Statins in therapy: Understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies

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European Journal of Medicinal Chemistry 85 (2014) 661-674
http://dx.doi.org/10.1016/j.ejmech.2014.08.037

Statins in therapy: Understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies.

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Summary

The atomic electrostatic potentials calculated by the CHELPG method have been shown to be sensitive indicators of the gas phase and solution properties of the statins. Solvation free energies in water, n-octanol and n-octane have been determined using the SMD solvent model. The percentage hydrophilicity and hydrophobicity (or lipophilicity) of the statins in solution have been determined using (a) the differences in solvation free energies between n-octanol and n-octane as a measure of hydrophilicity, and the solvation energy in octane as a measure of hydrophobicity (b) the sum of the atomic electrostatic charges on the hydrogen bonding and polar bonding nuclei of the common pharmacophore combined with a solvent measure of hydrophobicity, and (c) using the buried surface areas after statin binding to HMGCR to calculate the hydrophobicity of the bound statins. The data suggests that clinical definitions of statins as either "hydrophilic" or "lipophilic" based on experimental partition coefficients are misleading.

An estimate of the binding energy between rosuvastatin and HMGCR has been made using: (a) a coulombic electrostatic interaction model, (b) the calculated desolvation and resolvation of the statin in water, and (c) the first shell transfer solvation energy as a proxy for the restructuring of the water molecules immediately adjacent to the active binding site of HMGCR prior to binding. De-solvation and re-solvation of the statins before and after binding to HMGCR are major determinants of the energetics of the binding process.

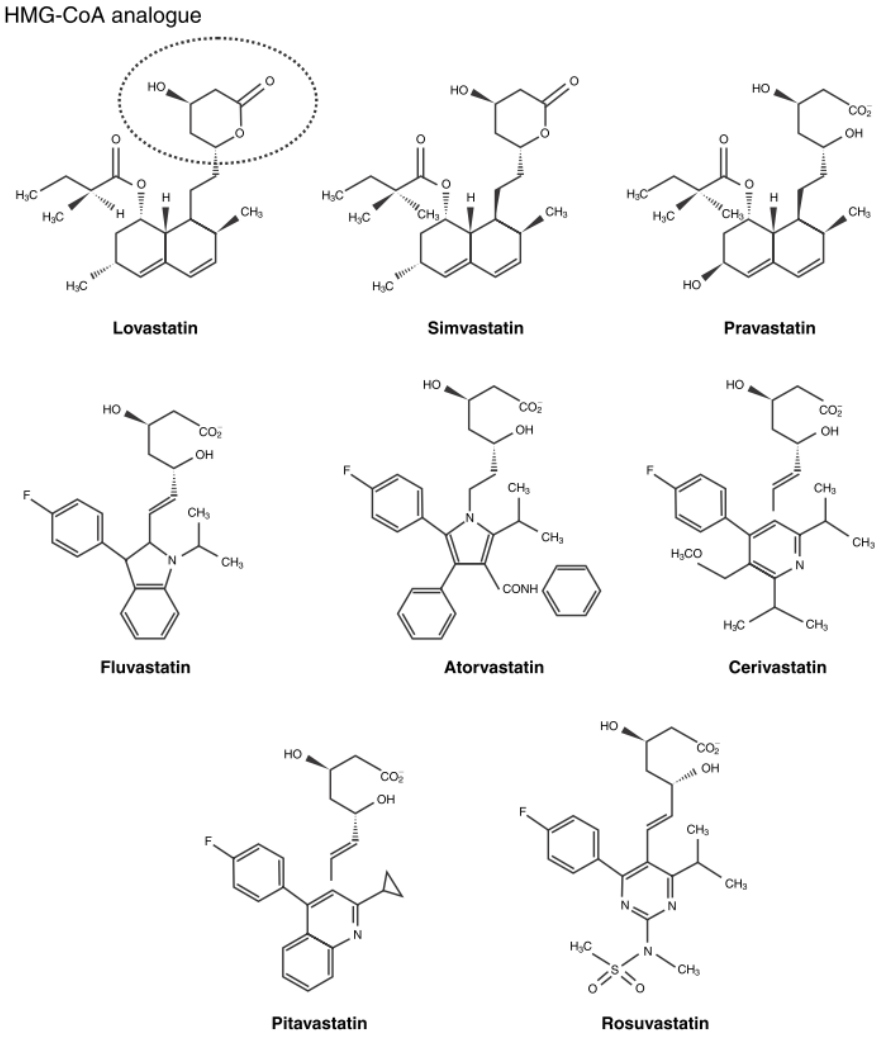
An analysis of the amphiphilic nature of lovastatin anion, acid and lactone and fluvastatin anion and their abilities to cross the blood brain barrier has indicated that this process may be dominated by desolvation and resolvation effects, rather than the statin molecular size or statin-lipid interactions within the bilayer.

The ionization energy and electron affinity of the statins are sensitive physical indicators of the ease that the various statins can undergo endogenous oxidative metabolism. The absolute chemical hardness is also an indicator of the stability of the statins, and may be a useful indicator for drug design.

Keywords: Statins, electrostatics, amphiphilicity, binding energies, metabolism.

1. Introduction

Statins are some of the most widely prescribed drugs in the world. Statins are HMG-CoA reductase inhibitors used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. HMG-CoA reductase (HMGCR) catalyses the conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol synthesis [1-3]. The associated reduction in intracellular cholesterol concentration induces LDL-receptor expression on the hepatocyte cell surface, which results in increased extraction of LDL-C from the blood and decreased circulating LDL-C concentrations. Statins also have beneficial effects on other lipid parameters, including increases in high-density lipoprotein cholesterol (HDL-C) concentration and decreases in triglyceride concentration. Increased cholesterol levels have been associated with cardiovascular disease, and statins have been found to prevent cardiovascular disease in those who are at high risk [4-5]. Statins also have beneficial cardiovascular effects independent of their lipid-modifying properties. These pleiotropic properties result from inhibition of the synthesis of nonsteroidal isoprenoid compounds, which are also produced from mevalonic acid [4-7].



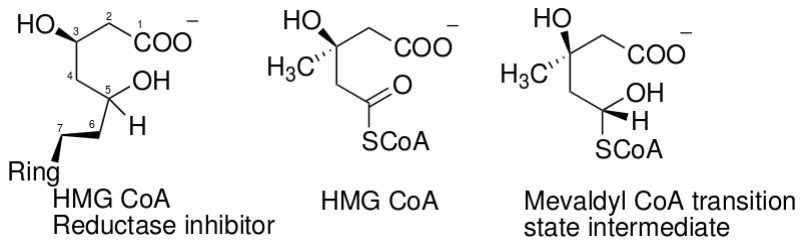


Figure 1. **Chemical structures of the statins, and structural similarities of the statins (HMG CoA Reductase inhibitors), HMG CoA, and mevaldyl CoA transition state for the conversion of mevalonate, a precursor in the synthesis of cholesterol in the liver. Lovastatin and simvastatin are shown in the lactone form, all other statins are shown in the acid anion form.**

The statin pharmacophore is modified 3,5 dihydroxyglutaric acid (DHGA) moiety, (a 3,5 dihydroxyheptanoic acid derivative for type 1 statins, like lovastatin, simvastatin, pravastatin, as well as atorvastatin, a type 2 statin, or a 3,5 dihydroxyhept-6-enoic acid derivative for type 2 statins like rosuvastatin, fluvastatin, pitastatin) which is structurally similar to the endogenous substrate HMG CoA and the mevaldyl CoA transition state intermediate (Figure 1). The statin pharmacophore binds to the same active site as the substrate HMG-CoA and inhibits the HMGCR enzyme. The HMGCR is stereoselective so all statins must have the required 3R,5R stereochemistry be effective inhibitors. The 3,5 dihydroxyhept-6-enoic acid derivative side chain for type 2 statins has an E configuration about the C_6-C_7 double bond. The statin pharmacophore is common to all statins, and molecular and clinical differences are due to the ring attached to the pharmacophore, which can be a partially reduced naphthalene (lovastatin, simvastatin, pravastatin), a pyrrole (atorvastatin), an indole (fluvastatin), a pyrimidine (rosuvastatin), a pyridine (cerivastatin), or a quinoline (pitavastatin). The substituents on the rings define the solubility of the statins and many of their pharmacological properties. Type 2 statins have a common 4-fluorophenyl substituent, with other polar substituents such as the methane sulphonamide group on rosuvastatin which allow stronger binding to HMGCR. Type 1 statins have a common hexahydro-napthalene ring.

The hydrophilicity of statins originates from the common pharmacophore (which has strongly polar hydroxyl, carboxylate substituents) plus other polar substituents (such as hydroxyl, fluoro, carboxy side chains, amide or sulphonamide) on the ring that can bind with polar amino acid side chains on the HMGCR enzyme through hydrogen or polar bonding. The hydrophobicity stems from the hydrocarbon ring structure or non-polar substituents (such as isopropyl, or phenyl groups). The hydrocarbon rings and non-polar substituents can form weak “hydrophobic” van der Waal’s or London type interactions with the non-polar amino acid side chains (leucine, valine, alanine etc) in the binding pocket of the HMGCR enzyme. While these interactions are much weaker than the hydrogen bonding or polar interactions formed between polar groups and the enzyme, there are many more such interactions possible, such that the sum of non-polar binding interactions can match overall the sum of the polar interactions in statin binding to HMGCR.

There are many clinical studies focussing on the differences between so-called hydrophilic and hydrophobic statins. Lipophilic versus hydrophilic statin therapy for heart failure has been assessed [8-9], and some of the pleiotropic (properties

independent of cholesterol lowering outcomes) effects of statins including effects on malignancies [4-7] may be related to use of hydrophobic statins.

The hepatoselectivity of the statins is related to their lipophilicity. The more lipophilic statins tend to achieve higher levels of exposure in non-hepatic tissues, while the hydrophilic statins tend to be more hepatoselective. The difference in selectivity is because lipophilic statins passively and non-selectively diffuse into both hepatocytic and non-heptatocytic tissue. The hydrophilic statins, such as rosuvastatin and pravastatin, rely largely on active transport (using the organic anion transporting polypeptide, OATP) into hepatocytic tissue to exert their effects. High hepatoselectivity is thought to reduce the risk of adverse side effects, such as myositis and myopathy, with the potential for rhabdomyolysis (the pathological breakdown of skeletal muscle) leading to acute renal failure.

Clinicians differentiate between fat soluble or hydrophobic (atorvastatin, simvastatin, fluvastatin, lovastatin, cerivastatin) and water soluble or hydrophilic statin (rosuvastatin, pravastatin) when prescribing statins depending on potential side effects from treating high cholesterol levels [10]. Lipophilic statins undergo hepatic and enteric metabolism via cytochrome P450 (CYP450 family of enzymes) whereas the water soluble statins are excreted largely unchanged. Pravastatin and rosuvastatin have therefore been not shown to participate in any clinically relevant drug-drug interactions with CYP450 agents. Lipophilic statins may have adverse metabolic consequences that include impaired insulin secretion and promotion of insulin resistance, whereas water soluble statins are better tolerated. For muscle related side effects water soluble statins (pravastatin, rosuvastatin) or modified release fluvastatin are preferred, but not for rhabdomyolysis where further statin treatment is contraindicated.

Statins have hydrophilic and hydrophobic regions, and are classed as *amphiphilic* drugs [11-14]. The hydrophobic region interacts with phospholipids (which are also amphiphilic) of membranes leading to myeloid debris imbibed within lysosomes appearing as autophagic vacuoles. Amphiphilic drugs don't require specific transport mechanisms to cross membranes, as they are soluble in aqueous biological fluids and lipid membranes, they simply diffuse through the body. The efficacy of such drugs depends on how fast they can partition into or cross the membrane. The rate of drug clearance through metabolism or specific pathways opposes the rate of accumulation. *Faster exchange and equilibration between aqueous and lipid phases means less drug is required to produce the desired effect*. Hence the ability to quantify the hydrophobic and hydrophilic proportion of a drug can be important in drug design.

Fat soluble statins are known to cross the blood brain barrier, whereas water soluble statins are thought not to cross the barrier. There is clinical evidence that highly lipophilic statins such as simvastatin and atorvastatin cross the blood brain barrier and cause cognitive impairment by affecting central nervous system cholesterol physiology [15]. Conversely, a recent population study suggests that patients who discontinue taking fat soluble statins may be more likely to develop Parkinson's disease than those who continue taking the statin [16(a)]. There is clinical evidence that the *dosage* of statins can cause lower or higher permeability [16(b)]. The ability of amphiphilic drugs like the statins to cross the blood brain barrier is thought to be

related to the cross sectional area of the drug in its membrane bound conformation, and lipophilicity as measured by log D partition coefficients [13].

The distinction between hydrophobic and hydrophilic statins is mainly based on experimental partition coefficients [17-21]. The log P partition coefficient (commonly water – n-octanol) for un-ionized drugs, or where the pH is adjusted to ensure the predominant species is un-ionized) or log D for ionized drugs are taken as measures of the lipophilicity of drugs. Log D is pH dependent. Log D is usually measured at pH = 7.4 (the physiological pH of blood serum). For un-ionized compounds, log P = log D at any pH. The lipophilicity (log D at pH 7.4) of some statins [23] are: cerivastatin 1.5-1.75, simvastatin 1.5-1.75, fluvastatin 1.0-1.25, atorvastatin 1.0-1.25, rosuvastatin -0.25-(-0.5) and paravastatin -0.75-(-1.0). Based on such data, rosuvastatin and pravastatin are commonly clinically referred to as hydrophilic statins, while cerivastatin, simvasatain, fluvastatin, and atorvastatin are called hydrophobic statins.

The partition coefficients of drugs like the statins between water and n-octanol have been very successful in mimicking biophasic behaviour. Proteins with their polar groups and lipids with their esters and phosphate groups can hydrogen and polar bond with drugs like statins, while the hydroxyl groups on octanol can act as hydrogen or polar bond acceptors or donors to mimic the behaviour of proteins interacting with the statins. Octanol also has a long hydrocarbon chain, so is overall hydrophobic, hence its use as a lipid bilayer mimicking solvent. The lipid solubility of drugs are widely acknowledged to have a major effect on bioavailability, bioactivity, and other pharmacological properties, and correlates with the ability to cross the blood brain barrier and other parts of the central nervous system [1-3].

Unfortunately, even though log P or log D in water–n-octanol (or other partitioning solvent combinations) is widely used to define drug lipophilicity, n-octanol contains 2.8M water in partitioning experiments at equilibrium, so most polar solutes would be solvated by this water, indicating that the log P, or log D values may be suspect. There are other significant experimental difficulties in drug partitioning experiments [21], such as solubility (especially poorly and partially soluble drugs), molecular sites (other than the pharamacophore of interest) that may be affected by pH, buffering agents, and the required equilibration times for widely different drug solvent partitions, that can lead to unknown experimental errors. These factors can be avoided by in silico methods, which can be used to supplement or validate experimental values. The published log P and Log D values of the statins may be suspect (or have significant errors) as a result of all these experimental factors.

It is possible to use widely available computational molecular orbital methods that incorporate sophisticated solvent models to accurately and quickly evaluate the hydrophilic and hydrophobic (or lipophilic) properties of potential drugs, and how such drugs might bind with proteins to produce desired pharmacological effects.

Electrostatic forces play a major role in biological processes, particularly in protein – ligand binding [24-28]. Cramer and Truhlar's SMD solvent model [29,30] which included water–octanol partition transfer energies, and hydrogen bonding interaction in the parameterization and optimization of their model, is well suited for biological

solvent modelling. The electrostatic potentials at nuclei [28,30] have been shown to be a powerful tool in examining chemical reactivity.

2. Aims of this study:

- To characterize the electrostatic surface properties of the statins, specifically the electrostatic potential at critical nuclei on the common pharmacophore moiety, as well as other polar and non-polar sites.
- Evaluate the factors determining statin hydrophilicity and lipophilicity critical to the pharmacokinetics of HMG-CoA reductase inhibition and the hepatoselectivity of the statins.
- Examine whether the traditional measures of hydophilicity and lipophilicity such as log P and log D partition coefficients are effective measures with respect to HMG-CoA reductase inhibition.
- Evaluate how the hydrophilicity and lipophilicity of statins influence their ability to cross the blood brain barrier.
- Examine whether atomic electrostatic charges on the pharmacophore can be used to evaluate hydrogen bonding and other polar interactions between the statins and HMGCR and whether the statin – HMGCR binding interaction energies can be calculated.
- Examine whether the ionization energy, electron affinity or absolute hardness of the statins have any predictive value in cytochrome (CYP450 family of enzymes) CYP2 or CYP3 (isoenzymes) primary metabolic reactivity, and any possible drug interactions with statins.

3. Results and Discussion

3.1 Electrostatic surface properties of statins

The CHELPG electrostatic atomic charges for the statins studied in various solvents are shown in Table 1 in volts (supplementary). CHELPG (CHarges from Electrostatic Potentials using a Grid based method) is an atomic charge calculation scheme in which atomic charges are fitted to reproduce the molecular electrostatic potential at a number of points on a grid surrounding the molecule [32]. The method is not well suited to large molecules particularly where the atoms of interest are buried deep within the molecule, and is also dependent of molecular conformation. However this study focuses on the *surface electrostatic properties* of statins. The method has been shown to be accurate for a wide range of neutral and charged species [33-36].

The nuclei of interest are the *polar* C_1-carboxy, C_3 and C_5 hydroxy groups of the common pharmacophore, and other polar groups such as the S=O of rosuvastatin, the C=O and C-O- of the 1-butanoyl-oxy side chain of type 1 statins, the 2-hydroxy group of pravastatin, and a representative group of *non-polar* elements such as the isopropyl methyls of the type 2 statins, the C_2 and C_6 methyls of hexahydro-napthalene moiety of type 1 statins, the C_2 and C_4 methyls of the 1-butanoyl-oxy side chain, and the C_1, C_7 and C_8 olefinic carbons of the hexahydro-napthalene moiety.

It is possible to identify each polar group of the statins, which can either hydrogen bond or polar bond with appropriate sites on solvents or HMGCR. It is then possible to calculate the total ability to engage in such bonding via the calculated electrostatic

charges, and hence calculate the total hydrophilic bonding capacity. It is more difficult to do the same for the hydrophobic interactions between a statin and a solvent or HMGCR, since there are many non-polar sites that can interact with other hydrophobic sites. Traditionally the hydrophobic bonding capacity of drugs has been assessed using proxy methods to mimic the hydrophobic interaction, including the widely used water-octanol partition coefficients.

The electrostatic surface charge distribution of some statins are shown in Figures 2 to 5, which give a visual representation of the some of the data in Table 1, noting that electrostatic charges in Table 1 (supplementary) are shown as positive for convenience, but are actually negative, so a low electrostatic atomic charge represents a nuclei of high electron density. The figures show nuclei of high negative electrostatic charge (or highest electron density) in blue, intermediate in light blue to green, and low negative charge in yellow, orange to red. The hydrophilic or most charged nuclei (deep blue) such as the carboxylate, hydroxyl, fluoro, sulphone, ester or amide groups, and least charged (red) hydrophobic nuclei (such a methyl, isopropyl, phenyl) are clearly evident, but the intermediately charged surface areas are also prominent. The common pharmacophore clearly shows the carboxylate group at C_1 with two blue high electron density oxygen atoms, and the two hydroxyl groups at C_3 and C_5 which appear as blue O atoms with red H atoms at the tips. It is clear that the phenyl side chains of the type 2 statins have some polar characteristics due to the **π** electron cloud. It is known from the Xray structures of the statin-HMGCR complexes [52], that significant hydrophobic bonding occurs between the statins and the leucine, valine and alanine amino acid residues of HMGCR. This hydrophobic bonding is thought to be an aliphatic-aliphatic group interaction, ie between the isopropyl groups of the leucine residue and the isopropyl groups of the type 2 statins. Hydrophobic bonding might also involve interaction between the **π** electron cloud of the atorvastatin phenyl or phenylcarbamoyl side chains and the charged arginine residue of HMGCR (see Figure 4). The relative bond strengths of these types of **π** electron cloud interactions [62] are about 1 kcal/mol which can be compared to van der Waals bonds 0.5-1 kcal/mol for aliphatic-aliphatic type interactions, dipole-dipole interactions such as R_3**N**---**C**(=O) 1 kcal/mol, hydrogen bonds such as N**H**—**O**H ca 1-10 kcal/mol, and purely ionic or electrostatic such as R_4**N**$^+$-- $^-$**O**-C(=O) ca 5 kcal/mol. Normal covalent bonds range from ca 40 to 140 kcal/mol.

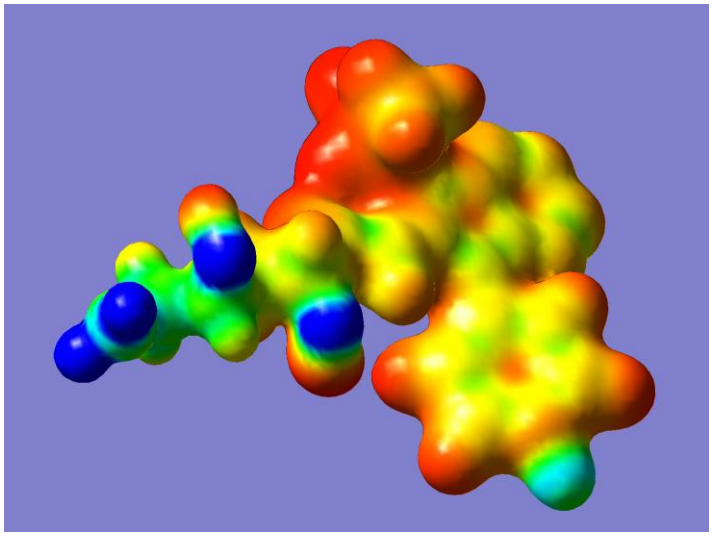


Figure 2. Electrostatic surface potential map of fluvastatin anion in water (with the 4-FC_6H_4 group at 30^o to the indole ring).

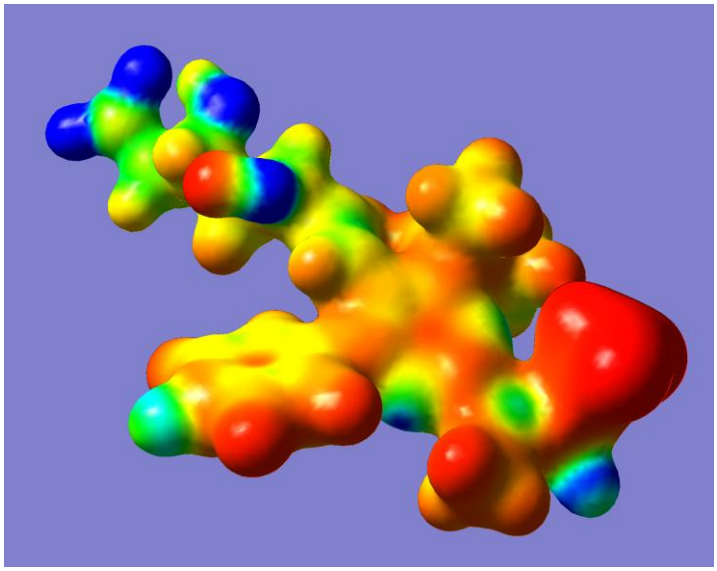


Figure 3. Electrostatic surface potential map of rosuvastatin anion in water (with the 4-FC_6H_4 group at 54^o to the pyrimidine ring).

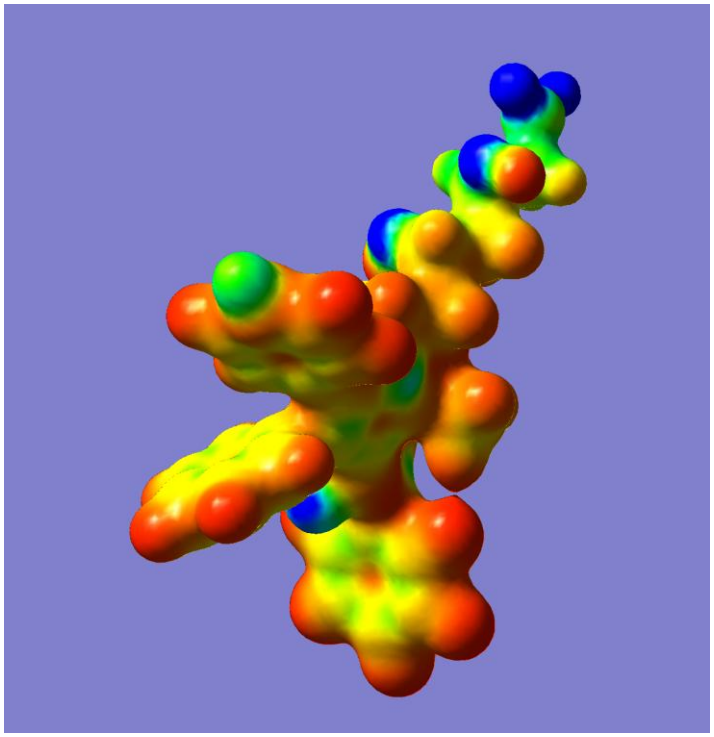


Figure 4. Electrostatic surface potential map of atorvastatin anion in water (with the 4-FC_6H_4 and phenyl groups at 54^o and 90^o to the pyrrole ring respectively).

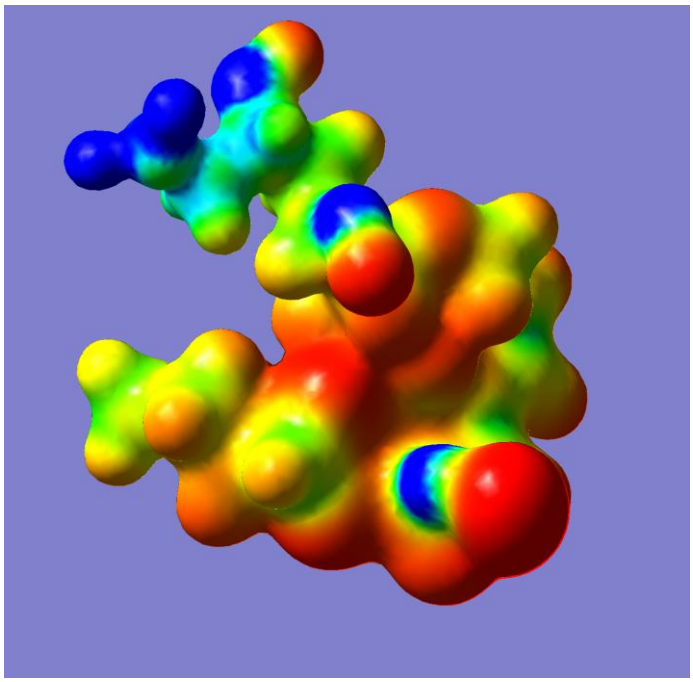


Figure 5. Electrostatic surface potential map of pravastatin anion in water.

The hydrophilic bonding capacity of the statins could be quantified by examining the atomic charges in water and n-octanol. These data would be reflective of water-octanol partition coefficients, which are discussed below in section 2. The octanol has been taken to be representative of a lipophilic environment in partition theory,

because of its long hydrocarbon chain. However, the data in Table 1 (supplementary) clearly shows that octanol does engage in significant hydrogen and polar bonding with statins, very similar to water's hydrophilic bonding capacity. Hence the data suggests that the *difference between octanol* (which has both hydrophilic + hydrophobic bonding capacity) *and octane* (only hydrophobic bonding capability) *may be a more useful measure of differentiating between hydrophilic and hydrophobic capability*. This is discussed below in section 2.

A comparison has been made between the anionic form of the statins (which predominate at the physiological pH = 7.4) and the acid form, which is often the clinically administered form of the drug. The data show a similar solvent trend for the acids compared to the anions, with the exception that the C_6 carboxy groups have lower (negative) electrostatic charges for the C=O and C-OH groups than the C=O and C—O^- groups of the anionic species, as expected.

The effect of conformational change has been investigated for rosuvastatin anion, where the 4F-C_6H_4- group can rotate with respect to the pyrimidine ring. Coplanarity has steric hindrance, and is not a stable conformer. The lowest energy conformation in water solution is 42^o from planar. The energy difference between a conformation which has the 4F-C_6H_4- group at 90^o to the pyrimidine ring and the 42^o conformation is 3.0 kcal/mol. Rosuvastatin in the bound state (complexed with HMGCR from the 1HWL PDB Xray structure) has a conformation where the 4F-C_6H_4- group is 63^o to the pyrimidine ring. The energy difference between the most stable 42^o conformation and the bound state 63^o conformation is 1.1 kcal/mol. Fluvastatin anion is the only statin that can have the 4F-C_6H_4- group in type 2 statins nearly coplanar (13^o from coplanarity with the indole ring suffers only a 1.6 kcal/mol penalty with respect to the lowest energy conformation which is 30^o from planarity in water, or a 3.3 kcal/mol penalty with respect to the 90^o conformation). The most stable (optimised) conformation of atorvastatin anion in water has the 4F-C_6H_4- group and the phenyl group attached to the pyrrole ring at 54^o and 52^o respectively to the pyrrole plane, such that the maximum distances (or steric relief) between these two rings is achieved. However, the energy difference for atorvastatin with a 90^o and the 54^o conformations for the 4F-C_6H_4- group is 3.4 kcal/mol. Atorvastatin acid has angles of 58^o and 68^o respectively, similar to the anion. It is highly likely that all the type 1 statins when complexed with HMGCR would seek to lower interaction energy with the amino acid residues by varying the conformation of the phenyl groups, and it is clear that fluvastatin can have the 4-fluorophenyl ring almost coplanar, but atorvastatin and rosuvastatin would have the phenyl rings far from coplanar, removing any resonance stabilization with the pyrrole and pyrimidine rings of the statins. The electrostatic atomic charges are sensitive to conformational change, as can be seen from Table 1 (supplementary).

A study of the conformations of the common 3,5 dihydroxyhept-6-enoic acid pharmacophore indicates that there can be many conformations possible, but the conformations of interest are those relating to the carboxylate group on C_1, and the hydroxyl group at C_3, particularly as inspection of the data in Table 1 (supplementary) indicates that electrostatic charges on this moiety are very sensitive to solvents. A comparison of two stable configurations of simvastatin where the C_3-OH bond angle is varied only with respect to the C(=O)-O^- plane shows that: (1) the more stable conformation is (where this angle is 60^o, with a Z configuration, and the distances

between the C-**O**H and C=**O** and C-**O**- are 2.6 A^o and 4.3A^o) 267 kcal/mol in water more stable compared with (2) the conformation which has an angle of 48^o, Z configuration, and bond distances of 2.8A^o and 3.5A^o respectively. The major difference between the two conformations (1) and (2) is the dramatic effect on the electrostatic charge on the C_3-**O**H group (see Table 1 simvastatin anion entry, a difference of 10 volts in water), where electron density on the O atom of the hydroxyl group indicates a strong electrostatic field effect is operating between the carboxylate group and the C_3-OH group. The differences in voltages between the charges on the C_3-OH and C(=O)-O^- of the two conformers is about 288 kcal/mol, indicating that energy difference can be predominantly attributed to the stabilization of the C_3-OH by the carboxylate group in the more stable conformer. This strong interaction would be expected to have a major impact on how statins can bind to appropriate receptors such as HMG-CoA reductase. However, the free energies of solvation in water, octanol and octane are very similar, and show parallel trends, with the more stable conformation (1) having solvation energies (84.5, 76.0, and 33.3 kcal/mol respectively) which are ca.3 kcal/mol lower than those for the less stable conformation in the three solvents. The solvation energies of the statins are discussed below in detail.

An interesting conformational effect is observed for rosuvastatin (Table 1, supplementary) where the one of the methyl groups of the 6-isopropyl group attached to the pyrimidine ring has an unusually large (negative) electrostatic atomic charge or high electron density at the C atom of the methyl group of the order of 4-6 volts compared to the other methyl group in the anion (the effect is smaller in the acids) which arises from a through space electrostatic interaction by the N at position 2 of the pyrimidine ring interacting with the H atoms of the methyl group. Clearly the dynamics of bond rotation within the isopropyl group would average out this effect, but the large energies involved in this effect would have significant consequences during binding of rosuvastatin to HMGCR, as the hydrophobic bonding effect ascribed to the isopropyl group interacting with hydrophobic amino acid residue (Leu^{562}) of HMGCR in the Xray structure [48] would be affected, possibly by a hyperconjugative effect. A similar effect is also seen for atorvastatin anion, where one methyl of the isopropyl group can electrostatically interact with the NH group of the C(O) –N(H)-C_6H_5 side chain through space.

Statin inhibition of HMGCR is highly stereoselective, and requires the 3R,5R dihydroxy configuration in the pharmacophore. The effect of an inactive 3S,5R configuration in the pharmacophore of rosuvastatin has been cursorily investigated. It was found that this configuration in water was less stable than the 3R,5R configuration by 57.2 kcal/mol overall. The active 3R,5R configuration has solvation free energies of 74.2, 68.6, 37.2 kcal/mol in water, n-octanol and n-octane compared with the 3S,5R configuration which has values of 103.4, 90.9 and 42.5 kcal/mol respectively. The large difference (103.4 – 74.2 kcal/mol) between the bulk solvation energies in water suggests that the known stereoselectivity of the statins required for binding to the HMGCR has a significant solvation and desolvation effect, see section 3 below on binding of statins to HMGCR).

3.2 Hydrophilic and hydrophobic (lipophilic) properties of statins

The SMD solvation model [29,30] has been applied to study the free energy of solvation, ΔG_S^o, of the statins. The model is based on $\Delta G_S^o = \Delta G_{ENP} + G_{CDS}$ where

ENP is the electronic nuclear polarization: the change in the solute free energy due to electrostatic interactions between the solute and the bulk solvent and distortion of the solute's electronic structure in solution. The solvent is modelled as a dielectric continuum. CDS is the cavitation dispersion structure, involving non-bulk solvent electrostatic contributions to the free energy of hydration. The CDS represents first solvation shell effects. It involves atomic surface tension (geometry dependent proportionality constants). The G_{CDS} term has been parameterized using extensive experimental data sets for optimization, and has the advantage of including a realistic experimentally based hydrogen bonding model. The CDS involves cavitation, dispersion, and as a collective "solvent structure contribution" estimates for partial hydrogen bonding, repulsion, and deviation of the dielectric constant from its bulk value.

Bulk solvent electrostatic interactions are long-range electrostatic polarization effects. The CDS covers *shorter-range* polarization effects and shorter-range non-electrostatic effects such as cavitation, dispersion, and solvent structural effects (which includes both hydrogen bonding) and exchange repulsion effects. The hydrogen bonding model uses Abraham's solvent model where α is the hydrogen bond acidity and β is the hydrogen bond basicity. The CDS contribution is a sum of terms (with atomic surface tensions) that are proportional to the solvent-accessible surface areas of the individual atoms of the solutes.

Implicit solvation models such as the SMD model have been extensively used as a basis for determining solute – solvent free energies in protein and enzyme folding processes. The solvation free energy is the energy required to transfer a solute molecule from a "vacuum" (or gas phase) into a solvent. Transfer free energies (the difference between the free energy of a solute in water and another solvent, eg n-octanol) have been used to quantify the hydophobicity of drugs and similar molecules [24-28]. Partition co-efficients of a non-polar solute (log S) or an ionized solute (log D) between water and n-octanol are experimentally determined, and the free energy, enthalpy and entropy can be determined by calorimetry.

The SMD model optimization and parameterization included extensive experimental transfer free energy data, which makes it a good model for examining transfer free energies of statins in various solvents. The model also treats ionized and neutral species well. Using the B3LYP/6.31G* level of theory, the SMD model achieves a mean unsigned error of 0.6-1.0 kcal/mol for neutrals and 4 kcal/mol for ions.

A solute's hydrophobicity is based on the free energy change required to bury a non-polar side-chain in the interior of a protein away from the water environment, and can be found by experiments in which a model compound is partitioned between water and a non-aqueous solvent. Transfer free energies are widely held to be proportional to the surface area of a non-polar solute. The transfer of an ion from water to a nonpolar media with dielectric constant of ~3 (lipid bilayer) or 4 to 10 (interior of proteins) costs significant energy [24-25].

A method to evaluate this transfer energy comes from Lee's [37,38] examination of Widom's [39] solute insertion model. In this model, initial rearrangement of solvent molecules occurs to form a cavity for the solute, with a free energy change, ΔG_c. Secondly, the solute enters the cavity, van der Waals interactions occur between

solute and solvent, and rearrangement of the solvent occurs at the cavity surface. The solute–solvent interaction energy is denoted E_a. The experimental transfer free energy $\Delta G_s°$ equals the sum of ΔG_c and E_a. The energetics of making a solute cavity in water compared to liquid alkanes have shown that significantly higher energy is expended to make a cavity in water compared to a liquid alkane, because of the small size of water, and the hydrogen bonding in water. ΔG_c for water is dominated by the entropy at room temperature, which is not so for liquid alkanes [40-46].

Table 2 (supplementary) shows the SMD solvent free energies for the bulk solvent, (using an electrostatic continuum model), and the non-bulk non-electrostatic CDS for the statins (anionic and neutral acid forms) in this study. The solvents used are water, n-octanol, and n-octane. Water and n-octanol are the basis of the widely used and documented log P partition coefficients, used to indicate the hydrophobicity or lipophilicity of numerous drugs and biological compounds. *In this study however, n-octane was chosen to compare with n-octanol, which is of a similar size and alkane length, and so should create a similar physical cavity to n-octanol, and more importantly, has no hydrogen bonding capability.*

It can be seen that the bulk solvent energies for water and n-octanol are very similar in magnitude (with the water solvation energies being generally some 10% higher for the anionic forms) and almost equal for the acid forms. This implies that n-octanol is equally as potent as water in forming hydrogen or polar bonds as water for these statins. Examination of the electrostatic potential at the polar sites on the DHGA pharmacophore (Table 1, supplementary) shows similar electrostatic atomic charges at the C_6-O, C_6=O, C_3-OH and C_5-OH sites for the statins in water and n-octanol, reflecting the similar bonding interactions with the two solvents. The dielectric constants for water (80) and n-octanol (9.9) influence the charges to some extent, but hydrogen bonding dominates (see Table 3(a) below).

There are large differences between the solvation energies for the acid statins compared to the much lower energies of the anionic statins, for all solvents, including n-octane. This observation reflects the significant polarization of the whole statin molecules, as reflected in the dipole moments shown in Table 1 (supplementary). So the negative charge on the carboxylic group clearly is delocalized over the DHGA pharmacophore moiety, by through space electrostatic field interaction, in all solvents. This can be seen visually in Figures 2-4.

The CDS data in Table 2 (supplementary) are remarkably constant for the anionic and acid forms, for all statins. The CDS solvation energies (kcal/mol) being about 10 +/- (2 to 4) for water, between 0 – 3 for n-octanol, and -10 +/- 2 for n-octane. As the CDS term includes hydrogen bonding, dispersion (van der Waals, London, etc), repulsion, and cavitation components, there is no straight forward way of evaluating various component energies using the SMD solvent model.

Using an alternate solvent model, PCM, with the same basis set, the non-electrostatic processes such as cavitation, dispersion, cavitation field effects, and repulsion energies between the statins and the solvents can be calculated. These calculations are shown in Table 5 for fluvastatin and rosuvastatin. These values can be directly compared with the data in Table 2 (supplementary) which used the PCM-SMD solvent model, as the basic PCM solvent model is common to both sets of data, and in

all cases, the values are differences between the gas state and solvent state. However the PCM/SMD model includes hydrogen bonding forces (and other solvent structural effects) besides cavitation, dispersion, cavitation field, and repulsive effects, which are calculated in Table 5 (summed in column 6). The CDS values in Table 2 (column 3) include all these factors *plus* hydrogen bonding and other polar structural solvent features.

It can be deduced from Tables 2 and 5 that the CDS term includes stabilizing hydrogen bonding and other polar interactions in the first solvent shell of the order of -32 to -38 kcal/mol for fluvastatin, lovastatin or rosuvastatin anions (as shown in column 7 of Table 5), or about -47 kcal/mol for atorvastatin, in both water or n-octanol. That is, the CDS first shell non-electrostatic values shown in column 3 of Table 2 (supplementary) are only consistent with the summed energies shown in column 6 of Table 2 (supplementary) if there are stabilizing hydrogen bonding and other polar interactions in this first solvent shell of the magnitude of -32 to -38 kcal/mol for fluvastatin, lovastatin or rosuvastatin (or -47 for atorvastatin). *The data also clearly shows that water and octanol can form hydrogen or polar bonds equally well for these statins.* In heptane/octane solvent, where no hydrogen bonding or polar interaction between the statins and the solvent is possible, the CDS hydrogen bonding + polar interactions shown in column 7 of Table 5 range from -0.8 to – 2.7 kcal/mol, which are close to zero (as they should be) within experimental error.

Table 3(a) shows an alternate method for calculating the hydrophilicity of statins by evaluating the hydrogen bond donor and acceptor ability of all polar groups on the various statins. Solvent hydrogen and polar donor acceptor bonding ability is the sum of the electrostatic atomic charges in volts taken from Table 1 (supplementary) on all polar groups, C-**O**$^-$, C=**O**, C-**O**-H, S=**O**, **O**-C(=**O**)-, C-**F**, **O**-Me which can interact with a polar solvent or with polar groups on HMGCR, The data shows that pravastatin has the most hydrogen bond donor or acceptor ability, followed by rosuvastatin, then lovastatin, then atorvastatin and cerivastatin. The data are virtually identical in water and n-octanol for the anionic statins, with the data for the acid form in both water and n-octanol are overall lower but shows precisely the same trend pattern indicating that n-octanol has parallel hydrogen and polar bonding capability as water. This data shows no correlation with log P (shown as relative lipophilicity), or calculated hydrophilic solvation energies, as expected since these indicators measure total molecular parameters.

As discussed above, the hydrophilicity has been calculated either from (1) the differences in solvation energies between n-octanol and n-octane, which assumes that the solvation energies in octane are a measure of the hydrophobicity of the statins, or (2) by directly examining the hydrogen bond donor and acceptor ability of all polar groups on the various statin. The traditional measure of lipophilicity of drugs has been to use log P or log D partition coefficients as transfer free energy measures. Various solvents have been used as proxies for evaluating hydrophobicity or lipophilicity, from octanol to non-polar solvents such as cyclohexane, heptane, n-hexadecane etc which are considered better choices than octanol [20].

The solvent surface accessible area (SSAA) has been used as a proxy for the hydrophobic effect. The hydrophobic effect is based on the free energy changes resulting from the burial of non-polar surface area of the solute or drug away from the

solvent inside the non-polar environment within a protein structure after complexation [24-28]. The hydrophobic effect is mostly entropic in nature at physiological temperatures and is driven by the properties of the solvent. The literature has a range of values regarding the magnitude of this effect [24-28,48-51], ranging from between 5 and 45 cal/($Å^2$ mol).

Table 3(b) shows the *surface accessible area* (SAA) taken from the Xray structure determinations of the bound statin-HMGCR complexes [48], hydrophobic solvation energies for the statins (taken from Table 2, supplementary), and literature log P and Log D for the statins [23,53].

The literature SAA data for the statins is remarkably similar, particularly the buried surface areas after statin binding to HMGCR, with only atorvastatin (1080 $Å^2$) being significantly greater than the normal range 870-880 $Å^2$ for all other statins. However the SAA data from the POPS data base shows that atorvastatin has almost the same hydrophilic percentage as the other statins (see paragraph below) suggesting that 1080 $Å^2$ value for atorvastatin may be incorrect. The calculated lipophilicity from the SAA data using a value of 0.045 kcal/mol/$Å^2$ correlates well with the values from octane solvation energies. However, it is noted that the choice of a SAA value of 0.045 kcal/mol/$Å^2$ (which is the top of the literature range) is arbitrary, but supports the notion that solvation energies in octane are a good proxy for hydrophobic bonding between the statins and HMGCR. The statins however are large molecules with significant hydrophobic elements, so a large hydrophobic interaction is expected.

The marked similarity of the SAA data for the statins is also reflected in the SAA analysis of the HMGCR-statin complexes taken from the PDB (structural data bank) using the POPS method [46]. The calculations show that the hydrophilic and hydrophobic SAA for the following HMGCR-complexed statins (rosuvastatin, fluvastatin, atorvastatin, cerivastatin, simvastatin, compactin) are very similar, with the hydrophobic surface area almost constant at 55.5 – 56.0%, and the hydrophilic area similarly constant at 44.0 - 44.5%.

The calculated data for lipophilicity for the statin anionic and acid forms in Table 3(b) do not correlate well with the log P or log D water-octanol partition coefficient data for statins. The acid forms of the statins are clearly more lipophilic than the anionic forms, as expected given the negative charge on the anionic form. Given that the solvent models and the electrostatic MO computational model used have been well authenticated for a range of neutral and charged molecules, and the known difficulties with the experimental determinations of log P and D, it suggests that the simple clinical definition of some statins as hydrophilic and others as hydrophobic may be misleading.

3.3 Calculating the hydrophilic interaction of the statin pharmacophore and estimating the binding interaction of rosuvastatin to HMGCR

The crystal structures of the statin-HMGCR complexes have been determined, and the structural interaction of the DHGA pharmacophore with the HMGCR enzyme is very similar for all statins [48]. *The differentiating factor is the size and shape of the hydrophobic ring structure in the various statins, and how these ring structures interact with the HMGCR enzyme*. The statins structurally block the active sites of the

HMGCR enzyme, by reversibly binding to the HMGCR enzyme in the nanomolar range, while the natural substrate HMG CoA's affinity is in the micromolar range. The conformational flexibility of the HMGCR enzyme can produce a shallow hydrophobic groove that the statins can enter. The specificity and the tight binding of statins is due to orientation and bonding interactions that form between the statin and the HMGCR enzyme. Hydrogen bonding and polar interactions (or charge – charge interactions) are formed between the DHGA pharmacophore and residues that are located in the cis loop of the enzyme. These hydrogen bonding and polar interactions are between Ser^{684}, Asp^{690}, Arg^{590}, Lys^{691} and Lys^{692}, Glu^{559}, Asn^{755}. The terminal carboxylate of the DHGA moiety forms a salt bridge with the cationic Lys^{735} of the enzyme. In rosuvastatin, Arg^{568}, and Ser^{565} form polar bonds to the two S=O bonds of the N-methyl-methylsulphonamide group. The guanidinium group of Arg^{590} forms a polar interaction between the arginine **ε** N atom and the p-F atom of the 4-fluorophenyl group, as well as interacting with the C_3 hydroxy group through the two ends of the δ-guanido group. The bond distances between the enzyme amino acid side chains and the polar groups of the pharmacophore are almost identical in all structures.

Van der Waals interactions are indicated between the hydrophobic side chains of the enzyme, which involve the Leu^{562}, Val^{683}, Leu^{853}, Ala^{856} and Leu^{857} and the statins. As the polar interactions DHGA pharmacophore with the HMGCR enzyme are very similar in both type 1 (Compactin, Simvastatin) and type 2 (Rosuvastatin, Fluvastatin, Atorvastatin, Cerivastatin) statins, it is very likely that the *differences* in statin clinical efficacy (eg IC_{50}, serum LDL – C reduction, etc) which result from *binding* to the HMGCR are due to these ***hydrophobic interactions*** (there are other drug effects such as solubility, bioavailabilty, binding residence times, rates of further metabolic removal pathways, half lives etc).

Type 2 statins form polar interaction between the fluorine atom on the 4-fluorophenyl group and the ε N atom of the guanidinium group of Arg^{590}. In addition to these interactions atorvastatin and rosuvastatin also form hydrogen bonds between Ser^{565} residue and either a carbonyl oxygen atom (Atorvastatin) or a sulfone oxygen atom (Rosuvastatin). Rosuvastatin also has a polar interaction between the Arg^{568} side chain and the electronegative sulfone group, making it the statin that has the greatest number of bonding interactions with HMGCR. The Xray structural determinations have identified the specific hydrophilic (hydrogen bonding and polar) interactions between the statins and the HMGCR enzyme, however the hydrogen bonding interactions are not exactly identified since the locations of the H atom are not known (a common problem for most lower to medium resolution Xray structural determinations [26]).

A significant portion of the binding interaction between an enzyme and a drug comes from the hydrophobic interaction (van der Waals, London forces) between the non-polar parts of the enzyme and drug, and entropically driven release of water that surrounds the free drug and the unoccupied enzyme binding site. At the same time, binding *selectivity* is driven by the *directional* hydrogen bonding and other polar interactions between the hydrogen bonding and polar sites on the enzyme and drug surfaces. The hydrophobic effect is widely accepted to be the major driving force in globular protein folding, and results in the burying of hydrophobic residues in the core of the protein.

Table 4 lists the hydrogen and polar bonding interactions between rosuvastatin and the HMGCR amino acid residues calculated directly from the electrostatic atomic charge potentials using the basic Coulombic formula with an assumed an effective dielectric **ε**=4 within the HMG CoA reductase enzyme interior binding pocket. The effective dielectric within a protein is a subject of debate, with values quoted as 4-6 commonly, occasionally 4-10, but **ε**=4 is the most widely used value, and which is believed to account for electronic polarization and small backbone fluctuations. Other authors believe that the value of **ε** depends on the computational method used. [24-28,48-51].

The geometry of hydrogen bonds in proteins has been analysed. Baker & Hubbard (1984), Hubbard (2001) [54,55] and Morozov (2004) [56] found that 90% of analysed NH -- O hydrogen bonds in the PDB structure data bank have bond angles about 158^{o}, and most analysed C=O – H hydrogen bonds have angles about 145^{o}. There was a normally broad spread of hydrogen bond donor to acceptor (O, N) distances of 1.7 to 2.4 A^{o} (usually greater than 1.6 but less than $2.5A^{o}$). The angle at the proton is between 130^{o} to 170^{o} and the angle at the acceptor was about 150^{o}. The Xray data indicates that a slightly bent linear hydrogen bond is normal in proteins. To model the rosuvastatin binding to HMGCR, the amino acid residues were set at the reported Xray determined distances to the statin (eg for a lysine to statin hydroxyl group interaction –**N**$^{+}$-H --- **O(H)**- the distance between the **N**--**O** atoms was set at the literature Xray structure distance). Since the Xray structures did not determine the position of the H atoms, a linear geometrical model was assumed for hydrogen bonding using (for example) the set N-O distance: the N**H**---**O** interaction is *linear*, so the actual NH--O bond is included within the Xray N---O distance, and the NH--O bonding distance is significantly shorter than the literature N--O distance. The actual hydrogen and polar bond distances used in the calculations are shown in parentheses in Table 4. For rosuvastatin – HMGCR hydrogen and polar bonding in the gas state, the total hydrophilic interactions is -32.1 kcal/mol (and -34.9 kcal/mol in water for comparison).

The estimated average hydrophilic interaction between HMG CoA Reductase amino acid residues and rosuvastatin calculated by summing the individual coulombic interactions in water (shown in Table 4) is -34.9 kcal/mol for the hydrophilic interactions, which compares to the value of -37.6 kcal/mol (*for the CDS first solvent shell only*, which excludes hydrophilic contributions from the bulk solvent) for rosuvastatin in water in Table 5, or a calculated value of -30.5 kcal/mol in column 4, Table 2 (supplementary). These data appear to confirm that drug (statin)-solvent transfer free energies are a reasonable proxy for estimating binding between enzymes and drugs such as statins in the interior of an enzyme or protein. It is noted that these data are a result of using 3 different methods to calculate the hydrophilic and hydrophobic components of rosuvastatin, using different solvent models and algorithms to separate out cavitation effects from bulk solvent effects.

Snyder, Whitesides et al [57] have recently investigated how the hydrophobic effect usually dominates the free energy of ligand –protein binding, noting that increasing the non-polar surface area of a ligand usually increases the binding interaction. The hydrophobic effect is thought to have its origin in the differences in characteristics of bulk water and water close to hydrophobic surfaces. Structured water molecules,

particularly those close to the surface of the protein—including both the molecules of water displaced by the ligands and those reorganized upon ligand binding—determine the thermodynamics of binding of these ligands at the active site of the protein.

The SMD solvent model does distinguish between the bulk solvent and the CDS first solvation shell effects, and so may give insights into reorganisation of solvent molecules around the statin ligand in various solvents. Transfer free energies (as partition coefficients) between water and octanol of various solutes or drugs have been taken as proxies for how proteins interact with the hydrophobic part of bound drugs. CDS - statin solvent data, could be *proxy indicators* of how HMGCR interacts with the statin in the binding pocket, where solvent rearrangement adjacent to the active site of the HMGCR is required before binding of the statin can occur.

Shoichet et al [58] have established the importance of solvation in ligand – protein interactions, and the strong impact on binding energies. The binding affinity of a ligand for a receptor depends on the interaction free energy of the two molecules relative to their free energies in solution: $\Delta G_{binding} = \Delta G_{interaction} - \Delta G_{solvation,L} - \Delta G_{solvation,R}$ where $\Delta G_{interaction}$ is the interaction free energy of the complex, $\Delta G_{solvation,L}$ is the free energy of desolvating the ligand, and $\Delta G_{solvation,R}$ is the free energy of occluding the receptor site from solvent. The estimated $\Delta G_{interaction}$ for rosuvastatin can be estimated from Table 4, which gives a value of -71.0 kcal/mol for the anionic form in the gas phase (-32.1 kcal/mol hydrophilic interaction, plus a hydrophobic bonding component, -38.9 kcal/mol). The $\Delta G_{solvation,R}$ can be estimated by assuming the CDS first shell solvation transfer free energy of rosuvastatin from n-octanol to water would be equivalent to the energy *expended* to rearrange water molecules around the surface of HMGCR to allow a cavity for rosuvastatin to bind to HMGCR in the binding pocket. These values are given in Table 2 column 2 for octanol 2.4 and water 10.5 kcal/mol, a transfer free energy of 8.1 kcal/mol. The polarity of the interior of a protein has been assumed to be that of n-octanol in studies of ligand binding in proteins, particularly when considering how water molecules in a binding pocket can rearrange to allow a ligand to bind to the protein's active site [24-26]. The $\Delta G_{solvation,L}$ is the reverse of the bulk solvation free energy (the change in energy from the gas state to the solvated state), -72.7 kcal/mol for the most stable 42^o conformation (compared with -74.2 kcal/mol for the 90^o conformation) from Table 2 (supplementary), then a rough preliminary estimate of the binding free energy between HMGCR and rosuvastin is -9.8 kcal/mol. No account has been made of any free energy contribution to the total binding energy by conformational processes involving HMGCR folding, however statins inhibitors block the active HMGCR site and so prevent any entropically driven folding processes related to mevalonate formation from occurring [59(a)]. Rosuvastatin anion in the bound state (complexed with HMGCR) has the 4-fluorophenyl group at a dihedral angle of 63^o to the pyrimidine ring, compared to the optimised conformation of rosuvastatin in water which has the 4-fluorophenyl group set at dihedral angle of 42^o. The difference in conformational *energy* is 1.1 kcal/mol, and the calculated configurational *entropy* [59(a), 59(b)] T**Δ**S *change* in water between the free optimised state and the bound state of rosuvastatin anion is 3.1 kcal/mol. Correcting for the *configurational entropy* (the loss of internal degrees of freedom by rosuvastatin upon binding to HMGCR) the estimated binding energy is then **-12.9 kcal/mol**. Carbonell and Freire [47(a)] and Sarver et al [47(b)] quote experimental values of -12.3 and -10.8 kcal/mol for the binding of rosuvastatin with HMG CoA reductase. The agreement between the

calculated value and the experimental value is reasonable, given the many assumptions in the calculation. While the estimated binding energy assumes an effective dielectric ε=4 within the binding pocket, it is noted that more *comprehensive* computations of binding energies in enzyme pockets assume similar values for the dielectric. Another uncertainty is the geometry of hydrogen bonds between the amino acid side chains and the statin polar groups is not known as the published Xray structures do not locate the H atoms. The purpose of this analysis is to show that using easily obtainable electrostatic potential computations, it is possible to get a quick *screening estimate* of the binding free energy contribution by a drug to the total binding energy between a drug in water solution and when bound within a protein cavity, using accepted drug design proxy methodologies. This analysis does suggest that solvation and desolvation processes dominate the binding energy process (see also section 1 above regarding the analysis of the 3S,5R versus 3R,5R dihydroxy pharmacophore configurations of rosuvastatin).

3.4 Analysing the amphiphilic (hydrophilic and lipophilic) nature of statins and implications for their ability to cross the blood brain barrier

The lipid bilayer is the diffusion barrier of biological membranes. The passive permeation of drugs across the blood brain barrier (BBB) has been probed with simple isotropic solvent/water partition models (e.g., octanol, hexadecane, octanol-hexadecane, 1,9-decadiene) as well as more sophisticated methods such as porcine brain lipid extract or in vivo rodent brain perfusion techniques. According to the solubility-diffusion theory, the passive permeability can be estimated as the product of the partition coefficient of the rate-limiting BBB boundary domain and water, and the BBB-phase diffusivity of the solute, divided by the thickness of the barrier domain. Diffusivity in the rate-limiting membrane phase is thought to be proportional to the minimum cross-sectional area of the solute [11].

Membrane permeation is thought to be dominated by the cross sectional membrane bound conformation of the drug, with the limiting cross sectional area for brain penetration is postulated to be 73 A^{o2}. The calculated octanol-water partition coefficient at pH 7.4, log D, which should be in the range of $-1.4 < \log D \text{ calc} < 7$ [13].

In vivo BBB permeability coefficients measurements of fluvastatin and lovastatin (acid and lactone forms) have been determined by Guillot et al [14a] as 2.5×10^{-4}, 1.4×10^{-4} and 2.3×10^{-2} cm/min respectively. Although lovastatin lactone undergoes in vivo hydrolytic conversion to the pharmacologically acid form, it exists long enough in blood to cross the BBB, whereas the fluvastatin and lovastatin administered acid forms would be in the anionic forms in the physiological pH 7.4 environment. Atorvastatin has also been shown to permeate the blood brain barrier and cause temporal lobe epilepsy in rats [14b].

The process of passively permeating a drug into a membrane can be portrayed [11,12] as: (a) desolvating the drug from the aqueous environment; (b) creating a cavity within the membrane for the drug, with the amount of energy to form the cavity being related to the energy needed to insert the drug into the membrane, and the drug is stabilized by electrostatic interaction between the drug and the polar head groups of

the lipids as well as hydrophobic interactions with lipid bilayer; and (c) resolvating the drug behind the lipid bilayer.

While n-octanol has known deficiencies for estimating the hydrophobic effect in proteins, mainly because of its relatively high water content at equilibrium in partitioning experiments, it has been widely accepted as a lipid bilayer mimicking solvent. There is a small amount of water in the bilayer core because of trans-bilayer transport processes, so an amount of water in the octanol layer at equilibrium is considered realistic [20].

Table 2 (supplementary) shows that the *bulk* solvation energies (calculated by the PCM/SMD solvent model) for lovastatin lactone (water 18.1, n-octanol 21.4 kcal/mol) and lovastatin acid (25.5, 26.9) are very similar in both solvents, whereas lovastatin anion (which would be the *predominant species at the physiological pH of 7.4*) has much higher values of water 87.9 and n-octanol 79.3 kcal/mol respectively. Fluvastatin anion has values of 76.4 and 70.5 kcal/mol by comparison respectively. These data suggest that desolvation and resolvation of lovastatin lactone compared to lovastatin anion (and fluvastatin anion similarly) would greatly facilitate lipid bi-layer permeation by almost 60-70 kcal/mol just for the desolvation, and roughly double that energy for desolvation-resolvation process. Thus the solvation-resolvation data is consistent with the experimental finding that the BBB permeation coefficients of fluvastatin anion and lovastatin anion are much lower than that for lovastatin lactone.

Table 5 shows calculated solvation energies (PCM solvent model) for creating a solvent cavity for the statins, and the dispersion, cavity field effects, and repulsion effects for lovastatin lactone and anion, as well as the values for the fluvastatin anion. It can be seen that the energies for *n-octanol* (and water) are very similar (see the sum of these values in column 6 which are 32.3, 31.3 and 29.7 kcal/mol respectively). These values can be compared with the CDS solvent values in Table 2, column 3, for lovastatin lactone, anion and fluvastatin anion in *n-octanol* of 1.0, 0.6 and 1.7 kcal/mol respectively. It should be noted that the PCM/SMD model does not allow separate calculation of cavity energies, as they are all included in the CDS energies along with solvent structural effects. If the *n-octanol solvent shells around these statins are reasonable proxies for creating cavities in the lipid membrane*, it indicates that the energies involved in creating a cavity for lovastatin lactone, anion and fluvastatin (along with the dispersion, repulsion, hydrogen and polar bonding energies between the statin and solvent within the cavity) in a lipid bilayer is about -32 kcal/mol. These energies are far less than the desolvation and resolvation energies required before and after permeation into the lipid bi-layer.

The magnitude of the hydrogen and polar bonding of the statins within the bi-layer are estimated in Table 5, column 7, using the values for n-octanol as a proxy for the lipid bi-layer: lovastatin lactone -32.9, lovastatin anion -32.3 kcal/mol.

It is also noted in Table 2 (supplementary) that the calculated molecular volumes (from ca. 300-330 cm^3/mol) of these statins in n-octanol are similar, so the physical sizes of the statins inside the lipid membrane would be similar if n-octanol is taken as a proxy for a lipid membrane. The polar surface area (calculated or experimentally determined) of a drug has been used as a measure of hydrogen bonding ability [15]. However, calculating the electrostatic atomic charge potential gives a direct measure

of a drug's hydrogen bonding sites. The electrostatic atomic surface charges for the lovastatin lactone, acid, anion in *n-octanol* and the charges for fluvastatin are given in Table 1 (supplementary): these values for the common pharmacophore are similar indicating that hydrogen bonding and polar interactions (as well as the hydrophobic bonding) between the statins and n-octanol are very similar (with the exception on the lactone element which is structurally different from the acid or carboxylate groups). The PCM/SMD solvent model has been parameterized by including hydrogen bonding interactions and water-octanol transfer free energies, so solvation energies calculated using this model are well suited to this analysis.

It can be predicted from the data in Table 2 (supplementary) that the acid (and lactone) forms of the statins would be permeate the lipid bi-layer easier than the anionic form, since in all cases, the bulk solvation energies in n-octanol are about 40-55 kcal/mol lower than the corresponding anionic form, while the CDS values in n-octanol are fairly constant at about 0-3 kcal/mol. Most statins are given in the orally active acid form, except lovastatin and simvastatin, which are administered as inactive lactone prodrugs. Both lactone and acid forms were observed in the human systemic circulation following oral administration of atorvastatin, lovastatin, simvastatin, and cerivastatin, indicating that some interconversion occurs between the lactone and acid forms of these statins [1,2]. However, the pH 7.4 environment of blood serum means that statin acids exist predominantly as the anionic species.

In summary, these above data are suggestive that experimentally observed differences in BBB permeability amongst lovastatin lactone, anion and fluvastatin are dominated by desolvation and resolvation of the statins, not statin molecular size or statin-lipid interaction processes within the lipid bi-layer.

3.5 Metabolism and possible drug interactions of statins

The cytochrome P450 family of mono-oxygenases (CYP) is a large group of enzymes that catalyze the oxidation of organic substances [1,60]. With the exception of pravastatin, which is transformed enzymatically in the liver cytosol, all statins undergo extensive microsomal metabolism by the cytochrome P450 (CYP) isoenzyme systems. About half of all drugs currently available in clinical practice are biotransformed in the liver primarily by the CYP450 3A4system. The CYP3A4 isoenzyme is responsible for the metabolism of lovastatin, simvastatin, and atorvastatin. Fluvastatin is metabolized primarily by the CYP2C9 enzyme,with CYP3A4 and CYP2C8 contributing to a lesser extent. Rosuvastatin is not extensively metabolized, but has some interaction with the CYP2C9 enzyme. The lactone form of the statins undergo rapid CYP metabolism [1]. Measures of the inherent chemical reactivity of the various statins are fundamental to the rate of metabolic reaction or statin-drug interactions in the body.

Induction or inhibition of CYP450 isoenzymes is an important cause of drug interactions. *Competitive inhibition between drugs at the enzymatic level is common and may serve to alter the disposition of statins, leading to increased plasma levels and greater risk of adverse*

events. The many possible interactions between the statins and other drugs has been documented [60], particularly those that may cause myopathy and rhabdomyolysis.

The ionization energy, IE, is the amount of energy required to remove one electron from an atom or molecule. Thus, it measures how strongly the outermost electron is attached to the atom. An atom may lose several electrons, and have multiple IE's, but the first electron is lost from the outer most orbitals and is the most easy to remove. The oxidation of a substrate involves the loss of electrons, so a lower IE generally means oxidation is more facile than a substrate with a higher IE. The electron affinity, EA, is the amount of energy needed to add an electron (ie chemical reduction) to an atom or molecule.

The *absolute hardness* $\eta = ½ (IE – EA)$ has been used as a measure of chemical inertness, the resistance to change in the electron distribution of a molecule [61]. Table 2 shows from the η values in water that atorvastatin (anion or acid 2.2, 2.25) is the most stable statin, followed by pravastatin (anion or acid 1.95, 1.95), lovastatin (anion or acid 1.90, 1.85), cerivastatin (anion or acid 1.85, 1.90), simvastatin (anion or acid 1.85, 1.85), rosuvastatin (acid, anion 1.80, 1.65), pitavastatin (anion or acid 1.60, 1.65), lovastatin lactone (1.20) and finally fluvastatin (anion or acid 0.95 0.40) is the most reactive statin by far. Fluvastatin (0.97) and pravastatin (0.81) have been reported [1] to have higher (renal) clearance rates ($l.hr^{-1}kg^{-1}$) than atorvastatin (0.25), cerivastatin (0.2), lovastatin (0.26-1.1), and simvastatin (0.45), although clearance rates include many influencing factors, including the facts that pravastatin is the only statin not bound to plasma proteins, so its circulating levels are high, and it is by far the most water soluble of all statins. Inhibitor efficacy is a function of many factors, with the rate of metabolic removal being only one factor, but the η value can be a guide to metabolic behaviour in drug design.

Fluvastatin which is primarily metabolized by CYP2C9, and rosuvastatin and pravastatin which are metabolized by other pathways, are more resistant to CYP450 metabolic removal, [1,60] which appears to be consistent with the IE or η data.

Drug-drug interactions would be expected to be influenced by the ease of electron transfer between the drugs, so the IE, EA or η should be indicators of the likelihood of such possible interactions.

The location of the highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) are shown in Table 2 (supplementary) as well. These locations vary according to the total electronic structure of the statins, and show significant differences between type 1 and 2 statins as expected. This observation reflects the importance of having a full understanding of the complete molecular structure and its inherent reactivity when designing drugs and their

possible interactions with endogenous substrates or other drugs.

Statins in the lactone form are known to undergo rapid metabolism via the microsomal CYP3A4 isoenzyme. It is interesting that lovastatin anion has a η value of 1.90, whereas the lovastatin acid and lovastatin lactone have values of 1.85 and 1.20, implying the lactone undergoes a faster rate of metabolic removal.

4. Conclusions

The atomic electrostatic potential calculated by the CHELPG method have been shown to be sensitive indicators of the gas phase and solution properties of the statins. The percentage hydrophilicity and hydrophobicity (or lipophilicity) of the statins in solution have been determined using (a) the differences in solvation free energies between n-octanol and n-octane as a measure of hydrophilicity, and the solvation energy in octane as a measure of hydrophobicity (b) the sum of the atomic electrostatic charges on the hydrogen bonding and polar bonding nuclei of the common pharmacophore combined with a solvent measure of hydrophobicity, and (c) using the buried surface areas after statin binding to HMGCR to calculate the hydrophobicity of the bound statins.

The data suggests that clinical definitions of statins as either "hydrophilic" or "lipophilic" based on experimental partition coefficients such as log P or D are misleading.

An estimate of the binding energy between rosuvastatin and HMGCR has been made using: (a) a coulombic interaction model to sum the hydrogen bonding and polar bonding interactions between HMGCR amino acid residues and the statins, (b) the calculated desolvation and resolvation of the statin in water, and (c) the first solvation shell (cavity dispersion structure of the SMD solvation model) solvation as a proxy for the restructuring of the water molecules immediately adjacent to the active binding site of HMGCR prior to binding. De-solvation and re-solvation of the statins before and after binding to HMGCR are major determinants of the energetics of the binding process.

An analysis of the amphiphilic nature of lovastatin anion, acid and lactone and fluvastatin anion and their abilities to cross the blood brain barrier has indicated that this process may be dominated by desolvation and resolvation effects, rather than the molecular size of the statin or statin-lipid interactions within the bilayer.

The ionization energy and electron affinity of the statins are sensitive physical indicators of the ease that the various statins can undergo oxidative metabolism. The absolute chemical hardness is also a physical indicator of the stability of the statins, the resistance to change in the electron distribution of a molecule. These physical properties

may be useful design guides to possible endogenous metabolic behaviour and drug-drug interactions.

Table 1. Electrostatic Potential at Nuclei (Volts) at key sites for Statins

Statin Type 2 *Ionized Anionic or Acid forms (where indicated)*	**3-OH**	**5-OH**	**1-C=O**	**1-C-O⁻ / C-OH**	**4-F C_6H_4F group**	**i-Pr CH_3 groups (positions where indicated)**	**2 S=O (Or C=O or O-Me as indicated)**	**Energies - AU Dipole (D)**
Rosuvastatin Anion **Gas 90°**	22.8	21.8	21.9	20.1	7.2	12.2, 5.2 (C6)	15.2, 14.3	1967.6134 34.8 D
Rosuvastatin Anion **Water** 90°	25.3	23.9	24.1	22.8	7.7	12.7, 5.3	17.2, 17.1	1967.7423 37.7 D
Rosuvastatin Anion **Octanol** 90°	24.6	23.3	23.6	22.1	7.6	12.7, 5.4	16.8, 16.4	1967.7305 37.4 D
Rosuvastatin Anion **Octane** 90°	23.1	22.0	22.3	20.5	7.4	12.5, 5.4	15.5, 14.8	1967.6742 36.0 D
Rosuvastatin Anion **Water** 90° **Di-anion**	25.4	24.2	24.1	22.8	7.7	12.5, 5.2	22.6, 21.8	1967.8191 29.3 D
Rosuvastatin Anion **Water** 90° **Neutral**	24.3	22.8	23.0	21.7	7.6	11.9, 5.6	20.0, 19.6	1967.5254 29.9 D
Rosuvastatin Acid **Gas 90°**	23.1	21.7	14.0	19.6	6.6	5.9, 4.9 (C6)	17.6, 17.1	1967.9188 8.3 D
Rosuvastatin Acid **Water 90°**	24.6	26.0	17.4	22.0	7.2	7.7, 4.9	20.0, 19.6	1967.9933 11.3 D
Rosuvastatin Acid **Octanol 90°**	23.9	25.3	16.6	21.5	7.1	7.3, 4.9	19.4, 18.1	1967.9859 10.8 D
Rosuvastatin Acid **Octane 90°**	22.1	23.5	14.6	20.0	6.9	6.4, 4.9	18.0, 17.6	1967.9538 9.0 D
Rosuvastatin Acid **Water 90° Anion**	24.8	26.0	17.8	22.1	7.2	7.0, 3.8	20.8, 20.6	1968.0519 17.4 D
Rosuvastatin Acid **Water 90° Cation**	23.2	25.3	17.1	22.0	7.1	7.6, 5.1	19.4, 19.0	1967.7661 19.8 D
Rosuvastatin Anion **Gas** 42°	19.4	19.3	19.6	18.5	5.6	10.9, 4.6	12.7, 11.9	1968.1574 32.1 D
Rosuvastatin Anion **Water** ring 42°	21.7	21.4	22.0	21.0	5.9	11.3, 4.6	14.4, 14.4	1968.2733 36.7 D
Rosuvastatin **Anion Octanol** 42°	21.1	20.8	21.4	20.4	5.9	11.4, 4.8	14.0, 13.7	1968.2650 35.1 D
Rosuvastatin Anion 42° **Octane**	19.7	19.5	20.0	18.9	5.7	11.1, 4.9	12.9, 12.3	1968.2163 33.5 D
Rosuvastatin	19.3	20.0	12.9	15.9	4.8	7.4, 4.9	13.8, 13.5	1968.5721

Acid **Gas** 42^{o}						(C6)		7.3 D
Rosuvastatin Acid **Water** 42^{o}	22.1	22.7	16.1	18.3	5.2	9.1, 4.8	15.8, 15.5	1968.6264 9.9 D
Rosuvastatin Acid **Octanol** 42^{o}	21.3	22.0	15.4	17.7	5.2	8.8, 4.9	15.3, 15.1	1968.6239 9.4 D
Rosuvastatin Acid **Octane** 42^{o}	19.7	20.4	13.4	16.3	5.0	7.8, 5.0	14.1, 13.9	1968.6036 7.9 D
Rosuvastatin 3S, 5S Anion **Gas 90^{o}**	20.2	21.7	19.0	20.3	6.7	6.5, 3.3 (C6)	17.5, 17.3	1967.3685 28.0 D
Rosuvastatin 3S. 5S Anion **Water 90^{o}**	22.4	24.8	22.1	25.5	7.1	6.9, 4.0	19.3, 20.1	1967.5485 35.7 D
Rosuvastatin 3S, 5S Anion **Octanol 90^{o}**	21.9	24.0	21.6	24.7	7.1	6.8, 3.8	19.5, 18.8	1967.5243 35.5 D
Rosuvastatin 3S, 5S Anion **Octane 90^{o}**	20.5	22.1	20.3	22.5	6.9	6.6, 3.5	17.9, 17.4	1967.4365 34.1 D
Rosuvastatin 3S, 5S **Water** 90^{o} ***Di-anion***	22.7	24.8	22.1	25.5	7.2	6.4, 3.2	21.2, 21.0	1967.6164 28.7 D
Rosuvastatin 3S, 5S **Water** Anion 90^{o} ***Neutral***	22.7	24.4	15.9	8.5	7,2	6.9, 3.9	20.1, 19.3	1967.3437 9.0 D
Fluvastatin 30^{o} Anion **Gas**	17.7	17.9	19.7	20.0	6.1	7.1, 4.9		1382.4102 31.9 D
Fluvastatin 30^{o} Anion **Water**	21.2	20.8	22.9	22.5	6.4	8.0, 5.4		1382.5319 36.3 D
Fluvastatin 30^{o} Anion **Octanol**	20.3	20.1	22.1	21.9	6.4	7.9, 5.3		1382.5226 35.7 D
Fluvastatin 30^{o} Anion **Octane**	18.2	18.4	20.3	20.5	6.2	7.5, 5.1		1382.4619 33.6 D
Fluvastatin Anion 90^{o} **Gas**	17.8	17.2	19.7	20.0	5.6	7.8, 6.0		1382.3968 35.3 D
Fluvastatin Anion 90^{o} **Water**	21.3	20.0	22.8	22.4	5.9	8.8, 6.6		1382.5272 39.4 D
Fluvastatin Anion 90^{o} **Octanol**	20.4	19.3	22.0	21.9	5.9	8.7, 6.5		1382.5186 38.8 D
Fluvastatin Anion 90^{o} **Octane**	18.3	17.6	20.2	20.5	5.7	8.1, 6.2		1382.4581 36.7 D
Fluvastatin Acid 30^{o} **Gas**	17.2	16.7	13.4	16.6	5.1	6.3, 5.4		1382.8731 6.6 D
Fluvastatin Acid 30^{o} **Water**	20.9	19.5	16.6	18.2	5.6	7.5, 5.8		1382.9099 10.3 D
Fluvastatin Acid 30^{o} **Octanol**	19.9	18.8	15.8	17.9	5.5	7.4, 5.7		1382.9138 9.5 D
Fluvastatin Acid 30^{o} **Octane**	17.7	17.1	13.9	16.8	5.3	6.6, 5.5		1382.8999 7.5 D

Atorvastatin Anion 90° **Gas**	20.8	18.0	20.0	18.6	5.6	8.9, 5.7	15.3 (C=O)	1863.4773 32.3D
Atorvastatin Anion 90° **Water**	23.0	20.8	22.2	20.8	6.0	10.1, 6.9	20.1 (C=O)	1863.5980 34.8 D
Atorvastatin Anion 90° **Octanol**	22.4	20.1	21.6	20.1	5.9	9.9, 6.6	18.2 (C=O)	1863.5947 34.6 D
Atorvastatin Anion 90° **Octane**	21.0	18.4	20.3	18.9	5.8	9.3, 5.9	15.9 (C=O)	1863.5422 33.4 D
Atorvastatin Acid 90° **Gas**	19.3	20.4	13.7	20.0	6.5	11.7, 5.6	14.2 (C=O)	1863.1770 6.4 D
Atorvastatin Acid 90° **Water**	23.5	23.7	17.4	22.5	7.0	12.8, 6.4	18.2 (C=O)	1863.2457 8.9 D
Atorvastatin Acid 90° **Octanol**	22.4	22.9	16.5	22.0	7.0	12.5, 6.2	17.1 (C=O)	1863.2463 8.3 D
Atorvastatin Acid 90° **Octane**	20.0	20.9	14.3	20.5	6.8	11.9, 5.8	14.8 (C=O)	1863.2160 6.9 D
Atorvastatin Acid 90° **Water Anion**	23.6	23.9	17.4	22.5	7.1	11.7, 6.1	23.5 (C=O)	1863.3149 6.9 D
Atorvastatin Acid 90° **Water Cation**	23.4	23.6	17.4	22.5	7.0	8.9, 6.1	15.9 (C=O)	1863.0651 22.6 D
Atorvastatin Anion 54° **Gas**	20.7	17.9	18.5	19.9	5.7	8.9, 5.3	15.9 (C=O)	1863.4808 32.9 D
Atorvastatin Anion 54° **Water**	23.0	20.6	22.0	20.7	6.0	10.1, 6.4	20.0 (C=O)	1863.6008 35.4 D
Atorvastatin Anion 54° **Octanol**	22.3	19.9	21.5	20.2	6.0	10.0, 6.1	18.8 (C=O)	1863.5978 35.2 D
Atorvastatin Anion 54° **Octane**	21.0	18.2	20.2	18.8	5.8	9.3, 5.5	18.3 (C=O)	1863.5456 33.9 D
Atorvastatin Acid 58° **Gas**	18.4	17.3	13.2	15.5	5.5	9.4, 8.3	15.0 (C=O)	1863.9996 8.5 D
Atorvastatin Acid 58° **Water**	20.8	20.5	16.1	17.7	6.0	10.6, 10.5	18.6 (C=O)	1864.0472 13.2 D
Atorvastatin Acid 58° **Octanol**	20.2	19.7	15.5	17.3	5.9	10.4, 10.0	17.7 (C=O)	1864.0545 12.1 D
Atorvastatin Acid 58° **Octane**	18.8	17.3	13.7	15.9	5.7	9.8, 8.8	15.6 (C=O)	1864.0367 9.6 D
Cerivastatin Anion 90° **Gas**	18.0	17.0	17.0	20.6	5.0	7.4, 3.7 (C2) 6.1, 5.4 (C6)	13.2 (**O**-Me)	1541.0062 40.1 D
Cerivastatin Anion 90° **Water**	21.5	19.8	19.8	24.4	5.2	6.4,6.0 (C2) 8.0, 3.6 (C6)	14.6 **(O**-Me)	1541.1569 46.5 D
Cerivastatin Anion 90° **Octanol**	20.6	19.1	19.2	23.6	5.2	7.8, 3.7 (C2) 6.4, 5.9 (C6)	14.1 (**O**-Me)	1541.1432 45.7 D
Cerivastatin Anion 90° **Octane**	18.5	17.4	17.5	21.5	5.1	7.6. 3.8 (C2) 6.3, 5.6 (C6)	13.2 (O-Me)	1541.0684 42.7 D

Cerivastatin Acid 90° **Gas**	17.8	16.5	12.5	16.5	4.8	5.6, 5.3 (C2) 8.1, 3.3 (C6)	16.5 (**O**-Me)	1541.5906 6.9 D
Cerivastatin Acid 90° **Water**	21.2	19.6	16.0	18.9	5.2	6.2, 5.5 (C2) 8.8, 3.5 (C6)	19.6 (**O**-Me)	1541.6351 10.0 D
Cerivastatin Acid 90° **Octanol**	20.3	18.8	15.2	18.4	5.2	6.1, 5.5 (C2) 8.7, 3.4 (C6)	14.2 (**O**-Me)	1541.6378 9.2 D
Cerivastatin Acid 90° **Octane**	18.3	17.0	13.1	16.9	5.0	5.8, 5.5 (C2) 8.4, 3.4 (C6)	17.0 (**O**-Me)	1541.6166 7.5 D
Pitastatin Anion 90° **Gas**	18.1	18.4	16.8	20.6	6.6	9.7, 4.5 Cyclopropyl		1421.7608 38.7 D
Pitastatin Anion 90° **Water**	21.6	21.3	19.8	24.5	5.2	11.2, 5.0 Cyclopropyl		1421.9104 45.0 D
Pitastatin Anion 90° **Octanol**	20.7	20.6	19.1	23.7	5.1	10.9, 5.0 Cyclopropyl		1421.8963 44.3 D
Pitastatin Anion 90° **Octane**	18.6	18.8	17.5	21.5	5.0	10.2, 4.7 Cyclopropyl		1421.8254 41.6 D
Pitastatin Acid 90° **Gas**	17.1	17.7	13.7	17.5	4.8	9.7, 4.5 Cyclopropyl		1422.3467 4.4 D
Pitastatin Acid 90° **Water**	20.3	20.7	16.7	19.4	5.3	11.4, 4.8 Cyclopropyl		1422.3887 5.3 D
Pitastatin Acid 90° **Octanol**	19.6	19.9	16.0	19.0	5.2	11.2, 4.8 Cyclopropyl		1422.3917 5.0 D
Pitastatin Acid 90° **Octane**	17.6	18.1	14.2	17.8	5.0	10.3, 4.7 Cyclopropyl		1422.3755 4.5 D
Statin Type 1 *Ionized or Acid forms (where indicated)*	**3-OH**	**5-OH**	**1-C=O**	**1-C-O⁻ / C-OH**	**C=O* C-O-***	**Me(C2)** **Me(C6)****	**Me(C2,4)#** **(C=C-C=C)**	**Energies AU** **Dipole (D)**
Lovastatin Anion **Gas**	17.7	18.2	18.2	20.2	12.8 11.2	7.0 (C2) 9.7 (C6)	6.8, 6.3 (5.6, 2.7)	1387.6908 17.2 D
Lovastatin Anion **Water**	21.4	19.8	20.4	23.7	14.6 12.1	7.7 (C2) 9.6 (C6)	7.5, 6.9 (5.6, 3.0)	1387.8237 21.9 D
Lovastatin Anion **Octanol**	20.5	19.4	20.1	22.8	14.2 11.8	7.6 (C2) 9.8 (C6)	7.4, 6.9 (5.6, 2.9)	1387.8131 20.7 D
Lovastatin Anion **Octane**	18.2	18.3	18.7	20.7	13.0 11.3	7.2 (C2) 10.0 (C6)	6.9, 6.5 (5.6, 2.7)	1387.7483 18.0 D
Lovastatin Anion **Water** ***Dianion***	23.0	25.6	22.0	25.7	22.7 22.0	6.8 (C2) +4.4 (C6)	8.8, 8.8 (15.7, 12.0)	1387.3436 22.4 D
Lovastatin Anion **Water** ***Neutral***	22.7	25.3	22.1	25.6	16.6 18.3	9.2 (C2) 6.1 (C6)	6.4, 9.9 (10.4, 4.9)	1387.1675 36.4 D
Lovastatin Acid **Gas**	19.3	17.0	15.7	16.9	15.4 11.5	6.1 (C2) 8.0 (C6)	6.4, 6.2 (5.6, 1.9)	1388.2528 4.2 D
Lovastatin Acid **Water**	21.7	19.4	16.8	17.4	21.4 13.1	6.6 (C2) 9.0 (C6)	6.8, 6.6 (6.5, 3.0)	1388.3437 22.2 D
Lovastatin Acid **Octanol**	21.1	18.8	16.4	17.3	19.5 12.6	6.5 (C2) 9.0 (C6)	6.9, 6.6 (8.6, 2.5)	1388.3387 18.1 D
Lovastatin Acid **Octane**	19.5	17.3	15.8	16.9	15.7 11.7	6.4 (C2) 8.3 (C6)	6.7, 6.4 (6.0, 2.2)	1388.2983 4.9 D
Lovastatin Acid **Water *Anion***	23.5	25.3	18.3	21.6	22.6 21.5	6.0 (C2) +3.6 (C6)	8.0, 8.6 (15.9, 12.8)	1387.8137 21.5 D
Lovastatin Acid **Water *Cation***	23.1	25.1	18.4	21.5	16.6 18.0	8.5 (C2) 6.9 (C6)	5.8, 9.6 (10.7, 5.6)	1387.6359 5.5 D

Lovastatin Lactone **Gas**	14.2 (**O**-C=**O**)	18.7	12.9 (O-C=**O**)		11.6 16.6	6.7(C2) 0.1 (C6)	5.2, 5.1 (12.9, 7.4)	1311.6824 3.8 D
Lovastatin Lactone **Water**	15.6 (**O**-C=**O**)	21.7	15.9 (O-C=**O**)		13.6 19.1	8.0(C2) +0.4 (C6)	5.8, 5.5 (14.5, 10.2)	1311.7211 6.6 D
Lovastatin Lactone **Octanol**	15.3 (**O**-C=**O**)	21.1	15.3 (O-C=**O**)		13.0 18.3	7.6 (C2) +0.2 (C6)	5.6, 5.6 (14.3, 9.6)	1311.7259 6.0 D
Lovastatin Lactone **Octane**	14.5 (**O**-C=**O**)	19.2	13.5 (O-C=**O**)		11.9 16.8	6.9 (C2) 0.1 (C6)	5.4, 5.3 (13.5, 8.1)	1311.7086 4.5 D
Pravastatin Anion **Gas**	17.4	17.7	14.1	14.0	12.0 10.5	18.2(OH,C2) 10.2(Me, C6)	6.5, 7.0 (9.6, 2.1)	1423.5035 12.4 D
Pravastatin Anion **Water**	21.1	19.1	20.5	23.7	14.7 11.6	20.8(OH,C2) 9.2(Me, C6)	7.2, 8.0 (10.3, 2.6)	1423.6093 25.5 D
Pravastatin Anion **Octanol**	22.7	23.6	21.8	23.8	15.6 15.0	23.2(OH,C2) 8.1(Me, C6)	6.0, 6.9 (11.0, 3.7)	1423. D
Pravastatin Anion **Octane**	17.8	17.0	15.9	16.6	12.4 10.6	18.5(OH,C2) 10.2(Me,C6)	5.7, 7.3 (9.8, 2.1)	1423.5381 16.4 D
Pravastatin Anion **Water *Di-anion***	23.2	24.7	22.3	24.7	23.6 16.1	23.8(OH,C2) 6.6(Me,C6)	6.1, 6.2 (11.1, 3.9)	1422.3677 16.6 D
Pravastatin Anion **Water *Neutral***	22.4	24.4	22.2	24.7	16.4 15.4	23.8(OH,C2) 7.9(Me,C6)	6.4, 7.1 (10.5, 2.6)	1423.2110 25.7 D
Pravastatin Acid **Gas**	17.2	16.4	14.2	16.8	13.7 12.2	18.5(OH,C2) 10.9(Me,C6)	6.7, 10.2 (10.9, 2.9)	1424.1506 3.5 D
Pravastatin Acid **Water**	20.4	19.0	16.3	19.0	15.7 13.7	21.0(OH,C2) 11.1(Me,C6)	7.1, 11.2 (11.7, 4.3)	1424.1952 5.9 D
Pravastatin Acid **Octanol**	19.6	18.4	15.9	18.5	15.2 13.3	20.4(OH,C2) 11.1(Me,C6)	7.1, 11.0 (11.6, 4.0)	1424.1972 5.4 D
Pravastatin Acid **Octane**	17.7	16.8	14.5	17.2	14.0 12.4	18.8(OH,C2) 11.1(Me,C6)	6.9, 10.4 (11.2, 3.3)	1424.1767 4.1 D
Pravastatin Acid **Water *Anion***	22.6	24.5	18.9	21.5	22.5 16.1	23.7(OH,C2) 7.3(Me,C6)	6.2, 6.2 (10.3, 3.2)	1423.8224 16.9 D
Pravastatin Acid **Water *Cation***	21.8	24.1	17.5	21.4	16.2 15.5	23.6(OH,C2) 8.6(Me,C6)	6.5, 7.3 (9.7, 1.9)	1423.6616 13.5 D
Simvastatin Anion **Gas**	21.5	20.8	19.7	21.6	13.0 12.2	5.4 (C2) 7.5 (C6)	8.4, 6.0, 6.0 3xMe Gps (10.0, 3.8)	1426.6209 18.1 D
Simvastatin Anion **Water**	23.2	24.5	22.3	24.8	13.7 14.9	6.4 (C2) 7.2 (C6)	9.1, 6.6, 6.8 3xMe Gps (10.1, 4.4)	1426.7622 23.1 D
Simvastatin Anion **Octanol**	22.7	23.6	21.8	23.9	14.4 13.2	6.1 (C2) 7.5 (C6)	9.0, 6.5, 6.7 3xMe Gps (10.1, 4.2)	1426.7498 21.9
Simvastatin Anion **Octane**	21.5	21.2	20.2	22.0	13.2 12.4	5.5 (C2) 7.7 (C6)	8.7, 6.2, 6.3 3xMe Gps (10.0, 3.8)	1426.6792 19.0 D
Simvastatin Anion **Water *Di-anion***	23.2	24.6	22/3	24.8	21.5 14.9	5.0 (C2) 6.5 (C6)	8.3, 6.9, 6.8, 3xMe Gps (10.4, 4.5)	1426.8081 57.9 D
Simvastatin Anion **Water *Neutral***	22.5	24.4	22.2	24.8	14.9 13.9	6.5 (C2) 7.8 (C6)	9.2, 6.8, 7.2 3xMe Gps (9.5, 2.9)	1426.6577 27.9 D
Simvastatin Acid **Gas**	19.3	17.0	14.9	16.3	15.4 12.7	6.4 (C2) 8.6 (C6)	8.6, 8.4, 5.8 3xMe Gps	1427.5631 5.2 D

							(5.9, 1.6)	
Simvastatin Acid **Water**	22.6	24.3	17.7	21.4	14.8 14.1	6.0 (C2) 7.2 (C6)	9.2, 6.2, 6.6 3xMe Gps (10.0, 3.7)	1427.2134 8.9 D
Simvastatin Acid **Octanol**	22.0	23.5	17.0	20.7	14.3 13.5	5.7 (C2) 7.4 (C6)	9.2, 6.2, 6.4 3xMe Gps (0.8, 3.2)	1427.2150 8.2 D
Simvastatin Acid **Octane**	20.4	21.2	15.0	18.9	13.1 12.5	5.0 (C2) 7.7 (C6)	8.8, 5.9, 6.0 3xMe Gps (9.2, 2.3)	1427.1885 6.4 D

Footnotes to Table 1:

Pharmacophore labelled as 3,5 dihydroxyhept-6-enoic (type 2) or 3,5 dihydroxyhept-6-anoic (type 1, plus atorvastatin) anion or acid

Energies are sum of electronic and thermal energies + thermal corrections

The nuclei for which the electrostatic charges are recorded are shown as underlined nuclei: eg **3-OH** refers to the charge on the oxygen atom of the 3 hydroxy group of the pharmacophore.

All voltages are negative except where preceded by (+) sign. Large negative values for **O**-H, C=**O**, C-**O**$^-$, S=**O** etc indicate nuclei of high electron density.

C=O and C-O- of 1-butanoyl-oxy side chain*

C2 + C6 Methyls of hexahydro-napthalene moiety**

C2 and C4 Methyls of 1-butanoyl-oxy side chain#

C=C-**C**=C are the C1 and C8 of hexahydro-napthalene moiety

The labels "***Dianion***" and "***Neutral***" for the anionic form of statins in column 1 indicate an electron has been "added" or "removed" from the starting anion.

The labels "***Anion***" and "***Cation***" for the acid and lactone form of statins in column 1 indicate an electron has been "added" or "removed" from the starting neutral acid species.

90^o etc refers to the angular rotation of the 4FC_6H_4 ring wrt to the pyramidine, indole, or quinoline rings for Type 2 statins.

Table 2. Solvation energies, ionization energies, electron affinities, and location site of HOMO and LUMO

Statin	**Solvation Energy** **Bulk solvent - kcal/mol**	**SMD CDS Non-Electrostatic Solvation Energy kcal/mol**	**Hydro-philic Solvation Energy - kcal/mol**	**IE & (EA) eV** **Water**	η ½ (IE – EA)	**HOMO Location Water**	**LUMO Location Water**
Rosuvastatin Anion 90^o 401 cm^3/mol Water 321cm^3/mol Octanol 305 cm^3/mol Octane	80.9 Water 73.5 Octanol 30.2 Octane	10.3 Water 2.5 Octanol -11.7 Octane	43.3 (58.9%)	5.0 (1.8)	1.60	C-O$^-$ hept-6-enoic carboxylate side chain	N-S of 2-[methyl(methyl sulfonyl)amino side chain
Rosuvastatin Acid 90^o 345 cm^3/mol Water 367cm^3/mol Octanol 279 cm^3/mol Octane	46.8 Water 42.1 Octanol 22.0 Octane	8.7 Water 2.2 Octanol -10.6 Octane	20.1 (47.7%)	5.20 (1.35)	1.90	C4 pyrimidine + C7 hept-6-enoic carboxylate side chain	C6 pyrimidine + C1 4F-Ph ring
Rosuvastatin Anion 42^o 367 cm^3/mol Water 331 cm^3/mol Octanol 308 cm^3/mol Octane	72.7 Water 67.5 Octanol 37.0 Octane	10.5 Water 2.4 Octanol -11.8 Octane	30.5 (45.2%)	4.9 (1.8)	1.55	C5 pyrimidine + C7 hept-6-enoic side chain	C2- pyrimidine – N methyl(methyl sulphonyl)amino side chain
Rosuvastatin Acid 42^o 245 cm^3/mol Water 314 cm^3/mol Octanol 359 cm^3/mol Octane	34.1 Water 32.5 Octanol 19.9 Octane	9.0 Water 2.2 Octanol -10.8 Octane	12.7 (39.1%)	5.2 (1.5)	1.35	C5 pyrimidine	C6 pyrimidine + C1 4F-Ph ring
~~Rosuvastatin~~ Anion 30^o 355 cm^3/mol Water	78.9 Water 72.0 Octanol 37.7 Octane	10.5 Water 2.4 Octanol -11.9 Octane	34.3 (47.6%)	4.9 (1.8)	1.55	C5 pyrimidine	C2 pyrimidine

355cm^3/molOctanol 336cm^3/mol Octane							
~~Rosuvastatin~~ Anion 20º 338 cm^3/mol Water 369 cm^3/mol Octanol 290 cm^3/mol Octane	78.3 Water 71.5 Octanol 37.4 Octane	10.6 Water 2.5 Octanol -11.8 Octane	34.1 (47.7%)			C4 pyrimidine + C7 hept-6-enoic side chain	C1 pyrimidine
~~Rosuvastatin Acid~~ 20º 352 cm^3/mol Water 321cm^3/mol Octanol 270 cm^3/mol Octane	45.9 Water 41.6 Octanol 21.9 Octane	9.0 Water 2.3 Octanol -10.8 Octane	19.7 (47.4%)	5.2 (1.6)	1.30	C5 pyrimidine	C4 pyrimidine
Rosuvastatin 3S,5S Anion 90º 365 cm^3/mol Water	113.0 Water 97.8 Octanol 42.7 Octane	8.0 Water 2.7 Octanol -9.8 Octane	55.1 (56.3%)	5.6 (1.9)	1.35	C1-N2-N6 pyrimidine	C3-C5 pyrimidine + C7 hept-6-enoic side chain
Fluvastatin Anion 30º 299 cm^3/mol Water 244 cm^3/mol Octanol 299 cm^3/mol Octane	76.4 Water 70.5 Octanol 32.5 Octane	11.0 Water 1.7 Octanol -10.5 Octane	38.0 (53.9)	4.4 (2.5)	0.95	C=O hept-6-enoic carboxylate side chain	C(=O) - C1-(C-O$^-$) hept-6-enoic carboxylate side chain
Fluvastatin Anion 90º 322 cm^3/mol Water 347 cm^3/mol Octanol 276 cm^3/mol Octane	81.8 Water 76.4 Octanol 38.5 Octane	11.4 Water 1.7 Octanol -10.6 Octane	37.9 (49.6)	4.5 (2.5)	1.00	C=O hept-6-enoic carboxylate side chain	C(=O) - C1-(C-O$^-$) hept-6-enoic carboxylate side chain
Fluvastatin Acid 30º 273 cm^3/mol Water 301 cm^3/mol Octanol 287 cm^3/mol Octane	23.1 Water 25.5 Octanol 16.8 Octane	11.0 Water 1.4 Octanol -10.1 Octane	8.7 (34.1%)	3.9 (3.1)	0.40	C3-C4-C9 indole ring	N1-C8 indole ring
Atorvastatin 90º Anion 374 cm^3/mol Water 425 cm^{-3}/mol Octanol 397 cm^{-3}/mol Octane	75.7 Water 73.7 Octanol 41.4 Octane	13.5 Water 0.7 Octanol -13.9 Octane	32.3 (43.8%)	4.6 (1.0)	1.80	C7 hept-6-enoic carboxylate side chain	C=O C(O)NHPh side chain
Atorvastatin 54º Anion 470 cm^3/mol Water 430 cm^{-3}/mol Octanol 527 cm^{-3}/mol Octane	75.3 Water 73.4 Octanol 40.7 Octane	13.5 Water 0.6 Octanol -13.8 Octane	32.7 (44.6%)	4.4 (1.0)	1.70	C7 hept-6-enoic carboxylate side chain	C=O C(O)NHPh side chain
~~Atorvastatin~~ Acid 90º 385 cm^3/mol Water 380 cm^3/mol Octanol 470 cm^3/mol Octane	43.1 Water 43.5 Octanol 24.5 Octane	12.6 Water 0.0 Octanol -13.4 Octane		4.2 (1.6)	1.30	C3-C4 (para) phenyl side chain	C=O C(O)NHPh side chain
Atorvastatin Acid 58º 340 cm^3/mol Water 435 cm^3/mol Octanol 345 cm^3/mol Octane	29.0 Water 34.5 Octanol 23.3 Octane	13.9 Water 0.3 Octanol -13.9 Octane	11.2 (32.5%)	4.5 (1.0)	1.75	C1-C2 phenyl side chain	C=O C(O)NHPh side chain
Cerivastatin 90º Anion 278 cm^3/mol Water 391 cm^3/mol Octanol 361 cm^3/mol Octane	94.1 Water 86.0 Octanol 39.0 Octane	10.9 Water 1.8 Octanol -8.7 Octane	47.0 (54.7%)	5.5 (1.0)	2.25	C-O$^-$ hept-6-enoic carboxylate side chain	N-C1 pyridine
Cerivastatin 90º Acid 416 cm^3/mol Water 308 cm^3/mol Octanol 355 cm^3/mol Octane	27.9 Water 29.6 Octanol 16.3 Octane	11.4 Water 1.3 Octanol -8.8 Octane	13.3 (44.8)	4.7 (0.9)	1.90	C1-C2 4F-C_6H_4 side chain	C=O hept-6-enoic carboxylate side chain
Pitastatin 90º Anion 299 cm^3/mol Water 346 cm^3/mol Octanol 355 cm^3/molOctane	93.8 Water 85.0 Octanol 40.5 Octane	8.5 Water 0.6 Octanol -10.4 Octane	44.5 (52.4%)	5.5 (1.8)	1.85	C10 quinoline ring	C9 quinoline ring

Pitastatin Acid 90º 339 cm^3/mol Water 365 cm^3/mol Octanol 235 cm^3/mol Octane	26.4 Water 28.3 Octanol 18.1 Octane	9.0 Water 0.2 Octanol -10.5 Octane	10.2 (36.0%)	4.8 (1.5)	1.65	C10 quinoline ring	C9 quinoline ring
Lovastatin Anion 306 cm^3/mol Water 300 cm^3/mol Octanol 318 cm^3/mol Octane	83.3 Water 76.7 Octanol 36.1 Octane	9.7 Water 0.8 Octanol -9.4 Octane	40.6 (52.9%)	3.6 (1.3)	1.15	C6 hept-6-enoic carboxylate side chain	C=O 2-methyl-1-oxobutoxy side chain
Lovastatin Acid 266 cm^{-3}/mol Water 313 cm^3/mol Octanol 325 cm^3/mol Octane	57.0 Water 53.9 Octanol 28.6 Octane	10.3 Water -0.3 Octanol -9.4 Octane	25.3 (47.0%)	3.0 (1.1)	0.95	C=O 2-methyl-1-oxobutoxy side chain	C=O hept-6-enoic carboxylate side chain
Lovastatin Lactone 251 cm^3/mol Water 290 cm^3/mol Octanol 299 cm^3/mol Octane	24.3 Water 27.3 Octanol 16.5 Octane	9.2 Water -0.9 Octanol -9.4 Octane	10.8 (39.6)	3.0 (1.1)	0.95	C6 Me hexahydro-napthalene moiety	C5-C6 hexahydro-napthalene moiety
Pravastatin Anion 374 cm^3/mol Water cm^3/mol Octanol 358 cm^3/molOctane	66.4 Water Octanol 21.7 Octane	9.6 Water Octanol -9.1 Octane	(%)	2.9 (1.4)	1.25	C-O^- hept-6-enoic carboxylate side chain	C=O butanoyl-oxy side chain
Pravastatin Acid 281 cm^3/mol Water 320 cm^3/mol Octanol 358 cm^3/mol Octane	28.0 Water 29.2 Octanol 16.4 Octane	11.3 Water 1.9 Octanol -9.4 Octane	12.8 (43.8%)	2.5 (1.2)	0.65	C3=C4 hexahydro-napthalene moiety	C3=C4 hexahydro-napthalene moiety
Simvastatin Anion 337 cm^3/mol Water 396 cm^3/mol Octanol	88.7 Water 80.9 Octanol 35.6 Octane	9.9 Water 0.4 Octanol -9.3 Octane	45.3 (56.0%)	2.8 (1.2)	0.80	C5-C6-C7 hept-6-enoic carboxylate side chain	C=O 2-dimethylbutanoate Side chain
Simvastatin Acid 312 cm^3/mol Water 331cm^3/mol Octanol 306 cm^3/mol Octane	32.6 Water 33.6 Octanol 16.9 Octane	10.4 Water 0.0 Octanol -9.3 Octane	16.7 (49.7%)	2.3 (1.2)	0.55	C5-C6-C7 hept-6-enoic carboxylate side chain	C=O 2-dimethylbutanoate

Footnotes to Table 2:

Solvation energies are calculated using the Polarizable Continuum Model (IEFPCM), Unified Force Field, scaled van der Waals surface cavity, with radii and non-electrostatic terms using the SMD solvation model. Solvation (free) energies are the differences between the energies of the statin in the gas phase and in the particular solvent.

Hydrophilic solvation energies are the differences between the solvation energies in n-octanol and n-octane. Values in (..) brackets are the hydrophilic solvation energies as percentages of the solvation energies in n-octanol.

SMD CDS (cavitation dispersion structure, non-bulk first solvation shell) solvent model: A. V. Marenich, C. J. Cramer, and D. G. Truhlar, *J. Phys. Chem. B*, 2009, 113, 6378-96. The CDS values in column 3 are included in the total solvation energies given in column 2.

IE, EA: Vertical ionization energy and electron affinity in eV. Calculated from by the SCF difference method for anionic form as IE = $E(M^-) - E(M)$ and EA = $E(M^-) - E(M^{2-})$ at the optimised geometry of M^-, or for the neutral acid form as IE = $E(M) - E(M^+)$ and EA = $E(M) - E(M^-)$ at the optimised geometry of M.

HOMO and LUMO: Highest occupied molecular orbital and lowest occupied molecular orbital.

Values in cm^3/mol are molecular volumes in water (or n-octanol or n-octane where indicated) defined as the volume inside a contour of 0.001 electrons/$Bohr^3$ density.

Absolute hardness, η = ½ (IE – EA), R.G. Pearson, J. Chem. Sci. 2005, 117, 369.

Table 3(a). Hydrogen and polar donor and acceptor bonding ability calculated from atomic electrostatic charge potentials, compared with hydrophilic solvation energies, and log P values.

Statin	**Water** **Donor Acceptor Ability -Volts**	**Octanol** **Donor Acceptor Ability -Volts**	**Hydrophilic Solvation Energy -kcal/mol**	**Relative Lipophilicity from Log P***
Rosuvastatin Anion	120.8 (0.88)	117.3 (0.88)	30.5	
Fluvastatin Anion	93.8 (0.68)	90.8 (0.68)	38.0	105 (pH 7.4)
Atorvastatin Anion	112.3 (0.82)	108.7 (0.82)	32.7	76 (pH 7.4)
Cerivastatin Anion	105.3 (0.77)	101.8 (0.76)	47.0	219 (pH 7.4)
Pitavastatin Anion	92.4 (0.67)	89.2 (0.67)	44.5	
Lovastatin Anion	116.7 (0.85)	113.0 (0.85)	44.5	71 (pH 7.4)
Pravastatin Anion	**137.6 (1.00)**	**133.3 (1.00)**	52.0	**1.0** (pH 7.4)
Simvastatin Anion	107.8 (0.78)	111.7 (0.84)	42.9	310 (pH 7.4)
Rosuvastatin Acid	115.7 (0.92)	112.0 (0.93)	12.7	
Fluvastatin Acid	80.8 (0.64)	77.9 (0.64)	8.7	73 (pH 2)
Atorvastatin Acid	99.7 (0.70)	96.3 (0.79)	11.2	100 (pH 2)
Cerivastatin Acid	100.5 (0.80)	92.1 (0.80)	13.3	0.1 (pH 2)
Pitastatin Acid	82.4 (0.65)	79.7 (0.66)	10.2	
Lovastatin Acid	105.3 (0.83)	101.4 (0.84)	12.0	72 (pH 2)
Pravastatin Acid	**126.2(1.00)**	**120.8 (1.00)**	17.2	**1.0** (pH 2)
Simvastatin Acid	107.8 (0.85)	99.4 (0.81)	12.0	194 (pH 2)
Lovastatin Lactone	81.1	78.7		

Footnotes to Table 3(a)

* Log P values from: H.N. Joshi, M.G. Fakes, A.T.M. Serajuddin, Pharm. Pharmacol. Commun. 1999, 5, 269.

Statin hydrogen and polar donor acceptor bonding ability is the sum of the electrostatic atomic charges in volts taken from Table 1 on all polar groups, C-**O**$^-$, C=**O**, **O**-H, S=**O**, **O**-C(**O**)-, C-**F**, **O**-Me which can interact with a polar solvent or with polar groups on HMGCR in water. Values in (brackets) are relative to pravastatin taken as 1.00.

The hydrophilic solvation energy is taken from Table 2, column 4.

4F-C_6H_4 ring of type 2 statins are lowest dihedral angle conformations see Table 1.

Table 3(B). Surface Accessible Areas, Log P, Log D and Calculated Hydrophobicity or Lipophilicity of Statins

Statin	**Surface Accessible Area** Å^2	**Relative Lipophilicity Log P *****	**Relative Lipophilicity Log D**** (pH 7.4)**	**Lipophilicity* from Solvation Energies in Octane -kcal/mol**	**Lipophilicity$^{\#}$ (from Surface Accessible Area) -kcal/mol**
Rosuvastatin anion 42^o	710, 130, 880		3.2	37.0	39.6
Fluvastatin anion 13^o	660, 80, 870	105 (pH 7.4)	100	38.8	39.2
Atorvastatin anion 54^o	840, 150, 1060	76 (pH 7.4)	100	40.7	47.7
Cerisvastatin anion 90^o	720, 100, 880	219 (pH 7.4)	316	39.9	39.6
Pitastatin anion 90^o				40.5	
Lovastatin$^{\#\#}$	670, 100,	71 (pH 7.4)		34.8	39.6

anion	880				
Pravastatin anion		1.0 (pH 7.4)	1.0	40.9	
Simvastatin anion	670, 110, 880	310 (pH 7.4)	316	36.7	39.6
Rosuvastatin acid 42^{o}				19.9	
Fluvastatin acid 30^{o}		73 (pH 2)		16.8	
Atorvastatin acid 54^{o}		100 (pH 2)		23.3	
Cerisvastatin acid 90^{o}		0.1 (pH 2)		16.3	
Pitastatin acid 90^{o}				18.1	
Lovastatin acid		72 (pH 2)		14.9	
Pravastatin acid		1.0 (pH 2)		16.9	
Simvastatin acid		194 (pH 2)		14.7	
Lovastatin Lactone				14.5	

Footnotes to Table 3(B):
* Values taken from Table 2, column 2: hydrophobicity solvation energies are the values in n-octane.
** Values from E. Istvan, J. Diesenhofer, Science, 2001, 292, 1160: for the unbound statins, the bound statins, and the buried surface areas after statin binding to HMGCR respectively (from Xray crystal structure determinations of bound statins-HMGCR)
*** Values from: H.N. Joshi, M.G. Fakes, A.T.M. Serajuddin, Pharm. Pharmacol. Commun. 1999, 5, 269.
**** Values from: M.C. White, J. Clinical Pharm. 2002, 42, 963.
[#] Lipophilicity calculated from Surface Accessible Energy multiplied by 0.045 kcal/mol/Å^2 see text discussion
[##] Surface accessible area values used for lovastatin are the literature values for compactin, which differs structurally from lovastatin only in not possessing a 6-methyl group on the hexahydro-napthalene moiety.
Conformations of 4F-C_6H_4 ring of type 2 statins shown in degrees with respect to the main ring of the statins.

Table 4. Estimated hydrophilic (and hydrophobic) interactions between HMG CoA Reductase amino acid residues and statin in interior binding pocket

HMG CoA Reductase Ligand -- Statin	**Bond Distances from Xray Literature Aº**	**Rouvastatin Anion Gas -kcal/mol Linear Bond**	**Rouvastatin Anion Water -kcal/mol Linear Bond**
Lysine–C-O$^-$	2.8	1.0 (1.7)	1.3 (1.7)
Serine–C=O Lysine--C=O	3.0	3.3 (1.6) 2.0 (2.1)	3.8 (1.6) 2.3 (2.1)
Arginine–C_3-OH Arginine–C_3-OH	3.5 3.2	4.3 (2.4) 4.9 (2.1)	4.1 (2.4) 4.3 (2.1)
Aspartic Acid—C_5-OH	2.9	3.6 (1.8)	3.8 (1.8)
Lysine—C_3-OH Asparagine—C_5-OH	2.9 3.0	0.9 (1.7) 1.7 (1.9)	1.1 (1.7) 2.0 (1.9)

Glutamic Acid^{-}C_5-OH	2.9	2.2 (1.8)	3.4 (1.8)
Arginine–FC_6H_4-	2.9	0.7 (1.9)	0.9 (1.9)
Arginine—S=O Serine—S=O	4.2 3.2	5.9 (3.1) 1.6 (1.7)	6.2 (3.1) 1.7 (1.7)
Total Calculated Hydrophilic Interactions		32.1	34.9
Estimated Hydrophobic Interaction**		38.9	42.3

Footnotes to Table 4

Estimated hydrophilic interactions calculated from Coulombic charge interactions between amino acid residue and statin polar group assuming an effective dielectric **ε**=4 within the HMG CoA reductase enzyme interior binding pocket. All values are negative - electrostatic interactions are attractive forces. Side chain amino acid residues were set at the reported Xray structurally determined distances from the relevant statin polar groups according to E.S. Istvan, J. Diesenhofer, Science, 2001, 292, 1160. The Xray bond distances are specified for O, N of the amino acid residues and the statin, and do not identify OH or NH distances. The values in columns 2 and 3 have the optimised linear NH or OH distances included within the specified Xray distances, where the literature Xray distances between the O or N atoms were set, then the H bond created between the atoms. For example the actual distance between the N^{+}-**H** --- **O**=C hydrogen bond between the HMGCR lysine residue to the statin carboxylate carbonyl oxygen atom in A^{o} used in the Coulombic calculations is shown in parentheses. Rosuvastatin calculated with 42^{o} dihedral angle between 4-fluorophenyl ring and pyrimidine ring. **Estimated hydrophobic interaction is the hydrophobic solvation energy calculated from Table 2, column 4, where the hydrophilic solvation energy is given as a percentage, and the remainder is taken to be the total hydrophobic solvation energy. The hydrophobic solvation energy is taken to be a proxy for the hydrophobic interaction between the HMG CoA reductase enzyme and the statin in the binding pocket. All values are negative.

Table 5. Cavitation, dispersion, cavity field effects, and repulsion solute-solvent interaction energies for selected statins

	Cavitation Energy*	**Dispersion Energy***	**Cavity Field Effects ***	**Repulsion Energy***	**Sum of Energies* (columns 2 to 4)**	**CDS Hydrogen Bonding +/or Polar Interaction Estimate**
Fluvastatin 90^{o} Anion Water	59.2	-35.7	0	-2.3	20.9	-32.3
Rosuvastatin 90^{o} Anion Water	66.6	-39.3	0	-2.5	24.8	-35.1
Rosuvastatin 42^{o} Anion Water	69.2	-39.6	0	-2.5	27.1	-37.6
Atorvastatin 90^{o} Anion Water	82.3	-45.4	0	-2.8	34.1	-47.6
Atorvastatin 54^{o} Anion Water	82.2	-45.8	0	-2.9	33.5	-47.0
Lovastatin Anion Water	61.0	-36.8	0	-2.1	22.1	-32.0
Lovastatin Lactone Water	61.1	-36.2	0	-2.1	22.8	-32.4
Fluvastatin Anion Octanol	66.1	-34.3	0	-2.1	29.7	-31.4
Rosuvastatin Anion Octanol	74.7	-37.7	0	-2.2	34.8	-37.2
Lovastatin Anion Octanol	68.6	-35.5	0	-1.8	31.3	-32.3

Atorvastatin 90° Anion Octanol	92.1	-43.4	0	-2.5	46.2	-46.9
Lovastatin Lactone Octanol	68.9	-34.8	0	-1.8	32.3	-32.9
Fluvastatin Anion Heptane/Octane	40.3	-30.6	0	-1.9	7.8	-2.7
Rosuvastatin Anion Heptane/Octane	45.4	-33.6	0	-1.9	9.9	-1.8
Atorvastatin 90° Anion Heptane/Octane	56.1	-38.8	0	-2.2	15.1	-2.2
Atorvastatin 58° Anion Heptane/Octane	56.1	-39.2	0	-2.3	14.6	-0.8

Footnotes to Table 5:
Solvent Model is the Polarizable Continuum Model (IEFPCM), solvation energies in kcal/mol.
Solute **cavitation energy** calculated by the model of R.A. Pierotti, *Chem. Rev.*, 1976, 76, 717.
Solute-solvent **dispersion interaction energy** calculated by the model of J. Florsi, F.Tomasi, and J. L. Pascual-Ahuir, *J. Comp. Chem.*, 1991, 12, 784.
Cavity-field interaction energy (also known as local field effect) calculated according to the model of R. Cammi, C. Cappelli, S. Corni, and J. Tomasi, *J. Phys. Chem. A*, 2000, 104, 9874-79.
Solute-solvent **repulsion interaction energy** calculated by the model of J. Florsi, F.Tomasi, and J. L. Pascual-Ahuir, *J. Comp. Chem.*, 1991, 12, 784.
n-Heptane used instead of n-octane as solvent input parameters not available for n-octane.
CDS "Hydrogen + Polar" interaction estimate in column 7 is calculated by adding the values for the various solvents from column 3 of Table 2 (SMD CDS Non-Electrostatic Solvation Energy) to the sum of energies in column 6 of Table 5.

Experimental

Electrostatic potential at nuclei were calculated using the CHELPG method in Gaussian 09. The atomic charges produced by CHELPG are not strongly dependant on basis set selection. Using the B3LYP level of theory, calculated atomic charges were almost invariant amongst the basis sets 6-31G, 6-31G(d), 6.311(d,p), 6-311+(2d,2p), 6-311G++(3df,3dp) [34,35]. Errors between calculated and experimental dipole moments were 3%. A potential weakness of CHELPG (and other methods to calculate electrostatic charges at nuclei from the molecular electrostatic potential, MEP, around the molecule) is the treatment of larger systems, in which some of the innermost atoms are located far away from the points at which the MEP is evaluated. However, this study is concerned with charges at the molecular surface, and how such charges interact with solvents, or other atomic charges on molecules near the surface of the statin molecules. High absolute computational accuracy is not the objective of this study, comparative differences, particularly in solution, are the foci of the study.

All calculations were at the B3LYP/6-31G*(6d, 7f) level of theory, using optimised geometries, as this level has been shown to give accurate electrostatic atomic charges, and was used to optimize the IEFPCM/SMD solvent model. With the 6-31G* basis set, the SMD model achieves mean unsigned errors of 0.6 - 1.0 kcal/mol in the solvation free energies of tested neutrals and mean unsigned errors of 4 kcal/mol on average for ions. [29]

Rizzo at al [33] have also used the 6-31G* basis set with CHELPG charges (compared with 7 other atomic charge models) to calculate absolute free energies of solvation and compare these data with experimental results for more than 500 neutral and charged compounds. The calculated values were in good agreement with experimental results across a wide range of compounds.

Adding diffuse functions to the 6-31G* basis set (ie 6-31^{+}*) had no significant effect on the solvation energies with a difference of ca 1% observed in solvents for the fluvastatin anion, which is within the literature error range for the IEFPCM/SMD solvent model. This is consistent with the finding [63] diffuse functions had a negligible effect on energy, geometry and charges for anions where conjugation or delocalisation of the negative charge was occurring. The statin anions have a fully conjugated and delocalised carboxylate group, with significant through space interaction with the 3-hydroxy group of the pharmacophore, as evident in the data in Table 1.

References

[1] P. Gazzerro, M.C. Proto, G. Gangemi, A.M. Malfitano, E. Ciaglia, S. Pisanti, A, Santoro, C. Laezza, M. Bifulco, Pharmacol. Rev. 2012, 64, 102.
[2] M. Schachter, Fund. Clin. Pharmacol. 2004, 19,117**.**
[3] L. Calza, Drug, Healthcare, Patient Safety, 2009, 1, 25.
[4] S. Zeichner, C.G. Mihos, O. Santana, J. Cancer Res. Therap. 2012, 8, 176.
[5] M. Kunzl, C. Wasinger, M. Hohenegger, World J. Pharmacol. 2013, 2, 100.
[6] C.Y.Yang, P.Y. Liu, J.K Liao, Trends Mol. Med. 2008, 14, 37.
[7] J.K. Liao, J. Clin. Invest., 2002, 110, 285.
[8] K.O. Bonsu, A. Kadirvelu, D.D. Reidpath, Sys. Revs. 2013, 2, 22.
[9] M.C. Kim et al, Kor. J. Int. Med., 2011, 26, 294.
[10] Water and Lipid Soluble Statins, http://www.gpnotebook.co.uk/simplepage.cfm?ID=x20120104193906598025
[11] O. Tsinman, K, Tsinman, N. Sun, A. Avdeef, Pharm. Res. 2011, 28, 337.
[12] M. Muehlbacher, G.M. Spitzer, K.R. Liedl, J. Kornhuber, J. Comput. Aided Mol. Des. 2011, 25, 1095.
[13] S. Güthe, A. Seelig, Model-based prediction of blood-brain barrier permeation of anticancer drugs, Mip Tec Abstracts, October 14, 2009; A. Seelig, J. Mol. Neurosci. 2007, 33, 32.
[14] (a) F. Guillot, P. Misslin, M. Lemaire, J Cardiovasc. Pharmacol. 1993, 21, 339; (b) E.A.van Vliet, L. Holtman et al, Epilepsia, 2011, 52, 1319.
[15] D.S.King, A.J. Wilburn, M.R.Wofford, T.K. Harrell, B.J. Lindley, D.W. Jones, Pharmacotherapy, 2003, 23 ,1663.
[16] (a) Y.C. Lee, C.H. Lin, R.M.Wu et al Neurology, 2013, 5, 410; (b) W.G. Wood, G.P. Eckert, U. Igbavboa, W.E. Muller, Ann. N.Y. Acad. Sci, 2010, 1199, 69.
[17] H.v.d.Waterbeemd, Physico-chemical approaches to drug design, in Drug Bioavailability, ed. H.v.d.Waterbeemd, H. Lennernas, P. Artusson, 2003, Ch. 1, Wiley-VCH, Weinheim.
[18] M.P. Edwards, D.A. Price, "Role of Physicochemical Properties and Ligand Lipophilicity Efficiency in Addressing Drug Safety Risks". Ann. Repts. Med. Chem. 2010, 45, 381.

[19] A. Leo, C. Hantsch, D.Elkins, Chem. Rev. 1971, 525.
[20] S. Balaz, Chem. Rev. 2009, 109, 1793–1899.
[21] F. Guillot, P. Misslin, M. Lemaire, J. Cardiovasc. Pharmacol. 1993, 21, 339.
[22] M. Earll, log P and pK_a measurements, 2006, showme.physics.drexel.edu/mirza/.../Logp%20and%20pKa%20uses.pdf
[23] M.C. White, J. Clin. Pharm. 2002, 42, 963.
[24] R.L. Baldwin, Weak Interactions in Protein Folding: Hydrophobic Free Energy, van der Waals Interactions, Peptide Hydrogen Bonds, and Peptide Solvation, Chapter 6, Protein Folding Handbook, ed. J. Buchner, T.Kiefhaber, published online: 31 Jan 2008,Wiley-VCH Verlag GmbH & Co. Weinheim. 2005, 127-162.
[25] R.L. Baldwin, J. Mol. Biol. 2007, 371, 283.
[26] P. Cozzini, M. Fornabaio, A. Marabotti, D.J. Abraham, G.E. Kellogg, A. Mozzarelli, Curr. Med. Chem. 2004, 11, 1345.
[27] I. Gitlin, J.D. Carbeck, G.M. Whitesides, Angew. Chem. Int. Ed. 2006, 45, 3022.
[28] P. Kukic, J.E. Nielsen, Future Med. Chem. 2010, 2, 647.
[29] V. Marenich, C. J. Cramer, and D. G. Truhlar, J. Phys. Chem. B, 2009, 113, 6378.
[30] G.D. Hawkins, J. Li, T. Zhu, C. Chambers, D. Giesen, D. Leotard, C.J. Cramer, D.J. Truhlar, Proc. Rational Drug Design, ACS, Symposium Series, 1998.
[31] B. Galabov, S. Ilieva, G. Koleva,W.D. Allen, H. F. Schaefer, P. von R.Schleyer, WIREs Comp. Mol. Sci. 2013, 13, 37.
[32] C.M. Breneman and K. Wiberg J. Comp. Chem. 1990, 11, 361
[33] R.C. Rizzo, T. Aynechi, D.A. Case, I.D. Kuntz, J. Chem. Theory Comput. 2006, 2, 128.
[34] F. Martin, H. Zipse, J. Comp. Chem. 2005, 26, 97.
[35] J. Kubelka, www.uwyo.edukubelka-chempopulation_analysis.pdf
[36] P. Thanikaivelan, J. Padmanabhan, V. Subramanian, T. Ramasami, Theor. Chem. Accts. 2002, 107, 326.
[37] B. Lee, Biopolymers, 31, 1991, 993.
[38] B. Lee, Methods Enzymol. 1995, 259, 555.
[39] B. Widom, J. Phys. Chem. 1982, 86, 869.
[40] N.T. Southall, K.A. Dill, A.D. Haymet, J. Phys. Chem. B 2006, 106, 521.
[41] G.A. Holdgate, W.H. Ward, F. McTaggart, Biochem. Soc. Trans. 2003, 13, 528.
[42] T. Zhang, D.E. Koshland, Protein Sci. 1996, 5, 348.
[43] P.L. Kastritis, A.M. Bonvin, J.R.Soc. Interface, 2013, 10, 20120835.
[44] J.L. MacCallum, D.P. Tieleman, Trends Biochem. Sci. 2011, 12, 653.
[45] K. Kahraman, J.M. Thornton, "Methods to characterize the structure of enzyme binding sites", in Computational Structural Biology, Ch 8, T. Schwede, M.C.Pietsch, eds, World Scientific, 2008.
[46] L. Cavallo, J. Kleinjung, F. Fraternali, Nucleic Acids Res. 2003, 31, 3364.
[47] (a) T. Carbonell, E. Freire, Biochem. 2005, 44, 11741, (b) R.W. Sarver, E. Bills, G. Bolton, L.D. Bratton, N.L. Caspers, J.B. Dunbar, M.S. Harris, R.H. Hutchings, R.M. Kennedy, S.D. Larsen, A. Pavlovsky, J.A. Pfefferkorn, G.Bainbridge, J. Med. Chem. 2008, 10, 3804.
[48] K.A. Sharp, A. Nicholls, R.F. Fine, B. Honig, Science, 1991, **252**, 106.
[49] K. Raha, K.M. Merz, Structural basis of dielectric permittivity of proteins, in Protein Folding and Drug Design, 2007, Societa Italiana de Fisica, Bologne, R.A. Broglia, G. Tiana eds. p. 193. (ε =4-6 is the usual value)

[50] L. Li, C. Li, Z. Zhang, E. Alexoz, J. Chem. Theory Comput. 2013, 9, 2126. (the most common value is **ε** =4, which is believed to account for electronic polarization and small backbone fluctuations).
[51] T. Simonson, C. L. Brooks III, J. Am. Chem. Soc. 1996, 118, 8452. A common simplifying approximation is to consider three separate regions of the system, each with a different dielectric constant: a) the core of the protein has a low dielectric constant (e = 2–4), b) the bulk solvent (water, buffer, or biological fluid) has a high dielectric constant (typically taken to be that of bulk water: e =80), and c) the surface of the protein and surrounding layer of solvent has intermediate values of dielectric constant (e =10–20).
[52] E. Istvan, J. Diesenhofer, Science, 2001, 292, 1160.
[53] H.N. Joshi, M.G. Fakes, A.T.M. Serajuddin, Pharm. Pharmacol. Commun. 1999, 5, 269.
[54] E. Baker, R. Hubbard, Prog. Biophys. Mol. Biol., 1984,**44**, 97.
[55] R.E. Hubbard, Encyclopedia of Life Sciences, 2001, 1, McMillan, www.els.net.
[56] A.V. Morozov, T. Kortemme, K. Tsemekhman, D. Baker, PNAS, 2004, 101, 6946.
[57] P.W. Snyder, G.M. Whitesides et al, PNAS, 2011, 108, 17889.
[58] B.K. Shoichet, A.R, Leach, I.D. Kuntz, Proteins: Structure, Function and Genetics, 1999, 34, 16.
[59] (a) C. N. Pace, Nature Struct. Molec. Biol. 2009, 16, 681; (b) M.K. Gilson, H.X. Zhou, Ann. Rev. Biophys. Biomol. Struct., 2007, 36, 21; (c) J. Kuriyan, B. Konforti, D. Wemmer, The Molecules of Life, Garland, 2008, Ch 12.
[60] S. Bellosta, R. Paoletti, A. Corsini, Circulation, 2004, 109, III-50.
[61] R.G. Pearson, J. Chem. Sci. 2005, 117, 369.
[62] C.D. Selassie, History of Quantitative Structure Activity Relationships, Ch. 1, in Burger's Medicinal Chemistry and Drug Discovery, 6th ed., vol. 1, D.J.Abraham, ed., 2003, Wiley Interscience.
[63] N. Treitel, R. Shenhar, I. Aprahamian, T. Sheradsky and M. Rabinovitz, Phys. Chem. Chem. Phys. 2004, 6, 1113.