Supplementary Materials

Figure Captions

Supplementary Figure 1.

(**A**) The DNA sequence of the synthetic gene coding for cAb-RN05 V_HH. The locations of CDR1, 2 and CDR3 are indicated as bars.

(**B**) A schematic drawing of the yeast surface display vector for cAb-RN05 V_HH used in this work.

Supplementary Figure 2. Water molecules found near the V_HH-RNaseA interface in the wild-type (**A**) and affinity-matured (**B**) complexes, depicted in stereoview. The V_HH and RNaseA backbone shown as ribbons are colored in cyan and pink, respectively. The water molecules that are found at the complex interface (14 and 13 molecules in panel A and B, respectively) are represented as a green spheres. Two spines of H-bonded water molecules are found and they are denoted as connected green bonds. The water molecules that form H-bonds to both V_HH and RNaseA residues are shown as larger and labeled (w1-w4 and w1-w5 in panel A and B, respectively). The protein residues that are involved in the interactions at the complex interface are shown as ball-and-stick models and labeled. Nitrogen and oxygen atoms are in blue and in red, respectively. Carbon atoms and covalent bonds involving a carbon atom are colored gray and yellow for V_HH and RNaseA residues, respectively. The hydrogen bonds involving the protein residues are shown as doted lines: water-protein and protein-protein interactions are in green and gray, respectively. The positions of ten water molecules (shown in bright green) are conserved (shift < 0.7 Å). The H-bond distances are within 2.6-3.4 Å and they are listed in Tables S1-S2. One of the two complexes in the asymmetric unit in the crystals of the affinity matured V_HH (chains A and B) is selected for presentation. The second conformation found for RNaseA residues S59 (panel A) and Y76 (panel B) is shown in gray.

Table S1. Direct H-bonds at the V_HH-RNaseA interface

V_HH		RNaseA		Distance (Å)	
Residue	Atom	Residue	Atom	Wild-Type	Mutant
Between V_HH and RNaseA					
CDR1					
Y27	OH	Q60	O	3.45	2.62
Y27	OH	N62	N	2.80	2.86
I32	N	N62	OD1	2.96	2.90
CDR3					
G95	O	Y73	OH	2.65	2.96
G95	O	Y115	OH	3.33	3.25
G96	O	T70	OG1	2.73	2.69
R100bT	O	G112	N	2.89	2.86
T100cR	O	Y115	OH	2.71	2.61
R100b	NE	E111	OE1	3.32	–*
T100cR	NE	E111	OE1	–*	3.06
Within V_HH					
Y97D	OD2	T100cR	NH2	–*	2.88
R45	NE	D100a	OD2	2.92	–*
R45	NH2	D100a	OD1	2.30	–*
Within RNaseA					
N71	ND2	G110	O	2.97	2.83
N71	OD1	Q69	NE2	3.03	2.80
Q69	OE1	N67	ND2	2.81	3.24
Q69	N	N67	OD1	2.84	2.97

*Present only in one structure

Table S2. Single water mediated H-bonds (Å) between V_HH and RNaseA at the complex interface as depicted in Figure S1.

Water molecule	Protein	Residue	Atom	Distance (Å)	
				Wild Type	Mutant
w1	V_HH	Y33	N	2.85	2.86
	V_HH	G96	O	2.88	2.86
	V_HH	G96	N	3.30[#]	2.92
	RNaseA	N62	OD1	2.69	2.86
	RNaseA	T70	O	3.17	2.87
w2	V_HH	T30	O	2.69	2.55
	V_HH	T30	N	3.57[#]	3.26
	RNaseA	N62	O	2.79	2.69
w3	V_HH	A95	O	2.95	2.80
	V_HH	G95	O	3.89[#]	2.86
	RNaseA	Y73	OH	2.70	2.77
w4	V_HH	Y100d	O	3.24	2.93
	RNaseA	G112	O	2.65	2.80

[#]The distance is longer than the H-bond length cutoff (3.5 Å) or the Donor-H···Acceptor angle is smaller than H-bond angle cutoff (100^{o}).

A

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CAAGTACAACTGGTAGAAAGCGGTGGTGGTCTGGTACAAGCAGGTGGTAGCCTGCGTCTG
 Q  V  Q  L  V  E  S  G  G  G  L  V  Q  A  G  G  S  L  R  L
 1                          10                          20

AGCTGCGCAGCAAGCGGTTACGCATACACCTACATCTACATGGGTTGGTTCCGTCAAGCA
 S  C  A  A  S  G  Y  A  Y  T  Y  I  Y  M  G  W  F  R  Q  A
                  CDR1   30                          40

CCAGGTAAAGAACGTGAAGGTGTAGCAGCAATGGACAGCGGTGGTGGTGGTACTCTGTAC
 P  G  K  E  R  E  G  V  A  A  M  D  S  G  G  G  G  T  L  Y
                           50    52 52a  CDR2

GCAGACAGCGTAAAAGGTCGTTTCACCATCAGCCGTGACAAAGGTAAAAACACCGTATAC
 A  D  S  V  K  G  R  F  T  I  S  R  D  K  G  K  N  T  V  Y
60                          70

CTGCAGATGGACAGCCTGAAACCAGAAGACACCGCAACCTACTACTGCGCAGCAGGTGGT
 L  Q  M  D  S  L  K  P  E  D  T  A  T  Y  Y  C  A  A  G  G
80       83 83a  b   c  84              90

TACGAACTGCGTGACCGTACCTACGGTCAATGGGGTCAAGGCACCCAAGTAACCGTAAGC
 Y  E  L  R  D  R  T  Y  G  Q  W  G  Q  G  T  Q  V  T  V  S
  CDR3  100 a  b   c   d 101                    110
AGCGGTGGC
 S  G  G
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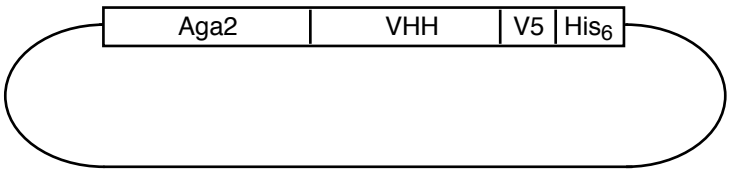
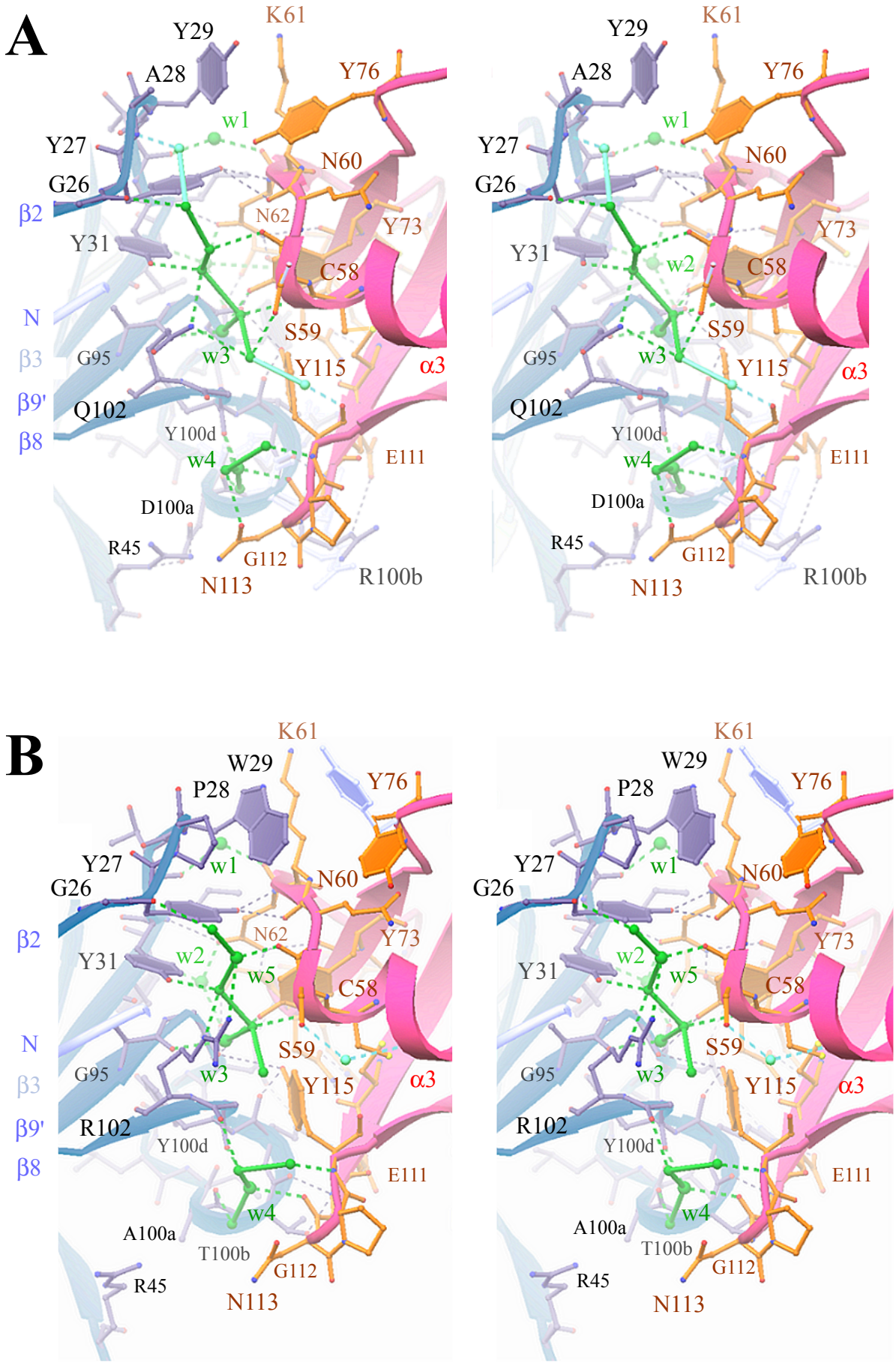
B



Supplementary Figure 1. Koide et al.



Supplementary Figure 2. Koide et al.