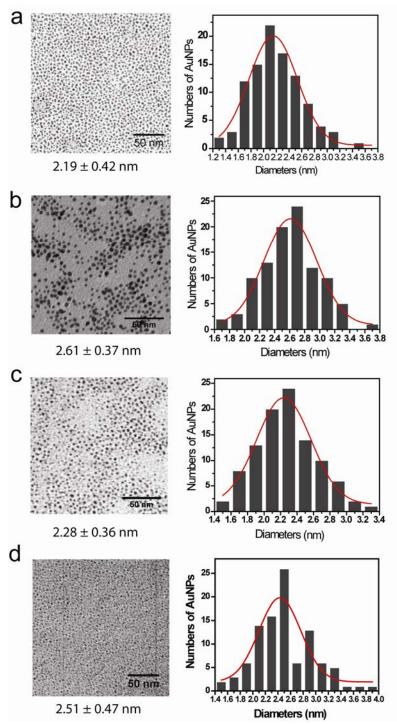
## Supporting Information for Small

Surface properties dictate uptake, distribution, excretion, and toxicity of nanoparticles in fish

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**Figure S1. TEM images and size distributions of AuNPs. a**, AuNP **1**; **b**, AuNP **2**; **c**, AuNP **3**; **d**, AuNP **4**. The size distributions are shown in the right panels as histograms and fitted Gaussian curves (red line). In each histogram, 100 nanoparticles were randomly measured.

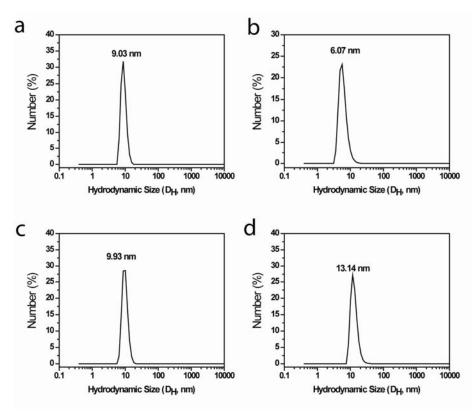
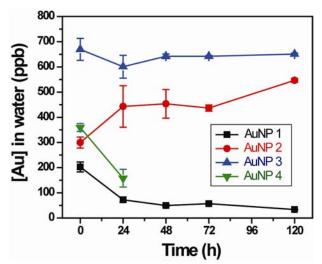


Figure S2. Hydrodynamic sizes of AuNPs in conditioned water. a, AuNP 1; b, AuNP 2; c, AuNP 3; d, AuNP 4.



**Figure S3.** Au concentration in tank water during the course of the exposure experiments. A single dose of 20 nM AuNPs was added to the water tank 30-60 minutes before adding the fish. During this 30-60 minute period, the water was aerated via an air-stone. During the aeration period, some of the AuNPs stick to the surface of the fish tanks, causing a drop in the total Au concentration. The time when the fish are added to the tank was set as 0 h. Aliquots (5 mL) of water were taken at 0, 24, 48, 72, and 120 h and analyzed by ICP-MS. Means in the figure are obtained from 3-6 measurements, and the errors bars represent the standard error of the mean.

Table S1. Biodistribution of AuNPs 1-3 in fish organs at 24 hr.

| Tuble 51. Blouistibution of flui (151 c m fish of gains at 21 m) |                      |                     |                    |                     |                    |                 |                    |
|--|----------------------|---------------------|--------------------|---------------------|--------------------|-----------------|--------------------|
| Organs   | Control <sup>#</sup> | AuNP 1 <sup>#</sup> |                    | AuNP 2 <sup>#</sup> |                    | AuNP 3#         |                    |
|  | Au (ng/mg)           | Au (ng/mg)          | p <sup>&amp;</sup> | Au (ng/mg)          | p <sup>&amp;</sup> | Au (ng/mg)      | p <sup>&amp;</sup> |
| Brain  | $0.35 \pm 0.14$      | $1.10 \pm 0.49$     | 0.162              | $0.27 \pm 0.08$     | 0.609              | $0.20 \pm 0.04$ | 0.186              |
| Heart  | $2.28\pm1.11$        | $19.0 \pm 9.59$     | 0.106              | $5.03 \pm 3.68$     | 0.485              | $1.57\pm0.77$   | 0.610              |
| liver  | $0.19 \pm 0.09$      | $1.12 \pm 0.57$     | 0.126              | $0.22 \pm 0.09$     | 0.777              | $0.18 \pm 0.04$ | 0.924              |
| Gonad  | $0.13 \pm 0.05$      | $12.1\pm11.1$       | 0.298              | $0.18 \pm 0.08$     | 0.573              | $0.09 \pm 0.03$ | 0.469              |
| Dorsal fin   | $1.35 \pm 0.76$      | $1.03 \pm 0.19$     | 0.684              | $3.41 \pm 2.69$     | 0.472              | $0.76 \pm 0.32$ | 0.485              |
| Gill   | $0.24 \pm 0.09$      | $5.07 \pm 1.38$     | 0.004              | $0.53 \pm 0.11$     | 0.054              | $0.16 \pm 0.03$ | 0.423              |
| Intestine  | $0.12 \pm 0.05$      | $35.2 \pm 9.10$     | 0.002              | $8.15 \pm 3.19$     | 0.025              | $7.35 \pm 3.68$ | 0.070              |

<sup>&</sup>lt;sup>#</sup> The concentration unit refers to ng of Au per mg of organ weight; means are averaged from eight fish (n=8), and the errors are reported as the standard error of the mean. <sup>&</sup> p-value of one-way ANOVA between control and AuNP treated groups.

Table S2. Biodistribution of AuNPs 1-3 in fish organs at 72 hr.

| Organs     | Control <sup>#</sup> | AuNP 1 <sup>#</sup> |          | AuNP 2 <sup>#</sup> |          | AuNP 3#           |          |
|------------|----------------------|---------------------|----------|---------------------|----------|-------------------|----------|
|            | Au (ng/mg)           | Au (ng/mg)          | $p^{\&}$ | Au (ng/mg)          | $p^{\&}$ | Au (ng/mg)        | $p^{\&}$ |
| Brain      | $0.47 \pm 0.16$      | $0.72 \pm 0.26$     | 0.240    | $0.54 \pm 0.16$     | 0.399    | $0.22 \pm 0.08$   | 0.409    |
| Heart      | $5.45 \pm 2.72$      | $14.66 \pm 7.27$    | 0.115    | $3.41 \pm 1.27$     | 0.512    | $1.43 \pm 0.41$   | 0.484    |
| liver      | $0.46 \pm 0.18$      | $0.74 \pm 0.27$     | 0.074    | $0.23 \pm 0.08$     | 0.736    | $0.17 \pm 0.07$   | 0.866    |
| Gonad      | $0.30 \pm 0.15$      | $1.45 \pm 0.55$     | 0.032    | $0.51 \pm 0.27$     | 0.195    | $0.54 \pm 0.31$   | 0.207    |
| Dorsal fin | $1.38 \pm 0.69$      | $5.21 \pm 2.06$     | 0.100    | $1.35 \pm 0.51$     | 0.999    | $0.62 \pm 0.17$   | 0.364    |
| Gill       | $0.25 \pm 0.12$      | $2.17 \pm 0.42$     | 0.0005   | $0.63 \pm 0.08$     | 0.005    | $0.33 \pm 0.09$   | 0.437    |
| Intestine  | $0.08 \pm 0.02$      | $144 \pm 25.6$      | 0.00006  | $11.19 \pm 2.75$    | 0.0013   | $40.85 \pm 13.71$ | 0.010    |

<sup>\*</sup> The concentration unit refers to ng of Au per mg of organ weight; means are averaged from eight fish (n=8), and the errors are reported as the standard error of the mean. \*p-value of one-way ANOVA between control and AuNP treated groups.

Table S3. Biodistribution of AuNPs 1-3 in fish organs at 120 hr.

| Organs     | Control <sup>#</sup> | AuNP 1 <sup>#</sup> |          | AuNP 2 <sup>#</sup> |          | AuNP 3#         |          |
|------------|----------------------|---------------------|----------|---------------------|----------|-----------------|----------|
|            | Au (ng/mg)           | Au (ng/mg)          | $p^{\&}$ | Au (ng/mg)          | $p^{\&}$ | Au (ng/mg)      | $p^{\&}$ |
| Brain      | $0.15 \pm 0.06$      | $0.34 \pm 0.07$     | 0.091    | $0.09 \pm 0.01$     | 0.364    | $0.04 \pm 0.02$ | 0.138    |
| Heart      | $0.77 \pm 0.32$      | $3.63 \pm 2.83$     | 0.355    | $0.41 \pm 0.10$     | 0.324    | $1.03 \pm 0.35$ | 0.618    |
| liver      | $0.05 \pm 0.03$      | $0.32 \pm 0.20$     | 0.226    | $0.11 \pm 0.04$     | 0.308    | $0.15 \pm 0.05$ | 0.168    |
| Gonad      | $0.08 \pm 0.06$      | $0.09 \pm 0.03$     | 0.889    | $0.08 \pm 0.03$     | 0.910    | $0.23 \pm 0.11$ | 0.260    |
| Dorsal fin | $0.27 \pm 0.14$      | $2.18 \pm 1.15$     | 0.151    | $1.11 \pm 0.50$     | 0.154    | $0.47 \pm 0.29$ | 0.566    |
| Gill       | $0.10 \pm 0.04$      | $1.09 \pm 0.08$     | 0.00003  | $0.48 \pm 0.09$     | 0.008    | $0.24 \pm 0.03$ | 0.021    |
| Intestine  | $0.03 \pm 0.01$      | $75.4 \pm 21.6$     | 0.013    | $9.95 \pm 3.00$     | 0.017    | $13.3 \pm 7.29$ | 0.118    |

<sup>\*</sup> The concentration unit refers to ng of Au per mg of organ weight; means are averaged from eight fish (n=8), and the errors are reported as the standard error of the mean. \*p-value of one-way ANOVA between control and AuNP treated groups.

**Table S4.** Biodistribution of AuNP **4** in fish organs.

| 0          | AuNP 4 <sup>#</sup> |                    |  |  |
|------------|---------------------|--------------------|--|--|
| Organs     | Au (ng/mg)          | p <sup>&amp;</sup> |  |  |
| Brain      | $1.29 \pm 0.15$     | 0.0004             |  |  |
| Heart      | $15.95 \pm 5.27$    | 0.024              |  |  |
| liver      | $0.99 \pm 0.23$     | 0.006              |  |  |
| Gonad      | $6.05 \pm 3.97$     | 0.159              |  |  |
| Dorsal fin | $14.30 \pm 3.44$    | 0.002              |  |  |
| Gill       | $25.45 \pm 5.71$    | 0.0004             |  |  |
| Intestine  | $0.86 \pm 0.29$     | 0.026              |  |  |

<sup>&</sup>quot;#" and "&" are the same as in Tables S1, S2, and S3. All of the fish died in 24 hrs after exposure to AuNP 4.