Resilin is a rubber-like protein found in specialized regions of insect cuticles, providing outstanding properties of high resilience and fatigue lifetime, where storage of kinetic energy is needed for skills such as flight and jumps \(^1\). Cellulose fibrils, the main strength component of the plant cell wall, consist of highly organized strong crystalline domains linked together by amorphous regions \(^2\). Inspired by the elasticity of insect's cuticle and the strength of the plant cell wall, we hypothesized that resilin (res) and cellulose nanocrystals (CNC) biocomposites would display enhanced mechanical properties featuring both strength and elasticity. Recombinant resilin variants were expressed in \(E.\ coli\) and further purified via affinity chromatography and inclusion bodies isolation techniques. Heat and acid treatments of cellulose enabled the production of colloidally stable suspensions of CNCs. Mechanical tests performed on recombinant res variants suggested similar elastic properties (resilience >95%) compared to the native resilin \(^3,4\). In addition, surface modification of res with catechol-amine resulted in novel DOPA-like adhesive resilin (DOPA-res), and adhesion to collagen-based matrices was shown. The latter modification did not hamper the elastic properties of the protein, and further supported our notion that resilin is intrinsically elastic at the nanometric level. Structural and physical analysis of the CNCs revealed liquid crystal behavior of negatively charged rod-like particles with typical dimensions of 150-200 nm (length) and 10-15 mm (width) \(^4,5\). In order to investigate mechanical and physical properties of resilin and CNC biocomposites, a cellulose binding domain (CBD) was fused to the protein c-termini (res-CBD), conferring intimate interaction between the res-CBD and the CNC with an optimal binding of 1:5 by mass. Res-CBD-CNCs were solvent cast into transparent films, and glycerol was studied as an additive to determine whether the addition of a wet
component to solvate the res-CBD improved the mechanical properties of composites. Res-CBD-CNC films displayed higher hydrophobicity compared to CNC films by advancing contact angle measurements (70–80° compared to 35–40°, respectively). Compared to the other films, res-CBD-CNCs responded more elastically to a given strain and exhibited higher Young's modulus values when glycerol was added in the range of 0.5–5 wt%. We assign the enhancement in the modulus to changes in CNC particles alignment within the composite films: In contrast to the multi-domain orientation typical of chiral nematic CNC films, res-CBD-CNC films exhibited long-range, uniaxial orientation driven by the CBD moiety. To the best of our knowledge, this is the first report of uniaxial alignment induced in CNC films prepared by solvent evaporation in the absence of external forces. Overall, films made of res-CBD-CNCs containing 0.5wt.% glycerol displayed synergistic effects of increased toughness (150%) and elasticity (100%) compared to their neat CNC counterparts. Finally, as a case study, Res-CBD-CNCs were inserted into a commercially available epoxy adhesive in order to assess their potential as bio-reinforcing agents in composite systems. The process involved binding of res-CBD to CNCs through the CBD domain, and a chemical reaction between the resin epoxide groups and res-CBD amine moieties. The resulting biocomposite showed a 50 % increase in the Young's modulus and a 20 % decrease in the tan (δ), compared to pristine epoxy.

This research is focused on the development and characterization of novel biocomposites based on resilin variants and CNCs. Our goal is to understand the material mechanical and physical behavior in relation to its molecular structure and properties.

Reference: