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Supplemental Material to:

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A fully synthetic human Fab antibody library based on fixed VH/VL framework pairings with favorable biophysical properties

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Supplementary Figure S1 to Tiller *et al*.

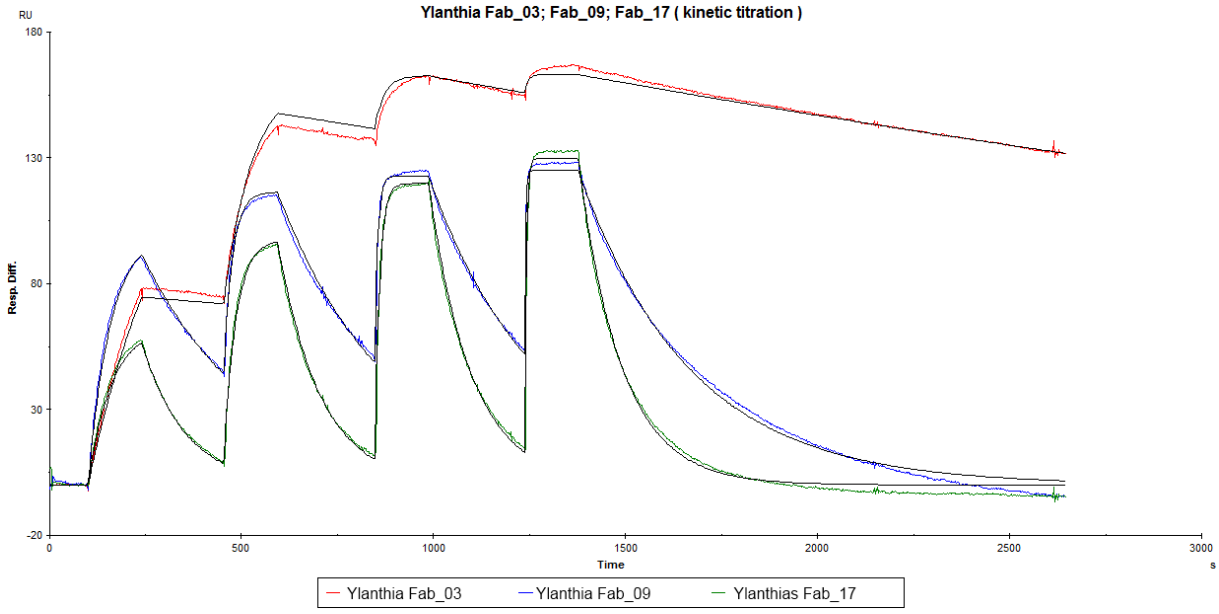

Figure S1. Sample sensorgrams (including applied fits) for three representative kinetic titration experiments.

Kinetic experiments to determine rate constants k_{on} and k_{off} were performed on a Biacore 3000 instrument (GE Healthcare, Uppsala, Sweden), according to the "kinetic titration" method published by Karlsson *et al.*[1]

Basically, protein antigen was immobilized on a Biacore CM5 sensor chip using standard EDC/NHS chemistry (immobilization level 190 RU). PBS containing 0.05% (v/v) Tween-20 was used as running buffer, with a flow of 30 µL/min applied throughout the experiment.

Monomeric Fab fragments (monomer content >90% as determined by HP-SEC) were used as samples. Each of these samples was injected in a 3^n concentration series (19 nM, 56 nM, 167 nM, 500 nM), for association phases of 140 s, intermittent dissociation phases of 215 s, and a final dissociation phase of 1200 s (20 min). A blank injection of running buffer following the same injection pattern was recorded and subtracted from each sensorgram. After each measurement, bound Fab was regenerated from the sensor surface by a 14 s injection of 10 mM Glycine / HCl pH 2.0.

The obtained sensorgrams were fitted according to a bimolecular (1:1) binding kinetics, using the method and model described by Karlsson *et al.*[1]

References

1. Karlsson R, Katsamba PS, Nordin H, Pol E, Myszka DG. Analyzing a kinetic titration series using affinity biosensors. Anal Biochem 2006; 349:136-47; PMID:16337141; http://dx.doi.org/10.1016/j.ab.2005.09.034.

Supplementary Table S1 to Tiller *et al*.

Table S1. Rate constants and affinities of 24 selected Ylanthia Fab molecules.

Monovalent affinities of 24 selected Ylanthia Fab molecules as determined by surface plasmon resonance (SPR) measurements against a protein target. In the analyzed subset, k_{on} values ranged between 2.9E+04 and 4.6E+06 [1/Ms] and k_{off} values between 8.1E-05 and 1.7E-01 [1/s].

[#] sensorgrams and applied fits presented in Figure S1

*slight deviation from bimolecular binding model / 1:1 binding

Sample	k_{on} [1/Ms]	k_{off} [1/s]	K_D [nM]
Fab_01	3.7E+05	1.1E-03	2.9
Fab_02	4.6E+06	1.4E-01	31
Fab_03[#]	2.4E+05	1.7E-04	0.7
Fab_04*	3.6E+05	3.2E-04	0.9
Fab_05	3.6E+05	1.1E-02	30
Fab_06	2.7E+05	6.4E-03	24
Fab_07	7.2E+05	1.3E-02	18
Fab_08	1.5E+05	1.1E-03	7.2
Fab_09[#]	7.9E+05	3.6E-03	4.6
Fab_10	9.6E+05	5.4E-03	5.6
Fab_11	1.5E+06	1.5E-01	96
Fab_12	1.1E+06	1.7E-02	16
Fab_13	5.8E+05	1.2E-02	20
Fab_14	4.1E+05	8.6E-03	21
Fab_15	7.1E+05	2.0E-02	29
Fab_16	5.9E+05	1.3E-03	2.2
Fab_17[#]	4.7E+05	1.0E-02	21
Fab_18	7.6E+05	3.5E-02	46
Fab_19	7.8E+05	3.2E-02	41
Fab_20	9.4E+05	1.7E-01	190
Fab_21	2.9E+04	8.1E-05	2.8
Fab_22*	2.1E+06	7.3E-02	35
Fab_23	3.9E+05	1.4E-02	37
Fab_24	7.0E+05	4.0E-02	57