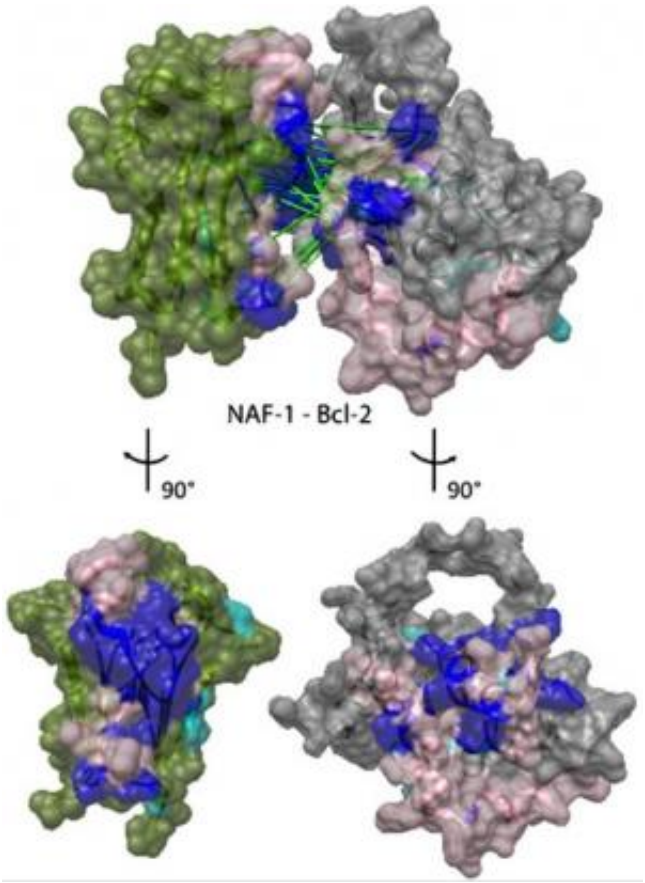
Cancer researchers find key protein link

March 27 2014



A combination of experimental and theoretical studies, in particular direct coupling analysis (DCA), revealed detailed interactions between NAF-1 (left) and Bcl-2 (right), two proteins involved in cellular life-and-death decisions. The green lines represent links between the proteins predicted by DCA. Credit: Rice/UCSD

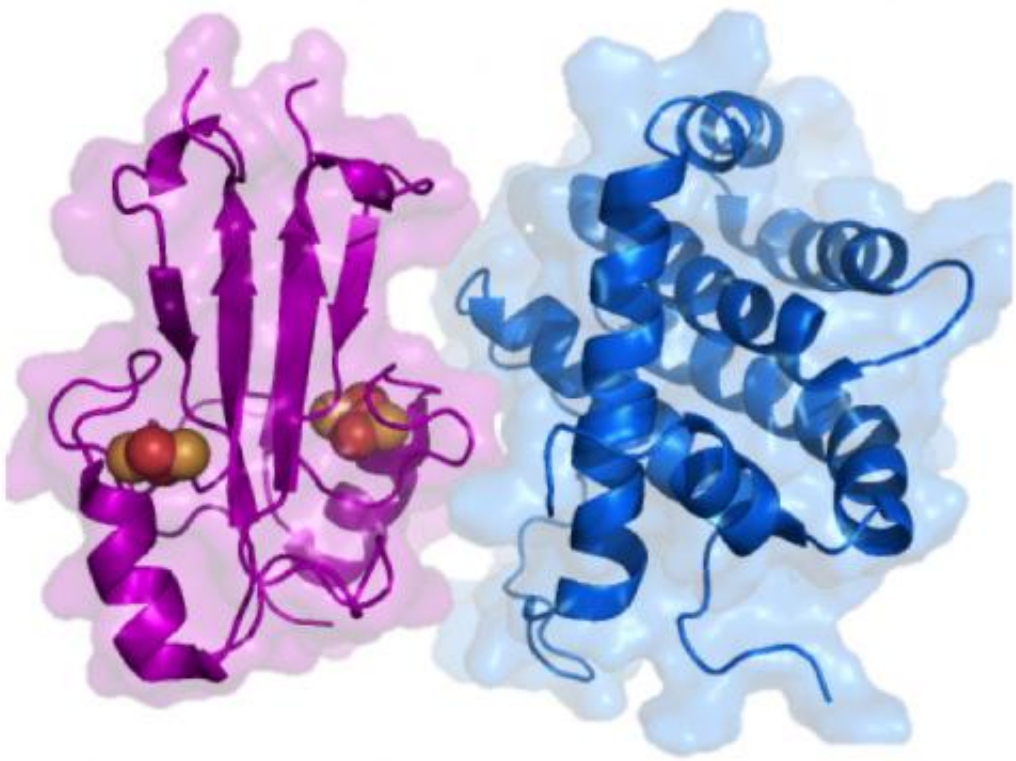
A new understanding of proteins at the nexus of a cell's decision to

survive or die has implications for researchers who study cancer and age-related diseases, according to biophysicists at the Rice University-based Center for Theoretical Biological Physics (CTBP).

Experiments and computer analysis of two key proteins revealed a previously unknown binding interface that could be addressed by medication. Results of the research appear this week in an open-source paper in the *Proceedings of the National Academy of Sciences*.

The proteins are Bcl-2, well-known for its role in programmed cell death , and NAF-1, a member of the NEET family that binds toxic clusters of iron and sulfur. How the two interact is now known as a major determinant in the cell processes of autophagy and apoptosis—literally, life and death. An ability to uncover binding sites on the proteins that send the cell one way or the other opens a path toward the regulation of those processes, according to José Onuchic, Rice's the Harry C. and Olga K. Wiess Chair of Physics and professor of physics and astronomy.

Pockets and folds in proteins exist to bind to other molecules and catalyze actions in a cell in signaling pathways. The ability to block a specific binding site or to enhance a desired interaction is critical to drug design, Onuchic said.



A combination of experimental and theoretical studies, in particular direct coupling analysis, revealed detailed interactions between NAF-1 (magenta) and Bcl-2 (blue), two proteins involved in cellular life-and-death decisions. Credit: Mark Paddock, Patricia Jennings/UCSD

"In our early work we have shown the link between NEET proteins and cancer. Now we can understand the molecular details of how these interactions are governed," Onuchic said. "Others have shown that NAF-1 is up-regulated in cancer cells, which leads us to believe that cancer may hijack control over the expression of this protein. This affects the cell's system of checks and balances. Understanding NAF-1 gives us a better idea of how to approach treatment."

The researchers found that NAF-1 binds to two specific regions of the protein Bcl-2 and that Bcl-2 binds to the NAF-1 groove formed between the beta cap and iron-sulfur cluster binding domains; the strongest

coupling is at the cluster binding domain and some contacts of interest are at the top of the beta-cap domain. Since the iron-sulfur cluster is the functional entity involved in NAF-1 activity, these findings clearly indicate that Bcl-2 interaction with NAF-1 affects its activity, Onuchic said.

The research team used a unique combination of experimental and theoretical methods, including peptide array binding studies with fragments of Bcl-2 to NAF-1; the researchers performed functional studies of cluster transfer and other full-length protein interactions with a spectrometer sensitive to hydrogen/deuterium exchange. They combined their results with a computer-based process created at CTBP called direct coupling analysis (DCA), through which interactions between proteins can be predicted by their genomic roots.

"Each of the three techniques not only confirmed the results of the other methods but also provided unique insights in their own right," said Patricia Jennings, a lead author and CTBP affiliate based at the University of California, San Diego (UCSD), where she is a professor of chemistry and biochemistry.

Jennings said the combined techniques are applicable to biomolecular interactions in general. "DCA helps us efficiently filter through massive amounts of data and does not require high-resolution structural studies, although those are desirable," she said. "Peptide array is powerful for localizing fragments that bind with high affinity, and hydrogen/deuterium exchange studies allow us to monitor parts of the intact protein that are not seen in structural studies and are not amenable to DCA analysis.

"Together, the techniques provide an exquisite synergy," she said.

Jennings is a co-lead author of the study with Onuchic; Rachel

Nechushtai, a professor at the Alexander Siberman Life Science Institute of the Hebrew University of Jerusalem; Assaf Friedler, a professor at the Hebrew University Institute of Chemistry; and Ron Mittler, a professor of biological sciences at the University of North Texas, Denton (UNT). Previous research by the team identified NAF-1 as one of two prime suspects in the proliferation of breast cancer.

"Once again, our international team of experts from different disciplines has shown that combined complementary efforts leads to innovative knowledge imperative for coping with cancer," Nechushtai said.

More information: www.pnas.org/content/early/201 …/1403770111.abstract

Provided by Rice University