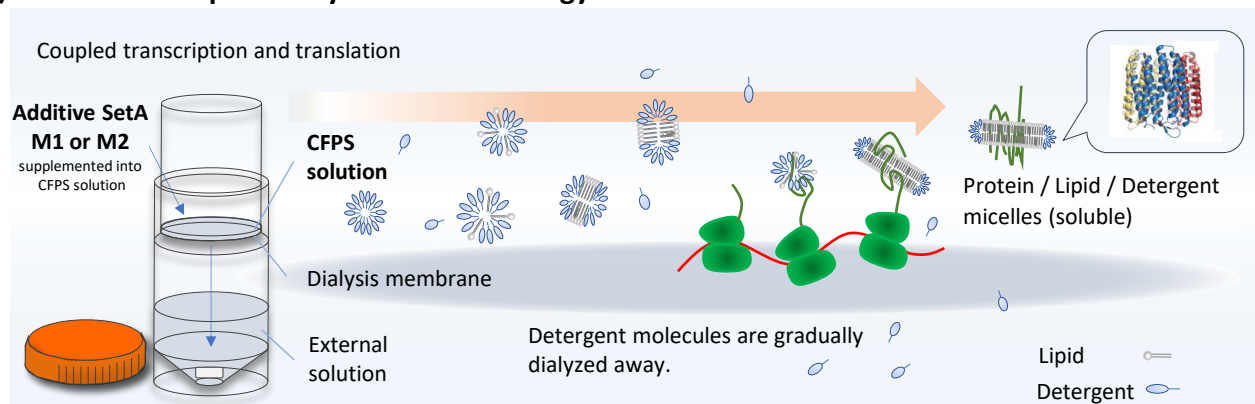


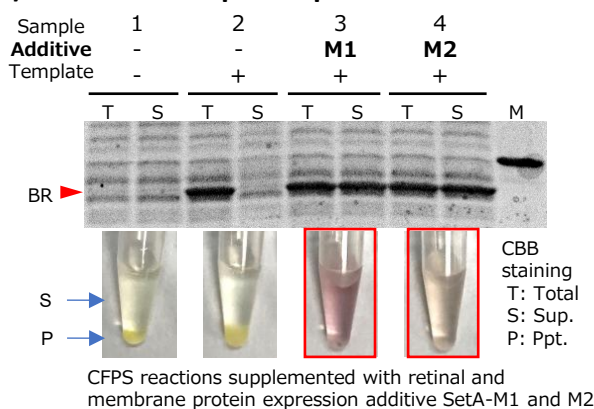
Application Note

Membrane proteins

(a) S-MF Cell-free protein synthesis technology



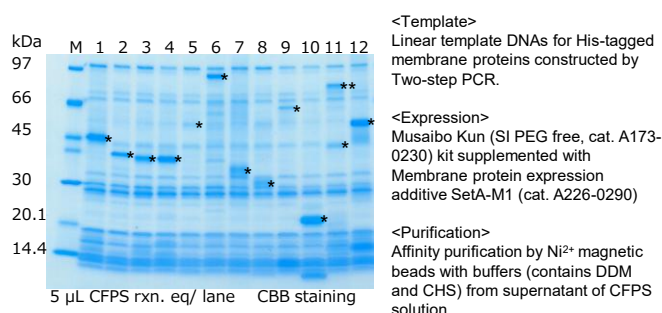
(b) Bacteriorhodopsin expression



Cat. no	Product	Size	Cat. price
A226-0290	Membrane protein expression additive SetA M1: PC + Digitonin M2: PC + Brij®S20	1 kit	28,000 JPY



(c) Human membrane protein expression



Lane	Category	Protein	UniProtID	MW (kDa)	TM ^{*1}
1	GPCR	ADRB2 β 2-adrenergic receptor	P07550	46.5	7
2		ET-AR endothelin receptor	P25101	46.5	7
3		CXCR1 interleukin-8 receptor	P25024	39.8	7
4		CCR7 C-C chemokine receptor	P32248	40.3	7
5	Non-GPCR	TNFR1 tumor necrosis factor receptor	P19438	48.3	1
6	Channel	GluR2 ionotropic glutamate receptor	P42262	96.2	4
7		Kir6.2 K ⁺ channel	Q14654	43.5	2
8		AQP9 H ₂ O channel	O43315	31.4	6
9	Transporter	BCRP ATP-binding cassette transporter, urate exporter	Q9UNQ0	72.3	6
10	Cell adhesion	claudin-4 tight junction protein	O14493	22.1	4
11	Other	SPP intramembrane protease (dimer)	Q8TCT9	41.5	9
12		PS1 a subunit of gamma-secretase complex	P49768	52.7	9

Summary

Bacteriorhodopsin (BR) and various human membrane proteins were successfully synthesized by S-MF cell-free protein synthesis (CFPS) technology developed by RIKEN^{*1}.

- The technology consists of dialysis-mode CFPS kit and membrane protein expression additives (M1 and M2) that include phospholipid and detergent. The additive is supplemented into the CFPS solution of dialysis-mode CFPS reaction, then the reaction is initiated. The synthesized membrane protein is folded with the help of lipids and solubilized by detergent in early phase. Then, detergent molecules are gradually dialyzed out into the external solution, forming proteomicelles (protein/lipid/detergent).
- BR was expressed using S-MF CFPS. BR expressed well even without any membrane additives and it was totally insoluble (sample2). In the presence of additive M1 or M2, BR expressed well and was soluble (sample 3 and 4). The purple color of the supernatant indicated that the BR was correctly folded and complexed with its co-factor retinal. In case of BR, sample 3 developed a deeper color than sample 4, thus additive M1 worked better than M2.
- Various human membrane proteins were expressed using S-MF CFPS with the M1 additive. Template DNAs were constructed by two-step PCR from human cDNAs (with human native codons). Proteins were synthesized by CFPS with the M1 additive, then roughly purified using His-affinity magnetic beads. The eluate from the beads was analyzed by PAGE. An asterisk indicates band for the target protein. For SPP, bands at double asterisks and single asterisk were supposed to be dimeric and monomeric products, respectively.

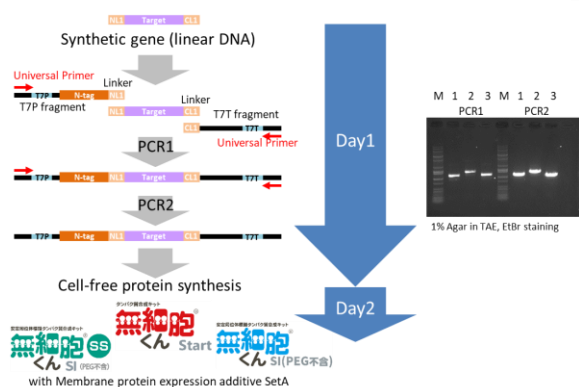
^{*1} Shinoda, T. et al., *Sci. Rep.* 6, 30442 (2016).

SARS-nCoV-2 proteins (Recombinant-free expression)

(a) Target protein

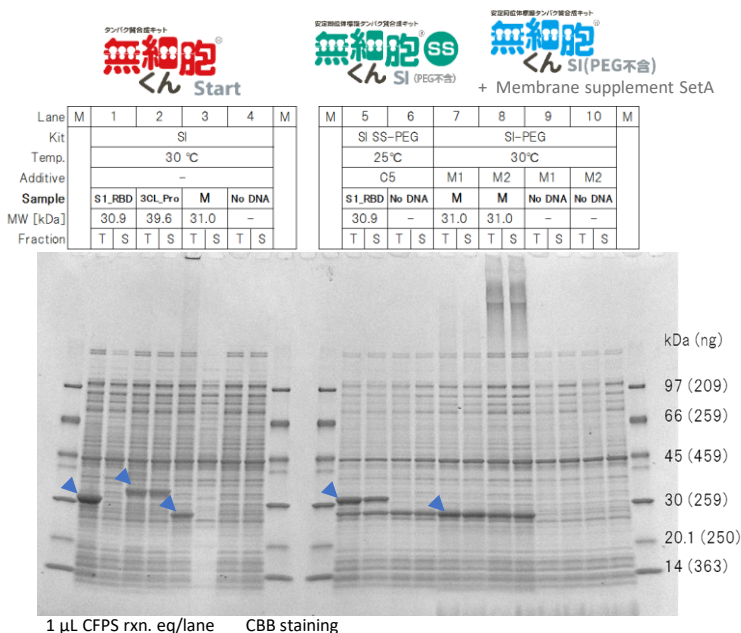
#	Name	UniProtID	Notes
1	S1_RBD	PODTC2(319-541)	223 a.a., Extracellular, with 4 SS bonds
2	3CL_Pro	PODTD1(3264-3569)	306 a.a., Cellular, with many Cys
3	M	PODTC5(1-222)	222 a.a., Membrane, 4TM

(b) Template preparation for CFPS



(c) Initial expression trial

(d) Optimization



Summary

Recombinant-free protein expression can be achieved by combining template construction by PCR and cell-free protein synthesis (CFPS). This procedure does not require the use of any recombinant bacterial cells, thus it is not restricted by Cartagena Protocol, and proteins can be safely and quickly expressed. As an example, SARS-nCoV2 proteins expression trials and CFPS condition optimization trials are shown.

- Target proteins are the S1_RBD extra cellular domain, 3CL protease domain, and membrane protein M of SARS-nCoV-2 virus.
- A gene fragment for each protein was prepared using a commercial gene synthesis service with codon optimization for *E. coli*. Then, transcription-translation control sequences, such as T7 promotor, RBS and T7 terminator, and N-Tag sequence were concatenated to the gene fragment by two-step PCR.
- As an initial expression trial, the three proteins were expressed using a CFPS kit (Musaibo Kun Start, cat. A183-0242), which is suitable for small-scale (100 μ L) expression trials. Expression of all the three proteins was confirmed, and S1_RBD and M were insoluble. The Musaibo Kun Start CFPS kit has a reductive reaction condition designed for intracellular protein, so it seemed not to match for S1_RBD (extracellular protein) and M (membrane protein).
- Reaction conditions for S1_RBD and M were further optimized. The solubility of S1_RBD was improved by a reaction condition for disulfide-bonded proteins using CFPS kit (Musaibo Kun SI SS PEG-free, cat. A241-0307) with a customized reduction condition C5 (fairly oxidative condition) at a low temperature (25°C). The membrane protein M was expressed solubly by reaction conditions for membrane proteins using CFPS kit (Musaibo Kun SI PEG-free, cat. A173-0230) supplemented with membrane protein expression additive SetA-M1 or -M2 (cat. A226-0290).

