Prof. Raphael Lamed's Research Interests

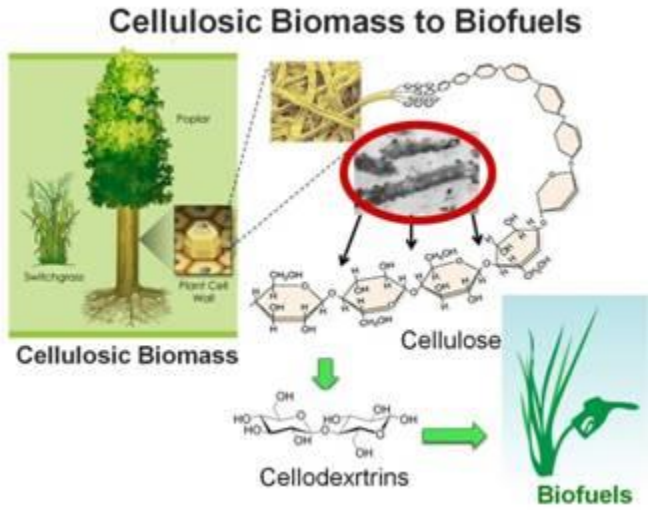
Biomass to Biofuels

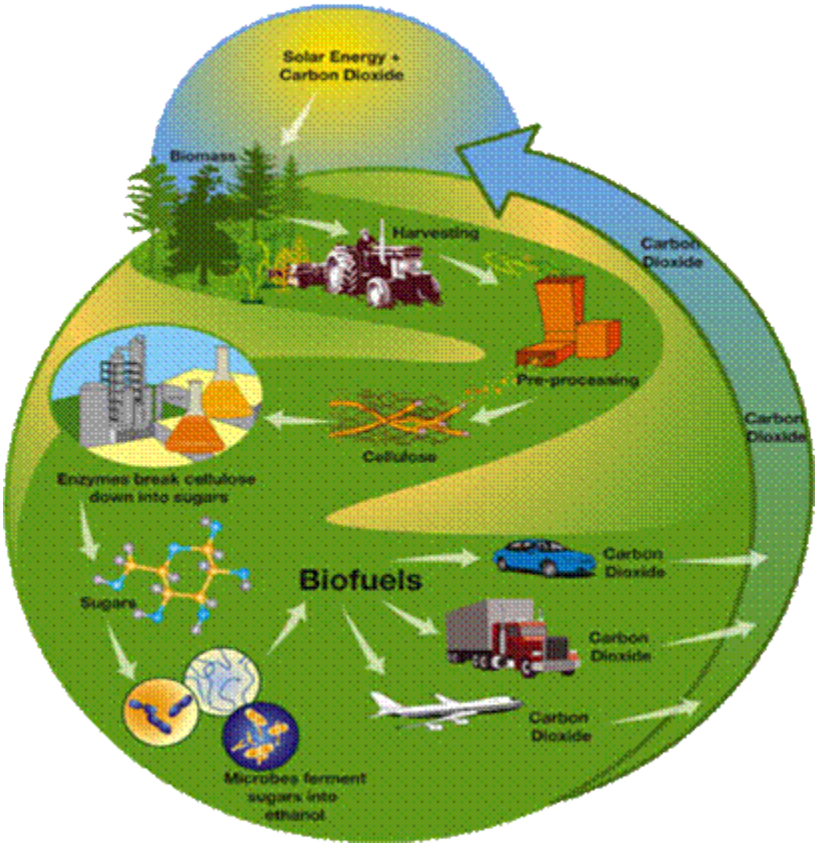
Biomass is the term used for all organic material originating from plants and is essentially the collection and storage of the sun's energy through photosynthesis. Biofuels are produced in processes that convert biomass into more useful intermediate forms of energy.

One of the important targets is to unlock the full potential of cellulosic biomass. Plants have evolved over several hundred million years to be recalcitrant—resistant to attacks from the likes of bacteria, fungi, insects, and extreme weather. Breaking down plants is no easy task. For cellulosic ethanol production, the primary challenge is breaking down (hydrolyzing) cellulose into its component sugars. We are exploring the causes of biomass recalcitrance and ways to overcome it using cellulases (enzymes that break down cellulose).

Our interest and research is in conversion of biomass to biofuels and also useful intermediate commodity chemicals.**The ability of cellulolytic bacteria to degrade cellulose can potentially be used for the degradation of organic matter in garbage and for production of biofuels from waste, which is a major environmental challenge of our time.** We are mainly involved in basic scientific research for a clean energy future and an alternative energy research initiative.

We have developed a novel approach to mimic a natural multi-enzyme protein complex – the cellulosome – and have achieved a new generation of designer cellulosomes for conversion of the biomass (ligno-cellulosic) to value added products. The cellulosome is essentially a macromolecular machine comprising different enzymes which can digest the biomass efficiently. Our research efforts can contribute towards the development of eco-friendly technologies and biotechnology products. Many of our research efforts have been recognized and cited by bio-based industries in their standard operation protocols.

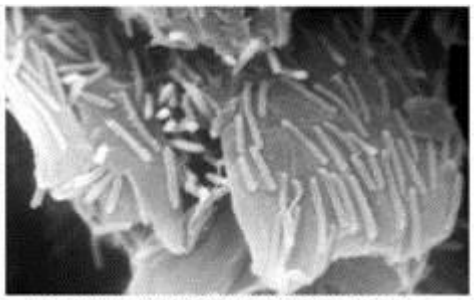




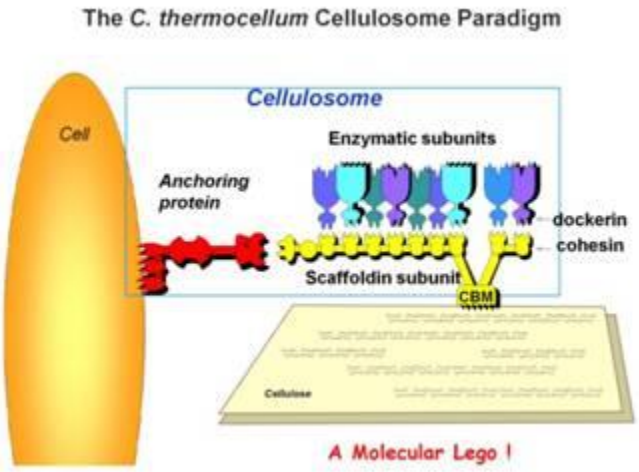
Bacteria can degrade cellulose via large multi-enzyme complexes – Cellulosomes

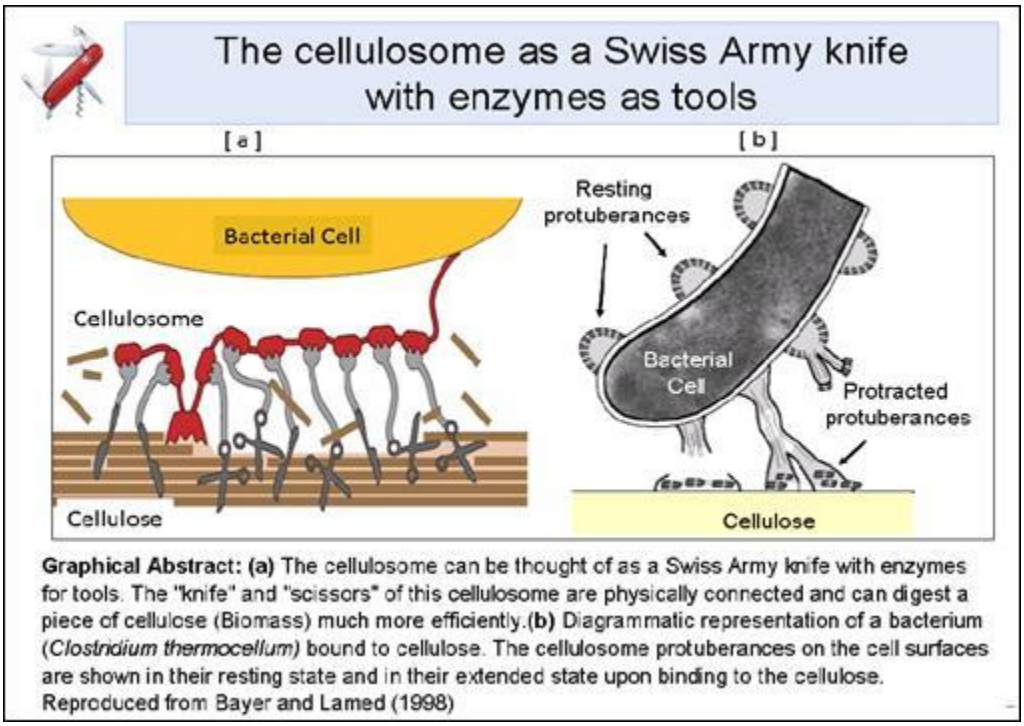
The cellulases of many cellulolytic bacteria were shown by us to be organized into discrete multienzyme complexes, called **cellulosomes.** The cellulosomes are associated with the cell surface and mediate cell attachment to the insoluble substrate and degrade it into soluble products which are then absorbed by the cell. The multiple subunits of cellulosomes are composed of numerous functional domains which interact with each other and with the cellulosic substrate. One of these subunits, a large glycoprotein, which we called **"scaffoldin"** , comprises a distinctive new class of noncatalytic scaffolding polypeptide. The scaffoldin subunit selectively integrates the various cellulases and xylanase subunits into the cohesive complex, by combining its **"cohesin"** domains with a typical " **dockerin** " domain present on each of the subunit enzymes.

Clostridium thermocellum
anaerobic, thermophilic bacterium
converts cellulosic substrate into ethanol.



Clostridium thermocellum Bound to Cellulose





Graphical Abstract: (a) The cellulosome can be thought of as a Swiss Army knife with enzymes for tools. The "knife" and "scissors" of this cellulosome are physically connected and can digest a piece of cellulose (Biomass) much more efficiently.**(b)** Diagrammatic representation of a bacterium (*Clostridium thermocellum)* bound to cellulose. The cellulosome protuberances on the cell surfaces are shown in their resting state and in their extended state upon binding to the cellulose. Reproduced from Bayer and Lamed (1998)

Microbial Biomass Sensors: A Novel Transcription Regulatory System in Cellulolytic Bacteria.

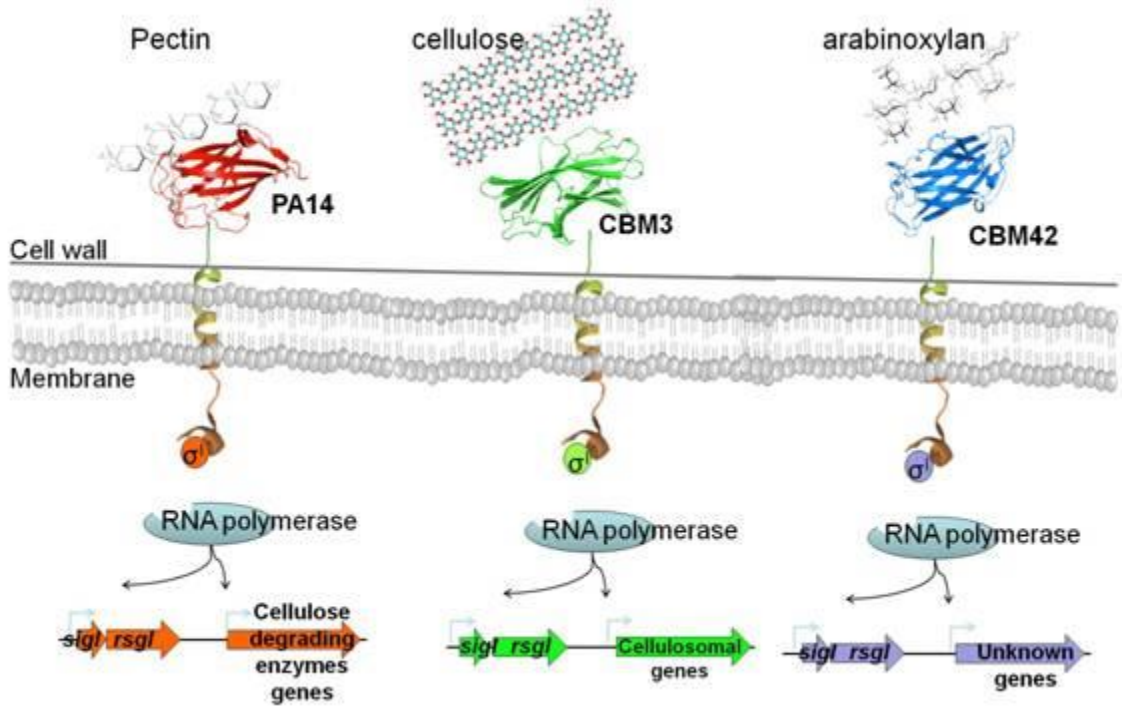
Some cellulolytic bacteria, such as *Clostridium thermocellum* produce extracellular multi-enzyme systems, highlighted by the cellulosome complex, for degradation of plant cell wall polysaccharides and cellulosic wastes. Such bacteria can produce over 70 different enzyme components, which include cellulases, xylanases, mannanases, arabinases,

galactanases, carbohydrate esterases and pectin-degrading enzymes. However, it is *not* known how the bacterium decides which enzymes are incorporated into the cellulosome.

We have recently discovered an incredible carbohydrate-sensing mechanism in *C. thermocellum* and other cellulolytic bacteria, which is purported to sense the distribution of the extracellular complex carbohydrates in the environment and regulate the activation of bacterial genes coding for polysaccharide-degrading enzymes . This mechanism includes a novel and unique set of membrane-associated anti-σ factors, which resemble RsgI, a negative regulator of the *Bacillus subtilis* alternative σ I factor. These putative RsgI-like anti-σ factors embody three domains: (i) a C-terminal carbohydrate-binding module (CBM) localized on the outer cell surface, (ii) an internal transmembrane/wall-spanning segment and (iii) an N-terminal cytoplasmic portion, which would bind the cognate σ-like factor.

When the σ factor is bound to the cytoplasmic portion of the anti-σ factor, the pair is considered to be in the off position. When the CBM binds to its target extracellular polysaccharide, the intracellular portion presumably undergoes a conformational change, whereby the cognate σ factor is released, binds to RNA polymerase, and then promotes expression of appropriate genes, including its own. This results in selective biosynthesis of the corresponding polysaccharide-degrading enzymes.

Understanding of these regulatory mechanisms is crucial for efficient biotechnological use of these bacteria for degradation of cellulosic matter and synthesis of value-added products.



Carbohydrate-sensing system - suggested mode of operation : The extracellular polysaccharides (cellulose, pectin, xylan, arabinose etc) interact with the respective CBM and protein modules, which in turn, induces a conformational change on the intracellular RsgI (anti-sigma) domain, The SigI (Sigma) factor is now free to interact with RNA polymerase and promote transcription of the SigI-dependent promoters.

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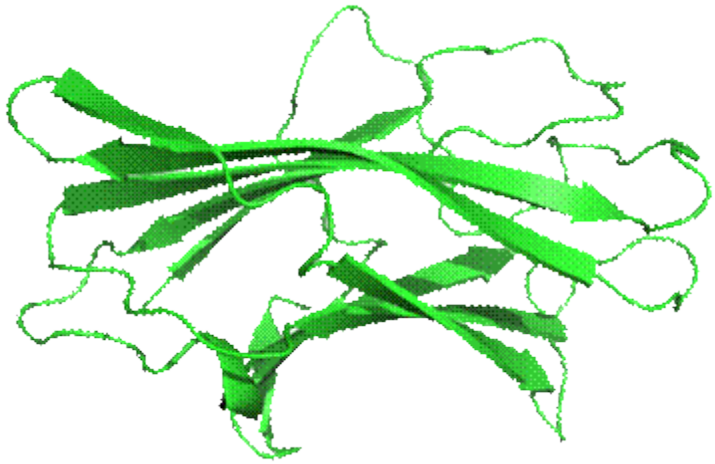
X-ray crystallography and 3-D structure solution

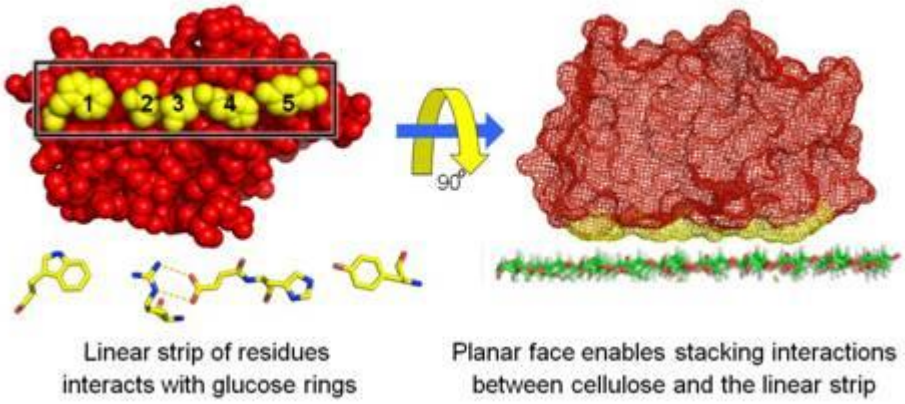
In collaboration with the laboratory of Prof. Felix Frolow

We are focusing on the crystallization and elucidation of the 3D structures of CBMs, enzymes, cellulosomal components and parts of the biomass-sensing regulatory system.
Resolving the 3D protein structure will help us to reveal its function and mode of action. Moreover it sheds light on the mechanism of protein-protein and protein-substrate interactions.

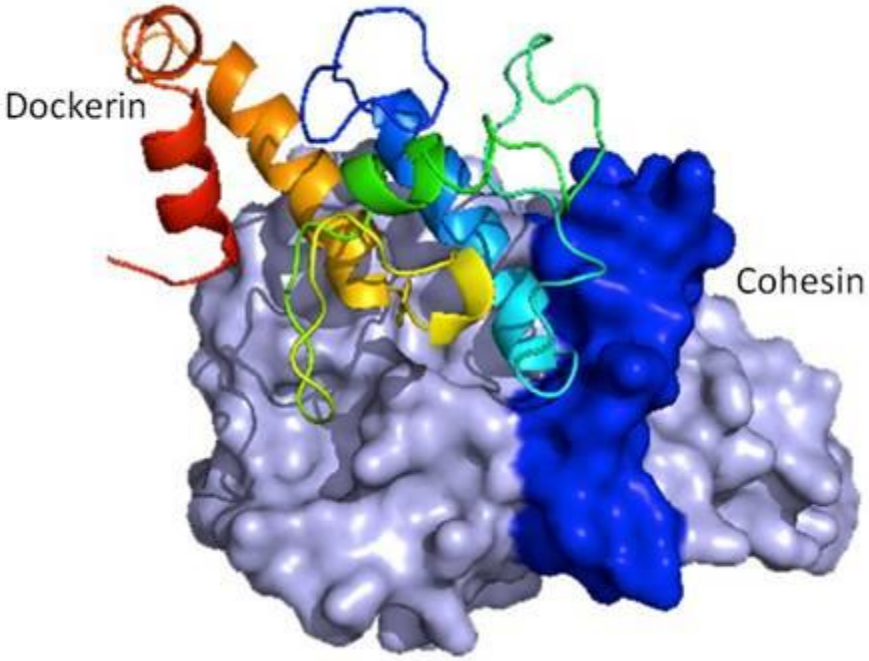
Carbohydrate Binding Module (CBM) from a novel transcription regulatory system:



Cellulose-binding strip of CBM – crystallography enables us to predict mode of interaction:



Cohesin- dockerin interactions between the cohesins and dockerins cellulosomal components


Dockerin
Cohesin