

A NEW PATH FOR FENTANYL TREATMENT

Fentanyl, a synthetic opioid 100 times stronger than morphine, offers effective pain management for cancer patients and other people managing debilitating, chronic conditions. However, the drug poses significant risk to those who abuse it by slowing, and sometimes stopping, breathing. Naloxone, a treatment known by the brand name Narcan, knocks opioids off receptors in the brain to quickly restore breathing in someone who has overdosed on fentanyl. The decline in fentanyl-related deaths in the United States—from 76,282 in 2023 to 48,222 in 2024, according to the Centers for Disease Control and Prevention—may be due, in part, to naloxone’s availability to first responders.

Nonetheless, risks from fentanyl consumption or exposure remain. Opioids can last longer in the body than naloxone, which has a half-life as short as 30 minutes. As a result, a patient treated with naloxone can potentially experience a second overdose from the circulating opioids that remain. Furthermore, more potent forms of fentanyl, such as carfentanil (100 times stronger than fentanyl) and remifentanyl, a faster-acting version, can be weaponized for chemical warfare. In 2002, for example, the Russian army released an aerosolized mixture of carfentanil and remifentanyl into a ventilation system to immobilize Chechen terrorists holding hostages in Moscow’s Dubrovka Opera House. The gas cocktail affected both the captors and their hostages, leaving more than 100 people dead and another 650 requiring hospitalization.

The cyclodextrin molecule subetadex-methyl (SBX-Me) can bind the opioid fentanyl (shown in light blue) in two different orientations, as shown above. SBX-Me remains bound to the opioid until it is excreted from the body. (Image by Edmond Lau.)

Looking beyond post-overdose treatment with naloxone, biotechnologies designed to generate an immune system response have proven too costly and ineffective. A team of Lawrence Livermore scientists imagined a better solution: a molecule that binds to fentanyl before it reaches receptors and then remains bound until the fentanyl is flushed out of the body. In research conducted over the course of 10 years, the team has developed a customized molecule, called subetadex-methyl (SBX-Me), to trap fentanyl and protect from the opioid’s effects for as long as the drug remains in someone’s system. SBX-Me could be used as both a treatment for fentanyl overdoses and a preventive measure, taken in advance of possible exposure to deadly bioagents.

Custom Countermeasure

From 2014 to 2016, Lawrence Livermore chemists Carlos Valdez and Brian Mayer and computational chemist Edmond Lau led a multidisciplinary team investigating potential medical countermeasures (MCMs) to synthetic opioids as part of a project funded by the Laboratory Directed Research and Development

(LDRD) Program. The researchers set out to design and synthesize a molecule tailored to meet three characteristics: high specificity for fentanyl and its analogs (compounds similar in structure), low toxicity, and a longer half-life in the body than fentanyl. The project supported Lawrence Livermore’s bioresilience mission focus area to rapidly identify, characterize, and counter natural and manmade chemical and biological threats.

Valdez, calling on his background in carbohydrate chemistry, turned to cyclodextrins as the main platform for the LDRD project’s focus. Cyclodextrins are molecules composed of glucose units joined in a circular fashion via glycosidic linkages. The result is a 3D structure resembling a truncated cone with a flexible interior cavity. The interior cavity enables cyclodextrins to act as a host to many “guest” organic molecules—synthetic opioids among them. Cyclodextrins can be produced in different sizes—specifically, different numbers of glucose units—to create the best match to trap a guest organic molecule. The hydrophilic exterior of cyclodextrins makes the guest soluble in water and, therefore, eliminated from the body rapidly. In addition to the size of the cyclodextrin, organic extensions can be attached to the end of the cone, modifying its overall propensity to form long-lasting inclusion complexes with the guest molecule.

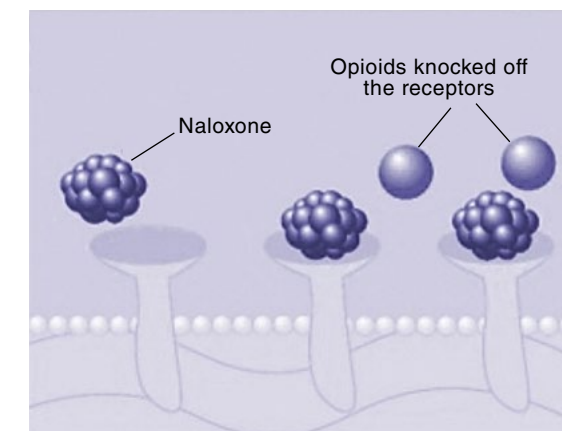
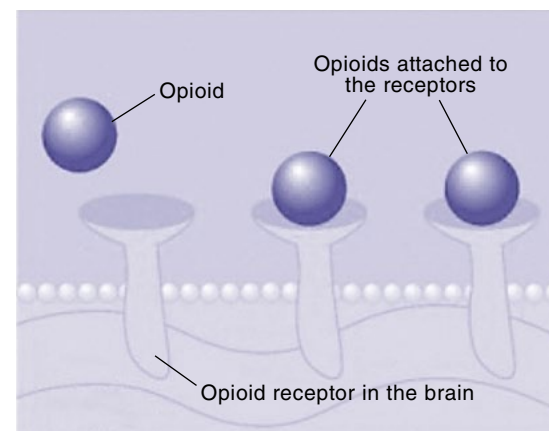
Over the course of the LDRD project, the research team established the great potential of cyclodextrins as fentanyl MCMs. Subetadex (SBX) was identified as a lead treatment candidate, but its evaluation did not extend beyond the project’s funding period. Then, in 2019, the United States Defense Threat Reduction Agency (DTRA) approached Valdez with an interest in both SBX, identified three years earlier, and further evaluation of potential fentanyl MCMs to address the increasing number of fentanyl overdoses in the nation. DTRA had forged a relationship in the past with Valdez’s group, based in the Laboratory’s Forensic Science Center, to identify potential nerve agent countermeasures. DTRA funded new work to revisit cyclodextrins as a treatment against fentanyl poisoning, and the

team picked up where the LDRD project left off, with the lead candidate being SBX.

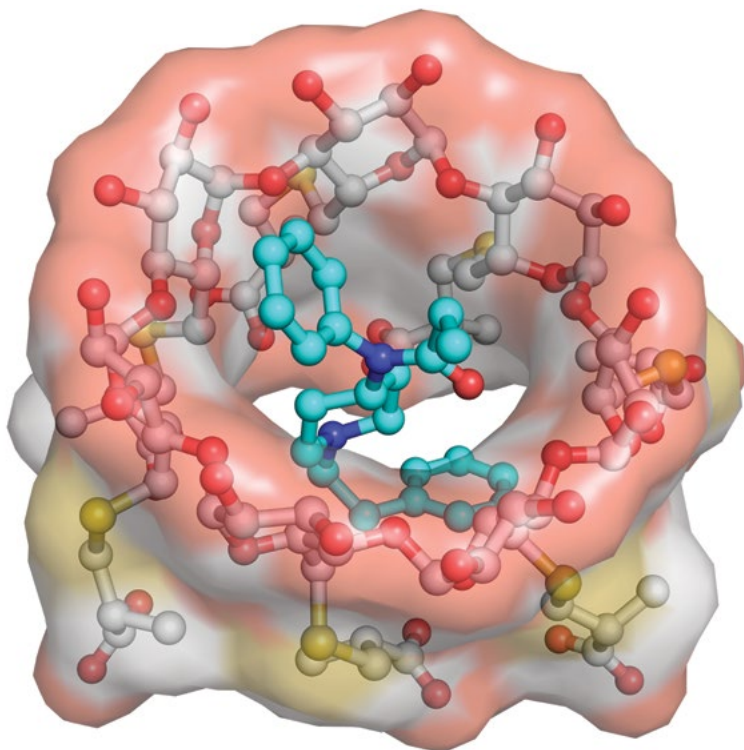
Reviving the Research

The team reviewed the findings from the 2014–2016 LDRD project. An early inspiration had been Sugammadex (SGX), the only cyclodextrin candidate to date with Food and Drug Administration (FDA) approval for clinical use. Developed in 2002 and marketed by Merck as Bridion, SGX is a chemically modified cyclodextrin that binds rocuronium, a powerful anesthetic used in hospitals during and after major surgeries. SGX removes rocuronium from circulation by effectively trapping it in its interior cavity and accelerating its clearance from the body of the patient. Unfortunately, SGX proved to be ineffective in terms of fentanyl as its structural cavity was too large to prevent the opioid from binding tightly and remaining in its interior. “We knew we were on the right track, so we decided to investigate other cyclodextrins with smaller cavities,” says Valdez. Given that SGX is an FDA-approved drug, the team specifically included SGX in their new round of studies as a gold standard for evaluating the toxicity of other cyclodextrin candidates.

SBX, a smaller version of SGX, was included in the revived project and used as a starting platform to launch additional discovery campaigns. Using nuclear magnetic resonance (NMR), the team confirmed that SBX formed a strong inclusion complex with fentanyl. Says Valdez, “When NMR revealed that SBX trapped fentanyl in its interior and did not let go of the opioid, we were very excited and started to work to discover cyclodextrins with equal or better binding affinity towards fentanyl.” The work on SBX was published in 2021, and DTRA agreed to fund the investigation into a customized molecule that would bind not only to fentanyl, but carfentanil and remifentanyl as well. “We had studied binding capabilities in the second year of the LDRD project and found that remifentanyl binds in a different way than carfentanil and fentanyl, which form complexes,” explains Lau.



Naloxone, known by the brand name Narcan, knocks opioids off receptors in the brain to quickly restore breathing in someone who has overdosed on fentanyl. However, someone treated with naloxone can experience a second overdose from opioids that remain in the body because naloxone has a shorter half-life than fentanyl.



Cyclodextrins, represented by the shaded area, form a 3D structure resembling a truncated cone with a flexible interior cavity that can host organic molecules. SBX-Me, above, comprised of oxygen atoms (red spheres), carbon atoms (light gray spheres), and sulfur atoms (yellow spheres), hosts a fentanyl molecule, shown in light blue. (Image by Edmond Lau.)

The search was on for a targeted treatment and preventative measure applicable to multiple forms of fentanyl, for use by first responders, law enforcement officials, and the military. A multidisciplinary team identified the ideal molecule based on promising results with SBX. Valdez designed and synthesized approximately 50 modified cyclodextrins to run through refining feedback loops for the evolving model. Lau computationally screened available chemicals to identify molecules for possible starting points and developed molecular dynamics simulations based on Valdez's designs and suggestions. "Carlos came up with new molecules faster than I could simulate them," says Lau. Responds Valdez, "And Ed greatly enhanced molecular dynamics, yielding candidates with promising high affinity toward fentanyl, carfentanil, and remifentanil."

Experimentalists, led by Derrick Kaseman, applied NMR to determine the binding mode and strength of the cyclodextrins and the opioids. Experiments were conducted at 37° C and pH 7.4 to mimic the environment where candidates would need to excel: the human body. "When we found a good cyclodextrin host for the opioids, we worked on its core to create analogs that offered better binding or reduced cell toxicity," says Valdez. Biologists, led by

Michael Malfatti and Heather Enright, ran toxicological screening and in vitro testing of the cyclodextrin candidates to either suggest further modifications, move to in vivo testing, or discard candidates altogether. Their results, in conjunction with the binding affinities determined by NMR, were continually implemented into the computational model created by Lau, improving the model's accuracy at predicting and constructing new cyclodextrin candidates with potentially better profiles than previous ones.

Crowning a Winner

In one variation created during this fine-tuning process, researchers added a methyl group to the extended carboxylic acid chain, which is anionic (negatively charged), lining up the upper rim of the SBX molecule. The chains, similar to spikes of a crown, remain separated due to their electrostatic interactions with each other, an interaction that also expanded the interior cavity of the original cyclodextrin. Combined, these characteristics amplified the molecule's binding capability and the capacity to accommodate and ultimately tightly bind the targeted opioids. Numerous NMR analyses proved that the resulting molecule, SBX-Me, trapped fentanyl more effectively than SBX.

"SBX-Me offered the binding ability for fentanyl we had sought. The final step toward cementing its place as a lead candidate was to obtain a favorable toxicological profile," says Valdez. The biologists' evaluation indicated toxicity levels low enough for SBX-Me to be a viable treatment. "The commercially available SGX was the gold standard to meet in terms of toxicity," says Malfatti. "The results for SBX-Me were close to those for SGX, which was a factor in choosing SBX-Me over other synthesized molecules, including SBX."

Follow-on in vivo testing further indicated SBX-Me's effectiveness. By tagging fentanyl, carfentanil, and remifentanil molecules and the SBX-Me molecule with carbon-14, Livermore biologists traced the movement of both the opioids and the treatment through animal tissues using mass spectrometry. SBX-Me cleared rapidly from all major organs and tissues with the SBX-Me excreted unchanged. Furthermore, SBX-Me featured a half-life of more than 7 hours in the hosts, longer than the half-life exhibited by naloxone. Recovery times were significantly

Treatment	Time to recovery without treatment	Time to recovery with SBX-Me treatment
Fentanyl	35 minutes	17 minutes
Carfentanil	172 minutes	59 minutes
Remifentanil	18 minutes	12 minutes

In vivo testing indicated significant reductions in recovery time for subjects treated with SBX-Me after exposure to fentanyl and its analogs, carfentanil and remifentanil.



Biologists David Baliu-Rodriguez (left) and Michael Malfatti review mass spectrometer results that quantify carbon-14-labeled SBX-Me in tissue. (Photo by Blaise Douros.)

improved compared to untreated cases. For fentanyl specifically, recovery time improved from 35 to 17 minutes. Carfentanil recovery was cut by two-thirds, from nearly 3 hours to less than 1 hour with SBX-Me. Recovery time from fast-acting remifentanil was reduced from 18 minutes when untreated to 12 minutes with the SBX-Me treatment.

Furthermore, SBX-Me doesn't bind naloxone, an important finding as the two can be used simultaneously to potentially reduce recovery time and require only one treatment, given SBX-Me's longer half-life. For warfighters, a treatment such as SBX-Me can be administered in advance, to provide systemic protection against fentanyl and related analogs when used as weapons. Similarly, emergency response personnel can benefit from administration of SBX-Me before responding to a situation in which accidental exposure to the opioids is likely.

The Livermore team's findings have been handed back to DTRA. In the meantime, the Laboratory's Innovation and Partnerships Office has filed a patent application for the SBX-Me molecule. More steps lie ahead before a commercial drug form would be available, starting with an Investigational New Drug application and subsequent FDA approval. However, Valdez anticipates that SBX-Me manufacturing could be scaled up rapidly given the commercial and ready availability of the starting materials available for its synthesis.

Future research may include development of MCM candidates for other illegal drugs dominating the public environment, such as ketamine. No doubt, team science—combining scientific expertise to solve challenging problems—will once again serve as the fastest route to discovery. The Laboratory stands ready with experts in chemical synthesis, pharmacokinetics (studying the journey and interactions of a drug as it moves through the body), computational modeling, chemical characterization, NMR, and biological testing to tackle new bioresilience projects. Among the SBX-Me team members, no one is surprised that research started 10 years ago opened a path to their success. As often happens in research, funding for one project ends and funding for other projects comes through. "We wrap up the results in a manuscript and move on," says Valdez. "By going back to the original project, however, we solved today's problem."

—Suzanne Storar

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