New insights into *Ruminococcus flavefaciens* cellulosome assembly

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The cellulosome is an elaborate multi-enzyme structure secreted by anaerobic microorganisms for the degradation of lignocellulosic substrates. Its assembly is mediated by high-affinity interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffoldins. The cellulosome of *Ruminococcus flavefaciens* is one of the most intricate described to date and its structure is assembled exclusively through single-binding-mode Coh-Doc interactions [1-3]. However, a set of R. *flavefaciens* Docs exhibits certain features associated with the classic dual-binding mode (DBM) [4-6].

To investigate DBM in ruminal cellulosomes, we have solved the structures of two Coh-Doc complexes through X-ray crystallography, one involving the Doc of the monovalent adaptor scaffoldin ScaH and another involving an atypical truncated dockerin with a single calcium-binding repeat. Our finding revealed that the DBM can be selectively incorporated into R. *flavefaciens* cellulosome through ScaH and that the single-repeat Docs can interact in three distinct conformations, including one with two Docs binding to a single Coh, a feature never previously reported.

These results suggest the existence of adaptor scaffoldins with the sole purpose of improving cellulosomal spatial conformation and of naturally occurring atypical dockerins with distinct binding mechanisms. We were also able to improve Coh-Doc affinity through structure-informed protein engineering, a key feature for the design of affinity-based technologies using tailored Coh-Doc interactions.

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