Supporting information------

Quantification of Proteins by Functionalized Gold Nanoparticles Using Click Chemistry

Kui Zhu,[a, b] Yi Zhang, [a] Sha He, [a] Wenwen Chen, [a] Zhuo Wang, * [a] Jianzhong Shen* [b] and Xingyu Jiang* [a]

[a] Key Lab for Biological Effects of Nanomaterials and Nanosafety, National Center for NanoScience and Technology, Chinese Academy of Sciences, Beijing 100190, China.
Tel: +86 010 8254 5558; Fax: +86 010 8254 5631;
E-mail: xingyujiang@nanoctr.cn, wangz@nanoctr.cn

[b] Department of Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China.
Tel: +86 10 6273 2803; Fax: +86 10 6273 1032;
E-mail: sjz@cau.edu.cn

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Experimental section

Bathocuproine disulfonic acid and bovine serum protein were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Casein, and Bradford kit were purchased from Solarbio Science and Technology Co., LTD (Beijing, China). BCA kit was purchased from Tiangen BIOTECH Co., LTD (Beijing, China). The sera of bovine, canine, duck, equine, feline and rabbit were kindly provided by Prof. Xin Guo (China Agricultural University). All the other used chemical reagents were analytical grade from Beijing Chemical Works. Milk samples were purchased from local supermarket in Beijing.

We prepared AuNPs, azide- and alkyne-functionalized AuNPs according previous work using thiol exchange reactions.[1,2] The prevention of protein adsorption on functionalized AuNPs was desired with a mole ration of PEG/ azide/ alkyne (5/1/1). The azide- and alkyne-functionalized AuNPs were mixed equally to obtain a fresh homogenous dispersed solution before use.

The Biuret reagent consist of potassium hydroxide and hydrated copper (II) sulfate, together with potassium sodium tartrate.[3] We first mixed proteins with Biuret reagents for five minutes at room temperature and then added appropriated functionalized AuNPs before test. All the experiments were repeated at least three times to ensure the accuracy of the measurement.

Statistical analysis: Normalized absorbance represents the ratio of various absorbance versus the maximum absorbance (5 mg/mL protein), respectively, in calibration curves of Bradford, BCA assay and CAP. A four parameter-logistic equation was used to fit the data. Calculations were performed using OriginPro 7.5 software (OriginLab Corporation, Northampton, MA).

Figures and Tables

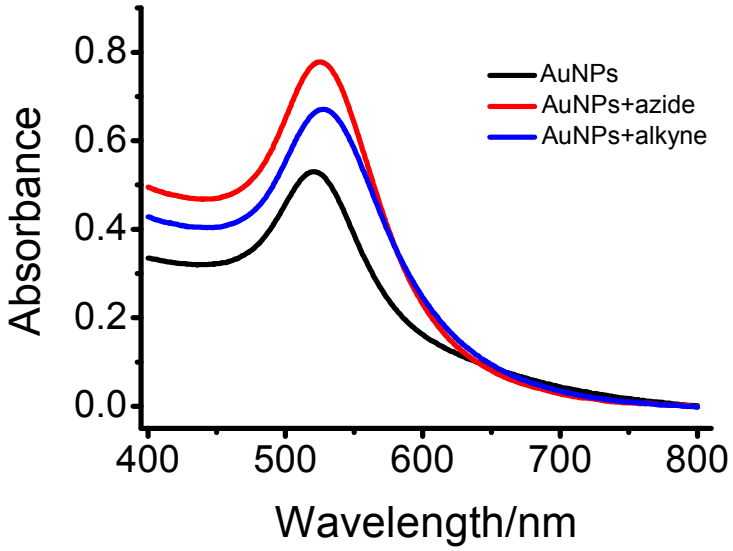
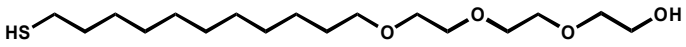


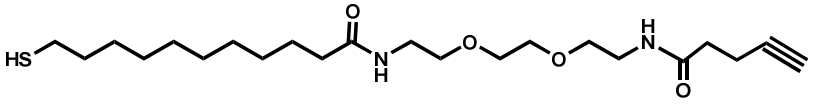
Figure S1 UV-vis spectra of the functionalized and undecorated AuNPs.

Note: The molecules on functionalized AuNPs are listed as below:

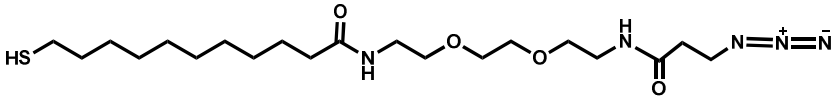
(1) thiol-PEG:

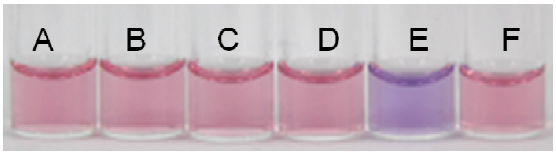


(2) azide functional ligands:



(3) alkyne functional ligands:





	A	B	C	D	E	F
AuNPs	√	√	√	√	√	√
BCDSA		√				√
Protein			√		√	√
Cu (II)				√	√	√

Figure S2 Typical photograph (A to F) shows the specificity of CAP for the determination of proteins at room temperature, and the various compounds are listed in the following table. The reagents added to each bottle are indicated below the image, and equal volume of alkaline solution was added to each bottle.

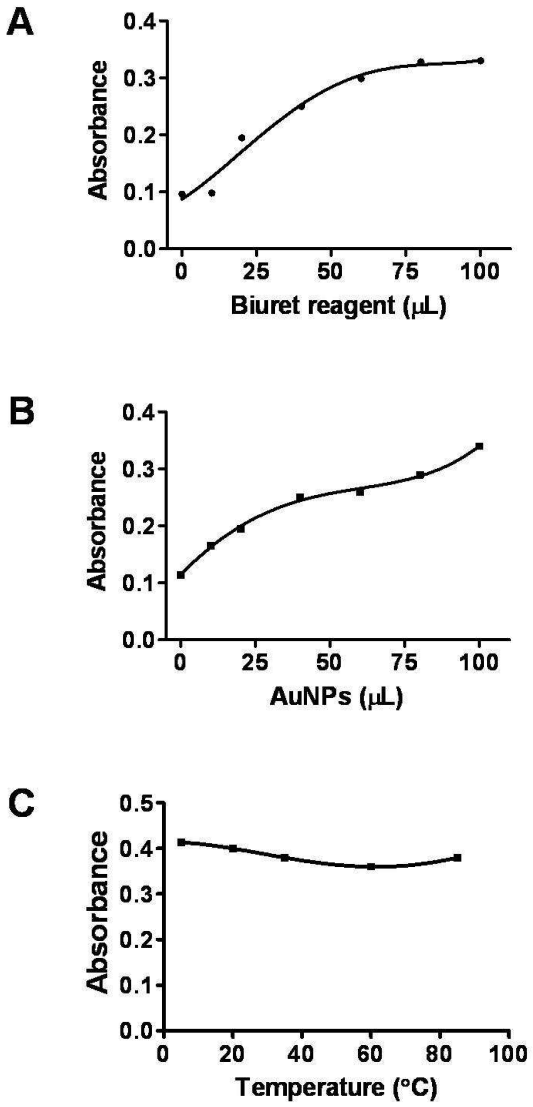


Figure S3 Optimal conditions of the CAP. (A) Effect of Biuret reagent; (B) Effect of the mixture of the functionalized AuNPs; (C) Effect of temperature of CAP.

	Pep	OVA	HSA	BSA	SPA	Hb	IgG	TRY	LYZ	SD
pI	1.0	4.6	4.7	4.7	5.1	7.1	8.0	10.3	11.0	—
Mw (kDa)	35.0	43.0	68.5	68.5	45.0	64.5	149.9	23.3	14.6	—
BCA	0.544	1.276	1.002	1.000	0.942	0.839	0.782	0.487	1.475	0.302
Bradford	0.030	0.999	1.015	1.000	0.677	1.019	0.272	0.255	0.912	0.342
CAP	0.624	1.312	1.008	1.000	0.911	0.853	0.713	0.507	1.421	0.301

Table S1 CAP for various purified proteins.

Note: The values of BCA, Bradford and CAP are the ratios of the OD values of various proteins and OD value of the standard (1 mg/mL BSA). Pep: pepsin; OVA: ovalbumin; HSA: human serum albumin; BSA: bovine serum albumin; SPA: staphylococcal protein A; Hb: hematoglobin; IgG: immunoglobulin G; TRY: trypsin; LYZ: lysozyme; SD: standard deviation. SDs are calculated row-wise, which evaluate the robustness of the three assays.

Herein, we give more elaborate details and add experiments to illustrate the discrepancy of the three assays in Table S1. First, according to the mechanism of CAP, Cu (II) is reduced to Cu (I) by proteins in the alkaline solution, which is similar to BCA. While Bradford assay is based on the dye-protein interactions. The discrepancy of the three assays is mainly due to the intrinsic properties of the protein individuals, such as the amino acid composition of the protein, glycosylated modification and the

stereo structures of the proteins of interest. [4-7] Of the amino acids normally found in proteins, the BCA reagents only reacts with cysteine, cystine, tyrosine and tryptophan.[8] For Bradford assay, the Coomassie dye binds to proteins with arginine residues on the basis of hydrophobic and ionic interactions.[5] Many types of carbohydrates hinder the binding of the dyes to hydrophobic and basic residues, and the hydrophilic sugar moieties can change the hydrophobicity of the glycoproteins so that less dye binds with proteins.[7] Because asparagine residues which are usually involved in carbohydrate linkage, are not involved with the dye binding while it prefers to arginine residues.[5]

Furthermore, the discrepancy in the values determined by the colorimetric assays may be partially due to the used standard protein (such as BSA) which may respond differently to the specific reagents which are used in different assays. The choice of standard protein is critical to the success of the assay. BSA is the original standard of choice, however, it has been noted that BSA has a significantly higher than "normal" response.[9] The BSA standard curve can only therefore be used to compare the relative protein concentration of similar protein solutions. As a result, there is a better agreement in the estimation of HSA and BSA in Table S1, for the two proteins sharing the conservative structure. So we applied BCA, Bradford and CAP methods to assay different proteins with different molecular weights and isoelectric points. Our choice of proteins is not arbitrary: these proteins represent a wide range of pIs (from 1.0 to 11.0). Moreover, OVA, HSA, BSA, Hb and IgM were widely used to investigate the discrepancy among Bradford, BCA and Lowry assays.[7] And we added several commonly used proteins to extend the pI range from1.0 to11.0. Smith *et al* used seven proteins to illustrate the protein-protein variation for the BCA method compared to the other method.[10] These proteins also represent a wide range of species from which they originate: bacteria, birds and mammals.

Interferents	Ratio of ODs	Interferents	Ratio of ODs	Interferents	Ratio of ODs
10% SDS	1.016	10mg/mL Lys	1.009	3M Urea	1.036
1% CTAB	7.089	10mg/mL Tyr	1.018	4M $NH_2OH·HCl$	0.920
1%Triton	1.170	1% Citric acid	1.046	0.5M Tris-base	0.795
1%Tween	1.027	10mg/mL DTE	0.705	1% Methanol	1.045
10% Lecithin	8.560	10mg/mL DTT	0.705	2M Na_2Ac	1.045
10mg/mL Thr	0.920	10mM Glucose	0.938	0.3% Melamine	0.714
10mg/mL Gly	0.839	40% Sucrose	0.991	20% $(NH_4)_2SO_4$	0.821
10mg/mL Ser	0.964	100mM EDTA	1.063	1M NaCl	1.071

Table S2 Effect of various laboratory reagents on CAP.

Note: ratio of ODs: ratios of the OD values of interferences and OD value of the standard (1 mg/mL BSA). SDS: sodium dodecyl sulfate; CTAB: cetyl trimethylammonium bromide; Thr:Threonine; Gly: Glycine; Ser: Serine; Lys: Lysine; Tyr: Tyrosine; DTT: dithiothreitol; DTE: dithioerythritol; EDTA: ethylenediaminetetraacetic acid;$NH_2OH·HCl$: hydroxylamine hydrochloride; Na_2Ac: sodium acetate; $(NH_4)_2SO_4$: ammonium sulfate; NaCl: sodium chloride. If the OD value around 1, it is considered normal data. Larger or small values indicate strong interference.

	Bovine	Canine	Anatine	Equine	Feline	Leporine (mg/mL)
BCA	73	55	26	68	75	64
Bradford	68	57	23	71	66	59
CAP	69	52	27	65	70	68

Table S3 Comparison of the three methods for the determination of protein concentrations in various sera.

Note: Least Signifficant Difference (LSD) test was introduced to analyze the results.

Samples	Fresh milk (mg /100 mL)						
	1	2	3	4	5	6	7
BCA [a]	3.1	3.1	3.3	3.5	3.4	3.4	3.1
CAP [b]	2.0	2.2	2.7	2.1	2.6	3.0	2.8
Claimed on the package [a]	2.9	2.9	3.2	3.0	3.1	3.4	2.9

Table S4 Comparison of the two methods in the determination of proteins in fresh milk samples.

Samples	Yogurt (mg /100 mL)				
	1	2	3	4	5
BCA	2.0	2.0	2.1	1.8	2.5
CAP	2.8	2.6	3.6	2.7	2.4
Claimed on the package	3.0	2.9	3.0	1.0	3.2

Table S5 Comparison of the two methods in the determination of proteins in yogurt samples.

Samples	Skim milk (mg /100 mL)		
	1	2	3
BCA	2.3	3.0	2.4
CAP	5.1	3.3	2.9
Claimed on the package	5.7	3.3	2.9

Table S6 Comparison of the two methods in the determination of proteins in skim milk samples.

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