

Routes to covalent catalysis by reactive selection for nascent protein nucleophiles

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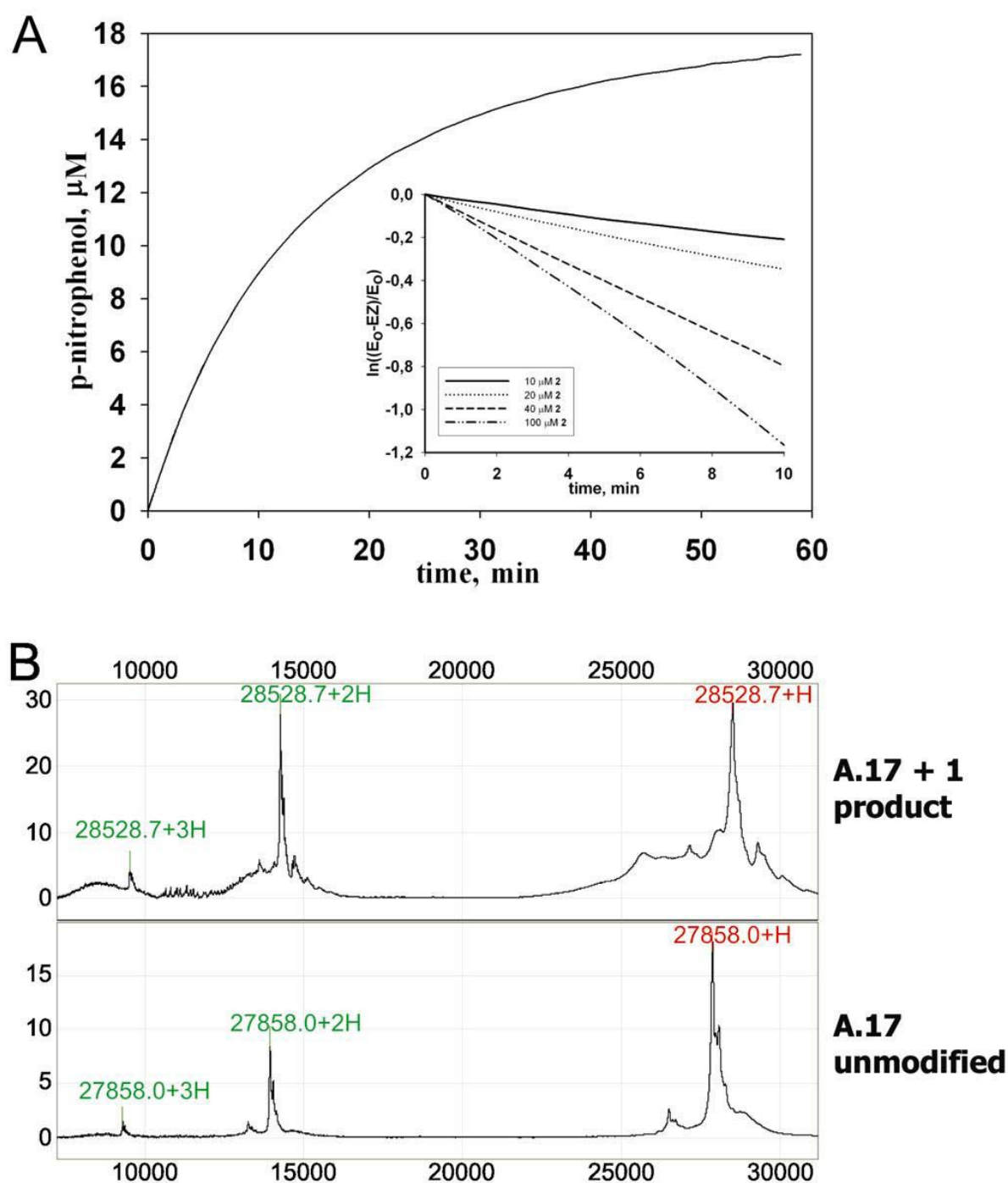
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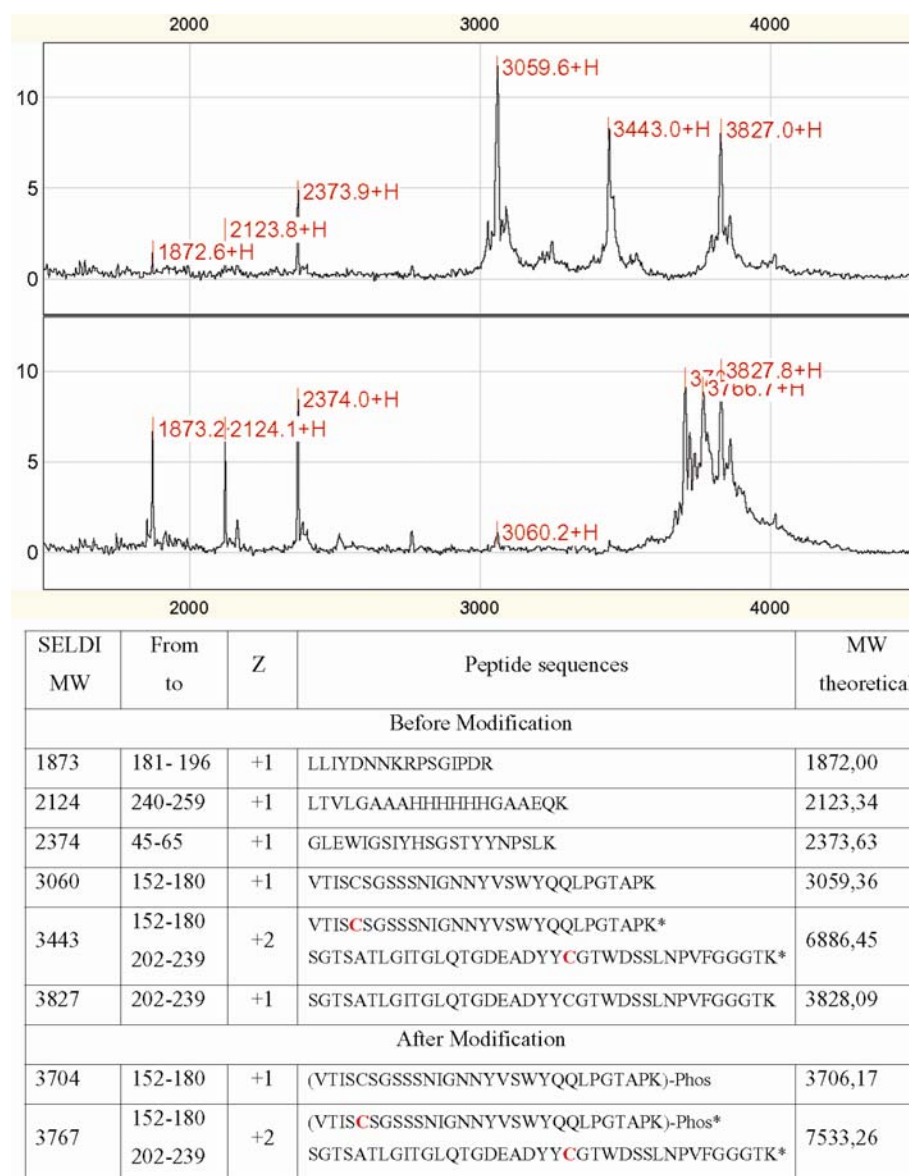
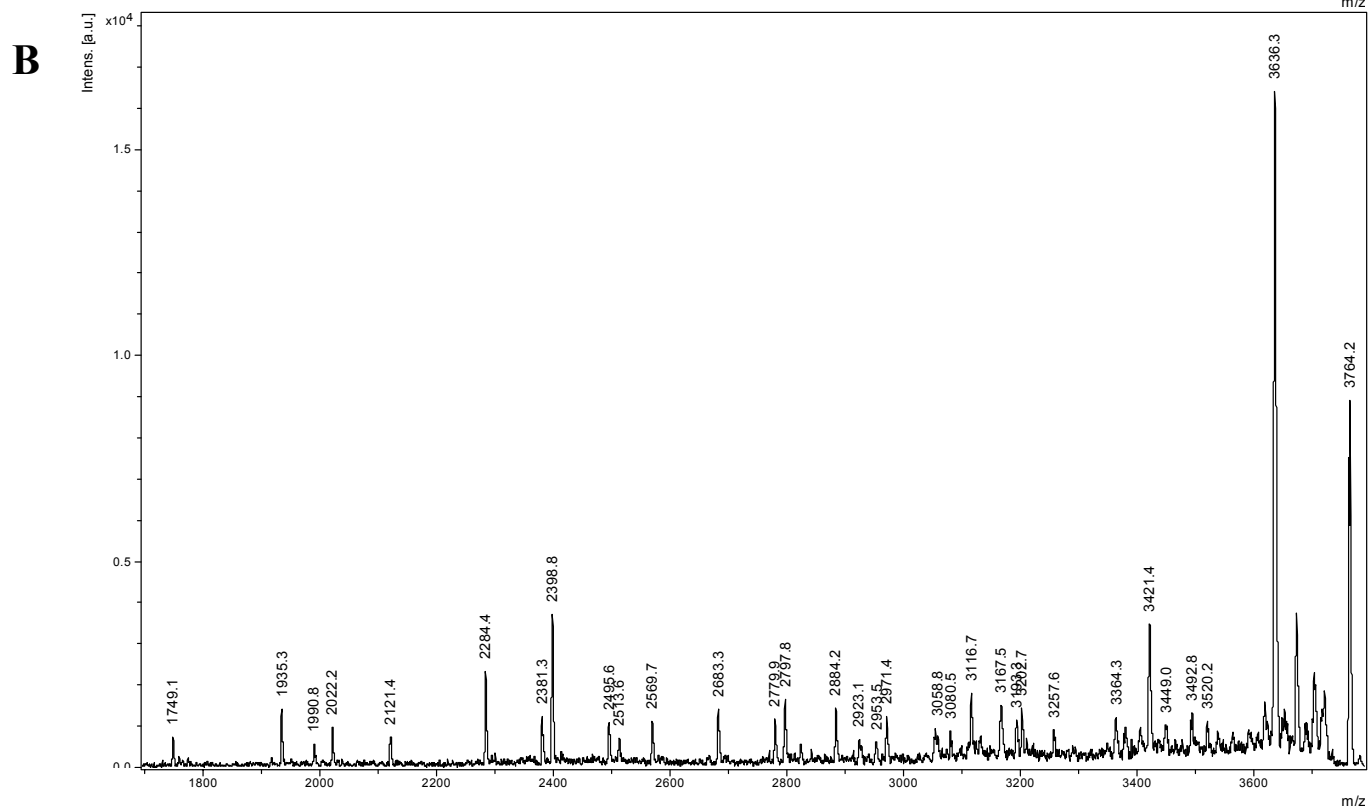
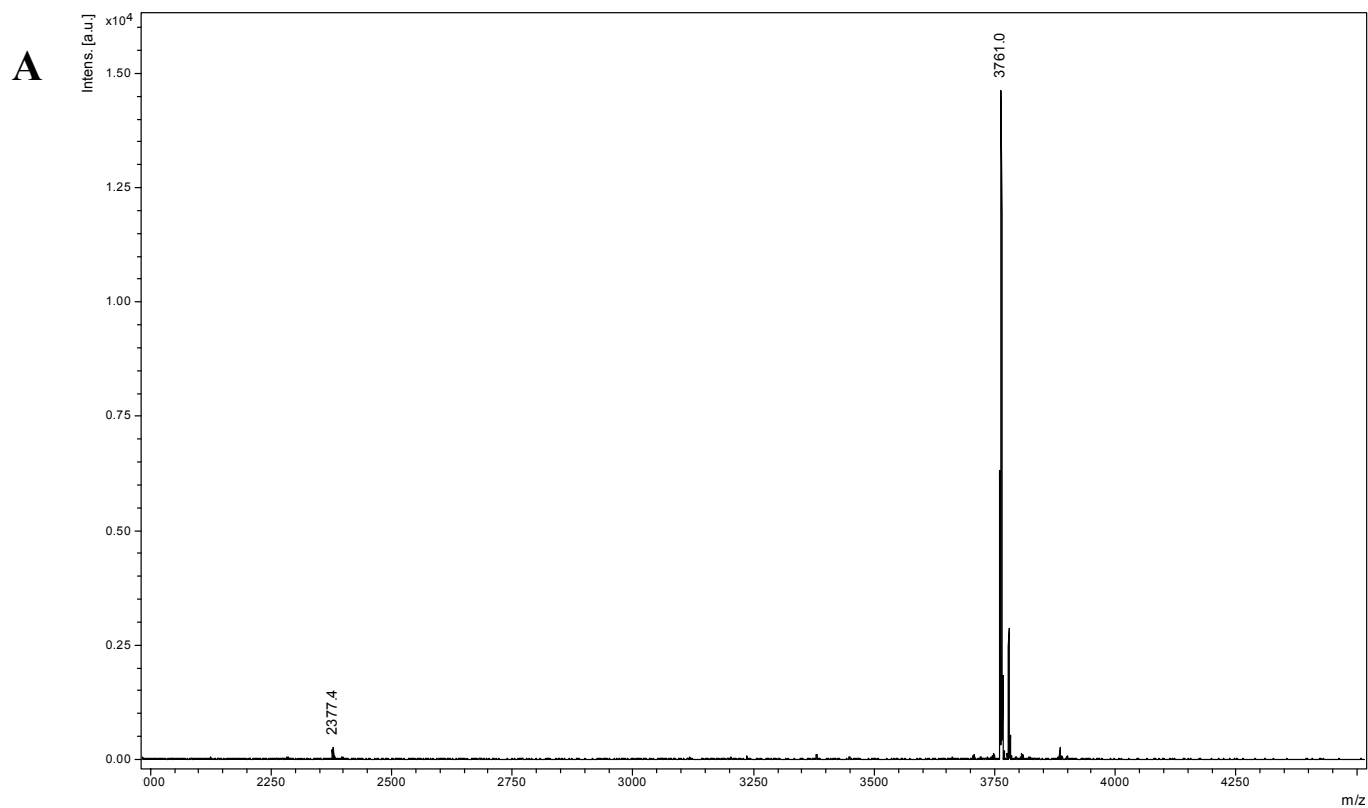


Figure S2. SELDI MS analysis of tryptic hydrolysate of **A.17** (upper spectrum) and **A.17** labeled with **1** (lower spectrum). Peptide sequences and theoretical and experimental molecular masses are presented in the table. The peptide sequences are identified according to scFv sequence numbering. Peptide 152 – 180 corresponded to CDR-L1 and flanking residues from FR-L1 and FR-L2 regions (VL 18-45 according to Kabat numbering).



C.

a	b	c''	Res:	x	y''	z
72,081	100,076	151,108	1 Val 29	-	-	-
173,129	201,124	252,156	2 Thr 28	3688,709	3662,730	3643,688
286,213	314,208	365,240	3 Ile 27	3587,662	3561,683	3542,640
373,245	401,240	452,272	4 Ser 26	3474,578	3448,599	3429,556
533,276	561,271	612,303	5 Cys 25	3387,546	3361,567	3342,524
620,308	648,303	699,335	6 Ser 24	3227,515	3201,536	3182,494
677,329	705,324	756,356	7 Gly 23	3140,483	3114,504	3095,462
764,361	792,356	843,388	8 Ser 22	3083,462	3057,482	3038,440
851,393	879,388	930,420	9 Ser 21	2996,429	2970,450	2951,408
938,425	966,420	1017,452	10 Ser 20	2909,397	2883,418	2864,376
1052,468	1080,463	1131,495	11 Asn 19	2822,365	2796,386	2777,344
1165,552	1193,547	1244,579	12 Ile 18	2708,323	2682,343	2663,301
1222,574	1250,569	1301,601	13 Gly 17	2595,238	2569,259	2550,217
1336,617	1364,612	1415,644	14 Asn 16	2538,217	2512,238	2493,196
1450,660	1478,655	1529,687	15 Asn 15	2424,174	2398,195	2379,153
1613,723	1641,718	1692,750	16 Tyr 14	2310,131	2284,152	2265,110
1712,791	1740,786	1791,818	17 Val 13	2147,068	2121,089	2102,046
1799,823	1827,818	1878,850	18 Ser 12	2047,999	2022,020	2002,978
1985,903	2013,898	2064,930	19 Trp 11	1960,967	1934,988	1915,946
2795,285	2823,280	2874,312	20 Tyr 10	1774,888	1748,909	1729,867
2923,344	2951,339	3002,371	21 Gln 9	965,506	939,526	920,484
3051,402	3079,397	3130,429	22 Gln 8	837,447	811,468	792,425
3164,486	3192,481	3243,514	23 Leu 7	709,388	683,409	664,367
3261,539	3289,534	3340,566	24 Pro 6	596,304	570,325	551,283
3318,561	3346,556	3397,588	25 Gly 5	499,252	473,272	454,230
3419,608	3447,603	3498,635	26 Thr 4	442,230	416,251	397,209
3490,645	3518,640	3569,673	27 Ala 3	341,182	315,203	296,161
3587,698	3615,693	3666,725	28 Pro 2	270,145	244,166	225,124
3715,793	3743,788	3794,820	29 Lys 1	173,093	147,113	128,071

Figure S3. MS analysis of peptide labeled with **1**, derived from clone **A.17**. (A) Direct MS analysis of the peptide absorbed on monomeric avidin resin from a tryptic hydrolysate of **A.17** reacted with **1**. (B) MS/MS analysis of this peptide. (C) Table of theoretical monoisotopic masses of MS/MS fragmentation of VTISC_{acetamide}SGSSSNIGNNYSWY_{phos}QLPGTAPK. Masses found in the MS/MS spectra are in bold with yellow highlight.

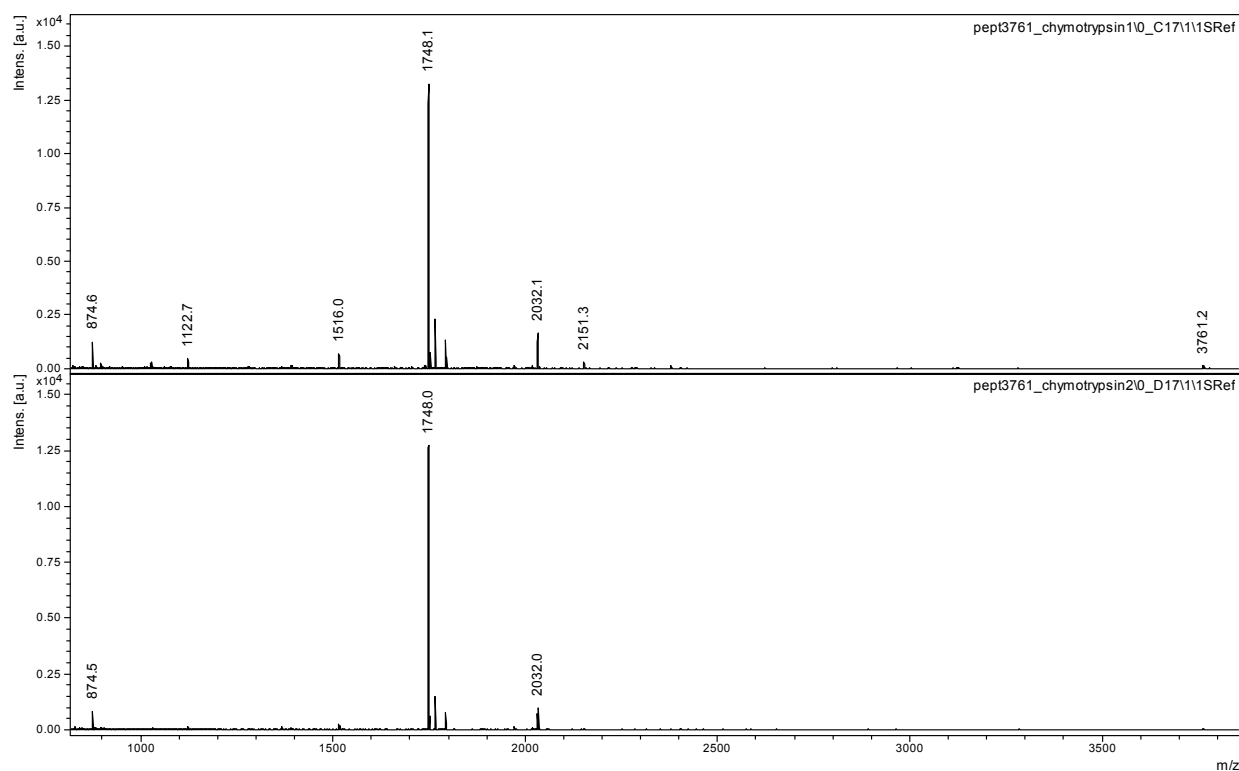


Figure S4. MALDI MS analysis of VL 18-45 (Kabat numbering) peptide digested with chymotrypsin. Two parts were observed $m/z = 2032.0$ - $\text{VTISC}_{\text{acetamide}}\text{SGSSSNIGNNYVSW}$ and $m/z = 1748.1$ - (YQQLPGTAPK+ phosphonate).

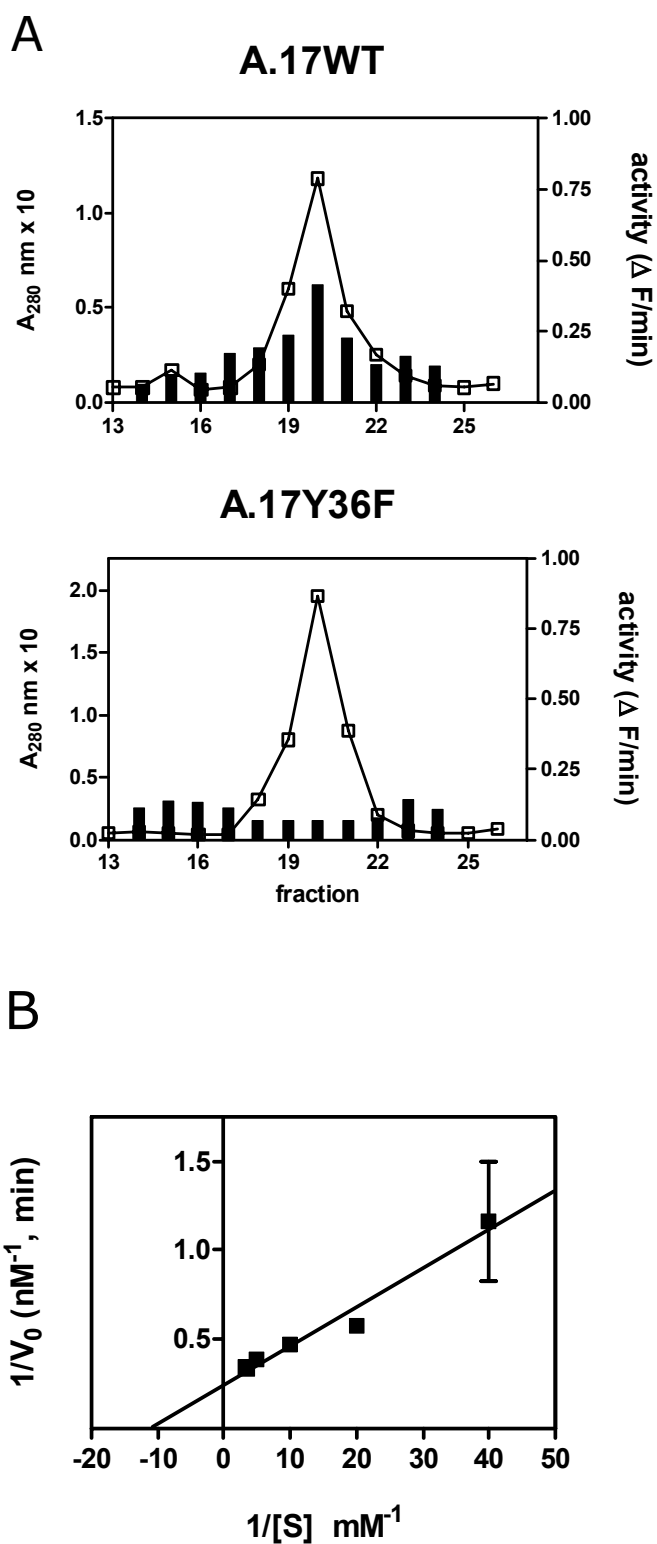


Figure S5. (A) A280 absorbance (\square) and superimposed reactivity of FPLC fractions (bars) as determined by F-mca fluorescence changes of scFv **A.17** and **A.17Y36F**. (B) Lineweaver-Burke plot of initial rates of F-mca hydrolysis in presence of 50 $\mu\text{g/ml}$ **A.17**.

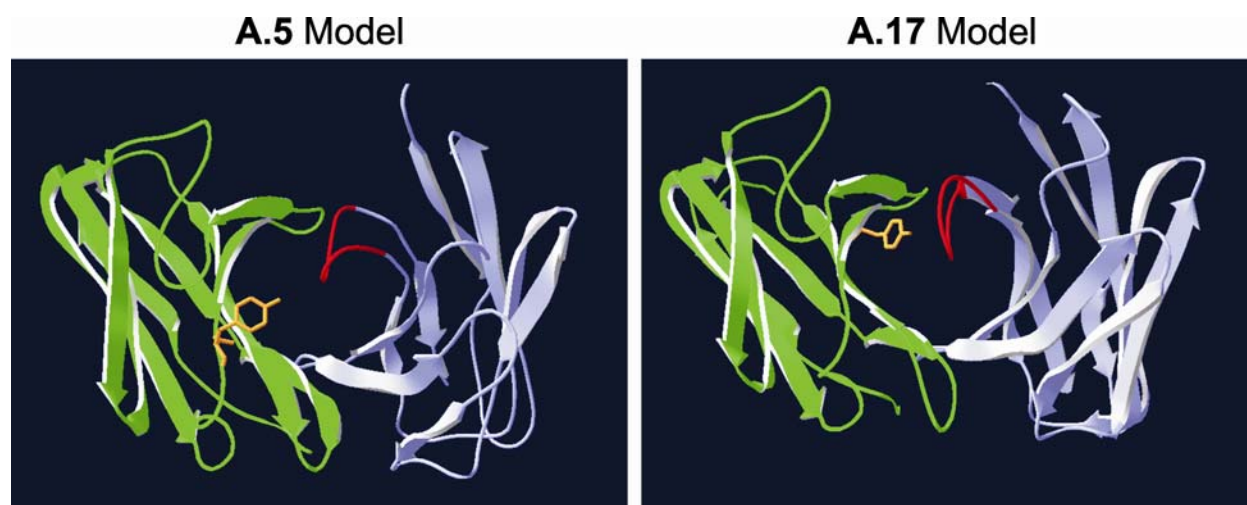


Figure S6. 3D modeling of reactive clones **A.5** and **A.17**. Preliminary models were derived from the three-dimensional structure of homologous antibody molecules (Protein Data Bank: 1F3R-B for VH and 2B0S-L for VL). Side chain replacements and relaxed backbone coordinates obtained by molecular dynamics provided the ternary structures for comparison of sites comprising potential Tyr nucleophiles. Light chains are colored green and heavy chains light blue. Active site Tyr residues are shown in yellow and CDR-H3 in red.