# A Dynamic Biomaterial-Ligand Tethering Strategy for Tissue Engineering

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## 1. Introduction

Tissue engineering is a **regenerative** approach that uses **material scaffolds** to repair or replace damaged tissues for the treatment of disease or injury.[1]

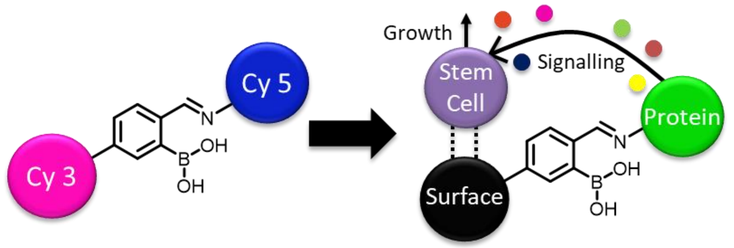


**Fig. 1** Schematic of *ortho*-boronobenzaldehydes and their use in conjugating proteins to a surface to allow for cell signalling which is needed for tissue development.

The effectiveness of a material depends on its ability to send signals to cells to direct tissue **growth** and **development**.[2]

Biochemical signalling can be synthetically emulated by **iterative** association of proteins to a material in a **reversible** and **dynamic** manner, which allows tissue growth to be **controlled**, recreating natural biochemical signalling pathways responsible for tissue development and maturation.[3]

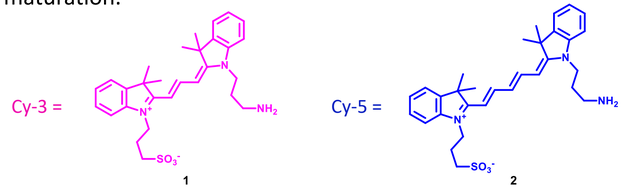


**Fig. 2** Schematic of colourful cyanine (Cy) dyes used in this work.

## 2. Use of Iminoboronates

We are particularly interested in the use of ***ortho*-boronobenzaldehydes** to attach bioactive proteins to materials due to their ability to form stabilised, yet **dynamic**, boronoimines.

A panel of *ortho*-boronoimines have been synthesised (**Fig. 3**) to understand their **functionality** and **interactions** when attached to a bioactive protein.

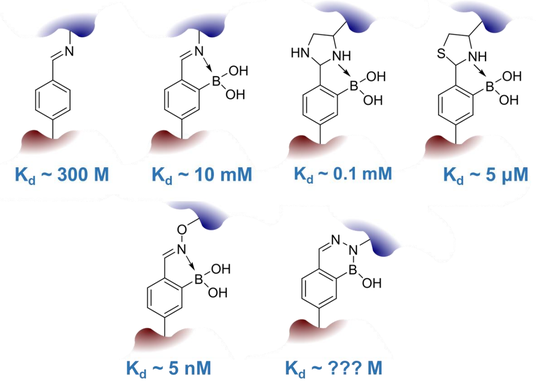


**Fig. 3** Schematic illustrating the various stabilities of iminoboronates for dynamic association of bioactive proteins.

## 3. Synthesis of Dye Analogues

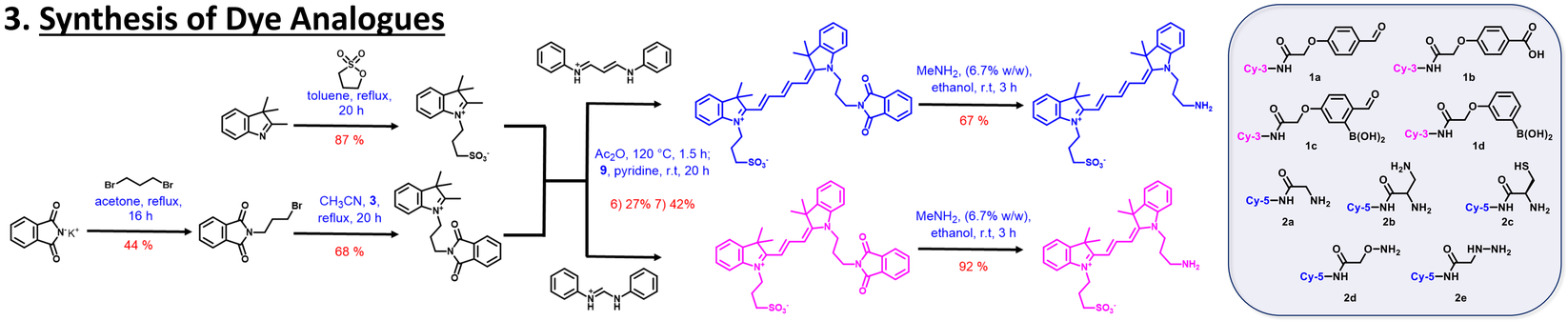


**Fig. 4** Synthesis of Cy-3 and Cy-5 dyes as well as corresponding target molecule analogues to form a library of *ortho*-boronoimine complexes for the formation of dynamic covalent networks.

In this work, we utilise the cyanine (**Cy-3** and **Cy-5**) dyes as they are very **strongly fluorescent** at low concentration, **chemically stable** with respect to **temperature** and **pH**. and undergo sufficient Förster resonance energy transfer (FRET) pairing.

These synthesised boronoimines will be screened using a **FRET assay**, to measure **rate of bond formation** and its **stability**. These stabilities can be converted to **reaction kinetics** which will later be used to **conjugate proteins** to biomaterial surfaces, and then direct cell behaviour.

## 4. FRET Assay

Förster resonance energy transfer (FRET) is a **non-radiative** energy transfer that occurs through **dipole-dipole** coupling from an excited donor fluorophore to a ground state acceptor (**Fig. 5**) when appropriate **spectral overlap** and **proximity** requirements are satisfied.[3]

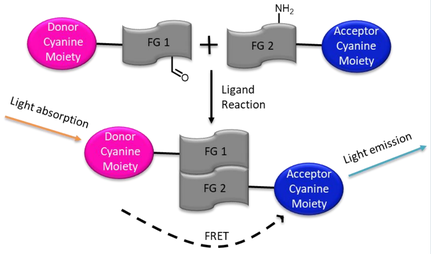


**Fig. 5** Ligation reaction between orthogonal Cy dyes resulting in acceptor emission due to energy transfer from an excited donor to the acceptor group.

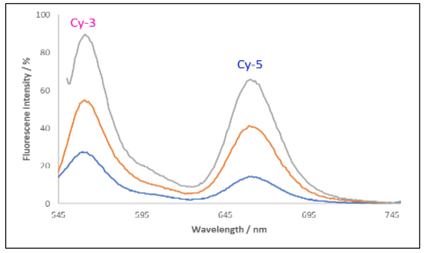


**Fig. 6** Emission spectra showing the non-radiative energy transfer from the donor Cy-3 excited state to the acceptor Cy-5 resulting in emission from the excited state of Cy-5.

Initial assays (**Fig. 6**) show significant Cy-5 emission which indicates that the iminoboronate bond between Cy-3 and Cy-5 dye is formed resulting in **sufficient proximity** for FRET to occur. This data can then be manipulated to determine **reaction thermodynamics**.

**References**

1. R. Langer et al., Science., 1993, 260, 920–926.
2. C. D. Spicer et al., Chem. Rev., 2018, 118, 7702–7743.
3. S. J. Forbes et al., Nat. Med., 2014, 20, 857–869.

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