Objective: To use nanobots for cell-based diagnosis, and as drug delivery vehicles in cancer. To do this we will:

- Develop (antibody & aptamer) conjugated bio-compatible metal-organic framework (MOF) nanobots for marker specific cell recognition and isolation (Aim 1).
- Use a computational model to characterise cell-nanoparticle interactions, predict novel structural combinations and optimise nanobot formulation (Aim 2).
- Use optimised cell targeting nanobots for circulating tumour cell (CTC) isolation and investigation (Aim 3).

Hypothesis: Autonomous nanobots (nanobots) can be used to target disseminated disease (CTCs) in cancer.

The problem: Early detection of disseminated disease is the key to effective cancer treatment. Current imaging methods lack resolution needed to detect early disease (<2mm) [2,3], while molecular methods lack the cellular context needed to ascertain treatment course [4]. In addition, current CTC isolation protocols lead to changes in cell phenotype, which makes data from further ex vivo analysis problematic. Finally, anticancer drugs are often cytotoxic and come with unwanted side effects, which makes application to all patients who might benefit problematic. The use of nanobot CTC isolation has the advantage of not relying on fixation protocols (cells stay viable), and unlike magnetic beads, nanobots can be easily removed for post capture analysis of the cells (Fig. 1A). In addition, because of their autonomous motion, neutrally charged nanobots have potential to be used for efficient drug delivery in patients to target disseminated disease (Fig. 1B).

Significance: In breast cancer the development of resistance to endocrine therapy, associated with loss of hormone receptors, is a key reason for patient mortality [5]. However, despite the cellular heterogeneity observed in breast tumours, no single cell-based assay has yet been developed that can identify changes in cancer cell receptor status in the blood of breast cancer patients [6].

Justification:

- Nanobots are effective at isolating specific cell types from a mixed cell population (Fig. 2).
- Nanobots can be dissolved in EDTA following isolation, leaving viable cells that can be investigated for malignant potential and determining specific drug treatment (Fig. 3).
- Nanobots can be used for the targeted delivery of cytotoxic cancer drugs (Fig. 4).

Novelty: Nanobots for targeting of CTCs is highly novel. The use of specific aptamers (rather than antibodies) in the field of CTC research has the potential to significantly advance the field.

Approach: There are currently four major platforms used for CTC isolation: (i) Density Gradient Centrifugation (DGC); (ii) Microfilters/membranes; (iii) Immune magnetic separation (e.g. CellSearch®); and (iv) Microfluidic chips [28]. These platforms are used separately or in conjunction with another, and each method has its own set of limitations and advantages. DGC on its own is non-specific (enriches all mononuclear cells), results in inadequate capture, and low purity and lack sensitivity. Filters will clog easily, are limited by small processing volumes, and low separation purity; they will capture large debris or cell clusters. Immune magnetic separation may also fail to retrieve captured cells as magnetic particles are permanently bound to CTCs, and there is low throughput as the process is time consuming; relying on trained pathologists to examine cells on slides. Microfluidic chips separate cells, however, it is difficult to retrieve captured cells as they are often permanently bound to substrate. While chips also suffer from problems of volume limitations and low throughput. In contrast to the older generation of CTC isolation methods, the use of nanobots has the potential to address all limitations in current techniques in one: (i) improve capture efficiency, purity, throughput, and release efficiency; (ii) lead to the analysis of almost unlimited sample volumes; (iii) be developed to be highly automated; and (iv) as nanobots are dissolved in EDTA, this will allow for the complete recovery of captured cells. As the recipient of a recent Nobel Prize in Chemistry (2016), self-propelled nanomachines represent a new paradigm in nanobiotechnology. These autonomous devices gather energy and navigate the surrounding environment, and can swarm to selectively search for specific cells or chemical species. Due to their unique advantages, synthetic nanomotors hold great promise for biomedical applications, such as targeted drug delivery, cell identification, biochemical sensing, nano surgery and bioimaging. Their use as bots for cell-based isolation has not been reported and seeks to fulfill a need not currently present in current CTC diagnostic methods.

Preliminary Data. Nanobot development. We have demonstrated a simple fabrication method for these nanobots, which allows the precise control of size, morphology, and functionality in a single-step process. Nanobots are fabricated by encapsulating Catalase in porous MOF matrices in the presence of hydrogen peroxide (H2O2, 50-200μM), nanobubbles are produced by a biocatalytic reaction (H2O2→O2). Bubbles made this way are retained by the MOF, thus shifting its buoyancy, and driving it vertically in a liquid column (Fig. 2).

In Fig. 2 we show that nanobots conjugated with mouse anti-human epidermal growth factor receptor 2 (Her2) antibody can be used to effectively separate Her2+ BT-474 cells from a mixed population, also containing Her2- MCF-7 cells [ratio 1:50; 50 μM H2O2 BT-474/MCF7]. After mixing with anti-Her2 conjugated nanobots and introduction of H2O2 (100 μM), targeted cells are carried to the top of the liquid column (Fig. 2). Importantly, for the first time we showed that the nanobots can be completely removed by EDTA and the recovered cells regain their full metabolic and proliferation potential [6]. We also demonstrated separation of CSGP4 positive cancer cells from a mixed population using house monoclonal antibody conjugated to nanobots (Fig. 4).

The focus of the grant will be to develop nanobots to identify and target two cell populations, that might be circulating in the blood of breast cancer patients, including Her2-receptor cells as well as those that have lost receptor expression. In this last case, a marker linked to TNBC will be investigated (chondroliatin sulphate proteoglycan/CSGP4).