BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Calin Plesa

eRA COMMONS USER NAME (credential, e.g., agency login): CPLESA

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Simon Fraser University, Burnaby, BC, Canada	BASc	06/2008	Engineering Physics
Delft University of Technology, Delft, the Netherlands	MSc	08/2009	Nanoscience
Chalmers University of Technology, Gothenburg, Sweden	MSc	06/2010	Nanoscience
Delft University of Technology, Delft, the Netherlands	PhD	01/2015	Biophysics
Delft University of Technology, Delft, the Netherlands	Postdoc	09/2015	Biophysics
University of California Los Angeles, Los Angeles, CA, USA	Postdoc	08/2019	Biochemistry

A. Personal Statement

The Plesa Lab focuses on accelerating the pace at which we understand and engineer biological systems, particularly proteins. Over the long-term, my goal is to uncover the rules for the engineering of novel protein function, particularly in the areas of sensing and interfacing with engineering. Towards this end, we develop new technologies for gene synthesis, multiplex functional assays, in-vivo mutagenesis, and genotype-phenotype linkages. These allow us to both access the huge sequence diversity present in natural systems as well as carry out testing of rationally designed hypotheses encoded onto DNA at much larger scales than previously possible. Using these approaches, we can quickly characterize and engineer entire protein families, rather than focusing on individual proteins.

B. Positions and Honors

Positions and Employment

2006 (3 months) Undergraduate researcher with Prof. Markku Sopanen, Dept. of Electronics and Nanoengineering, Aalto University, Helsinki, Finland
2007 (4 months) Undergraduate researcher with Prof. Glenn Chapman, Engineering Science, Simon Fraser University, Burnaby, BC, Canada
2007-2008 Undergraduate researcher with Prof. Ash Parameswaran, Engineering Science, Simon Fraser University, Burnaby, BC, Canada
2010 (6 months) Undergraduate researcher with Prof. Bengt Nordén, Dept. Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, Sweden
2010 - 2015 Graduate Student with Prof. Cees Dekker, Kavli Institute of Nanoscience, Dept. of Bionanoscience, Delft University of Technology, Delft, the Netherlands
2015 (8 months) Postdoctoral Fellow with Prof. Cees Dekker, Kavli Institute of Nanoscience, Dept. of Bionanoscience, Delft University of Technology, Delft, the Netherlands
2015 - 2019 Postdoctoral Fellow with Prof. Sri Kosuri, Dept. of Chemistry and Biochemistry, University of California Los Angeles
2019 - present Assistant Professor, Knight Campus, University of Oregon, Eugene, OR
2019 - present Affiliated Professor, Dept. of Biomedical Engineering, Oregon Health & Science University

Other Experience and Professional Memberships

2018 - present Member, American Society for Microbiology
2018 - present Member, Protein Society

Honors

2002	Summit scholarship, Simon Fraser University
2007	Undergraduate Student Research Award, NSERC (Natural Sciences and Engineering Research Council of Canada)
2008	Erasmus Mundus Scholarship, European Commission
2009	Best Information Processing Project Award, iGEM
2014	Outstanding Poster Award at Significance of Knotted Structures conference, Biophysical Society
2015	PhD awarded with highest distinction, Cum Laude, TUDelft
2016	Rubicon Postdoctoral Fellowship, NWO
2016	Long-Term Fellowship, HFSP (Human Frontier Science Program)
2018	UCLA Chancellor's Award for Postdoctoral Research
2018	Career Awards at the Scientific Interface, BWF (Burroughs Wellcome Fund)

C. Contribution to Science

1. Developed DropSynth, a scalable, low-cost method to build thousands of defined gene-length constructs in a pooled (multiplexed) manner (a). DropSynth uses a library of barcoded beads that pull down the oligonucleotides necessary for a gene's assembly, which are then processed and assembled in water-in-oil emulsions. This was used to successfully assemble >7,000 synthetic genes that encode phylogenetically-diverse homologs of two essential genes in E. coli. As a proof of concept, the ability of homologs of an essential enzyme (PPAT) to complement a knockout E. coli strain was tested in multiplex, revealing core functional motifs and reasons underlying homolog incompatibility. (b) Further optimizations to the DropSynth protocol now allow single pot assemblies of up to 1536 genes with yield >20%. DropSynth coupled with multiplexed functional screens facilitate the exploration of sequence-function relationships at unprecedented scale.

    a. **Plesa C***, Sidore AM*, Lubock N, Zhang D, Kosuri S. Multiplexed Gene Synthesis in Emulsions for Exploring Protein Functional Landscapes. Science. 2018 Jan 19;359(6373):343-347. PubMed PMID: 29301959.
    b. Sidore AM*, **Plesa C***, Samson JA, Kosuri S. DropSynth 2.0: high-fidelity multiplexed gene synthesis in emulsions. BioRxiv 740977 [Preprint]. 2019 [cited 2019 Aug 21]. Available from: https://doi.org/10.1101/740977.

2. Polymer Physics. Solid-state nanopores are small nanometer-scale holes in thin membranes which can be used to probe biomolecules such as DNA and protein. My graduate studies focused on extending this technique to probe a wide range of different phenomena including knotting, polymer relaxation, and translocation. Long DNA molecules contain knots, but no techniques existed to observe these at such long lengths (>10 kbp), where they are most prevalent. I showed that nanopores could be used to observe knots present in DNA molecules as long as 166 kbp and provided the first ever data on knotting occurrence at long lengths (a). Another unresolved problem in this field is how to convert the recorded current signals from the time domain into useful positional information (spatial domain). This transform requires knowledge of how the local velocity changes as the molecule translocates through the pore. To address this, I used DNA origami molecules containing protrusions at well-defined positions to determine the local velocity, as well as intra- and inter-molecular velocity fluctuations present during the translocation process (b). In these types of measurements, each molecule passes through the pore only once and then diffuses away. I demonstrated that it is possible to recapture one individual DNA molecule through a nanopore over 1000 times by quickly switching the electric field, which allowed me to probe the relaxation time of the molecule (c).

    a. **Plesa C**, Verschueren D, Pud S, van der Torre J, Ruitenberg JW, Witteveen MJ, Jonsson MP, Grosberg AY, Rabin Y, Dekker C. Direct observation of DNA knots using a solid-state nanopore. Nat Nanotechnol. 2016 Dec;11(12):1093-1097. PubMed PMID: 27525473.
    b. **Plesa C**, van Loo N, Ketterer P, Dietz H, Dekker C. Velocity of DNA during translocation through a solid-state nanopore. Nano Lett. 2015 Jan 14;15(1):732-7. PubMed PMID: 25496458.

c. **Plesa C**, Cornelissen L, Tuijtel MW, Dekker C. Non-equilibrium folding of individual DNA molecules recaptured up to 1000 times in a solid state nanopore. Nanotechnology. 2013 Nov 29;24(47):475101. PubMed PMID: 24177388.

3. Biosensing. Solid-state nanopores are a promising technology for biosensing. I investigated novel ionic solutions for slowing down the translocation velocity (a) and demonstrated that it is possible to use nanopores to detect individual protein bound along a long DNA molecule using a new model system with anti-DNA antibodies (b). The high velocity of translocation is of particular concern when measuring proteins. By carrying out measurements on proteins of various size and charge I revealed the fundamental limits encountered in these types of measurements (d). I also developed a new analysis technique for nanopore data and released software now in use by many research groups (c).

   a. **Plesa C**, van Loo N, Dekker C. DNA nanopore translocation in glutamate solutions. Nanoscale. 2015 Aug 28;7(32):13605-9. PubMed PMID: 26206066.
   b. **Plesa C**, Ruitenberg JW, Witteveen MJ, Dekker C. Detection of Individual Proteins Bound along DNA Using Solid-State Nanopores. Nano Lett. 2015 May 13;15(5):3153-8. PubMed PMID: 25928590.
   c. **Plesa C**, Dekker C. Data analysis methods for solid-state nanopores. Nanotechnology. 2015 Feb 27;26(8):084003. PubMed PMID: 25648179.
   d. **Plesa C**, Kowalczyk SW, Zinsmeester R, Grosberg AY, Rabin Y, Dekker C. Fast translocation of proteins through solid state nanopores. Nano Lett. 2013 Feb 13;13(2):658-63. PubMed PMID: 23343345.

4. DNA nanostructures. DNA nanostructures assembled using DNA origami can be docked on top of a solid-state nanopore and allow for complete control over the aperture geometry and biochemical functionalization, which is important in numerous applications. I characterized a number of different DNA nanoplate designs using nanopores in order to determine their mechanical properties and ionic permeabilities (a). This revealed that DNA nanoplates are typically very permeable to ions and exhibit a number of unexpected mechanical properties including bending and buckling in the presence of an electric field. In my master's work, I carried out a stepwise assembly of a polycyclic DNA hexagon nanonetwork using tripodal building blocks (b).

   a. **Plesa C**, Ananth AN, Linko V, Gülcher C, Katan AJ, Dietz H, Dekker C. Ionic permeability and mechanical properties of DNA origami nanoplates on solid-state nanopores. ACS Nano. 2014 Jan 28;8(1):35-43. PubMed PMID: 24295288.
   b. Lundberg EP, **Plesa C**, Wilhelmsson LM, Lincoln P, Brown T, Nordén B. Nanofabrication yields. Hybridization and click-fixation of polycyclic DNA nanoassemblies. ACS Nano. 2011 Sep 27;5(9):7565-75. PubMed PMID: 21827213.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/calin.plesa.1/bibliography/53764478/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Career Awards at the Scientific Interface, Burroughs Wellcome Fund — Plesa (PI) — 07/01/18-07/01/23
Ubiquitous biosensing through engineered histidine kinases

Completed Research Support

Long-Term Fellowship, HFSP (Human Frontier Science Program) — Plesa (Postdoc/PI) — 09/01/16-08/01/19
Engineering novel signaling pathways in human cells

Rubicon postdoctoral fellowship, NWO — Plesa (Postdoc/PI) — 03/01/16-02/28/18
Engineering novel signaling pathways in human cells