Open Access

Research Article

Prec. Nanomed. 2019;2(2):256-269

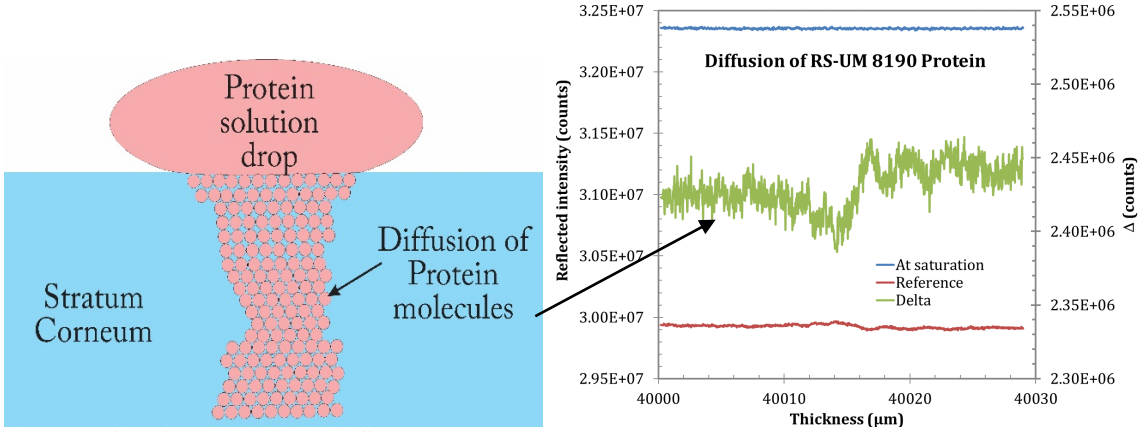
Critical evaluation of the interaction of special proteins with human stratum corneum via terahertz scanning reflectometry and spectrometry

Kariah Crosby[1], Aunik Rahman[1], Kera F. Crawford[2], Zia Shariat-Madar[2], Bozena Michniak-Kohn[3], Donald A. Tomalia[4,5,6] and Anis Rahman[1,7]*

[1]*Applied Research & Photonics, Harrisburg, PA 17111*
[2]*University of Mississippi, University, MS, 38677*
[3]*Rutgers University, Piscataway, NJ 08854*
[4]*NanoSynthons, Mt. Pleasant, MI 48858*
[5]*University of Pennsylvania, Philadelphia, PA 19104*
[6]*Virginia Commonwealth University, Richmond, VA 23284*
[7]*Harrisburg University of Science and Technology, Harrisburg, PA 17101*

Submitted: December 18, 2018 Accepted: March 7, 2019 Published: March 17, 2019

Graphical Abstract



Thickness profile of UM8190 protein diffusion into stratum corneum (green curve, right Y-axis) indicates that the concentration of UM8190 increases towards the depth of the Stratum Corneum (SC) beginning at 14 µm depth (at the stage position ~4014 µm). The concentration of UM8190 reaches the maximum at 16 µm depth (the stage position ~4016 µm) and remains steady throughout the remaining thickness of the SC.

Abstract

Many patients with chronic skin disease develop hemostatic abnormalities. The blood coagulation factor XII is a multifunctional protease, which is involved in thrombosis, fibrinolysis, and inflammatory processes. The aim of this investigation was to assess the autoactivation of FXII that leads to the generation of FXII fragments and their subsequent cell penetration compared to UM8190, a lipophilic selective prolyl carboxypeptidase inhibitor compound. Terahertz scanning reflectometry (TSR) and terahertz spectrometry (TS) were used to study the surface-mediated FXII activation, as well as penetration of the FXII and UM8190, their retardant property, diffusion kinetics and fragmentation profiles into human stratum corneum (SC). From the diffusion kinetics and profiling experiments it was found that FXII does not penetrate the SC but remains mostly on the surface. Compound UM8190 indicates penetration into the SC, as indicated by the increased reflected intensity of T-ray. The terahertz spectral analysis via absorbance spectra indicates that at a low frequency of 0.56 THz a prominent peak occurs due to water or moisture for the SC alone. This peak, however, exhibits a shift for post-diffusion samples of both FXII saturated SC and UM8190-saturated SC. This is indicative of adhesion of these proteins onto the SC. Though this process corroborates the binding of FXII to the cell membrane surface

* Corresponding author. email: a.rahman@arphotonics.net

as reported in the in vitro findings, it does not appear to be activated and degraded. It was also found that there are a number of absorbance peaks characteristic for each molecule and these peaks are uniquely shifted relative to each other when compared with the SC alone. Thus, these absorbance peaks may be utilized for assigning identifying features of the protein and peptides in this present study. Further investigation will be conducted for assigning the absorbance peaks to the specific proteins and their resonances.

Keywords

Terahertz spectroscopy, terahertz reflectometry, hemostasis, blood coagulation factor XII, UM8190, protein diffusion, concentration distribution, stratum corneum, dendrimer

Abbreviations

TSR: Terahertz scanning reflectometry
TS: terahertz spectrometry
FXII: blood coagulation factor XII also known as Hageman factor (EC 3.4.21.38)
PK: plasma pre-kallikrein, (Fletcher factor)
HK: kininogen
FXIIa: activated Hageman factor
SC: human stratum corneum
PCRP: prolyl carboxypeptidase
UM8190: (*S*)-N-dodecyl-1-((*S*)-pyrrolidine-2-carbonyl) pyrrolidine-2-carboxamide, a selective prolyl carboxypeptidase inhibitor
KKS: kallikrein-kinin system
BK: bradykinin, a potent inflammatory peptide
HAE: hereditary angioedema
S 2302: Chromogenic substrate for plasma kallikrein and factor XIIa.

Rationale and Purpose

The purpose of this investigation is to analyze and study biocompatibilities of certain organic molecules such as the FXII and UM8190 proteins and their interactions with human stratum corneum. FXII is activated by multiple mechanisms. In the absence of any activator, FXII has a long half-life, but in the extracellular matrix-bound or membrane-bound conformation, it is activated and initiated the activation of the plasma kallikrein-kinin system (KKS), in turn leading to clot formation via the extrinsic pathway. Based on the accumulated evidence using TSR-TS, we propose that the FXII undergoes desorption-adsorption fluctuation in the Vroman layer, and the protonation of FXII due to acidity of stratum corneum (known to have a pH of around 4 to 5.5) does not induce a conformational change in FXII to be converted to activated FXII (FXIIa) in real time.

Introduction

The protein FXII is considered to be a key initiator in the intrinsic process leading to blood coagulation that involves high molecular weight kininogen (HK) and plasma prekallikrein (PK). It is synthesized as an inactive proenzyme, which can be cleaved not only proteolytically but also non-proteolytically into FXIIa after exposure to negatively charged surfaces in vitro. Substantial interest is focused on the interaction of FXII with negatively charged ligands. The surface of the stratum corneum (SC) carries a negative charge. It is known that such a positively charged molecule can percolate through the stratum corneum leading to enhanced penetration of the plasma membrane [1]. The plasma KKS consists of several proteins that react with each other and in concert with the extrinsic and common coagulation pathways augment the propagation of clots and induce an inflammatory response that helps to promote the healing process [1]. Factor XII and PK (Fletcher factor), two KKS proteins, are proteases that are activated by proteolysis. FXII, a water-soluble zymogen, is considered a potential prothrombotic, proinflammatory and profibrinolytic protease, and is active when in association with extracellular matrix or negatively charged surfaces [2]. The contact activation hypothesis suggests that the binding of FXII to the negatively charged surfaces results in the activation of FXII to produce activated FXII

(FXIIa), with the eventual formation of a fibrin clot. Once activated, FXIIa can catalyze PK to kallikrein which further amplifies the initial signal. It also acts on HK to generate bradykinin (BK, a potent inflammatory peptide) [1]. HK serves as a carrier protein for PK and Factor XI (activated plasma thromboplastin antecedent) which binds to cells as well as to negatively charged surfaces.

Uncontrolled FXII-dependent PK activation can result in the development of pathological pain and angioedema. Patients with this deficiency of C1-inhibitor or mutations in the coagulation FXII gene have elevated kallikrein and BK. This can be a life-threatening issue when the larynx is involved and is referred to as a form of hereditary angioedema (HAE) [3]. V. vulnificus protease, a major toxic factor causing skin damage, can activate FXII [4]. Moreover, FXII deficient patients have prolonged activated partial thromboplastin time, which relates to the artificially created compartment of hemostasis. These clinical and experimental observations provide support for the hypothesis that FXII plays a significant role in thrombin generation, which leads to thrombus formation without any effect on hemostasis. The accumulation of activated FXII at sites of vascular injury or negatively charged surfaces provides an important mechanism for the formation of thrombus and BK. Since many patients with chronic skin disease develop hemostatic abnormalities, investigations were performed to assess the autoactivation of FXII leading to the generation of FXII fragments and their subsequent cell penetration compared to UM8190, a lipophilic compound [5]. Recent advances have given rise to the development of terahertz reflectometry, terahertz spectrometry and reconstructive imaging [6–8]. This technology has allowed the assessment of changes in structure and potential penetration of the FXII, its retardant property, diffusion kinetics and fragmentation profile of FXII in SC. This study extends knowledge of FXII interactions with human stratum corneum and helps to reveal its association with negatively charged surface-dependent shape changes and hence activation of the FXII in the native membrane.

Background

Currently many drugs are delivered orally; however, they are often degraded prior to reaching the targeted site due to low pH in the stomach resulting in inefficient treatment. Ultimately this method of administration has led physicians to prescribe large quantities of antibiotics in order to cure infections and alleviate symptoms. Alternate methods such as drug-infused topical solutions, aid with the direct exposure to the antigen and provide high-efficiency transport through the bloodstream, thus minimizing exposure to proteases and hazardous environmental factors. The purpose of this investigation is to analyze and study biocompatibilities of certain organic molecules such as the FXII and UM8190 proteins and their interactions with human stratum corneum [6]. These results will then be used to guide further characterization of macromolecules as topical agents for permeation in the SC including their interaction and morphology *via* terahertz imaging and also for tissue health monitoring. It has been demonstrated that this imaging technique is useful for visualizing the level of tissue penetration [7] using a nano-scanner with nanomaterials and solutions that are in direct contact with human flesh. If a positive correlation is detected, then one will have the ability to target and eliminate skin conditions from acne, psoriasis, etc. at the source with high efficiency while permeating to the bloodstream.

Principle

The principle behind measuring diffusion kinetics is to assess the ability of an analyte to permeate into a membrane. With this knowledge, physicians are then able to prescribe appropriate drug doses for the formulation of transdermal drug delivery, such as a topical solution or a patch.

Functionality of a Terahertz Scanning Reflectometer (TSR)

Measurement of the concentration gradient in a non-invasive (non-destructive) fashion is important in several areas including penetration of an active ingredient through human skin or other tissues. However, to our knowledge, there is no direct method to obtain two critical factors in such studies, namely: the concentration distribution of permeating ingredient across the thickness of a substrate (e.g., skin) and the kinetics (or rate) of such permeation even though these two parameters are essential for quantitative assessment using Fick's Laws of Diffusion, which are defined as the ability of a solution to diffuse into a substrate based on the

concentration gradient of that particular solution in a given substrate [6]. In one dimension, Fick's first law relates the flux, *J*, directly to the concentration gradient $\left(j = -D\frac{\partial C}{\partial x}\right)$, where *C* is the concentration and *D* is the diffusion coefficient, and Fick's second law relates the kinetics of diffusion with the second derivative of concentration $\left(\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}\right)$. Therefore, direct measurement of the quantities $\frac{\partial C}{\partial x}$ and $\frac{\partial C}{\partial t}$ are of practical importance [6]. Here, we describe a technique for measuring both the concentration gradient and the kinetics of diffusion in real time. Thus, the capabilities will impact many areas of life sciences and physical sciences including Franz-cell characterization of skin and other biological tissues, transdermal drug delivery, personal care products, and other substrate/active ingredient characterizations where effect of an active ingredient on a substrate is important. Examples of experimental results of diffusion for an active ingredient in the human SC are discussed herein.

The experimental setup of the TSR has been discussed elsewhere [6]. A continuous wave terahertz source is used that generates the terahertz radiation from an electro-optic dendrimer via dendrimer dipole excitation mechanism [9]. The terahertz beam is focused onto the specimen at a 90° angle via an off-axis parabolic reflector (normal incidence). The beam reflected by the substrate is directed to the detection system via a beam splitter/combiner. The specimen cell is comprised of a scanning platform that is controlled by a 1-d motion controller. This arrangement as described elsewhere [6], allows direct measurements as follows. The off-axis parabolic reflector is adjusted such that initially, the terahertz beam remains focused on the substrate (specimen) surface. At this position the motion control can be engaged for scanning the substrate to interrogate the reflectance across its thickness; this gives the $\frac{\partial C}{\partial x}$ when the blank substrate reflectance is subtracted from the reflectance of the same substrate treated with a desired ingredient. However, when the beam remains focused at the surface and the motion control is locked at that position, then the ingredient may be applied on the substrate to let it permeate across the thickness while the reflectance is measured in real time. In this case, the reflectance is directly proportional to the rate of permeation of the ingredient across the substrate, $\frac{\partial C}{\partial t}$.

Terahertz Time-Domain Spectroscopy

Terahertz time-domain spectroscopy is a novel technique for probing molecular interactions with the highest sensitivity possible [8]. Here, a time-evolution of the signal is acquired corresponding to the zeroth order constructive interference when a probe beam scans a stationary beam in the time-domain that interrogates the specimen. This signal is known as an interferogram in the time-domain. A suitable Fourier transform yields the terahertz spectra that may be presented in terms of absorbance and other parameters. Because the terahertz radiation can penetrate almost all non-metallic objects, it helps to probe both the surface and sub-surface regions. Additionally, the terahertz signal is sensitive to resonances such as the vibrational, rotational, translational and torsional motions in a molecule. For this reason, terahertz spectra produce many characteristic absorbance peaks that are not available using other protocols. Some details of the technique have been reported elsewhere [8].

Materials and Methods

Materials

SC samples were collected from the New York Firefighter's skin bank. They were kept frozen until prior to the experiments. The proteins used were obtained as follows; FXII (Enzyme Research Laboratory, South Bend, IN) and UM8190 (an in-house compound) [5]. Other laboratory instruments used during the experiments are Ovation micropipette and micro-tips, laboratory microbalance, 4 mL glass bottles, TSR (Applied Research & Photonics, Harrisburg, PA), software application suite for the TSR, a nano-scanner built into the TSR, digital calipers, Plexiglas mount, ruler, spatula, tape, and other customary lab supplies.

Methods

The experimental setup is shown in Appendix 2 for the terahertz reflectometer and the terahertz spectrometer; both from Applied Research & Photonics, Harrisburg, PA 17111.

Assay for FXIIa hydrolytic activity.

The activity of FXIIa was monitored by adding the chromogenic substrate S-2302 (D-Pro-Phe-Arg-p-nitroanilide, Enzyme Research, USA) to 100 µL of reaction mixtures containing various concentrations of FXII or FXIIa in HEPES-carbonate buffer (137 mMNaCl, 3 mM KCl, 12 mM$NaHCO_3$, 14.7 mM HEPES, 5.5 mM dextrose and 0.1% gelatin, pH 7.1, containing 10 µM$CaCl_2$, and 1 mM $MgCl_2$). Following one hour of incubation at 37°C, the reaction was terminated by addition of 1 mM phosphoric acid and the activity of coagulation factors was measured by paranitroanilide production. The absorbance of 405 nm wavelength light at 37 C was recorded. Paranitroanilide generation was normalized to control (buffer containing no FXII or FXIIa), and the optical density of paranitroanilide generation (y-axis) was plotted as a function of varying concentrations of FXIIa or FXII (x-axis). These data were the mean ± S.E.M. of three experiments.

Chromogenic monitoring of FXIIa activity over time.

S2302 assay was performed and initiated by the addition of FXII or FXIIa to a 100 µL 96 microtiter plate containing HEPES-carbonate buffer. After addition of the enzyme, the solution was gently mixed, and the time-dependent changes in optical intensity was discontinuously monitored with BioTek microplate absorbance reader. The release of paranitroanilide was detected by a lab spectrophotometer at 405 nm. Time course of paranitroanilide generation in the presence of either FXIIa (20 nM) or FXII (20 nM) was monitored from hydrolysis of S2302. These data were the mean ± S.E.M. of three experiments.

Nanoscanning reflectometer study.

The nanoscanning reflectometer [6] was calibrated by manipulating the positioning stage to the appropriate Z-axis location *via* the control software of the built-in front-end interface. Off-axis parabolic mirrors were tuned to receive the maximum power from the detection system. The position, where the reflected power was the most linear as a function of Z-variation was selected as Z-position for the measurements. All measurements were done at room temperature under ambient conditions.

The SC (a thin lateral segment of the outermost portion of the human skin) has a known thickness of ~30 microns [6]. Four different Plexiglas mounts were made to mount four pieces of SC; measurements were carried out one at a time. FXII protein solution was prepared using a 16.125 µM sample that was diluted with 200 µL of deionized water. UM8190 solution was prepared with 1.18 mg compound and 200 µL of deionized water. For the determination of the thickness profile, the scanning range was fixed from 40.000 mm to 40.030 mm for a total of 30 µm scan.

The thickness profile (i.e., thickness vs. the reflected intensity) was collected three times per analyte (FXII, UM8190 and deionized water, respectively). The obtained average was used for subsequent analysis for each sample.

Next, the permeation kinetics were measured. For this step, 100 µL of the above prepared FXII solution was dropped on the bare SC while the time-dependent trace was being acquired. Kinetics was followed until the trace reached saturation, thus, indicating the completion of diffusion of the protein into the SC. It took approximately 72 hours for each sample to reach saturation after applying the protein on the SC (not shown).

In order to compute the permeation profile of each of the proteins, thickness profiles were also measured after saturation of kinetics for the respective protein (i.e., post saturation). The difference of the thickness profile of the blank SC (pre-diffusion) and that after saturation of the kinetics (post-diffusion) yielded the distribution of a given protein across the thickness of the SC. In addition, the data for permeation kinetics for FXII and UM8190 into the SC, respectively, were also acquired (see Appendix 2). The next step of the experiment involved terahertz time-domain spectrometry. Each SC sample was mounted on the nanoscanner on the TeraSpectra (Applied Research & Photonics) which remained stationary. The sample was aligned with the kinematic mount's knobs for the highest reflected intensity at a vertical incidence of the terahertz beam. An interferogram was then generated using the built-in Interferogram-Pulse software. For each sample, four interferograms were taken and the average was used for subsequent spectral analysis. Fourier analysis was conducted *via* an algorithm for

unevenly sampled data for generating the absorbance spectra, known as the Lomb periodogram [10, 11].

Results

Hydrolysis of S2302 as a function of coagulation factor concentration.

To study the autoactivation and degradation of FXII on SC, it is very important to use a pure and zymogen form of FXII for the TSR-TS study. We initially addressed whether the commercially purchased FXII was a pure zymogen and had not undergone autolysis or degradation during the purification process by amidolytic activity measurement and SDS-PAGE. The concentration dependence of FXII or activated FXIIa was measured in the presence of a saturating concentration of S2302 (0.5 mM) (Fig. 1).

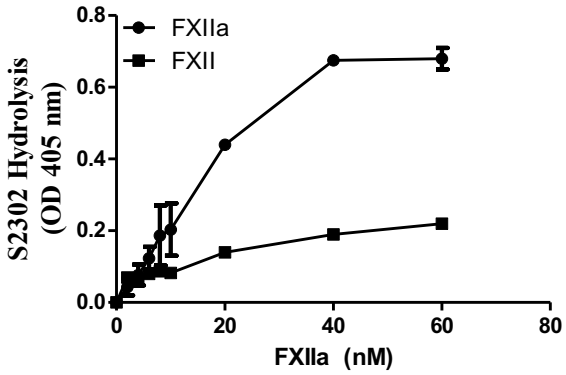


Fig. 1. Concentration dependence for the S2302 hydrolysis in the presence of Factor XIIa.

Diffusion kinetics.

To demonstrate the technique, Fig. 3 shows the diffusion kinetics of different solvents (viz., deionized [DI] water, methanol, and acetone) into a paper substrate. Reference points (red curve, methanol) indicate the blank substrate when an analyte is applied. The saturation point indicates when all analyte has permeated into the substrate causing saturation of the analyte across the thickness of the substrate. The The thickness profiles of a blank SC (reference), and SC with DI water at saturation are shown in Fig. 4 (left Y-axis), and the difference (Δ) is shown on the right Y-axis of Fig. 4, and Fig. 5 displays the thickness profiles

Adding purified FXIIa to S2302 increased substrate hydrolysis in a concentration-dependent manner. Although slight hydrolytic activity was obtained with FXII under our experimental condition, FXII did not give the expected low molecular mass products (α-FXIIa, the two-chain active enzyme) according to SDS-PAGE (not shown). Taken together, our data show that FXII zymogen has none to a very little FXIIa activity.

Time-course analysis of FXII and FXIIa.

In discontinuous assays in which S2302 was incubated with FXIIa or zymogen FXII, we found that FXIIa amidolytic activity was substantially greater than the ability of zymogen FXII (Fig. 2).

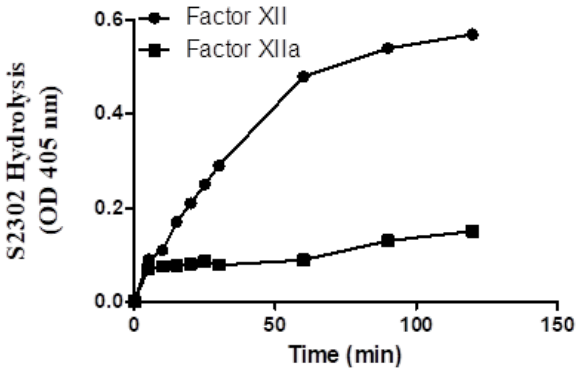


Fig. 2. The time-course analysis of Factor XII and activated Factor XIIa.

difference (Δ) of the thickness profiles at the reference point and at the saturation point gives the concentration gradient of the analyte in the substrate. This is the key step for the direct measurement of the diffusion parameters for a given system. This technique is applicable to any substrate/analyte combination.

Relationship between SC thickness profiles and FXII.

of FXII protein diffusion in to SC (right Y-axis). UM8190, a peptide which competitively inhibits the proteolytic cleavage of substrates by prolyl carboxypeptidase (PRCP) [5] was used as a control.

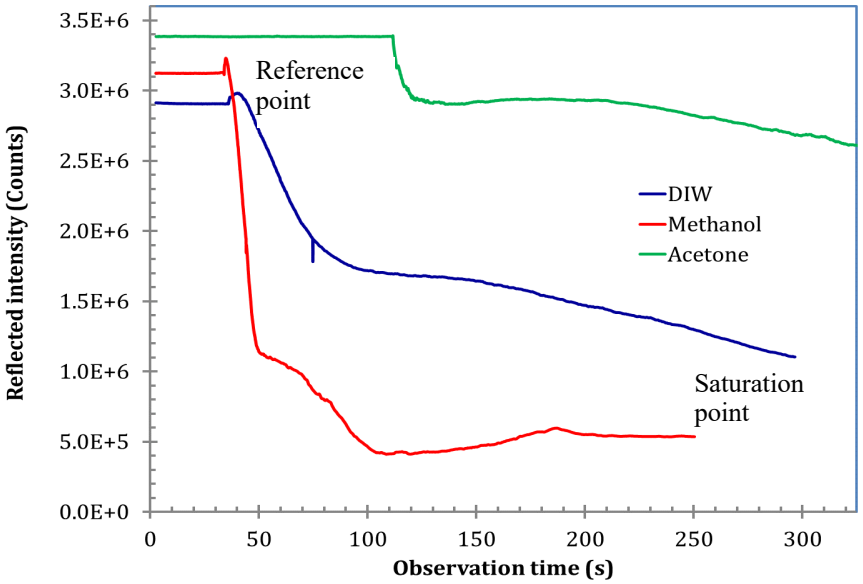


Fig. 3. Diffusion kinetics of different solvent into a paper substrate. The reference point (red curve, methanol) indicates the blank substrate when an analyte is applied. The saturation point indicates when all analyte has permeated in to the substrate causing saturation of the analyte across the thickness of the substrate.

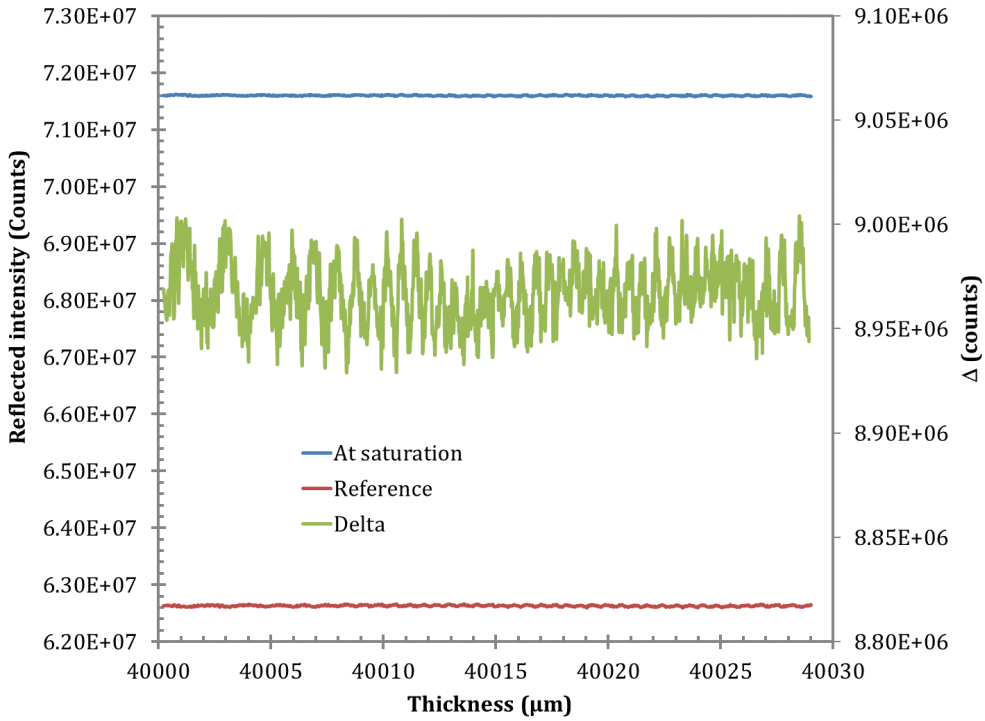


Fig. 4. Thickness profile of blank stratum corneum (reference) and with deionized water at saturation. The difference (Δ) is shown on the right Y-axis.

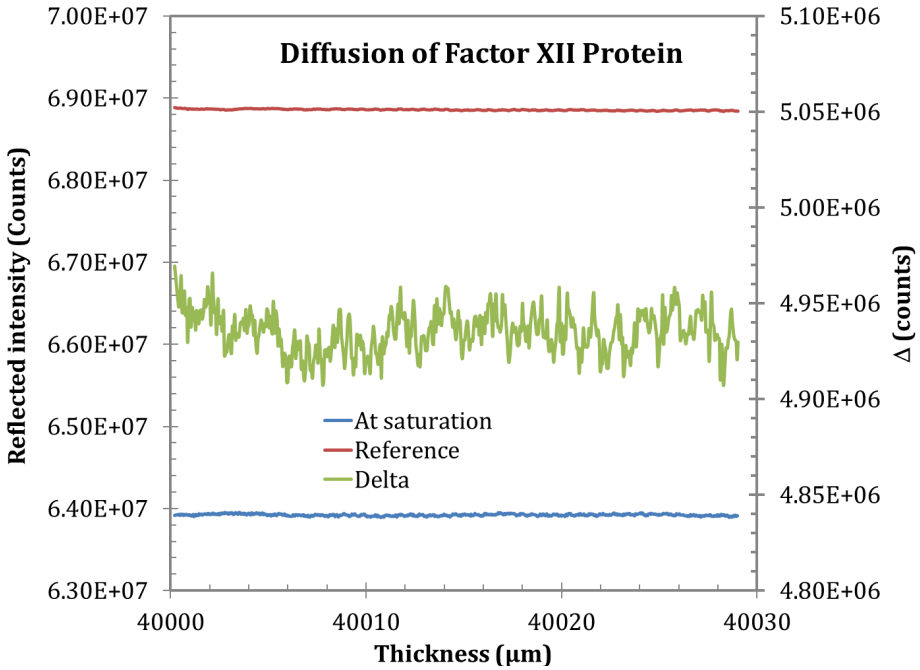


Fig. 5. Thickness profile of FXII protein diffusion into stratum corneum (right Y-axis)

.

The chemical characteristics of UM8190 (i.e., the molecular mass of less than 500 Dalton, no more than 10 hydrogen acceptors and no more than 5 hydrogen bond donors [5] predict that this molecule most likely penetrates the SC. As expected, Fig. 6 shows the thickness profile of UM8190 protein diffusion in to SC (right Y-axis) and Fig. 7 compares the diffusion of FXII (left Y-axis) and UM8190 (right Y-axis) proteins into the stratum corneum.

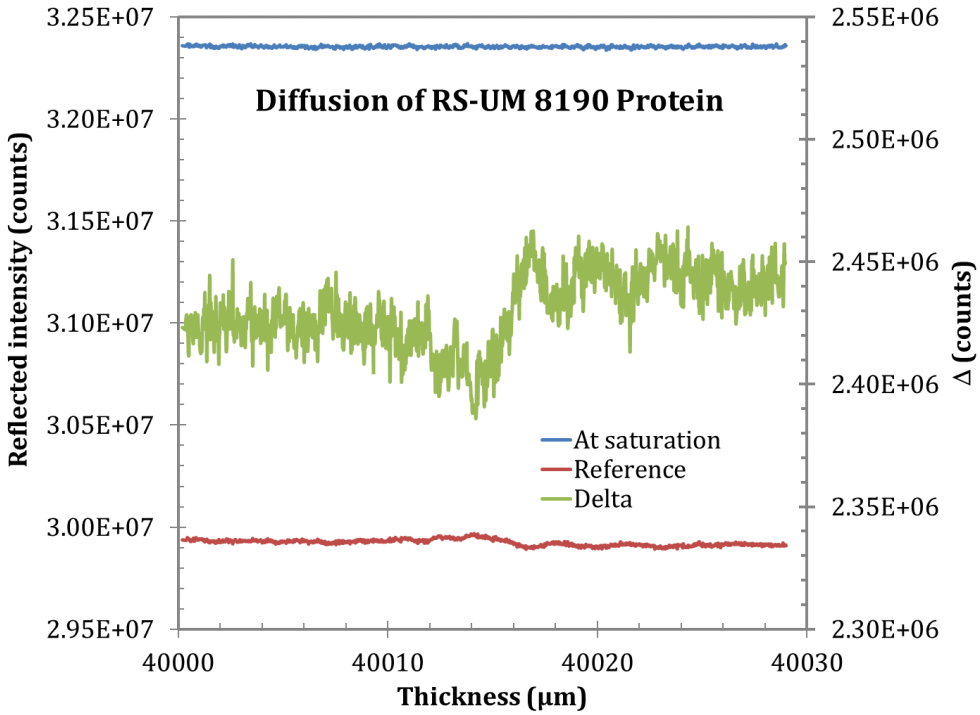


Fig. 6. Thickness profile of UM8190 protein diffusion into stratum corneum (right Y-axis).

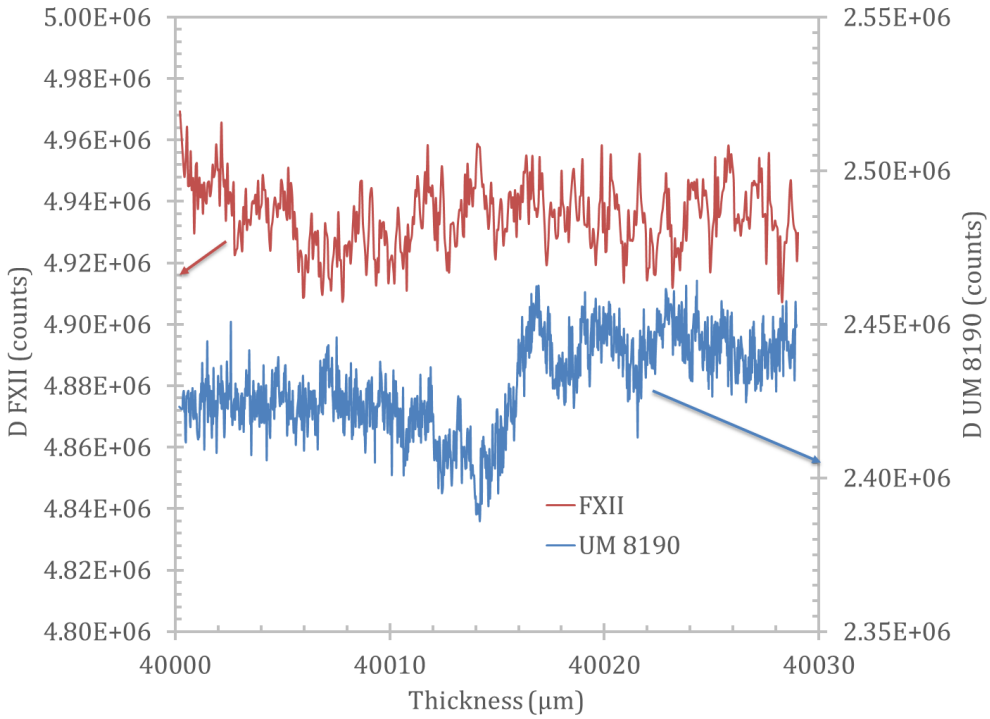


Fig. 7. Comparison of FXII (left Y-axis) and UM 8190 (right Y-axis) proteins' diffusion into the stratum corneum

As seen in Fig. 7, the distribution of FXII across the SC remains almost unchanged with a slightly higher concentration at the surface (stage position = 40000 µm) and shows a slightly decaying trend towards the depth; indicating poor permeation into the SC. However, the concentration of UM8190 increases towards the depth of the SC beginning at 14 µm depth (at the stage position ~4014 µm). The concentration of UM8190 reaches the maximum at 16 µm depth (the stage position ~4016 µm) and remains steady throughout the remaining thickness of the SC. The apparent noisy behavior in both protein profiles (Fig. 7) is assumed to be due to the percolating nature of permeation of the proteins [6] into the SC.

Spectral Analysis

The terahertz time-domain signals (aka interferograms) were acquired for the SC alone, SC after the diffusion experiments with the proteins FXII (SC-FXII), and UM8190 (SC-UM8190), respectively (not shown). Four different interferograms were taken for each sample and the average interferogram obtained from all four time-domain signals was used for Fourier analysis of the respective samples. However, since the experimental data are not always equally spaced in time, therefore, a special transform known as the Lomb periodogram [10, 11] was used in the present spectral analysis. Lomb (1976) and Scargle (1982) developed a novel type of periodogram (Fourier spectrum) analysis, quite powerful for finding, and testing the significance of, weak periodic signals in otherwise random, unevenly sampled data [10, 11].

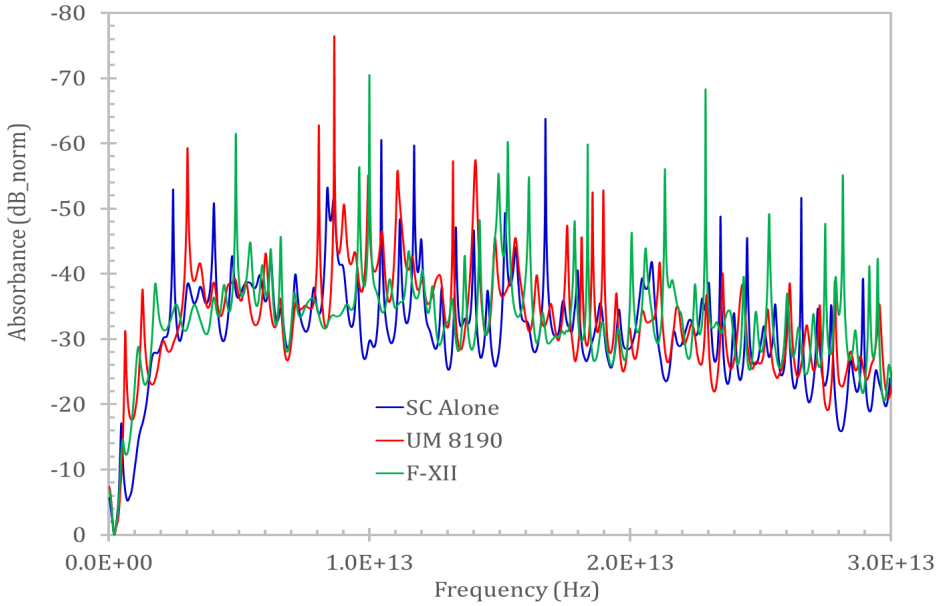


Fig. 8. Lomb periodogram absorbance spectra of stratum corneum (SC) alone, SC saturated with UM8190 (SC-UM8190), and SC saturated with FXII (SC-FXII).

Fig. 8 shows the absorbance spectra over 0.1 THz to ~30 THz for all three samples: SC alone, SC_UM8190 and SC-FXII. As seen, there are a great number of distinguishable frequencies where all samples have strong absorption. Some absorbance peaks for all three samples are shifted with respect to each other which is clearly visible from the closeup plot of Fig. 9 over 0.1 THz to 20 THz. At a lower frequency of 0.56 THz a prominent peak is observed for SC alone due to water content but for both SC-FXII and SC-UM8190, there is a shift of this low-frequency peak indicating that protein molecules have adhered to the SC.

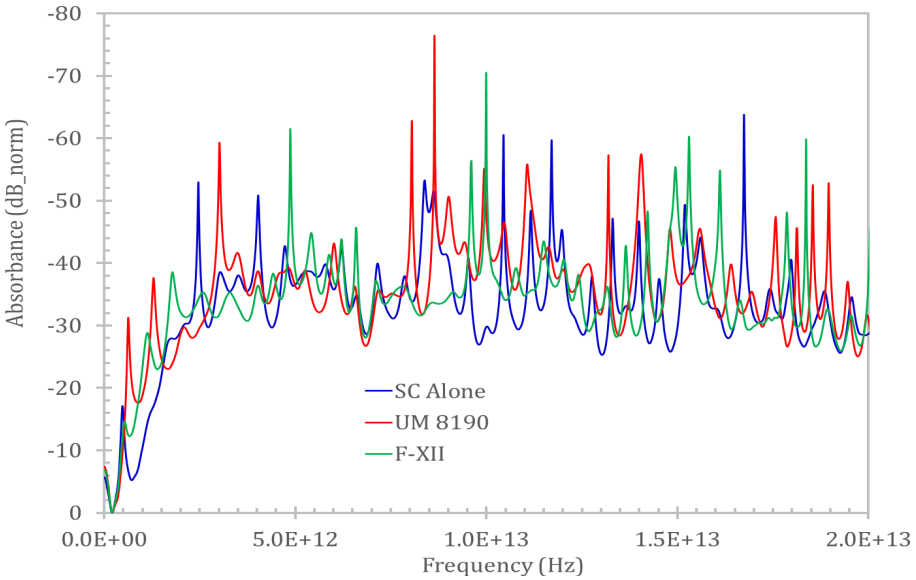


Fig. 9. Closeup of Lomb periodogram absorbance spectra up to 20 THz of stratum corneum (SC) alone, SC-UM8190, and SC-FXII.

Discussion

FXIIa is a multifunctional protease which can activate multiple blood coagulant and proinflammatory pathways. A growing body of research indicates that zymogen FXII is converted to FXIIa through autoactivation via contact to charged surfaces or procoagulant surface (activated endothelial cells or apoptotic cells). However, the exact mechanism is still controversial. Given the surface-mediated FXII activation as proposed by the standard

paradigm, this paper reports a novel method to demonstrate surface-mediated zymogen FXII activation on SC using TSR-TS.

Our study focused on the surface-mediated FXII activation on SC and its potential role in FXII degradation in order to understand the mechanism of surface-mediated KKS activation on apoptotic cells. TSR-TS study showed that the spectrum analysis of FXII did not appreciably change on SC. UM8190 was found to be a diffusible molecule, whereas FXII was not activated and did not seem to be degraded into diffusible low molecular weight products. Moreover, our results demonstrated that the diffusion, saturation, kinetics, and spectrum analysis of FXII and compared with those of UM8190.

The FXII protein which possesses both coagulant and proinflammatory abilities in biological systems, understanding the mechanism of surface-mediated FXII activation is essential in order to assist in manufacturing key therapeutics for a host of medical diseases, including inflammation and stroke or the development of an ideal biocompatible surface. In summary, we presented a new approach for surface-mediated FXII activation detection. We demonstrated the effectiveness of the TSR-TS approach to detect the molecular diffusivity of UM8190 in SC. However, further investigations are needed to determine the clinical utility of TSR-TS approach in characterizing the mechanism of surface-mediated contact activation.

Verification of functional states of FXII and FXIIa.

The unregulated proteolytic activity of FXIIa could compromise cell function, as seen in patients with hereditary angioedema type III (HAE-III). Patients with HAE-III have recurrent skin swelling, swelling of the tongue, and pharynx. Here, we initially explored the measurement of both FXII and FXIIa functional activity before incubation with the SC. For full proteolytic activity, FXIIa was used as positive control. FXIIa activity was verified by S2302 hydrolysis. FXIIa hydrolyzed S2302 in a concentration (see Fig. 1) and incubation time-dependent manner (see Fig. 2), and this effect was completely abolished by corn trypsin inhibitor (data not shown). However, FXII had no significant proteolytic activity. Characterization of FXII confirmed its zymogen action.

Interaction of FXII with the stratum corneum.

To date, little is understood about the fate of FXII on SC or whether FXII surface activation exhibits different shape characteristics, which may lead to FXIIa proteolysis and whether the fragments of the subsequent proteolytic processes pass into the cytosol. We used UM8190 as a positive control. FXII did not undergo shape changes after only one minute with the initial involvement of SC. After the interaction of FXII with the cells, there was no proteolytic product or the penetration of FXII or FXIIa into the SC. These data also suggested that the UM8190 compound was able to diffuse, permeate and penetrate the SC. In conclusion, FXII contact activation on SC does not produce proteolytic products using terahertz reflectometry and spectrometry protocols. In summary, the potential of this technology to better understand the interaction of macromolecules on the biological surface was demonstrated in real time.

Conclusions

From the diffusion experiments it is concluded that protein FXII does not penetrate the SC, it remains on the surface. However, peptide UM8190 exhibits some penetration into the stratum corneum, as indicated by the increased reflected intensity of the T-ray.

The terahertz spectral analysis via Lomb periodogram absorbance spectra indicates that at a low frequency (0.56 THz) there is a prominent peak for the SC alone, indicating the presence of water and/or moisture in this sample. However, this peak shifts for both SC-FXII and SC-UM8190 post-diffusion samples, which shift indicates adhesion of these proteins to the SC. Additionally, there are a number of frequencies at which distinguishable absorbance peaks are present and these peaks are also shifted for each sample as compared to others. We hypothesize that these peaks may be utilized for assigning further features of the proteins used in the present study [7, 8], even though further investigations are necessary to confirm assignations of absorbance peaks to a specific protein signature as related to their interactions with the SC.

Acknowledgement

Some principle of the present work was presented at the 48th Silicon Symposium, June 7-9, 2017, Philadelphia, PA and at the 252nd ACS National Meeting, Philadelphia, PA, August 18 - 26th, 2016.

Conflict of Interests

The authors declare no conflicts of interest. For signed statements, please contact the journal office: editor@precisionnanomedicine.com

Quote this article as Crosby K, Rahman A, Crawford KF, Shariat-Madar Z, Michniak-Kohn B, Tomalia DA, and Rahman A, Critical evaluation of the interaction of special proteins with human stratum corneum via terahertz scanning reflectometry and spectrometry, Precis. Nanomed. 2019;2(2):256-269, https://doi.org/10.33218/prnano2(2).181218.2

References

1. Bryant, J. W. and Shariat-Madar, Z., (2009) "Human plasma kallikrein-kinin system: physiological and biochemical parameters," Cardiovasc Hematol Agents Med Chem. 7(3): 234-50.
2. Brunnee, T., Reddigari, S. R., Shibayama, Y., Kaplan, A. P., Silverberg, M., (1997) "Mast cell derived heparin activates the contact system: a link to kinin generation in allergic reactions," Clin Exp Allergy, 27(6):653-63.
3. Miranda, A. R., Ue, A. P., Sabbag, D. V., Furlani Wde, J., Souza, P. K., Rotta, O., (2013) "Hereditary angioedema type III (estrogen-dependent) report of three cases and literature review," 88(4): 578-584.
4. Shinoda, S., (2005) "Pathogenic factors of vibrios with special emphasis on Vibrio vulnificus," Yakugaku Zasshi, 125(7):531-47.
5. M Rabey, F., SVS Gadepalli, R., Diano, S., Cheng, Q., Tabrizian, T., Gailani, D., J.M. Rimoldi & Shariat-Madar, Z., (2012) "Influence of a novel inhibitor (UM8190) of prolylcarboxypeptidase (PRCP) on appetite and thrombosis," Current medicinal chemistry, 19(24), 4194-4206.
6. Rahman, A., Frenchek, S., Kilfoyle, B., Pattarkine, L., Rahman, A., & Michniak-Kohn, B. B. (2012), "Diffusion Kinetics & Permeation Concentration of Human SC Characterization by Terahertz Scanning Reflectometry," Drug Development and Delivery, May 2012, v. 12(4), pp 43–49.
7. Rahman, A., Rahman, A. K., & Rao, B. (2016), "Early detection of skin cancer via terahertz spectral profiling and 3D imaging," Biosensors and Bioelectronics, v. 82 pp. 64–70. DOI: 10.1016/j.bios.2016.03.051
8. Rahman, A. (2011), "Dendrimer based terahertz time-domain spectroscopy and applications in molecular characterization," Journal of Molecular Structure, 1006, 59–65. DOI: 10.1016/j.molstruc.2011.07.004
9. Rahman, A., Rahman, A. K., and Tomalia, D. A., "Engineering dendrimers to produce dendrimer dipole excitation based terahertz radiation sources suitable for spectrometry, molecular and biomedical imaging," Nanoscale Horiz., 2017, 2, 127-134. DOI: 10.1039/C7NH00010C.
10. Scargle, J.D. (1982) "Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data," The Astrophysical Journal, v. 263, 835–853, 1982 December 15.
11. Press, W.H and Rybicki, G.B. (1989) "Fast algorithm for spectral analysis of unevenly sampled data," The Astrophysical Journal, v. 338, 277-280,1989 March 1.

Appendix-1

The experimental setup is shown in Figure A1-1 [6] and A1-2 [8]. Figure A1-1 (left panel) exhibits the terahertz reflectometer instrument while the right panel exhibits the optical circuit implementing the optical reflectometer functionalities. A 3D motion control system is used to position the sample at the focal point of the beam. The Z-axis of the positioning system is used to scan the beam through the substrate's thickness with a hardware resolution of ~24 nm.

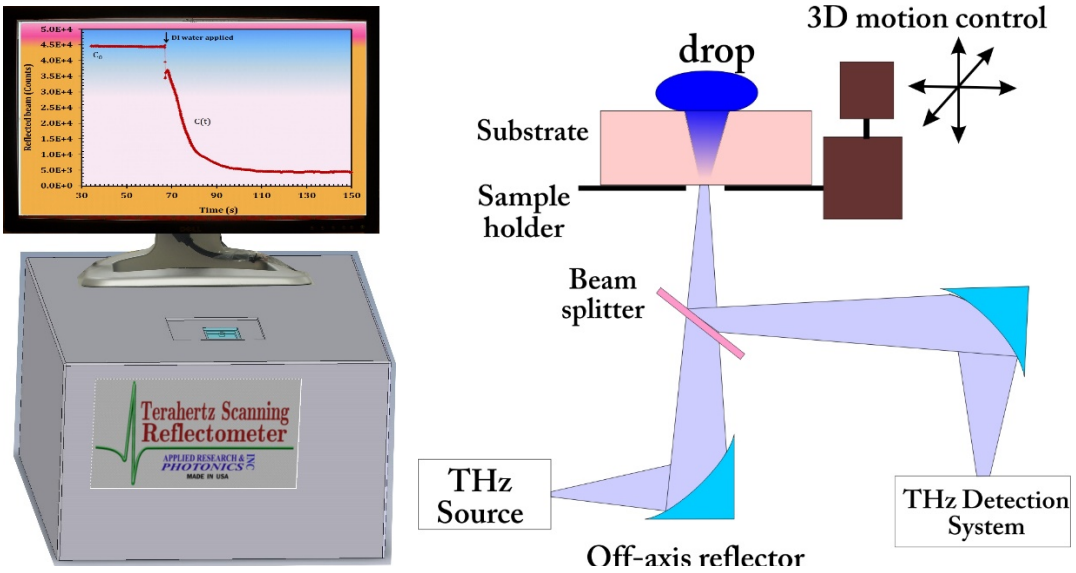


Figure A1-1. Left – the terahertz reflectometer instrument. Right – The optical circuit implementing the optical reflectometer functionalities. The 3D motion control system is used to position the sample at the focal point of the beam. The Z-axis of the positioning system is used to scan the beam through the substrate's thickness with a hardware resolution of ~24 nm. Adapted from ref. [6].

The terahertz time-domain spectrometer instrument is displayed in Figure A1-2 where the left panel shows the actual instrument and the right panel display the optical circuit implementing the time-domain spectrometer functionalities. The pump laser is split in to two arms; a pump beam that goes through a time-delay generator and probe beam that remains stationary. Details principle of the terahertz time-domain spectroscopy is explained elsewhere [8].

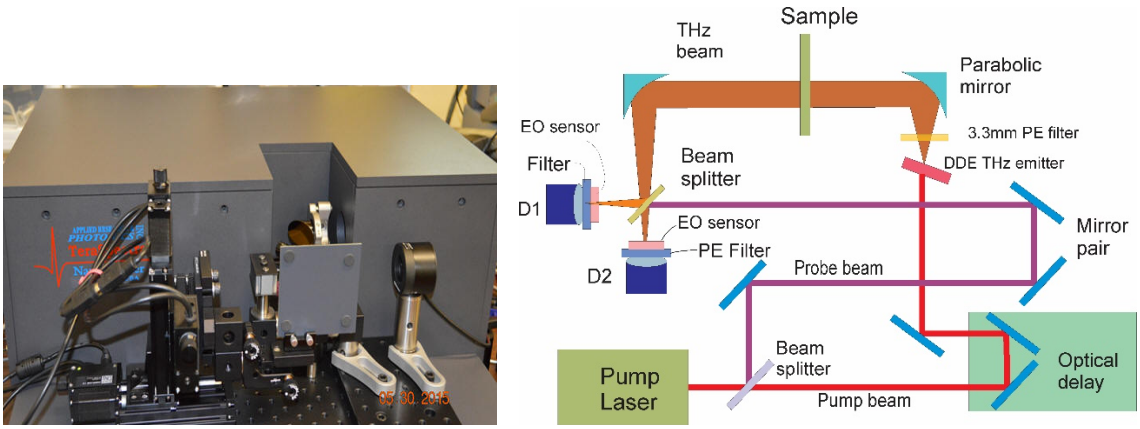


Figure A1-2. Left – terahertz time-domain spectrometer instrument. Right – The optical circuit implementing the time-domain spectrometer functionalities. The pump laser is split in to two arms; a pump beam that goes through a time-delay generator and probe beam that remains stationary. Adapted from ref. [8].

Appendix-2: Kinetics data

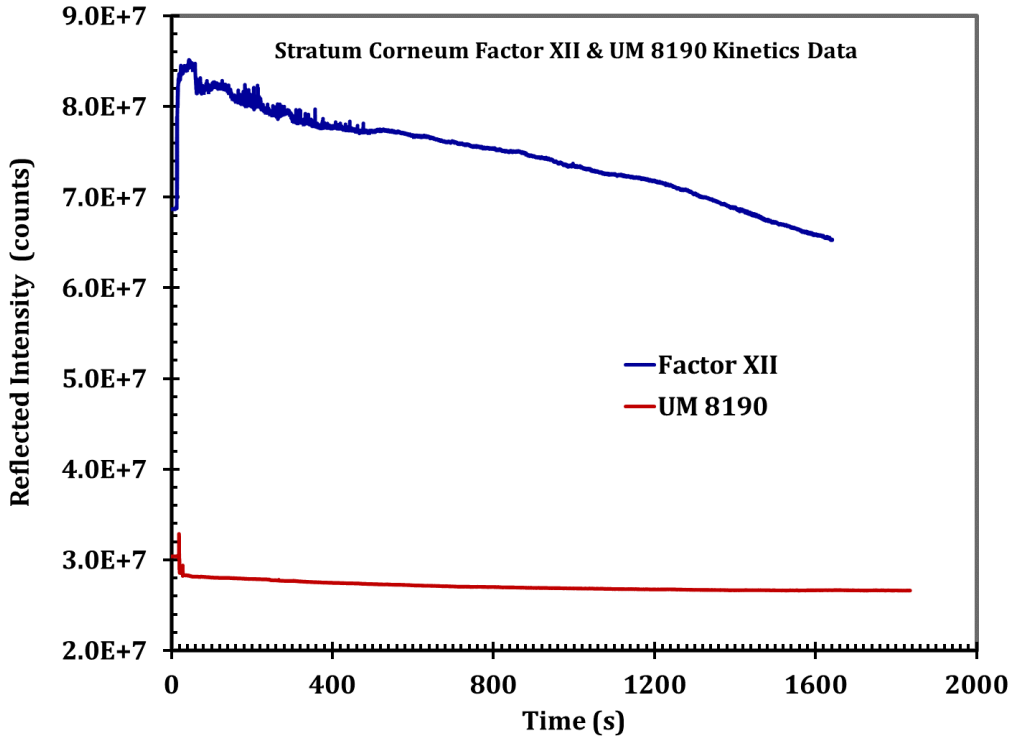


Fig. A2-1. Kinetics of permeation of the respective proteins in to the stratum corneum.

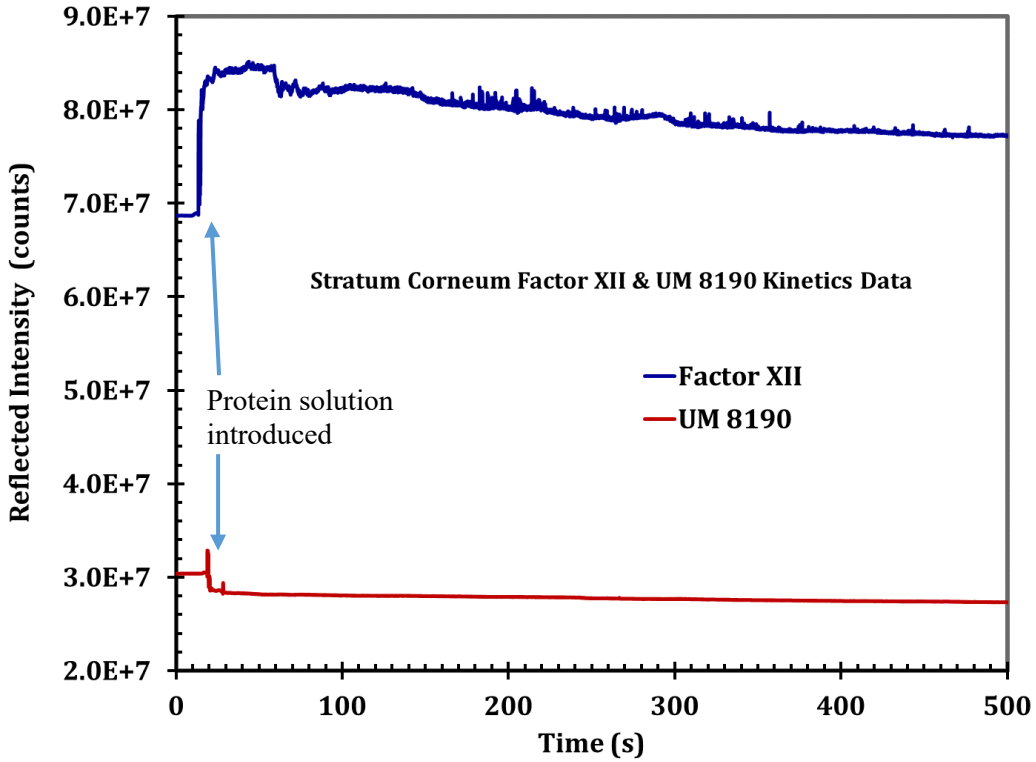


Figure A2-2. A close up of Figure A1-1 showing the decay of the reflected intensity after application f the protein solution on to the stratum corneum.