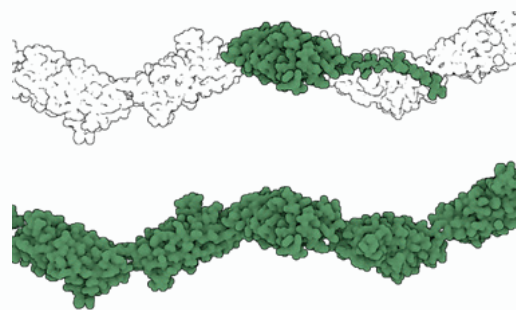


# Caf1 Bioactive Proteins The Core Technology

**What is Caf1?** Caf1 is a protein which is produced by bacteria, forming a gel-like coat that protects them from phagocytosis [1,2]. The Caf1 protein monomer is a small 15 kDa subunit that forms polymers by inserting the extended N-terminal strand of one subunit into the “acceptor cleft” of another subunit [3-5]. This process leads to very long polymers (up to 250 subunits) of Caf1 that can reach up to 1.5  $\mu\text{m}$  in length and MDa in size [6]. These polymers can also efficiently refold and self-assemble *in vitro* following thermal denaturation, allowing subunits from different polymers to be recombined at will [7].

**Why use Caf1?** Caf1 has a remarkable set of properties, which allow it to be used for a vast array of biological purposes. In its unmodified, “wild-type” form it has a “non-stick” nature, i.e. mammalian cells do not stick to or interact with it [8]. However, as the protein is produced recombinantly

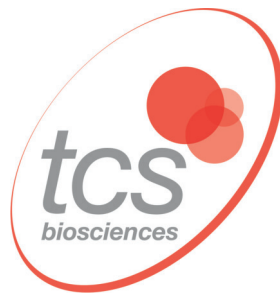


In *E. coli*, we are able to genetically encode bioactive sequence motifs (e.g. adhesion motifs from extracellular matrix proteins [7-9], or sequences from growth factors [10]), allowing us to precisely define how the protein interacts with cells. Each modified Caf1 subunit will present its engineered protein sequence at a known position on the protein, so that a Caf1 polymer, which consists of tens or hundreds of subunits, will present multiple copies of the bioactive motif to cells at the same time.

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MARRABIO The logo for MARRABIO, consisting of the word 'MARRABIO' in a bold, black, sans-serif font, followed by a stylized icon of three interlocking puzzle pieces in green and yellow.

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# Caf1

## The Core Technology

These bioactive Caf1 modules can then be mixed both with the inert wild-type protein and with each other to easily produce materials with complex and defined bioactivities [7,10]. Crucially, no chemical modification or change in the production process is required, and the resulting polymers are both highly stable [2, 11] and free from animal materials.

Caf1 polymers are ultra stable, with a melting temperature of  $> 80^{\circ}\text{C}$ . This means that they can be used for long periods of time at room temperature and above and are easy to work with. Users find that the procedure for coating cell culture plates is quick and easy. Different bioactive Caf1 modules can be easily mixed and matched to produce multifunctional materials. As Caf1 is a bacterial protein, it is completely animal free, avoiding batch to batch variation, and can be produced scalably to meet demand.

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