PEOPLE

MARIE CURIE ACTIONS

Intra-European Fellowships (IEF)

Call: FP7-PEOPLE-2012-IEF

“CLEDEPOLY”

FINAL REPORT

(Final Publishable Summary Report)

FINAL PUBLISHABLE SUMMARY REPORT

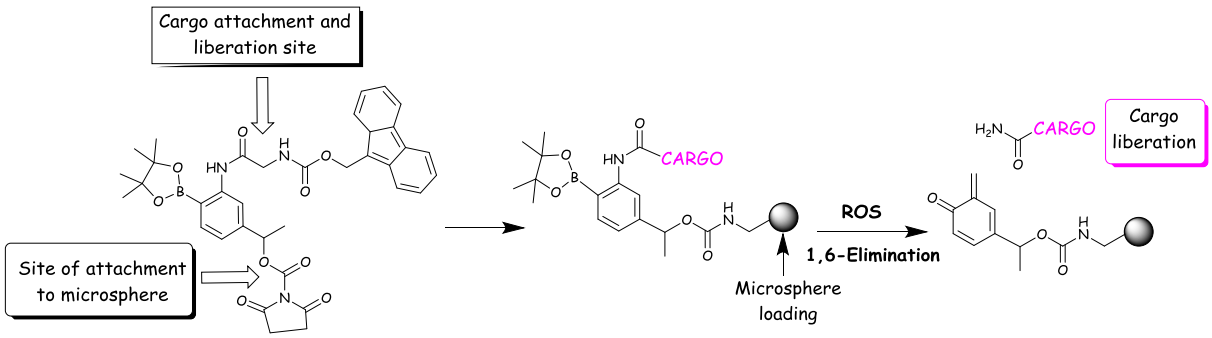
The aim of the project was the generation of monomers with original modes of perturbation, designed to respond to specific biological or chemical stimuli, and their application in the discovery of highly specific, sensing, modulating and bio-responsive polymers. The project proposed provide an efficient mechanism by which tissue specific delivery of cargos will be achieved by the use of bio- or chemical- responsive polymers, thereby allowing the control or modulation of specific biomedical processes.

The longer term aims were the development of a targeted drug delivery system, which is especially desirable in the treatment of cancer. Although potent chemotherapeutic agents are used to treat cancer, their therapeutic window remains small owing to their toxicity, which leads to severe side effects. Therefore, a directed approach needs to distinguish between healthy and cancer cells, which are inherently difficult to discriminate, in order to ensure the specific intracellular delivery of toxic molecules to degenerated tissue. Thus polymers can be designed to be cleaved or fragmented in the proximity of diseased tissues, cancers or regions of high oxidative stress. The nature of the environment (biological or chemical stimulation), will subsequently trigger the delivery of a cargo; entrapped within the polymer; into cells or tissues at specific sites of infection, injury or disease.

Objective (first section of the project):

(1). Reactive Oxygen Species (ROS) have been demonstrated to be involved in a number of pathological processes. The aims of the first section of the research project were the design and synthesis of reactive oxygen species "activatable-polymers" that can "detect" *in vivo* reactive oxygen species and undergo efficient degradation under conditions of oxidative stress and use them to subsequently release cargos into adjacent tissue or inside cells.

In this project the synthesis and characterization of a ROS cleavable linker and its attachment to a polymeric model support (a microsphere) has been carried out (Scheme 1).



Scheme 1. Reactive Oxygen Species (ROS) cleavable linker attached to a microsphere.

The efficiency of the release of the cargo from the cleavable linker was evaluated by HPLC, fluorescence analysis and flow cytometry analysis (following cellular uptake of the microspheres). The results showed that the linker was cleaved in the presence of H_2O_2 (ROS). I am currently writing a communication based on these results.

Objective (second section of the project):

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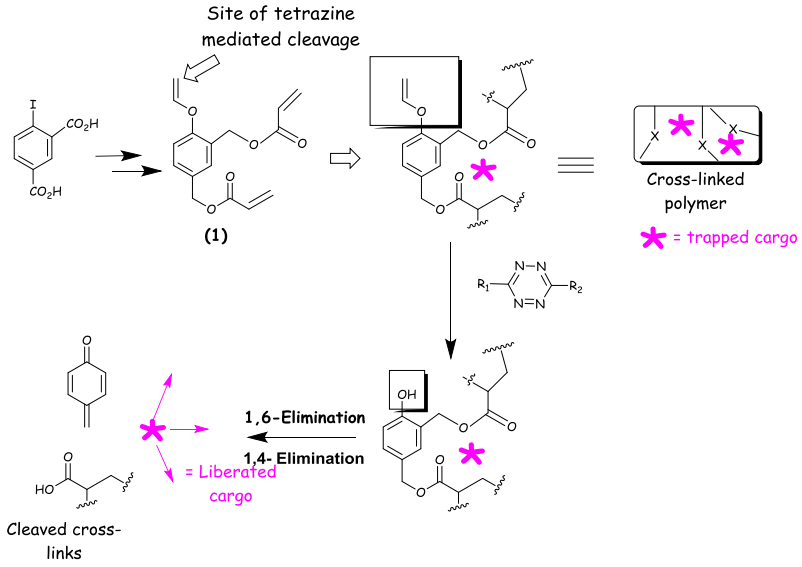
(2). The second aim of this project was to synthesize a drug-polymer conjugate which could be implanted in the proximity of the cancer, with liberation of the drug by interaction with a tetrazine. This will allow highly localized and controlled un-caging of the cytotoxic drug into the proximity of the cancer, with minimal adverse effects on normal tissues.

Many cancer drugs work by being cytotoxic towards the cancer cells, this leads to severe toxicity towards healthy tissues and organs. One effective strategy to circumvent these side effects is to cage a cytotoxic drug with a "caging group", which will supress the cytotoxicity of the drug. It has been established that 1,2,4,5-tetrazines (s-tetrazines) are powerful dienes in inverse Diels-Alder (iDA) reactions of dienophiles. Tetrazine Diels-Alder chemistry has been shown to be mild, water compatible and has been applied *in vivo,* showing its non-toxic and highly specific chemistry. Thus caging of a drug with a dienophile and its de-caging via tetrazine chemistry, using Diels-Alder chemistry, will allow controlled un-caging of the cytotoxic drug in the proximity of the cancer (scheme 2).



Scheme 2. Tetrazine activatable polymers.

During this part of the project a series of tetrazines containing pyridyl, phenyl and/or pyrimidyl moieties have been synthesized. Two fluorescents cargos were "dienophile caged" to evaluate the cargo trapping and release *in vitro*. The releasing of the corresponding dienophile caged-fluorophores by tetrazines was evaluated by HPLC, NMR and Fluorescence Detection. As a "proof-of-concept", we have successfully shown that our probes can be de-caged by tetrazines. . A patent filing based on this work has been approved by the University of Edinburgh (intellectual property advisory group) and is currently being written with filing expected in June/July 2015. Following this publication will take place.