Nanoscience enables ultrasensitive detection of Alzheimer’s biomarker

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Alzheimer’s disease (AD), which afflicts an estimated 16 million people worldwide, is a neurodegenerative dementia characterized by memory loss and cognitive impairment (1). Symptoms begin with mild cognitive impairment that cannot be distinguished from other more benign forms of age-related dementia. Even as the disease progresses, clinical diagnosis can be made with only 65–90% accuracy. AD cannot be definitively diagnosed until after death, when brain tissue can be examined for the senile plaques and neurofibrillary tangles characteristic of the disease (2). The plaques result from aggregation of amyloid-β peptides and were long thought to be responsible for AD pathogenesis; however, their presence does not always correlate with neurological symptoms. Smaller, soluble oligomers of these peptides, referred to as amyloid-β derived diffusible ligands (ADDLs), have recently been hypothesized as the causative agent in AD-related memory loss (3). Support for the role of ADDLs comes from their neurotoxicity (4), presence at elevated levels in the brains of AD patients as compared with age-matched controls (5), and mouse studies that indicate a reversal of memory loss upon injection of amyloid-β antibodies (6, 7). The work of Klein, Mirkin, and coworkers (8, 11) makes clever use of nanoparticles as DNA carriers to improve sensitivity.

The bio-barcode amplification strategy makes clever use of nanoparticles as DNA carriers to improve sensitivity.

DNA strands as well as the thermocycling and enzymatic amplification common to PCR. Variations have been reported in which improved reagents or other forms of enzymatic amplification provide improvements in sensitivity, quantification, or ease of use (e.g., by avoiding thermocycling) (14–21). For example, IPCR using oligovalent streptavidin-DNA assemblies has been shown to provide 1,000-fold increases in sensitivity as compared with traditional ELISA (18, 19).

The BCA strategy used by Klein, Mirkin, and coworkers (8, 11) makes clever use of nanoparticles as DNA carriers to enable millionfold improvements over ELISA sensitivity. Fig. 1 illustrates the approach. CSF is first exposed to monovalent anti-ADDL antibodies bound to magnetic microparticles. After ADDL binding, the microparticles are separated with a magnetic field and washed before addition of secondary antibodies bound to DNA:Au nanoparticle conjugates. These conjugates contain covalently bound DNA as well as complementary “barcode” DNA that is attached via hybridization. Unreacted antibody:DNA:Au nanoparticle conjugates are removed during a second magnetic separation, after which elevated temperature and low-salt conditions release the barcode DNA for analysis. Importantly, each Au nanoparticle carries hundreds of identical barcode DNA strands, providing substantial amplification. A second advantage is the ability to perform the BCA assay in homogeneous suspension, where faster kinetics are possible and where excess binding sites can be used to drive protein adsorption simply by addition of more particles. In addition, because the resulting DNA is separated from the particles and sample matrix before quantification, it can be detected by any method ranging from gel electrophoresis to electrochemistry (11, 22).

Klein, Mirkin, and coworkers (8, 23) took advantage of the “scanometric” DNA detection method to quantify the DNA produced by ADDL BCA. This approach, introduced by Mirkin and coworkers in 2000 (23), provides ultrasen-
sitive DNA analysis in a surface-based sandwich hybridization assay for which the probe strands are arrayed on a solid support and the detection strand is bound to an Au nanosphere (Fig. 2). The selectively assembled Au nano-spheres then act as nucleation sites for Ag deposition upon the chemical reduction of Ag⁺ from solution. The resulting Ag deposits, which can be quantified by a simple desktop scanner such as is used to scan documents for computer manipulation (hence scannometric), indicate the presence and amount of target DNA.

Fig. 2. Scannometric detection.