Published in final edited form as:

Supramol Chem. 2015 ; 27(1-2): 123–126. doi:10.1080/10610278.2014.914627.

Probing the Protein–Nanoparticle Interface: The Role of Aromatic Substitution Pattern on Affinity

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Abstract

A new class of cationic gold nanoparticles has been synthesized bearing benzyl moieties featuring -NO₂ and -OMe groups to investigate the regioisomeric control of aromatic nanoparticle-protein recognition. In general, nanoparticles bearing electron withdrawing group demonstrated higher binding affinities towards green fluorescent protein (GFP) compared to electron-donating groups. Significantly, a ~7.5 and ~4.3 fold increase in binding with GFP was observed for -NO₂ groups in *meta*- and *para*-position respectively, while *ortho*-substitution showed similar binding compared to the unsubstituted ring. These findings demonstrated that nanoparticle-protein interaction can be controlled by the tuning the spatial orientation and the relative electronic properties of the aromatic substituents. This improved biomolecular recognition provides opportunities for enhanced biosensing and functional protein delivery to the cells.

Keywords

gold nanoparticles; aromatic substitution; nanoparticle-protein affinity

Introduction

Aromatic interactions, i.e. non-covalent forces involving the presence of aromatic moieties, play a critical role in the development of variety of biochemical processes including DNA duplexing (1), thermophilic protein stability (2) and biomacromolecular recognition (3). Arene interactions involved in substrate recognition by enzymes have also been of interest in *de novo* drug design (4) and synthetic biology (5). In particular, the modification of the electron density on the planes of the aromatic ring as well as the formation of dipoles by the introduction of a substituent, are determinant factors in aromatic interactions (6). Relevant results have also been indicated the importance of the geometry and the directionality of the aromatic groups, such as edge-face (H- π or “T-shaped” interaction), offset stacking (C- π or “parallel-dispaced” interaction) and face-to-face stacking (π - π stacking or “sandwich”) (7).

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Despite the fact that these interactions have been extensively studied in model systems, understanding these interactions in biological systems is challenging.

Monolayer protected nanoparticles (NPs) provide a versatile platform for biomolecular surface recognition (8) owing to their commensurate size and tunable surface functionalities for selective and/or specific interaction with the target biosystems (9). In prior studies, we have demonstrated the charge-complementary surface recognition and activity inhibition of chymotrypsin by monolayer protected gold NPs (10). Furthermore, the importance of surface hydrophobicity (11) on the stability of NP-protein complexes has also been established. However, how spatial orientation of aromatic groups on the NP surface and their relative electronic properties dictate the interactions with proteins has not been systematically explored.

Herein, we report the regioisomeric effect of the electron-withdrawing and electron-donating groups on the aromatic NP-protein interactions. To study this effect, we have synthesized seven positively charged ~2 nm core-diameter gold NPs (NP1-NP7) that feature benzyl group-terminated monolayers with nitro groups of strong electron-withdrawing character (NP2, NP3, NP4), and methoxy groups that are pi-electron-donating (NP5, NP6, NP7) (Figure 1). All of the NPs feature similar physicochemical properties (charge and size, see supporting information). A critical point in the design of these NPs is the inclusion of a non-interacting biocompatible spacer that prevents aggregation, and more importantly, allows specific chemical groups to be exposed to the NP surface (the interacting zone) (12). Based on this model, ligands having aromatic rings featuring different electron density profiles on the NP surface should provide a direct platform to examine both the electronic and regioisomeric effect on particle-protein interactions.

Results and discussion

Green fluorescent protein (GFP) was chosen as the target protein to probe the NP-protein complex stability profile. GFP is a beta barrel-shaped protein that features negatively charged surface at physiological pH (pI 5.92) (13). As gold NPs are positively charged, they can efficiently bind with GFP resulting in the fluorescence quenching of this protein (14). Moreover, as GFP dimer contacts consist of a core of hydrophobic/aromatic residues (15), it is expected that a change in the aromatic configuration of NPs can affect the NP-GFP interaction. To study this model system, we performed fluorescence titration studies between GFP and the different NPs in 5 mM sodium phosphate buffer (pH= 7.4), monitoring the change in fluorescence intensity of GFP at 510 nm (λ_{em} = 475 nm). The resulting titration plots were analysed using nonlinear least-squares curve-fitting analysis to obtain the physicochemical parameters of the binding process, namely the binding constant (K_s) and the stoichiometries (n) (16). The concentration of GFP was kept constant at 125 nM and the fluorescence was recorded while varying the concentration of NPs from 0–400 nM (titrations performed in triplicate, see supporting information). Figure 2a depicts a typical titration plot of GFP with NP1 evidencing the quenching capabilities of these particles that allows the study of the binding affinity.

The physicochemical data of the binding process for NP1-NP7 towards GFP are shown in Figure 1. By comparing the magnitude of the binding constants, it is evident that the presence of methoxy groups (NP2, NP3, and NP4), in general, exhibits a larger binding affinity compared to the nitro moieties (NP5, NP6, and NP7). This difference in affinity can be rationalized by the fact that electron-withdrawing substituents (nitro) reduce the electron density of the aromatic ring (as observed in Figure 1), thereby presumably enhancing the interaction with negatively charged GFP. On the other hand, the methoxy groups do not affect the electron density of the aromatic ring at the same extent as nitro groups, providing lower binding affinities comparative to these groups.

Among the NPs with methoxy groups (NP5-NP7), an increase in affinity towards GFP is expected in the *ortho-meta-para* series, as the electronic effect generated by the quaternary nitrogen at C-1 is compensated less efficiently (less electron density over the quaternary group) (Figure 1). However, this trend is followed only by NP5 and NP7, while NP6 has the lowest affinity in this group. A computational analysis revealed that in the most stable conformation of these aromatic residues, the potentially-interacting aromatic planes are less exposed due to a steric effect provided by the methoxy group, reducing their capability of interaction. (Figure 1, side view). Such effect introduces another variable to the system, changing significantly the extent of the interactions.

In the case of the electron-withdrawing groups (NP2-NP4), steric considerations are not to blame for the differences in the *ortho-meta-para* series as the -NO₂ aligns with the plane of the ring. However, the exposure of the -NO₂ group that can participate in dipole/dipole interactions on its own, may induce additional binding ability in the *meta*- and *para*-positions, interactions that may be reduced in the *ortho*-position, due to the relative low exposure. Such difference elicits a significant impact on the binding affinity; conferring NP3 and NP4 the largest values of K_s compared to NP1 (~7.5 and ~4.3 fold respectively) (Figure 2b). This result mirrors the case of flavin recognition via dipole-containing aromatic systems, where electron-withdrawing fluorophenyl ring showed favourable interaction with flavin ring when fluorine atom was placed in the *meta*- and *para*-positions but interacted less favourably when the fluorine was placed in *ortho*-position (compared to unsubstituted phenyl) (17). Significantly, Rashkin and Waters also demonstrated that the electronegative substituent in *meta*-position showed largest offset π - π stacking interactions between two phenyl rings in water due to direct interaction of the substituents to the aromatic systems (18). This direct interaction of substituents can significantly alter the binding process and can favourably influence the NP-protein complexation (*vide supra*). Nonetheless, both the nature of the substitution and their relative position on the aromatic NP surface can lead to substantial alterations in the particle affinity towards a given protein.

Conclusions

In summary, we have synthesized a series of cationic gold NPs having electron-donating and electron-withdrawing benzyl moieties and investigated their interaction with GFP. In general, electron-withdrawing substituents showed higher binding affinities compared to electron-donating groups. Significantly, electron-withdrawing groups in the *meta*- and *para*-positions showed ~7.5 and ~4.3 fold increase in binding while *ortho*-substitution showed

similar binding compared to the unsubstituted ring, demonstrating a regioisomeric control over aromatic NP-protein interactions. This study demonstrates that NPs can be engineered to harness improved binding affinity to a particular protein by simply changing the aromatic substitution on their functional headgroup. The significance of this work is twofold: 1) harnessing specific molecular recognition by tuning NP surface can help in develop strategies for biosensing and functional protein delivery inside the cells.; 2) higher binding affinity of NPs towards a given protein provides enhanced thermodynamic stability of NP-protein conjugate in complex matrices including cell culture media, serum, and plasma. In particular, this regioisomeric control on the stability and the formation of a 'protein-corona' (19) on NP surface in serum is currently under investigation and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the NIH: (GM077173 and EB014277, VR) and MRSEC facilities (DMR-0820506). Zeynep Ekmekci acknowledges TUBITAK for supporting her stay at the University of Massachusetts Amherst through Program 2214. She also acknowledges her Ph.D. supervisor Prof. Dr. Metin Balci.

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Figure 1.

Chemical structures of the benzyl-terminated gold nanoparticles used in protein recognition studies. The ligand design features a hydrophobic interior for particle stability, a tetra(ethylene glycol) spacer for biocompatibility and solubility, and the interacting aromatic headgroup. The electrostatic potential maps (EPM) of the different benzyl NP headgroups depict the effect of the substituent over the aromatic electron density. The side view of NP5-NP7 evidence the steric hindrance of these particles. These regioisomeric parameters affect the binding parameters, (binding constant (K_s)) and the stoichiometries (n)) of each NP towards GFP. Errors represent the standard error of the mean. Computational calculations

were performed using Gaussian 03 under a DFT/B3LYP level of theory and 6-311G++** basis set, and the EPMs were generated using a total density cube (coarse, SCF) and a mapped surface with an isovalue of 0.0004.

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Figure 2.

a) Fluorescence titration plot for the complexation of GFP with NP1. The change in GFP emission was monitored at 510 nm (excitation= 475 nm) with increasing NP concentrations (0–250 nM, in case of NP1). b) Fold increase of the binding affinities for NP2-NP7 towards GFP, compared to NP1.