Self-Assembly of Enzyme Responsive Peptide-Functionalised Gold Nanoparticles

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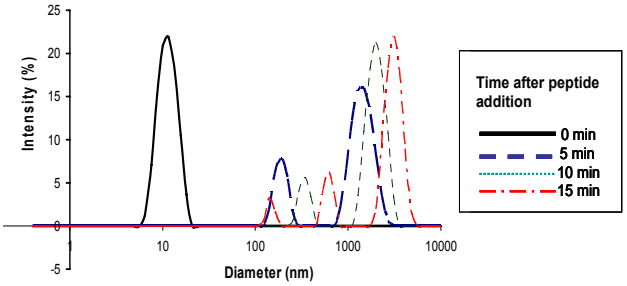
Statement of Purpose:
The use of biomolecules to organize the assembly of inorganic nanoparticles has many potential applications in the biomedical fields, for example in the development of new sensor technologies [1].

We have used N-fluorenyl-9-methoxycarbonyl (Fmoc)-protected peptides to induce the aggregation of gold nanoparticles in a one-step reaction under mild conditions. The aggregation is driven by self-assembly of peptides through attractive π–π stacking interactions. Fmoc-protected peptides are common intermediates in the synthesis of peptides and their versatility in sequence benefits from the availability of a large pool of amino acids. Fmoc-protected peptides have also been reported to be anti-inflammatory [2], and hence have possible applications in drug delivery. Here we investigated a series of Fmoc-peptide-gold nanoparticle assemblies tailored to exhibit different physical properties and the sequence Fmoc-Ser-Ser-Phe-Tyr-Ser-Gly-Gly-Gly-Cys was tested for its ability to sense free prostate specific antigen (fPSA). fPSA was reported to exhibit enzymatic activity towards Ser-Ser-Phe-Tyr-Ser [3] and should cause the dis-assembly of the system through enzymatic hydrolysis which releases a positive amine group.

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. fPSA was purchased from Scipac. For characterization of the early stages of aggregation, synthesized peptides were added to the stabilized gold nanoparticles to final concentrations of 100nM in 100mM NaCl, 10mM KPO_4, pH 8 *in situ* and monitored every 5 minutes for the first 15 min using dynamic light scattering and Halo LM10^{TM3}. For characterization of the late stages of aggregation, the peptide-functionalised gold nanoparticles were characterized using transmission electron microscopy and UV-visible spectroscopy after 48h. Raman spectroscopy (200mW, 785nm laser) was used to confirm the chemical attached of peptides on gold nanoparticles. The functionalized-peptide gold nanoparticles were centrifuged and re-dispersed in 50mM Tris-HCl buffer, pH 7.8, 0.1M NaCl before the addition of fPSA (4, 10, 20 ng/ml) at 37 ^{0}C.

Results/Discussion:
Halo LM10^{TM} is a nanoparticle technique which allows individual particles to be sized and viewed. Dynamic light scattering sizes particles using an average value. Results obtained using dynamic light scattering measured an increase in size of the nanoparticle assembly from 9.89 nm to 190-1480nm after 5 min of peptide addition (Fig 1). Aggregation was viewed by Halo LM10^{TM} and an increase in particle size from a range of 80-100nm to 80-780nm after 5 min of peptide addition was measured. The larger apparent size of unassembled nanoparticles (80 nm apparent versus 10 nm actual) as sized by Halo LM10^{TM} is thought to be due to the particles adsorbing on the optical glass surface which renders them immobile, hence being interpreted as a larger particle.
Figure 1 Results from dynamic light scattering showing an increase in nanoparticle size upon addition of Fmoc-protected peptides from 0-15 min.

UV-visible spectroscopy and transmission electron



microscopy, confirm the observations on the assembly of the nanoparticles. The UV-visible spectrum of peptide functionalized gold nanoparticles showed a red-shift in the plasmon resonance peak from 525nm to 565nm as the particles assembled into an aggregated state. Surface-enhanced Raman spectroscopy of the peptide functionalized gold nanoparticles confirmed the attachment of Fmoc-peptides on gold nanoparticles.
Upon addition of fPSA, a blue-shift in the UV-visible spectrum to 525 nm was monitored after 4 hours. Transmission electron microscopy images of the peptide-functionalised gold nanoparticles after enzyme incubation revealed well-dispersed nanoparticles.

Conclusions:
We have also successfully created an assembly of peptide-functionalised gold nanoparticles by one-step reaction under mild conditions, which could be viewed and characterised from early time points (5-15 min). The ability to sense fPSA was also demonstrated.

References:
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