University of Wollongong

Research Online

Australian Institute for Innovative Materials -
Papers

Australian Institute for Innovative Materials

1-1-2013

Surface and biomolecular forces of conducting polymers

Michael J. Higgins

University of Wollongong, mhiggins@uow.edu.au

Gordon G. Wallace

University of Wollongong, gwallace@uow.edu.au

Follow this and additional works at: <https://ro.uow.edu.au/aiimpapers>

Part of the [Engineering Commons](#), and the [Physical Sciences and Mathematics Commons](#)

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Surface and biomolecular forces of conducting polymers

Abstract

In this review, we provide insight into the surface forces of conducting polymers, a class of intelligent materials that offer unique strategies for controlling biomolecular interactions in wide-ranging biomedical applications. Critical to the success of these applications is that the polymer interface is exposed to biological fluids whose interactions are controlled through the polymer surface chemistry and electrochemical switching of the surface properties. There is, however, little known about the intermolecular and surface forces that govern these interactions. Therefore, the purpose of this review is to more closely examine the forces that mediate interactions with biological entities, including forces such as van der Waals, electrostatic, hydrophobic, and hydrogen bonding. We introduce relevant surface properties such as surface energy and surface potential, and demonstrate how they manifest as forces. In particular, we highlight the emerging use of Atomic Force Microscopy for directly measuring these forces at the single molecule level; a unique capability that is enabling deconvolution of complex biomolecular interactions with conducting polymers. Finally, we provide an overview of biomolecular interactions, namely model proteins and DNA, and conclude by discussing a growing area of interest; the spatio-temporal and reversible control of biomolecular forces via electrical stimulation.

Keywords

conducting, polymers, forces, surface, biomolecular

Disciplines

Engineering | Physical Sciences and Mathematics

Publication Details

Higgins, M. J. and Wallace, G. G. (2013). Surface and biomolecular forces of conducting polymers. *Polymer Reviews*, 53 (3), 506-526.

Surface and Biomolecular Forces of Conducting Polymers

Michael J. Higgins & Gordon G. Wallace

ARC Centre of Excellence for Electromaterials Science (ACES),

Intelligent Polymer Research Institute (IPRI),

AIIM Facility, Innovation Campus,

University of Wollongong,

Squires Way, Fairy Meadow, NSW, 2519, Australia

Received Date

Assoc. Prof. Michael Higgins

Email: mhiggins@uow.edu.au

Tel: +61-2-4221-3989

Fax: +61-2-4221-3114

Abstract

In this review, we provide insight into the surface forces of conducting polymers, a class of “intelligent” materials that offer unique strategies for controlling biomolecular interactions in wide-ranging biomedical applications. Critical to the success of these applications is that the polymer interface is exposed to biological fluids whose interactions are controlled through the polymer surface chemistry and electrochemical switching of the surface properties. There is however little known about the intermolecular and surface forces that govern these interactions. Therefore, the purpose of this review is to more closely examine the forces that mediate interactions with biological entities, including forces such as van der Waals, electrostatic, hydrophobic and hydrogen bonding. We introduce relevant surface properties such as surface energy and surface potential, and demonstrate how they manifest as forces. In particular, we highlight the emerging use of Atomic Force Microscopy for directly measuring these forces at the single molecule level; a unique capability that is enabling deconvolution of complex biomolecular interactions with conducting polymers. Finally, we provide an overview of biomolecular interactions, namely model proteins and DNA, and conclude by discussing a growing area of interest; the spatio-temporal and reversible control of biomolecular forces via electrical stimulation.

Keywords: Forces, Biomolecular Interactions, Polypyrrole, PEDOT, Conducting Polymers, Atomic Force Microscopy

1.0 Introduction

Conducting polymers such as polypyrrole, poly(3,4-ethylenedioxythiophene) and polythiophene are rapidly developing as electromaterials for use in biomedical applications¹. Through their integration with other materials, solution processing and fabrication capabilities, the discovery of unique functions and applications (e.g. drug delivery) and improved stability, conducting polymers are poised to have a major impact. It is conceivable that in the not too distant future they will form an integral component in a clinically applied medical device (e.g. electrode coating) and become ubiquitous in the longer-term as polymer electronics continues to advance. As an electrode for stimulation and recording, conducting polymers are attractive due to their controllable surface roughness and high porosity that significantly increases charge injection capacity and lowers impedance². Their low modulus enables development of flexible electrodes more suited to soft biological tissues³, whilst their solution processing capabilities are amenable to additive fabrication techniques which are envisaged to overcome the complex fabrication of esoteric structures demanded by medical applications (e.g. scaffolds for tissue regeneration)⁴.

Beyond their operation as a stimulating or recording electrode, much of the research interest has been in their unique properties that mimic biological functions and enable true integration with the biological environment⁵. A principle mechanism of several biomedical applications is their electronic-to-ionic current conversion through oxidation and reduction that enables electrically controlled ion/water transport and exchange at the polymer-liquid interface. Small, charged species such as drugs incorporated into the polymer can be electrically released for delivery of therapeutics to the local environment⁶. Oxidation and reduction of the polymer also has dramatic effects on surface properties, thus providing a means to control the adsorption of proteins and other biomolecules native to cellular and

tissue environments⁷. The above attributes as well as the ease at which conducting polymers can be functionalized with ligands/recognition molecules, either through physical entrapment, non-covalent and covalent binding has led to application in several areas, including implantable flexible electrodes⁸, scaffolds for tissue regeneration and engineering⁹, drug release polymers and implants¹⁰, in vitro cell culture systems¹¹, biosensing¹², bioseparation and bioremediation¹³.

Critical to this review is that in many of the above applications the polymer surface is exposed to interstitial fluids and blood and will most often come into contact with various biological entities such as salts, polysaccharides, fatty acids, enzymes, hormones, neurotransmitters and cell adhesion proteins. In applications such as biosensing, bioseparation and bioremediation, the purpose is to selectively and then reversibly bind one or more of these biological entities to enable a multi-use device. For better electrode integration and performance, conducting polymer coatings on implantable electrodes or tissue regeneration scaffolds aim to promote an optimal host tissue response through selective recruitment and adhesion of cells¹⁴. This is done by controlling the rate, extent and type of host protein adsorption, which triggers monocyte/macrophage recruitment and activation, proliferation, and activation of other cell populations in the inflammatory response. Similarly for in vitro applications, conducting polymers are used as substrates in cell culture systems to control cell adhesion, proliferation and differentiation, by controlling the binding of serum proteins or growth factors at the polymer surface¹⁵.

A common thread is the necessity to control interactions by the processes of adsorption and binding of biomolecules to the polymer surface. Therefore, the purpose of this review is to more closely examine these processes with a special focus on the intermolecular

and surface forces that mediate the biological interactions, including forces such as van der Waals, electrostatic forces, hydrophobic and hydrogen bonding¹⁶. These forces are typically categorized into two different groups, Liftshitz-van der Waals (non-polar, dispersive forces) and Lewis acid-base or polar forces. Some of these forces act over a shorter range to determine the adhesion strength and binding energies, while others are longer range and influence the path-finding and docking of molecules onto a surface. Generally, these forces are considered to be non-specific (they occur between many different biological entities) and, for example, may lead to non-specific adsorption of proteins onto a conducting polymer electrode. Specific interactions arise from a unique combination of forces that act cooperatively in a directional manner (e.g. complimentary bonds) to produce even stronger, non-covalent binding. This type of interaction results in formation of molecular complexes, or enables bioactivity of a protein through its binding in a specific orientation. The interactions of biomolecules, particularly proteins, often occur immediately upon initial exposure, or “first kiss”, of the material-liquid interface and are critical for the success of an implanted device. However, a major limitation is non-specific binding of interfering species or host proteins resulting in poor outcomes such as loss of selectivity in biosensors or increased impedance of implanted electrodes. Given the extensive work on the development of conducting polymer biosensors^{12,17}, an area that greatly depends on specific binding, there has been surprisingly little foray into directly quantifying or modelling the intermolecular and surface forces of conducting polymers. Understanding forces in this area will provide a deeper insight the interactions that control biological events *in vitro* and *in vivo*, particularly as conducting polymers are further modified, functionalized and electrically stimulated to control non-specific and specific interactions for use in biomedical applications.

In this review, we first define the chemical structure of typical conducting polymers and some biologically relevant dopants. It is the chemical structures, the interactions

between the polymer and dopants, and redox states that determine surface properties most relevant to intermolecular and surface forces. We provide a summary of the surface properties, including surface energy and surface potential, which govern the adhesion and interaction energies of conducting polymers. Based on known values for surface energy and zeta potential, one can use theory to predict the magnitude of forces and binding energies; examples of theoretical values calculated for conducting polymer nanoparticle-nanoparticle and protein-conducting polymer interactions are provided. To verify such forces, force measuring techniques are used to directly quantify the forces between different surfaces at the nanoscale and molecular levels. We specifically detail the growing use of Atomic Force Microscopy (AFM) for characterizing the interaction forces of conducting polymers, including those involving adhesion, electrostatic forces, entropic forces of single polymer chains and specific binding of proteins. Furthermore, the interaction forces are often measured as a function of the polymer redox state. Finally, we provide overview of biomolecular interactions for different biomolecules, namely model proteins and DNA. We conclude with a perspective on our current understanding of the forces and approaches for improving the design of conducting polymers by drawing on our knowledge in this area.

1.1 Chemical Structure and Interfacial Groups

Conducting polymers have a repeating ring structure with conjugated backbone structure. In their neutral form they are non-conducting and become conducting when oxidized. The charge associated with the oxidized form is generally delocalised over 2-3 repeating units and in the form of radical cations. Oxidation and reduction results in the reversible intercalation of anions termed dopants that maintain electroneutrality, as shown in Figure 1A for polypyrrole (PPy) doped with chloride (Cl^-) ions. The charge from oxidation is again

confined over 2-3 repeating units localized closest to the chloride ion. At the polymer-water interface, the translational diffusion coefficient of Cl^- ions diminishes by two orders of magnitude as they move from bulk water to the PPy interior¹⁸. The Cl^- ions progressively lose their hydration shell, which is compensated by coordination around charge sites on the polymer. The PPy^+/Cl^- polymer in its oxidized form thus presents a hydrophilic, zwitterionic surface that can be electrochemically reduced to a neutral surface of comparable lower surface energy. Unlike mobile Cl^- ions, larger dopants such as anionic surfactants remain entrapped in the polymer during oxidation and reduction (Figure 1B). In the oxidized form, sulfate groups of the dodecylbenzenesulfonate (DBS^-) coordinate with charged sites, while the fatty acid groups extend into the polymer-liquid interface^{19,20}. The orientation of the DBS^- is reversed for the reduced form as it is more energetically favourable for the fatty acids to coordinate through hydrophobic interactions with the neutral polymer backbone. This use of less mobile dopants provides an approach to stably and reversibly switch between low and high energy surfaces. Surface properties such as charge, energy and their related interactions are thus determined by the oxidation state of the polymer and physiochemical interactions of the dopants.

1.2 Surface Energy and Zeta Potential

Surface energy determines the wetting and adhesion properties of a material surface. Inverse gas chromatography (IGC) and contact angle (CA) measurements are common techniques used to measure surface energy of conducting polymers. For IGC, a chromatographic column is packed with the material under study and molecular probes (e.g. series of alkanes) are injected at an infinite dilution. The thermodynamics of the polymer-probe interaction is given by a measured retention volume, which describes the elution behaviour of the solute and used

to quantify surface energies^{21,22}. CA measurements are much simpler and quicker but still enable very high sensitivity. The contact angle can be related to the surface tension or energy via Young's Equation, $\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\cos\theta$ (eq.1), where θ is the measured contact angle and γ is the surface energy of the solid-vapour (sv), solid-liquid (sl) and liquid-vapour (lv) interface. γ_{lv} and θ are measureable parameters and several models using expressions combined with eq. 1 are applied to determine the desired γ_{sl} of the material^{23, 24}. Depending on the model, the procedure typically involves measuring the contact angle of two or three test liquids to differentiate the Liftshitz-van der Waals (dispersive) and Lewis acid-base (polar) properties components of the surface energy.

Table 1 shows the surface energies of conducting polymers and their different dopants, their dispersive and polar contributions where available (dispersive are most common) and temperatures used to obtain the measurements. For the main types of different conducting polymers, a range of surface energies exists due to dependencies on the type of polymer, dopant and different temperatures used in experiments. PPy, poly(3,4-ethylenedioxythiophene) (PEDOT) and polyaniline (PANI) can generally be considered medium to high surface energy materials, while poly(3-hexylthiophene) (P3HT) and poly(3-octylthiophene) (POT) have much lower surface energies^{25,26, 27}. This is highlighted in Figure 2 where a general range of PPy and PANI surface energies is compared with values obtained for conventional insulating polymers²⁸ and other materials/liquids such as metal/metal oxides, carbons, organic solvents and water^{29,30,31, 32}. Dedoped polymers have similar surface energies to conventional insulating polymers whereas the surface energy of doped polymers is a function of the dopant type and concentration²⁴. Doped polymers have high surface energies as a consequence of their conductivity, namely the presence of radical cations and ions. Strong hydrophobic groups (e.g. tolyl groups) of the dopants, or those bearing hydroxyl and carboxyl groups that can form intermolecular hydrogen bonds, will nevertheless vary the

wetting properties of the polymer. For example, the low surface energy of POT even after doping is likely due to the octyl pendent groups²⁵, which have similar surface energies of 23 mJ/m. Liftshitz-van der Waals (dispersive) and non-polar interactions mainly contribute to the lower surface energy of P3HT, as is plausible due to segregation of alkyl chains at the surface²⁴. In contrast, the high surface energy character of PPy and PANI is suggested to occur from Lewis acid-base contributions, giving them the ability to bind both acidic and basic species²⁵. PPy has predominately Lewis acidity, with the acidic sites possibly being the most energetic and due to N-H bonds on the pyrrole acting as electron-pair acceptors and /or positively charged backbone. PANI on the other hand behaves as a Lewis base (n-donor). As expected, Lewis acid-base interactions significantly contribute to the PEDOT surface energy, especially acidic groups, which indicate exposure of dopant anions at the surface.

A change in doping levels also modifies the surface energy. An increase from 15% up to 30% in doping levels for PPy⁺/nitrate (NO₃⁻) results in a doubling of the surface energy (from 50 to 110 mJ/m²)²⁵. Alternatively, the surface energy significantly decreases by increasing the ratio of PPy to a hydrophobic surfactant dopant such as sodium *bis*(2-ethylhexyl) sulfosuccinate³³. An interesting but important phenomenon to consider when working with some conducting polymers is that the surface energy shows a decline over days or weeks. PPy⁺/Cl⁻ and PPy⁺/sulfate (SO₄⁻) show decreases of up to 17-50 mJ/m² compared to PPy⁺/tosylate (TOS⁻) (5.2 mJ/m²) and PANI (1.4 mJ/m²), which are relatively stable³⁴. Similar studies reveal temporal effects on surface energy decline, with the related to an increase in C=N bond defects³⁵. Though not yet fully understood, one other explanation is that the decline in surface energy may be due to irreversible binding of adsorbents (e.g. hydrocarbons) on high energy sites until saturation, as is common occurrence for contamination of high energy surfaces. Whilst high surface energy polymers easily adsorb molecular species in air, they inherently form low energy interfaces in fluid.

A special feature of conducting polymers is their ability to dynamically and reversibly switch the surface energy using electrical stimulation. In situ dynamic CA measurements with Wilhelmy balance in an electrochemical cell show the contact angle of P3HT⁺/perchlorate (ClO₄⁻) decreases (higher surface energy) and increases (low surface energy) with oxidation and reduction, respectively³⁶. In their neutral dedoped state these films are hydrophobic and become more hydrophilic as charge is injected and ClO₄⁻ ions move into the polymer during the oxidation. This process is reversible during reduction. PEDOT⁺/TOS⁻ films unexpectedly show the opposite behaviour. The decreasing CA during reduction is related to the reorganization of ion distributions at the interface³⁶; in this case sulfate groups of the TOS⁻ switch their orientation to face out at the polymer-liquid interface. This is similar to the situation for the PPy/DBS⁻ (Figure 1B). Therefore, electrical switching of surface energy is dependent on the interplay between dopant type, dopant concentration, doping potential, time, electrolyte ions. Although combined effects of topography and surface chemistry are not discussed here, it is possible to significantly enhance the changes in surface energy by producing highly porous conducting polymer structures³⁷.

The measured potential across an interface consists of contributions from two layers. The layer nearest to the material is that of fixed or bound surface charges termed the Stern Layer and the other from free ions in the diffuse double layer. The double layer is made up of the shear plane and Gouy-Chapman plane and well-known for governing electrostatic forces between charged surfaces and controlling stability of colloidal dispersions. Electrophoresis and streaming potential measurements give the potential value of the shear plane (usually close to the double layer potential) termed the zeta potential. Double layer theory, which is discussed further below, relates the electrostatic force between surfaces to their zeta potential and charge density.

The zeta potential of conducting polymers is dependent on the type of dopant and especially pH. Firstly, the isoelectric points (iep) are generally found either at very high or low pH^{38,39,40,41,42}. This means that the zeta potential, either positive or negative, remains reasonably constant over a large pH range and then abruptly changes at the iep. For example, PPy coated glass beads have a constant zeta potential of +40mV from pH4-10 and iep just over 10 after which the potential drops rapidly to -40mV⁴². These changes in the zeta potential as a function of solution pH are caused by deprotonation and protonation of the polymer. Charged -NH⁺ groups are deprotonated in alkaline solutions and become less positively charged, while acidic solutions restore the positive surface charge due to reprotonation. However, the presence of oxygen groups on dopants such as dodecyl sulfate (DS⁻) and octadecyl sulfate (OS⁻) may dictate the surface charge, leading to a lower iep and negative zeta potentials over a greater range of increasing pH⁴⁰. Therefore, because the iep of PPy particles are typically in the low or high pH range, they will most often carry a significant charge under neutral pH conditions relevant to biological systems and have a propensity to bind oppositely charge proteins (Figure 3).

Surface potential values of electropolymerized films are more difficult to obtain though kelvin probe force microscopy (a variant of AFM) shows highly localized variations in surface potential that correlate with the characteristic morphology of conducting polymer films^{43,44}. These measurements reveal that the surface potential is influenced by numerous factors, including polymerization times, film thickness, prior exposure to electrolytes and the underlying electrode substrate.

1.3 Intermolecular and Biological Forces

Measuring forces is important for understanding the interaction between molecules, particles and surfaces. Information on when they arise, whether they are attractive or repulsive, their magnitude and the distances over which they occur can shed light on interactions that control biological events *in vitro* and *in vivo*. Various thermodynamic expressions and theories exist for calculating different types of energies and forces and can be used to reasonably predict their magnitude and range between surfaces of interest¹⁶. The variation in energy, $E(D)$, and force, $F(D)$, as a function of distance between two interacting particles or surfaces are related by $F(D) = -dE(D)/dD$ (eq.2) which defines the force at a specified distance as the negative of the energy gradient at D . Covalent bonds with energies of ≈ 1 electron-Volt ($1\text{eV} = 1.6 \times 10^{-19}$ Joules) occur over short distances (0.1 nanometres, nm), thus forces required to break their bonds are on the order of $1.6 \times 10^{-19} \text{ J}/0.1 \text{ nm} \approx 1.6$ nanonewtons (nN). Non-covalent interactions of similar bond energies are much weaker as they typically extend over longer distances ($\approx 1\text{nm}$), thus giving forces of $1.6 \times 10^{-19} \text{ J}/1 \text{ nm} \approx 160$ piconewtons.

Van der Waals (VDW) forces are always present between molecules and surfaces and are either attractive or repulsive but always attractive between similar materials. The VDW force has a power law dependence on D and when related to the Hamaker constant, A , which reflects the strength of interaction between two bodies, can be calculated for various interacting geometries. For example, the VDW force and energies for two interacting particles of radius, R , separated by D is given by:

$$F(D) = \frac{-A}{6D^2} \left(\frac{R_1 R_2}{R_1 + R_2} \right) \quad \text{eq.3}$$

The Hamaker constant is related to the dispersive component of the surface energy, γ_d , between two flat surfaces where $\gamma_d = A/24\pi D_0^2$ (eq.4). D_0 is the cut-off distance for their

effective separation at molecular contact and given as 0.165 nm. Based on typical surface energy (γ_d) values from Table 1, the Hamaker constant for a conducting polymer such as PPy and PANI is $\approx 10 \times 10^{-20}$ J (obtained using a surface energy of 50 mJ m²). Higher values of 25-50 $\times 10^{-20}$ J have been reported for PPy particles³⁹ and are comparable to metals. Therefore, one can estimate the typical VDW force occurring between two PPy nanoparticles of 50 nm in diameter at molecular contact (≈ 0.4 nm = two water layers present) using eq. 3 and obtain a value of -1.3×10^{-9} N or -1.3 nN. The minus sign represents an attractive force. The force increases to -2.6 nN for the same particle interacting with a surface ($F(D) = -AR/6D^2$) (eq.5).

These particles will be subject to long range electrostatic forces that are repulsive for similarly charged surfaces and roughly decay exponentially as a function of D . Again these forces are dependent on the geometry where particle-particle interactions can be given by:

$$F(D) = \kappa \left(\frac{R_1 R_2}{R_1 + R_2} \right) Z e^{-\kappa D} \quad \text{eq.6}$$

$$Z = 64\pi\epsilon\epsilon_0(kT/e_c)^2 \tanh^2(z e_c \psi_o / 4kT) = (9.38 \times 10^{-11}) \tanh^2(\psi_o / 107) \quad \text{eq.7}$$

The Debye length, κ , in eq.6 is the characteristic decay of the interaction determined only by the solution conditions such as type and concentration of ions and temperature. κ falls with increasing ionic strength and for monovalent salt solutions at 37°C has a decay length in nanometres given by $\kappa^{-1} = 0.3/[\text{NaCl in moles/litre}]^{0.5}$. Thus, for physiologically concentrations of NaCl (0.15M) the decay length is 0.78nm. The constant, Z (J m⁻¹), is analogues to the Hamaker constant for the VDW force and is given in eq.7 where ϵ is the dielectric constant of the medium, ϵ_0 is the vacuum permittivity, k is Boltzmann's constant, T is temperature, e_c is unit charge, z is the valency of ions in solution ($z = 1$ for monovalent salts) and ψ_o is the surface potential. If the surface potential is known, one can calculate the

double layer forces, or vice versa. An estimate of the double layer force of PPy particles under various solution conditions is possible by taking typical zeta potentials from Figure 3. For example, for 50nm diameter particles with a zeta potential of +30 mV in 150 mM NaCl ($\kappa^{-1} = 0.78$ nm) at pH7, eq.7 gives 7×10^{-12} J m⁻¹ for the Z constant that when inserted into eq.6 gives $+6.7 \times 10^{-11}$ N or 67 pN for the repulsive force. This force is significantly less than the above VDW force showing that attractive forces will dominate the interaction at very short range for these PPy particles. This is generally the case for conducting polymers that have strong VDW interactions and require modifications to improve the solubility/processibility of particulate dispersions.

Similarly, the electrostatic force between oppositely charged PPy surfaces and proteins can be estimated. An estimation of the electrostatic force for an interaction between bovine serum albumin protein ($\psi_1 = +25$ mV, $R \approx 7.5$ nm) and PPy surface (-30mV) in 150mM at neutral pH is achieved using:

$$F(D) = 4\pi\epsilon\epsilon_0\kappa\psi_1\psi_2Re^{-\kappa D} \quad \text{eq.8}$$

where ψ_1 and ψ_2 are now the different surface potentials of the protein and PPy surface. Eq.8 effectively gives a linear approximation for the double layer force that lies between boundary conditions for constant potential and charge conditions. It also considers the geometry of particle (e.g. protein) interacting with a flat surface. Therefore, an electrostatic force of -51 pN (attractive due to negative sign) is obtained when computing eq.8 with the known zeta potentials. All of the above calculate forces of are similar order to those directly measured with AFM on different conducting polymer systems, as discussed further below.

Thermodynamic expressions and forces laws provide a means to calculate energies and forces based on knowledge of the interacting surfaces yet the ability to make comparisons with direct measurements using force measurement techniques is invaluable⁴⁵.

The direct measurement of intermolecular and surface forces is a relatively unexplored area for conducting polymers, with AFM so far being the main tool used. The principle of AFM force measurements are shown in Figure 4A. They involve measuring the change in deflection of a flexible cantilever with sharp tip whilst bringing the tip into contact and then withdrawing it from a surface. Tip-surface interaction forces, F , acting on the cantilever are easily measured using simple Hooke's Law, $F = kd$, where, k , is the cantilever stiffness (spring constant) and, d , is the cantilever deflection.

AFM force measurements between a silicon tip and sulfonated polyaniline (SPANI) in 1mM KCl at pH 2.5 and 25°C show interaction forces that are dependent on the applied potential⁴⁶. At low pH, an interaction between a slightly negatively charged tip and SPANI film with negatively applied bias produces a repulsive force that extends out to 20 nm (Figure 4B, i). As the applied potential is increased towards positive values the repulsive force diminishes eventually to the point where a net attractive force and tip-polymer “pull-off” adhesion of 2.0–2.5 nN is present (Figure 4B, ii).

Force measurements can be performed where a known potential is also applied to the AFM tip and polymer (Figure 4C). In this case, the interaction between a gold coated tip with applied -200 mV bias and PPy/hyaluronic acid (HA⁻) films in 0.005mM NaCl at neutral pH and room temperature similarly produces interactions that are dependent on prior charging of the polymer⁴⁷. The interaction however becomes more complex as a function of the lateral position of the tip across the surface. For uncharged (as-grown) films, a purely repulsive interaction occurs on nodules of the characteristic “cauliflower” polymer morphology (Figure 4C, i) but additional short range attractive forces and ‘pull-off’ adhesion of 0.5 nN appear within the peripheries of the nodules (Figure 4C, ii). When the polymer is charged prior to the measurements with +200mV, the repulsive force significantly diminishes and the

attractive force and pull-off adhesion are again present, as in Figure 4C (ii), yet on this occasion the interaction is not dependent on the lateral position of the tip (i.e. nodule versus periphery). The magnitude of the electrostatic repulsive forces for the uncharged and charged films is $\approx 10\text{-}100$ pN which corresponds to surface potentials of $\approx -5\text{mV} - 50\text{mV}$ when calculated by fitting the force-distance curves to double layer theory related to eq. 8. An interesting aspect of this research is that charging of these polymers related to the ability of living stem cells to adhere more to the surface⁴⁷. Because the charging did not significantly change the topography and modulus of the polymer, it is expected that electrostatic forces play a role in promoting the binding and bioactivity of extracellular proteins involved in cell adhesion.

Chemical modification of AFM tips and surfaces enables the interactions of functional groups (e.g. $-\text{COOH}$, $-\text{NH}_2$, $-\text{OH}$ and $-\text{CH}_3$) (Figure 4D). This approach has been applied to model surfaces (e.g. self-assembled monolayers)^{45, 48} and more recently to conducting polymers. For example, a series of functional groups such as those used for glutaraldehyde crosslinking of proteins are introduced onto the AFM silicon tip and force measurements are performed after each functionalization step to assess their involvement in the interaction with the conducting polymer⁴⁹. Plasma treated silicon nitride tip (SiN_3) bearing $-\text{OH}$ groups, which are hydrophilic and negatively charged at neutral pH, show a small repulsive force and no adhesion to as-grown PPy/chondroitin sulfate (CS^-) films with no applied potential (Figure 4D, i). In contrast, 3-ethoxydimethylsilylamine propyl (3-EDSPA) treated tips terminated with protonated NH_3^+ groups at neutral pH show an attractive force during approach followed by a “pull-off” adhesion of 2.0 nN (Figure 4D, ii). This net attractive force and adhesion between the positively charged tip and negatively charged polymer surface indicates the presence of anionic sulfate groups of CS^- at the polymer surface. Glutaraldehyde (GAH) functionalized tips bearing carbonyl groups are

reactive for primary amines to enable protein crosslinking. Due to the presence of carbonyl groups these tips are negatively charged and show a small repulsive force but unlike the silica tips a “pull-off” adhesion of 0.5 nN is present (force curves not shown). These tips could potentially undergo a Schiff’s base reaction to couple with –NH groups of the polymer, however, the magnitude of the adhesion forces does not suggest the formation of covalent bonds.

Individual and multiple chains of the polymer can be extended between the tip and surface and shows a different adhesion force profile. The extension of a PANI chain between an AFM tip and PANI surface in acetic acid-sodium acetate (HaC-NaAc) buffer at pH 2.8 shows a non-linear increase in force with increasing distance⁵⁰, which is typical behaviour for an elastic chain response when being ‘stretched’ (Figure 4E). Depending on the number of chains that are picked up by the tip, the force can range from $\approx 200 - 800$ pN until their elastic restoring force overcomes non-covalent interactions (with the tip) causing them to detach. The focus of these measurements is on the use of entropic models such as the Freely Jointed Chain (FJC) model that describes the properties of single polymer chains. $K_{segment}$ which is a measure of chain stiffness is obtained by fitting the non-linear response of the force. For PANI polymers, $K_{segment}$ of single chains increases in the order of doped PANI < reduced PANI < oxidized PANI. These studies do well in explaining the molecular level origin of macroscopic mechanical properties of PANI. More recently, an AFM tip modified with an integrated platinum electrode and subsequently coated with PPy/para-toluene sulfonate (pTS⁻) via pulsed polymerization show similar interactions involving the stretching of PPy chains⁵¹. When a potential of +0.4V is applied to the PPy electrode tip its interaction with a glass surface shows repulsive forces and also “pull-off-adhesion of ≈ 0.5 nN but the

latter is not present at -0.4V. Over-oxidising the PPy tip with +1.0V induces an interaction similar to Figure 4E, indicating the liberation and extension of PPy chains originating from the integrated PPy electrode tip.

An underlying interest in many of these studies is extrapolating the forces to biomolecular interactions such as protein adsorption. A direct approach is to covalently functionalize the AFM tip with a protein of interest. The tip is brought into contact usually for a delayed period (1-5 secs) to initiate binding to the conducting polymer surface. AFM tips functionalized with fibronectin (Fn), a well-known extracellular matrix protein that facilitates cell adhesion, show “pull-off” adhesion on PPy doped with glycoaminoglycans (GAGs)⁵². This adhesion is due to bulk interactions (e.g. multiple proteins) of the protein functionalized tip and specific forces involved are difficult to identify even though these dopants are known to specially interact with Fn. Interestingly, nanoscale mapping of the “pull-off” adhesion across the surface shows that the density of adhesion and its lateral dependence correlates with the distribution of more doped, conductive regions across the films, suggesting that Fn adhesion is mediated by interactions with the dopants⁵². After “pull-off” adhesion, other types of adhesion events occur, including sawtooth-forces due to sequential unfolding of folded FN domains as the tethered molecules are stretched (Figure 4F, i) or plateau forces that involve the desorption or “peeling” of FN molecules from the polymer surface (Figure 4F, iii)⁵². The former has a very characteristic profile during the extension of single proteins in that the forces to unfold individual domains (peaks) is $\approx$ 100-200 pN and spacing between peaks is equal to the fully extended length of unfolded domain (i.e. 28 nm = $\approx$ 70 peptides).

Multiple sawtooths also arise when there are multiple binding sites along the length of the protein. The interaction of Fn with PPy doped CS, Dextran Sulfate, HA and pTS shows sawtooths due to multiple binding sites with peak spacings of ≈ 60 nm (Figure 4F, i & ii) that correlate with the distance between heparin-binding domains of Fn, suggesting their involvement in the interaction⁴⁹. Heparin-binding domains are highly positively charged regions known to selectively interact with sulfate and anionic groups of GAGs. A specific sequence of the domains may bind due to disruption of their hydrogen bonds, which maintain the folded protein conformation. Subsequent coordinated presentation of a domain sequence enables their binding to anionic groups of the different dopants⁴⁹. Binding of individual domains gives average forces ranging from 100-150 pN, which correspond to relatively high interaction energies (given by integrated area under binding peak), particularly when acting in series⁴⁹. Thus, the Fn freely interacts along its length, allowing binding at heparin domains, and is able to extend up to its contour length (≈ 175 nm) under tensile forces. Repeating experiments using Fn functionalized AFM tips on more hydrophobic, polythiophene films show that extension of the Fn is greatly reduced to distances of ≈ 25 nm which correlates with dimensions of Fn in its folded conformation, suggesting that this conformation is retained, e.g. hydrogen bonds are not disrupted, during interactions with these low surface energy polymers⁵³.

Effects of electrical stimulation on nanoscale and molecular interactions are of significant interest yet relatively unexplored. In the above experiments, assessment of the “pull-off” adhesion force between the silicon tip and SPANI films shows that it tracks the electrochemically induced charge at the polymer surface⁴⁶. An adhesion versus electrode potential curve exhibits a titration-like curve response (Figure 5A) where the minimum and

maximum of the adhesion represents a surface that is saturated with positive or negative charge. Least squares fitting to these curves exhibit an inflection point that corresponds to the potential where the electrostatic force transforms from repulsive to attractive. For this silicon tip-SPANI interaction, a measured inflection point of -125 mV is approximately equal to the half-way potential in cyclic voltammetry measurements. For the Fn-PPy interaction, applying a positive bias to the PPy causes strong electrostatic attraction between the majority of negatively charged Fn domains and positively charged polymer, resulting in order of magnitude higher adhesion forces of $\approx 1\text{-}2\text{ nN}$ (Figure 5B)⁴⁹. In contrast to the weaker interactions via heparin domains, this electrochemically induced adhesion is stronger and non-specific but can be reversibly switched to smaller piconewton adhesion forces by applying an opposite negative bias to the polymer. Rather than applying a constant potential, this is demonstrated using cyclic voltammetry where the pull-off adhesion is plotted as a function of the change in voltage and current (Figure 5B) and becomes kinetically dependent on the scan-rate⁴⁹.

1.4 Protein Interactions

Adsorption isotherms describe the adsorption capacity of a specific protein-material system and can be modelled to provide quantitative information on the saturation adsorbed amount and protein affinity for the surface^{40,54}. Measurements are typically done with model proteins such as bovine serum albumin (BSA), human serum albumin (HSA) and fibrinogen (Fbn) and at different pH to examine the effect of iep's and changes in the zeta potential of both protein and material.

BSA is negatively charged in buffer solutions with $\text{pH} > 4.8$ and consequently subject to increasing electrostatic repulsive forces on PPy films that are generally considered to become more negatively charged (deprotonated) at increasing pH ⁵⁵. However, Figure 3 shows that BSA will be either electrostatically attracted or repelled depending on the iep of the polymer composition⁴⁰. In any case, selective adsorption of proteins on conducting polymers is generally driven by long range electrostatic interactions but their magnitude at a specific pH and for a particular polymer composition may be influenced by other factors such as contributions from intermolecular forces (e.g. hydrophobic) and competing protein-protein interactions. For example, greater BSA adsorption on PPy occurs around the iep of the protein due to fewer net charges that reduce electrostatic repulsion between proteins, enabling incoming proteins to more easily adsorb in the presence of existing adsorbed proteins⁴⁰. Adsorption isotherms also show that HSA adsorption increases on surfaces, i.e. $\text{PPy}^+/\text{TOS}^- > \text{PPy}^+/\text{DS}^- > \text{PPy}^+/\text{Cl}^-$, with increasing hydrophobicity⁵⁶. Further CA measurements to calculate the interfacial interaction energy, E_{int} , for these different PPy-HSA systems show that a decreasing E_{int} correlates with decreasing surface hydrophobicity. Separating the E_{int} into dispersive and Lewis acid-base components shows that in the case of PPy^+/Cl^- and PPy^+/DS^- acid-base forces contribute more than van der Waals, while for $\text{PPy}^+/\text{TOS}^-$ the van der Waals predominately contributes to E_{int} . There may also be unexpected protein adsorption on the polymer even when the net electrostatic interaction between them is repulsive. The adsorption of BSA above its iep to negatively charged PPy is explained by the presence of -NH^+ terminated alkyl chains of a PPy polymer that extend out into solution to produce a dominant electrostatic attractive interaction with the protein⁴⁰.

BSA, and similarly HSA, adsorption isotherms typically show a one-step process in the formation of a monolayer⁵⁴, though recently two-step processes associated with protein

conformational changes are induced by rougher surfaces⁵⁷. Proteins such as Fbn and Fn show more complex adsorption processes that are not well described by simple Langmuir isotherm models^{54,57}. These proteins undergo significant conformational changes at the surface, exposing specific peptide sequences, which may lead to polymerization and assembly of complex fibre networks.

Of particular interest is potential-assisted control of protein adsorption, which has been shown to either enhance or resist protein adsorption^{58,59}. Potential assisted adsorption is generally described by electrostatic attractive forces of negatively charged proteins (typically isoelectric point < neutral pH) to a positively charged electrode. However, oxidation of conducting polymers shows varied effects on protein adsorption; Fn adsorption decreases on some oxidized films^{60,15} but increases on others⁵⁷. Many factors such as the redox process (Figure 1) and effects on the parameters described above (surface energy and zeta potential) for a given composition and protein system must be considered. In addition to adsorption, controlling the protein conformation is especially appealing for extracellular matrix proteins such as Fn that mediate cell adhesion. Fn can be electrochemically switched from a folded conformation to an unfolded conformation that exposes cell binding motifs to promote cell adhesion^{61,62}. It is possible to direct specific ligand–receptor interactions via electrical control for biosensing systems such as selective and reversible control of antibody–antigen interactions in polymers doped with antibodies. Oxidation of antihuman Fn antibody–doped PPy promotes selective binding of Fn whereas reduction of films facilitates Fn dissociation⁶³. Staircase potential electrochemical impedance microscopy measurements suggest that the binding reversibility is not due to the suppression of secondary, hydrophobic forces but attributed to the minimization of charge in the polymer films. Reversible binding is also possible using short pulse potentials, as the time scale for potential-assisted adsorption is

suggested to be too short for the antibody–antigen complex to establish complete, irreversible binding through secondary hydrogen and hydrophobic forces⁶⁴.

1.5 DNA Interactions

The binding of double-helical DNA to PPy is generally considered to be due to electrostatic attractive forces between the negatively charge DNA and positively charged (oxidized) groups on the polymer. The type of dopant in the polymer significantly affects DNA adsorption. DNA interactions with PPy that show a low binding activation energy is consistent with being mostly in a reversibly bound state⁶⁵. PPy–DNA affinity constants are in the range of 10^6 M^{-1} for PPy^+/Cl^- and $\text{PPy}^+/\text{NO}_3^-$, indicating a stronger interaction that contrasts with that of a DNA– $\text{PPy}^+/\text{SO}_4^-$ system⁶⁶. Strong DNA adsorption is obtained at low pH and high ionic strength. Acidic condition increase positive charge on PPy, while increases in salt concentrations are suggested to decrease electrostatic repulsion between DNA molecules and thus favour their adsorption to the surface⁶⁶.

XPS measurements and dielectric monitoring of DNA adsorption onto PPy/Cl suggests the DNA initially adsorbs in a flat-on configuration and then shifts to an end-on orientation as more molecules adsorb⁶⁷. DNA adsorption to PPy^+/Cl^- in buffer is also accompanied by ion exchange. Outermost layers of the positively charged PPy^+/Cl^- initially liberate Cl^- dopant ions as the negatively charged DNA begins to form electrostatic interactions⁶⁸. As the amount of adsorbed DNA increases, the PPy surface is screened and necessitates the co-adsorption of sodium ions in order to compensate excess negative charge of the DNA.

Interesting details have emerged on interactions between specific DNA nucleotide sequences and conducting polymers, suggesting that highly directional and specific interactions requiring hydrogen bonding are present. For example, the DNA-PPy interaction shields the activity of restriction enzymes targeting specific sequences 50-G/AATTC-30 (target for EcoRI) and 50-G/GATCC-30 (target for BamHI)^{69,70}. In particular, PPy and PEDOT contain good donor/acceptors (-NH group) of hydrogen bonds for specific interactions at these sequences sites, while polythiophene derivatives without hydrogen bond donor/acceptors show weaker interactions.

An overall model proposes that the interaction with the PPy initially induces an alteration in the double helix that exposes nitrogen bases and subsequently allows formation of hydrogen bonds and intercalation of the polymer. Experimental data is supported by modelling and simulation calculations that reveal the formation of specific N-H---O hydrogen bonds, N-H---S bonds, π -- π stacking, and N-H--- π interactions, in addition to the expected electrostatic interactions^{71,72}. In general, N-H---O hydrogen bonds were found to be abundant and to have relatively large accumulated lifetimes.

1.6 Conclusion

Due to their radical cations, negatively charged dopants and organic polymer backbone, conducting polymers possess strong amphoteric characteristics and propensity for acid-base interactions, as well as hydrophobic interactions, that make them very attractive for studying protein and biomolecular interactions. Controlling these interactions remains quite challenging as a range of competing forces, including van der Waals, electrostatic, hydrophobic and hydrogen bonding, makes it difficult to control the selectivity or complete reversibility of binding. For example, the adsorption of proteins is easily promoted through

longer range electrostatic forces however completely reversing this adsorption may be hindered by the presence of stronger hydrophobic forces or cooperative hydrogen bonding. It is possible to evaluate interaction energies and their dispersive and non-dispersive components from theory and surface energy and zeta potential values. However, extracting such parameters for complex proteins, different material structures/configuration and varying environmental conditions (different electrolytes) may not be straightforward. The interactions may also become complex as the biomolecules undergo surface-induced conformational changes and vary significantly in their interactions across non-homogenous surfaces.

Further insight into the complexities of these biomolecular interactions can be gained from force measurement techniques. Importantly, these techniques reveal how the surface parameters manifest as forces that ultimately govern the interactions; it is the magnitude, range (distance) and combination of these forces that determine many biological processes. AFM provides the flexibility to measure forces between different biomolecules, surfaces and materials with a relatively wide range in force sensitivity (10^{-10} – 10^{-6} N). Nearly any molecule of choice can be studied through chemical modification of an AFM tip and their interactions resolved at the single molecule level whilst moving the tip across the surface to correlate with nanoscale surface topography and chemistry. Single molecule force sensitivity is helpful for deconvoluting complex interactions and highly complementary to atomic/molecular modelling and simulation approaches that operate on comparable lengthscales and, together with AFM, are expected to play a critical role in fundamental advances this area. Practical uses can also be found, for example, by supporting high-throughput bacteriophage library screening methods with AFM as a next step in assessing binding kinetics and mechanistics of those ligands identified as having a high binding

affinity⁷³. This is a rational approach but not as yet truly implemented as part of a discovery pipeline in the quest for new high affinity ligands.

There is a considerable amount on the effect of electrical stimulation that we still do not understand: How does electrically modulating the surface potential and charge density of conducting polymers affect the energy and force profile of interactions with other nanomaterials, biomolecules and gels? What are the changes in the magnitude, range and contribution of the different forces? How does electrical stimulation affect highly specific, complementary forces that occur at very short range such as growth factor or ECM interactions with their receptors of living cells? And how can electrical stimulation, in combination with well-defined chemical surface architectures, be used to reversibly and temporally modulate specific forces without interference from other species? Effects of electrically-induced local environmental changes at the interface such as pH and ionic concentration must also be considered.

Conducting polymers provide a fascinating material to explore this area, as electrical switching of physical properties, chemical surface groups, protonation/deprotonation and ion exchange offers a variety of routes for modulating surface interactions. There is still much to be discovered in regard to the forces at play in biomolecular interactions with conducting polymers. Bringing together high-throughput screening assays, force measuring techniques, atomic and molecular modelling and novel assembly of conducting polymers and their surface ligands/molecular constituents will also lead to the design of highly advanced materials that are increasingly required to provide dynamic and reversible control of biomolecular interactions for biomedical applications involving DNA, protein, bacterial and stem cell interactions.

Acknowledgements:

This work has been supported by the Australian Research Council under the Australian Research Fellowship and DP110104359 (Dr Michael Higgins) and ARC Australian Laureate Fellowship of Prof. Gordon Wallace. We also greatly acknowledge the ARC Centre of Excellence for Electromaterials Science (ACES) and Australian National Fabrication Facility (ANFF).

Figure Legends

Figure 1. The electrochemical oxidation and reduction processes of PPy in the presence of two different dopants. (A) Small mobile dopants such as Cl^- ions are incorporated into the polymer upon oxidation (left side). The Cl^- ions are typically released upon reduction of the polymer (right-side). (B) Large immobile dopants such as DBS^- remain entrapped and instead switch their orientation in response to the doping/dedoping processes. During oxidation (left-side), sulfonate groups of DBS^- coordinate with positive charges, but alkyl chains of the DBS^- then physically orientate to interact with the neutral polymer during reduction (right-side). Figure adapted from [20] and [37]

Figure 2. Range of dispersive (γ_d) surface energy values for various conducting polymers, conventional polymers, metals and metal oxides, carbon, water and organic liquids. Figure from [25]

Fig. 3. Zeta potentials of PPy-based adsorbents at different solution pH values.

Figure 3. Zeta potential of different doped polypyrrole (PPy) adsorbents (powders) at different pH. The different dopants are chloride (Cl), dodecyl sulfate (DS), octadecyl Sulfate (OS) and aminated-termination (N). Figure from [40]

Figure 4. A) Schematic of AFM force curve. a) tip approaches; b) attractive force and tip-surface contact; c) repulsive contact force; d) tip-sample adhesion; e) “pull-off” adhesion and tip withdrawal. B) Force curves for interaction between silicon tip and SPANi-coated electrode in 1 mM KCl at 25 °C and pH 2.5 at applied potentials of (i) -350 mV and (ii) +250mV(vs AgQRE) [46]. C) Force curves for interaction between gold coated tip (biased at -200mV) uncharged PPy-HA film in 0.005 M NaCl(aq) electrolyte at the location of (i) nodule structures and (ii) peripheries of nodules structures of the polymer morphology [47]. D) AFM force curves for interaction between (i) plasma treated AFM silicon nitride tip (SiN) and (ii) aminosilinated tip (3-EDSPA) and PPy/CS in phosphate buffer saline [52]. E) Force

curves for interaction between silicon tip and doped PANI in pH 2.8 HAc-NaAc buffer solution showing adhesion and extension of PANI chains. The force curves have been inverted and fitted using a Freely Jointed Chain model to give values of segment elasticity and length [50]. F) Force curves for the interaction of FN with non-electrically stimulated PPy/CS. The force curves have been inverted. The peak at (i) corresponds to initial detachment of the tip and fibronectin molecules from the surface. The two subsequent peaks (1st and 2nd dashed lines) and their spacing of ≈ 28 nm at (ii) and (iii) correspond to sequential unfolding of FNIII modules (~ 75 amino acid residues). The peak spacings at points (iv) and (v) are greater than that for FN unfolding and correlate with multiple detachment of FN–polymer binding sites. The binding forces for these sites are represented by peaks with remaining dashed lines. The detachment of the protein can also proceed less commonly via ‘non-specific’ desorption that show a constant force independent of the extension length, i.e., plateau forces (vi). [49]

Figure 5. A) Pull-off adhesion between silicon tip and SPANi electrode as a function of the electrochemical potential applied to the substrate in 1 mM KCl at 25 °C and pH 2.5. The solid lines represent least squares fits to a titration curve. Each data point represents the average of 256 measurements and the error bar is the standard deviation. These curves give

E° values of -100 mV [46]. (B) Pull-off adhesion force (dashed curve) versus voltage and corresponding cyclic voltammograms (solid curve) for PPy/CS films. Adhesion values (black circles) represent an average from individual force curves collected at each time point during 3 cyclic voltammogram cycles performed at a scan rate of 50 mV/s. No changes in pull-off adhesion were observed for slower scan rates of 5mV/sec (data not shown) [49].

Materials	γ_s	γ_d	γ_p	T (°C)	Ref
Dedoped P3HT	37.5	30.2	7.3		[24]
Doped P3HT	54	31.7	22.3		[24]
Dedoped PPY	44.2	32.1	12.2		[24]
POT		32.2		65	[25]
FeCl ₃ -doped PPY	55.4	35.8	19.6		[24]
Dedoped PANI	44.6	36.9	7.7		[24]
ETSO ₃ -doped PANI	69.9	38.1	31.7		[24]
HF-doped PANI	64.4	38.7	25.7		[24]
HCl-doped PANI,	57.9	39	18.9		[24]
TSA-doped PANI	62.8	39.1	23.7		[24]
SSA-doped PANI	67.4	39.6	27.8		[24]
PPyCl		42		47.7	[22]*
PANI		42.6		58	[28]
PPyDS		53		120	[27]
PPy-SO ₄		57.8		50	[33]
PPyNO ₃		61.2		47.7	[22]
PANI		87.3		68	[25]
PPyTOS		89.2		57.6	[34]
PTFE		19		20	[28]
CP-SIL		30.4		47.7	[22]
PE		33		20	[28]
PMMA		40		20	[28]
PVC		41.5		20	[28]
C fibre		46.5		29	[32]
Pb, Sn, Fe		100		25	[30]
Graphite		129		44.5	[29]
Smectite		159		180	[31]
Hg		200		20	[30]

Table 1. List of surface energy values (mJ/m²) for different conducting polymers. Total (γ_s), dispersive (γ_d) and polar (γ_p) surface energies and temperatures (T°C) used for measurements.

The values are listed from lowest to highest for the dispersive (γ_d) component. Bottom part of table shows comparative values for conventional insulating polymer and other materials. References for values are found in far right column. Abbreviations – polypyrrole (PPy), poly(3-hexylthiophene) (P3HT), poly(3-octylthiophene) (POT), polyaniline (PANI), polytetrafluoroethylene (PTFE), polyethylene (PE), poly(methyl methacrylate) (PMMA), polyvinyl chloride (PVC), polysiloxane-based (CP-SIL), carbon (C), ferric chloride (FeCl_3), ethane sulfate (ETSO_3), hydrofluoric acid (HF), hydrochloric acid (HCL), p-toluene sulfonic acid (TSA), sulfosalicylic acid (SSA), chloride (Cl), dodecyl sulfate (DS), sulfate (SO_4), nitrate (NO_3), tosylate (TOS).

Polymers, in *Organic Bionics*. 2012, Wiley-VCH Verlag GmbH & Co. KGaA. p. 81-112

² Cogan, S.F. “Neural stimulation and recording electrodes, in Ann. Rev. Biomed. Eng.”, Annual Reviews. 2008, p. 275-309

³ Kim D.H.; Richardson-Burns S.; Povlich L.; et al. “Soft, Fuzzy, and Bioactive Conducting Polymers for Improving the Chronic Performance of Neural Prosthetic Devices”. In: Reichert WM, editor. *Indwelling Neural Implants: Strategies for Contending with the In Vivo Environment*. Boca Raton (FL): CRC Press; 2008. Chapter 7.

⁴ Guimard, N.; Gomez, N.; and Schmidt, C. “Conducting polymers in biomedical engineering”. *Prog. Poly. Sci.*, 2007, 32, 876-921.

⁵ Wallace, G.; Spinks, G. “Conducting polymers- bridging the bionic interface”. *Soft Matter*, 2007, 3, 665-671.

⁶ Thompson, B.C; Moulton, S.E; Richardson, R.T; Wallace G. G. “Optimising the incorporation and release of a neurotrophic factor using conducting polypyrrole”. *J. Control. Release*, 2006, 116, 285-294.

⁷ Higgins, M. J.; Molino, P. J.; Yue, Z.; Wallace, G. G. “Organic Conducting Polymer–Protein Interactions”. *Chem. Mater.* 2012, 24, 828-839.

⁸ Khodagholy, D., Doublet, T; Gurfinkel, M.; Qiulichini, P.; Ismailova, E.; Herve, T.; Sanaur, S.; Bernard, C.; Malliaras, G. G.; “Highly conformable conducting polymer electrodes for in vivo recordings”. *Adv. Mater.*, 2011, 23, H268-H272.

-
- ⁹ Pelto, J.; Björninen, M.; Pälli, A.; Talvitie, E.; Hyttinen, J. A. K.; Mannerström, B.; Suuronen Seppanen, R.; Kellomäki, M.; Miettinen, S.; Haimi, S., “Novel Polypyrrole Coated Polylactide Scaffolds Enhance Adipose Stem Cell Proliferation and Early Osteogenic Differentiation”. *Tiss. Eng*, 2012
- ¹⁰ Richardson, R. T.; Wise, A. K.; Thompson, B. C.; Flynn, B. O.; Atkinson, P. J.; Fretwell, N. J.; Fallon, J. B.; Wallace, G. G.; Shepherd, R. K.; Clark, G. M.; O’Leary, S. J., “Polypyrrole-coated electrodes for the delivery of charge and neurotrophins to cochlear neurons”. *Biomaterials* 2009, 30, 2614-2624.
- ¹¹ Persson, K. M.; Karlsson, R.; Svennersten, K.; Löffler, S.; Jager, E. W. H.; Richter-Dahlfors, A.; Konradsson, P.; Berggren, M., “Electronic Control of Cell Detachment Using a Self-Doped Conducting Polymer”. *Adv. Mater.* 2011, 23, 4403-4408.
- ¹² Gerard, M.; Chaubey, A.; Malhotra, B.D. “Application of conducting polymers to biosensors” *Biosens. Bioelectron.*, 2002, 17, 345 – 359.
- ¹³ Yang, X.; Too, C.O.; Sparrow, L.; Ramshaw, J.; Wallace, G.G. “Polypyrrole–heparin system for the separation of thrombin”. *React. Func. Polym.* 2002, 53, 53-62.
- ¹⁴ Cui X.; Lee V.A.; Raphael Y.; Wiler J.A; Hetke J. F.; Anderson D. J.; Martin D. C. “Surface modification of neural recording electrodes with conducting polymer/biomolecule blends”. *J. Biomed. Mater. Res.* 2001,56, 261-72.

-
- ¹⁵ Saltó C.; Saindon E.; Bolin M.; Kanciurzevska A.; Fahlman M.; Jager E. W.; Tengvall P.; Arenas E.; Berggren M. "Control of neural stem cell adhesion and density by an electronic polymer surface switch". *Langmuir*, 2008, 24, 14133-8
- ¹⁶ Leckband, D.; Israelachvili J. "Intermolecular forces in biology". *Quart. Rev. Biophys.* 2001, 34, 105-267.
- ¹⁷ Cosnier, S.; Holzinger, M. "Electrosynthesized polymers for biosensing" *Chem. Soc. Rev.* 2011, 40, 2146-2156.
- ¹⁸ Cascales, J. L.; Otero, T. F. "Molecular dynamic simulation of the hydration and diffusion of chloride ions from bulk water to polypyrrole matrix" *J. Chem. Phys.* 2004, 120, 1951.
- ¹⁹ Halldorsson, J. A.; Wu, Y.; Brown, H. R.; Spinks, G. M.; Wallace, G. G. "Surfactant-controlled shape change of organic droplets using polypyrrole." *Thin Solid Films*, 2011, 519, 6486-6491.
- ²⁰ Causley, J.; Stitzel, S.; Brady, S.; Diamond, D.; Wallace, G. "Electrochemically-induced fluid movement using polypyrrole" *Syn. Met.*, 2005, 151, 60-64.
- ²¹ Voelkel, A.; Strzemiescka, B.; Adamska, K.; Milczewska, K. "Inverse gas chromatography as a source of physiochemical data" *J. Chromatog. A*. 2009, 1216, 1551-1566.

-
- ²² Chehimi, M. M.; Abel, M. L.; Pigois-Landureau, E.; Delamar, M. "Characterization of conducting polymers by inverse gas chromatography Part II. Effect of dopant on the dispersive and specific properties of polypyrrole". *Syn. Met.*, 1993, *60*, 183-194
- ²³ Fadda, E.; C. Clarisse. "Characterization of the surface modifications of conducting poly (3-octylthiophene) films by contact angle measurements." *Syn. Met.* 1995, *72*, 99-103.
- ²⁴ Liu, M. J.; Tzou, K.; Gregory, R. V. "Influence of the doping conditions on the surface energies of conducting polymers". *Syn. Met.* 1994, *63*, 67-71.
- ²⁵ Chehimi, M. M.; Abel, M. L.; Perruchot, C.; Delamar, M.; Lascelles, S. F.; & Armes, S. P. "The determination of the surface energy of conducting polymers by inverse gas chromatography at infinite dilution". *Syn. Met.* 1999, *104*, 51-59.
- ²⁶ Bailey, R. A.; Persaud, K. C.; "Application of inverse gas chromatography to characterisation of a polypyrrole surface" *Anal. Chim. Acta*, 1998, *363*, 147-156.
- ²⁷ Ge, H.; Wallace, G. G. "Properties of thermally treated polypyrroles" *Polymer*, 1992, *33*, 2348-2352.
- ²⁸ Wu, S.; "Polymer interface and adhesion", Marcel Dekker, New York, 1982.
- ²⁹ Donnet, J. B.; Park, S. J.; Balard, H. "Evaluation of Specific Interactions of Solid Surfaces by Inverse Gas Chromatography" *Chromatographia*, 1991, *31*, 434-440.

³⁰ Fowkes, F. M. "Attractive forces at interfaces" *Int. Eng. Chem.* 1964, 56, 40-52.

³¹ Bandosz, T. J.; Jagiello, J.; Andersen, B.; Schwarz, J. A. "Comparison of methods to assess surface acidic groups on activated carbons" *Anal. Chem.* 1992, 64, 891-895.

³² Schultz, J.; Lavielle, L.; Martin, C. "The role of the interface in carbon fibre-epoxy composites" *J. Adhes.* 1987, 23, 45-60.

³³ Boukerma, K.; Mičušík, M.; Mravčáková, M.; Omastová, M.; Vaulay, M. J.; Beaunier, P.; Chehimi, M. M. "Surfactant-assisted control of the surface energy and interfacial molecular interactions of polypyrrole". *Coll. and Surf. A: Physicochem. Eng. Asp.* 2007, 293, 28-38.

³⁴ Chehimi, M. M.; Abel, M. L.; Sahraoui, Z.; Fraoua, K.; Lascelles, S. F.; Armes, S. P. "Time-dependent variation of the surface energy of conducting polypyrrole". *Int. J. Adhes. Adhes.* 1997, 17, 1-8.

³⁵ Chehimi, M. M.; Abdeljalil, E. "A study on the degradation and stability of polypyrrole by inverse gas chromatography, X-ray photoelectron spectroscopy, and conductivity measurements" *Syn. Met.* 2004, 145, 15-22.

³⁶ Wang, X.; Ederth, T.; Inganäs, O. "In situ Wilhelmy balance surface energy determination of poly (3-hexylthiophene) and poly (3, 4-ethylenedioxythiophene) during electrochemical doping-dedoping". *Langmuir*, 2006, 22, 9287-9294.

-
- ³⁷ Xu, L., Chen, W., Mulchandani, A., & Yan, Y. "Reversible conversion of conducting polymer films from superhydrophobic to superhydrophilic" .Angew. Chem. Int. Ed., 2005, 44, 6009-6012.
- ³⁸ Zhang, X.; Bai, R. "Surface electric properties of polypyrrole in aqueous solutions". Langmuir, 2003,19, 10703-10709.
- ³⁹ Markham, G.; Obey, T. M.; Vincent, B. "The preparation and properties of dispersions of electrically-conducting polypyrrole particles". Coll. Surf., 1990 51, 239-253.
- ⁴⁰ Zhang, X.; Bai, R.; Tong, Y. W. "Selective adsorption behaviors of proteins on polypyrrole-based adsorbents". Sep. Purify. Tech., 2006, 52, 161-169.
- ⁴¹ Butterworth, M. D.; Corradi, R.; Johal, J.; Lascelles, S. F.; Maeda, S.; Armes, S. P. "Zeta potential measurements on conducting polymer-inorganic oxide nanocomposite particles". J. Coll. Inter. Sci., 1995, 174, 510-517.
- ⁴² Bai, R.; Zhang, X. "Polypyrrole-coated granules for humic acid removal". J. Coll. Inter. Sci., 2001, 243, 52-60
- ⁴³ Semenikhin, O. A.; Jiang, L.; Iyoda, T.; Hasimoto, K.; Fujishima, A.; Serra Moreno, J.; Panero, S.; Materazzi, S.; Martinelli, A.; Sabbieti, M. G.; Agas, D.; Materazzi, G. "Atomic Force Microscopy and Kelvin Probe Force Microscopy Evidence of Local Structural Polypyrrole-polysaccharide thin films characteristics: electrosynthesis and biological properties". J. Phys. Chem. 1996, 100, 9-11.

⁴⁴ Barisci, J. N.; Stella, R.; Spinks, G. M.; Wallace, G. G., Characterisation of the topography and surface potential of electrodeposited conducting polymer films using atomic force and electric force microscopies. *Electrochim. Acta* 2000, 46, 519- 531.

⁴⁵ Butt, H.-J.; Cappella, B.; Kappl, M. “Force measurements with the atomic force microscope :Technique , interpretation and applications”. *Surf. Sci. Rep.* 2005, 59, 1-152.

⁴⁶ Campbell, S. D.; Hillier, A. C. “Nanometer-scale probing of potential-dependent electrostatic forces, adhesion, and interfacial friction at the electrode/electrolyte interface”. *Langmuir*, 1999, 15, 891-899.

⁴⁷ Pelto J. M.; Haimi S.P.; Siljander A. S; Miettinen S.S.; Tappura K. M.; Higgins M. J.; Wallace G. G.; “Surface properties and interaction forces of biopolymer-doped conductive polypyrrole surfaces by atomic force microscopy”. *Langmuir*. 2013, 29, 6099-108.

⁴⁸ Thomas, R. C.; Houston, J. E.; Crooks, R. M.; Kim, T.; Michalske, T. A.; “Probing adhesion forces at the molecular scale” *J. Am. Chem. Soc.*, 1995, 117, 3830-3834.

⁴⁹ Gelmi A.; Higgins M. J.; Wallace G. G. “Resolving sub-molecular binding and electrical switching mechanisms of single proteins at electroactive conducting polymers”. *Small*. 2013, 9, 393-401.

⁵⁰ Yu, Y.; Zhang, Y.; Jiang, Z.; Zhang, X.; Zhang, H.; Wang, X. “Full View of Single-Molecule Force Spectroscopy of Polyaniline in Oxidized, Reduced, and Doped States”. *Langmuir*, 2009, 25, 10002-10006.

⁵¹ Knittel, P.; Higgins, M. J.; Kranz, C. Polypyrrole-modified Atomic Force-Scanning Electrochemical Microscopy Probes. (2013) *Angew. Chem.* (submitted)

⁵² Gelmi A.; Higgins M.J.; Wallace G. G. “Quantifying fibronectin adhesion with nanoscale spatial resolution on glycosaminoglycan doped polypyrrole using Atomic Force Microscopy”. *Biochim. Biophys. Acta*. 2013, Mar 24. doi:pii: S0304-4165(13)00083-4.

⁵³ Gelmi, A.; Zaroni, M.; Higgins, M. J.; Gambir, S.; Officer, D. L.; Diamond, D.; Wallace, G. G. “Optical Switching of Protein Interactions on Photosensitive–Electroactive Polymers measured by Atomic Force Microscopy” *J. Mater. Chem. B*. 2013, 1, 2162-2168.

⁵⁴ Liu, M.; Zhang, Y.; Wang, M.; Deng, C.; Xie, Q.; Yao, S. “Adsorption of bovine serum albumin and fibrinogen on hydrophilicity-controllable surfaces of polypyrrole doped with

dodecyl benzene sulfonate—A combined piezoelectric quartz crystal impedance and electrochemical impedance study". *Polymer*, 2006, 47, 3372-3381.

⁵⁵ Zhang, Z.; Li, G.; Yan, F.; Zheng, X.; Li, X. "Towards understanding of protein adsorption behavior on plasma polymerized pyrrole film". *Cent. Euro. J. Chem.*, 2012, 10, 1157-1164.

⁵⁶ Azioune, A., Chehimi, M. M., Miksa, B., Basinska, T., & Slomkowski, S. "Hydrophobic protein-polypyrrole interactions: The role of van der Waals and Lewis acid-base forces as determined by contact angle measurements". *Langmuir*, 2002, 18, 1150-1156.

⁵⁷ Molino, P. J.; Higgins, M. J.; Innis, P. C.; Kapsa, R. M.; Wallace, G. G. "Fibronectin and Bovine Serum Albumin adsorption and conformational dynamics on inherently conducting polymers: A QCM-D study". *Langmuir*, 2012, 28, 8433-8445.

⁵⁸ Khan, G. F.; Wernet, W. "Adsorption of proteins on electro-conductive polymer films." *Thin Solid Films*, 1997, 300, 265-271.

⁵⁹ Li, Y.; Neoh, K. G.; Kang, E. T. "Plasma protein adsorption and thrombus formation on surface functionalized polypyrrole with and without electrical stimulation". *J. Coll. Inter. Sci.* 2004, 275, 488-495.

⁶⁰ Gumus, A.; Califano, J. P.; Wan, A. M. D.; Huynh, J.; Reinhart-King, C. A.; Malliaras, G. G. "Control of cell migration using a conducting polymer device". *Soft Matter* 2010, 6, 5138-5142.

⁶¹ Wan, A. M. D.; Schur, R. M.; Ober, C. K.; Fischbach, C.; Gourdon, D.; Malliaras, G. G.

“Electrical Control of Protein Conformation”. *Adv. Mater.* 2012, 24, 2501-2505.

⁶² Svennersten, K.; Bolin, M. H.; Jager, E. W. H.; Berggren, M.; Richter-Dahlfors, A.

“Electrochemical modulation of epithelia formation using conducting polymers”.

Biomaterials 2009, 30, 6257-6264.

⁶³ O'Grad, M. L.; Parker, K. K. “Dynamic control of protein-protein

interactions”. *Langmuir*, 2008, 24, 316-322.

⁶⁴ Gooding, J. J.; Wasiowych, C.; Barnett, D.; Hibbert, D. B.; Barisci, J. N.; Wallace, G. G.

“Electrochemical modulation of antigen–antibody binding” .*Biosens. Bioelectron.*, 2004, 20, 260-268.

⁶⁵ Minehan, D. S.; Marx, K. A.; Tripathy, S. K.; “Kinetics of DNA binding to electrically

conducting polypyrrole films”. *Macromolecules*, 1994, 27, 777-783.

⁶⁶ Saoudi, B.; Jammul, N.; Abel, M. L.; Chehimi, M. M.; Dodin, G. “DNA adsorption onto

conducting polypyrrole”. *Synth.Met.*, 1997, 87, 97-103.

⁶⁷ Saoudi, B.; Despas, C.; Chehimi, M. M.; Jammul, N.; Delamar, M.; Bessière, J.; Walcarius,

A. “Study of DNA adsorption on polypyrrole: interest of dielectric monitoring”. *Sens. Act. B: Chem.* 2000, 62, 35-42.

⁶⁸ Saoudi, B.; Jammul, N.; Chehimi, M. M.; Jaubert, A. S.; Arkam, C.; & Delamar, M. “XPS study of the adsorption mechanisms of DNA onto polypyrrole particles”. *Spectroscopy: An International Journal*, 2004, 18, 519-535.

⁶⁹ Alemán, C.; Teixeira-Dias, B.; Zanuy, D.; Estrany, F.; Armelin, E.; del Valle, L. J. “A comprehensive study of the interactions between DNA and poly (3, 4-ethylenedioxythiophene)”. *Polymer*, 2009, 50, 1965-1974.

⁷⁰ Teixeira-Dias, B.; del Valle, L. J.; Estrany, F.; Armelin, E.; Oliver, R.; Alemán, C. “Specific interactions in complexes formed by polythiophene derivatives bearing polar side groups and plasmid DNA”. *Euro. Polym. J.*, 2008, 44, 3700-3707.

⁷¹ Preat, J.; Teixeira-Dias, B.; Michaux, C.; Perpète, E. A.; Alemán, C. “Specific Interactions in Complexes Formed by DNA and Conducting Polymer Building Blocks: Guanine and 3, 4-(Ethylenedioxy) thiophene”. *J. Phys. Chem. A*, 2011, 115, 13642-13648.

⁷² Preat, J.; Zanuy, D.; Perpète, E. A.; Alemán, C. “Binding of Cationic Conjugated Polymers to DNA: Atomistic Simulations of Adducts Involving the Dickerson’s Dodecamer”. *Biomacromolecules*, 2011, 12, 1298-1304.

⁷³ Sanghvi, A. B.; Miller, K. P. H.; Belcher, A. M.; Schmidt, C. E. “Biomaterials functionalization using a novel peptide that selectively binds to a conducting polymer”. *Nat. Mater.*, 2005, 4, 496-502.