Researchers eliminate drug discovery bottleneck

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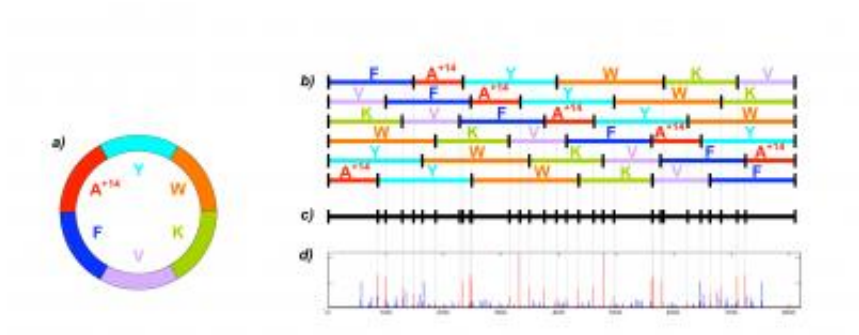

Determining the structure of unknown natural compounds is a slow and expensive part of drug screening and development -- but this may now change thanks to a new combination of experimental and computational protocols developed at the University of California, San Diego and presented at RECOMB 2008 (Research in Computational Molecular Biology) on March 31 in Singapore. Credit: UC San Diego

Determining the structure of unknown natural compounds is a slow and expensive part of drug screening and development – but this may now change thanks to a new combination of experimental and computational protocols developed at the University of California, San Diego and presented at RECOMB 2008 (Research in Computational Molecular Biology) on March 31 in Singapore.

UC San Diego researchers have devised a way to cut the time it takes to determine the structure of peptides derived from natural compounds

from six months or a year to as little as one day. This advance may assist drug discovery researchers – who need to know as much as possible as quickly as possible about the natural products with antibiotic, antiviral and other pharmacologically interesting properties that they are probing.

According to the researchers, it is currently difficult, time consuming and costly to determine the molecular structure of a class of natural compounds called nonribosomal peptides (NRPs) that are intensely studied for their drug potential.

To address this issue, UCSD researchers developed a quick, automated and inexpensive way to determine the structure of NRPs through an innovative collaboration between mass spectrometry experts at the UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences and bioinformatics experts and computer scientists from UCSD's Jacobs School of Engineering.

If you imagine the structure of an NRP as a cyclic string of beads, then the new algorithms both decipher the mass of each bead based on the mass spectrometry and determine the order of the beads within the ring – crucial pieces of information for uncovering both the structure of the molecule and its pharmacological activities.

In addition to screening for new drugs and studying natural compounds, the authors say this work may aid biosynthetic engineering efforts to reprogram E. coli strains in order to turn them into NRP assembly lines, now that researchers have a rapid method for characterizing the resulting NRPs.

NRPs such as penicillin, and other natural products, have an unparalleled track record in pharmacology: nine out of the top 20 best-selling drugs were either inspired by or derived from natural products, the authors say.

Nonribosomal peptides evolved over millions of years and often serve chemical defense and communication purposes for the organisms that manufacture them, explained first author Nuno Bandiera, a UCSD postdoctoral researcher and successful Ph.D. candidate from the computer science department at UCSD's Jacobs School of Engineering.

It is notoriously difficult to determine the structure of NRPs because the usual peptide sequencing tools do not work. The cyclic structures of NRPs, the prevalence of non-standard amino acids that thwart database lookups, and the lack of structural information directly inscribed in the genomic DNA due to the nonribosomal nature of the peptides are all major contributors to the roadblock. Researchers have had to rely on slow, manual, expensive and not always reliable approaches to deciphering the structure of NRPs.

"This work removes a particularly troublesome bottleneck in the drug discovery pipeline for this class of therapeutics," said Pieter Dorrestein, assistant professor in the Skaggs School of Pharmacy and Pharmaceutical Sciences and the Departments of Pharmacology, Chemistry and Biochemistry. "We have shown a way to quickly, structurally characterize nonribosomal peptides. Our next step is to replicate our findings with newly discovered, potentially therapeutic peptides."

The UCSD researchers have shown that it is possible to break NRP rings apart and then break the resulting peptide strings into smaller and smaller subunits of the original ring using multiple passes with a mass spectrometer. This approach – called multistage mass spectrometry – allowed the UCSD Skaggs School researchers to collect data on the weights of ring fragments as these fragments got progressively shorter and more numerous with each pass of the mass spectrometer.

The UCSD Jacobs School computer scientists designed algorithms that

literally pick up the pieces from here. The algorithms glue the overlapping pieces together until they have reassembled a series of possible original ring structures, explains Julio Ng, a graduate student in UCSD's Interdisciplinary Bioinformatics Ph.D. program and RECOMB 2008 paper co-author.

The algorithms make use of data on the weights of the various NRP ring fragments collected at each stage using mass spectrometry. This work is an extension of an award-winning automated approach Bandeira and colleagues used to reconstruct snake venom peptides.

"Our Recomb 2008 paper represents the first demonstration of de novo sequencing of nonribosomal peptides. Without knowing the structure of the original compound, we can determine it," explains computer science professor Pavel Pevzner, the last author on the RECOMB 2008 paper and the director of UCSD's Center for Algorithmic and Systems Biology which is part of the UCSD Division of the California Institute for Telecommunications and Information Technology (Calit2).

In their RECOMB 2008 paper, the researchers document how they used de novo sequencing to determine the structure of two different nonribosomal peptides. In order to be able to verify their results, the researchers chose peptides that had been independently sequenced using a slow, labor intensive, costly and somewhat inconsistent nuclear magnetic resonance (NMR) approach. NMR provides information on the position of specific atoms within a molecule by using the magnetic properties of nuclei. The team is now working on more than ten additional compounds and has filed a provisional patent for the technique.

This project arose after Roger Linington from UC Santa Cruz, a co-author on the RECOMB 2008 paper, approached Dorrestein with the hope that Dorrestein's group would be able to use mass spectrometry to

obtain the molecular structure of a natural compound that is very effective against malaria. When Dorrestein found that the data being collected from a strictly mass spectrometer approach was getting extremely complicated – in large part due to the cyclic structure of the compound, he contacted Pevzner. What followed was a fruitful back and forth between the mass spectrometry team and the computer scientists that eventually led to this novel and creative solution.

Source: University of California - San Diego