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Creating Robust and Reversible Cell-Gel Networks using Boronic Acid Chemistry

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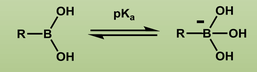
Abstract

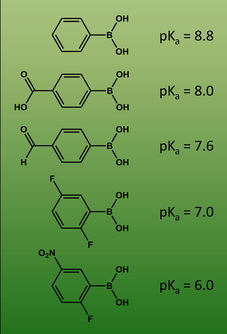
Current biosensor technologies typically use platforms mimicking intrinsic cell detection and response pathways (i.e., antibody-based detection). These approaches require signal amplification steps, resulting in decreased discrimination between pathogenic and non-pathogenic species. In contrast, cells have evolved highly efficient discriminatory mechanisms for detecting small quantities of pathogenicity factors and evoking biological responses. Due to the inability to retain the high-level sensitivity and selectivity of these mechanisms in *ex vivo* systems, the development of cell-based biosensors (CBB) is becoming increasingly explored, but often lack longevity and stability. The model organism, *Saccharomyces cerevisiae*, offers advantages for use in CBBs due to its robustness, controllable growth cycle, and ability to be lyophilized and stored. Yeast encapsulation strategies have been explored to increase stability and longevity; encapsulation, however, is often lengthy and requires several chemical additives. We present a simple approach to directly and rapidly form reversible cell-gel networks using a synthetic peptide displaying boronic acid (BA) residues. BAs covalently bind to most saccharides, in which binding is favored at high pH and reversible by lowering the pH. We hypothesized that the BA peptide would form cell-gel networks by binding to the various polysaccharides and glycoproteins decorating the cell wall. Here, we present that through binding of the BA groups to cell surfaces, yeast cells indeed act as BA crosslinkers, creating an infinite network of chemically-linked, viable cells. Furthermore, the gelation process is versatile with other organisms and fully reversible. Overall, this unique approach to forming viable cell-gel networks provides a promising path forward toward the development of useful CBBs.

Introduction to Boronic Acids (BAs)

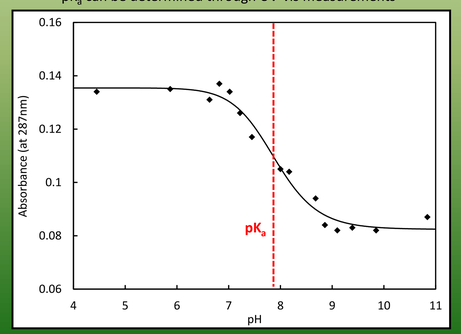
BAs are Lewis acids with pK_a values tunable across physiological pH



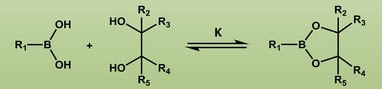
Phenyl BAs (PBAs) are useful due to their stability and synthetic versatility



pK_a can be determined through UV-vis measurements



BAs can reversibly bind to compounds bearing two or more hydroxyl groups (including most saccharides)



Binding affinity (K) is a complex function of:

1. BA (pK_a)

Binding affinity between PBA derivatives and glucose at pH 6.5

Substituents	pK_a	K
None	8.8	0.84
4-Br	8.3	5.6
3-Cl-4-F	7.8	7.6
2-F-5-F	7.6	33
3-F-4-F-5-F	6.8	17
2-F-5-NO_2	6.0	25

2. Saccharide

Binding affinity between PBA and saccharides at pH 7.4

Saccharide	K
fructose	160
sorbose	120
mannose	13
glucose	4.6
lactose	1.6
sucrose	0.67

3. pH

Binding affinity between PBA and glucose at various pH

pH	K
6.5	0.84
7.0	2.0
7.4	4.6
8.0	7.2
8.5	11

Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291-5300. Yan, J. et al. *Tetrahedron* **2004**, *60*, 11205-11209.

Introduction to Gelation and Networks

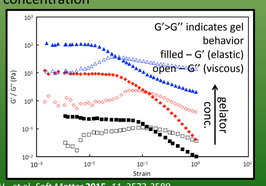
- **Gels are comprised of infinite 3D chemically- or physically-crosslinked networks**
- **Chemically-crosslinked networks can form in mixtures of binding species**
- **An infinite 3D network requires an average of > 2 binding sites per molecule**

Simple gel test – Inverted vial

- Shown is a 1% solution of peptide in H_2O
- The peptide forms a physically-crosslinked network of nanofibers upon addition of salt

Rheological Measurements

- Shown are small amplitude oscillatory shear of peptide gels with variable gelator concentration



Swelling Equilibrium

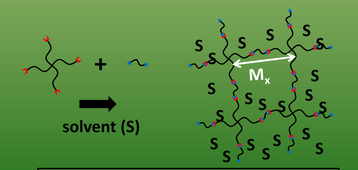
$$\ln(1-\phi_e)+\phi_e+\chi\phi_e^2=\frac{M_s}{M_x}\left(\frac{\phi_e}{2}-\phi_e^{1/3}\right)$$

$\phi_e \equiv$ volume fraction solvent (*e.g.*, H_2O)

$\chi \equiv$ interaction parameter (solvent/network compatibility)

$M_s \equiv$ molar mass of solvent

$M_x \equiv$ molecular weight between crosslink points (cell size)



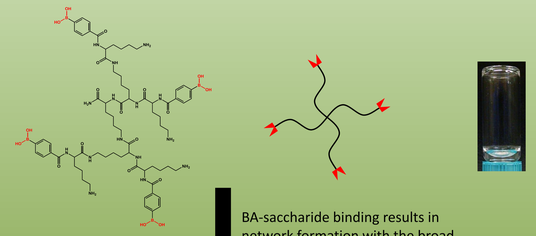
The amount of solvent a gel can imbibe is increased by increasing cell size

Jones, B.H., et al. *Soft Matter* **2015**, *11*, 3572-3580.
Jones, B.H., et al. *Chem. Comm.* **2015**, DOI: 10.1039/C5CC05207F.

BA-Based Gels

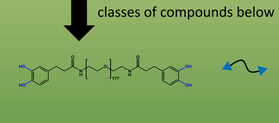
We have established chemistry for the preparation of multi-functional BA peptides and polymers, and have used these compounds to create reversible hydrogels from saccharide-containing (and related) entities

Tetra-BA peptide dendrimer prepared by solid-state peptide synthesis

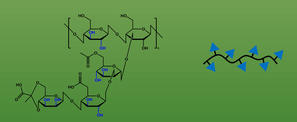


BA-saccharide binding results in network formation with the broad classes of compounds below

End-functional synthetic polymers (*e.g.*, PEG-dicatechol)



Polysaccharides (*e.g.*, xanthan gum)



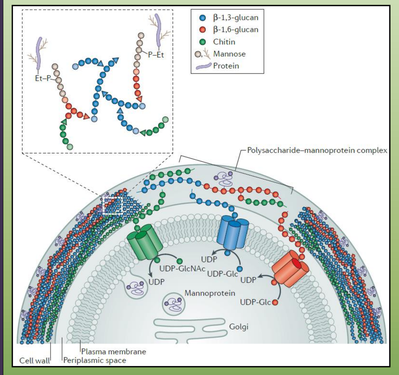
Glycoproteins (*e.g.*, avidin)

All systems undergo reversible sol-gel transitions by adjusting pH from 5-9!

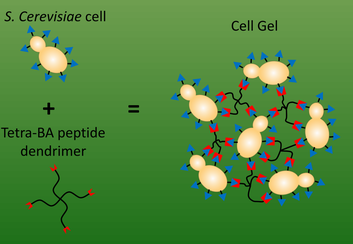
Making Live-Cell Gels

The cell wall is decorated with saccharide moieties

Shown below is a diagram of a yeast cell wall

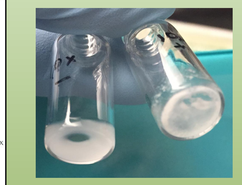


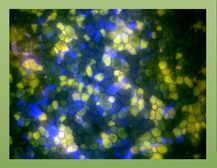
Cells should behave as crosslinkers for multi-BA compounds!

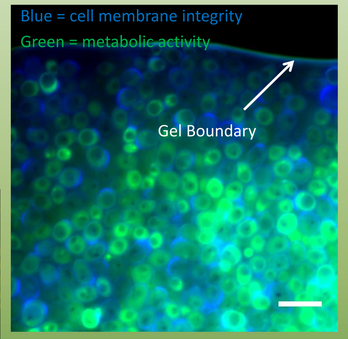


Boronic acid peptides cause cell gelation

S. cerevisiae can be gelled and are viable

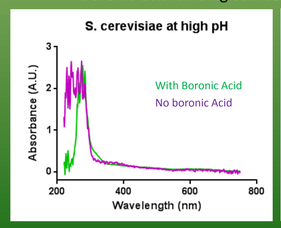


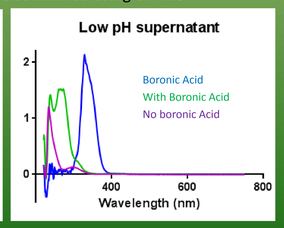




Boronic acid binds to the cells

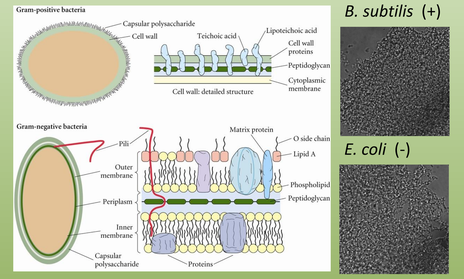
Boronic acid binding can be confirmed using UV-VIs





Boronic acid peptides cause bacterial cell gelation

Both Gram – and + bacteria can be gelled

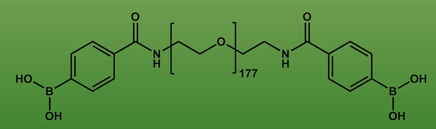


B. subtilis (+)

E. coli (-)

Building a boronic acid library

Synthesize different boronic acids to be compatible with different types of cells (e.g. mammalian cells)



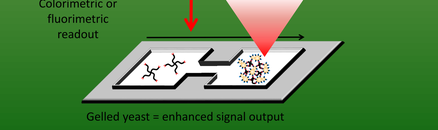
Cabib, E.; Arroyo, J. *Nat. Rev.* **2013**, *11*, 648-655.

Conclusions/Next Steps

- **Boronic acid chemistry offers a novel method for directly linking cells together in a reversible manner**
- **Cells remain viable and metabolically active when gelled using boronic acid peptides**
- **Build a library of boronic acid compounds to gel mammalian cells together**
- **Test compatibility of boronic acids with different cell types**
- **Confirm chemical binding of boronic acid compounds to cells**

Implications

- **Gelling live cells can be useful for building cell-based biosensors**
- **Cell-gel networks can be used to study *in vitro* wound-healing**



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