Development of an AlphaLISA® protein-protein interaction assay to screen for re-purposed drugs as targeted disruptors

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- **Drug repurposing is an intriguing option to quickly respond to an emerging disease**
- **AlphaLISA® assays provide a flexible platform to investigate protein-protein interactions linked to infection**
- **Important optimization, proof of performance and counter-screen assay steps are described**

Introduction

For nearly two decades the scientific community has warned us of the need to be prepared for a pandemic, whose likelihood has increased due to several factors related to globalization[1]. A major part of this pre-paredness is our ability to quickly identify the cause of the emerging disease and its route of infection. Once these characteristics are identified our ability to quickly exploit this knowledge becomes paramount. To this end, drug repurposing provides the tools of approved or at least highly tested drugs that might be applicable to the treatment of the disease.

Taking the example of SARS-CoV-2, it was quickly realized, that, similar to related corona viruses, the point of interaction with cells was angiotensin-converting enzyme 2 (ACE2)[2]. With this knowledge the screen described herein was performed[3]. This serves as an example of using recent advances in assay technology and the importance of assay optimization and validation. This screen further employs a useful, commercially available counter-assay.

Assay Principle

To search for disruptors of the protein-protein interaction (PPI) between ACE2 and the SAR-CoV-2 Spike Protein (S1) Receptor Binding Domain (RBD) an AlphaLISA assay was selected. This proximity-based assay has several advantages, including the commercial availability of streptavidin donor beads and protein A acceptor beads (figure 1 A). These allow for testing of various candidate protein combinations with appropriate Avi and Fc tags. Using this approach, it should be possible to identify existing drugs that disrupt the interaction between the 2 proteins leading to a decrease in emission signal produced.

For this AlphaLISA assay there exists a useful counter-screen that can be used to eliminate some false-positives (figure 1 B). Since there is essentially a direct interaction between the donor and acceptor beads, this counter-screen is useful in eliminating compounds that interfere with the AlphaLISA assay by, for example, quenching singlet oxygen or absorbing either excitation or emission light.

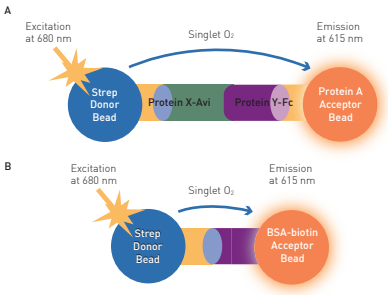

Fig. 1: Assay Principles for approaches used
A) PPI AlphaLISA Assay Principle B) Tru-Hits counter-screen principle

Materials & Methods

- White 384 & 1536 microplates (Aurora)
- BMG PHERAstar *FSX* microplate reader
- ACE2-His-Avi (ACROBiosystems)
- RBD-Fc, ACE2-His & S1-His (Sino Biological)
- Streptavidin donor beads, Protein A acceptor beads & TruHits kit (PerkinElmer)

Experimental Procedure

For complete experimental details please refer to Hanson et al[3]. For all experiments a final concentration of 10 µg/mL for both donor and acceptor beads was employed.

Cross-titration experiment

Cross-titration of ACE2-His-Avi (300-0.1 nM) against RBD-Fc (300-0.1 nM) was performed in 384 well plates. Resulting combinations were mixed and incubated for 30 minutes at 25 °C. Donor and acceptor beads were added and plates read.

Competition binding assays

ACE-His concentration gradient (1 µM to 0.01 nM) was mixed with RBD-Fc (4 nM) and incubated at 25 °C for 30 minutes. ACE2-His-Avi (4 nM) was added, followed by incubation at 25 °C for 30 minutes. Donor/acceptor beads were added, and the final mix incubated for 40 minutes at 25 °C then read.

TruHits Assay

All reagents except compounds are from the TruHits kit. Twenty nL of compounds were dispensed into 1536 well plates followed by 3 µL of Biotin-BSA acceptor beads. Mixture was incubated at RT with 200 rpm shaking for 30 minutes. Three µL of Streptavidin donor beads were added and incubated as before then read.

Rev. 04/2021
Keywords: Protein-Protein Interaction; AlphaLISA; High-Throughput Screening

Instrument Settings

Optic settings	AlphaScreen, Endpoint	
	Module	AS 680 615
General settings (fastest)	Settling time	0.0 s
	Excitation time	0.05 ms
	Integration start	0.08 ms
	Integration time	0.10 ms

Results & Discussion

Protein concentrations were optimized using a cross titration experiment. The results in figure 2 show that maximal signal was obtained by combination of both, RBD-Fc and ACE2-Avi at 3.7 nM. Thus, a concentration of 4 nM was used for the remaining experiments.

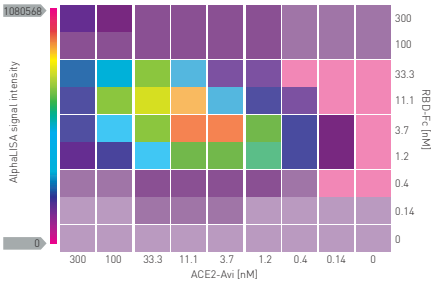

Fig. 2: Assay optimization: Cross titration of proteins

Indicated concentrations of RBD-Fc and ACE2-Avi were combined and intensity of signal production determined after AlphaLISA acceptor/donor beads were added.

Signal consistency was also tested with a high-signal control (ACE2-Avi + RBD-Fc + both AlphaLISA beads) and a low-signal control (ACE2-Avi + RBD-Fc + streptavidin donor beads). Results indicated satisfactory performance with Z' = 0.73 and signal to background = 268.7 (data not shown)[3].

For this assay to be useful, one must be able to disrupt the interaction between the proteins. Therefore, as a proof of principle, unlabeled versions of the interacting proteins were employed in competition binding assays. Figure 3 shows the results of adding increasing ACE2-His. As hoped, AlphaLISA signal decreases with increased ACE2-His. Similar results were obtained with His-S1, a version of the SARS-CoV-2 spike protein (data not shown)[3].

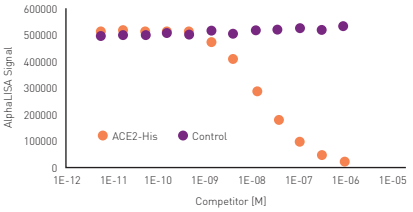

Fig. 3: Proof of performance: untagged ACE2 lowers AlphaLISA signal
Addition of increasing ACE2-His exhibits a dose-dependent decrease in AlphaLISA signal. The average of 3 replicates is displayed.

Finally, figure 4 depicts the utility of the TruHits Assay. If a test compound leads to a decrease in this counter-screen signal it need not be further characterized.

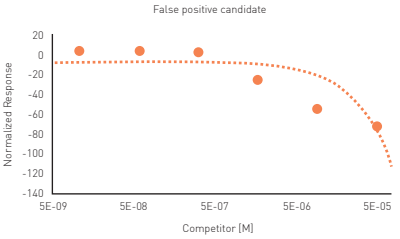

Fig. 4: Counter-screen: TruHits Assay
The compound employed exhibits a dose-dependent decrease in AlphaLISA signal indicating that this compound directly interferes with the AlphaLISA assay.

Conclusion

A PPI assay suitable for HTS was developed. This provides an example of how improved assay and microplate reader technologies can be leveraged to develop new screens to improve our ability to respond to emerging diseases.

References

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2. Gheblawi, M et al. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System. Circ. Res. (2020) **126**: 1456-1474
3. Hanson, QM et al. Targeting ACE2-RBD interaction as a platform for COVID19 therapeutics: Development and Drug-Repurposing Screen of an AlphaLISA Proximity Assay. ACS Pharmacol Transl Sci Article ASAP