

**Supplementary material**

**Integrating Single Domain Antibodies into Field-Deployable Rapid Assays**

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Table S1. Tail names and amino acid sequences

Table S2. Sequences of sdAb used in the VFA

Figure S1. Evaluation of different ACVE capture formats

Figure S2. Evaluation of two ACVE-based constructs on AuNPs

**Table S1.** Tail names and amino acid sequences

Tail name	Tail sequence
Hop tail	GAGGSGGAPASNRCSQGSCWN
Spy tag (ST)	AHIVMVDAYKPTK
Gold binding peptide (gbp) 1	MHGKTQATSGTIQS
Gbp2	LKAHLPPSRLPS
Synthetic zipper (synzip) 5	NTVKELKNYIQELEERNAELKNLKEHLKFAKAELEFELAAHKFE
synzip 6	QKVAQLKNRVAYKLENAKLENIVARLENDNANLEKDIANLEKDIANLERDVAR
synzip 17	NEKEELKSKKAELRNRIEQLKQKREQLKQKIANLRKEIEAYK
synzip 18	SIAATLENDLARLEENARLEKDIANLERDLAKLEREEAYF
E34	ITIRAAFLEKENTALRTEIAELEKEVGRSENIIVSKYETRYGPL
Rhizavidin (RZ)	FDASNFKDFSSIASASSWQNSGSTMIIQVDSFGNVSGQYVNRAGTGCQN SPYPLTGRVNGTFIAFVSGWNNSTENCNSATGWTGYAQVNGNNTIEIVTSWNL AYEGGSGPAIEQQQDTFQYVPTTENKSLKLD

**Table S2.** Sequences of sdAb used in the VFA

SdAb name	SdAb sequence
ACVE	DVQLVESGGGLVQPGGSLRLTCAASGLIFGSYAMGWFRQAPGKAREFVAAISWSGGDTYADSVK GRFTISRDNANTVYLQMNLSLEPEDTAVYSCAAVGSKYISKDAKDYGYWGQGTQVTVSS
A3H2	EVQLVESGGGLVQAGDSLRLSCTASGRIFSRVAVMGWFRQAPGKREFVAAISAAPGTAYYAFYADS VRGRFIISADSAKNTVYLQMNLSLKPEDTAVYYCAADLKMQVAAYMNQRSVDYWGQGTQVTVSS
D12f	EVQLQASGGGLVQDGGSLRLSCAVAGRPLSDYGVGWFRQASGKREFVAVISGSGIVTDYANSVK GRFTISRDVAKNVVHLQMNLSLKPEDTAVYYCAALNPVYAASRNSNDYGYWGQGTQVTVSS
F6H2Y	QVQLVESGGGLVQAGGSLRLSCATSGGTFSDYGMGWFRQAPGKREFVAAIRNRNGNGNGIEYA DSVKGRFTISRDNANTVYLQMNLSLTPEDTAVYYCAASISGYAYNTIERYNYWGQGTQVTVSS
E2	EVQLQASGGGLVQAGGSLRLSCAASGRTDSTQHMAWFRQAPGKREFVTAIQWRGGGTSYTDS VKGRFTISRDNANTVYLEMNSLKPEDTAVYYCATNTRWTFYFPTVPDRYDYWGQGTQVTVSS
C2	EVQLQASGGGLVQAGGSLRLSCAASGFTFSSYAMMWVRQAPGKLEWVSAINGGGGTSYADS VKGRFTISRDNANTLYLQMNLSLKPEDTAVYYCAKYQAAVHQEKEDYWGQGTQVTVSS
B6	EVQLQASGGGLVQAGDSLRLSCVAVSGRTISTFAMGWFRQAPGKREFVATINWSGSSARYADPV EGRFTISRDDAKNTVYLEMSSLKPGDSAVYYCASGRYLGGITSYSQGFAPWGQGTQVTVSS

**Figure S1.**



Evaluation of different ACVE capture formats. Upper left spot = ACVE; upper right spot = ACVE-ACVE; lower left spot = SCVE-ST; lower right spot = ACVE-synthetic zipper 17

**Figure S2.**



Evaluation of two ACVE-based constructs on AuNPs. Left is ACVE-hop and right is the trivalent construct ACVE-ACVE-ACVE. Miriad test cartridges were spotted with a monoclonal anti-SEB (mAb 2F2) on the top, and SEBv on the bottom. Next 100  $\mu\text{L}$  of SEBv (10  $\mu\text{g}/\text{mL}$ ) was applied to the cartridge, followed by a wash and the AuNPs. Cartridges were washed again and photographed.