Supporting information for

Nanoparticle Size and Coating Chemistry Control Foliar Uptake Pathways, Translocation and Leaf-to-Rhizosphere Transport in Wheat

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Table S. 1: Gold nanoparticle characteristics depending on their size and their coating. Different lower-case letters indicate significant differences between the mean of the groups (ANOVA test followed by Fisher's LSD test for multiple comparisons, $p \leq 0.05$).

Size denomination	Coating	Primary diameter (nm) *	Hydrodynamic diameter (nm)	Zeta potential (mV) **
3	Citrate	2.6±1.0 [c]	5.3±1.5 [c]	-69.2 ±1.3 [f]
	PVP	2.7±1.2 [c]	6.6 ± 1.4 [c]	-56.8 ± 0.6 [d]
10	Citrate	9.5±0.9 [b]	11.3 ± 2.9 [b]	-50.9 ± 0.6 [c]
	PVP	9.2±1.5 [b]	16.6 ±5.1 [b]	-47.6 ± 2.0 [b]
50	Citrate	53.4±11.4 [a]	61.0 ± 22.4 [a]	-60 ±1.2 [e]
	PVP	59.1±14.8 [a]	80.3 ± 25.8 [a]	-31.1 ±1.0 [a]

* measured with TEM images of two different magnifications on at least 100 particles
** 10 mg/l AuNPs, 1mM KCl, pH 6.8.

Table S. 2: Au count changes in the transversal sections of µ-XFM images of leaves exposed to 12nm citrate- or PVP-AuNPs over time. Fluorescence counts were normalized by I0. Slits and detector position were similar between measurements.

Treatment	Au fluorescence counts in the transversal section				Au % leaving the transversal section between 12h and 36h	
	Total		Associated with Zn counts > $1.2x10^8$		Total	Associated with Zn counts > $1.2x10^8$
	12h	36h	12h	36h		
Citrate-AuNPs	$1.94 x10^{10}$	$1.14 x10^{10}$	$1.48x10^{10}$	$7.21x10^9$	31%	94%
PVP-AuNPs	$7.48 x10^{10}$	$6.12 x10^{10}$	$1.76 x10^{10}$	$8.51 x10^9$	18%	67%

Table S. 3: Growth conditions and treatments for different type of analysis

Growth matrix	Application	Time of exposure	Measurement	Type of analysis
Hydroponic	Spraying of 30µl	12 and 36 h	Uptake and translocation	Large scale µ-XRF mapping at the AS synchrotron
	Deposition of 4 drops of 7µl	48 h		High resolution µ-XRF mapping at NSLS-II synchrotron
Lufa 2.2 soil		7 days	Transport and impact	Total metal analysis and plant health measurement

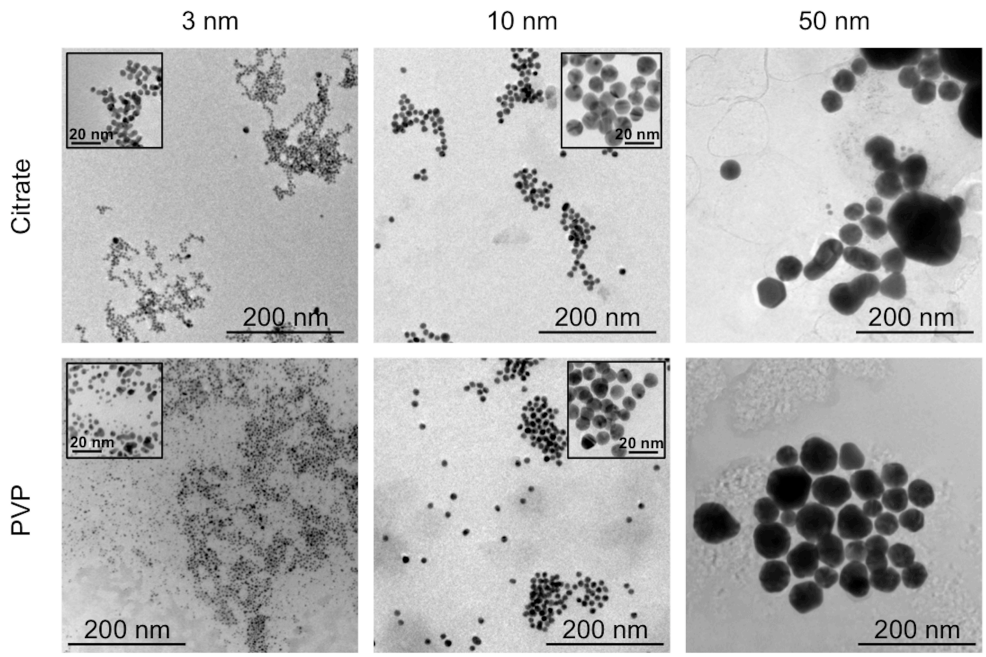

Figure S. 1: TEM images of different AuNPs. A 7µl drop of a 10 mg/L AuNP suspension was deposited and dried on a Cu grid with a carbon film before imaging.

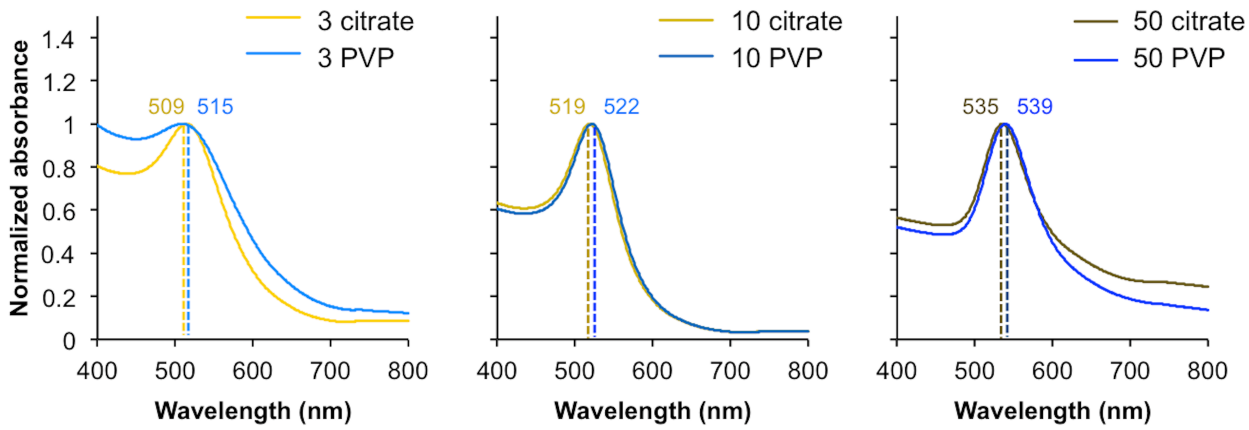

Figure S. 2: UV absorption spectra for suspensions of AuNPs with different sizes and coatings collected at 100 mg/L.

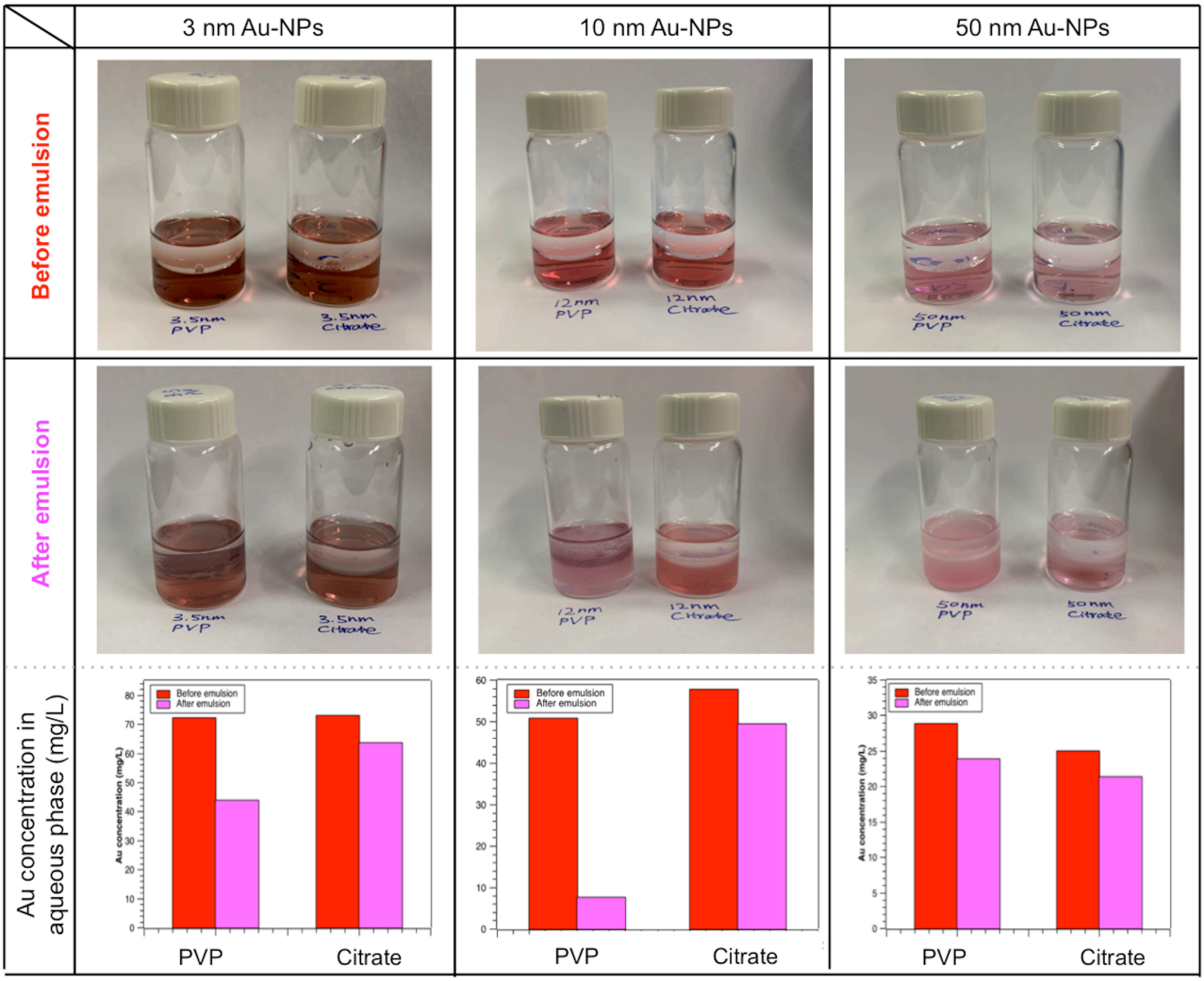

Figure S. 3: AuNP dodecane-water partitioning. 4ml of AuNPs suspension in water with different coatings and sizes were probe sonicated with 4ml deodecane and allowed to settle for 24hs. Au concentrations in the water phase were measured by ICP-MS before sonication and 24hs after sonication.

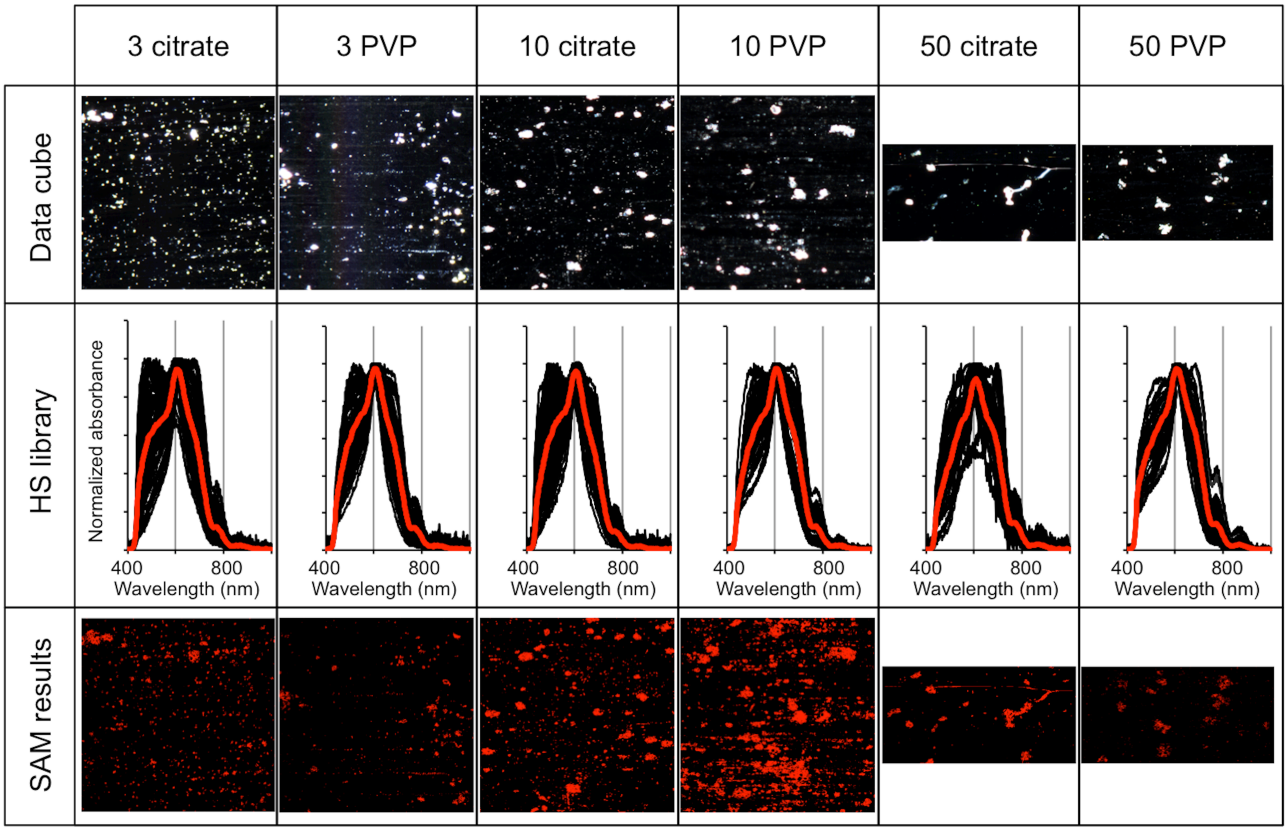

Figure S. 4: Enhanced dark-field microscope data cubes containing the hyperspectral signal in each pixel of AuNPs suspensions of different sizes and coatings. Hyperspectral libraries of the materials were built as previously described[1], after *(i)* spectral data reduction using coherent minimum noise fraction images to separate pixels containing noise and *(ii)* endmember identification using a pixel purity index to find and group the pixels containing similar AuNPs hyperspectral signals. These AuNPs hyperspectral libraries were used to highlight the pixels containing similar hyperspectral signal using a spectral angular mapping algorithm (SAM) and an angle of 0.085 rad.

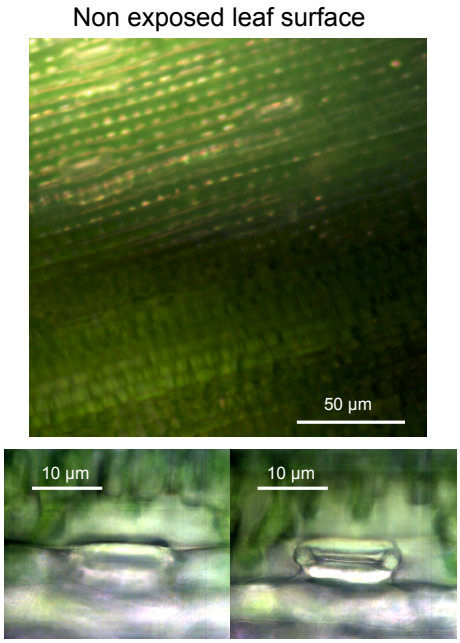

Figure S. 5: EDF-HSI data cube of a control leaf exposed to DIW. No pixels were found to contain the AuNP hyperspectral library signatures. (SAM, 0.085 rad)

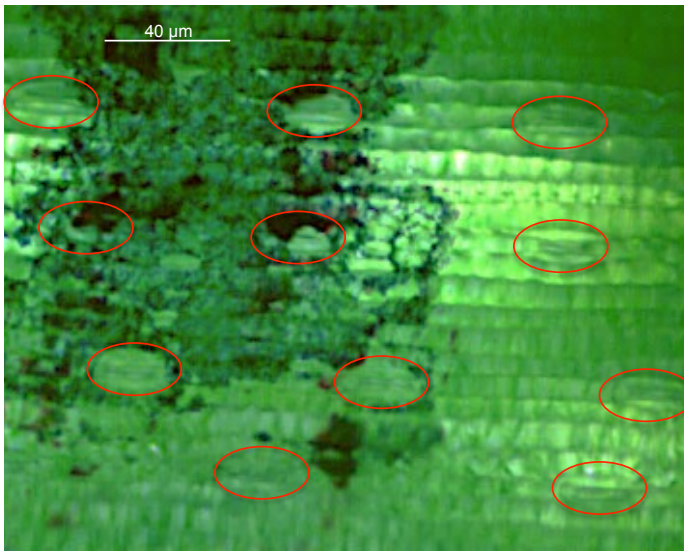

Figure S. 6: Image of the leaf surface exposed to 3.5 nm citrate-AuNPs for 7 days without rinsing. The circles indicate the locations of the stomata.

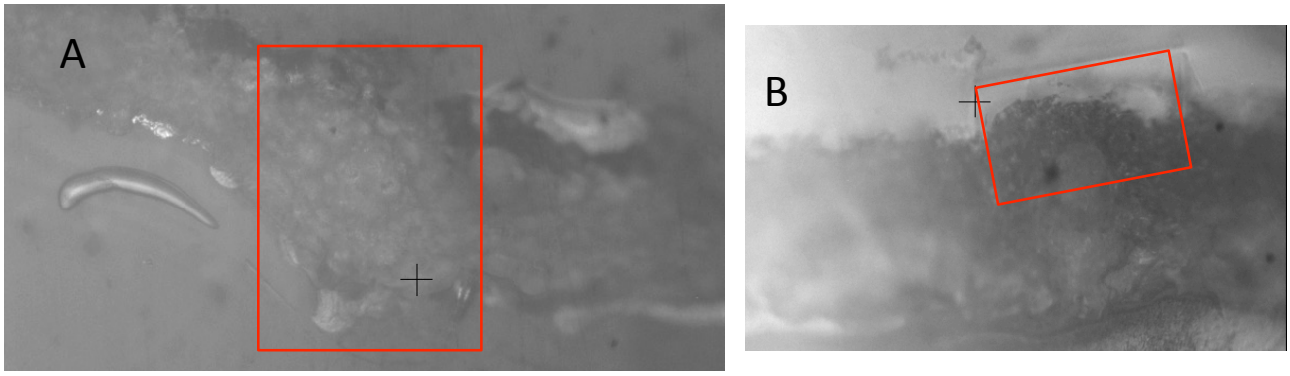

Figure S. 7 Cross sections of leaves exposed to (A) citrate-AuNPs or (B) PVP-AuNPs used for XFM mappings. Red rectangles show the area scanned

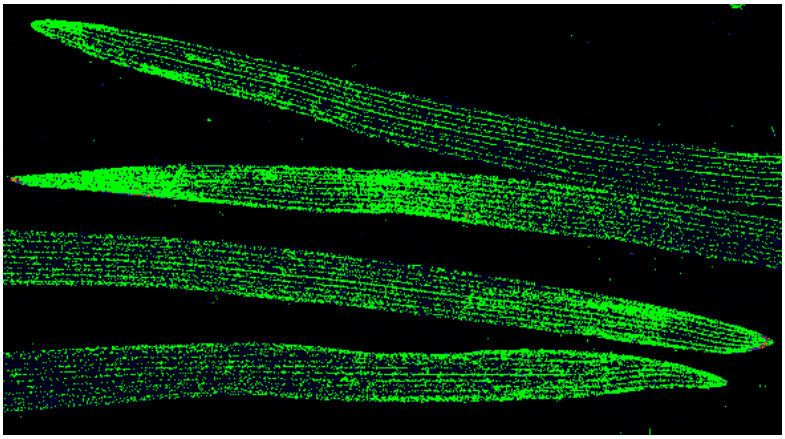

Figure S. 8: Pixels showing Zn Kα fluorescence counts higher than 1.2E8 are mainly present in the leaf vasculature.

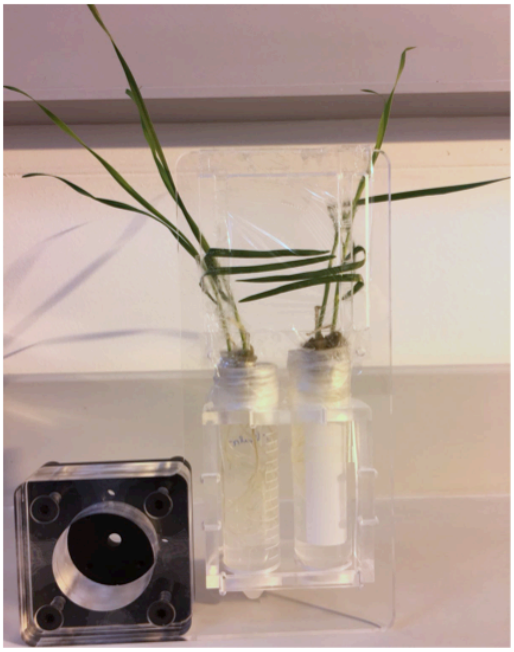

Figure S. 9: Wheat plant mounting setup for µ-XFM measurement over time. Leaves exposed to AuNPs were mounted in between ultralene layers, while the plants were kept alive in hydroponic solution

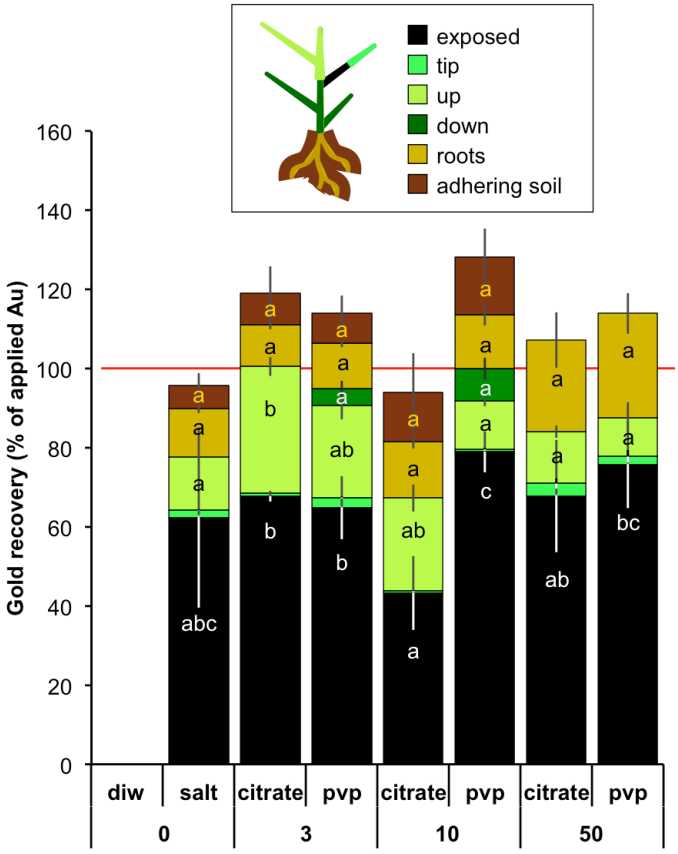

Figure S. 10: Gold nanoparticle (AuNPs) transport in wheat. Au mass balance in wheat 7 days after foliar exposure to 10µg/l Au suspensions (4 x 7µl drop deposition). Au percentage is calculated based on the mass of Au added in the drop. Different lower-case letters indicate significant differences between the mean of the groups (ANOVA test followed by Fisher's LSD test for multiple comparisons, $p \leq 0.05$).

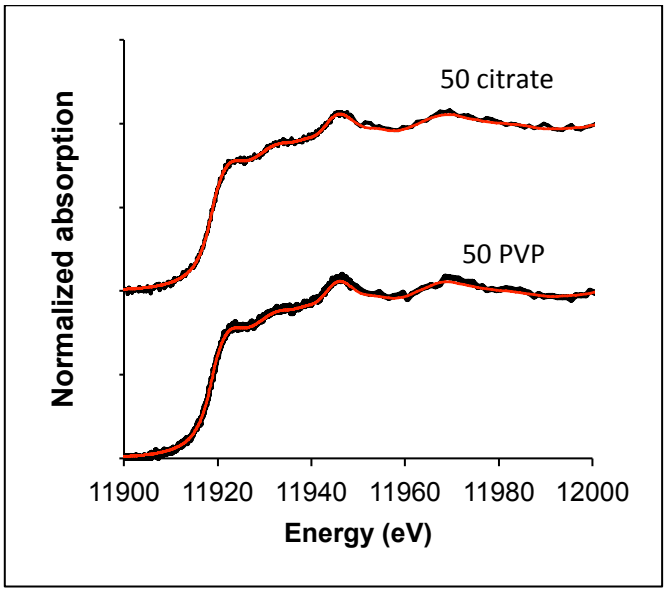

Figure S. 11: X-ray absorption near edge structure at Au L_{III}-edge of wheat roots foliar exposed to 50 nm citrate-AuNPs or PVP-AuNPs for 7 days. Linear combination fitting with a reference metallic Au^0 nanoparticle (in red) indicated that 100% of the Au associated to wheat roots were metallic Au. Fit quality factors were for citrate- and PVP-coated AuNPs exposure, respectively: R_{factor}= 7x10^{-4} and 13x10^{-4} , χ^2= 5x10^{-2} and 9x10^{-2}.

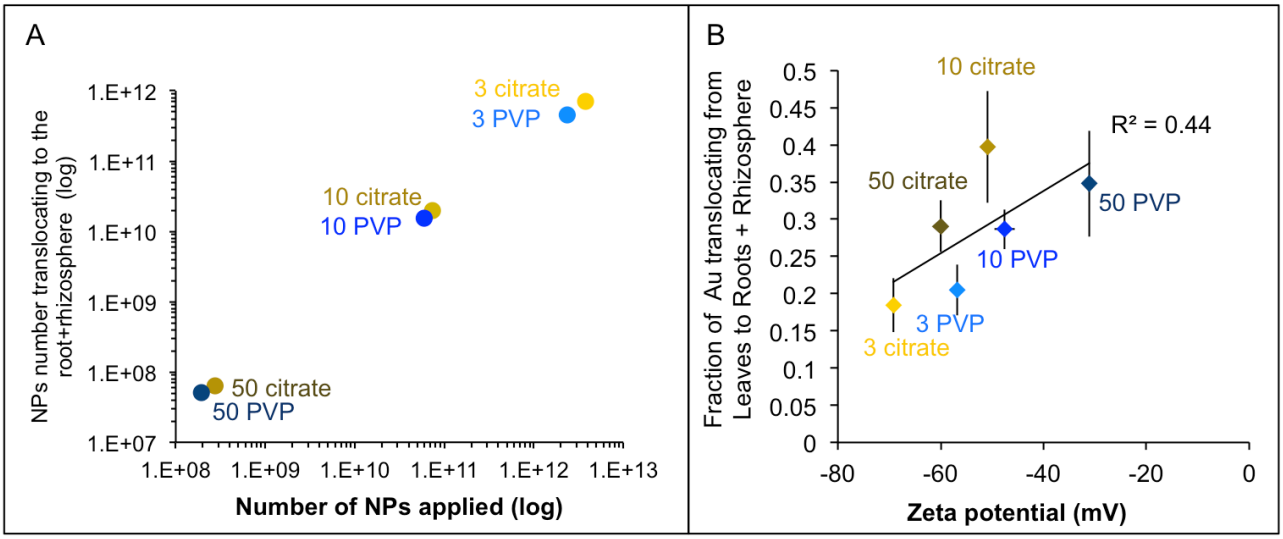

Figure S. 12: A. Number of AuNPs translocated to roots after foliar application *vs.* the number of AuNPs applied. The number of AuNPs was calculated based on the mass of Au measured in the tissues, the average primary diameter of each type of AuNP, and gold density (19.3 g.cm^{-3}). B. Correlation between the translocation of AuNPs from the leaves to the root system (roots + rhizosphere) and the zeta potential of the AuNPs of different sizes and coating.

Cited references

(1) Avellan, A.; Schwab, F.; Masion, A.; Chaurand, P.; Borschneck, D.; Vidal, V.; Rose, J.; Santaella, C.; Levard, C. Nanoparticle Uptake in Plants: Gold Nanomaterial Localized in Roots of Arabidopsis Thaliana by X-Ray Computed Nanotomography and Hyperspectral Imaging. *Environ. Sci. Technol.* 2017, *51*, 8682–8691.