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Technology Offer

Automated Stability Screening of Protein Complexes Using ProteoPlex

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Abstract

ProteoPlex is an advanced analytical platform designed to optimize the stability of macromolecular protein complexes. It integrates automated differential scanning fluorimetry (DSF) with a proprietary deconvolution algorithm and robotic liquid handling to screen diverse chemical conditions. This system identifies ideal buffer compositions, pH values, and ligand interactions that preserve the native quaternary structure of complex protein assemblies. Unlike conventional DSF, which is limited to single-domain proteins, ProteoPlex is tailored to handle the complexity of multisubunit assemblies. It offers high-throughput, low-sample consumption, and automated workflows, thus accelerating biochemical and structural studies. The technology enables rapid stabilization of fragile complexes for downstream applications in X-ray crystallography, cryo-electron microscopy, and drug discovery.

Background

Large macromolecular complexes play essential roles in biological processes, but their structural analysis is hindered by low stability and conformational heterogeneity upon purification. Conventional stabilization approaches are often manual, protein-specific, and require extensive biochemical knowledge. Traditional DSF methods fail to interpret overlapping unfolding transitions typically observed in multicomponent complexes. This limits their utility in structural biology, especially for challenging targets like ribosomes, spliceosomes, or ATPase assemblies. There is a critical need for a general, automated, and sensitive method to assess and enhance the stability of these biomolecular machines under varying chemical environments.

Technology

ProteoPlex automates stability screening through a novel extension of DSF tailored for protein complexes. The system uses a thermocycler, fluorescence detection, and liquid-handling robotics to perform thermal unfolding assays across a matrix of buffer and ligand conditions. During thermal denaturation, the dye Sypro Orange binds exposed hydrophobic regions, generating fluorescence profiles indicative of protein unfolding. A key innovation lies in ProteoPlex's analytical software, which applies a thermodynamic unfolding model capable of distinguishing cooperative versus non-cooperative transitions. This model estimates enthalpy, entropy, and cooperativity parameters for each condition. The platform supports both manual and fully automated modes, including a Protein Concentration Determination (PCD) module, and can screen up to 88 buffer conditions or ligands in a single run. Resulting data enable identification of conditions that yield monodisperse, stable samples, verified through downstream structural methods such as electron microscopy.

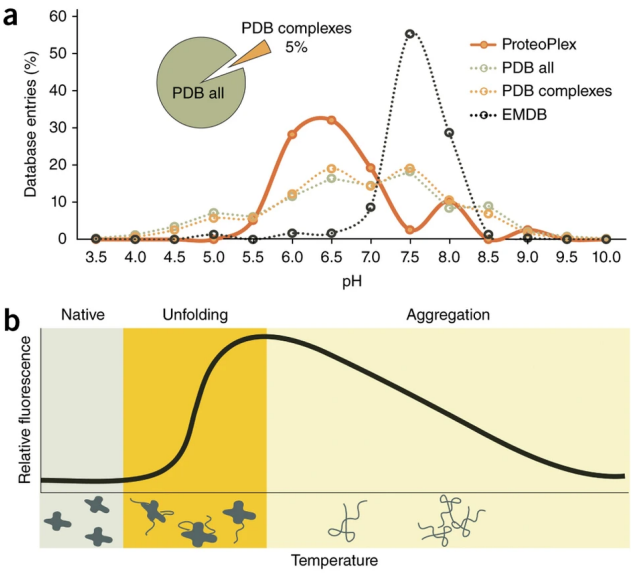

Figure 1: Stability Screening of Protein Complexes with ProteoPlex. **(a)** Comparison of pH values used in structural biology (PDB and EMDB) with those identified by ProteoPlex. ProteoPlex covers a broader pH range, revealing optimal conditions often missed in conventional studies. **(b)** Schematic of a typical DSF thermal unfolding curve, illustrating the transition from native (low fluorescence), to unfolded (high fluorescence due to dye binding), to aggregated (fluorescence decrease).

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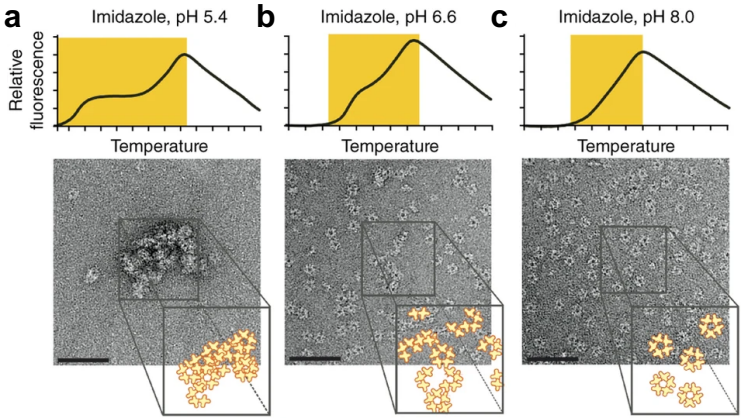

Figure 2: Experimental DSF curves and corresponding electron microscopy (EM) images of a protein complex at different imidazole pH values. At pH 5.4, strong aggregation is observed **(a)**; pH 6.6 shows partial aggregation **(b)**; and at pH 8.0, a nearly ideal two-state transition corresponds to a monodisperse, stable sample suitable for structural analysis **(c)**.

Advantages

- **Automated high-throughput buffer and ligand screening** for protein complexes without prior biochemical knowledge.
- **Advanced deconvolution algorithm** resolves overlapping thermal unfolding transitions unique to macromolecular assemblies.
- **Minimal sample volume requirements**, enabling screening of scarce or precious protein preparations.
- **Enhanced structural homogeneity and stability**, verified through direct correlation with EM or crystallization success.
- **Fully integrated robotic system** allows unattended operation from setup to analysis.

Potential applications

- Structural biology: stabilization of complexes for **cryo-EM or X-ray crystallography.**
- Drug discovery: screening **ligand-induced stabilization** for therapeutic target validation.
- Protein engineering: evaluating **mutant stability** in multimeric protein systems.
- Biochemistry: optimizing **buffer conditions** for functional assays or reconstitution studies.
- Academic research: characterization of **novel or poorly understood macromolecular machines**.

Patent Information

PCT application (*WO2013034160A1*; 06.09.2011), active in EP, US, JP, CN

Publications

Chari, A., Haselbach, D., Kirves, J. M., Ohmer, J., Paknia, E., Fischer, N., ... & Stark, H. (2015). ProteoPlex: stability optimization of macromolecular complexes by sparse-matrix screening of chemical space. *Nature methods*, *12*(9), 859-865.

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