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14. ABSTRACT Mutational events that drive a normal cell to become a cancer cell require the coordinated overexpression of multiple biomarkers. Unique biomarker combinations can create dynamic, physiologic patterns at different stages of cancer development. The assignment of protein expression patterns, or protein profiling, to delineate differences between normal tissues and developing cancer is gaining momentum as a critical instrument in aiding diagnosis, tailoring therapeutics, and predicting clinical outcomes. These proteomic studies, however, investigate changes in protein expression in cell lines and bulk tissue specimens at the gross proteomic level. While powerful, this technique fails to account for the heterogeneity of most tumors since the histopathology associated with many cancers encompasses only a small fraction of the total number of cells present in a tissue section. To date, it is impossible to visualize these cancer biomarker patterns <i>in situ</i>, which define the status of the cell, both non-invasively and <i>in vivo</i>. We present a major leap in cancer imaging ideology to develop a novel molecular imaging paradigm that utilizes multiple cellular targets to generate imageable signals in the same cell thereby achieving chemical resolution. <i>The objective of our proposed research is to develop a new imaging platform consisting of targeted-trans-complementing reporter fragments to simultaneously image the cancer signature in vivo and in real time.</i> A number of unique advantages of this imaging platform are envisaged, such as: 1) increased specificity; 2) multi-marker imaging at the cellular level; and 3) ability to recognize distinct genetic patterns among different tumor types and grades. In the proposed experiments, we will conjugate each reporter fragment to one of two different ligands to simultaneously target and image <i>in vivo</i>, via enzyme reporter re-construction <i>in situ</i>, a molecular signature consisting of two or more different targets. If the cells contain only one or none of the targets, no reporter signal will be observed. The feasibility of this imaging paradigm will be conducted <i>in vivo</i> with animals bearing tumors that express either a subset or all of the biomarkers required for enzyme <i>trans</i>-complementation.		

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INTRODUCTION

Targeted-reporter platforms answer a critical unmet need and have real application for imaging the multi-step progression of cancer growth that requires the coordinated overexpression of multiple biomarkers. The development of these platforms to investigate molecular signatures associated with disease creates the next frontier in *in vivo* imaging. The importance for imaging molecular signatures is underscored by the almost weekly publication of sets of genomic markers that are diagnostic or predictive of disease states. By exploiting multi-marker imaging, we ultimately seek to image combinations of biomarkers that will uniquely identify cancers from normal tissue and report on the biochemical status of cancer cells. These expression patterns can thus be indicative of the type, stage, or severity of disease. However, the application of imaging molecular signatures is far more expansive than just applications for cancer diagnosis or disease detection. Once developed, we will be able to transform molecular imaging from single biomarker identification to imaging and interrogating cellular dynamics during other complex processes such as embryonic development, growth, proliferation, and differentiation in addition to cellular changes that occur early in the course of disease.

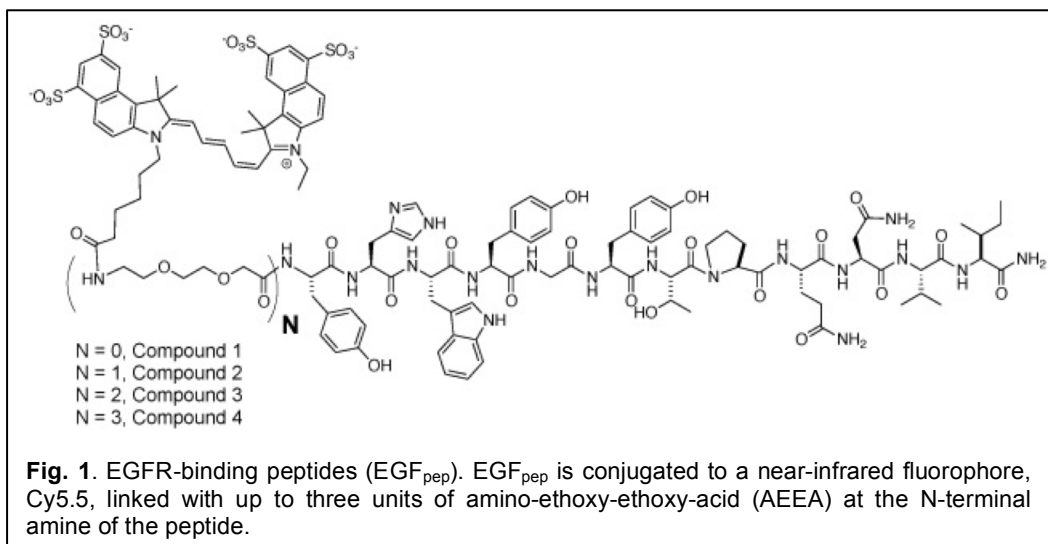
Our research seeks to utilize relatively innocuous building blocks to create an exquisitely intuitive platform. By linking a reporter fragment to a targeting moiety, we are only constrained by the number of fragments with which the reporter can be cleaved. The underlying hypothesis of this work is: trans-complementing subunits of image-able enzymes can be exploited to design a system so that multiple changes in a cancer cell's diagnostic signature can be imaged simultaneously. This approach is only achievable by utilizing subunit complementation, which provides a chemical resolution that is far more precise than the physical or anatomical-based resolution currently employed to produce data using non-invasive imaging hardware. The pioneering utility of the technique significantly increases specificity, decreases background artifacts, and promotes our ability to interrogate the status of cells rather than just the presence of cancer biomarkers. Thus, by expanding the number of biomarkers available to non-invasive diagnostic and therapeutic assessment, we envision an innovative platform-based approach to disease identification, staging, and treatment.

BODY

Our research aims to utilize inactive subunits of an image-able enzyme that are added exogenously to the targeted cells or tissue via intravenous injection into the animal. No tinkering with the genome of the diseased tissue is required and these bio-probes can be utilized to report on the physiologic expression levels of important biomarkers in any tissue to which they are administered. The development of such a non-invasive imaging system that bases disease detection on biochemical differences rather than on anatomical differences between normal and diseased tissues is clearly innovative and provides an intelligent-design strategy to pursue non-invasive assessment of diagnostic and prognostic genetic signatures *in vivo*. Eventually, this technology will have the ability to accurately assess the entire mass of a diseased tissue rather than a limited biopsy of the tissue, providing a more comprehensive knowledge of genetic changes than either individual genetic or proteomic analyses can achieve.

To develop an epidermal growth factor receptor (EGFR) targeted imaging complex that would cross the Blood Brain Barrier (BBB) and selectively bind to brain tumor cells overexpressing EGFR, we created a peptide-based near infrared (NIRF) probe to compare to full-length EGF. This allowed us to assay specificity, kinetic behavior, and binding affinity of the receptor-targeted peptides as outlined in **Specific Aim 1 Milestone 1**. For these studies, we employed a peptide discovered through phage display screening against purified human EGFR. The peptide was modified to include linkers and a NIRF dye (**Fig. 1**). To determine the optimal space between the NIRF dye and the peptide, we designed and synthesized a series of peptides to include increasing numbers of discrete ethylene glycol units to serve as linkers between a Cy5.5 and the N-terminal end of the peptide. Cy5.5 and EGF_{pep} were either directly linked or linked via 1, 2, or 3 units of discrete ethylene glycol (AEEA) moieties (**Fig. 1**). To determine which of the compounds optimally interacts with cells

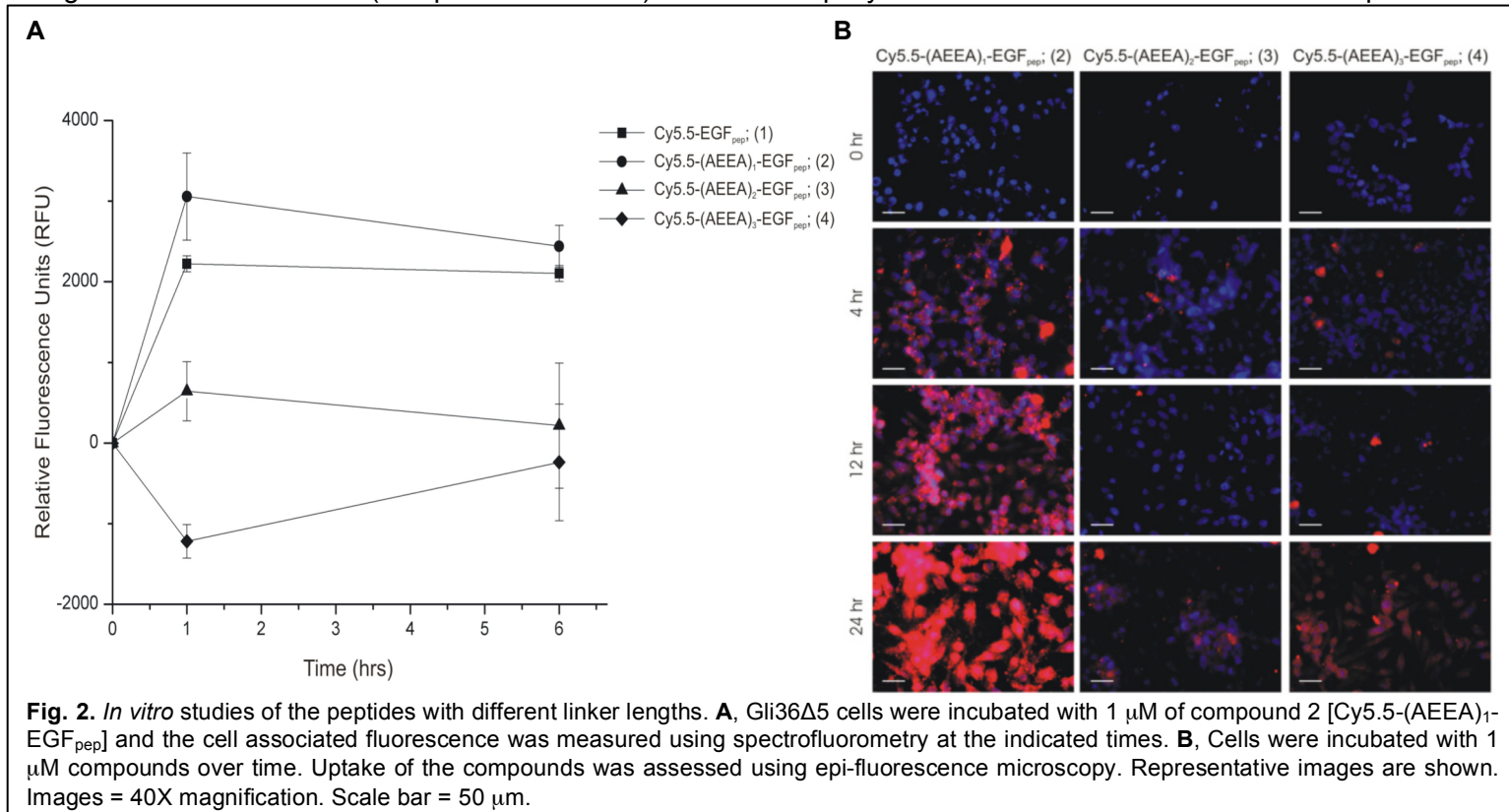
expressing EGFR, the apparent binding for each bioconjugate was fluorometrically determined from a saturation binding assay in vitro using a human GBM cell line overexpressing EGFR, Gli36Δ5, (**Table 1**). Compounds 1, 2, 3, and 4 all bound to the cells with affinities in the micromolar (μM) range. Compound 2, which had a single linker, had the highest apparent affinity with a K_d at least 2-fold better than compound 1, which had no ethylene linker, $8.9 \mu\text{M}$ to $18.5 \mu\text{M}$ respectively. Compounds 3 and 4 have significantly higher affinities for binding, $64.4 \mu\text{M}$ and $123.0 \mu\text{M}$, respectively.



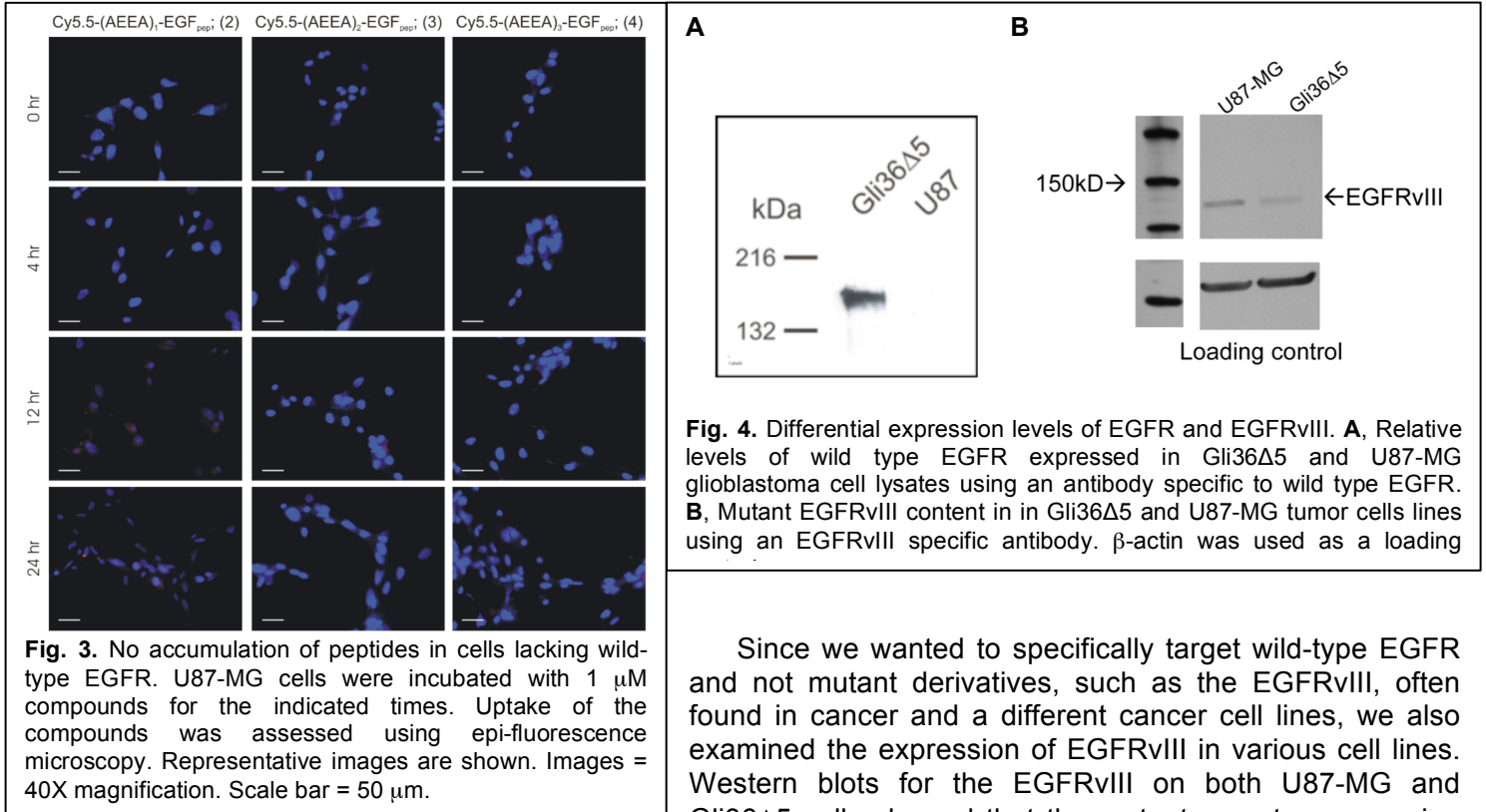
Compound	Peptide (EGF_{ppep})	K_d (μM)
1	Cy5.5- YHWYGYTPQNVI-amide	18.5 ± 3.9
2	Cy5.5-(AEEA) ₁ -YHWYGYTPQNVI-amide	8.9 ± 3.7
3	Cy5.5-(AEEA) ₂ -YHWYGYTPQNVI-amide	64.4 ± 24.6
4	Cy5.5-(AEEA) ₃ -YHWYGYTPQNVI-amide	123.0 ± 174.0
5	YHWYGYTPQNVI-amide (GE11-amide)	ND
6	Cy5.5-(AEEA) ₁ -NYQTPVYGWYH-amide (scrambled)	ND

Table 1. Binding affinities of the EGF peptides.

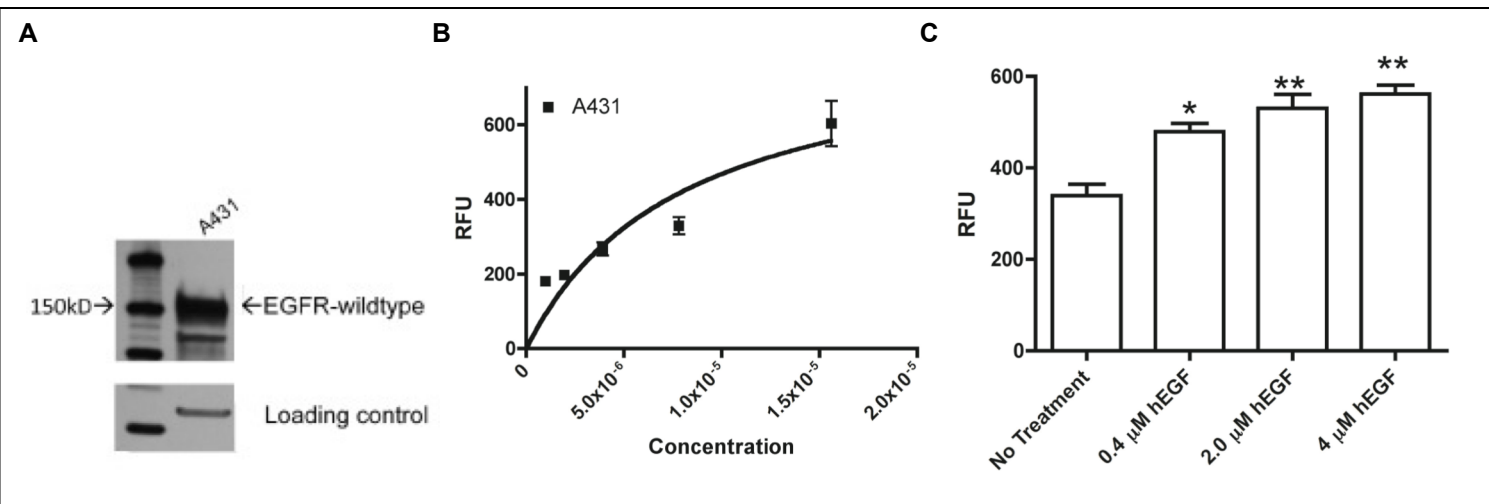
We next used immunofluorescence microscopy to determine the fate of the peptide complexes once they bound to glioblastoma cells expressing EGFR. As predicted from the affinity measurements, compound 2, which had the highest affinity, also showed the greatest accumulation of fluorescence after incubation with Gli36Δ5 cells (**Fig. 2A**). Neither compound 1 (no linker) nor either of the molecules with greater linker numbers (compounds 3 and 4) was taken up by the cells to the same extent as compound 2.



Interestingly, cells took up the peptide with the longest linker and the worst binding affinity, compound 4, better than compound 3 (**Fig. 2B**). Compound 4 compares most closely to commercially available full-length EGF by linker numbers; however, the presence of the hydrophilic Cy5.5 fluorophore appears to impact binding affinity. When tested against U87-MG cells, which express much lower levels of the EGFR (**Fig. 4A**), no cellular uptake for any of the compounds was observed (**Fig. 3**).



is similar (**Fig. 4B**) for both cell lines. In addition, saturation binding studies conducted with A431 cells, a squamous carcinoma cell line, that expresses high levels of only wild type EGFR (**Fig. 5A**), indicated that the



Kd for binding was similar to that measured with Gli36Δ5 cells (**Fig. 5B**). Further, incubation of compound **2** with A431 cells displayed increasing fluorescence in the presence of the EGF ligand, which increases cycling of wild type EGFR in cells (**Fig. 5C**).

Consequently, we were able to determine the optimal peptide length and degree of functionalization of each linker length. We were also able to create NIR-conjugated peptides that targeted with variable receptor affinities and demonstrated that the linker length affected uptake and accumulation using the wild type EGFR specifically.

During the time period covered in this progress report, we were able to characterize a series of engineered *in vitro* cancer signature glioma cell lines as outlined in **Specific Aim 1 Milestone 3**. Rat glioma cells (9L) were stably transfected with pcDNA3.1 plasmids containing the full length coding region of the human EGFR or the human transferrin receptor (TfR). To create a cell line overexpressing both receptors, 9L cells overexpressing TfR were stably transfected with pcDNA3.1-EGFR and selected using antibiotics. Western blotting was used to show the relative expression of each human receptor protein in engineered cell lines (**Fig. 6A**). Immunofluorescence staining localizes the receptors both at the cell membrane (arrows) and within the cytoplasm of the cells (**Fig. 6B**). We also examined the expression of EGFR and TfR in the human glioma cell lines, Gli36Δ5 and U87, described in detail above.

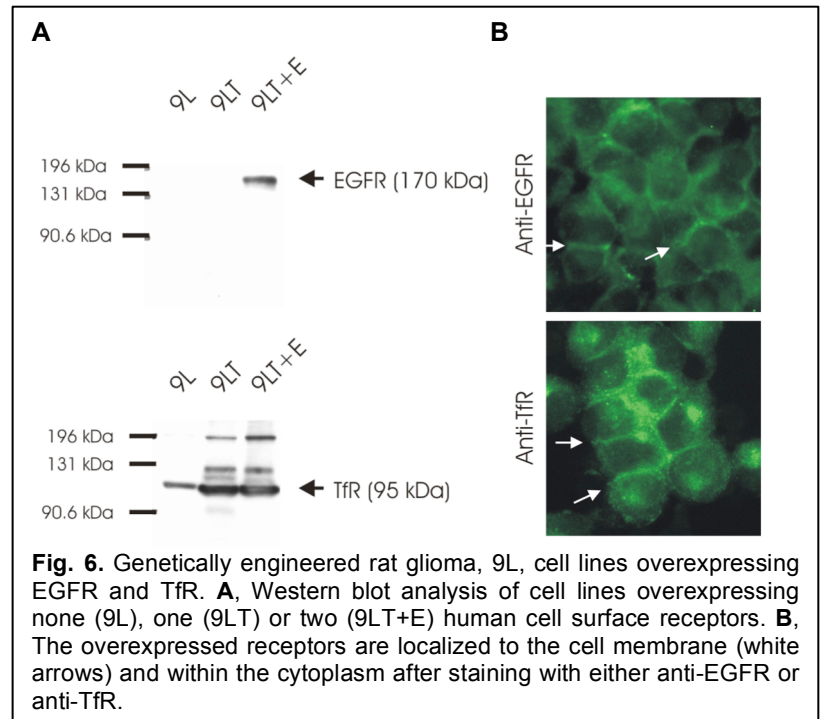


Fig. 6. Genetically engineered rat glioma, 9L, cell lines overexpressing EGFR and TfR. **A**, Western blot analysis of cell lines overexpressing none (9L), one (9LT) or two (9LT+E) human cell surface receptors. **B**, The overexpressed receptors are localized to the cell membrane (white arrows) and within the cytoplasm after staining with either anti-EGFR or anti-TfR.

To prepare the β -galactosidase (β -gal) complexes, we linked biotinylated EGF for the EGFR to biotinylated β -gal using fluorophore-conjugated streptavidin. Ligand, linker, and reporter fragment were mixed in a molar ratio 1:1:3 at room temperature for 1 hr. Excess D-biotin was added to block any remaining unbound streptavidin sites. In the case of control assays, untargeted reporter complex was prepared with D-biotin, in place of the ligand, mixed with linker and reporter fragment in a molar ratio 1:1:3.

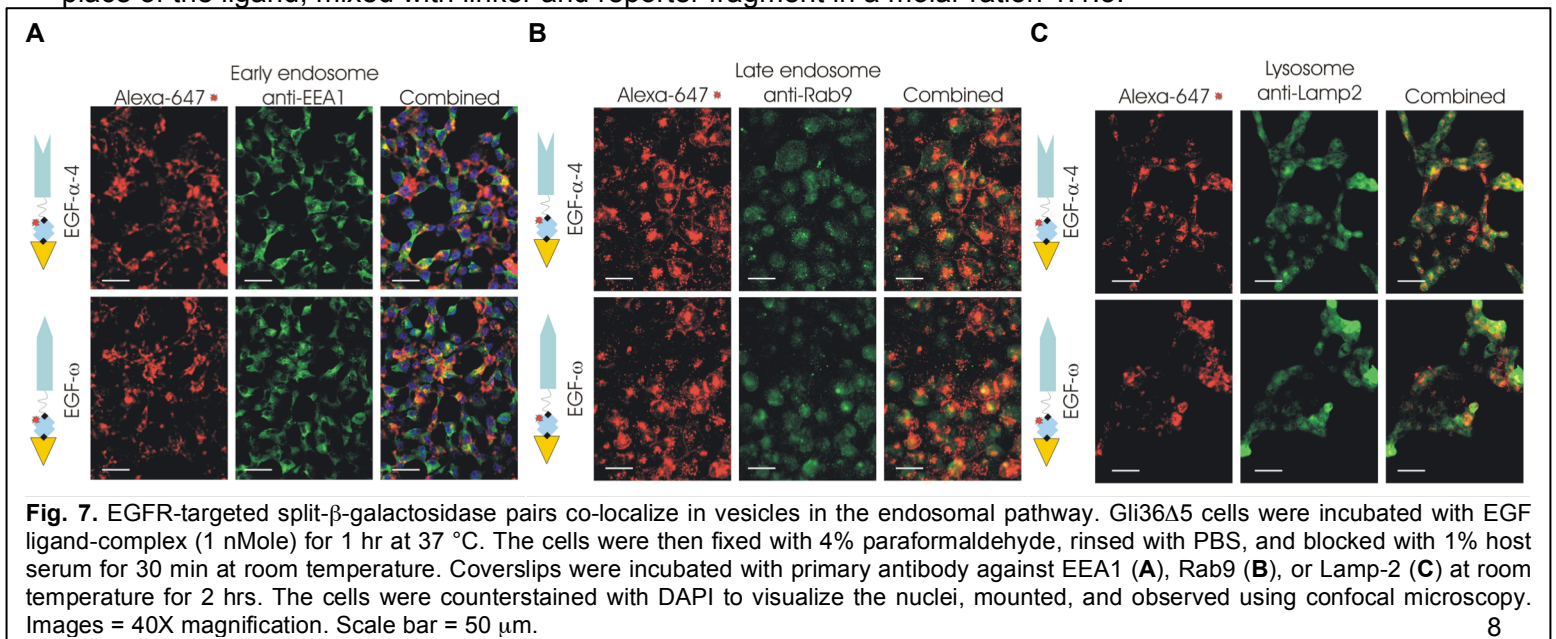
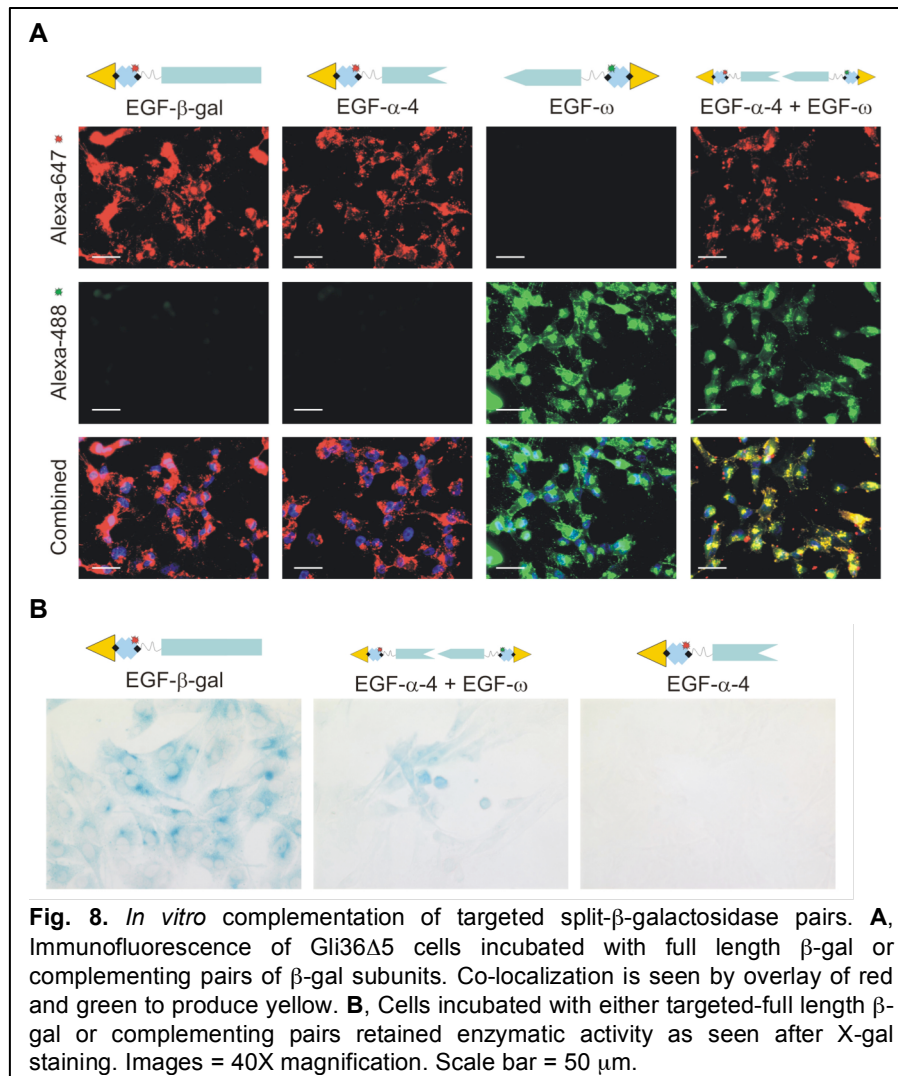


Fig. 7. EGFR-targeted split- β -galactosidase pairs co-localize in vesicles in the endosomal pathway. Gli36Δ5 cells were incubated with EGF ligand-complex (1 nMole) for 1 hr at 37 °C. The cells were then fixed with 4% paraformaldehyde, rinsed with PBS, and blocked with 1% host serum for 30 min at room temperature. Coverslips were incubated with primary antibody against EEA1 (**A**), Rab9 (**B**), or Lamp-2 (**C**) at room temperature for 2 hrs. The cells were counterstained with DAPI to visualize the nuclei, mounted, and observed using confocal microscopy. Images = 40X magnification. Scale bar = 50 μm.

In our studies, complementation of the β -gal subunits involves a multiplicity of molecules; thus, tracking the uptake and accumulation of each single targeted-complex is necessary to evaluate the bioavailability of the imaging agents as outlined in **Specific Aim 1 Milestone 4**. We used fluorescence microscopy to follow the spatial and temporal distributions of targeted- β -galactosidase subunits during internalization of the target, EGFR. Full-length EGF, the targeting moiety, was linked to either the α -4 subunit (EGF- α -4) or ω subunit (EGF- ω) of β -gal. Human glioblastoma cells, Gli36 Δ 5, overexpressing the EGFR were incubated for 4 hrs at 37 °C with each complex singly. An intrinsic fluorophore conjugated to the linker allowed us to examine the sub-cellular localization of each targeted-complex. As expected, cells that overexpress the EGFR bind and take up the EGF-targeted β -gal subunits. The targeted-complexes can be found throughout the functional EGFR internalization pathway, primarily in early endosomes (**Fig. 7A**) and lysosomes (**Fig. 7C**). Very little of either EGF-targeted complex was found in late endosomal vesicles (**Figure 7B**). Addition of the EGFR-targeted complexes did not adversely affect the viability of the cells as assayed by trypan blue staining (**not shown**).



Next, we examined whether concomitant incubation of cells with both targeted β -gal subunits altered either the uptake or location of the targeted-complexes (**Fig. 8A**). Gli36 Δ 5 cells were incubated for 4 hrs at 37 °C with EGF- β -gal, EGF- α -4 singly, EGF- ω singly, or the combination of EGF- α -4 and EGF- ω . In this case, the EGF- α -4 complex was labeled with Alexa 647 fluorophore and the EGF- ω complex was labeled with Alexa 488 fluorophore to distinguish between the two complexes. EGFR-targeted α -4 and ω complexes each sequestered in a manner similar to that of targeted-full length β -gal. The combination of both targeted-complexes did not negatively impact the uptake of either complex. The targeted-complexes co-localized to the same location predominately, although EGF- α -4 was observed in disparate locations approximately 20% of the time as measured by relative fluorescence densitometry. Enzymatic activity was visualized by overnight X-gal staining (**Fig. 8B**).

Incubation of EGF- α -4 or EGF- ω with its non-targeted, complementing partner, i.e. B- ω or B- α -4, respectively,

further demonstrated the specificity of the targeting moiety to drive the uptake and accumulation of the complex (**Fig. 9**). Non-targeted complementing fragments were not internalized as shown by a lack of intrinsic fluorescent signal; also, the presence of the non-targeted fragment did not inhibit the uptake of the targeted-complex. As a final control, uptake specificity of EGF- α -4 or EGF- ω was competitively inhibited in the presence

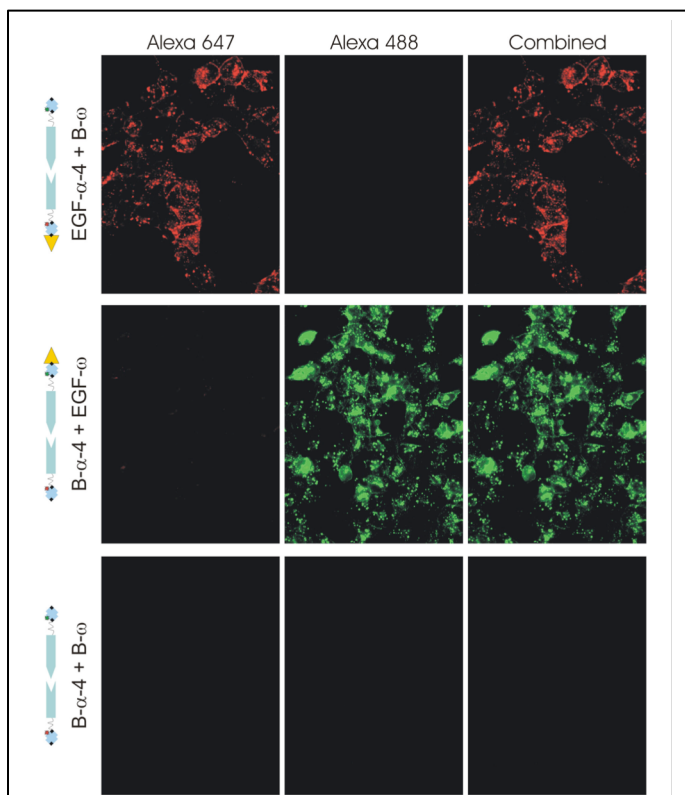


Fig. 9. Non-targeted β -galactosidase fragments do not accumulate within the cells. Gli36 Δ 5 cells incubated with 1 nM targeted-subunit simultaneously with the non-targeted complementing subunit for 1 hr at 37C only accumulated the targeted-subunit as observed by confocal microscopy. Images = 40X magnification.

of increasing concentrations of free EGF ligand (**Fig. 10**). Uptake of EGF- α -4 is dramatically decreased with both a 1:1 (200 nM) and 1:5 (1000 nM) ratio of complex to free ligand as visualized by fluorescence microscopy (**Fig. 10A**). EGF- ω is also inhibited, but not to the same extent as EGF- α -4. The relative fluorescence of each treatment regime was quantified and graphed. EGF- α -4 binding was reduced by 68% at the highest concentration of free ligand and EGF- ω was reduced by approximately 40% (**Fig. 10B**).

Several different combinations of complementing pairs of β -gal were examined for uptake and enzymatic activity as proposed in **Specific Aim 1 Milestone 5**. The most robust pair to date is the α -4 and ω combination. The α -4 and 1- ω pair also demonstrated relatively strong enzymatic activity, but staining took longer the optimal 18 hrs. The only inefficient pair was the α -1 and 1- ω pair. This is not unexpected since the α -1 fragment is very small and rapidly diffuses away, out of the cellular compartments where complementation could be optimized. In the short term, we will move forward into *in vivo* studies with the α -4 and ω complementing pair.

We next tested the utility of the construct to accumulate in tumors expressing EGFR using an orthotopic mouse model for brain tumors. Glioma cells,

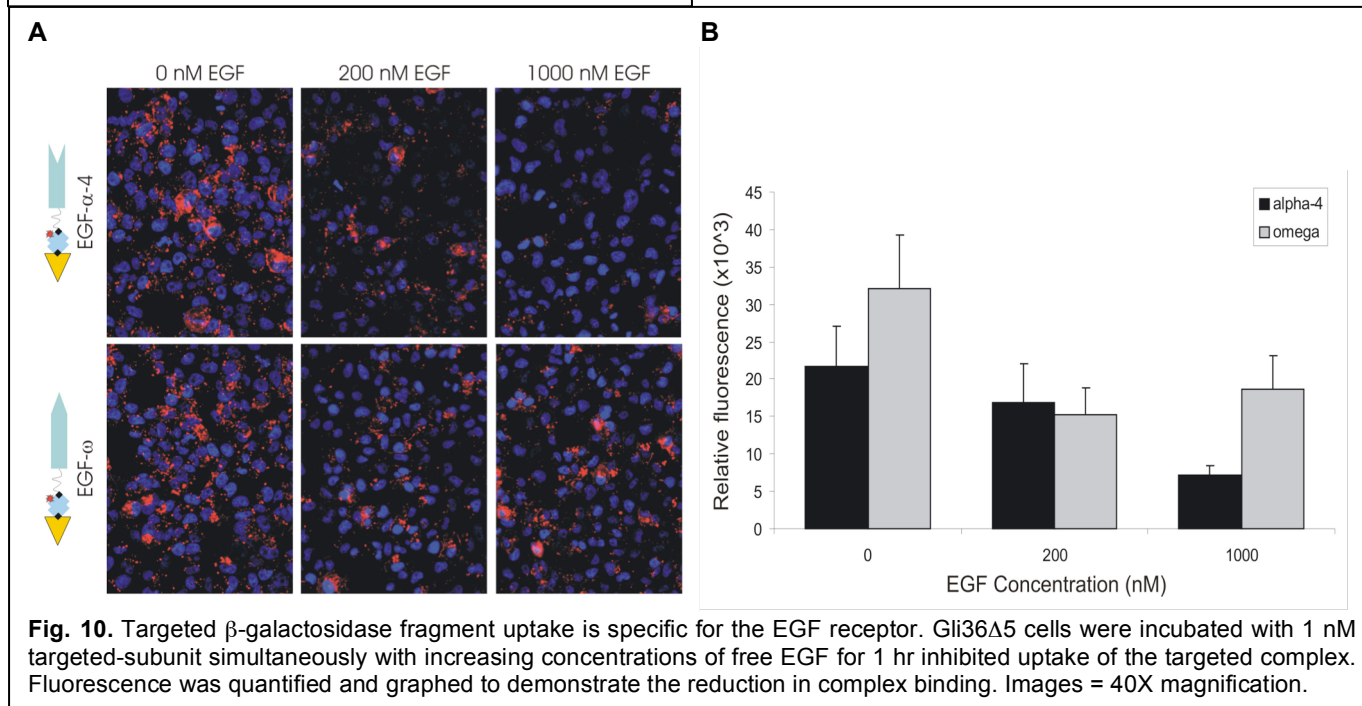


Fig. 10. Targeted β -galactosidase fragment uptake is specific for the EGF receptor. Gli36 Δ 5 cells were incubated with 1 nM targeted-subunit simultaneously with increasing concentrations of free EGF for 1 hr inhibited uptake of the targeted complex. Fluorescence was quantified and graphed to demonstrate the reduction in complex binding. Images = 40X magnification.

Gli36Δ5, were stereotactically implanted in the brains of mice and grown for approximately 10 days as per IUCAC approved protocols (**Specific Aim 2 Milestone 2**). Mice were intravenously injected with 1 mg/kg body weight of either EGF-β-gal (targeted) or B-β-gal (non-targeted), both of which incorporated Alexafluor 647-labeled streptavidin to easily visualize targeted-complex uptake following tissue preparation (**Fig. 11A**). Targeted-β-gal crossed the BBB as measured by fluorescence molecular tomography in living mice (**Fig. 11B**). Approximately 3-5% (~133 nM) of the injected dose accumulated within the tumor within 4 hours. After 4 hours, the mice were euthanized and the brains were removed and imaged whole and then serially transected into 2 mm sections and imaged again *ex vivo* using an *in vivo* Maestro fluorescence imaging system (**Fig. 11C**, top panels). EGF-β-gal specifically accumulates in the tumor within 4 hours. In contrast, non-targeted B-β-gal did not accumulate in the tumor as indicated by a lack of fluorescent signal, but presumably remained in the ventral cerebral and cerebellar arteries of the brain (**Fig. 11C**, bottom panels).

Serial sections of the brains were cryosectioned and counterstained with DAPI to delineate cell nuclei. Fluorescence images captured at 100X magnification clearly showed significant EGF-β-gal uptake within the tumor and internalization within the cells'

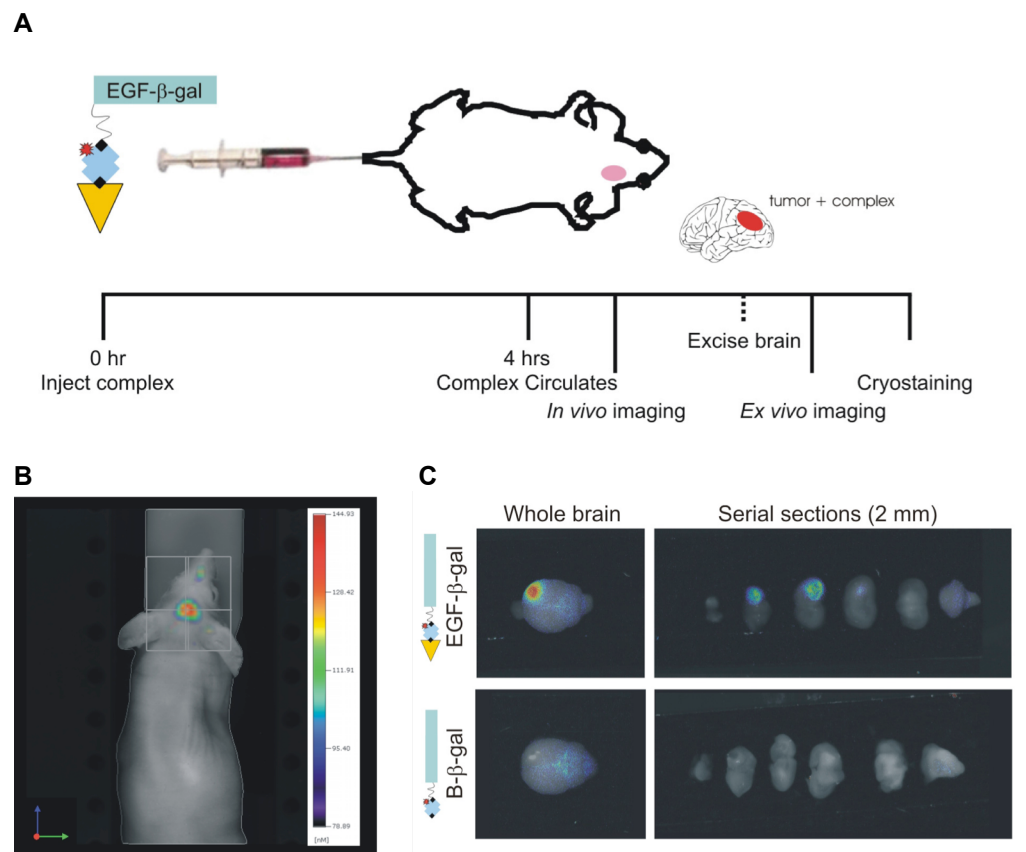


Fig. 11. Fluorescence imaging of targeted-β-galactosidase complex in orthotopic brain tumors. Gli36Δ5 cells were implanted in mice and injected with either EGF-β-gal or B-β-gal.

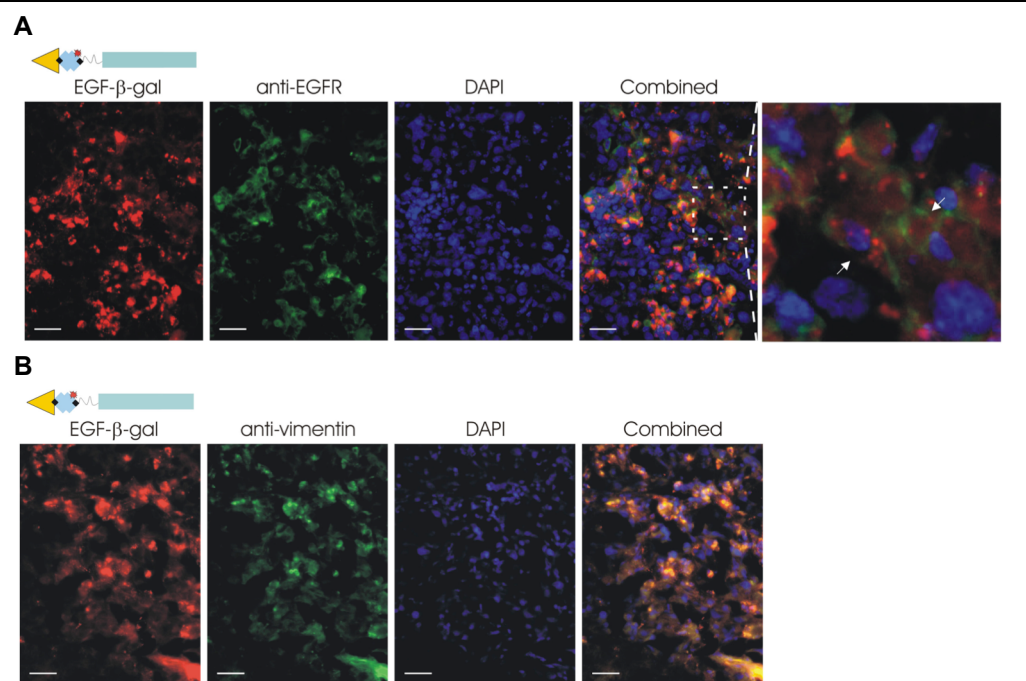
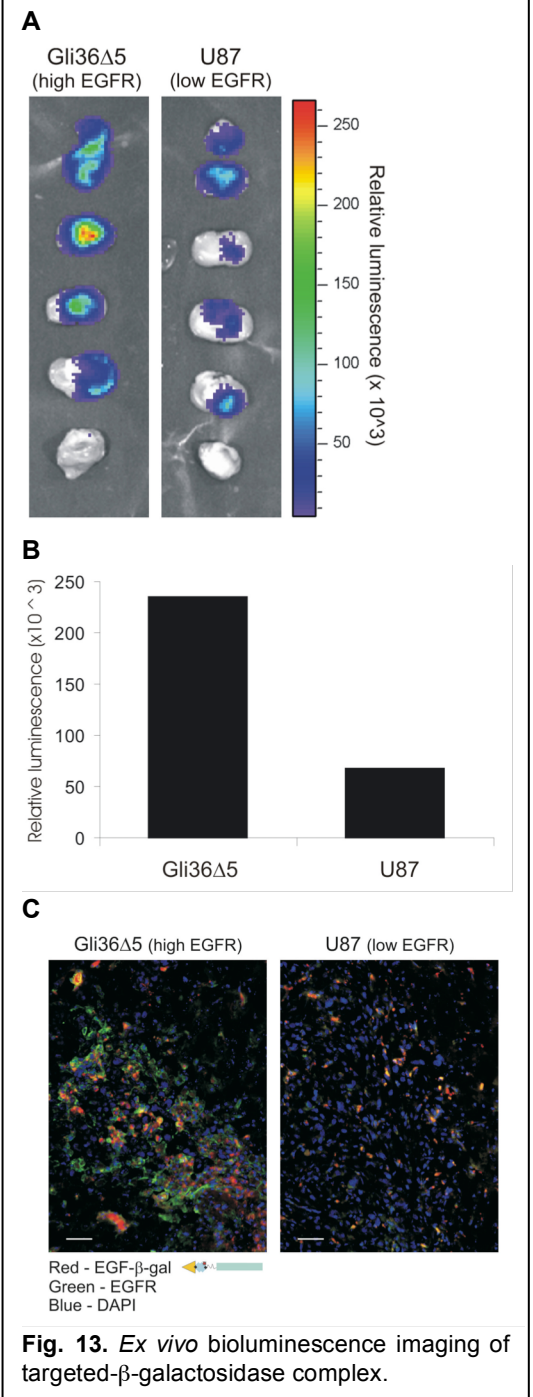
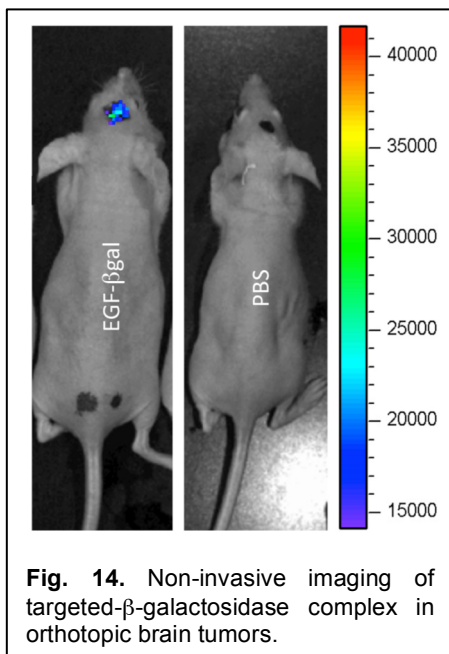


Fig. 12. Validation of targeted-β-galactosidase complex in orthotopic brain tumors.

cytoplasm. Cryosections of brain containing the tumor region were then counterstained with anti-EGFR (**Fig. 12A**) or anti-vimentin (**Fig. 12B**) and visualized using epi-fluorescence microscopy. These studies indicated that EGFR is heterogeneously expressed within the tumor (green) and that targeted- β -gal complex (red) accumulates specifically in glioma cells overexpressing EGFR (**Fig. 12A**). Vimentin staining, a standard pro-invasive intermediate filament tumor marker, identified implanted cells within the orthotopic mouse model which were of human origin, i.e. Gli36 Δ 5 cells, and demonstrated that targeted- β -gal complex (red) only co-localized within human cells expressing vimentin (green) (**Fig. 12B**). Little to no β -gal complex was found within other regions of the brain (data not shown).

We next sought to determine if β -gal activity was maintained by the targeted complex for **Specific Aim 2 Milestone 3**. For these studies orthotopic brain tumors implanted as described above were used. After approximately 10 days of growth tumors were harvested by removing the intact mouse brain and then sectioning it into 2 mm sections. Following sectioning, a bioluminescent β -gal substrate, Galacton-Plus, was topically applied to the *ex vivo* brain serial sections to evaluate the delivery and integrity of enzyme across the BBB (**Fig. 13A**). Further, this targeting ability and enzymatic activity in Gli36 Δ 5-derived brain tumors, expressing high levels of EGFR, was compared to brain tumors implanted with U87 cells expressing low levels of EGFR. Robust bioluminescence was captured within the tumor overexpressing EGFR after 3 minutes, indicating that β -gal maintained its activity. Minimal β -gal activity was observed in U87-derived tumors, which express very low levels of the EGFR protein as previously determined by Western blot. The luminescence was quantified and Gli36 Δ 5 tumors possessed 3-4-fold more enzymatic activity than U87 tumors (**Fig. 13B**). Tumor cryosections counterstained with EGFR antibody (green) and DAPI (blue) demonstrated the differences in both the level of EGFR expression and EGFR-targeted uptake of the β -gal complex within the two tumor types, high EGFR expression and low EGFR expression, respectively (**Fig. 13C**).



The next step in these studies was to demonstrate that complex formation could be imaged *in vivo* in living mice as outlined in **Specific Aim 3 Milestone1**. Mice containing orthotopically-implanted Gli36 Δ 5 cells were intravenously injected with 1 mg/ kg body weight of EGF- β -gal, (**Fig. 14**). Four hours later, Galacton-Plus, bioluminescence β -gal substrate, was stereotactically injected into the brain cavity through the original burr-

hole used to implant the tumors. Targeted- β -gal crossed the BBB as measured by bioluminescence imaging in living mice. Little to no bioluminescence was observed in sham treated mice.

KEY RESEARCH ACCOMPLISHMENTS

The following lists the key research accomplishments emanating from this research during this period:

- Synthesis of NIRF-EGF peptide probe (EGF-Cy5.5) with high affinity for the EGFR and specific accumulation in cells overexpressing EGFR.
- Demonstration that mutant EGFR, EGFRvIII, is not required to effectively image cancer cells *in vitro*.
- Characterization of linker length on uptake and accumulation of EGF-Cy5.5 using kinetic dissociation assay and immunofluorescence microscopy.
- Creation of genetically engineered rat glioma cell lines overexpressing no human receptors, one human receptor, or combination of two human receptors.
- Evaluation of EGFR and TfR pattern of overexpression in human glioma cell lines, Gli36 Δ 5 and U87.
- Self-assembly of targeted β -gal complexes.
- Track uptake and accumulation of EGFR targeted-complex into the endosomal pathway.
- *In vitro* complementation of targeted split- β -galactosidase pairs.
- Recognition that the optimal complementing β -gal pairs, α -4 and ω , are the most robust pair to move forward into *in vivo* animal tumor model studies.
- *In vivo* imaging of EGFR targeted-complex in orthotopic mouse model of brain tumor.
- *Ex vivo* validation of EGFR targeted-complex uptake in orthotopic mouse model of brain tumor corresponds with upregulated EGFR expression.
- *Ex vivo* imaging of EGFR targeted-complex in orthotopic mouse model of brain tumor using bioluminescence.

REPORTABLE OUTCOMES

Original research papers currently in preparation:

Agnes, R.S.; **Broome, A-M.**; Kavik, K.; Verma, A.; Wang, J.; & Basilion, J.P. (2012) An optical probe for noninvasive molecular imaging of orthotopic brain tumors overexpressing epidermal growth factor receptor. *Molecular Cancer Therapeutics*. **11**(10): 2202-11. [PMCID: PMC3829608] Impact factor: 5.23

Broome, A-M.*; Ramamurthy, G.; Lavik, K.; Liggett, A.; Verma, A.; & Basilion, J.* (2014) Optical imaging of targeted beta-galactosidase in brain tumors. *PloSOne*. (*corresponding author) Impact factor: 3.2

Invited lectures:

- Molecular imaging gets personal: The future of personalized diagnostics and therapeutics. (2011) *Malone University Alpha Gamma Chapter Induction Ceremony*.
- Molecular imaging: The future of personalized medicine. (2011) *CWRU School of Medicine Radiology Grand Rounds*.
- Novel molecular imaging platforms. (2011) *University of Akron Chemistry Seminar Series*.
- Molecular imaging gets personal: targeting the cancer signature. (2011) *MUSC Center for Biomedical Imaging Seminar Series*.
- Imaging the tumor microenvironment with novel bio-molecule constructions. (2011) *Tumor Microenvironment and Metastasis Focus Group*.

- Building targeted-split enzyme nanomolecule complexes using directed self-assembly. (2011) *Foundations of Nanoscience: Self-Assembled Architectures and Devices, 8th Annual Meeting*.
- Molecular imaging gets personal: The future of personalized diagnostics and therapeutics. (2012) *Wayne State University School of Medicine Radiology Grand Rounds*.
- Size does matter in pre-clinical imaging: Challenges and other barriers with brain imaging. (2013) *Perkin Elmer and Sandford Burnham Pre-clinical Imaging Methods Seminar*.
- Overcoming the barriers of theranostic delivery to the brain. (2013) *Clemson University Page Morton Hunter Distinguished Lecture Series*.
- Treating gliomas with nanotechnology-based drug delivery systems (2013) *Medical University of South Carolina Hollings Cancer Center Spring Retreat*.
- Big things come in nano-sized packages: Bridging nanotechnology and molecular imaging. (2013) *Medical University of South Carolina MSTP Seminar Series*.
- Small solutions for big problems: Nanotechnology in an imaging world. (2014) *University of Maryland, Baltimore County Chemistry Seminar Series*.

Oral presentations at international conferences:

Broome, A-M.; Ramamurthy, G.; Lavik, K.; Liggett, L.A.; & Basilion, J.P. (2011) Targeting split-enzyme reporter fragments to achieve chemical resolution for molecular imaging. *SPIE BiOS: Biomedical Optics Symposium*.

Poster presentations at international conferences and workshops:

- **Broome, A-M.**; Ramamurthy, G.; Lavik, K.; Pinter, M.; Kinstlinger, I.; & Basilion, J.P. (2012) Targeted-split enzyme complementation to interrogate the cancer signature using molecular imaging. *World Molecular Imaging Congress*.
- **Broome, A-M.**; Ramamurthy, G.; Lavik, K.; Verma, A.; Pinter, M.; Basilion, J.P. (2012) Molecular imaging of the cancer signature using targeted-split enzyme complementation. *American Association for Cancer Research, 103rd Annual Meeting*.
- Verma, A.; Ramamurthy, G.; Chung, S.; **Broome, A-M.**; and Basilion, J.P. (2011) Targeted-enzyme complementation to image cancer receptors. *BMES Annual Meeting*.
- **Broome, A-M.**; Ramamurthy, G.; Lavik, K.; Verma, A.; & Basilion, J.P. (2011) Targeted-split enzyme nanomolecule complexes for molecular imaging of the coordinated expression of cell surface receptors in glioblastomas. *World Molecular Imaging Congress. Best Poster in Category*.
- Agnes, R.S.; **Broome, A-M.**; & Basilion, J.P. (2011) Differential fluorescence molecular imaging of EGFR in brain tumors. *World Molecular Imaging Congress*.
- Agnes, R.S.; **Broome, A-M.**; & Basilion, J.P. (2011) Targeted, non-invasive optical imaging agent for fluorescence molecular tomography studies. *American Peptide Society, 22nd American Peptide Symposium*.
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12/01/2012 - 11/30/2016

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Proteomic Guided In Vivo Molecular Imaging

Role Investigator: Multi-Principal Investigator

Total Direct Costs: \$5,000,000

Action: Not funded; resubmit

Employment applied for and received based on experience supported by this award:

Associate Professor

Director of Molecular Imaging, Center for Biomedical Imaging

Director of Small Animal Imaging, Hollings Cancer Center

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CONCLUSION

Targeted-reporter platforms have real utility for imaging the multi-step progression of cancer growth that requires the coordinated overexpression of cell surface biomarkers. The development of these platforms to investigate molecular signatures associated with disease creates the next frontier in *in vivo* imaging. The exponential number of genomic marker sets that are considered diagnostic or predictive of disease states underscores the importance of imaging molecular signatures. By exploiting multi-marker imaging, we ultimately seek to image combinations of biomarkers that will uniquely identify cancers from normal tissue and report on the biochemical status of these cells. These expression patterns can thus be indicative of the type, stage, or severity of disease. The application of imaging molecular signatures is therefore critical for cancer and disease detection.

Our research utilizes inactive subunits of an image-able enzyme that are driven to complementation by targeting specific biomarkers on the surface of cells. By linking a reporter fragment to a targeting moiety, this approach provides a cellular resolution that is far more precise than the physical/ anatomical-based resolution currently employed. The pioneering utility of the technique significantly increases specificity, decreases background artifacts, and, combined with a cancer systems approach, promotes our ability to interrogate the status of cells rather than just the presence of cancer biomarkers. In the studies described here, we will utilize a bi-complementation strategy.