SUPPLEMENTAL INFORMATION

UNUSUAL WATER-MEDIATED ANTIGENIC RECOGNITION OF THE PRO-INFLAMMATORY CYTOKINE INTERLEUKIN-18

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Cavity Analysis

We sought to address whether the water-filled cavity observed in the IL-18/125-2H Fab complex crystal structure was unusual, and if so, how unusual. We thus analyzed all antibody/protein complexes present in the Protein Data Bank (http://www.rcsb.org/pdb) as of mid-January, 2009.

Our search strategy was necessarily tripartite, because current RCSB structural annotations do not allow retrieval of *all* antibody structures with confidence by using a simple search strategy. We considered as "antibody" any of: intact antibodies; antibody Fab, Fab', $F(ab)'_2$, and F_V (including scF_V) fragments; camelid V_{HH} domains; and shark antigen-binding domains. When we refer to "antibody" below, we mean this expanded set. We excluded from analysis other immunoglobulin(-like) domain-containing proteins such as antibody F_C domains, T cell receptors, major histocompatibility complexes, and other (generally extracellular) proteins (although many such proteins were inadvertently "caught" by our search "nets").

Query A was based on the antibody light chain variable region SCOP classification. We required at least two entities to be present in order to exclude, for example, unliganded scF_V structures; this approach did allow, however, many unliganded Fab structures to be included. Use of three entities would have undesirably excluded scF_V (binary) complexes. The search was also restricted to x-ray structures:

RCSB QUERY A

Experimental Method Search : Experimental **Method=X-RAY** and **ScopTree Search for V set domains (antibody variable domain-like)** and Number of Entities Search : **Min Number of Entities=2**

Query B searched for any of a variety of antibody-like terms present in the *titles* of the PDB entries. Due to limitations on the implementation of Boolean searches at the RCSB, experimental method and number of entities could not be restricted:

RCSB QUERY B

```
StructTitleQuery: struct.title.comparator=contains
struct.title.value=antibody  or StructTitleQuery:
struct.title.comparator=contains struct.title.value=Fab  or
StructTitleQuery: struct.title.comparator=contains
struct.title.value=camelid  or StructTitleQuery:
struct.title.comparator=contains struct.title.value=scFv  or
StructTitleQuery: struct.title.comparator=contains
struct.title.value=FV  or StructTitleQuery:
struct.title.comparator=contains
struct.title.value=immunoglobulin  or StructTitleQuery:
struct.title.comparator=contains struct.title.value=IgG  or
StructTitleQuery: struct.title.comparator=contains
struct.title.value=IgM
```

Similarly, Query C searched the PDB entry *structural description* with an expanded set of keywords:

RCSB QUERY C

```
StructDescQuery: entity.pdbx_description.comparator=contains
entity.pdbx_description.value=antibody  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=antibodies  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=Fab  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=camelid  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=scFv  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=Fv  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=immunoglobulin  or
StructDescQuery: entity.pdbx_description.comparator=contains
entity.pdbx_description.value=IgG  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=IgM  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=IgE  or StructDescQuery:
entity.pdbx_description.comparator=contains
entity.pdbx_description.value=IgA
```

Search results were exported to Excel spreadsheet format and combined, after sorting by PDB ID, to eliminate duplicates. A record was retained of which query(ies) identified each entry. Next, using both the title of the entry and, when (often) necessary, examining the PDB entry and/or the structure itself (using PyMOL), the ~2,500 hits were classified according to the following scheme:

Classification Scheme

Y = Relevant (i.e. an antibody), PROTEIN complex
N = Relevant, NOT complex
H = Complex, hapten or saccharide
P = Complex, peptide
D = Complex, DNA or RNA
X = Irrelevant (e.g., Fc, TCR, other Ig-fold proteins, or just random entries caught by the net (like FABx proteins, sIGMa factor, trIGGer factor, lIGAnd, non-complexed antIGEn, etc.).

Of the 1,003 *bona fide* antibody structures thus identified, we restricted our attention to antibody/protein complexes, and excluded complexes between antibodies and haptens (or transition state analogues or inhibitors or substrates, i.e. all catalytic antibody complexes), (oligo)saccharides, DNA, RNA, and peptides. Arguably, we could have included antibody/peptide complexes, but visual analysis of a random sampling of these suggested that their binding sites typically consist of an interface much more restricted compared to antibody/protein complexes.

Analysis of pockets and cavities ("pockets" are open to bulk solvent, whereas "cavities" are completely buried) present in the 316 antibody/protein complex structures made use of the CASTp (1) (http://sts-fw.bioengr.uic.edu/castp/index.php) and GPSS (2) (http://gpss.mcsg.anl.gov) web servers. Water molecules were ignored in all surface and cavity *calculations*; results were visualized with PyMOL (DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. http://www.pymol.org), and water molecules present in and around cavities were examined. Cavity volume was defined as the interior volume of the molecular surface of the cavity, i.e. number "**G**" below; the cavity volume observed in the IL-18/125-2H Fab complex is ~215 Å^3. "Relevant" cavities possessed a volume greater than 100 Å^3 present at the antibody/protein interface. Finally, notations were made on the CASTp volume results, the number of waters observed in each cavity that was identified, and any other observations relevant to pockets, cavities or waters at the antibody/protein interfaces. A typical CASTp entry is shown below (additional details are available at http://sts-fw.bioengr.uic.edu/castp/inout.php):

```
POC: 1bql  73   0  61.152  233.69  17.563  210.94  98.31  62
      A    B    C    D       E       F       G      H    I
A = PDB ID
B = Cavity/pocket ID
C = Number of mouths (by definition, cavities have no
    "mouths", i.e. C = 0)
D = Solvent-accessible surface area (Å²; Richard's surface)
E = Molecular surface area (Å²; Connolly's surface)
F = Solvent-accessible cavity volume (Å³; Richard's surface)
G = Molecular surface cavity volume (Å³; Connolly's surface)
H = Arc length (Å)      (H & I represent irrelevant CASTp
I = Number of corners    algorithm information)
```

These classified results are available as an Excel spreadsheet on the *Journal of Biological Chemistry* web site as file PDB_Antibody_Cavity_Search_MASTER_LIST.xls. It is sorted by "Complex?" and then "PDB ID". The "Query" field indicates which query(ies) identified a given entry: a "1" in the hundreds place represents Query A; the tens place, Query B; and the ones place, Query C (e.g., 1bgx, "110", was identified in Queries A and B, but not Query C). The spreadsheet contains HTML links to both the RCSB entry (column A) and, when available, the PubMed citation (column Z). The CASTp cavity results are summarized in "Cavity Information" (column E). Interfacial cavities >100 Å^3 in volume are summarized in Supplementary **Table S1**.

Table S1. Large cavities found at antibody/protein complex interfaces. Only structures possessing cavities with volumes greater than 100 Å^3 at the antibody/protein interface are shown. Following the IL-18/125-2H Fab complex entry, the table is sorted by PDB ID. Volumes >200 Å^3 are in **boldface**. Structures discussed in the main text are shown in **blue boldface**.

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
2vxt	**N/A**	**IL-18/125-2H Fab Complex**	**1.50**	**19.6**	**215**	**10**	**This work**	**N/A**
1bql	111	Structure of an Anti-HEL Fab Fragment Complexed with Bobwhite Quail Lysozyme	2.60	29.1	**211**	1	OPEN to solvent	8880929
1bvk	110	Humanized Anti-Lysozyme Fv Complexed with Lysozyme	2.70	29.7	150 165			9463398
1egj	111	Domain 4 of the Beta Common Chain in Complex with an Antibody	2.80	28.8	110			10753826
1ezv	111	Structure of the Yeast Cytochrome Bc1 Complex Co-Crystallized with an Antibody Fv-Fragment	2.30	25.4	**243**	2	Almost not a cavity	10873857
1fbi	111	Crystal Structure of a Cross-Reaction Complex Between Fab F9.13.7 and Guinea-Fowl Lysozyme	3.00	0.0	111 178			7629116
1fdl	111	Crystallographic Refinement of the Three-Dimensional Structure of the Fab D1.3-Lysozyme Complex at 2.5-Angstroms Resolution	2.50	0.0	141	0		1712773
1fe8	111	Crystal Structure of the Von Willebrand Factor A3 Domain in Complex with a Fab Fragment of IgG Ru5 That Inhibits Collagen Binding	2.03	26.4	125 130 142		3 mol/AU. Barely a cavity; mostly internal to VWF	11098050
1fj1	111	Lyme Disease Antigen OspA in Complex with Neutralizing Antibody Fab La-2	2.68	28.1	125 121	3 3		11183781
1g7h	111	Crystal Structure of Hen Egg White Lysozyme (Hel) Complexed with the Mutant Anti-HEL Monoclonal Antibody D1.3(VLW92A)	1.85	0.0	151	4		11112523
1g7i	111	Crystal Structure of Hen Egg White Lysozyme (Hel) Complexed with the Mutant Anti-HEL Monoclonal Antibody D1.3 (VLW92F)	1.80	24.8	139	4		11112523
1g7j	111	Crystal Structure of Hen Egg White Lysozyme (Hel) Complexed with the Mutant Anti-HEL Monoclonal Antibody D1.3 (VLW92H)	1.75	22.8	143	4		11112523
1g7l	111	Crystal Structure of Hen Egg White Lysozyme (Hel) Complexed with the Mutant Anti-HEL Monoclonal Antibody D1.3 (VLW92S)	2.00	24.0	129	4		11112523
1g7m	111	Crystal Structure of Hen Egg White Lysozyme (Hel) Complexed with the Mutant Anti-HEL Monoclonal Antibody D1.3 (VLW92V)	1.90	22.6	145	3		11112523

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
1gc1	111	Hiv-1 Gp120 Core Complexed with Cd4 and a Neutralizing Human Antibody	2.50	30.2	188	2	OPEN to solvent	9641677
1hez	110	Antibody-Antigen Complex	2.70	27.8	119	2	This cavity is NOT at the Ab combining site, but rather on side of Fab (kappa light chain, VL), where Protein L binds to Ab. Only one of two Protein L/VL interfaces has this cavity (interfaces are asymmetrical; read paper to determine which is/are physiological).	11587642
1i9r	111	Structure of CD40L in Complex with the Fab Fragment of Humanized 5c8 Antibody	3.10	28.5	**512** **561** **553**		Quite large, deep almost-cavity at interface, but OPEN to solvent (trimer in AU; these are repetitions of the same pocket)	11525169
1jps	111	Crystal Structure of Tissue Factor in Complex with Humanized Fab D3h44	1.85	22.4	185	5		11601848
1jto	111	Degenerate Interfaces in Antigen-Antibody Complexes	2.50	24.0	103			11676532
1kb5	**111**	**Murine T-Cell Receptor Variable Domain/Fab Complex**	**2.50**	**0.0**	**199** **718**	**2** **7**	**Larger pocket has 7 waters (8 stated in Mazza G. *et al.* (1999) *J. Mol. Biol.* 287:773), at TCR/Fab interface. Association constant Ka ~2.4 x 10^8 M^{-1}**	**9250664**
1kb9	101	Yeast Cytochrome bc1 Complex	2.30	24.9	172	2	Just barely a cavity	11726495
1kip	111	Fv Mutant Y(B 32)A (VH Domain) of Mouse Monoclonal Antibody D1.3 Complexed with Hen Egg White Lysozyme	2.10	0.0	152	3	Two adjacent large pockets at interaface, with waters. Equilibrium binding constant Kb ~7.0 x 10^7 M^{-1}	8952503
1kiq	111	Fv Mutant Y(B 101)F (VH Domain) of Mouse Monoclonal Antibody D1.3 Complexed with Hen Egg White Lysozyme	1.85	0.0	155	4	Two adjacent large pockets at interaface, with waters. Equilibrium binding constant Kb ~4.0 x 10^6 M^{-1}	8952503
1kxq	111	Camelid VHH Domain in Complex with Porcine Pancreatic Alpha-Amylase	1.60	21.9	141		Not actually a cavity (but close). This is the "correct", CDR-bound orientation VHH to amylase (which has a large pocket open to solvent).	11960990
1kxt	111	Camelid VHH Domains in Complex with Porcine Pancreatic Alpha-Amylase	2.00	23.6	172		Very similar to 1KXV, with unusual side-on binding of VHH to amylase. "Cavities" OPEN to solvent.	11960990

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
1kxv	111	Camelid VHH Domains in Complex with Porcine Pancreatic Alpha-Amylase	1.60	22.9	**230**	5	Unusual side-on binding of VHH to amylase. OPEN to solvent in other complex in AU (which CASTp lists as a "cavity": POC: 1kxv 194 0 72.770 244.21 21.340 229.88 104.71 70).	11960990
1kyo	101	Yeast Cytochrome bc1 Complex with Bound Substrate Cytochrome c	2.97	26.8	**231** **257**	0	Similar to 1KB9. 2 mol/AU. Smaller pocket is OPEN (not a cavity).	11880631
1lk3	110	Engineered Human Interleukin-10 Monomer Complexed to 9d7 Fab Fragment	1.91	24.0	97 96	3 3	Just under 100 Å^3 cutoff	12121653
1mel	111	Crystal Structure of a Camel Single-Domain VH Antibody Fragment in Complex with Lysozyme	2.50	32.3	109 116	1 0	Smaller cavity OPEN to solvent via narrow neck	8784355
1nma	101	N9 Neuraminidase Complexes with Antibodies NC41 and NC10: Empirical Free-Energy Calculations Capture Specificity Trends Observed with Mutant Binding Data	3.00	0.0	111	0	Between VH & neuraminidase	7517697
1ob1	111	Crystal Structure of a Fab Complex with Plasmodium Falciparum Msp1-19	2.90	28.8	95 99	0 0		12729744
1p84	101	HDBT Inhibited Yeast Cytochrome bc1 Complex	2.50	25.2	180	1	OPEN to solvent	12782631
1qfu	111	Influenza Virus Hemagglutinin Complexed with a Neutralizing Antibody	2.80	28.4	168 114	0 2	Larger cavity OPEN to solvent	10360354
1rjl	111	Structure of the Complex Between OspB-CT and Bactericidal Fab-H6831	2.60	23.5	**223**	5		15713683
1s78	101	Insights Into ErbB Signaling From the Structure of the ErbB2-Pertuzumab Complex	3.25	26.8	111 106	0 0		15093539
1tzh	111	Crystal Structure of the Fab YADS1 Complexed with H-VEGF	2.60	27.1	114	2	OPEN to solvent	15306681
1uac	110	Crystal Structure of HYHEL-10 FV Mutant SFSF Complexed with Turkey White Lysozyme	1.70	24.8	**318**	3	Not actually a cavity; narrow neck to solvent	12709438
1uj3	111	Crystal Structure of a Humanized Fab Fragment of Anti-Tissue-Factor Antibody in Complex with Tissue Factor	2.10	22.7	109	~8	Not actually a cavity; rather an intricate series of necks, all OPEN to solvent	14646147
1vfb	**111**	**Bound Water Molecules and Conformational Stabilization Help Mediate an Antigen-Antibody Association**	**1.80**	**0.0**	**156** **770**	**4** **~10**	**Larger pocket OPEN to solvent by two necks, at the interface**	**8302837**
1w72	111	Crystal Structure of HLA-A1:MAGE-A1 in Complex with Fab-HYB3	2.15	24.8	290 297	7 4	These cavities are between the MAGE-A1 peptide and HLA, **not** the HLA/peptide complex and the Fab. (2 complexes/AU)	15537658
1yqv	**111**	**The Crystal Structure of the Antibody Fab HyHEL5 Complex with Lysozyme at 1.7 A Resolution**	**1.70**	**23.4**	**136**	**4**		**15858274**

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
1yy9	11	Structure of the Extracellular Domain of the Epidermal Growth Factor Receptor in Complex with the Fab Fragment of Cetuximab/Erbitux/IMC-C225	2.61	28.9	101 **238**	1 6	Larger pocket OPEN to solvent	15837620
1zmy	1	cAbBCII-10 VHH Framework with CDR Loops of cAbLys3 Grafted on It and in Complex with Hen Egg White Lysozyme	3.00	25.4	100	0		16095608
2aep	11	An Epidemiologically Significant Epitope of a 1998 Influenza Virus Neuraminidase Forms a Highly Hydrated Interface in the NA-Antibody Complex.	2.10	22.4	N/A	N/A	No relevant cavities >100 Å^3 Paper mentions 33 buried waters, of which 13 are buried by protein only (i.e., other 20 buried by protein & *other* waters). Careful examination of this cryogenic structure reveals several invaginations and lots of waters, but no significant cavities. Part of the difference between the reported results and those observed with CASTp/PyMOL may be related to the fact that the Ab seems to bind NA right at a large hydrated crevice in neuraminidase. Binding Kd is ~12 nM. Related entry 2aeq, determined at room temperature (note resol. & R_{free} increase) has some cavities, but none truly significant.	16384583
2aeq	11	An Epidemiologically Significant Epitope of a 1998 Influenza Virus Neuraminidase Forms a Highly Hydrated Interface in the NA-Antibody Complex.	3.00	31.2	106 150	0 0	Smaller pocket just barely open to solvent	16384583
2cmr	10	Crystal Structure of the HIV-1 Neutralizing Antibody D5 Fab Bound to the gp41 Inner-Core Mimetic 5-Helix	2.00	25.8	**261**	3	Cavity also includes a glycerol molecule	16862157
2dd8	11	Crystal Structure of SARS-CoV Spike Receptor-Binding Domain Complexed with Neutralizing Antibody	2.30	26.1	148	5	OPEN to solvent via small neck	16597622
2dqf	110	Crystal Structure of HyHEL-10 FV Mutant (Y33A, Y53A) Complexed with Hen Egg Lysozyme	2.50	26.8	115	1	OPEN to solvent	17166830
2dtg	11	Insulin Receptor (IR) Ectodomain in Complex with Fab's	3.80	31.0	**244**	0		16957736
2ibz	111	Yeast Cytochrome bc1 Complex with Stigmatellin	2.30	25.6	173	2	Not quite a cavity (but very narrow neck)	17337272
2iff	111	Structure of an Antibody-Lysozyme Complex: Effect of a Conservative Mutation	2.65	0.0	173	2		7531245
2j6e	11	Crystal Structure of an Autoimmune Complex Between a Human IgM Rheumatoid Factor and IgG1 Fc Reveals a Novel Fc Epitope and Evidence For Affinity Maturation	3.00	28.8	133 125	1		17395205

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
2j88	111	Hyaluronidase in Complex with a Monoclonal IgG Fab Fragment	2.60	24.7	134	2	Narrow OPENing to solvent	17374540
2nyy	111	Crystal Structure of Botulinum Neurotoxin Type A Complexed with Monoclonal Antibody CR1	2.61	24.6	123 100	0 0	Smaller pocket OPEN to solvent; adjacent to larger cavity	17173035
2nz9	111	Crystal Structure of Botulinum Neurotoxin Type A Complexed with Monoclonal Antibody AR2	3.79	27.8	131 109	0 0	Cavities adjacent. to one another	17173035
2q8a	11	Structure of the Malaria Antigen AMA1 in Complex with a Growth-Inhibitory Antibody	2.40	24.4	**461**	9	Slightly OPEN to solvent via narrow neck. Affinity of 1F9 antibody is not specified; estimate ~100 μM from Fig. 2A of Coley, AM *et al.* (2006) *Infection & Immunity* **74**:2628.	17907804
2q8b	11	Structure of the Malaria Antigen AMA1 in Complex with a Growth-Inhibitory Antibody	2.30	25.6	**461**	10	Slightly OPEN to solvent via narrow neck	17907804
2qad	11	Structure of Tyrosine-Sulfated 412d Antibody Complexed with HIV-1 YU2 gp120 and CD4	3.30	26.9	140 141	0 0	OPEN to solvent	17901336
2r0l	11	Short Form HGFA with Inhibitory Fab75	2.20	24.8	186 **321**	3 3		18077410
2r69	10	Crystal Structure of Fab 1A1D-2 Complexed with E-DIII of Dengue Virus at 3.8 Angstrom Resolution	3.80	36.3	106	0	Quite OPEN to solvent	18264114
2vwe	11	Crystal Structure of Vascular Endothelial Growth Factor-B in Complex with a Neutralizing Antibody Fab Fragment	3.40	31.0	115	0	OPEN to solvent	18930733
3b2u	11	Crystal Structure of Isolated Domain III of the Extracellular Region of the Epidermal Growth Factor Receptor in Complex with the Fab Fragment of IMC-11F8	2.58	29.1	**237** **277** **264** **254** **254** **222** **294**	1 2 1 0 0 1 0	Largest cavity almost OPEN to solvent	18275813
3bn9	11	Crystal Structure of MT-SP1 in Complex with Fab Inhibitor E2	2.17	26.7	138 129	1 2	Long tunnel (OPEN at both ends) between Fab & protease is filled with water. Tunnel created by long, arching CDR H3. Ab has pM affinity.	18514224
3cvh	11	How TCR-Like Antibody Recognizes MHC-Bound Peptide	2.90	29.6	135 114	0 0		18703505
3cx5	1	Structure of Complex III with Bound Cytochrome c in Reduced State and Definition of a Minimal Core Interface for Electron Transfer	1.90	26.3	**245** **250**	3 3	OPEN to solvent	18390544

PDB Link	Query	Description	Resol. (Å)	R_{free} (%)	Cavity Volume (Å^3)	Water Molecules	Notes	PubMed Link
3cxh	1	Structure of Yeast Complex III with Isoform-2 Cytochrome c Bound and Definition of a Minimal Core Interface for Electron Transfer	2.50	25.6	131 **214**	0 1	Smaller pocket OPEN to solvent. Larger cavity almost OPEN to solvent.	18390544
3eo1	11	Structure of the Fab Fragment of GC-1008 in Complex with Transforming Growth Factor-Beta 3	3.10	27.9	131 131	0 0		19073914
3hfm	111	Structure of an Antibody-Antigen Complex. Crystal Structure of the Hy/HEL-10 Fab-Lysozyme Complex	3.00	0.0	121	0		2762305

Supplemental Figures

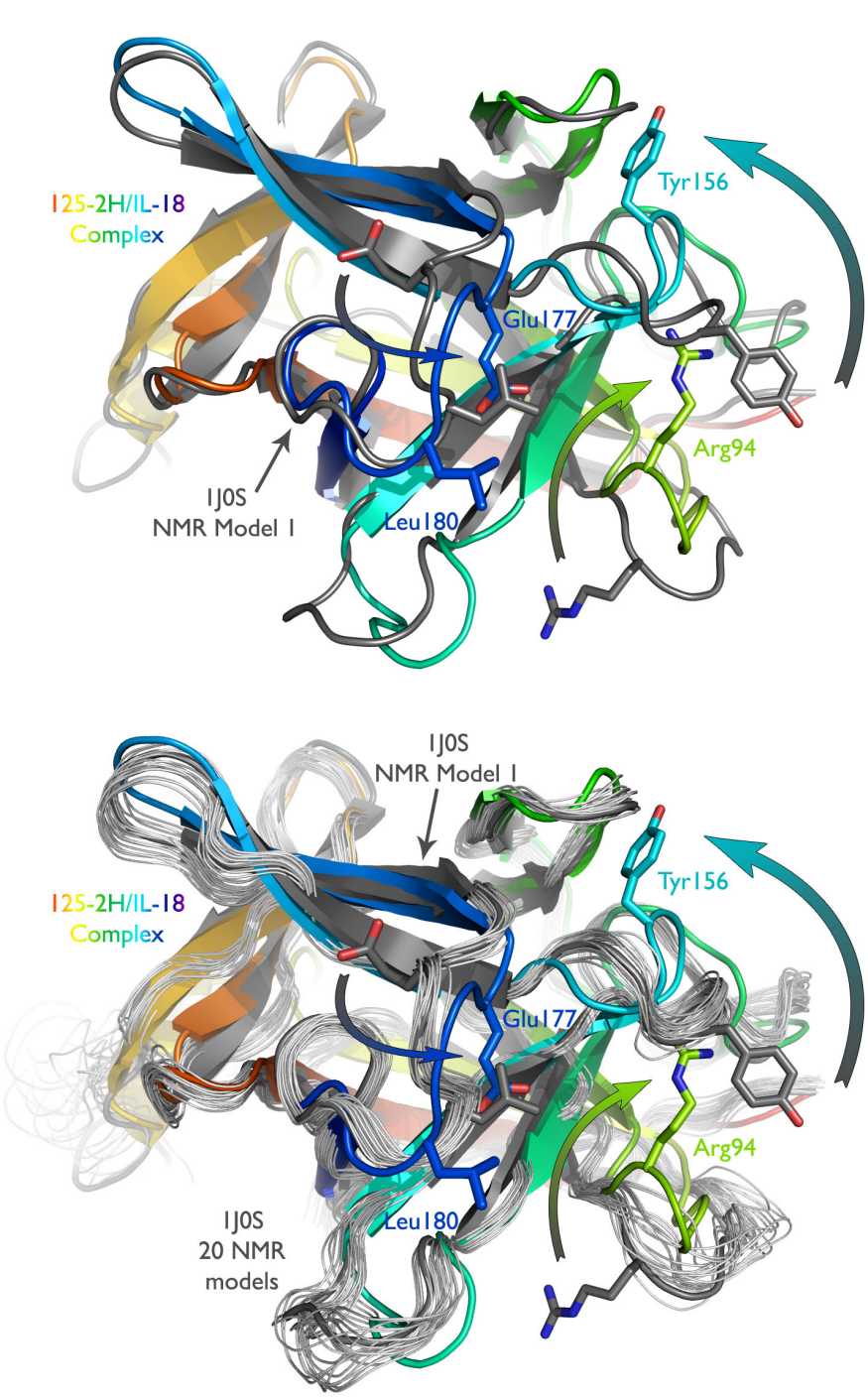

Figure S1. Free- and 125-2H-bound IL-18 exhibit large conformational differences. Free- (grey; NMR, PDB entry 1J0S) and 125-2H Fab-bound (rainbow, *N/C*-termini red/blue; x-ray, this work) IL-18 are superimposed. (a) The best NMR model and the x-ray structure are shown as cartoons. (b) All 20 NMR models (tubes; light grey), the best NMR model (cartoon), and the x-ray structure are shown. Arg94, Tyr156, Glu177, and Leu180, and associated surface loops, exhibit significant movement which far exceeds the observed NMR positional variability.

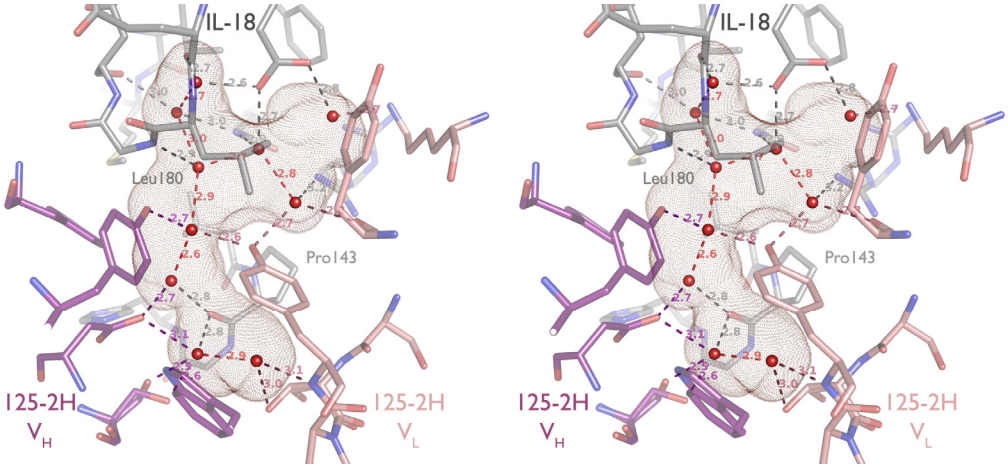

Figure S2. Ice-like hydrogen bonds fill the buried cavity between IL-18 and 125-2H. In this relaxed-eye stereoview, IL-18 (grey) and the 125-2H Fab fragment (purple [V_H] and pink [V_L]) are shown as sticks, the water molecules sequestered between the two proteins are shown as red spheres, and the molecular surface of the buried cavity is shown as a brown dotted surface. All hydrogen bonds (<3.2 Å) between these water molecules and either the proteins or other water molecules are shown as dotted lines; the colors of the lines and associated distances match those of the hydrogen bond partners.

Acknowledgements

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References

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2. Binkowski, T. A., and Joachimiak, A. (2008) *BMC structural biology* **8**, 45