Novel opportunities for non-invasive diagnostics for cancer patients

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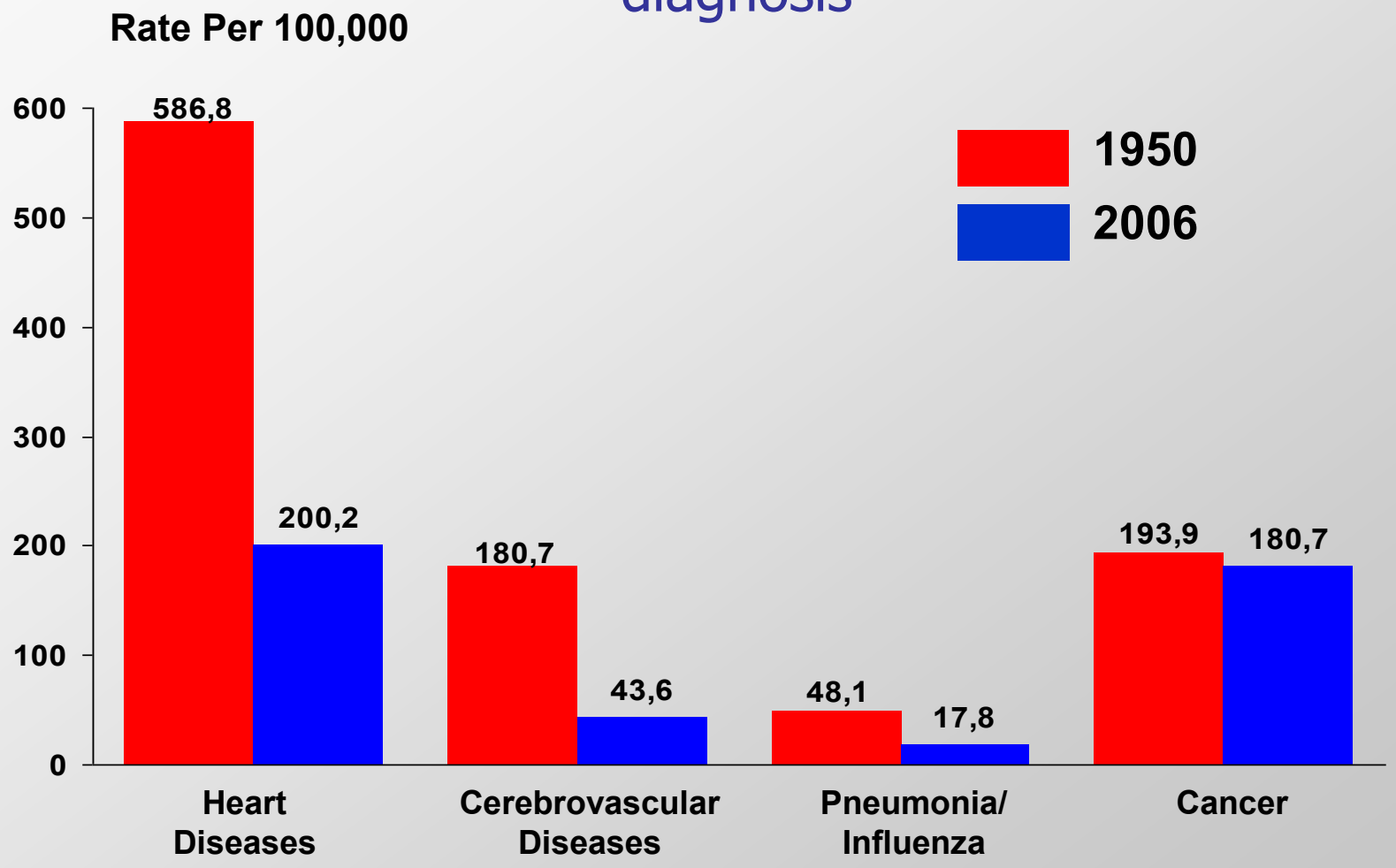
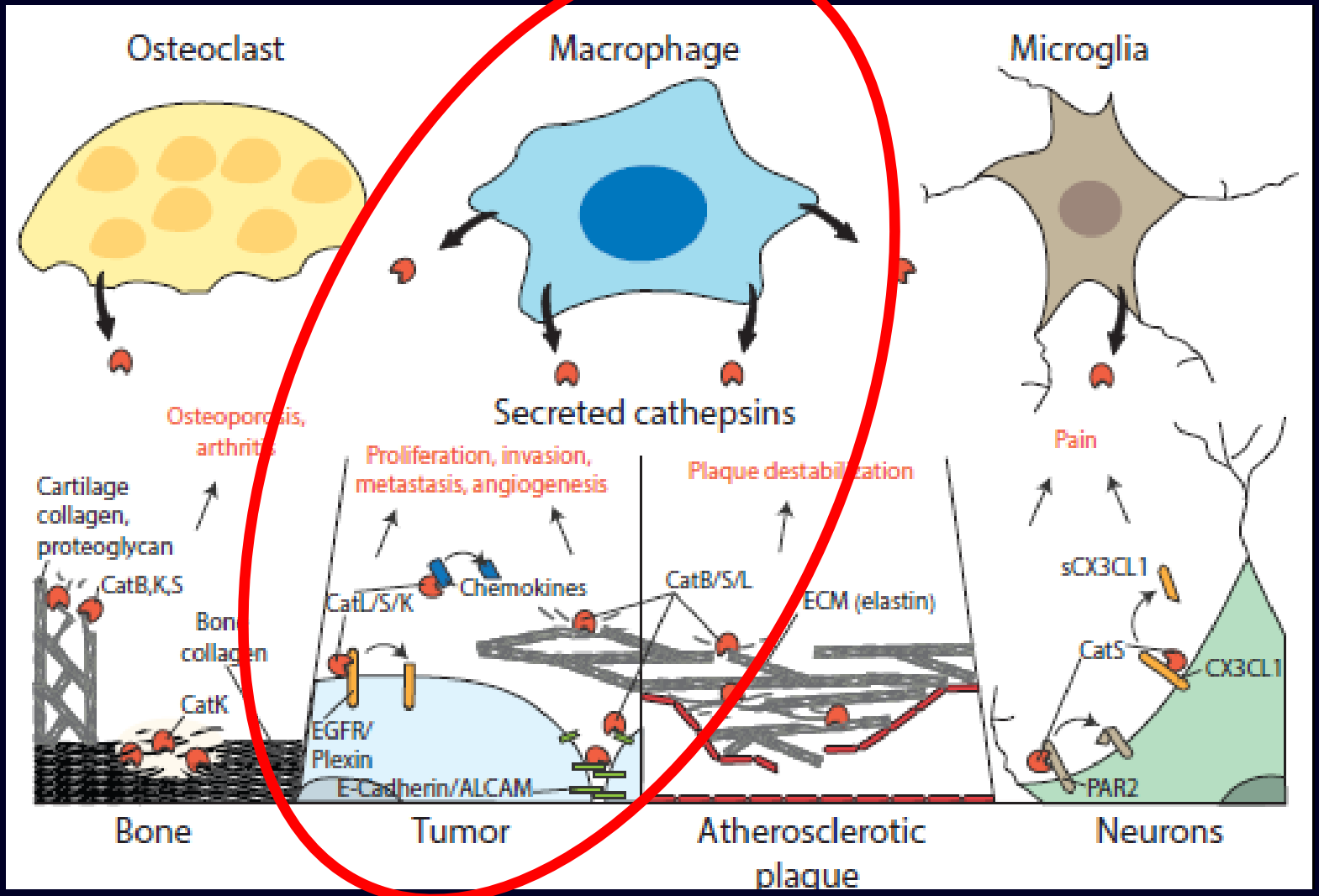
Cancer is still one of the leading causes of mortality in developed countries, which is at least partially linked to late diagnosis



Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised. 2002 Mortality Data: US Mortality Public Use Data Tape, 2002, NCHS, Centers for Disease Control and Prevention, 2004

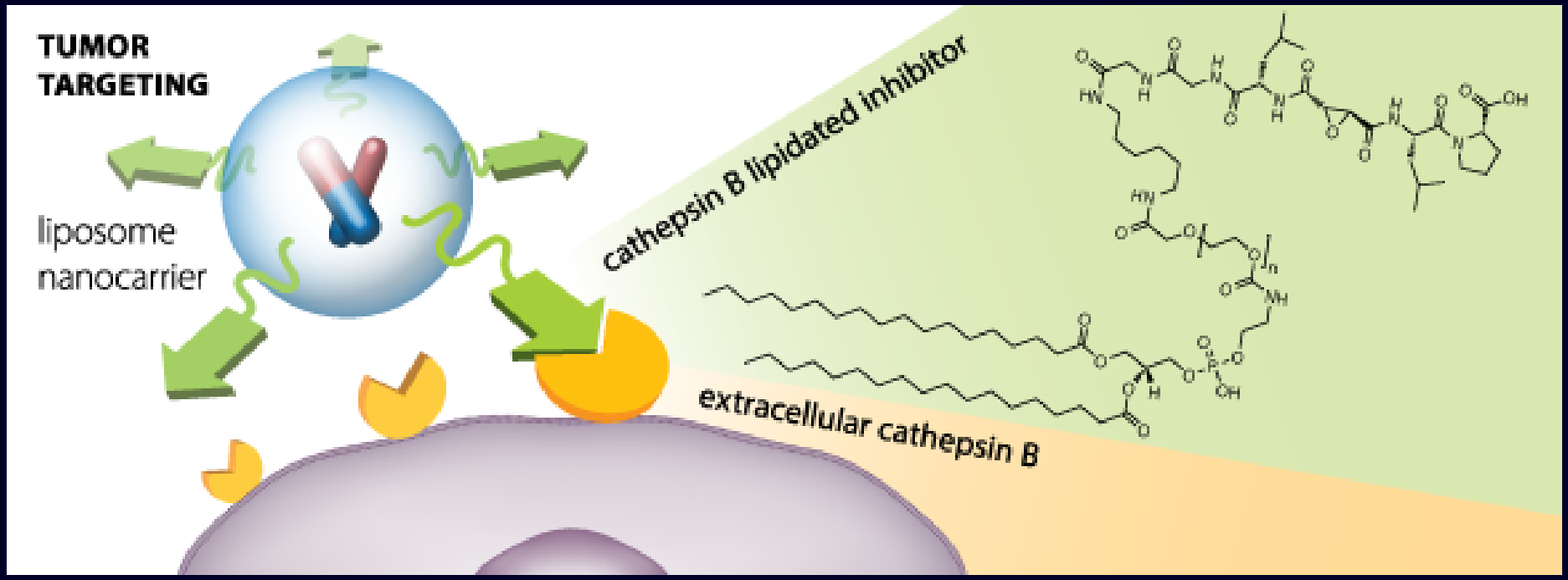
Proteolytic enzymes (proteases) are often overexpressed in cancer: which opportunities this offers for diagnosis?

- Proteases, in particular cysteine cathepsins and matrix metalloproteases are often secreted from infiltrated immune cells – extracellular cathepsins markers of inflammation and inflammation-associated diseases such as many cancers;
- Especially cathepsins highly suitable imaging targets as they offer good signal-to-noise ratio due to their cancer overexpression and secretion.
- Extracellular cathepsins also highly suitable targets for targeted drug delivery



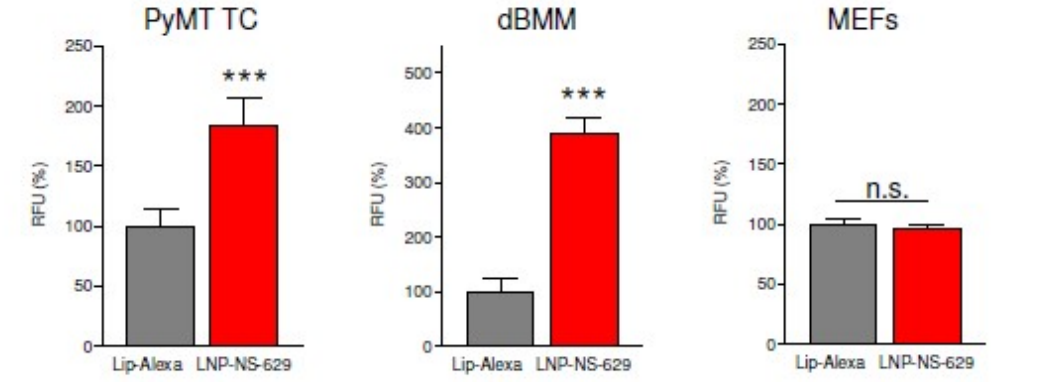
Kramer et al (2017) Trend Pharm Sci

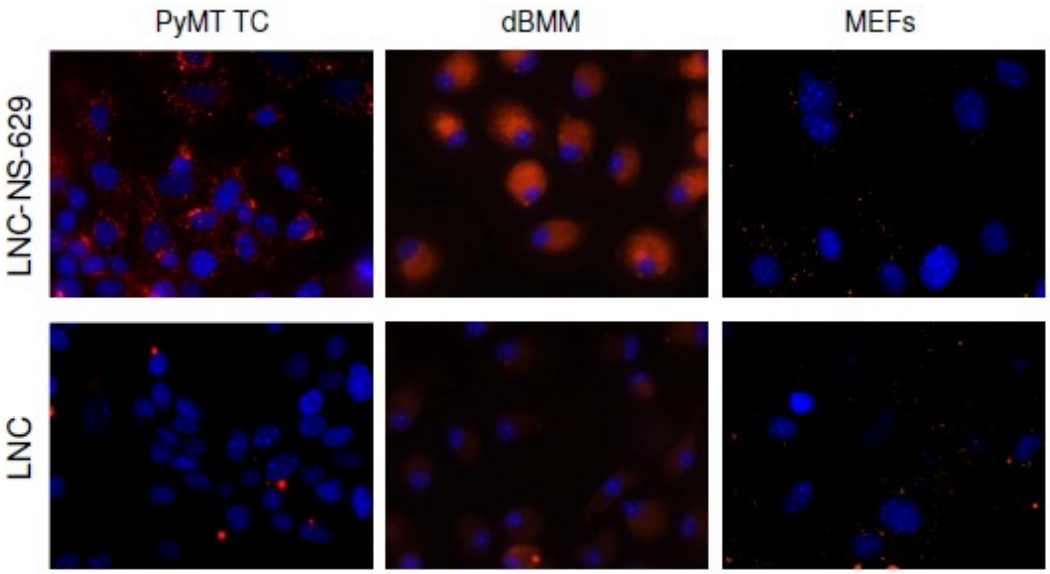
Active targeting of cathepsins – potential for theranostic applications



Mikhaylov et al., (2014) Angewandte Chemie Intl Ed.

Tumor cells (PyMT TC) and macrophages (dBMM) express and secrete much more catB than normal cells (MEFs), which can be successfully targeted by the LNC-NS-629 system





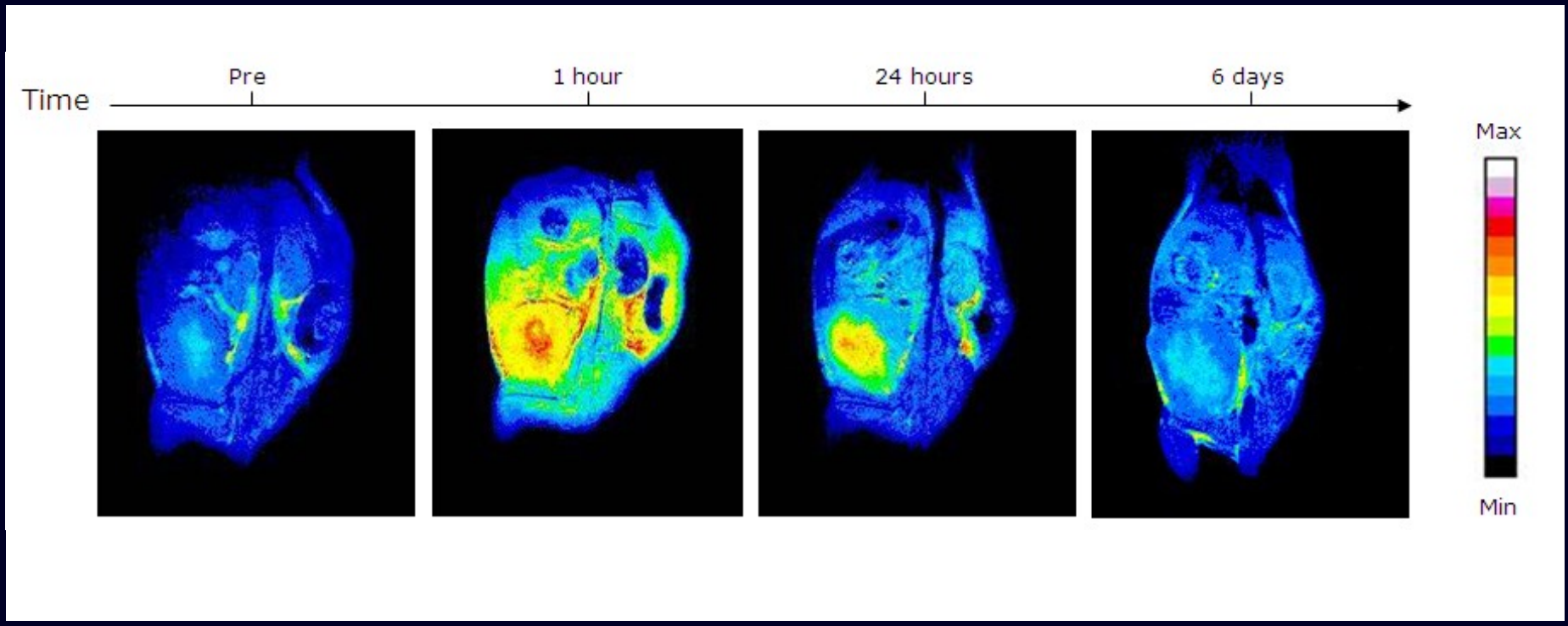
- LNC-AF555
- LNC-NS-629-AF555

Table 1: A list of proteins identified in conditioned media from primary PyMT tumor cells (PyMT TC) and differentiated bone marrow derived macrophages (dBMM).The proteins are ordered by relative abundance as estimated from spectral counting in tumor cells.

Protein	Uniprot accession	Number of peptides	Peptide spectral counts	
			PyMT TC	dBMM
Cathepsin B	P10605	15	34	94
MCF-1	P07141	5	20	4
Cathepsin L	P06797	9	16	8
MMP-12	P34960	10	0	11
Ferritin light chain	Q3THE6	8	0	22

Mikhaylov et al., (2014) Angewandte Chemie Intl Ed.

LNC-NS-629 system successfully targets tumors in vivo: MRI in breast cancer mouse model



T_1–weighted MR images of orthotopic breast cancer mouse model after administration of LNC-NS-629 containing MRI contrast agent, Magnevist®

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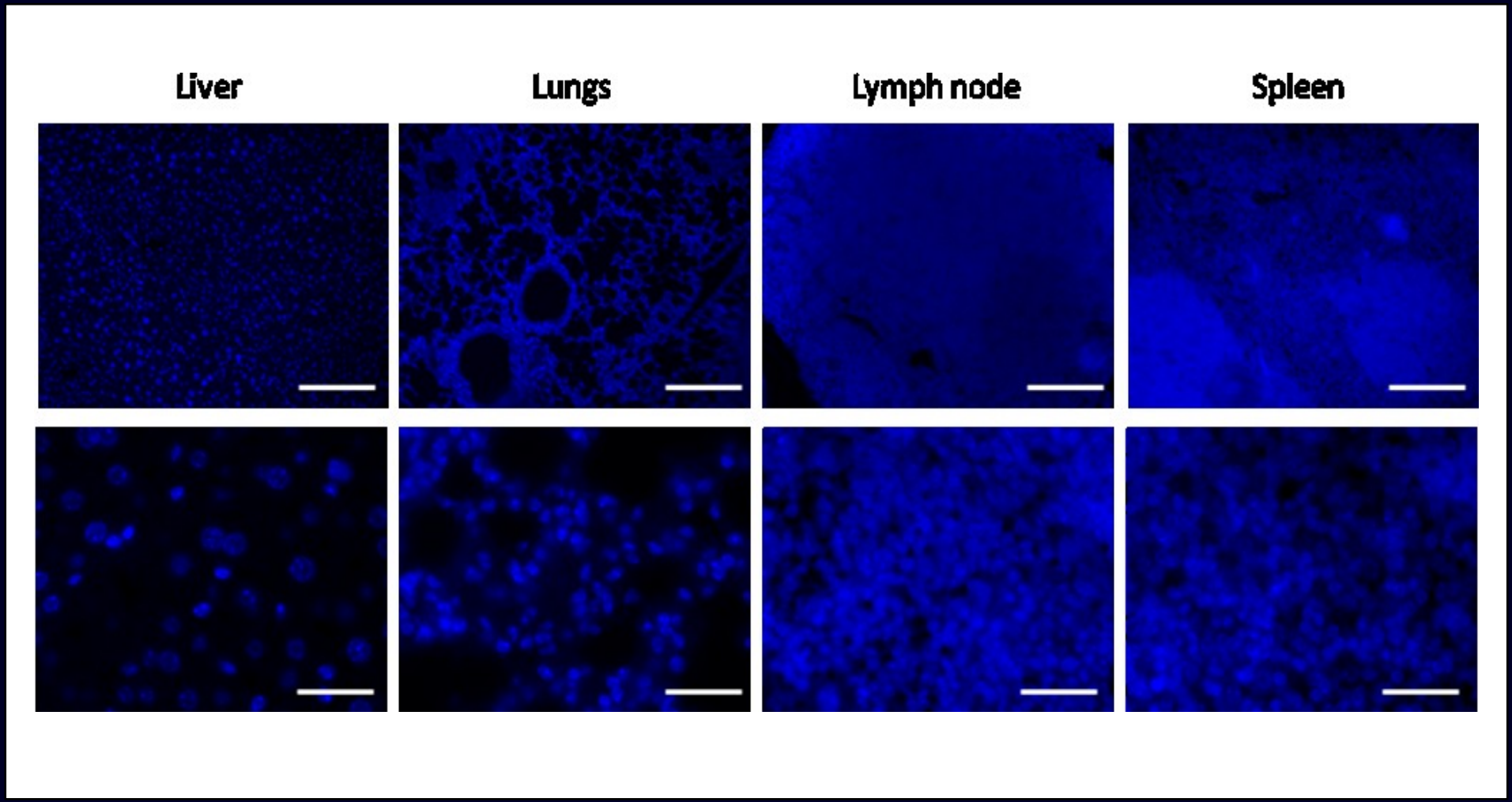
LNC-NS-629 system does not target normal tissues: no visible on- or off-target effect



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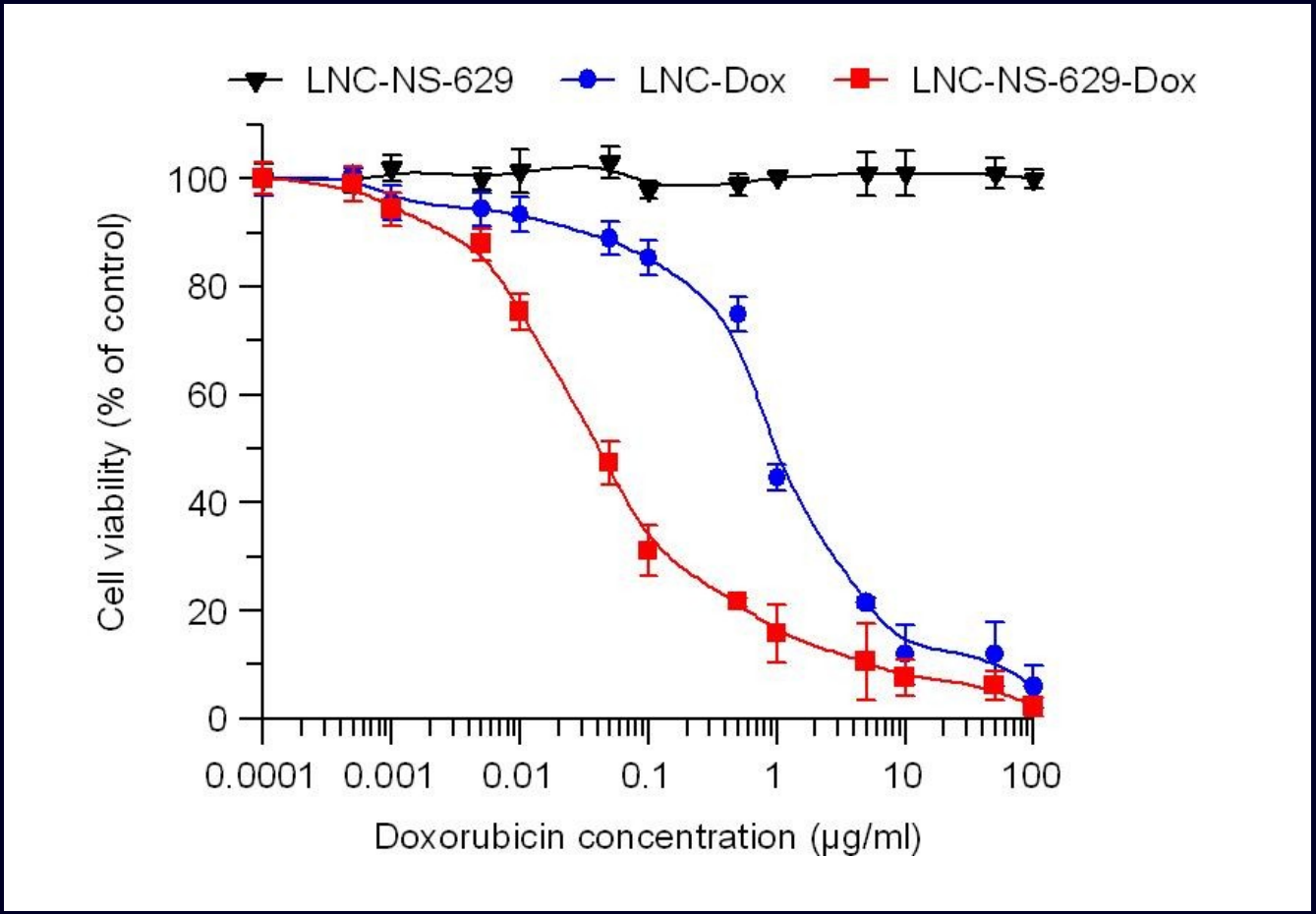
LNC-NS-629 encapsulated doxorubicin much more efficiently killing PyMT mouse breast cancer cells than doxyl (liposome encapsulated doxorubicin wo targeting)



PyMT cells were treated with doxorubicin encapsulated in naked LNC (LNC-Dox) and CtsB targeted LNC-NS-629 (LNC-NS-629-Dox) liposomes at varying [illegible]

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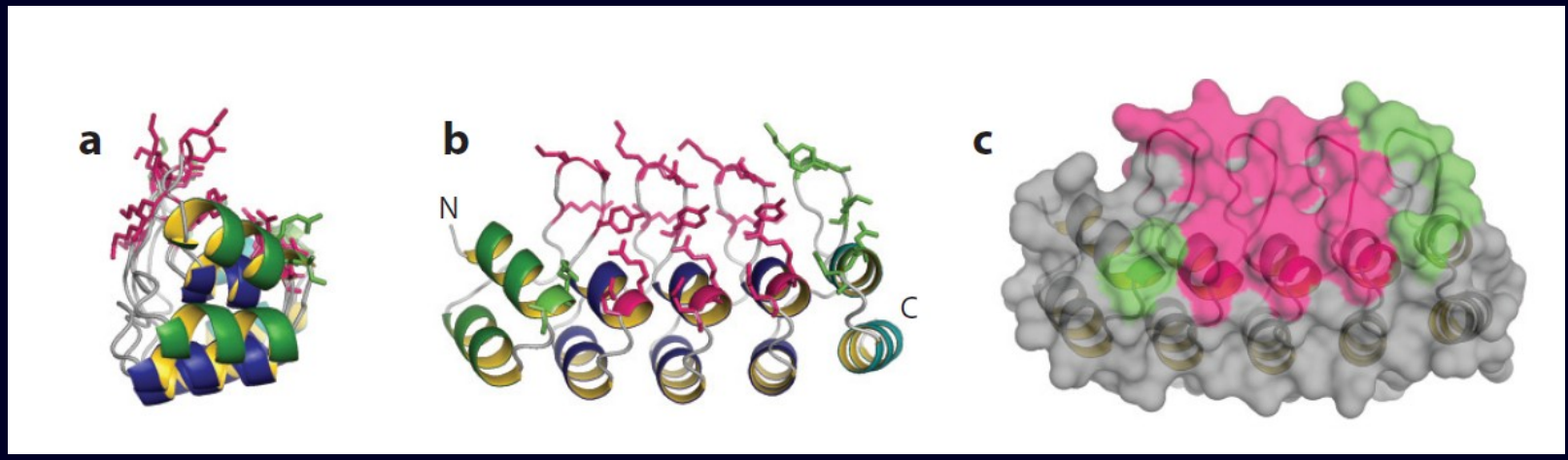
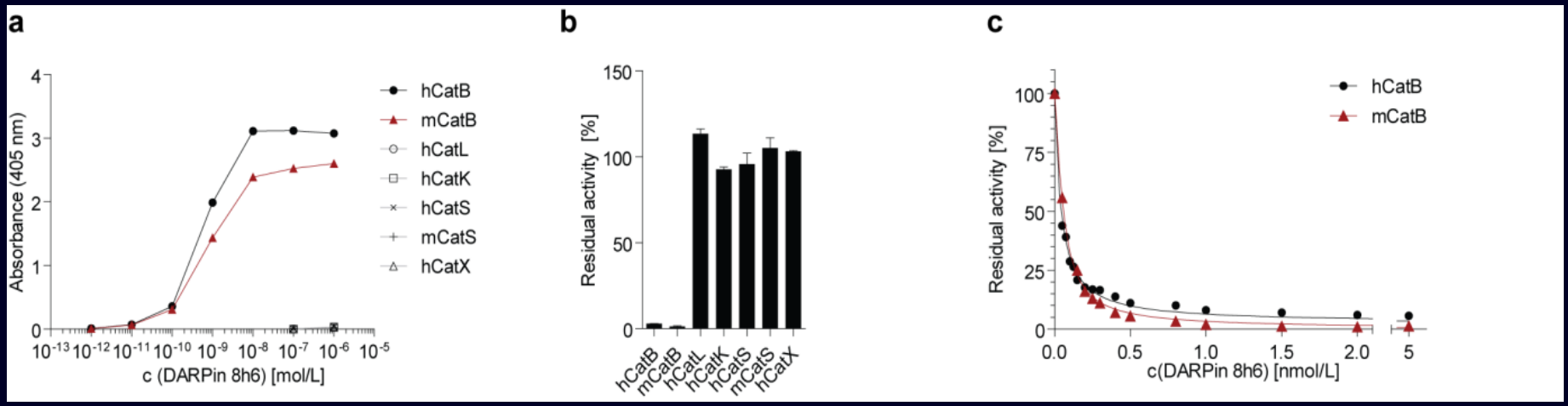
In addition to small molecules, protein molecules can be used in imaging: antibodies or engineered proteins (DARPin's, …)



- DARPin: designed ankyrin repeat proteins: very stable, easy to make in E. coli, ribosome display typically generates cca 10^{12} binders, …
- Many uses: biochemical tools (assist in crystallization, …), diagnosis tools, therapeutics

Pluckthun (2015) Annu Rev Pharmacol Toxicol

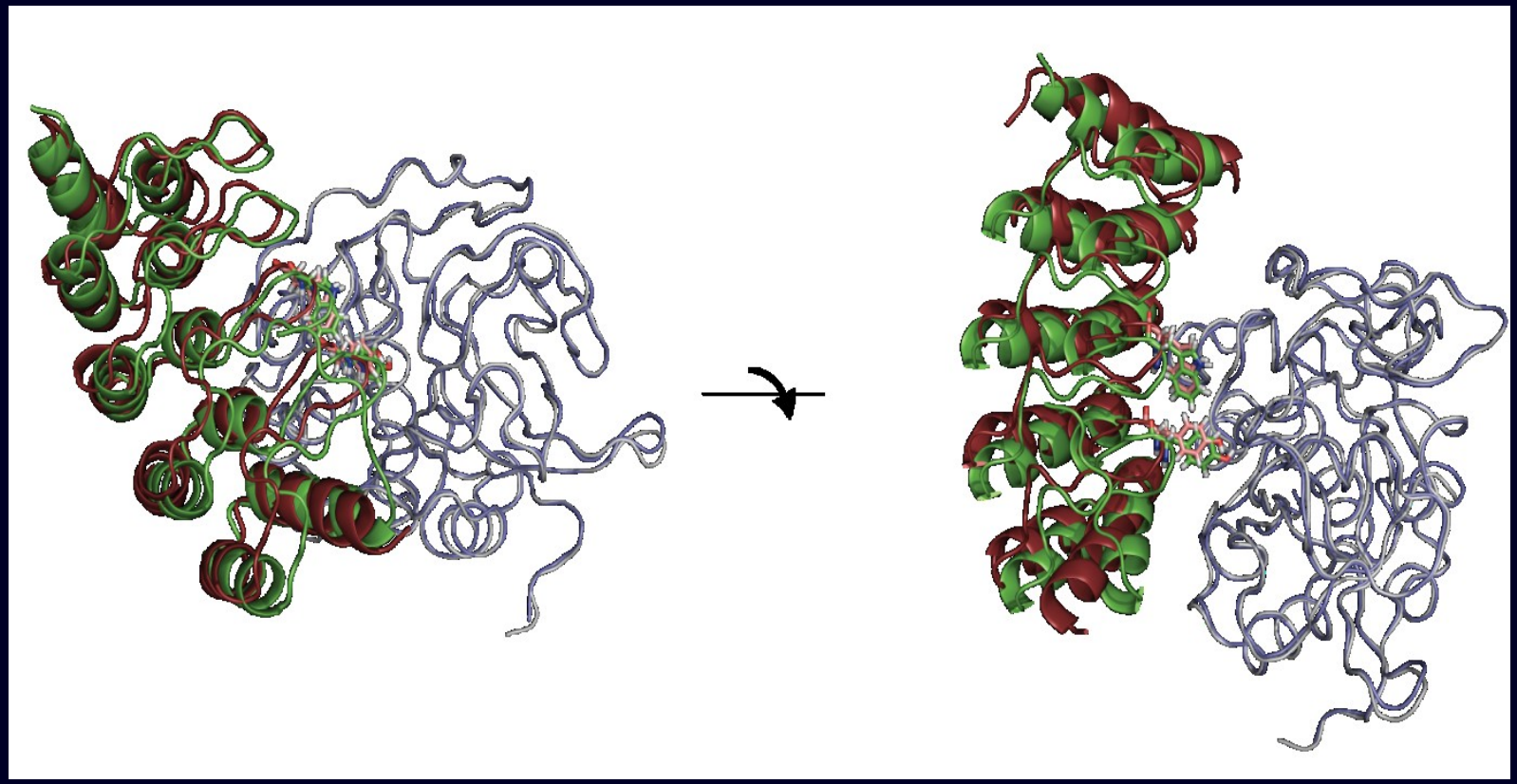
Targeting cancer-associated cathepsin B by DARPins



Over 40 binders obtained after 8 rounds of selection
(4 against human, 4 against mouse cat B)
DARPin 8h6 binds and inhibits human and mouse cat B with pM affinity
(Ki ~ 35 pM)

Kramer et al (2017) Theranostics

Crystal structure of DARPin 8h6 (red) and 81 (green) in complex with cathepsin B



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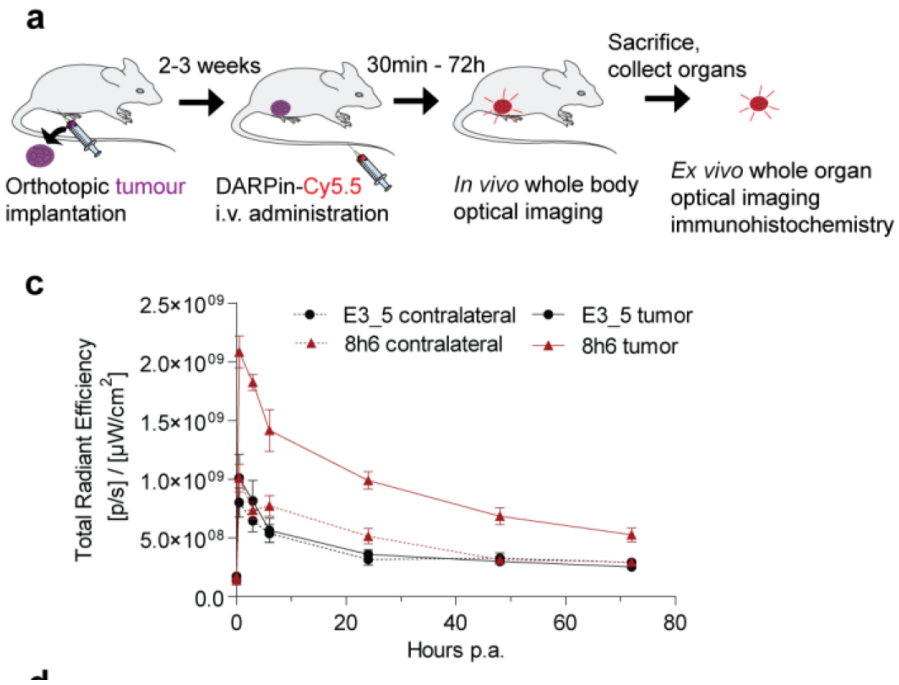
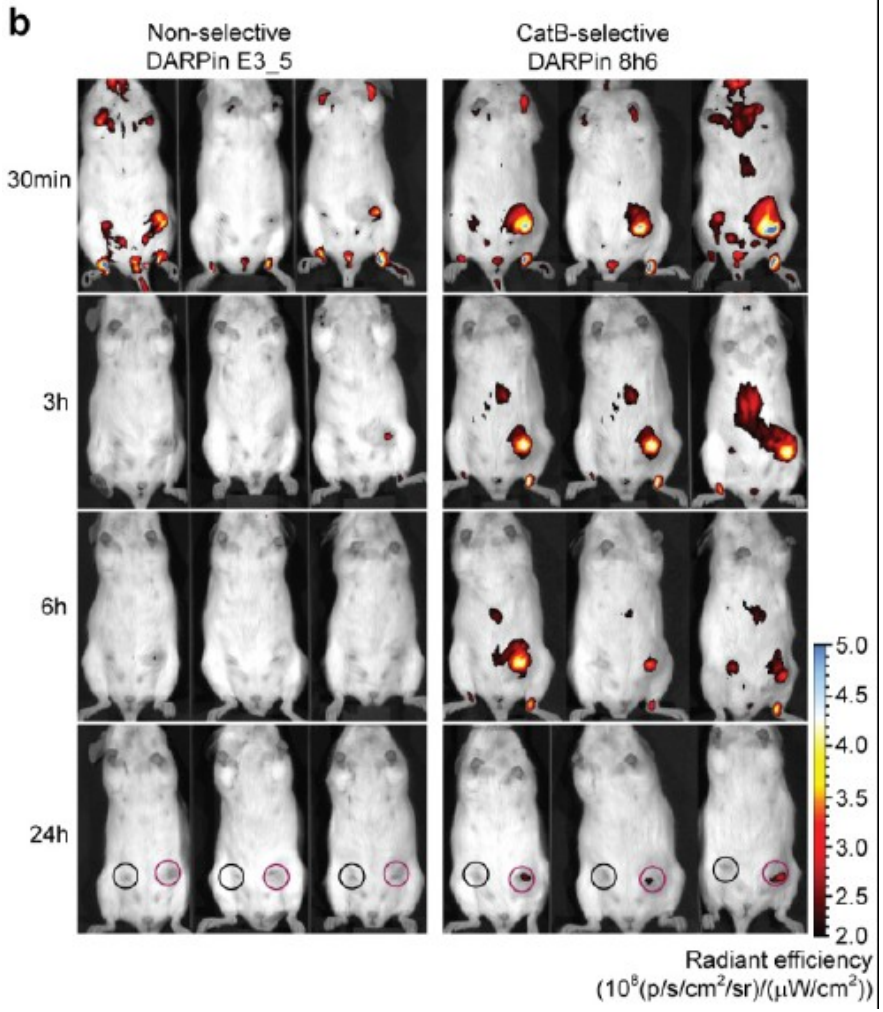
Cathepsin B in mouse model of breast cancer can be succesfully imaged with 8h6 DARPin (PyMT and 4T1)





Kramer et al (2017) Theranostics

Conclusions

- Proteolytic enzymes, such as cysteine cathepsins B and S, are highly suitable as targets for diagnostic imaging in cancer and other inflammation- associated diseases
- In addition to small molecule imaging agents (substrates, ABPs) engineered proteins (DARPins) offer an alternative in diagnostic imaging of cathepsins in cancer
- Proteolytic enzymes such as cysteine cathepsins are highly suitable as **targets for targeted drug delivery** in cancer and other inflammation-associated diseases (therapy) – paradigm shift; proteases are not only therapeutic targets!

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