The binding affinity of molecularly imprinted polymers (MIPs) relies on the mechanisms and the extent of the functional monomer-template interactions present in the prepolymerization mixture. Thus, a clear understanding and optimizing the physiochemical parameters governing these interactions is key in designing and modeling MIPs with good selectivity. Quantum chemical method was applied here for the theoretical investigation into the interaction between cortisol and pyrrole in a molecularly imprinted prepolymerization mixtures. Since polypyrrole (PPy) is one of the most extensively used conducting polymers in design of bioanalytical sensors, pyrrole is chosen as a functional monomer. The pre-assembly system of possible conformations of cortisol/pyrrole monomer systems have been optimized with the use of density functional theory (DFT) at B3LYP/6-31G(d) level using Gaussian 09 software. The binding energy calculations of a range of structurally related steroids (cortisol, progesterone, prednisolone, 21-deoxycortisol and 6-methyprednisolone) with functional monomer have been analyzed through computational modelling. The most stable configurations of cortisolfunctional monomer complexes have been optimized and selected. Based on the conformational analysis and the calculated binding energies of steroid/pyrrole molecular imprinted systems, we have concluded that the interactions between cortisol and pyrrole are more specific and stronger in comparison to the interactions between other steroid hormones (progesterone, prednisolone, 21-deoxycortisol and 6-methyprednisolone) and pyrrole. 

© The Author(s) 2017. Published by ECS. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/2.0101705jes] All rights reserved.

In the recent years, molecularly imprinted polymers (MIPs) have become one of the most useful materials for designing new analytical methodologies as they are selective molecular recognition phases. They are similar to immunosorbents in that MIPs can be tailored for different target analysis. During the molecular imprinting process highly cross-linked co-polymers are formed around analyte molecules acting as cavity-creating templates. After extractive removal of the template, the remaining molecularly imprinted polymer matrix contains 3-dimensional binding cavities facilitating rebinding due to complementary shape and functionality for the template molecule (Figure 1). Depending upon the nature of chemical bonds involved, MIPs synthesis techniques can be classified into two common approaches: (i) covalent imprinting and (ii) non-covalent imprinting. Non-covalent imprinting has been most widely adopted in many laboratories due its flexibility in choice of functional monomers and template molecules. The main non-covalent interactions responsible for molecular recognition in biomimetic systems are hydrogen bonding, ion-pairing, and π-π interactions. Hydrogen-bonding interactions is a strong interaction that plays a crucial role in biological recognition systems and in determining the structures of proteins and nucleic acids. The combination of utilizing strong hydrogen-bonding interactions with MIPs will create imprinted polymers with specific recognition properties.

The corticosteroids (Figure 2) are a group of chemically related natural steroid hormones and synthetic steroid compounds that resemble the structure of human adrenal hormone cortisol. Natural corticosteroids are secreted by the adrenal cortex. Cortisol is an important natural steroid hormone and released into circulation in response to stress and low blood-glucose. Synthetic corticosteroids are important therapeutic drugs that are widely used in a diverse range of conditions, for example, asthma, inflammation and pain associated with joint disease and arthritis. The assessment of steroid hormones and their metabolites in clinical settings is essential for diagnosing a wide variety of endocrine disorders and therapeutic applications. The major limitation of currently available corticosteroid immunoassays is cross-reactivity with structurally similar steroid hormones. One potential alternate to address the cross-reactivity issue is designing a synthetic receptor with tailor-made recognition sites that are able to bind preferentially to specific target molecules. This will eliminate cross-reactivity to similarly structured compounds. In past years, several papers have been published dealing with molecular imprinting of corticosteroids such as cortisol and corticosterone. However, attempts have not yet been made to study corticosteroids-monomer interactions, and affinities to the binding mechanism by using quantum chemical modelling. This study seeks to investigate the binding characteristics of the complex formed between different steroids and pyrrole, a monomer widely used in the literature for molecularly imprinted polymers. Since polypyrrole (PPy) is one of the most extensively used conducting polymers in design of bioanalytical sensors, pyrrole is chosen as a functional monomer. Moreover, the MIP films of PPy can be easily deposited onto the biosensors and electrodes using electropolymerization technique for creating biosensors with good reproducibility.

**Experimental**

**Computational details.**—In order to understand the properties of MIP at molecular level and to understand the binding mechanism of the prepolymerization mixture, the template-monomer model complexes were set up. All calculations were performed using the Gaussian 09 electronic structure program. Steroid hormone templates and functional monomer, pyrrole were drawn using GaussView, version 5.0.8. The electronic energies were calculated through DFT using the popular hybrid functional B3LYP. The geometry optimization was performed at the B3LYP/6-31G(d) level. Further all transition states (TS) formed in this study are traced at PM6 level implemented in Gaussian 09. Harmonic vibrational frequencies have been computed for all the species and are characterized as either minima or TS on the
potential energy surface. All minima have real frequencies, while TS have a single imaginary frequency. Animating the imaginary vibrational frequency and computing the intrinsic reaction coordinate aided in connecting the TS to the products and reactants. Each complex has been optimized separately, that is, complexes with one pyrrole as well as those multiple pyrrole (based on the number of binding sites). Cortisol derived steroids (prednisolone and 6-methylprednisolone) present five important binding sites that are, C3, C11, C17, C20, and C21 atoms possessing hydroxyl or ketone group implicated in the interaction with pyrrole. Binding energies were computed as the difference between the energy of the complex and the energy of each molecule Table I.

\[
\Delta E = E(\text{Template} - \text{Monomer complex}) - E(\text{Template}) - E(\text{Monomer})
\]

Results and Discussion

For a family of compounds such as the corticosteroids, several factors may play a part in determining the nature of their biological actions, including size of the molecule, degree of unsaturation and the disposition and nature of substituents. Conformations of the steroid molecules may also affect the binding energy. From the structures of various corticosteroids analyzed (Figure 2), the implications of various functionalities for recognition by MIPs can be deduced. Gas phase binding energy calculation of cortisol with pyrrole show binding energy of 10.9 kcal/mol. Whereas, compounds such as progesterone and 21-dehydroxycortisol with no –OH residue on 21st position,) have binding energies that are representative of the steroid-monomer complex that is reduced (8.2 and 8.4 kcal/mol, respectively). This reduction in the binding energy can be explained by the loss of hydrogen bonding between the steroid hormones and the polymer caused due to the removal of –OH group. A higher binding energy is seen in 21-dehydroxycortisol (8.4 kcal/mol) when compared to progesterone (8.2 kcal/mol). This is due to the presence of –OH group at 17th position, which increase its binding affinity toward the polymer through the formation of hydrogen bonding.

As can be seen from Figure 2, cortisol, prednisolone, and 6-methylprednisolone have almost similar chemical structures. Any slight change in the structure of these steroid hormones brings out a difference in binding energy of the complex. For example, 6-methylprednisolone only differ from prednisolone by a –CH3 group at 6th position yet this is enough to signal an energy change. The binding energy calculation revealed that the 6-methylprednisolone (9.7 kcal/mol) has lesser binding affinity than prednisolone (10.7 kcal/mol). This presence of the extra –CH3 group at the 6th position causes the increase in steric constrains of the 6-methylprednisolone structure compared to prednisolone.

The difference in binding is very close between cortisol and prednisolone as they have similar structural characteristics except for a double bond between C1 and C2 position in prednisolone. The computational results are in good agreement with the previously published research on homology modelling of the ligand-binding domain of glucocorticoid receptor.13 As reported, binding to the specific hormone receptors is acutely dependent on the presence of the 3-one in the A-ring, which is the characteristic structural feature of all biologically active glucocorticoid, where both the entities differ by a double bond. The double bond in prednisolone decreases the binding energy (10.7 kcal/mol) compared to cortisol (10.9 kcal/mol). Earlier Ramstrom et al.,10 Baggiani et al.,12 investigated that steroidal structural motifs able to increase or decrease the molecular recognition of

### Table I. Binding energies of template-monomer complex.

| Steroid             | Optimized energy (kcal/mol) | Optimized energy of complex (kcal/mol) | Monomer: steroid ratio | ΔEC₃ (kcal/mol) |
|---------------------|-----------------------------|--------------------------------------|------------------------|----------------|
| Cortisol            | −740719.67                  | −871031.41                           | 1:1                    | 10.9           |
| Prednisolone        | −739979.78                  | −870291.3765                         | 1:1                    | 10.7           |
| 6-methylprednisolone| −764272.6211                | −870179.2780                         | 1:1                    | 9.7            |
| Progesterone        | −600593.34                  | −730902.4009                         | 1:1                    | 8.2            |
| 21-dehydroxycortisol| −693955.6250                | −824227.4441                         | 1:1                    | 8.4            |
| Pyrrole             | −130300.8137                | -                                    | -                      | -              |
corticosteroids. Their study indicates that the presence of a double bond on the steroidal ring A (between C1 and C2 position in prednisolone) decreases the flexibility of ring A (Figure 3) by reducing the binding affinity of prednisolone slightly. It can be seen from the results that a small change in the number and position of functional groups on the steroidal nucleus results in a significance difference in the binding energy. Biological activity of the steroids also varies with considerably with small structural changes. Combining the structural details of steroidal hormones with biochemical knowledge showed how variations in the functional groups substituted at specific positions around their scaffold were related to specific biological activity; hence allowing new medicines to be developed.

Experimental results also leads to the conclusion that the interference of prednisolone is higher in commercial cortisol affinity binding assays. Moreover, as recently reviewed by Krasowski et al., commercially available cortisol affinity assays still have cross-reactivity of more than 100% with the analogs, especially prednisolone. An important finding from this study is the increasing H-bond strength and binding energy with increasing number of double bonds around the carbonyl group at C3, which could be used as a basic clue to design specific imprinted polymers.

Conclusions

Computational modelling coupled with DFT calculations were used to evaluate the capability of polypyrrole to bind steroid hormones for future applications. Five different steroids with the functional group changes at C1, C17, and C21 were studied. The results show that the functional group changes (C1, C17, and C21) brings out the changes in binding energy. An important finding is the decrease in the binding energy with the inclusion of in the double bond in ring A. Though the difference in binding energy of the material will help to improve the

![Figure 2](image1.png)

Figure 2. Structural formula of corticosteroid derivatives and pyrrole. The A, B, C, and D in cortisol structure represents the four different rings in steroids.

![Figure 3](image2.png)

Figure 3. Most stable complexes of steroids with pyrrole at optimized conditions. Geometry optimization was performed at the B3LYP/6-311G (d) level using Gaussian 09.
affinity toward the detection particular steroid, in clinical samples the complete removal of interference from structurally similar steroids needs to be analyzed further with complicated polymer substrates.

Acknowledgments

This work was supported by the National Science Foundation (NSF) ASSIST Nanosystems ERC (EEC-1160483). The authors acknowledge the Instructional & Research Computing Center (IRCC) at Florida International University for providing HPC computing resources that have contributed to the research results reported within this paper, web: http://ircc.fiu.edu.

References

1. G. Vasapollo, R. Del Sole, L. Mengola, M. R. Lazzoi, A. Scardino, S. Scorrano et al., “Molecularly imprinted polymers: present and future prospective.”, Int. J. Mol. Sci. 12, 5908 (2011).

2. A. Poma, A. P. F. Turner, and S. a. Piletsky, “Advances in the manufacture of MIP nanoparticles,” Trends Biotechnol. 28, 629 (2010).

3. K. Haupt, “Imprinted polymers-tailor-made mimics of antibodies and receptors.”, Chem. Commun. (Camb), 171 (2003).

4. K. Haupt and K. Mosbach, “Molecularly imprinted polymers and their use in biomimetic sensors.”, Chem. Rev. 100, 2495 (2000). (accessed June 10, 2015).

5. G. Wulff, “The role of binding-site interactions in the molecular imprinting of polymers.”, Trends Biotechnol. 11, 85 (1993).

6. Y. Xing, M. A. Edwards, C. Ahlem, M. Kennedy, A. Cohen, C. E. Gomez-Sanchez et al., “The effects of ACTH on steroid metabolomic profiles in human adrenal cells.”, J. Endocrinol. 209, 327 (2011).

7. R. Fraser, M. C. Ingram, N. H. Anderson, C. Morrison, E. Davies, and J. M. C. Connell, “Cortisol Effects on Body Mass, Blood Pressure, and Cholesterol in the General Population.”, Hypertension. 33, 1364 (1999).

8. J. E. Graham, L. M. Christian, and J. K. Kiecolt-Glaser, “Stress, age, and immune function: toward a lifespan approach.”, J. Behav. Med. 29, 389 (2006).

9. M. D. Krasowski, D. Drees, C. S. Morris, J. Maakestad, J. L. Blau, and S. Ekins, “Cross-reactivity of steroid hormone immunoassays: clinical significance and two-dimensional molecular similarity prediction.”, BMC Clin. Pathol. 14, 33 (2014).

10. O. Ramström, L. Ye, and K. Mosbach, “Artificial antibodies to corticosteroids prepared by molecular imprinting.”, Chem. Biol. 3, 471 (1996).

11. C. Baggiani, G. Giraudi, F. Trotta, C. Giovannoli, and A. Vanni, “Chromatographic characterization of a molecular imprinted polymer binding cortisol.”, Talanta. 51, 71 (2000).

12. C. Baggiani, P. Baravalle, C. Giovannoli, L. Anfossi, and G. Giraudi, “Molecularly imprinted polymers for corticosteroids: Analysis of binding selectivity.”, Biosens. Bioelectron. 26, 590 (2010).

13. R. Dey, P. Roychowdhury, and C. Mukherjee, “Homology modelling of the ligand-binding domain of glucocorticoid receptor: binding site interactions with cortisol and corticosterone.”, Protein Eng. 14, 565 (2001).

14. S. Tunn, G. Pappert, P. Willnow, and M. Krieg, “Multicentre evaluation of an enzyme-immunoassay for cortisol determination.”, J. Clin. Chem. Clin. Biochem. Zeitschrift Ft Klin. Chemie Und Klin. Biochem. 28, 929 (1990). (accessed December 25, 2015).

15. I. A. Ionita, D. M. Fas, and F. Akhlaghi, “Development of a sensitive and selective method for the quantitative analysis of cortisol, cortisone, prednisolone and prednisone in human plasma.”, J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 877, 765 (2009).