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Role of Surface Proteins in Magnetotaxis-based Applications

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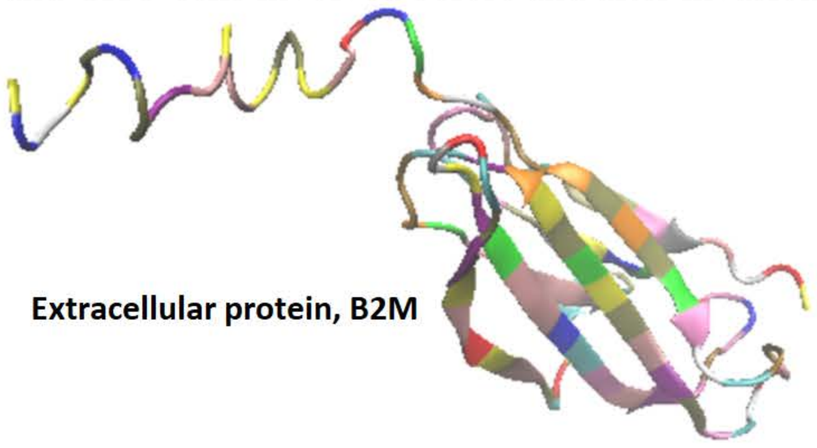
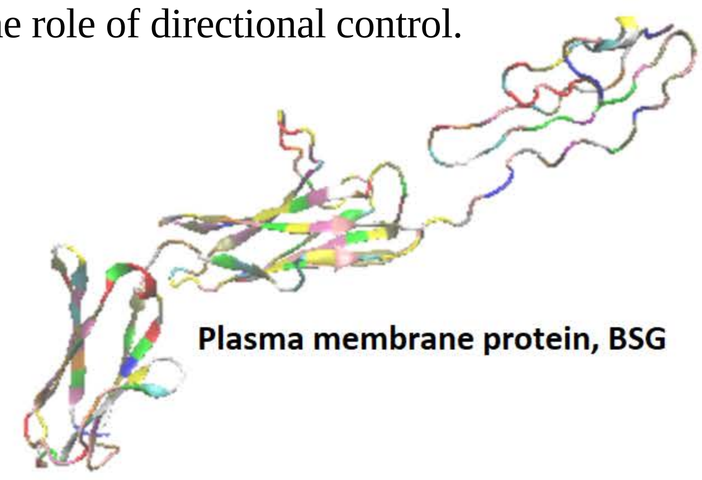
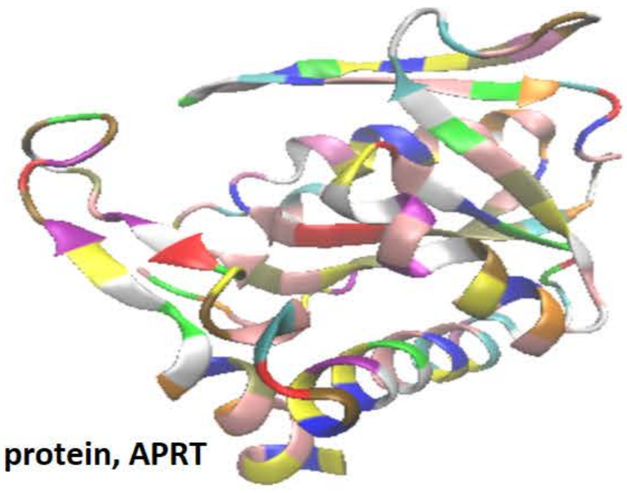
Magnetospirillum magneticum[1] (AMB-1) are a species of magnetotactic bacteria that are capable of orienting along the earth's magnetic field lines through their organelles called magnetosomes. Many studies have shown that certain engineered-bacteria can infect the tumor cells resulting in a controlled death of a tumor. This work deals with a technique utilizing AMB-1 along a predefined path through magnetotaxis, which can pave a way for selective doping as well as isolation of the tumor cells from a group of healthy cells through a magnetic invasive assay (MIA). For such a control, tiny mesh of vertical electrical coils each having a diameter of ~ 5 mm is fabricated, which establishes the path for the bacteria to move along the magnetic field lines. The molecular dynamics simulations at the interface of the bacterial cell surface proteins (MSP-1 & flagellin) and Chinese Hamster Ovary[2] (CHO) cell surface containing cytoplasmic and extracellular proteins (BSG, B2M, SDC1, AIMP1, and FOS) will establish an association between the invading AMB-1 and the host CHO cells. The experimental demonstration will involve the CHO invasion by the AMB-1 and isolation of selected CHO cells. Statistical analysis along with the relevant electron and force microscopy data will confirm the number of AMB-1 and CHO cells involved before and after invasion and the role of directional control.



Extracellular protein, B2M



Plasma membrane protein, BSG



Cytosol protein, APRT

Materials and Methods

- Experimental:
  - *32 AWG magnetic wire*, *cavity slide* [20 (dia.) x 30 mm (deep)], *current source* and *gaussmeter* for the experimental setup.
  - *Light Microscopy*: To check the morphology of AMB-1 and magnetotaxis verification.
  - *Scanning/ Transmission Electron and Atomic Force Microscopy*: To be done to confirm the *CHO cells* invasion by *AMB-1* through a typical invasive assay protocol.
  - *Inverted Microscope:*
    - *i. Isolation of the invaded CHO cells* from the non-invaded cells using vertical coil based arrangement through magnetotaxis.
    - *ii. Real time invasion of the CHO cells* using the magnetically guided and directionally controlled AMB-1 cells - *magnetic invasive assay* (MIA).
- MD Simulations:
  - Interactive protein system modeling using *VMD*[3] and simulations using *NAMD*[4].
  - All simulations will be carried out for a *time period of 100ns*.
  - *CHARMM force field* and *TIP3 water model* with ions including Na, Cl, Mg and K according to the experimental procedure.
  - *Periodic boundary conditions* based on a *constant temperature* of 300K (MIA) and 310K (incubation) at a *constant pressure* of 1 Atm.
  - *Data analysis* for the interactions taking place between the AMB-1 cell surface proteins and CHO proteins in the extracellular, plasma membrane and cytosol region.

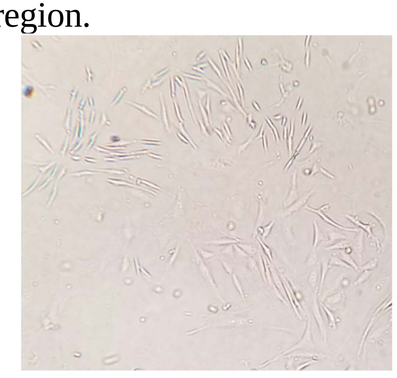


Fig.1 CHO Cells under 400x

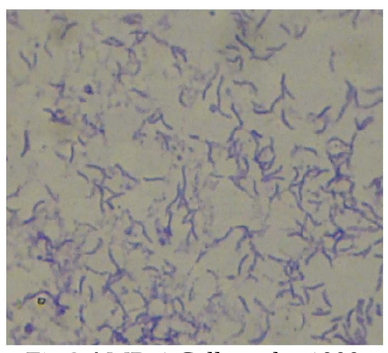


Fig.2 AMB-1 Cells under 1000x

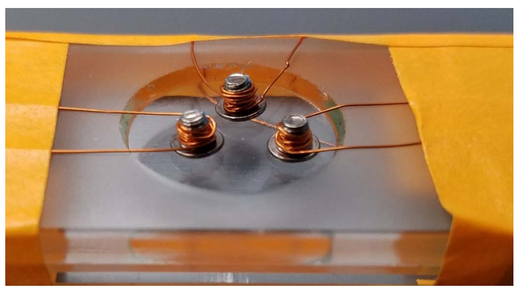


Fig.3 Vertical coils in the cavity

No.	Proteins	Location	
1.	SDC1	Extracellular Region	CHO proteins
2.	B2M	Extracellular Region	
3.	AIMP1	Extracellular Region	
4.	HTR1B	Plasma Membrane	
5.	ATP7A	Plasma Membrane	
6.	BSG	Plasma Membrane	
7.	CDH2	Plasma Membrane	
8.	CD44	Plasma Membrane	
9.	GJC1	Plasma Membrane	
10.	LAMP1	Plasma Membrane	
11.	AIMP2	Cytosol	
12.	ASNS	Cytosol	
13.	FOS	Cytosol	
14.	MSP-1	Surface protein	AMB-1 Proteins
15.	Flagellin	Flagellar protein	

Key Points

- The AMB-1 was found to be sensitive to the magnetic field after four days of the sub-culture until seven days whereas the CHO cells are found to be more confluent by the 4th day of culture.
- The movement of the AMB-1 cells is found to be towards the nearest pole when the current is supplied and random otherwise.
- A controlled switching of the current through the multiple coils provides a guided path for the AMB-1 and invaded CHO cells.
- Molecular dynamics simulations quantify the interactive energies between the AMB-1 cell surface proteins and the three sets of CHO proteins.

Future Work

The quantitative analysis of the AMB-1 and CHO cells, pre and post analysis would determine the number of AMB-1 per CHO cell statistically. We built the coil mesh for the AMB-1 and observed them under the field for a complete directional control. The NAMD simulations and VMD data analysis would justify the interactions of the surface proteins giving more credibility to our aim.

Combinations	Current	Coil	0^0	90^0	180^0	270^0	Center
1 0 0	0.50	A	8.2	10.2	9.5	8.9	3.2
		B	0.1	0.3	0.2	0.5	0
		C	0.4	0.1	0.2	0.9	0
	0.75	A	10.2	11.2	7.8	10.5	5.2
		B	0.3	0.2	0.1	0.6	0
		C	0.2	0.4	0.3	0.2	0
0 1 0	0.50	A	0.2	0	0.1	0.4	0.6
		B	8.2	8.5	8.9	10.2	2.3
		C	0.3	0.1	0.1	0	0
	0.75	A	0.1	0.2	0	0	0.5
		B	19.9	20.0	19.3	18.8	9.0
		C	0.2	0.1	0	0.2	0
0 0 1	0.50	A	0	0	0	0	0.2
		B	0	0.1	0.1	0	0
		C	7.4	6.9	7.9	7.2	1.6
	0.75	A	0	0	0	0	0
		B	0	0	0	0	0
		C	13.2	14.6	13.6	14.0	2.6

1 1 0	0.50	A	8.2	8.4	9.2	8.8	2.9
		B	9.5	9.8	10.3	10.2	3.2
		C	1.1	0.7	0.6	0.4	0.5
	0.75	A	14.4	15.2	14.6	15.0	5.0
		B	12.2	13.0	13.6	14.2	5.2
		C	0	0.9	0.4	0.6	0.9
1 0 1	0.50	A	6.6	7.1	6.4	6.2	1.4
		B	0.2	O.1	0.3	O.7	0.8
		C	7.5	6.9	6.8	6.6	1.6
	0.75	A	16.8	17.4	16.0	15.8	2.8
		B	0.4	0.3	0.9	0.8	1.0
		C	13.5	13.8	14.0	14.4	2.3
0 0 1	0.50	A	0.3	0.6	0.5	0.8	0.3
		B	9.2	10.2	10.7	11.1	0.9
		C	10.8	11.0	11.7	10.9	1.2
	0.75	A	0.3	0.7	0.1	0.9	1.1
		B	15.5	16.4	15.9	16.9	3.3
		C	14.4	15.2	14.4	13.9	3.5
1 1 1	0.50	A	8.2	8.5	8.8	8.7	1.4
		B	9.7	10.2	9.9	10.2	1.5
		C	8.8	9.0	9.5	9.9	1.8
	0.75	A	16.3	16.8	16.7	16.2	2.8
		B	18.8	20.2	19.2	19.8	3.3
		C	14.9	15.2	14.7	15.9	3.0

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4. Phillips JC, Braun R, Wang W, et al. Scalable molecular dynamics with NAMD. *J Comput Chem*. 2005;26(16):1781-1802.