

Supporting information for:

**SOURCES OF BIASES IN THE IN VITRO TESTING OF
NANOMATERIALS: THE ROLE OF BIOMOLECULAR CORONA**

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Supplementary Figures

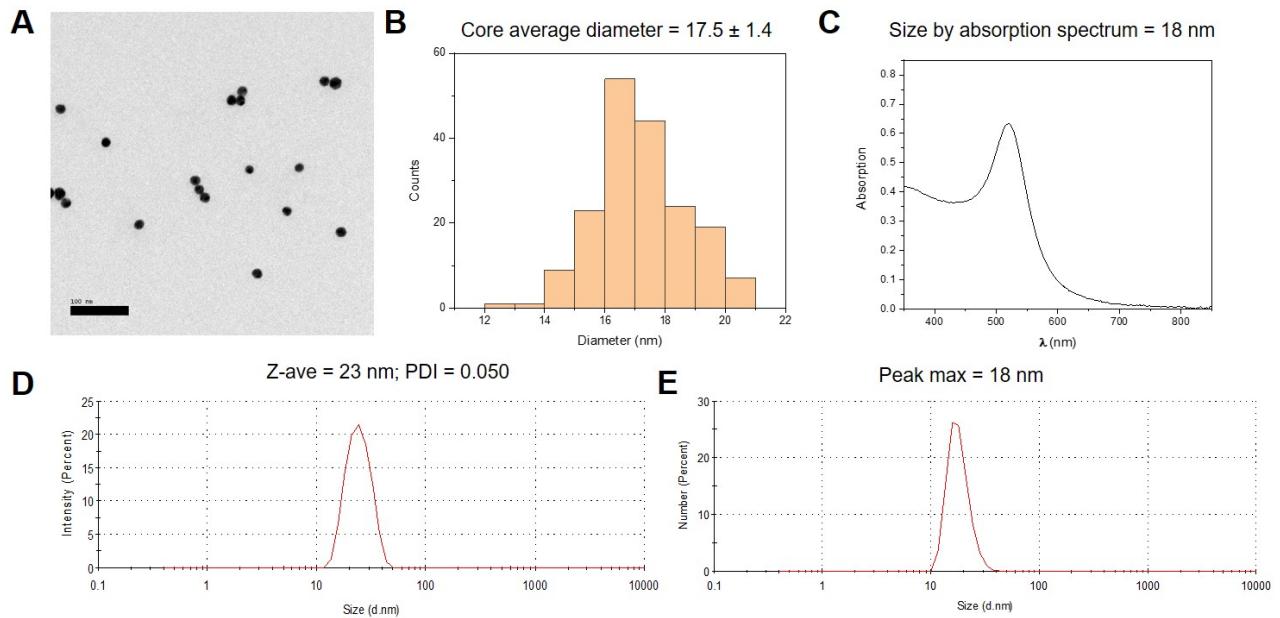


Figure S1. Characterization of GNP. A) Representative TEM micrograph (scale bar, 100 nm). B) Statistical size analysis from TEM imaging. At least 100 NPs from 10 different images were considered. C) UV-Vis absorption spectrum. D,E) DLS measurements expressed as Intensity (D) and Number (E).

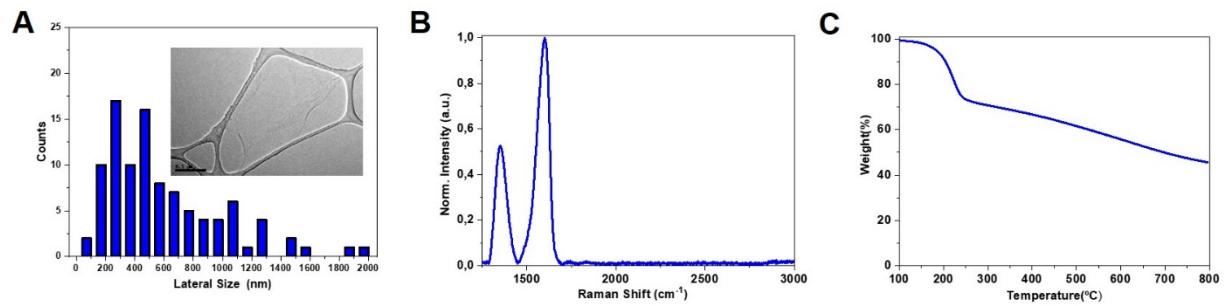


Figure S2. Characterization of GNP. A) Statistical size analysis from TEM imaging. *Inset:* representative TEM micrograph (scale bar, 500 nm). B) Raman spectrum. C) TgA in nitrogen atmosphere. Data from *Nano Letters* 23.7 (2023): 2981-2990.

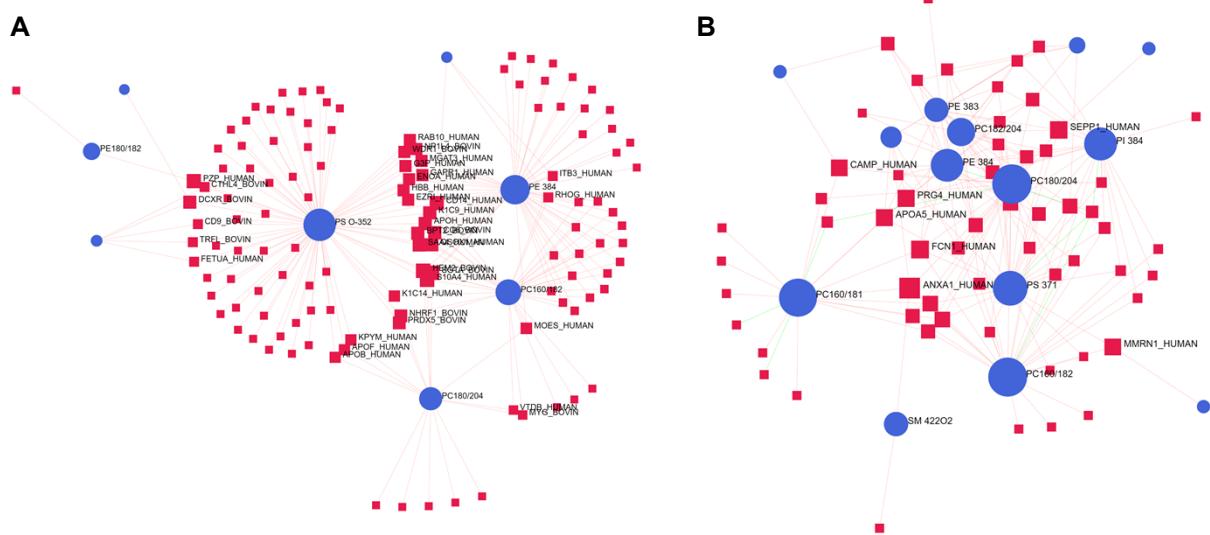


Figure S3. Association analysis between the lipid and protein components of the BMC. A) GNP, B) GO. Proteins are represented as red squares and lipids as blue dots. Only significant correlations with $r^2 \geq 0.9$ are reported.

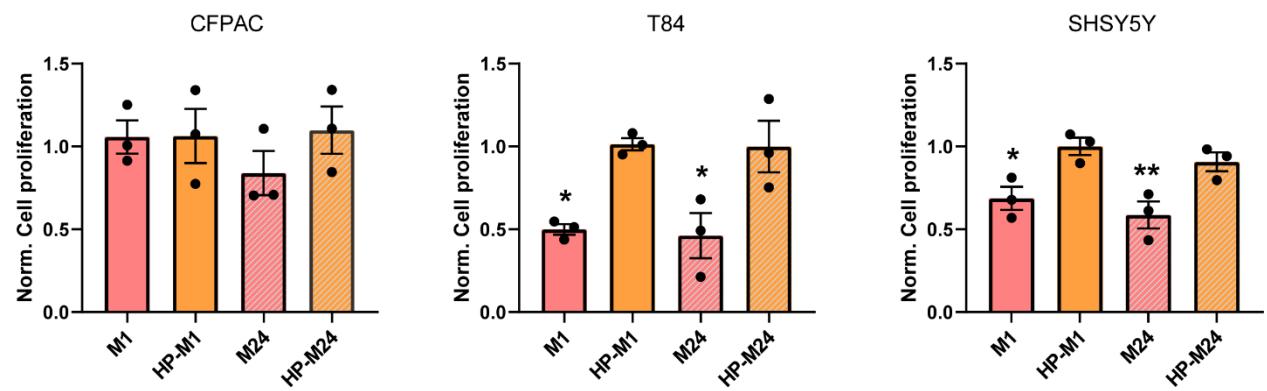


Figure S4. Cell proliferation upon incubation with 10 µg/mL GO for 1 and 24 h under different BMC conditions. Data are normalized over untreated control conditions at both 1 and 24 h and expressed as means \pm SEM of N = 3 biological replicates. Statistical analysis: *p<0.05, **p<0.01, one-way ANOVA/Tukey's tests.

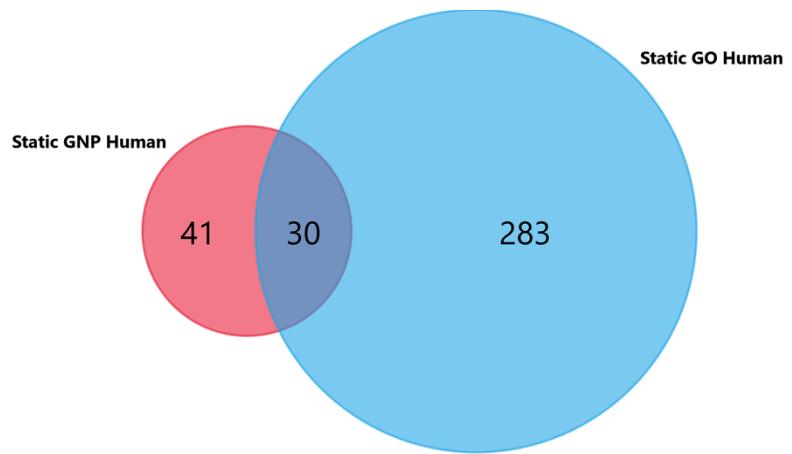


Figure S5. Venn overlap analysis for only the human proteins present in the static list of features of GNP (pink) and GO (blue).

Supplementary Tables

Table S1. Top 20 proteins (relative abundance from LC-MS) for the BMC of GNP in the four BMC conditions.

HP		M	
VTNC_HUMAN	Vitronectin	APOE_BOVIN	Apolipoprotein E
ALBU_HUMAN	Albumin	A1AT_BOVIN	Alpha-1-antiproteinase
APOA1_HUMAN	Apolipoprotein A-I	ALBU_BOVIN	Albumin
KNG1_HUMAN	Kininogen-1	HBBF_BOVIN	Hemoglobin fetal subunit beta
IGKC_HUMAN	Immunoglobulin kappa constant	FETUA_BOVIN	Alpha-2-HS-glycoprotein
APOB_HUMAN	Apolipoprotein B-100	APOA1_BOVIN	Apolipoprotein A-I
IGHM_HUMAN	Immunoglobulin heavy constant mu	HBA_BOVIN	Hemoglobin subunit alpha
IGHG1_HUMAN	Immunoglobulin heavy constant gamma 1	A2MG_BOVIN	Alpha-2-macroglobulin
APOA_HUMAN	Apolipoprotein(a)	CASA1_BOVIN	Alpha-S1-casein
APOE_HUMAN	Apolipoprotein E	FETUB_BOVIN	Fetuin-B
ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2	APOC3_BOVIN	Apolipoprotein C-III
IGHA1_HUMAN	Immunoglobulin heavy constant alpha 1	TSP1_BOVIN	Thrombospondin-1
FIBA_HUMAN	Fibrinogen alpha chain	APOA2_BOVIN	Apolipoprotein A-II
FIBB_HUMAN	Fibrinogen beta chain	THRΒ_BOVIN	Prothrombin
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	IPB2_BOVIN	Insulin-like growth factor-binding protein 2
APOC3_HUMAN	Apolipoprotein C-III	A2AP_BOVIN	Alpha-2-antiplasmin
IGHG2_HUMAN	Immunoglobulin heavy constant gamma 2	C1QA_BOVIN	Complement C1q subcomponent subunit A
CLUS_HUMAN	Clusterin	KNG1_BOVIN	Kininogen-1
APOC1_HUMAN	Apolipoprotein C-I	ITIH4_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H4
APOA2_HUMAN	Apolipoprotein A-II	URP2_BOVIN	Fermitin family homolog 3
HP-M1		HP-M24	
ALBU_BOVIN	Albumin	ALBU_BOVIN	Albumin
A1AT_BOVIN	Alpha-1-antiproteinase	A1AT_BOVIN	Alpha-1-antiproteinase
FETUA_BOVIN	Alpha-2-HS-glycoprotein	FETUA_BOVIN	Alpha-2-HS-glycoprotein
ALBU_HUMAN	Albumin	HBA_BOVIN	Hemoglobin subunit alpha
VTNC_HUMAN	Vitronectin	A2MG_BOVIN	Alpha-2-macroglobulin
A2MG_BOVIN	Alpha-2-macroglobulin	APOA1_BOVIN	Apolipoprotein A-I
APOA1_BOVIN	Apolipoprotein A-I	ALBU_HUMAN	Albumin
HBA_BOVIN	Hemoglobin subunit alpha	VTNC_HUMAN	Vitronectin
MP2K1_HUMAN	MAP kinase kinase 1	TRFE_BOVIN	Serotransferrin
TRFE_BOVIN	Serotransferrin	MP2K1_HUMAN	MAP kinase kinase 1
FETA_BOVIN	Alpha-fetoprotein	HBBF_BOVIN	Hemoglobin fetal subunit beta
FETUB_BOVIN	Fetuin-B	FETA_BOVIN	Alpha-fetoprotein
HEMO_BOVIN	Hemopexin	FETUB_BOVIN	Fetuin-B
IGKC_HUMAN	Immunoglobulin kappa constant	HEMO_BOVIN	Hemopexin
HBBF_BOVIN	Hemoglobin fetal subunit beta	A1AG_BOVIN	Alpha-1-acid glycoprotein
A1AG_BOVIN	Alpha-1-acid glycoprotein	A2AP_BOVIN	Alpha-2-antiplasmin
APOA1_HUMAN	Apolipoprotein A-I	TTHY_BOVIN	Transthyretin
ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2	SPA37_BOVIN	Serpin A3-7
A2AP_BOVIN	Alpha-2-antiplasmin	A1BG_BOVIN	Alpha-1B-glycoprotein
A1BG_BOVIN	Alpha-1B-glycoprotein	CO3_BOVIN	Complement C3

Table S2. Top 20 proteins (relative abundance from LC-MS) for the BMC of GO in the four BMC conditions.

HP		M	
ALBU_HUMAN	Albumin	APOA1_BOVIN	Apolipoprotein A-I
FA11_HUMAN	Coagulation factor XI	ALBU_BOVIN	Albumin
VTNC_HUMAN	Vitronectin	HBBF_BOVIN	Hemoglobin fetal subunit beta
HRG_HUMAN	Histidine-rich glycoprotein	A2MG_BOVIN	Alpha-2-macroglobulin
FIBA_HUMAN	Fibrinogen alpha chain	FETUA_BOVIN	Alpha-2-HS-glycoprotein
APOA1_HUMAN	Apolipoprotein A-I	A1AT_BOVIN	Alpha-1-antiproteinase
SAA4_HUMAN	Serum amyloid A-4 protein	HBA_BOVIN	Hemoglobin subunit alpha
APOE_HUMAN	Apolipoprotein E	F12AI_BOVIN	Factor XIIa inhibitor
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	APOH_BOVIN	Beta-2-glycoprotein 1
KNG1_HUMAN	Kininogen-1	APOE_BOVIN	Apolipoprotein E
IGHM_HUMAN	Immunoglobulin heavy constant mu	PEDF_BOVIN	Pigment epithelium-derived factor
IGHG1_HUMAN	Immunoglobulin heavy constant gamma 1	GELS_BOVIN	Gelsolin
PLMN_HUMAN	Plasminogen	APOA2_BOVIN	Apolipoprotein A-II
FIBB_HUMAN	Fibrinogen beta chain	THRBL_BOVIN	Prothrombin
KLKB1_HUMAN	Plasma kallikrein	ITIH1_BOVIN	Inter-alpha-trypsin inhibitor heavy chain H1
APOC4_HUMAN	Apolipoprotein C-IV	FA5_BOVIN	Coagulation factor V
LYSC_HUMAN	Lysozyme C	CFAH_BOVIN	Complement factor H
APOA2_HUMAN	Apolipoprotein A-II	TTHY_BOVIN	Transthyretin
FA12_HUMAN	Coagulation factor XII	VTDB_BOVIN	Vitamin D-binding protein
IBP3_HUMAN	Insulin-like growth factor-binding protein 3	KNG1_BOVIN	Kininogen-1
HP-M1		HP-M24	
ALBU_BOVIN	Albumin	ALBU_BOVIN	Albumin
A1AT_BOVIN	Alpha-1-antiproteinase	A1AT_BOVIN	Alpha-1-antiproteinase
APOA1_BOVIN	Apolipoprotein A-I	FETUA_BOVIN	Alpha-2-HS-glycoprotein
FIBA_HUMAN	Fibrinogen alpha chain	ALBU_HUMAN	Albumin
VTNC_HUMAN	Vitronectin	HRG_HUMAN	Histidine-rich glycoprotein
FETUA_BOVIN	Alpha-2-HS-glycoprotein	APOA1_BOVIN	Apolipoprotein A-I
HRG_HUMAN	Histidine-rich glycoprotein	VTNC_HUMAN	Vitronectin
ALBU_HUMAN	Albumin	TRFE_BOVIN	Serotransferrin
FA11_HUMAN	Coagulation factor XI	FA11_HUMAN	Coagulation factor XI
APOA1_HUMAN	Apolipoprotein A-I	FETUB_BOVIN	Fetuin-B
FETUB_BOVIN	Fetuin-B	A2MG_BOVIN	Alpha-2-macroglobulin
SAA4_HUMAN	Serum amyloid A-4 protein	FIBA_HUMAN	Fibrinogen alpha chain
IGHG1_HUMAN	Immunoglobulin heavy constant gamma 1	HEMO_BOVIN	Hemopexin
A2MG_BOVIN	Alpha-2-macroglobulin	KNG1_HUMAN	Kininogen-1
KNG1_HUMAN	Kininogen-1	APOA1_HUMAN	Apolipoprotein A-I
TRFE_BOVIN	Serotransferrin	A1BG_BOVIN	Alpha-1B-glycoprotein
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	HBBF_BOVIN	Hemoglobin fetal subunit beta
HEMO_BOVIN	Hemopexin	FETA_BOVIN	Alpha-fetoprotein
APOA2_BOVIN	Apolipoprotein A-II	FA12_HUMAN	Coagulation factor XII
APOE_HUMAN	Apolipoprotein E	APOA_HUMAN	Apolipoprotein(a)