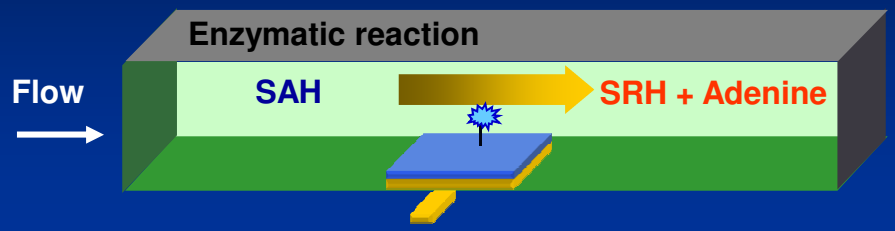
Reproducible Enzyme Assembly and Catalytic Activity in Reusable BioMEMS

Rubloff Research Group Accomplishments

Reproducible Enzyme Assembly and Catalytic Activity in Reusable BioMEMS

Accomplishment

- Pro-tagged Pfs enzymes are spatially assembled in microfluidic channels with biochemical assembly strategy mediated by chitosan
- Enzymatic reaction products are collected and conversion substrate SAH is analyzed with HPLC



Significance

- Simple, robust and covalent enzyme assembly in the post-fabricated microfluidic environment
- Enzyme assembly under mild aqueous conditions with spatial and temporal programmability and orientational control
- Assembled enzyme exhibits enzymatic activity, assembly reversibility and stability over extended periods of time.

People involved

Xiaolong Luo, Angela Lewandowski, Bill Bentley, Gary Rubloff

Collaboration with Hyunmin Yi, Gregory F. Payne and Reza Ghodssi

Links

Enzyme Assembly and Enzymatic Reaction in Microfluidic

Publications

X. L. Luo, A. T. Lewandowski, H. M. Yi, R. Ghodssi, G. F. Payne, W. E. Bentley and G. W. Rubloff, "Reproducible Assembly and Catalytic Activity of a Metabolic Pathway Enzyme in Reusable BioMEMS Devices ", *Lab on a Chip*, Submitted.

X. L. Luo, J. J. Park, H. Yi, A. T. Lewandowski, W. E. Bentley, G. F. Payne, R. Ghodssi, and G. W. Rubloff, "Chitosan-mediated Enzyme Assembly toward Rebuilding a Metabolic Pathway in the Microfluidic Environment," *Materials Research Society 2007 Spring Meeting*, San Francisco, CA, April 9-13, 2007.

J. J. Park, X. L. Luo, H. Yi, R. Ghodssi, and G.W. Rubloff, "In situ Biomolecule Assembly and Activity within Completely Packaged Microfluidic Devices", *IEEE/NLM Life Science Systems and Applications Workshop*, Bethesda, MD, July 13-14, 2006.

Presentations

X. L. Luo, A. T. Lewandowski, G. F. Payne, R. Ghodssi, W. E. Bentley, and G. W. Rubloff, "Enzyme Assembly and Catalytic Activity in a Reusable BioMEMS Plateform for Metabolic Engineering", Proceedings of the 11th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Paris, France, October 7-11, 2007.

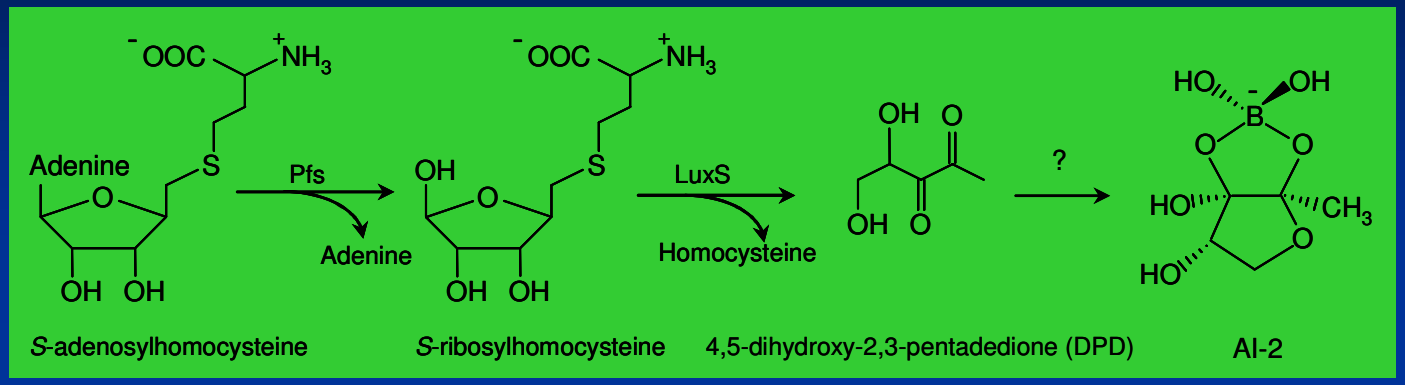
X. L. Luo, J. J. Park, H. Yi, A. T. Lewandowski, W. E. Bentley, G. F. Payne, R. Ghodssi, and G. W. Rubloff, "Chitosan-mediated Enzyme Assembly toward Rebuilding a Metabolic Pathway in the Microfluidic Environment," *Materials Research Society 2007 Spring Meeting*, San Francisco, CA, April 9-13, 2007.

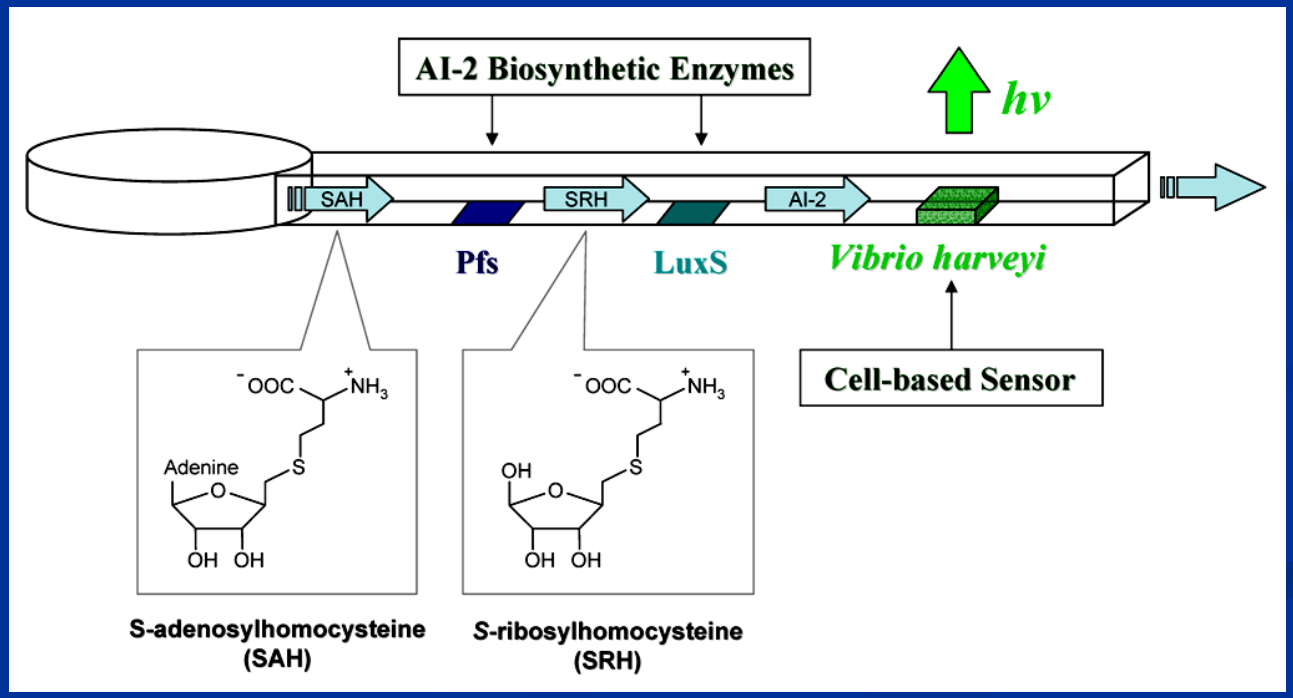
X. L. Luo, J. J. Park, H. Yi, R. Ghodssi, G. W. Rubloff, "Biomolecule Assembly and Functionality in Completely Packaged Microfluidic Devices," *American Vacuum Society 53rd International Symposium*, San Francisco, CA, November 12-17, 2006.

J. J. Park, X. L. Luo, H. Yi, R. Ghodssi, and G.W. Rubloff, "In situ Biomolecule Assembly and Activity within Completely Packaged Microfluidic Devices", *IEEE/NLM Life Science Systems and Applications Workshop*, Bethesda, MD, July 13-14, 2006.

Our Vision—

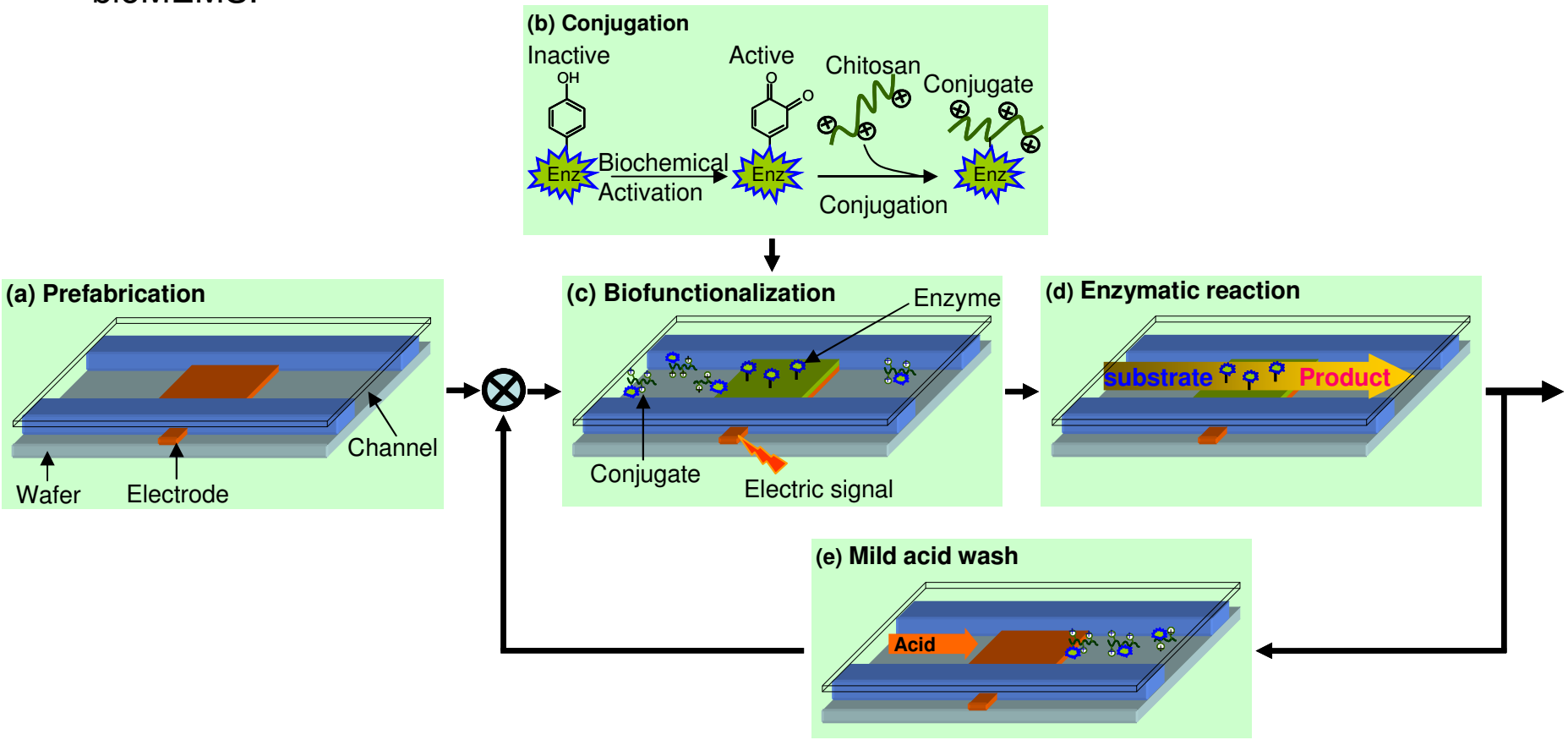
Metabolic Pathway in Microfluidics





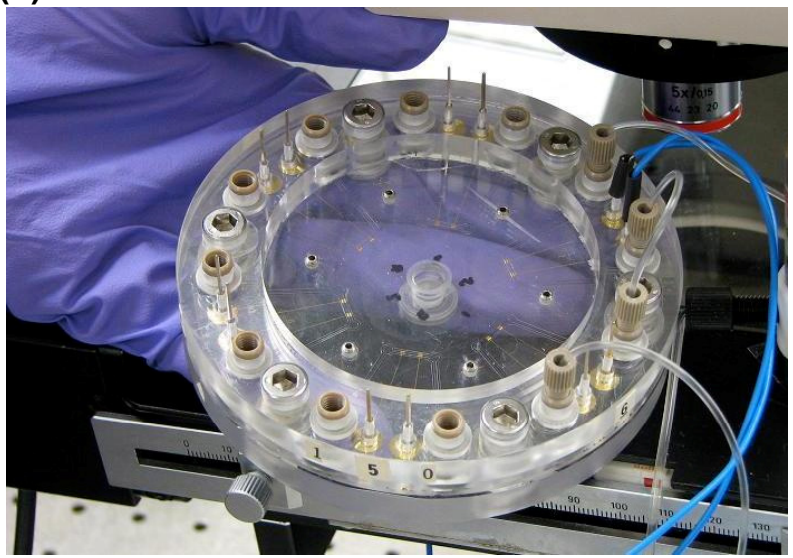
Reproducible Enzyme Assembly in Reusable BioMEMS

1. Reproducible assembly of a bio-catalytically active enzyme is achieved in a reusable bioMEMS.
2. Assembly is based on covalent conjugation of enzyme followed by electrodeposition of the conjugate onto electrodes in microfluidic channels.
3. The assembled conjugate can be removed by a mild acid wash without harming the bioMEMS.

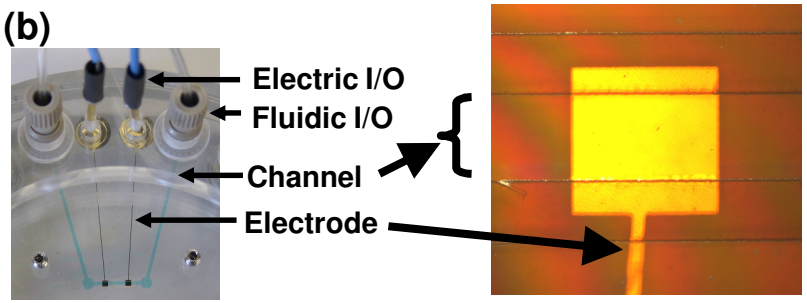


BioMEMS and Control System

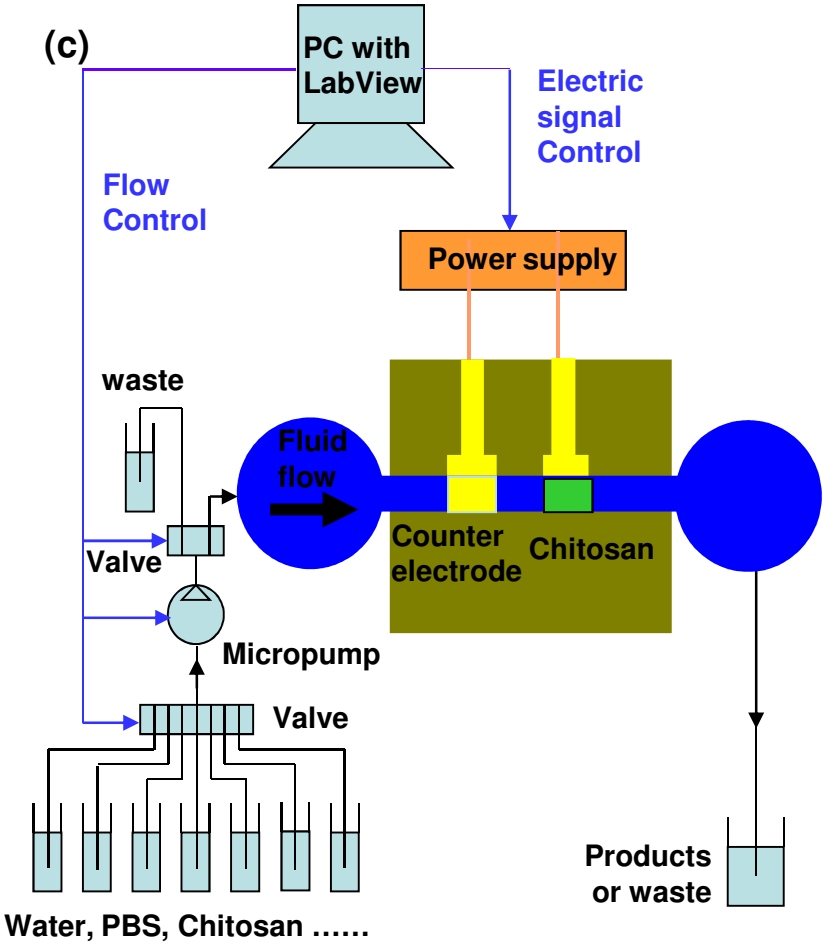
(a)



(b)



(c)



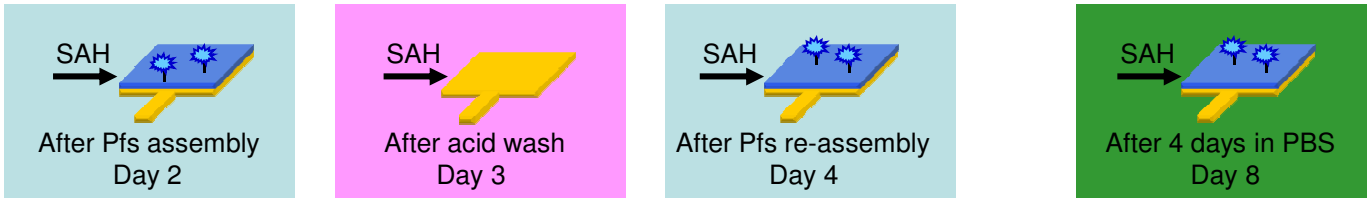
Enzyme Assembly and Catalytic Activity

- Pfs enzyme was assembled (day 1), removed by acid (day 3) and re-assembled (day 3).
- Substrate SAH was introduced after enzyme was assembled (day 2), removed (day 3), re-assembled (day 4) and left in PBS buffer for 4 days (day 8).
- The background colors in each step in (a) correspond to the background colors in (b) and (c).
- **(a)** Experimental process. **(b)** Schematic flow of enzymatic reactions. **(c)** % conversion (red square) vs. flow rate (black line).

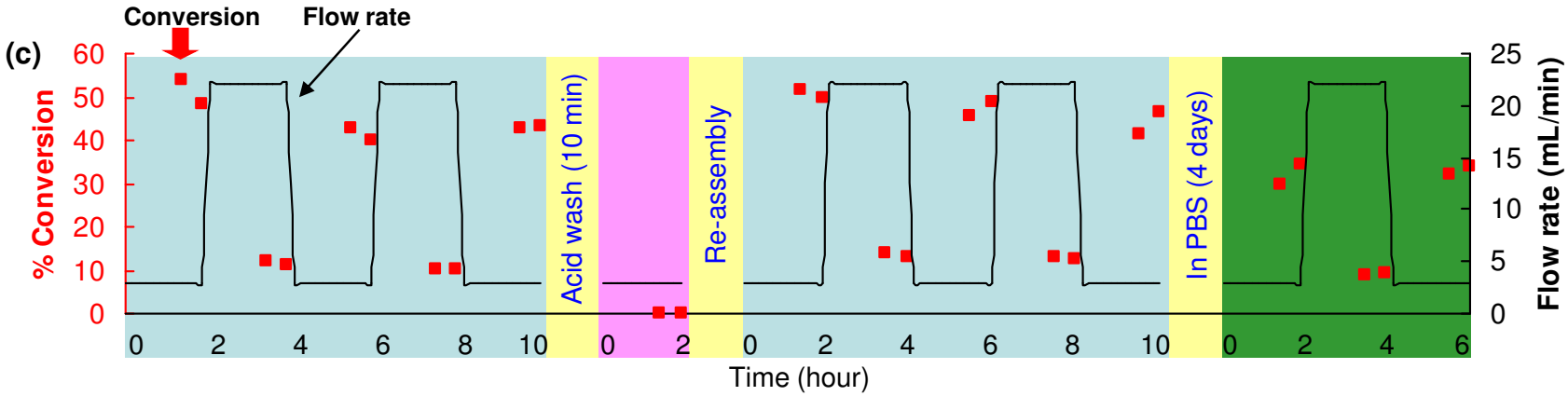
(a)

	Step #	Procedure	Flow rate (μL/min)	Time (min)
Day 1	1	DI water cleaning	50	
	2	BSA	3	120
	3	PBS buffer	3	30
	4	**Pfs-chitosan assembly**	**static**	**4**
	5	PBS buffer	5, 20	30
Day 2	**6**	**Enzymatic reaction (SAH)**	**3, 22**	**600**
	7	HCl wash	22	10
	8	DI water cleaning	50	90
Day 3	**9**	**Enzymatic reaction (SAH)**	**3**	**120**
	10	DI water cleaning	50	60
	11	BSA	5	120
	12	PBS buffer	5	30
	13	**Pfs-chitosan re-assembly**	**static**	**4**
	14	PBS buffer	5, 20	30
Day 4	**15**	**Enzymatic reaction (SAH)**	**3, 22**	**600**
Day 5-7	16	In PBS buffer	static	3 days
Day 8	**17**	**Enzymatic reaction (SAH)**	**3, 22**	**360**
	18	Cleaning	50	

(b)

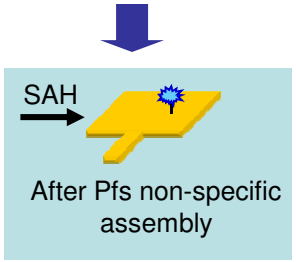


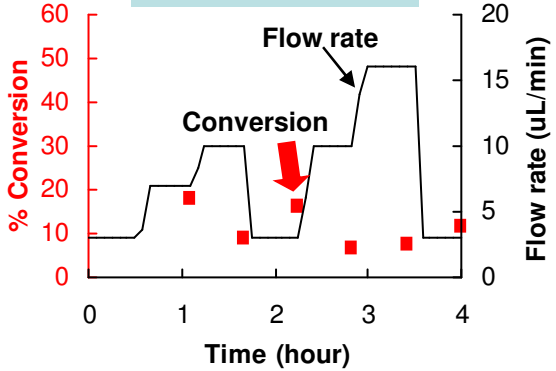
(c)

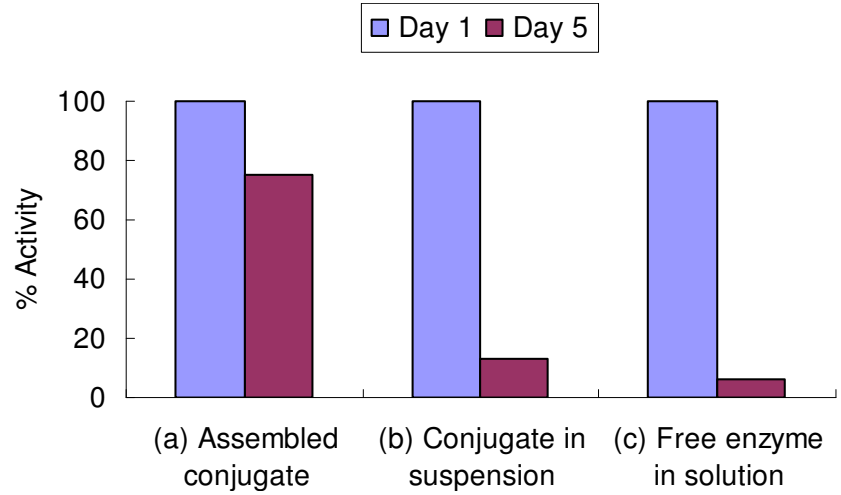
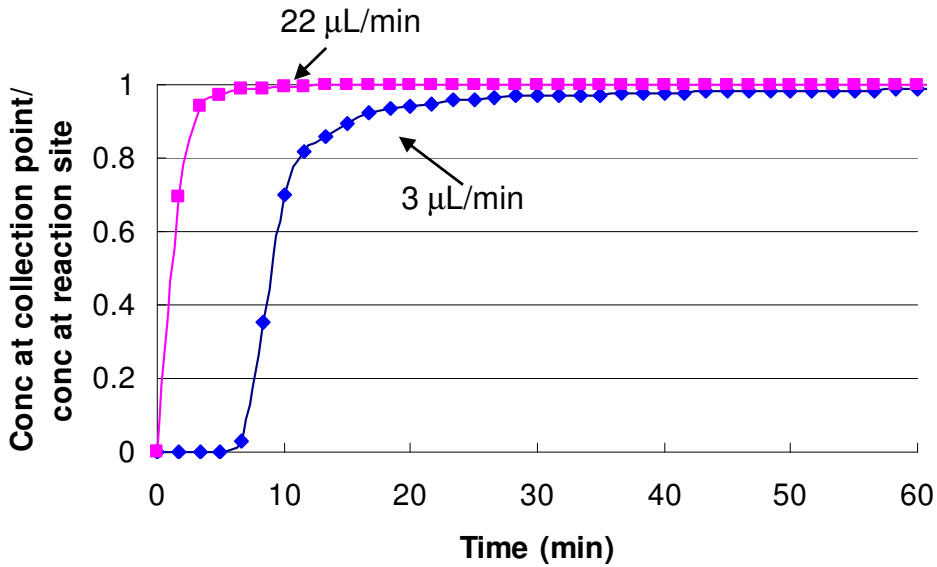


Enzyme Assembly and Catalytic Activity

- Negative control to determine Pfs non-specific binding within microfluidic channel.
- Pfs was introduced into microfluidic channel without the activating enzyme tyrosinase and without chitosan.





The transient concentration response at sample collection point to the concentration change at reaction site.

Analysis of enzyme tability after 4 days in PBS buffer