# Site-specific targeting of enterovirus capsid by functionalized monodisperse gold nanoclusters

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## Introduction







- Enteroviruses are about 30 35 nm icosahedral bioparticle without a lipid envelope.
- Contain numerous clinically important human pathogens belonging to coxsackie viruses, echoviruses and polioviruses.
- Cause a wide range of acute diseases, from mild rash to viral meningitis, heart muscle failure and paralysis.
- Thus it is important to understand the pathogenesis of virus infection in vitro and in vivo.
- Hence reliable means of tracking the virus are crucially needed.

- Development of precise protocols for accurate site-specific conjugation of monodisperse inorganic nanoparticles to large biomolecules and bionanoparticles, providing new tools for bioimaging and tracking in biological systems, is one of the challenges in contemporary bionanoscience and nanomedicine.
- Recent applications to study viruses and their distribution in cells and tissues have usually involved the use of fluorescent markers for optical imaging or colloidal gold markers for enhancing image contrast in electron microscopy.
- But in these cases long-term stability of the markers may be compromised by uncontrolled processes such as photobleaching.
- Furthermore, as the markers link to viruses indirectly via antibodies, the antibody-antigen interactions may not be strong enough to withstand harsh conditions.
- Therefore, reliable tracking continues to be a challenge, and more direct and robust tracking strategies are desirable.

#### In this paper...

- A successful site-specific covalent conjugation of functionalized atomically monodisperse gold clusters with 1.5-nm metal cores to viral surfaces is reported.
- Water-soluble Au<sub>102</sub>(para-mercaptobenzoic acid)<sub>44</sub> clusters, functionalized by maleimide linkers to target cysteines of viral capsid proteins, were synthesized and conjugated to enteroviruses echovirus 1 (EV1) and coxsackievirus B3 (CVB3).
- They observed that the clusters enhance the icosahedral topology of the viruses in TEM.

#### **Results and Discussions**

- Au<sub>102</sub>(pMBA)<sub>44</sub> clusters were synthesized by previously reported method and characterized by TEM, NMR, UV-vis spectroscopy and gel electrophoresis.
- Then carboxylic acid terminal of ligand is bound to the six carbon alcohol with maleimide functionality, i.e., N-(6-hydroxyhexyl)maleimide through an esterification reaction to give Au<sub>102</sub>(pMBA)<sub>44-m</sub>[N-(6-hexyl 4mercaptobenzoate)maleimide]<sub>m</sub> – Au102-MI.
- The success of the functionalization is confirmed by gel electrophoresis, UV-vis and IR spectroscopy.
- > The functionalized clusters were then conjugated to free thiols of surface cysteines of EV1 and CVB3 via the Michael addition reaction.







### Optical absorption spectrum and PAGE analysis of functionalized $Au_{102}$ -MI and pure $Au_{102}$ (pMBA)<sub>44</sub> in H<sub>2</sub>O.



images of CVB3 TEM viruses (A-C) treated with functionalized and nonfunctionalized gold clusters. (A) After incubation for 2 days with Au102-MI clusters without column purification, (B) after column purification, (C)control with conventional negative staining of virus sample incubated with nonfunctionalized  $Au_{102}(pMBA)_{44}$ , and (D) EV1 incubated with Au102-MI for 1 day. Negative staining has been used also in D, resulting in the dark gray halo around the cluster.



Quantitative and positions analysis of the Au102-MI clusters in the TEM images of cluster-virus conjugates. (A) Cluster-cluster distances compared with the thiol-thiol distances of cysteine residues in the known structure of EV1. In the panels on the right, "Exp." shows the experimental data for the distribution of the cluster centers (marked by red dots on the left) and "Model" shows the calculated distance distribution of the thiol binding sites in the cysteines close to the viral surface. (B) Identification of binding patterns of the gold clusters in EV1, compared with thiol positions denoted by yellow spheres.



A clear correlation of the cluster positions to the known sites of the cysteines is found, to the extent that the most likely orientation of the virus particle with respect to the TEM grid can be deduced, and several features of pentagonal and trigonal rotational symmetries of the viral surface can be identified.

The spatial distribution and cysteine–cysteine distance distributions are different in EV1 and CVB3. The fact that the cluster–cluster distributions reflect well these differences is the most convincing evidence of cysteine-specific binding of the functionalized clusters to both virus types.

#### Virus



The amount of infective quantified by the endpoint dilution assay. EV1 or CVB3 was treated with Au102-MI gold clusters and control clusters for 24 or 48 h at 37 °C and serially diluted on GMK cells to evaluate the infectivity of the virus preparations. Control virus was either fresh virus directly from the stock or virus that was incubated at 37 °C without gold clusters.

### Summary

- A unique procedure for achieving robust, direct, site-specific labelling of enteroviruses is demonstrated.
- Quantitative analysis of transmission electron microscopy images and the known virus structures showed high affinity and mutual ordering of the bound gold clusters on the viral surface and a clear correlation between the clusters and the targeted cysteine sites close to the viral surface.
- Infectivity of the viruses was not compromised by loading of several tens of gold clusters per virus.
- These advances allow for future investigations of the structure-function relations of enteroviruses and enterovirus-related virus-like particles, including their entry mechanisms into cells and uncoating in cellular endosomes.

- The functionalized clusters of this kind can be used for water testing/water purification.
- If we have a cluster material which effects the infectivity of viruses, can they be used to destroy them?

