Joint Graduate Presentation
Department of Microbiology

# Nanogold technology in Microbiology

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# 1. Introduction

Current Applications of Nanotechnology:

Applications in Medical Sciences:

- 1. Antimicrobial (Silver)
- 2. Biomolecule detection (Gold)
- 3. Drug delivery (Carbon)

# 2. Underlying principles

- Physical properties of gold nanoparticles

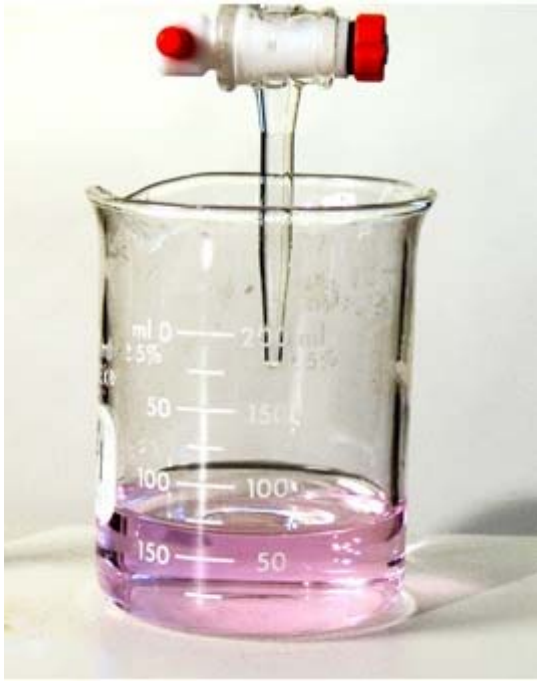
- Detection is based on:
- For large nanogold particles (>200nm)
  - Precipitation of gold on target molecules
  - Net result: gold is seen on surface as deposits

# 2. Underlying principles

- Large nanogold particles
  - Similar physical properties as its bulk material
    - Yellow in colour
    - The principle of detection is the precipitation of gold particles onto target
    - Have advantage of ease in detection (can be detected by a CCD camera alone)

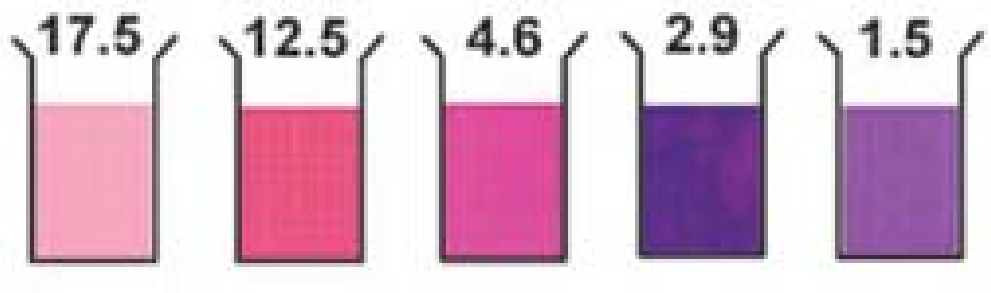
# 2. Underlying principles

- For small nanogold particles (2-100nm)
  - Change in absorption wavelength by plasmon resonance (i.e. colour is not gold anymore)
  - Net result: As a powerful red dye, or act as a distance dependent reporters



# 2. Underlying principles

- For small nanogold particles (2-100nm)
- Give different physical properties than its bulk material
  - Change from red to blue when gold particles bring close together (i.e. distance dependent)




- Martin Holtzhauer, Michael Rudolph, Application of colloidal gold for characterization of supports used in size-exclusion chromatography, Journal of Chromatography A, Volume 605, Issue 2, 17 July 1992, Pages 193-198
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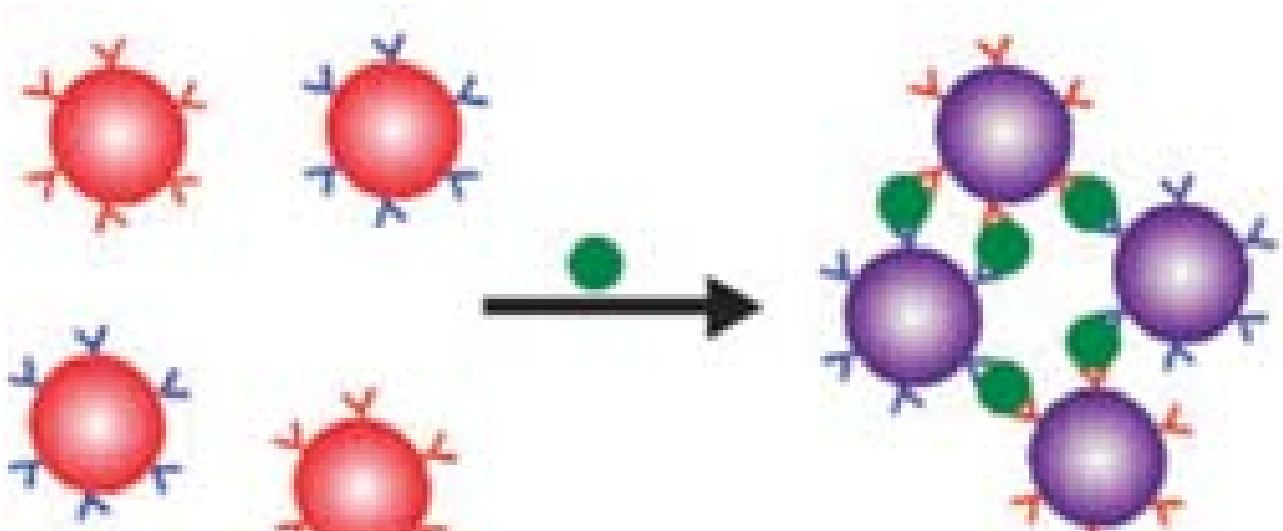
# 3. Applications in biochemical methods

- Nanogold particles can be conjugated to antibodies, primers, or oligopeptides by interaction of thiol groups

- It can also conjugate to chemicals like aminodextran

# 3 Applications in biochemical methods

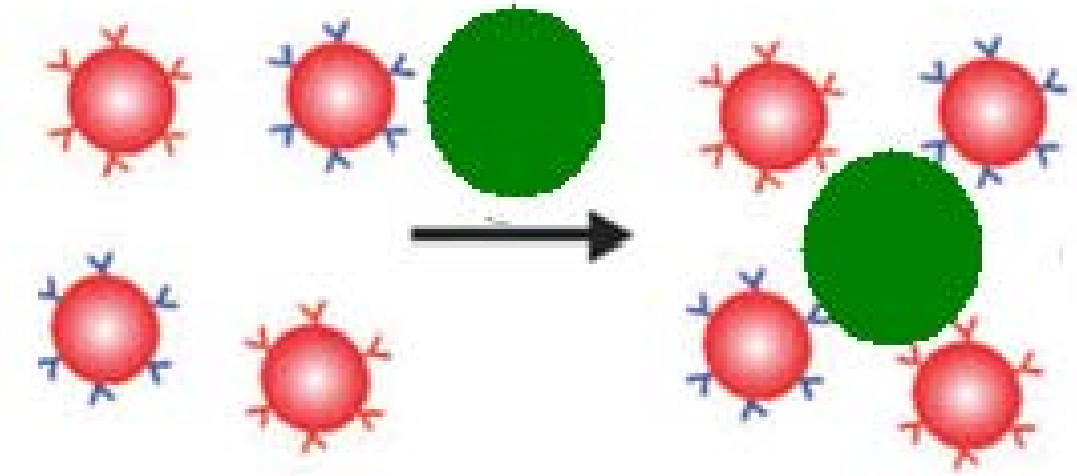
- Distance dependent detection of antigens
  - Conjugate to antibodies
  - Antigen + Au-Antibodies → Nanogold particles bring close together



- Martin Holtzhauer, Michael Rudolph, Application of colloidal gold for characterization of supports used in size-exclusion chromatography, Journal of Chromatography A, Volume 605, Issue 2, 17 July 1992, Pages 193-198

# 3 Applications in biochemical methods

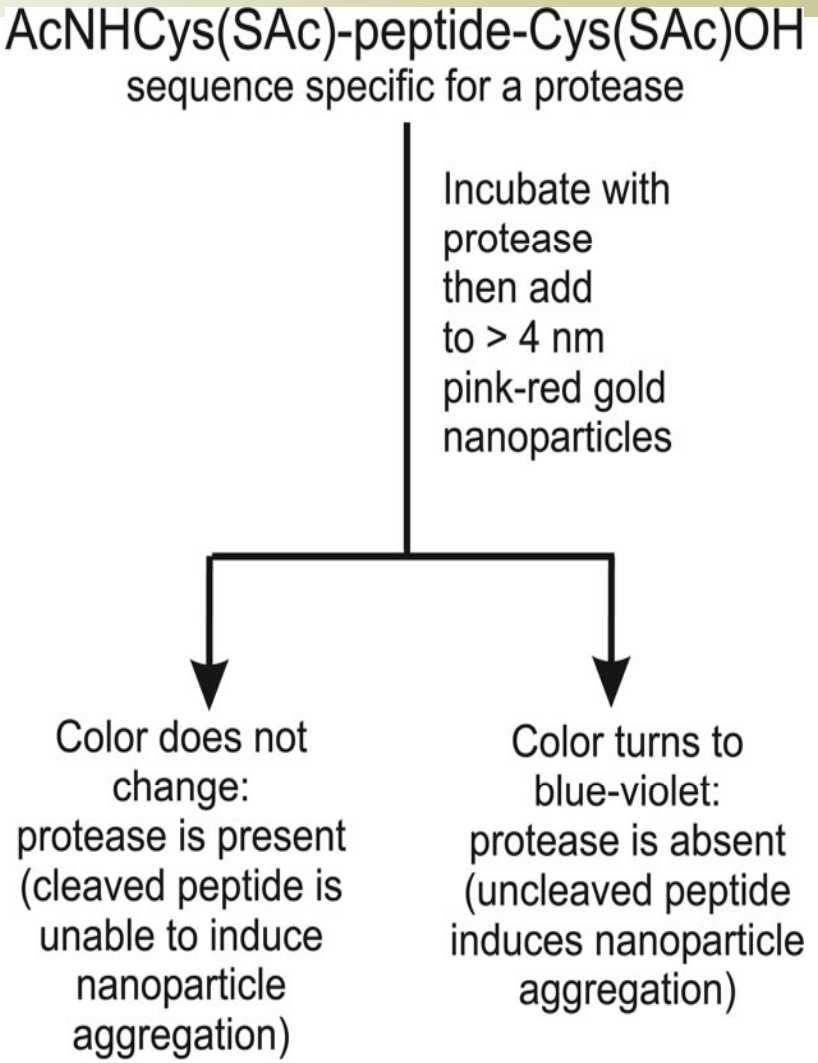
- Large antigens…



- Solution: Use bigger size nanogold particles

# 3 Applications in biochemical methods

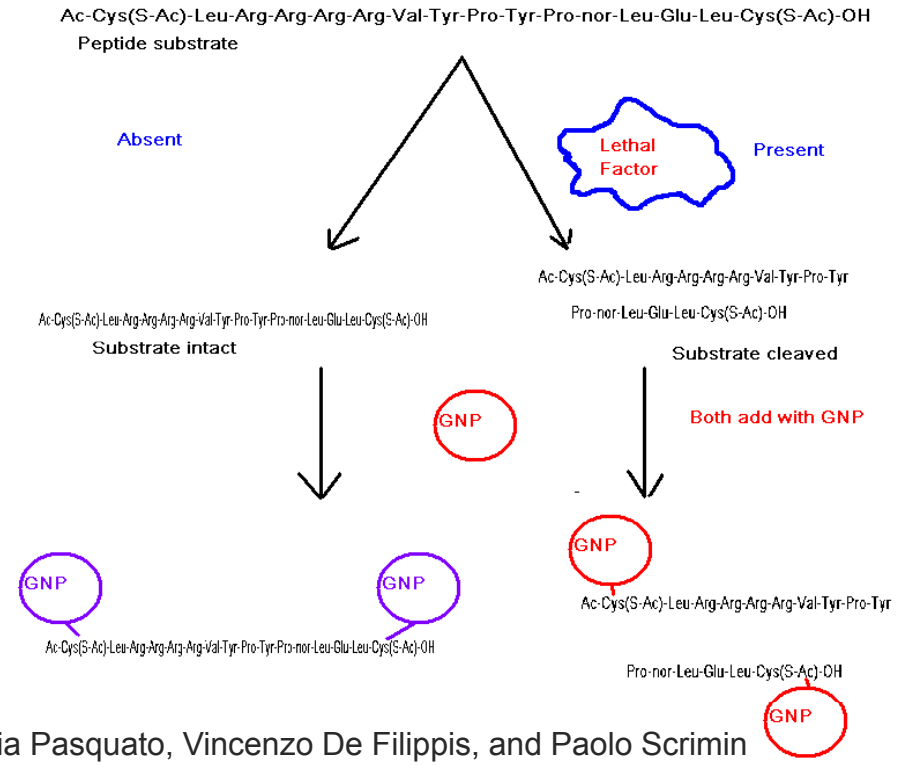
- Detection of enzymatic activity
  - E.g. Substrate specific protease:




- Cristian Guarise, Lucia Pasquato, Vincenzo De Filippis, and Paolo Scrimin
  **Gold nanoparticles-based protease assay**
  PNAS 2006 103: 3978-3982

# Applications in Microbiology

- Detection of enzyme activity
  - Anthrax Lethal factor – Detection of *Bacillus anthracis*




  - Cristian Guarise, Lucia Pasquato, Vincenzo De Filippis, and Paolo Scrimin
    **Gold nanoparticles-based protease assay**
    PNAS 2006 103: 3978-3982

# 3 Applications in biochemical methods

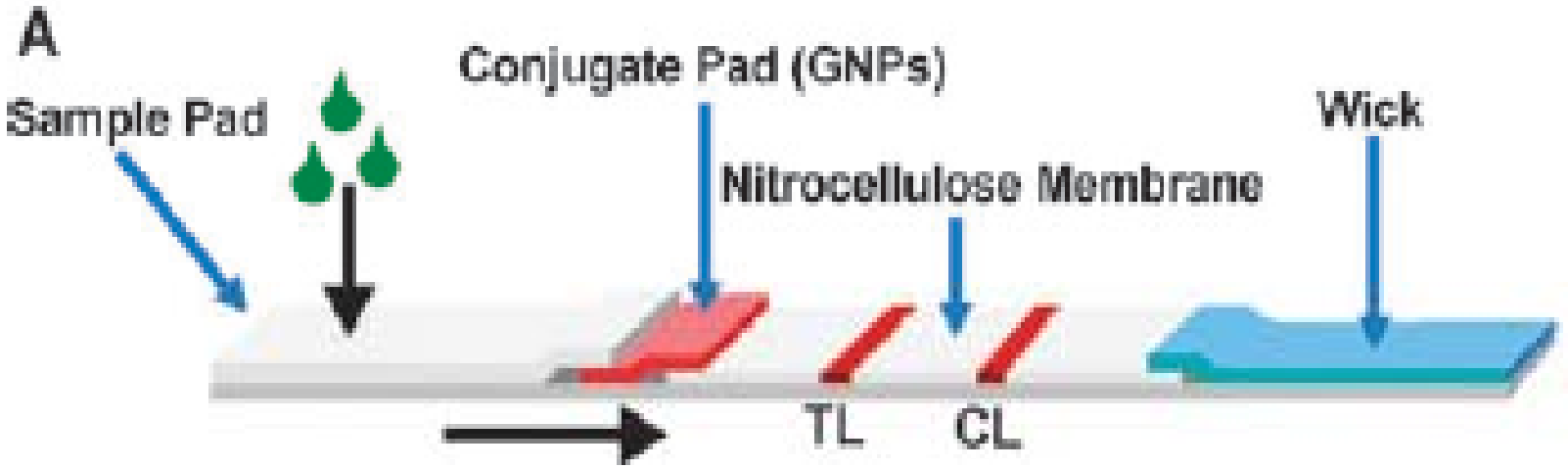
- Under investigations:
  - SNP detection by ligase reaction
  - Detection of amplified nucleic acid products

A colorimetric method for point mutation detection using high-fidelity DNA ligase
Jishan Li, Xia Chu, Yali Liu, Jian-Hui Jiang, Zhimin He, Zhiwei Zhang, Guoli Shen, and Ru-Qin Yu
Nucleic Acids Res. 2005; 33(19): e168

Eric Tan, Barbara Erwin, Shale Dames, Karl Voelkerding, and Angelika Niemz Isothermal DNA Amplification with Gold Nanosphere-Based Visual Colorimetric Readout for Herpes Simplex Virus Detection , Clin Chem 2007 53: 2017-2020

# 3 Applications in biochemical methods

- Nanogold as a dye
  - Lateral flow device




R **Wilson** - Chemical Society Reviews, 2008

# 3 Applications in biochemical methods

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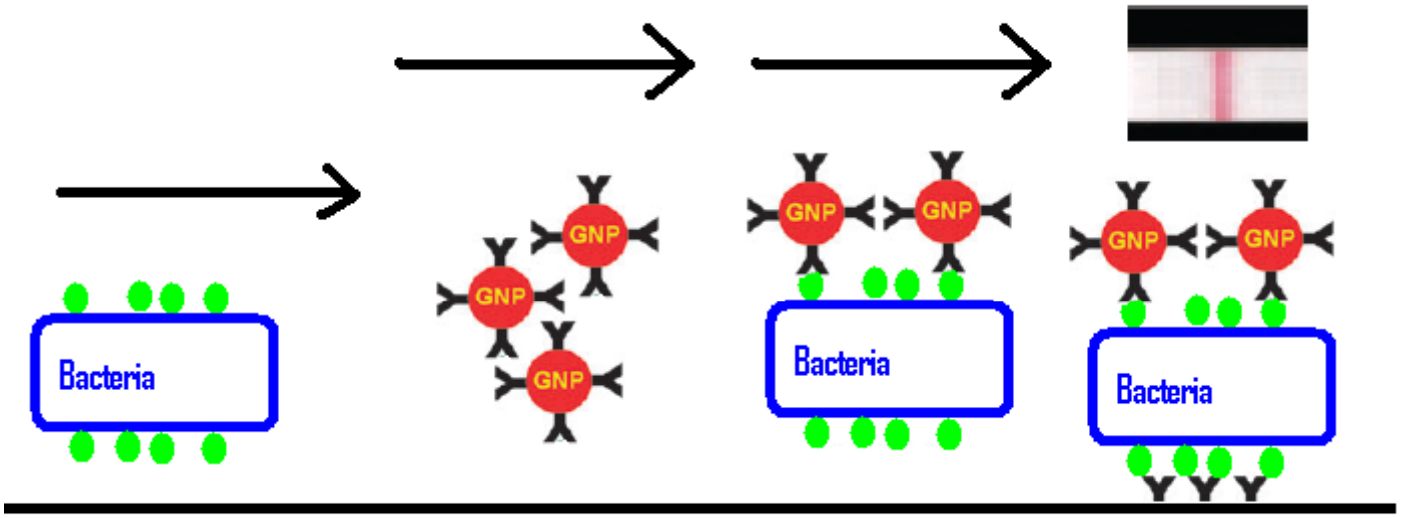
  - Lateral flow device




# Applications in Microbiology

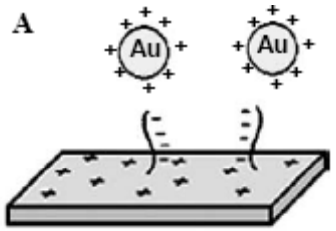
- Lateral flow devices:

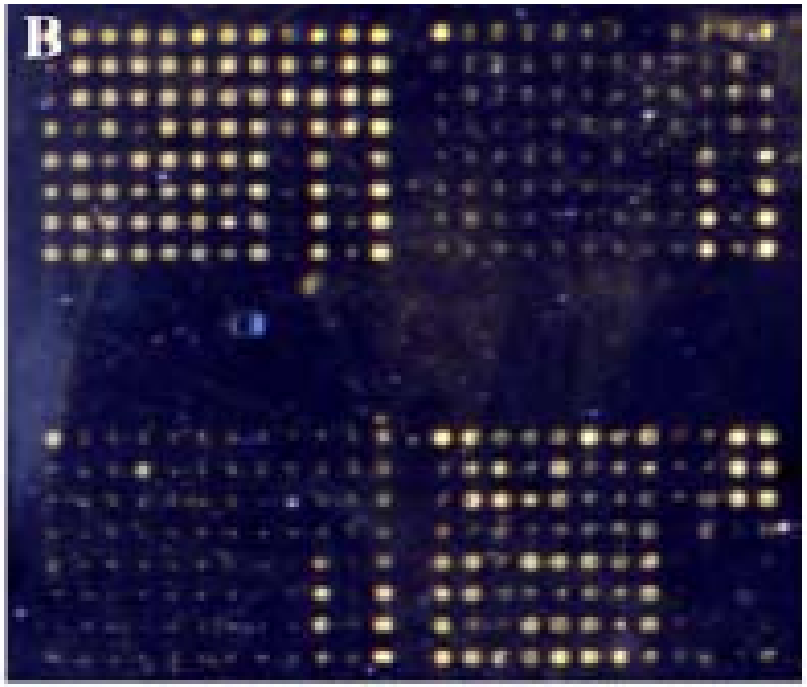
- Immunochromatographic test strip against *V. harveyi* in aquaculture
- immunochromatographic assay for the detection of *S.aureus* in food


- Paisarn Sithigorngul, Sombat Rukpratanporn, Nilawan Pecharaburanin, Pornthip Suksawat, Siwaporn Longyant, Parin Chaivisuthangkura, Weerawan Sithigorngul, A simple and rapid immunochromatographic test strip for detection of pathogenic isolates of Vibrio harveyi, Journal of Microbiological Methods, Volume 71, Issue 3, December 2007, Pages 256-264
- Su-Hua Huang, Gold nanoparticle-based immunochromatographic test for identification of Staphylococcus aureus from clinical specimens, Clinica Chimica Acta, Volume 373, Issues 1-2, November 2006, Pages 139-143

# 3 Applications in biochemical methods

- Cationic 250nm nanogold particles
- Stronger attraction to Hybridized nucleotides (more negative)

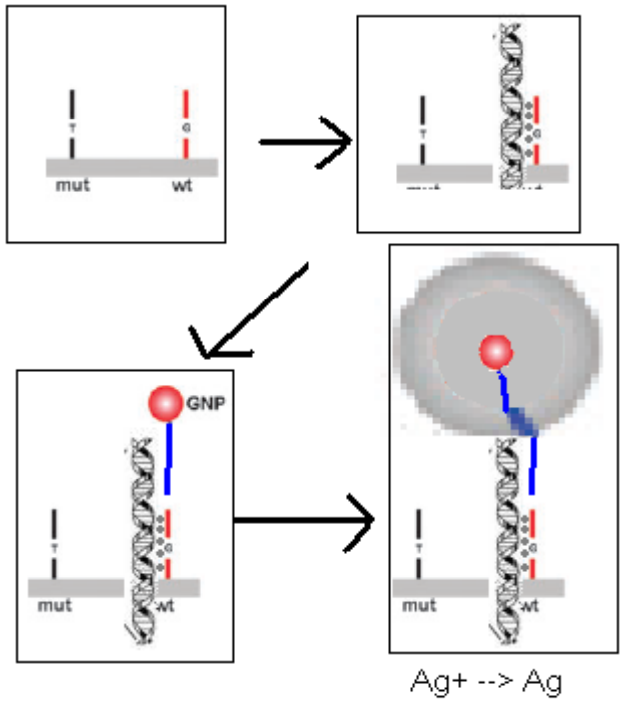



- Y **Sun**, WH Fan, MP McCann, V Golovlev - Analytical biochemistry, 2005

# 3 Applications in biochemical methods

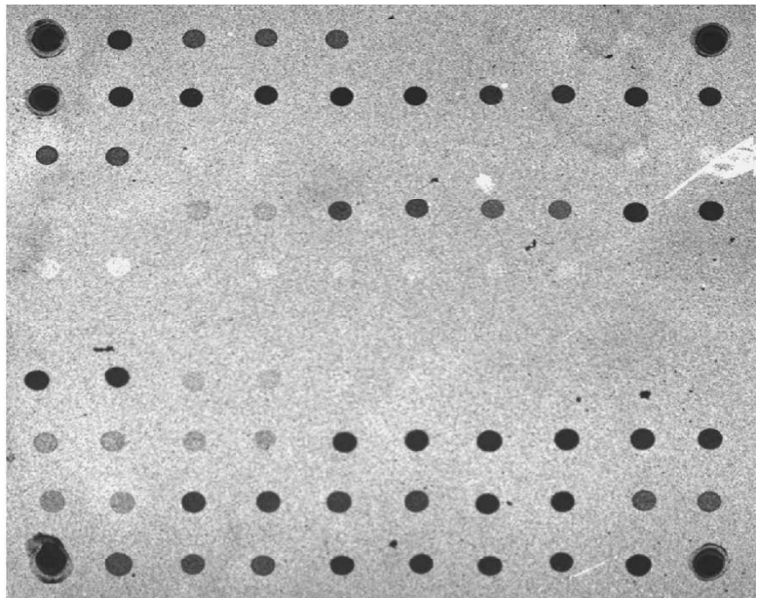
- Detection of unamplified sequence from genomic DNA, with silver enhancement



# Applications in Microbiology

- Microarray for the screening of antibiotic resistance of *S.Aureus* and *M.tuberculosis*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 91: POSITION MARKER | 92: aphA3, 2 | 93: aphA3, 3 | 94: aphA3, 3 | 95: aphA3, 4 | 96: linA, 1 | 97: linA, 2 | 98: linA, 3 | 99: linA, 4 | 100: POSITION MARKER |
| 81: POSITION MARKER | 82: sat, 1 | 83: sat, 2 | 84: sat, 2 | 85: sat, 3 | 86: sat, 3 | 87: sat, 4 | 88: sat, 4 | 89: aphA3, 1 | 90: aphA3, 1 |
| 71: norA, 4 | 72: norA, 4 | 73: tst-1, 1 | 74: tst-1, 1 | 75: tst-1, 2 | 76: tst-1, 2 | 77: tst-1, 3 | 78: tst-1, 3 | 79: tst-1, 4 | 80: tst-1, 4 |
| 61: tetK, 3 | 62: tetK, 3 | 63: tetK, 4 | 64: tetK, 4 | 65: norA, 1 | 66: norA, 1 | 67: norA, 2 | 68: norA, 2 | 69: norA, 3 | 70: norA, 3 |
| 51: tetM, 2 | 52: tetM, 2 | 53: tetM, 3 | 54: tetM, 3 | 55: tetM, 4 | 56: tetM, 4 | 57: tetK, 1 | 58: tetK, 1 | 59: tetK, 2 | 60: tetK, 2 |
| 41: aphD, 1 | 42: aphD, 1 | 43: aphD, 2 | 44: aphD, 2 | 45: aphD, 3 | 46: aphD, 3 | 47: aphD, 4 | 48: aphD, 4 | 49: | 50: |
| 31: ermC, 4 | 32: ermC, 4 | 33: ermA, 1 | 34: ermA, 1 | 35: ermA, 2 | 36: ermA, 2 | 37: ermA, 3 | 38: ermA, 3 | 39: ermA, 4 | 40: ermA, 4 |
| 21: blaZ, 3 | 22: blaZ, 3 | 23: blaZ, 4 | 24: blaZ, 4 | 25: ermC, 1 | 26: ermC, 1 | 27: ermC, 2 | 28: ermC, 2 | 29: ermC, 3 | 30: ermC, 3 |
| 11: coA, 1 | 12: coA, 2 | 13: coA, 3 | 14: coA, 3 | 15: coA, 4 | 16: coA, 4 | 17: blaZ, 1 | 18: blaZ, 1 | 19: blaZ, 2 | 20: blaZ, 2 |
| 1: POSITION MARKER | 2: mecA, 1 | 3: mecA, 2 | 4: mecA, 2 | 5: mecA, 3 | 6: mecA, 3 | 7: mecA, 4 | 8: mecA, 4 | 9: coA, 1 | 10: POSITION MARKER |



- S. Monecke, I. Leube and R. Ehricht, Genome Lett., 2003, 2, 106

# Conclusion

- When the size of the gold nanoparticles decrease, they possess different optical and physical characteristics

- Such characteristics can be utilized in many biochemical tests, and therefore useful in microbiology

# References


- S. Monecke, I. Leube and R. Ehricht, Genome Lett., 2003, 2, 106
- Paisarn Sithigorngul, Sombat Rukpratanporn, Nilawan Pecharaburanin, Pornthip Suksawat, Siwaporn Longyant, Parin Chaivisuthangkura, Weerawan Sithigorngul, A simple and rapid immunochromatographic test strip for detection of pathogenic isolates of Vibrio harveyi, Journal of Microbiological Methods, Volume 71, Issue 3, December 2007, Pages 256-264
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- Y Sun, WH Fan, MP McCann, V Golovlev - Analytical biochemistry, 2005

# References


- A colorimetric method for point mutation detection using high-fidelity DNA ligase, Jishan Li, Xia Chu, Yali Liu, Jian-Hui Jiang, Zhimin He, Zhiwei Zhang, Guoli Shen, and Ru-Qin Yu, Nucleic Acids Res. 2005; 33(19): e168
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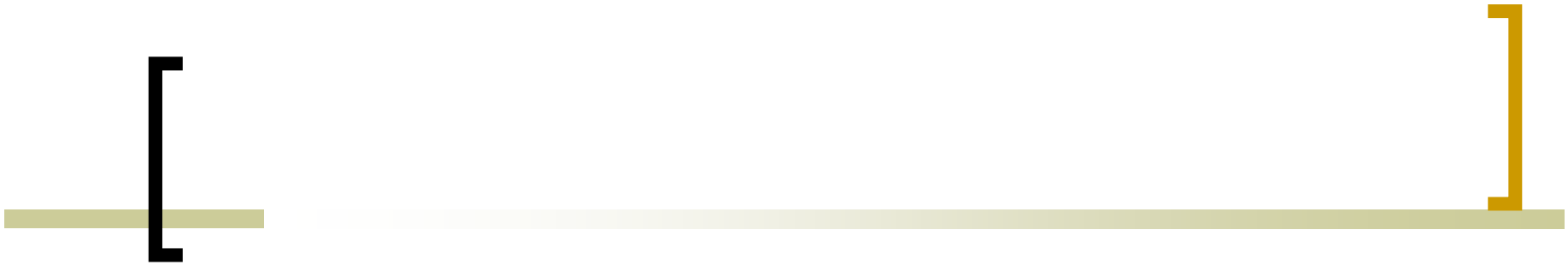
- R Wilson - Chemical Society Reviews, 2008



End

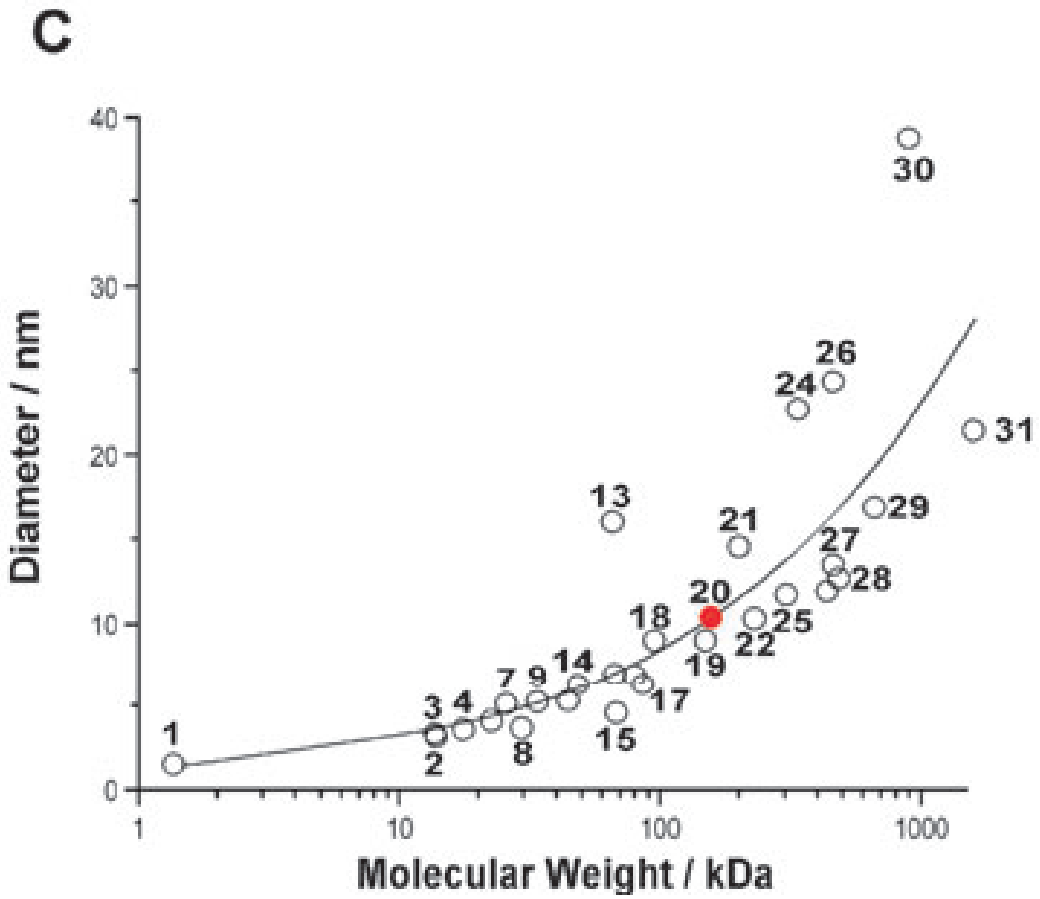
# Detection sensitivity

- Protease assay: 25nM LF(by eye)
- Lateral flow device: 100 thousand cfu
- DNA probe: $10^{-11}$mol target DNA (eye)

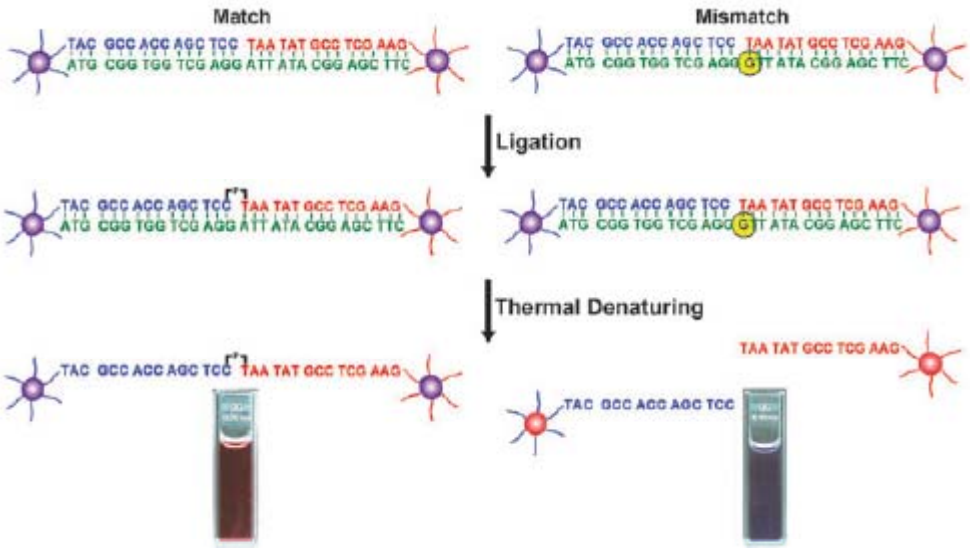
# Colour Intensity

- Sybr safe: 7.3 x $10^{4}$M
- Nanogold (10nm):~1 x $10^{8}$M
- Nanogold (80nm): 5 x $10^{10}$M

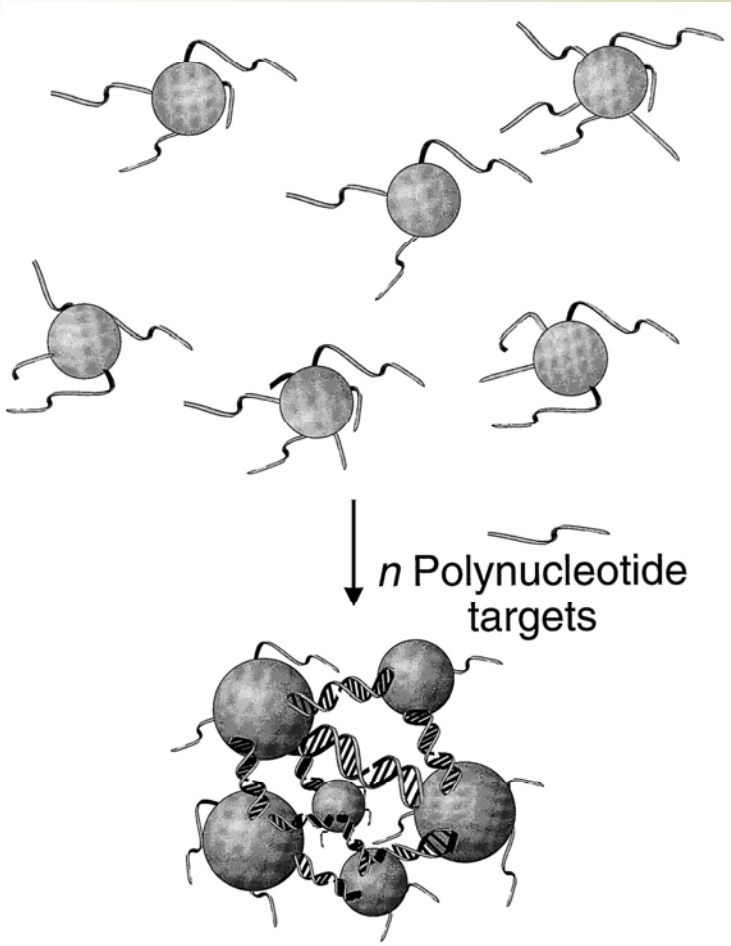
# Size and max. distance



# SNP detection



# Amplified products



# DIY nanogold (10-20nm)

- Use: conjugate to antibody
- 1. Disintergrate

- Aqua regia (nitro-hydrochloric acid) +
- Gold
- → Chloroauric acid

# DIY nanogold (10-20nm)

- 2. Assemble
- Take $5.0 \times 10^{-6}$ mol of $HAuCl_4$, dissolve it in 19 ml of deionized water (the result should be a faintly yellowish solution).
- Heat it until it boils.
- Continue the heating and, while stirring vigorously, add 1 ml of 0.5% sodium citrate solution; keep stirring for the next 30 minutes.
- The colour of the solution will gradually change from faint yellowish to clear to grey to purple to deep purple, until settling on wine-red.
- Add water to the solution as necessary to bring the volume back up to 20 ml (to account for evaporation).

# Specificity and sensitivity