Table S1. The amino acid sequence of PSMA nanobody

Name	Amino acid sequence
NB	EVQLLEEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKWLEWVSDISWN GGSTYYADSVKGRFTISRDNAENTLYLQMNSLKPDDTAVYYCAKMGEGGWGANDYWGQGT QVTVSS
NB-hing	EVQLLEEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKWLEWVSDISWN GGSTYYADSVKGRFTISRDNAENTLYLQMNSLKPDDTAVYYCAKMGEGGWGANDYWGQGT QVTVSSEPKSCDKTHTCP
NB-flag-hing	EVQLLEEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKWLEWVSDISWN GGSTYYADSVKGRFTISRDNAENTLYLQMNSLKPDDTAVYYCAKMGEGGWGANDYWGQGT QVTVSSDYKDDDDKEPKSCDKTHTCP
NB-cmyc-hing	EVQLLEEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKWLEWVSDISWN GGSTYYADSVKGRFTISRDNAENTLYLQMNSLKPDDTAVYYCAKMGEGGWGANDYWGQGT QVTVSSEQKLISEEDLEPKSCDKTHTCP

Table S2. The results of the homology modeled structures evaluations

NBs	Z-score	QMean	Molprobity	Ramachandran plot regions		
				Favored%	Allowed%	Disallowed%
NB	-6.27	0.77±0.08	2.79	85.90	14.10	-
NB-hing	-5.75	0.74±0.07	2.59	87.90	11.20	0.90
NB-flag-hing	-6.67	0.71±0.07	2.65	85.20	13.10	1.70
NB-cmyc-hing	-5.62	0.71±0.07	2.32	94.00	5.10	0.9

TableS3. The PIPER pose energies and PIPER pose scores of the obtained docking poses

NBs	PIPER pose energy	PIPER pose score
NB	-194.195	-251.991
NB-hing	-176.623	-161.509
NB-flag-hing	-225.179	-335.131
NB-cmyc-hing	-213.503	-180.176

Table S4. Nanobody-antigen interaction analysis of the best docking pose

NB							
PSMA Residues	NB Residues	Distance (A)	PSMA Chain	CDR	Interaction type	Surface complementary	Buried SASA %
	54:Asn	2.9	B	2	1x hb to 54:Asn		
137:Glu	58:Thr	3.1		2	1x hb to 58:Thr	0.71	54.80
138:Ile			B	-	-	0	6.40
139:Phe	34:Tyr	2.9	B	1	-	0.4	93.10
	34:Tyr	2.9	B				
	55:Pro	3.7					
140:Asn	35:Ser	4.0			1x hb to 34:Tyr	0.61	72.00
141:Thr	34:Tyr	3.6	B	1		0.66	80.20
	100:Tyr	3.0	B	3			
142:Ser	34:Tyr	3.9		1	-	0.73	85.50
144:Phe	33:Glu	3.8	B	1	-	0.04	35.50
146:Pro			B	-	-	0.08	72.60
147:Pro	28:Arg	3.0	B	1	1x hb to 28:Arg	0.75	30.00
148:Pro	28:Arg	3.8	B	1	-	0	1.60
149:Pro	28:Arg	3.3	B	1	-	0.86	36.50
152:Glu	28:Arg	2.9	B	1	2x hb, 1x salt bridge to 28: Arg	0.71	17.20
241:Ser	3:Glu	2.9	B	Non-CDR	1x hb, to 3:Glu	0.68	78.10
251:Gly	100:Tyr	3.7	B	3	-	0.8	84.00
	100:Tyr	2.8	B	3			
	102:Tyr	2.8		3			
300:Tyr	3:Glu	3.0		Non-CDR	1x pi stack to 102:Tyr	0.73	93.80
301:Asp	100:Tyr	3.5	B	3	-	0.75	100.00
303:Gln	101:Gly	3.2	B	3	-	0.88	52.00
	101:Gly	3.4	B	3			
304:Lys	100:Tyr	3.9		3	-	0.57	79.70
324:Lys	3:Glu	3.0	B	Non-CDR	1x hb to 3:Glu	0.13	19.20
NB-hing							
PSMA Residues	NB Residues	Distance (A)	PSMA Chain	CDR	Interaction Type	Surface Complementary	Buried SASA %
123:Thr	2:Gln	3.7 A	B	Non-CDR	1x hb to 2:Gln	0.88	80.60%
	3:Glu	3.4 A	B	Non-CDR			
124:His					1x hb to 3:Glu	0.9	78.30%

	103:Arg	3.6 A	B	3			
125:Pro	101:Gly	4.0 A				0.89	86.10%
	103:Arg	3.2 A	B	3			
	101:Gly	3.4 A					
	102:Tyr	3.8 A					
127:Tyr	99:Gly	4.0 A				0.89	95.50%
	54:Asn	2.9 A	B	2			
137:Glu					1x hb to 54:Asn	0.69	94.10%
	103:Arg		B	3			
141:Thr		2.7 A			2x hb to 103:Arg	0.71	100.00%
	103:Arg	3.5 A	B	3			
142:Ser						0.91	84.90%
	104:Gly	3.2 A	B	3			
143:Leu	103:Arg	3.3 A				0.81	93.10%
	41:Gln	2.9 A	B	Non-CDR			
	96:Tyr	3.0 A			1x hb to 41:Gln		
144:Phe					1x pi stack 96:Tyr	0.78	65.70%
	126:Cys	3.0 A	B	Non-CDR			
147:Pro	127:Pro	3.0 A				0.81	67.30%
	126:Cys	3.2 A	B	Non-CDR			
149:Pro	127:Pro	3.7 A			1x hb to 126:Cys	0.82	34.50%
	124:His	3.0 A	B	Non-CDR	1x hb to 124:His		
	125:Thr	3.0 A			2x hb to 125:Thr		
152:Glu	126:Cys	3.2 A			1x hb to 126:Cys	0.86	61.90%
	47:Leu	3.0 A		Non-CDR			
301:Asp	46:Gly	3.1 A			1x hb to 47:Leu	0.77	93.20%
	48:Glu	3.0 A		Non-CDR	1x hb to 47:Leu		
304:Lys	47:Leu	3.1 A			1x hb, 1x salt bridge to 48:Glu	0.9	50.00%
345:His	101:Gly	3.7 A		3		0.82	47.20%

NB-flag-hing

PSMA Residues	NB Residues	Distance (A)	PSMA Chain	CDR	Interaction Type	Surface Complementary	Buried SASA %
143:Leu			A			0.58	31.40
144:Phe	102:Tyr	3.0	A	3			
	28:Arg	3.4		1			
	29:Phe	4.0		1	1x pi stack 102:Tyr	0.89	89.60

146:Pro	100:Tyr	3.3	A	3			
	29:Phe	3.7		1	1x pi alkyl to 100:Tyr, 1x pi alkyl		
	34:Tyr	3.8		1	to 29:Phe, 1x pi alkyl to 34:Tyr	0.79	96.80
147:Pro	100:Tyr	2.5	A	3			
	101:Gly	3.3		3			
	102:Tyr	3.5		3	1x pi alkyl to 102:Tyr	0.79	82.00
149:Pro	101:Gly	3.3	A	3		0.86	38.30
152:Glu	103:Arg	2.9	A	3			
	101:Gly	3.3		3	1x hb, 1x salt bridge to X:103:Arg	0.87	66.30
153:Asn	5:Gln	3.7	A	Non-CDR			
	1:Leu	3.8		Non-CDR		0.62	38.60
243:Pro	100:Tyr	3.7	A	3	1x pi alkyl to 100:Tyr	0.88	42.90
249:Pro	100:Tyr	3.0	A	3	1x pi alkyl to 100:Tyr	0.79	83.60
251:Gly			A	1		0.89	
	34:Tyr	3.2					97.60
300:Tyr	33:Glu	3.3	A	1			
	34:Tyr	3.3			1x pi stack 34:Tyr	0.89	76.40
304:Lys			A				
	33:Glu	3.0		1	1x hb, 1x salt bridge, to X:33:Glu	0.63	49.80
556:Tyr	100:Tyr	2.7	A	3	1x hb to X:100:Tyr	0.8	100.00

NB-cmyc-hing

PSMA Residues	NB Residues	Distance (A)	PSMA Chain	CDR	Interaction Type	Surface Complementary	Buried SASA %
235:Phe	58:Thr	3.6	A	2		0.8	64.30
237:Pro	56:Ala	3.5	A	2		0.85	31.30
238:Gly	56:Ala	3.9	A	2		0.78	24.30
239:Val			A	-		0.64	15.20
314:Pro	100:Tyr	2.7	A	3	1x hb to 100:Tyr	0.77	59.80
316:Asp	100:Tyr	3.5	A	3		0.92	0.50
317:Ser	30:Met	3.2	A	1		0.76	55.20
	30:Met	3.0	A	1			
	100:Tyr	3.2		3			
	33:Glu	3.4		1	2x hb to 30:Met		
320:Arg	31:Ile	3.7		1		0.76	94.20
	35:Ser	2.5	A	1	1x hb to 35:Ser		
	54:Asn	3.1		2	1x hb to 54:Asn		
322:Ser	55:Pro	3.8		2		0.75	96.10
323:Leu	35:Ser	3.3	A	1		0.48	80.80

	60:Asp	2.9	A	Non-CDR			
324:Lys	37:His	3.5		Non-CDR	1x salt bridge to 60:Asp	0.79	59.90
	34:Tyr	3.0	A	1			
325:Val	37:His	3.7		Non-CDR		0.65	82.30
326:Pro	34:Tyr	3.0	A	1		0.81	57.40