再構成型無細胞タンパク質合成系 (PURE*frex*®) を用いた糖タンパク質合成

Synthesis of glycosylated proteins using PURE*frex*®

◦松本 令奈[1], 丹羽 達也[2], 田口 英樹[2], 金森 崇[1]
([1]ジーンフロンティア株式会社、[2]東工大・研究院・細胞センター)

PUREfrexは、PURE systemを基にした大腸菌でのタンパク質合成に関与する因子のみから再構成した無細胞タンパク質合成系である。PUREfrexでは、ポリペプチドの合成のみが行われるが、適切な追加因子を使用することで翻訳後修飾も可能である。本発表では、糖転移酵素および基質となる糖供与体を用い、PUREfrexでN型糖鎖修飾タンパク質の合成を行った。糖鎖修飾反応はJewettらの方法を参考にした(Kinghtlinger et al.(2019))。初めに、大腸菌Colicin-E7 immunityproteinにN型糖鎖付加配列を挿入したモデルタンパク質(Im7-6)、ならびに数種類の糖転移酵素をPUREfrexでそれぞれ合成し、これらと糖供与体を混合してIm7-6の糖鎖修飾反応 (in vitro glycosylation (IVG)) を行った。反応後のSDS-PAGEおよび質量分析により、糖鎖付加配列中のアルギニン残基へのグルコース付加を起点とし、ガラクトース、GlcNAc、シアル酸の付加を確認した。さらに、Im7-6と糖転移酵素の合成および糖鎖修飾反応をone-potで同時に行ったところ、この方法でも糖鎖修飾されたIm7-6を確認できた。また、ジスルフィド結合を含むタンパク質や膜タンパク質に対しても、糖鎖付加が可能であることを確かめた。この結果より、PUREfrexでも糖タンパク質を合成できることが示された。

◦ Rena Matsumoto, Tatsuya Niwa, Hideki Taguchi and Takashi Kanamori
([1]GeneFrontier Corp., [2]Cell Biology Center, IIR, Tokyo Tech)

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PURE*frex*®

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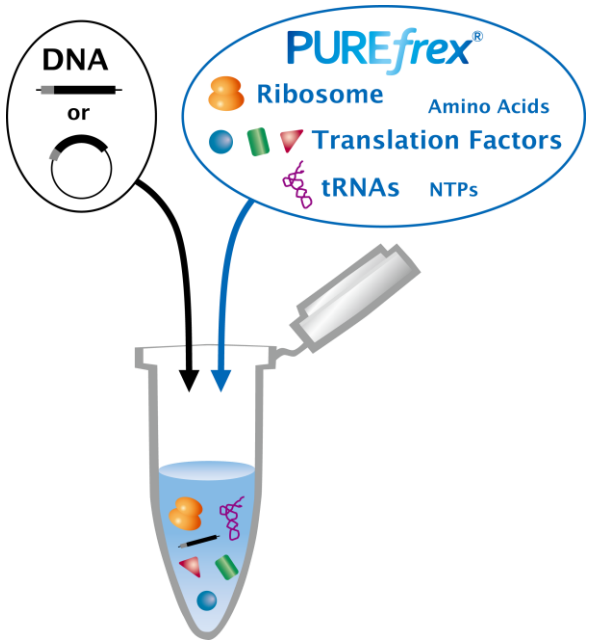
Translation process



Reconstituted cell-free protein synthesis system based on the PURE system technology

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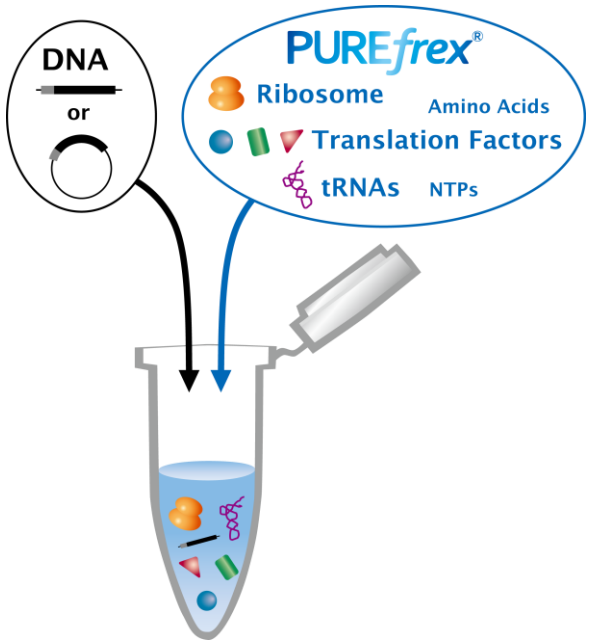
Translation process



Reconstituted cell-free protein synthesis system based on the PURE system technology

Polypeptides

Polypeptide ≠ Functional protein

Synthesis of functional proteins using PURE*frex*®

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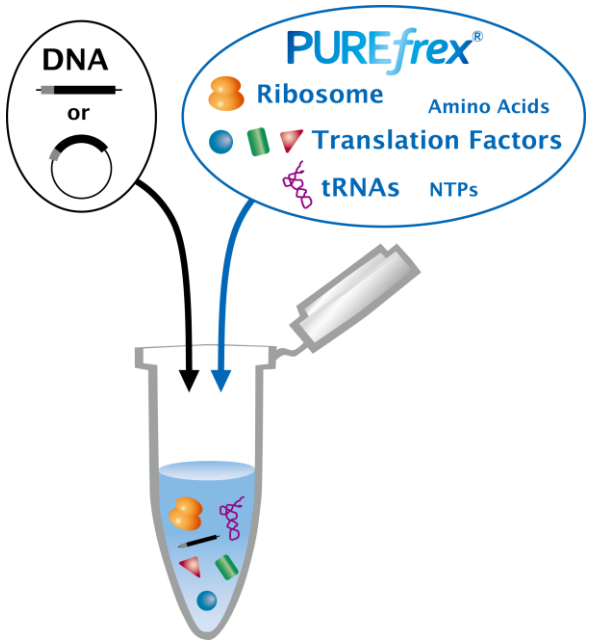
Translation process



Reconstituted cell-free protein synthesis system based on the PURE system technology

Maturation process

- Formation of higher-order structure
  - Folding of polypeptide
  - Formation of complex
- Post-translational modification
  - Formation of disulfide bonds
  - Phosphorylation
  - Glycosylation
  - Lipidation
  - Processing
- Localization
  - Insertion into membrane
  - Translocation across membrane

Functional proteins

Synthesis of functional proteins using PURE*frex*®

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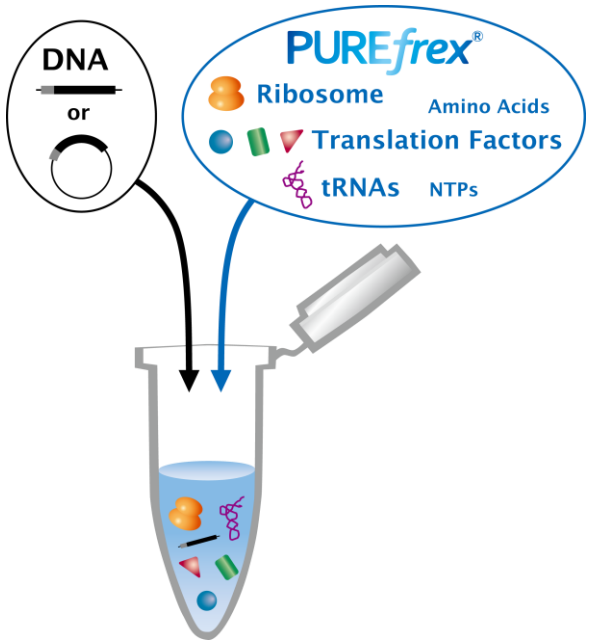
Translation process



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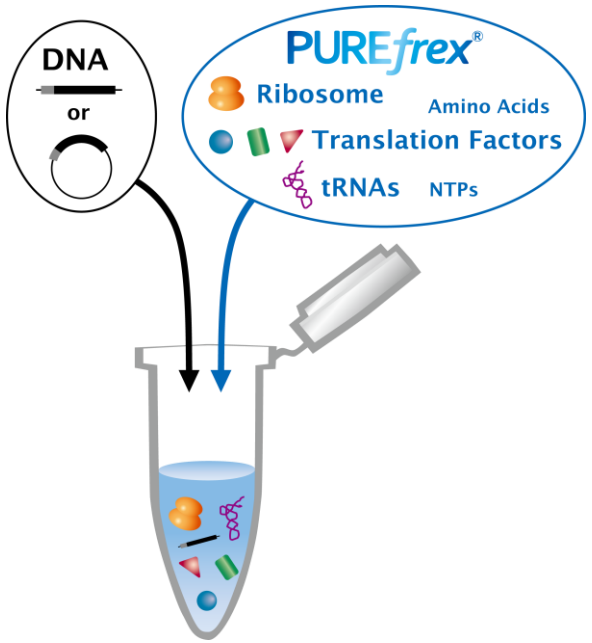
Synthesis of functional proteins using PURE*frex*®

GeneFrontier

Translation process



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Functional proteins

in vitro Glycosylation (IVG) of target proteins combined with Cell-Free Protein synthesis (CFPS) by PURE*frex*®

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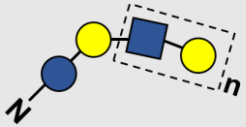
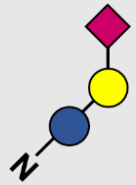
Glycosylation		Glycosyltransferase	Sugar donor
Glc		ApNGT (*A. pleuropneumoniae*)	
Glc/Gal		ApNGT (*A. pleuropneumoniae*)	UDP-Glucose
		NmLgtB (*N. meningitidis*)	UDP-Galactose
Glc/Gal/ GlcNAc		ApNGT (*A. pleuropneumoniae*)	UDP-Glucose
		NmLgtB (*N. meningitidis*)	UDP-Galactose
		LgtA (*N. meningitidis*?)	UDP-GlcNAc
Glc/Gal/Sia (α2-6)		ApNGT (*A. pleuropneumoniae*)	UDP-Glucose
		NmLgtB (*N. meningitidis*)	UDP-Galactose
		PdST6 (*P. damsale*) or ST6Gal1 (*H. sapiens*)	CMP-Sialic acid
Glc/Gal/Sia (α2-3)		ApNGT (*A. pleuropneumoniae*)	UDP-Glucose
		NmLgtB (*N. meningitidis*)	UDP-Galactose
		CjCST-I (*C. jejuni*)	CMP-Sialic acid

in vitro Glycosylation (IVG) of target proteins combined with Cell-Free Protein synthesis (CFPS) by PURE*frex*®

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Cell-free Protein Synthesis (CFPS)

PURE*frex*®

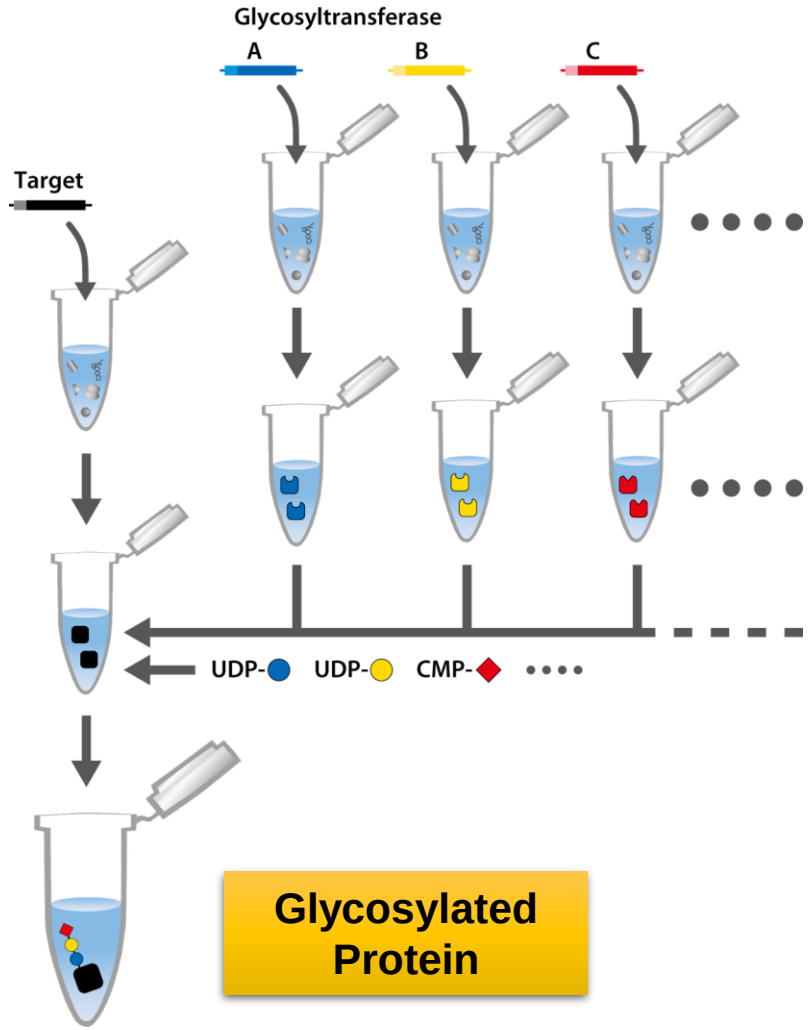
↓

in vitro Glycosylation (IVG)

↓

The glycosylation reaction was based on the method of Jewett et al. (Kinghtlinger et al. (2019) *Nat. Commun*., 10, 5404).

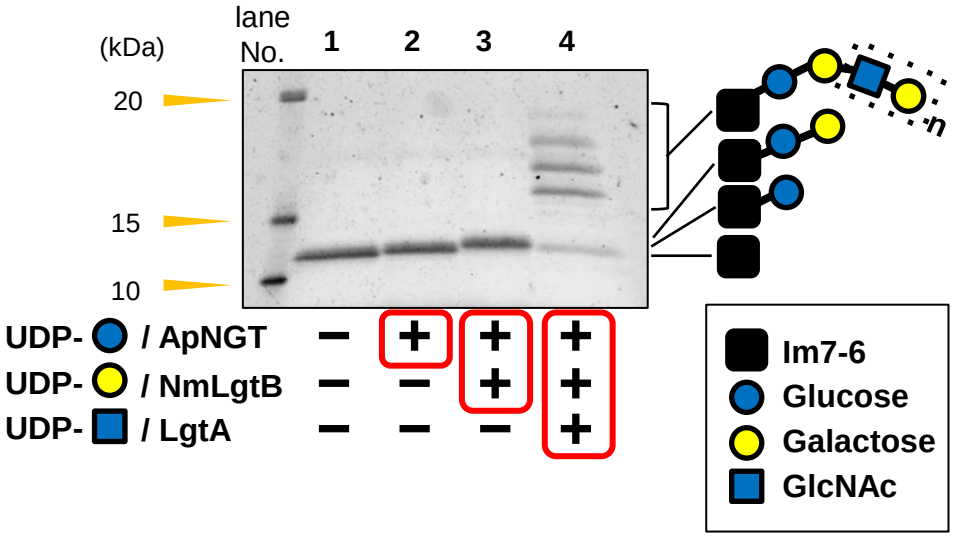
Glycosylated Protein



in vitro Glycosylation of model protein (IVG)

Colicin-E7 immunity protein mutant (Im7-6) (*E. coli*)

Length	101 a.a.
Molecular weight	11,366 Da



MS analysis

Im7-6 [21-49] EIEKEATTGGNWTTAGGDVLDVLLEHFVK

Modification	Theo. MH+ [Da]	Number of PSMs*			
		lane 1	lane 2	lane 3	lane 4
-	3129.5739	50	1	8	2
Glc	3291.6267	0	33	7	9
Glc-Gal	3453.6795			106	48
Glc-Gal-(GlcNAc-Gal)$_1$	3818.8117			0	29
Glc-Gal-(GlcNAc-Gal)$_2$	4183.9439			0	49
Glc-Gal-(GlcNAc-Gal)$_3$	4549.0761			0	30

*Number of PSMs (Peptide Spectrum Matches) was calculated as the average value of three measurements.

(The same applies to the following experiments.)

IVG with PURE*frex*® could provide the N-glycosylation of Im7-6.

in vitro Glycosylation of model protein (IVG)

Colicin-E7 immunity protein mutant (Im7-6) (*E. coli*)

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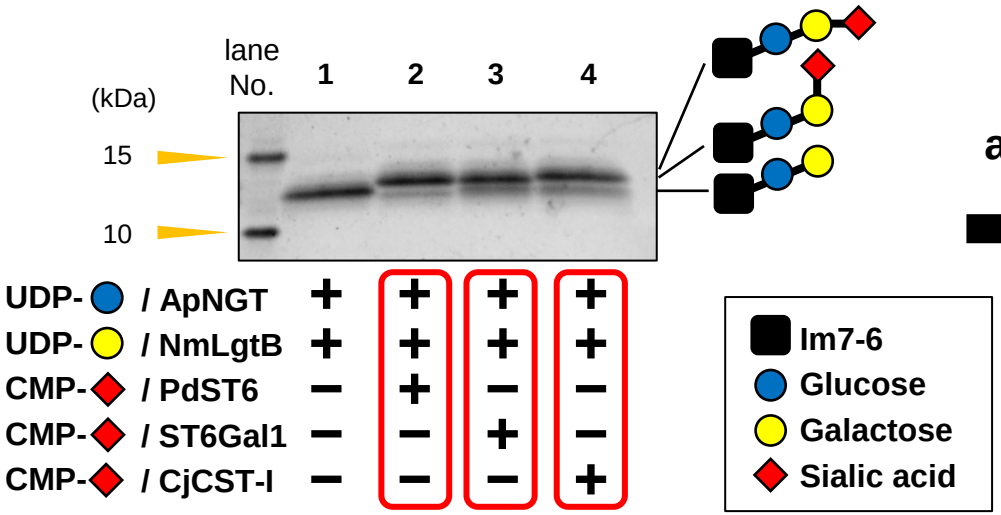

MS analysis

Im7-6 [21-49] EIEKEATTGGNWTTAGGDVLDVLLEHFVK

Modification	Theo. MH+ [Da]	Number of PSMs			
		(control)	lane 2	lane 3	lane 4
-	3129.5739	92	7	5	4
Glc	3291.6267	0	30	25	30
Glc-Gal	3453.6795	0	36	74	69
Glc-Gal-Sia	3744.7750	0	77	74	72

IVG with PURE*frex*® could provide the addition of sialic acid to Im7-6.

One-pot IVG

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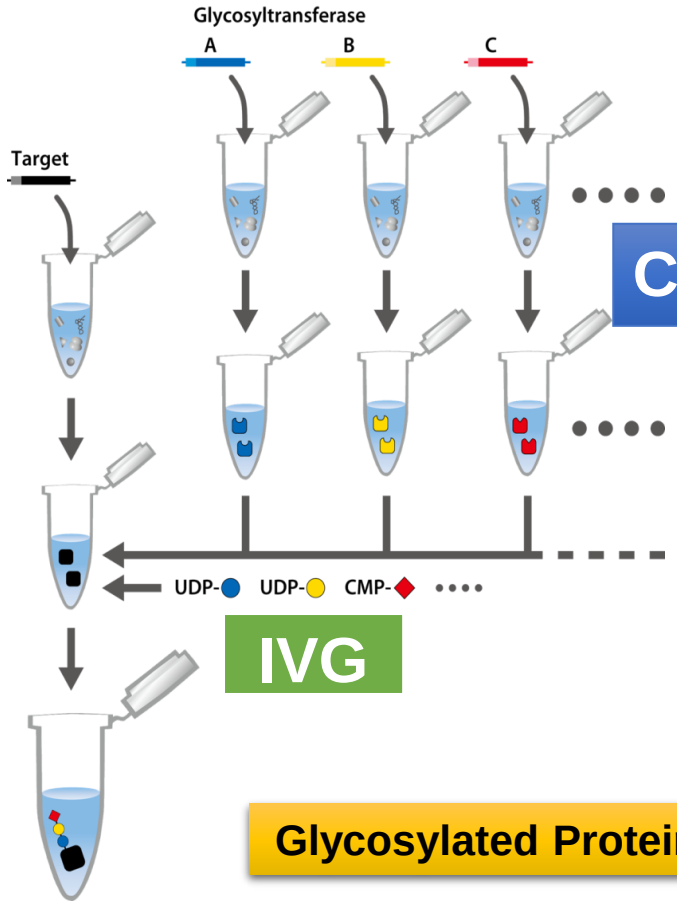
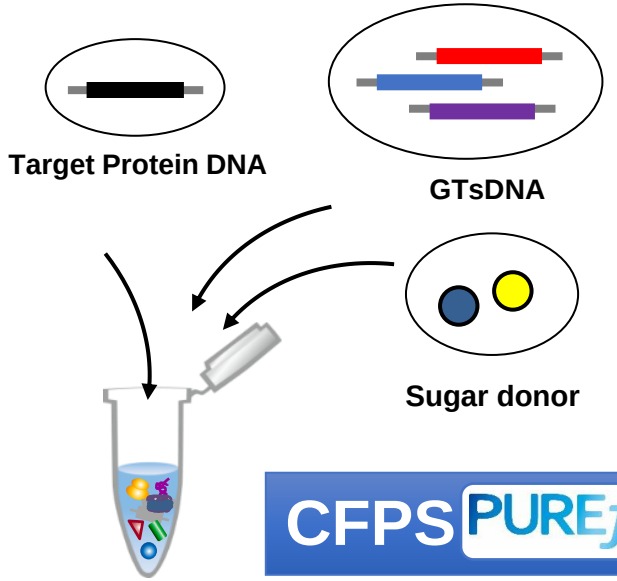
IVG



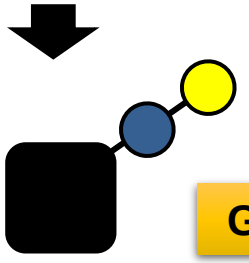
CFPS PURE*frex*®

Glycosylated Protein

One-pot IVG



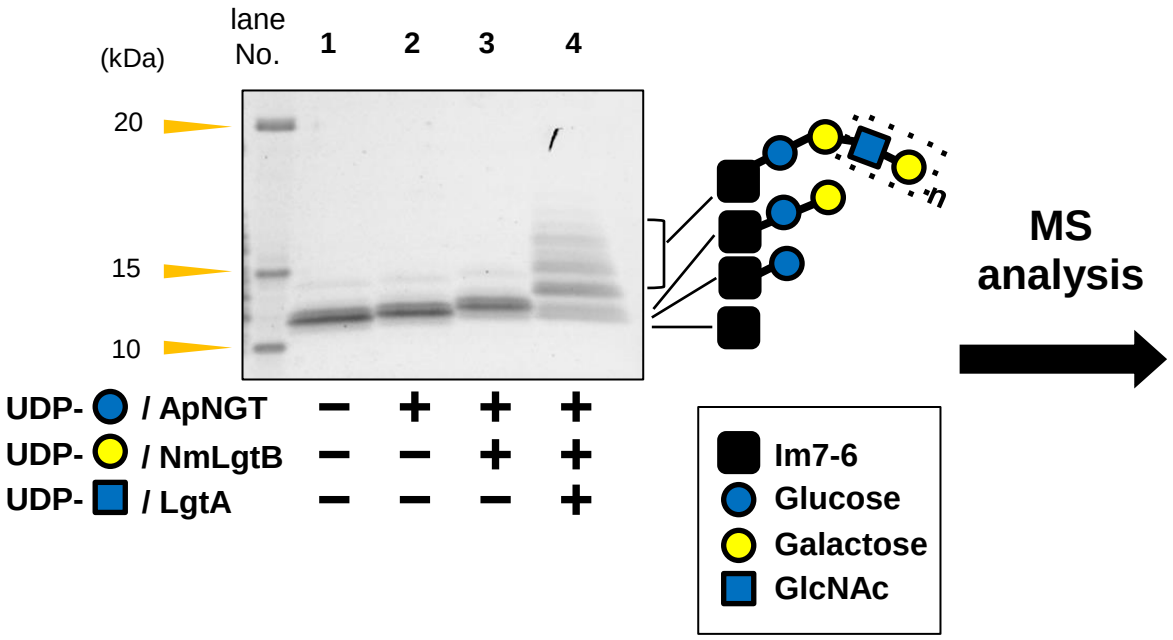
CFPS PURE*frex*® + IVG



Glycosylated Protein

One-pot IVG

Colicin-E7 immunity protein mutant (Im7-6) (*E. coli*)



MS analysis

Im7-6 [21-49] EIEKEATTGGNWTTAGGDVLDVLLEHFVK

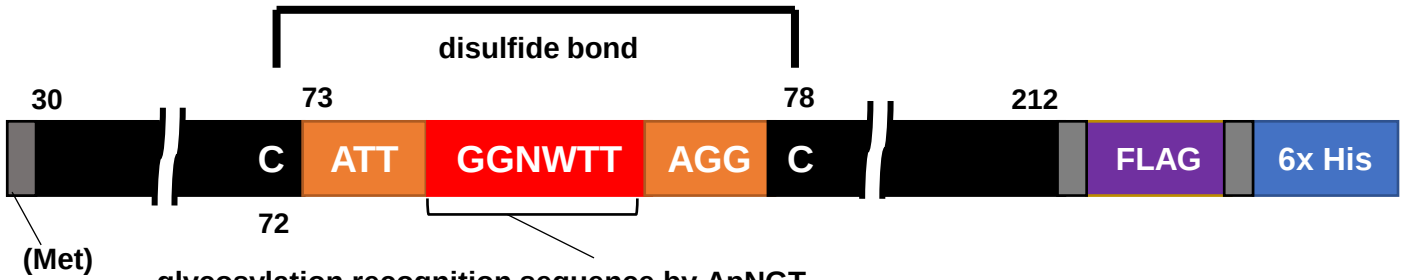
Modification	Theo. MH+ [Da]	Number of PSMs			
		lane 1	lane 2	lane 3	lane 4
-	3129.5739	90	7	50	70
Glc	3291.6267	1*	114	31	21
Glc-Gal	3453.6795	1*	0	102	51
Glc-Gal-$(GlcNAc-Gal)_1$	3818.8117	0	0	0	71
Glc-Gal-$(GlcNAc-Gal)_2$	4183.9439	0	0	0	34
Glc-Gal-$(GlcNAc-Gal)_3$	4549.0761	0	0	0	8

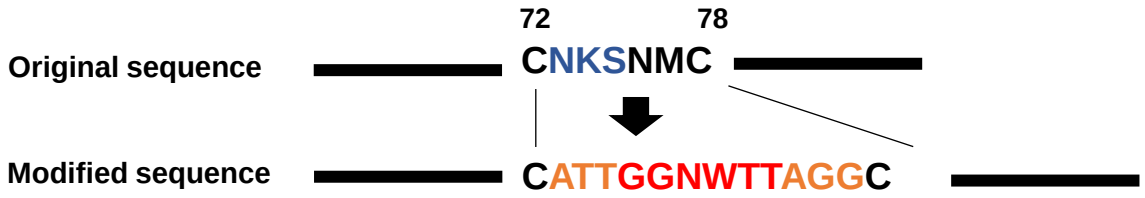
*It is most likely detecting a carryover from the previous measurement.

One-pot IVG by PURE*frex*® could provide the N-glycosylation of Im7-6.

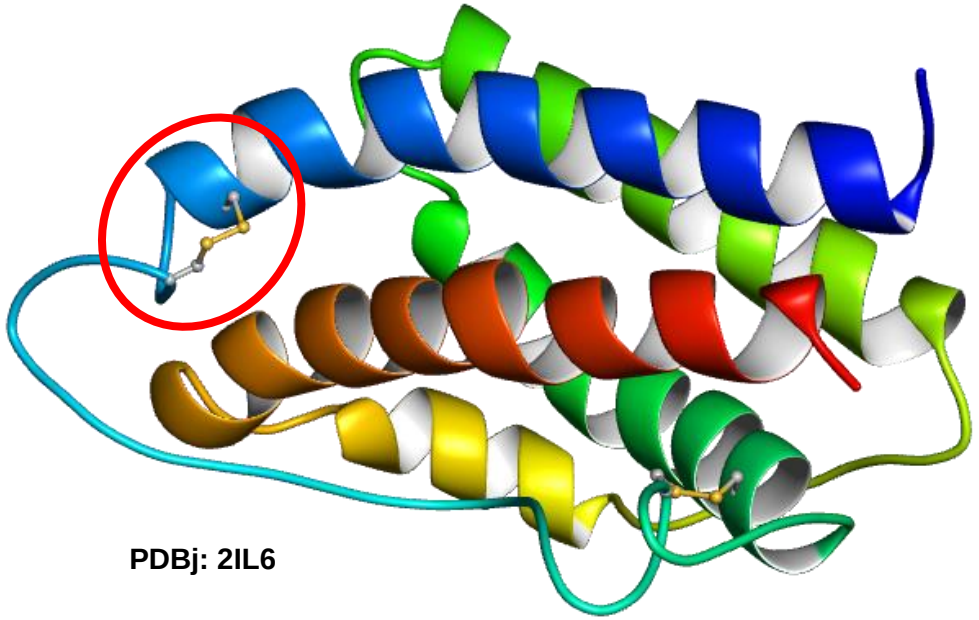
in vitro Glycosylation of model protein (IVG)

Human Interleukin-6 (hIL6)





Length	209 a.a.
Molecular weight	23,550 Da



PDBj: 2IL6

in vitro Glycosylation of model protein (IVG)

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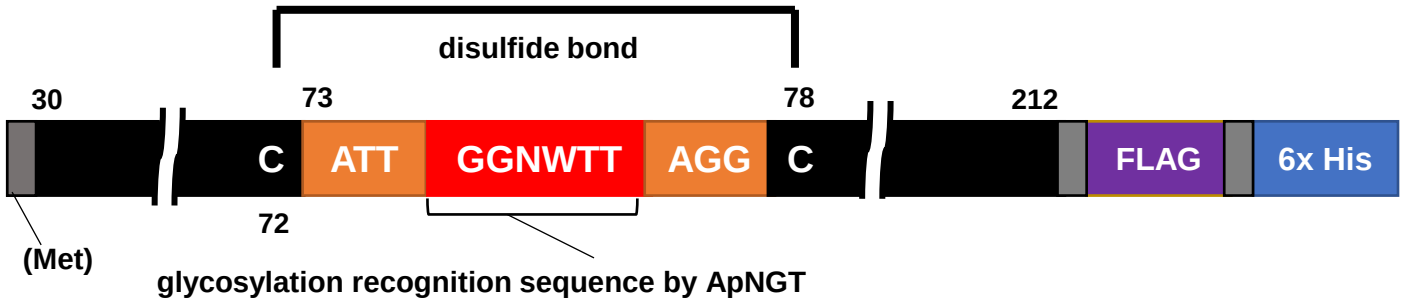
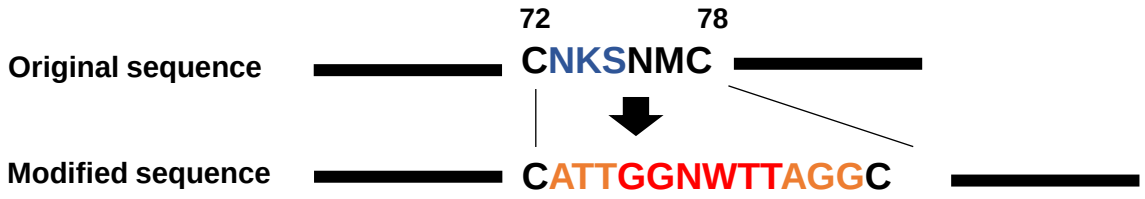
Human Interleukin-6 (hIL6)





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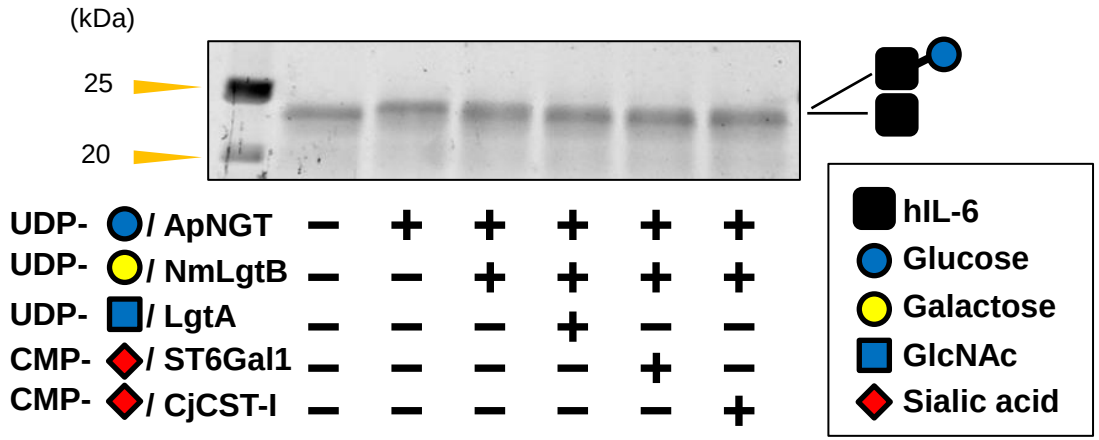
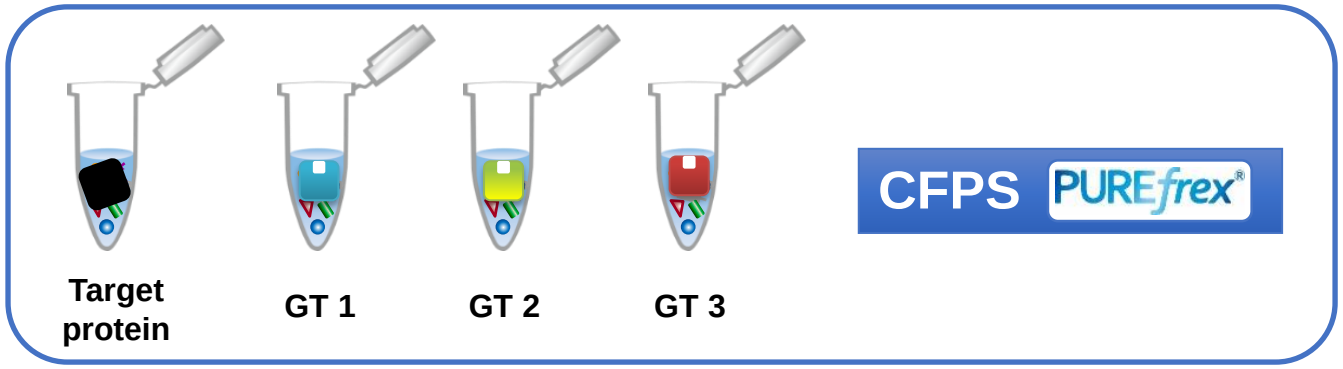
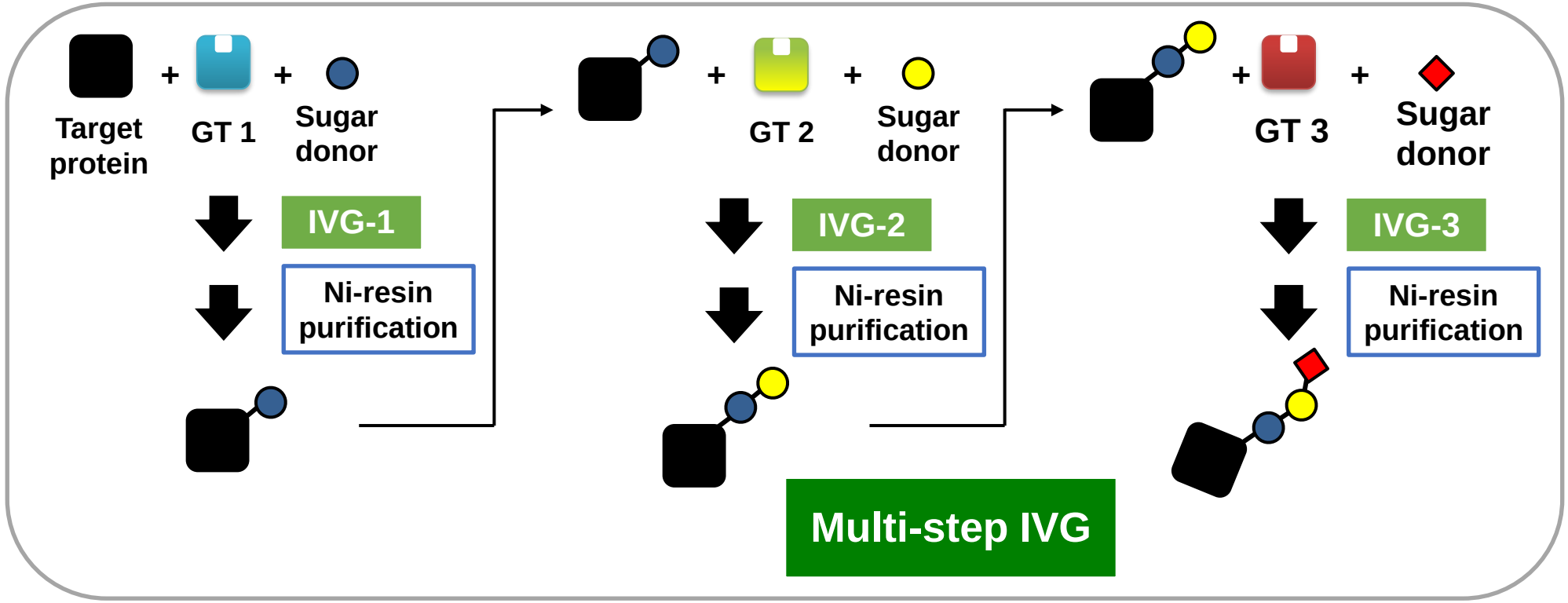


IVG by PURE*frex*® could not provide the N-glycosylation of Im7-6.

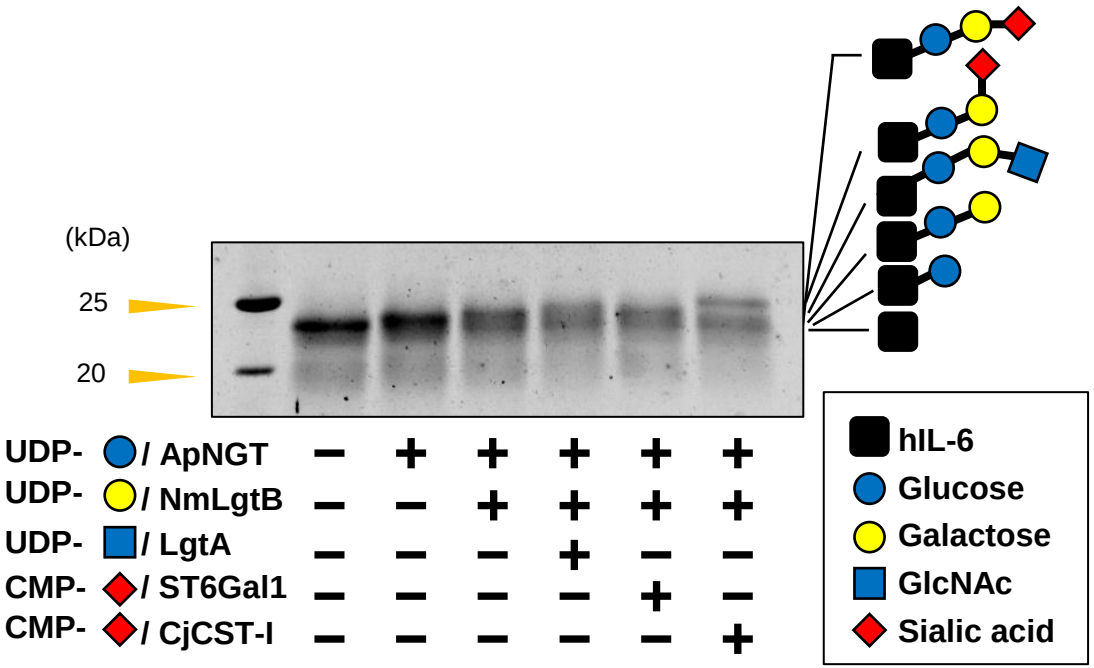
Multi-step IVG



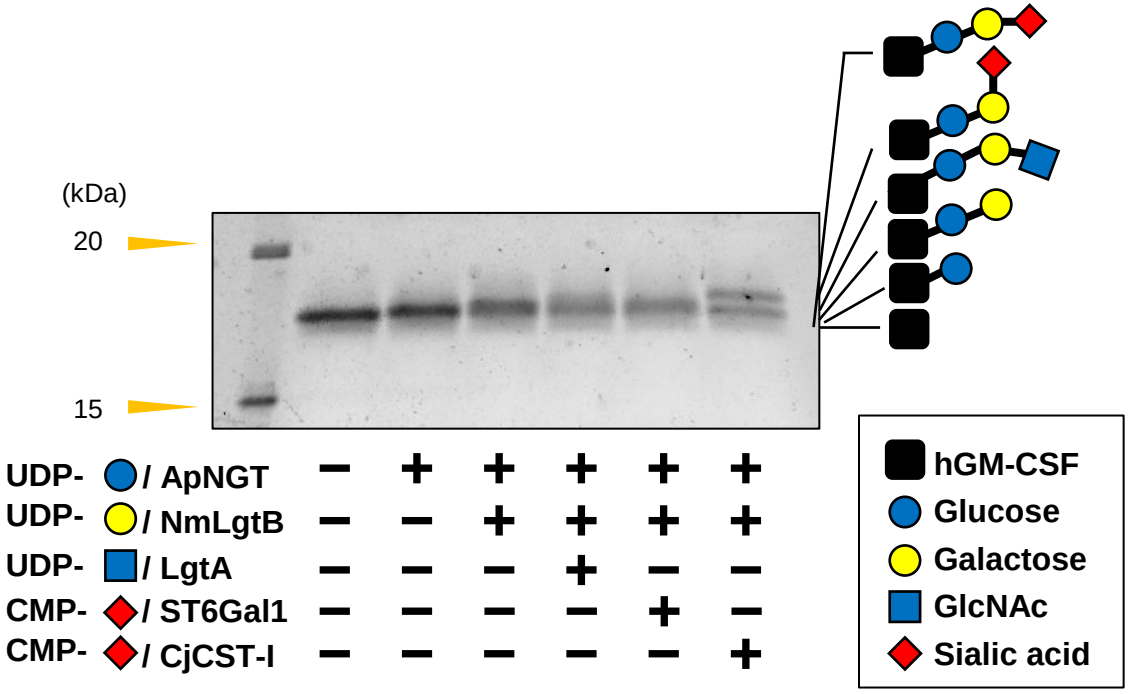


Multi-step IVG

Human Interleukin-6 (hIL6)



Human Granulocyte-macrophage colony-stimulating factor (hGM-CSF)

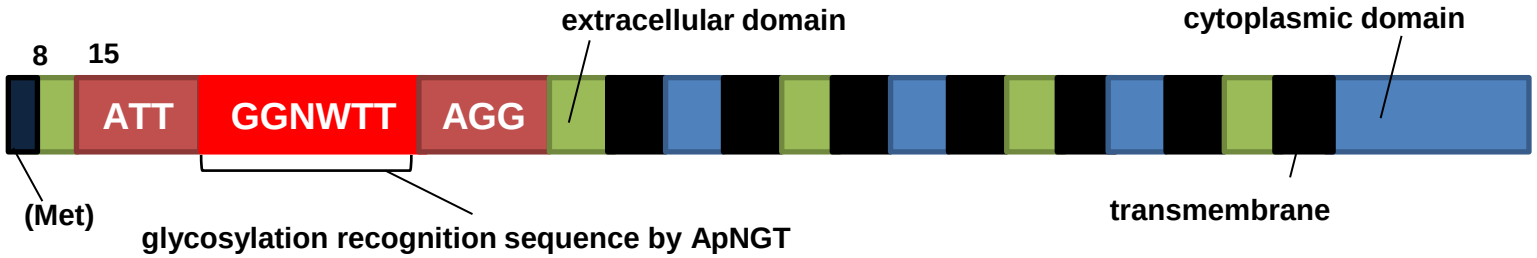


Multi-step IVG with PURE*frex*® was effective for disulfide-bonded proteins.

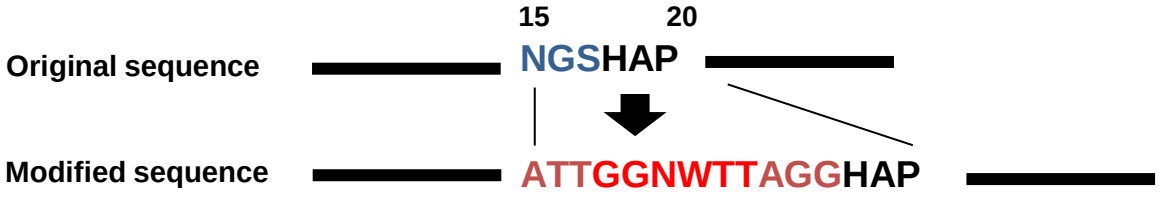
Multi-step IVG

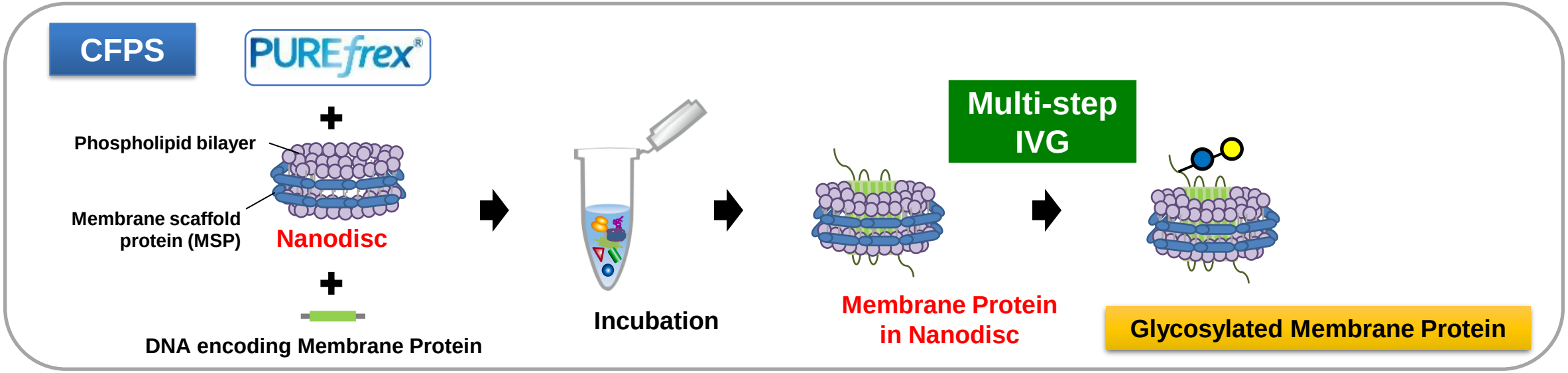
GeneFrontier

Human Beta-2 adrenergic receptor (hADRB2)



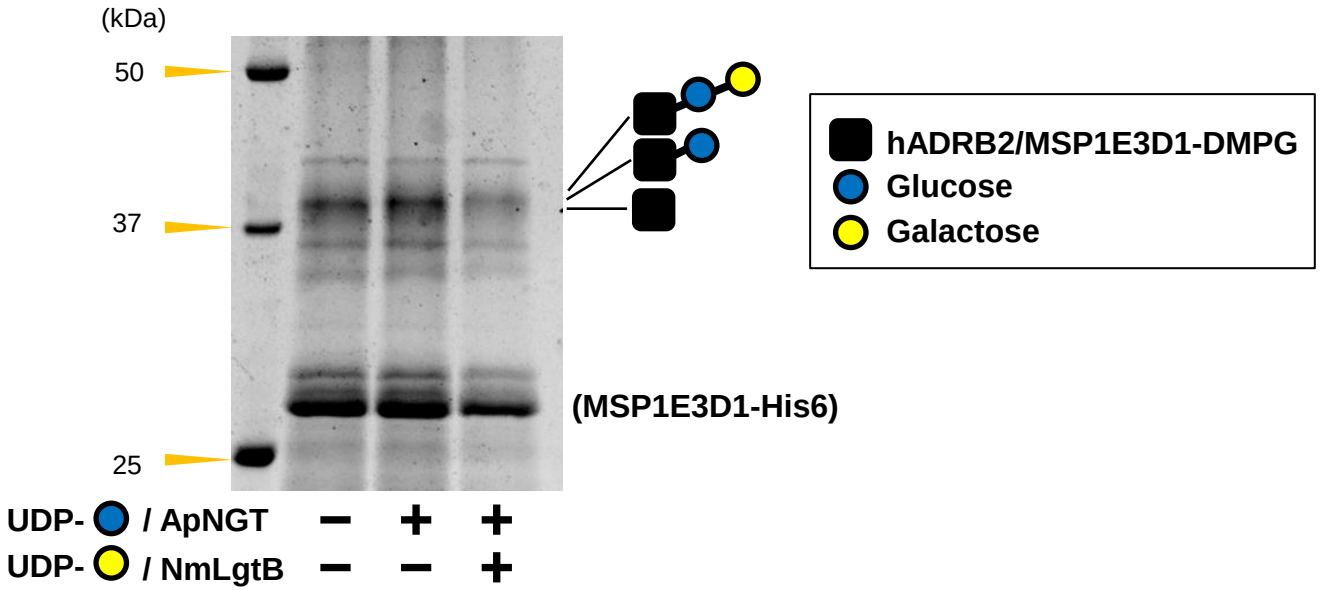
Length	416 a.a.
Molecular weight	46,764 Da

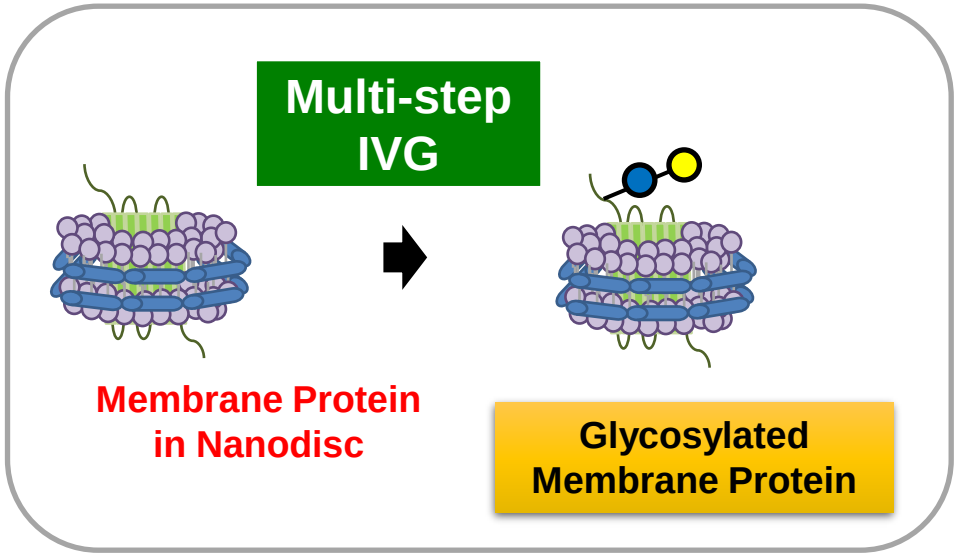




Multi-step IVG

Human Beta-2 adrenergic receptor (hADRB2)





Multi-step IVG with PURE*frex*® was effective for membrane proteins.

Summary

- ***N*-glycosylation of Im7-6 were succeeded IVG and One-pot IVG with PURE*frex*®.**
- **Depending on the target protein, glycosylation could be achieved by arranging the method as Multi-step IVG.**

Glycoproteins can be synthesized by IVG using PURE*frex*®.