Peptide-Functionalised Gold Nanoparticle Assembly for Rapid and Sensitive Protease detection

L.Koh, [a] A.Laromaine, [a] M.Murugesan, [a] R.V.Ulijn [c+], M.M. Stevens [a,b+]

[a] *Department of Materials, Imperial College, Exhibition Road, London, UK.*

[b] *Institute of Biomedical Engineering, Imperial College, Exhibition Road, London, UK*

c Manchester Materials Science Centre, University of Manchester &UMIST.

+corresponding authors m.stevens@imperial.ac.uk, *R.Ulijn@manchester.ac.uk*

Statement of Purpose:

Protease screening is beneficial to the development of biomedical sciences as proteases are involved in disease states such as HIV, Alzheimer's disease, Hepatitis C, Candida infections and pancreatitis [1]. The ability to produce a colorimetric protease sensor is an attractive one. Nanomaterials based on peptide-functionalised gold nanoparticles offer an approach for the development of a simple, selective, sensitive colorimetric sensor. The optical properties of gold nanoparticles are such that changes in their aggregation state can be monitored [2]. Peptides are suitable tethers for gold nanoparticle assembly as attachment to the gold surface can be easily achieved by the incorporation of a cysteine residue. Peptide-functionalised gold nanoparticle hybrid systems can be tailored for different physical properties by changing the peptide sequence. We propose that by designing an aggregated peptide-nanoparticle system which dis-assembles upon encounter of the target protease, we would detect a change in colour of the peptide-nanoparticle solution from blue to pink.

We demonstrate the proof-of-concept with Thermolysin. Thermolysin is known to be selective for hydrophobic residues for P_2 and non-selective for residues in the P_1 position (P_1-P_2, where P_1 is the N-terminus) [3]. Peptide N-Fluorenyl-9-methoxycarbonyl (Fmoc)-Gly-Phe-Cys was designed to have this specificity; the incorporation of Cys facilitates attachment to the gold nanoparticle surface via a gold-thiolate bond [4] and π–π interactions [5] between the Fmoc groups are designed to cause an aggregation of peptide-functionalised gold nanoparticles. The selective hydrolysis of Gly-Phe bond would produce a positive charge at the N-terminus, resulting in a dispersion of peptide-functionalised gold nanoparticles.

Methods: 10nm gold nanoparticles (BBI International, UK) were stabilized by complexation with dipotassium bis(p-sulfonatophenyl)phenylphosphine dihydrate (Strem Chemicals, MA). Peptides were synthesized by standard Fmoc solid phase peptide synthesis using ABI 433A peptide synthesizer. Synthesized peptides were added to the stabilized gold nanoparticles final concentrations of 100nM in 100mM NaCl, 10mM KPO_4, pH 8 overnight. Peptide-functionalised gold nanoparticle solution was centrifuged and re-dispersed thrice in 10mM KPO_4, pH 8 before adding Thermolysin solutions (7.2nM, to 2.08zM) from *B. thermoproteolyticus Rokko* (Sigma-Aldrich, UK). The peptide-functionalised gold nanoparticle assembly was characterized by UV-visible spectrometry (200-800nm) and transmission electron microscopy. An aggregation/dispersion parameter (A/D ratio) was computed from each UV-visible spectrum and taking the area under the curve from 550 to 700nm divided by the area under the curve from 490 to 540nm.

Results/Discussion:

Functionalization of the gold nanoparticles with peptides produced an aggregated blue peptide-functionalised gold nanoparticle solution characterized by TEM images and UV-visible spectroscopy. Introduction of Thermolysin (7.2nM to 2.08zM) caused the peptide-functionalised gold nanoparticle solution to turn pinkish-red (Fig 1). TEM images revealed a well-dispersed system after Thermolysin addition. A/D ratio calculations detected a change after 5 min. 7.2nM Thermolysin produced a blue-shift in the plasmon resonance peak from 565 nm to 532nm in 6 hours (Fig 1). Single molecule (2.08 zM) detection was also demonstrated which took 23h to produce a well dispersed system. Control experiments using Fmoc-Gly-d-Phe-d-Cys as the control peptide and trypsin as the control protease did not result in the dis-assembly the system.

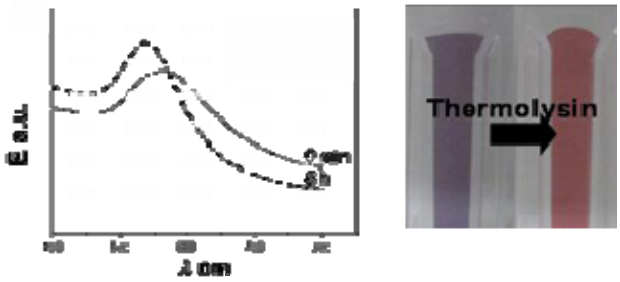

Figure 1 (left) UV-visible spectra of Fmoc-Gly-Phe-Cys-gold nanoparticles (solid line) prior to addition of Thermolysin. +Phe-Cys-gold after 6h incubation with 7.2nM Thermolysin. (right) Digital pictures of peptide-functionalised gold nanoparticle solution showing the colour change from a blue solution to pinkish-red after addition of Thermolysin.

Conclusions:

We have successfully designed a protease sensor using peptide-functionalised gold nanoparticle assemblies, which enables rapid protease detection with a much higher sensitivity (single molecule) than previously reported. Our approach offers dynamic control over the assemblies under mild conditions, which could prove useful for the development of a new class of biologically-controlled materials with applications in drug delivery and monitoring of enzymes.

References

[1] Maly D.J. ChemBioChem. 2002;3(1):16-37

[2] Chowdhury,M.H. J. Biomed.Opt. 2004;9(6):1347-1357

[3] Ron H.P.Doeze, Angew. Chem. Int. Ed. 2004;43:3138-3141

[4] Niemeyer.C.M., Angew. Chem. Int. Ed;2001(40):4128-4158

[5] Toledano, S. JACS. 2006;128(4):1070-1071