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Supplementary Materials

Surface Modification of Polypyrrole via Affinity Peptide: Quantification and Mechanism

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Figure S1

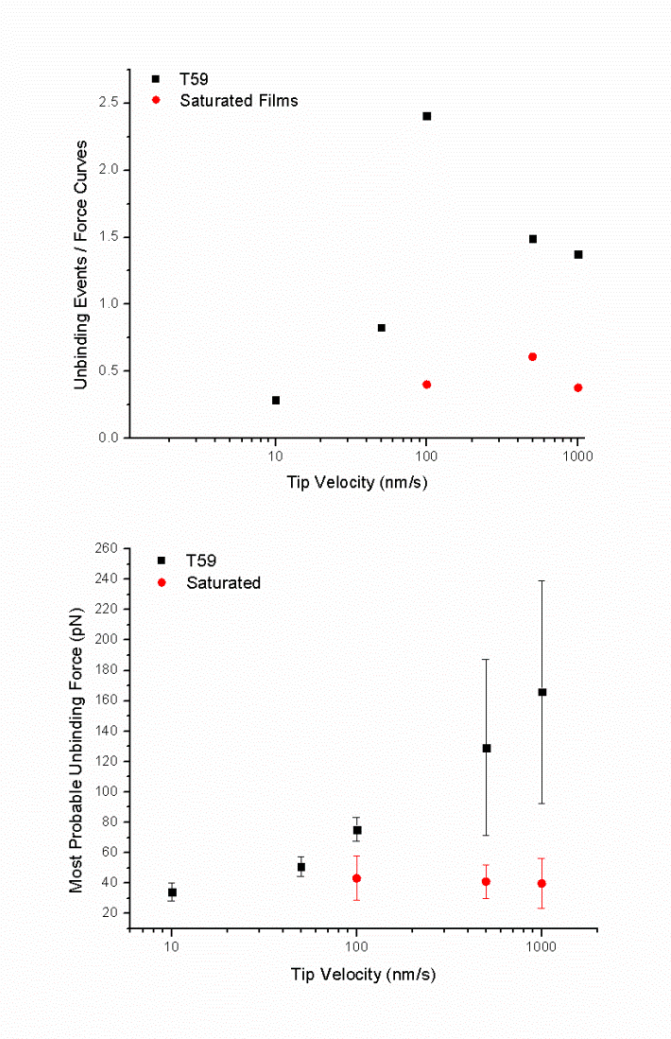

Figure S1. AFM force spectroscopy study of T59 functionalized tips with PPyCl slides. The red circles denote the results with the addition of 1 µM T59 to solution prior to measuring, the black squares indicate no free T59 in solution. The top chart shows the ratio of unbinding events to force curves, showing that fewer if any binding events are observed when free T59 is added to solution. The bottom chart shows the most probable unbinding force based on a Gaussian fit. The films which were treated with free T59 show weaker and non-specific, interactions with the functionalized tip.

Experimental: The protocol for this experiment was identical to the one for the AFM studies described in the main text, with the exception that the PPy film was incubated in 10 µM T59 in an effort to saturate the PPyCl surface. The film was incubated in 10 µM T59 for two hours immediately prior to the analysis being performed. The 10 µM T59 solution was also used as the buffer during the AFM pulling experiment. Analysis was also performed in an identical fashion to the earlier study, however this was limited to the three largest loading rates.

Figure S2

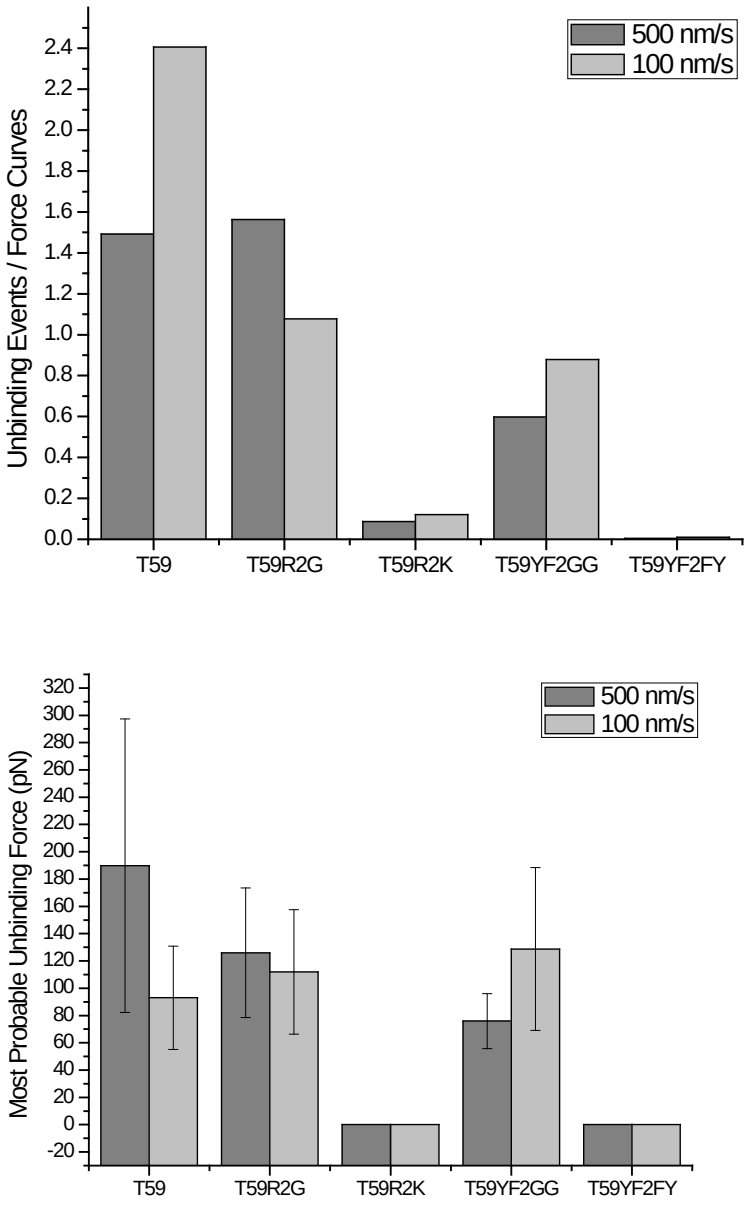

Figure S2. Results of the AFM force study with T59 variants and PPyCl films. The top chart shows the ratio of unbinding events and force curves. The second chart shows the most probable unbinding force based on a Gaussian fit. The R3 residue has been removed and replaced with either a glycine (T59R2G)

or lysine (T59R2K). This study shows that removal of the R3 residue disrupts, but does not eliminate binding, whereas replacement with lysine totally disrupts binding. Additionally we looked at the role of residues Y9 and F10. We removed these residues and replaced them with glycine, T59YF2GG and reversed their order, T59YF2FY. We can see that the replacement of these residues with glycine changes, but does not completely disrupt binding; however, the inversion of the order does disrupt binding, indicating some, as of yet undetermined, role of these resides.

Experimental: Silicon nitride tips functionalized with maleimide via a poly(ethylene glycol) spacer and alkane thiol were purchased from NovaScan with a nominal spring constant of 60 pn/nm. Two T59 peptide variants were purchased which had Gly-Gly-Ser-Ser-Cys sequence added at the C-terminus (New England Peptide) as a linker. The four peptide variants were: THGTSTLDYFVIGGSSC, which is referred to as T59R2G, THKTSTLDYFVIGGSSC, which is referred to as T59R2K, THRTSTLDGGVIGGSSC, which which is referred to as T59YF2GG, and THRTSTLDFYVIGGSSC, which is referred to as T59YF2FY. Tip functionalization and force curve measurement were performed as described in the main text.

Figure S3

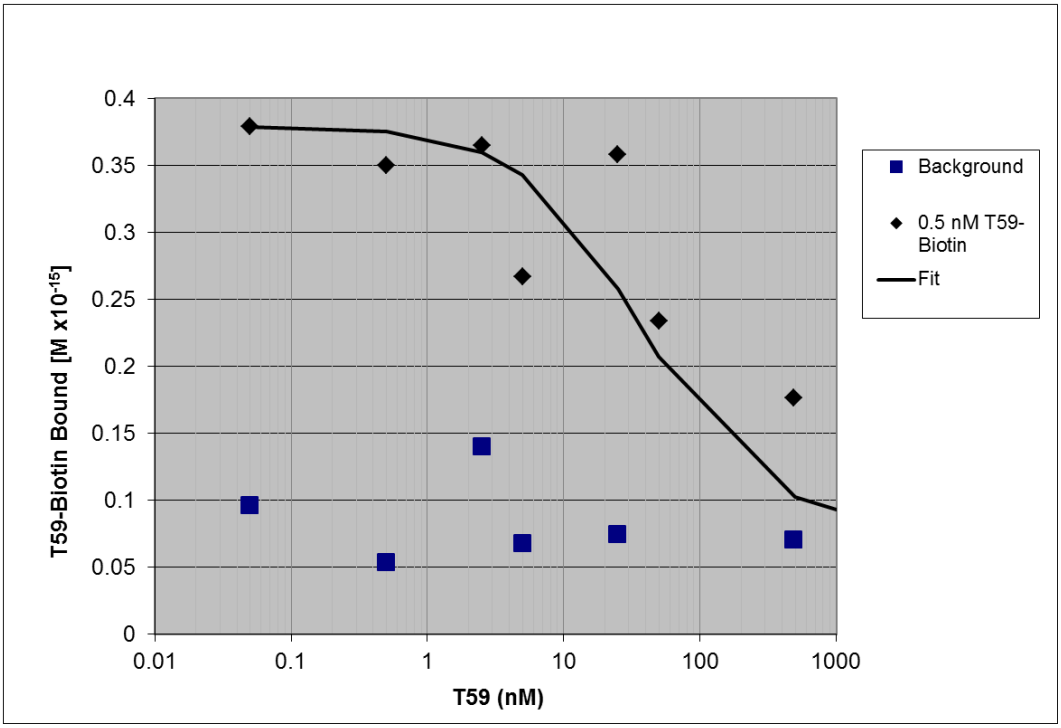

Figure S3: Results of the ELISA based homologous competition assay. The plot shows the amount of T59-Biotin (T59-GGGSK-biotin) bound to polypyrrole as a fraction of saturation level (black diamonds). Free T59 was added to compete with the biotinylated version of T59, NeutrAvidin-HRP was used to detect surface bound T59-Biotin. There is a non-specific background evident when no T59-B was added at about 10% of saturation (blue squares). This was used as the baseline when fitting the decay bound T59-B with increased free T59. We can see that the IC50 is in the range of 50 nM, which is in agreement with the equilibrium binding assay finding of KD = 92.6 nM.

Experimental: A homologous competition was performed in parallel to the equilibrium binding assay described in the main text. PPy films were prepared as described in the text and treated with a 5 nM solution of T59-B in 3% BSA to which was added increasing amounts of free T59 which were allowed to

incubate for 1 hour. These were then washed and the assay performed as described in the text for the equilibrium binding assay.

Figure S4

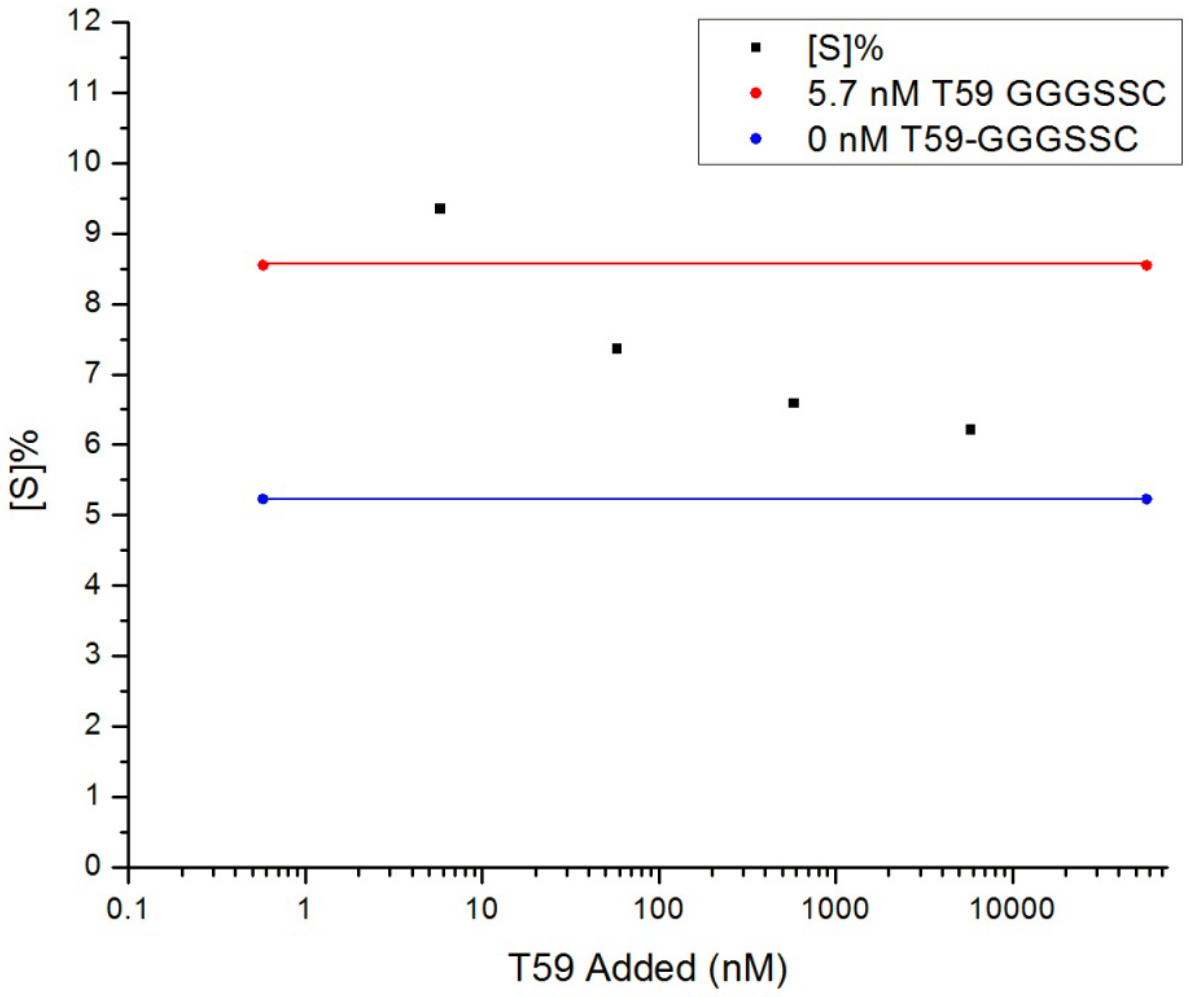

Figure S4: Homologous competition assay performed with the T59-GGGSSC as the labeled molecule. XPS was used to follow the sulphur elemental content (the sulfur in the additional cysteine) in an effort to quantify the decrease in T59-GGGSSC upon the addition of T59. The red line indicates the level of sulphur found when only labeled T59 is added, and the blue line is the background, a result of a film incubated in PBS (in blue). Taken with the result of figure S3 and the equilibrium binding assay in the main text, the data support an IC_{50} on the order of 100 nM.

Experimental: A homologous competition binding curve was generated using the T59 peptide (New England Peptide) and a cysteine labeled version of the T59 peptide, T59-Gly-Gly-Gly-Ser-Ser-Cys (T59-GGGSSC), also aquired from New England Peptide. The wells were incubated with 100 µl of 5.7 nM T59-GGGSSC in DI water for one hour. The solution was then aspirated from the wells and each well, rinsed three times with DI water. Each well was then incubated for 60 minutes with 100 µl of increasing concentrations of T59 in DI water. Wells were then rinsed three times with 150 µL of DI water. Wells were then dried in a vacuum over night, films removed from the ITO substrate and mounted on carbon tape for XPS analysis.

XPS analysis was performed at the Center for Nano-Molecular Sciences at the University of Texas at Austin on the Xylos XPS instrument with the assistance of Dr. Hugo Celio, and previous training by Dr. Yang Min Sun. Samples were analzyed for carbon, nitrogen, chloride, and sulphur.