- Structure of an anti-PEG antibody reveals an open ring that captures highly flexible PEG polymers

- 5 Huckaby et al.



8 Supplementary Figure 1: Representative electron density for the Fab-PEG

# 9 interface in the crystal structure

- 10 Image showing the 2Fo-Fc electron density map for the crystal structure contoured at a
- 11 1sigma level to show the quality of the data in the vicinity of the PEG molecule and the
- 12 correspondingly bound Fab.





# 16 Supplementary Figure 2: PEG polymer antigen colored by semi-circular domain

#### 17 with relevant solvent molecules added

- 18 Water solvent molecules make alternating hydrogen bonds to ether oxygen atoms of the
- 19 PEG polymer chain, contributing to the various semi-circular domains (colored yellow,
- cyan, and magenta) and overall spiral shape of the PEG molecule while in complex with
- the anti-PEG Fab.



### 25 Supplementary Figure 3: Superposition of all antibody-antigen structure

- 26 complexes
- 27 Superimposing all complexes from both of the crystal structures reveals strong
- alignment for protein atoms of the Fab molecules as well as atoms along the PEG
- 29 antigens, but alignment begins to diverge near the terminal ends of the polymer chains.
- 30 This divergent behavior is likely a result of inherent PEG polymer chain flexibility distal
- to the antigen-binding interface region of the complex.
- 32

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а	6-3   VH 3-3   VH E11   VH PEG.2 6A9   VH 157G29D1   VH 15-2   VH	102040QIQLVQSGPELKKPGETVKISCKASGYTFKNYGMNWVKQAPGKGLKWMGWINTYTGQPEVKLEESGGGLVQPGGSMKLSCAASGFIFSDAWMDWVRQSPERGLEWVAEIRSKANGLAPQVQLQESGAELARPGASVMMSCKASGYTFTTYTMNWVKQRPGQGLEWIGYIIPSSGYVEVKFEESGGGLVQPGGSMKLSCAASGFTFSDAWMDWVRQSPEKGLEWVAEIRSKANNHAIQVQLQQPGAELVKPGASVKLSCKASGYIFTNYWINWVKQRPGQGLEWIGNSYPGSSSTEVKLEESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQSPEKGLEWVTEIRSKSNNYAT
	6-3   VH 3-3   VH E11   VH PEG.2 6A9   VH 157G29D1   VH 15-2   VH	608096110IYANDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARDWGPYWGQGTLVIVYYAESVKGRFTISRDDSKSSVYLQMNNLRSEDTGIYYCTSTLYYFDYWGQGTLVTVDYNQKFKGKTILTTDKSSSTAYMQLSSLTSEDSAVYYCVRS-LDGYFWFAYWGQGTLVTVYYAESVKGRFTISRDDSKSSVYLQMNSLRAEDTGIYYCTRGWYPYYFDYWGQGTLLTVNYNEKFKSKATLTVDTSSSTAYMQLSSLTSDDSAVFYCARSGPTGTAWFASWGQGTLVTVHYAESVKGRFTISRDDSKGSVYLQMNNLRAEDTGIYYCSNRY
	6-3   VH 3-3   VH E11   VH PEG.2 6A9   VH 157G29D1   VH 15-2   VH	SA SS SA SA SA
b	6-3   VL 3-3   VL E11   VL PEG.2 6A9   VL 157G29D1   VL 15-2   VL	102040NIMMTQSPSSLAVSAGEKVTVNCKSSQSVLYSSNQMNYLAWYQQKPGQSPKLLIYWASTRQIVLTQSPAIMSAFPGERVTLTCSASSSVRSSYLCWYQQKPGSSPKLWIYSTSNLDVLMTQSPLSLPVSLGDHASISCRSSKSIVH-SNGNTYLEWFLQKPGQSPKLLIYKVSNRDVLMTQTPLSLPVSLGDQASISCRSSQSIVH-SDGNTYLEWYLQKPGQSPKLLIYKVSNRDVLMTQTPLSLPVSLGDQASISCRSSQSIVH-SNGNTYLDWYLQKPGQSPKLLIYKVSNRDIVMTQSHKFMSTSVRDRVTITCKASQDVNTSVAWYQQKPGQSPKLLIYWASTR
	6-3   VL 3-3   VL E11   VL PEG.2 6A9   VL 157G29D1   VL 15-2   VL	60 80 100 ESGVPDRFTGSGSGTDFTLTISSVQTEDLAVYYCLQYLS-SWTFGGGTKLEIK ASGVPARFSGSGSGTSYSLTISSMEAEDAASYFCHQWSSYPRTFGGGTKLEIK MSGVPDRFSGSGSGTDFTLKIRRVEAEDLGVYYCSQGSHVPPTFGGGTKLEIK FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIK FSGVPDRFSGRGSGTDFTLKITRVEAEDLGVYYCFQGSHVPLTFGAGTKLELKRA HTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQYINYPYTFGGGTKLEIK
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#### Supplementary Figure 4: Variable domain amino acid sequence alignments for 35

#### 36 various APA clones

- **a** Variable heavy (VH) domain amino acid sequence alignments for six different APA 37
- clones (6-3, 3-3, E11, PEG.2 6A9, 157G29D1, and 15-2) with Kabat numbering and 38
- CDRs highlighted (CDR1, green; CDR2, yellow; CDR3, pink). The 96<sup>th</sup> residue is 39
- labeled and highlighted blue to show conservation of the ring-forming tryptophan (W) or 40

- 41 similarly bulky and hydrophobic tyrosine (Y) residue across other APA clones in the VH
- 42 domain.
- **b** Variable light (VL) domain amino acid sequence alignments for six different APA
- 44 clones (6-3, 3-3, E11, PEG.2 6A9, 157G29D1, and 15-2) with Kabat numbering and
- 45 CDRs highlighted (CDR1, green; CDR2, yellow; CDR3, pink).





49 Supplementary Figure 5: Overlay of two different APA Fab clone structures

### 50 reveals different binding mechanisms to PEG backbone

- a Ribbon (magenta) and surface model representations of clone 6-3 Fab structure in
- 52 complexation with PEG backbone antigen (carbon, cyan; oxygen, red).
- **b** Ribbon (green) and surface model representations of clone 3.3 Fab structure in
- 54 complexation with PEG backbone antigen (carbon, orange; oxygen, red).
- **c** Overlay of clone 6-3 Fab (magenta) and clone 3.3 Fab (green) structures represented
- as ribbon models in complex with PEG backbone antigens. Complementarity-
- 57 determining regions (CDRs) in the variable domains at the antigen-binding interface are
- 58 labeled to show structural differences in the hypervariable regions leading to different
- 59 PEG binding mechanisms.
- 60



# 63 Supplementary Figure 6: Size and purity of wild type (w.t.) and mutant anti-PEG

- 64 **Fabs**
- 65 SDS-PAGE stained with Coomassie revealed high purity (single stained protein band
- 66 per sample well) and similar molecular sizes (~ 50 kDa) across all APA Fabs expressed,
- 67 including wild type (w.t.) and mutant samples.
- 68



# 71 Supplementary Figure 7: Anti-PEG Fab binding kinetics and affinity to PEG

- 72 polymer backbone antigen
- 73 Bio-layer interferometry (BLI) was used to measure the binding affinity constant (K<sub>D</sub>),
- association rate constant (kon), and dissociation rate constant (koff) between APA Fab
- and PEG antigen. Binding to immobilized PEG was measured at various APA Fab
- concentrations above and below the calculated binding affinity constant.

# Supplementary Table 1: Crystallographic data collection and model refinement statistics 77

# 78

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	6VL9	6VL8			
Space group	P 21 21 2	P 1 21 1			
Unit cell parameters $a, b, c$ (Å)	90.711, 169.788, 69.394	112.108, 87.791, 116.074			
Unit cell parameters $\alpha$ , $\beta$ , $\gamma$ (°)	90.00, 90.00, 90.00	90.00, 113.66, 90.00			
Resolution (Å)	43.819-2.634 (2.700-2.634) <sup>1</sup>	41.925-2.423 (2.484-2.423)			
No. of reflections collected	189233	477285			
No. of unique reflections	31825	78203			
CC1/2	0.973 (0.863)	0.981 (0.916)			
CC*	0.993 (0.963)	0.995 (0.978)			
Mean I/ $\sigma$ I	11.9 (3.08)	17.07 (2.42)			
Rpim	0.86 (0.238)	0.075 (0.262)			
Completeness (%)	98.0 (100.0)	99.8 (98.6)			
Redundancy	5.9 (6.4)	6.1 (4.5)			
$R_{\text{work/}} R_{\text{free}}$ (%)	20.95/26.28 (27.33/33.31)	20.76/25.67 (28.57/31.00)			
Number of protein atoms (non- hydrogen)	6545 (2 Fabs in the AU)	13106 (4 Fabs in the AU)			
Number of ligand atoms	59	144 unique (288 total)			
Number of solvent atoms (non- hydrogen)	78	168			
Average B value for protein atoms $(Å^2)$	38.10	44.78			
Average B value for ligand atoms $(Å^2)$	36.98	41.34			
Average B value for solvent atoms $(Å^2)$	31.81	37.63			
RMSD bond lengths (Å)	0.002	0.003			
RMSD bond angles (°)	0.542	0.583			
Clashscore	3.77	3.85			
Ramachandran favored (%)	96.20	96.33			
Ramachandran outliers (%)	0.36	0.36			
<sup>1</sup> Values in parentheses correspond to the highest resolution shell.					