

Supplementary Table 1. Properties of bacterial IBs adjustable through process conditions (extended version of Table 1).

Parameter	Culture conditions	Recombinant protein ( <i>E. coli</i> strain)	S vs. IS (%)	IBs structural features	Ref.
Culture time	Bioreactor, fed-batch (37 °C), IPTG induction	$\beta$ -lactamase (K12, HMS174-DE3)	5-95	IBs increased in median diameter from 325 nm to 410 nm, 2 and 6 h after induction.	(Margreiter, <i>et al.</i> , 2008)
	Batch (37 °C), IPTG induction	hGH (M15)	N.D.	IB size incremented from 200 up to 800 nm after 4 h of induction. IBs showed strong binding with CR and Th-T, and were more resistant to proteolysis and denaturation.	(Upadhyay, <i>et al.</i> , 2012)
	Bioreactor, batch (37 °C), IPTG induction	Sphingomyelinase-D (BL21-Gold-DE3)	3–97	IBs increased in a median diameter from 450 nm (1 h post-induction) to 600 nm (3 h post-induction).	(Castellanos-Mendoza, <i>et al.</i> , 2014)
Inductor concentration	Bioreactor, fed-batch (37°C), 1.0 $\mu$ M IPTG / g dry biomass	$\beta$ -lactamase (K12, HMS174-DE3)	2–98	Similar aggregation, increasing ~25 % RP content in IBs.	(Margreiter, <i>et al.</i> , 2008)
	Bioreactor, fed-batch (37°C), 20 $\mu$ M IPTG / g dry biomass		5–95	Similar aggregation, lower RP content in IBs.	
	Shake flask, batch (37 °C) 0.01 mM IPTG	XynB (BL21)	13–87	Decreased aggregation.	(Jhamb & Sahoo, 2012)
	Shake flask, batch (37 °C) 1.0 mM IPTG		4-96	Increased IB formation.	
	Batch (37°C), 0.1 mM IPTG	GFP	N.D.	Lower hydrodynamic diameter.	(Luo, <i>et al.</i> , 2006)
	Batch (37°C), 2.0 mM IPTG		N.D.	Hydrodynamic diameter increased from 550 to 645 nm.	
Growth rate	Shake tubes, batch (37 °C)	EGFP (DH5- $\alpha$ )	N.D.	~5-10 times more RP are in IBs at the fastest growth rate.	(Iafolla, <i>et al.</i> , 2008)
Temperature	Batch (30 °C), <i>trp</i> promoter	IFN- $\alpha$ 2 (HB101)	73-27	Less RP aggregation	(Schein & Noteborn, 1988)
	Batch (37 °C), <i>trp</i> promoter		5-95	Increase in temperature promoted RP aggregation	
	Batch (30 °C), <i>trp</i> promoter	IFN- $\gamma$ (C600/ $\lambda$ -lys)	95-5	Less RP aggregation	
	Batch (37 °C), <i>trp</i> promoter		18-82	Increase in temperature promoted RP aggregation	
	Bioreactor, batch (30°C in growth phase), thermoinduction at 39 °C	SpA- $\beta$ gal (RR1 lacZAM15)	N.D.	Lower IBs formation at initial post-induction time.	(Strandberg & Enfors, 1991)
	Bioreactor, batch (30°C in growth phase), thermoinduction at 42°C		N.D.	The highest accumulation of IBs, formed mainly during the first hours after induction.	
	Shake flask, batch (30°C), 0.1 mM IPTG		99–1	Less RP aggregation.	

	Shake flask, batch (37 °C), 0.1 mM IPTG	OmpA- $\beta$ -lactamase (RB791)	60-40	Impurities were lower in IBs from cultures at 37 °C vs. 42 °C. The highest IBs concentration.	(Valax & Georgiou, 1993)
	Shake flask, batch (42°C), 0.1 mM IPTG		83-17	Temperature increase promoted IB formation.	
	Batch (37 °C by 4 h), after 1 mM IPTG cultures were set to 18 °C	A $\beta$ 42(F19D)-GFP	N.D.	Less RP aggregation and high specific fluorescence.	(de Groot & Ventura, 2006)
	Batch (37 °C by 4 h), after 1 mM IPTG cultures were set to 25°C		N.D.	The formed fluorescent IBs were solubilized and denatured faster by proteases and chaotropic agents, than those from cultures at 42 °C.	
	Batch (37 °C by 4 h), after 1 mM IPTG cultures were set to 37 °C		N.D.	Increase in temperature promoted IB formation and decreased their fluorescence	
	Batch (37 °C by 4 h), after 1 mM IPTG cultures were set to 42 °C		N.D.	Increase in temperature promoted IB formation and depleted their fluorescence.	
	Shake flask, batch (25°C), 0.4 mM IPTG	G-CSF	2-98	Proteins inside IBs formed at 25°C presented similar structure as the native versions, with increased extractability in mild detergents, compared with those from 37°C and 42°C.	(Jevsevar, <i>et al.</i> , 2005, Peternel, <i>et al.</i> , 2008)
		GFP	33-77		
		His7dN6TNF- $\alpha$	60-40		
	<b>pH</b>	Bioreactor, batch (30°C in growth phase, thermoinduction at 39°C)	SpA- $\beta$ gal (RR1 lacZAM15)	N.D.	When pH decreased to ~5.5, IB accumulation was triggered, reaching up to 30 % of cell dry weight.
Bioreactor, batch (30°C in growth phase, thermoinduction at 42°C)		N.D.		When pH decreases to ~5.5, IBs formation increased vs. 39°C, reaching up to 75 % of cell dry weight.	
Bioreactor, batch (37 °C), 0.1 mM IPTG. pH 7.5		Sphingomyelinase-D (BL21-Gold-DE3)	0-100	IBs were more resistant to proteolysis and denaturation.	(Castellanos-Mendoza, <i>et al.</i> , 2014)
Bioreactor, Batch (37 °C), 0.1 mM IPTG. pH uncontrolled (reach pH 8.5)			0-100	IBs grown faster and were less resistant to proteolysis and denaturation.	
Bioreactor, batch (37 °C), 0.1 mM IPTG. pH 7.5, and set to 8.5 after induction		Phospholipase A2 (Origami™)	0-100	IBs presented more $\alpha$ -helices, were solubilized faster by proteinase-K and bonded less Th-T.	(Calcines-Cruz, <i>et al.</i> , 2018)
Bioreactor, batch (37 °C), 0.1 mM IPTG. pH 7.5, and set to 6.5 after induction		Phospholipase A2 (Origami™)	0-100	IBs presented less $\alpha$ -helices, were less solubilized by proteinase-K and bonded more Th-T.	
<b>Agitation</b>	Shake flask, batch (37 °C), 0.1 mM IPTG, orbital (200 rpm)	Phospholipase A2 (BL21-Gold-DE3)	0-100	IBs showed sizes of ~400 nm with less $\alpha$ -helices fraction, compared with those formed under resonant acoustic.	(Valdez-Cruz, <i>et al.</i> , 2017)
	Shake flask, batch (37 °C), 0.1 mM IPTG, resonant acoustic (20 g)		0-100	Diffused protein clusters were seen inside cells. IBs at 20 g were the most degraded after 120 min.	

Human interferon- $\alpha$ 2 (IFN- $\alpha$ 2); Interferon- $\gamma$  (IFN- $\gamma$ ); Human growth hormone (hGH); Xylanase (XynB); Alzheimer-related peptide Ab42 mutant fused to green fluorescent protein (Ab42(F19D)-GFP); Green fluorescent protein (GFP); Protein A from *Staphylococcus aureus* and  $\beta$ -galactosidase (SpA  $\beta$ -gal); Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG); Not determined (N.D.); guanidinium chloride. (GnCl); CR: Congo red, Thioflavin-T (Th-T); S vs. IS: Soluble versus insoluble protein fractions; Inclusion bodies (IBs); Recombinant protein (RP).

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