# Cancer nanotechnology using elastin-like polypeptides

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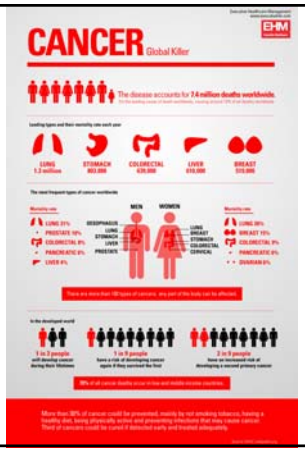
## Presentation outline

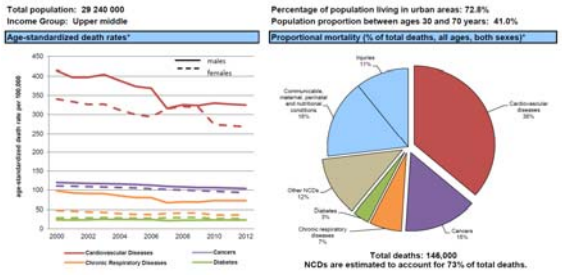
1. **Introduction**
2. **Protein polymers - Elastin-like polypeptides (ELP)**
   - Synthesis and characterization
   - Biodistribution of ELP protein polymers using positron emission tomography (PET)
   - Cancer targeting nanoparticles and evaluation with PET
3. **Conclusion**
4. **Acknowledgements**

## Cancer facts

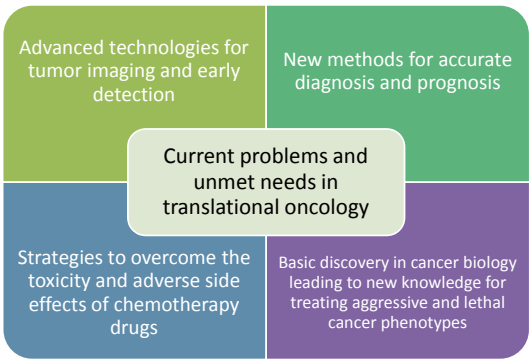
- 1 in 8 deaths due to cancer.
  - More deaths than AIDS, TB and malaria combined.
- International Agency for Cancer Research estimates 12.7million new cancer cases in 2008.
  - 7.6 million deaths (~21000 cancer deaths a day)
- Deaths worldwide projected to continue rising, with an estimated 13.2 million deaths in 2030.
- Cancer will overtake heart disease as the leading cause of death worldwide by 2010.

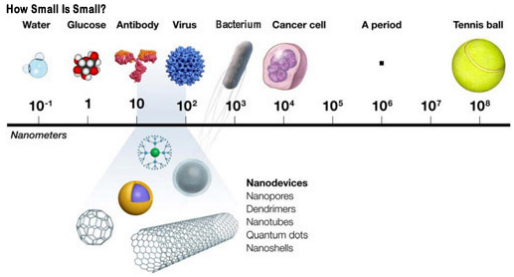



## Malaysia health facts




World Health Organization - Noncommunicable Diseases (NCD) Country Profiles, 2014.

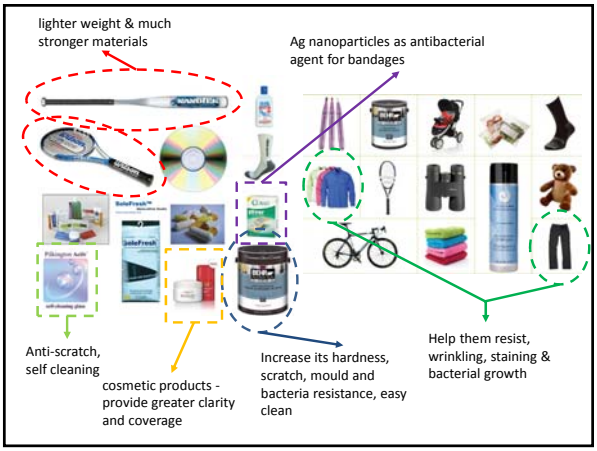





"…the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, [for] novel applications."

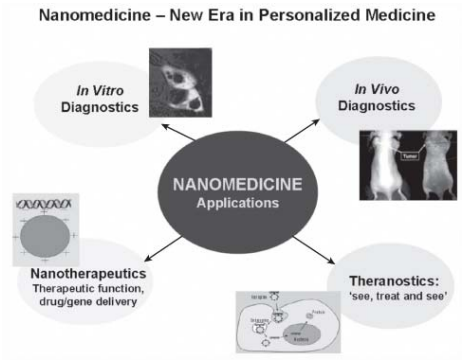
**US National Nanotechnology Initiative**

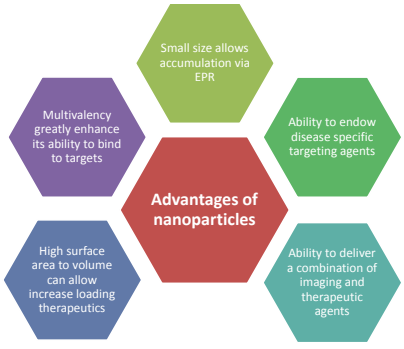
## Nanotechnology and Nanomedicine

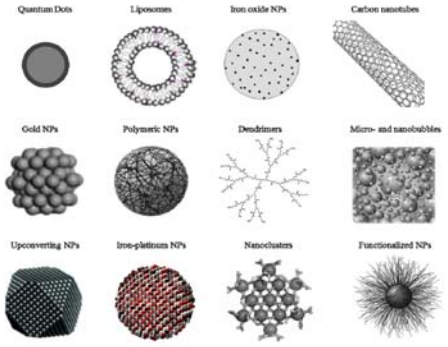




## The 'nano' soldier


- Durable & lightweight
- $SiO_2$ nanoparticles in a liquid polymer hardens on ballistic impact



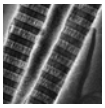



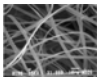



F Re et al 2012 J. Phys. D: Appl. Phys. 45 073001

## Protein polymers have attractive properties for use in nanotechnology

1. Compatibility with genetic engineering
   - Ease of synthesis, large macromolecules, uniformity
2. Produced in high yields
   - Readily expressed bacteria
   - Ease of purification
3. No bioconjugate chemistry is required to link fusion proteins to polymers
4. Non-immunogenic, biocompatible and biodegradable

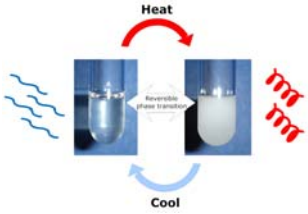

collagen


elastin


Spider silk

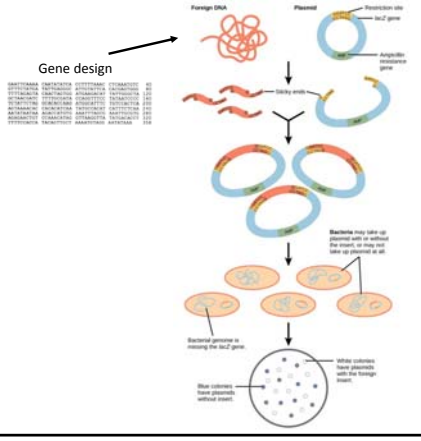
## Elastin-like polypeptides (ELPs)

- $(VPG\underline{X}G)_n$
- inverse phase transition temperature, $T_t$
- $T_t$ is a function of
  - guest residues, X
  - Length, n
  - MW

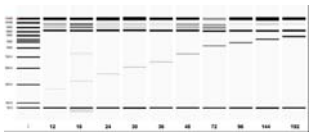


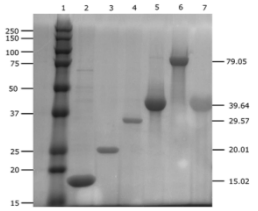

## Attributes of ELPs

- genetically engineered precision
  - length
  - sequence
  - monodispersity
- high yields (50-500 mg/L culture)
- self-assembly of block copolymers
- biocompatible
- biodegradable




## ELP DNA and protein library obtained genetic engineering

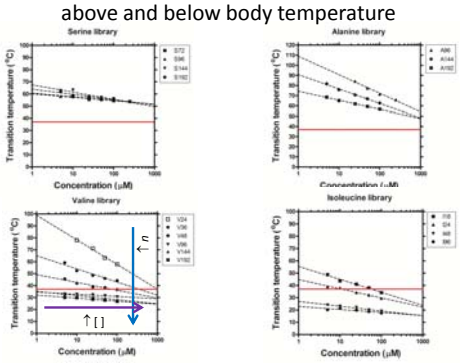

Virtual gel of the Serine library as obtained with the BioRad Experion



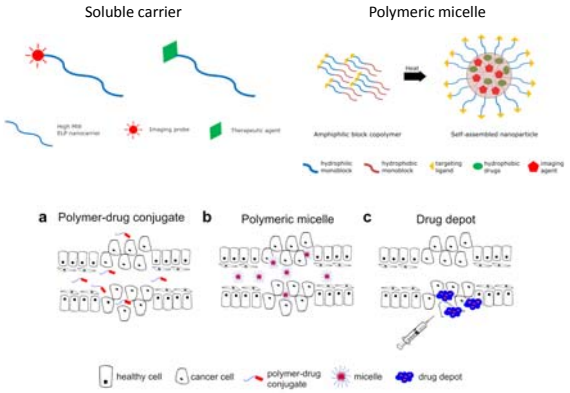
SDS PAGE library of purified ELP library

S. Mohd Janib *et al.*, (2014), *Polym. Chem.*, **2014**, 5:1614-1625

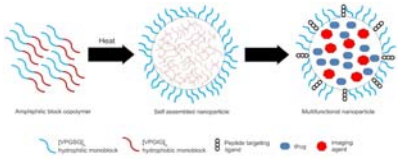
## ELP libraries generated exhibit phase transition above and below body temperature




- Exhibit concentration and length dependence
- Serine> alanine > valine > isoleucine

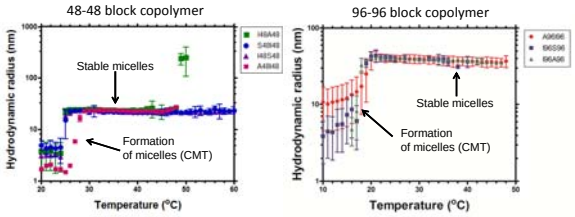



## ELP block copolymer library




| Length (pentamer) | Amino acid sequence | Abbreviated form | Expected MW (kDa) |
|---|---|---|---|
| 36 | $G(VPGIG)_{18}(VPGSG)_{18}Y$ | I18S18 | 15.02 |
| 48 | $G(VPGIG)_{24}(VPGSG)_{24}Y$ | I24S24 | 20.01 |
| 72 | $G(VPGIG)_{36}(VPGSG)_{36}Y$ | I36S36 | 29.57 |
| 96 | $G(VPGIG)_{48}(VPGSG)_{48}Y$ | I48S48 | 39.64 |
| 192 | $G(VPGIG)_{96}(VPGSG)_{96}Y$ | I96S96 | 79.05 |

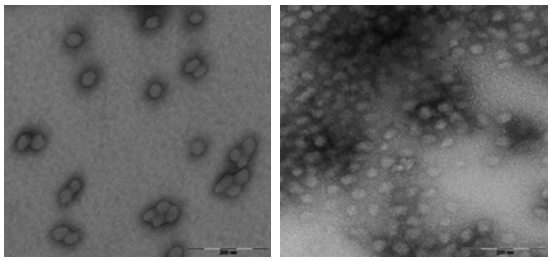
## ELP diblock copolymers assemble into nanoparticles




S. Mohd Janib *et al.*, (2014), *Polym. Chem.*, **2014**, 5:1614-1625

## Micelles observed under TEM

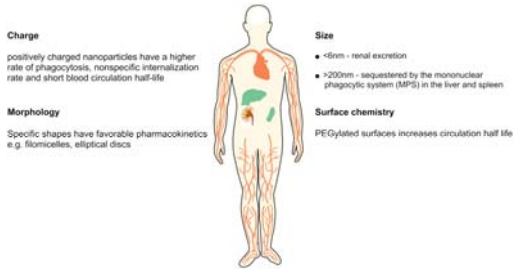
I48S48 I96S96



40.22 ± 4.50 nm 48.32 ± 3.99 nm

S. Mohd Janib *et al.*, (2014), *Polym. Chem.*, **2014**, 5:1614-1625

## Physical characteristics of NP effect its *in vivo* biodistribution



## PET allows non-invasive imaging of ELP biodistribution

- Clinical imaging technique which produces 3D images of functional processes in the body.
  - Processes visualized by injecting small amounts of radioactive tracer
- Advantages of PET:
  - Non-invasive
  - Quantitative
  - High sensitivity and specificity

## Radionuclide selection crucial for optimal imaging efficacy

1. A physical half-life paralleling the biological half-life of the biomolecule
2. Decay characteristics appropriate for PET imaging
   a) High positron branching with no or weak accompanying radiation ($\beta^-$, $\gamma$)
      - ❖ High sensitivity PET imaging
      - ❖ Reducing radiation burden to patient
   b) Low $\beta^+$-energy to allow high-resolution PET imaging
3. The availability of the radionuclide
   – Cost effective, fast separation strategy – automation
4. Not complicated, fast labelling procedure

## Traditional PET radioisotopes have limitations in nanoparticulate-based molecular imaging

- Common radionuclides for PET
  – F-18, C-11, N-13, and O-15
  – 'Organic' radioisotopes – make up biological molecules
  – Short half-lives, lengthy radiosynthesis under organic conditions, fast clearance
  – Needs onsite cyclotron


18F-FDG


18F-DOPA


$^{11}$C-choline

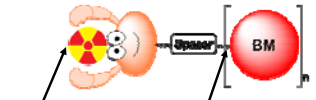

$^{11}$C- methionine

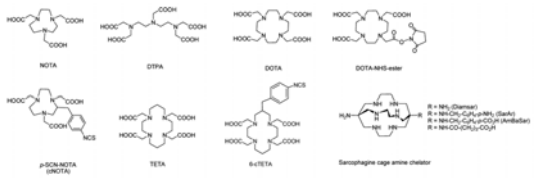
## Increasing use of radiometals for nanoparticulate-based imaging agents

- Metallic radioisotopes have advantages over non-metallic isotopes
  – Radiometals are more easily available
  – Longer half-life
  – Labelling done under mild conditions, suitable for biological molecules

**Table 3** Selected radiometals for imaging and relevant physical decay data

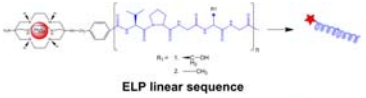
| Nuclide | Half-life | Emission (branching ratio) | $E_{max}$ | $E_{avg}$ | Production | Modality | Ref. |
|---|---|---|---|---|---|---|---|
| $^{64}$Cu | 12.7 h | $\beta^+$ (18%) | 653 keV | 278 keV | Cyclotron, $^{64}$Ni(p,n)$^{64}$Cu | PET | 6, 8, 13 |
| $^{68}$Ga | 67.7 m | $\beta^+$ (89%) | 1.899 MeV | 836 keV | Generator, $^{68}$Ge/$^{68}$Ga | PET | 6, 12, 13 |
| $^{86}$Y | 14.7 h | $\beta^+$ (32%) | 1.221 MeV | 535 keV | Cyclotron, $^{86}$Sr(p,n)$^{86}$Y | PET | 6, 7, 13 |
| $^{89}$Zr | 3.3 d | $\beta^+$ (23%) | 902 keV | 396 keV | Cyclotron, $^{89}$Y(p,n)$^{89}$Zr | PET | 6, 13 |
| $^{111}$In | 2.8 d | EC (100%) | | 171 keV<br>245 keV | Cyclotron, $^{111}$Cd(p,n)$^{111m,g}$In<br>$^{112}$Cd(p,2n)$^{111m,g}$In | SPECT | 6, 7 |



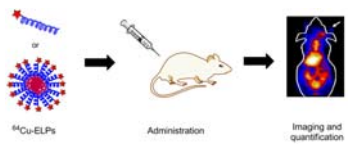




**Copper-64**
- $T_{1/2}$ = 12.7h
- Facile labeling
- low positron energy β+ (0.655mEv) to give high resolution images

## Conjugation with chelator, followed by Cu-64 chelation confers radioactive properties


ELP linear sequence


ELP block copolymer [VPGAG-VPGIG]

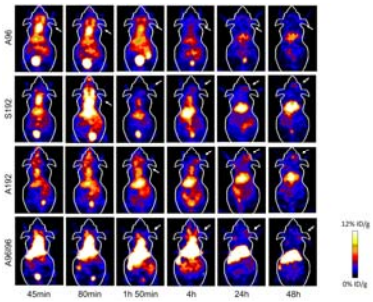



Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.

## Does MW, guest residue identity and nanostructure adopted affect biodistribution?

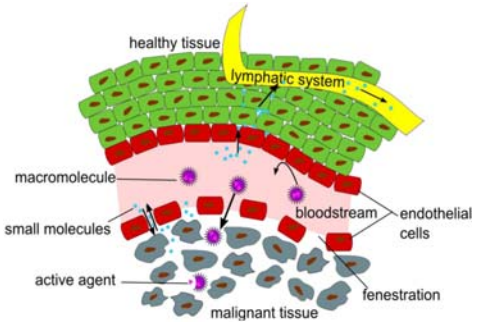
| ELP | Amino acid sequence | MW (kDa) | $T_t$ at 25μM (°C) | $R_h$ (nm) | Construct |
|---|---|---|---|---|---|
| A96 | $G(VPGAG)_{96}Y$ | 37.0 | 84.3 | 5.2 ± 0.6 | |
| A192 | $G(VPGAG)_{192}Y$ | 73.6 | 61.9 | 7.1 ± 0.5 | |
| S192 | $G(VPGSG)_{192}Y$ | 76.6 | 57.4 | 7.4 ± 0.4 | |
| A96I96 | $G(VPGAG)_{96}(VPGIG)_{96}Y$ | 77.7 | 20.6 | 37.4 ± 2.5 | |

## Tumor accumulation of ELP via EPR




- Intense heart signal indicate protein polymers are circulating. A96 only up to ~4h.
- A96 – smallest polymer, kidney and bladder staining
- Micelle forming A96I96 – high liver signal

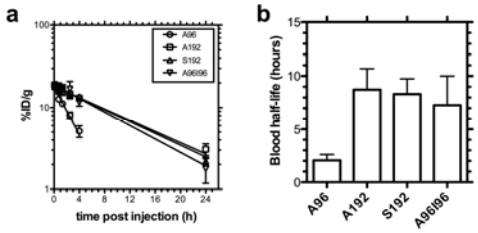
Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.




- ❖ discontinuous endothelium
  - increased permeability of vasculature
- ❖ leakage of lymphatic drainage

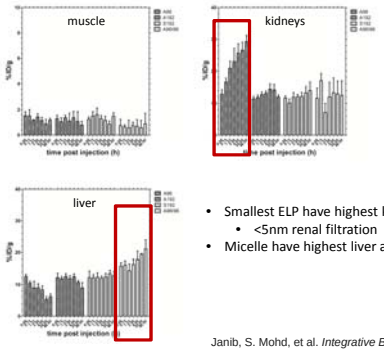
Stockhofe, Katharina, et al. *Pharmaceuticals* 7.4 (2014): 392-418.

## Heart ROI enables determination of biological half-life




Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.

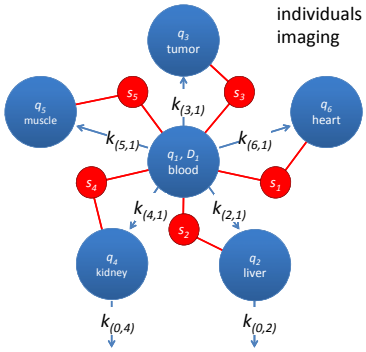
## Varying biodistribution profile was observed for different ELP construct




- Smallest ELP have highest kidney sequestration
  - <5nm renal filtration
- Micelle have highest liver accumulation

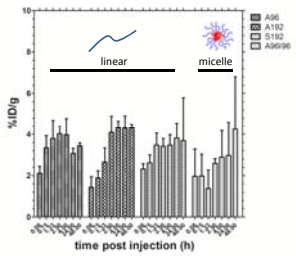
Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.

## Pharmacokinetic modeling in individuals based on microPET imaging




Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.

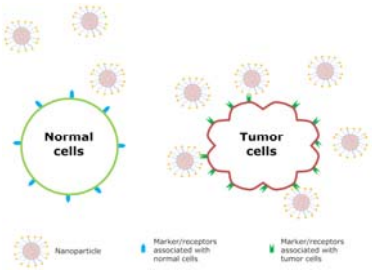
## No significant difference of tumor accumulation between constructs




- a steady increase in tumor uptake is observed
- tumor concentration 3-4 %ID/g

Janib, S. Mohd, et al. *Integrative Biology* 5.1 (2013): 183-194.

## Targeted peptide nanoparticles for increased efficacy

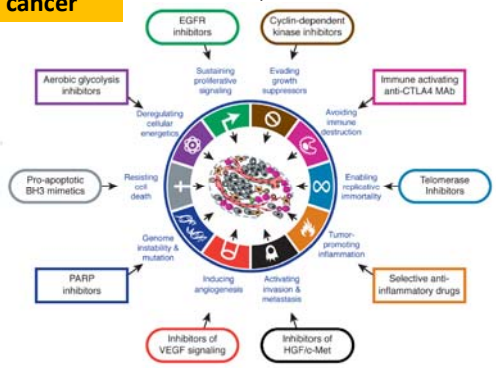



Choice of markers:
- ❖ Can differentiate between normal and tumor cell
  - Cancer processes
  - Upregulated in tumor progression

Why?
- ❖ Reducing toxicity to normal cells → ↓ side effects
- ❖ Better treatment outcome

## Hallmarks of cancer

Exploiting cancer specific processes improves chances of early detection and treatment




Hanahan & Weinberg. *Cell* 144.5 (2011): 646-674.

**TABLE 1**
Peptide Receptors, Disease Indications, and Radiopeptide Probes in Clinical Trials in Europe

| Peptide | Receptor | Disease indication | Radiopeptide probe |
|---|---|---|---|
| Somatostatin | sst2 | NETs; gastroenteropancreatic NETs | $^{111}$In-DTPA-octreotide* |
| | | | $^{111}$In-DOTA-lanreotide |
| | | | $^{111}$In-/$^{90}$Y-/$^{177}$Lu-/$^{68}$Ga-DOTATOC |
| | | | $^{177}$Lu-/$^{68}$Ga-DOTATATE |
| | | | $^{111}$In-DOTA-BASS† |
| | | | $^{99m}$Tc-HYNIC-TOC/-TATE |
| | | | $^{99m}$Tc-N$_4$-TATE |
| | | | $^{99m}$Tc-depreotide |
| | | | $^{18}$F-deoxyfructosyl-TATE |
| | sst2/sst3/sst5 | | $^{68}$Ga-DOTANOC |
| Bombesin | GRP receptor | Prostate cancer, breast cancer | $^{99m}$Tc-RP527 |
| | | Gastrointestinal stromal tumor | $^{68}$Ga-BZH3 |
| | | | $^{64}$Cu-CBC-AR06 |
| | | | $^{68}$Ga-/$^{177}$Lu-AMBA |
| Cholecystokinin/gastrin | Cholecystokinin 2 | Medullary thyroid cancer | $^{111}$In-DTPA-o-Glu-minigastrin |
| | | | $^{99m}$Tc-demogastrin 2 |
| RGD peptides | $\alpha_v\beta_3$ integrin | Various | $^{18}$F-galacto-RGD |
| | | | $^{18}$F-RGD-K5 |
| | | | $^{18}$F-AH111585 |
| Substance P | Neurokinin 1 | Glioblastoma | $^{111}$In-DOTA-substance P |
| | | | $^{111}$In-/$^{90}$Y-DOTAGA-substance P |
| GLP-1/exendin | GLP-1 receptor | Insulinomas | $^{111}$In-[Lys$^{40}$(Ahx-DTPA)NH$_2$]-exendin-4 |
| | | | $^{111}$In-[Lys$^{40}$(Ahx-DOTA)NH$_2$]-exendin-4 |

*Approved.
†First radiolabeled somatostatin-based antagonist in clinic.
BASS = pNO$_2$-Phe-cidCys-Tyr-dTrp-Lys-Thr-CysidTyrNH$_2$; CBC = cross-bridged cyclam (4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane); AMBA = DO3A-CH$_2$CO-Gly-(4-aminobenzoyl)-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$.

J Nucl Med 2011; 52:425–55S

## Exploiting cancer specific processes for early detection and treatment - Angiogenic process

- Formation of new blood vessels
  - To supply nutrients and oxygen for tumor growth
- Integrin
  - Implicated in the process of angiogenesis and tumor metastasis.
  - upregulated during angiogenesis but not on quiescent endothelial cells.
  - integrins including $\alpha v\beta_3$ recognize the tripeptide RGD on the ECM to facilitate adhesion.
  - RGD specific antagonists targeting integrins can induce apoptosis and inhibit angiogenesis.

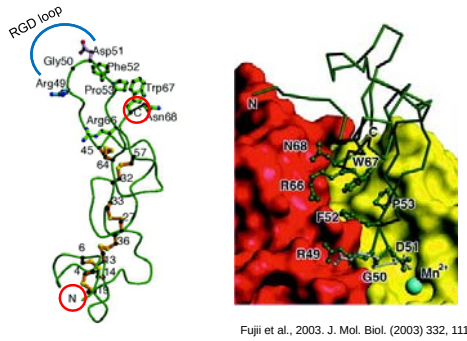
## Disintegrins

❖Disintegrins are peptides that are isolated from snake venom
- These peptides vary in size from 5000-14,000 Da.
- They are disulfide rich.
- Can be classified into several categories,
  - They are either mono- or dimeric.
  - Can either be RGD-containing or non-RGD containing.

❖Most studied – controstatin (homodimer) and echistatin (monomer).
- Have a high affinity for α5β1, αvβ3 and αvβ5.
- Through RGD sequence exert potent anti-angiogenic activity → stops tumor growth

Swenson, S., et al. *Curr. Pharm. Design.* **2007**, *13*, 2860-2871.

## Proper folding and RGD-loop display essential for activity




Fujii et al., 2003. J. Mol. Biol. (2003) 332, 1115–1122

## Improving therapeutic activity of VCN with ELP

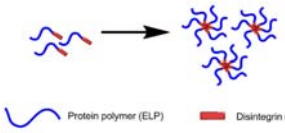
- Vicrostatin – a chimeric recombinant disintegrin
  - ❖ Fusion of the C-terminal tail of echistatin to the disintegrin contortrostatin.
- VCN (7kDa) is rapidly cleared.
  - – Limited biodistribution and therapeutic activity.
- Increase the molecular weight of compound by fusing ELP with VCN to alter its PK
  - – reduced renal clearance
  - – Improve EPR effect

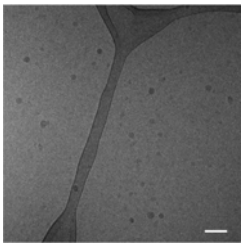
## Various ELP-VCN constructs generated for investigation

| Label | Amino acid sequence | MW (kDa) | Construct |
|---|---|---|---|
| S96-VCN | G(VPG**S**G)$_{96}$-VCN | 45.3 | N—S96—VCN—C |
| V96-VCN | G(VPG**V**G)$_{96}$-VCN | 46.6 | N—V96—VCN—C |
| A192-VCN | G(VPG**A**G)$_{192}$-VCN | 80.7 | N—A192—VCN—C |
| I96A96-VCN | G[VPG**I**G]$_{96}$[VPG**A**G]$_{96}$-VCN | 84.7 | N—I96—A96—VCN—C |

- ❖ MG[VPG**A**G]$_{192}$DAPANPCCDAATCKLTTGSQCADGLCCDQCKFMKEGTVCRRARGDDLDD YCNGISAGCPRNPHKGPATYstopstop
  - Express in Origami B cells
  - Yield = 20-30mg/L
  - Molar extinction coefficient = 3760
- ❖ MG[VPGAG]$_{192}$YRGDGG was also generated

## Disintegrin drives nanoparticle assembly



A192-VCN multimers have spherical morphology. CryoTEM of A192-VCN and with radii of ~15 nm with a relatively narrow distribution of morphologies

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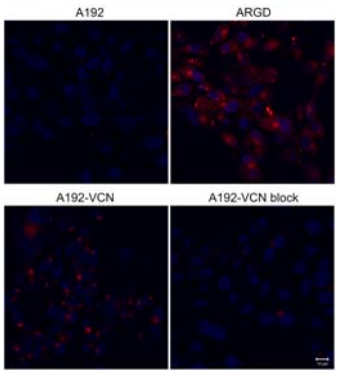


ELP with RGD-containing sequence taken up by breast cancer cell line

- Little to no association with A192
- Reduction in signal intensity observed after blocking with VCN

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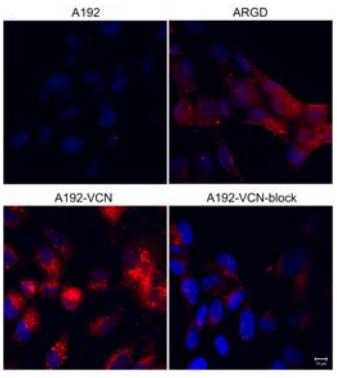


ELP with RGD-containing sequence taken up by HUVECs

- Little to no association with A192
- Reduction in signal intensity observed after blocking with VCN

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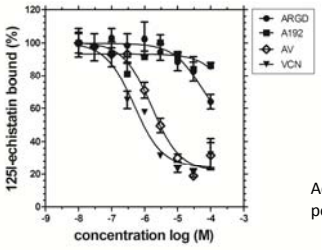
## *In vitro* binding assay showed varied affinities and specificities of peptides




| Peptide | IC 50 (μM) |
|---|---|
| VCN | 0.48 |
| A192-VCN | 1.64 |
| ARGD | 51.82 |
| A192 | ND |

Adding high MW carrier reduces potency of VCN!!

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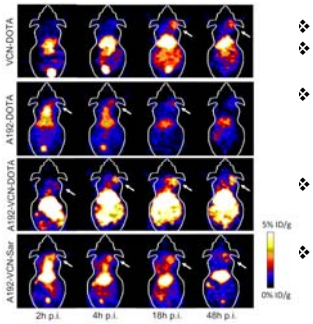
## A192-VCN actively targets and accumulates in the tumor




- Circulating nanoparticles
- Liver uptake is apparent due to sequestration by the RES.
- Tumor sequestration by EPR for A192-DOTA. Slight tumor accumulation was observed with vicrostatin alone.
- Significant liver and intestinal uptake of A192-VCN-DOTA due to instability of DOTA chelator.
- Improvement in image and signal was obtained with Sar.

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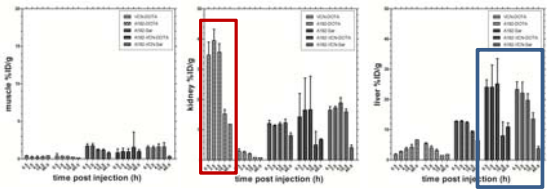
## Protein polymer modification of VCN switches route of clearance from kidneys to liver




- Minimal muscle accumulation
- VCN exhibited highest kidney uptake and cleared.
- Liver accumulation for the fusion protein is more prominent

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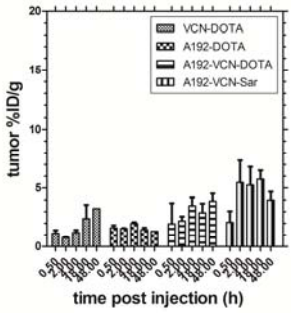
## ELP-VCN-Sar exhibited higher tumor uptake compared to other constructs




- Minimal tumor signal is observed for VCN
- Accumulation via the EPR effect was seen with A192-DOTA.
- AV-DOTA and AV-Sar steady increase in accumulation over time

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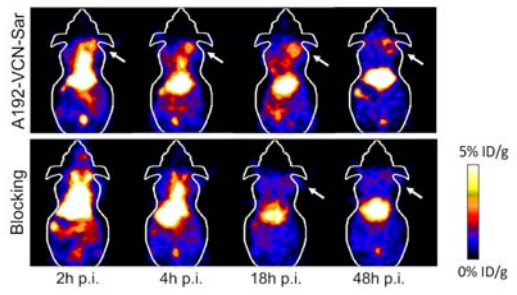
## A192-VCN uptake can be saturated, suggesting receptor mediated uptake

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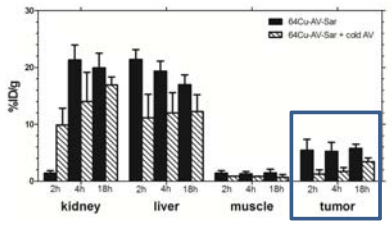
## Blocking reduces tumor uptake of A192-VCN




- Tracer uptake in the tumor was reduced in the presence of cold, unlabeled A192-VCN.
- The uptake of radiolabeled fusion protein in other organs was also lower.

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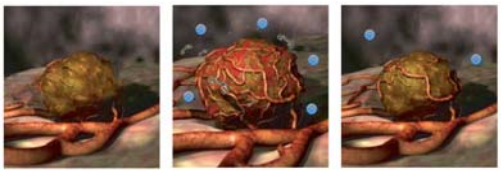
## Targeting markers in the angiogenic process allows for early detection

**Early detection** **Treatment**



Tumor needs new blood vessels to supply it with oxygen, nutrients for survival

Starts giving out signals for blood vessels to grow towards it

Nanoparticles sensitive towards this process preferentially accumulates at the site

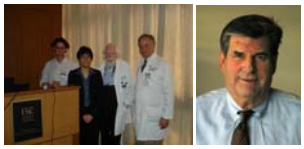
If the nanoparticles are carrying drugs can 'starve' the tumor leading to its tumor shrinkage

Figures adapted from Genentech

## Conclusion

- Tunable property of ELP makes it easy to tailor a peptide with the desirable characteristic
- Used image-guided biodistribution studies to aid in the determination of the ideal protein characteristics with the desired PK profile
- Developed a targeted fusion protein strategy that is specific and selective, has improved pharmacokinetics and the ability to monitor tumor-targeting efficacy.

**Mentor and Committee Members**



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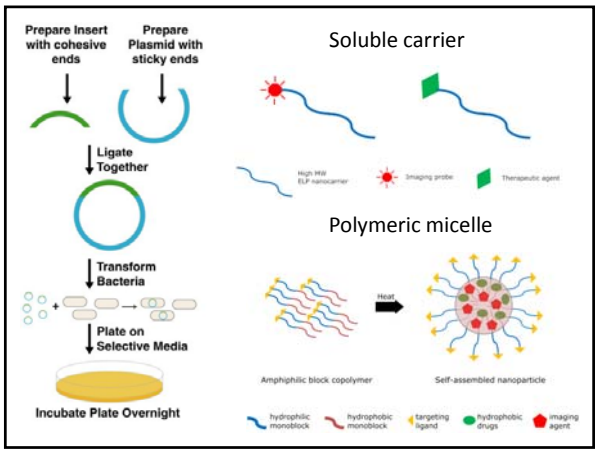
- USC Whittier Foundation


## The problem with cancer

- Selectivity
  - Selectively treating cancer cells, while avoiding toxicity normal cells

- Early detection
  - Treating cancer early, to increase the chances of success.


Prepare Insert with cohesive ends
Prepare Plasmid with sticky ends
Ligate Together
Transform Bacteria
Plate on Selective Media
Incubate Plate Overnight
Soluble carrier
Polymeric micelle
Heat
Amphiphilic block copolymer
Self-assembled nanoparticle