# The thermal fluctuations of red blood cells

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

.....John Hale

# Abstract

In this thesis, we describe the development of a new technique for determination of the mechanical properties of the red blood cell membrane from measurement of its thermal fluctuations. Experimentally, the shape fluctuations of the equatorial contours of red blood cells are recorded using fast phase-contrast video microscopy, from which the Fourier fluctuation spectrum is obtained and analysed. The experimentally obtained fluctuation spectra are interpreted using a coarse grained particle dynamics simulation which models the thermal fluctuations of an elastic mesh endowed with bending and shear elasticity, and constant volume and surface area. We demonstrate that the simulation correctly describes the mean shape of the red cell as well as the membrane thermal fluctuations. Comparison between theory and experiment leads to physically sound values for the relevant membrane elastic moduli and helps distinguish between the contributions of the lipid bilayer and the membrane skeleton.

We extend this technique to investigate the mechanical response of red blood cells to oxidative stress. We show that it is possible to discriminate between the actions of different oxidising agents from their distinctive effects on the membrane thermal fluctuation spectrum. This allows comparative measures of the membrane material properties to be extracted using an approximate analytical model thus discriminating between the effects of each oxidising agent on different structural components of the membrane.

This technique was also applied to investigate the response of the red blood cell to oxidative stress under simulated hyperglycemic conditions characteristic for disease

states such as diabetes. We established that the membrane elasticity of glycated cells deteriorate much fasted under administration of hydrogen peroxide, which may be related to the observed microvascular complications in diabetes, characterised by disproportionately high levels of reactive oxidative species. We demonstrate that metformin, one of the most widely prescribed anti-diabetic drugs, has an ameliorative effect on the membrane mechanical properties, which is probably due to its anti-glycating effects.

The technique provides a reproducible means to assess the effects of reactive oxidative species on the red blood cell membrane mechanical properties and distinguish between effects on the protein membrane skeleton and the lipid bilayer. This makes the new method of potential value in monitoring the effects of drug induced changes hence assessing progress of treatment in terms of the antioxidant or anti-glycation properties of administered drugs in diabetes or other conditions characterised by high levels of oxidative stress.

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