# Engineered Protein Based Delivery Agents for the Treatment of Osteoarthritis


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## Abstract


Osteoarthritis (OA) is a disease that brings about joint degradation and affects the normal function involved in the joints. OA is hypothesized to be induced by excessive levels of all-*trans* Retinoic acid (*at*RA). In order to combat this up pathological up-regulation of *at*RA, another small molecule is needed to compete for receptors involved in *at*RA-induced OA. The molecule being studied to compete is a pan retinoic acid receptor (RAR) inverse agonist known as BMS 493. A delivery vessel is then needed to transport BMS 493 to a joint. Our group is researching the development of a biomolecular delivery vehicle to facilitate the transport of BMS 493 to affected chondrocytes. The vessel being studied is the coiled coil domain of cartilage oligomeric matrix protein (COMP), which is a non-collagenous extracellular matrix glycoprotein that exists in cartilage, tendons, and ligaments. The coiled-coil domain (COMPcc) is known to self-assemble into a homopentamer with a hydrophobic pore at its center, within which smalls molecules are also known to bind within hydrophobic pore. For example, it has been shown to bind curcumin, vitamin D, all-trans retinol, and retinoic acid. Two specific variations of COMPcc protein are studied, the first being based off of the wild-type sequence, the second is a mutant of COMPcc where the residue Q54 is mutated to an alanine (Q54A). Previous studies have shown that Q54A has a higher affinity to bind small molecules such as all-trans retinol, which is structurally similar to *at*RA. The variants were generated in this study and used in binding studies to measure its affinity towards BMS 493 within its pore. We intend to compare the binding capacity of both these proteins with *at*RA as well as the inverse agonist, BMS 493.


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## Osteoarthritis




Little, C. B.; Hunter, D. J. *Nat. Rev. Rheumatol.*, **2013**, *9*, 485-497.

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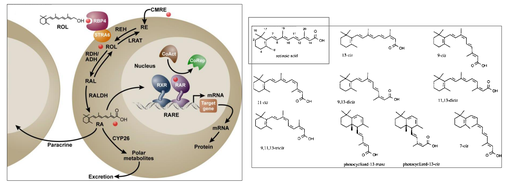
## All-*trans* Retinoic Acid (*at*RA)

- A derivative of Vitamin A.
- High levels of *at*RA has inidicated an association with osteoarthritis by increasing degradative enzymes MMP-13 (matrix metalloproteinase 13) and aggrecanase and thus down-regulating several matrix molecules in cartilage, *i.e.* type I, II, IX and XI collagen, proteoglycans, aggrecan, and link protein.




Davies M. R. *et al., Arth & Rheum*, **2009**, 60(6), 1722-1732.
Clagett-Dame M. & Knutson D., *Nutrients*, **2011**, *3*, 385-428.
Motto M. G. *et al., J Chroma*, **1989**, *481*, 255-262.

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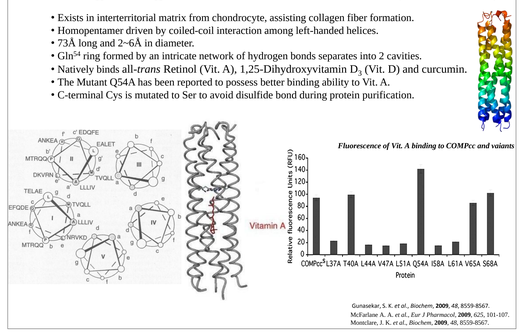
## Cartilage Oligomeric Matrix Protein (COMPcc)

- Exists in interterritorial matrix from chondrocyte, assisting collagen fiber formation.
- Homopentamer driven by coiled-coil interaction among left-handed helices.
- 73Å long and 2~6Å in diameter.
- $Gln^{54}$ ring formed by an intricate network of hydrogen bonds separates into 2 cavities.
- Natively binds all-*trans* Retinol (Vit. A), 1,25-Dihydroxyvitamin $D_3$ (Vit. D) and curcumin.
- The Mutant Q54A has been reported to possess better binding ability to Vit. A.
- C-terminal Cys is mutated to Ser to avoid disulfide bond during protein purification.




Gunasekar, S. K. *et al., Biochem*, **2009**, *48*, 8559-8567.
McFarlane A. A. *et al., Eur J Pharmacol*, **2009**, 625, 101-107.
Montclare, J. K. *et al., Biochem*, **2009**, *48*, 8559-8567.

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## BMS493

- An Anti agonist to retinoic acid receptor (RAR).




•Arrows indicate BMS493 as best possible Anti Agonist

le Maire, A. *et al. Nat. Struct. Mol. Biol.*, **2010**, *17*, 801-807.

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## Optimization and Expression




•6.9 kDa Indicating presence of COMPcc post expression

Studier, F. "Protein Production by Auto-induction in High-density Shaking Cultures." *Protein Expression and Purification* 41.1 (2005): 207-34.

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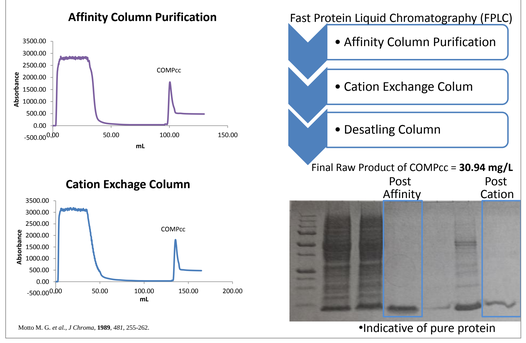
## Purification




Motto M. G. *et al., J Chroma*, **1989**, *481*, 255-262.

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## Binding

Ultra Performance Liquid Chromatography (UPLC)




•Zonal Elution Method
•Indicative of small molecule (BMS493) binding to protein (COMPcc)

Hage, David S., and Arundhati Sengupta. *Analytical Chemistry* 70.21 (1998): 4602-609. Print.

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## Conclusion

- Optical Density for expression was able to be optimized to produce large amounts of protein.
- Purification via affinity column, cation exchange column, and de-salting column was performed while maintaining high yield and concentrations.
- BMS 493 was shown to bind to COMPcc via UPLC

## Future Work

- 24-hour binding and releasing study of COMPcc to BMS493.
- Determination of dialysis and releasing rate with 1:1 volumetric ratio.
- Solid and liquid phase extractions for quantification of BMS493 from treated cell culture media.
- Q54A, expression, purification, and binding and releasing studies.


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