In vivo fate of systemically administered encapsulin protein nanocages and implications for their use in targeted drug delivery

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Background: Encapsulins are proteins which self-assemble from protein subunits into identical nanocage structures.[1] Encapsulins are a novel and promising tool for targeted therapeutic delivery, enhanced medical imaging and vaccine delivery.[2-5] This is due to the relative ease in which they can be bioengineered to display or encapsulate specific proteins, and/or RNA.[6] While encapsulins are being increasingly investigated *in vitro*, to be effectively utilised as a drug/therapeutic delivery system, there remains a need to understand the biological fate and effect of encapsulin proteins *in vivo.* Here, we determine the composition of the protein corona, the biodistribution to tissues, as well as the toxicity of encapsulin.

Experimental: *Thermotoga maritima* (Tm) encapsulin (Tm-Enc) was recombinantly expressed, purified and conjugated to a fluorescent tag (Cy7). Biophysical characteristics were determined using microscopy (TEM), spectroscopy and protein gel analysis. Blood compatibility was determined *via* haemolytic assay. The composition of the protein corona was determined *via* LC-MS. Biodistribution of Tm-Enc was determined in BALB/c mice and visualised using an IVIS imaging system over 72 hours. Finally, un-tagged Tm-Enc was administered (5mg/kg) to determine toxicity over 14 days.

Results and Discussion: Tm-Enc showed no haemolytic effect up to the highest tested concentration. The protein corona was investigated to determine how the composition of the protein corona changed. We showed that the composition dynamically changed from 1hr incubation vs. 6hr. Further, we investigated the biodistribution profile of Tm-Enc *in vivo* and show that Tm-Enc accumulated exclusively in the liver and kidneys (Fig 1A) by 3hrs, and remains in the liver up to 72hrs. We have shown that Tm-Enc is phagocytosed by liver Kupffer cells (Fig. 1B). Finally, we administered untagged Tm-Enc to determine any toxic effects *in vivo.* We show no gross weight changes, no pathological changes in the liver, kidney, heart, spleen or lung, and no pro-inflammatory response up to 14 days post administration.

Conclusions: Tm-Enc is non-toxic to RBCs and tissues, and localises to the Kupffer cells in the liver over 72 hours. The long biodistribution time may be attributed to the reduced number of opsonins adsorbing to the surface, and the lack of pro-inflammatory immune response following administration. This work will inform the design/engineering of encapsulins for future use in drug delivery.

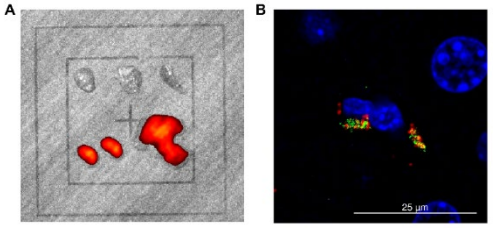

Figure 1: (A) Biodistribution of Tm-Enc to the liver and kidney at 12hrs post administration. **(B)** Association of Tm-Enc (red) with Kupffer cells in the liver (green) at 12 hrs post administration.

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