METHODS FOR CAPTURE AND ANALYSIS OF MACROMOLECULAR COMPLEXES

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. Nos. 62/970,035 and 62/970,015, filed Feb. 4, 2020, which are hereby incorporated by reference in their entirety.

FIELD

[0002] The present application relates to methods for capture and analysis of macromolecular complexes.

BACKGROUND

[0003] Characterizing macromolecular complexes (MCs) and their interactions is essential for understanding any biological process at the molecular level. With increased resolution and throughput of mass spectrometry (MS) in the last decade, MS-based proteomic analyses following co-immunoprecipitations have been extensively utilized to identify interacting partners of many proteins (DeBlasio et al., "Insights into the Polerovirus-plant Interactome Revealed by Coimmunoprecipitation and Mass Spectrometry," *Mol. Plant Microbe Interact.* 28:467-481 (2015); Budayeva et al., "A Mass Spectrometry View of Stable and Transient Protein Interactions," *Adv. Exp. Med. Biol.* 806: 263-282 (2014); Bauer et al., "Affinity Purification-mass Spectrometry. Powerful Tools for the Characterization of Protein Complexes," *Eur. J. Biochem.* 270: 570-578 (2003)). These assays rely on the availability of antibodies that are well characterized, highly specific, and high-affinity against the protein of interest (POI) or a peptide tag that allow the binding partners of the (tagged)-target protein to be co-precipitated while the antibody is immobilized on beads/resins. Even for a single antibody, significant lot-to-lot variability affects the purity, specificity and yield of (co-) immunoprecipitations. The (co-) immunoprecipitated proteins are subsequently eluted by denaturation (i.e. with heat, SDS or combinations) or by on-bead proteolytic digestion and analyzed by MS. However, contaminating peptides derived from the antibody/serum or Protein-A/G are routinely found at an order of magnitude higher abundance than the POI (DeBlasio et al., "Insights into the Polerovirus-plant Interactome Revealed by Coimmunoprecipitation and Mass Spectrometry," *Mol. Plant Microbe Interact.* 28:467-481 (2015); Budayeva et al., "A Mass Spectrometry View of Stable and Transient Protein Interactions," *Adv. Exp. Med. Biol.,* 806:263-282 (2014)). This can hinder the identification of interacting partners, particularly if they are rare or substoichiometric. In some cases, eluates are further fractionated by gel electrophoresis and individual protein bands are excised to exclude heavy and light chains of the antibody prior to MS. This method limits the number of proteins that can be identified and prevents analysis of proteins below the limit of detection for a given electrophoresis/protein staining technique (Jafari et al., "Comparison of In-gel Protein Separation Techniques Commonly Used for Fractionation in Mass Spectrometry-based Proteomic Profiling," *Electrophoresis* 33:2516-2526 (2012)) while increasing the likelihood of keratin contamination and the length of time needed for sample preparation.

[0004] The present application is directed to overcoming these and other deficiencies in the art.

SUMMARY

[0005] The present application relates to a method for analyzing a molecular target in a sample. The method involves providing an aptamer, wherein the aptamer is a high affinity binding partner to at least a portion of the molecular target in the sample. The aptamer is contacted with the sample containing the molecular target under conditions effective for the molecular target and aptamer to bind to each other. The molecular target is separated from the sample to form a molecular target enriched sample. The separated molecular target of the enriched sample is then analyzed.

[0006] In some embodiments, the separated molecular target is analyzed using mass spectrometry. In some embodiments, the separated molecular target is analyzed using cryo-electron microscopy. In some embodiments, the separated molecular target is analyzed using nucleotide sequencing. In some embodiments, the separated molecular target is analyzed using any combination of these techniques.

[0007] To provide an alternative to immunoprecipitations, an RNA aptamer-based affinity purification method has been developed using the highly-specific and high-affinity Green Fluorescent Protein (GFP)-aptamer (Shui et al., "RNA Aptamers that Functionally Interact with Green Fluorescent Protein and its Derivatives," *Nucleic Acids Res.* 40:e39 (2012), which is hereby incorporated by reference in its entirety) to co-purify GFP-tagged target proteins and their binding partners for identification by mass spectrometry (MS) (AptA-MS), nucleotide sequencing, and/or cryo-electron microscopy. Nucleic acid aptamers can be selected against a wide variety of targets and synthesized in unlimited quantities by cost-effective methods. These properties, in addition to their high specificity and affinity, make aptamers attractive reagents for affinity purification. Indeed, aptamers have been used for affinity purification of targets from biological mixtures followed by MS, but mainly for target detection and biomarker discovery (Gulbakan, "Oligonucleotide Aptamers: Emerging Affinity Probes for Bioanalytical Mass Spectrometry and Biomarker Discovery," *Anal. Methods* 7:7416-7430 (2015), which is hereby incorporated by reference in its entirety). These detection assays were developed with a handful of aptamers and demonstrated to work by proof-of-principle experiments with little or no biological applications. General and simple affinity-capture methods using RNA aptamers are lacking, especially those that allow for quantitative analysis of protein interactions and protein complex formation directly from cellular lysates and can be applied to address a broad array of biological questions in a wide range of species, tissues, and cell types.

[0008] The GFP protein in combination with the high-affinity and high-specificity GFP-aptamer (Shui et al., "RNA Aptamers that Functionally Interact with Green Fluorescent Protein and its Derivatives," *Nucleic Acids Res.* 40:e39 (2012), which is hereby incorporated by reference in its entirety) serves as a suitable affinity tool to identify, for example, protein-protein interactions by MS, three-dimensional structure by cryo-electron microscopy, and DNA binding sites of proteins within a complex using nucleotide sequencing techniques. The methods described herein allow use with a broad collection of existing GFP-fusion proteins in human cells and other model organisms including *Drosophila* and yeast. Here, it is demonstrated that AptA-MS is superior to conventional co-immunoprecipitations for subsequent MS analysis, because it is devoid of immunoprecipitation-derived protein contaminants, and provides a dra-