Characterization of Self-Assembled Monolayers of Peptide Mimotopes of CD20 Antigen and Their Binding with Rituximab

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Supporting Information:

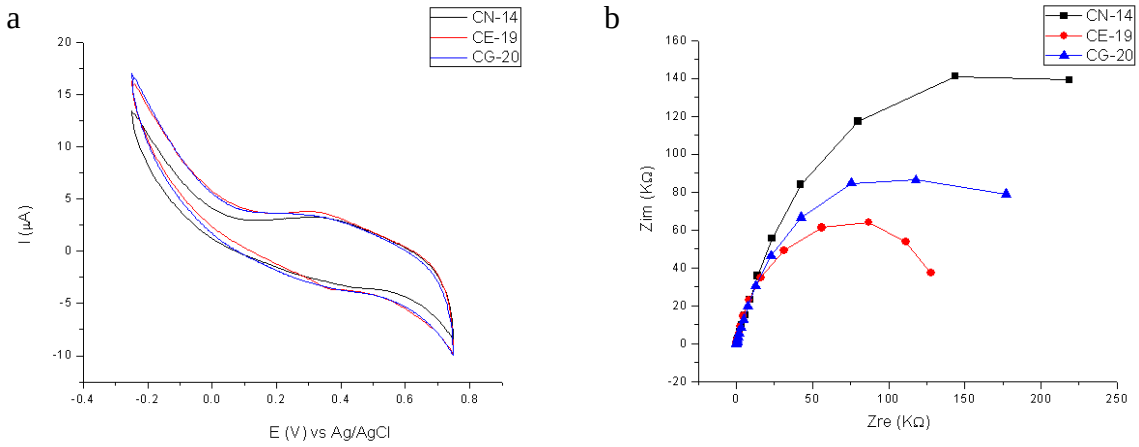


Figure S1. Characterization of immobilized peptides by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). (a) The CV measurements across CN-14, CE-19, and CG-20 showed greatly dampened Epa and Epc peaks. This is consistent with an insulated surface resulting from the binding of peptides to the gold. (b) The high impedance from the measured peptides support that immobilization was successful.

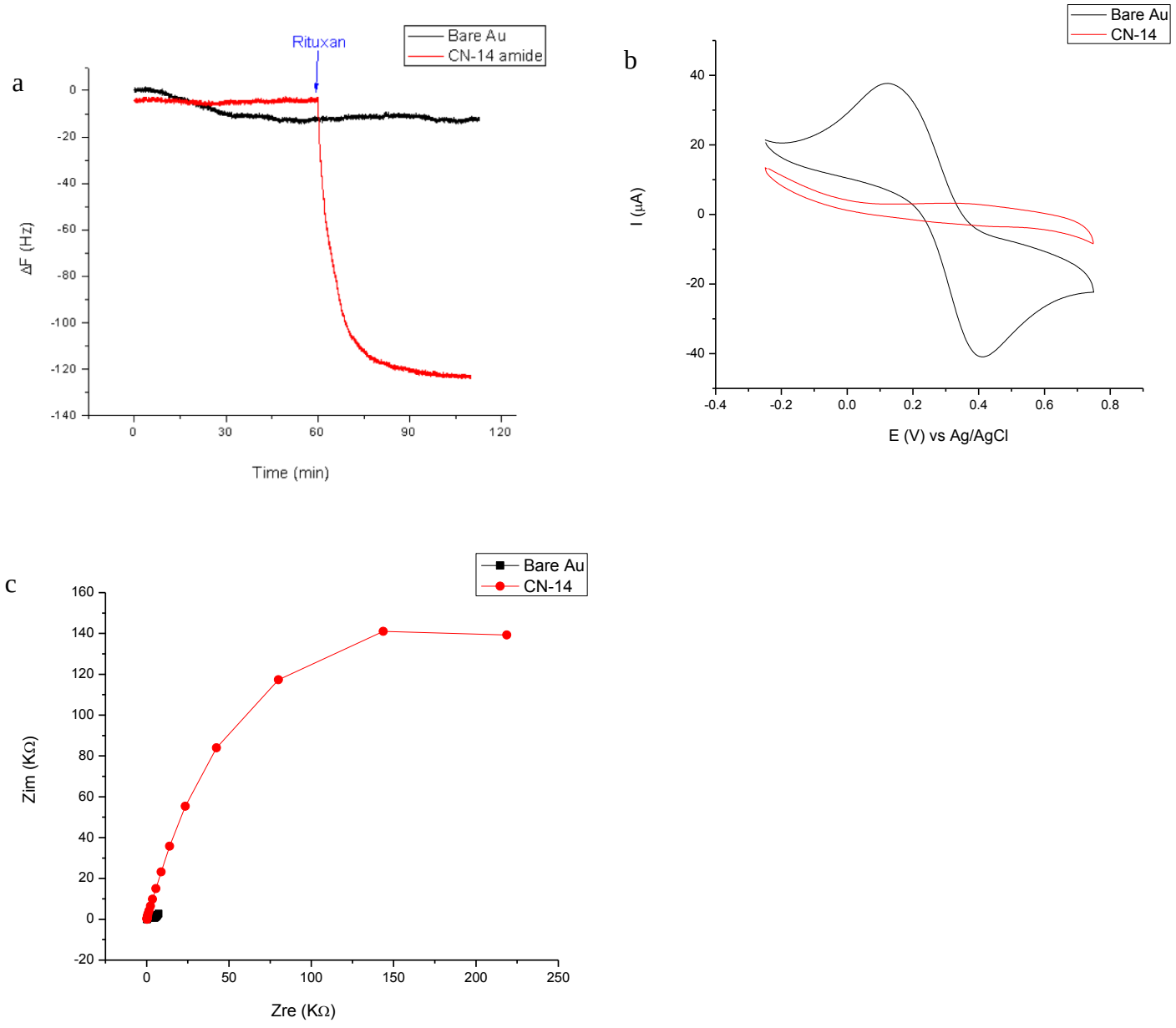


Figure S2. (a) The frequency change vs time curve: bare gold electrode (black), and CN-14 amide (red) modified Au QCM electrodes were exposed to small amount (1 µl spiking in 1 ml PBS) of Rituxan (Rituximab) (1µg/µl). **(b)** CVs of 1 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ in 0.1 M $NaClO_4$ on bare gold electrode (black) and CN-14 modified electrode surfaces. Scan rate, 50 mV/s. **(c)** EIS Nyquist plots. Frequency range was 0.01 Hz-100 kHz, ac amplitude 5 mV.

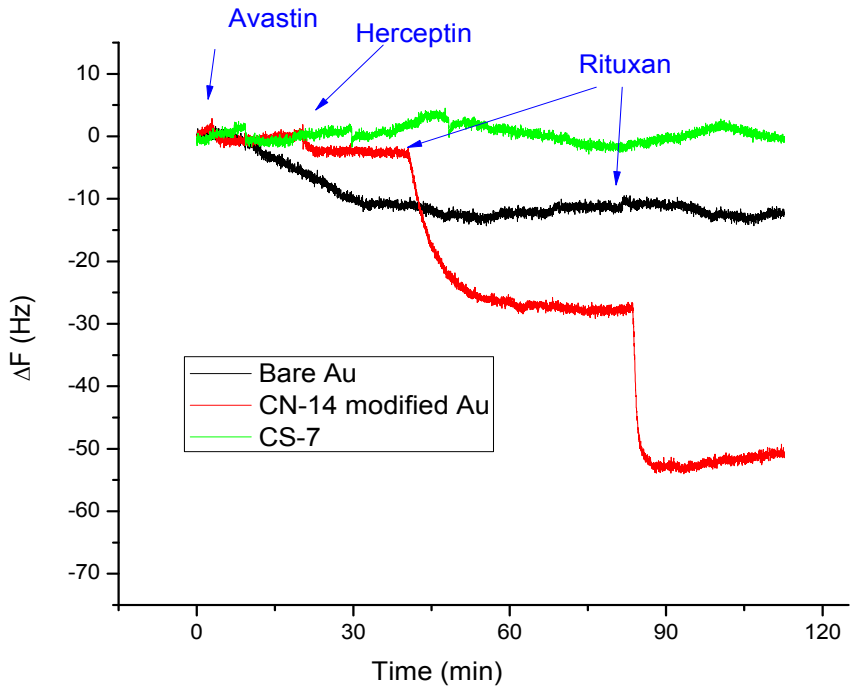
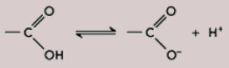
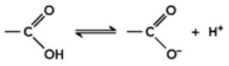
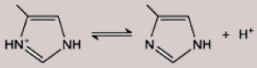
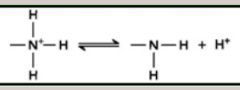
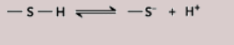
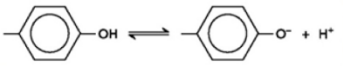
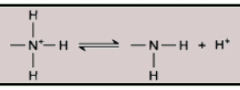
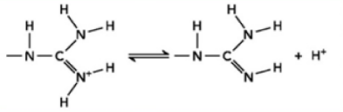


Figure S3. The frequency change vs time curve: bare gold electrode (black), CN-14 (red), and CS-7 (green) modified Au QCM electrodes were exposed to Avastin (Bevacizumab), Herceptin (Trastuzumab), and Rituxan (Rituximab) sequentially.

Table S1. Titratable residues refer to amino acids that contain sites which can be protonated or deprotonated. Depending on the protonation state of these sites, the peptide can contain a positively or negatively charged region. Whether titratable sites can ionize depend on their pK_a and the solution pH.[1]

Group	Acid ⇌ Base + H^+	pK_a
Terminal Carboxyl		3.1
Aspartic acid and Glutamic acid		4.4
Histidine		6.5
Terminal amino		8.0
Cysteine		8.5
Tyrosine		10.0
Lysine		10.0
Arginine		12.0

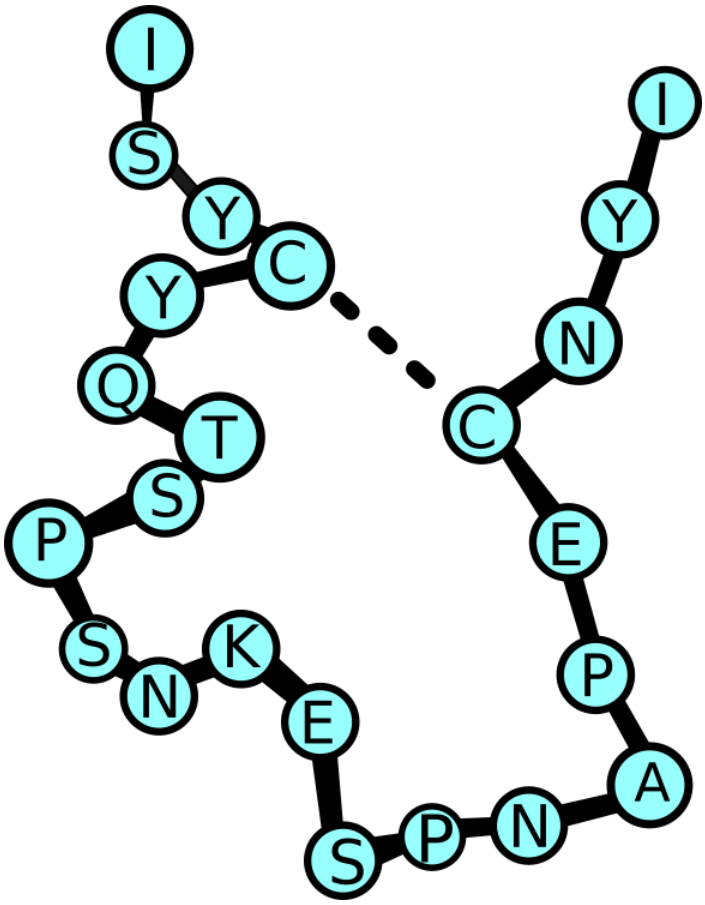


Figure S4. Drawing of CD20 crystallized epitope from crystal binding data of CD20 with Rituximab[2]

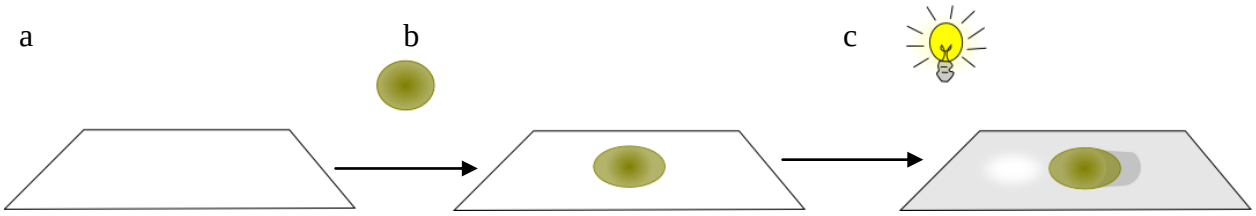


Figure S5. The atomic force microscopy (AFM) images were enhanced by applying them as textures to a 2D plane. The data in the images were used to displace the geometry according to the pixel colors of the AFM textures. A light source was added to illuminate and cast shadows across the distorted geometry of the plane to achieve greater variations between the higher and lower regions of the AFM images. (a) The plane is made in 3D space. (b) The AFM texture is mapped to the 3D plane. (c) The AFM texture is applied as normal mapping and a light source is introduced above the plane to add variation between elevated regions.

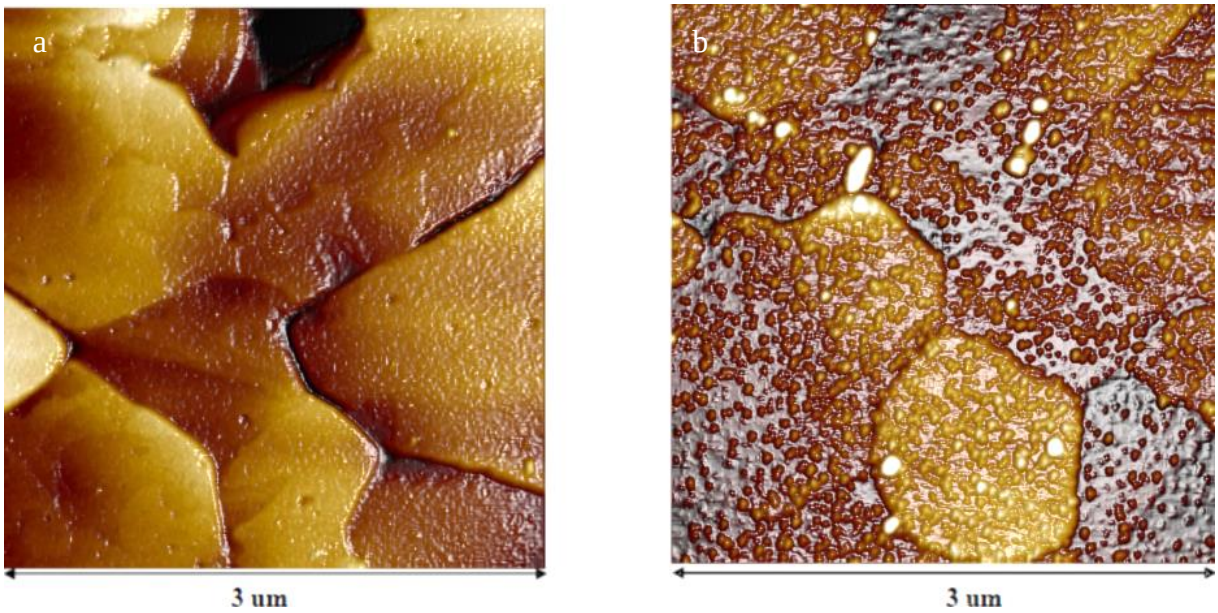


Figure S6. In order to establish more contrast between the higher and lower areas of the SAM, the image was enhanced by applying normal mapping with the use of a light source. This allowed for the more subtle topology of the SAM to be magnified for interpretation.

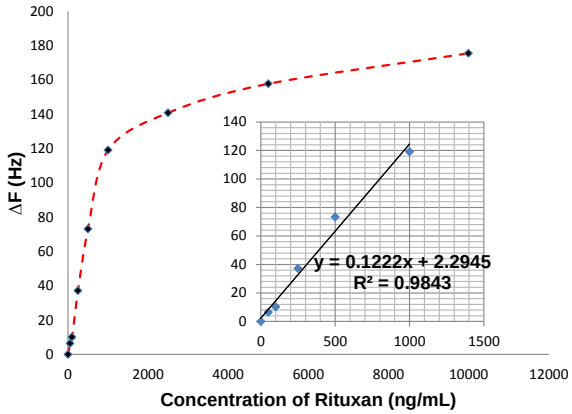


Figure S7. Plot of Rituximab / ΔM vs Rituximab of CN-14 amide modified Au QCM electrode for the CN-14 amide/Rituximab binding association constant (K_a) determination: The CN-14 amide/Rituximab binding amount at the nanogram level (ΔM) can be obtained from the time relationship of frequency shift (ΔF) at various Rituximab concentrations. The CN-14 amide/Rituximab binding activity is described in Equation 1.

$$\text{CN-14 Amide} + \text{Rituximab} \underset{K_{Off}}{\overset{K_{On}}{\rightleftharpoons}} \text{CN-14 Amide/Rituximab Complex} \qquad (1)$$

Based on a Langmuir adsorption isotherm, the association (K_a) constant for CN-14 Amide/Rituximab interactions can be determined by Equation 2.

$$[\text{Rituximab}]/\Delta M = [\text{Rituximab}]/\Delta M_{max} + 1/(\Delta M_{max} - K_a) \qquad (2)$$

Where, the ΔM_{max} is the maximum amount that Rituximab can be bound and the ΔM_{max} is the characteristics of the modified QCM electrode surface, which is equivalent to the binding of Rituximab when all the CN-14 Amide is bonded to Rituximab as the CN-14 Amide/Rituximab Complex. ΔM is the measured Rituximab binding amount at equilibrium, which is a function of the Rituximab concentration and will not change with the time, and [Rituximab is the concentration of Rituximab injected into the QCM cell.

Based on the results from Figure 7, Rituximab/ΔM was plotted as a function of Rituximab concentration (Figure S4). Applying equation, the ratio of the slope to the intercept gives the association constant of CN-14 Amide 5.32 x 10^7 M^{-1}**.**

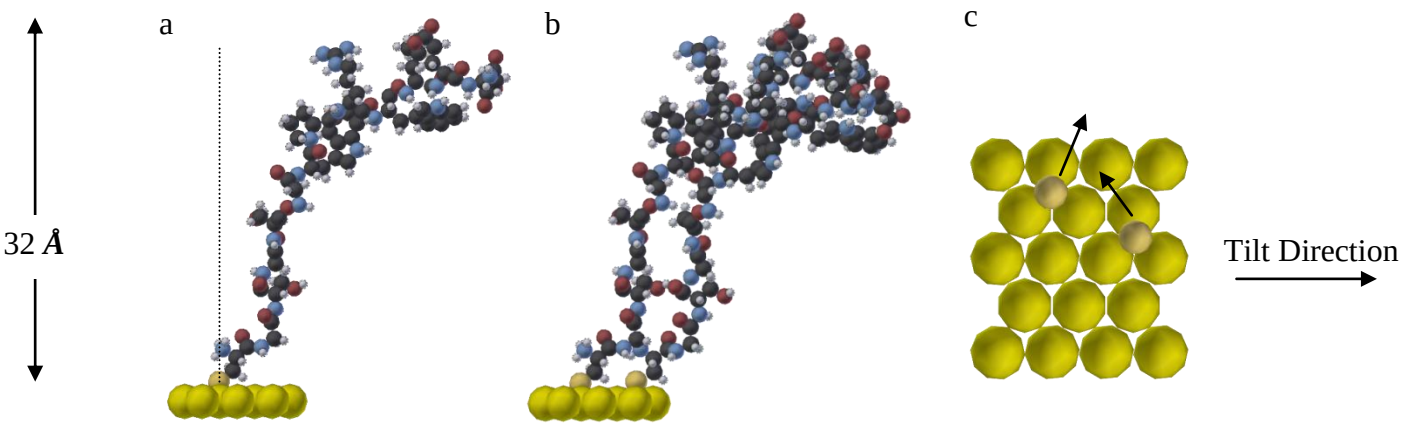


Figure S8. (a) Model of CN-14 on a gold (111) surface. (b) Model of two CN-14 peptides to demonstrate self-assembled monolayer folding on a gold (111) surface. (c) The position and orientation of the two CN-14 peptides on gold (111) from (b)**.**

References

(1) Srivastava , J.; Barber, D.; Jacobson, M. Intracellular pH Sensors: Design Principles and Functional Significance. *Physiology* **2007,** *22*, 30-39.
(2) Du, J.; Wang, H.; Zhong, C.; Peng, B.; Zhang, M.; Li, B.; Huo, S.; Guo, Y.; Ding, J. Structural Basis for Recognition of CD20 by Therapeutic Antibody Rituximab. *J. Biol. Chem.* **2007,** *282*, 15073-15080.