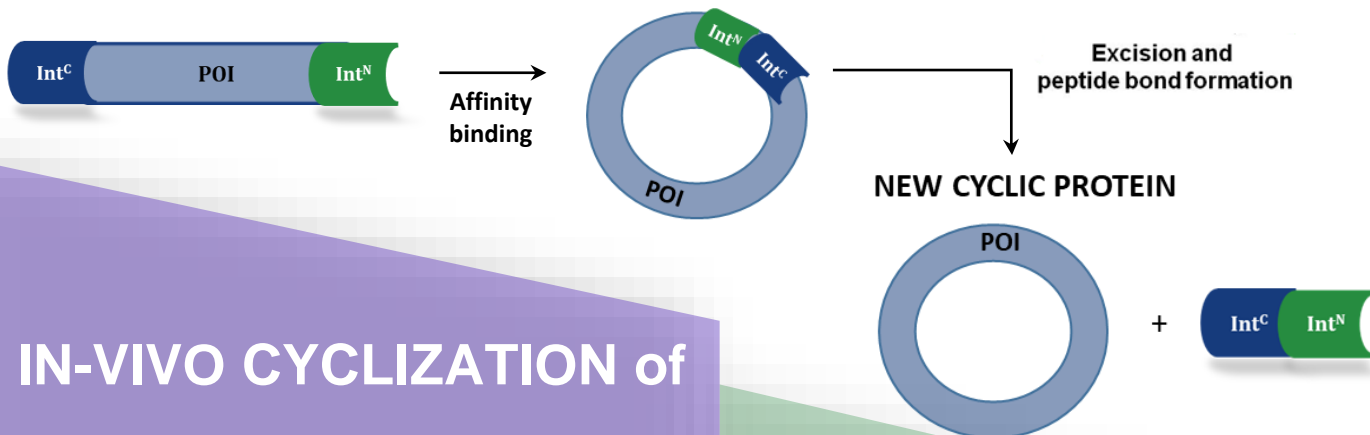


## Cyclic Proteins and Peptides

Split-intein mediated Protein/Peptide Cyclization



### IN-VIVO CYCLIZATION of PEPTIDES and PROTEINS

- Co-translational reaction
- No precursors detected after reaction
- Possibility to generate libraries of cyclic peptides

### Four different split intein pairs

- Efficient and highly specific
- Fast

Trans-splicing efficiency and kinetics\*  
Obtained at 25°C and pH 7

Intein	Yield (SP %)	k (s <sup>-1</sup> )	t <sub>1/2</sub> (s)
G1	80 – 90	5.7·10 <sup>-2</sup>	12
G8	85 – 95	1.7·10 <sup>-2</sup>	40
N1	85 – 95	6.2·10 <sup>-2</sup>	11
I1	90 - 95	2.0·10 <sup>-2</sup>	34

\* Data collected in a model system with natural exteins

### Broad extein specificity

- ZIP Inteins need a **Ser** at position +1 but they are quite **promiscuous** regarding the other extein positions. **No scar** left in cyclic products.



### Business Model

- Technology evaluation agreement
- Joint development agreement
- R&D license
- Commercial license



### Intellectual property

- SPLITTERA technology
- Fully owned and patented (WO2013/045632)

#### Specificity for the -1 position

Splicing activity (%) in the extein context SGX-gp41.1-SGG (wild-type: SGY-gp41.1-SSS)

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
gp41.1	77	74	80	80	76	66	78	47	90	84	81	77	25	78	92	76	37	43	68	71*

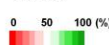
\* Wild-type aa

#### Specificity for the +2 position

Splicing activity (%) in the extein context EGS-gp41.1-SXG (wild-type: SGY-gp41.1-SSS)

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
gp41.1	86	82	67	58	70	61	82	70	83	78	78	69	13	69	78	71*	77	74	78	74

\* Wild-type aa



Data from Oeemig et al., 2020

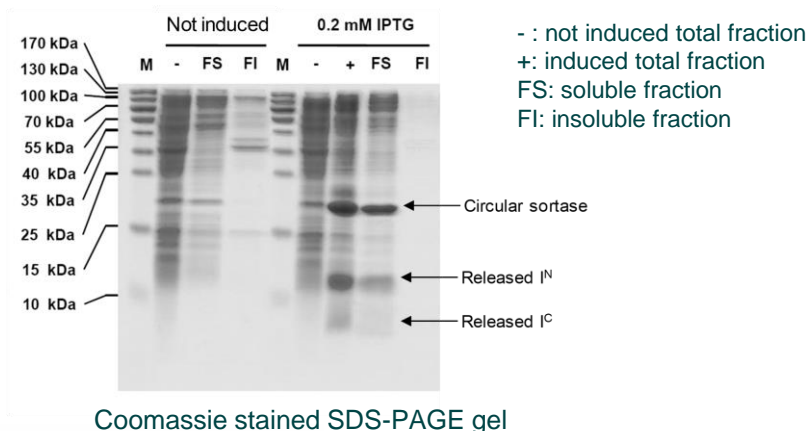
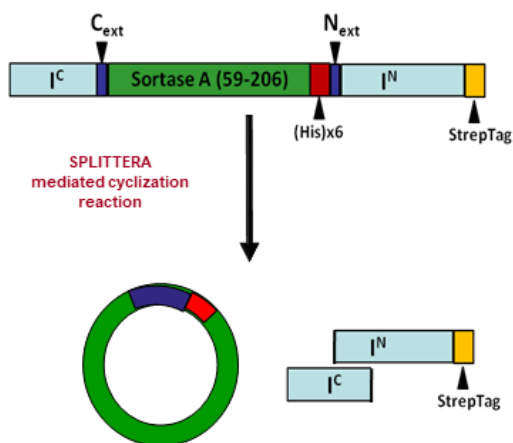
# SPLITTERA Platform

## Cyclic Proteins and Peptides

### EXAMPLES OF CYCLIC PROTEINS OBTAINED VIA TRANS-SPLICING

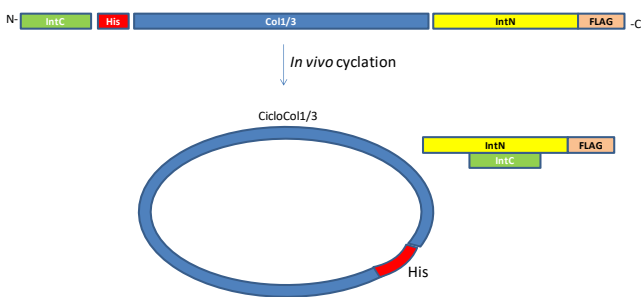
#### Cyclic SORTASE

Cyclic sortase was recovered by IMAC

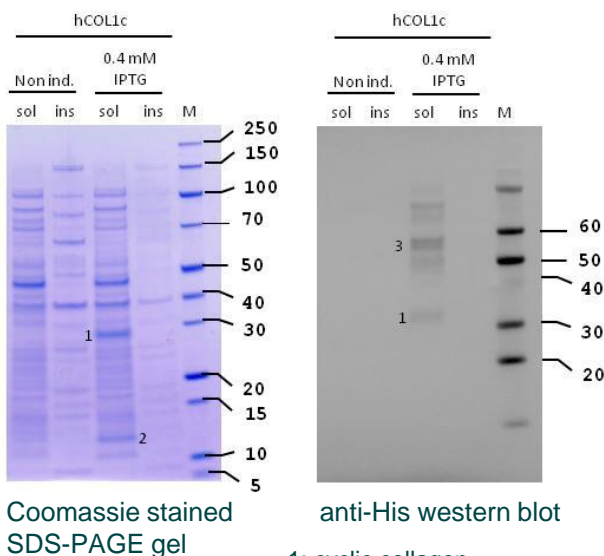


#### Cyclic COLLAGEN

Cyclic collagen was recovered by IMAC



- Cyclic COLLAGEN production doubles production of linear collagen
- Cyclic COLLAGEN is more stable to degradation caused by temperature



- 1: cyclic collagen  
2: released I<sup>N</sup>  
3: cyclic collagen multimers