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Allostery in Drug Discovery

Yeast Surface Display Library Screening for the Discovery of Allosteric Antibodies

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Abstract: This protocol describes a screening approach using yeast surface display libraries to facilitate the discovery of allosteric antibodies. The platform leverages multiparametric fluorescence-activated cell sorting (FACS) to select antibody fragments that bind to allosteric sites on target proteins. The procedure includes target and ligand preparation, yeast library staining, and a FACS-based sorting strategy. This approach enables the efficient identification of antibody fragments with allosteric binding modes at early stages of the discovery process.

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Experimental Protocol for Early Selection of Non-Competitive Antibody Fragments Using Yeast Surface Display and FACS

1. **Protein Preparation**
    - Prepare or purchase the recombinant target protein with a tag.
    - Prepare or purchase the ligand, preferably as an Fc-fusion protein.
    - Confirm ligand binding to the target protein via Bio-Layer Interferometry (BLI).
    - Determine K_D, k_{on}, and k_{off}, and calculate the equilibrium time.
2. **Yeast Library Generation**
    - Generate a yeast surface display library, the displayed portion should contain a different tag than the one on the target protein.
    - Culture and induce yeast cells.
3. **Pre-Incubation**
    - Pre-incubate 1 µM of the target protein with 3 µM of the ligand at room temperature for at least the equilibrium time to allow complex formation.
        - If k_{off} is too fast, skip the pre-incubation step and immediately perform the staining step using target and ligand proteins along with detection antibodies.
4. **Staining for FACS**
    - Wash yeast cells with PBS.
    - Incubate cells with the target-ligand complex.
    - Wash and stain (using different fluorophores) for:
        - Surface display
        - Target binding
        - Ligand binding
5. **FACS Sorting**
    - Use a suitable sorter with different lasers available.
    - Check controls to ensure surface display, target binding (alone), and no direct binding to the ligand.
    - If all conditions are okay, apply sorting gates:

Cells → Yeast cells → Single cells → Target binding$^+$ → Ligand binding$^+$

- Select yeast cells that bind to the target in the presence of the ligand, and adjust the gate to select for ligand modulation.
    - Positive allosteric modulators (high ligand signal)
    - Negative allosteric modulators (low ligand signal)
- Enrich for allosteric binders as long as necessary.

6. **Clone Analysis**

- Sequence sorted cells.
- Cluster sequences and select hit candidates.
- Express selected candidates.
- Purify candidates and confirm binding to the target via BLI.
- Perform competition and ligand modulation assays to confirm allosteric binders.