NIST quantifies low levels of 'heart attack risk' protein

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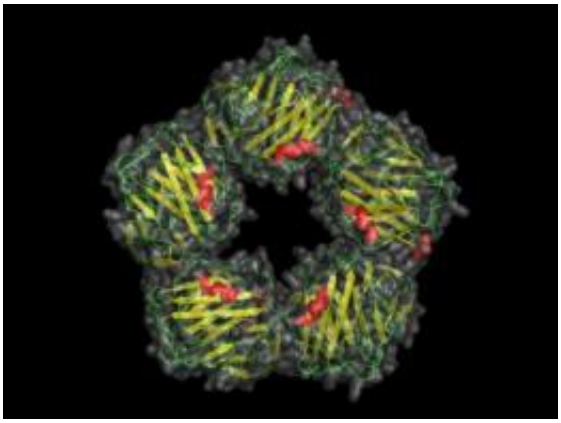

This is a computer-generated image of the structure for C-reactive protein. Credit: S. Kolstoe, Center for Amyloidosis, University College, London, UK

Searching for a needle in a haystack may seem futile, but it's worth it if the needle is a hard-to-detect protein that may identify a person at high risk of a heart attack circulating within a haystack of human serum (liquid component of blood).

C-reactive protein (CRP), a molecule produced by the liver in response to inflammation, normally accounts for less than 1/60,000 of a person's total serum protein, or about 1 milligram per liter (mg/L) of serum. Recent evidence suggests that a CRP level between 1 and 3 mg/L indicates a moderate risk of cardiovascular disease while a level greater than 3 mg/L predicts a high risk. A clinical diagnostic procedure known

as the high-sensitivity CRP (hsCRP) test has been used to detect higher-than-normal levels of the protein and warn a patient about elevated risk for cardiovascular disease.

However, there is no certified reference material—in this case, a sample of human serum with accurately determined amounts of the CRP for various risk levels—against which the accuracy of methods for measuring CRP can be evaluated. The problem: normal, low-risk of cardiovascular disease CRP levels are so low that even mass spectrometry (a very sensitive technique for separating and identifying molecules based on mass) cannot easily quantify them.

In a recent paper in *Analytical Chemistry,* NIST researchers Eric Kilpatrick and David Bunk describe the first steps toward development of a certified reference material that can be used to assess the accuracy of routine clinical laboratory tests for CRP. The researchers accomplished this by isolating the minute amounts (less than 1 mg/L) of CRP circulating at normal levels in serum prior to measurement. Using a protein isolation technique called affinity purification, Kilpatrick and Bunk added polystyrene beads coated with anti-CRP antibodies to normal human serum. The antibodies bind tightly to any circulating CRP, allowing it to be easily removed from solution. The researchers then cleave the purified protein they isolated into its component parts, known as peptides, using enzyme digestion. The peptides are more readily measured by the mass spectrometer, resulting in a very precise determination of the total CRP.

To see if their purification method yields CRP that can serve as a reference material, Kilpatrick and Bunk will next mix purified CRP with genetically engineered CRP containing a heavy isotope of nitrogen (nitrogen 15) and then run the combined pool through affinity purification, enzyme digestion and mass spectrometry. The peptides with the heavy nitrogen 15 atoms will be easily detected and precisely

quantified by the mass spectrometer. If the measurements for the nitrogen 15-tagged peptides compare favorably to those made for the purified serum CRP, then that will validate the use of the affinity purification method for quantifying extremely low levels of the protein. In turn, this validation will clear the way for purified serum CRP derived by the NIST method to be eventually used as a quality control and calibration tool by manufacturers for the hsCRP test.

More information: E.L. Kilpatrick and D.M. Bunk. Reference measurement procedure development for C-reactive protein in human serum. Analytical Chemistry, Vol. 81, No. 20, pages 8610-8616. Oct. 15, 2009

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