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ABSTRACT

The in vivo partitioning behavior of ionogenic organic chemicals (IOCs) is of paramount importance for their toxicokinetics and bioaccumulation. Among other proteins, structural proteins including muscle proteins could be an important sorption phase for IOCs, because of their high quantity in the human and other animals' body and their polar nature. Binding data for IOCs to structural proteins are, however, severely limited. Therefore, in this study muscle

protein-water partition coefficients ($K_{MP/w}$) of 51 systematically selected organic anions and cations were determined experimentally. A comparison of the measured $K_{MP/w}$ with bovine serum albumin (BSA)-water partition coefficients showed that anionic chemicals sorb stronger to BSA than to muscle protein (up to 3.5 orders of magnitude), while cations sorb similarly to both proteins. Sorption isotherms of selected IOCs to muscle protein are linear (i.e., $K_{MP/w}$ is concentration independent) and $K_{MP/w}$ is only marginally influenced by pH value and salt concentration. Using the obtained dataset of $K_{MP/w}$ a polyparameter linear free energy relationship (PP-LFER) model was established. The derived equation fits the data well (R^2=0.89, RMSE=0.29). Finally, it was demonstrated that the in vitro measured $K_{MP/w}$ of this study have the potential to be used to evaluate tissue-plasma partitioning of IOCs in vivo.

Introduction

Assessing the toxicity and the bioaccumulation potential of chemicals in the environment requires reliable information on their partitioning into biological compartments such as lipids and proteins. In the past, environmental scientists mainly focused on neutral organic chemicals and predictive tools with different levels of complexity have been developed for these chemicals. In simple models it is often assumed that the predominant sorption phase is the lipid fraction of an organism and that octanol is an appropriate surrogate for lipids. Models with more mechanistic insight explicitly consider different types of lipids and proteins as sorbing phases[1-3] and/or use more sophisticated approaches to predict relevant partition coefficients. For example polyparameter linear free energy relationships (PP-LFERs)[4, 5] have been established for different biological phases including storage lipid,[6] phospholipids,[7] and proteins.[8, 9]

Although there is a need for predictive models that are capable of predicting the toxicity and the bioaccumulation potential of ionogenic organic chemicals (IOCs) as well, only a few attempts have been made so far to include IOCs in environmental partitioning models.[10] For a better understanding of bio-partitioning of IOCs, the relevant sorption phases for organic ions have to be identified. In contrast to neutral chemicals, IOCs are not expected to primarily partition to storage lipid, but rather to polar lipids (phospholipid membranes) and proteins.

At least two classes of protein may serve as significant sorption phases for IOCs in vivo. First, serum albumin and other serum proteins: Serum albumin is well-known for its ability to bind a broad range of chemicals, especially organic anions, reducing their freely dissolved fraction in plasma and increasing the sorption capacity of the plasma.[11, 12] Additionally, for cationic and neutral chemicals a significant contribution of another plasma protein (α1-acid glycoprotein) to the sorption capacity of the plasma has also been described.[13] In our previous study with bovine serum albumin (BSA), we measured serum albumin binding for a set of systematically selected organic anions and cations, in order to reveal the mechanisms that underlie the partitioning.[14] We pointed out the influence of the molecular structure, especially the three-dimensional (3D) shape, on the sorption of organic ions to BSA and discussed the requirements for successful modeling of serum albumin binding of IOCs.[14]

The second class of proteins that could be an important sorption phase for IOCs are structural proteins. Structural proteins make up the majority of the proteins in the human body, with a fraction of 10% by mass of the whole body. About half of the structural proteins (4% of the whole body mass) are muscle proteins (e.g., actin and myosin). The other half (6% of the whole body mass) include keratin and collagen.[15, 16] Generally, structural proteins are not expected to be a target site for toxic actions, but similar to serum albumin, sorption to structural protein can

62 be substantial and affect the transport, distribution, and freely dissolved concentration of
63 chemicals in the body. There are studies that focused on the sorption of organic chemicals to
64 different structural proteins (i.e., gelatin, muscle protein, collagen, and keratin), [9, 17-19] but their
65 targets were mainly neutral organic chemicals. Only a few studies have included some binding
66 data for IOCs as well.[18, 19]

67 Because for many chemicals partitioning data for proteins other than serum albumin are not
68 readily available, partitioning models often consider the bulk protein fraction of an organism as
69 one sorption phase, using serum albumin as a representative protein for all proteins.[2] Endo et al.
70 have shown in their study that for neutral chemicals, sorption to muscle protein and serum
71 albumin is profoundly different.[9] This difference is reasonable, because sorption to serum
72 albumin generally happens at specific binding sites,[9, 11, 20] while this is presumably not the case
73 for structural proteins. A further study demonstrated that structural proteins can be a more
74 important sorption phase than lipids for H-bond donor neutral chemicals in lean tissues.[1] Such
75 general conclusions could not be made for IOCs so far, because data for binding of IOCs to
76 structural proteins are rare.

77 As an extension of our previous work with serum albumin,[14] the aim of this study was to
78 investigate how the molecular structure of IOCs influences their sorption to structural protein
79 and to elucidate the sorption mechanism. For this purpose, muscle protein isolated from chicken
80 breast filet was used as a model protein and muscle protein-water partition coefficients ($K_{MP/w}$)
81 were determined for organic anions and cations with diverse molecular structure (e.g., with
82 different charged functional groups and basic structure) using an equilibrium dialysis method.
83 Because partitioning processes of ions are potentially influenced by pH value and salt
84 concentration, the dependence of log $K_{MP/w}$ on pH value and the concentration of other inorganic

(phosphate and K^+) and organic ions (lactate) were investigated for selected test chemicals. Furthermore, sorption isotherms were measured and the reversibility of binding to muscle protein was checked. Using the obtained dataset for muscle protein binding of IOCs, polyparameter linear free energy relationships (PP-LFERs) were tested for their suitability as a predictive tool. The measured log $K_{MP/w}$ values were also compared with serum albumin-water partition coefficients from our previous study. Finally, we evaluated how far partitioning to serum albumin and structural protein can explain muscle tissue-plasma partition coefficients that were measured in vivo.

Materials and methods

Materials

Muscle proteins were isolated from chicken breast filet as described by Endo et al.[9] (for details on protein preparation and lipid extraction, see the supporting information (SI)). The protein content of typical muscle-meat is approximately 19% according to ref 21. Because the amount of muscle protein required for the experiments was larger than originally expected, two lots of protein had to be prepared. The two lots sometimes exhibited slightly different $K_{MP/w}$ by up to 0.2 log units, but without any clear trend. That is, three out of seven chemicals for which a comparison is possible showed lot 2 > lot 1 and the remaining four chemicals lot 1 > lot 2. Thus, we assumed that the lot difference in terms of sorption properties is negligible and, therefore, combined data measured with the two lots. Table S1 in SI shows which lot was used for each experiment. The water used for the experiments was purified with a Milli-Q Gradient A10 system from Millipore. Methanol (Suprasolv) and acetonitrile (gradient grade) were acquired from Merck and Sigma Aldrich, respectively. Lactic acid, NaN_3, KH_2PO_4 and $K_2HPO_4 \cdot 3H_2O$ were purchased from Merck or Sigma Aldrich. The test chemicals chosen for the experiments

with muscle protein were various organic anions and cations. In particular, differently substituted benzoic acids, naphthoic acids, and sulfonates were chosen to elucidate the influence of the molecular structure on the sorption to muscle protein. Several pesticides and pharmaceuticals were added to the dataset, because of their environmental relevance. We especially considered the chemicals from our previous work with BSA[14] to ensure a good overlap of the datasets for the two proteins. All test chemicals measured in this study have only one ionizable functional group and are predominantly present as their ionic species at pH 7.0 (>97%). Note that the term "ionogenic organic chemical" (IOC) could refer to weak acids and bases that are primarily neutral as well, but within this study IOCs only mean chemicals that are mainly present as their ionic species in an aqueous phase at physiological pH values. Although all data reported here are for ionic species, the chemical names of neutral species are often used for acids and bases (e.g., 3-chlorobenzoic acid instead of 3-chlorobenzoate), because they are more common. Additional information on the test chemicals (e.g. CAS number, provider, chemical structure, pK_a values and recovery from control experiments) is summarized in Table S2, SI. Unless otherwise specified below, all dialysis experiments were conducted using 30 mM phosphate buffer at pH 7.7, which gave the targeted pH of 7.0-7.1 in the presence of the muscle protein sample (more details on the dialysis experiments and on the choice of buffer are provided in the SI). Controlling the ion composition and pH value of the buffer in the dialysis experiments was found to be difficult, because salts and acids (particularly lactic acid) from the muscle protein sample were found to contribute significantly to the salt concentration and the pH value of the buffer. Therefore, the actual salt composition and pH value in the solution after dialysis experiments were determined (Table 1). The change in pH and salt concentrations upon addition of muscle protein could be avoided in future experiments by an additional purification step, for example by

131 dialyzing the protein against a large amount of buffer solution. To prevent microbial growth all
132 solutions contained 300 mg/L NaN_3 (i.e. 4.6 mM NaN_3).

133 **Table 1.** Measured salt concentrations and pH value of buffer solution used for the dialysis
134 experiments with muscle protein. All values are from a single measurement (except for
135 phosphate, for which the mean of 2 measurements is shown); pH value was checked regularly
136 after dialysis and was always within ± 0.1 log unit.

Analyte	**Buffer before dialysis** c [mmol/L]	**Buffer after dialysis[a,b]** c [mmol/L]	**Buffer after dialysis[a,c]** c [mmol/L]	**Intracellular concentration[d]** c [mmol/L]
Chloride	n.a.	1.8	2.0	5-15
Phosphate	34	44	39	-
Lactate	n.a.	14	9.7	-
Ca	n.a.	0.1	0.1	10^{-4}
Fe	n.a.	1.3×10^{-3}	1.0×10^{-3}	-
K	47	67	61	140
Na	8.9	6.6	6.7	5-15
Mg	n.a.	2.2	1.7	0.5
pH	7.7	7.0	7.1	7.1

[a] amount of muscle protein: 500 mg/10 mL buffer

[b] first lot of muscle protein

[c] second lot of muscle protein

[d] from ref 22

n.a. not analyzed

137 ## Dialysis experiments

138 An equilibrium dialysis approach was chosen to determine the extent of binding to muscle
139 protein, expressed as muscle protein-water partition coefficient (log $K_{MP/w}$). Log $K_{MP/w}$ is defined
140 as the ratio of the concentration of the target chemical in muscle protein (C_{MP}) to the freely
141 dissolved concentration in water (C_{free}):

142 $$K_{MP/w}\left[\frac{L_{water}}{kg_{MP}}\right] = \frac{C_{MP}}{C_{free}} \qquad (1)$$

The experimental setup for the dialysis experiments was similar to that in our previous studies with BSA,[14, 23] where buffer solutions with and without protein were separated with dialysis membrane and the concentration of the chemical in the buffer-only solution (= C_{free}) was measured after equilibration (see SI for more details). The main difference between BSA and muscle protein in the experiments was the solubility in water. Unlike serum albumin the muscle protein did not completely dissolve in water. Therefore, instead of pipetting a protein solution into the dialysis cells, a fixed amount of muscle protein was directly weighed into one half cell of the dialysis cells and 5 mL of a diluted solution of the test chemical in buffer were added. For all test chemicals 3-4 replicates of the muscle protein cells were prepared and additionally 3-4 control samples without protein. The recovery of the test chemicals from the control samples was on average 87-105%. Because of the high amount of undissolved protein, stirring of the muscle protein suspension in the dialysis cells was not possible. Instead, the cells were equilibrated on an orbital shaker at 200 rpm at 37°C. As with our experiments with BSA, concentration measurements were always performed after 48 and 72 hours, indicating no difference (data not shown). Hence, data for both time points were combined and used to calculate the mean. The same amount of muscle protein (500 mg) was used for all experiments, because the final salt composition of the medium was found to be dependent on the amount of muscle protein (see also Table 1). Spectra\Por 3 dialysis membrane from Spectrum Laboratories was used (for more information on the membrane and quantification of leaking, see SI).

Reversibility of binding to muscle protein

Because irreversible binding of the test chemicals to muscle protein would complicate the $K_{MP/w}$ measurement, reversibility of muscle protein binding was determined using the method described in our previous work with BSA.[14] In short: muscle protein samples were prepared as described above and the dialysis experiment was performed. After three-day equilibration, the

buffer containing half-cell was emptied completely and was refilled with fresh buffer. A small shift in pH (from 7.0 to 7.3) was observed upon addition of fresh buffer in the first reversibility tests (with anionic chemicals) and we improved the experimental setup for the cationic chemicals by adding 10 mM lactate to the refill buffer (i.e., making the buffer more similar to the dialysate). Additional three days were given for equilibration and the buffer phase was sampled again. If binding to muscle protein is completely reversible and no mass loss of the chemicals occurred, the partition coefficients calculated from the equilibrium concentrations measured after three and six days should be the same. If a test chemical is irreversibly bound to muscle protein or degraded in the presence of protein, log $K_{MP/w}$ determined after six days should become higher than after three days.

Isotherm experiments

Because log $K_{MP/w}$ for the test chemicals of this study were measured at different concentration levels, it is important for their comparability that the sorption to muscle protein is linear (i.e., $K_{MP/w}$ is independent of the concentration of the chemical) or that the degree of non-linearity is known. Linearity of sorption was checked for five anionic (2-naphthoic acid, 3,4-dichlorobenzoic acid, 4-butylbenzoic acid, ketoprofen, and 2,3,5,6-tetrachlorophenol) and one cationic test chemical ((S)-(-)-propranolol). Dialysis cells were prepared as described above, 3-5 concentration levels were measured for each chemical and the initial concentrations of the respective test chemicals was varied by 1.5 to 3.5 orders of magnitude.

Influence of pH value, lactate and salt concentration

The partitioning of organic ions to proteins can be influenced by pH value and salt concentration. Previous studies with serum albumin found no clear trend in pH dependence [14, 24] but a significant competing effect of chloride on the partitioning of organic anions.[14, 25, 26] For log $K_{MP/w}$ the dependence on pH value and the influence of the concentration of other ions has not

been investigated so far. Therefore, log $K_{MP/w}$ of 3,4-dichlorobenzoic acid and benzyldimethyloctylammonium were measured in 30 mM phosphate buffer adjusted to pH 6, 8 and 9 (at equilibrium with muscle protein) in addition to our regular solution with pH 7. The partitioning of 3,4-dichlorobenzoic acid, coumachlor, and benzyldimethyloctylammonium to muscle protein was also measured at two additional concentrations of K^+ and phosphate (8 and 105 mM phosphate, corresponding to 16 and 157 mM K^+, respectively, measured at equilibrium), while pH was kept between 6.5 and 7.1 at equilibrium.

The isolated muscle protein used for this study contained considerable amounts of lactic acid. Because the lactate concentration in our test system after equilibration with muscle protein was higher than the concentration of lactate in muscle in vivo (for calculation see SI), we also investigated the influence of lactate on log $K_{MP/w}$. For this purpose sorption of 3,4-dichlorobenzoic acid, coumachlor and benzyldimethyloctylammonium was additionally measured at equilibrium lactate concentrations of 42 and 96 mM (final pH adjusted to 7.0 with KOH).

Instrumental analysis

All test chemicals were measured with an HPLC system from JASCO, equipped with either a UV detector (UV-970 M, JASCO) or a fluorescence detector (RF-10AXL, Shimadzu). Depending on the chemicals, either an Eclipse Plus C18 column (4.6 mm × 100 mm, 5 μm particle size) or a ZORBAX Extend C18 column (4.6 mm × 150 mm, 5 μm particle size), both from Agilent, was used. The eluent was a mixture of acetonitrile and water (both containing 0.1 wt% orthophosphoric acid) at a flow rate of 1 mL/min. For four anionic (3-chlorobenzoic acid, 3-methoxy-2-naphthoic acid, 2,4,6-trimethylbenzene sulfonate and 4-bromobenzene sulfonate) and three cationic test chemicals (verapamil, difenzoquat and dibenzylamine) the influence of the

dissolved small molecules from the protein sample on the quantification was checked. Calibration standards prepared in clean phosphate buffer (for cations, phosphate buffer with lactate) and in buffer that was dialyzed against muscle protein were measured and compared. No difference between the two sets of standards was found, indicating that the components in the dialysate did not interfere with the quantification of the test chemicals in the dialysis experiments.

Results and discussion

Reversibility tests

The results from the reversibility tests are presented in Table S3, SI. Interestingly, log $K_{MP/w}$ determined from samples taken at day six are slightly smaller than log $K_{MP/w}$ determined for day three (on average 0.09 log units). Since irreversible binding or a mass loss should result in a higher $K_{MP/w}$ on day six than on day three, binding to muscle protein seems to be reversible for the eleven chemicals tested. While the cause of the difference between log $K_{MP/w}$ determined on days three and six is unknown, several explanations may be conceivable. First, salt concentrations are slightly different between days three and six, because the composition of fresh buffer added after three days is not the same as that of the dialysate removed (see also Table 1). For the anionic test chemicals, a small shift in pH (from 7.0 to 7.3) was also measured (see method section). However, small shifts in pH and salt composition should not have a significant influence on log $K_{MP/w}$ (see next section). Second, a volume shift due to the osmotic pressure caused by muscle protein is possible, but was found to be small (only up to 10% of the total volume). Third, because of the prolonged duration of the experiment, the muscle protein might have partly degraded or structurally reorganized. In any case, the differences observed were small and do not have an influence on the following discussions.

Influence of pH value, lactate and salt concentration

For 3,4-dichlorobenzoic acid and benzyldimethyloctylammonium log $K_{MP/w}$ was measured at pH 6, 7, 8 and 9 (Figure S3, SI). Increasing the pH value from 6 to 9, decreases log $K_{MP/w}$ for 3,4-dichlorobenzoic by 0.5 log units, while for benzyldimethyloctylammonium log $K_{MP/w}$ increases 0.4 log units. This small change in the partitioning behavior with pH could be explained by assuming that the protein carries more positive net charges at lower pH (attractive for 3,4-dichlorobenzoic acid) and more negative net charges at higher pH (attractive for benzyldimethyloctylammonium), yet actual sorption mechanisms are unknown.

Muscle protein binding of 3,4-dichlorobenzoic acid, coumachlor and benzyldimethyloctylammonium was measured at different concentrations of K^+, phosphate and lactate (Figure S2, SI). The concentration of other organic or inorganic ions was expected to influence the sorption of the test chemicals to muscle protein for various possible reasons such as competition for possible binding sites for ions and ion pair formation. However, a factor of 10 difference in the K^+, phosphate or lactate concentration caused only a factor of ≤1.6 difference in $K_{MP/w}$ (or ≤0.2 log units) for the three chemicals tested, indicating no substantial influence of salt concentrations on the sorption. From these results it can be concluded that it is unlikely an ion exchange process or ion pair partitioning that dominates the partitioning of organic ions to muscle protein. The results indicate that minor variations in experimental pH and salt concentrations do not influence $K_{MP/w}$. Nevertheless, some control is still needed, because shifts in pH value can change the speciation of ionizable chemicals, and neutral and ionic species of a chemical can exhibit different partitioning behavior toward the protein. In future experiments this is especially important for chemicals with a pK_a value close to 7, for which ionic and neutral species are present in equal distribution.

Sorption isotherms

Sorption isotherms were measured for six test chemicals (2-naphthoic acid, 3,4-dichlorobenzoic acid, 4-butylbenzoic acid, ketoprofen, 2,3,5,6-tetrachlorophenol and (S)-(-)-propranolol). All isotherms were fitted using the log-transformed Freundlich model, $\log C_{MP} = n_f \cdot \log C_{free} + \log K_{Fr}$, where n_f and K_{Fr} are the Freundlich exponent and Freundlich coefficient, respectively. The Freundlich exponent is 1.00 ± 0.08 (mean ± standard deviation) for the tested

chemicals, indicating linear sorption to muscle protein (Figure S4, SI). We, therefore, surmise that log $K_{MP/w}$ measured at a single concentration (Table 2) can represent the sorption behavior of the test chemicals at different concentrations.

Table 2. Determined logarithmic muscle protein-water partition coefficients (log $K_{MP/w}$) at 37°C, final pH 7.0-7.1 and final salt concentration as stated in Table 1. Log $K_{MP/w}$ was calculated based on the dry weight of muscle protein. The concentration of muscle protein in the experiments was always 5% by weight.

Test chemical	**log $K_{MP/w}$ [L_{water}/kg_{MP}]**	**SD**
Benzoic acids		
3-chlorobenzoic acid	0.72	0.02
4-chlorobenzoic acid	0.72	0.03
3,4-dichlorobenzoic acid	1.31	0.02
4-bromobenzoic acid	0.91	0.01
4-butylbenzoic acid	1.39	0.01
4-hexylbenzoic acid	2.17	0.04
2-cyclohexylbenzoic acid	1.14	0.02
Naphthoic acids		
2-naphthoic acid	1.14	0.01
2-naphthaleneacetic acid	1.09	0.02
1-naphthoic acid	0.87	0.02
1-naphthaleneacetic acid	0.98	0.02
4-fluoro-1-naphthoic acid	1.00	0.02
1-bromo-2-naphthoic acid	1.19	0.02
6-bromo-2-naphthoic acid	1.89	0.02
2-methoxy-1-naphthoic acid	0.70	0.01
2-ethoxy-1-naphthoic acid	0.74	0.02
3-methoxy-2-naphthoic acid	0.85	0.02
Phenoxy acids		
4-(2,4-dichlorophenoxy)butyric acid	1.53	0.01
2,4,5-trichlorophenoxyacetic acid	1.61	0.03
mecoprop[a]	1.17	0.02
Arylpropionic acids		
ketoprofen[a]	1.21	0.02
ibuprofen[a]	1.48	0.04
fenoprofen[a]	1.62	0.03

(S)-(+)-naproxen	1.33	0.02
Anthranilic acid derivatives		
N-phenylanthranilic acid	1.86	0.04
flufenamic acid	2.40	0.07
mefenamic acid	2.40	0.02
Phenols		
2,3,5,6-tetrachlorophenol	2.63	0.02
pentachlorophenol	3.36	0.20
dinoseb[a]	2.45	0.02
dinoterb	2.46	0.04
bromoxynil	1.74	0.02
Coumarines		
coumachlor	2.21[b]	0.02
coumafuryl	1.14	0.07
Others		
diclofenac	2.47	0.08
1-pyrenecarboxylic acid	2.30[b]	0.02
Sulfonates		
4-n-octylbenzenesulfonate	2.81[b]	0.04
2,4,6-trimethylbenzenesulfonate	0.72[b]	0.02
4-bromobenzenesulfonate	0.86[b]	0.04
naphthalene-2-sulfonate	1.03[b]	0.05
1-pyrene sulfonate	2.40[b]	0.03
Cations		
(S)-(-)-propranolol	1.48[b]	0.02
alprenolol[a]	1.17[b]	0.06
fluoxetine[a]	2.04[b]	0.01
dibenzylamine	1.29[b]	0.02
imipramine	1.83[b]	0.03
verapamil[a]	1.54[b]	0.07
difenzoquat	0.92[b]	0.07
benzyltributylammonium	0.89[b]	0.09
benzyldimethyloctylammonium	1.44[b]	0.02
tetraphenylphosphonium	1.80[b]	0.01

[a] Chiral compounds. Because no information on the enantiomeric composition was available, we assumed the racemic mixture.

[b] Determined using the second lot of muscle protein.

Muscle protein-water partition coefficients

Muscle protein-water partition coefficients (log $K_{MP/w}$) were determined for 41 anionic and 10 cationic chemicals on a single concentration level (Table 2). Compared to the previously determined partition coefficients to BSA, the measured partition coefficients of this study are rather low. For the majority of the test chemicals (38 out of 51) log $K_{MP/w}$ is smaller than 2 and many chemicals could not be measured because of too weak sorption (fraction bound <20%, which corresponds to log $K_{MP/w} \leq 0.6$; Table S4, SI). Two primary amines (4-phenylbutylamine and 1-naphthylmethylamine) had to be removed from the dataset, because results for log $K_{MP/w}$ were not reproducible.

Especially compared to the BSA-water partition coefficients (log $K_{BSA/w}$) measured in our previous study,[14] log $K_{MP/w}$ of this study fall within a lower range (0.70 to 3.36). Furthermore, for BSA a much stronger sorption of 2-naphthoic acids compared to 1-naphthoic acids was found,[14] whereas for muscle protein all naphthoic acids have similar partition coefficients. This indicates steric effects for binding to BSA, but not for binding to muscle protein. As was observed with BSA, sorption to muscle protein for ortho-substituted benzoic acids seems to be lower than for other benzoic acids. For example, log $K_{MP/w}$ for 3-chlorobenzoic acid, 4-chlorobenzoic acid, and 3,4-dichlorobenzoic acid is 0.72, 0.72, and 1.31, respectively, while binding of 2-chlorobenzoic acid, 2,6-dichlorobenzoic acid, and 2,4,6-trimethylbenzoic acid was too weak to be measurable (fraction bound <20%, log $K_{MP/w}$ <0.6). The dataset for muscle protein includes three pairs of chemicals with the same non-ionic substructure, but with either a sulfonate or a carboxylate group as charged functional group: 4-bromobenzenesulfonate and 4-bromobenzoic acid, naphthalene-2-sulfonate and 2-naphthoic acid, and 1-pyrene sulfonate and 1-pyrenecarboxylic acid. The difference between log $K_{MP/w}$ measured for sulfonates and carboxylates is small (≤0.1 log units), which is again similar to the observations with BSA.

Theoretical contribution of neutral species to determined log $K_{MP/w}$

As stated in the method section, all test chemicals were more than 97% present as ions in the experiments. Hence, it is assumed that the measured log $K_{MP/w}$ represent the partition coefficients of the ionic species and that the contribution of the neutral species can be neglected. To verify this assumption the following calculation was done. Using PP-LFERs log $K_{MP/w}$ can be calculated for the corresponding neutral species of the test chemicals (see also next section and SI). The calculated log $K_{MP/w}$ of the neutral species (Table S8, SI) are on average 0.6 log units higher than the measured log $K_{MP/w}$ of the ionic species. The highest positive difference was calculated for 2-cyclohexylbenzoic acid (1.47 log units) and the highest negative difference for coumafuryl (0.3 log units). With the log $K_{MP/w}$ of the neutral species and the pK_a value of the chemical and the pH value at which the partition coefficients were measured, the contribution of the neutral species to the measured log $K_{MP/w}$ can be calculated. A contribution of ≤7% was calculated for all test chemicals except for only one chemical (Table S8, SI). Hence, the influence of the neutral species on the measured log $K_{MP/w}$ is estimated to be generally negligible. The exception was dibenzylamine, for which 40% contribution of the neutral species was calculated. The pK_a value of dibenzylamine used for the calculation was 8.54 (predicted using Calculator Plugins from Marvin 15.8.17.0, 2015, ChemAxon) and agrees with an experimental pK_a value reported in the literature (8.52).[27] However, no experimentally determined PP-LFER descriptors were available for dibenzylamine and the reliability of the calculation cannot be further evaluated. As the major contribution (60%) is from the ionic species, in the following discussions we assume that the measured log $K_{MP/w}$ for dibenzylamine is attributable to its ionic species, bearing in mind that the value used might be subject to a relatively large error.

Polyparameter linear free energy relationships (PP-LFERs)

For neutral organic chemicals a PP-LFER model for muscle protein-water partition coefficients based on equation 2 is already available from the literature (eq. 4 in Table 3).[9]

$$\log K_{MP/w} = c + eE + sS + aA + bB + vV \qquad (2)$$

In eq. 2 the capital letters represent the substance properties and the small letters the corresponding system properties. E is the excess molar refraction, S is the polarizability/dipolarity parameter, A represents the H-bond donor properties, B the H-bond acceptor properties, and V is the molar volume. This PP-LFER approach is applicable for neutral chemicals only.

Abraham et al.[28, 29] have proposed a PP-LFER equation that can be used for ionic chemicals as well (eq. 3).

$$\log K_{MP/w} = c + eE_i + sS_i + aA_i + bB_i + vV_i + j^+J_i^+ + j^-J_i^- \qquad (3)$$

Note that in this approach ionic chemicals do not only have specific descriptor values for E, S, A, B and V (denoted with subscript i), but also require two additional descriptors for their charge (J_i^+ and J_i^-). The flow chart in Figure S5 shows how the PP-LFER equation for the organic ions in this study was derived. Descriptors for the neutral species of all test chemicals (E, S, A, B, V) were required for the calculation of the descriptors of the corresponding ionic species. If available, the experimentally determined descriptors of neutral species collected in the UFZ-LSER database[30] were used. For many chemicals no experimental substance descriptors were found and the descriptors for such chemicals were predicted using the ABSOLV module from the ACD/Percepta software (2015 release). Using the empirical equations from Abraham et al.[28, 31] and the descriptors of the neutral species, descriptors of the ionic species (E_i, S_i, A_i, B_i, V_i, J_i^+, J_i^-) were calculated. Because such equations are only available for phenols, carboxylic acids, pyridines, and amines, eleven chemicals had to be excluded from the calculation (e.g., sulfonates,

coumarines, quaternary ammonium compounds). $K_{MP/w}$ data for 46 neutral chemicals from Endo et al.[9] were added for extension of the dataset.

Table 3. Regression equations derived for log $K_{MP/w}$ [L_{water}/kg_{MP}], values in parentheses denote standard errors.

Eq.	*e*	*s*	*a*	*b*	*v*	*c*	j^-	j^+	*n*	R^2	RMSE
eq. 4[a]	0.51 (0.10)	-0.51 (0.17)	0.26 (0.17)	-2.98 (0.24)	3.01 (0.21)	-0.79 (0.25)	-	-	46	0.95	0.22
eq. 5	0.675 (0.067)	-0.764 (0.083)	-0.196 (0.146)	-2.285 (0.120)	2.511 (0.143)	-0.237 (0.157)	2.890 (0.209)	-0.682 (0.188)	86	0.887	0.288

[a] equation taken from Endo et al.[9] calibrated with neutral chemicals only.

All chemicals (46 neutral, 34 anionic, 6 cationic, 86 in total) were used to derive all system descriptors (*e*, *s*, *a*, *b*, *v*, j^+, j^-), leading to eq. 5 (Table 3). As depicted in Figure 1, eq. 5 fits the data well for all chemicals (RMSE = 0.29). Eq. 5 calculates log $K_{MP/w}$ of neutral chemicals almost as well as eq. 4 (RMSE = 0.26 for eq. 5 for neutral chemicals only, compared to RMSE = 0.22 for eq. 4). Hence, eq. 5 may provide a predictive tool for partitioning of both neutral and ionic chemicals to structural proteins. The good performance of eq. 5 is surprising, keeping in mind that empirical formulas were used to calculate the descriptors of ionic species from the descriptors of neutral species and that the descriptors of the neutral species themselves are also predicted for many chemicals (23 out of 40 ionic chemicals). In the Supporting Information two additional fits are presented (eqs. 6, 7 in Table S5). For the first fit the system descriptors *e*, *s*, *a*, *b*, and *v* were derived by using only neutral chemicals. These descriptors (*e, s, a, b, v*) were then set as fixed and j^+ and j^- were calculated using the data for ions. The second fit demonstrates how the fit changes if only ionic chemicals were used for the calculation of all system descriptors. Both equations predict the data worse than eq. 5, with the RMSE being 0.50 and 0.36 for the first and second fit, respectively.

The data for $K_{MP/w}$ were also compared with logarithmic octanol-water partition coefficients of the neutral species of the test chemicals (log K_{ow} neutral). A correlation with log K_{ow} neutral also fits the data worse (RMSE = 0.41, R^2 = 0.53) than eq. 5 (Figure S6, SI).

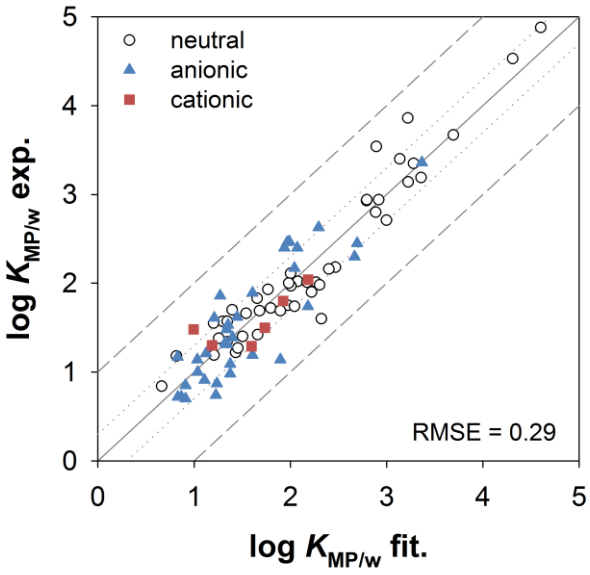

Figure 1. Comparison of PP-LFER fitted (log $K_{MP/w}$ fit.) using eq. 5 and experimentally determined muscle protein-water partition coefficients (log $K_{MP/w}$ exp.). The solid line denotes the 1:1 line, and dotted and dashed lines indicate a deviation of 0.3 and 1 log unit, respectively.

Comparison with serum albumin

Figure 2 shows a comparison of experimental log $K_{MP/w}$ and experimental BSA-water partition coefficients (log $K_{BSA/w}$). For 31 anionic and 4 cationic chemicals of this work log $K_{BSA/w}$ from our previous study on BSA binding are available.[14] Data for 39 neutral chemicals from Endo et al.[8, 9] were included for comparison. While the data scatter considerably, some clear trends do exist. For the neutral chemicals log $K_{MP/w}$ is on average one log unit smaller than log $K_{BSA/w}$. For the anionic chemicals log $K_{MP/w}$ is much smaller than log $K_{BSA/w}$ by up to 3.7 log units (for 2-naphthaleneacetic acid and naphthalene-2-sulfonate). For the four cations log $K_{MP/w}$ and log $K_{BSA/w}$ are similar.

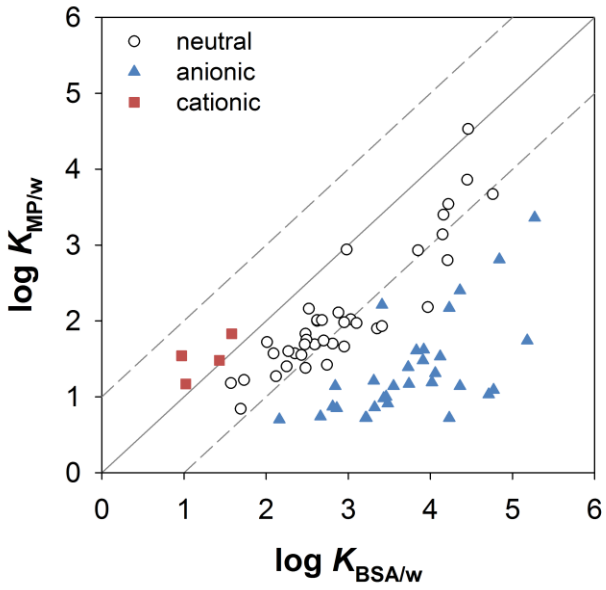

Figure 2. Comparison of experimentally determined muscle protein-water partition coefficients (log $K_{MP/w}$) and BSA-water partition coefficients (log $K_{BSA/w}$) for 39 neutral, 31 anionic and 4 cationic chemicals.

Combining the findings of this and the previous study,[14] we shortly summarize here the similarities and differences in sorption properties between BSA and muscle protein. First, anionic chemicals sorb more strongly to BSA than to muscle protein, while cationic chemicals seem to show similar partition coefficients to both proteins, as shown just above. Here, it should be noted that a comparison of both proteins was only possible for four cationic chemicals (two secondary and two tertiary amines) and could be specific for these chemicals. Second, a high influence of the 3D molecular structure was found for sorption to BSA, while this was of minor importance for muscle protein. Third, sorption to BSA is influenced by the concentration of competing ions, whereas sorption to muscle protein is not. All in all, it is evident that sorption to muscle protein and serum albumin can differ substantially (particularly for anionic and neutral chemicals) and that for partitioning models (e.g., for the assessment of the bioaccumulation

potential of chemicals) both proteins should be approached separately, instead of considering the bulk protein fraction of an organism as one sorption phase.

Implications for partitioning of IOCs in vivo

As an application of the dataset obtained in this study, partition coefficients between muscle tissue and plasma were estimated for different chemicals based on their partitioning to muscle protein and serum albumin. In the literature[32] it has been reported that in vivo measured muscle tissue-plasma steady-state distribution ratios ($K_{muscle/plasma}$) show the following trend: cationic chemicals have the highest values for $K_{muscle/plasma}$ (0.5 - 29), followed by neutral chemicals (0.4 - 6), while anionic chemicals show relatively small $K_{muscle/plasma}$ (< 0.2). In Figure S7A selected literature data are plotted for 11 neutral chemicals and 42 acids and bases that are predominantly present in their ionic form (>95%) and have bound fractions of more than 20% in plasma.

Using a simple model (eq.8) equilibrium $K_{muscle/plasma}$ were estimated for all chemicals for which experimental data for $K_{BSA/w}$ and $K_{MP/w}$ were available (4 cationic and 31 anionic chemicals of this study and 39 neutral chemicals from Endo et al.[8, 9]) .

$$K_{muscle/plasma} = \frac{f_{SA,muscle} \cdot K_{BSA/w} + f_{MP,muscle} \cdot K_{MP/w} + f_{w,muscle}}{f_{SA,plasma} \cdot K_{BSA/w} + f_{protein,plasma} \cdot K_{MP/w} + f_{w,plasma}} \qquad (8)$$

In the calculation it is assumed that the only relevant sorption phases in muscle tissue and plasma are structural proteins, serum albumin, and water. The volume fractions of serum albumin, muscle protein and water in muscle and plasma were calculated from physiological data collected in refs [15, 16, 33] and are denoted with f_{SA}, f_{MP}, and f_w, respectively; $f_{protein,plasma}$ is the fraction of other serum proteins. Serum proteins other than albumin are represented by muscle protein, because partition coefficients to these proteins are not available for the test chemicals of this study. The calculated values for $K_{muscle/plasma}$ are presented in Table S9 and Figure S7B. Interestingly, these theoretical partition coefficients show the same trend and dimension as the

418 literature data for $K_{muscle/plasma}$. The highest values for $K_{muscle/plasma}$ were calculated for the four
419 cationic chemicals (2.5 - 4.3). Neutral chemicals generally show lower values than cations (0.1 -
420 4.3) and for all anionic chemicals a very low $K_{muscle/plasma}$ was calculated ($\leq$ 0.4). Experimental
421 log $K_{muscle/plasma}$ for (S)-(-)-propranolol and verapamil are 0.63 and 0.54, respectively, and agree
422 well with the calculated partition coefficients of 0.47 and 0.64, respectively. While more data are
423 clearly required to assess partitioning of IOCs into biological tissues, the good prediction of the
424 trends for in vivo $K_{muscle/plasma}$ is encouraging, as it suggests that the in vitro partitioning data for
425 isolated serum albumin and muscle protein are relevant for the assessments of the in vivo
426 behavior of IOCs.
427 It should be noted that the calculation of $K_{muscle/plasma}$ above is very simplified, because it
428 considers only serum albumin and muscle protein. Significant partitioning to phospholipids
429 would increase the sorptive capacity of both plasma and muscle tissue. Because muscle tissue
430 has more phospholipids than plasma,[33] $K_{muscle/plasma}$ would increase with increasing sorption to
431 phospholipids. Specific sorption to plasma proteins other than serum albumin would increase the
432 sorptive capacity of the plasma and thus decrease $K_{muscle/plasma}$. If α_1-acid glycoprotein binding is
433 significant, for example, a lower $K_{muscle/plasma}$ is expected for neutral and cationic chemicals.
434 Furthermore, the partition coefficients measured in this study represent a mixture of the different
435 structural proteins found in muscle tissue. For a future extension of the modeling approach
436 discussed above to other tissues than muscle tissue, partitioning behavior of organic ions towards
437 other types of structural protein (e.g., keratin and collagen) should also be evaluated.
438 ASSOCIATED CONTENT

439 **Supporting Information**. Further information on the test chemicals, preparation of muscle
440 protein, salt concentration and pH dependence of log $K_{MP/w}$, PP-LFER modeling and predicted

441 muscle tissue-plasma partition coefficients; 6 additional figures (including sorption isotherms for
442 selected chemicals) and 9 additional tables. This material is available free of charge via the
443 Internet at http://pubs.acs.org.

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447 ACKNOWLEDGMENT

448 We thank Trevor N. Brown for the collection of physiological data and Grit Weichert for help
449 in the preparation of muscle protein. Special thanks go to Andrea Pfennigsdorff for her
450 assistance in laboratory work. Satoshi Endo acknowledges the support by the MEXT/JST Tenure
451 Track Promotion Program.

452 REFERENCES

453 1. Endo, S.; Brown, T. N.; Goss, K.-U., General Model for Estimating Partition Coefficients
454 to Organisms and Their Tissues Using the Biological Compositions and Polyparameter Linear
455 Free Energy Relationships. Environ. Sci. Technol. 2013, 47, (12), 6630-6639.

456 2. van der Heijden, S. A.; Hermens, J. L. M.; Sinnige, T. L.; Mayer, P.; Gilbert, D.; Jonker,
457 M. T. O., Determining High-Quality Critical Body Residues for Multiple Species and Chemicals
458 by Applying Improved Experimental Design and Data Interpretation Concepts. Environ. Sci.
459 Technol. 2015, 49, (3), 1879-1887.

460 3. Escher, B. I.; Cowan-Ellsberry, C. E.; Dyer, S.; Embry, M. R.; Erhardt, S.; Halder, M.;
461 Kwon, J.-H.; Johanning, K.; Oosterwijk, M. T. T.; Rutishauser, S.; Segner, H.; Nichols, J.,

462 Protein and Lipid Binding Parameters in Rainbow Trout (Oncorhynchus mykiss) Blood and
463 Liver Fractions to Extrapolate from an in Vitro Metabolic Degradation Assay to in Vivo
464 Bioaccumulation Potential of Hydrophobic Organic Chemicals. Chem. Res. Toxicol. 2011, 24,
465 (7), 1134-1143.

466 4. Poole, C. F.; Ariyasena, T. C.; Lenca, N., Estimation of the environmental properties of
467 compounds from chromatographic measurements and the solvation parameter model. J.
468 Chromatogr. A 2013, 1317, 85-104.

469 5. Endo, S.; Goss, K.-U., Applications of Polyparameter Linear Free Energy Relationships
470 in Environmental Chemistry. Environ. Sci. Technol. 2014, 48, (21), 12477-12491.

471 6. Geisler, A.; Endo, S.; Goss, K.-U., Partitioning of Organic Chemicals to Storage Lipids:
472 Elucidating the Dependence on Fatty Acid Composition and Temperature. Environ. Sci. Technol.
473 2012, 46, (17), 9519-9524.

474 7. Endo, S.; Escher, B. I.; Goss, K. U., Capacities of Membrane Lipids to Accumulate
475 neutral Organic Chemicals. Environ. Sci. Technol. 2011, 45, 5912-5921.

476 8. Endo, S.; Goss, K. U., Serum Albumin Binding of Structurally Diverse Neutral Organic
477 Compounds: Data and Models Chem. Res. Toxicol. 2011, 24, (12), 2293-2301.

478 9. Endo, S.; Bauerfeind, J.; Goss, K.-U., Partitioning of Neutral Organic Compounds to
479 Structural Proteins. Environ. Sci. Technol. 2012, 46, (22), 12697-12703.

480 10. Armitage, J. M.; Arnot, J. A.; Wania, F.; Mackay, D., Development and evaluation of a
481 mechanistic bioconcentration model for ionogenic organic chemicals in fish. Environ. Toxicol.
482 Chem. 2013, 32, (1), 115-128.

11. Peters Jr, T., All About Albumin. Academic Press: San Diego, 1995.

12. Kragh-Hansen, U., Molecular aspects of ligand binding to serum albumin. Pharmacol. Rev. 1981, 33, (1), 17-53.

13. Kremer, J. M.; Wilting, J.; Janssen, L. H., Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol. Rev. 1988, 40, (1), 1-47.

14. Henneberger, L.; Goss, K. U.; Endo, S., Equilibrium Sorption of Structurally Diverse Organic Ions to Bovine Serum Albumin. Environ. Sci. Technol. 2016, 50, (10), 5119-26.

15. ICRP, Report of the Task Group on Reference Man. ICRP Publication 23 1975.

16. ICRP, 2002. Basic Anatomical and Physiological Data for Use in Radiological Protection Reference Values. ICRP Publication 89. Ann. ICRP 32 (3-4).

17. Xing, B.; McGill, W. B.; Dudas, M. J., Sorption of benzene, toluene, and o-xylene by collagen compared with non-protein organic sorbents. Can. J. Soil Sci. 1994, 74, (4), 465-469.

18. Hansen, S.; Selzer, D.; Schaefer, U. F.; Kasting, G. B., An extended database of keratin binding. J. Pharm. Sci. 2011, 100, (5), 1712-1726.

19. Wang, L.; Chen, L.; Lian, G.; Han, L., Determination of partition and binding properties of solutes to stratum corneum. Int. J. Pharm. 2010, 398, (1–2), 114-122.

20. Sudlow, G.; Birkett, D. J.; Wade, D. N., The Characterization of Two Specific Drug Binding Sites on Human Serum Albumin. Mol. Pharmacol. 1975, 11, (6), 824-832.

21. Cheung, P. C. K.; Mehta, B. M., Eds. Handbook of Food Chemistry. Springer-Verlag: Berlin Heidelberg, 2015; Vol. 1.

22. Alberts, B., Molecular Biology of the Cell. Garland Pub.: 1989.

23. Oemisch, L.; Goss, K.-U.; Endo, S., Ion exchange membranes as novel passive sampling material for organic ions: Application for the determination of freely dissolved concentrations. J. Chromatogr. A 2014, 1370, 17-24.

24. Bischel, H. N.; MacManus-Spencer, L. A.; Zhang, C.; Luthy, R. G., Strong associations of short-chain perfluoroalkyl acids with serum albumin and investigation of binding mechanisms. Environ. Toxicol. Chem. 2011, 30, (11), 2423-2430.

25. Wilting, J.; van der Giesen, W. F.; Janssen, L. H.; Weideman, M. M.; Otagiri, M.; Perrin, J. H., The effect of albumin conformation on the binding of warfarin to human serum albumin. The dependence of the binding of warfarin to human serum albumin on the hydrogen, calcium, and chloride ion concentrations as studied by circular dichroism, fluorescence, and equilibrium dialysis. J. Biol. Chem. 1980, 255, (7), 3032-3037.

26. Honoré, B.; Brodersen, R., Detection of carrier heterogeneity by rate of ligand dialysis: Medium-chain fatty acid interaction with human serum albumin and competition with chloride. Anal. Biochem. 1988, 171, (1), 55-66.

27. Christensen, J. J.; Izatt, R. M.; Wrathall, D. P.; Hansen, L. D., Thermodynamics of proton ionization in dilute aqueous solution. Part XI. pK, $\Delta H°$, and $\Delta S°$ values for proton ionization from protonated amines at 25°. J. Chem. Soc. A 1969, 1212-1223.

28. Abraham, M. H.; Acree, W. E., Equations for the Transfer of Neutral Molecules and Ionic Species from Water to Organic phases. J. Org. Chem. 2010, 75, (4), 1006-1015.

29. Abraham, M. H.; Zhao, Y. H., Determination of Solvation Descriptors for Ionic Species: Hydrogen Bond Acidity and Basicity. J. Org. Chem. 2004, 69, (14), 4677-4685.

30. Endo, S.; Watanabe, N.; Ulrich, N.; Bronner, G.; Goss, K.-U., UFZ-LSER database v 2.1 [Internet]. In Helmholtz Centre for Environmental Research-UFZ: Leipzig, Germany, 2015; Vol. v 2.1.

31. Abraham, M. H., The permeation of neutral molecules, ions, and ionic species through membranes: Brain permeation as an example. J. Pharm. Sci. 2011, 100, (5), 1690-1701.

32. Yun, Y. E.; Edginton, A. N., Correlation-based prediction of tissue-to-plasma partition coefficients using readily available input parameters. Xenobiotica 2013, 43, (10), 839-852.

33. White, D. A., The phospholipid composition of mammalian tissues. In Form and function of phospholipids, Ansell, G. B.; Hawthorne, J. N.; Dawson, R. M. C., Eds. Elsevier Scientific Publishing Company: Amsterdam, London, New York, 1973.

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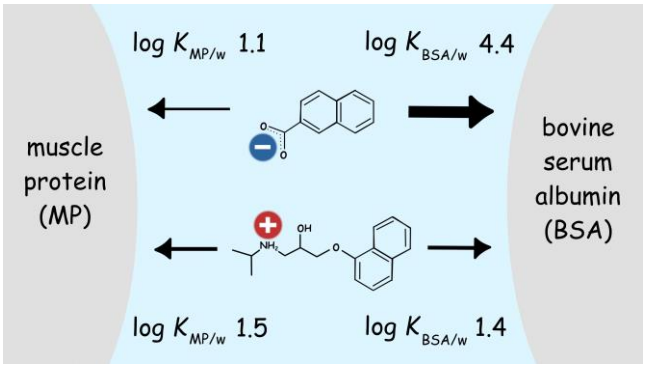
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log $K_{BSA/w}$ 4.4
muscle protein (MP)
bovine serum albumin (BSA)
log $K_{MP/w}$ 1.5
log $K_{BSA/w}$ 1.4