

S3 Table. Correlative Parametric Analysis of Amino Acid Distributions - Input Matrices.

Library design can be guided by information regarding each position's mutational tolerance and naturally evolved sequence to reduce the prevalence of overly destabilizing mutations as well as identifying structurally stabilizing mutations. Additionally, the chemical diversity found at the interfaces of well characterized natural binders, such as the complementarity determining regions (CDR) of antibodies, can be applied to protein scaffolds to accommodate for strong binding interactions. Here, a model for library design was built based on a linear combination of (A) computational stability, (B) natural homolog sequence frequency, and (C) CDR diversity input matrices. These three elements were weighted based on the (D) target exposure (i.e. proximity to the binding interface) and solvent exposed surface area (i.e. orientation and packing) of each site.

A. Computational sitewise assessment of changes in stability ($\Delta\Delta G$, kcal/mol) upon mutation. Conducted in FoldX.

	D23	A24	P25	A26	V27	T28	V29	R30	Y31	G52	S53	K54	S55	T56
A	0.34	-	0.28	-	(0.31)	(0.30)	1.67	0.23	0.06	1.70	(0.09)	0.73	(0.23)	0.26
C	0.37	0.29	0.42	0.01	(0.24)	(0.21)	1.28	0.86	(0.15)	3.38	(0.22)	0.86	0.07	0.03
D	-	0.73	0.72	(0.08)	(0.03)	(0.34)	3.28	0.65	1.51	5.39	0.00	0.93	(0.00)	0.97
E	0.18	0.64	0.56	(0.25)	(0.25)	(0.48)	3.24	0.39	0.29	5.24	(0.48)	0.55	(0.02)	(0.13)
F	(0.22)	0.51	0.75	(0.50)	(0.16)	(0.44)	4.17	0.18	(0.33)	6.16	(0.28)	(0.06)	(0.36)	(0.16)
G	1.11	0.35	0.15	0.21	0.02	(0.33)	2.54	0.76	0.34	-	0.20	1.70	0.05	0.75
H	0.59	0.73	1.50	0.10	0.33	(0.15)	4.09	0.91	0.75	6.14	0.14	0.96	0.03	0.54
I	0.30	0.98	0.64	(0.07)	(0.07)	(0.01)	(0.31)	0.81	0.40	11.73	(0.40)	0.95	0.16	(0.03)
K	(0.04)	0.13	0.49	(0.37)	(0.38)	(0.57)	3.01	(0.00)	(0.46)	4.76	(0.73)	-	(0.56)	(0.56)
L	(0.24)	0.38	0.02	(0.47)	(0.56)	(0.48)	0.82	(0.06)	(0.47)	6.31	(0.71)	(0.31)	(0.38)	(0.25)
M	(0.19)	0.04	(0.47)	(0.57)	(0.58)	(0.53)	0.81	0.07	(0.84)	4.41	(0.78)	(0.32)	(0.45)	(0.91)
N	0.35	0.32	0.52	(0.07)	(0.24)	(0.30)	2.13	0.64	0.65	4.25	(0.04)	0.75	(0.54)	0.63
P	2.35	(0.59)	-	(0.08)	1.28	(0.06)	3.89	2.73	2.37	3.52	0.28	3.58	(0.08)	1.71
Q	0.18	0.40	0.49	(0.26)	(0.25)	(0.35)	3.04	0.11	0.06	5.13	(0.39)	0.49	(0.29)	(0.10)
R	0.12	0.16	0.74	(0.32)	(0.22)	(0.51)	4.14	-	(0.40)	5.01	(0.53)	(0.12)	(0.83)	(0.51)
S	0.62	0.24	0.19	0.19	(0.27)	(0.23)	2.43	0.79	0.03	3.21	-	0.84	-	(0.17)
T	0.71	0.73	0.61	0.37	(0.18)	-	1.16	1.12	1.16	8.01	0.32	1.23	0.70	-
V	0.62	0.82	0.71	0.20	-	0.12	-	0.90	0.86	8.15	0.03	1.25	0.40	0.07
W	0.17	1.03	1.87	(0.50)	0.69	(0.19)	7.81	0.49	0.45	7.10	(0.17)	0.03	(0.08)	(0.14)
Y	(0.12)	0.45	1.36	(0.45)	0.01	(0.39)	6.36	0.29	-	6.31	(0.27)	0.00	(0.29)	(0.10)

	T76	G77	R78	G79	D80	S81	P82	A83	S84	S85	K86
A	(0.07)	0.46	0.45	0.37	0.02	(0.04)	(0.70)	(0.15)	(0.34)	(0.06)	0.34
C	(0.07)	0.38	0.68	0.44	0.04	0.06	(0.65)	0.04	(0.20)	(0.13)	0.32
D	1.17	0.68	0.60	0.15	-	(0.03)	(0.61)	(0.01)	0.05	(0.15)	0.51
E	0.58	0.72	0.17	0.27	(0.15)	(0.12)	(0.79)	(0.04)	0.02	(0.12)	0.23
F	(0.69)	(0.14)	0.03	0.11	(0.24)	(0.30)	(1.13)	(0.30)	(0.36)	(0.23)	(0.36)
G	0.45	-	0.70	-	(0.39)	(0.51)	(0.60)	0.14	0.17	0.41	0.19
H	0.97	0.91	0.44	0.40	0.13	0.27	(0.58)	0.04	0.31	0.49	0.31
I	(0.98)	0.68	0.47	1.00	0.62	0.30	(0.92)	(0.14)	(0.47)	0.03	0.79
K	(0.11)	0.15	(0.02)	(0.15)	(0.44)	(0.29)	(1.08)	(0.38)	(0.38)	(0.17)	(0.06)
L	(1.16)	0.06	(0.09)	0.01	(0.27)	(0.25)	(1.03)	(0.39)	(0.95)	(0.49)	(0.10)
M	(1.37)	(0.27)	0.05	0.14	(0.29)	(0.23)	(1.06)	(0.45)	(1.28)	(0.63)	(0.33)
N	0.38	0.24	0.45	0.12	(0.14)	(0.09)	(0.66)	(0.10)	(0.08)	(0.17)	0.41
P	1.30	1.43	0.64	2.26	1.17	1.70	-	0.53	2.03	0.40	1.32
Q	0.18	0.57	0.03	0.20	(0.13)	(0.20)	(0.93)	(0.29)	(0.31)	(0.16)	0.14
R	0.37	0.18	-	0.09	(0.23)	(0.22)	(0.76)	(0.23)	(0.55)	(0.09)	(0.00)
S	0.26	0.34	0.57	0.41	(0.18)	-	(0.36)	0.00	0.02	(0.08)	0.38
T	-	0.74	0.69	0.84	0.40	0.33	(0.38)	0.10	0.02	0.01	0.86
V	(0.55)	0.99	0.64	0.98	0.54	0.43	(0.57)	0.04	(0.19)	0.04	0.92
W	1.10	0.52	0.45	0.32	(0.08)	0.07	(0.89)	(0.16)	(0.19)	0.29	(0.20)
Y	0.03	0.03	0.10	0.19	(0.17)	(0.22)	(1.07)	(0.24)	(0.18)	0.06	(0.23)

B. Natural sequence frequency for the tenth type III domain of human fibronectin: pfam database summary. Below the full amino acid table, the four most frequent residues at each site are listed.

	V	S	D	V	P	R	D	L	E	V	V	A	A	T	P	T	S	L	L
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	13.1%	12.6%	3.2%	28.6%	1.6%	4.5%	4.1%	2.1%	4.3%	15.0%	4.6%	11.2%	10.1%	2.0%	10.4%	1.7%	6.6%	11.2%	2.9%
C	0.5%	-	-	1.2%	-	-	-	-	-	2.2%	0.7%	0.6%	0.7%	-	0.6%	0.6%	1.0%	3.5%	0.5%
D	1.5%	0.8%	16.0%	-	0.7%	1.0%	13.4%	-	3.7%	0.9%	3.9%	15.8%	1.8%	4.3%	8.8%	13.9%	1.8%	-	3.1%
E	3.5%	1.1%	9.6%	0.8%	0.8%	6.9%	6.2%	-	13.3%	2.2%	5.6%	11.8%	3.1%	2.7%	8.6%	5.5%	5.1%	-	4.7%
F	0.8%	-	-	1.2%	-	1.6%	0.8%	5.0%	1.1%	5.1%	1.9%	0.7%	0.9%	0.9%	-	1.3%	1.2%	4.4%	2.5%
G	7.1%	22.2%	3.8%	3.0%	4.1%	8.8%	9.1%	-	1.6%	2.1%	2.7%	6.7%	4.7%	7.2%	5.0%	2.9%	4.5%	0.6%	1.2%
H	0.6%	-	1.6%	-	-	2.1%	1.9%	-	4.8%	-	1.5%	1.6%	1.1%	0.9%	2.3%	2.8%	1.9%	-	2.4%
I	2.7%	-	0.6%	7.4%	1.2%	2.1%	2.3%	11.8%	2.2%	11.7%	7.2%	1.4%	13.1%	0.8%	1.0%	1.0%	-	21.8%	4.3%
K	1.8%	3.6%	4.0%	1.9%	0.6%	8.5%	5.1%	-	11.5%	1.2%	4.7%	3.3%	3.9%	2.8%	11.7%	2.8%	3.3%	-	5.4%
L	13.2%	0.6%	1.3%	7.6%	0.8%	5.2%	1.4%	42.5%	1.5%	8.4%	6.5%	1.5%	7.8%	1.8%	1.1%	1.1%	-	19.6%	12.1%
M	3.9%	-	-	1.4%	-	1.2%	-	2.0%	0.9%	0.9%	1.4%	0.8%	1.3%	0.6%	0.9%	-	0.6%	5.6%	2.0%
N	0.8%	2.0%	4.3%	0.8%	1.2%	1.8%	25.6%	-	3.6%	1.0%	2.4%	11.1%	2.2%	8.5%	3.5%	11.8%	3.3%	-	3.9%
P	24.0%	14.3%	41.0%	11.1%	81.9%	1.8%	6.4%	8.7%	2.0%	2.0%	1.0%	4.5%	2.9%	0.6%	12.9%	-	-	-	0.6%
Q	2.1%	2.5%	5.1%	1.5%	0.9%	9.6%	3.4%	0.6%	9.2%	0.9%	2.3%	3.2%	2.4%	2.1%	3.3%	2.5%	3.5%	-	4.8%
R	1.9%	2.4%	2.7%	1.1%	-	14.0%	3.1%	-	11.1%	0.9%	3.6%	3.0%	3.7%	3.4%	4.9%	5.2%	2.7%	-	7.9%
S	4.3%	28.4%	3.1%	3.1%	2.0%	10.4%	9.0%	0.8%	9.2%	3.1%	14.0%	13.1%	6.8%	22.1%	16.2%	14.8%	45.8%	-	9.8%
T	4.4%	7.3%	1.5%	5.2%	1.5%	12.4%	5.4%	1.7%	14.0%	5.2%	18.7%	5.2%	7.4%	35.6%	4.9%	27.6%	13.4%	0.9%	21.5%
V	11.6%	0.7%	1.4%	22.8%	1.1%	5.7%	1.5%	21.7%	4.6%	34.8%	14.7%	3.3%	23.4%	1.5%	1.4%	2.6%	1.3%	28.7%	7.8%
W	-	-	-	-	-	-	-	-	-	0.7%	-	0.7%	1.3%	0.9%	0.8%	-	1.1%	1.0%	1.2%
Y	1.9%	-	-	0.6%	-	1.6%	-	-	0.7%	1.2%	2.2%	0.5%	1.1%	0.8%	1.2%	1.1%	1.9%	-	1.4%
Most Frequent																			
1	P (0.24)	S (0.28)	P (0.41)	A (0.29)	P (0.82)	R (0.14)	N (0.26)	L (0.43)	T (0.14)	V (0.35)	T (0.19)	D (0.16)	V (0.23)	T (0.36)	S (0.16)	T (0.28)	S (0.46)	V (0.29)	T (0.21)
2	L (0.13)	G (0.22)	D (0.16)	V (0.23)	G (0.04)	T (0.12)	D (0.13)	V (0.22)	E (0.13)	A (0.15)	V (0.15)	S (0.13)	I (0.13)	S (0.22)	P (0.13)	S (0.15)	T (0.13)	I (0.22)	L (0.12)
3	A (0.13)	P (0.14)	E (0.1)	P (0.11)	S (0.02)	S (0.1)	G (0.09)	I (0.12)	K (0.12)	I (0.12)	S (0.14)	E (0.12)	A (0.1)	N (0.08)	K (0.12)	D (0.14)	A (0.07)	L (0.2)	S (0.1)
4	V (0.12)	A (0.13)	Q (0.05)	L (0.08)	A (0.02)	Q (0.1)	S (0.09)	P (0.09)	R (0.11)	L (0.08)	I (0.07)	A (0.11)	L (0.08)	G (0.07)	A (0.1)	N (0.12)	E (0.05)	A (0.11)	R (0.08)

	I 20	S 21	W 22	D 23	A 24	P 25	A 26	V 27	T 28	V 29	R 30	Y 31	Y 32	R 33	I 34	T 35	Y 36	G 37	E 38
A	1.9%	6.8%	-	2.6%	18.2%	2.2%	15.0%	9.2%	4.5%	6.9%	2.0%	3.9%	3.1%	2.1%	1.4%	2.9%	1.3%	4.8%	3.0%
C	-	1.0%	-	-	-	-	-	1.8%	-	0.7%	-	0.7%	1.0%	-	-	0.8%	1.8%	1.8%	0.7%
D	-	1.3%	-	16.5%	2.1%	3.7%	4.4%	6.4%	17.5%	6.2%	10.9%	2.2%	2.2%	4.2%	-	3.0%	0.6%	2.5%	7.2%
E	-	5.8%	0.5%	15.2%	6.2%	1.6%	9.4%	4.8%	5.6%	2.1%	6.3%	4.4%	2.9%	9.6%	0.6%	13.6%	1.6%	2.3%	23.9%
F	2.1%	0.6%	1.6%	-	-	-	-	3.0%	0.6%	4.0%	-	2.9%	8.9%	1.2%	1.0%	2.0%	6.0%	1.6%	0.9%
G	-	2.4%	0.5%	3.0%	4.2%	3.1%	13.5%	11.1%	4.8%	11.5%	18.1%	30.7%	4.5%	2.9%	1.3%	5.9%	1.1%	13.5%	-
H	-	2.7%	-	1.8%	1.5%	0.7%	1.2%	2.1%	1.6%	0.7%	1.9%	5.3%	1.4%	1.7%	-	1.5%	2.2%	2.4%	1.6%
I	16.1%	1.0%	-	0.8%	2.3%	-	2.2%	6.5%	1.9%	24.3%	1.7%	3.0%	3.7%	10.9%	37.3%	2.7%	3.4%	5.1%	4.0%
K	-	6.3%	-	8.9%	8.5%	1.6%	4.9%	3.0%	3.3%	0.6%	6.3%	3.4%	1.6%	9.5%	0.7%	4.6%	3.4%	8.8%	7.3%
L	44.5%	1.3%	0.7%	2.1%	2.6%	0.8%	4.1%	8.4%	1.8%	6.8%	5.1%	3.5%	2.3%	6.0%	17.6%	5.1%	5.4%	3.2%	4.4%
M	1.3%	1.3%	-	0.6%	1.1%	-	0.6%	1.6%	-	0.9%	0.5%	1.0%	-	1.0%	1.1%	0.9%	1.8%	1.3%	0.7%
N	-	4.7%	-	8.4%	2.2%	2.4%	5.3%	5.1%	12.1%	3.2%	5.4%	6.6%	1.7%	3.9%	0.5%	4.1%	0.9%	5.0%	2.2%
P	0.7%	1.1%	0.7%	3.7%	22.6%	73.0%	6.8%	5.6%	9.9%	3.0%	1.3%	1.2%	3.5%	3.7%	0.6%	0.8%	-	1.4%	8.5%
Q	-	6.5%	-	7.8%	2.5%	1.3%	4.3%	3.1%	3.7%	0.9%	6.8%	3.1%	1.2%	6.1%	0.6%	7.9%	2.1%	4.9%	5.3%
R	-	6.0%	-	3.8%	5.1%	0.6%	3.0%	2.3%	2.6%	0.6%	10.6%	3.1%	0.8%	15.5%	-	5.7%	4.5%	12.2%	7.1%
S	-	31.6%	-	9.6%	8.4%	4.4%	13.9%	8.0%	14.7%	2.5%	7.4%	11.5%	6.5%	3.7%	0.6%	10.4%	0.9%	9.1%	3.8%
T	0.6%	16.1%	-	12.7%	4.8%	1.4%	4.8%	4.0%	11.7%	2.7%	12.3%	1.9%	1.4%	5.8%	1.9%	17.6%	0.8%	5.5%	3.2%
V	29.7%	2.3%	-	1.4%	6.6%	1.9%	4.9%	8.4%	2.1%	20.4%	2.0%	2.2%	3.3%	9.2%	31.6%	5.6%	4.6%	6.7%	8.2%
W	0.9%	-	92.4%	-	-	-	-	-	-	-	-	0.9%	1.0%	0.6%	-	0.5%	4.8%	2.0%	1.0%
Y	-	0.8%	0.8%	-	-	-	0.8%	5.2%	0.8%	1.4%	0.5%	8.4%	48.4%	2.4%	1.8%	4.3%	52.4%	5.7%	6.4%

Most Frequent

1	L (0.45)	S (0.32)	W (0.92)	D (0.17)	P (0.23)	P (0.73)	A (0.15)	G (0.11)	D (0.17)	I (0.24)	G (0.18)	G (0.31)	Y (0.48)	R (0.15)	I (0.37)	T (0.18)	Y (0.52)	G (0.14)	E (0.24)
2	V (0.3)	T (0.16)	F (0.02)	E (0.15)	A (0.18)	S (0.04)	S (0.14)	A (0.09)	S (0.15)	V (0.2)	T (0.12)	S (0.12)	F (0.09)	I (0.11)	V (0.32)	E (0.14)	F (0.06)	R (0.12)	P (0.09)
3	I (0.16)	A (0.07)	Y (0.01)	T (0.13)	K (0.09)	D (0.04)	G (0.14)	V (0.08)	N (0.12)	G (0.11)	D (0.11)	Y (0.08)	S (0.06)	E (0.1)	L (0.18)	S (0.1)	L (0.05)	S (0.09)	V (0.08)
4	F (0.02)	Q (0.07)	P (0.01)	S (0.1)	S (0.08)	G (0.03)	E (0.09)	L (0.08)	T (0.12)	A (0.07)	R (0.11)	N (0.07)	G (0.04)	K (0.1)	T (0.02)	Q (0.08)	W (0.05)	K (0.09)	K (0.07)

	T	G	G	N	S	P	V	Q	E	F	T	V	P	G	S	K	S	T	A
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
A	6.3%	5.8%	6.1%	2.8%	6.5%	4.1%	6.5%	4.3%	7.4%	3.9%	4.9%	6.3%	5.4%	8.6%	6.5%	5.7%	4.3%	2.6%	16.7%
C	1.6%	1.5%	1.1%	0.6%	1.5%	-	1.0%	0.6%	1.3%	1.7%	1.5%	1.7%	0.7%	2.0%	1.2%	-	2.8%	1.2%	4.4%
D	3.8%	9.9%	7.5%	13.3%	6.2%	6.8%	2.2%	4.1%	8.0%	1.5%	4.9%	1.6%	7.6%	5.3%	7.8%	6.1%	4.8%	2.6%	1.4%
E	7.7%	5.0%	6.7%	12.4%	6.7%	11.4%	4.2%	8.7%	23.7%	2.8%	5.4%	2.8%	7.7%	3.1%	5.3%	12.6%	3.6%	9.0%	3.1%
F	1.6%	1.3%	1.1%	1.2%	1.0%	1.4%	3.3%	0.6%	0.5%	10.6%	0.5%	1.6%	0.6%	0.8%	0.8%	0.8%	1.6%	3.0%	6.4%
G	0.7%	19.6%	27.6%	6.1%	9.7%	5.4%	2.6%	4.9%	2.9%	2.4%	3.1%	1.5%	3.6%	32.9%	3.3%	2.2%	2.9%	1.6%	2.8%
H	0.9%	1.5%	1.2%	2.6%	1.5%	1.4%	0.6%	3.5%	2.1%	2.0%	1.4%	0.8%	1.7%	1.8%	2.6%	2.3%	1.8%	2.1%	4.2%
I	6.8%	3.3%	1.6%	1.8%	1.4%	2.2%	11.9%	1.6%	1.4%	8.4%	2.8%	12.1%	1.7%	2.8%	2.1%	2.2%	1.5%	2.3%	6.2%
K	8.4%	8.4%	5.6%	8.0%	7.4%	6.0%	2.7%	13.3%	7.2%	2.9%	5.2%	2.6%	5.9%	3.9%	4.2%	15.5%	2.6%	4.0%	3.4%
L	7.3%	4.0%	2.9%	2.8%	2.9%	2.8%	10.0%	4.2%	1.5%	11.7%	3.7%	11.2%	2.2%	2.2%	2.7%	3.9%	5.2%	3.6%	5.1%
M	2.1%	1.4%	1.2%	1.3%	0.8%	0.7%	3.1%	1.5%	0.9%	2.9%	1.2%	1.7%	0.7%	0.6%	0.6%	1.0%	1.3%	1.2%	2.0%
N	2.8%	6.6%	2.8%	14.5%	3.6%	2.7%	2.0%	4.7%	6.3%	3.4%	6.4%	1.9%	5.0%	5.7%	9.2%	4.8%	8.9%	5.1%	2.1%
P	2.9%	2.1%	2.7%	2.7%	1.3%	22.7%	0.8%	2.0%	2.2%	0.9%	3.6%	0.7%	28.2%	3.3%	3.8%	2.3%	3.0%	2.7%	2.1%
Q	2.6%	4.5%	3.5%	4.2%	5.2%	4.2%	1.2%	16.5%	3.9%	2.0%	4.6%	1.4%	3.2%	1.6%	3.0%	9.5%	3.3%	4.1%	2.5%
R	5.5%	5.3%	7.4%	5.9%	6.9%	4.1%	4.2%	9.3%	6.4%	3.6%	6.7%	2.4%	3.3%	3.3%	4.0%	10.4%	5.6%	5.5%	2.1%
S	9.2%	8.2%	9.1%	6.2%	18.8%	6.9%	4.3%	7.1%	6.7%	3.3%	12.6%	3.3%	8.4%	8.1%	18.9%	4.8%	17.1%	17.2%	4.1%
T	14.9%	3.3%	5.2%	6.5%	10.8%	7.1%	8.9%	5.2%	8.1%	4.0%	23.6%	6.9%	7.5%	5.8%	16.9%	7.7%	24.0%	23.3%	5.5%
V	11.8%	4.1%	2.7%	2.4%	3.7%	2.1%	21.5%	2.2%	5.2%	14.5%	5.9%	36.2%	5.3%	5.5%	4.7%	5.0%	3.3%	4.5%	11.5%
W	0.6%	1.1%	1.4%	1.3%	1.7%	6.2%	5.5%	3.1%	2.9%	8.9%	1.0%	1.1%	-	1.9%	1.0%	1.9%	0.6%	2.1%	1.1%
Y	2.5%	3.3%	2.5%	3.4%	2.5%	1.5%	3.5%	2.4%	1.3%	8.7%	1.0%	2.2%	0.8%	0.8%	1.3%	0.9%	1.8%	2.3%	13.2%

Most Frequent

1	T (0.15)	G (0.2)	G (0.28)	N (0.15)	S (0.19)	P (0.23)	V (0.21)	Q (0.16)	E (0.24)	V (0.14)	T (0.24)	V (0.36)	P (0.28)	G (0.33)	S (0.19)	K (0.15)	T (0.24)	T (0.23)	A (0.17)
2	V (0.12)	D (0.1)	S (0.09)	D (0.13)	T (0.11)	E (0.11)	I (0.12)	K (0.13)	T (0.08)	L (0.12)	S (0.13)	I (0.12)	S (0.08)	A (0.09)	T (0.17)	E (0.13)	S (0.17)	S (0.17)	Y (0.13)
3	S (0.09)	K (0.08)	D (0.07)	E (0.12)	G (0.1)	T (0.07)	L (0.1)	R (0.09)	D (0.08)	F (0.11)	R (0.07)	L (0.11)	E (0.08)	S (0.08)	N (0.09)	R (0.1)	N (0.09)	E (0.09)	V (0.12)
4	K (0.08)	S (0.08)	R (0.07)	K (0.08)	K (0.07)	S (0.07)	T (0.09)	E (0.09)	A (0.07)	W (0.09)	N (0.06)	T (0.07)	D (0.08)	T (0.06)	D (0.08)	Q (0.1)	R (0.06)	R (0.06)	F (0.06)

	T 58	I 59	S 60	G 61	L 62	K 63	P 64	G 65	V 66	D 67	Y 68	T 69	I 70	T 71	V 72	Y 73	A 74	V 75	T 76
A	1.9%	3.0%	3.3%	3.3%	1.0%	3.5%	6.7%	6.1%	5.3%	3.8%	1.7%	2.7%	3.0%	2.1%	2.1%	3.2%	45.5%	2.2%	2.7%
C	0.8%	0.9%	0.5%	-	-	-	0.7%	-	1.6%	-	-	0.7%	0.9%	1.1%	-	1.1%	1.1%	1.5%	1.1%
D	4.0%	2.9%	6.3%	11.7%	0.9%	3.6%	2.4%	7.2%	-	11.5%	-	3.3%	1.0%	2.1%	1.3%	0.9%	1.8%	0.5%	2.2%
E	5.9%	1.0%	7.3%	4.1%	1.0%	10.9%	9.9%	3.2%	3.1%	21.7%	1.9%	11.0%	1.3%	6.4%	3.3%	3.1%	6.0%	3.8%	5.9%
F	1.1%	6.1%	1.1%	0.8%	3.2%	1.1%	1.4%	3.0%	1.1%	0.5%	4.4%	1.3%	24.9%	0.8%	0.8%	11.4%	-	8.2%	3.2%
G	0.6%	-	3.1%	38.1%	-	0.9%	1.5%	41.5%	0.8%	1.6%	1.4%	2.6%	1.8%	2.5%	2.8%	1.2%	5.5%	0.9%	1.1%
H	2.0%	-	2.5%	2.6%	-	2.3%	0.9%	2.1%	2.2%	2.0%	2.9%	1.8%	-	1.8%	0.5%	2.9%	1.0%	1.2%	1.1%
I	4.3%	22.4%	0.9%	0.7%	4.5%	5.1%	1.9%	0.5%	4.2%	1.1%	1.2%	2.5%	18.2%	0.9%	12.5%	3.5%	1.1%	13.3%	2.0%
K	6.7%	0.9%	7.4%	4.3%	0.5%	16.6%	3.9%	2.4%	4.5%	7.4%	1.8%	8.4%	0.9%	6.1%	1.6%	5.5%	3.9%	1.1%	4.3%
L	5.7%	21.9%	1.7%	1.2%	71.2%	5.8%	1.9%	3.0%	11.6%	4.8%	4.2%	6.0%	13.9%	2.8%	15.3%	8.2%	2.2%	12.6%	2.7%
M	1.3%	2.0%	0.8%	0.5%	1.8%	1.6%	0.6%	0.6%	2.1%	0.8%	0.8%	0.9%	2.8%	0.7%	1.6%	1.8%	0.5%	1.9%	0.9%
N	4.6%	1.1%	5.3%	13.6%	0.7%	4.0%	3.0%	10.9%	2.7%	6.6%	1.2%	6.9%	0.9%	4.9%	2.4%	1.7%	2.6%	1.1%	19.9%
P	2.5%	0.8%	4.2%	1.9%	0.5%	1.4%	45.8%	-	-	2.7%	1.0%	1.9%	0.6%	1.2%	1.4%	1.1%	3.9%	1.0%	1.7%
Q	3.5%	1.5%	4.7%	2.7%	0.7%	8.3%	2.6%	1.6%	3.2%	6.2%	0.9%	6.4%	-	5.4%	0.9%	5.2%	4.1%	0.9%	4.1%
R	6.5%	0.8%	5.2%	3.0%	1.3%	9.5%	2.1%	2.3%	5.3%	4.5%	2.2%	5.7%	-	21.1%	1.1%	8.7%	4.8%	0.9%	5.4%
S	6.1%	0.8%	17.6%	5.4%	0.8%	7.9%	5.0%	7.5%	5.7%	9.2%	1.1%	10.5%	0.7%	11.7%	1.0%	4.7%	7.5%	1.6%	10.1%
T	31.6%	2.3%	23.2%	2.4%	3.3%	10.4%	6.0%	2.6%	31.5%	11.6%	1.4%	20.9%	1.5%	22.4%	1.9%	4.9%	5.0%	3.0%	24.3%
V	9.4%	28.2%	2.4%	1.3%	6.3%	5.3%	2.3%	0.8%	13.1%	2.2%	2.0%	3.9%	21.3%	1.5%	46.6%	4.4%	1.9%	34.7%	2.9%
W	-	-	0.7%	-	-	-	-	0.7%	-	0.6%	0.5%	-	-	1.1%	-	2.0%	-	0.8%	0.5%
Y	1.1%	2.2%	1.9%	1.7%	0.9%	1.4%	1.1%	3.1%	0.7%	-	68.5%	2.0%	4.5%	3.3%	2.0%	24.5%	0.8%	8.7%	3.9%

Most Frequent

1	T (0.32)	V (0.28)	T (0.23)	G (0.38)	L (0.71)	K (0.17)	P (0.46)	G (0.41)	T (0.31)	E (0.22)	Y (0.69)	T (0.21)	F (0.25)	T (0.22)	V (0.47)	Y (0.25)	A (0.46)	V (0.35)	T (0.24)
2	V (0.09)	I (0.22)	S (0.18)	N (0.14)	V (0.06)	E (0.11)	E (0.1)	N (0.11)	V (0.13)	T (0.12)	F (0.04)	E (0.11)	V (0.21)	R (0.21)	L (0.15)	F (0.11)	S (0.07)	I (0.13)	N (0.2)
3	K (0.07)	L (0.22)	K (0.07)	D (0.12)	I (0.04)	T (0.1)	A (0.07)	S (0.08)	L (0.12)	D (0.12)	L (0.04)	S (0.1)	I (0.18)	S (0.12)	I (0.13)	R (0.09)	E (0.06)	L (0.13)	S (0.1)
4	R (0.06)	F (0.06)	E (0.07)	S (0.05)	T (0.03)	R (0.09)	T (0.06)	D (0.07)	S (0.06)	S (0.09)	H (0.03)	K (0.08)	L (0.14)	E (0.06)	E (0.03)	L (0.08)	G (0.05)	Y (0.09)	E (0.06)

	G 77	R 78	G 79	D 80	S 81	P 82	A 83	S 84	S 85	K 86	P 87	I 88	S 89	I 90	N 91	Y 92	R 93	T 94
A	18.6%	6.7%	8.8%	1.1%	8.1%	5.6%	42.2%	10.0%	6.6%	2.6%	8.7%	11.4%	7.0%	6.1%	4.8%	4.2%	8.4%	9.4%
C	0.7%	-	1.1%	0.7%	2.0%	0.8%	1.1%	2.6%	1.0%	-	-	1.6%	0.7%	1.0%	-	-	-	-
D	2.6%	0.6%	0.6%	22.8%	0.7%	1.3%	-	1.7%	4.3%	1.3%	2.3%	0.7%	1.2%	3.4%	8.8%	2.2%	2.1%	1.0%
E	5.3%	7.6%	1.5%	17.5%	0.8%	5.3%	2.7%	10.2%	2.4%	19.6%	4.3%	1.2%	1.3%	5.3%	7.8%	10.5%	14.6%	1.8%
F	4.5%	2.0%	4.6%	3.5%	3.7%	2.2%	2.6%	2.5%	1.7%	0.5%	-	7.1%	-	4.0%	-	14.1%	2.5%	0.6%
G	14.6%	-	49.6%	1.1%	9.1%	2.5%	8.6%	13.9%	23.6%	7.0%	8.3%	4.0%	32.0%	6.1%	36.5%	4.7%	2.3%	2.8%
H	1.7%	3.0%	0.8%	1.1%	0.8%	1.2%	-	1.9%	0.6%	2.2%	0.8%	0.5%	-	-	0.6%	3.4%	1.0%	-
I	2.9%	2.3%	2.1%	4.3%	3.4%	6.5%	5.8%	1.8%	1.3%	0.7%	2.4%	13.2%	-	14.8%	-	1.3%	-	1.1%
K	4.9%	13.9%	0.8%	2.8%	3.9%	5.6%	1.0%	3.4%	1.0%	21.3%	1.7%	2.0%	0.8%	1.3%	2.7%	4.1%	9.0%	1.0%
L	2.8%	3.0%	2.3%	4.0%	2.5%	4.0%	6.7%	2.7%	1.2%	1.9%	0.6%	14.2%	-	15.4%	0.7%	4.4%	1.6%	2.1%
M	1.5%	1.1%	1.2%	0.6%	1.0%	3.2%	2.3%	0.8%	-	0.6%	-	3.7%	-	2.5%	-	1.2%	-	0.5%
N	7.6%	1.7%	5.5%	13.4%	6.4%	1.7%	1.4%	5.0%	15.6%	10.2%	3.3%	1.1%	2.5%	0.9%	11.5%	1.1%	1.0%	0.7%
P	3.4%	-	1.0%	-	-	23.6%	-	-	-	0.5%	55.1%	0.6%	-	2.0%	3.5%	11.1%	19.0%	6.4%
Q	3.3%	5.2%	1.4%	4.7%	3.0%	5.4%	1.6%	4.9%	2.0%	9.7%	1.8%	1.2%	1.2%	1.4%	2.6%	2.2%	5.4%	0.8%
R	1.9%	31.2%	0.8%	5.7%	7.4%	8.2%	2.6%	5.7%	1.4%	12.1%	1.3%	0.8%	1.0%	1.3%	1.4%	1.9%	16.3%	0.5%
S	7.6%	1.7%	3.5%	1.7%	19.4%	2.0%	1.2%	15.8%	21.7%	2.8%	2.9%	0.7%	45.9%	2.5%	14.9%	5.0%	7.6%	55.5%
T	3.0%	2.5%	3.7%	3.0%	11.4%	4.3%	1.6%	7.7%	7.9%	3.0%	3.0%	2.6%	4.5%	4.2%	2.8%	1.6%	3.0%	13.0%
V	6.8%	5.4%	3.1%	7.2%	12.0%	12.7%	13.6%	4.9%	4.7%	1.3%	1.8%	24.1%	-	22.6%	-	3.5%	0.8%	1.4%
W	0.6%	-	0.5%	-	-	-	0.7%	-	-	-	-	1.4%	-	2.1%	-	9.5%	2.3%	-
Y	5.7%	10.9%	7.1%	3.7%	4.3%	3.3%	3.2%	4.0%	2.1%	2.1%	0.6%	7.8%	-	2.6%	-	13.6%	1.8%	-

Most Frequent

1	A (0.19)	R (0.31)	G (0.5)	D (0.23)	S (0.19)	P (0.24)	A (0.42)	S (0.16)	G (0.24)	K (0.21)	P (0.55)	V (0.24)	S (0.46)	V (0.23)	G (0.37)	F (0.14)	P (0.19)	S (0.55)
2	G (0.15)	K (0.14)	A (0.09)	E (0.18)	V (0.12)	V (0.13)	V (0.14)	G (0.14)	S (0.22)	E (0.2)	A (0.09)	L (0.14)	G (0.32)	L (0.15)	S (0.15)	Y (0.14)	R (0.16)	T (0.13)
3	N (0.08)	Y (0.11)	Y (0.07)	N (0.13)	T (0.11)	R (0.08)	G (0.09)	E (0.1)	N (0.16)	R (0.12)	G (0.08)	I (0.13)	A (0.07)	I (0.15)	N (0.11)	P (0.11)	E (0.15)	A (0.09)
4	S (0.08)	E (0.08)	N (0.06)	V (0.07)	G (0.09)	I (0.07)	L (0.07)	A (0.1)	T (0.08)	N (0.1)	E (0.04)	A (0.11)	T (0.04)	A (0.06)	D (0.09)	E (0.11)	K (0.09)	P (0.06)

C. Complementarity distribution mimicking CDRH3 of antibodies (CDR'), modified from previous work (Hackel et al., 2010).

CDR' amino acid frequency distribution:

A	0.06
C	0.05
D	0.11
E	0.02
F	0.03
G	0.04
H	0.06
I	0.02
K	0.02
L	0.02
M	0.00
N	0.09
P	0.04
Q	0.01
R	0.03
S	0.13
T	0.05
V	0.03
W	0.01
Y	0.17
Z	0.03

Nucleotide composition of codon design:

A1	C1	G1	T1	A2	C2	G2	T2	A3	C3	G3	T3
0.20	0.15	0.25	0.40	0.50	0.25	0.15	0.10	0.00	0.45	0.10	0.45

D. Target exposure and solvent exposed surface area (SASA) for the BC, DE, and FG loops of fibronectin.

Position	Target Exposure	SASA
D23	0.68	0.35
A24	0.90	0.32
P25	0.70	0.10
A26	0.79	0.75
V27	0.69	0.57
T28	0.84	0.75
V29	0.46	0.03
R30	0.90	0.49
Y31	0.37	0.43
G52	0.54	0.12
S53	0.94	0.83
K54	0.68	0.64
S55	0.85	0.41
T56	0.93	0.48
T76	0.39	0.08
G77	0.60	0.48
R78	0.79	0.81
G79	0.72	0.77
D80	0.70	0.74
S81	0.54	0.69
P82	0.59	0.76
A83	0.76	0.81
S84	0.52	0.54
S85	0.61	0.14
K86	0.58	0.88