Supporting information

Engineering non-covalently assembled protein nanoparticles for long-acting gouty arthritis therapy

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Figures

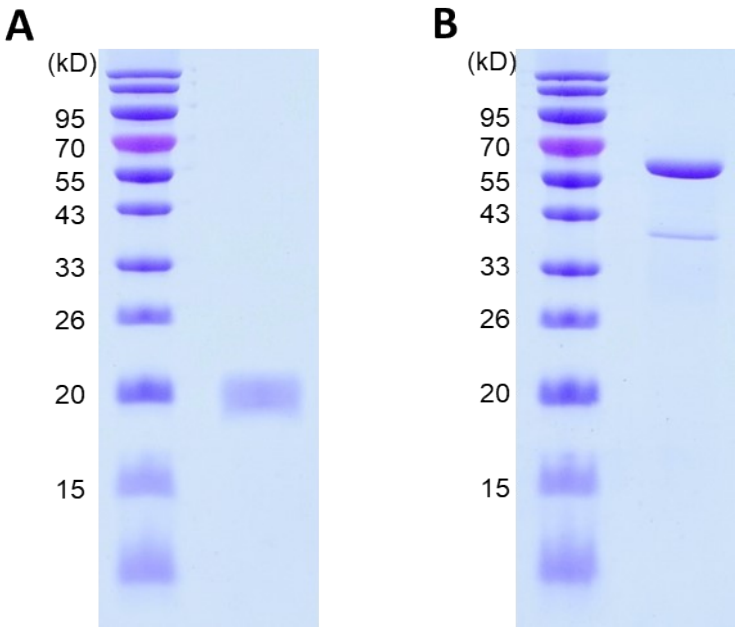

Figure S1. SDS-PAGE analysis of (A) the IL-1Ra protein and (B) the IK-Pr. The protein purity was 97% and 91%, respectively.

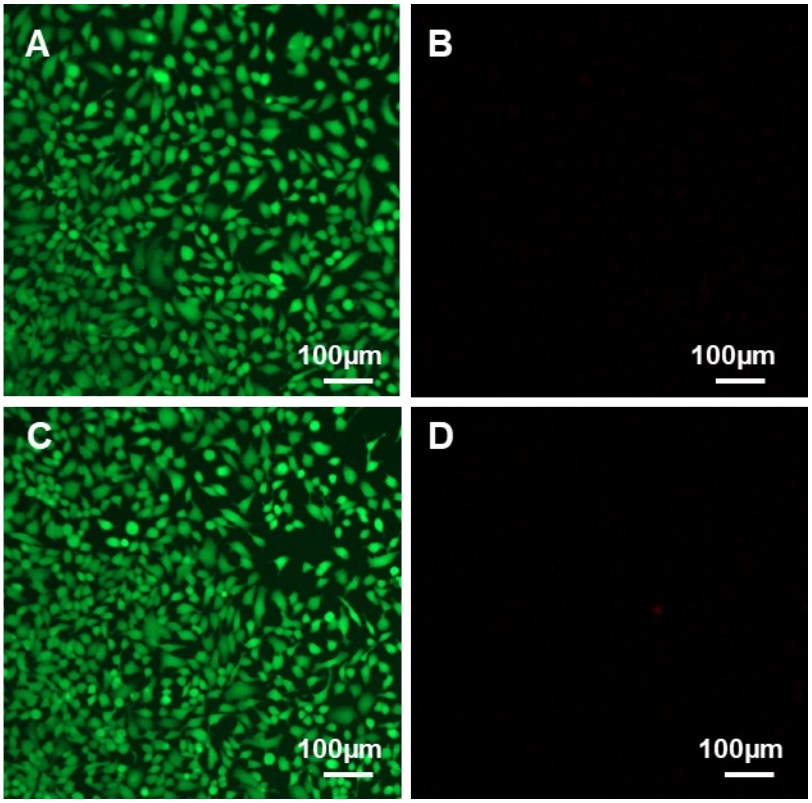

Figure S2. Confocal laser scanning microscope (CLSM) was used for analysis of the biocompatibility and cytotoxicity of the IK-Np. (A) and (B) showed BMSCs in blank control group stained by calcein-AM and pripodium iodide (PI), respectively. (C) and (D) showed BMSCs stained by calcein-AM and PI after co-incubated with 400 μg/mL of the IK-Np for 48 hours, respectively. Scale bar: 100 μm. The results illustrated excellent biocompatibility of the IK-Np.

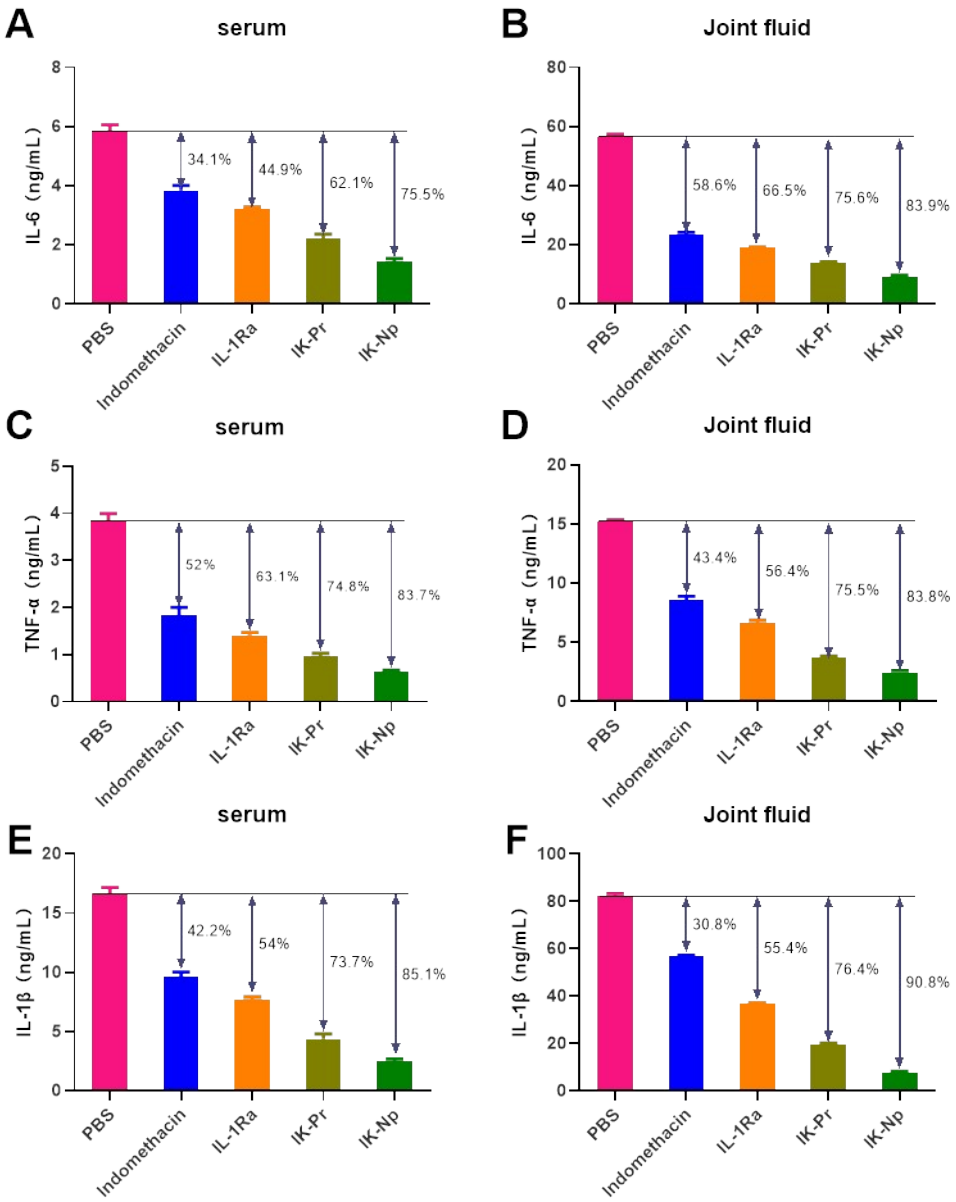

Figure S3. Cytokines in the plasm and joint fluid of rats after therapy were detected by ELISA. (A) and (B) showed the levels of IL-6 in serum and joint fluid of rats, respectively. (C) and (D) showed the levels of TNF-α in serum and joint fluid of rats, respectively. (E) and (F) showed the levels of IL-1β in serum and joint fluid of rats, respectively.

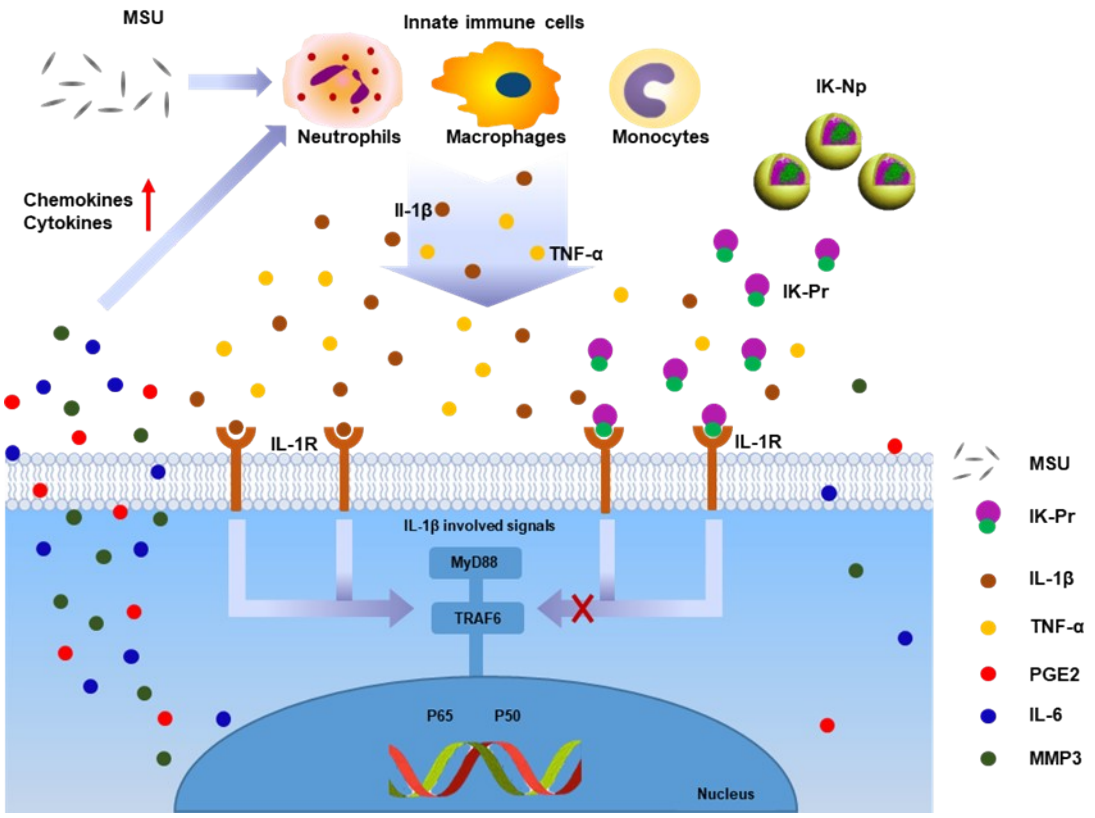

Figure S4. Illustration of potential mechanism of the IK-Np involving the inhibition of IL-1β signal pathway. MSU crystals stimulated innate immune cells including macrophages, monocytes and neutrophils, facilitating the production of active IL-1β and TNF-α. IL-1β further binds to IL-1 receptor (IL-1R) of surrounding synoviocytes and chondrocytes, resulting in inflammation process. Downstream cytokines (such as PGE2, MMP3 and IL-6) subsequently were secreted, promoting the inflammatory cascades and causes tissue damage through various signal pathways. The IK-Prs are slowly released from the nanoassembled IK-Np and reached to nidus during systemic circulation, where the IK-Pr can competitively bind to IL-1R and suppress the IL-1β signal pathway, resulting in alleviation of inflammatory reaction.

Table S1. Half-lives of different therapeutics based on IL-IRa

Therapeutics based on IL-1Ra	Modification strategy	Half-life (hour)	Formation	Mode of administration	Source
IK-Np	**Fusing of IL-1Ra with ELP and PEGylation**	**27.16**	**Nanoparticle**	**Subcutaneous injection**	**This study**
Anakinra	Human IL-1Ra expressed in *E. coli*	4-6	Protein	Subcutaneous injection	FDA-approved drug (https: //www.drugs.com/ kineret.html)
IL-1Ra-PF127	IL-1Ra loaded in Pluronic F-127 gels	12.53±2.48	Polymer-based gel	Subcutaneous injection	Akash M. S. H. et al., 2013 [1]
IL-1Ra-HSA	Fusing human serum albumin (HSA) to the carboxyl terminal of IL-1Ra	9.8 ± 1.7	Recombinant protein	Intravenous injection	Liu M. et al., 2012 [2]
dAbm16-IL-1Ra	Fusing the Anti-serum albumin domain antibodies (dAbs) to the amino terminal of IL-1Ra	4.3	Recombinant protein	Intravenous injection	Holt L. J. et al., 2008 [3]
IL-1Ra-MPs	IL-1Ra loaded by mineral coated microparticles (MPs)	detectable in serum within 24 hours	Microparticle	Local bolus injection	Clements A. E. B. et al., 2018 [4]

References

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