Identification of a collagen-binding peptide for nano theranostics modification applied on enhanced imaging and regeneration of osteoarthritic articular cartilage

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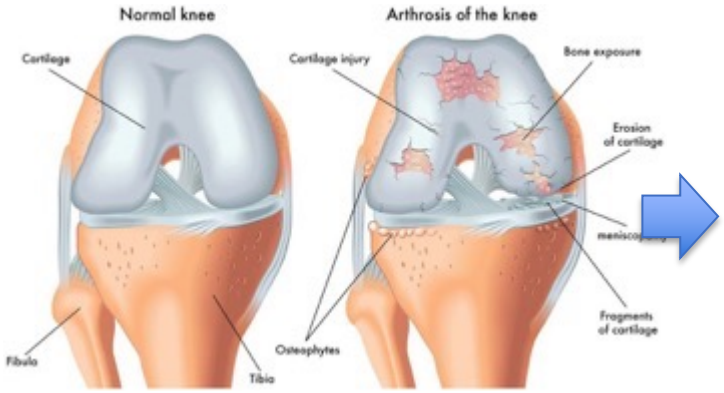
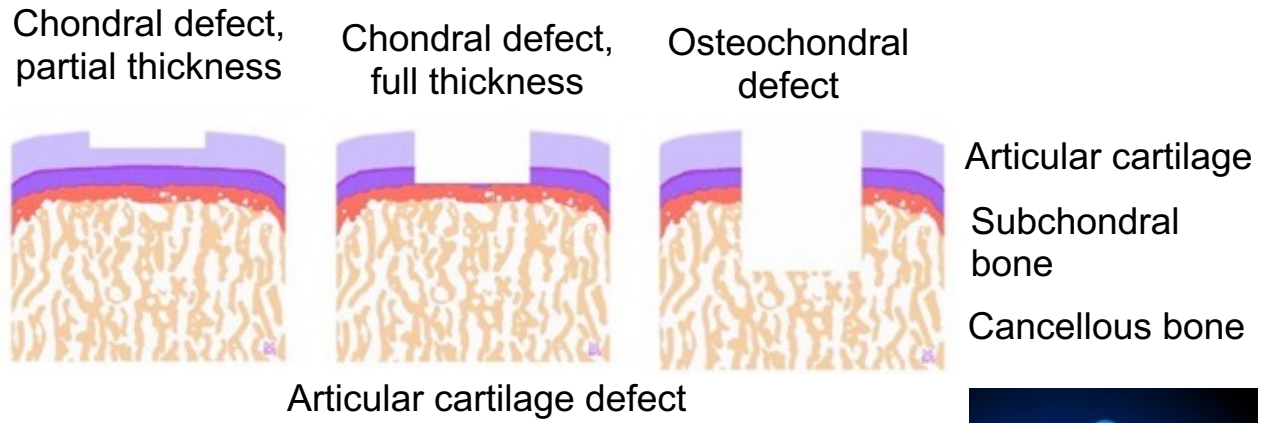
INTEGRATING
Delivery Science
ACROSS DISCIPLINES

Osteoarthritis (OA)

- Unmet medical need—the initial stage of OA without any pain or uncomfortable feeling, and difficult to detect from imaging examination. Currently, no disease modified OA drug, (DMOAD)

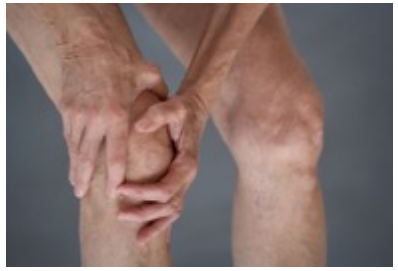




Articular cartilage defect

➢ Significance:
- Smooth and intact surface
- No self-healing capability at post-trauma

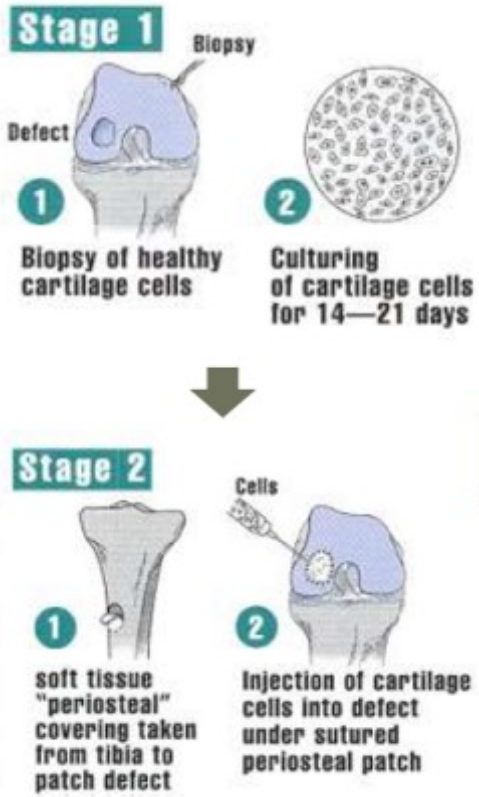
➢ Symptoms:
- OA, combined with chronic inflammation, and disability
  - Pain
  - Locking

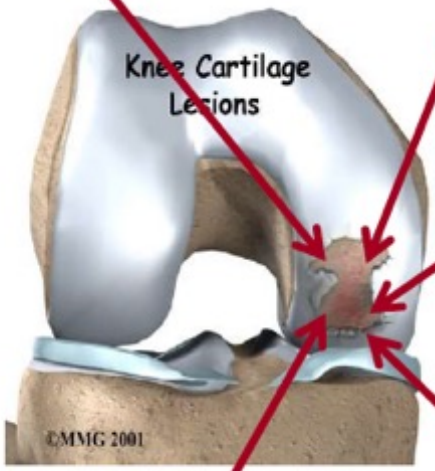


Alternative therapy

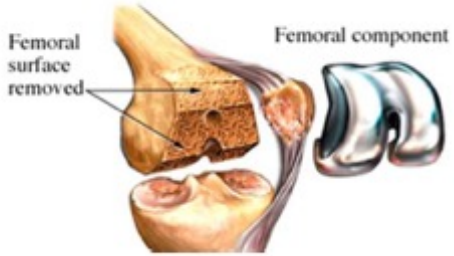
Autologous Chondrocyte Implantation (自體細胞移植)

Carticel/MACI (Vericel, US &EU)
ChondroCelect (TiGenix, DE)



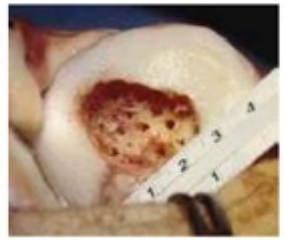


Total Knee Replacement



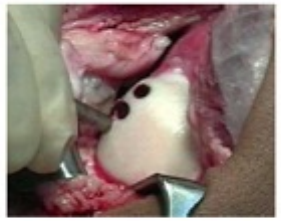
Marrow Stimulation

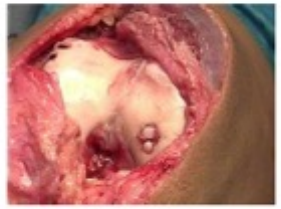
Microfracture



Osteochondral Grafting

Mosaicplasty





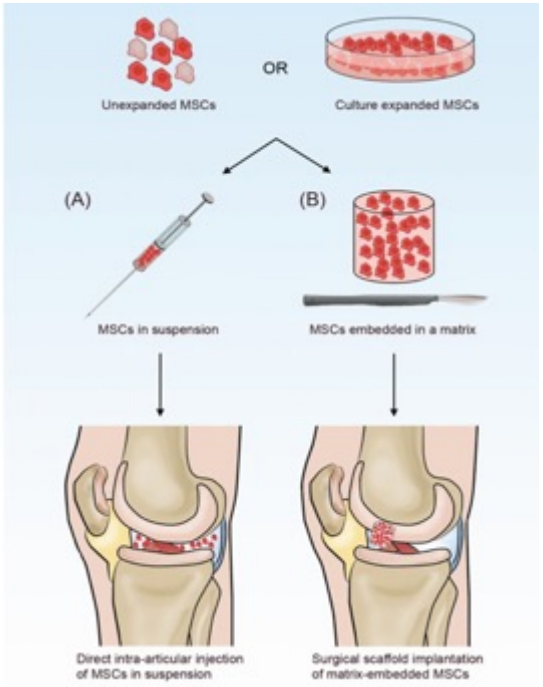
Allograft Cartilage (異體移植)

DeNovo NT (Zimmer, US)
BioCartilage (Arthrex, US)

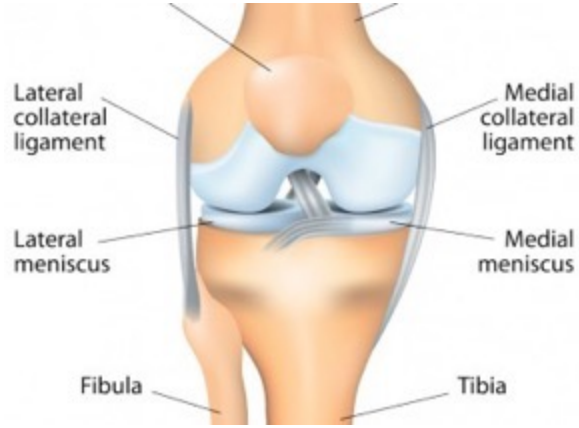
Current situation

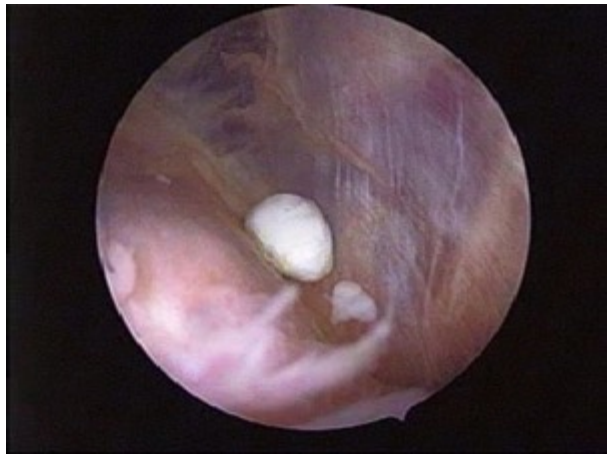
How will the outcomes of the study resolve the issues?

MSC for OA treatment (intra-articular injection)



- Resides in the gutter not in the articular joint surface
- Clinical trial suggests up to 10^8 cells per injection has effect in increasing joint space



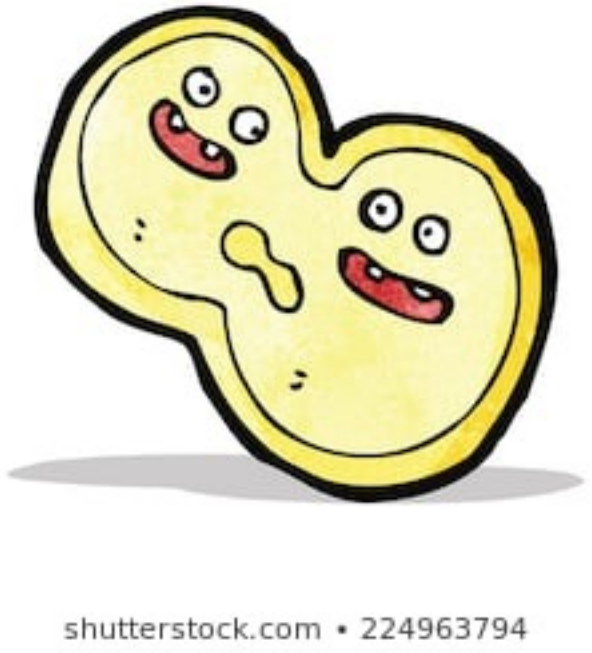


Will the study resolve underlying mechanism of the biological problem?

The key products and technology background.

Fundamental problem Target...

Targeting delivery (materials, drug and cells)

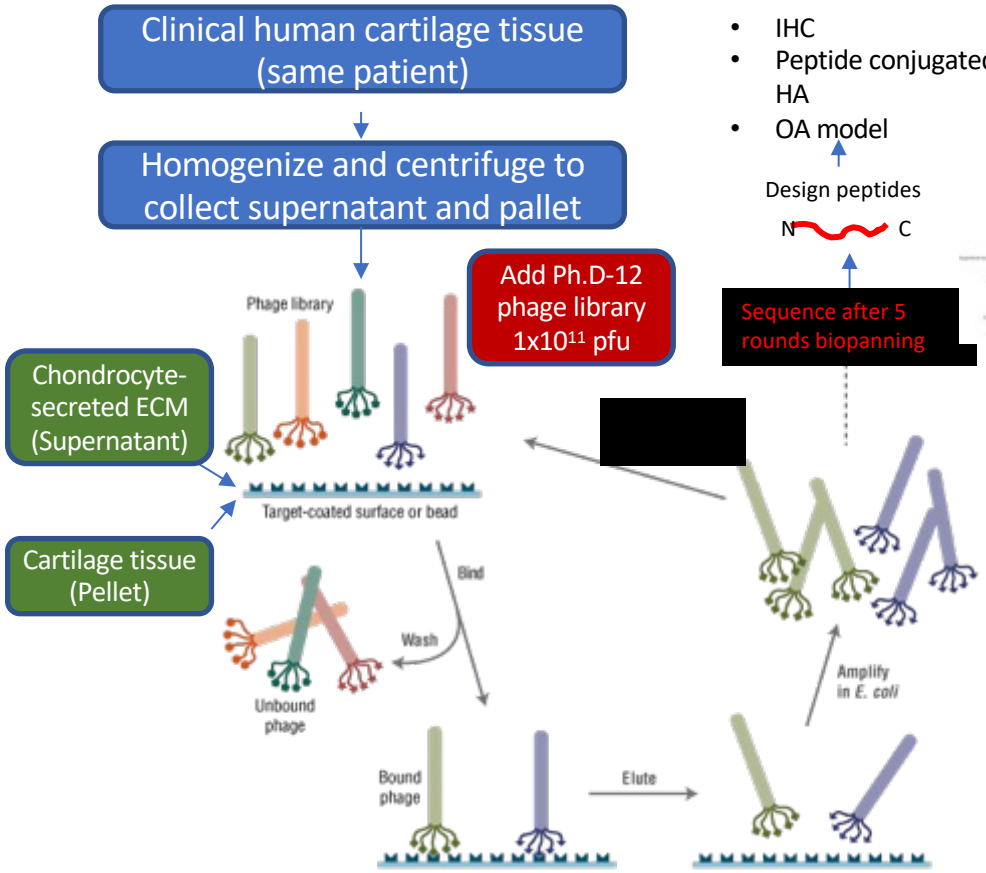


Functional cells precisely home to the disease target

Identification of OA-targeting peptides

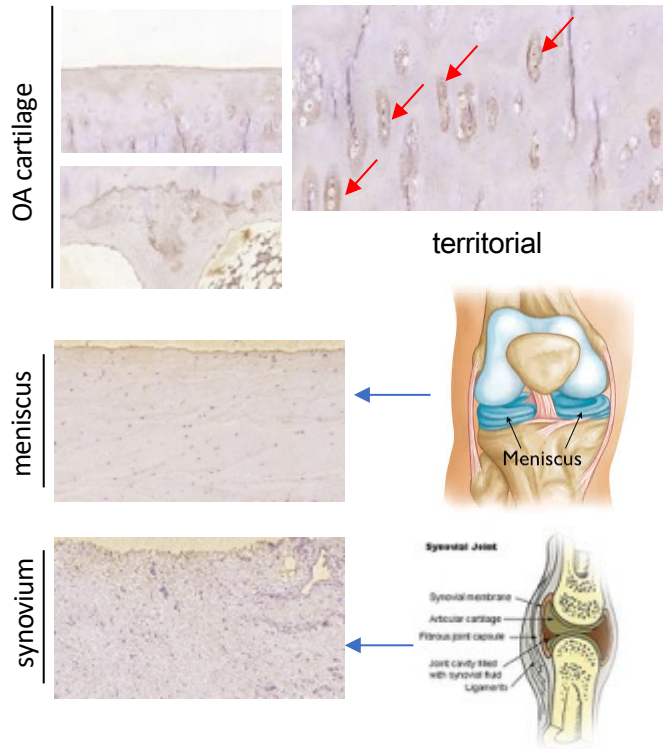
Technology development

Explore the OA-targeting peptide



IHC analysis in the binding activities of selected phage clone to human OA cartilage, synovium and meniscus section

C5-24 peptide



Analyze the specificity

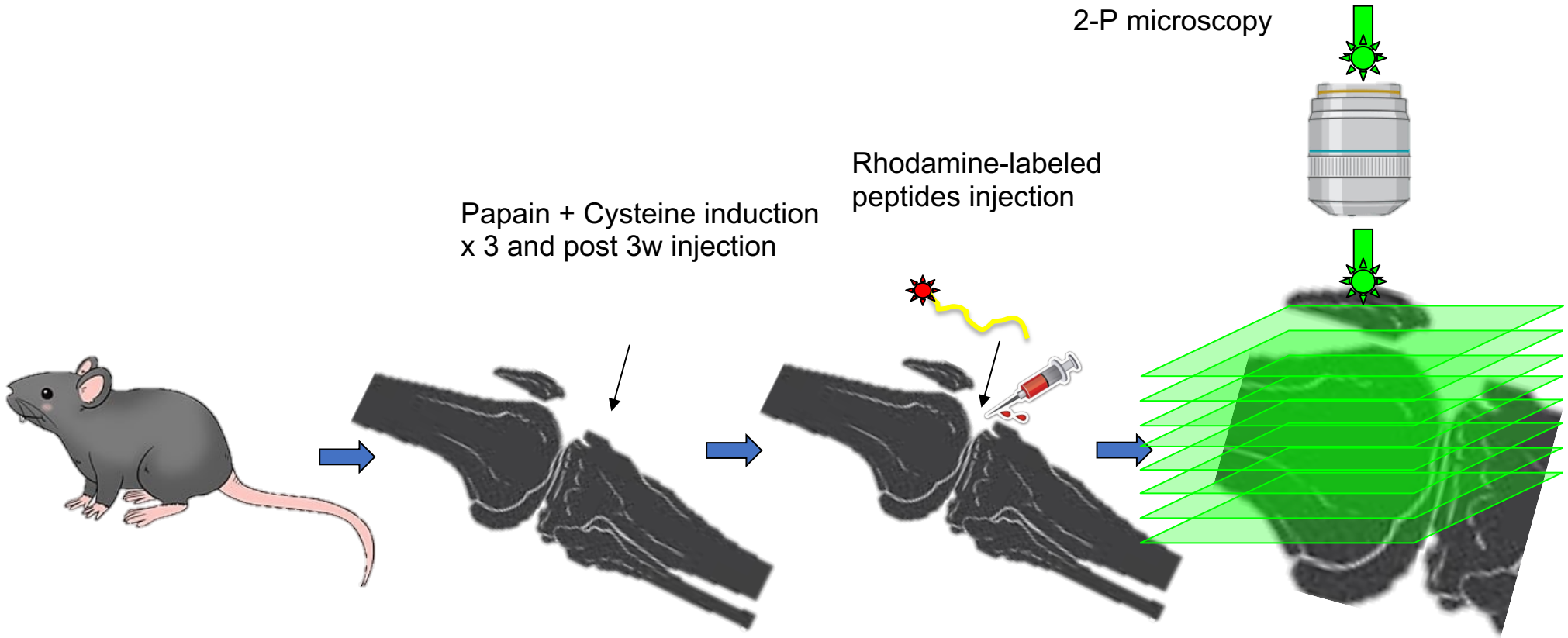
- IHC analysis in the binding specificity

	Help phage	C5-87	C5-66	C5-83	C5-91	C5-24	E5-8	C5-46
territorial				+	++	+++		
Inter-territorial		+++	+++	++	+	+	+++	+++
Tidemark		+++	+++	++	+	+	+++	+++
Calcified cartilage		+++	+++	++	+	+	+++	+++
trabecula		+++	+++	++	+	+	+++	+++
meniscus		+++	+++	++			+++	+++
synovium		++	++	+			+++	++

Intravital imaging of OA targeting

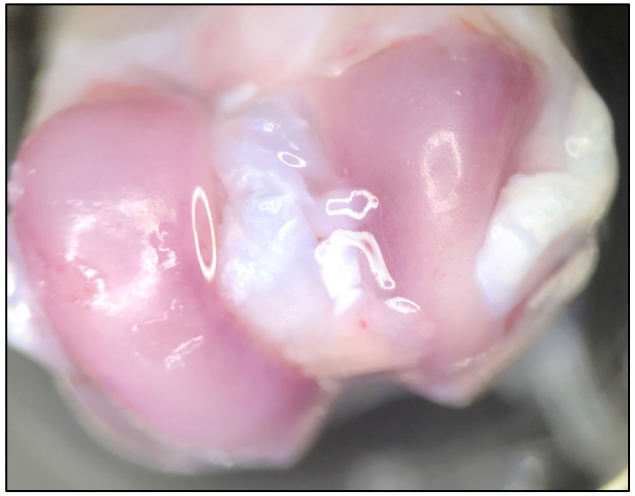
Intravital microscopic observation

To observe the peptides binding in the OA joint in vivo, an OA joint model was induced by enzyme injection in rats, followed by rhodamine-labeled C5-24 peptides injection and 2-P microscopic observation.

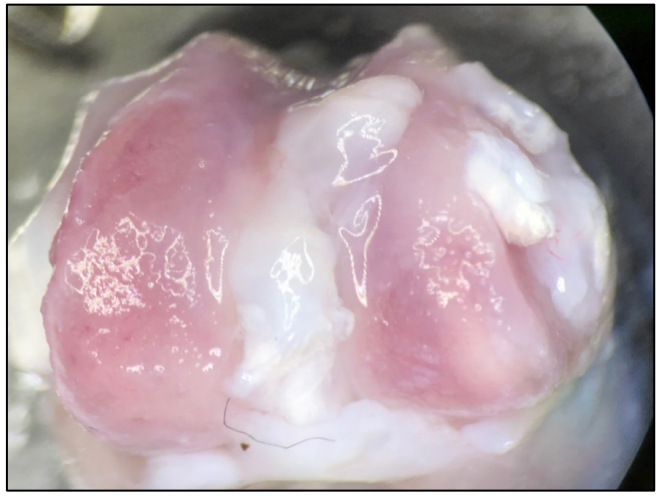


Gross picture of peptide injection in joint

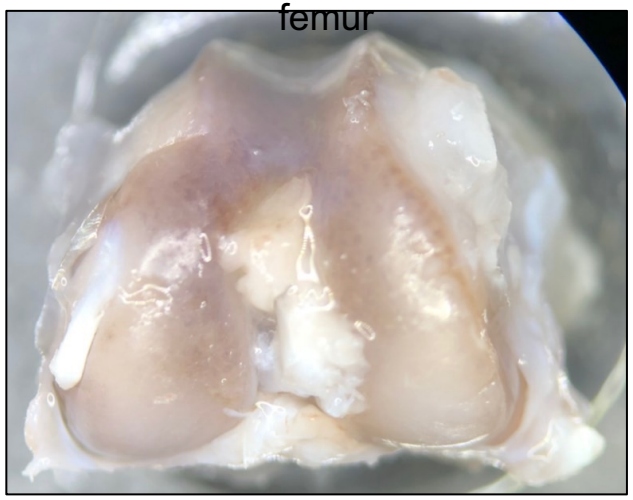
C5-24 peptide inj. control femur



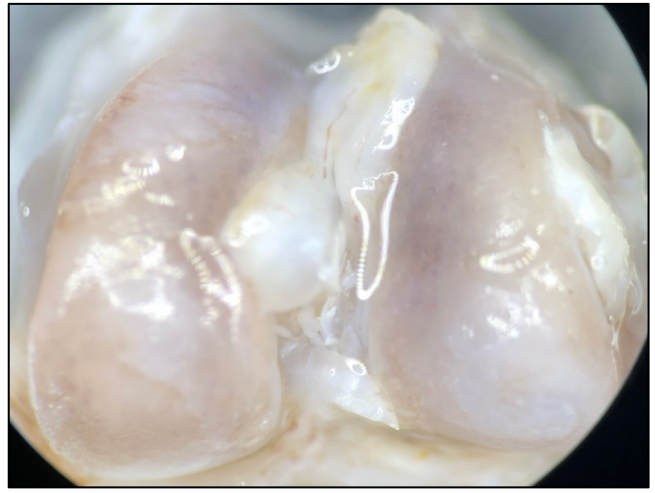
C5-24 peptide inj. OA femur



Scramble peptide inj. control femur

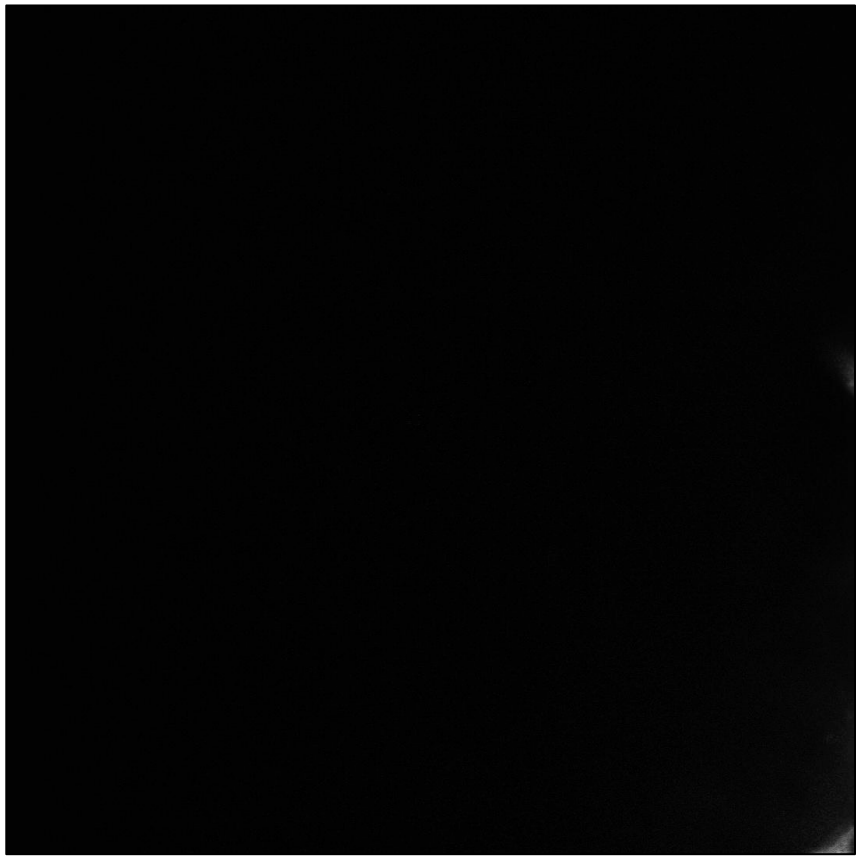


Scramble peptide inj. OA femur



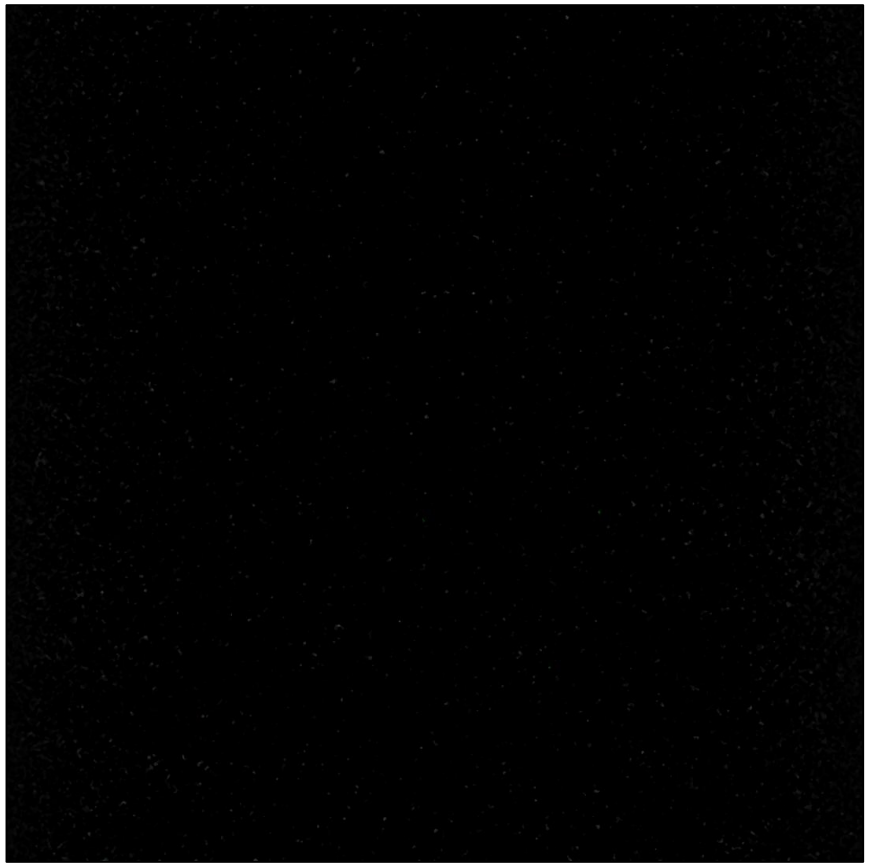
Intravital microscopy of C5-24 peptide injection

C5-24 peptide inj. control femur



SHG shows intact collagen II inter-structure.

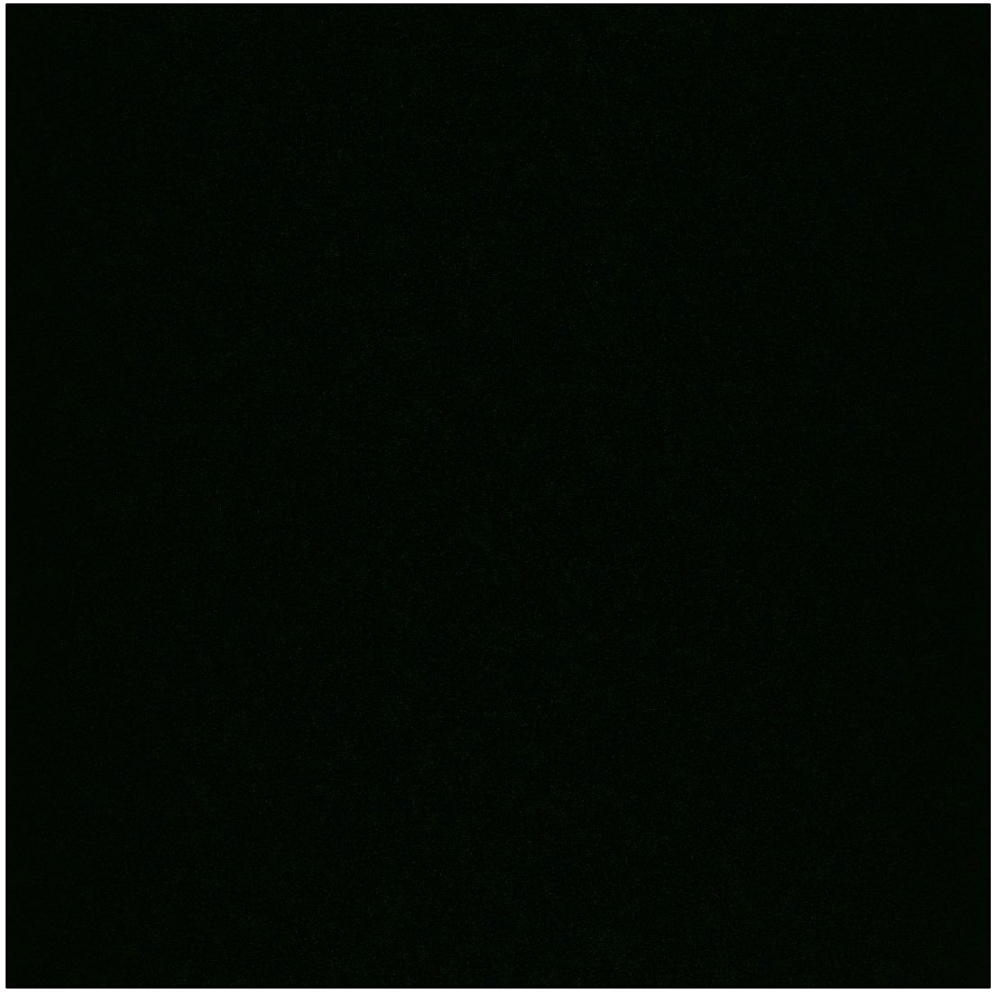
C5-24 peptide inj. OA femur



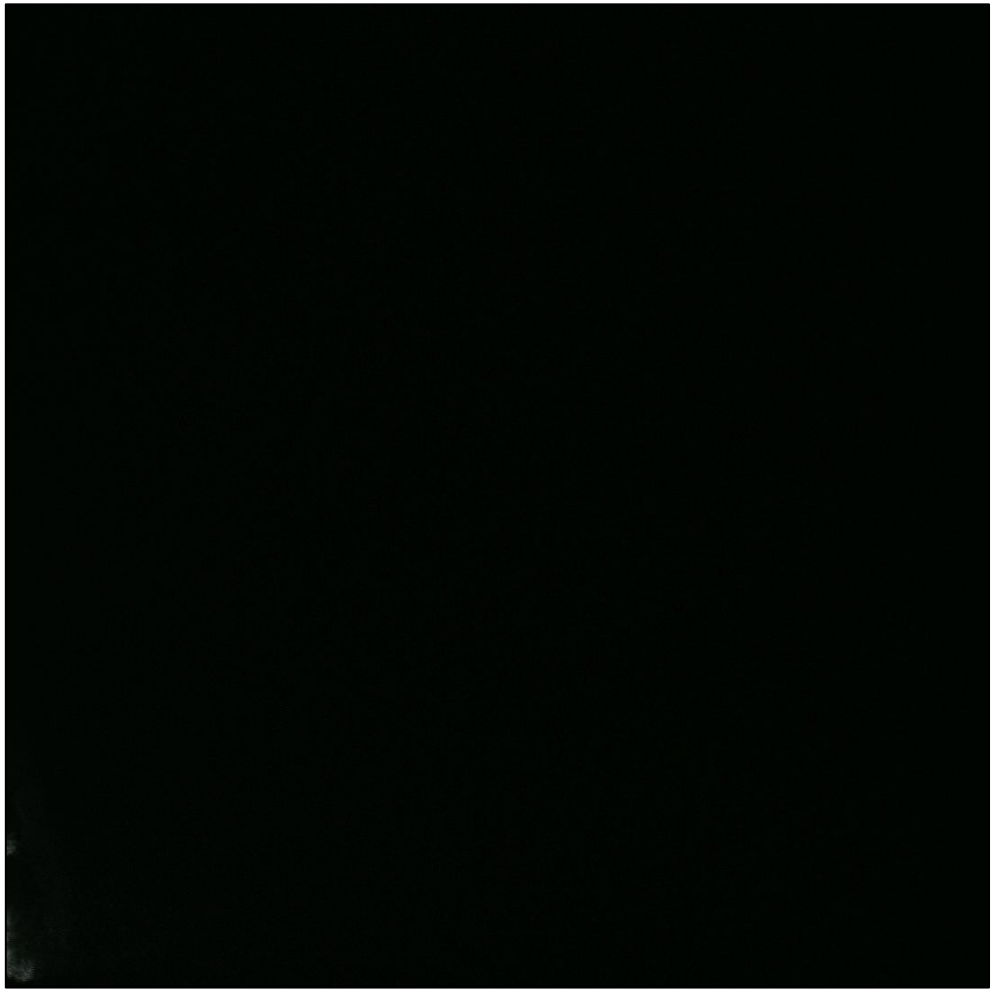
SHG shows disrupted collagen II inter-structure.

Intravital microscopy of scramble-peptide injection

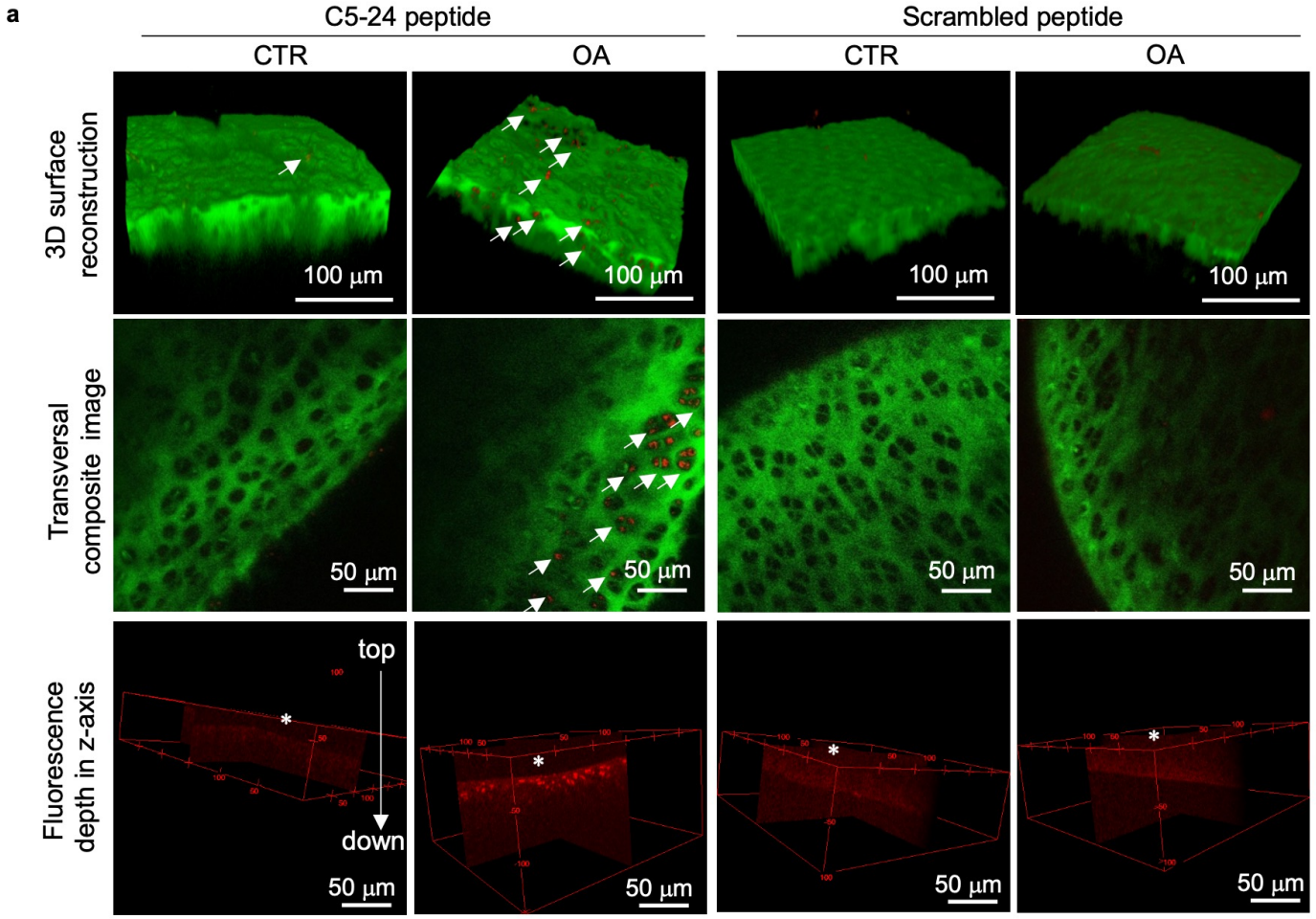
Scr-peptide inj_control joint femur



Scr-peptide inj_OA joint femur



Intravital imaging demonstrates the binding capability of C5-24 peptides to OA cartilage



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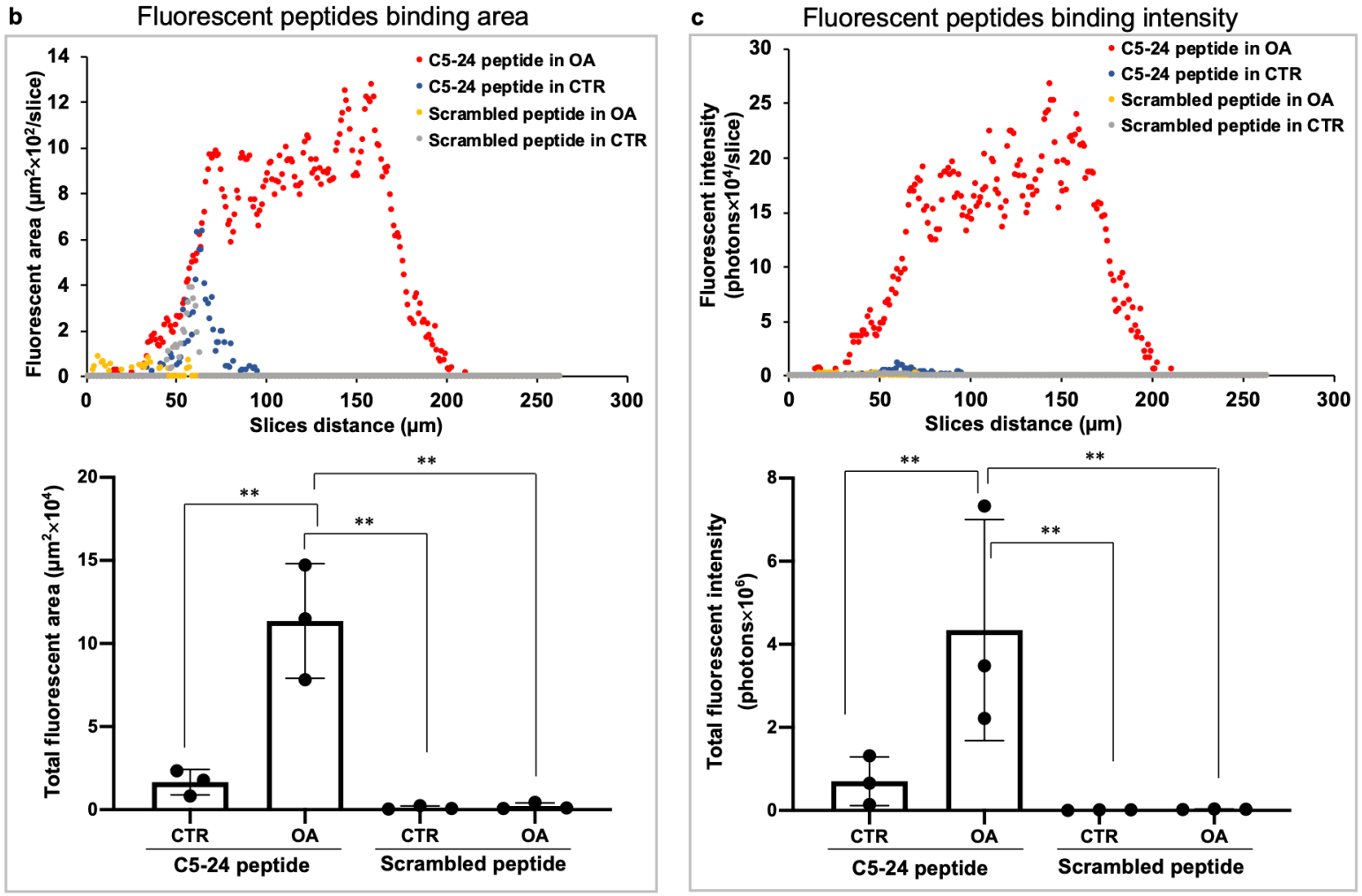
Overall fluorescent peptide binding intensity in each slice (upper panel) and all groups (lower panel)



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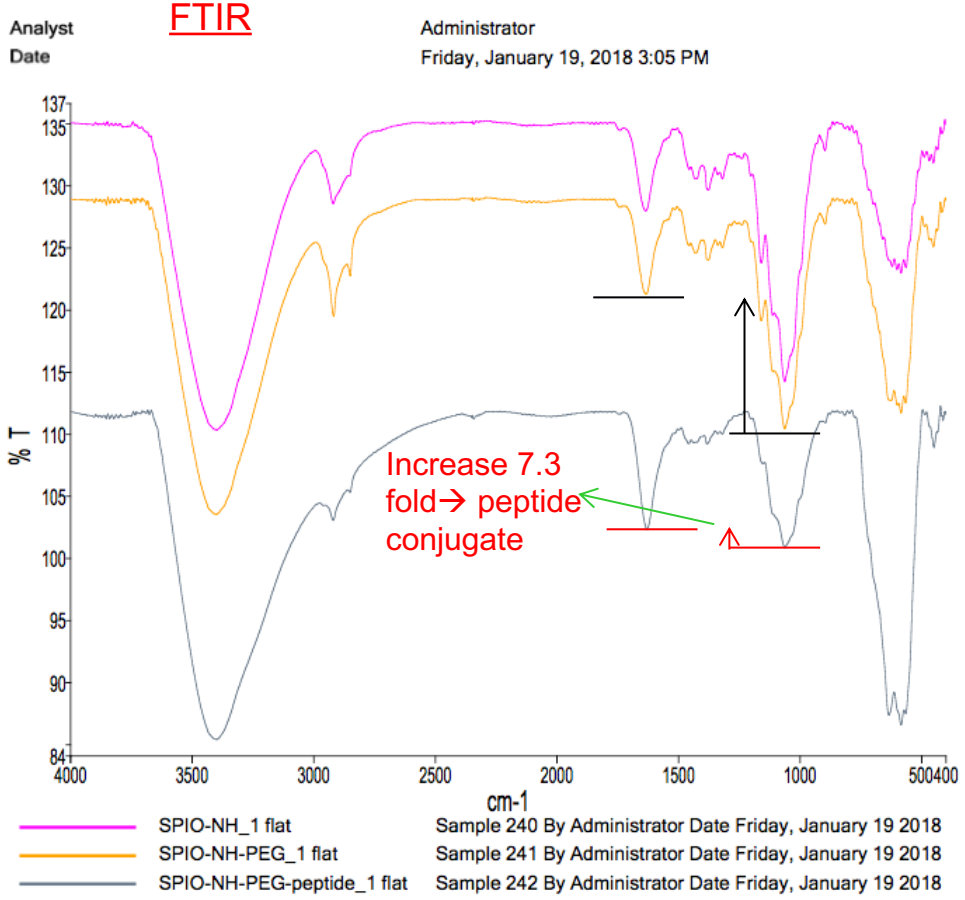
Application in early OA diagnosis

OA-SPIO

- Superparamagnetic iron oxide (SPIO) surface modified with C5-24 peptide
- Micro-injected in the OA joint in rat model

FTIR



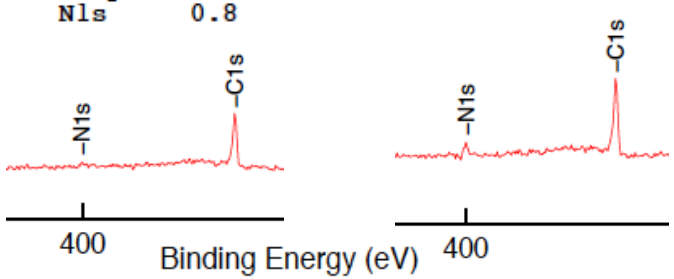
XPS (X-ray photoelectron spectroscopy)

SPIO

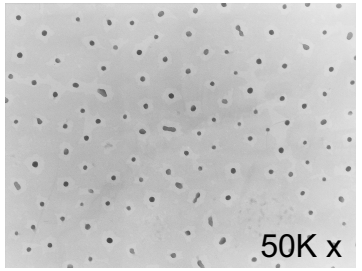
Atomic %	
Ols	58.2
Cls	22.9
Fe2p3	18.1
Nls	0.8

OA-SPIO

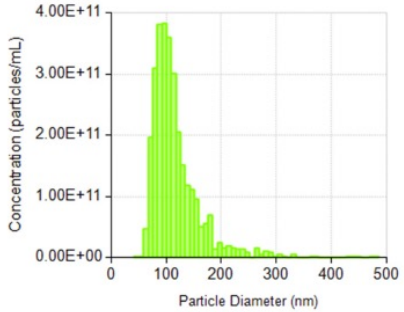
Atomic %	
Ols	57.5
Cls	26.7
Fe2p3	13.3
Nls	2.6



EM



Sizer scattering



➢ OA-targeted peptide conjugated SPIO, mean 118.2 nm diameter, average current:92.45 nA

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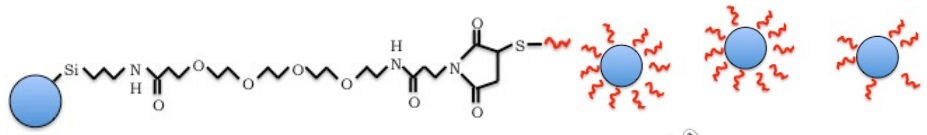
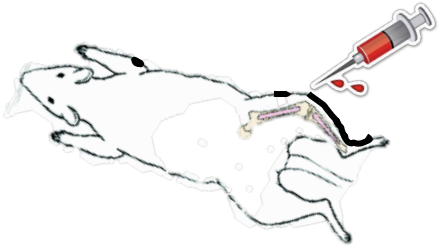
Application of C5-24 peptides in early OA diagnosis

MRI detecting OA through C5-24 peptide conjugated SPIO particle

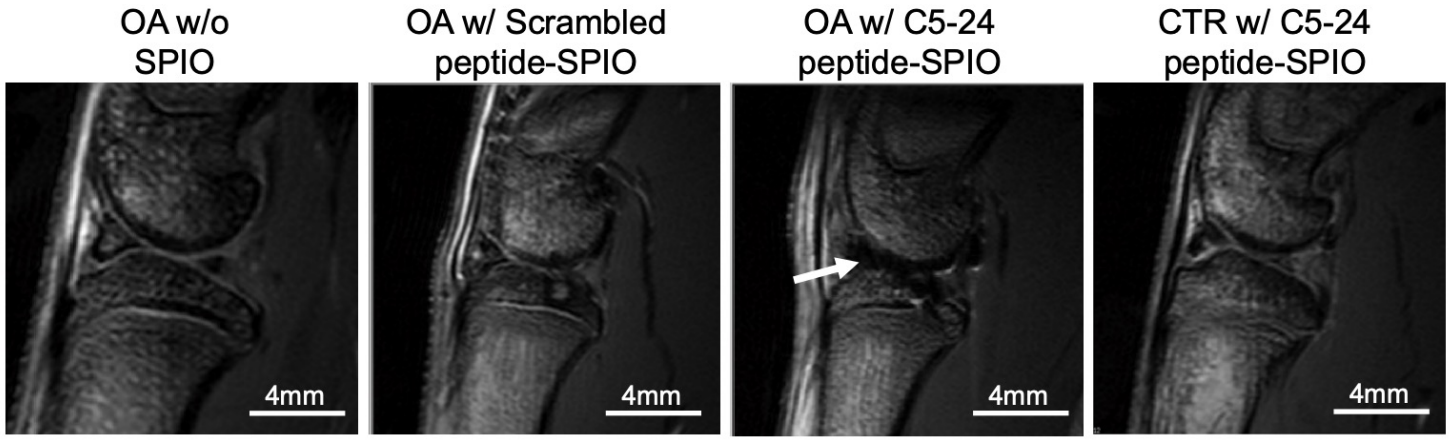


Papain and cysteine induced mild OA (intra-articular inj.)



MRI

SD Rat OA model



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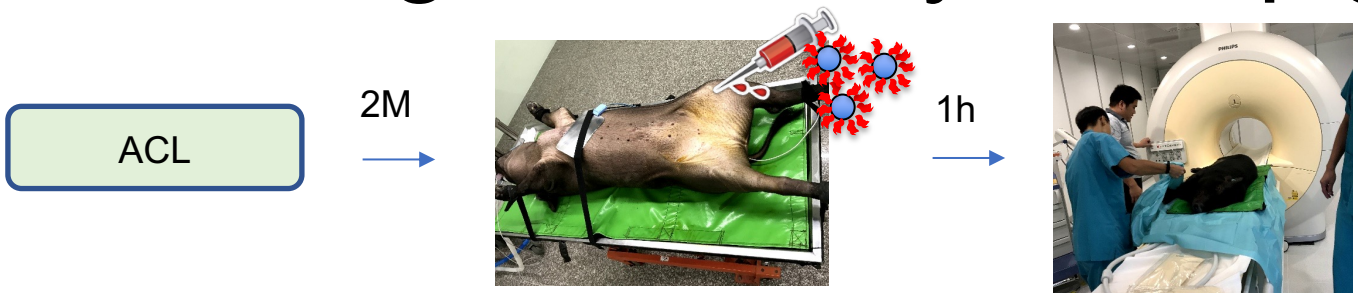
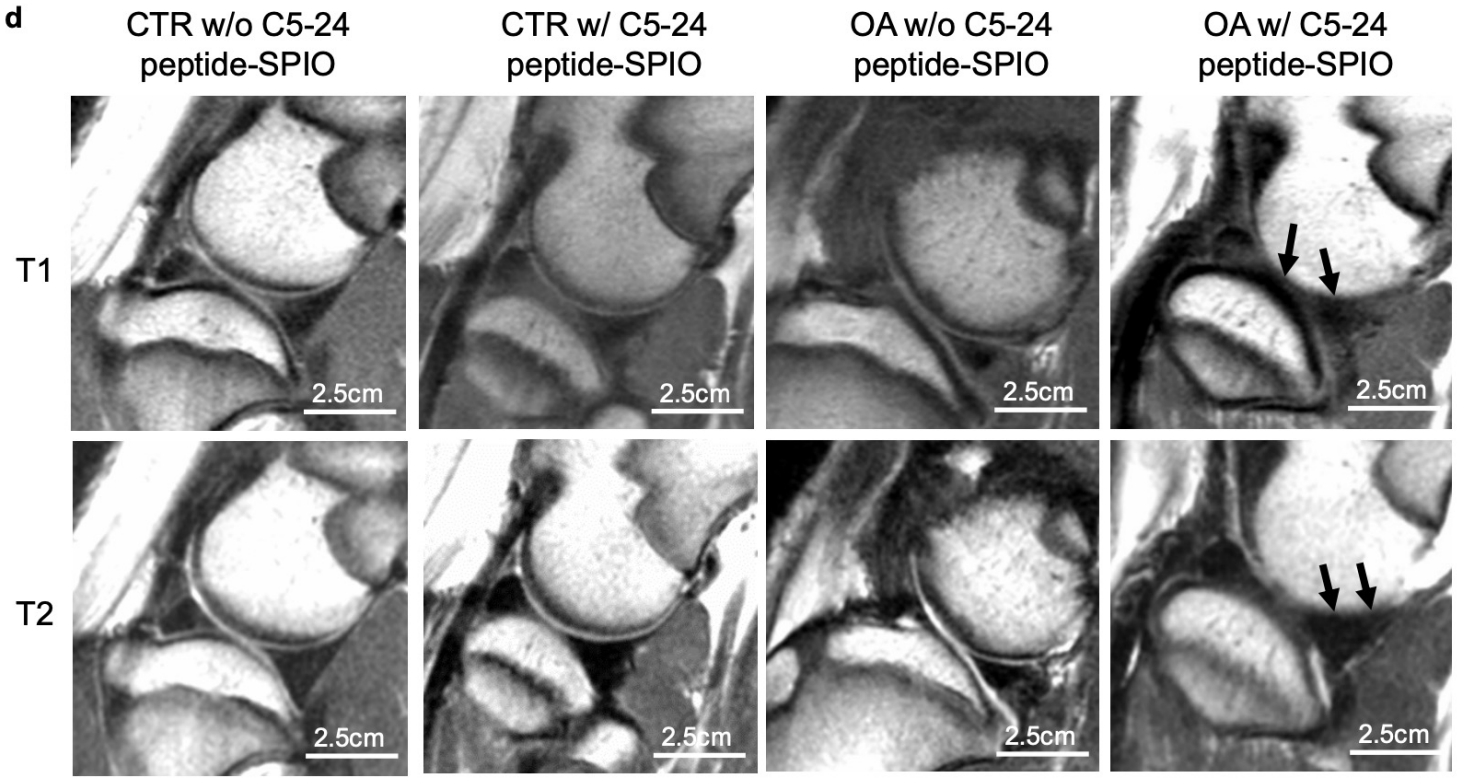
MR images of SPIO-conjugated C5-24 peptides bound to OA cartilage in a Lanyu minipig model

Lan-Yu pig ACL OA





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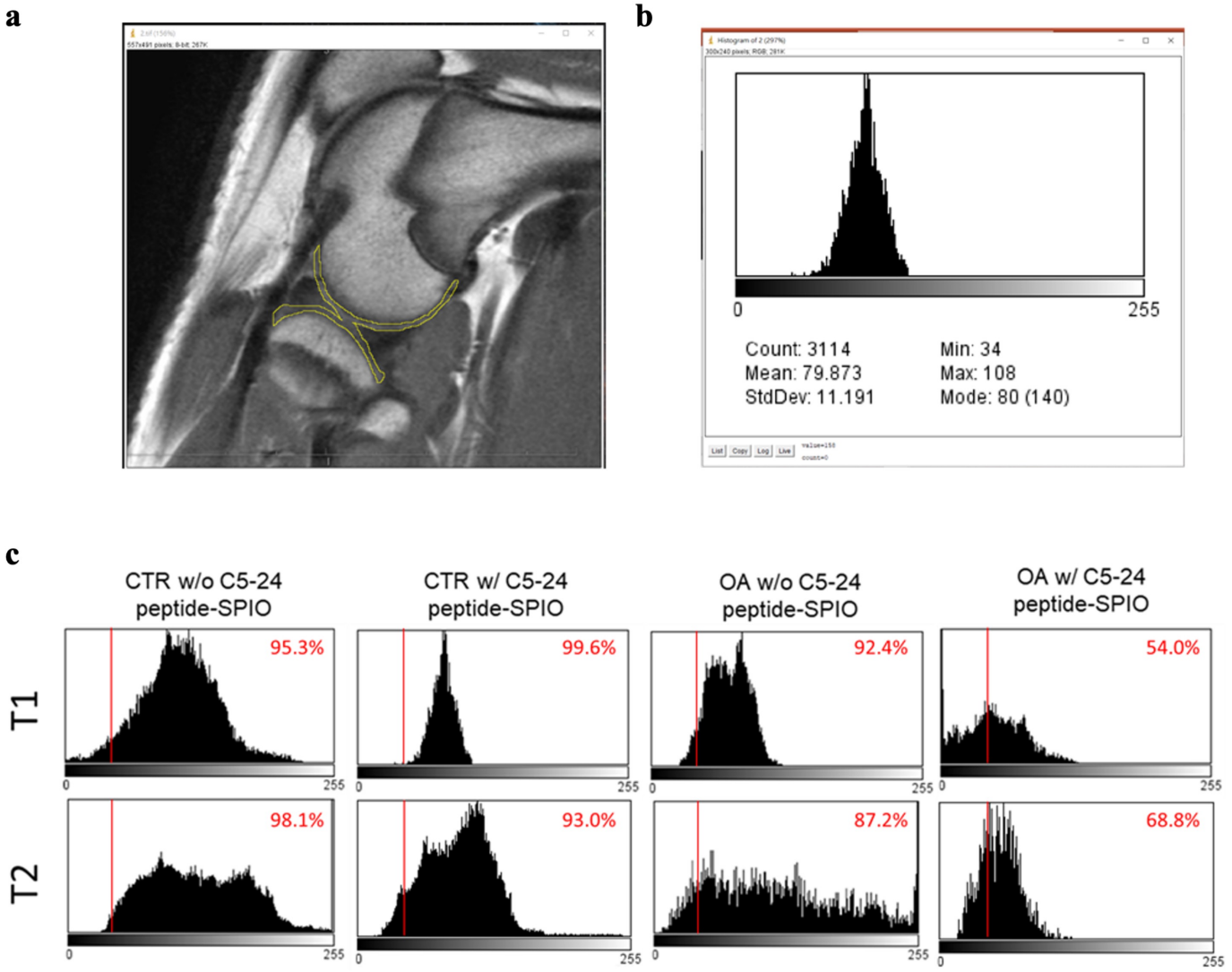
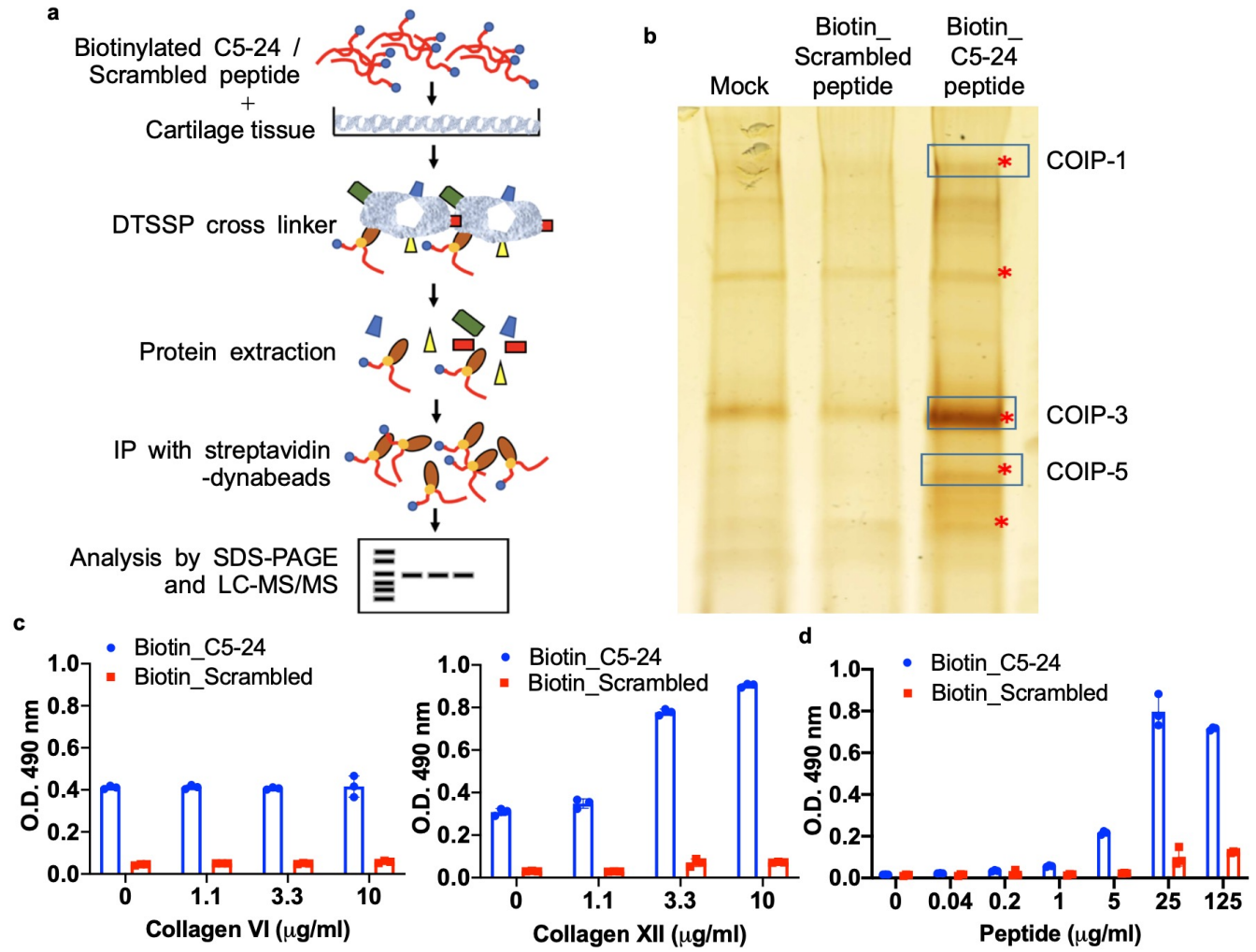
Quantification of Pig MR imaging



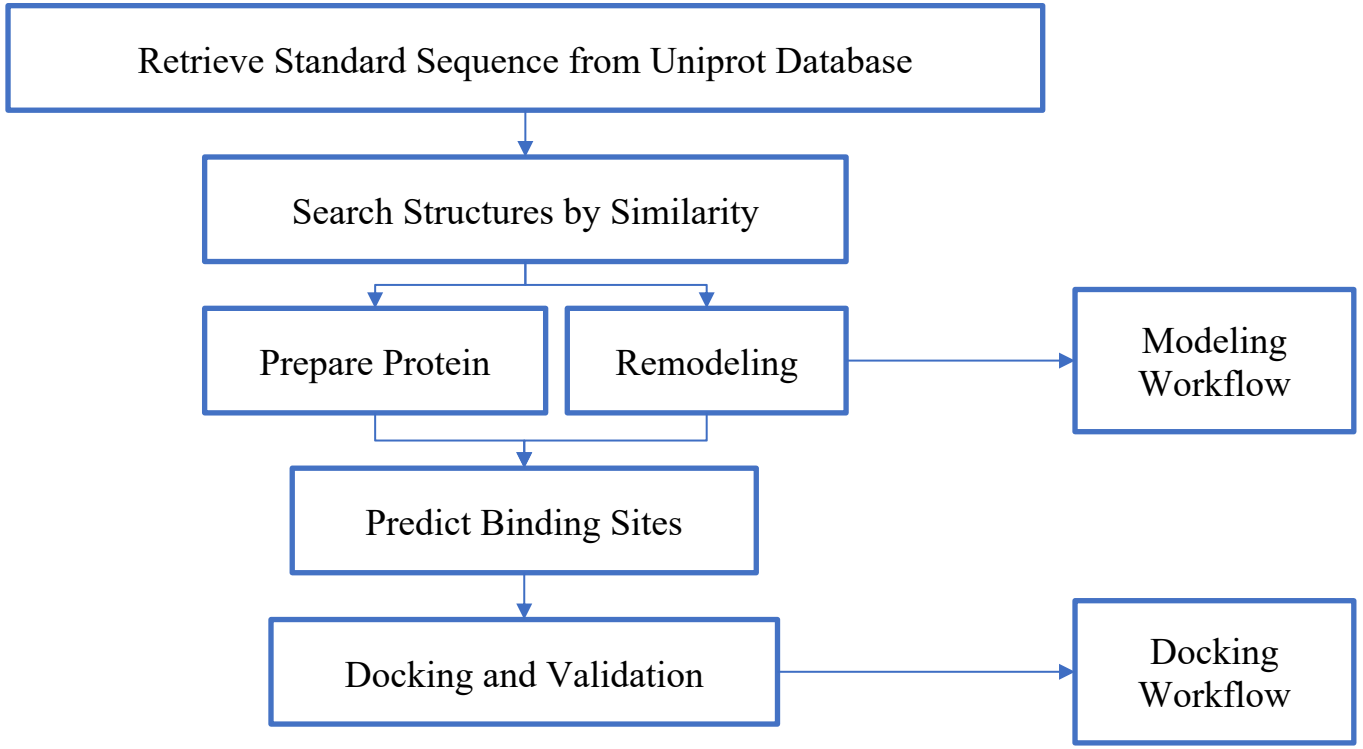
T1- and T2-weight gray-scale images, the cut-off value was set to 45 in the 0-255 gray-scaled value

Identification of binding proteins

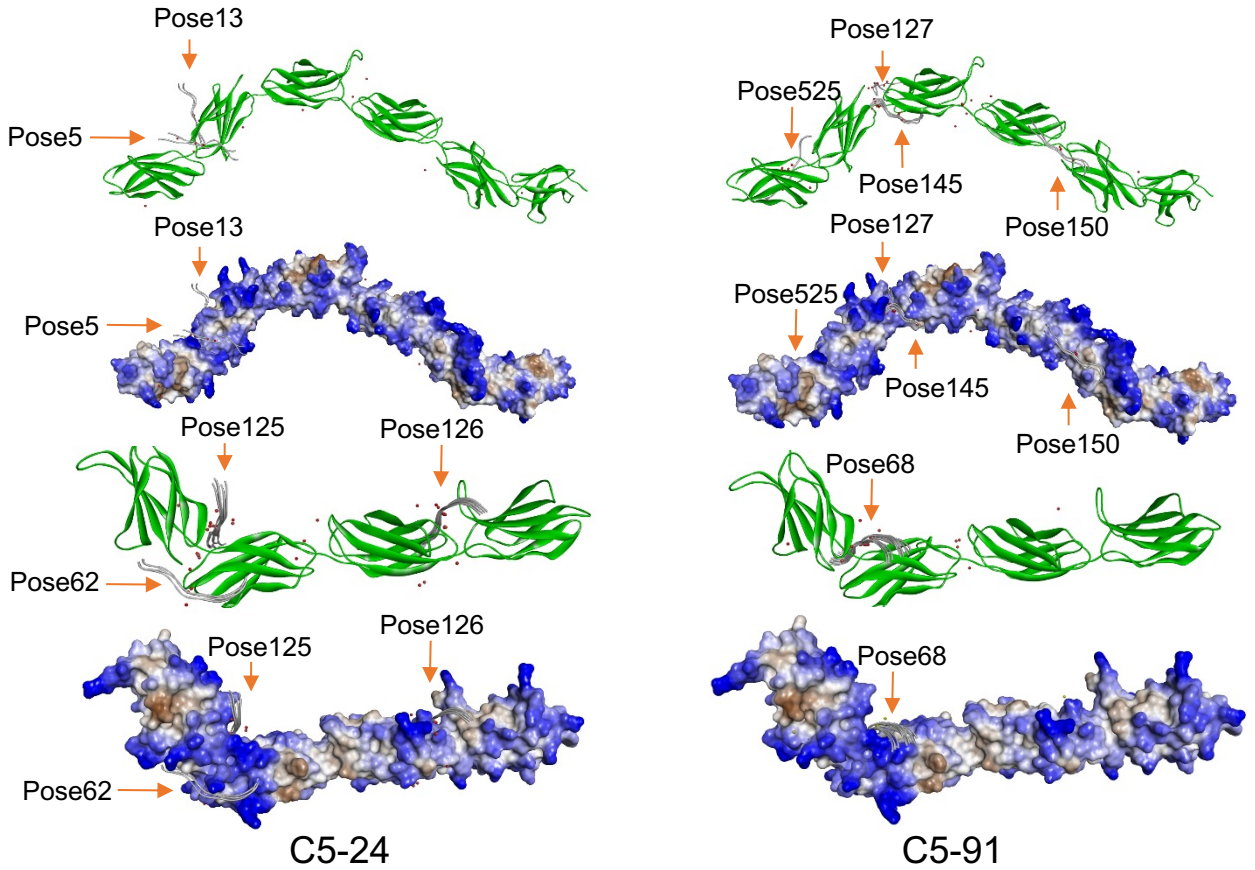
Identification of the binding protein of C5-24 peptides



Workflow to validate the peptide docking with the OA ECM protein



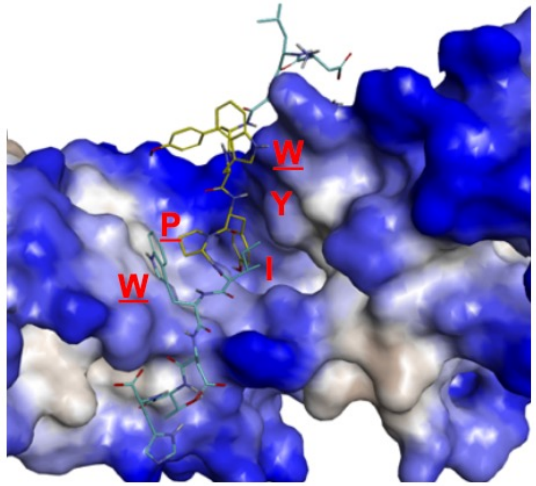
Peptide docking to the particular OA ECM protein



- Identify potential docking poses with solid interaction bonding eg. H-, Van der Waals forces, hydrophobic rx….etc. through 2-D and surface modeling.

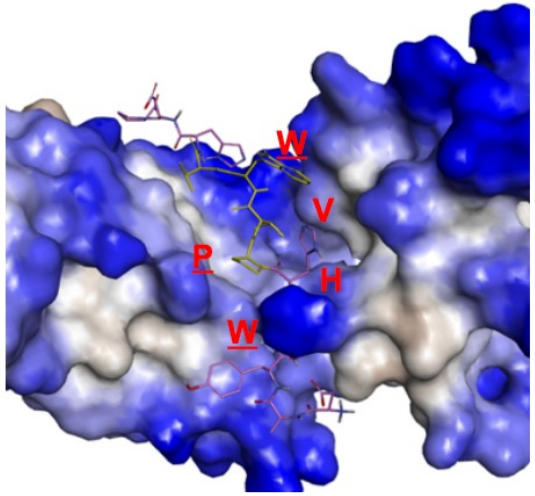
Interactions of peptides with OA ECM

C5-24 docking L1385-S2285 pose 125
DLQYWYPIWDTH



C5-24 Pose 125

C5-91 docking L1385-S2285 pose 68
DAYWHPVWVHDP



C5-91 Pose 68

	ZDockScore	E_RDock
C5-24 Pose 125	14.1	-11.9716
C5-91 Pose 68	16.16	-26.3188

- Peptide docking model showed particular interaction a.a. in peptide sequence → WX_1PX_2W

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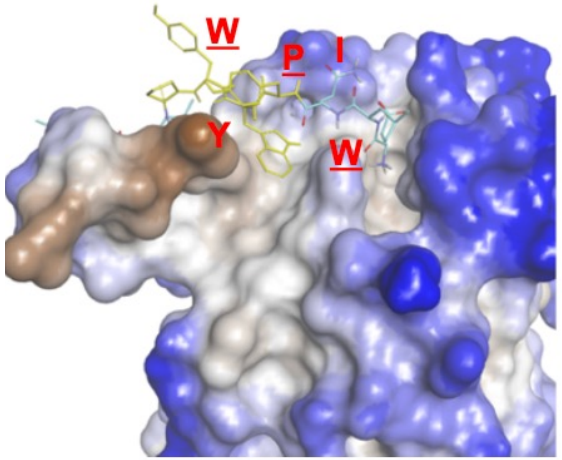
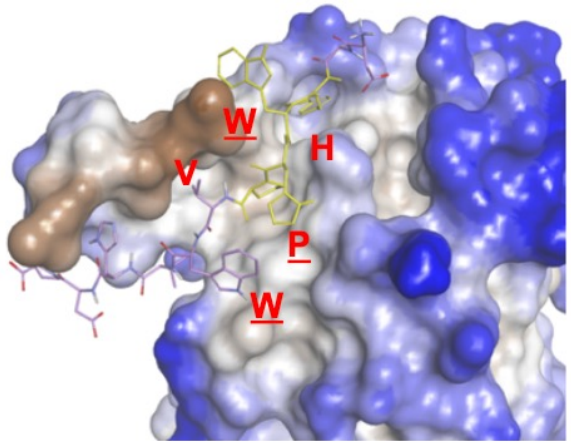
Interactions of peptides with OA ECM

C5-24 docking S2506-P2724 C-terminus pose 34
DLQYWYPIWDTH

C5-91 docking S2506-P2724 C-terminus pose 42
DAYWHPVWVHDP



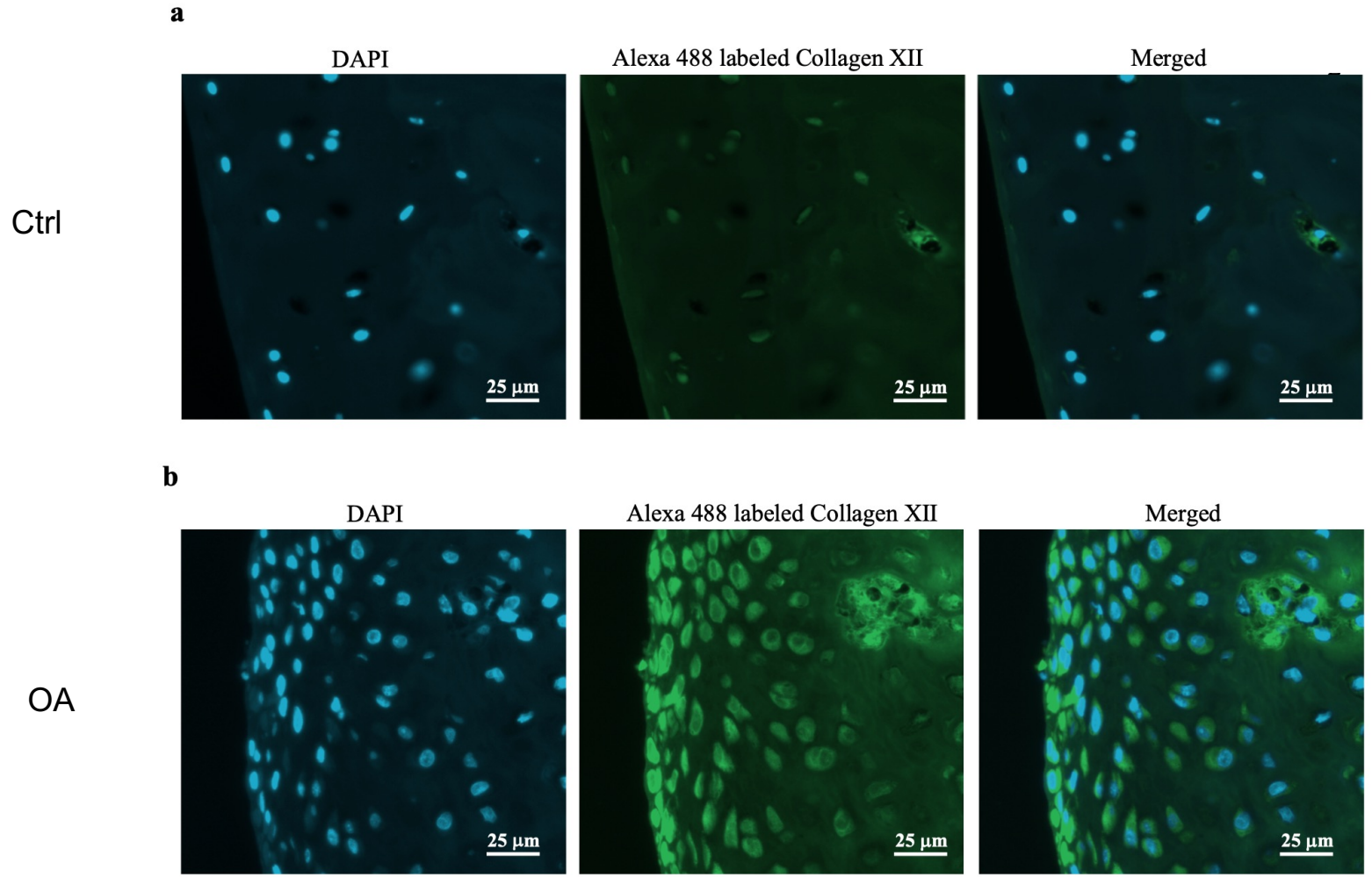
C5-24 Pose 34



C5-91 Pose 42

	ZDockScore	E_RDock
C5-24 Pose 34	13.82	-11.0487
C5-91 Pose 42	15.88	-20.8773

Expression of collagen XII in rat normal and OA joints



Normal and enzyme-induced OA knee joints were subjected to immunofluorescence for collagen XII.

Peptide sequence selected by bio-panning of human cartilage tissue and chondrocyte-secreted protein

Table S1. Alignment of phage-displayed peptide sequences selected by biopanning of human cartilage tissue and chondrocyte-secreted protein (grouping according to motifs similarity).

	Phage clone	Amino acid sequence	Frequency
1	C5-3, C5-50, C5-84, C5-87	GDYVIDWNFIEW	4/24
	C5-37	TVGSFFVEWMMH	1/24
	C5-66	DIGGWFVEWSLA	1/24
2	C5-22, C5-83, C5-92	DWGYFSWAYDSA	3/24
	C5-43	DWYTVSWLTDSN	1/24
3	C5-42, C5-91	DAYWHPVWVHDP	2/24
	C5-24	DLQYWYPIWDTH	1/24
	C5-12	HVYQKPSYWWYP	1/24
	C5-21	TWHFVDFSADTH	1/24
4	E5-8, E5-48	DYFTLDFTFDSW	2/24
	C5-46	NQVYFHYFDLDF	1/24
5	E4-14, E5-24	SPWWLWKAHNEA	2/24
6	C5-38	EVFNHYIQYSTE	1/24
7	E4-1	LPGMELFWNVAN	1/24
8	E4-4	DTFVFGSSKWRA	1/24
9	E4-15	SNNMRAPVNEIY	1/24

C: Phage clones targeting human cartilage tissue lysates.

E: Phage clones targeting human cartilage tissue pieces.

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Summary

- Confirmed the phage clones that specifically bind to OA cartilage rather than synovium and meniscus.
- Identification of the target binding protein of C5-24 peptide, ColXII in OA ECM.
- Modeling of cross-species homology of C5-24 peptide docking target.
- Collagen-binding peptides identified via phage display can be used to enhance the homing of mesenchymal stem cells to osteoarthritic tissue, its lubrication by hyaluronic acid, and its visualization via magnetic resonance imaging (MRI), and its targeting-delivery of drug carrier.

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Thank You

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