Supplementary Information for
Tryptophan regulates Drosophila zinc stores

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Food intake quantifications

Food intake quantifications were performed as in (1). Larvae of the \( w^+ \) or \( \nu^l \) genotypes were raised under normal diet for 72 h post-hatching under non-crowded conditions and then transferred to plain agar for 45 minutes, then for another 45 minutes to normal diet supplemented with 0.2% artificial blue color (Dosicolor) with or without 2.4 mM kyn. Each larva was then imaged and scored for the presence of blue food in their intestines. The experiment involved 10 individuals per genotype and condition and was repeated 6 times. As slight differences were observed between the coloration of the intestines between genotypes, we quantified food uptake by grinding 30 larvae in 10 µL water, centrifuged at 18,800 g for 1 min, recovered the supernatant, diluted 1:10 and read the absorbance spectrum in duplicates at a Nanodrop reader. An absorbance peak was observed at 630 nm, where comparative quantifications are shown from three independent biological replicates. In an alternative version of the protocol, we also had the \( w^+ \) or \( \nu^l \) larvae grow on diet supplemented with 2.4 mM kyn throughout their development.

Purity of synthesized chemicals

\( \text{Zn(XA)}_2 \) and 3HK-Zn-Cl were prepared as previously reported (2); elemental analysis was performed to check the purity of the synthesized complexes. Calculated values for \( \text{C}_{20}\text{H}_{11}\text{N}_2\text{O}_8\text{ZnNa·2H}_2\text{O} \) were: C 45.18%; H 2.84%; N 5.27%; Zn 12.30%. Found values were: C 44.86%; H 2.75%; N 4.97%; Zn 14.5%. For the 3HK-Zn complex, calculated for \( \text{C}_{10}\text{H}_{12}\text{ClN}_2\text{O}_4\text{Zn} \) (in brackets the percentages of the free ligand \( \text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_4 \)): C 37.07% (53.57%); H 3.42% (5.39%); N 8.64% (12.49%); Zn 20.18% (0%). Found: C 53.63%; H 5.55%; N 11.72%; Zn 0.15%. While for XA the results are consistent with the formation of a 1:2 complex with Zn(II), in the case of 3HK the results are very similar to those obtained for the free ligand, with a low percentage of Zn found by ICP-OES analysis. This result underscores the difficulty of obtaining 3HK-Zn-Cl in solid state, nevertheless sufficient product was synthesized for the purpose of XAS studies.

Electrospray ionization mass spectrometry (ESI-MS) analysis

Aliquots of the solutions of free ligands and Zn complexes were diluted with water and directly infused into the mass spectrometer, an Agilent 6530 Accurate-Mass Quadruple Time-of-Flight (Q-TOF) system equipped with an electrospray interface. Ionization was achieved in the positive or negative ion mode by application of 4.0 kV at the entrance of the capillary; the pressure of the nebulizer gas was 20 psi. The drying gas was heated to 325 °C at a flow rate of 10 µL/min. Full-scan mass spectra were recorded in the mass/charge (m/z) range of 50–3000. Isotopic distributions were calculated with the program Molecular Weight Calculator (https://omics.pnl.gov/software/molecular-weight-calculator). Fig. S8 shows ESI-MS spectra for 3HK complexes, whereas Fig. S12 for XA complexes.

Spectrophotometric competition assays

A 1.6 mM stock solution of Zincon was prepared by dissolving 43.5 mg of the chelator (sodium salt, 85% dye content) in 1 mL of NaOH (1 M) prior to dilution to 50 mL with water. Then, samples (2 mL total volume) were prepared by adding 50 µL of Zincon stock solution to 1900 µL of sodium borate buffer (50 mM, pH 9.0) or HEPES buffer (10 mM,
pH 7.5) with 100 mM NaCl. The final concentration of Zincon in the samples was 40 μM. Complexation was initiated by the addition of ZnCl₂ solution (1.6 mM) to achieve a final concentration of 80 μM. Reagent blanks were prepared analogously except for the substitution of the metal salt stock solution with water. Competition studies between Zn ligands and Zincon were performed by adding different concentrations of 3HK or XA (from 10 mM stock solutions) to Zincon both in borate buffer and in HEPES with 100 mM NaCl. After each addition, the samples were incubated for 5 minutes at room temperature. Absorption spectra were recorded from 200 to 750 nm on a PerkinElmer Lambda 2 Bio UV/Visible Spectrophotometer (Waltham, MA, USA) using a cuvette with a 1 cm pathlength (Fig. S19).

The equilibrium of Zn exchange between Zincon and kyn metabolites can be expressed as:

\[
\text{Zn} - \text{Zincon} + 3\text{HK} \rightleftharpoons \text{Zincon} + \text{Zn} - 3\text{HK}
\]

\[
\frac{K_{d,\text{app}}(\text{Zn} - \text{Zincon})}{K_{d,\text{app}}(\text{Zn} - 3\text{HK})} = \frac{[\text{Zincon}][\text{Zn} - 3\text{HK}]}{[\text{Zn} - \text{Zincon}][3\text{HK}]}
\]

and

\[
\text{Zn} - \text{Zincon} + 2\text{XA} \rightleftharpoons \text{Zincon} + \text{Zn} - (\text{XA})_2
\]

\[
\frac{K_{d,\text{app}}(\text{Zn} - \text{Zincon})}{K_{d,\text{app}}(\text{Zn} - (\text{XA})_2)} = \frac{[\text{Zincon}][\text{Zn} - (\text{XA})_2]}{[\text{Zn} - \text{Zincon}][\text{XA}]^2}
\]

Assuming that zinc is bound to either Zincon or kyn metabolites, it can be deduced that, at half-maximal absorption, [Zincon], [3HK] and [XA] can be calculated by subtracting the Zn-bound fractions from the analytical concentrations \(C_{\text{Zincon}}, C_{3\text{HK}}\) and \(C_{\text{XA}}\), respectively:

\[
[Z\text{n-Zincon}] = [Z\text{n}-3\text{HK}] = C_{\text{Zn}}/2
\]

\[
[Z\text{incon}] = C_{\text{Zincon}} - [Z\text{n-Zincon}] = C_{\text{Zincon}} - C_{\text{Zn}}/2
\]

\[
[3\text{HK}] = C_{3\text{HK}} - [Z\text{n}-3\text{HK}] = C_{3\text{HK}} - C_{\text{Zn}}/2
\]

and

\[
[Z\text{n-Zincon}] = [Z\text{n-(XA)}_2] = C_{\text{Zn}}/2
\]

\[
[X\text{A}] = C_{\text{XA}} - 2[Z\text{n-(XA)}_2] = C_{\text{XA}} - C_{\text{Zn}}
\]

The apparent Zn-Zincon (ZnL) dissociation constant at pH 7.5 was obtained by fitting the absorption data to the following equation using the Solver add-in of Excel 2013 that yielded a \(K_{d,\text{app}}\) of 2.2·10⁻⁶ M (Fig. S19D).

\[
\Delta A_{\text{obs,620 nm}} \propto [\text{ZnL}] = \frac{C_L + C_{\text{Zn}} + K_{d,\text{app}} - \sqrt{(C_L - C_{\text{Zn}} - K_{d,\text{app}})^2 - 4C_L C_{\text{Zn}}}}{2}
\]

where \(C_L = [\text{L}_T] + [\text{ZnL}]\) = total ligand concentration, \(C_{\text{Zn}} = [\text{Zn}] + [\text{ZnL}]\) = total Zn concentration and \(\Delta A_{\text{obs,620 nm}} = \Delta A_{\text{max,620 nm}} [\text{ZnL}] / C_L\). To obtain \(\Delta A_{\text{obs,620 nm}}\), the tailing
from the absorption band at 470 nm due to free Zincon was subtracted from the Zn-Zincen band at 620 nm.

**Spectrophotometric pKa determination**

3HK was dissolved in NMP1 buffer (5 mM N-Ethylmaleimide, 5 mM 2-(N-morpholino)ethanesulfonic acid, 5 mM Piperazine-N,N'-bis(2-ethanesulfonic acid)). XA was dissolved in NMP2 buffer (same three chemicals as above at a concentration of 2 mM). The pKa values of 3HK and XA were determined by titrating a solution containing 0.1 mM for 3HK or 0.05 mM XA, between pH 1.4 and 10.5, using HCl or NaOH to adjust pH. To determine the impact of Zn coordination on the ligand pKa values, 0.025 mM ZnCl₂ was added to the ligand solutions and the pH titration was performed. The absorption spectra of the solutions were determined using an Agilent 8453 diode array UV-Vis spectrophotometer (Fig. S14). The glass electrode for pH measurement was calibrated using a three-point calibration with standard buffer solutions at pH values: 4.0, 7.0 and 10.0. All titrations were performed in duplicate at room temperature (approximately 25°C). For estimation of the pKas of 3HK, the absorbance obtained at 420 nm was plotted as a function of pH and the data were fitted into equation 1 (for 2 species) using the ‘fittype’ function of matlab.

Equation:

$$\frac{m1 \times 10^{-2 \cdot M0} + m3 \times 10^{-m2-M0} + m5 \times 10^{-m2-m4}}{10^{-2 \cdot M0} + 10^{-m2-M0} + 10^{-m2-m4}}$$

where M0 is the pH, m1 is the absorbance of the protonated species, m3 the absorbance of the unprotonated species, m2 is the pKa between the species m1 and m3, m4 is the pkₐ₂ and m5 is the absorbance of the unprotonated species found at higher pH. For XA we used the absorbance at 255 nm and the same 2-species model.

**Fluorescence measurements**

XA (100 μM) was dissolved in water (pH 12), sodium borate buffer (50 mM, pH=9), HEPES buffer (50 mM, pH 7.5) with 100 mM NaCl, or 20 mM ammonium acetate buffer (pH 5.6). Then different aliquots of ZnCl₂ (up to 2 equivalents with respect to XA) were added from a stock solution of 3 mM concentration (Fig. S13B-G). 3HK (200 μM) was dissolved in HEPES buffer (50 mM, pH 7.5) with 100 mM NaCl or in 20 mM ammonium acetate buffer (pH 5.6). Then aliquots of ZnCl₂ (up to 1 eq with respect to 3HK) were added (Fig. S10B-E). Fluorescence assays were performed by adding the samples on a Greiner 96 well plate black that was loaded into a Tecan Infinite M1000 PRO instrument. Multiple fluorescence intensity spectra were acquired with iControl software using excitation wavelengths of 280, 330, 368 and 405 nm by setting a step size and bandwidth of 5 nm and a manual gain of 199.

**pH dependence of Zn binding to kyn metabolites**

In water, 3HK displays two pKa values, pKₐ₁=1.9 and pKₐ₂=9.0 (Fig. S14). However, in DMSO-d₆, NMR analysis of the pD-dependence of chemical shifts allows to obtain the pKas of four protic groups (Fig. S15). In particular, the chemical shift of H2 is very sensitive to deprotonation of the carboxylic group whose pKa increases from 1.9 (in water) to 7.15 (in DMSO), indicating that the pD must be raised above this last value to favor Zn
binding. Similarly, XA displays only two apparent pKa values in water, pK_{a,1}=2.5 and pK_{a,2}=7.55 (Fig. S14), corresponding to the carboxylic and the phenol/quinoline nitrogen groups, respectively, as confirmed by NMR titration (Fig. S16). 1D ¹H NMR spectra of XA recorded at pH values ranging from 12 to 3, indicated complete loss of Zn at acidic pH, as inferred from the similarity of chemical shifts in the spectra of the complex and the free ligand.

**DFT studies of the Zn complexes with kyn metabolites**

A model for the 3HK-Zn complex was constructed considering all the experimental observations derived from ESI-MS and NMR. Namely, the coordination of a chloride ion to Zn was considered, as well as participation of the aliphatic region of 3HK, having the metal ion coordinated by the aliphatic amine, the carboxylate and the carbonyl group (Fig. S18A). Geometry optimization yielded a nearly tetrahedral Zn structure with metal-ligand distances that compare well with those derived from the EXAFS fits (Table S2). The lowest energy structure after geometry optimization corresponds to an orientation where the aliphatic carbonyl and the aromatic amine are in close proximity, which is in line with a short and symmetric distance between the aliphatic CH₂ (C3 in Fig. 4A) and the aromatic CH (C10 in Fig. 4A) as evidenced by the similarity of chemical shifts of the two geminal protons and by the dipolar connectivity derived from 2D ¹H-¹H ROE SY maps (Fig. S10A). From the broadening of ¹³C signals of 3HK-Zn-Cl (Fig. 4A), it is deduced that the aliphatic chain of 3HK is involved in chemical exchange between at least two different conformations, which correspond to the two stereoisomers depicted in Fig. S18A, whose calculated structural models give identical XAS features.

For the case of the Zn(XA)₂ complex, a model was constructed where the metal ion is coordinated by the quinoline nitrogen and the carboxylate group of each ligating XA, while the phenolic OH are not directly ligated to Zn (Fig. S18B). The Zn ion is a four-coordinate site in a distorted square planar geometry. A hydrogen bond between the carboxylate of one XA molecule and a phenyl hydroxyl group of a second XA stabilizes this structure. Comparing the metal-ligand distances of these two Zn(XA)₂ complexes (Table S3) to those derived from the EXAFS fits (Table S2), it becomes clear that the four-coordinate Zn model provides the best agreement with the experimentally derived data. While the phenol OH oxygens are not directly ligated to the Zn ion, they are at a long distance that models well the long Zn-O distances observed in the EXAFS fits. Solutions studies of the Zn(XA)₂ complex provide evidence for the formation of a deprotonated form of the complex at pH values above pK_{a,2}. A model was built for this species, where the two phenolic OH groups are deprotonated. The geometry optimization yielded an hexacoordinate Zn ion where the quinoline nitrogens, carboxylates and phenolic groups of each ligating XA are coordinated to the metal ion (Fig. S18C). The hexacoordinate Zn model yields a set of four Zn-O distances that are shorter than those observed for the protonated form of the complex (Fig. S18B), however metal ligand distances of the hexacoordinate structural model cannot be directly compared to experimental data, since the EXAFS spectrum of this species cannot be deconvoluted. Total energies and corresponding smallest vibrational frequencies of the optimized structures are presented in Table S4.
Fig. S1. (A) Flies raised under normal diet (black bar), under normal diet supplemented with 2.4 mM Trp (red bar) and under normal diet supplemented with 2.4 mM glutamate (Glu), 2.4 mM glycine (Gly), 2.4 mM phenylalanine (Phe), or 2.4 mM histidine (His) as indicated (blue bars). Trp addition increased Zn accumulation (asterisk indicates p<0.05; one-way ANOVA followed by post-hoc Tukey test). Addition of other amino acids had no effect. (B) Amino acid supplementation had no effect on Fe accumulation. See also Data S1. (C) Early third instar larvae of the v^{+} (black bars) or v^{-} (red bars) genotypes, raised previously under normal diet, were transferred to plain agar for 45 minutes, then for another 45 minutes to normal diet supplemented with 0.2% artificial blue color (Dosicolor) with or without 2.4 mM kyn as indicated. Each larva was then scored for the presence of blue food in their intestines. The experiment involved 10 individuals per genotype and condition and was repeated 6 times. Data are expressed as the percentage of larvae with presence of blue food in their intestines. Statistical analysis with 2-way-ANOVA showed no difference between genotype or treatment groups. (D) Initial procedure was as in (C), except 30 individuals per genotype and condition were grinded in water and blue absorbance was recorded as an indicator of ingested food. Statistical analysis with 2-way-ANOVA indicated difference between genotype (p<0.05). The presence of kyn in the diet did not affect feeding behavior. (E) Same as (D) except that the 2.4 mM kyn treatment was present throughout larval growth.
Fig. S2. (A) Zn : P ratio determined in organs dissected out of third instar larvae of indicated genotypes indicates that wild type larvae also accumulate Zn in MTs, just as previously shown for the adult flies (3). For metal determinations in dissected MTs, 8 pairs of MTs were digested in 200 µL of concentrated (65%) metal-free Suprapur® nitric acid.
at 60 °C for 12 hours and then brought to a final volume of 1 mL with Milli-Q water. (B) Portion of an anterior MT dissected out of a 3rd instar larva of the w+; ZnT35C/Cyo genotype grown for 24 h on a diet containing 1 mM Zn sulfate. Note that ZnT35C expression is particularly high in the transitional segment, present throughout the main segment, but absent in the distal segment. (C) MTs of wandering 3rd instar v1/ mutant larvae grown in the presence or absence of 2.4 mM kyn were treated with 1 µM Fluozine3 and imaged directly at the confocal microscope. Autofluorescence of granules coincides with Fluozin3 fluorescence. (D) MTs of early 3rd instar v1/ mutant larvae grown in the presence of 1 mM Zn were cultured ex vivo in the presence or absence of 2.4 mM kyn. The samples were also treated with DAPI to visualize nuclei (red). Note that addition of kyn ex vivo induces the formation of granules (arrows) while reducing the expression of ZnT35C (green). Treatment of 2nd instar larvae to induce the expression of ZnT35C was with 1 mM Zn sulfate for 24 h, 3 pairs of MTs from early 3rd instar larvae were placed in sterile 24-well plates containing Schneider’s medium supplemented with 1% fetal calf serum, 50 IU penicillin and 50 µg/ml streptomycin. MTs were treated for 18 h with 0.5 mM kyn at 25°C.
**Fig. S3.** Error bar plot of the normalized fluorescence spectrum of the granules of control (black) and vermilion (blue) larvae that were raised for 24 hours at 2nd instar stage on normal diet and diet supplemented with 2.4 mM kyn, respectively. Fluorescence images of malpighian tubules across the emission range 410 to 650 nm were obtained at each 5nm step with the Olympus FluoView™ FV1000 upon excitation at 405 nm. ImageJ was used to extract the integrated fluorescence density of 15 granules for each condition. To normalize the fluorescence of each granule in the range 0 to 1 the formula \[
\frac{(\text{fluorescence at } x \text{ nm} - \min(\text{fluorescence}))}{(\max(\text{fluorescence}) - \min \text{fluorescence})}
\] was applied. The inset corresponds to a scatter plot of the integrated density data showing the relative intensities before and after kyn treatment of \(v^1\) mutant larvae.
Fig. S4. (A) Normalized XANES and (B) $k^3$-weighted EXAFS of indicated MTs collected at SuperXAS beamline at SLS (black lines) and KMC-3 beamline at BESSY (red dashes). These spectra show the remarkable reproducibility of our biological samples as a function of mutation and alimentation.
**Fig. S5.** Normalized XANES of individual measurements from (A-D) indicated MTs or (E) MTP show no sign of radiation damage throughout the measurements. Shown are the first (red), a middle (blue) and the last (green) scan of a series from the same sample as well as the final average (black) seen in Fig. 3. For more clarity, insets show a zoom of the Zn absorption edge. Note that each scan is almost perfectly superimposable. MTs shown were measured at the SuperXAS beamline at SLS, whereas MTP was measured at the KMC-3 beamline at BESSY-II. Each scan from SuperXAS corresponds to roughly 20 minutes of X-ray exposure at \( \sim 10^{11} \) photons/s while scans at KMC-3 correspond to roughly 8 minutes at \( \sim 10^{11} \) photons/s.
Fig. S6. Unnormalized XANES of MTs show a drastic decrease of absolute Zn in $v^l$ compared to $w^+$ while addition of dietary kyn results in complete recovery. (A) Kα fluorescence detected Zn absorption relative to photon flux before the sample. The resulting XANES intensity, in a first approximation, corresponds to the absolute amount of Zn present in the sample. Spectra are background corrected with a linear fit and were collected at the SuperXAS beamline at SLS. (B) Integral of XANES from A quantifies the decrease in Zn in $v^l$ compared with $w^+$ at above 90%, in agreement with measurements from ICP-OES (Fig. S2A). Relative integrals compared to $w^+$ are indicated. Integrals were computed between 9600eV and 9750eV.
Fig. S7. Spectral composition analysis from normalized XANES and $k^3$-weighted EXAFS. Indicated spectra from MTs containing Zn granules (black lines) were reproduced (red dashes) by utilizing indicated spectra from MT samples without Zn storage granules (blue lines) and solid state reference compounds 3HK-Zn-Cl and Zn(XA)$_2$ (Fig. 3C,D). Spectral weights are indicated (see Table S1) and difference spectra are shown in grey.
Fig. S8. ESI-MS spectra of (A) 3HK with ZnCl\textsubscript{2} in DMSO-d\textsubscript{6} (pD 8.0); (B) 3HK with ZnCl\textsubscript{2} in H\textsubscript{2}O (pH 4.7); (C) L-Kyn with ZnCl\textsubscript{2} in DMSO-d\textsubscript{6} (pD 7.4); (D) L-Kyn with ZnCl\textsubscript{2} in H\textsubscript{2}O (pH 6.6); (E) 3HK with ZnI\textsubscript{2} in H\textsubscript{2}O (pH 6.6); (F) 3HK with CdCl\textsubscript{2} in H\textsubscript{2}O (pH 6.0). The ligand concentration is indicated on each spectrum with the assignment of the most relevant peaks. The calculated isotope mass distribution for the specified molecular formula is shown in the inset of each panel.
Fig. S9. (A) 1D $^{13}$C NMR spectra of free 3HK (red contours) and 3HK-Zn-Cl (black contours) in DMSO-$d_6$ (pD = 8.0). (B) 1D $^{13}$C NMR spectra of L-Kynurenine-Zn-Cl in D$_2$O (pD = 7.0). The structural formulas with atom numbering are shown at the top of each panel. $^{13}$C signals exchange broadened upon Zn binding are marked with red dots. The $^{13}$C signal of DMSO-$d_6$ and TSP are marked with asterisks.
Fig. S10. (A) Overlay of 2D $^1$H-$^{13}$C HSQC NMR spectra of free 3HK (red contours) and 3HK-Zn-Cl (black contours) in DMSO-d$_6$ (pD 8.0). The structural formula is shown as inset. UV-vis absorption (B, C) and fluorescence emission spectra (upon excitation at 368 nm) (D, E) of 3HK at the indicated concentrations, in the absence and presence of 1 equivalent of ZnCl$_2$, recorded in (B, D) 20 mM ammonium acetate buffer (pH 5.6) and (C, E) 50 mM HEPES with 100 mM NaCl (pH 7.5).
**Fig. S11.** 1D 1H NMR titration of XA with ZnCl₂ in D₂O (pD 12.3). The spectrum of the synthesized XA-Zn complex, dissolved in D₂O under the same conditions, is shown at the top.
Fig. S12. ESI-MS spectra of XA with ZnCl₂ in (A) 20 mM ammonium hydrogencarbonate (pH 8) and in (B) 20 mM ammonium acetate (pH 7) buffers. The ligand concentration is indicated on each spectrum with the assignment of the most relevant peaks. The calculated isotope mass distribution for the specified molecular formula is shown in the inset of each panel.
**Fig. S13.** (A) Overlay of 2D $^1$H-$^{13}$C HSQC NMR spectra of free XA (red contours) and XA-Zn (black contours) in D$_2$O (pD 12.3). The structural formula is shown as inset. UV-vis absorption (B, C, D) and fluorescence emission spectra (upon excitation at 368 nm) (E, F, G) of XA, in the absence and presence of up to 2 equivalents of ZnCl$_2$, recorded in (B, E) 50 mM sodium borate buffer (pH 9.0), (C, F) 50 mM HEPES with 100 mM NaCl (pH 7.5), (D, G) 20 mM ammonium acetate buffer (pH 5.6).
Fig. S14. pH titration of kyn metabolites XA (50 μM in NMP2 buffer) and 3HK (100 μM in NMP1 buffer), and their corresponding Zn complexes, as followed by electronic absorption spectroscopy. Following the drastic changes in absorption intensity (at 255 nm for XA and 420 nm for 3HK) as a function of pH, two pKa values can be identified in each case. 3HK displays two pKa values, $pK_{a,1} = 1.9$ and $pK_{a,2} = 9.0$, which are not perturbed upon addition of 25 μM Zn. XA displays two pKa values, $pK_{a,1} = 2.5$ and $pK_{a,2} = 7.55$; the latter is shifted to 7.2 in the corresponding Zn complex.
Fig. S15. (A) 1D $^1$H NMR pD titration of 3-HK in DMSO-d$_6$ as a function of added DCIO$_4$. (B) pKa values of different 3HK protic groups (identified by colored dots), as obtained by fitting the NMR chemical shift changes of the indicated protons as a function of pH. The low field resonance of OH (C7) reflects the formation of an intramolecular O–H⋯N hydrogen bond with NH$_2$ in ortho position. This implies that the hydroxyl proton is stably bound and there is little intermolecular exchange with residual water molecules in DMSO-d$_6$. By lowering the pD, the protonation of NH$_2$ (C6) causes a deshielding (due to decreased electron density and increased positive charge) and a new signal appears corresponding to NH$_3^+$ (C6). This is accompanied by the breakdown of the intramolecular hydrogen bond and the broadening of the hydroxyl proton, which begins to exchange efficiently with water and shifts further downfield.
Fig. S16. (A) 1D $^1$H NMR pD titration of XA and (B) Zn(XA)$_2$ in D$_2$O as a function of added DCIO$_4$. 
Fig. S17. (A) Normalized XANES, $k^3$-weighted EXAFS and Fourier transform of EXAFS of 3HK-Zn-Cl and Zn(XA)$_2$ reference compounds in solution and in solid state. Compounds in solution were prepared identically as for NMR experiments. The two solid state Zn(XA)$_2$ compounds shown in (B) were precipitated at different pH as indicated.
Fig. S18. DFT-derived geometry optimized structures for the Zn complexes with kyn metabolites: (A) 3HK-Zn, (B) Zn(XA)$_2$ and (C) Zn(XA)$_2$ in its deprotonated form. Notice in A the 3HK-Zn-Cl stereoisomer which yields the exact same ligand-metal distances for both. Structures were optimized with the BP functional in implicit solvation. See Table S3 for a comparison of different functionals with and without implicit solvation, Table S4 for the smallest vibrational frequencies of the optimized structures and Data S2 for the cartesian coordinates.
Fig. S19. Competition of 3HK and XA with Zn-bound Zincon. Absorption spectra were recorded at 25 °C in (A, B, C) 50 mM sodium borate buffer (pH 9.0) and (D, E, F) 10 mM HEPES with 100 mM NaCl (pH 7.5), using Zincon at a final concentration of 40 µM. The free Zincon shows an absorption band at 470 nm and the Zn-bound Zincon shows an absorption band at 620 nm. The color transition upon Zn binding is shown in the inset of panel (A). The plot of panel (D) shows the change in absorbance at 620 nm (ΔA_{obs,620 nm}) for Zincon at pH 7.5 as a function of Zn concentration (C_{Zn}). Experimental values are shown as blue dots and the fitted curve is in orange.
**SUPPLEMENTARY TABLES**

**Table S1.**
Weights of spectral composition shown in Fig. 3 resulting from minimization of sum of squared differences (SSD) between composition of indicated XANES or EXAFS spectra.

| Spec1  | Spec2  | Spec3  | Ref     | XANES | EXAFS |
|--------|--------|--------|---------|-------|-------|
|        |        |        |         | % Spec1 | % Spec2 | % Spec3 | SSD | % Spec1 | % Spec2 | % Spec3 | SSD |
| MT v1  | 3HK-Zn |        | MT w⁺   | 100%   | 0.02   | 100%   | 428 |
| MT v1  |        | XA-Zn  |         | 100%   | 0.27   | 100%   | 126 |
| MT v1  | 3HK-Zn |        | MT w⁺   | 32%    | 1.16   | 100%   | 539 |
| MT v1  |        | XA-Zn  |         | 27%    | 0.06   | 73%    | 80  |
|        | 3HK-Zn |        |         | 70%    | 0.07   | 30%    | 418 |
| MT v1  | 3HK-Zn | XA-Zn  | MT w⁺   | 19%    | 0.97   | 67%    | 20% |
| MT v1  |        |        |         | 70%    | 75%    | 30%    | 72  |
| MT p   | 3HK-Zn |        | MT w⁺   | 100%   | 0.93   | 100%   | 465 |
| MT p   |        | XA-Zn  |         | 28%    | 0.07   | 72%    | 73  |
| MT p   | 3HK-Zn |        | MT w⁺   | 25%    | 1.13   | 75%    | 41% |
| MT p   |        | XA-Zn  |         | 70%    | 59%    | 30%    | 397 |
| MT p   | 3HK-Zn |        | MT w⁺   | 14%    | 0.06   | 70%    | 15% |
| MT v1  | 3HK-Zn |        | MT w⁺   | 100%   | 0.33   | 100%   | 349 |
|        |        |        |         | 100%   | 0.33   | 100%   | 145 |
| MT p   | 3HK-Zn |        | MT w⁺   | 36%    | 0.07   | 64%    | 430 |
| MT p   |        | XA-Zn  |         | 66%    | 0.88   | 34%    | 331 |
| MT p   | 3HK-Zn |        | MT w⁺   | 66%    | 0.08   | 34%    | 55  |
| MT p   |        |        |         | 66%    | 0.08   | 34%    | 55  |
| MT p   | 3HK-Zn |        | MT w⁺   | 22%    | 0.06   | 64%    | 54  |
| MT p   |        |        |         | 22%    | 0.06   | 64%    | 54  |
| MT p   | 3HK-Zn |        | MT w⁺   | 100%   | 1.26   | 100%   | 417 |
| MT p   |        | XA-Zn  |         | 36%    | 0.07   | 64%    | 73  |
| MT p   | 3HK-Zn |        | MT w⁺   | 28%    | 1.03   | 72%    | 322 |
| MT p   |        | XA-Zn  |         | 66%    | 0.08   | 34%    | 33% |
| MT p   | 3HK-Zn |        | MT w⁺   | 18%    | 0.06   | 64%    | 50  |
|        |        |        |         | 18%    | 0.06   | 64%    | 50  |
Table S2. EXAFS Simulation Parameters

Interatomic distances from EXAFS analysis from solid state samples of XA-Zn (top) and 3HK-Zn (bottom). Spectra were collected at SuperXAS beamline and are shown, together with EXAFS simulation, in Figure 3D. Shown are elements of scattering shell, number of backscattering atoms in that shell (N), interatomic distance (Å) and Debye-Waller factor (σ). The shell column indicates backscatters which share the same Debye-Waller factor. For XA-Zn, coordination numbers were restricted to yield a sum of 2 representing two conformations corresponding to the protonated and unprotonated form (see pH titration in Fig. S14 and DFT geometry optimization in Fig. S18). For 3HK-Zn, the number of backscattering atoms (N) were fixed, motivated by results from DFT geometry optimizations and NMR.

| XA-Zn   | Element | N  | R / Å | 2σ² / Å² | Shell |
|---------|---------|----|-------|----------|-------|
| Zn-O    | 1.81    | 1.89| 0.004 | 1        |
| Zn-N    | 1.81    | 2.10| 0.004 | 1        |
| Zn-O    | 0.20    | 2.05| 0.004 | 1        |
| Zn-N    | 0.20    | 2.04| 0.004 | 1        |
| Zn-O    | 1.81    | 2.93| 0.004 | 2        |
| Zn-O    | 0.20    | 2.35| 0.004 | 2        |
| Zn-C    | 1.81    | 2.72| 0.004 | 2        |
| Zn-O    | 1.81    | 2.78| 0.004 | 2        |
| Zn-C    | 1.81    | 3.20| 0.004 | 2        |
| Zn-C    | 1.81    | 3.34| 0.004 | 2        |
| Zn-O    | 1.81    | 3.79| 0.004 | 2        |
| Zn-O    | 0.97    | 2.35| 0.007 | 3        |
| Zn-O    | 0.90    | 3.56| 0.007 | 3        |
| Zn-O    | 3.56    | 4.35| 0.007 | 3        |

| 3HK-Zn  | Element | N  | R / Å | 2σ² / Å² | Shell |
|---------|---------|----|-------|----------|-------|
| Zn-O    | 2       | 2.00| 0.004 | 1        |
| Zn-N    | 1       | 2.01| 0.004 | 1        |
| Zn-Cl   | 1       | 2.28| 0.004 | 1        |
| Zn-O    | 2       | 2.75| 0.007 | 2        |
| Zn-C    | 2       | 2.85| 0.002 | 3        |
Table S3

Metal ligand distances for the structural models of the Zn complexes with kyn metabolites (Fig. S18). The 3HK-Zn-Cl stereoisomers yield the exact same ligand-metal distances. Utilized functionals and use of implicit solvation are indicated.

|                 | Functional | Basis set | Solvation   | O(Carbonyl) | O(Carboxylate) | N (Amine) | Cl  | Cl  | C1  | C2  | C3  | C4  |
|-----------------|------------|-----------|-------------|-------------|----------------|-----------|-----|-----|-----|-----|-----|-----|
| 3HK-Zn-Cl       | BP         | def2-TZVP | gas phase   | 2.17        | 1.94           | 2.14      | 2.15| 2.71| 2.79| 3.45| 3.14|
|                 | B3LYP      | def2-TZVP | gas phase   | 2.16        | 1.94           | 2.15      | 2.16| 2.71| 2.79| 3.45| 3.13|
|                 | wB97X-D3   | def2-TZVP | gas phase   | 2.14        | 1.93           | 2.14      | 2.14| 2.69| 2.77| 3.43| 3.10|
|                 | BP         | def2-TZVP | SMD (water) | 2.22        | 2.07           | 2.11      | 2.25| 2.79| 2.82| 3.48| 3.18|
|                 | B3LYP      | def2-TZVP | SMD (water) | 2.26        | 2.06           | 2.12      | 2.28| 2.80| 2.84| 3.51| 3.21|
|                 | wB97X-D3   | def2-TZVP | SMD (water) | 2.22        | 2.05           | 2.10      | 2.25| 2.77| 2.82| 3.49| 3.17|

|                 | Functional | Basis set | Solvation   | O(Carboxylate) | N (Quinol) | O (Phenol) | C9  | C10 | C2  | C  | C (Carboxylate) | O(Carboxylate) |
|-----------------|------------|-----------|-------------|----------------|------------|------------|-----|-----|-----|----|----------------|----------------|
|                 | BP         | def2-TZVP | gas phase   | 1.97           | 2.06       | 3.09       | 3.53| 3.16| 2.78| 2.76| 3.96           |
|                 | B3LYP      | def2-TZVP | gas phase   | 1.96           | 2.07       | 3.03       | 3.51| 3.17| 2.79| 2.77| 3.95           |
|                 | wB97X-D3   | def2-TZVP | gas phase   | 1.96           | 2.04       | 2.96       | 3.46| 3.13| 2.76| 2.75| 3.94           |
|                 | BP         | def2-TZVP | SMD (water) | 2.03           | 2.11       | 3.12       | 3.54| 3.18| 2.84| 2.82| 4.03           |
|                 | B3LYP      | def2-TZVP | SMD (water) | 2.03           | 2.14       | 3.08       | 3.50| 3.17| 2.84| 2.82| 4.02           |
|                 | wB97X-D3   | def2-TZVP | SMD (water) | 2.02           | 2.09       | 2.98       | 3.47| 3.14| 2.82| 2.80| 4.00           |

|                 | Functional | Basis set | Solvation   | O(Carboxylate) | N (Quinol) | O (Phenol) | C9  | C10 | C2  | C  | C (Carboxylate) | O(Carboxylate) |
|-----------------|------------|-----------|-------------|----------------|------------|------------|-----|-----|-----|----|----------------|----------------|
|                 | BP         | def2-TZVP | gas phase   | 2.20           | 2.05       | 2.36       | 3.05| 2.97| 2.91| 2.98| 4.22           |
|                 | B3LYP      | def2-TZVP | gas phase   | 2.21           | 2.07       | 2.32       | 3.02| 2.97| 2.92| 2.99| 4.22           |
|                 | wB97X-D3   | def2-TZVP | gas phase   | 2.21           | 2.05       | 2.28       | 2.99| 2.95| 2.90| 2.98| 4.20           |
|                 | BP         | def2-TZVP | CPCM (water)| 2.60           | 2.08       | 2.14       | 2.88| 2.89| 3.05| 3.26| 4.53           |
|                 | B3LYP      | def2-TZVP | CPCM (water)| 2.68           | 2.11       | 2.10       | 2.88| 2.89| 3.06| 3.26| 4.58           |
|                 | wB97X-D3   | def2-TZVP | CPCM (water)| 2.59           | 2.07       | 2.10       | 2.86| 2.88| 3.04| 3.25| 4.49           |
Table S4
Total energies and smallest vibrational frequencies for the structural models of the Zn complexes with kyn metabolites (Fig. S18). Exchange-correlation functionals, basis sets, and the implicit solvation used are indicated. Total energies are in Hartree (a.u.) and frequencies in cm⁻¹.

| 3HK-Zn-Cl | Level of theory | Functional | Basis set | Solvation | Total Energies | Frequencies |
|-----------|-----------------|------------|-----------|------------|----------------|-------------|
|           |                 | BP         | def2-TZVP | gas phase  | -3038.462104   | 26.9        |
|           |                 | B3LYP      | def2-TZVP | gas phase  | -3038.215429   | 27.6        |
|           |                 | wB97X-D3   | def2-TZVP | gas phase  | -3037.947521   | 24.3        |
|           |                 | BP         | def2-TZVP | SMD (water)| -3038.524097   | 34.6        |
|           |                 | B3LYP      | def2-TZVP | SMD (water)| -3038.283004   | 40.8        |
|           |                 | wB97X-D3   | def2-TZVP | SMD (water)| -3038.014477   | 32.3        |

| XA-Zn-XA | Level of theory | Functional | Basis set | Solvation | Total Energies | Frequencies |
|----------|-----------------|------------|-----------|------------|----------------|-------------|
|          |                 | BP         | def2-TZVP | gas phase  | -3261.079475   | 15.6        |
|          |                 | B3LYP      | def2-TZVP | gas phase  | -3260.843598   | 16.1        |
|          |                 | wB97X-D3   | def2-TZVP | gas phase  | -3260.356366   | 15.6        |
|          |                 | BP         | def2-TZVP | SMD (water)| -3261.148331   | 14.5        |
|          |                 | B3LYP      | def2-TZVP | SMD (water)| -3260.920542   | 21.0        |
|          |                 | wB97X-D3   | def2-TZVP | SMD (water)| -3260.432460   | 13.0        |

| XA-Zn-XA deprotonated | Level of theory | Functional | Basis set | Solvation | Total Energies | Frequencies |
|-----------------------|-----------------|------------|-----------|------------|----------------|-------------|
|                       |                 | BP         | def2-TZVP | gas phase  | -3259.951543   | 16.1        |
|                       |                 | B3LYP      | def2-TZVP | gas phase  | -3259.708934   | 15.9        |
|                       |                 | wB97X-D3   | def2-TZVP | gas phase  | -3259.211319   | 12.9        |
|                       |                 | BP         | def2-TZVP | SMD (water)| -3260.233759   | 14.0        |
|                       |                 | B3LYP      | def2-TZVP | SMD (water)| -3259.997825   | 22.6        |
|                       |                 | wB97X-D3   | def2-TZVP | SMD (water)| -3259.503818   | 21.5        |
Data S1. (separate file)
Raw data for elemental determinations (Zn, Fe, Cu, Mn & P) including those presented in
Fig. 1 and S1, organized in different spreadsheets as per experiment (Fig. 1B, C - “genes”; 
Fig. 1D - “diet rescue”; Fig. 1E - “RNAi”; Fig. 1F-I - “defined media”; Fig. S1 - “aa 
supplements”; Fig. S2 “tissues”). The number of separate determinations (n) is provided 
first, followed by the genotype, followed by the diet used or the type of sample analyzed 
as appropriate. Mean values and standard deviation from the mean have been calculated 
for easy reference.

Data S2. (separate file)
Cartesian coordinates for the four optimized structures depicted in Fig. S18 copied into a 
single txt file. The structures were optimized using the BP exchange-correlation functional, 
the def2-TZVP basis set, and the SMD implicit solvation model with the dielectric constant 
for water. The corresponding total energies and smallest vibrational frequencies are 
reported in Table S4.

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