

VersaTile: A high-throughput DNA assembly method for the rapid construction and evaluation of cellulosome components

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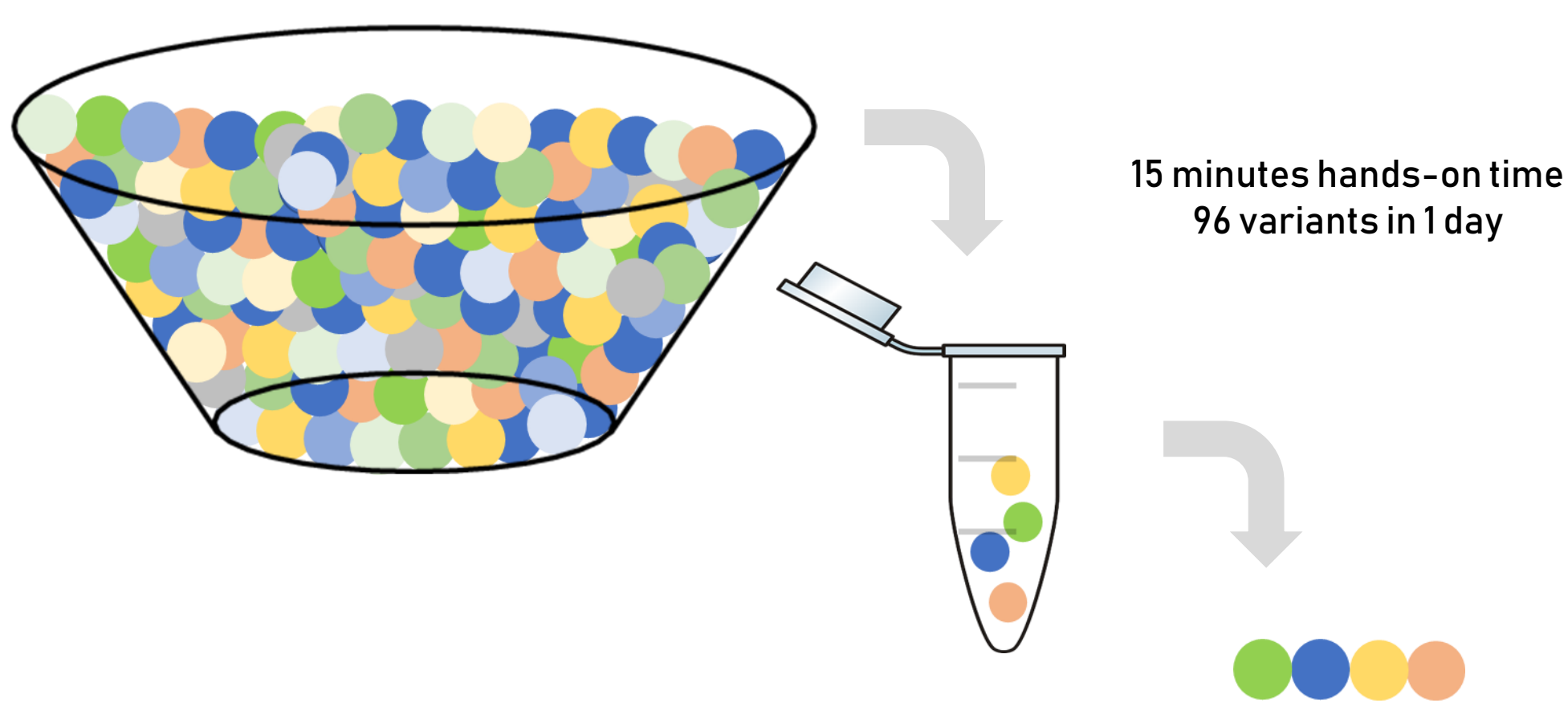
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1. VersaTile Shuffling

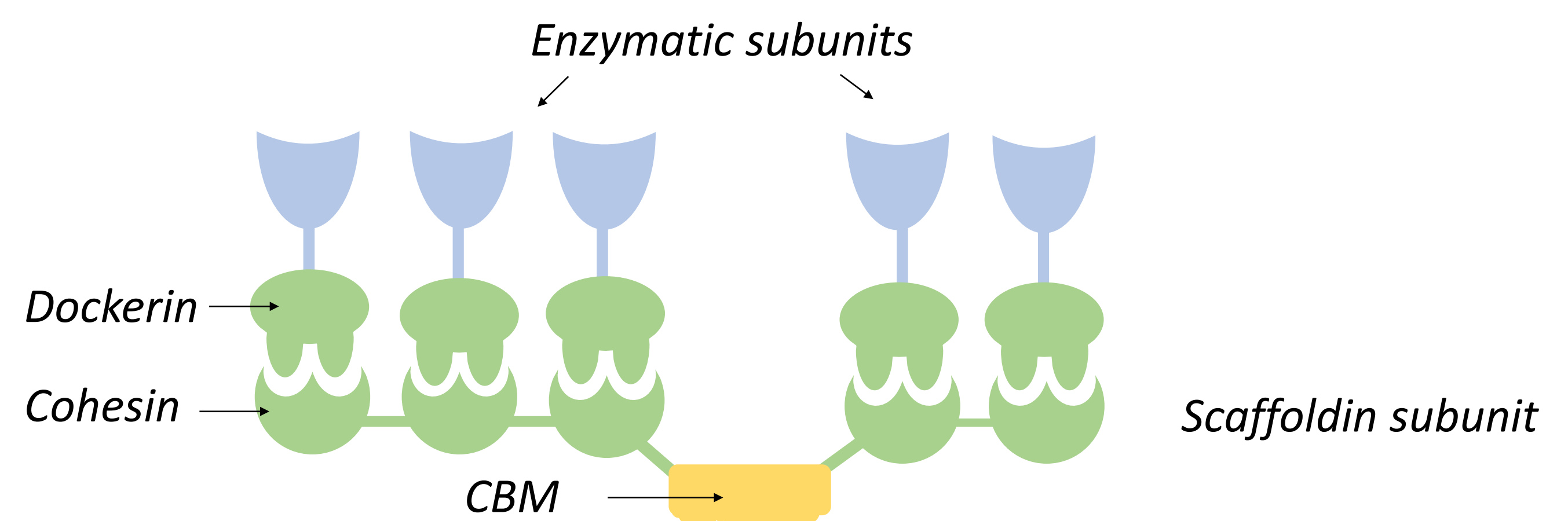
VersaTile Shuffling (VTS) is a **DNA shuffling method** for the assembly of non-homologous coding sequences, enabling us to create **modular proteins** at a high rate.

VTS involves the construction of a **repository of 'tiles'** or modules that one wants to shuffle (Step I), followed by the **assembly of any combination** of these tiles (Step II).



2. Designer cellulosomes

Designer cellulosomes are multi-enzyme complexes composed of a backbone molecule carrying several complementary CAZymes. The close proximity of all complementary enzymes enhances their synergy.

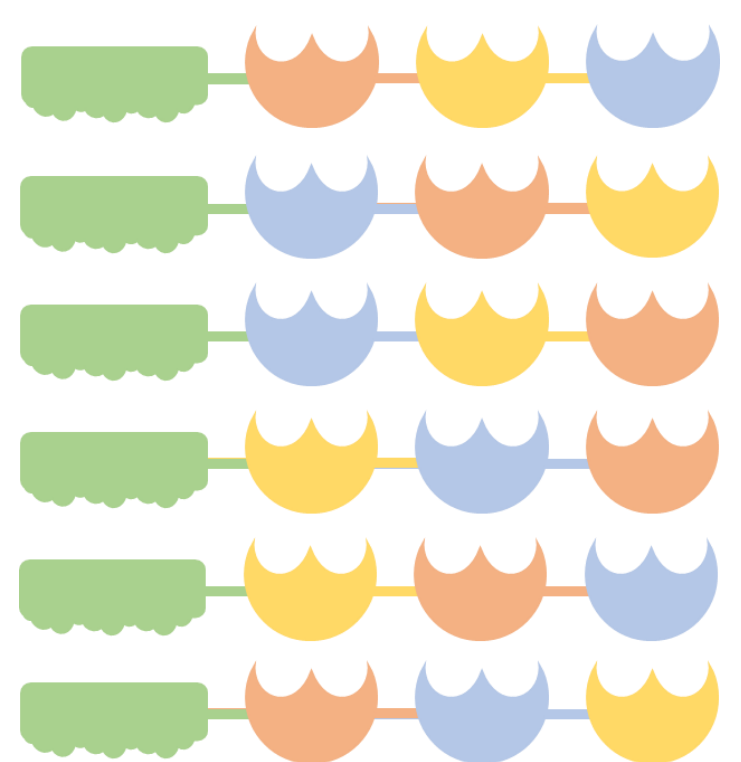


Current drawbacks:

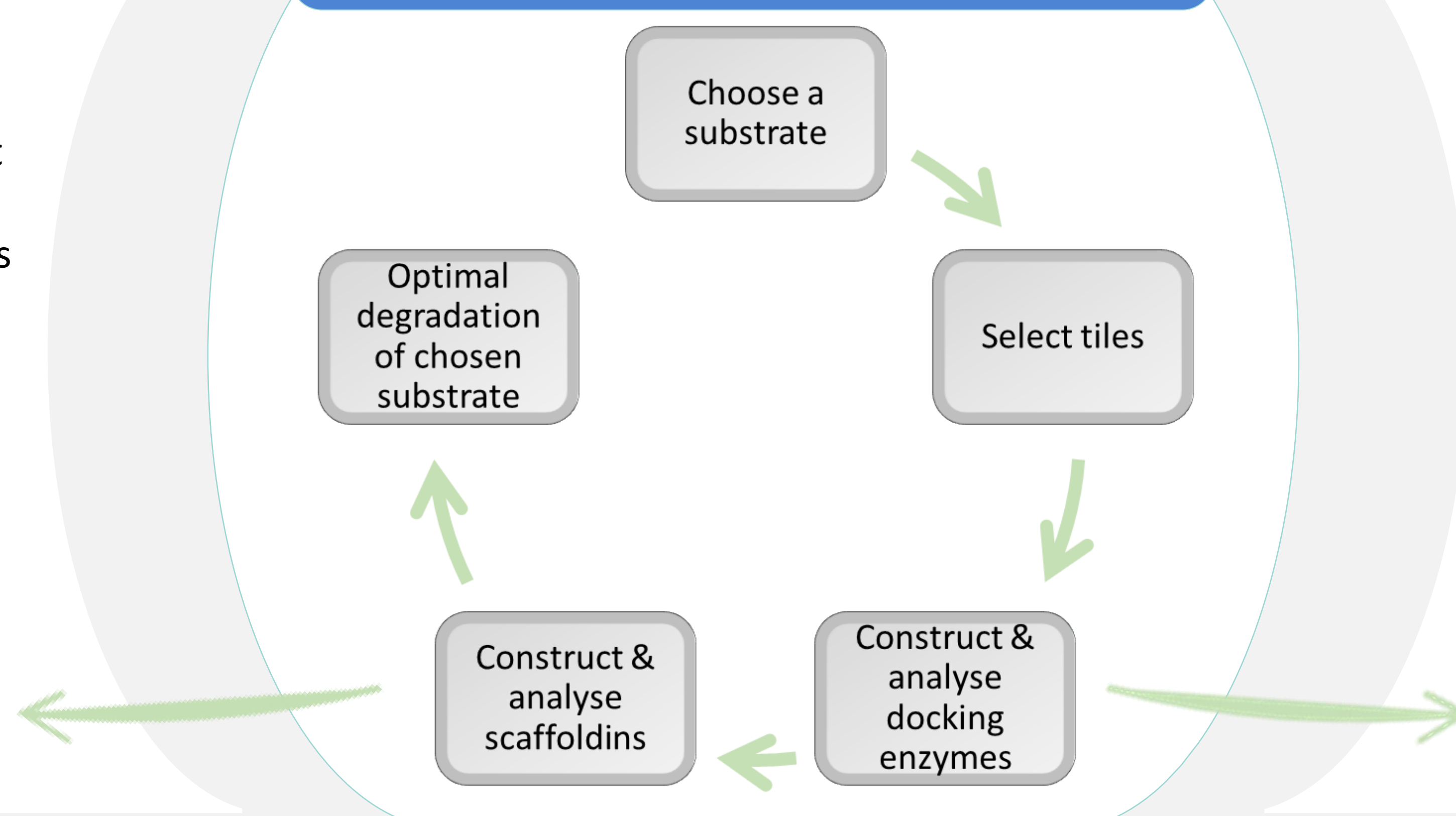
- Use of **standard cloning techniques** makes the construction process tedious and allows the creation and analysis of only one or a few designer cellulosome(s) at a time.
- A **high number of variables** is influencing the performance of designer cellulosomes.

Scaffoldins

- High-throughput construction & expression of scaffoldins
- Switching cohesin positions = changing relative position of different docking enzymes
- VersaTile Shuffling destination vectors allow expression with different tags.

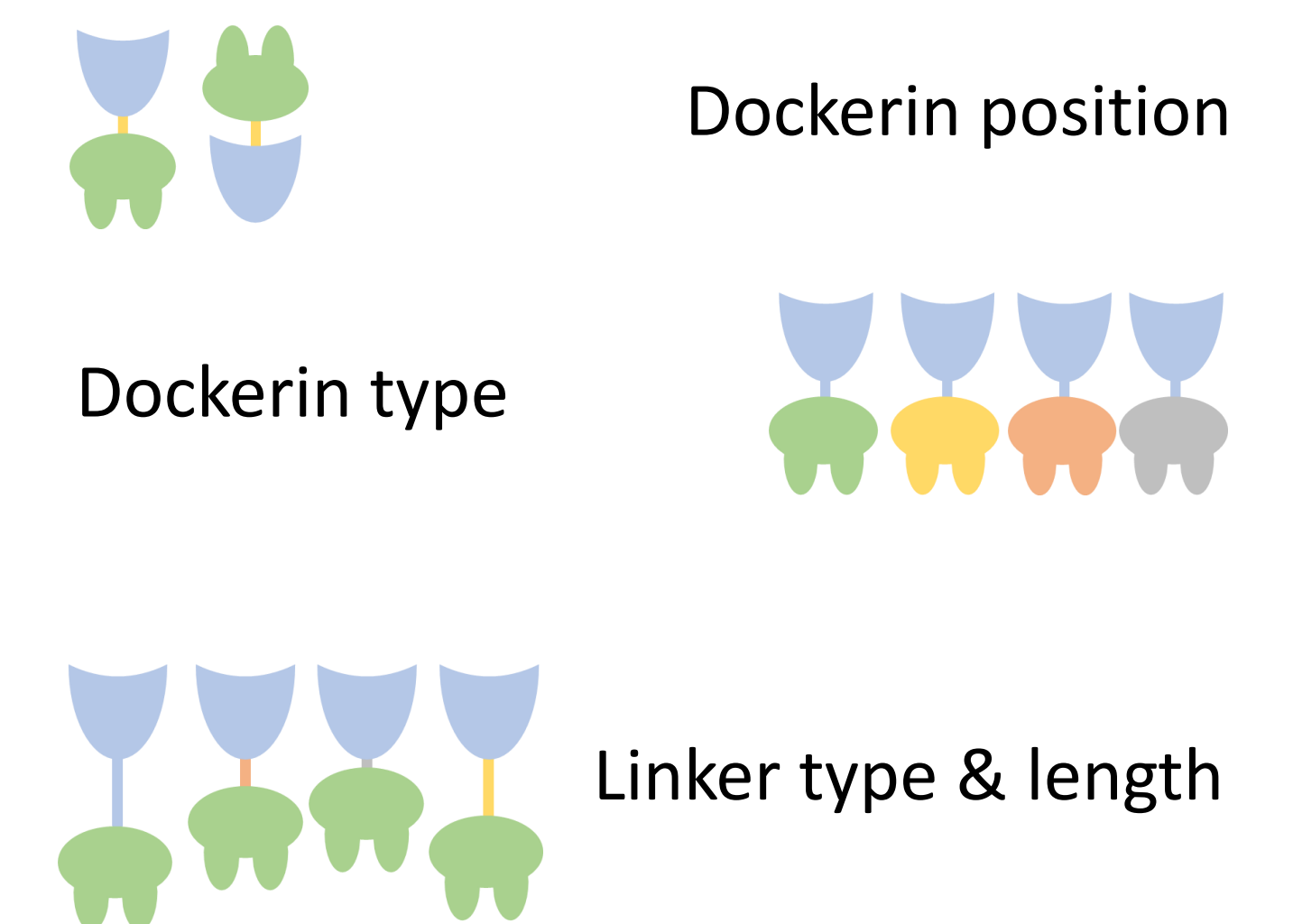


3. Construction of optimized designer cellulosomes



Docking enzymes

- High-throughput construction & expression of docking enzymes
- Investigate effect of different parameters:



4. Case study: xyloglucanosome

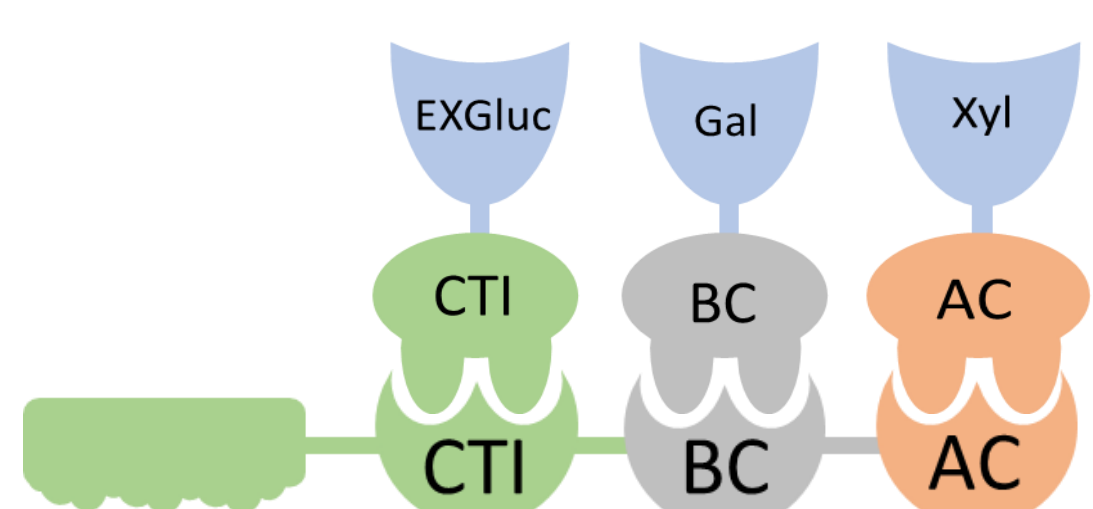
Construction and analysis multi-modular proteins

Xyloglucan degradation:

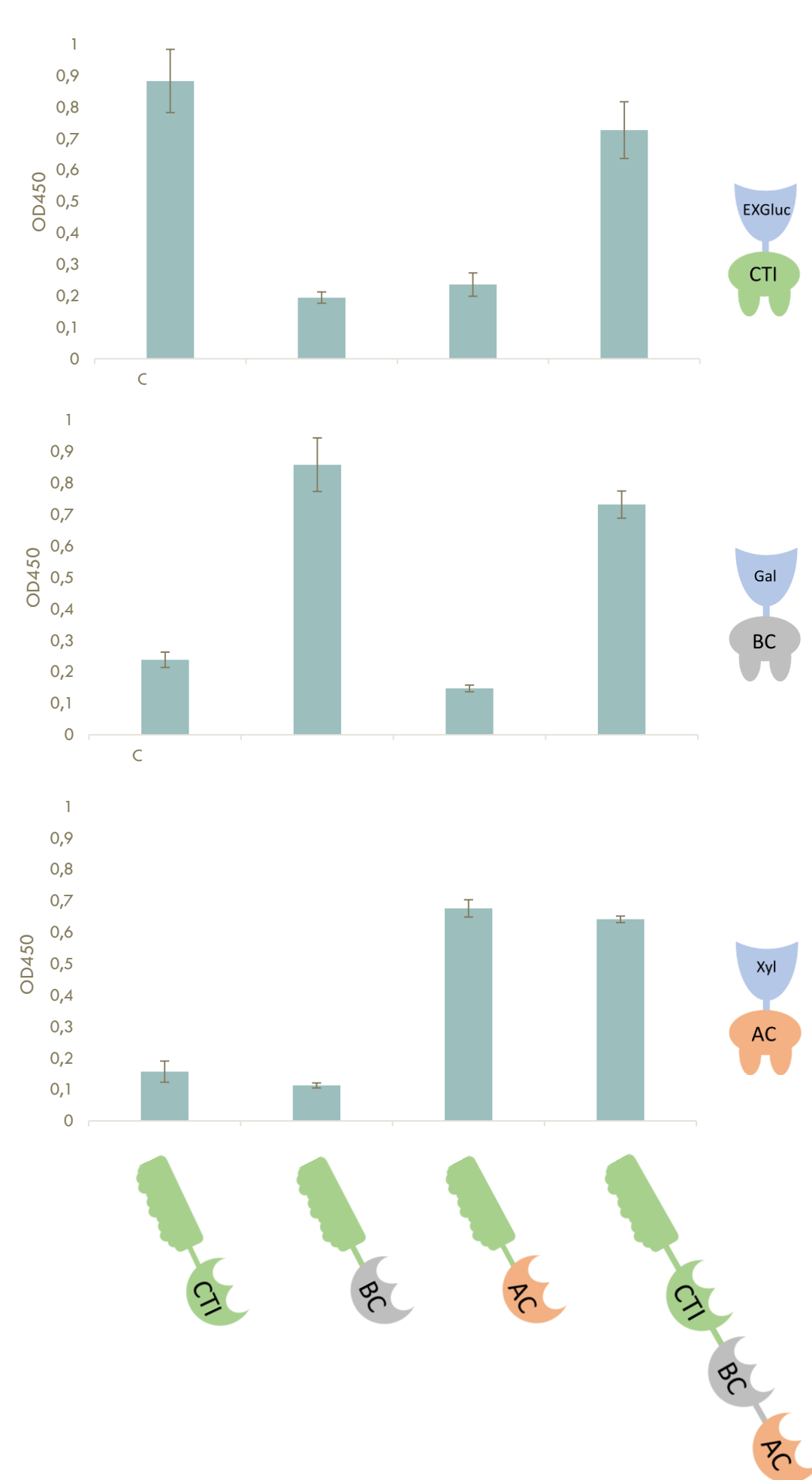
- Endoxyloglucanase
- β -1,2-galactosidase
- α -1,6-xylosidase

Fuse dockerins & analyse activity

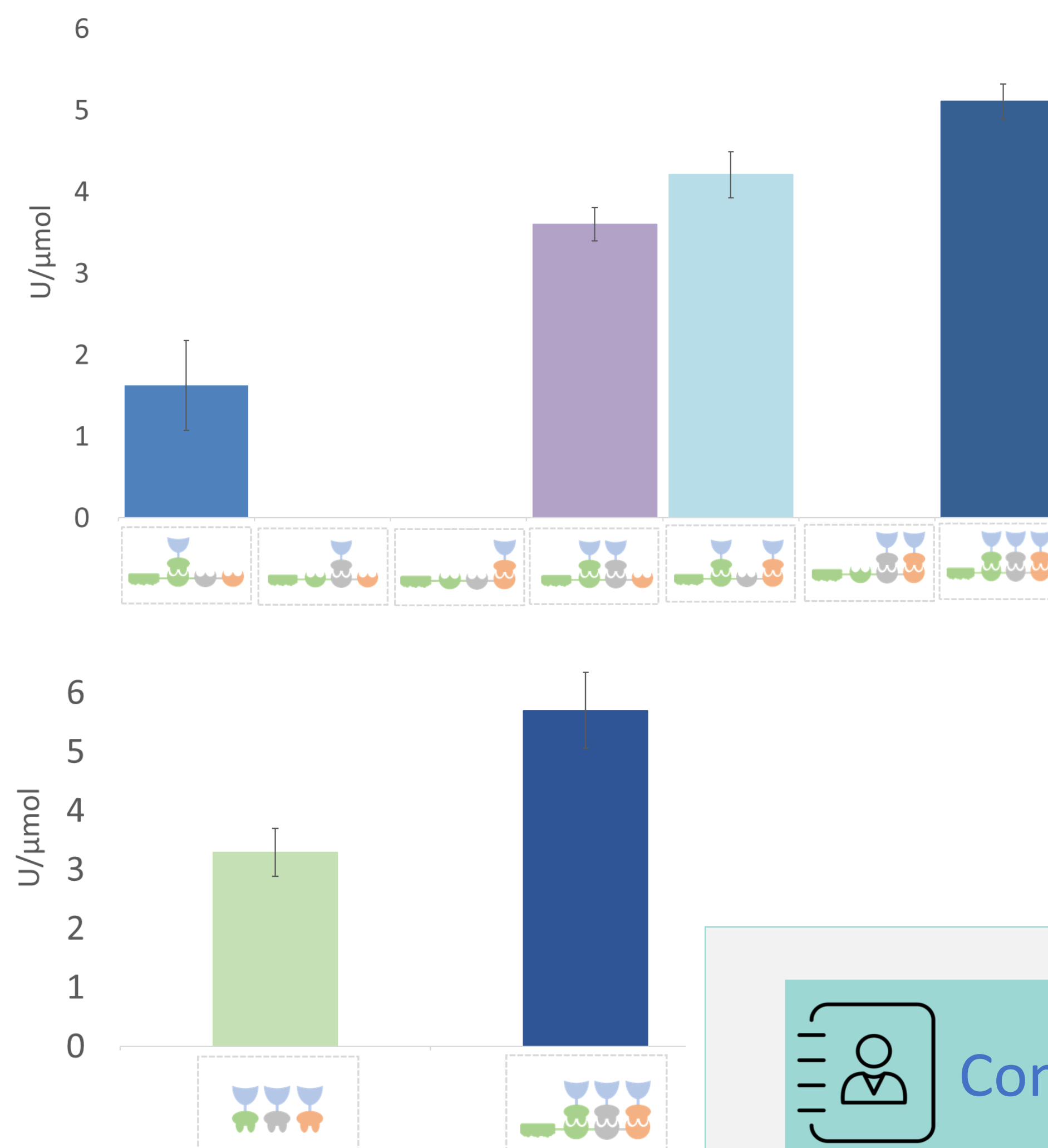
Construct scaffoldin & form complex



Interaction assay



Activity analysis multi-enzyme complex



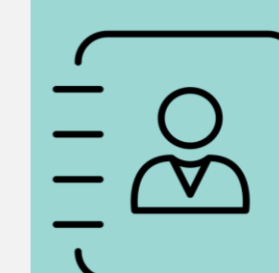
5. Take home messages

Designer cellulosome construction

- ✓ Each combination of enzymes can be converted to multiple possible cellulosomes.
- ✓ Preliminary assays to determine the optimal modular architecture require a quick and easy assembly method.

VersaTile Shuffling

- ✓ High-throughput method
- ✓ Rapid construction
- ✓ Designer cellulosomes or other modular proteins



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