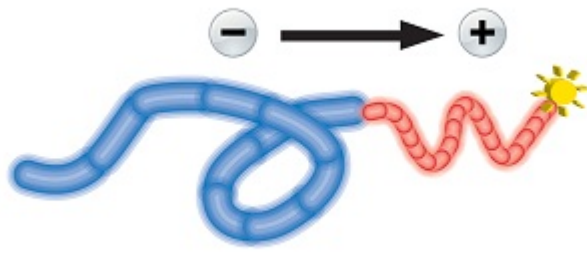


Method for producing completely monodisperse, highly repetitive polypeptides for use with Free-Solution Conjugate Electrophoresis and other bioanalytical applications

Researchers from Prof. Annelise Barron's laboratory have developed a novel method for purifying large, biologically produced protein polymers for DNA sequencing and genotyping. This technique is used to generate truly monodisperse, highly repetitive polypeptides that can be used, for instance, as DNA “drag-tags” in Free-Solution Conjugate Electrophoresis (“FSCE”). These large, protein-based polymers enable significantly longer sequencing reads than the smaller drag-tags to which the technology was previously restricted by purity problems. This technology is particularly applicable for DNA separations on microfluidic platforms because it facilitates exon-length sequencing in an aqueous solution, without the need for a viscous sieving matrix.

The general clone construction/protein design and accompanying purification protocols can be used for generating protein-based polymers for pharmaceutical, biomaterials, or other research applications where precise characterization and true monodispersity of a protein modifier is important.



This schematic of FSCE shows the monodisperse protein drag-tag (blue) conjugated to the DNA sequencing fragment (red) with fluorescent dye attached for detection (yellow star). Covalent attachment of the drag-tag to the DNA molecule allows for size-based separation in an electric field with no sieving matrix.

Stage of Research

The inventors have purified drag-tags as long as ~ 500 amino acids. They used a 267-amino acid drag tag in FSCE to read 265 bases of DNA by capillary electrophoresis in 30 minutes, with no sieving matrix.

Applications

- **DNA sequencing and separation** - generating monodisperse drag-tags for FSCE-based DNA separation, sequencing, and genotyping technologies
- **Microfluidics** - drag-tags enable DNA separation and sequencing in an aqueous solution (without a viscous sieving matrix), which is ideal for implementation on microfluidic devices
- **Biomaterials research** - these clone/protein designs and purification methods could be used to generate other protein polymers for applications in which precise characterization and true monodispersity is important (e.g., any FDA-regulated product)

Advantages

- **Pure protein polymers** - FSCE is a highly sensitive analytical method that provides high resolution and can thus determine the true monodispersity of a protein polymer sample

- **Longer sequencing reads** - larger protein polymer drag-tags have the potential to achieve single-base resolution sequencing of up to 400 contiguous DNA bases (compared to previous, smaller drag-tags that gave read lengths of ~180 bases or less)
- **Advantages of FSCE** - less time, lower reagent cost, and simpler preparation than matrix-based separation, particularly in microfluidic chips

Publications

- Wang XX, Albrecht JC, Lin JS, Barron AE, [Monodisperse, "Highly" Positively Charged Protein Polymer Drag-Tags Generated in an Intein-Mediated Purification System Used in Free-Solution Electrophoretic Separations of DNA](#), *Biomacromolecules*, Jan. 9, 2012, 13, 117-123.
- Jennifer S. Lin, Jennifer Coyne Albrecht, Robert J. Meagher, Xiaoxiao Wang, and Annelise E. Barron, [Completely Monodisperse, Highly Repetitive Proteins for Bioconjugate Capillary Electrophoresis: Development and Characterization](#), *Biomacromolecules*, May 9, 2011, 12(6), pp. 2275-2284.
- Albrecht JC, Lin JS, Barron AE., [A 265-base DNA sequencing read by capillary electrophoresis with no separation matrix](#), *Anal Chem.* 2011 Jan 15;83(2):509-15. Epub 2010 Dec 23.

Patents

- Published Application: [20120202948](#)
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