

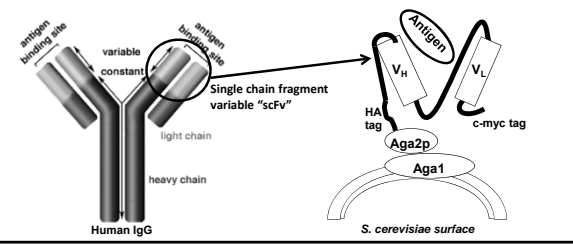
Isolation of Alternative Affinity Reagents for *Entamoeba histolytica* Detection Utilizing an Accelerated Pipeline

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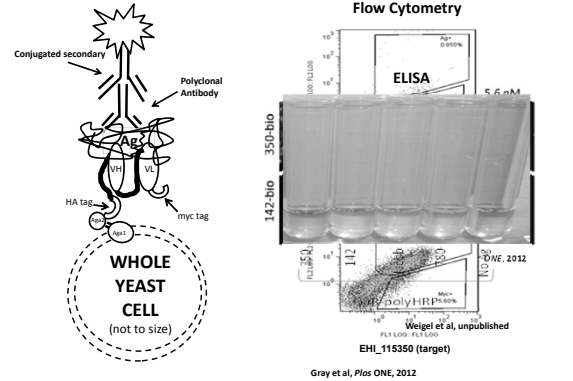
The Accelerated Molecular Probe Pipeline

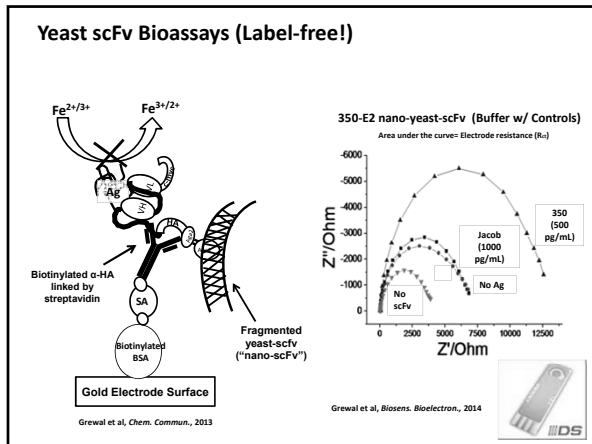
GOAL: Making alternative antibody-like molecules a viable and cost-effective substitute to animal monoclonal antibodies

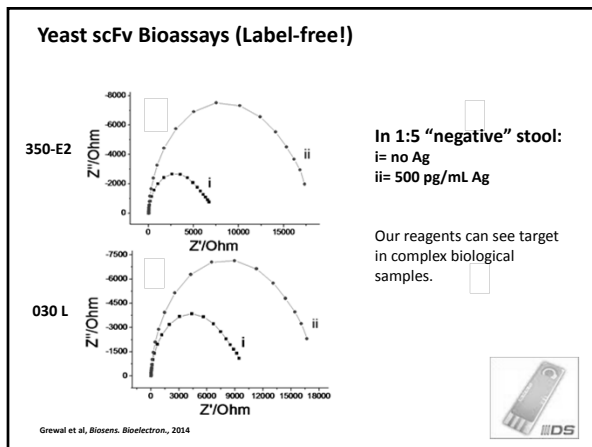
ADVANTAGES: scFv cloned off display surface of cell
 * 2-3 week production
 * Unique selection environment in which they were selected
 * Unique selection environment in which they were selected
 - 1-2 rounds of FACS

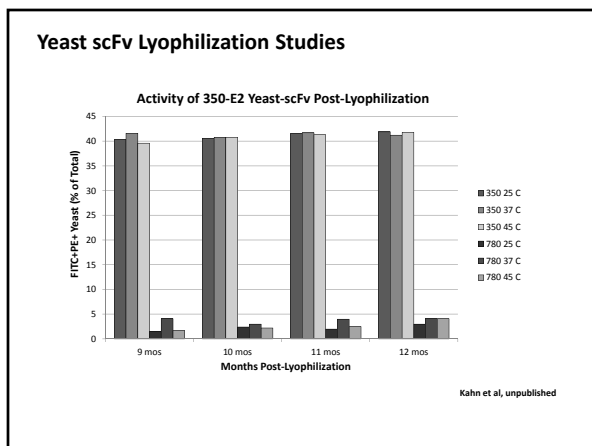


Yeast scFv Bioassays





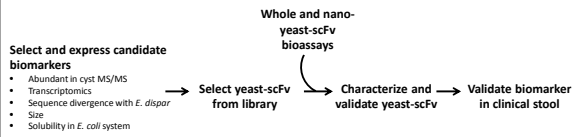
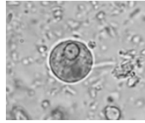




Entamoeba histolytica Biomarker Validation

Attempting to validate a new biomarker for *Entamoeba histolytica*,

- Tricky pathogen to diagnose in settings in which is endemic
- Focusing on the **cyst stage**, which is shed and transmitted, yet is not the focus of current tests



scFv Inventory

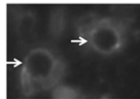
Target	Annotation	Unique scFv	LOD (pM) Whole Yeast Sandwich	LOD (pM) Nano-scFv SPE Electrode
EHI_006810	14-3-3 Protein 3	3	156-400	ND
EHI_104630	Filamin 2	3	ND	ND
EHI_033250	Polyadenylate-binding protein	7	ND	ND
EHI_070730	Rho GTPase	14	ND	ND
EHI_182030	Rab GTPase F2	3	156	ND
EHI_115350	Chromodomain DNA-binding protein	1	313	0.59
EHI_044500	Cell-wall protein Jacob2	3	20-70	ND
EHI_101240	Staphylococcal nuclease-like protein	TBD	--	--
200.m00090	Clathrin heavy chain	TBD	--	--

scFv Inventory: Spotlight on Jacob2

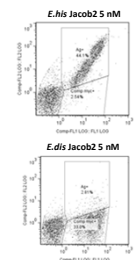
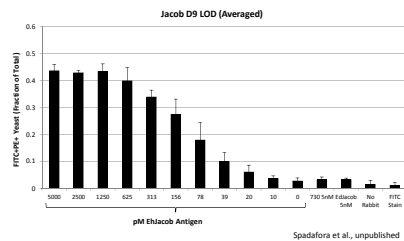
Jacob2 is a known outer cyst wall lectin.

It has a very divergent spacer which has been expressed in the lab.

We have 3 scFv that bind recombinant *E. histolytica* Jacob2, but not to the recombinant *E. dispar* Jacob2.



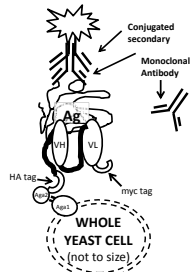
Ali et al, PLOS Negl. Trop. Dis., 2012



Jacob2 scFv Epitope Binning

Do the 3 scFv have unique epitopes? Mouse monoclonals we have developed in parallel aided us in that investigation.

Conclusion: Each scFv has a unique antigen epitope, though the Jacob D9 epitope seems to overlap the other two.



Jacob scFv Epitope Binning: Ag-Binding Cells (% of Total)

	1A4	1D3	4A4	2A7	2A12	2B10	2C7	2F1	3D2	4A3	4D2	4E12
Jacob D9	28.78%	20.94%	24.01%	3.46%	21.74%	18.61%	3.70%	5.63%	21.75%	19.50%	18.40%	21.57%
Jacob C9	46.26%	34.57%	39.50%	2.11%	31.30%	23.83%	13.64%	16.11%	43.11%	32.11%	36.29%	33.23%
Jacob D8	14.73%	34.45%	14.23%	12.27%	4.03%	0.27%	3.60%	3.52%	14.08%	0.12%	10.62%	5.13%
350-E2	0.98%	0.65%	0.54%	0.55%	0.70%	0.49%	0.42%	0.40%	0.36%	0.35%	0.44%	0.50%

Summary

- We can isolate scFv molecules specific to antigens of interest within 2-3 weeks, completely *in vitro*.
- Whole or fragmented (“nano-”) yeast-scFv are maintained in the environment in which they are selected and have been successfully integrated in a number of assays.
- Freeze-dried yeast-scFv still function after 11-12 months of storage.
- We have 34 yeast-scFv to 7 recombinant *E. histolytica* cyst biomarkers, 3 of which are specific to the pathogen’s outer wall protein.
 - Future directions will investigate the performance of these scFv in complex samples (purified parasite, spiked stool, clinical stool)

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