Supporting Information

for

Multifaceted Peptide Assisted One-pot Synthesis of Gold Nanoparticles for Plectin-1 Targeted Gemcitabine Delivery in Pancreatic Cancer

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This document contains

- 1. Supplementary Methods
- 2. Supplementary References
- 3. Supplementary Figures (S1 and S2) with legends
- 4. Supplementary Movie legends (SM1 and SM2)

Supplementary Methods:

Model Building

A gold nanoparticle (GNP) was built to a dimension of approximately 60 Å in diameter consisting of 4,896 Au atoms. The gold lattice was built within Visual Molecular Dynamics (VMD) using a tcl-script and code to generate a gold lattice that could be subtracted using a spherical function from the origin.¹⁻³ This gold sphere was imported into Maestro for further molecular modeling. Nonapeptides were chosen with the sequence N-terminal-KTLLPTPYC C-term to decorate the surface of the gold sphere via thiol linkages evenly spaced. The –SH linked Au bonds are described (see 'Nanoparticle preparation'). The optimal number of peptides chosen was 53 with the N-termminal lysine facing into aqueous solution away from the GNP. The entire peptide fused GNP (53pep-GNP) consists of 12,696 atoms. Additionally 53 Cl- counterions were added to the system to neutralize the NH³⁺ charge from the terminal lysine. Further, 200 Gemcitabine drug molecules were introduced into the system (see Nanoparticle preparation), which consists of 5,799 atoms. Finally physiological milieu of Na⁺ and Cl⁻ were added to a solvated box surrounding the 53pep-GNP using SPC water model. In total the final simulation contains 205,500 atoms, which forms a cubic cell of 1,728 nm³ (or 2.1x10⁶ Å³) (**Figure 3A-C**).

Peptides were examined using Procheck and What-If.^{4,5} The side chains and rotamers were examined using refinement protocol and verified.⁵ The final system was subjected to energy optimization with PR conjugate gradient with an R-dependent dielectric for 100,000 steps with relaxing restraints. Each model was exported to the following formats: Maestro (MAE), VMD (PDB) (Supplemental Material). Model manipulation was done with Maestro (Macromodel, version 9.8, Schrödinger, LLC, New York, NY, 2010), or Visual Molecular Dynamics (VMD).¹ The refinement modeling was built as a using Schrodinger Maestro and VMD.¹⁻³

NANOPARTICLE PREPARATION

Following Maestro/VMD building of the Set up to steps to restrain the positions of Au atoms and SH⁻ groups from both Desmond minimization and MD simulations. The following steps were taken to prep the nanoparticles (NP) + peptide + drug system:

- 1. Removed all Au-Au bonds in NP to ease the force field (FF) setup stages
- 2. Added hydrogens to peptide chains
- 3. Used disordered system builder* to sprinkle 200 drug molecules

Disordered system builder requires Materials Science Suite, which we used Desmond system builder to set solvent, PBC, and FF.⁶⁻⁸ The first key here is to assign OPLS3 FF, which would automatically assign UFF parameters for gold atoms. The second key here is to set the position restraints for Au atoms and SH- groups. Here the following steps were taken:

1. Run Desmond system builder to set PBC (orthorhombic with minimal volume) and OPLS3 FF. 9

2. Load the system builder output from Desmond minimization panel.

3. From 'Advanced Options', select 'Restraints', and set force constant of 10 for all Au atoms and SH- groups.

4. Run minimization.

5. Once minimization is done, repeat 2-3 with molecular dynamics panel to run MD (NVT-310K).

I. Final system characteristics:

- Au NP with peptide chains with SH terminations/constraint bonds at Au surface

- 200 drug molecules
- 52 Cl⁻ ions to neutralize charges

-physiological milieu ions (Na⁺/Cl⁻)

- Water molecules (SPC)

The constructed model using the structure assembled in VMD and Maestro,¹⁻³ dumped the drug molecules using disordered system builder (described above), set up the box with SPC water and Cl- ions, minimized and ran NpT MD for 1 ns with positional applied force restraints applied for Au and S atoms using the OPLS3 FF.⁹ We modified the S atoms to be thiol (SH) groups parameter so a proper FF definition for it was in place, but the steric positioning at the gold mimics –S-Au connectivity. Since all S atoms are restrained, the Au-binding site being represented by -SH group near Au atoms would have nearly the same effect as setting the explicit S-Au covalent bonds at the surface using a QM parameterization.

II. Equilibrating Simulations

Using the super-fast version of GPU-Desmond, we performed a minimal equilibration for 1.0 ns NVT simulation over the 250,000-atom system in just under 3 hours and the nanoparticle stays intact. We then started longer molecular dynamics simulations (MDS) using NVE/NPT ensembles for >400 ns.

Molecular Dynamics Simulation

The system was minimized with relaxed restraints using Steepest Descent and Conjugate Gradient PR, and equilibrated in solvent with physiological salt conditions, as described in the literature.¹⁰⁻¹³ After equilibration was established, each system was allowed to run an additional MD production length of >10 nanoseconds. The primary purpose of MD for this study was conformational stability, refinement, and interaction calculations that may occur at drug-GNP interface. The protocol for refinement includes the following steps: (1) Minimization with explicit water molecules and ions, (2) Energy minimization of the entire system, and (3) MDS for >10 ns to relax to the force field (OPLS3/Amber).^{9,14} Following the refinement protocol, production simulations were completed to collect data.

I. Molecular dynamics protocol

OPLS3(Desmond)/Amber(NAMD2) force fields were used with the current release of NAnoscale Molecular Dynamics 2 engine.^{14,15} The system simulated, including hydrogens, consist of 2.5 x10⁵ atoms with solvation using SPC water and ions. In all cases, we neutralized with counterions, and then created a solvent with 150 mM Na⁺ Cl⁻ to recreate physiological strength. SPC water molecules were added around the protein at a depth of 15-18 Å from the edge of the molecule depending upon the side.¹⁶ Our protocol has been previously described in the literature.¹² Simulations were carried out using the particle mesh Ewald technique with repeating boundary conditions with a 9 Å nonbonded cut-off, using SHAKE with a 2-fs timestep. Preequilibration was started with three stages of minimization with 10,000 steps of SD, PRCG, relaxing restraints, then followed by 1000 ps of heating under MD, with the atomic positions of nucleic and protein fixed. Then, two cycles of minimization (5000 steps each) and heating (1000 ps) were carried out with soft restraints of 10 and 5 kcal/(mol·Å²) applied to all backbone atoms and metals. Next, 5000-steps of minimization were performed with solute restraints reduced to 1 kcal/(mol Å²). Following that, 400 ps of MDS were completed using relaxing restraints (1 kcal/(mol·Å²)) until all atoms are unrestrained, while the system was slowly heated from 1 to 310 K using velocity rescaling upon reaching the desired 310K during this equilibration phase. Additionally, NPT equilibration based MD was used with velocity rescaling for >10 ns. Finally, production runs of MD were carried out with constant pressure boundary conditions (relaxation time of 1.0 ps). A constant temperature of 310 K was maintained using the Berendsen weakcoupling algorithm with a time constant of 1.0 ps. SHAKE constraints were applied to all hydrogens to eliminate X-H vibrations, which yielded a longer simulation time step (2 fs). Our methods for equilibration and production run protocols are in the literature.^{10,11,17,18} Translational

and rotational center-of-mass motions were initially removed. Periodically, simulations were interrupted to have the center-of-mass removed again by a subtraction of velocities to account for the "flying ice-cube" effect.¹⁹ Following the simulation, the individual frames were superposed back to the origin, to remove rotation and translation effects.

Supplementary References:

- 1. W. Humphrey, A. Dalke and K. Schulten, *J Mol Graph*, 1996, **14**, 33-38, 27-38.
- 2. Schrödinger, *Journal*, 2012.
- 3. Schrödinger, *Journal*, 2013.
- 4. E. Krieger, G. Koraimann and G. Vriend, *Proteins*, 2002, **47**, 393-402.
- 5. R. A. Laskowski, M. W. MacArthur, D. S. Moss and J. M. Thornton, *J Appl Cryst*, 1993, **26**, 283-291.
- 6. D. Shivakumar, J. Williams, Y. Wu, W. Damm, J. Shelley and W. Sherman, *J Chem Theory Comput*, 2010, **6**, 1509-1519.
- 7. R. A. Lippert, K. J. Bowers, R. O. Dror, M. P. Eastwood, B. A. Gregersen, J. L. Klepeis, I. Kolossvary and D. E. Shaw, *J Chem Phys*, 2007, **126**, 046101.
- 8. Z. Guo, U. Mohanty, J. Noehre, T. K. Sawyer, W. Sherman and G. Krilov, *Chem Biol Drug Des*, 2010, **75**, 348-359.
- 9. E. Harder, W. Damm, J. Maple, C. Wu, M. Reboul, J. Y. Xiang, L. Wang, D. Lupyan, M. K. Dahlgren, J. L. Knight, J. W. Kaus, D. S. Cerutti, G. Krilov, W. L. Jorgensen, R. Abel and R. A. Friesner, *J Chem Theory Comput*, 2016, **12**, 281-296.
- 10. T. Caulfield and B. Devkota, *Proteins*, 2012, **80**, 2489-2500.
- 11. T. Caulfield and J. L. Medina-Franco, *J Struct Biol*, 2011, **176**, 185-191.
- 12. T. R. Caulfield, B. Devkota and G. C. Rollins, *J Biophys*, 2011, **2011**, 219515.
- 13. T. R. Caulfield, *Journal of molecular graphics & modelling*, 2011, **29**, 1006-1014.
- 14. D. A. Case, T. E. Cheatham III, T. Darden, H. Gohlke, R. Luo, K. M. Merz Jr, A. Onufriev, C. Simmerling, B. Wang and R. Woods, *Journal of Chemical Chemistry*, 2005, **26**, 1668-1688.
- 15. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. Skeel, L. Kale and K. Schulten, *Journal of Computational Chemistry*, 2005, **26**.
- 16. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *Journal of Chemical Physics*, 1983, **79**, 926-935.
- 17. K. Reblova, F. Lankas, F. Razga, M. V. Krasovska, J. Koca and J. Sponer, *Biopolymers*, 2006, **82**, 504-520.
- 18. K. Reblova, E. Fadrna, J. Sarzynska, T. Kulinski, P. Kulhanek, E. Ennifar, J. Koca and J. Sponer, *Biophys J*, 2007, **93**, 3932-3949.
- 19. T. E. Cheatham, III and M. A. Young, *Biopolymers*, 2001, **56**, 232-256.

Supplementary Figure



Figure 1: A. Hydrodynamic size distribution of GNP-PTPC (Black) and GNP-Delta-PTP(Red). **B.** UV-vis spectra of GNP-PTPC (Blue) and GNP-Delta-PTPC (Red). **C.** Optical images of GNP-Delta-PTP (left) and GNP-PTPC (right) aqueous solution.



Figure 2: A. Optical images of GNPs incubated with increasing concentrations of NaCl for 1 hour. **B.** Stability analysis of the GNPs by monitoring hydrodynamic diameter after incubation with various concentration of NaCl.



Figure 3: UV-vis absorption spectra were collected form GNP-sup, GNP-Gem-soup, GNPwash-sup and GNP- Gem-wash-sup. Absorption peak for gemcitabine was clearly monitored in the GNP-PTP1-Gem-soup sample demonstrating the presence of unbound gem. After washing this sample further, no peak for gem was observed in the washing buffer. This confirms that the gemcitabine strongly absorbed on the GNP surface.



Figure 4: Size distribution of GNP-Gem from TEM.



Figure 5: Hydrodynamic size distribution of GNP-Gem.

Supplementary Movies

Movie S1. Simulation for nonapeptide bound gold nanoparticle with drug capturing.

Movie S2. Simulation of the 'zoom' into region from Figure 1B for nonapeptide bound gold nanoparticle with drug capturing.