High-throughput Protein Expression Screening of Synaptic Cell Surface Protein Families

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Introduction

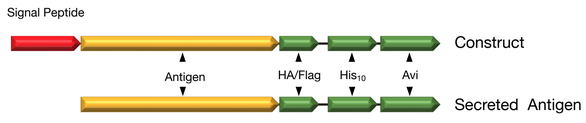
IPI's Antibody Discovery Platform is dependent on availability of high-quality antigens. Cell surface receptors, the focus of targets at IPI, are particularly challenging to recombinantly express and purify in part due the customization necessary to design each construct. One can circumvent this problem by designing multiple constructs for each and assessing them in parallel. At IPI, we have developed a high-throughput screening technique that streamlines the process of construct design and protein expression evaluation. Using this method, we successfully evaluated constructs for the synaptic proteins from the cell surface receptor families neurexin, neuroligin, latrophilin and teneurin.

Neurexins (NRXNs) and teneurins (TENMs) are pre-synaptic proteins that interact with their post-synaptic partners neuroligins (NLGNs) and latrophilins (LPHNs or ADGRLs), respectively. These proteins play important roles in neurotransmission,[1] axon targeting and neural wiring,[2] movement regulation and associated memories.[3] Absence or mutation of these proteins leads to neurodevelopmental or cognitive disorders, including attention deficit hyperactivity disorder (ADHD).[4] However, regulatory mechanisms of these proteins in neurotransmission are not completely understood due to the lack of protein tools to effectively differentiate between the post- and pre-synaptic components. The availability of recombinant antibodies can be an asset in such studies.

We leveraged our high-throughput small-scale expression method to screen 32 human and murine constructs. The estimated yields from small-scale expression translated successfully to our large-scale antigen production platform, resulting in the purification of 27 high-quality antigens. These and evaluation of other targets show that the technique has significant potential for improving production strategies for difficult-to-purify proteins.

Small-Scale Expression Screen

Antigen Design



- HA/FLAG differentiated between human and murine proteins.
- His-tag and Avi-tag facilitated affinity purification and site-specific biotinylation for antibody discovery, respectively.

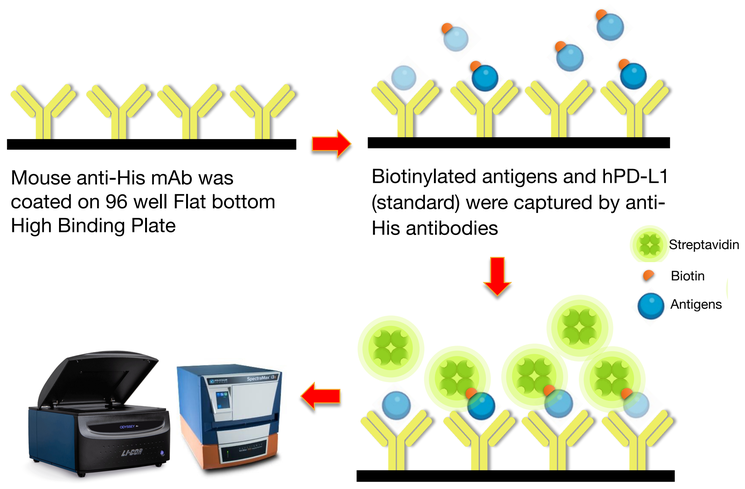
Small-Scale Expression

Miniprepped Constructs

Small-Scale Culture

- Constructs were miniprepped from DH5α.
- DNA was normalized for transfection using the Lynx LM900 liquid handler.
- Biomek i7 was used for the high throughput transfection of Expi293 HEK cells.
- Cell supernatant with expressed antigens were harvested after five days and biotinylated.

ELISA



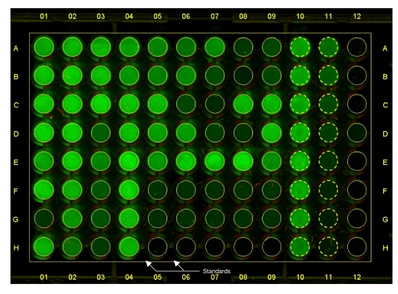
Mouse anti-His mAb was coated on 96 well Flat bottom High Binding Plate

Biotinylated antigens and hPD-L1 (standard) were captured by anti-His antibodies

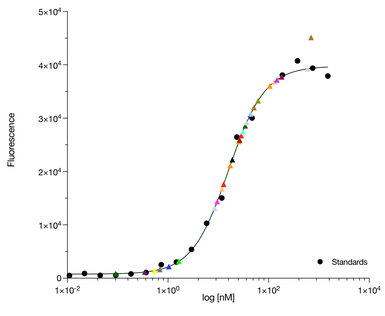
Captured antigens bound to IRDye 800CW Streptavidin or AlexaFlour488 Conjugated Streptavidin

To assess expression level, Fluorescence was measured by LI-COR Odyssey CLx and SpectraMax i3x

ELISA Results Analysis



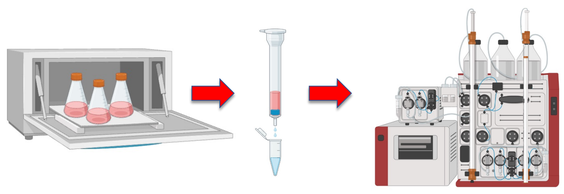
- Expressed proteins were detected by luminescence measurements in the LI-COR Odyssey CLx at 800 nm
- Higher luminescence signal indicated increased protein expression.



- The standard curve was generated by fluorescence measurements and 4 parameter logistic curve fit from the SpectraMax i3x.
- Estimated yields of expressed protein were calculated from this standard curve (colored triangles).

Transition to the Large-Scale Platform

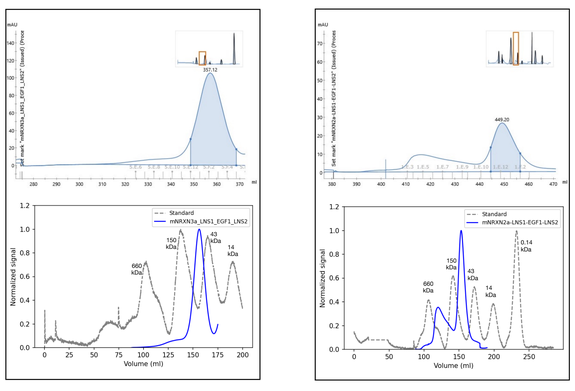
Large-Scale Expression & Purification



- Constructs estimated to express well in small-scale were transfected in Expi293 HEK cells and grown for five days.
- Expressed antigens were purified by Nickel affinity and high-throughput Size Exclusion Chromatography (SEC).

High producer: mNRXN3a_LNS1_EGF1_LNS2 (50kDa)

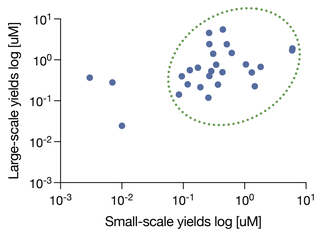
Low producer: mNRXN2a_LNS1_EGF1_LNS2 (54kDa)



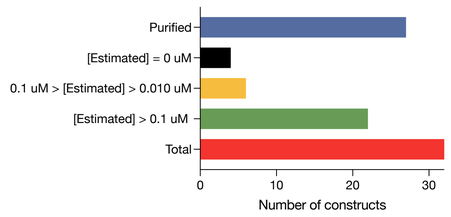
- Expressed antigens eluted at volumes consistent with their molecular weights in SEC (see overlay with calibration curves).

Small-scale Predictions Translate To Large-scale Success

Correlation of yields from small-scale expression screen with the large-scale purification platform



Summary: Number of high, medium, and low yield constructs in small-scale screen and purified constructs.



- Yield of protein from large-scale purification was well correlated with estimates from the small-scale screen.
- In general, constructs exhibiting expression levels > 0.1 μM could be reliably purified.

Caveat:

The small-scale screen analysis assumed complete biotinylation of antigens in the cell culture supernatant.

However, we observed lower labeling levels in the supernatant compared to purified antigen samples.

This could result in an underestimation of expression levels.

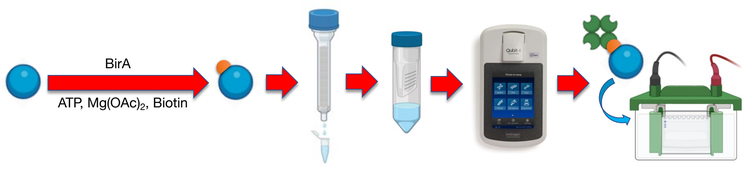
It is worth noting that constructs lacking fluorescence signal did not yield usable antigen during scale-up.

Final yields of antigens after large-scale purification (colors from [Estimated] in summary graph above)

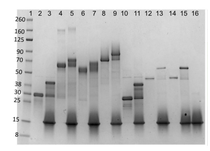
Protein Family	Species	Construct	Final yields (μM)
Neurexin 1a	Human	ECD_noSS1234	1.2
		LNS1_EGF1_LNS2	3.1
		LNS2_crystal	5.4
	Mouse	LNS1_EGF1_LNS2	3.4
		LNS2_6PNP	13.9
Neurexin 1b	Human	ECD	8.9
	Mouse	3B3Q	21.3
Neurexin 2a	Mouse	LNS1_EGF1_LNS2	9.1
		LNS2	0.0
Neurexin 2b	Human	ECD	2.0
Neurexin 3a	Mouse	LNS1_EGF1_LNS2	4.9
		LNS2	28.4
Neurexin 3b	Human	ECD	1.2
	Mouse	ECD	7.8
Neuroligin 1	Human	5OJK	3.0
	Mouse	3B3Q	1.8
Neuroligin 2	Human	5XEQ	1.5
	Mouse	ECD	0.9
Neuroligin 3	Mouse	7CEE	1.4
Neuroligin 4	Human	4X-3BE8	3.7
		4Y-ECD	0.0
	Mouse	ECD	0.0
Latrophilin 1	Human	ECD	2.8
	Mouse	6SKA	1.2
Latrophilin 2	Human	ECD	0.0
	Mouse	6SKE	20.8
		ECD	0.0
Latrophilin 3	Human	6VHH	1.9
	Mouse	4YEB	4.6
		5AFB	8.9
		crystal	2.3
Teneurin-1	Human	CTERM	0.0

Biotinylation & Quality Assessment

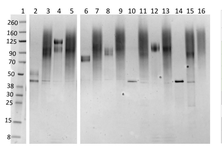
- Antigens that successfully eluted from SEC were usually fit for downstream processing for biotinylation and Discovery.
- These were biotinylated with BirA, assessed for labeling by Streptavidin shifts in SDS-PAGE followed by Dynamic Light Scattering (DLS) to determine monodispersity and homogeneity.



BirA Biotinylation

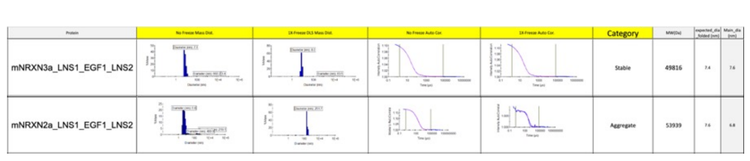


Ln	Sample name	Mol. Wt. (Da)
1	Molecular weight marker	
2,3	hNRXN1a_LNS2_crystal	26080
4,5	hNRXN1a_LNS1_EGF1_LNS2	57531
6,7	mNRXN3a_LNS1_EGF1_LNS2	49816
8,9	hNRXN3b_ECD	61072
10,11	mNRXN2a_LNS2	24658
12,13	MBP-Avi	44846
14,15	biot-MBP-Avi	44846
16	2ug monostrep	15500



Ln	Sample Name	Mol. Wt. (Da)
1	Duo	
2,3	mADGRL3_5AFB	47097
4,5	mADGRL3_crystal	98821
6,7	mNLGN1_3B3Q	67095
8,9	mNLGN3_7CEE	76597
10,11	C1_MBP-Avi	44846
12,13	hADGRL3_6VHH	98788
14,15	C+_Biot-MBP-Avi	44846
16	Strep	60000

Biotinylated antigens, when incubated with Streptavidin or Monoavidin exhibited a molecular weight shift towards higher values in SDS-PAGE



Fully biotinylated antigens were further assessed by DLS for oligomerization status and aggregation level. Non-aggregated antigens were used for antibody discovery

Conclusions

- Small-scale expression coupled with ELISA is an effective technique to screen out low expressing constructs.
- The extrapolation of estimated expression levels from the small-scale screen to our large-scale antigen production platform demonstrated consistent translation in terms of required volumes to achieve protein yields suitable for downstream applications.
- Most antigens that showed good yields in the small-scale screen could be biotinylated successfully for antibody discovery.
- Limitation: The high throughput small-scale expression screening technique does not differentiate between well-behaved intact antigens and/or degraded/aggregated proteins.

References

1. Chen F, Venugopal V, Murray B, Rudenko G. ***Structure*** (2011). 19, 779-89.
2. Jackson, V.A., Meijer, D.H., Carrasquero, M. et al. ***Nat Commun*** (2018). 9, 1079.
3. Richard Sando et al. ***Science*** (2019). 363, eaav7969.
4. Samantha L. Regan, Michael T. Williams, Charles V. Vorhees, ***Neurosci. Biobehav. Rev.*** (2021). 127, 619-629.

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