Supplementary Information

Appendix 1. Independent variables used in RSM

Variables	**Levels**		
	-1	0	+1
A. Post-induction temperature (°C)	25	31	37
B. Cell density at induction time (OD 600 nm)	0.2	0.5	0.8
C. IPTG concentration (mM)	0.1	0.55	1

Appendix 2. Stereochemical quality of predicted 3D models of designed CD20 molecules by PROCHECK

Proteins	**ProSA Z-score**	**Ramachandran plot quality (%)**				**Verify 3D (%)**	**ERRAT (%)**	**Molprobity score (%)**
		Most favored	**Additionally allowed**	**Generously allowed**	**Disallowed**			
CD20/20aa PAS	-3.71	80.0%	10.0%	1.4%	8.6%	78.20%	75.58%	71%
CD20/40aa PAS	-2.97	80.0%	10.0%	1.4%	8.6%	80.61%	81.13%	71%
CD20/60aa PAS	-3.31	79.2%	17.7%	2.1%	1.0%	84.92%	88.13%	85%
CD20/80aa PAS	-2.86	65.1%	25.7%	4.6%	4.6%	71.92%	71.01%	74%
CD20/G_4S	-1.42	91.7%	8.3%	0%	0%	36.59%	76.6%	100%

ProSA Z-score determines the overall quality of the models. ERRAT, Verify3D, and Molprobity values range between 0 to 100 percent in which 100% is the best and 0 is the worst.

Appendix 3. Stereochemical quality of predicted 3D model of Trx-CD20/G$_4$S by PROCHECK

Protein	**ProSA Z-score**	**Ramachandran plot quality (%)**				**Verify 3D (%)**	**ERRAT (%)**	**Molprobity Score (%)**
		Most favored	**Additionally allowed**	**Generously allowed**	**Disallowed**			
Trx-CD20/G$_4$S	-4.27	91.3	6.8	0.6	1.2	87.82	86.7	69

ProSA Z-score determines the overall quality of the models. ERRAT, Verify3D, and Molprobity values range between 0 to 100 percent in which 100% is the best and 0 is the worst.

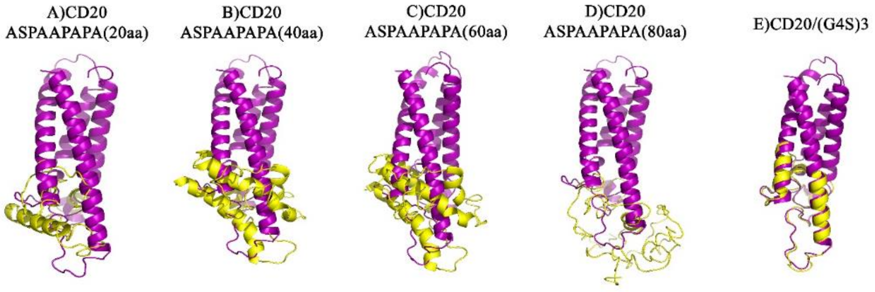
Appendix 4. Physicochemical properties of Trx-tagged CD20

Proteins	**pI**	**Estimated half-life (h)**			**Gravy**	**II**[1]	**AI**[2]	**SC**[3]
		Mammalian	Yeasts	*E. coli*				
Trx-CD20	5.82	30	>20	>10	-0.30	25	81.32	0.575
CD20/G$_4$S	7.92	30	>20	>10	-0.57	52	57.2	0.589

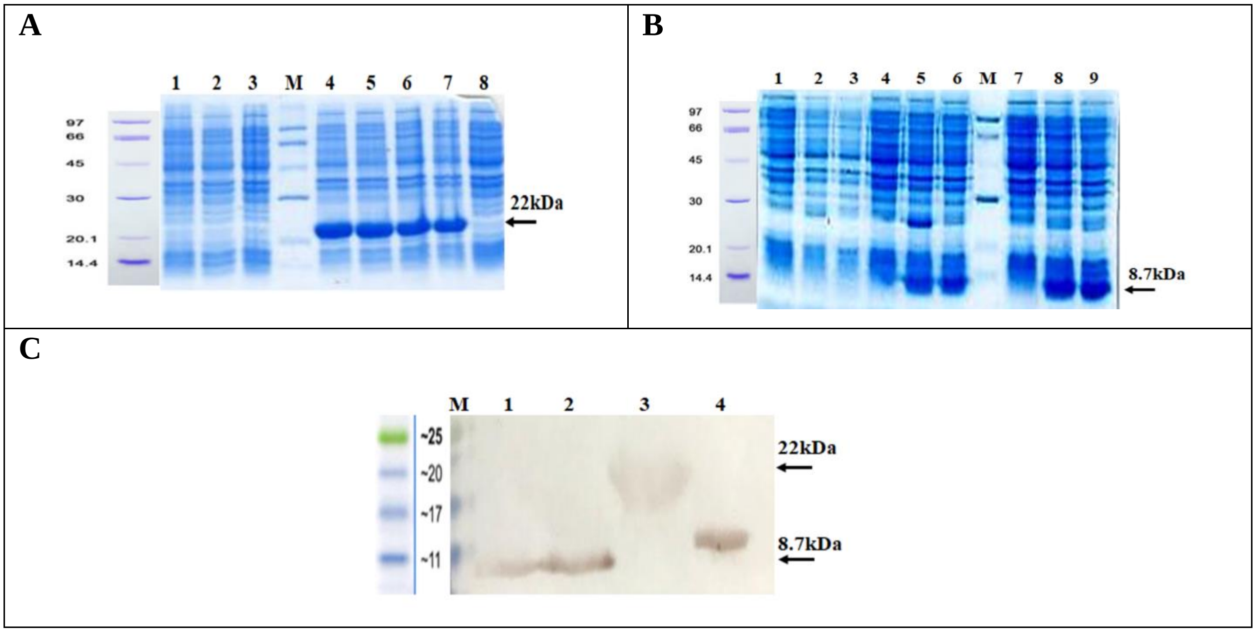
[1] II: instability index; [2] AI: Aliphatic index; [3] SC: Solubility score.

Appendix 5. Analysis of ANOVA for response surface quadratic model

Proteins	**Std. Dev.**	**Mean**	**C.V. (%)**	**R^2**	**Adj R2**	**Pred R^2**	**Adeq Precision**
Trx-CD20	0.28	6.65	4.15	0.9496	0.8588	0.7113	7.979
CD20/G$_4$S	1.59	33.18	4.78	0.9764	0.9340	0.7323	15.85

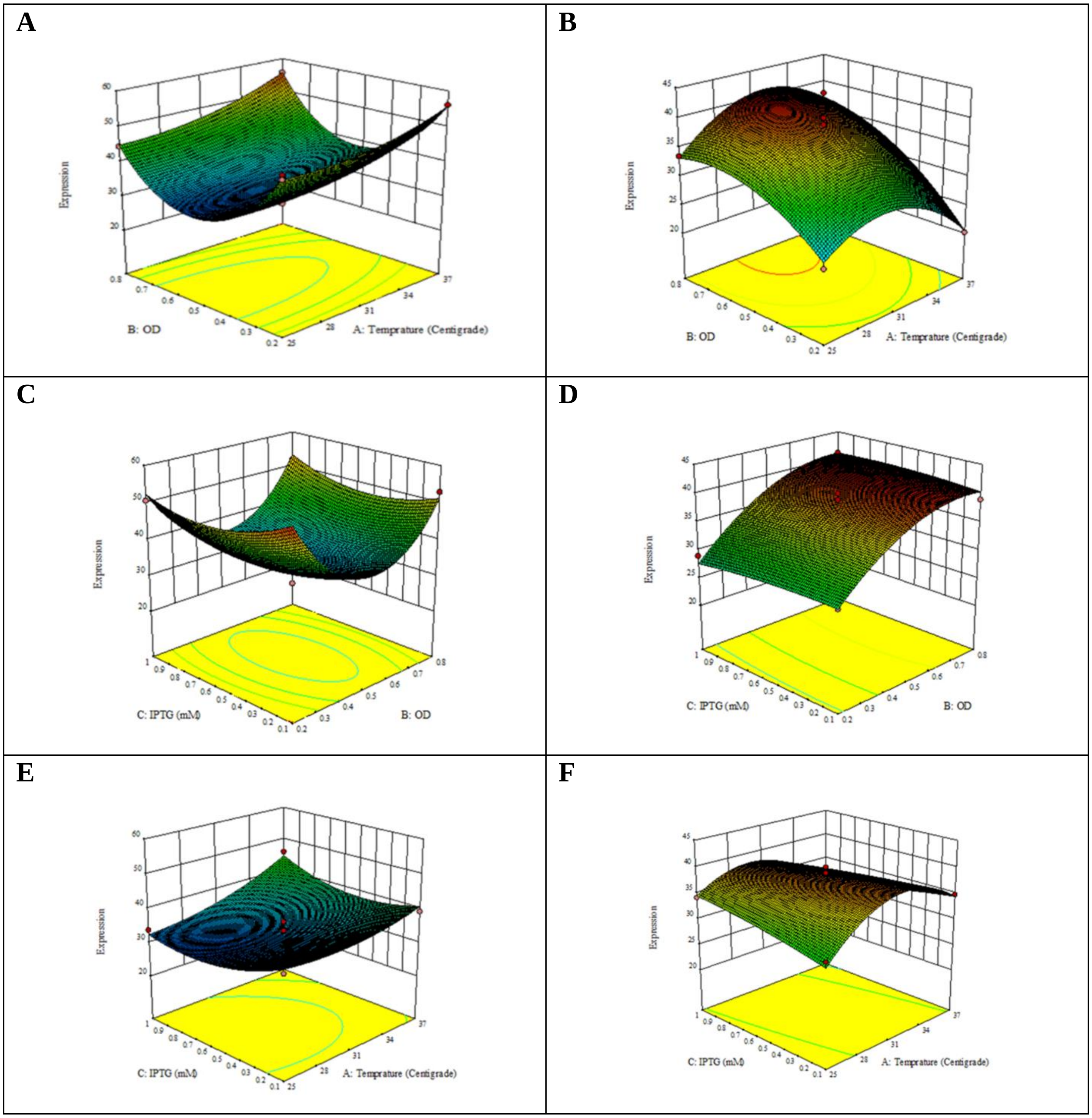


Appendix 6. Structural alignment of C-terminal His-tagged CD20 constructs (Yellow) against full-length CD20 protein (Purple)



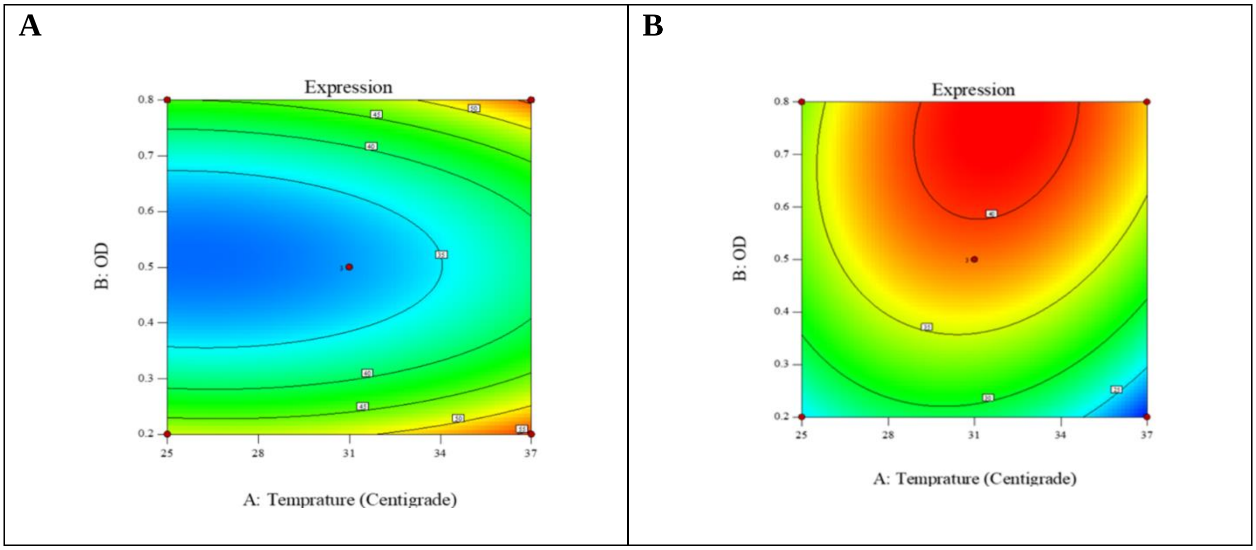
Appendix 7. Protein expression analysis. **A,** Trx-CD20: #1, 2: Lysate of *E. coli* cells harboring pET24a expression vector before and after induction (Negative control); #3, 8: Lysate of recombinant *E. coli* cells before induction; M: Protein Marker; #4-7: Lysate of recombinant *E. coli* cells 4h after induction. **B,** CD20/G$_4$S: #1-3: Lysate of *E. coli* cells harboring pET28a expression vector before and after induction (Negative control); #4, 7: Lysate of recombinant *E. coli* cells before induction; M: Protein Marker; #5, 6, 8, 9: Lysate of recombinant *E. coli* cells 4h after induction. **C,** Western blotting using anti-His antibody: M: Protein marker; #1, 2: Lysate of recombinant *E. coli* cells expressing CD20/G$_4$S protein (8.7kDa); #3: Lysate of recombinant *E.*

coli cells expressing Trx-CD20 protein (22kDa); #4: His-tagged protein (positive control, 13kDa)

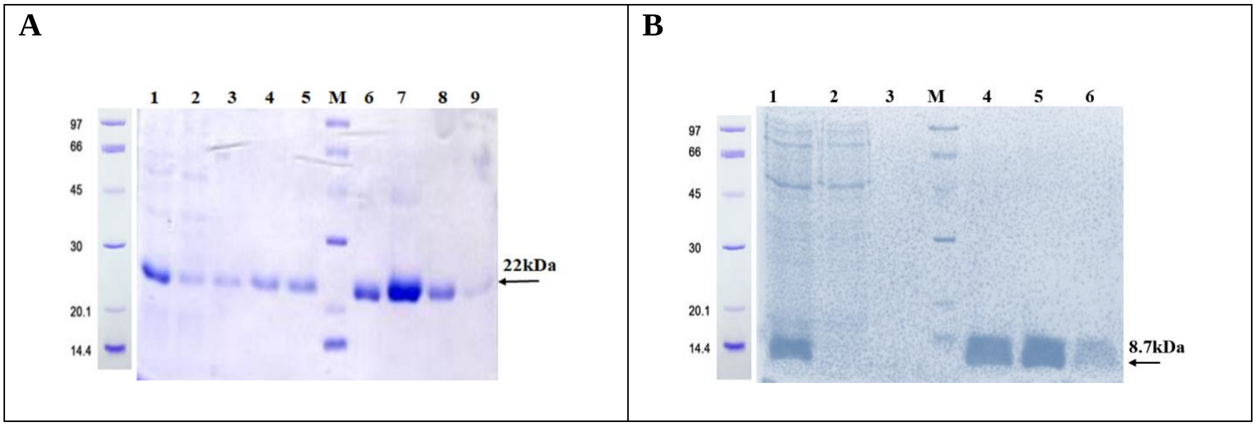


Appendix 8. The response surface plots (3D) demonstrating densitometric measurement of Trx-CD20 (A, C, E) and CD20/G$_4$S (B, D, F) expression level by optimizing the following pairs of parameters when other studied parameters were kept constant at zero levels: **A, B,** Temperature

and cell density at the time of induction; **C, D,** Optical density and IPTG; **E, F,** Temperature and IPTG



Appendix 9. Contour plots showing the effect of temperature and cell density in the case of 0.55mM IPTG on: **A,** Trx-CD20; **B,** CD20/G_4S expression levels



Appendix 10. Ni-NTA affinity purification. **A,** Trx-CD20: #1 Initial sample (IS); #2: Flow through sample (FT); #3: Washing sample; #4-9: Elution samples; M: Protein ladder; **B,** CD20/G_4S: #1: IS; #2: FT; #3: Washing sample; M: Protein Mw marker; #4-6: Elution samples