

assay, AgCNTs at a concentration of 50 $\mu\text{g}/\text{mL}$ showed an inhibition zone of 24.1 ± 4.14 mm for Salmonella, while the growth curve assay showed that the lag phase was prolonged, and bacterial growth was reduced by 29.5%, as compared to the negative control. The standard plate count assays showed that the Minimum Inhibitory concentration of AgCNTs was between 50 - 100 $\mu\text{g}/\text{mL}$. Quantitative real-time PCR analysis showed that there was a dose-dependent reduction in Salmonella trrRBCA locus DNA concentration after exposure to AgCNTs. By comparison to AgCNTs, commercial silver nanoparticles or CNTs alone did not inhibit bacterial growth or gene expression significantly as tested by the assays mentioned above. SEM studies showed that the AgCNTs damaged the bacterial membranes with marked changes in morphology; TEM studies also confirmed the presence of fewer bacterial cells with damaged membranes. AFM studies are currently being conducted to further assess the mechanism by which AgCNTs inhibit Salmonella enterica serovar Typhimurium.

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Detection of Salmonella from Food using UV-Laser Induced Breakdown Spectroscopy

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Salmonella infections have increasingly become a major public health problem, and early detection is crucial to prevent economic and human losses. Laser-Induced Breakdown Spectroscopy, or LIBS, is the newest technique that could perhaps be used as a rapid and sensitive technology for the detection of Salmonella in foods. To investigate this hypothesis we used a Nd:YAG laser with operating wavelength of 266 nm to generate spectral fingerprints from brain heart Infusion (BHI), and chicken broth artificially inoculated with a range of concentrations (10 - 10⁸ cfu/ml) of Salmonella enterica serovar Typhimurium. These results were then compared with standard detection methods including PCR and qPCR. LIBS could detect up to 105 CFU/ml of Salmonella from BHI, and 107 CFU/ml from chicken broth without extended sample processing. By comparison 102 CFU/ml could be detected by PCR from BHI, and up to 104 CFU/ml from chicken broth. qPCR was the most sensitive technique with detection limits of 10 cfu/ml from BHI and 102 CFU/ml from chicken broth. Our findings suggest that LIBS may be a potentially useful technique to detect bacterial foodborne contamination without extended sample processing. LIBS has fast analysis time, and is easy to perform as compared to competing techniques. However, the sensitivity and specificity of the assay needs to be improved for field applications.

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Molecular Hydrogen Formation by Escherichia Coli Hydrogenase 3 during Fermentation of Glucose at Slightly Acidic pH

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Molecular hydrogen (H_2) can be produced by *Escherichia coli* during mixed-acid fermentation of sugars (glucose) or glycerol via membrane-bound formate hydrogen lyase complex (FHL), which is composed by formate dehydrogenase H and hydrogenases (Hyd). This bacterium possesses different hydrogenases. pH can influence on activity of hydrogenases which might determine formate metabolism and H_2 production.

In this study it's shown that at slightly acidic pH (pH 6.5), during fermentation of glucose *E. coli* wild type produces H_2 . The H_2 production yield and its rate determined by redox measurements was decreased in $\Delta fhlA$ mutant with deletion of transcriptional activator of Hyd-3 or Hyd-4, and stimulated in $\Delta hybB$ or $\Delta hybC$ mutants (with defective Hyd-1 or Hyd-2, respectively) and $\Delta hybC \Delta hybB$ double mutant (with defective Hyd-1 and Hyd-2). H_2 production rate was not inhibited in $\Delta hyfG$ mutant with defective Hyd-4. *N,N'*-dicyclohexylcarbodiimide (DCCD) or sodium azide, the inhibitors of proton translocating F_0F_1 -ATPase, suppressed H_2 production in wild type and mutant strains studied. Thus, at pH 6.5 Hyd-3 but not Hyd-4 may participate in the H_2 formation whereas Hyd-1 and Hyd-2 probably operate in H_2 oxidation mode. During glucose fermentation at pH 6.5 hydrogenases have relationship with the F_0F_1 -ATPase. The relationship has been clearly demonstrated for the cells grown at alkaline pH (pH 7.5) that is playing an important role in generation of $\Delta\mu_{\text{H}^+}$, or detoxification of formic acid formed during fermentation and neutralization of the cytoplasm.

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Chirality-Mediated Structure-Properties Relationship in Biomaterials

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The phenomenon of the homochirality of life, its origin and implications remains one of the most enigmatic fields of science. It has been shown that homochirality provides necessary control for proper protein dynamics (folding) and is a fundamental basis of selectivity in all biochemical processes. Little is known, though, about the implications of the structural phenomenon of homochirality on the

strength, elasticity, and other relevant mechanical characteristics of biopolymers. However, these effects could be profound and lead to significant morphological differences of the resulting biomaterial which is relevant to a number of severe human pathologies. Here, we present the detailed study of the peptide hydrogels assembled from a pair of self-repulsive but mutually attractive oppositely-charged oligopeptides with identical (homochiral) or mirror (heterochiral) chirality. Nanoscale structural and morphological characteristics derived from the SANS data demonstrate the distinctions between homochiral and heterochiral hydrogels as seen from the 2D cross-sections of the fibers, pair-wise distance distribution functions and the mass-fractal and correlation length analysis. 1H NMR was used to monitor faster gelation kinetics for heterochiral gels as compared to the homochiral ones, and to observe the differences in the diffusion coefficients and T1 and T2 relaxation times. Studies at the nanoscale and molecular level have allowed us to suggest how these differences translate into the distinctive mechanical strength and elasticity of such homochiral and heterochiral hydrogels observed by means of dynamic rheometry. Our findings show that homochiral biomaterials are characterized by much better viscoelastic properties, thus possessing evident stability advantages over the heterochiral ones.

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Do Cellulases Exhibit Diffusion Along Cellulose Surfaces? Evidence from FRAP and Single Molecule Experiments

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Cellulases are important enzymes in biofuel production because they hydrolyze cellulose into soluble oligosaccharides. Cellulase structure is modular, typically composed of one or more cellulose binding modules (CBM) and a catalytic domain (CD). The current view of cellulase-cellulose interactions is that cellulases first adsorb onto the cellulose surface via the CBM, increasing their local concentration at the surface and facilitating the attachment of the CD for catalysis. Based on earlier FRAP experiments, it has been further assumed that cellulases can steadily diffuse along the cellulose surface until a catalytic site is found. To test these notions we conducted FRAP and single molecule tracking (SMT) experiments for *Thermobifida fusca* cellulases adsorbed onto bacterial micro-crystalline cellulose. Our findings show that cellulase surface diffusion cannot be accurately determined by FRAP measurements alone, due to their highly dynamic binding/unbinding, especially at temperatures where catalytic activity occurs. FRAP data shows that under constant buffer flow, where rebinding of enzymes is removed, cellulases exhibit limited fluorescence recovery, arguing that surface diffusion plays a minor role in the recovery observed in previous experiments. These observations were further confirmed by SMT, where limited surface diffusion was observed for endocellulases, and no significant surface diffusion was observed for exocellulases. Our observations are consistent with a model where surface diffusion occurs when cellulases are bound to the surface solely by their CBM. Thus, exocellulases which have the cellulose fibrils threaded into their CD cannot exhibit fast long range displacements, while endocellulases which have open catalytic clefts can exhibit surface diffusion based on the loose attachment to the surface via the CBM. These experiments shed light onto the molecular mechanisms of cellulase-cellulose interactions and challenge some of the notions of surface diffusion of cellulases.

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Investigating Cellulase Synergistic Binding and Activity on Simple and Complex Cellulose Morphological Structures

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Cellulases are an important class of cell-wall degrading enzymes that biochemically convert cellulosic feedstocks to fermentable sugars. Understanding the molecular basis of cellulase interactions responsible for synergistic behavior observed between cellulases is critical for rationally designed enzyme cocktails. Traditional biochemical techniques that take indirect bulk solution measurements of free protein to determine bound protein concentration are limited in resolving spatial and temporal patterns of enzyme diffusion and binding that are key to understanding synergism. Integrating advanced imaging techniques like epi-fluorescent microscopy with the existing biochemical data will allow us to overcome these limits and offer the potential for extracting greater insight into the molecular mechanisms that govern cellulase synergism. Binding kinetic data for multiple cellulases with different catalytic actions colocalizing on simple and complex cellulose morphologies can be determined with a high degree of spatial and temporal resolution that has not previously been possible; thus, allowing for a more in-depth exploration of the heterogeneous catalytic interactions between synergistic cellulases. Pure fluorescently labeled populations of *Thermobifida fusca* endocellulase Cel5A, and exocellulase Cel6B, were applied to immobilized cellulose.