers). We will do our best to get to the rest of the unanswered questions at a later date.

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[Here is a write-up of our research!](#) - A great write-up without all of the technical detail; also not behind a paywall!

[Here is our paper!](#)

Find Benjamin Bratton | [Twitter](#) | [Google Scholar](#)

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CORRESPONDENCE:

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You mentioned this appears to be activated by quorum sensing. Disrupting quorum sensing has been a therapeutic goal for a while now. How is that going?

[FUZZY_BUNNY](#)

I am not a quorum sensing (QS) expert but I will do my best to answer. Being across the hall from Bonnie Bassler's lab gives me a bit of context. :)

For those with no context, QS just means that bacteria produce small molecules that they then sense (called autoinducers). As bacterial numbers increase, especially in confined spaces, autoinducer concentration increases; bacteria integrate this information to do surprising things, like coordinating

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group behaviors (including virulence, in some bacteria), and preparing for transitions (such as the transition between surface-attached and free-swimming lifestyles). Of course, QS is not the only cue that bacteria use to know that their environment is changing. Interfering with bacterial environment sensing is a cool way to try to trick bacteria into adopting the wrong strategy, and QS-inhibition might play an important role there. (For the record, my lab thinks more about interfering with surface-sensing, which also activates virulence in a lot of pathogens like *Pseudomonas aeruginosa*).

To answer your question: people have discovered small molecules that act as both agonists and antagonists of canonical QS receptors. However, *V. cholerae* activates its virulence and attachment regime at low cell density, and actually turns *off* virulence at high-cell density. It seems like maybe *V. cholerae* has evolved to always be prepared to find a biological surface to attach to and infect, and then once it has established quorum, to escape to its next host. Consistent with this, cholera a) causes massive diarrhea, and b) switches from attached to free-swimming behaviors at high cell density. Our work suggest that changes in cell shape may play a role in all of this, possibly aiding in colonization steps, or in dispersal from the host.

Could this be used to trick cholera into doing the wrong thing at the wrong time? Perhaps. For better or for worse, cholera is a treatable disease. This is obviously a good thing, because countries with sanitary water don't have issues with infection. On the other hand, it has some downside – clever ideas like this that might limit outbreaks or infections are unlikely to be seriously pursued. It's just too expensive to bring a drug to market, narrow-spectrum treatments in particular. That goes double for tropical diseases that are “solved” in rich nations, and that generally infect people who can't afford fancy narrow-spectrum treatments.

Thanks for the great question! - TMB

Appreciate the hard work and the results, guys. 2 questions:

1) Do you think future medical applications to avoid outbreaks such as this should tackle the diseases themselves or the host bodies to optimize immunity? One worries that even with advanced molecular engineering techniques, chances for mutation or pleiotropic consequences of altering or excising a genetic sequence could strengthen bacteria such as this.

2) A big, general question: How do you think we can best go about improving scientific literacy in America?

[kingdomundersiege](#)

Thanks for two (really three) interesting questions. They are a little outside of my expertise, but I'll give it my best shot. If I screw anything up, apologies in advance... maybe Ben can get it in editing. ;)

1a) I think it depends on the disease. Given the choice between treatment and prevention, prevention is usually more optimal. I think the two biggest improvements to human health (at least as far as infectious disease is concerned) are sanitation and vaccination, which are both preventative measures. Antibiotics and similar tools are fantastic, but for various reasons bacteria are great at evolving resistance. Thus, perhaps pursuing host-based options (e.g., vaccines) is better than pursuing pathogen-based options (e.g., antibiotics). However, not a lot of work has been done to target particular aspects of infection biology besides killing pathogens outright. Narrow-spectrum treatment options that target things like virulence, attachment, dispersal, etc. may have some of the advantages of preventative medicine, but require a better understanding of the pathogens themselves.

1b) This seems to be a different question, so I'm going to give you a whole new answer! I'm not 100% sure what you're asking, but I wholeheartedly support laboratory work on pathogens. I understand the “dual-use” concerns (we might accidentally engineer a worse bug), but I think the benefits far outweigh

the cost. If you're asking about genetically modifying environmental populations of pathogens, I think that's a riskier proposition. There is an analogous idea used in sexually reproducing populations, the "gene drive." It has a lot of potential merit for reducing parasite burdens, but genetically modifying breeding populations does obviously carry some risk. I wouldn't rule out the idea, but I think it's definitely risky and should be done only with caution.

2) I think about this a lot. I have thought about going into science writing and science communication, and while I love research, I suppose it's still a possibility. I don't have an expert answer here. I think that it's obvious that we're failing, and that most of the best communication ends up preaching to the choir. I've read that emotional appeals are more effective than rational appeals when arguing with people who disagree with you. Maybe that's a source of our difficulty, as a community? I would imagine that many scientists feel uncomfortable making emotionally-based arguments, and would rather just discuss hypotheses/observations. Not sure if this is a great answer, and even if it is, not sure how to change it. I suppose it's obvious, but championing reason and empiricism, and supporting science education.

Hope I at least somewhat answered your questions! - TMB

Great work, guys! I had a few questions for you:

1. Does inhibiting or mutagenizing CrvA render the bacterium avirulent or more susceptible to antibiotics?
2. Are there attempts to identify CrvA inhibitors that could be used to treat environments like *V. cholerae* contaminated water?
3. Are there other medical applications for CrvA (ie. could you clone it into other bacteria and use it to treat other diseases)?

Thanks!

[footiebuns](#)

Thanks! the project was a blast.

1. Deleting CrvA lessens virulence in host models, and also slows them down when swimming through gels. Interestingly, it doesn't seem to slow them down when swimming in liquids. We think that maybe the curvature helps them to "corkscrew" or "drill" their way through gels, but haven't directly tested that. We also haven't looked at how CrvA impacts antibiotic resistance, but it's a good question.
2. Not yet, but we have thought about screening a library of small molecules for potential CrvA inhibitors. This has worked historically with other bacterial cytoskeletons, like MreB (which is an essential protein under most realistic conditions). Screens identified the small molecule A22, which prevents MreB polymerization. A22 has been a great research tool, but doesn't seem to be likely drug, as it is effective only at extremely high concentrations.
3. We haven't really tried that yet! CrvA may be able to curve other bacteria. If so, this would be exciting from a basic research and bio-engineering angle. Not sure if it would have any obvious medical applications, unless curvature turns out to be a universal gel motility factor, and you really need to engineer a bacterium to swim through gels.

Thanks again! - TMB

Hello, so is there a TL;DR of why these cells form the shapes they do? How will this help other researchers develop ways of preventing Cholera? Do the cells have any internal clock or do they become more and less active in a 24 hour period?

[afirewallguru](#)

"Why" questions are dangerous. We can test things in the lab, and show that they confer advantages, but it's hard to really know *why* something evolved a certain way. We did our best to try to understand the advantages associated with curvature, and here's what we saw:

1. Curved and straight cells are both perfectly healthy. They grow really quickly, doubling in biomass in under 20 minutes (faster than *E. coli*)!
2. Curved and straight cells both swim very well through liquid. They are very fast, swimming about 30 body lengths per second!
3. Curved cells are better at swimming through gels, especially really dense gels. Gels are actually crosslinked polymers filled with fluid (kind of like a wet sponge or a balled up net in the ocean). Curved cells have a real advantage when the density of polymer is high, and the pore size of the gel is smaller. We don't really know why, but it may be as simple as the curvature helps them corkscrew through the gel.
4. Curvature helps cells colonize the gut (mouse models) and also to pathogenize the gut (rabbit models). We hypothesize that this is because curvature helps cells to "burrow" into the gel that lines the gut. It's been demonstrated before that the mucus gel lining the gut is a barrier to bacterial entry and close association with gut epithelium. Also, bacteria like *Helicobacter* and *Campylobacter* are corkscrew shaped, and straight mutants of those bacteria have a similar motility and colonization defect. So it seems like curvature helping gel motility and thus gut colonization may be a common theme!

Thanks for the question! Also, thanks to [u/Nietzschemouse](#) for a great answer! - TMB

This QuASAR assay (the pulse chase) seems pretty powerful... it seems like you can use it to help answer just about any question about how bacteria grow.

So what major questions do you think the assay can be applied to? And what are its limitations?

[subito_lucres](#)

Thanks, we think it is pretty powerful too. The use of fluorescent D-amino acids (FDAAs) has exploded over the past few years but they have not yet been used extensively for quantitative experiments. Not all bacterial species incorporate all of the probes in the same way, so the technology is not entirely plug-n-play. In addition to species-specific labeling issues, quantitative fluorescence microscopy is tricky. To do it properly requires an understanding of all of the components of the data collection pipeline from sample preparation, image acquisition and data processing. Finally, developing the appropriate biophysical model to compare/fit your data with is usually a non-trivial process.

Thanks! -BPB

Are there homologs for CrvA in other bacterial taxa (especially non-pathogenic bacteria), and if so how different are they functionally?

[IYKWIM_AITYD](#)

We haven't identified CrvA homologs in distantly related bacteria. However, it's pretty well conserved in the *Vibrio* genus. All of the curved *Vibrios* we've looked at have a CrvA homolog, and none of the straight ones (of which there are a few) have a CrvA homolog. thus, we feel pretty comfortable concluding that CrvA is necessary for curvature in the *Vibrio* genus. That's exciting, because *Vibrios* are huge marine/aquatic pests and human pathogens.

Thanks for the great question! - TMB

All eukaryotic cells share a common cytoskeletal architecture (actin, tubulin, etc.) Are there similar highly conserved structural features in bacteria? Is there a lot of diversity?

[FUZZY_BUNNY](#)

Great questions! [u/adr007](#) is correct; the answer to both your questions is "yes!"

People used to think of bacteria as bags of enzymes. I even learned in undergrad that bacteria have "no membrane-bound organelles" and there was never any talk of bacterial cytoskeletons.

Bacteria have homologs to the canonical eukaryotic cytoskeletons - MreB is an actin homolog, FtsZ is a tubulin homolog, and Crescentin (and now CrvA!) is an intermediate filament homolog. There is actually a decent [Wikipedia page about the prokaryotic cytoskeleton](#), worth a read if you are new to the field. There is also tons of [reviews!](#)

Hi, I'm a microbiology major and hearing stuff like this is exciting. Glad to see studies on microorganisms like this still show up in today's news. Keep up the good work.

I'm not much informed on curved bacteria but are there any other that are curved and not cocci or bacilli? Is it a sickle cell curvature or sever curvature like in helical bacteria?

[MisterChao](#)

Thanks for your kind words! I'm a bacteriologist through-and-through, and I am also very pleased by the reception.

There are many curved bacteria.

- Most of the *Vibrio* genus is curved.
- *Helicobacter* is curved.
- *Campylobacter* is curved.
- *Caulobacter crescentus* is curved.
- *Bdellovibrio* is curved.

(There may be exceptions in the above groups, but I'm only an expert on *Vibrio*)

To our knowledge (I just double-checked with Ben), *all* curved bacteria are actually helical (although there are mutants that are exceptional). Again, this is just to our knowledge. Also, the vast majority of these helices are right-handed, and we don't really know why. It may suggest a near-universality of mechanism, or it could reflect something more mundane, like the chirality of peptidoglycan.

As far as other bacterial shapes? [Here is the seminal review](#) (IMHO) of bacterial cell shape. It's a great read. There are all kinds of bacterial shapes, and many variations on helical/vibrioid curvature.

Thanks! - TMB

First of all, I noticed you had uploaded your MATLAB scripts for QuASAR to GitHub, so I would like to thank you for contributing towards reproducibility!

Couple of questions:

1. What other bacteria do you think also exploit curvature to increase pathogenesis? Do you have plans to explore these bacteria?
2. Do you have a physical explanation for why curvature improves motility? It is not obvious to me why curved cells move faster than straight cells do.

[edwinks!](#)

You're welcome! We use GitHub internally for version and bug tracking as well.

1) There are a variety of other pathogens that have curved shapes relevant to pathogenesis, including the genres *Campylobacter* (meaning curved rod) and *Helicobacter* (meaning helical rod). Where they have been tested, the straight-rod mutants of these species are impaired in their ability to colonize hosts. We don't have any Campy or Helicobacter in the lab, they are microaerobes and harder to work with than hardy Cholerae.

2) Our current thought is that curvature enhances motility in matrices. *V. cholerae* is a flagellated bacteria uses the rotation of its helical flagella to propel itself forward. The body then counter-rotates, and in the case of curved rods, their curvature allows them to "corkscrew" through the meshwork and push off the matrix. In aqueous media, we don't see a difference in swimming speeds between straight and curved cells. One of the first observations and descriptions of this was in 1979 by Berg and Turner <http://www.nature.com/nature/journal/v278/n5702/abs/278349a0.html>

Thanks! - BPB

How did you decide to study this?

[yankcanuck](#)

Great question!

when I came to grad school, I wasn't really sure what I was going to study. I did a rotation Bonnie Bassler's lab, working on cholera biofilm stuff with Carey Nadell.

My next rotation was with Zemer Gitai (my current mentor), and his lab was interested in *Caulobacter crescentus*. *Caulobacter* is also a curved rod, like cholera. However, cholera curvature was completely mysterious. So, Zemer and I, along with my rotation mentor Alex Persat (who is also an author on the paper) just thought it would be fun to try to figure it out.

Taking on a project from scratch is hard and it's risky. But man, is it fun! - TMB

ps - Ben is the undisputed master of 3D imaging and microscopy in our lab. I begged Ben to help me with the project when I got in over my head with the image analysis. He took mercy, and it really elevated the work. He may have a different answer, but that's my recollection!

Since Cholera cells are curved, does that change the inside of the cell, or is it still the same?

[Jakobihaskell](#)

In most bacteria, the rigid cell wall is what defines the shape of the cell. Within our resolution, we did

not find any difference in the chemical composition of the cell wall. We have not yet measured all of the cellular contents using tools like proteomics, metabolomics, lipidomics, etc. but our assumption is that the cellular contents are almost the same in straight and curved cells.

Thanks! -BPB

Awesome work guys. Simple question, have you been able to clone the curvature gene into other bacteria and if so do they display similar characteristics?

[LabLinebacker](#)

We haven't really tried that yet! CrvA may be able to curve other bacteria. If so, this would be exciting from a basic research and bio-engineering angle. It could be tricky because it would need to (1) be transported (2) assemble and (3) interact with the appropriate cell wall synthesis and remodeling enzymes. Since we don't know its interaction partners, it is hard to know which species we should try to express it in.

Thanks -BPB

How does the protein know on which "side" of the bacteria to congregate? A free floating protein would diffuse and express equally on all sides. This protein is expressed near the midpoint between the poles, which seems like a sophisticated reaction. It seems like there just be some mechanism controlling it. Its not mentioned in the summary article, but maybe you can explain?

[phylaxer](#)

CrvA has a signal sequence which the cell uses as a cue to export it to the periplasm (the space between the inner and outer membranes in a Gram negative bacterium). Because purified CrvA assembles on its own *in vitro*, our model is that it assembles into a polymer once it is in the periplasm. This stable polymer is then able to promote one face of the cell as the *inner* face or join the already formed polymer at that face. We were able to find a media condition where CrvA did not assemble as polymers. Cells grown in this media were straight and, following a media swap to LB, developed polymers before they became curved.

Thanks! -BPB

I assume you must have used some interesting selection strategies for CRV mutants. How did you do it?

[OldGuyzRewl](#)

I'm not sure I would call it a **selection**, but we imaged roughly 10,000 transposon mutants and looked for something that was straight. One trick we used was to include cefalexin while growing the cells. This is a drug that inhibits cell division and helps accentuate morphology defects.

Thanks! -BPB

How will this have a significant effect on the way we combat cholera?

[PapaTimbs](#)

I am not sure if it will! See the answer to a similar question:

If people are interested in inhibiting CrvA in particular, a small molecule screen for a CrvA inhibitor could be useful. I don't think it's likely that this will stop cholera infections altogether. Sadly, that probably won't happen until modern sanitation becomes more common in areas with endemic cholera.

We think the research is exciting from multiple angles. Human health is one of them, but so is the basic bacterial cell biology. - TMB

I'm an environmental engineer dealing in the design of water, wastewater, and water reuse systems. Is cholera a disease we as a species can eradicate (either now with more resource allocation or in the future), or only monitor and manage the risks?

[GreenWithENVE](#)

Cholera will be difficult to eradicate because it is perfectly happy as an "environmental" microbe. It lives in estuaries and consumes chitin, which is very abundant. Even less dangerous strains can be transformed into epidemic forms by lysogenic infection with a Ctx phage, which confers a few new attributes upon the cell (including deadly cholera toxin and a new type-IV pilus that mediates gel attachment, TCP). This seems to have happened several times historically, leading to different epidemic strains (e.g., Classical biotype vs. El Tor biotype). - TMB

What evolutionary advantage does curvature confer? Why are the vibrios so pathogenic?

[TheWangernumbCode](#)

Evolutionary advantage is tough to answer, but in the lab it seems to help with gel motility and host colonization/pathogenesis.

I am not sure why the *Vibrios* are so pathogenic. Maybe because so many of them are curved?

In all seriousness, the clade seems to have a lot of adaptations that help it to be a finely tuned pathogen. They generally swim fast, they grow very fast (*V. natriegens* is the fastest growing bacterium I know of, doubling every ten minutes or so). Many eat chitin, which is a ubiquitous marine biotic surface. They are very good at their job! - TMB

CrvA has an annotated neighbour, VCA1076, that appears to be in the same operon and is only 41 amino acids long! Is this a correct annotation? Have you considered what the role, if any, VCA1076 has in *Vibrio* curvature?

[biograf_](#)

We don't think it is a correct annotation, but we aren't really sure. The transcriptome of *V. cholerae* is published, I don't remember seeing any transcript of it.