Synthesis and Cytotoxicity Studies of Gold Nanoparticle Systems

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Declaration

I declare that this dissertation titled ***Synthesis and Cytotoxicity Studies of Gold Nanoparticle Systems*** is my own work except where otherwise acknowledged. It is submitted to the Department of Chemistry, University of Zululand for a PhD degree in Chemistry. I declare that all the sources used or quoted have been indicated and acknowledged by means of full references.

Sosibo, Ndabenhle Mercury

_____ _____

Signature Date

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ABSTRACT

In this work, the immobilisation of different biomolecules on thioalkylated polyethelene glycol (PEG)-capped gold monolayer protected clusters (MPCs) has been successfully conducted. This followed a series of aqueous and nonaqueous synthetic protocols carried out to synthesize gold nanoparticles and MPCs of sizes ranging between 4 – 200 nm. Hetero-bifunctional PEG ligands possessing functionalities such as carboxyl, hydroxyl, biotin and nitrilotriacetic acid (NTA) were introduced resulting in stable, biocompatible gold MPCs, templates for biomolecular functionalisation reactions. Biomolecular functionalisation strategies such as carbodiimide coupling, biotin-avidin interaction and Ni-NTA-histidine interactions following the introduction of the bivalent hexadentate Ni(II) onto the NTA matrix of the MPCs, were conducted to formulate the biomolecular hybrid systems. A range of biomolecules including the cell-penetrating TAT peptide (YGRKKRRQRRR), mitogen-activated protein kinase (MAP kinase), streptavidin and fluorescent-labelled FAM-TAT peptide were successfully immobilised on the gold MPCs. The simplicity of the synthetic approaches and the stability of the resultant biomolecular systems strengthened their potential applications in targeted drug delivery, molecular recognition tools for diagnostics and in the purification, quantification and beneficiation of tagged fusion biomolecules.

The colloidal gold nanoparticles, MPCs and bioconjugates were further investigated for inherent biologic effects through a series of end-point based *in vitro* assays. The cytotoxicity, namely the causation of necrotic cell death was studied using the neutral red assay on CHO22 cell line. All three system types showed benign cytotoxicity properties; demonstrating minor dose-dependence decline in cell viability through necrotic cell death. Additionally, dose-dependent patterns were also observed in the apoptosis-induction effects of these gold systems on CHO22 and CD4 expressing Jurkat cell lines. Overall, this work demonstrated facile protocols of synthesis for colloidal gold nanoparticles, MPCs and bioconjugates, and subsequently through *in vitro* cellular interaction assays, demonstrated these systems as useful tools for application in life sciences and related fields.

Publications and Conference

1. **Sosibo N.M.**, Tshikhudo R.T. and Revaprasadu N., Stable, Hydrophilic Nitrilotriacetic Acid (NTA) Capped Gold Monolayer Protected Clusters, in *Quantum-Dot and Nanoparticle Bioconjugates – Tools for Sensing and Biomedical Imaging*, edited by J. Cheon, H. Mattoussi, C.M. Niemeyer, and G. Strouse (Mater. Res. Soc. Symp. Proc. **Volume 1064E**) Warrendale, PA, 2008, 1064-10.

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3. **Sosibo N.M.**, Dodgen C., Meyer M., Tshikhudo R.T., Rees J., van der Lingen E. and Revaprasadu N., Size-Dependent Cytotoxicity of Some Colloidal Gold Nanoparticles, *JACS Comunications*, Manuscript in preparation.

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Contents

List of Figures xiii

List of Tables xix

List of Abbreviations xx

Dedication xxii

CHAPTER 1: INTRODUCTION AND LITERATURE SURVEY 1-48

1.1. INTRODUCTION 1

1.1.1. Aims and Approach of the Study 1

1.1.2. Gold: A Historical Perspective 1

1.1.3. The Nanoscale of Things 2

1.1.4. Nanostructured Effects 3

1.1.5. Bottom-up and Top-down Approaches 5

1.2. SYNTHESIS OF NANOPARTICLES 5

1.2.1. Citrate Reduction Method 6

1.2.2. Two-Phase Reduction: Brust-Schiffrin Method 7

1.2.3. Polyol Reduction Method 8

1.2.4. Sonochemical Route 9

1.2.5. Synthesis in Confined Matrices 10

1.2.6. Chemical Vapour Deposition (CVD) Route 10

1.2.7. Solvothermal/ Hydrothermal Route 11

1.2.8. Biosynthesis Route 12

1.3. FORMATION OF THE NANOPARTICLE 13

1.3.1. Precipitation of Nanoparticles 13

1.3.2. Stabilisation of Nanoparticles 14

1.3.3. Full-Shell ‘‘Magic Number’’ Clusters 15

1.4. APPLICATIONS OF NANOPARTICLES 16

1.4.1. Biological Applications 16

1.4.2. Chemical and Materials Applications 17

1.5. PROPERTIES OF METAL NANOPARTICLES 18

1.5.1. Optical Properties 18
1.6. FUNCTIONALISATION OF GOLD NANOPARTICLES 20
1.6.1. Monolayer Protected Clusters (MPCs) 20
1.6.2. Poly(ethylene glycol) (PEG) 21
1.6.2.1. Introduction 21
1.6.2.2. Synthesis of PEG 22
1.6.3. Monolayer Protected Nanoparticle Reactions 23
1.6.3.1. Ligand Place-Exchange Reactions 23
1.6.3.2. Acid-Amine Coupling Reactions 24
1.6.3.3. Polymerisation Reactions 24
1.6.3.4. Acid-Base Reactions 25
1.6.3.5. Other Reactions of the ω-Functionalities 25
1.7. BIOMOLECULAR FUNCTIONALISATION OF MPCs 25
1.7.1. Electrostatic (Charge) Attachment 26
1.7.2. Specific Affinity Immobilisation 27
1.7.3. Covalent Attachment 28
1.8. INTERFACING NANOPARTICLES WITH CELLS 28
1.8.1. Nanoparticle-Based Targeting Strategies 30
1.8.2. Mechanism of Uptake 30
1.8.2.1. Passive Targeting 31
1.8.2.2. Active Targeting 31
1.8.3. Fate of Nanoparticles in the Body 32
1.9. CHARACTERISATION TECHNIQUES 33
1.9.1. UV-vis Spectroscopy 33
1.9.2. Transmission Electron Microscopy 33
1.9.3. Agarose Gel Electrophoresis 34
1.9.4. Flow Cytometry 34
1.10. THESIS LAYOUT 35
1.11. REFERENCES 36

CHAPTER 2: SYNTHESIS OF COLLOIDAL GOLD NANOPARTICLES

AND MONOLAYER PROTECTED CLUSTERS IN AQUEOUS
AND NONAQUEOUS MEDIA 49-82
2.1. INTRODUCTION 49
2.2. The Gold-Sulphur Bond 51
2.2.1. Chapter Strategy 53
2.3. EXPERIMENTAL MATERIALS AND METHODS 54
2.3.1. Materials 54
2.3.2. Instrumentation 54
2.3.3. Synthesis of Citrate-Capped Colloidal Nanoparticles 55
2.3.4. Synthesis of Borohydride-Capped Colloidal Nanoparticles (SM10) 55
2.3.5. Synthesis of 3-Thiopheneacetic Acid-Capped MPCs (SM11) 56
2.3.6. Synthesis of 4-Aminothiophenol-Capped MPCs (SM12) 56
2.3.7. Synthesis of 2-Mercaptoethanol-Capped MPCs (SM13) 56
2.3.8. Synthesis of L-Cysteine-Capped MPCs (SM14) 56
2.3.9. Synthesis of L-Glutathione-Capped MPCs (SM15) 57
2.3.10. Synthesis of Alkylthiol-Capped MPCs (SM16-18) 57
2.4. RESULTS AND DISCUSSION 58
2.5. SUMMARY AND CONCLUSIONS 79
2.6. REFERENCES 80

CHAPTER 3: SYNTHESIS OF GOLD MONOLAYER PROTECTED CLUSTERS AND BIOMOLECULAR FUNCTIONALISATION 83-128
3.1. INTRODUCTION 83
3.1.1. Chapter Strategy 83
3.1.1.1. Surface PEGylation: MPC Synthesis 84
3.1.1.2. Carbodiimide Coupling Strategy 85
3.1.1.3. Biofunctionalisation via the biotin-avidin (BAB)

Protocol 85
3.1.1.4. Hexahistidine-tagged Protein Immobilisation 85
3.2. EXPERIMENTAL MATERIALS AND METHODS 86
3.2.1. Materials 87
3.2.2. Instrumentation 87
3.2.3. Synthesis of Gold Nanoparticles 87
3.2.4. PEG-OH Gold MPCs 87
3.2.5. PEG-COOH Gold MPCs 87
3.2.5.1. Part I: Synthesis 87
3.2.5.2. Part II: Biofunctionalisation with Streptavidin 88
3.2.6. PEG-biotin MPCs 88
3.2.6.1. Part I: Synthesis 88
3.2.6.2. Part II: Biofunctionalisation with Streptavidin 89
3.2.6.3. Part III: Biomolecular Functionalisation with Biotin-TAT 89
3.2.6.4. Part III: Biomolecular Immobilisation with FAM-TAT 89
3.2.7. PEG-NTA Gold MPCs 90
3.2.7.1. Part I: Synthesis 90
3.2.7.2. Part II: Synthesis of PEG-NTA-Ni Conjugates 90
3.2.7.3. Part III: Immobilisation of Hexahistidine-tagged MAP kinase 90
3.3. RESULTS AND DISCUSSION 91
3.4. SUMMARY AND CONCLUSIONS 124
3.5. REFERENCES 125

CHAPTER 4: CYTOTOXICITY EVALUATION OF COLLOIDAL GOLD NANOPARTICLES, MONOLAYER PROTECTED CLUSTERS AND BIOMOLECULAR CONJUGATES 129-147
4.1. INTRODUCTION 129
4.1.1. Chapter Strategy 132

4.2. EXPERIMENTAL MATERIALS AND METHODS 133
4.2.1. Tissue Culture Media and Cell Lines 133
4.2.2. Cell Culturing 133
4.2.2.1. Thawing of Cells 133
4.2.2.2. Trypsinization of Cells 134
4.2.2.3. Cell Seeding 134
4.2.2.4. Morphological Analysis 134
4.2.2.5. Cryo-presevation of Cells 134
4.2.2.6. Neutral Red Cytotoxicity Assay 135
4.2.2.7. APOPercentage™Assay 135
4.3. RESULTS AND DISCUSSION 136
4.3.1. Gold NPs, MPCs and Bioconjugates 136
4.3.2. Neutral Red Cytotoxicity Assay on CHO22 Cell Lines 137
4.3.3. Apoptosis Induction Studies on CHO22 Cell Lines 140
4.3.3.1. The Effect of Gold Nanoparticle Size on Apoptosis 140
4.3.3.2. The Effect of MPCs Functionality of Apoptosis 142
4.3.3.3. The Effect of Biomolecular Functionality 142
4.3.4. Apoptosis Induction Studies on Jurkat Cell Lines 142
4.3.4.1. Dose-Dependent Apoptosis Induction in Jurkat Cell Lines 143
4.3.4.2. Cellular Morphological Changes of Jurkat Cells 144
4.4. SUMMARY AND CONCLUSIONS 145
4.5. REFERENCEES 145

CHAPTER 5: CONCLUSIONS AND FUTURE OUTLOOK 148-152
5.1. CONCLUSIONS 148
5.1.1. Colloidal Gold Nanoparticles and Monolayer Protected Clusters 148
5.1.2. PEGylated Gold Monolayer Protected Clusters 148
5.1.3. Gold Bioconjugates 149

5.1.4. Cellular Interactions of Colloidal Gold Nanoparticles, MPCs and Bioconjugates 150
5.2. FUTURE OUTLOOK 151

List of Figures

Figure 1.1.	Representation of the transformation of the electronic density of states (DOS) of the valence and conduction bands in metal and semiconductor nanoparticles with comparison to their relevant bulk and atomic states respectively. In metals, the semi-continuous density of states in valence and conduction bands splits into two discrete electronic levels. The level spacing increases with the decreases particle sizes [22].	4
Figure 1.2.	The LaMer diagram, showing the time dependence of the concentration required to achieve monodispersity [112]. I. Prenucleation phase, II. Nucleation stage and III. Growth step.	14
Figure 1.3.	Electrostatic stabilisation (A), charged ions adsorb on the surface of the metal nanoparticle creating an electrostatic double layer providing a Coulombic repulsion between the nanoparticles. Steric-stabilisation (B), of the nanoparticles by bulky ligands or polymers: (i) entropic effect and (ii) osmotic effect.	15
Figure 1.4	Idealised representation of hexagonal close packed full-shell 'magic number' clusters [119].	16
Figure 1.5.	Origin of the surface plasmon resonance caused by the interactions of the nanoparticle electron in the conduction band with electromagnetic radiation. (A) A dipole is induced, oscillating in phase with the electric field of the incoming light. (B) Transverse and longitudinal oscillation of electrons in a metal nanorod [142].	19
Figure 1.6.	Some examples of nanoparticles in biological applications: (A) in vitro labelling of HEK cells by CdS/ZnS core-shell quantum dots [217] and (B) in vitro imaging using a quantum dot-encoded multicolour bead of a live rat [218].	29
Figure 2.1.	Synthesis of gold nanoparticles by the reduction of the gold(III) salt; by (i) citrate reduction/capping, (ii) the Brust biphasic method and (iii) the direct reduction route in the presence of a bi-/multifunctional capping ligand.	50
Figure 2.2.	Various proposed modes of binding of sulphur on gold surfaces [14], B: gold-sulphur binding modes of a 102 gold-atom cluster system [15] and C: well-characterised gold(I) thiolate complex.	52
Figure 2.3.	Optical absorption spectra of the citrate-capped colloidal gold nanoparticles synthesised at different citrate-to-gold ratios.	61

Figure 2.4.	TEM micrographs and the corresponding size distribution analysis of the citrate-capped colloidal nanoparticles: A) SM1, B) SM2, C) SM3 and D) SM4.	62
Figure 2.5.	TEM micrographs and the corresponding size distribution analysis of the citrate-capped colloidal nanoparticles: A) SM5, B) SM6, C) SM7 and D) SM8.	63
Figure 2.6.	TEM micrographs of the citrate-capped colloidal nanoparticles (SM9).	64
Figure 2.7.	The A) optical absorption spectrum, B) a TEM micrograph and C) the size distribution analysis of the borohydride-capped colloidal nanoparticles.	66
Figure 2.8.	The A) absorption spectrum, B) TEM micrograph and C) the particle size distribution graph of the 3-TA-capped gold MPCs.	67
Figure 2.9.	The A) optical absorption spectrum, B) TEM micrograph of the as-prepared gold MPCs and C) a TEM micrograph of the MPCs dispersed in water at pH 2.0.	69
Figure 2.10.	Optical absorption spectrum (A), TEM micrograph and size distribution analysis of the 4-aminothiophenol-capped gold MPCs synthesised at pH 1.5.	70
Figure 2.11.	Optical absorption (A) and TEM micrograph of 2-mercaptoethanol-capped gold MPCs.	71
Figure 2.12.	Optical absorption spectrum A), B) TEM micrograph and C) the particle size distribution graph of L-cysteine-capped gold MPCs.	72
Figure 2.13.	Optical absorption spectrum(A), B) TEM micrograph and C) size distribution analysis graph of the GSH capped MPCs.	74
Figure 2.14.	Optical absorption (A) and (B) TEM micrograph of the GSH capped MPCs after heating at 80 °C.	75
Figure 2.15.	Optical absorption spectra of the alkylthiol-capped gold MPCs.	77

Figure 2.16.	TEM micrographs of the alkylthiol-capped gold MPCs, hexanethiol (A1), octanethiol (B1) and dodecanethiol; and the corresponding size distribution quantifications, for hexanethiol- (A2), octanethiol- (B2) and dodecanethiol-capped gold MPCs.	78
Figure 3.1.	A generic design of functional PEGylated AuMPCs.	84
Figure 3.2.	Absorption and structural analysis of the PEG-OH functionalised gold MPCs. UV-Vis absorption spectra (A1) [ME1, 14 nm], (A2) [ME2, 5.5 nm] and TEM micrographs, (B1) [ME1, 14 nm] and (B2) [ME2, 5.5 nm].	93
Figure 3.3.	Agarose gel electrophoresis of PEG-OH functionalised AuMPCs; lane 1: ME1 [14 nm] and lane 2: ME2 [5.5 nm].	93
Figure 3.4.	A schematic representation of the simultaneous incorporation of multiple functionalities on the surface of the gold core.	94
Figure 3.5.	Optical and structural analysis of PEG-COOH and PEG-OH mixed monolayer functionalised gold MPCs; A1) and A2) 1% PEG-COOH [ME3A, 14 nm], B1) and B2) 10% PEG-COOH [ME3B, 14 nm] and C1) and C2) 50% PEG-COOH [ME3C, 14 nm] functionalised MPCs.	95
Figure 3.6.	Optical absorption (A) and the EM micrograph (B) of the 100% PEG-COOH functionalised MPCs [ME3D, 14 nm].	96
Figure 3.7.	Agarose gel electrophoresis of PEG-COOH functionalised AuMPCs [14 nm] (lanes 1 = 1% (ME3A), 2 = 10% (ME3B), 3= 50% (ME3C) and 4= 100% (ME3D)).	97
Figure 3.8.	Optical absorption (A) and the TEM micrograph (B) of the 100% PEG-COOH functionalised gold MPCs [ME4, 5.5 nm].	98
Figure 3.9.	A generic strategy for the introduction of biomolecules by employing the EDC/NHS coupling route.	99
Figure 3.10.	UV-vis optical absorption of the streptavidin conjugates, A1) ME5A, B1) ME5B and the TEM micrographs, A2) ME5A and B2) ME5B.	100

Figure 3.11.	Agarose gel electrophoresis of PEG-COOH functionalised MPCs and the corresponding streptavidin conjugates; lane 1: streptavidin conjugates (ME5A), lane 2: 1% PEG-COOH (ME3A), lane 3: streptavidin conjugates (ME5B) and lane 4: 10% PEG-COOH (ME3B).	101
Figure 3.12.	A schematic represnteation of the introduction of PEG-biotin on the gold core.	102
Figure 3.13.	Absorption and structural analysis of PEG-biotin functionalised AuMPCs. A1) and A2) 1% [ME6A,14 nm] and B1) and B2) 10% [ME6B, 14 nm] PEG-biotin MPCs.	103
Figure 3.14	Agarose gel electrophoresis of 14 nm PEG-biotin functionalised AuMPCs; lane 1 = 1% (ME6A) and 2 = 10% (ME6B).	103
Figure 3.15.	Optical absorption and structural analysis of the 5.5 nm PEG-biotin functionalised AuMPCs. A1) and A2) 1% [ME7A, 5.5 nm] and B1) and B2) 10% [ME7B, 5.5 nm] PEG-biotin MPCs.	104
Figure 3.16.	Agarose gel electrophoresis of PEG-biotin gold MPCs; lane 1: 1% (ME7A) and lane 2: 10% PEG-biotin (ME7B).	104
Figure 3.17.	The streptavidin molecule (Protein Data Bank code 1SWE), showing the four biotin-binding sites [37]. The image was generated using the Accelrys® Discovery Studio Visualizer 2.0, Accelrys Software Inc.	105
Figure 3.18	Biomolecular immobilisation of streptavidin onto biotiylated gold MPCs via the biotin-streptavidin interaction.	106
Figure 3.19.	Optical absorption and structural analysis of streptavidin-AuMPC bioconjugates formed through the BA method. A1) and B1) ME8 (14 nm) and B1) and B2) ME9 (5.5 nm).	107
Figure 3.20.	Agarose gel electrophoresis of streptavidin-AuMPC bioconjugates. (A) lane 1: STV bioconjugates (ME8, 5.5 nm)and lane 2: corresponding PEG-biotin MPCs (ME6A, 1%, 14 nm; (B) lane 1: streptavidin bioconjugates (ME9, 5.5 nm) and lane 2: corresponding PEG-biotin MPCs (ME7A, 1%, 5.5 nm).	107
Figure 3.21.	The BAB strategy for the introduction of the TAT CPP onto the surface of streptavidin-MPC conjugates.	108

Figure 3.22.	Structural analysis of the TAT-immobilised bioconjugates. A1 ME10 (14 nm), and B: ME11 (5.5 nm).	110
Figure 3.23.	Agarose gel electrophoresis of TAT-immobilised bioconjugates, (A) lane 1: streptavidin conjugates [ME8, 14 nm], lane 2: corresponding TAT bioconjuagates (ME10) and (B) lane 1: streptavidin conjugates [ME9, 5.5 nm] and lane 2: the corresponding TAT bioconjugates (ME11)	110
Figure 3.24.	Optical and structural analysis of the FAM-TAT gold bioconjugates; A1) and A2) ME12 (14 nm) and B1) and B2) ME13 (5.5 nm).	112
Figure 3.25.	Agarose gel electrophoresis of the FAM-TAT gold bioconjugates; A) lane 1: streptavidin conjugates (ME8, 14 nm), lane 2: corresponding FAM-TAT bioconjugates (ME12); B) lane 1: streptavidin conjugates (ME9, 5.5 nm) and lane 2: corresponding FAM-TAT bioconjugates (ME13).	112
Figure 3.26.	Analysis of PEG-NTA functionalised gold MPCs (14 nm). A1, B1 and C1, optical absorption of 1% [ME14A, 14 nm], 10% [ME14B, 14 nm] and 50% [ME14C, 14 nm] and A2, B2 and C2, corresponding TEM micrographs of 1%, 10% and 50% PEG-NTA gold MPCs.	114
Figure 3.27.	Agarose gel electrophoresis of PEG-NTA functionalised MPCs; lane 1: ME14A (1%), lane 2: ME14B (10%) and lane 3: ME14C (50%).	115
Figure 3.28.	Analysis of PEG-NTA, PEG-COOH costabilised gold MPCs (14 nm). A1, B1 and C1, optical absorption of 1% [ME15A, 14 nm], 10% [ME15B, 14 nm] and 50% [ME15C, 14 nm] and A2, B2 and C2, corresponding TEM micrographs of 1%, 10% and 50% PEG-NTA gold MPCs.	116
Figure 3.29.	Agarose gel electrophoresis of PEG-NTA functionalised MPCs costabilised with PEG-COOH; lane 1: ME15A (1%), lane 2: ME15B (10%) and lane 3: ME15C (50%).	117
Figure 3.30.	TEM micrograph of PEG-NTA MPCs, costabilised with PEG-OH (A) ME16A and (B) ME16B.	118
Figure 3.31.	Agarose gel electrophoresis; lane 1: 1% [ME16A, 5.5 nm] and lane 2: 10% PEG-NTA MPCs [ME16B, 5.5 nm].	118
Figure 3.32.	The introduction of the Ni^{2+} cations into the NTA of the PEG-NTA gold MPCs.	119

Figure 3.33.	UV-Vis absorption spectrum for the PEG-NTA-Ni (B,ME17) compared with the 1% PEG-NTA MPCs (B, ME14A) from which it was synthesised. Inset: the distinct physical changes on addition of the nickel (II).	120
Figure 3.34.	The attachment strategy of 6xHis-tagged MAP kinase onto the surface of the Ni(II) bound gold MPCs.	121
Figure 3.35.	Optical absorption measurements for the 6xHis-tagged MAP kinase bioconjugates (ME18, 14 nm). Inset: Visual comparison of (A) PEG-NTA conjugates, (B) PEG-NTA-Ni gold conjugates (ME17) and (C) the MAP kinase gold bioconjugates.	122
Figure 3.36.	Agarose gel electrophoresis analysis of the 6xHis-tagged MAP kinase bioconjugates. Lane 1: MAP kinase bioconjugates (ME18, 14 nm) and lane 2: 1% PEG-NTA gold MPCs (ME14A, 14 nm	123
Figure 3.37.	TEM structural analysis micrograph of the 6xHis-tagged MAP kinase bioconjugates (ME18, 14 nm).	123
Figure 3.38.	A schematic demonstration of the different functionalities that have been successfully incorporated onto the gold core, using various protocols.	124
Figure 4.1.	Quantitative representation of the gold MPCs and bioconjugates induced cytotoxicity responses for the CHO22 cells. Functionality-dependent responses of MPCs, 5.5 nm (A) and 14 nm (B), biomolecular functionality comparisons for 14 nm particles (C) and size comparisons for MPCs (1% carboxy of 5.5 and 14 nm) (D).	138
Figure 4.2.	Apoptosis quantification histograms of gold nanoparticles showing size effects (A), MPCs demonstrating the effect of functionality (B) and different bioconjugates (C) on CHO22 cells.	141
Figure 4.3.	Apoptosis measurements of gold MPCs at two doses on Jurkat cells.	143
Figure 4.4.	Cellular morphology changes in Jurkat cells treated with 2.5 x 10^{11} NPs over a period of 24 hours. PBS treated cells (negative control, A), and the corresponding -OH functionalised (B), -COOH functionalised (C) and 1% biotin functionalised (D) MPCs responses.	144

List of Tables

Table 2.1.	Reagents and quantities used for the investigation of the citrate-capped gold nanoparticles.	55
Table 2.2.	Summary of the results in the citrate-capped gold nanoparticles investigation.	64
Table 3.1.	PEGylated ligands for the synthesis of gold monolayer protected clusters.	84
Table 3.2.	Sample codes and their short descriptions.	91
Table 4.1.	Cell lines.	133
Table 4.2.	Sample codes of the investigated materials.	136

Symbols and abbreviations

7-AAD	7-aminoactinomycin D
AGE	Agarose gel electrophoresis
BAB	Biotin-avidin-biotin
CHO	Chinese hamster ovary cells
CPP	Cell penetrating peptide
CVD	Chemical Vapour Deposition
2D	Two dimension
3D	Three dimension
Da	Dalton
DMME	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
EDC	N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride
fcc	Face centred cubic
FCS	Foetal calf serum
FSC	Forward scatter
FACS	Fluorescence-Activated Cell Sorting
FAM	6-Carboxyfluorescein
g	Gram
GSH	L-Glutathione
GST	Glutathione-*S*-transferase
6xHis	Hexahistidine
kDa	Kilo Dalton
LD_{50}	Median lethal dose
LDH	Lactate-dehydrogenase
M	Molar
mL	Milliliter
μL	Microliter
MAP	Mitogen-activated protein
mM	Milimolar
MMPCs	Mixed monolayer protected clusters
MPCs	Monolayer protected clusters

MPS	Mononuclear phagocytic system
MRI	Magnetic resonance imaging
MTT	3-(4,4-dimethylthiozol-2-yl)-2,5 diphenyl tetrazonium bromide test
NHS	*N*-hydroxysuccinimide
μm	Micrometer
nm	Nanometer
NTA	Nitrilotriacetic acid
OD_{540}	Optical density at 540 nm
PBS	Phosphate buffered saline
PEG	Poly(ethylene glycol)
PEG-SH	Thioalkylated PEG
PI	Propidium iodide
PS	Phosphatidylserine
PVP	poly(vinyl pyroolidone)
r	Radius
ρ	Density
R	Organic group
RES	Reticuloendothelial system
RME	Receptor mediated endocytosis
ROS	Reactive oxidative species
rpm	Revolutions per minute
SAMs	Self assembled monolayers
SDS	Sodium dodecyl sulphate
SPION	Superparamagnetic iron oxide nanoparticles
SPR	Surface plasmon resonance
SSC	Side scatter
TOABr	Tetraoctylammonium bromide
TEM	Transmission Electron Microscopy
UV-vis	Ultraviolet-visible