The novel Fh8 tag:

a novel fusion system for recombinant protein production and purification in *Escherichia coli*

Fh8 and H tags

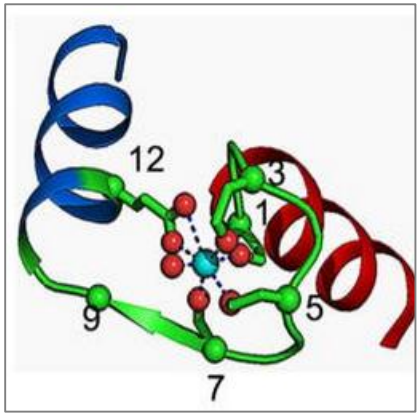
Fh8: 8-kDa protein from *Fasciola hepatica*

- Excreted-secreted antigen by the parasite *F. hepatica* in the early stages of infection
- Located on the surface of the parasite → useful tool for the diagnosis, vaccine and drug development against *F. hepatica* infections
- Immunogen → strong immune response
- Belongs to the calmodulin-like EF-hand Calcium Binding Protein family

Fh8 and H tags

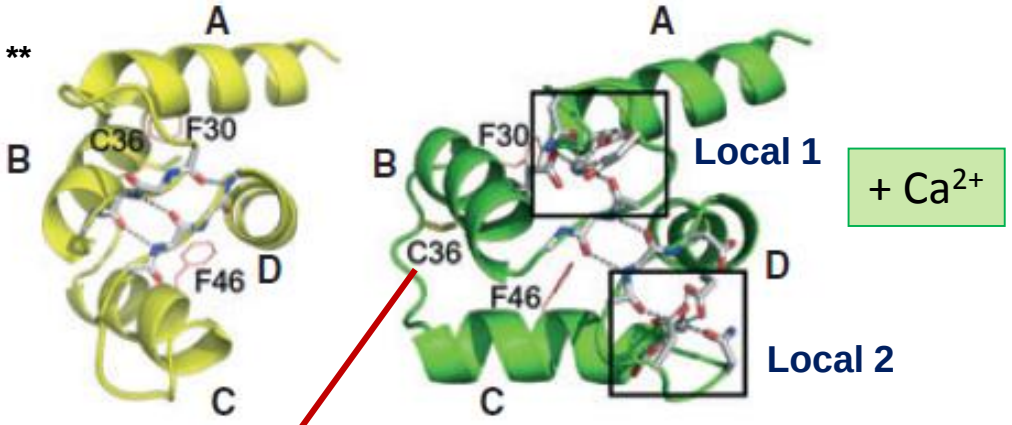
Fh8: 8-kDa protein from *Fasciola hepatica*

*



- Ca^{2+}

**



+ Ca^{2+}

* Zhou, Y., Frey, T.K. and Yang, J.J. (2009) Viral calciomics: interplays between Ca^{2+} and virus. Cell Calcium 46, 1-17

** Fraga, H., Faria, T.Q., Pinto, F., Almeida, A., Brito, R.M.M. and Damas, A.M. (2010) FH8-a small EF-hand protein from *Fasciola hepatica*. Febs Journal 277, 5072-5085.

EF-1 **EF-2**

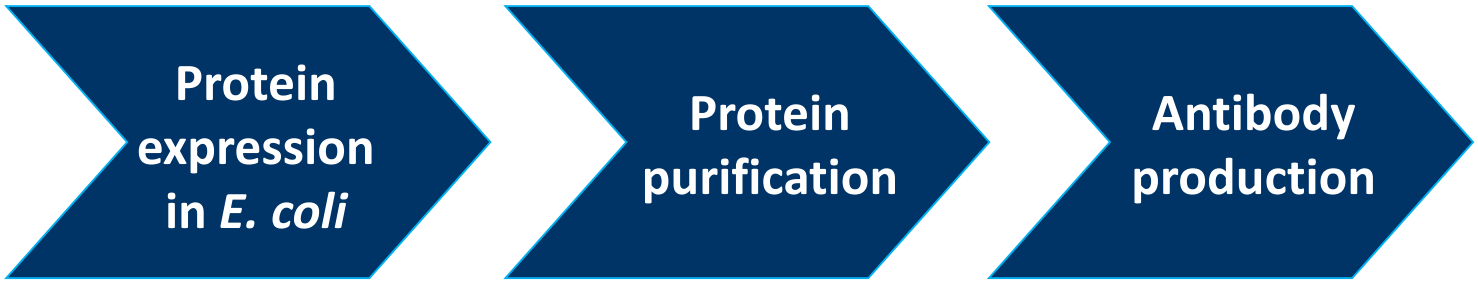
M S P S V Q E V E K L H V L **D R N G D G K V S A E E** L K A F A D D S K **C** P L D S N K I K A F I K E H **D K N K D G K L D L K E** L V S I L S S

H

→ Hypotheses:

- Important for Fh8's stability and production;
- Critical for the immunological response of the Fh8 antigen.

Fh8 and H tags



➡ **Initially:**

- Fh8 and H tags ⬆ **protein expression** in *E. coli*
- H-fused proteins elicited a humoral response: ⬆ **production of polyclonal antibodies**

Fh8 and H tags

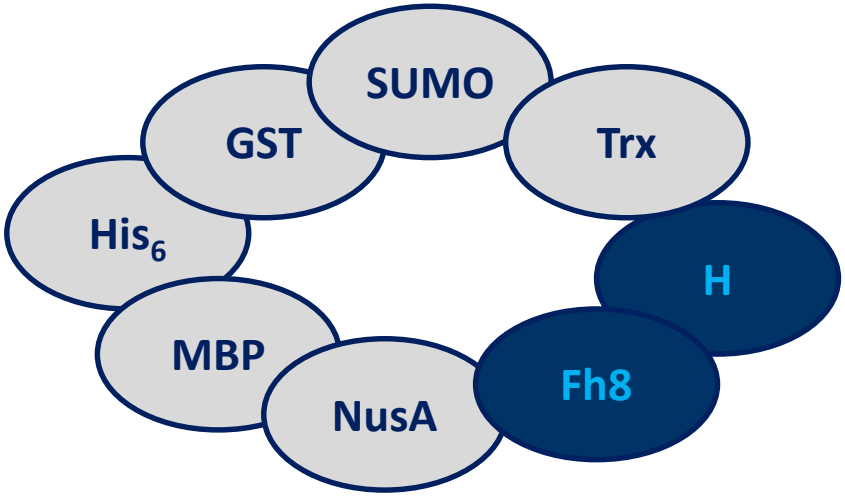
➡ **More recently:**

- **Fh8 and H peptides** as **solubility enhancer partners**, and comparison to the traditionally used solubility tags;
- The **Fh8** usefulness as a **purification handle**.

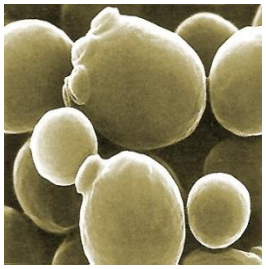
Comparison with traditional fusion tags

Costa, S. J., et al. (2013). *Applied Microbiology and Biotechnology* 97(15): 6779-91.

CP12 - 1 Surface protein from *Cryptosporidium parvum* parasite



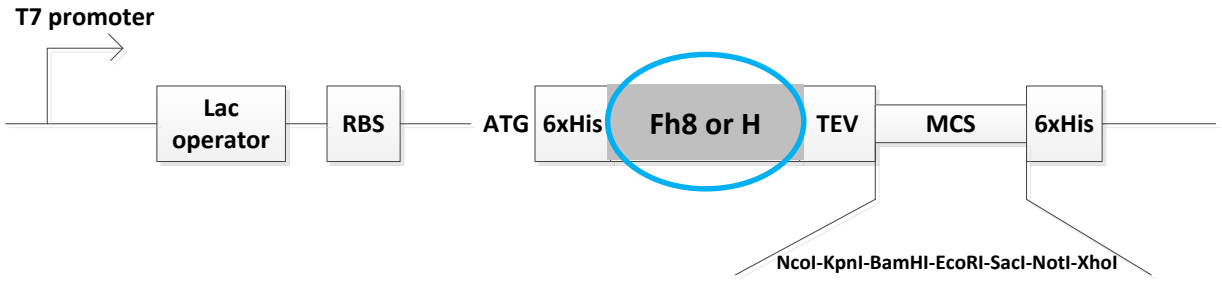
FTL - 1 Lectin from *Artocarpus incisa* plant



YPK1, YPK2, SPO14, RVS167 - 4 proteins from *Saccharomyces cerevisiae* (membrane, cytoplasm, and nucleus)

Comparison with traditional fusion tags

Costa, S. J., et al. (2013). *Applied Microbiology and Biotechnology* 97(15): 6779-91.



pETM Fh8: 10 kDa

pETM H: 3.6 kDa

- **8 tags** (3.4 – 57 kDa) X **6 target proteins** (8.4 – 78 kDa)
- 10 and 500 mL cultures
- 4 *E. coli* strains and 2 induction conditions
- Purification by **nickel affinity**
- Tag removal using the **TEV protease** (or **SenP2** protease for SUMO fusions)

Comparison with traditional fusion tags

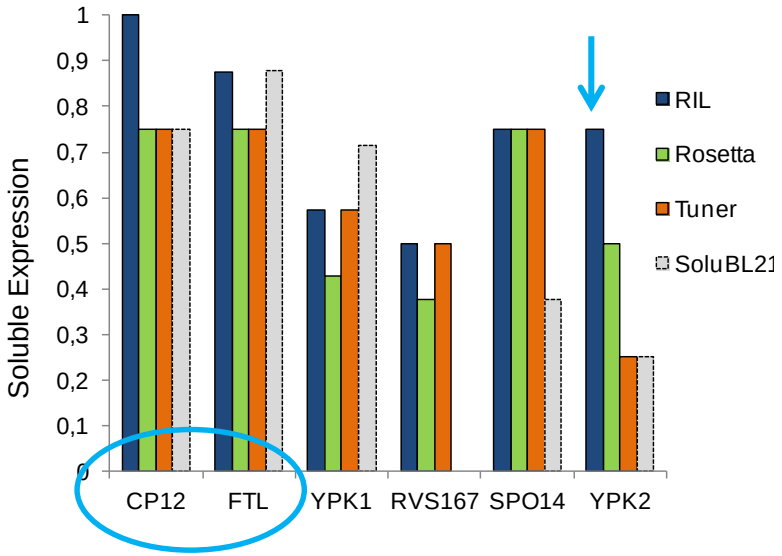
- **Small scale:**

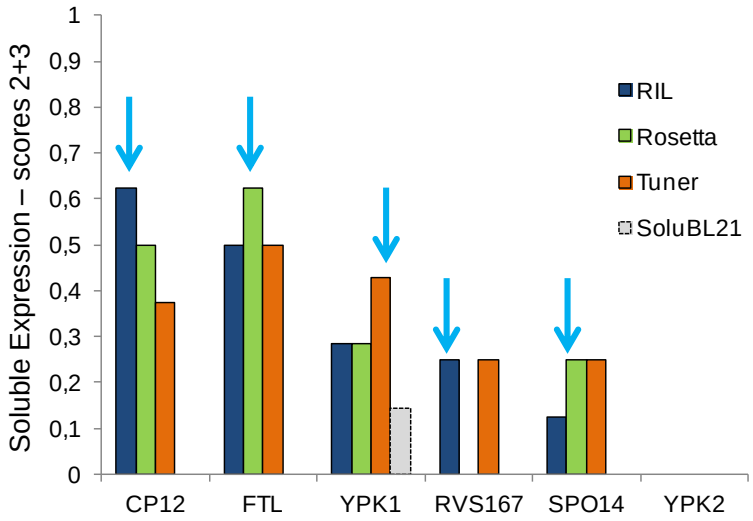
  - SDS-PAGE

  - Bradford

  - Scale 0-3

Costa, S. J., et al. (2013). *Applied Microbiology and Biotechnology* 97(15): 6779-91.





➡**Best induction conditions**: 0.2 mM IPTG, 18 ºC, 20 h (except CP12: 0.5 mM, 28 ºC, 3 h)

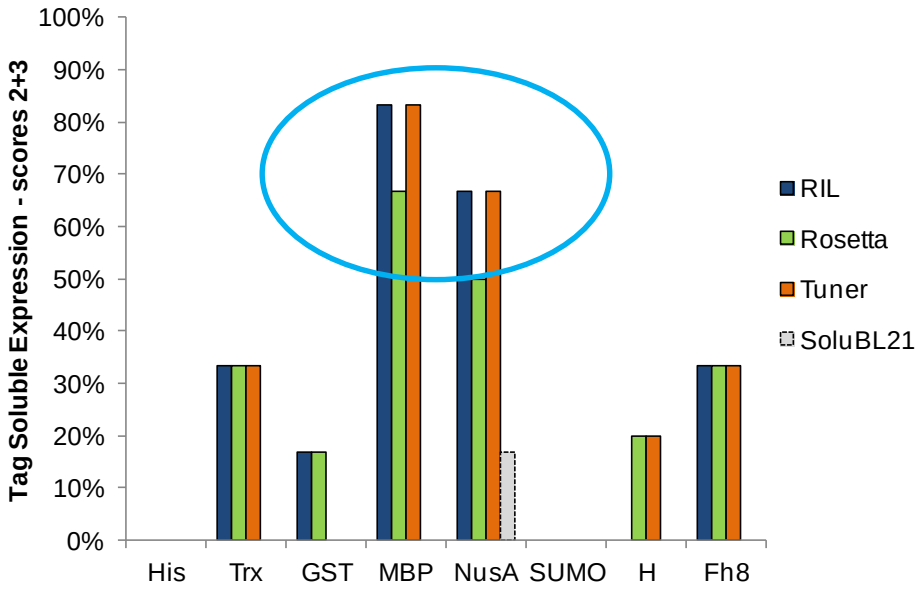
Comparison with traditional fusion tags

- **Small scale:**

Costa, S. J., et al. (2013). *Applied Microbiology and Biotechnology* 97(15): 6779-91.

➡ **Expression**: **MBP ~ NusA ~ Trx > Fh8 ~ H ~ His**

➡ **Solubility**: **MBP > NusA > Fh8 ~ Trx > GST ~H > His**

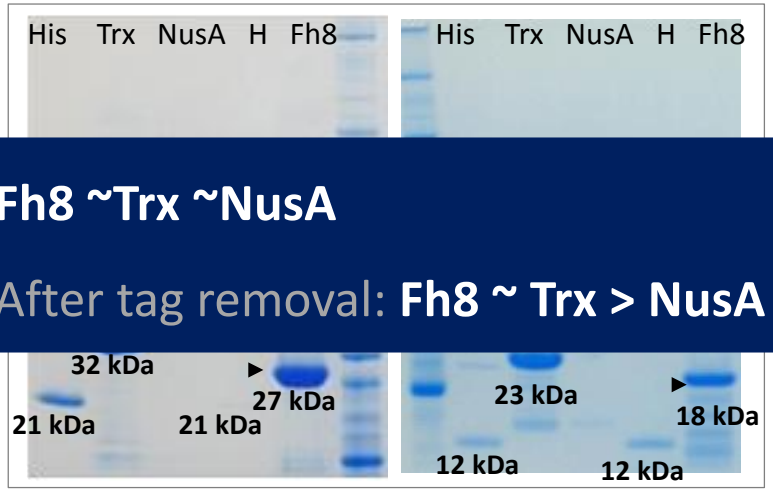


Comparison with traditional fusion tags

- **Large scale:**

Costa, S. J., et al. (2013). *Applied Microbiology and Biotechnology* 97(15): 6779-91.

FTL

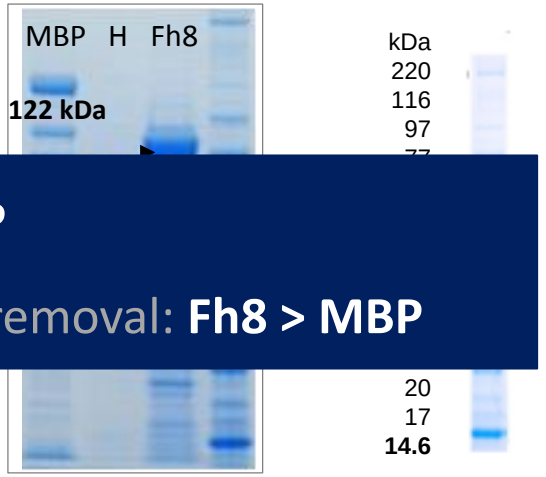


CP12

Fh8 ~Trx ~NusA

After tag removal: **Fh8 ~ Trx > NusA**

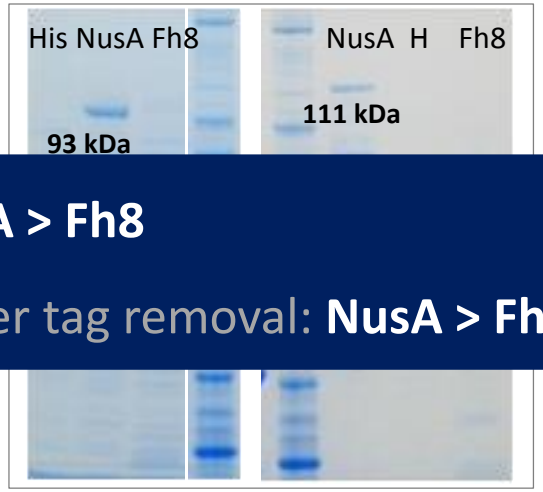
YPK2



Fh8 > MBP

After tag removal: **Fh8 > MBP**

YPK1



RVS167

NusA > Fh8

After tag removal: **NusA > Fh8**

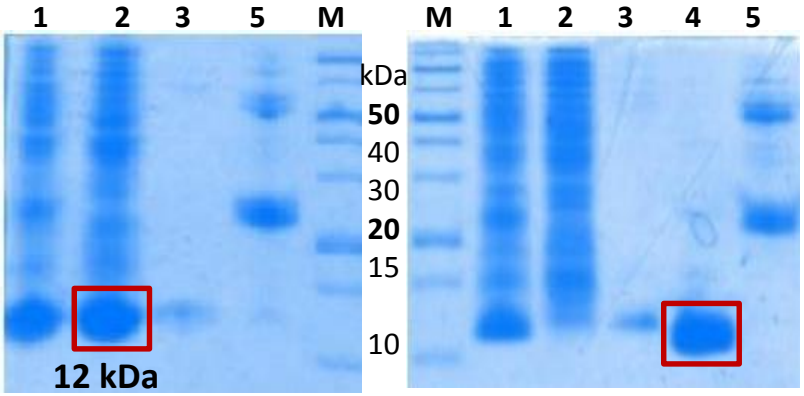
The Fh8 tag as purification handle

Costa, S. J., Coelho, E., et al. (2013). *Protein Expression and Purification*. doi: 10.1016/j.pep.2013.09.013

- **GFP** (green fluorescent protein) and **SOD** (superoxide dismutase)
- **Hydrophobic interaction** *vs* Nickel affinity

➡ **Fh8:**

Without Ca^{2+} **With Ca^{2+}**



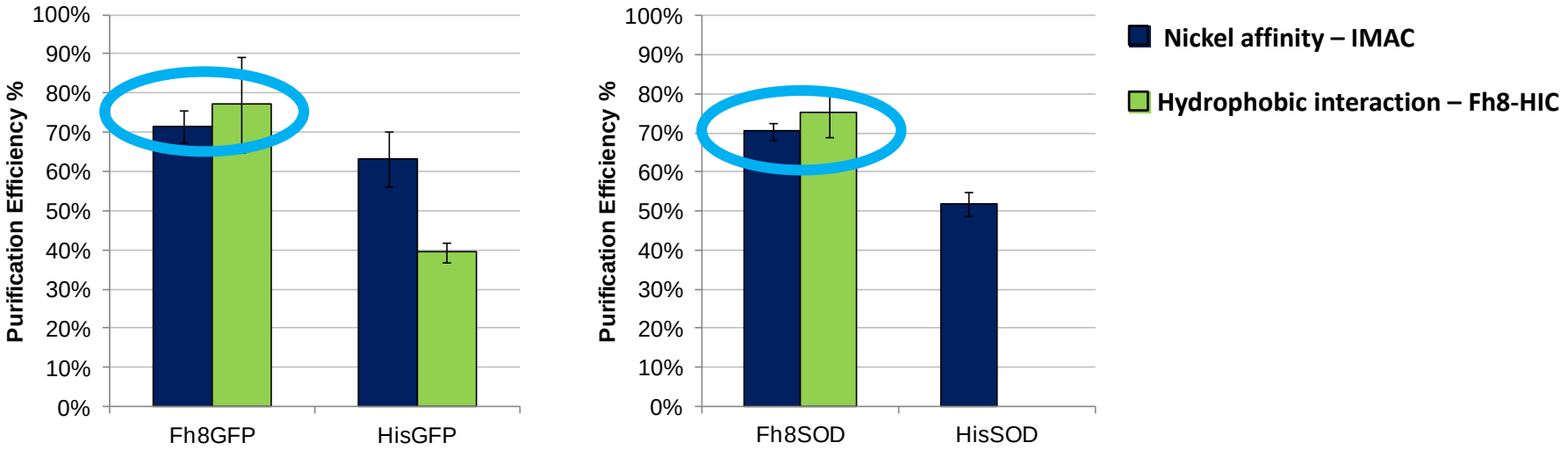
1 –sample loaded onto the purification column
2 – flow-through sample
3 – washing sample
4 – elution with EDTA
5 – elution with pH 10

Ca^{2+}-dependent binding

The Fh8 tag as purification handle

Costa, S. J., Coelho, E., et al. (2013). *Protein Expression and Purification*. doi: 10.1016/j.pep.2013.09.013

➡ **Fh8GFP and Fh8SOD:**

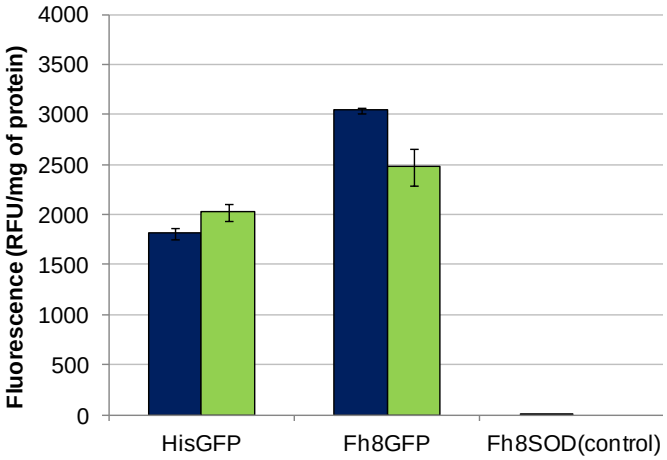


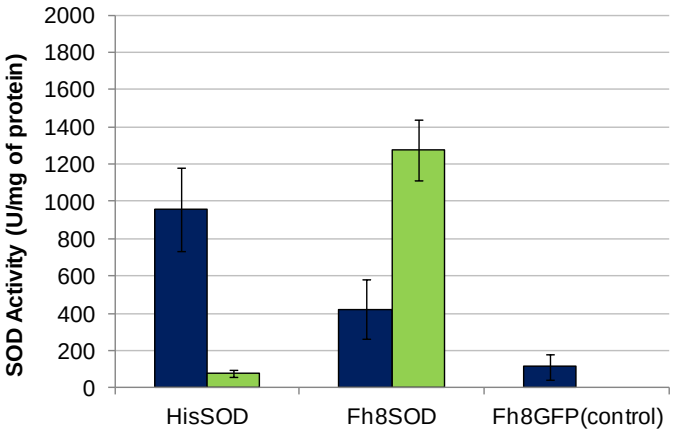
➡ IMAC/HIC or HIC/IMAC: ↑ protein **purity**

The Fh8 tag as purification handle

Costa, S. J., Coelho, E., et al. (2013). *Protein Expression and Purification*. doi: 10.1016/j.pep.2013.09.013

➡ **Fh8GFP and Fh8SOD:**

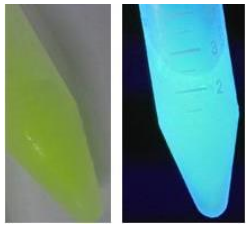




Nickel affinity – IMAC

Hydrophobic interaction – Fh8-HIC

➡ Proteins kept their **biological activity** after purification by Fh8-HIC.



Conclusions

➡ The novel Fh8 tag:

- incresases protein **expression** levels in *E. coli*
- promotes/improves protein **solubility**
- is ranked **among the best** traditionally used solubility enhancer tags
- offers an efficient and economical protein **purification** → one of the few existing tags to **combine solubility enhancer and purification abilities**

Conclusions

➡ **The novel Fh8/H fusion system:**

into the **two lowest molecular weight fusion partners** described so far

Thank you for your attention!

Email: **sofiajuditecosta@gmail.com**

about.me/sofiacosta

Questions?