References 12

Links, Abstracts, Articles, etc.

These links should work as of 2014; sometimes you have to click on them several times; if they don't work, then Google/search the titles

Albumin –Related References

http://en.wikipedia.org/wiki/Human_serum_albumin Wiki Albumin

http://albumin.org/ Excellent Albumin source

Fluoroquinolone – HSA Binding

http://www.ncbi.nlm.nih.gov/pubmed/21417790 **Effect of metal ions on some pharmacologically relevant interactions involving fluoroquinolone antibiotics.** *"Complexation of five metal cations, Fe(3+), Al(3+), Zn(2+), Cu(2+) and Mg(2+) with four fluoroquinolones, levofloxacin, sparfloxacin, ciprofloxacin hydrochloride and enrofloxacin and human serum albumin (HSA) has been studied for better understanding of bioavailability of drugs interacting with metals and proteins*.

http://www.ncbi.nlm.nih.gov/pubmed/21273024 **Lomefloxacin promotes the interaction between human serum albumin and transferrin: a mechanistic insight into the emergence of antibiotic's side effects**
Chamani_j@yahoo.com

http://www.ncbi.nlm.nih.gov/pubmed/21142219 **Comparison of binding interactions of lomefloxacin to serum albumin and serum transferrin by resonance light scattering and fluorescence quenching methods.**

http://www.sciencedirect.com/science/article/pii/S002223131000181X **Complexation of fluoroquinolone antibiotics with human serum albumin: A fluorescence quenching study**. *"Mechanism of interaction and detailed physico-chemical characterization of the binding of four fluoroquinolones: levofloxacin, sparfloxacin, ciprofloxacin HCl and enrofloxacin with human serum albumin has been studied at physiological pH (7.4) using fluorescence spectroscopic technique. The stoichiometry of interaction was found to be 1:1 for all the drugs used. The association constants for the interaction were of the order of 10 4rth in most cases. At low drug:protein ratios, a significant fraction of the added drug was bound. The predominant interactions involved are hydrogen bonding and Van der Waal's interactions in the case of levofloxacin, hydrophobic interactions in the case of ciprofloxacin hydrochloride and enrofloxacin and hydrogen bonding, hydrophobic and electrostatic interactions in the case of sparfloxacin. The drug binding region did not coincide with that of the hydrophobic probe, 1-anilinonaphthalene-8-sulfonate (ANS). From the displacement of site-specific probes and site-marker drugs, it was concluded that ciprofloxacin hydrochloride is site II-specific while enrofloxacin is a site I-specific drug. Levofloxacin binds at both site I and site II with equal affinity. Sparfloxacin had higher affinity for site II than site I. It is also possible that sparfloxacin binds at the interface between site I and site II. Stern–Volmer analysis of the data showed that the quenching mechanism is predominantly collisional for the binding of ciprofloxacin HCl and enrofloxacin while both*

static and collisional quenching mechanisms are operative in the case of levofloxacin and sparfloxacin. High magnitude of the rate constant for quenching showed that the process is not entirely diffusion controlled. Circular dichroism (CD) spectroscopic studies showed that the presence of drugs did not cause any major changes in the secondary structure of HSA." nseedher@yahoo.com

http://www.arkat-usa.org/get-file/20046/ **Photoinduced modifications by fluoroquinolone drugs in bovine serum albumin (BSA) and ribonuclease A (RNAse) as model proteins**. *"Photosensitized protein oxidation by drugs, with the consequent modification of their structure is thought to be responsible for the occurrence of phototoxic phenomena such as photoallergy and loss of biological functions. In this paper we have investigated in detail the interaction of four fluoroquinolones namely ciprofloxacin, lomefloxacin, norfloxacin and ofloxacin with two proteins such as Bovine Serum Albumin (BSA) and Ribonuclease A (RNAse A) chosen as models . . . The obtained results showed that the four drugs are able to photooxidize proteins with the formation of protein-protein cross-link. This effect was also confirmed in isolated erythrocyte membranes. Furthermore the effect of the fluoroquinolones was also evaluated on isolated aromatic aminoacids. In this context the four drugs are able to photodamage in particular tyrosine and histidine. These results are important in the light of the growing interest for the comprehension of the mechanism of phototoxicity induced by these antibacterial drugs . . . The results presented in this paper clearly demonstrated that fluoroquinolones are able to bind protein and upon UVA irradiation to induce various chemical changes in these macromolecules. In particular they are able to photooxidize proteins and to induce protein-protein cross-links both in isolated proteins (RNAse) or in erythrocyte membranes. In this case the formation of intermolecular cross-link induces an increase of the rigidity of the plasmatic cellular membranes with a consequent alteration of the cellular functions. It is known that protein cross-link occurs prevalently between His and Lys residues or with the formation of dityrosine bridges. In this context we have demonstrated that the fluoroquinolones are able to photodamage some aminoacids in particular Tyr and His. An important aspect to clarify with further experiments is the photohaptenic property of fluoroquinolones by which is originated their photoallergenicity. The experiments carried out with LMX suggest the formation of a covalent adduct between the proteins and the drug. In conclusion proteins can be considered a major target at molecular level in the mechanism of action of fluoroquinolones."* E-mail: Francesco.dallacqua@unipd.it

http://onlinelibrary.wiley.com/doi/10.1002/bio.2494/abstract **Competitive binding of fluoroquinolone antibiotics and some other drugs to human serum albumin: a luminescence spectroscopic study**. *"Co-administration of several drugs in multidrug therapy may alter the binding of each to human serum albumin (HSA) and hence their pharmacological activity. Thirty-two frequently prescribed drug combinations, consisting of four fluoroquinolone antibiotics and eight competing drugs, have been studied using fluorescence and circular dichroism spectroscopic techniques. Competitive binding studies on the drug combinations are not available in the literature. In most cases, the presence of competing drug decreased the binding affinity of fluoroquinolone, resulting in an increase in the concentration of free pharmacologically active drug. The competitive binding mechanism involved could be interpreted in terms of the site specificity of the binding and competing drugs. For levofloxacin, the change in the binding affinity was small because in the presence of site II-specific competing drugs, levofloxacin mainly occupied site I. A competitive interference mechanism was operative for sparfloxacin, whereas competitive interference as well as site-to-site displacement of competing drugs was observed in the case of ciprofloxacin hydrochloride. For enrofloxacin, a different behavior was observed for different combinations; site-to-site displacement and conformational changes as well as independent binding has been observed for various drug combinations. Circular dichroism spectral studies showed that competitive binding did not cause any major structural changes in the HSA molecule."*

http://link.springer.com/article/10.1365/s10337-008-0814-0 **Characterization of Interactions Between Fluoroquinolones and Human Serum Albumin by CE–Frontal Analysis.** *"The percentages of protein binding (PB)*

for fluoroquinolones to HSA were between 8.6 and 22.2%, while the PB percentages for fluoroquinolones to human plasma were between 10.2 and 33.1%. It can be found that the PB percentages for fluoroquinolones to HSA are mostly lower than those for fluoroquinolones to human plasma. It suggests that HSA is the primary protein responsible for the binding of fluoroquinolones in human plasma. The thermodynamic parameters were obtained by CE–FA. The positive ΔH and ΔS values obtained by CE–FA showed that the binding reaction was an endothermic process, and the entropy drive the binding and hydrophobic interaction played major roles in the binding of fluoroquinolones to HSA."

http://link.springer.com/article/10.1007%2Fs10953-010-9527-8 **Investigation of the Interaction Between Ofloxacin and Bovine Serum Albumin: Spectroscopic Approach**

http://jac.oxfordjournals.org/content/61/3/561.full **Plasma protein binding of fluoroquinolones affects antimicrobial activity**

http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=55424&XNSPRACHE_ID=1&XNKONGRESS_ID=65&XNMASKEN_ID=900 **Plasma protein binding DOES influence bacterial killing by fluoroquinolones – investigation by use of an improved method**

http://pubs.rsc.org/en/Content/ArticleLanding/2013/NJ/c2nj40798a#!divAbstract **Zinc(II) complexes with the quinolone antibacterial drug flumequine: structure, DNA- and albumin-binding**

http://www.ncbi.nlm.nih.gov/pubmed/24029748 **Metal Complexes of Quinolone Antibiotics and Their Applications: An Update** (My note: nice paper for detail summaries of mechanisms).

https://www.jstage.jst.go.jp/article/analsci/22/12/22_12_1515/_article **Interaction between Fluoroquinolones and Bovine Serum Albumin Studied by Affinity Capillary Electrophoresis**

Need to Google this title: **A New Detection Technique for Fluoroquinolone-Conjugated Proteins by High Performance Liquid Chromatography with UV/Fluorescence Detectors**. "This study was aimed to develop a simple, fast, and reliable technique to detect fluoroquinolones (FQs) -conjugated bovine serum albumin (BSA). Four tested FQs, enrofloxacin, ofloxacin, danofloxacin, and orbifloxacin, were conjugated with BSA by following the N-hydroxysuccinimide ester method. The technique was designed according to the different absorption characteristics of the FQs and BSA; FQs can be detected by both UV and fluorescence detectors, but BSA can only be detected by UV. The results demonstrated that the developed method was efficient in detecting FQs-BSA conjugates. In addition, the method not only trace the FQs and BSA conjugation responses but also can be used to estimate the level of FQs-BSA conjugation. Therefore, this technique is a valuable tool for the detection drug-carrier-conjugated antigens, especially for FQs-BSA-conjugates during the production of anti-FQs monoclonal antibodies . . . Serum albumins are the major proteins in the circulatory system of animals and contribute to the osmotic blood pressure. They play a dominant role in drug disposition and efficacy. Many drugs can bind reversibly to albumins, which then function as carriers. Serum albumins often increase the apparent solubility of hydrophobic drugs in plasma and thus modulate their delivery to cells. Furthermore, the molecular weight (MW) of some drugs, like antibiotics, is too low to raise their antibodies. Therefore, for these low MW drugs, also called haptens, albumins can act as carrier proteins. When haptens are conjugated with carrier proteins, their MWs become larger and thus can stimulate the production of anti-hapten antibodies. It is therefore necessary to investigate the conjugation of FQs and bovine serum albumin (BSA) in order to gain a better understanding of the processes involved. Drug residues in animal tissues and milk pose a potential threat to human health. There is an obvious need for a rapid and accurate method to detect the residual drug in edible animal tissues in order to protect consumers' health. There are many techniques currently being employed to analyze drug residues . . . Therefore,

the purpose of this study was to estimate the extent of FQ-BSA conjugations by HPLC with UV/fluorescence detectors . . . FQs are extensively used in veterinary medicine, so it is very important to detect their residues in edible animal tissues due to public health concerns. However, currently no simple and reliable detection kits are available for residual FQs. Because FQs are haptens, they need to be conjugated with high MW carrier proteins to raise anti-FQs antibodies. Therefore, a detection method for monitoring the conjugation of FQs and BSA during the combination stage is needed . . . In this study, the conjugation of FQs with BSA was successfully established by N-hydroxysuccinimide ester method and analyzed by HPLC with both fluorescence and UV detectors. Compared with the method employed by Duan and Yuan, our technique was much easier and cheaper for detecting FQs-BSA conjugated proteins and can be used to estimate the degree of conjugation according to integrated areas in the chromatograms. The technique would be of value in the production of anti-FQs antibodies and the development of a commercial detection kit for FQs." (My note: this needs to be done studying FQ-HSA conjugates during the acute phase. Once target areas on HSA's are known, start looking for antibodies in affected patients against specific HSA locations – might be with cations, fatty acids, thyroid or other steroid hormones).

http://informahealthcare.com/doi/abs/10.1080/15376510309835?journalCode=txm **Photo-Induced Interaction of Antibacterial Quinolones With Human Serum Albumin**

http://core.ac.uk/download/pdf/140669.pdf **Labeless Immunosensor Assay for Fluoroquinolone Antibiotics based upon an AC Impedance Protocol.** The monitoring of fluoroquinolones within both food and the environment is important since these antibiotics have potential health and environmental damaging effects. Ciprofloxacin concentrations in hospital wastewaters were monitored and correlations with DNA damaging effects made (Hartmann et al 1999). Levels of ciprofloxacin in hospital outflow water between 0.7-124.5 ng ml-1 were measured using HPLC (Hartmann et al 1999) and shown to display genotoxicity at levels as low as 5.2 ng ml-1. Similar work (Batt et al 2006) measured wastewater ciprofloxacin using LC/MS/MS and found levels between 0.031-5.6 ng ml-1 (even after treatment) with a limit of detection of 0.030 ng ml-1. Levels in vivo have also been widely studied with the therapeutic ranges typically being between 0.57-2.30 Pg ml-1 in serum and 1.26-4.03 Pg g-1 in tissue (Licitra et al 1987) . . . A recent publication (Torriero et al 2006) details the use of a horseradish peroxidase based biosensor for the detection of ciprofloxacin due to its inhibition of the oxidation of catechol, however other piperazine based compounds could potentially interfere with this determination. Linear responses were obtained between 0.02-65 PM with the limit of detection being 0.4 nM. We have within this work developed a labeless immunosensor for ciprofloxacin as a typical fluoroquinolone. The sensor utilises screenprinted carbon electrodes, modified by deposition of first, a conducting polymer (polyaniline) which is then modified with biotinylating reagent. Complexion of the immobilised biotin with avidin allows the further binding of biotinylated antibodies via standard avidin-biotin interactions (Figure 2). The resultant electrodes are capable of detecting low levels of the antigen - ciprofloxacin. Control (My note: using horse radish peroxidase as a biosensor for Cipro – hr peroxidase shows cross reactivity with TPO, MPO, LPO, possibly others. Also note that biotin and avidin are often part of the hypersensitivity rxn's flox victims experience).

http://www.nature.com/jid/journal/v117/n5/full/5601271a.html **Quinolone-Photoconjugated Major Histocompatibility Complex Class II-Binding Peptides with Lysine are Antigenic for T Cells Mediating Murine Quinolone Photoallergy.** *"We have investigated the mechanisms of FQ photoallergy in mice. The photoallergenicity of FQ is mainly derived from their photohaptenic moiety. FQ are covalently coupled to protein by UVA exposure (Tokura et al, 1996b). Because of this ability, epidermal cells are photoderivatized with FQ upon UVA irradiation, thereby initiating immunologic consequences (Tokura et al, 1996b,1998). Among epidermal cells, the Langerhans cells are critical, which are photomodified with a systemically given FQ upon UVA irradiation of the skin and become photohapten-bearing, T cell stimulatory cells (Tokura et al, 1998;Ohshima et al, 2000). Murine FQ photosensitivity involves the activation of major histocompatibility (MHC) class II-restricted, quinolone-*

photoadduct-specific, CD4+ Th1 cells expressing T cell receptor Vbeta13 (Tokura et al, 1998). There exists broad cross-reactivity among FQ, suggesting that FQ carry the same haptenic epitope recognized by Vbeta13+ T cells. The presence of T cells that react with FQ-photomodified syngeneic cells is also proven in patients with FQ photosensitivity. There are some relationships between the chemical structure of FQ and photoallegenicity. The piperazinyl (or methylpiperazinyl) group, the major side chain of FQ linked at C7, is altered by UVA irradiation (Yoshida and Moroi, 1993;Tiefenbacher et al, 1994), and thus protein may be covalently bound to the piperazinyl ring during its photodegradation to form an allergic FQ–protein complex (Tokura, 1998) . . . Here, using ofloxacin (OFLX) as a representative FQ (Schiefe et al, 1993;Ronnau et al, 1997), we demonstrate that FQ attaches preferentially to lysine in the peptides, and photoadducts between FQ and MHC-binding peptides are responsible for the induction and elicitation of murine FQ photoallergy . . . OFLX and BSA were mixed in a solution at a molar ratio of 40:1 and irradiated with UVA at 2 J per cm2. An amino acid composition analysis of BSA photoreacted with OFLX revealed that the amount of lysine was decreased by 33% after photoreaction compared with untreated BSA Figure 2. No substantial change was found in the other amino acids examined, except for methionine, which was decreased by 12%. This suggested that OFLX preferentially photobinds to lysine, leading to alteration of the structure of lysine and the resultant reduction of its amount . . . This study demonstrated that OFLX photobinds preferentially, but not exclusively, to lysine. Because of the presence of two amino residues, lysine gives a free amino residue when situated in peptides. Providing that OFLX is photoreacted with free amino groups to form covalent photoadducts, both the amino residues of lysine and N-terminus in peptides may be the binding sites. It is thus assumed that H6KH5 and H12 carry two and one photocoupling sites, respectively, for OFLX, which is consistent with the result obtained from our ST-Q-9 affinity chromatographic analysis. Lysine has also been reported to be the major amino acid bound to haptenic drug penicillin (Weltzien and Padovan, 1998) and ordinary hapten trinitrophenyl (Ortmann et al, 1992) . . . We, nevertheless, found that the lysine-containing motif with affinity to I-Ad, as represented by OVA9(K+), evoked the T cell proliferative responses when presented by Langerhans cells, whereas class II-unmatched or lysine-lacking peptide did not. It is unlikely that OFLX-photoderivatized proteins in the photomodified murine epidermal cells used as immunogen share peptide sequences with the synthetic peptides derived from species other than mice, implying that the peptide is not substantially involved in the antigenic epitope . . . This study suggests that OFLX-peptide photodeterminants formed by virtue of a photoreactive role of lysine are responsible for the induction and elicitation of OFLX photoallergy. In clinical photoallergy to FQ, we can assume that the drug photobinds to self peptides on MHC class II molecules of Langerhans cells, thereby sensitizing and restimulating T cells. The development of FQ less photoaffinitive to peptide/protein may avoid the photosensitive adverse effect of these widely used drugs."

http://cmr.asm.org/content/23/4/858.full **Penetration of Drugs through the Blood-Cerebrospinal Fluid/Blood-Brain Barrier for Treatment of Central Nervous System Infections** Fluoroquinolones: Most fluoroquinolones are moderately lipophilic drugs with a molecular mass of around 300 Da and low binding to plasma proteins (approximately 20 to 40%) (21). Around the physiological pH, most agents are uncharged, which favors their CNS penetration. Based on the AUCCSF/AUCS ratio, their penetration into the CSF in the absence of meningeal inflammation is much higher than that of β-lactam antibiotics (see Table S1.1 in the supplemental material). Fluoroquinolones are of great value for the treatment of CNS infections by Gram-negative aerobic bacilli (ciprofloxacin) and by Mycobacterium tuberculosis (moxifloxacin) (4, 5). The activity of most fluoroquinolones is too low to treat Streptococcus pneumoniae meningitis (176). Because of their relatively high CNS toxicity, a strong increase of their systemic dose as with β-lactam antibiotics is not feasible. Since several fluoroquinolones enter the CNS readily, intrathecal injections of fluoroquinolones are unnecessary.

http://www.ncbi.nlm.nih.gov/pubmed/23529676 **Quinolones and non-steroidal anti-inflammatory drugs interacting with copper(II), nickel(II), cobalt(II) and zinc(II): structural features, biological evaluation and perspectives.**

http://www.hindawi.com/journals/ijac/2012/408057/ **Interaction of Avelox with Bovine Serum Albumin and Effect of the Coexistent Drugs on the Reaction**. "Most drugs are able to bind to plasma protein when they entrance in blood plasma system of organism, and serum albumin is the most abundant protein in blood plasma and serves as a depot protein and transport protein for numerous endogenous and exogenous compounds [1]. Generally speaking, drugs could bind with serum albumin mostly through the formation of noncovalent complexes reversibly. The drug-protein complex can be regarded as a form of drug in the biology temporary storage, it can effectively avoid drug elimination from metabolism so quickly that it can maintain the total concentration and effective concentrations of blood medicine in plasma. In addition, binding of drugs to plasma proteins controls their free, active concentrations and provides a reservoir for a longer action, the binding of drugs is responsible for the protective role of albumin. Therefore, interaction of a drug with, and competition for, the binding sites on plasma proteins might strongly affect its distribution, elimination, as well as its pharmacodynamics and toxic properties [2]. Competition between two drugs for their binding to plasma protein can strongly affect the drug disposition of both drugs, with possible serious physiological consequences. Binding parameters are indeed fundamental factors in determining the overall pharmacological activity of a drug, and in this context the determination of the binding parameters of drugs to albumin has become essential to understand their pharmacokinetic, pharmacodynamic, and toxicological profile . . . 3.4. Identification of the Binding Sites of Avelox on the BSA: Participation of tyrosine (Tyr) and tryptophan (Trp) groups in drug-serum albumin complexes is assessed using different excitation wavelengths. At 280 nm wavelength, the Trp and Tyr residues in serum albumin are excited, whereas the 295 nm wavelength excites only Trp residues. In the subdomains of BSA, IIA subunit (Trp and Tyr) and IIIA (Tyr) are also considered as the main binding sites for small-drug molecule [15]. Based on the Stern-Volmer equation, comparing the fluorescence quenching of protein excited at 280 nm and 295 nm allows to estimate the participation of Trp and Tyr groups in the complex [16]. As seen in Figure 4, the quenching curves of BSA excited at 280 nm and 295 nm in the presence of Avelox overlap below the molar ratio Avelox : BSA = 80 : 1. This means that if there are fewer than 80 Avelox molecules for 1 BSA molecules, only Trp residues take part in the interaction of Avelox with BSA, and protein fluorescence was quenched by almost 80% at the molar ratio 80 : 1. The quenching of BSA fluorescence exited at 280 nm above this molar ratio was slightly higher than that excited at 295 nm which means that Tyr residues began to take part in the interaction. According to this conclusion, it could be inferred that Avelox molecules only take interaction with Trp residues of BSA at low concentration, whereas both Trp and Tyr residues at high concentration, this conclusion can also expound why the value of is slightly more than 1, which has been shown in Table 1 . . . BSA has two Trp moieties (Trp-134 and Trp-212) located in subdomains IB and IIA, respectively, and only Trp-212 is located within a hydrophobic binding pocket of the protein which usually bind many small ligands, especially heterocyclic ligands of average size and small aromatic carboxylic acids [17]. In this way, it could confirm that the primary binding site for Avelox was located in subdomain IIA of BSA . . . For the synchronous fluorescence spectra of protein, when the value between the excitation and emission wavelengths is stabilized at either 15 or 60 nm, the synchronous fluorescence gives characteristic information for Tyr residues or Trp residues [26]. Because of the red shifts of maximum emission wavelengths of both Tyr and Trp with the less hydrophobic environment, blue shifts of maximun emission wavelengths with the more hydrophobic environment ocurr. These red or blue shifts indicated that the conformation of BSA has been changed

http://nar.oxfordjournals.org/content/39/11/4808.abstract **Use of divalent metal ions in the DNA cleavage reaction of topoisomerase IV**. (Osheroff paper). " It has long been known that type II topoisomerases require divalent metal ions in order to cleave DNA. Kinetic, mutagenesis and structural studies indicate that the eukaryotic

enzymes utilize a novel variant of the canonical two-metal-ion mechanism to promote DNA scission. However, the role of metal ions in the cleavage reaction mediated by bacterial type II enzymes has been controversial.

http://mt.china-papers.com/2/?p=229214 **Studies on the Antagonistic Action Between Fluoroquinolones and Chloramphenicol by Spectroscopy**

http://www.pharmazie.uni-wuerzburg.de/PharmChem/AKHg/Papers/science2.pdf **Protein binding in a congeneric series of antibacterial quinolone derivatives**

Albumin

http://www.sciencedirect.com/science/article/pii/S0161589012002842 **Structural and immunologic characterization of bovine, horse, and rabbit serum albumins.** *"Serum albumin (SA) is the most abundant plasma protein in mammals. SA is a multifunctional protein with extraordinary ligand binding capacity, making it a transporter molecule for a diverse range of metabolites, drugs, nutrients, metals and other molecules. Due to its ligand binding properties, albumins have wide clinical, pharmaceutical, and biochemical applications. Albumins are also allergenic, and exhibit a high degree of cross-reactivity due to significant sequence and structure similarity of SAs from different organisms. Here we present crystal structures of albumins from cattle (BSA), horse (ESA) and rabbit (RSA) sera. The structural data are correlated with the results of immunological studies of SAs. We also analyze the conservation or divergence of structures and sequences of SAs in the context of their potential allergenicity and cross-reactivity. In addition, we identified a previously uncharacterized ligand binding site in the structure of RSA, and calcium binding sites in the structure of BSA, which is the first serum albumin structure to contain metal ions.* maks@iwonka.med.virginia.edu, wladek@iwonka.med.virginia.edu

http://sbkb.org/update/research/serum-albumin-diversity **Serum albumin diversity**

http://www.pubfacts.com/detail/2011121/Acetylsalicylate-human-serum-albumin-interaction-as-studied-by-NMR-spectroscopy--antigenicity-produc **Acetylsalicylate-human serum albumin interaction as studied by NMR spectroscopy--antigenicity-producing mechanism of acetylsalicylic acid.**

Google book: **The Plasma Proteins:** *Albumin roles: metabolism of fats, fatty acids. Also acts as a buffer pool to stabilize the plasma concentrations of Ca, tryptophan, and hormones, including cortisol, testosterone, and estrogens. Tryptophan binding varies inversely with the load of fatty acids. This causes the concentration of free tryptophan to rise when fatty acid levels rise and may have important consequences in the levels of tryptophan in the brain and the production of serotonin. . . .glutathione from the gut may also travel with albumin . . . metabolism of albumin also serves as important source of amino acids when these are low . . . Albumin is synthesize by the liver, and maybe in trace amounts by the thyroid gland.*

http://peds.oxfordjournals.org/content/24/11/845.full **Exploiting bias in a non-immune human antibody library to predict antigenicity.** *Biologics that induce an adaptive immune response can cause significant toxicities, including inflammation, anaphylaxis, complement-mediated cell lysis and result in altered pharmacokinetics leading to both limited efficacy and toxicity (Schellekens, 2002; Subramanyam, 2006). While 'foreign-ness' is a primary factor in immunogenicity (van den Berg and Rand, 2004; Kanduc, 2008), the numerous cases in which even completely human proteins have led to immune stimulation (Peces et al., 1996; Zang et al., 2000) indicate that immunogenicity is a complex phenomenon with many contributing factors. With the introduction of new therapies consisting of novel recombinant and completely non-human proteins, immunogenicity, i.e. the tendency of a*

protein drug to be recognized as foreign by the immune system, has become a significant concern in the drug development, approval and monitoring process (Shankar et al., 2006; Ponce et al., 2009). Accordingly, strategies to reduce immunogenicity have been pursued with great interest . . . . As both arms of the adaptive immune system function in determining the immunogenicity of a therapeutic protein, significant effort has been devoted to developing tools for evaluating both B and T cell immunogenicity. Although there is sometimes significant overlap between the epitopes recognized by both T and B cells, in most cases the reactive epitopes are completely distinct (Brons et al., 1996; Rosenberg and Atassi, 1997), and must therefore be addressed independently. Similarly, while T cells contribute significantly to the production of a strong B cell response, anti-drug antibodies are primarily responsible for the altered pharmacokinetics and complications resulting from administering an immunogenic drug (Ponce et al., 2009). Therefore, both classes of epitopes are significant and the most complete means to assess clinical immunogenicity would involve the study of both. E-mail: Margaret.E.Ackerman@Dartmouth.edu. I have the full paper, look up her lab.

http://jb.oxfordjournals.org/content/147/2/191 **Multiple conformational state of human serum albumin around single tryptophan residue at various pH revealed by time-resolved fluorescence spectroscopy.** *Human serum albumin (HSA) plays important roles in transport of fatty acids and binding a variety of drugs and organic compounds in the circulatory system. This protein experiences several conformational transitions by the change of pH, and the resulting conformations were essential for completing the physiological roles in vivo*

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2755717/ **Structure, Properties, and Engineering of the Major Zinc Binding Site on Human Albumin.** *Zinc is not only required for hundreds of essential extra- and intracellular proteins and enzymes but is also recruited by toxins such as anthrax lethal factor (1) and staphylococcal enterotoxin (2). There is a need to understand how zinc transport and distribution is controlled (3). Although considerable progress has been made in the identification and study of membrane-bound zinc transporters, the molecular mechanism of extracellular zinc transport is still obscure. The total concentration of zinc in blood is high, ~15–20 μm (4), and plasma zinc concentrations are maintained at a relatively constant level, except during periods of dietary zinc depletion and acute responses to stress or inflammation, when they are depressed (5). In humans, ~98% of so-called "exchangeable" zinc in blood plasma (9–14 μm) is bound to serum albumin (6). Studies on perfused rat intestine have implicated albumin in the transport of newly absorbed zinc in portal blood, from the intestine to the liver (5). Albumin has also been shown to promote zinc uptake by endothelial cells, with receptor-mediated endocytosis as the most likely mechanism (7).*
http://www2.warwick.ac.uk/fac/sci/chemistry/research/blindauer/blindauergroup/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2781501/ **Ibuprofen Impairs Allosterically Peroxynitrite Isomerization by Ferric Human Serum Heme-Albumin.** *Human serum albumin (HSA),[3] the most abundant protein in plasma (reaching a blood concentration of about 7.0 × 10^{-4} M), is characterized by an extraordinary ligand binding capacity, providing a depot and carrier for many compounds. HSA affects the pharmacokinetics of many drugs; holds some ligands in a strained orientation, providing their metabolic modification; renders potential toxins harmless, transporting them to disposal sites; accounts for most of the antioxidant capacity of human serum; and displays (pseudo)enzymatic properties (1,–14).*

http://onlinelibrary.wiley.com/doi/10.1111/j.1423-0410.2002.tb05326.x/abstract;jsessionid=54E0A77DA3F1135E734539BF53753BDA.f03t01 "As a plasma transporter molecule, HSA displays truly bewildering binding . His lab: http://www.bio.ph.ic.ac.uk/~scurry/Research.html#HSA unfortunately, he's not really studying HSA and drug binding anymore

http://www.biochemj.org/bj/423/bj4230023.htm **Structural basis of transport of lysophospholipids by human serum albumin**. *Lysophospholipids play important roles in cellular signal transduction and are implicated in many biological processes, including tumorigenesis, angiogenesis, immunity, atherosclerosis, arteriosclerosis, cancer and neuronal survival. The intracellular transport of lysophospholipids is through FA (fatty acid)-binding protein. Lysophospholipids are also found in the extracellular space. However, the transport mechanism of lysophospholipids in the extracellular space is unknown. HSA (human serum albumin) is the most abundant carrier protein in blood plasma and plays an important role in determining the absorption, distribution, metabolism and excretion of drugs.*

http://www.febsletters.org/article/S0014-5793(08)00396-7/abstract **The antioxidant properties of serum albumin**

http://www.translationalres.com/article/S1931-5244(09)00300-4/abstract **Albumin Benkovac (c.1175 A > G; p.Glu392Gly): a novel genetic variant of human serum albumin**

http://onlinelibrary.wiley.com/doi/10.1002/bip.21314/abstract **Exploration of human serum albumin binding sites by docking and molecular dynamics flexible ligand–protein interactions.** *These results serve to explain the high ligand-promiscuity of HSA*

http://www.ncbi.nlm.nih.gov/pubmed/23602811 **Novel insights into the pleiotropic effects of human serum albumin in health and disease.** chungeun@hawaii.edu

http://www.ncbi.nlm.nih.gov/pubmed/9188698 **Mutagenesis studies of thyroxine binding to human serum albumin define an important structural characteristic of subdomain 2A**.

http://www.ncbi.nlm.nih.gov/pubmed/8702585 **Mutations in a specific human serum albumin thyroxine binding site define the structural basis of familial dysalbuminemic hyperthyroxinemia.**

http://www.ncbi.nlm.nih.gov/pubmed/10430791 **Structural investigations of a new familial dysalbuminemic hyperthyroxinemia genotype.**

http://www.ncbi.nlm.nih.gov/pubmed/7937417 **Elevated thyroxine levels in a euthyroid patient. A search for the cause of euthyroid hyperthyroxinemia.** *"A clinically euthyroid man with a family history of hyperthyroidism presented for evaluation of an elevated thyroxine (T4) level and an increased free T4 index with a normal thyrotropin (TSH) level. Results of thyroid hormone-binding protein tests confirmed the diagnosis of familial dysalbuminemic hyperthyroxinemia. This disorder should be considered in patients who have a normal serum TSH level, despite an elevated total T4 concentration. Accurate diagnosis is essential to avoid inappropriate treatment. Affected family members also should be identified. No treatment is required, because patients remain euthyroid and maintain a normal free T4 level."* (My note: Consider for all the patients on NDT, and even synthetics, who require elevated medication doses to feel normal)

http://www.ncbi.nlm.nih.gov/pubmed/1907423 **Evaluating thyroid gland function in patients with protein anomalies.** *"The euthyroid hyperthyroxinemia (EHT) is characterized on the one hand by a normal basal THS or TRH-TSH response but also by high plasma values of total thyroxine (TT4) on the other. However if only TT4 is assessed, "hyperthyroidism" may be diagnosed erroneously. EHT may be caused by an increase of specific thyroxine binding proteins which may be hereditary (permanent) or acquired (transient). The most frequent disturbance is due to an estrogen induced increase of thyroxine binding globulin (TBG) in the course of pregnancy, anticonceptive drugs or estrogen treatment. The albumin associated HT (FDH syndrome), first reported in 1979, has autosomal*

dominant traits. 144 patients with FDH syndrome were observed during the period between 1984 and 1990, i.e. 7% (1986) of all hyperthyroid patients explored. Family screening is required to prevent unjustified treatment. Additionally existing disturbances of thyroid function as well as other protein binding anomalies may both cause problems in differential diagnosis. Prealbumin associated hyperthyroxinemia (PAAH), first published in 1982, may be due to an inherited increase in affinity, but may also be the consequence of a true elevation of prealbumin plasma concentration in the course of an islet cell cancer; both conditions are extremely rare. ***Nearly as rare are patients with plasma autoantibodies directed against T4 and/or T3 (5 cases); yet, a reverse T3 autoantibody could be observed in merely 1 case.*** *By means of our modified radio-thyroxine-agarosegel-iceelectrophoresis all such protein anomalies may be diagnosed and differentiated in 1 procedure. Moreover, all other types of EHT must be taken into consideration by differential diagnosis."*

http://www.ncbi.nlm.nih.gov/pubmed/8767512 **Diagnosis of thyroid hormone transport protein anomalies: an overview**. "Inherited or acquired variations in the serum concentrations of TBG, TTR or albumin are rather common. Clinical studies have shown that most patients with transport protein variations were referred for thyroid testing because of incidentally detected "unusual" thyroid function tests."

http://www.ncbi.nlm.nih.gov/pubmed/23811338 **Binding of transition metal ions to albumin: sites, affinities and rates.**

http://journals.sbmu.ac.ir/jps/article/viewFile/5021/4424 **Drug comparison and categorizing regarded with human serum albumin from years 2006 to 2012** *" Human Serum Albumin (HSA), an important globular protein, is considered as carrier, responsible for blood osmotic pressure and several other important functions and also has three high affinity specific binding sites (I, II and III) for drugs. X-ray crystallography analyses have revealed that the drug binding sites are located in sub domains IIA and IIIA [1,2] . . . The drug-protein binding affinity is an important factor for regulation of drug concentration in blood. So, this binding property can change the activity and half life of the drug in the body [5]. HSA involves in distribution, transportation and metabolism of a wide variety of ligands including different metabolites and pharmaceutical reagents and also maintenance of the blood osmotic pressure [6]. Due to its importance and characteristics, researchers usually use HSA as a protein model for study the complexes of proteins and drugs [7]. HSA binds to many poorly water soluble drugs and carry them through the blood stream [8, 9]. Following this binding, metabolism distribution, free concentration and elimination rate of various drugs can be significantly regulated."*

Evolution

The characteristic 3-domain structure of albumin and alpha-fetoprotein has been conserved throughout mammalian evolution. Thus, 35.2% amino acid homology is found between bovine serum albumin and murine AFP. Ohno (1981) addressed the vexing question of why this conservation occurs despite the nonessential nature of serum albumin as indicated by cases of analbuminemia. Minghetti et al. (1985) found a high rate of both silent substitutions and effective substitutions with amino acid changes in serum albumin. Although the rates of effective substitution in amino acid changes were not as high in albumin as in alpha-fetoprotein, they were still faster than those of either hemoglobin or cytochrome c. This high evolutionary change rate for albumin may be consistent with the fact that inherited

analbuminemia produces surprisingly few symptoms despite the virtually complete absence of albumin.

Enzymatic properties of albumin

The most pronounced enzymatic activities of albumin are different types of hydrolysis. Key examples are esterase-like activities involving 411Tyr or 199Lys and the thioesterase activity of 34Cys. In the first case, hydrolysis involves water and both products are released, whereas in the latter cases one of the products is set free, and the other stays covalently bound to the protein. However, the modified 34Cys can be converted back to its reduced form by another compound/enzymatic system. Among the other activities are glucuronidase, phosphatase and amidase as well as isomerase and dehydration properties. Albumin has great impact on the metabolism of, for example, eicosanoids and xenobiotics. For a review, see **Kragh-Hansen, U.** (2013) Molecular and practical aspects of the enzymatic properties of human serum albumin and of albumin-ligand complexes. Biochim. Biophys. Acta 1830, 5535-5544.

Protection of a cytokine

Albumin can stabilize different endogenous compounds. For example, it stabilizes a hydrophobic, recombinant cytokine [**Hawe, A. & Friess, W.** (2007) Stabilization of a hydrophobic recombinant cytokine by human serum albumin. J. Pharm. Sci. 96, 2987-2999].

Nerve agents phosphylate a tyrosine residue in albumin

The organophosphorus nerve agents sarin, soman, cyclosarin and tabun phosphylate a tyrosine residue on albumin in human blood. These adducts may offer relatively long-lived biological markers of nerve agent exposure [**Williams, N.H., Harrison, J.M., Read, R.W. & Black, R.M.** (2007) Phosphylated tyrosine in albumin as a biomarker of exposure to organophosphorus nerve agents. Arch.Toxicol, 81, 627-639].

Albumin interaction with cell membrane receptors

Glycoprotein 60 (gp60), also called albondin, is a plasma membrane component of most continuous endothelia responsible for binding albumin and for its internalization and subsequent transcytosis. Cubilin and megalin, and complexes thereof, can internalize albumin in, for example, the proximal tubules of the kidneys. Gp18 and gp30 are found on a variety of cells, and these scavenger receptors bind conformationally-modified albumin. For a review, see **Merlot, A.M., Kalinowski, D.S. & Richardson, D.R.** (2014) Unraveling the mysteries of serum albumin – more than just a serum albumin. Front. Physiol. 5, Article 299. Finally, the FcRn receptor interacts with albumin in a pH-dependent manner, see above.

Albumin degradation in the cytosol - the N-end rule

The very small amounts of albumin found in the cytosol, probably arising by leakage from cell organelles, are apparently degraded along with other soluble proteins via the "N-end rule". An arginine residue is first attached to an N-terminal aspartic or glutamic acid, leading to multiple attachments of ubiquitin and then degradation in a proteasome [**Varshavsky, A.** (1996) The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. USA 93, 12142-12149; **Graciet, E., Hu, R.G., Piatkov, K., Rhee, J.H., Schwarz, E.M. & Varshavsky, A**. (2006) Aminoacyl-transferases and the N-end rule pathway of prokaryotic/eukaryotic specificity in a human pathogen. Proc. Natl. Acad. Sci. USA 103, 3078-3083]. The binding of arginine to the aspartic acid of bovine albumin by arginine-tRNA-protein transferase was first noted in 1963 (**Kaji, A., Kaji, H., & Novelli, G. D**. A soluble amino acid incorporating system from rat liver. Biochim. Biophys. Acta 76, 474-477) and studied extensively by **Soffer, R.L**. in 1973 (Post-translational modification of proteins catalyzed by aminoacyl-tRNA-protein transferases. Mol. Cell. Biochem. 2, 3-14).

Biochim Biophys Acta. 2013 Dec;1830(12):5535-44. doi: 10.1016/j.bbagen.2013.03.015. Epub 2013 Mar 23.

Molecular and practical aspects of the enzymatic properties of human serum albumin and of albumin-ligand complexes.

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Abstract

BACKGROUND:

Human serum albumin and some of its ligand complexes possess enzymatic properties which are useful both in vivo and in vitro.

SCOPE OF REVIEW:

This review summarizes present knowledge about molecular aspects, practical applications and potentials of these properties.

MAJOR CONCLUSIONS:

The most pronounced activities of the protein are different types of hydrolysis. Key examples are esterase-like activities involving Tyr411 or Lys199 and the thioesterase activity of Cys34. In the first case,

hydrolysis involves water and both products are released, whereas in the latter cases one of the products is set free, and the other stays covalently bound to the protein. However, the modified Cys34 can be converted back to its reduced form by another compound/enzymatic system. Among the other activities are glucuronidase, phosphatase and amidase as well as isomerase and dehydration properties. The protein has great impact on the metabolism of, for example, eicosanoids and xenobiotics. Albumin with a metal ion-containing complex is capable of facilitating reactions involving reactive oxygen and nitrogen species.

GENERAL SIGNIFICANCE:

Albumin is useful in detoxification reactions, for activating prodrugs, and for binding and activating drug conjugates. The protein can be used to construct smart nanotubes with enzymatic properties useful for biomedical applications. Binding of organic compounds with a metal ion often results in metalloenzymes or can be used for nanoparticle formation. Because any compound acting as cofactor and/or the protein can be modified, enzymes can be constructed which are not naturally found and therefore can increase, often stereospecifically, the number of catalytic reactions. This article is part of a Special Issue entitled Serum Albumin.

Arch Toxicol. 2007 Sep;81(9):627-39. Epub 2007 Mar 8.

Phosphylated tyrosine in albumin as a biomarker of exposure to organophosphorus nerve agents.

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Abstract

The organophosphorus nerve agents sarin, soman, cyclosarin and tabun phosphylate a tyrosine residue on albumin in human blood. These adducts may offer relatively long-lived biological markers of nerve agent exposure that do not 'age' rapidly, and which are not degraded by therapy with oximes. Sensitive methods for the detection of these adducts have been developed using liquid chromatography-tandem mass spectrometry. Adducts of all four nerve agents were detected in the blood of exposed guinea pigs being used in studies to improve medical countermeasures. The tyrosine adducts with soman and tabun were detected in guinea pigs receiving therapy 7 days following subcutaneous administration of five times the LD(50) dose of the respective nerve agent. VX also forms a tyrosine adduct in human blood in vitro but only at high concentrations.