RESEARCH PROFILE

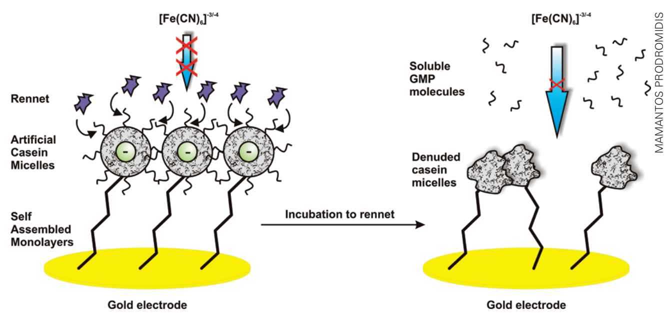
Electrochemical impedance spectroscopy says "cheese!"

Cheese is a gastronomic icon in many countries such as Switzerland, Italy, and of course, France, where Charles de Gaulle once quipped, "How can you govern a country in which there are 246 kinds of cheese?" The production of many cheeses relies on rennet, a natural enzymatic mixture that coagulates milk. But monitoring rennet activity can be difficult. In a recent *Analytical Chemistry* paper (DOI 10.1021/ac1017925), Mamantos Prodromidis and colleagues at the University of Ioannina (Greece) describe a sensor based on electrochemical impedance spectroscopy (EIS) that uses a different approach to analyze rennet.

Milk is a colloidal suspension. The colloids, called micelles, are festooned on the surface with a κ-casein protein layer, the top of which looks like wild, curly hair. The micelles are negatively charged, and repulsive forces keep them in suspension. Rennet contains the protease chymosin, which chews off κ-casein, decreasing the repulsion between the micelles. The micelles can then aggregate to form curds.

Sources of commercial rennet include cows, goats, camels, and genetically-engineered microbes. Because the amount of chymosin in these sources varies, scientists have been developing ways to evaluate rennet's clotting capabilities since the end of the 19th century. Most methods rely on visual observation of milk as it coagulates, which can be subjective. A commercial bench instrument, called the Optigraph, is also available to more quantitatively measure the NIR attenuation as milk clots.

But the investigators wanted to develop a cheaper and simpler approach that doesn't involve milk because, as Prodromidis points out, milk may differ by source, age, season, location, and other factors. Thus they designed a single-use, EIS-based sensor to test rennet activity. Prodromidis explains that they chose to use EIS because there is the potential to exploit the low cost of electrode manufacturing and the capabilities of miniaturization and multiplexing in future generations of the sensor.



When rennet cleaves κ-casein in the artificial casein micelles, $[Fe(CN)_6]^{-3/-4}$ can better access the gold electrode, causing a drop in the charge-transfer resistance. GMP is the soluble glycomacropeptide that is produced from the cleavage of κ-casein.

The investigators attached artificial casein micelles onto a gold electrode modified with a thiol self-assembled monolayer. They measured the resistance to an AC potential at the electrode−electrolyte interface before and after the sensor was immersed in a rennet sample. As rennet degraded the artificial casein micelles on the electrode surface, it produced neutral structures without the hair-like layer that could cluster. The clustering further exposed the electrode surface, allowing a redox probe in bulk solution, $[Fe(CN)_6]^{-3/-4}$, more access to the electrode. The increased interaction with $[Fe(CN)_6]^{-3/-4}$ decreased the charge-transfer resistance at the electrode−electrolyte interface.

Changes in the charge-transfer resistance before and after sampling, expressed as a relative decrease, gave a quantitative number for the rennet's milk-clotting ability. The more dramatic the drop in charge-transfer resistance, the more active the enzyme.

The investigators tested how negatively charged, positively charged, and neutral thiol self-assembled monolayers affected the sensor's performance. The neutral monolayer was the most advantageous because it was hydrophilic and chemically reactive towards the amino groups on the micelles, bypassing the need for coupling agents or activators to attach the micelles. Prodromidis and colleagues found that the sensor's performance was also strongly affected by the size of the artificial casein micelles and the concentration and ionic strength of the immobilization solution.

Experts expressed enthusiasm for the work. "This paper shows that clever programming of modified gold electrodes can be used in new and interesting biosensing applications based on EIS," says Roland De Marco of Curtin University (Australia). He adds the sensor "can be easily used for spot analyses during production for the analytical monitoring of cheese clotting." Heinz-Bernhard Kraatz at the University of Western Ontario (Canada) says the work's "novelty lies in the complexity of attaching micelles to a surface and using an enzymatically-driven reaction to alter the properties of the sensing surface."

The investigators tested the sensor on various commercial powdered or liquid rennet samples and measured values that matched those calculated with a reference method. They observed reliable signal changes within 5 min of incubation, suggesting that the sensor would be useful in routine analysis.

The investigators are now busy further developing the sensor. "So far, experiments have been conducted in the lab in combination with a bench-type electrochemical analyzer," says Prodromidis. "However, in combination with portable instrumentation, the sensors in their current form—or even better, as flat, interdigitated electrodes—can be used as disposable sensors for on-site measurements.

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