Protein-Polymer Block Copolymer Thin Films for Detection of Small Proteins in Biological Matrices via Size-Exclusion

Justin M. Paloni, Eric A. Miller, Hadley D. Sikes, and Bradley D. Olsen
Massachusetts Institute of Technology, Department of Chemical Engineering

Introduction

Biosensor sensitivity is often vastly reduced for measurements in biological fluids due to nonspecific binding effects from off-target molecules. In this work, we demonstrate that protein-polymer block copolymer thin films can exclude many of these off-target molecules via an apparent size-exclusion mechanism, resulting in a two order of magnitude improvement in sensitivity.

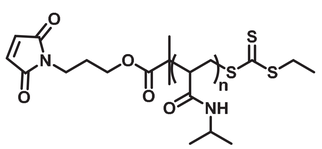
Bioconjugate Synthesis

A protein with high binding affinity for streptavidin is modified with an N-terminal cysteine group to introduce a free thiol onto the protein surface. This thiol group is then conjugated to a maleimide-functionalized poly(N-isopropylacrylamide) (PNIPAM) molecule via a thiol-Michael addition to form bioconjugates.

Bioconjugation Scheme

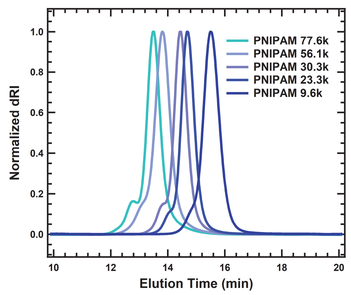
N-Cys 3x rcSso7d.SA Maleimide-PNIPAM 3xSP Conjugate

PNIPAM Structure & Protein Sequence



MGSIHHHHHHSSGLVPRGSHMCATVKFTY
QGEEKQVDISKIKIVARDGQYIDFKYDEGGG
AYGYGWVSEKDAPKELLQMLEKQGGGGSG
GGGSMATVKFTYQGEEKQVDISKIKIVARD
GQYIDFKYDEGGGAYGYGWVSEKDAPKELL
QMLEKQGGGGSGGGGSMATVKFTYQGEE
KQVDISKIKIVARDGQYIDFKYDEGGGAYGY
GWVSEKDAPKELLQMLEKQGG

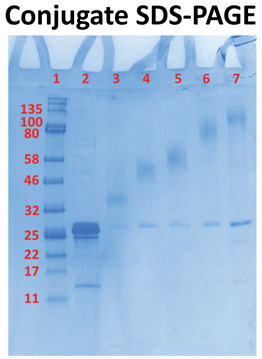
PNIPAM GPC Traces



Conjugate Composition

Conjugate	PNIPAM M_n (kDa)	PNIPAM Đ	Domain Spacing (nm)
3xSP9.6k	9.6	1.05	18.4
3xSP23.3k	23.3	1.06	26.9
3xSP30.3k	30.3	1.08	27.5
3xSP56.1k	56.1	1.11	35.9
3xSP77.6k	77.6	1.13	43.5

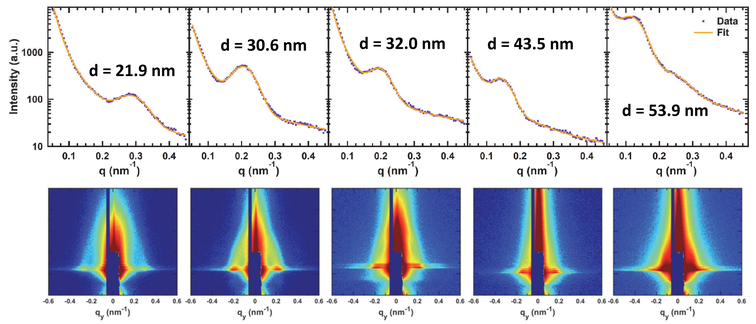
Conjugate SDS-PAGE



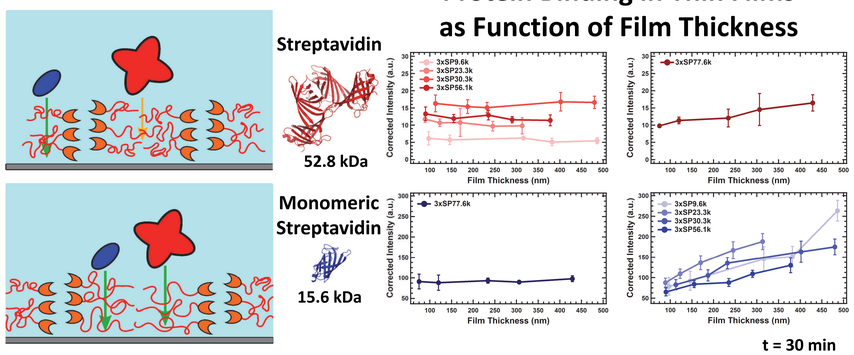
1: Ladder
2: 3x rcSso7d.SA
3: 3xSP9.6k
4: 3xSP23.3k
5: 3xSP30.3k
6: 3xSP56.1k
7: 3xSP77.6k

Protein Diffusion into Thin Films

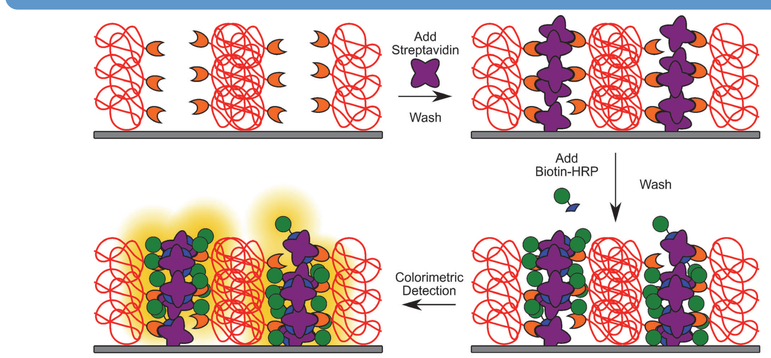
Wide Range of Domain Spacings in Thin Films

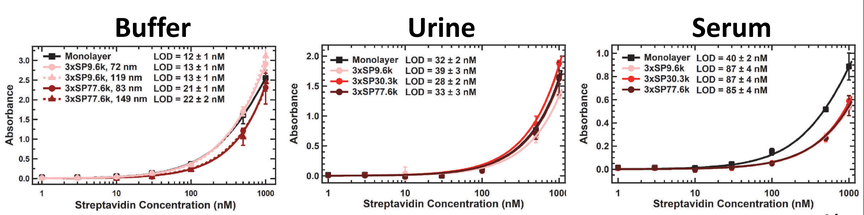


Protein Binding in Thin Films as Function of Film Thickness



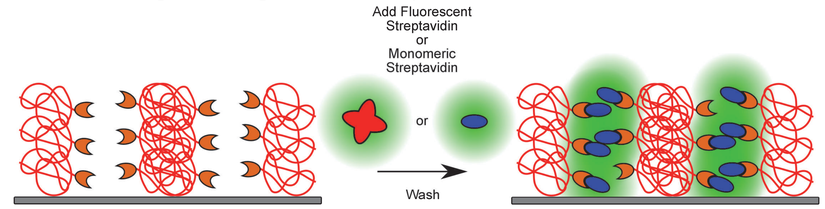
ELISA Assays Compatible with Thin Films

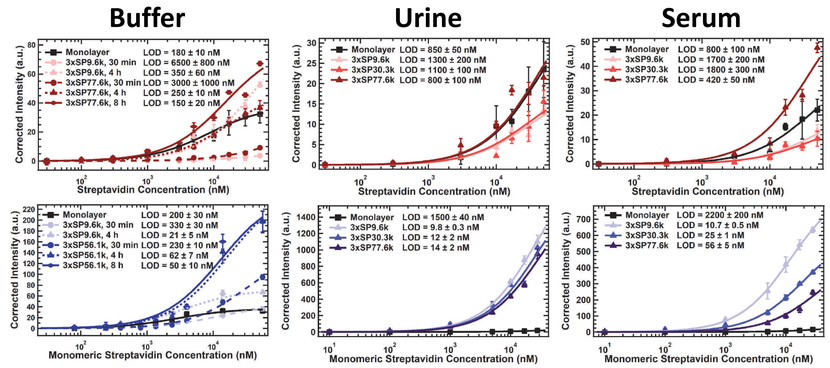




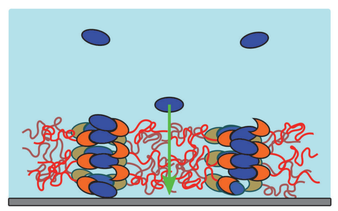
Biosensing in Buffer and Biological Fluids

Fluorescent assays performed in buffer indicate an order of magnitude enhancement in sensitivity compared to a monolayer sensor. This improvement increases to two orders of magnitude in biological fluids, presumably due to the exclusion of larger, off-target molecules by the films.

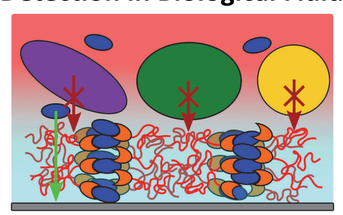




Detection in Buffer



Detection in Biological Fluid



Conclusions

The constructed protein-polymer conjugate thin film biosensors are demonstrated to significantly improve sensitivity in biological fluids when detecting small proteins. Comparisons between sensitivities for two proteins with different sizes but the same affinities for the binding protein in the films suggest that the polymer domains in these thin films are able to effectively screen molecules based on their size. Because this thin film architecture was found to permit detection by both fluorescence and ELISA-based techniques, these materials represent a platform technology with the potential to greatly improve small protein detection methods.

Acknowledgements

This work was supported by a grant from the National Science Foundation (DMR-1253306). We thank Dr. Zhang Jiang for GISAXS experimental assistance at APS beamline 8-ID-E at Argonne National Laboratory. We also thank Dr. Masafumi Fukuto and Dr. Ruipeng Li for SAXS experimental assistance at NSLS-II beamline 11-BM at Brookhaven National Laboratory. The Biophysical Instrumentation Facility for the Study of Complex Macromolecular Systems (NSF-0070319) is gratefully acknowledged for use of a CD spectrometer.