The art of fusion: a synthetic approach to create cross-kingdom hybrids

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Cross-kingdom hybrids

Lewis Thomas said of **cell fusion**: "*it is the most unbiologic of all phenomena, violating the most fundamental myths of the last century, for it denies the importance of specificity, integrity, and separateness in living things*"(1).

Cross-kingdom fusion raises **ontological**, **ethical** and **poetic questions**: how do multi-kingdom cell fusions challenge our categories and understandings of life? Where do they belong within both biological and cultural realms? How does their existence impact the environment and society?

In this interdisciplinary collaboration between **artists** (SymbioticA, University of Western Australia), **scientists** and **social scientists** (SynthSys, University of Edinburgh), we propose to create yeast-mammalian cell fusions, while exploring such questions through art.

We are engineering these fusion hybrids and observing their formation as they assemble into a new cell type. The work-in-progress was presented as an **art exhibition** at the Edinburgh International Science Festival in April 2018.

References & Acknowledgments

References:

(1) **The Lives of a Cell: Notes of a Biology Watcher.** 1974. Thomas, L. The Viking Press, Inc, New York, 148.
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(3) **A library of mammalian effector modules for synthetic morphology.** 2014. Cachat E, Liu W , Hohenstein P, Davies JA. *Journal of Biological Engineering* 8:26
(4) **Efficient size-independent chromosome delivery from yeast to cultured cell lines.** 2017. Brown DM, Chan YA, Desai PJ, Grzesik P, Oldfield LM, Vashee S, Way JC, Silver PA, Glass IJ. *Nucleic Acids Res*. 45(7):e50

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Synthetic fusion

Engineering fusion between *S. cerevisiae* and HEK293 cells

Fusions between mammalian cells (e.g. to produce hybridomas) are often performed with chemical fusogens such as Polyethylene glycol (PEG). Here we use a synthetic approach where a biological fusogen is expressed on the surface of mammalian cells and confers fusogenic properties to HEK293 cells (human embryonic kidney cells).

p14FAST is a reptilian reoviral protein(2) that induces cell-cell fusion by creating pores at contact sites between apposed cell membranes (**Fig. 1**).

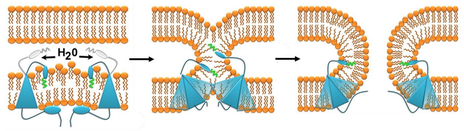


Figure 1
Model for the p14FAST protein (blue) membrane fusion reaction(2)

A fusion cell line was engineered previously in TREx cells(3), where p14FAST expression induced fusion between neighbouring cells. We used clone THFU-10 from this cell line to drive fusion between mammalian and yeast cells.

The yeast *Saccharomyces cerevisiae* possesses a thick polysaccharide cell wall that prevents the plasma membranes from both species coming into contact. To allow fusion, we prepared **spheroplasts** from yeast as described by Brown *et al*.(4).

Detecting fusion events

Various microscopy techniques were used to detect fusion events between THFU-10 cells and *S. cerevisiae* spheroplasts (see **Fig. 4**, opposite) Although cell-cell fusion could be observed between THFU-10 cells, forming large multinucleated cells (as described previously(2)), inter-species fusion events between yeast and mammalian cells were **difficult to detect** or distinguish from close contact events.

To monitor inter-species fusion events we are now concentrating on detecting fused cells sharing cytoplasms originating from both cell types. To this end, we are engineering each cell type with separate domains from a **dimerization-dependent fluorescent protein** (ddFP), localised in their respective cytoplasm. This way, only in the event of inter-species cell-cell fusion and cytoplasmic merge, will fused cells emit fluorescence (**Fig. 2**)

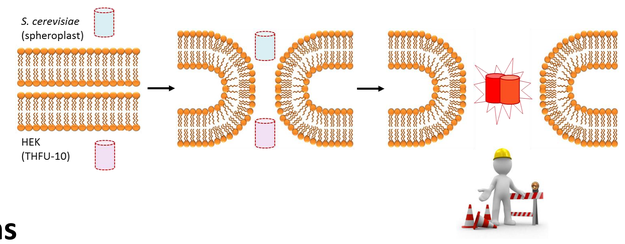


Figure 2
Upon fusion, yeast spheroplasts and mammalian cells merge their cytoplasmic content, allowing dimerization of the ddFP and fluorescence emission.

Safety considerations

The fusion cells THFU-10 express fusogens when induced with tetracycline, and as such might fuse with other cell membranes and in particular with cells of living humans. Therefore, rigorous precautions should be taken when handling them.

Edinburgh International Science Festival

The work-in-progress was presented as an art exhibition at the Edinburgh International Science Festival (**Fig. 3**).

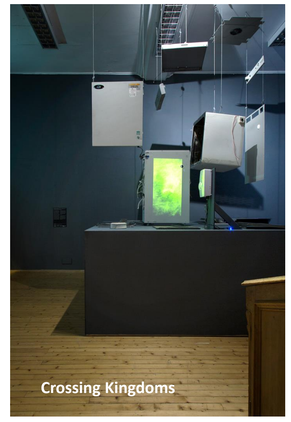
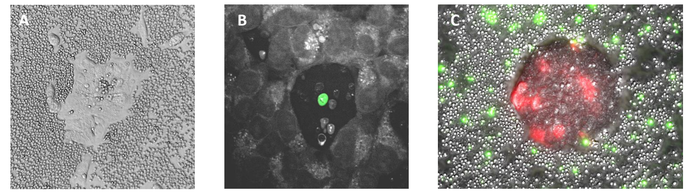


Figure 3
'Crossing Kingdoms' exhibit by Oron Catts, Ionat Zurr and Tarsh Bates @ SummerHall (31/03-13/05 2018)

To document fusion events, we use various microscopy techniques. Thus far, we have only likely observed 'close- contact' events (**Fig. 4**).

Figure 4 (below)
Live microscopy images showing contact between mammalian syncitia and yeast spheroplasts. A) bright field - , B) coherent anti-Stokes Raman (CARS) - , C) fluorescence microscopy. Red: mammalian, green: yeast cells.



Science, Art and Society

The project is the result of an interdisciplinary experiment: this poster has co-authors from **science**, **engineering**, **social science** and **art**. The questions the artists had about how to fuse mammalian and yeast cells acted as a nexus for unexpected interdisciplinary encounters. It challenged everyone involved to think in new ways, and resulted in something that would not have happened otherwise.

The creation of new life forms raises questions in the contexts of **responsible innovation** and **ethics**. Rather than commenting on these issues from a distance, they are incorporated in- and explored alongside- the actual manipulation of life forms. This is a direct way of examining, questioning and critiquing the new engineered constructions that are being created through synthetic biology.

The art of fusion: this project not only fuses different cell types, it also fuses questions, techniques and disciplines, producing hybrid entities, like this poster.