Chapter 1

Introduction

The outcome of a chemical or biochemical reaction crucially depends on the environment in which the reaction is carried out. Proton transfer constitutes a ubiquitous example where key factors such as reactivity, yield and rate of the reaction are controlled by the medium [1, 2]. The latter may range from simple bulk solvents to complex systems such as proteins, membranes and micelles [1, 3]. In many cases of biological relevance, unlike bulk solvents, the reaction occurs within the confines of active site cavity of an enzyme or a membrane pore. Alternatively, the reacting groups may be attached, for instance, to the surface of a protein, thus deriving important contributions to the reaction from water and other molecules/ions bound to the surface or residing in the Stern layer. It is therefore of fundamental importance to understand the changes in reactivity and energetics of proton transfer reaction while constrained exo- or endohedrally to any such complex media.

A major challenge in this problem is to resolve the effects of constraints/confinements decoupled from the inherent multi-scale complexities associated with systems such as a membrane channel. In this thesis, we have chosen to work with single walled carbon nanotube (CNT) as a simple model of the constraining medium. The size and width of its inner core make the CNT an idealized mimic of hydrophobic cavities [4, 5]. Anchoring reacting groups on the outer surface of CNTs help us in exploring effects of the hydrophobic surface and the solvent molecules nearby. The present work focuses on *in silico* covalent functionalization followed by detailed investigation of simple proton transfer reactions between different donor and acceptor pairs in exo- and endohedral CNTs.

1.1 Carbon Nanotubes: An Overview

The past couple of decades have witnessed a sudden growth in the number of known allotropes of carbon with the discovery of fullerenes, carbon nanotubes and graphene followed by a large number of other nanostructures such as nano-horns, nano-onions, nano-buds and nano-tori [6]. Of these, single and multi-walled carbon nanotubes (CNTs) are quintessential nanomaterials on account of a host of novel properties with potential applications in diverse fields of science and technology [6]. Although the existence of CNTs was reported earlier, synthesis of single walled nanotube independently by the research groups of Iijima [7] and Bethune [8] in 1991 set in motion the modern nanotechnology revolution. Carbon nanotubes are now recognized as the carbon fiber having the highest strength of any material and the highest thermal conductivity. Metallic carbon nanotubes transport electric current ballistically without dissipating heat. They can also function as active semiconductors in nanoscale devices [9], all as a result of their unique topologically controlled electronic properties [10, 11, 12, 13, 14, 15, 16]. The phenomenal progress in research on carbon nanotubes has been extensively documented in several review articles [6, 17, 18, 19, 20]. We shall highlight next the major advancements in the study of single walled carbon nanotubes (SWCNTs) pertinent to the present discussion.

1.1.1 Structure of Single Walled Carbon Nanotubes (SWCNT)

CNT exists in a solid fluffy powdered form in its physical state. A wide range of methods such as arc-evaporation, laser ablation, chemical disproportionation and chemical vapor deposition have been used to synthesize single- and multi-walled CNTs [21, 22, 23, 24]. Typical diameters of SWCNTs may range from one to tens of nanometer and length upto a few centimeter. Use of higher temperatures during synthesis are found to be conducive to the growth of CNTs with larger diameters [23]. Depending on the method of synthesis, it may be open-ended or closed at the end by a hemispherical cap similar to fullerene [25]. The latter is obtained when the pentagonal ring tips of open-ended SWCNTs are nucleated at some stage in the growth and initiate the closure mechanism [26].

The fundamental chemical unit of CNT is graphene that is composed of a hexagonal ring network of sp^2 hybridized carbon atoms similar to benzene rings [27]. The three-dimensional structure of SWCNT may be viewed as a graphene sheet rolled up into a seamless cylindrical structure [28] as shown in Fig. 1.1. The symmetry of wrapping is characterized in terms of a pair of integers (n, m) corresponding to the chiral vector, $\vec{C}_h = n\vec{a}_1 + m\vec{a}_2$ in the graphene plane. The chiral vector thus determines the orientation of six-membered carbon rings with respect to the nanotube axis leading to the classification of CNTs as achiral (armchair or zigzag) and chiral. For example, zigzag and armchair CNTs are represented by $(n, 0)$ and (n, n), respectively. In a typical sample of SWCNT, there is a continuous distribution of tubes with different chiral vectors. Rolling up of a two-dimensional graphite sheet to a quasi-one dimensional nanotube causes the formation of so-called van Hove singularities, i.e. energy levels with very high density of states. For example, a finite density of states occurs at the Fermi level if the value of $(n - m)$ is zero (armchair) or an integer multiple of 3 (zigzag) resulting in metallic and semi-metallic conductivity respectively. Otherwise the CNTs are semiconductors [29]. The electronic properties of SWCNTs are independent of their length, but dependent on the pair (n, m) [29].

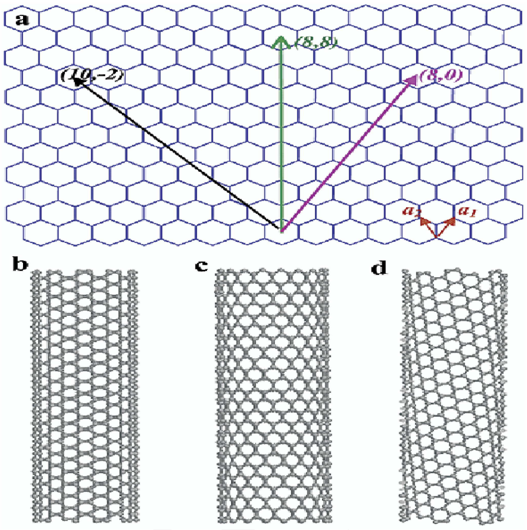

Fig. 1.1: (a) A two dimensional graphene sheet that on rolling along the basis vectors a_1 and a_2 leads to the formation of single walled carbon nanotubes. Rolling along $(8, 8)$, $(8, 0)$, and $(10, -2)$ vectors gives rise to different types of SWCNTs such as armchair (b), zigzag (c), and chiral (d) respectively. This picture has been reproduced with permission from *Acc. Chem. Res.*, **2002**, *35*, 1035-1044.

Multiwalled CNTs are comprised of more than one single-walled cylindrical tubes weakly bound to the neighboring tubes through van der Waals forces [28]. The constituent cylinders are likely to have different chirality independent of other walls [30].

1.1.2 Solubility and Reactivity of SWCNTs

The properties of SWCNTs are determined by their structure and composition including chirality and defects of the wall. However, practical applications of pristine SWCNTs are most severely affected by their poor solubility in aqueous and organic solvents [17]. A wide range of chemical modifications have been devised to enhance their solubility and expand their technological applications [17, 19, 20, 31, 32]. It is has been found that SWCNTs exhibit higher chemical reactivity compared to multiwalled carbon nanotubes [33, 34] providing greater opportunities of modification. Chemists have achieved functionalization of SWCNTs by groups such as acids, esters, aldehydes, amines, thiols, macro- and biomolecules resulting in marked improvement in their solubilities with better results produced by relatively larger groups [35, 36, 37]. CNTs functionalized with polar groups are found to be energetically stable in extended configurations while those with non-polar groups remain folded to maintain their stability in water [38].

Designing of functional SWCNTs and their potential applications depend on the varying reactivities and local curvatures of different available sites in these systems, *viz.* the sidewall C-atoms and the end caps, if present [39].

- *Reactivity of end-caps*: The end caps exhibit similarity to fullerenes. It is well known that the reactivity of fullerenes depend strongly on the curvature of their carbon framework [40]. For C_{60}, the outer convex surface is highly susceptible towards addition reactions while the inner concave surface is inert towards endohedral additions [40]. Similarly the chemical reactivities of SWCNTs with closed ends derive mainly from the caps as evidenced by selective opening of nanotube tips by air or wet chemical oxidation [41].
- *Reactivity of sidewall C-atoms*: In the context of open-ended SWCNTs used as models in this thesis, it is pertinent to discuss the reactivity of sidewall C-atoms that closely resemble aromatic benzene rings. A perfect SWCNT does not have chemically active bonds dangling at the open edges as observed in graphenes. So its chemistry stems primarily from the sidewall C-sites. The radius of curvature imposed on graphene network in nanotubes causes the three normally planar sp^2 hybridized C-atoms to undergo distortion resulting in bond angles closer to those characteristic of genuine sp^3 hybridization. Such distortion is likely to be the major source of strain in SWCNTs making sites being functionalized relatively more reactive compared to analogous sites in planar graphene faces. For example, the high reactivity of smaller SWCNTs with radius of curvature 3.5 Å has been attributed to 10% sp^3

character of wall carbon atoms [41]. The reactivites of sidewall C-atom sites are generally gauged in terms of angles of pyramidalization and π-orbital mis-allignment. Compared to fullerenes, SWCNTs typically have larger diameters and lower surface curvature leading to much lower reactivity towards addition reactions [27]. It has been shown from density functional and semi-empirical calculations that the convex surface of SWCNT is more reactive than the concave one and this difference in reactivity increases with increasing pyramidalization angle and decreasing diameter of the tube [39, 41, 42, 43]. Additional enhancement of reactivity may occur when sidewall defects such as Stone-Wales defect are present [19, 44, 45, 46]. Similarly, the tip of open ended SWCNTs with pentagonal rings and benzyne termination were also found to be potential reactive site [34, 41, 47].

It is evident from the discussion above that the overall chemical reactivity of SWCNTs and subsequent schemes of derivatization depend strongly on how they are synthesized. For example, SWCNTs prepared by arc discharge method are found to be less reactive than those synthesized by catalytic chemical vapor deposition (CCVD) method as the latter appear to contain more structural defects [19, 32, 41].

1.1.3 Chemical Modifications of SWCNTs

The variation of reactivity at different sites of SWCNTs has been optimally utilized in devising a wide range of methods for their chemical modifications as shown in Fig. 1.2 [12, 19, 20, 32, 48, 49, 50, 51]. These methods may be broadly classified as follows.

A. *Covalent functionalization*: In view of the low reactivity of carbon nanotubes compared to fullerenes, functionalization of the sidewalls and defective open ends by covalent bond formation requires highly reactive reagents and rather drastic reaction conditions [49, 52]. This is accomplished by three different approaches, namely, thermally activated chemistry, electrochemical modification and photochemical functionalization [50]. Since it is very difficult to achieve control over chemo- and regio-selectivity of these reactions, theoretical calculations are found to complement the experimental observations effectively.

Exohedral bond formation: Substantial degrees of exohedral covalent functionalization have been achieved starting with fluorine and reactive species such as nitrenes, carbenes and different radicals [49]. Several oxygen-containing functional groups, such as carbonyl, carboxyl and hydroxyl groups have also been attached covalently to the outer surface of the sidewall and open ends of SWCNTs through oxidation [31, 53, 54, 55]. Diazonium reactions are reported to yield high degree of functionalization of the CNT surface with $-F$, $-Cl$, $-CO_2H$, $-NO_2$ and reactive amine moieties [52]. Many of these serve as the precursors for a host of derivatives including SWCNTs functionalized with alkyl or aryl groups, amides and esters [16, 56, 57, 58, 59]. It has been possible to attach N-terminal protected amino acids and a series of bioactive peptides onto modified CNT surfaces [52, 60]. These reactions in most cases drastically alter the electronic properties of SWCNTs. But microwave assisted modification of SWCNTs by the Bingel reaction not only improved their solubilities, but also preserved their intrinsic electronic properties even with moderate levels of modification [61]

Endohedral bond formation: As discussed earlier, the inner concave surface of SWCNTs exhibit much lower reactivity compared to its outer surface making endohedral covalent bond formation a formidable synthetic challenge. Current studies indicate [62] that bulkier molecules prefer to retain their chemical identities inside SWCNTs with small diameter. On the other hand, for $(10, 10)$ and $(12, 12)$ SWCNTs with moderate diameters, small groups like methyl radicals can form a stable covalent bond with the inner sidewall [42, 62]. Calculations based on PM3 methods show that the minimum energy configuration of an endohedral F-adduct is higher in energy compared to its exohedral analogue by $43.0\,kcal\ mol^{-1}$ [42]. It is also predicted that addition of F-atom on the outer convex surface of a $(10,10)$ SWCNT preceding an inner wall attachment of F produces a more stable endohedral covalent bond in the mixed exo/endohedral functionalization. Under such circumstances, the endohedral adduct is predicted to be more stable than a non-covalent complex with a free F-atom located in the tube center [42]. Park and coworkers [63] then succeeded in functionalizing the inner layer of multiwalled CNTs with hydroxyl group in a progressive outer to inner attachment to the sidewall in the presence nitric acid and supercritical water. IR and Raman spectroscopic studies also indicate covalent attachment of groups such as $-CH_2$, $-C-OH$ and $-C-O-C$ to sp^3 C-atom of the inner sidewall [63]. These studies demonstrate the effectiveness of supercritical water as a reaction medium for endohedral covalent functionalization.

B. *Noncovalent functionalization*: This is a rapidly expanding field whereby solubilities of SWCNTs are remarkably improved while preserving the much valued properties of the pristine nanotubes. Several methods of non-covalent functionalization have been reported based on small aromatic molecules, surfactants, polymers and biomacromolecules [64, 65, 66]. In the presence of surface-active molecules, the nanotubes are believed to be at the hydrophobic interiors of micelles resulting in stable dispersions [49]. If the hydrophobic part of the amphiphile or the polymer wrapping around the nanotube surface contains an aromatic group, efficient π-π stacking and van der Waals interactions drive the formation of a non-covalent complex minimizing unfavorable interaction of a non-polar nanotube surface and the solvent water. Formation of protein-nanotube conjugate by the enzyme bovine serum albumin provides an important example in this class [17]. Known examples for the application of these SWCNT hybrids include the fabrication of flourescence energy transfer (FET) devices, chemical sensors, and photovoltaic devices. Endohedral functionalization may also be carried out by trapping guest molecules such as fullerenes inside the CNT core [49]. Encapsulation of biomolecules such as proteins and nucleic acids by trapping them into the inner cavities of CNTs has resulted in a new class of compounds [17, 67]. Some of the vital applications of these bio-CNT compounds include drug delivery and utility as biosensers [67].

It may be noted that the present thesis investigates the energetics of reactions between groups that are endo/exohedrally attached to the surface of the SWCNTs.

1.2 SWCNT as a Model of Hydrophobic Nanopore

Hydrophobic interactions at the molecular level between water and non-polar solutes are widely known to drive several biological and self-assembly processes such as protein folding and formation of micelles and membranes in aqueous solutions. However, weakly polar or non-polar pores play a crucial role in the unique biological function of a large number of systems including aquaporin water channels, proton pumps, cytochrome P450 and the gating region of potassium channels [68]. Consequently, the last decade has witnessed important experimental [69, 70, 71, 72] and theoretical studies [18, 73] on the structure and dynamics of water within hydrophobic confinement. A biological ion channel is characterized by an internal radius of $1-2$ Å and length of ~ 30 Å that closely compares to the size of a CNT [74, 75]. This analogy along with a relative simplicity of its structure led to an extensive use of SWCNTs as the prototype of hydrophobic nanopores. It has been pointed out by Alexiadis and Kassinos [18] that even if the carbon nanotubes turned out to have no practical applications at all, the study of water confinement inside CNTs would still be of very high relevance to understand the role of water-filled and water-permeable pores in chemistry, biology and material science. Narrow water-filled pores have been suggested as building blocks for high-selectivity/high-flux membranes for molecular devices and fuel

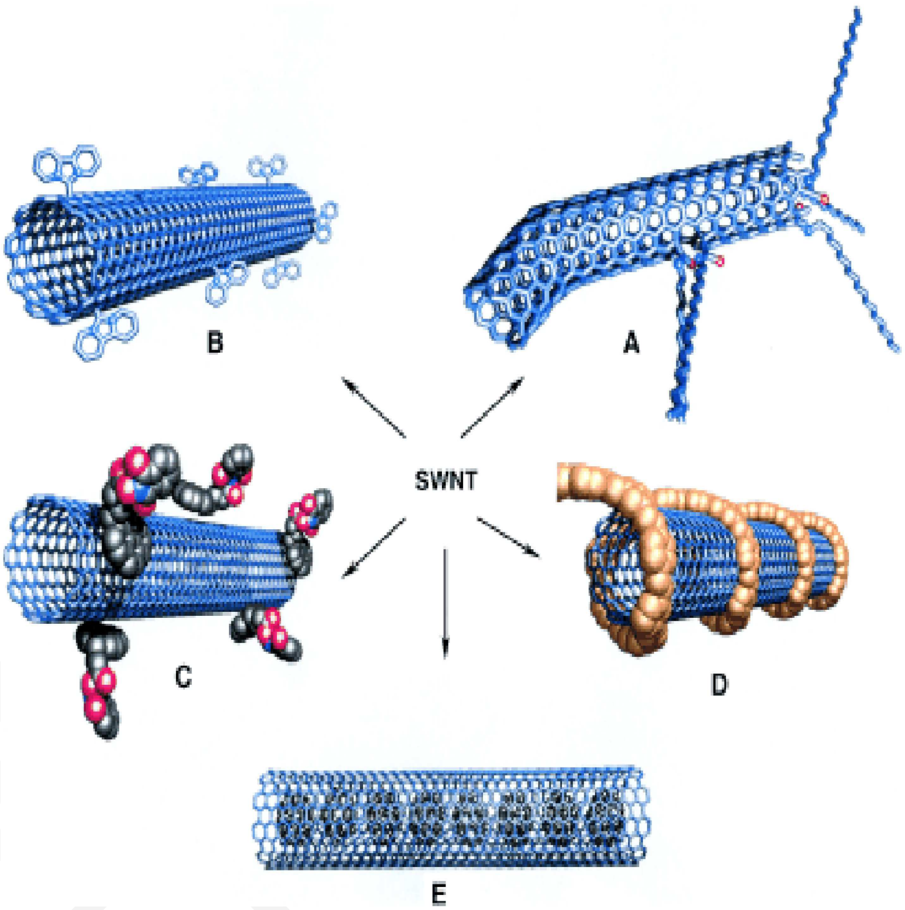

Fig. 1.2: Different routes to functionalize single walled carbon nanotubes. Covalent functionalization can be carried out at (A) ion defective sites, such as end caps (B) sidewall with chemical groups such as surfactants (C) and polymers (D). Endohedral functionalization using noncovalent methods (E) is also reported. Non-covalent functionalization at the outer surface is possible. This picture has been reproduced with permission from *Angew. Chem. Int. Ed.*, **2002**, *41*, 1853-1859.

cells [76]. The choice of CNTs are further substantiated by the recent reports that they may be used as nanosyringes to inject drugs across cellular membranes or to transport ions and water selectively into biosensor devices [4]. Let us highlight below some of the intriguing aspects of water and ions inside single walled carbon nanotubes.

1.2.1 Filling of CNT pores by water

The inert hydrophobic nature of the inner cavity of CNTs has been known to the scientific community for several years [75, 77, 78, 79]. With the increase of diameter to moderately high values, the hydrophobic character of the inner cavity of a CNT becomes more pronounced [15] although it does not depend on its chirality [18]. It has also been shown that increasing the flexibility of the CNT increases their apparent hydrophobic character, while the presence of water inside the channel makes them more resistant to radial collapse [77]. Water filling of an empty nanotube is strongly cooperative with suggested coexistence of dry and wet states [4, 44, 80, 81, 82] with experimental indications of wetting of the inner cavity of CNT using liquids having surface tension less than 200 mNm^{-1} [81, 82]. A small reduction in the van der Waals attraction between water and carbon atoms may trigger drying of a previously water-filled pore of a solvated CNT [73]. In addition, the number of water molecules filling the pore is found to depend on factors such as local polarity of the cavity, internal electric fields and shape fluctuations [68] along with the presence of defects and/or attached hydroxyl/carbonyl groups [83]. Gogotsi and co-workers [84] also reported the encapsulation of aqueous fluids inside hydrothermal CNTs.

1.2.2 Structure of water confined to SWCNT

Several properties including structure of water inside SWCNTs depend crucially on the method by which they are probed [85]. In particular, details of the force field parameters may affect the results of molecular dynamics simulations that have been extensively used to investigate the structure of water confined within SWCNTs of varying sizes [18, 86]. Most of these studies, to ensure computational efficiency, employed CNT models of dimensions smaller than those typically obtained in a standard sample [4]. However, independent of the models used, it is evident that water inside the nanotube differs considerably from the bulk [87].

If the diameter of the nanotube is comparable to the size of water molecules, as in the case of narrow $(6, 6)$ SWCNT, there is only sufficient space for the formation of a single-file water chain aided by strong hydrogen bonding interactions between confined water molecules and their weak interaction with the wall [68]. Each water molecule is estimated to have $1-1.5$ hydrogen bonds on an average compared to $2-4$ of such bonds in bulk water [73]. A pictorial representation of the molecular dynamics simulation of water in CNT by Hummer and coworkers is shown in Fig. 1.3. A similar reduction in the number of hydrogen bonds as function of temperature and density has also been observed in the molecular dynamics simulations of supercritical water inside carbon nanotubes [88]. The formation of single-file water chain is signified by the appearance of a single peak in the radial water density profile [73]. The energetics of such arrangement derive major contribution from the dipole-dipole ordering interaction which may persist even upto macroscopic lengths [89]. With the increase in CNT diameter, a second layer of water is allowed inside the tube as evident from the second peak in the radial density profile of water [18, 90] shown in Fig. 1.4. For very large CNTs at normal temperatures, formation of three or four layers gradually lead to a decorrelation of the innermost water molecules with the wall and the bulk properties are restored [90, 91, 92, 93]. Hansaki and co-workers [94, 95] also reported the formation of water layers from their molecular dynamics simulations of CNTs with diameters ranging from 5 to 53.5 Å.

Formation of ordered water arrays inside CNTs have been debated for long [96]. Under ambient conditions of 1 bar and 300 K, water confined inside CNTs of a critical size of 8.6 Å was reported to exhibit ice-like symmetric structure [97]. Although no systematic attempt has been made so far to interprete the behavior of confined water in terms of a phase diagram, water structure in CNTs is experimentally found to undergo a structural transition from a liquid-like to a solid-like state between 200 to 250 K depending on pressure and CNT geometry [18, 87]. As identified recently in a combined neutron scattering and molecular dynamics study [71], a 'shell+chain' configuration is formed at temperatures below $\sim$ 210 K. The shell consists of a sqaure ice sheet rolled into a hollow concentric cylinder inside the nanotube parallel to the long tube axis with a gap of $\sim$ 3 Å between the shell and CNT wall [71]. In the center of the tube, the single file of water chain extends along the tube axis. At $\sim$ 210 K, drastic fluctuations transform the shell-chain structure into a liquid-like state resembling supercooled water [71].

1.2.3 Dynamics of water inside SWCNTs

Several molecular dynamics studies [18] report the formation of orientationally ordered water wire permitting exceptionally rapid water flow through short SWCNTs [98, 89]. Hummer *et al* reported the water conduction through an uncapped, single-walled $(6, 6)$ SWCNT of length 13.4 Å and diameter 8.1 Å with an average of about 17 water molecules passing through the tube per nanosecond [73] that compared well with the measured rate of water flow in aquaporin-I channel. Longer molecular dynamics simulations (460 ns) of water transport through CNTs with radii of 7 to 20 Å also confirm fluctuations of water density inside the core between an almost empty (vapor-like) and filled (liquid-like) states

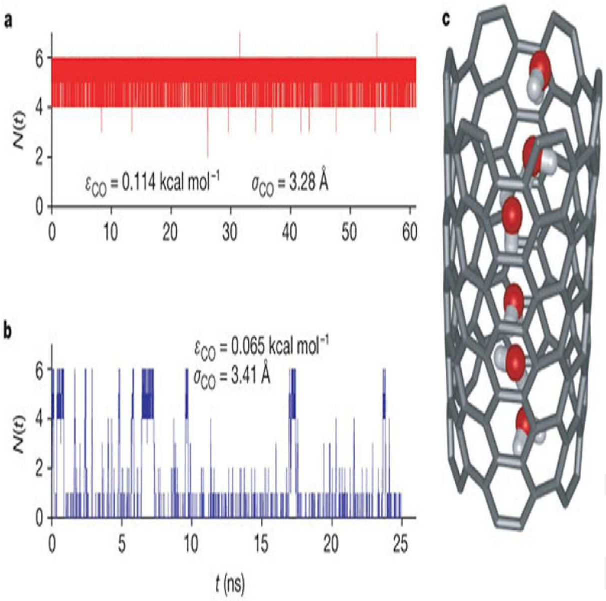

Fig. 1.3: Representative snapshot of molecular dynamics simulation of CNT in water. (a) The number of water molecules confined to carbon nanotube with time $N(t)$ (b), Interactions between C-atoms of CNT and water molecules and 1-D array of water molecules connected by hydrogen bonds (c) are shown in the figure. This picture has been reproduced with permission from *Nature*, **2001**, *414*, 188-190.

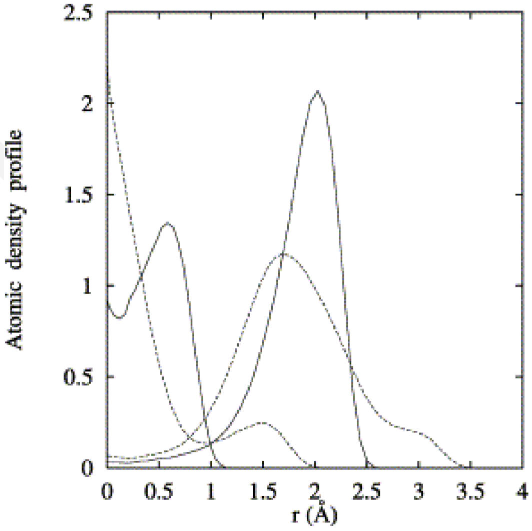

Fig. 1.4: The structure of water confined to (8 : 8) and 6 : 6 CNTs is presented in terms of $O - CNT$ atomic density profile (full lines) and $H - CNT$ profile (dashed lines). This picture has been reproduced with permission from *Chem. Phys. Lett.*, **2000**, *329*, 341-345.

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within $\sim$ 30 ps in an avalanche-like fashion independent of the pore size [99]. Such behavior may be mapped to capillary evaporation alternating with capillary condensation [73, 99] and it is reported to persist even under continuous deformation of the tube wall [100].

It was also noted that water conduction occurs in pulses/bursts with peaks of about 30 water molecules per nanosecond [73]. In narrow CNTs, bursts occuring from the filled state contribute upto 77 % of the total flux [99]. Such concerted, unidirectional water motion has been described in terms of a continuous-time random walk model [101, 102]. With diameters of CNTs in the range of $10 - 20$ Å, experimentally observed rates of water flow exceed the rates predicted from hydrodynamics by factors of $\sim$ 1000 or more [76, 103]. Molecular dynamics simulations of diffusion of water molecules through $(6, 6)$ and $(8, 8)$ nanotubes indicate an initial ballistic transport changing into Fickian diffusion in the long run [104].

Formation of water layers with stacked-ring structure inside SWCNTS are found to be associated with unusual hydrogen bonding features using vibrational spectroscopy [105]. Moreover, confinement within narrow SWCNTs compel the water molecules to remain longer in each other's vicinity leading to longer lifetimes and rapid reformation of hydrogen bonds in these systems [88, 95]. The reorientational dynamics of confined water molecules were reported to exhibit bistability involving angular jumps between the two stable states [106]. The angular jump of a water molecule in the bulk involves the breaking of a hydrogen bond with one of its neighbours and the formation of a hydrogen bond with a different neighbour. In contrast, the angular jump of a confined water molecule corresponds to an interchange of the two hydrogen atoms that can form a hydrogen bond with the same neighbour. The free energy barrier between these two states at temperature T measures only a few k_BT (k_B: Boltzmann constant).

1.2.4 Water confined inside functionalized SWCNTs

Functionalizations of SWCNTs at present focus mainly on tailoring the chemistry of carbon nanotubes. However, little or no attempt has been made so far to harvest the immense opportunities presented by these modifications. Functionalization is expected to add a new perspective to the storage and permeation of fluids by nanotubes [18]. For example, molecular dynamics studies on ionic flow in CNTs functionalized at both open ends with $-COOH$ groups amply demonstrate how selectivity between cation and anion permeation may be acheieved [107]. Anchoring of the carboxylic groups shown in Fig. 1.5 at the inner wall substantially alters the hydrophobicity of the inner cavity and was found to allow faster permeation of a mixture of water and methanol [108] in a continuous flow instead of the pulsed behavior discussed earlier in this section. Recently, by embedding a $(9, 9)$ SWCNT vertically between two graphite sheets and endohedrally attaching eight or sixteen carbonyl groups, Gong *et al* presented a theoretically designed molecular sieve in an attempt to mimic the structure of potassium channels in membrane spanning proteins (e.g., KcsA) [109]. Molecular dynamics studies of Na^+ and K^+ ion transport through these nanopoes demonstrated remarkable selectivity dependent on the functionalization pattern of the carbonyl groups [109]. For instance, the passage rate of K^+ ion is 4.6 times that of Na^+ ion for one type of functionalization of the nanopore, while Na^+ ions are transported 2.2 times faster than K^+ ions in a differently modified core. These observations have been primarily attributed to highly specific alteration of hydration structures of these ions under the model conditions [109].

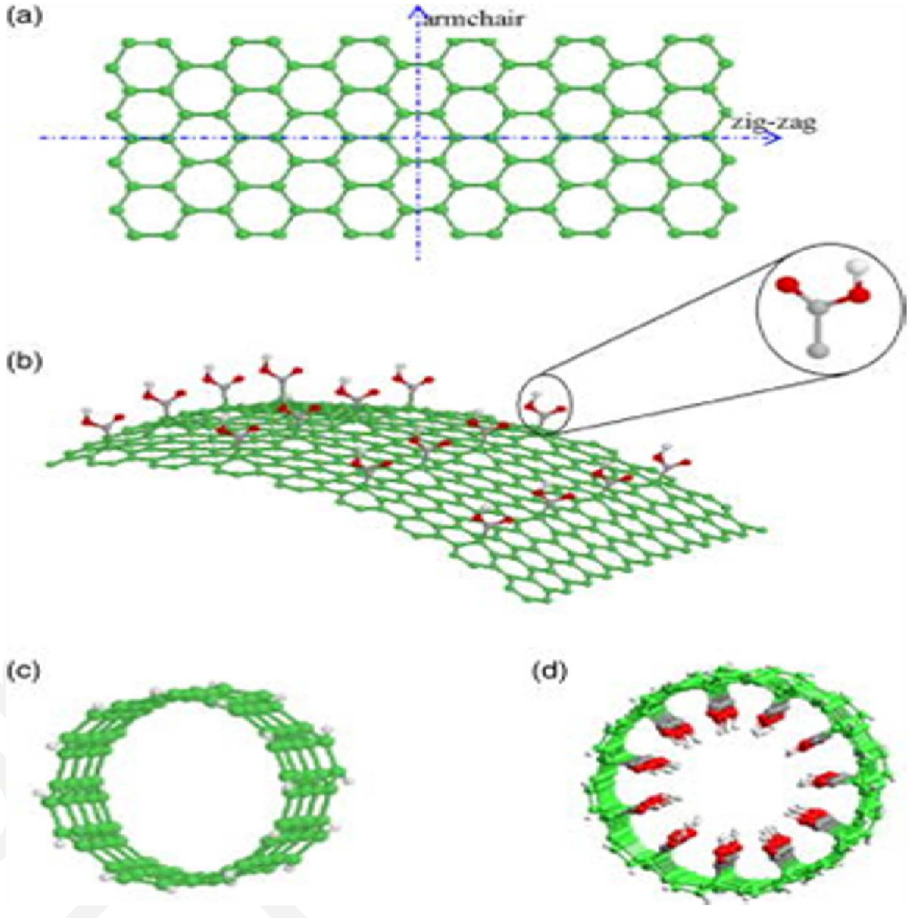

Fig. 1.5: (a) A hexagonal graphene sheet that is needed as precursor to obtain zig-zag/armchair single walled carbon nanotubes. (b) The graphene sheet with active Carboxylic acid groups. (c) The graphene sheet rolled into an armchair SWCNT with a diameter of 8.15 Å. (d) A hydrophilic SWCNT with endohedrally anchored -COOH groups. This picture has been reproduced with permission from *J. Chem. Phys.*, **2005**, *122*, 214702-214709.

1.3 Modeling Biological Proton Transfer with the Help of SWCNTs

Understanding the mechanism of simple charge transfer processes such as proton transfer is a fundamental problem in bio-enzymatic catalysis [1]. Derivation of any such mechanism usually requires as input detailed informations such as high resolution structures of the active site, protonation states of the reacting groups and results of structural and kinetic studies on mutation at one or more points at or near the active site. Often energetics of analogous proton transfer reactions in a simpler medium provide useful insight into the mechanism. Once again, SWCNTs appear to be the ideal model of choice to mimic proton conduction in biological nanopores with minimal complexity arising from the confining nanotube itself.

1.3.1 Overview of biological proton transfer

The molecular mechanism of proton transport through a polar medium such as water is generally discussed in terms of the Grotthuss mechanism [110]. It is interesting to note that during the original proposal by Grotthuss, not even the correct composition of water was known [111, 112]. According to this mechanism, proton transport process consists of two elementary steps, *viz.* hopping of the proton between donor and acceptor sites and turn or reorientation of the donor-acceptor pair to optimize the distance separating them for an efficient hopping. At present, it is well established that in bulk water, there is no individual proton, H^+ or hydronium cation, H_3O^+ that migrates through the hydrogen bonded network [113, 114]. Instead, proton transfer involves a series of isomerization between the Eigen and the Zundel cations, the two species being separated by an activation barrier of about 2.5 $kcal\ mol^{-1}$ [112, 113].

Biological proton transport generally involves long range translocation of protons in and through proteins in numerous enzymatic catalyses and bioenergetic processes [1, 115, 116, 117]. At the simplest level, based on a model proposed originally by Onsager [2, 118] an excess proton may be assumed as being relayed in a protein via a Grotthus-like mechanism [111] involving hydrogen bonded network of polar amino acid side chains and water molecules present inside the protein. The driving force behind long range transport of proton generally originates from the difference in pK_a values of amino acid sidechains as ΔG_0 ($kcal\ mol^{-1}$) = 1.38 $[pK_a(DH^+) - pK_a(AH^+)]$ [117]. However, these proton paths may be long-lived or transient in nature on account of complex dynamics of the protein matrix [68]. We will briefly highlight here important aspects of biological proton transfer in confinement with reference to three extensively studied systems.

Carbonic anhydrase

An interesting yet complicated case study is presented by the isozymes of carbonic anhydrase (CA). In the highly efficient isozyme human carbonic anhydrase (HCA) II, the rate determining step involves an intramolecular proton transfer between a zinc-bound water (hydroxide) molecule and His-64 with a maximal value of the rate constant $k_B = 800\ ms^{-1}$ [119]. This key histidine residue is located in a narrow channel leading from the surface of the enzyme into the active site and is shown in Fig. 1.6. It is characterized by a pK_a value of 7.2 [119] facilitating proton transfer both from and to His as required by the reversible nature of the catalysis. This residue is also found in the isozymes CA IV and VII at the same location and has been connected to the high catalytic turnovers of these isozymes as well [120]. A much lower efficiency of the isozyme HCA III ($k_B = 3\ ms^{-1}$ [119]) has been attributed to the presence of a Lys residue ($pK_a \sim 9$) at the position 64 that is too

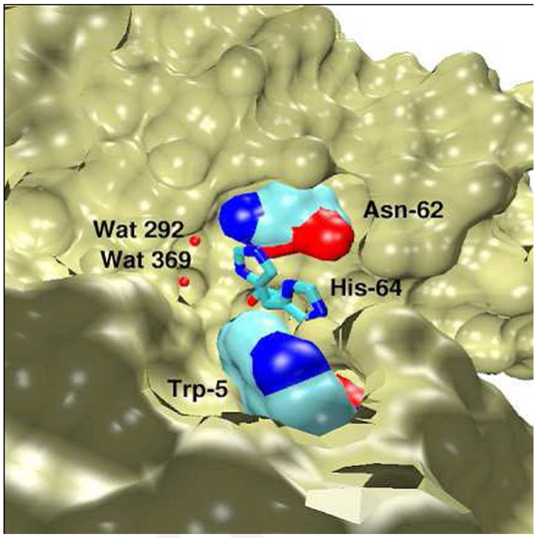

Fig. 1.6: A key His sidechain located at the active site which is in a confined hydrophobic channel with neighbouring Asn and Trp residues and surrounding water molecules. The involvement of His sidechain in the proton transfer aided by hydrogen bond network is indicated in the pictorial representation. This picture has been reproduced with permission from *J. Phys. Chem. B*, **2007**, *111*, 10563-10576.

basic at physiological pH to serve as an efficient proton shuttle [121]. However, mutation of Lys by His (K64H) in HCA III results in a marginal recovery of the catalytic rate with $k_B = 20.0\ ms^{-1}$ in spite of the His residue having a pK_a of 7.5. A somewhat higher efficiency of the proton shuttle may be obtained if a Glu or Asp residue is inserted at the location 64 in HCA III resulting in $k_B \sim 40.0\ ms^{-1}$ where both the residues exhibit pK_a values equal to 6.4 and 5.7 respectively. [122].

The observed variations in efficiency of the putative proton shuttle as discussed above thus appear to depend not only on the pK_a values, but also on various other factors. Rotation of the residue 64 through the channel-like pore and a non-equilibrium coupling of the protonation state of the side chain to its fluctuating orientation may be some of the major contributors [3, 113, 123, 124]. As already indicated, the presence of specific neighboring residues and internal water molecules may also play a crucial role [3, 113, 119, 123, 124]. However, the variation of reactivity of different residues such as His, Asp, Glu at the position 64 restricted inside a channel-like environment is not completely understood.

Like His, Tyr sidechain is known to play a crucial role in a wide range of chemical and biochemical processes primarily through (a) deprotonation of the phenolic OH group present in its sidechain and (b) formation hydrogen bond in both protonated and deprotonated states of the sidechain [125]. In human carbonic anhydrase II, a crucial role is played by a tyrosine residue, Tyr-7, in the active site, not only in providing a translocation path for an excess proton, but also in stabilizing the fluctuating conformations of a neighboring His residue that is widely believed to be a proton shuttle between the active site and the bulk solvent [126]. Although most of the current analyses of HCA II mechanism are based on Tyr-7 sidechain being in its protonated state, recent neutron diffraction studies seem to indicate that Tyr-7 may be present in the active site of HCA II with its sidechain in an unprotonated phenoxide form [124]. Needless to say, any such alteration of the protonation state of Tyr-7 sidechain would crucially affect the free energetics of the alternative pathways that have been proposed to supplement the His-mediated shuttle to exchange proton between the active site and the protein surface. The importance of Tyr in several other enzymes, including carboxypeptidase A [125, 127] is also well documented.

Aquaporin

Aquaporins are membrane proteins that facilitate the transport of water across the cell membrane. These water pores, however, are specifically designed to be impermeable to charged species such as protons and thus help in the conservation of membrane's electrochemical potential. Different experimental and theoretical studies on the paradoxical selectivity of aquaporin confirm that there are two stages of filter inside the channel [128, 129, 130, 131, 132, 133, 134]. The first stage of the filter is located in the middle of the channel at the asparagine/proline/alanine (NPA) motif and the other is located on the extracellular face of the channel in the aromatic/arginine region. It has been suggested that protein-water interactions dominate only in the NPA and aromatic/arginine regions of the pore. In contrast, water-water interactions dominate the energetics of water permeation in the other regions of the channel. From a proton free energy and electrostatic profile, it was observed that there is a high barrier located at the NPA region which is the dominant factor preventing proton transfer across this region. However a secondary peak on the free energy profile also appears near the aromatic/arginine region [130, 131, 134]. There is a general conclusion that electrostatic interactions, rather than proton wire interruption effects, dominate the mechanism of proton exclusion in aquaporins [134].

Gramicidin A channel

Gramicidin A is the smallest known, naturally-occurring transmembrane ion channel constituted by a 15-residue linear peptide of alternating D- and L-amino acids. The high proton conductance through this channel has been investigated by both experimental and theoretical studies [135, 136, 137, 138, 139]. In Fig. 1.7, a chain of water molecules buried in the gramicidin A system is shown. A fully microscopic empirical valence bond (EVB) model and a simplified EVB-based model with Langevin dynamics simulations reveal that proton transfer is controlled by the change in solvation energy of the transferred proton along the channel axis in gramicidin [136]. However, from a molecular dynamics simulation with potential-of-mean force calculation, it was concluded that the mobility of protons in gramicidin A is essentially determined by the fine structure and the dynamic fluctuations of the hydrogen-bonded network [137]. Recently another study of the gramicidin channel using a sequential dynamical Monte Carlo algorithm suggests that proton desolvation as well as water rotations are equally important for proton transfer through the channel [139].

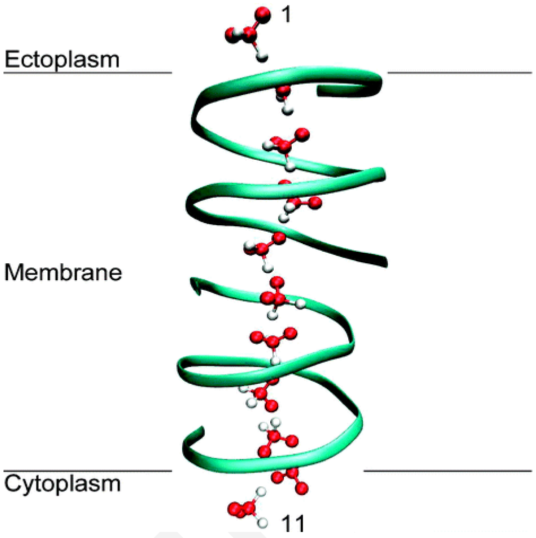

Fig. 1.7: A typical example of membrane channel such as Gramicidin A is shown. The water molecules confined to the membrane channel are depicted using ball and stick representation. This picture is reproduced with permission from *J. Phys. Chem. B* **2008**, *112*, 13401-13410.

1.3.2 Proton transfer through carbon nanotubes

The highly orientated single-file arrangement of water wire through SWCNTs is expected to facilitate fast proton transport through the hydrophobic nanopore with a near-ideal scenario to understand the Grotthuss mechanism in a biological perspective. Charge transport through carbon nanotubes by itself is of utmost importance in view of numerous potential applications including ion-selective molecular sieves [109]. Use of SWCNTs to model biological confinement and membrane transport, however, needs accurate mapping of the CNT pore to the system being investigated under appropriate conditions [75, 140, 141].

The conduction of an additional proton through the ordered water chain inside CNT is found to mediate proton transfer upto 40 times faster than in bulk water [142, 143]. It has also been found that an excess proton is hydrated by the oriented water wire inside the nanotube mainly in a distorted Zundel form unlike the distorted Eigen form encountered

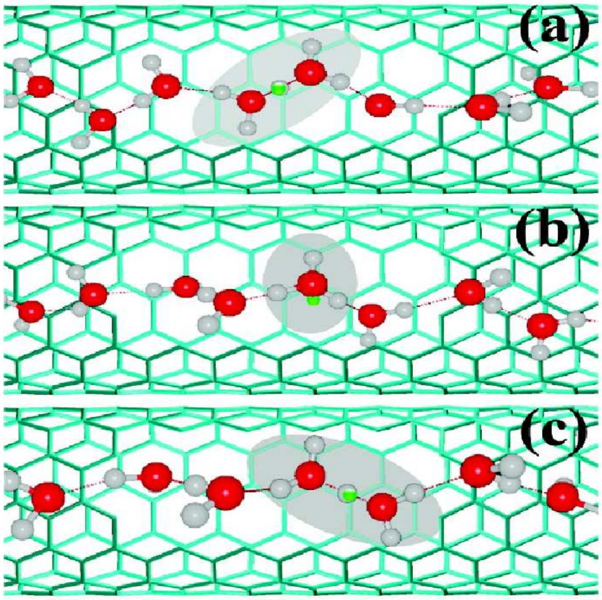

Fig. 1.8: Proton transfer of an excess proton through a $(6, 6)$ single walled carbon nanotube from MSEVB simulation. The proton transfer is facilitated by water chain connected by hydrogen bonds. The excess proton is in a Zundel cationic form at -0.17 ps (a), Eigen cationic form at 0.03 (b) and again in a Zundel form at 0.05 ps (c). Center of charge of the protonated water is depicted as green ball. This picture is reproduced with permission from *J. Am. Chem. Soc.* **2010**, *132*, 11395-11397.

in bulk water [144, 145]. Moreover, the fast proton transfer has been suggested to involve a Zundel-Zundel mechanism through the transient intermediate $H_7O_3^+$ in contrast to the Eigen-Zundel-Eigen mechanism found in bulk water [145]. The underlying dynamics is represented in Fig. 1.8. It may be noted that the scenario described in these studies refers to proton conduction through a perfect $(6, 6)$ SWCNT that is free from any electrostatic effects or other environmental effects. Results from *ab initio* simulations also support the above findings [146].

Electrostatics is expected to play an important role in proton conduction through narrow hydrophobic nanopores. The effects of electrostatics on the rate of proton transport in narrow CNTs have been analyzed using various quantum mechanical-molecular mechanical approaches including empirical valence bond (EVB) [141], multistate empirical valence

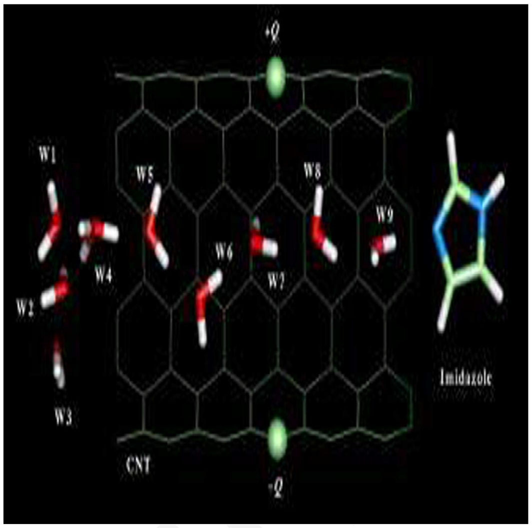

Fig. 1.9: A snapshot of the $QM - MM$ simulation of proton transport through SWCNT with a imidazole group at one end of the tube and an external perturbation created by opposite charges (green ball) on the CNT wall perpendicular to the axis of the tube. This picture is reproduced with permission from *J. Chem. Phys.* **2006**, *124*, 204510-204518.

bond (MSEVB) [145], Carr-Parrinello molecular dynamics (CPMD) [142] and *ab initio* molecular dynamics (AIMD) [147] simulations. The interactions between polar sidechains and water molecules play an important role, for example, in the function of aquaporin channels [140, 141]. Such interactions have been suggested to control the electrostatic barrier to proton transfer thereby determining if proton conduction or blockage would take place across the tube [141]. Hassan *et al* studied the effect of external electric fields applied perpendicular to the tube axis to mimic effects of polar amino acid sidechains in close proximity [148]. At lower field, such perturbation has been found to produce a to and fro shuttling motion of the proton within the tube before being delivered to the acceptor in a few picoseconds. For field above ~ 0.5 V/Å, proton transfer is reported to be blocked with the excess proton trapped at the center of the tube shown in Fig. 1.9 [148]. It is also expected that changes in the protonation state of a functional group attached to the inner wall of the CNT, resulting from proton transfer, may also trigger a further influx of water molecules inside hydrophobic regions sustaining the formation of crucial proton connectivities through the tube [146]. In the presence of hydrogen bond defects and large desolvation penalty inside the CNT, the rate of proton conduction is found to reduce to about one proton per hour per tube at neutral pH [143].

1.4 Aim of the Thesis

It is evident from the preceding discussion that computational modeling of functionalized carbon nanotubes (FCNTs) is an emerging area that has hardly been explored so far. In view of the rich chemistry that such systems demonstrate and the large number of derivatives that are already available in literature, a prudent choice of the system to be investigated seems in order. In this thesis, we have chosen to work with SWCNTs functionalized endo- and exohedrally with analogues of polar amino acid sidechains and/or one or more hydroxyl group(s). Our choice serves two major purposes. First, we are able to generate *in silico* FCNTs that are otherwise difficult to synthesize. Model structures with functional group(s) attached to sites of our choice are expected to complement those systems that may not be amenable to experimental separation on account of poor regio- or stereoselectivity. Second, we can design the model structures in a way to be able to mimic at the simplest level confinement of these groups in membrane channel or an enzyme active site. In the thesis, we have investigated the following two classes of covalently functionalized SWCNTs.

- Endohedral functionalization: In this model, the functional groups are suspended from the inner wall of the nanotube and extend into the core of CNT,
- Exohedral functionalization: The functional groups in this model are attached to the outer surface of CNT and orient away from the wall of the nanotube.

The other important aspect of our work concerns the choice of proton transfer reaction as a probe of the effects of hydrophobic confinement on one of the most fundamental chemical/biochemical processes. To the best of our knowledge a fully microscopic description of a proton transfer process between active residues while suspended inside the core of CNT or on its surface has not been reported earlier.

In this thesis, we present a detailed investigation on the generation of optimized structures of functionalized carbon nanotubes using density functional based theories in the gas phase. While the donor is selected from a set of polar amino acid sidechains (including His, Glu, Asp, Ser, Thr and Tyr), a hydroxyl (-OH) group has been chosen as the acceptor of proton in the reaction being studied. The effect of confinement on reactivity has been explored first by calculating proton affinity values of the reactants in gas phase. Variation in water structure around the reactant pair is then studied to understand the critical contribution of hydrogen bonding to neighboring groups on the observed reactivity patterns. We finally report the effect of confinement on free energy changes associated with proton tranfer reactions using the empirical valence bond theory. The findings of our research with single walled carbon nanotubes thus elucidate the effects of confinement decoupled from the inherent complexities associated with systems such as a membrane channels.