Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Implications of intracrystalline OC17 on the protection of lattice incorporated proteins

Dot blot analysis for the optimization OC17 expression

Optimization of OC17 expression was tested both in LB medium and TY medium supplemented with 1% glucose. The reason for the addition of glucose depends on the fact that basal protein expression in pET plasmids could be interfered by glucose.[1] Testing at 25 °C and 30 °C and using different IPTG concentrations in a range between 0.1 and 1mM, it was determined that the expression of OC17 is achieved only in LB medium whereas there is no expression in the TY medium. There is negligible expression at in TY medium 30 °C (Fig. S1). Because the *pelB* sequence was used to direct the expressed protein to the periplasmic fraction, it was expected that for the protein to be negligible in the medium. An is seen in the Fig S1, although at low quantities, there is some amount of protein in the medium. This might arise because of the small size of the target protein OC17 which has a molecular weight of around 15 kDa. It should be noted that the secretion into periplasmic space is observed primarily at 30 °C with no protein secretion at 25 °C in both media. As explained in more detail in the manuscript, the expression of OC17 is clearly efficient in LB medium particularly at 30 °C using an IPTG concentration of an 0.1 M. Further optimization with lower IPTG concentrations resulted in enhanced protein expression as described in the main text.

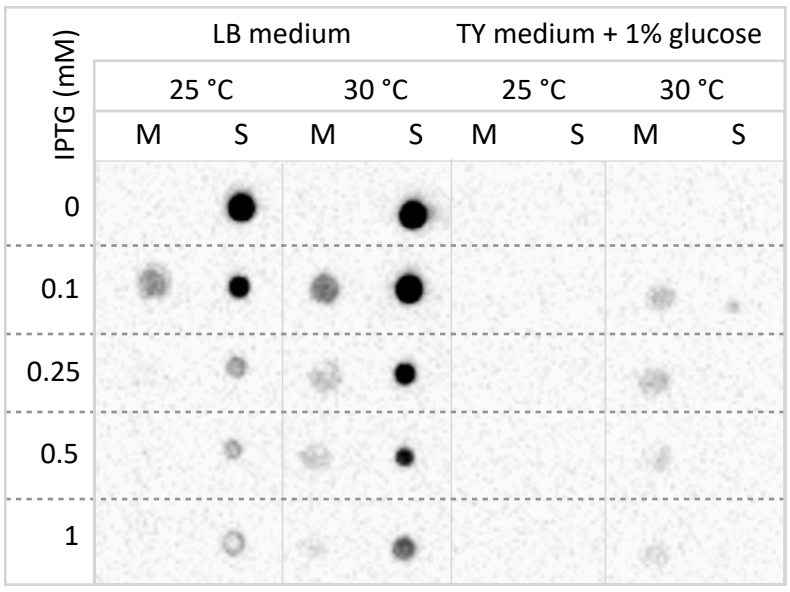

Fig S1. The expression of OC17 in E.coli BL21 cells was tested in two media at two temperatures. The existence of the pelB sequence in the plasmid allows protein secretion into the periplasmic fraction, however, protein was detected in the medium, probably because of the small size of the protein with a molecular weight of 15.3 kDa. IPTG concentrations between 0.1 and 1 mM were tested and detection was done using anti-His tag antibody.

Primers used for OC17 expression and cDNA encoding *OC17* gene

The cDNA encoding OC17 was obtained from the GenBank (KF835610). The primers used for the cloning are given below:

OC-17 forward primer:

caccctcagttcgaaaaaagcgcAGATCCGGATGGTTGTGGTCC

OC-17 reverse primer:

gtcattaatggtgatggtggtgatgGGCTGCTGCTTTACAAACAAATGC

pET26b forward primer:

catcaccaccatcaccattaatgac

pET26b reverse primer:

tgcgctttttttcgaactgagggtg.

The lowercase letters in the OC17 primers show the complementary regions with the plasmid primers.

OC17 cDNA used in this work is given below. Note that some amino acids were optimized for *E.coli* expression.

ATCCGGATGGTTGTGGTCCTGGTTGGGTTCCGACACCTGGTGGTTGTCTGG
GTTTTTTTAGCCGTGAACTGAGCTGGTCACGTGCAGAAAGCTTTTGTCGTC
GTTGGGGTCCGGGTAGCCATCTGGCAGCAGTTCGTAGCGCAGCCGAACTG
CGTCTGCTGGCAGAACTGCTGAATGCAAGCCGTGGTGGTGATGGTAGCGG
TGAAGGTGCAGATGGTCGTGTTTGGATTGGTCTGCATCGTCCGGCAGGTAG
CCGTAGCTGGCGTTGGAGTGATGGCACCGCACCGCGTTTTGCAAGCTGGCA
TCGTACCGCAAAAGCACGTCGTGGTGGTCGTTGTGCAGCACTGCGTGATGA

AGAAGCATTTACCAGCTGGGCAGCACGTCCGTGTACCGAACGTAATGCATTT
GTTTGTAAAGCAGCAGCC

Reference

1. R. Novy, B. Morris, Use of glucose to control basal expression in the pET system, *inNovations*. 2001, 13, 8-10. (Newsletter of Novagen Inc. Online access 18 November 2022)
https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/386/947/innovations-013-nvg.pdf