

João Conniot^{1,2}, Sheiliza Carmali², Teresa S. Barata², Liana C. Silva¹, Rogério Gaspar¹, Steve Brocchini², Helena F. Florindo¹

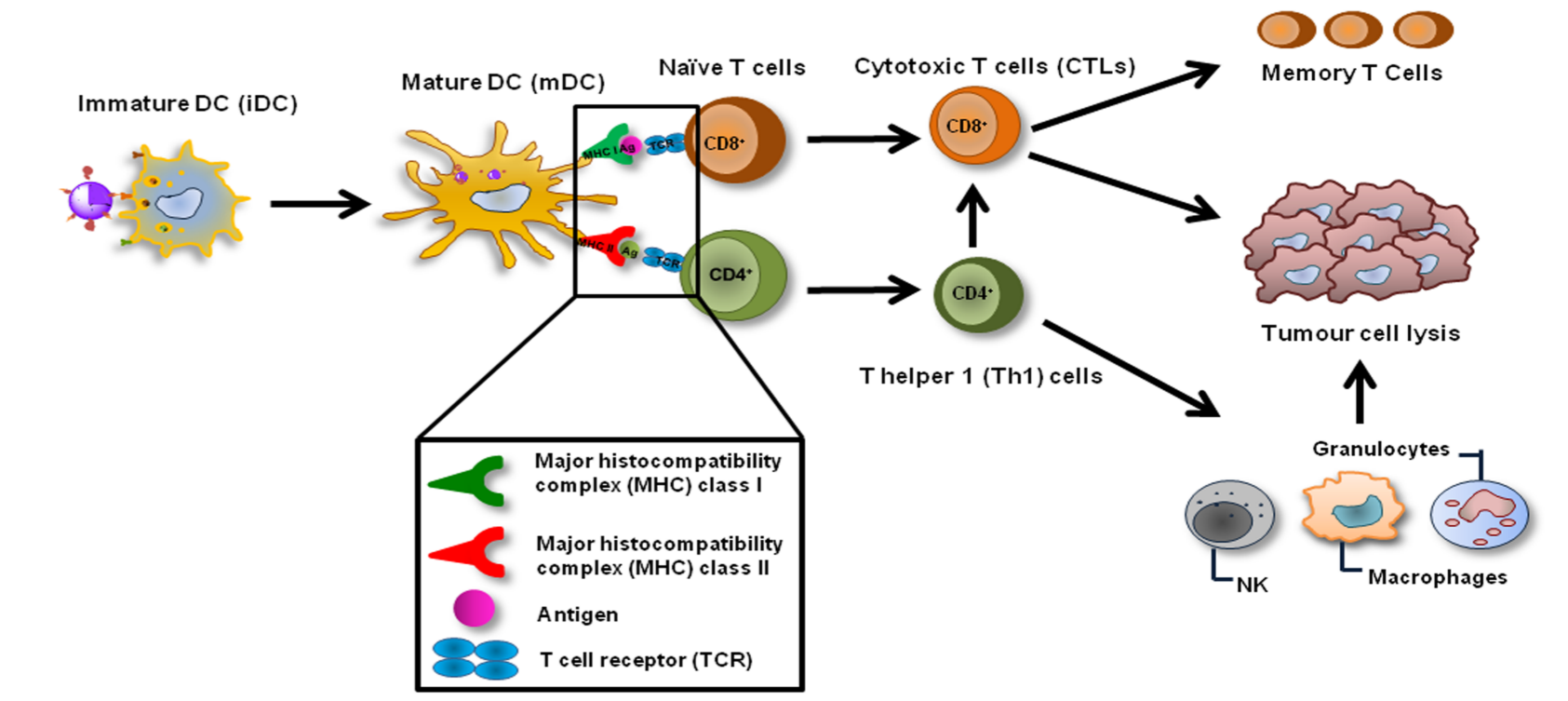
¹Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal;

²EPSRC Centre for Innovative Manufacturing in Emergent Macromolecular Therapies, UCL School of Pharmacy, London, UK.

jmoconniot@ff.ulisboa.pt

Background

- Conventional cancer treatments are generally unspecific and poorly effective.
- Dendritic cell (DC)-based cancer vaccines are expected to elicit a highly specific immune response against tumour cells.
- New efficient platform for specific DC targeting and for modulation of the intracellular trafficking of antigens need to be designed.

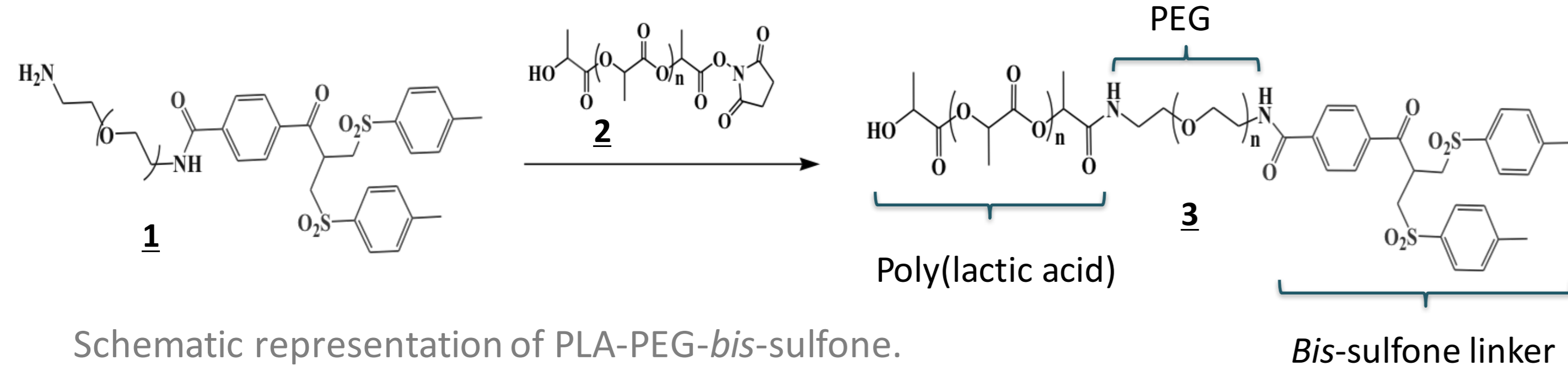


Purpose

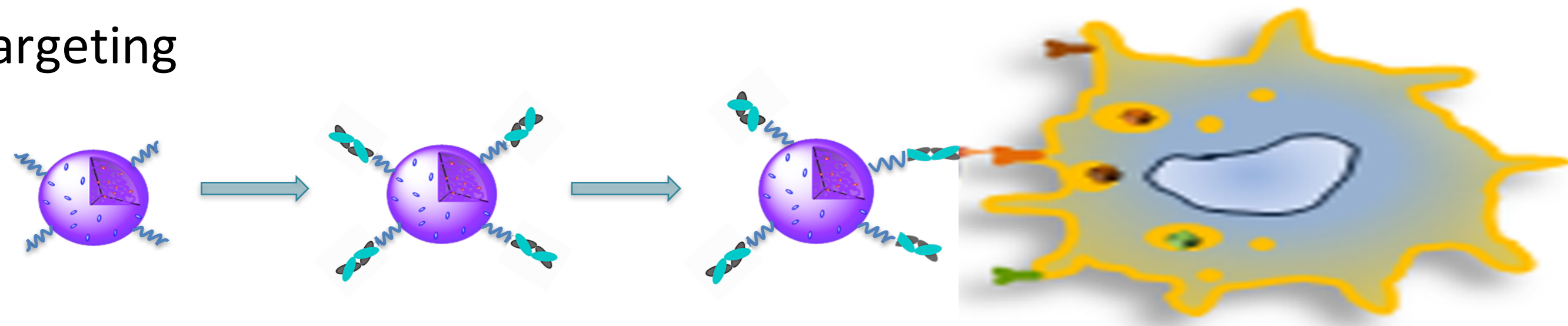
Development of PLA-PEG-*bis*-sulfone polymeric nanoparticles (NPs) for site-specific functionalization with ligands for DC targeting.

Tasks

A – Synthesis and characterization of PLA-PEG-*bis*-sulfone **3**



B – Preparation and characterization of PLA-PEG-*bis*-sulfone NPs for DC targeting



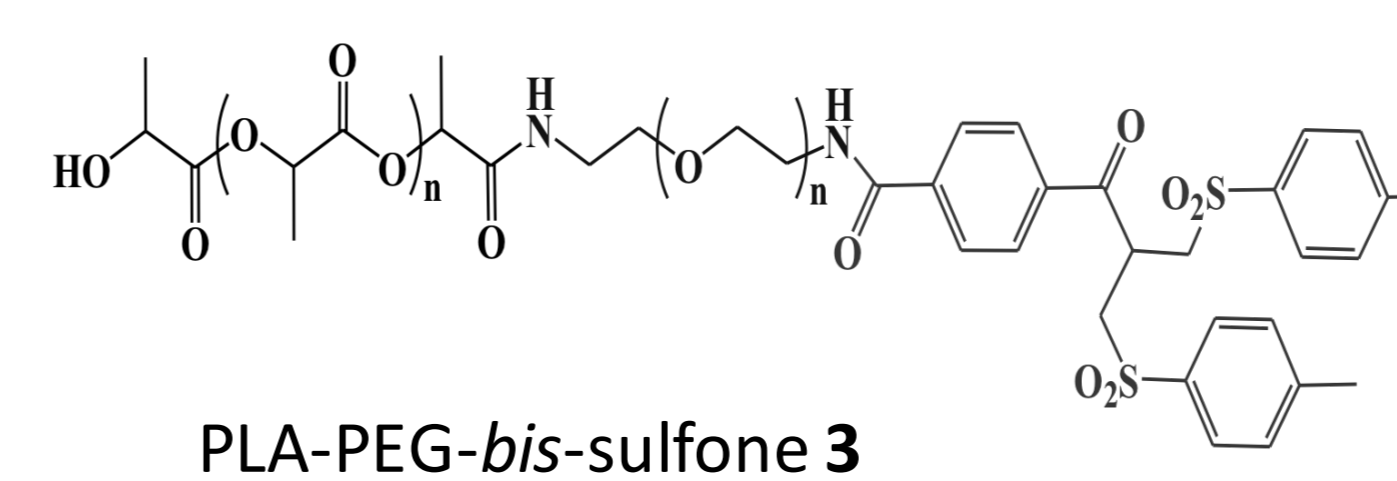
Methods

- Preliminary characterisation of PLA-PEG-*bis*-sulfone **3** performed by ¹H-NMR. Conjugation of compound **3** to DTT-reduced antigen-binding fragments (Fabs) confirmed by SDS-PAGE.
- PLA-PEG-*bis*-sulfone NPs were prepared by the DESE method, using a blend of PLA-PEG-*bis*-sulfone/PLA. mPEG-PLA/PLA NPs were used as control.
- NPs were characterized using DLS, zeta potential (ZP) and TEM.
- Fabs were allowed to react overnight with *bis*-sulfone moieties of prepared NPs, under gentle stirring. The association of Fabs to PLA-PEG-*bis*-sulfone NPs was assessed by Ellman's reagent assay.

Results

Preliminary characterization of PLA-PEG-*bis*-sulfone **3**

¹H-NMR Spectroscopy

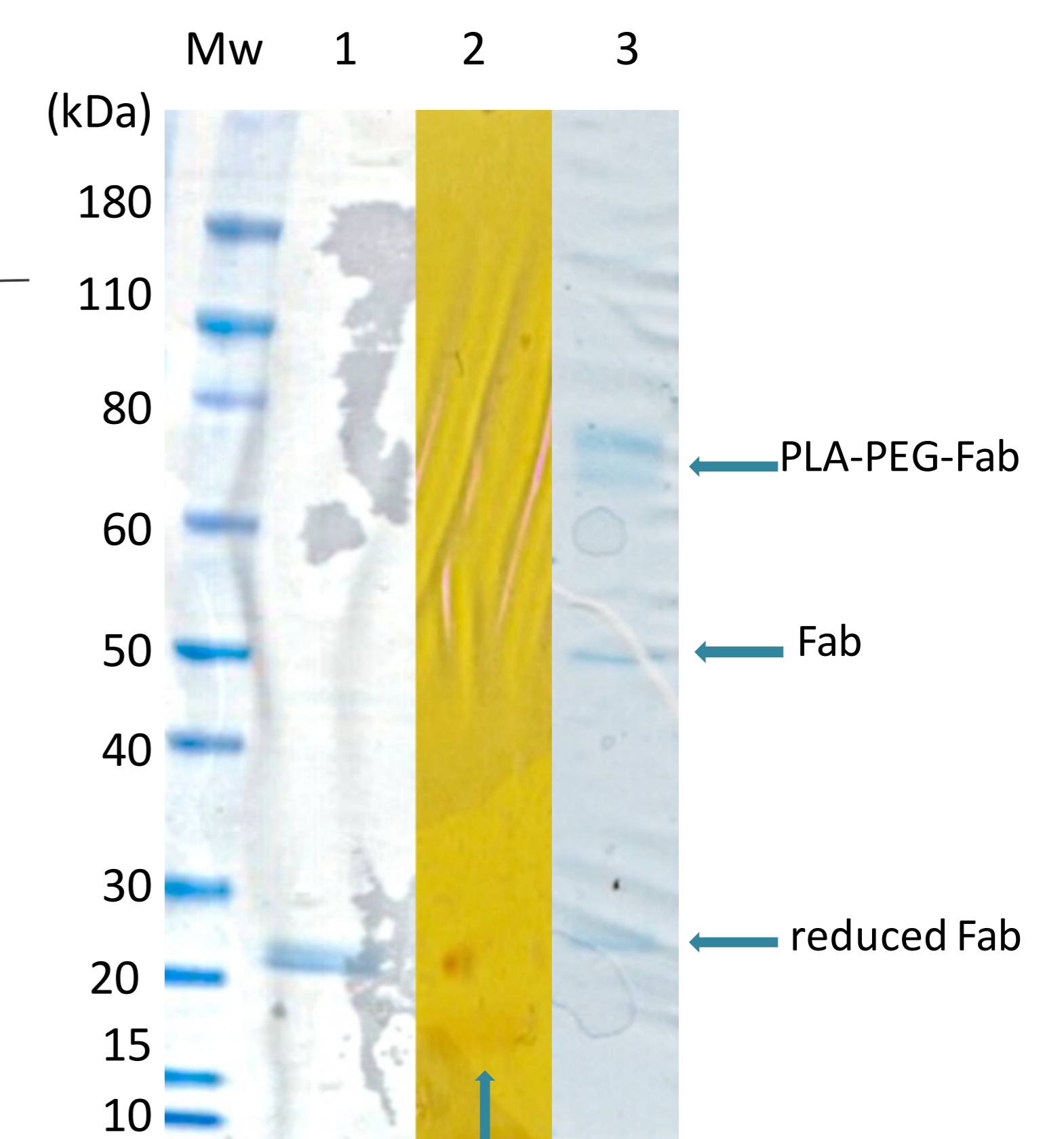


Characteristic peaks

¹H-NMR (CDCl₃, 400 MHz)

PLA	1.58 – 1.64 ppm
PEG	3.48-3.7 ppm
<i>Bis</i> -sulfone (Ar)	7 - 8 ppm

SDS-PAGE of PLA-PEG-Fab conjugate



Lanes: 1 – DTT-reduced Fab; 2 – PLA-PEG-*bis*-sulfone (Mw(PEG)= 10 kDa); 3 – Fab conjugation with PLA-PEG-*bis*-sulfone **3**.

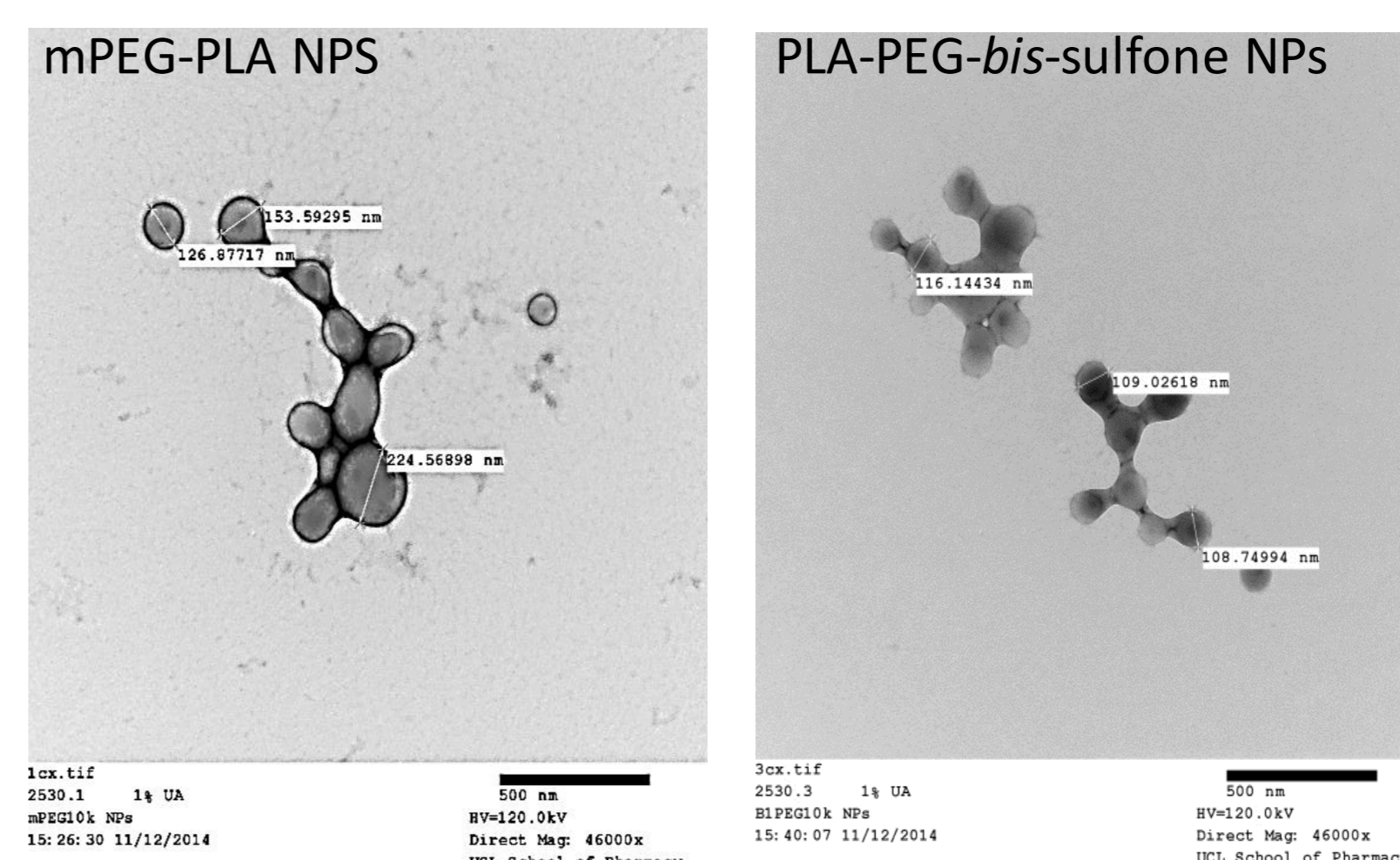
Characterization of PLA-PEG-*bis*-sulfone NPs

DLS, Pdl and ZP

NP Formulation	Size (nm)	Pdl	ZP (mV)	
			0.1 M PBS + 0.15 M NaCl pH 7.6	50 mM PBS + 20 mM EDTA pH 7.8
mPEG-PLA NPs	265.5 ± 20.4	0.08 ± 0.02	-26.24 ± 1.21	0.76 ± 0.55
PLA-PEG- <i>bis</i> -sulfone NPs	275.2 ± 15.1	0.14 ± 0.06	-28.24 ± 1.30	0.92 ± 0.81

(N=3, mean ± SD)

TEM



Association efficiency of Fabs to NPs

NP Formulation	Association efficiency (%)
mPEG-PLA NPs	70.6
PLA-PEG- <i>bis</i> -sulfone NPs	83.2

(Preliminary assay, N=1)

Conclusions & Future Perspectives

- Synthesis of PLA-PEG-*bis*-sulfone **3** was proven and the polymer is able to bind to reduced Fabs.
- Both PLA-PEG-*bis*-sulfone and mPEG-PLA NPs exhibited spherical morphology, similar surface charge and size variability.
- The conjugation of Fabs to PLA-PEG-*bis*-sulfone NPs presented promising results, and optimization procedures will be explored in collaboration with Professor Nicholas Peppas' lab.
- NPs will be explored for DC targeting to potentiate tumor antigen delivery for the promotion tumor cell eradication.

References & Acknowledgements



Conniot J. *et al.*; (2014) Front. Chem.; 2: 105.
Brocchini S. *et al.*; (2006) Nat. Protoc; 1: 2241-2252.

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