

Supporting Information

Interaction of Colloidal Gold Nanoparticles with Urine and Saliva Biofluids: An Exploratory Study

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A. Characterization of AuNPs

A.1 Physicochemical characterization of Au NPs

Table S1: Particle dimensions, hydrodynamic diameter (HD) and respective polydispersity index (PDI), zeta potential (ZP) and respective pH of as synthesized Au NPs.

	Size (nm) ^a	HD (nm)	PDI	ZP (mV)	pH
AuNSs	55.5 ± 8.2	46.3 ± 0.7	0.323 ± 0.002	-61.0 ± 0.3	6.3
AuNFs	89.6 ± 15.2	77.9 ± 1.3	0.199 ± 0.004	-58.1 ± 1.0	3.9
AuNRs	20.5 ± 7.3 ^b	79.6 ± 6.6	0.192 ± 0.030	71.3 ± 4.9	2.6
	7.1 ± 1.3 ^c				

a) Assessed by transmission electron microscopy analysis; b) length; c) width

A.1. Optical spectra of AuNPs before and after incubation with biofluids

Figure S1 shows the optical spectra of AuNSs, AuNFs and AuNRs before (as synthesized) and after incubation for 15, 30 and 60 minutes with saliva and urine samples.

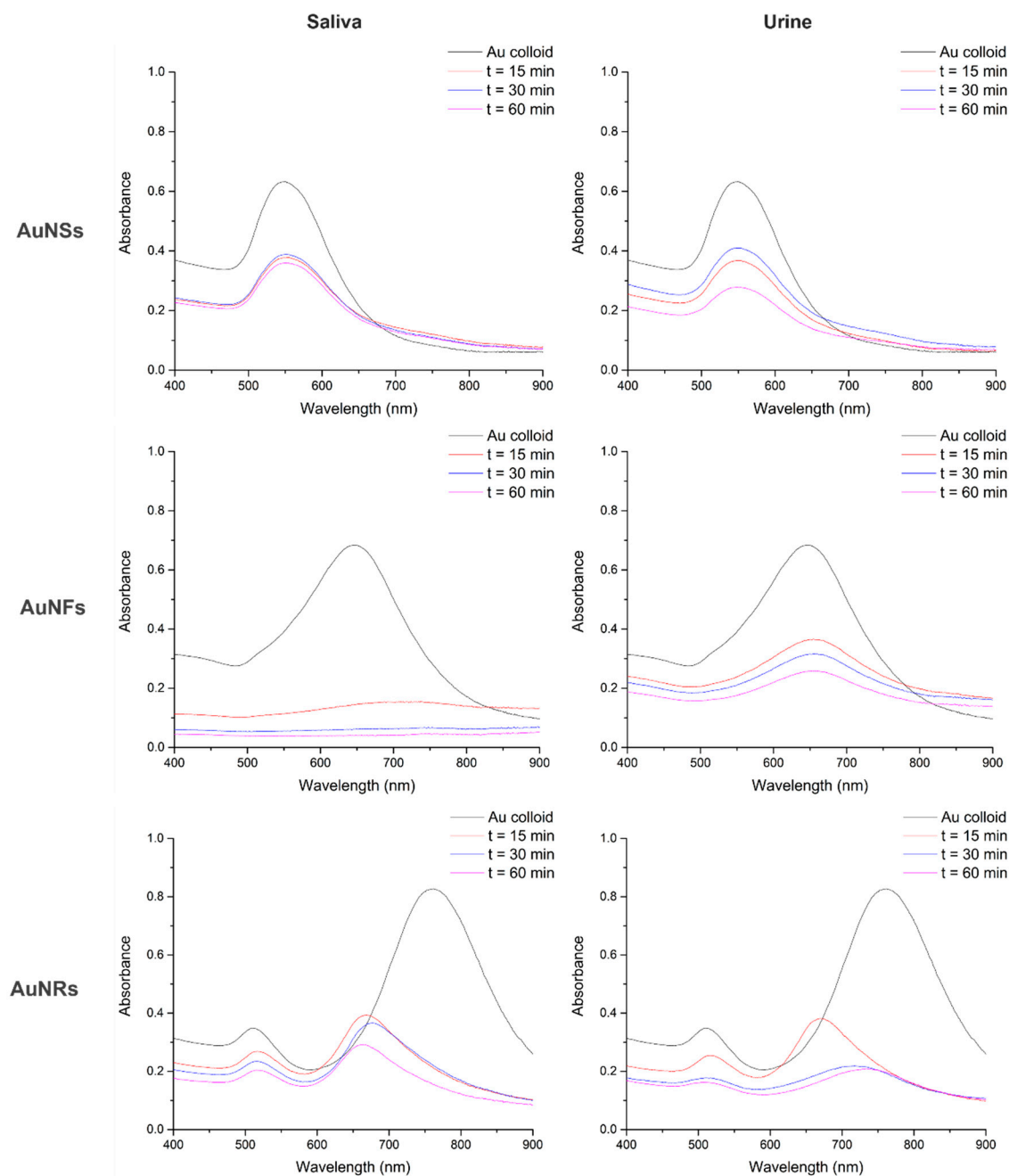


Figure S1: Optical spectra of AuNSs, AuNFs and AuNRs before and after incubation with saliva and urine for 15, 30 and 60 minutes.

A.2. Zeta potential and hydrodynamic diameter AuNPs

The AuNFs demonstrate a high aggregation tendency over time when incubated with saliva, consequently, they were characterized through polydisperse index (PDI), as table S1 presents.

Table S2: PDI of AuNPs before and after being in contact with saliva.

Sample	Time (minutes)	PDI
AuNFs	0	0.199 ± 0.015
	15	0.886 ± 0.047
	30	1 ± 0.001
	60	0.586 ± 0.049

B. Protein analysis by LC-MS/MS

Table S3: Protein ID according to Uniprot code and protein respective name identified on the surface of AuNSs and AuNRs.

Protein ID	Name
P15515	Histatin-1
P05109	Protein S100-A8
P01040	Cystatin-A
P04080	Cystatin-B
P0DOY2	Immunoglobulin lambda constant 2
P01834	Immunoglobulin kappa constant
P01700	Immunoglobulin lambda variable 1-47
P01619	Immunoglobulin kappa variable 3-20
P01624	Immunoglobulin kappa variable 3-15
A0A0A0MRZ8	Immunoglobulin kappa variable 3D-11
P01764	Immunoglobulin heavy variable 3-23
A0A0C4DH38	Immunoglobulin heavy variable 5-51
A0A0B4J1X5	Immunoglobulin heavy variable 3-74
P01599	Immunoglobulin kappa variable 1-17
A0A0C4DH31	Immunoglobulin heavy variable 1-18
P01780	Immunoglobulin heavy variable 3-7
A0A0C4DH68	Immunoglobulin kappa variable 2-24
P06702	Protein S100-A9
A0A0B4J1Y9	Immunoglobulin heavy variable 3-72
P06310	Immunoglobulin kappa variable 2-30
P01614	Immunoglobulin kappa variable 2D-40
P01824	Immunoglobulin heavy variable 4-39
P69905	Hemoglobin subunit alpha
P01034	Cystatin-C
P02766	Transthyretin
P68871	Hemoglobin subunit beta
P28325	Cystatin-D
P01036	Cystatin-S
P09228	Cystatin-SA
P01037	Cystatin-SN
P61626	lysozyme c
P12273	Prolactin-inducible protein
P01591	Immunoglobulin J chain
P80188	Neutrophil gelatinase-associated lipocalin
Q6MZM9	Proline-rich protein 27
Q96DA0	Zymogen granule protein 16 homolog B
P0DOX8	Immunoglobulin lambda-1 light chain
P01033	Metalloproteinase inhibitor 1
P09211	Glutathione S-transferase P
P0DOX7	Immunoglobulin kappa light chain
Q96DR5	BPI fold-containing family A member 2

P06870	Kallikrein-1
P54108	Isoform 3 of Cysteine-rich secretory protein 3
P25311	Zinc-alpha-2-glycoprotein
P23280	Carbonic anhydrase 6
P01859	Immunoglobulin heavy constant gamma 2
P04406	Glyceraldehyde-3-phosphate dehydrogenase
P01876	Immunoglobulin heavy constant alpha 1
P07858	Cathepsin B
Q6P5S2	Protein LEG1 homolog
Q8TAX7	Mucin-7
P60709	Actin, cytoplasmic 1
P07339	Cathepsin D
P29508	Serpin B3
P01009	Alpha-1-antitrypsin
P06733	Alpha-enolase
Q04695	Keratin, type I cytoskeletal 17
P20061	Transcobalamin-1
P0DOX2	Immunoglobulin alpha-2 heavy chain
Q8N4F0	BPI fold-containing family B member 2
P0DOX5	Immunoglobulin gamma-1 heavy chain
P13646	Keratin, type I cytoskeletal 13
P80303	Nucleobindin-2
P08779	Keratin, type I cytoskeletal 16
P02533	Keratin, type I cytoskeletal 14
Q02818	Nucleobindin-1
P02774	Isoform 3 of Vitamin D-binding protein
Q14CN4	Keratin, type II cytoskeletal 72
P04745	Alpha-amylase 1
P10909	Isoform 2 of Clusterin
P07602	Isoform Sap-mu-9 of Prosaposin
P13645	Keratin, type I cytoskeletal 10
P48668	Keratin, type II cytoskeletal 6C
Q7Z794	Keratin, type II cytoskeletal 1b
P35527	Keratin, type I cytoskeletal 9
P13647	Keratin, type II cytoskeletal 5
Q5T749	Keratinocyte proline-rich protein
Q08380	Galectin-3-binding protein
P35908	Keratin, type II cytoskeletal 2 epidermal
P04264	Keratin, type II cytoskeletal 1
P02768	Serum albumin
Q14515	SPARC-like protein 1
P02787	Serotransferrin
P02788	Lactotransferrin
P22079	Lactoperoxidase
P14923	Junction plakoglobin
P01833	Polymeric immunoglobulin receptor

P09958	Furin
P05164	Isoform H7 of Myeloperoxidase
P32926	Desmoglein-3
Q02413	Desmoglein-1
P01024	Complement C3
Q9UGM3	Deleted in malignant brain tumors 1 protein
P15924	Desmoplakin
Q9Y6R7	IgGFc-binding protein
Q9HC84	Mucin-5B

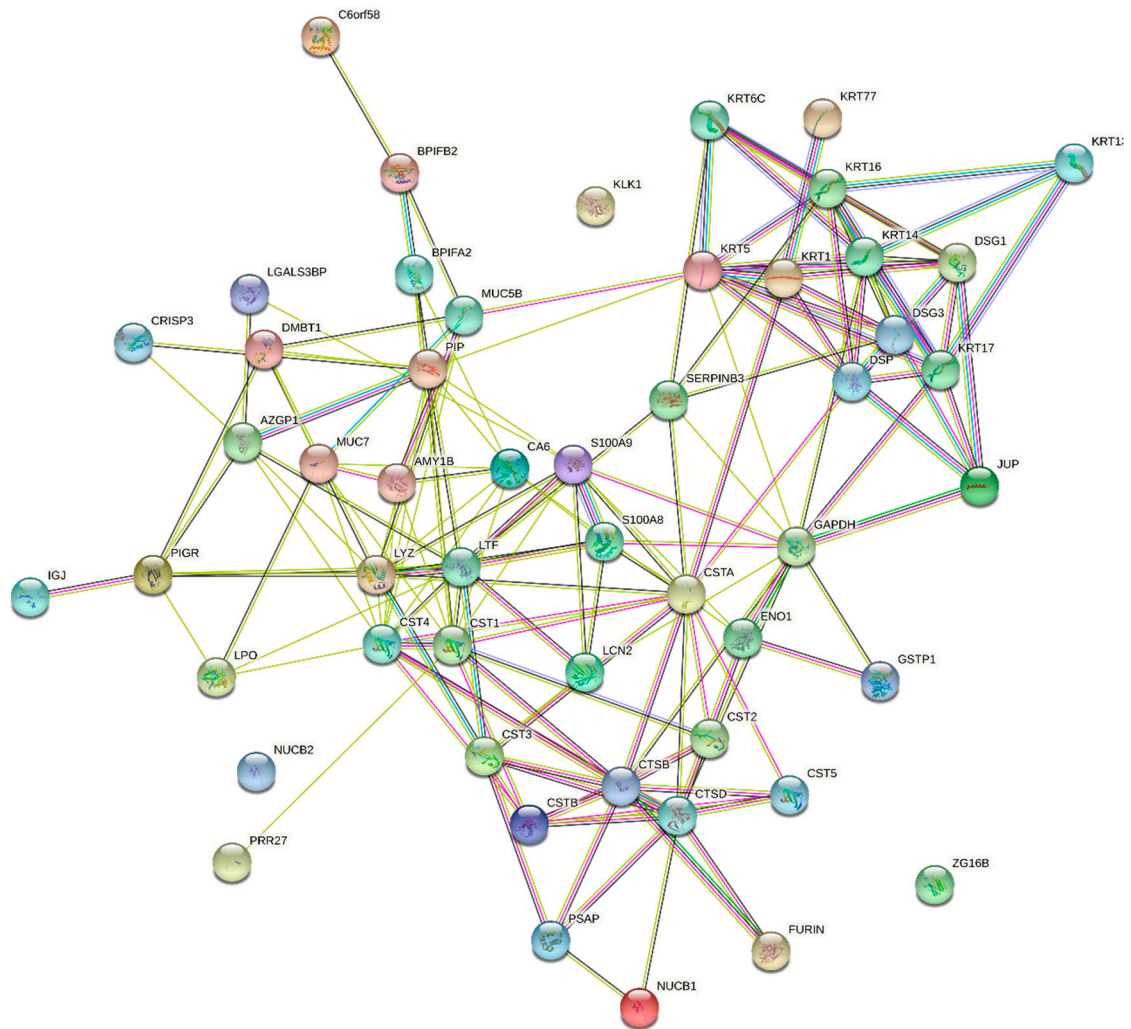


Figure S3: Analysis of PPI network of the 56 proteins common to AuNPs and identified in the Human Salivary Proteome Wiki.

Table S4: The 20 most abundant proteins on the surface of AuNSs and AuNRs and their respective molecular weight (kDa), pI, gravity value and relative abundance.

Protein ID	Name	MW (kDa)	pI	Gravy value	Abundance (10 ⁸)
<i>AuNSs</i>					
P01036	Cystatin-S	16.2	5.02	-0.399	9.03
P01876	Immunoglobulin heavy constant alpha 1	37.6	6.51	-0.206	6.27
P04745	Alpha-amylase 1	57.7	6.93	-0.435	4.45
P04264	Keratin, type II cytoskeletal 1	66	8.12	-0.626	3.29
P01833	Polymeric immunoglobulin receptor	83.2	5.74	-0.341	2.23
P01037	Cystatin-SN	16.4	7.21	-0.440	2.16
P13645	Keratin, type I cytoskeletal 10	58.8	5.21	-0.623	2.04
P02768	Serum albumin	69.3	6.28	-0.353	1.93
P35527	Keratin, type I cytoskeletal 9	62	5.24	-0.700	1.40
P61626	Lysozyme c	16.5	9.16	-0.195	1.26
P35908	Keratin, type II cytoskeletal 2 epidermal	65.4	8	-0.471	1.24
P01834	Immunoglobulin kappa constant	11.8	6.52	-0.537	1.24
Q96DA0	Zymogen granule protein 16 homolog B	22.7	7.39	-0.163	0.776
P12273	Prolactin-inducible protein	16.6	8.05	-0.175	0.589
P01034	Cystatin-C	15.8	8.75	-0.111	0.493
P01591	Immunoglobulin J chain	18.1	5.24	-0.338	0.419
P0DOX8	Immunoglobulin lambda-1 light chain	22.8	6.76	-0.447	0.367
P0DOX2	Immunoglobulin alpha-2 heavy chain	48.9	6.81	-0.235	0.302

P28325	Cystatin-D	16.1	7.17	-0.242	0.214
P02533	Keratin, type I cytoskeletal 14	51.5	5.16	-0.537	0.158
<i>AuNRs</i>					
P04264	Keratin, type II cytoskeletal 1	66	8.12	-0.626	4.37
P13645	Keratin, type I cytoskeletal 10	58.8	5.21	-0.623	2.93
P04745	Alpha-amylase 1	57.7	6.93	-0.435	2.60
P35527	Keratin, type I cytoskeletal 9	62	5.24	-0.700	2.11
P01036	Cystatin-S	16.2	5.02	-0.399	1.64
P02768	Serum albumin	69.3	6.28	-0.358	1.62
P35908	Keratin, type II cytoskeletal 2 epidermal	65.4	8	-0.471	1.44
P01876	Immunoglobulin heavy constant alpha 1	37.6	6.51	-0.206	0.787
P01037	Cystatin-SN	16.4	7.21	-0.440	0.403
P23280	Carbonic anhydrase 6	35.3	7.02	-0.465	0.388
P06870	Kallikrein-1	28.9	4.83	-0.113	0.332
P25311	Zinc-alpha-2-glycoprotein	34.2	6.05	-0.576	0.321
P12273	Prolactin-inducible protein	16.6	8.05	0.175	0.310
P01833	Polymeric immunoglobulin receptor	83.2	5.74	-0.341	0.279
P02533	Keratin, type I cytoskeletal 14	51.5	5.16	-0.537	0.267
Q96DA0	Zymogen granule protein 16 homolog B	22.7	7.39	-0.163	0.224
P01834	Immunoglobulin kappa constant	11.8	6.52	-0.537	0.213
Q8TAX7	Mucin-7	39.1	8.78	-0.597	0.172
P13647	Keratin, type II cytoskeletal 5	62.3	7.74	-0.428	0.126
P48668	Keratin, type II cytoskeletal 6C	60	8	-0.432	0.114

Table S5: Uniprot code and respective names of the proteins that interact with citrate according to SwissTargetPrediction.

Protein ID	Name
P53396	ATP-citrate synthase
P37268	Squalene synthetase (<i>by homology</i>)
P36544	Neuronal acetylcholine receptor protein alpha-7 subunit (<i>by homology</i>)
Q9GZT9	Eglnine homolog 1
P04035	HMG-CoA reductase
Q4U2R8	Solute carrier family 22 member 6 (<i>by homology</i>)
P49354/P49356	Protein farnesyltransferase
P43005	Excitatory amino acid transporter 3
P43116	Prostanoid EP2 receptor
P43088	Prostanoid FP receptor
Q9UHC9	Niemann-Pick C1-like protein 1
Q9UJM8	Hydroxyacid oxidase 1
Q9Y2K7	Lysine-specific demethylase 2A
Q9UPP1	Histone lysine demethylase PHF8
P41229	Lysine-specific demethylase 5C
Q14832	Metabotropic glutamate receptor 3
Q14416	Metabotropic glutamate receptor 2
P39086	Glutamate receptor ionotropic kainate 1
P42261	Glutamate receptor ionotropic, AMPA 1
P0DMS8	Adenosine A3 receptor
Q16478	Glutamate receptor ionotropic kainate 5
Q14833	Metabotropic glutamate receptor 4
P48058	Glutamate receptor ionotropic, AMPA 4
P41594	Metabotropic glutamate receptor 5
O00222	Metabotropic glutamate receptor 8
Q13002	Glutamate receptor ionotropic kainate 2
Q13003	Glutamate receptor ionotropic kainate 3
Q13255	Metabotropic glutamate receptor 1
Q14831	Metabotropic glutamate receptor 7

P42262	Glutamate receptor ionotropic, AMPA 2
O15303	Metabotropic glutamate receptor 6
P16662	UDP-glucuronosyltransferase 2B7
Q04609	Glutamate carboxypeptidase II
P52209	6-phosphogluconate dehydrogenase
P15090	Fatty acid binding protein adipocyte
Q07869	Peroxisome proliferator-activated receptor alpha
P05413	Fatty acid binding protein muscle
Q01469	Fatty acid binding protein epidermal
Q03181	Peroxisome proliferator-activated receptor delta
O14842	Free fatty acid receptor 1
P12104	Fatty acid binding protein intestinal
Q96RI1	Bile acid receptor FXR
P11413	Glucose-6-phosphate 1-dehydrogenase
Q8TDU6	G-protein coupled bile acid receptor 1
P50416	Carnitine O-palmitoyltransferase 1, liver isoform (<i>by homology</i>)
P28845	11-beta-hydroxysteroid dehydrogenase 1
P80365	11-beta-hydroxysteroid dehydrogenase 2
P10275	Androgen Receptor
P11473	Vitamin D receptor
P11511	Cytochrome P450 19A1
P47869/P47870 /P18507	GABA A receptor alpha-2/beta-2/gamma-2
P30304	Dual specificity phosphatase Cdc25A
O60218	Aldo-keto reductase family 1 member B10
P06746	DNA polymerase beta (<i>by homology</i>)
O15379	Histone deacetylase 3
P43004	Excitatory amino acid transporter 2
P00915	Carbonic anhydrase I
Q16790	Carbonic anhydrase IX
Q9H4A4	Aminopeptidase B
O14684	Prostaglandin E synthase
P08185	Corticosteroid binding globulin
P04278	Testis-specific androgen-binding protein

P37058	Estradiol 17-beta-dehydrogenase 3
Q9UBS5	GABA-B receptor (<i>by homology</i>)

Table S6: Molecular weight (kDa), pI, gravity value and relative abundance of the 18 proteins identified only on the surface of AuNSs or AuNRs.

AuNPs	Protein ID	Name	MW (kDa)	pI	Gravy value	Abundance (10 ⁵)
AuNSs	P16870	Carboxypeptidase E	53.1	5.14	-0.449	2.05
	P11021	Endoplasmatic reticulum chaperone BiP	72.3	5.16	-0.486	0.458
	P02741	C-reactive protein	25	5.63	-0.0321	20.6
	P00450	Ceruloplasmin	122.1	5.72	-0.536	0.0689
	A8K2U0	Alpha-2-macroglobulin-like protein 1	161	5.73	-0.157	0.620
	P01871	Immunoglobulin heavy constant mu	51.9	6.15	-0.319	33.1
	P04792	Heat shock protein beta-1	22.8	6.4	-0.567	2.11
	P01782	Immunoglobulin heavy variable 3-9	12.9	7.08	-0.0271	1.37
	P04259	Keratin, type II cytoskeletal 6B	60	8	-0.451	0.390
	A0A0C4D H34	Immunoglobulin heavy variable 4-28	13.1	9.29	-0.00513	0.156
AuNRs	Q8NBJ4	Golgi membrane protein 1	45.3	4.97	-0.983	0.178
	Q8NFT8	Delta and Notch-like epidermal growth factor-related receptor	78.4	5.17	-0.151	0.0999
	Q6S8J3	POTE ankyrin domain family member E	121.3	6.2	-0.682	1.85
	P30740	Leukocyte elastase inhibitor	42.7	6.28	-0.248	2.15
	P00738	Haptoglobin	45.2	6.58	-0.421	1.86
	P04083	Annexin A1	38.7	7.02	-0.419	0.0756
	P02790	Hemopexin	51.6	7.02	-0.434	0.189
	O00584	Ribonuclease T2	29.5	7.08	-0.582	0.451