

Supplementary Material: Evaluation of Solid Supports for Slide- and Well-Based Recombinant Antibody Microarrays

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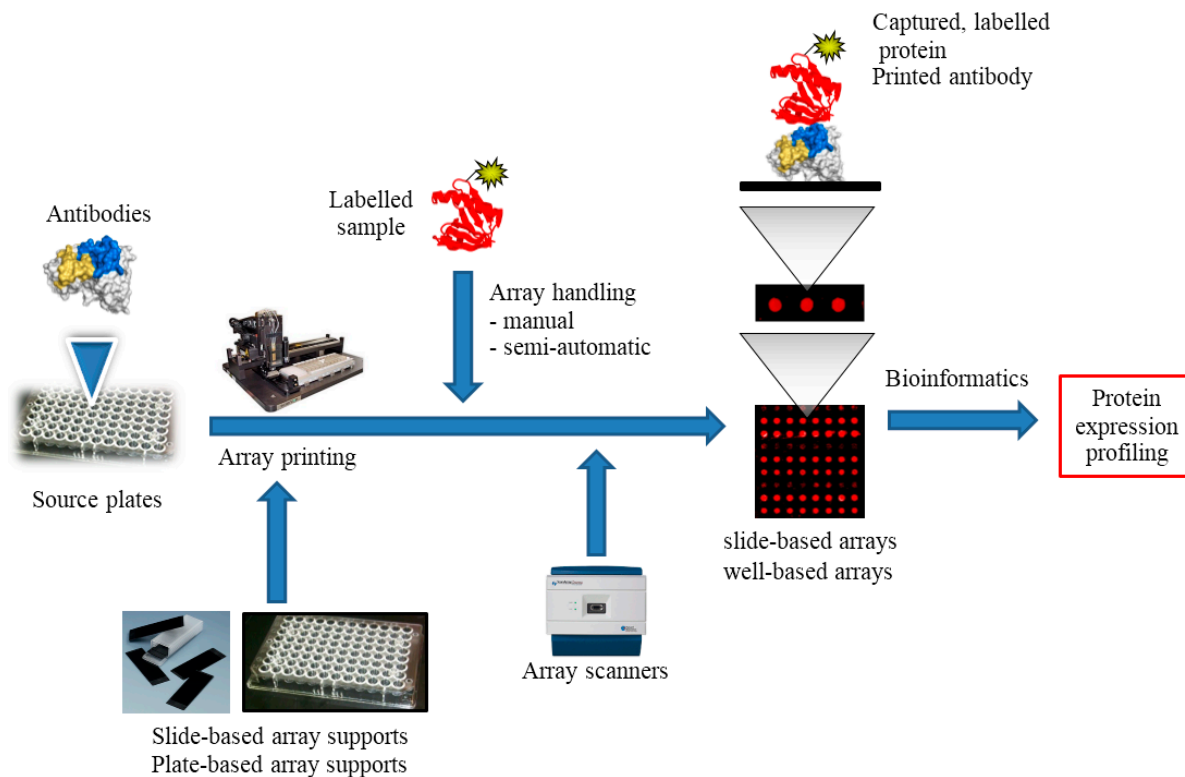


Figure S1. Schematic illustration of the recombinant antibody microarray technology platform, also highlighting the main technical issues addressed in the study.

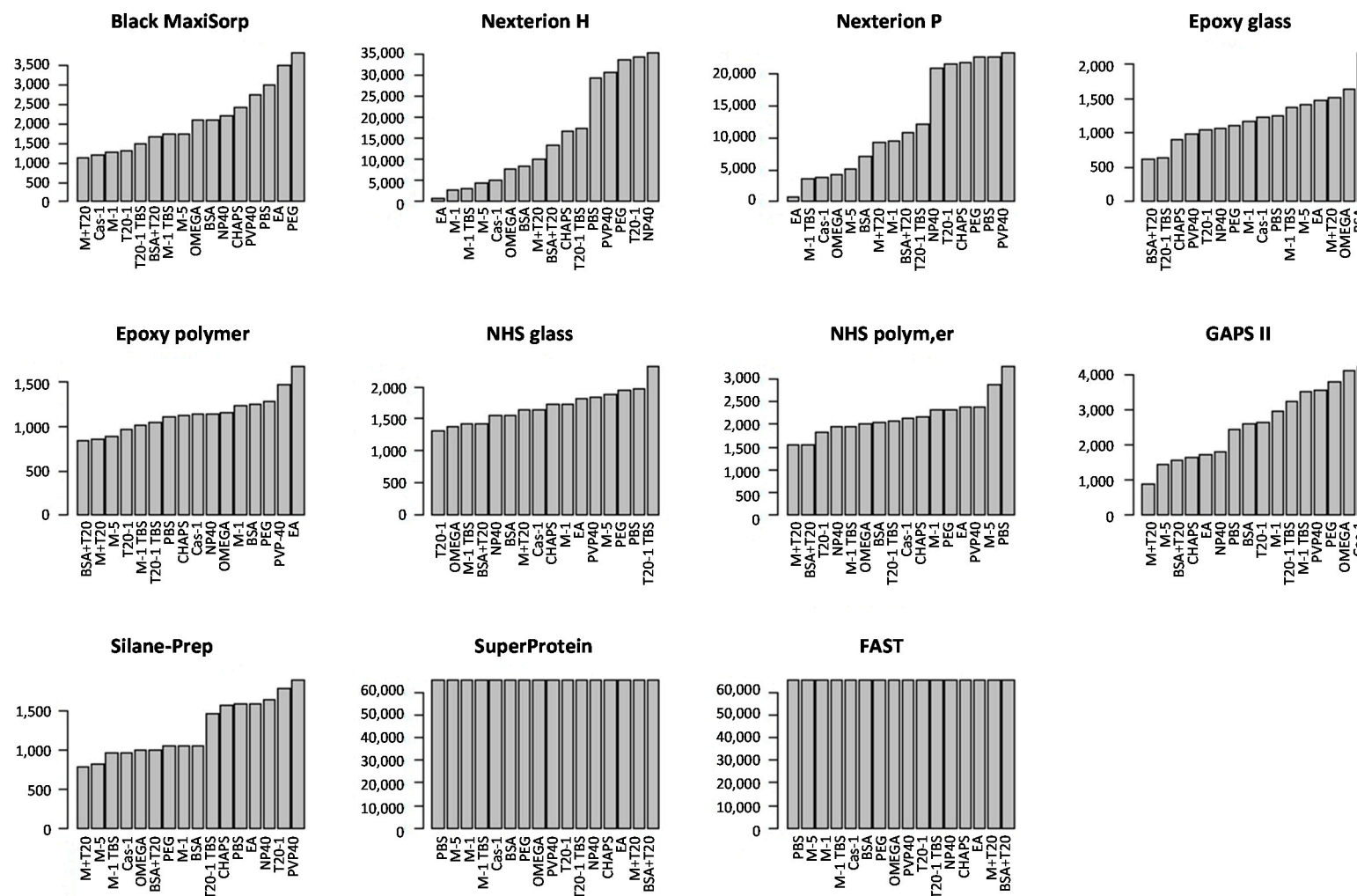


Figure S2. Evaluation of blocking buffers for slide-based solid supports. The slides were blocked with 16 different blocking solutions (Table S2) and then exposed to crude, directly-biotinylated serum sample, whereafter any non-specific background binding (surface fouling) was detected.

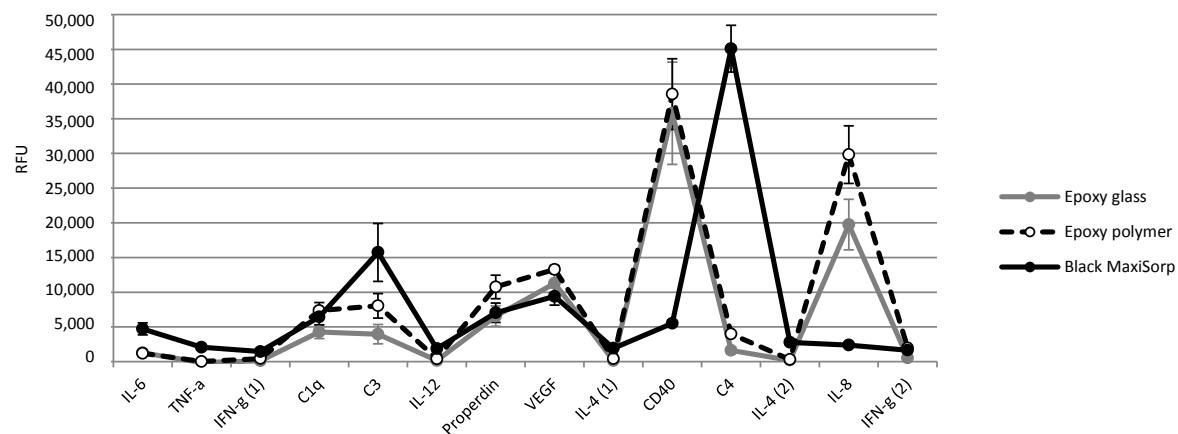


Figure S3. Comparison of antibody binding pattern on three different slide-based solid supports. A serum sample was profiled on a 14-plex antibody microarray printed on slide-based supports, including black MaxiSorp, epoxy polymer and epoxy glass. Quantified signals were collected from the highest possible scanner setting without any spot saturation (60% PMT gain/90% LP for black MaxiSorp slides and 50% PMT gain/90% LP for the epoxy-coated slides).

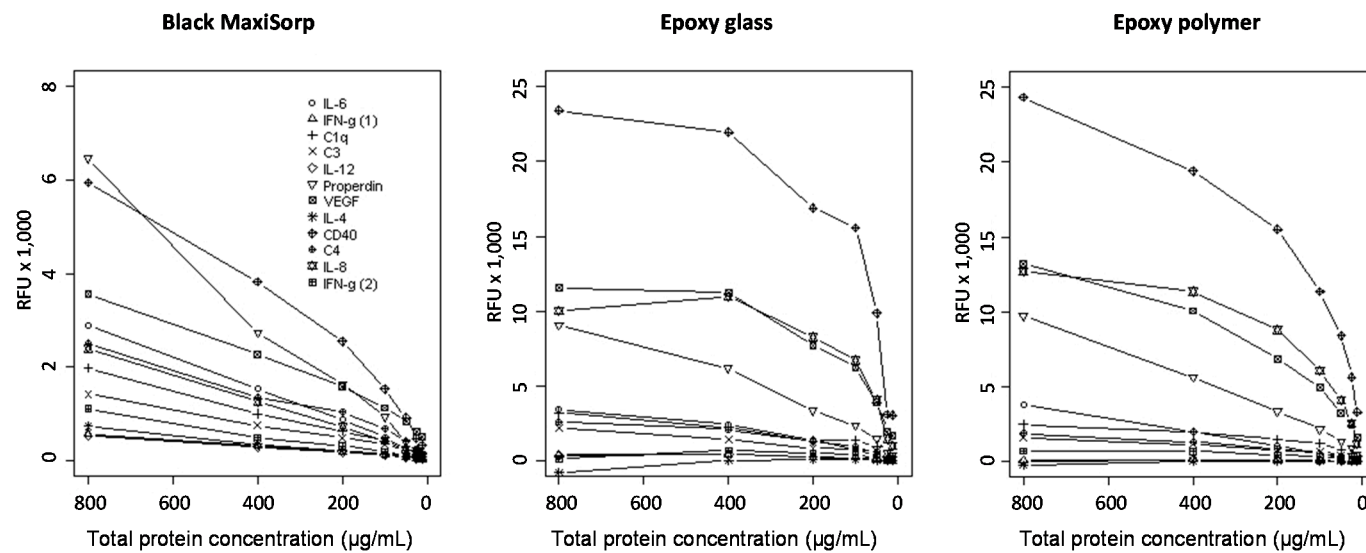


Figure S4. Comparison of assay sensitivity and the dynamic range of slide-based antibody microarrays. Serial dilutions of a serum sample, ranging from a total protein concentration of 12.5–800 $\mu\text{g/mL}$, was profiled on black MaxiSorp, epoxy polymer and epoxy glass.

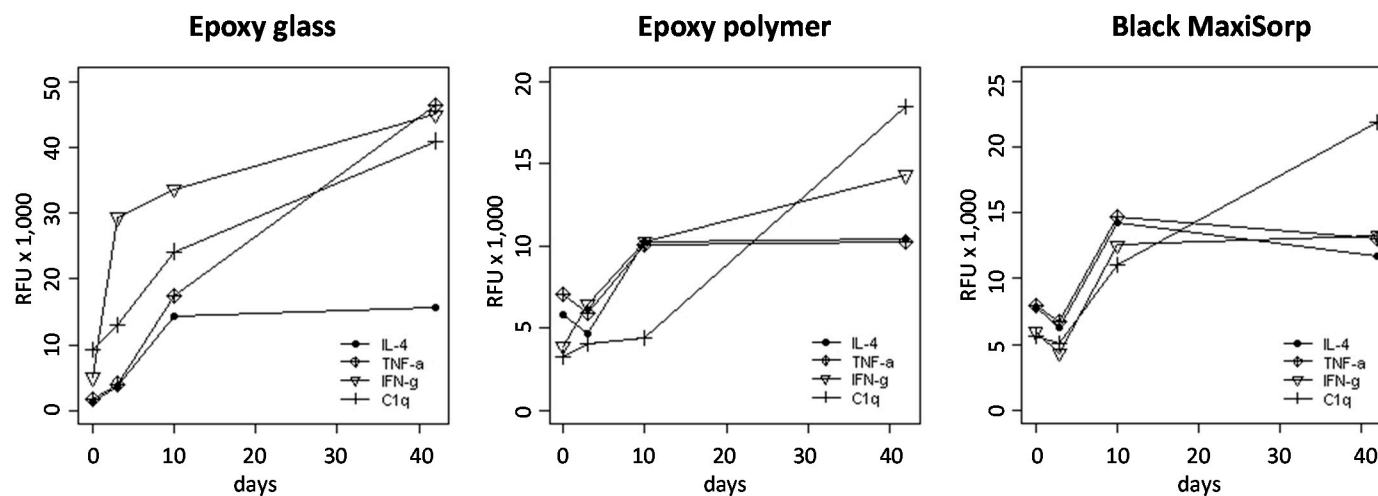


Figure S5. Evaluation of long-term storage on the activity of slide-based antibody microarrays. Arrays were printed on black MaxiSorp, epoxy polymer and epoxy glass slides. All antibody microarrays were printed on Day 0 and then stored dried out for 0, 3, 10 or 42 days prior to array handling (blocking, washing, sample incubation, *etc.*). The antibody activity for four representative antibodies, targeting IL-4, TNF- α , IFN- γ and C1q, was assessed.

Table S1. Antibodies used.

Antigen	No. of Antibody Clones	Antigen	No. of Antibody Clones
Angiotenin	1	IL-9	2
Apolipoprotein A1	1	IL-10	3
Bruton's Tyrosine Kinase	1	IL-11	2
C1 esterase inhibitor	2	IL-12	3
C1q	1	IL-13	3
C1s	1	IL-16	3
C3	12	IL-18	2
C4	4	Integrin α -10	1
C5	9	Integrin α -11	1
CD40	2	LDL	2
CD40 ligand	1	Leptin	1
Cystatin C	1	Lewis x	1
Factor B	2	Lewis y	1
GLP-1R	1	MCP-1	2
GM-CSF	1	MCP-3	1
IFN- γ	2	MCP-4	1
IgM	1	Mucin 1	1
IL-1 α	2	Procathepsin	1
IL-1 β	2	Properdin	1
IL-1ra	1	RANTES	2
IL-2	1	TGF- β	1
IL-3	3	TM peptide	1
IL-4	3	TNF- α	1
IL-5	2	TNF- β	2
IL-6	3	VEGF	2
IL-8	1		

Table S2. Blocking agents evaluated for blocking of slide-based solid supports.

Name	Blocking Agent	Buffer	Type
PBS	None	PBS	Control
BSA-1	1% (<i>w/v</i>) BSA	PBS	Protein
BSA-5	5% (<i>w/v</i>) BSA	PBS	Protein
Cas-1	1% (<i>w/v</i>) casein	PBS	Protein
Cas-5	5% (<i>w/v</i>) casein	PBS	Protein
M-1	1% (<i>w/v</i>) non-fat dry milk	PBS	Protein
M-1 TBS	1% (<i>w/v</i>) non-fat dry milk	TBS	Protein
M-5	5% (<i>w/v</i>) non-fat dry milk	PBS	Protein
M-5 TBS	5% (<i>w/v</i>) non-fat dry milk	TBS	Protein
OMEGA ^(a)	1% (<i>w/v</i>) OMEGA	PBS	Polymer
PEG	1% (<i>w/v</i>) PEG2000	PBS	Polymer
PVP40	1% (<i>w/v</i>) PVP 40	PBS	Polymer
CHAPS ^(b)	1% (<i>w/v</i>) CHAPS	PBS	Detergent
NP40 ^(c)	1% (<i>v/v</i>) NP-40	PBS	Detergent
T20-1	1% (<i>v/v</i>) Tween-20	PBS	Detergent

Table S2. *Cont.*

Name	Blocking Agent	Buffer	Type
T20-1 TBS	1% (v/v) Tween-20	TBS	Detergent
TX100	1% (v/v) Triton-X-100	PBS	Detergent
BSA + T20	1% (w/v) BSA + 1% (v/v) Tween-20	PBS	Mix
M + T20	1% (w/v) non-fat dry milk + 1% (v/v) Tween-20	PBS	Mix
EA	25 mM ethanolamine	100 mM sodium borate	Other

^(a) OMEGA= *N*-Octanoyl-*N*-methylglucamine; ^(b) CHAPS= 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate; ^(c) NP40 = 4-Nonylphenyl poly(ethylene glycol)