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Informatics Update

Screen Reveals Compounds Against ALS

Using a cluster of old computers researchers identified a set of virtual compounds that they believe could lead to promising therapies for inherited forms of the muscle-crippling disease amyotrophic lateral sclerosis (ALS). When synthesized and tested, the compounds prevented the mutant protein associated with familial forms of ALS, called SOD1, from clumping.

The mutant SOD1, a two-part, or dimeric, structure is unstable, and its tendency to fall apart is believed to contribute to the aggregation of ALS. In a recent paper published in the Proceedings of the National Academy of Sciences (PNAS), Soumya Ray, an instructor in neurology at Brigham and Women's Hospital, Harvard Medical School, Boston, used a computer platform that he developed, an 18-node "Beowulf" Linux cluster (each node having a 2.0 GHz Pentium processor) to identify compounds that would target a cavity between the two halves of the dimer, which they believed might be the place of SOD1's instability.

The *in silico* screen searched for compounds from a public database that would bind to the SOD1 dimer interface and stabilize its structure. A trial version of the FirstDiscovery suite, from Schrödinger,

Portland, Ore., was used for database preparation and high-throughput docking. The suite includes a program called Glide, which was the primary tool for performing the docking calculations, including the docking of ligands and scoring of hits. Ray and his colleagues then purchased 100 of the most promising compounds and mixed them with mutant SOD1 to see if they would inhibit aggregation, which 15 of them did.

Peter Lansbury, PhD, professor of neurology, Harvard Medical School, was first skeptical of the effectiveness of the *in silico* screening approach. "I am obviously a big believer now," he says, "but you still have to have very good secondary screens because not all of the hits have activity."

The unstable nature of the mutant SOD1 structure was investigated by Lansbury and others who believed that the propensity to aggregate may be what led to disease. Working with Lansbury on the theory, Ray then designed a mutant SOD1 in which the two halves were held together by a disulfide bond, but this molecule did not aggregate *in vitro*. A comparison of his version and the crystal structure of the most common SOD1 variant, called A4V, solved by a group of British researchers, revealed the cavity that became their target.

The *in silico* screen worked by first hitting the cavity of a computer model of the A4V mutant with 2 million virtual molecules, drawn from a public database. Not only did the resulting 15 compounds slow or inhibit aggregation, they also made it more difficult for the A4V mutant to unfold in the presence of a denaturing agent.

The compounds were further validated when they did not have that effect on Ray's early cavity-occluded SOD1 version. This confirmed that the compounds were binding specifically at the site they chose. They also found that the molecules appear to bind to other SOD1 mutants.

Lansbury and his colleagues are preparing to conduct animal trials of the molecules. He says that they hope to point the way to a viable strategy for identifying hits for diseases where the etiology is unclear. "If we can validate this strategy in an animal, we will have many companies interested in developing this as an ALS therapeutic."

They also have other plans for using Beowulf. "We are constructing a modern, state-of-the-art facility for *in silico* screening at our main center's neurological diseases lab," says Lansbury. "We believe that this approach has great potential and are looking for compounds that bind to several Parkinson's disease gene products."

