

## High Content Screening of Combinatorial Library for Identifying Biomarkers

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#### **Outline**

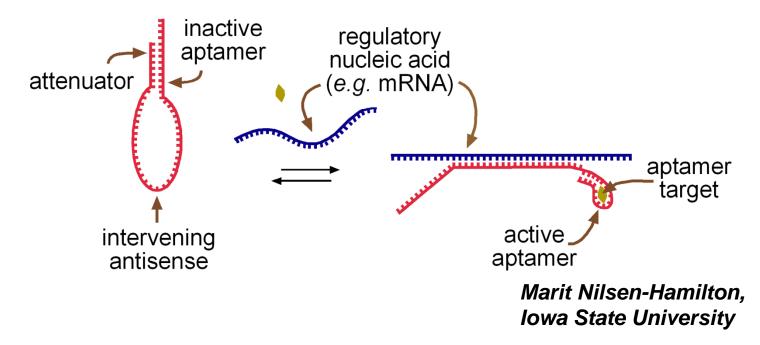


- Motivation
- Issues
- Approach
  - Model systems
  - Ligand library
  - Chemoinformatics
- Results

#### **Motivation**



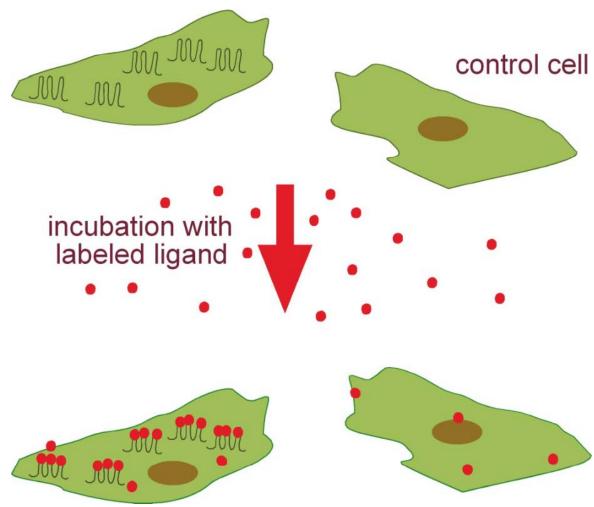
- Develop technology for in vivo imaging of gene expression
  - Mammalian as well as plant species
- Molecular Methodology



#### **Experimental Design**



aptamer-expressing cell



#### **Issues**



- Identify improved ligands with
  - Lower background
    - Improved in- and out- fluxes
  - Lower toxicity
  - High signal to noise ratio
- Design new aptamer constructs that bind to ligand

#### **Approach**



- Use a combinatorial fluorescent library of small molecules
- Develop a screening protocol against 3 mammalian cell lines
  - Chemoinformatics system
- Screen candidate ligands against the model system
  - Plant species
  - Higher level mammalian model system
- Improve properties of screened ligand by local perturbation

#### **Combinatorial library**



#### R1 Building Block (S5):



# 

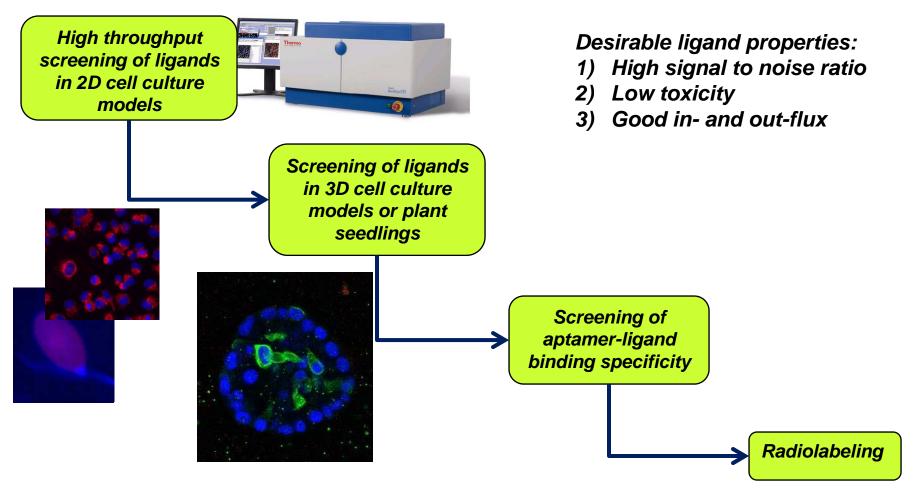
#### **Protocol**



- Live cell imaging on a well-by-well basis
  - Nuclear is labeled with vibrant red ruby
  - Two time points
    - Immediately after incubation
    - 60 minutes after washout
  - Approximately 1000 wells
  - Cellomic instrument
- Quantitative analysis on a cell-by-cell basis
  - segmentation
  - Registration
- Tight integration with the BioSig extension on Chemoinformatics
- Validation with confocal microscopy

#### Pipeline for screening ligands

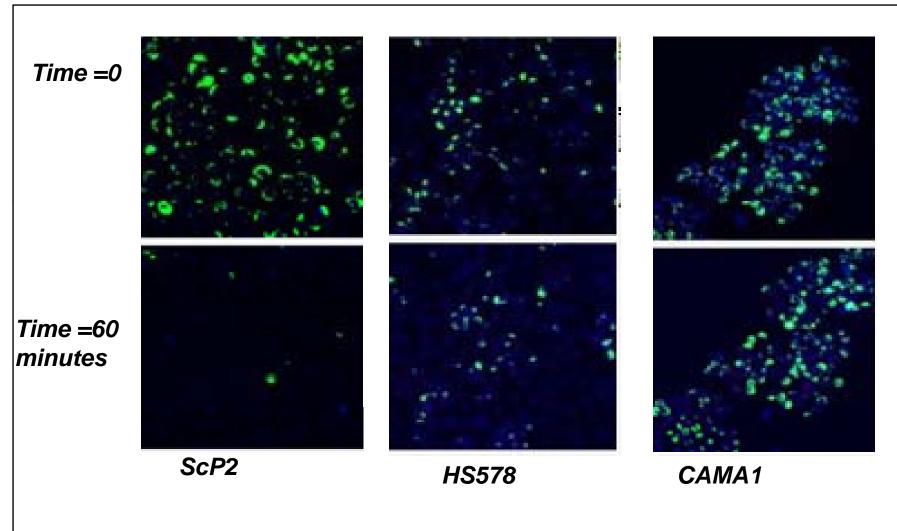




Optimizing of ligand properties for nuclear imaging of aptamer RNA

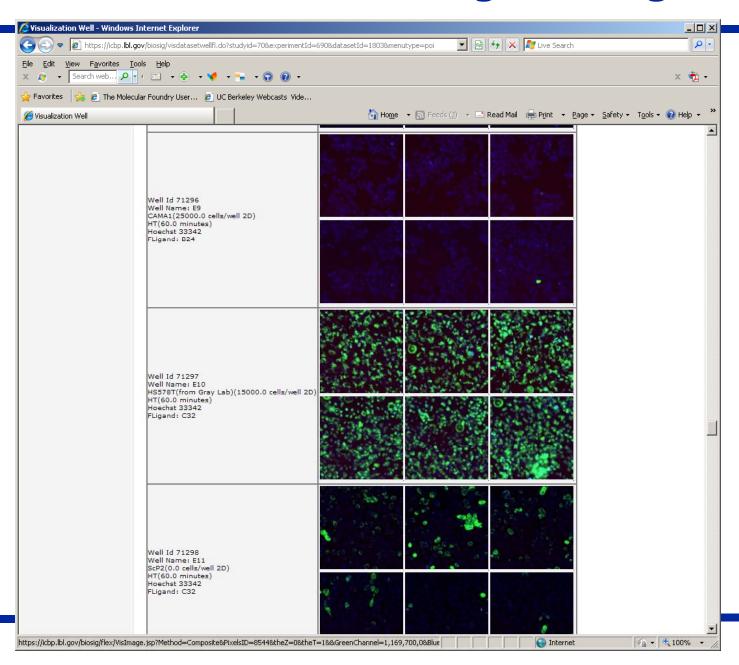
#### Ligand response is heterogeneous .....





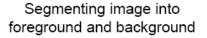
#### Data access through BioSig





#### **Segmentation**



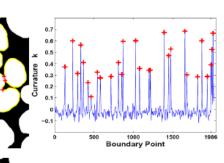


Detecting points of maximum curvature along the contour

Adaptive Thresholding



$$k = \frac{x'y'' - y'x''}{(x'^2 + y'^2)^{3/2}}$$



Triangulating points of maximum curvature by Delaunay triangulation

Reduce Hypothesis space







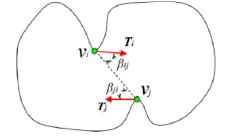
Applying geometric constrains for edge inference





- 1) No intersection with background;
- The angle between T<sub>i</sub> and T<sub>j</sub> should be maximized;
- 3)  $\beta_{ii}$  and  $\beta_{ii}$  should be as close as  $\pi/2$
- 4) No intersection with other edges

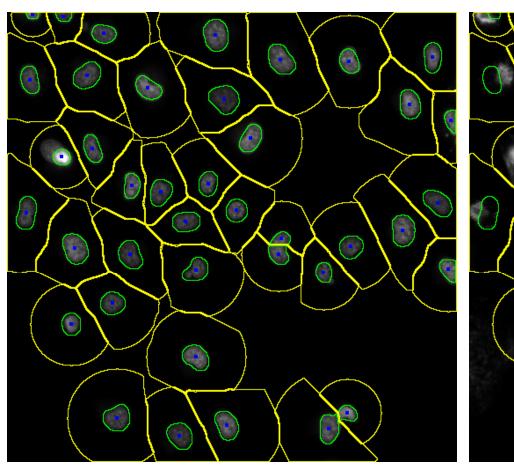
- Let E<sub>in</sub> be the edge set after edge pruning, and E<sub>out</sub>← ∅.
- 2. While  $E_{in} \neq \emptyset$ 
  - (a) In  $V_{in}$ , if  $deg(v_i) = 1$ , then  $E_{in} \leftarrow E_{in} \setminus e_{ij}$  and  $E_{out} \leftarrow E_{out} \cup e_{ij}$ .
  - (b) In  $E_{out}$ , if  $e_{ij} \in E_{out}$ , then  $E_{in} \leftarrow E_{in} \setminus e_{i*} \setminus e_{j*}$ , where \* stands for vertices.
  - (c) If  $e_{ij} \in E_{in}$ ,  $e_{jk} \in E_{in}$ , and  $e_{ki} \in E_{in}$ , with  $deg(v_i) = 2$ ,  $deg(v_j) = 2$ , and  $deg(v_k) = 2$ , then  $E_{in} \leftarrow E_{in} \setminus e_{ij} \setminus e_{jk} \setminus e_{ki}$ , and  $E_{out} \leftarrow E_{out} \cup e_{ij} \cup e_{jk} \cup e_{ki}$ .
- 3. For  $v_i \in V$  with no  $e_{i*} \in E_{out}$ . Use its tangent normal direction to generate an edge into  $E_{out}$ .
- 4. For e<sub>ij</sub> ∈ E<sub>out</sub>, e<sub>jk</sub> ∈ E<sub>out</sub>, and e<sub>ki</sub> ∈ E<sub>out</sub>, with deg(v<sub>i</sub>) = 2, deg(v<sub>j</sub>) = 2, and deg(v<sub>k</sub>) = 2, choose the two edges which produce the minimum convexity after decomposition, and delete the remaining one from E<sub>out</sub>.

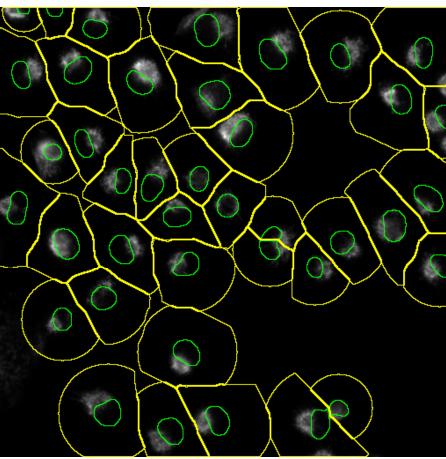


Q.Wen , etc "A Delaunay Triangulation Approach for Segmenting Clumps of Nuclei". IEEE International Symposium on Biomedical Imaging, 2009

#### Fluorescent signal measurement

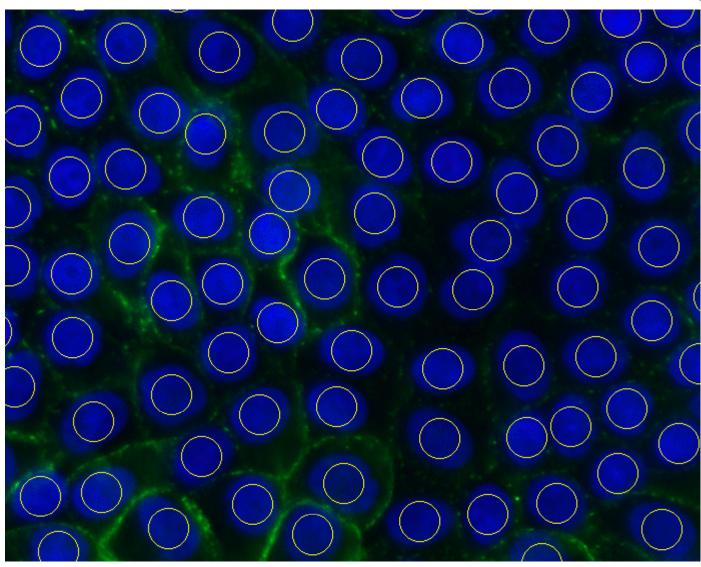






## **Evolving Fronts for Local Neighborhood Estabilshment**





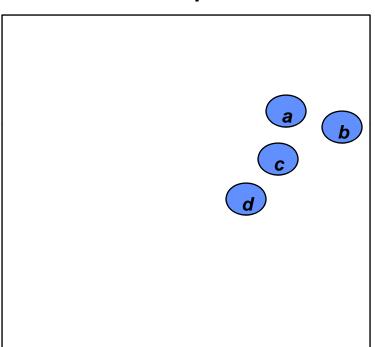
#### Registration



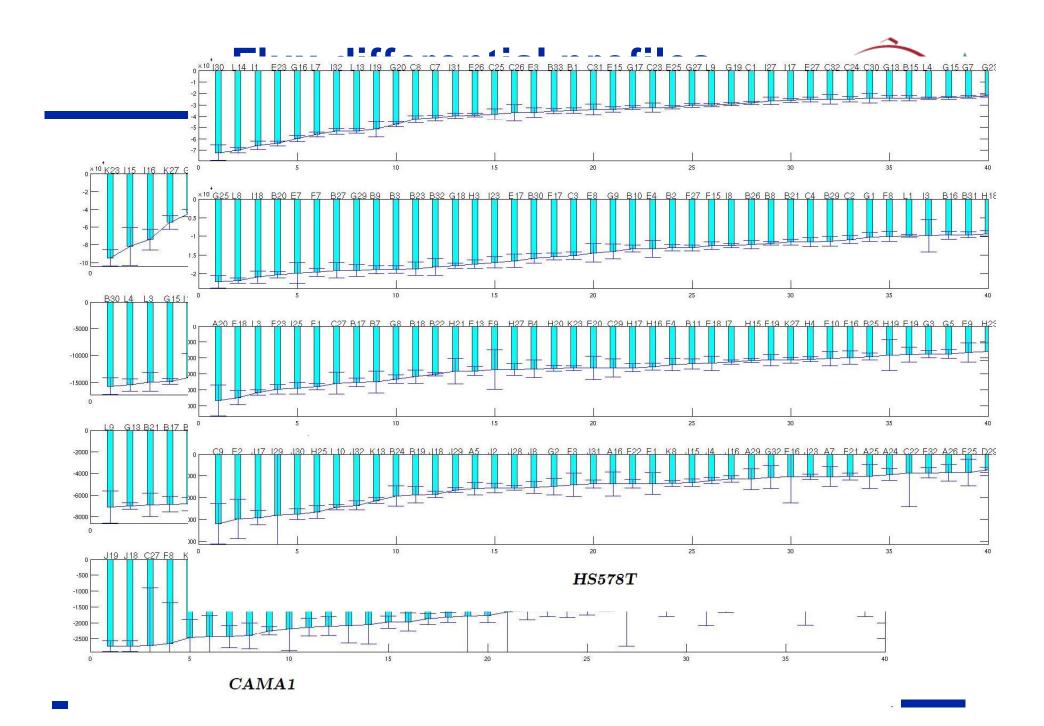
 $T_{o}$ 

1 2 3

 $T_1$ 

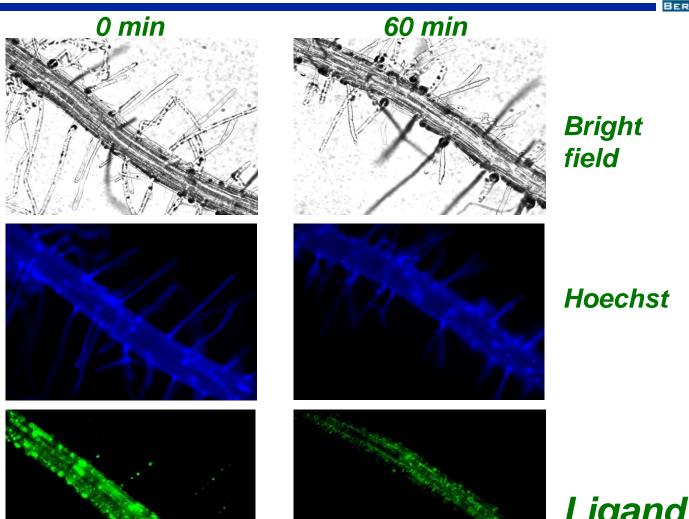


$$a = 1$$
;  $b = 2$ ;  $c = 3$ ;  $d = 4$ 



### Identify candidate ligands and validate





Ligand E14

#### Results



- A number of ligands with desirable properties have been identified
- Multiple mammalian cell lines are crucial for screening ligands
- Mammalian lines can serve as a proxy for other model system (e.g., plant species)
- BioSig extension for Chemoinformatics analysis has expedited throughput

#### Acknowledgement



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