I-bodies: human single domain antibodies that antagonize chemokine receptor CXCR4

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Supplementary Data

FIGURE LEGENDS

S1. Biophysical characterization of i-body scaffold

(**A.**) Circular dichroism spectroscopy of 21H-5 at 20 °C and 80 °C, and at 20 °C after repeated heating to 80 °C (Recool 1-4). (**B.**) Thermal stability of crude periplasmic extract of AD5G8-5. Lane 1: untreated, Lane 2: heat treated at 40 °C, Lane 3: heat treated at 50 °C, Lane 4: heat treated at 60 °C, Lane 5: heat treated at 70 °C, Lane 6: heat treated at 80 °C, Lane 7: heat treated at 90 °C, Lane 8: heat treated at 99 °C. Arrow indicates AD5G8-5. (**C.**) Retention of active AD5G8-5 protein following incubation at various pHs for 4 weeks at 4 or 37 °C. (**D.**) Analytical ultracentrifugation of 21H-5 and AD5G8-5. The quality of the *c(M)* distribution best-fits are demonstrated by the random distribution of residuals obtained for each analysis.

S2. Analysis of a shark : i-body loop graft clone, 23B-2.

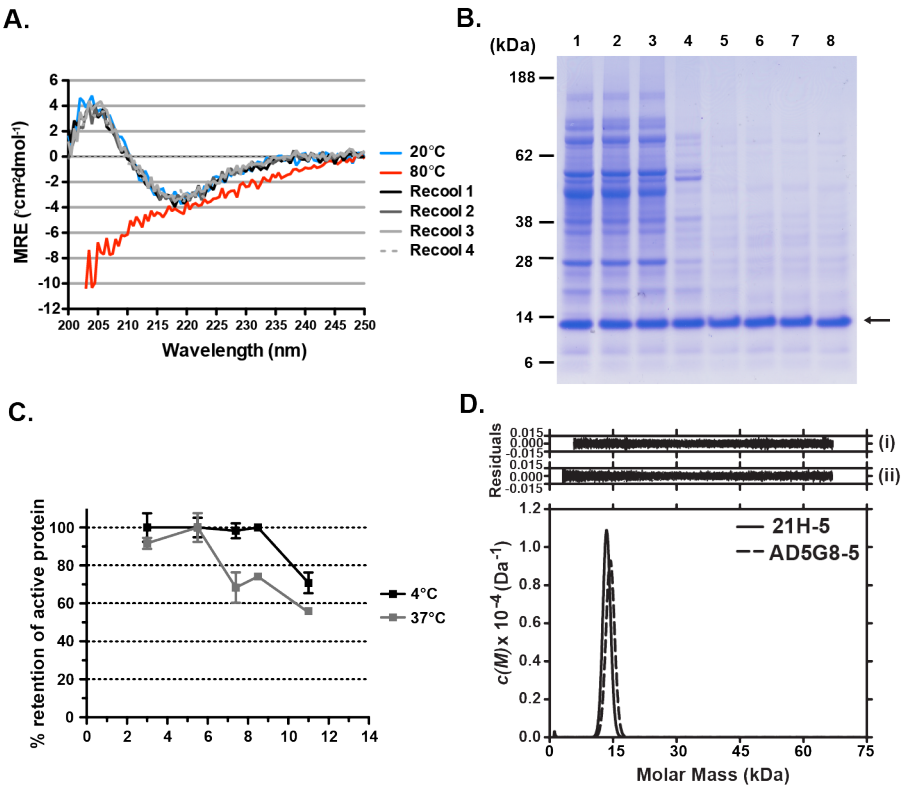
I-body clone 23B-2 was engineered by replacing the 21H-5 residues ^{82}EDGS^{85} with the V_{NAR} clone 1A-7 residues ^{88}SDAMSNYSYPIS^{99}. V_{NAR} clone 1A-7 binds the monoclonal antibody 5G-8. (**A.**) Protein expression and purification of 23B-2 showed that the resulting recombinant protein was soluble by size-exclusion analysis. (**B.**) ELISA revealed that specificity for 5G-8 was transferred to clone 23B-2, as compared with the original V_{NAR} clone 1A-7. 23B-2 did not bind lysozyme, which was included as a negative control.

S3. (A. - G.) Kinetic data set collected for i-bodies binding to immobilized CXCR4. I-bodies were diluted three-fold and injected (**A.**) from 2187 nM to 27 nM, (**B.** to **G.**) 81 nM to1 nM. (**A**.) ADCX-99 [**a. inset** shows fit of the responses at equilibrium (plotted against injected i-body concentration) to simple binding isotherm]. (**B.**) AM1-126, (**C.**) AM1-320 (**D.**) AM4-272, (**E.**) AM3-523, (**F.**) AM4-746, (**G.**) AM4-1121, Binding responses (black sensorgrams) are overlayed with fits of a simple 1:1 kinetic interaction model (orange lines). (**H. - L.**) **SPR experiments showing binding competition between CXCL12 ligand and various i-bodies.** (**H.**) Sensorgram showing scheme of the SPR based competition assay. I-body injection (#1) was followed by CXCL12 ligand injection (#2). Injection of running buffer (RB) prior to CXCL12 ligand injection provides a baseline (no inhibition) response (black sensorgram). Sensorgrams shown in green represent injection controls used for baseline-response subtraction: light green = i-body + RB, dark green = RB + RB. Panels (**I. - L.**) show appropriately subtracted SPR responses arising from CXCL12 ligand binding to CXCR4 with no pre-bound i-body (black sensorgrams) or with pre-bound i-body injected at 50 nM (red sensorgrams) or 200 nM (blue sensorgrams) (**I.**) AM1-126 (**J.**) AM3-114 (**K.**) AM4-272 (**L.**) AM3-523. CXCL12 ligand was injected at 13 nM.

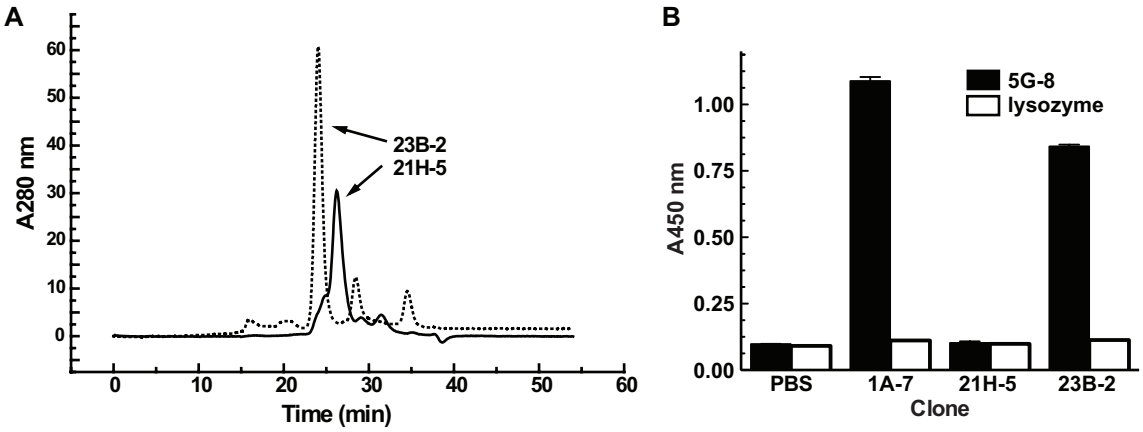
S4. Inhibition of entry of viruses in the presence of CXCR4-binding i-bodies.

(**A.**) % inhibition of entry of reporter viruses pseudotyped with VSVG by CXCR4 i-bodies. (**B.**) % inhibition of entry by CXCR4 i-bodies of HIV reporter viruses pseudotyped with the CCR5-using YU-2 Env. Error bars show s.d.

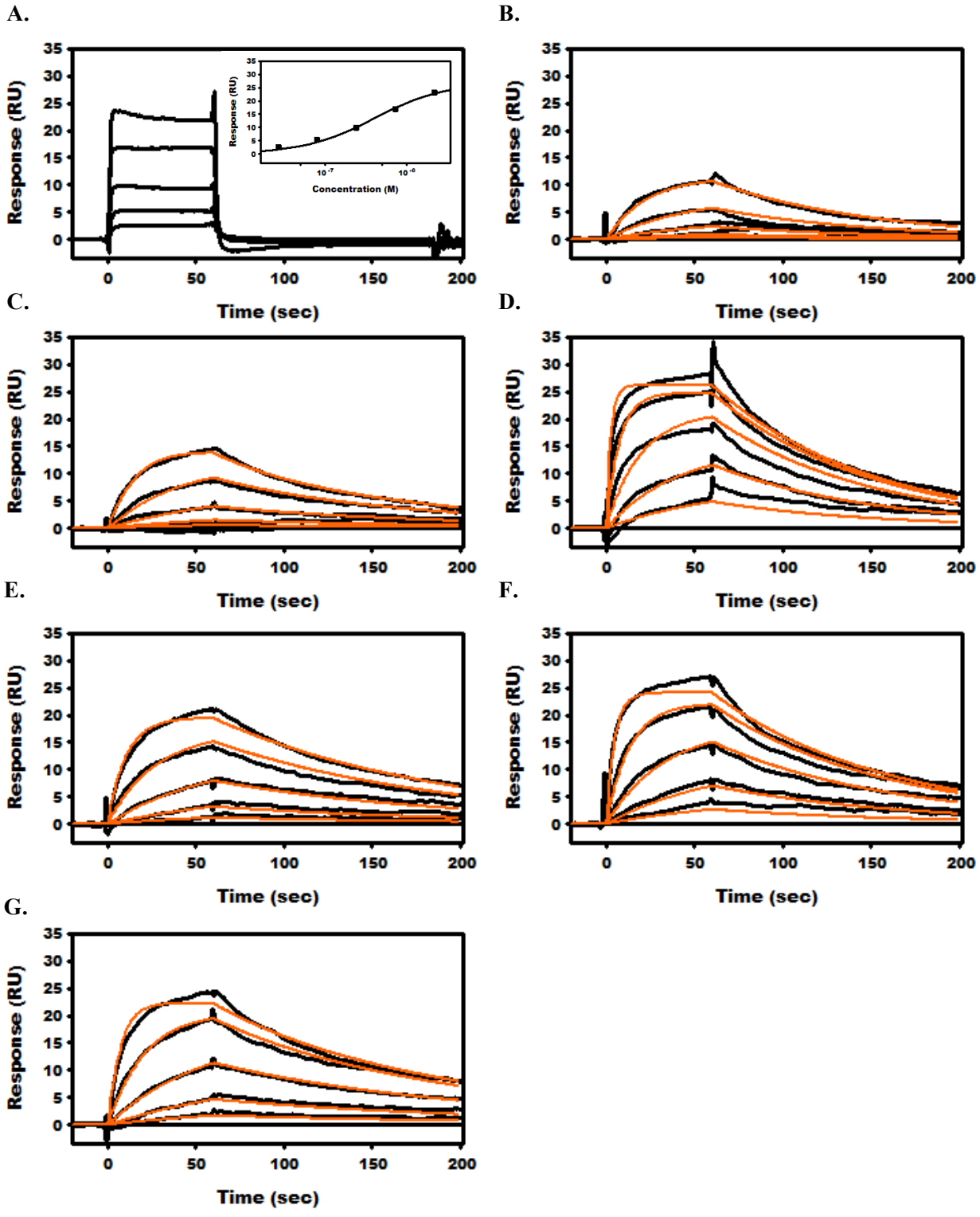
S1.


A.
MRE (°cm²dmol⁻¹)
Wavelength (nm)
20°C
80°C
Recool 1
Recool 2
Recool 3
Recool 4
B.
(kDa)
1 2 3 4 5 6 7 8
188
62
38
28
14
6
C.
% retention of active protein
4°C
37°C
D.
Residuals
(i)
(ii)
c(M)x 10⁻⁴ (Da⁻¹)
21H-5
AD5G8-5
Molar Mass (kDa)

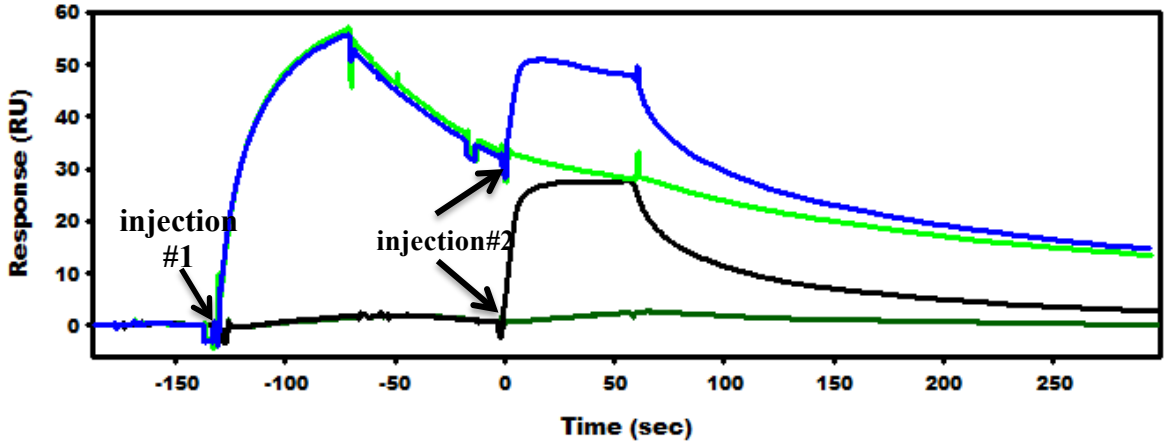
S2.


A
A280 nm
Time (min)
23B-2
21H-5
B
A450 nm
5G-8
lysozyme
PBS
1A-7
21H-5
23B-2
Clone

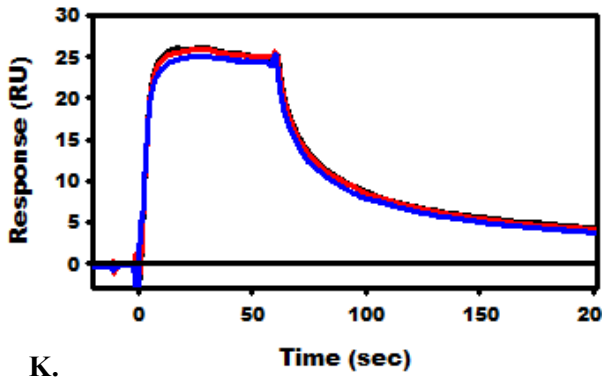
S3.



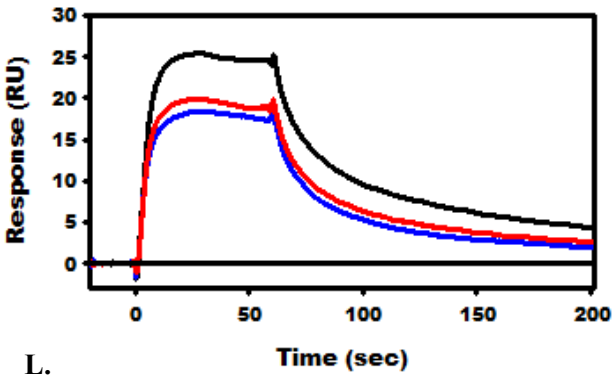
H.


Response (RU)
Time (sec)
injection #1
injection#2

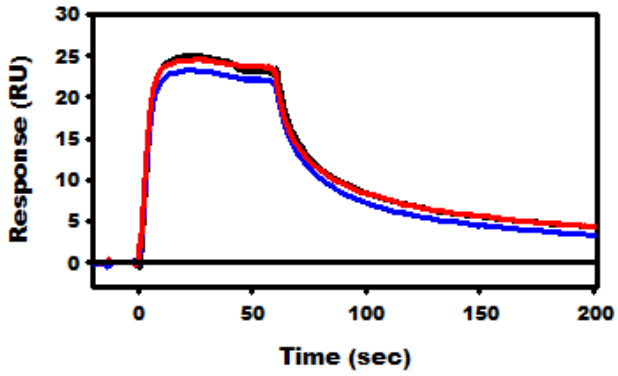
I.


Response (RU)
Time (sec)

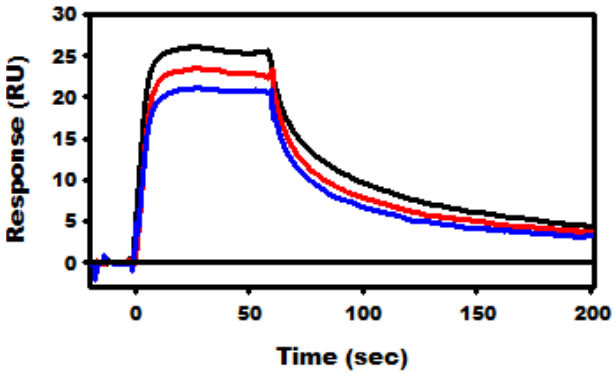
J.


Response (RU)
Time (sec)

K.


Response (RU)
Time (sec)

L.


Response (RU)
Time (sec)

S4.

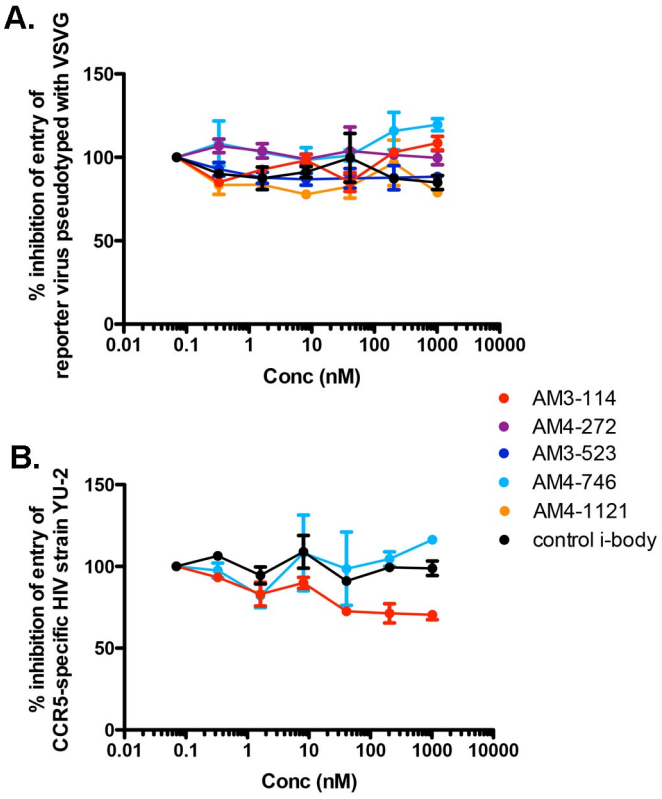

A.
% inhibition of entry of reporter virus pseudotyped with VSVG
150
100
50
0
0.01
0.1
1
10
100
1000
10000
Conc (nM)
B.
% inhibition of entry of CCR5-specific HIV strain YU-2
150
100
50
0
0.01
0.1
1
10
100
1000
10000
Conc (nM)
AM3-114
AM4-272
AM3-523
AM4-746
AM4-1121
control i-body

Table S1. 21H-5 structure data collection and refinement statistics

	Native
Data collection	
Space group	*P* 21
Cell dimensions	
a, *b*, *c* (Å)	23.85, 107.33, 41.75
α, β, γ (°)	90.00, 99.57, 90.00
Wavelength (Å)	0.9537
Resolution (Å)*	40.00-1.9 (2.00-1.90)
R_{sym} or R_{merge}*	0.107 (0.852)
$I / \sigma I$*	15.2 (2.8)
Completeness (%)*	99.9 (100)
Redundancy*	7.6 (7.6)
Refinement	
Resolution (Å)	41.23-1.4
No. reflections	17174
R_{work} / R_{free}	0.172/0.199
No. atoms	
Protein	1521
Ligand/ion	1
Water	163
B-factors	

*Values in parentheses are for highest-resolution shell.

Table S2. Sequences of peptides and V_{NAR}s isolated against mAb 5G-8, compared with the sequence of i-body AD5G8-5. AYP or SYP is a common motif among these binders.

Binder type	*Clone*	*Peptide[1] or CDR3[2] sequence*	*Reference*
Peptide	M1	[1]DRHSRIVILMPLAYP	(1)
Peptide	E2	[1]EDENTLQHAYPID	(1)
V_{NAR}	1A-7	[2]YFSDAMSNYSYPIPGEKG	(2)
V_{NAR}	1A-11	[2]DYSPSCYSYPSLESAVEG	(2)
V_{NAR}	1A-14	[2]SAALSPNSYYCPSCLEKG	(2)
i-body	AD5G8-5	[2]THSANTKSYPTEDFT	-

REFERENCES

1. Coley, A. M., Campanale, N. V., Casey, J. L., Hodder, A. N., Crewther, P. E., Anders, R. F., Tilley, L. M., and Foley, M. (2001) Rapid and precise epitope mapping of monoclonal antibodies against *Plasmodium falciparum* AMA1 by combined phage display of fragments and random peptides. *Protein Eng.* **14**, 691-698
2. Simmons, D. P., Streltsov, V. A., Dolezal, O., Hudson, P. J., Coley, A. M., Foley, M., Proll, D. F., and Nuttall, S. D. (2008) Shark IgNAR antibody mimotopes target a murine immunoglobulin through extended CDR3 loop structures. *Proteins* **71**, 119-130