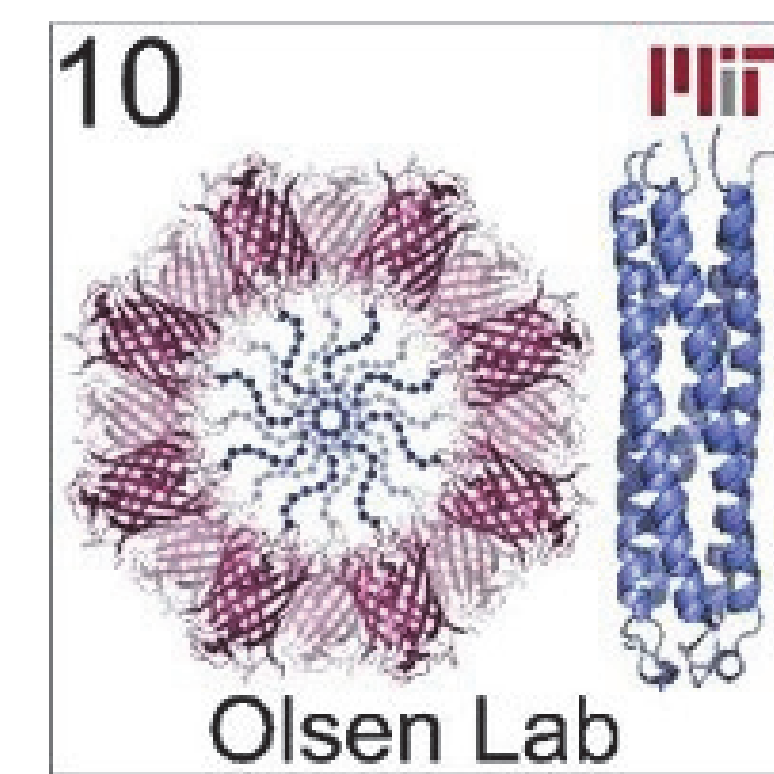




# Protein-Polymer Block Copolymer Thin Films for Detection of Small Proteins in Biological Matrices via Size-Exclusion

Justin M. Paloni, Eric A. Miller, Hadley D. Sikes, and Bradley D. Olsen

Massachusetts Institute of Technology, Department of Chemical Engineering



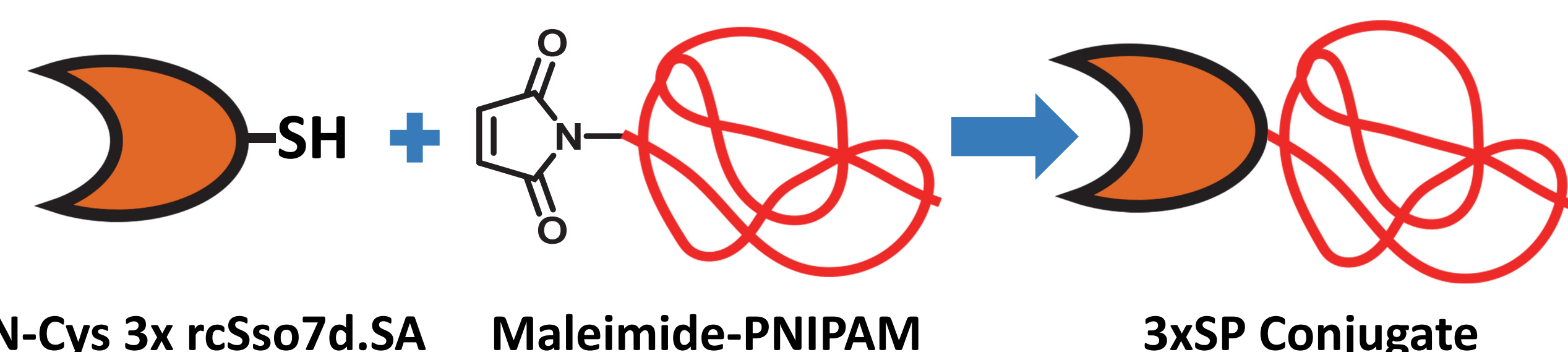
## Introduction

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.

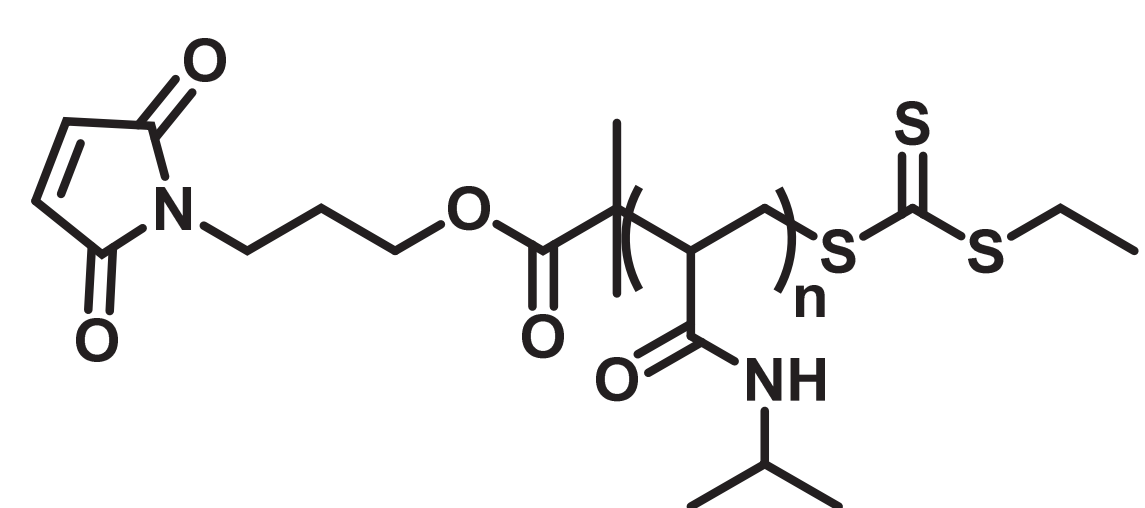
## Bioconjugate Synthesis

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



### PNIPAM Structure & Protein Sequence

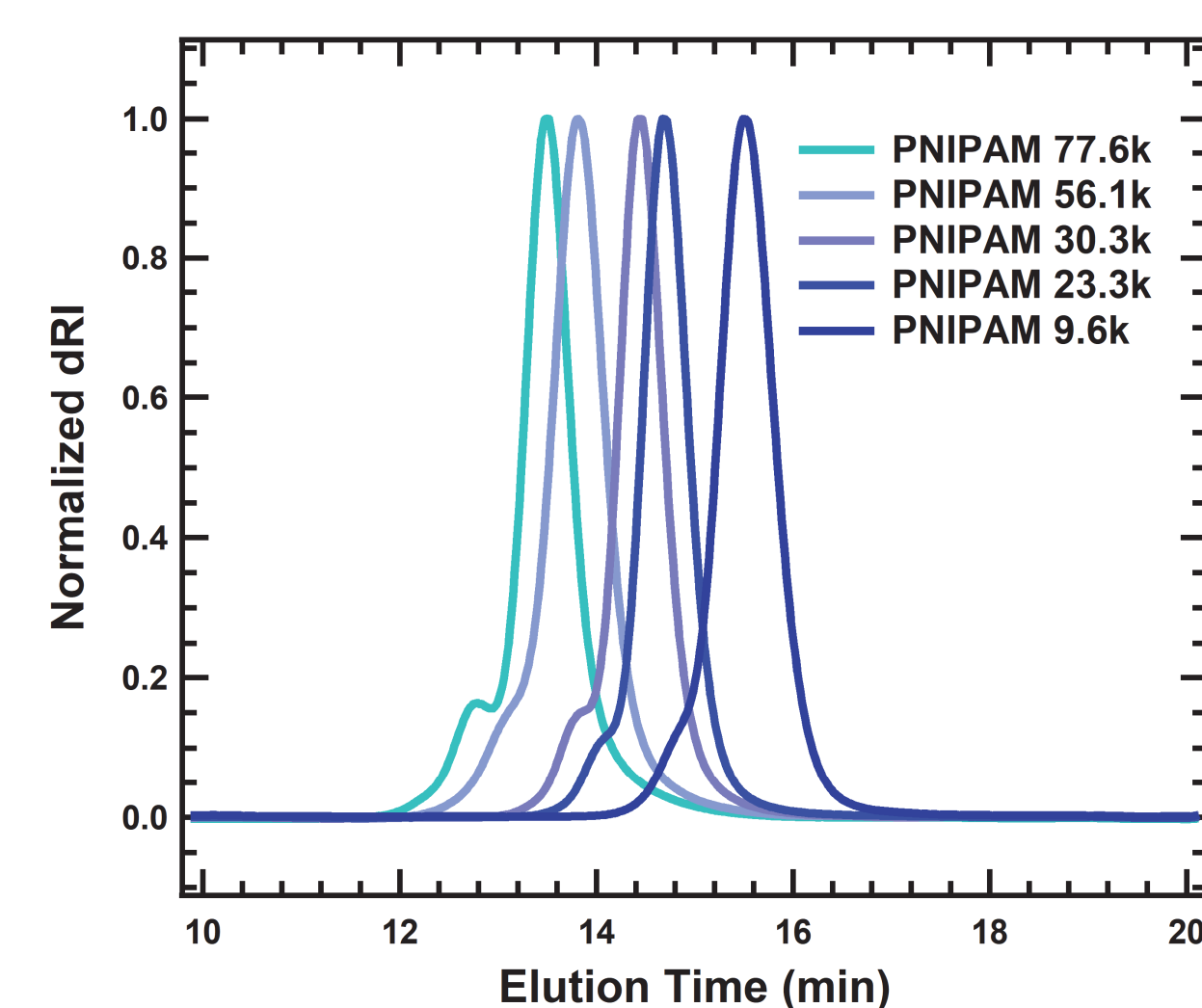


MGSIH...HHSSGLVPRGSHM...CATVKFTY  
 QGEEKQVDISKIKIVARDGQYIDFKYDEGGG  
 AYGWVWSEKDPKELLQMLEKQGGGGGG  
 GGGMATVKFTYQGEKQVDISKIKIVARD  
 GQYIDFKYDEGGGAYGYWVSEKDPKELL  
 QMLEKQGGGGGGGGMATVKFTYQGEE  
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 GWVSEKDPKELLQMLEKQGGG

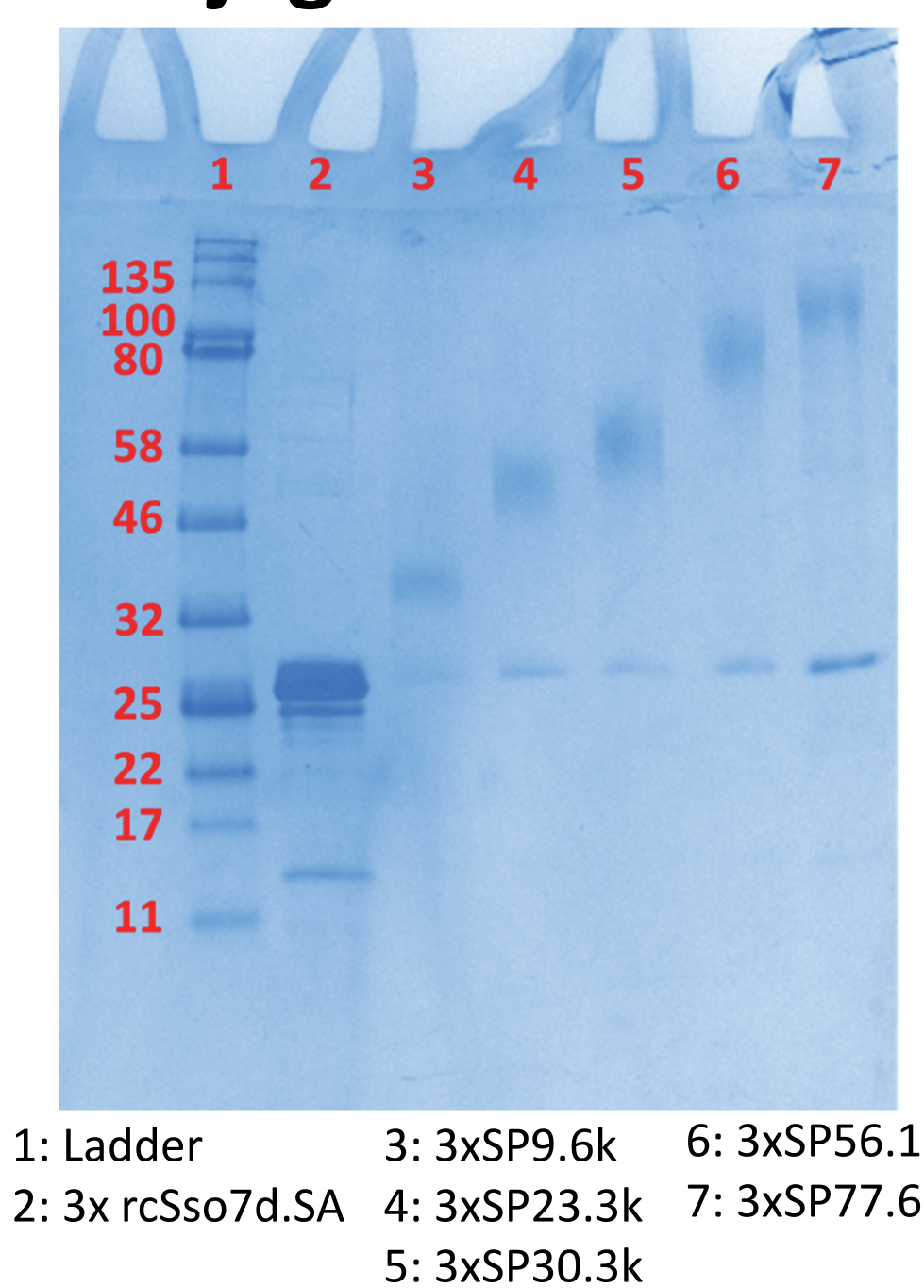
### Conjugate Composition

Conjugate	PNIPAM $M_n$ (kDa)	PNIPAM $\bar{D}$	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

### PNIPAM GPC Traces

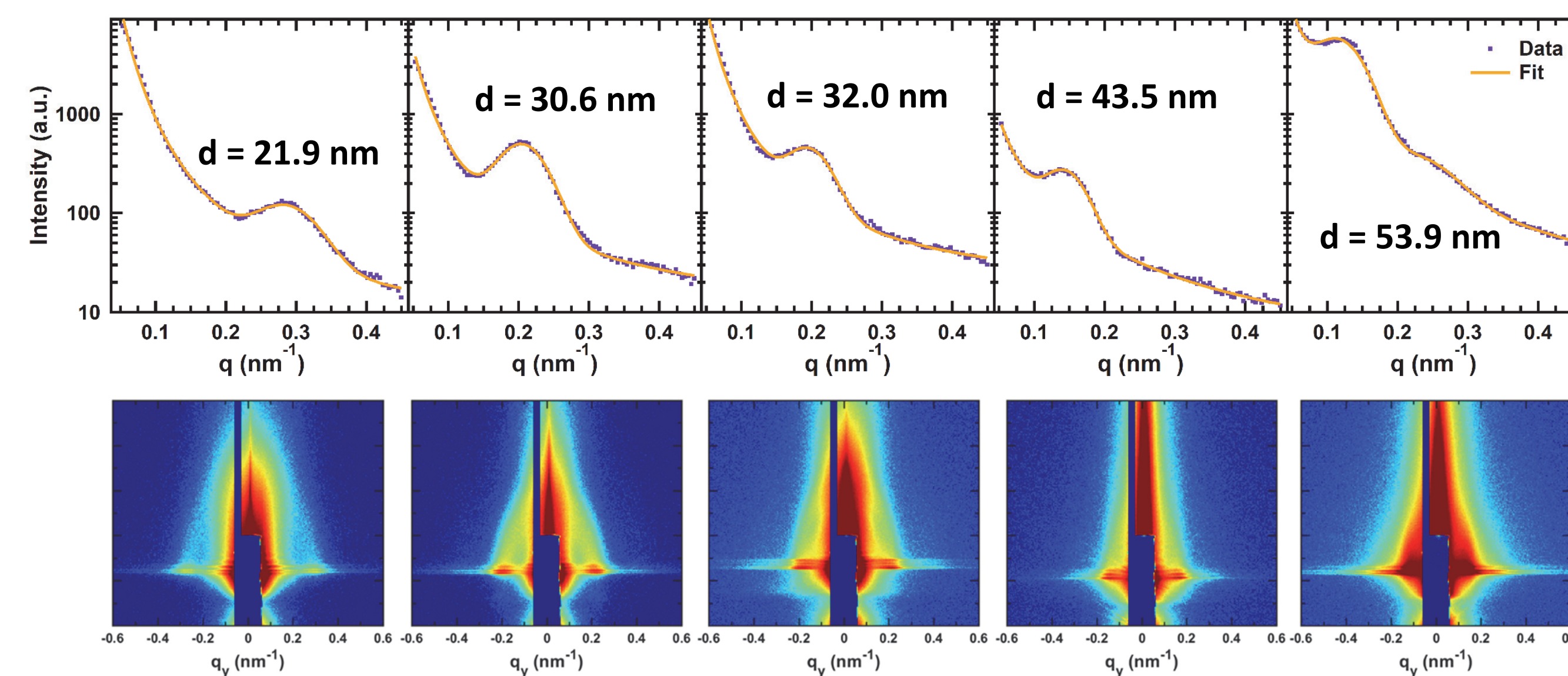


### Conjugate SDS-PAGE

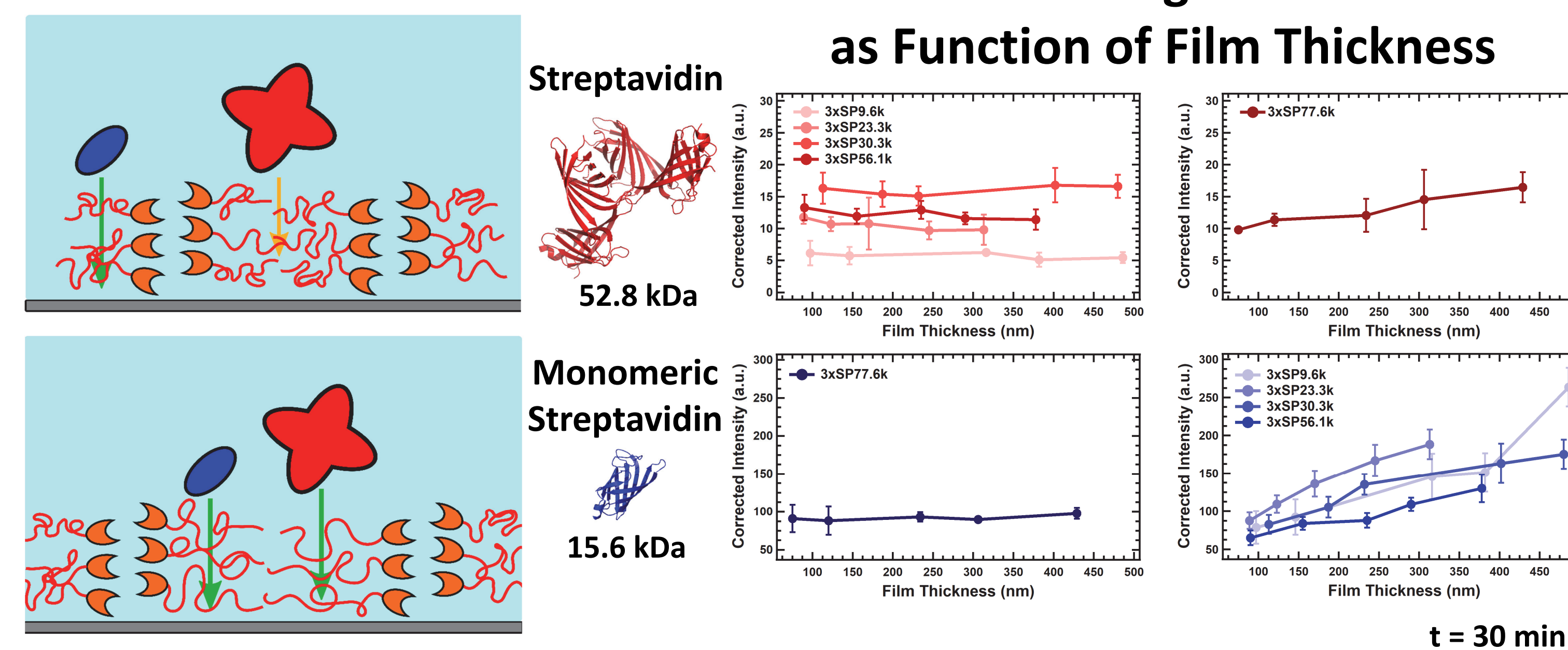


## Protein Diffusion into Thin Films

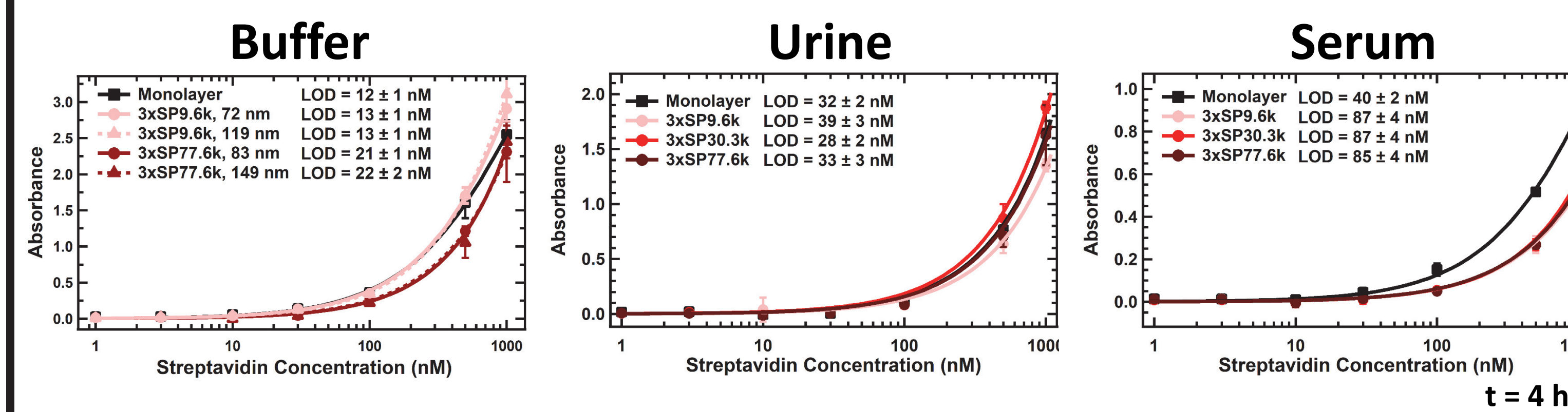
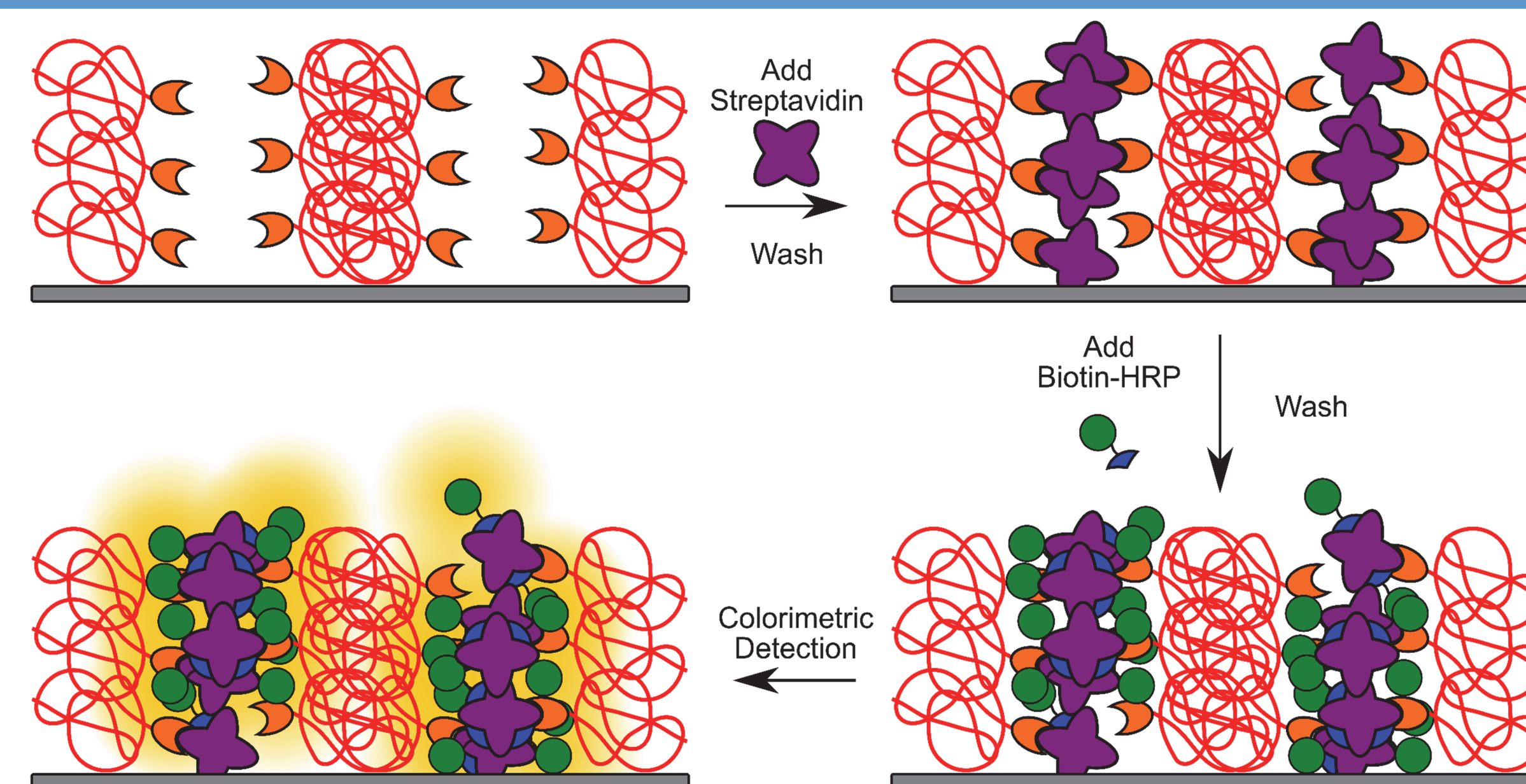
### Wide Range of Domain Spacings in Thin Films



### Protein Binding in Thin Films as Function of Film Thickness

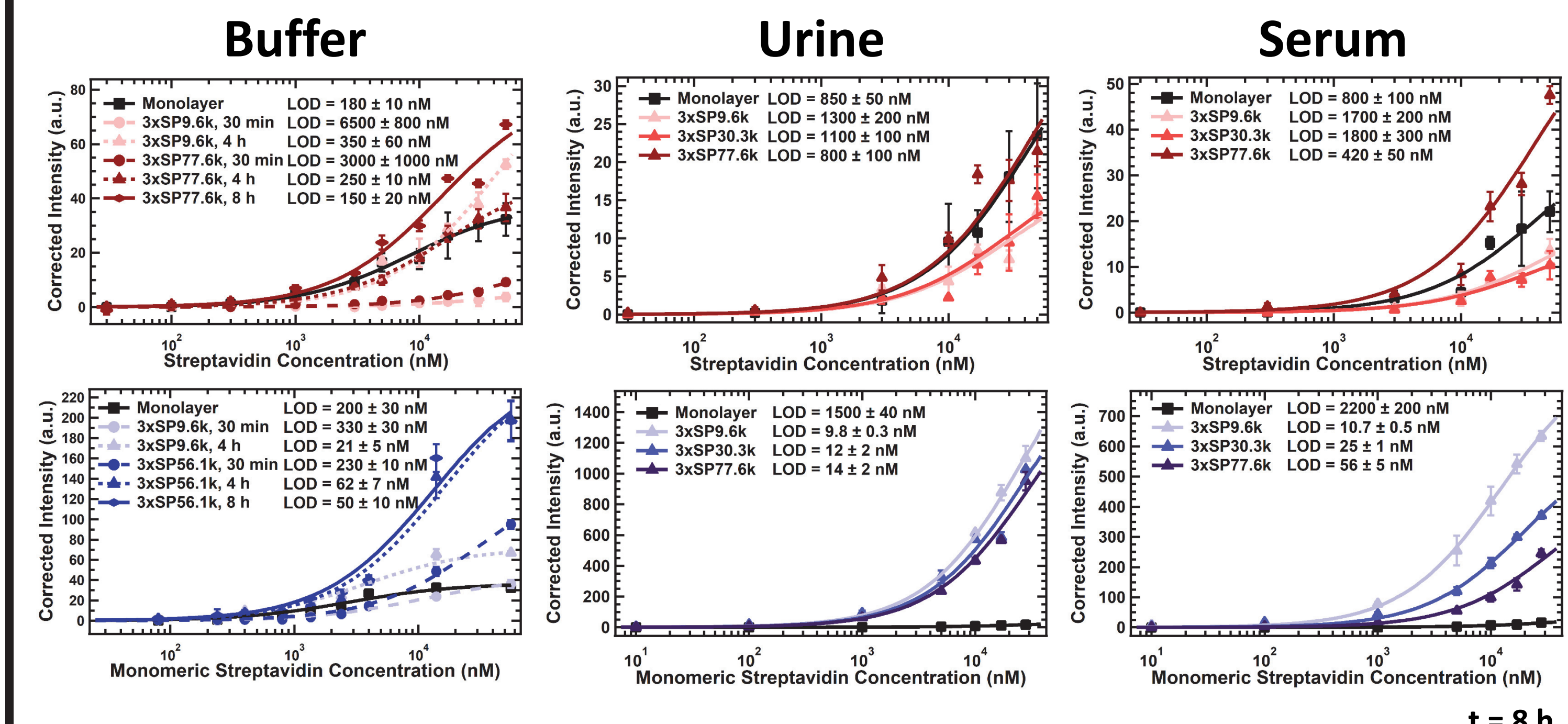
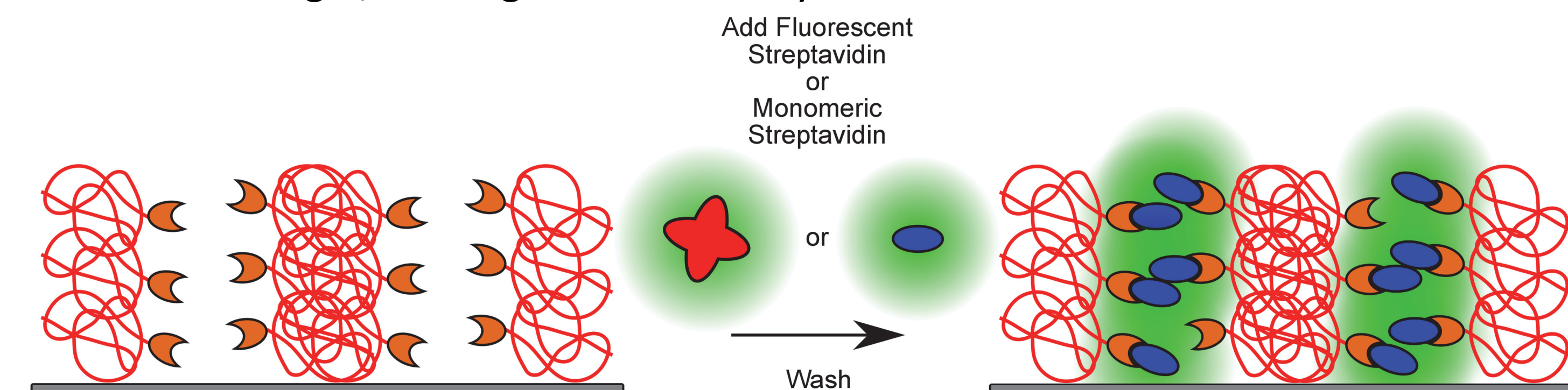


## ELISA Assays Compatible with Thin Films

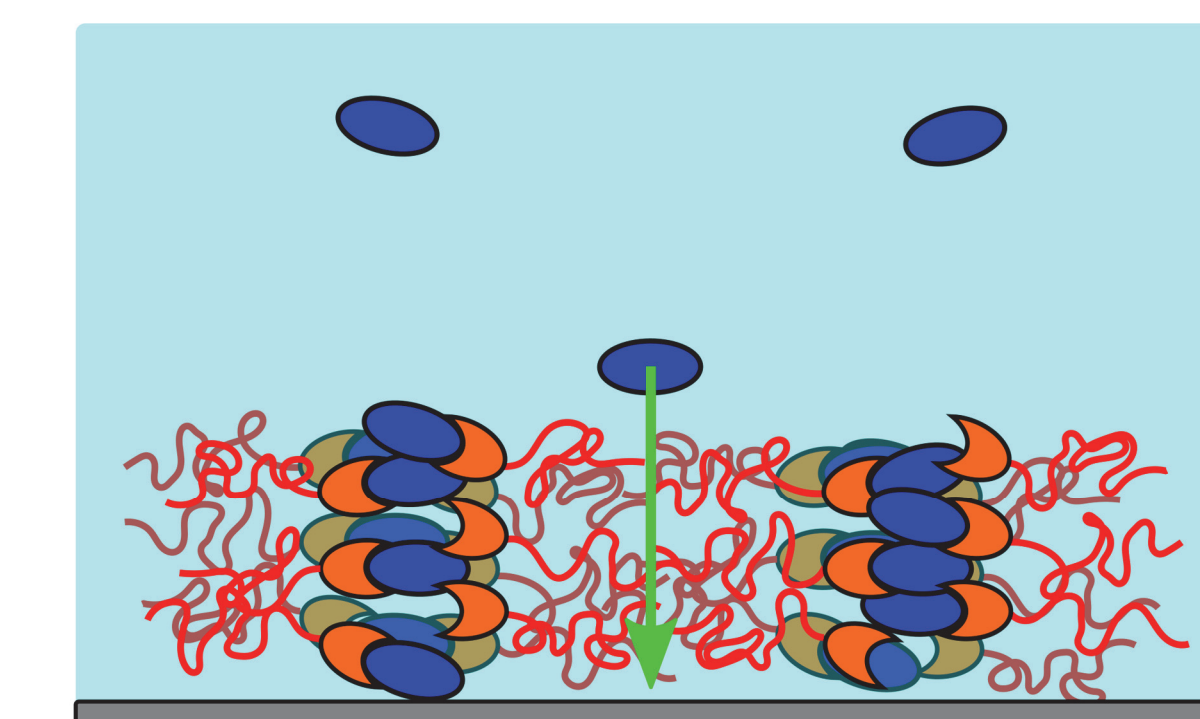


## Biosensing in Buffer and Biological Fluids

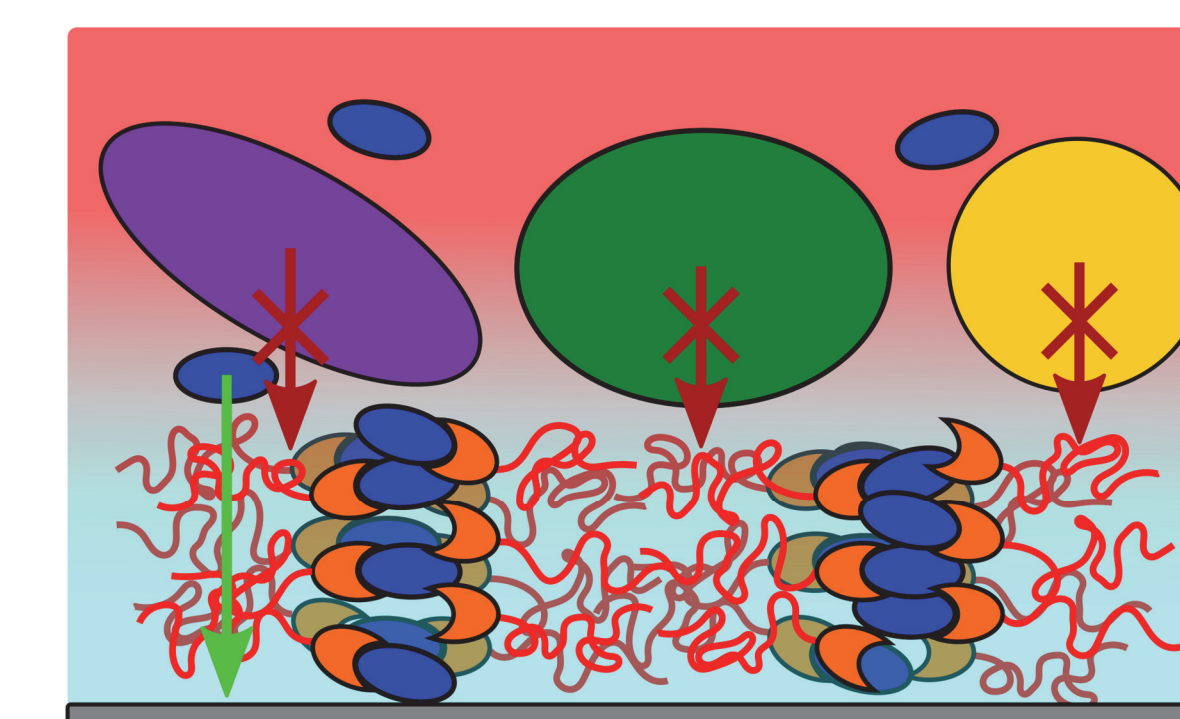
Fluorescent assays performed in buffer indicate an order of magnitude enhancement in sensitivity compared to a monolayer sensor. This improvement increases to two orders of magnitude in biological fluids, presumably due to the exclusion of larger, off-target molecules by the films.



### 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

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