

**Additional file 2: Table S1.** Positively-charged patches size profile of rHuEPO WT from the charged patch calculator. Complete screening of posQ ratio scores for the modified rHuEPO WT (PDB: 1EER) is shown. The largest positive patches are represented by blue (ratio > 1.0). Those proteins with ratio above 1.0 are predicted as insoluble and below 1.0 as soluble. The three targeted residues in this study are highlighted in red. Ratio: largest positively-charged patch (posQ) value from the charged patch calculator [29]. Charge patches: HYD, hydrophobic (non-charged); NEG, negatively-charged; POS, positively-charged.

Residue		Ratio	
ALA	1	POS	1.492
PRO	2	POS	1.492
PRO	3	HYD	
ARG	4	POS	1.492
LEU	5	POS	1.492
ILE	6	POS	0.003
CYS	7	POS	1.492
ASP	8	HYD	
SER	9	POS	1.492
ARG	10	HYD	
VAL	11	HYD	
LEU	12	HYD	
GLU	13	POS	1.492
ARG	14	HYD	
TYR	15	NEG	
LEU	16	HYD	
LEU	17	NEG	
GLU	18	HYD	
ALA	19	POS	0.477
LYS	20	POS	1.492
GLU	21	NEG	
ALA	22	POS	0.477
GLU	23	POS	1.492
ASN	24	HYD	
ILE	25	HYD	
THR	26	HYD	
THR	27	POS	1.492
GLY	28	HYD	
CYS	29	POS	1.492
ALA	30	POS	1.492
GLU	31	HYD	
HIS	32	NEG	
CYS	33	NEG	
SER	34	POS	1.492
LEU	35	NEG	
ASN	36	HYD	
GLU	37	HYD	
ASN	38	HYD	
ILE	39	NEG	
THR	40	NEG	
VAL	41	HYD	
PRO	42	POS	0.022
ASP	43	HYD	
THR	44	POS	1.492
LYS	45	POS	1.492
VAL	46	HYD	
ASN	47	POS	1.492
PHE	48	POS	1.492
TYR	49	POS	1.492
ALA	50	HYD	
TRP	51	HYD	
LYS	52	NEG	
ARG	53	HYD	
MET	54	HYD	
GLU	55	HYD	
VAL	56	NEG	
GLY	57	NEG	
GLN	58	NEG	
GLN	59	NEG	
ALA	60	NEG	
VAL	61	HYD	
GLU	62	HYD	
VAL	63	HYD	
TRP	64	HYD	
GLN	65	HYD	
GLY	66	HYD	
LEU	67	HYD	
ALA	68	HYD	
LEU	69	NEG	
ARG	70	NEG	
SER	71	HYD	
LEU	72	HYD	
THR	73	NEG	
THR	74	HYD	
LEU	75	HYD	
LEU	76	HYD	
ARG	77	NEG	
ALA	78	NEG	
LEU	79	HYD	
GLY	80	NEG	
ALA	81	NEG	
GLN	82	NEG	
LYS	83	HYD	
GLU	84	HYD	
ALA	85	NEG	
ILE	86	NEG	
SER	87	HYD	
PRO	88	POS	1.492
PRO	89	NEG	
ASP	90	HYD	
ALA	91	HYD	
ALA	92	HYD	
ALA	93	HYD	
ALA	94	POS	0.477
PRO	95	POS	0.477
LEU	96	NEG	
ARG	97	HYD	
THR	98	POS	0.477
ILE	99	HYD	
THR	100	HYD	
ALA	101	NEG	
ASP	102	HYD	
THR	103	POS	0.477
PHE	104	HYD	
ARG	105	POS	0.477
LYS	106	POS	0.477
LEU	107	POS	0.477
PHE	108	HYD	
ARG	109	HYD	
VAL	110	POS	0.014
TYR	111	HYD	
SER	112	NEG	
ASN	113	NEG	
PHE	114	NEG	
LEU	115	POS	0.014
ARG	116	HYD	
GLY	117	HYD	
LYS	118	NEG	
LEU	119	NEG	
LYS	120	NEG	
LEU	121	NEG	
TYR	122	NEG	
THR	123	NEG	
GLY	124	HYD	
GLU	125	NEG	
ALA	126	HYD	
CYS	127	HYD	
ARG	128	HYD	
THR	129	HYD	
GLY	130	POS	0.025
ASP	131	HYD	
ARG	132	POS	0.022
	133	POS	1.492
	134	POS	1.492
	135	POS	1.492
	136	POS	1.492
	137	POS	1.492
	138	NEG	
	139	POS	1.492
	140	POS	1.492
	141	POS	1.492
	142	POS	1.492
	143	HYD	
	144	POS	1.492
	145	HYD	
	146	HYD	
	147	POS	1.492
	148	POS	1.492
	149	HYD	
	150	POS	1.492
	151	POS	1.492
	152	POS	1.492
	153	HYD	
	154	POS	1.492
	155	HYD	
	156	HYD	
	157	POS	1.492
	158	POS	1.492
	159	POS	1.492
	160	NEG	
	161	POS	1.492
	162	POS	1.492
	163	POS	1.492
	164	POS	1.492
	165	NEG	
	166	NEG	