Electronic Supplementary Information

Probing the *Qi* of Traditional Chinese Herbal Medicines by the Biological Synthesis of Nano-Au

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1. Materials

1.1.Chemicals

HAuCl₄·4H₂O (99.99%) was purchased from Sigma-Aldrich, Germany, and used as received. Deionized water purified by a Milli-Q System (Millipore, Billerica, MA, USA) was used as the universal solvent.

Yin & Yang	"Si Qi"	TCHMs*	Chinese Name	Scientific Name	
	Hot	a1	Gan Jiang	Zingiberis Rhizoma	
	Hot	a2	La Jiao	Red Chili	
	Hot	a3	Bi Ba	Piper longum L	
Vana	Hot	a4	Rou Gui	Cinnamomi Cortex	
Tang	Warm	b1	Gui Zhi	Cmnamomi Mmulus	
	Warm	b2	Ai Ye	Artemisiae Argyi Folium	
	Warm	b3	Cang Shu	Atractlodis Rhizoma	
	Warm	b4	Rou Cong Rong	Cistanches Herba	
	Cool	c1	Tu Da Huang	Rumex madaio Makino	
	Cool	c2	Bo He	Menthae Haplocalycis Herba	
	Cool	с3	Xi Yang Shen	Panacis Quinquefolii Radix	
Yin	Cool	c4	Lv Cha	Rizhao Green tea	
	Cold	d1	Jin Yin Hua	Lonicerae Japonicae Flos	
	Cold	d2	Pu Gong Ying	Mongolian Dandelion Herb	
	Cold	d3	Ye Ju Hua	Chrysanthemi Indici Flos	
	Cold	d4	Shi Hu	Dendrobii Caulis	

1.2. List of TCHMs used in this study

*The selection was based on the consultation of two authoritative classical texts of TCM (i.e. *Shennong's Classic of Materia Medica* and *Compendium of Materia Medica*) and the confirmation of the representativeness of the "*Si Qi*" properties by TCM practitioners. All TCHMs were purchased from a local Chinese medical hall, Tonrentang Chinese Medicine, Singapore Branch.

2. Supplementary Text

Statistical Analysis and Detailed Calculations

2.1. Attributes of the Qi-related features used for classification

(i) Solution color attribution: warm color (*w*), and cool color (*c*).

(ii) SPR intensity: increasing (*i*), and decreasing (*d*).

(iii) Reaction time: t < 1 h, 1 h $\leq t < 2$ h, and $t \geq 2$ h.

(iv) Morphology of nano-Au as examined by TEM: NPs & NFs, NPs only, NP & SA-

SNs, and SA-SNSs only (including the SA-NSs and SA-NFs).

2.2. Detailed calculations of the Discrimination Probabilities of each *Qi*-related feature between two different *Qi* families

2.2.1. hot vs. warm

$$\begin{split} P_{color} &= P_w^{hot} \times P_c^{warm} + P_c^{hot} \times P_w^{warm} = 1 \times \frac{1}{4} + 0 \times \frac{3}{4} = \frac{1}{4} \\ P_{SPR} &= P_i^{hot} \times P_d^{warm} + P_d^{hot} \times P_i^{warm} = 1 \times 0 + 0 \times 1 = 0 \\ P_{Time} &= P_{t < 1h}^{hot} \times P_{1h \le t < 2h}^{warm} + P_{t < 1h}^{hot} \times P_{t \geq 2h}^{warm} + P_{1h \le t < 2h}^{hot} \times P_{t < 1h}^{warm} + P_{1h \le t < 2h}^{hot} \\ &\times P_{t \geq 2h}^{warm} + P_{t \geq 2h}^{hot} \times P_{t < 1h}^{warm} + P_{t \geq 2h}^{hot} \times P_{1h \le t < 2h}^{warm} \\ &= 0 \times 0 + 0 \times 0 + \frac{1}{4} \times 1 + \frac{1}{4} \times 0 + \frac{3}{4} \times 1 + \frac{3}{4} \times 0 = 1 \\ P_{TEM} &= P_{NPs \& NFs}^{hot} \times P_{NPs only}^{warm} + P_{NPs \& NFs}^{hot} \times P_{NPs \& NFs}^{warm} \\ &+ P_{NPs only}^{hot} \times P_{NPs \& NFs}^{warm} + P_{NPs \& SA-NSs}^{hot} + P_{NPs \& SA-SNS only}^{hot} \\ &+ P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& NFs}^{warm} + P_{NPs \& SA-SNS}^{hot} + P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& SA-SNS}^{warm} \\ &+ P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& NFs}^{warm} + P_{NPs \& SA-SNS}^{hot} + P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& SA-SNS}^{warm} \\ &+ P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& NFs}^{warm} + P_{NPs \& SA-SNS}^{hot} + P_{NPs \& SA-SNS}^{hot} \times P_{NPs \& SA-SNS}^{warm} \\ &= 1 \times \frac{1}{2} + 1 \times 0 + 1 \times \frac{1}{4} + 0 \times 0 + 0 \times \frac{1}{4} +$$

2.2.2 *hot* vs. *cool*

$$\begin{aligned} P_{color} &= P_w^{hot} \times P_c^{cool} + P_c^{hot} \times P_w^{cool} = 1 \times \frac{3}{4} + 0 \times \frac{1}{4} = \frac{3}{4} \\ P_{SPR} &= P_i^{hot} \times P_d^{cool} + P_d^{hot} \times P_i^{cool} = 1 \times \frac{1}{4} + 0 \times \frac{3}{4} = \frac{1}{4} \\ P_{Time} &= P_{t < 1h}^{hot} \times P_{1h \le t < 2h}^{cool} + P_{t < 1h}^{hot} \times P_{t \geq 2h}^{cool} + P_{1h \le t < 2h}^{hot} \times P_{t < 1h}^{cool} + P_{t > 2h}^{hot} \times P_{1h \le t < 2h}^{cool} \\ &\times P_{t \geq 2h}^{cool} + P_{t \geq 2h}^{hot} \times P_{t < 1h}^{cool} + P_{t > 2h}^{hot} \times P_{1h \le t < 2h}^{cool} \\ &= 0 \times \frac{1}{4} + 0 \times \frac{1}{4} + \frac{1}{4} \times \frac{1}{2} + \frac{1}{4} \times \frac{1}{4} + \frac{3}{4} \times \frac{1}{2} + \frac{3}{4} \times \frac{1}{4} = \frac{3}{4} \end{aligned}$$

$$\begin{split} P_{TEM} &= P_{NPs \ \& \ NFs}^{hot} \times P_{NPs \ only}^{cool} + P_{NPs \ \& \ NFs}^{hot} \times P_{NPs \ \& \ SA-NSs}^{cool} + P_{NPs \ \& \ NFs}^{hot} \times P_{SA-NSs \ only}^{cool} \\ &+ P_{NPs \ only}^{hot} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-NSs}^{hot} + P_{NPs \ only}^{hot} \times P_{SA-SNSs \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs}^{hot} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNS}^{hot} + P_{NPs \ \& \ SA-SNSs \ only}^{hot} \times P_{SA-SNSs \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs}^{hot} \times P_{NPs \ \& \ SA-SNS}^{cool} \times P_{NPs \ \& \ SA-SNS}^{cool} + P_{NPs \ \& \ SA-SNS \ Sonly}^{hot} \times P_{NPs \ \& \ SA-SNS \ Sonly}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs \ only}^{hot} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \\ &+ P_{SA-SNSs \ only}^{hot} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNS \ only}^{hot} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \times P_{NPs \ \& \ SA-SNS \ only}^{cool} \\ &= 1 \times \frac{1}{4} + 1 \times \frac{1}{4} + 1 \times \frac{1}{2} + 0 \times 0 + 0 \times \frac{1}{4} + 0 \times \frac{1}{2} + 0 \times 0 + 0 \times \frac{1}{4} + 0 \times \frac{1}{4} = 1 \end{split}$$

2.2.3. hot vs. cold

$$\begin{aligned} P_{color} &= P_w^{hot} \times P_c^{cold} + P_c^{hot} \times P_w^{cold} = 1 \times 1 + 0 \times \frac{1}{2} = 1 \\ P_{SPR} &= P_i^{hot} \times P_d^{cold} + P_d^{hot} \times P_i^{cold} = 1 \times \frac{1}{2} + 0 \times 0 = \frac{1}{2} \\ P_{Time} &= P_{t \leq 1h}^{hot} \times P_{1h \leq t < 2h}^{cold} + P_{t \leq 1h}^{hot} \times P_{t \geq 2h}^{cold} + P_{1h \leq t < 2h}^{hot} \times P_{t \leq 1h}^{cold} + P_{1h \leq t < 2h}^{hot} \\ &\times P_{t \geq 2h}^{cold} + P_{t \geq 2h}^{hot} \times P_{t \leq 1h}^{cold} + P_{t \geq 2h}^{hot} \times P_{1h \leq t < 2h}^{cold} \\ &= 0 \times \frac{1}{4} + 0 \times 0 + \frac{1}{4} \times \frac{3}{4} + \frac{1}{4} \times 0 + \frac{3}{4} \times \frac{3}{4} + \frac{3}{4} \times \frac{1}{4} = \frac{15}{16} \\ P_{TEM} &= P_{NPs \& NFs}^{hot} \times P_{NPs & nly}^{cold} + P_{NPs \& NFs}^{hot} \times P_{NPs \& SA-NSs}^{cold} + P_{NPs & nly}^{hot} \times P_{SA-SNSs & nly}^{cold} \\ &+ P_{NPs & only}^{hot} \times P_{NPs \& NFs}^{cold} + P_{NPs \& SA-SNs}^{hot} \times P_{NPs & SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{hot} \times P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{hot} \times P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{hot} + P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{hot} \times P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{hot} + P_{NPs \& SA-SNs}^{cold} + P_{NPs \& SA-SNs}^{cold}$$

2.2.4. warm vs. cool

$$\begin{aligned} P_{color} &= P_{w}^{warm} \times P_{c}^{cool} + P_{c}^{warm} \times P_{w}^{cool} = \frac{3}{4} \times \frac{3}{4} + \frac{1}{4} \times \frac{1}{4} = \frac{5}{8} \\ P_{SPR} &= P_{i}^{warm} \times P_{d}^{cool} + P_{d}^{warm} \times P_{i}^{cool} = 1 \times \frac{1}{4} + 0 \times \frac{3}{4} = \frac{1}{4} \\ P_{Time} &= P_{t < 1h}^{warm} \times P_{1h \le t < 2h}^{cool} + P_{t < 1h}^{warm} \times P_{t \geq 2h}^{cool} + P_{1h \le t < 2h}^{warm} \times P_{t < 1h}^{cool} + P_{t \geq 2h}^{warm} \times P_{1h \le t < 2h}^{cool} \\ &\times P_{t \geq 2h}^{cool} + P_{t \geq 2h}^{warm} \times P_{t < 1h}^{cool} + P_{t \geq 2h}^{warm} \times P_{1h \le t < 2h}^{cool} \\ &= 1 \times \frac{1}{4} + 1 \times \frac{1}{4} + 0 \times \frac{1}{2} + 0 \times \frac{1}{4} + 0 \times \frac{3}{4} + 0 \times \frac{1}{4} = \frac{1}{2} \end{aligned}$$

$$\begin{split} P_{TEM} &= P_{NPS \& NFs}^{warm} \times P_{NPs \text{ only}}^{cool} + P_{NPS \& NFs}^{warm} \times P_{NPs \& SA-NSs}^{cool} + P_{NPs \& NFs}^{warm} \times P_{SA-SNSs \text{ only}}^{cool} \\ &+ P_{NPs \text{ only}}^{warm} \times P_{NPs \& NFs}^{cool} + P_{NPs \text{ only}}^{warm} \times P_{NPs \& SA-NSs}^{cool} + P_{NPs \text{ only}}^{warm} \times P_{SA-SNSs \text{ only}}^{cool} \\ &+ P_{NPs \& SA-SNs}^{warm} \times P_{NPs \& NFs}^{cool} + P_{NPs \& SA-SNs}^{warm} \times P_{NPs \& SA-SNs}^{cool} + P_{NPs \& SA-SNs}^{warm} \times P_{NPs \& SA-SNS}^{cool} + P_{NPs \& SA-SNs}^{warm} \times P_{NPs \& SA-SNs}^{cool} + P_{NPs \& SA-SNs}^{warm} \times P_{NPs \& NFs}^{cool} + P_{NPs \& NFs}^{warm} + P_{NPs \& NFs}^{warm} + P_{NPs \& NFs}^{warm} + P_{NPs \& NFs}^{warm} + P_{NPs \& NFs}^{cool} + P_{NPs \& NFs}^$$

2.2.5. warm vs. cold

$$\begin{aligned} P_{color} &= P_{w}^{warm} \times P_{c}^{cold} + P_{c}^{warm} \times P_{w}^{cold} = \frac{3}{4} \times 1 + \frac{1}{4} \times 0 = \frac{3}{4} \\ P_{SPR} &= P_{i}^{warm} \times P_{d}^{cold} + P_{d}^{warm} \times P_{i}^{cold} = 1 \times \frac{1}{2} + 0 \times \frac{1}{2} = \frac{1}{2} \\ P_{Time} &= P_{t<1h}^{warm} \times P_{1h\leq t<2h}^{cold} + P_{t>2h}^{warm} \times P_{t>2h}^{cold} + P_{1h\leq t<2h}^{warm} \times P_{1h\leq t<2h}^{cold} + P_{t>2h}^{warm} \times P_{t>2h}^{cold} + P_{t>2h}^{warm} + P_{t>2h}^{warm}$$

2.2.6. *cool* vs. *cold*

$$P_{color} = P_{w}^{cool} \times P_{c}^{cold} + P_{c}^{cool} \times P_{w}^{cold} = \frac{1}{4} \times 1 + \frac{3}{4} \times 0 = \frac{1}{4}$$

$$P_{spR} = P_{i}^{cool} \times P_{d}^{cold} + P_{d}^{cool} \times P_{i}^{cold} = \frac{3}{4} \times \frac{1}{2} + \frac{1}{4} \times \frac{1}{2} = \frac{1}{2}$$

$$P_{Time} = P_{t<1h}^{cool} \times P_{1h\leq t<2h}^{cold} + P_{t<1h}^{cool} \times P_{t\geq 2h}^{cold} + P_{t\geq 2h}^{cool} \times P_{t\leq 1h}^{cool} + P_{t\geq 2h}^{cool} \times P_{1h\leq t<2h}^{cool} + P_{t\geq 2h}^{cool} \times P_{1h\leq t<2h}^{cool} + P_{t\geq 2h}^{cool} \times P_{t\leq 1h}^{cool} + P_{t\geq 2h}^{cool} + P_{t\geq 2h}^$$

$$\begin{split} P_{TEM} &= P_{NPs \ \& \ NFs}^{cool} \times P_{NPs \ only}^{cool} + P_{NPs \ \& \ NFs}^{cool} \times P_{NPs \ \& \ SA-NSs}^{cold} + P_{NPs \ \& \ NFs}^{cool} \times P_{SA-SNSs \ only}^{cool} \\ &+ P_{NPs \ only}^{cool} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNs}^{cool} + P_{NPs \ \& \ SA-SNSs}^{cool} \times P_{SA-SNSs \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs}^{cool} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNs}^{cool} \times P_{NPs \ \& \ SA-SNss \ only}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs}^{cool} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNs \ NFs}^{cool} \times P_{NPs \ \& \ SA-SNs \ NFs}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs \ NFs}^{cool} \times P_{NPs \ \& \ NFs}^{cool} + P_{NPs \ \& \ SA-SNs \ NFs}^{cool} \times P_{NPs \ \& \ SA-SNs \ NFs}^{cool} \\ &+ P_{NPs \ \& \ SA-SNs \ Only \ NFs \ \& \ NFs \ N$$

3. Supporting Figures



Figure S1. (a) The evolution of the color of the nano-Au solution in a *Bi Ba* extract (from the *hot* family), and (b) the intensity of Au SPR peak at 550 nm plotted against reaction time.



Figure S2. TEM images of the nano-Au products synthesized by Bi Ba with different extract concentrations. (a) a fresh stock solution, (b) a 5-fold diluted solution, and (c) a 10-fold diluted solution.



Figure S3. (a) XPS survey spectrum and (b, c) high-resolution N 1s and Au 4f spectra of the nano-Au product synthesized by the *Bi Ba* extract.



Figure S4. EDX spectrum of the nano-Au product synthesized by the *Bi Ba* extract.



Figure S5. Characterization of the nano-Au products synthesized by the TCHMs from the *hot* family: (a1) *Gan Jiang*, (a2) *La Jiao*, (a3) *Bi Ba*, and (a4) *Rou Gui*.



Figure S6. Characterization of the nano-Au products synthesized by the TCHMs from the *cold* family: (d1) *Jin Yin Hua*, (d2) *Pu Gong Ying*, (d3) *Ye Ju Hua*, and (d4) *Shi Hu*.



Figure S7. Characterization of nano-Au products synthesized by the TCHMs from the *warm* family: (b1) *Gui Zhi*, (b2) *Ai Ye*, (b3) *Cang Shu*, and (b4) *Rou Cong Rong*.



Figure S8. Characterization of nano-Au products synthesized by the TCHMs from the *cool* family: (c1) *Tu Da Huang*, (c2) *Bo He*, (c3) *Xi Yang Shen*, and (c4) *Lv Cha*.



Figure S9. A schematic illustration of the overlaps in Qi-related features in the biosynthesis of nano-Au with TCHMs of different Qi properties. The three-dimensional spatial coordinates are used to represent the three measured Qi-related features: (*x*) solution color attribution as observed by the naked eyes, (*y*) solution SPR properties and reaction times as measured by UV-vis spectroscopy, and (*z*) the morphological features of nano-Au as observed by TEM.

4. Supporting Table

Test	Hot	Warm	Cool	Cold
	100%: Warm	75%: warm	25%: warm	0%: warm
Solution color attribute	0%: cool	25%: cool	75%: cool	100%: cool
Time for complete reaction	75%: $t \ge 2$ h 25%: 1 h $\le t < 2$ h	100% : <i>t</i> < 1 h	50%: $t < 1$ h 25%: 1 h $\leq t < 2$ h 25%: $t \geq 2$ h	75%: $t < 1$ h 25%: 1 h $\leq t < 2$ h
SPR peak intensity	100%: Increasing	100%: Increasing	75%: Increasing 25%: Decreasing	50%: Increasing 50%: Decreasing
Morphological forms of nano- Au	100%: NPs & NFs	25%: NPs & NFs 50%: NPs only 25%: SA-NSs only	25%: NPs only 50%: SA-NSs only 25%: NPs & SA-NSs	100%: SA-SNSs (75%: SA-NSs & 25%: SA-NFs)

Table S1: Frequency distributions of various Qi classification attributes from the biosynthesis of nano-Au