Specificity of multivalent constructs is concentration dependent

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Statement of Purpose: A novel approach to circumvent the issue of non-specific treatment is achieved by targeting drugs to cells via conjugating them or an imaging agent to an antibody or ligand for a cell surface receptor that is over-expressed by the target cell population (Balthasar, Michaelis et al. 2005). In this study we investigate the binding specificity of a polymeric, multivalent construct, comprised of three peptide segments (TWYKIAFQRNRK) linked by poly(ethylene glycol) spacers. The individual peptide has been demonstrated to bind to the $\alpha_6\beta_1$-integrin (Nakahara, Nomizu et al. 1996). The binding specificity of the construct is calculated by investigating the binding on two cell types, target cells (glioma cells, SF 767) and non-target cells (normal human astrocytes, NHA). The hypothesis of this study is that multivalent constructs exhibit greater specificity than dodecameric peptide at concentrations of construct less than the affinity of the receptor-ligand bond.

Methods: The construct was synthesized using typical solid state Fmoc chemistry. The dodecameric peptides were linked with three poly(ethylene glycol) chains each 20 atoms long and a FITC molecule was added at the amine end of the construct. Binding was analyzed via imaging of the fluorescent binding to both cell types at 4 °C.

Results/Discussion: When fluorescently-labeled constructs are incubated with cancer and normal cells (Figures 1A and 1B respectively) the results indicate increased binding on the target cells in comparison to non-target cells at a concentration of 0.6 μM (Fig 1).

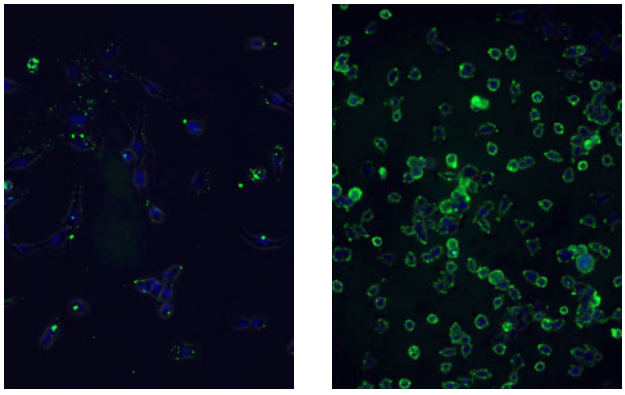

Figure 1. Binding of fluorescently labeled multivalent construct to astrocytes (left panel) and gliomas (right panel) at a concentration of 0.6 μM.

Figure 2 shows a quantitative analysis of the ratio of binding between cancer and normal cells (specificity) as concentration of the construct is varied. These data exhibit a sharp increase in specificity at concentrations less than the affinity of the receptor-ligand bond (4.28 μM).

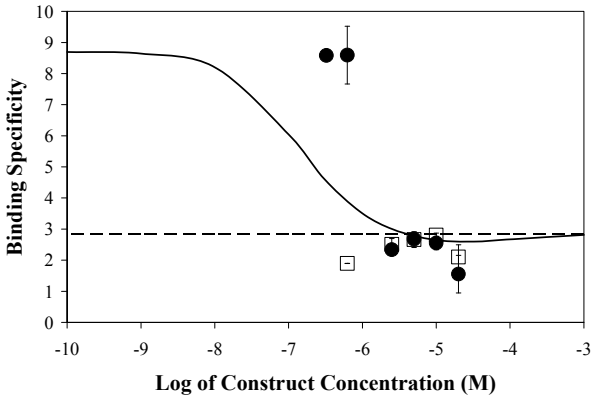

Figure 2. Binding specificity of multivalent construct (circle) versus monovalent construct (dodecamer peptide, squares). Continuous lines indicate predicted values by biophysical model, dashed line corresponds to monovalent construct, solid line to trivalent construct.

Conclusions: Previous theoretical biophysical modeling predicted that specificity of multivalent constructs increases as concentration decreases. In this study we confirm that the trend holds experimentally as well. The predicted values do not correlate with the experimental values exactly, however the overall trend is the same. This disconcordance is probably due to finite non-specific binding in the experimental system. In this study we have found a concentration (0.6 μM) at which the tradeoff between specificity (enhanced at low concentration) and contrast is optimal; yielding acceptable levels of both resulting in effective targeting (specificity ~8:1) of glioblastoma cells vs. normal astrocytes.

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References: Balthasar, S.,et al. (2005). Biomaterials 26(15): 2723-32.
Nakahara, H., et al. (1996). J Biol Chem 271(44): 27221-4.