# Towards Rational *de novo* Design of Peptides for Inorganic Interfaces

M.J. Biggs† and M. Mijajlovic

Institute for Materials and Processes, University of Edinburgh
King's Buildings, Mayfield Road, Edinburgh, UK.

## ABSTRACT

Protein adsorption at inorganic surfaces is highly relevant to nano- and bionanotechnology. Just two examples of great significance are the use of specific peptide sequences to control cell deposition on tissue scaffolds, and the tethering of bio-photosynthetic reaction centers on electrodes to harvest light for power generation and hydrogen production. Understanding of the behavior of proteins in such situations and the design of surface-binding proteins for technological applications is currently limited by the largely empirical approaches used. We are, therefore, developing the application of molecular modeling to the elucidation of the behavior of peptides at fluid/solid interfaces – in this talk, we will provide details of these models and their application to the study of peptides at fluid/solid interfaces and their *de novo* rational design.

***Keywords***: biosensors, nanoelectronics, nanophotonics, surface binding peptides, tissue engineering.

## 1 INTRODUCTION

Protein adsorption at inorganic interfaces occurs across science, engineering, medicine and nature [1]. Protein adsorption is, for example, the first step in the body's response to implants such as artificial heart valves [2] that ultimately may lead to complications and even life-threatening reactions such as emboli. Improvements in our understanding of this response are now being exploited to develop new implant technologies [2]. Similar approaches are also underpinning the next generation of tissue scaffolds to improve spatial control over cell adhesion, which is essential for growing all but the simplest tissue [3]. Protein adsorption and migration on solid surfaces are central to bioseparations [4], fouling in the processes industries and beyond [5], and biosensors and bioarrays [6-8]. Examples of protein adsorption important in the natural world include antifreeze proteins (AFPs) that allow some species to survive at sub-zero temperatures by inhibiting ice crystal growth [9], and proteins that aid biomineralization, a process responsible for the formation of all natural inorganic materials such as bones, teeth and egg shells [10]. Such examples from nature are now inspiring new 'biomimetic' technologies. By borrowing ideas from AFPs, a number of groups have in recent years developed peptides that control crystal growth [11], for example, whilst some are developing the use of peptides to self-assemble nanoscale entities to form complex multiscale structures [11].

Experimental challenges mean fundamental understanding of the behaviour of proteins at inorganic interfaces is still in its infancy [11]. For example, the role and actions of various proteins during biomineralization is still much debated [12] – this in part arises from our current inability to determine the three-dimensional conformation of biomolecules at solid interfaces and the associated interactions. This lack of fundamental understanding means the design of peptides that recognise specific inorganic surfaces for technological uses is also very much empirical (*e.g. ref.* [13]), albeit sometimes supported by sequence analysis methodologies [14]. Whilst experiment must undoubtedly play a central role in improving fundamental understanding and in any design of peptide/surface systems, molecular simulation can also play a major role.

In this talk, we will outline how we are applying molecular simulation to the elucidation of the behavior of peptides at fluid/solid interfaces and the rational *de novo* design of peptides that recognize such interfaces with specificity.

## 2 METHODS

The methodology being developed at Edinburgh for the rational *de novo* design of peptides that preferentially bind at a target solid-fluid interface is shown in Fig. 1. Briefly, a physicochemical-based *in silico* evolutionary process is used to identify sets of peptide sequences that may potentially bind to the target interface. Once identified, these sequences are synthesized and subject to experimental characterization to determine the validity of the predictions. If the predictions are poor, the experimental data is used to improve the models underpinning the *in silico* evolutionary process. If, on the other hand, the predictions prove reasonable, data obtained from sequence analysis is fed back to inform further searches if this is warranted. Data from *in vivo* or *in vitro* combinatorial searches can also be used as input to the process.

An essential component of the *in silico* evolutionary process is the prediction of the binding energy of candidate sequences using physicochemical models of the binding process. As a typical *in silico* evolutionary simulation requires many 1000s of such predictions, an *ab initio* structure prediction approach [16] is used to determine the global free energy minimum of candidate sequences in the bulk phase and at the target interface. The semi-explicit

† Fax: +44-131-650-6551. E-mail: m.biggs@ed.ac.uk.

Langevin dipole approach [17] is used to model solvents [18], which we have shown to be O($10^2$) times faster than explicit solvent methods yet is still able to resolve phenomena such as hydrogen bond bridging and solvent structuring between the peptide and solid surface.

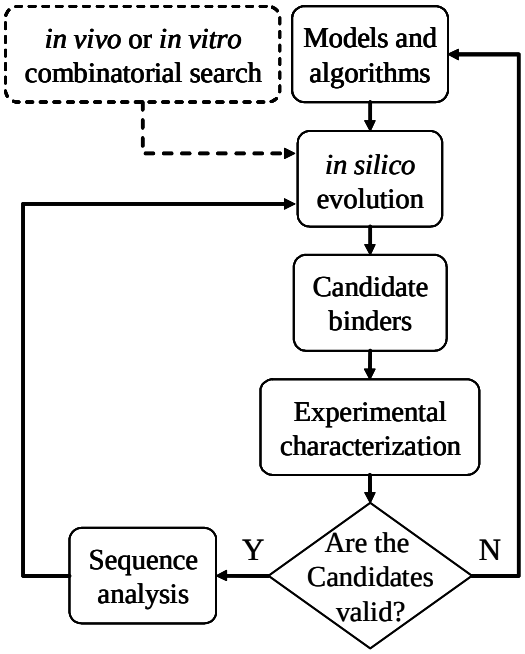


**Figure 1.** The rational *de novo* design methodology being developed.

## 3 RESULTS AND DISCUSSION

We have applied the *ab initio* structure and free energy prediction method to a variety of peptides in the gas and liquid phases, and at fluid/solid interfaces [19, 20]. A recent example is the pentapeptide met-enkephalin at the water/graphite interface [20]. The intrapeptide and peptide-graphite interactions were modeled using the Amber94 PE model [21] with the graphite carbon atoms being assumed to be equivalent to the aromatic carbon atoms of this PE model. The peptide-graphite and water-graphite interactions were modeled using the LD-Amber method [22], with the graphite carbon atoms once again being modeled as $sp^2$ hybridized C atoms; the evaluated water-graphite interfacial energy obtained using this approximation was found to be inline with experimental data.

Fig. 2 shows the lowest energy structure of zwitterionic met-enkephalin in water. The extended structure seen here is in good agreement with other simulation studies [23] and is inline with experimental results which suggest this molecule can take a range of extended structures in dilute solutions [24-28]. The experimental studies also suggest met-enkephalin in water is flexible. Although the EA cannot offer direct evidence for such flexibility or otherwise, the hydrogen-bonding extant in the structure predicted here suggests it is likely to be flexible – there is a single hydrogen bond (HB) between the CO-group of the first glycine and NH-group of the phenylalanine residue, shown as a dashed red line in Fig. 2, whilst the only groups that can support HB bridging are the CO-groups of the phenylalanine residue and C-term, and the NH-groups of the N-term and first glycine residue.

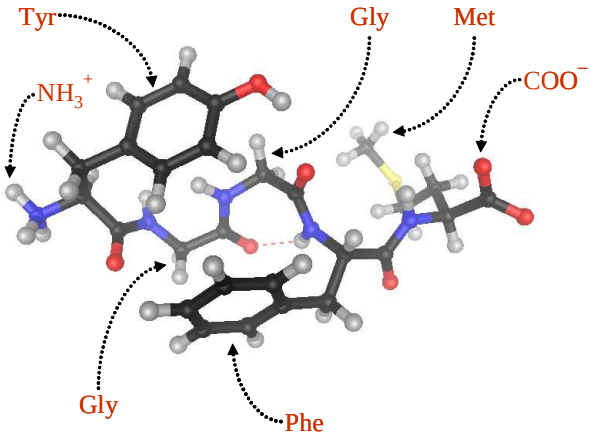


**Figure 2.** Predicted conformation of zwitterionic form of met-enkephalin in water.

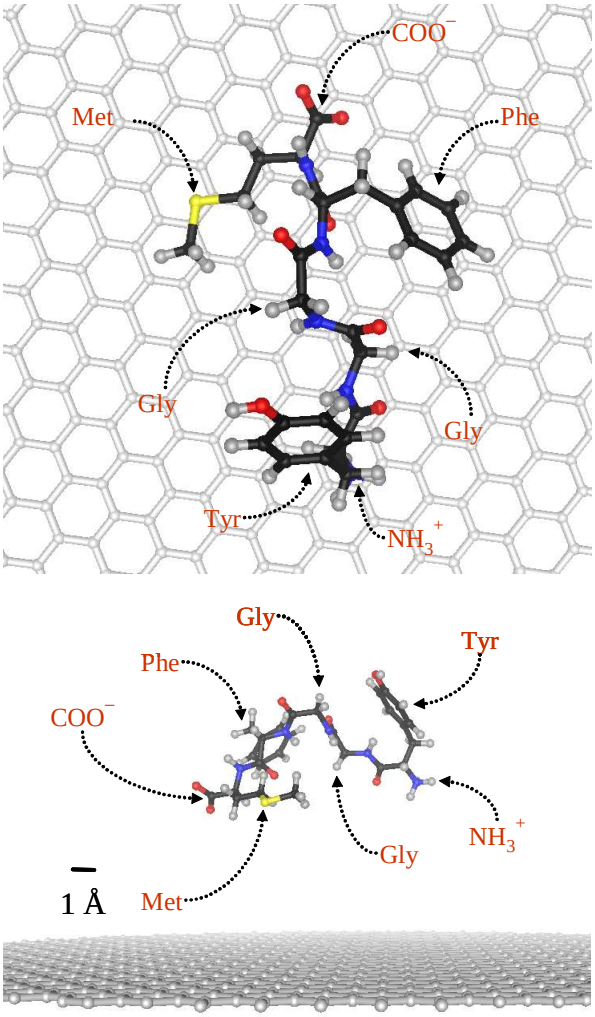


**Figure 3.** Predicted structure of zwitterionic form of met-enkephalin at water/graphite interface.

Fig. 3 shows the predicted structure of the zwitterionic form of met-enkephalin at the water/graphite interface. This figure shows that the sole HB of the solvated structure in Fig. 2 is absent, leading to an even more extended structure. Although this conformation is preferred to the bulk phase structure by slightly less than 20 kcal/mol, the peptide is located approximately 9 Å from the graphite surface, which corresponds to three layers of water between the surface and the peptide. As suggested by this degree of separation and confirmed in Fig. 4, the adsorbed state is water-mediated rather than *via* dispersive interaction between the peptide and graphite.

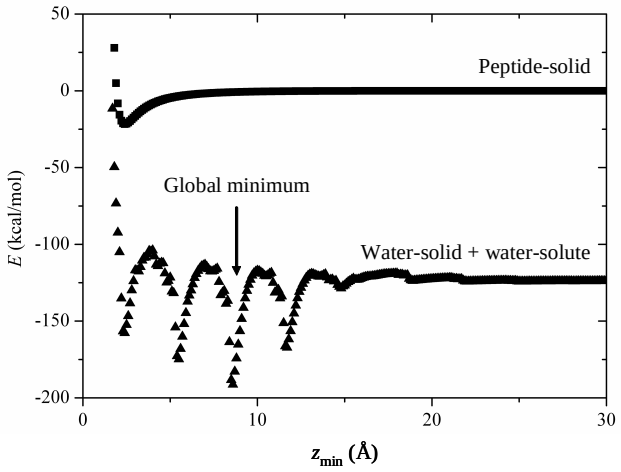


**Figure 4.** The variation of the energy associated with the peptide-graphite and water-graphite + water-peptide interactions with the shortest distance between the graphite and the peptide in its adsorbed conformation.

Upon removal of the water, Fig. 5 shows that met-enkephalin flattens out and becomes more closely associated with the graphite surface via π-bonding between the graphite and the benzene rings of the phenylalanine and tyrosine residues. The structure is also less extended due to the strong interaction between the oppositely charged N- and C-terms that are no longer shielded by water.

## 4 CONCLUSIONS

We have developed a means of rapidly predicting the structure and binding energy of peptides at fluid/solid interfaces. We are now extending the approach to various surfaces (e.g. metals where electron polarization is likely to be important) and looking to apply it to identify solid surface binding peptides of high specificity that will facilitate, amongst other things, the self-assembly of nanoelectronic devices such as carbon nanotube based field effect transistors and nanoelectronic systems based on such devices.

## ACKNOWLEDGMENTS

MM thanks the University of Edinburgh and UUK for PhD scholarships. MJB thanks the Royal Academy of Engineering and the Leverhulme Trust for partial support of this research.

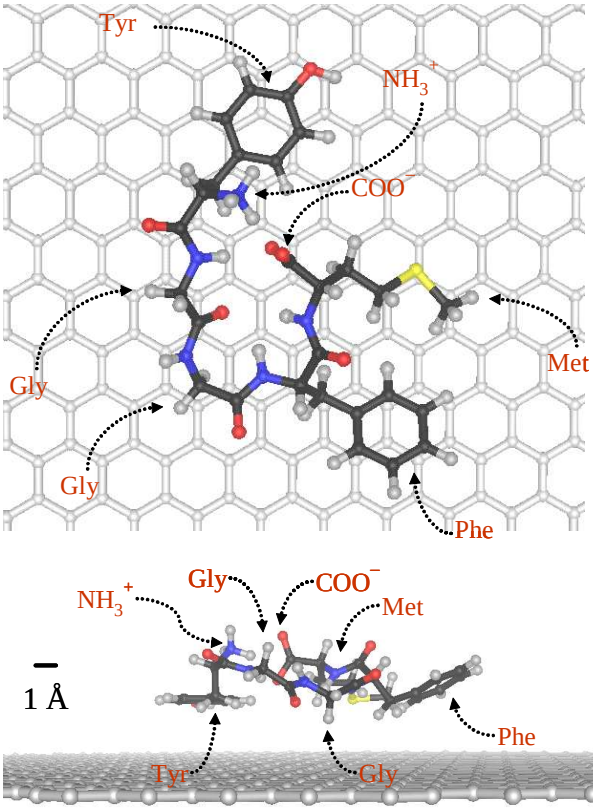


**Figure 5.** Predicted structure of zwitterionic form of met-enkephalin at water/graphite interface.

## REFERENCES

[1] B. Kasemo, Surf. Sci. 500, 656, 2002.
[2] B.D. Ratner and S.J. Bryant, Annu. Rev. Biomed. Eng. 6, 41, 2004.
[3] H. Shin, S. Jo, and A.G. Mikos, Biomaterials 24, 4353, 2003.
[4] T.M. Przybycien, N.S. Pujar and L.M. Steele, Curr. Op. Biotech. 15, 469, 2004.
[5] H.-C. Flemming, Appl. Microbiol. Biotechnol. 59, 629, 2002.
[6] J. Castillo, S. Gáspár, S. Leth, M. Niculescu, A. Mortari, I. Bontidean, V. Soukharev, S.A. Dorneanu, A.D. Ryabov and E. Csöregi, Sensors & Actuators B 102, 179, 2004.
[7] C. Hultschig, J. Kreutzberger, H. Seitz, Z. Konthur, K. Büssow and H. Lehrach, Curr. Op. Chem. Biology 10, 4, 2006.
[8] K.E. Sapsford, Y.S. Shubin, J.B. Delehanty, J.P. Golden, C.R. Taitt, L.C. Shriver-Lake and F.S. Ligler, J. Appl. Microbiol. 96, 47, 2004.
[9] Y.-C Liou, A. Tocilj, P.L. Davies and Z. Jia, Nature 406, 322, 2000.
[10] S. Weiner and L. Addadi, Mater. Chem. 7, 689, 1997.
[11] M. Sarikaya, C. Tamerler, A.K.Y. Jen, K. Schulten and F. Baneyx, Nature Mat. 2, 577, 2003.

[12] S. V. Patwardhan, G. Patwardhan and C.C. Perry, J. Mat. Chem. 17, 2875, 2007.
[13] P. Schaffner and M.M. Dard, Cell. Mol. Life Sci. 60, 119, 2003.
[14] C. Tamerler and M. Sarikaya, Acta Biomat. 3, 289, 2007.
[15] D.J. Osguthorpe, Curr. Op. Struct. Biol. 10, 146, 2000.
[16] D.P. Djurdjevic and M.J. Biggs, *J. Comp. Chem.* 27, 1177, 2006.
[17] J. Florián and A. Warshel, J. Phys. Chem. B 101, 5583, 1997.
[18] M. Mijajlovic and M.J. Biggs, J. Phys. Chem. B 111, 7591, 2007.
[19] D.P. Djurdjevic, "*Ab initio* Protein Fold Prediction Using Evolutionary Algorithms", PhD Thesis, University of Edinburgh, United Kingdom, 2006.
[20] M. Mijajlovic, "*Ab initio* prediction of the conformation of solvated and adsorbed proteins", PhD Thesis, University of Edinburgh, United Kingdom, 2008.
[21] W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, K.M. Merz, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell and P.A. Kollman, J. Am. Chem. Soc., 117, 5179, 1995.
[22] M. Mijajlovic and M.J. Biggs, J. Phys. Chem. B, 111, 7591, 2007.
[23] M. Kinoshita, Y. Okamoto and F. Hirata, J. Am. Chem. Soc., 120, 1855, 1998.
[24] B.P. Roques, C. Garbay-Jaureguiberry, R. Oberlin, M. Anteunis and A.K. Lala, Nature, 262, 778, 1976.
[25] C.R. Jones, V. Garsky and W.A. Gibbons, Biochem. Biophys. Res. Commun., 76, 619, 1977.
[26] M.A. Khaled, M.M. Long, W.D. Thompson, R.J. Bradley, G.B. Brown and D.W. Urry, Biochem. Biophys. Res. Commun., 76, 224, 1977.
[27] M.A. Spirtes, R.W. Schwartz, W.L. Mattice and D.H. Coy, Biochem. Biophys. Res. Commun., 81, 602, 1978.
[28] W.H. Graham, E.S.I. Carter and R.P. Hicks, Biopolymers, 32, 1755, 1992.