

DNA Conjugation to Nanoparticles

Sunho Park

Abstract

Nanoparticle–DNA (NP–DNA) conjugates have been highlighted due to their versatility in diverse science and engineering fields. The protocol of DNA conjugation to gold nanoparticles (AuNPs), which are among the most popular NPs in bio-applications, is thus described here. This protocol also includes ligand exchange of AuNP to make AuNPs suitable for conjugation process and a fluorescence technique to evaluate the average number of DNA strands attached to single AuNP.

Key words Nanoparticle, Gold nanoparticle, DNA, Conjugation, Ligand exchange, Gel electrophoresis, Lyophilization, DNA staining, Fluorescence, DNA coverage ratio

1 Introduction

DNA–nanoparticle conjugates have attracted great interest as there are numerous opportunities to utilize the properties of the nanomaterial and the DNA synergistically on small length scales [1–5]. In particular, gold nanoparticles (AuNPs) are widely used in biomedical applications such as delivery, sensing, and imaging due to their exceptional biocompatibility [6–9]. AuNPs have a relatively inert surface but can be easily conjugated to biomolecules by covalent linkage via the sulfur atom in a thiol. Therefore, thiol-capped DNA oligos have been popular for constructing AuNP–DNA conjugates.

Thiol-aided conjugation is considered feasible for the laboratory; however, some important issues are often ignored in practice. The first is the stability of the nanoparticles during the conjugation process. Conjugation via a thiol group is typically performed in concentrated ionic buffers which screen the surface charge of the particles and DNA, so that electrostatic repulsion is diminished, and therefore increasing the collision rate between the two species. Using concentrated samples also raises this collision rate. Lyophilization is a method that can elevate both the ionic strength of solution and the concentration of each species extremely high as

the water content of the solution dries out. Citrate-stabilized AuNPs [10] are commonly used in laboratories but they irreversibly aggregate during lyophilization due to their low stability. A chemical ligand bis(*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt is thus used to replace the citrate molecules on the AuNP surface, and allows AuNPs to sustain stability under more severe ionic conditions.

Another issue is the evaluation of the exact coverage ratio of AuNP–DNA conjugates. The average number of conjugated DNA strands per each particle is lower than the number from DNA:AuNP incubation ratio in the reaction due to <100 % efficient conjugation. To quantify the coverage, the sample must be cleaned up by removing the free DNA strands from the solution, and then the conjugated DNA strands are completely liberated from the AuNPs to count their population. In this method, 6-mercapto-1-hexanol is used for the DNA displacement [11]. Fluorescence measurement of the stained DNA strands is performed afterwards to quantify the DNA concentrations. AuNPs should be excluded from the solution before the measurement to avoid fluorescence quenching [12].

This chapter includes the protocols of ligand exchange of AuNP, AuNP–DNA conjugation, and evaluation of DNA coverage in detail (Fig. 1). Purification of samples relies only on typical biochemical lab techniques such as centrifugation and agarose gel electrophoresis. The protocols provide most biochemists and nanoparticle researchers with an easier and more accurate AuNP–DNA conjugate preparation that does not require extensive effort or specialized skills.

2 Materials

All solutions are prepared with 0.5× tris-borate-EDTA buffer (0.5× TBE, pH=8.3) unless specified otherwise.

2.1 Ligand Exchange

1. 0.5× Tris-borate-EDTA buffer (TBE): 45 mM tris, 45 mM boric acid, and 1 mM EDTA in ultrapure deionized water (resistivity of 18 MΩ cm at room temperature) (*see Note 1*).
2. Gold nanoparticle (AuNP): Synthesized or commercially achieved citrate-stabilized aqueous solution (*see Note 2*).
3. Bis(*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BPS) (*see Note 3*).
4. Sodium Chloride (NaCl).
5. 1 % Agarose gel: 0.5 g of agarose powder in 50 mL 0.5× TBE. Cast in an appropriate agarose gel electrophoresis device. Use 0.5× TBE as gel running buffer (*see Note 4*).

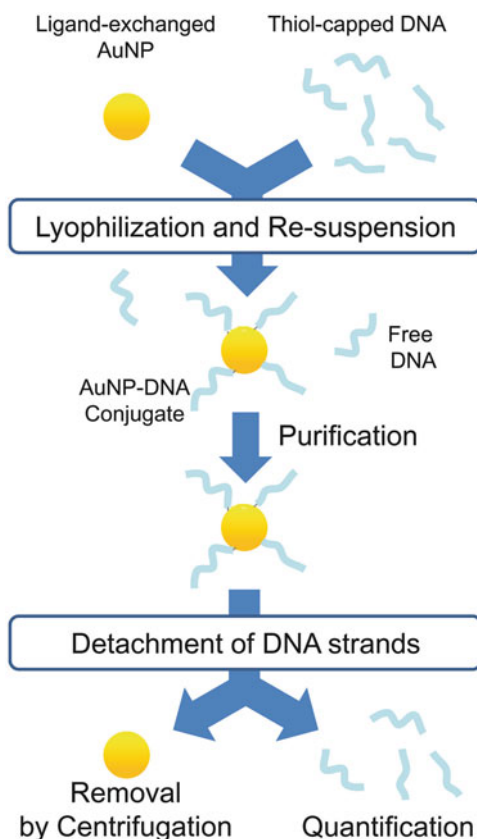


Fig. 1 An overview of the protocol of nanoparticle–DNA (NP–DNA) conjugation and coverage ratio quantification

2.2 Conjugation and Evaluation of Coverage Ratio

1. DNA oligonucleotide: commercially available thiol-capped DNA strands (this protocol works best for 10–50 mer DNA oligos). Adjust the concentration to 1 $\mu\text{g}/\mu\text{L}$ (*see Note 5*).
2. 100 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP): 0.0287 g of TCEP in 1 mL 0.5 \times TBE (*see Note 6*).
3. 10 mM 6-Mercapto-1-hexanol (MCH): 1 μL of pure MCH (~ 7.3 M) into 729 μL of 0.5 \times TBE (*see Note 7*).
4. DNA staining solution: 100 \times SYBR[®] Gold (Invitrogen). Dilute 1 μL of stock solution (10,000 \times) with 99 μL of 0.5 \times TBE (*see Note 8*).

3 Methods

3.1 Ligand Exchange and Purification of AuNPs

1. Put a pinch amount (~ 0.1 g) of BPS into ~ 100 mL of aqueous citrate stabilized AuNP solution, which is typically at $\sim 10^{-9}$ M concentration and pale red. Place the solution on an orbital shaker and gently mix it for an overnight (*see Note 9*).

2. Put 1 g of NaCl into the AuNP–BPS solution. Stir and mix well with a disposable pipette. The color of the solution will turn dark gray in a few seconds. Separate the solution evenly into two 50 mL centrifuge tubes or eight 15 mL tubes. Place the tubes in a centrifuge and spin them for ~5 min at rcf $\sim 1,000 \times g$ (*see Note 10*).
3. Remove the clear supernatant from all of the tubes with a pipette and add ~100 μL of 0.5 \times TBE with 200 μL pipette and tip right onto the sediment at the bottom of one of the tubes. Re-disperse the particles well by pressing and releasing the pipette, take out the dark red solution, and put it right into the sediment in another one of the tubes. Repeat this step until all of the AuNP aggregates are recovered (*see Note 11*).
4. Place the thick red AuNP solution into a long single well in 1 % agarose gel. Apply an electric field of ~4 V/cm to the gel. The AuNP band will move from the anode (–) to cathode (+). Stop running the gel after the band shifts by ~3–4 cm (taking ~1 h) (*see Note 12*). Cut out the narrow AuNP band using a clean razor blade, chop it into many small pieces, and put the sliced pieces into a 15 mL tube. Pour several milliliters of 0.5 \times TBE into the tube until the agarose gel pieces are fully soaked. Tighten the cap and place the tubes in a 4 °C refrigerator for ~2 days (*see Note 13*).
5. Take out the red solution and discard the solid pieces left in the tube. Distribute the solution into 1.5 mL tubes (300 μL each) and place the tubes in a microcentrifuge. Spin the tubes for ~10 min at rcf $\sim 10,000 \times g$. Gently remove the supernatant (pale pink) and collect the red oily layer at the bottom of the tubes (*see Note 14*).
6. Use a UV–VIS spectrometer to collect an absorbance spectrum. Confirm the peak absorbance is at ~520 nm and record the peak absorbance value. Determine the concentration of the stock solution using Beer–Lambert law (*see Note 15*).

3.2 Conjugation of DNA Oligos with AuNPs

1. Determine a target coverage ratio (average number of conjugated DNA strands per AuNP) and the amount of AuNP stock solution to be used. Calculate the necessary amount of DNA solution (*see Note 16*). Mix the same volume of 100 mM TCEP solution with the DNA solution. Leave the mixture on the bench for ~1 h (*see Note 17*).
2. Mix the TCEP+DNA solution well with AuNP solution. Separate evenly the mixture into 1.5 mL tubes (~100 μL per tube) and lyophilize the samples until they are dried out (*see Note 18*).

3. Re-disperse the dried sample in one of the tubes by adding $\sim 50\ \mu\text{L}$ $0.5\times$ TBE into it, and use this solution to recover all the other samples. Place the concentrated solution in a $4\ ^\circ\text{C}$ refrigerator for ~ 1 day to allow further conjugation (*see Note 19*).
4. Add $\sim 1\ \text{mL}$ of $0.5\times$ TBE to the sample and separate the diluted solution evenly into $1.5\ \text{mL}$ tubes ($\sim 200\ \mu\text{L}$ per each tube). Spin the tubes with a microcentrifuge ($\text{rcf} \sim 10,000\times g$) for $10\ \text{min}$ to collect AuNP–DNA conjugates at the bottom and leave unconjugated DNA strands in supernatant. Discard the supernatant and collect the red layer at the bottom of the tubes. Repeat this whole washing step at least three times. The red solution from the final washing is the stock solution of AuNP–DNA conjugates.
5. Take UV–VIS absorbance spectrum to achieve the concentration of the stock AuNP–DNA conjugate solution (*see Note 20*).

3.3 Determination of Coverage Ratio

1. Dilute the AuNP–DNA stock solution to $0.1\ \mu\text{M}$ in $1\ \text{mM}$ MCH and leave the sample undisturbed for ~ 1 day (*see Note 21*).
2. Spin the AuNP–DNA+MCH solution with a microcentrifuge ($\text{rcf} \sim 10,000\times g$, $10\ \text{min}$). Carefully transfer the clear supernatant to a new $1.5\ \text{mL}$ tube and discard the original tube containing dark gray residue at the bottom (*see Note 22*). Put $10\ \mu\text{L}$ of this solution into $89\ \mu\text{L}$ of $0.5\times$ TBE, and add $1\ \mu\text{L}$ $100\times$ SYBR[®] Gold to the mixture. Make three or four identical samples for better accuracy of coverage measurement. Do the following step (staining of reference DNA) immediately (*see Note 23*).
3. Dilute the stock DNA solution to $1\ \mu\text{M}$ (*see Note 24*). Make reference DNA solutions of 1×10^{-8} , 5×10^{-8} , 1×10^{-7} , 2×10^{-7} , and $4\times 10^{-7}\ \text{M}$, in $0.1\ \text{mM}$ MCH and $1\times$ SYBR[®] Gold (*see Note 25*). These reference samples and the samples from the previous step should be kept in the dark for $\sim 1\ \text{h}$.
4. Take fluorescence spectrum of the samples from **step 2** and reference solutions from **step 3**. The excitation wavelength is at $495\ \text{nm}$ and the emission peak is around $537\ \text{nm}$ for SYBR[®] Gold (Fig. 2a). Collect the peak emission intensity of each solution. Fit the data from DNA references into a linear function, $I = a \cdot C + b$, where I is the intensity of fluorescence [cps; counts per second], C is the concentration of DNA solution [M], and a , b are constants (Fig. 2b). Calculate the DNA concentration of the samples from **step 2** by use of the measured intensity and the fitted equation. Divide this value by $1\times 10^{-8}\ \text{M}$ (*see Note 26*) to get the coverage ratio of the stock AuNP–DNA.

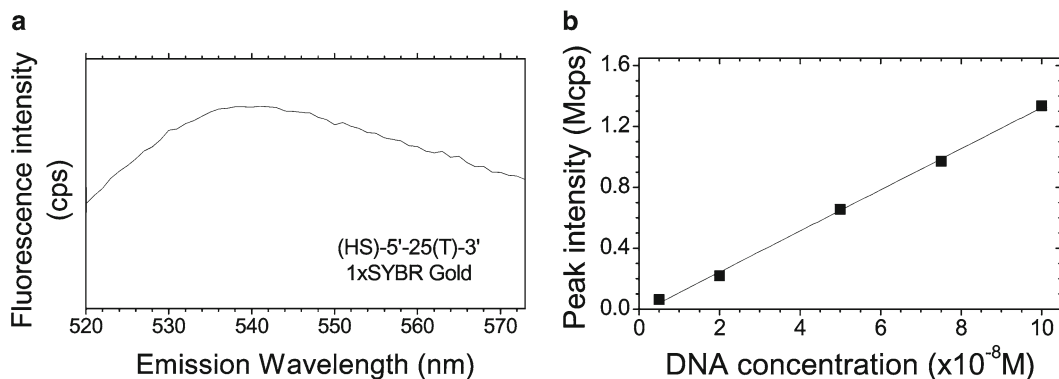


Fig. 2 Intensity of fluorescence emission from 1× SYBR® Gold-stained DNA oligo (HS-5'-TTTTT TTTT TTTT TTTT TTTT-3') in 0.5× TBE and 0.1 mM MCH was measured. Excitation wavelength was 495 nm. **(a)** An example of emission spectrum, **(b)** A linear relationship between peak emission intensity and concentration of stained DNA

4 Notes

1. 0.5× TBE is made by either diluting commercially available concentrated TBE buffer or dissolving powdered TBE in water.
2. This protocol works for AuNP of diameter in 5–20 nm, while it is most suitable for ~10 nm AuNPs. Aqueous AuNP colloids are synthesized mostly by reducing Au³⁺ ions in sodium citrate solution; however, citrate-capped AuNPs are not stable enough to endure the conjugation process suggested here. Most failures, including irreversible aggregation of AuNPs, happen during the centrifugation or lyophilization steps.
3. BPS molecules form dipolar bonds between their own oxygen atoms and gold atoms on AuNP's surface. They are negatively charged and generate sufficiently high zeta-potential in ionized buffer.
4. Agarose gel electrophoresis is used to exclude unbound BPS molecules and residues from AuNP synthesis. Add 0.5 g agarose powder to 50 mL 0.5× TBE (or 1 g agarose to 100 mL) in 250 mL glass beaker and mix well using a disposable pipette. Pull up and push out the mixture with the pipette several times to make the agarose solution homogenized. A typical gel comb has multiple teeth to make separate wells in a gel. For this purification purpose, put some electrical tape on the teeth to cover the empty spaces between the teeth so that the comb will form a long, single well in the gel. After the gel casting device is assembled, heat up the agarose solution in the

beaker with a microwave oven. Interrupt the heating process a few times to take out and shake the beaker to re-disperse any possible sediment at the bottom. The solution is finished heating when it boils and turns clear. Take the beaker carefully out of the microwave oven, pour the hot solution quickly into the gel tray, and place the gel comb at a right position. Remove small bubbles floating on the surface level of the hot gel solution before the gel starts hardening. A 10 μL or 20 μL pipette and tip works well for pulling up the bubbles. It takes 30 min to 1 h for full hardening of the gel. Remove the gel comb and pour into the tank 0.5 \times TBE as gel running buffer. The gel must be fully immersed.

5. Thiol-capped DNA strands typically form dimers by making disulfide bonds, or are capped with an additional protecting group by the manufacturer. Thus, they need to be reduced before conjugation with AuNPs.
6. It is preferred to use fresh solution. Dithiothreitol (DTT) is often used for the same purpose; however, TCEP is preferable over DTT in that it is an irreversible and more powerful reducing agent.
7. It is preferred to use fresh solution. Handle the stock MCH bottle in a fume hood as it has a strong odor.
8. It is preferred to use fresh solution. This product is a DNA staining dye and stored at $-20\text{ }^{\circ}\text{C}$. Thaw the stock in a dark place at room temperature. Put the stock bottle back into the freezer right after taking out the necessary amount.
9. The concentration of 0.1 g of BPS [$M_w=534.6$] in 100 mL solution is $\sim 1.9\text{ mM}$, which is much higher than a typical concentration of aqueous AuNP solution. An overnight reaction is sufficient to ensure the BPS content on AuNP surface has reached an equilibrium state.
10. Excessive amounts of NaCl can make the ionic strength of the solution very high. This fully screens the surface charge of the AuNPs in solution and can result in precipitation of the AuNPs. Tune the centrifugal force and centrifugation time depending on the size and surface charge density of AuNP as the precipitated AuNPs may aggregate permanently under too strong centrifugation conditions.
11. Limit the final volume of the re-dispersed AuNP solution within a few hundred microliters so that the volume does not exceed the capacity of the gel loading well.
12. These gel running times and electric field strengths are based on lab experience. You may need to run the gel for longer if the particles are larger, ionic strength of running buffer is greater, or the electric field strength is low.

13. AuNPs in gel band will diffuse into 0.5× TBE buffer and the concentration distribution of AuNPs will reach an equilibrium state inside and outside gel. Slicing of the gel band facilitates this because it increases the surface area for diffusion flux. More AuNPs are recovered by transferring the remaining solid gel pieces into another tube filled with clean 0.5× TBE. It takes less time for diffusion for smaller AuNPs.
14. Spin the tubes for an extended time if not much of the AuNPs collect at the bottom. This is the stock solution of AuNP to be used for DNA conjugation, and the concentration is typically in the order of ~1 μM . You may adjust the concentration exactly to 1 μM using 0.5× TBE for convenience of later use.
15. $A = \epsilon cl$, where A [OD, optical density] is the peak value of the absorbance spectrum, ϵ [$\text{M}^{-1} \text{cm}^{-1}$] is the extinction coefficient, l [cm] is the path length, and c [M] is the concentration of the solution. ϵ of AuNPs depends on their size and ligand types, and values can be found in literature ($\sim 10^8 \text{ M}^{-1} \text{cm}^{-1}$ for ~10 nm AuNP) [13]. Dilute the stock AuNP solution to make the measured absorbance peak lie within a typical working range of most UV–VIS spectrometers, 0.1–10 O.D.
16. If 50 μL of $1 \times 10^{-6} \text{ M}$ AuNP stock solution is used to make 1:10 AuNP:DNA conjugates, for example, the exact amount of DNA strands necessary is $10 \times (50 \mu\text{L}) \times (1 \times 10^{-6} \text{ M}) = 500 \times 10^{-6} \mu\text{mol}$. When M_w [$\mu\text{g}/\mu\text{mol}$] is the molecular weight of DNA oligo, this amount is equivalent to $(500 \times 10^{-6} \mu\text{mol}) \times M_w$ [μL] of 1 $\mu\text{g}/\mu\text{L}$ stock DNA solution. Due to the nature of conjugation process, however, use two or three times as much of the exact amount. Not all the DNA used will be conjugated.
17. Keep the mixture in a dark place if the DNA strands have fluorescent markers.
18. An easy way of lyophilization is to put the samples in 1.5 mL tubes and place them in a vacuum chamber with mild centrifugation. Use a pushpin to make an air hole on the lid of the tubes. Centrifugation helps the samples stay at the bottom of the tube during the drying process. Spinning the samples too strongly sometimes results in irreversible aggregation. Higher success rates are usually associated with running weak centrifugation for the first ~10 min only while under vacuum.
19. This refrigeration step for further conjugation may be skipped if a higher amount of extra DNA (*see Note 16*) is used during the TCEP treatment step.
20. The concentration of AuNP–DNA conjugates is equivalent to the concentration of core AuNPs. DNA absorbance spectra have a peak at 260 nm and vanish in most visible light wavelength range; therefore, the AuNP absorbance peak value at 520 nm is rarely affected by conjugated DNA.

21. The sulfur atom of MCH forms a covalent bond with a gold atom on the AuNP surface, the same way of DNA conjugates to the AuNP. Due to the excessive population of MCH molecules in solution, the surface of AuNP becomes densely covered by MCH, replacing the DNA strands. The AuNP eventually becomes neutral as the negatively charged DNA and BPS are displaced in this process. If the concentration of the stock AuNP–DNA solution is 1 μ M, for example, add 10 μ L AuNP–DNA and 10 μ L 10 mM MCH to 80 μ L 0.5 \times TBE in a 1.5 mL tube (100 μ L in total).
22. AuNPs have been aggregated and discarded by centrifugation. Remember that the DNA strands in the supernatant are in 1 mM MCH and have been detached from 0.1 μ M AuNP–DNA.
23. The sample is now in 0.1 mM MCH, 1 \times SYBR[®] Gold. DNA strands in the solution were released from 1×10^{-8} M AuNP–DNA. SYBR[®] Gold, a staining agent of DNA, is added after all other substances have been mixed. DNA staining is fully saturated in about half an hour; however, it starts degrading after a few hours. Thus, perform the fluorescence measurement quickly in the following steps.
24. 1 μ g/ μ L DNA stock is at molar concentration of $1/M_w$. Dilute properly with 0.5 \times TBE.
25. Mix together 1 μ L of 1 μ M DNA, 1 μ L of 10 mM MCH, and 97 μ L of 0.5 \times TBE, then put 1 μ L of 100 \times SYBR[®] Gold. This makes 1×10^{-8} M DNA in 0.1 mM MCH, 1 \times SYBR[®] Gold, and 0.5 \times TBE. Change the amount of 1 μ M DNA appropriately for each target DNA concentration, and reduce the amount of 0.5 \times TBE by the increased amount of 1 μ M DNA to equalize the volumes of DNA reference solutions at 100 μ L.
26. Note that the DNA strands have been detached from 1×10^{-8} M AuNP–DNA.

References

1. Alivisatos P (2004) The use of nanocrystals in biological detection. *Nat Biotechnol* 22: 47–52
2. De M, Ghosh PS, Rotello VM (2008) Applications of nanoparticles in biology. *Adv Mater* 20:4225–4241
3. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H (2005) Quantum dot bioconjugates for imaging, labelling and sensing. *Nat Mater* 4:435–446
4. Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M (2009) Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater* 8:543–557
5. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, Frangioni JV (2010) Design considerations for tumor-targeted nanoparticles. *Nat Nanotechnol* 5:42–47
6. Chen C-C, Lin Y-P, Wang C-W, Tzeng H-C, Wu C-H, Chen Y-C, Chen C-P, Chen L-C, Wu Y-C (2006) DNA-gold nanorod conjugates for remote control of localized gene expression by near infrared irradiation. *J Am Chem Soc* 128:3709–3715
7. Ghosh PS, Kim C-K, Han G, Forbes NS, Rotello VM (2008) Efficient gene delivery vectors by tuning the surface charge density of amino acid-functionalized gold nanoparticles. *ACS Nano* 2:2213–2218

8. Thomas M, Klibanov AM (2003) Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. *Proc Natl Acad Sci U S A* 100: 9138–9143
9. Macfarlane RJ, Lee B, Jones MR, Harris N, Schatz GC, Mirkin CA (2011) Nanoparticle superlattice engineering with DNA. *Science* 334:204–208
10. Frens G (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nat Phys Sci* 241:20–22
11. Park S, Brown KA, Hamad-Schifferli K (2004) Changes in oligonucleotide conformation on nanoparticle surfaces by modification with mercaptohexanol. *Nano Lett* 4:1925–1929
12. Dulkeith E, Ringler M, Klar TA, Feldmann J (2005) Gold nanoparticles quench fluorescence by phase induced radiative rate suppression. *Nano Lett* 5:585–589
13. Liu X, Atwater M, Wang J, Qun H (2007) Extinction coefficient of gold nanoparticles with different sizes and different capping ligands. *Colloids Surf B* 58:3–7

Nanomaterial Interfaces in Biology

Methods and Protocols

Bergese, P.; Hamad-Schifferli, K. (Eds.)

2013, XI, 293 p. 73 illus., 53 illus. in color., Hardcover

ISBN: 978-1-62703-461-6

A product of Humana Press