

Adsorption-Induced Changes in Ribonuclease A Structure and Bioactivity on Solid Surfaces

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The supporting information contains (i) experimental methodology and (ii) results, for the different techniques used in current study to determine the surface coverage, adsorbed configuration, and bioactivity of adsorbed protein. The experimental methodology contains description on the adsorption procedure, XPS technique, ellipsometry technique, CD spectroscopy, and AAL/MS technique. The result section contains description on the (a) surface coverage and (b) helix content of the protein when adsorbed from different solution concentrations and surface chemistries, (c) the effect of labeling on the structure of protein in solution and adsorbed state, (d) surface coverage following trypsin treatment, and (e) raw data on the extent of modification in solution, and profile of the target residues in adsorbed RNase A.

S.1 Experimental Methodology

S.1.a Adsorption Procedure. For conformational analysis of the adsorbed protein structure using CD spectroscopy, transparent substrates such as glass are required. Therefore, HDPE and PMMA surfaces were prepared on these substrates in order to be used with a CD spectropolarimeter. Prior to protein adsorption, the substrates were equilibrated in the buffer solution for half an hour, using the adsorption scheme shown in Figure S.1. Depending on the

type of adsorbent surface, different adsorption schemes were required in order to ensure that protein adsorbed only to the substrate of interest. While the adsorption scheme in Figure S.1.a was used with the glass substrates, the adsorption scheme in Figure S.1.b was used with the polymeric substrates (HDPE and PMMA), as the polymer samples were coated on only one side of the glass substrates. Similar adsorption schemes were also used for preparing the samples for mass spectrometry. Both sides of the glass substrates were adsorbed with protein, while only one side of the polymer samples was exposed to protein solution.

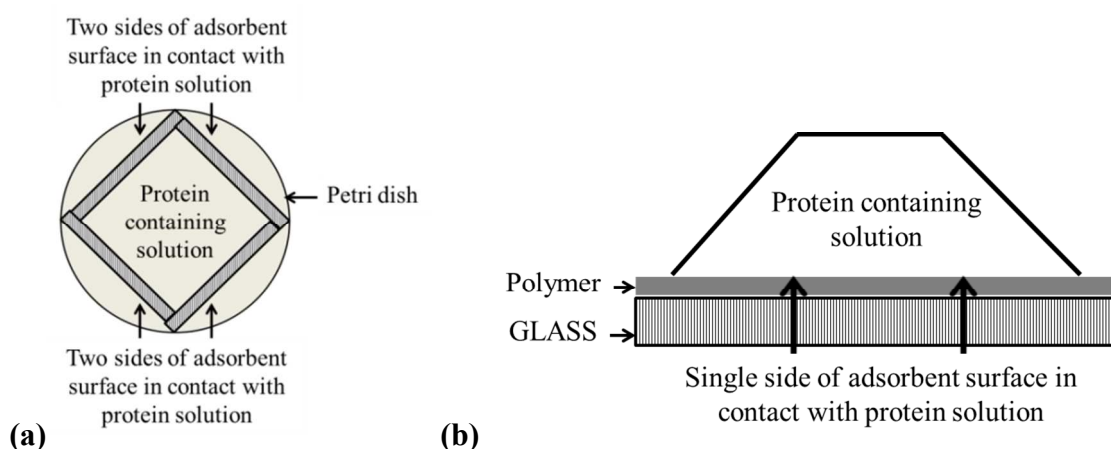


Figure S.1. The adsorption scheme used for the current study is shown (a) Protein adsorbed on both sides of the adsorbent surface and (b) Protein adsorbed on a single side of the adsorbent surface.

S.1.b Methodology to Acquire the XPS spectra. XPS spectra were taken on a Surface Science Instruments S-probe spectrometer. This instrument has a monochromatized Al K α X-ray and a low energy electron flood gun for charge neutralization of non-conducting samples. The samples were fastened to the sample holder with double sided tape and run as insulators. X-ray spot size for these acquisitions was approximately 800 μm . Pressure in the analytical chamber during spectral acquisition was less than 5×10^{-9} Torr. Pass energy for survey and detail scans was 150eV. The take-off angle (the angle between the sample normal and the input axis of the energy

analyzer) was $\sim 55^\circ$ (55° take-off angle $\approx 50 \text{ \AA}$ sampling depth). Service Physics Hawk Data Analysis 7 Software was used calculate surface atomic concentrations using peak areas above a linear background and elemental sensitivity factors. The binding energy scales of the high-resolution spectra were calibrated by assigning the most intense C1s high-resolution peak a binding energy of 285 eV.

S.1.c Methodology to determine the Thickness of the Protein Film by Ellipsometry.

Ellipsometry measurements were performed using a Sopra GES5 variable angle spectroscopic ellipsometer (Sopra Inc., Palo Alto, CA) and the accompanying GESPack software package. Briefly, the total of six spectra for at least two test points on each sample in deionized water were scanned from 250 nm to 850 nm at 10 nm intervals using an incident angle of 75° and the thicknesses of each layer on surface were fitted and calculated using the regression method in Sopra's Winelli (ver. 4.08) software. The areal densities of RNase A on each adsorbent surface were determined using the de Feijter's equation in eq s.1.¹

$$\text{Surface coverage } (\mu\text{g}/\text{cm}^2) = 0.1 * d_f * (n_f - n_b) / (dn/dc) \quad (\text{s.1})$$

In equation (s.1), d_f describes the film thickness (in nm), n_f describes the refractive index of the adsorbed protein film, n_b describes the refractive index of the buffer, and dn/dc refers to the increment of refractive index of protein solution versus protein solution concentration and is considered to be constant for any protein. The following parametric values, $n_f=1.42$, $n_b = 1.33$ and $dn/dc = 0.188 \text{ ml/g}$ were used, to measure the thickness of the protein layers.

S.1.d Methodology to Determine the Secondary Structure of the Proteins in Solution and Adsorbed State using Circular Dichroism Spectropolarimetry (CD).

The CD spectra (consisting of the ellipticity and absorbance values over wavelengths ranging from 190 to 240 nm) for the substrate containing protein were recorded before and after protein adsorption were

obtained at room temperature using a Jasco J-810 spectropolarimeter (Jasco, Inc., Easton, MD).²
³ Throughout the study, slides remained hydrated in buffer solution. The CD spectrum for protein in solution was measured in 0.10 mm path length demountable quartz cuvette (Starna) at 1.00 mg/ml solution concentration using parameters and techniques previously described. Briefly, the background-corrected solution CD spectra were recorded from 190 nm to 300 nm at a scan rate of 50 nm/min with a response time of 0.25 s using six accumulations. In case of adsorbed proteins, the CD spectra were recorded from 190 nm to 300 nm at a scan rate of 10 nm/min with a response time of 2s, and a bandwidth of 0.5 nm. The spectra was averaged from six such accumulations.² However, in order to accurately determine the structure of the adsorbed protein, the amount of the protein on the substrate must be known, it is essential to determine the molar extinction coefficient of protein.

Once the CD signals (θ_{mol}) were converted to their respective molar ellipticity units (equation s.2), the spectra were then deconvoluted to predict secondary structure using the CONTIN/LL, SELCON3, and CDSStr methods provided with the CDPro package using the SP43 and SP48 protein reference datasets. Each of the deconvoluted spectra was then assessed for quality by analyzing the R-fit using non-linear regression. The final secondary structures represent the averaged structures obtained from all of the reliable outputs (R-fit < 10) resulting from the above described data analysis methods, which are consistent with the data analysis recommendations for CD.

$$\theta_{\text{mol}} = \frac{\theta_{\text{raw}} * M}{10000 * Q_{\text{ads}}} \quad (\text{s.2})$$

where, θ_{raw} is the background corrected raw CD signal, ' L ' is the path length of the cuvette (cm), and ' M ' is the mean residue molecular weight (equation s.3).

$$M = \frac{\text{Theoretical molecular weight of the protein}}{\text{Total number of amino acids in the protein}} \quad (\text{s.3})$$

All CD experiments were done under a steady stream of nitrogen flow (~ 10 liters/minute) and the HT voltage was kept below 600 V, in order to maximize the signal to noise ratio.

S.1.e. Methodology to Determine the Solvent Exposure of Residues within the Protein using the Amino-Acid Labelling and Mass Spectrometric (AAL/MS) Technique. AAL/MS technique was used to determine the final configuration of the protein to gain insights into the protein's adsorbed orientation, areas that are involved in protein-protein interactions, and areas within the protein that underwent tertiary unfolding.

S.1.e.1 Target Residues within RNase A for Labeling. A total of 34 residues were targeted in the current study by side-chain modification of the Arg, Lys, Tyr, His, Asp, and Glu amino acid groups within RNase A (Figure S.2.)

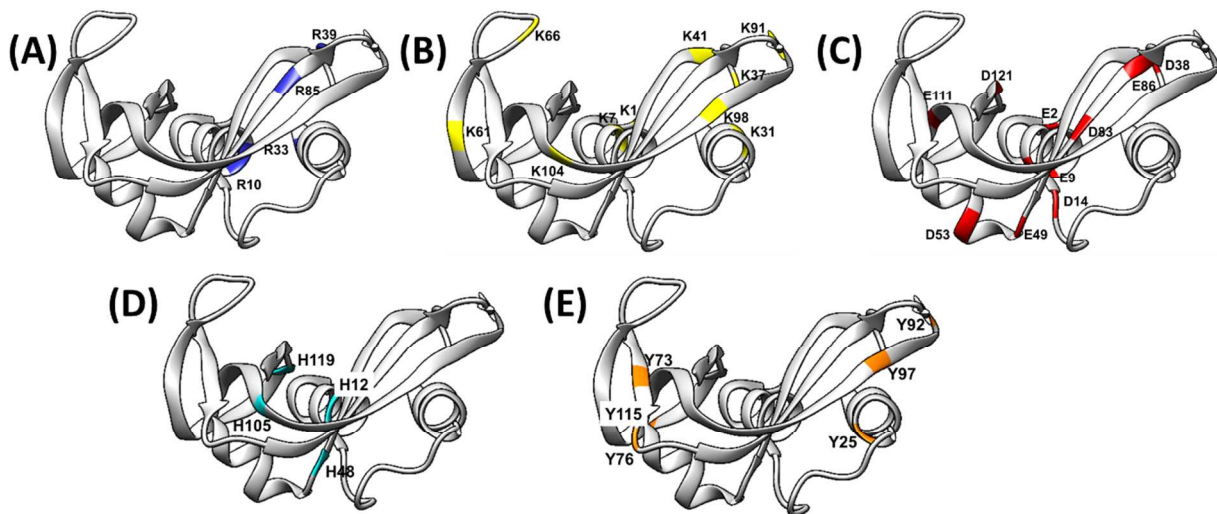


Figure S.2. The residue distribution within RNase A for (A) arginine residues (R), (B) lysine residues (K), (C) aspartic and glutamic acid residues (D, E), (D) histidine residues (H), and (E) tyrosine residues (Y). Each of the targeted residues are represented by the single letter amino acid code and its primary sequence position.

S.1.e.2 Labeling Agents for Modification and Target Amino Acid Distribution in RNase A.

The chemical modification of RNase A (5 mg/mL) was done to identify the extent of solvent exposure for Arg, Lys, Tyr, His, Asp, and Glu residues in RNase A's native state. All the target residues were labeled under a common reaction condition to facilitate direct comparison of the labeling profiles. In the current study, irrespective of the type of modification process, reaction between the labeling agent and its target amino acid were carried out at 5x the overall molar concentration of reacting amino acids in the dark at 25°C for 3 hours in PPB. The solution pH was maintained at 7.4 by adding required amounts of monobasic potassium phosphate (Sigma, P8708) or dibasic potassium phosphate (Sigma, P8508). Following the modification, solution samples were dialyzed against PPB (10 kDa cut-off) for 6 hours to remove the unbound labeling agents. Unmodified RNase A samples (5 mg/mL) under identical conditions were used as the negative control. Identical labeling conditions that were used to label the native state of the protein, was also used in its adsorbed state.

S.1.e.2.a. Arg modification: Arg accounts for 4 of the 124 residues in the native structure of RNase A (Figure S.2.A). Arg modification was carried out in a two-stage reaction process in which the primary reacting agent, 2, 3-butanedione (Sigma, B85307) reacts with the side-chain of solvent-accessible Arg residues, after which the secondary reacting agent, 3-acetamidophenylboronic acid (Sigma, 566012) was added in 1:2 molar ratio to form an aryl complex with an expected mass increase of 172.069 Da per modified Arg residue.⁴

S.1.e.2.b. Lys modification: Lys accounts for 10 of the 124 residues within the native RNase A (Figure S.2.B). The acylation of Lys in proteins using acetic anhydride (Sigma, 320102) is a single-stage reaction process with the resulting product showing an expected mass increase of 43.018 Da per modified Lys residue.⁵

S.1.e.2.c. Asp and Glu modification: Asp and Glu account for 10 of the 124 residues in the native structure of RNase A (Figure S.2.C). The COO⁻ modification of RNase A was carried out in a two-stage reaction process in which the primary reacting agent, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (Sigma, E6383) reacts with solvent accessible COO⁻ functional groups of Asp and Glu following which the secondary reacting agent, N-hydroxysuccinimide (Sigma, 130672) was added in 1:4 molar ratio to form an amide cross-link with an expected mass increase of 97.016 Da per modified Asp and Glu residue.⁶

S.1.e.2.d. His modification: His accounts for 4 of the 124 residues in the native structure of RNase A (Figure S.2.D). The His modification of RNase A was carried out using Diethyl pyrocarbonate in a single stage reaction in which the primary reacting agent was added in 1:2 molar ratio to form a product with a mass increase of 146.14 Da.⁷

S.1.e.2.e. Tyr modification: Tyr accounts for 6 of the 124 residues in the native structure of RNase A (Figure S.2.E). The reaction of RNase A with tetranitromethane was carried out in a single stage process by reacting in 1:4 ratio. The nitration process results in product with a mass increase of 44.99 Da.⁷

S.1.f. Tryptic Digestion and Desorption of the Labeled Protein. As a pre-requisite to analyze the mass shift in target residues of a protein by mass-spectrometry, proteolytic digestion of the modified and unmodified protein in solution and in its adsorbed state are necessary. Proteolytic digestion of RNase A was done using sequence-grade porcine trypsin (Promega) which was diluted in 10 mM hydrochloric acid.

S.1.f.1 Tryptic Digestion of Labeled and Unlabeled RNase A: Protease digestion of RNase A in solution was performed according to previously reported methods.⁷ Briefly, 4.0 μL of each protein solution was added to 100 μL of 1.0 mM ammonium bicarbonate. To reduce the disulfide

bonds in RNase A, 3.0 μL of 45 mM dithiothreitol (DTT) was added, and the sample was incubated at 37°C for 20 min. After the samples were cooled to room temperature, the reduced cysteines were alkylated by adding 4.0 μL of 100 mM iodoacetamide (IAA). The reaction was allowed to proceed in the dark for 20 min. The excess reagents were removed by lyophilizing to completion in SpeedVac (Savant Instruments Inc.) for 1h. RNase A samples were then digested by trypsin, at a protease-to-substrate ratio of 1:50 (w/w) using 0.04 $\mu\text{g}/\mu\text{L}$ protease solutions in their respective buffers at 37°C for 18 h. Following incubation, 1.5 μL of 0.1% (v/v) trifluoroacetic acid was added to stop the digestion. The solutions containing the peptide fragments were collected after digestion, lyophilized, and processed for MS.

S.1.f.2 Tryptic Digestion and Desorption of Labeled RNase A on Different Material

Surfaces. Following the labeling process, excess reagents were removed by rinsing the surfaces with pure buffer after which the adsorbent surfaces were dried under a steady stream of nitrogen.

The surfaces with the adsorbed RNase A were initially placed in a digestion box filled with solution 1 (0.2 M NH_4HCO_3 in 50% acetonitrile (v/v), pH 7.8) following which the adsorbed protein layers were reduced (45 mM DTT) and alkylated (100 mM IAA) prior to being tryptic digested (0.04 $\mu\text{g}/\text{mL}$) overnight in a temperature-controlled chamber. Digested protein samples were recovered and excess reagents were removed by lyophilizing overnight. Samples were then reconstituted in 50 μl injection solution (50% acetonitrile, 0.1% formic acid), for data acquisition.

S.1.g. Procedure for Mass Spectrometry:

Trypsin-digested peptides were analyzed using an Ultra Performance Liquid Chromatography System (UPLC, Waters) coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF MS, Waters) with electrospray ionization in both ESI^+ -MS and ESI^+ -MS/MS (SetMass without fragmentation) mode operated by Masslynx software (V4.1). Each sample in methanol was directly injected into the C18 column (Waters) with a 150

$\mu\text{L}/\text{min}$ flow rate of mobile phase, consisting of solution A (95% H_2O , 5% acetonitrile, 0.1% formic acid) and solution B (95% acetonitrile, 5% H_2O , 0.1% formic acid) in a 15 min gradient starting at 95% of solution A to 30% of solution A for 10 min and back to 95% of solution A for 12 min. The ion source voltages were set at 3 KV, the sampling cone at 37 V, and the extraction cone at 3 V. In both modes, the source and desolvation temperatures were maintained at 120°C and 225°C , respectively, with the desolvation gas flow at 200 L/h.

The Q-TOF MS scanning was done from 50 m/z to 1000 m/z at 1 s with a 0.1-s inter-scan delay using extended dynamic range acquisition with centroid data format. For real-time mass calibration, direct infusion of sodium formate solution (10% formic acid/0.1 M sodium hydroxide/acetonitrile at a ratio of 1:1:8) at 1 s/10 s to the ion source at 2 $\mu\text{L}/\text{min}$ was used. Tryptic peptides were acquired from 200 to 2000 m/z, then mass was calibrated against lockmass manually, and then deconvoluted to single charge by MaxEnt 3. The resulting peptide list was copied into GPMW (ver. 8.20) and searched against known protein sequences to identify potential modifications at 0.1% precision with maximum number of modifications per peptide set at 2 and Check-Fit enabled for trypsin. The intensities obtained from mass matching were subsequently used in quantifying the extent of solvent exposure for the targeted residues in RNase A. The mass spectra for different surfaces for different modifications for each of the different adsorption conditions were obtained with signal to-noise ratios good enough to resolve the respective peptides. For each of the surfaces, multiple elutions of the adsorbent surface were carried out to ensure that almost all of the peptide containing the target residue was recovered from the tryptic digest. In the current study, all the peptides with the target residue of interest were recovered for which the mass shift was estimated at 0.1% precision.

S.1.h. Analysis of Mass Spectrometric Data. Sample-to-sample variation in the ionization process even within unmodified peptides is typically high, and would be further compounded when the target peptides are modified by different chemical labels.^{8,9} A very straight-forward approach to dealing with this problem is to normalize the intensity of peptide of interest to an internal standard, which is usually another peptide that does not contain a modifiable residue.⁸ Such a strategy has been reported to minimize ionization efficiency concerns and can provide semi-quantitative measurements of the extent of modification.⁸

Trypsin is known to cleave peptide chains with high specificity at the carboxyl side of Lys and Arg, except when either one is followed by proline. [11] A central step essential to tryptic digestion of a given peptide with positively charged Lys and Arg amino acids involves the binding of the peptide to the negatively charged catalytic sites of the trypsin. However, most side-chain modifying agents that are available to label Lys or Arg in the protein neutralize the positive charge on these amino acids, resulting in the alteration of the specificity with which the peptides are cleaved. [8] Additional alterations in the specificity of trypsin can also be introduced when these positively charged amino acids are sterically blocked by the adsorbent surfaces.

Following the methods presented by Xu and Bowden [5] under these circumstances the baseline reference in the acquired mass spectra for the protein is determined as the effective sum of the intensities from the internal standard and its variants as represented by equation S.5. The overall contribution of the intensities from internal standards was therefore accounted by considering the contribution of the internal standards generated as a result of tryptic digestion plus those generated as a result of missed cleavage. In the event of a missed cleavage, peptides undergoing a mass shift due to the modification process were given an added weighting to

partially account for any variation in the ionization efficiency due to the labeling process, as represented by equation S.6.[5]

$$\text{"Baseline reference} = \Sigma \text{ Intensities from Internal Control " } \quad (\text{s.2})$$

$$\Sigma \text{ Intensities from Internal Control} = \Sigma(\text{N} * \text{I})_{\text{modified}} + \Sigma(\text{I})_{\text{unmodified variants}} \quad (\text{s.3})$$

where N is the weighting factor and is defined as the number of the modifying agents on the peptide containing the residue of interest, which is estimated using equation s.4.

$$\text{N} = (\text{Mass (modified peptide)} - \text{Mass (unmodified peptide)}) / (\text{Mass (labeled product)}) \quad (\text{s.4})$$

The signal intensities for peptide fragments containing the residue of interest were subsequently normalized to obtain the normalized intensities (I_{norm}) using the baseline reference as shown in equation s.5.

$$I_{\text{norm}} = \text{Intensity of peptides with the residues of interest} / \text{Baseline reference} \quad (\text{s.5})$$

The absolute extent of modification for a target residue in its solution (I_{soln}) and adsorbed state (I_{ads}) was subsequently estimated from a given mass spectrum by calculating the ratio of the weighted intensity of peptide fragments containing the labeled target amino acid to the total weighted intensities of all peptide fragments, as shown in equation s.6.[5, 8]

$$I_{\text{soln}} \text{ or } I_{\text{ads}} = (\Sigma(\text{N} * I_{\text{norm}})_{\text{modified}}) / (\Sigma(\text{N} * I_{\text{norm}})_{\text{modified}} + \Sigma(I_{\text{norm}})_{\text{unmodified}}) \quad (\text{s.6})$$

In the current study, the intensity of peptide without the target residue of interest and generated as a byproduct of tryptic digestion was used as the internal control. The absolute extent of modification of target amino acids in proteins were then quantified from the normalized spectral intensities acquired for the individual modification process. Table S.1 provides the

detailed listing of the internal reference standards used in the current study for each amino acid modification.

Table S.1. Internal controls used in the current study to directly compare the labeling profile of multiple amino acids in RNase A were targeted via different batch experiments.

Target residue	Internal control	Start ID	End ID
Arg, Asp & Glu	TTQANK	98	103
Lys, Tyr, His	FER	8	10

Although all of the reagents used in current study are well-characterized, and are expected to be highly specific to the targeted amino acid with minimal cross-reactions, possible side-reactions were assessed using mass spectrometry based on the signal-to-noise ratio of the spectra when a threshold was applied to investigate possible side-reactions. Since minimal-to-no side reactions were observed, the internal controls were considered to be relatively less affected by the labeling agents, and therefore their ion abundances within a given spectrum was considered to ideally serve as the baseline to scale intensities of peptide segments undergoing modification. Nevertheless, table S.2 shows the possible side reactions that could occur when different labeling agents were applied for a wide range of conditions. In the event of such cross-reaction, the results of the current study could be impacted if the internal controls are affected. As a result, all possible variants and ionization efficiency corrections for the internal controls (TTQANK and FER) due to primary amine modifications were pre-considered in the mass spec analysis. (Table S.2)

Table S.2. The labeling reagent used in the current study, along with the target amino acid and side-reaction to quantify the adsorption-induced structural changes in protein by AAL/MS technique.

Labeling reagent	Target amino acid	Side-reaction ^{4, 8, 10, 11, 12}
Acetic anhydride	Lysine	Histidine, Tyrosine, Cysteine
1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride + N-hydroxysuccinimide (EDC/NHS)	Aspartic acid and Glutamic acid	Primary amines
2, 3-butanedione + 3-acetamido phenylboronic acid	Arginine	None
Diethylpyrocarbonate	Histidine	Lysine
Tetranitromethane	Tyrosine	None at pH < 8

S.2 Results and Discussion

S.2.a. Surface coverage of adsorbed RNase A. Figure S.3 presents a plot of the surface coverage of the adsorbed RNase A on each of our three surfaces for each solution concentration. As these plots clearly show, 2h of exposure to the protein solutions resulted in very similar areal densities for each surface, which fall within the areal densities corresponding to the theoretical limits for a saturated surface of a monolayer of RNase A organized in a close-packed side-on orientation ($0.21 \mu\text{g}/\text{cm}^2$) and close-packed end-on orientation ($0.28 \mu\text{g}/\text{cm}^2$). It is also important to note that the surface densities at this time-point generally increased with increasing solution concentration for each surface between these two theoretical values, with PPI effects considered to increase in magnitude with increased surface density. The fact that the distribution of initial areal densities of adsorbed RNase A was quite similar for each of our three materials also indicates that, on average, the PPI effects were initially quite similar for each type of surface.

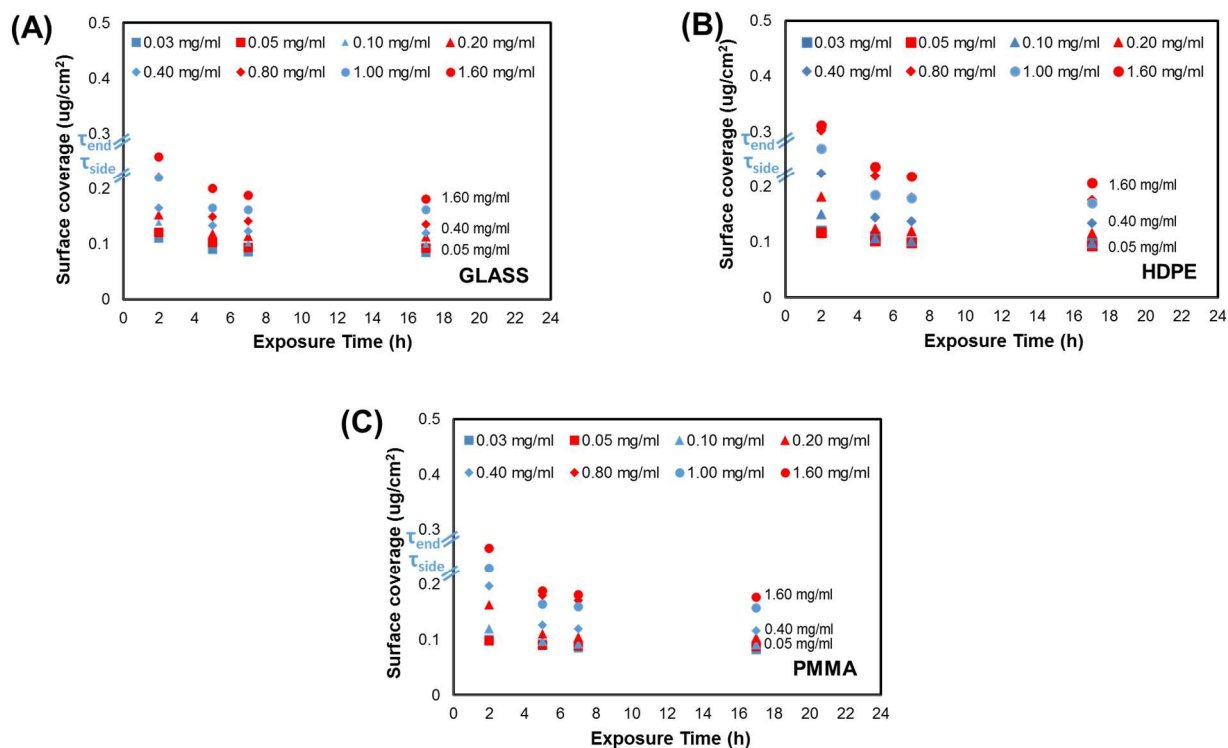
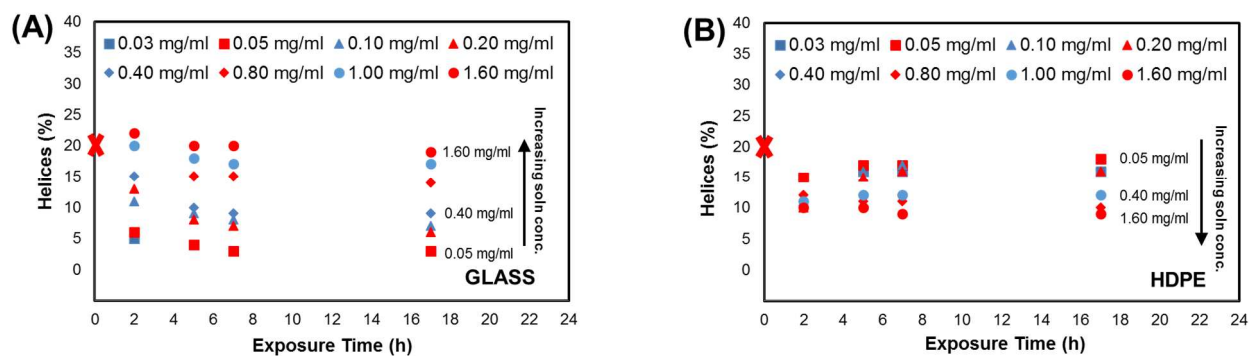


Figure S.3: Effect of varying exposure time on the surface density of RNase A adsorbed on (a) glass, (b) HDPE, and (c) PMMA surfaces. (Exposure time point (e.g., n hours; $n \geq 2$) represents 2 h exposure under the designated protein solution concentration followed by (n-2) hours of equilibration in PPB) ($N=3$; averaged 95% C.I. was $\pm 0.018 \mu\text{g}/\text{cm}^2$ for surface coverage measurements). τ_{side} ($0.21 \mu\text{g}/\text{cm}^2$) and τ_{end} ($0.28 \mu\text{g}/\text{cm}^2$) refers to the theoretical full surface coverage of RNase A for adsorption in ‘side-on’ and ‘end-on’ orientations, respectively.

As indicated by the 5h time point in Figure S.3, when the layers of adsorbed RNase A were allowed to relax for 3h in PPB, the surface coverage of each layer spontaneously decreased to values at or below that for a close-packed side-on orientation. Following these shifts, the surface coverage were still found to be widely distributed, thus continuing to provide a range of PPI for each surface type. The surface coverage then appeared to stabilize with relatively little further change with continued exposure time of 5–10h and 10–17h, thus showing that the RNase

A was irreversibly adsorbed to the surface. However, variation in bulk solution concentrations provided a broad range of surface coverage, and was assumed to proportionally correspond to a broad range of PPI effects within the adsorbed protein layers.

S.2.b. Adsorption-induced changes in Secondary Structure of RNase A. The influence of adsorption conditions on the helical content of adsorbed RNase A is presented in figure S.4. The percent helical structure shown in Figure S.3 corresponding to the 0h exposure times represents the native helical content of the protein in solution ($20\% \pm 2$), with the subsequent time points representing the average helical structure of the protein layers in the adsorbed state. The 2h time point represents the structure of the saturated layers of the adsorbed protein after 2h exposure to their respective protein solution concentrations followed by rinsing in pure buffer to remove loosely bound proteins, while the time points after 2h represent the time given the protein to equilibrate following adsorption while immersed in pure buffer solution (e.g., 5h time point represents 2h exposure under the designated protein solution concentration followed by 3h of equilibration in PPB).



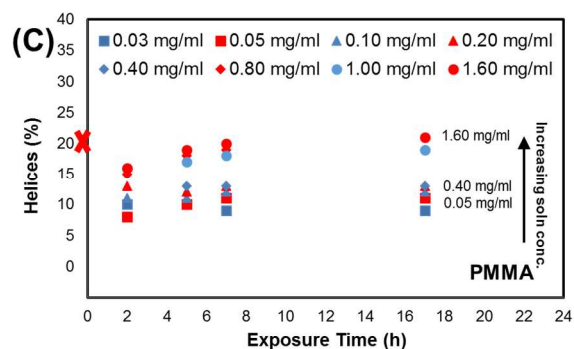


Figure S.4: Helical content of adsorbed RNase A on (a) glass, (b) HDPE, and (c) PMMA surface as a function of exposure time. Symbols represent different protein solution concentrations that were used to adsorb the RNase A to each surface. Zero time point represents the native helical structure of RNase A in solution, which was 20 % ($\pm 2\%$), consistent with the reported secondary structure. ($N = 3$, averaged 95% C.I. values = $\pm 4\%$ helicity for each data point). The black arrows indicate the direction of increasing solution concentration from which the protein was adsorbed.

As shown in Figure S.4, the adsorption of RNase A to each surface following 2h exposure to the protein solution resulted in significant reduction of helical content on each surface and for each solution concentration, which reflects the combined influences of protein-surface interaction, PPI, and internal protein stability effects upon RNase A. Comparisons of the adsorption response at 2h between these three surfaces show some interesting differences. In particular, the solution concentration from which RNase A was adsorbed had a very strong influence on the adsorbed structure on glass and PMMA surfaces, with greater helicity being retained when adsorbed from increased solution concentration. In distinct contrast to this, while significant loss in helical structure upon adsorption occurred on the HDPE surface, the increase

in solution concentration resulted in significant reduction of the RNase A's helical structure when adsorbed from solutions of higher protein concentration.

The data shown in Figure S.4 for the exposure times of 5, 10, and 17h represent the structural response of RNase A during the 15h of equilibration in the pure buffer following the 2h adsorption period. As shown in Figure S.4, the structure of the RNase A on each of these surfaces underwent significant changes between the 2h and 5h overall exposure times ($p < 0.05$ non-parametric test), but then generally tend to stabilize post 5h time point. Subsequent changes in the percent helicity between the 5h, 10h, and 17h time points was not significantly different ($p > 0.05$) for any of the surfaces.

S.2.c Effect of Labeling on the Secondary Structure of Proteins in Solution and adsorbed State. The effect of the respective chemical modifications on the secondary structure of RNase A was verified using CD spectroscopy in both the native (Figure S.5) and adsorbed (Table S.3) state of the protein.

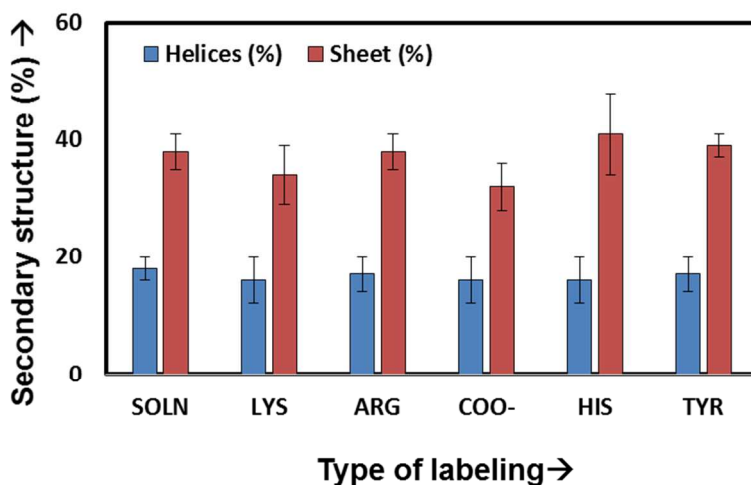


Figure S.5. The effect of chemical labeling on the secondary structure of native RNase A (N = 3, error bar represents the mean \pm 95% C.I.). Soln refers to the solution state of the RNase A when

none of the amino acids were labeled. LYS refers to lysine labeling, ARG – arginine labeling, COO⁻– carboxyl labeling, HIS – histidine labeling, TYR – tyrosine labeling

Table S.3. Helical content (%) within the adsorbed RNase A before and after chemical labeling (N =3, error bar presents the mean \pm 95% C.I.).

RNase A Solution Concentration (mg/ml)		Unlabeled (%)	Lys (%)	Arg (%)	COO ⁻ (%)	His (%)	Tyr (%)
GLASS	0.03	5 \pm 2	6 \pm 3	4 \pm 3	6 \pm 2	6 \pm 3	4 \pm 2
	1.00	19 \pm 4	17 \pm 4	16 \pm 4	18 \pm 5	19 \pm 4	18 \pm 5
HDPE	0.03	18 \pm 2	16 \pm 4	17 \pm 4	16 \pm 4	17 \pm 3	17 \pm 4
	1.00	9 \pm 2	10 \pm 3	8 \pm 3	10 \pm 2	9 \pm 2	9 \pm 2
PMMA	0.03	8 \pm 2	10 \pm 4	9 \pm 3	8 \pm 2	10 \pm 4	9 \pm 3
	1.00	18 \pm 3	19 \pm 4	18 \pm 4	18 \pm 3	20 \pm 5	20 \pm 4

S.2.d Effect of Trypsin Treatment on the Surface Coverage of the Protein. The amount of protein adsorbed on each surface, before and after trypsin treatment, as determined by ellipsometry is given in Table S.4.³

Table S.4. Surface coverage of RNase A on different surfaces for different conditions before and after trypsin treatment (N = 3, error bar presents the mean \pm 95% confidence interval, C.I.).

RNase A Solution Concentration (mg/ml)		Surface coverage before trypsin treatment ($\mu\text{g}/\text{cm}^2$)	Surface coverage after trypsin treatment ($\mu\text{g}/\text{cm}^2$)
GLASS	0.03	0.08 \pm 0.01	0.021 \pm 0.008
	1.00	0.16 \pm 0.03	0.033 \pm 0.007
HDPE	0.03	0.10 \pm 0.01	0.015 \pm 0.005
	1.00	0.17 \pm 0.03	0.034 \pm 0.006
PMMA	0.03	0.08 \pm 0.02	0.014 \pm 0.007
	1.00	0.16 \pm 0.03	0.043 \pm 0.005

The proteins/peptide fragments remaining on the adsorbent surface could be either from the RNase A or the trypsin that was used to digest the protein. However, as it can be seen from the table, since only about 10 % of the initially adsorbed amount of protein was found to be on

the adsorbent surface. It was therefore assumed that almost all of the RNase A has been recovered from the adsorbent surface.

S.2.e Mass Spectrometric Analysis of Adsorbed RNase A with Chemically Modified Residues. Raw data on the profile is provide in Table S.5.

Table S.5. Raw data on the residue profile values for the adsorbed and solution phases of the protein that were targeted in the current study using either TTQANK and FER or both as the internal control. For each of the surface, tryptic digests of the protein were pooled from four different samples.

Residue #	I _{soln}	Concentration (mg/ml)	Glass	HDPE	PMMA
1	1	0.03	-1	-0.10	-0.10
		1.00	-0.301	-1	-1
2	0.339	0.03	0.169	0.47	0.47
		1.00	0.169	0.47	0.345
7	0.54	0.03	-0.334	0.210	0.188
		1.00	-0.210	-0.732	-0.732
9	0.447	0.03	-0.127	0.094	0.049
		1.00	-0.252	0.299	0.119
10	0.359	0.03	-0.555	0.445	0.093
		1.00	0.32	0.32	0.445
12	0.1	0.03	0.673	0.523	0.673
		1.00	0.845	0.523	0.699
14	0.1	0.03	0.61	0.75	0.523
		1.00	0.398	0.954	0.813
25	0.1	0.03	0.731	0.578	0.632
		1.00	0.778	0.319	0
31	0.608	0.03	-0.562	0.158	0.137
		1.00	0.102	-0.784	-0.784
33	0.268	0.03	0.095	0.467	0.133
		1.00	0.309	0.309	-0.428
37	0.506	0.03	-0.102	0.12	0.216
		1.00	0.12	-0.482	-0.248
38	0.943	0.03	-0.385	-0.099	-0.151
		1.00	-0.276	-0.196	-0.19
39	0.724	0.03	-0.86	-0.559	-0.258
		1.00	-0.115	-0.115	-0.337
41	0.283	0.03	0.247	-0.452	0.247
		1.00	0.109	0.004	-0.151
48	0.1	0.03	0.786	0.808	0.786
		1.00	0.523	0.824	0.699
49	0.279	0.03	0.102	-0.446	0.253
		1.00	0.333	0.291	0.121
53	0.623	0.03	-0.247	-0.794	-0.096
		1.00	-0.016	-0.058	-0.162
61	0.569	0.03	-0.056	-0.755	-0.056
		1.00	-0.194	-0.755	-0.357

66	0.706	0.03	0.151	-0.849	-0.15
		1.00	-0.451	-0.849	-0.849
73	0.156	0.03	0.682	-0.193	0.409
		1.00	0.108	0.409	0.029
76	0.593	0.03	0.102	-0.773	-0.171
		1.00	-0.472	-0.171	-0.551
83	0.2	0.03	0.155	0.699	0.699
		1.00	0.331	0.363	0.444
85	0.597	0.03	-0.776	-0.077	0.048
		1.00	-0.01	-0.01	-0.776
86	0.291	0.03	0.235	0.536	0.536
		1.00	0.013	0.156	0.332
91	0.891	0.03	0.05	-0.95	-0.251
		1.00	-0.126	-0.95	-0.251
92	0.465	0.03	0.333	-0.145	-0.667
		1.00	0.156	0.032	-0.145
97	0.1	0.03	1	0.523	0
		1.00	0.824	0.699	0.523
98	0.738	0.03	-0.169	-0.868	-0.169
		1.00	0.132	-0.868	0.007
104	0.447	0.03	0.35	-0.65	0.35
		1.00	-0.65	-0.65	0.049
105	0.324	0.03	0.188	0.489	0.188
		1.00	-0.511	-0.511	-0.511
111	0.569	0.03	-0.755	0.245	-0.755
		1.00	0.245	-0.01	-0.153
115	0.1	0.03	0	0	0
		1.00	0.398	1	0
119	0.487	0.03	0.011	0.312	0.011
		1.00	-0.688	-0.688	-0.688
121	0.1	0.03	0	1	0
		1.00	1	0.745	0.602

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